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RESEARCH ON THE EFFECTS OF ALTERATION OF THE INDIGENOUS MICROFLORA OF THE MONKEY

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FOREWORD

This is the final report of a study conducted at the Gnotobiology Laboratory of the Biosciences Operation, Bioastronautics Section of General Electric's MOL Department, Valley Forge Space Technology Center, King of Prussia, Pennsylvania. The work was done for the Life Support Division of Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio, under Contract AF 33(615)-5242 during the period 1 July 1966 to 15 June 1966 and was technically monitored by Dr. Alton Prince of the Biotechnology branch of the Aerospace Medical Research Laboratories. This study was made in support of Project No. 6373 Aerospace Life Support.

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Dr. T. D. Luckey, Professor of Biochemistry, was a consultant on the project and furnished much valuable advice and guidance. Table XIII from his book "Germfree Life and Gnotobiology" was reprinted with the permission of the publisher, Academic Press, New York, N. Y.

This technical report has been reviewed and is approved.

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ABSTRACT

The feasibility of changing the bacterial and fungal flora of monkeys undergoing biological confinement was studied. The significance to the host of an altered ecological relationship was examined with special attention to the feasibility and consequences of requiring microbial compatibility of astronauts for extended space mission. It was determined that while it would be extremely desirable to have microbial compatibility among crew members, tampering with the indigenous flora poses special problems for which there are as yet no answers. A data and information retrieval system, designed to aid in solving some of the problems mentioned above, has been designed and is presented.

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SECTION I

INTRODUCTION

Previous studies have been shown that the indigenous body microflora in the normal animal are not in a stable situation with regard to the numbers and kinds of species. What is relatively stable is the total number of microorganisms found in and on the animal.

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

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

Considerable effort has been expended to define the indigenous microflora of laboratory animals as well as the human. In spite of a large body of literature, the very immensity of the task forces us to make gross (and often erroneous) assumptions. This study is an attempt to define some of the conditions that alter the normal balances and keep the natural condition from being reestablished.

Among the major variables that determine the composition of the body microflora are diet, exposure to other living creatures, microorganisms in the atmosphere, food and surroundings, body cleanliness, and perhaps the physical state of the individual concerned. In normal life manipulation of these variables is possible only in the grossest context--yet enormous forward progress in the well being of the human race has been made, even with the crude controls now practiced. For example, food sterilization, sewage treatment, potable water treatments, drugs and general sanitation have been shown to be effective means for general controls.

The possibility of changing the indigenous bacterial flora for an extended period, the effect on the host of the altered ecological relationships, the techniques that would be involved in such a study and the possibility of showing compatibility requirements (in the sense of a similar body microflora) for normal humans or animals has never been systematically studied, although individual parts of the above have been reported in gnotobiotic investigations. These are vital questions when considering long-term space flight. The study, therefore, while confined to infra-human primates, was primarily designed to establish more definite parameters to be used during evaluations of the microbiological considerations of manned space flight.

SECTION II

CONDUCT OF THE EXPERIMENT

A. HISTORICAL

Six male rhesus, macaca mulata, post puberty and about six pounds each, were quarantined as a group for four weeks at the vendor's animal colony.* During the period, the animals were examined for tuberculosis, malaria, ova and parasites and overt pathogens (Shigella-Salmonella). No evidence of any of the above was found. Upon delivery of the animals to GE, the animals were placed in special animal quarters apart from other animals. They were caged in pairs and a random pairing system established. Every few days, the pairs were changed to insure all animals continued exposure to the microflora of the entire group. All attendents and laboratory workers were given x-ray examinations and a general health survey to insure that specific pathogens would not be transmitted accidentally to the experimental group. Additional health precautions for the personnel are listed in Appendix II.

B. THE ISOLATORS

Figures 1, 2 and 3 illustrate the isolator system used during the course of the experiments. The isolators were specially designed by GE Bioscience Operation and Frank Matthew, of Matthew's Research, the isolator vendor. Appendix I further discusses and illustrates the isolators and their technical details.

During the last two weeks of gentling and pairing, the animals were switched to an autoclaved diet. This was done to accustom them to the taste change which occurs upon sterilization and to verify that the diet planned for use during the isolation period was nutritionally adequate. Since the vitamin loss during autoclave sterilization is relatively severe, supplementary vitamins were fed via the water supply. The water was also autoclave sterilized. Table I gives the nutritional content of the diet, Table II the contents of the vitamin supplement and Table III, the amount of vitamins given contrasted to the minimum daily requirement of each Vitamin required by humans. The vitamins were filter sterilized by millipore filter techniques. The filtering was done within a sterile flexible Trexler type isolator. During this pretest period, the animals were kept in open grill cages, exposed to normal atmosphere, with the usual temperature (75°F and relative humidity of 50 percent) of the primate holding center. The food after autoclaving was not kept sterile other than enclosure in sealed kraft paper bags.

C. THE GNOTOBIOTIC EXPERIMENT

Animals (No. 119**, 116**, 120** and 118***) were randomly selected from the six possible subjects for placement in the isolators. Animals 115 and 121 were kept in the holding center

*Primate Import Corporation, 34 Munson St., Port Washington, N.Y.

**Primary Subjects

***Isolator Control Subjects

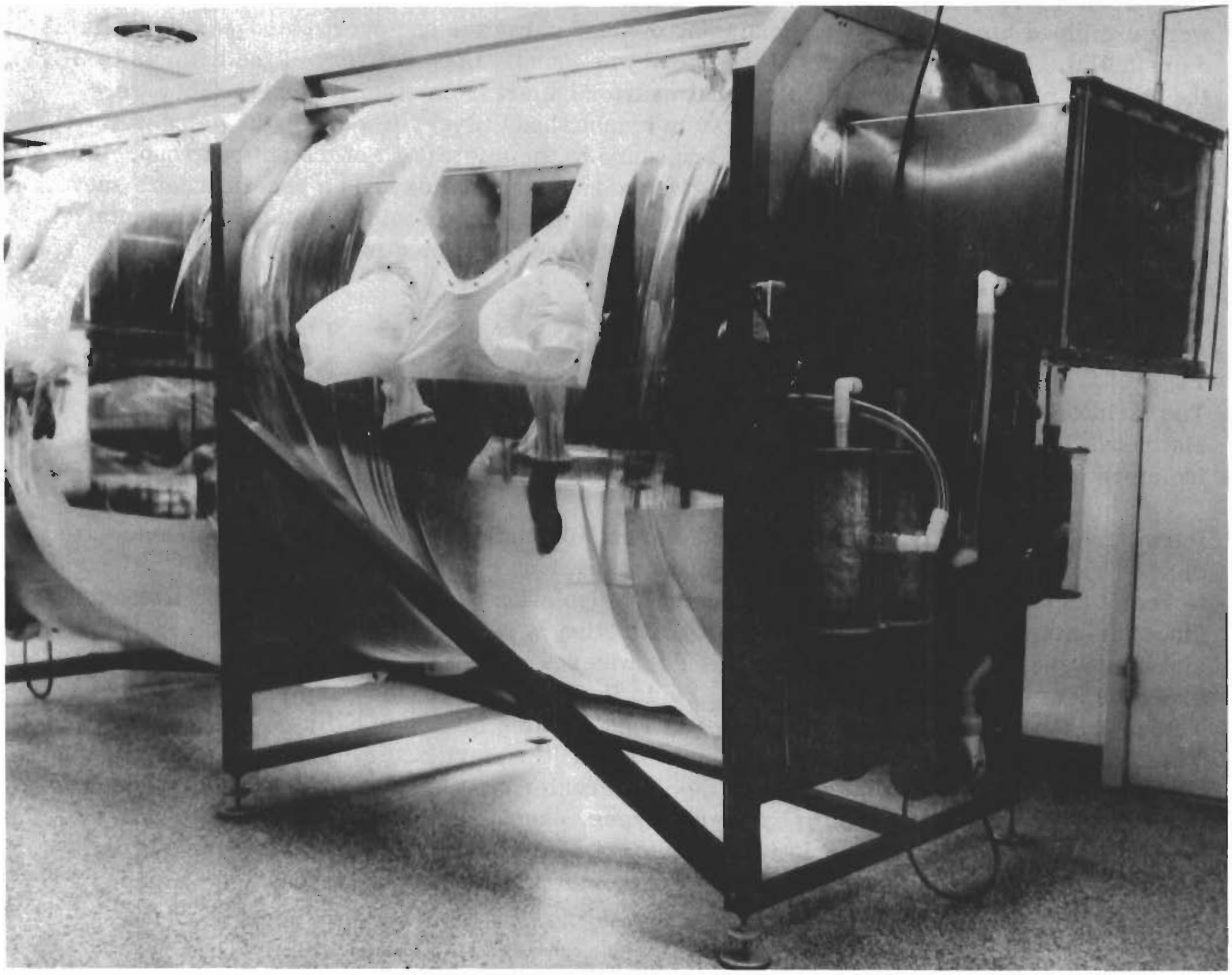


Figure 1. Isolator System

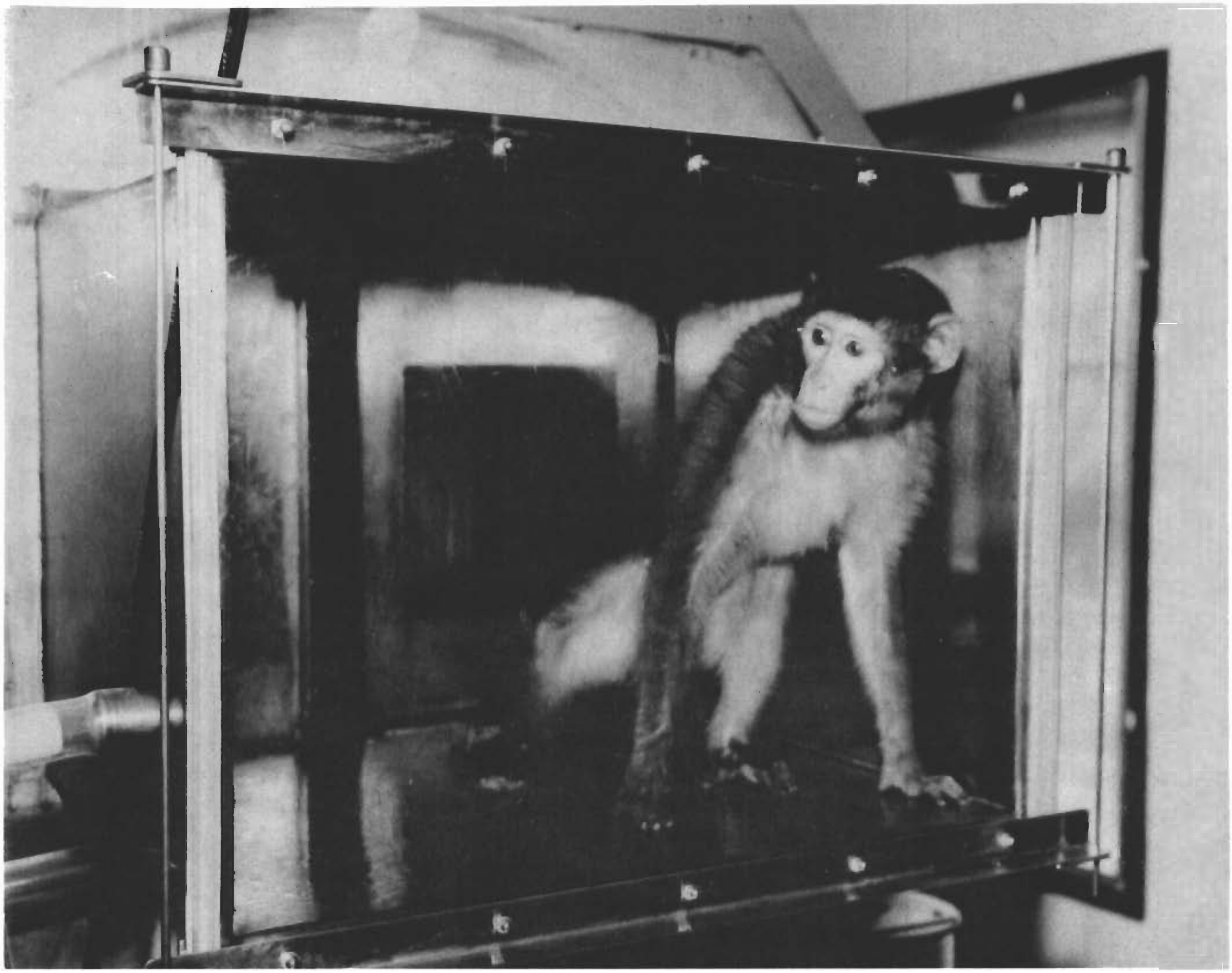


Figure 2. Holding Isolator and Pass-Through

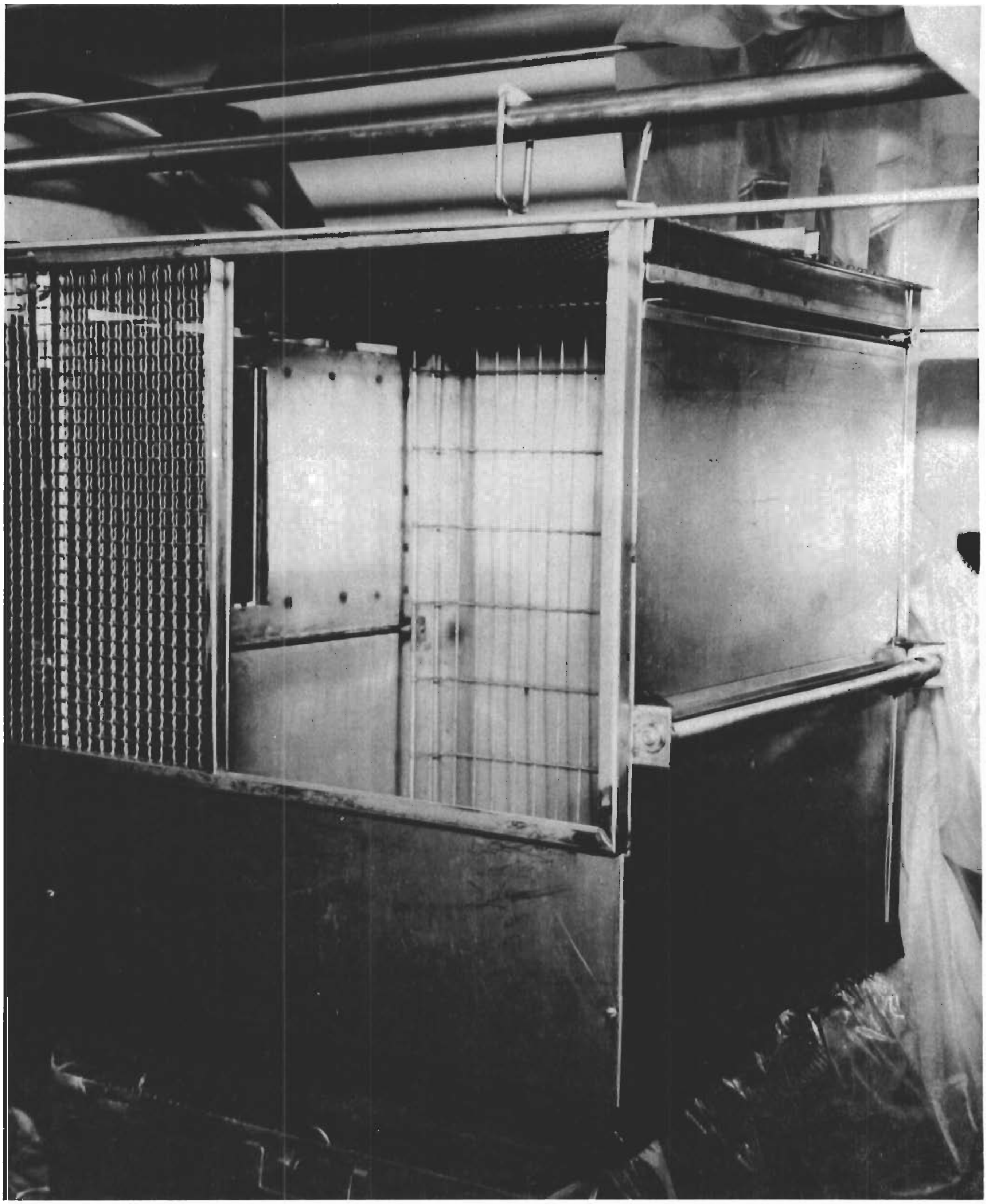


Figure 3. Cage Inside Main Isolator

TABLE I.

CONSTITUENTS OF PRIMATE DIET BEFORE AUTOCLAVE STERILIZATION ROCKLAND PRIMATE DIET

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

Ground Yellow Corn	Pyridoxine Hydrochloride
Dried Skimmed Milk	Thiamine Hydrochloride
Dehulled Solvent Extracted Soybean Meal	Vitamin A Palmitate
Animal Fat (Preserved with Propylene Glycol, BHT, Citric Acid)	D-Activated Plant Sterol (Source of Vitamin D-2)
Ground Whole Wheat	Vitamin E Supplement
Dehydrated Alfalfa Meal	Choline Chloride
Brewer's Dried Yeast	Ascorbic Acid and Traces of Manganese Sulphate
Cane Sugar	Iron Carbonate
1.5 percent Calcium Carbonate	Iron Oxide
0.75 percent Salt	Copper Oxide
Vitamin B-12 Supplement	Cobalt Carbonate
Ribolflavin Supplement	Potassium Iodide
Calcium Patothenate	Zinc Sulphate
Niacin	
Folic Acid	

TABLE II.

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

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

TABLE III.

DAILY VITAMIN SUPPLEMENT

Vitamin	Proportion of Minimum Daily Requirement
A	4
D	3
C	6
B_1	8
B_2	2
Niacinamide	*
*MDR not established	

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

as controls. During this holding period all animals continued in good health and exhibited normal weight gains. Immediately following the introduction of the autoclaved diet, the animals went on a self-imposed fast, for about three days, but upon resumption of eating, quickly learned to like the sterilized diet as evidenced by a lesser wastage of food. The vitamin supplement was delivered in the water supply. The water dispensers were brown bottles (to reduce the vitamin loss from ambient light). Appendix III discusses the diet further.

When the animals (primary subjects) were placed in the isolators, it was found that timing of the feeding would quickly induce the animals to travel from isolator to isolator as desired. Thus the separate sections of the isolators could be opened, cleaned, stool and urine specimens removed, food and water placed into the isolators and the whole resterilized with 2% peracetic acid solution at times of our choice.

A numerical sampling of the microflora of selected body sites of the animals was performed just before the subjects were isolated. Animals 119, 116 and 120 were well shaven before being isolated. Animal 121 was also shaven.

The temperatures inside the isolators were at all times essentially the same as those experienced by the controls. (Normal laboratory temperature was kept at 75°F.) When for any reason the air temperature dropped below 75°F, quartz lamp heating units were automatically activated directly onto the animal's cage areas until the air temperature was reestablished to the desired point. The relative humidity within the laboratory, control center and interior of the isolators was automatically controlled, additional moisture sometimes being required during the colder months of the year. Our desired RH was 50 percent. The isolators were placed near windows so that the animals at their option, would get both sun and shade during the day. To insure ease in handling during body and skin sampling, the animals had been thoroughly gentled, accustomed to the sounds of voices and personnel and a radio played 24 hours a day.

The animals were not cleaned before placement within the isolator systems in order to remove one possible shock variable from the transfer. No outward evidence of emotional stress was noted at any time during the experiment, which would be traced to isolation. The animals quickly became attentive when visitors or staff approached the isolators and seemed to evidence as much interest in the humans as the humans evidenced in the animals. No loss of appetite or refusal to take liquid ever occurred during the period of isolation except following the baths discussed later.

In Table IV the numbers, subject to the cycling phenomena, which will be discussed later, represent average counts and baseline data for the animals. In Table V, microorganism population and moisture content of primate feces at times of isolation includes an estimate of feces moisture content.

A sampling of the animals shortly after introduction into the isolator, showed Animal 119 with a rapidly spreading surface infection of *Pseudomonas*. Table VI gives the microorganisms identified at this time. The Pseudomonas replication rate seemed unaffected by Phisohex,® and Vesphene,® but peracetic acid killed the cultures effectively during test.

TABLE IV.

BACTERIAL POPULATIONS – SELECTED BODY SITES OF ANIMAL IN ISOLATORS

Body Area	Aerobic Flora		Anaerobic Flora	
	Animal No. 116	Animal No. 119	Animal No. 116	Animal No. 119
Conjunctiva	2×10^6	2×10^5	3.5×10^6	1.59×10^8
Throat	3.46×10^8	3.7×10^7	7.20×10^8	7.20×10^8
Gingiva	2.48×10^8	5.32×10^8	Overgrown Fungi 10	TNTC 10^8
Axilla	1.19×10^7	3×10^6	3.5×10^6	zero $\times 10^5$
Groin	6×10^6	7.5×10^7	1×10^6	9.00×10^8
Glans Penis	7×10^6	9.2×10^7	$3. \times 10^6$	3.68×10^8

All counts are on Blood Agar plates. Sampling and dilutions follow the procedures of Gall. Fluid Thioglycollate has been used for the anaerobic dilutions instead of Galls' Broth. Aerobic dilutions are prepared in Brain Heart Infusion Broth. The swabs from the given areas are placed in 10 ml and this is arbitrarily taken as the 10^{-3} dilution in accordance with previous work by West, Gall and others.

TABLE V.

MICROORGANISM POPULATION AND MOISTURE CONTENT OF PRIMATE FECES AT TIME OF ISOLATION

Animal No.	Sample	Total Feces (Grams)***	Weight Used for Moisture Determination (Grams)	Weight Lost (Grams)	Percent Moisture	Wet Weight Diluted/ 100 ml for Counts (Grams)	Counts	
							Aerobic	Anaerobic
115	1	3.88	3.88	1.22	31.44	3 mm loop/10 ml*	3.30×10^{10}	3.80×10^{10}
115	2	3.50	2.89	1.69	58.47	0.35	2.30×10^{10}	1.83×10^{10}
116	1	4.66	4.10	2.65	64.63	0.48	zero $\times 10^{8}$**	1.1×10^{9}
116	2	10.19	10.00	4.53	45.30	0.17	3×10^{8}	zero $\times 10^{8}$**
118	1	3.13	3.09	1.76	56.95	0.13	6×10^{8}	1×10^{8}
119	1	7.96	7.06	5.28	74.78	0.89	2×10^{8}	2×10^{8}
119	2	5.20	Weighing Error			0.12	1.7×10^{9}	zero $\times 10^{8}$**
120	1	6.60	6.51	4.74	72.81	0.50	7×10^{8}	9×10^{8}
121	1	2.69	2.69	0.73	27.13	3 mm loop/10 ml*	1.65×10^{10}	1.22×10^{10}
121	2	2.32	1.60	1.10	68.75	0.35	1.41×10^{10}	1.23×10^{10}

*Feces counts done following procedure of Gall eg. a standard loop full of material into 10 ml followed by serial dilutions. The initial tube is arbitrarily taken as 10^{-3}.

**No growth at 10^{8} dilution, lower dilutions accidently destroyed.

***Feces as delivered from the animal, collected within minutes of defecation.

Animals being fed sterilized Rockland primate diet with supplemental vitamin. Water given ad-libitum.

TABLE VI.

PREDOMINANT AEROBIC ORGANISMS ISOLATED FROM SELECTED BODY LOCATIONS SHORTLY AFTER ISOLATION

Organism	Location and Animal					
	Eye	Throat	Gingiva	Axilla	Groin	Glans Penis
Staph*	A	B, C	A, B	A, C	B	C
Staph**	B, C	A, C	C	B	A, C	
α Hemo Strep	B	A, B, C	A, B, C	B	A, B	A
γ Hemo Strep	C				C	C
Pseudomonas		B	B		B	
Undiff. Fungi				C		
Lactobacillus					C	
Bacillus Undiff.		A, B, C	A, B, C	A, B, C	A, B, C	A, B, C

*Non-pathogenic

**Potential pathogen (based on Mannitol-salts agar)

A = Animal No. 116

B = Animal No. 119

C = Animal No. 120

Other organisms isolated but not identified at this date

The animal was completely immersed in 1/10 percent peracetic acid for several seconds and the fur and skin thoroughly wetted down. The bathing was done inside the large plastic section of the isolator and then the animals were dried with sterile toweling. Twenty-four hours and subsequent skin and fur sampling did not indicate the presence of Pseudomonas. For the first few days after the bath, the fur and skin of the animals reflected the loss of the microorganism population. Sampling of several points on the body gave a negligible count even after 24 hours.

Shortly thereafter a new and different population took over. The animals occasionally are able to reach their feces and this new population reflected the genera found in the feces. The new population quickly reached the approximate numerical levels previously found. Table VII is a summary of the organisms isolated before and after Cloxacillin® administration, discussed later, but also shows the loss of Pseudomonas, from surface sites, before the administration of the penicillin.

Following the success of the peracetic acid baths, all isolated animals were bathed in similar fashion with similar results.

Using a mixed culture of monkey feces microflora, antibiotic sensitivity determinations were run using 10^3 dilution on blood agar plates grown both aerobically and anaerobically. Antibiotics used were BBL sensi-discs. Table VIII shows the antibiotics tested.

The most effective antibiotic on the mixed fecal flora, as tested in vitro, is Penicillin.

Sodium Cloxacillin monohydrate, pediatric dosage equivalent to 50 mg/Kg/24 hour for one week was administered to reduce microflora. Cloxacillin was chosen over penicillin G for the following reasons: higher acid stability, penicillinase resistance, enteric absorption is not as adversely influenced by the food in the stomach.

The disinfection process preceded administration of Cloxacillin; this antibiotic, acting primarily through the blood stream changed the character of the intestinal microflora on animal nos. 119, 116 and 120. Not all the microflora were affected (Tables VII and VIII), but Streptococci, Leptothrix and others disappeared or at least were not recovered in following analyses. The Proteus and Staphylococcus were also affected by the dose although not in the level that detection was no longer possible. The animals, both suffering moderate diarrhea, were kept isolated for several weeks to ascertain if a new balance had been struck. Outwardly, the health of the animals was unaffected. About four weeks after administration of Cloxocillin had ceased, neomycin was administered to animal nos. 119 and 120 for three days. Animal no. 116 was held as an isolator control and animal nos. 115 and 121 as an open colony control. Table IX compares the results. By a succession of antibiotics, it appears that the internal balance can also be affected.

Within two days, following the administration of neomycin, the monkeys 119 and 120 were suffering very badly from loose stools. Refusal to take food and general malaise indicated they were not feeling well. An isolation of Candida in the feces of animal no. 119 following administration confirmed our belief that the microorganism was present in the body but at

TABLE VII.

SUMMARY OF ORGANISMS ISOLATED AND IDENTIFIED BEFORE AND AFTER CLOXACILLIN ADMINISTRATION, AND AFTER BATHING IN PERACETIC ACID

Genera	Feces		Conjunctiva		Throat		Gingiva		Axilla		Groin		Glans Penis	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Staphylococcus	A, B, C	A	B, C		B		C		A, C		A, B	A, B, C	B, C	B
Streptococci	C		C		A, B		A, B, C		C		C		A	
Pheumococci													A	
Lactobacilli											C			
Clostridia	A, B, C		C											
Corynebacteria	A, C	A, C	A, C	A, C	A, C	B	A, C			A	B, C		A, C	
Escherichia		A												
Proteus		A, B									C		C	B
Klebsiella														
Pseudomonas					C		B	B						
Alcaligenes						B		B						
Bacillus Sp.			C		A									
Leptothrix					A									
Bacteroides	B, C													
Aspergillus									C					
Salmonella-Shigella*		A, B											C	
Candida		B, C												

A = Animal No. 116

B = Animal No. 119

C = Animal No. 120

*The presence of some organisms, found after antibiotic administration may be due to the fact only small numbers were present to begin with but these multiplied rapidly after competition was lessened.

TABLE VII-a.

COMPARISON OF COUNTS JUST PRIOR TO AND TWO DAYS AFTER BATHING WITH 0.1 PERCENT PERACETIC ACID

Animal No. 119	Prewash Counts		Post-Wash and After Two-Day Counts	
Body Sites	Aerobic	Anaerobic	Aerobic	Anaerobic
Axilla	3×10^{6}	5×10^{6}	1.2×10^{6}	negligible at 10^{5} dilution
Groin	1.0×10^{7}	1.8×10^{7}	1.5×10^{7}	1.45×10^{7}

It will be noted that no permanent change in total count was observed in the groin area, however, Pseudomonas is no longer isolated from the animal. Loss of this microorganism which was considered the marker organism for body and fur decontamination indicates that other microorganisms also were eliminated. Recontamination is from fecal sources or from sub-surface skin sites. The presence of subcutaneous microorganisms has not been demonstrated for <u>Macaca mulatta.</u>

TABLE VIII.

RESULTS OF SENSI-DISC TESTS ON FECAL FLORA

Antibiotic	Units Tested	Relative Results
Streptomycin	2 mcg	
Penicillin	2 units	
Colymycin	2 mcg	
Kynex	0.25 mg	
Tetracycline	5 mcg	
Kantrex	5 mcg	
Neomycin	5 mcg	
Chloromyatin	5 mcg	
Polymyxin	50 units	

TABLE IX.

SUMMARY OF AVERAGE COUNTS BEFORE AND AFTER A-CLOXACILLIN; B-PERACETIC ACID BATHING; C-NEOMYCIN

Animal		Feces A Aerobe	Feces A Anaerobe	Feces C Aerobe	Feces C Anaerobe	Conjunctiva B Aerobe	Conjunctiva B Anaerobe
Animal No. 116	Before	3.9×10^8	3.1×10^8			2.0×10^6	3.5×10^6
	After	3.3×10^9	6.6×10^9			1.2×10^7	2.3×10^7
		Throat B Aerobe	Throat B Anaerobe	Gingiva B Aerobe	Gingiva B Anaerobe	Axilla B Aerobe	Axilla B Anaerobe
	Before	3.5×10^8	7.2×10^8	2.5×10^8	1.0×10^7	1.2×10^7	3.5×10^6
	After	1.2×10^9	7.4×10^9	1.5×10^9	4.1×10^9	2.6×10^6	7.7×10^6
		Groin B Aerobe	Groin B Anaerobe	Glans Penis B Aerobe	Glans Penis B Anaerobe		
	Before	6.0×10^6	1.0×10^6	7.0×10^6	3.0×10^6		
	After	7.5×10^7	2.4×10^8	9.5×10^7	1.4×10^8		
		Feces A Aerobe	Feces A Anaerobe	Feces C Aerobe	Feces C Anaerobe	Conjunctiva B Aerobe	Conjunctiva B Anaerobe
Animal No. 119	Before	2.0×10^8	2.0×10^8	1.6×10^9	1.7×10^{10}	1.2×10^5	1.6×10^5
	After	2.9×10^8	4.7×10^8	6.0×10^3	1.4×10^8	< 1000	6.0×10^3
		Throat B Aerobe	Throat B Anaerobe	Gingiva B Aerobe	Gingiva B Anaerobe	Axilla B Aerobe	Axilla B Anaerobe
	Before	2.7×10^5	7.2×10^5	5.3×10^6	$>3.0 \times 10^6$	3.0×10^4	5.0×10^4
	After	2.0×10^5	4.0×10^5	2.2×10^6	1.3×10^7	< 1000	< 1000
		Groin B Aerobe	Groin B Anaerobe	Glans Penis B Aerobe	Glans Penis B Anaerobe		
	Before	7.5×10^5	9.0×10^5	9.2×10^5	3.7×10^5		
	After	9.4×10^4	1.3×10^5	1.7×10^6	2.2×10^6		

TABLE IX.

