SPACE SYSTEMS

VOLUME II

Research on Long Term Biological Isolation of Primates and Mice

APPENDICES

PREPARED FOR

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
MANNED SPACECRAFT CENTER
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GENERAL ELECTRIC COMPANY
SPACE SYSTEMS ORGANIZATION
BIOSCIENCES OPERATION
VALLEY FORGE SPACE CENTER

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BY

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T. D. LUCKEY

APPENDIX A

PHOTOGRAPHS OF LABORATORY OPERATIONS AT VALLEY FORGE SPACE CENTER

APPENDIX A

FIGURE NUMBER	DESCRIPTION
A-1	Miss Judy Bushay, Laboratory Technician, prepares "Candle Jars" employed in the incubation of certain bacterial cultures which require reduced oxygen tension for growth. The candle burns out in the closed jar leaving an atmosphere of approximately 5% CO_2 plus nitrogen.
A-2	Culture plates are labeled before use as primary nutrient media for isolating bacterial organisms from monkeys in our animal colony. These plates were required for one-day's testing on five monkeys. View is of rear laboratory.
A-3	A technician prepares a feces sample for a moisture determination The instrument in the background weighs the sample before and after moisture removal.
A-4	Bob Weidner, Animal Handler/Technician, checks the monkeys in our primate holding cages. Overhead are heat lamps, automatically set to restore temperature of the cage should a drop occur not compensated by the normal heating system.
A-5	Dick Ruby, Bacteriologist, clamps the door on the transfer cabinet of the primate isolators. Note the arrangement of the air filtration system to the isolator: one intake and one outlet filter each for the transfer cabinet and the main isolator. All exhaust air is also sent through a charcoal deodorizer.
A-6	Culture medium ready for use. The picture also illustrates the arrangement of the isolators in the rear laboratory with a material storage cabinet and the door to the office at the far end.
A-7	Primary bacteriological work area in central laboratory showing some of the incubators in the background.
A-8	Transferring sterile food into isolator from stainless steel sterilization drum. This drum, designed for autoclaving, is sealed with a thermally resistant plastic. After the end of the drum is inserted into a transfer sleeve and the interior sterilized with peracetic acid, the plastic end is slit and the contents transferred to the isolator.
A-9.	Transfer of materials and animals from one isolator to another under sterile conditions.
A-10	Dr. T. D. Luckey is weighing germ-free mouse for study of food efficiency. Visible are polycarbonate mouse cages and sterile supply bottles.

FIGURE NUMBER	DESCRIPTION
A-11	These particulates were found on aluminum glove rings and under rubber sleeves attached to isolator. They are razor sharp and if not carefully removed before equipment is used, would destroy sleeves and allow contamination. Reliance upon equipment, as it comes from vendor, is often cause for failure. Each item must be individually tested before use in the experiment.
A-12	Inspecting anaerobic cultures during baseline studies (John Geating).
A-13	Injecting "Sernylan", primate tranquilizer, into animal prior to sampling for hematology studies. We have found this drug to be extremely effective and easy to use.
A-14	View of central mouse laboratory showing "bunk bed" isolator set up.
A-15	John Geating, Supervisor of the primate laboratory tests, squeezes bar action on monkey in new cage. Note trolley attachment for moving stainless steel cage about isolator during primate transfer operations.
A-16	Handling mice wearing gloves under sterile conditions is slippery work.
A-17	Transferring sterile supplies into mouse isolator. The individual performing the task is Mr. H. Kaplan, assigned to supervise the area of the laboratory for Dr. Luckey.

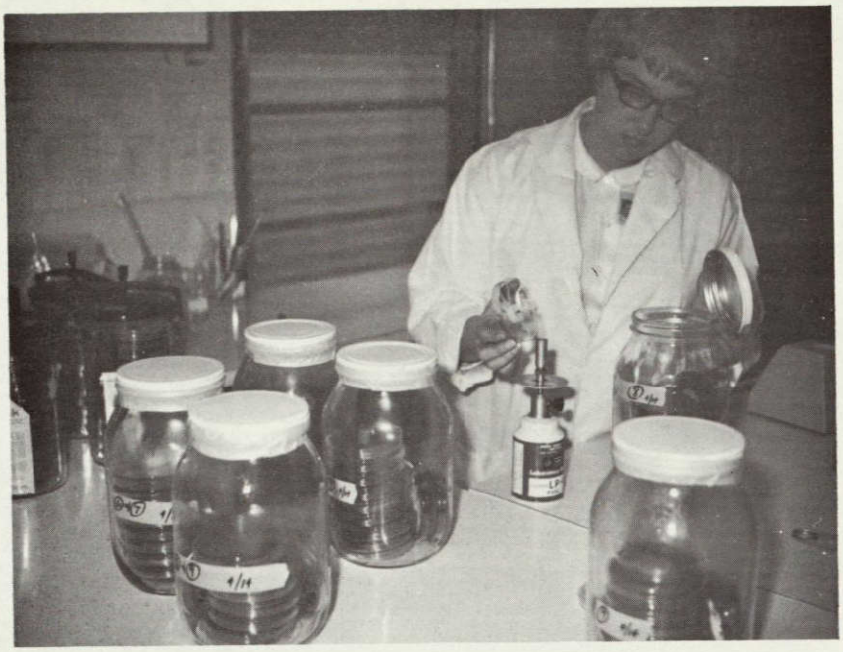


FIGURE A-1

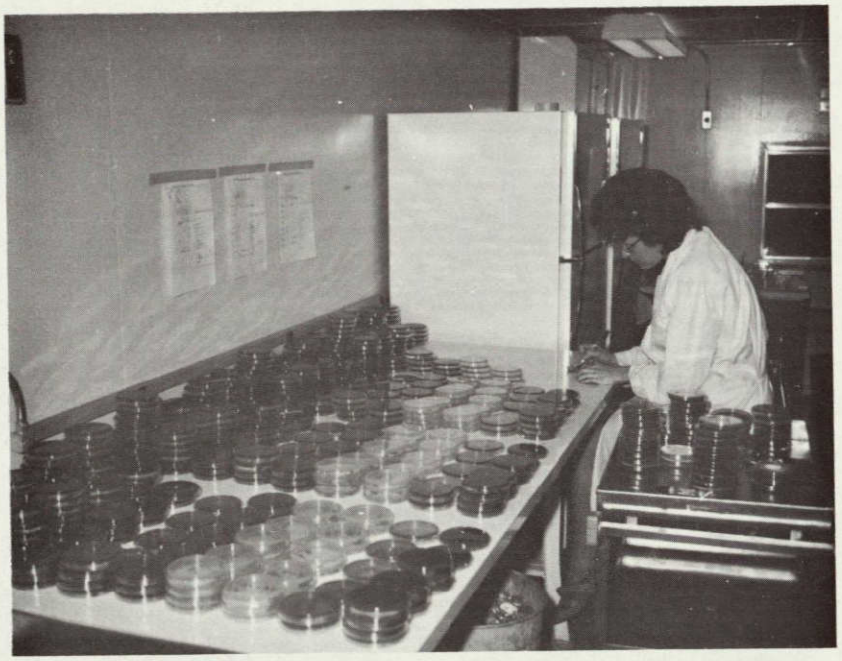


FIGURE A-2

FIGURE A-3

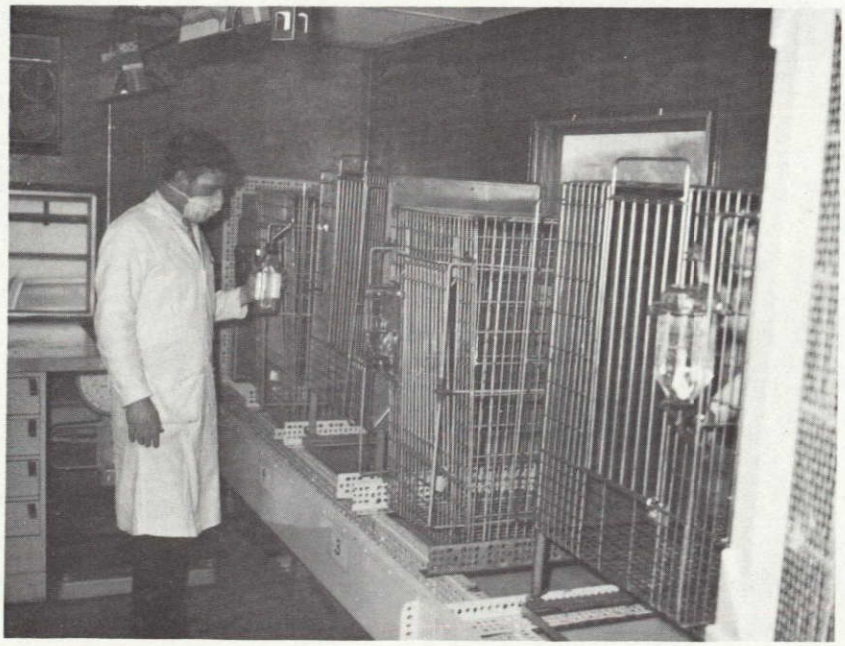


FIGURE A-4

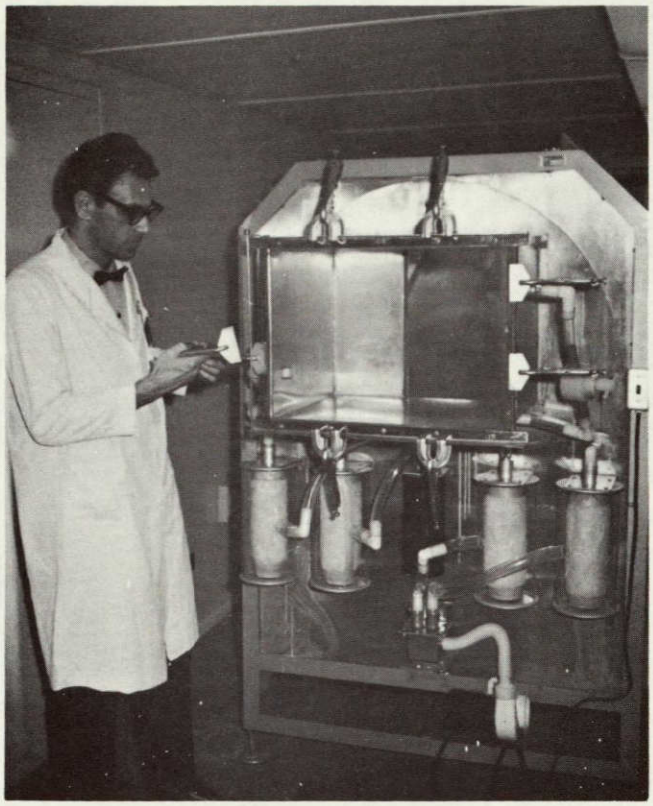


FIGURE A-5

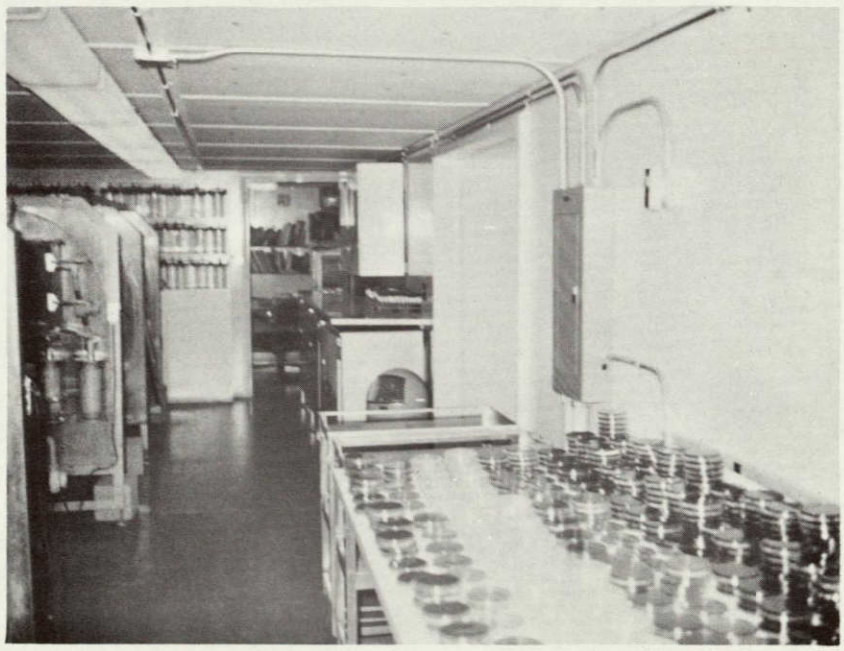


FIGURE A-6

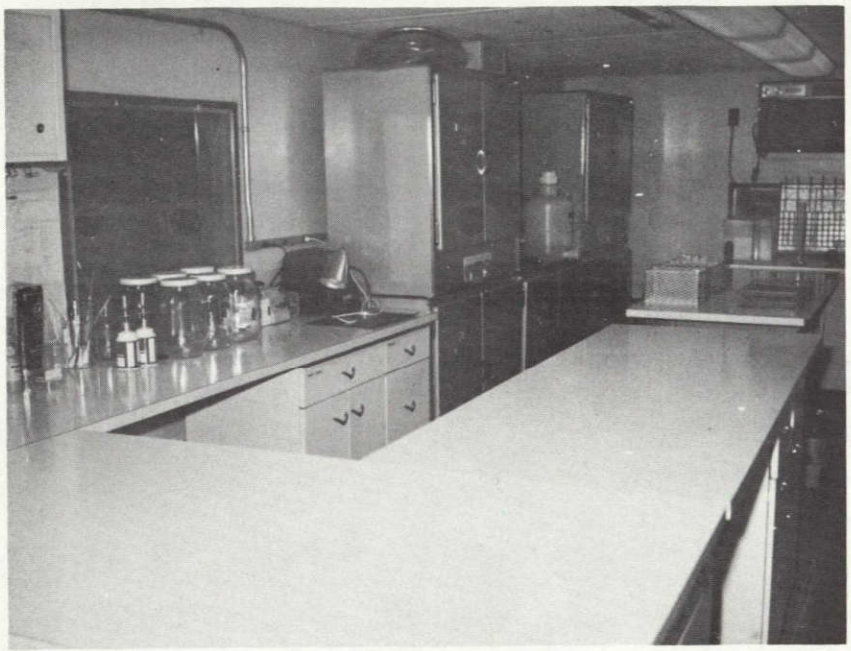


FIGURE A-7

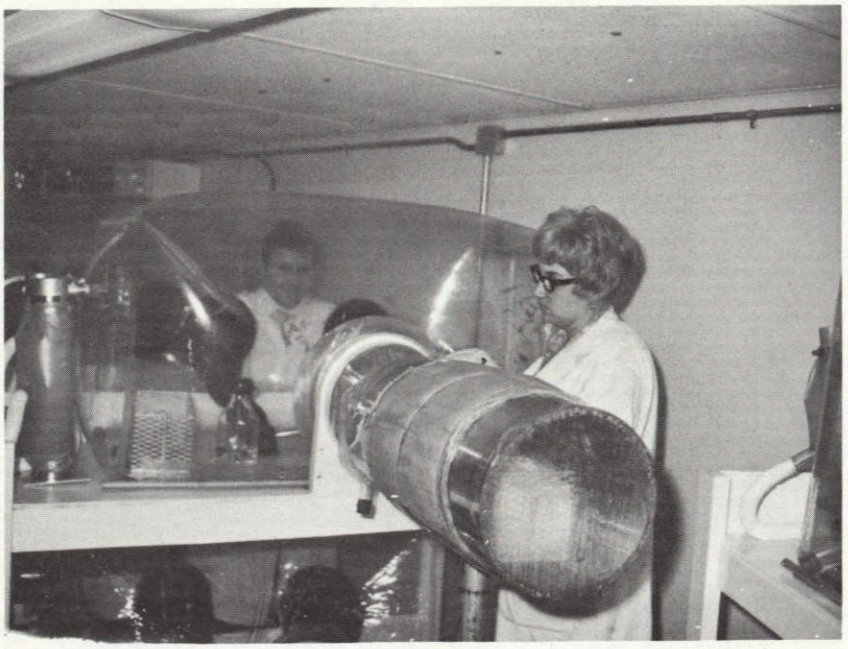


FIGURE A-8

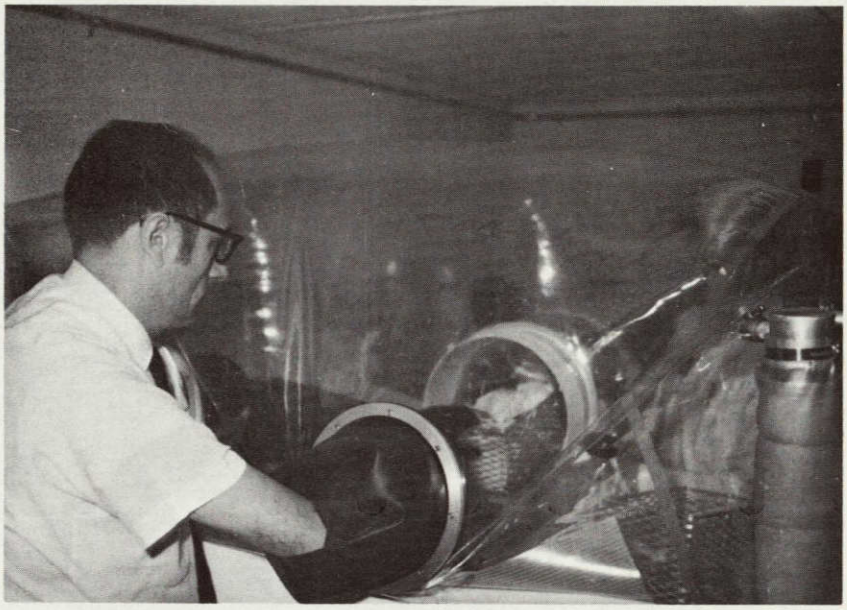


FIGURE A-9

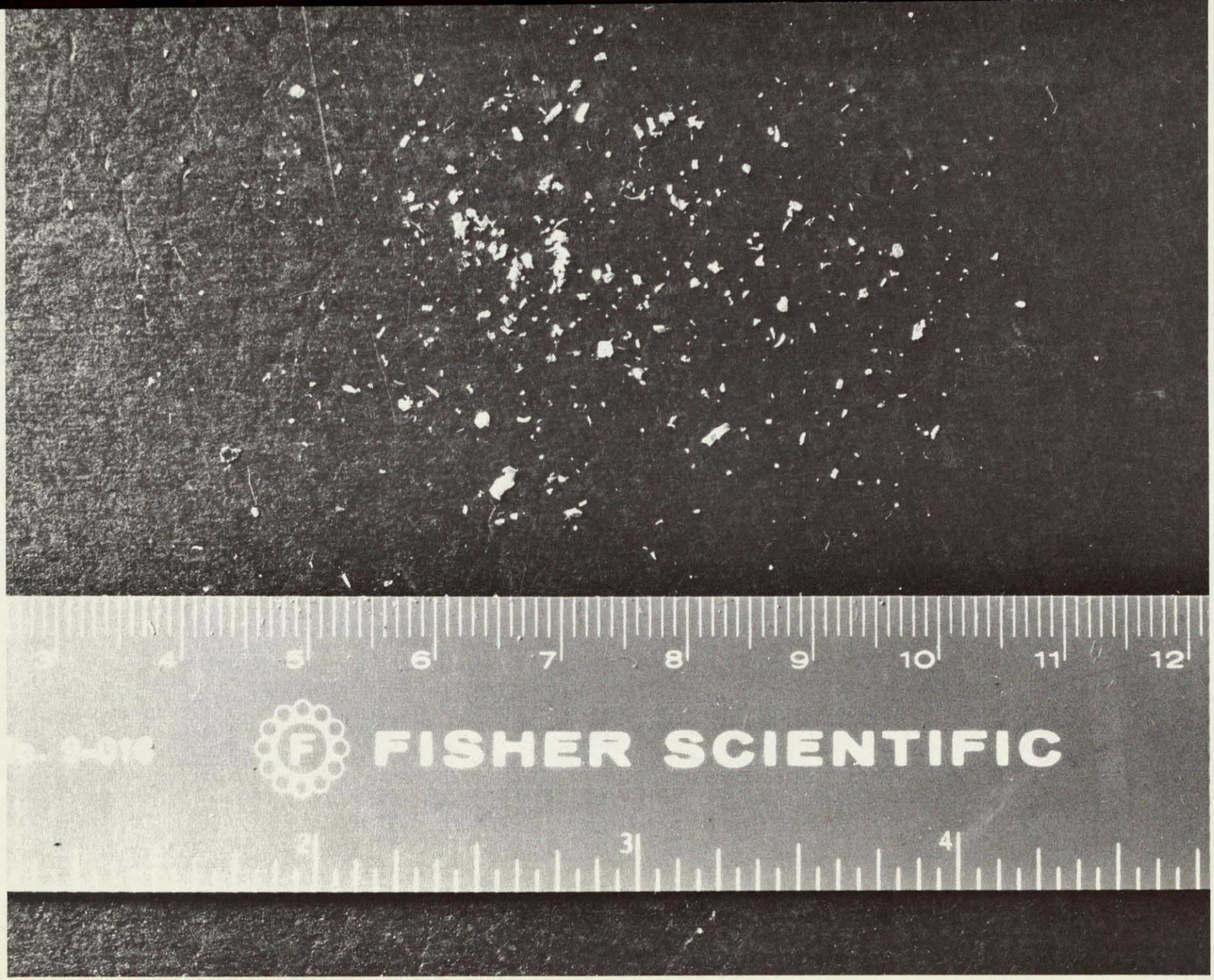


FIGURE A-11

STERILE • DISPOSABLE
PLASTIC SYRINGE

FIGURE A-13

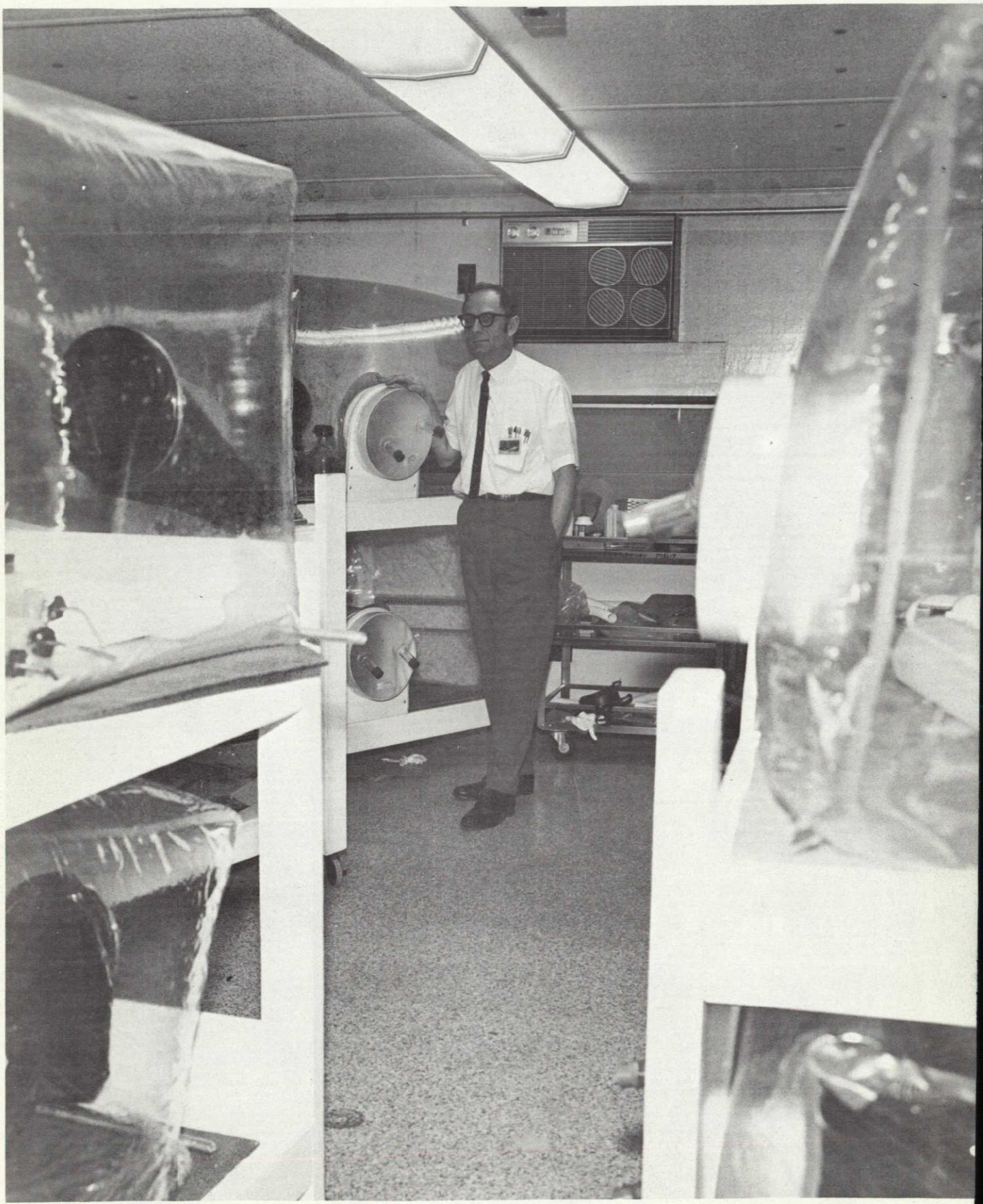


FIGURE A-14

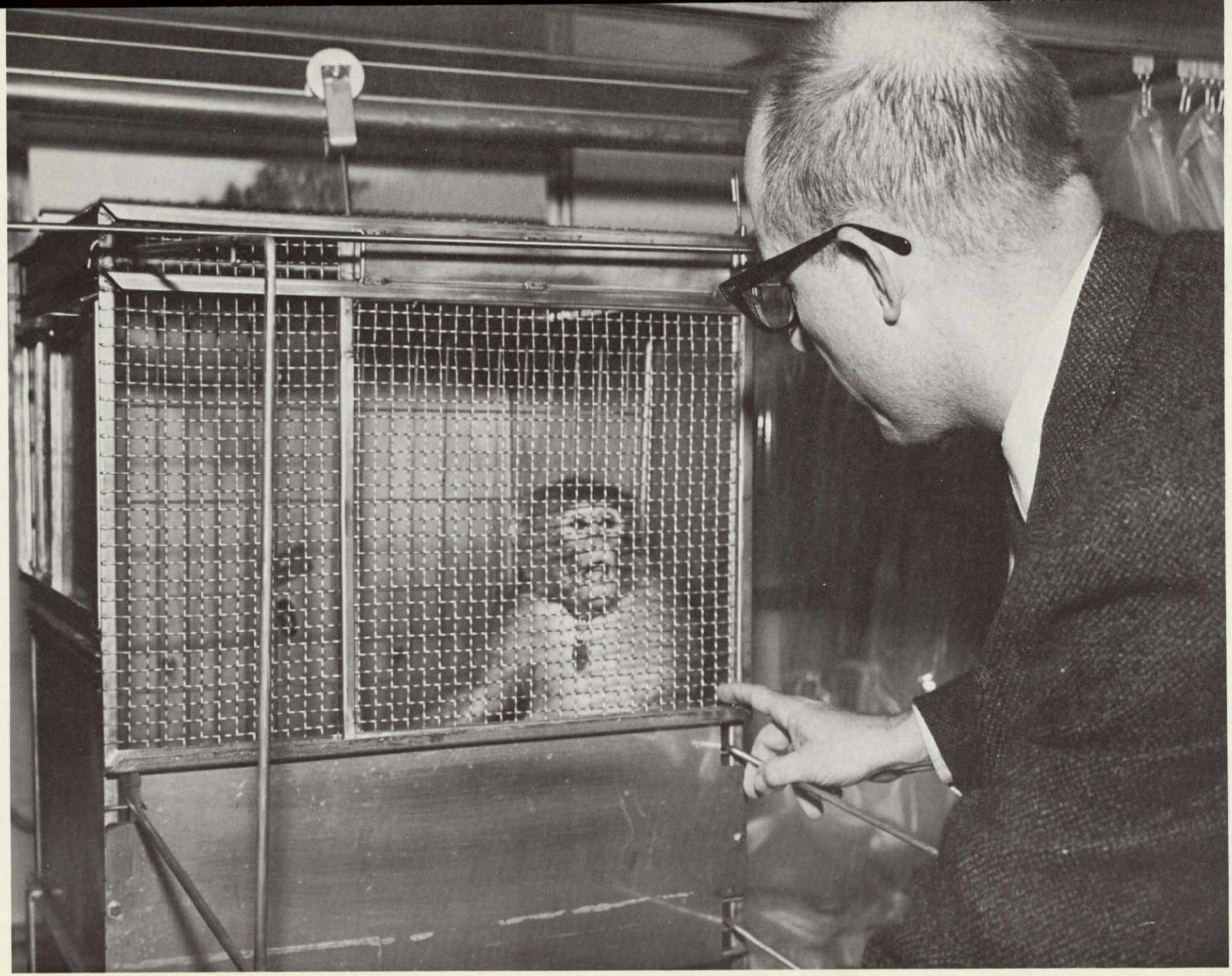


FIGURE A-15

FIGURE A-17

APPENDIX B

SUMMARY OF MICROBIOLOGICAL DATA ON PRIMATES DURING ISOLATION EXPERIMENT

TABLE B-I

MICROFLORA DATA

TOTAL VIABLE AEROBIC AND ANAEROBIC COUNTS, LOG VALUES OF MARKER ORGANISMS

(PER DRY WEIGHT OF FECES OR PER SWAB SAMPLE)

