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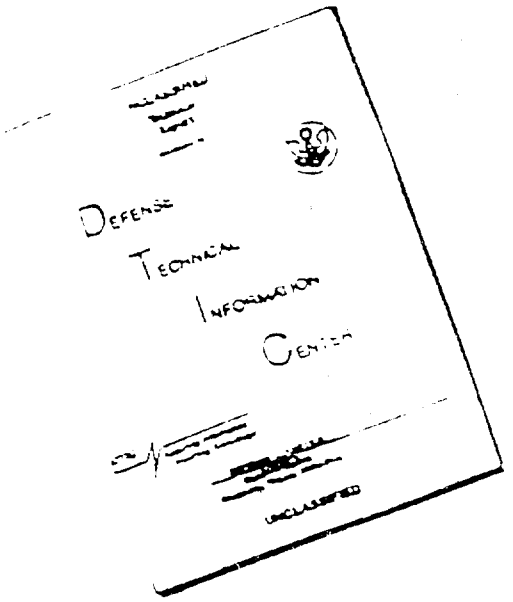

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Report 991-Final

ARTIFICIAL ATMOSPHERES IN SEALED ENVIRONMENTS

Fred A. Hitchcock (retired), John H. Dines,
and Edwin P. Hiatt

Department of Physiology

Department of the Navy
Office of Naval Research

Contract Nonr 495(19)

RF Project 991
Report - Final

FINAL

R E P O R T

by

THE OHIO STATE UNIVERSITY
RESEARCH FOUNDATION

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On: ARTIFICIAL ATMOSPHERES IN SEALED ENVIRONMENTS

For the period: 1 September 1959 - 31 August 1962

Submitted by: Fred A. Hitchcock (retired 1961), John H. Dines, and Edwin P. Hiatt
Department of Physiology

Date: September 1962

ABSTRACT

There are two parts to this report. Part I deals with the design, construction, and testing of a facility used to investigate the effect of exposing small animals to abnormal gaseous environments for relatively long periods of time. This facility consisted of two 1000-liter plexiglass chambers which were designed to be completely closed, with provision for handling the animals and replenishing their food and water via glove ports without altering their gaseous environment. There was also an arrangement for regulating temperature and humidity, for absorbing carbon dioxide and for measuring oxygen consumption. In one chamber the pressure could be controlled below atmospheric pressure.

Part II deals with the use of this facility to study the reaction of young, white rats to an atmosphere almost free of nitrogen but with the same partial pressure of oxygen in their lungs as the control animals breathing air. This was achieved by keeping the experimental animals in almost pure oxygen at a reduced pressure. Thus, there were actually two experimental variables, the lack of nitrogen and the decreased total pressure. After some preliminary experimental trials which were not satisfactory because of failure of control of the environment or intercurrent disease in the rats, a successful experiment lasting 24 days was completed. This 24-day period was preceded and followed by several days of observation with both experimental and control animals in the same environment. No effect of the nitrogen-free environment was observed. The experimental rats gained weight, ate, drank, and consumed oxygen at the same rate. A decrease in their rate of urine production was attributed to the increased rate of evaporation at the lower pressure.

PART I. DESIGN, CONSTRUCTION, AND TESTING OF AN ENVIRONMENTAL FACILITY

In recent years systems have been described in which animals were maintained in various atomospheres, either at ground level for short periods or at simulated altitudes for many days (ref. 1-6). None of these systems, however, combinded the characteristics of a closed system with the ability to examine the animals *in situ* at all times.

DESCRIPTION

The systems(one experimental and one control) described in this paper were used to study the prolonged effect of artificial atmospheres on small animals. They were constructed so that the animals could be examined, excreta collected, oxygen consumption measured, and exchange of materials accomplished without altering the animals' environment. The control system was kept at ground level and was, therefore, subjected to daily variations of barometric pressure, the pressure within the experimental system was held constant at any predetermined value between 730 and 196 mm Hg.

The experiments for which the systems were designed also called for a recirculated atmosphere of constant composition, temperature controlled at 24°C ± 1°, and a relative humidity of 50-55%.

Each system consisted of an animal chamber and circulation ducts to hold the environmental gas. The animal chamber was connected to a spirometer containing oxygen. As both systems were identical, except for pressure control, only the experimental one will be described (illustrated diagrammatically in Fig. 1) although references will be made to the control system when necessary.

The animal chamber (approximately 1000 liters in volume) was made of half-inch thick plexiglass. Plastic tubes inserted through the walls and cemented in position with ethylene dichloride formed supports for all hose connections. Small tubes for sampling were inserted in the same way.

The circulation duct, which included two interconnected plexiglass boxes, contained the centrifugal circulation fan and motor (A), dehumidifying coils (B), and heating units (C). The aluminum circulation fan was driven by a 1/20th h.p. explosion-proof motor. (American Blower Co. Model 45H). At 1725 rpm and 1/4-inch H_2O static pressure this fan produced a flow of 3226 liters (114 cu.ft.) per minute.

