DEVELOPMENTS OF ADVANCED RESEARCH ANIMAL HOLDING FACILITY (RAHF) FOR SPACE STATION:

I. DEVELOPMENTS OF FUNCTIONAL MODEL

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ABSTRACT

An advanced Research Animal Holding Facility (RAHF) will be required for life science experiments in the Space Station for the study of space medicine and biology, etc. Key requirements for the RAHF include double isolation and biocontamination in the Space Station modules.

The model developed here, consists of Environmental Control and Life Support System (ECLSS), experimental equipment and a mini-work station. The ECLSS is of fully-enclosed environmental control type. Physiological equipment is a telemetry Physiological Monitoring System (PMS).

The mini-work station is used for integrated control of ECLSS and data management of physiological monitoring for the model of basic telescience execution.

They have been conducted as mission verification experiments with the model equiped from 1986 to 1988.

The experiments are duration feeding tests (for one and two months) in an enclosed environment using golden hamsters. Trace contaminants/odor data, hamster activity data, etc. are collected and analyzed.

It was concluded that the fully-enclosed experiments were feasible with the flight model by using succesful waste managing systems.

INTRODUCTION

This report deals with the functions and operations of the animal holding facility for space missions with a functional model (Bread Board Model), and the description of the results of the experiments from 1986 to 1988 (duration holding tests under fully-enclosed environment for four and eight weeks).

Major problem of the animal holding facility is how to keep atmosphere clean inside without mentenance and how to design it compact, low electric consumption and user-friendly for develop a flight model.

In order to solve these requirements, feeding tests including physiological measurements and environmental control tests with golden hamsters were planned to conduct by using functional model developed under automatical conditions.

This model has become a first step of the development of the experimental RAHF equipment for space missions.

MATERIALS AND METHODS

(1) TEST EQUIPMENT

The schematic view of this model is shown in Fig. 1. The right part of Fig. 1 is a holding facility (glove-box), the central part a mechanical section including an air circulator and others and the left part a control section.

This model is composed of three subsystems; a fully-enclosed environment life support subsystem for holding animals, an experiment subsystem for conducting basic experiments and an integrated control subsystem for controlling these two units (see Fig. 2).

Fig. 1 Front view of functional model of advanced RAHF for Space Station

(a) Fully-enclosed environment life support subsystem:

This subsystem is designed to be fully enclosed so that animals can be held completely apart from external atmosphere (equivalent to air in the cabin of a spacecraft) for over three months. The subsystem has functions of recycling air inside the unit to keep the pressure, temperature, humidity, O_2 concentration and CO_2 concentration in the holding facility within the specified range, removing contaminants/odors like ammonia, micro-organisms, dust and disposing of feces, urine, and leftovers of animal feeding.

The outline of the configuration of this subsystem is shown in Fig.3. The data of main operations are shown in Table 1.

The holding facility also provides a glove box which offers easy access to the animals and instruments inside with gloves, and allows for transportation between the holding facility outside by using the air lock without being contaminated.

Fig. 2 Schematic diagram of integrated control and management system

Fig. 3 Block diagram of fully enclosed ECLSS of functional model

(b) Animal holding/physiological subsystem:

This subsystem receives fundamental data concerning monitor of physiology and metabolic changes of animals especially exposed to influenced under micro-gravity measuring and analyzing, behaviors, circadian rhythm, weight, and some kinds of physiological signals (electroencephalogram, electrocardiogram, body temperature).

These physiological signals are obtained by radio wave telemeters from the electrodes and amplifiers permanently implanted in animal's abdominal cavity.

(c) Integrated control subsystem:

The animal experiment facility on orbit features complexity of the system using a variety of equipments, diversity in experiment operations and control system, and changes in experiment contents with the lapse of time. This requires efficient performance of a series of experiment work in a minimum of the crew's worktime, strengthening of material assistance for reducing the crew's work, and full use of the telepresence concepts for increasing the number of opportunities for ground scientists to participate in space experiments.

Table 1 Characteristics of functional model of advanced RAHF

Temperature control	: Typically 20 to 25°C, ±2°C
Humidity control	: Typically 50 to 60% RH ±5%RH
Pressure control	: External pressure ±0.005 atmosphere
O2 concentration	: 21.0 ±1.0%
CO2 concentration	: less than 0.5%
Removal of toxic gas/odor	: NH3 ... less than 5 ppm Others ... not specified
Degree of cleanliness	: Class 100,000 (according to Fed. Std. 209B)
Degree of biocleanliness	: Class 100,000 (according to NHB5340.2)
Animal holding	: Objects ... Hamsters, rats, mice, squirrel monkeys, mini-rabbits, etc. Numbers ... equivalent to 10 to 20 numbers of hamster

Designed to solve the problems mentioned above, this subsystem has two sections. Section 1 is used for assistance in operation, monitoring, detection of malfunctions and self-diagnosis and resource management.