MONKEY NUMBER 2

Samples Taken ← 9/8/59

	WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
	FISCAL WEEK	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
FECES	TOTAL AEROBES	1×10^{6}		3×10^{6}			3×10^{6}		1×10^{6}		6×10^{5}		1×10^{6}		5×10^{5}		7×10^{6}		8×10^{5}		1×10^{6}			3×10^{6}			1×10^{6}	8×10^{5}		3×10^{7}		1×10^{6}		9×10^{6}		5×10^{6}		2×10^{7}	
	TOTAL ANAEROBES	2×10^{6}		6×10^{6}			9×10^{6}		3×10^{6}		8×10^{5}		2×10^{6}		9×10^{5}		8×10^{5}		1×10^{6}		1×10^{6}			8×10^{6}			9×10^{6}	2×10^{6}		7×10^{6}		5×10^{6}		2×10^{6}		9×10^{6}		9×10^{6}	
	MARKER ORGANISMS																																							
	E. coli	7		5			5				4		4		<3		<3		<3					<3						<3		<3		<3		<3		<3	
	S. aureus	4					4		3		3		4		4		3		4		4			5			5			5		6		3		3		<3	
	S. epidermidis	3					4		3		4		4		4		4		4					5			4	5		4		5				3		<3	
	Alpha Streptococcus (Group D)	9					10		10		10		10		9		9		9		9			10			10	10		9		9		9		10		10	
	Lactobaci III	10					10		7		8		9		8		9		7		9			9			9	8		8		9		9		9		10	
	OBLIGATE ANAEROBES																																							
GINGIVA	TOTAL AEROBES	1×10^{6}					2×10^{6}		1×10^{6}		1×10^{7}		3×10^{7}		3×10^{6}		9×10^{6}		9×10^{6}		9×10^{6}			6×10^{6}			5×10^{6}	9×10^{6}		1×10^{7}		2×10^{7}		5×10^{6}		9×10^{6}		9×10^{6}	
	TOTAL ANAEROBES	2×10^{6}					2×10^{6}		9×10^{6}		3×10^{7}		2×10^{7}		4×10^{6}		2×10^{6}		3×10^{6}		2×10^{6}			1×10^{7}			1×10^{7}	10^{6}		1×10^{7}		2×10^{7}		2×10^{6}		2×10^{7}		9×10^{6}	
	MARKER ORGANISMS																																							
	S. aureus						2		3		2		3		4		4		3		4			5			4	5		4		3		5		3		4	
	S. epidermidis						3		3		3		4		3		4		3		4			4			5	6				4		3		4		4	
	Alpha Streptococcus I	5					6		7		7		7		6		5		6		5						6	5		6		6		5					
	Alpha Streptococcus II						5										5																						
	Gamma Streptococcus												6		6		5																						
	Leptotrichia innominata						5		5		6		5		5		4				4			5			6	6		6		5		6		6		6	
	Haemophilus																6				6			6			6	6		6				6		6		7	
	Mycoplasma	2					4		4		4		5		4						3			4			4	4		3		4				6		5	
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme						2		6		6		5		4		5				5			5			5	6						5		6		6	
	Actinomyces israelii										5		5		4												4	6								4		4	
THROAT	TOTAL AEROBES	7×10^{6}					5×10^{6}		3×10^{6}		2×10^{6}		7×10^{6}		6×10^{6}		1×10^{6}		1×10^{6}		3×10^{6}			5×10^{6}			1×10^{6}	4×10^{6}		1×10^{6}		4×10^{6}		2×10^{6}		1×10^{7}		5×10^{6}	
	TOTAL ANAEROBES	8×10^{6}					2×10^{6}		6×10^{6}		3×10^{6}		7×10^{6}		1×10^{7}		2×10^{6}		9×10^{6}		3×10^{6}			1×10^{7}			2×10^{6}	9×10^{5}		1×10^{6}		6×10^{6}		9×10^{6}		2×10^{6}		5×10^{6}	
	MARKER ORGANISMS																																							
	S. aureus (coag +)						2		2		4		4		4		4		4		5			>3			4	3		3		4		5				3	
	S. epidermidis						2		2		3		3		3				3									4				3						3	
	Alpha Streptococcus I	6					6		6		6		6		6		5		5		5						6	5		6		5		6					
	Alpha Streptococcus II																																							
	Gamma Streptococcus												4		6																									
	Leptotrichia innominata						5		5		5		5		5		3		4		5						4	4		4		5		5		5		5	
	Haemophilus																										5	5		5		6				7		7	
	Mycoplasma	3					4		<3		2		4		3		2		2		2			3			2	2		2		2				3		2	
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme						<		<		4		4		5		4		4		4																5		5	
	Actinomyces israelii										<		4																											
EYE	TOTAL AEROBES	5×10^{6}					7×10^{6}		1×10^{6}		2×10^{6}		4×10^{6}		3×10^{6}		3×10^{6}		1×10^{6}		4×10^{6}			9×10^{6}			9×10^{6}	1×10^{6}		7×10^{6}		7×10^{6}		1×10^{6}		9×10^{6}		9×10^{6}	
	TOTAL ANAEROBES	2×10^{6}					7×10^{6}		2×10^{6}		2×10^{6}		3×10^{6}		2×10^{6}		1×10^{6}		1×10^{6}		5×10^{6}			1×10^{6}			3×10^{6}	4×10^{6}		2×10^{6}		9×10^{6}		$<10^{6}$		3×10^{6}		9×10^{6}	
	MARKER ORGANISMS																																							
	S. epidermidis	2							2		3		3		4		4		8		2			3			3	4		3		3		3		5		4	
GROIN	TOTAL AEROBES	8×10^{5}					1×10^{6}		3×10^{6}		5×10^{6}		1×10^{6}		2×10^{6}		1×10^{5}		3×10^{5}		3×10^{5}			$>10^{7}$			$>10^{7}$	$>10^{7}$		4×10^{6}		1×10^{7}		6×10^{6}		5×10^{7}		10^{7}	
	TOTAL ANAEROBES	6×10^{5}					9×10^{6}		1×10^{6}		4×10^{6}		2×10^{6}		1×10^{6}		9×10^{5}		4×10^{5}		2×10^{5}			$>10^{7}$			$>10^{7}$	5×10^{6}		1×10^{6}		9×10^{6}		3×10^{6}		1×10^{7}		10^{7}	
	MARKER ORGANISMS																																							
	S. epidermidis	3					5		4		4		5		6		5		5		5			>5			>10^7	7		6		6		5		6		6	

FOLDOUT FRAME 1

FOLDOUT FRAME 2

TABLE B-II

MICROFLORA DATA

TOTAL VIABLE AEROBIC AND ANAEROBIC COUNTS, LOG VALUES OF MARKER ORGANISMS

(PER DRY WEIGHT OF FECES OR PER SWAB SAMPLE)

MONKEY NUMBER 3

← SAMPLES TAKEN

SAMPLES TAKEN 5/31/49

	WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
	FISCAL WEEK	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
FECES	TOTAL AEROBES	5×10^{6}		5×10^{6}				3×10^{6}	2×10^{6}	9×10^{6}	4×10^{6}	3×10^{6}	1×10^{7}	2×10^{7}	9×10^{6}	4×10^{6}	2×10^{6}	5×10^{7}	2×10^{7}	9×10^{6}				3×10^{6}	9×10^{6}	5×10^{7}	2×10^{7}	4×10^{6}	4×10^{6}	1×10^{7}	5×10^{7}	2×10^{7}	4×10^{6}	4×10^{6}	1×10^{7}	5×10^{7}	2×10^{6}	2×10^{7}	6×10^{6}	
	TOTAL ANAEROBES	1×10^{6}		1×10^{6}				4×10^{10}	2×10^{10}	3×10^{10}	1×10^{10}	1×10^{10}	2×10^{10}	2×10^{10}	1×10^{10}	6×10^{9}	4×10^{9}	2×10^{10}	1×10^{10}	3×10^{9}	2×10^{10}			7×10^{9}	2×10^{10}	3×10^{10}	5×10^{9}	1×10^{10}	6×10^{9}	4×10^{9}	2×10^{9}	4×10^{9}	3×10^{10}	4×10^{9}	9×10^{9}	5×10^{9}	2×10^{10}	9×10^{9}	6×10^{9}	
	MARKER ORGANISMS																																							
	E. coli	7		7				6	5	6	5	4	4	4	3																					<3	<3	<3		
	S. aureus	4								5	6		5			3			3	3				3			3				2		3	2					
	S. epidermidis	4						4	6	6	6	4	5			4	4	3			4			5		4	5	4		4	2	4	3	4	4	3	3	3	3	
	Alpha Streptococcus (Group D)	10						9	8		7	7	7	7	7	9	8	8	9	8				8	9		7	6		6	7	6	8	–	6	7	8		
	Lactobacilli	10						8	9	8	8	9	7	7	8	8	8	8	7	8	7			10	8	8	8	6	<4	7	7	6	8	7	6	7	8	7	8	
	Anaerobic diphtheroid								8				9	8	9	9	9	7	9	8				9	9	10	9	9		8	7	7	6	7	7	6	9	8	9	
	OBLIGATE ANAEROBES																																							
GINGIVA	TOTAL AEROBES	5×10^{6}						9×10^{6}	4×10^{6}	2×10^{7}	7×10^{6}	6×10^{6}	2×10^{8}	3×10^{8}	2×10^{6}	1×10^{7}	9×10^{6}	3×10^{8}	3×10^{8}	2×10^{8}				5×10^{6}		1×10^{8}		4×10^{6}		4×10^{7}		2×10^{8}		6×10^{7}		9×10^{6}		8×10^{6}		
	TOTAL ANAEROBES	3×10^{7}						3×10^{9}	1×10^{8}	1×10^{8}	5×10^{7}	1×10^{10}	8×10^{8}	6×10^{8}	6×10^{8}	1×10^{10}	4×10^{9}	6×10^{8}	3×10^{8}	2×10^{8}				9×10^{7}		1×10^{9}		5×10^{7}		2×10^{8}		4×10^{8}		2×10^{8}		5×10^{7}		6×10^{7}		
	MARKER ORGANISMS																																							
	S. aureus	3						2																								2								
	S. epidermidis	3						2	4	4	4	4	5	3	4	4	4	3	3					4		3		4		4		4		3			4			
	Alpha Streptococcus I	6						6	6	6	6	7	6	6	6	6	6	6	6							6		5		6		6		1			1			
	Alpha Streptococcus II	✓						5	5		6	5		5	4	5	4	5	5							5		5		6		6		1			1			
	Gamma Streptococcus									5	6		5		4	6		5																						
	Lactobacillus salivarius							5		4		5		5	4	5	4	4	4							5		6		5		6		5			5			
	Haemophilus													5	6	6	6	6	5					4		6		5		5		5		8			6			
	Mycoplasma spp.	4							4	3			5	4	3	3	<2	<2	<2	<2				3		3		4		4		<2		–			4			
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme										6	5	5	5	4	5		4	5	4				6		6		4		4		5					5			
	Actinomyces israelii										5		5		–	5			4	–				4		4		4		4		4					5			
THROAT	TOTAL AEROBES	4×10^{6}						3×10^{6}	1×10^{7}	7×10^{6}	1×10^{8}	3×10^{6}	3×10^{8}	2×10^{8}	2×10^{8}	3×10^{8}	4×10^{8}	2×10^{8}	2×10^{8}	2×10^{8}				2×10^{6}		2×10^{8}		2×10^{8}		5×10^{8}		3×10^{8}		3×10^{8}		4×10^{8}				
	TOTAL ANAEROBES	1×10^{6}						5×10^{8}	2×10^{10}	4×10^{8}	1×10^{8}	2×10^{8}	6×10^{8}	3×10^{8}	2×10^{8}	1×10^{8}	4×10^{8}	3×10^{8}						3×10^{8}		2×10^{8}		5×10^{8}		9×10^{8}		1×10^{9}		4×10^{8}		5×10^{8}				
	MARKER ORGANISMS																																							
	S. aureus	2						2	2																															
	S. epidermidis	2							4	4	4	3	4	4	4	2	4	2	2		2				2		2		4		2		3		2					
	Alpha Streptococcus I	5						5	6	5	5	5	5	6	5	4	6	5	5		5				6		5		5		5		5						
	Alpha Streptococcus II	✓						6	4			4	4																											
	Gamma Streptococcus								5				5		4	5	6	5	5								6		5		6		7		1					
	Lactobacillus salivarius	6						5	3	5		6	5	4	4	3	3	3			4						4		4		4		–		3		4			
	Haemophilus												4	6	6	6	5	6	5		5				24		6		6		–		5		6					
	Mycoplasma spp.								4	4			4	4	3	3	<2	<2	<2		3				3		2		<2		2		<2		<2					
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme									4		4	5	4	4	5	4	4	4					4		5		4						0		4				
	Actinomyces israelii												4								4												4		0					
EYE	TOTAL AEROBES	5×10^{6}						3×10^{8}	1×10^{8}	5×10^{8}	2×10^{8}	1×10^{8}	4×10^{8}	1×10^{8}	2×10^{8}	1×10^{8}	1×10^{8}	2×10^{8}	1×10^{8}	2×10^{8}				2×10^{8}		3×10^{8}	2×10^{8}	1×10^{8}		2×10^{8}		1×10^{8}		2×10^{8}		1×10^{8}		2×10^{8}		2×10^{8}		
	TOTAL ANAEROBES	1×10^{8}						6×10^{8}	1×10^{8}	4×10^{8}	1×10^{8}	1×10^{8}	1×10^{8}	2×10^{8}	1×10^{8}	1×10^{8}	1×10^{8}	2×10^{8}	0	1×10^{8}				2×10^{8}		1×10^{8}	1×10^{8}	1×10^{8}		1×10^{8}		1×10^{8}		1×10^{8}		1×10^{8}		1×10^{8}		1×10^{8}		
	MARKER ORGANISMS																																							
	S. epidermidis	3						3	5	4	5	3	3	3	3	2	4	3	2	3	2			4	4	5	4	5	4	4	4	4	5	4	3	5	5	5		
GROIN	TOTAL AEROBES	5×10^{6}						2×10^{8}	2×10^{8}	3×10^{8}	3×10^{8}	4×10^{8}	8×10^{8}	2×10^{8}	2×10^{8}	4×10^{8}	4×10^{8}	2×10^{8}	2×10^{8}	3×10^{8}	2×10^{8}			2×10^{8}	1×10^{8}	2×10^{8}	5×10^{8}	2×10^{8}	1×10^{8}	4×10^{8}	3×10^{8}	2×10^{8}	2×10^{8}	1×10^{8}	1×10^{8}	2×10^{8}	2×10^{8}	4×10^{8}	2×10^{8}	
	TOTAL ANAEROBES	4×10^{6}						2×10^{9}	8×10^{8}	2×10^{8}	9×10^{8}	3×10^{8}	6×10^{8}	2×10^{8}	8×10^{8}	4×10^{8}	2×10^{8}	3×10^{8}	6×10^{8}	2×10^{8}				2×10^{8}	3×10^{8}	6×10^{8}	2×10^{8}	8×10^{8}	1×10^{8}	2×10^{8}	1×10^{8}	3×10^{8}	9×10^{8}	2×10^{8}	2×10^{8}	1×10^{8}	3×10^{8}	2×10^{8}	4×10^{8}	2×10^{8}	
	MARKER ORGANISMS																																							
	S. epidermidis	4						5	5	6	5	4	5	4	4	3	4	4	4	3	5			5	5	4	4	5	3	4	4	4	3	4	3	3	3	4		

FOLDOUT FRAME 1

FOLDOUT FRAME 2

TABLE B-III

MICROFLORA DATA

TOTAL VIABLE AEROBIC AND ANAEROBIC COUNTS, LOG VALUES OF MARKER ORGANISMS

(PER DRY WEIGHT OF FECES OR PER SWAB SAMPLE)

MONKEY NUMBER 4

SAMPLES TAKEN 2-3/31/69

DOSED IN DIET 4-5/21/69

SAMPLES TAKEN 5-5/5/69

	WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
	FISCAL WEEK	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
FECES	TOTAL AEROBES	3×10^{10}		4×10^{10}			3×10^{10}	1×10^{10}	2×10^{10}	5×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	1×10^{10}	2×10^{10}	2×10^{10}	6×10^{10}	3×10^{10}	5×10^{10}	2×10^{10}	1×10^{11}			2×10^{10}	1×10^{10}	2×10^{10}	2×10^{10}	1×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	
	TOTAL ANAEROBES	5×10^{10}		8×10^{10}			2×10^{10}	1×10^{10}	2×10^{10}	5×10^{10}	2×10^{10}	1×10^{10}	4×10^{10}	2×10^{10}	2×10^{10}	6×10^{10}	5×10^{10}	2×10^{10}	4×10^{10}	2×10^{10}	1×10^{11}			2×10^{10}	1×10^{10}	2×10^{10}	2×10^{10}	1×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	2×10^{10}	
	MARKER ORGANISMS																																							
	E. coli	8		4																	<2			<2		<2				<3						<3	<3	<2		
	S. aureus	7					4	6	4	7	4	8	7	3	5	4	4	2	4		6			3		4	4	5		3	3	3	2	3	3		3		3		
	S. epidermidis						5	6	6		4			6	5	5	5	4	3	4	5			5	4	5	4	3	5	5	2	3	3	4	5	4	3		4		
	Alpha Streptococcus (Group D)	10					10	11	10	8	9	11	9	8			9	6	9										4	6		7	7	6	6				6		
	Lactobacilli	10					10	9	10	8	9	8	8	8	7	9	8	7	9	7	9			8	9	6	7	6	6	7	7	8	7	6	7	7	7	7	7	7	
	Anaerobic diphtheroid													6	8	9	6	6	9					9	10		7	6	8	6	7	7	6	4	4		7	7			
	OBLIGATE ANAEROBES																																							
GINGIVA	TOTAL AEROBES	5×10^{9}					1×10^{9}	2×10^{9}	2×10^{9}	5×10^{9}	2×10^{9}	2×10^{9}	4×10^{9}	3×10^{9}	5×10^{9}	2×10^{9}	2×10^{9}	3×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}			1×10^{9}		1×10^{9}		3×10^{9}		2×10^{9}		5×10^{8}		1×10^{9}		2×10^{9}		2×10^{9}		
	TOTAL ANAEROBES	2×10^{9}					1×10^{9}	3×10^{8}	2×10^{9}	2×10^{9}	5×10^{8}	2×10^{9}	7×10^{8}	8×10^{8}	5×10^{9}	2×10^{9}	3×10^{8}	2×10^{9}	2×10^{9}	2×10^{9}	2×10^{9}					5×10^{8}		2×10^{9}				1×10^{8}		2×10^{9}		2×10^{9}		2×10^{9}		
	MARKER ORGANISMS																																							
	S. aureus	2					6	3	3		4	5	3	3	3	4	4	4	3	4				4		4		3		4		6		5		7		3		
	S. epidermidis	5						4	4	3	4	5	5	4	3	4	4	5	4	3				4		4		3		7		6		5		8		4		
	Alpha Streptococcus I	6					4	6	6	6	6	6	5	6	6	6	6	6	6	6						4		7		7		7		7		8				
	Alpha Streptococcus II						5	6							5	6												4												
	Gamma Streptococcus						6	6	6		6		4	6			5	6								4				7		9		8						
	Leptotrichia innominata							4	4	4		4	4		4		3	5	6	7						7		7		6		5		5		8		6		
	Haemophilus						~6		5	3	5	5	4	6	4	5	5	5	4	4				4		5		4		5		5		5		9		7		
	Mycoplasma													6	4	5	5	5	4	4				5		5		4		5		7		5		7		5		
	OBLIGATE ANAEROBES																																							
	Eubacterium fusiforme						✓	✓	✓	4	6	4	4											5						5								6		
	Actinomyces israelii										4		4						4											4								5		
THROAT	TOTAL AEROBES	3×10^{9}					1×10^{9}	4×10^{9}	4×10^{9}	2×10^{9}	4×10^{9}	5×10^{9}	2×10^{9}	1×10^{9}	1×10^{9}	2×10^{9}	6×10^{9}	2×10^{9}	1×10^{9}		1×10^{9}				5×10^{8}		2×10^{9}		5×10^{8}		2×10^{9}		1×10^{9}		2×10^{9}		2×10^{9}		
	TOTAL ANAEROBES	1×10^{9}					2×10^{9}	1×10^{9}	2×10^{9}	5×10^{8}	2×10^{9}	5×10^{9}	3×10^{8}	1×10^{9}	5×10^{8}	2×10^{8}	1×10^{9}	2×10^{9}	6×10^{8}		5×10^{8}				7×10^{8}		4×10^{9}		2×10^{9}				2×10^{9}		1×10^{9}				
	MARKER ORGANISMS																																							
	S. aureus	2					6	3	3	3	4	4	3	6	4	3	2		3		2				2		3		3		4		5		4		9				
	S. epidermidis	3					4	4	4		3	4	3	3	4	3	4	3	3						2		3		3				4		5		3				
	Alpha Streptococcus I	6					5	5	5	5	6	5	5	6	5	5	6	6	6		6				6				6		6		7		7						
	Alpha Streptococcus II							3						4	5	4																								
	Gamma Streptococcus						5	5	6				4			5			5								6		5		6										
	Leptotrichia innominata							4	3	3			5	3	3	4	4	5	4		4						4		6		6		7		5						
	Haemophilus												✓	5	5	7	6	5	4		6				6		5		6		7		5		6						
	Mycoplasma	2					6		4	3	5	4	4	3	5	4	4	4	4		3				3		4		3		4				4						
	OBLIGATE ANAEROBES																																							
	Eubacterium fusiforme								5		4																4														
	Actinomyces israelii														4																						4				
EYE	TOTAL AEROBES	2×10^{9}					2×10^{9}	4×10^{9}	4×10^{9}	4×10^{9}	1×10^{9}	4×10^{9}	3×10^{9}	3×10^{9}	2×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	2×10^{9}	1×10^{9}	2×10^{9}			1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	2×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	2×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	2×10^{9}	2×10^{9}	
	TOTAL ANAEROBES	2×10^{9}					1×10^{9}	6×10^{8}	2×10^{9}	1×10^{9}	5×10^{8}	2×10^{9}	5×10^{8}	3×10^{8}	2×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	2×10^{9}	1×10^{9}	1×10^{9}			1×10^{9}	2×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	2×10^{9}	2×10^{9}	
	MARKER ORGANISMS																																							
	S. epidermidis	3					3	4	4	3	4	4	3	4	5	3	3	3	2	3	4			4	4	4	4	4	4	4	4	6	5	5	5	5	4	4			
GROIN	TOTAL AEROBES	5×10^{9}					1×10^{9}	2×10^{9}	2×10^{9}	3×10^{9}	4×10^{9}	2×10^{9}	2×10^{9}	4×10^{9}	2×10^{9}	2×10^{9}	3×10^{9}	4×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}			2×10^{9}	2×10^{9}	1×10^{9}	1×10^{9}	3×10^{9}	2×10^{9}	2×10^{9}	1×10^{9}	2×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	2×10^{9}	1×10^{9}	1×10^{9}	3×10^{9}		
	TOTAL ANAEROBES	2×10^{9}					2×10^{9}	1×10^{9}	2×10^{9}	3×10^{9}	1×10^{9}	2×10^{9}	2×10^{9}	2×10^{9}	2×10^{9}	1×10^{9}	2×10^{9}	2×10^{9}	2×10^{9}	2×10^{9}	2×10^{9}			2×10^{9}	1×10^{9}	2×10^{9}	1×10^{9}	2×10^{9}	2×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	1×10^{9}	2×10^{9}		
	MARKER ORGANISMS																																							
	S. epidermidis	4					4	4	5	4	4	5	4	3	4	4	4	4	3	4	5			4	4	4	5	4	4	5	6	6	5	5	6	4	3			

FOLDOUT FRAME 1

FOLDOUT FRAME 2

TABLE B-IV
MICROFLORA DATA
TOTAL VIABLE AEROBIC AND ANAEROBIC COUNTS, LOG VALUES OF MARKER ORGANISMS
(PER DRY WEIGHT OF FECES OR PER SWAB SAMPLE)

MONKEY NUMBER 5

Samples taken 12/5/62

Placed in Isolation 1/17/63

Samples taken 2/26/63

	WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
	FISCAL WEEK	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
FECES	TOTAL AEROBES																																							
	TOTAL ANAEROBES																																							
	MARKER ORGANISMS																																							
	E. coli	5					4								3																									
	S. aureus	5					3	3	3	3	6	4	4	4	4	3	7		4	6	8			4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	S. epidermidis							4	3	4	4	4	4	3	3	5	4	5	4	4	4			4	4	4	4	4	4	5	4	5	4	5	4	4	4	4	4	4
	Alpha Streptococcus (Group D)	10					10	10		10	9	9	10			10	10	9	10	10	9			10	10		9	9	9	9	9	9	9	9	9	9	9	9	9	9
	Lepto. biflexa	10					10	9	10	10	9	10	9	10	10	10	10	9	9	9	7			10	7	7	9	6	6	7	6	7	7	6	7	7	6	10	10	10
	Haemophilus diphtheroid															7			10	10	7				10		9	10	9	7		7		6	6	6	10	10	10	10
	Proteus																	4		4	4			4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4
	OBLIGATE ANAEROBES																																							
GINGIVA	TOTAL AEROBES																																							
	TOTAL ANAEROBES																																							
	MARKER ORGANISMS																																							
	S. aureus	2					3	2	3	2		3	3	3	3	2	2	2	3	2				4		4		2		6		5		3		3		3	
	S. epidermidis	3						3	3	3	2	3	4	3	3	2	2	2	3	2				3		4		3		4		5		3		3		4	
	Alpha Streptococcus I	3					5	6	7	6	5	5	6	5	5	6	5	6	4	5				4				4						4		6			
	Alpha Streptococcus II						5	4												6																			
	Gamma Streptococcus						5				4	4	4	6					6	6								6						7					
	Lepto. interrogans icterohaemorrhagiae							3	5	3	4	5	5	6	3			4								5		4		6				6		5		6	
	Haemophilus															5	4		5	6				4		6		6		6		8		7		6		7	
	Mycoplasma						5		3	2	2		3	3	3	4		5	3	2				4		3		4		5		6						4	
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme						4	4		4	4	5	6	5	4	6	4	4		6								7		5						5		6	
	Bacteroides (Bacteroides) ruminicola											4		4	4	6	6							4				4		4						5		6	
	TOTAL AEROBES																																							
	TOTAL ANAEROBES																																							
	MARKER ORGANISMS																																							
	S. aureus						4	5	3			3	2	3	4										3															
	S. epidermidis	3					3	3	3	3	2	3	3	3	4	2	2	2	3	2					3		3		4				3		3		4		
	Alpha Streptococcus I	6					4	6	6	4	6	6	6	6	6	6	6	6	6	6					6		8		6				6		6				
	Alpha Streptococcus II						4		4																															
	Gamma Streptococcus						5					4		4				3	5											4				5				6	
	Lepto. interrogans icterohaemorrhagiae						5	5	5	3	3	5	5	5	3			3	5											4				4		6			
	Haemophilus													4	4	6	6	4	2						6		6						7					
	Mycoplasma						5			2			3	3	3										62		<2		<2		2								
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme											4	4	4	4	6																							
	Bacteroides (Bacteroides) ruminicola													4	4																								
	TOTAL AEROBES																																							
	TOTAL ANAEROBES																																							
	MARKER ORGANISMS																																							
	S. epidermidis	3					3	2	3	3	4	4	3	2	2	2		3	2		2				3	4	3	4	3	4	3	4	~	3	3	3	4	4		
	TOTAL AEROBES																																							
	TOTAL ANAEROBES																																							
	MARKER ORGANISMS																																							
	S. epidermidis	3					3	2	3	4	3	4	4	3	3	3		2		3	3				2	3	4	5	4	4	4	4	4	4	5	4	3	4	5	

