BIOLOGICAL EVALUATION OF VARIOUS
SPACECRAFT CABIN ATMOSPHERES*, I

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Abstract. The physiological consequences of exposure to several possible spacecraft atmospheres were evaluated. Each atmosphere contained oxygen at a partial pressure of 180 mm Hg. Rabbits and rats were exposed at 1 atm abs. for one week each to atmospheres containing nitrogen, helium, argon or neon; and to pure oxygen at 200 mm Hg. In addition rats were exposed at a total pressure of 474 mm Hg to atmospheres containing nitrogen, helium or neon.

Metabolic rates were increased in animals exposed to helium-oxygen at sea level, and reduced in those exposed to the low pressure, pure oxygen environment. Rates during sea-level exposures to argon and neon, and during the altitude exposures, did not differ appreciably from results obtained in air at sea level. Rabbits sustained a significant loss of hemoglobin (9%) and red blood cells during their exposure to helium-oxygen.

These responses are consistent with the thermal characteristics of the several gaseous environments. A good correlation was found to exist between the calculated relative convective heat transfer in the various atmospheres and the observed metabolic rates. The possibility of an effect of helium at the molecular level has not been ruled out completely.

After saturation with the inert gases studied, rats decompressed to 100 mm Hg showed the most severe symptoms of decompression sickness; nitrogen produced less damage; animals exposed to helium or neon were free of serious symptoms.

The data provide the first experimental support for several theoretical advantages of neon for use in space cabin atmospheres.

1. Introduction

The current state of the art of space suit design does not permit extravehicular activity with a reasonable degree of free body movement in the presence of pressure differentials of more than a few pounds per square inch. A space cabin environment of air at 1 atm pressure, therefore, carries with it a time-consuming and potentially dangerous decompression obligation before extravehicular activity may commence and exposes the crew to the hazard of decompression sickness in the event of an uncontrolled loss of cabin pressure. On the other hand, experimental evidence indicates that a pure oxygen environment, even at a total pressure of less than 1 atm. may give rise to physiological problems. Because of the potential physiological unsuitability of pure

oxygen as the space cabin atmosphere for extended manned missions, consideration must be given to the problem of selecting synthetic, two-gas space cabin environments.

If some gas other than the obviously acceptable nitrogen is used as the diluent for oxygen in such a two-gas environment, then there is a possibility that prolonged exposure may prove to be deleterious. Since any two-gas system is inherently fraught with potential decompression hazards, selection of the inert gas diluent will also depend to a significant degree on what advantages a prospective diluent may offer in terms of reducing the possibility of decompression sickness on reduction of pressure.

The physiological consequences of exposing mammals to oxygen-inert gas mixtures for prolonged periods of time had not been systematically explored prior to the time when the present study was planned. As one attempt to begin to eliminate this information deficit, we proposed to conduct comparative physiological studies on rabbits and rats exposed for a period of seven days at normal and subnormal pressures to atmospheres containing helium, neon, nitrogen or argon. These studies, to be conducted in the presence of nonlimiting levels of oxygen, were designed to provide parametric information concerning the effects of these gases on standard indices of physiologic normalcy as a first step in the development of a comprehensive understanding of inert gas physiology as it applies to the selection of space cabin atmospheres.

2. Exposure of Rabbits at a Constant Oxygen Tension to Atmospheres Containing Nitrogen, Helium, Argon, Neon, or no Inert Gas

A. INTRODUCTION

Part I of this project is an overall evaluation of several possible inert gas diluents for use in spacecraft atmospheres. Using rabbits, we have measured many physiological and biochemical responses to one-week exposures in atmospheres containing either nitrogen, helium, argon, neon or no inert gas at all. The rabbits were exposed at one atmosphere total pressure and at a consistent nearly normal oxygen tension in a closed, controlled environmental system. Salient results suggest that for as long as one week any of the inert gas atmospheres tested is safe and probably better than pure oxygen alone and that for very long exposures helium may not be the best choice of diluent gas.

Rabbits were chosen for these experiments primarily because of their broad thermal neutrality. The choice was appropriate for other reasons also since their size permits an adequate amount of blood to be drawn for analysis, and they have readily accessible veins and arteries in their ears.

The purpose of the experiment was to compare the physiological responses to the various gases. We chose a sea-level exposure as a convenient compromise between high pressure which might exaggerate the effects of the gases and reduced pressure which might be found in a spacecraft.

The partial pressure of oxygen selected for all experiments was 180 mm Hg. This value was chosen because it is low enough to avoid toxic manifestations of oxygen even during extended exposures and high enough to permit the intermittent manometric

measurement of oxygen consumption in the closed system without risking hypoxia in the experimental animals.

At best we expected the effects of the 'inert' gas diluents to be subtle and possibly difficult to detect. We chose therefore to examine a wide assortment of physiological and biochemical parameters which might serve as clues for further, more detailed examination. Not all the tests can be justified on the basis of their sensitivity to subtle disturbances or because they have been shown in other experiments to be altered by the gaseous medium; some are included mainly because they help to round out a thorough surveillance.

Since helium has been shown repeatedly to cause an increase in metabolic rate in mammals unless temperature compensations are made (Cook, 1950; Schreiner et al., 1965; Konza, 1965)*, it seemed appropriate to measure metabolic rate in all environments tested. To date there have been no comprehensive experiments involving the effect of neon on oxygen consumption (Roth, 1966).

Many studies of egg hatchability have shown prominent and sometimes disastrous effects when nitrogen was replaced by some other gas (Allen, 1963; Weiss et al., 1965; Volskii, 1959; Boriskin et al., 1962), but for the most part growth has been shown to be normal in helium (Barach, 1934; Schreiner, 1964; Schreiner et al., 1965). We felt that growth was a good though nonspecific measure of the general stress of an environment and we monitored gain in body weight of all animals as well as food and water consumption and total waste production.

Since the effects of helium on metabolism in mammals are presumably due to its thermal properties (Epperson et al., 1966), we monitored body, skin and ambient temperature in all rabbit exposures. The ambient temperature was adjusted as closely as possible to 26 °C in all circumstances.

Exposures to operational spacecraft atmospheres (or to some other undetermined stress) have resulted in disturbances of the red blood cell system in both chamber tests (Helvey et al., 1965; Brooksby et al., 1966) and actual spaceflights (Swisher and Fisher, 1966). The effects seen have been attributed in part to the increased PO_2. We felt that hematological disturbances are likely in any unusual atmospheric environment and because of the dual possibility here of either general toxicological or specific inert gas effects, we carried out an extensive hematological surveillance. This surveillance included count and morphology of the formed elements of blood, blood volume, hematocrit and hemoglobin content, red cell survival and erythropoietic activity.

Other observations were made as part of a general program but without specific justification. We measured blood gases and acid-base balance; serum sodium, potassium and glucose; and blood urea nitrogen. Heart rate and respiratory frequency were determined regularly on some animals from each group and an electrocardiogram was recorded. Selected animals from each group were autopsied and certain tissues were preserved for histological inspection.

* References will be published with Part II in Space Life Sciences, Vol. 2, Number 4.

Three general types of control information were available. First, in some cases experimental responses could be compared to the same parameters measured on the same animal before the exposure. Next, along with each group of 6 animals exposed in the chamber, an additional 3 were kept in regular cages and subjected to most of the same tests. Finally, the 'nitrogen' exposure was for all practical purposes an air control itself in which all parameters were measured in the same way as in the other experimental runs.
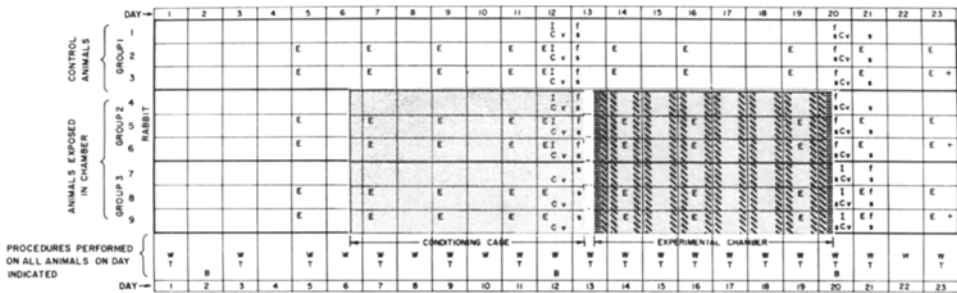
B. PROCEDURES AND METHODS

The basic experimental plan consisted of seven-day chamber exposures of rabbits to each of five different atmospheres with the objective of comparing physiological responses in the different atmospheres.

Each experimental run involved a unit of nine rabbits which was divided into three groups. One group of three was maintained as a control group; these were kept in regular rabbit cages. The other two groups were given the same exposure in the test environment but were subjected to different blood analyses. Each different run was carried out with new, previously unexposed animals.

A calendar showing the scheduled events for a typical run is given in Figure 1.

An experiment lasted 23 days. Each unit of nine rabbits was ordered from the supplier so as to arrive on the eve of day 1. All animals were kept in regular rabbit cages for 5 days after arrival, and from the 6th to the 13th day the experimental groups were housed in a conditioning cage having the same configuration as the chamber cage. From the 13th day to the 20th day the test animals were exposed to the experimental

Fig. 1.

atmosphere, and at the completion of the exposure they were observed for three more days. Throughout the experimental period the rabbits were fed a standard pelletized diet (Rockland Rabbit Ration, Teklad, Inc., Monmouth, Illinois).

TABLE I

Chamber gas composition
(Rabbits)

Inert gas	Total pressure mm Hg	PO_2 mm Hg	PCO_2 mm Hg	PN_2 mm Hg	PCH_4 mm Hg	Temperature °C	Relative humidity %
N_2	758 ± 2	177 ± 4	< 1.5	–	0.6	26.2 ± 0.3	55–65
He	758 ± 2	180 ± 4	2	3	2	26.5 ± 1	50–60
Ar	758 ± 3	180 ± 2	< 1.5	0.9	0.8	26.5 ± 0.5	60–65
Ne	755 ± 5	176 ± 5	1	0.8	1.3	27 ± 1	55–60
(O_2)	$200 \pm 1*$	180 ± 2	< 1	0.5	1	27 ± 0.5	~ 60

* During the first 48 hours of the oxygen run, total pressure was maintained at 190 ± 1 mm Hg with a PO_2 of 172 ± 3 mm Hg.

