

## STUDY REPORTS

9. Title: Nutritional and Health Requirements for Development and Maintenance of Conventional Animal Colonies.

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### OBJECTIVE

The objective of this study is to produce and maintain healthy conventional laboratory animals for the support of investigative programs and to establish procedures and breeding standards for optimum production and animal health within the colonies.

### DESCRIPTION

Surveys have been instituted on all rodent production colonies to determine the incidence and extent of parasitic infestations, latent virus infections, bacterial infections, and presence of other pathological conditions which may affect the outcome of investigations utilizing these animals as biological models. The information obtained from this disease screening program is used to continually evaluate the quality of animals bred in the laboratory and assure their uniformity. Procedures are employed to insure that breeding stock is systematically randomized and selected for increased litter size, growth, and weaning rates. All animals purchased for investigative work are examined at the time of purchase and all subhuman primates are quarantined and undergo a standardized conditioning program prior to issue to investigators. Individual clinical records are maintained on all subhuman primates and necropsies are performed on all animals that die within the colonies to determine the cause of death and to discover spontaneous abnormalities.

Those animals not in production colonies are purchased locally and consist mostly of the following primates: gibbons (Hylobates lar), rhesus monkeys (Macaca mulatta), cynomolgus monkeys (Macaca fascicularis), and pig tailed macaques, (Macaca nemestrina). The remainder of the animals housed consist of rabbits, sheep and goats, several types of poultry, dogs, cats, bandicoots, and tree shrews. All animals are observed daily by technicians and veterinarians to detect possible problems.

### PROGRESS

Primates. The primates continue to receive the highest amount of professional attention of any of the laboratory animals, chiefly because of the intensive quarantine and preventive medicine program they are subjected to.

Special problems were encountered during June, 1968, with young gibbons which had been purchased for dengue virus studies. Six out of twelve animals purchased died acutely over a three week period. Necropsies on each of the animals showed varying degrees of pneumonia and enteritis. The remainder of the animals were closely watched and treated twice daily with terramycin for two weeks and no further deaths occurred. Ten primates, all gibbons, were necropsied during August. Most died spontaneously but several were euthanized because pathological conditions precluded their use for further research work. Three animals died from malignant lymphomas; these cases are described in the Gibbon Leukemia Study included in this section. Infections of the respiratory system, particularly bronchopneumonia, continued to be the most serious problem in the colony. Five gibbons out of the ten that died had respiratory infections, although in two animals the findings were incidental.

One male was removed from Koh Kled Kaeo island after finding it had an enlarged abdomen. When an exploratory laparotomy was performed a massive liver which contained multiple abscesses was found to be the cause of swelling. The animal was sacrificed and the pathological findings showed that the liver contained both amoebic abscesses and hydatid cysts. The lungs and brain also contained cysts of Cystercercus cellulosae, the larval form of the human tapeworm Taenia solium.

Two deaths in gibbons resulted from infections with Enterobacter cloacae. These cases and the subsequent follow up work is described in the "Enterobacter cloacae Infections in Gibbons" study included in this report.

The most troublesome clinical problem among gibbons is dermatitis caused by Microsporum canis. As many as 15 animals have cases of ringworm that require constant attention. Griseofulvin has some beneficial effect on the course of infection. However, resistance seems to develop and levels of this antibiotic that were high enough to cause blindness in one animal are not sufficient to effect a cure. The troublesome nature of this problem brought about the study on "Dermatomycoses in Gibbons" which is included in this report. Additional information is included in Table VIII.

Rodents. A disease screening program was developed in July, 1968, and commenced in August. The program involves post mortem, bacteriological, virological, and parasitological examinations for animals randomly sampled from one of the four production colonies each week. This program has had the immediate effect of systematically identifying diseases endemic to the colony as well as indirectly improving animal quality through early detection of newly introduced diseases.

Mice. The most significant finding in the mouse colony resulted directly from the disease screening program. Endemic murine pneumonia was discovered in approximately five percent of the female breeders. This disease does not seem to resemble any of the described respiratory diseases of the mouse. Grossly, the large portions of the lungs are reddish gray and consolidated. Ordinarily, one to two lobes are involved; but, frequently it is difficult to find any normal tissue. Severe acute pneumonia and chronic peribronchiolitis are consistently diagnosed from sections of the lungs submitted and are reported to closely resemble lesions caused by Pneumocystis carinii in pneumonias of children. A more detailed treatment of this disease is given in the study on "Mouse Pneumonia" which is included in this report. Other findings are summarized in Table 1.