FOLDOUT FRAME 1

FOLDOUT FRAME 2

TABLE B-V

MICROFLORA DATA

TOTAL VIABLE AEROBIC AND ANAEROBIC COUNTS, LOG VALUES OF MARKER ORGANISMS

(PER DRY WEIGHT OF FECES OR PER SWAB SAMPLE)

MONKEY NUMBER 6

Samples Taken ← 9/19/69

	WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
	FISCAL WEEK	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
FECES	TOTAL AEROBES																																							
	TOTAL ANAEROBES																																							
	MARKER ORGANISMS																																							
	L. acid.			6			7	7		7		7		7		7		6		6					7		5-		6		5-		5		5-		5-		5	
	S. aureus			5			4	4		4		4		4		4				4					3		4		2		3		3		4		6		7	
	S. epidermidis						4	5		4		3		3				4							3		4				3		4						
	Alpha Streptococcus (Group D)			11			9	10		11		10				11		8		10					10		9		9		10		10		10				
	Lactobacilli			10			9	9		9		9		8				7		8					10		9		9		5-		9		9		9		9	
	X																								10															
	OBLIGATE ANAEROBES																																							
VAGINA	TOTAL AEROBES																																							
	TOTAL ANAEROBES																																							
	MARKER ORGANISMS																																							
	S. aureus			4			4	3		2		3		5		3		4		4					4		4		4		4		3		4		4			
	S. epidermidis			3			3			2								3											2		3				3		4			
	Alpha Streptococcus I			5				6		6		6		5		5		6		6							4		6		6				6					
	Alpha Streptococcus II			5			5	6																			3-				2-									
	Gamma Streptococcus			6			6	7				6		6		6		4									4				4									
	Lepto. trichia innominata						5	6		4		4		5				3									3-		3-		2-		6		6		6			
	Haemophilus													6											5-		5-		6				6		7		6			
	Mycoplasma			4			6			4		3		5				2		3					4		3		4		4				4					
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme			-			-	-		5		6		6		5									6		4		6		6		6		7		7			
	Actinomyces israelii							-				5		5		4									5-		4						5-		6					
THROAT	TOTAL AEROBES																																							
	TOTAL ANAEROBES																																							
	MARKER ORGANISMS																																							
	S. aureus			4			3	3				5		5		3		3							3		4		4		4		4		4		4			
	S. epidermidis						3			2		2		3		2																		3						
	Alpha Streptococcus I			5			5	4		6		6		6		5									4		4		6-		6-		6-							
	Alpha Streptococcus II			5			4			6																														
	Gamma Streptococcus						5	4								4																								
	Lepto. trichia innominata			3			4	4		3		3		5				3											4		4		4		5		5			
	Haemophilus													5				6							6-		5-		5-				5-		6		6			
	Mycoplasma			3			5			3				4				3							<2		3		3		2				4		4			
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme						-	-		4				5													4				4		4		5		6			
	Actinomyces israelii																																4							
EYE	TOTAL AEROBES																																							
	TOTAL ANAEROBES																																							
	MARKER ORGANISMS																																							
	S. epidermidis			2			4	3				2		2		2		<2		<2					3		3		3		4		3		3		4			
NOSE	TOTAL AEROBES																																							
	TOTAL ANAEROBES																																							
	MARKER ORGANISMS																																							
	S. epidermidis			4			3	3		2		3		3		4		4		<2					26		6		5-		6		6		5		5-			

FOLDOUT FRAME

FOLDOUT FRAME 2

TABLE B-VI

MICROFLORA DATA

TOTAL VIABLE AEROBIC AND ANAEROBIC COUNTS, LOG VALUES OF MARKER ORGANISMS

(PER DRY WEIGHT OF FECES OR PER SWAB SAMPLE)

MONKEY NUMBER 7

Annotations above columns: week 2 — "SAMPLES TAKEN 4/14/67"; week 4 — "PAIRED IN ISOLATION 5/2/67"; week 5 — "SAMPLES TAKEN 5/5/67"

FECES

WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
FISCAL WEEK	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
TOTAL AEROBES			10×10^{8}			2.3×10^{8}	2×10^{8}	5×10^{10}	2×10^{10}	3×10^{7}	1×10^{8}	5×10^{10}	1×10^{8}	9×10^{7}	4×10^{10}	5×10^{10}	6×10^{9}	5×10^{10}	9×10^{10}	1×10^{11}			1×10^{10}	2×10^{8}	3×10^{8}	9×10^{8}	1×10^{7}	4×10^{7}	1×10^{9}	3×10^{8}	2×10^{9}	4×10^{7}	5×10^{8}	5×10^{7}	6×10^{8}	2×10^{8}	3×10^{8}	7×10^{9}	
TOTAL ANAEROBES			3.1×10^{10}			1.2×10^{8}	5×10^{10}	5×10^{10}	4×10^{10}	2×10^{10}	2×10^{10}	5×10^{10}	5×10^{10}	2×10^{10}	2×10^{11}	1×10^{10}	4×10^{10}	3×10^{10}	1×10^{11}	2×10^{11}			3×10^{10}	2×10^{8}	5×10^{8}	9×10^{8}	3×10^{8}	6×10^{9}	5×10^{7}	6×10^{9}	7×10^{7}	7×10^{9}	9×10^{8}	3×10^{10}	2×10^{11}	6×10^{9}	6×10^{9}	8×10^{10}	
MARKER ORGANISMS																																							
E. coli			7			8	7	5	6	6	6	6	4	6	6	7	7	4	6	6			<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	<3	
S. aureus			4			3	5	4	4	3	6		3	4	3	5				4			5	6	5	7	6	5	6	6	5	5	5	6	5	5	4	5	
S. epidermidis			4			3	5	5	6	5	4				3	6	4	5	4	5			6	6	6	7	6	5	5	6	5	6	6	6	4	5	5	5	
Alpha Streptococcus (Group D)			11				10	10	10	10	10	9	10	10	10	10	9	9	9	10			9	10	7	9	9	7	9	8	8	7	8			8	8	9	
Lactobacilli			10			8	10	10	8	7	9	8	8	8	9	10	8	8	8				8	9	8	6	6	<3	4	4	6	5	6	7	6	4	5	5	
Anaerobic diphtheroid																																							
OBLIGATE ANAEROBES																																							

GINGIVA

WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
TOTAL AEROBES			4.0×10^{5}			1×10^{7}	7×10^{5}	4×10^{6}	3×10^{6}	2×10^{6}	1×10^{7}	4×10^{6}	2×10^{6}	3×10^{6}	5×10^{5}	2×10^{6}	6×10^{6}	4×10^{6}	1×10^{7}				7×10^{6}		1×10^{6}		6×10^{7}		2×10^{7}		6×10^{6}	5×10^{6}	7×10^{7}		3×10^{7}		2×10^{7}		
TOTAL ANAEROBES			7.6×10^{5}			1×10^{8}	3×10^{6}	2×10^{7}	2×10^{7}	1×10^{7}	3×10^{7}	1×10^{7}	1×10^{7}	3×10^{6}	2×10^{7}	1×10^{7}	7×10^{6}	1×10^{7}	5×10^{7}				1×10^{7}		5×10^{6}		5×10^{6}		1×10^{6}		5×10^{6}	1×10^{7}	3×10^{6}		1×10^{6}		2×10^{6}		
MARKER ORGANISMS																																							
S. aureus			2			4	3	3	3	4	4	4	2	2	2	2	4	3					<4		5		5		6		3	3	5				4		
S. epidermidis			2				4	4	4	3	5	3		2	2	2	3	4	4				<4		5		6		6		3	5			4		4		
Alpha Streptococcus I			5			5	6	6	6	6	6	5	5	5	5	5	5	6	6						5		6		5		6								
Alpha Streptococcus II			5				6																				7												
Gamma Streptococcus			5			7	5	7		5	5	5	4		4	6			5								7		6				6						
Leptotrichia buccalis						4	5		5	4	4	4	4				4								5		5				4	4	5		5		5		
Haemophilus													5				3	5	6				4									5	6		6		6		
Mycoplasma spp.			3			4		5	4	4	3	4	4	4		3	3	2	2				4		3		4		3		2				2		3		
OBLIGATE ANAEROBES																																							
Fusobacterium fusiforme						✓	✓	6	6	5	6	6	5	6		5													4						7		6		
Actinomyces israelii			✓				✓			5	5	5	4	6	4	4			4																		5		

THROAT

WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
TOTAL AEROBES			8.6×10^{5}			1×10^{6}	4×10^{6}	2×10^{6}	2×10^{6}	1×10^{6}	1×10^{6}	8×10^{5}	1×10^{6}	5×10^{5}	6×10^{5}	8×10^{4}	5×10^{5}			1×10^{6}				2×10^{6}		9×10^{5}		2×10^{5}		1×10^{6}				6×10^{6}		1×10^{8}			
TOTAL ANAEROBES			3.6×10^{5}			1×10^{7}	8×10^{6}	1.8×10^{6}	8×10^{6}	5×10^{6}	5×10^{6}	1×10^{6}	2×10^{6}	2×10^{6}	4×10^{6}	3×10^{5}	3×10^{6}			5×10^{6}				5×10^{5}		4×10^{6}		4×10^{6}		5×10^{6}				3×10^{7}		3×10^{7}			
MARKER ORGANISMS																																							
S. aureus			2			5	3	3	3	4	4	3	3	2		2				2				5		4		4		4				4		4			
S. epidermidis			2				2	3	3		5	3	2	2	2		3			3				5		5		4		4				4		3			
Alpha Streptococcus I			5			5	6	6	6	5	5	5	5	5	5	4	5			6				5		4		5		4				5					
Alpha Streptococcus II			4				5																																
Gamma Streptococcus			5			5	6	5		4	4	4	4		4	3																							
Leptotrichia buccalis			2			4	5	4	5	4	3	4	5				3			4								4		4						5			
Haemophilus												✓	6	6	5	4	5			5				7		5		4		4				5		7			
Mycoplasma			3			4		4	4	4		4	3	3		2	2			2				2		2		2		2						2			
OBLIGATE ANAEROBES																																							
Fusobacterium fusiforme						✓		5	6		5	5	5				4																			4			
Actinomyces israelii											4			4	4																			4					

EYE

WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
TOTAL AEROBES			4.7×10^{3}			3×10^{3}	8×10^{2}	1×10^{4}	1×10^{4}	9×10^{3}	2×10^{4}	1×10^{3}	1×10^{4}	6×10^{2}	1×10^{2}	8×10^{2}	1×10^{3}	5×10^{3}	2×10^{4}	1×10^{3}			2×10^{4}	5×10^{4}	5×10^{3}	1×10^{5}	1×10^{6}	5×10^{5}	1×10^{6}	8×10^{4}	2×10^{4}	6×10^{3}	5×10^{5}	4×10^{4}	3×10^{5}	3×10^{5}	3×10^{5}		
TOTAL ANAEROBES			8.5×10^{3}			2×10^{3}	6×10^{3}	2×10^{4}	1×10^{3}	2×10^{4}	2×10^{4}	2×10^{3}	1×10^{2}	9×10^{2}	2×10^{2}	1×10^{3}	5×10^{2}	5×10^{3}	4×10^{2}	5×10^{2}			2×10^{4}	6×10^{4}	1×10^{3}	5×10^{4}	1×10^{6}	2×10^{5}	2×10^{4}	2×10^{4}	5×10^{4}	3×10^{3}	8×10^{5}	4×10^{3}	3×10^{5}	4×10^{5}	4×10^{5}		
MARKER ORGANISMS																																							
S. epidermidis			3			3	4	4	4	3	4	2	2	2		2	3	3	2	3			<4	5	5	5	6	5	4	4	3	4	–	4	3	4	4		

GROIN

WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
TOTAL AEROBES			3.3×10^{5}			5×10^{4}	2×10^{4}	4×10^{4}	5×10^{4}	2×10^{5}	2×10^{5}	4×10^{3}	2×10^{5}	4×10^{3}	1×10^{5}	2×10^{4}	7×10^{4}	3×10^{5}	6×10^{5}	4×10^{4}			2×10^{5}	2×10^{7}	3×10^{7}	1×10^{6}	4×10^{5}	1×10^{6}	9×10^{3}	2×10^{7}	4×10^{7}	8×10^{7}	9×10^{7}	1×10^{6}	5×10^{7}	2×10^{7}	6×10^{7}		
TOTAL ANAEROBES			9.6×10^{5}			2×10^{4}	1×10^{4}	6×10^{4}	2×10^{4}	1×10^{5}	2×10^{5}	6×10^{2}	3×10^{5}	4×10^{5}	2×10^{5}	2×10^{4}	5×10^{5}	4×10^{5}	3×10^{5}	4×10^{4}			2×10^{5}	2×10^{7}	1×10^{7}	4×10^{7}	7×10^{7}	4×10^{6}	3×10^{7}	5×10^{7}	1×10^{6}	4×10^{7}	5×10^{7}	1×10^{6}	4×10^{7}	1×10^{8}	5×10^{7}		
MARKER ORGANISMS																																							
S. aureus			4			3	3	3	2		3	2	3	4	4	4										7	8	7	6	7	5	5	6	6	6		7		
S. epidermidis			4			4	4	4	4	4	4	3	4	5	4	4	5	4	5	5			[illegible]	[illegible]	1×10^{6}	7	7	7	7	7	6	6	6	7	7	6	7		

FOLDOUT FRAME 1

FOLDOUT FRAME 2

TABLE B-VII

MICROFLORA DATA

TOTAL VIABLE AEROBIC AND ANAEROBIC COUNTS, LOG VALUES OF MARKER ORGANISMS

(PER DRY WEIGHT OF FECES OR PER SWAB SAMPLE)

MONKEY NUMBER 8

Samples Thrown ← 4/11/69

	WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
	FISCAL WEEK	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
FECES	TOTAL AEROBES			3×10^{11}			5×10^{11}		3×10^{11}		7×10^{9}	6×10^{7}	4×10^{9}		6×10^{9}		3×10^{9}		5×10^{8}		1×10^{8}		1×10^{8}		7×10^{6}		2×10^{6}		1×10^{9}		1×10^{11}		9×10^{8}		3×10^{9}		3×10^{9}		
	TOTAL ANAEROBES			3×10^{11}			6×10^{10}		1×10^{10}		1×10^{10}	2×10^{7}	1×10^{10}		1×10^{10}		6×10^{7}		4×10^{9}		7×10^{8}		1×10^{8}		3×10^{6}		6×10^{8}		1×10^{11}		3×10^{10}		2×10^{9}		3×10^{10}		2×10^{10}		
	MARKER ORGANISMS																																							
	E. coli			6							3	4			6		3		4		5			4				3		< 2		< 2		< 3		< 3		< 3		
	S. aureus			5			4		4		4	5	5		4		3		3		3			4		4		4		4		4		4		4		3		
	S. epidermidis			3			4				3	2	4											6		4		3		4				3		3				
	Alpha Streptococcus (Group D)			11			10		10		9	7	9		9		8		8		9			10		11		10		10		8		10		10		9		
	Lactobacilli			10			10		9		8	9	9		8		8		7		9			10		7		8		8		6		8		8		9		
	OBLIGATE ANAEROBES																																							
GINGIVA	TOTAL AEROBES			3×10^{6}			1×10^{7}		2×10^{7}		6×10^{6}		7×10^{6}		2×10^{6}		3×10^{6}		2×10^{6}		6×10^{6}			3×10^{6}		3×10^{6}		2×10^{6}		3×10^{5}		1×10^{7}		2×10^{7}		3×10^{7}		3×10^{7}		
	TOTAL ANAEROBES			1×10^{7}			2×10^{9}		2×10^{8}		2×10^{8}		1×10^{8}		1×10^{8}		2×10^{8}		5×10^{8}		3×10^{8}			2×10^{8}		6×10^{8}		4×10^{8}		6×10^{8}		4×10^{8}		7×10^{8}		5×10^{8}		4×10^{8}		
	MARKER ORGANISMS																																							
	S. aureus			4			4		3		3		5		4		6		5		3			2		4		3		3		3		4		3				
	S. epidermidis			2			2		2		2										4					3		4											
	Alpha Streptococcus I			6			6		6		7		5		5		5		5		5					5		5		4									
	Alpha Streptococcus II			5			5																					6		4									
	Gamma Streptococcus			4							5				4						4							5		6									
	Leptotrichia innominata						4		4				5		4											4		5		6		5		6		6		6	
	Haemophilus														5																								
	Mycoplasma			5			6		4		4		4		3		3		4		4			4		4		5		4		4		6		6		4	
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme			1			1				4		6		5		6		5		5			6		5		6		7		5		7		7		7	
	Actinomyces israelii														4				4		4			6		4		6		6		3		6		6		6	
THROAT	TOTAL AEROBES			3×10^{6}			7×10^{5}		2×10^{6}		9×10^{5}		2×10^{6}		6×10^{4}		3×10^{6}		1×10^{6}		2×10^{6}			2×10^{6}		1×10^{6}		2×10^{6}		2×10^{6}		1×10^{6}		1×10^{6}		5×10^{5}		2×10^{6}	
	TOTAL ANAEROBES			8×10^{6}			2×10^{8}		6×10^{8}		1×10^{8}		3×10^{8}		1×10^{8}		2×10^{8}		2×10^{8}		6×10^{8}			4×10^{8}		1×10^{8}		1×10^{8}		3×10^{8}		2×10^{8}		1×10^{8}		2×10^{8}		2×10^{8}		
	MARKER ORGANISMS																																							
	S. aureus			4			3		3		4		5		4		6		5		4			4		5		3		5		4		2		3		3		
	S. epidermidis																				3					4				4									
	Alpha Streptococcus I			5			5		7		5		5		6		6		5		5					5		5		6		6		4					
	Alpha Streptococcus II			5			4																					5											
	Gamma Streptococcus			4																																				
	Leptotrichia innominata			4			4						4		4													4		4		4		4		4		6	
	Haemophilus												6		6		6				6			6		6		5		6		6		6		7			
	Mycoplasma			3			4		4		3		4		4		3		3					4		3		3		3		2		3		3			
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme													5	5				5		5					4		4		4						4		6	
	Actinomyces israelii										4				5																					4			
EYE	TOTAL AEROBES			3×10^{3}			2×10^{3}		2×10^{3}		1×10^{3}		3×10^{3}		$<10^{3}$		$<10^{3}$		4×10^{4}		1×10^{4}			3×10^{3}		2×10^{3}		1×10^{3}		2×10^{4}		2×10^{3}		2×10^{3}		1×10^{4}		5×10^{3}	
	TOTAL ANAEROBES			1×10^{4}			2×10^{3}		5×10^{3}		1×10^{3}		1×10^{3}		1×10^{3}		$<10^{3}$		4×10^{4}		2×10^{4}			2×10^{4}		1×10^{3}		1×10^{3}		7×10^{4}		4×10^{3}		2×10^{3}		1×10^{3}		6×10^{3}	
	MARKER ORGANISMS																																							
	S. epidermidis						2				3		2		2		$<10^{2}$		2		2			24		43		3		2		3		2		4		4		
GROIN	TOTAL AEROBES			2×10^{3}			2×10^{3}		1×10^{3}		1×10^{3}		2×10^{3}		6×10^{3}		1×10^{3}		2×10^{2}		5×10^{3}			1×10^{3}		7×10^{2}		1×10^{3}		2×10^{3}		2×10^{3}		2×10^{3}		1×10^{3}		2×10^{3}	
	TOTAL ANAEROBES			2×10^{3}			8×10^{3}		1×10^{3}		2×10^{3}		6×10^{3}		8×10^{3}		2×10^{3}		2×10^{2}		2×10^{3}			1×10^{3}		6×10^{3}		3×10^{3}		2×10^{4}		1×10^{3}		2×10^{3}		1×10^{3}		2×10^{3}	
	MARKER ORGANISMS																																							
	S. epidermidis						2		3		2		2		3		3		2		3			3		4		3		6		6		5		2		3		

FOLDOUT FRAME 1

FOLDOUT FRAME 2

TABLE B-VIII
MICROFLORA DATA
TOTAL VIABLE AEROBIC AND ANAEROBIC COUNTS, LOG VALUES OF MARKER ORGANISMS
[PER DRY WEIGHT OF FECES OR PER SWAB SAMPLE]

MONKEY NUMBER 9

Samples Taken ← Weekly

	WEEKS FROM START	START	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
	FISCAL WEEK	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
FECES	TOTAL AEROBES			4×10^{7}				1×10^{10}		2×10^{9}		5×10^{9}		7×10^{9}		5×10^{9}		1×10^{9}		8×10^{9}			1×10^{10}		8×10^{9}		2×10^{10}		3×10^{9}		3×10^{10}		3×10^{10}		9×10^{9}		3×10^{9}	
	TOTAL ANAEROBES			3×10^{10}				4×10^{10}		1×10^{10}		2×10^{10}		1×10^{10}		3×10^{11}		8×10^{9}		2×10^{10}			6×10^{10}		2×10^{10}		3×10^{10}		3×10^{10}		6×10^{10}		8×10^{10}		6×10^{10}		4×10^{10}	
	MARKER ORGANISMS																																							
	E coli			6				6		4				2		5		3		3					4.3		4.3		3		4.3		4.3		4.3		4.3		4
	S aureus			4								5		3		3		4		4					4		3				2		4		4		4	
	S epidermidis			3				4		3		4		3		3		4		5					4		3				3		4		4		4	
	Streptococcus spp			1				9		8		9		9		8		7		10					9		10				10		9		9		5	
	Lactobacillus			10				9		7		9		9				6		8					9		10		8		8		9		9		8		7	
	OBLIGATE ANAEROBES																																							
GINGIVA	TOTAL AEROBES			6×10^{7}				6×10^{8}		1×10^{8}		5×10^{8}		5×10^{8}		4×10^{8}		4×10^{8}		6×10^{8}					2×10^{7}		4×10^{7}		2×10^{7}		3×10^{7}		2×10^{7}		3×10^{7}		8×10^{7}			
	TOTAL ANAEROBES			9×10^{7}				1×10^{8}		6×10^{8}		1×10^{8}		8×10^{7}		1×10^{8}		3×10^{8}		2×10^{8}					1×10^{8}		2×10^{8}		6×10^{7}		3×10^{8}		2×10^{8}		2×10^{8}		7×10^{7}			
	MARKER ORGANISMS																																							
	S aureus			2						4				3		3		3		6					4		4				2		3		3					
	S epidermidis			3										3		5		4		4					4		4				2		3		3		4			
	Alpha Streptococcus II			1				6		5		6		6		6		6		7					5		5		6		6		5							
	Alpha Streptococcus III													6																										
	Gamma Streptococcus			1								5		5				6							6		6		6		7		6							
	Leptotrichia buccinalis			5				4		4		3		5											5		5		5		5		5		6		6			
	Haemophilus													6				5		6					7		7		7				6		7		6			
	Mycoplasma			5						2		4		5		4		4		3					6		5		5		5				5					
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme			1				1		4		6		6		5		5		5					6		5						6		6		6			
	Actinomyces israelii													4											5		4								5		6			
THROAT	TOTAL AEROBES			5×10^{7}				2×10^{8}		5×10^{7}		2×10^{8}		2×10^{8}		6×10^{7}		3×10^{8}							7×10^{6}		3×10^{6}		4×10^{6}		2×10^{6}		4×10^{6}		4×10^{6}		3×10^{6}			
	TOTAL ANAEROBES			2×10^{8}				3×10^{8}		1×10^{8}		2×10^{8}		2×10^{8}		1×10^{8}		2×10^{8}							3×10^{8}		5×10^{8}		5×10^{8}		6×10^{8}		3×10^{8}		6×10^{8}		5×10^{8}			
	MARKER ORGANISMS																																							
	S aureus			2						2		2		2		2		3		3					3		3				2		2		2					
	S epidermidis			1				6		5		5		5		2		2		4					3		3						2		2		3			
	Alpha Streptococcus II			1				4																																
	Gamma Streptococcus													4		4		5																						
	Leptotrichia buccinalis			4				4		4		3		4													4		5		5		5		5		5			
	Haemophilus													5				5							5		6		6				6		6		6			
	Mycoplasma			4						3		2		6		3		3							3		3		3		2				4					
	OBLIGATE ANAEROBES																																							
	Fusobacterium fusiforme							1																	4								4		5		5			
	Actinomyces israelii																																							
EYE	TOTAL AEROBES			3×10^{6}				1×10^{7}		2×10^{7}		4×10^{6}		1×10^{8}		1×10^{8}		4×10^{7}		2×10^{7}					4×10^{6}		2×10^{6}		1×10^{6}		1×10^{6}		1×10^{6}		2×10^{7}		1×10^{6}			
	TOTAL ANAEROBES			1×10^{8}				2×10^{8}		3×10^{8}		4×10^{8}		1×10^{8}		3×10^{8}		1×10^{8}		7×10^{8}					2×10^{8}		2×10^{8}		4×10^{8}		1×10^{8}		6×10^{8}		2×10^{8}		2×10^{8}			
	MARKER ORGANISMS																																							
	S epidermidis			2								3		2		–		3		2					4		3		–		–		3		4		4			
GROIN	TOTAL AEROBES			5×10^{6}				3×10^{8}		4×10^{8}		7×10^{8}		1×10^{8}		3×10^{8}		1×10^{8}		1×10^{8}					7×10^{8}		9×10^{8}		3×10^{8}		2×10^{8}		7×10^{8}		9×10^{8}		2×10^{8}			
	TOTAL ANAEROBES			6×10^{8}				5×10^{8}		9×10^{8}		7×10^{8}		2×10^{8}		1×10^{8}		1×10^{8}		1×10^{8}					1×10^{8}		1×10^{8}		5×10^{8}		1×10^{8}		6×10^{8}		7×10^{8}		1×10^{8}			
	MARKER ORGANISMS																																							
	S epidermidis			3				2		3		2		3		3		4		4					4		4		2		3		4		4		4			

FOLDOUT FRAME 1

FOLDOUT FRAME 2

APPENDIX C

PRIMATE HEMATOLOGY DATA

ISOLATED ANIMALS NUMBER 3, 4, 5, AND 7

CONTROL ANIMALS NUMBER 2, 6, 8, AND 9

TABLE C-I

PRIMATE ISOLATION STUDIES

MONKEY NUMBER 2

HEMATOLOGY DATA - SUMMARY CHART

FISCAL WEEK/WEEKS FROM START

MEASUREMENT	19/1	20/2	21/3	22/4	23/5	24/6	25/7	26/8	27/9	28/10	29/11	30/12	31/13	32/14	33/15	34/16	35/17
HGB GM/100 ML NV = 11 - 12.5			10.6				10.6		8.8		9.1		9.0		8.2		
PVC % NV = 39 - 43			31.0				32.0		31.0		34.8		30.0		26.0		
RBC x 10^6/MM^3 NV = 5 - 6			6.9				6.35		4.23		4.35		4.2		3.7		
WBC x 10^3/MM^3 NV = 7 - 13			12.8				14.3		10.5		9.1		8.0		6.2		
% BANDS			3				6		—		—		—		—		
% SEGS			30				26		17		41		17		27		
% TOTAL NEUTROS. NV = 20 - 56			33				32		17		41		17		27		
% LYMPHS NV = 40 - 76			55				58		73		47		75		62		
% MONOS NV = 0.5 - 2.0			4				3		6		5		2		6		
% EOS NV = 1 - 3			8				6		4		7		5		4		
% BASOS NV = 0 - 2							1						1		1		
RBC INDICES MCV μ^3 NV = 65 - 78			45				50		73		80		71		70		
MCH $\mu\mu$GM NV = 18 - 23			15				17		21		21		21		22		
MCHC GM/100 NV = 27 - 31			34				33		22		26		33		32		
NV = NORMAL VALUE																	

