Vol. I

APOLLO DIET EVALUATION

FINAL REPORT
NAS9-10955

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VOLUME I

APOLLO DIET EVALUATION

FINAL REPORT

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FORWARD

This is the final report of the Apollo Diet Evaluation conducted at the Department of Biochemistry of the University of Missouri School of Medicine in Columbia, Missouri. This study was done for the National Aeronautics and Space Administration Manned Spacecraft Center at Clearbrook, Texas under contract NAS9-10955 during the period June 7, 1970 to June 7, 1971. The technical monitor of the program was Dr. Paul Rambaut of the NASA Manned Spacecraft Center.

It is a pleasure to acknowledge the valuable contribution of Dr. Ahmed Kotb. His collaboration made possible Volume II which is a critical evaluation of markers in nutrition.

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I. INTRODUCTION

A pragmatic view of conquest of space in manned vehicles suggests there are two major components aside from any given mission and its specific goals. Ecologically, the space craft and the astronauts are the "nature", or in another sense, the hardware; while the food, water and oxygen (air) are the "nurture" (the software) of the system. Both have equal importance. In the success of any mission reliability has been the keynote to the hardware of the U.S. manned space program. All possible contingencies and failures have been examined and the space craft has many-fold factors of safety built into it, while the astronauts have years of training and practice in each anticipated operation. Yet, nurture has virtually no factor of safety and may be marginal, particularly when considered as an integral component of man, food (including water and air) and microbes. This report deals with food and provides a small input to management for a synchronized ability concept to make the software (nurture) and the hardware (spacecraft and man) meet comparable performace specifications. Our previous work (Bengson and Luckey, 1970) indicated that composite Apollo diet was adequate for three generations of classic mice in the open laboratory. However, under a variety of specific gnotobiotic conditions, this diet was found to be nutritionally inadequate (Luckey, 1970). Although the conclusion of this report was that paper analysis of the diets were more exacting than biological evaluation, this same diet fed to dignotophoric mice was inadequate for life.

The approach to the present study is a continued office evaluation of Apollo diets plus experimental work on mice to look at morphologic changes. Herein was developed a new approach for the determination of the exact nutrient intake of astronauts during space flights. A major part of this study will be concerned with this latter concept as an important component of future understanding of the needs and results of space flights. Nutritional safety factors are as essential as are mechanical safety factors. An exact knowledge of the nutritional status of astronauts during space flight cannot be obtained until a good knowledge of the intake of specific nutrients

is made available. The concept of dietary nutritional markers will allow an evaluation of daily nutrient intake to be made on an exact basis. Feasibility studies for this multiple approach to the overall nutritional status of the individual are underway.

II. BACKGROUND

A combination of the lack of emphasis and support for nutritional programs, the changing dietary patterns for inflight feeding, the lack of exact data of food intakes, and the fact that man's total reaction cannot be predicted from experimental conditions or "trial" runs has made the nurture of man in space one of the most critical components of any proposed long manned space project. The possibility of a changed microflora increases the hazard of man in prolonged space flight. This concept is seen in a comparison of the two papers provided in appendix A. The first, entitled "Apollo Diet Evaluation: A Comparison of Biological and Analytical Methods Including Bioisolation of Mice and Gamma-radiation of Diet", indicates that Apollo diet is adequate for mice in isolation and that sterilization of this diet shows no adverse affect. Even here, mice in bioisolation had increased mortality compared to comparable mice fed in the open laboratory. This paper has been submitted to the Journal of Clinical Nutrition for publication. The second paper entitled, "Gnotobiology is Ecology", shows a variety of adverse reactions when the same diet is fed to mice which have a limited microflora. A possible malfunction could be noted in every parameter studied: (a) the body weight of mice was decreased, (b) the growth rate of mice was decreased and stunted adults resulted when certain microflora were fed, (c) the white blood count of some mice was decreased drastically and in others it appeared to be increased compared to the control when different microflora were present and (d) the decreased hemoglobin noted with certain microflora such as E. coli leads to a dramatic suggestion that normal components of our intestinal microflora may contribute to incipient anemia. The anemia was so bad that it was judged to have contributed to the decreased survival of mice. Anatomical changes such as hair loss and change in cecum weight were also noted. The most important parameter studied was survival. Under certain conditions with

relatively innocuous indigenous microflora (i.e., Escherichia coli, Lactobacillus leichmanii or E. coli plus Candida albicans) it was found that only 20% of the mice survived during a two month period compared to 80-100% of different control groups surviving. Such evidence suggest that more studies must be made on nutritional requirements of astronauts under different conditions. This work has also been presented at the Xth International Congress of Microbiology in August in Mexico City; the abstract entitled "Gnotobiologic Evaluation of Apollo Diet" is given in Appendix A.

The importance of this work is indicated in the Introduction above, this gives an overview of the concepts involved. More specifically, this study provides methodology for a correlation of biological and chemical testing more exact than that which has been done before. This will provide evidence of the need for future astronauts to apply good nutritional principles. This information will be utilized to devise suitable corrective measures for malfunction of nurture systems. The most important single aspect of the work was the concept of providing specific data on the dietary intake for each astronaut for any nutrient specified. The feasibility studies of this concept are being examined. This use of nutrient indicators to allow fecal examination for the determination of specific nutrient intake and/or balance has led to a broader consideration of the use of fecal indices of health and disease. This is given as appendix B. If a daily fecal sample can be obtained from each astronaut, dietary markers with specific analyses will give much information to study health and disease parameters in the astronaut. These fecal indices come from three major vectors: the undigested food, the host contribution and the microbic contribution. It is not easy to separate these three vectors; therefore, the use of nutrient indicators is reinforced; for example, it is estimated that live bacterial cells make up 1/3 of the total fecal bulk and that material which has been processed by approximately 10^{11} bacteria per gram makes up 90 or more percent of the fecal mass. This illustrates the difficulty of drawing conclusion from fecal analyses. Nevertheless, ways and means are suggested for evaluating health and disease status of the astronaut through fecal indices. This general concept will be brought to fruition during the course of this report when specific nutrient indicators are used to determine the nutrient intake of animals.

III. STUDIES

1. Fiber in the Diet

Since the previous study with Apollo diet had showed rather dramatic effects when fiber was available to the animals (as bedding), a specific experiment was run to determine the occurrence of these effects when fiber was added to the diet of some mice and not to others. The composition of the syntype diet is: casein, 20%; corn oil, 5%; salt mixture, 3.6%; vitamin mix, 5%; choline chloride, 0.2%; corn starch, 60.2%; and fiber, 6.0%. When fiber was taken from the diet the corn starch quantity was 66.2%. In other words, fiber was substituted for corn starch in the syntype diet. All other details were essentially the same as those reported in the papers, Appendix A. The most interesting results are provided in Table 1. When mice receiving fiber are compared to those receiving none, fiber had no effect upon body weight at the 1% level of significance. It is noted that germ-free animals are somewhat heavier than classic animals fed the same diet whether the classic animals were inside or outside the isolator; this was most noticeable when the classic animals were inside the isolator. When the body weight less the full cecum was considered, no statistical differences were found except between groups 7 and 8; here the actual difference seems to be negligible despite the fact that it was significant at the 5% level. In germ-free animals fed either the Apollo diet or the syntype diet the weight of the cecum full was greater in mice fed no fiber than in those fed fiber; however, the difference between groups 3 and 4 was not significant at the 1% level although it was significant at the 5% level. There were no differences of statistical significance between cecum weights of any mice fed under classic conditions. This same pattern was seen when the cecum was calculated as a percent of body weight; in this consideration the body weight was corrected for the cecum weight. Since most of the cecum weight is cecal contents, the cecal contents showed the same differences as did the cecum full. Finally, the weight of the cecum wall was statistically greater in germfree mice fed Apollo diet without fiber than in those fed with fiber at the 1% confidence level. Other than this the only difference which

was found was that all germfree mice had a significantly higher cecum wall weight than did the classic mice; this was true at the 1% confidence level. A similar pattern was noted when the percent of the empty cecum compared with the body weight minus the cecal contents, the penultimate line on Table 1. At the 5% level of significance the mice in groups 7 and 8 had more hemoglobin than had germfree mice fed the same syntype diet. Incidentally, it should be noted that the hemoglobin values of the germfree mice and the classic mice in isolators seem to be lower than that of classic mice outside the isolator. Particularly for groups 9 and 10, this statement is statistically valid. No significant differences were seen when comparing any of the mice fed fiber to those strict controls fed no fiber in the following parameters: 1) intestinal length; 2) intestinal length per unit body weight; 3) intestinal length per unit body weight corrected for the cecum content; 4) hemoglobin content of the blood or 5) body weight.

2. Paper Evaluations

Most reports indicate weight loss and possibly calcium loss as being the two major problems in astronaut flights (Berry and Smith, 1969). The dehydrated foods of early Apollo food systems required one hour or more to prepare each meal. On this basis one reason for the weight loss could be a low calory intake due to excessive time for meal preparation. Therefore, the study on food preparation time was made (Appendix C-1) to determine whether an astrochef would be an asset for any flights. Note in Table 1 of this report that the total meal preparation plus eating time for early Apollo flights would be approximately 6 hours per day, for seven days per week. On an 8 hour work week, it would be impossible to do the work required and to spend three hours each day in food preparation. Therefore, it was assumed that the astronauts would be working either a 60 hour or an 84 hour week. Calculations were made for both of these. As shown in the figures, an astrochef would provide a net work efficiency on a 40 hour week if only two persons were in a flight team. He would probably provide more work if two persons were working a 60 hour week because he

would probably do more than simply be a chef. An astrochef would probably provide more work with a 3-man crew on an 84 hour work week. However, if the efficiency of food preparation changed from 3 hours per day to 2 hours, then the astrochef would be equivalent to the fifth astronaut as far as work efficiency is concerned. If a high efficiency of one hour per day for three meals plus snacks could be obtained, then a chef would not be efficient until a 10-man crew were assembled. Figures 5 and 6 provide a different way of looking at the same patterns and illustrate the work weight equivalent on a per man basis when an astrochef is added. It was concluded that an astrochef would be a savings of weight for any flight with a crew of more than four if the total days food preparation took only two hours. If three hours were involved then a chef on board would provide more work when the total crew, including the chef, were three persons. This illustrates another advantage of recent changes to TV type and wet pack foods. A work efficiency study with these new diet patterns has not been made; the study given in Appendix C-1 shows how such a problem could be approached.

In another study it appeared that potassium, iron and calcium might become a limiting factor in astronaut efficiency during prolonged space flights. Therefore, a study of potassium, iron and calcium in Apollo diets was made (see Appendix C-2). One assumption made in this study is that the food intake is accurately known. This is a problem which has not been resolved because it appears that on some flights some astronauts report their food intake very conscientiously while on other flights the food intake is not verifiable. Using this data as the best available, it became obvious from this report that the low potassium, high sodium diets could cause a toxicity from excess sodium. The loss of potassium may represent tissue damage or it could cause tissue damage if it were continued very long. The iron imbalance could lead to severe anemia on flights of more than two months. Surprisingly, the intended diet was low in calcium and had a low calcium-phosphorus ratio. This combined with the low calcium intake suggested that the diet as designed and as taken was very poor for maintaining calcium balance. It should also be remembered that potassium and sodium as well as calcium and phosphorus are important

in neuromuscular junction reactions; therefore, it would be anticipated that the low calcium might increase the neuromuscular irritability and the irritable response to sudden stimuli by subjects fed this diet. Calcium is important in muscle contraction as well as nerve stimulation and in bone composition. This particular study suggests that it is important to determine much more accurately the nutrient intake of each astronaut and that their diets be fortified and monitored for reliability under conditions other than ideal before prolonged space flights are undertaken.

All available data on the nutrient intakes of astronauts in flight was utilized to form an opinion regarding which were satisfactory and which were not. Using criteria discussed above and in the report entitled "Apollo Diet Evaluation" (Appendix C-3), eighteen astronauts were given a nutritional rating. Data from flight No. 12 and later flights were not available; the best flight appeared to be No. 11 and the worst No. 8, from a nutritional viewpoint.

3. Nutrient Markers

The problems of nutrient imbalance, getting maximum utilization of food for astronauts and logistics to determine how much food is needed for any given flight makes it imperative to determine nutrient requirements and the exact food intake in astronauts. Individual reporting, the counting of food packages and the total food balance of a space ship do not provide undisputable evidence of the food and nutrient intake of any given astronaut. Therefore, a method of nutrient indicators was suggested and was accepted in principle. Feasibility tests are being run. The concept and an outline of the preliminary work and experimental background for safety and human use was presented on February, 1971 (see Nutrient Indicators in Appendix D-1). When it was determined to undertake experimental work in this area, the Nutrient Indicator Feasibility Protocol (Appendix D-2) was submitted. This has formed a guide for experimental work.

The preliminary work was aimed at use of heavy metals which could be analyzed by activation analysis. It was thought that a single analytical procedure for multiple elements would give the most efficient and least expensive method for the overall work. Although this concept has not been tested thoroughly, we are presently searching for acceptable markers which

may be analyzed by any reasonable method.

Seven separate experiments with rats have been completed; the analytical data is not yet available. 1) The first is the use of a fecal marker. Brilliant blue dye was fed to two rats by placing o.5% of the dye in the drinking water, ad libitum, for five weeks; this is approximately 10 times more than would normally be used for one day. At the end of five weeks the rats were sacrificed. The dye was not appreciably absorbed as judged from the lack of color in fat deposits and from the natural color of the internal organs, muscle, blood and urine. Digesta in the gastrointestinal tract was deeply marked with the blue dye and the lining of the mouth, the cardia stomach and the ileum seemed to have adsorbed some dye. Since this could be washed out by two minutes of running water over the tissue, it did not seem to be absorbed into the cells lining the tract. This simple experiment suggests that brilliant blue dye would be one of the best colored markers to begin or to end an experiment.

Cerium was developed as the collection marker. We had considerable difficulty getting this material into solution and in order to have good data a method was devised by which a known amount of the collection marker could be given each day to rats. This method is to feed the rats ad libitum twice a day for two hours. They were fed from 9 to 11 in the morning and at night. However, before they were given any food, they were given a small pellet containing a known quantity of the collection marker. This pellet must be eaten before the animal received any other food; after eating this, the food was provided. The rats learned to eat this pellet fast after one feeding. A variety of nutrient markers can be used. Each is placed into the diet in proportion to a given nutrient which one desires to follow or to make a balance study. 2) The second experiment involved the determination of the recovery of one dose (25γ) of known quantity of each element. All feces were collected for six days following ingestion of this material and the samples submitted to activation analysis. 3) The third experiment was to determine the stabilization point of the excretion of markers given twice daily in the food (see the Balance Study Model figure at the end of Appendix D-2).

This experiment also was run six days (it was anticipated that equilibrium would be reached at 3-4 days). The samples are being analyzed. The data obtained will give the slope of the curve indicating the amount of marker excreted and the break in that curve to indicate when the excretion pattern could be considered to be stable. 4) The fourth experiment was a simple balance study during the collection period of 6 days. 5) Incorporated into this study was the fifth study which was to test three diets with variable compositions. In experiment five, a classic balance study with complete collections were made and data from these will be compared with results from the marker study. This should give an index of the reliability of the nutrient indicator concept. 6) The sixth experiment was a modification of the fourth experiment; however, instead of having a six-day collection period, a one-day collection period was made on six individual animals. We will determine whether or not a one-day collection period with six animals will give reliable data. This data will be compared with those obtained on the six days immediately preceeding. If one day will give adequate results, it will greatly simplify experimental work with this method. 7) The seventh experiment was a determination of diurnal variation during a three-day period. This experiment should help to determine at what time of day collections should be made and for how long a period would any collection be representative of the total day. All of the animal work for the seven experiments is completed; however, the analytical data has not been obtained. Therefore, these seven experiments will be reported at a later date in composite report. The summary of the two nutrient indicator conferences held on this campus are given in Appendix D-3. This will illustrate the fine collaboration which we are getting from the University of Missouri Nuclear Reactor Facility with Drs. Jim Vogt and Mike Kay.

A list of suggested nutrients to be marked and the markers which might be tried is given in Table 2. It should be noted that analytical methodology has been ignored and only about 1/3 of these have been utilized in studies thus far. A complete literature search with information on toxicity, absorbability, ease of analysis and utilization of individual markers is needed.

4. Literature Review of Markers in Nutrition

A thorough review of markers in nutrition was made and is submitted as An Evaluation of Nutritional Markers (Appendix E). It is comprehensive in that all articles on the subject have been evaluated; many application articles

have not been incorporated into this review. The review provides information on all compounds which have been used as fecal markers in nutrition and all papers of importance from a theoretical or methodology viewpoint are considered. This review gives an evaluation of methods, markers and procedures to the extent that it is possible. This review has been accepted for publication by Nutrition Abstracts and Reviews. It will probably appear in two parts. Part 1 in the January 1972 issue and Part 2 in the April issue. It is appended as Volume 2 of this report.

The review indicates that few markers have been shown to be quantitatively accurate and few have been thoroughly examined. This adds considerable significance to our feasibility studies as providing a theoretical base and a practical system for future work in this area.

IV. RECOMMENDATIONS

Astronauts on short term flights usually showed a loss of weight and occasional dehydration. The possibility of a low calory, low calcium, low potassium and low iron diet has made nutrition a major consideration for longer space flights. Although many nutritional difficulties could be proposed on prolonged space flights (for example, microbic shock) it is difficult to assess exact nutritional problems because food intake records are totally inadequate for any accurate estimation. Therefore, a method has been proposed which will allow the intake of the total amount of food or any given nutrient to be monitored accurately. The method is simple, in principle, the nutrient or the total amount of food simply carries an indicator which is not absorbed and meets other criteria for nutritional markers. A collection marker must be ingested twice each day and the feces should be preserved from each day for each astronaut. Analysis of the feces for the collection marker will tell what proportion of the food and feces is represented from the collection of each day and how much of each nutrient under consideration was taken in accordance to each nutrient indicator. A variety of 15 nutrient indicators would be made available in ppm quantities in the diet. Presently, one (1/10 to 10) ppm of these indicators can be analyzed. It is anticipated that this quantity might decrease by 1 to 3 logs as the state of the art improves.

Feasibility tests with rats are presently under way and a complete literature search has been finished as a part of Vol. II of this report. The characteristics of a variety of markers have been examined. The background data for specific markers was obtained. The marking of food has yet to be designated. There should be experiments with toxicity with mice as indicated on page 4 of the measurement of nutrient intake given in Appendix F1. Further work needs to be done as noted on page 4 with monkey feasibility and with human feasibility as noted on page 6. If these basic experiments are completed satisfactorily, then it will be judged that the method is applicable for use by man in space. For the information suggested, a complete nutritional understanding of the astronauts could be obtained. This information would be very useful for individual astronauts who may be in trouble or who are anticipating problems on prolonged space flights, and for the logistics of longer space flights. This would stem from a more exact knowledge of the amount of calories needed, and nutrients (i.e., protein) needed, the efficiency of the utilization of calories and other nutrients, and the waste vs. storage capacity needed for the craft. Although 15 nutrient indicators have been suggested more or fewer could be utilized. An individual marker could be incorporated into the sample to simplify human recording.

The specific nutritional problems of an astronaut during two ± one month space flights is outlined as Appendix F-2. In retrospect it is noted that the 1964 Tampa Meeting of the Conference on Nutrition in Space and Related Waste Problems held at Tampa considered the nutritive requirements of the lunar space flights of one to two weeks duration. The general conclusion was that the nutritive intake of the astronauts could vary considerably and was not particularly of importance on such a short term flight. Now that we are on the threshold of flights of one to three months, it is imperative that another conference of nutritionally recognized experts be held to consider the needs of man in space for 1-3 months. Following this, predictably another conference should be arranged for man in space for one to two years. There is need for a conference involving the man-food-microbe interrelationships which should be held with our Russian counterparts. Now, at the end of the Apollo flights, is an appropriate time to propose that the American and

Russian working scientists would relate to each other in conference and in publication, the methodology, materials used, the problems and results from space flights thus far. This should include the evolution of food types used, food preparation, nutritional standards and food presentations. Interrelationships should be presented on the microflora and waste problems, microflora studies of man in space and whatever is known of the bacteriology of food, water, waste and the space vehicle. This would make a good two-week conference with one or two monographs being published from the conference. It is proposed that this be held during the next fiscal year and that the Americans provide the major cost for five days in Helsinski, while the Russians would provide the cost for five days in Leningrad. If more than one year elapses, the personnel involved would not be readily available, the data will be largely forgotten, or misplaced; therefore, preliminary work on this meeting should begin within the next few months.

Finally, it is recommended that a short course in nutrition be incorporated into all astronaut training programs. A 10 hour course is outlined in Appendix G. This would provide each astronaut with basic information about the utilization of foods during the flights and provide a base for communication with the nutrition staff. Although this was of little importance for flights of 1-2 weeks, it becomes important for flights of one or more months.

V. SUMMARY

Manned space flights of 1 - 3 months duration will demand much more preparation in the areas of food, water and oxygen(the nurture of the system) in order to bring the reliability of this part of the program equal to the man-machine(the nature) component of the program. Previous work has indicated that present diets are satisfactory for short term flights as long as no contingencies arise. Present work also indicates that there was virtually no safety factor built into the Apollo diet program. The first steps in thwarting any and all possible malfunctions are to learn what is presently being utilized and what is required. Unfortunately, the records from Apollo flights are poor. The excreta provide a good index for health and disease which can be used to determine the nutrient intake of and/or balance of each astronaut. Using this as a data base, one could then predict

what might be needed for long term space flights with good reliability.

Experimental work with the Apollo (1968) comminuted diet indicated that this diet was marginal for anything but short term flights. A study with fiber added to this diet showed that certain intestinal parameters such as the wall of the cecum and the cecum size of mice are affected favorably by the addition of fiber in the diet. A series of paper evaluations suggested that one reason for the deficient caloric intake on the early Apollo diets was the excessive food preparation time demanded by the system. A second study suggested that the diets as designed and as eaten were deficient in potassium, iron and calcium. Using the information available, a nutritional evaluation of Apollo diets was made; the best appeared to be Apollo 11. Data from Apollo 12 and 14 were not available. Nutritionally, Apollo 8 was the worst.

Since there is a serious problem involved in getting reliable food intake data, a new system for obtaining this information was proposed. This system involves the addition of a nutrient marker in the parts per million range for each nutrient to be studied. In addition to this, a daily intake marker would be utilized. This intake marker could be individually identified in order to save astronaut marking of fecal bags. After obtaining daily samples of the feces from each astronaut, the indicators would show how much of any given nutrient was taken for that day and from this information a balance study could be run. The variety of indicators which are under study include heavy metals which are virtually not absorbed and do not affect the utilization of dietary ingredients. Feasibility studies with rats are underway with 7 experiments. The first is an evaluation of brilliant blue dye as a fecal marker. This seems to be quite satisfactory. The second is the study of the recovery of a single dose of a known quantity of 5 markers. The third is the determination of the number of days the marker must be given before the excretion is stabilized when the marker is given twice daily. The fourth experiment is a simple balance study to compare the marker system with the classic gross analysis system. The fifth is a study with variable composition of diets to be sure that the markers will detect changes in the diet. The sixth experiment is a one day collection period to see if data from it gave comparable reliability to a six day collection. The seventh experiment was a study of diurnal variation.

When these experiments are finished, it is anticipated that toxicity studies in mice and monkeys will be made. Satisfactory results in these two tests would lead to taste testing, recovery testing, and further feasibility testing in humans prior to use in Apollo flights.

It is recommended that these feasibility studies be completed. It is also recommended that a meeting be held for an evaluation of the nutritional problems which might arise in a one to three month flight. It is also recommended that a Russian-American collaboration be made to record the progress in diet, the interaction between diet and microbe in man, diet and microbe in food, diet and microbe in waste, and man and microbe. This conference should take approximately two weeks and should result in two monographs at the close of the Apollo program. Finally, it is recommended that the astronauts have more information about nutrition for good rapport between the astronauts and the nutrition personnel. The goal for future manned space flights is to make diet as reliable as all other components of the spacecraft with safety factors and crew understanding comparable to those existing for astronaut-machine capabilities.

TABLE I

EFFECT OF DIETARY FIBER IN MICE

State	GERMFREE				CLASSIC					
Environment	ISOLATOR				ISOLATOR		OUTSIDE			
Diet	APOLLO		SYNTYPE		SYNTYPE		SYNTYPE			STOCK
Diet Sterile	YES		YES		YES		YES		NO	NO
Fiber in Diet	NO	YES	YES	NO	YES	NO	YES	NO	YES	YES
Group Number	1	2	3	4	5	6	7	8	9	10
Mice/group	8	7	4	5	5	6	5	6	5	6
Weight, gm	28.3	26.8	27.8	29.1	23.1	21.6	25.4	24.8	23.7	23.3
Body Less Cecum, gm	24.4	24.4	27.9	26.8	22.9	21.9	25.2	24.4	23.5	22.6
Cecum, Full, gm	2.86	1.87	1.71	2.25	0.25	0.31	0.24	0.31	0.21	0.45
Cecum, % of Body	10.81	7.09	5.95	8.38	1.04	1.32	0.80	1.25	0.88	2.00
Cecum Wall, gm	0.25	0.17	0.19	0.19	0.08	0.10	0.10	.009	0.08	0.17
Cecum Wall, % of Body	0.94	0.64	0.42	0.70	0.40	0.42	0.34	0.37	0.36	0.73
Hb, % of Blood	13.0	11.8	13.5	12.6	13.6	13.6	14.9	15.0	15.4	15.3

TABLE II

Suggested Nutrients to be Marked and Markers

Nutrient	Marker
Calories	Yb-Ytterbium
Protein	Au-gold
Fat	Eu-Europium
Carbohydrate	Dy-Dysprosium
Ca	Tb-Terbium
P	Ho-Holmium
Mg	Er-Erbium
Fe	Lu-Lutetium
Cu	T-Titanium
Na	Sc-Scandium
K	Ir-Iridium
B_{12}	Gd-Gadolinium
Folate	La-Lanthanum
Cr	Zr-Zirconium
Collection	Ce-Cerium
H_2O (in food)	PEG-polyethylene glycol
Fecal	BB-Brilliant Blue
Others	Cr-Edta=Chromium EDTA
	La-Lanthanum
	Y-Yttrium
	Ru-Ruthinium
	Cr_2O_3-Chromic oxide
	ZrO-Zirconium Oxide
	T_iO-Titanium oxide
	$BaSO_4$-Barium Sulfate

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VII. APPENDICES

APOLLO DIET EVALUATION: A COMPARISON OF BIOLOGICAL AND ANALYTICAL METHODS INCLUDING BIOISOLATION OF MICE AND γ RADIATION OF DIET[1]

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SPACE DIET EVALUATION

Abstract

Previous inadequacies in the evaluation of practical diets suggested a comparison of analytical and biological methods should be made for Apollo diet. Rations eaten during 34 man-day preflight training exercises in 1968 were compiled and averaged to give a representative Apollo diet. Nominal flight foods were ground and comminuted before being packaged under vacuum in polyethylene bags which were then sealed in nitrogen filled cans and 2/3 were sterilized with 5×10^6 rads of cobalt gamma radiation. Analyses indicated 21% loss in histidine occurred during the radiation sterilization of this dry diet; other nutrient losses were less than 12%. A comparison of the analyses to recommended allowances for man and mice suggested the diet would be adequate for man for a short time. Since mouse requirements are proportionally higher, the diet would be expected to be grossly inadequate for mice in both mineral and B-vitamin content. However, feeding mice exclusively this diet for three generations showed that the diet was adequate to maintain life, reproduction, lactation and good general appearance. Growth was slow in the first generation mice; subsequently it was somewhat better. Food efficiency patterns changed with each generation and were generally low. General observation indicated the alopecia and skin problems of first generation mice in isolation were not found in the second and third generation mice. Mice fed non-sterilized Apollo diet showed more susceptibility to infection with histologic evidence of infection and to anemia than did mice fed the irradiated diet. Despite the fact that calculations indicated that the diet would be grossly inadequate for them, mice survived when fed Apollo diet. Thus the analytical evaluation of Apollo diet is more rigorous than is the biological evaluation. This suggests that safety factors incorporated into some nutrient allowances are high for classic mice in the open laboratory. Although feeding γ radiation sterilized diet showed no adverse effect, bioisolation of mice appeared to increase mortality.

The evaluation of practical diets by comparing the quantitative nutrient requirements of the species to be fed with the analytical data taken from the diet is a logical, inexpensive and much used procedure. However, it involves the following important and potentially precarious assumptions: all nutrients required by the species to be fed the diet are known; all nutrient quantities are revealed by the analytical data; the conditions of use are not meaningfully different from the conditions used to establish the nutrient requirements within the limits of the safety factor built into the recommended allowance used; and the availability of each nutrient is, or can be adjusted to be, equivalent in the analytical work to that of the species being fed. The concept is restated: Are our analytical methods adequate to give assurance of biological value without bioassay?

Historically, 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 (1). Tables of the protein, carbohydrate, lipid, ash and energy content of feeds were accumulated and used widely. However, discerning experimenters began to doubt the validity of this concept[2]. During the first decade of this century the work of Hopkins in England and Osborn and Mendel of U.S.A. showed that knowledge of the total nitrogen of feeds did not provide a reliable index of the biological value of proteins because it did not reflect the content of individual essential amino acids. During the next two decades it was found that proximate analysis did not reveal the presence of essential vitamins and trace elements. Thereafter food tables were developed which included amino acid, vitamin and mineral (including some trace elements) data. The concept was still premature as shown by the dramatic failures which resulted from feeding U.S. Army K rations to rats by Silber[3] and Lepkovsky (2) and rats and monkeys by Register et al. (3). One fallacy was revealed when Snell (4) showed that elements of the vitamin B_6 complex were more than one thousand times more active for certain assay microorganisms than for animals.

This background made it important to evaluate astronaut diet biologically. The diet was designed to be adequate according to calculations from food analysis; it was developed for Apollo flights and was a vital part of the continuous development of space food for the future. But was it biologically a complete food for mammals? Are our knowledge of nutrient requirements and our analytical methods adequate to give assurance of biological value without bioassay? This question forms the conceptual basis for the major hypothesis tested here.

A second hypothesis is involved: prolonged isolation may cause a simplification of the flora in the intestine with the formation of a dominant flora which will markedly alter the nutritive requirements of the host. The base for this hypothesis has been previously reviewed (5). Therefore, it was pertinent to feed Apollo diet to classic animals which were maintained in bioisolation using gnotobiotic techniques. Mice were chosen as the test animal and a variety of parameters of health were used in the biological evaluation of Apollo diet for three successive generations under three conditions: a) in the open laboratory with untreated diet; b) in the open laboratory with irradiated diet; and c) in strict bioisolation. Mice in bioisolation were provided with sterile air, water and food, and groups of control mice were fed the radiation sterilized and untreated diet under open laboratory conditions. Control groups of mice were fed a laboratory chow. This communication provides the base for other reports on the effect of specific microbial floras upon mice fed Apollo diet.

METHODS AND MATERIALS

Diet

The formulation of a composite Apollo diet was made from the daily average of rations eaten during 1968 preflight tests by astronauts, W. C. (4 days), J. K. (10 days), V. B. (10 days) and J. E. (10 days). The amount of each item eaten gave the relative quantities to be used in the composite Apollo diet (Table 1). Each item was prepared according to production guide specifications using refrigeration for any storage of igredients or product (6). Adequate quantities of each item were granulated to pass a No. 20 mesh and blended under nitrogen. This gave

a mix with particles small enough to prevent mice from picking individual parts. This comminuted Apollo diet[4] was granular and light brown in color; it packed easily, tasted sweet and was readily accepted by mice. It was somewhat hygroscopic. The granulated diet was vacuum packed in 400 ± 4 g quantities into polyethylene bags; these were sealed under nitrogen and placed with wax paper packing into No. 2 1/2 tin cans. The cans were packed into cartons and frozen to -45°C for storage. All shipping was completed with continuous cooling below 0°C under insulated blankets.