Rats. Chronic murine pneumonia was found to exist in the rat colony as it does in most conventional rat colonies. The most significant finding was the presence of the larval, tapeworm, Cystercercus fasciolaris in the liver of 6% of the rats necropsied. Since this tapeworm is only transmitted to rats when ova passed in the feces of infected cats are ingested, this finding illustrates a problem that can result from using unsterilized bedding. Other findings are summarized in Table 2.

Hamsters. The most significant finding in the hamster colony occurred during July and September when Salmonella bovismorbificans was found. Although production figures or pathological findings do not suggest an adverse effect on production by this infection, it is well known that Salmonella infections are activated by various environmental and induced stresses and are therefore extremely undesirable to have in a colony of laboratory animals. This finding justifies the need for developing a new colony of hamsters.

Twenty five pregnant hamsters were purchased from Con-Olson Co. of Wisconsin to replenish the Salmonella infected colony. However, during shipment, or upon arrival in December, 1968, the hamsters developed ileitis believed to be caused by Proteus mirabilis. This organism destroyed 75% of the weanlings produced by the new breeders. Research is presently underway concerning the pathogenicity of the Proteus mirabilis organism. A separate study on Hamster Ileitis is included in this report. Additional findings are summarized in Table 3.

Guinea Pigs. Guinea pig production was reduced from 44 litters in April, 1968, to 8 litters in June, 1968. This drop in production was due to a Salmonella weltevreden epizootic. A new colony of 75 pregnant guinea pigs arrived in January to replace the old colony. Additional findings are summarized in Table 4.

Colony Operations: Production statistics in each of the nine breeding colonies are shown in Tables 5, 6 and 7. Although guinea pigs and hamster production was depressed due to Salmonella infections, the colony still exceeded 1967's animal production by 8.5%.

Since the disease screening program showed that Salmonella exists in the guinea pig and hamster colonies, these colonies are in the process of being replaced with Salmonella free breeding stock imported from the States.

A shipment of guinea pigs from Walter Reed Army Institute of Research arrived in February and will be of sufficient size to entirely meet the experimental needs by August, 1969.

Because of the high incidence of murine pneumonia, the rat colony is being replaced with Sprague Dawley and inbred Fischer stock from the U.S. Naval Radiological Defense Laboratory in San Francisco whose colony is free from this widespread disease.

The opening of the 3rd floor of the new laboratory building made it possible to provide isolated holding rooms for each of the imported species where they may remain temporarily isolated and conditioned to their new environment.

In response to needs of the Department of Parasitology, four strains of inbred mice, consisting of three breeding pairs each, were procured from Jackson Laboratory. These strains will serve as a nucleus for a BALB/C, C57BL/6J, HRS/J, and HG/Hu inbred mouse colony. The latter two strains carry a recessive mutant for hairlessness, a characteristic that may make their adaptation as breeders in Thailand more successful. The BALB/CJ and C57BL/6J are very popular and prolific strains. In addition to having these two strains available for experimental work, it is hoped that the hybrid stock resulting from cross breeding these strains will be useful as a vigorous isogenic research animal.

Table 1  
ANNUAL MOUSE PRODUCTION

Month	Litter Born	Number Born	Average Litter Size	% Conception Rate
April 1968	2,915	26,882	9.22	81.69
May 1968	3,143	28,487	9.06	80.86
June 1968	2,427	21,824	9.00	76.55
July 1968	2,479	22,318	9.00	79.91
August 1968	2,801	25,980	9.27	78.55
Sept 1968	2,306	21,885	9.49	79.94
Oct 1968	2,712	26,501	9.77	81.30
Nov 1968	2,461	24,241	9.85	82.43
Dec 1968	2,501	25,793	9.91	82.68
Jan 1969	2,626	26,836	10.21	81.06
Feb 1969	2,508	25,693	10.24	84.46
Mar 1969	2,826	26,988	9.54	83.38
Total	31,705	302,418	9.56	81.7

Table 2  
ANIMALS BORN IN HAMSTER AND GUINEA PIG COLONIES

Animals	Number of Litters	Number of Animals	Average/Litter
HAMSTERS	2,258	16,226	7.92
GUINEA PIGS	189	645	3.41

Table 4  
ANIMALS ISSUED

Animals	Produced			Purchased		
	Suckling	Juvenile	Adult	Suckling	Juvenile	Adult
Mice	84,344	65,894	10,221			
Rats	382	3,319	540			
Hamsters		7,986	557			
Guinea Pigs		535	129		1,905	
Rabbits				54		531
Monkeys					5	30
Poultry					874	41
Cats						22
Tree shrews						40
Squirrel						21
Gibbons					10	

Total animals issued: 176,442  
 Animal blood issued: 85,230 cc  
 Embryonated eggs issued: 9,735