Table I is a summary of the gas compositions involved in the various experimental runs. There were slight fluctuations in all chamber parameters from day to day; the values given here are weighted averages and represent the 'effective' gas composition in the chamber over the course of a week-long exposure.

There were a few exceptions to the planned protocol. Two animals were injured in handling (broken backs) and had to be replaced by animals from the control group, and consequently had only one day in the conditioning cage. Of the argon-exposed animals there are 4 in group 2 and only 2 in group 3. There were some variations in the time spent in the conditioning cage – these are indicated in the graphical summaries of our experimental results.

Individual runs were overlapped so as to use the chamber efficiently. Because of the structure of the experiment a simple listing of physiological parameters measured may be misleading; we therefore list them in groups according to the scope of the particular analysis.

EXPOSURE:

    Group 1: controls, kept in regular cages.
    Group 2 and 3: in conditioning cage days 6–13, in experimental atmosphere days 13–20.

Determined on all animals, as individuals, periodically throughout the run and daily during the exposure period:
    Body weight,
    Relative activity,
    Body (rectal) temperature,
    Skin temperature,
    Environmental temperature,
    Twenty-four hour food consumption.

Determined on two animals in each group, periodically:
  Respiratory frequency,
  Cardiac frequency,
  Electrocardiogram.

Determined on blood of all animals on days 2, 12 and 20:
  Red blood cell count,
  White blood cell count,
  Differential count,
  Reticulocyte count,
  Hematocrit,
  Hemoglobin,
  Sodium,
  Potassium,
  Glucose,
  Urea nitrogen,
  pH,
  Arterial blood CO_2 tension (P_aCO_2),
  Standard bicarbonate,
  Arterial blood oxygen tension (P_aO_2).

Determined on each animal, days 12–13 and days 20–21:
  Total red cell volume,
  Twenty-four hour red cell survival.

Determined on each animal in groups 1 and 2:
  Twenty-four hour iron uptake, pre-exposure, days 12–13,
  Eight day iron uptake, days 12–20.

Determined on each animal in group 3:
  Twenty-four hour iron uptake, post-exposure, days 20–21.

Determined on animals in conditioning cage and chamber, as a total for all 6 animals:
  Daily water consumption,
  Daily waste production,
  Oxygen consumption breathing air, day 13 and 20,
  Oxygen consumption breathing experimental atmosphere, twice daily, days 14–19.

Performed on one animal in each group:
  Autopsy,
  Histological examination.

Exposure to experimental atmospheres was carried out in an environmental chamber especially designed for this experiment. The chamber is a vertical cylinder approximately 110 cm (42 inches) in diameter and about the same height, with a conical base and a plexiglass dome cover. The volume of the system is about 800 liters. An environmental control system circulates the atmosphere through scrubbers designed to remove CO_2 and ammonia and heavy volatile contaminants, and to control water vapor. A catalytic burner can be placed on line intermittently to remove the lighter organic contaminants such as methane and carbon monoxide. Gas flow was adjusted to provide a molar flow rate of about 115 liters/min S.T.P. for all atmospheres used.

The control system operates to maintain a constant pressure, venting when necessary and adding oxygen or inert gas according to the signal of a polarographic oxygen detector. This method of control is conservative of inert gas and therefore permits long runs with a minimum usage of inert gas. Total pressure is maintained within one-

half per cent of the selected value and PO_2 within ± 3 mm Hg. Chamber temperature is controlled by adjusting the temperature of the laboratory.

Inside the chamber is a wire cage divided into six pie-shaped compartments for individual rabbits. Animals are handled through 'dry-box' gloves sealed into portholes. The cage can be rotated to facilitate handling of all animals through one set of gloves. Handling and transfer operations must be performed with the chamber at sea level pressure. The chamber is equipped with a lock for passing equipment in and out; this is used for entry of the animals after a new atmosphere has been established inside.

This experiment was carried out in the autumn, when the length of the daylight was changing. To prevent possible seasonal metabolic fluctuations, we left the room lights on at all times. This also prevented the increase in activity of the animals that would ensue each morning when the lights were turned on; such changes in activity disturb measurements of oxygen consumption.

The male New Zealand albino rabbits used in the experiment were inbred and constituted as uniform a population as could be attained practically. They were to be delivered at a weight of between 2.0 and 2.5 kg on the day before the beginning of the run. We felt that this size rabbit would be old enough to be metabolically 'stable' and still young enough to have a consistent rate of growth. Specifying the initial weight range resulted in all animals starting the experiment at about the same age as well, about 3 months. Because of an administrative error, the animals obtained for the oxygen exposure were all females, and by the time this was noticed the bulk of the experiment had been completed.

C. RESULTS

This section includes responses attributable to the different experimental atmospheres followed by a comparison of the effects of the different gas species on the various physiological parameters.

1. *Summary*

This comprehensive survey of the physiological responses of rabbits to week-long exposures in each of several experimental atmospheres shows in general that none of the atmospheres tested caused any portentious physiological consequences. Results of measurement on animals exposed to atmospheres containing argon or neon in place of nitrogen were essentially indistinguishable from results on animals exposed to air. Helium caused a general increase in metabolism, evidenced by increases in oxygen and food consumption and an increased rate of weight gain. There were changes in the blood of animals exposed to helium – a reduction in red blood cell count, hematocrit and hemoglobin, and some evidence of an increase in iron uptake during and immediately after the exposure. Animals exposed to a low-pressure, pure oxygen environment showed less dramatic blood changes and showed a slight weight loss.

2. *Metabolism*

a. *Body Weight.* Figure 2 compares the daily weights of the animals in each of the

RABBIT WEIGHTS
EXPOSURE TO EXPERIMENTAL ATMOSPHERES

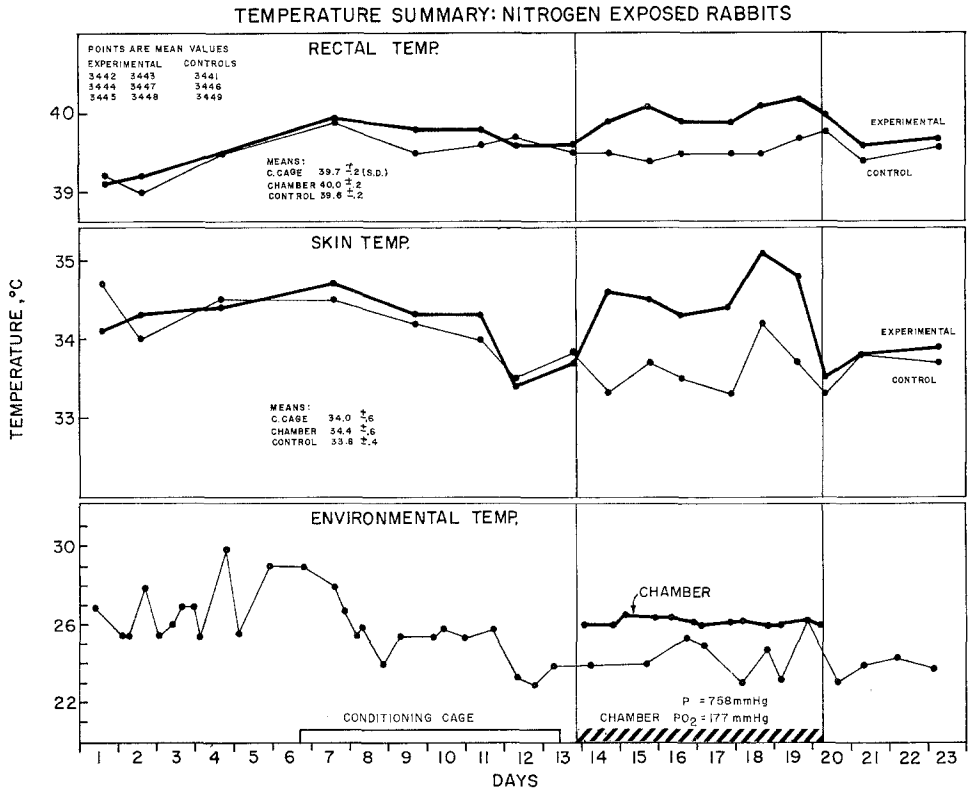TEMPERATURE SUMMARY: NITROGEN EXPOSED RABBITS
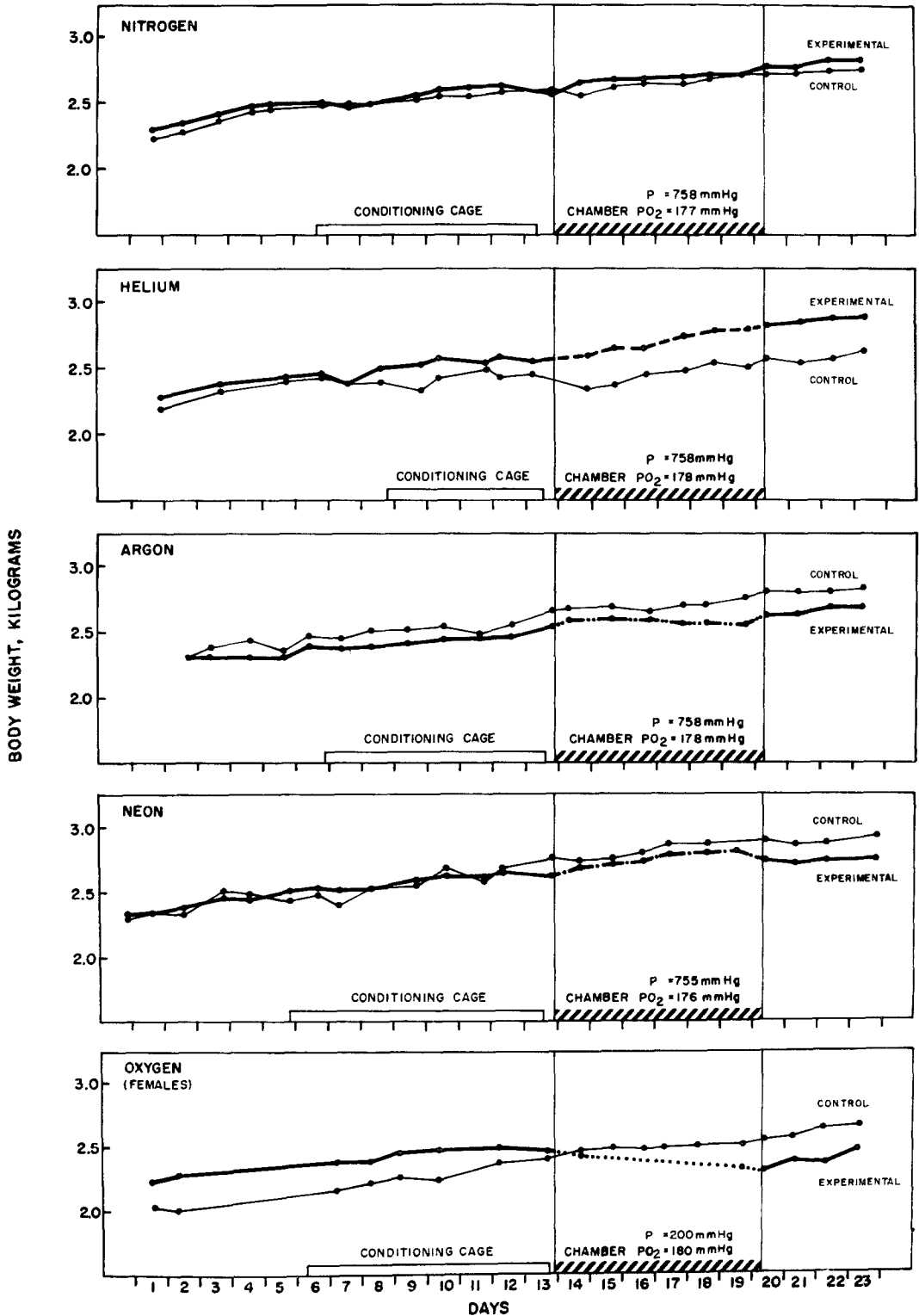
Fig. 3.   Temperatures during nitrogen experiment. Heavy lines apply to those animals which were exposed to the chamber atmosphere; thin lines signify control animals in regular cages.