MEASUREMENT	36/18	37/19	38/20	39/21	40/22	41/23	42/24	43/25	44/26	45/27	46/28	47/29	48/30	49/31	50/32	51/33	52/34
HGB GM/100 ML NV = 11 - 12.5	8.3				8.5		6.6		7.5		8.7		9.9		9.7		
PVC % NV = 39 - 43	30.0				29.6		22.8		29.2		30.0		30.0		30.0		
RBC x 10^6/MM^3 NV = 5 - 6	4.36				5.28		5.78		6.61		5.94		5.82		8.78		
WBC x 10^3/MM^3 NV = 7 - 13	6.71				5.39		4.40		6.60		4.84		5.50		9.4		
% BANDS	—				3				2		4		2		—		
% SEGS	13				17		20		27		16		22		28		
% TOTAL NEUTROS. NV = 20 - 56	13				20		20		29		20		24		28		
% LYMPHS NV = 40 - 76	85				70		80		69		80		76		72		
% MONOS NV = 0.5 - 2.0	2				5				1						0		
% EOS NV = 1 - 3					5				1						0		
% BASOS NV = 0 - 2																	
RBC INDICES MCV μ^3 NV = 65 - 78	69				56.2		34.6		44.2		50.6		51.7		34.2		
MCH $\mu\mu$GM NV = 18 - 23	19				16.2		11.4		11.3		14.7		17.1		11.0		
MCHC GM/100 NV = 27 - 31	27.7				28.7		28.9		22.7		29.0		33.0		32.4		
NV = NORMAL VALUE																	

TABLE C-II

PRIMATE ISOLATION STUDIES

MONKEY NUMBER 3

HEMATOLOGY DATA · SUMMARY CHART

FISCAL WEEK/WEEKS FROM START

MEASUREMENT	19/1	20/2	21/3	22/4	23/5	24/6	25/7	26/8	27/9	28/10	29/11	30/12	31/13	32/14	33/15	34/16	35/17
HGB GM/100 ML NV = 11 – 12.5			12.3	12.3		10.9	12.4	11.6			9.6				10.9		10.0
PVC % NV = 39 – 43			34.9	33.4		32.0	34.8	32.3			33.9				29		35
RBC x 10^6/MM^3 NV = 5 – 6			6.45	5.3		5.7	6.15	5.75			4.26				4.35		4.68
WBC x 10^3/MM^3 NV = 7 – 13			8.0	7.4		12.8	8.6	8.0			3.4				4.6		5.28
% BANDS			6	5		6	4	3									1
% SEGS			33	34		48	23	24			22				34		8
% TOTAL NEUTROS. NV = 20 – 56			39	39		54	27	27			22				34		9
% LYMPHS NV = 40 – 76			54	46		40	65	65			68				56		91
% MONOS NV = 0.5 – 2.0			2	6		1	2	3			5				5		
% EOS NV = 1 – 3			4	7		4	4	5			5				4		
% BASOS NV = 0 – 2			1	2		1	2								1		
RBC INDICES MCV μ^3 NV = 65 – 78			54	63		56	57	56			79				67		75
MCH μμ GM NV = 18 – 23			19	23		19	20	20			23				39		21
MCHC GM/100 NV = 27 – 31			35	36		34	36	36			28				26		28
NV = NORMAL VALUE																	

MEASUREMENT	36/18	37/19	38/20	39/21	40/22	41/23	42/24	43/25	44/26	45/27	46/28	47/29	48/30	49/31	50/32	51/33	52/34
HGB GM/100 ML NV = 11 – 12.5	13.3	10.0		9.0	8.5	10.4	9.7	10.3	8.5	9.7	10.3	9.9	9.6		9.9	11.6	
PVC % NV = 39 – 43	40	31.5		40.2	29.4	33.3	33.7	33.6	34.4	33.0	31.5	32.1	31.2		30.0	35.6	
RBC x 10^6/MM^3 NV = 5 – 6	4.36	4.12		5.5	6.12	6.89	5.67	6.51	6.16	6.75	4.86	7.02	6.30		7.75	6.42	
WBC x 10^3/MM^3 NV = 7 – 13	4.51	3.52		5.5	2.53	3.33	6.71	4.73	2.20	2.53	3.85	3.85	4.4		4.40	5.61	
% BANDS	—				1				8				12				
% SEGS	15	20		12	17	6	12	15	18	22	8	10	38		28	4	
% TOTAL NEUTROS. NV = 20 – 56	15	20		12	18	6	12	15	26	22	8	10	50		28	4	
% LYMPHS NV = 40 – 76	83	71		86	68	94	88	80	72	78	92	86	48		68	95	
% MONOS NV = 0.5 – 2.0	2	3		2	1			5	2						4	1	
% EOS NV = 1 – 3		6			13							4	2				
% BASOS NV = 0 – 2																	
RBC INDICES MCV μ^3 NV = 65 – 78	91.7	76.5		73	48.1	48.4	59.5	51.7	55.9	48.9	64.9	45.8	49.6		38.7	55.4	
MCH μμ GM NV = 18 – 23	30.5	24.3		16	13.8	15.2	17.2	15.8	13.9	14.4	21.2	14.1	15.4		12.8	18.1	
MCHC GM/100 NV = 27 – 31	33.3	31.7		22	28.9	31.3	28.8	30.7	24.7	29.4	32.7	30.8	30.8		33.0	32.4	
NV = NORMAL VALUE																	

TABLE C-III

PRIMATE ISOLATION STUDIES

MONKEY NUMBER 4

HEMATOLOGY DATA - SUMMARY CHART

FISCAL WEEK/WEEKS FROM START

MEASUREMENT	19/1	20/2	21/3	22/4	23/5	24/6	25/7	26/8	27/9	28/10	29/11	30/12	31/13	32/14	33/15	34/16	35/17	36/18	37/19	38/20	39/21	40/22	41/23	42/24	43/25	44/26	45/27	46/28	47/29	48/30	49/31	50/32	51/33	52/34
HGB GM/100 ML NV = 11 - 12.5	13.6		13.1				12.6		11.8	12.2	10.5		10.2	10.5			9.6	9.4	15.4		12.0	11.5	10.6	10.4	11.4	10.0	10.9	10.8	10.8	12.1		13.4	11.8	
PVC % NV = 39 - 43	39.9		39.2				35.8		37	35	35.8		34	35			37.5	31.5	30		36.2	39.6	35.1	35.1	37.8	37.6	37.8	39.3	38.6	37.1		40.1	36.1	
RBC x 10^6/MM^3 NV = 5 - 6	6.23		6.75				5.45		5.9	5.0	5.25		5 0	5.16			6.26	4.18	4.82		6.21	6.42	6.14	5.67	5.73	6.58	6.09	6.91	7.28	6.56		8.23	6.51	
WBC x 10^3/MM^3 NV = 7 - 13	8.6		3.5				7.5		5.4	5.6	7.4		5.2	3.9			2.42	3.08	1.54		2.20	2.20	2.42	1.76	2.53	1.87	1.65	1.76	2.97	3.74		5.50	3.41	
% BANDS	7		1														6				4	3	2			16	2	2	2	2				
% SEGS	42		30				42		47	45	76		44	50			38	18	42		36	55	26	40	10	36	30	50	22	6		36	30	
% TOTAL NEUTROS. NV = 20 - 56	49		31				42		47	45	76		44	50			44	18	42		40	58	28	40	10	52	32	52	24	8		36	30	
% LYMPHS NV = 40 - 76	42		49				51		42	44	17		46	43			48	76	56		60	39	68	60	90	48	56	44	76	88		62	64	
% MONOS NV = 0.5 - 2.0	2		5				1		5	6	1		5	2			6	6	2			1	3				2			4				
% EOS NV = 1 - 3	6		12				5		6	4	7		5	5			2					2					10	4				2	6	
% BASOS NV = 0 - 2	1		3				1			1	1						0						1											
RBC INDICES MCV μ^3 NV = 65 - 78	64		58				66		63	70	68		69	68			60	75.5	62.4		58	61.7	57.2	62.0	65.1	57.2	62.2	56.8	53.1	57.6		48.8	55.4	
MCH $\mu\mu$ GM NV = 18 - 23	22		19				23		20	24	20		20	20			15	22.5	31.9		19	17.9	17.6	18.7	19.8	15.2	17.8	15.8	14.8	18.5		16.3	18.1	
MCHC GM/100 NV = 27 - 31	34		33				35		32	35	29		30	30			26	29.8	51.3		33	29.1	30.2	29.7	30.2	26.6	28.8	27.5	28.0	32.6		33.5	32.7	
NV = NORMAL VALUE																																		

TABLE C-IV

PRIMATE ISOLATION STUDIES

MONKEY NUMBER 5

HEMATOLOGY DATA - SUMMARY CHART

FISCAL WEEK/WEEKS FROM START

MEASUREMENT	19/1	20/2	21/3	22/4	23/5	24/6	25/7	26/8	27/9	28/10	29/11	30/12	31/13	32/14	33/15	34/16	35/17	36/18	37/19	38/20	39/21	40/22	41/23	42/24	43/25	44/26	45/27	46/28	47/29	48/30	49/31	50/32	51/33	52/34
HGB GM/100 ML NV = 11 – 12.5	11.8	12.9	12.6			10.4	11.9				9.9		9.6	10.0			9.3	9.3	8.5		9.3	9.9	10.1	9.8	10.7	7.6	9.5	8.7	9.9	8.5		10.6	11.4	
PVC % NV = 39 – 43	34.0	36.8	35.5			33.0	33.8				30.9		34	32			34.4	30.1	31.6		33.4	32.2	32.1	31.5	32.1	29.2	29.2	28.2	32.0	26.5		32.2	36.0	
RBC x 10^6/MM^3 NV = 5 – 6	5.4	6.5	5.95			4.6	5.05				4.69		4.8	6.24			4.44	3.38	4.30		5.27	5.51	6.82	6.0	6.80	5.87	5.41	5.85	6.55	5.86		6.21	7.13	
WBC x 10^3/MM^3 NV = 7 – 13	6.6	11.0	7.6			7.0	6.7				7.5		8.3	6.4			6.82	6.49	4.84		3.41	4.51	2.97	7.04	5.72	3.63	4.29	1.98	4.07	41.8		4.40	7.70	
% BANDS	1	3				3	2						1				4	2	1				1			8							1	
% SEGS	24	18	2			26	16				41		23	40			26	14	17		22	16	12		28	28	8	6	12	8		16	13	
% TOTAL NEUTROS. NV = 20 – 56	25	21	2			29	18				41		24				30	16	18		22	16	13		28	36	8	6	12	8		16	14	
% LYMPHS NV = 40 – 76	62	64	76			61	70				49		65	54			69	83	82		76	83	79		64	60	90	94	84	92		82	82	
% MONOS NV = 0.5 – 2.0	6	8	3			3	5				3		4				1					1				2			2				1	
% EOS NV = 1 – 3	6	6	18			6	5				6		6	5				1			2		8		8	2	2		2			2	3	
% BASOS NV = 0 – 2	1	1	1			1	2				1		1	1																				
RBC INDICES MCV μ^3 NV = 65 – 78	62	57	58			72	67				66		71	52			77	89.1	73.6		64	58.5	47.1	52.6	46.6	49.8	54.1	48.3	48.8	45.3		51.8	50.5	
MCH $\mu\mu$ GM NV = 18 – 23	22	20	21			23	24				21		20	16			21	28.5	19.8		18	17.9	14.8	16.7	15.5	12.9	17.6	14.9	15.1	14.5		17.1	16.0	
MCHC GM/100 NV = 27 – 31	34	35	35			32	35				32		28	31			27	30.9	26.9		28	30.7	31.5	31.1	33.4	25.6	32.5	30.8	30.9	32.1		32.9	31.7	
NV = NORMAL VALUE																																		

TABLE C-V

PRIMATE ISOLATION STUDIES

MONKEY NUMBER 6

HEMATOLOGY DATA - SUMMARY CHART

FISCAL WEEK/WEEKS FROM START

MEASUREMENT	19/1	20/2	21/3	22/4	23/5	24/6	25/7	26/8	27/9	28/10	29/11	30/12	31/13	32/14	33/15	34/16	35/17	36/18	37/19	38/20	39/21	40/22	41/23	42/24	43/25	44/26	45/27	46/28	47/29	48/30	49/31	50/32	51/33	52/34
HGB GM/100 ML NV = 11 − 12.5	11.5	12.2	12.1			10.8		11.0		11.4		11.4		11.2			7.3		11.7				11.6		12.2		12.7		12.2			12.9	11.8	
PVC % NV = 39 − 43	35.9	36.3	35.1			33.5		30.9		34		33		31			30.6		34.2				38.0		38.0		39.5		38.0			36.4	37.5	
RBC x 10^6/MM^3 NV = 5 − 6	6.3	5.9	5.9			5.95		6.3		4.25		4.24		5.52			3.85		3.97				6.30		6.61		7.72		6.99			6.99	7.38	
WBC x 10^3/MM^3 NV = 7 − 13	6.7	7.2	8.0			11.8		11.1		10.3		13.1		10.2			6.49		8.58				50.6		9.13				7.26			10.56	7.59	
% BANDS	2	4	2			1		1						1			2		1				1		6								4	
% SEGS	19	27	15			14		21		35		55		24			25		26				13		28		18		10			22	20	
% TOTAL NEUTROS. NV = 20 − 56	21	31	17			15		22		35		55		25			27		27				14		34		50		74			70	68	
% LYMPHS NV = 40 − 76	50	45	57			65		56		51		27		57			71		66				69		60									
% MONOS NV = 0.5 − 2.0	5	2	5			2		3				2		1			2		4				3		6									
% EOS NV = 1 − 3	23	22	19			18		18		14		15		16					3				14		2		32		16			8	8	
% BASOS NV = 0 − 2	1		2					1				1		1																				
RBC INDICES MCV μ^3 NV = 65 − 78	57	62	59			56		49		80		77		56			79		86.2				60.2		57.5		51.3		54.4			52.1	50.8	
MCH $\mu\mu$GM NV = 18 − 23	18	21	21			18		18		25		25		20			19		29.5				18.4		18.5		16.5		17.5			18.5	16.0	
MCHC GM/100 NV = 27 − 31	32	33	34			32		36		33		34		36			24		34.2				30.5		32.1		32.2		32.2			35.4	31.5	
NV = NORMAL VALUE																																		

TABLE C-VI

PRIMATE ISOLATION STUDIES

MONKEY NUMBER 7

HEMATOLOGY DATA - SUMMARY CHART

FISCAL WEEK/WEEKS FROM START

MEASUREMENT	19/1	20/2	21/3	22/4	23/5	24/6	25/7	26/8	27/9	28/10	29/11	30/12	31/13	32/14	33/15	34/16	35/17	36/18	37/19	38/20	39/21	40/22	41/23	42/24	43/25	44/26	45/27	46/28	47/29	48/30	49/31	50/32	51/33	52/34
HGB GM/100 ML NV = 11 - 12.5	10.7	10.6	10.9	10.6		10.3	10.3				8.9			9.0			6.6	9.8	9.7			8.9	9.1	9.7	9.6	8.2	9.8	7.8	9.8	9.6		9.6	9.7	
PVC % NV = 39 - 43	31.8	32.0	32.2	30.0		30.0	31.4				28.1			28			30.1	31	30.1			31.0	30.0	30.7	33.0	31.7	31.1	25.0	30.7	31.0		30.8	31.0	
RBC x 10^6/MM^3 NV = 5 - 6	5.57	5.4	5.75	5.35		5.4	6.0				4.26			4.37			4.63	3.76	4.25			5.97	6.71	3.96	7.32	7.23	5.77	4.88	5.79	6.45		5.33	5.76	
WBC x 10^3/MM^3 NV = 7 - 13	8.1	7.9	8.7	10.7		7.6	11.3				9.7			10.8			9.9	9.24	7.92			7.59	8.36	11.0	7.81	5.83	10.02	4.40	7.04	10.2		7.81	7.04	
% BANDS	1	2				1	2										1					2					4			4				
% SEGS	37	27	20	14		19	12				20			18			26	35				22	12	20	16	28	42	26	28	24		18	28	
% TOTAL NEUTROS. NV = 20 - 56	38	29	20	14		20	14				20			18			27	35				24	12	20	16	28	46	26	28	28		18	28	
% LYMPHS NV = 40 - 76	53	64	67	81		76	80				74			75			69	62				76	80	80	82	70	52	72	72	58		76	68	
% MONOS NV = 0.5 - 2.0	8	5	6	1		3	4				2			4			2	3							2	2							4	
% EOS NV = 1 - 3		2	4	2		1	1				4			3			2						8				2	2		14		6		
% BASOS NV = 0 - 2	1		3	2			1																											
RBC INDICES MCV μ^3 NV = 65 - 78	57	59	56	56		55	52				66			64			65	82.4	70.8			52.0	44.8	77.5	45.1	43.9	54.0	51.3	53.2	48.2		57.9	53.8	
MCH $\mu\mu$ GM NV = 18 - 23	19	19	19	19		19	17				21			18			14	26.1	22.8			14.9	13.5	24.5	13.1	11.4	16.9	16.0	16.8	14.9		18.0	16.8	
MCHC GM/100 NV = 27 - 31	33	33	33	35		34	33				32			36			22	36.1	32.2			28.7	30.3	31.6	28.1	25.9	31.5	31.2	31.9	30.9		31.2	31.3	
NV = NORMAL VALUE																																		

TABLE C-VII

PRIMATE ISOLATION STUDIES

MONKEY NUMBER 8

HEMATOLOGY DATA - SUMMARY CHART

FISCAL WEEK/WEEKS FROM START

MEASUREMENT	19/1	20/2	21/3	22/4	23/5	24/6	25/7	26/8	27/9	28/10	29/11	30/12	31/13	32/14	33/15	34/16	35/17	36/18	37/19	38/20	39/21	40/22	41/23	42/24	43/25	44/26	45/27	46/28	47/29	48/30	49/31	50/32	51/33	52/34
HGB GM/100 ML NV = 11 – 12.5			11.9				11.3		9.4		9.3		10.0					9.5				9.5		11.2		9.4		10.0		11.2		9.7		
PVC % NV = 39 – 43			35.6				34.9		30		32.1		34					33.8				35.1		36.5		34.1		36.0		33.5		30.4		
RBC x 10^6/MM^3 NV = 5 – 6			5.1				6.85		5.1		4.74		4.8					4.8				6.58		5.98		7.30		6.82		5.75		5.56		
WBC x 10^3/MM^3 NV = 7 – 13			7.4				8.4		7.35		6.2		8.3					8.47				6.27		6.49		4.84		4.73		6.27		3.41		
% BANDS			1				1															2				2				4				
% SEGS			21				39		21		18		20									41		20		28		20		24		18		
% TOTAL NEUTROS. NV = 20 – 56			22				40		21		18		20									43		20		30		20		28		18		
% LYMPHS NV = 40 – 76			67				50		69		76		73									55		80		60		76		56		78		
% MONOS NV = 0.5 – 2.0			4				4		6		6		5									2				2								
% EOS NV = 1 – 3			5				5		3				2													8		4		16		4		
% BASOS NV = 0 – 2			2				1		1																									
RBC INDICES MCV μ^3 NV = 65 – 78			69				51		59		68		71					70				53.4		61.1		46.8		52.8		58.3		54.8		
MCH μμ GM NV = 18 – 23			23				17		18		20		21					19.6				14.4		18.8		12.9		14.7		19.5		17.5		
MCHC GM/100 NV = 27 – 31			33				33		31		29		32					28.1				27.1		30.7		27.6		27.8		33.4		31.9		
NV = NORMAL VALUE																																		

TABLE C-VIII

PRIMATE ISOLATION STUDIES

MONKEY NUMBER 9

HEMATOLOGY DATA - SUMMARY CHART

FISCAL WEEK/WEEKS FROM START

MEASUREMENT	19/1	20/2	21/3	22/4	23/5	24/6	25/7	26/8	27/9	28/10	29/11	30/12	31/13	32/14	33/15	34/16	35/17	36/18	37/19	38/20	39/21	40/22	41/23	42/24	43/25	44/26	45/27	46/28	47/29	48/30	49/31	50/32	51/33	52/34
HGB GM/100 ML NV = 11 - 12.5		12.6				11.3		10.9		10.6		11.2					7.0		11.7		9.1		10.4		10.4		10.0		9.6			10.3	9.9	
PVC % NV = 39 - 43		35.3				32.8		30.8		32		31					30.3		34.8		32.1		31.4		29.3		30.6		28.0			31.3	31.1	
RBC x 10^6/MM^3 NV = 5 - 6		6.25				5.3		5.45		5.14		4.25					4.59		4.78		5.04		5.94		5.84		6.27		5.53			6.12	5.11	
WBC x 10^3/MM^3 NV = 7 - 13		6.5				7.9		8.8		10.6		9.9					10.34		11.0		6.82		3.96		6.16		6.71		38.5			5.39	2.53	
% BANDS		1						1											1				1		1		2						1	
% SEGS		12				7		12		14		16					9		9		12		14		23		8		10			8	19	
% TOTAL NEUTROS. NV = 20 - 56		13				7		13		14		16					9		10		12		15		24		10		10			8	20	
% LYMPHS NV = 40 - 76		73				85		82		83		74					89		90		88		83		70		88		88			92	72	
% MONOS NV = 0.5 - 2.0		5				5		1		1		2					2						2		3		2						4	
% EOS NV = 1 - 3		9				3		3		2		5													3				2				4	
% BASOS NV = 0 - 2								1				3																						
RBC INDICES MCV μ^3 NV = 65 - 78		56				62		57		63		74					66		72.8		64		52.8		50.2		48.8		50.7			51.2	60.9	
MCH $\mu\mu$ GM NV = 18 - 23		20				21		20		21		26					15		24.5		18		17.5		17.8		15.9		17.7			16.9	19.4	
MCHC GM/100 NV = 27 - 31		36				35		36		33		36					23		33.7		28		33.1		35.5		34.8		34.3			32.9	31.8	
NV = NORMAL VALUE																																		

APPENDIX D

AN ANALYSIS OF MONKEY MICROFLORA DATA

APPENDIX D

Data tested is to be found in Tables D-I through D-IV in Appendix D.

In addition to basic data on the tables, the following information was given the statistician.

(1) Four test monkeys, labeled henceforth as M3, M4, M5, and M7 were placed in biological isolation in the fourth week of a test period. At intervals, the aerobic and anaerobic microflora were counted in the feces in terms of counts per gram.

(2) Four other monkeys, identified as M2, M6, M8, and M9 were set up as a control group and consequently were placed in a "normal" non-isolated environment. Their microflora count was also maintained over the same period totaling 36 weeks.

(3) Even though the test time was the same in both groups, the experimental group was generally assayed weekly (with a few missing data points) and the control group every two weeks.

(4) The primary objective of the test was to determine the effect of biological clinical isolation.

(5) The eight monkeys were randomly selected and the controls selected at random from the group of eight.

Rationale and Conclusions

It seems reasonable to test the trends, that is, the growth or decay rate of the microflora during the 36-week period.

Two of the experiment (bio-isolated) group demonstrated significantly detectable linear trends (decay rates) in the aerobic count.

The control (not bio-isolated) group demonstrated no significant trend either way for both the aerobic and anaerobic count.

The anaerobic count for the experimental group exhibited no significant trends.

From this sample of four, one could conclude that clinical isolation does indeed effect the aerobic trend in a proportion of the monkeys.

From this small sample, it is possible to project the sample size necessary to determine more precisely the proportion of the monkeys that would exhibit an aerobic linear decay rate. Since the sample is small, the true proportion may likely be quite different than .5. For example, we may be 90% certain that we could predict within 20% with 70 monkeys, within 10% with 280 monkeys, and within 6% with 780 monkeys. To be 95% certain, we would have to increase the sample sizes to 100, 400 $>$ 1,000 respectively and for 99% a further increase to 170, 680, $>>$ 1,000 respectively.

These figures are undoubtedly conservative but do provide an estimate of the size of any projected future experiment if high certainty were required.

One monkey in the experimental group exhibited a marginal decay rate which indicates further that the figures (sample sizes) are conservative.

Statistical Analysis

The bulk of the computational effort was provided by the regression program "XREG" and the binomial distribution program "BINOM" on the 605 timesharing system at the General Electric Valley Forge Space Center. The descriptions of these utility programs can be found in the Engineering and Scientific Computer Applications Library Manual also available at General Electric.

The first step in the analysis was to determine if there was a statistically detectable trend in the 16 separate groups of microflora count.

By "statistically detectable" is meant whether the trend is large compared to the variation about the trend line.

By "large" is meant unlikely if due to chance variation. More precisely, a trend that is significant at the α % level means that if the data comes from a population with no trend, trends larger (absolutely) can occur by chance α % of the time for a number of samples. Therefore, large $(1 - \alpha)$% values indicate increasingly significant trends. Trends (counts per gram feces per week) were computed for each of the 8 monkeys for both aerobic and anaerobic data.

More than one computation (regression calculation) was performed; therefore, the significance level must be altered to conservatively "protect" the possibility that significant trends do not exhibit themselves by chance more than anticipated. For example, if it is desired that this occur, no more than 10% of the time then the level for each individual run should be set at $(1 - \sqrt[8]{1 - \alpha})\% = 98.69\%$. More conservative, if we want this to occur, no more than 5% of the time the level should be adjusted to $(1 - \sqrt[8]{1 - \alpha})\% = 99.36\%$. At this latter level, two of the monkeys from the experimental group exhibited significant trends for aerobic data:

M3 at -5.31×10^8 and M4 at -11.4×10^8 counts per week.

The calculated trends and their $(1 - \alpha)$% significance levels are presented in Tables D-I and D-II. Further calculations reveal that we are 95% certain that the true trend for M3 lies in the interval $(-8.9 \times 10^8, -1.17 \times 10^8)$. For monkey M4, the interval is $(-18.5 \times 10^8, -4.35 \times 10^8)$. The width of these intervals depends on the amount of residual variation about the trend.

TABLE D-I

AEROBIC TRENDS AND SIGNIFICANCE LEVELS

MONKEY	TREND x 10^{8}*	SIGNIFICANCE LEVEL %
M3**	-5.31	99.48
M4**	-11.4	99.75
M5**	.453	9.79
M7**	-13.2	98.36
M2***	4.52	74.03
M6***	-20.0	88.99
M8***	-27.7	84.32
M9***	-6.78	71.65

*Counts per gram feces per week.

**Bioisolated

***Not Isolated

TABLE D-II

ANAEROBIC TRENDS AND SIGNIFICANCE LEVELS

MONKEY	TREND x 10^{8}*	SIGNIFICANCE LEVELS %
M3**	1.78	10.38
M4**	10.1	23.31
M5**	-33.4	28.44
M7**	-48.1	84.46
M2***	11.5	56.40
M6***	-63.2	78.81
M8***	43.1	19.26
M9***	-23.3	78.89

*Counts per gram feces per week.

**Bioisolated

***Not Isolated

Tables D-III and D-IV represent time sharing computer results for the two significant trends (Aerobic Data for M3, M4).

The first page of each table presents the estimates of the trend, constant term (intercept) and a check on numerical fidelity.

The second page illustrates the exercising of option 1, the listing of the raw data (counts per week), calculated for the regression (trend line), residual and % deviation of the actual data from the trend line.