Table 5  
Animals Born in Rodent Colonies

Animals	Number of Litters	Number of Animals	Average/Litter
Mice	31,705	302,418	9.6
Rats	2,543	15,551	10.2
Hamsters	2,258	16,226	7.92
Guinea Pigs	189	645	3.41
Total	34,695	335,886	7.8

Table 6

Production figures for C57BL/6J, HRS/J, HG/Hu, BALB/C Inbred Mice  
 Fischer and Rando bred Sprague Dawley rats,  
 and Walter Reed Rando bred Guinea Pigs  
 (Imported Nucleus Colonies)

Animals	No. of litters	No. of animals	Average/litter	Conception rate
Mice C57BL/6J	6	35	6.4	100%
HG/Hu	3	21	6	100%
HRS/J	4	19	4	100%
BALB/C Inbred	1	6	2	20%
Fischer Rats Inbred	7	45	6.7	75%
Guinea Pigs Rando bred	70	299	3	95%
Sprague Dawley Rats Rando bred	20	192	7.5	100%

Table 7 (Cont.)

Animal Number	Mode of Death (sacrificed, etc.)	Most Significant Primary Lesions (organ and brief description)	Secondary Lesions (by organ)	Significant Lab or Clinical Findings	Diagnosis
S-68	Sacrificed	<u>Liver</u> : numerous focal areas of small mononuclear cell infiltrates around the small bile ducts. <u>Lung</u> : a few petechial hemorrhages. <u>Mesentery</u> : Caseous nodules are degenerating parasites surrounded by foreign body granulomatous reaction.			TB reactor
S-75	Sacrificed		<u>Brain</u> : diffuse, mild encephalitis. <u>Intestine</u> : diffuse, mild enteritis. <u>Lung</u> : mild, interstitial pneumonitis.		Sacrificed following Atlatoxin fungus suspension feeding.
S-77	Sacrificed	<u>Lung</u> : alveolar walls filled with masses of adult and immature lymphocytes. <u>Kidney</u> : interstitial infiltration of lymphocytes. <u>Liver</u> : capsule, sinusoids & vessels are infiltrated with lymphocytes with islands of the cells in the periportal areas. <u>Brain</u> : vessels of meninges, cerebrum & cerebellum filled with lymphocytes.			Sacrificed due to severe generalized malignant lymphoma.
S-8	Sacrificed				inoculated with Dengue suspensions.

Table 7 (Cont.)

Animal Number	Mode of Death (sacrificed, etc.)	Most Significant Primary Lesions (organ and brief description)	Secondary Lesions (by organ)	Significant Lab or Clinical Findings	Diagnosis
S-13	Sacrificed				inoculated with Dengue suspen-sions
B-31S	Natural Death	Lung: discolored, congested. Heart: right auricle swollen with chicken fat clot adherent to endocardium. Lymph glands: enlarged, reddened. Bone marrow: considerably reddened.			Acute Malaria with interstitial nephritis and hepatitis.
B-60	Natural Death	Liver: yellow, swollen Spleen: yellow, swollen Lung: congestion, focal hemorrhage. Intestine: hemorrhage submucosal		Enterobacter cloacae (Experimentally induced)	septic thrombo-embolic encephalitis.
S-7	Natural	Lung: lobes dark red to black; adhesions between lung and parietal pleura. Liver: fatty, enlarged	Intestine: numerous adhesions between the intestine and abdominal wall		Hemorrhagic verminous Pneumonia, <u>Strongyloides</u> sp. <u>Strongyloidosis</u> intestine.
S-17	Natural Death	Lung: adhered to sternum, Liver: spotted and soft. Kidney: yellowish, tough.			Malignant lymphoma.

Table 7 (Cont.)

Animal Number	Mode of Death (sacrificed, etc.)	Most Significant Primary Lesions (organ and brief description)	Secondary Lesions (by organ)	Significant Lab or Clinical Findings	Diagnosis
S-72	Natural	<p><u>Liver</u>: yellow, swollen</p> <p><u>Lung</u>: hemorrhages in the omentum, focal areas of pneumonia.</p>		<p><u>Enterobacter cloacae</u> (Experimentally induced)</p>	Septicemia. ✓
S-91	Natural	<p><u>Liver</u>: yellowish cast</p> <p><u>Lymph nodes</u>: swollen, discolored.</p> <p><u>Pancreas</u>: pancreatic hemorrhage.</p> <p><u>Lungs</u>: mottled with red to black focal lesions.</p>			Septicemia pneumonia interstitial nephritis.