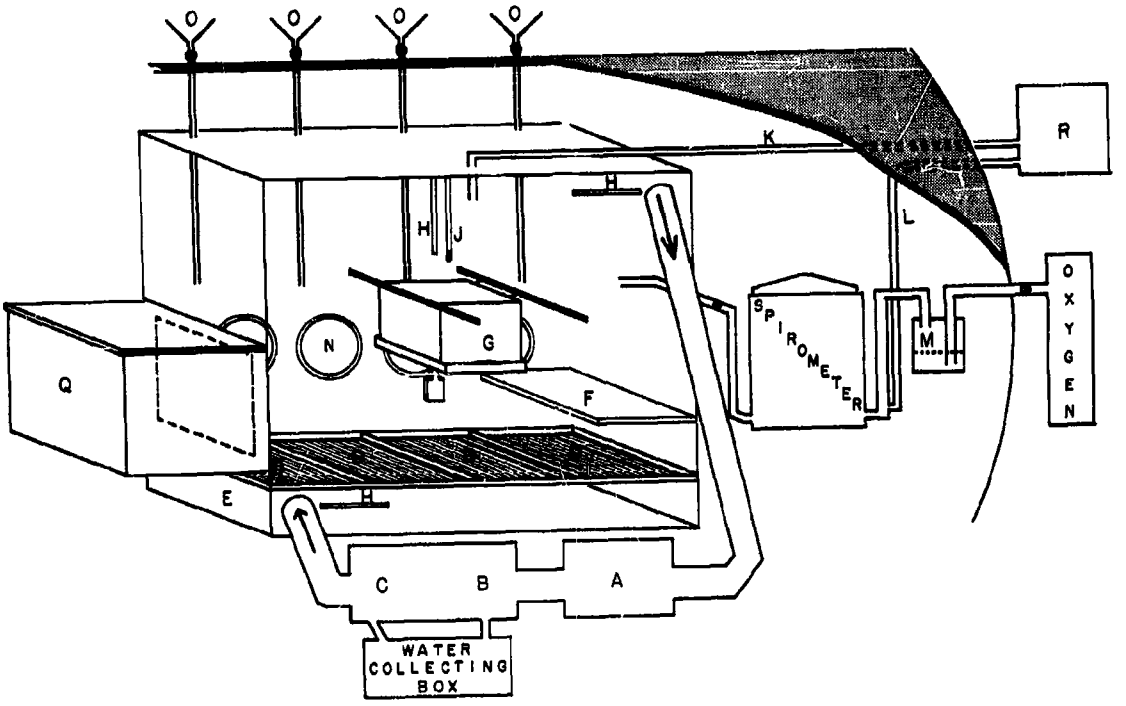

Fig. 1. Experimental closed system within altitude chamber

(A) Circulation fan (B) Dehumidifier (C) Heating coils (D) Filter beds (E) Position for activated carbon filter (F) Work platform (G) Rat cage (H) Thermometers (J) Wet bulb thermometer (K and L) Lines from animal chamber and spirometer to evacuation system (R) (M) Humidifier (N) Holes for rubber gloves - gloves not shown in diagram (O) Graduated water containers with line to drinking boxes on animal cages (Q) Hatch and gas lock. Arrows indicate direction of circulation. Controls for dehumidifier and heating coils placed outside the altitude chamber

The total capacity of the dehumidifier was between 1500 and 1700 B.T.U. per hour. The temperature of the coils was controlled by an expansion valve and could be calculated from the suction pressure. The heating units provided up to 400 watts and were controlled by a variable transformer.

Within the animal chamber shallow wire mesh trays (D) contained CO_2 absorbent. A cylindrical filter (E) containing activated carbon could be fitted over the circulation inlet for the absorption of other evolved gases. A work platform(F) positioned at one end of the chamber held additional food, scales, and collecting vessels. Four animal cages, positioned as the one (G) in Fig. 1, were supported in such a way that they could be easily handled. (In experiments so far each cage has held three rats). Other cage arrangements were tried, particularly one with individual cages for each rat arranged on a large wheel, but the design portrayed proved most satisfactory.

Thermometers (H) indicated inlet, outlet, and mid-chamber temperatures, the last named being positioned so that it indicated the temperature experienced by the animals. The humidity was measured by wet- and dry-bulb thermometers placed on a platform together with a small fan and positioned as in Fig. 1 (J). All electrical leads were run into the system through rubber stoppers. Pressure regulation was obtained by placing the complete system within a 16-man altitude chamber, which had an air lock. The various system controls were on the outside. The spirometer was so positioned that its scale would be read through an observation port.

A rubber tube (K) running through the wall of the altitude chamber connected the animal chamber to a special evacuating system (Fig. 2 and Fig. 1 R). A similar connection (L) was made for the spirometer, which also has its own oxygen supply line passing through a humidifier (M). This arrangement made it possible to adjust the gas composition. Obtain samples for analysis, and to maintain pressure equilibrium between the closed system and the altitude chamber.

The pressure within the altitude chamber was controlled by adjustment of the usual inlet and vacuum valves. An altitude controller was designed but only used occasionally. It consisted of a photocell which sensed the height of the mercury column in the Hess barometer attached to the altitude chamber, and electrically controlled a bleed valve into the chamber. With this it was possible to maintain a chosen absolute pressure setting within 0.2 mm Hg.

All operations within the animal chamber were carried out through two pairs of long rubber gloves (N). These were fitted on to plastic rings (American Sterlizer Co. Plastic Dry Box Rings), which were cemented with ethylene dichloride around 6-½-inch holes cut in one side of the chamber. As yet attempts to close the holes with plexiglass discs when the gloves are not in use have proved unsatisfactory. Instead, stiff plastic "caps" were fitted over the gloves and around the glove rings in order to reduce the diffusion of gases through the rubber and to minimize volume changes in the system due to glove movement.

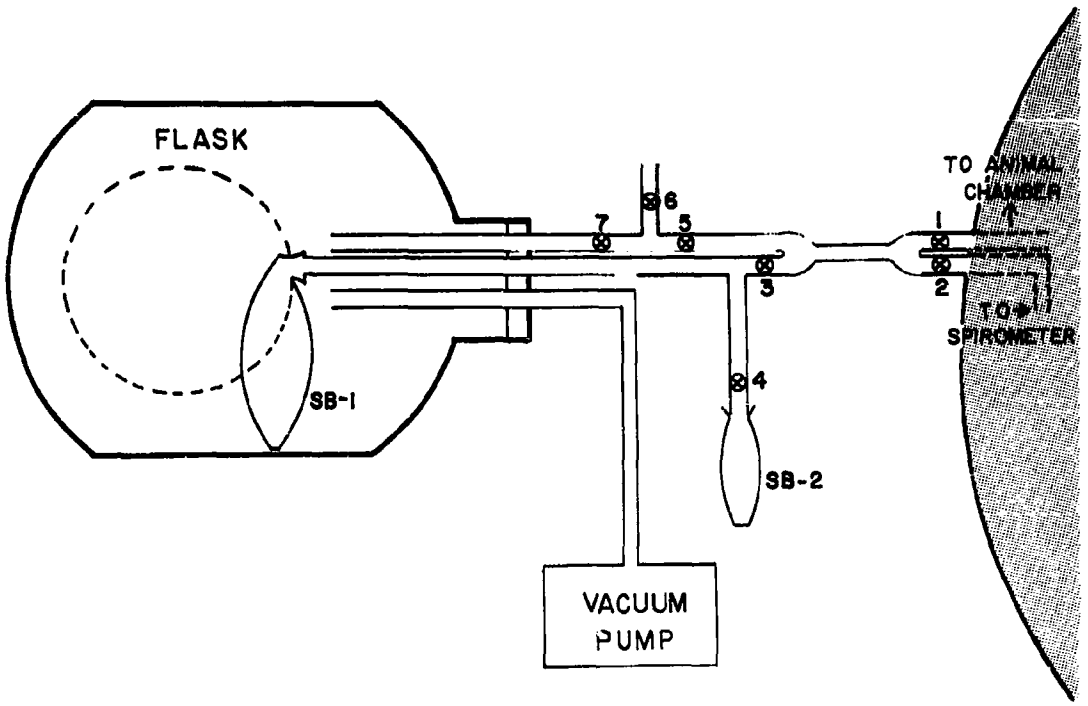

Fig. 2. Evacuation System: SB-1, First Sample Bag, SB-2 Second Sample Bag

Tap 7 is open at all times unless gas is being introduced into the closed system, in which case the gas enters via taps 6, 5, and 1 or 2. For normal operation, flask pressure is reduced by a vacuum pump. By opening taps 1 and 5 gas is removed from the animal chamber and vented to atmosphere. By opening taps 1 and 3 a sample is obtained in the first sample bag (tap 3 is then closed). Flask pressure is increased to ambient by opening tap 6. Sample is transferred to second sampling bag by blowing in through 6 to increase flask pressure above ambient and opening tap 4.

