BIOLOGICAL EVALUATION OF VARIOUS
SPACECRAFT CABIN ATMOSPHERES, II*

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3. Exposure of Rats at a Constant Oxygen Tension – But at Various Pressures – To Atmospheres Containing Nitrogen, Helium, Argon, Neon or no Inert Gas; Effects of Decompression to 100 mm Hg

A. INTRODUCTION

Experiments described in Part I of this report were concerned with a broad surveillance of metabolic, biochemical and pathological factors which might be affected by exposure to each of several synthetic atmospheres. Rabbits were used in those experiments. In this section, we present similar experiments with rats. Here our primary purpose was to compare nitrogen, helium, argon and neon with respect to altitude decompression and as a secondary task to provide an interspecies comparison of some of the metabolic effects of these gases.

B. PROCEDURES AND METHODS

The experimental arrangement of our rat studies was similar to that described in Part I. Animals were exposed in the same chamber, which is described in Part I. Eighteen rats per experiment were conditioned for one week, exposed to air in the chamber for one day, and to the experimental atmosphere for 6 days. Six of the rats in each unit were removed after 3 days of exposure to the experimental atmosphere and decompressed in order to test the effect of the specific inert gas on their susceptibility to decompression sickness.

During the exposure, we monitored several metabolic indexes such as oxygen consumption and body temperature.

1. *Experimental Plan*

The experimental atmospheres used in this series of experiments were the same ones as used in Part I, plus some others. As before, oxygen tension was constant in all exposures at 180 mm Hg. At one atmosphere total pressure rats were exposed to environments containing nitrogen (i.e., essentially air), helium, argon and neon. At an arbitrarily chosen 473 mm Hg total pressure, the inert diluent gases employed were nitrogen, helium and neon; a pure oxygen exposure was made at a total pressure of 200 mm Hg. A summary of the chamber parameters pertinent to the experiment is given in Table VIII. Values in this table are weighted means, calculated in order to

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TABLE VIII

Chamber gas compositions
(rats)

Inert gas	Total pressure mm Hg	PO₂ mm Hg	PCO₂ mm Hg	PN₂ mm Hg	PCH₂ mm Hg	Temperature °C ± 1	Relative humidity %
N₂	755 ± 5	177 ± 4	~ 1	–	<1	24.5	55–60
	473 ± 3	178 ± 2	~ 1.5	–	<1	26	~ 40
He	755 ± 5	176 ± 4	~ 1	~ 1	0.7	26	~ 50
	474 ± 3	176 ± 4	~ 1	~ 2	<1	27	~ 45
Ar	758 ± 3	177 ± 3	~ 1	~ 1.3	<1	26	55–60
Ne	757 ± 3	178 ± 4	~ 1	~ 0.8	<0.5	25	50–55
	473 ± 5	178 ± 3	~ 1	~ 0.7	<1	25.5	~ 50
(O₂)	192 ± 1	180 ± 5	~ 0.7	0.4	<0.5	26	~ 40

Time-integrated mean values and approximate range of daily variation are shown.

present the value that best represents the experimental conditions from the point of view of their biological effectiveness. The ranges given are the extremes of daily variation, excluding momentary deviations or those intentionally caused as part of the experiment.

A calendar showing the plan of the experiments is given in Figure 12. During the conditioning period, the animals had time to adjust to the cage configuration and drinking nipples; weights, temperature, food and water consumption, and activity were monitored. On day 8, the animals were placed into the chamber with air as the atmosphere and an initial measurement of oxygen consumption was made on the entire unit. This was repeated twice the following morning (still on air), once before and once after a determination of CO_2 consumption.

The chamber was then opened, temperatures and weights were taken and the animals were introduced into the experimental atmosphere. An oxygen consumption determination was performed after an hour of equilibration. The next morning (day 10) and on day 12 and 15, 2 one-hour oxygen comsumption determinations were performed, separated by a half-hour measurement of CO_2 consumption.