SUMMARY OF AVERAGE COUNTS BEFORE AND AFTER A-CLOXACILLIN; B-PERACETIC ACID BATHING; C-NEOMYCIN (Cont)

Animal No. 120		Feces A		Feces C		Feces B	
		Aerobe	Anaerobe	Aerobe	Anaerobe	Aerobe	Anaerobe
	Before	7.0×10^7	9.0×10^7	2.4×10^7	4.3×10^7	2.7×10^4	1.4×10^5
	After	2.4×10^7	4.3×10^7	1.0×10^4	1.1×10^8	2.6×10^4	2.2×10^4
		Throat B		Gingiva B		Axilla B	
		Aerobe	Anaerobe	Aerobe	Anaerobe	Aerobe	Anaerobe
	Before	4.4×10^6	5.2×10^6	2.7×10^6	3.7×10^6	1.2×10^5	2.7×10^4
	After	5.1×10^6	6.9×10^6	6.2×10^6	4.1×10^6	2.0×10^4	< 1000
		Groin B		Glans Penis B			
		Aerobe	Anaerobe	Aerobe	Anaerobe		
	Before	3.7×10^5	2.2×10^4	2.4×10^5	1.3×10^5		
	After	1.4×10^5	1.4×10^5	1.4×10^4	1.0×10^4		

All reported counts were performed on blood Agar plates using the spread plate technique.

Lowest dilution used required 1000 microorganisms or more to read (10^3) reported as less than 1000, in the third serial dilution, no colonies were found.

a level too low for isolation by routine techniques. To prevent spread, Nystatin (700,000 units/day for three days) was administered. Shortly thereafter, feces samples from the affected animal did not exhibit Candida. The administration of Nystatin appeared to have no affect on the total numerical counts of microorganisms in the feces.

In that the total counts of anaerobic microorganisms did not change substantially after neomycin, which was contrary to all expectations, it was determined that initial dosages were insufficient to cause the changes desired. Following a stabilization period of one month, Tetrex F*, was administered for 14 days. Samplings of selected body sites and feces of the animals during the following restabilization show the predominant skin and body microflora to be Corynebacteria and Staphylococcus. Table X gives average numerical values for both aerobic and anaerobic microorganisms during this period. Tetrex F is a propietary combination of neomycin and Nystatin. Our laboratories are currently evaluating this antibiotic. The animals were rebathed in peracetic acid seven days after start of dosage. The dosage was 10 cc/animal/day**. The data is presented in Tables XI and XII.

*Furnished by J.P. Brochetti of Bristol Myers Laboratories
**Equivalents to 250 mg Tetracycline Hcl and 250,000 units of Nystatin

TABLE X.

NUMERICAL COUNTS OF SELECTED BODY AREAS*

Conjunctiva

Animal No.	Atmosphere	Average Mixed Count Blood Agar	Average Corynebacteria Muller Serum Tellurite	Average Staphylococcus Mannitol Salts
115 (Control)	Aerobic	1.80×10^6	1.5×10^6	1.08×10^6
	Anaerobic	1.45×10^6	---	---
116	Aerobic	1.22×10^6	9.7×10^5	1.74×10^6
	Anaerobic	1.67×10^6	---	---
118	Aerobic	1.80×10^6	1.54×10^6	2.16×10^6
	Anaerobic	4.86×10^7	---	---
119	Aerobic	1.32×10^6	1.41×10^6	2.04×10^6
	Anaerobic	2.52×10^6	---	---
120	Aerobic	6.56×10^6	4.07×10^6	Not Determined
	Anaerobic	1.32×10^7	---	---
121 (Control)	Aerobic	5.2×10^5	3.7×10^5	1.89×10^5
	Anaerobic	4.3×10^5	---	---

Throat

Animal No.	Atmosphere	Average Mixed Count Blood Agar	Average Corynebacteria Muller Serum Tellurite	Average Staphylococcus Mannitol Salts
115 (Control)	Aerobic	1.00×10^8	3.9×10^6	2×10^4
	Anaerobic	1.88×10^7	---	---
116	Aerobic	2.10×10^8	2.10×10^8	6.8×10^4
	Anaerobic	2.94×10^8	---	---
118	Aerobic	4.86×10^8	3.88×10^7	1.5×10^4
	Anaerobic	4.86×10^8	---	---
119	Aerobic	4.37×10^8	4.98×10^7	7×10^3
	Anaerobic	4.32×10^8	---	---
120	Aerobic	5.94×10^7	3.66×10^7	Not Determined
	Anaerobic	1.02×10^7	---	---
121 (Control)	Aerobic	1.22×10^9	8.9×10^6	1.74×10^7
	Anaerobic	4.30×10^8	---	---

*From 25 May to 24 June 1967

TABLE X.

NUMERICAL COUNTS OF SELECTED BODY AREAS* (Cont)

Gingiva

Animal No.	Atmosphere	Average Mixed Count Blood Agar	Average Corynebacteria Muller Serum Tellurite	Average Staphlococcus Mannitol Salts
115 (Control)	Aerobic	2.09×10^7	$3. \times 10^5$	3.06×10^6
	Anaerobic	1.62×10^8	---	---
116	Aerobic	7.29×10^8	4.18×10^7	1.64×10^6
	Anaerobic	4.86×10^8	---	---
118	Aerobic	4.86×10^8	3.87×10^7	2.5×10^4
	Anaerobic	7.02×10^8	---	---
119	Aerobic	6.48×10^8**	4.41×10^7	4.1×10^4
	Anaerobic	over-run Pseudomonas 5th serial dilu.	---	---
120	Aerobic	4.59×10^8	7.70×10^7	Not Determined
	Anaerobic	1.32×10^8	---	---
121 (Control)	Aerobic	3.82×10^7	3.7×10^7	2.30×10^6
	Anaerobic	2.30×10^7	---	---

Axilla

Animal No.	Atmosphere	Average Mixed Count Blood Agar	Average Corynebacteria Mueller Serum Tellurite	Average Staphlococcus Mannitol Salts
115 (Control)	Aerobic	1.80×10^7	1.16×10^7	Not Determined
	Anaerobic	8.7×10^6	---	---
116	Aerobic	2.11×10^6	4.03×10^6	3.34×10^6
	Anaerobic	2.59×10^6	---	---
118	Aerobic	2.1×10^4	8×10^3	1.2×10^4
	Anaerobic	1.1×10^5	---	---
119	Aerobic	2×10^3	5×10^3	6×10^3
	Anaerobic	4×10^3	---	---
120	Aerobic	2.67×10^5	1.93×10^5	Not Determined
	Anaerobic	1.20×10^5	---	---
121 (Control)	Aerobic	6×10^5	5×10^5	9×10^4
	Anaerobic	10^6	---	---

*From 15 May to 24 June 1967

**Pseudomonas Present

TABLE X.

NUMERICAL COUNTS OF SELECTED BODY AREAS* (Cont)

Groin

Animal No.	Atmosphere	Average Mixed Count Blood Agar	Average Corynebacteria Muller Serum Tellurite	Average Staphlococcus Mannitol Salts
115	Aerobic	10^5	No growth at 10^{-5} dilu.	No growth at 10^{-5} dilu.
(Control)	Anaerobic	10^5	---	---
116	Aerobic	1.72×10^6	7.3×10^5	1.10×10^6
	Anaerobic	2.39×10^6	---	---
118	Aerobic	1.77×10^6	1.13×10^6	1.63×10^5
	Anaerobic	2.64×10^6	---	---
119	Aerobic	5.31×10^6	3.33×10^6	4.2×10^5
	Anaerobic	2.35×10^6	---	---
120	Aerobic	1.96×10^6	10^6	Not Determined
	Anaerobic	1.28×10^7	---	---
121	Aerobic	7.7×10^7	5×10^5	3.12×10^7
(Control)	Anaerobic	6.0×10^7	---	---

Glans Penis

Animal No.	Atmosphere	Average Mixed Count Blood Agar	Average Corynebacteria Muller Serum Tellurite	Average Staphlococcus Mannitol Salts
115	Aerobic	3.92×10^8	1.36×10^7	Not Determined
(Control)	Anaerobic	4.50×10^8	---	---
116	Aerobic	3.64×10^7	3.46×10^7	7.53×10^6
	Anaerobic	1.63×10^7	---	---
118	Aerobic	4.86×10^8	9.8×10^5	7.89×10^6
	Anaerobic	7.02×10^8	---	---
119	Aerobic	4.05×10^7	3.8×10^5	5.22×10^7
	Anaerobic	4.32×10^7	---	---
120	Aerobic	9.30×10^7	1.28×10^7	Not Determined
	Anaerobic	over-run probably Proteus	---	---
121	Aerobic	1.16×10^8	1.1×10^6	4.20×10^7
	Anaerobic	2.54×10^8	---	---

*From 15 May to 24 June 1967

TABLE X.

NUMERICAL COUNTS OF SELECTED BODY AREAS* (Cont)

Feces

Animal No.	Atmosphere	Average Mixed Count Blood Agar
116	Aerobic	1.19×10^8
	Anaerobic	1.24×10^8
119	Aerobic	1.30×10^8
	Anaerobic	1.08×10^8

*From 15 May to 24 June 1967

TABLE XI.

TOTAL BODY COUNTS FOLLOWING TETREX F ADMINISTRATION AND BATHING WITH PERACETIC ACID SOLUTION

	Area	Animal No. 118			Animal No. 119			Animal No. 121		
		Blood Agar	Mueller Tellurite	Mannitol Salts	Blood Agar	Mueller Tellurite	Mannitol Salts	Blood Agar	Mueller Tellurite	Mannitol Salts
Aerobic Counts	(1) Conjunctiva	4.92×10^6	2.11×10^6	7.28×10^6	5.0×10^3	No growth 10-3	1.0×10^3	1.3×10^4	4×10^3	3×10^3
	(2) Throat	2.90×10^7	1.4×10^4	5.1×10^7	5.52×10^7	1.17×10^7	Ng 10^{-3}	1.08×10^9	1.86×10^7	2×10^4
	(3) Gingiva	5.1×10^7	1.8×10^7	9.4×10^4	6.48×10^7	3.92×10^6	Ng 10^{-3}	5.0×10^8	2.31×10^7	1×10^3
	(4) Axilla	1.0×10^4	2.0×10^3	1.0×10^3	Ng 10^{-3}	Ng 10^{-3}	1×10^3	2.1×10^4	1.6×10^4	1.3×10^4
	(5) Groin	7.5×10^6	6.0×10^3	3.1×10^4	1.80×10^6	4.5×10^5	1.33×10^6	3.3×10^6	2.33×10^5	2.01×10^6
	(6) Glans Penis	3.72×10^7	1.8×10^5	2.94×10^7	1.14×10^7	3.72×10^6	2.4×10^7	1.40×10^7	5.2×10^4	8.2×10^6
Anaerobes	(1) Conjunctiva	4.80×10^6			3×10^3			1.9×10^4		
	(2) Throat	2.22×10^7			1.14×10^7			1.2×10^9		
	(3) Gingiva	1.38×10^7			1.86×10^7			7.8×10^8		
	(4) Axilla	1.4×10^4			$1 \quad 10^3$			1.5×10^4		
	(5) Groin	1.38×10^7			1.8×10^6			3.2×10^6		
	(6) Glans Penis	7.8×10^6			6.48×10^7			4.2×10^6		

Animal Nos. 118, 119 and 121 were bathed in 0.1 percent peracetic acid by total immersion for approximately 15 seconds. They were sampled on the various body areas approximately 24 hours following this bath. Animal Nos. 118 and 119 are both in isolation and received a daily dosage of 250 mg tetracycline hydrochloride and 250,000 units Nystatin for seven days prior to the bath. Animal No. 121 is in an open cage with full exposure to the normal atmosphere and received no antibiotic. Counts were performed on Blood Agar aerobically and anaerobically from all sites. Also counts were performed on Mueller Tellurite (specific for corynebacteria) agar and Mannitol salts (specific for straphylococci) agar aerobically.

TABLE XII.

FECES COUNTS FOLLOWING AND DURING TETREX F ADMINISTRATION

Aerobic Counts - Animal No. 116

Sampling Day	Blood Agar	Mueller Tellurite	Eosin Methylene Blue
1	3.7×10^7	1.3×10^6	2.2×10^6
3	1.0×10^8	1.5×10^8	2.7×10^6
5	3.6×10^7	4.1×10^7	4.3×10^6
7	4.9×10^7	1.1×10^8	2.2×10^7
9	4.2×10^7	2.8×10^7	1.1×10^7
11	1.7×10^6	1.2×10^5	2.2×10^7
Anaerobes			
1	1.0×10^8		
3	7.7×10^7		
5	3.8×10^8		
7	1.9×10^8		
9	8.4×10^7		
11	1.2×10^8		

The colonies on the EMB plates were atypical of Coliform organisms. There appears to be an initial upsurge of aerobic growth with a very gradual reduction following. This might be due to the slow build up of residual antibiotic action whereby a small amount initially acts as a stimulus.

The aerobic Blood Agar plates from samples taken 9 July 67 and 11 July 67 had a spreader which is probably proteus. This may have an effect of masking some colonies thus the counts could conceivably be higher. First samples started 18 hours after administration of antibiotics.

TABLE XII.

FECES COUNTS FOLLOWING AND DURING TETREX F ADMINISTRATION (Cont)

Aerobic Counts - Animal No. 118

Sampling Day	Blood Agar	Mueller Tellurite	Eosin Methylene Blue
1	1.5×10^6	No growth 10^{-4}	1×10^4
3	2.6×10^7	1×10^4	Ng 10^{-4}
5	2.91×10^7	Ng 10^{-4}	4.7×10^6
7	8.0×10^6	Ng 10^{-4}	1.3×10^5
9	1.3×10^6	Ng 10^{-4}	Ng 10^{-4}
11	9.8×10^6	Ng 10^{-4}	1×10^4
	Anaerobes		
1	5.0×10^6		
3	3.8×10^7		
5	3.8×10^7		
7	3.3×10^7		
9	5.6×10^7		
11	2.5×10^7		

The colonies on the EMB plates were typical of Escherichia sp.

TABLE XII.

FECES COUNTS FOLLOWING AND DURING TETREX F ADMINISTRATION (Cont)

Aerobic Counts - Animal No. 119

Sampling Day	Blood Agar	Mueller Tellurite	Eosin Methylene Blue
1	2.8×10^{7}	No growth 10^{-4}	Ng 10^{-4}
3	1.2×10^{7}	6×10^{5}	2.5×10^{6}
5	4.8×10^{7}	4.8×10^{5}	2.8×10^{6}
7	3.3×10^{7}	3.5×10^{5}	3.5×10^{6}
9	1.4×10^{8}	3.6×10^{5}	3.6×10^{7}
11	4.6×10^{7}	4×10^{4}	3.1×10^{7}
	Anaerobes		
1	3.3×10^{7}		
3	7.1×10^{7}		
5	1.7×10^{8}		
7	1.2×10^{8}		
9	2.0×10^{8}		
11	1.4×10^{8}		

The EMB colonies were atypical. All of the aerobic blood agar plates after 1 July 67 had a spreader believed to be proteus, which may have a masking effect. This is unavoidable by the spread plate technique used.

SECTION III
DISCUSSION

A. BACKGROUND

"Medical microbiology", writes Dubos (1967), "has concerned itself primarily with the potentially pathogenic members of the indigenous microbiota. Yet, the symbiotic species are of at least equal importance because.....they are essential to the well being of their host." The differentiation of "potential pathogens" from other organisms is hardly a cause for quarrel with Dubos, in that he has also stressed the demarcation between pathogen and nonpathogens is frequently dependent upon the physiologic condition of the host. His thesis that the autochthonous bacterial flora have achieved a semi-symbiotic status with the mammalian species through a long period of evolutionary association--as compared with those of more recent association which possess various degrees of pathogenicity concerns us only to the extent that in the context of space flight--any new association of microflora and astronaut will probably be detrimental to the astronaut.

It is known that some microorganisms commonly thought of as nonpathogens and certainly in the class of those considered common to life and major contributors to the gastrointestinal balance, can be lethal to species other than their normal host. Often "strange" microorganisms cannot be established and in other cases establishment causes unexpected effects both good and bad for the host. To the concept of microfloral balance must be added species specificity. Individual susceptibility has long been recognized and is probably present in many cases not reported simply because of temporary conditions that prohibit establishment of the new species in the host. An area of disagreement among bacteriologists studying the gastro-intestinal microflora is the stability of the microflora population in the host healthy state. Some claim there is approximately constant composition and others hold the position that the microfloral population is anything but constant. Attempts to get beyond the semantics, species variation and exact definitions reveal more the regions of agreement than disagreement. For normal humans, it is generally agreed that the total numbers of microorganisms are approximately constant (except for the cycling phenomena described by Prince and Gall (1966) and the possibilities for temporarily altering the normal state are many and can be rapidly demonstrated.

Physiological disturbances, drugs, environmental changes and foods are among the factors which have been shown to result in an altered state. One form of altered state is, of course, imbalance, where imbalance is thought of as predominance of one or few forms of micro-organisms in the gut. Although seemingly perfectly healthy animals have been shown to have intestinal microflora of only one or two bacteria, Luckey (1963) in his monograph Germ Free Life and Gnotobiology describes many instance of this observed by various workers. Generally these examples are gnotobiotes that have been germfree and have become contaminated by accident or design. The bodily defenses and the physiology of the these gnotobiotes is only superficially the same as conventional animals--every day the literature reports more differences between germfree animals and conventional animals. Table XIII from Luckey,

TABLE XIII.

SUMMARY OF THE DEVELOPMENT OF POTENTIAL "ANTIMICROBIAL DEFENSE SYSTEMS" IN GERMFREE ANIMALS*

Good	Intermediate	Little or None
Skin	Mucosa of Gastrointestinal	Cilia and squamous epithelium in sinuses
Thymus	Liver	
Bursa Fabricius		Sinus and nasal lymphatic tissue
Spleen	Spleen lymphopoiesis	
	Spleen secondary reaction centers	Tissue secondary reaction centers
	Plasma cells	Guinea pig plasma cells
Tissue monocytes, basophils, eosinophils, and heterophils	Tissue reticuloendothelial cells	Guinea pig plasms cells
Periodic vaginal exudat-ion, inflammation, and neutrophil infiltration	Tissue exudation (in cuts)	
Lymphocytes production	Lymph nodes (number and size) Lumphocytes in lymph nodes Circulating lymphocytes	
Serum heterophils	Serum monocytes	
Serum α and β globulins	Serum γ and α-globulins	
Heterochemagglutinins	Antibacterial agglutinins	Dietary protein antibodies
Antibody production system complement	Properidin	Foreign Tissue antibody production. Serum bactericidal activity
Block clearing mechanism "Shock" defense system Defecation	Reparative processes	Phagocytosis Blood vessel formation in new tissue

*Luckey, T. D., "Germfree Life and Gnotobiology" Academic Press, N. Y. N. Y., 1963. Copyrighted. Reprinted with permission of publisher.

is illustrative of some of the differences in defense mechanisms. Many other abnormalities have been reported, Sprintz (1962), Wostman (1959), Miyakawa (1958), Tanami (1959).

Early experiments by Reyniers and his Lobund group have shown that simplification of intestinal flora occurs when an animal is subjected to a sterile air, sterile food and water regime, Reyniers (1946). This change from the normal environment has been discussed at length by Bengson and Thomae (1965, Luckey (1966) and Reyniers (1946).

It appears that the extent of simplification and the speed at which it occurs are dependent upon a number of variables. It has been estimated, Gordon (1967), that the number of individuals involved in a group under the sterile conditions would probably have to be less than 12 and perhaps less than 6. With this we concur. The number of individuals in the group may be the prime factor for all practical purposes in a situation that is not drug altered. Previous experiments in our laboratory, using rats, have demonstrated simplification under sterile air, sterile food, and water regimes, with six individuals. The size of the animals, the bacterial load, the health, the variety of species and perhaps very importantly, in light of the experience cited, the compatibility of the individual hosts to the varied burden. In the Lobund experiments, the rats died while the controls, living under normal conditions, survived, Reyniers (1946).

Very little is known about the body and cellular changes that occur when simplification or alteration of flora is evidenced. Microbial shock has been postulated as a consequence of simplification, long term isolation and reintroduction of species, Luckey (1966). New, to the particular host animal, species of microflora may cause infection, Tanami (1966), with severity beyond that to be expected as a result of previous host (species) experience with the microorganism.

Phillips (1966), Seelig (1966), Altemeier (1963), Andriole (1962) and Dubos (1966), have described numerous cases of rampant invasions by normally "harmless" microorganisms following drug therapy. The lethality, of such secondary invasions has been amply demonstrated. In our experiments with the rhesus, we were constantly on the alert for such secondary invasions, in particular those caused by Staphlococci and Candida.

Our choice of primates was dictated by the knowledge accumulated on the rhesus. The compatibility question was side stepped, at least during the early stages of the experiment, by the dose association and pairings. The animals did differ in their indigenous microflora. It is to be doubted if in the normal state, whatever that may be, that any two animals would ever be exactly alike as to microfloral species throughout the entire body and as for numbers, the concept of gnotobiotic exactness is patently ridiculous except in the case of microorganisms equalling zero. The shifting of the populations of the gut, cycling, appears a normal sequence of life. Sufficient identities of species were found among the subject animals to provide what we considered reliable marker guides as to the numerical changes of the gut flora. The isolation of the individual subjects then could reasonably be expected to produce simplification if carried out for a sufficient period of time, Reyniers (1946); Bengson (1965); Luckey (1966); and Morillo (1964). The sterile food diet alone could certainly induce alteration of numbers if not species, Gibbons (1964). For the above reasons, an antibiotic administration program as described previously in the historical section, was begun in order to achieve as common a microflora as possible in as short a time as possible. While at first though, changing the microflora by removing the same organisms from all the subjects is not the way to a common flora, yet it becomes readily apparent that a series of antibiotics, in this case Cloxocillin, Neomycin and Nystatin not only removes certain of the commonalities but also removes many susceptible microorganisms that are peculiar to individual animals. Reinoculation of desired bacterial species--desired here, and referring to what we wanted, not necessarily what was good for the animal--is one way to a very similar if not an exactly common microflora.

We found that regardless of the antibiotic administered, that a new (resistant) population grew to fill the void. In very short times, the numerical population was close to the original numbers found in the gut. The alteration of the flora, internal or external, is fraught with problems. In addition to the types of secondary invasions we were alerted for, the Pseudomonas spread almost brought the experiments to a premature close. Our ability to decontaminate the animals exceeds that practical for human astronauts. The peracetic acid bath is not liable to be a tolerated feature, even among volunteers. As noted, following peracetic acid bathing, and after a seeming absence of several months, Pseudomonas infection reoccurred. In that the re-infection was not universal, to the experiment group, it must have been harbored within the animal (No. 119) or on his fur or somewhere in or on the isolator.

The isolators were scrubbed and sterilized by peracetic acid daily. No leaks were detected in the isolator before or after infection, and Pseudomonas is not common within the laboratory. The only reasonable conclusion is that the animal was always internally contaminated by Pseudomonas, original source unknown, and that the number of Pseudomonas organisms was very small. Less certain, but at least reasonable, is the assumption that the Pseudomonas was dormant, non-virulent, or held in check by other microorganisms in the population and that the alteration of the population enabled its resurgence.

The validity of comparing infra-human primate data with humans is always a question yet the question must be raised. Can the same thing happen to the astronaut?

It probably is impossible to sterilize an individual human. The work by Uhlrich (1966) describing subcutaneous microflora, the studies of the sterilization groups working out of the NASA Office of Planetary Quarantine and reports of many other bacteriologists, have shown that surface decontamination of humans is possible only for an extremely short time. Studies on burn patients and the infections of the burn sites, even under isolation, indicate the body is a vast reservoir of probably non-reachable bacteria.

With animals, a different story seems possible. Vander Waaij (1967) of Holland has reported obtaining germ-free mice by antibiotic therapy. Mouse skin and human skin are so different that a comparison seems futile. Monkey skin appears like mouse skin to the extent that the peracetic acid baths did permanently, at least while in the isolator, change the flora. The Corynebacteria and the fur and skin population seemed to originate from the feces following the bath. If the bath had merely removed the surface indigenous population and the subsurface sites had renewed the population, the general nature of the species isolated would have been similar to the original findings. As shown by Table VII, such was not the case.

It is doubtful if responsible scientists would, at this stage of knowledge, call for a sterile astronaut, were this possible. The microbic shock Luckey mentioned, alone would deter such a proposal. In the primates studies, we continually found that the supposed static conditions were not static. To achieve a static state, we must go to the zero condition mentioned afore. The diet fed, Appendix III, was adequate as evidenced by the continued good health of the subjects and the controls. Yet the diet must have exerted some effect on the bacterial population and thus affected the fungi and other physiological parameters. The effect of diet, on the "normal" animal and human has had some work performed but certainly only enough to show intriguing possibilities for microbial population control, Gall and Riely (1967); Thomae (1967); Lyght (1966); Gall (1964); Reyniers (1946); Porter (1940); and Winitz (1966).

The survival of human skin populations, following decontamination by washing, has been studied by Uhlrich (1966); Skinefield (1965); Evans (1950); and Updegraff (1964). The general findings of these investigations, that normal levels of bacterial population may be increased temporarily by bathing or showering and decreased by use of germicidal detergents, supports Uhlrich's contention that human skin has a built in control mechanism which maintains levels of population peculiar to each individual. The mechanism of the control is unknown but is effected by residual action of such decontaminants as hexachloraphene when long term use of the chemical has been shown. This has pertinance to the astronaut hygiene problem. Until enough experiments have been done and data correlated between animal skin and human skin, the decontamination of monkey skin in our experiments can only be cited as indicative of what could happen to surface populations. Litsky and Litsky have evaluated single bar soaps, Litsky and Litsky (1967). They have confirmed and extended Uhlrich's work. In addition to the residual action of hexachloraphene type soaps, they have evaluated new products and reported substantial improvement in reducing microbial populations, with greatly extended times for population recovery.

This work has important connotations. The possibility of developing products to further extend the recovery time is encouraging. The repopulation of the monkey skin surfaces, from sources other than skin bacteria, may thus have validity, in the consideration of an astronaut hygienic problem. Yet, if it should be possible to reduce the normal body surface microflora to a very low level by extended use of efficient bacterial soaps, then resistant bacteria and fungi from other sources may develop into the predominant skin microorganisms. Monkey 119 was affected by the rapidly spreading Pseudomonas infection, was washed with a hexachloraphene product. Little if any effect was noted on the Pseudomonas. Tests with other common decontaminating agents were equally ineffective. We resorted to the 1/10% peracetic acid bath to eliminate the infection. Luckey has used 1/10% peracetic acid solution routinely to decontaminate his own hands following laboratory work involving pathogens. He has not reported ill effects from this relatively harsh treatment but it must be carefully considered that this was not a continuous daily or even frequent procedure. Skin sensitivity, like bacterial populations, varies from person to person. As an emergency measure, however, this treatment should be considered. Also to be taken into account is the fact that the very condition, abnormally low population of the normal microflora because of the decontaminant process, may have led to the infection susceptibility originally. This situation will be repeated as long as the atmosphere and cabin contains decontaminant-resistant pathogens. A case for pre-flight cabin sterilization is thus presented. This is an area that must be investigated before long flights. Gnotobiotic study of the spacecraft defined here as "knowledge of living organisms aboard the spacecraft" then may be required. The term gnotobiotic does not limit the number of species or the numerical value of the count.

SECTION IV

SAMPLING, CODING, RETRIEVAL AND ANALYSIS OF MICROBIOLOGICAL DATA

A. GENERAL

It has become apparent that the ultimate utility of the study results is dependent upon the statistical reliability of the sampling procedures and the ease with which the data can be handled and compared with that obtained from isolation and confinement tests on man and other animals.

The computer facilities of the Missile and Space Division, Valley Forge Space Technology Center, including the General Electric proprietary Desk Side Computer System (DSCS) have been employed in a program to develop accurate and readily usable procedures to handle data generated during the experimentation. The methodology is available for application to past, present, and future experiments dealing with the isolation and confinement aspects of manned space flight.

Historically, the way in which biologists have attempted to develop the theories and practice regarding the maintenance of man in good physiological condition, is to use lower animals as test subjects. As animals higher in the phylogenetic scale are used, the opportunity increases to extrapolate experimental results and apply them to man.

The contract, "Research on the Effects of Alteration of the Indigenous Microflora of the Monkey" will, it is hoped, furnish guidelines for the control and management of the indigenous microflora of space vehicle crews. By using monkeys, greater risks can be taken; in this instance, confinement for several months and then exposure to a normal "classic" animal. The simplest possible experimental design which met the contractual requirements resulted in large amounts of raw data, thus celerity in data reduction is necessary. The data obtained during the course of experimental work was suitably coded for retrieval and analysis.

B. REQUIREMENTS

In order for the data to be accurate and precise, they must be obtained by the use of carefully controlled standardized procedures; in order to be useful, they must be subjected to rigorous statistical procedures, such as T-testing, analyses of variance, regression analyses, etc. Before large volumes of data can be handled efficiently, however, it is necessary to translate the experimental results into computer language. Then as experimental work progresses, data can be stored and periodically analyzed.

Unfortunately, much biological data does not lend itself to precise quantitation. Because of the judgment factor, each type of data has to be considered separately, on the basis of its susceptibility to expression in precise terms. For example, motility is an attribute present in some microorganisms and absent in others. The presence or absence of this characteristic can be expressed easily. However, the morphology of a microorganism is not simply rod, sphere or spiral. If morphological information were important, it would be necessary to actually measure a sample of each organism isolated and establish size limits.

After a codification procedure was established, the data was stored so that retrieval of the information could be made in a manner which answers the specific questions of an investigator. Such a data retrieval system is now working at the Valley Forge Space Technology Center, for use with the General Electric proprietary remote time-sharing computer system to answer inquiries from anywhere in the United States about documents, piece parts and program items. The real problem in utilization of such retrieval systems for the monkey flora data handling is in the proper and complete indexing of the test results.

Available programming techniques and data storage capacity resulting from the DSCS have been used in this study to evaluate the microbial data that is being collected in the model (monkey) manned space flight experiments. The basis of this computerized system is a microbial data file which contains a series of indexed, coded, data records. Each record contains all the essential information that is generated from the samples that are taken from a particular animal at one time. The data file resides in an auxiliary memory area (magnetic disc) that can be randomly accessed by a computer.

The data are arranged in such a manner that analyses can be performed from virtually any standpoint that relates to the data. Furthermore, the capability exists to perform these analyses from a remote location. Therefore, it is possible for someone in a remote location using a teletype terminal to access the computer system (facility) which contains the data file, and request that a particular analysis be performed. The computer system then performs the analysis and communicates the results back to the remotely located terminal.

In general, to establish an information system of this type, four phases of activity must be considered:

- Data Gathering - The collecting of data by mechanical, electrical or human means.
- Data Organization - The arrangement of data into a workable form and storage of the data in a computer system in a compact, readily accessible form.
- Data Processing - Electronic manipulation of the data in order to provide the required information.
- Data Communication - The transmission of the data from the source to the computer and the transmission of the ordered data or analytical results from the computer to the user.

Each of these four steps, as they relate to this particular task, is briefly explained in the following paragraphs.

1. DATA GATHERING

Data Gathering, or data collection, involving the microbial populations of six male, rhesus monkeys, has been in process for approximately nine months.

The following information was collected each time samples were made:

a. The animal that was sampled

b. The date and time when the sample was made

c. Temperature, pressure and humidity at the time of the sample, if different than normal, ambient environment

d. Antibiotics, if any, that were used since the last sample

e. Antiseptic washes, if any, that were used since the last sample

f. Type diet (name) and whether sterile or non-sterile

g. Whether or not the animal was in isolation

h. Length of time in isolation

i. Whether or not other animals were present (which ones, how long)

j. The classes of organisms and genera that were found at each body site that was sampled

2. DATA ORGANIZATION

Data Organization was done in the following manner. Each time an animal was sampled, a physical record was generated on a source document similar in form to the one shown in Figure 4. (Actual examples of forms used are shown in Appendix V, Figures 22 and 23.) From the source document (physical record) computer records of coded information were generated. This task involved developing a computer program which accepts as input the coded and empirical information from the source document, and "loads" this information into an auxiliary computer memory area for permanent storage. As a result of this process, a computerized microbial data file which contains a series of indexed, coded, data records was created.

