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AERO MEDICAL LABORATORY

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Statement A
Approved for Public Release

WRIGHT AIR DEVELOPMENT CENTER

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RDO No. 695-72
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Wright Air Development Center
Air Research and Development Command
United States Air Force
Wright-Patterson Air Force Base, Ohio

FOREWORD

This project was originated under Research and Development Order No. 695-72 entitled "Physiology of Rocket Flight" and continued as a sub-task under Research and Development Order No. 695-77 entitled "Physiological Effects of Gravitational Stress" with M. F. Lee serving as Project Engineer. It was assigned to the Acceleration Section, Biophysics Branch, Aero Medical Laboratory, Wright Air Development Center, Directorate of Research. This is a logical continuation of the studies initiated under "Physiology of Rocket Flight" and published in the Journal of Aviation Medicine as "Animal Studies of the Subgravity State during Rocket Flight" (Volume 23, October 1952).

ABSTRACT

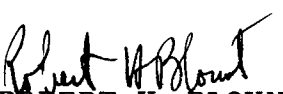
This report presents the results of a series of experiments conducted to determine the basic requirements of mice in a sealed atmosphere. The studies were undertaken as a preliminary to the design of a small container suitable for placement in an orbital rocket.

Oxygen consumption of a mouse was determined in the resting state and during various states of activity for time periods ranging from 1 hour to 2 weeks. The basic requirements of food, water, oxygen, and soda lime were also established. Evidence suggests that a mouse can live under fairly comfortable conditions in a sealed compartment for a thirty-day period.

PUBLICATION REVIEW

This report has been reviewed and is approved.

FOR THE COMMANDER:



ROBERT H. BLOUNT
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INTRODUCTION

The development of a small instrumented orbital satellite now lies within the economic and technical competence of our society. Such a device would present an environment in which the weightless state would exist for an indefinite period of time. Hitherto, studies of the possible effect of the weightless state upon behavior and physiology could be carried out for only very brief periods in aircraft and for not more than two to four minutes in rockets with a parabolic trajectory. Such rocket studies have been reported by groups working in the Aero Medical Laboratory (1). Mice were employed in these tests because in the very limited space available for biological work a small animal has the advantage of having the maximum freedom of movement. No significant disturbance resulted from the brief exposures and it became evident that far more prolonged periods would have to be employed to study the possible deleterious effects of loss of gravity. Pending the development of an instrumented orbital rocket of the type mentioned by Haber (2), much work is necessary in the laboratory to develop a closed self-contained environment in which the mouse could be left untended for periods of as long as a month. The first stage in the development of such a device would be to establish the food, water, oxygen, and carbon dioxide elimination needs of a mouse. With this knowledge, planning of the actual experimental mockup could proceed. If the apparatus were in the form of a drum then the amount of gravity to which the subject was to be exposed could be adjusted to any desired value by rotating the drum at various rates. This report describes the results of a series of experiments designed to secure basic information on the survival requirements of mice in a sealed environment such as would exist in an orbital satellite.

SECTION I

EQUIPMENT AND METHODS

Oxygen was supplied to a mouse and the amount consumed was measured. The original method consisted of placing the mouse in a small jar which was attached to a mercury manometer and flushed with oxygen. As the oxygen was used up by the mouse the mercury column rose and the volume of oxygen consumed was measured from the resulting pressure difference. Carbon dioxide was absorbed by soda lime. A more accurate method employed a 30 cc oiled syringe connected with the sealed jar by means of a needle passing through a rubber stopper. The needle was attached to the syringe by means of a three-way stopcock, the other end of which connected with an oxygen tank. A glass tube was also inserted through the rubber stopper and was attached to a water manometer. The bottle was flushed with oxygen and the syringe, the content of which could be replenished by turning the stopcock, was also filled with oxygen. As the mouse consumed the oxygen in the jar, a pressure differential developed in the water manometer, so that the oxygen used could then be measured by the replacement of a known amount from the syringe until the water columns were equalized. Carbon dioxide was absorbed by a layer of soda lime covered with wire mesh on the bottom of the jar which was sealed with paraffin.

Experiments were run from one to seven hours. In two of the experiments the jar containing the mouse was covered so that the animal was in complete darkness for a period of from one to three hours. The oxygen content of the jar was determined by the Scholander (3) method. Later, since continuation of an experiment over a week-end was not feasible with the small water bottle, a five gallon bottle calibrated in cc's was substituted and volume instead of the weight recorded. Food, water and soda lime were supplied in quantities large enough to provide the needs of the mouse for the five to six day period of the experiment.

