Contract No. NAS-9-11941

STUDIES ON DISEASE TRANSMISSION IN SPACECRAFT ENVIRONMENTS

FINAL REPORT

October 1974

Submitted by

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C. Olmsted, J. and Kenyon, A. J. 1971. Lymphoproliferative Diseases of Fowl - Radiologic Evidence for Transplantable Nature of Lymphoblastic Leukemia (JM-V). Avian Dis. 15:672.

D. Kenyon, A. J., Gander, J. E., Lopez, C. and Good, R. A. 1972. Virus Isolation by Affinity Chromatography: Isolation of Aleutian Mink Disease Agent. AALAS, abstract, 23rd Annual, p. 26.

E. Kenyon, A. J. 1973. Isolation of Aleutian Disease Virus From Mink. Northeastern Mink Farmer 146:6.

F. Kenyon, A. J., Gander, J. E., Lopez, C. and Good, R. A. 1973. Isolation of Aleutian Mink Disease Virus by Affinity Chromatography. Science 179:187.

G. LeDoux, R. G. and Kenyon, A. J. 1973. Protides of Mustelidae: Comparative Study of the Plasma Lactate Dehydrogenases. Comp. Biochem. Phys. 45B:225.

H. Yoon, J. W., Kenyon, A. J. and Good, R. A. 1973. Demonstration of Aleutian Mink Disease Virus in Cell Culture. Nature New Biol. 245:205.

I. Kenyon, A. J., Yoon, J. W. and Dunker, A. K. 1974. Isolation of Picornaviruses by Affinity Chromatography. AALAS, Abstract, 25th Annual, p. 1.

III. ABSTRACT OF ACCOMPLISHMENTS

During the total period of this contract and its extensions, a logical progression of events--through preliminary experiments then design and construction of a low pressure facility--has resulted in studies on the effects of the Skylab gas mixtures on general health and immunocompetence of mice and ferrets subjected to the Skylab space cabin environment (SCE). Preliminary experiments were performed at the Illinois Institute of Technology, Research Institute (IITRI) to determine initial tolerability of ferrets maintained at gas ratios of 30% nitrogen and 70% oxygen under 5 psia. Depression of immunologic responses related to stress of the initial event (decompressing to 5 psia) were evident at 7 days after introduction into Skylab environment. Recovery to normal response was apparent by 21 days exposure. This evidence consisted of decreased lymphoid organ weight and reduced response to antigenic stimulation. Also in preliminary experiments, it became apparent that histologic changes in the mucociliary system of the upper respiratory tree were attributable to high levels of ammonium arising from animal waste due to low turnover rates of gas environment.

Thus, a stainless steel low pressure facility which consisted of 2 subchambers, that permitted mutual isolation of experimental groups and/or selective removal of animals without return of the entire cabin to ambient pressure was developed. Throughout experimental tests, the chamber environment was stable, fluctuating only 4-6 mm Hg in pressure and 1-3^{o}C in temperature. Upon ascent to altitude, neither young adult mice, ferrets, nor suckling mice indicated signs of bends.

Mice subjected to the 70:30 O_2:N_2 and 5 psi cabin environment, had severely reduced thymic weights 3 days after exposure to cabin atmosphere.

In ferrets, spleen weights were most affected by ascent to altitude; thymus weights remained relatively unchanged. Control ferrets housed in rigid glass isolators with absolute filters also showed a similar trend in splenic weights due to stress factors related to the enclosed environment. Animals housed in the open did not show these changes.

Murine hepatitis virus (MHV_3) infection in SCE did not appear to be enhanced, whereas influenza virus infection was. Since humoral antibody response is a major factor in MHV_3, control of this hepatitis may be a response of primarily B-type lymphocytes. The fact that mice which had reduced thymic weights and not reduced splenic weights indicate a prerequisite for reduced resistance to influenza virus. This apparently involves T-cell or combined T- and B-cell function as opposed to MHV_3 which is known to elicit humoral antibody. In mice, as indicated by normal spleen weights and normal humoral antibody response, this limb of the immune system was unaffected by SCE.

Our studies have clearly demonstrated that ferrets immunized with Brucella Strain 19 prior to being housed in SCE had decreased synthesis of IgG compared to their respective controls. Separation of IgG from IgM was accomplished by inactivation of IgM with mercaptoethanol. Chamber stresses, therefore, had affected humoral antibody activity in the ferrets, as indicated by reduced spleen weights and decreased IgG synthesis.

Because of the possibility of latent infections being responsible for stress-induced upper respiratory diseases of astronauts, the role of neutralizing antibody as a function of antibody affinity/avidity was investigated. The model consisted of Aleutian disease virus (ADV) which infects ferrets and mink resulting in nonneutralized immune complexes. These

studies demonstrated that early antibody to ADV had lower affinity/avidity than late antibody with respect to chronicity. These studies culminated in a description of antibody affinity, first isolation of ADV and its cultivation in vitro.

Since passive immune aggregation represented an approach that may be used to detect subclinical viral infections in astronauts, this procedure was studied as a possible means of rapid detection of lymphoproliferative viruses. Preliminary analyses have indicated the procedure described in contract NAS 9-11371, Microbial Ecology Measurement System, was unworkable due to dissociation of antibody from insoluble supporting matrix. As in affinity chromatography (immunoadsorption) the antibody must be covalently bound to the supporting material to prevent new adsorption equilibria from being established when exposed to antigen.

IV. INTRODUCTION - PRELIMINARY ANALYSES AND EVALUATION OF SPACE CABIN ENVIRONMENTS ON FERRET RESPIRATORY SYSTEM.

The frequency of "minor colds" experienced by astronauts in flight and the experimental evidence of altered susceptibility to respiratory disease in mice exposed to space cabin environments prompted consideration of depressed physiologic and immunologic responses as a result of spacecraft environmental stress. Factors of importance in the induction of respiratory disease are the depression of normal immune mechanisms and the function of the mucociliary system. The muscosal movement of inhaled particles by ciliated cells could be impaired in the spacecraft environment. Reduced atmospheric pressures cause differences in aerodynamic equivalent diameters of particles. This, coupled with aerosal aggregation which occurs at zero gravity, alters the pathogenic character of aerosols.

In the initial considerations of disease transmission, it seemed important to study the immunologic and physiologic effects of small animals exposed to a simulated spacecraft environment. In these considerations, it also seemed important to dilineate the function of pressure in respiratory physiologic response to inhaled particles; to experimentally establish the effect of the spacecraft environment on the median aerodynamic mass of aerosolized inert and infectious particles; and to measure the rate of elimination of inhaled organisms in relation to reduced mucociliary clearance and altered alveolar macrophage function.

Although the methodology was available to undertake a study of factors relating to respiratory physiology and disease transmissions, the following preliminary study was developed at the Illinois Institute of Technology, Research Institute (IIIRI).

The objectives of this study were:

1. Obtain evidence of signs of stress associated with the chamber environment.
2. Relate stress factors to physiologic and immunologic predisposition to disease.

The experimental design consisted of placing 14 ferrets in simulated spacecraft environment as proposed and used in the Skylab missions. The environmental conditions were 30% nitrogen, 70% oxygen, 5 psia, relative humidity 50%, temperature 26°C. Control subjects (14 ferrets) were housed in animal in similar cages, but at ambient conditions. At time zero, when the animals entered the chamber, 2 ferrets from each group were sacrificed, and the following analyses made. On blood, a complete hemogram was obtained, while on serum hemagglutination of sheep RBCs, serum protein profile (electrophoresis) and serum enzyme levels were determined. At necropsy, body weights and all organ weights were obtained. Tissues were fixed in 10% formaldehyde as well as frozen. On fixed tissues, H and E stains and mucicarman stain were performed. Particular enphasis was placed on the respiratory system using mucicarman stain and analysis of respiratory mucociliary system. The tests performed are listed in Table 1.

Animals and the animal caretaker entered the chamber together to set up cage arrangements (the animal caretaker used an oxygen mask and prebreathed 100% oxygen during ascent to altitude (30 minutes). The configuration of the chamber is shown in Figure 1 and control panel in Figure 1'. During the test period, chamber performance was as indicated in Table 2. The experimental design is shown in Table 3.

TABLE 1

TESTS PERFORMED

Blood	Serum
Hematocrit	Hemagglutination
WBC	Serum protein
RBC	Electrophoresis
	LDH Isozymes
	ALK Phosphotase

Intact Ferret - Body wt.
Organ weights on necropsy

Tissues (Fixed)	Tissues (Frozen)
H & E stain on section	Immunofluorescence
Mucicarman stain	(lung and lymph nodes)

Figure 1

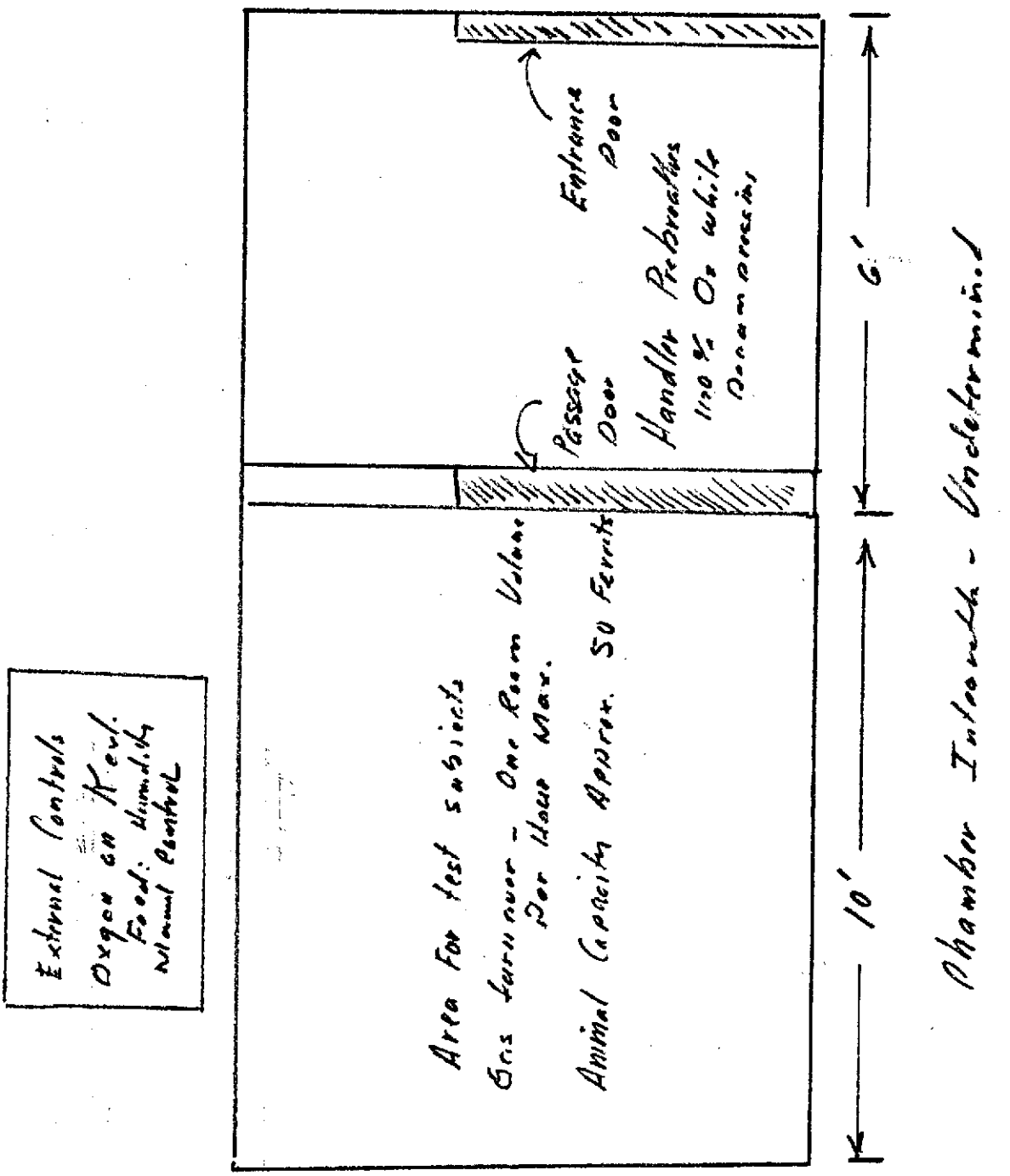


Figure 1'

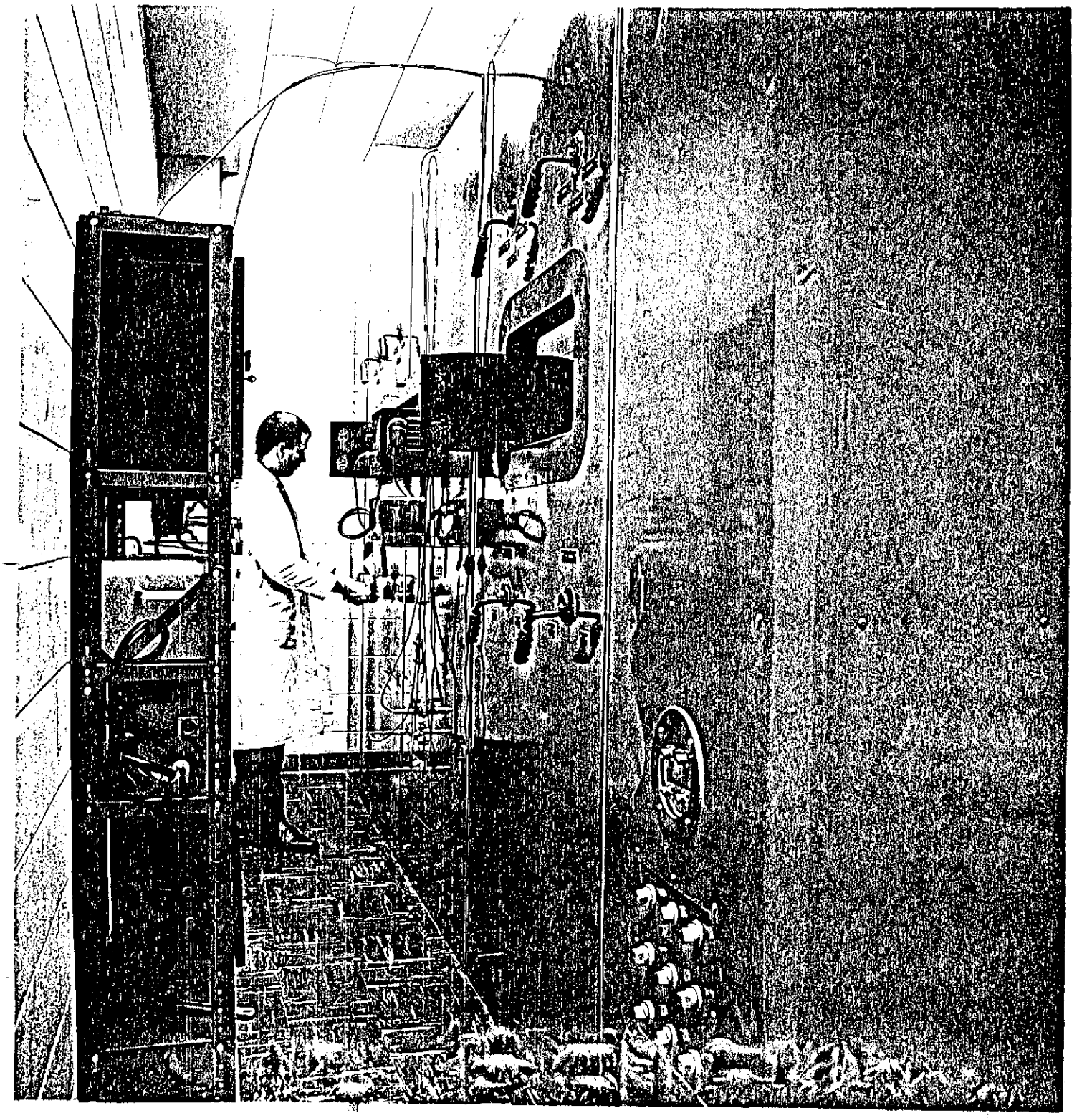


TABLE 2

RECORD OF ENVIRONMENTAL CONDITIONS IN TEST CHAMBER

A simulated altitude of 27,000 feet ± 500 feet (5 psia) was maintained.*

Date	O_2%	CO_2%	Temp. °C	R.H. %
7-27-71	68	2.0	26	48
7-28-71	69	2.1	23.5	50
7-29-71	72	2.0	25	48
7-30-71*	70	2.1	23	65
8-02-71	76	2.1	18	49
8-03-71	66	2.1	24	46
8-04-71	65	2.0	20	62
8-05-71	69	1.9	23	58
8-06-71	72	1.9	23	52
8-09-71	72	2.0	21	51
8-10-71	68	2.0	22	52
8-11-71	71	1.8	21	51
8-12-71	76	1.7	21	51
8-13-71	73	1.8	21	51
8-16-71	75	1.7	21	54
8-17-71	72	1.7	21	51
8-18-71	72	1.7	21	51
8-19-71	70	1.6	21	51
8-20-71	70	1.4	22	48
8-23-71	75	1.1	22	52
-	71.1	1.8	21.9	52.1 average

*8-31-71 Malfunction of automatic vacuum control. Chamber lost vacuum to sea level.

TABLE 3

EXPERIMENTAL DESIGN

Day	Open	Chamber
0	2	
1	2	2
3	2	2
7	2	2
14	2	2
21	2	2
28	1	1

The results of this study were as follows:

Body weight - In both control and test subjects, the ferrets gained weight over the 28-day period. The average weight for both groups was 550 g and at the end of the test period, the control was 880 g, while the test group was only 680 g. Early in the course of the chamber exposure (day 1) the average weight was less than the starting weight which may have resulted from dehydration (Figure 2).

Lymphoid organ weight - Both the thymus and spleen weights decreased markedly in the chamber group. This reduction in weight was evident in the first week of test which suggests that induction into the SCE was stressful. The values shown were placed on percent of body weight which tends to mask the reduction because of overreduced body weight. However, these changes in lymphoid organ weight are still significant (Figures 3 and 4).

Weight of control organs - Liver and kidney weights were also taken so as to monitor weight changes of organs which have relatively little stress-association. As shown in Figures 5 and 6, as the ferrets gained weight; the relative size of the liver and kidney decrease with no noticeable change occurring in the first week of exposure.

Red blood cell count - The RBC level averaged 5.9×10^6 at zero time and rose to 6.1×10^6 for the chamber group. This rapid increase may be associated with dehydration of the test subjects. At day 7, both groups had similar levels (7.4 chamber; 7.6 controls); however, at the end of the period, the test group had 5.9 and the control 7.4 (Figure 7).

White blood cell count - During the first week of exposure, the test group had a lower WBC count than did the control groups (at days 3 and 7, test had 4,000 and 4,800, respectively; whereas the ambient group had 5,100 and 7.900, respectively). Again, as anticipated under stressful conditions, the WBC count had decreased (Figure 8).

Figure 2

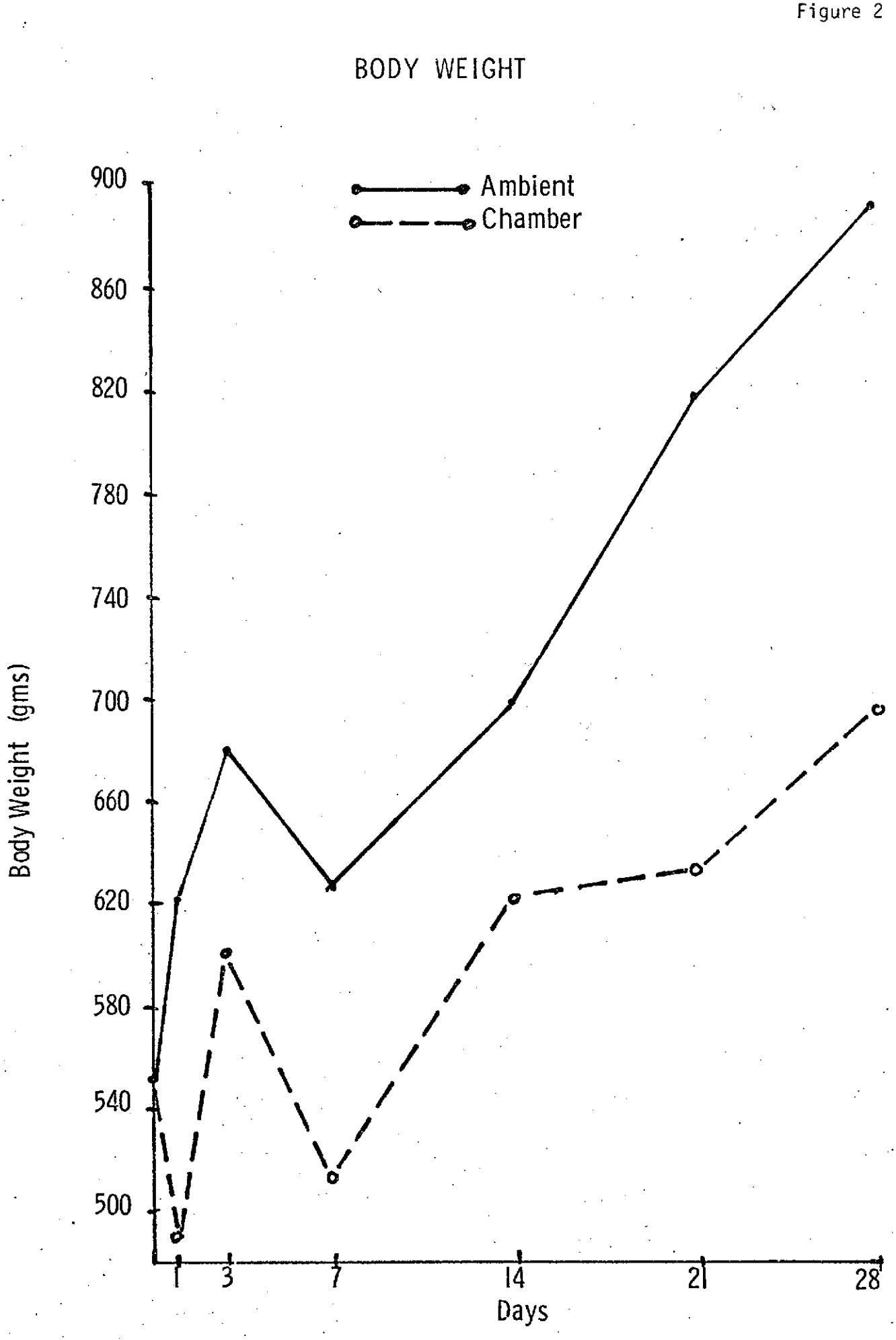

BODY WEIGHT
Ambient
Chamber
900
860
820
780
740
700
660
620
580
540
500
Body Weight (gms)
1
3
7
14
21
28
Days

Figure 3

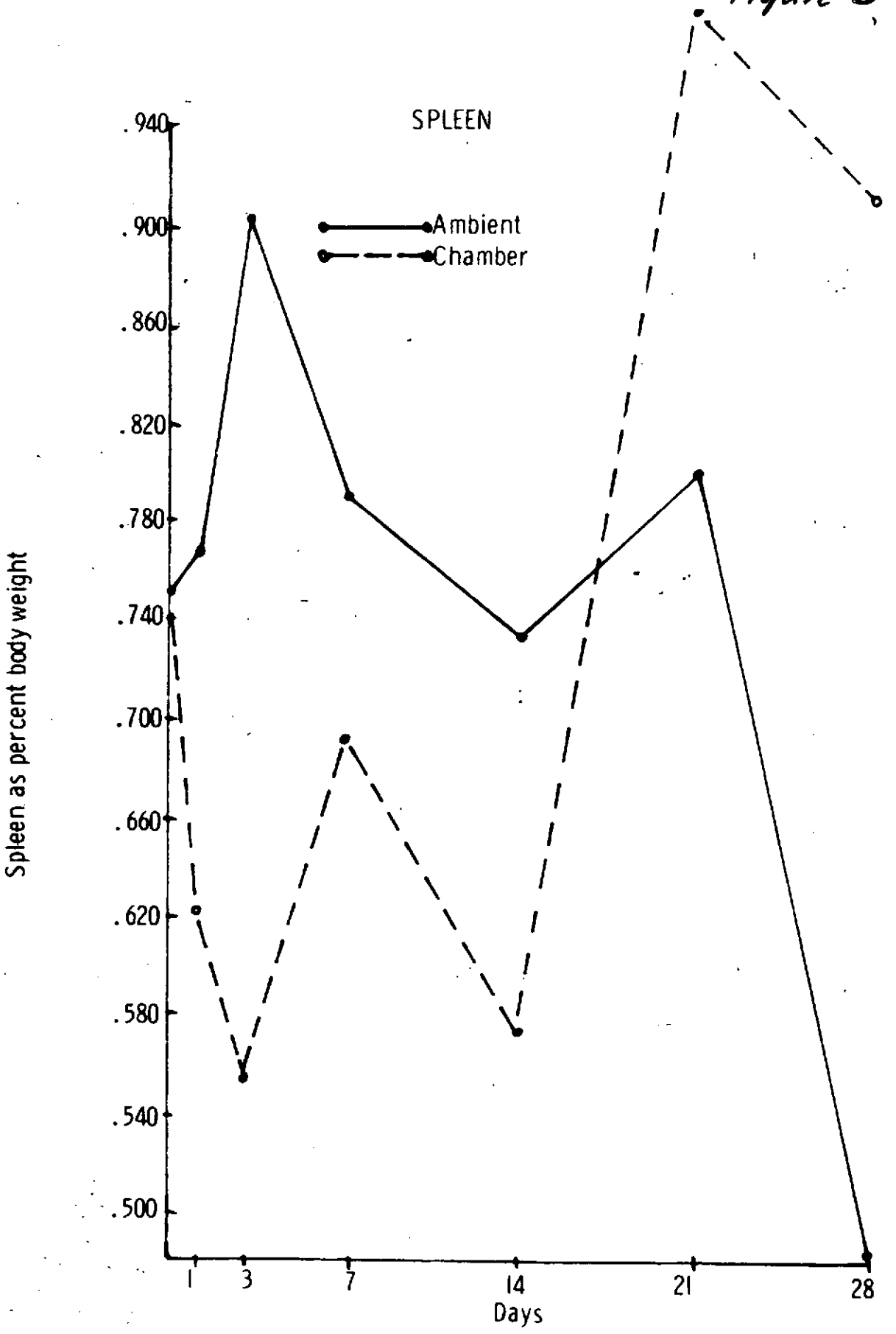

SPLEEN
Ambient
Chamber
.940
.900
.860
.820
.780
.740
.700
.660
.620
.580
.540
.500
Spleen as percent body weight
1
3
7
14
21
28
Days

Figure 4

THYMUS

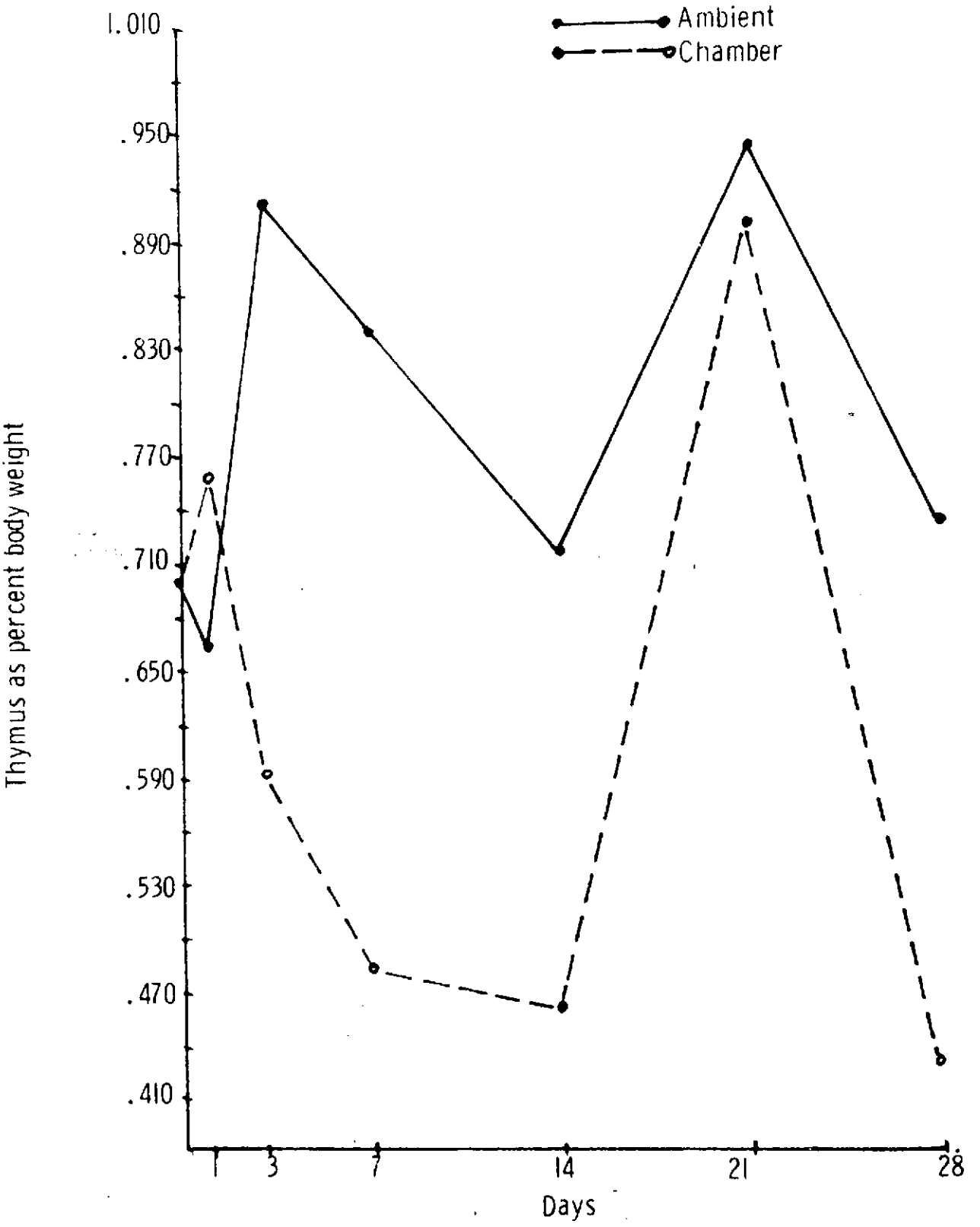


Figure 5

LIVER

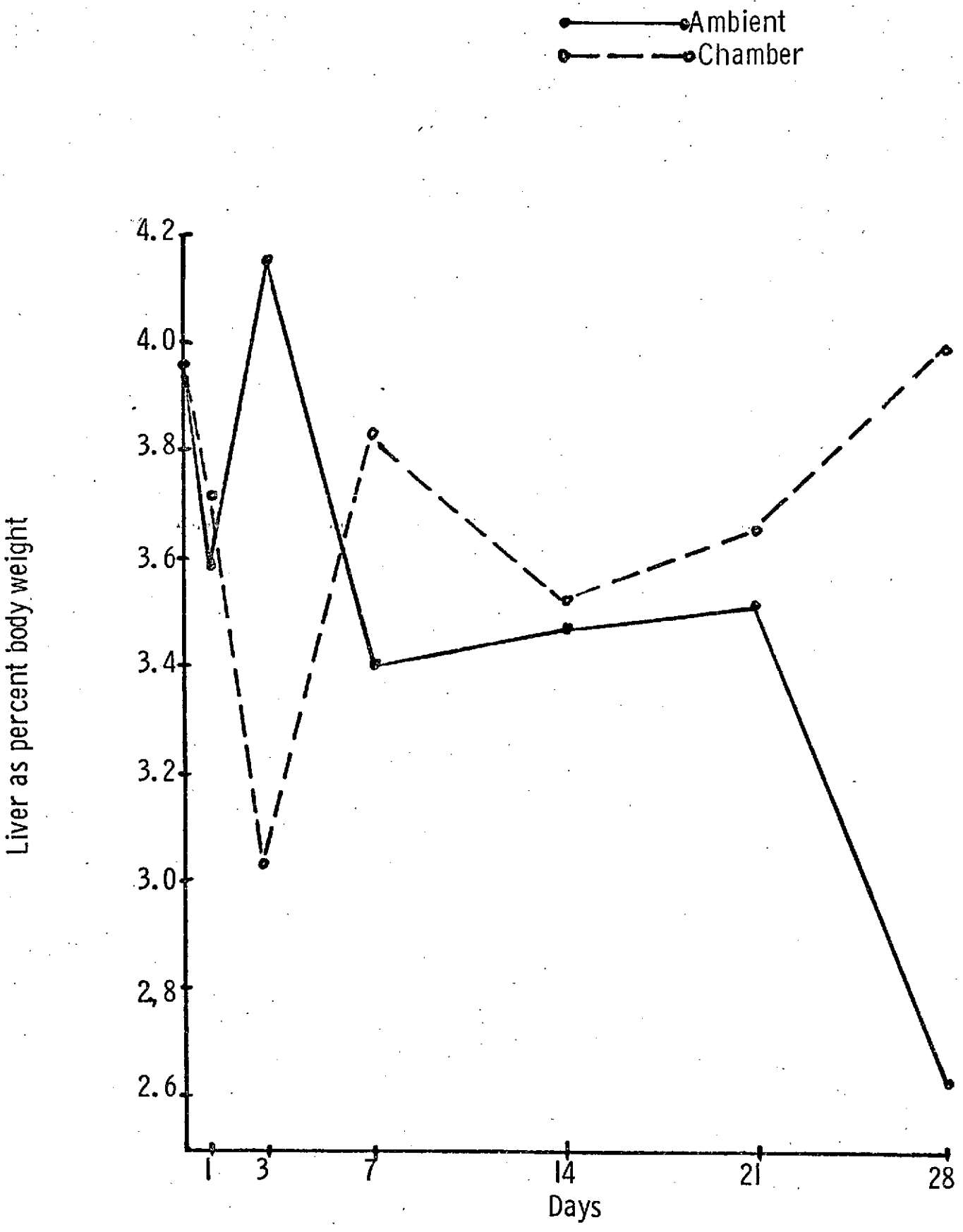


Figure 6

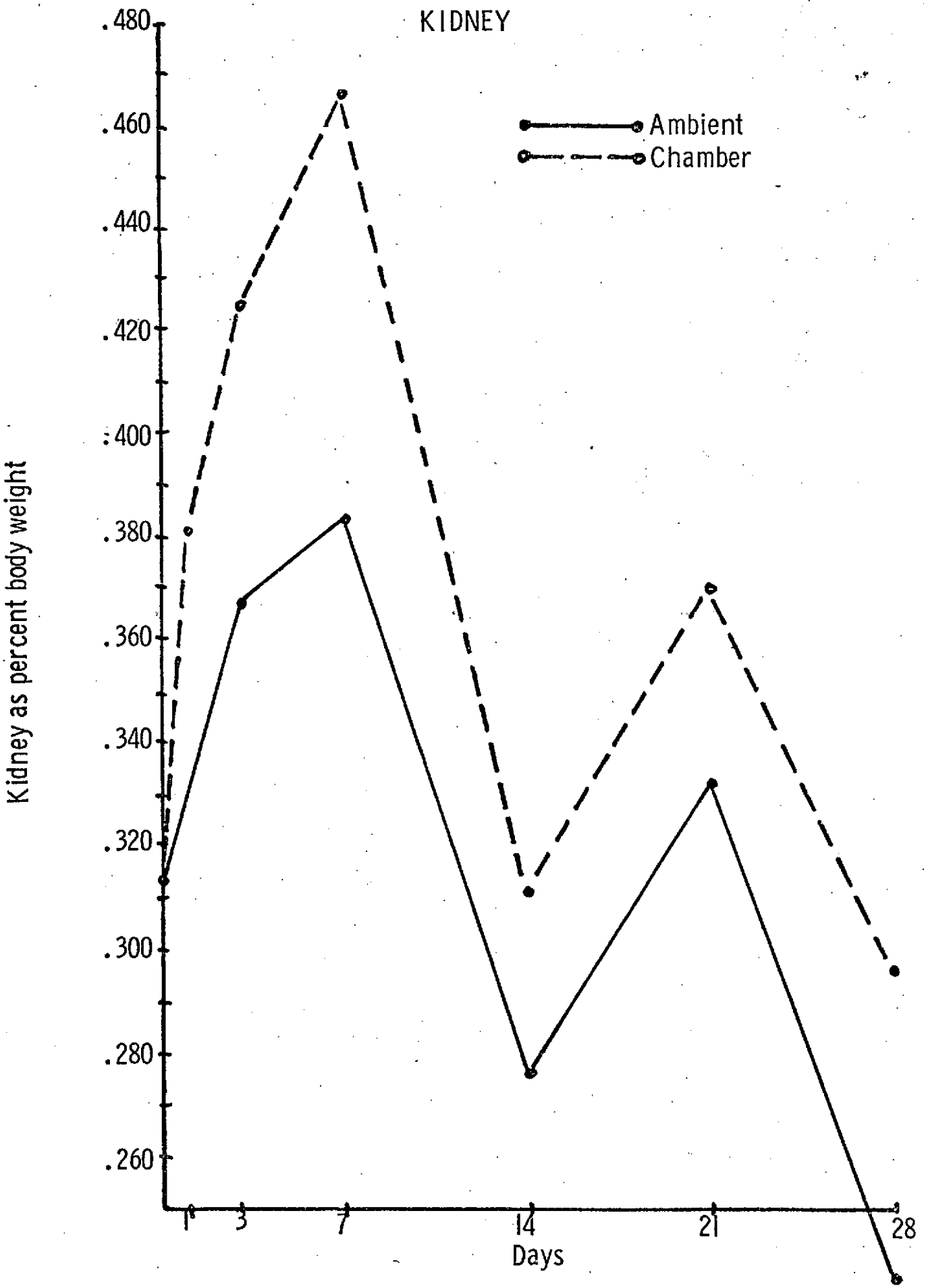

KIDNEY
Ambient
Chamber
.480
.460
.440
.420
.400
.380
.360
.340
.320
.300
.280
.260
Kidney as percent body weight
1
3
7
14
21
28
Days