five different runs. The heavy line shows the mean of the six experimental animals, and the thin line shows the mean of a control group that was maintained at the same time on a parallel basis in regular cages. The period of exposure is indicated by a code that is characteristic of each gas. This set of symbols is used throughout the report.

The body weight curves on all units seem to dip slightly just after the animals enter the conditioning cage. This is not surprising in view of the change in cage configuration and the change from a watering bowl to a drinking nipple. This slight but consistent effect warns us to be cautious about making direct comparisons of experimentally exposed animals with those which have just entered the conditioning cage.

A comparison of the chamber exposures reveals two apparent differences. Animals exposed to helium seemed to gain weight faster than the helium control group, and there is a clear weight loss in the animals exposed to the low pressure, pure oxygen

Fig. 2.   Average daily weights of animals are shown by solid line, interrupted during the period of exposure to the experimental atmosphere. Thin solid lines are animals in appropriate control groups, held in regular cages and breathing air. Animals exposed to oxygen lost wieght, those exposed to helium appeared to gain weight faster than usual.
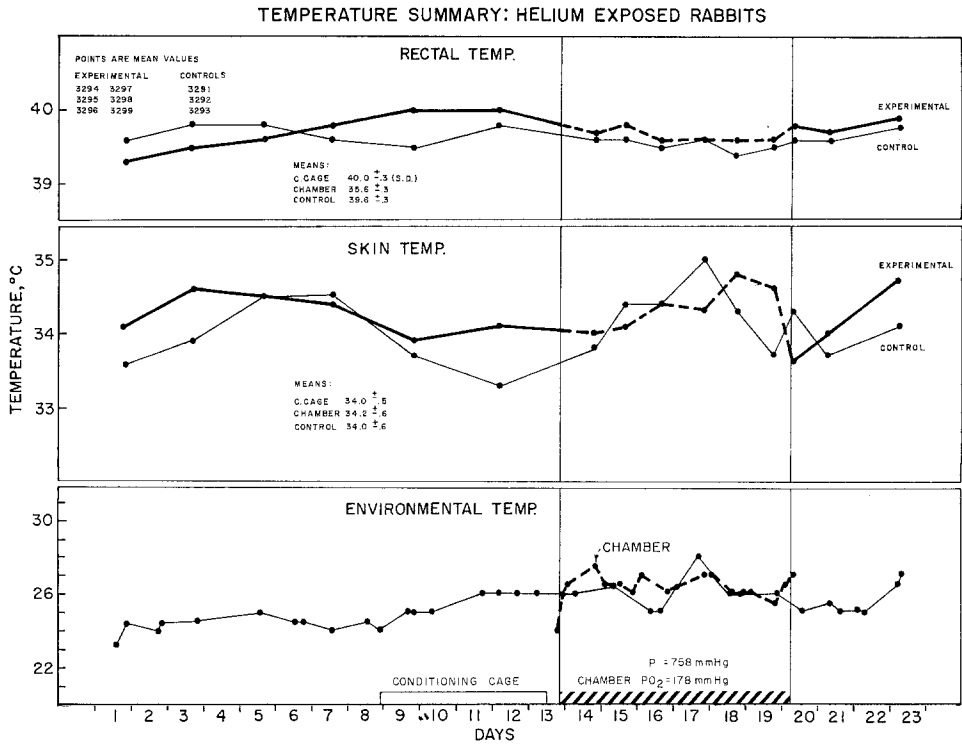
Fig. 4.   Temperatures during helium experiment. Heavy lines apply to animals exposed to helium in chamber; thin lines signify control animals in regular cages. Despite other differences, helium animals maintained their body and skin temperatures at the same level as the controls.

environment. The argon animals also seem to have gained weight during the exposure a little more slowly than usual. These possibilities were tested statistically. One-week gains were calculated for all animals during the chamber run and for all exposed groups during the week in the conditioning cage. The helium animals gained more weight than the controls ($p < 0.05$), the oxygen animals lost weight($p < 0.001$) and the argon animals had no significant change from the controls.

b. *Body, Skin and Environmental Temperature.* Rectal, skin and environmental temperatures are given in Figures 3 through 7. Each of these graphs shows mean rectal and skin temperatures of all animals in the experimental groups along with temperatures of the control group. Environmental temperature is included also – both for the exposed animals while they were in the chamber and for the remaining control animals throughout the experiment.

The most prominent differences on these graphical summaries (Figures 3–7) are the cases where experimental values diverge from control values during the chamber phase. The mean body temperature of nitrogen-exposed animals seems to be slightly elevated during the exposure, and the skin temperature of the nitrogen, neon and pure oxygen groups also seems to be slightly elevated at that time. But equally as prominent as the
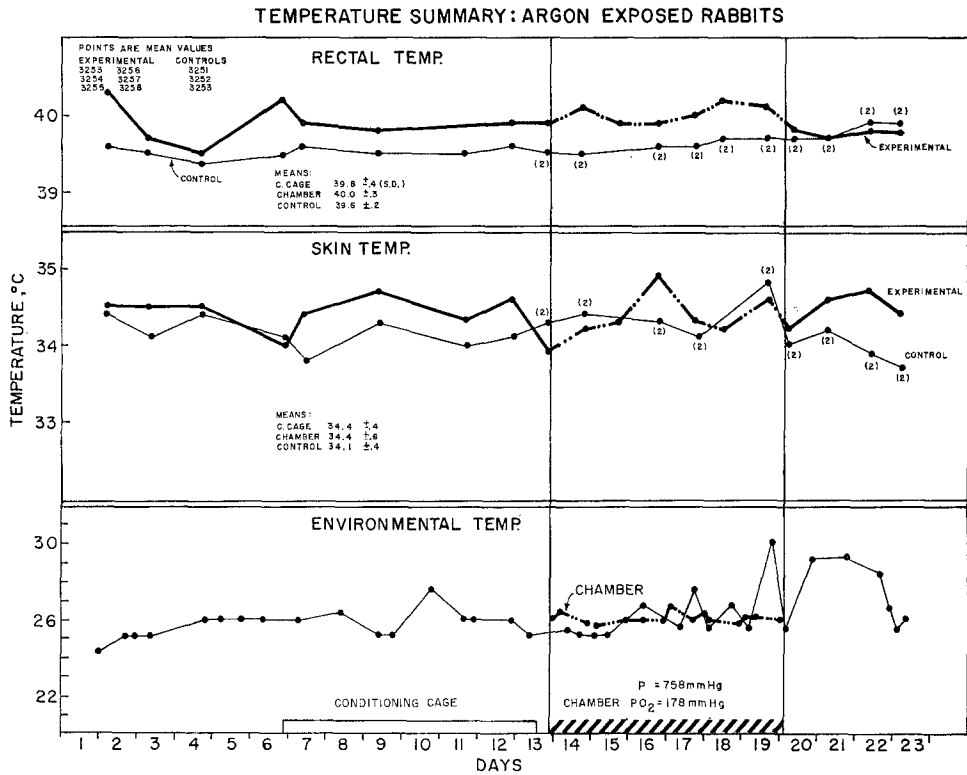
Fig. 5.   Temperatures during argon experiment. Heavy lines apply to animals exposed to argon in chamber; thin lines signify control animals in regular cages.

differences is the lack of differences shown by parallelism between control and experimental body temperatures in the helium, argon, neon and oxygen runs.

c. *Oxygen Consumption*. Values of this parameter for all five experimental units are shown in Figure 8. The points determined twice daily during the exposure are connected by the appropriately coded lines. The control values taken at the beginning and end of each experimental run are shown by symbols at either side of the figure. Units are milliliters of oxygen per kilogram of rabbit weight per minute. The values were obtained collectively at one time on all animals in each group and converted to the per-kilogram basis.