For a third type of experiment intended to determine the difference in oxygen consumption of an active and an inactive mouse, an air tight revolving drum measuring 15 cm. in diameter, (Figure 1) was fabricated. A syringe was attached by means of rubber tubing to the front of the

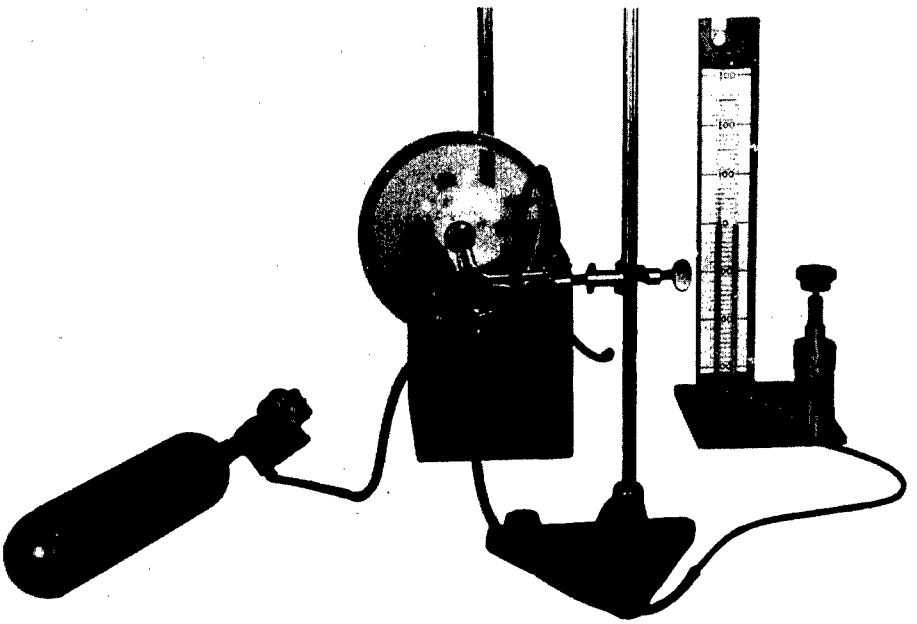

Figure 1 - Revolving drum for measuring consumption during activity

drum and the oiled barrel of the syringe with both ends removed was in turn attached by rubber tubing to stopcocks which served as connectors for the water manometer and the 30 cc syringe containing oxygen which was replenished from a tank connected to the syringe. Soda lime was placed in a gauze bag and attached to the rear of the drum which could be revolved at either 2-3/4 or 7 rpm.

The mouse was weighed and placed in the drum, which then remained stationary for the first hour, was revolved during the second hour and, in most cases, was again stationary for the third hour. Meanwhile, other mice were placed in separate containers so that the amount of food eaten, the amount of food scattered, and the amount of waste material deposited per day could be determined. Purina Lab Chow in bar form was used as food. The resulting approximations of the various metabolic requirements of a mouse were considered adequate for the fulfillment of the purpose of this project.

The next step was to confine a mouse in an enclosed environment which contained proper amounts of these items necessary to sustain life under such a condition. For this purpose a bell jar containing a mouse living space of 13 x 26 cm was used. This device has the advantage for experimental work of having a simple and quick seal. Figures 2 and 3 show typical examples of bell jars at the onset of a thirty day confinement and at the termination of such a period. Six of this type of experiment were run, various modifications being introduced with each one, until a point was reached where a mouse could live under fairly comfortable conditions for a period of thirty days. In its final form the bell jar, including modifications, contained an overhead rack which allowed soda lime to be extended above as well as below the mouse. This was found to be necessary because of the scattering of food which would fall onto and cover the greater portion of the soda lime in the bottom of the jar. A wire gauze rack supported the mouse, clear of this layer of soda