Figure 4, the coding format, was derived from the following questions and answers:

1. Q. What is the maximum possible number of body sites?

   A. 15

   Q. What number of body sites is presently used?

   A. 7

Measurement Number *****

Animal Sampled **

Date **/**/** Time ****

CONDITIONS

Temp **°C Hum **% Pres *** mm of Hg

Isolation * (yes/no) Time in Isolation *** days

Diet * (Sterile or Non-sterile)

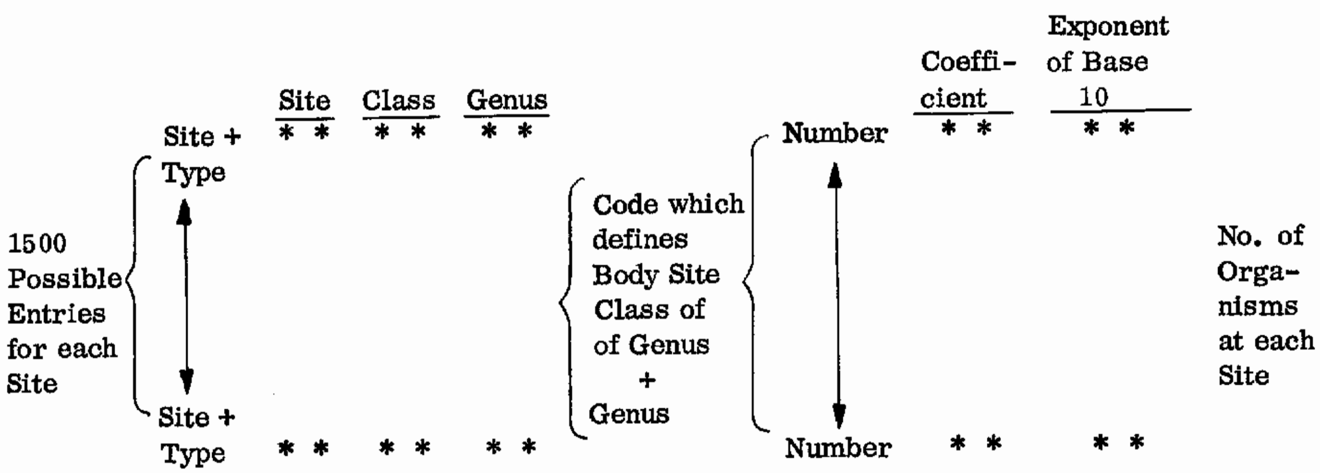
Diet Name *

Organisms Ingested ******

Other Animals Present * (Number)

Which One * How Long *** (Days)
Which One * How Long ***
Which One * How Long ***
Which One * How Long ***

ORGANISMS



* = 1 Character of Code

Figure 4. Record Identification Information

Q. What is the location of these seven sites?

A. Feces (considered a body site for coding purposes)

Conjunctiva

Throat

Gingiva

Axilla

Groin

Glans Penis

2. Q. How many different kinds (kind was defined as taxonomic class) of organisms have been found?

A. 3

Q. What is the maximum number considered possible?

A. 15

Q. How many different genera have been found?

A. 19

Q. What is the maximum possible number of genera?

A. 100

3. Q. How many antibiotics and combinations thereof have been used?

A. (1) Tegopen (Sodium Cloxacillin)

(2) Mycifradin Sulfate (Neomycin Sulfate)

(3) Nystatin

(4) Tetrex-F (Tetracycline and Nystatin)

(5) Chloramphenicol and Furoxone

Q. What is the maximum number of antibiotics and combinations thereof anticipated?

A. 15

Q. How many different antiseptic washes have been used?

A. (1) Peracetic acid (0.1%)

Q. What is the maximum number of different antiseptic washes that could be used?

A. 5

4. Q. What is the number of days since the start of the experiment and how long have the animals been in isolation?

A.

Animal No.	115	116	118	119	120	121
Arrival at GE	303	303	303	303	303	303
Sampling Begun	151	176	135	214	190	214
Placing in Isolation	None	188	127	197	188	None

Q. What was the sampling rate?

A. Nominal 2/week

Range 0–5 times/week

Q. What is the number of animals in the experiment?

A. In isolation (4) }
In cage colony (2) } (6)

5. Q. What are the environmental conditions?

A. The assumption is made that temperature, relative humidity and barometric pressure is constant

Temperature: $70^{\circ}F \pm 5^{\circ}F$

R.H.: $50\% \pm 5\%$

(Note: Pressure is higher in isolators than in lab colony at constant ΔP)

6. Q. What number of measurements has been made to date?

(One measurement defined as all sampling made on one animal in a single day)

A. Total number to date: 79

7. Q. What number of changes has been made in the diet?

A. One control and one animal in isolation had their diets changed for a single period (one month) during the course of experiment. They were then placed back on the regular diet.

3. DATA PROCESSING

Data Processing required that computer programs be developed which could interact with the microbial data file and perform the following analyses:

a. The variation in numbers of organisms of various types on a given body site sampled at different times and between different body sites sampled at the same time.

b. The degree of transfer of organisms between subjects.

c. The bionomic interrelationships among organisms.

d. The effect of the presence or lack of biological isolation, antiseptic washes, antibiotics and other environmental factors on organisms.

e. Indigenous controlling or stabilizing effect that certain organisms have on the microbial population.

f. Exogenous stabilizing and controlling effects that certain organisms have on the microbial populations.

g. Extent of microbiological compatibility required for group confinement.

Programs were developed to perform analyses a. and d. above. They are also stored in auxiliary computer memory area (magnetic disc). Programs to perform the other analyses can be written when data is available, and similarly stored. As a result, when an analysis is required, an individual informs the computer system by code of the analyses he requires. See Appendix V for the sequence of events occurring when the real time computer system is contacted by teletype and a program is requested. The proper program is then located in the auxiliary memory, translated from source language (GE modified Fortran II) by a compiler into machine-object language, and stored ready for use in the computer's core memory. The computer then executes the program on command from the requestor. During the execution of the program, the computer's core memory interacts with the records in the Microbial Data File or Animal Log File specified by the Program. For other programs, data can be entered by hand on the remote teletype or by punched paper tape. A fourth alternate is the use of magnetic tape physically stored and accessible in the central computer, which has been previously written via the teletype or a punched card-to-tape reader. This interaction continued until all the instructions that make up the program had been executed to completion and the desired results obtained. These results were then available for transmission to the requestor.

Flexibility exists to modify the programs that are presently stored and to add entirely new programs if they are required. As a result, as the requirements and priorities for analysis change, the overall computerized system will have the capability to conform to these changes.

4. DATA COMMUNICATION

Data Communication has two channels.

From source to computer, is the channel through which information on the source documents (physical records) that has been collected has been converted to punched cards or punched tape. The card or tape information was then converted to magnetic tape and the tape information loaded into the auxiliary memory area, using standard batch processing techniques. As a result, the Microbial Data File and Animal Log File were created. From this point on, the capability exists to transmit file-update information to these files from a teletype terminal or from punch cards.

From computer to user is the channel through which, upon a user's request, printouts and analyses of these files are performed, and the resultant information is transmitted to the user, via a telephone line to a teletype terminal or by standard batch reports.

Having delineated the steps necessary to apply information retrieval techniques to microbiological data, a detailed examination of how the information was coded follows. Normally, in the conduct of any laboratory work, a laboratory notebook is used to record an experimental design, results, and other observations. If the experiment, such as this one on monkey flora, runs for any length of time, the amount of recorded data increases and laboratory notebooks for the one experiment begin to fill up shelves. When comparisons are made between the results obtained on two days (widely separated in time), an appreciable amount of time is spent fumbling through pages, looking for the data specifically needed. Furthermore, should a number of pieces of information be required, such as the numbers and kinds of organisms found at four body sites on two monkeys for twelve weeks, an inordinate amount of time is spent finding the data in notebooks, re-recording the data and then manually sorting and comparing some 200,000 pieces of information (2 monkeys x 2 samplings/week x 12 weeks x 4 body sites x total population at each site, e.g., 192 populations x 5 genera found at each site).

The structuring and coding of the data blank enables one to develop an insight into the experimental frame and the voids that exist in the data banks. These voids may then be filled by further experimentation and/or literature searches.

Much of the data collected in biological experiments is subjective and qualitative in nature. The field has not yet been developed to the point where all of the parameters of a biological system can be expressed in objective, precise, quantitative terms (i.e., numeric). Statistical analyses in depth are therefore difficult to make, inasmuch as certain of the inputs to a data bank are subjective and based on judgment.

The incidence or absence of species migration from one individual to another can be demonstrated through time-distribution plots for individuals, and possibly within groups. Additional information may be forthcoming from a specific treatment of the data, but cannot be predicted easily until the data are suitably coded and available for retrieval procedures.

Remaining variables, both qualitative and quantitative must be collected, sorted, grouped and compared in an effort to discover significant changes, effects and correlations. The most efficient methodology available, which can be used to accomplish these tasks, was that encompassed by information retrieval techniques.

5. EQUIPMENT USED

The General Electric Desk-Side Computer System (DSCS) is a remotely controlled, business and scientific data processing system. It comprises a standard tele-typewriter for communication via telephone lines to a General Electric DATANET-30 and a GE-235 computer at Valley Forge Space Technology Center (VFSTC), Pennsylvania. The programming technique used was a version of Fortran II - GE Card Fortran. Additional programming techniques are continually being developed for the system. Special programs may be stored on the random access disc at VFSTC.

The GE-DSCS is an ever-evolving system. Uses to date have included solution of engineering design problems, statistical analyses, library searches and a number of research applications.

C. HANDLING THE DATA

A format was determined which would arrange raw data from laboratory notebooks in a manner permitting the data to be readily placed in the computer. This involved the choice of a data cataloging method which would systematize the actual transfer of information from the laboratory notebook to a computer disc within a framework, flexible enough to accommodate changes in experimental design, yet distinctive enough to furnish a key to the stored data. This key is comprised of the following Record Identification Information:

a. Record Number

b. Animal Sampled

c. Date

d. Time

Record Number encompasses all data obtained from any one site on an animal on one day. Thus, if on one day one site on six animals or six sites on one animal are sampled, six measurements will have been made. With the Measurement Number as a key, all other pertinent information on one animal can be included in the record, both on the day the sampling was done and several days or weeks later as subcultures, counts and identifications of organisms are made.

The next heading on the record "Conditions" includes:

a. Temperature (^{o}C)

b. Relative humidity (percent RH)

c. Atmospheric pressure (mm Hg)

d. Isolation (Yes/No)

e. Time in isolation (days)

f. Other animals present (number) which one (animal number - how long (days) (Repeated for Number of Animals Present)

g. Diet (name) - (sterile or non-sterile)

h. Water (sterile or non-sterile)

i. Antiseptic Wash (name/no.)

j. Antibiotics (Name(s))

k. Organisms ingested

During this experiment, atmospheric conditions of temperature, pressure and humidity are essentially constant but should data on human chamber subjects at altitude be analyzed, this data would be of importance. The next items of interest concern the isolation (within a biological barrier) of the animals. Water and food sterility and type of food are also recorded.

Antiseptic washes used must be noted because of their effects on the skin flora. Antibiotics have been administered singly and in pairs. At this point, it did not seem necessary to include the time elapsed since the last administration of an antibiotic, but this could be calculated by the computer at a later time if subsequent iterations of one or more analyses showed that the information was significant. Affecting the floral population is the oral administration of organisms and this, too, can be noted.

Finally, the microorganisms themselves are listed by:

a. Body Site

b. Type (Class to which Genus belongs and Genus)

c. Number

Two problems arose: (1) How to classify the organisms of interest and (2) How inclusive the classification should be? The latter question arose because monkeys do have intestinal parasites such as worms and protozoa. By specifying on our purchase order that our monkeys would be free of worms and protozoa as well as TB and S&S, the animals received in our laboratories had no discernible signs of worms and protozoa. However, these fauna may have had an effect on the flora and possibly would be included in future data analyses.

The former problem is one of taxonomy. In the Plant Kingdom, the Class Bacteria and the Subphylum Fungi (or Division Mycota, depending on which authority is quoted) contain organisms of pertinence to this study. The Phylum Protozoa and possibly Phylum Nematoda in the Animal Kingdom also contain organisms of interest and, had they been found during the course of this experiment, would be included in data analyses even though they cannot be considered flora, but indeed, are fauna.

Because taxonomists do not seem to be able to agree on how to classify the Fungi, and certain of the Protozoa and Bacteria, a decision was made to categorize the organisms found in this study by Class and Genus. Regardless of whether there are four or nine classes of fungi, the coding system can be adapted to accept either. For the time being, we are assuming that Genera belonging to four Classes of Fungi and four Classes of Protozoa in addition to the Class Bacteria, may be found.

In order to size the disc storage capacity required, the number of characters utilized in the coding of the raw data had to be calculated. We were not dealing here with bits or "words" but with characters. In Figure 4, the number of characters required to code each item of information is shown by the number of asterisks. For example, time requires four characters. The number of characters needed for both Record Identification Information and Conditions can be determined with relative ease. The only place where the number of characters could increase significantly, is for Organisms Ingested since, if thirty different organisms were to be used, the number of characters required would be 120. Under "Organisms", sufficient storage capacity had to be allotted for the worst possible case, namely that at each body site every possible genus would be found. We have assumed, based on our own experience plus a literature search, that a maximum of 100 genera belonging to a maximum of 15 taxonomic classes could be found. Thus far, we have sampled at seven body sites (for coding purposes, a feces sample is considered to be a body site sample), and are assuming that not more than 15 different sites will be utilized.

Thus, for each body site, 1500 (15 Classes x 100 genera) entries are possible. To denote the 100 genera, space for three hundred characters must be allotted. (In the simplest form, if we wish to code 100 of the same kind of thing, the total number, 100, which has three digits, establishes the number of digits or characters required to denote each of the total 100. Number 1 would be 001, 52 would be 052, etc.) The 1500 possible entries for each site, then, would require 300 x 30 or 9000 characters. For all body sites, assuming a maximum of 15, 9000 x 30 or 270,000 would be required for microbial data alone. Since this capacity would be required only is 100 genera belonging to 15 Classes were found at 15 body sites, considerably less capacity is actually required. Based on examinations of the data, we are presently using 500,000 character capacity to store, retreive and analyze the data. As more experience is gained this capacity can be increased or decreased as needed.

Another advantage of the present system is that once current data has been analyzed, it does not have to be left in the computer, but can be transferred to magnetic or punched tape for permanent storage. At some later time, if new analyses are desired or if the current data are to be compared with data obtained a year or two hence, the data on the tape can be fed back into the computer at that time. Our present estimate of computer storage time with a 500,000 character capacity limit is about six months.

In drawing together and coding data which have been gathered to date, it became obvious that the format which has been described would not be sufficiently flexible to store this, plus ancillary, information for retrieval and analysis. The problem which was encountered can be stated: some of the data referred to things that were done to the animal or which described the animal's condition while other data was purely microbial and described the population of microorganisms at a given site on a given date. The former data could be called "Animal Log Information" while the latter is "Microbial Data". For example, on a given date, X milligrams of antibiotic C were given to monkey Y. But no microbial sampling was done till three days later. This information could not be entered on the format shown in Figure 5 because the two events occurred at different times.

Two files, therefore, were used. One was a log of significant events which might have an effect on the microorganisms of an animal (Animal Log Information) while the other dealt with the results of microbial sampling exclusively (Microbial Data). Thus two files could be stored separately in the computer auxiliary memory (later to be transferred to magnetic tape for permanent storage or to core memory for computation). The significance of any event or series of events in the animal's life could then be determined by comparing the two files and looking for correlations between events and trends in microbial data. All of the analyses which were discussed before can be done using the revised format. Intervals between events can be determined automatically (i.e., "how long" in days). In addition, flexibility is gained by use of this double format so that the effect of significant events, such as administration of antibiotics or antiseptic wash, upon the microorganism population of an animal can be determined.

The format currently being used and the number of characters involved are represented in Figures 5 and 6. A new category has been added called "Observations" on the Animal Log (Figure 5). This represents events which may affect microbial data or the way the animal is treated but which are not listed as a separate category. An example is diarrhea. If an animal develops diarrhea, he may be treated with antibiotics and/or ingestion of microorganisms in the form of Lactinex. The other categories of the Animal Log have been broken away from the original format. Whether or not the animal is "in isolation" is answered by a "yes" or "no" in the Name or Presence Column. Then the category stays in the same isolated or non-isolated position until the record is updated by a change to the opposite condition. The same is true of the category "Diet (Sterile or Non-Sterile)". "Diet Name" is another category which stays the same in the computer record until a change is effected.

Record No. ****

Animal ***
Date **/**/**

Categories	Name or Presence	Amount
Isolation (Yes/No)	*	
Diet (Sterile or Non-Sterile)	*	
Diet Name	*	***
Organisms Ingested	*	***
Antiseptic Wash	*	
Antibiotic	*	***
Observation	*	***

Other Animal Information

Animal **

Added or Removed *

* = 1 Character of Code

Figure 5. Animal Log Information

Record No. ***

Animal Sampled **
Date **/**/**

Temperature ** Humidity ** Pressure **

Organisms

Site	Aerobes	Anaerobes		Coefficient	Exponent of Base Ten
**	*	*	Number	**	**

	Site	Class	Genus		Coefficient	Exponent of Base Ten
Site and Type	**	**	**			
↕				↕		
Site and Type	**	**	**	Number	**	**

* = 1 Character of Code

Figure 6. Microbial Data

On the other hand, "Organisms Ingested", "Antiseptic Wash", "Antibiotics" and "Observations" are unique events which must be coded into the computer record every time an animal is fed microorganisms, is bathed in antiseptic solution, is given antibiotics or is observed to behave in a way or be in a condition which may alter his indigenous microorganisms. "Other Animal Information" pertains to the presence of other animals with the animal coded and being described. Entries in this category also stay the same in the computer record until some change takes place with the addition or removal of an animal from the same isolator or lab colony as the animal under consideration.

The second part of the revised format is the Microbial Data itself (Figure 6). This is similar to the original format (Figure 5) except that the total number of aerobes and anaerobes present at a given site on a given date has been added as a category and the time of day the sample is taken has been deleted because it was felt that it played no important role in the data obtained during this program. If it should become important later, in this program or in another program, it can be added.

By changing the format in which the microbial and animal data are kept in the computer, two separate records are thus generated. Information can now be entered into either record separately as data is gathered, and correlations between the two records can be made as desired. Such correlations may well illuminate the significant variables which affect the microbial flora of primates and man.

D. RESULTS

1. CODING AND RETRIEVAL

A major part of the effort was spent in coding the Microbial Data and Animal Log Information, writing programs to "place" this data in the computer auxiliary memory and writing programs to retrieve the data for subsequent examination and analysis. The data itself, both log and microbial, was stored in two secondary files on the magnetic disc which could be accessed by their record addresses. A primary file of record addresses was then created which was indexed by key-word descriptors refering to the content of the records. (A listing of these descriptors is shown in Appendix V, Figure 24.) For example, a typical Animal Log Record might have reference to diet name - Rockland Primate Diet, diet-sterile, animals added, animals removed, isolation - yes, diarrhea, antibiotics-Tegopen. The primary file stored this record address under all these descriptors. A second program could examine the primary file by any one or combination of descriptors (see Appendix V).

The record address is found as a "hit" and printed out if called for with other record addresses containing information to which these descriptors applied. These addresses may be sent to a "scratch" area where other programs can examine them, retrieve the records themselves and print out their contents in a variety of ways. A detailed discussion of this search facility and examples of its operation are given in Appendix V.

The key programs were written to display the data in the secondary files. For animal log information, the Log Report Generator Program can retrieve and display log information for any animal in its entirety or between any two dates. Further modifications of this program made it even more flexible, permitting summaries by activities (e.g., diet or antibiotics used) and by elements within activities (e.g., Purina Monkey Chow or Tetrex-F) and permitting display of the data by activity, by date or by date and activity. A report of any one activity is also possible and the period for which a report is generated can be changed. (Examples of the operation of these programs and the programs themselves are shown in Appendix V, Figures 21 and 27). This program thus allowed an investigator to examine the log data for any animal or group of animals in a variety of ways, in part or in toto, depending on the kinds of questions to be answered. The ease and flexibility of this computerized approach as opposed to manually shuffling through stacks of laboratory notebooks cannot be over-emphasized. Even more important, pieces of significant information that might be overlooked using a manual approach because of the sheer mass and disorganization of the raw lab notebooks data, cannot escape notice when the computer is used.

A second key program was created to display the contents of microbial data records in the secondary file. This is called the Report Generator Program. It permits the display of the quantitative results of microbial sampling in 24 possible matrix arrays determined by animal, date of sampling, sites sampled, and class-genus found. A detailed exploration of the operation of this program plus examples are found in Appendix V.

The Report Generator Program,as on the Log Report Generator Program, gives an investigator a great deal of flexibility in displaying the sampling data. Depending on the kind of question he wishes to ask, one of the 24 types of displays will permit him to answer it easily. He first searches the primary file for the type of data which interests him and then automatically sends the record addresses found to a scratch area of the auxiliary magnetic disc memory. He next calls for the Report Generator Program by means of the appropriate code. This then retrieves the records themselves in the actual secondary file by looking up the addresses stored in the scratch area, and prints the stored data out in the matrix called for by the investigator.

These two major programs thus permit retrieval of all data in both the animal log file and the microbial data file, in part or in toto. In addition, they allow an investigator at the teletype, remote to the central computer where both programs and data are stored, to display the data in a variety of ways which are useful to him in answering questions about what has been done to the animals, what the results of sampling have been, how the manipulations of the animals have affected the sampling results, and how the microbial data varies with time, site, animal and class-genus. The versatility which this gives an investigator in manipulating data and animals and in learning how to modify his experimental protocol is unexcelled by any manual non-computer technique available.

2. STATISTICAL ANALYSIS

Certain of the statistical analyses outlined in the previous section could not be performed due to a lack of pertinent data. With the microbial data on hand the following statistical evaluations were possible:

a. Limits of variation could be established:

   1. On a given body site sampled at different times.

   2. Between different body sites averaged over the period of the period of the experiment.

b. An attempt could be made to identify environmental factors affecting microorganisms studied, e.g., isolation, antibiotics, antiseptic washes, diet, etc.

Comparisons between animals along these lines could also be made.

A number of statistical analyses which were projected initially could not be performed due to the lack of data in certain areas. Additional sampling, microbial counts, identification of microorganisms, and manipulation of the animals would be required as outlined below to complete the analyses orginally desired:

a. Limits of variation

   1. Identification of all genera present in every sample

   2. Numerical counts of number of organisms of each genus present in every sample

b. Degree of transfer of microorganisms between subjects

   1. Exposure of isolated animals to each other and to control animal(s)

   2. Exhaustive sampling of animals involved for several months following exposure on a regular basis.

   3. Identification of all genera present in every sample

   4. Numerical counts of number of organisms of each genus present in every sample

c. Bionomic interrelationships among organisms

   1. Regular sampling from each site over an extended period of time

   2. Identification of all genera present in every sample

   3. Numerical counts of number of organisms of each genus present in every sample

d. Environmental factors affecting microorganisms studied

1. Identification of all genera present in every sample

2. Numerical counts of number of organisms of each genus present in every sample

3. Further manipulation of environmental factors as dictated by the analyses of changes in microflora of animals.

e. Indigenous controlling or stabilizing microorganisms

1. Identification of all genera present in every sample

2. Numerical counts of number of organisms of each genus present in every sample

3. Analysis of 1 and 2 to determine which organisms are exerting a controlling or stabilizing influence.

f. Exogenous organisms enhancing stabilization and control

1. Introduction of potentially controlling organisms into or onto experimental animals.

2. Long term analyses of microflora as in a1 and a2 and e3.

3. Repeated introduction of potentially controlling organisms into or onto experimental animals.

4. Repeat of f2.

5. Introduction of other potentially controlling organisms.

6. Repeat of f2.

g. Extent of microbiological compatibility required for group confinement.

1. Completion of experimental protocol calling for contact between animals already in isolation and exposure to control animal(s).

2. Observation to determine development of pathological conditions in experimental animals.

3. Extensive microbial sampling on a regular basis over the period of several months.

4. Identification of all genera present in every sample.

5. Numerical counts of number of organisms of each genus present in every sample

6. Addition or removal of still more animals of similar or different microfloral populations until the size of the confined group and the individual microfloral populations is optimized.

7. Extensive sampling a1 and a2.

The above outline presents the ideal experimental procedures for permitting valid statistical treatment of microbial sampling data. The minimum experimental requirements for effective minimum statistical treatment of data are outlined below:

a. Regular sampling of all sites on all animals including controls. This can be once a day, once every three days, once a week or once every two weeks, depending on facilities and personnel available, for a period of one year.

b. Identification of all genera present from all samples taken in the first month.

c. Numerical counts for at least five marker genera plus total aerobes and anaerobes throughout the sampling period. (Each dilution plated in triplicate.)

d. Manipulation of environmental factors: e.g. isolation, antibiotics, antiseptic w ses, organisms ingested as dictated by the results of sampling data. Controls should include:

   1. One animal in isolation which receives a sterile diet by no treatment of any kind.

   2. One animal in the lab colony which receives sterile diet, antibiotics, antiseptic washes, organisms ingested, etc. on the same schedule as the experimental animals in isolation.

   3. One animal in the lab colony which receives no treatment of any kind, however, it should receive a non-sterile diet.

e. Identification of all genera present from all samples taken during the sixth month.

f. Re-exposure of isolated animals to each other during the seventh month. This should include rotating pairs of animals in the same cage so that all are exposed to each other.

g. Identification of all genera present during the ninth month.

h. Exposure of all isolated animals to a control animal. This should consist of sharing of air supply for the tenth month and intimate contact as above between all experimental animals and control animal for the eleventh month.

i. Identification of all genera present during the tenth, eleventh and twelfth months and numerical counts of potentially pathogenic organisms as well as marker organisms.

The statistical analyses which were possible were performed using the GE Time-Sharing Computer. The programs required were written and edited using the computer, and finally stored in auxiliary memory for later use when necessary. Organisms identified during the course of the experiment for each animal and site are presented in Table XIV.

Limits of variation were attacked from a number of directions. Maximum and minimum counts for aerobes and anaerobes and for genera for which numerical data was available were extracted from the data and are presented in Table XV.

Another approach to the determination of limits of variation which also gave information bearing on the effect of environmental conditions on microbial populations was to employ linear regression analysis on microbial counts (dependent variable) for a given organism at a given site with time in days as the independent variable. This allowed us to ascertain whether there was a measurable change with a tendency to increase or decrease over the period of sampling. The program employed also permitted transformation of the dependent variable in a variety of ways including logarthmic transformation. This type of transformation allowed us to test for the presence of an expotential change in microbial numbers. Analysis of residuals, however, (difference between actual value of dependent variable and computed value by the best fitted linear regression line) showed that the data followed a sinusoidal pattern about an untransformed regression line with a general tendency to increase or decrease. The results of linear regression using untransformed data are presented in Table XVI. Comparison between animals yields interesting results. Animal no. 115, a laboratory colony control, shows no significant change in its number of fecal aerobes or anaerobes over the period of sampling. The fecal aerobes in animal nos. 116, 118 and 119 are decreasing. These animals were all receiving antibiotics over an extended period of time. Animal no. 120's fecal aerobes increased with time. This animal had been in isolation but had not been receiving antibiotics. Although no. 116's fecal anaerobes do not show a significant change, animal nos. 118 and 119's fecal anaerobes also decreased. Animal no. 120's fecal anaerobes increased at the same rate as his aerobes. Analyses of fecal Corynebacteria present an incomplete picture due to the sparcity of data. Animal no. 116 shows no significant change while animal no. 119 shows a decreasing curve. Axilla and groin aerobic and anaerobic counts shows no clearly defined pattern. Animal no. 119 had received more antiseptic washes than no. 116, but only axilla anaerobes were decreasing significantly for no. 119. It is interesting to note, however, that the rate of increase for animal no. 119's groin-anaerobes is less than that for no. 116's by two orders of magnitude.

To determine site-to-site differences within animals and between animals exhaustive T-testing was performed using the computer. Examples of the extensive results of these analyses are given in Tables XVII A and XVII B. The results are summarized in two ways in Table XVII C and XVII D. In Table XVII C, significant differences betwen mean counts at the 90 percent confidence level are noted. In Table XVII D, mean counts whose probability of being different is less than 50 percent are listed. In other words, the means in this latter case probably arise from count distributions which are the same.

Despite the sparcity of data, certain similarities and differences are evident. Within one animal, for example, fecal counts are frequently different from other body sites. When they are similar, it is usually to throat, gingiva and glans penis. This is not surpirsing since these animals are known to be coprophagous. Throat and gingiva are also frequently similar. But they can be either different or the same as conjunctiva, axilla, groin, and glans penis. This is also true of conjunctival counts when compared to other body sites.

As for similarities and differences between animals, control animals (nos. 115 and 121) are frequently different than some experimental animals for all types of organisms at all sites. But an anomalous result is that sometimes the control animal counts are significantly different from each other. Between experimental animals themselves, animal no. 116 shows up most frequently as differing from one or the other of the remaining experimental animals. Similarities between experimental animals are very frequent, animal nos. 118 and 119 being most often similar at all sites and for all organisms. Another anomalous result is that sometimes the experimental animals are found to have counts similar to the controls. In all cases, however, more frequent sampling would help to make these distinctions or similarities clearer.

3. SUMMARY

The power and flexibility of using a digital computer for storing and retrieving microbial data and animal log information and displaying it in a variety of useful ways, has been amply demonstrated. The use of statistical routines on the computer has also resulted in enormous time saving and ease of operation as compared with the effort which would have been required to perform these analyses manually. One of the main accomplishments of this approach has been to show what kind of experimental protocol would be necessary to accomplish all the aims of this area of experimentation. The combination of man and high-speed computer is a tool of unexcelled merit for studying the effects of isolation on the microbial ecology of groups of animals and for collating the results of such experiments for application to the problem of confinement of man during space flight.