Figure 7

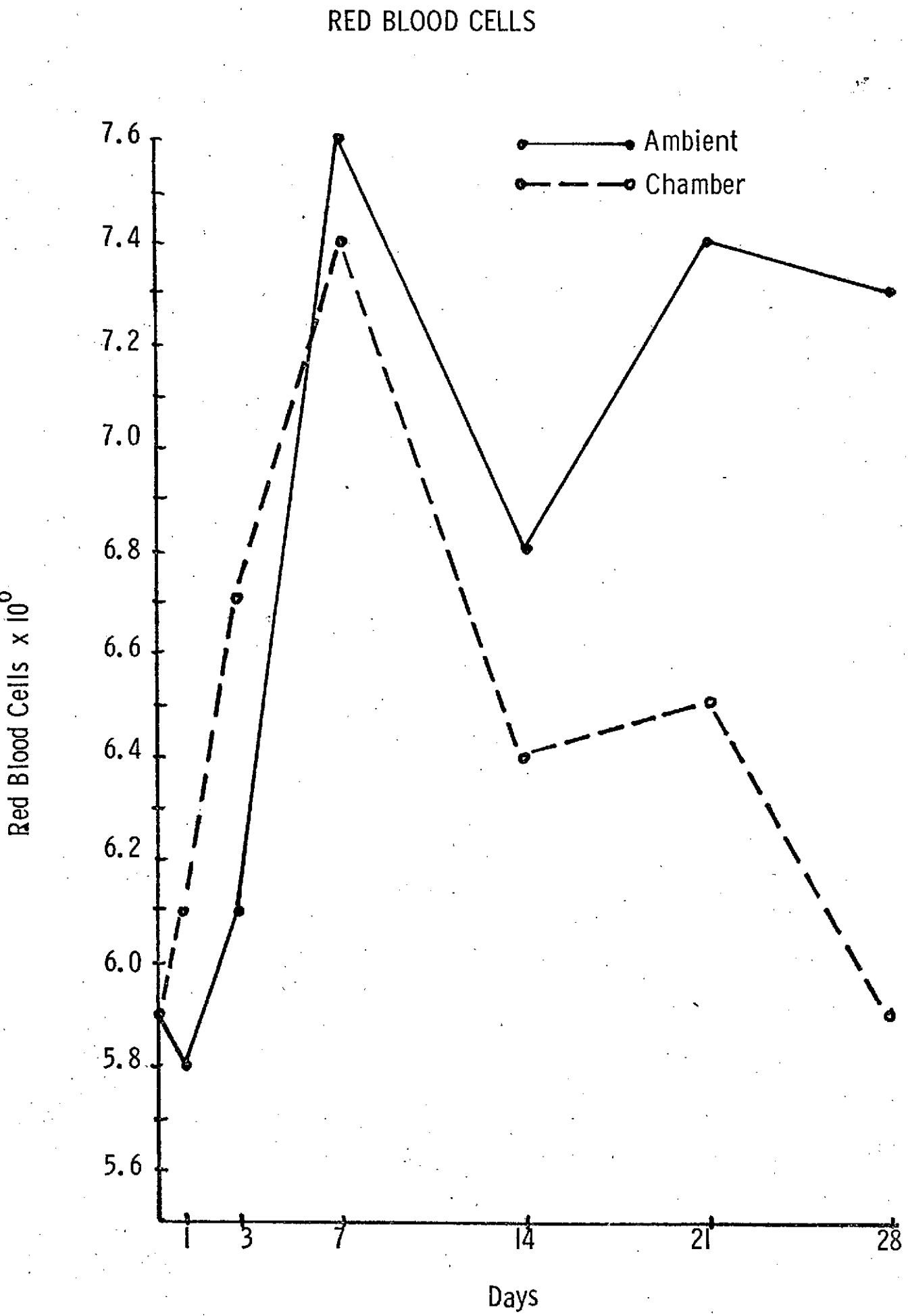
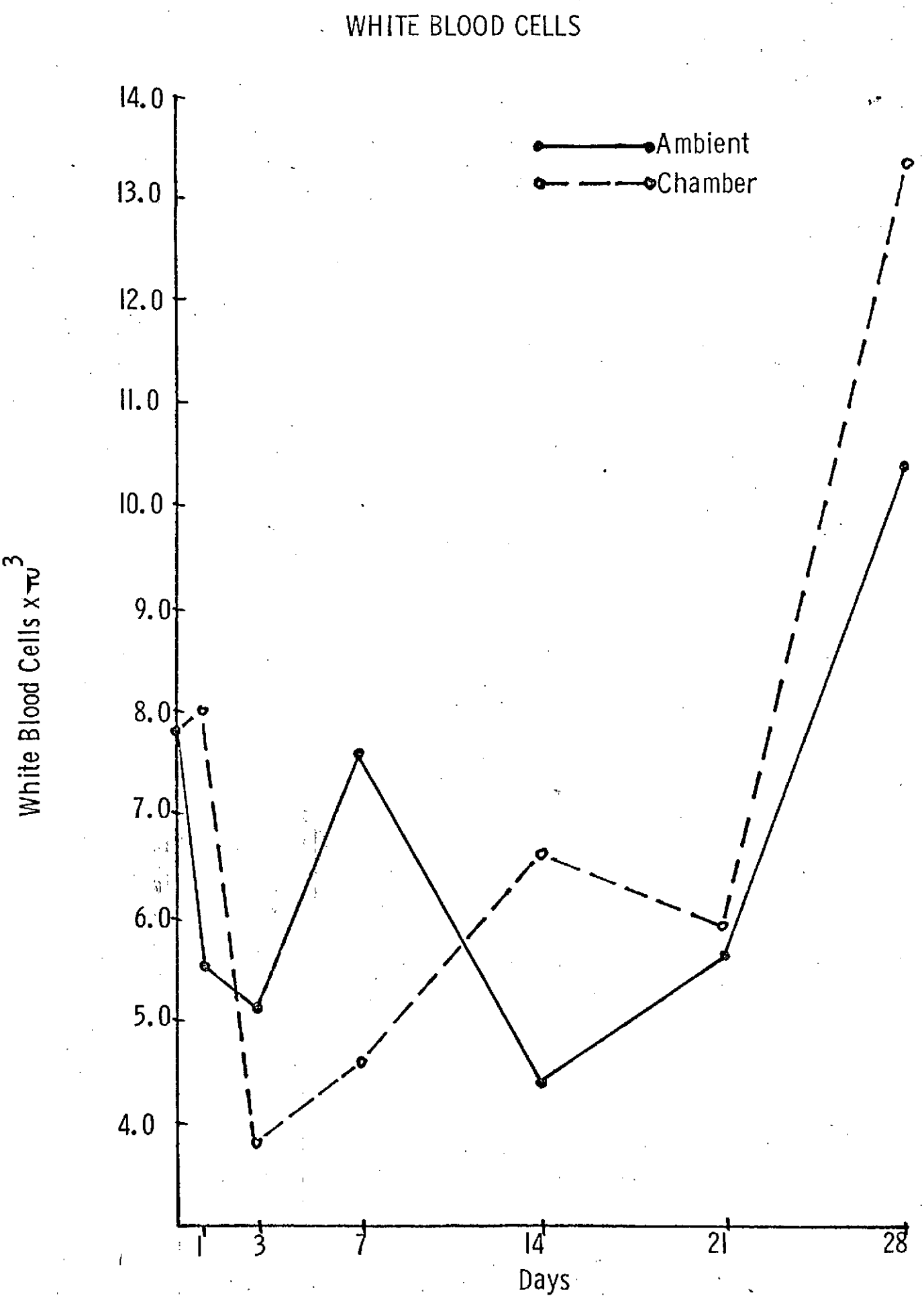

RED BLOOD CELLS
Ambient
Chamber
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
Red Blood Cells x 10^6
1
3
7
14
21
28
Days

Figure 8


WHITE BLOOD CELLS
Ambient
Chamber
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
White Blood Cells x 10^3
1
3
7
14
21
28
Days

Enzyme assays - The enzyme assays shown in Table 2 were performed on both groups. No significant changes were observed in serum enzyme levels.

Hemagglutination assay - A pronounced difference existed in antibody response of the chamber animals compared to controls. At day 3 to day 14, the antibody production to sheep RBCs was reduced to zero due to lack of IgG synthesis. The antibody level of the control group remained relatively constant through the 28-day period; whereas the chamber group reacted to the stress by cessation of antibody synthesis and then recovery by day 21 through day 28 (Figure 9).

Histologic evaluation of lymphoid tissue - Sections made of splenic tissue on 3 and 7 days post-exposure to SCE revealed a greatly reduced number of germinal centers being formed in the splenic follicles; whereas the control had strong signs of antigenic stimulation. These changes support the concept of a reduction in immunocompetence as the result of stress and support other observations such as reduced hemagglutination level, reduced WBC count, and reduced lymphoid organ weight (Figure 10).

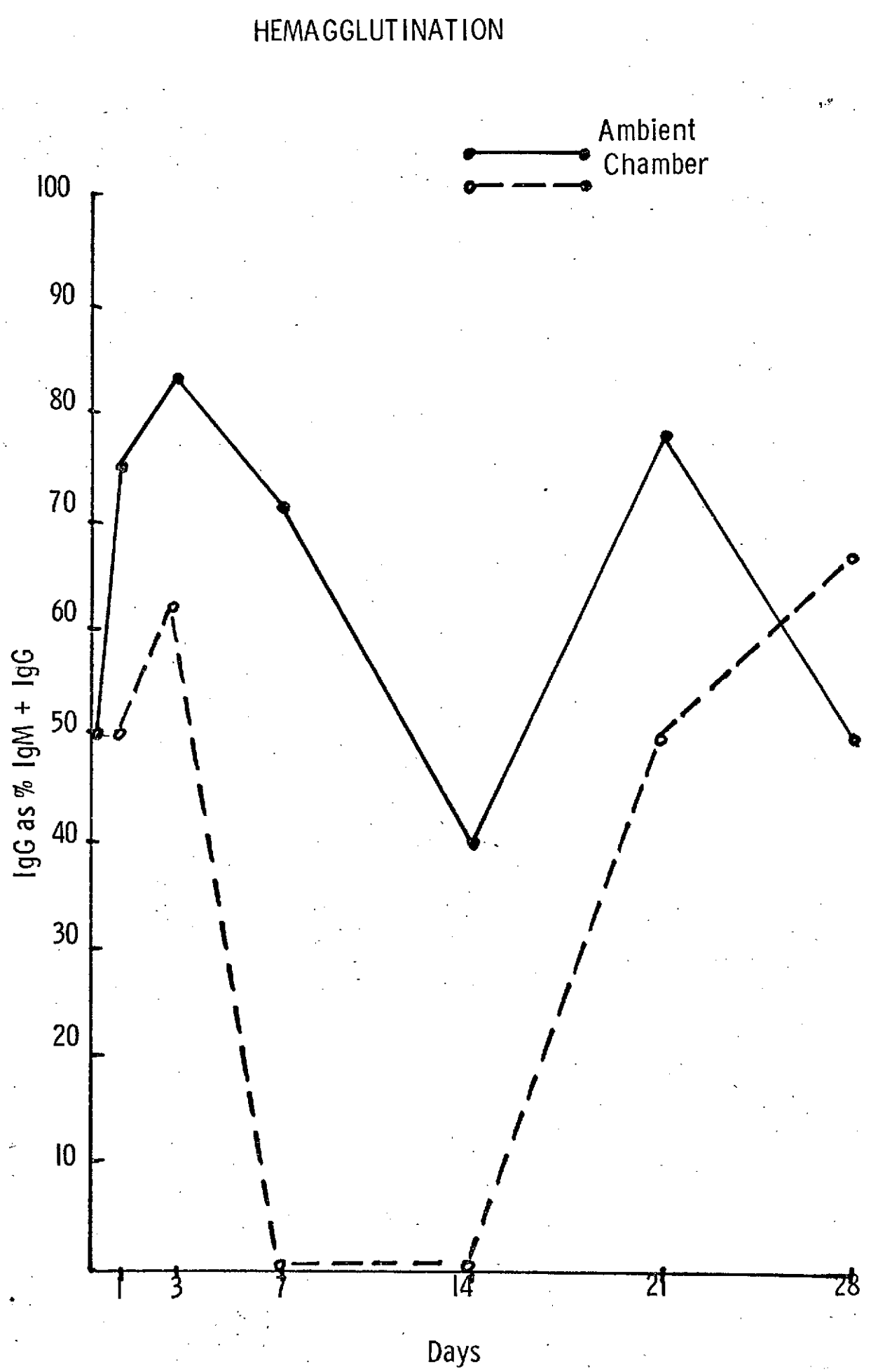
Histologic changes of mucociliary system - During the course of this mission, sections were prepared from representative areas of the respiratory tree and lung. In sections taken at 7, 14 and 21 days post-exposure to SCE, it became apparent that there was reduced secretion of the goblet cells as demonstrated by mucicarman stain. Also, these appeared to lessen height to the ciliary blanket. These changes were very apparent as seen in Figures 11 and 11' of bonchus sections from chamber and control animals. During these experiments it became obvious that the IITRI chamber had inadequate gas/chamber volume turnover or circulation of filtered gas. The ammonium level reached objectional levels in the antichamber (ambient lock) during removal of animals on test days when the lock was brought to ambient

conditions. Thus, many of the changes associated with the respiratory system may be directly related to ammonia toxic from urinary waste.

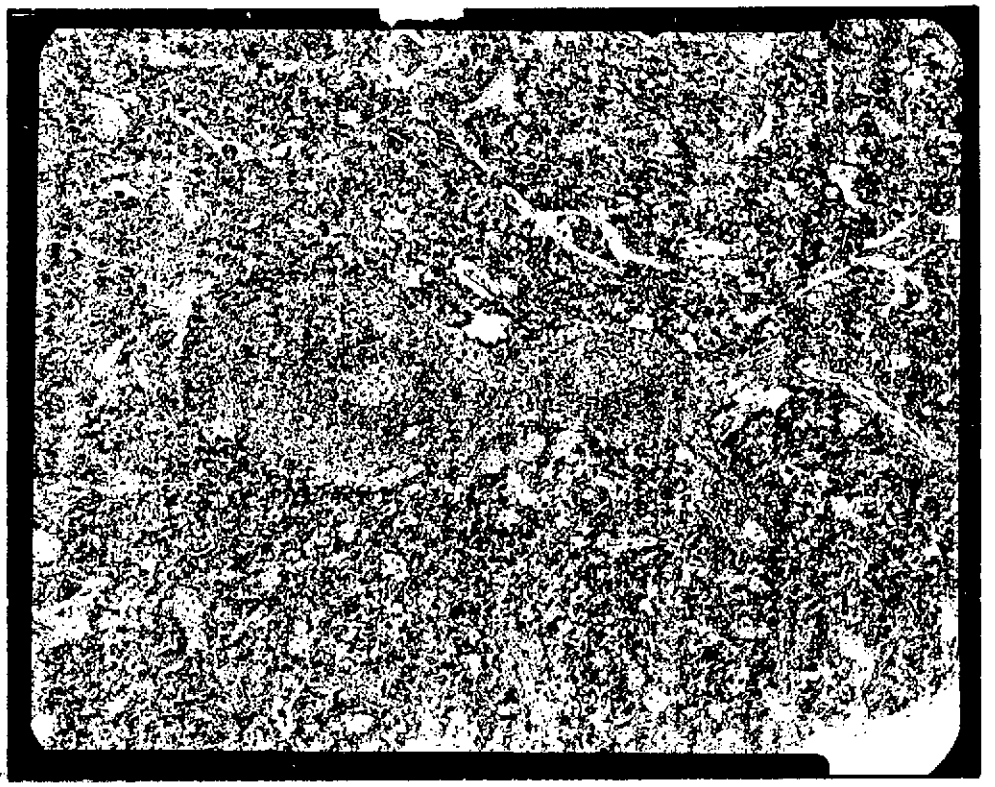
In conclusion, regarding preliminary experiments conducted at IITRI, the following became apparent:

1) The induction of ferrets into a SCE was stressful.
2) There occurred a reduced immunologic responsiveness associated with the initial events.
3) These changes could be easily documented in organ weights, serology and histology.
4) The histologic changes observed in the upper respiratory tree are likely the result of low gas/volume exchange in the space cabin simulator.

Figure 9


HEMAGGLUTINATION
Ambient
Chamber
100
90
80
70
60
50
40
30
20
10
IgG as % IgM + IgG
1
3
7
14
21
28
Days

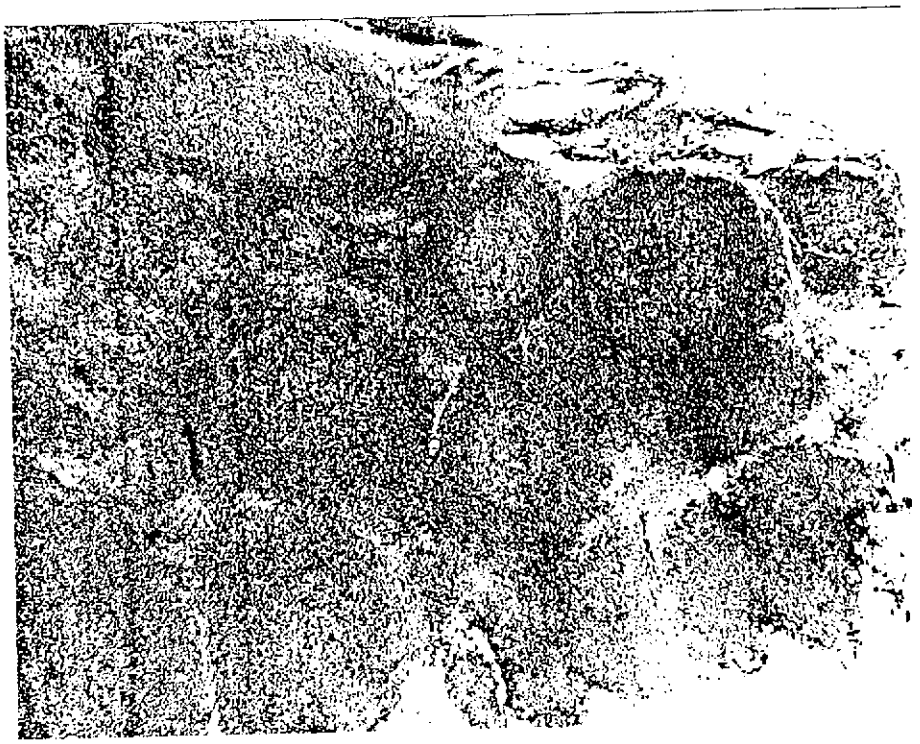
Splenic Follicle from Control Group



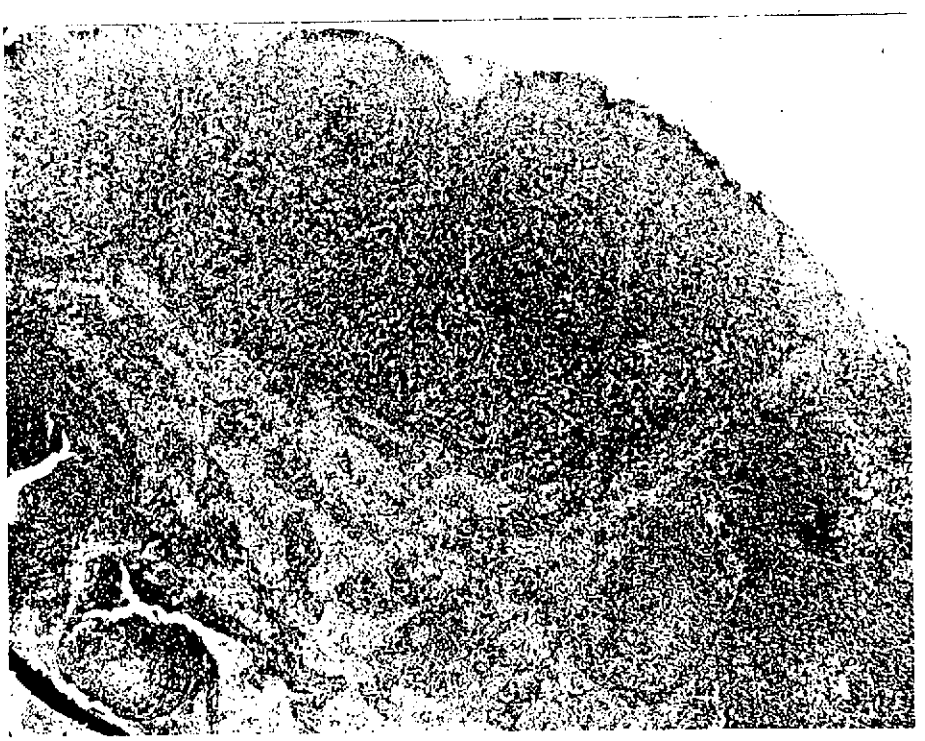
Splenic Follicle from Chamber Group

This figure depicts the lack of immunologic reactivity of splenic follicle of ferrets SCE after 7-14 days.

Lymph Node Control



Lymph Node Chamber Group



This figure depicts the lack of immunologic reactivity of lymph node follicle of ferrets SCE after 7-14 days.

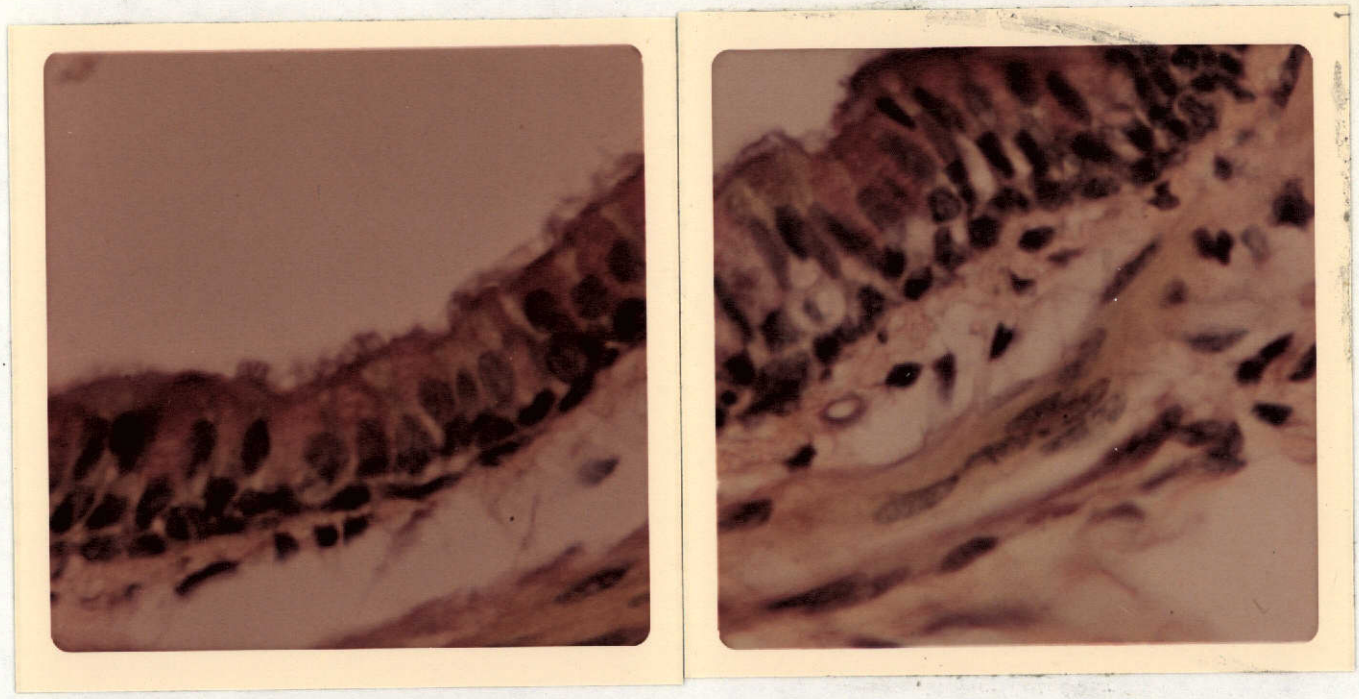
Figure 11

Bronchus Control

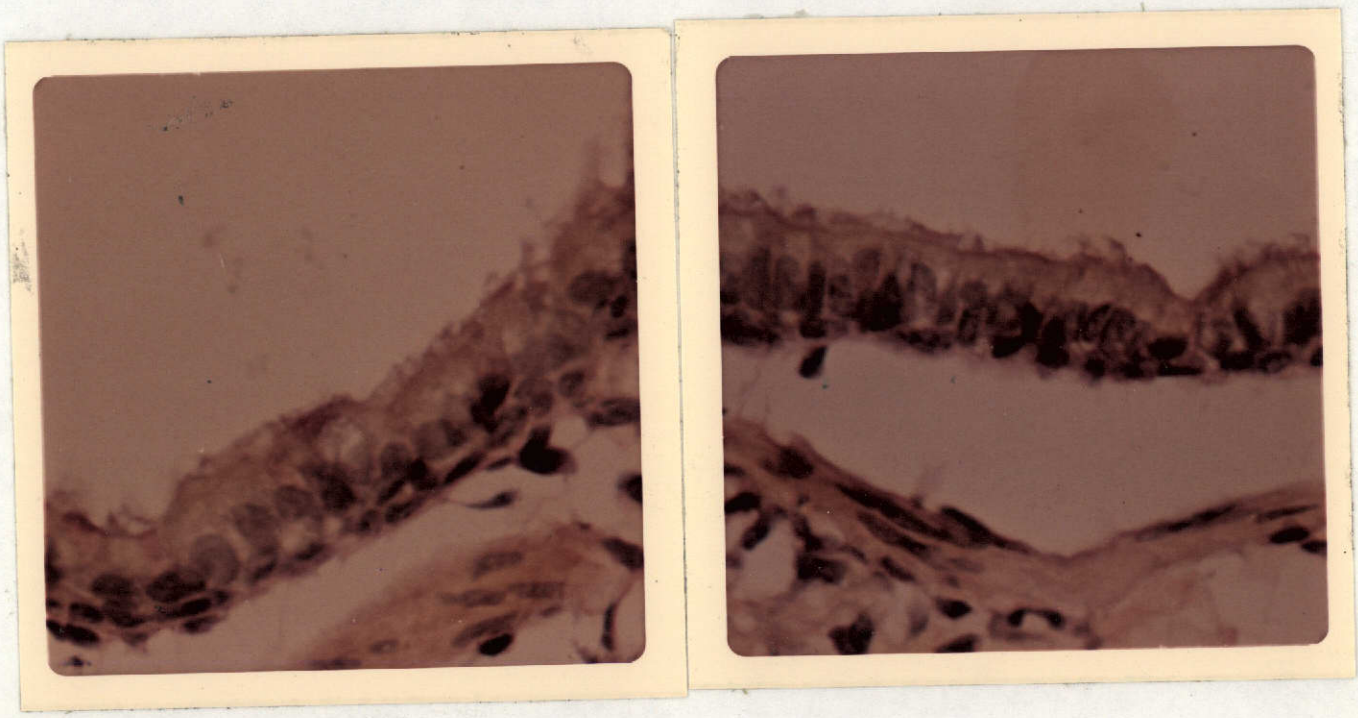
Bronchus Chamber Group

The top histologic section (mucicarman stain) shows normal development of mucociliary system, whereas in the bottom photpgraph the goblet cell secretion is greatly reduced.

Bronchus Control



Bronchus Chamber Group



The top histologic section (mucicarman stain) shows normal development of mucociliary system, whereas in the bottom photograph the goblet cell secretion is greatly reduced.

V. CHAMBER DEVELOPMENT

A. SPECIFICATIONS SOUGHT IN CHAMBER DESIGN

1.0 GENERAL

The purpose of this specification is to provide guide lines in the construction of a reduced pressure test facility to be installed at the University of Connecticut. The statements as to function are the minimum requirements and standard components or changes that will economically improve performance incorporated.

1.1 PURPOSE OF SYSTEM:

This unit is designed to hold at least 20 ferrets, either free or in cages at test conditions for a minimum period of 180 days. The animals must be maintained at the test conditions during feeding, cleaning, and selective removal of animals for examination. The system will operate on an open cycle at present but must be capable of easy modification to a closed cycle.

1.2 OPERATING PARAMETERS:

The chamber will operate with an atmosphere varying from air to 70% oxygen and 30% nitrogen supplied from an outside pressure source. The operating temperature will be 20 to 37^{o}C and relative humidity from 50 to 75%. The operating pressure will be from 760 to 200 mm Hg. A gas input of up to 2 scfm of an oxygen - nitrogen will be used that will be dry. The capacity to remove animals, move them from one chamber to another, feed and clean them is required. Manipulators as outlined in Section 3 will be used. The present system will operate under semi-automatic control as outlined in sections 5, 6, and 7 but will be converted to automatic control and recording at a later date.

1.3 RELIABILITY:

Due to the length of the test (180 days) and operating conditions, the unit must be designed to fail safe. The value of the test subjects increase with time and all equipment of utility failures should return or maintain the animals in a liveable atmosphere. It must be possible to isolate a failed component and replace it without aborting the test. Types of failure to be anticipated are, but not limited to:

a. Controller failure

b. Gas supply failure

c. Valve failure, open or closed

d. Power failure

1.4 PERFORMANCE SCHEDULE:
(After date of award)

0 Weeks	
2 "	Preliminary sketches
4 "	Schematics, flow sheets, material ordered
8 "	Full design drawings
12 "	Construction complete
16 "	Delivery
18 "	Acceptance

2.0 MAIN LOCK:

The main lock (2 required) will hold the 20 loose or caged ferrets.

2.1 SIZE:

The lock will have a working space of 30 x 30 x 48 inches long.

2.2 ACCESS:

One end will have a full opening door and the other a lock. Eight (8) 2 inch spare couplings will be on the rear wall for instrumentation and future use. Sufficient couplings are to be provided for necessary operating connections.

2.3 CONSTRUCTION:

The interior will be of 304 SS with all welds ground smooth and passivated. All internal fitting will be noncorroding. One wall will be transparent, and there will be a 3-inch view port in the main door.

2.4 Each lock will have manipulators as outlined in Section 4 and an air handling sytem as outlined in Section 5.

3.0 INTERCONNECTING LOCK:

Between the two main locks is an interconnecting lock. This unit is to facilitate the transfer of cages, animals and food so that the one main lock can be evacuated and ther other raised to atmospheric pressure.

3.1 This unit must handle a cage of 11 x 11 x 23 inches. Animals must be able to walk through or cages pushed through the lock.

3.2 The lock door should pressure-seal, if possible, to reduce leakage and swing completely out of the way to provide mobility for the animals and cages.

3.3 If the lock is longer than 12 inches, a view port must be provided.

4.0 MANIPULATORS:

Two types of manipulators are required for each lock. The units must be easy to use and functional. When not in use, they must not protrude into the interior or exterior of the chambers.

4.1 Two (2) manipulators will be provided for each main lock. Their purpose will be to pick up animals and place them in cages, move cages or transfer them in and out of the interconnecting lock. They must sweep the entire floor area of the chamber.

4.2 One (1) manipulator will be provided that will force free animals within the chamber to move from the main lock to the interconnecting lock. This unit should be flush with the front door and sweep the

entire floor area, pushing the animals into the lock. When not in use, the operating mechanism should be removed and the interior section should be flush with the front door.

5.0 TEMPERATURE, HUMIDITY CONTROL:

Due to the fact that the gas flow will be low, humidity is expected to rise; however, for small animal populations, the humidity may drop due to the dry gas input.