Section 2 is for integrated control of various experiments and measurements and for systematical processing and packaging various kinds of data, and has functions of monitoring, analyzing and controlling these pieces of information via telecommunication lines from remote locations. All this make the subsystem a user-friendly system to be handled easily including, high reliability and good safety.

(2) METHODS

This model was used, for three years from 1986 to 1988, to feed golden hamsters in a fully-enclosed environment for four consecutive weeks and eight consecutive weeks), and to measure data concerning the environment inside the holding facility, generation of contaminants and odors, and their behavioral activities and metabolism of animals.

(a) Environmental conditions:

List of environmental conditions of the RAHF is shown in Table 1. Evaluation experiments were conducted particularly on three items : waste management, water flush and flushing water pH control (neutralization with 0.1 N/H₂SO₄ solutions), these problems for the development of flight model. Evaluation tests conditions are shown in Table 2.

(b) Feeding method:

Four hamsters as experimental group were individually housed in cages inside the holding facility. Another four as control group were placed in each cage under an open environment. Both groups could take food and water any time.

(c) Measurement items:

- * Environmental conditions inside the holding facility: Temperature, humidity, pressure, atmospheric pressure (inside and outside the holding facility), O₂ concentration, CO₂ concentration.
- * Contaminants/odors, microorganisms (bacteria/eumycetes), dust in the air inside the holding facility and flushing water.

Organic nitrogen compounds,	
Organic sulfur compounds	: gas chromatography
Hydrocarbons	: gas chromatography and mass spectrometry
NH ₃ , CO	: portable detecting instrument
Ammonium ion, sulfate ion	: titrimetric methods
Microorganism	: culture medium method
Dusts	: light-scattering photometry
- * Behavioral activities of hamsters:
Observations and wheel running activities (number of revolutions)
- * Metabolic data:
Weight, feed intake, water intake, feces, O₂ consumption

RESULTS

Oxygen , O₂, concentration control and CO₂ removal of the atmosphere in the cage are controled easily which are varied with animal metabolic activities. But trace contaminants/odors are difficult to keep lower range below specific concentrations (see Table 1), so that, not only gas analysis but evaluation of generating sources are very valuable. This report mainly describes such environmental data (for animal behavioral data, see Tanaka et al.1989).

(1) Components of gas produced by holding hamsters in the facility:

Components of gas produced without waste management or removal of contaminants and odors (Case 6) are shown in Table 3.

They include mainly organic nitrogen compounds (ammonia, trimethylamine) organic sulfur compounds(hydrogen sulfide, methylsulfide) and hydrocarbons.

Table 2 Evaluation tests for trace contaminants/odors removal systems

Confirmed Experimental No.	Exp. Condition Exp. case	Water flush	Waste Management	pH cont.	Confirmed item	Data
1	6	X	X		Generation amount of contaminants/odor Generation amount of ammonia	TABLE 3 FIG. 11
2	2	o	X	X	Effectiveness of water flush	FIG. 5 FIG. 6
3	1, 3	o	o	X	Effectiveness of waste management + water flush	FIG. 7 FIG. 8
4	4	o	X	o	Effectiveness of water flush/pH control	FIG. 7 FIG. 8

Mark o : Use/Treatment = applicable
Mark X : Use/Treatment = not applicable

Among these, ammonia and trimethylamine, which are harmful and malodorous, match quite high concentration compared with other components. These gases derive mainly from secretes, feces and urine, fermented gas inside intestines of animals being fed, especially when they are fermented. Gases produced by food, its fermented products and the feeder (equipment and repair materials), though making up an extremely small quantity, are included.

Table 3 Trace contaminant concentration in holding unit
(Case 6: no waste management without trace contaminants removal)

		Case 6 (10 days later)	Case 3 (8 weeks later)	MAC in Space Station	Recognized threshold value (Japan Environmental & Hygiene Center)
Organic nitrogen compounds	Ammonia [ppm]	200	7	25	0.59
	Trimethylamine [ppm]	3.6	0.0059		1.4
Organic sulfur compounds	Methylmercaptan [ppb]	0.15	N.D.	100	0.65
	Hydrogen sulfide [ppb]	4.1	0.0032	2000	5.6
	Methylsulfide [ppb]	10.9	N.D.	1000	2.3
	Methyldisulfide [ppb]	17.5	N.D.		2.9
Hydrocarbons	Carbon monoxide [ppm]	15	18	25	
	Acetone [ppm]	0.80	0.0033	300	110
	Tetrahydrofuran [ppmT**]	0.33	0.0018		
	2-butanone [ppm]	0.36	0.0003		
	4-methyl. 2-pentanone [ppm]	0.11	0.094		
	Toluene* [ppm]	1.80	0.013	20	4.8
	Xylene* [ppm]	0.22	0.0011		
	Benzene* [ppm]	0.003	N.D.		
	Dichloromethane [ppmT**]	0.58	0.058		

Note : * These gases depending upon something other than breeding animals.
** ppmT : Toluene conversion value

The changes of the amount of ammonia measured in Case 6 are shown in Fig.4. Ammonia has its unique properties, increasing rapidly after a certain periods.