The data recorded for M3 were at the weeks: 0, 2, 6, through 19, 22 through 36.

For M4, the data was recorded at the weeks: 0, 2. 5 through 19, 22 through 36.

The third page of each table contains the second and third options of the regression program. The second is the standard analysis of variance and the third contains confidence bands for the trend at the 90%, 95%, 99% and 99.5% levels.

TABLE D-III

REGRESSION ON M3 AEROBIC DATA

```
     MULTIPLE REGRESSION

WISH TO SEE OPTION CODE? YES OR NO=YES


    OPTION CODE
1-LISTING OF OBSERVED, PREDICTED VALUES & DIFFERENCES
2-ANOVA
3-MOMENT MATRIX
4-COVARIANCE MATRIX-MEANS/STANDARD DEVS
5-CORRELATION MATRIX
6-SIMULTANEOUS CONFIDENCE BANDS FOR E(Y)
7-CONFIDENCE BANDS FOR E(Y) & PREDICTION INTERVAL FOR MEAN
8-CONFIDENCE BANDS FOR A PARAMETER
9-CONFIDENCE BANDS FOR RESIDUAL VARIANCE

IS DATA TO BE READ FROM A FILE, TYPE YES NO OR STOP=YES

TYPE FILENAME, 1 TO 8 CHARACTERS=M3

INTERCEPT? YES OR NO=YES


DEP., NO. & IND. VARIATES?=2,1,1

   INTERCEPT =  1.35750E+10

****PARAMETER ESTIMATES & CHECK****

 1 -5.31202E+08 -6.29265E+03
```

TABLE D-III (Continued)

	OBSERVED	PREDICTED	RESIDUAL	% DEVIATION
1	4.00000E+10	1.35750E+10	2.64250E+10	194.66
2	5.00000E+10	1.25126E+10	3.74874E+10	299.60
3	3.00000E+09	1.03878E+10	-7.38777E+09	-71.12
4	2.00000E+08	9.85657E+09	-9.65657E+09	-97.97
5	4.00000E+07	9.32537E+09	-9.28537E+09	-99.57
6	4.00000E+07	8.79417E+09	-8.75417E+09	-99.55
7	3.00000E+07	8.26297E+09	-8.23297E+09	-99.64
8	1.00000E+07	7.73176E+09	-7.72176E+09	-99.87
9	7.00000E+07	7.20056E+09	-7.13056E+09	-99.03
10	4.00000E+07	6.66936E+09	-6.62936E+09	-99.40
11	4.00000E+07	6.13816E+09	-6.09816E+09	-99.35
12	9.00000E+07	5.60696E+09	-5.51696E+09	-98.39
13	2.00000E+08	5.07575E+09	-4.87575E+09	-96.06
14	8.00000E+07	4.54455E+09	-4.46455E+09	-98.24
15	9.00000E+07	4.01335E+09	-3.92335E+09	-97.76
16	2.00000E+08	3.48215E+09	-3.28215E+09	-94.26
17	3.00000E+08	1.88854E+09	-1.58854E+09	-84.11
18	9.00000E+08	1.35734E+09	-4.57343E+08	-33.69
19	5.00000E+07	8.26141E+08	-7.76141E+08	-93.95
20	2.00000E+07	2.94939E+08	-2.74939E+08	-93.22
21	4.00000E+06	-2.36263E+08	2.40263E+08	101.69
22	4.00000E+06	-7.67464E+08	7.71464E+08	100.52
23	1.00000E+07	-1.29867E+09	1.30867E+09	100.77
24	5.00000E+07	-1.82987E+09	1.87987E+09	102.73
25	1.00000E+07	-2.36107E+09	2.37107E+09	100.42
26	9.00000E+07	-2.89227E+09	2.98227E+09	103.11
27	8.00000E+07	-3.42347E+09	3.50347E+09	102.34
28	5.00000E+07	-3.95467E+09	4.00467E+09	101.26
29	6.00000E+06	-4.48588E+09	4.49188E+09	100.13
30	2.00000E+07	-5.01708E+09	5.03708E+09	100.40
31	5.00000E+06	-5.54828E+09	5.55328E+09	100.09

SUM OF RESIDUALS = 6.40000E+01

TABLE D-III (Continued)

```
CODE?=2


   SOURCE          DF      SS             MS
 REGRESSION         1   9.11972E+20   9.11972E+20
    ERROR          29   2.90249E+21   1.00086E+20
    TOTAL          30   3.81446E+21

    F-RATIO =  9.11189E+00 A   99.4750 % VALUE
    MULTIPLE CORRELATION COEFFICIENT =  4.88961E-01

CODE?=8


   CONFIDENCE BANDS FOR A PARAMETER

PARAMETER INDEX, NO. LEVELS & LEVELS?=1,4,.90,.95,.99,.995

 CONFIDENCE  LOWER       PREDICTED     UPPER         WIDTH
   90.00 -8.30208E+08 -5.31202E+08 -2.32195E+08  5.98013E+08
   95.00 -8.91114E+08 -5.31202E+08 -1.71289E+08  7.19825E+08
   99.00 -1.01626E+09 -5.31202E+08 -4.61421E+07  9.70119E+08
   99.50 -1.06583E+09 -5.31202E+08  3.42360E+06  1.06925E+09
```

TABLE D-IV

REGRESSION ON M4 AEROBIC DATA

```
IS DATA TO BE READ FROM A FILE, TYPE YES NO OR STOP=YES

TYPE FILENAME, 1 TO 8 CHARACTERS=M4

INTERCEPT? YES OR NO=YES


DEP., NO. & IND. VARIATES?=2, 1, 1
AWAITING FILE ACCESS

   INTERCEPT =  3.15065E+10
****PARAMETER ESTIMATES & CHECK****
  1 -1.14144E+09 -1.91700E+04
```

TABLE D-IV (Continued)

CODE?=1

AWAITING FILE ACCESS

	OBSERVED	PREDICTED	RESIDUAL	% DEVIATION
1	3.00000E+10	3.15065E+10	-1.50653E+09	-4.78
2	4.00000E+10	2.92236E+10	1.07764E+10	36.88
3	2.00000E+10	2.57993E+10	-5.79933E+09	-22.48
4	1.00000E+11	2.46579E+10	7.53421E+10	305.55
5	2.00000E+10	2.35164E+10	-3.51644E+09	-14.95
6	3.00000E+08	2.23750E+10	-2.20750E+10	-98.66
7	4.00000E+09	2.12336E+10	-1.72336E+10	-81.16
8	8.00000E+10	2.00921E+10	5.99079E+10	298.17
9	2.00000E+09	1.89507E+10	-1.69507E+10	-89.45
10	1.00000E+09	1.78092E+10	-1.68092E+10	-94.38
11	4.00000E+07	1.66678E+10	-1.66278E+10	-99.76
12	8.00000E+07	1.55264E+10	-1.54464E+10	-99.48
13	5.00000E+07	1.43849E+10	-1.43349E+10	-99.65
14	2.00000E+07	1.32435E+10	-1.32235E+10	-99.85
15	5.00000E+08	1.21020E+10	-1.16020E+10	-95.87
16	2.00000E+07	1.09606E+10	-1.09406E+10	-99.82
17	4.00000E+09	9.81915E+09	-5.81915E+09	-59.26
18	9.00000E+08	6.39483E+09	-5.49483E+09	-85.93
19	1.00000E+07	5.25339E+09	-5.24339E+09	-99.81
20	1.00000E+06	4.11195E+09	-4.11095E+09	-99.98
21	1.00000E+07	2.97051E+09	-2.96051E+09	-99.66
22	5.00000E+06	1.82907E+09	-1.82407E+09	-99.73
23	4.00000E+06	6.87629E+08	-6.83629E+08	-99.42
24	3.00000E+07	-4.53812E+08	4.83812E+08	106.61
25	5.00000E+07	-1.59525E+09	1.64525E+09	103.13
26	7.00000E+08	-2.73669E+09	3.43669E+09	125.58
27	8.00000E+07	-3.87813E+09	3.95813E+09	102.06
28	3.00000E+07	-5.01958E+09	5.04958E+09	100.60
29	3.00000E+07	-6.16102E+09	6.19102E+09	100.49
30	7.00000E+07	-7.30246E+09	7.37246E+09	100.96
31	3.00000E+06	-8.44390E+09	8.44690E+09	100.04
32	7.00000E+06	-9.58534E+09	9.59234E+09	100.07

SUM OF RESIDUALS = 7.93600E+03

TABLE D-IV (Continued)

```
CODE?=2

    SOURCE          DF      SS             MS
 REGRESSION          1   4.48515E+21   4.48515E+21
      ERROR         30   1.23667E+22   4.12222E+20
      TOTAL         31   1.68518E+22

    F-RATIO =  1.08804E+01 A   99.7491 % VALUE
    MULTIPLE CORRELATION COEFFICIENT =  5.15900E-01

CODE?=6

    CONFIDENCE BANDS FOR A PARAMETER

PARAMETER INDEX, NO. LEVELS & LEVELS?=1,4,.90,.99\5,.99,.995

 CONFIDENCE  LOWER          PREDICTED        UPPER          WIDTH
   90.00 -1.72877E+09 -1.14144E+09 -5.54115E+08  1.17465E+09
   95.00 -1.84816E+09 -1.14144E+09 -4.34726E+08  1.41343E+09
   99.00 -2.09306E+09 -1.14144E+09 -1.89823E+08  1.90324E+09
   99.50 -2.18988E+09 -1.14144E+09 -9.29992E+07  2.09688E+09
```

APPENDIX E

APOLLO DIET PREPARATION

APPENDIX E

A composite diet similar to that used in pre-flight tests by W. Cunningham (4 days in August, 1968), Kerwin (10 days), Brand (10 days) and Engle (10 days dated March 6, 1968) was developed (Table E-I). This was corrected for an error in apricot cereal cubes to give the composition summarized in Table E-II. This composite diet was prepared by the Whirlpool Corporation of St. Joseph, Michigan. The chocolate cubes and strawberry cubes were obtained as GFP from NASA. Other items were prepared to conform to the production guide specifications approved by the NASA-MSC Nutrition Group. All items were then granulated to pass as No. 20 mesh and blended. Certain items, such as fruit cakes, were dried more than usual and frozen for ease of granulation. The blend gave the desired characteristics; a complete mix with a particle size small enough that mice would not pick out individual pieces (Figure E-1). This material could be fed to mice with assurance that they were getting a representative composite of Apollo diet as of August, 1968. The material was brown, packed easily into any desired form, was not unduly hygroscopic and tasted and looked much like the ginger powder sometimes used on graham crackers. It was sweet and the mice ate it readily.

Based upon estimates of need for the experiment (Table E-III), the quantity purchased was 110 Kg plus a small sample of each food used. The granulated (20 mesh), blended diet was processed in a manner to minimize contact with air. The materials and finished product were maintained at refrigeration temperatures wherever possible. The granulated diet was vacuum packed in 400 gm $\pm$1% lots into polyethylene bags and these placed with wax paper packing and under nitrogen into No. 2½ commercial tin cans (Figure E-2). These were placed into

TABLE E-I

PRE-FLIGHT CONSUMPTION OF APOLLO FOOD ITEMS - 1968

FOOD	AVERAGE OF FOUR MEN	UNIT WGT. (gm)	GRAMS USED	% OR Kg/ 100 Kg	UNITS/ 100 Kg	NEEDED UNITS/ 110 Kg
Applesauce	1.25	35.0	43.75	1.48	42.3	46.5
Apricot Cereal Cubes	8	6.3	38.0	1.71	271	298
Bacon Square	22	5.0	110.0	3.73	746	821
Banana Pudding	2	70.0	140.0	4.75	68.0	74.8
Beef, Barbecue Bites	4	3.6	14.4	0.49	136	150
Beef and Gravy	1.5	35.0	52.5	1.78	50.8	55.9
Beef, Hash	0.25	28.8	7.2	0.24	8.3	9.1
Beef, Pot Roast	1.25	27.0	33.75	1.14	42.2	46.4
Beef, Sandwich	15.5	3.1	48.05	1.63	52.6	579
Beef, Stew B.	2	3.5	7.0	0.24	68.5	75.4
Beef and Vegetable	1	22.0	22.0	0.75	34.1	37.5
Brownies	8	6.5	52.0	1.76	271	298
Butterscotch Pudding	0.75	70.0	52.5	1.78	25.4	27.9
Canadian Bacon & Applesauce	0.5	29.0	14.5	0.49	16.9	18.6
Cheese Sandwich	7.5	4.1	30.8	1.04	252	277
Chicken and Gravy	0.25	24.5	6.1	0.21	8.6	9.5
Chicken Salad	1.25	41.0	51.25	1.74	42.5	46.8
Cinnamon Toast Bread	39	6.3	245.7	8.33	1322	1454
Chocolate Cake	13.5	6.0	81.0	2.75	458	504
Chocolate Pudding	1.25	70.0	87.5	2.97	42.4	46.6
Cocoa	3.75	42.0	157.5	5.34	127	140
Corn Chowder	1.5	56.0	84.0	2.85	50.8	55.8
Corn Flakes, S.C.	0.75	36.8	27.6	0.94	25.6	28.2
Cream of Chicken Soup	0.25	27.5	6.9	0.24	8.7	9.6
Date Fruit Cake	2.5	13.5	33.75	1.14	87.7	96.5
Drink, Breakfast	1	8.5	8.5	0.29	34.1	37.5
Drink, Grapefruit	2.75	46.0	126.5	4.29	93.2	102.5
Drink, Orange	3	40.1	120.3	4.08	102	112
Drink, Orange-Grapefruit	1.5	40.1	60.2	2.04	50.9	56.0
Drink, Pineapple-Grapefruit	2.25	40.1	90.2	3.06	76.3	83.9
Fruit Cocktail	1	22.5	22.5	0.76	33.8	37.2
Gingerbread Cubes	5	7.0	35.0	1.19	170	187
Peaches	1	23.0	23.0	0.78	33.9	37.3
Pea Soup	1.5	49.0	73.5	2.49	50.8	55.9
Pineapple Fruit Cake	6.0	13.5	81.0	2.75	204	224
Potato Salad	0.5	25.5	12.8	0.44	17.3	19.0
Potato Soup	1.75	40.0	70.0	2.37	59.3	
Sausage	1.8	40.0	72.0	2.44	61.0	67.1
Salmon Salad	1	40.0	40.0	1.35	33.8	37.1
Shrimp Cocktail	0.75	31.0	23.25	0.79	25.5	28.1
Strawberry Squares	6	6.0	36.0	1.22	203	223
Sugar Cookies	8	6.0	48.0	1.63	272	299
Toasted Bread Cubes	21.5	6.3	135.45	4.59	728	801
Toasted Oat Cereal	0.75	24.0	18.0	0.61	25.5	28.1
Tuna Salad	1	40.0	40.0	1.36	34.0	37.4
TOTAL			2583.95			

TABLE E-II

APOLLO DIET - 1968

CATEGORY	NUMBER*	ITEM	PERCENT OF DIET	SUB-TOTALS
Meat	3B	Beef and Gravy	2.03	21.26
	19B	Beef Sandwich	1.86	
	5B	Beef Pot Roast	1.31	
	6B	Beef and Vegetables	0.85	
	4C	Beef Barbecue Bites	0.56	
	3C	Beef Hash	0.28	
	4C	Beef Stew Bites	0.27	
	11B	Canadian Bacon & Applesauce	0.56	
	1B	Bacon Squares	4.26	
	10B	Chicken Salad	1.98	
	7B	Chicken and Gravy	0.24	
	4-2	Cream of Chicken Soup (SLF)	0.27	
	12B	Sausage Patties	2.79	
	16B	Salmon Salad	1.55	
	14B	Shrimp Cocktail	0.90	
	16B	Tuna Salad	1.55	
Cereals	24B	Cinnamon Toasted Bread Cubes	9.51	19.77
	24B	Toasted Bread Cubes	5.24	
	30B	Toasted Oat Cereal	0.70	
	30B	Corn Flakes, Sugar Coated	1.07	
	38B	Corn Chowder	3.25	
Vegetables	38B	Pea Soup	2.84	6.05
	13B	Potato Salad	0.50	
	49	Potato Soup	2.71	
Fruit	1A	Strawberry Cubes**	1.39	21.62
	27B	Peach Bars	0.89	
	46A	Applesauce	1.69	
	23B	Apricot Cereal Cubes	1.46	
	53	Drink, Grapefruit	4.90	
	53	Drink, Orange	4.60	
	53	Drink, Orange-Grapefruit	2.33	
	53	Drink, Pineapple-Grapefruit	3.49	
	28B	Fruit Cocktail	0.87	
Dairy	18B	Cheese Sandwich	1.19	7.62
	26B	Cocoa	6.10	
	54	Drink, Breakfast	0.33	
Sweets	29C	Banana Pudding	5.42	23.63
	32B	Brownies	2.01	
	29C	Butterscotch Pudding	2.03	
	29C	Chocolate Pudding	3.39	
	1A	Chocolate Cubes**	3.13	
	34B	Date Fruitcake	1.31	
	33B	Gingerbread Cubes	1.35	
	34B	Pineapple Fruitcake	3.13	
	1-1	Sugar Cookies (SLF)	1.86	

*Production Guide Number

**Not supplied by Whirlpool

FIGURE E-1

APOLLO DIET SHOWING INNER BAG PACK AND FINE BLEND

TABLE E-III

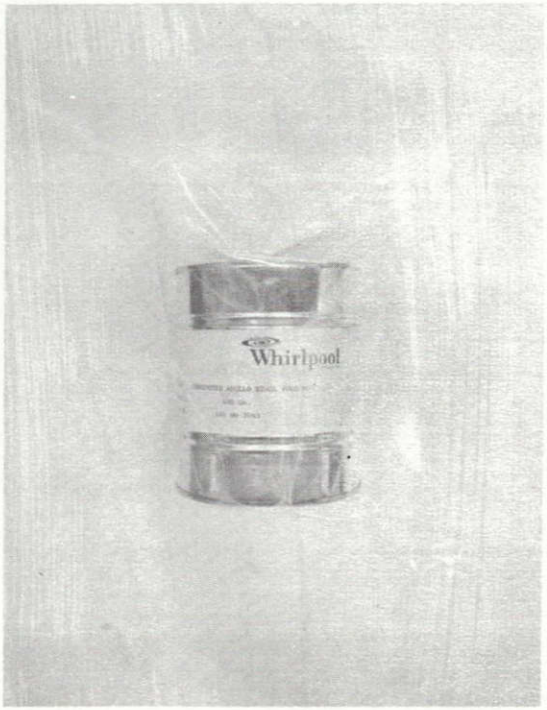
ASTRONAUT DIET EVALUATION

GENERATION	MICE IN TEN GROUPS	DAYS	MOUSE-DAYS
1	200 Weanling	40	8,000
2	40 Litters ≅ 80 Mice	20	1,600
	200 Weanling	40	8,000
3	40 Litters ≅ 80 Mice	20	1,600
	200 Weanling	40	8,000
		TOTAL	27,200

27,200 MOUSE DAYS x 4 grams FOOD PER DAY = 108,800 grams

FIGURE E-2

CANNED APOLLO DIET WITHIN PROTECTIVE PLASTIC BAG



corrigated cardboard boxes packed with ice under insulated blanket and taken to Valley Forge in an air conditioned car. The documentation on moisture analysis and microbiology was satisfactory; these were confirmed independently on samples as received (Table E-IV). The individual items showed good quality with the exception of the fecal Streptococcus in some cereal items, cocoa, chocolate pudding and especially cream of chicken soup.

Each can was heat sealed in a polyethylene bag and this placed into a second polyethylene bag and taped shut (Figure E-3). The outer bag provided a high degree of cleanliness to the inner bag which was later sterilized with peracetic acid. Spore strips were placed into the center of the diet in five cans which were subsequently resealed without nitrogen.

Twenty-eight of the 275 cans and some of the samples of food were refrigerated at the Valley Forge Space Center. The remainder of the material was taken to Brookhaven National Laboratory at Upton, New York in an air conditioned car with ice and thermal blanket to keep it cool. It was stored in the Biology Walk-in Refrigerator, and frozen at dry ice temperature prior to radiation sterilization by Mr. Frank Rizzo and associates. The sterile diet was returned to the Valley Forge Space Center under refrigeration and placed in a walk-in refrigerator. Ninety cans were taken out to start the first ten groups of mice. These were held at room temperature for the remainder of the test (about two months) to simulate the temperature conditions presently used in space flights. The logistics plan is given in Table E-V.

Both the spore strips and the diet were found to be sterile following radiation sterilization. The radiation sterilized and the untreated samples of food items were submitted to NASA-Houston for taste testing.

TABLE E-IV

APOLLO TYPE SPACE FOOD FOR ANIMAL FEEDING EXPERIMENT
Quality Assurance Provisions

Food Item	Whp. Lot No	Moisture Analysis %	Microbiological Analyses: Total Plate Count	Total Coliform Count	Fecal Coliform Count	Fecal Streptococcus Count	Coagulase (+) Staphylococcus Count	Salmonella Count
Apricot Cereal Cubes	LW 327	3.0,3.1	640	0	Negative	0	Negative	Negative
	LW 444	2.4,2.6	60	0	Negative	0	Negative	Negative
	LW 507	2.4,2.4	240	0	Negative	0	Negative	Negative
Cinnamon Toasted Bread Cubes	LW 443	2.6,2.6	20	0	Negative	0	Negative	Negative
	LW 484	2.4,2.4	0	0	Negative	1	Negative	Negative
	LW 512	2.0,2.2	280	0	Negative	0	Negative	Negative
	LW 535	1.8,2.2	100	0	Negative	0	Negative	Negative
	LW 537	1.6,1.6	100	0	Negative	0	Negative	Negative
Toasted Bread Cubes	LW 190	3.2	100	0	Negative	0	Negative	Negative
	LW 232	2.0	120	0	Negative	0	Negative	Negative
	LW 374	2.2,2.5	20	0	Negative	0	Negative	Negative
	LW 403	2.6,2.9	120	0	Negative	1	Negative	Negative
	LW 462	2.4,2.6	120	0	Negative	3	Negative	Negative
	LW 513	2.6,3.2	100	0	Negative	0	Negative	Negative
	LW 583	2.2,2.3	220	0	Negative	0	Negative	Negative
Sugar Cookie Cubes	LW 282	2.5,3.2	5660	0	Negative	0	Negative	Negative
	LW 407	2.2,2.2	160	0	Negative	1	Negative	Negative
	LW 464	2.9	60	0	Negative	1	Negative	Negative
	LW 576	2.1,2.1	40	0	Negative	0	Negative	Negative
	LW 596	2.2,2.5	40	0	Negative	3	Negative	Negative
Brownies	LW 278	3.6	5020	0	Negative	1	Negative	Negative
	LW 426	4.1,4.6	480	0	Negative	0	Negative	Negative
	LW 514	5.4,5.4, 5.6	1780	0	Negative	0	Negative	Negative
Gingerbread	LW 237	8.1	700	0	Negative	0	Negative	Negative
	LW 371	7.6,8.0	740	0	Negative	0	Negative	Negative
	LW 394	7.3,7.3	340	0	Negative	0	Negative	Negative
	LW 468	6.6,6.9	320	0	Negative	0	Negative	Negative
	LW 509	8.9,9.4	2080	0	Negative	0	Negative	Negative

APOLLO TYPE SPACE FOOD FOR ANIMAL FEEDING EXPERIMENT
Quality Assurance Provisions

Food Item	Whp. Lot No.	Moisture Analysis %	Microbiological Analyses: Total Plate Count	Total Coliform Count	Fecal Coliform Count	Fecal Streptococcus Count	Coagulase (+) Staphylococcus Count	Salmonella Count
Date Fruitcake	LW 283	8.4	860	0	Negative	0	Negative	Negative
	LW 405	7.2,7.5, 8.9	140	0	Negative	0	Negative	Negative
	LW 418	4.8,5.0, 5.8	620	0	Negative	0	Negative	Negative
	LW 480	7.7,7.8	220	0	Negative	0	Negative	Negative
	LW 515	6.8,7.0, 8.0	120	0	Negative	0	Negative	Negative
	LW 549	9.0,9.1	200	0	Negative	0	Negative	Negative
	LW 618	7.2,7.8	20	0	Negative	0	Negative	Negative
Pineapple Fruitcake	LW 337	5.8,8.2	220	0	Negative	0	Negative	Negative
	LW 376	6.4,6.8	80	0	Negative	0	Negative	Negative
	LW 377	5.8,6.4	460	0	Negative	0	Negative	Negative
	LW 481	8.4,9.2	60	0	Negative	0	Negative	Negative
	LW 516	7.9,8.2, 8.4	400	0	Negative	0	Negative	Negative
	LW 530	8.8,8.9	2540	0	Negative	0	Negative	Negative
	LW 553	6.6,8.4	780	0	Negative	0	Negative	Negative
	LW 574	8.6,9.5	60	0	Negative	0	Negative	Negative
	LW 595	7.8,9.7	1680	0	Negative	0	Negative	Negative
	LW 607	8.8,9.2, 9.2,9.6, 9.8	280	0	Negative	0	Negative	Negative
	LW 620	7.5,8.1	140	0	Negative	0	Negative	Negative
Chocolate Cubes	LN 376	2.2	30,000-33,000	< 10	Negative	0,10	Negative	Negative
	LN 428	2.2	22,000	< 10	Negative	0	Negative	Negative
Strawberry Cubes	LN 538	2.9	1,400	0	Negative	0	Negative	Negative
	LN 594	2.5	600	0	Negative	0	Negative	Negative

TABLE E-IV (Continued)

APOLLO ..PE SPACE FOOD FOR ANIMAL FEEDING EXPERIMENT

Quality Assurance Provisions

Food Item	Whp. Lot No.	Moisture Analysis %	Microbiological Analyses: Total Plate Count	Total Coliform Count	Fecal Coliform Count	Fecal Streptococcus Count	Coagulase (+) Staphylococcus Count	Salmonella Count
Bacon Bars	LS 253	3.1,3.7	330;360	0	Negative	0	Negative	Negative
	LS 287	6.2,8.5, 10.4,10.6, 11.9	20;180	0	Negative	0	Negative	Negative
	LS 296	9.8,11.1, 12.7,13.1, 13.6	180;240 420;460 560	0	Negative	0	Negative	Negative
Barbecue Beef Bites	LS 289	0.9,1.0	520;560	0	Negative	0	Negative	Negative
	LS 336	0.6,0.7	60;800	0	Negative	0	Negative	Negative
Beef Stew Bites	LS 265	0.3,0.3	3200; 7000	0	Negative	0	Negative	Negative
	LS 291	0.6,0.9	640;740	0	Negative	0	Negative	Negative
Beef Sandwiches	LS 255	1.7,1.9	50;70	0	Negative	0	Negative	Negative
	LS 338	0.6,0.7, 0.8	280;340 620	0	Negative	0	Negative	Negative
Cheese Sandwiches	LS 241	1.1,1.1	170;260	0	Negative	0	Negative	Negative
	LS 256	1.2,1.2	600;600	2,2	Negative	0	Negative	Negative
	LS 297	1.2,1.9	2000; 2100	0	Negative	0,5	Negative	Negative
	LS 312	1.3,1.8	1200; 1200	0	Negative	0	Negative	Negative
Beef and Gravy	LS 264	1.8,1.8	5000; 5000	0	Negative	0	Negative	Negative
	LS 278	0.1,0.2, 0.5	240;500	0	Negative	0	Negative	Negative
	LS 299	0.3,0.3	340;540	0	Negative	0	Negative	Negative
	LS 307	0.5,0.6	100;320	0	Negative	0	Negative	Negative

