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Final Report to the



National Aeronautics and Space Administration



Title of Project: Study of the Murine Viruses Present in "Germfree" Mice

For the Period: September 1, 1968 to June 30, 1970

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Contract Number: NAS 9-8641

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Synopsis

The NASA germfree mouse colony has been established and monitored for indigenous virus infection. Starting with a breeding nucleus and continuing through the $P_3$ generation to the expansion colony no evidence has been found for infection with the following viruses: pneumonia virus of mice, mouse pneumonitis, polyoma, Sendai, minute virus of mice, ectromelia, mouse adenovirus, mouse hepatitis, lymphocytic choriomeningitis, Riley, mouse salivary gland virus, thymic or epidemic diarrhea of infant mice. Some equivocal data has been obtained with regard to reovirus type 3, and Theiler's encephalomyelitis virus infection, however, it is likely that the mice are not infected with either of these agents. There is infection throughout the colony with mouse leukemia virus.

A virus profile analysis was performed on two groups of 150 germfree mice, each taken from a sample of 750 germfree mice used on the Apollo 11 and Apollo 12 missions.

## Introduction

The objective of the study was to characterize, identify, and determine the prevalence of indigenous murine viruses which might have been present in the "germfree" (gnotobiotic) mice used for the detection of extraterrestrial agents by the National Aeronautics and Space Administration (NASA), Lunar Receiving Laboratory (LRL).

The examples of occult murine viruses which interfere with research efforts are quite numerous. While no attempt will be made here to enumerate them, it is safe to say that these viruses have caused the invalidation of research projects valued at hundreds of thousands or perhaps millions of dollars. Indigenous viruses act in an insidious manner by contaminating a variety of biologic systems such as: tissue cultures, transplantable solid tumors, virus leukemias, and other virus reagents. After contaminating these systems the viruses are then free to cause a variety of secondary effects. These may be and most often are interpreted by the investigator as primary effects, and he is brought to the threshold of what may be, and often is, a very long, painful, and expensive misadventure in science.

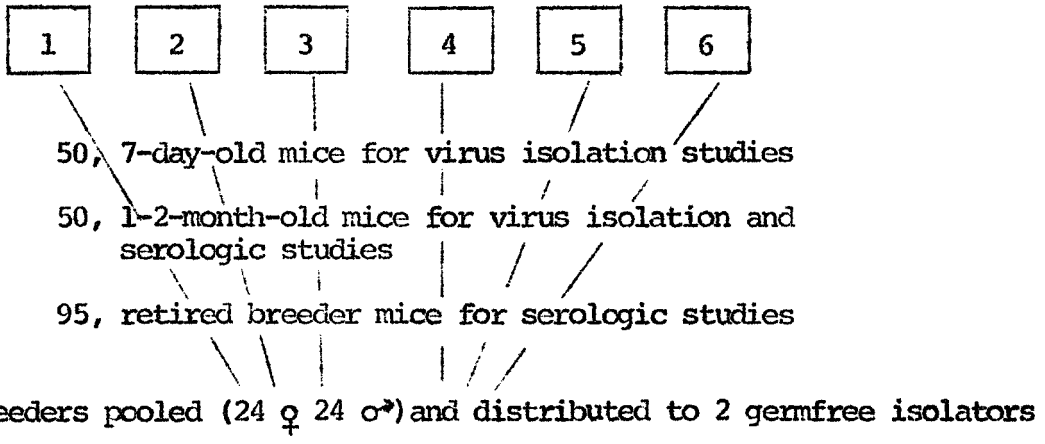
## Exhibit A

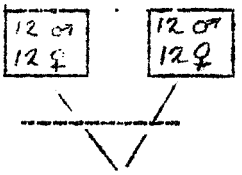
Table I outlines the steps leading up to the establishment of the NASA germfree mouse colony and indicates the tests which were carried out at each step. Table 2 and Table 3 detail the results of the virus monitoring which was carried out at each generation step. All germfree mice in exhibit A and exhibit B were tested for infection by the following viruses: pneumonia virus of mice (PVM), reovirus type 3 (Reo 3), Theiler's encephalomyelitis (strain GDVII), mouse pneumonitis (K), polyoma, Sendai, minute virus of mice (MVM), ectromelia, mouse adenovirus (M.Ad.), mouse hepatitis virus (MHV), lymphocytic