Ground food was supplied in non-spill feeders based on the design reported by Fregly (ref. 7) (Fig. 3). These contained 500 gm. of available food and were provided with a device to prevent bridging of the food.

Water was supplied in drinking devices as seen in Fig. 4. The box was supported outside the animal cage while the drinking cup rested inside it. The boxes were filled from sources outside the altitude chamber (Fig. 1(O)) and, although known volumes of water could be added to each box from individual graduated containers, accurate measurement of the water level in the boxes was difficult.

Feces were collected in removable metabolism trays suspended beneath the cages and urine was allowed to drain into bottles attached to the trays.

A sealed hatch and air lock (Q), placed at one end of the animal chamber, made possible the removal or replacement of bulky materials before or during experimental runs.

OPERATIONAL PROCEDURES

It must be pointed out that once the experimental system was at reduced pressure operators could enter the altitude chamber only through its lock and carry out their work at the reduced pressure. This required a team experienced in altitude chamber operation.

When a mixture of gases was contained within the animal chamber, pressure changes brought about by manipulation through the rubber gloves caused these gases to mix with the oxygen in the spirometer. This was overcome either by shutting off the spirometer and attaching an expansion bag to the animal chamber (to accommodate the pressure changes) or by temporarily filling the spirometer with the gas mixture. When 100% oxygen was being used throughout, this problem did not arise.

Since 20 pounds of soda lime lasted approximately 10 days in experiments carried out so far using young rats, renewal was necessary during prolonged runs. The use of the sealed hatch and air lock was time consuming, so as long as the materials for exchange could be contained in a vessel with a diameter less than 6-½ inches, they were passed through the glove holes in the following manner.

The first operator could close the glove hole by holding a suitably sized disc against it on the inside of the animal chamber. The second operator would take off the glove, put the vessel inside it, and replace the glove. The first operator would then take the vessel into the chamber. The procedure was confined to experiments where the very small influx of outside air was not important.

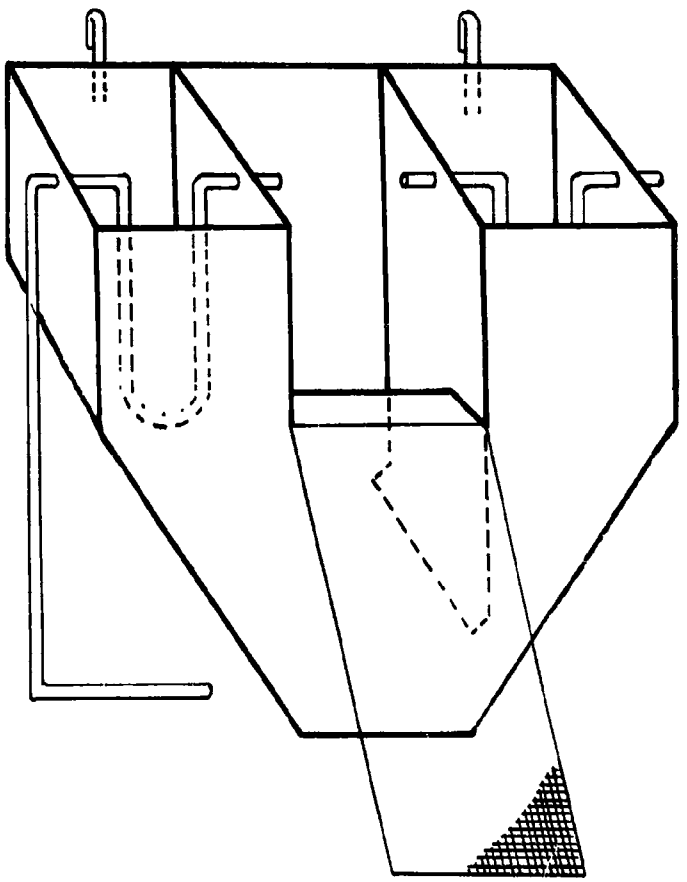

Fig. 3. Non-Spill Rat Feeders Based on Design of Fregley (7)

Rats feed from central platform. Bent wires ensure free flow of ground food from hoppers because rats often knock against them.

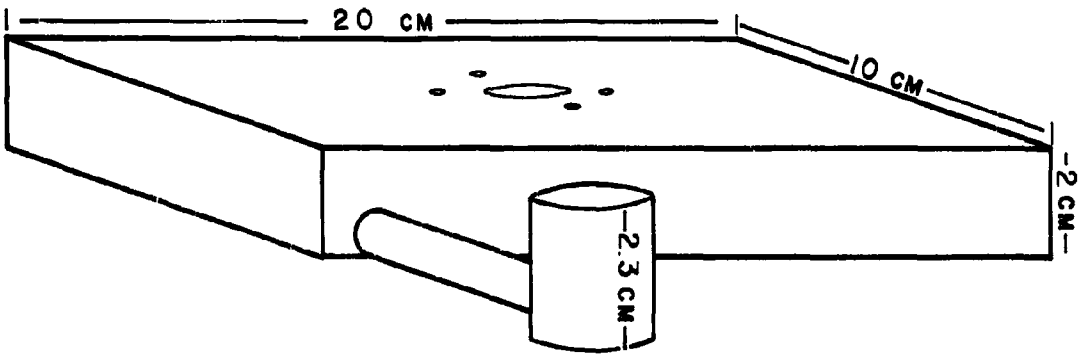

Fig. 4. Drinking Box

Large hole in top for refilling. Small holes for pressure equalization

ATMOSPHERIC CIRCULATION

The circulation fan produced an air velocity of 275 meters (900 ft.) per minute through the animal chamber outlet with all the filters in position. However the low working pressure of the fan was a disadvantage, especially when the system was at reduced pressure (ref. 8), because very low resistance filters had to be used. A 3450-rpm motor would probably be more satisfactory.