One point to be considered in interpreting these data is that the group of rabbits exposed to pure oxygen at reduced pressure were, due to an error, all females. The significance of this error is indicated by the distinct difference in the air control values of the oxygen group. The mean control oxygen consumption of these animals is 8.56 ml/kg-min while the pre-plus-post average of the other control values is 13.1 ml/kg-min. For this reason we have not made any further comparisons of the oxygen unit with the others. This error is slightly mitigated by the knowledge that the pure oxygen at reduced pressure environment has been extensively studied elsewhere; appropriate comparisons are considered in the discussion.
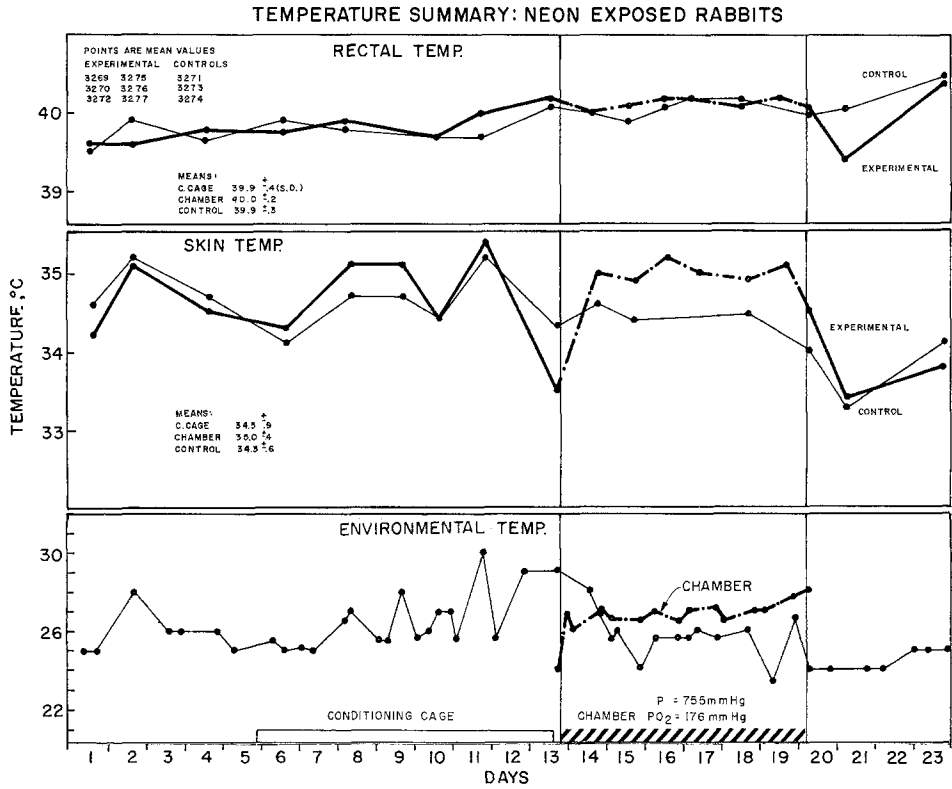
Fig. 6.   Temperatures during neon experiment. Heavy lines apply to animals exposed to neon in chamber; thin lines signify control animals in regular cages.

Visual inspection of Figure 8 suggests that the animals breathing helium had an increased oxygen consumption and that there was not much difference in oxygen consumption among the other groups.

Looking first at the nitrogen group, it is clear that the control values determined before the exposure are significantly higher than those determined with nitrogen in the chamber. This difference is in fact significant by the t-test ($p < 0.001$). Since there is essentially no difference between air and the 'nitrogen' atmosphere in the chamber, there should be no such difference in oxygen consumption. We are confident that the reason for the high pre-exposure control value is that the animals were excited after having been installed in the chamber only an hour before. An additional possible explanation is that the system may not have been completely in equilibrium this soon after being started anew. Because of this, we have chosen the nitrogen unit and the post-exposure controls as basis of comparison of oxygen consumption rates in the various gaseous environments tested. There is no statistically significant difference between the mean of oxygen consumption during the nitrogen exposure and the post-exposure controls, so this pooling is justified.
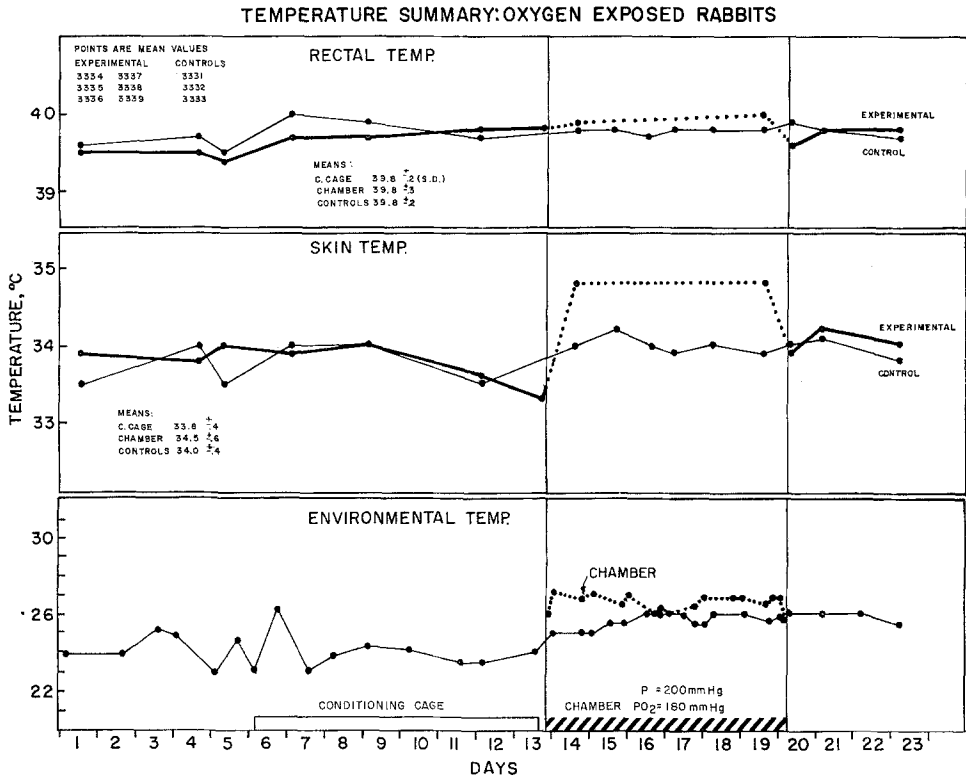
Fig. 7. Temperatures during oxygen experiment. Heavy lines apply to those animals which were exposed to a pure oxygen atmosphere at reduced pressure; thin lines signify control animals in regular cages. These animals had increased skin temperatures and appeared to perspire heavily. (The 'chamber mean' value reflects the two temperature measurements made during exposure as well as the immediate post-exposure temperature measurements.)

A summary of mean values and t-test comparisons of each gas with both the nitrogen run and the mean of all post-exposure control values is given in Table II.

The results indicate that helium caused an increase of 27% in the oxygen consumption of these rabbits compared to similar conditions in an atmosphere essentially the same as air. There is an indication that a similar increase of 7% is caused by neon. It must be remembered that these comparisons represent different groups of animals, although the different groups showed a striking (and probably fortuitous) uniformity in their control values.

d. *Food Consumption.* This was one of the least precise parameters monitored in this experiment. Daily averages are shown in Figure 9 and despite the crudeness of the measurement one may derive certain impressions from an inspection of this figure. One consistent detail is the drop in consumption of all five experimental groups on the first day in the conditioning cage. This helps to emphasize the importance of a 'conditioning' exposure in this type of experiment. There was some tendency for a reduction also on the first chamber day.
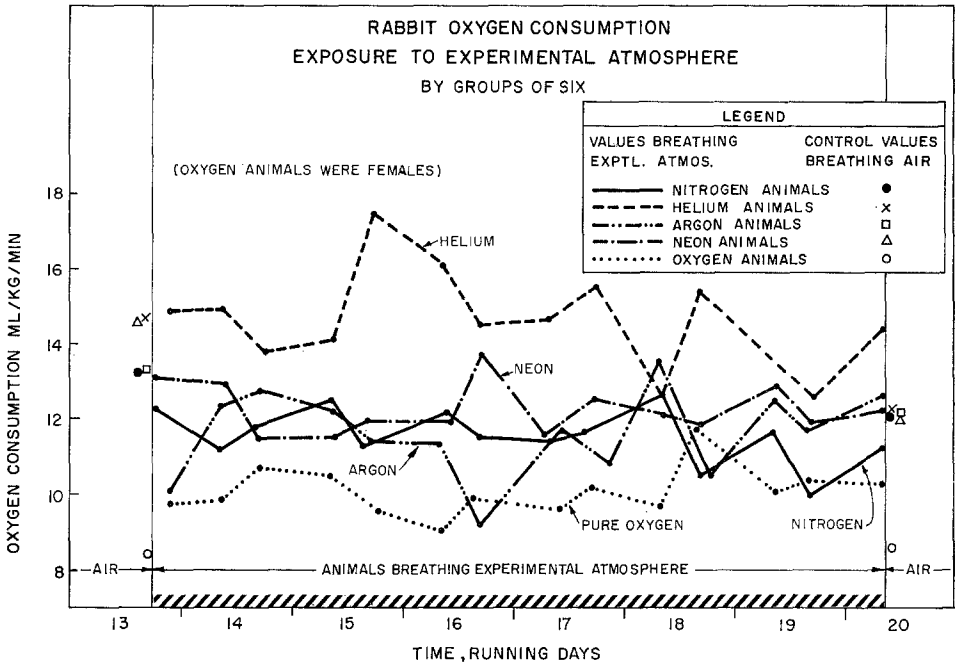
Fig. 8. Twice daily oxygen consumption measurements of rabbits exposed to various atmospheres for one week. Symbols on sides are control values with animals breathing air. Hatched bar denotes period of exposure. PO₂ in chamber was 175–180 mm Hg, total pressure 1 atm except for the pure oxygen exposure. Animals in oxygen run were female, all others male.

TABLE II

Statistical comparison of oxygen consumption data

	n	Mean ± Std. Dev.	vs Nitrogen			vs Post-exposure controls		
			t	$d.f.$	p	t	$d.f.$	p
Pre-exposure controls	4	14.00 ± 0.78	5.84	16	< 0.001	4.809	6	< 0.001
Nitrogen	14	11.53 ± 0.74		–		1.53	16	n.s.
Helium	13	14.66 ± 1.33	7.63	25	< 0.001	3.7	15	< 0.005
Argon	14	11.47 ± 1.13	0.17	26	n.s.	1.11	16	n.s.
Neon	14	12.22 ± 0.67	2.58	26	< 0.01	0.32	16	n.s.
Oxygen at Reduced Pressure	14	10.06 ± 0.66		–			–	
Post-exposure controls	4	12.11 ± 0.10	1.53	16	n.s.		–	

About the only other information supplied by these data is the increase in consumption of the helium animals during their exposure. The increase is qualitatively consistent with the 27% increase in oxygen consumption.