APOLLO .Æ SPACE FOOD FOR ANIMAL FEEDING EXPERIMENT
Quality Assurance Provisions

Food Item	Whp. Lot No.	Moisture Analysis %	Microbiological Analyses: Total Plate Count	Total Coliform Count	Fecal Coliform Count	Fecal Streptococcus Count	Coagulase (+) Staphylococcus Count	Salmonella Count
Beef Pot Roast	LS 227	1.7,1.7	110;190	0,2	Negative	0	Negative	Negative
	LS 277	0.4,0.5	250;260	0	Negative	0	Negative	Negative
Beef Hash	LS 319	0.9,0.9	4700; 7500	0	Negative	0	Negative	Negative
Beef with Vegetables	LS 226	1.1,1.2	30;1600	0	Negative	0	Negative	Negative
Canadian Bacon and Apple-sauce	LS 234	1.9,2.1	4000; 10000	2-4	Negative	0	Negative	Negative
	LS 276	1.0,1.1	460;490	0	Negative	0	Negative	Negative
	LS 310	1.1,1.4	120;280	0	Negative	0	Negative	Negative
Sausage Patties	LS 246	0.4,0.4	30;40	0	Negative	0	Negative	Negative
	LS 258	1.5,1.6	230;280	0	Negative	0	Negative	Negative
	LS 295	0.1,0.1	30;70	0	Negative	0	Negative	Negative
	LS 309	0.4,0.4 0.5,0.5, 0.5	180; 1700	0	Negative	0	Negative	Negative
	LS 340	0.2,0.4	180;360	0	Negative	0	Negative	Negative
Chicken and Gravy	LS 318	1.3,1.4	20;80	0	Negative	0	Negative	Negative
Chicken Salad	LS 244	0.7,0.7	1700; 5300	0,2	Negative	0	Negative	Negative
	LS 259	0.8,1.0	100; 1200	0	Negative	0	Negative	Negative
	LS 283	0.2,0.6, 0.8	640; 1800	0	Negative	0	Negative	Negative
	LS 314	0.7,0.7	340;600	0	Negative	0	Negative	Negative
Potato Salad	LS 263	1.5	500; 1000	0	Negative	0	Negative	Negative
	LS 339	0.7,0.8	1800; 4900	0	Negative	0	Negative	Negative

TABLE E-IV (Continued)

APOLLO TYPE SPACE FOOD FOR ANIMAL FEEDING EXPERIMENT
Quality Assurance Provisions

Food Item	Whp. Lot No.	Moisture Analysis %	Microbiological Analyses: Total Plate Count	Total Coliform Count	Fecal Coliform Count	Fecal Streptococcus Count	Coagulase (+) Staphylococcus Count	Salmonella Count
Salmon Salad	LS 260	0.3,0.3	2600; 3400	0	Negative	0	Negative	Negative
Shrimp Cocktail	LS 216	1.6,1.7	2300; 2500	0	Negative	0	Negative	Negative
Tuna Salad	LS 261	1.3,1.3	70;80	0	Negative	0	Negative	Negative
Sugar Coated Corn Flakes	LW 668	2.5	560	0	Negative	0	Negative	Negative
Toasted Oat Cereal	LW 669	3.8	780	0	Negative	0	Negative	Negative
Applesauce	LW 567	1.0,1.3	20	0	Negative	0	Negative	Negative
	LW 646	0.2,0.5, 0.6,1.0, 1.3	0	0	Negative	0	Negative	Negative
Fruit Cocktail	LW 517	3.0,3.0, 3.2,3.2, 3.2	100	0	Negative	0	Negative	Negative
	LW 606	2.3,2.4, 2.4,2.4, 2.4	640	0	Negative	0	Negative	Negative
Peaches	LW 631	1.5,1.5, 1.6,1.6, 1.6,1.7, 1.8,2.0	200	0	Negative	0	Negative	Negative
Cream of Chicken Soup	LW 541	2.6,2.9	3040	1	Negative	42	Negative	Negative
Corn Chowder	LW 205	3.7	1140	0	Negative	0	Negative	Negative
	LW 391	1.5,1.5, 1.6	880	0	Negative	0	Negative	Negative

APOLLO TYPE SPACE FOOD FOR ANIMAL FEEDING EXPERIMENT
Quality Assurance Provisions

Food Item	Whp. Lot No.	Moisture Analysis %	Microbiological Analyses: Total Plate Count	Total Coliform Count	Fecal Coliform Count	Fecal Streptococcus Count	Coagulase (+) Staphylococcus Count	Salmonella Count
Pea Soup	LW 545	2.6,3.2,3.2	460	0	Negative	0	Negative	Negative
Potato Soup	LW 300	2.4,3.0	6320	0	Negative	0	Negative	Negative
	LW 449	1.7,1.7	6160	0	Negative	0	Negative	Negative
	LW 455	2.2,2.8	5640	0	Negative	0	Negative	Negative
	LW 496	2.1,2.1	2320	0	Negative	0	Negative	Negative
Banana Pudding	LW 166	1.6,1.8	300	0	Negative	0	Negative	Negative
	LW 299	1.3,1.3	340	0	Negative	0	Negative	Negative
	LW 382	1.7,1.8	300	0	Negative	0	Negative	Negative
	LW 489	1.0,1.2	240	0	Negative	0	Negative	Negative
	LW 520	1.8,1.9,1.9	260	0	Negative	0	Negative	Negative
	LW 610	1.8,1.8,1.8, 2.0,2.2	140	0	Negative	2	Negative	Negative
Butterscotch Pudding	LW 313	2.0,2.0	220	0	Negative	0	Negative	Negative
	LW 380	2.2,2.2	0	0	Negative	0	Negative	Negative
	LW 521	1.6,1.8,1.8	200	0	Negative	0	Negative	Negative
Chocolate Pudding	LW 421	1.6,1.6,1.7	360	0	Negative	52,102	Negative	Negative
Cocoa	LW 704	1.8	1380	0	Negative	2	Negative	Negative
Breakfast Drink	LW 678	0.8	440	0	Negative	0	Negative	Negative
Grapefruit Drink	LW 564	0.2,0.4,0.8	80	0	Negative	0	Negative	Negative
	LW 671	0.1	0	0	Negative	0	Negative	Negative
Orange Drink	LW 563	0.2,0.2,0.2	60	0	Negative	0	Negative	Negative
Orange-Grapefruit Drink	LW 562	0.4,0.6,0.6	20	0	Negative	0	Negative	Negative
Pineapple-Grapefruit Drink	LW 566	0.1,0.4,0.4	20	0	Negative	0	Negative	Negative
Anhydrous Calcium Lactate	LW 706	0.5	< 100	<10	Negative	0	Negative	Negative

TABLE E-IV (Continued)

APOLLO TYPE SPACE FOOD FOR ANIMAL FEEDING EXPERIMENT
Quality Assurance Provisions

		Microbiological Analyses					
	Headspace Oxygen, Percent	Total Plate Count	Total Coliform Count	Total Coliform Count	Fecal Streptococcus Count	Coagulase(+) Staphyloccus Count	Salmonella Count
Comminuted Apollo Space Food Mix	2.0,2.0	4540	0	Negative	2	Negative	Negative

FIGURE E-3

APOLLO DIET PACKAGING SHOWING IN SERIES (LEFT TO RIGHT)

(a) Double bag can as before entering isolator.

(b) Single bag can as after first peracetic acid sterilization.

(c) Can in sterile isolator.

(d) Opened can showing inner pack.

TABLE E-V

APOLLO DIET LOGISTICS

WEEK	Kg	CASES*	ITEM
10	--	--	Lab diet controls (11-14) started.
17	108	8	Diet delivered
	8	2/3	Sample
	10	1	Control, non-sterile
	34	2-3/4	4°C, Store non-sterile
	66	5½	Sterilze - Use 32 Kg at RT.
	33	2-3/4	4°C Store until second generation
28	33	2-3/4	Second Generation food to RT
	34	2-3/4	Sterilize diet for third generation
29	34	2-3/4	4°C Store for third generation
37	34	2-3/4	Third generation food to RT

	DIET			WEEK	
	CASES	Kg	TYPE	FROM	TO
Refrigerated	2-3/4	34	Non-sterile	17	28
	2-3/4	33	Sterile	17	28
	2-3/4	34	Sterile	29	37
Refrigeration Total	5½	67		17	28
	2-3/4	33		29	37

*Assume 24 Cans (500 gm each) per case.

Preliminary work on radiation sterilization of spore strips and simulated Apollo diet is given as Appendix E.

Tables E-VI to E-IX provide data about the composition of the Apollo-68 diet and the changes from sterilization with 5 (4.6-6.2) million rads γ rays from a 500 K Curie cobalt source. The temperature rose from -64° to + 5°C during the 55 minute radiation (about 10°C increase per megarad). None of the gross constituents changed significantly during sterilization (Table E-VI). The water and fiber contents of the diet were low by design. The ash content is unexpectedly low while the fat, protein and carobhydrate content is adequate for either man or mouse. The energy content is about 4.4 Cal./gm.

Although no significant loss of minerals occurred from the radiation (Table E-VII), several of the elements are seriously low when compared to the mouse recommended allowances. Iron, magnesium and possibly calcium may be borderline for man during prolonged periods. These would not be expected to present any problem in short flights.

Vitamin analyses* showed that of seven representative vitamins only riboflavin had a significant loss (11%) during radiation sterilization (Table E-VIII). The other B-vitamins were not affected (0-2% loss). Vitamin C and Vitamin A also showed no loss. This indicates free radical and oxidation reactions to be minimal. The B-vitamin content of Apollo diet is seriously low when compared to the requirements for man and mouse. This has little meaning for a short time but could be very serious for a prolonged flight.

The amino acids in Apollo diet (Table E-IX) are adequate for man and mouse both before and after radiation sterilization. The average loss was 7%.

*Analyses performed by Wisconsin Alumini Research Foundation, Madison, Wisconsin.

TABLE E-VI

PROXIMATE ANALYSIS (PERCENT)

ITEM	NON-STERILE	IRRADIATED	DIFFERENCE
H_2O	2.9	3.2	+0.3
Fiber	0.9	0.7	-0.2
Fat	14.4	14.4	0.0
Ash	3.5	3.5	0.0
N_2	2.89	2.83	-0.06
Protein (Crude)	18.1	17.7	-0.4
Total	39.8	39.5	
Carbohydrate (Difference)	60.2	60.5	
Energy Cal/gm	4.43	4.42	

Courtesy of C. W. Gehrke, and associates, Argricultural Chemistry Department, University of Missouri, Columbia, Missouri.

TABLE E-VII
APOLLO DIET - ELEMENTS

MOUSE				APOLLO DIET***			MAN		
REMARKS	ALLOWANCE mg/DAY*	APOLLO DIET mg/DAY**	ELEMENT	NON-IRRADIATED mg/Kg	IRRADIATED mg/Kg	LOSS %	ALLOWANCE mg/DAY****	APOLLO DIET mg/500 gm**	REMARKS
	13	35	Na	8700	8700, 8800	0		4350	
	15	15	K	4700	4700, 4800	0		2350	
Low	23	8.6	P	1900	2000, 2300	0	800	1075	
Low	23	7.6	Ca	2000	2000, 1800	5	800	950	?
	1.5	1.6	Mg		411		350	206	Low
		8.2	S		2040			1020	
	20	46	Cl		11400			5700	
Low	0.75	0.084	Fe		22.2		10	11	Border
Low	0.18	0.009	Cu		2.3			1.2	Low
Low	0.01	0.00002	Co		0.018			0.009	
	0.008	.076	Zn		19.0			9.5	
Low	0.13	.016	Mn		4.0			2.0	
		0.0005	Mo		0.12			0.06	
			V		0.05			0.03	

*Albrittin (1969) for a 25 gm mouse.
**Calculated for the average of the values from irradiated diet: 500 gm provides about 2,200 calories.
***The first four minerals were determined by chemical methods, the others by spectrography by Drs. G. W. Gehrke, and E. Pickett, Department of Agriculture Chemistry, University of Missouri, Columbia, Missouri
****NRC daily recommended allowance, 1968.

TABLE E-VIII
VITAMIN CONTENT AND IRRADIATION LOSSES

MOUSE			VITAMINS	APOLLO DIET - mg/100 gm[3]			MAN		
REMARKS	mg/DAY[1]	mg/gm[2]		NON-IRRADIATED	IRRADIATED	LOSS %	ALLOWANCE[4] mg/DAY	mg/500g[5]	REMARKS
		3.2	Ascorbic Acid	79.6, 79.6 Ave. 79.6	79.3, 79.6 Ave. 79.45	0.19	60	397	O.K.
Low	0.5	0.010	Riboflavin	0.286, 0.288 Ave. 0.287	0.252, 0.258 Ave. 0.255	11.2	1.7	1.28	Low?
Low	0.025	0.006	Thiamin	0.15, 0.15 Ave. 0.15	0.15, 0.15 Ave. 0.15	0	1.4	0.75	Low
Low	0.025	0.007	Vitamin B_6	0.166, 0.168 Ave. 0.167	0.162, 0.166 Ave. 0.164	1.8	2.0	0.82	Low
I.U.	25	37	Vitamin A, I.U.	755, 776 Ave. 776	773, 800 Ave. 787	0	5000	4960	O.K.
Very Low	0.25	.019	Pantothenate	0.488, 0.496 Ave. 0.492	0.466, 0.496 Ave. 0.481	2.2	---	0.24	
	0.0125	0.0009	Folate	0.0236, 0.0220 Ave. 0.0229	0.0236, 0.0236 Ave. 0.0236	0	0.4	0.12	Low

(1) Recommended Allowance for 25 gm mouse from Handbook of Biological Data. W. S. Spector, W. B. Saunders, 1956, p. 196.
(2) Calculated from irradiated Apollo Diet assuming 4 gm diet/day.
(3) Data from Warf Analyses.
(4) NRC Recommended Allowance 1968 for men 22-35 years of age.
(5) This is equivalent to a caloric intake of 2,000 cal.

AMINO ACIDS IN APOLLO DIET

MOUSE				APOLLO DIET %(3)			MAN		
REMARKS	mg/DAY(1) ALLOWANCE	mg/DAY(2) APOLLO DIET	AMINO ACID	NON-IRRADIATED	IRRADIATED	LOSS	ALLOWANCE(4) gm/DAY	gm/500(5) APOLLO DIET	REMARKS
	1	21.6	Histidine	0.68	0.54	20.7	-	2.70	
	6	22.8	Isoleucine	0.60	0.57	5.0	1.4	2.85	
	4	52.4	Leucine	1.41	1.31	7.1	2.2	6.55	
	2	44.4	Lysine	1.19	1.11	2.5	1.6	5.55	
	2	14.8	Methionine	0.40	0.37	7.6	2.2	1.85	O.K. with
		6.4	Cysteine	0.17	0.16	5.8		0.80	Cysteine
	1	29.6	Phenylalanine	0.76	0.74	3.8	2.2	3.70	
		19.2	Tyrosine	0.49	0.48	2.1		2.40	
	2	23.2	Threonine	0.65	0.58	10.7	1.0	2.90	
	1		Tryptophane				0.5		No Data
	4	26.0	Valine	0.68	0.65	4.4	1.6	3.25	
		36.8	Arginine	1.02	0.92	9.8	-	4.60	
			Alanine	0.78	0.75	3.8			
			Aspartate	1.34	1.24	7.3			
			Glutamate	2.98	2.70	9.3			
			Glycine	0.99	0.91	8.0			
			Ornithine	0.00	Trace				
			Hydroxyproline	0.34	Trace				
			Proline	0.65	0.74	0			
			Serine	0.70	0.64	8.4			
			NH_3	0.10	0.10	0			
			Total	15.59	14.51	6.7			

(1) Based on 1/5 rat minimum requirement, (Spector, 1956)
(2) Present in 4 gm irradiated Apollo Diet
(3) Analyses from G. W. Gehrke, Dept. of Agriculture Chemistry, Univ. of Missouri using gas chromatography on acid hydrolysate, excepting tryptophane.
(4) NRC allowance from data of Rose, et al. (1955)
(5) This provides 2200 Calories of irradiated Apollo diet.

Histidine, arginine and threonine were the most labile of the amino acids in this diet during radiation. The relatively low level of methionine and cysteine (the data include cystine) and the 6-8% loss during sterilization make methionine a remotely possible problem. Problems such as this would be greatly magnified if individual astronauts ate a less well chosen diet; current information suggests this (our) diet is better than that used on some Apollo flights due to the relative ease with which the drink, cereal and sweet items could be consumed.

Analyses of our Apollo-68 diet indicate it is not adequate for mice and few items would be borderline for man on prolonged flights.

APPENDIX F

RADIATION STERILIZATION OF DIET: HISTORICAL AND CURRENT

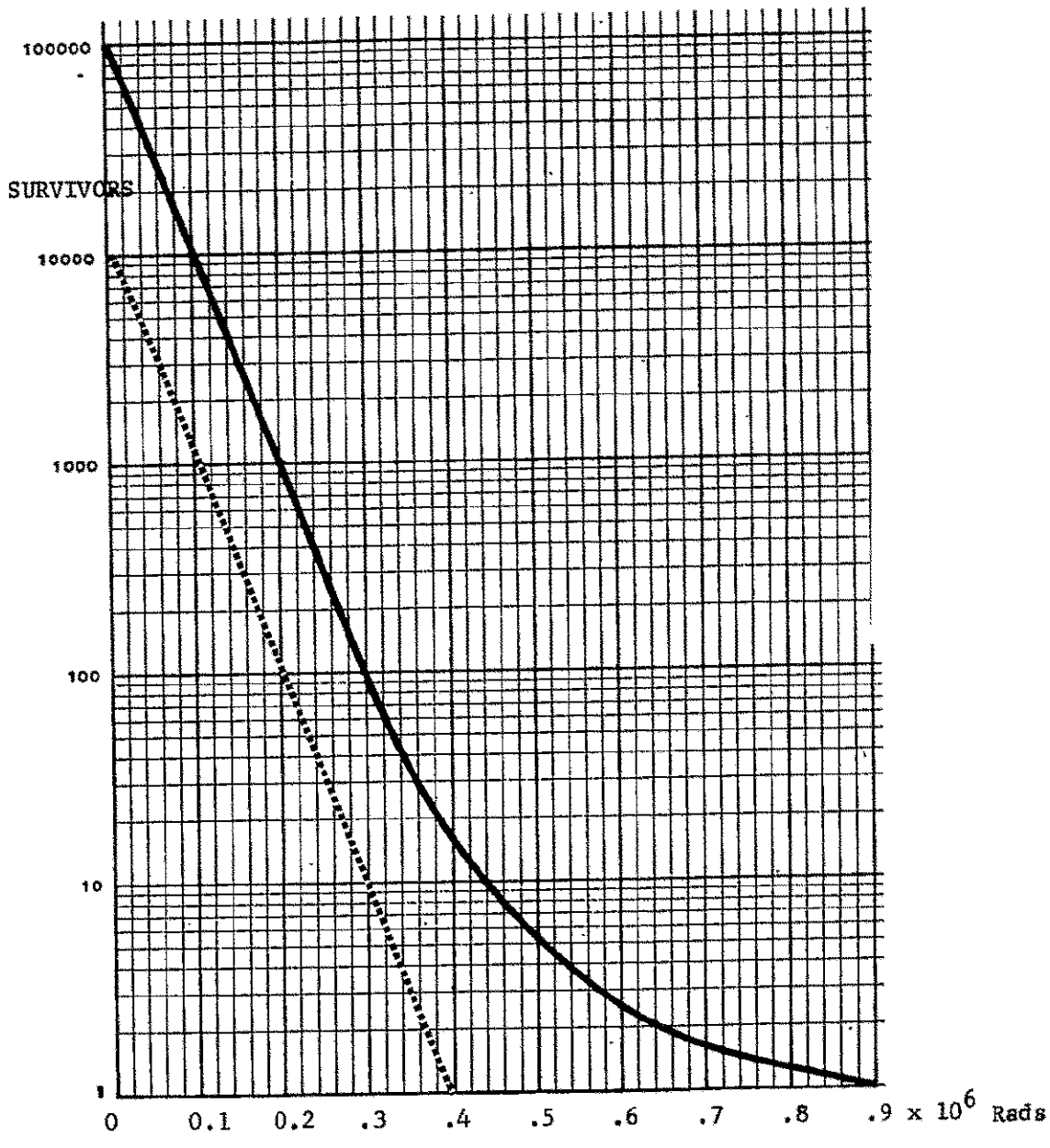
APPENDIX F

Radiation was the preferred method for sterilization of Apollo diet for feeding gnotobiotic mice. Filtration was deemed impractical because the diet was not completely soluble; dry and wet heat would be expected to destroy more vitamins and amino acids than does radiation (Luckey, T. D., et. al., Food Res., 20:180-185); and chemical sterilization would cause many reactions giving harmful products. Initial arrangements for radiation sterilization were made with the University of Missouri at their Columbia reactor, but it was decided that a better place would be Brookhaven National Laboratory. With the cooperation of Dr. John Cusack and his co-workers, Mr. Frank Rizzo of the Radiation Division of Brookhaven National Laboratories, the diet was sterilized at Brookhaven National Laboratories. A preliminary experiment was designed to give information regarding the radiation death curve for microbial spores, especially the "tail" (Figure F-1) dealing with the last survivors. This figure is exaggerated to illustrate the potential problems around one survivor. The dotted line illustrates the increased effectiveness of the kill when the number of initial microbes is decreased one log from those represented by the solid line.

The suggested quantity of radiation for reliable sterilization of the Apollo diet was 5×10^6 rads. This is somewhat more than Luckey (1955) had used for mouse feed (3×10^6 rads) and more than the British use (4.0×10^6 rads: J. S. Paterson and R. Cook, ILAR News, 12:21-22, 1969) (R. E. Horton and J. L. S. Hickey, Proc. Animal Care Panel, 11:93-106, 1961) for sterilization of animal diet. This information, combined with the knowledge that the Apollo diet as delivered should have very low inherent bacterial and spore contents, suggested that 5×10^6 rads provided a good safety factor.

FIGURE F-1

SURVIVAL - RADIATION ESTIMATE ON SPORE STRIPS



The suspected tailing of the survivor curve made it important to obtain more information related to our specific problem. The preliminary experiment was outlined as shown in Table F-I and F-II. The experiment and results did not follow this exact protocol because the spore strips available and those donated by Baltimore Biological Laboratories (Mr. R. Schmidt) were found to be different from those outlined. The design used is given in the first part of Table F-III. Samples of simulated Apollo diet (SAD) and spore strips were radiated at Brookhaven National Laboratory.

The data from the spore strips is given in Table F-III and plotted in Figure F-2. Standard bacteriological procedures were used to grow the spores under ideal conditions and count the resultant agar colonies from surviving individuals. It is noted that only 1% or less of the original spores were found, due to inability to free them from the paper. In spite of this, the curves show good agreement with each other and with the expected, from theory wherein lower numbers of individual spores are examined. No tailing was noted (Figure F-2) and the frequency with which zero counts were observed with the several low doses of radiation suggested that tailing was not of major importance. Serendipitously, the data fell into the most meaningful range because the comminuted Apollo diet was found by us to have 1×10^3 microorganisms per gram and 5×10^3 by Whirlpool Corporation. Thus, excepting possible effects of nutrients upon the spores in the dry Apollo diet, it is reasonable to expect the diet to be sterile with a minimum of 1×10^6 rads of Cobalt gamma radiation; this point is obtained by extrapolating a line which incorporates the three points showing the most resistance in Figure F-2.

The limited data given in Table F-III on SAD (simulated Apollo diet) show that 2×10^5 rads was inadequate to give sterilization while 1×10^6, 3×10^6,

TABLE F-I

EXPERIMENT OUTLINE FOR SPORE RADIATION

SPORES*	SAMPLES	DOSE
10^5	10	5×10^6
10^5	10	3×10^6
10^5	10	2×10^6
10^5	20	1×10^6
10^5	20	6×10^5
10^5	10	4×10^5
10^5	10	2×10^5
10^5	10	0
10^4	10	5×10^6
10^4	10	3×10^6
10^4	10	1×10^6
10^4	10	4×10^5
10^4	10	2×10^5
10^4	10	1×10^5
10^4	10	0

*B. subtilis var. niger
*B. stearothermophilus

TABLE F-II

SPORE DEATH ESTIMATES (4-7-69)

1. Curve gives 90% kill at 10^5 rads (see Figure F-1).

2. Source gives 5×10^6 rads per 30 minutes.

3. Data run on Spore Strips

TIME (Minutes)	RADS x 10^6	SPORE STRIP EXPECTED COUNT
0	0	10,000
1	0.1 x 67	220
3	.5	0-2
6	1.0	0
30	5	0

4. Data run on SAD (Simulated Apollo Diet)

TIME (Minutes)	RADS x 10^6	BACTERIA/gm
0	0	1,000,000
1	0.167	100
3	0.5	0
6	1.0	0
30	5	0

5. Sample placement in can (10^4 spores each)

   a. Center

   b. Bottom Center

   c. Side, One-Half Way to the Top

BROOKHAVEN NATIONAL LABORATORY, HIGH INTENSITY LABORATORY
EXPERIMENT WITH FRANK RIZZEL AND BOB MOCK ON APRIL 10, 1969
BACTERIOLOGY BY HERB KAPLAN AT VALLEY FORGE SPACE TECHNOLOGY CENTER