Summary of Deaths Occurring in the Monkey Colony from April 1968 — March 1969

Animal	Most Significant Lesion	Organs Affected	Secondary Findings	Pathological Diagnosis	No. of Animals Necropsied
Rhesus Monkey <u>Macaca mulatta</u>	Congestion, Necrosis Fatty degeneration	Liver	pneumonia nephritis	Hepatitis	4
	Fatty degeneration	Kidney Liver	Nephritis	Tuberculosis (unable to recover organism)	1
Cynomologous Monkey <u>Macaca fascicularis</u>	Congestion, discoloration	Lungs	Septicemia	Pneumonia	2
Pig-Tailed Macaque <u>Macaca nemestrina</u>	Parasitic lesions Enlarged mesentery	Stomach	Pneumonia	Verminous gastritis	1

Table 8.  
MICE  
Bacterial Flora (180 examined)

	<u>Intestines</u>	<u>Lungs</u>	<u>Liver</u>
<u>Proteus</u>	55%		1.3%
<u>Bacillus</u>		36%	
<u>E. coli</u>	54%	2.5%	1.3%
<u>Pseudomonas</u>	9.2%		
<u>alpha Streptococcus</u>		12.8%	1.3%
<u>Micrococcus</u>		6.3%	
<u>Staph. epidermidis</u>		10%	
<u>Diphtheroid</u>		3.8%	
<u>Paracolon</u>	1.3%		
<u>Providencia</u>	7.2%		
<u>E. Freundii</u>	1.3%		

Endoparasites (60 examined)

<u>Hymenolepis nana</u> (tapeworm)	18%
<u>Coccidia species</u>	7.3%
<u>Syphacia obvelata</u> (pinworm)	4.5%
<u>Strongyloides</u> (nematode)	2.3%

Ectoparasites (60 examined) none

Occurrence of Pathologic Lesions in Various Organs

Lung	1.6%
Intestine	3.2%
Liver	13.7%
Kidney	3.3%

Antibodies to Ectromelia and Polyoma Viruses

None

Table 9.  
GUINEA PIGS

<u>Bacterial Flora (60 examined)</u>		
	<u>Intestines</u>	<u>Lungs</u>
<u>Proteus</u>	35	
<u>Bacillus</u>		22.6%
<u>E. coli</u>	66%	
<u>Pseudomonas sp.</u>	18.3%	
<u>Alpha Streptococcus</u>		12.6%
<u>Staphylococcus epidermidis</u>	15%	10%
<u>Paracolon</u>	13.3%	
<u>Providencia</u>	10%	
<u>Aerobacter cloacae</u>	5.6%	
<u>A. aerogenoides</u>	4.3%	
<u>Salmonella tananarine</u>	3.3%	
<u>Endoparasites—Negative</u>		
<u>Ectoparasites</u>		
Lice	100% (presently unidentified)	
<u>Occurrence of Lesions in Various Organs</u>		
Lungs	31%	
Liver	22%	
Ovary	6.6%	

Table 10.  
HAMSTERS

<u>Bacterial Flora (135 examined)</u>		
	<u>Intestines</u>	<u>Lung</u>
<u>Proteus</u> sp.	61%	9%
<u>Bacillus</u> sp.	1.3%	
<u>E. coli</u>	8.6%	
<u>Pseudomonas</u> sp.	17.8%	
<u>alpha Streptococcus</u>		15.9%
<u>Staphylococcus epidermidis</u>		14.3%
<u>Paracolon</u>	7.7%	
<u>Providencia</u>	9.3%	
<u>A. aerogenoides</u>	.73%	
<u>Aerobacter</u> sp.	8%	
<u>Salmonella bovis</u> <u>morbificans</u>	4%	
<u>Endo—and Ectoparasites</u>		
Negative		
<u>Occurrence of Lesions in Various Organs</u>		
Lungs		11.3%
Liver		5.7%
Intestines		7.3%
Kidneys		1.3%

Table 11.  
RATS  
Bacterial Flora (135 examined)

	<u>Intestines</u>	<u>Lungs</u>
<u>Proteus</u>	55.5%	3.3%
<u>Bacillus</u>		7.7%
<u>E. Coli</u>	20%	.8%
<u>Pseudomonas</u>	10.8%	
<u>Streptococcus &amp;</u>	2.7%	12%
<u>Staphylococcus epidermidis</u>	1%	8%
<u>Providencia</u>	.7%	
<u>A. aerogenoides</u>	1.8%	

Endoparasites (45 examined)

<u>Hymenolepis nana</u> (tapeworm)	3.3%
<u>Cystercerus fasciolaris</u> (tapeworm)	6%
<u>Coccidiosis</u>	1.3%

Ectoparasites

None

Occurrence of Lesions in Various Organs

Lung	15%
Intestines	7.8%
Liver	5.5%
Adrenals	2.3%