CONTROL OF TEMPERATURE AND HUMIDITY

With 400 watts of available heat, temperatures throughout the animal chamber could be held uniform and a level which maintained the rats at about 24° as long as the laboratory temperature was between 20°-22°C. High external heat loads required low inlet temperatures in order to maintain the mid-chamber temperature. The resultant temperature gradient was not linear and it was difficult to assess the average temperature of the animal chamber in these circumstances. The difficulty was aggravated when the system was at reduced pressure, presumably because of the effects of the lower gas density.

It was found that the relative humidity for the specified temperature could be well controlled within the desired range. Once the expansion valve was set, it required little adjustment unless the atmospheric pressure within the closed system was greatly altered.

Relative humidity was determined for ground level conditions from dry- and wet-bulb temperature differences using sea level psychrometric charts. The same dry bulb - wet bulb - humidity relationship is not valid for high altitude or for gas mixtures other than air, because in these circumstances the wet-bulb temperature may not be the same as the adiabatic saturation temperature. We are indebted to Dr. Charles D. Jones of the Department of Mecanical Engineering for calculating the tables from which the relative humidity was obtained when atmospheres of pure or enriched oxygen were used at reduced pressure.

We feel, however, that it would be advantageous to use sensors which registered water-vapor pressure rather than wet- and dry-bulb thermometers. Such sensors would be independent of total pressure and gas mixture, and could be calibrated by a Dew Point Hygrometer.

OXYGEN CONSUMPTION DETERMINATIONS

Because both the volume of the spirometer and animal chamber with its ducts were known, the oxygen content of the system could be calculated, given pressure, temperature, humidity, and oxygen concentration. The difference in oxygen content at the beginning and end of a chosen period gave the

oxygen consumption for that time. If either the experimental or control system was at ground level pressure, samples were obtained from the animal chamber after the spirometer was closed off from it, by attaching two rubber bags to sampling tubes. The first bag was empty and the second contained about a liter of gas of approximately the same mixture as that in the chamber. By squeezing the second bag a sample was displaced into the first. This was analyzed in a Beckman E2 Oxygen Analyzer and a Custom Engineering and Development Co. Model 300 A R Nitrogen Analyzer. Samples were obtained from the spirometer at the same time.

When the experimental system contained almost 100% oxygen at reduced pressure, samples were obtained through the evacuating system. In this case the spirometer did not have to be shut off from the animal chamber. Accuracy of oxygen consumption measurements were improved by increasing the time over which measurements were made and by ensuring uniformity of chamber temperature and pressure. It became a practice to allow one spirometer full of oxygen to be consumed for each determination. Sample taking was not a satisfactory method of monitoring the closed environment when a mixture of gases was used at reduced pressure. In the present design plans are being made to install remotely reading gas-analyzing equipment within the animal chamber to correct this defect.

MANAGEMENT OF VARIOUS GAS MIXTURES WITHIN THE ANIMAL CHAMBER

The experimental system so far has held almost pure oxygen at a pressure of 196 mm Hg for up to 24 days. At the beginning of such a run some enrichment of the environmental gas with oxygen was necessary as nitrogen diffused out of various containers. During the experiment the oxygen concentration in the animal chamber would fall from 99% to 98% over a period of 24 hours, most probably because of diffusion through the rubber gloves, and would necessitate oxygen enrichment. (More efficient ways of sealing off the glove holes when the gloves are not in use are being tested). Mixtures of oxygen and nitrogen were held more constant at pressures of 282 mm Hg for several days, as was a mixture of helium and oxygen at ground level pressures for eight days.

Although changes in pressure, temperature, and humidity caused the animal chamber gas mixture to enter the spirometer, or oxygen from the spirometer to enter the animal chamber, the problem was mostly confined to the control system because of the daily variations of barometric pressure and the very different composition of the gas mixtures on the two compartments. Increased barometric pressure caused oxygen enrichment of the animal chamber atmosphere and vice versa.

When oxygen enrichment of the environmental gas occurred in the control system, the spirometer was shut off and a rubber bag containing the inert gas of the mixture was attached to a sampling tube in the animal chamber. As oxygen was consumed by the animals, it was replaced by the inert gas until the original composition was restored. This procedure did not interfere with measurements of oxygen consumption.

If, however, the oxygen concentration became low in the animal chamber, it was almost impossible to correct it without sacrificing some other requirement (e.g., by lowering the temperature or humidity in the chamber, oxygen could be drawn into it from the spirometer). As accumulation of specific toxins was not being studied, oxygen was introduced from the spirometer and allowed to displace an equal volume of gas mixture which escaped through a sampling tube. Ten liters of oxygen introduced in this way would raise the oxygen concentration from 20% to 20.8% in a 1000-liter chamber containing 20% O_2 and 80% N_2).

When pressures were reduced and held constant, as in the experimental system, little change in composition of environmental gas mixtures was experienced. Diffusion of diluent gas from the animal chamber into the spirometer was negligible under our operating conditions.

If, because of inadvertent loss of control, adjustment of the environmental gas mixture must be made when the system is at reduced pressure, two courses of action are possible. If the animal chamber needs to be enriched with oxygen, gas can be withdrawn from it by using the evacuating system. This will cause oxygen to be drawn into it from the spirometer. Alternatively either oxygen or the diluent gas can be introduced into the animal chamber via the evacuating system (see Fig. 2) and displacement can take place into spirometer. As long as there is a remotely operated shut-off valve between the spirometer and the animal chamber, the former can then be emptied and refilled with oxygen.

To obtain an oxygen diluent gas mixture for the animals such that the alveolar P_{O_2} was similar to that obtained when breathing air at ground level, the following procedure was adopted. Air was withdrawn from the animal chamber and oxygen was added to it via the spirometer, at the same time as the altitude chamber was "taken up" to 33,000 feet (196 mm Hg). (In order to avoid oxygen toxicity and/or dysbarism in the rats this usually took at least five hours). Once 100% oxygen content had been established at this pressure the chamber was brought down at a rate not greater than 300 feet per minute and the diluent gas introduced into the system at such a rate that pressure equilibrium was maintained. This was found to be the most economical way of obtaining an oxygen concentration within 5% of that which was desired. Once this approximation had been reached, a known volume of diluent gas was added to the animal chamber, after which the spirometer was emptied and refilled with oxygen.

It is believed that the system described possesses certain advantages, especially for the less well endowed establishments. It may be used to study artificial atmospheres without any reduction of pressure. It lends itself to easy modification and can be constructed to meet individual needs. It is robust, simple in operation and, finally, inexpensive. The complete animal chamber without labor or spirometer, cost approximately $1,000.