Thus, contaminants and odors contained in the air inside the holding facility become highly concentrated in a quite short period if waste management or removal of harmful gases are not carried out. Contrarily, as shown in Table 3, proper waste management and removal of contaminants (with water flush and adsorbents) keep their concentrations at an extremely low level even after eight weeks of feeding.

Further material selections are important because Toluene, Xylene, Benzene are detected in the atmosphere inside as shown Table 1 which seem to be generated from not animals but materials of cage, (plastics, adhesives, foods and others).

(2) Water flush effect:

As shown in Table 3, especially water soluble ammonia and trimethylamine have a higher concentrations than other gases. The method of removing them in the atmosphere inside with water flush is extremely effective, which is proved in Figs. 5 and 6.

(3) Waste management effect:

In the case without waste management, an amount of contaminants and odors is accumulated as the feeding period lasts longer, which is quite remarkable compared with the case waste management (see Figs. 6 and 7).

This implies that removal of feces and urine is extremely effective and essential to control the generation of contaminants and odors.

(4) Flush water pH control (neutralization with 0.1N/H₂SO₄ solution) effect:

In Case 4 (with pH control and without waste management), the ammonia concentration both in the air inside the holding facility and the flushing water was kept at an extremely low level for a comparatively long period of time (see Figs.7 and 8).

This phenomenon, found exclusively in Case 4, is peculiar compared with to the case the pH control of flushing water is provided adequately.

From this result, it may be suggested that ammonia is adsorbed in the flushing water, becoming (NH₄)₂SO₄ by neutralizing the water with 0.1N/H₂SO₄ solution, ammonia has been treated by microorganisms in the flushing water coupled with the environmental conditions of the water, such as temperature, pH and others.

Fig. 4 Ammonia(NH₃) accumulation in animal holding unit(Case 6: no waste management without trace contaminants/odors removal)

Fig. 5 Trace contaminants/odors accumulation in animal holding unit (Case 2:without waste management)

Fig. 6 Ammonia(NH_4^+) concentration in flushing water (with and without waste management)

Fig.7 Ammonia(NH_4^+) accumulation in animal holding unit (Case 3 and Case 4: with and without waste management)

Fig. 8 Ammonia (NH_3) accumulation in animal holding unit (Case 4: without waste management)

Ammonia can be treated in the following two ways by the action of microorganisms

i) Biological nitrification and denitrification (usable for sewage treatment)

ii) Denaturation by bacteria (denaturation into protein)

The quantity of each bacterium were measured in the flushing water (pH 8.5) after six weeks of feeding in Case 4. The results are shown as Table 4.

No nitrified bacteria (oxidized ammonia bacteria, oxidized nitrite bacteria) are detected, ammonia in the flushing water is not led to nitrification or denitrification, by bacteria i.e. $\text{NH}_4 \rightarrow \text{N}_2$.

Presumably ammonia sulfate may be ingested, by bacteria as a nutrient in Case 4, while other neutralization substance such as trichloro-aceticammonium is not ingested by the bacteria.

The species of bacteria in general were identified as pseudomonas paucimobils and pseudomonas maltophilia, and further examination will be needed to confirm only the bacteria of the above-mentioned types have contributed to the treatment of ammonia.

Further experimental data need to be accumulated on the material balance concerning the propagation of bacteria, the relation between the types of bacteria and the treatment of ammonia, etc. in order to reflect the ammonia treatment process by bacteria upon the design of a fully-enclosed environment life support subsystem.

Table 4 Quantity of bacteria in the flushing water (pH 8.5)

No.	Bacteria	Incubation temp. (°C)	Quantity of bacteria (per 100 ml)
1	bacteria in general	25 °C	2.6×10^7
2	bacteria in general	35 °C	2.1×10^7
3	ammonium oxydizing bacteria	30 °C	below 30
4	nitrite oxydizing bacteria	30 °C	below 30
5	denitrifier bacteria	30 °C	27.9×10^7

CONCLUSION

As a result of this feeding experiment under a fully-enclosed life support system over long durations of four weeks and eight weeks, the confirmed points are followings;

- (1) Judging the environment inside the holding facility from the results of analyses of contaminants and odors, and the data on behavioral activities and metabolism of the animals being fed, the feeding environment of this model is considered to be almost equivalent to that of open cages.
- (2) The generation of contaminants and odors can be controlled at an extremely low level even in the case of long-duration feeding through proper waste management and water flush.
- (3) Therefore, fundamental feeding experiments of animals are considered to be possible under the conditions of the fully-enclosed environment introduced into this model.

ACKNOWLEDGEMENT

The authors thank S. Takagi for his technical assistance.

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