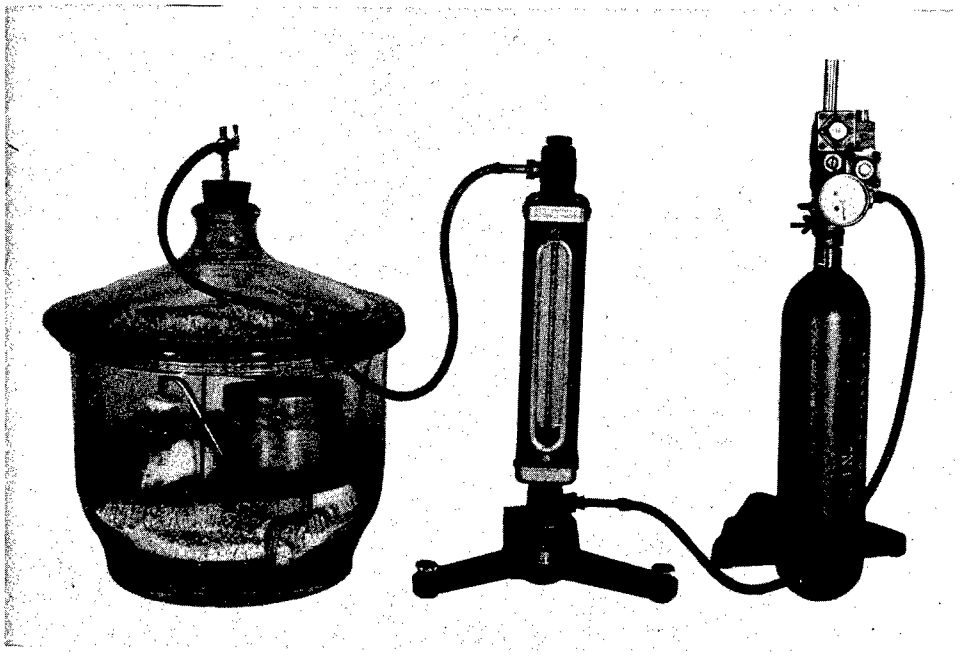

Figure 2 - Typical bell jar at onset of 30 day confinement period

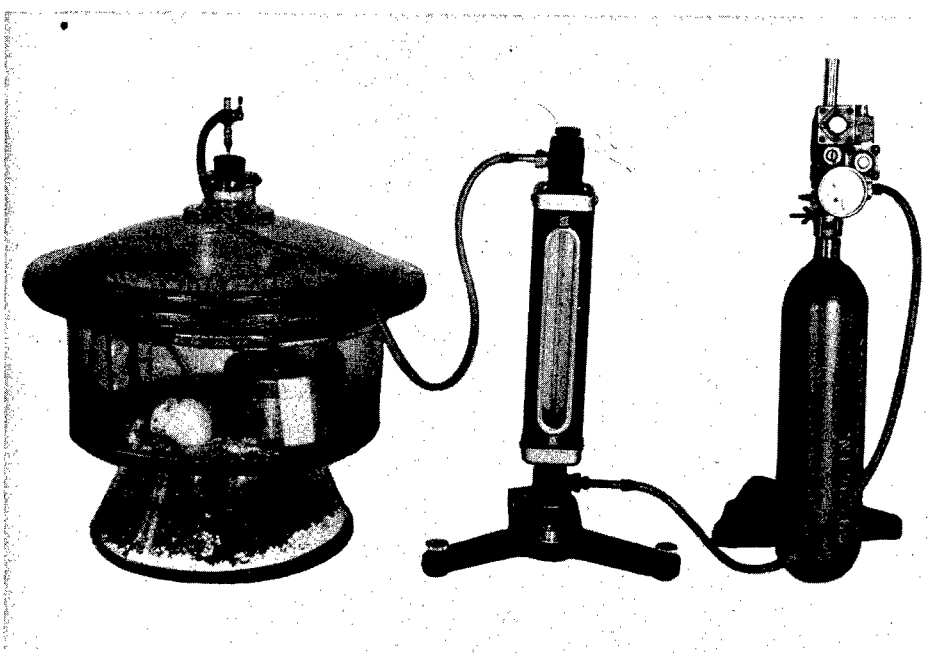

Figure 3 - Typical bell jar at termination of 30 day confinement period

lime. The food under these sealed conditions tended to mold rapidly and was, therefore, pretreated with a 0.2% solution of sodium benzoate and dried at room temperature. Water was supplied in a bottle which contained a wick of nylon cord. The jar was attached to a hanging bead flow meter which indicated the amount of oxygen being consumed per minute and which also served as a check for leakage in the system. In conjunction with these experiments, a bell jar was set up under conditions identical to the above except that the lid was left ajar and the contents exposed to room air rather than sealed with oxygen. The growth curves of the mice in air and those sealed in oxygen were compared. Normal growth curves were also established for a further group of mice

housed in a small animal cage. Also, in order to check the effect of the sodium benzoate, the growth rate of a group of mice sustained on regular food was compared with that of a second group which was fed the treated food.