Some groups were fed sterile diet in anticipation of feeding germfree and microbially associated gnotobiotes the Apollo diet. Available techniques for sterilization were steam under pressure, dry heat, filtration, chemicals and radiation. Filtration was not acceptable because the diet was not soluble; dry or wet heat would be expected to destroy more vitamins and amino acids than does radiation (7); and chemical sterilization could cause many reactions giving harmful products (8). Therefore, radiation was the method chosen for sterilization of Apollo diet for those mice to be fed the sterile diet. We have previously used 3×10^6 rads of γ radiation to sterilize syntype diets for mice, lambs (9), and chicks (10). Since a curve showing the extent of radiation death of bacteria may "tail" near the 100% kill area, spore strips[5] were placed within cans with simulated Apollo diet and irradiated to provide information on the reliability of the dose chosen. The data from 3 series of resistant spore strips (figure 1) shows that the non-irradiated strips were found to have 10^3 bacteria each when standard bacteriological procedures were used to grow the spores on thioglycollate agar to count the resultant colonies from surviving individuals. The curves show good agreement with each other, no tailing was noted and the frequency with which zero counts were observed with the several relatively low doses of radiation suggested that tailing was not of major importance. Serendipitously, those data fell into the most meaningful range because the comminuted Apollo diet was found to have a low microbial content: 1×10^3 microorganisms per gram by us and 5×10^3 by others[4]. Thus, excepting possible effects of nutrients upon the spores in the dry Apollo diet, it was reasonable to expect the diet could be sterilized with 1×10^6 rads of Cobalt gamma radiation. This conservative estimate was also obtained by extrapolating

a line which incorporates the three points from the most resistant spores. When the data were used in a calculation of death values and the sterilization dose using the method of Bruch et al. (11) a value of 1.2×10^6 rads was obtained. This agrees well with the data showing no survivors at 0.8×10^6 rads. When about 1/2 g of irradiated, simulated Apollo diet was placed in 10 ml of enriched culture media the data showed that 2×10^5 rads was inadequate to give sterilization while 1×10^6, 3×10^6 and 5×10^6 rads produced a product from which no viable microorganisms were recovered. This preliminary work suggests that 5×10^6 rads gives a 5-fold safety factor for Apollo sterilization.

Each can of diet to be sterilized was heat sealed in a polyethylene bag and this placed into a second polyethylene bag which was taped shut. The outer bag provided a high degree of cleanliness to the inner bag which must later be sterilized with peracetic acid for the mice reared in bioisolation. Spore strips were placed into the center of the diet in five cans which were subsequently resealed without nitrogen and replaced into cartons. This diet was then treated with $5.4 \pm 0.8 \times 10^6$ rads of γ radiation from a 500 K Curie source. The temperature of the diet rose from -64° C to 5° C during the 55 minute exposure (plus 7 minutes to stop the radiation, manually turn the cases in order to obtain a more uniform dosage in all cans, and bring the source into radiation position). Incubation spore strips in culture media or feeding the diet to germfree mice (to be reported later) showed the diet was free from all viable microorganisms.

Analyses[6] of non-irradiated and irradiated Apollo diet provided information about the effect of radiation sterilization upon nutrients in a dry diet and provides data for comparison with the quantitative nutrient requirements of mice and men. Mice fed non-irradiated Apollo diet were the control for groups fed irradiated diet in gnotobiotic or open environments. Other control mice were fed untreated or steam sterilized laboratory chow[7] in different environments as indicated. Food, in conical ceramic feeders, and water were available to the mice ad libitum. All water was deionized, autoclaved and stored in "hard" glass bottles. The waterers were rubber-stoppered, glass bottles with stainless steel nipples; they were changed weekly. Samples of water drawn periodically from the water bottles gave resistance values from 0.2-3 million ohms.

Animals

Each regimen was started with 20 weanling white mice[8]. Groups of three females and two males were placed together in standard polycarbonate cages fitted with stainless steel wire lids. Expectant and lactating dams were provided individual cages. Two regimens were maintained in the open laboratory in standard animal racks: one received radiation sterilized diet, the other non-irradiated diet. Their cages were fitted with stainless steel wire floor raised about 1.5 cm and having 6 mm openings and were changed weekly. Animals in the third regimen were housed in a standard germfree polyethylene isolator fitted with neoprene gloves, a sterile port and air filters; the sterile polycarbonate cages of these mice had no raised wire floor and the cages were changed daily excepting for food efficiency studies or suckling litters. This daily change to sterile cages was intended to keep the mice clean, dry and free from any extrinsic source of microbes other than freshly voided feces. Coprophagy was thus encouraged as a means to help them maintain a normal flora in bioisolation. Although classic mice were placed into these isolators, strict gnotobiotic procedures were followed: the isolator, cages, food, water, air, and all materials were sterilized. Bacteriologically they were as isolated as astronauts in space ships. The mice fed laboratory chow were maintained on sterilized bedding in the polycarbonate cages. All mice were maintained on a 12 hour light cycle, at a temperature of $26 \pm 3^{\circ}$ C in air-conditioned trailer-laboratories.

Food efficiency data were obtained during a 5 day period after one week acclimatization to the diet. Weighed quantities of food were placed in conical feeders which were placed in 15 cm Petri plate tops. Aluminum foil was placed under the 1 cm wire mesh floor of cages having this floor. This provided adequate provisions for the minimizing of waste and the collection of waste 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 food presented. The total food consumption and the weight increment of the mice provided the data needed to obtain the food efficiency. Food efficiency = $\frac{\text{g gain x 100}}{\text{g food}}$. Standard procedures for observations of health, autopsy, histology, hematology and immunology were followed.

Brachial blood was collected under anesthesia. Autopsy of 5 males, with an occasional female, was performed at 60 days of age for the first generation, 90 days for the second and 30 days for the third generation. Statistical data were taken from those animals which were within 3σ of the means. Probability values (P) were obtained from calculated t values.

RESULTS AND DISCUSSION

Diet Analyses

Radiation-sterilized comminuted Apollo diet was indistinguishable from non-radiated diet by appearance, taste or odor. A pilot study with 17 individual food items indicated only in one-fourth could irradiation changes be detected by a taste panel[6]. Triplicate proximate analysis of a single sample of comminuted Apollo diet (Table 2) indicates water, fiber and ash to be low when compared to our previous experimental diet (7). The first two items were low by design and the low ash was examined further through its constituents. The total protein and calorie content appear acceptable for either man or mice, the 1800 Calories contained in 400 g diet is more than many astronauts have eaten (12). As expected, irradiation caused no significant change in ash, fat, fiber, nitrogen and water.

Elemental analysis indicated that no significant loss of major elements occurred during the radiation procedure (Table 3). When the quantity of each element in the diet is compared to the recommended allowances given for man (13)(assuming about 400 g with about 1800 Calories are consumed per day) the quantity of Mg may be low for prolonged flights. When a like comparison is made for the mouse (assuming 4 g of food were provided daily), several elements appear to be very deficient. These are listed with the increase needed to bring the element to the mouse recommended allowance (14); phosphorus, 3 fold; calcium, 3 fold; iron, 8 fold; copper, 2 fold; cobalt, 140 fold; and manganese, 9 fold.

Amino acid analyses of the Apollo diet by gas chromatography indicated that a low loss generally occurred during radiation sterilization (Table 4). Histidine, threonine and arginine losses were 21, 11 and 10% respectively. Losses in all other amino acids were less than 10%. Tryptophan was not determined because an acid hydrolysate was used. The increase reported for proline was confirmed by column chromatography of two other samples by a different laboratory. When the amount of essential

amino acids recommended for man (13) is compared to the amount in 400 g of diet, no serious differences are noted. Methionine is not seriously low when 1/3 of the cystine-cysteine values are included. When the percentage of amino acids in the Apollo diet is compared to the percent "required" by mice using rational consideration (15) (no amino acid recommended allowances for mice are available), the isoleucine, methionine-cysteine-cystine and valine contents of the irradiated diet appear to be somewhat low. The phenylalanine requirement seems to be adequate when 1/3 of the tyrosine is added. Threonine is questionable. Excepting tryptophan, all other essential amino acids appear to be present in adequate amounts on a "percent of diet" basis.

Analyses of representative vitamins (Table 5) indicated that riboflavin was the vitamin most labile to gamma-radiation under the conditions described. The 11% riboflavin loss is of minor significance when compared to vitamin losses generally noted with heat or chemical sterilzation of diets. Radiation changed 10% of the reduced ascorbate into the dehydro form. As reflected by the lack of change in total ascorbate, the increase in dehydroascorbate mirrored the decrease in reduced ascorbate. The stability of such easily oxidized compounds as vitamin A and ascorbic acid and such radiation labile compounds as riboflavin, pyridoxine and thiamin during gamm-ray sterilization of the dry diet suggest that a minimum of free-radical oxidation occurred with amino acids, fatty acids or other compounds. The dryness of this diet undoubtedly discouraged chain reactions and other chemical reactions which occur readily when more moisture is present or is added immediately after irradiation.

When compared to the recommended allowances for man (Table 5), the vitamin content of 400 g of Apollo diet appears to be marginal in vitamin A, riboflavin, thiamin and vitamin B_6 and low in folate. This would be expected to have little effect on a mature man during short space flights. When compared with the allowances suggested for the mouse, 5 g of Apollo diet appears to be seriously low in riboflavin, thiamin, vitamin B_6, pantothenate and folate.

Animal Observations

The survival, appearance, reproduction and lactation performance of the mice (Table 6) indicates that mice in isolation lived the most hazardous existence. Survival was 97% in the open laboratory with mice fed radiation sterilized Apollo diet, 85% with mice fed the non treated diet and only 40% in mice in bioisolation. When the floor became wet (with no puddles) on two occasions, some mice in isolation died within 1-2 days although no infectious disease could be established from blood or organ cultures. This experience is also noted in the mice fed laboratory chow. Mice fed the non-treated Apollo diet looked well in the first generation (in contrast to the histology report) but several individuals were visibly ill and died in the second generation. This pattern contrasts strikingly with the sudden death of all animals in one group (with no previous symptons) which occurred in bioisolation. The general appearance of the mice was generally good in the open laboratory while first generation mice in isolation appeared wet much of the time; some became almost completely nude and had considerable skin erythema with mild edema. After filter paper bedding was added, the mice in isolation improved their appearance considerably. Reproduction was generally slow in the first generation mice fed the non-treated Apollo diet. The litter which survived longest did not appear to be healthy; their appetite was poor and several died between the first and second month of life. The poor reproduction in animals fed the non treated diet made it necessary to transfer young mice from group 9 to continue the experiment for non-treated diet in the open laboratory for the second generation. This information plus autopsy observations indicated that mice fed the non-treated diet appeared to be less healthy than those fed the gamma irradiated diet in the open laboratory. Newborn mice of group 8 survived poorly but the number weaned was adequate for continuing successive generations. Reproduction of mice fed laboratory chow was not better than that of mice fed Apollo diet. Subsequent generations were not continued for these groups.

Growth and Food Efficiency

The mice fed laboratory chow grew satisfactorily (Figure 2a). Both males and females fed autoclaved diet consistently grew at a slightly decreased rate when compared to those fed untreated diet. The growth

of all mice fed Apollo diet was somewhat slow the first week of the experiment. It was acceptable during the second week for mice reared in the open laboratory but it remained slow for mice reared in isolation (Figure 2b). Thereafter, the males in isolation appeared to grow satisfactorily while the females did not; the high mortality in this group may have skewed the growth data in favor of the heavier males. The females appeared to be stunted and the surviving males lost weight at 2 months. In the open laboratory there appeared to be no essential difference between mice fed the irradiated diet and those fed non-irradiated Apollo diet. The growth of second generation mice showed a different pattern of response (Figure 2c); the mice in isolation were heavier at weaning and maintained their weight advantage throughout the experiment. Second generation mice fed the irradiated diet in the open laboratory generally grew less well than those fed the non-irradiated diet.

The food efficiency data (Table 7) indicated the non-irradiated Apollo diet was utilized more efficiently than the irradiated diet. Mice fed Apollo diet grew more and showed much better food utilization than did mice fed laboratory chow; unfortunately the latter mice had finished their growth spurt by the 28th day when the food efficiency experiment started (see Figure 2). Technical difficulties prevented accumulation of precise data from the second and third generations.

Autopsy and Histology

In general all of the organs appeared to be normal at autopsy excepting the skin of the first generation mice which were reared in isolation. Histologic examination of the epithelium showed that there were mild acanthosis, acatholysis, hyperkeratosis, parakeratosis, and spongiosis. The skin of isolated mice fed the same diet in the second and third generations were normal. The first generation mice appeared to be continually wet although no water accumulated on the floor excepting as noted and each group was placed in a dry, clean cage every day. The first generation mice started on May 20, the second on September 4 and the third on November 18; the relative humidity appeared to decrease with each generation. Filter paper bedding was added to all mice on October 13 when the second generation dams gave birth to litters which were used in the 3rd generation study. Both humidity and filter paper may have had a profound influence upon the skin condition

and other parameters. The third generation mice reared in isolation had the same good appearance as those reared in the open laboratory. All mice fed the laboratory chow appeared to be healthy throughout the experiment excepting the one group which became wet and died. The dead mice showed multiple infection from cultures of blood and lungs.

The ovaries of animals in group 10 were somewhat small but showed no pathology. Livers of all animals appeared to have normal histological appearance. The mucosa of the ileum, cecum and lymph nodes of the large intestine showed a decreased number of lymph cells (sometimes no lymph nodes were seen in mice of group 8). Mice in group 10 had reduced numbers of reticuloendothelium system (RES) cells, reduced Peyers patches and very few solitary lymph nodes: these deficiencies were sometimes very striking. These effects were less noticeable in mice in group 9 although some of them had a RES deficiency. The deficiency of white cells was not general throughout the body; the spleen showed an abundance of lymph cells and germinal centers.

The lungs of some animals in group 10 showed some alveoli which were distended and still others which had collapsed. There was a suggestion of pneumonia in some of these animals. This was not seen in groups 8 and 9. In histologic summary, mice in group 9 were the most healthy during the first generation. Those in isolation (group 8) showed skin lesions in the first generation which were not evident in the second and third generation. Animals fed the non-treated Apollo diet in the open laboratory showed some evidence of decreased ability to react against microbial attack. Mice fed the laboratory chow were not examined histologically.

Hemoglobin

The mineral and B vitamin analyses of the Apollo diet provided evidence to suspect severe anemia in these mice. The appearance of ears and eyes of the animals sometimes suggested anemia was present, but this was not consistently seen. The hemoglobin values showed that first generation mice fed non-treated Apollo diet were anemic while the mice fed irradiated Apollo diet were not (Table 8). When these

two groups (groups 10 and 9) were compared a difference was found, $p = 0.026$. The variation was greatest when anemia was found: i.e., mice fed non-irradiated Apollo diet were found to have 5.9, 7.4, 11.6, 12.6, and 13.2 percent hemoglobin, while those fed irradiated diet in the open laboratory were consistently high (13.3 - 14.2%). No difference was found when the first and second generation mice fed non-treated diet were compared. Anemia was not present in the third generation mice; when the first or second generation mice were compared with the third generation mice it is noted that the differences were statistically significant ($p = 0.009$ for comparison of groups 10 and 36, and $p = 0.001$ for groups 34 and 36). The anemia of the mice fed non-treated Apollo diet was further evident when they were compared with mice fed laboratory chow ($p = 0.005$ when groups 10 and 14 are compared).

When hemoglobin values of first and second generation animals fed the gamma irradiated diet in the open laboratory were compared, no statistical difference was noted due to the great variability in the mice in the second generation (individual data are 3.8, 7.5, 11.1, 13.6 and 14.6 for group 33). However, the uniformity of the first (group 9) and third generations (group 35) did allow a statistical difference ($p = < 0.001$) to indicate that the first generation mice were slightly anemic. This anemia was confirmed when these first generation mice were compared with mice fed autoclaved laboratory chow ($p = 0.016$ for groups 9 and 13). Since some second generation mice fed gamma irradiated diet appeared to be anemic, the difference between second and third generation mice (groups 33 and 35) fed gamma irradiated diet appears to be real despite the p value of 0.078; variability is very high in groups where some mice are anemic.

In the first, second and third generations no statistical differences were noted between hemoglobin values of mice fed irradiated diet in isolated conditions. A similar statement could be made for the three groups fed laboratory chow. The slight anemia of the first generation mice in isolation was confirmed when group 8 and 12 were compared ($p = 0.041$). No statistical differences were noted between

any groups fed irradiated diet excepting groups 9 and 35. No differences were noted between gamma irradiated diet and untreated diet in the second or third generation in the open laboratory (compare group 33 to 34 and 35 to 36 respectively).

White Blood Cells

The white blood cell count of first generation mice fed non-treated Apollo diet was higher than that of second or third generation mice fed the same diet or first generation mice fed the gamma irradiated diet or mice fed laboratory chow (Table 8); the highest p value in these comparisons was 0.017. The white blood cell count of first generation mice fed the gamma irradiated diet in the open laboratory (group 9) was somewhat higher than that of the second generation mice in group 33 ($p = 0.025$) and it was definitely lower than that of the third generation mice ($p = < 0.001$): the white cell count in mice of group 35 was so high that all comparisons with it gave statistically significant differences excepting with mice fed autoclaved laboratory chow ($p = 0.320$ for the comparisons of groups 8 and 13). The first generation mice fed gamma radiation sterilized diet in isolation had a lower white blood count than did those fed the same diet in the open laboratory ($p = <0.001$ for groups 8 and 9): any possible difference in the second generation was masked by the very high variability in the second generation. No change in this respect was noted in the laboratory chow fed animals ($p = 0.62$ for groups 12 and 13). All mice fed gamma irradiated Apollo diet in isolation showed a consistent low white blood count with no significant change from one generation to the next. In the second generation no difference was noted between mice fed untreated and gamma irradiated diet.

Serum Protein

Data from the electrophoretic patterns (Table 9) indicated that the total serum protein of mice fed gamma-irradiated diet in the open laboratory seemed to vary more than did those fed untreated diet or those fed irradiated diet in the isolator. The first generation of these mice (group 9) showed a somewhat high quantity of total serum protein while the second generation (group 33) had a low serum protein and that of the third generation mice (group 35) was somewhat low compared

to the other groups in the third generation. This lowered serum protein seemed to be reflected in both albumin and globulin fractions on a gram basis. The alpha-2 globulin of the first generation mice fed irradiated diet in isolation (group 9) appeared to be somewhat low, and on a percentage of total protein basis the alpha-1 globulin of these mice was low: this fraction was lowest in second and third generation mice fed the irradiate diet (groups 33 and 35). Both second and third generation mice fed the gamma-irradiated diet in the open laboratory showed a low total beta-globulin pattern. In these mice it was difficult to separate beta-1 from beta-2 globulins. The beta-1 globulin of mice fed gamma-sterilized diet in isolation appeared to be low; when compared to that of the first generation, the second generation was definitely low. In all generations mice fed gamma-irradiated diet in the open laboratory showed a lower quantity of gamma globulin than was found in the other two regimens. The albumin/globublin ratio was somewhat high for the third generation mice fed the gamma-irradiated diet in the open laboratory. This was not found to be true for second generation mice or for mice fed gamma-irradiated diet in the isolator. Pooled samples were used for this work; therefore, individual variation and statistical significance could not be determined.

Parameters of Defense

A variety of defense parameters were studied in the mature young mice (Table 10). The relative weights of the liver plus spleen increased with each generation excepting that of group 32. The phagocytic index of mice in all regimens showed a somewhat decreasing pattern with the second and third generation being lower than the generations preceding them. It is probable that sheep red blood cells hemagglutinin titers were the same throughout the three generations, excepting the possibility that mice fed the gamma-irradiated diet in the open laboratory consistently had a slightly lower titer than the other groups. The serum complement titers of animals fed untreated and gamma-irradiated diets in the open laboratory were comparable. The serum complement of mice in isolation reached a higher titer in all three generations than was found in other

regimens. The reason for this difference is not obvious. The negative interferon titers found throughout all regimens in all generations in mice which have not been challenged with a virus infection was expected.

Comparison of Analytical and Biological Evaluation

The analytical data indicate that radiation of this dry Apollo diet did not seriously decrease its nutrient content. Histidine was the only nutrient which sustained a loss greater than 12%. A paper comparison of recommended allowances with nutrients present suggested that the diet would probably be adequate for man; however, it would appear to be definitely deficient in minerals and vitamins for mice. Many nutrient requirements for mice have not been well established and it seems probable that some of the recommendations are too high.

The general results of the biological test suggest that both gamma irradiated and untreated Apollo diets were adequate for mice over three generations. In general each succeeding generation of mice appeared to be better than their predecessors and the radiation sterilized diet provided fewer abnormalities than did the non-sterile diet (this refers primarily to the poor reproduction and the histologic suggestions of infection with lowered microbial defense parameters seen in group 10). The anemia observed in the first generation mice fed non-treated diet was apparently alleviated when fiber was added to the diet. The added variable of strict bioisolation did not seem to adversely affect specific results excepting in the first generation mice when humidity may have been high. Although many statistically valid differences were noted when comparing results from several parameters, the overall view remains: three generations of mice were successfully fed comminuted Apollo diet with or without irradiation sterilization. Similar results with irradiated diets were reported by Luckey et al. (7), Metlikskii et al. (16), Ley et al. (17) and Paterson and Cook (18).

A study of radiation sterilized diet is relevant to space foods since some sterilized food items were used in the Russian Space Program of Soyuz 4 and 5 and other irradiated foods are planned (19). The bulk of literature shows high nutrient loss occurs during irradiation in diets with relatively high water content. This was confirmed for vitamin losses by Coates et al. (20). Unless other factors such as stress or

microflora changes are involved, the remaining problem would be astronaut acceptance of the diet. This has been a problem with the Apollo program and as Lepkovsky (2) indicated, acceptance of the diet is influenced both by motivation and by conditions. His conclusion was that acceptability of a diet cannot be tested except under the conditions of use.

The results suggest that presently the evaluation of diets by comparison of analytical data with nutrient allowances is a more rigorous test than is the biological evaluation. This is partially due to the safety factors built into the nutrient allowances and the adaptability of living organisms to somewhat adverse conditions. Nevertheless, the results suggest mouse dietary allowances are high. The conclusion that the analytical method of evaluation is satisfactory reverses the concensus of the evaluation of K-ration 25 years ago. This mark of maturity of nutritional science reflects the increased knowledge since that date and leads to confidence that diets which are calculated to be adequate will be nutritionally satisfactory under normal conditions. Diet sterilization by gamma radiation did not change this result. However, biosiolation of mice resulted in high mortality.

Bioisolation deaths previously reported have been attributed to poor diets or to changes in the intestinal flora (5). About 30% of our mice in isolation died when fed the autoclaved stock diet. Therefore, specific deficiencies of the Apollo diet cannot be held entirely responsible for these deaths which are characterized by the suddeness and completeness with which all animals caged together will die. Although some animals have shown infection when examined after death, most animals, moribund or dead, were found to have no infection. Since germfree animals fed the Apollo diet did not die (21), the environment, a specific diet and infection are effectively ruled out as individual causes of death. The dramatic changes in hemaglobin values caused by specific microflora in gnotobiotic mice (21) mirrored the effect of adding filter paper bedding in these experiments. Although evidence of shock was not seen at autopsy, the speculation remains that the combination of a marginal diet plus the changes in the microflora plus the confined environment (with high humidity) must have surpassed some limit of adaptability and/or viability to cause the sudden bioisolation deaths.

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FOOTNOTES

1. This work was performed in the General Electric Valley Forge Space Technology Center while the senior author was Visiting Scientist. It was supported in part, by NASA grant, NAS9-9000.
2. Babcock related to Atwater as reported by E. V. McCollum in "From Farm Boy to Scientist, University Kansas Press, 1964, p. 116.
3. D. R. Silber, in a personal letter, 1970.
4. From the Whirlpool Corporation, St. Joseph, Michigan.
5. Obtained through the courtesy of Mr. R. Schmidt of the Baltimore Biological Laboratories, Baltimore, Maryland.
6. Deposited with the American Society for Information Service. Standard, accepted procedures used throughout are referenced. Vitamin analyses were performed by the Wisconsin Alumni Research Foundation, Madison, Wisconsin and all other analyses were obtained through the courtesy of Dr. C. W. Gehrke of the Agricultural Chemistry Department, University of Missouri, Columbia, Missouri.
7. Purina Lab Chow 5010C Autoclavable from Purina Mills, St. Louis, Missouri.
8. CRL-CD-1 (HdM/ICR) Swiss mice obtained from the Charles River Breeding Laboratory, Wilmington, Massachusetts.

FIGURE 1

EFFECT OF COBALT GAMMA RADIATION UPON SPORES OF *BACILLUS STEAROTHERMOPHILUS* AND *BACILLUS SUBTILIS*

The fourth line from the ordinate indicates the most resistant variants from the results of all experiments.

Fig. 1

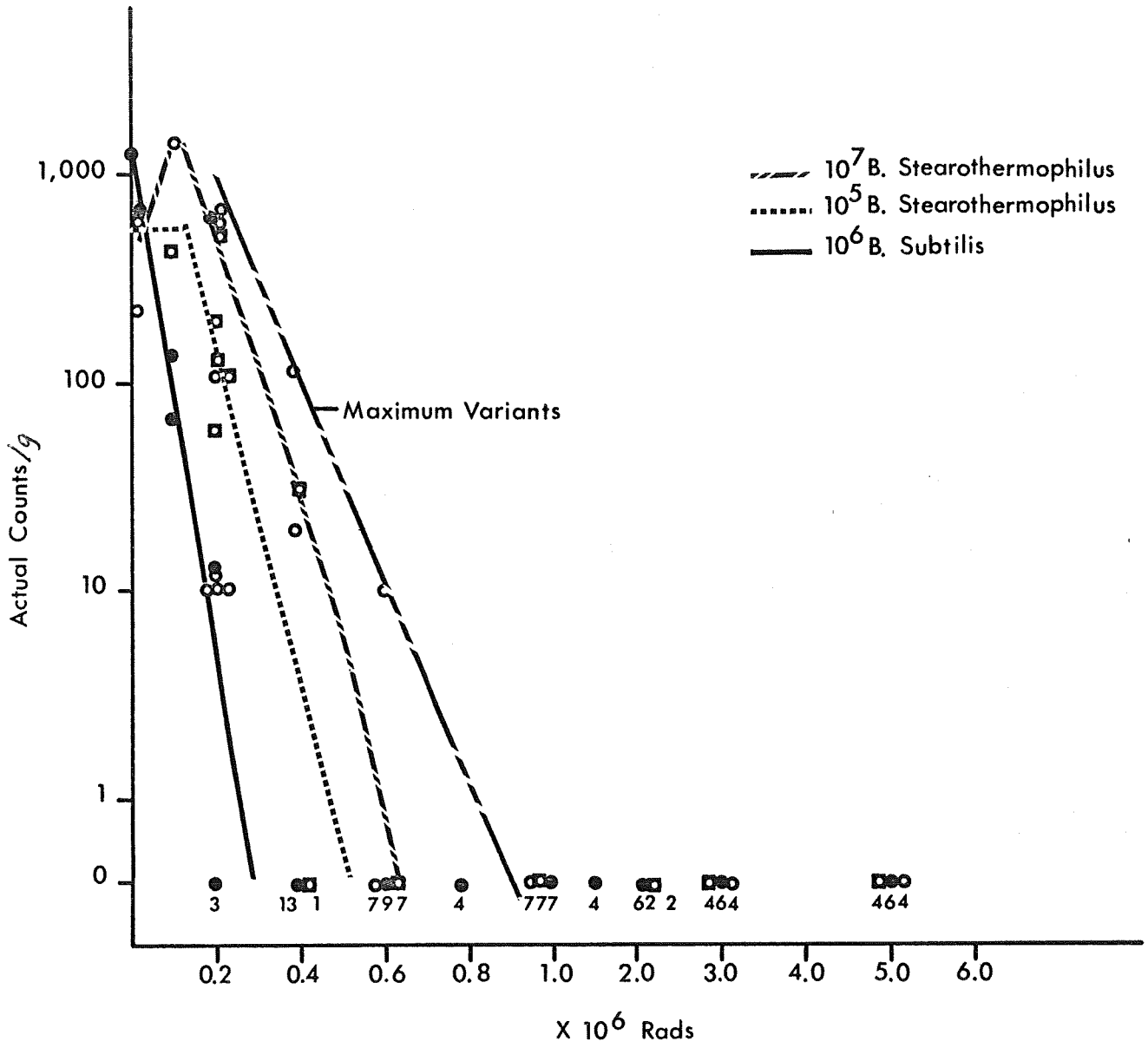
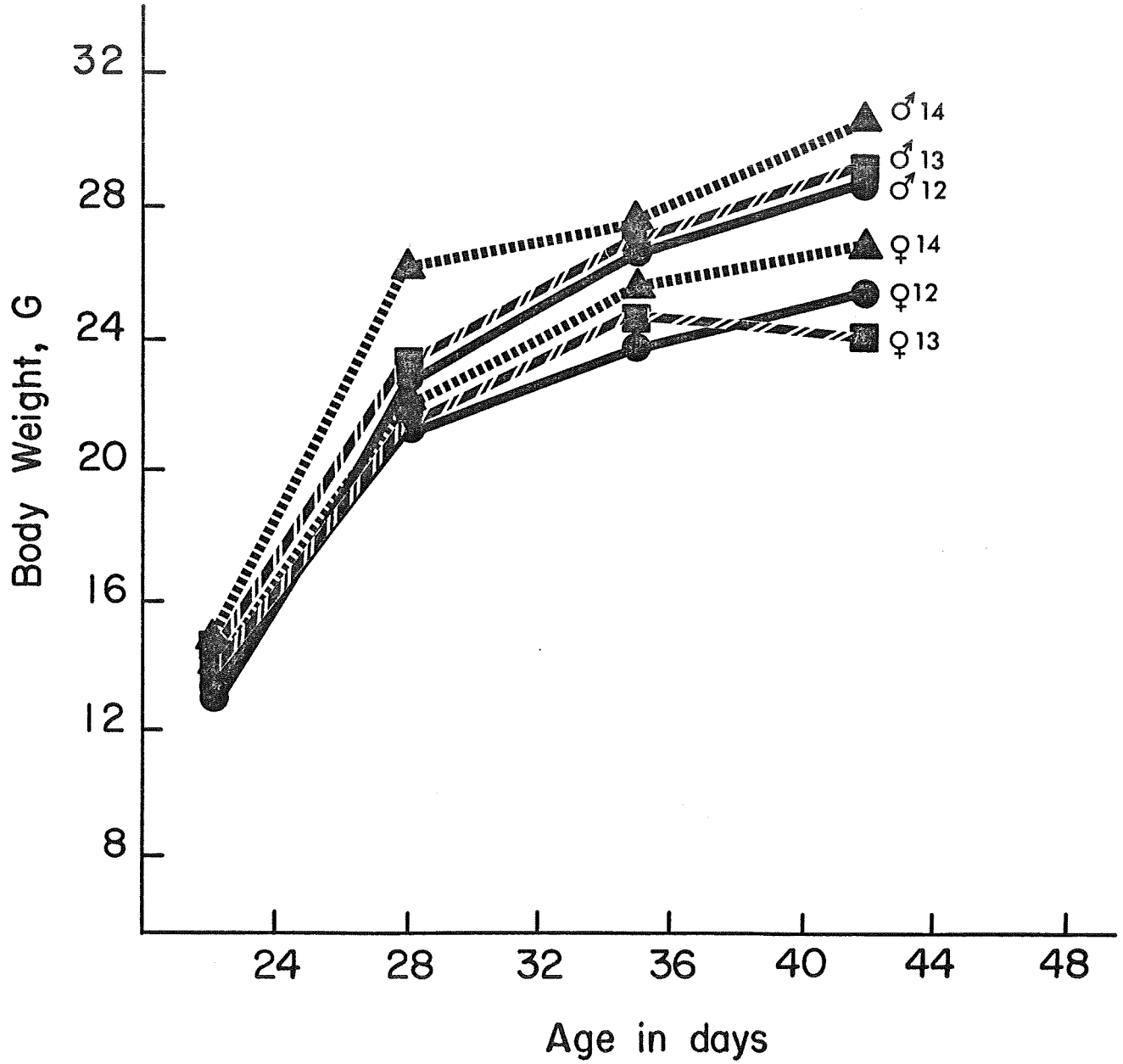

1,000
100
10
1
0
Actual Counts/g
Maximum Variants
10^7 B. Stearothermophilus
10^5 B. Stearothermophilus
10^6 B. Subtilis
3
13 1
797
4
777
4
62 2
464
464
0.2
0.4
0.6
0.8
1.0
2.0
3.0
4.0
5.0
6.0
X 10^6 Rads