RABBIT FOOD CONSUMPTION
EXPOSURE TO EXPERIMENTAL ATMOSPHERES
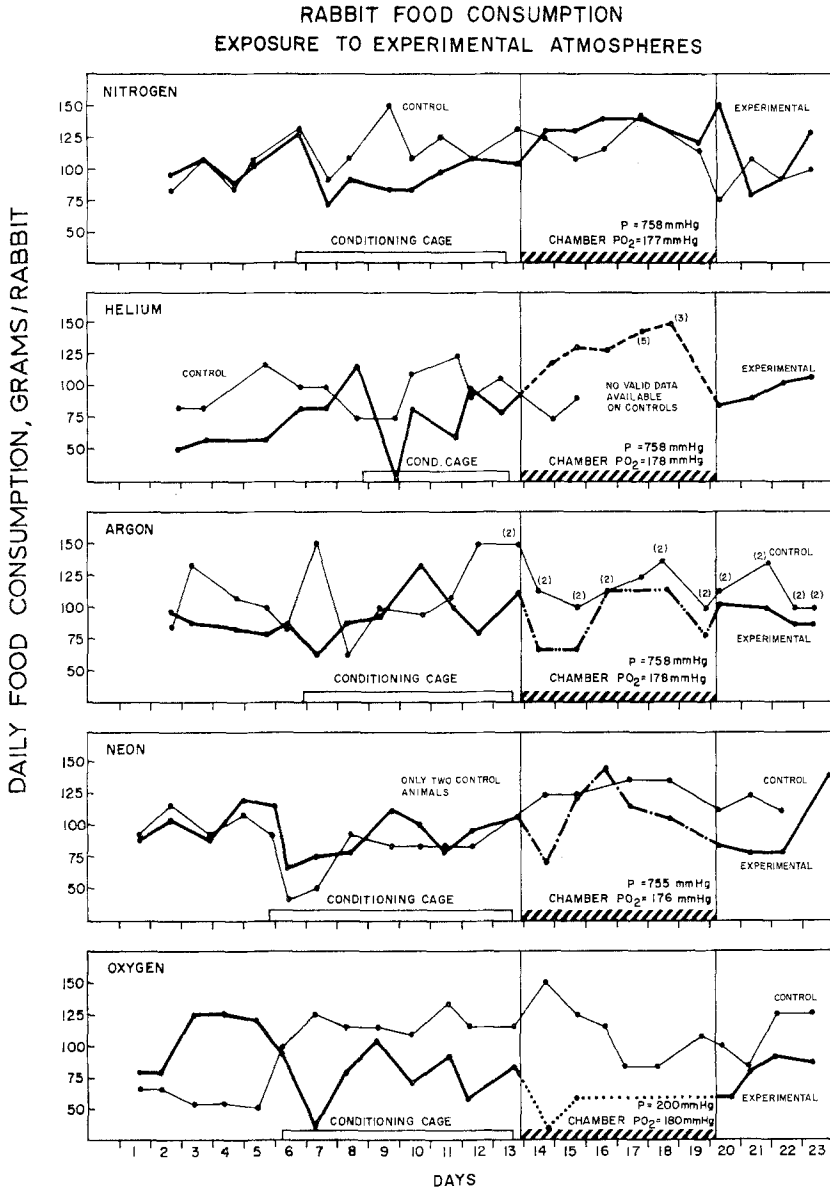
Fig. 9.   Mean daily values of food consumed by rabbits exposed to each experimental atmosphere. Despite the large fluctuation, it can be seen that the animals ate less the first day in the conditioning cage, and that the helium-exposed animals had an increased appetite.

e. *Water Consumption and Waste Production.* Estimates of both water consumption and waste production are shown in Table III.

f. *Relative Activity.* No quantitative measure of activity was attempted, but subjectively there seemed to be differences between the animals exposed to different gases. Most prominent was the lethargic state of the oxygen animals; they were universally

TABLE III

Estimate of rabbit water consumption and waste production

| | Day | Liters | | | | | | | | | |
| | | N₂ | | He | | Ar | | Ne | | O₂ | |
		H₂O	Waste	H₂O	Waste	H₂O	Waste	H₂O	Waste	H₂O	Waste
Conditioning cage	7	2.0	1.8			L*a	L	2.3	1.7	2.5	2.8
	8	2.0	1.8			L	L	2.5	2.1	L	L
	9	3.1	2.2	2.5	–	L	L	L	L	3.5	3.0
	10	2.7	2.1	3.3	3.5	3.1	2.8	L	L	3.2	2.6
	11	2.9	2.4	L	L	2.9	2.9	2.8	2.7	3.4	3.1
	12	2.9	2.1	3.3	2.2	2.1	1.8	2.9	2.0	2.5	2.2
	13	2.7	2.4	L	L	2.1	1.7	3.4	3.1	3.0	2.6
	Mean	2.6	2.1	3.0	2.8	2.5	2.3	2.8	2.3	3.0	2.7
Chamber	14	1.8	1.4	2.7	2.4	2.0	1.1	3.0	2.5	1.0	1.0
	15	2.5	2.5	–	2.0	1.7	2.9	3.0	2.9	1.8	1.7
	16	2.5	2.2	3.5	2.1	2.5	2.4	2.6	3.2	2.2	1.7
	17	2.6	2.9	3.0	2.9	1.9	2.0	2.3	2.5	2.6	2.7
	18	2.8	2.6	2.9	3.5	L	L	2.7	3.4	2.0	1.6
	19	L	L	3.3	3.2	L	L	2.9	2.8	2.7	3.0
	20	2.5	2.5	2.5	2.3	3.5	2.8	2.6	2.7	1.6	1.4
	Mean	2.5	2.4	3.0	2.6	2.3	2.2	2.7	2.9	2.0	1.9

a 'L' represents a leaking valve.

rated as hypoactive during the chamber run. Despite the apparent inactivity, these animals were considerably more difficult to handle while in the chamber. It is not known whether this is related to the fact that these animals were all females, but the possibility does exist. They behaved normally and displayed normal activity during the pre- and post-exposure phases. All of the animals showed a reduction of activity when first put in the conditioning cage; they appeared to become acclimated in a few days, then slowed down again when transferred to the chamber. All animals were less active in the chamber cages than in the regular rabbit cages. While in the chamber the nitrogen and helium animals were more active than those exposed to argon and neon.

g. *Cardiac Frequency.* Heart rates of all five experimental units are shown in Figure 10. The mean heart rate of these normal rabbits under control conditions is about 220 beats per minute. The only deviations that appear to be related to the experimental exposure are a slight decrease in the oxygen-exposed animals and a possible increase in the animals exposed to helium. These are in agreement with observations on other parameters such as activity and oxygen consumption.

h. *Respiratory Frequency.* Values for respiratory frequency were highly variable and are not displayed graphically.

The most interesting aspect of these data is their disagreement with some values in the literature. Our values are considerably higher than expected, running greater than 250 breaths per minute, while literature values are 38–69 breaths per minute (Altman and Dittmer, 1964; Dittmer and Grebe, 1958). This is undoubtedly a

panting-type respiration, and could possibly be a function of the handling and restraint techniques we used. If, however, it was due only to momentary excitement, we would expect an occasional low value in a calm rabbit, and none was found. Possibly handbook values are based on rabbits anesthetized or maintained at a lower temperature, or different strains.

There do not seem to be any detectable changes in respiratory frequency as a function of the gas species. Most rates were down slightly and were quite uniform between animals on the first day after coming out of the chamber. This was not related to the type of exposure.

i. *Electrocardiograms.* Electrocardiograms recorded between an ear and a chest electrode were taken from animals exposed to each gas environment. No differences were found that could in any way be related to the gas being breathed, except the frequency changes already mentioned.

3. *Biochemistry*

a. *Red Blood Cell Indices.* A summary of the routine clinical determinations that bear on erythrocyte economics is given in Table IV.

Inspection of the red cell data reveals changes only in the helium-exposed rabbits. Hematocrit, hemoglobin and red cell count all appear to be reduced, possibly as a result of the seven-day exposure to a predominately helium atmosphere. In addition, these animals have a reticulocyte count that appears to be increased following exposure.

To test these differences statistically, the helium values were compared with pooled controls from all gases. The mean differences between the average pre-exposure values and those of day 20 were compared using the *t*-test. The decreases in hematocrit, hemoglobin and red cell count were all found to be highly significant ($p < 0.005$, 0.005 and 0.001, respectively).

Since there were no pre-exposure reticulocyte counts available for the helium animals, differences cannot be compared. The mean value for all control animals is 1.56%, while the post-exposure helium animals (day 20) had a mean value of 2.25%. The difference between these means is not significant ($p > 0.1$, $d.f. = 38$), although the apparent increase is in the proper direction to be consistent with the idea that erythropoiesis was stimulated by the observed loss of red cells. The animals exposed to pure oxygen seemed to have a slightly reduced reticulocyte count, but we could not show this difference to be significant ($p > 0.3$). Normal rabbits have between 1 and 2% reticulocytes (Scarborough, 1931).

b. *Isotope Studies.* These studies using radioactive iron and chromium were carried out in order to examine the possible effects of the experimental atmospheres on red blood cell formation and destruction. The rationale was to examine the effect of the exposures on the rate of iron incorporation, by testing some animals before and through the exposure, and by testing others immediately following the exposure. This resulted in the division of each unit of six exposed rabbits into two groups of three, an uncomfortably small sample size.