TABLE XIV. ORGANISMS IDENTIFIED DURING THE COURSE OF THE EXPERIMENT

Animal No. 115

Sites

Conjunctiva	Throat	Gingiva	Axilla	Groin	Glans Penis	Feces
Streptococcus			Streptococcus			Streptococcus
Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium
						Proteus
						Escherichia
Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
						Aerobacter

Animal No. 116

Conjunctiva	Throat	Gingiva	Axilla	Groin	Glans Penis	Feces
					Streptococcus	
Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium
			Proteus	Proteus		Proteus
						Escherichia
Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
						Shigella
Escherichia-Aerobacter Group	Escherichia-Aerobacter Group	Escherichia-Aerobacter Group		Escherichia-Aerobacter Group	Escherichia-Aerobacter Group	

Animal No. 118

Conjunctiva	Throat	Gingiva	Axilla	Groin	Glans Penis	Feces
						Streptococcus
Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium
Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	
						Aerobacter
						Shigella
	Escherichia-Aerobacter Group				Escherichia-Aerobacter Group	Escherichia-Aerobacter Group

Animal No. 119

Conjunctiva	Throat	Gingiva	Axilla	Groin	Glans Penis	Feces
	Streptococcus	Streptococcus				
Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium
				Proteus	Proteus	Proteus
Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
		Escherichia-Aerobacter Group		Escherichia-Aerobacter Group	Escherichia-Aerobacter Group	
	Pseudomonas	Pseudomonas		Pseudomonas		Pseudomonas
						Salmonella
	Alcaligenes	Alcaligenes				

Animal No. 120

Conjunctiva	Throat	Gingiva	Axilla	Groin	Glans Penis	Feces
Streptococcus		Streptococcus	Streptococcus	Streptococcus		Streptococcus
Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium
				Proteus	Proteus	
Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus
				Escherichia-Aerobacter Group	Escherichia-Aerobacter Group	
	Pseudomonas					
					Salmonella	
				Lactobacillus		
			Bacteroides	Bacteroides		
			Clostridium	Clostridium		
			Aspergillus	Aspergillus		

Animal No. 121

Conjunctiva	Throat	Gingiva	Axilla	Groin	Glans Penis	Feces
						Streptococcus
Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	Corynebacterium	
Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	Staphylococcus	
Escherichia-Aerobacter Group	Escherichia-Aerobacter Group	Escherichia-Aerobacter Group				

TABLE XV. LIMITS OF VARIATION

	Animal No.	Per Gram of Sample	
		Maximum Count	Minimum Count
Conjunctiva			
Aerobes	115	1.3×10^7	1.8×10^6
	116	1.2×10^7	1.2×10^6
	118	4.9×10^6	1.0×10^4
	119	1.3×10^6	$< 1 \times 10^3$
	120	2.6×10^7	2.7×10^4
	121	5.2×10^5	1.3×10^4
Anaerobes	115	1.5×10^6	7.9×10^6
	116	2.3×10^7	1.7×10^6
	118	4.9×10^7	4.0×10^3
	119	2.5×10^6	3.0×10^3
	120	2.6×10^7	1.4×10^5
	121	1.9×10^4	4.3×10^5
Corynebacterium	115	1.5×10^6	---
	116	6.4×10^6	5.5×10^5
	118	2.1×10^6	$< 1 \times 10^3$
	119	1.4×10^6	$< 1 \times 10^3$
	120	4.1×10^6	1.4×10^5
	121	4.0×10^3	3.7×10^5
Staphylococcus	115	1.1×10^6	---
	116	8.9×10^6	1.7×10^6
	118	7.3×10^6	$< 1 \times 10^3$
	119	2.0×10^6	$< 1 \times 10^3$
	120	1.4×10^7	1.1×10^5
	121	1.9×10^5	3.0×10^3

TABLE XV. LIMITS OF VARIATION (Cont)

	Animal No.	Per Gram of Sample	
		Maximum Count	Minimum Count
Conjunctiva (Cont)			
Escherichia-Aerobacter Group	115	---	---
	116	2.3×10^{6}	---
	118	$< 1 \times 10^{5}$	---
	119	$< 1 \times 10^{3}$	---
	120	$< 1 \times 10^{4}$	---
	121	6.0×10^{3}	---
Throat			
Aerobes	115	5.1×10^{9}	9.9×10^{7}
	116	1.2×10^{9}	6.3×10^{6}
	118	4.9×10^{8}	2.9×10^{7}
	119	2.4×10^{8}	2.0×10^{5}
	120	5.9×10^{7}	4.4×10^{6}
	121	1.2×10^{9}	1.1×10^{7}
Anaerobes	115	3.7×10^{9}	1.6×10^{8}
	116	7.4×10^{9}	1.3×10^{7}
	118	4.9×10^{8}	2.2×10^{7}
	119	4.3×10^{8}	6.0×10^{5}
	120	6.9×10^{7}	5.2×10^{6}
	121	1.2×10^{9}	1.9×10^{7}
Corynebacterium	115	3.9×10^{6}	---
	116	7.2×10^{8}	$< 1 \times 10^{5}$
	118	3.9×10^{7}	1.4×10^{4}
	119	5.0×10^{7}	2.0×10^{5}
	120	7.7×10^{7}	3.5×10^{6}
	121	1.9×10^{7}	7.0×10^{6}

TABLE XV. LIMITS OF VARIATION (Cont)

	Animal No.	Per Gram of Sample	
		Maximum Count	Minimum Count
Throat (Cont)			
Staphylococcus	115	2.0×10^4	---
	116	2.7×10^6	6.8×10^4
	118	5.1×10^7	$< 1 \times 10^4$
	119	7.0×10^3	$< 1 \times 10^3$
	120	1.4×10^5	2.0×10^4
	121	1.7×10^7	2.0×10^4
Escherichia-Aerobacter Group	115	---	---
	116	7.6×10^7	---
	118	1.0×10^3	---
	119	$< 1 \times 10^3$	---
	120	$< 1 \times 10^4$	---
	121	1.9×10^4	---
Gingiva			
Aerobes	115	1.5×10^9	2.1×10^7
	116	1.5×10^9	1.8×10^8
	118	4.9×10^8	2.0×10^5
	119	6.5×10^8	2.2×10^6
	120	4.6×10^8	2.7×10^6
	121	5.0×10^8	1.9×10^7
Anaerobes	115	7.2×10^8	1.6×10^8
	116	4.1×10^9	4.8×10^8
	118	7.0×10^8	1.3×10^6
	119	1.9×10^7	3.0×10^6
	120	4.5×10^8	3.7×10^6
	121	7.8×10^8	9.7×10^6

TABLE XV. LIMITS OF VARIATION (Cont)

	Animal No.	Per Gram of Sample	
		Maximum Count	Minimum Count
<u>Gingiva</u> (Cont)			
Corynebacterium	115	3.0×10^6	---
	116	4.6×10^8	1.0×10^6
	118	3.9×10^7	2.6×10^5
	119	4.4×10^7	1.5×10^6
	120	7.7×10^7	$< 1 \times 10^5$
	121	2.3×10^7	3.7×10^6
Staphylococcus	115	3.1×10^6	---
	116	3.6×10^6	1.0×10^5
	118	9.4×10^4	$< 1 \times 10^3$
	119	4.1×10^4	$< 1 \times 10^3$
	120	1.5×10^7	$< 1 \times 10^4$
	121	2.3×10^6	1.0×10^3
Escherichia-Aerobacter Group	115	---	---
	116	2.3×10^7	---
	118	$< 1 \times 10^3$	---
	119	1.0×10^3	---
	120	$< 1 \times 10^4$	---
	121	1.0×10^3	---
<u>Axilla</u>			
Aerobes	115	6.6×10^7	4.3×10^7
	116	1.5×10^4	6.0×10^4
	118	2.1×10^4	9.0×10^3
	119	2.8×10^5	$< 1 \times 10^3$
	120	1.1×10^7	$< 1 \times 10^4$
	121	6.0×10^5	2.0×10^4

TABLE XV. LIMITS OF VARIATION (Cont)

	Animal No.	Per Gram of Sample	
		Maximum Count	Minimum Count
Axilla (Cont)			
Anaerobes	115	3.0×10^7	8.7×10^6
	116	2.9×10^7	1.0×10^4
	118	1.1×10^5	1.4×10^4
	119	5.0×10^4	$< 1 \times 10^3$
	120	1.7×10^7	$< 1 \times 10^4$
	121	1.0×10^6	$< 1 \times 10^4$
Corynebacterium	115	1.7×10^7	---
	116	1.0×10^7	2.2×10^6
	118	1.8×10^4	2.0×10^3
	119	5.0×10^3	$< 1 \times 10^3$
	120	1.2×10^6	1.0×10^4
	121	8.0×10^5	$< 1 \times 10^4$
Staphylococcus	115	---	---
	116	1.6×10^7	9.0×10^5
	118	1.0×10^3	$< 1 \times 10^3$
	119	5.0×10^3	$< 1 \times 10^3$
	120	1.8×10^7	$< 1 \times 10^4$
	121	1.7×10^5	1.3×10^4
Escherichia-Aerobacter Group	115	---	---
	116	$< 1 \times 10^5$	---
	118	$< 1 \times 10^3$	---
	119	$< 1 \times 10^3$	---
	120	$< 1 \times 10^4$	---
	121	$< 1 \times 10^3$	---

TABLE XV. LIMITS OF VARIATION (Cont)

	Animal No.	Per Gram of Sample	
		Maximum Count	Minimum Count
<u>Groin</u>			
Aerobes	115	2.7×10^7	1.0×10^5
	116	7.5×10^7	1.0×10^5
	118	7.5×10^6	1.0×10^3
	119	8.6×10^6	1.6×10^4
	120	2.0×10^6	1.0×10^4
	121	7.7×10^6	1.1×10^5
Anaerobes	115	2.2×10^8	1.0×10^5
	116	4.2×10^8	6.0×10^4
	118	1.4×10^7	$< 1 \times 10^3$
	119	2.4×10^6	1.0×10^4
	120	1.3×10^7	2.2×10^4
	121	6.0×10^7	5.0×10^4
Corynebacterium	115	$< 1 \times 10^5$	---
	116	3.3×10^7	7.2×10^5
	118	1.1×10^6	$< 1 \times 10^3$
	119	3.3×10^6	6.0×10^3
	120	1.4×10^6	5.3×10^5
	121	5.0×10^5	$< 1 \times 10^4$
Staphylococcus	115	$< 1 \times 10^5$	---
	116	4.4×10^7	1.1×10^5
	118	1.6×10^5	$< 1 \times 10^3$
	119	1.6×10^6	1.1×10^4
	120	2.2×10^6	6.0×10^5
	121	8.2×10^4	3.1×10^7

TABLE XV. LIMITS OF VARIATION (Cont)

	Animal No.	Per Gram of Sample	
		Maximum Count	Minimum Count
Groin (Cont)			
Escherichia-Aerobacter Group	115	---	---
	116	7.0×10^5	---
	118	$< 1 \times 10^3$	---
	119	5.0×10^3	---
	120	1.0×10^4	---
	121	$< 1 \times 10^3$	---
Glans Penis			
Aerobes	115	3.9×10^8	1.2×10^8
	116	9.5×10^7	7.0×10^6
	118	4.9×10^8	6.1×10^4
	119	4.0×10^7	9.2×10^5
	120	9.3×10^7	1.4×10^5
	121	1.2×10^8	2.2×10^5
Anaerobes	115	4.5×10^8	4.9×10^7
	116	1.4×10^8	3.0×10^6
	118	7.0×10^8	5.3×10^4
	119	6.5×10^7	3.7×10^5
	120	2.2×10^8	1.0×10^4
	121	2.5×10^8	8.0×10^4
Corynebacterium	115	1.4×10^7	---
	116	1.0×10^8	4.6×10^6
	118	9.8×10^5	4.6×10^4
	119	3.7×10^6	2.2×10^5
	120	1.3×10^7	3.0×10^5
	121	1.1×10^6	1.0×10^4

TABLE XV. LIMITS OF VARIATION (Cont)

	Animal No.	Per Gram of Sample	
		Maximum Count	Minimum Count
Glans Penis (Cont)			
Staphylococcus	115	---	---
	116	1.2×10^{8}	7.5×10^{6}
	118	2.9×10^{7}	2.6×10^{4}
	119	5.2×10^{7}	1.2×10^{5}
	120	1.4×10^{7}	$< 1 \times 10^{4}$
	121	4.2×10^{7}	2.4×10^{5}
Escherichia-Aerobacter Group	115	---	---
	116	9.7×10^{6}	---
	118	2.8×10^{5}	---
	119	2.0×10^{5}	---
	120	1.0×10^{4}	---
	121	$< 1 \times 10^{3}$	---
Feces			
Aerobes	115	3.1×10^{11}	7.1×10^{7}
	116	1.0×10^{10}	1.7×10^{6}
	118	4.0×10^{10}	1.5×10^{5}
	119	6.1×10^{9}	7.6×10^{3}
	120	1.0×10^{10}	$< 1 \times 10^{3}$
	121	1.7×10^{8}	1.5×10^{8}
Anaerobes	115	3.9×10^{12}	2.3×10^{8}
	116	1.5×10^{10}	3.4×10^{7}
	118	7.6×10^{12}	5.0×10^{6}
	119	1.5×10^{10}	7.1×10^{6}
	120	7.8×10^{9}	2.5×10^{7}
	121	1.2×10^{8}	---

TABLE XV. LIMITS OF VARIATION (Cont)

	Animal No.	Per Gram of Sample	
		Maximum Count	Minimum Count
<u>Feces</u> (Cont)			
Corynebacterium	115	---	---
	116	1.5×10^8	1.2×10^5
	118	1.0×10^4	$< 1 \times 10^4$
	119	6.0×10^5	$< 1 \times 10^4$
	120	---	---
	121	---	---
Escherichia-Aerobacter Group	115	---	---
	116	---	---
	118	4.7×10^6	$< 1 \times 10^4$
	119	$< 1 \times 10^4$	---
	120	---	---
	121	---	---

TABLE XVI. STATISTICAL ANALYSIS OF DATA

Linear Regression Analysis of Changes in Number of Organisms at a Given Site for a Given Class Genus over the Period Samples were Taken

Animal No.	Slope (b) (Organisms/Day)	T Value (Test whether b is significantly different from 0)	Correlation Coefficient (r)	Change
Feces-Aerobes				
115	6.4×10^8	0.211	0	None
116	-3.5×10^7	3.74	0.694	Decreasing
118	-5.9×10^7	1.31	0.238	Decreasing
119	-1.2×10^7	1.93	0.392	Decreasing
120	3.0×10^7	1.36	0.418	Increasing
Feces-Anaerobes				
115	1.1×10^{10}	0.330	0	None
116	-1.2×10^7	0.733	0	None
118	-9.6×10^9	1.10	0.131	Decreasing (Slight Tendency)
119	-1.7×10^7	0.916	0	Decreasing (Slight Tendency)
120	3.0×10^7	3.55	0.812	Increasing
Feces-Corynebacterium				
116	-4.3×10^6	0.541	0	None
119	-6.2×10^4	4.74	0.918	Decreasing
Axilla-Aerobes				
116	8.5×10^3	0.116	0	None
119	-2.9×10^2	0.267	0	None
Axilla-Anaerobes				
116	9.9×10^4	1.42	0.449	Increasing
119	-3.0×10^2	7.07	0.971	Decreasing
Groin-Aerobes				
116	4.07×10^4	0.0166	0	None
119	2.9×10^4	1.72	0.496	Increasing
Groin-Anaerobes				
116	1.6×10^6	1.34	0.409	Increasing
119	1.0×10^4	2.35	0.429	Increasing

TABLE XVIIA. RESULTS OF T-TESTING SAMPLING DATA (BETWEEN SITES ON A GIVEN ANIMAL -119)

	Sampling Data						
Site	1	2	3	4	5	6	7
Aerobes							
1(Conjunctiva)		*60%<P<80%	60%<P<80%	60%<P<80%	<50%	60%<P<80%	99.5%
2(Throat)			<50%	<50%	<50%	60%<P<80%	60%<P<80%
3(Gingiva)				<50%	<50%	60%<P<80%	60%<P<80%
4(Axilla)					<50%	80%<P<90%	80%<P<90%
5(Groin)						80%<P<90%	80%<P<90%
6(Glans Penis)							60%<P<80%
7 (Feces)							
Anaerobes							
1(Conjunctiva)		60%<P<80%	95%<P<97.5%	60%<P<80%	<50%	80%<P<90%	99.5%<P<99.9%
2(Throat)			<60%	60%<P<80%	<50%	<60%	60%<P<80%
3(Gingiva)				<50%	<50%	<50%	95%<P<97.5%
4(Axilla)					<50%	80%<P<90%	60%<P<80%
5(Groin)						95%<P<97.5%	80%<P<90%
6(Glans Penis)							60%<P<80%
7(Feces)							

*Numerical values in percent denote the probability (P) that the mean microbial counts arise from different populations.

TABLE XVIIA. RESULTS OF T-TESTING SAMPLING DATA (BETWEEN SITES ON A GIVEN ANIMAL-119) (Cont)

	Sampling Data						
Site	1	2	3	4	5	6	7
Corynebacterium							
1(Conjunctiva)	*	<50%	<50%		<50%	<50%	<50%
2(Throat)			<50%	60%<P<80%	60%<P<80%	60%<P<80%	80%<P<90%
3(Gingiva)				60%<P<80%	60%<P<80%	60%<P<80%	60%<P<80%
4(Axilla)					<50%	<50%	<50%
5(Groin)						<50%	60%<P<80%
6(Glans Penis)							60%<P<80%
7(Feces)							
Staphylococcus							
1(Conjunctiva)		<60%	<60%	<60%	<50%	60%<P<80%	
2(Throat)			90%<P<95%	<50%	<50%	60%<P<80%	
3(Gingiva)				<60%	<50%	<60%	
4(Axilla)					<50%	60%<P<80%	
5(Groin)						80%<P<90%	
6(Glans Penis)							
7(Feces)							

*Numerical values in percent denote the probability (P) that the mean microbial counts arise from different populations

TABLE XVIIB. FECES RESULTS OF T-TESTING SAMPLING DATA (BETWEEN ANIMALS ON A GIVEN SITE)

Aerobes

Animal No.	115	116	118	119	120	121
115		*80%<P<90%	80%<P<90%	90%<P<95%	60%<P<80%	> 99.9%
116			< 50%	60%<P<80%	< 50%	60%<P<80%
118				60%<P<80%	< 50%	> 99.9%
119					60%<P<80%	95%<P<97.5%
120						80%< P<90%

Anaerobes

Animal No.	115	116	118	119	120	121
115		80%<P< 90%	<50%	80%<P<90%	60%<P<80%	97.5%<P<99%
116			60%<P<80%	<50%	<50%	> 99.9%
118				60%<P<80%	<60%	99.5%<P<99.9%
119					<50%	> 99.9%
120						90%<P<95%

Coryne-bacterium

Animal No.	115	116	118	119	120	121
119		90%<P< 95%				

*Numerical values in percent denote the probability (P) that the mean microbial counts arise from different populations.

TABLE XVIIC. RESULTS OF T-TESTING SAMPLING DATA

(SUMMARY OF MEAN MICROBIAL COUNTS WHICH ARE SIGNIFICANTLY DIFFERENT AT THE 90 PERCENT CONFIDENCE LEVEL)

Between Sites on a Given Animal

Animal No.		Sites*
116	Aerobes	1-3, 3-4, 3-5, 3-6, 4-6
116	Anaerobes	1-7, 4-6, 4-7, 5-7, 6-7
116	Corynebacterium	1-2
118	Aerobes	1-7, 2-7, 3-7, 4-7, 5-7, 6-7
118	Anaerobes	1-7, 2-7, 3-7, 4-7, 5-7, 6-7
119	Aerobes	1-7
119	Anaerobes	1-7, 3-7, 5-6
119	Staphylococcus	2-3
120	Aerobes	2-4, 2-5
120	Anaerobes	3-4
120	Corynebacterium	1-3
120	Staphylococcus	2-3, 2-4, 2-6, 5-6
121	Aerobes	1-7, 4-7, 5-7
121	Anaerobes	1-7, 4-7, 5-7
121	Corynebacterium	1-2, 2-4, 2-5, 2-6

*Site Codes

1	Conjunctiva	3	Gingiva	5	Groin
2	Throat	4	Axilla	6	Glans Penis
				7	Feces

TABLE XVIIC. RESULTS OF T-TESTING SAMPLING DATA (Cont)

Between Animals on a Given Site	Animal No.
Conjunctiva - Aerobes	120-121
Conjunctiva - Anaerobes	120-121
Conjunctiva - Corynebacterium	118-121
Conjunctiva - Staphylococcus	116-121, 118-121
Throat - Aerobes	119-121, 120-121
Gingiva - Staphylococcus	116-120, 118-120
Axilla - Aerobes	116-118, 116-119, 116-121
Axilla - Anaerobes	116-118, 116-119, 116-121
Axilla - Corynebacterium	116-118, 116-121
Axilla - Staphylococcus	118-120, 119-120
Groin - Corynebacterium	120-121
Feces - Aerobes	115-119, 115-121, 118-121, 119-121
Feces - Anaerobes	115-121, 116-121, 118-121, 119-121, 120-121
Feces - Corynebacterium	116-119

TABLE XVIIID. RESULTS OF T-TESTING SAMPLING DATA

(SUMMARY OF MEAN MICROBIAL COUNTS WHOSE PROBABILITY OF BEING DIFFERENT IS LESS THAN 50 PERCENT)

Between Sites on a Given Animal		Sites
Animal No.		
115	Aerobes	1-5, 1-7, 2-3, 2-7, 3-6, 3-7, 4-7, 5-7, 6-7
115	Anaerobes	1-7, 2-7, 3-6, 3-7, 4-7, 5-6, 5-7, 6-7
116	Aerobes	1-4, 1-5
116	Anaerobes	1-4, 2-3, 2-7, 3-7, 5-6
116	Corynebacterium	4-5, 6-7
116	Staphylococcus	1-4, 2-3, 4-5
118	Aerobes	1-5, 2-3, 2-6, 3-6
118	Anaerobes	1-5, 2-3, 2-6, 3-6, 5-6
118	Corynebacterium	1-2, 2-3, 5-6,
118	Staphylococcus	1-2, 1-6, 2-6, 3-5
119	Aerobes	1-5, 2-3, 2-4, 2-5, 3-4, 3-5, 4-5
119	Anaerobes	1-5, 2-5, 3-4, 3-5, 3-6, 4-5
119	Corynebacterium	1-2, 1-3, 1-5, 1-6, 2-3, 4-5, 2-6, 4-7, 5-6
119	Staphylococcus	1-3, 1-5, 2-4, 2-5, 3-5, 4-5
120	Aerobes	2-6, 4-5
120	Anaerobes	1-4, 4-5
120	Corynebacterium	1-5, 2-3
120	Staphylococcus	1-3, 1-6, 3-5, 4-5
121	Aerobes	1-4, 3-7
121	Anaerobes	2-3, 3-6, 4-5, 4-6, 6-7
121	Corynebacterium	1-6, 2-3, 4-5, 4-6, 5-6
121	Staphylococcus	1-4, 2-5, 5-6

TABLE XVIIID. RESULTS OF T-TESTING SAMPLING DATA (Cont)

Between Sites on a Given Animal	Animal No.
Conjunctiva - Aerobes	115-116, 115-120, 116-118, 116-120, 119-121
Conjunctiva - Anaerobes	115-116, 115-118, 115-120, 116-118, 116-120, 118-120, 119-121
Conjunctiva - Corynebacterium	116-118, 116-119, 116-120, 118-120, 119-120
Conjunctiva - Staphylococcus	116-118, 116-120, 118-120, 119-121
Throat - Aerobes	118-119
Throat - Anaerobes	115-116, 118-119, 118-121
Throat - Corynebacterium	118-119, 118-121, 119-121
Throat - Staphylococcus	116-121, 119-121, 120-121
Gingiva - Aerobes	115-116, 118-119, 118-120, 118-121, 119-120, 119-121, 120-121
Gingiva - Anaerobes	115-118, 115-121, 118-120, 118-121, 120-121
Gingiva - Corynebacterium	116-120, 118-119, 118-121, 119-121
Axilla - Aerobes	118-119
Axilla - Anaerobes	115-116, 116-120, 118-119
Axilla - Corynebacterium	118-119, 119-120, 119-121, 120-121
Axilla - Staphylococcus	118-121, 119-121
Groin - Aerobes	115-116, 118-119, 118-121
Groin - Anaerobes	115-116, 118-119, 118-120, 118-121
Groin - Corynebacterium	118-119, 118-120, 118-121, 119-120, 119-121
Groin - Staphylococcus	116-121, 118-119, 119-120, 120-121
Glans Penis - Aerobes	115-118, 116-120, 116-121, 119-120, 120-121
Glans Penis - Anaerobes	115-116, 115-118, 115-121, 116-118, 116-120, 116-121, 118-120, 118-121, 119-120, 120-121
Glans Penis - Corynebacterium	118-121
Glans Penis - Staphylococcus	116-120, 118-120, 118-121, 119-120, 119-121, 120-121
Feces - Aerobes	116-118, 116-120, 118-120
Feces - Anaerobes	115-118, 116-119, 116-120, 119-120

APPENDIX I

BACTERIOLOGICAL PROCEDURES AND SAMPLING

The methods of West and Gall were followed as applicable. Figures 7 through 9 illustrate the dilution schemes, aerobic and anaerobic isolations. As the marker organisms were identified and the experiment proceeded only those procedures applicable to the problem at hand were followed. Thus only a few samplings were sent through the isolation scheme in that we were more interested in following the fate of a few critical genera.

Notes on Procedures and Isolates

1. All samples were analyzed as soon as possible after they were taken from the animal.

2. All media and diluent were prewarmed at 37°C.

3. All incubation were carried out at 37°C unless otherwise stated.

4. Cultures were identified to the genera level only.

5. Isolates were stored at 4°C on appropriate culture media.

6. Periodicity of sampling can only be determined in the natural course of sampling.

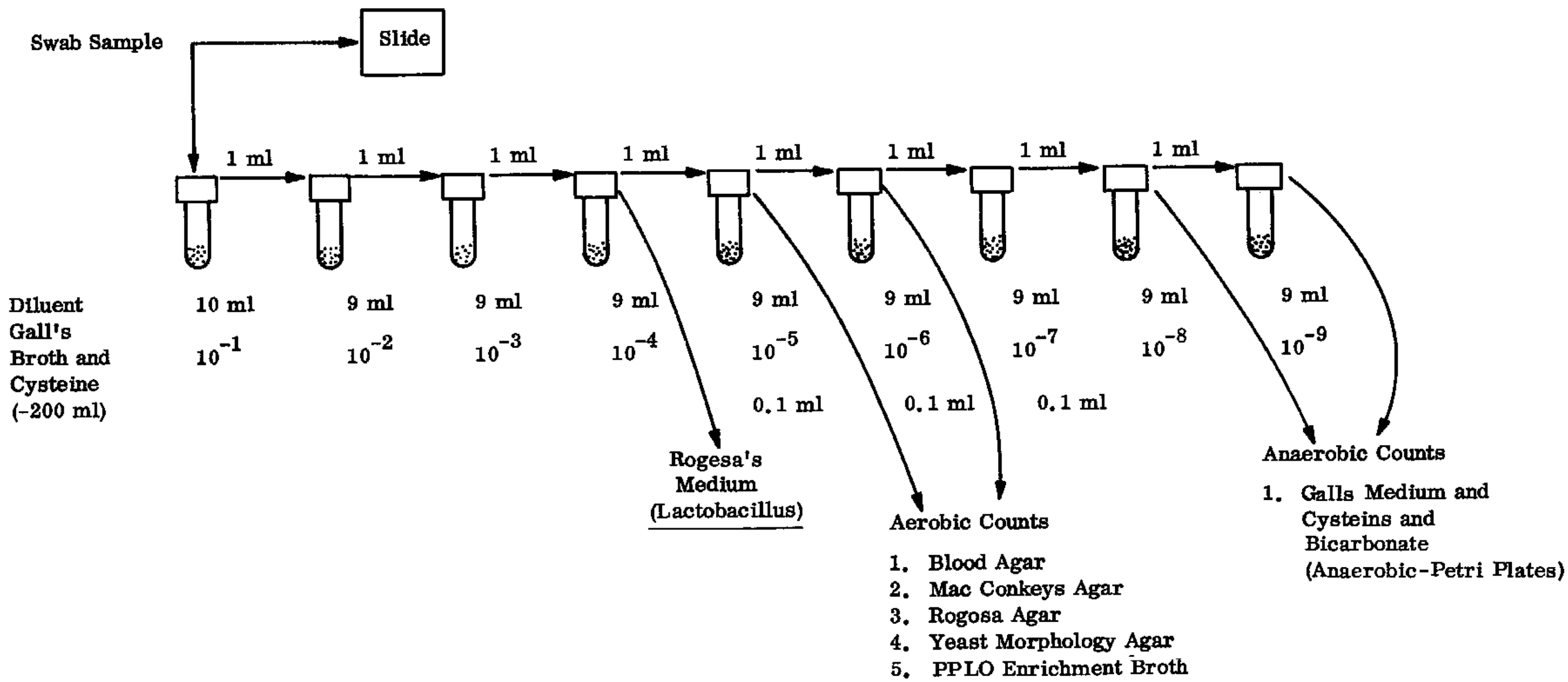


Figure 7. Sample Dilution Scheme*

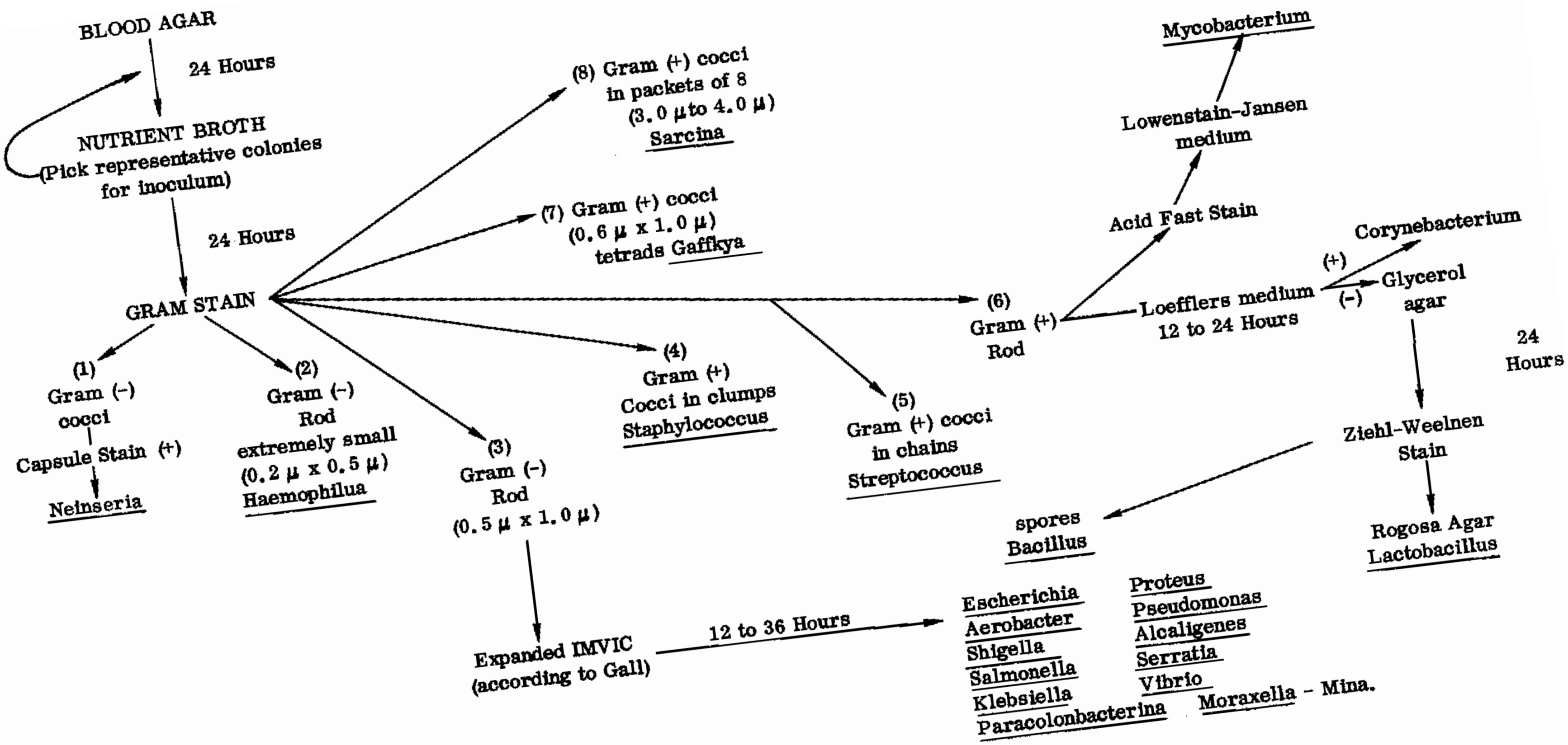


Figure 8. Aerobe Isolation

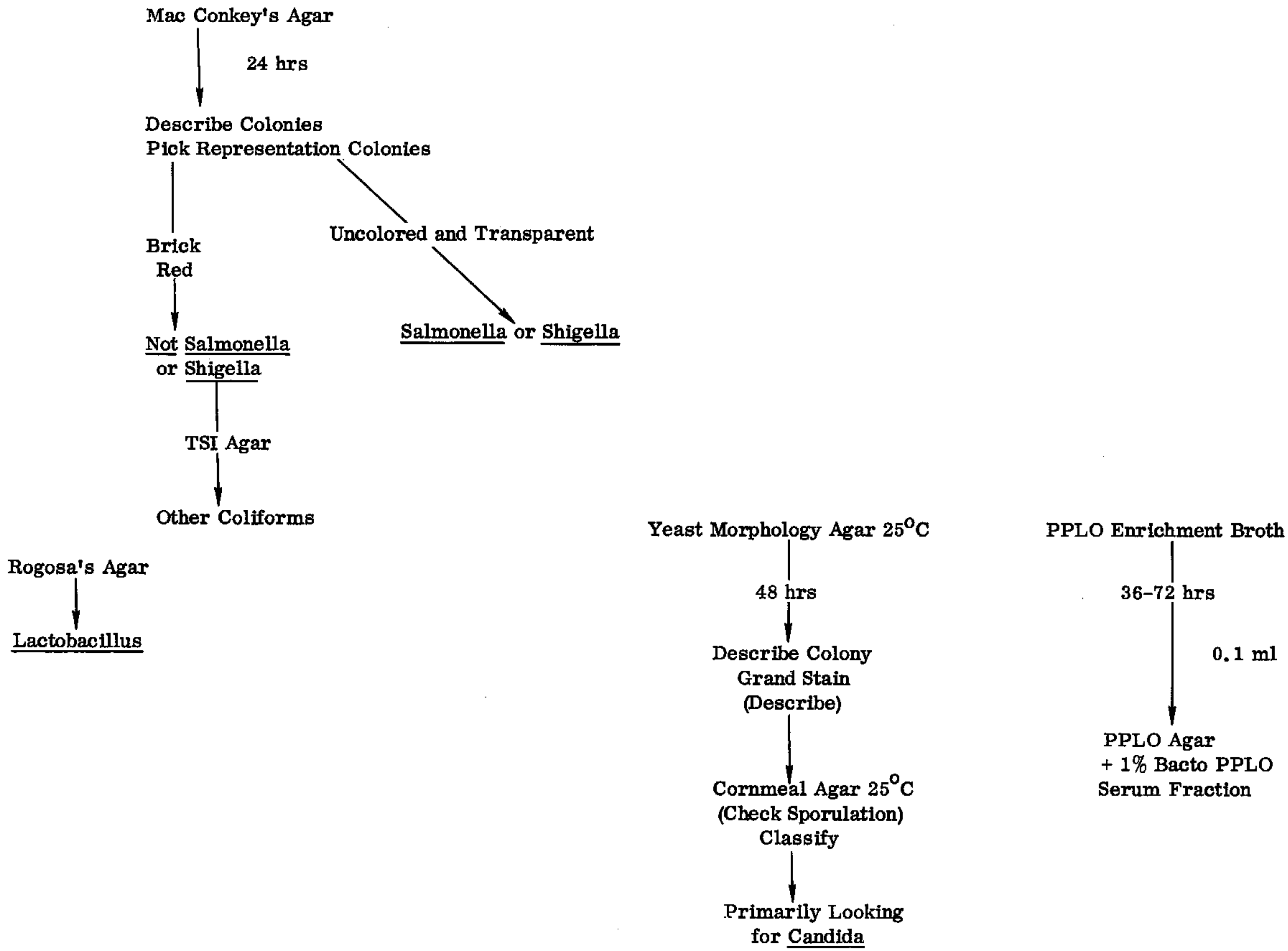


Figure 8. Aerobe Isolation (Cont)