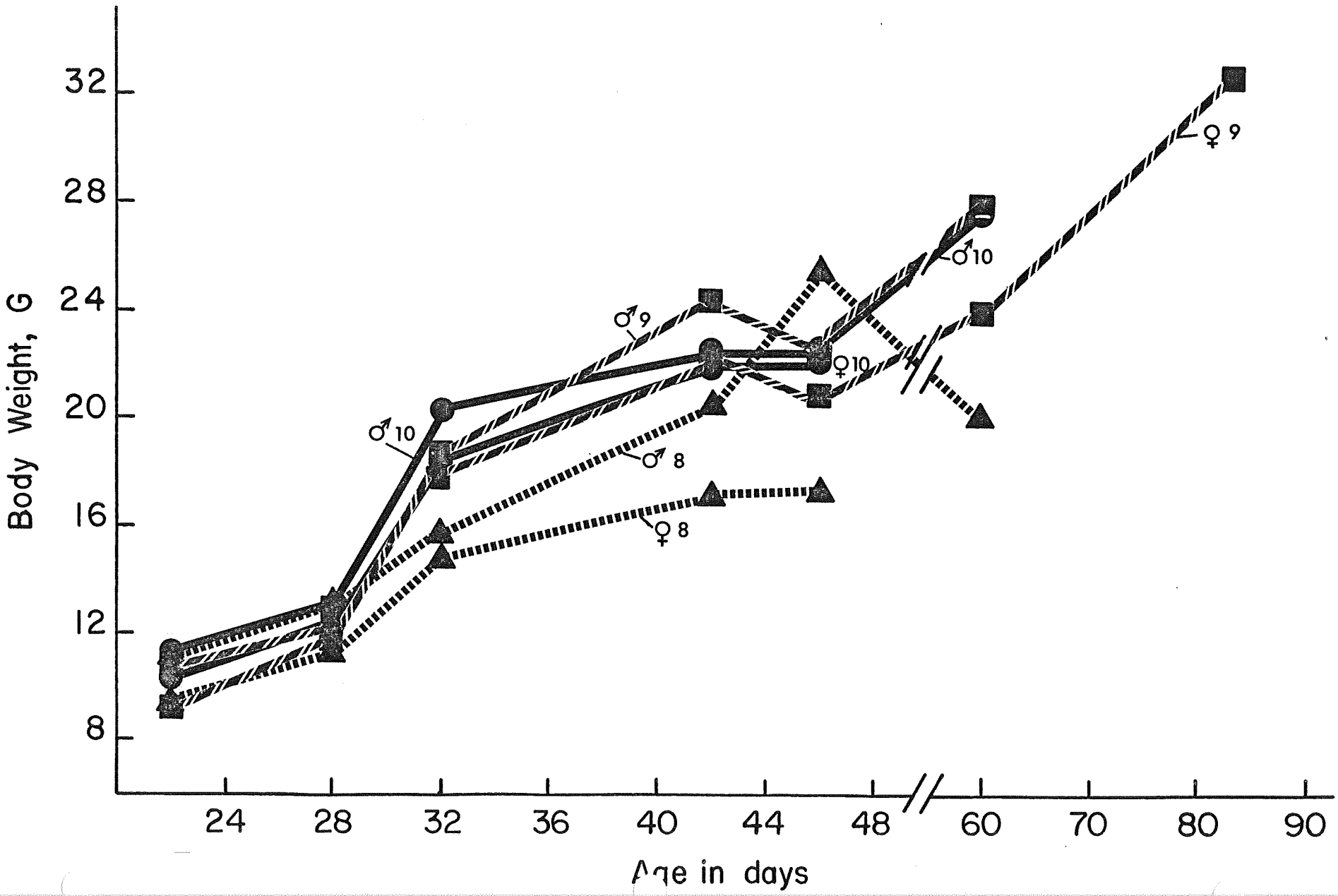
FIGURE 2

GROWTH CURVES OF MICE

The first generation started with 8 males and 12 females per group; the second generation had 22, 23 and 9 mice for groups 34, 33 and 31 respectively. Average values for body weight are plotted on the ordinate with time on the abscissa. The groups are identified below:

Groups	Symbol	Diet	Diet treatment	Environment
8, 31		Apollo	γ Radiation	Bioisolation
9,33		Apollo	γ Radiation	Open
10, 34		Apollo	Untreated	Open
12	X	Lab Chow	Autoclaved	Bioisolation
13	o	Lab Chow	Autoclaved	Open
14	.	Lab Chow	Untreated	Open


32
28
24
20
16
12
8
Body Weight, G
♂14
♂13
♂12
♀14
♀12
♀13
24
28
32
36
40
44
48
Age in days


Body Weight, G
32
28
24
20
16
12
8
24
28
32
36
40
44
48
60
70
80
90
Age in days
♂9
♂10
♀10
♀9
♂10
♂8
♀8

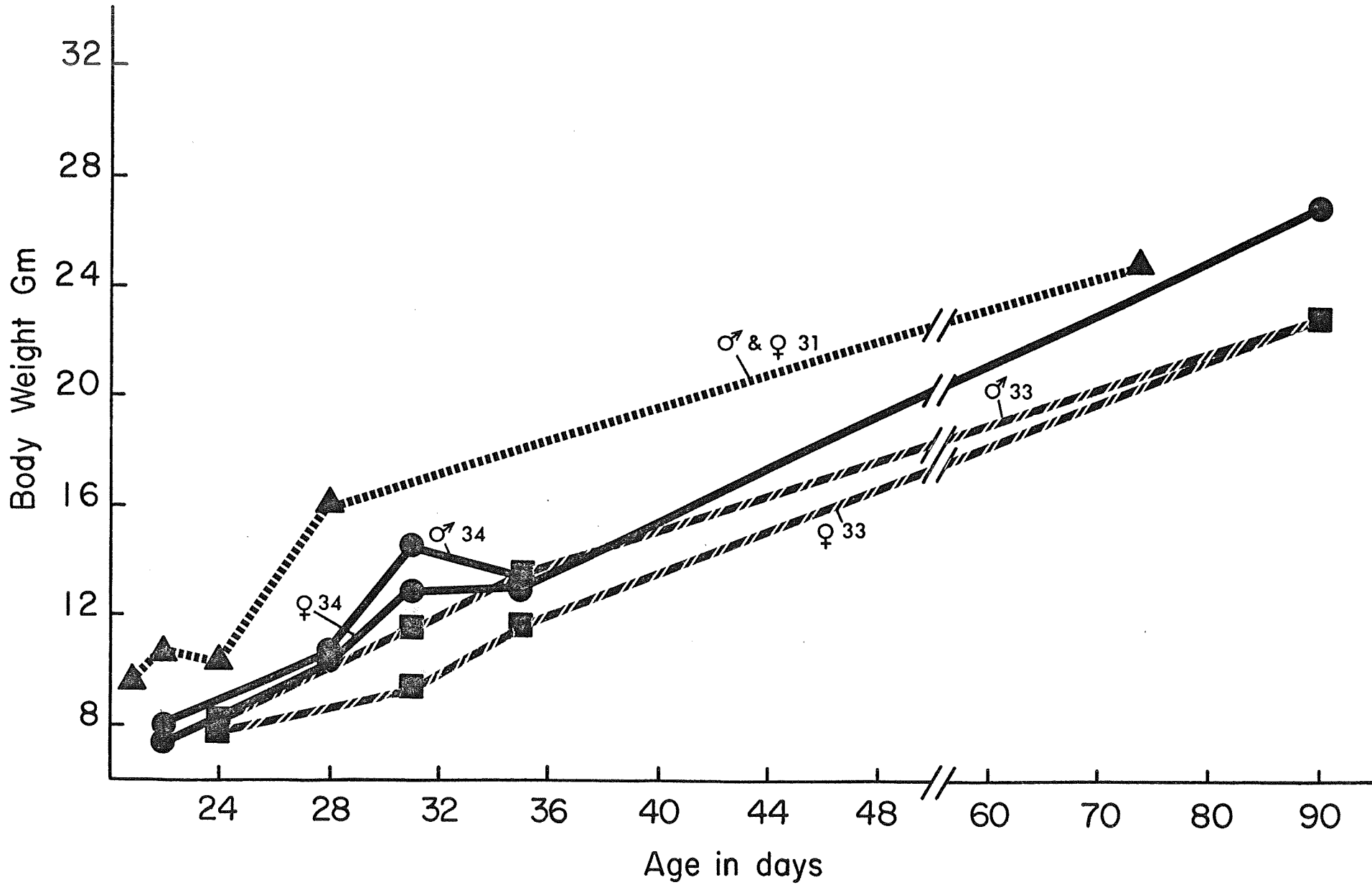

Body Weight Gm
32
28
24
20
16
12
8
♂ & ♀ 31
♂ 33
♀ 33
♂ 34
♀ 34
24
28
32
36
40
44
48
60
70
80
90
Age in days

TABLE 1

REPRESENTATIVE APOLLO DIET - 1968

CATEGORY	ITEM	PERCENT OF DIET	SUB-TOTALS
Meat	Bacon Squares	4.26	21.26
	Beef and Gravy	2.03	
	Beef Sandwich	1.86	
	Beef Pot Roast	1.31	
	Beef and Vegetables	0.85	
	Beef Barbecue Bites	0.56	
	Beef Hash	0.28	
	Beef Stew Bites	0.27	
	Canadian Bacon and Applesauce	0.56	
	Chicken Salad	1.98	
	Chicken and Gravy	0.24	
	Cream of Chicken Soup	0.27	
	Sausage Patties	2.79	
	Salmon Salad	1.55	
	Shrimp Cocktail	0.90	
	Tuna Salad	1.55	
Cereals	Cinnamon Toasted Bread Cubes	9.51	19.77
	Corn Flakes, Sugar Coated	1.07	
	Corn Chowder	3.25	
	Toasted Bread Cubes	5.24	
	Toasted Oat Cereal	0.70	
Vegetables	Pea Soup	2.84	6.05
	Potato Salad	0.50	
	Potato Soup	2.71	
Fruit	Strawberry Cubes	1.39	21.95
	Peach Bars	0.89	
	Applesauce	1.69	
	Apricot Cereal Cubes	1.46	
	Drink, Breakfast	0.33	
	Drink, Grapefruit	4.90	
	Drink, Orange	4.60	
	Drink, Orange-Grapefruit	2.33	
	Drink, Pineapple-Grapefruit	3.49	
	Fruit Cocktail	0.87	
Dairy	Cheese Sandwich	1.19	7.29
	Cocoa	6.10	
Sweets	Banana Pudding	5.42	23.63
	Brownies	2.01	
	Butterscotch Pudding	2.03	
	Chocolate Pudding	3.39	
	Chocolate Cubes	3.13	
	Date Fruitcake	1.31	
	Gingerbread Cubes	1.35	
	Pineapple Fruitcake	3.13	
	Sugar Cookies	1.86	

TABLE 2

PROXIMATE ANALYSIS OF APOLLO DIET

Item	Non-Sterile	Irradiated	Difference
H_2O	2.9	3.2	+ 0.3
Fiber	0.9	0.7	- 0.2
Fat	14.4	14.4	0.0
Ash	3.5	3.5	0.0
N_2	2.89	2.83	- 0.06
Prot. (Crude)	18.1	17.7	+ 0.4
Total	39.8	39.5	
CHO (Diff.)	60.2	60.5	
Energy Cal/gm	4.43	4.42	

TABLE 3

ELEMENTAL ANALYSIS OF APOLLO DIET

Mouse		Element	Apollo Diet (3)			Man	
Allowance mg/day(1)	Apollo Diet mg/day(2)		Non-Irradiated mg/Kg	Irradiated mg/Kg	Loss %	Allowance mg/day(4)	Apollo Diet mg/400 gm(2)
13	35	Na	8700	8700, 8800	0		3490
15	19	K	4700	4700, 4800	0		1880
24	7.6	P	1900	2000, 2300	0	800	760
22	7.8	Ca	2000	2000, 1800	5	800	800
1.7	1.6	Mg		411		350	164
	8.2	S		2040			815
20	46	Cl		11400			4560
0.75	0.089	Fe		22.2		10	8.9
0.018	0.009	Cu		2.3			0.92
0.01	0.00007	Co		0.018			0.007
0.008	.076	Zn		19.0			7.6
0.14	.016	Mn		4.0			1.6
	0.0005	Mo		0.12			0.05

(1) Albrittin (14) for a 25 gm mouse.
(2) Calculated from the average of the values from the non-irradiated diet were data are available: assume 400 gm per day for one man and 4 gm for one mouse.
(3) The first 4 minerals were determined by chemical methods, the others by spectography by Dr. G. W. Gehrke. and E. Pickett, Department of Agriculture Chemistry, University of Missouri, Columbia. See footnote 6.
(4) NRC daily recommended allowance (13).

TABLE

AMINO ACIDS IN APOLLO DIET

Mouse	Amino Acid	Apollo Diet %(2)			Man	
%(1) Allowance		Non-Irradiated	Irradiated	Loss	Allowance(3) gm/day	gm/400(4) Apollo Diet
0.5	Histidine	0.68	0.54	20.7	-	2.72
1.0	Isoleucine	0.60	0.57	5.0	1.4	2.40
1.6	Leucine	1.41	1.31	7.1	2.2	5.64
1.2	Lysine	1.19	1.11	2.5	1.6	4.75
0.9	Methionine	0.40	0.37	7.6	2.2	1.60
	Cystine	0.17	0.16	5.8		0.68
1.0	Phenylalanine	0.76	0.74	3.8	2.2	3.04
.8	Tyrosine	0.49	0.48	2.1		1.96
0.8	Threonine	0.65	0.58	10.7	1.0	2.60
0.3	Tryptophane				0.5	
1.1	Valine	0.68	0.65	4.4	1.6	2.72
1.0	Arginine	1.02	0.92	9.8		4.08
	Alanine	0.78	0.75	3.8		
	Aspartate	1.34	1.24	7.3		
	Glutamate	2.98	2.70	9.3		
	Glycine	0.99	0.91	8.0		
	Ornithine	0.00	Trace			
	Hydroxyproline	0.34	Trace			
	Proline	0.65	0.74	0		
	Serine	0.70	0.64	8.4		
	NH_3	0.10	0.10	0		
	Total	15.59	14.51	6.7		

(1) "Requirements" for 20% protein diet (15).
(2) Analyses from G. W. Gehrke, Dept. Agr. Chemistry, Univ. of Missouri using gas chromatography on acid hydrolysate; see footnote 6 for methodology.
(3) NRC allowance (13)
(4) This provides about 1800 calories of non-Irradiated Apollo diet.

TABLE 5

VITAMIN CONTENT OF APOLLO DIET

Mouse		Vitamins	Apollo Diet, mg/100 gm[3]			Man	
Allowance mg/day(1)	mg/4 gm diet (2)		Non-Irradiated	Irradiated	Loss %	Allowance (4) mg/day	mg/400g diet
		Reduced Ascorbate	56.0, 57.0	51.0, 50.5	10.1		
	3.2	Ascorbic Acid	79.6, 79.6 Ave. 79.6	79.3, 79.6 Ave. 79.45	0.19	60	320
0.07	0.012	Riboflavin	0.286, 0.288 Ave. 0.287	0.253, 0.258 Ave. 0.255	11.2	1.7	1.2
0.035	0.006	Thiamin	0.15, 0.15 Ave. 0.15	0.15, 0.15 Ave. 0.15	0	1.4	0.60
0.035	0.007	Vitamin B_6	0.166, 0.168 Ave. 0.167	0.162, 0.166 Ave. 0.164	1.8	2.0	0.66
0.007	0.012	Vitamin A[5]	0.25, 0.26 Ave. 0.29	0.26, 0.27 Ave. 0.27	0	1.7	1.2
0.35	0.020	Pantothenate	0.488, 0.496 Ave. 0.492	0.466, 0.496 Ave. 0.481	2.2	-	2.0
0.0175	0.0009	Folate	0.0236, 0.0220 Ave. 0.0228	0.0236, 0.0236 Ave. 0.0236	0	0.4	0.092
0.035	0.076	Vitamin K		0.019			0.076

(1) Recommended Allowance for 25 gm mouse (14).

(2) Calculated from irradiated Apollo Diet assuming 4 gm diet/day.

(3) Data from WARF Analyses; see footnote 6.

(4) NRC Recommended Allowance 1968 for men 22-35 years of age (13).

(5) IU have been converted to mg using the ratio of 1 IU = 0.3 ug.

TABLE

SURVIVAL, APPEARANCE, REPRODUCTION AND LACTATION

Regimen	Group	No.(1)	Wgt.Ave., gm 21 days	Deaths(2)	Fur(3)	Skin(4)	Litters	Mice Weaned	Health Observations(5)
1st Gen.									
Open Lab.	10	20	10.7	1	0-1	0	2	11(6)	Good and active throughout. Some look anemi
Open + γ Diet	9	20	10.3	2	0-1	0	6	35	As above. Fur looks best of all at 2 mo. Maturing well.
Isol + Diet	8	20	10.6	11	3-5	2-4	6	9	Good at start. Look sick at one week. Improvement at 2 months. Sporadic deaths.
2nd Gen.(7)									
Open Lab	34	22	7.6	7	2-3	0	7	12(8)	OK at start. Look ill at 2 months.
Open + γ Diet	33	23	7.6	0	0	0	4	12	OK at start. Look anemic. Better than GP
Isol + γ Diet	31	9	10.5	2	0	0	3	20	Good throughout.
3rd Gen.									
Open Lab	36	4	5.9	0	0	0			Good throughout.
Open + γ Diet	35	12	5.9	0	0	0			Good throughout.
Isol + γ Diet	32	20	11.0	18	0	0			OK until castastrophic deaths at 2 weeks when 19 died.
1st Gen.									
Open + Lab Chow	14	19	14.0	0	0	0	7	34	Normal
Open + Claved Chow(9)	13	20	13.9	0	0	0	5	14	Normal
Isol + Claved Chow	12	20	13.7	6	0	0	2	12	OK until bedding in one cage became wet; then all 5 died in 24 hours. One died after cage became wet.

(1) Number of mice started; low number reflect poor reproduction or lactation in previous generation.
(2) Deaths prior to autopsy at about 2 months corrected to 20 mice/gp.
(3) Fur loss scale: 0 = normal, 5 = completely nude.
(4) Skin erythema scale: 0 = none, 4 = severe erythema with edema.
(5) Health is a summary of recorded weekly observations including general appearance, posture, size, fur coat, skin, ears, eyes, nares, tail, limbs, rectal-genital area and response to irritation. These were not recorded for mice fed laboratory chow.
(6) This litter of 11 was born when dam was 104 days of age. This litter did not appear to be healthy and several died before 2 months.
(7) These mice were provided with bedding at reproduction: filter paper for those fed Apollo diet and sani-cell for those fed laboratory chow.
(8) Eleven animals from group No. 9 were used to begin the second generation group No. 34
(9) Claved designates autoclaved.

TA 7

FOOD EFFICIENCY DATE

(Five mice per cage taken during their 5th week of life)

~~GROUP~~ GROUP		BODY WEIGHT			FOOD, G				G GAIN X 100 / FOOD	AVERAGE AND STD. DEVIATION
	CAGE	START	END	CHANGE	START	WASTE	END*	USED		
8	A	53.6	71.2	17.6	110.8	66.5	66.5	44.3	39.7	
γ Diet	B	63.9	84.3	20.4	104.7	49.1	49.1	55.6	36.7	36.9
+	C	55.2	66.1	10.9	128.0	85.1	85.1	42.9	25.4**	2.71
Bioisolation	D	59.8	81.6	21.8	111.2	39.7	47.6	63.6	34.3	
9	A	63.8	95.1	31.3	108.8	32.6	32	77	40.6	
γ Diet	B	57.1	83.2	26.1	112.3	41.7	41	71	36.7**	39.6
	C	64.1	94.9	94.9	119.4	41.8	41	78	39.5	0.91
	D	66.2	96.5	96.5	111.6	35.1	34	78	38.8	
10	A	56.1	94.1	38.0	103.8	31.8	31	73	52.7	49.8
Diet un-	B	56.9	93.1	36.2	116.3	41.1	40	76	47.6	2.62
treated	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	
12	A	108.3	120.3	12.0	128.5	45.2	44	85	14.2	
Claved	B	108.3	105.5	-2.7***	177.6	84.4	82	96	-	
Bioisolation	C	108.3	121.7	12.9	172.6	74.4	72	101	12.8	12.7
	D	116.2	126.7	10.5	157.9	65.6	64	94	11.2	1.5
13	A	111.8	134.1	22.3	199.2	99.8	92.4	106.8	20.9	
Claved	B	111.4	128.8	17.4	160.7	65.3	54.9	105.8	16.5	18.1
	C	103.1	118.9	15.8	183.5	96.6	89.4	94.1	16.8	
	D	119.6	130.5	10.9	168.5	77.3	69.2	99.3	11.0**	2.5
14	A	123.2	130.8	7.6	129.6	42.4	34.0	95.6	8.0	
Chow untreated	B	119.8	127.5	7.7	111.7	23.4	16.4	95.3	8.1	
	C	115.9	122.3	6.4	120.0	30.8	21.6	98.4	6.5	7.5
	D	95.5	104.1	8.6	121.7	52.6	46.6	75.1	11.5**	0.9

*END = Waste corrected for fecal contamination. Sometimes wated food could be collected with no contamination. Where data could not be obtained, 3% of the dried waste was deducted (groups 9, 10 and 12).

**These values were deleted in further calculations because they were more than 3σ from the mean.

***Water not available last day.

TABLE 8

HEMATOLOGY OF MICE

Diet Environs	Hb, %				WBC/mm^3		
	Gp	N	M	σ	N	M	σ
1st Generation Apollo Diet							
Untreated, open	10	5	10.1	3.28	3	4987	256
γ Radiated, open	9	6	14.0	0.40	3	3997	319
γ Radiated, isolation	8	4	13.9	0.47	3	1540	110
2nd Generation Apollo Diet							
Untreated, open	34	4	10.8	1.85	4	2195	1341
γ Radiated, open	33	5	10.9	4.47	3	2676	635
γ Radiated, isolation	31	3	13.8	1.31	3	2127	606
3rd Generation Apollo Diet							
Classic	36	5	15.6	0.52	3	2457	337
Classic + γ Diet	35	4	16.1	0.77	3	7333	445
Classic + γ + isolation	32	2	16.3	1.77	2	2070	650
1st Generation Lab Chow							
Untreated, open	14	2	16.4	0.21	2	3550	495
Autoclaved, open	13	3	15.7	1.09	3	6833	3818
Autoclaved, isolation	12	3	16.0	1.01	3	5400	1411

TABLE 9

SERUM PROTEINS

L PROTEIN EIGHT ms) %	ALBUMIN		GLOBULINS												ALBUMIN GLOBULIN RATIO
			α_1		α_2		β Total		β_1		β_2		γ		
	(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%	
							1st Generation								
4.6	2.51	54.5	.51	11.1	.25	5.5	** .97	21.1	.51	11.0	.55	12.0	.36	7.8	1.20
5.2	2.01	57.9	.49	9.5	.28	5.3	**1.09	21.0	.54	10.5	.55	10.5	.33	6.3	1.38
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	1.21
							2nd Generation								
4.6	2.59	56.2	.51	11.2	.31	6.7	.83	18.0	-	-	-	-	.36	7.9	1.29
3.7	2.08	56.3	.42	11.2	.23	6.3	.65	17.5	-	-	-	-	.32	8.7	1.28
4.3	2.25	52.4	.51	11.9	.25	5.9	.87	20.3	.36	8.3	.51	12.0	.41	9.5	1.11
							3rd Generation								
4.6	2.54	55.1	.51	11.2	.28	6.1	.91	19.8	-	-	-	-	.36	7.9	1.23
4.0	2.39	59.8	.40	10.0	.25	6.1	.63	13.8	-	-	-	-	.33	8.2	1.48
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	1.16

peak in this region

TABLE 9

SERUM PROTEINS

REGIMEN	GROUP	TOTAL PROTEIN WEIGHT (gms) %	ALBUMIN		GLOBULINS											
					α_1		α_2		β Total		β_1		β_2		γ	
			(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%	(gms)	%
1st Generation																
Open-untreated	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
Open- γ diet	9	5.2	2.01	57.9	.49	9.5	.28	5.3	**1.09	21.0	.54	10.5	.55	10.5	.33	6.3
Iso.- γ diet	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
2nd Generation																
Open-untreated	34	4.6	2.59	56.2	.51	11.2	.31	6.7	.83	18.0	-	-	-	-	.36	7.9
Open- γ diet	33	3.7	2.08	56.3	.42	11.2	.23	6.3	.65	17.5	-	-	-	-	.32	8.7
Iso.- γ diet	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
3rd Generation																
Open-untreated	36	4.6	2.54	55.1	.51	11.2	.28	6.1	.91	19.8	-	-	-	-	.36	7.9
Open, γ diet	35	4.0	2.39	59.8	.40	10.0	.25	6.1	.63	13.8	-	-	-	-	.33	8.2
Iso.- γ diet	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

** Homogeneous double peak in this region

DEFENSE PARAMETERS

Regimen	Phagocytic Index[1]			Hemagglutinin		Complement					Interferon
	Group	LS/100 gm[2]	PI[3]	Dilution	Log 2	0	100	200	500	1000[4]	Titer[5]
1st Generation											
Open, untreated diet	10	4.96	4.75	1:64	6	+2	+2	0	0	0	Neg.
Open, γ diet	9	5.61	5.18	1:32	5	+2	+2	0	0	0	Neg.
Iso. - γ diet	8	5.61	5.33	1:64	6	+2	+2	+2	+2	0	Neg.
2nd Generation											
Open, untreated diet	34	5.17	4.96	1:64	6	+2	+2	0	0	0	Neg.
Open, γ diet	33	5.80	4.14	1:32	5	+2	+2	0	0	0	Neg.
Iso. - γ diet	31	5.85	4.19	1:64	6	+2	+2	+2	+2	0	Neg.
3rd Generation											
Open untreated diet	36	5.84	3.56	1:64	6	+2	+2	0	0	0	Neg.
Open - γ diet	35	6.01	3.72	1:32	5	+2	+2	0	0	0	Neg.
Iso. - γ diet	32	5.26	4.11	1.64	6	+2	+2	+2	+2	0	Neg.
Injested Control						+2	+2	+2	+2	+1	4.3 logs

(1) Average from 2 mice per group
(2) Weight of liver and spleen per 100 gm body
(3) Corrected phagocytic Index with 8 mg carbon per 100 gm body
(4) Dilution factor
(5) Tested at dilutions of 10, 100 and 1000

APOLLO DIET EVALUATION: A COMPARISON OF BIOLOGICAL AND ANALYTICAL METHODS INCLUDING BIOISOLATION OF MICE AND RADIATION OF DIET

by

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19104 and Manned Spacecraft Center, NASA, Houston,
Texas - 77058

Methods Deposited with the Editor of the J. Nutr. for the American Society for Information Service (ASIS).

Proximate Analysis:	Fat, fiber, ash, water. Na+, K+, Ca++, Phosphores and nitrogen: "Book of Methods", Association of Official Agricultural Chemistry, Washington, D. C. 10th ed., 1965.
Mo and Co:	R. L. Mitchell, "The Spectrographic Analysi of Soils, Plants, and Related Materials", Tech. Comm. 44, Commonwealth Bu. Soil Sci., Harpenden, Herts., England, 1948, and E. E. Pickett and B. E. Hankins, Anal. Chem. 30, 47-50 (1958).
Zn, Fe, Mn, Mg:	W. T. Elwell and J. A. F. Gidley, "Atomic-Absorption Spectrophotometry", Pergamon Press, London, 2nd ed., 1966.
S, Thiamine and Riboflavin:	"Official Methods of Analysis", A.O.A.C., Washington, D. C., 10th ed., 1965.
Cl:	"Standard Methods of Clinical Chemistry", Vol. 3, D. Seligson, ed., p. 81. Academic Press, New York, 1961.
Vitamin B-6:	Atkins, Schultz, Williams and Frey, Ind. and Eng. Chem., Anal. Ed., 15, 141 (1943) (S. carlsbergensis).
Pantothenic acid:	Nielands and Strong, Arch. of Bio. 19, 2 (1948).
Folic acid:	A.O.A.C., 830 (1955) 8th ed.
Vitamin A:	Carr-Price Blue Color, Methods of Vitamin Assay, Assn. of Vitamin Chemists, Inc., Interscience Publishers, Inc., N. Y., 25 (1951).
β-carotene:	Moore and Ely, Ind. Eng. Chem., Anal. Ed., 13, = 600 (1941).
Ascorbic Acid:	J. Biol. Chem. 160, 217 (1945).

Amino Acids: Roach, D. and C. W. Gehrke 1969 The GLC Chromatography of amino acids. J. Chromat. 43:303.

Histology was performed with tissues fixed in 10% formalin and stained with either MacCallum-Goodpasture stain or Hematoxylin and Eosin. Hemoglobin was determined by the direct iron determination.

Serum proteins were determined by integration of the curves obtained by paper electrophoresis (Spinco Manual).

Compliment titer was determined by the Brucella abortus reaction with mouse sera and human O Rh+ cells. Nishioka, K. J. Immunol. 90: 96, 1963. Phagocytic Index was determined by carbon clerance.

Inteferon - Determined on a non-challenge basis by Dr. R. R. Rafajko, Director of Research of North American Biologicals, Inc., Rockville, Md. 20852, using the assay with vesicular stomatitis virus reduction by the method of Habel, C. and N. Salzman, "Fundamental Techniques in Virus Research", Academic Press, N. Y., 1970.

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Texas - 77058

Food panel data deposited with the Editor of the J. Nutr. for the American Society for Information Service (ASIS).

TABLE I

TASTE PANEL EVALUATION OF IRRADIATED AND NON-IRRADIATED FOODS

Item	Difference Category	Mean	Median	Standard Deviation	Range
Salmon Salad #1	0	7.1	7	.99	6-8
Salmon Salad #2		7.3	7	1.16	5-9
Cream of Chicken Soup #1	0	5.4	6	1.84	1-8
Cream of Chicken Soup #2		5.7	6	1.95	1-8
Sausage Patties #1	0	7.25	7	.75	6-8
Sausage Patties #2		7.17	7	1.11	5-9
Pineapple-Grapefruit Dr. #1	0	6.57	7	.53	6-7
Pineapple-Grapefruit Dr. #2		6.86	7	.38	6-7
Butterscotch Pudding #1	1	5.71	6	1.38	4-8
Butterscotch Pudding #2		7.14	7	.69	6-8
Shrimp Salad #1	1	6.66	7	1.66	4-8
Shrimp Salad #2		6	6	.87	4-7
Fruit Cocktail #1	1	7.4	8	.97	6-9
Fruit Cocktail #2		6.8	7	1.68	4-9
Beef Pot Roast #1	1	7.3	7	1.34	5-9
Beef Pot Roast #2		7.7	8	1.25	5-9
Orange-Grapefruit Dr. #1	1	6	7	1.83	2-8
Orange-Grapefruit Dr. #2		7.22	8	1.64	4-9
Banana Pudding #1	1	6	7	1.56	3-8
Banana Pudding #2		5.4	6	1.67	3-8
Orange Juice #1	1	6.75	7	1.04	5-8
Orange Juice #2		6.55	6	1.42	4-9
Applesauce #1	1	6.7	7	.9	5-8
Applesauce #2		6.1	6	.99	4-7
Bacon Bars #1	1	6.4	6	1.07	1-8
Bacon Bars #2		7.4	7	1.07	6-9
Toasted Bread Cubes #1	2	4.2	4	1.87	1-7
Toasted Bread Cubes #2		6.2	6	1.55	4-8
Corn Chowder #1	2	4.71	5	1.79	2-8
Corn Chowder #2		6.75	7	.88	6-8
Cocoa #1	3	5.12	4	1.64	4-8
Cocoa #2		6.5	7	1.41	4-8
Cheese Cracker Cubes #1	3	3.7	4	1.16	2-6
Cheese Cracker Cubes #2		6.6	7	1.35	4-9

#1 - Irradiated
#2 - Non-Irradiated

17 Tested

TECHNOLOGY INCORPORATED
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8531 NORTH NEW BRAUNFELS AVENUE
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PHONE: 512 824-7373
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January 14, 1970

MEMORANDUM TO: Dr. M. C. Smith

FROM : Dr. C. S. Huber

SUBJECT : Taste Panel Evaluation of Irradiated and Non-irradiated Foods

Samples of irradiated and non-irradiated foods, which were used to feed mice in an investigation conducted by General Electric under Contract NAS 9-9000, were evaluated by a series of taste panels in the Food and Nutrition Laboratory. Two samples, irradiated and non-irradiated, were evaluated for each specific food item. The results of the evaluation are included in Table I.