Table I

Summary of Virus Monitoring of the NASA Germfree Mouse Colony at Charles River Breeding Laboratories

1. Baseline Study - selection of breeders for nucleus colony - mice for initial testing sampled at random from each isolator




2. Establishment of NASA Colony

$P_0$ generation - 48 breeders for serologic studies

$P_1$ generation - 48 7-day-old sucklings for virus isolations
48 7-week-old mice for virus isolations and serologic studies
48 breeder mice for serologic studies

$P_2$ generation - 48 7-day-old sucklings for virus isolation
48 6-week-old mice for virus isolations and serologic studies
48 breeder mice for serologic studies

3. Expansion Colony at the 3rd generation -

58 sucklings for virus isolations
100 3-to 6-week-old mice for virus isolations and serologic studies
60 9-week-old mice for virus isolations and serologic studies

Table 2

Virus Serologic Testing of Germfree Mice From the NASA Germfree Mouse Colony

| | Serologic Test | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hemagglutination-Inhibition | | | | | | | | Complement Fixation | | |
| | PVM | Reo3° (RDE Rx) | GDVII° | K | Polyoma | Sendai | MVM | Ectromelia | H.Ad. | MHV | LCM |
| Baseline Mice | | | | | | | | | | | |
| 1-2 mo. old | 0/50* | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 |
| breeders | 0/95 | 0/92 | 0/95 | 0/95 | 0/95 | 0/95 | 0/95 | 0/95 | 0/95 | 0/95 | 0/95 |
| $P_o$ Generation | | | | | | | | | | | |
| breeders | 0/48 | 0/46 | 1/41 | 0/48 | 0/48 | 0/48 | 0/48 | 0/39 | 0/48 | 0/48 | 0/48 |
| $P_1$ Generation | | | | | | | | | | | |
| 7-weeks-old | 0/48 | 1/45 | 1/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 |
| breeders | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 |
| $P_2$ Generation | | | | | | | | | | | |
| 6-weeks-old | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 | 0/48 |
| breeders | 0/48 | 0/48 | 0/48 | 0/47 | 0/48 | 0/48 | 0/48 | 0/31 | 0/48 | 0/47 | 0/46 |
| Expansion Colony | | | | | | | | | | | |
| 3-6 weeks-old | 0/64 | 0/57 | 0/64 | 0/64 | 0/57 | 0/57 | 0/57 | 0/40 | 0/64 | 0/64 | 0/57 |
| 9-weeks-old | 0/60 | 0/60 | 0/60 | 0/60 | 0/60 | 0/60 | 0/60 | 0/60 | 0/60 | 0/60 | 0/60 |

*No. of sera positive/No. of sera tested
°See text for discussion of the significance of inhibitors

Table 3

Testing of NASA Germfree Mice by Virus Isolation Techniques

| | Virus | | | | | |
|---|---|---|---|---|---|---|
| | MSGV | Thymic | LDH | Leukemia | LCM | EDIM |
| Baseline Mice | | | | | | |
| 1-2 month old | 0/10* | 0/10 | 0/10 | 6/9 | 0/10 | |
| sucklings | | | 0/10 | 1/10 | 0/10 | 0/10 |
| $P_1$ Generation | | | | | | |
| 7-weeks-old | 0/10 | 0/10 | 0/10 | 10/10 | 0/10 | |
| sucklings | | | 0/10 | 2/10 | 0/10 | 0/10 |
| $P_2$ Generation | | | | | | |
| 6-weeks-old | 0/10 | 0/10 | 0/10 | 9/10 | 0/10 | |
| sucklings | | | 0/10 | 1/10 | 0/10 | 0/10 |
| Expansion Colony | | | | | | |
| sucklings | 0/6** | 0/12 | 0/12 | 10/12 | 0/12 | 0/12 |
| 3-6-weeks-old | 0/18 | 0/20 | 0/20 | 4/19 | 0/20 | 0/19 |
| 9-weeks-old | 0/8 | 0/12 | 0/12 | 12/12 | 0/12 | 0/12 |