TABLE F-III

DETAILS OF SPORE AND DIET RADIATION

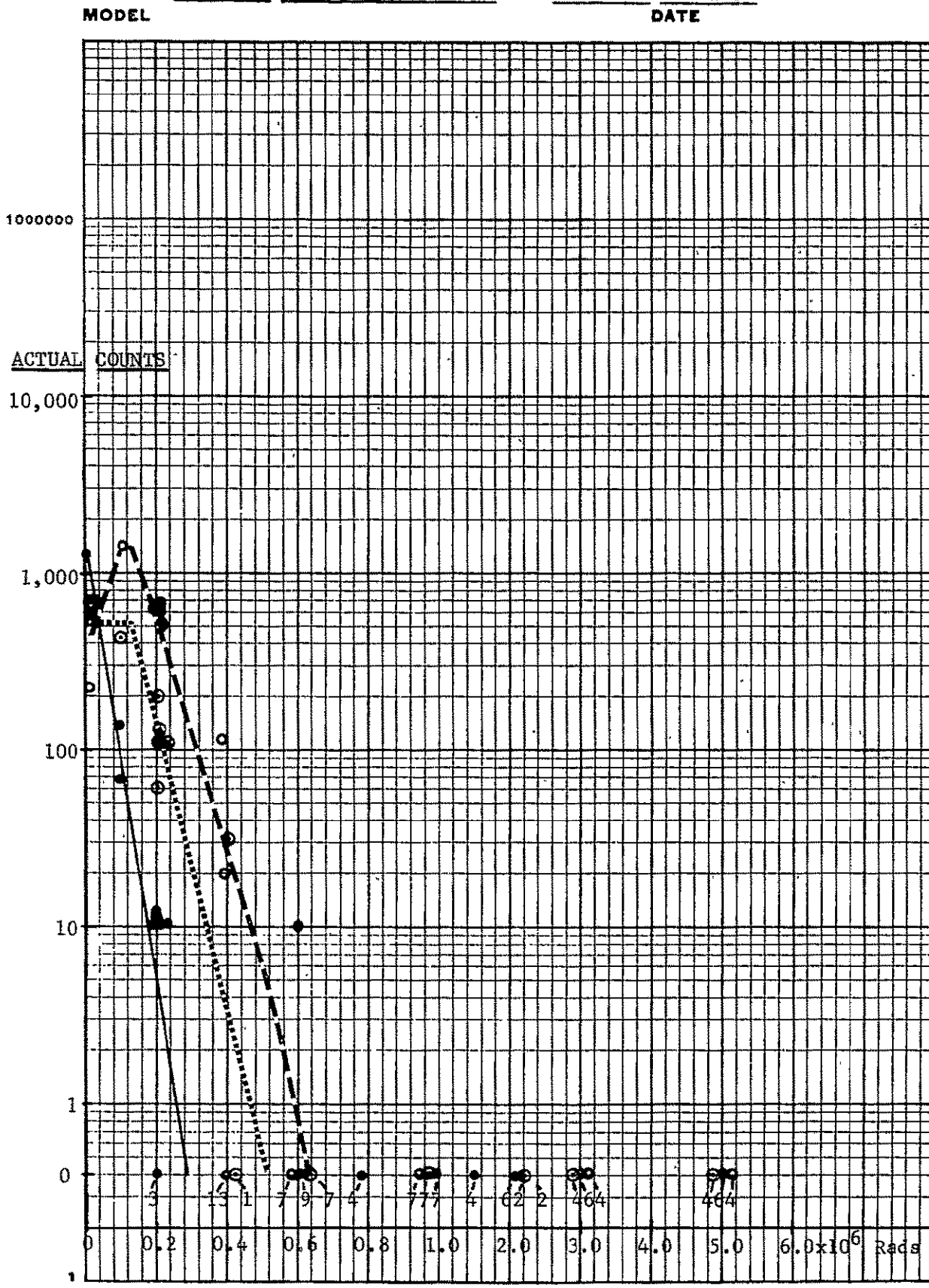
SAMPLE NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
0.2 -.3 Diet Sample in Small Closed Bag + 10^5 + 10^7 B. stearo. Spores	X		X	X		X		X		X	X		X	X		X	X		X		X	X		X				X	X		X		X	
Spore Strip, Bacillus subtilis var. niger, 10^6 spores		X	X		X	X	X	X	X			X	X		X	X		X	X	X	X		X		X	X	X	X	X	X	X	X	
10^6 B. subtilis Spores + 10^7 B. stearo. Thermal Strip + 10^5 B. stearo.	X		X	X		X		X		X	X		X	X		X	X		X		X	X		X				X	X		X		X	
Intended Radiation Rads x 10^5	50	50	50	30	30	30	20	20	10	10	10	6	6	4	4	4	2	2	2	1	1	0	0	0	2	4	4	6	6	4	4	2	2	
Cobalt Source Tube No.	9	9	9	9	9	9	9	9	9	9	9	7	7	7	7	7	1	1	1	1	1	-	-	-	7	9	1	7	7	7	7	1	1	
Time Irradiated (Minutes)	31	31	31	18.5	18.5	18.5	12.5	12.5	6	6	6	15.5	15.5	10	10	10	15.5	15.5	15.5	7.5	7.5	-	-	-	20	10	30.5	15.5	15.5	10	10	15.5	15.5	
Rads per Minute	←				161,500				→	←				9,200		→	←		13,030		→	0	0	0	←			Same by Tube Number				→		
Actual Dose, Rads x 10^5	50.1	50.1	50.1	29.3	29.3	29.3	20.2	20.2	9.7	9.7	9.7	6.1	6.1	3.9	3.9	3.9	2.0	2.0	2.0	.98	.98	0	0	0	7.8	16.2	4.0	6.1	6.1	3.9	3.9	2.0	2.0	
Envelopes Only Identifying Code:																																		
A = 10^5 B. stearo.	A		A	A		A		A		A	A		A	A		A	A		A		A	A		A				A	A				A	
B = 10^7 B. stearo.		B	B		B	B	B	B	B	B		B	B		B	B		B	B	B	B		B		B	B	B	B	B	B	B	B	
C = 10^6 B. subtilis	C		C	C		C		C		C	C		C	C		C	C		C		C	C		C				C	C				C	
Number of Samples Irradiated	2	2	2	1	3	3	4	2	2	6	1	1	1	1	1	1	1	1	1	1	1	3	1	1	5	5	5	3	4	4	4	4	4	
Bact.** Found In: A (No. of Tubes Tested Follows Dash)	0-2		0-2	0		0-3		0-2		0-5	0		0	0		10^1	10^2		10^2		10^2	10^2 -3		10^2				0-3	0-4				10^2 -4	
B		* 0-3	* 0-3		* 0-3	* 0-3	0-4	0-2	0-2	0-5		0	0		0	0		0	10^1	10^2	10^2		10^3		0-4	0-4	0-4	0-3	0-4	0-4	0-4	0-2 10^1-2	0-3 10^1	
C	0-2		0-2	0		0-3		0-2		0-5	0		0	10^1		10^2	10^1		10^3		10^3	10^2 -3		10^2				0-2 10^1	0-4				10^2 -4	
Diet (- or + for Each Tube)	=			-							-						÷					+ +												
Diet Sterile at 1, 3 and 5 x 10^6 Rads																																		
* CONTAMINATED																																		
** UNINOCULATED BACTERIOLOGIC CONTROLS WERE NEGATIVE										-																								

FOLDOUT FRAME

FOLDOUT FRAME 2

FIGURE F-2

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



and 5 x 10^6 rads produced a product from which no viable microorganisms were recovered (using about 1/2 gm in 10 ml of enriched cultured media).

Table F-IV presents a summary of the information provided in a partial survivor evaluation. This data allows calculation of D γ (death) values, the dosage of radiation needed for 90% kill or a one log reduction in the number of spores; and the doses needed to sterilize, the F γ values, were calculated from the D γ values according to procedure of C. F. Schmidt (Dose Requirements for the Radiation Sterilization of Food, presented at the European Meeting on the Microbiology of Irradiated Foods, Paris, April 20-23, 1960) and Bruch, et al. (Dev. Ind. Microbiol., 4:334-342, 1963). The most stringent condition, using 10^5 spores of Bacillus stearothermophilus, gave a calculated sterilization of 1.2 x 10^6 rads. This is remarkably close to the value obtained from the curves (using 10^7 spores).

This preliminary experiment indicates that 5 x 10^6 rads gives about a 5-fold safety factor for Apollo diet sterilization.

In order ot obtain radiation dosimetry and geometry on the Apollo diet prepared for germfree operations, some astronaut diet (GFE from Houston-MSC) was processed (pulverized, mixed and packaged) at General Foods Corporation at Tarrytown, New York on 3 April 1969 through the courtesy of Dr. D. E. James, Director of Research Engineering and Dr. B. F. Buchanan, in Research Management. Mr. Tom Johnston, Mr. Fred Patrizio and their assistants, particularily Mr. J. Festa and Mr. W. M. Fallon, were most helpful in the preparation. Their knowledge of the processing problems involved was great enough that very simple procedures were successfully used.

The astronaut food made available by MSC differed somewhat from that to be used in our mouse feeding experiment (Table F-V). When the items available were

TABLE F-IV

DIET AND SPORE STERILIZATION SUMMARY

RADS x 10^6	B. stearothermophilus				B. subtilis		Diet	
	10^5		10^7		10^6			
	FOUND	REPL.	FOUND	REPL.	FOUND	REPL.	FOUND	REPL.
0	500	4	600	4	1310	1	+	4
0.10	460	1	1360	1	200	2		
0.20	520 100	1 5	1090 500	1 5	10 0	4 6	+	1
0.39 0.40	30 0	1 1	70	2	0	14		
0.61	0	8	10 0	1 7	0	9		
0.78					0	4		
0.97	0	6	0	6	0	7	-	1
1.62					0	4		
2.02	0	2	0	2	0	6		
2.93	0	4	0	4	0	6	-	1
5.01	0	4	0	4	0	6	-	2

D_γ= The 90% Death Value = $\frac{\text{Rads}}{\log A - \log B}$

where A = Total Samples x Spores per Sample and

B = Number of Samples not Sterile (where some are sterile)

$D_{\text{B. subt.}} = \frac{2 \times 10^5 \text{ Rads}}{\log (10 \times 130) - \log 4} = 8 \times 10^4 \text{ Rads}$

$D_{\text{B. stearo.}} = \frac{6 \times 10^5 \text{ Rads}}{\log (8 \times 136) - \log 1} = 2 \times 10^5 \text{ Rads}$

TABLE F-IV (Continued)

CALCULATED DEATH RATES

$$D_\gamma = \frac{t}{\log A - \log B}$$

D = (time for) 90% reduction (Rads)

t = time $\simeq$ Rads

A = No. Samples x Spores/Sample

B = No. Samples Not Sterile

B. subtilis $D_\gamma = \frac{2 \times 10^5 \text{ Rads}}{\log (10 \times 130) - \log 4} = \frac{2 \times 10^5 \text{ Rads}}{\log 1300 - 0.602}$

$3.11394 - 0.60206$

$= 2.51188$

$= \frac{2 \times 10^5}{2.5111} = \underline{8 \times 10^4 \text{ Rads}}$

B. stearo. $D_\gamma = \frac{6 \times 10^5 \text{ Rads}}{\log (8 \times 136) - \log 1} = \frac{6 \times 10^5}{\log 1088} = 3 \log 1.088$

$\frac{6 \times 10^5}{3.03663} = 2 \times 10^5$

$= \underline{2 \times 10^5 \text{ Rads}}$

TABLE F-IV (Continued)

CALCULATED STERILITY (F)

$F_\gamma = D_\gamma(\log M + 1)$

where D_γ = Rads for 90% Kill (one log)

and M = Number of Spores per Samples x Number Samples

B. subtilis $F_\gamma = 8 \times 10^4 (\log [131 \times 1] + 1)$

$(2 \log 1.31 + 1)$

$(2.11727 + 1)$

$= 8 \times 10^4 (3.11727)$

$= 23.81 \times 10^4 = \underline{2.5 \times 10^5 \text{ Rads}}$

B. stearo. $F_\gamma = 2 \times 10^5 (\log [136 \times 1] + 1)$

$(2 \log 1.36 + 1)$

(3.13354)

$= 2 \times 10^5 (3.1335)$

$= 6.267 \times 10^5 = \underline{6.3 \times 10^5 \text{ Rads}}$

Assume 10^5 Spores in place of the 136 found

$F_\gamma = 2 \times 10^5 (\log 10^5 + 1)$

$= 2 \times 10^5 (6) = 1.2 \times 10^6$ Rads

NOTE: D_γ and F_γ are used to express the 90% reduction by gamma radiation. More specific would be $D_{Co\,\gamma}$ and $F_{Co\,\gamma}$.

TABLE F-V

COMPARISON OF APOLLO DIET WITH THAT USED IN

ESTIMATING DENSITY OF APOLLO DIET FOR RADIATION DOSIMETRY

CATEGORY	ITEM	USED, SAD		APOLLO, %
		gm	%	
MEAT AND DAIRY			17	27
	Beef Pot Roast	59.5		
	Chicken Bites	42.6		
	Chicken Sandwich	39.0		
		106.0		
CEREALS			15	19
	Cinnamon Toast	14.1		
	Corn Flakes	29.4		
	Corn Chowder	49.6		
		93.1		
VEGETABLES			5	6
	Pea Soup	30.7		
FRUIT			0	5
DRINK (Mostly Sugar)			27	21
	Grapefruit	27.0		
	Orange	88.0		
	Pineapple-Grapefruit	26.1		
	Cocoa	28.9		
		170.0		
SWEETS			36	23
	Pudding	95.9		
		111.5		
	Fruitcake*	0.87		
	Cookies	12.1		
		220.4		

*Dried and difficulty in process gave little material.

passed through a 20 mesh screen and blended, the specific gravity of the mix was 0.574. The specific gravity of dry foods was determined (Table F-VI) to calculate a mix to give the same density. Subsequently, items were purchased at the supermarket and blended to give a simulated Apollo diet (SAD).

The SAD (Simulated Apollo Diet) mixture had specific gravity of 0.58 which seemed to be adequate to simulate a diet of Specific Gravity 0.57. It is anticipated that the complete Apollo diet will give a specific gravity of 0.50; this estimate is based upon the increased sugar and decreased mean and fruit of SAD compared to Apollo diet. However, SAD does have comparable foods of low water content, comparable trace elements and adequate Ca^{++}, P and other major minerals.

SAD was packaged in polyethylene bags and fitted into a No. 2½ can. It was found that 522 gm would fit into the can when jarred 3-4 times. Eighteen packages were made. Field oats were placed in other cans to provide adequate numbers of cans to simulate the geometry to be used.

N_2 and vacuum seals were not used in this preparation of SAD because the cans could not be sealed -- the dosimeter is to be placed inside. Since these bags will rapidly pass O_2 and N_2, they would equilibrate with air over 1-2 days, whereas the Apollo diet used to feed mice were processed and sealed in metal cans under N_2.

Upon return to the Valley Forge Space Center, the simulated diets used were calculated and estimated to have: (1) high Specific Gravity due to about 4% H_2O compared to 2-3% H_2O in Apollo diet; and (2) too high Specific Gravity due to more sugar and less meat and dairy products in SAD compared to Apollo diet. The effect of vacuum packing upon Specific Gravity is not known; after two days the N_2 in the can will infiltrate the bag and vacuum packing will

TABLE F-VI

SUPERMARKET ITEMS BLENDED TO PROVIDE

SAD (SIMULATED APOLLO DIET) SPECIFIC GRAVITY

ITEM	AMOUNT		SP. G.
	POUNDS	OUNCES	
Skim Milk Powder	4	0	0.31
Gelatin, Orange Flavored	3	2	--
Graham Cracker Crumbs		14	0.48
Oatmeal, Instant Flakes	6	0	0.20
Sugar (≅ drink and sweets)	7	0	0.86
Tang	1	11	0.89
Jello - Pudding, Chocolate		4½	0.84
Jello - Pudding, Fudge		4½	0.84
Jello - Pudding, Vanilla		4	0.84
Jello - Pudding, Pineapple - Cream		4	0.84
Bread Crumbs		10	--
Hamburger Seasoning		1	--
Chicken Gravy Powder		2	--
UNUSED ITEMS			
Potato Buds	--	--	0.27
Total, General Mills	--	--	0.14
Grape Nuts, Post	--	--	0.47
Cream of Wheat, Nabisco	--	--	0.74
Sparkleen	--	--	0.86
Composite of Mixture Used	24	9	0.584
ADDITIONAL SAD - VFSTC (4/7/69)			
Mixed Cereal with Banana	2	--	0.20
Skim Milk Powder	2	--	0.31
Sugar	2½		0.86
Final Composite	27	9	0.5

have negligible effect on the long term basis. It may affect the amount placed in the can. More oatmeal, skim milk powder and sugar were added to provide 28 cans of SAD. One case of 24 cans was needed for the radiation dose-geometry and max-min study.

The estimates provided a basis for the radiation sterilization studies prior to receipt of Apollo diet. Quantitative data of survivors from the spore strips were used to estimate the efficiency (of over-kill) in dry diet sterilization, and to correlate the mechanical dosimeters with biological activity in 0.57 Specific Gravity material.

Plans for Apollo diet radiation sterilization were made following conversations with Mr. J. D. Kaylor, the Supervisory Food Technologist at the Technology Laboratory of the Bureau of Commercial Fisheries USDI at Gloucester, Massachusetts. It was considered that Brookhaven National Laboratory could do this work more efficiently in their High Intensity Radiation Development Laboratory. Dr. John Cusack, Chief of Brookhaven High Intensity Radiation Development Laboratory, was consulted and arrangements made for preliminary study with Frank Rizzo of the Radiation Division. The diet and appropriate spore strips were taken to Brookhaven at Upton, New York on 9 April.

Radiation dose-heat relationships were worked out using one can of simulated Apollo diet and one can of Apollo diet. The constant rise of $7^{o}C$ per 10^{6} rads was found over the range from 10^{5} to 10^{8} rads in SAD and $5^{o}C$ per 10^{6} rads for the Apollo diet. This information was helpful to estimate the heat absorbed when the final diet was sterilized for the mice.

APPENDIX G

MOUSE HEMATOLOGY DATA

TABLE XVIII

SUMMARY OF MOUSE GROWTH DATA

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

GNOTOBIOTIC MICE - *E. coli* - APOLLO DIET

GROUP 2	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	22	12	34	66	0	0	1540	7.4	17.8	4.4	M
	2	8	9	17	77	5	1	440	8.7	20.2	2.3	M
	3	20	10	30	68	2	0	5280	5.4	17.7	3.25	M
	4	22	2	24	72	4	0	770	5.4	22.7	3.0	M
GROUP 21												
	1	12	52	64	36	0	0	2420	13.9	23.5	2.6	F
	*2	-	-	-	-	-	-	-	-	25.4	1.6	M
	3	10	25	35	65	0	0	660	16.0	24.0	-	F
	4	8	66	74	26	0	0	1980	13.9	23.2	2.1	M
	5	0	36	36	64	0	0	1430	17.0	21.4	1.2	M

*Died before bleeding.

GNOTOBIOTIC MICE - L. leichmannii - APOLLO DIET

GROUP 3	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	22	5	27	64	5	4	5610	12.8	20.4	3.4	M
	2	20	9	29	66	5	0	1760	8.3	15.0	3.1	M
	3	25	21	46	49	5	0	3300	7.7	19.0	3.85	M
	4	25	12	37	60	3	0	2310	12.2	14.7	4.75	M
	5	17	6	23	67	6	4	5060	11.3	18.5	3.4	M
GROUP 23												
	1	5	31	36	64	0	0	1210	13.9	35.2	1.3	F
	2	2	6	18	82	0	0	3630	13.2	30.3	2.5	F
	3	0	6	6	90	4	0	1540	13.6	36.7	2.0	F
	4	2	22	24	76	0	0	1760	15.6	30.9	2.0	M
	5	0	8	8	92	0	0	2970	13.2	24.3	2.7	F

GNOTOBIOTIC MICE - C. albicans - APOLLO DIET

GROUP 4	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	17	16	33	62	4	1	5060	8.5	14.3	4.7	M
	2	29	41	70	25	5	0	1925	11.9	15.2	2.2	?
	3	24	40	64	30	4	2	2860	6.7	14.1	2.9	M
	4	19	45	64	33	3	0	5170	10.6	16.4	2.9	M
	5	36	42	78	20	2	0	6490	11.9	13.2	2.2	M
GROUP 24												
	1	2	36	38	62	0	0	4070	12.8	23.6	1.7	F
	2	0	40	40	56	4	0	8140	14.3	25.4	0.8	F
	3	2	60	62	34	4	0	4510	16.0	32.5	0.6	F

GNOTOBIOTIC MICE - *E. coli* AND *L. leichmannii* - APOLLO DIET

GROUP 5	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	19	22	41	52	6	1	2420	5.6	18.0	1.75	?
	2	18	36	54	42	4	0	1430	5.9	23.2	2.00	M
GROUP 26												
	1	0	8	8	92	0	0	1980	17.0	34.0	2.3	M
	2	0	16	16	84	0	0	2530	15.1	30.6	1.5	F
	3	4	14	18	82	0	0	2090	13.6	38.2	1.5	F
	4	0	8	8	92	0	0	3080	16.0	27.1	1.4	F
	5	2	14	16	84	0	0	1430	14.6	31.1	1.7	F

GNOTOBIOTIC MICE - *E. coli* AND *C. albicans* - APOLLO DIET

GROUP 6	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	18	31	49	49	2	0	5390	6.7	13.7	1.5	M
	2	23	49	72	24	4	0	3740	9.0	18.0	4.0	M
GROUP 28												
	1	4	8	12	88	0	0	CLOTTED		33.2	1.0	F
	2	0	4	4	78	18	0	3520	16.8	35.1	0.9	F
	3	0	32	32	68	0	0	880	16.0	30.0	1.8	M
	4	2	36	38	62	0	0	3630	14.2	31.1	1.6	M
	5	1	5	6	94	0	0	2310	15.6	36.2	1.5	F

TABLE XIX (Continued)

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

* Only 3 animals carried to end.

Group 20 not applicable

CLASSIC MICE - GNOTOBIOTIC ISOLATION - APOLLO DIET

GROUP 8	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	23	12	35	62	2	1	1650	14.2	15.1	0.20	F
	2	19	14	33	65	1	1	1430	12.1	19.8	0.30	M
	3	20	13	33	61	4	2	1540	13.9	19.5	0.20	M
	4	13	4	17	81	2	0	3630	13.2	24.6	0.40	M
	5	15	14	29	66	2	3	2750	14.2	20.0	0.30	M
GROUP 31												
	1	4	28	32	68	0	0	2530	13.2	36.1	0.60	F
	2	0	40	40	50	10	0	1430	15.3	20.3	0.55	M
	3	-	-	-	-	-	-	-	-	20.3	0.80	F
	4	4	12	16	80	4	0	2420	12.9	28.7	0.60	M
	5	-	-	-	-	-	-	-	-	16.7	0.55	M
GROUP 32												
	1	2	14	16	84	0	0	1610	17.5	22.0	0.60	M
	2	6	24	30	70	0	0	2530	15.0	22.2	0.50	M

CLASSIC MICE - NON-STERILE - APOLLO DIET

GROUP	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
10	1	8	1	9	89	2	0	2640	7.4	29.6	0.35	M
	2	12	6	18	72	8	2	5280	13.2	25.2	0.25	M
	3	7	2	9	89	2	0	4840	5.9	26.3	0.20	F
	4	24	11	35	63	2	0	3960	12.6	27.6	0.55	M
	5	10	6	16	80	4	0	4840	11.6	29.5	0.30	M
34												
	1	0	20	20	80	0	0	880	8.7	23.7	0.40	M
	2	2	12	14	86	0	0	3830	13.0	29.2	0.50	M
	3	4	15	16	74	10	0	2750	11.5	32.8	0.50	M
	4	9	41	50	42	8	0	1320	10.1	21.8	0.55	M
36												
	1	6	22	28	72	0	0	6490	15.3	29.3	0.90	M
	2	0	20	20	80	0	0	2090	15.0	25.2	0.90	F
	3	8	12	20	80	0	0	2750	15.6	38.3	0.90	M
	4	2	12	14	86	0	0	3520	16.3	32.5	0.90	M
	5	2	20	22	78	0	0	2530	16.0	32.6	1.0	M

GERMFREE MICE - PURINA LABORATORY CHOW 5010C - AUTOCLAVED

GROUP 11	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	3	5	8	73	15	4					
	1	4	5	9	79	10	2	6900	14.2	-	-	-
	2	4	6	10	84	4	2					
	2	4	9	13	77	8	2	4500	15.6	-	-	-
	3	5	5	10	82	6	2					
	3	1	0	1	95	4	0	5200	15.9	-	-	-
	4	POOR SMEAR										
	4	6	7	13	80	4	4	5200	14.9	-	-	-

CLASSIC MICE - GNOTOBIOTIC ISOLATION - PURINA LABORATORY CHOW 5010C - AUTOCLAVED

GROUP 12	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	4	3	7	87	6	0					
	1	6	4	10	87	3	0	6700	16.2	-	-	-
	2	4	2	6	91	2	1					
	2	4	5	9	84	5	2	3900	14.9	-	-	-
	3	4	10	14	77	7	2	5600	16.9	-	-	-
	4	POOR SMEAR										
	4	7	6	13	83	4	0	-	-	-	-	-
	5	5	10	15	69	14	2	-	-	-	-	-

CLASSIC MICE - PURINA LABORATORY CHOW 5010C - AUTOCLAVED

GROUP 13	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	2	5	7	85	6	2					
	1	1	4	5	83	12	0	9700	14.9	-	-	-
	2	-	-	-	-	-	-	8300	16.9	-	-	-
	3	3	5	8	88	3	1					
	3	2	10	12	84	3	1	2500	15.2	-	-	-

CLASSIC MICE - PURINA LABORATORY CHOW 5010C - UNTREATED

GROUP 14	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	-	-	-	-	-	-	3200	16.2	-	-	-
	2	4	4	8	82	9	1					
	2	2	3	5	88	6	1	3900	16.5	-	-	-

GNOTOBIOTIC MICE - S. epidermidis - APOLLO DIET

GROUP 15	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	17	4	21	77	1	1	5390	12.3	28.9	3.0	M
	2	33	13	46	48	3	3	3190	12.9	16.8	2.2	M
	3	34	14	48	47	5	0	1320	12.6	20.7	3.3	M
	4	18	8	26	73	1	0	6710	11.8	29.4	3.2	M
	5	17	6	23	73	4	0	12,760	13.0	31.8	3.1	M

TABLE XXIV

CONFIRMATION COUNTS OF BACTERIAL SPECIES INTRODUCED

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

GNOTOBIOTIC MICE - Bacterioides sp. - APOLLO DIET

GROUP 17	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	-	-	-	-	-	-	-	-	25.8	1.3	F Gravid
	2	6	24	30	68	2	0	1430	17.4	25.6	1.1	M
	3	4	14	18	80	2	0	3630	15.1	35.4	1.0	F Gravid
	4	0	42	42	54	4	0	1760	14.2	30.2	0.9	F Gravid

GERMFREE MICE - PURINA LABORATORY CHOW 5010C - AUTOCLAVED

GROUP 37	MOUSE	BANDS	SEGMENTED NEUTROPHILES	TOTAL NEUTROS	LYMPHOCYTES	MONOCYTES	EOSINOPHILES	WBC	Hgb	WEIGHT	CECUM WEIGHT	SEX
	1	0	26	26	72	2	0	4420	16.2	27.8	1.10	M
	2	0	18	18	80	2	0	3850	16.0	25.8	0.90	F
	3	-	-	-	-	-	-	-	-	15.8	0.30	F
	4	0	52	52	48	0	0	2310	15.6	25.6	0.70	M

APPENDIX H

MOUSE INTERFERON DATA

MOUSE INTERFERON TITRES **

GROUP	DILUTIONS TESTED	INTERFERON TITER
1	-1, -2, -3	Negative*
2	-1, -2, -3	Negative*
3	-1, -2, -3	Negative*
4	-1, -2, -3	Negative*
5	-1, -2, -3	Negative*
6	-1, -2, -3	Negative*
7	-1, -2, -3	Negative*
8	-1, -2, -3	Negative*
9	-1, -2, -3	Negative*
10	-1, -2, -3	Negative*
15	-1, -2, -3	Negative*
16	-1, -2, -3	Negative*
17	-1, -2, -3	Negative*
19	-1, -2, -3	Negative*
21	-1, -2, -3	Negative*
23	-1, -2, -3	Negative*
24	-1, -2, -3	Negative*
26	-1, -2, -3	Negative*
28	-1, -2, -3	Negative*
30	-1, -2, -3	Negative*
31	-1, -2, -3	Negative*
32	-1, -2, -3	Negative*
33	-1, -2, -3	Negative*
34	-1, -2, -3	Negative*
35	-1, -2, -3	Negative*
36	-1, -2, -3	Negative*
37	-1, -2, -3	Negative*
NABI (Control)	-3.5, -4.0, -4.5	4.3 Logs

*Less than 1.0 log, e.g., less than 1:10

**Determinations by North American Biologicals, Rockville, Maryland, using GD VII yield reduction assay technique.

APPENDIX I

MOUSE PHAGOCYTIC INDEX DATA

PHAGOCYTIC INDEX (α) OF MICE*

BASED ON CARBON DOSE OF 8 mg/100 mg MOUSE WEIGHT READ AT 700 mμ

GROUP	ANIMAL WEIGHT GRAMS	LIVER & SPLEEN WEIGHT GRAMS	WLS/100 GRAMS MOUSE	K_8 PHAGOCYTIC INDEX AT GIVEN DOSE	α CORRECTED INDEX
1	22.8	0.9555	4.19	.009	4.93
2	10.8	0.5700	5.28	.006	3.46
2	14.2	0.6215	4.38	.021	6.29
3	31.9	1.5068	4.72	.034	6.81
4	19.0	0.8369	4.40	.024	6.51
5	29.0	1.3062	4.50	.029	6.76
5	22.8	0.9555	4.19	.014	5.66
6	20.1	0.9768	4.86	.029	6.29
6	16.2	0.9375	5.79	.025	5.02
7	28.7	1.0597	3.69	.045	9.65
7	23.4	1.0226	4.37	.330	15.8
8	30.2	1.5181	5.03	.033	6.36
8	31.0	1.5462	4.99	.018	5.29
9	31.3	1.7618	5.63	.012	4.09
9	35.1	1.9577	5.58	.043	6.26
10	37.0	2.0033	5.41	.021	5.10
10	30.0	1.3546	4.51	.008	4.40
15	26.9	1.3723	5.10	.067	3.67
16	24.1	1.0255	4.25	.004	3.71
16	26.2	1.3521	5.16	.003	2.79
17	34.6	1.7295	5.00	.010	4.30
19	26.4	1.8361	6.95	.029	4.42
21	29.5	1.5577	5.28	.023	5.39
21	26.5	1.1239	4.24	.006	4.32

GROUP	ANIMAL WEIGHT GRAMS	LIVER & SPLEEN WEIGHT GRAMS	WLS/100 GRAMS MOUSE	K_8 PHAGOCYTIC INDEX AT GIVEN DOSE	α CORRECTED INDE
23	31.0	1.2489	4.03	.012	5.68
23	41.0	2.2511	5.49	.006	3.35
24	21.5	1.1888	5.53	.007	7.46
24	22.1	1.6938	7.66	.007	4.39
26	31.0	1.5874	5.12	.005	7.21
26	34.2	1.7642	5.16	.006	5.16
28	31.1	1.5940	5.12	.006	3.55
28	32.0	1.3949	4.36	.003	7.12
30	25.0	1.5873	6.35	.013	3.72
31	32.2	1.5336	4.76	.008	4.22
31	32.4	2.2441	6.93	.024	4.15
32	22.2	1.1677	5.26	.010	4.11
33	33.2	2.0566	6.19	.009	3.03
33	31.2	1.6853	5.40	.023	5.25
34	28.1	1.2101	4.31	.026	6.89
34	31.2	1.8808	6.03	.006	3.03
35	29.2	1.7104	5.86	.010	3.68
35	28.1	1.7290	6.15	.012	3.76
36	25.0	1.4062	5.62	---	-----
36	31.8	1.7916	5.65	.008	3.56

*Animal Designation identified in Table XVI-PART B entitled Final Experimental Design.