SECTION II

RESULTS AND DISCUSSION

In the short term oxygen consumption experiments a mouse weighing between 25 and 30 grams consumed from 2.7 to 3.6 cc/gm/hr in the initial two hours, while one weighing less than 12 grams consumed approximately twice as much oxygen per gram in the same period. When the experiments were run for six or seven hours, the oxygen consumption decreased after the third or fourth hour. It was not unusual for a mouse to use 3.6 cc/gm/hr the first two hours and then decrease consumption until by the seventh hour oxygen intake was only between 1 or 2 cc/gm/hr. In the experiments in which the mouse was in darkness for a period of from one to three hours, the oxygen consumption was the same as that for the mice exposed to light. Oxygen content of the animal container was approximately 20% and after three hours dropped to about 16.5%. In two cases in which the early oxygen content was approximately 20%, it dropped to 16.5% and 11.6% respectively after seven hours. When the jar was initially flushed with oxygen, the oxygen content after four to six hours was 87% and 92% respectively. Some of the rather high oxygen consumption figures obtained during this period may have been due to leakage of the screw cap jars employed in this phase of the work.

Subsequent experiments of 2 to 4 weeks duration employed bell jars with a flow meter attached to eliminate that possibility. Results with this set-up confirmed those of the initial experiments and established the oxygen consumption to be somewhere between 1 and 3 cc per minute, varying with the activity of the mouse. Oxygen consumption over periods of from three to seven days averaged between 3.25 and 5.6 cc/gm/hr. Table 1 summarizes the data obtained from the bell jar experiments.

Table 2 gives the figures on the oxygen consumption experiments performed with the revolving drum. It can be seen from this table that the oxygen consumption of the mice in all cases increased during the second hour while the drum was revolving. This increase varied from 0.3 to 2.5 cc/gm/hr. During the third hour, when the drum was stationary the oxygen consumption increased in two cases and decreased in two cases. In one experiment which was run for four hours, the third and fourth hour showed no change.

In Table 3 the results of the work done to determine the amount of food eaten and scattered and the amount of waste material are indicated. The average size mouse required about .26 gm food/gm mouse. Of this amount approximately .16 gm/gm mouse/day was eaten while the remainder was scattered. These figures rose appreciably for a very young mouse. The waste material amounted to approximately 1.4 gm/day. Water intake varied, but averaged approximately 5 cc per day.

The metabolic requirements of an average size mouse for a thirty day confinement period are summarized in Table 4.

TABLE 1

DATA OBTAINED FROM BELL JAR AND ALLIED EXPERIMENTS

Bell Jar	No. of Days	Wt. before Gm	Wt. after Gm	Change Gm	H_2O loss/day cc	Food loss/day Gm
2	20	24.3	21.1	-3.2		5.7
3	53	14.5	22.3	7.8	5.5	8.0
4	54	12.2	11.5	-0.7	1.9	14.5
5 *	37	7.0	15.0	8.0	4.1	18.0
6 *	37	7.1	17.0	9.9	5.0	13.0
7 #	17	7.4	16.6	9.2		5.6
8 #	60	13.9	20.0	6.1		9.5
						6.5
Food treated with sodium benzoate						5.9
Food untreated						8.5
Normal growth in cage						

* Tight seal on jar - opened only two or three times
No. 5 closed for 10 day period. No. 6 closed for 17 days

\# Lid ajar and so exposed to room air

TABLE 2

OXYGEN CONSUMPTION STUDIES INVOLVING THE USE OF THE REVOLVING DRUM

Mouse gm	1st hour Drum stationary cc/gm/hr	2nd hour Drum revolving cc/gm/hr	3rd hour Drum stationary cc/gm/hr	4th hour Drum stationary cc/gm/hr
15.2	3.6 *	4.0	5.5	
16.6	2.1 #	4.2		
17.9	4.4 *	6.3	5.5	5.6
21.6	5.4 *	7.9	5.6	