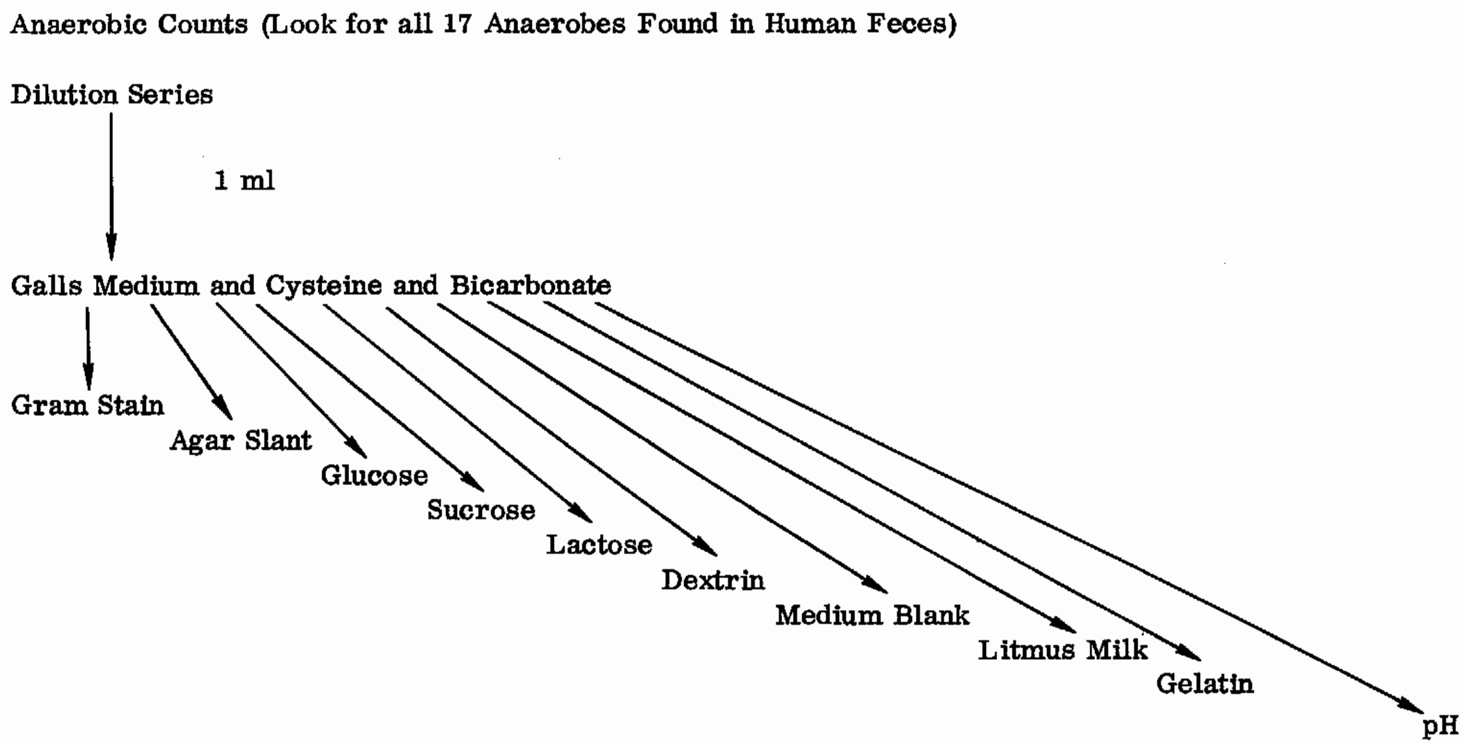


Figure 9. Anaerobe Isolation

APPENDIX II

MEDICAL PROCEDURES FOR EMPLOYEES HANDLING/WORKING WITH MONKEYS

I. Annual Chest X-ray

Tuberculin skin testing every six months for those individuals who are tuberculin negative.

II. Immunization Program

Smallpox Vaccine
Oral Polio Vaccine
Tetanus Toxoid Vaccine

III. Treatment of Injury (Monkey Bite)

Clean wound thoroughly
Administer tetanus toxoid booster
Administer gamma globulin 15 cc., intramuscularly

IV. Any individual with an open wound on the exposed part of his body is not permitted to handle a monkey.

V. Individuals handling monkeys are to wear a protective respiratory mask (surgical type) and protective gloves. Individuals working around monkeys but not handling monkeys are to wear a protective respiratory mask.

APPENDIX III

DIET AND WATER

Visual estimates, average food intake and feces measurements indicate that all six animals were very close regarding size throughout the experiment. The apparent state of good health was reflected in the activities, the appetites and other more subtle indications such as alertness to sounds, recognition of their animal caretakers, and response to learned activities such as isolator transfer. Experimental complexities made it extremely difficult to weigh the animals in the isolators. The control animals grew about a kilogram during the past year.

The GE diet (Table I) contains 17 percent crude protein. One estimate of protein required is 7.5 percent, May et. al (1950) with about three grams per day per kilogram of body weight intake. Recognizing the denaturing effect of autoclave sterilization, the animals consumed more than the required amount. We doubt that more than 50 percent of the protein, available before autoclaving, was destroyed. The control animals, when fed this diet, exhibited every sign of robust health.

The rhesus required fat in the diet, Greenberg (1949) or at least essential fatty acids. The polyunsaturated fatty acids required are certainly to be found in the animal fat portion of the feed mixture (Table I). Although mineral requirements, iron, calcium, phosphorus, etc. are constituent parts of the daily portion, the quantitative requirements are largely unknown, Day (1966a). To replace the Vitamins lost during autoclaving, a vitamin supplement was added. Of particular interest were A, B and C.

Known requirements for Vitamins include A, D, E, C, B_1, B_2, B_6, Niacin, Pantothemic acid, Brotin, Folic Acid and B_{12}, Day (1966b). Cyanocobalamin, B_{12}, has been shown to increase growth rate, May (1951). This does not prove an absolute need but in the absence of literature data to prove or disprove the requirement, B_{12} was added. The vitamin additive, prepared by Vitarine Co. of Springfield Gardens, New York, does not contain B_{12} but tocopherols are present in the solid materials fed.

Table II lists the composition of the daily sterile vitamin formulation of the drinking water which was prepared daily. Following autoclave sterilization of the water (using the method of Heumpner, 1967), the Millipore filter sterilized vitamin supplement was added. The entire mixing process was done inside a sterile, Trexler type, flexible isolator. The water containers (brown bottles), were attached to sterile stainless steel water dispensers.

The narrow necked containers were filled as nearly to the brim as possible following the addition of the vitamins, in order to reduce the exposure to the air. All water containers were kept in the darker section of the isolator. The animals were given free access to the water dispenser at all times.

There were individual, and consistent, differences in the total water requirement of the different animals. Although as stated, the activity level of the monkies appeared similar, at least one animal constantly exceeded the others in total intake. Each animal finished about

760 ml of liquid a day except No. 119. This animal emptied his initial fortified liquid usually at the end of eight or nine hours. (A second container more than satisfied him for the next sixteen hours.) The water in the second container was not fortified, and it is speculated that the vitamin supplement contained some feature or taste that the animal unconsciously craved or desired.

The individuality of this animal cannot be explained by any data that GE has nor did his external actions and characteristics seem different from the other animals.

APPENDIX IV

DISCUSSION OF THE ISOLATOR SYSTEM

The isolator system, shown in Figure 1, 2 and 3 was designed by the authors and Mr. Frank Matthews, President of Matthew Research, Alexandria, Virginia. Each system is comprised of two separate isolators connected by a stainless steel tunnel. One isolator consists of stainless steel with a large plastic window and the other isolator consists of 30 gauge transparent plastic. Two systems comprise a unit and two units were used during the experiment.

The flexible plastic isolator (polyvinyl chloride) enclosed a stainless steel monkey cage.* The monkey cage was equipped with a squeeze panel, operated by the turning of a crank. Provision was made for separating the animal from his urine and feces. The urine and feces were collected separately in a manner similar to that employed by standard metabolism cages. The cage was equipped with locks and an automatic watering device which was movable (at the desire of the researcher), within the isolator. Figure 10 and Figure 11 illustrate these features.

To keep the animals under observation at all times, the flexible isolator was equipped with rigid plexiglass windows (Figure 12). Glove ports, zipper closers, equipment holding trays, cage support locks and minor items common to gnotobiotic isolators were included. Air was furnished to the animals after passing through a HEPA filter designed to remove all microorganisms from the air supply. The atmosphere was exhausted from the isolators into the laboratory through a similar system and was charcoal-filtered to remove any odors.

The glove ports, one set on each side of the flexible isolator worked well. Particularly useful was a sliding feature that enabled the operator to reach any portion of the isolator without uncomfortable stretching. Air jets inside the glove mitigated some of the discomfort and sweating normally occuring to an operator while working for an extended period using rubber gloves.

One set of glove ports on each side was found to be inadequate. On many occasions, another set of hands (three people) was needed to accomplish a particular task and this was, of course, impossible. Bathing the animals was a particularly unpleasant and difficult task for two people working remotely. When sampling certain body areas, the addition of another person would have considerably reduced the time factor.

The flexible isolator could be easily zipped open for cleaning. Closing the isolator was not as simple and plastic zippers such as GE used have their disadvantages, one disadvantage being they fail to hold tight. After one zipper failure, (fortunately when the animal was in the stainless steel section), GE resorted to closing the zipper on the isolator and reinforcing the entire

*Manufactured by Matthews Research Co., Alexandria, Va.

zipper with two-inch vinyl tape. A steel zipper such as that used on astronaut space suits is recommended.

The isolators were kept under constant positive pressure when not actually opened for cleaning. Sterilization was accomplished using freon powered peracetic acid sprayers. The isolators could then be leak-tested immediately after sterilization. Figures 13, 14, and 15 illustrate other pertinent features of the system. Figure 16 shows the tunnel entrance from main isolator to the stainless steel holding section.

The system's air supply can be interconnected by ports built into the isolator walls. During the major portion of the experiment, these were taped shut but during the last phase, opening the ports enables GE to reunite the animals in the sense of transferring their microflora from one animal to another via the atmosphere.

GE's overall impression of the isolator system, in spite of the drawbacks reported, was superior.

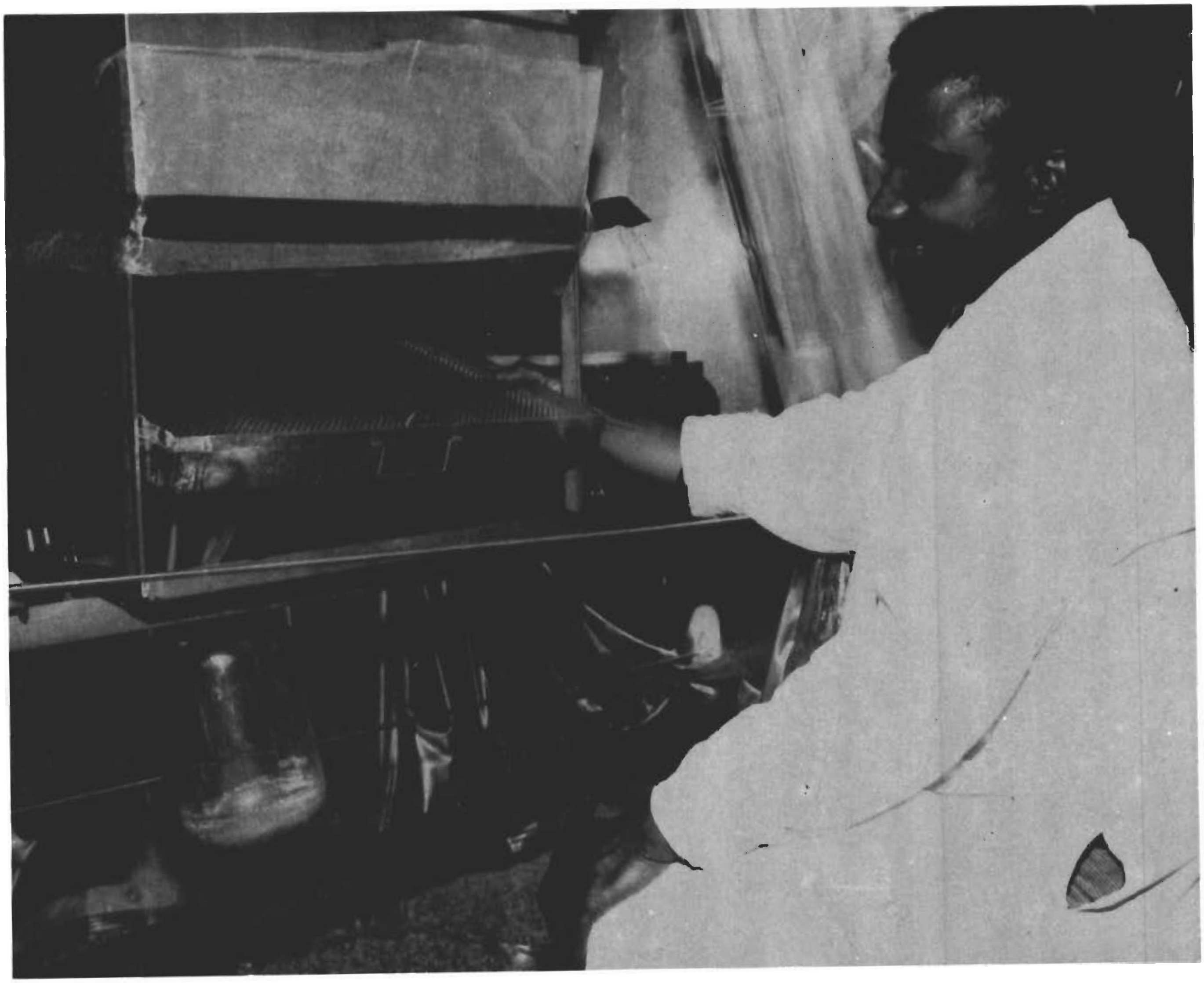


Figure 10. Technician Removing Feces Tray for Cleaning, Urine Collection Bottle Below

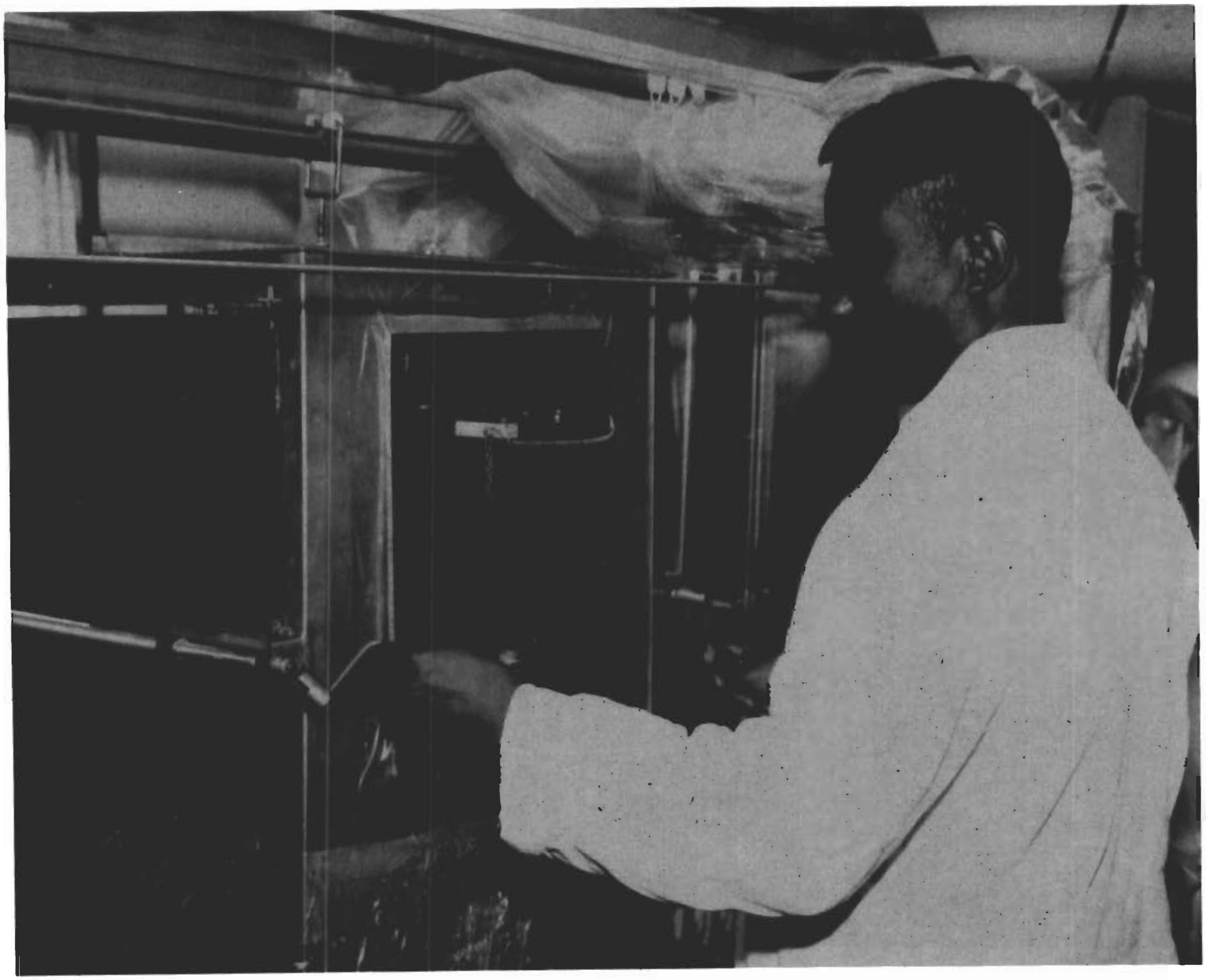


Figure 11. Technician Cranks to Operate Squeeze Cage Panel

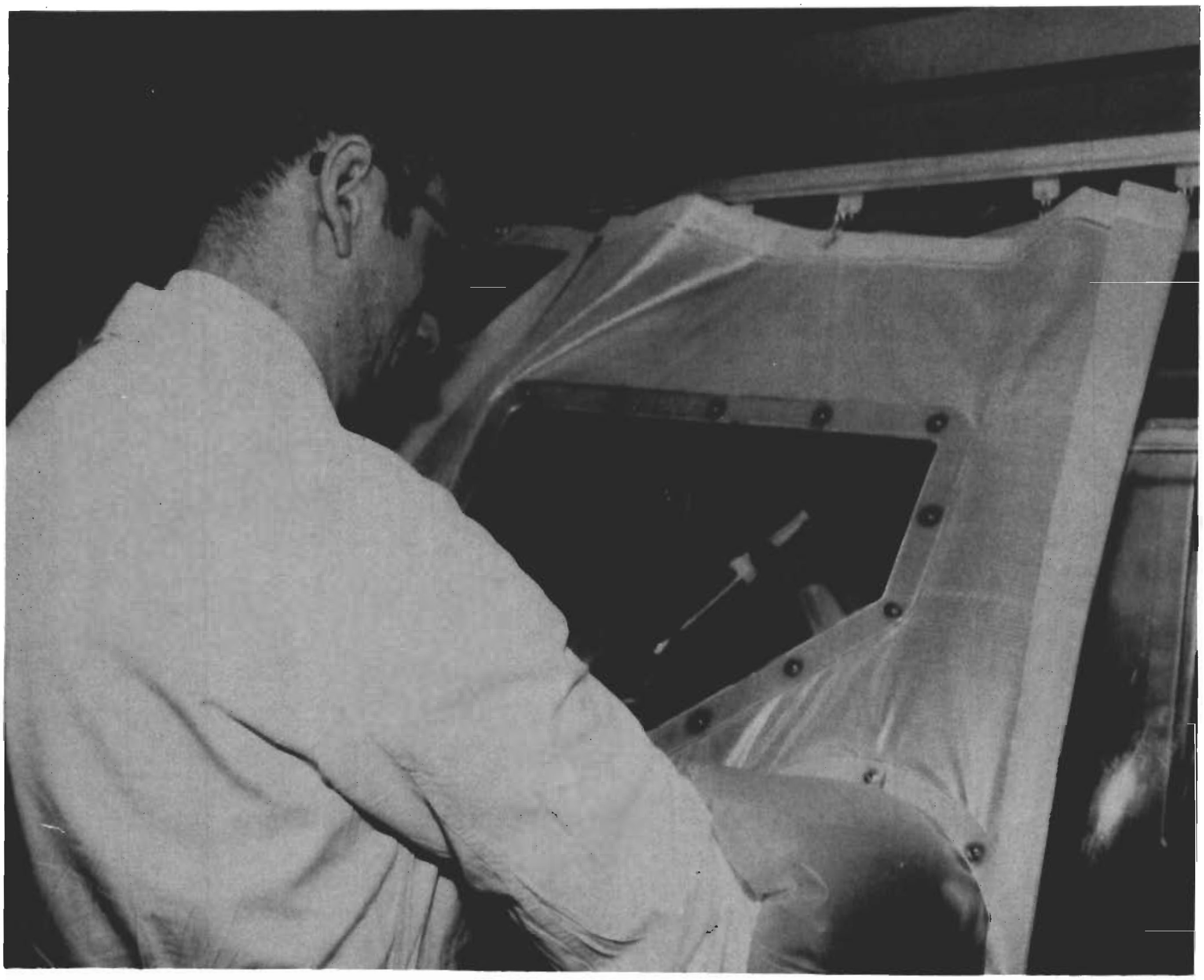


Figure 12. Rigid Plexiglas Window in Isolator-Bacteriologist Preparing Swab for Sampling