RABBIT HEART RATE

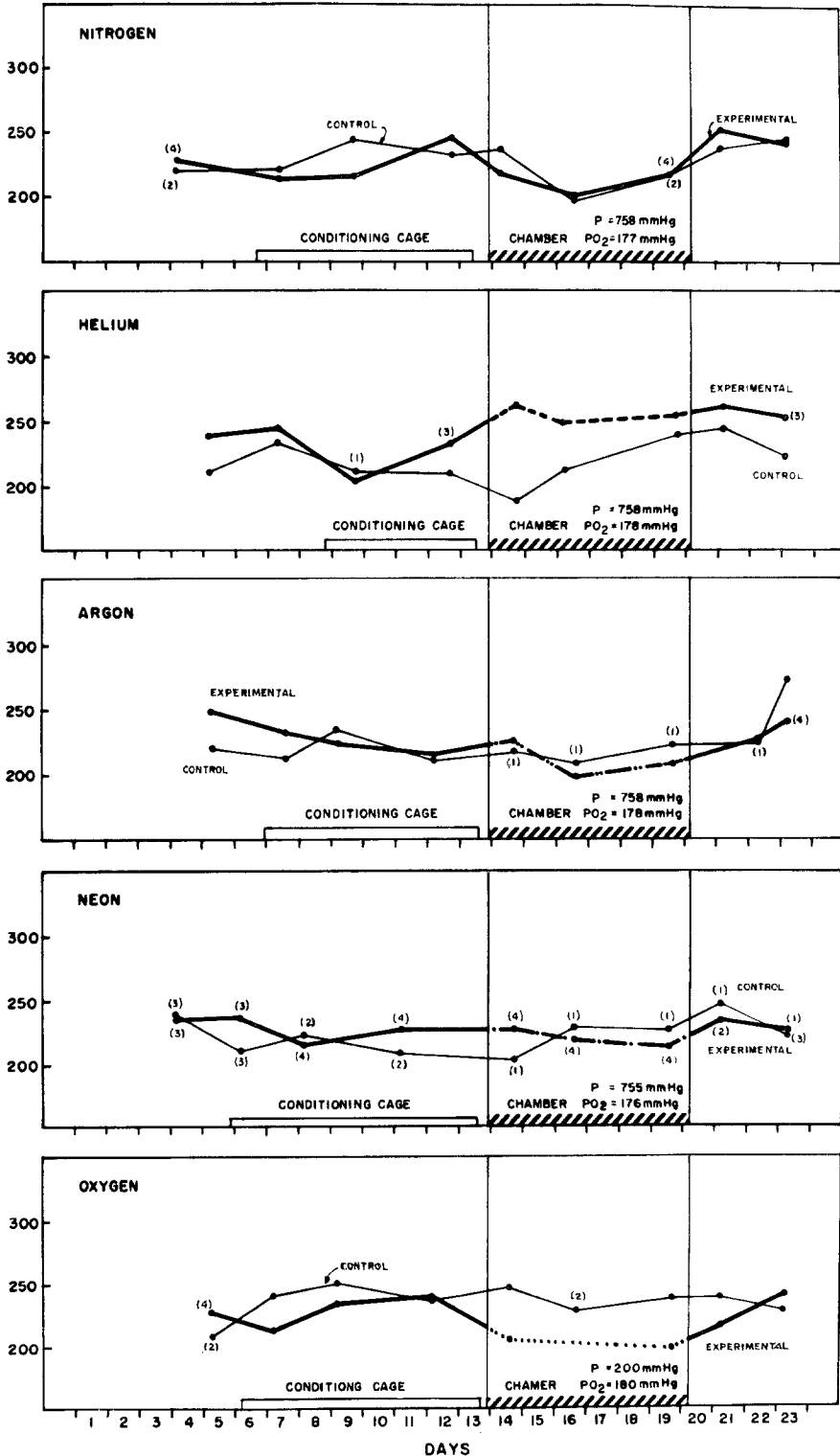## EXPOSURE TO EXPERIMENTAL ATMOSPHERES

Table V summarizes this information as mean values for each group. Where two groups were given the same test, the mean of both is given. Values are shown plus and minus one standard deviation. The small superscripts show the number of animals in each group or the number of valid determinations reflected by a particular mean value.

We feel that reduction and interpretation of the data presented in Table V must be done cautiously. The large variation and small numbers in each group preclude the use of routine parametric statistical techniques. The entire isotope study was subjected to analysis of variance with a singularly uneventful result, and with no meaningful interpretations afforded by this approach. Likewise, we did not feel that valid conclusions could be based on the t-test. We have therefore based our interpretation on a critical inspection of the data, which is supported to a certain extent by a non-parametric rank test.

There do not appear to be any important differences in the animals exposed to nitrogen, neon or argon, except for some apparent differences in red cell survival and a possible slight decrease in one-day iron uptake following neon exposure. The neon-exposed animals had the lowest percentage of red blood cells surviving after 8 days, (48%,) but the one control value available for comparison was still lower. It must be remembered that each new injection of tagged cells (used primarily for the determination of red cell volume and only incidentally for red cell survival) was taken from a different donor rabbit. Because of this the only valid comparison that can be made with regard to this parameter is the difference between groups injected at the same time. There is no evidence from other sources to suggest increased red cell destruction in the neon groups and the post-exposure iron uptake is, if anything, down instead of up.

The most interesting results are the post-exposure iron uptake values of the helium and oxygen animals. The data suggest that the exposure to helium prompted an increased erythropoiesis, since the post-exposure 24-hour Fe^{59} uptake is 52% in group 3 while the comparable pre-exposure values for the other two helium groups were 36% and 37%.

Another approach was used to seek evidence of a difference in iron uptake as a result of the exposure. Each pre-exposure 24-hour iron uptake value was subtracted from that rabbit's 8-day uptake, yielding differences which are based on each animal as his own control. The differences for rabbits in the two groups (1 and 2, exposed and non-exposed) were then compared by means of the Wilcoxon rank test (Diem, 1962, p. 191). Helium-exposed animals had iron uptakes consistently greater than non-exposed animals with a p value of 0.1. This is the minimum p value obtainable with this test on the number of data points available. It does not establish statistical significance, but indicates a definite trend. The p value of 0.1 means that for groups this size the possibility of all three exposed animals having greater uptakes than all three controls will occur by chance less than 10% of the time.

Fig. 10. Mean heart rates of two rabbits from each group and four from each experimental unit. Beats per minute were counted from electrocardiograms recorded while animals were sitting quietly, but only a few minutes after they had been handled.

TABLE IV

Red blood cell summary

Mean, standard deviation and number of samples are shown in each block. Group 1 were controls, Groups 2 and 3 were exposed to experimental atmosphere on days 13–20.

Group	Day 2		Day 12		Day 20	
	1	2 + 3	1	2 + 3	1	2 + 3
Hematocrit, %						
Nitrogen	39.3 ± 1.3 (3)	38.8 ± 1.0 (6)	37.5 ± 0.5 (3)	36.2 ± 2.6 (6)	38.6 ± 2.7 (3)	37.3 ± 2.6 (6)
Helium	40.1 ± 0.5 (3)	38.8 ± 3.3 (4)	38.4 ± 2.7 (3)	38.2 ± 2.3 (6)	39.8 ± 1.9 (3)	35.4 ± 2.6 (5)
Argon	41.2 ± 2.9 (3)	38.6 ± 2.6 (6)	40.2 ± 3.0 (3)	38.6 ± 1.4 (6)	37.6 ± 2.7 (2)	36.8 ± 3.1 (6)
Neon	35.4 ± 1.8 (3)	37.4 ± 2.1 (6)	37.3 ± 2.3 (3)	39.4 ± 2.3 (6)	39.0 ± 0.7 (2)	38.3 ± 1.3 (6)
Oxygen	38.4 ± 1.2 (3)	36.6 ± 2.7 (6)	36.7 ± 2.4 (3)	37.1 ± 3.5 (5)	38.6 ± 2.3 (3)	37.3 ± 3.7 (6)
Hemoglobin, mg/100 ml						
Nitrogen	12.3 ± 0.5 (3)	12.7 ± 0.6 (6)	11.4 ± 0.5 (3)	11.4 ± 0.7 (6)	11.4 ± 0.6 (3)	11.2 ± 0.5 (6)
Helium	12.2 ± 0.7 (3)	12.0 ± 1.0 (4)	11.9 ± 0.7 (3)	11.9 ± 0.7 (6)	12.4 ± 0.2 (3)	10.9 ± 1.1 (6)
Argon	12.5 ± 1.3 (3)	11.8 ± 0.9 (6)	12.6 ± 1.2 (3)	12.0 ± 0.5 (6)	11.7 ± 0.8 (2)	11.9 ± 0.9 (6)
Neon	11.1 ± 0.7 (3)	11.6 ± 1.0 (6)	11.7 ± 0.8 (3)	12.2 ± 0.7 (6)	11.8 ± 0.2 (2)	11.7 ± 0.6 (6)
Oxygen	12.4 ± 0.2 (3)	12.0 ± 0.8 (6)	11.8 ± 0.6 (3)	11.8 ± 1.0 (6)	12.5 ± 0.6 (3)	12.1 + 1.2 (6)
Red Cells, $10/mm^3$						
Nitrogen	5.9 ± 0.3 (3)	6.2 ± 0.2 (6)	5.7 ± 0.5 (3)	6.1. ± 0.7 (6)	5.5 ± 0.1 (3)	5.4 ± 0.3 (6)
Helium	6.8 ± 1.4 (3)	6.0 ± 0.2 (3)	–	6.3 ± 0.4 (6)	6.7 ± 0.8 (33)	5.0 ± 0.8 (6)
Argon	6.1 ± 0.8 (2)	6.1 ± 0.5 (4)	5.9 ± 0.6 (3)	5.9 ± 0.4 (6)	5.9 ± 0.3 (2)	5.6 ± 0.3 (5)
Neon	6.7 ± 0.3 (3)	6.9 ± 1.1 (6)	5.7 (1)	6.4 ± 1.0 (5)	5.9 ± 0.3 (2)	5.9 ± 0.7 (6)
Oxygen	5.1 ± 0.2 (3)	5.8 ± 0.9 (6)	5.5 ± 0.6 (3)	5.7 ± 0.6 (6)	5.6 ± 1.0 (3)	5.8 ± 0.8 (6)
Reticulocytes, %						
Nitrogen	1.2 ± 0.4 (3)	1.1 ± 0.5 (6)	1.2 ± 0.2 (3)	1.4 ± 0.3 (5)	1.7 ± 0.9 (3)	1.3 ± 0.2 (6)
Helium	–	–	–	–	1.3 ± 0.5 (3)	2.2 ± 1.2 (6)
Argon	–	–	1.4 ± 0.7 (3)	1.7 ± 0.7 (6)	1.6 ± 0.2 (2)	1.3 ± 0.3 (5)
Neon	2.2 ± 0.7 (3)	2.2 ± 1.1 (6)	1.4 ± 0.6 (3)	1.5 ± 0.6 (5)	1.7 ± 0.2 (2)	1.4 ± 0.6 (6)
Oxygen	1.5 ± 0.5 (3)	1.5 ± 0.4 (5)	1.6 ± 0.2 (3)	1.6 ± 0.6 (6)	2.0 ± 0.8 (3)	1.2 ± 0.6 (5)