5.1 TEMPERATURE CONTROL:

20 to 37^{o}C plus/minus 2^{o}, the temperature will be maintained close to the ambient room temperature 22^{o}C 65% RH, however, slight deviations are expected. A temperature control and readout are to be provided utilizing a coil and blower assembly inside the main lock not occupying more than 1/2 cubic foot.

5.2 Humidity control 50 to 75% RH plus/minus 5% RH must be maintained at 250 mm Hg pressure. Humidity will be reduced through the use of cold coil and raised by adding moisture to a pad in the inlet gas line. The cold coil will have a drain that will be emptied to atmosphere. A humidity controller and readout will be provided reading in % RH.

6.0 ATMOSPHERIC PRESSURE CONTROL:

This system will maintain the unit at a test point from 250 mm Hg to 760 mm Hg plus/minus 10 mm Hg.

6.1 A vacuum pump, pressure controller and readout will be provided with the system.

6.2 The unit will be capable of pumping the system down in 15 minutes and handle a steady flow of 2 scfm for 180 continuous days.

6.3 A readout of pressure will be available for all three locks if required.

6.4 Changes in pressure will be modulated and capable of hand control.

6.5 The vacuum pump will not back stream oil and be compatible with 100% oxygen.

6.6 The vacuum pump can be isolated from the system and serviced if required.

6.7 The pressure of the chamber cannot go below 20 mm Hg of set point under any circumstances.

6.8 The pressure of the chamber cannot go above 780 mm Hg.

7.0 GAS CONCENTRATION CONTROL:

The purpose of this system is to maintain a liveable atmosphere for the test subjects.

7.1 The operating atmosphere of 70% oxygen and 30% nitrogen will be supplied from a pressure source supplied by the Laboratory at a rate from 0 to 2 scfm of either gas.

7.2 The oxygen concentration will be controlled by an oxygen sensor that has an accuracy of plus/minus 2% over a range of 2 to 200 mm of oxygen in any background gas.

7.3 The controller will have a readout in mm oxygen and an off-on control for admitting the correct gas to maintain operating conditions.

7.4 The sensor will not require replacement during the 180-day test or be continuously recalibrated and be insensitive to pressure changes.

7.5 The system will be compatible with conventional recorders.

7.6 The gas valving shall be such that on a controller failure, the atmosphere will remain habitable for the animals.

7.7 The gas valving shall permit operation of the system manually with empirical data secured from flow meters supplied with the system. Micrometering valves and pressure compensated flow meters will be provided.

8.0 APPLICABLE CODES:

8.1 The pressure portion of the system will be designed and constructed in accordance with applicable ASME Code Section VIII Unfired Pressure Vessels.

8.2 Electrical portions of the chamber will be in accordance with NFPA Code 56 and 70 and NEMA Standards.

9.0 ACCEPTANCE:

9.1 Acceptance tests outlined in Appendix 1 will be run and test data furnished to the chief investigator for final release.

9.2 One (1) reproducible copy of wiring schematics, flow sheets, and piping diagrams will be provided.

9.3 Assembly drawings and schematics will be approved prior to construction and any changes will be approved by the chief investigator.

9.4 Utility requirements will be established 30 days after date of award.

9.5 The manufacturer's engineer will be present during start-up of all tests and be available for training personnel on the use of the equipment until final acceptance.

10.0 APPENDIX 1 - ACCEPTANCE TESTS:

The purpose of these tests is to assure the customer that the unit will perform as requested.

10.1 VESSEL INTEGRITY:

The chamber will be evacuated to 250 mm of Hg and all valving closed. Pressure will not rise more than 100 mm Hg in 15 minutes.

10.2 The chamber will be controlled at a pressure of 250 mm Hg for 48 hours and there will not be a variation of more than 20 mm of Hg during that time.

10.3 The chamber will be set at 30°C and 65% RH and held at that point for 48 hours at 250 mm Hg within specifications.

10.4 The gas concentration will be set at 70% O_2 and 30% N_2 at 250 mm Hg and held for 48 hours with a constant flow of less than 2 scfm.

Note: Tests 10.2, 10.3 and 10.4 can be run simultaneously.

10.5 MANIPULATOR FUNCTION:

With the chamber at 250 mm Hg pressure, animals will be transferred from lock to lock and placed in the cages utilizing the manipulators.

10.6 FAILURE TESTS:

Unit will be operating at 250 mm Hg 70% O_2 and 30% N_2.

10.6.1 Power turned off - the system will return slowly to atmospheric pressure.

10.6.2 Inlet gas supply fails - the unit will return to atmospheric pressure.

10.6.3 Oxygen controller will fail high and low.
The oxygen concentration will not go below 160 torr.

10.6.4 The vacuum control will fail open. The pressure will not drop below 20 torr of set point.

10.6.5 On all above modes of failure, alarm contacts will close and remain closed until reset.

B. GENERAL DESCRIPTION OF CONSTRUCTED HYPOBARIC CHAMBER

The hypobaric chamber was capable of holding small animals (mice or ferrets) for long-term studies at partial vacuums to 5 psi for at least 180 days. A gas mixture of 70% O_2 and 30% N_2 was maintained by an oxygen analyzer (described below); temperature (range: 15-37^{o}C) and humidity (range: 50-75%) were controlled by appropriate regulators (described below).

The chamber was 9-feet long and fabricated from stainless steel (304 B SS) to comprise of 2 subchambers, each 4-feet long, joined by an interconnecting lock (see Figure 12, photograph of chamber). A subchamber permitted great flexibility in experimental designs. Namely, a control group could be housed on one side of the chamber opposite from the test group and retained at ambient pressure, thereby subjecting controls to identical conditions of confinement. Further, animals could be selectively removed without returning the entire cabin to ambient pressure (see Figures 13-17 for schematics).

The chamber was constructed to allow for minimum leakage--all welds had been ground smooth and passivated. Internal fittings were non-corroding to prevent moisture and/or ammonia from causing chamber deterioration. The front wall of each subchamber was reinforced 1-inch thick aircraft grade Plexiglas, to facilitate surveillance of experimental animals. Subchambers had a working space of 30" x 30" x 48" joined by an interconnecting lock, containing 2 inner doors; during operation of a single subchamber, one door of the lock was closed to insure that the gasket was pressure-sealing. External access to each subchamber was a full-opening door fitted with 2 pressure-sealing gaskets with a middle anulus.

Movement of animals between subchambers was accomplished by a pair of finger-like manipulators (sealed with "O" rings). The manipulators

Figure 12

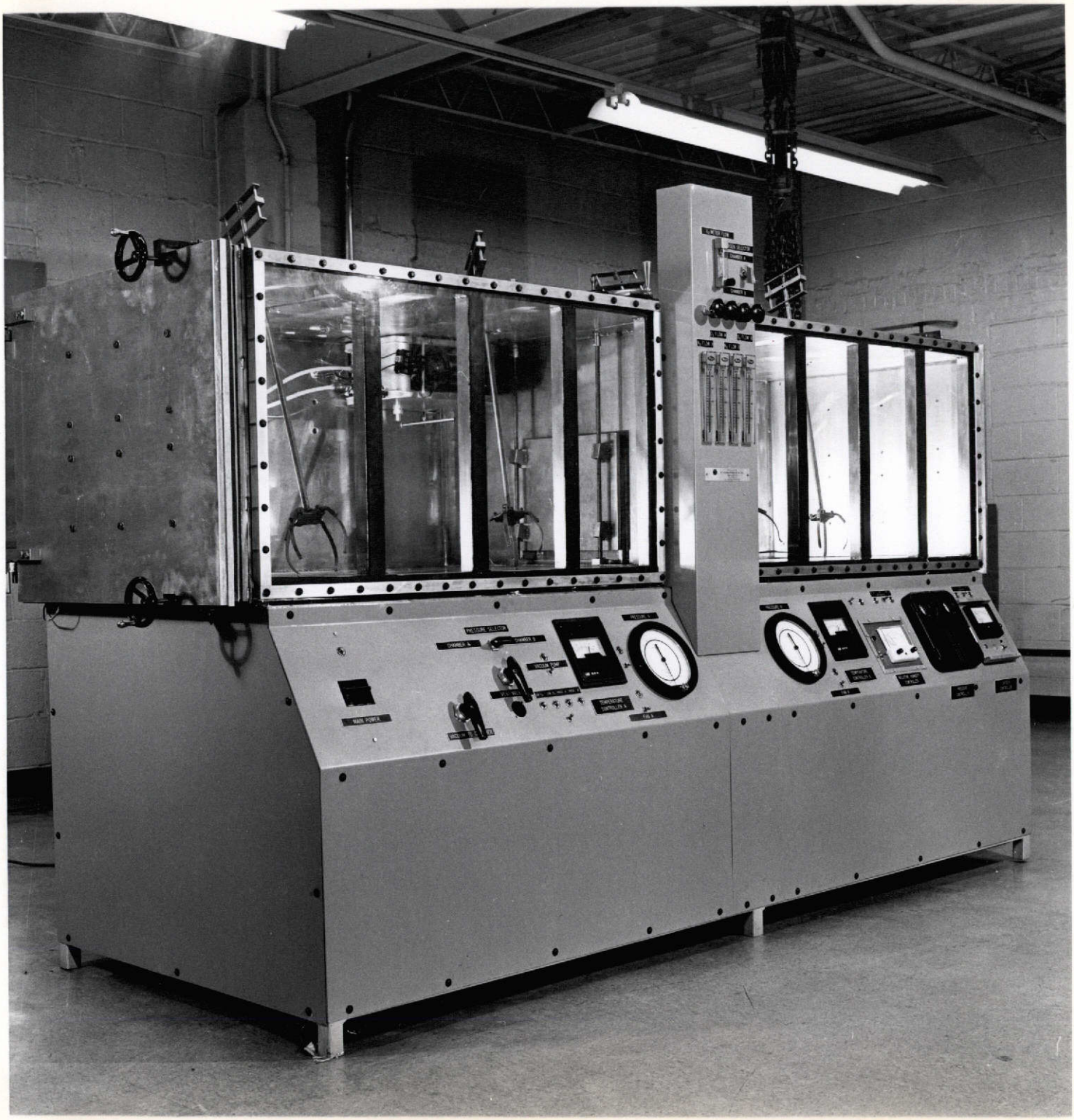
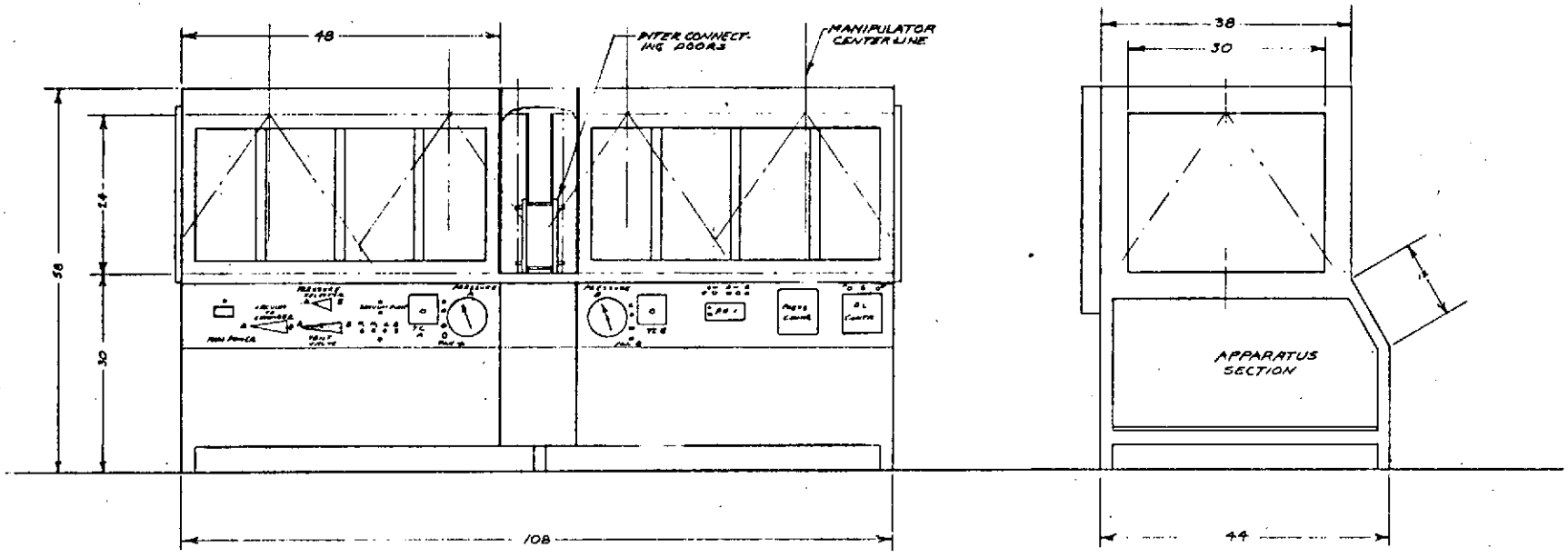
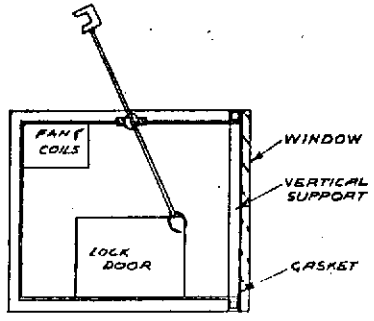

PRESSURE SELECTOR
CHAMBER A
CHAMBER B
MAIN POWER
VACUUM PUMP
TEMPERATURE CONTROLLER A
TEMPERATURE CONTROLLER B

Figure 13. Chamber Schematic:
General Arrangment





SECTION

REV. No.	No. OF REV.	DESCRIPTION OF REVISIONS	DATE	BY	BOMB
			DRAWN		DATE
			CHECKED		DATE
			APPROVED		DATE 7/27/71
			SHOP ORDER	EC-101	
			SCALE	1/2" = 1"	

TITLE: GENERAL ARRANG.

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Tel Area Code 215 - 838-7834

DRWG. No. UC-5 REV. NO

Figure 13

were spring-loaded, and protruded from the top of each subchamber; these units had the capability of sweeping the entire floor, and could be lifed from the subchamber when not in use.

Two drainage locks were installed in the bottom of the chamber which permitted draining the chamber and relative humidity controller overflow pans. Numerous safety devices had been included on the chamber, such as safety valves, lights, and alarms, which were activated by pressure-sensitive switches whenever loss of electrical power and/or unsafe reduction in either O_2 or N_2 occurred.

Since the major systems are complex, following is a description of the operating parameters used in the simulated Skylab cabin, after which the major component systems will be described in more detail.

C. SKYLAB ENVIRONMENT - OPERATING PARAMETERS

Gas was supplied by external tanks with a 70:30 O_2:N_2 mixture controlled by an oxygen analyzer. Air turnover rate was 2-4 changes per hour which at 5 psi is equivalent to 6-12 changes/hour. During most experiments, 17.0 standard cubic feet per hour (scfh) of O_2 was used, and approximately 7.5 scfh N_2. Nitrogen was also used to drive the valve on the vacuum pump, bringing total N_2 consumption to approximately 16.0 scfh. Routinely, temperature was maintained between 18 and 24°C, and relativehumidity between 30 and 60%. Both the temperature and relative humidity deivces had self-contained regulator switches, which were set to operate at desired experimental conditions. Temperature was adjusted to meet species requirements, but also fluctuated directly with changes in relative humidity. Chamber pressure 504 mm Hg $\pm$ 3 mm when operating, which is equivalent to approximately 34% of an atmosphere, or 5 psi.

D. MAJOR SYSTEMS COMPONENTS

The major components of the hypobaric chamber were a vacuum pump, temperature and humidity control devices, and an oxygen analyzer (Figs. 4-7). Overriding mechanical control was included on all these components to insure stability within the chamber, but adjustments could be made manually during chamber operation.

The vacuum pump evacuated the chamber to the pressure selected on the pressure controller. A hand valve on the chamber front determined which chamber was connected to the vacuum system, and a vent valve was included for rapidly returning the chamber to ambient pressure. If the pump was turned off or power was lost, a solenoid valve closed, thereby temporarily preventing loss of vacuum to the chamber. Repairs were made on the vacuum pump by shutting the gas inflow valves; vacuum was held for 12 hours during chamber performance tests of this nature. During normal operation, the pressure controller was set at 504 mm Hg; as an additional control, when the vacuum was above 524 mm Hg, a solenoid valve turned off the vacuum pump. During usual operation, when the desired vacuum was attained, a throttling valve restricted the flow of gas to the pump, thereby maintaining the desired cabin pressure.

Temperature was regulated by a controller which activated either a heater, or, upon high temperature, contacts closed an energizing solenoid valve allowing cooling water to flow through the coil. Shut-off of the internal heaters occurred when the temperature reached above 85^{o}C. Closely related to temperature control was the relative humidity system. Relative humidity was increased by admitting distilled water into the chamber, and was decreased by activating the cooling coil of the temperature system, which then condensed the excess moisture. If, as a result of this compensation for high humidity, the temperature dropped, the heater was activated

and air reheated. Both temperature control and humidity control systems have switches which determine which side of the chamber if monitored.

Oxygen concentration was controlled by an oxygen sensor which had and accuracy of $\pm$ 2% over a range of 2 to 200 mm of oxygen in combination with any gas. Concentrations of O_2 were regulated by a solenoid valve which admitted N_2 whenever necessary to maintain the planned gas mixture. When the controller was operating, internal fans were running to permit mixing of the gases; these fans also served to stabilize the cabin atmosphere. Safety features which were included so that in the event of controller failure, the atmosphere would remain habitable by shutting off N_2 flow. Similarly, if the vacuum pump failed, O_2 continued to flow into the chamber, slowly bringing the chamber to ambient pressure.

E. OPERATION MANUAL

1.0 GENERAL

This operating manual describes the function and operation of the components. Every attempt has been made to furnish full and complete instructuon. Should conditions arise that are not covered, please contact the contractor at the Sloan-Kettering Institute, New York for further information or consultation. Full brochures covering the subassemblies had been provided. Failure to read and observe their requirements may result in damage to the components of the entire system. The manufacturer recommended that the entire manual be read prior to operation.

1.1 PURPOSE OF SYSTEM

The chamber is designed to house animals for long-term studies at partial vacuums to 5 psia. The oxygen concentration should not exceed 70% at 5 psia. The temperature should be maintained from 18^{o} to 37^{o}C and the relative humidity at approximately 65%.

1.2 METHOD OF OPERATION OF SYSTEMS

A vacuum pump evacuates the chamber to a pressure selected by the pressure controller. When the set pressure is reached, a throttling valve restricts the flow of gas to the pump.

Oxygen input is set by adjusting a flowmeter on the control panel. If the oxygen content of the chamber is high, the oxygen controller admist nitrogen into the system. During normal operation there is always a flow of oxygen into the chamber. Temperature is regulated by a controller activating either a heater of valve admitting cooling water to a coil. A high temperature limit cutout is provided.

Relative humidity is controlled by admitting distilled water to a pad on low humidity or activating the cooling coil on high humidity to condense the moisture. Should the temperature drop, the heater will reheat the air.

1.3 METHOD OF OPERATION OF LOCK

The inner doors raise when the handles on the top of the chamber are rotated clockwise. During operation, only one door should be closed, so that the gasket is pressure-sealing.

1.4 MANIPULATORS

The chamber has 4 manipulators to facilitate the moving of the cages or animals about. These units are sealed with "O" rings and can be extended or retracted. Springs force the fingers to open and can be reversed or removed if desired.

1.5 DRAINAGE LOCKS

Below the chamber are 2 drainage locks. They permit draining of the chamber, pan and relative humidity overflow without venting the chamber. The upper valve is normally open and the lower valve is closed.

2.0 INSTALLATION

The unit should have the following utilities:

1. 200 volt - 60 cycle - 30 amp service.
2. 3 to 5 gallon/minute cooling water 50 psia "3/8" for vacuum pump and cooling coils.
3. 50 psig min nitrogen supply 10 cfm 1/4 tube.
4. 50 psig min oxygen supply 10 cfm 1/4 tube.
5. Distilled water supply for relative humidity control. 1/4" tube.
6. Vent to exterior for vacuum pump exhaust 1/2" hose.

7. Drain for cooling water out and for drain locks on chamber 1-1/2" line.

3.0 START UP

3.1 Be sure all the installation steps have been followed.

3.2 Check level of oil in vacuum pump - at least one inch should show in sight glass.

3.3 Turn on cooling water.

3.4 Close main switch. All other switches should be in the "off" position. All warning lights should be on. (See 4.0)

3.5 At this point all valves on panel should point vertically up. This is the closed position.

3.6 Turn on vacuum pump; pump should start and cooling water begin to flow.

3.7 Select pressure to be operated at (refer to controller instruction manual).

3.8 If only one chamber is to be drawn down, close the required doors.

3.9 Turn vacuum to chamber valve (HV-16) and pressure selector valve HV-17 to point to the left or right determined by the choice of chamber to be evacuated. NOTE: Both valves must always point in the same direction. Failure to do so will prevent the controller from monitoring the correct chamber and possibly damage the system.

3.10 The chamber will now lower in pressure.

3.10 At this time the setting of the other controllers should be checked out and the rest of the system turned on.

4.0 ALARMS AND SAFETY DEVICES

4.1 There are 4 warning lights on the console and a bell that is activated after an emergency condition arises for more than 3 minutes. Two lights are effected by chamber pressure opening when the vacuum exceeds 520 mm. Hg. Two lights open when the O_2 or N_2 supply pressure drops below 50 psig.

Upon alarm (after 3 minutes) the control valve will close and cannot open until the defect is corrected. Refer to wiring diagram UC-4.

4.2 There are 2 pressure-receiving valves at the rear of the chamber that prevent positive pressure from occurring inside the chamber.

4.3 There are two high temperature cutout thermal switches that will disconnect the heater if chamber temperature goes above $85^{o}F$.

All of the above devices can be regulated by referring to the instructions supplied.

5.0 VACUUM SYSTEM

5.1 Referring to drawing UC-2:

The vacuum is drawn by the pump through control valve CV-1. The selector valve HV-16 determines which chamber will be connected.

There are two throttling valves HV-12 and 13 which can regulate the flow thru the central valve.

On alarm, SV-2 is de-energized removing the signal from the control valve and closing it.

Should control valve CV-1 be inoperative, there are two bypass hand valves that can provide manual function.

When the pump is turned off or power is lost, SV-1 opens, venting the vacuum pump to permit easy starting and prevent oil back-streaming.

HV-17 determines what chamber pressure the instrument will see:

NOTE: Both HV-17 and HV-16 must be pointed in the same directions on the control panel at all times.

Vent valve HV-7 is available to rapidly vent the chamber.

5.2 The controller received its controlled air from the nitrogen supply via regulator R-3 mounted next to the control valve.

6.0 GAS SUPPLY SYSTEM

6.1 Oxygen and nitrogen are supplied from K or liquid cylinders at 50-60 psig through the rear panel. The gas is then passed through filters (mounted below the chamber).

6.2 The nitrogen line has a bleed connection (item 25) connected to pressure switch PS-1 and regulator R3 and then goes to the solenoid valves SV-7 and 8 with their bypass valves HV-1 and 2.

The panel of mounted flowmeters indicate flow into the chamber as regulated by HV-3 and 4. To set the flow, open the correct bypass valve and adjust the panel-mounted valve. When the oxygen controller calls for nitrogen, the solenoid valve will operate.

6.3 The oxygen line has a bleed connection (item 25) connected to pressure switch PS-2 and then goes to the panel-mounted flowmeters. This is set by simply adjusting panel-mounted HV-5 and HV-6.

7.0 TEMPERATURE CONTROL SYSTEMS

7.1 Switch 6 turns on the fan in chamber A and switch 8 energizes the temperature controller on low temperature; contacts close energizing heater H-1 mounted inside the chamber. On high temperature, contacts close energizing solenoid valve SV-3 allowing cooling water to flow through the coil. A hand valve is provided to adjust the flow for rate of temperature drop.

7.2 Switch 7 and 9 perform similar functions for chamber B.

7.3 High temperature cutout: shut off the internal heaters if the temperature is above 85°F.

8.0 RELATIVE HUMIDITY CONTROL SYSTEM

8.1 Switch 10 turns the humidity controller to a functioning position, switch 11 selects the appropriate chamber.

8.2 After setting the controller limits, when the R.H. is above the high set point, the cooling coil water flows, condensing moisture. When the R.H. is below the lower set point, water flows into the coil area.

8.3 Should the cooling coil during its dehumidification cycle drop the temperature, the heating coil will automatically be engaged.

9.0 OXYGEN CONTROL SYSTEM

9.1 Set the oxygen partial pressure upper limit. Using hand valve 24, select the chamber to be monitored. Turn on controller. Referring to the directions, adjust the flow after the warm-up period by the valve on the panel-mounted flowmeter.

9.2 When the controller is operating (refer to instructions), set the oxygen input flowmeter to the desired flow. When the concentration of oxygen exceeds the set point, the nitrogen SV-7 or 8 will open. Adjust Nitrogen flow. During the oxygen control, the internal fans must be running to permit mixing of the gases.

9.3 When the oxygen meter is turned on, a pump will start. This pump raises the gas from partial vacuum to ambient so that an accurate reading of the meter is obtained. The partial pressure reading on the meter is referenced at ambient pressure.

10.0 MAIN COMPONENTS

1. Vacuum Pump Main

   Beach-Russ Size #1-1/2-D Type SS rotary single stage. 1 HP Motor Unit has been filled with: Houghto Safe 1010 manufactured by E. F. Houghton & Co., 303 W. Lehigh Avenue, Philadelphia, Pennsylvania 19133, Telephone 215-739-7100. Use only this type - phosphate ester, due to oxygen service.

2. Vacuum Pump Oxygen Sensor Supply, Metal Bellows Corporation, Pump MB-155, SN #15-194, 1075 Proficence Highway, Sharon, Massachusetts 02067.

3. Fisher F4152-2C9, Low Pressure Controller, 4150X2-A1-B1-D6-E10-F1-G1-H1-J1-K1-L1-N1-P1.

4. Fisher Valve with F5513R-13, Activator, 1/2" type 513R-GS G5X1-A1-B17-C50-D1-E1-F1.

   Fisher Controls Company, Marshalltown, Iowa 50518

5. Temperature Controller -40 to 50°C, UE Type 802P Model 4BS

6. Vacuum Switches, 0-30" vacuum, UE Type H54 model 22

7. Pressure Switches, 10 to 100 psig, UE Type H54 model 25

   United Electric Controls Company, 85 School Street, Watertown, Massachusetts 02172, Telephone 617-926-1000.

8. Flowmeters, F. W. Dwyer Mfg. Co., Inc., P.O. Box 373, Michigan City, Indiana 46360, Telephone 219-872-9141 No. RMB-54 range 20-200 scfh air.

9. Gauges, Heise Bourdon Tube Co., Inc., South Main Street, Newtown, Connecticut 06470, Telephone 203-426-4406 #CM-7779-7780.

10. High Temperature Cutout Model AIS Code 161, Burling Instrument Company, P.O. Box 298, Chatham, New Jersey 07928, Telephone 201-635-9481.

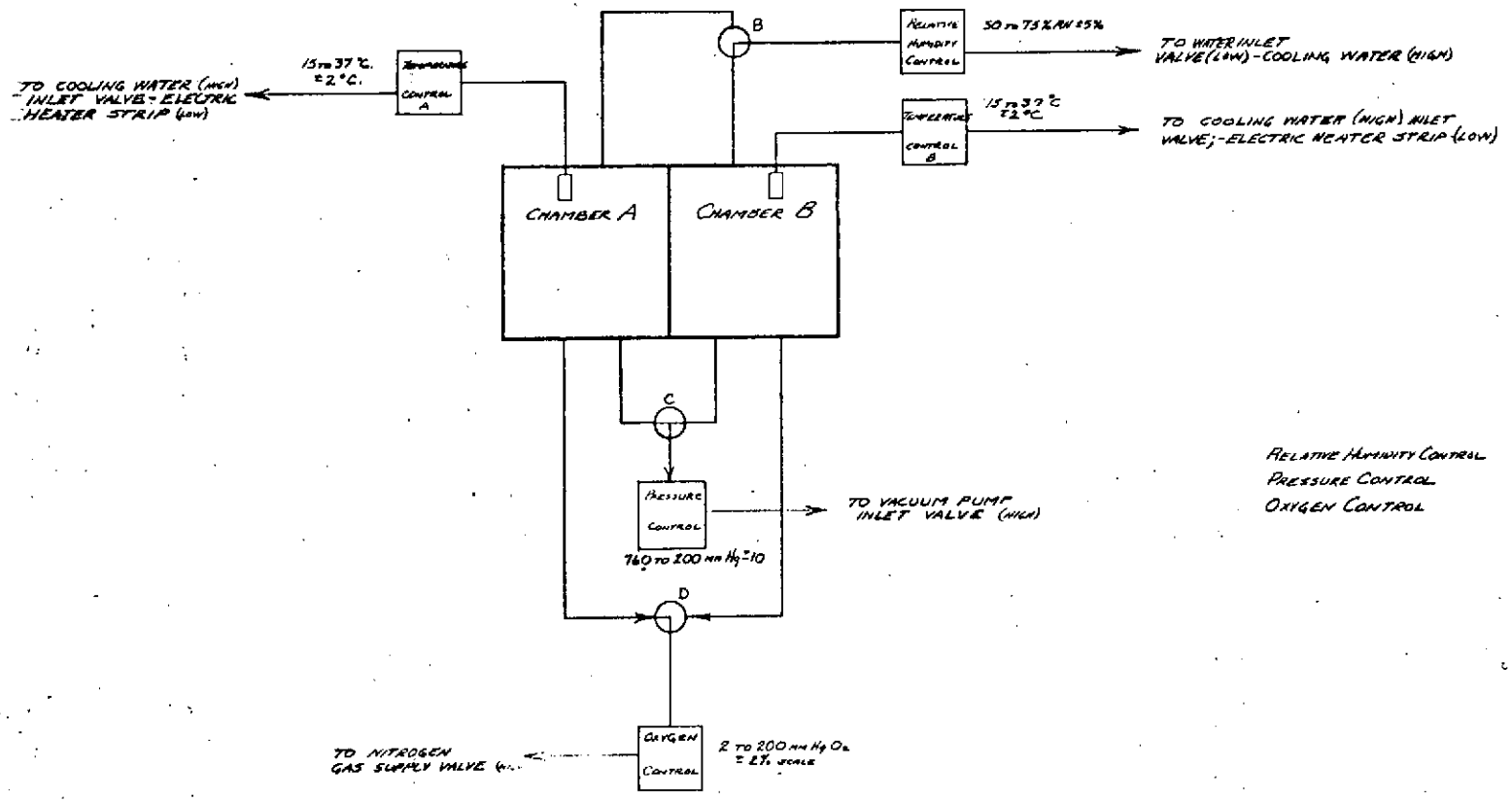
11. Relative Humidity Controller, Environmental Devices, Inc. Amlab. Instrument Division, Ivoryton, Connecticut 06442, Model HIC-2 with 2 sensors, Zach Abbey.

12. Oxygen Analyzer, Model 11 System/pump, Thermo-Lab Inst., Inc. 1308 Wm. Flynn Highway, Rt. 8, Glenshaw, Pennsylvania 15116, Don Sayles.

(For details see schematics, Figures 13-17.)

Figure 14. Chamber Schematic:
Controller Flow Sheet

Figure 14



	Valve	Selection
Relative Humanity Control	B	" A or B
Pressure Control	C	" A or B - A & B
Oxygen Control	D	" A or B

REV. NO.	NO. OF REV.	DESCRIPTION OF REVISIONS	DATE	BY	CHKD
			DRAWN		DATE
			CHECKED		DATE
			APPROVED	SJJ	DATE 7/24/81
			SHOP ORDER	EC-101	
			SCALE	NONE	
			DWG. NO.	UC-1	REV. NO.