Seventeen sets of samples were evaluated. The non-irradiated sample received a higher preference rating for 11 food items. The difference between treatments was quite noticeable for the cube items. The irradiated cheese cracker cubes and toasted bread cubes were darker in color than the non-irradiated samples. The irradiated banana pudding and milk were also darker in color. Some of the darkening may be attributed to the heat which is generated during the irradiation process.

The irradiated salmon salad and shrimp salad had a very bright color and also a more pronounced fishy flavor.

The irradiated milk sample was not evaluated by a taste panel because it had a very offensive odor. It was difficult to rehydrate both samples of milk.

Clayton —
Did panels know which samples were treated?

Dr. Smith —
The panel participants did not know that the samples were treated.

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Gnotobiology is Ecology[1,2]

T. D. Luckey, PH.D.

Gnotobiology involves only studies where all living species are known to the investigator; this provides a more exact definition of the total environment than was previously possible. The new potential that gnotobiology brings to ecology is to open the biological component of the environment to exact qualitative and quantitative description; this allows biological standardization equivalent to that available for physical and chemical control of the environment. This paper explores the interaction of microorganisms inoculated singly and in pairs into germfree mice.

Some components of the environment and their relationships are presented in Fig. 1. The state of macrobes separate from external physical or chemical environment is represented by embryos; their environment comprises material that is transmitted through a macrobe of the same species with no external physical or chemical environment separate from mother-self. This experimental model is useful in immunology. When mammals are delivered into a germfree isolator via cesarean operation, the macrobe is taken from *state 1** into *2** where it transiently remains until the amnion is removed; then it is in *state 3**. Experimentally *state 2** is equivalent to the embryo in an egg: temperature is controlled exogenously, whereas outside gases and other chemicals must pass through and be "processed" by outer structures before reaching the embryo. *State 3** is also used in immunology. When diet is administered, the macrobe enters *state 4**, which is exemplified by germfree research. Other experimental designs are readily seen from the figure. Finally, to reach *state 5* the germfree macrobe is inoculated with one or more species of microbes, or a newborn classic animal eats and becomes contaminated with a multiplicity of microbes. When one or more pure cultures of microorganisms or metazoan parasites are successfully established in a germfree host, this association may continue to be defined by gnotobiotic criteria. The inoculated host is no longer germfree; it is one other class of gnotobiotic organisms, a gnotophore, which carries one or more known microbic species with the absence of all other viable species. The interaction of an individual with all of these five components of the environment (*state 1** may be either the individual under consideration or all associated macrobes) comprises his ecology. The outer circle represents time in the sense of both maturation and scientific manipulation, a reminder that each observation represents one instant in the lifetime of the individual.

Reviews of the effect of microorganisms in monognotophoric hosts (which carry only one species of microbe) are available both by host *1*) and microbe *2*) classification. Less work has been done using more than one microorganism in a given host. Phillips (3) and Wescott (4) have explored the interrelationships of bacteria–protozoa combinations in gnotobiotic animals. The classic work of Tanami (5) showing bacteria–bacteria interaction in gnotobiotic guinea pigs is reviewed to provide a background for more recent work.

[1] From the Department of Biochemistry, University of Missouri Medical School, Columbia, Missouri 65201.

[2] Supported by National Aeronautical and Space Administration (NASA-9000).

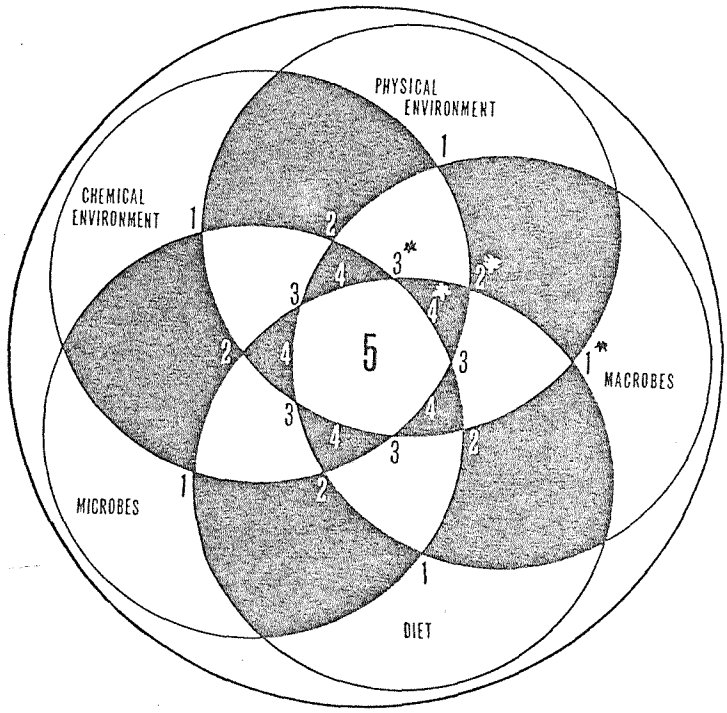

FIG. 1. Environmental components may be studied separately and in various combinations as indicated Some of the states, represented by numbers, are not useful without special delineation. The asterisks show one of many approaches to the complexities represented by both classic and gnotophoric animals in *states*. An embryo is in *state 1** with little input from any environmental component other than its mother. A germfree colony is in *state 4**. The outer circle represents time.

When Tanami inoculated germfree guinea pigs with a guinea pig strain of *Escherichia coli,* M_1, this microbe produced necrotic and hemorrhagic enteritis in the intestine and could be recovered from blood; all three guinea pigs died within 10 days (*experiment 1*, Table 1). When a human strain of *E. coli,* F_6, was inoculated into germfree guinea pigs no tissue damage was noted; Tanami found 10^9 bacteria/g feces and the guinea pigs survived. When equal quantities of each strain were inoculated into a single germfree guinea pig (*experiment 3*), the human strain rapidly disappeared and could not be found in the feces after 4 days; the guinea pig died with the same symptoms caused by the M_1 strain. When the two strains were simultaneously introduced into three germfree guinea pigs with the human strain predominating (*experiment 4*), the human strain survived, whereas the M_1 strain disappeared from the feces within 4 days and the guinea pigs survived. A comparable result was found when either *Lactobacillus bifidus* or *Lactobacillus acidophilus* was inoculated with *E. coli* being given in equal numbers on the following day; *E. coli* did establish itself within 8 days but apparently it could not compete well, as shown by the decreasing fecal counts during the next 2 weeks; again the guinea pigs showed no abnormalities and survived. When 6×10^7 cells of *L. bifidus* were inoculated with 3×10^3 cells of M_1 strain of *E. coli,* results from *experiment 6* showed *L. bifidus* established itself at 10^9/g feces, whereas *E. coli* could not establish itself and disappeared completely within 12 days. A

TABLE 1

Microbial interactions in gnotophoric guinea pigs (5)

Experiment	Oral inoculum		Feces count, log/g				Histopathology	Gross observations
	Microorganism	Number	Day 4	Day 8	Day 15	Day 24		
1	*E. coli*, M_1[a]	10^4		9			Enteritis and infection[b]	All dead in 10 days
2	*E. coli*, F_6[a]	10^4		9			None	Diarrhea only
3	*E. coli*, M_1	10^5	9	9				
	E. coli, F_6	10^5	0	0			Enteritis	All dead in 10 days
4	*E. coli*, M_1	10^3	0	0	0		None	Healthy
	E. coli, F_6	10^6	7	7	9			
5	*E. coli*, M[c]	10^7	7	9	8	7	None, excepting lymph node activation and increased phagocytic activity	Healthy
	L. bifidus	10^7	9	9	9	9		
6	*E. coli*, M_1	10^3	6	4	0	0	As *experiment 5*	Healthy
	L. bifidus	10^7	9	9	9	9		
7	*E. coli*, M_1	10^3	9	9	9	9	As *experiment 5*	Healthy
	S. faecalis[d]	10^7	9	9	9	9		

[a] The M_1 strain was taken from guinea pigs and the F_6 strain from a human. [b] Necrotic and hemorrhagic. [c] The *E. coli* was given 1 day after *L. bifidus*; similar results were noted with *L. acidophilus*. [d] When added separately, the only reaction noted was a slight lymph node stimulation.

more interesting phenomenon was found in *experiment 7* when 3×10^7 cells of *Streptococcus faecalis* were inoculated with 3×10^3 cells of *E. coli*. Both microorganisms established themselves at 10^9/g feces. The continued health of the guinea pigs suggested that either the potential of the M_1 strain for invasiveness or the guinea pig's defense mechanisms were changed by the presence of the *S. faecalis* to allow survival of guinea pigs associated with the otherwise lethal strain of *E. coli*.

Biologic evaluation of Apollo diets showed that they were adequate for three generations of mice in the open laboratory. When classic mice were confined in a germfree isolator and fed the sterile Apollo diet, excessive deaths suggested that either the stress of isolation or some combination of microorganisms was detrimental (paper in preparation). The following experiments illustrate the effect of mono- and di-inoculation of microbes into germfree mice; different parameters were evaluated to determine which microbic species might be the most compatible with the host and which species were most compatible with each other.

EXPERIMENTAL

Classic white mice provide control data for each parameter; they were fed: *a*) nonsterile Apollo diet in the open laboratory; *b*) gamma-irradiated Apollo diet in the open laboratory; and *c*) gamma-irradiated Apollo diet using gnotobiotic procedures; i.e., classic mice were maintained free from any microorganisms not associated with them at entry into the sterile isolator. The three germfree groups were given *a*) autoclaved laboratory chow; *b*) gamma-irradiated Apollo diet with paper bedding, and *c*) gamma-irradiated Apollo diet with no bedding on a stainless steel screen floor. The last provided a control for all of the gnotophoric groups that were fed gamma-irradiated Apollo diet and maintained on stainless screen floor with no bedding. Weanling germfree white mice were inoculated orally with about 10^4 cells of mouse-adapted microbes; the exception was the *Staphylococcus epidermidis*, a contaminant that oc-

TABLE II

Numbers of fecal microbes

Microbe	Log count/g
Monoassociated Mice	
E. coli	12
L. leichmannii	7
S. epidermidis	11
C. albicans	10
Diassociated Mice	
E. coli	11
L. leichmannii	6
E. coli	12
C. albicans	9
C. albicans	9
L. leichmannii	6
S. epidermidis	11
C. albicans	9

cured at weaning. Most of the data were taken when five male mice were killed at 2 months of age.

RESULTS

Fecal counts from the gnotophoric mice are given in Table II. It should be noted that inoculation of two microorganisms gave individual counts within one log of that obtained when either was inoculated singly. In spite of this apparent lack of a large effect of any one microbe upon the numbers of any other, some parameters measured did show dramatic effects.

The body weight at 2 months of age is indicated in Fig. 2, *A*. The germfree mice fed the Apollo diet were small when compared with those fed lab chow or those having filter paper bedding. The *S. epidermidis* and possibly *E. coli* monofloras gave increased body weight. A *Candida albicans* monoflora gave a decreased body weight. An *E. coli–Lactobacillus leichmannii* diflora gave results similar to that of *E. coli* monoflora. The *L. leichmannii–C. albicans* diflora was stimulatory when compared with either monoflora. The *E. coli–C. albicans* diflora gave results comparable to that obtained with a *C. albicans* monoflora. Similarly the result from the *C. albicans–S. epidermidis* diflora resembled that obtained from *S. epidermidis* monognotophoric mice. Such comparisons allow an evaluation of the dominance of different microorganisms according to the results given by the body weight of the animal. In this sense *S. epidermidis* dominated *C. albicans, C. albicans* dominated *E. coli, E. coli* dominated *L. leichmannii,* and *L. leichmannii* with *C. albicans* gave a growth stimulation not seen in mice monoassociated with either microbe. The preferred microorganism was *S. epidermidis;* both fecal count and body weight data from these monoassociated mice were surprisingly high. Tanami (5) reported that *L. bifidus* gave growth stimulation in germfree guinea pigs, and Schaedler *et al.* (6) showed that *Bacteroides* and other components of the indigenous flora were beneficial in the development of gnotobiotic mice.

The total white blood count of germfree animals was lower than that of *S. epidermidis* monoflora mice or *S. epidermidis–C. albicans* diflora mice (Fig. 2, *B*). The *C. albicans–L. leichmannii* diflora mice had lower white blood counts than mice having either of these respective monofloras, and *E. coli–L. leichmannii* diflora mice had lower white blood counts than those of either of these monoflora groups. The combination of *C. albicans* with *E. coli* was comparable to that of *C. albicans* alone. Most interesting is that phenomena in which *L. leichmannii,* as one number of a diflora, effected a lowering of the white blood cell count.

When hemoglobin was the parameter of evaluation (Fig. 2, *C*), germfree mice given filter paper bedding were found to be normal whereas those on stainless screen were somewhat anemic. Again it was noted that mice monoassociated with *S. epidermidis* were the best of all monoflora mice and equivalent to those associated with both *S. epidermidis* and *C. albicans. C. albicans*

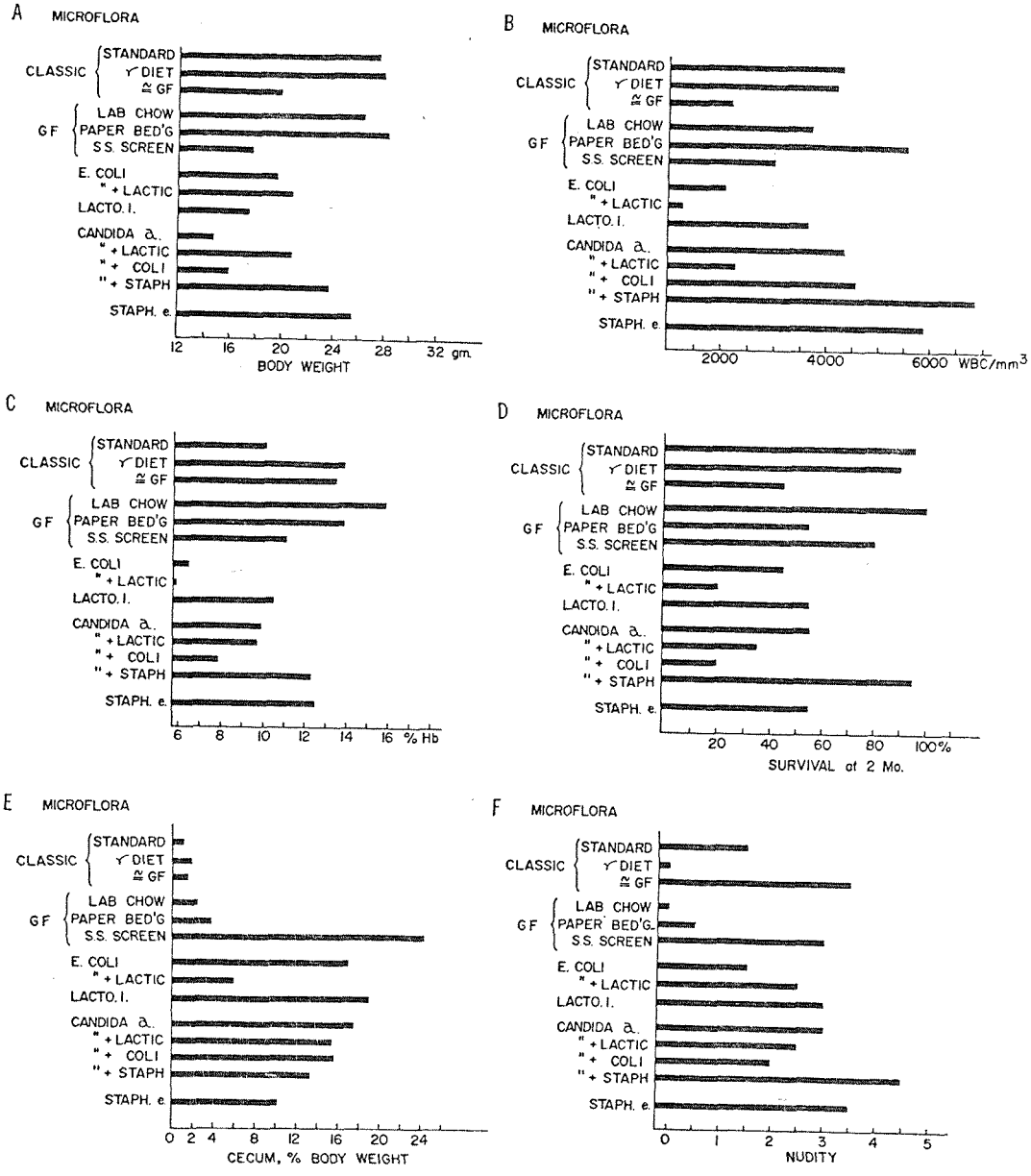

FIG. 2. *A*. This and the following figures present average values taken from predominantly male mice, 2 months old. All mice were obtained at weaning from Charles River Breeding Laboratory, Wilmington, except the group "Gf-paper bedding" was second generation from those reared on wire screen. At the start the classic mice were larger and looked more healthy than the germfree mice. The gnotophoric mice were inoculated orally at 21 days of age except those with *S. epidermidis* that arrived monocontaminated. *B*. Total white blood count. *C*. Percent hemoglobin. *D*. Data present the percent survival at 2 months from 8 males and 12 female mice/group. *E*. Relative cecum weight. *F*. Alopecia in mice fed the Apollo diet. The scale of nudity is: 0 = good hair coat; 5 = completely nude.

and *L. leichmannii* singly or together produced slightly lowered hemoglobin in mice, whereas those having *L. leichmannii* plus *E. coli* showed a dramatic anemia comparable to *E. coli* monoflora mice. The diflora mice with *E. coli* and *Candida*

gave an intermediate value; these two were judged to be equivalent to each other in their effect upon hemoglobin values. Hemoglobin data provide a slightly different picture of microbial dominance when compared with the above parameters.

The survival data (Fig. 2, *D*) reflect the poor state of the mice when they were received as well as their marginal diet. Some of the germfree mice died during the 1st week. No gnotophoric mice showed adequate survival excepting the *C. albicans–S. epidermidis* diflora mice; these survived better than mice with either of the microbes singly. *C. albicans–L. leichmannii* diflora mice survived poorly compared with *C. albicans* or *L. leichmannii* monoflora mice. Diflora mice with *L. leichmannii* and *E. coli* survived less well than mice carrying either of these microorganisms alone. The *E. coli* and *C. albicans* in combination gave poorer survival than was obtained with either of those microbes singly. In three of the four groups examined a combination of organisms allowed less survival than was obtained in the respective monoflora mice. The similarity of results with survival to those obtained with white blood cells adds significance to the lowered white blood cell counts noted in diflora mice with *L. leichmannii*.

The cecum size of germfree animals maintained on stainless screen floor is obviously much greater than that of the control animals (Fig. 2, *E*). Germfree mice having filter paper bedding had dramatically reduced ceca, which mirrors those obtained when fiber was added to the diet of germfree rats (7). The *E. coli* or *L. leichmannii* monoflora were not very helpful but in diflora these microbes were quite helpful in reducing the cecum size. *Candida albicans* alone or in any combination was somewhat intermediate with no specific combination showing an advantage. The *S. epidermidis* monoflora mice had smaller ceca than did other gnotophoric mice excepting the *E. coli–L. leichmannii* diflora mice.

Germfree mice reared on stainless screen exhibited alopecia (Fig. 2, *F*); neck, shoulders, back, sides, and abdomen were affected in that order. This was alleviated by filter paper bedding or the laboratory chow. Monognotophoric mice carrying *S. epidermidis, C. albicans,* or *L. leichmannii* showed alopecia comparable to germfree mice. The *E. coli* monoassociated mice and *E. coli–C. albicans* diflora mice tended to be less nude. The diflora of *C. albicans* with *S. epidermidis* increased alopecia; some of these mice were almost completely hairless.

Although no in vivo microbial antagonism was noted from the fecal microbe counts, a form of microbial dominance is seen in the effects of microbes upon the mice. Those discussed above and two not presented are summarized in Table III. The patterns of dominance suggest an average as noted at the bottom. In four of the eight parameters studied *S. epidermidis* dominated *C. albicans* and they were equivalent (the effect observed in diflora mice was intermediate between the two monoflora groups of mice) in two. *Candida albicans* dominated *E. coli* in three parameters; the two microbes were equivalent in two and gave a depression in two. *Escherichia coli* dominated *L. leichmannii* in two or three parameters; the two caused a depression in two or three parameters, and they were equivalent in two when compared with the effects seen in the respective monoflora mice. The main effect noted with the *L. leichmannii–C. albicans* combination was a depression in half of the parameters being studied and in two parameters the microbes were equivalent. Although these generalities might be useful in anticipating results from other parameters, the general lack of predictability of the effects of two microbes from the results in monoflora mice would suggest the information obtained from diflora

TABLE III

Qualitative microbial dominance in vivo

Parameter	Interactions
Weight	Staph > candida > coli > lactic ∧ candida
WBC	Staph > candida > coli ∨ lactic ∨ candida
Hemoglobin	Staph > candida ≅ coli > lactic ≅ candida
Survival	Staph ∧ candida ∨ coli ∨ lactic ∨ candida
Cecum size	Staph ≅ candida ≅ coli ∨ lactic ≅ candida
Nudity	Staph ∧ candida ≅ coli ≅ lactic ∨ candida
Food efficiency	Staph > candida ∨ coli ≅ lactic ∨ candida
Lymphocytes	Staph ≅ candida > coli ∨ lactic < candida
Majority	Staph > candida > coli ∨ lactic ∨ candida
Minority	≅ ≅,∨ ≅, > ≅

Key: ≅ = equivalent; > = greater than; < = less than; ∨ = depression when the two were combined, and ∧ = stimulation when both were present.

studies would not be a good basis to predict results in trignotophoric mice.

DISCUSSION

These preliminary studies illustrate the range of action of two microbic species as components of host ecology. Also illustrated is microbic interaction in vivo under gnotobiotic conditions. Such bignotobiotic experiments have been little explored in the past. Using another path of the experimental design in Fig. 1, one could undertake a study of microbes in vivo to compare metabolic and physiologic reactions with those in vitro.

The interrelationships presented herein outline possible mechanisms by which the intestinal flora can act as a part of the host defense system. A summary of the effects of *E. coli* is illustrative. Harmful strains of *E. coli* produced poor survival or death in gnotobiotic mice and guinea pigs. *L. acidophilus* apparently overgrew the *E. coli*—it could not be found in fecal cultures after 6 days. When added with *E. coli* to germfree guinea pigs, *S. faecalis* neutralized the effects of *E. coli* without inhibiting its growth. When *L. leichmannii* was added with *E. coli* to germfree mice more deaths occurred than were obtained with *E. coli* monoflora. When *C. albicans* was added with *E. coli* to germfree mice the diflora was more harmful than either monoflora for survival and food efficiency. The in vivo effect of a second microbe added to the harmful strain of *E. coli* may be *a*) overgrowth *b*) neutralization, or *c*) increased harmfulness. Surprisingly, species of *Lactobacillus* were found in both categories *a*) and *c*).

Recent work (8, 9) has shown an energy contribution to animals from cecal microbic fermentation. Such studies should be extended to the colon.

The fecal flora probably reflects the status of microbes in the lumen of the rectum, large intestine, and possibly the cecum. It is presently a poor guide to microbes intimately associated with the mucosal lining of any of the alimentary tract. Each component of the alimentary tract has its own distinct flora. Rosebury's (10) review gives the status of the oral flora. The flora of the crop of the chicken is predominantly a single species of *Lactobacillus* (11). This homogenous flora is maintained in intimate association with the mucosal wall by virtue of the nucleic acids, a nutritive requirement for this species, supplied by epithelial disintegration. The rumen has its distinctive flora and the stomach of monogastric animals has an intimate yeast layer over the cardiac mucosa and lactobacilli associated with the

fundus mucosa. Tapered rods, spirochetes, and filamentous microbes provide an intimate lining to the mucosal cells of the cecum and colon and are not usually reported in fecal counts (12), yet they are present in high concentrations. These ecologic niches within the alimentary tract for special microbes raise questions regarding the present work. Did the microbes used herein occupy any or all of these niches? Would any or all of these mucus-associated microbes have altered or prevented the effects of microbes on hosts in this study? Work needs to be done with more species of microbes. The histologic techniques of the Dubos school must be combined with the anaerobic culture methods of the Hungate–Moore school to give more complete microbic information for each alimentary tract niche. Such information must be combined with that presented herein and with knowledge of chemical mediators of microbic interaction to provide a realistic view of microbic–host interactions. The approach of Raibaud and associates is salutory (13).

SUMMARY

Gnotobiology allows a more exact exploration of ecology by separating macrobes from microbes and providing an experimental base for qualitative and quantitative understanding of the interaction of microbes in and with the host. Mono- and diflora studies with *E. coli, L. leichmannii, S. epidermidis,* and *C. albicans* indicated that each microbe exerted profound effects upon the host and upon any coinhabitant of the host. The *S. epidermidis* monoflora mice were better than other gnotobiotic mice for the following parameters: growth, hemoglobin, and cecum size. The *S. epidermidis–C. albicans* diflora mice were the most nude and showed the best survival of all the groups compared. The *E. coli* monoflora gave the best fur coat and the worst survival. For most parameters measured in gnotophoric mice *S. epidermidis* dominated *C. albicans*, *C. albicans* dominated *E. coli*, and *L. leichmannii* gave a depressing action with either *C. albicans* or *E. coli*. None of this information was reflected in the fecal microbe counts.

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GNOTOBIOLOGIC EVALUATION OF APOLLO DIET

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Radiation sterilized, comminuted 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 30 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 di-flora *C. albicans* reduced the food efficiency of mice. *C. albicans* or *Bacteroides* monoflora provided protection from the alopecia observed in other groups. Details of observed microbial counts will be presented. It is concluded that the nurture and well being of mice is profoundly affected by their intestinal microflora.

Ae-6

MECHANISM OF ALUMINUM ALLOYS CORROSION IN JET AIRCRAFT FUEL TANKS BY THE GROWTH OF *CLADOSPORIUM RESINAE*

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It has been recognized that microbial contamination of jet fuel is a cause of aluminum alloys corrosion by aero-space microbiologists recently. Fungus, *Cladosporium resinae* and *Micrococcus caseolyticus*, *Vibrio fecalis*, bacteria have been isolated from jet aircraft fuel systems. Aluminum alloys corrosion test has performed in the laboratory for confirmation of identified strains. *Cladosporium resinae* is the most powerful for aluminum alloys corrosion has been observed. More than 20 anti-microbial compounds have been proved satisfactorily to remove the contamination from jet fuel. Furtheremore investigation of the evaluation of practicability with these compounds are under the way. Mechanism of aluminum alloys corrosion by the growth of fungus than bacteria has been emphasized in this paper.

CHANGES IN INDIGENOUS MICROFLORA DURING BIO-ISOLATION SIMULATING LONG TERM SPACE FLIGHT

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After four 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 as 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 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 a increase in the anaerobic count. Some microorganisms, originally present in numbers of less than 10^7/gram of sample, increased in the bioconfined animals to 10^7/gram of sample count. After five months, the *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 marker organisms. Associated physiological studies included hematology and serum proteins. Implications of these results, in the context of long term space flight are possibly deleterious changes in the immunity mechanisms, potential pathogen runaway and loss of the benefits of a protective mixture of microflora.

Fecal Indices of Health and Disease

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The feces (equivalent words are stool, excrement, egista and ordoure) are a reflection of the interaction of the environment, intestinal microbes and host. This interaction makes the digesta of the intestinal tract and the feces equivalent to blood in that both reflect something of all organs with which they come into contact. However, the stool has been less well utilized and is less well understood. The digesta and feces remain one of the last frontiers of man's understanding of himself. It should be considered as a dynamic and integral part of the whole man.

Environmental factors to be considered in feces composition are the relatively obvious nurture and the less obvious nature. Nurture provides the food, drugs and drink of the host, and to some degree the inhalations of the host and material absorbed into the skin. Components of any or all of these may be reflected in the excreta. Although other means of excretion form a part of the total pattern of excretory materials, feces and urine remain the most important. This report will be concerned with feces but it is obvious that urinary studies should be correlated with many of the problems suggested herein. The other component of the environment is less well considered in the formation of feces; the nature of the environment in which the feces are formed. The pH of the intestinal tract is rarely on the alkaline side, the pH of the feces may be within a one-half unit pH of neutrality. The temperature at 37° is ideal for bacterial action; fever or chilling should have a remarkable effect on the intestinal mucosa and microflora. (I've seen no good verification of this). This temperature change may also be reflected in the amount of cellular debris and the amount of absorption which takes place in the lower intestine. The eH of the lower intestine has not been well studied. It is usually in the range of -0.2 mv. The eH of feces must play a tremendous part on the dominance of different species of intestinal flora. This flora plays an important part in health and is

one defense mechanism of the host. The amount of water in the lumen is adequate for good microbial growth. The quantity of water will be considered under physical characteristics. The element of time is important in considering the physical chemical characteristics of the stool. The amount of time it is held in the rectum is longer for man than for most other species; i.e., farm animals show an almost constant hourly passage of food into the GI tract and feces from the GI tract. The variety of actions within the stool while it is held in the rectum may be quite important depending upon the amount of time the stool is held in the rectum. Equally important should be considered the amount of time the stool is held following passage. A new and changing gas, temperature, water content, redox potential and pH are presented to the external stool.

The contribution of the host to the feces is considerable. The saliva, gastric juice and intestinal juices all contain a variety of enzymes, minerals, hormones and excellular debris which can be reflected in the composition of the feces. Although the secretion, excretions and cell debris of the host contribute to the digesta as it passes through the tract, the major action to be observed is the absorption of food material from the intestinal millieu. The residue of digestion is the unabsorbed food and the host contribution residues with microbial processing. During the passage of food through the GI tract the material becomes concentrated between 5 and 10 times by the action of bioincrassation. Bioincrassation is the act of increasing the concentration of any material by taking other material away from it, in a biological system. Thus the absorption of carbohydrate, protein and fat leaves a concentrated mineral and vitamin mixture in the feces.

The third major vector of feces formation is the microbic contribution. Feces consists of mostly living microbial cells, dead cells and microbial processed material. This is an area of active investigation but little useful understanding has been presented in a literature to date. Without going into detail in this area it should be recalled that the total numbers of living microorganisms approached approximately 10^{11} living microbes per gram of feces. The great majority of these are small anaerobes about which we know little from a chemical and metabolic view-

point. These resident cells are most important in the normal individual; however there are showers of transient microorganisms and organisms, such as yeasts, which are constantly in the fecal material in small quantities; these do not become established in the intestinal tract under normal conditions. Some yeasts and potential pathogens are always present. Very often pathogenic organisms are also found in normal stools. It is estimated that live cells may make up 30 or more percent of the total fecal bulk. The total bacterial contribution of live cells, dead cells and microbial processed material must constitute well over 90 percent of the feces. This shows what a difficult job it will be to determine cause and effect of host metabolic health and disease processes from analysis of the feces alone.

In spite of the above problems, certain information on the health and/or disease state of the individual is derivable from physical, chemical examination of feces. The following brief consideration of the normal excretory products in feces of man will be followed by ways and means of understanding health and disease through fecal examination.

PHYSICAL CHARACTERISTICS

The physical characteristics to be examined are quantity, appearance, physical consistency, temperature, and pH.

The variation in the normal daily excretion is so great that the average daily excretion value of 100 grams is of little importance unless careful observation of diet and individual characteristics are observed. The adult male will excrete somewhat more than this on an average, approximately 140 $\pm$ 30 grams of solids, with dry matter ranging between 25 and 45 grams per day. The amount will vary depending upon the individual characteristics, the amount of food taken in, the size of the host, the frequency of voiding, the type of food and the health status. Indigestible hemecelluloses (such as agar-agar, psyllium seed, bassirom) and modern bulk polymers are nondigestible and absorb water readily. Therefore their ingestion considerably increases the bulk of feces. The use of low residue food, a decrease in food ingested and some times the normal metabolic efficiency of the host will lead to small quantities of feces.