(Continued)

TABLE 2 - Continued

Mouse gm	1st hour Drum stationary cc/gm/hr	2nd hour Drum revolving cc/gm/hr	3rd hour Drum stationary cc/gm/hr	4th hour Drum stationary cc/gm/hr
23.5	4.7 *	5.1	5.9	
23.7	4.5 #	5.1 (1/2 hour)	4.6	
30.0	4.0 #	4.9		
Mean 21.2	4.1	5.4	5.4	

* Rate of rotation 2-3/4 rpm

\# Rate of rotation 7 rpm

TABLE 3

FOOD CONSUMPTION, FOOD SCATTERING, AND FECAL ELIMINATION

Mouse gm	Food eaten and scattered gm/day	Food eaten and scattered gm/gm/mouse	Food eaten gm/day	Food eaten gm/gm/mouse	Scattered gm/day	Waste gm/day
8.2	7.9	.963	4.8	.585	3.1	1.2
17.5	4.9	.280	3.5	.20	1.4	1.3
23.0	7.4	.187	3.7	.161	3.7	1.7
25.4	6.5	.255	4.1	.166	2.4	1.4
31.0	6.3	.203	3.9	.126	2.4	1.3
Mean 17.0	6.6		4.0		2.6	

TABLE 4

REQUIREMENTS FOR SURVIVAL OF A MOUSE FOR THIRTY DAYS IN A SEALED ENVIRONMENT OF LIMITED SPACE

	per day	per thirty days
Water	5 cc	150 cc
Food	6.6 gm	198 gm
scattered	2.6 gm	78 gm
eaten	4.0 gm	120 gm
Oxygen	5 cc/gm/hr	72,000 cc
Soda lime		1,300 gm

The mouse survival experiments represent an attempt to study the requirements of an animal confined within a sealed environment of limited space for a thirty day period. The quantity of food, water and soda lime required to sustain a mouse for this length of time was first determined in short term tests and the indicated amounts were then enclosed with the animal in an airtight compartment. As long as there was no breakdown of oxygen supply, the mouse survived.

The amount of food consumed per day under the conditions of these experiments (approximately 3.5 grams for a 20 gram mouse) is in agreement with the figures obtained by M. Maqsood and E. P. Reineke (4) in their work on the influence of environmental temperature and thyroid status on food consumption. However, when the mice in our experiments were allowed free access to the food, they scattered a large amount of it, and for an undetermined reason tended to scatter more while in undisturbed solitary confinement for a period of days than those mice which were disturbed by weighing every second day. Boredom was thought to be a possible explanation for this occurrence, since the mice did not consume food excessively during these periods.

No difficulty was experienced in training a mouse to take water from a wick. Even when very young, they adapted quickly to this method in most cases. The water intake of 5 cc per day is about 2 cc less than that reported by Maqsood and Reineke and it is believed that this discrepancy may be due to the wick method of supply.

The oxygen consumption of mice was found to vary with size and activity, the intake being as high as 8 cc per gram per hour in one instance in the revolving drum. In P. R. Morrison's study of mammalian oxygen consumption (5) a number of workers were reported to have found consumption under basal conditions to range between 1.6 cc per gram per hour and 2.8 cc per gram per hour. An allowance of 5 cc per gram per hour should, therefore, fully satisfy the demands of an active growing mouse. A standard Air Force high pressure bailout bottle weighing 2.75 pounds and measuring 27 cm by 7 cm, would more than meet the demands of a thirty day period.

The next phase of this project would employ the above data to design a mockup of a small rotating container of a type which could eventually be housed in an instrument-carrying orbital rocket. Figure 4 illustrates the amounts of food, water, soda lime and oxygen needed for stocking such a capsule to sustain a single mouse for thirty days. Figure 5 shows the first castings made for the capsule mockup together with the drive motor and oxygen tank. The diameter of this drum is only 14 inches and the total weight of the loaded thirty day capsule should not exceed thirty pounds.