Chamber pressure was adjusted, if necessary, on day 12 to laboratory ambient
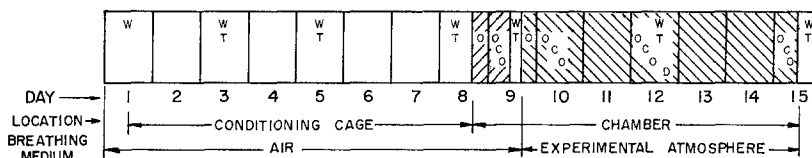
RAT EXPERIMENTAL CALENDAR

Fig. 12.   Procedures: Daily: Food consumption (per compartment of 3 animals); water consumption (per unit of 18 animals); Activity of animals; environmental temperatures.
Periodic: symbols: W – Body weights (all animals); T – rectal and skin temperatures (6 animals from each unit); O – oxygen consumption (per unit of 18 animals); C – carbon dioxide production (per unit of 18 animals) D – decompression (6 animals from each unit, sea level runs only).

pressure by the addition of inert gas while weight, etc., measurements were made. The chamber was then returned to the specified condition, usually in less than $\frac{1}{2}$ hour. At the end of the exposure temperatures and then weights were obtained, as soon as possible after opening the chamber.

Male Sprague-Dawley rats were used. To increase susceptibility to decompression, we ordered the largest rats available. These were classified as retired breeders and ranged in weight from 400 to over 600 g.

2. Methods

Physiological variables were measured on the rats by straightforward methods similar to those used in Part I, except as noted here.

a. *Body Temperature.* Matched thermistor probes, the same ones as mentioned in Part I, were used inside and outside the chamber for measuring rectal temperature. We were careful to insert the probe at least 6 cm (Lomax, 1966) and the probe was left inside for the 2 min empirically determined to be sufficient for a stable reading. Temperature readings were made on 6 rats from each experimental unit. They were chosen at random except that temperatures were not determined on animals to be decompressed.

b. *Skin Temperature.* We felt that the surface probe used for measuring skin temperatures on rabbits was too large and difficult to use for rats. We therefore obtained a surface-mounted thermistor probe (YSI No. 520). This probe has a thermistor bead exposed at the end of a 1 mm diam teflon tube. It can measure surface temperatures without much disturbance, and because of the insulating properties of teflon has very little stem effect. We measured skin temperatures at several locations on a group of rats in order to select a suitable measurement site. We found the base of the tail near the hairline to be the location that offered the best combination of reproducibility and sensitivity, and all reported measurements were made at that site. Measurements were made by touching the thermistor firmly to the skin and recording the stable reading (10–20 sec). The measurement was repeated, if necessary, until two consecutive readings agreed to within 0.1 °C. This particular type of thermometer required calibration, which was done daily.

c. *Environmental Temperature.* The temperature that was most representative of that to which the rats were exposed was recorded several times daily. This was read on a nearby wall-mounted thermometer or on the chamber recording thermometer, depending on where the rats were at the time.

d. *Oxygen Consumption.* The method was the same as that used in the rabbit experiments. Over a one-hour period the temperature, pressure and PO_2 in the chamber were maintained at as constant level as practicable and the amount of oxygen added to maintain the constant PO_2 was recorded. Appropriate corrections were made to compensate for small deviations in the 'constant' parameters. This value of oxygen used by the entire unit during that hour was converted to ml per minute per kilogram of rat. Ordinarily, 2 one-hour determinations were made, separated by sufficient time to perform a CO_2 production measurement.

e. *Carbon Dioxide Production.* For this measurement the sodium hydroxide scrubber and Purafil odor absorber were taken off line and CO_2 was allowed to build up in the chamber for half an hour. Duplicate samples for gas analysis were taken 5 min after the beginning and 20 min later. Gas chromatographic analyses of the CO_2 content of chamber effluent gas were used, along with the system volume, to calculate the amount of CO_2 produced during the time period. This small buildup of inspired CO_2 usually reached 7 mm Hg and never exceeded 11 mm Hg and therefore was judged not to be of any significance as a toxic contaminant. The accumulated CO_2 was of course quickly removed as soon as the scrubbers were switched back into the circuit. The Purafil had to be taken off line, as our experience in the rabbit experiments showed, because otherwise it would adsorb enough CO_2 to invalidate the determination.

f. *Food and Water Consumption.* These animals were fed large pellets of standard rat diet (Rockland Mouse/Rat Diet) from overhead feeders. These could be removed and weighed and thus provided a fair estimate of the actual amount of food eaten by the rats. Weighing was done usually at the time temperatures were taken and daily consumption was calculated from these figures. Far less food was lost than in the rabbit experiments.