The normal shape is fusiform not unlike a large, fat cigar. The appearance is generally honogeneous although gross undigested particles, i.e., corn, may often be seen. The fecal mass is usually a formed, cohesive material. The microscopic appearance will show both live and dead bacteria and other microbic forms. It will show many particles of digesta of all sizes and many starch granules and sometimes the eggs or other particles from protozoa or other invertebrates. Microscopic observations may include fat, connective tissue, fibre, formed elements from the epithelial lining of the tract, erythrocytes, pus, corpuscles, mucosus, parasites and sometimes crystalline deposts such as coprosterol, soaps, fatty acids, inorganic crystals and pancreatic or other calculi.

The principle pigment of the feces is sterobilin which is formed by oxidation of stercobilinogen. This compound comes from bilirubin which is reduced by intestinal bacteria to mesobilirubinogen. This is the normal breakdown product from hemoglobin and sometimes both bilirubin and biliverdin may be seen in fecal material, particularly in nursing infants or during diarrhea. The dark color of the stool may be due to excessive oxidation of stercobilin before or after voiding. Dark stools are noted with diets high in meat whereas a light stool may come from a milk diet. Dark stools may also be obtained following the ingestion of bismuth or iron drugs and light stools may result from barium meals or excessive fat excretion. Oral administration of antibacterial drugs tend to give light colores stools. Other food characteristics may be noted following their ingestion, (i.e., calomel ingestion leads to a green stool due to the lack of bacterial action; this provides biliverdin in unmetabolized form). The odor of the stools are rarely due to material being eaten; it is usually due to bacterial action upon the food residues. The normal odor of feces comes from skatole and indole. Other compounds such as methane, methylmercaptan or hydrogen sulfide may also be regularly produced by bacterial fermentation. This variety of compounds occurs more markedly with a meat diet or a mixed diet; less odor is obtained when a vegetable diet is used and little odor is detected in a stool from a strictly milk diet.

Single strains of microorganisms in the intestinal tract of rats or other animals will produce characteristic odors in the excreted gases. Thus a pure lactic acid culture will be detected in the odor of the exhaust gas of an isolator housing monoassociated rats.

The appearance may be further characterized by the consistency of the stool. If it is liquid it indicates diarrhea. It may be soft which is normal for the food intake or it may be toward the diarrhea state. The consistency is normally solid; it may be hard due to partial constipation or it may be very hard or even pelleted and extra dry from constipation. Although the fluidity of feces is an important physical characteristic, it is normally related so much to the proportion of dry weight that this factor will be taken up under the chemical characteristics.

The temperature is normally 37° when voided and may decrease to room temperature quite rapidly due to water evaporation and/or heat convection to the new medium. The eH has been little explored. It is expected to be in the neighborhood of -0.2 ± 0.1 mv.

CHEMICAL CHARACTERISTICS

The normal pH of feces is approximately 7: it may vary within 1/2 unit pH of this and is often on the alkaline side of 7. The total acidity or alkalinity have not been well investigated and are not normally considered, as they have been in urine. Dietary lactose favors an acidic stool.

The enclosed tables from Altman and Dittmer and from Geigy Tables give the normal excretion pattern of compounds in the feces of man. Gross analysis of feces shows a remarkable similarity to that of food and the whole body. Minerals and vitamins are more concentrated and carbohydrate is considerably less in feces than in food.

ENZYME AND ANTIBODY

Protein constituents should be considered, although neither have been well examined in either normal or abnormal conditions. Normally there are few free proteolytic digestive enzymes in feces. The digestive enzymes of the host apparently have been processed by the bacteria; therefore, the appearance of such enzymes indicates an abnormal state. Copra-antibodies have been studied by bacteriologists and immunologists to an

extent. What has not been studied is the appearance of normal serum antibodies in the feces. The detection of these antibodies may be difficult but they are apparently active and functioning in the GI tract. Thus, W. J. Visek presented a paper entitled "Effect of Urea Hydrolysis Upon the Cell Life Span and Metabolism" at the 1971 Federation Meetings. It should soon appear in the Symposium Volume. He gave evidence that tissue injection of urease into an animal allowed the antibody of urease to appear in the intestinal tract where it prohibited the intestinal microorganisms from producing ammonia. He related this to cancer production. This suggests that intensive tests for antibodies of a variety of sources might be made in feces.

METABOLIC STUDIES

Feces are very important in metabolic studies in which the original compound of interest or its metabolites may be found in the feces. Of equal importance are balance studies of any nutrient or drug or other compound of interest, i.e., cholesterol; these may be studied using the diet, feces and urine as the prime places for intake and excretion. It is recognized that cholesterol may be synthesized in the body and therefore such metabolites are not ideal for balance studies. As has been previously discussed, nutritional markers are most useful to determine dietary intake in balance studies.

ABNORMAL CONDITIONS DETECTABLE BY FECAL ANALYSIS

1. Nutritional Status

A poor nutritional status can be detected by the disproportion of any given nutrient or a variety of nutrients in the feces when compared to normal. This generalization is exemplified by energy: a nutritional balance for energy may be done by direct or indirect calorimetry or by examination of the protein, fat and carbohydrate balance of the individual. Aside from the balance study, a simple excess of any one component in the feces is adequate criterion to suspect improper digestion. For example, a fatty stool is clear evidence that fat absorption is not occurring properly and the total energy

intake of the individual should be questioned. Oser mentions the uptake of orally administered vitamin B-12 as a good test for pernicious anemia.

2. Alimentary Tract

The consistency and dry weight of the stool gives a good index of the overall function of the GI tract. If the output of feces is unusually variable this suggests the possibility of a partial blocking of the intestinal tract. If the output is small, this suggests starvation. If the fecal material is hard or low in dry matter, this suggests constipation. If there is an excess of water, the diarrhea may be caused by many possibilities such as vitamin deficiency or a local or generalized infection throughout the tract. At such a time an examination of the fecal material for microorganisms is indicated. Hook worms, tape worms or other parasites or their ova may be found. A pure culture or excessive quantity of any one species of microorganism may indicate infection. This may happen with a variety of microorganisms.

A search for blood should be made to determine whether or not the lining of the intestinal tract is intact. A fatty stool would suggest steatorrhea or poor absorption of fats; this might be related to the GI tract problems per se or to the bile. The pH should be $7.0 \pm .5$. It is usually slightly acid in children or babies and slightly alkaline in adults. It should not be very far from 7, if it becomes too alkaline then an ammonia test should be run and total fecal urease should be determined. Too much ammonia could be absorbed and become toxic and according to some hypothesis it may cause tumor indirectly. A search for host digestive enzymes can be made. These should be present in negligible quantity under normal conditions. If the intestinal tract has been stimulated and is passing digesta too fast, then the digestive enzymes may be found. At the same time more bilirubin and biliverdin may be noted. Prolonged diarrhea will continually bring the digestive enzymes through the tract and cause anal itching.

The types and kinds of bacteria present should be studied to learn to control and maintain a well balanced flora in the healthy individual; dietary sources of microbes and/or microbial nutritive material may eventually be incorporated into the diet. The amount of skatole and indole can be determined to give an index of microbial putrefaction.

The determination of volatile fatty acids would indicate how much antibacterial action was present in the feces. A determination of Vitamin B_{12} would indicate how much activity was going on in the production of B vitamins and particularly this important vitamin.

3. Blood

Localized or generalized bleeding of the alimentary tract will be reflected by blood in the stool. Microscopic examination might show pus, red blood cells or excessive white cells. Tests for ocult blood should be run and a combination of bilirubin and biliverdin and urobilinogen would show whether excess hemoglobin breakdown has occurred either in the intestine or in the body. Vitamin K should be present in the feces. Its presence may be correlated with blood clotting time. When drugs or certain sickness occurs vitamin K may disappear and blood clotting be prolonged. Copper and iron determinations may be needed to determine the balance of these two nutrients for blood formation. Other balances which might be done are folic acid, vitamin B_{12}, riboflavin and vitamin B_6; all are needed for blood formation.

4. Liver

Examination of stools for bile acids, coprosterol, cholesterol derivatives, excessive fat, bilirubin and biliverdin can be made to determine whether bile production and hemoglobin breakdown by the liver is adequate. This gives an index of the state of health of the liver. The presence of gall stones or excess cholesterol suggest malfunction of the liver. The stool loses its color during obstructive jaundice and the stools decrease drastically the content of bilirubin, urobilin, urobilinogens, cholesterol (from the body) and alkaline phosphatase.

5. Vascular Health

Cholesterol could be determined to indicate and give a rough indication of the state of the vascular health of the individual. Admittedly other criteria would be more exact and better. The size and saturation of fatty acids excreted should be observed.

6. Bone

Calcium soaps and excessive fat in the feces indicate that bone formation will be eventually disturbed. Both indicate a general activity of intestinal function and health. More particularly, if these are excreted in large quantities, vitamin D will be carried with the lipid excreted. The calcium soaps form an insoluble material which prevents the calcium absorption. Other calcium salts as phytates, oxalate or citrate could be determined. A total balance for calcium, phosphorous and magnesium should be run for the determination of the potential for building and maintaining bone and calcium stores. Vitamin D may be run directly on feces. A sophisticated system to determine calcium binding protein could be done (as per the work of Corradinora and R. H. Wasserman, 1971, Vitamin D: Induction of Calcium Binding Protein in Embryonic Chick Intestine In Vitro, Science, 172, pp. 731-733).

7. Muscle

Since much of the muscle mass is enzymatic and structural protein, a total nitrogen balance would be of importance to study muscle metabolism. At the same time creatinine and creatine should be determined in the feces and correlated with urinary excretion of these compounds. A dietary survey of zinc may be important from the total muscle mass viewpoint. The determination of potassium and sodium would indicate the state of tissue health in the intestine and tissue draining into intestine. This might best be run with a sodium and/or potassium balance since the dietary intake may have an important reflection upon the excretion of these two elements.

8. Pancreas

Pancreatic calculi may sometimes be seen in the feces. Pancreatic enzymes could be tested following anything given to accelerate the passage of digesta through the GI tract. This may be normally observed during period of diarrhea.

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IX. METABOLIC END PRODUCTS

102. EXCRETION PRODUCTS IN FECES: MAN

Values are based on "normal" dietary intake, including approximately 10 g nitrogen/day. In reducing values to mg/kg or μg/kg, a body weight of 70 kg was assumed, unless specific weight was reported in the literature. Values in parentheses are ranges, estimate "c" (*see* Introduction).

	Constituent (Synonym)	Amount Excreted per kg body wt per day	Reference
	General Chemical Constituents, mg		
1	Solids	394(140-560)	46
2	Water	(910-1820)	40
	Electrolytes, mg		
3	Aluminum	0.0006	26
4	Arsenic	0.033(0.001-0.116)	36
5	Calcium	(5-10)	16,38
6	Chlorine	(0.21-0.50)	4
7	Cobalt	(0.000002-0.000020)	20
8	Copper	0.027(0.023-0.037)	26
9	Iron	120(65-208)	9
10	Lead	0.0042	26
11	Magnesium	2.5(1.510-3.185)	29
12	Manganese	(0.018-0.120)	26,27
13	Mercury	0.00014	39
14	Nickel	(0.0012-0.0025)	27
15	Phosphorus, total	0.00986(0.00710-0.02000)	9
16	Potassium	6.7	7
17	Silver	0.0008	26
18	Sodium	1.7	7
19	Sulfur, total	2.0	7
20	Tin	(0.17-0.45)	7,26
21	Zinc	0.100(0.058-0.144)	41
	Vitamins & Related Compounds, μg		
22	Thiamine	7.80(0.67-18.00)	10
23	Riboflavin	14.7(8.0-23.0)	10
24	Nicotinic acid	52(12-124)	10
25	Biotin	1.90(0.63-6.64)	10
26	Pantothenic acid	31.40(3.85-63.40)	10
27	Folic acid	4.3(1.8-7.7)	10
28	*p*-Aminobenzoic acid	3.50(1.01-8.20)	10
29	Ascorbic acid	(60-70)	6
30	Vitamin E	308(226-391)	28
31	Xanthophyll	(8-100)	42
32	Xanthophyll + carotene	(20-600)	42
	Lipids & Miscellaneous Organic Acids, mg		
33	Fats, total	56(30-100)	46
34	Neutral	(10-45)	15
35	Unsaponifiable	33(22-38) [1]	45
36	Fatty acids, total	(41-92)	3,18,25
37	linoleic	(1.6-3.6)	18
38	oleic	(5-11)	18
39	palmitic	(13-30)	18
40	stearic	(14-33)	18
41	Soaps, total	53(40-66) [1]	45
42	*n*-dodecanoic	0.3 [2]	34
43	*n*-tetradecanoic	1.9 [2]	34
44	*n*-pentadecanoic	0.4 [2]	34
45	*n*-hexadecanoic	38.8 [2]	34
46	*n*-heptadecanoic	1.3 [2]	34
47	*n*-octadecanoic	49.2 [2]	34
48	$\Delta^{9,10}$-hexadecanoic	1.1 [2]	34
49	$\Delta^{9,10}$-octadecanoic	7.0 [2]	34
	Neutral steroids		
50	Total	(9-14)	2,12
51	Campesterol (24α-Methyl-cholest-5-en-3β-ol)	0.6	12
52	Cholesterol (cholest-5-en-3-α-ol)	1.4	12
53	Coprostanol (5β-cholestan-3β-ol)	6	12
54	Coprostanone (5β-cholestan-3-one)	0.6	12
55	β-Sitosterol (24β-ethyl-cholest-5-en-3β-ol)	0.6	12
56	Stigmasterol (24β-ethyl-cholest-5,22-dien-3β-ol)	2	12
57	Hydrocarbons	3.9(1.4-5.6)	2
58	Mono- & di-glycerides	1.2(0.4-1.7)	2
59	Triglycerides	3.9(1.4-5.6)	2
60	Long-chain alcohols	1.9(0.7-2.6)	2
61	Long-chain esters	1.9(0.7-2.6)	2
62	Phospholipids	2.3(0.8-3.4)	2
63	Bile acids, total	3.9(1.4-5.6)	3
64	individual [3]	Trace	5,8,11,13,19,23,24,32
65	Phenol, total	(0-3)	14

[1] At 8-12 years old. [2] Expressed as % of total fatty acids. [3] Includes lithocholic; 3β-hydroxy-5β-cholanoic; chenodeoxycholic; 3α,7α-dihydroxy-5β-cholanoic; 3α,7β-dihydroxy-5β-cholanoic; 3β,7α-dihydroxy-5β-cholanoic; deoxycholic; 3α,12β-dihydroxy-5β-cholanoic; 3β,12α-dihydroxy-5β-cholanoic; 3β,12β-dihydroxy-5β-cholanoic; cholic; 3α,7α,12α-trihydroxy-5α-cholanoic; 3α,7β,12α-trihydroxy-5β-cholanoic; 3β,7α,12α-trihydroxy-cholanoic; 3β,7β,12α-trihydroxy-cholanoic; 3-keto-5β-cholanoic; 3,12-diketo-5β-cholanoic; 3-keto-7α-hydroxy-5β-cholanoic; 3-keto-12α-hydroxy-5β-cholanoic; 3α-hydroxy-7-keto-5β-cholanoic; 3α-hydroxy-12-keto-5β-cholanoic; 3β-hydroxy-12-keto-5β-cholanoic; 3α,7α-dihydroxy-12-keto-5β-cholanoic; and 3α,12α-dihydroxy-7-keto-5β-cholanoic acids.

continued

	Constituent (Synonym)	Amount Excreted per kg body wt per day	Reference
	Nitrogenous Substances, mg		
66	Imidazole derivatives	(0-0.2)	30
	Porphyrins		
67	Bilirubin	0.14	44
68	Coproporphyrin	(0.005-0.014)	35
69	Protoporphyrin	0.014	17
70	Urobilinogen + stercobilinogen	2	43,44
71	Uroporphyrin	(0.00014-0.00060)	35
72	Purine bases	(2-3)	31
73	Amino acids, arginine, total	3.8(2.9-5.0)	37
74	histidine, total	1.7(1.4-2.1)	37
75	isoleucine, total	4.3(3.3-5.5)	37
76	leucine, total	5.6(4.3-6.9)	37
77	lysine, total	5.7(4.5-6.9)	37
78	threonine, total	4.0(3.3-5.2)	37
79	valine, total	4.6(3.6-6.2)	37
80	Nitrogen, total	(11.4-36.0)	21
81	ammonia	(0.36-1.2)	33
	Enzymes		
82	Chymotrypsin	Consult references	1,22
83	Trypsin		1,22

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(For references see page 660)

Faeces consist of a complex mixture of food residues, digestive-tract secretions, and cells shed from the intestinal wall; representatives of the intestinal flora are also present. Unlike the faeces following food intake, the fasting faeces – on which only a few older studies exist[1] – contain no food residues; the meconium discharged from the bowel of the newborn infant is also free of bacteria. Unless otherwise stated the data here given for faeces apply to adults on a mixed diet. Detailed studies of the faeces have been published by KRZYWANEK and FLASCHENTRÄGER[1] and by HINSBERG et al.[2], and there is a tabular summary due to ALBRITTON[3].

	Mean	95% range (extreme range in brackets)	s	Reference	Remarks
Physicochemical data					
Appearance	...	...	...	...	Meconium: Soft, sticky, homogeneous mass, odourless and *greenish brown* to *black* in colour. Infants' stools: *Golden yellow* (bilirubin) on breast milk, turning *green* (biliverdin) on long standing; *brown* (stercobilin) on cow's milk. Adult stools: *Brown* (stercobilin, bilifuscin, mesobilifuscin), darkening on exposure to air; darker on low-residue meat diets, lighter on high-residue vegetable diets. *Black* from plant juices (whortleberries), charcoal, iron (ferric sulphide). *Pitchblack* when haematin content is high (black pudding, in haemorrhage of stomach or upper intestinal tract). *Light grey* when fat content is high (colour not due to fat but to breakdown products of bile pigments[4]).
Odour	...	...	...	...	Typical odour is due to volatile degradation products of protein.
Amount					
Meconium (g)	–	(70–90)	–	5	Values from (a) 44 children, (b) 24 male adults. The amount excreted daily depends on the amount and nature of the diet: infants on breast milk excrete less faeces (ca. 15–25 g/24 h[5]) than those on cow's milk (ca. 30–40 g/24 h[5]); for adults the daily amount after long fasting falls to 9.5–22 g, on a purely meat diet it is 54–64 g, on a purely vegetable diet ca. 370 g; in disease daily amounts of 500–1200 g or more occur[6].
(a) Children's stools, 2 months–6 years (g/24 h)	–	(6.6–54.1)	–	6	
(b) Adult stools (g/24 h)	115.3	33.1–197.5	41.1	7	
Number of stools per day					
Children					
1 day	–	(3–4)	–	8	69% of 500 healthy newborn infants had their first stools within 12 hours, 94% within 24 hours[10].
1 week	–	(4–5)	–	9	
2 weeks	–	(3–4)	–	9	
3–6 weeks	–	(2–3)	–	9	
7–13 weeks	–	(1–2)	–	9	
Water					
(a) Meconium (g/kg)	774	712–836	31	11	Values from (a) 12, (b) 44, (c) 7 subjects.
(b) Children's stools, 2 months–6 years (g/kg)	–	(623–857)	–	6	
(c) Adult stools					
(g/kg)	750	–	–	12	
(g/24 h)	111	–	–	12	
Dry substance					
Meconium (g/kg)	276	–	–	13	Values from (a) 44, (b) 24, (c) 7 subjects. In adult stools[1,8,12,14] 14–30% or more of the dry substance consists of dead bacteria, 25–40% of food residues (cellulose, muscle fibres, etc.); about $^1/_3$ of the dry substance is inorganic material, $^1/_3$ nitrogenous substances, $^1/_4$ lipids, $^1/_6$ cellulose and similar substances.
(a) Children's stools, 2 months–6 years (g/24 h)	–	(2.0–12.9)	–	6	
(b) Adult stools (g/24 h)	34.0	1.6–66.4	16.2	7	
(c) Adult stools (g/24 h)	21	11–31	5	12	
Ash (% of dry substance)					
Meconium	4.0	–	–	13	
Adults	20	–	–	1	
Calorific value					
(kcal/g dry substance)	5.15	(4.21–5.99)	–	16	Values of over 6 kcal/g dry substance are pathological and indicate inadequate utilization of food.
(kcal/24 h)	139	< 213 (upper limit of normal)	–	16	
pH value					
Meconium	6.1	(5.7–6.4)	–	16	The pH depends on the type of food and its rate of passage through the intestinal tract as well as on the intestinal flora, etc. Infants on breast milk have acid stools, those on cow's milk neutral or alkaline stools[16]. Adult stools with the pH given are soft and formed, whereas acid stools are rather unformed, alkaline stools rather hard[15].
Infants' stools, 6 days (on breast milk)	4.9	(4.6–5.2)	–	16	
Adult stools	7.15	5.85–8.45	0.65	17	

(For references see page 660)

	Mean	95% range (extreme range in brackets)	s	Reference	Remarks
Ions					
(mEq/kg) (anions or cations in solution)	–	(180–220)	–	20	Among the cations in solution are the sodium, potassium and ammonium and part of the calcium and magnesium, among the anions organic acids, free fatty acids, bicarbonate, chloride and part of the phosphate[21–23].
Inorganic substances					
Bicarbonate (mEq/kg)	< 30	–	–	22	
Chloride (mEq/24 h)	–	(0.5–3.0)	–	24	With an average chloride intake of 50–150 mEq/24 h.
Phosphorus					
(a) Meconium (mmol/kg)	5.28	2.38–8.18	1.45	11	Values from (a) 12 infants, (b) persons with an average phosphorus intake of 25–50 mmol/24 h. Most of the phosphorus is present as calcium phosphate, a small part as phosphate ion in solution[22, 23].
(b) Adult stools (mmol/24 h)	–	(10–25)	–	24	
Sulphate	0	–	–	22	
Fluoride (mg/24 h)	–	(0.5–2.2)	–	25	With an average fluorine intake of 1.5–4.7 mg/24 h.
Iodine (µg/24 h)	–	(10–57)	–	26	Values from 7 persons.
Potassium					
(a) Meconium (mEq/kg)	31.4	11.8–51.0	9.8	11	Values from (a) 12 infants, (b) persons with an average potassium intake of 50–75 mEq/24 h, (c) 7 adults.
(b) Adult stools (mEq/24 h)	–	(5–15)	–	24	
(c) Adult stools (mEq/24 h)	11.3	3.3–19.3	4	12	
Sodium					
(a) Meconium (mEq/kg)	136	90–182	23	11	Values from (a) 12 infants, (b) persons with an average sodium intake of 50–150 mEq/24 h, (c) 7 adults.
(b) Adult stools (mEq/24 h)	–	(0.5–5.0)	–	24	
(c) Adult stools (mEq/24 h)	6.5	0.5–12.5	3	12	
Calcium					
(a) Meconium (mEq/kg)	23.2	6.5–39.9	8.35	11	Values from (a) 12 infants, (b) persons with an average calcium intake of 25–75 mEq/24 h. In adult stools about 10 mEq/24 h is of endogenous origin (intestinal secretions)[27].
(b) Adult stools (mEq/24 h)	–	(15–65)	–	24	
Magnesium					
(a) Meconium (mEq/kg)	39.2	18.2–60.2	10.5	11	Values from (a) 12 infants, (b) persons with an average magnesium intake of 20–40 mEq/24 h.
(b) Adult stools (mEq/24 h)	–	(10–30)	–	24	
Iron					
(a) Meconium (mg/kg)	16.8	(12.0–27.1)	–	28	Values from (a) 6 infants, (b) persons with an average iron intake of 7 mg/24 h
(b) Adult stools (mg/24 h)	–	(5.7–6.7)	–	29	
Copper					
Meconium (mg/kg)	17.0	(9.5–24.7)	–	28	Values from 6 infants.
Adult stools (mg/24 h)	1.96	0–4.62	1.33	30	
Zinc					
Meconium (mg/kg)	65.0	(38.8–117)	–	28	
Adult stools (mg/24 h)	–	(5.1–10.3)	–	31	
Cobalt (µg/24 h)	–	(0.19–1.21)	–	32	
Manganese (mg/24 h)	3.69	0–8.29	2.30	30	
Other elements	...	...	...	...	The amounts of aluminium, lead and tin are of the same order as those in the food[30]. On strontium excretion see SCHMID and ZIPF[33], on strontium in meconium see WIDDOWSON[11].
Nitrogenous substances					
Nitrogen					
Meconium (g/kg)	19	–	–	13	Values from (a) 24, (b) 7 persons. The nitrogenous components are from mucus and epithelial cells of the intestinal wall and from digestive juices, bacteria and food. 17% of the nitrogen is in the bacterial fraction; about 47% of it is water-soluble[12]. During fasting about 0.25 g nitrogen per day is excreted in the stools[1]. The nitrogen content is *increased* in some types of diarrhoea as well as in pancreatic disease and steatorrhoea.
Infants' stools (g/24 h)					
On breast milk	0.16	–	–	1	
On cow's milk	0.4	–	–	1	
Adult stools (g/24 h)					
(a)	1.8	–	0.2	7	
(b)	1.1	–	–	12	

	Mean	95% range (extreme range in brackets)	s	Reference	Remarks
Proteins	...	...	...	...	The proteins consist mainly of undigested nutrient proteins and bacterial proteins, with only a very small proportion of plasma proteins, most of which enter the intestine with the digestive secretions and are broken down by bacteria and absorbed. The following have been demonstrated immunologically in meconium and to some extent in children's faeces[34]: prealbumin, albumin, γG-globulin, α_2-macroglobulin and siderophilin. Faeces, and especially meconium[35], also contain mucopolysaccharides, including blood-group specific substances.
Amino acids	...	...	...	...	In children's stools free amino acids represent only a small part of the total nitrogen[36].
Ammonia (mg/kg)	–	(251–884)	–	37	Ammonia arises in the terminal intestine from bacterial action.
Porphyrins					
Coproporphyrin (mg/24 h)	0.422	0.012–0.832	0.205	38	Deutero- and mesoporphyrin are also present[38]. The porphyrin content is often *increased* in idiopathic steatorrhoea[38] and some porphyrias[39].
Protoporphyrin (mg/24 h)	0.955	0–2.09	0.567	38	
Bilirubin					
Meconium (mg/kg)	585	(252–1020)	–	40	The bilirubin content of the meconium falls as that of the plasma rises (the values given are for a plasma bilirubin level of less than 50 mg/l). Towards the end of the first year of life, when the intestinal flora has developed, the bilirubin content of the stools reaches the adult level[42]. Disturbances of the intestinal flora by broad-spectrum antibiotics cause an increase in the bilirubin content of adult stools.
Adult stools (mg/24 h)	–	(5–20)	–	41	
Urobilinogen (mg/24 h)					
Men	101	(57–200)	–	43	As determined, 'urobilinogen' includes various colourless and coloured bacterial breakdown products of bilirubin (particularly stercobilinogen and stercobilin). Urobilinogen is rarely found in the stools in the first week of life and is present only in small and fluctuating quantity during the first year[41]. On bilirubin breakdown see page 362.
Women	40	(80–150)	–	43	
Purine bases					
As nitrogen (mg/24 h)	–	(63–73)	–	44	Uric acid is also present in small amount in the stools and meconium[7].
Enzymes	...	...	...	...	The enzymes arise from digestive secretions, cells of the intestinal wall and bacteria.
Trypsin (mg/g)	0.065	–	–	45	Meconium contains no trypsin[45]. In chronic pancreatitis the trypsin and chymotrypsin content of the stools is often lowered[45].
Chymotrypsin (mg/g)	0.421	–	–	45	
Non-nitrogenous substances					
Carbohydrates (g/kg)					
Children, up to 1 year	–	(< 8)	–	47	In the faeces of healthy adults these consist solely of indigestible polysaccharides from food, such as cellulose and hemicellulose. Mono- and disaccharides are found occasionally in infants' stools, glucuronic acid in the stools of newborn.
Adults	0	–	–	47	
Organic acids (mEq/kg)	150	(100–400)	–	22	Organic acids make up rather more than 50% of the anions of faeces and arise from bacterial decomposition of carbohydrates. See also below under 'Volatile fatty acids' and 'Lactic acid'.
Lactic acid (mg/24 h)					
(a) Children	160	(4.5–370)	–	36	Values from (a) 11, (b) 28 subjects. Often increased when absorption of carbohydrate is disturbed.
(b) Adults	32.4	0–76.4	22	48	
Phenols (mg/24 h)	–	(20–80)	–	49	Breakdown products of aromatic amino acids.
Lipids	...	...	...	...	The lipid fraction consists of free (41.9%) and saponified fatty acids, mono-, di- and triglycerides (15.9%), phospholipids (6.3%), free sterols (28.7%), sterol esters (7.2%)[62], bile acids, carotenoids, higher alcohols and hydrocarbons. The fatty acids of faeces are to a large extent endogenous[50]; their composition depends on the fatty acid composition of the diet[63].

	Mean	95% range (extreme range in brackets)	t	Reference	Remarks
Total fats (g/24 h)					
(a) Children, 2 months–6 years	–	(0.29–1.79)	–	6	Values from (a) 44 children by the SPERRY method, (b) 14 and (c) 24 adults by the method of VAN DE KAMER et al.[52]. On a fat-free diet the daily fat excretion is about 2 g[53]. In adult stools about 15% of the lipids are in the bacterial fraction[12]. The fat content *is increased* in various forms of malabsorption, in too rapid passage of food through the intestine, in biliary and pancreatic disease and in obstruction of the flow of lymph from the intestine. Steatorrhoea is better diagnosed by determining the fat content of the 24-hour stools than from the dry substance[4]; the microscopic examination of undigested food residues is diagnostically useless[4, 54].
(b) Adults	5.54	0.14–10.94	2.7	51	
(c) Adults	4.0	0.8–7.2	1.6	7	
As percentage of dry substance					
(a) Children, 2–6 months	–	(5.2–43.1)	–	6	
(a) Children, 6 months–6 years	–	(6.1–25.8)	–	6	
(c) Adults	13.3	–	8.07	7	
Free fatty acids (g/24 h)					
(a) Children, 2 months–6 years	–	(0.14–1.38)	–	6	
(b) Adults	3.96	–	2.28	50	
Volatile fatty acids (mEq/24 h)	–	(9.8–31.2)	–	55	Acetic, propionic, butyric, valeric and other volatile fatty acids arise from bacterial decomposition of carbohydrates in the intestine; they are *increased* in sprue.
Neutral sterols					
Meconium (g/kg)	7.9	–	–	56	The ratio of sterol esters to free sterols is about 0.15. In adults the sterols consist of about 60% coprosterol, 15% cholesterol + cholestanol, 4% 7-dehydrocholesterol + Δ^7-cholestenol, 17% plant sterols[63].
Children's stools (g/24 h)					
1st week	0.24	–	–	56	
7 weeks–10 months	0.10	–	–	56	
Adult stools (g/24 h)	–	(0.39–0.76)	–	57	
Bile acids (g/24 h)	–	(0.27–0.48)	–	57	Estimated range. The following bile acids have been identified in faeces[58]: chenodeoxycholic acid, cholic acid, deoxycholic acid and lithocholic acid. The bile acid content varies with the nature of the nutrient fat[59].
Vitamins					
Vitamin B_6 (mg/24 h)					
Infants	–	(0.15–0.30)	–	60	
Adults	–	(0.7–0.9)	–	60	
Vitamin B_{12} (µg/24 h)	~10	–	–	61	
Ascorbic acid (mg/24 h)	–	(< 10)	–	62	

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FOOD PREPARATION TIME

A. Introduction

A part of the weight loss of astronauts in space is attributed to a decreased eating. Part of the weight loss may be due to the time it takes to prepare food, particularly in the early Apollo flights. At the time this report was prepared, two years ago, the assumption was made that three meals per day plus snacking was equivalent to four meals per day. This report was made with the suggestion that each meal took one hour to prepare and about 30 minutes to eat. This preparation included finding the food bin, determining the right package for the day, separating the packages, hydrating the food (including the time for it to set in water or to be massaged), and time to mix the anti-bacterial pill with food residues and put away the dirty material. Although 1/2 hour for eating may seem long compared to what actually happened on a realistic time scale, 1/2 hour for eating is not too much when the meal is eaten in a relaxed atmosphere with small talk and/or reading or relaxation, music etc. Therefore in the following we have presumed four meals per day and have suggested ways of looking at efficiency in food preparation.