*No. pools virus positive/No. pools tested: each pool consists of 5 mice
**10 mice per pool MSGV sucklings only

choriomeningitis (LCM), mouse salivary gland virus (MSGV), thymic, Riley (LDH), leukemia, and epidemic diarrhea of infant mice (EDIM). Serologic tests were used to detect antibody to 11 of the indigenous murine viruses: PVM, Reo3, GDVII, K, polyoma, Sendai, MVM, ectromelia, M.Ad., MHV, and LCM. The mice were individually bled at a 1:5 serum dilution and tested by either the hemagglutination-inhibition (HI) or complement fixation (CF) test. Virus isolation attempts were carried out using pools of tissue extracts prepared, in most cases, from 5 mice. The appropriate organ or organs were harvested and 10% extracts prepared. These tissue extracts were inoculated into tissue cultures or animals according to established protocols.

An agent causing necrosis of the thymus was isolated while carrying out the thymic virus protocol on a pool of $P_2$ generation 6-week-old mice. The gross and histologic pathology of the thymus of mice inoculated with the isolate virus was indistinguishable from mice infected with thymic agent and the growth curve of the agent in suckling mice was similar to thymic agent. However, a diarrhea noted in the mice inoculated with the isolate has not been previously observed associated with thymic virus infection. Studies were undertaken to determine whether the virus was infecting the NASA mice or the test mice. Attempts to reisolate the virus from the original mouth swab (a NASA mouse) material were not successful. Also no other isolation was made from approximately 100 mouth swab pools from 500 NASA axenic mice. Mouth swabs were taken from the test mice (NIH swiss mice), however no isolations were made. However since the virus could not be reisolated from the NASA mice and since the thymic agent is prevalent in conventionally reared mice it is likely that the virus was an accidental contaminant of the isolation system and is not present in the NASA germfree mouse stocks.

Although Reo 3 and GDVII hemagglutination inhibitors were present in the germfree mouse sera it is likely that they are nonviral in nature and represent nonspecific inhibitors. Extensive observation and testing of the inhibitor for Reo 3 revealed the following information: 1) The inhibitor was usually of low titer, was never associated with CF antibody and did not stain Reo 3 in the indirect immunofluorescent antibody test (Table 4). 2) In most instances the inhibitor, unlike antibody, was destroyed completely by chemical treatment with the enzyme RDE. 3) The epidemiology of the inhibitor was not typical of a reovirus infection. 4) Virus was never isolated from any of the NASA mice. 5) A similar nonspecific hemagglutination inhibitor is sometimes observed in other colonies of mice which are not infected with the virus. 6) If reovirus infection were present in the isolators the infection would have spread, clinical disease would have become apparent and the number of sero-positive animals would have increased; this did not occur.