TABLE V

Summary of Isotope Study

Test Atmosphere	Group	Day 12-13			Chamber	Day 20-21				
		Total RBC Volume ml/kg	24-hr. RBC Survival %	24-hr. Iron Uptake %		Total RBC Volume ml/kg	8-Day RBC Survival %	24-hr. RBC Survival %	8-Day Iron Uptake %	24-hr. Iron Uptake %
Nitrogen	1	18.8±1.3	92.1±2.6	30.3±8.5		17.7±0.9	69.2±6.8	87.0±4.7	68.3±5.3	-
	2	17.3±1.1	89.5±4.6	39.6±5.1		17.6±1.2	68.0±2.2	91.4±3.7	77.7±5.6	-
	3			-						39.6±10.2
Helium	1	17.1±1.6	85.3±1.8	35.7±12.6		16.6±1.2	56.9±3.6	84.9±3.6	66.2±11.2	-
	2	15.9±2.1	87.5±5.0	37.1±1.6		15.2±1.3	62.0±4.8	85.0±4.8	76.1±3.9	-
	3			-						52.1±8.4
Argon	1[2]	18.0±3.5	83.8±3.5	33.0±4.7		17.1±2.3	57.0±3.3	85.2±1.3	70.0±4.4	-
	2[4]	17.5±1.1	84.9±5.9	41.7±9.8		15.8±1.2	58.0±2.7	89.9±2.3	72.7±4.3	-
	3[2]			-						48.1±10.0
Neon*	1[2]	17.8±0.1	82.6±8.5	44.6±2.4		17.3[1]	39.5[1]	90.4[1]	81.1±1.6	-
	2	17.9±1.2	82.9±7.4	41.7±3.7		18.5±1.7	48.4±12.8	86.0±6.5	79.5±12.5	-
	3			-						33.2±12.6
Oxygen	1	17.7±1.3	90.3±3.1	32.7±6.8		17.6±0.2	64.9±7.0	86.3±4.9	66.4±3.8	-
	2	16.6±2.6	92.9±3.9	33.1±3.0		15.9±1.6	72.7±10.7	84.6±6.5	61.1±5.0	-
	3			-						28.9±6.0

n = 3 per group except where noted by superscript

* "8-day" RBC survival and iron uptake were actually 9-day values on neon-animals

The animals exposed to oxygen in the absence of inert gas showed the opposite tendency with respect to iron uptake. The three rabbits in group 3 appear to have assimilated less Fe^{59} on the day after emerging from the chamber than did the unexposed animals over a comparable period. The individual differences between one-day and eight-day uptake were again compared in groups 1 and 2 and again the Wilcoxon test produced a p value of 0.1. This suggests that the exposure to 180 mm Hg PO_2 in the absence of inert gas caused a slight inhibition of erythropoiesis.

c. *Blood Chemistry.* A comparison of individual rabbit data on plasma sodium, potassium, glucose, urea nitrogen, pH, bicarbonate and gas tension showed no meaningful differences as a function of the inert gas diluent, so only a summary table is included here. Table VI gives pre- and post-exposure averages for the six animals exposed to each experimental atmosphere.

TABLE VI

Blood chemistry summary

Exposure condition	Na^+ meq/1.	K^+ meq/1.	Glucose mg/ 100 ml	Urea nitrogen mg/100 ml	pH	Bicarb. meq/1.	P_aCO_2 mm Hg	P_aO_2 mm Hg
N₂ Pre-	147	6.0	77	22	7.41	24.6	38	81
Post-	148	5.9	58	18	7.45	24.6	34	98
He Pre-	144	5.0	77	21	7.43	27.6	41	93
Post-	148	4.7	72	24	7.45	28.5	41	81
Ar Pre-	142	4.7	89	28	7.43	25.6	38	86
Post-	158	4.8	89	27	7.46	25.7	36	93
Ne Pre-	149	5.1	75	23	7.42	24.3	36	93
Post-	141	5.0	69	22	7.45	25.9	37	82
O₂ Pre-	154	4.9	81	21	7.40	23.0	35	97
Post-	129	2.6	56	20	7.44	24.0	32	89

Several parameters in this table appear to show differences in pre- and post-exposure values and therefore deserve comment. In a number of cases the control animals not exposed to the experimental atmosphere (and not shown on the table) showed changes similar to those seen in the treated animals. These changes may have occurred spontaneously in a group of rabbits, may have been caused by events unrelated to the experiment, or may reflect systematic analytical inaccuracies. In any case they cannot be seriously considered in evaluating effects of the inert gas atmosphere. Changes that belong in this category are the increase in sodium in the argon animals, the P_aO_2 differences in the nitrogen animals, and most of the differences seen in glucose. The evidence of a reduction in glucose in the oxygen animals may be valid.

Differences in blood gas values were systematic. Many factors will excite an animal – the type of handling, the room temperature, and even the personality of the technician. Excitement will in turn affect his ventilation rate, and hence his P_aCO_2, pH and P_aO_2. The standard bicarbonate, however, is relatively independent of this

perturbation, and showed reasonable stability in all groups. Post-exposure animals had been confined for a week and were probably more excitable on removal from the chamber. The tendency seems to be toward a reduced P_aCO_2 at that point. A reduction in arterial oxygen tension may reveal a diffusion barrier and perhaps other types of pulmonary damage, but conditions were not well enough controlled in these experiments for this interpretation to be made.

The sodium and potassium values are considerably reduced in six female rabbits following exposure to the low-pressure, pure oxygen environment. The control animals did not respond in a parallel way, but maintained normal levels. However, the initial blood samples drawn out of all animals soon after their arrival were low in sodium and potassium in just the same way. This suggests that some other environmental stress, possibly dehydration, may have caused the change. As mentioned, the oxygen animals were females and therefore do not provide a completely valid comparison.

Both sodium and potassium values of the oxygen unit are on occasion well below normal and may reveal analytical errors, although Spector (1956) lists normal rabbit values of serum potassium between 2.7 and 5.1 meq/liter.

4. Pathology

This section considers leucocyte counts and both gross and microscopic pathology. A summary of leucocyte information is given in Table VII. It includes only values on exposed animals before and after the chamber exposure.

The leucocyte responses seen in the nitrogen, helium and oxygen animals suggest that the animals may have been under some degree of stress, but these data are not a suitable index for any sort of quantitative comparison. All values are normal and are consonant with the thesis that the exposures were generally innocuous (Farr *et al.*, 1948).

Post-mortem examination found all animals essentially normal. One argon animal had minor atelectic spots on its lungs but it is not necessary to associate this symp-

TABLE VII

Leucocyte counts

Exposure condition	Leucocytes, 10^3/cu mm	% Polymorphonuclear	% Mononuclear
N₂ Pre-	8.5	28.3	71.7
Post-	10.1	28.5	71.5
He Pre-	8.8	32.0	68.0
Post-	9.3	40.2	59.8
Ar Pre-	7.5	32.8	67.2
Post-	7.4	47.0	53.0
Ne Pre-	8.4	33.0	67.0
Post-	8.3	32.3	67.7
O₂ Pre-	8.4	33.8	66.2
Post-	10.5	45.7	54.2

tom with the exposure. The oxygen animals had been equilibrated with an atmosphere devoid of inert gas and if any atelectasis had developed it should have been in these rabbits, but their lungs were not atelectatic. An important point to be considered is that the necropsy examinations were performed three days after the animals had been removed from the chamber – a sufficient period of time for possible transient disturbances to return to normal.

Likewise, histological examination of liver, spleen, lung, thryoid, kidney and adrenal tissues did not reveal any lesions that could be attributed to the experimental atmospheres. Several pathological conditions were noted but in no cases were they limited to the exposed rabbits. One of the oxygen-exposed rabbits (out of two examined) showed slight reticuloendothelial hyperplasia in the spleen; it was not noted in either of the helium-exposed animals.

E. DISCUSSION

The above presentation of the results was organized according to the parameters involved. Since the purpose of the experiment was to compare effects of different gases, we will now discuss the experimental results in terms of gases.

1. *Nitrogen*

The animals breathing the atmosphere containing nitrogen were essentially breathing air, and this run therefore may serve as a baseline for the experiment. The nitrogen run was not the first one to be done so the results should not reflect the learning process.

Although air is tested and proven as a suitable breathing medium, the situation in a closed system provides specific stresses not normally encountered; namely, thermal stress and exposure to contaminants.

Figure 3, showing body temperatures of the nitrogen groups, shows that the body temperature of the animals was raised slightly during the period of exposure in the chamber. The difference is less than half a degree between the exposed and control animals but it may reflect differences in environment. Relative humidity of the room containing the control animals and animals in the conditioning cage was not measured but was presumed to be 40–50%. The chamber relative humidity was higher (Table II), about 60%. The chamber temperature was higher than the control temperatures by about one degree C. These differences are in the proper direction to account for the observed differences in body and skin temperature, but we feel they are not a sufficient explanation.

It appears that subtle physiological factors affecting maintenance of body temperature may obscure the equally subtle effects on temperature caused by the surrounding gas. During the course of the entire experiment, a great deal of effort went into temperature monitoring, partly in the hope that metabolic differences in the gases might be revealed. Since the most prominent changes were seen in the nitrogen run, we are forced to conclude that body and skin temperatures are not the best possible indexes to use in evaluating the effects of inert gases. Some temperature monitoring is essential in chronic exposure experiments as an indicator of spontaneous infection, etc.