TITLE: CONTROLLER FLOW SHEET

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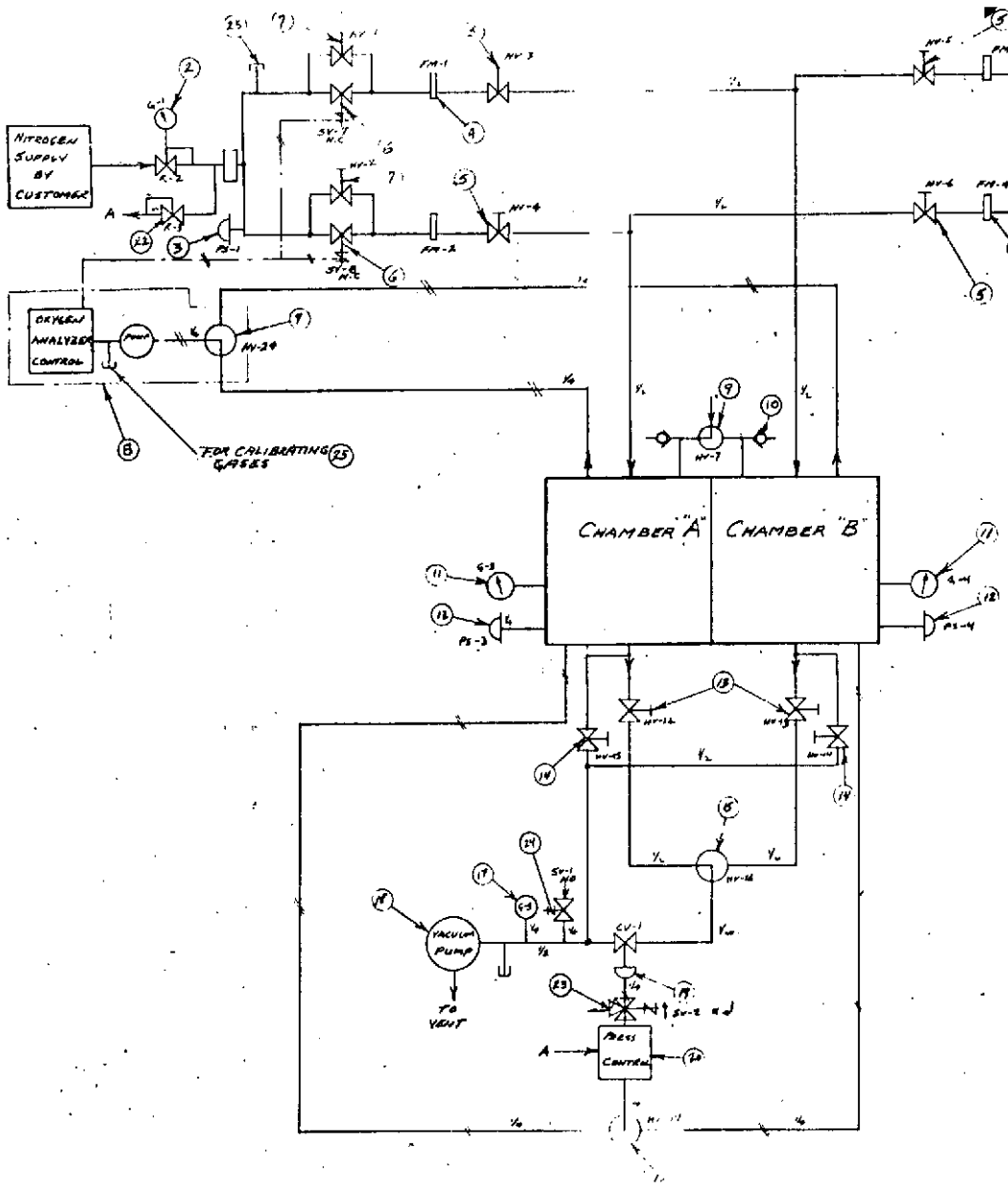
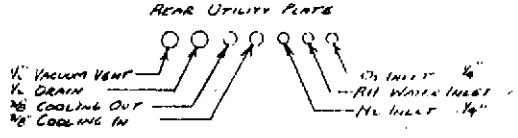
Figure 15. Chamber Schematic: Gas and Pressure Flow Diagram

Figure 15



ITEM	NAME	SPECIFICATION
1	REGULATOR	100-3000 PSIG INPUT 50 PSIG OUTPUT OXYGEN
2	"	" " NITROGEN
4	FLOWMETER 0-10 SLPM	OXYGEN 50 PSIG INPUT 5-15 PSIG OUTPUT
5	"	NITROGEN "
6	SOLENOID	CONTROL N2 FLOW INTO CHAMBER
8	O2 ANALYZER CONTROLLER	CLOSES CONTACT O2 RISE 0-760 MM Hg CONTROL
10	CHECK VALVE	SET TO RELIEVE ON POSITIVE INTERNAL PRESSURE
12	PRESSURE SWITCH	OPEN CONTACT PRESSURE DOWN Hg BELOW SET POINT 100-760 MM H_2 - CLOSE WHEN SET CONTACT FOR ALARM SET AT 520 MM Hg RESETS AT 760 MM
20	PRESSURE CONTROLLER	3-0 PSI OUTPUT 100-760 MM Hg CONTROL ETC.
22	REGULATOR INSTRUMENT	50 PSI INPUT 20 OUTPUT.
23	SOLENOID 3 WAY	VENT CONTROL VALVE OPERATING ON ACTS AS RELIEF.
24	SOLENOID VALVE	2 WAY - VENT VACUUM PUMP IN OFF CONDITION.
3	PRESSURE SWITCH	N2 & O2 SUPPLY - TRIPS SUPPLY BELOW 50 PSIG

REAR UTILITY PLATE



REV. No.	No. OF REV.	DESCRIPTION OF REVISIONS	DATE	BY	CHNG
1	-	GENERAL	2/15/72	SST	

TITLE: GAS & PRESSURE FLOW DIAGRAM

DRAWN — DATE
CHECKED — DATE
APPROVED SST DATE 9/10/71
SHOP ORDER
SCALE

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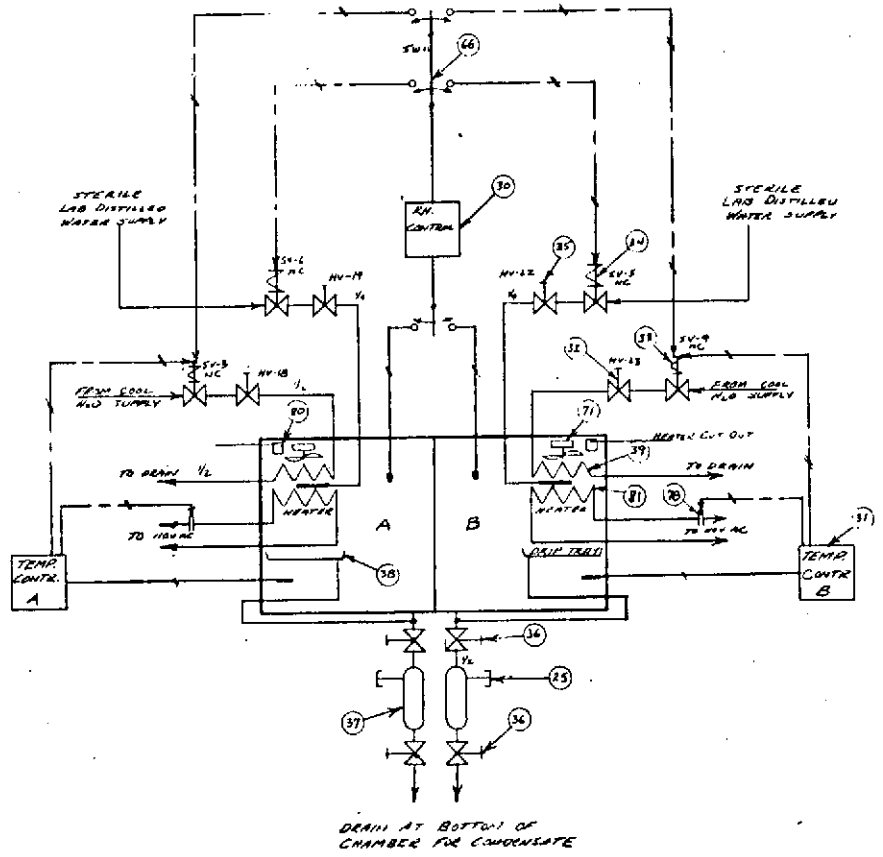
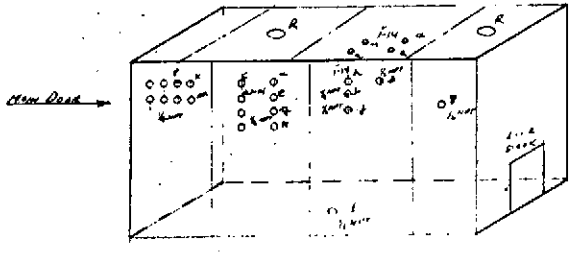
DRWG. No. UC-2 REV. No. 1

Figure 16. Chamber Schematic: Temperature and Humidity Systems

Figure 16





REAR VIEW OF CHAMBER SHOWING FEED THRU LOCATION

FEED THRUS REQUIRED

CHAMBER "A"				CHAMBER "B"			
Nos	Size	Use	Location	Nos	Size	Use	Location
6	1-14	SPARE	a		1-14	SPARE	a
1	½ NPT	PRESS. H2O TRANS.	b	1	½ NPT	PRESS. H2O TRANS.	b
1	1-14	HELIUM RH SENSOR	c	1	1-14	HELIUM RH SENSOR	c
1	½ C	O2 SENSOR	d	1	½ C	O2 SENSOR	d
1	1-14	COOLING WATER IN	e	1	1-14	COOLING WATER OUT	e
1	½ NPT	DRAIN	f	1	½ NPT	DRAIN	f
1	½ NPT	FAN DRAIN	g	1	½ NPT	FAN DRAIN	g
1	1-14 C	HELIUM RH SENSOR	h	1	1-14 C	HELIUM RH SENSOR	h
1	½ NPT	HTEMP CUT OUT	i	1	½ NPT	HTEMP OUTLET	i
1	½ C	PRESSURE SENSING	k	1	½ C	H2O2 VAPOR SENSING	k
1	¾ NPT	RH WATER IN	L	1	¾ NPT	RH WATER IN	L
1	½ NPT	O2 RH PILOT	m	1	½ NPT	O2 RH PILOT VENT-DEVICE	m
1	½ NPT	VENT-RELIEF	n	1	½ NPT		n
1	½ NPT	MAIN DOOR INFLATION SOLENOID	P	1	½ NPT	MAIN DOOR INFLATION SAFETY SOLENOID	P
1	½ NPT	VACUUM OUTLET	q	1	½ NPT	VACUUM OUTLET	q
1	2½	TOP MANIFOLD	R	1	2½	TOP	R
5	¼	SPARE		5			
2	½	SPARE		2			

REV. No.	No. OF REV.	DESCRIPTION OF REVISIONS	DATE	BY	DRWG
2		GENERAL	2/25/72	SEF	
1		GENERAL	10/15/71		

TITLE: TEMP. HUMIDITY SYS.

DRAWN: DATE
CHECKED: DATE
APPROVED: JJ DATE 7/6/72
SHOP ORDER: EC-1101

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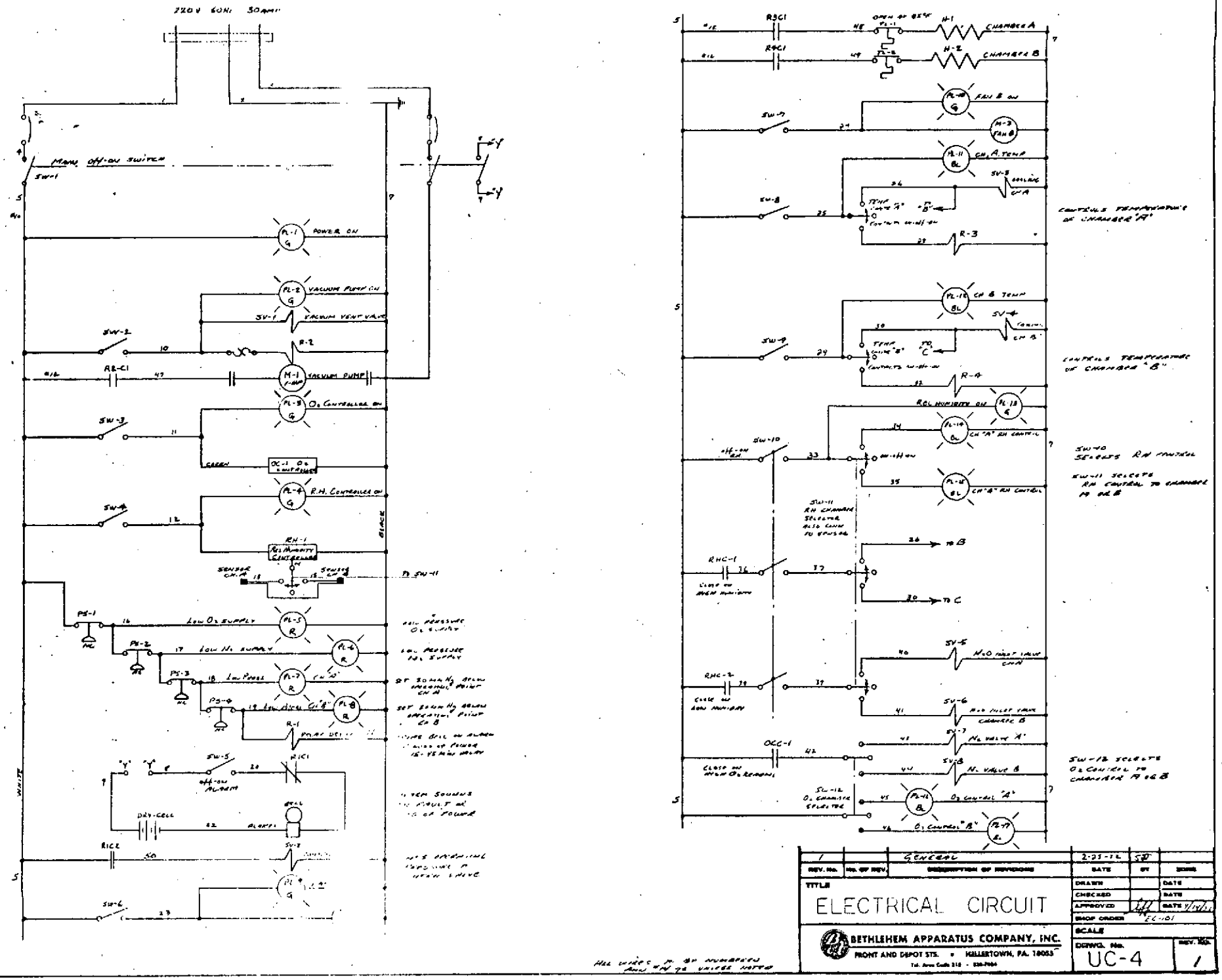
SCALE
DRWG. No. UC-3
REV. No. 2

Figure 17. Chamber Schematic:
Electrical Circuit

Figure 17



		General	2-23-72	SD	
REV. No.	NO. OF REV.	DESCRIPTION OF REVISIONS	DATE	BY	BOARD

TITLE: ELECTRICAL CIRCUIT

DRAWN | DATE
CHECKED | DATE
APPROVED | DATE 3/24/72
SHOP ORDER EC-101
SCALE

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DWG. No. UC-4 | REV. NO. 1

ALL WIRES #18 AWG UNLESS NOTED

VI. SPACECRAFT ENVIRONMENT STUDIES

A. INTRODUCTION

Heightened susceptibility to infection, although having been suggested by numerous investigators, had not been clearly established with the proposed gas mixtures for Skylab. The parameters planned for use in the Skylab mission were a 70:30 O_2:N_2 mixture, which at 5 psi, is equivalent to a partial pressure of O_2 similar to sea level conditions. The studies were undertaken to establish the effect of reduced pressure on the respiratory clearance system, which was followed by clearance of titanium dioxide (TiO_2) as inert dust. General immunocompetence of animals housed in SCE was evaluated by the pattern of antibody production to Brucella abortus and by in vivo pathogenesis of influenza and hepatitis virus. Finally, it was of interest to ascertain whether the apparent stress phenomena is a generalized stress or specifically related to diseases of the respiratory tract.

B. MATERIALS AND METHODS

1. EXPERIMENTAL MATERIALS

a. Mice - CD-1 strain mice which were uniform in size (24-26g) were obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts 01887). For most experiments, mice selected were 3-4 weeks old; when suckling mice were used to conduct studies using hepatitis virus, they were 10 days old and the litters kept with their dams.

b. Ferrets - All ferrets utilized in hypobaric tests were obtained from Marshall Research Animals, Inc. (North Rose, New York 14516). Animals were pre-bled to assure normalcy, and on the basis of normal immunoglobulin levels, were determined to be free of Aleutian disease. In the studies conducted, young female (dark phase) ferrets, weighing approximately 400 g were used.

c. Animal Maintenance - Mice were fed dry, pelleted Purina Mink Chow (Ralston Purina Co., St. Louis, Missouri). Water was provided by Ancare plastic water bottles (Ancare Corporation, Manhasset, Long Island, New York 11030); watering tubes were used in one experiment, but later they were replaced with special "demand" valves which were essentially leakproof. Finally, with mice, it was considered necessary to use commercially prepared moist shipping diet to eliminate the use of water bottles entirely; this sterile feed was obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts).

A group of 6 mice (or 10 suckling mice plus dam) was kept to an 11-1/2" x 7-1/2" x 5" polystyrene disposable cage (Maryland Plastics, Inc., New York, New York 10016); cage tops were Maryland Plastic's #28 "Econoclid", which were slightly modified with a larger food hopper. Sawdust and wire inserts were placed in the bottom of the cages; the wire inserts keeping

all mice visible even upon death of an animal. Control mice were kept in the same manner; in the case of the first hypobaric test, the controls were placed in rigid plastic isolators (Ambient Domes, described below).

Ferrets were maintained on dry, pelleted mink food (G'n Dry Mink Food, SDR Enterprises, Inc., Andover, Connecticut). Water was admitted from an external source into a watering dish; water could be admitted at any time by a switch. Ferrets were "group-housed" in a large, wire cage. It was equipped with an aluminum sliding door which opened into a pass-through, connecting the cage of one subchamber to its mirror-image or counterpart in the other subchamber. Animals were moved from one subchamber to the other for cleaning. Selective removal of animals was accomplished by individually trapping the chosen animals in weighted wire baskets. Animals intended for later kill periods were retained in the other subchamber. After the pass-through was removed, the appropriate inner door was closed, and the selected side of the chamber brought to ambient pressure. This design permitted maintenance of the experimental groups at altitude, while still allowing for removal of animals. Control groups were housed in rigid plastic isolators (Ambient Domes, described below).

2. VIRAL INOCULA

a. Murine hepatitis virus - Murine hepatitis virus (MHV_3 strain) was obtained from Dr. T. N. Fredrickson (Department of Pathobiology, University of Connecticut, Storrs, Connecticut 06268). The virus had been passaged in peritoneal macrophage cultures, and contained 7.6×10^7 plaque-forming units/ml. Prior to use in this study, it was passaged once in newborn CD-1 mice. Inoculum used in this study was a 10% homogenate of infective livers in Medium-199 (Grand Island Biological, Grand Island, New York).

b. Influenza virus - Mouse-adapted influenza A/PR-8 was obtained from Dr. James D. Fenters (I.I.T. Research Institute, 10 West 35th Street, Chicago, Illinois 60616). The MLD_{50} (mouse lethal dose) was certified to be $10^{5.84}$/0.04 ml.

3. BRUCELLA AGGLUTINATION ASSAY

Sera from ferrets were harvested from blood samples obtained by cardiac puncture, and frozen in order that all assays be performed simultaneously. Antibody titer was determined by standard tube agglutination tests as follows: Brucella abortus tube antigen (U.S.D.A. Animal Disease Laboratory, Ames, Iowa) was prepared according to the manufacturer's instructions. To each tube 0.50 ml serum, plus 1.50 ml tube antigen was added; subsequent tubes contained 1.0 ml tube antigen, and 1.0 ml was transferred, making doubling dilutions through 4096. Tubes were incubated at 37°C and titer determined after 24 hours of incubation and at 48 hours.

For differtiating the classes of immunoglobulins, sera were incubated for 24 hours in 0.10M mercaptoethanol (i.e. 2 µl/0.50 ml) which reduced the disulfide groups of the 19S components resulting in subunits. Dilutions and incubation of the sera were identical to that described above.

4. AMBIENT DOMES

Ambient* control animals (mice and ferrets) for the hypobaric studies were housed in rigid, 6-foot plastic isolators (6 MP-TmP, The Germfree Laboratories Inc., Miami, Florida 33133). These units are described in the Final Report of Contract No. NAS 9-10844, Mod. No. 1S-Studies of Effects of Closed Microbial Ecology, 180-Day Test Period. The ambient domes

*The term "ambient" refers to the atmospheric pressure encountered at Storrs, Connecticut.

were used to provide animals with air flow and temperature equivalent to that of the hypobaric chamber. Sterility was not maintained in ambient domes because it was not relevant to the problems being investigated, namely, pressure changes and gas mixtures.

C. EXPERIMENTAL DESIGN

1. HYPOBARIC EFFECT ON MICE

Five experimental groups were included in this experiment (see Table 1 for description of groups). Groups I and IV were placed in nest boxes in the hypobaric chamber; in each test the ascent rate was near 1,000 ft/min for the first 3 minutes, then slowed to approximately 500 ft/min until the 27,000 foot altitude was attained. Groups II, III, and V were held in ambient pressure domes, having environmental parameters (temperature and air movement) similar to the space cabin, except that pressure in the domes was near that of sea level. Prednisolone (Wolins Pharmaceutical Co., Melville, New York 11746; 5 mg/mouse) was given to Group II subjects on days 0 and 12, in an attempt to establish a maximum stress effect with corticosteroids. Group IV test subjects had been previously aerosolized (on days minus 4 through minus 1) intermittently, for a total 24 hour exposure. Six mice each were scheduled to be killed on days 1, 3, 7, 11, 21 and 28 from the hypobaric groups (Groups I and IV). However, due to mechanical failure* of the vacuum system on day 4, the experimental design was slightly modified, as follows: When the chamber was returned to ambient after 104 hours of operation, the mice planned for the 21 and 28 day kills in Groups I and IV were used, giving a total of 12 test subjects in Groups I and IV on day 4 (Table 4 clarifies the modified experimental plan). Despite return of the chamber groups to ambient pressure on day 4, kill periods were continued through day 11 for Groups I and IV, and through day 21 for the other 3 groups, as it was thought that a stress event, if occurring at all, would take place early, and further, this effect could be seen as

*At 96 hours of operation, the seals of high vacuum pump began leaking diffusion oil, which resulted in lower pumping efficiency, causing repeated changes in pressure inside the chamber. It was the manufacturer's belief that appropriate seals had not been installed to accommodate an aryl-phosphate ester oil, thus the chamber was brought to ambient pressure after 104 hours into the run.

TABLE 4. Experimental Design for Hypobaric Test in Mice

Group	Day 1	3	4	7	11	21	28
I[a]	6[b]	6	12	6	6	0	0
II	6	6	6	6	6	6	0
III	6	6	6	6	6	6	0
IV	6	6	6	6	6	0	0
V	6	6	6	6	6	6	0

[a]Experimental Groups

I Chamber
II Prednisolone
III Control
IV TiO_2 - Chamber
V TiO_2 - Control

[b]Numbers represent the number of mice per kill period

easily upon descent to ambient pressure as ascent to atitude. At kill periods, complete postmortems were performed on all subjects, body weight, major organ weights were recorded, and tissues were fixed for histopathological examination. Portions of lung from Groups IV and V were assayed for TiO_2 concentration by the hydrogen peroxide method (see Final Report: Contract No. NAS 9-11575; Biological Activity of Lunar Soil).

2. HYPOBARIC EFFECT ON FERRETS

In a similar experiment (as described above) a 28-day test run was completed using ferrets (see Table 5 for experimental design). All ferrets were immunized with 0.10 ml (16.50×10^9 cells/ml) Brucella abortus, Strain 19 vaccine (Colorado Serum Co., Denver, Colorado 80216) intramuscularly on day minus 5 (5 days prior to introduction into SCE). Group IV animals had been aerosolized with TiO_2 intermittently for 24 hours (days minus 4 through minus 1), and these animals were marked by tags on chain "necklaces", to distinguish these animals from the Group I ferrets. Groups III and V were controls to the Groups I and IV animals, respectively, and were individually caged, and then placed in the ambient pressure dome. As in the mouse experiment described above, a group of ferrets was given Prednisolone (10 mg), intramuscularly, on day 0, and they were also placed in isolators. Aerosolized controls (Group V) to the TiO_2-aerosolized animals (Group IV) were maintained with other control groups at ambient pressure in isolators. On the given kill periods, gross postmortems were performed, including body weight, major organ weight, histopathology, antibody titer to Brucella, and in Groups IV and V, TiO_2 assays were performed on portions of lung. Five normal ferrets were killed on day 0 to establish control values; two TiO_2 aerosolized ferrets were killed 24 hours after the last aerosolization period, to establish baseline values for TiO_2 clearance.

TABLE 5. Experimental Design for Hypobaric Test in Ferrets

Group[a]	Days at Space Cabin Environment					
	1	3	7	12	21	28
I	3[b]	4	3	3	3	2
II	1	1	1	1	1	1
III	2	2	2	2	2	2
IV	2	2	2	2	1	0
V	1	1	1	1	1	1

[a]Experimental Groups

I Chamber
II Prednisolone
III Control
IV TiO_2 - Chamber
V TiO_2 - Control

[b]Numbers represent the number of ferrets per kill period

3. MURINE HEPATITIS VIRUS INFECTION IN SCE

This experiment was performed in an attempt to evaluate response to an infectious agent with a nonrespiratory predilection. Litters consisting of 10 CD-1 pups were given 0.10 ml MHV_3 intraperitoneally; dilutions were 10^{-1}, 10^{-2}, or undilute (1 litter per dilution). The litters were maintained in individual nest boxes and placed in the hypobaric chamber. Ambient pressure controls were treated the same, and maintained in the same room (not in isolators). Mortality was closely observed, and recorded 4 times/day for both chamber and control groups. The experiment was terminated and the chamber returned to ambient pressure 2 days after the last chamber test subject succumbed to the infection.

4. INFLUENZA A/PR-8 INFECTION IN SCE

These tests were performed to establish whether viruses affecting the respiratory system are enhanced under hypobaric, normoxic chamber conditions. In the first experiment, 3-week-old CD-1 mice were anesthetized with pentabarbital and given 0.50 ml A/PR-8, intranasally. The following levels of virus were instilled: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} (6 mice used per dilution). Once the animals recovered from the pentabarbital, they were brought to 5 psi (34% atm.) at the ascent rate of 1000 ft/min. Ambient pressure controls were identically treated and maintained in standard nest boxes in the same room. Mortality was recorded as in the MHV experiment described above, and, as in that experiment, the chamber was returned to ambient pressure 2 days after the last chamber subject died.

This experiment was repeated, but a few changes in animal maintenance were made. Moist food was used to avoid humidity problems associated with water bottles; also the chamber temperature was increased from the 18-20°C range to 20-22°C range. Further, since a very high mortality was observed in the first A/PR-8 experiment, a slightly smaller dosage of virus was given. Two additional control groups were included to allay fears that spontaneous pneumonia resulted in the high mortality of the first experiment. Six untreated mice were placed in chamber A; six other mice were anesthetized and given isosaline intranasally, and they, too, were then placed in Chamber A. Test subjects instilled with influenza A/PR-8 were housed in Chamber B, with air flowing from Chamber A to Chamber B to minimize airborne spread of the agent (inner doors of the lock were slightly opened, and the entire chamber was operated as 1 large cabin). Control animals were identically treated and housed in the same room using similar maintenance practices. the first experiment. Six untreated mice were placed in chamber A; six other mice were anesthetized and given isosaline intranasally, and they, too, were then placed in chamber A. Test subjects instilled with influenza A/PR-8 were housed in Chamber B, with air flowing from Chamber A to Chamber B to minimize airborne spread of the agent (inner doors of the lock were slightly opened, and the entire chamber was operated as 1 large cabin). Control animals were identically treated and housed in the same room using similar maintenance practices.

D. RESULTS

1. HYPOBARIC EFFECT ON MICE

As mentioned earlier, due to failure of the vacuum system, the 28-day mission was aborted on day 4, after approximately 104 hours of operation. The initial experimental design was continued as planned, because it was felt that the initial ascent was the stressful period, and also, that descent was probably stressful regardless of the length of the hypobaric, normoxic treatment.

Organ weight assays reflected some differences between chamber animals and controls (Groups I and III, respectively). It is important to notice that both spleen and thymus weights were affected by descent on day 4, but the stress effect was more pronounced on the thymus (Figs.18 and 19). On day 7, 3 days after descent, the thymus weight of controls was 0.50% of body weight, while on the other hand the chamber animals had average thymus weights that were only 0.22% of body weight. Prednisolone treatment depressed lymphoid organ weights -- spleen, liver, (Fig. 20), and thymus weights of this group were notably smaller than all other groups. Body weight was also lower in the Prednisolone-treated group (Fig. 21). As anticipated, kidney weights did not establish any trend (Fig. 22).

The combined effect of TiO_2 aerosolization and exposure to SCE (Group IV) resulted in lower spleen and thymus weights on most kill days, as compared to their controls (Figs. 18 and 19, lower graphs). Aerosolized TiO_2 was cleared at similar rates from chamber and control animals (Fig. 23).

2. HYPOBARIC EFFECT ON FERRETS

The ascent to altitude did not outwardly harm the test subjects--no signs of bends or other discomfort was noticed in the hypobaric groups. All 5 experimental groups gained weight (Fig. 24) during the test run, as expected of young animals.

Figure 18. Hypobaric Effect on Mice;
Thymus Weight Assay

Group I: Chamber

Group II: Prednisolone

Group III: Chamber control

Group IV: TiO_2 - Chamber

Group V: TiO_2 control

Figure 18

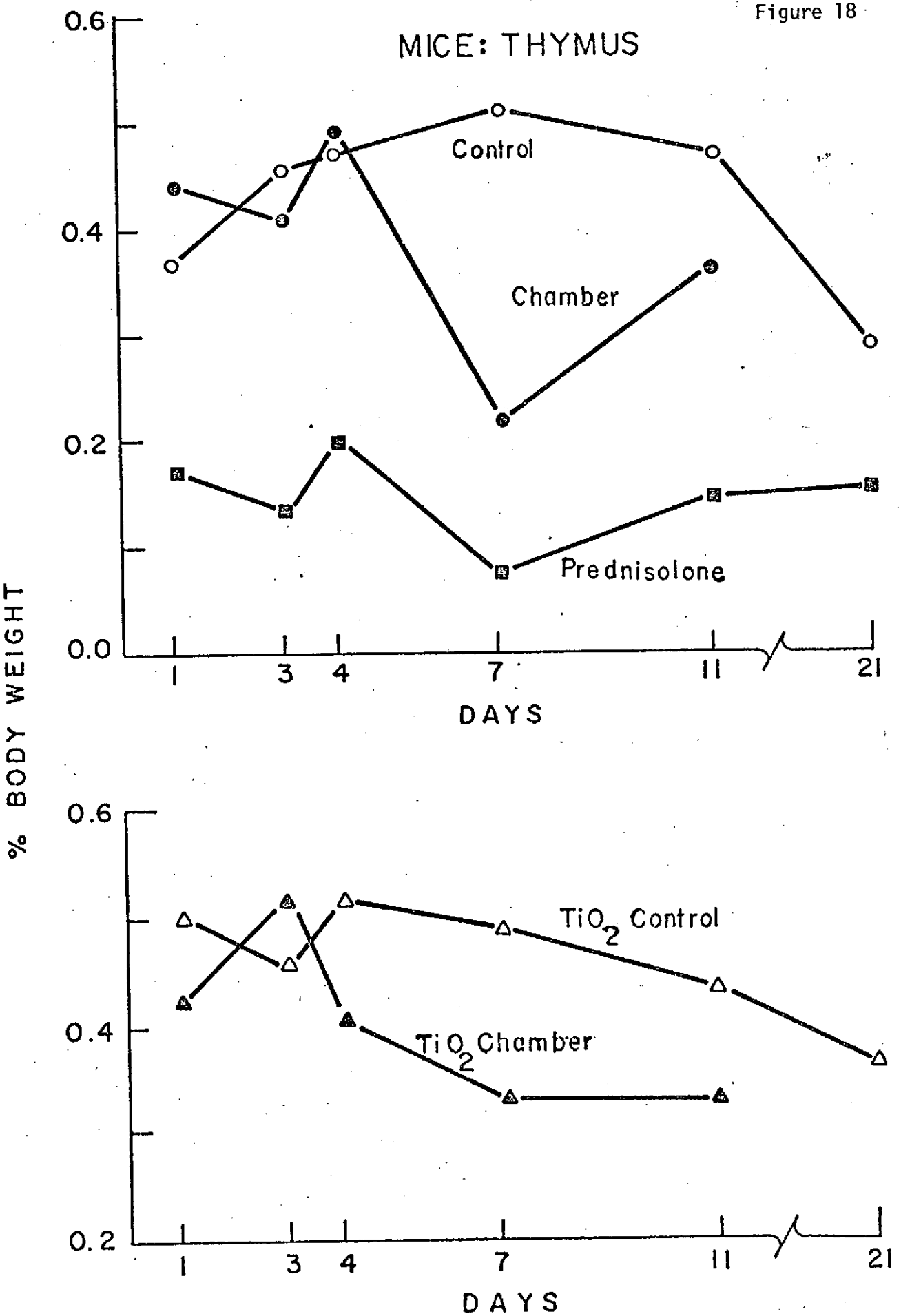

MICE: THYMUS
Control
Chamber
Prednisolone
0.6
0.4
0.2
0.0
1
3
4
7
11
21
DAYS
% BODY WEIGHT
TiO_2 Control
TiO_2 Chamber
0.6
0.4
0.2
1
3
4
7
11
21
DAYS

Figure 19. Hypobaric Effect on Mice;
Spleen Weight Assay

Group I: Chamber

Group II: Prednisolone

Group III: Chamber control

Group IV: TiO_2 - Chamber

Group V: TiO_2 control

Figure 19

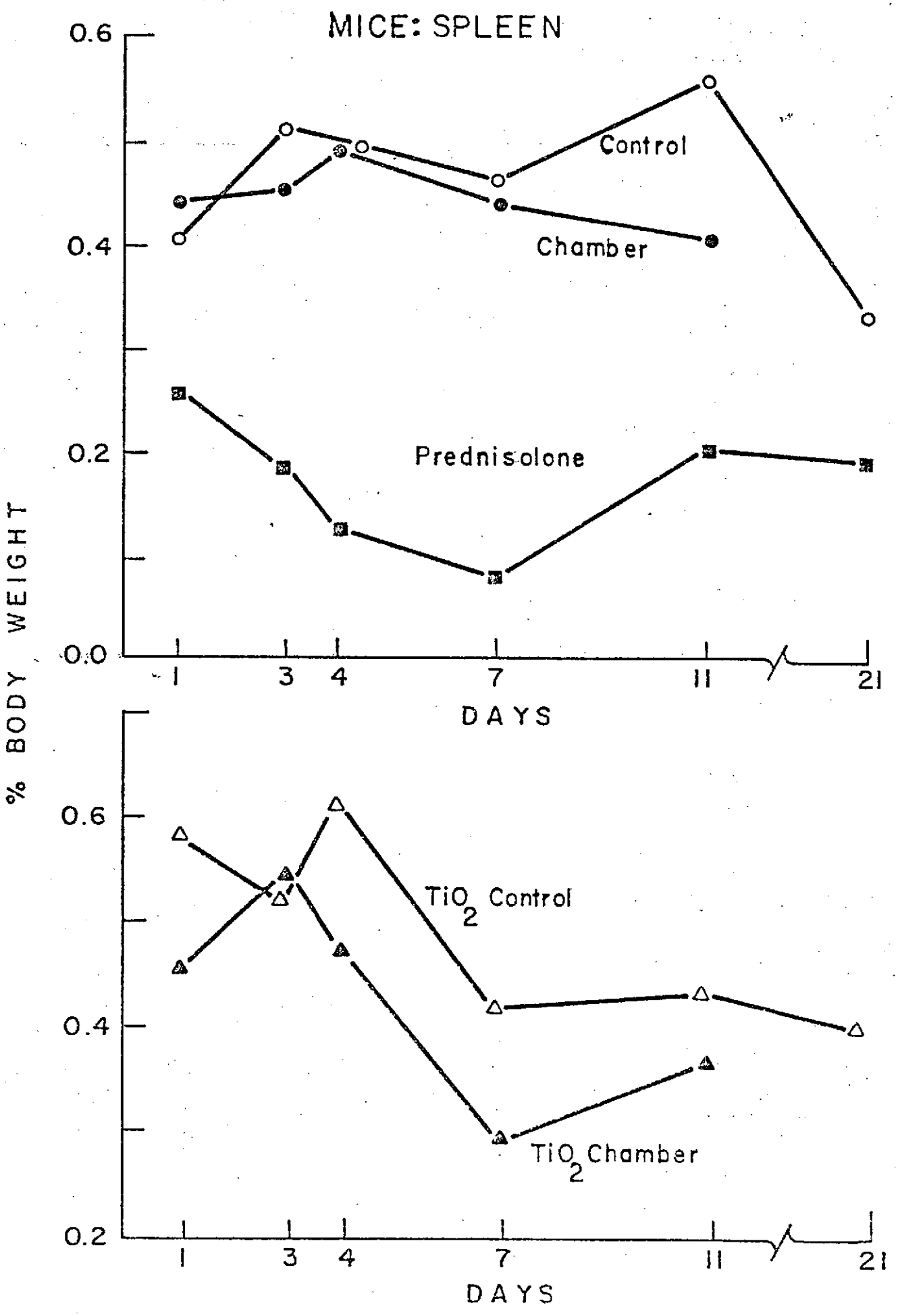

MICE: SPLEEN
0.6
0.4
0.2
0.0
Control
Chamber
Prednisolone
% BODY WEIGHT
1
3
4
7
11
21
DAYS
0.6
0.4
0.2
TiO_2 Control
TiO_2 Chamber
1
3
4
7
11
21
DAYS