During the course of the contract changes were made in several of the isolation techniques. The method used for leukemia virus isolations were changed from the COMUL to the XC test; a tissue culture cytopathic test developed by Drs. Hartley and Rowe, NIH. The XC test became the technique of choice for the following reasons: it requires only 14 days for completion compared to 42 days for the COMUL, it is a technically simpler test and is less espensive to perform than the COMUL, and the XC test is as sensitive or perhaps more sensitive than the COMUL test. Changes also were made in the source of mice utilized for several of the isolation techniques. When a spontaneous diarrhea of unknown etiology was found to be associated with National Laboratory Animal Company mice (NLW) the colony could not be used for EDIM isolations. Area 68, Charles River Breeding Laboratories mice were selected as the alternative source since they were sensitive to

Table 4

Properties of a Reovirus Type 3 Hemagglutination Inhibitor in the $P_2$ Generation Breeder Germfree Mouse Sera

| Serum No. | Serologic Test: Hemagglutination-Inhibition, Untreated[δ] | Hemagglutination-Inhibition, RDE* Treated | Complement Fixation | Fluorescent Antibody |
|---|---|---|---|---|
| 1 | 160** | -[+] | - | - |
| 2 | 80 | - | - | - |
| 3 | 80 | - | - | - |
| 4 | 80 | - | - | - |
| 5 | 80 | - | - | - |
| 6 | 80 | - | - | - |
| 7 | 40 | 20 | - | - |
| 8 | 40 | 20 | - | - |
| 9 | 40 | 20 | - | - |
| 10 | 40 | 20 | - | - |
| 11 | 40 | - | - | - |
| 12 | 40 | - | - | - |
| 13 | 40 | - | - | - |
| Initial Serum Dilution | 1:20 | 1:20 | 1:5 | 1:10 |

*Receptor Destroying Enzyme
[+]Negative at <1:20 serum dilution
**Reciprocal antibody titer
[δ] All sera were heated at 56°C for 30 min. prior to test

EDIM infection, were readily available, had been screened for their virus spectrum, and had been observed for several years without any evidence of diarrhea. An alternative source of mice to National Laboratory also was sought for use in LCM and Riley virus isolations. NIW mice often were not available in sufficient quantitites to fulfill contract obligations. The following colonies producing ICR and Swiss Webster mice were tested for LCM and Riley virus sensitivity; Charles River Breeding Laboratories (area 68), Microbiological Associates, Inc., Cumberland View Farms and Brookside Farm. The Charles River Breeding Laboratories, area 68, mice were chosen as the alternative source to National Laboratory.

Exhibit B

NASA's decision to advance the Apollo mission dates necessitated an addendum to the contract. The "new" germfree colony was not yet sufficiently established to release mice for use in the LRL vertebrate (mammalian) protocol for analysis of lunar samples brought back by Apollo 11 and Apollo 12. A decision was made to use mice from back-up germfree breeding isolators being maintained at CRBL for use in such a situation, however mice from these isolators had not been tested for virus profile. LRL received 600 weanling mice from the back-up isolators on July 1, 1969, and again on October 29, 1969. Use of these mice for testing lunar samples for extraterrestrial agents required that a virus profile analysis be completed on a group of 150 weanling mice randomly selected from the same isolators as the LRL sample. Preliminary tests were completed within one month on the same 16 indigenous murine viruses monitored for in the NASA germfree mouse colony analysis. The isolation and serologic techniques used were the same as for the NASA germfree colony. Results of the serologic tests are presented in Tables 5 and 6. All

Table 5

Virus Serologic Testing of 150 Apollo 11 Mice

| Germfree Isolator No. | No. of Sera Tested | Serologic Test | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hemagglutination-Inhibition | | | | | | | | Complement Fixation | | |
| | | PVM | Reo3 | GDVII | K | Polyoma | Sendai | MVM | Ectromelia | M.Ad. | MHV | LCM |
| 4A ♀ | 10 | -* | - | - | - | - | - | - | - | - | - | - |
| 6A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 7A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 11A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 18A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 28A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 29A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 30A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 33A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 2A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 8A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 9A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 25A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 27A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 34A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| Total | 150 | - | - | - | - | - | - | - | - | - | - | - |