B. Problem Statement

In Table 1 the daily meal preparation and eating time is compared for someone eating out in a cafeteria or restaurant and not counting the time waiting for the food to be served. We assume that approximately 2 hours per day are used in eating (3 meals plus "snacks"). In a modern home with a TV type meal using electronic cooking; as little as one hour per day might be used by the housewife to prepare meals which would take two hours per day to eat. The third bar indicates conventional cooking time at home when as much as four hours per day is used in food preparation. This is equivalent to what was being done in the Apollo flight according to an early report. Each meal would take 90 minutes to completely prepare, eat and clean up. The next column indicates the astronaut needs are simply to eat in a somewhat leisure fashion, approximately 2 hours per day. Since the total flight plan must include some preparation, the astronaut aim should be only one or possible two hours of preparation per day, as indicated

on the lower bar; these preparation times are designated "high efficiency" and "efficient" respectively..

The effect of the time involved in food preparation upon the astronaut work schedule is given in the next three tables. Table 2 suggests what would happen in a forty hour week on earth compared to on Apollo flights, ~~on earth~~. The meal is ready when the earth man walks to the table and he spends two hours per day eating or 14 hours per week eating. The astronaut on early Apollo flights, spent 14 hours per week eating (not counted as work time) and 28 hours per week in food preparation (seven days at 4 hours per day). This left him only 12 hours effective working time, less than 2 hours per day of working time since he must eat seven days per week and the 40 hour week assumed only 5 days. A similar calculation is given in Table 3 for a 60 hour week. Here the net work on Apollo flights would be 32 hours per week or 4 1/2 hours per day of work on a seven day week. In Table 4, the Army system of 12 hours on and 12 hours off duty is illustrated with all of the eating being done during the work day. This would allow 42 hours of effective work separate from food preparation and eating. This allows six hours per day work on a 7-day basis. It is recognized that this is only 50% efficiency for such an operation. In Table 5 the assumptions are made that the early Apollo diet was not efficient and that an efficient food preparation would utilize only 2 hours per day of a food preparation. One could aim at the third column, a high efficiency food preparation in which only one hour per day is used in food preparation. The applied work in each case is given as 42, 56 and 63 hours per week which are not involved in preparation and eating during an 84 hour week. The last line gives the "astrochef point"; the point at which the total crew members including the astrochef provides the same work time as if the astrochef were a working man. Thus, on early Apollo flights under such a system, a third crew member who was the full-time chef would have allowed more time for effective work by the other two than having three crew members prepare their meals separately.

C. Chef Equivalent - Evaluation

Figure 1 presents the effect of an astro chef using an assumption of a 40 hour work week. The next work which would be obtained during one week with different numbers of astronauts. The abcissa gives the total crew including the chef. From the two lines on the figure it is obvious that beginning with two total crew would allow an increase in work produced if one of the two were a chef. With three total crew, a chef would provide more than double the amount of net working time. This figure and the data in Table 2 both assume that eating would be done outside the 40 hour working week.

In Figure 2 a similar presentation suggests what would happen using a 60 hour work week. Again assuming that the 14 hours a week devoted to eating would be done outside of working hours. With a 60 hour week two astronauts would do the same net work if one were a chef or if both were working crew members. With 3 astronauts there is slight advantage if one of the three were a chef. This time saving continues dramatically as the crew members increase.

Surprisingly the picture does not change too much with a 84 hour work week. Parenthetically one notes that if one works 84 hours per week and eating is included that this is little different from a 60 hour work week with eating excluded (84 hours compared to 74 hours). When there are three members in the crew, approximately the same amount of work would be done, a small increment is shown in favor of having the astro chef.

Using the concepts of Table 5 with more efficient food preparation would give data plotted in Figure 4. The astro chef point with the effi~~ct~~ient food preparation of 2 hours per day per person is reached with a total crew of 5. Five persons would give the same amount of work whether one was a chef or crew member and a crew of 6 gives a slight work advantage if one member is a chef. These two curves are more nearly parallel and thus the result is not as dramatic as shown on figure 3 in which the food preparation time was 4 hours per day for a crew man. If the work week were only 60 or 40 hours instead of the 84 hours illustrated, the increase in work time would be much greater. A third study with the 84 hour work week with using a high efficiency food preparation of one hour per day would indicate that little would be

gained by having a chef on board unless the total crew were greater than 10 or 12. The general mathematical model indicates that chef time is equivalent to food preparation time multiplied by the number of total crew.

From an engineering viewpoint the work involved in food preparation is equivalent to weight. In Figure 5 a 40 hour work week is shown in which the addition of a chef to a crew of astronauts would increase the work as shown by the connecting lines. In other words the addition of one more member (the astrochef) increases the work effectiveness of the astronauts in spite of having one less crew member. This can be translated into increased weight of equipment, food, water, material and astronauts for a given work schedule. In Figure 6, the same study presents a 60 hour working week. Here the advantage of a chef increases as the number of crew is increased. A chef gives no advnatage to one astronaut. With two working crew members, there is a slight gain in work which probably would be made up by the weight of and needs of the added member. In other words the missing factor is how much weight the cook would add compared to the increased work which would result as shown.

D. Solution to the Problems.

The thought processes above, the concepts and example given should be utilized for an actual plan of any given flight. What needs be done for each flight would be to define the expected work hours, the eating time and the estimated food preparation time. From these three one could construct charts or figures as above to determine at what point an astrochef would be advantageous.

T. D. Luckey
University of Missouri, Columbia
February, 1971

Table I

DAILY MEAL PREPARATION AND EATING TIME

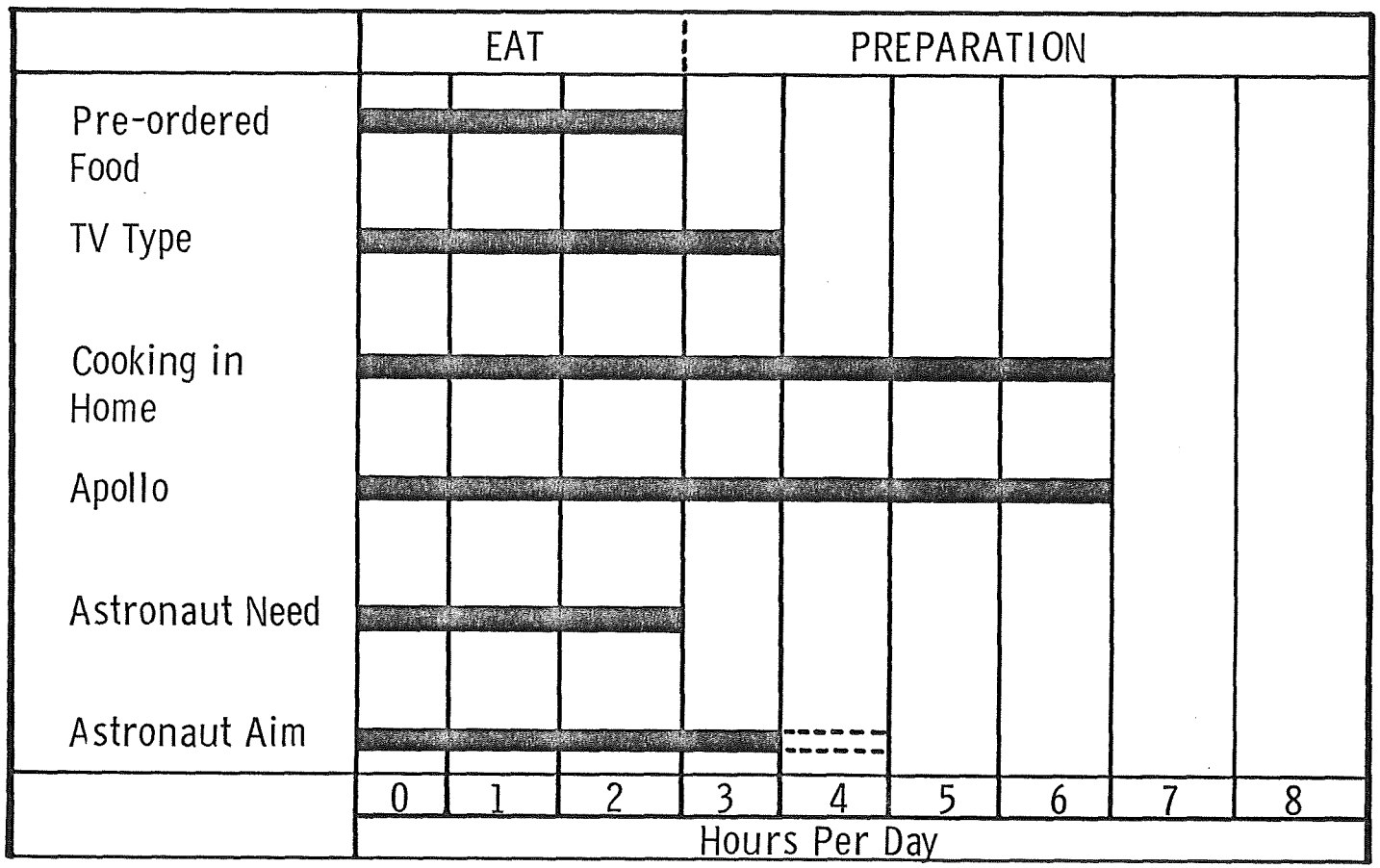


Table 2

ESTIMATED TIME STUDY - 40 HOUR WEEK

	HOURS PER WEEK	
	EARTH	APOLLO
WORK	40	40
EAT*	14	14
"FOOD PREPARATION"	0	28
EFFECTIVE WORK	40	12

*Earth man spends about 2 hours/day eating.
Apollo man spends about 1 1/2 hours/meal or 6 hours/day including 2 hours/day eating and 4 hours preparation time (for four meals).

Table 3

ESTIMATED TIME STUDY - 60 HOUR WEEK

	HOURS PER WEEK	
	EARTH	APOLLO
WORK	60	60
EAT*	14	14
"FOOD PREPARATION"	0	28
EFFECTIVE WORK	60	32

CONCLUSION:

Approximately one-half of astronaut 60 hour/week work time is concerned with food preparation.

*Earth man spends about 2 hours/day eating.
Apollo man spends about 90 minutes/meal or 6 hours/day including 2 hours eating and 4 hours preparation and handling.

Table 4

TIME STUDY - 84 HOUR WEEK

	HOURS PER WEEK	
	EARTH	APOLLO
WORK	84	84
EAT*	14	14
"FOOD PREPARATION"	0	28
EFFECTIVE WORK**	70	42

*Earth man spends about 2 hours/day eating and "coffee".
Apollo man uses about 90 minutes per meal or 6 hours/day including 2 hours eating and 4 hours preparation time for four meals.

**Acceptable field work with eating as part of day.

Table 5

WORK - EAT - FOOD PREPARATION TIME STUDY

	HOURS PER WEEK			
	APOLLO*	EFFICIENT	HI EFFICIENT	MAXIMUM
WORK WEEK	84	84	84	84
EAT 2 HOURS/DAY	14	14	14	14
NET WORK	70	70	70	70
FOOD PREPARATION	28	14	7	0
APPLIED WORK	42	56	63	70
Astro Chef-Point**	2 1/2	5 1/4	9	--

* Assumptions: (1) 84 hour week; (2) 4 meals/day; (3) 90 minutes/meal (1 hour preparation and 30 minutes eating time).

**The crew number at which the presence of the astro chef gives the same work time as a "working" man.

Figure 1

NET WORK - 40 HR. WEEK

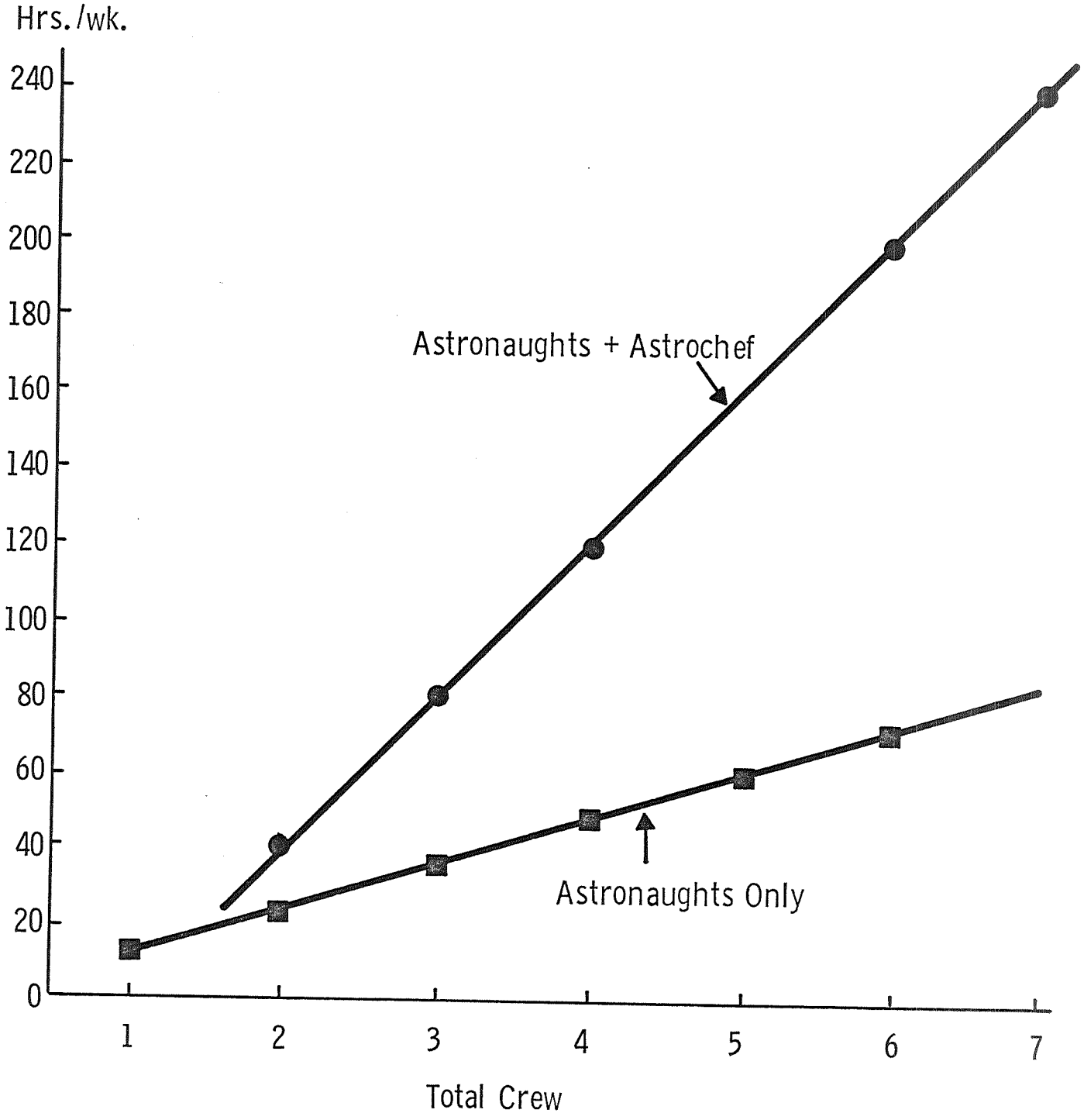


Figure 2

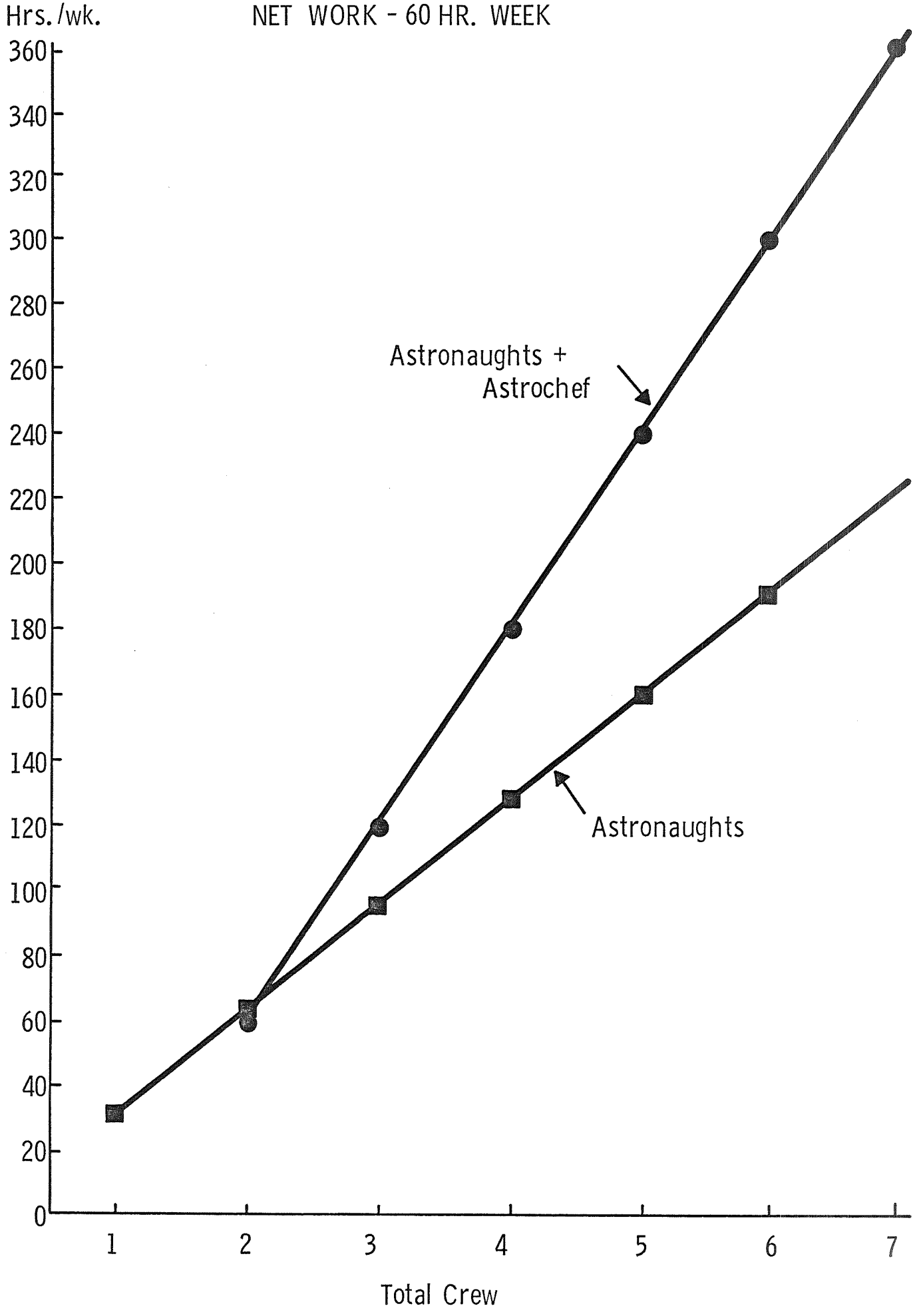

Hrs./wk.
NET WORK - 60 HR. WEEK
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
Astronaughts +
Astrochef
Astronaughts
1
2
3
4
5
6
7
Total Crew

Figure 3

NET WORK - 84 HR. WEEK

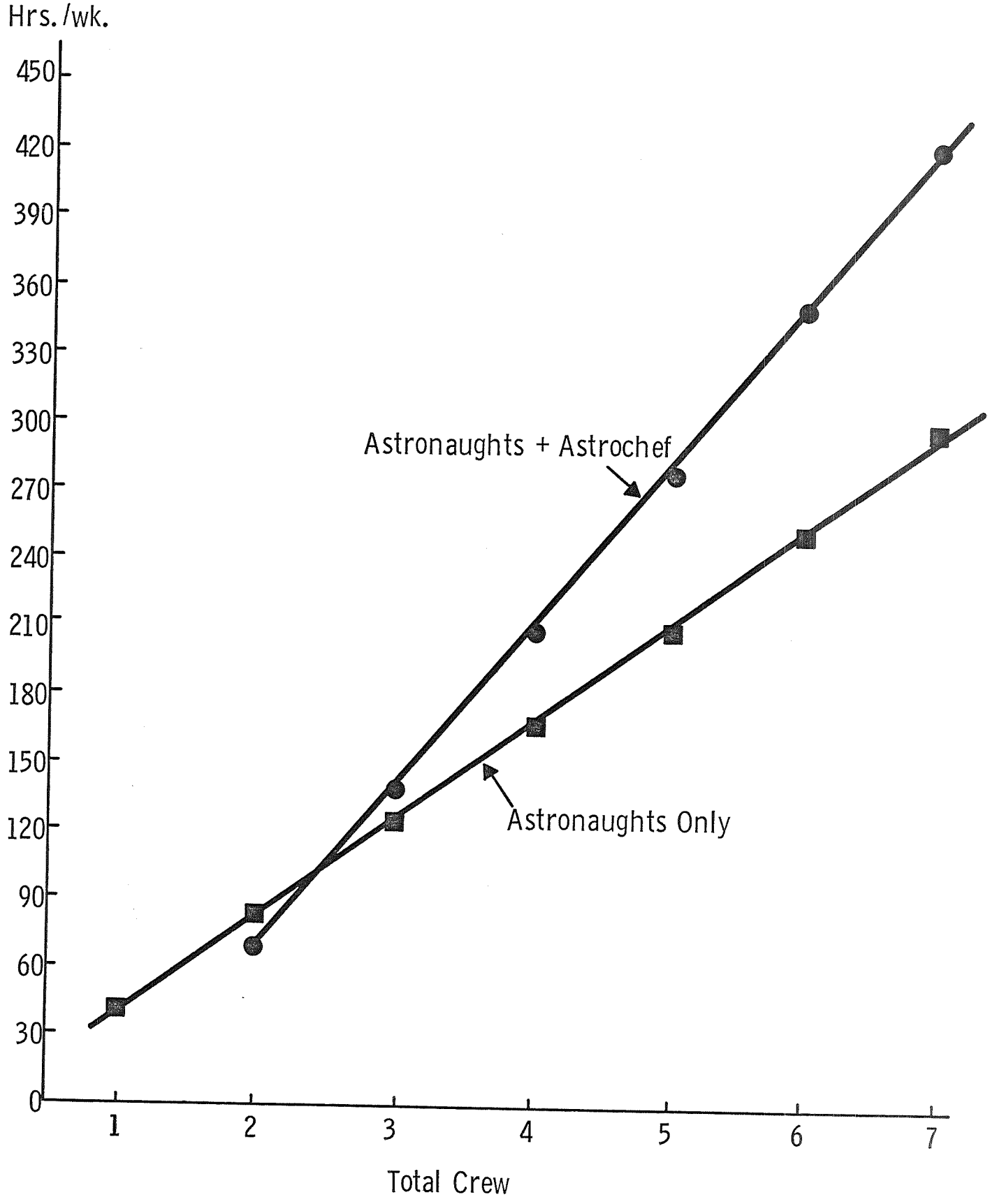


Figure 4

TIME STUDY - 84 HOUR WEEK

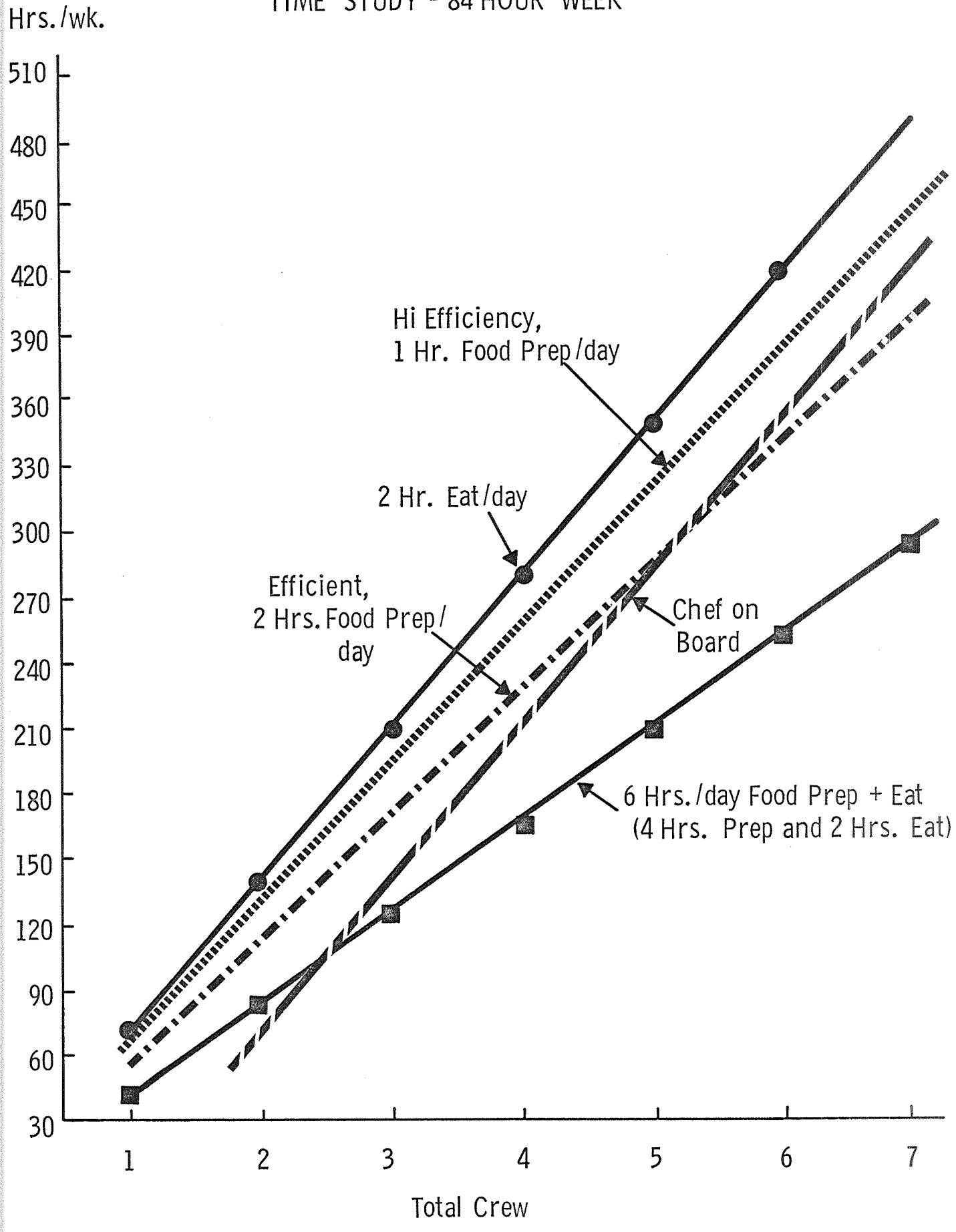


Figure 5

NET WORK - 40 HR. WEEK

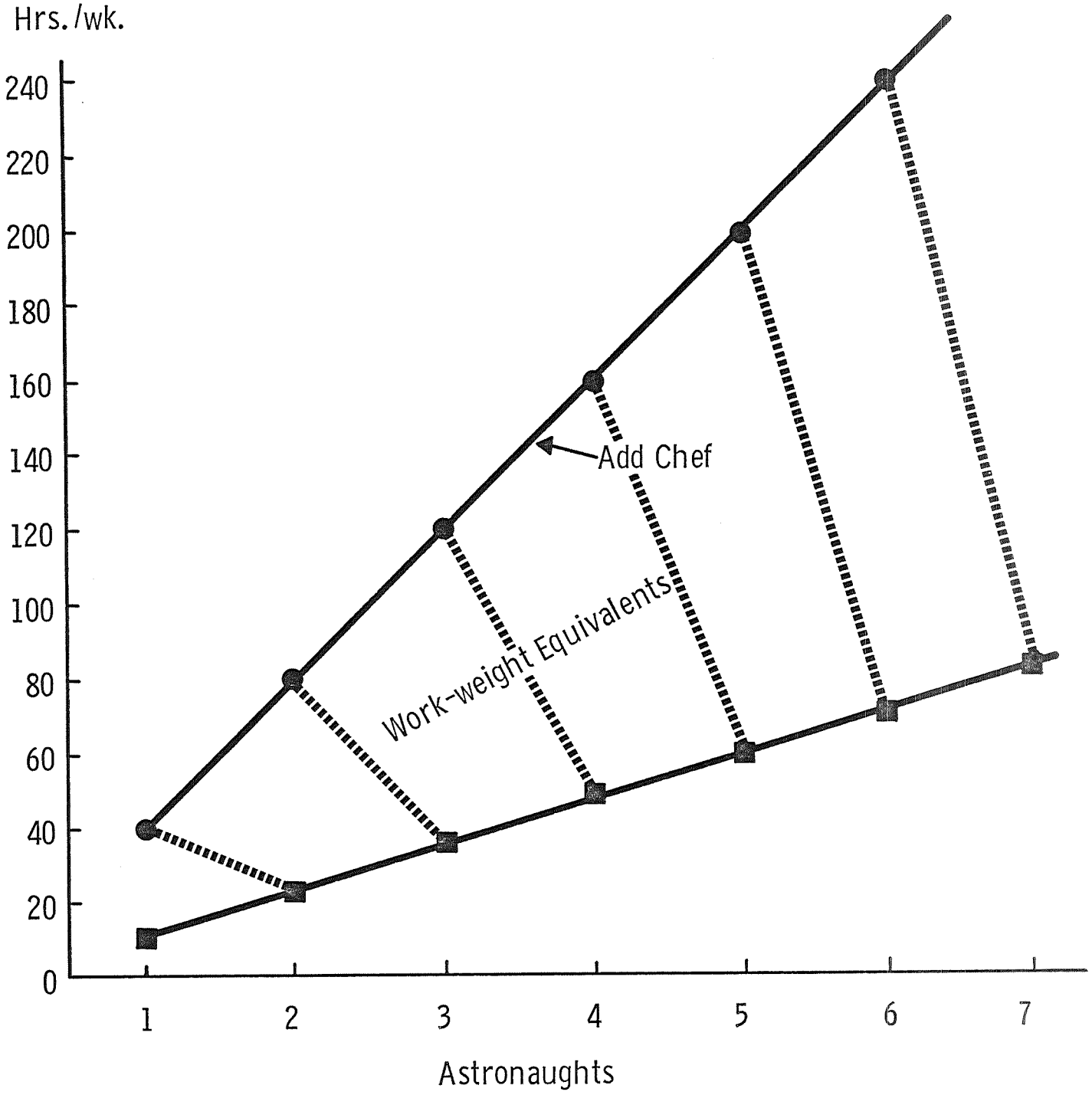
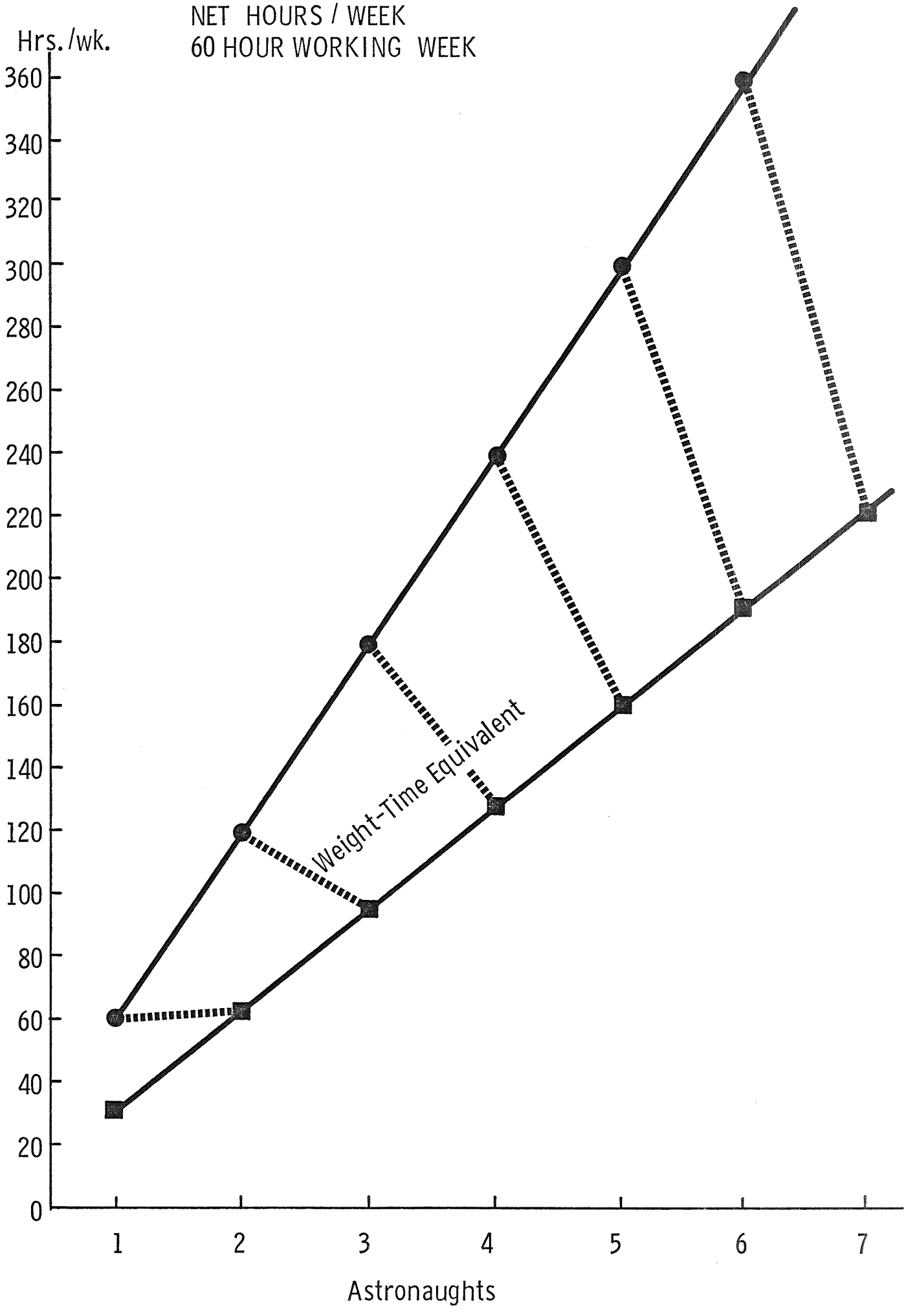


Figure 6

NET HOURS / WEEK
60 HOUR WORKING WEEK



POTASSIUM, IRON AND CALCIUM IN APOLLO DIETS

T. D. Luckey - February 1971

The following summary of data obtained from Dr. Huber in his annual report (September 30, 1969) to you and the Brodzinski, et al. report suggests that a similar set of data should be made available from the Gemini and Mercury flights. This report must accept the data as given with the realization that the greatest error by far must reside in estimates of the food intake. This is very damaging to any concepts using those data as a base, i.e., the balance studies. Although iron intake values are low, there was no problem whatsoever. On prolonged flights, iron would obviously become a problem and anemia would set in, depending to some extent upon the flora according to our report. Note also the high loss of iron in the balance study. This should be correlated to hemoglobin and hematocrit data. Was there hemo concentration from water deprivation? However, since Apollo flights were short there should be no problem with iron deficiency at the present time.