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PART II. PROLONGED EXPOSURE OF YOUNG RATS TO A NITROGEN-FREE ATMOSPHERE AT REDUCED PRESSURE

INTRODUCTION

As a result of the interest in space and submarine exploration much thought has been given to the problem of supplying animals in closed environmental systems with a favorable gaseous atmosphere.

In some instances this part of the environment is maintained close to that of the ambient air to which the animals are accustomed. But in other instances artificial atmospheres are used, as in the case of the Mercury Project capsules where the occupants breathe pure oxygen at a reduced pressure of about one-third of sea level atmospheric pressure (ref. 8).

Experiments exposing men to almost nitrogen-free, low-pressure environments have been carried out. Michel, Langevin, and Gell (ref. 14) describe the effects on six men maintained in a pure oxygen environment at a pressure of 418 mm of Hg for 168 hr. Some of these men showed evidence of pulmonary irritation. Morgan, Welch, and Ulvedal (ref. 16) reported on two subjects kept at a simulated altitude of 33,500 ft. in essentially 100% oxygen for almost 17 days in a closed chamber. These subjects showed only minor physiological effects of their sojourn in this environment.

MacHattie and Rahn (ref. 10) maintained mice for as long as 51 days in an atmosphere of oxygen at a total pressure of 197 mm Hg (10). Some of the animals died within the first 48 hours, apparently of atelectasis, but most of them survived and some litters of young were raised in the environment. Since oxygen was flushed through the chamber to remove CO_2, this was not a closed system.

Berry and Smythe (ref. 4) also studied the influence of pure oxygen atmospheres at pressures equivalent to 30,000 or 34,000 ft. of altitude on the metabolism of mice. These mice were exposed for a period of three to five weeks but not continuously since they were returned to ground level for a few minutes each day when they were removed for feeding, watering and cleaning. In this process they were exposed to rapid changes in pressure. However, they showed no metabolic abnormality and responded to cortisone injections in the same manner as their controls. Like the MacHattie and Rahn experiments, this was a flow-through rather than a closed system.

Allen (refs. 1a, b) has reported that fertilized chicken eggs do not develop normally in atmospheres which are low in nitrogen, whether they are pure oxygen at reduced pressure or mixtures of helium and oxygen at normal pressures.

The investigation described in this paper was planned to study the growth rate and O_2 consumption of young white rats under the influence of continuous exposure to a low pressure atmosphere consisting almost entirely of oxygen.

METHODS

The design of the experimental animal chambers has been described in Part I. In essence, two identical plexiglass chambers were arranged so that the contained gas was circulated over dehumidifying coils, heating units, and filter beds of soda lime to control the temperature and humidity and to absorb carbon dioxide. Each chamber had a volume of about 1000 liters and was connected to a large spirometer containing oxygen so that, as the animals utilized oxygen from the chamber, it was replaced from the spirometer, thus maintaining an almost constant gaseous composition in the closed system. One animal chamber, hereafter called the control chamber, contained air at ambient barometric pressure. The other, termed the experimental chamber, was placed inside an altitude chamber. During the experimental period the experimental animal chamber contained oxygen at a pressure of about 196 mm Hg. Under these circumstances the alveolar P_{O_2} of rats in the experimental chamber was practically the same as that of the rats in the control chamber but the atmosphere was almost nitrogen free. Twelve weanling Sprague-Dawley rats, weighing about 65 gm. each, were placed in each chamber at the beginning of an experiment, three to a cage. Special watering and feeding devices were provided and long rubber gloves attached to ports in the plexiglass chambers made it possible to weigh the rats, replenish their food and water, and remove wastes without altering their gaseous environment.

Several trials of duration varying from 4 to 17 days were made before the 24-day experiment to be reported in detail here. These preliminary experiments had various flaws. In some there was failure to maintain equivalent conditions in the two chambers. In an experiment lasting 17 days several rats from each group died of an intercurrent respiratory infection. In general, we will report only data obtained in our last 24-day experiment, but, where appropriate, we will refer to confirmatory data from previous experiments.

At the beginning of the rats were weighed, marked, and placed three in a cage in the plexiglass chambers which were then sealed. Both chambers contained air and were maintained at ground-level pressure were temperature and humidity controlled for a preliminary observation period lasting six days.

On the seventh day the pressure in the experimental chamber was gradually reduced by evacuating the surrounding altitude chamber. Oxygen was flushed through so as to maintain a partial pressure of oxygen in the alveoli of the experimental rats at least equivalent to that in the alveoli of the control rats until a pressure of 196 mm Hg and a composition of 99% O_2 was reached. In order to reduce the possibility of dysbarism for the rats as a result of the change in pressure this procedure was carried out very gradually over several hours.

During the ensuing 24 days the experimental chamber was maintained at a total pressure of 196-200 mm Hg, the oxygen concentration from 98 to 99.8%, carbon dioxide below 0.1%, the mid-chamber temperature at 22-24°C, and the

relative humidity close to 50%. (The humidity was measured by wet-dry bulb thermometer difference. That of the control chamber was read from a standard table for converting such readings to relative humidity in air at approximately sea level. For the experimental chamber it was necessary to prepare a special graph taking into account the reduced pressure and the oxygen atmosphere. Later observations have caused us to believe that the latter factor is smaller than we orginally estimated so that the humidity in our experimental chamber was actually slightly lower than that of the control chamber). Illumination was continuous and approximately equal in the two chambers and both groups of rats were visually isolated from the rest of the laboratory.

During this 24-day experimental periods the usual practice was to weigh the animals and measure food, feces, and urine twice a week. However they were inspected several times each day for changes in behavior as the enviromental control was monitored. When the experimental animals were weighed and replenished it was necessary for two operators to denitrogenate by breathing oxygen for an hour in the lock of the altitude chamber. Then the lock was evacuated to the pressure level of the main chamber to permit the operator to enter and examine the animals.

After the 24-day experimental period the experimental chamber was gradually returned to ground-level pressures over a period of several hours while nitrogen was admitted. Then followed another observation period of eight days with both chambers at atmospheric pressure and the rats breathing a mixture corresponding to ambient air.

Oxygen consumption measurements were carried out on days when the rats were not handled. They were determined by measuring the difference in the total volume of oxygen in the chamber-spirometer system before and after an experimental run which lasted at least two hours. The oxygen concentration was measured with a Beckman E2 oxygen analyzer in duplicate samples of the chamber and spirometer contents withdrawn into rubber bags. It was necessary to measure the oxygen percentage in both the chamber and the spirometer because changes in pressure or temperature during a period of measurement could cause gas to move from the chamber to the spirometer, or the reverse, thus altering the composition, especially in the control chamber. The volumes of oxygen were then corrected to standard temperature and pressure (dry) and expressed as mℓ/kgm/min. Measurements on the two chambers were made simultaneously, but not always at the same time of day.