Water usage was monitored by recording the daily depletion of the reservoir at the time of refilling. Fewer leaks were noticed than in the rabbit experiments, possibly due to the smaller size and better seal of the rat nipples.

g. *Relative Activity.* Two indexes were used in estimating activity of the rats. The animals were checked several times a day, and subjective estimates of the general temper of the entire unit were recorded if there were any unusual observations. Any special ease or difficulty in handling was noted. In addition, during the oxygen consumption determinations, the number of animals moving around the cages, preening, etc., was noted several times during the run and a representative activity value was recorded.

3. *Decompression*

The method used for studying the susceptibility of our experimental animals to altitude decompression was adapted from that of Philp and Gowdey (1962, 1964). These authors decompressed rats from hyperbaric pressures to a simulated altitude of 10000 ft and were able to induce 'bends' in rats and score the decompression symptoms observed in a reasonably reproduceable manner. This method has been used successfully in our laboratory (Schreiner *et al.*, 1966), but again in the case of decompression from high pressures. In adapting this technique to altitude decompression we tried several modifications of the procedure without notable success. Use of large female animals, guinea pigs and a faster treadmill all failed to enhance the bends incidence in animals equilibrated with air at sea level pressure. The reasonably successful procedure we selected is described here.   ·

Schreiner *et al.* (1966) found that female rates were more susceptible to decompression sickness than males and that they had more fat than males of the same size. We found, however, that the largest females we could obtain were about 400 g and that

it was quite easy to obtain males as large as 600 g. Our animals were all in the weight range of 548 to 620 g. All were quite fat.

Our apparatus is the same as that of Philp and Gowdey (1964). A compartmented 'squirrel cage' treadmill forces the rats to walk at 1.8 meters per minute. This is not 'exercise' as such but serves merely to elucidate leg paralysis. The treadmill is a motor driven cylinder 38 cm in diameter which rotates at 1.5 rpm. It fits into a plexiglass vacuum chamber for decompression.

Decompression was carried out on day 12, which means that the animals had been breathing the experimental atmosphere for 3 full days and were completely saturated with the inert gas in question. Only rats exposed at a full atmosphere of pressure were decompressed. None had been previously decompressed. The rats were decompressed 2 at a time. The decompression chamber was purged with oxygen to at least 95%, and the end covered with a polyethylene flap. A flow of oxygen was maintained to minimize entry of nitrogen. The lock on the main animal chamber was purged with chamber gas and 2 animals were 'locked out'. They were quickly placed into the treadmill via the polyethylene flap, the cover was replaced and decompression commenced. The chamber was decompressed to 100 mm Hg (47000 ft) within $2\frac{1}{2}$ to 3 min. A faster rate was avoided in order to prevent complications due to trapped gases. Timing began when animals were removed from the chamber atmosphere.

The treadmill was started* and the animals were observed for 20 min, longer if decompression symptoms were present. Most rats showed symptoms normally attributed to hypoxia and most recovered from these within a few minutes. Usually the rats became tired and sluggish after 20 min whether or not they showed symptoms of decompression sickness but unless they had such symptoms, recovery was prompt on return to sea level.

The symptoms used to assess decompression sickness were graded according to an arbitrary scale:

  0 – No symptoms;
  1 – abnormal actions – scratching, sneezing, etc.;
  2 – mild disturbance in walking;
  3 – paralysis of hind legs;
  4 – hind leg paralysis and partial paralysis of forelegs;
  5 – total paralysis – animal slides or tumbles;
  6 – Death.

The animals were scored according to the highest grade attained during the first 20 min of observation. The usual procedure was for one observer to write a word description of the symptoms and for another to assign the score. The criteria of decompression sickness used are not completely objective nor was this a blind study. Our experience has shown us, however, that unexplained individual variations between

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* The induction motor and gear case are both lubricated with silicone lubricant because of the danger of fire in the 95 % oxygen environment.

animals in susceptibility to decompression sickness are so great that our criteria can be accepted as a reasonable yardstick of individual responses of the experimental animals to decompression.

A preliminary run in which rats were exposed to the nitrogen-containing atmosphere was incomplete for other reasons but the decompression was performed in the prescribed manner and the data on these six rats are included with the others.

C. RESULTS

1. *Summary*

Of the several metabolic indexes measured before and during the exposure of rats to synthetic gaseous environments at sea level and at altitude, body and skin temperature, oxygen consumption, carbon dioxide production and the rate of food consumption showed demonstrable changes.