Figure 20. Hypobaric Effect on Mice; Liver Weight Assay

Group I: Chamber

Group II: Prednisolone

Group III: Chamber control

Group IV: TiO_2 - Chamber

Group V: TiO_2 control

Figure 20

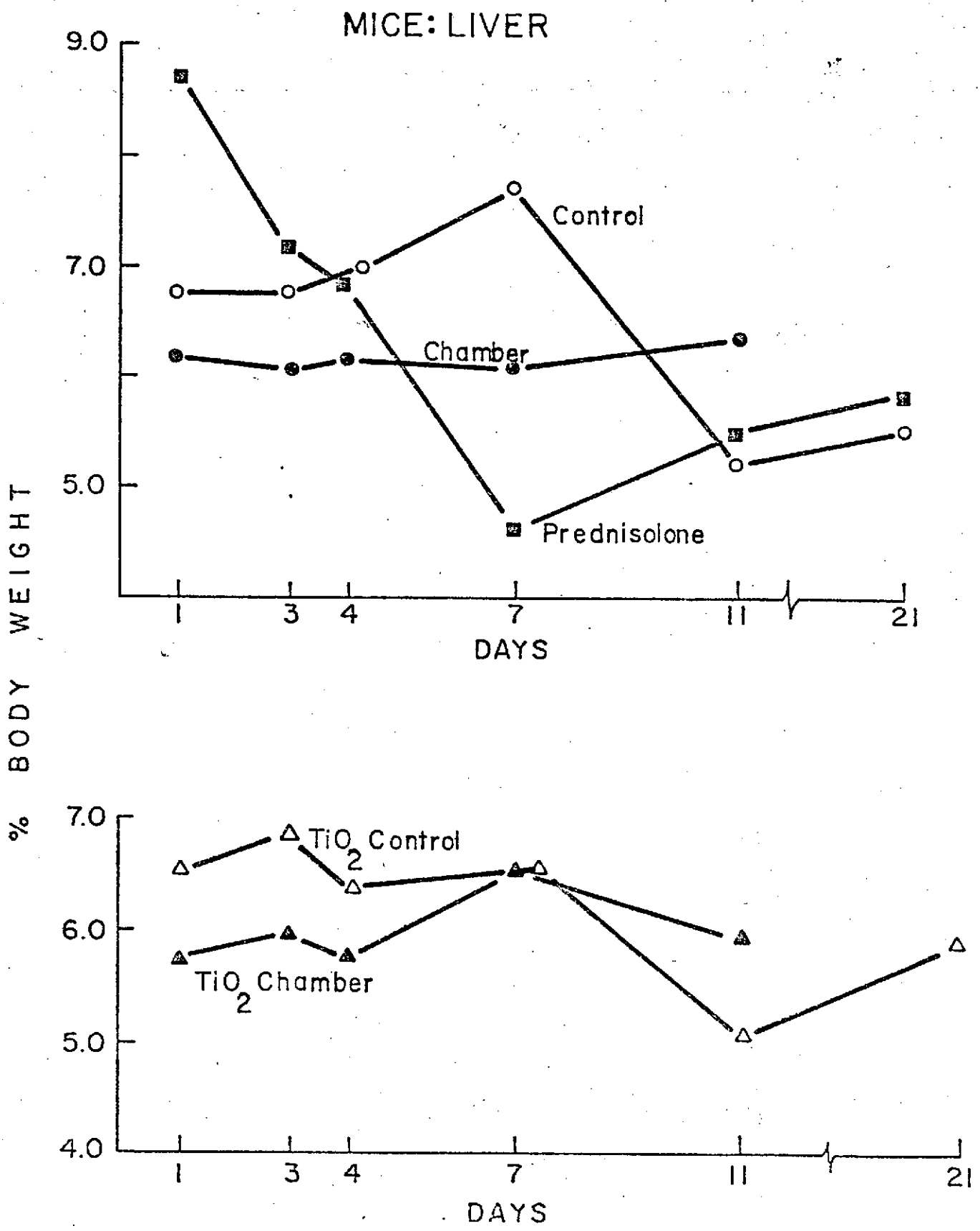

MICE: LIVER
9.0
7.0
5.0
Control
Chamber
Prednisolone
1
3
4
7
11
21
DAYS
% BODY WEIGHT
7.0
6.0
5.0
4.0
TiO2 Control
TiO2 Chamber
1
3
4
7
11
21
DAYS

Figure 21. Hypobaric Effect on Body Weight in Mice

Group I: Chamber

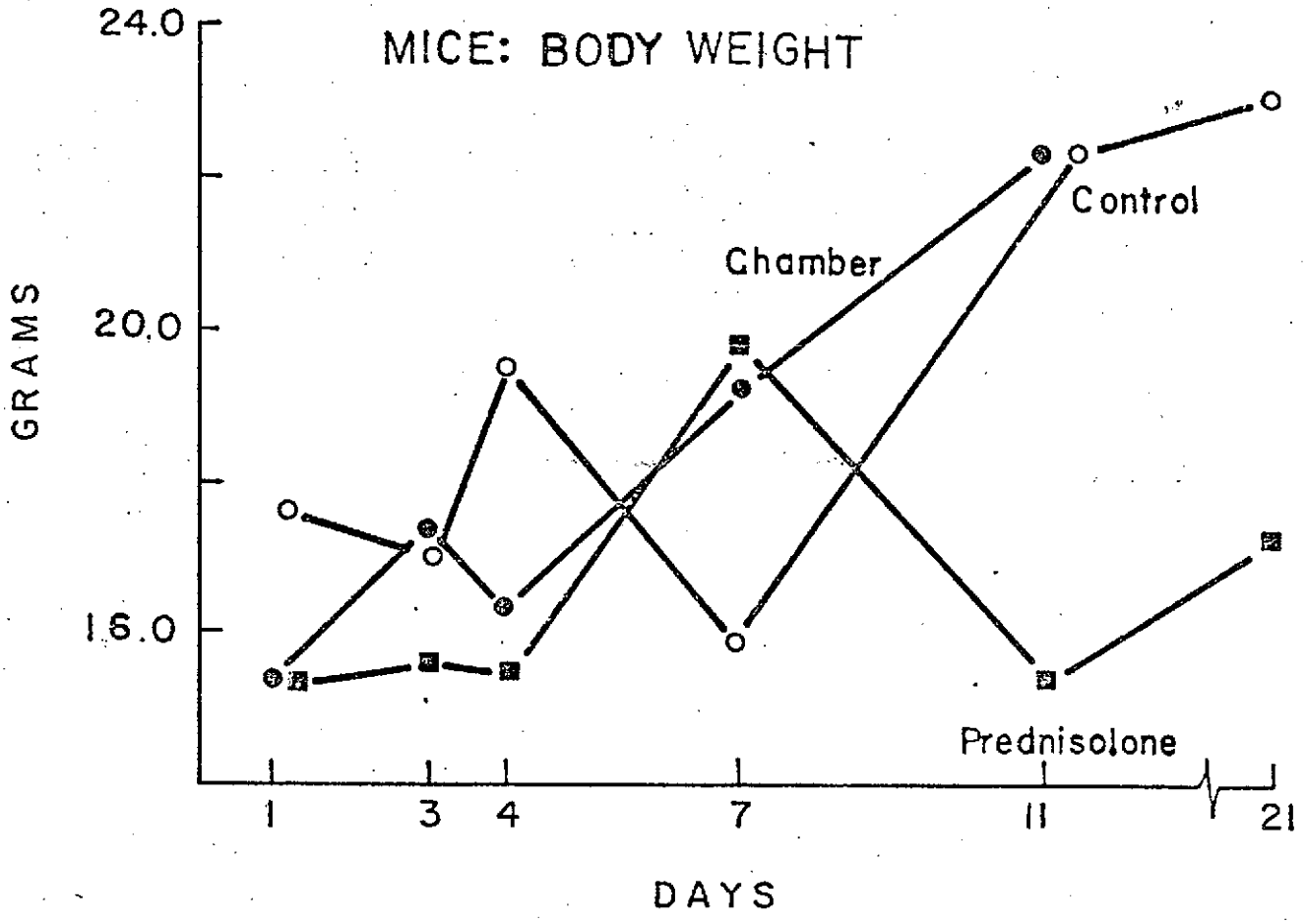
Group II: Prednisolone

Group III: Chamber control

Group IV: TiO_2 - Chamber

Group V: TiO_2 control

Figure 21


MICE: BODY WEIGHT
24.0
20.0
16.0
GRAMS
Control
Chamber
Prednisolone
1
3
4
7
11
21
DAYS

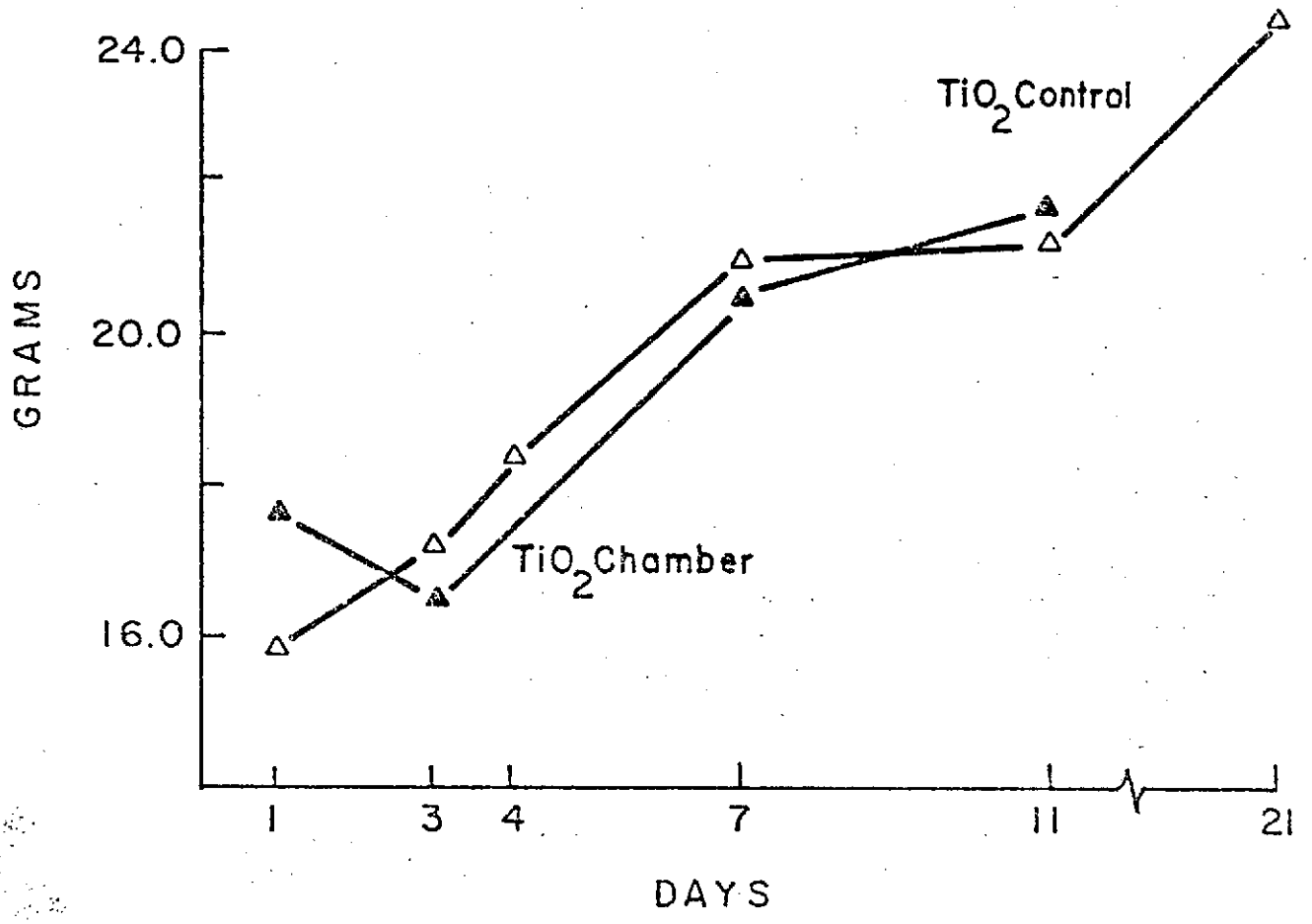

24.0
20.0
16.0
GRAMS
TiO2 Control
TiO2 Chamber
1
3
4
7
11
21
DAYS

Figure 22. Hypobaric Effect on Mice;
Kidney Weight Assay

Group I: Chamber

Group II: Prednisolone

Group III: Chamber control

Group IV: TiO_2 - Chamber

Group V: TiO_2 control

Figure 22

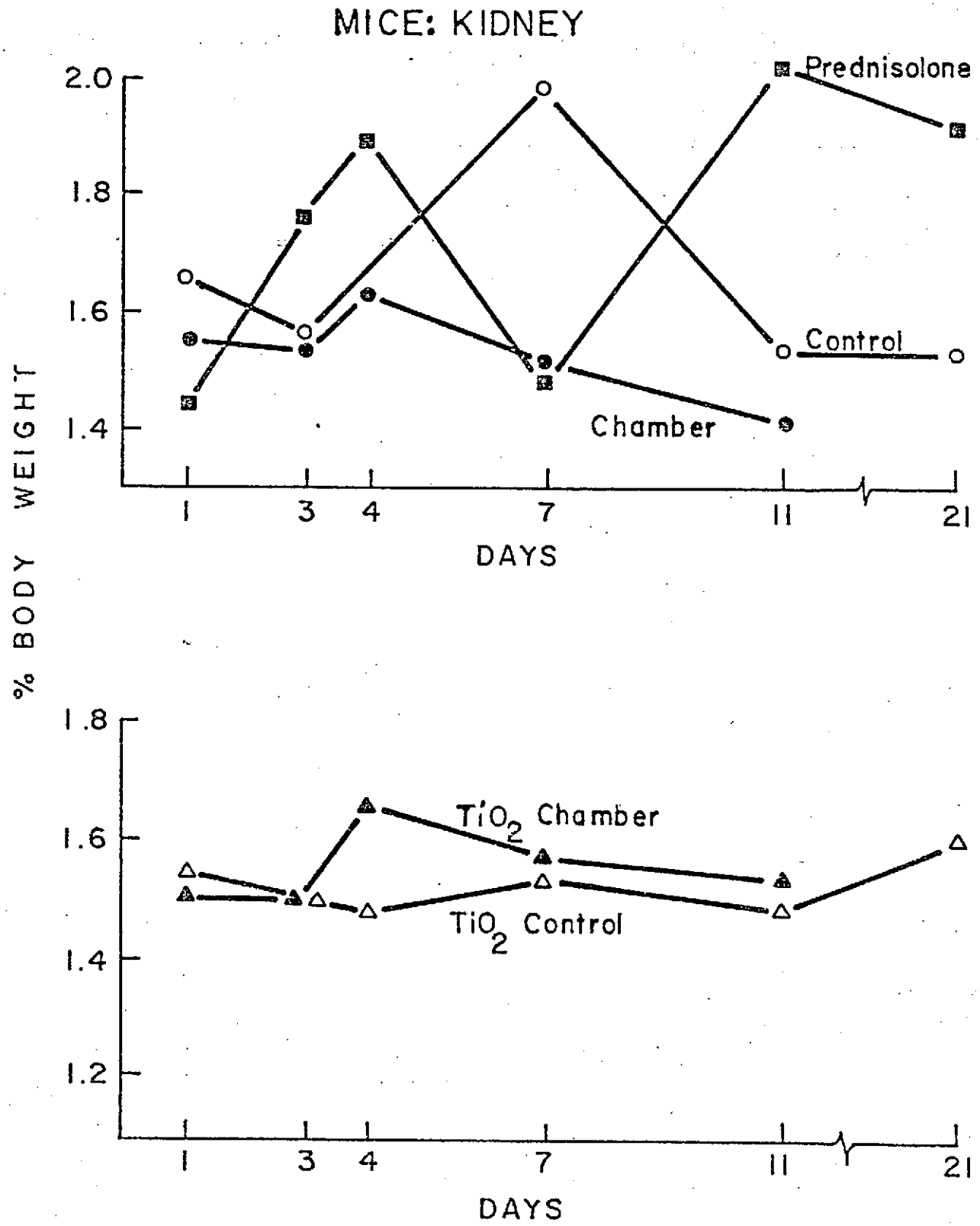

MICE: KIDNEY
% BODY WEIGHT
2.0
1.8
1.6
1.4
Prednisolone
Control
Chamber
1
3
4
7
11
21
DAYS
1.8
1.6
1.4
1.2
TiO_2 Chamber
TiO_2 Control
1
3
4
7
11
21
DAYS

Figure 23. Hypobaric Effect on Mice; TiO_2 Clearance

Figure 23

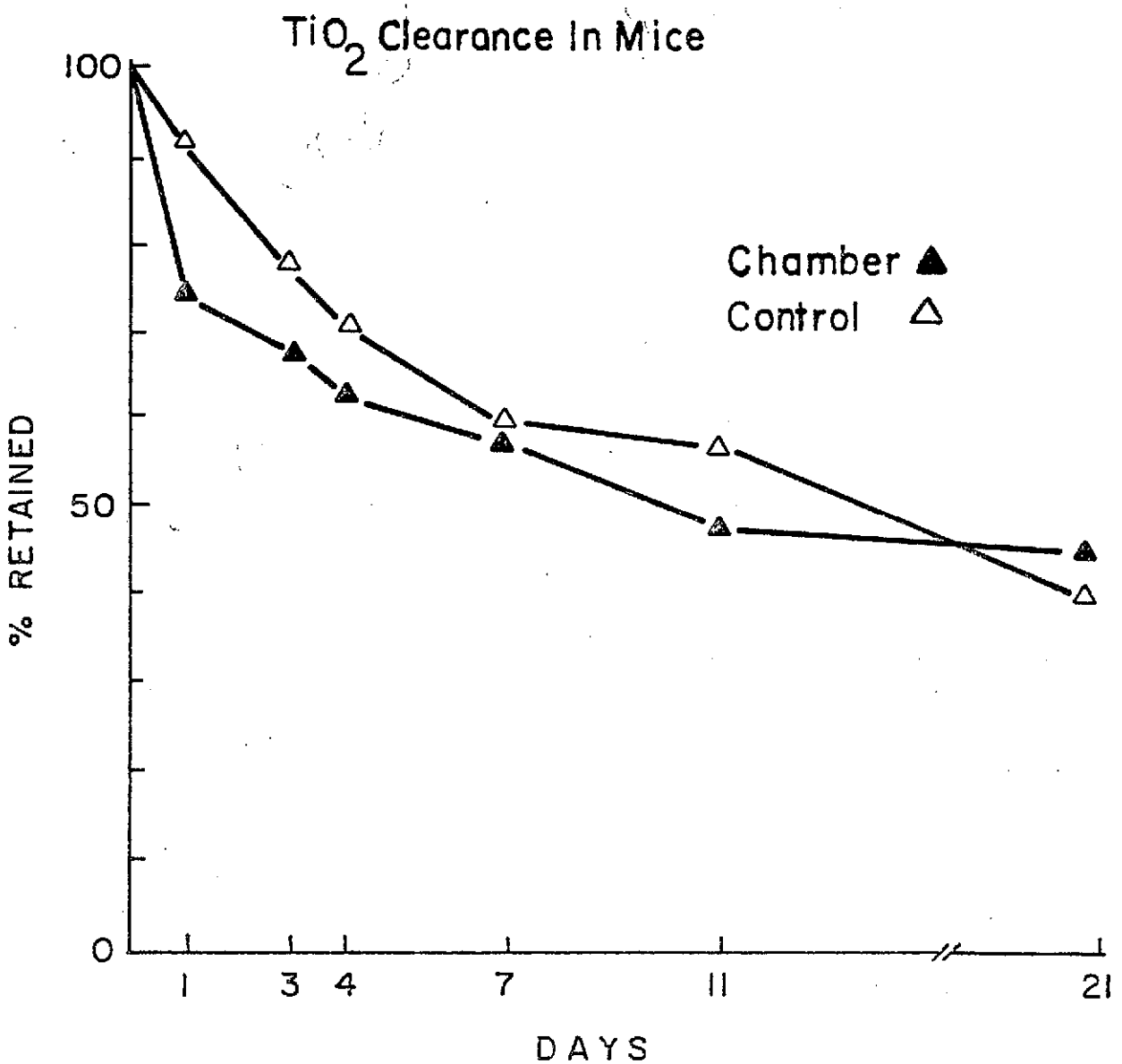

TiO2 Clearance In Mice
100
50
0
% RETAINED
Chamber
Control
1
3
4
7
11
21
DAYS

Figure 24. Hypobaric Effect on Body Weights in Ferrets

Group I: Chamber

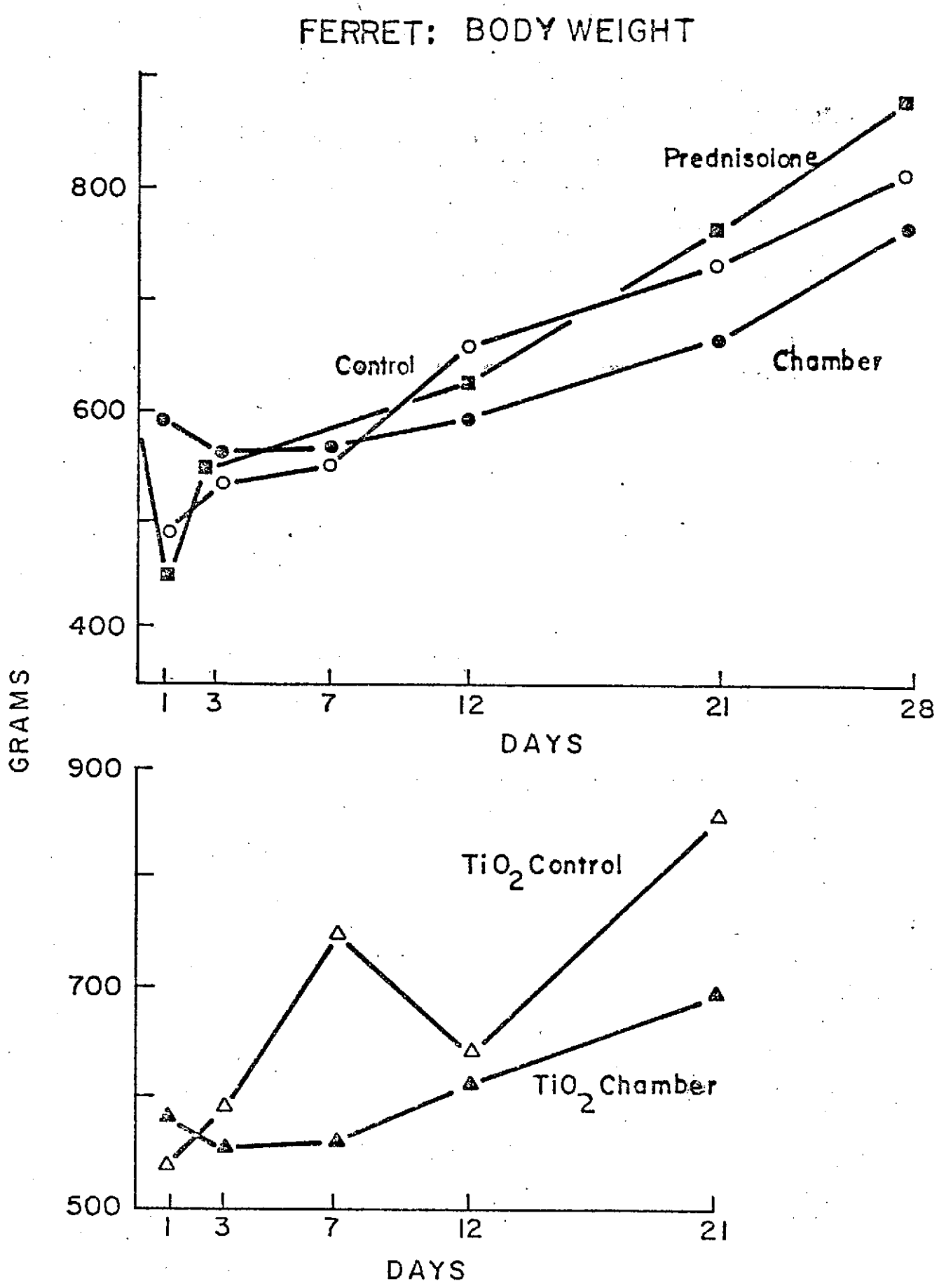
Group II: Prednisolone

Group III: Chamber control

Group IV: TiO_2 - Chamber

Group V: TiO_2 control

Figure 24


FERRET: BODY WEIGHT
GRAMS
800
600
400
Prednisolone
Control
Chamber
1
3
7
12
21
28
DAYS
900
700
500
TiO2 Control
TiO2 Chamber
1
3
7
12
21
DAYS

Organ weight assay trends (Figs. 24-28) were similar to the pilot experiment in mice described above. Corticosteroid hormone treatment depressed both spleen and thymus weights, and to a lesser extent liver and kidney weights. For example, spleen weights of the Prednisolone-treated animals stabilized through the course of the experiments at 0.40% of body weight, while the control spleen weight value was 0.70% of body weight.

The effect of hypobarism on spleen weight resulted in reduced spleen size, from a value of 1.3% (of body weight) on day 0 to 0.81% on day 3; control animals showed a similar reduction in spleen weight values, decreasing from 1.3% on day 0 to 0.78% on day 3. Actual spleen weights on day 0 ranged from 5.2 g to 11.6 g, averaging 7.8 g, larger spleens than found in unstimulated ferrets.

Thymus weights of ferrets were not greatly affected by hypobaric treatment, unlike mice, which had severely depressed thymus weights on day 7. In the ferret, the ambient pressure controls (Group III) had slightly smaller thymus weights on days 12, 21, and 28 than the hypobaric test group (Group I), but these differences are probably not significant.

Hypobaric test subjects cleared TiO_2 at the same rate as the ambient controls (Fig. 29); these results indicate that respiratory cellular functioning was not malaffected by SCE exposure. However, TiO_2 aerosolized animals which were subjected to SCE had lower body, spleen, thymus, and liver weights on the majority of the kill periods, compared to the aerosolized controls (Group V). Relative to the other three groups, both aerosolized groups (Groups IV and V) had organ and body weight values within the normal range.

Humoral antibody production to Brucella abortus was used as an indicator immune system functioning in the SCE. Standard tube agglutination tests revealed a decrease in overall titer (Fig. 30) which suggests that the later stage of antibody synthesis, responsible, for IgG production, was suppressed. This

Figure 25. Hypobaric Effect on Ferrets; Spleen Weight Assay

Group I: Chamber

Group II: Prednisolone

Group III: Chamber control

Group IV: TiO_2 - Chamber

Group V: TiO_2 control

Figure 25

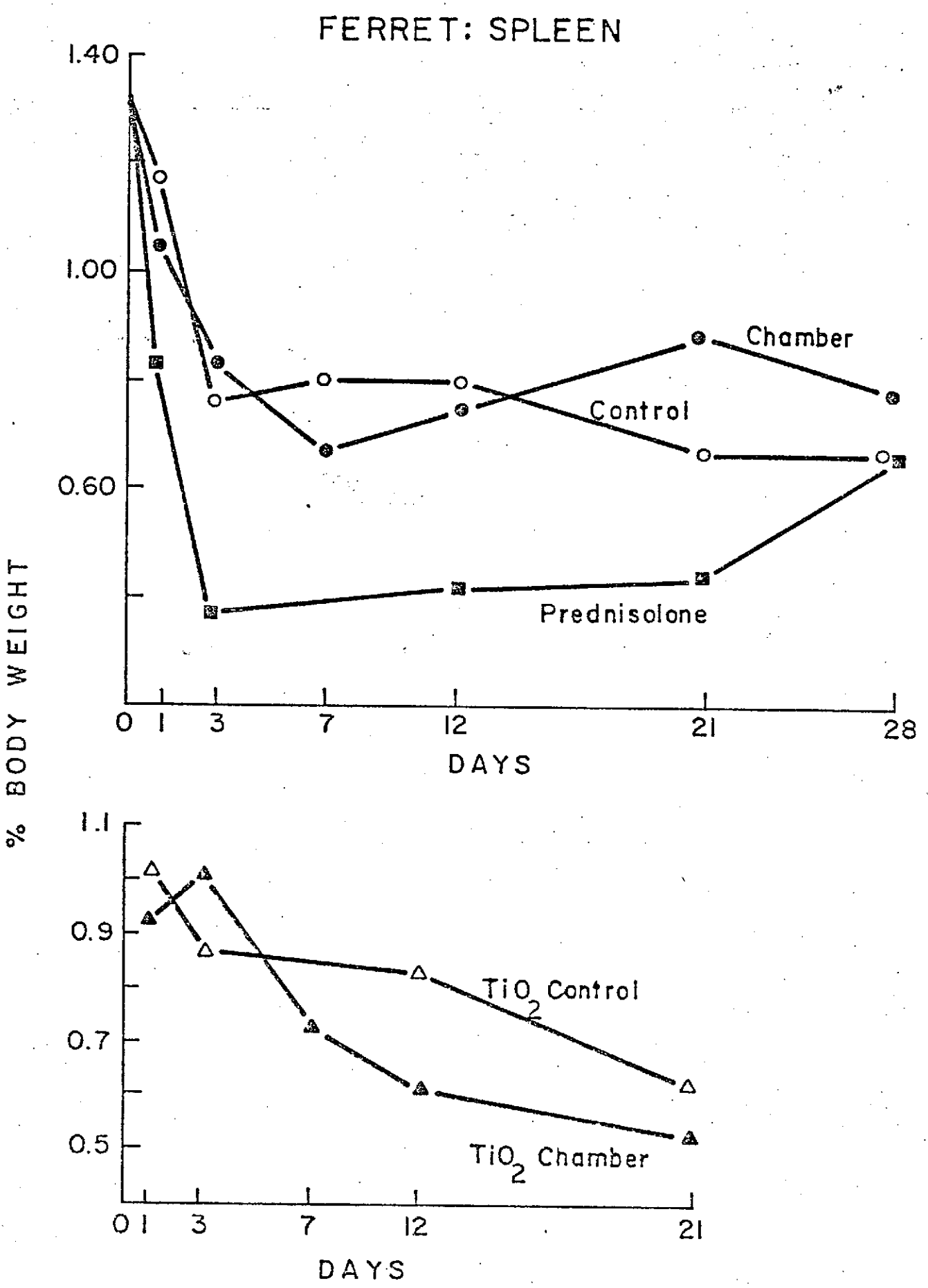

FERRET: SPLEEN
1.40
1.00
0.60
% BODY WEIGHT
Chamber
Control
Prednisolone
0 1 3 7 12 21 28
DAYS
1.1
0.9
0.7
0.5
TiO2 Control
TiO2 Chamber
0 1 3 7 12 21
DAYS

Figure 26. Hypobaric Effect on Ferrets; Thymus Weight Assay

Group I: Chamber

Group II: Prednisolone

Group III: Chamber control

Group IV: TiO_2 - Chamber

Group V: TiO_2 controls

Figure 26

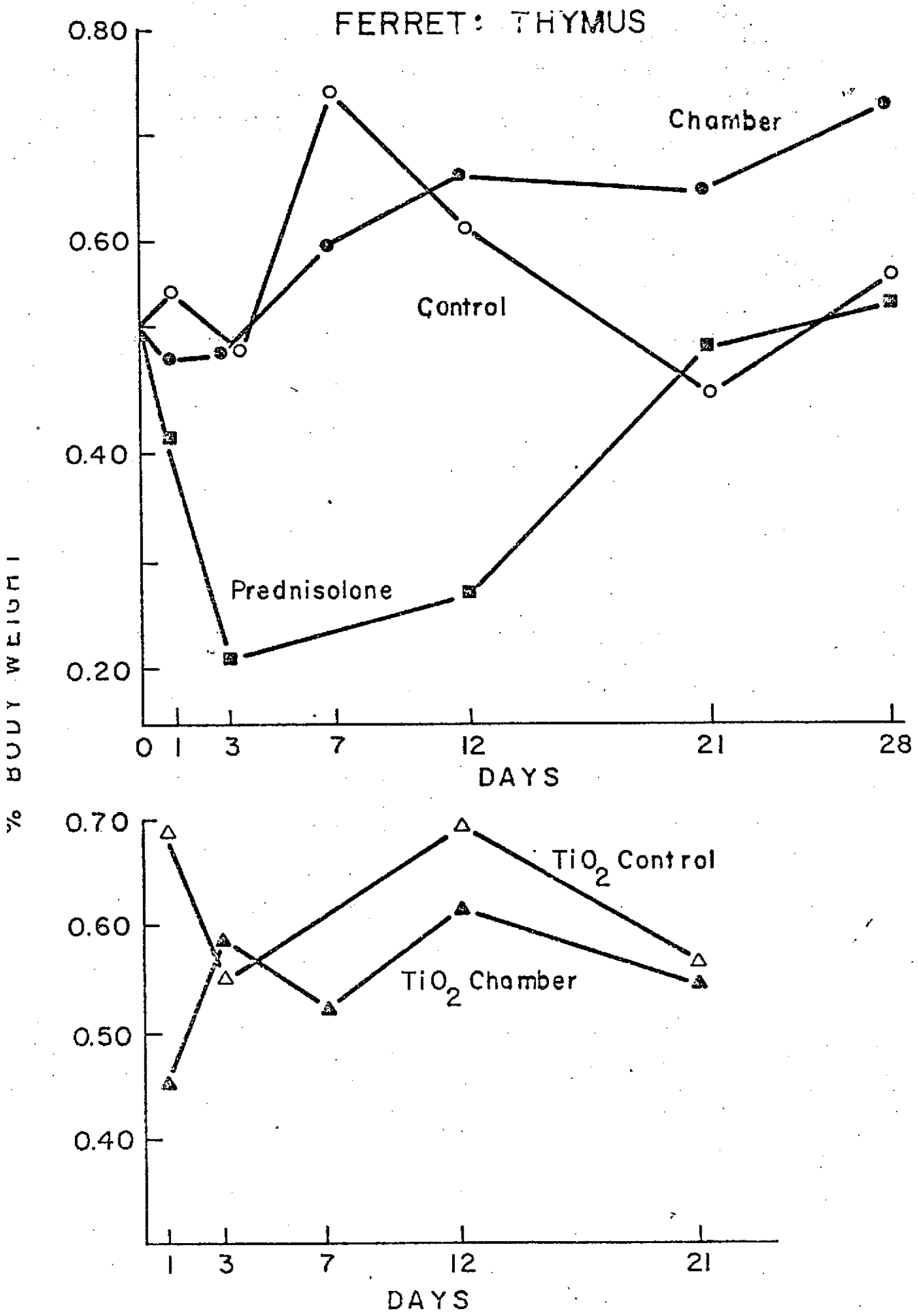

FERRET: THYMUS
0.80
0.60
0.40
0.20
Chamber
Control
Prednisolone
0 1 3 7 12 21 28
DAYS
% BODY WEIGHT
0.70
0.60
0.50
0.40
TiO2 Control
TiO2 Chamber
1 3 7 12 21
DAYS