The same gas samples utilized for measuring oxygen concentration were used to analyze for C_{O_2} (with a Beckman LB-1 carbon dioxide analyzer) and for nitrogen (with a 300 AR Nitralyzer).

As a check on the influence of the closed systems, a second group of control rats, known as open controls, was set up in similar cages but exposed to the ambient air of the laboratory. Though an attempt was made to keep their gaseous environment near the same temperature and humidity as the other rats, they were exposed to much wider variations in both.

It should be mentioned that the chambers were not actually completely closed environments during the whole period of the experiment. In order to maintain the concentration of oxygen in the experimental chamber, it was necessary to flush the chamber with 100% oxygen for several minutes every other day or so to remove nitrogen admitted either through leaks or in transferring materials via the glove ports. This was accomplished by withdrawing gas from the chamber while admitting oxygen at the rate which maintained a constant pressure. The control chamber was opened to the outside air at similar intervals but there was no attempt to equate the flushing volume in the two chambers. Because they were not completely closed during the whole period, no conclusions can be drawn about accumulation of toxins during this period

RESULTS

Within the limits of our observations there was little effect on the experimental rats of exposure for 24 days to an atmosphere virtually lacking in nitrogen and at approximately one-fourth atmospheric pressure. All of the animals survived and no difference in their appearance or behavior was noted from that of the closed controls.

The growth rates for the two groups of rats are essentially the same (Fig. 5). The analysis of data consisted of evaluating the growth rates of the two groups, during the period when the expermental rats were in the nitrogen-free atmosphere, by means of co-variance analysis. Although the regression line for the experimental rats was significantly elevated above that for the control rats, the slopes of the lines were virtually the same. Regression coefficients were 7.28 gm. per day for the experimentals and 7.00 gm. per day for the controls (Table 1).

The open control rats gained weight at the same rate as the closed controls and experimentals, but a fourth group kept under ordinary animal room conditions has a much lower rate of growth (4.5 gm/day).

Measurements of oxygen consumption were begun after the experimental animals had been in the atmosphere of oxygen at reduced pressure for a couple of days. These were repeated at least three times a week during the time the experimental animals were at reduced pressure and for eight days after they had been returned to ground-level pressure and and air atmosphere like that of the controls. There was no consistent difference in the oxygen consumption of the control and experimental groups. Figure 6 shows the data on oxygen consumption.

They are in good agreement with the measurements made in the previous 17-day experiment, and in general agreement with the literature when allowance is made for the fact that our animals were not fasted and that no attempt was made to reduce their activity (refs. 3, 5, 17).

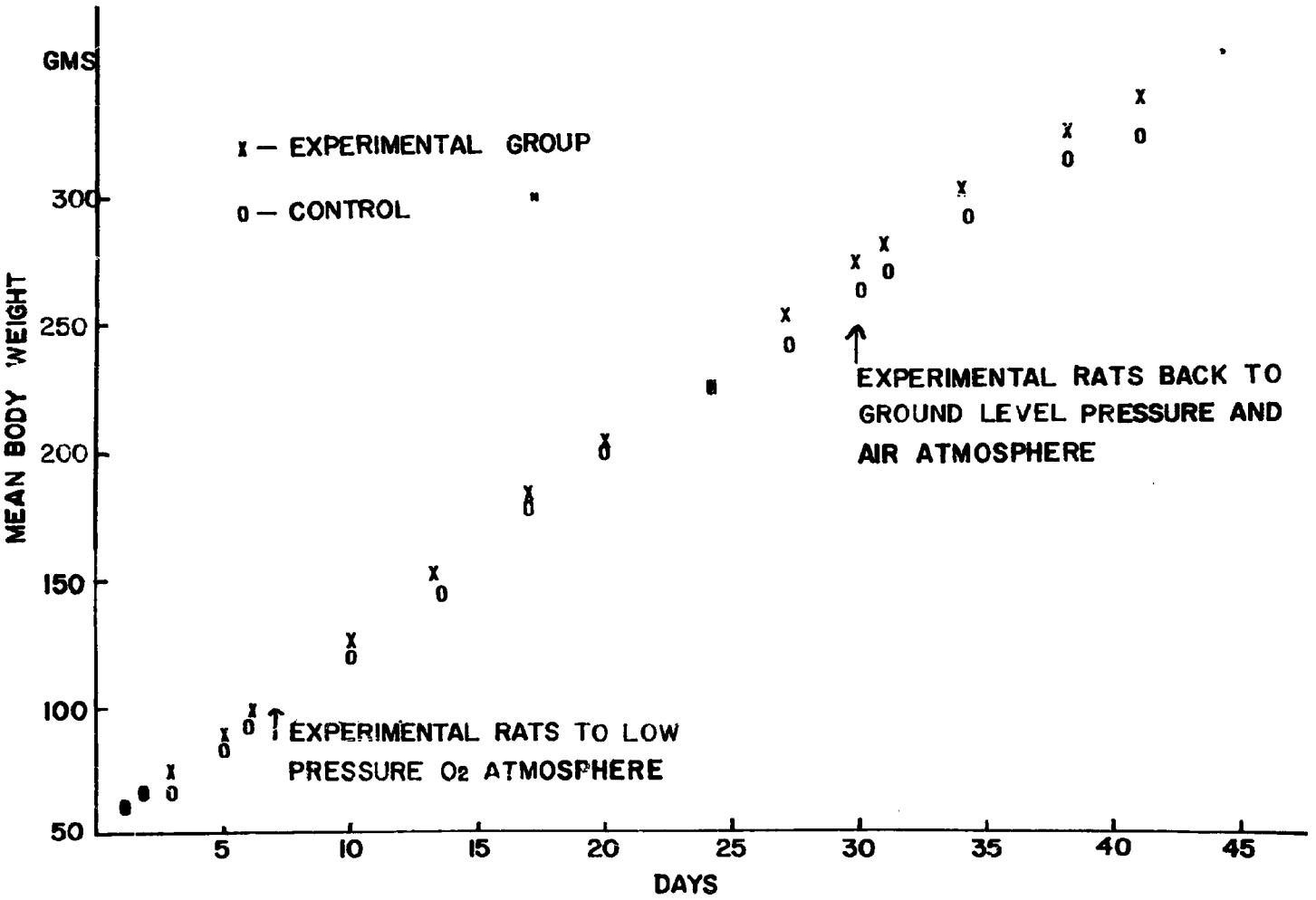

Fig. 5. Rate of Growth by Weight of Experimental and Control Rats Including 24-Day Period when Experimental Rats Were in Nitrogen-Free Atmosphere