The same may or may not be true with certain of the Apollo personnel with potassium losses. Note particularly Apollo 8 CDR had about 2 grams of potassium loss per day; however, this cannot be ignored although it was an exceptional occurrence. A 70 Kg man should have about 250 gm of potassium. A loss of 2 gm/day for 12 days (not actual Apollo 8 time) would represent a loss of 10% of the total body potassium. This could lead to nausea, weakness, confusional states, cardiac irregularities and muscle paralysis as the cellular functions of potassium were changed. Incidentally, severe potassium depletion brings increased toxicity from sodium when dietary sodium-potassium balance is too high (Cannon et al., Metabolism 2:297, 1953). Surprisingly, the potassium balance for Apollo 10 was positive while the reported intake of potassium was next to lowest of all flights. The negative potassium balances may reflect tissue losses. Thus a fat and water balance is needed to determine how much of the weight loss is water, fat and/or tissue. If most is tissue loss, the caloric intake may be satisfactory under zero G conditions. The negative potassium balance may be a breakdown of cells due to stresses during the flight.

This report also ignores the low calorie intake which was serious only for Apollo 8 and 10 and marginal for all of the others. The protein intake on CMP of Apollo 12 and all personnel of Apollo 13, all of Apollo 10 and two of the 3 in Apollo 8 are marginal. This is probably not serious for a short flight. Calcium is the most serious problem noted from the average daily nutrient intake of Apollo 7 through 13.

The beginning of the problem is noted in the ash, see either Table 1 of this (the Huber) report or Table 3 from another report. In Apollo 10 the ash is recorded to be very low (3%): this is not compatible with the amount of calcium, phosphorus, potassium and sodium which are reported for that flight compared with any other flight. This ash value must have something wrong with it. Secondly, as noted in the report, the calcium is generally low on the early flights particularly Apollo 8 and 9. There is seen to be a dramatic and consistent difference between the first flights 7, 8, 9 and the later flights 10, 11, 12 and 13. In the last four flights only 2 persons of the 9 had adequate calcium intakes. CDR of Apollo 7 was somewhat low. The worst calcium intake certainly was LMP of Apollo 8. Although Apollo 8 was very low and Apollo 9 was generally low, on a short term flight this would not seem to be serious. However, it is made particularly bad because of the low calcium/phosphorus ratio. A ratio of 1-1 is recommended by NRC for man. The Ca/P ratios for Apollo 7 were not unreasonable, those for Apollo 8 were bad, those for Apollo 9 varied with CMP being the worst of any and again a very dramatic change is noted between the first three Apollo flights and the subsequent flights where the Ca/P ratios were about 1.

The low calcium intakes of Apollo 8 and 9 could be made much more detrimental by the low calcium/phosphate ratio. This would tend to provide much less calcium absorbed than would occur with diets having a good calcium/phosphorus ratio. It would be anticipated that people in this condition would be in serious difficulties within a few months. Table 1 from the Brodzinski report, 1970, shows the average daily calcium intake and excretion in Apollo astronauts. Only on Apollo 10 was the average value reasonable. Even on Apollo 11, 1/3 of a gram of calcium was lost each day. On Apollo 7 the average loss was over half a gram daily. One would anticipate that the commander of this flight lost much more than

did the other two crew members. Therefore the average is biased and does not show what happened to the commander. The data do show CDR for Apollo 8 was very bad with 1 gram being lost each day. This could not continue for many weeks before calcium loss became serious. It would be more interesting to find the balance data for LMP of Apollo 8 since his was the lowest calcium intake of any astronaut on all flights and he had a calcium/phosphorus ratio of 0.48. This man must have lost more than did the CDR man. It could be predicted that the CMP was quite comparable to CDR in his calcium loss. If this data were available, it would be worthwhile to check the calcium balance. The most interesting balance data are given for Apollo 9: here individual data are available. Both the CDR and LMP are low in calcium intake and both have a low calcium/phosphorus ratio; both lost approximately 1 gram per day during the flight. The most interesting is CMP of Apollo 9. Here the calcium intake was bad the the calcium/phosphorus ratio was the worst of all the astronauts. This individual lost over 2 grams of calcium per day. This is as much or more than a pregnant or lactating mother would expect to lose and could not go on more than a few weeks before calcium deficiency was shown by hyperirritability and poor muscle contraction. Decreased bone deposition would occur. Permanent skeletal damage could result unless it were corrected within one or two months. Another parameter to consider is that of Ca^{++} as it affects the state of hydration of blood proteins and thus blood viscosity.

The low magnesium intakes noted generally throughout all Apollo flights would tend to accentuate the deficiency of a calcium low diet. Less magnesium in the lumen leaves more calcium to chelate or attach to insoluble salts. This accentuates the calcium deficiency. For example, the simple inorganic chemistry involved in the gut lumen would suggest that the ions which can form insoluble salts such as sulfate, phosphate, long chain fatty acids and phytate can take up a greater amount of calcium when the magnesium of a diet is low than when the magnesium content is high. Thus calcium-magnesium phytate would have a higher percentage of calcium when the magnesium content of the diet was low than when the magnesium content would be high. This makes less calcium available to the intestinal

mucosa. Secondly, the absorption of calcium through the intestinal mucosa is inhibited by magnesium deficiency in man (the data is less clear in animals). Whether or not a low magnesium diet which has 1/3 to 2/3 of the recommended allowance would have any effect on calcium absorption is open to speculation.

The correlations of the calcium balance data are roughly related to the calcium intake and the calcium phosphorus ratio. The correlation of Ca low intake or loss with the amount of food eaten is not present, i.e., the calorie intake of Apollo 8 and Apollo 9. It is seen that calorie intake in Apollo 9 is adequate and yet the calcium intake is quite poor. In both of the flights calcium supplementation in the drinks was in force. Supplementation of drink powders appeared to be routine for the first three Apollo flights and became less routine in the last flights.

It is important that the calcium/phosphorus ratio should approach one in both man and animals. For example, rats are very refractile to vitamin D deficiency unless the calcium/phosphorus ratio is upset. Generally a ratio outside of the range of 2 to 1 to 1 to 2 is needed to obtain a vitamin D deficiency on any diet in a rat.

As noted from the table attached, the proposed daily nutrient intake for Apollo 7 through 10 are given with a calculation of the proposed calcium phosphorus ratios. There is a rough correlation with what was seen in the average daily intake; particularly was the calcium/phosphorus ratio very low in Apoll 8 and 9. Beginning with Apollo 10 the calcium/phosphorus ratio of the intended food was quite adequate. This proposed calcium intake and the low calcium/phosphorus ratios occur in diets which have been supplemented with calcium in the drinking powders. Apparently more calcium addition to other foods should be considered for any future fortification; or, as may be apparent from Apollo 10, 11, 12 and 13, more calcium foods such as sardines, calcium cheeses or milk products should be incorporated into the foods used.

These data should be correlated with the serum calcium and bone loss data and with the determination of calcium in different flights and on different individuals. It would be particularly important not to generalize

from averages, or from one individual to another, or from one flight to another since each individual situation makes a great difference. When a low calcium and a poor calcium/phosphorus ratio is combined, any individual may be on the brink of a disastrous calcium balance as shown by CMP in Apollo 9. Here the calcium/phosphorus ration seems to be more important than the actual calcium intake; CDR on Apollo 8 actually received less calcium than did CMP on Apollo 9. Individual data on LMP Apollo 8 would be most interesting, if it were available. In comparing the detection of the effect of calcium loss in different methods it would be important to use the same individual. The individuals that should be looked into with most detail are those which were mentioned above. Based on an old paper in which the calcium metabolism and bone ash were correlated to the ash of toenails of chicks, one could propose that a sensitive index of calcium intake, loss and balance could be made from daily finger nail clippins. These could be analyzed and related to the day of production of that part of the nail. Presumably this could be done with hair but it has actually been worked out with toenails, (Campbell, J.A., B. B. Migicovsky and A. R. G. Emslie, 1945, Studies on the chick assay for vitamin D. I. Precision of tibia and toe ash as creteria of response, J. Poult. Sci., 24:3-7).

Recommendations

The obvious problems with calories, calcium, potassium and iron in astronaut feeding recommend these four nutrients as guides for tagging dietary intake on Apollo 15.

The calories are of obvious importance and interest. As discussed above the weight loss may or may not be due to calories.

Calcium is also of obvious importance and interest. In addition to this, the negative calcium balance makes it of prime importance on longer flights. For example, an average loss of .635 milligrams (Apollo 7 through 11) per day on a 60 day flight would amount to 38 grams. This amount of calcium is equivalent to about 108 grams of inorganic bone and represents 3% of the total body supply of calcium.

The negative potassium balance reported makes this element of importance due to the fact that potassium indicates the state of health of

the tissues of the body. In previous Apollo flights an average loss of 300 milligrams per day (Apollo 8 through 11) could be translated into a 60 day flight to anticipate a total loss of about 18 grams. Since the total body supply of potassium is 250 grams this 18 grams represents 7% of the total body calcium. If this were lost the astronaut would be in a precarious state with respect to his sodium/potassium ratio and to the functioning of potassium in all tissues. He would also be very susceptible to potassium deficiency symptoms, particularly during times of nausea, sickness or excess diarrhea or urination.

A normal adult has about 4 grams of iron in his body. The average lost on the Apollo flights 7 through 11 was 6.4 milligrams per day. During a 60 day flight this would represent 384 milligrams. This loss would be 9% of the total body iron supply. Although this slight anemia which might result would probably not be serious, here is a problem which should be watched very carefully. This negative iron balance would not be acceptable on prolonged space flights.

For the above reasons it is recommended that exact intakes and careful balance studies by made for calories, calcium, potassium and iron. These nutrients should be marked in each food available to Apollo 15 astronauts and all individual feces should be returned to earth for balanced studies of these nutrients. This can be done using heavy metal nutrient indicators in the p.p.m. to p.p.b. range.

Luckey Evaluation of Ca^{++} Problem - February 1971

Apollo 7	Proposed Daily Nutrient Intake		Ca/P	Ave. Daily Inflight Nutrient Intake		
	Ca (gm)	P (gm)	Ratio	Ca (gm)	P (gm)	Ca/P Ratio
CDR	802	1289	.62	644	1060	.61
LMP	983	1200	.82	925	841	1+
CMP	1089	1376	.79	938	1125	.83
Apollo 8						
CDR	590	1340	.44	427	847	.50
LMP	590	1345	.44	366	760	.48
CMP	590	1345	.44	479	983	.49
Apollo 9						
CDR	696	1652	.42	562	1146	.49
LMP	687	1653	.42	494	892	.55
CMP	624	1513	.45	489	1073	.45
Apollo 10						
CDR	1137	1227	1+	836	814	1+
LMP	1164	1176	1-	854	701	1+
CMP	1137	1197	1-	808	746	1+
Apollo 11						
CDR	1194	1155	1+	1036	1050	1-
LMP	1203	1190	1+	1114	1225	1-
CMP	1192	1153	1+	851	901	.94
Apollo 12						
CDR				1095	1090	1
LMP				1022	1028	1
CMP				1291	905	1+
Apollo 13						
CDR				870	780	1+
LMP				871	720	1+
CMP				786	716	1+

(Assume Red = CDR, Blue = LMP and White = CMP).

Proposed Daily Nutrient Intakes
Apollo VII, VIII IX X

	Water gms.	Calories	Protein gms.	Fat gms.	Carbo. gms.	Ash gms.	Ca mgs.	P mgs.	Fe mgs.	K mgs.	Na mgs.	Mg mgs.	Vit C mgs.	Weight gms.
Apollo VII								coffee 62						
Red	15.7	2335	96.1	85.5	308.2	18.5	802.0	1289	10.2	4579	2214	225.8	9.9	582.4
Blue	16.1	2495	118.4	92.1	314.5	19.0	983.0	1200	10.2	4546	2327	245.5	10.4	556.2
White	14.8	2534	109.3	87.4	343.17	19.7	1089.0	1376	9.9	4693	2341	217.3	10.4	570.9
Apollo VIII							lower fat							
Red	31.5	2393	103.3	70.0	347.7	20.2	589.7	1340	9.2	5340	2008	201.7	11.5	571.7
Blue	31.5	2404	103.3	70.3	349.7	20.3	589.8	1345	9.2	5342	2015	201.7	11.5	574.2
White	31.5	2404	103.3	70.3	349.7	20.3	589.8	1345	9.2	5342	2015	201.7	11.5	574.2
Apollo IX														
Red	40.14	2751	131.2	89.7	364.3	23.6	695.9	1652	11.3	6146	2432	239.9	13.22	647.3
Blue	40.08	2744	129.8	89.8	363.8	23.7	686.9	1653	11.4	6169	2420	235.7	13.26	645.4
White	38.56	2501	121.1	87.8	327.9	21.87	624.3	1513	10.4	5701	2232	221.9	12.33	590.8
Apollo X*														
Red	13.0	2076	80.1	66.7	299.8	18.9	1137.0	1227	7.2	4442	2003	177.0	9.73	478.2
Blue	11.1	1945	81.9	64.4	268.5	19.3	1164.0	1176	6.9	4643	1929	166.8	10.10	444.9
White	12.0	2046	75.7	64.8	302.3	18.3	1137.0	1197	6.9	4325	1934	175.3	9.34	472.7
Apollo XI*														
Red	10.3	2043	64.6	64.4	313.8	16.1	1194.0	1155	6.3	3601	1733	160.6	7.36	468.8
Blue	10.5	2063	71.0	64.1	312.5	16.2	1203.0	1190	6.3	3668	1727	162.8	7.47	473.9
White	10.6	2043	62.7	63.5	317.7	15.9	1192.0	1153	6.1	3550	1737	158.4	7.22	470.2

* Meals were supplemented with snack foods which provided additional nutrients not included here.

TABLE 1

AVERAGE DAILY NUTRIENT INTAKES
APOLLO VII, VIII, IX, X, XI

	*Water gms.	Calories	Protein gms.	Fat gms.	Carb. gms.	Ash gms.	Ca mgs	P mgs.	Fe mgs.	Na mgs.	K mgs.	Mg mgs.	NaCl mgs.	Weight gms.
Apollo VII														
Red	13.6	1966	81.2	72.4	259.1	15.5	644.0	1060.0	8.5	3808	1879	191.9	8.4	441.2
Blue	11.6	1804	73.8	56.1	267.7	14.1	925.0	841.0	7.2	3477	1336	141.3	7.8	418.7
White	13.2	2144	95.9	75.5	279.6	17.5	938.0	1125.0	8.6	4004	1958	184.6	8.7	481.4
Apollo VIII														
Red	23.0	1477	58.6	39.3	231.1	11.1	427.2	846.7	5.0	3170	1229	112.7	6.7	360.5
Blue	24.7	1339	52.2	32.7	217.0	9.8	365.8	760.0	5.0	2730	986	97.1	5.8	333.3
White	12.1	1687	80.4	48.6	240.0	15.0	479.0	983.1	6.6	3985	1571	145.0	8.8	408.7
Apollo IX														
Red	34.5	1924	86.0	59.7	280.2	15.3	562.5	1146.3	7.1	3996	1677	157.4	8.85	485.1
Blue	30.8	1639	66.2	47.3	252.4	13.0	494.3	892.4	5.9	3412	1386	128.6	7.1	400.8
White	32.4	1715	77.8	53.2	239.6	14.5	489.0	1073.0	6.5	3768	1708	145.6	7.96	404.3
Apollo X														
Red	69.6	1346	57.6	34.3	212.6	3.4	836.4	814.1	5.5	2972	1463	107.1	6.29	389.3
Blue	53.6	1246	48.7	30.4	203.5	3.1	854.0	700.9	4.9	2670	1182	95.6	5.38	351.9
White	64.8	1265	45.5	30.1	212.8	3.3	808.2	745.8	4.8	2291	1376	103.7	4.39	366.0
Apollo XI														
Red	87.9	2040	79.3	65.2	290.3	17.1	1035.8	1049.9	8.3	2770	1751	137.8	7.89	538.4
Blue	108.5	2278	93.5	73.3	321.5	18.9	1114.0	1224.7	9.1	3218	2061	165.9	8.85	604.4
White	73.3	1645	71.1	54.4	224.5	13.9	851.2	901.4	6.6	2065	1441	119.3	6.89	447.7

* This represents the water content in the food prior to rehydration or consumption.

TABLE 1

AVERAGE DAILY CALCIUM INTAKE AND EXCRETION BY APOLLO ASTRONAUTS

Mission	Astronaut	Intake[6] (mg)	Fecal Excretion (mg)	Total Excretion * (mg)	Ratio of Excretion To Intake	Mass Balance (mg/day)
Apollo 7	Average	836	1140	1430	1.70	- 590
Apollo 8	CDR	427.2	1150	1440	3.36	- 1010
Apollo 9	CDR	562.5	1190	1490	2.64	- 930
Apollo 9	LMP	494.3	1100	1380	2.78	- 880
Apollo 9	CMP	489.0	2260	2830	5.78	- 2340
Apollo 10	Average	832.9	730	910	1.10	- 80
Apollo 11	Average	1000.3	1090	1360	1.36	- 360
Averages		767.7	1120	1400	1.83	- 635

* Based on 80% fecal excretion

TABLE 2

AVERAGE DAILY POTASSIUM INTAKE AND EXCRETION BY APOLLO ASTRONAUTS

Mission	Astronaut	Intake (6) (mg)	Fecal Excretion (mg)	Total Excretion * (mg)	Ratio of Excretion To Intake	Mass Balance (mg/day)
Apollo 8	CDR	1229	499	3020	2.46	- 1795
Apollo 9	CDR	1677	253	1540	.916	+ 141
Apollo 9	LMP	1386	276	1670	1.21	- 286
Apollo 9	CMP	1708	403	2440	1.43	- 732
Apollo 10	Average	1340	176	1070	.797	+ 272
Apollo 11	Average	1751	350	2120	1.21	- 367
	Averages	1527	300	1820	1.19	- 296

* Based on 16.5% fecal excretion

TABLE 3

AVERAGE DAILY IRON INTAKE AND EXCRETION BY APOLLO ASTRONAUTS

Mission	Astronaut	Intake[6] (mg)	Excretion* (mg)	Ratio of Excretion To Intake	Mass Balance (mg/day)
Apollo 7	Average	8.1	15.7	1.9	- 7.6
Apollo 8	CDR	5.0	13.3	2.7	- 8.3
Apollo 9	CDR	7.1	11.6	1.6	- 4.5
Apollo 9	LMP	5.9	13.2	2.2	- 7.3
Apollo 9	CMP	6.5	17.2	2.6	- 10.7
Apollo 10	Average	5.1	6.7	1.3	- 1.6
Apollo 11	Average	8.0	16.4	2.1	- 8.4
Averages		6.8	13.2	1.9	- 6.4

* Assuming 100% fecal excretion

Low Fe, Intake
Low Fe in diet
Rabbit & Anemic Rat

Reliability of data - good.

BLE II Average Daily Nutrient Intakes Apollo XIII

Nutrient	CDR	CMP	LMP
Calories	1581	1535	1524
Protein (gm)	58.8	56.8	57.4
Fat (gm)	50.2	47.2	48.9
Carbohydrate Total	238.6	235.2	227.9
Carbohydrate - Fiber	4.59	4.50	4.48
Ash (gm)	15.41	14.96	14.64
Calcium (mg)	870	871	786
Phosphorus (mg)	780	720	716
Iron (mg)	8.36	8.15	8.12
Sodium (mg)	3629	3495	3350
Potassium (mg)	2036	1942	1964
Magnesium (mg)	107.4	98.3	102.2
Chloride (gm)	5.9	5.7	5.5

APOLLO DIET EVALUATION

T. D. Luckey
UMC - March 24, 1971

Using all the data available on food intakes for Apollo flights 7 through 11 and 13, astronaut diets were evaluated from the viewpoint of their deficiences in specific nutrients, the balance of nutrients in the diet and whatever data was available on the nutrient intake per day and nutritional balance studies. These were grouped from the viewpoint of an estimate of what might happen to survival, maintenance, growth, reproduction and health through three generations. The five categories were (A) is the best, where we would anticipate health through several generations, (B) we would expect poor reproduction and lactation performance during the first generation and almost certainly after the second generation (C) we would expect poor growth and (D) we would expect the animals could not maintain themselves well and (E) we would expect poor survival. We might expect deformities to appear in both 4 and 5 categories. Certainly 5 would exhibit clinical symptoms after some time. These are all valued judgments. Others would see these in a somewhat different light but any qualified nutritionist should find the major ratings to be approximately the same.

On Table 1, a rating with 11 LMP the best and 8 LMP the worst. The average were the Apollo flights CDR or CMP. Table 2 lists the rating of different Apollo flights. It is seen that astronauts in Apollo 11 appeared to be the best fed. Apollo 8 and 9 astronauts were the worst fed. Apollo 7 was mixed while Apollo 10 was intermediate. From a nutritional viewpoint the group on Apollo 13 seemed to be somewhat low in protein, those in Apollo 10 were definitely low in protein and were low in magnesium. The others listed below them would not be nutritionally acceptable from the data given. Thus only five astronauts ate well according to this paper evaluation.

The above reasoning gave three Apollo diets to be tried on the mouse test: namely, the best was 11 LMP, the worst was 8 LMP and the medium was 10 CMP. These diets will be simulated and fed to mice with a controlled diet in order to see how the growth, reproduction and lactation compare.

Criticisms of the individual diets are given from what data has been made available.

Eleven LMP is the best of all the diets evaluated. No criticism is offered since both the calories and the protein were up and the mineral balances seemed to be satisfactory.

Apollo 7 CMP was rated second. This man was fed equivalent to Apollo 11 CDR. They both had about the same amount of calories and adequate calcium. The protein intake was better for 7 CMP than it was for 11 CDR. The iron lost on both flight 7 and 11 was something which would not be satisfactory over a long period of time, but this was not considered to be as serious as some of the other problems noted for other Apollo astronauts.

Apollo 7 LMP had a protein intake equivalent to that of Apollo 11 CDR. This caloric value was below all three of the astronauts. This is probably not a serious fault.

Apollo 11 CMP showed a definite low calory intake. The protein was somewhat low. While this diet is not rated as unsatisfactory, it is not considered to be excellent.

All of Apollo 13 astronauts seemed to be in a less good category than the ones above. They were all very similar and the protein and caloric intakes were apparently comparable throughout. The calcium intake and the calcium/phosphate balance was not bad; however it was not good. These could not be rated excellent. There was iron loss in this flight as there was in flight 11 generally. The low protein and the low caloric intake makes all the diets of Apollo 13 rated as not acceptable for prolonged flights.

The Apollo 10 astronauts were found to be grouped together with CDR somewhat better than CMP, and this somewhat better than LMP. CDR had a somewhat low calcium intake, ver definitely low caloric intake, a low protein intake and a low magnesium intake. The iron loss did not seem to be significant. Apollo 10 CMP had less satisfactory caloric and protein intakes then had Apollo 10 CDR. Apollo 10 LMP had a low magnesium intake as well as unsatisfactory caloric and protein intake. It is doubtful if any of this group could have survived in good health with such a nutritionally poor diet during a 1 or 2 month flight.

Apollo 7 CDR had a very low calcium intake anda very poor calcium/phosphorus ratio. The caloric intake was adequate and the protein intake

was adequate. The iron loss could have caused trouble but this would not be serious for man for many weeks.

Apollo 9 CDR had adequate calory intake, the iron loss was not bad and the potassium seemed to be adequate. The protein was adequate but the calcium intake and the calcium/phosphorus ratio seemed to be very poor.

Apollo 8 CMP had a very poor calcium intake and a poor calcium/phosphorus balance. The protein was adequate, the calcium was somewhat low but this diet must be downgraded because of the poor calcium intake.

Apollo 9 LMP was rated 15th of the group. The calcium was very bad, the iron showed a negative balance, the protein was low and the calories were somewhat low.

The diet of Apollo 8 CDR astronaut had low calories, low potassium, low calcium and a low calcium phosphorus balance. The potassium loss of 1 gram per day could have been very serious for this person in a short time.

Apollo 9 CMP ate a diet which was very low in calcium and had a very poor phosphorus balance. The astronaut was losing 7 mg of iron per day and up to 2 grams of potassium per day. The caloric and protein intakes were not unsatisfactory but this did not seem to be a real problem. The major problem here must have been the potassium loss and therefore the water balance and also the state of the tissues in the body. The high potassium losses of these two astronauts would indicate that vomiting and/or diarrhea may have occurred during part of the flight. It may be noted that this analysis was done with no reference to individuals or to flight information other than diet; therefore, the author has no idea as to which men were involved nor what problems they had nor in what condition they came back to earth following their flight.

The diet Apollo 8 LMP was the worst of all. The calcium intake is very bad, the calcium/phosphorus ratio is very bad and the magnesium intake is very bad. Here was a low calory diet with a low protein intake. All of these suggest that this man could not have been effective during a flight of more than a few weeks. However, for this short time he may have felt better than both Apollo 9 CMP and Apollo CDR astronauts. He is rated below them due to the caloric, protein and calcium problems which would develop serious deficiencies on a long flight.

TABLE 1

Numberical Rating of Apollo Nutrition*

Rating	
1	11-LMP
2	7-CMP
3	11-CDR
4	7 LMP
5	11-CMP
6	↑
7	13
8	↓
9	10-CDR
10	10-CMP
11	10-LMP
12	8-CDR
13	9-CDR
14	8-CMP
15	9-LMP
16	8-CDR
17	9-CMP
18	8-LMP

TABLE 2

Apollo Flights Rated Nutritionally*

No.	CRD	LMP	CMP
7	12	4	2
8	16	18	14
9	13	15	17
10	9	11	10
11	3	1	5
13	6	7	8

* No. 1 is best and No. 18 is the worst

NUTRIENT INDICATORS - Outline

- T. D. Luckey, February 1971

A. INTRODUCTION: Problem and solution statements.

B. LITERATURE SEARCH AND TABLE OF POSSIBILITIES.

C. PRELIMINARY WORK:

1. Marker characteristics and analysis.
2. Background data: amount of proposed markers present in diets, water and stools.
3. Marking Food:
   a. form
   b. material
   c. quantity
   d. methods to add to food types

D. EXPERIMENTAL BACKGROUND FOR SAFETY IN HUMAN USE.

1. Toxicity in rats: long term using 100 X expected quantities
2. Feasibility in rats to test the concept and methods.
3. Pilot study in rats and monkeys to confirm multiple marker concept and to provide data for human safety.
4. Human tests
   a. taste
   b. absorption and storage - 100 day
   c. Blind food test

E. WRITTEN STANDARDS:

1. Food preparation
2. Testing methods
3. Future foods

F. KNOWLEDGE DISSEMINATION: written and oral reports and publications.

NUTRIENT INDICATORS

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A. INTRODUCTION

A method is proposed which will allow the intake of the total amount of food or any given nutrient to be accurately monitered. Total food intake, and any specific nutrient, is difficult to obtain accurately in experimental animals and/or humans. Most studies with humans are subject to errors by the subject. Animal studies are open to error through wasted food which is difficult to recover, through contamination of wasted and or presented food with excreta or stresses of the animal with restricted movement.

This method incorporates a nutrient indicator in all food available, a nonabsorbed marker would be added to the food in direct proportion to the amount of nutrient in the food. Collection of all feces, homogenization and analysis for the marker would give a direct means to determine how much of the nutrient was ingested. It is proposed to develop the method with heavy metals which would allow direct determination of multiple markers using activation analysis.

The work required is outlined on the following pages. Calories will be the subject using fat, carbohydrate and protein as the multiple approach to the subject. Thus, a multiple marker system will be developed using three markers for these three nutrients. Specifically the method will allow the accurate determination of one or more nutrients consumption from analysis of stools even while the subjects are fed pantry style.

B. LITERATURE

1. A complete literature search.
2. Information summarized in seminar.
3. Publication of a complete, critical review of literature on markers in nutrition.
4. Evaluate possible types of marker materials possible and select representative of the best type for testing. Table 1 indicates those considered. For multipe nutrient indicators to be used in humans as well as animals, heavy metals which can be determined by activation analysis appear to be the best choice.

C. EXPERIMENTAL

1. Characteristics of markers

Record the characteristics of the specific markers to be used. This shall include methods to add each marker to the food and method to be used for testing. The use of multiple markers will require testing for compatibility which would not be needed if only one or two were to be used. Ten to fifteen of the most promising markers should be examined.

2. Background data.

a. Determine the amount of background traces of the 10-20 markers occur naturally in Apollo diets. This can begin with the Composite 1968 diet.

b. Determine background traces in Apollo water (spent fuel supply).

c. Determine background traces of the 10-20 markers in stools from Apollo diet eaters.

3. Marking Food

a. Form of Markers (1) collodial suspension
(2) solution
(3) adsorbed on to injesta
(4) capsules, diluted as desired in lactose or methyl cellulose

b. Materials - examples are listed in Table 2.

Three would give calories and other valuable information.