Figure 27. Hypobaric Effect on Ferrets; Liver Weight Assay

Group I: Chamber

Group II: Prednisolone

Group III: Chamber control

Group IV: TiO_2 - Chamber

Group V: TiO_2 controls

Figure 27

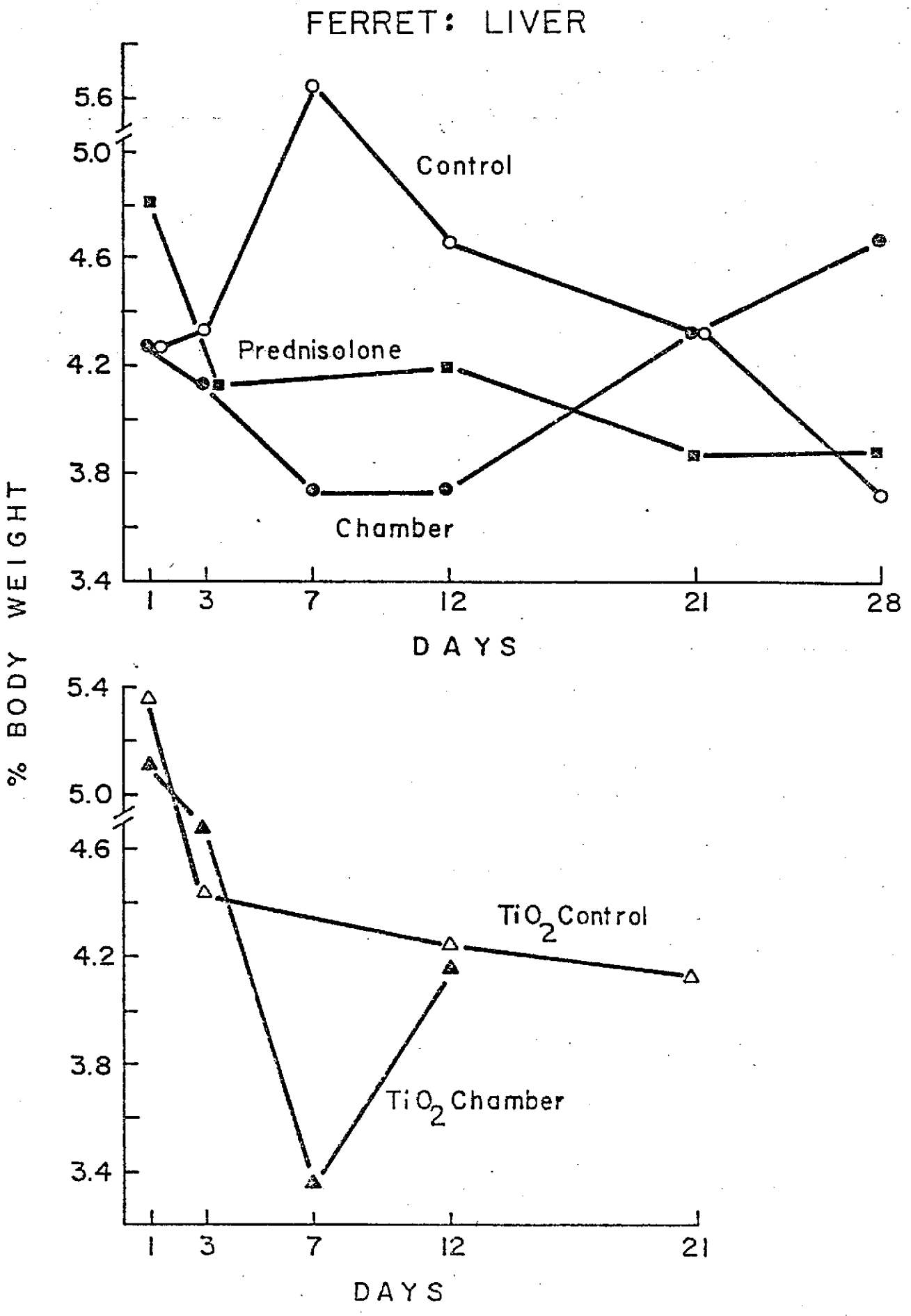

FERRET: LIVER
% BODY WEIGHT
5.6
5.0
4.6
4.2
3.8
3.4
Control
Prednisolone
Chamber
1
3
7
12
21
28
DAYS
5.4
5.0
4.6
4.2
3.8
3.4
TiO2 Control
TiO2 Chamber
1
3
7
12
21
DAYS

Figure 28. Hypobaric Effect on Ferrets; Kidney Weight Assay (Left and Right Kidneys Together as Percent Body Weight)

Group I: Chamber

Group II: Prednisolone

Group III: Chamber controls

Group IV: TiO_2 - Chamber

Group V: TiO_2 controls

Figure 28

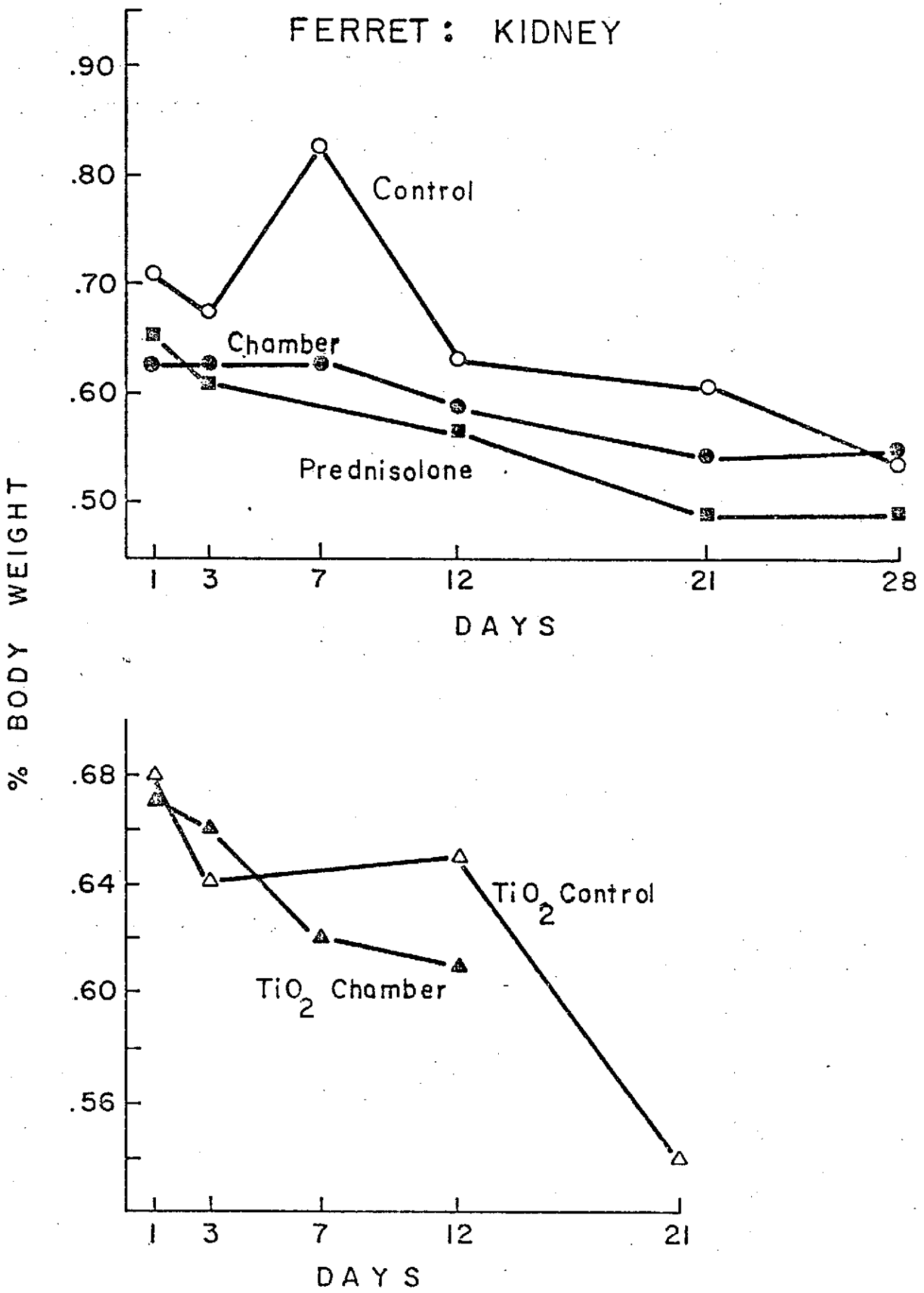

FERRET : KIDNEY
.90
.80
.70
.60
.50
Control
Chamber
Prednisolone
1
3
7
12
21
28
DAYS
% BODY WEIGHT
.68
.64
.60
.56
TiO2 Control
TiO2 Chamber
1
3
7
12
21
DAYS

Figure 29. Hypobaric Effect on Ferrets; TiO_2 Clearance.

Figure 29

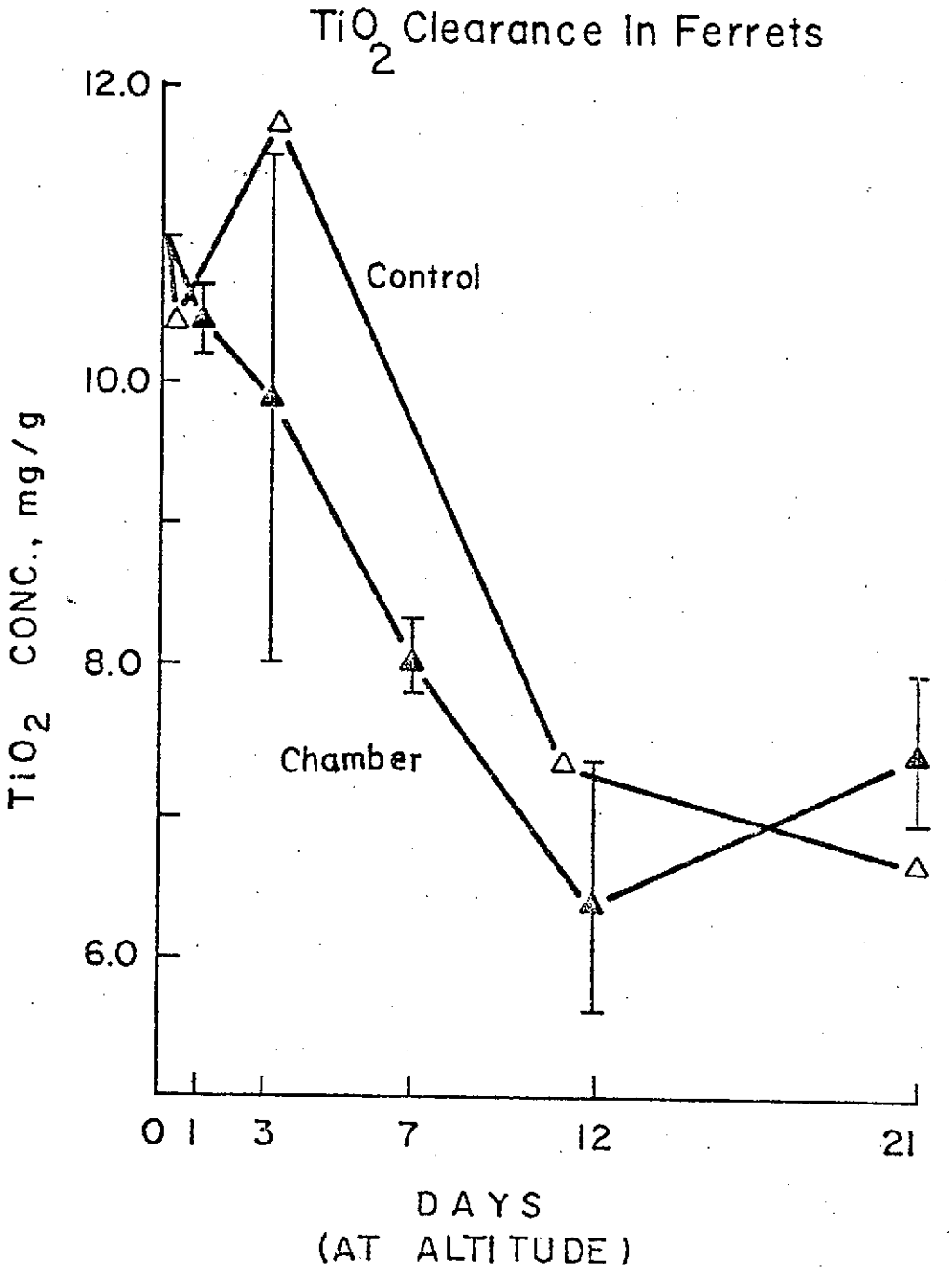
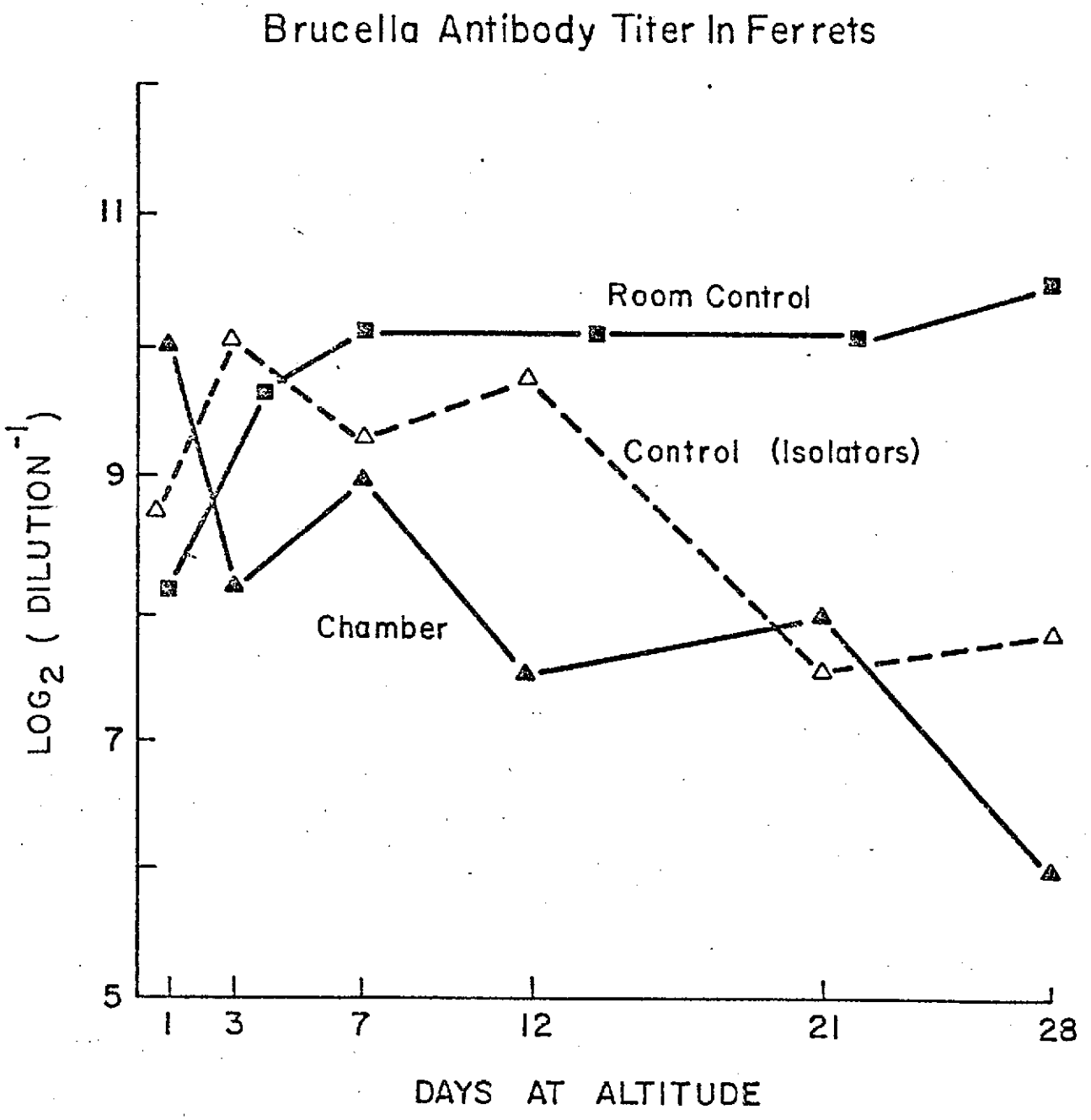

TiO2 Clearance In Ferrets
12.0
10.0
8.0
6.0
TiO2 CONC., mg/g
Control
Chamber
0 1 3 7 12 21
DAYS
(AT ALTITUDE)

Figure 30. Hypobaric Effect on Ferrets; Brucella antibody titers determined by tube agglutination assay


Brucella Antibody Titer In Ferrets
11
9
7
5
LOG2 (DILUTION-1)
Room Control
Control (Isolators)
Chamber
1
3
7
12
21
28
DAYS AT ALTITUDE

cessation of antibody production was also noticed in the ambient controls, which were housed in isolators. In order to establish a typical antibody pattern for ferrets housed in a nonrestrictive environment, a group of immunized animals were maintained in standard wire cages at ambient pressure (Fig. 30, "Room Control"). One sees that these ferrets exhibited the expected rise in antibody titer, as defined by standard tube agglutination tests.

To determine whether IgG synthesis was actually depressed in chamber animals, the sera were treated with mercaptoethanol. Subsequent readings of the tubes showed that IgG production was severely depressed in the chamber group, which exhibited no detectable titer on days 12 and 28, and a titer of 6 (reciprocal of dilution) on day 21. Ambient control animals also showed a decrease in IgG synthesis, but titers were appreciably higher throughout the experiment; for example, on days 12 and 28, this group had titers of 16, and on day 21, a titer of 64 (Fig. 31).

3. MURINE HEPATITIS INFECTION IN SCE

As it was previously thought that respirtator infections are enhanced by exposure to SCE, an experiment was included to evaluate response of an infectious agent with a nonrespiratory predilection, such as mouse hepatitis virus. Experimental data show that chamber subjects began to die one day earlier than ambient dome controls, but the overall mortality patterns were similar (Fig. 32).

4. INFLUENZA VIRUS INFECTION IN SCE

In the first pilot experiment performed, mice in both hypobaric and ambient groups began to die 3 days after instillation with virus. However, the death rate of the hypobaric subjects was much more severe than in the controls, and by day 4, more than 50% of the hypobaric subjects, but only 6.6% of the controls, had died. At the termination of the experiment on day 11 (2 days after the last chamber subject had died), 90% of the hypobaric group, but only

Figure 31. Hypobaric Effect on Ferrets; Brucella Antibody Titers Determined by Tube Agglutination With or Without Mercaptoethanol (ME) Treatment

Tube Agglutination - Chamber Group

Tube Agglutination - Ambient Control

Tube Agglutination & M.E. - Chamber Group

Tube Agglutination & M.E. - Ambient Controls

Figure 31

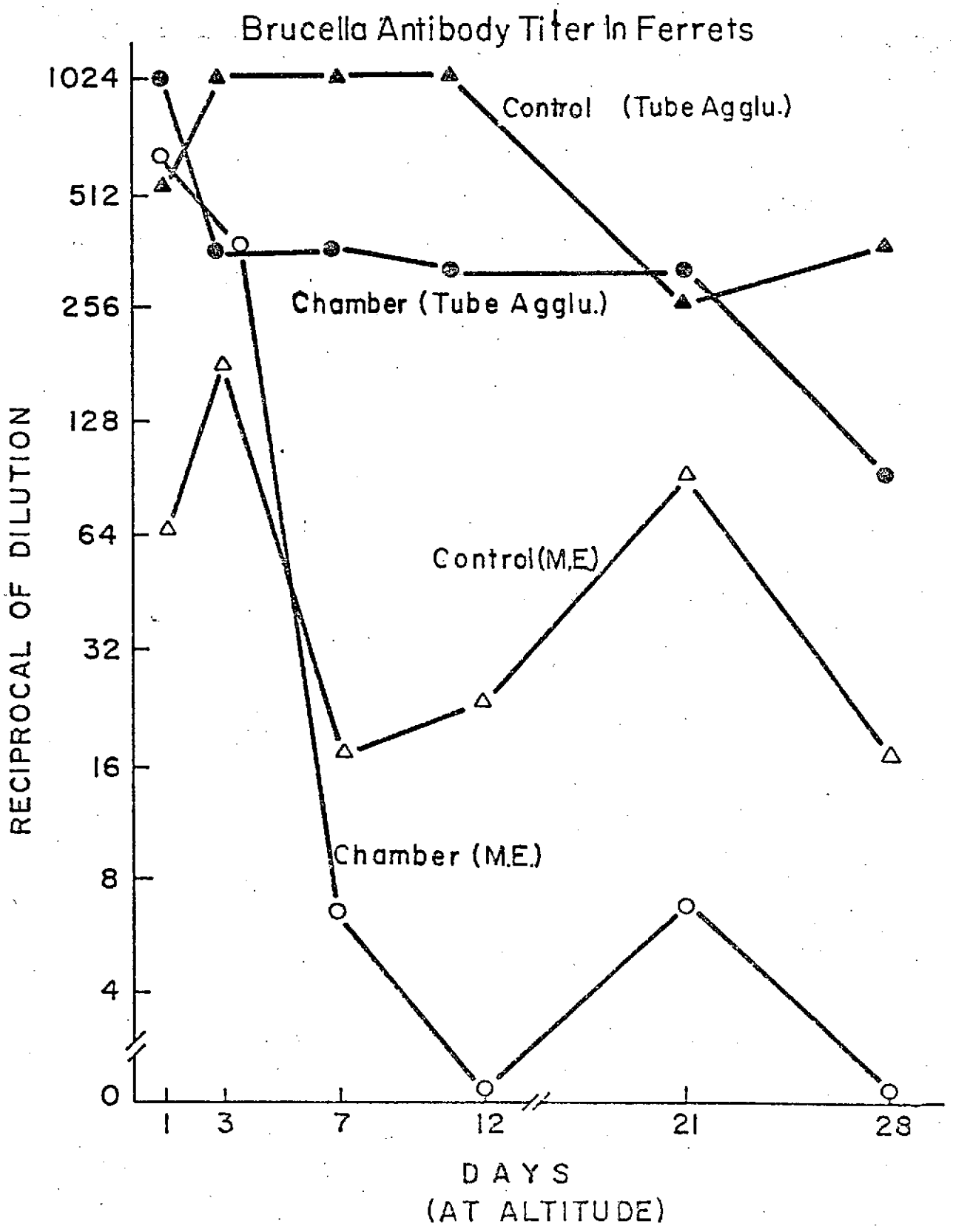
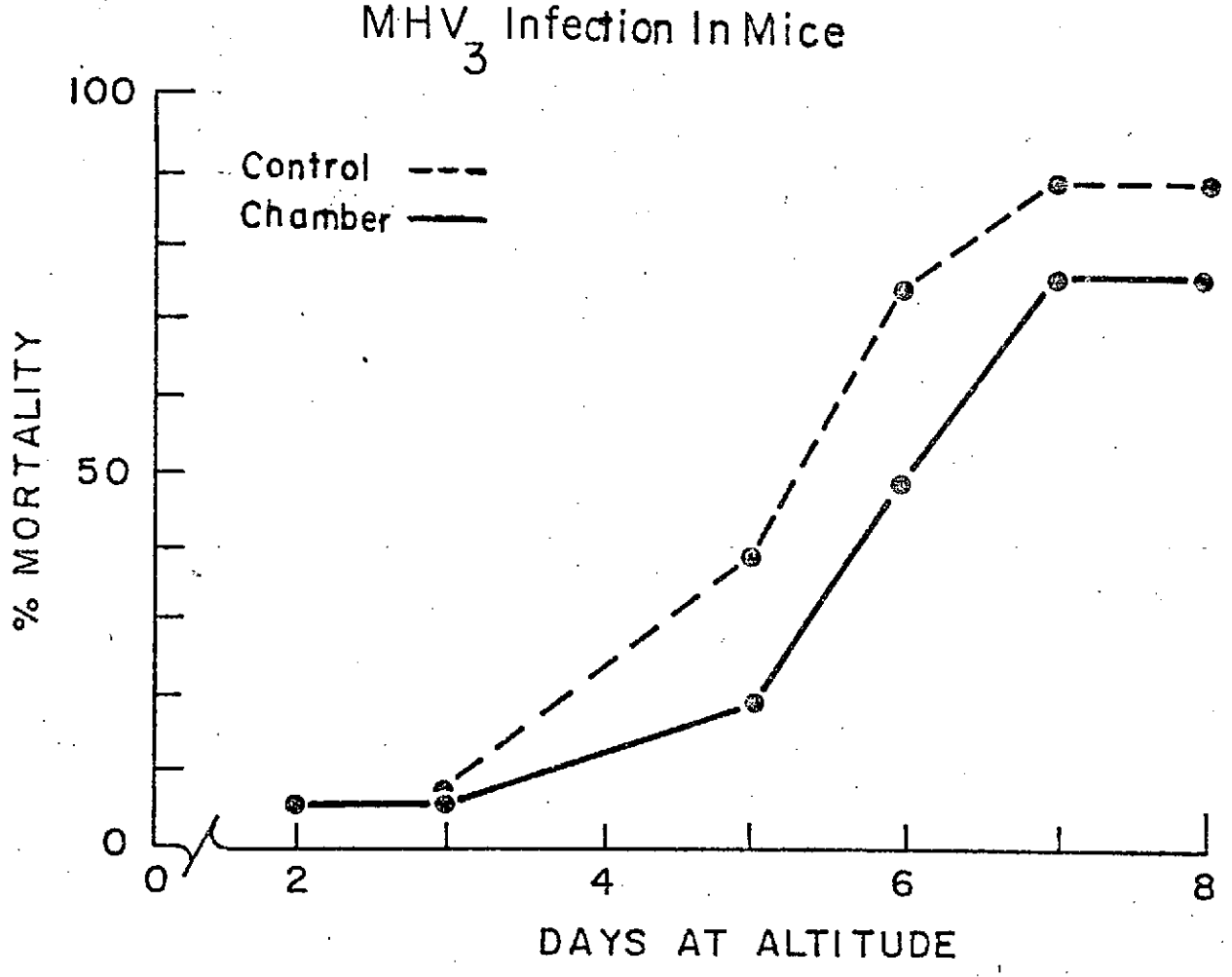

Brucella Antibody Titer In Ferrets
1024
512
256
128
64
32
16
8
4
0
RECIPROCAL OF DILUTION
Control (Tube Agglu.)
Chamber (Tube Agglu.)
Control(M.E.)
Chamber (M.E.)
1
3
7
12
21
28
DAYS
(AT ALTITUDE)

Figure 32. Response of Mice Housed in SCE to MHV_3 Infection (Mortality is expressed cumulatively)

Figure 32


MHV3 Infection In Mice
100
50
0
% MORTALITY
Control
Chamber
0
2
4
6
8
DAYS AT ALTITUDE

43.3% of the ambient controls, had died (Fig. 33). (Values given are the average cumulative mortality of the 5 dilutions tested.)

Due to the severity of illness incurred during the first experiment, when it was repeated, a slightly smaller dosage of virus was given, and the chamber temperature was increased to provide a more suitable mouse environment (20-24^{o}C). Moisture-rich sterile food was used, which eliminated the problem of leaky valves in water bottles. With these modifications, the experiment was continued as before. Animals from both hypobaric and ambient pressure control groups started to die on day 4, at which time the experimental results were similar to the first experiment, i.e., initially the morality was more severe in the hypobaric animals (Fig. 34). Specifically, on day 7, approximately 23% of the chamber animals, but only 10% of the controls, had died.

Figure 33. Response of Mice Housed in SCE to Influenza A/PR-8 Infection (Trial 1). (Percent Mortality is Cumulative.)

Figure 33

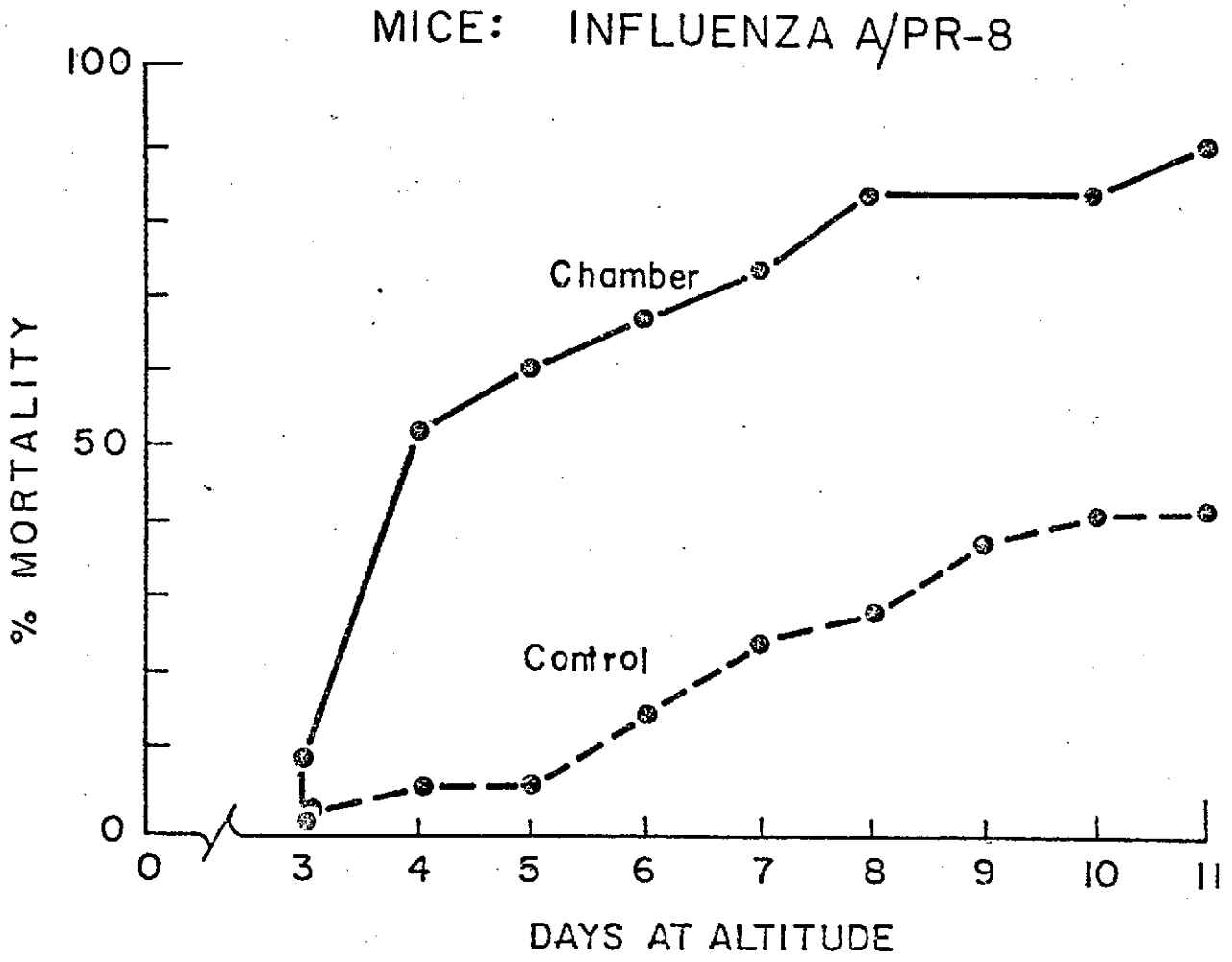
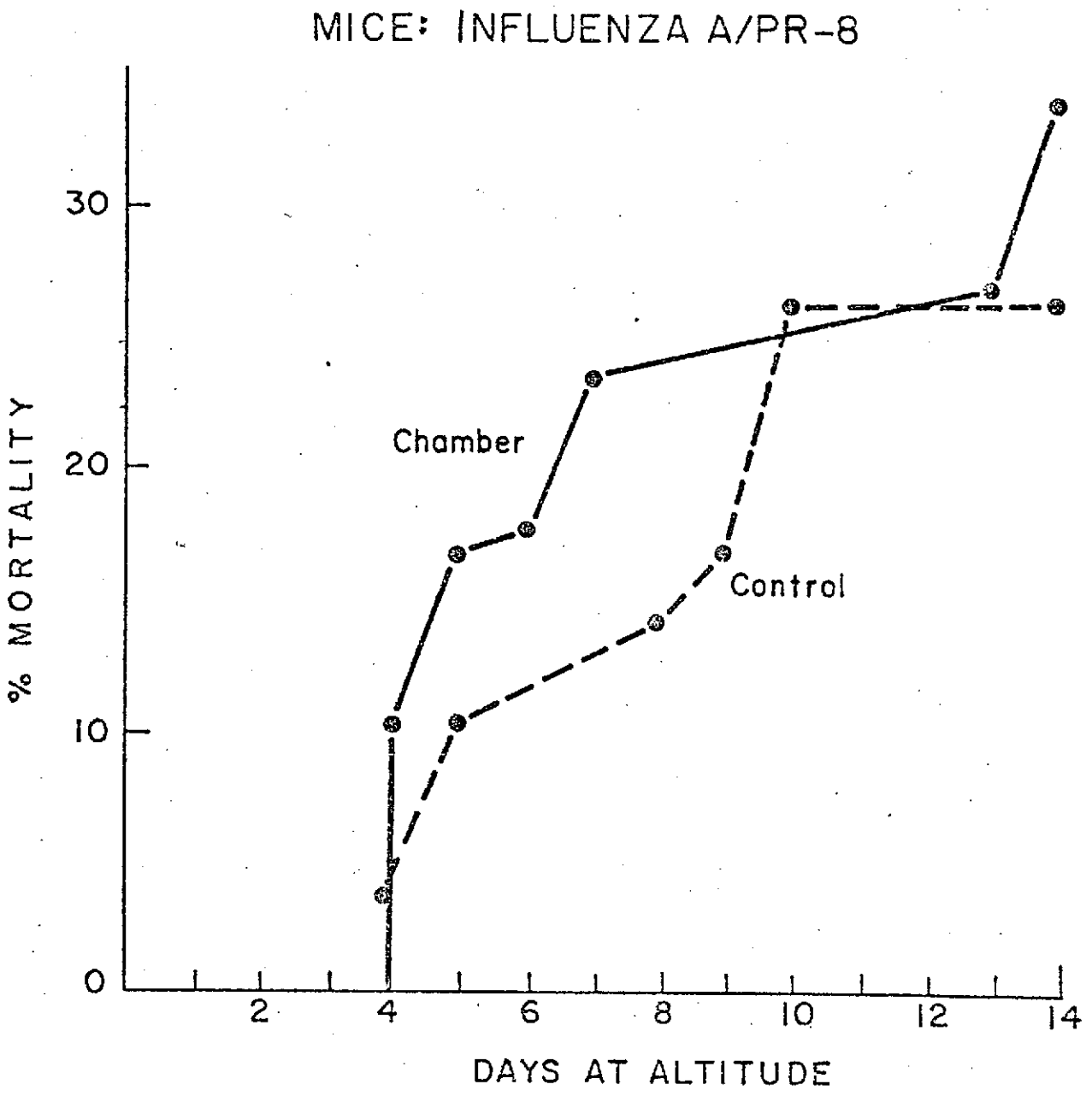

MICE: INFLUENZA A/PR-8
100
50
0
% MORTALITY
Chamber
Control
0
3
4
5
6
7
8
9
10
11
DAYS AT ALTITUDE

Figure 34. Response of Mice Housed in SCE to Influenza A/PR-8 Infection (Trial 2)

Figure 34


MICE: INFLUENZA A/PR-8
% MORTALITY
30
20
10
0
Chamber
Control
2
4
6
8
10
12
14
DAYS AT ALTITUDE

E. DISCUSSION

Increased susceptibility to infectious diseases as the result of factors associated with spacecraft environments has not been clearly established. However, the frequency of "minor colds" experienced by astronauts in flight, and the experimental evidence for altered susceptibility (Aerospace Med. 38:382, 1967; 40:176, 1969) prompts consideration of depressed physiologic and immunologic responses as a result of environment stress. Depression of normal immune mechanisms at the lymphoid level by pituitary-adrenal homones has been well established (Immunology 15:643, 1968; J. Immunol. 98:1076, 1967) and associated with the central role of these hormones in physiologic stress (The Physiology and Pathology of Exposure to Stress, Selye, H., 1956, ACTA, Inc., Montreal).