TABLE I. Analysis of Co-variance of Rat Growth Rate

X = days, Y = wt. in grams

Condition	f	Ex^2	Exy	Ey^2	Regress Coef., gm/day	Deviations from Regression		
						f	$Ey^2 - (Exy)^2/Ex^2$	Mean Square
Control	7	497.9	3,485.0	24,460.4	7.00	6	67.5	11.25
Experimental	7	497.9	3,623.4	26,454.7	7.28	6	85.9	14.32
Within						12	153.4	12.78
Regress. Coef.						1	19.2	19.20
Common	14	995.8	7,108.4	50,915.1	7.14	13	172.6	13.28
Adjusted Means						1	104.6	104.60
Total	15	995.7	7,108.4	51.024.8		14	277.2	

1. F (for difference between the two sample regress. coefs.

$$F = \frac{19.20}{12.78} = 1.50^* \quad (f = 1, 12; F05 = 4.75)$$

*not significant

2. F (for sample difference in elevation - adj. means)

$$F = 104.60 = 7.88^* \quad (f = 1, 13; F05 = 4.67)$$

*significant beyond the .05 level

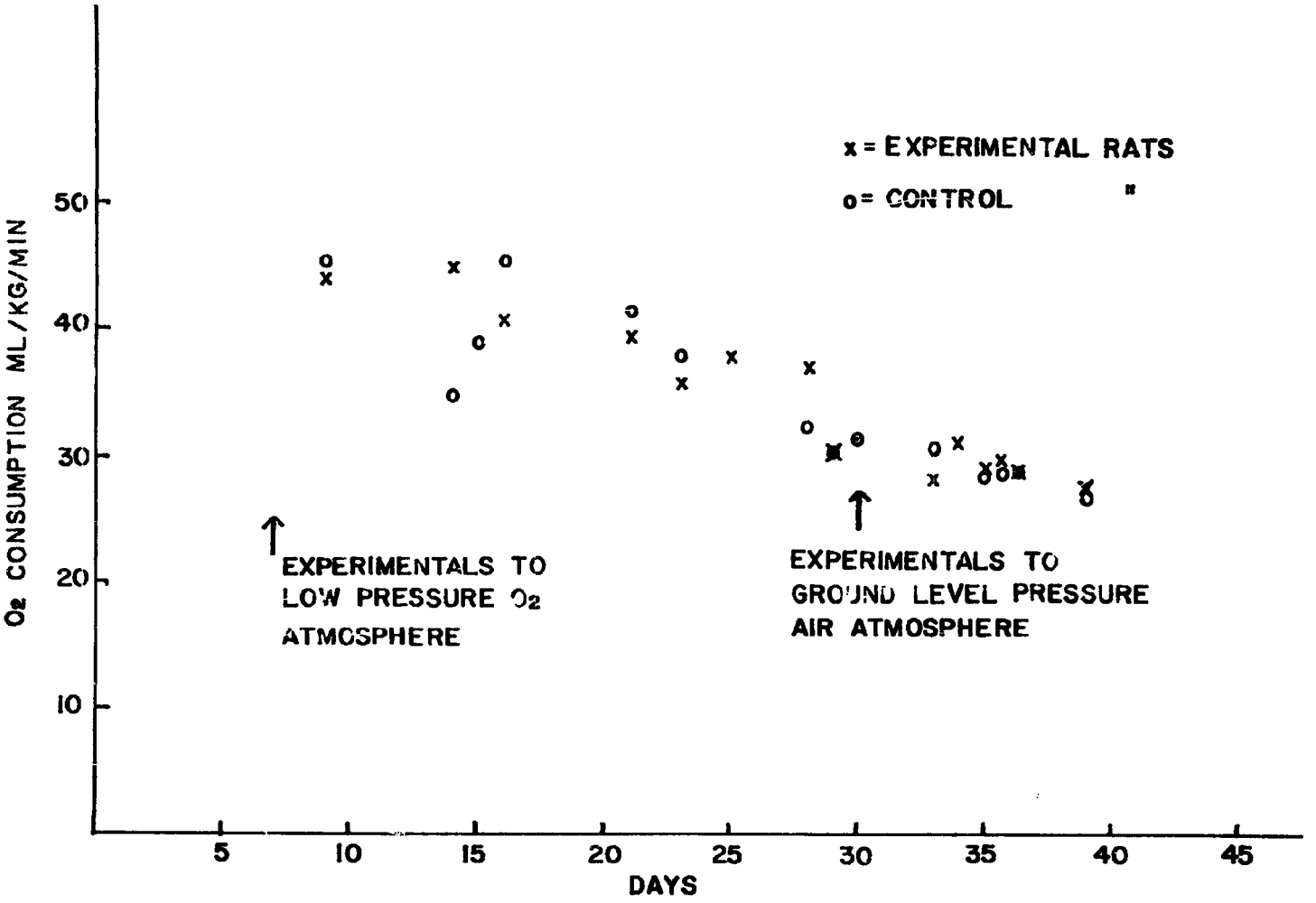

Fig. 6. Plot of Oxygen Consumption Measurement on Experimental and Control Rats Beginning During Period when Experimental Rats Were in Nitrogen-Free Atmosphere and Continuing Through the Period when They Were Returned to an Atmosphere Like That of the Controls

It was observed that the urine volume produced by the experimental animals while they were in the nitrogen-free environment was about one-third that of the controls, being of the order of 1 to 2 ml per rat per day. Urine volume could not be measured with consistent accuracy with the arrangement used because of occasional water spillage and a variable amount of feces through which the urine passed to the collection bottles. It was felt that the reduced urinary volume was due to an increased extra-renal loss of water due partly to an increased rate of evaporation at reduced atmospheric pressure (refs. 11, 13, 19). This point is under study.

The total food consumption of the two groups of rats was almost identical during the 24 days while the experimentals were at stimulated altitude. It was 5966 gm. for the closed controls and 1 gm. less for the experimentals. That of the open controls was 5668 gm. Very little spillage of food occurred from the special feeders, being less than 1 gm. per rat per day. Feces output was also very similar in weight and appearance for the three groups but, since there was some variation in the moistness of the feces and we did not obtain dry weights, no exact figure can be given.

At the end of the period of observation hemoglogin determinations were carried out on tail blood obtained from the experimental, the closed control, and the open control rats by the method of Sheard-Sanford (ref. 20). The results are presented in Table II. There was no significant difference in the values for animals in the three groups.