Figure 13. Scale and Cage System Used for Recording Animal Weights

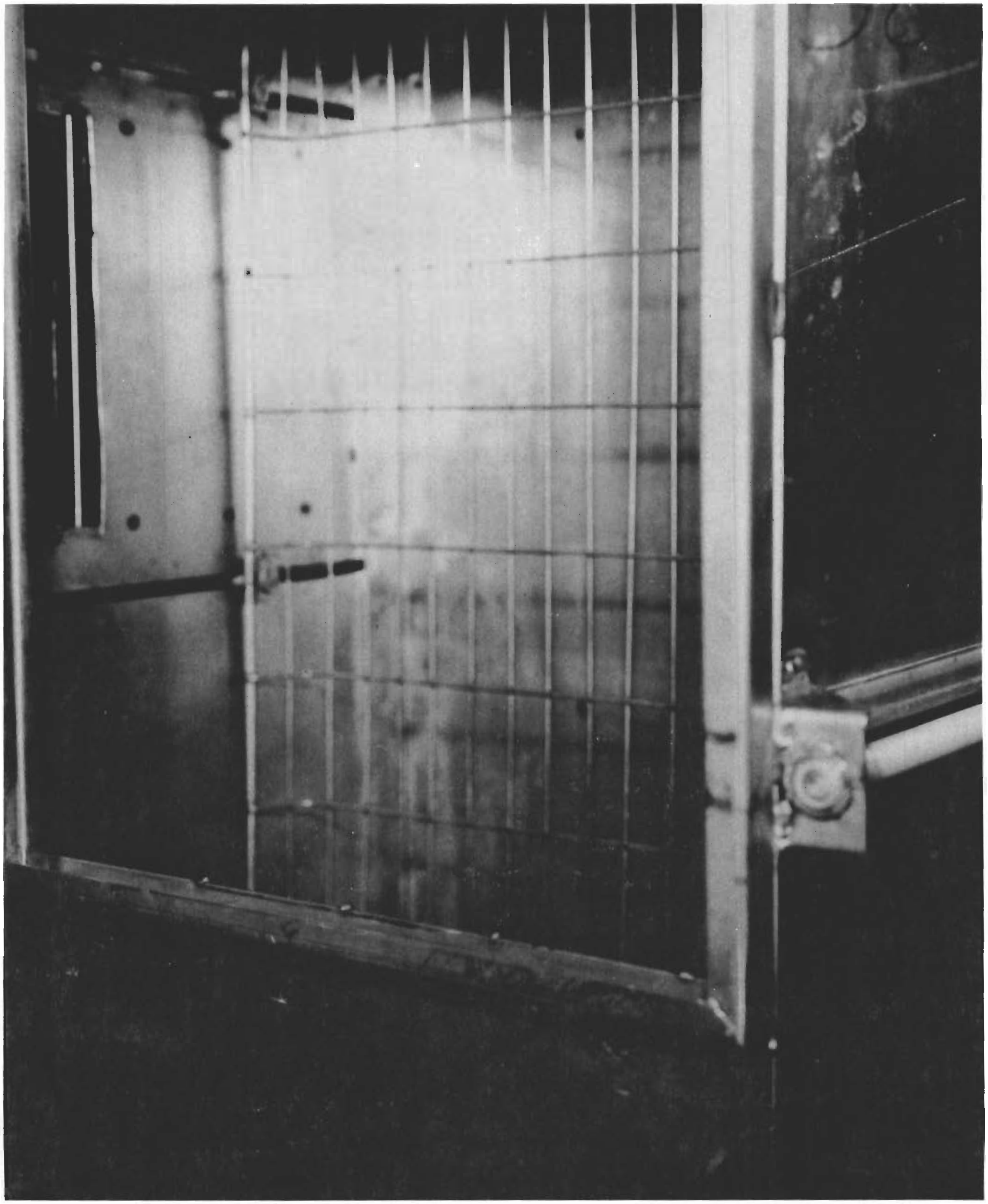


Figure 14. Stainless Steel Cage Illustrating Crank-Operated Squeeze Panel

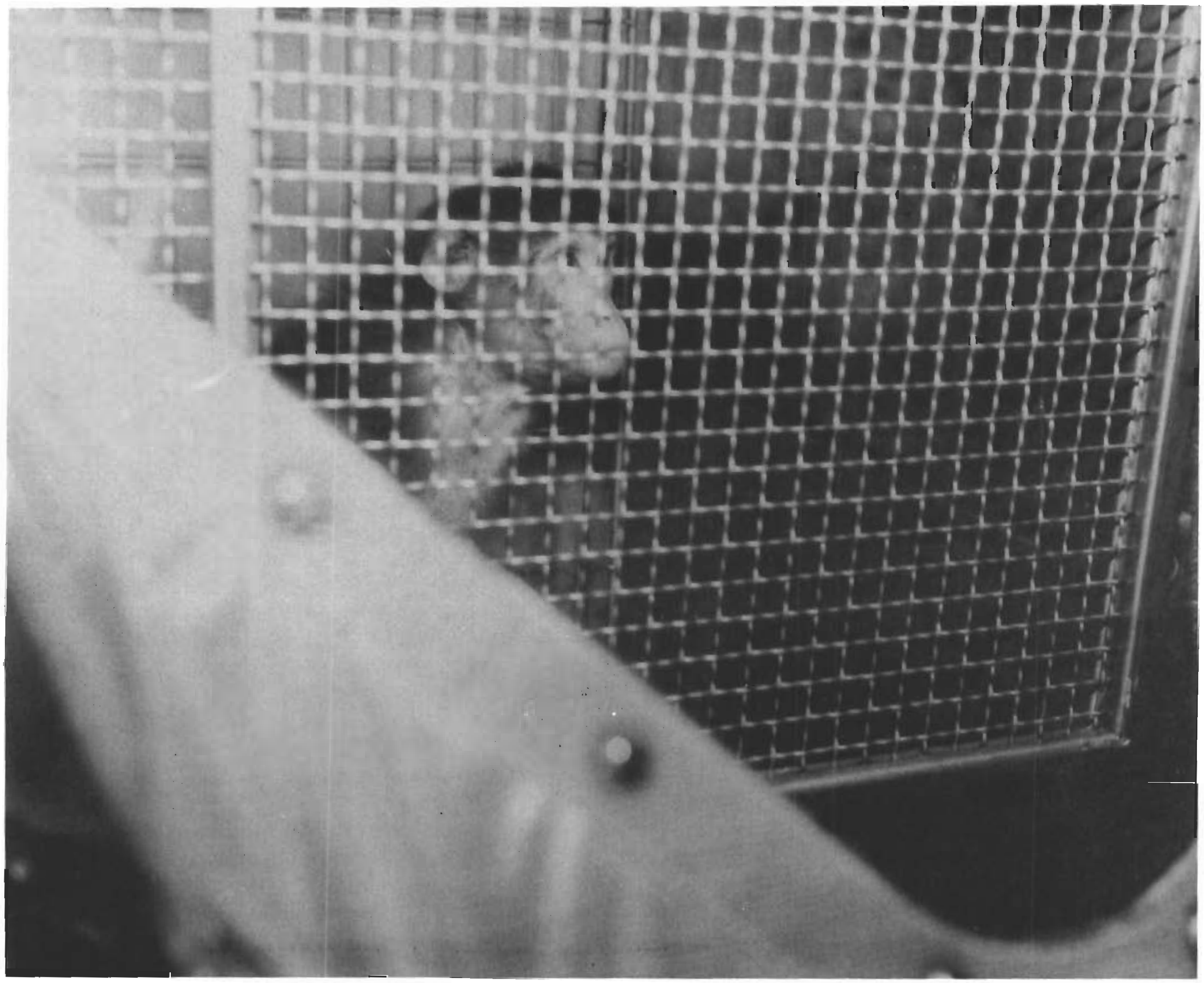


Figure 15. View Through Clear Plastic Section of Animal in Cage

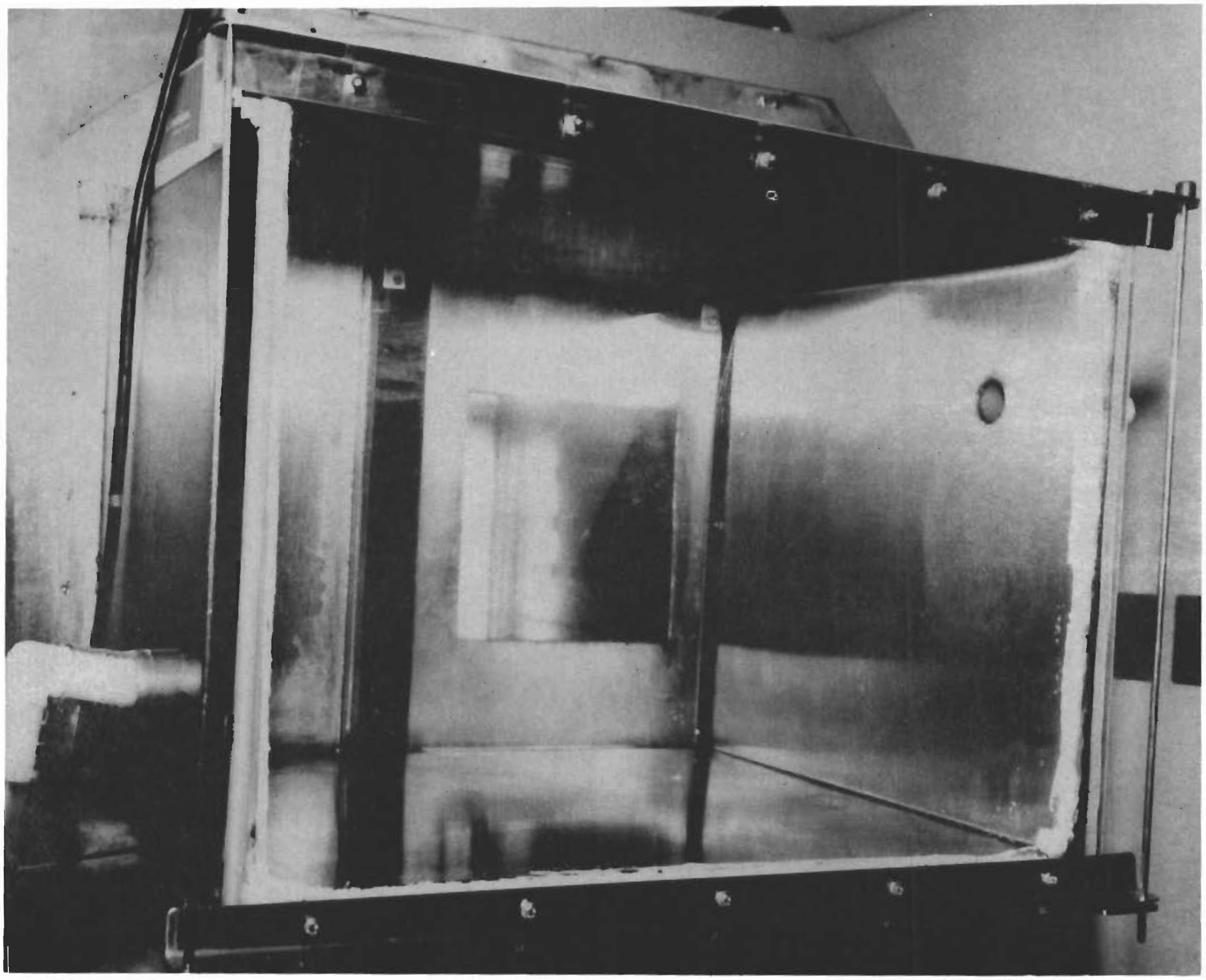


Figure 16. Stainless Steel Holding Isolator Showing Connecting Tunnel Entrance

APPENDIX V

COMPUTER PRINTOUTS

Introduction and Explanation

Appendix V presents some of the actual printouts from the GE DSCS. Figure 17 illustrates the "LOGREP" Program in the modified Fortran II compiler language used by the GE DSCS. This program was developed especially for this study to report any segment of the log information on any one of the monkeys studied. The operation of the program is demonstrated in Figure 18. This shows the entire sequence of events when the computer is dialed via regular telephone lines from the DSCS teletype terminal. After the telephone connection is made, the computer types out on the terminal "THIS IS THE MSD 265 SYSTEM" and requests authorization to proceed by asking for a legitimate user number which can be billed for time-sharing usage: " USER NUMBER -- ." The operator at the terminal replies with the number which has been assigned. Then the computer asks for the system: "SYSTEM--."

The operator replies in this case with: "$FORTRAN." The computer next asks whether it is to prepare itself for compilation of a new program or is to call up some old (old means previously stored) Fortran program stored in the auxiliary memory (magnetic disc area) assigned to this user number. Since the program of interest is already stored, the operator replies: "$OLD." The computer types out "WAIT" while it switches all the users on-line in and out of control until this operator's turn comes up again. It then searches its auxiliary memory for the program called for and when it finds the "LOGREP" program it signals the operator at the remote terminal by typing out "READY." Since the operator wishes now to use the program as it is, he types in "$RUN." He receives another "WAIT" signal in return, and, when his turn comes up again, it proceeds to run the program. First, it types out the memory area which is available for use by the program. This is the difference, in octal arithmetic, between the two numbers following "LOAD LIMITS" and is determined by the memory storage space used up by the Fortran compiler and by the machine "object" program in machine language generated by the user "source" program in Fortran. Next, it asks for input data from the operator by calling for an animal number. Then it queries the operator as to whether the entire animal log is desired. If the answer were "YES," the computer would then proceed to type out the entire log for the animal designated. The operator in this case replies "NO" so the computer proceeds to ask for the time interval between which log data is desired. The operator replies August 15, 1966 to September 7, 1966 in the format dictated by the computer: "60815, 60907." The computer then proceeds to type out the answer and stops itself when completed.

Some excerpts from the log of animal No. 118 are shown in Figure 19. An example of the operation of the updated, more flexible form of the Log Report Generator program is found in Figure 21. All programs involved are listed in Figures 17 and 20.

As has been described in the body of the report, all of the log data and microbial data (microbial identifications and numerical counts resulting from sampling of specific sites) was stored in the computer in auxiliary memory (magnetic disc storage). Copies of the forms used for collecting these data are shown in Figures 22 and 23. A major facility was developed for the purposes of searching a list of descriptors, and pulling out of a primary file the record numbers to which these descriptors applied. (The list of descriptors is shown in Figure 24.) These record numbers (addresses) can then be printed out and a secondary file containing the actual records can be examined word by word, in part or in full by a number of programs written for these purposes. A second part of this facility allows the operator to "SEND" these addresses to another part of auxiliary memory storage (Scratch Area) where they are used by another major program (REPORT GENERATOR) to go back and search the secondary file for actual microbial data and to display the data in twenty-four possible matrices depending upon the hierarchy used.

The search of the primary file employs any combination of descriptors employing a programmed logical format. A slash (/) is equivalent to "OR;" a plus sign (+) is equivalent to "AND;" a minus sign (-) is equivalent to "AND NOT;" and a period (.) is equivalent to "END OF LOGICAL STATEMENT."

The operation of the search program is illustrated in Figure 25. The same sequence of steps takes place when the telephone connection is made to the computer as described above. In reply to the computer's demand for a "SYSTEM --," however, the operator at the remote teletype calls for "$INØØ1" which is the routine for searching the primary file. The computer replies "READY" and the operator types in a logical combination of descriptors. He wants to know what the computer has stored on animal Nos. 115 or 121, which contains information on Corynebacteria but not Staphylococci in its sampling data. The operator, in effect states his demand by using the logical statement shown in Figure 25. Translated, this reads: (Animal No. 115 or Animal No. 121) and Corynebacterium, but not Staphylococcus, and Sampled. The operator then types "$RUN." The computer replies: "0007HITS" which means that it has searched its entire primary file containing the record addresses for all the data and has found seven records which contain data which fit the logical statement of descriptors. It then types out "PRINT: =," which is a demand to know whether it should type out these record addresses. If "NONE" were typed in by the operator, it would go on to the next step. But in this case, the operator tells the computer to type out the addresses by replaying "ALL." The computer then types out the seven addresses. It goes on to type "SEND:=." This is a request it asks the operator which means, do you want these addresses sent to a separate area of auxiliary memory (Scratch Area) where they can be examined by other programs. In this case, the operator does not want this done, so he replies "NONE." He could have any or all of these addresses sent to this memory area by typing in the addresses separately or by replying "ALL" in which case all seven addresses would be sent. (This last facility will later be seen to be essential to the operation of the Report Generator Program.) After this sequence is completed, the computer indicates that it has carried out the operation called for by replying "QUESTION COMPLETED." It is now ready for the next search.

The Report Generator Program was a major accomplishment of this effort. It permits the display of microbial data records whose addresses have been "sent" to an area of auxiliary memory (Scratch Area) by the search routine in 24 different ways according to the hierarchy requested. The four members of the hierarchy are animal (No. 1), date (No. 2), site of sampling (No. 3) and class-genus found (No. 4). The format of the printout of microbial data is determined by the order in which these categories are typed into the computer from the remote terminal. The best way to understand this program is to study the examples in Figure 27. The program including its subroutines is listed in the GE DSCS's version of Fortran II in Figure 26.

Some abbreviations in the examples of output from the Report Generator Program require explanation: a number in the form "XE+Z" means $X \cdot 10^Z$; "NC" means the genus in question was identified qualitatively but no count is available; a number in the form "N-Y" means that no growth was observed at a dilution of 10^{-Y}. Site codes are as follows:

1 = Conjunctiva

2 = Throat

3 = Gingiva

4 = Axilla

5 = Groin

6 = Glans Penis

7 = Feces

Class-Genus codes are as follows:

CODE	CLASS	GENUS
101	Bacteria	Streptococcus
102	Bacteria	Corynebacterium
103	Bacteria	Proteus
104	Bacteria	Excherichia
105	Bacteria	Staphylococcus
106	Bacteria	Aerobacter
107	Bacteria	Shigella
108	Bacteria	Escherichia-Aerobacter Group
109	Bacteria	Pseudomonas
110	Bacteria	Salmonella
111	Bacteria	Lactobacillus
112	Bacteria	Bacteroides
113	Bacteria	Clostridium
214	Ascomycetes	Aspergillus
115	Bacteria	Alcaligenes

Dates are printed out in the form 70314 where the first digit refers to year (1967), the next two digits refer to month (March) and the last two digits refer to day (14th). To make clear what input is typed in by the operator and what output is printed out by the computer, the operator input has been underlined. To make new inputs of hierarchy stand out, "HIERARCHY RECORDED" has been printed beside the new orders of numbers.

The data in any records whose addresses are sent to the Scratch Area can be read directly (whether animal log or microbial data records) by means of the secondary file search program. This program also permits the teletype operator to read the contents of any record requested by its address. The program and examples of its operation are listed in Figures 28 and 29.

Examples of coded data records as actually stored in auxiliary memory (magnetic disc) are shown in Figures 30 and 31. The interaction of the Primary File, Secondary File, Scratch Area and Fortran Programs, which as a whole compose the Data Management System, is diagrammed in Figure 32.

```
$LIST

      COMMON DUM(27),KR(64),K(5,300,1),KCD1(9,6),KCD2(120,10)
      IRAM=1545
      CALL SYSL01(IRAM,KR)
      NA=1
      DO 60 I=1,9
      DO 60 J1=1,6
      KCD1(I,J1)=KR(NA)
      NA=NA+1
   60 CONTINUE
      IRAM=3040
   62 IRAM=IRAM+1
      NB=1
      CALLSYSL01(IRAM,KR)
      IF(IRAM-3041)63,63
      NB=1
      GO TO 61
   63 DO 61 I=1,100
      DO 61 J1=1,10
   65 KCD2(I,J1)=KR(NB)
      NB=NB+1
      IF(NB-61)61,62,61
   61 CONTINUE
   64 PRINT14
   14 FORMAT("TYPE ANIMAL NUMBER FOR WHICH LOG INFORMATION "
     1"IS DESIRED"/)
      READ:N1
      PRINT16
   16 FORMAT("DO YOU WISH THE ENTIRE LOG (YES/NO)")
      READ17,N2
   17 FORMAT(A3)
      IF(N2-/454660)66,18,66
   66 IF(N2-/702562)19,19,19
   18 PRINT20
   20 FORMAT("TYPE STARTING AND ENDING DATES OF THE DESIRED"
     1"PRINT OUT IN THE FORM--70114,71105--WHERE 7 REPRESENTS"
     2" THE YEAR, 01 AND 11 REPRESENT THE MONTHS JAN.(01) AND "
     2"NOV.(11) AND 14 AND 05 REPRESENT THE DAYS OF THOSE MONTHS"/
      READ:N3,N4
   19 IF(N1-115)26,21,26
   26 IF(N1-116)27,22,27
   27 IF(N1-118)28,23,28
   28 IF(N1-119)29,24,29
   29 IF(N1-120)70,25,70
   70 IF(N1-121)18,71,18
   21 IRAM1=1561
      GO TO30
   22 IRAM1=1801
      GO TO 30
```

Figure 17. LOGREP Program (Original Form) (Sheet 1 of 3)

```
 23   IRAM1=2241
      GO TO 30
 24   IRAM1=2321
      GO TO 30
 25   IRAM1=2561
      GO TO 30
 71   IRAM1=2801
 30   IRAM2=IRAM1+30
      II=0
      DO 31 I2=IRAM1,IRAM2
      IF(IRAM1-I2)75,47,75
 75   IF(NX-9)48,47,47
 47   NX=0
      CALL SYSL01(I2,KR)
      NH=NH+1
      DO 32 J=1,57,7
      IF(KR(J))32,33,32
 32      CONTINUE
      LS=63
      GO TO 51
 33   LS=J-1
 51   DO 31 JJ=1,LS,7
      II=II+1
      K(2,II)=KR(JJ)
      K(3,II)=KR(JJ+1)
      K(4,II)=KR(JJ+5)
      K(5,II)=KR(JJ+6)
      IF(K(2,II)-9)101,102,101
 102  K(5,II)=/514560
 101  AA=KR(JJ+4)/10.-6
      K(1,II)=AA*100000+KR(JJ+2)*100+KR(JJ+3)
      NX=NX+1
      IF(K(3,II)-30)31,900,31
 900  K(4,II)=/003301
      K(5,II)=/472360
 31   CONTINUE
 48   IF(N3)36,35,36
 36   DO 40 II1=1,II
      IF(K(1,II1)-N3)40,42,42
 40   CONTINUE
 42   DO 41 II2=II1,II
      IF(K(1,II2)-N4)41,43,43
 41   CONTINUE
      GO TO 43
 35   II1=1
      II2=II
 43   DO 45 II=II1,II2
      KYR=K(1,II)/10000+60
      KMO=XMODF(K(1,II),10000)/100
      KDA=XMODF(K(1,II),100)
      CALL CONVERT(KYR,KMO,KDA,IYR,IMO,IDA)
      IF(K(4,II)-/003301)84,82,84
```

Figure 17. LOGREP Program (Original Form) (Sheet 2 of 3)

```
84    IF(K(4,II)-/606060)81,82,81
82    PRINT83,IMO,IDA,IYR,(KCD1(K(2,II),J),J=1,6),(KCD2(K(3,II),L)
     1,L=1,10),K(4,II),K(5,II)
      GO TO 45
81    PRINT80,IMO,IDA,IYR,(KCD1(K(2,II),J),J=1,6),(KCD2(K(3,II),L)
     1,L=1,10),K(4,II),K(5,II)
80    FORMAT(1X,A2,"/",A2,"/",A2,2X,6A3,2X,10A3,1X,I7,A3)
83     FORMAT(1X,A2,"/",A2,"/",A2,2X,6A3,2X,10A3,2X,A6,A3)
45    CONTINUE
      STOP
      END
      SUBROUTINE CONVERT(KYR,KMO,KDA,IYR,IMO,IDA)
      K=10
      IOCTA=/010000
      IOCT=/000100
      IMO=IDA=/000000
      DO 100 I=1,31
      IF(I-K)600,500,600
500   IDA=IDA+IOCTA-/001100
      K=K+10
      GO TO 400
600   IDA=IDA+IOCT
400   IF(KDA-I)100,110,100
100   CONTINUE
110   K=10
       DO200 I=1,12
      IF(I-K)150,140,150
140   IMO=IMO+IOCTA-/001100
      GO TO 170
150   IMO=IMO+IOCT
170   IF(KMO-I)200,210,200
200   CONTINUE
210   IF(KYR-66)230,220,230
230    IF(KYR-67)250,240,250
250   PRINT270
270   FORMAT("ERROR IN YEAR INPUT ")
240   IYR=/060700
      RETURN
220   IYR=/060600
      RETURN
      END
```

Figure 17. LOGREP Program (Original Form) (Sheet 3 of 3)

```
THIS IS THE MSD 265 SYSTEM

15  USER NUMBER--
SYSTEM--$FORTRAN
RUN TYPE-- $OLD
OLD PROGRAM NAME--LOGREP
WAIT.

READY.

$RUN
WAIT.

LOAD LIMITS 07523 12275

TYPE ANIMAL NUMBER FOR WHICH LOG INFORMATION IS DESIRED
:=115

DO YOU WISH THE ENTIRE LOG (YES/NO):=NO

TYPE STARTING AND ENDING DATES OF THE DESIRED
PRINT OUT IN THE FORM--70114,71105--WHERE 7 REPRESENTS
 THE YEAR, 01 AND 11 REPRESENT THE MONTHS JAN.(01) AND
NOV.(11) AND 14 AND 05 REPRESENT THE DAYS OF THOSE MONTHS
:=60815,60907

 08/15/66  ANTIBIOTICS      CHLORAMPHENICOL+FUROXONE        110MG
 08/16/66  ANTIBIOTICS      CHLORAMPHENICOL+FUROXONE        110MG
 08/17/66  OBSERVATIONS     ANTIHELMINTHIC TRET.(TRIBNDZE)   75MGK
 08/17/66  OBSERVATIONS     TB TESTED NEGATIVE
 08/22/66  OBSERVATIONS     STOOL CULTURE NEGATIVE(SAL+SH)
 08/22/66  OBSERVATIONS     BLOOD SMEAR NEGATIVE(MALARIA)
 09/01/66  OBSERVATIONS     TB TESTED NEGATIVE
 09/07/66  OBSERVATIONS     ANIMAL RECEIVED AT GE
*STOP     0 AT 07250
```

ABBREVIATIONS: MG = MILLIGRAMS; MGK = MILLIGRAMS PER KILOGRAM

Figure 18. Example of Operation of LOGREP Program

09/07/66	OBSERVATIONS	ANIMAL RECEIVED AT GE	
09/07/66	DIET (STERILE/NON)	NON STERILE	
09/07/66	DIET NAMES	PURINA MONKEY CHOW+BANAS.+ORS.	
09/07/66	OTHER ANIMALS	ANIMAL ADDED	115AN
09/07/66	OTHER ANIMALS	ANIMAL ADDED	116AN
09/07/66	OTHER ANIMALS	ANIMAL ADDED	119AN
09/07/66	OTHER ANIMALS	ANIMAL ADDED	120AN
09/07/66	OTHER ANIMALS	ANIMAL ADDED	121AN
03/02/67	ISOLATION (YES/NO)	YES	
03/02/67	DIET (STERILE/NON)	STERILE	
03/02/67	DIET NAMES	SIMILAC+IRON+VITAMINS	
03/02/67	OTHER ANIMALS	ANIMAL REMOVED	115AN
03/02/67	OTHER ANIMALS	ANIMAL REMOVED	121AN
03/03/67	SAMPLES TAKEN	FECES	182171RN
03/06/67	SAMPLES TAKEN	FECES	182172RN
03/10/67	ORGANISMS INGESTED	LACTINEX	1GM
03/23/67	DIET (STERILE/NON)	STERILE	
03/23/67	DIET NAMES	ROCKLAND PRIMATE DIET+VITAMINS	
06/23/67	SAMPLES TAKEN	CONJUNTIVA	182065RN
06/23/67	SAMPLES TAKEN	THROAT	182066RN
06/23/67	SAMPLES TAKEN	GINGIVA	182067RN
06/23/67	SAMPLES TAKEN	AXILLA	182068RN
06/23/67	SAMPLES TAKEN	GROIN	182069RN
06/23/67	SAMPLES TAKEN	GLANS PENIS	182070RN
06/30/67	ANTIBIOTICS	TETREX F	250MG
07/01/67	ANTIBIOTICS	TETREX F	250MG
07/01/67	SAMPLES TAKEN	FECES	182174RN
07/02/67	ANTIBIOTICS	TETREX F	250MG
07/03/67	ANTIBIOTICS	TETREX F	250MG
07/03/67	SAMPLES TAKEN	FECES	182175RN
07/04/67	ANTIBIOTICS	TETREX F	250MG
07/05/67	ANTIBIOTICS	TETREX F	250MG
07/05/67	SAMPLES TAKEN	FECES	182176RN
07/06/67	ANTIBIOTICS	TETREX F	250MG
07/06/67	ANTISEPTIC WASHES	PERACETIC ACID	0.1PC
07/07/67	ANTIBIOTICS	TETREX F	250MG
07/07/67	SAMPLES TAKEN	FECES	182177RN
07/07/67	SAMPLES TAKEN	CONJUNTIVA	182073RN
07/07/67	SAMPLES TAKEN	THROAT	182074RN
07/07/67	SAMPLES TAKEN	GINGIVA	182075RN
07/07/67	SAMPLES TAKEN	AXILLA	182076RN
07/07/67	SAMPLES TAKEN	GROIN	182077RN
07/07/67	SAMPLES TAKEN	GLANS PENIS	182078RN

ABBREVIATIONS: AN = ANIMAL NUMBER; RN = RECORD NUMBER; GM = GRAM; MG = MILLIGRAM; PC = PERCENT.

Figure 19. Excerpts From Log of Animal No. 118

```
OLD PROGRAM NAME--LOGREP

$LIST

        COMMON DUM(27),KR(64),NS(9),K(5,225,1),KCD1(9,6),KCD2(99,10)
       1N1(6)
        IRAM=1545
        CALL RAM(IRAM)
   64   PRINT 402
  402   FORMAT("TYPE THE NUMBER OF ANIMALS FOR WHICH LOG INFORMATION
       1" IS DESIRED (6 MAX.)"/)
        READ:NN1
        PRINT403
  403   FORMAT("TYPE ANIMAL NUMBERS (115,116,120,ECT.)"/)
        READ:(N1(I),I=1,NN1)
        DO 45 I=1,NN1
        N3=N4=0
        PRINT485,N1(I)
  485   FORMAT(/"ANIMAL"1X,I4)
        PRINT16
  16    FORMAT("DO YOU WISH THE ENTIRE LOG (YES/NO)")
        READ17,N2
   17   FORMAT(A3)
        IF(N2-/454660)66,18,66
  66    IF(N2-/702562)19,19,19
  18    PRINT20
  20    FORMAT("TYPE STARTING AND ENDING DATES OF THE DESIRED"
       1"PRINT OUT IN THE FORM--70114,70223--"//
       2"DATE CODE EXPLANATION"//3X"FOR DATE CODE 70114"/
       37X"7=YEAR(1967)"/6X"01=MONTH(JAN.)"/6X"14=DAY(JAN.14)"/)
        READ:N3,N4
  19    CALL LOG(I,IRAM1,IRAM2)
        II=0
        DO 31 I2=IRAM1,IRAM2
        IF(IRAM1-I2)75,47,75
  75    IF(NX-9)48,47,47
  47    NX=0
        CALL SYSL01(I2,KR)
        NH=NH+1
        DO 32 J=1,57,7
        IF(KR(J))32,33,32
   32      CONTINUE
        LS=63
        GO TO 51
  33    LS=J-1
        IF(LS)51,48,51
  51    DO 31 JJ=1,LS,7
        II=II+1
        K(2,II)=KR(JJ)
        K(3,II)=KR(JJ+1)
        K(4,II)=KR(JJ+5)
        K(5,II)=KR(JJ+6)
        IF(K(2,II)-9)101,102,101
  102   K(5,II)=/514560
  101   AA=KR(JJ+4)/10.-6
        K(1,II)=AA*100000+KR(JJ+2)*100+KR(JJ+3)
        NX=NX+1
        IF(K(3,II)-30)31,900,31
  900   K(4,II)=/003301
        K(5,II)=/472360
  31    CONTINUE
  48    IF(N3)36,35,36
  36    DO 40 III=1,II
        IF(K(1,III)-N3)40,42,42
  40    CONTINUE
        GO TO 42
```

Figure 20. Listing of the Main Program and Subroutines Which Make Up the Final Form of Log Report Generator Program (Sheet 1 of 6)

```
42    DO 41 II2=II1,II
      IF(K(1,II2)-N4)41,41,310
310   II2=II2-1
      GO TO 43
41    CONTINUE
      GO TO 43
35    II1=1
      II2=II
      N3=K(1,II1)
      N4=K(1,II2)
43    CALL SUM(II1,II2)
      CALL DATE(N3,KYR,KMO,KDA)
      CALL CONVERT(KYR,KMO,KDA,IYR,IMO,IDA)
      CALL DATE(N4,KYR,KMO,KDA)
      CALL CONVERT(KYR,KMO,KDA,IYR1,IMO1,IDA1)
      PRINT309,N1(I),IMO,IDA,IYR,IMO1,IDA1,IYR1,NS(1),NS(2),NS(4),
     1NS(5),NS(6),NS(7),NS(9)
309   FORMAT(///,28X,"LOG INFORMATION",///,30X,"ANIMAL",1X,I4,///
     119X"PERIOD  FROM ",A2,"/",A2,"/",A2," TO ",A2,"/",A2,"/",A2/
     2/23X"TOTALS FOR THIS PERIOD"//19X"ACTIVITIES"20X"FREQ."//
     316X"ENVIRONMENT CHANGED"11X,I3,"  TIMES"/16X"DIET CHANGED"
     418X,I3,"  TIMES"/16X"ORGANISMS INGESTED"12X,I3,"  TIMES"
     5/16X"ANTISEPTIC WASHES USED"8X,I3,"  TIMES"/16X"ANTIBIOTICS"
     6" USED"14X,I3,"  TIMES"/16X"OBSERVATIONS MADE"13X,I3,"  TIME
     7/16X"SAMPLES TAKEN"17X,I3,"  TIMES"/)
      CALL TUMS(II1,II2)
      PRINT486
486   FORMAT(//"DO YOU WISH DETAILED LOG INFORMATION (YES/NO)"/)
      READ505,NX2
505   FORMAT(A3)
      IF(NX2-/454660)524,45,524
524   IF(NX2-/702562)506,506,506
506   PRINT507
507   FORMAT(//"INDICATE WHICH OF THE FOLLOWING PRINT OUTS YOU DES
     1/1X"1"3X"COMPLETE REPORT BY ACTIVITY"/1X"2"3X"COMPLETE RE"
     2"PORT BY DATE"/1X"3"3X"COMPLETE REPORT OF ONE ACTIVITY"/
     31X"4"3X"COMPLETE REPORT BY DATE AND ACTIVITY"/
     41X"5"3X"CHANGE PERIOD"/1X"6"3X"NEXT LEVEL"/)
      READ:NX3
      GO TO(508,514,510,508,18,45),NX3
510   PRINT512
512   FORMAT(//"INDICATE ONE OF THE FOLLOWING ACTIVITIES"/
     1/1X"1"3X"ENVIRONMENT"/1X"2"3X"DIET"/1X"3"3X"ORGANISMS IN"
     2"GESTED"/1X"4"3X"ANTISEPTIC WASHES"/1X"5"3X"ANTIBIOTICS"
     3/1X"6"3X"OBSERVATIONS"/1X"7"3X"SAMPLES TAKEN"/)
      READ:IZ1
      GO TO (394,394,395,395,395,395,396),IZ1
394   IZ2=IZ1
      GO TO 513
395   IZ2=IZ1+1
      IZ1=IZ2
      GO TO 513
396   IZ1=IZ1+2
      IZ2=IZ1
      GO TO 513
508   IZ1=1
      IZ2=9
513   CALL SUMM(II1,II2,I,IZ1,IZ2,IMO,IDA,IYR,IMO1,IDA1,IYR1)
      IF(NX3-4)506,514,506
514   PRINT515,N1(I),IMO,IDA,IYR,IMO1,IDA1,IYR1
515   FORMAT(///31X"ANIMAL"1X,I4,///19X"PERIOD  FROM ",A2,"/"
     1,A2,"/",A2," TO ",A2,"/",A2,"/",A2,//24X"DETAILED LOG IN"
     2"FORMATION"//3X"DATES"5X"ACTIVITIES"10X"ELEMENTS"24X"NUMBERS
509   DO 45 II=II1,II2
      CALL DATE(K(1,II),KYR,KMO,KDA)
      IF(KMM-KMO)516,517,516
```

Figure 20. Listing of the Main Program and Subroutines Which Make Up the Final Form of Log Report Generator Program (Sheet 2 of 6)

```
516     PRINT518
518     FORMAT(" ")
517     KMM=KMO
        CALL CONVERT(KYR,KMO,KDA,IYR,IMO,IDA)
        IF(K(4,II)-/003301)84,82,84
84      IF(K(4,II)-/606060)81,82,81
82      PRINT83,IMO,IDA,IYR,(KCD1(K(2,II),J),J=1,6),(KCD2(K(3,II),L)
       1,L=1,10),K(4,II),K(5,II)
        GO TO 46
81      PRINT80,IMO,IDA,IYR,(KCD1(K(2,II),J),J=1,6),(KCD2(K(3,II),L)
       1,L=1,10),K(4,II),K(5,II)
80      FORMAT(1X,A2,"/",A2,"/",A2,2X,6A3,2X,10A3,1X,I7,A3)
83       FORMAT(1X,A2,"/",A2,"/",A2,2X,6A3,2X,10A3,2X,A6,A3)
46      IF(II-II2)45,506,45
45      CONTINUE
        STOP
        END
```

```
$OLD
OLD PROGRAM NAME--SUM
WAIT.

READY.

$LIST
```

```
        SUBROUTINE CONVERT(KYR,KMO,KDA,IYR,IMO,IDA)
        K=10
        IOCTA=/010000
        IOCT=/000100
        IMO=IDA=000000
        DO 100 I=1,31
        IF(I-K)600,500,600
500     IDA=IDA+IOCTA-/001100
        K=K+10
        GO TO 400
600     IDA=IDA+IOCT
400     IF(KDA-I)100,110,100
100     CONTINUE
110     K=10
         DO200 I=1,12
        IF(I-K)150,140,150
140     IMO=IMO+IOCTA-/001100
        GO TO 170
150     IMO=IMO+IOCT
170     IF(KMO-I)200,210,200
200     CONTINUE
210     IF(KYR-66)230,220,230
230      IF(KYR-67)250,240,250
250     PRINT270
270     FORMAT("ERROR IN YEAR INPUT ")
240     IYR=/060700
        RETURN
220     IYR=/060600
        RETURN
        END
        SUBROUTINE SUM(II1,II2)
        COMMON DUM(27),KR(64),NS(9),K(5,225,1)
        DO 594 JJX=1,9
594     NS(JJX)=0
        DO 300 J=1,9
```