*Negative

Table 6

Virus Serologic Testing of 150 Apollo 12 Mice

| Germfree Isolator No. | No. of Sera Tested | Serologic Test: Hemagglutination-Inhibition PVM | Reo3 | GDVII | K | Polyoma | Sendai | MVM | Ectromelia | Complement Fixation H.Ad. | MHV | LCM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18A ♀ | 10 | -* | - | - | - | - | - | - | - | - | - | - |
| 25A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 26A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 27A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 31A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 6A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 7A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 28A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 29A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 30A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 5A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 3A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 4A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 8A ♀ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| 14A ♂ | 10 | - | - | - | - | - | - | - | - | - | - | - |
| Total | 150 | - | - | - | - | - | - | - | - | - | - | - |

*Negative

sera were negative for antibody to the test viruses. The virus isolation results are presented in Tables 7 and 8. With the exception of mouse leukemia virus all virus isolation attempts were negative. Mouse leukemia was isolated from 15 of 30 Apollo 11 tissue extract specimens and 18 of 30 Apollo 12 tissue extract specimens.

Table 7

Attempts to Demonstrate Latent Virus in

a Sample of 150 Apollo 11 Mice

| Germfree Isolator No. | No. Mice Tested | Virus | | | | | |
|---|---|---|---|---|---|---|---|
| | | MSGV | Thymic | LDH | Leukemia | LCM | EDIM |
| 4A ♀ | 10 | -* | - | - | + | - | - |
| 6A ♀ | 10 | - | - | - | + | - | - |
| 7A ♂ | 10 | - | - | - | + | - | - |
| 11A ♂ | 10 | - | - | - | + | - | - |
| 18A ♂ | 10 | - | - | - | + | - | - |
| 28A ♂ | 10 | - | - | - | + | - | - |
| 29A ♀ | 10 | - | - | - | + | - | - |
| 30A ♀ | 10 | - | - | - | + | - | - |
| 33A ♂ | 10 | - | - | - | + | - | - |
| 2A ♀ | 10 | - | - | - | - | - | - |
| 8A ♂ | 10 | - | - | - | - | - | - |
| 9A ♂ | 10 | - | - | - | - | - | - |
| 25A ♀ | 10 | - | - | - | - | - | - |
| 27A ♀ | 10 | - | - | - | - | - | - |
| 34A ♂ | 10 | - | - | - | - | - | - |
| Total | 150 | - | - | - | 9** | - | - |

*Each negative is a test on 2 pools of 5 mice each
**Leukemia virus was isolated from mice from 9 isolators. A total of 15 pools, consisting of 5 mice each, yielded virus.

Table 8

Attempts to Demonstrate Latent Virus in

a Sample of 150 Apollo 12 Mice

| Germfree Isolator No. | No. Mice Tested | Virus | | | | | |
|---|---|---|---|---|---|---|---|
| | | MSGV | Thymic | LDH | Leukemia | LCM | EDIM |
| 18A ♀ | 10 | -* | - | - | + | - | - |
| 25A ♀ | 10 | - | - | - | + | - | - |
| 26A ♀ | 10 | - | - | - | + | - | - |
| 27A ♀ | 10 | - | - | - | + | - | - |
| 31A ♀ | 10 | - | - | - | + | - | - |
| 6A ♂ | 10 | - | - | - | + | - | - |
| 7A ♂ | 10 | - | - | - | + | - | - |
| 28A ♂ | 10 | - | - | - | + | - | - |
| 29A ♂ | 10 | - | - | - | + | - | - |
| 30A ♂ | 10 | - | - | - | + | - | - |
| 5A ♂ | 10 | - | - | - | + | - | - |
| 3A ♀ | 10 | - | - | - | - | - | - |
| 4A ♀ | 10 | - | - | - | - | - | - |
| 8A ♀ | 10 | - | - | - | - | - | - |
| 14A ♂ | 10 | - | - | - | - | - | - |
| Total | 150 | - | - | - | 11** | - | - |

*Each negative is a test on 2 pools of 5 mice each
**Leukemia virus was isolated from mice from 11 isolators. A total of 18 pools, consisting of 5 mice each, yielded virus.