Another factor of near equal importance in induction of respiratory disease is the function of the mucociliary system (Health Physics 2:379, 1960). Studies of pulmonary clearance (J. Clin. Invest. 43:4, 1964) indicate a two-phase response involving a rapid phase and a slow phase following exposure to environmental pollutants; i.e., microbiologic aerosols, tobacco smoke or radioactive aerosols. The rapid phase is primarily associated with the cephalad movement of the mucous blanket while the slow phase involves alveolar clearance via the reticuloendothelial and lymphatic transport system. Thus, it is clear that factors which alter the "viscidity" of "excessive fluidity" impair the non-Newtonian mucosal movement of inhaled particles by the ciliated cells.

In general, particles considered as being "lung damaging" in size range from 0.25 to 10 μ. Particles of less than 0.25 μ are seldom retained in the lungs and particles with diameters greater than 10 μ are lodged in the mucociliary systems of the upper respiratory tract and hence do not reach the bronchi (Pulmonary Deposition ofInhaled Aerosols, Hatch, T. and Gross, P., 1964, Academic Press, New York). At sea-level the majority of "free-formed" aerosolized

virus particles are of a size range which would not be retained in the lungs. However, this is not true at reduced atmospheric pressures. The difference in aerodynamic equivalent diameters of a given particle at New York as opposed to Denver is 20% larger (Raabe, O. G., In: Inhalation Carcinogenesis (M. G. Hanna, Jr., P. Nettesheim and J. R. Gilbert, editors), U. S. Atomic Energy Commission, Oak Ridge, Tennessee, 1970). This coupled with aerosol aging (aggregation) which would still occur at zero gravity in actual space flight alters the pathogenic character of biologic aerosols.

Utilizing the low-pressure facility designed to house mice and ferrets for periods up to 180 days, the environmental conditions proposed for the Skylab mission, namely, a gas mixture of 70:30 O_2:N_2 and an atmospheric pressure of 5 psi (34% atm.), were tested. The SCE tested in this study has a partial pressure of O_2 equivalent to that normally found at sea level, and thus is hypobaric, normoxic. During chamber tests, overall health of experimental animals was evaluated by clinical appearance of test subjects. In the first 2 hypobaric tests, in which young mice and ferrets were used, and even when suckling mice were used as in the MHV experiment, no signs of bends or other discomfort were noted upon ascent or descent in altitude. Organ (lymphoid) weight assays were used as indicators of stress. The SCE effect on alveolar macrophages was evaluated by following clearance rates of TiO_2 in animals which had been aerosolized prior to SCE exposure. Activity of the immune system was investigated by quantitation of humoral antibody to Brucella, and then IgG activity was separated from IgM activity by mercaptoethanol inactivation of IgM. Finally, response to viral disease - influenza and hepatitis - was evaluated to confirm or deny reports that SCE is particularly enhancing to those viruses having a respiratory predilection.

In both the mouse pilot experiment, and the study using ferrets, spleen and thymus weights of chamber animals were reduced. Control ferrets, which were housed in isolators, also had reduced spleen and thymus weights, apparently due to confinement stress. In the ferret, the spleen was more affected by SCE and isolator confinement, whereas in the mouse, the thymus was the target organ. Both mice and ferrets exposed to SCE also exhibited slightly reduced kidney and liver weights throughout the experiment. The data suggests that there is a stressful period at the time of ascent or descent in altitude, and that the effect may vary according to the species. The stress of confinement and hypobarism probably stimulates corticosteroid hormone production, which may have alternate effects on mice and ferrets; the effect of corticosteroids would account for reduced spleen weights in ferrets, and reduced thymus weights in mice.

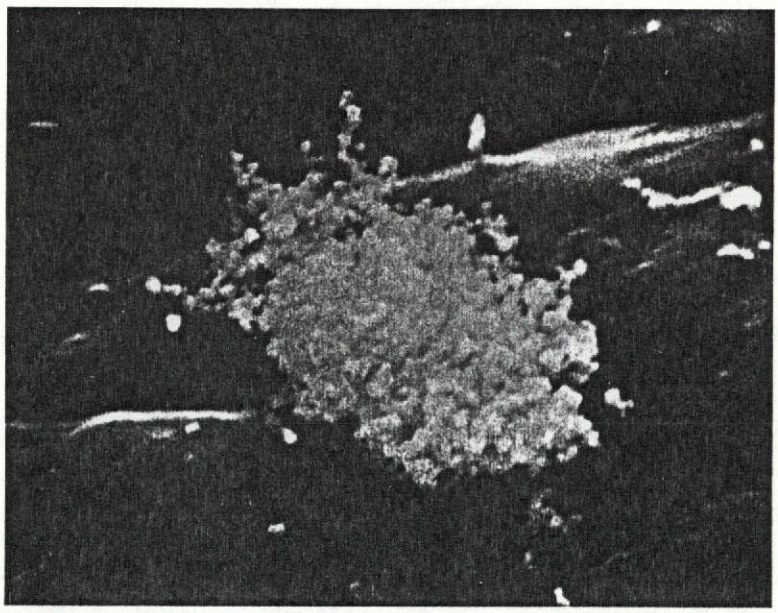
A Prednisolone-treated group was included in both mouse and ferret experiments in order to establish a maximum stress effect of the hormone on lymphoid and other organs. The reasoning was that if the effect was not detectable after a relatively high dosage of corticosteroids were administered, then stress due to factors associated with SCE in the chamber which resulted in corticosteroid release, could probably not be detected. In mice, Prednisolone caused a marked depression in lymphoid organ size: spleen, liver, and thymus weights were lower in this group than in any other. Ferrets also showed depressed spleen and thymus weights after Prednisolone treatment; to a lesser extent, liver and kidney weights were reduced. (By 21 and 28 days, the Prednisolone group caught up with the controls.) At the level of hormone given a species distinction, regarding the mode of action of the drug, was not observed - virtually all organ and body weights of mice and ferrets were lower in this group than in the other 4 groups. The depression in lymphoid organ weights suggests that the

drug affects transformation of stem cells along the plasma line, and thus a reduced population of these cells was detected in lymphoid organs.

Earlier NASA programs had developed methodology and established baseline parameters for lung clearance of inert dust, specifically, TiO_2 (Contract No. NAS 9-11575, Biological Activity of Lunar Soil). These preliminary experiments had shown that aerosolized TiO_2 was in the submicron range, and therefore is deposited mainly in the acini of the lung; particle measurement was accomplished by light microscopy, sedimentation analysis, and most recently, by Scanning Electron Microscopy (Fig. 35). TiO_2 clearance was used as a model to determine whether environmental conditions in the chamber, such as gas mixutre, humidity, temperature, and ammonia fumes, would alter the alveolar clearance rate. Mice and ferrets exposed to SCE after 24 hours of aerosolization, cleared TiO_2 at approximately the same rate as their ambient controls, suggesting that the SCE was not detrimental to alveolar phagocytosis. The slope of the clearance curves imply a rapid clearance of TiO_2; however, the data is somewhat misleading. Due to increase in body weight and, therefore, lung weight, concentration of TiO_2 per gram of wet tissue would naturally show a decreasing trend. An earlier study with mature ferrets had shown that over 90% of deposited TiO_2 remained after 30 days.

During both mouse and ferret experiments, the ambient controls were held in rigid plastic isolators, in order to maintain similar air flow and temperature to the chamber. An earlier study (Coyne, R. V. and Ackerman, G. A., Aerospace Med. 40:1219-1223, 1969) has reported that spleen weights and serum antibody titers to Brucella abortus were decreased in animals subjected to 100% O_2 at 5 psi, as did mice held in ambient pressure domes. Perhaps the comparison between chamber subjects and controls would have been more meaningful if the controls were held in conventional cages, which was done in the influenza virus and hepatitis virus experiments.

Figure 35. Scanning Electronmicrograph of TiO_2

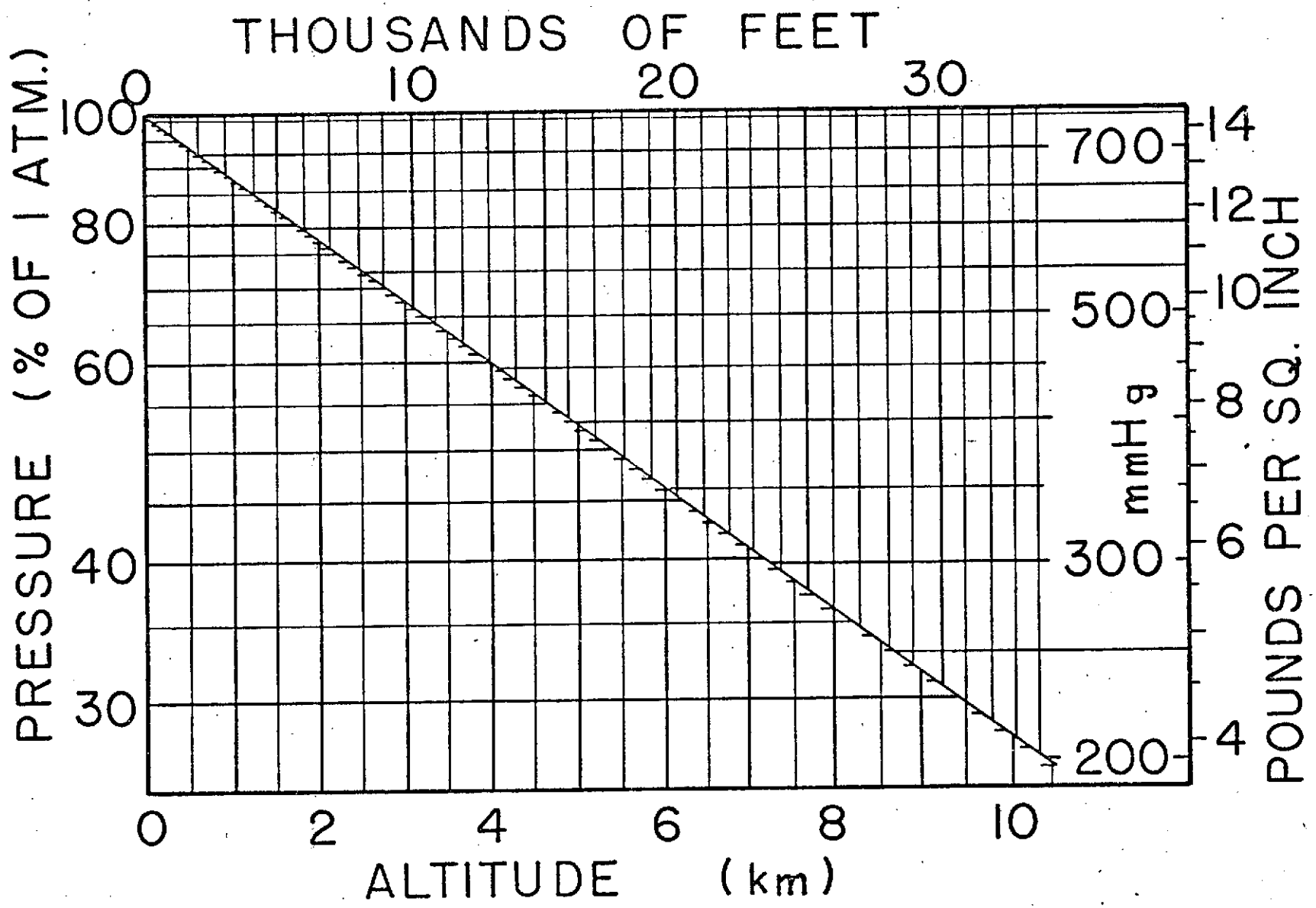


Ferrets immunized with Brucella abortus 5 days prior to SCE exposure, as well as their controls, revealed a decrease in antibody titer during the 28-day test. Such a decrease in antibody titer suggests that the later stage of antibody production which synthesizes IgG, was suppressed. Animals given an identical inoculation, but housed in standard wire cages, exhibited an increase in titer, as expected after a primary immunization. To separate IgM antibody activity from IgG, both of which are detectable by standard tube agglutination tests, sera from chamber animals and their controls (housed in ambient pressure domes) were incubated with mercaptoethanol. Mercaptoethanol reduces the disulfide bonds which covalently binds holds together the IgM polymers, thus inactivating it. Immunoglobulin in IgM, which is synthesized primarily during the early stage of the immune response, is mercaptoethanol sensitive, while IgG under these conditions, is not. When the sera were so treated, the total serum titers decreased, most notably in the chamber group; the decreasing trend suggests that the initial antibody produced was catabolized, and that synthesis of IgG was either depressed or inhibited. The IgG levels for both groups were much lower than the combined IgG and IgM titers, was expected.

Response to viral infection in the simulated spacecabin was performed because IgG synthesis appeared to be suppressed, and also to establish whether the SCE enhanced only respiratory infections, as had been intimated in the literature. Bacterial and viral infections have been reported either enhanced or unaffected by various spacecabin conditions. J. P. Schmidt (Fed. Proc. 28:1099-1103, 1969), for example, had observed heightened infection with mengovirus, but not influenza virus, when conditions were hypobaric and normoxic at the time of infection. These results also support the belief that the stressful period is at the time of change in barometric pressure. (For correlation of studies see altitude conversion graph on Figure 36.)

Figure 36. Pressure Conversion Graph

Figure 36


THOUSANDS OF FEET
0
10
20
30
PRESSURE (% OF 1 ATM.)
100
80
60
40
30
700
500
300
200
mmHg
14
12
10
8
6
4
POUNDS PER SQ. INCH
0
2
4
6
8
10
ALTITUDE (km)

Since it seemed probable that the effect of SCE depended on the response evoked by the agent, a virus having a predilection for the respiratory system, mouse-adapted influenza, was chosen, and for comparison, murine hepatitis virus was also studied. The course of influenza virus infection was much more severe in the chamber animals. A preliminary experiment showed that the chamber animals had nearly twice the mortality of the controls; when repeated the mortality rate of the chamber animals was enhanced, suggesting a trend of shortened time of disease onset, but the cumulative mortality at the end of the experiment was similar in both groups. The possibility of spontaneous pneumonia being the primary cause of deaths was discounted; uninoculated controls maintained in the chamber did not die or appear ill. When hepatitis virus was given, however, the difference in mortality between chamber and control subjects was negligible.

Enhanced influenza infection, but not hepatitis, can be explained since murine hepatitis virus is known to elicit humoral antibody production. This virus replicates in the liver Kupffer cells, which process the antigen and start the sequence of events resulting in lymphocyte transformation into the plasma cell series. It is not yet clear how influenza virus is processed, but it is thought to evoke a T-cell or combined T- and B-cell response. In mice, the thymus size was decreased by SCE exposure, while the spleen remained similar in size to controls. One can postulate that murine hepatitis virus infection was not enhanced due to normal activity of the humoral immunity system, as suggested by normal spleen weights. However, a defect in the T-cell system would impair response to an antigen which probably involves action of both T- and B-cells. In the ferret there is evidence that B-cell function was impaired as a result of SCE exposure because 1) spleen weights decreased and 2) IgG synthesis was suppressed.

VII. ANTIBODY AFFINITY AS A FACTOR IN CHRONICITY OF VIRAL DISEASES (CONTRACT SUPPLEMENT, NAS 9-11941, MOD. No. 4S).

A. SUMMARY OF WORK COVERED BY MOD No. 4S.

1. Studies on development of the methodology for preparation and use of immunoadsorbents may be summarized as follows:

a) No advantage was obtained in using immobile supporting material with aliphatic side arms such as Affi-Gel 10, which is an agarose bead with a 10 angstrom aliphatic side arm terminating in a carboxy N-hydroxy-succinimide ester, over procedures using covalent binding of antibody directly to the polyhydric backbone of Sepharose 4B through the cyanogen bromide reaction.

b) A salt gradient ranging from 0.05 to 3.0 M NaCl displaced virus from immunoadsorbents prepared with rat anti-EMC and mink anti-ADV but not from mink anti-EMC.

c) Acid gradients ranging from pH 7 to pH 2 (glycine-HCl in 1.0 M NaCl) dissociated virus at pH 5.0 from immunoadsorbents prepared with mink anti-ADV and not others.

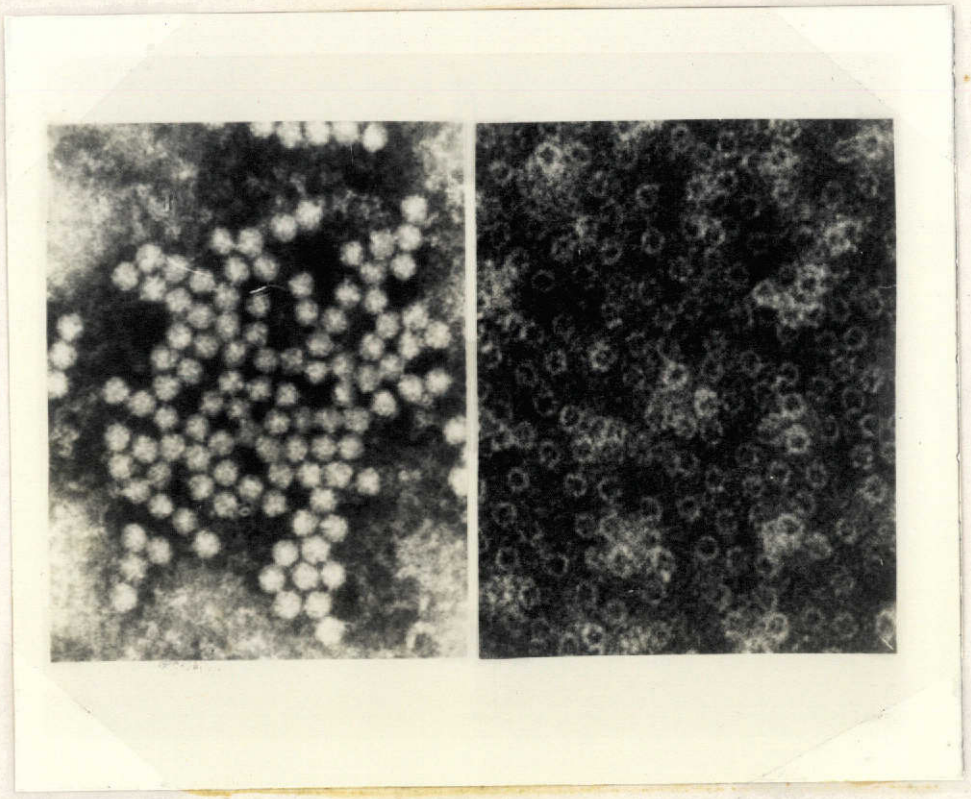
d) However, in all cases when the pH of the immunoadsorbent column was returned to near neutrality, virus was dissociated from all columns tested, mink anti-ADV, mink anti-EMC and rat anti-EMC.

2. Virus characterization based on studies with immunoadsorbents.

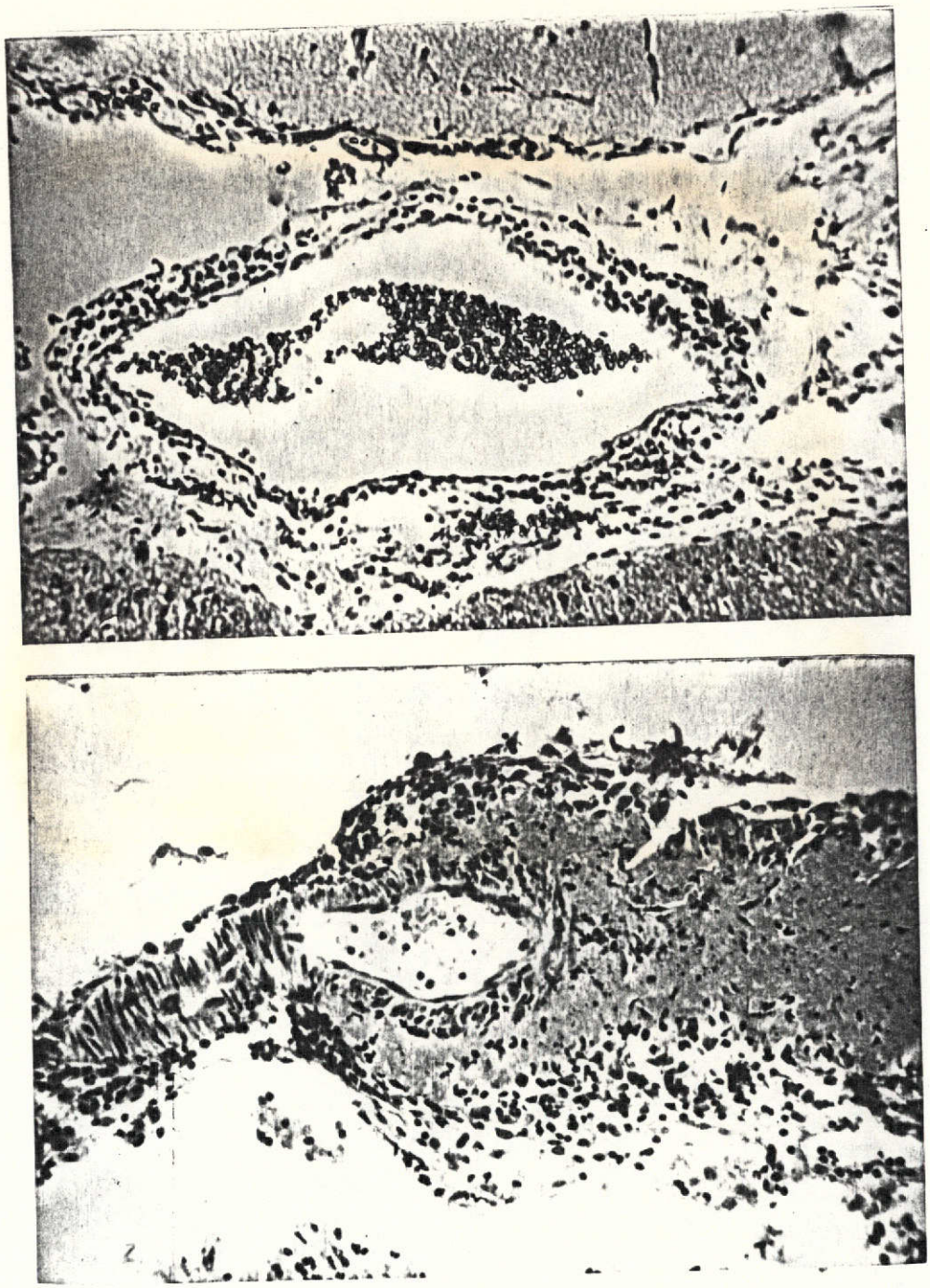
Studies on the characterization of the ADV may be summarized as follows:

a) Measurements from electron micrographs indicate ADV to be 25 nm in diameter and appear as an icosahedral particle (see Fig. 37 below).

Figure 37



Shown above are ADV particles from a mink kidney cell culture. The intact particles on the left have a buoyant density of 1.34 and the empty particles on the right, 1.29 in cesium chloride gradient. If this virus is classified as a picornavirus (based on ^{3}H-uridine uptake; capsid polypeptide pattern resembling other picornavirus; apparent cytoplasmic replication as indicated by Tsai and co-workers), the presence of empties in cell culture would further suggest that it belongs to the enterovirus subgroup.



Depicted (top) is typical periarteritis nodosa with medial necrosis in ADV infected mink. In a survey of 100 commercially raised mink possessing gammapathies, 17% had severe vascular lesions. Bottom photograph depicts arteritis (cerebellum) in ADV infected ferret. Aleutian disease in ferrets results in a higher incidence of monoclonal type gammapathies under ranch conditions.

b) The virus has been shown to incorporate isotopically-labeled uridine and not thymidine.

c) When analyses were conducted on mink kidney cell cultures, both empty and full particles were obtainable in separate fractions; intact particles have a density of 1.34 and empty particles, 1.29 (cesium chloride gradients).

d) Characterization of the peptides by SDS-gel electrophoresis has revealed 5 polypeptide components.

e) When purified virus is inoculated into mink, typical AD develops.

f) In L-cell cultures, CPE (as cell death) resulted 5 days after inoculation.

g) In mink kidney cell cultures, similar changes occur in established monolayers of cells.

h) Using capsid labeled (^{3}H mixed amino acids) intact and empty particles, empty particles bind to affinity columns at 91% level as opposed to 52% level for intact particles.

B. DETAILED REPORT OF WORK COVERED BY THIS PERIOD:

1. Aleutian disease as a model for studies on antibody affinity and chronicity patterns of virus diseases.

Aleutian disease (AD) of mink is caused by a virus possessing picornaviral characteristics and is manifested as a systemic proliferation of lymphocytes/plasmacytes with concomitant hypergammaglobulinemia, segmental vasculitis and fibrinoid deposition in the arterial walls and renal glomeruli. Since Helmboldt and Jungherr first described AD (Am. J. Vet. Res. 19, 212, 1958), considerable attention has been given to the development of vasculitis ranging from observations of isolated field cases to studies on the sequential development of lesions in infected ranch mink (Henson et al., Pathol. Vet 3, 289, 1966). However, this and other studies lacked definitive precision since the actual time course was not established in experimental studies, and the inocula used were crude tissue extracts from chronically infected mink (Henson et al., Lab. Invest. 19, 153, 1968). The virus may have existed in complex or aggregate form when inoculated.

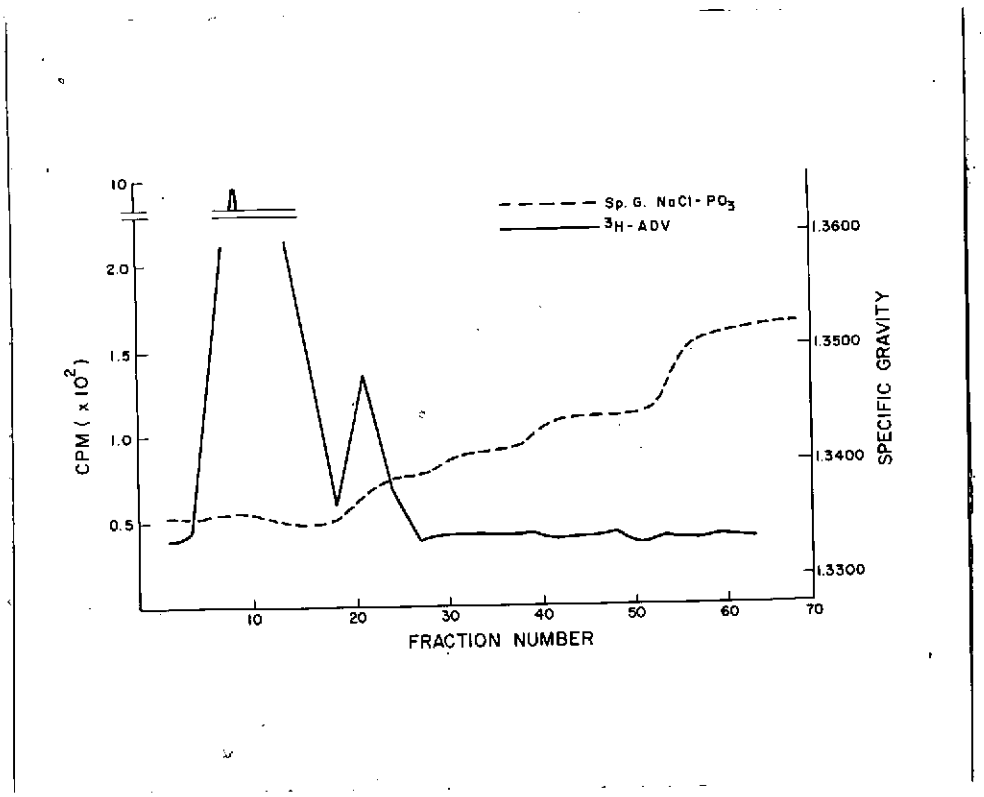
As shown below, in mink and ferrets (Fig. 38) with Aleutian disease (Kenyon et al., Am. J. Vet. Res. 28, 1167, 1967), vasculitis becomes a striking feature in mustelids during chronic stages of the disease. In both mink and ferrets this may be associated with a transition from heterogenous IgG gammopathy (polyclonal) to a highly homgeneous IgG (monoclonal). It has not been established whether the vascular lesions in mink and ferrets are a time dependent phenomena unrelated to specific features of the immunoglobulin elicited in response to the virus or are a function of antibody affinity/avidity.

In diseases such as AD, where viral chronicity is a prominent characteristic, an ideal situation exists for studies on the development of

immune complex disease and vasculitis. First, the virus is antigenic, and it may be replicating in endothelial cells of infected mink (Tsai et al., Can. J. Microbiol. 15, 135, 1969). This consideration is based on the description cytoplasmic crystalline arrays of particles in vascular tissue from AD infected mink. Porter and co-workers found AD antigenic material in phagocytes (Von Kupffer cells) during the first 10 days postinoculation with direct immunofluorescence using immunoglobulin from chronically infected mink (Porter et al., J. Exp. Med. 130, 575, 1969). However, the presence of virus in these cells may have been primarily the function hepatic immune clearance. Secondly, in AD as in other viral infections, the agent is self-replicating, thus producing a continuous supply of antigen. Third, the immune response results in nonneutralized complexes which retain their ability to infect and perpetuate the infection (Porter and Larsen, Proc. Soc. Exp. Biol. Med. 126, 680, 1967). These points were brought out by Notkins in a study of infectious virus-antibody complexes in which he suggests that virus-antibody complexes like other antigen-antibody complexes activate biological mediators which result in immunopathologic changes, such as increased capillary permeability and migration of polymorphonuclear leukocytes (Notkins, J. Exp. Med. 134, 41, 1971).

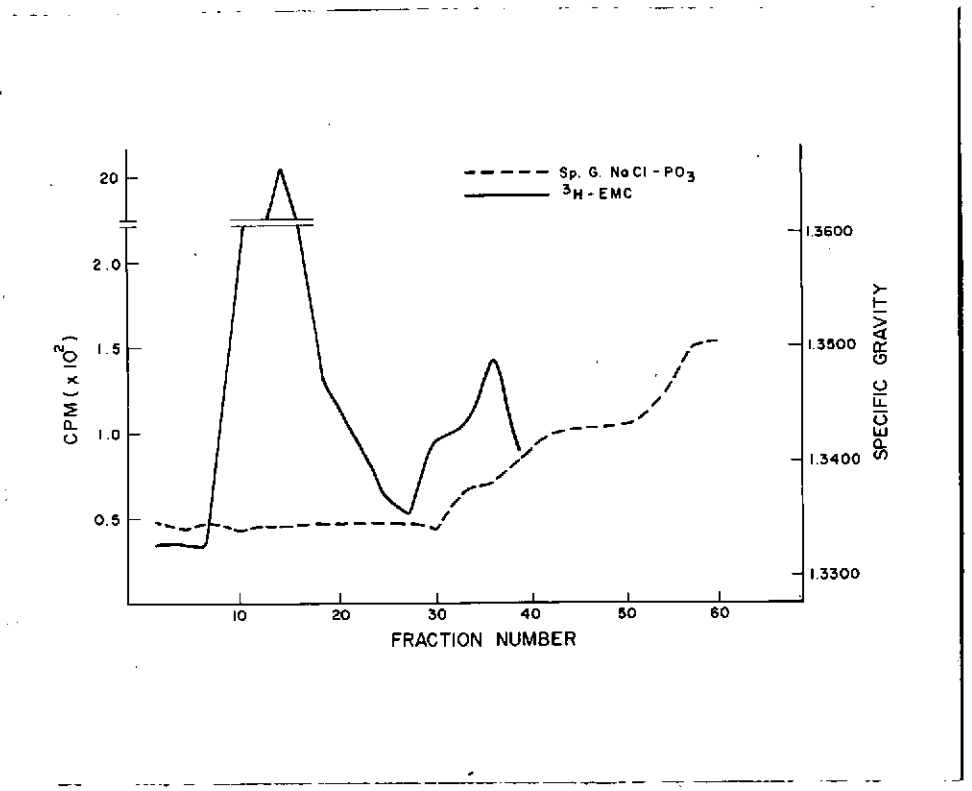
It is important to note that in AD antibodies of greatly different affinities have been described (Fig. 39-41 below) and that the role of low affinity or low avidity antibody has not been experimentally studied in the development of vasculitis and glomerulonephritis. In mink as well as other species, the usual response to immunizing agents is a neutralizing antibody of high affinity and with apparently increased avidity upon repeated immunization. This has been shown with mink immunized with the encephalomyocarditis virus (EMC).