RESULTS OF HEMAGGLUTININ PRODUCTION EXPERIMENT

Antigen - Sheep Red Blood Cells (S-RBC).

Amount and Nature of Immunization - I.P. injection 0.5 cc of 10% suspension of S-RBC in N-saline or approximately $1 \propto 10^8$ S-RBC

Assay - Four days following immunization by brachial bleeding and serial dilution of hemagglutination

Sera diluted as follows:

Undiluted - 1:1 - 1:2 - 1:4 - 1:8 - 1:16 - 1:32 - 1:64 - 1:128 - 1:256 - 1:512 $\rightarrow \infty$

$\text{Log}_{\text{base 2}} =$ 0 1 2 3 4 5 6 7 8 9

HEMAGGLUTININ TITRES*

GROUP	DILUTION	LOG_2
1	1:32 - 1:64	5 - 6
2	1:64	6
3	1:256	8
4	1:64	6
5	1:64	6
6	1:128	7
7	1:64 - 1:128	6 - 7
8	1:64	6
9	1:32	5
10	1:64	6
15	1:64	6
16	1:64 - 1:128	6 - 7
17	1:64	6
19	1:64	6
21	1:64	6
23	1:256 - 1:512	8 - 9
24	1:64 - 1:128	6 - 7
26	1:64	6
28	1:128 - 1:256	7 - 8
30	1:164 - 1:128	6 - 7
31	1:64	6
32	1:64	6
33	1:32	5
34	1:64	6
35	1:32	5
36	1:64	6

*Animal Designation identified in Table XVI-PART B entitled Final Experimental Design.

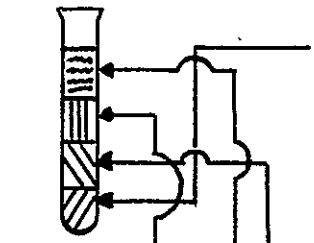
COMPLEMENT TITRES

The numerous reports (Winn, 1966) of complement produced in mice measured by the 50% hemolytic reactivity technique (Mayer, 1946) essentially report that the mouse has negligible quantities of complement. The reason for this is uncertain. The 50% hemolysis technique is one where various dilutions of mouse serum are added to reaction mixtures containing human erythrocytes and varying concentrations of Brucella abortus antigen and antisera with the amount of hemoglobin released being measured spectrophotometrically, following a standard incubation period. This analysis is based on the fact that complement has a series of eleven components; all eleven being required to achieve hemolysis. When one or more of these components or co-factors are low or absent, there is little or no hemolysis.

The immune-adherence test, as described by Nishioka (1963) and indicated in Figure I-1, offers many unique advantages. One of these being that it is extremely sensitive and very minute amounts of complement can be assayed. In the mouse, apparently one or more of the co-factors are low and as a result, you get very little hemolysis by the 50% hemolytic reactivity. The accompanying results (see Table I-I) will illustrate that the mice apparently have the first four factors in sufficient levels. By the immune-adherence technique, a positive result is any dilution in which a +2 or greater response of hemagglutination is recorded. Control values using serum from guinea pigs showed a positive titre of 1 to 1,000 dilutions in this laboratory. Nichioka indicated they obtained guinea pig serum complement titres of 1 to 2,000.

FIGURE I-1

SCHEMATIC DIAGRAM OF NISHIOKA COMPLEMENT PROCEDURE



13x75 mm Glass Tube

(1) Particulate Antigen (Ag), i.e., Brucella abortus Strain Number 1119-3 from Sylvania Chemical Company. .2 cc of Veronal Buffer with Serum Albumin, Ca^{++} and Mg^{++} ($SAVB^{++}$) solution containing 5.0×10^7 B. abortus (Stock 1:10 with $SAVB^{++}$) particles

(2) Antisera (Ab), i.e., Anti B. abortus .2 cc of 0.277 mg Ab N/ml appropriately diluted (Stock 1:320 with $SAVB^{++}$) from rabbit (immune sera optimal dilution approximately 0.173 µg Ab N/ml heated at 56°C for 30 minutes)

(3) Complement (C^1), i.e., test sera appropriately diluted 0.5 ml with $SAVB^{++}$

(4) Human O Rh^+ cells 0.1 ml of 2% solution in $SAVB^{++}$

(a) Shake in H_2O bath at 37°C for 10 minutes

(b) Stand in H_2O bath at 37°C for 55 minutes

(c) Read 0, +, ++, +++ or ++++
Consider ++ or greater significant

CONTROL: Ag + Hu O Rh^+ ⟶ ibid procedure

SAVB: 5 x stock 200 ml + 800 ml H_2O ⟶ 1 Liter + 1 g

Bovine Serum Albumin (BSA) from Armour Laboratories, Chicago, Illinois

TABLE I-I

COMPLEMENT TITRES BY IMMUNE-ADHERENCE*

GROUP	UNDILUTED	DILUTIONS				
		1:100	1:200	1:500	1:1000	1:2000
1	+3	+2	+2	0	0	0
2	+3	+2	+2	0	0	0
3	+3	+2	+2	0	0	0
4	+3	+2	+2	0	0	0
5	+2	+2	0	0	0	0
6	+2	+2	0	0	0	0
7	+2	+2	0	0	0	0
8	+2	+2	+2	+2	0	0
9	+2	+2	0	0	0	0
10	+2	+2	0	0	0	0
15	+2	+2	+2	0	0	0
16	+2	+2	+1	0	0	0
17	+2	+2	+1	0	0	0
19	+3	+2	+2	0	0	0
21	+3	+2	+2	0	0	0
23	+3	+2	+2	0	0	0
24	+3	+2	+2	0	0	0
26	+2	+2	0	0	0	0
28	+2	+2	0	0	0	0
30	+2	+2	0	0	0	0
31	+2	+2	+2	+2	0	0
32	+2	+2	+2	+2	0	0
33	+2	+2	0	0	0	0
34	+2	+2	0	0	0	0
35	+2	+2	0	0	0	0
36	+2	+2	0	0	0	0
37	+2	+2	+2	+1	+1	0
BALB/C (Control)	+2	+2	+2	+2	+1	0
G-P (Control)	+4	+3	ND	ND	+2	+1

*Procedure of Nishioka (1963)

REFERENCES

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3. Winn, H. J. (1966) Immune Functions (Chapter 31). In: Biology of the Laboratory Mouse, E. L. Green (ed), 2nd edition, The Blakiston Div., McGraw-Hill Book Company.

APPENDIX K

OUTLINE OF MOUSE STANDING OPERATING PROCEDURES

APPENDIX K

1. INTRODUCTION

2. DIET

   a. Composition or type and physical state

   b. Sterilization

   c. Storage (1) before; (2) after b; and (3) in isolator (time, temp. gas)

   d. Feeding

   e. Container in each section above

   f. Cleaning of receptacle and renewal frequency

3. WATER, DRINKING

   a. Source and composition

   b. Sterilization: (1) first; (2) interim; and (3) second

   c. Time and amount

   d. Container in each of above (type of lines)

   e. Cleaning

   f. Sterility checking

4. PERACETIC ACID

   a. Source

   b. Mix (detergent, water, other)

   c. Storage

   d. Container in above

   e. Sprayer

5. ANIMALS

   a. Source

   b. Special characteristics

   c. Shipping time and food

   d. Age, sex

   e. Appearance

   f. Record until sacrifice

   g. How sacrificed

   h. Autopsy

   i. Tissues

   j. Discard

6. ISOLATORS

   a. Source

   b. Type, material, size, specs

   c. Description (air inlet and outlet)

   d. History of usage and breaks

7. STERILIZING UNITS

   a. Autoclave

   b. Tunnel Entry

8. GLOVES

   a. Source

   b. Primary

   c. Protection gloves

   d. Treatment (washing, sterilization, powder)

9. FILTERS

   a. Source

   b. Type and specs

10. ACCESSORIES

   a. Cages

   b. Waste

   c. Balance studies

11. GENERAL SUPPLIES

12. PHYSICAL CONDITIONS

   a. Light cycles

   b. Pressure, noise, etc.

13. AIR

   a. Source

   b. Pollutants

   c. Sterilization

   d. Pressure and rate

14. PROCEDURES

   a. Receipt and storage of shipped animals

   b. Animal insertion into isolators and cages

      (1) Gloves or tweezers

      (2) Weight and character of animals

   c. Isolator sterilization

      (1) Preparation

      (2) Cleaning

      (3) Sterilization

      (4) Monitoring and testing

   d. Isolator care

e. Isolator decontamination and cleaning

f. Isolator leaks and repair

g. Gloves

(1) Care

(2) Leaks

(3) Replacement and/or repair

h. Air Filter

(1) Preparation and source

(2) Sterilization

(3) Care and repair

i. Material preparation and storage

(1) Solid

(2) Liquid

(3) Diet

j. Material Sterilization

(1) Solid

(2) Liquid

(3) Diet

k. Sterile transfer

(1) Animals

(2) Material

l. Sterility testing routine

(1) Isolator

(2) Gloves

(3) Cages

(4) Diet

(5) Animal and Wastes

(6) Air

(7) Water

m. Monitoring of Sterility

(1) Viable culture

(2) Chemical

(3) Sensitive tape

n. Animal Handling

o. Records

(1) Labeling

(2) Animal marking

(3) Time and performance records

p. Microbiology

q. References

r. Persons and work list

APPENDIX L

PRELIMINARY EXPERIMENT, FOOD EFFICIENCY

APPENDIX L

A COMPARISON OF GROWTH RATES AND FERTILITY OF CONVENTIONAL AND GERMFREE MICE MAINTAINED UNDER GNOTOBIOTIC ISOLATION*

H. I. Kaplan,**, M. H. Bengson,** and T. D. Luckey***

INTRODUCTION

A study was performed to obtain baseline data in mice for use in evaluating the overall efficiency of diets under different gnotobiotic conditions. One parameter to be determined was the food efficiency based on weight gain per weight of food utilized over a given time. Food Efficiency = grams gained/ grams food utilized (Figure L-1). Another parameter was the fertility of the animals on the diet.

Newton (1966) studied the effect of environment on labor in parturient mice and found that births are somewhat affected. This effect was primarily a slowing of the delivery rate, but not necessarily accompanied by a reduction in the percentage of pregnancies in a population, nor a change in the average litter size.

Mirone (1953) studied the effect of vitamin B_{12} and cobalt chloride on growth and reproduction on four strains of mice. She found addition or subtraction of B_{12} and cobalt chloride afforded no significant weight gains over her controls, nor any great differences in the size of the litters. The weaning rates in both test and control groups were equivalent. This finding was believed to be due to possible storage of a growth factor passed from the parents who were reared on a stock diet. In a subsequent study, Mirone (1954) reported that dba, C_{57}, GF1 and C3H strains of mice on choline deficient diets were not changed as to

*This study was done under NASA Contract NAS 9-9000.
**Biosciences Operation, Space Systems Organization, General Electric Company, King of Prussia, Pennsylvania.
**Department of Biochemistry, University of Missouri, Columbia, Missouri.

FIGURE L-1

FOOD EFFICIENCY = GRAMS GAINED/GRAMS FOOD UTILIZED

their growth rates, nor did anemia develop, as in other studies on pigs and rats. The mice did, however, have a lower conception rate as compared with the control mice. Also found was a high incidence of maternal death due to profuse bleeding at parturition. These deaths were accompanied by incomplete expulsion of the fetus which Mirone attributed to the loss of contractility in the choline deficient mice.

These studies were conducted on conventional mice only. Tennant (1968) in a starvation study utilizing both conventional and germfree mice, found that conventional mice survive total starvation longer then germfree mice of the same age, sex and strain. He also concluded that E. coli gnotophoric mice showed conflicting results in that one group with total food withdrawal outlived the germfree controls. Two other E. coli gnotophoric groups, one receiving thiamine and the other water, succumbed faster then the germfree controls.

Baker (1966) did a large scale study somewhat similar to ours, and the results will be discussed as comparative data later in this report.

The experimental design of our study was as follows: eighty CRL-CD-1 (HdM/ICR Swiss) mice, of which 20 were germfree, were obtained from Charles River Breeding Laboratories.

At 22 days, they were distributed into four groups of 20 each. The germfree group of twenty and twenty conventional mice were placed into separate germfree isolators. All groups were housed in four cages each with two males and three females. The other 40 mice were sub-divided into two groups in like manner but kept in an open colony.

The germfree group and the conventional group under gnotobiotic isolation designated as Group A and B respectively were fed Purina(R) Lab Chow 5010C Autoclavable which was sterilized by autoclaving 20 minutes at 121°C in sealed

ATI(R) steriline syringe bags inside a standard autoclave. The adequacy of the sterilization process and sterility of the food was monitored by use of at least three spore strips of 5×10^6 Bacillus stearothermophilus in each drum and also by placing aliquots of the food into thioglycollate broth medium. The spore strips were incubated in Trypticase soy broth at 55°C. Replicate cultures of the food samples were incubated at 28 ±3°, 35° and 55°C for 7 days.

The other conventional animals in open colony, Group C, was fed the sterilized diet which was handled in the same manner as the food for Groups A and B. Group D received the same diet untreated. Figure L-2 illustrates the groupings.

All animals received ad libitum, sterile deionized water having a conductivity after sterilizing of about 0.205 megohms at 23°C as determined on a Barnstead(R) purity meter model FM-4.

The mice were weighed initially, at age 22 days upon distribution into groups (Table L-I), and after a ten day period of acclimation, the food efficiency study was begun. At age 31 days, Group D was reduced to 19 mice as one female was in ill health and was discarded.

The mice were placed in clean cages equipped with 5/16 inch mesh screen floors approximately 1 cm above the cage bottom to limit coprophagy. All food offered each group was weighed to the nearest tenth of a gram before addition to the food hoppers on the cages. At the end of four days, the remaining food in each cage was weighed and recorded.

The mice were weighed at the beginning and end of this period. The waste under the screens was weighed and an estimate of the food in the fecal residue recovered made.

The average of each total group (Table L-II) was then determined.

FIGURE L-2

GROUP	TREATMENT CRL:CD-1 (HdM/ICR SWISS) MICE
A	GERMFREE - STERILE DIET - STERILE ISOLATION
B	CONVENTIONAL - STERILE DIET - STERILE ISOLATION
C	CONVENTIONAL - STERILE DIET - NON-STERILE ISOLATION
D	CONVENTIONAL - NON-STERILE DIET - NON-STERILE ISOLATION

TABLE L-I

INITIAL WEIGHTS IN GRAMS

GROUP	CAGE	SEX					AVERAGE WEIGHTS	
		MALE	MALE	FEMALE	FEMALE	FEMALE	CAGE	GROUP
A*	1	7.8	8.0	10.3	5.8	6.4	7.7	8.4
	2	9.4	10.6	8.8	7.8	8.5	9.0	
	3	7.5	9.5	9.4	10.3	6.1	8.6	
	4	8.3	8.0	8.3	7.3	9.4	8.3	
B**	1	11.4	13.6	10.2	15.4	14.1	12.9	13.7
	2	12.5	13.5	13.2	12.7	16.2	15.6	
	3	12.4	14.3	13.0	15.7	12.4	13.6	
	4	13.3	13.0	13.7	12.6	12.0	12.9	
C***	1	14.0	14.5	15.5	13.3	14.3	14.3	13.9
	2	16.9	15.5	12.4	14.0	13.4	14.4	
	3	12.6	12.3	13.0	12.4	13.4	12.7	
	4	15.7	14.0	13.9	14.1	12.6	14.1	
D****	1	16.4	17.4	13.4	17.2	12.7	15.4	14.0
	2	13.2	13.1	12.7	13.6	13.1	13.1	
	3	14.6	15.4	11.5	13.5	12.5	13.5	
	4	12.7	15.1	13.1	15.6	*****	14.1	

*A = Germfree
**B = Conventional Mice, Sterile Diet, Sterile Isolation
***C = Conventional Mice, Sterile Diet, Non-Sterile Isolation
****D = Conventional Mice, Non-Sterile Diet, Non-Sterile Isolation
*****Mouse ill - discarded

TABLE L-II

MICE FOOD EFFICIENCY SUMMARY - FOUR DAYS (WEEKS 4-5)

CAGE	GROUP	BODY WEIGHT (GMS)			FOOD (GMS)				EFFICIENCY	
		START	END	CHANGE	START	END	WASTE	USED	GM GAIN/GM FOOD x 100	AVERAGE
11 (A)	A	60.4	76.1	15.7	99.8	40.0	6.0	53.8	29.2	23.2
	B	83.4	100.6	17.2	173.9	77.1	16.2	80.6	21.4	
	C	92.0	106.2	14.2	102.2	13.6	13.6	75.0	19.0	
	D	84.1	55.4*	----	94.4	73.4	7.2	3.8	----	
12 (B)	A	108.3	120.3	12.0	128.5	43.7	8***	76.8	15.6	13.9
	B	108.3	105.6	2.7**	177.6	82.0	8	87.6	----	
	C	108.8	121.7	12.9	172.6	72.3	8	92.3	14.0	
	D	116.2	126.7	10.5	157.9	63.8	8	86.1	12.1	
13 (C)	A	111.8	134.1	22.3	199.2	92.4	7.4	99.4	22.4	17.7
	B	111.4	128.8	17.4	160.7	54.9	10.4	95.4	18.2	
	C	103.1	118.9	15.8	183.5	89.4	7.2	86.9	18.2	
	D	119.6	130.5	10.9	168.5	69.2	8.1	91.2	12.0	
14 (D)	A	123.2	130.8	7.6	129.6	34.0	8.7	86.9	8.7	9.3
	B	119.8	127.5	7.7	111.7	16.4	7.0	88.3	8.7	
	C	115.9	122.3	6.4	120.0	21.6	9.2	89.2	7.2	
	D	95.5	104.1	8.6	121.7	46.6	6.0	69.1	12.4	

*Water Not Used.

**Water Not Available??

***Average Food Wasted in Groups 12 and 14 is 8.0 gm.

11 = Germ-Free

12 = Classic Mice, Maintained as Germ-Free

13 = Classic Mice, Normal Air, Sterile Food and Water

14 = Classic Mice, Normal Air, Non-Sterile Food and Sterile Water

Each cage contains 3 females and 2 Males

Age of Mice - 4 Weeks at Start, ICR Strain White

Source - Foster Charles River

From Table L-II, it can be seen that Group D, consisting of conventional mice fed the non-sterile diet but sterile water in a conventional exposed cage, had the lowest average efficiency. Group B, the conventional mice reared on sterile diet under gnotobiotic conditions, utilized their food better. Group C, the conventional mice on the sterile diet exposed to open laboratory conditions, had a greater increase in food efficiency and Group A, consisting of the germ-free mice under germfree isolation on the sterile diet, showed the greatest increase in food efficiency.

There is, in the case of Group A, a significant difference in the average initial weight of the mice (Table L-I) which must be considered in the interpretation of the results. It is possible that the germfree mice gain weight at a more rapid rate regardless of initial weight. Baker (1968) reports this in a study for the National Cancer Institute in which he used a total of 327 CFW mice in the same age range as our mice. The average starting weight of the axenic mice in Baker's study was 12 grams compared to 8 and 9 grams for the conventional mice receiving autoclaved and non-autoclaved diet respectively. Baker's conventional mice were all reared in open colony. This finding of higher initial weights for like-aged axenic and conventional mice is unusual. We, and others, have found that axenic animals generally are of lighter weight than conventional counterparts, at least with regard to the strain used in this experiment.

The effect of isolation on fertility in this study presents an interesting phenomenan.

The mice in Groups A, C and D show no significant differences (Table L-III). Group B had only two litters opposed to six litters for Group A, five litters for Group C and seven litters for Group D.

TABLE L-III

TOTAL LITTERS

GROUP	TOTAL MALES	TOTAL FEMALES	TOTAL LITTERS
A	8	12	6
B	8	12	2
C	8	12	5
D	8	11	7

The germfree mice were not adversely affected, and the diet did not deter pregnancies in this group nor in the two conventional control groups. The isolated conventional group had only two pregnancies throughout the experiment even though this was an equal opportunity experiment. Though Newton (1966) in his study showed some delay could be expected in delivery when mice were transferred from a familiar cage to a second cage of different design, our mice were all housed in similar cages throughout, and the isolation of the mice into discrete groups occurred just past weaning.

One possible cause for the reduced number of pregnancies might be a shifting of microflora which could cause an imbalance in the isolated group. This, however, is highly speculative in as much as such shifts were not measured in this study. It was noted that food efficiency measurements made before microbial shifts would be expected showed the isolated group doing better than the non-isolated group receiving the non-sterile diet, but poorer than the other two groups. The Baker study did not consider conventional mice under gnotophoric isolation so the reason for this phenomenum must be determined in subsequent studies with larger samplings.

SUMMARY

In this limited experiment, axenic mice fed a sterilized commercial diet evidenced the greatest food efficiency. This difference was also evident when calculated as weight gain divided by food accepted. The mice were of equal ages when the experiment began, but the axenic mice were substantially smaller than their conventional counterparts. The results given may have been influenced by the disparity in initial size between the groups. Results of differing environmental conditions and sterilization of diet indicate further work should be carried out to determine the effect of initial weight on food efficiency.

REFERENCES

1. Baker, D. E. J.: Methods and Equipment for Gnotobiotics - Final Report. National Cancer Institute Contract PH 43-62-461, Section 12, 1966.

2. Mirone, Leonora and Wade, E. M.: Vitamin B_{12} and Cobalt Chloride in growth and reproduction of four strains of mice. Am. J. Physiol. 175:11-12, 1953.

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4. Newton, N. et al.: Parturient mice: Effect of environment on labor. Science 151(3717):1560, 1966.

5. Tennant, B., Malm, O. J., Horowitz, R. E. and S. M. Levenson: Response of germfree, conventional, conventionalized and E. coli monocontaminated mice to starvation. J. Nutr., Vol. 94:151-160, 1968.

APPENDIX N

OTHER PROGRAM RESULTS

APPENDIX N

In addition to the accomplishment of the primary technical purposes of these experiments, a number of other benefits have been achieved.

(1) Three technical papers have thus far been generated.

(a) Kaplan, H. I., M. H. Bengson, and T. D. Luckey. A comparison of growth rates and fertility of conventional and germfree mice maintained under gnotobiotic isolation. Presented at American Association for Laboratory Animal Science, 20th Annual Technical Meeting, Dallas, Texas, October 14, 1969.

(b) Luckey, T. D., M. Smith, H. Kaplan, and M. H. Bengson. Gnotobiotic evaluation of an Apollo diet. To be presented at X International Congress for Microbiology, Mexico City, Mexico, August, 1970.

(c) Bengson, M. H., J. K. Ferguson, J. A. Geating, and J. McQueen. Changes in indigenous microflora during bio-isolation simulating long term space flight. To be presented at X International Congress of Microbiology, Mexico City, Mexico, August, 1970.

At least three more are due in the planning stage for publication in 1970.

(2) An International Symposium has been organized at the University of Missouri. This Symposium, "Ecology of the Intestinal Flora in a Changing Environment", will bring together some of the outstanding authorities in microbial ecology. Some of the problems discovered during the research will be given the attention of this group. The program and participants are given in Figures N-1 and N-2.

(3) The opportunity for combined government-industry-university research on a daily intimate working basis afforded by Professor T. D. Luckey's Sabbatical Leave spent in the General Electric Laboratories has proved of special benefit to all concerned.

The experience gained by the opportunities afforded Dr. Luckey to observe and actively participate in the industrial requirements and approach to the technical problems encountered will certainly be passed down to his

FIGURE N-1

ECOLOGY OF THE INTESTINAL FLORA IN A CHANGING ENVIRONMENT

First International Symposium
Presented by:

The University of Missouri-Columbia School of Medicine and Extension Division with the cooperation of the School of Veterinary Medicine, The Space Sciences Research Center and the Graduate School and held in connection with the Spring meeting of the Missouri Branch of the American Society for Microbiology.

MEDICAL CENTER AUDITORIUM

MARCH 30-31, 1970

Monday, March 30

A.M.

8:15 Registration and Coffee

8:45 Welcome -- Dean Kingrey

NORMAL FLORA
Moderator -- Rolf Freter

8:50 Introduction -- Don Luckey

9:00 Human Normal and Abnormal Flora
Helmut Haenel

9:30 Fecal Flora of Man -- Lorraine Gall

9:50 Coffee Break

10:00 Pathogen-Normal Flora Interactions
Dave Hentges

10:20 Rumen Microbes -- Marv Bryant

10:50 Discussion

*12:00 Lunch and Tour -- Space Sciences Research Center -- John McKenna

P.M. EFFECT OF ANTIBIOTICS AND DIET
Moderator -- Herb Goldberg

2:00 Effect of Antibiotic Therapy
Sydney Finegold

2:30 Ecologic Consequences of Resistance Transfer Factors -- Sidney Cohen

3:00 Coffee Break

3:15 Antibiotics Influence Microflora and Drug Resistance in Domestic Animals
Williams Smith

3:45 Human Fecal Flora Under Controlled Diet Intake -- Stan Speck

4:05 Discussion

Tuesday, March 31

A.M.

8:50 Welcome -- Dean Bloomfield

ACTIVITIES OF MICRO FLORA
Moderator -- Russ Schaedler

9:00 Metazoa-Protozoa-Bacteria Interrelationships
Dick Wescott

9:20 Bacteria-Mucosa Interactions --Dwane Savage

9:50 Coffee Break

10:10 Energy Metabolism in Anaerobes
Lee Baldwin

10:40 Metabolic Contributions of the Cecal Flora
Richard McBee

11:00 Discussion

12:00 Lunch (on your own)

P.M. EFFECT OF ISOLATION
Moderator -- Jim McQueen

1:30 Changes During Hibernation -- Ella Barnes

2:00 Effect of Bioisolation -- Bang Bengson

2:20 Coffee Break

2:35 Gnotobiology as Ecology -- Don Luckey

2:50 Discussion

3:20 Summary and Perspective
Moderator -- Bill McCulloch with Rolf Freter, Herb Goldberg, Russ Schaedler, Jim McQueen, and Frank Engley

"May there never develop in me the notion that my education is complete but give me the strength and leisure and zeal continually to enlarge my knowledge".

--Maimonides

MONDAY EVENING

6:00 P.M. - RAMADA INN - Social Hour - Dinner Meeting
Welcome: Bob Schiffman - Collegium Musicum: Andy Minor
"Women in Space": Dick Lawton

FIGURE N-2

PARTICIPANTS

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SOME FUTURE CONFERENCES

April 15-16	Urology Seminar (Kansas City)
April 27-May 1	Pediatric Radiology (Kansas City)
May 6-7	Relationships of Rehabilitation to Disability Determination
May 13-14	Spring Clinical-Conference

students. In return, the insights, experience, and dispassionate viewpoints of the professional scholar greatly affected the conduct of our work and broadened the outlook of our entire staff. The "Visiting Scientist" concept is useful. We would welcome opportunities to again participate in such a joint project. The NASA's gain while directly measurable in dollars is hopefully best shown by the quantity and quality of the results as presented in this report.

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