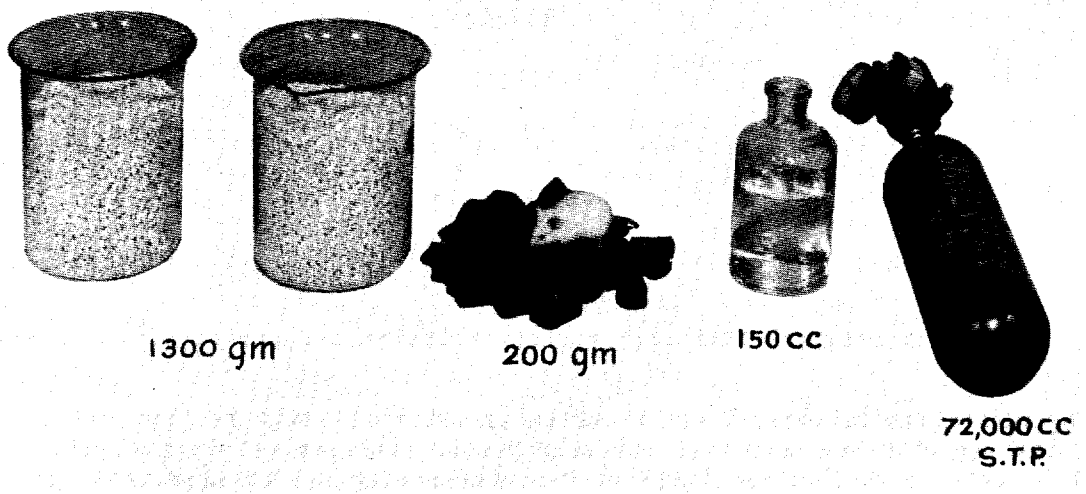

Figure 4 - Soda lime, food, water and oxygen required to sustain an average size mouse during a 30 day confinement period

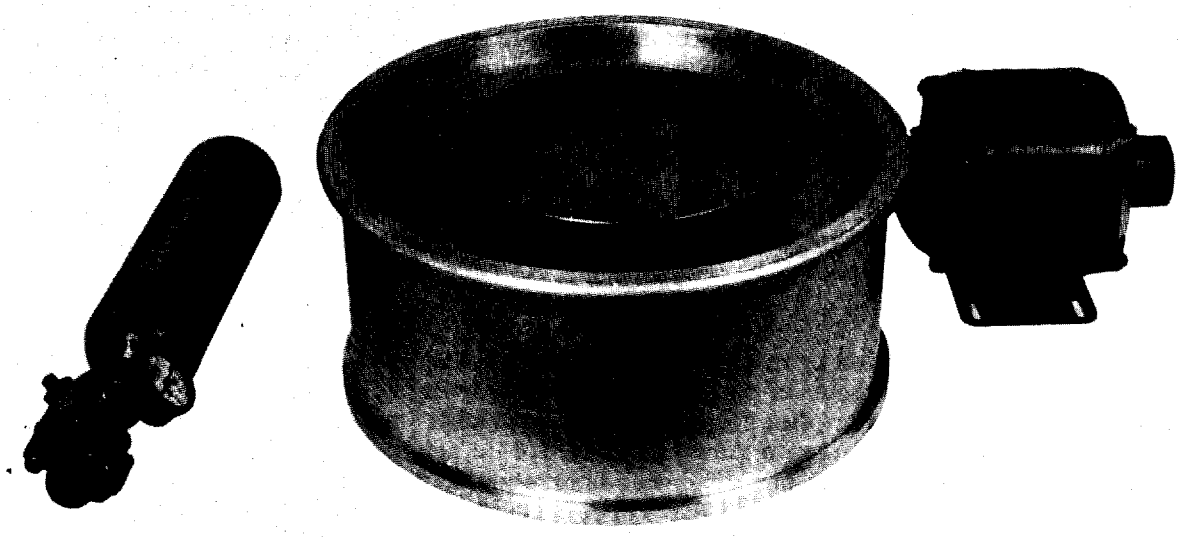

Figure 5 - Castings of a small animal capsule mockup with oxygen tank and drive motor

The initial steps in determining the physiological needs of a small biological system are described in this report. However, with the development of a capsule suitable for installation in a rocket and the application of the data reported herein to this container, fresh problems of survival will arise, since only the general needs have been considered up to this point. Now the necessity of making this general information conform to the specific needs of a particular system arises. The effects of the weightless state upon such factors as the amount of water evaporation, scattering of food and suspension of soda lime will vary with the design of the closed container. These more specific requirements could well be determined in a fresh series of experiments with a new device more explicitly designed for possible use in the instrumentation section of a rocket.

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