DISCUSSION

These studies apply to the question of whether the nitrogen in air serves any physiologic purpose for mamals. If it is true, as has been suggested, that nitrogen at high pressure can cause narcosis, then one might wonder if its removal would have the opposite pharmacological effect. However the concept of nitrogen narcosis is still questionable in that some investigators think the depressed state seen in divers breathing air under several atmospheres of pressure is really due to an accumulation of carbon dioxide. This is attributed in part to the increased work of respiration due to the increased density of the ventilated gas, especially as turbulence increased the viscous resistance to flow in the respiratory passages. If this is true, then the narcosis is only indirectly due to high nitrogen pressure and is not a pharmacologic but a physical effect (refs. 2,12,21).

Another physical effect of the nitrogen in the air is that of a filler or diluent substance to keep areas of lung from collapsing when oxygen is absorbed from the alveoli. Such a collapse or atelectasis is known to occur in animals breathing pure oxygen when respiratory passages are obstructed or when areas of the lungs are congested by acceleration (ref. 9).

TABLE II. Hemoglobin Concentration (gms/100 ml blood)

Rat No.	Experimentals	Closed Controls	Open Controls
1	12.2	12.8	11.0
2	12.2	12.5	12.5
3	11.8	12.5	12.2
4	12.2	12.2	11.2
5	11.1	11.8	11.8
6	12.8	lost sample	11.8
7	12.2	12.2	12.2
8	11.8	11.8	11.2
9	12.8	11.8	8.8*
10	10.8	11.1	12.2
11	12.2	11.8	12.7
12	11.5	12.2	11.5
Mean	11.9	12.0	11.8

* This animal had hemorrhaged from a lacerated foot the previous day.

The experimental rats in this study showed no evidence of any deleterious effect of the lack of nitrogen whether pharmacological or physical. No attempt was made to determine whether atelectasis occurred but, if it was present, it had no inhibitory effect on inhibitory effect on the growth or behavior of these young rats.

It was believed that the growth rate of these young rats from the weanling state to young adults would be a sensitive indication of any inhibitory or stimulating factor in their environment. Such animals have been much studied and their nutritional and environmental requirements are well known. Furthermore, the measurement technique (weighing) was relatively easy to carry out with fair accuracy in the limiting circumstances and the number of animals and observations reduced the importance of chance error. It was somewhat surprising to find that all three groups of rats grew at rates which are much greater than the rates usually considered quite good for animals of the same strain (refs. 6,7). This is probably due to the easy availability of ground food and water at all times and the unusually stable temperature and humidity.

It is difficult to find values in the literature with which to compare our results for oxygen consumption since most such measurements are carried out on fasted rats sedated by high humidity, and for relatively short periods of time. Benedict and MacLoed (ref. 3) reported in 1929 that intermittent activity could increase oxygen consumption by 15-20% and that young rats may have a decrease of 28% on fasting. The values reported here are somewhat higher than those reported by Davis and Hastings (ref. 5) for fasted, dosing rats of similar age but almost twice as high as the values reported by Moses (ref. 17) for fasted, sleeping rats.

The greatest source of difficulty in our measurements of oxygen consumption, leaving out occasional mistakes in reading the spirometer scale or in analysis, was in determining the mean temperature of the gas in the chambers, especially when there was a large gradient from inlet to outlet. This happened particularly in the experimental chamber when, because of a high ambient temperature, the heaters were turned off so that air from the dehumidifying coils entered the chamber at a low termpeature. As the experiment progressed we learned to minimize the temperature gradient in the chamber during the period of a determination. This may account for the decreased scatter of our results toward the end of the experiment.

The chief difference observed between the experimental and the control rats was the decreased urine output of experimentals. Since they did not drink less water, it is assumed that they lost more water by evaporation. McCutcheon and Taylor (ref. 11) have shown the increased evaporation rate in human subjects at reduced ambient pressures with a constant vapor pressure. Miller (ref. 15) has dicussed the theoretical aspects of this and suggested that the increased evaporation may be due to the demands of temperature regulation since heat loss by convection is reduced in the rarified atmosphere. However, the rate of evaporation from any liquid is increased at reduced pressures and in the system under discussion where the humidity is regulated by the condensation of water in the dehumidifier one would expect a greater loss by evaporation in the experimental animals on a purely physical basis. Quimby, et. al, (ref. 19) have shown that rats exposed to reduced

pressures with sea level oxygen partial pressures do not show any change in body temperature, though their temperatures decrease in rarified air, i.e., with hypoxia, especially at low humidities. Picon-Reategui, et.al., have shown that rats maintained at an altitude of 15,000 ft. in 60% relative humidity and given water ad lib lost 20% of their body water in the first week and do not recover completely in 30 days. It is apparent that the dehydrating effect of lowered barometric pressure particularly with hypoxia could be of great practical importance and is worthy of more study.

SUMMARY

1. Twelve weanling male albino rats were kept in an atmosphere almost free of nitrogen consisting of 99% oxygen at a pressure of 196 mm Hg for 24 days. Temperature and humidity were closely regulated. Their rate of growth, food and water consumption, urine output and oxygen consumption were compared with a control group in an identical closed chamber but breathing air at ambient atmospheric pressure. The alveolar partial pressure of oxygen was calculated to be equivalent in the two groups. Special provision was made for handling the animals without altering their gaseous environment.

2. The animals were observed for six days before the experimental period and for eight days afterward during which time both groups were kept in the control atmosphere.

3. Both groups of animals showed excellent rates of growth during the experimental period (7 gm/day) but there was no significant difference between experimentals and controls. A second group of control animals kept under similar conditions except that their cages were open to ambient air showed essentially the same rate of growth.

4. There was no consistent difference in the rate of oxygen consumption between the two groups.

5. No difference was observed in behavior, food consumption, water intake or feces production.

6. The urine output of the experimental rats was about one-third that of the controls. This was attributed to an increase in evaporative loss of water in the rarified atmosphere.

7. No difference was noted in the blood hemoglobin concentration between the two groups of rats at the end of the experiment.

8. It was concluded that the lack of nitrogen and the decreased pressure had no effect on young male white rats within the limitations of our methods of observation.

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FUTURE PLANS

We have already carried out some pilot experiments substituting helium for nitrogen in the breathing mixture of young rats and plan to continue these studies.

Because of reports of effects of low nitrogen atmosphere on embryonic tissues we will include breeding animals in some of our future experiments.

Efforts will continue toward improving the control of temperature, humidity, pressure and gas composition in our experimental chambers.

The National Aeronautics and Space Administration has given us a research grant to continue these investigators for the next three years beginning September 1, 1962.

Part I and Part II will be submitted separately for publication in a scientific journal.

Investigator _____ Date 10 23 62

Supervisor _____ Date 10/23/62

For The Ohio State University Research Foundation

Executive Director _____ Date 10/23/62

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