Figure 20. Listing of the Main Program and Subroutines Which Make Up the Final Form of Log Report Generator Program (Sheet 3 of 6)

```
      IF(J-3)299,300,299
  299 IF(J-8)298,300,298
  298 DO 300 II=II1,II2
      IF(K(2,II)-J)304,301,304
      PRINT:NS(J)
  301 NS(J)=NS(J)+1
  304 IF(J-2)303,300,300
  303 IF(K(2,II)-(J+7))300,302,300
  302 NS(J)=NS(J)+1
  300 CONTINUE
      RETURN
      END
      SUBROUTINE DATE(KX,KYR,KMO,KDA)
      KYR=KX/10000+60
      KMO=XMODF(KX,10000)/100
      KDA=XMODF(KX,100)
      RETURN
      END
      SUBROUTINE SUMM(II1,II2,I,IZ1,IZ2,IMO,IDA,IYR,IMO1,IDA1,IYR1)
      COMMON DUM(27),KR(64),NS(9),K(5,225,1),KCD1(9,6),KCD2(99,10)
     1N1(6)
      PRINT702,N1(I),IMO,IDA,IYR,IMO1,IDA1,IYR1
  702 FORMAT(///31X"ANIMAL"1X,I4,///19X"PERIOD   FROM ",A2,"/"
     1,A2,"/",A2," TO ",A2,"/",A2,"/",A2//25X"DETAILED LOG IN"
     2"FORMATION"//)
  790 DO 703 J=IZ1,IZ2
      IF(J-3)761,703,761
  761 IF(J-8)715,703,715
  715 PRINT716,(KCD1(J,L),L=1,6)
      PRINT745
  716 FORMAT(/2X,6A3)
      GO TO(712,714,703,717,719,721,722,703,723),J
  745 FORMAT(//11X"ELEMENTS"23X"DATES"5X"NUMBERS"/)
  712 NK1=1
      NK2=3
      GO TO 739
  714 NK1=3
      NK2=14
      GO TO 739
  717 NK1=15
      NK2=29
      GO TO 739
  719 NK1=30
      NK2=35
      GO TO 739
  721 NK1=36
      NK2=49
      GO TO 739
  722 NK1=50
      NK2=90
      GO TO 739
  723 NK1=93
      NK2=99
  739 M=0
      DO 703 NK=NK1,NK2
      IF(M)775,776,775
  775 PRINT774
  774 FORMAT(" ")
      M=0
  776 DO 703 II=II1,II2
      IF(J-1)763,768,763
  768 IF(NK-3)763,764,763
  763 IF(K(3,II)-NK)703,705,703
  705 CALL DATE(K(1,II),KYR,KMO,KDA)
      CALL CONVERT(KYR,KMO,KDA,IYR,IMO,IDA)
      IF(K(4,II)-/003301)765,766,765
```

Figure 20. Listing of the Main Program and Subroutines Which Make Up the Final Form of Log Report Generator Program (Sheet 4 of 6)

```
765   IF(K(4,II)-/606060)762,766,762
766   PRINT767,(KCD2(NK,NZ4),NZ4=1,10),IMO,IDA,IYR,K(4,II),K(5,II)
      M=M+1
      GO TO 703
762   PRINT730,(KCD2(NK,N),N=1,10),IMO,IDA,IYR,K(4,II),K(5,II)
730   FORMAT(9X,10A3,1X,A2,"/",A2,"/",A2,2X,I7,A3)
767   FORMAT(9X,10A3,1X,A2,"/",A2,"/",A2,3X,A6,A3)
      M=M+1
      GO TO 703
764        NK1=91
      NK2=92
      GO TO 739
703   CONTINUE
      M=0
      RETURN
      END
      SUBROUTINE TUMS(II1,II2)
      COMMON DUM(27),KR(64),NS(9),K(5,225,1),KCD1(9,6),KCD2(99,10)
      PRINT809
809   FORMAT(/23X"SUBTOTALS FOR THIS PERIOD"//8X"ACTIVITIES"
     112X"ELEMENTS"22X"FREQ.")
      KRR1=0
      DO 804 NK5=1,99
      KRR=0
822   DO 804 II=II1,II2
827   IF(K(3,II)-NK5)803,802,803
802   KRR=KRR+1
817   IF(K(2,II)-8)819,820,819
820   KRR2=1
      GO TO 803
819   KRR2=K(2,II)
803   IF(II-II2)804,814,804
814   IF(KRR)805,804,805
805   IF(KRR1-KRR2)811,812,811
811   PRINT813
813   FORMAT(" ")
812   KRR1=KRR2
      PRINT806,(KCD1(KRR2,M),M=1,6),(KCD2(NK5,L),L=1,10),KRR
806   FORMAT(6X,6A3,2X,10A3,I3,"  TIMES")
804   CONTINUE
      RETURN
      END


$OLD
OLD PROGRAM NAME--RAM
WAIT.


READY.


$LIST

      SUBROUTINE RAM(IRAM)
      COMMON DUM(27),KR(64),NS(9),K(5,225,1),KCD1(9,6),KCD2(99,10)
      CALL SYSL01(IRAM,KR)
      NA=1
      DO 60 I=1,9
      DO 60 J1=1,6
      KCD1(I,J1)=KR(NA)
      NA=NA+1
60    CONTINUE
      IRAM=3040
62    IRAM=IRAM+1
      NB=1
```

Figure 20. Listing of the Main Program and Subroutines Which Make Up the Final Form of Log Report Generator Program (Sheet 5 of 6)

```
      CALLSYSL01(IRAM,KR)
      IF(IRAM-3041)63,63
      NB=1
      GO TO 61
 63   DO 61 I=1,99
      DO 61 J1=1,10
 65   KCD2(I,J1)=KR(NB)
      NB=NB+1
      IF(NB-61)61,62,61
  61  CONTINUE
      RETURN
      END
      SUBROUTINE LOG(I,IRAM1,IRAM2)
      COMMON DUM(27),KR(64),NS(9),K(5,225,1),KCD1(9,6),KCD2(99,10)
     1N1(6)
      IF(N1(I)-115)26,21,26
 26   IF(N1(I)-116)27,22,27
 27   IF(N1(I)-118)28,23,28
 28   IF(N1(I)-119)29,24,29
 29   IF(N1(I)-120)70,25,70
 70   IF(N1(I)-121)71,71,71
 21   IRAM1=1561
      GO TO30
 22   IRAM1=1801
      GO TO 30
 23   IRAM1=2241
      GO TO 30
 24   IRAM1=2321
      GO TO 30
 25   IRAM1=2561
      GO TO 30
 71   IRAM1=2801
 30   IRAM2=IRAM1+30
      RETURN
      END
```

Figure 20. Listing of the Main Program and Subroutines Which Make Up the Final Form of Log Report Generator Program (Sheet 6 of 6)

```
RUN TYPE-- $LOAD LOGRFP,SUJ-M,RAM

LOAD LIMITS 13134 13367

TYPE THE NUMBER OF ANIMALS FOR WHICH LOG INFORMATION IS DESIRED (6 MAX.)
:=11++3

TYPE ANIMAL NUMBERS (115,116,120,ECT.)
:=115,116,118

ANIMAL  115
DO YOU WISH THE ENTIRE LOG (YES/NO):=NO

TYPE STARTING AND ENDING DATES OF THE DESIRED
PRINT OUT IN THE FORM--70114,70223--

DATE CODE EXPLANATION

   FOR DATE CODE 70114
       7=YEAR(1967)
      01=MONTH(JAN.)
      14=DAY(JAN.14)
:=60815,60907


                          LOG INFORMATION

                           ANIMAL  115

                   PERIOD  FROM 08/15/66 TO 09/07/66

                       TOTALS FOR THIS PERIOD

                   ACTIVITIES                 FREQ.

                 ENVIRONMENT CHANGED         5  TIMES
                 DIET CHANGED                1  TIMES
                 ORGANISMS INGESTED          0  TIMES
                 ANTISEPTIC WASHES USED      0  TIMES
                 ANTIBIOTICS USED            2  TIMES
                 OBSERVATIONS MADE           6  TIMES
                 SAMPLES TAKEN               0  TIMES

                       SUBTOTALS FOR THIS PERIOD

       ACTIVITIES          ELEMENTS                          FREQ.

      DIET            NON STERILE                         1  TIMES

      DIET NAMES      PURINA MONKEY CHUW+BANAS.+ORS.      1  TIMES

      ANTIBIOTICS     CHLORAMPHENICOL+FUROXONE            2  TIMES

      OBSERVATIONS    ANTIHELMINTHIC TRET.(TRIBNDZE)      1  TIMES
      OBSERVATIONS    TB TESTED NEGATIVE                  2  TIMES
      OBSERVATIONS    STOOL CULTURE NEGATIVE(SAL+SH)      1  TIMES
      OBSERVATIONS    BLOOD SMEAR NEGATIVE(MALARIA)       1  TIMES
      OBSERVATIONS    ANIMAL RECEIVED AT GE               1  TIMES

      ENVIRONMENT     ANIMAL ADDED                        5  TIMES
```

Figure 21. Example of Operation of Final Form of Log Report Generator Program (Characters Underlined Indicate Input to Computer from Teletype Terminal) (Sheet 1 of 6)

```
DO YOU WISH DETAILED LOG INFORMATION (YES/NO)
:=YES

INDICATE WHICH OF THE FOLLOWING PRINT OUTS YOU DESIRE

 1    COMPLETE REPORT BY ACTIVITY
 2    COMPLETE REPORT BY DATE
 3    COMPLETE REPORT OF ONE ACTIVITY
 4    COMPLETE REPORT BY DATE AND ACTIVITY
 5    CHANGE PERIOD
 6    NEXT LEVEL
:=1

                        ANIMAL  115

              PERIOD  FROM 08/15/66 TO 09/07/66

                  DETAILED LOG INFORMATION

ENVIRONMENT

     ELEMENTS                           DATES      NUMBERS

    ANIMAL ADDED                       09/07/66     116AN
    ANIMAL ADDED                       09/07/66     118AN
    ANIMAL ADDED                       09/07/66     119AN
    ANIMAL ADDED                       09/07/66     120AN
    ANIMAL ADDED                       09/07/66     121AN

DIET

     ELEMENTS                           DATES      NUMBERS

    NON STERILE                        09/07/66

    PURINA MONKEY CHOW+BANAS.+ORS.     09/07/66
```

Figure 21. Example of Operation of Final Form of Log Report Generator Program (Characters Underlined Indicate Input to Computer from Teletype Terminal) (Sheet 2 of 6)

ANTIBIOTICS

ELEMENTS	DATES	NUMBERS
CHLORAMPHENICOL+FUROXONE	08/15/66	110MG
CHLORAMPHENICOL+FUROXONE	08/16/66	110MG

OBSERVATIONS

ELEMENTS	DATES	NUMBERS
ANTIHELMINTHIC TRET.(TRIBNDZE)	08/17/66	75MGK
TB TESTED NEGATIVE	08/17/66	
TB TESTED NEGATIVE	09/01/66	
STOOL CULTURE NEGATIVE(SAL+SH)	08/22/66	
BLOOD SMEAR NEGATIVE(MALARIA)	08/22/66	
ANIMAL RECEIVED AT GE	09/07/66	

Figure 21. Example of Operation of Final Form of Log Report Generator Program (Characters Underlined Indicate Input to Computer from Teletype Terminal) (Sheet 3 of 6)

INDICATE WHICH OF THE FOLLOWING PRINT OUTS YOU DESIRE

1 COMPLETE REPORT BY ACTIVITY
2 COMPLETE REPORT BY DATE
3 COMPLETE REPORT OF ONE ACTIVITY
4 COMPLETE REPORT BY DATE AND ACTIVITY
5 CHANGE PERIOD
6 NEXT LEVEL

:=2

ANIMAL 115

PERIOD FROM 09/07/66 TO 09/07/66

DETAILED LOG INFORMATION

DATES	ACTIVITIES	ELEMENTS	NUMBERS
08/15/66	ANTIBIOTICS	CHLORAMPHENICOL+FUROXONE	110MG
08/16/66	ANTIBIOTICS	CHLORAMPHENICOL+FUROXONE	110MG
08/17/66	OBSERVATIONS	ANTIHELMINTHIC TRET.(TRIBNDZE)	75MGK
08/17/66	OBSERVATIONS	TB TESTED NEGATIVE	
08/22/66	OBSERVATIONS	STOOL CULTURE NEGATIVE(SAL+SH)	
08/22/66	OBSERVATIONS	BLOOD SMEAR NEGATIVE(MALARIA)	
09/01/66	OBSERVATIONS	TB TESTED NEGATIVE	
09/07/66	OBSERVATIONS	ANIMAL RECEIVED AT GE	
09/07/66	DIET	NON STERILE	
09/07/66	DIET NAMES	PURINA MONKEY CHOW+BANAS.+ORS.	
09/07/66	OTHER ANIMALS	ANIMAL ADDED	116AN
09/07/66	OTHER ANIMALS	ANIMAL ADDED	118AN
09/07/66	OTHER ANIMALS	ANIMAL ADDED	119AN
09/07/66	OTHER ANIMALS	ANIMAL ADDED	120AN
09/07/66	OTHER ANIMALS	ANIMAL ADDED	121AN

Figure 21. Example of Operation of Final Form of Log Report Generator Program (Characters Underlined Indicate Input to Computer from Teletype Terminal) (Sheet 4 of 6)

```
INDICATE WHICH OF THE FOLLOWING PRINT OUTS YOU DESIRE

 1    COMPLETE REPORT BY ACTIVITY
 2    COMPLETE REPORT BY DATE
 3    COMPLETE REPORT OF ONE ACTIVITY
 4    COMPLETE REPORT BY DATE AND ACTIVITY
 5    CHANGE PERIOD
 6    NEXT LEVEL
:=3


INDICATE ONE OF THE FOLLOWING ACTIVITIES

 1    ENVIRONMENT
 2    DIET
 3    ORGANISMS INGESTED
 4    ANTISEPTIC WASHES
 5    ANTIBIOTICS
 6    OBSERVATIONS
 7    SAMPLES TAKEN
:=6


                         ANIMAL   115

              PERIOD   FROM 09/07/66 TO 09/07/66

                    DETAILED LOG INFORMATION


 OBSERVATIONS

       ELEMENTS                                DATES      NUMBERS

     ANTIHELMINTHIC TRET.(TRIBNDZE) 08/17/66         75MGK

     TB  TESTED NEGATIVE            08/17/66
     TB  TESTED NEGATIVE            09/01/66

     STOOL  CULTURE NEGATIVE(SAL+SH) 08/22/66

     BLOOD  SMEAR NEGATIVE(MALARIA)  08/22/66

     ANIMAL  RECEIVED AT GE          09/07/66
```

Figure 21. Example of Operation of Final Form of Log Report Generator Program (Characters Underlined Indicate Input to Computer from Teletype Terminal) (Sheet 5 of 6)

```
INDICATE WHICH OF THE FOLLOWING PRINT OUTS YOU DESIRE

 1    COMPLETE REPORT BY ACTIVITY
 2    COMPLETE REPORT BY DATE
 3    COMPLETE REPORT OF ONE ACTIVITY
 4    COMPLETE REPORT BY DATE AND ACTIVITY
 5    CHANGE PERIOD
 6    NEXT LEVEL
:=6

ANIMAL  116
DO YOU WISH THE ENTIRE LOG (YES/NO):=$STOP
READY.
```

Figure 21. Example of Operation of Final Form of Log Report Generator Program (Characters Underlined Indicate Input to Computer from Teletype Terminal) (Sheet 6 of 6)

ANIMAL LOG INFORMATION

ANIMAL ________________ (code) RECORD NUMBER ______________

DATE ________________

CATEGORIES	NAME OR PRESENCE	AMOUNT
Isolation (yes/no)	________	
Diet (sterile or non)	________	
Diet Name	________	________
Organisms Ingested	________	________
Antiseptic Wash	________	
Antibiotics	________	________
Observations	________	________

OTHER ANIMAL INFORMATION

ANIMAL ________________

ADDED OR REMOVED ______________

Figure 22. Animal Log Information Data Collecting Form

MICROBIAL DATA

RECORD NUMBER ________

ANIMAL SAMPLED ____________

DATE ______________

TEMPERATURE __________ HUMIDITY __________ PRESSURE __________

SITE

____

TOTAL NUMBER

AEROBES ____________________

ANAEROBES ____________________

CLASS	GENUS	NUMBER

Figure 23. Microbial Data Collecting Form

ANIMAL 115
ANIMAL 116
ANIMAL 118
ANIMAL 119
ANIMAL 120
ANIMAL 121
ISOLATION YES
ISOLATION NO
DIET STERILE
DIET NON-STERILE
DIET CHANGES
PURINA MONKEY CHOW
BANANAS ORANGES
VITAMINS
ROCKLAND PRIMATE DIET
SIMILAC
ORGANISMS INGESTED
LACTINEX
ANTISEPTIC WASH
PERACETIC ACID
ANTIBIOTICS
CHLORAMPHENICOL FUROXONE
TEGOPEN
TETREX F
NEOMYCIN
NYSTATIN
ANTIHELMINTHIC TREATMENT
THIBENDAXOLE
DIARRHEA
TB TESTED NEGATIVE
STOOL CULTURE NEGATIVE SAL SHI
ASPERGILLUS
ALCALIGENES
NO COUNT AEROBES
NO COUNT ANAEROBES
NUMBER
NO GROWTH
BLOOD SMEAR NEGATIVE MALARIA
ANIMAL RECEIVED
ANIMAL WEIGHED
FECES YELLOW
FECES NORMAL
EYES IRRITATED
OTHER ANIMALS PRESENT
ANIMAL REMOVED
SAMPLED
FECES
CONJUNCTIVA
THROAT
GINGIVA
AXILLA
GROIN
GLANS PENIS
BACTERIA
ASCOMYCETES
STREPTOCOCCUS
CORYNEBACTERIUM
PROTEUS
ESCHERICHIA
STAPHYLOCOCCUS
AEROBACTER
SHIGELLA
ESCHERICHIA AEROBACTER GROUP
PSEUDOMONAS
SALMONELLA
LACTOBACCILLUS
BACTEROIDES
CLOSTRIDIUM

Figure 24. Thesaurus of Descriptors from Primary File

```
THIS IS THE MSD 265 SYSTEM


10  USER NUMBER--
SYSTEM--$IN001
READY.


(ANIMAL*115/ANIMAL*121)+CORYNEBACTERIUM-STAPHYLOCOCCUS+SAMPLED.
$RUN
 00007  HITS
  PRINT:=ALL
 151610 151611 151620 151622 151723 151726 151728
 SEND:=NONE

QUESTION COMPLETED
```

Figure 25. Example of Primary File Research

LISTING OF THE THREE SUBPROGRAMS WHICH TOGETHER MAKE UP THE REPORT GENERATOR PROGRAM.

```
THIS IS THE MSD 265 SYSTEM

12  USER NUMBER--FWT212
SYSTEM--$FORTRAN
RUN TYPE-- $OLD
OLD PROGRAM NAME--RPG
WAIT.

READY.

$LIST

      DIMENSION J(4),LINH(40),KT(10),JT(4)
      COMMON NDUM(55),ID(512),KR(64)
      COMMON K(5,250,1),ISB(250)
    2 CALL HIER(J)
      KS=4
      DO 50 L=1,250
      ISB(L)=L
      DO 50 I=1,5
   50 K(I,L)=0
      L=1
   80 CALL READUM(NH)
      SENSE LIGHT 4
   90 IF(J(4))95,91,95
   91 DO 94 I=2,4
   94 JT(I)=J(I-1)
      JT(1)=4
      SENSE LIGHT 6
      NL=3
      GO TO 97
   95 DO 96 I=1,4
   96 JT(I)=J(I)
      NL=4
   97 CALL SORT(JT,NH)
      CALL COLS(NH,J,LINH,LC)
      IF(LC-1)3,3,1
    3 PRINT 11,J(1)
   11 FORMAT("1DO NOT USE CODE NUMBER"I2" AS COLUMN HEADER--"/
     2"THERE IS ONLY ONE REPRESENTATIVE OF THAT VARIABLE."//)
      GO TO 200
    1 DO 100I=1,4
  100 KT(I)=K(I,ISB(L))
      CALL PF(KS,J,KT,NL)
      CALL HDR(J,LC,LINH)
      CALL LINR(J,LC,L,LINH,KS,NL,NH)
      IF(J(4))198,198,199
  198 L=L+1
  199 IF(L-NH)4,4,200
  200 PRINT 10
   10 FORMAT("1END REPORT")
      SENSE LIGHT 0
      SENSE LIGHT 4
      GO TO 2
```

Figure 26. Report Generator Program (Sheet 1 of 5)

```
    4 IF(J(4))1,5,1
    5 L=L-1
      GO TO 1
      END


$OLD
OLD PROGRAM NAME--REPORT
WAIT.


READY.


$LIST

      SUBROUTINE SORT(J,NH)
      DIMENSIONJ(4)
      COMMON NDUM(55),ID(512),KR(64)
      COMMONK(5,250,1),ISB(250)
      DO 110 L=1,250
  110 ISB(L)=L
      N=NH
  115 DO 400 L=2,NH
      DO 350 II=1,4
      I=5-II
      IF(K(J(I),ISB(L))-K(J(I),ISB(L-1)))320,350,400
  320 ITM=ISB(L-1)
      ISB(L-1)=ISB(L)
      ISB(L)=ITM
      SENSE LIGHT 1
      GO TO400
  350 CONTINUE
  400 CONTINUE
      IF(SENSE LIGHT1)115
      RETURN
      END
      SUBROUTINE HIER(J)
      DIMENSION J(4)
      IF(SENSE LIGHT 4)100
      PRINT 10
   10 FORMAT("CODING OF VARIABLES"//" 1---ANIMAL"/" 2---DATE"/
     2" 3---SITE"/" 4---CLASS-GENUS"///"TYPE CODE NUMBERS OF "
     2"EACH VARIABLE IN ORDER"/"STARTING WITH THE HIGHEST ORDER,"
     3/"I.E.,MAJOR GROUP TO MINOR GROUP (COLUMN HEADER)"/)
      GO TO 101
  100 SENSE LIGHT 4
  101 READ:(J(I),I=1,4)
      DO 110 I=1,2
      JT=J(I)
      J(I)=J(5-I)
  110 J(5-I)=JT
      IF(J(2)-4)200,150,200
  150 JT=J(1)
      J(1)=J(2)
      J(2)=JT
      PRINT 11,J(4),J(3),J(2),J(1)
   11 FORMAT("HIERARCHY REORDERED TO EQUIVALENT: "4I2)
  200 RETURN
       END
      SUBROUTINE READUM(NH)
      COMMON NDUM(55),ID(512),KR(64)
      COMMON K(5,250,1)
      IRAM=1537
      NREC=8
      CALL SYSL01(IRAM,ID,NREC)
```

Figure 26. Report Generator Program (Sheet 2 of 5)

```
      NREC=1
      DO 68 I=1,512
      IF(ID(I))69,69,68
68    CONTINUE
69    NH=I-1
      PRINT:NH
      L=1
      DO 500 M=1,NH
      IRAM=XMODF(ID(M),10000)
      CALL SYSL01(IRAM,KR,NREC)
      LS=L
      ND=2*KR(7)+6
      K(5,L)=KR(5)
      K(4,L)=-2
      K(5,L+1)=KR(6)
      K(4,L+1)=-1
      L=L+2
      IF(KR(7))70,70
      DO 20 I=8,ND,2
      K(4,L)=KR(I)
      K(5,L)=KR(I+1)
      L=L+1
20    CONTINUE
70    LN=L-1
      DO 30 I=1,3
      DO 30 LL=LS,LN
30    K(I,LL)=KR(I+1)
500   CONTINUE
      NH=LN
      RETURN
      END
```

```
$OLD
OLD PROGRAM NAME--FORMS
WAIT.

READY.

$LIST
```

```
      SUBROUTINE PF(KS,J,KT,NL)
      DIMENSION J(4),KT(4)
   10 FORMAT("ANIMAL NUMBER--"I5)
   11 FORMAT("DATE--"I7)
   12 FORMAT("SITE CODE--"I3)
   13 FORMAT("CLASS-GENUS CODE--"I5)
   14 FORMAT("AEROBE")
   15 FORMAT("ANAEROBE")
   90 FORMAT(//)
      PRINT 90
      PRINT 90
      KS=XMIN0F(KS,NL)
  100 DO 200 I=3,KS
      IR=KS-I+3
      IB=J(IR)
      GO TO(110,120,130,140),IB
  110 PRINT 10,KT(1)
      GO TO 200
  120 PRINT 11,KT(2)
      GO TO 200
  130 PRINT 12,KT(3)
      GO TO 200
  140 IF(KT(4)+1)150,160,170
```

Figure 26. Report Generator Program (Sheet 3 of 5)

```
150 PRINT 14
    GO TO200
160 PRINT 15
    GO TO 200
170 PRINT 13,KT(4)
200 CONTINUE
    PRINT 90
300 RETURN
    END
    SUBROUTINE LINR(J,LC,L,LINH,KS,NL,NH)
    DIMENSION J(4),LINE(40,2),LINH(40)
    COMMON NDUM(55),ID(512),KR(64)
    COMMON K(5,250,1),ISB(250)
    LL=1
100 DO 110 I=1,40
    LINE(I,1)=0
110 LINE(I,2)=0
    KS=0
    DO 200 I=1,LC
    IF(SENSE LIGHT 6)130
118 IF(J(1)-4)150,150,800
130 SENSE LIGHT 6
    IF(K(4,ISB(L)))150,600,140
140 L=L+1
    GO TO 130
150 IF(LINH(I)-K(J(1),ISB(L)))190,160,800
190 I=I+1
    GO TO 150
160 LINE(I,1)=K(5,ISB(L))
    IF(SENSE LIGHT 6)161,169
161 SENSE LIGHT 6
    L=L+1
    LINE(I,2)=K(5,ISB(L))
    LL=2
169 DO 170 II=2,NL
    IF(K(J(II),ISB(L))-K(J(II),ISB(L+1)))165,170,165
165 KS=II
170 CONTINUE
    L=L+1
    IF(KS-2)200,300,300
200 CONTINUE
300 PRINT 10,K(J(2),ISB(L-1))
10  FORMAT(I6,2X)
    DO 400 I=1,LC
    DO 400 L2=1,LL
    IF(LINE(I,L2))310,311,310
311 LINE(I,L2)=/606060
310 IF(LINE(I,L2)-99999)350,350,320
320 PRINT 11,LINE(I,L2)
 11 FORMAT(1H+,3X,A3,2X)
    GO TO 380
350 FLN=LINE(I,L2)/100
    MANT=XMODF(LINE(I,L2),100)
    FLN=10.**MANT*FLN
    PRINT 12,FLN
 12 FORMAT(1H+,1PE8.1)
380 IF(XMODF(I*L2,8))400,390,400
390 PRINT 13
 13 FORMAT(8X)
400 CONTINUE
420 IF(KS-2)800,100,600
600 RETURN
800 IF(L-NH)801,601,600
801 PRINT 15,L,NH
 15 FORMAT("SEQ.ERR. AT"I4" FOR"I4////)
    STOP 1
```

Figure 26. Report Generator Program (Sheet 4 of 5)

```
  601 L=L+1
      GO TO 600
      END
      SUBROUTINE HDR(J,L,LINH)
      DIMENSION LINH(40),J(4)
      FORMAT IFØ(" ANIMAL DATE  SITE CL-GEN")
      IF(J(1)-4)200,100,200
  100 PRINT 10
   10 FORMAT(15X,"CLASS-GENUS CODE"/)
      PRINT 11,IFØ(2*J(2)),IFØ(2*J(2)+1),(LINH(KK),KK=3,L)
   11 FORMAT(2A3"    AEROBE   ANAEROBE"I5,5I8,5(/6X,8I8))
      GO TO 500
  200 J1F=5*(J(1)-1)+2
      J1L=J1F+4
      FORMAT ICGOH(" ANIMAL NUMBERS DATES            SITE CODES
      PRINT 13,(ICGOH(KK),KK=J1F,J1L)
   13 FORMAT(15X,5A3/)
      IF(J(3)-4)210,300,210
  210 IF(J(4)-4)220,300,220
  220 PRINT 14,IFØ(J(2)*2),IFØ(J(2)*2+1),(LINH(KK),KK=1,L)
   14 FORMAT(2A3,2X,I11,3I16/9(I19,3I16/))
      PRINT 15
   15 FORMAT(7X,4(4X,4HAERB,4X,4HANRB))
      GO TO 500
  300 PRINT 16,IFØ(2*J(2)),IFØ(2*J(2)+1),(LINH(KK),KK=1,L)
 16   FORMAT(2A3,8I8,5(/6X,8I8))
 500  PRINT20
 20   FORMAT(" ")
      RETURN
      END
      SUBROUTINE COLS(NH,J,LINH,LC)
      DIMENSION J(4),LINH(40)
      COMMON NDUM(55),ID(512),KR(64)
      COMMON K(5,250,1)
      DO 50 I=1,40
   50 LINH(I)=0
  100 LC=0
      DO 120 L=1,NH
      DO 110 I=1,LC
      IF(K(J(1),L)-LINH(I))110,120,110
  110 CONTINUE
      LC=LC+1
      LINH(LC)=K(J(1),L)
  120 CONTINUE
  130 DO 200 L=2,LC
      IF(LINH(L)-LINH(L-1))150,200,200
  150 LT=LINH(L)
      LINH(L)=LINH(L-1)
      LINH(L-1)=LT
      SENSE LIGHT 2
  200 CONTINUE
      IF(SENSE LIGHT 2)130
      RETURN
      END

ELAPSED TIME IN HUNDREDTHS OF HOURS 029
```

Figure 26. Report Generator Program (Sheet 5 of 5)

```
THIS IS THE MSD 265 SYSTEM

 4  USER NUMBER--
SYSTEM--$IN001
READY.

(ANIMAL*115/ANIMAL*118/ANIMAL*121)*(CONJUNCTIVA/THROAT/GINGIVA)*
SAMPLED.
$RUN
 00024  HITS
  PRINT:=ALL
 151609 151610 151611 151617 151618 151619 182057 182058 182059 182065
 182066 182067 182073 182074 182075 212881 212882 212883 212889 212890
 212891 212897 212898 212899.
SEND:=ALL

QUESTION COMPLETED

$HELLO
 4  USER NUMBER--
SYSTEM--$FORTRAN
RUN TYPE-- $LOAD RPG,REPORT,FORMS

LOAD LIMITS 11676 13643

CODING OF VARIABLES

 1---ANIMAL
 2---DATE
 3---SITE
 4---CLASS-GENUS

TYPE CODE NUMBERS OF EACH VARIABLE IN ORDER
STARTING WITH THE HIGHEST ORDER.
I.E.,MAJOR GROUP TO MINOR GROUP (COLUMN HEADER)
:=1 2 3 4

       24

ANIMAL NUMBER--  115
DATE--  70314

                CLASS-GENUS CODE

 SITE     AEROBE   ANAEROBE  101      102      105      108

     1    1.3E+07  7.9E+06   NC                NC
     2    5.1E+09  3.7E+09            NC
     3    1.5E+09  7.2E+08            NC
```

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 1 of 15)

DATE-- 70623

CLASS-GENUS CODE

SITE	AEROBE	ANAEROBE	101	102	105	108
1	1.8E+06	1.5E+06		1.5E+06	1.1E+06	
2	9.9E+07	1.6E+08		3.9E+06	2.0E+04	
3	2.1E+07	1.6E+08		3.0E+06	3.1E+06	

ANIMAL NUMBER-- 118
DATE-- 70405

CLASS-GENUS CODE

SITE	AEROBE	ANAEROBE	101	102	105	108
1	1.0E+04	4.0E+03		N-3	N-3	N-5
2	3.8E+07	3.2E+07		1.0E+06	N-4	1.0E+03
3	2.0E+05	1.3E+06		2.6E+05	N-3	N-3

DATE-- 70623

CLASS-GENUS CODE

SITE	AEROBE	ANAEROBE	101	102	105	108
1	1.8E+06	4.9E+07		1.5E+06	2.2E+06	
2	4.9E+08	4.9E+08		3.9E+07	1.5E+04	
3	4.9E+08	7.0E+08		3.9E+07	2.5E+04	

DATE-- 70707

CLASS-GENUS CODE

SITE	AEROBE	ANAEROBE	101	102	105	108
1	4.9E+06	4.8E+06		2.1E+06	7.3E+06	
2	2.9E+07	2.2E+07		1.4E+04	5.1E+07	
3	5.1E+07	1.4E+07		1.8E+07	9.4E+04	

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 2 of 15)

ANIMAL NUMBER-- 121
DATE-- 70510

CLASS-GENUS CODE

SITE	AEROBE	ANAEROBE	101	102	105	108
1	1.2E+05	7.0E+04		9.0E+03	1.5E+05	6.0E+03
2	1.1E+07	1.9E+07		7.0E+06	2.3E+04	1.9E+04
3	1.9E+07	9.7E+06		4.3E+06	1.1E+04	1.0E+03

DATE-- 70623

CLASS-GENUS CODE

SITE	AEROBE	ANAEROBE	101	102	105	108
1	5.2E+05	4.3E+05		3.7E+05	1.9E+05	
2	1.2E+09	4.3E+07		8.9E+06	1.7E+07	
3	2.8E+07	2.3E+07		3.7E+06	2.3E+06	

DATE-- 70707

CLASS-GENUS CODE

SITE	AEROBE	ANAEROBE	101	102	105	108
1	1.3E+04	1.9E+04		4.0E+03	3.0E+03	
2	1.1E+09	1.2E+09		1.9E+07	2.0E+04	
3	5.0E+08	.8E+08		2.3E+07	1.0E+03	

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 3 of 15)

END REPORT:=1 3 2 4   Hierarchy Reordered.