1) Gold and its salts

2) Dysprosium and its salts

3) Europium and its salts

c. Quantity - to be determined by analysis method. It is anticipated that 10 ng of marker would be added for each gm of fat or protein in the diet and 1 ng for each gm of carbohydrate. For example, 80 gm protein eaten in one day would supply 800 ng gold to about 160 gm stool; when dried this would approximate 10 ng/gm (10 p.p.b) and one nanogram of dysprosium per gm of dietary carbohydrate would be satisfactory

d. Methods to add the marker to food include dilution in powdered foods (i.e. flour) and natural juices (i.e. canned peaches), solution in prepared foods (i.e., jellow), adsorption onto particulates (i.e., colloidal resins or food coatings) and absorption into the outer layer of solid foods (i.e., rice, sliced meat). Thiamin and iron enrichment food practices show the basic methods.

D. EXPERIMENTAL BACKGROUND

1. Toxicity in rats - Feed 20 rats 100 times the expected quantity with controls fed none. Determine:

   a. Growth rate for three weeks

   b. Appearance: general - autopsy with histology of anomalies

   c. Blood study

      1) Hb
      2) WBC
      3) Differential
      4) Serum proteins

   d. Urine

      1) Appearance
      2) Protein
      3) A.A.N

   e. Reproduction and lactation - 3 generations

   f. Food efficiency (5 days at 4 weeks of age).

2. Feasibility - 10 rats fed expected level
   - test analytical system
   - test food preparation + eating in rats

3. Pilot study with (1) 20 rats and (2) 3 monkeys

   a. Singly - 6 markers ± control
   b. Multiple - 6 markers ± control
   c. Multiple markers in multiple foods ± control

      1) Test recovery
      2) Food efficiency
      3) Growth, reproduction effects (1 generation complete)
      4) Blood, urine, tissues for markers

   NOTE: Much of items D- 1, 2, and 3 could be accomplished in one protocol, as outlined herein:

Details of the need for monkeys are outlined below since this is work which could be carried out in a routine monkey colony; i.e., that of the Sinclair Research Farm.

Animals - rats and monkeys

Age - 4 young and 4 young adults/gp.

Duration - 1 month - intensive

3 months - extensive

1 year -chronic

1 week - Food efficiency study

Diet - Standard

Marker quantities o, ppb and 100X this.

Design

Rats						Marker Amount	Monkeys					
0		1 ppb		100 ppb			0		1 ppb		100 ppb	
2/3	5	2/3	5	2/3	5	mo(Age) yr	1/2±	4±	1/2±	4±	1/2±	4±
1,3	1,3,12	1,3	1,3	1,3	1,3,12	Test, mo	1,3	1,3,12	1,3	1,3	1,3	1,3,12
a-d		a-d		a-d		Analyses	a-c+e		a-d+e		a-d+e	
X	X	X	X	X	X	Food Utilize Study??	X	X	X	X	X	X

Analyses

a = Food at 0, 1 and 3 months

b = Feces

c = Urine

d = Whole body

e = Tissues separately: liver, kidney, muscle, brain, bone and blood

Monkey Experiments

1. Recovery: (a) three adult monkeys fed 6 rare earth metals singly and six (or 10) together to test recovery of orally administered markers in feces during the next 10 days. These monkeys can be their own controls before and after the test period.
   b) Do this with dye and CrO_2 markers to compare "known standards" with the above.
   c) Compare results of (a) and (b) with data from known food consumption and food composition.
2. Food efficiency: Feed 3 young female monkey 6 (to 10) heavy metal markers at ten times the p.p.b. level expected to be used and test food efficiency for 4 weeks, where:

   $$\text{Food efficiency} = \frac{\text{gm growth}}{\text{gm food}}$$

   This requires exact measurements of the total food intake and an accounting of wasted food. Determine if this is changed compared to these monkeys two weeks prior to administration of the markers and when compared to 3 control animals during a comparable period.
3. The monkeys used in No. 2 should be continued through reproduction and lactation. The usual observations for health should be recorded. Blood, feces, urine, hair and milk should be examined for markers each six months.
4. Three adult monkeys should be fed 10 times the highest levels expected to be used. After two months they will be sacrificed and examined for markers: feces, urine, hair, slavia, skin, muscle, eyes, bone, liver, spleen, lungs, kidney, brain and each alimentary tract compartment contents (concentration and total) and washed wall.
5. Human tests.
   a. Taste test. Different types of foods will have marker added and compared to control foods treated in the like manner with no marker added. These will be evaluated in a taste panel.
   b. Four humans will take the estimated quantity of markers each day for 100 days. Medical examination and determination of the markers will be made in blood, urine, hair, and nails at 25 day intervals.

c. Blind food test. Ten young, mixed sex adults will be paid to eat at a diet table for 30 days. They will not know exact dates of the protocol. All of a variety of food available to each subject will contain nonabsorbed markers. Complete collection of feces each day will allow the quantitative determination of the amount of each marker. This quantity will provide an accurate record of this specific nutrient intake if the marker were incorporated into the food in direct proportion to the amount of the specific nutrient. A protocol is indicated:

Day	Activity
1	24 hour collection every day
	Physical examination this date and once each 7 days thereafter
2-3	Diet (no marker)
4-10	Diet plus marker
11-17	Diet (no marker)
18-24	Diet plus marker
25-30	Diet plus marker

Subjects will eat only at the diet table. They can eat anything available in any quantity in quantities to be measured exactly and to provide 1500-2500 Kcal daily.

E. Written Standards. The methods developed for food preparation with nutrient indicators will be written for production adaptation. The standards methods for testing for the markers will be given in detail. An evaluation will be given of the usefulness of the concept developed for future astronaut flights.

Table 1

Types of Nutrient Indicators

A. Elements
   1. Heavy, inert metals
   2. Enrichment of natural isotopes (K, D_2O)
   3. Artificial isotopes

B. Compounds
   1. Inorganic
      CrO
   2. Organic
      a. Natural dyes
         Beet Juice
         Carmine
      b. Synthetic dyes
         Fluorescene
      c. Excess Nutrients, bone, fat, fe
      d. Other
         (1) Unnatural Fa, AA? steroid, Carotenoids
         (2) Lignin
         (3) Wax
         (4) Nitrogen

C. Particulates:
   1. Polymers: Polystyrene, rubber, glass
   2. Cells: Yeast $\pm$ death
      Bacteria (spores) $\pm$ death
   3. Charcoal
   4. Other - corn, sized metal or plastic particles in the micron range.

D. Other: combinations as gold absorbed onto 200 mesh resin.

Table 2

SUGGESTED NUTRIENTS TO BE MARKED AND MARKERS

The nutrients	Marker
Protein	Au-gold
Fat	Eu-Europium
Carbohydrate	Dy-Dysprosium
Ca	Gd-Gadolinium
P	Ho-Holmium
Mg	Er-Erbium
Fe	Y-Yttrium
Cu	T-Titanium
Na	Se-Scandium
K	Ce-Cerium
B_{12}	Tb-Terbium
Folate	La-Lanthanum
H_2O (food)	Zr-Zirconium

The above tentative lists suggests the elements to be considered with nutrients of interest. Drs. E. Cox and Jim Vogt in the Research Reactor are collaborating in a survey of possible elements

Nutrient Indicator Feasibility Protocol

February 1971

I. Animal - Rats, Sprague-Dawley Strain (or suitable substitute) young, adult, male (about 200g)

II. Housing - Individual wire bottom or metabolism cages.

III. Water - Distilled, ion exchange water with 1 million ohms resistance placed in plastic waterers with plastic covered stoppers and stainless steel nipples.

IV. Food - A commercial rat chow in fine granular form ± nutrient indicators as needed.

V. Use of markers

A. Fecal Marker - Mark beginning and end of each collection period with brilliant blue dye as a fecal marker in food. Begin collection at first sign of dye. End collection with first sign of second dye application.

B. Collection Marker - The use of this marker will allow the determination of total fecal output. It is given 2 times daily beginning prior to collection by:

4 days in small lab animals
5 days in dogs and small primates
6 days in humans and large primates
8-10 days in ruminants and large animals

C. Nutrient Marker (s) - These are indicators placed in the diet in known proportion to any desired nutrients. They are given at same time as collection marker. The markers will be received at a concentration of 10mg/ml. An appropriate quantity will be added to a small quantity of diet as diluent and this mixed into more diet using no greater than 10 fold dilution. Three replicates will be analyzed to establish homogeneity.

VI. Rat Feasibility - A Methodology Study — diet adaptation 4-5 days.

A. Excretion Stabilization: to determine how many days are required to give a uniform excretion pattern in rats fed a commercial chow.

1. Feed lab chow with fecal marker, collection marker, and 4 nutrient markers to 4 male, young adult rats.

2. Collect all feces daily for 6 days. Discard those of day 5. Record the subjective observations of the dye.

3. Weigh the daily fecal collection for each individual. Dry overnight at °C. Grind with mortar and pestle (to approximate 20 mesh), mix, weigh and take duplicate samples and run activation analysis for desired elements.

4. Calculate dialy excretion of collection and nutrient markers. Plot excretion curves.

5. Repeat as desired with different diets.

B. Diurnal Variation - to determine if there is a specific diurnal excretion pattern for the rat and if it is possible to select suitably sampling times.

1. Continue experiment VI-A for 3 extra days with the 4 rats fed same diet and markers.

2. Collect feces of each rat four times daily at the following specific times: 9 a.m., 3 p.m., 9 p.m. and 3 a.m.

3. Keep collections for different periods (4 periods), different rats (4 rats), and different days (3 days) separate.

4. Process samples for analysis as previously described.

5. Analyze diets for intra- and inter-day variations and between animals variations.

C. Random Sample Study - to determine if the use of the collection marker will allow satisfactory use of random sampling without need for total collection.

1. Feed lab chow with fecal, collection and 4 nutrient markers to 6 male young, adult rats.

2. After a stabilization period, collect all daily stools for 6 days. Weigh, dry overnight at °C. Weigh the dried stools and accurately but randomly divide the feces of each animal into 4 samples which consist of (a) 10%, (b) 20%, (c) 30% and (d) 40%. Composite samples of same percentage for each rat. Grind, weigh and take 3 replicates for analysis.

3. Submit to activation analysis. Calculate quantities of each of the five markers in each sample.

4. Calculate % of total in each of the four main samples or combinations as follows: (a) = 10%, (b) = 20%, (c) = 30%, (d) = 40%, (a) + (b) = 30%, (a) + (c) = 40%, (a) + (d) = 50%, (b) + (c) = 50%, (b) + (d) = 60%, (c) + (d) = 70%, (a) + (b) + (c) = 60%, (a) + (b) + (d) = 70%, (b) + (c) + (d) = 90%, (a) + (b) + (c) + (d) = 100%.

D. Reliability for Diet Variations

Exact food intakes will be recorded for the following diets and will be fed to 6 yound adult male rats for a period of 4 days prior to the collection of feces:

1. Laboratory chow powder (Purina No. 56).

2. As no. 1 plus a fecal marker at day 4 plus a collection marker twice daily (10-12 hrs apart) and 4 nutrient markers in known proportion to calories, Ca, K, Fe content of diet.

3. As no.1 with 10% fat added plus markers in the appropriate proportions.

4. As no. 1 with 20% fiber added plus markers in the appropriate proportions.

5. "Bad" Apollo diet plus the same markers in appropriate proportions. The feces of each rat for 6 days will be combined, dried, mixed and triplicate samples taken for activation analysis. Actual and estimated nutrient intakes will be compared to establish reliability of the method.

E. Analyze total carcass of 3 control and 3 experimental rats from D.

VII.

A. 4 rats
5 days
2 samples

40 samples

B. 4 rats
3 days
4 samples

48 samples

C. 6 rats
4 samples
3 replicates

72 samples

D. 6 groups
6 rats/group

36 rats
3 replicates

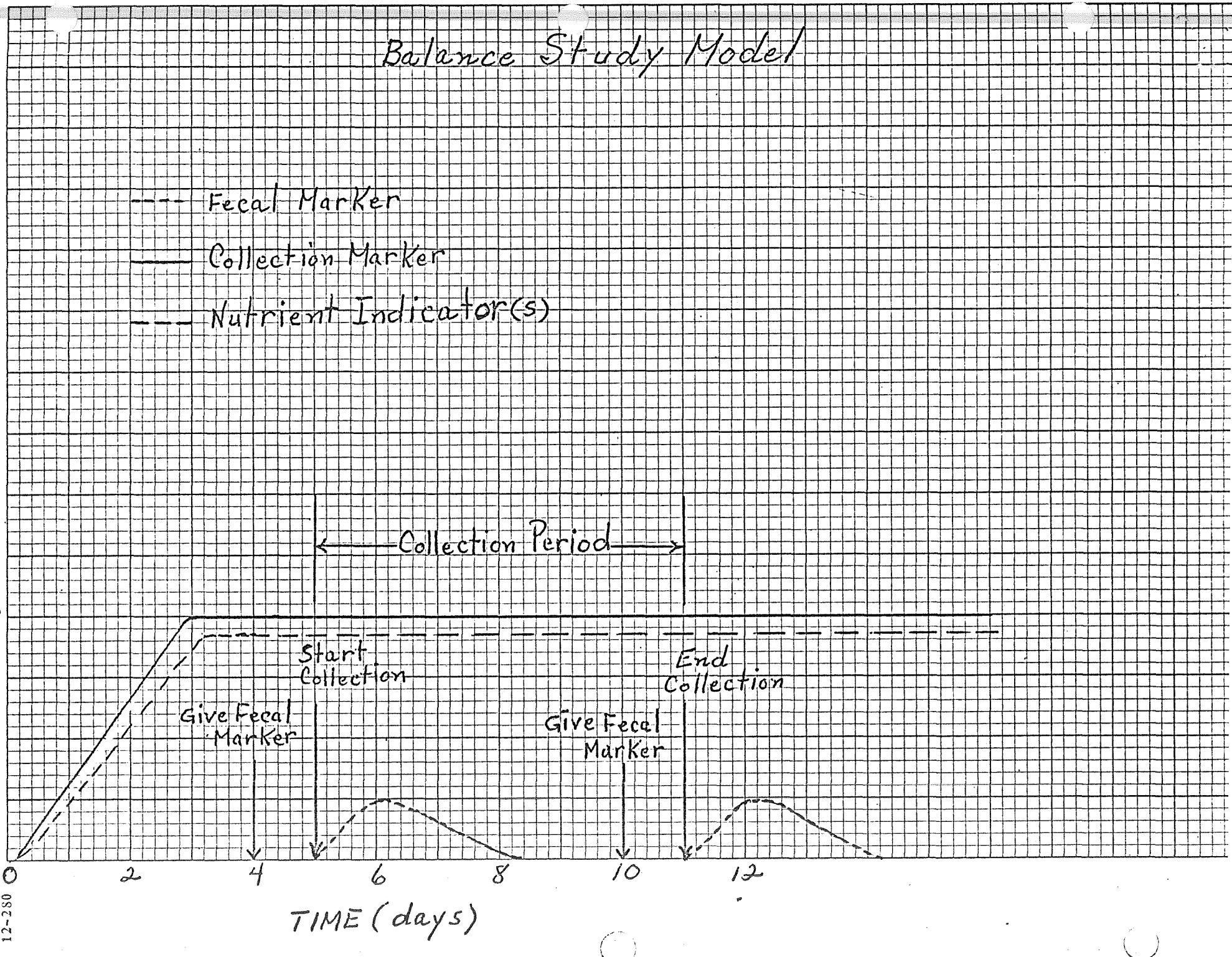
108 samples of feces

6 diets
3 replicates

18 samples

E. Tissue content - 6 rats

Total 40 + 48 + 72 + 108 + 18 + 6 292


Balance Study Model
Fecal Marker
Collection Marker
Nutrient Indicator(s)
Collection Period
Start Collection
End Collection
Give Fecal Marker
Give Fecal Marker
Amount of Marker Excreted
0
2
4
6
8
10
12
TIME (days)

Nutrient Indicator Conference

On Wednesday at 10:00 a.m., February 17, 1971, Drs. Luckey and A. Kotb met in the Nuclear Reactor Facility of UMC with Drs. Jim Vogt and Mike Kay and Don Gray. The subject was ways and means in which we could use ~~the~~ nuclear activation analysis for the determination of five elements which would be used as markers in a diet of animals and eventually humans. The immediate problem was to determine if cooperation would be available for rat experiments to begin within ten days and whether or not it seemed feasible that 5 elements would be available for use in Apollo 15 diet. The attached letter was the result of our discussion with Dr. Kay on January 12 and two or three telephone conversations with him and Dr. Vogt since that date. This formed the background to our meeting.

Immediate application was made of the analyses of Brodzinski et al. on 17 elements of feces of Apollo astronauts, the references and pertinent figure from that report are attached. Much of the conversation revolved around the quantity of element which could be determined. It was agreed that the letter contained values which were at least 10 times higher than they needed to be. Using 1 gram sample size as a standard and 5 micrograms of the element to be determined, it was estimated that the diet could contain approximately 1/2 microgram per gm since bioincrassation can be expected to give a 10 fold concentration of anything in the diet which is not absorbed. The lower quantities would give somewhat less accuracy in the counting statistics but it was believed that the overall precision in the analytical method would be within a 5% error. Rather than use the elements suggested in the February 15 letter, it was agreed that further search would be made for more favorable elements. Arbitrarily, it seems best to use smaller quantities if sensitivity and accuracy were equal. A major problem is to determine a group of elements which can be analyzed simulataneously in the same sample, thus there would be no major interference with anyone from any other. This tends to rule out europium which has many radiations at different energy levels. Others which will be looked at will be iridium, terbium and scandium. Dysprosium, gadolinium, yttrium and titanium still seem to be possibilities. Based on the outline of nutrient indicators presented in February, 1971, page 9, Table 2, it was felt that a total of 15 indicators would be the maximum sought in our broad search.

Immediately, we will concern ourselves with the five which seem most feasible and readily available. Activation at UMC will be used for the immediate experiments and routine work will be sent to Handford via directions from Houston. Recent publicity on the Handford plant suggests that this may not be a reliable source for activation analysis work. Dr. Vogt's letter suggests otherwise.

It was anticipated that a short exposure in our reactor followed by one week decay with counting thereafter would give adequate data for the results which we are seeking. Arrangements were made for the comminuted 68 Apollo diet to be analyzed as soon as possible. It was suggested that the "high" gold in astronaut feces comes from the gold used in their equipment rather than from a dietary source. The meeting adjourned on a note that we would meet in 10 days with more information and that animal experiments could begin in the first week of March.

The attached animal experiments protocol was developed following this meeting.

NUTRIENT INDICATORS

SECOND CONFERENCE ON ACTIVATION ANALYSIS

On Friday, February 26 at 10:00 a.m., Drs. Don Luckey and Ahmed Kotb met for the second time in the Nuclear Reactor Facility with Drs. Jim Vogt and Mike Kay.

1. Dr. Kay presented a list of five elements with the desired concentration of each in the feces. The choice of these five he said was based on experimental evidence:

   Terbium $\geq$ 1 µg
   Ytterbium $\geq$ 1 µg
   Lutetium $\geq$ 0.1 µg
   Tantalum $\geq$ 1 µg
   Iridium $\geq$ 0.5 µg

2. From the discussion, it was suggested that:
   a. counting would start one week after irradiation of samples to allow for Na decay.
   b. drying of samples should be carried out at 80°C.
   c. the 1-ml capacity Holland vials should be tried first for holding samples.

3. Dr. Luckey discussed the "Balance Study Model" and the number of samples to be analyzed in the rat feasibility study. It was agreed that the design looked good.

4. It was decided that samples of the lab chow diet should be irradiated for analysis of their contents of the five mentioned elements.

5. Dr. Kay agreed to deliver solutions of these elements to Dr. Luckey on Monday, March 1, 1971 so that exact amounts can be mixed with lab chow to check the accuracy of the technique of activation analysis when samples of the diet containing these markers are submitted for activation analysis.

6. Dr. Luckey suggested that cerium be included in the list since it has been widely used and accepted. Dr. Vogt agreed to have Ce at a concentration of $\pm$ 4 µg/gm of feces to replace tantalum.

THE MEASUREMENT OF NUTRIENT INTAKE: Calories, Ca, K and Fe

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March - 1971

INTRODUCTION

A method is proposed which will allow the intake of the total amount of food or any given nutrient to be accurately monitered. Total food intake, and any specific nutrient, is difficult to obtain accurately in experimental animals and/or humans. Most studies with humans are subject to errors by the subject. Animal studies are open to error through wasted food which is difficult to recover, through contamination of wasted and or presented food with excreta or stresses of the animal with restricted movement.

The most exact method of determining the intake of a given nutrient without personnel feeding each subject would be to add a marker to the food which could be accurately determined in the collected feces. This would be applicable to experimental and practical work. One experimental use we are pursuing is to determine the contents of the alimentary tract compartments. A practical use would be to determine how much protein or calories are consumed by an astronaut during a flight. Ultimate application in man demands considerable experimental work to give an appreciation of what might work, how efficient the marker is and whether or not it could be harmful

THEORY

All of a variety of foods available to the subject will contain nonabsorbed markers. Complete collection of feces each day will allow the quantitative determination of the amount of each marker. This quantity will provide an accurate record of this specific nutrient intake if the marker were incorporated into the food in direct proportion to the amount of the specific nutrient. One to ten markers will be sought which are virtually not absorbed and can be used in the ppb-ppm range to indicate how much of a given nutrient, i.e. protein, is ingested by the subject each day.

The new method incorporates a nutrient indicator in all food available, a nonabsorbed marker would be added to the food in direct proportion to the amount of nutrient in the food. Collection of all feces, homogenization and analysis for the marker would give a direct means to determine how much of the nutrient was ingested. Using a collection marker Dr. Kotb and I have developed a method with heavy metals which would allow the determination of multiple markers with only partial collection of excreta.

The work required is outlined on the following pages. Calories, Ca, K and Fe will be determined directly and with markers in a multiple approach to the subject. The two methods will be compared to indicate the validity of the new method and to provide a protocol for future balance studies. The method will allow the accurate determination of the consumption of one or more nutrients from analysis of stools even while the subjects are fed pantry style.

B. LITERATURE

1. A complete literature search is in progress and will be finished in June 1971.
2. Information summarized in seminar
3. Publication of a complete, critical review of literature on markers in nutrition
4. Evaluate possible types of marker materials possible and select representative of the best type for testing. Table 1 indicates those considered. For multiple nutrient indicators to be used in humans as well as animals, heavy metals which can be determined by activation analysis appear to be the best choice. This seems superior to a series of dyes and/or particles to be identified by calorimetry or microscopic observation, i.e., colored bacteria.

C. PRELIMINARY WORK:

1. Characteristics of markers.

Record the characteristics of the specific markers to be used. This shall include methods to add each marker to the food and method to be used for testing. The use of multiple markers will require testing for compatibility which would not be needed if only one or two were to be used. Ten to fifteen of the most promising markers should be examined.

The criteria sought for a successful nutrient marker will be: 1) not absorbed during digestion; 2) not radioactive; 3) readily dispersed in a variety of foods; 4) not volatile during feces drying; 5) detectable in small quantities; 6) readily detected with a minimum of manipulations; 7) have low material abundance in diet; and 8) be neither toxic to the host nor affect the digestability of any foodstuff. Heavy metals and particularly some of the rare earth metals could best be used to give a multiple approach through a single assay with activation

2. Background data.
   a. Determine the amount of background traces of the 10-20 markers which occur naturally in Apollo diets. This can begin with the Composite 1968 diet.
   b. Determine background traces in Apollo drinking water.
   c. Determine background traces of the 10-20 markers in stools from Apollo diet eaters.

3. Marking Food.
   a. Form of Markers (1) collodial suspension
      (2) solution
      (3) adsorbed on the injesta
      (4) capsules, diluted as desired in lactose or methyl cellulose.
   b. Materials suggested are listed in Table 2. Five of these are presently being used. The others are merely suggestions. The most interesting nutrients are suggested. This proposed work will provide capability to measure any nine nutrients.
   c. The nutrient intake will be determined by the analysis method. For example 10 ng of marker would be added for each gm of protein in the diet and 1 ng of another for each gm of carbohydrate. For example, 80 gm protein eaten in one day would supply 800 ng to about 160 gm stool; when dried this would approximate 10 ng/gm (10 p.p.b) and one nanogram of dysprosium per gm of dietary carbohydrate would be satisfactory (these figures are examples only).

d. Methods to add the marker to food include dilution in powdered foods (i.e. flour) and natural juices (i.e. canned peaches), solution in prepared foods (i.e. jello), adsorption onto particulates (i.e. colloidal resins or food coatings), filling of foods, and absorption into the outer layer of solid foods (i.e. rice, sliced meat). Thiamin and iron enrichment food practices show the basic methods used which include use of simulated food particles (i.e. rice).

D. EXPERIMENTAL WORK

1. Feasibility Data - The work on rats given as Addendum A, is in progress. This data confirms the feasibility of the method and provides a model system for future work.

2. Toxicity in mice - Feed 20 mice 100 times the expected quantity for three generations, with controls fed none. Determine in fifteen females:

   a. Growth rate for three weeks

   b. Appearance: general - autopsy with histology of anomalies

   c. Blood study

      1) Hb
      2) WBC
      3) Differential
      4) Serum proteins

   d. Urine

      1) Appearance
      2) Protein
      3) A.A.N

   e. Reproduction and lactation - 3 generations

   f. Sacrifice and analyze the total carcass less blood and GI tract for marker elements in six adult,(3 males and 3 females) at 2 months of feeding.

3. Monkey Feasibility Protocol.

   Diet will be monkey chow treated as desired.

   A. Balance Study - Feed 3 adult monkeys 10 times the amount of 10 markers for 10 days with 5 day adaptation period.

(a) Food intake to be measured

(b) Fecal and urinary excreta to be collected quantitatively for analysis.

(c) Activation and chemical analysis of (1) diet and (2) excreta:
(1) 2 x 3 (triplicates) = 6 samples.
(2) 3 x 3 x 2 x 3 (three 5 day collections) = 54 samples

b. Reproduction

Three mature female monkeys are to be fed 8-10 markers at 10 times expected level of use and 3 fed no markers. They will be bred. The usual observations of health will be made during pregnancy, and lactation of 3 monkeys and one control. Blood, feces, urine, hair and milk will be examined each 6 months.

5 samples x 6 monkeys x 2 examination x triplicates = 180 samples.

c. Absorption of markers will be checked in 3 adult monkeys fed 10 times the level of use expected during a 2 month period. The following tissues will be analyzed for the markers: feces, urine, skin, muscle, bone, liver, blood, lungs, kidney, brain and each alimentary tract compartment and washed wall. These compartments are oral-pharyngeal, esophagus, stomach, duodenum, jejunum, ileum, cecum-appendix, colon and rectum (20 compartments and 2 composite walls). One control monkey will be sacrified.

4 x 3 x 22 = 264 samples

d. Analysis: part 1 = 60 samples
2 = 180 samples
3 = 264 samples
Total 504

e. Markers and Quantities

Element	Amount per Kg diet	Toxicity Levels/Kg	
		10 X	100 X
Ce	500 ug	5000	50,000
Lu	99 ug	990	9,900
Ir	82 ug	820	8,200
Tb	260 ug	2600	26,000
Yb	212.5 ug	2125	21,250

5 others to be named as soon as calculations are completed.

4. Human Feasibility Studies

a. Recovery - Two adult males will take four times the expected daily intake at one dose. Fecal collections before and during the next 8 days will be analyzed to determine the loss curve and the proportion recovered. Eight days x triplicates x 2 mend = 54 samples.

b. Useage - Three adult males will take the daily expected intake of markers each day for 100 days. Physical examinations will be made before and following this period. One day and 6 day fecal samples will be taken twice during this test. Blood and urine samples will be analyzed at 1, 2 and 3 months. Fourteen samples x triplicates x 2 men = 84 samples for analysis.

c. Taste - 10 representative foods will be prepared with and without the expected markers. Food without markers will be treated with an equal quantity of distilled water. These will be submitted to taste panels in order to determine any difference in taste.

d. Balance Study - Thirty different foods will be prepared with appropriate quantities of markers. These will be dispensed at a food table for 10 young adult males. The following protocol will be followed:

Day	Activity
1-3	Diet acclimatization period
4-10	Collection on daily basis

24 Hour collection on days 4 through 9.

Physical examination on days 0 and 10

30 foods + (10 subjects x 6 collections) = 90 samples

e. Analyses: Part 1 = 54 samples
2 = 84 samples
3 = 0 samples
4 = 90 samples
228

f. Balance study: Calories, calcium, potassium, protein

E. Written Standards - The methods developed for food preparation with nutrient indicators will be written for production adaptation. The standards methods for testing for the markers will be given in detail. An evaluation will be given of the usefulness of the concept developed for future astronaut flights.

F. Dissemination of Knowledge: Review publication, Publication of data, Seminars and International Symposium.

Table 1

Types of Nutrient Indicators

A. Elements
   1. Heavy, inert metals
   2. Enrichment of natural isotopes (K, D_2O)
   3. Artifical isotopes

B. Compounds
   1. Inorganic
      CrO
   2. Organic
      a. Natural dyes
         Beet Juice
         Carmine
      b. Synthetic dyes
         Fluorescence
      c. Excess Nutrients, bone, fat, fe
      d. Other
         (1) Unnatural Fa, AA? steroid, Carotenoids
         (2) Lignin
         (3) Wax
         (4) Nitrogen

C. Particulates:
   1. Polymers: Polystyrene, rubber, glass
   2. Cells: Yeast $\pm$ death
      Bacteria (spores) $\pm$ death
   3. Charcoal
   4. Other - corn, sized metal or plastic particles in the micron range

D. Other: combinations as gold absorbed onto 200 mesh resin.

Table 2

Suggested Nutrients to be Marked and Markers

The nutrients	Marker
Calories	Yb-Ytterbium
Protein	Au-gold*
Fat	Eu-Europium*
Carbohydrate	Dy-Dysprosium*
Ca	Tb-Terbium
P	Ho-Holmium*
Mg	Er-Erbium*
Fe	Lu-Lutetium
Cu	T-Titanium*
Na	Se-Scandium*
K	Ir-Iridium
B_{12}	Gd-Gadolinium*
Folate	La-Lanthanum*
H_2O (food)	Zr-Zirconium*
Collection	Ce-Cerium

The above tentative lists suggests the elements to be considered with nutrients of interest. Drs. M. Kay and J. Vogt in the Research Reactor, UMC, are collaborating in a survey of possible elements. Those with asterisk have not been confirmed.

Astronaut Short Course in Nutrition

- A 10 hour lecture-discussion approach.

Time	Topic
1 Hr.	Introduction, Dietary Allowances, Use of Food Tables, Digestion, Absorption, Metabolism and Excretion
1 Hr.	Discussion
1 Hr.	Forty Nutrients become you - Identification, needs, analysis, presence in foods, additives.
1 Hr.	Discussion
1 Hr.	Conditioning Factors and Food Intake - Nutrient balance, Environment, Individual Character, Stress, Digestive actions and Weight control
1 Hr.	Discussion
1 Hr.	Problems: Ontogenetic - Child (Food Faddist - Teenage - Coke and chips; Pot and Pregnancy - Elderly - limited and set menu USA $\simeq$ poor: Indians, Eskimos, Mexicans, Applelachia
1 Hr.	Discussion
1 Hr.	Space Feeding - Beginning and Mercury + Gemini - Apollo - short flight problems - Post Apollo - medium (1-2 month) flight problems - Use of in flight and experimental information
1 Hr.	Discussion

Reading:
1. Nutrition Manual - Stare
2. Text, Pike and Brown
3. Current articles

Astronaut Nutrition for two (± one) Months Space Flights

I. Review development of short term diets (1-10 days).
   - A. Planned
   - B. Provided
   - C. Used
   - D. Evaluation
   - E. Summary of nutritionists suggestions and response

II. Predictable needs and criteria of adequate nutrition for 2 ± 1 months space flights.
   - A. Air exchange and cleansing
   - B. Water requirements and balance
   - C. Energy needs
   - D. Fat-carbohydrate - Roughage
   - E. Protein-Amino Acid needs
   - F. B-vitamin needs
   - G. Fat-soluble vitamin needs
   - H. Macro-mineral needs
   - I. Trace element needs
   - J. Intestinal Flora problems
   - K. Microbial aspects of food, water and waste
   - L. Waste systems and/or utilization
     - (1) Urine
     - (2) Feces
     - (3) Gas
     - (4) Containers

III. Preflight adaptation to diet

IV. Summation