Figure 39



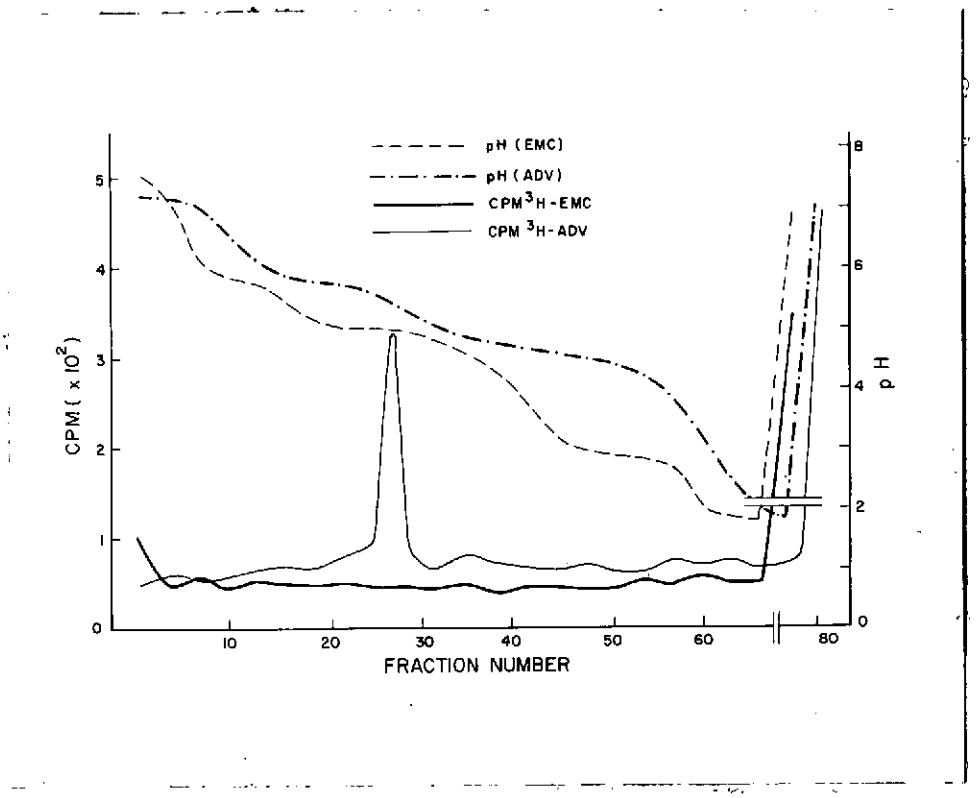
Depicted in this graph is the elution profile of capsid-labeled ADV from an immunoadsorbent column prepared with a mixture of monoclonal and polyclonal immunoglobulins from ADV infected mink. The column bed volume was 5 ml and was prepared with Affi-gel 10 adsorbent. A total of 20,000 cpm as ^{3}H-capsid-labeled virus were applied to the column; elution consisted of NaCl gradient (0.1 - 3.0 M at pH 7.6). The first large peak consists of the percolate containing unbound virus, the second peak represents the virus displaced at 0.5 M NaCl.

Figure 40



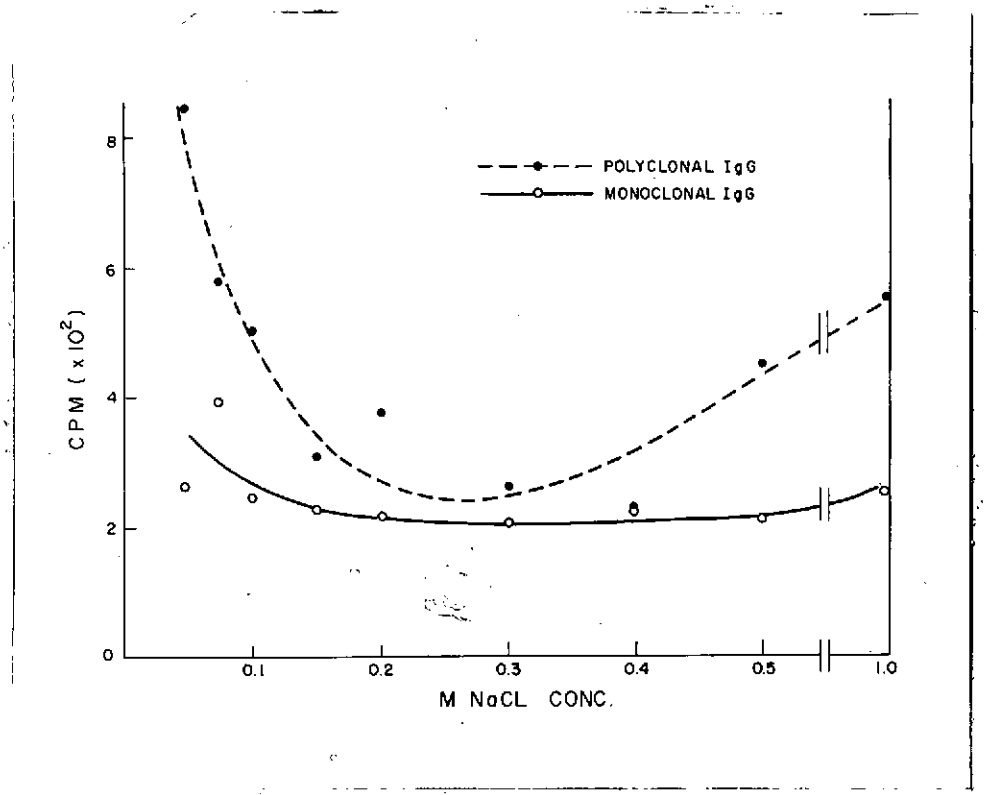
Depicted in this graph is the elution profile of encephalomyocarditis virus (EMC) with ^{3}H-labeled capsid displaced from an immunoadsorbent column prepared with rat anti-EMC IgG. The conditions for column preparation and chromatography were like that shown in Figure 2. As in Figure 2 with mink anti-ADV system, labeled material was desorbed from the immunoadsorbent at 0.5 M NaCl. In a similar experiment using mink anti-EMC as the immunoadsorbent, no components were detectable in the salt eluate. In these experiments it became apparent that mink develop a high titer-high affinity antibody when injected intracranially with EMC (10^{4} mouse LD_{50}).

Figure 41



Shown above are graphs of acid eluates from immunoadsorbent columns subjected to salt gradients (Figs. 2 and 3). The broken lines represent the pH gradient established and glycine-HCl (0.2 M) in 1.0 M NaCl. The heavy solid line is the elution profile for EMC fractions and the lighter line represents fractions from ADV system. Two observations are apparent in this figure: a) only with ADV were components obtainable at approximately pH 5.0 and b) in all cases, when the pH was restored to neutrality, addition virus was desorbed. In the case of EMC, these fractions desorbed by neutralization were infections. In the system using mink anti-EMC immunoadsorbent, no virus was eluted until the column was restored to neutrality.

Figure 42



Depicted above are graphs of labeled virus in percolates showing the relative binding affinities of polyclonal (broken line) and monoclonal (solid line) anti-ADV IgG immunoglobulins in varying concentrations of NaCl. Immunoadsorbent columns were prepared by binding each kind of IgG to Sepharose 4B with cyanogen bromide. (Monoclonal IgG was isolated from pooled sera having gamma globulins with electrophoretic homogeneity equal to that of serum albumin on cellulose acetate membranes; IgG was classed as polyclonal when the heterogeneity was 3 times as broad as serum albumin.) Columns (1.5 ml of immunoadsorbent) were used for each salt concentration and equilibrated against that salt solution before ADV (^{3}H-capsid-labeled, 1400 cpm in 20 µl) was added to each of the 2 types of immunoadsorbent. The concentration of label in the percolates is inversely proportional to the binding power of each kind of immunoglobulin at a given salt concentration. The data suggests that polyclonal immunoglobulins are suppressed by salt concentrations above and below the physiologic range.

2. Virus isolation and characterization.

Since the first description of AD by Helmboldt and Jungherr, the virus has remained unclassified and the various strains used in research uncharacterized. The virus that this investigator passaged since 1961 has a target-size molecular weight of 1.5×10^6 (estimated from inactivation by ionizing irradiation) and produced typical infiltrated of lymphocytes and plasmacytes in many organs with a doubling of serum gamma globulin concentration by 30 days after inoculation.

The approach which proved successful in isolation of the virus from mink tissue extracts (Kenyon et al., Science 179, 187, 1973) and later, by this laboratory, from L-cell cultures (Yoon et al., Nature 245, 205, 1973) and from mink kidney cells (Yoon et al., Fed. Proc. (Abst. 2227) 605, 1974) was affinity chromatography. Briefly, the use of an immunoadsorbent for ADV isolation consisted of covalent binding IgG anti-ADV with cyanogen bromide to Sepharose 4B (other solid phase covalent linked systems have been used; see below). Virus containing extracts from in vivo or in vitro systems were percolated through the immunoadsorbent (viral sorption) followed by washing of the immunoadsorbent until the effluent buffer is identical before and after in radioactivity or detectable chemical composition. Elution (desorption) systems used consisted of salt gradients and pH gradients superimposed on 0.75 - 1.0 M NaCl with glycine-HCl buffers ranging from pH 7.0 to 2.0. Eluate fractions are collected until the immunoadsorbent is returned to neutrality. This procedure has been widely used to isolate dilute antigens, enzymes, haptens and ligands (as reviewed by Cuatrecasas, Adv. Enzymol. 36, ed. Merster, J., Wiley and Sons, New York., 1972). However, viruses have not been isolated by this technique, presumably because of the difficulty in obtaining sufficient quantities of specific

antibody and lack of development of conditions which allow desorption of intact virus. Aleutian disease virus seemed suitable for isolation by immunoadsorbents since large amounts of presumably specific antibody are produced during the disease (Porter et al., J. Exp. Med. 130, 575, 1969) (see Fig. 37).

Other applications of affinity chromatography in isolation of animal viruses (by this laboratory) concerned refinement of procedures using IgG immunoglobulins produced in rats and mink by immunization with EMC since this virus has been extremely well characterized (Reuckert, Picornaviral Architecture, Comp. Virol. Ch. 8, Academic Press, N. Y., 1971), and it has a relatively simple capsid configuration, 3 major peptides and 1 minor. The fact that the virus is similar to ADV and can be easily quantitated by hemagglutination, plaque assay, bioassay in mice and elicits high antibody titers in mink as well as rats, it was selected for comparative analyses and development of standard procedures.

Since in AD a tremendous gammapathy exists, there is little likelihood of an antigen excess occurring in the peripheral circulation. Thus, another mechanism such as low affinity/avidity antibody may be responsible for lack of immune complex clearance. Recent studies on antibody affinities directed towards viral determinants (Kenyon et al., Fed. Proc. p. 605 (Abst. 2228), 1974) has shown that at least several broad classes of affinities exist. In these experiments it was shown that mink immunized with EMC produced high titers of antibody with high affinity, i.e., labeled virus (^{3}H-amino acids) could not be eluted in salt gradients from 0.1 M to 3.0 M NaCl or with glycine-CHl gradients (pH 7 to 2) in 1 M NaCl. On the other hand, mink anti-ADV immunoadsorbent systems showed components in salt eluates at 0.5 M NaCl and again at pH 5.0 on the acid gradients. Rats

immunized with EMC produced antisera with high neutralization titers; however, when evaluated on immunoadsorption columns, a component was eluted with 0.5 M NaCl but not in the acid gradient. Graphs of these data are shown above. If one compares early and late (polyclonal and monoclonal) anti-ADV for ability to adsorb virus on a series of immunoadsorbent columns equilibrated in various salt concentrations (ranging from 0.05 M to 1.0 M NaCl), it was apparent that polyclonal columns in salt extremes (hypo- and hypertonic) did not adsorb virus as well as monoclonal columns (see Fig. 42 below). In all experiments, when the columns were restored to neutrality, labeled capsids/ virus appeared in the eluates. This material in experiments with EMC was still infectious after desorption when assayed for infectivity in CD-1 mice.

Experiments performed by Nicklin and Stephen (Immunochemistry 11, 35, 1974) on recovery of protein antigens from immunoadsorbent columns prepared with non-avid antisera, indicated elution profiles similar to those seen with AD, i.e., protein components eluted at pH ranges of 4 to 5. They found that columns of non-avid sera adsorbed and released homologous antigen 3-4 times more efficiently than columns prepared with the avid counterparts (antisera). For virus isolation, in procedures where antigenic structure is a function of the capsid configuration, it becomes important to dissociate or desorb the virus under conditions which will not denature it. Thus, the efficiency of the procedure may be greatly enhanced using the properties of a subpopulation of antibodies possessing a lesser binding affinity/avidity for homologous antigen without preliminary removal of antibodies of higher binding strength. When columns are recycled, this is achieved at the expense of an initial loss of antigen in the first cycle. The necessity of sharp gradient formation in securing effective desorption

of protein antigens using pH gradients has been reported (Crook et al., Immunochemistry 9, 945, 1972) and has been observed by this laboratory with ADV (Kenyon et al., Science 179, 187, 1973).

In AD it is unknown whether the antibody subpopulation which binds virus with greater affinity/avidity represents neutralizing antibodies, and the weak binding subpopulation represents that species which forms infectious immune complexes. With antibodies to enzymes, it has been shown that as the affinity of the antibody increases so does the degree of enzyme inactivation, supposedly induced by changes in steric configuration (Erickson, Immunochemistry 11, 41, 1974). Similarly, it appears that as the affinity of antiviral-antibody increases, so does the viral inactivation rate and reduction in human complex formation as shown with mink anti-EMC and with rat anti-EMC.

Another possible factor in AD contributing to the pool of immune complexes is the formation of defective particles. In many virus infections the number of defective particles outnumbers infectious particles by as much as 100 to 1 (Notkins, J. Exp. Med. 134, 41, 1974).

VIII. PASSIVE IMMUNE AGGREGATION (EXTENSION OF CONTRACT NAS 9-11941 MOD. No. 4S).

A. SUMMARY OF WORK COVERED BY EXTENSION:

Studies on passive immune aggregation using the MEMS with antibody prepared in mink and rats to encephalomyocarditis virus.

1. Work performed at NASA using instrumentation fabricated by Aerojet Medical and Biological Systems.

a) Rate of decay (spontaneous aggregation) of antibody coated beads balanced against uncoated beads showed no consistent trend.

b) Reactions of 3 levels of IgG anti-EMC treated beads (10 mg, 6 mg and 1 mg) in total volume of 10 GBS with 0.1 ml EMC titered from 10^{-1} to 10^{-6} produced no consistent trends in readings. (Although Dr. J. L. McQueen, contract monitor, felt the approach to selection of bead concentration and other factors seem empirical, the conditions used were those described by the manufacturers of the test bed system, NAS 9-11371.

c) Determination of decay rates of only virus added to uncoated beads indicated no significant effect.

2. Work performed at Sloan-Kettering Institute using Beckman Acta III.

a) The Acta III was set up with water in the reference holder and various bead concentrations in the sample holder (quartz cuvettes) with wavelength at 470 and slit width at 0.4 to provide a concentration giving an optical density of 1.0.

b) Decay rate or settling was determined overtime. A constant rate (linear OD function against time) was Δ 0.5 O.D./15 min.

c) During the following experiments, no consistent trends were obtained. The factors tested were:

i) Variations of antibody concentration 0.001 mg/10 ml bead solution as described on p. 11, Final Report, Contract NAS 911371.

ii) Range of temperature for sensitizing beads 26°C, 37°C, and 56°C.

iii) Length of time of sensitizing beads 0.5, 1.0, 3.0 and 6.0 hr.

iiii) Time and temperature of holding sensitizing beads before reading.

B. DETAILED REPORT

The procedure used for preparation of sensitized latex beads was as initially recommended on page 11, Final Report, Contract NAS 9-11371, Aerojet Medical and Biological Systems.

The stock suspension (10%) of beads was washed in distilled water and resuspended in 10 volumes of glycine buffered saline, pH 9.0 (GBS) 0.1 M glycine in 0.05 M NaCl at pH 9.0. A volume of antibody in GBS equivalent to the original volume of stock beads, ranging in concentration from 1.25 to 5.0 mg/ml of antibody protein depending on bead size. The mixture was agitated gently for 30 minutes at room temperature after which an equal volume of GBS was added and the incubation was continued for an additional 30 minutes.

The supernatant was removed after centrifugation at 3000 x g for 5 minutes. The resultant beads were reconstituted to 25 times original volume in GBS containing 0.2% crystalline bovine albumin.

The antisera used was prepared in either mink or rats to encephalomyocarditis virus (EMC). The mink were administered 1 injection intracranially of 10^4 ID (mouse infectious doses) and at 3 weeks later, another injection of 10^4 ID given intraperitoneally. The intracranial inoculation produced a transient encephalopathy which lasted 7 days. At the conclusion of this immunization, the neutralization titers in vivo exceeded 10^6 ID_{50} and HAI titer of 11 doubling dilutions. Antisera was also raised in the rat by similar immunization routine; in which case, the titer was not as high (10^5 ID_{50} in vivo).

The IgG immunoglobulin fraction was isolated by DEAE cellulose chromatography and concentrated to 1-2 mg/ml in phosphate buffer pH 7.6. The efficiency of these antisera were also determined in a series of

immunoadsorbent experiments using Sepharose 4B or Affi-Gel 10. Prior to use of aliquots of the immunoglobulin, it was stored at -70°C. In each case, the titer the potency and quality of the immunoglobulin preparation was excellent.

In each experiment, the linearity of "readout" was determined over a range of bead concentrations, and found to be linear as seen in Figure 43 (plot on semi-logarithmic paper) and Figure 44 on linear paper.

It must be stressed as seen in Figure 45, that all assays conducted with latex beads to which the antibody was noncovalently bound, no consistent or reproducible trend was observed between aggregation and virus dilution. This was true over all parameters studied, i.e., effect of antibody concentration, effect of incubation temperature, effect of length of incubation, effect of bead concentration and effect of diluent used for virus. It appears that the antibody must be bound covalently to the supporting matrix.

Figure 43

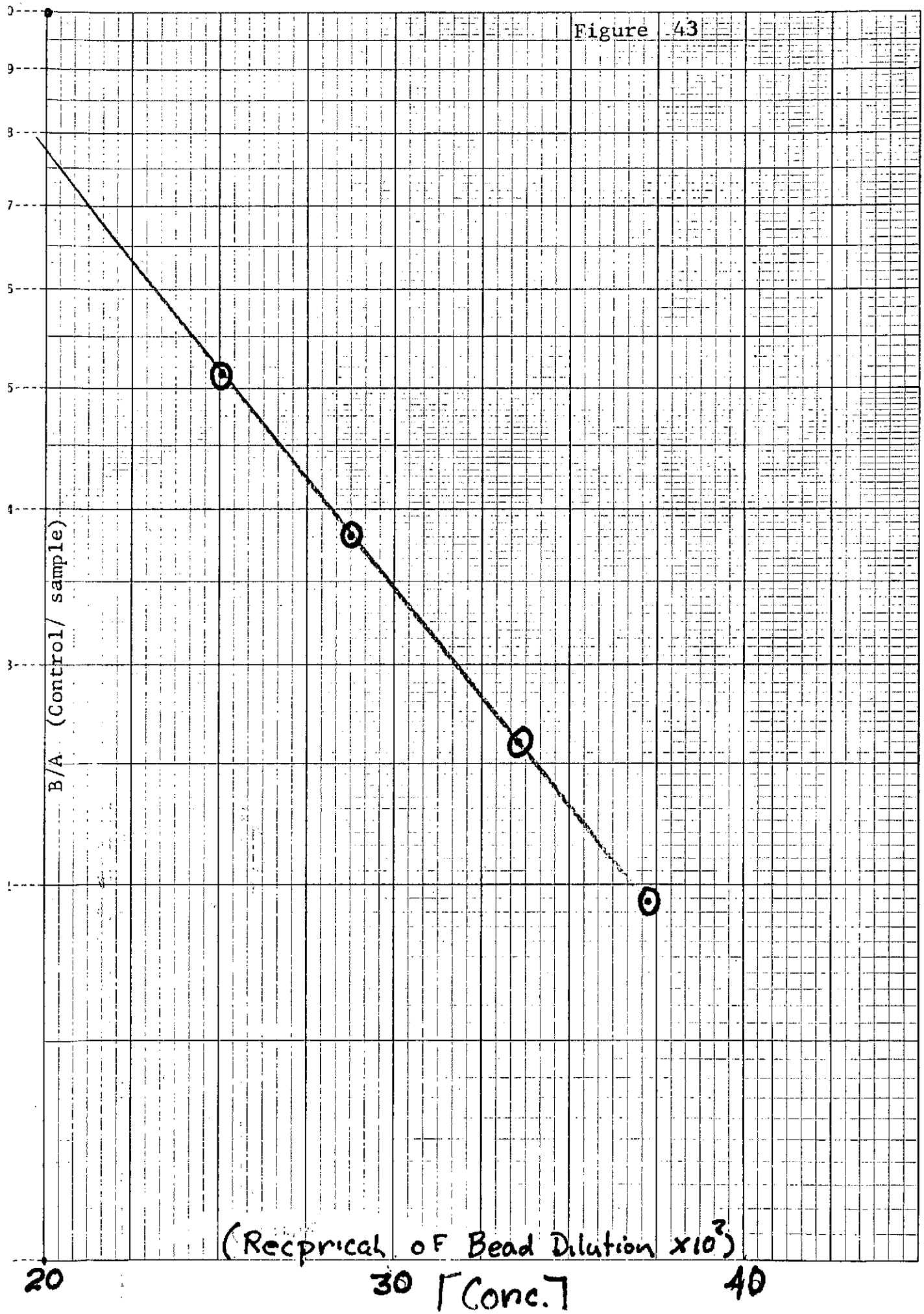

B/A (Control/ sample)
(Recprical of Bead Dilution x10³)
20
30
[Conc.]
40

Figure 44

Bead Concentration vs. Read Out

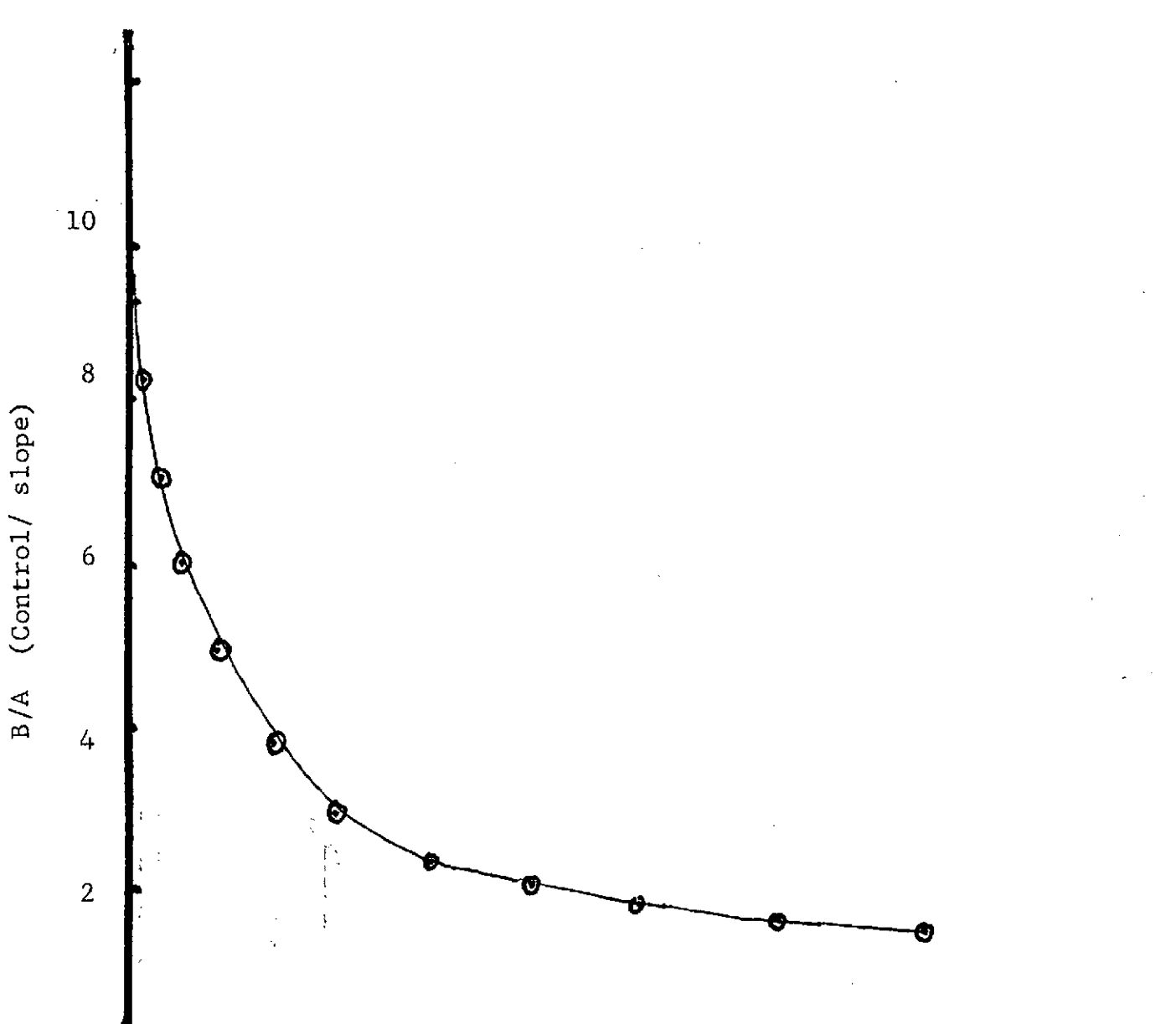
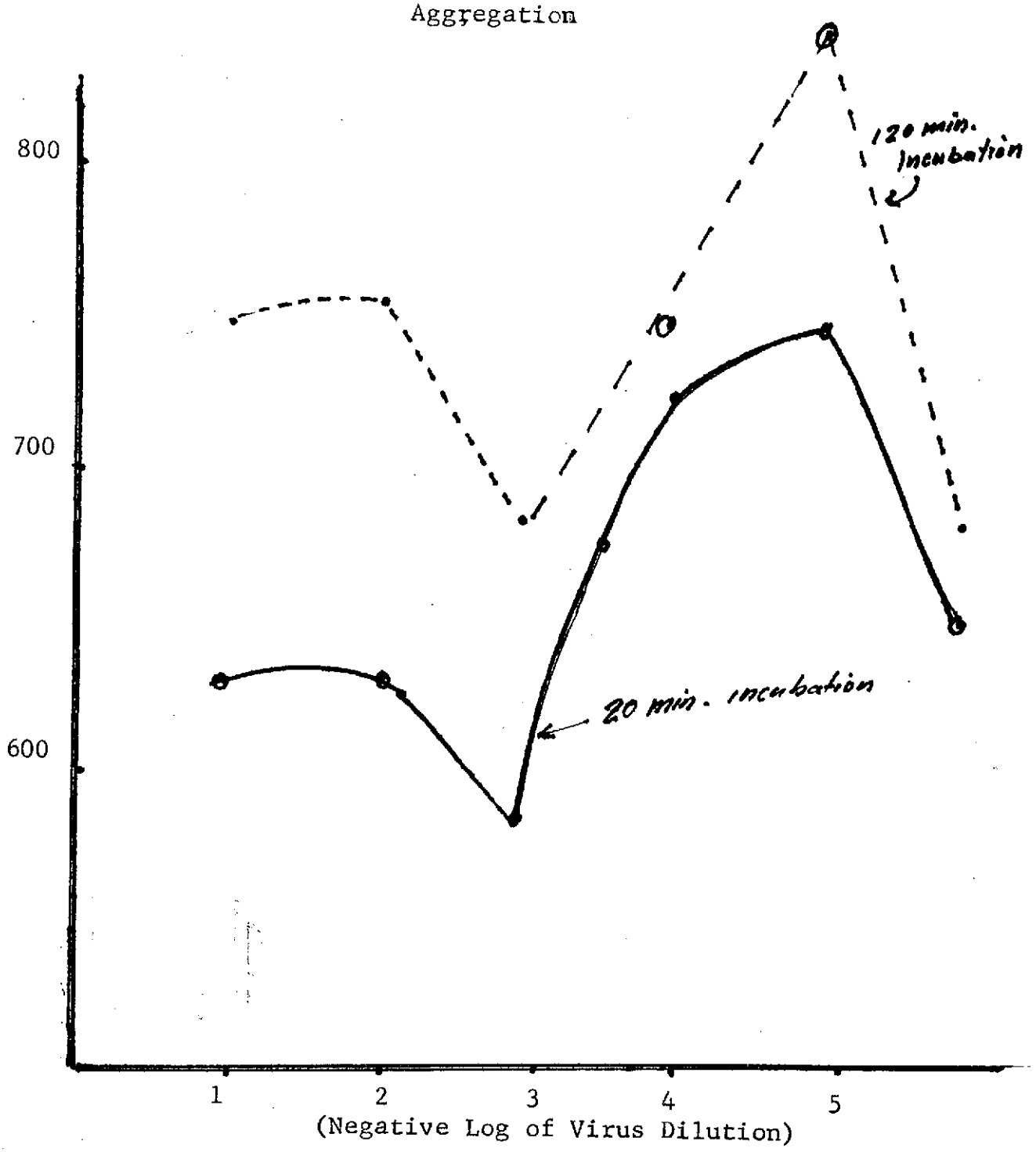


Figure 45

Effect of Incubation Time on Passive Immune Aggregation



IX. SUMMARY

Repeatedly, it has been suggested that there was a stressful event associated with ascent or descent in altitude (Schmidt, Fed. Proc. 28:1099, 1969). In this study and in those conducted by Erlich and Mieszkuc (Dev. Ind. Microbiol. 5:207, 1964) using animal models, they did not establish the nature of the enhancement of disease in experimental subjects under SCE.

Thus, a series of experiments were conducted starting with preliminary studies performed at the Illinois Institute of Technology, Research Institute (IITRI) to determine initial tolerability of ferrets to gas ratios of 30% nitrogen and 70% oxygen under 5 psia. Depression of immunologic responses related to stress of the initial event (decompressing to 5 psia) were evident at 7 days after introduction into Skylab environment. Recovery to normal response was apparent by 21 days exposure. This evidence consisted of decreased lymphoid organ weight and reduced response to antigenic stimulation. Also in preliminary experiments, it became apparent that histologic changes in the mucociliary system of the upper respiratory tree were attributable to high levels of ammonium arising from animal waste due to low turnover rates of gas environment.

Thus having extended the work previously reported in the sense that disease enchancement could be a factor of stress mediated through immunodeficiency, a program was initiated with a better designed chamber which would not compromise animal health by accumulation of animal waste.

For this purpose, a stainless steel low pressure facility was designed which consisted of 2 subchambers, that permitted mutual isolation of experimental groups and/or selective by removal of animals without

return of the entire cabin to ambient pressure. Throughout experimental tests, the chamber environment was stable, fluctuating only 4-6 mm Hg in pressure and 1-3^{o}C in temperature. Upon ascent to altitude, neither young adult mice, ferrets, nor suckling mice indicated signs of bends.

Mice subjected to the 70:30 O_2:N_2 and 5 psi cabin environment, had severely reduced thymic weights 3 days after exposure to cabin atmosphere. In ferrets, spleen weights were most affected by ascent to altitude; thymus weights remained relatively unchanged. The fact that control ferrets housed in rigid Plexiglas isolators with absolute filters showed a similar trend in splenic weights due to stress factors related to the enclosed environment. Animals housed in the open did not show these changes.

It is important to note that ferrets housed in the chamber described in the text of this report were free of lesions in the mucociliary system. Although rodents have a less distinct goblet cell and mucociliary blanket, no changes were observable in these animals that could be attributed to accumulation of ammonium in the SCE.

Murine hepatitis virus (MHV_3) infection in SCE did not appear to be enhanced, whereas influenza virus infection was. Since humoral antibody response is a major factor in MHV_3, it may be a response of primarily B-type lymphocytes. Mice which had reduced thymic weights and not reduced splenic weights indicate a prerequisite for reduced resistance to influenza virus, which apparently involves T-cell or combined T- and B-cell function as opposed to MHV_3 which is known to elicit humoral antibody. In mice, as indicated by normal spleen weights and humoral response, this limb of the immune system was unaffected by SCE.

Our studies have clearly demonstrated that ferrets immunized with Brucella Strain 19 prior to being housed in SCE had decreased synthesis of

IgG from IgM was accomplished by inactivation of IgM with mercaptoethanol. Chamber stresses, therefore, affected humoral antibody activity in the ferrets, as indicated by reduced spleen weights and decreased IgG synthesis.

Because of the possibility of latent infections being responsible for stress induced upper respiratory diseases of astronauts, the role of neutralizing antibody as a function of antibody affinity/avidity was investigated. The model consisted of Aleutian disease virus (ADV) which infects ferrets and mink resulting in nonneutralized immune complexes. These studies demonstrated that early antibody to ADV had lower affinity/avidity than late antibody with respect to chronicity. These studies culminated in a description of antibody affinity, first isolation of ADV and its cultivation in vitro.

With the prospective development of antiviral agents and the possible viral etiology of cancer, there is an increasing need for the rapid detection of viruses prior to overt disease. Present methods of virus isolation and identification commonly involved the use of cell culture systems in order to increase the virus population to readily detectable levels or the formation of cytopathic changes in the cultured cells. A minimum of 72 hours and a maximum of upwards to a month or more are necessary to accomplish the isolation procedures.

However, the use of immunoadsorbents as applied above, this ADV has greatly facilitated detection and isolation from cell culture systems. The prime factor required is either incorporation of an isotopic label or an assay for the agent in eluates during desorption. By other methods, the identification of the particular virus in question may involve 2 to 24 hours for the more rapid tests such as complement fixation and hemagglutination inhibition to 2 - 7 days for neutralization tests.

Thus it had been proposed that an efficient and rapid method of detection be available, one which could detect viruses in an astronaut before the symptoms of the disease were evident and the afflicted person or persons could be isolated. With this knowledge, of course, space flights could be aborted or astronauts substituted.

The method proposed for viral detection utilized passive immune aggregation (PIA) technique. When viruses and certain types of homologous antibodies react, the viruses are agglutinated and this agglutination detected by a number of means. In the PIA technique, recommended by the contract monitor, latex spheres (beads), on the order of 1 to 2 μm in size were coated with specific antibody. When they react with viruses, agglutination occurred. This agglutination was detected by absorbency of transmitted light.

Bead agglutination tests are in common usage to detect antigen-antibody reactions. In some the antigen is attached to the beads to detect antibody and in others antibody is coated on the beads and will react antigen. Such tests are available commercially to detect rheumatoid factor, C-reactive protein, antinucleoprotein factors associated with systemic lupus erythematosis, and fibrinogen levels among others. These tests the patients specimen is reacted with the sensitized beads and macroscopic agglutination usually indicates a positive test.

Since then, passive immune aggregation represented an approach that may be used to detect subclinical viral infections in astronuats or possibly even cancer patients, this procedure was studied as a means of rapid detection of viruses in animal systems. Preliminary analyses have indicated the procedure described in contract NAS 9-11371, Microbial Ecology Measurement System, was unworkable due to dissociation of antibody from

insoluble supporting matrix. As in affinity chromatography (immunoadsorption) the antibody must be covalently bound to the supporting material to prevent new adsorption equilibria from being established when exposed to antigen.