ANIMAL NUMBER-- 115
SITE CODE-- 1

CLASS-GENUS CODE

DATE	AEROBE	ANAEROBE	101	102	105	108
70314	1.3E+07	7.9E+06	NC		NC	
70623	1.8E+06	1.5E+06		1.5E+06	1.1E+06	

SITE CODE-- 2

CLASS-GENUS CODE

DATE	AEROBE	ANAEROBE	101	102	105	108
70314	5.1E+09	3.7E+09		NC		
70623	9.9E+07	1.6E+08		3.9E+06	2.0E+04	

SITE CODE-- 3

CLASS-GENUS CODE

DATE	AEROBE	ANAEROBE	101	102	105	108
70314	1.5E+09	7.2E+08		NC		
70623	2.1E+07	1.6E+08		3.0E+06	3.1E+06	

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 4 of 15)

ANIMAL NUMBER-- 118
SITE CODE-- 1

CLASS-GENUS CODE

DATE	AEROBE	ANAEROBE	101	102	105	108
70405	1.0E+04	4.0E+03		N-3	N-3	N-5
70623	1.8E+06	4.9E+07		1.5E+06	2.2E+06	
70707	4.9E+06	4.8E+06		2.1E+06	7.3E+06	

SITE CODE-- 2

CLASS-GENUS CODE

DATE	AEROBE	ANAEROBE	101	102	105	108
70405	3.8E+07	3.2E+07		1.0E+06	N-4	1.0E+03
70623	4.9E+08	4.9E+08		3.9E+07	1.5E+04	
70707	2.9E+07	2.2E+07		1.4E+04	5.1E+07	

SITE CODE-- 3

CLASS-GENUS CODE

DATE	AEROBE	ANAEROBE	101	102	105	108
70405	2.0E+05	1.3E+06		2.6E+05	N-3	N-3
70623	4.9E+08	7.0E+08		3.9E+07	2.5E+04	
70707	5.1E+07	1.4E+07		1.8E+07	9.4E+04	

ANIMAL NUMBER-- 121
SITE CODE-- 1

CLASS-GENUS CODE

DATE	AEROBE	ANAEROBE	101	102	105	108
70510	1.2E+05	7.0E+04		9.0E+03	1.5E+05	6.0E+03
70623	5.2E+05	4.3E+05		3.7E+05	1.9E+05	
70707	1.3E+04	1.9E+04		4.0E+03	3.0E+03	

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 5 of 15)

SITE CODE-- 2

CLASS-GENUS CODE

DATE	AEROBE	ANAEROBE	101	102	105	108
70510	1.1E+07	1.9E+07		7.0E+06	2.3E+04	1.9E+04
70623	1.2E+09	4.3E+07		8.9E+06	1.7E+07	
70707	1.1E+09	1.2E+09		1.9E+07	2.0E+04	

SITE CODE-- 3

CLASS-GENUS CODE

DATE	AEROBE	ANAEROBE	101	102	105	108
70510	1.9E+07	9.7E+06		4.3E+06	1.1E+04	1.0E+03
70623	2.8E+07	2.3E+07		3.7E+06	2.3E+06	
70707	5.0E+08	7.8E+08		2.3E+07	1.0E+03	

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 6 of 15)

END REPORT:=1 4 2 3   Hierarchy Reordered.

24

ANIMAL NUMBER-- 115
AEROBE

SITE CODES

DATE	1	2	3
70314	1.3E+07	5.1E+09	1.5E+09
70623	1.8E+06	9.9E+07	2.1E+07

ANAEROBE

SITE CODES

DATE	1	2	3
70314	7.9E+06	3.7E+09	7.2E+08
70623	1.5E+06	1.6E+08	1.6E+08

CLASS-GENUS CODE-- 101

SITE CODES

DATE	1	2	3
70314	NC		

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 7 of 15)

CLASS-GENUS CODE-- 102

SITE CODES

DATE	1	2	3
70314		NC	NC
70623	1.5E+06	3.9E+06	3.0E+06

CLASS-GENUS CODE-- 105

SITE CODES

DATE	1	2	3
70314	NC		
70623	1.1E+06	2.0E+04	3.1E+06

ANIMAL NUMBER-- 118
AEROBE

SITE CODES

DATE	1	2	3
70405	1.0E+04	3.8E+07	2.0E+05
70623	1.8E+06	4.9E+08	4.9E+08
70707	4.9E+06	2.9E+07	5.1E+07

ANAEROBE

SITE CODES

DATE	1	2	3
70405	4.0E+03	3.2E+07	1.3E+06
70623	4.9E+07	4.9E+08	7.0E+08
70707	4.8E+06	2.2E+07	1.4E+07

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 8 of 15)

CLASS-GENUS CODE-- 102

	SITE CODES		
DATE	1	2	3
70405	N-3	1.0E+06	2.6E+05
70623	1.5E+06	3.9E+07	3.9E+07
70707	2.1E+06	1.4E+04	1.8E+07

CLASS-GENUS CODE-- 105

	SITE CODES		
DATE	1	2	3
70405	N-3	N-4	N-3
70623	2.2E+06	1.5E+04	2.5E+04
70707	7.3E+06	5.1E+07	9.4E+04

CLASS-GENUS CODE-- 108

	SITE CODES		
DATE	1	2	3
70405	N-5	1.0E+03	N-3

ANIMAL NUMBER-- 121
AEROBE

	SITE CODES		
DATE	1	2	3
70510	1.2E+05	1.1E+07	1.9E+07
70623	5.2E+05	1.2E+09	2.8E+07
70707	1.3E+04	1.1E+09	5.0E+08

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 9 of 15)

ANAEROBE

SITE CODES

DATE	1	2	3
70510	7.0E+04	1.9E+07	9.7E+06
70623	4.3E+05	4.3E+07	2.3E+07
70707	1.9E+04	1.2E+09	7.8E+08

CLASS-GENUS CODE-- 102

SITE CODES

DATE	1	2	3
70510	9.0E+03	7.0E+06	4.3E+06
70623	3.7E+05	6.9E+06	3.7E+06
70707	4.0E+03	1.9E+07	2.3E+07

CLASS-GENUS CODE-- 105

SITE CODES

DATE	1	2	3
70510	1.5E+05	2.3E+04	1.1E+04
70623	1.9E+05	1.7E+07	2.3E+06
70707	3.0E+03	2.0E+04	1.0E+03

CLASS-GENUS CODE-- 108

SITE CODES

DATE	1	2	3
70510	6.0E+03	1.9E+04	1.0E+03

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 10 of 15)

END REPORT:=3 4 2 1   Hierarchy Reordered.

24

SITE CODE-- 1
AEROBE

ANIMAL NUMBERS

DATE	115	118	121
70314	1.3E+07		
70405		1.0E+04	
70510			1.2E+05
70623	1.8E+06	1.8E+06	5.2E+05
70707		4.9E+06	1.3E+04

ANAEROBE

ANIMAL NUMBERS

DATE	115	118	121
70314	7.9E+06		
70405		4.0E+03	
70510			7.0E+04
70623	1.5E+06	4.9E+07	4.3E+05
70707		4.8E+06	1.9E+04

CLASS-GENUS CODE-- 101

ANIMAL NUMBERS

DATE	115	118	121
70314	NC		

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 11 of 15)

CLASS-GENUS CODE-- 102

ANIMAL NUMBERS

DATE	115	118	121
70405		N-3	
70510			9.0E+03
70623	1.5E+06	1.5E+06	3.7E+05
70707		2.1E+06	4.0E+03

CLASS-GENUS CODE-- 105

ANIMAL NUMBERS

DATE	115	118	121
70314	NC		
70405		N-3	
70510			1.5E+05
70623	1.1E+06	2.2E+06	1.9E+05
70707		7.3E+06	3.0E+03

CLASS-GENUS CODE-- 108

ANIMAL NUMBERS

DATE	115	118	121
70405		N-5	
70510			6.0E+03

SITE CODE-- 2
AEROBE

ANIMAL NUMBERS

DATE	115	118	121
70314	5.1E+09		
70405		3.8E+07	
70510			1.1E+07
70623	9.9E+07	4.9E+08	1.2E+09
70707		2.9E+07	1.1E+09

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 12 of 15)

ANAEROBE

ANIMAL NUMBERS

DATE	115	118	121
70314	3.7E+09		
70405		3.2E+07	
70510			1.9E+07
70623	1.6E+08	4.9E+08	4.3E+07
70707		2.2E+07	1.2E+09

CLASS-GENUS CODE-- 102

ANIMAL NUMBERS

DATE	115	118	121
70314	NC		
70405		1.0E+06	
70510			7.0E+06
70623	3.9E+06	3.9E+07	8.9E+06
70707		1.4E+04	1.9E+07

CLASS-GENUS CODE-- 105

ANIMAL NUMBERS

DATE	115	118	121
70405		N-4	
70510			2.3E+04
70623	2.0E+04	1.5E+04	1.7E+07
70707		5.1E+07	2.0E+04

CLASS-GENUS CODE-- 108

ANIMAL NUMBERS

DATE	115	118	121
70405		1.0E+03	
70510			1.9E+04

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 13 of 15)

SITE CODE-- 3
AEROBE

ANIMAL NUMBERS

DATE	115	118	121
70314	1.5E+09		
70405		2.0E+05	
70510			1.9E+07
70623	2.1E+07	4.9E+08	2.8E+07
70707		5.1E+07	5.0E+08

ANAEROBE

ANIMAL NUMBERS

DATE	115	118	121
70314	7.2E+08		
70405		1.3E+06	
70510			9.7E+06
70623	1.6E+08	7.0E+08	2.3E+07
70707		1.4E+07	7.8E+08

CLASS-GENUS CODE-- 102

ANIMAL NUMBERS

DATE	115	118	121
70314	NC		
70405		2.6E+05	
70510			4.3E+06
70623	3.0E+06	3.9E+07	3.7E+06
70707		1.8E+07	2.3E+07

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 14 of 15)

CLASS-GENUS CODE-- 105

ANIMAL NUMBERS

DATE	115	118	121
70405		N-3	
70510			1.1E+04
70623	3.1E+06	2.5E+04	2.3E+06
70707		9.4E+04	1.0E+03

CLASS-GENUS CODE-- 108

ANIMAL NUMBERS

DATE	115	118	121
70405		N-3	
70510			1.0E+03

Figure 27. Example of Search of Primary File, Sending of Report Addresses to Scratch Area and Operation of Report Generator Program With Several of the 24 Hierarchies Possible (Sheet 15 of 15)

```
OLD PROGRAM NAME--SEARCH

READY.


$LIST

      COMMON DUM(27),KR(64),ID(512),K(5,225,1),KCD1(9,6),
     1KCD2(99,10),KCD3(2,7),KCD4(15,10)
      IRAM=1545
      CALL RAM(IRAM)
429   IRAM=1537
      NREC=8
      CALL SYSL01(IRAM,ID,NREC)
      DO 10 I=1,512
      IF(ID(I))10,11,10
10    CONTINUE
11    NH=I-1
      PRINT12
12    FORMAT(//"DO YOU WISH TO ENTER A RECORD ADDRESS"/
     1"AND HAVE THAT RECORD SENT BACK TO THE TTY OR"/
     2"DO YOU WISH TO HAVE RECORDS WHICH ARE IN THE"/
     3"SCRATCH AREA SENT TO THE TTY"/
     4"     TYPE 1 FOR ENTERING AN ADDRESS AND"/
     5"          2 FOR READING THE SCRATCH AREA"/)
      READ:NTY
      IF(NTY-1)35,36,35
36    PRINT37
37    FORMAT(/"TYPE THE RECORD ADDRESS IN THE FORM 151629"/)
      READ:ID(1)
      NH1=1
      NH2=1
      GO TO 38
35    NH2=NH
      NH1=1
38    DO 14 I=NH1,NH2
      II=0
      PRINT 425,ID(I)
425   FORMAT(//2X"RECORD NUMBER"2X,I7)
      ID1=ID(I)/10000+100
      IRAM=XMODF(ID(I),10000)
      CALL SYSL01(IRAM,KR)
      IF(KR(2)-100)21,21,20
21    DO 32 J=1,57,7
      IF(KR(J))32,33,32
32       CONTINUE
      LS=63
      GO TO 51
33    LS=J-1
51    DO 31 JJ=1,LS,7
      II=II+1
      K(2,II)=KR(JJ)
      K(3,II)=KR(JJ+1)
      K(4,II)=KR(JJ+5)
      K(5,II)=KR(JJ+6)
      IF(K(2,II)-9)101,102,101
102   K(5,II)=/514560
101   AA=KR(JJ+4)/10.-6
      K(1,II)=AA*100000+KR(JJ+2)*100+KR(JJ+3)
      NX=NX+1
      IF(K(3,II)-30)31,900,31
900   K(4,II)=/003301
      K(5,II)=/472360
31    CONTINUE
      PRINT322,ID1
322   FORMAT(///31X"ANIMAL"1X,I4,//3X"DATES"5X"ACTIVITIES"
```

Figure 28. Listing of Main Program and Subroutines of the Secondary File Search Program Which will Print Out Secondary File Data From Record Addresses Sent to Scratch Area of Requested at Teletype Terminal (Sheet 1 of 4)

```
     110X"ELEMENTS"24X"NUMBERS")
      II1=1
      II2=II
509   DO 45 II=II1,II2
      CALL DATE(K(1,II),KYR,KMO,KDA)
      IF(KMM-KMO)516,517,516
516   PRINT518
518   FORMAT(" ")
517   KMM=KMO
      CALL CONVERT(KYR,KMO,KDA,IYR,IMO,IDA)
      IF(K(4,II)-/003301)84,82,84
84    IF(K(4,II)-/606060)81,82,81
82    PRINT83,IMO,IDA,IYR,(KCD1(K(2,II),J),J=1,6),(KCD2(K(3,II),L)
     1,L=1,10),K(4,II),K(5,II)
      GO TO 45
81    PRINT80,IMO,IDA,IYR,(KCD1(K(2,II),J),J=1,6),(KCD2(K(3,II),L)
     1,L=1,10),K(4,II),K(5,II)
80    FORMAT(1X,A2,"/",A2,"/",A2,2X,6A3,2X,10A3,1X,I7,A3)
83     FORMAT(1X,A2,"/",A2,"/",A2,2X,6A3,2X,10A3,2X,A6,A3)
45    CONTINUE
      GO TO14
90    NA=0
      DO 206IN=2,6
      NA=NA+1
206   K(1,NA)=KR(IN)
      IF (K(1,3)-7)450,451,450
451   KS=93
      GO TO 454
450   KS=K(1,3)+93
454   CALL DATE(K(1,2),KYR,KMO,KDA)
      CALL CONVERT(KYR,KMO,KDA,IYR,IMO,IDA)
      IF(KR(7))226,229,226
226   NA=0
      ND=2*KR(7)+6
      DO 225 NT=8,ND,2
      NA=NA+1
       K(2,NA)=KR(NT)/100
      K(3,NA)=XMODF(KR(NT),100)
      K(4,NA)=KR(NT+1)
225   CONTINUE
229   PRINT220,K(1,1),IMO,IDA,IYR,(KCD2(KS,L),L=1,10)
      IF(K(1,4)-99999)351,351,352
352   PRINT221,K(1,4)
      GOTO423
351   CALL ANA(K(1,4),XNA3)
      PRINT235,XNA3
423   IF(K(1,5)-99999)354,354,353
353   PRINT223,K(1,5)
      GO TO 227
354   CALL ANA(K(1,5),XNA3)
      PRINT224,XNA3
227   IF(KR(7))421,14,421
421   JRR=KR(7)
      PRINT336
336   FORMAT(/7X"CLASS"16X"GENUS"27X"COUNT"/)
      DO 222 KRR=1,JRR
      IF(K(4,KRR)-99999)320,320,350
350   PRINT232,(KCD3(K(2,KRR),LL),LL=1,7),(KCD4(K(3,KRR),LO),LO=1
     1,K(4,KRR)
      GO TO 222
320   CALL ANA(K(4,KRR),XNA3)
      PRINT233,(KCD3(K(2,KRR),LL),LL=1,7),(KCD4(K(3,KRR),LO),LO=1
     1,XNA3
222   CONTINUE
14    CONTINUE
      GO TO 429
```

Figure 28. Listing of Main Program and Subroutines of the Secondary File Search Program Which will Print Out Secondary File Data From Record Addresses Sent to Scratch Area of Requested at Teletype Terminal (Sheet 2 of 4)

```
220   FORMAT(//31X"ANIMAL"1X,I4,//31X"DATE ",A2,"/",A2,"/",A2,//
     131X"SITE"2X,10A3)
221   FORMAT(//27X"AEROBES"6X,A3)
235   FORMAT(//27X"AEROBES"2X,E10.3)
223   FORMAT(/25X"ANAEROBES"6X,A3)
224    FORMAT(/25X"ANAEROBES"2X,E10.3)
232   FORMAT(5X,7A3,10A3,6X,A3/)
233   FORMAT(5X,7A3,10A3,2X,E10.3/)
      STOP
      END
      SUBROUTINE ANA(KX,XNA3)
      NA1=KX/100
      NA2=XMODF(KX,100)
      XNA3=NA1*10.**NA2
      RETURN
      END

$OLD
OLD PROGRAM NAME--TAM
WAIT.


READY.


$LIST

       SUBROUTINE RAM(IRAM)
       COMMON DUM(27),KR(64),ID(512),K(5,225,1),KCD1(9,6),
      1KCD2(99,10),KCD3(2,7),KCD4(15,10)
       CALL SYSL01(IRAM,KR)
       NA=1
       DO 60 I=1,9
       DO 60 J1=1,6
       KCD1(I,J1)=KR(NA)
       NA=NA+1
 60    CONTINUE
       IRAM=3040
 62    IRAM=IRAM+1
       NB=1
       CALLSYSL01(IRAM,KR)
       IF(IRAM-3041)63,63
       NB=1
       GO TO 61
 63    DO 61 I=1,99
       DO 61 J1=1,10
 65    KCD2(I,J1)=KR(NB)
       NB=NB+1
       IF(NB-61)61,62,61
  61   CONTINUE
       IRAM=3058
       CALL SYSL01(IRAM,KR)
       NA=1
       DO 70 I=1,2
       DO 70 JI=1,7
       KCD3(I,JI)=KR(NA)
       NA=NA+1
 70    CONTINUE
 72    IRAM=IRAM+1
       CALL SYSL01(IRAM,KR)
       NB=1
       IF(IRAM-3059)73,73
       NB=1
       GO TO 71
 73    DO 71 I=1,15
```

Figure 28. Listing of Main Program and Subroutines of the Secondary File Search Program Which will Print Out Secondary File Data From Record Addresses Sent to Scratch Area of Requested at Teletype Terminal (Sheet 3 of 4)

```
      DO 71 JI=1,10
      KCDA(I,JI)=KR(NB)
      NB=NB+1
      IF(NB-61)71,72,71
71    CONTINUE
      RETURN
      END
      SUBROUTINE CONVERT(KYR,KMO,KDA,IYR,IMO,IDA)
      K=10
      IOCTA=/010000
      IOCT=/000100
      IMO=IDA=000000
      DO 100 I=1,31
      IF(I-K)600,500,600
500   IDA=IDA+IOCTA-/001100
      K=K+10
      GO TO 400
600   IDA=IDA+IOCT
400   IF(KDA-I)100,110,100
100   CONTINUE
110   K=10
       DO200 I=1,12
      IF(I-K)150,140,150
140   IMO=IMO+IOCTA-/001100
      GO TO 170
150   IMO=IMO+IOCT
170   IF(KMO-I)200,210,200
200   CONTINUE
210   IF(KYR-66)230,220,230
230    IF(KYR-67)250,240,250
250   PRINT270
270   FORMAT("ERROR IN YEAR INPUT ")
240   IYR=/060700
      RETURN
220   IYR=/060600
      RETURN
      END
      SUBROUTINE DATE(KX,KYR,KMO,KDA)
      KYR=KX/10000+60
      KMO=XMODF(KX,10000)/100
      KDA=XMODF(KX,100)
      RETURN
      END
```

Figure 28. Listing of Main Program and Subroutines of the Secondary File Search Program Which will Print Out Secondary File Data From Record Addresses Sent to Scratch Area of Requested at Teletype Terminal (Sheet 4 of 4)

PRIMARY FILE SEARCH

```
ANIMAL*115+DIARRHEA.
$RUN
 00002  HITS
  PRINT:=ALL
 151561 151565
SEND:=ALL

QUESTION COMPLETED
```

Figure 29. Examples of the Operation of the Secondary File Search Program (Sheet 1 of 3)

```
$LOAD SEARCH,TAM


LOAD LIMITS 11056 12142


DO YOU WISH TO ENTER A RECORD ADDRESS
AND HAVE THAT RECORD SENT BACK TO THE TTY OR
DO YOU WISH TO HAVE RECORDS WHICH ARE IN THE
SCRATCH AREA SENT TO THE TTY
    TYPE 1 FOR ENTERING AN ADDRESS AND
         2 FOR READING THE SCRATCH AREA
:=2


  RECORD NUMBER    151561


                                        ANIMAL   115

   DATES      ACTIVITIES          ELEMENTS                              NUMBERS

 08/03/66  ENVIRONMENT          NO ISOLATION
 08/03/66  DIET                 NON STERILE
 08/03/66  OBSERVATIONS         ANTIHELMINTHIC TRET.(TRIBNDZE)           75MGK
 08/03/66  OBSERVATIONS         TB TESTED NEGATIVE
 08/14/66  ANTIBIOTICS          CHLORAMPHENICOL+FUROXONE                110MG
 08/14/66  OBSERVATIONS         DIARRHEA
 08/15/66  ANTIBIOTICS          CHLORAMPHENICOL+FUROXONE                110MG
 08/16/66  ANTIBIOTICS          CHLORAMPHENICOL+FUROXONE                110MG
 08/17/66  OBSERVATIONS         ANTIHELMINTHIC TRET.(TRIBNDZE)           75MGK

  RECORD NUMBER    151565


                                        ANIMAL   115

   DATES      ACTIVITIES          ELEMENTS                              NUMBERS

 03/07/67  DIET                 STERILE
 03/07/67  DIET NAMES           SIMILAC+IRON+VITAMINS
 03/09/67  OBSERVATIONS         DIARRHEA
 03/09/67  OBSERVATIONS         FECES YELLOW
 03/13/67  SAMPLES TAKEN        FECES                               151725RN
 03/14/67  SAMPLES TAKEN        FECES                               151726RN
 03/14/67  SAMPLES TAKEN        CONJUNCTIVA                         151609RN
 03/14/67  SAMPLES TAKEN        THROAT                              151610RN
 03/14/67  SAMPLES TAKEN        GINGIVA                             151611RN
```

Figure 29. Examples of the Operation of the Secondary File Search Program (Sheet 2 of 3)

```
DO YOU WISH TO ENTER A RECORD ADDRESS
AND HAVE THAT RECORD SENT BACK TO THE TTY OR
DO YOU WISH TO HAVE RECORDS WHICH ARE IN THE
SCRATCH AREA SENT TO THE TTY
    TYPE 1 FOR ENTERING AN ADDRESS AND
         2 FOR READING THE SCRATCH AREA
:=1

TYPE THE RECORD ADDRESS IN THE FORM 151629
:=161884


  RECORD NUMBER    161884

                              ANIMAL   116

                              DATE 04/21/67

                              SITE  AXILLA

                           AEROBES    0.260E+07

                         ANAEROBES    0.770E+07

       LASS                  GENUS                          COUNT

     BACTERIA            ESCHERICHIA-AEROBACTER GROUP        N-5

     BACTERIA            STAPHYLOCOCCUS                   0.900E+06

     BACTERIA            CORYNEBACTERIUM                  0.220E+07
```

Figure 29. Examples of the Operation of the Secondary File Search Program (Sheet 3 of 3)

1561						
1	2	8	3	66	Ø	Ø
2	4	8	3	66	Ø	Ø
7	5Ø	8	3	66	75	Ø
7	52	8	3	66	Ø	Ø
6	36	8	14	66	11Ø	Ø
7	51	8	14	66	Ø	Ø
6	36	8	15	66	11Ø	Ø
6	36	8	16	66	11Ø	Ø
7	5Ø	8	17	66	75	Ø
Ø						

Figure 30. Animal Log Record as Stored on Disc

2Ø59						
2Ø59	118	7Ø4Ø5	3	2Ø5	13Ø5	3
1Ø8	Ø	1Ø5	Ø	1Ø2	26Ø4	Ø
Ø	Ø	Ø	Ø	Ø	Ø	Ø
Ø	Ø	Ø	Ø	Ø	Ø	Ø
Ø	Ø	Ø	Ø	Ø	Ø	Ø
Ø	Ø	Ø	Ø	Ø	Ø	Ø
Ø	Ø	Ø	Ø	Ø	Ø	Ø
Ø	Ø	Ø	Ø	Ø	Ø	Ø
Ø	Ø	Ø	Ø	Ø	Ø	Ø
Ø						

Figure 31. Microbial Data Record as Stored on Disc

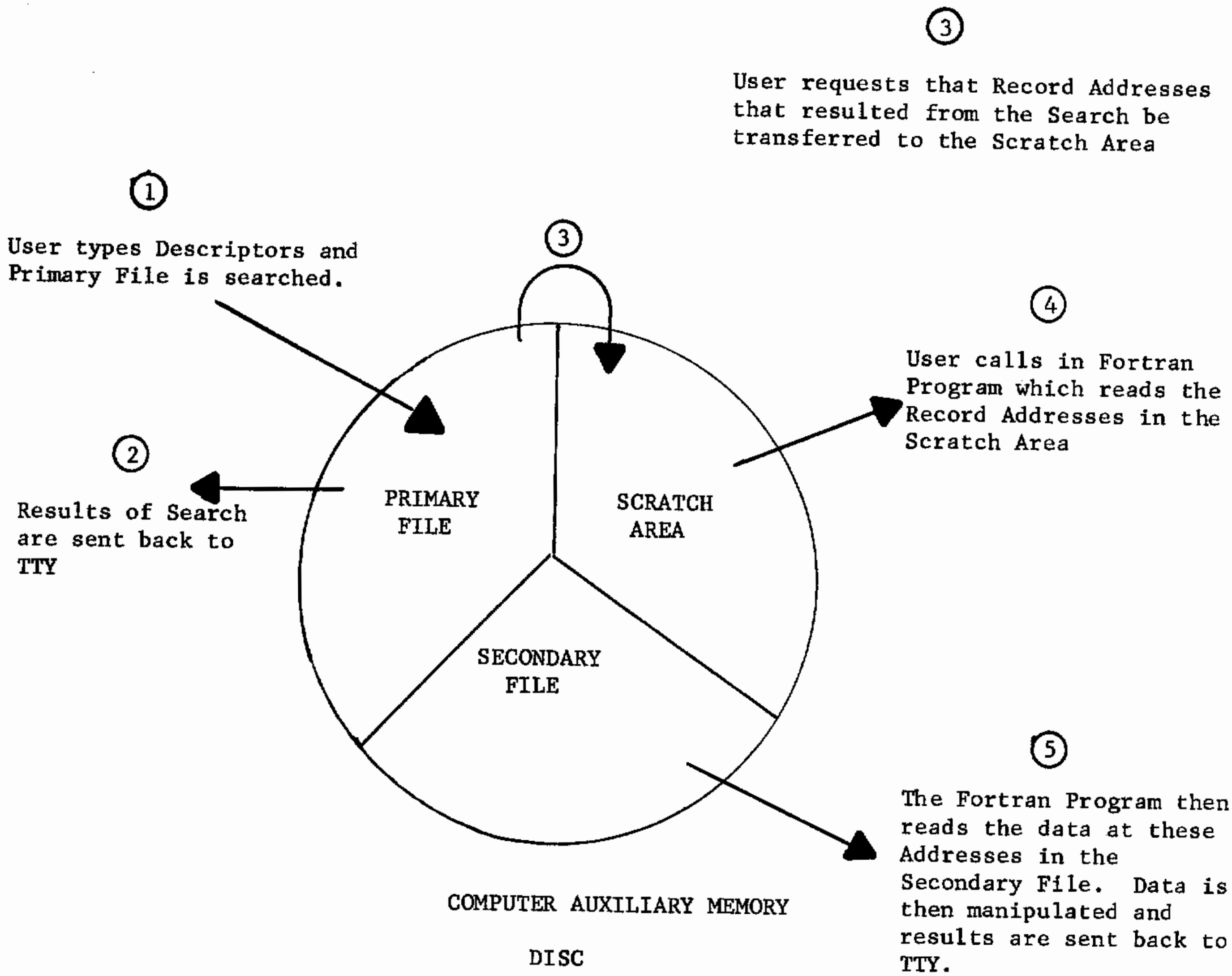


Figure 32. Microbial Data Management System

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13. ABSTRACT

The feasibility of changing the bacterial and fungal flora of monkeys undergoing biological confinement was studied. The significance to the host of an altered ecological relationship was examined with special attention to the feasibility and consequences of requiring microbial compatibility of astronauts for extended space mission. It was determined that while it would be extremely desirable to have microbial compatibility among crew members, tampering with the indigenous flora poses special problems for which there are as yet no answers. A data and information retrieval system, designed to aid in solving some of the problems mentioned above, has been designed and is presented.

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Computer Coding, Bio Data						
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