Other metabolic indexes measured on the nitrogen animals must be considered normal. Body weight showed a steady consistent gain throughout the experiment. Food, water and oxygen consumption were consistent with control values; heart rate suggested that animals were less active while in the chamber, as did the subjective observations of activity. Handbook values for rabbit oxygen consumption (Dittmer and Grebe, 1958) show a range of 10.5–14 ml/kg/min, and we recorded 11.5.

Hematological and biochemical data are all normal and internally consistent.

2. *Helium*

By far the most prominent changes were seen in rabbits exposed to helium. This was to be expected on the basis of previous experimentation (Cook *et al.*, 1951; Leon and Cook, 1960; Dianov, 1964; Konza, 1965; Wright *et al.*, 1965) but we found only one other report in which rabbits had been studied. Galvin *et al.* (1966) found a 13% increase in oxygen consumption of helium exposed rabbits compared to air controls under circumstances somewhat similar to ours. Our increase was 27%. This extra oxygen requirement is undoubtedly related to the increased heat loss in helium about which more will be said in the discussion, Part 4.

Almost all metabolic indexes were affected by the helium exposure. These animals gained weight steadily, perhaps even faster than their controls. Their heart rate and activity level were higher than the other groups and their food consumption was up.

In contrast, body temperature and skin temperature (Figure 5) both failed to deviate from the pre-exposure control values or those of the parallel controls. This stability of skin temperature was not expected in view of the increased helium heat loss (Fox *et al.*, 1966; Epperson *et al.*, 1966).

The surprising changes in red blood cell economics are summarized in Figure 11.

Hematocrit, hemoglobin, red cell count and red cell values are all down following helium exposure. The possibility of a compensating erythropoiesis is suggested by the trends towards an increased reticulocyte count and greater iron uptake values. A possible hypothesis would be that some sort of red cell destruction or loss took place and that in response new cells were being formed at an increased rate. Not consistent with this hypothesis is the fact that no change was seen in survival of injected donor red cells during the week-long exposure to helium. Other biochemical parameters were normal, indicating that major fluid shifts may not be involved, but such a possibility is not ruled out.

One factor that may be involved is brought out in a recent paper by Szkutnik (1965). He reports that summer rabbits exposed to cold showed a decrease in erythrocyte count and hemoglobin, along with increased food consumption, weight, and body temperature. If we assume our indoor rabbits to be 'summer' acclimated and that helium acts as a cold stress, the response might be the same. The mechanism for this response is probably part of a generalized adaptation to cold stress.

Despite the apparent consistency of this erythrocyte disturbance, the complete picture could have been caused by only a single confounding factor in the experiment, one completely unrelated to helium. And even if this response is normal, it might be
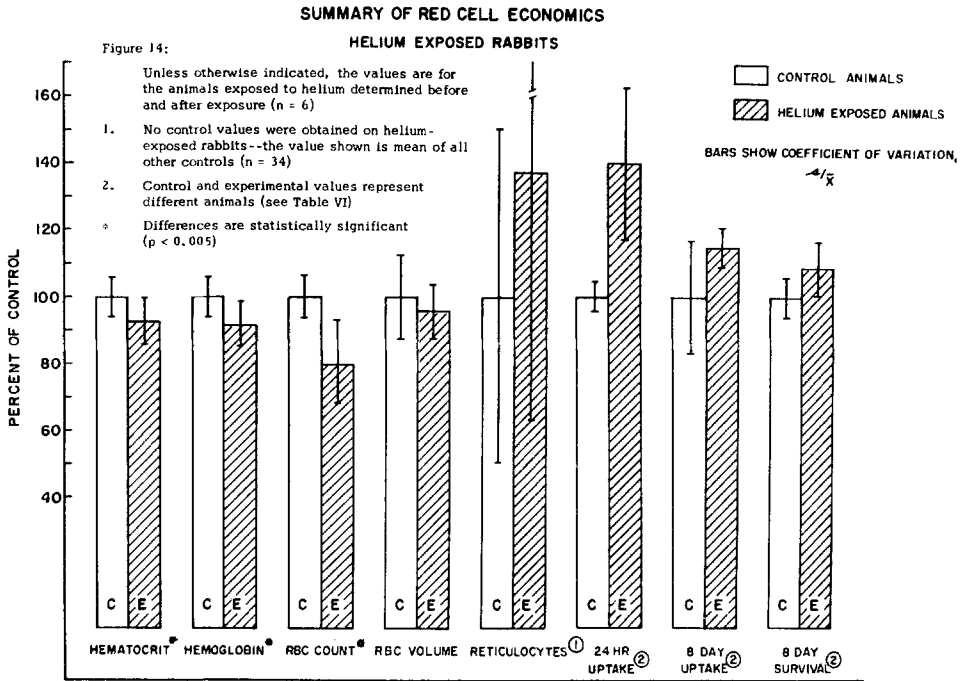
Fig. 11.

difficult to reproduce since many adaptation phenomena are intermittent and return to baseline after a time (Jordan et al., 1966a, b).

Some studies on humans exposed to helium have failed to show any effects on red cell dynamics (Hamilton et al., 1966a; Zeft et al., 1966). On the other hand Hock (1966) reports somewhat sketchy data from Sea Lab II personnel that indicate a definite drop in red cell count; this subjects were exposed to cold and had greatly increased appetites.

Contaminants might be present in any closed system and indeed have caused suspicions in the past (Helvey et al., 1965). Methane was allowed inadvertently to build up to several thousand parts per million during part of our helium exposure and although this value exceeds the ideal 'space maximum allowable concentration' (Auerbach and Russell, 1966), we have found no evidence whatsoever that suggests that methane has any toxic properties at all at these levels (Webb, 1964; Gerarde, 1963).

We therefore conclude that some sort of disturbance took place in the red cell economics of rabbits exposed to four-fifths of an atmosphere of helium for one week. Further experiments are called for to ascertain if this phenomenon is a real and predictable effect of exposure to helium, if it can assume a serious magnitude and what its mechanism might be.

3. Argon

Animals exposed to argon did not respond in a way significantly different from nitrogen animals but there were trends or indications that deserve to be mentioned. The argon

rabbits failed to gain weight during part of the week in the chamber, but not enough to merit statistical signfiicance; food consumption was down slightly during the first few days. Oxygen consumption was the same for the nitrogen animals and there were no remarkable temperature deviations. These animals exhibited normal activity and had a heart rate pattern similar to that of the nitrogen unit.

Red cell data are remarkably uniform and show no deviations from normal. A high post-exposure sodium value is shown in Table VI but the same sort of increase was seen in the controls; no other deviations are apparent.

On the basis of these rabbit exposures, we find no grounds for objecting to the use of argon as a diluent gas in a closed system. There are other objections to argon, however, the primary one being its unfavorable decompression characteristics (see Part II).

4. Neon

Neon is the favored space cabin diluent gas from a theoretical point of view (Roth, 1959, 1965; Welch and Robertson, 1965). Our experiments do not offer any distinct metabolic, physiological or pathological objections to its use.

Neon effects on rabbit metabolism are indistinguishable from those of nitrogen with the exception of a 7% increase in oxygen consumption, significant at the 0.01 level (Table II). However, there was no difference between the neon unit and the average of the post-exposure control values. The comparison that shows significance is between two different groups of animals and must be considered accordingly.

The only red cell measurement of note in the neon experiment is the low iron uptake in three rabbits following the exposure. None of the other values add confidence to this point, so it most likely represents an analytical variation. The high '8-day'uptake is due in part to the fact that in this experiment the actual uptake period was 9 days instead of 8. The 24-hour uptake in question was started correctly at the end of the exposure.

We have seen no references to any long duration experiments in which neon has been used as a major component of the atmosphere although it has been successfully breathed in diving chambers without ill effects (Bennett, 1966; Schreiner et al., 1966). Its possible advantages with respect to decompression are considered experimentally in Part II.

5. Oxygen

One purpose of the oxygen exposure was to establish a comparison between our inert gas experiments and previous space cabin experiments using pure oxygen at reduced pressures. Because we inadvertently used a metabolically different experimental animal (the female of the species), we cannot make strict comparisons of many of these data, in particular, the measurement of oxygen consumption.

We do not see in these experiments any evidence that suggests that nitrogen plays a specific metabolic or physico-chemical role apart from its action as a diluent for oxygen. It appears that a gas with thermal properties similar to nitrogen such as neon or argon can fill the role of diluent quite adequately without derangement of ordinary

homeostatic mechanisms. Our experiments do not offer any new evidence that a diluent gas for oxygen is really essential in the situation where the total pressure is kept low. No lung atelectasis was seen in animals autopsied three days after returning to the air environment.

Routine hematological values were not affected by the exposure (Table IV) although there was the indication that the animals exposed to pure oxygen had a lower iron incorporation as a result of the exposure (Table V). If the exposure to pure oxygen acted to suppress erythropoiesis, this type of response might be anticipated.

A number of human exposures to a similar atmosphere at greater PO_2 levels (Michel et al., 1960; Morgan et al., 1963; Zalusky et al., 1964; Kellett and Coburn, 1966) have failed to show significant changes in red cell economics, but red cell disturbances have been reported in astronauts exposed to a pure oxygen atmosphere ($P_IO_2 = 258$ mm Hg) in addition to their other stresses (Swisher and Fischer, 1966).

It does not seem likely that oxygen toxicity may have been the cause of our observations made on the oxygen animals. By assuming an R.Q. of 0.82 and a constant alveolar PCO_2 of 40 mm Hg, we have calculated that an alveolar oxygen tension of about 90 mm Hg should have prevailed during our pure oxygen experiment (Boothby et al., 1954). This is below the normal 105 mm Hg but probably not low enough to cause a significant hypoxic response. It certainly does not seem compatible with the notion of oxygen toxicity. Animals in the exposures containing inert gas should have had an average P_aO_2 of 118 mm Hg.

The rabbits showed indications of a thermal stress during the 180 mm Hg pure oxygen exposure. They were quite lethargic and inactive, had an increased skin temperature, a reduced appetite, low potassium and consumed less water. Much of the time their fur appeared to be damp. This environment provided principally because of the reduced density, a 50% reduction in convective heat loss and it is possible that this could have been responsible for the symptoms observed.