A slight upward trend of rectal temperature was noted in the nitrogen and to a lesser extent in the argon runs at sea level. In the 3 altitude runs where it was determined (helium, neon, and oxygen) skin temperature apparently rose during the exposure period.

Oxygen consumption was noticeably increased in both the nitrogen and helium sea level runs relative to pre-exposure determinations made in air. A decrease in food consumption was seen in the animals of the oxygen unit during their altitude exposure, while helium seemed to cause an increase in appetite both at sea level and at altitude.

Decompression of rats equilibrated at sea level pressure with the several inert gases of this study showed remarkable differences in the degree of susceptibility to decompression sickness of these animals.

Animals equilibrated with either helium or neon were successfully decompressed from sea level to 100 mm Hg for 20 min and except for one animal of the neon group they remained free of serious symptoms. A significant fraction of nitrogen-exposed animals and most of those equilibrated with argon suffered severe decompression sickness or death.

2. *Metabolism*

This section includes measurements of the following parameters:

            a. Body weight;
            b. Body, skin and environmental temperature;
            c. Oxygen consumption and carbon dioxide production;
            d. Food and water consumption;
            e. Relative activity;

a. *Body Weight*. Because mature rats were used in these experiments the body weight of the animals was stable and, therefore, was not a sensitive index of the general condition and response of the animals to stress. Data summarized in Figure 13 show that this was indeed the case. The mean body weights of all groups of rats remained re-
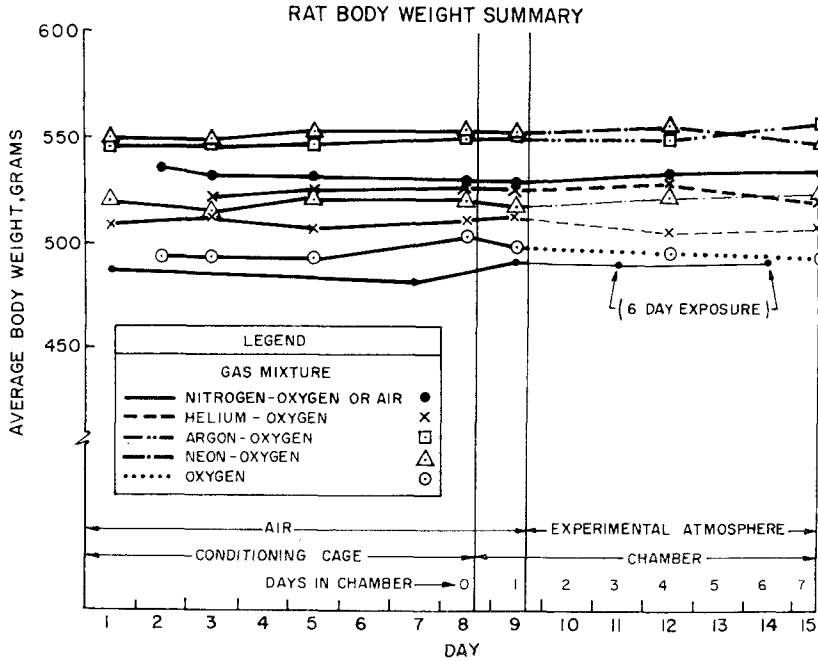
Fig. 13.   Mean body weights of all rats in each of 3 experiments. Heavy lines show animals at sea level pressure, light lines and symbols signify reduced pressure. Broken lines signify period of exposure.

markably constant throughout the period of observation. Changes in mean body weight observed during the experimental exposure were no greater than those seen during the pre-exposure period.

b. *Body, Skin and Environmental Temperature.* Results of temperature measurements on animals exposed to experimental atmospheres at sea level pressure are summarized in Figure 14, and Figure 15 summarizes results of temperature measurements on animals exposed at reduced pressures. Mean values of rectal and skin temperatures are given for 6 animals of each experimental population. The environmental temperatures given are those that apply to each unit of animals regardless of their physical location; they were determined several times daily but only average values are plotted. During the course of the experiment the delicate thermistor used for skin temperature measurements was damaged and no skin temperatures were recorded during exposure to argon-oxygen at sea level pressure; likewise, this probe was unavailable before and during the low pressure exposure to nitrogen-oxygen.

Concentrating first on the rectal temperatures, we find a remarkable uniformity in the animals exposed to the low pressure environments, and among the sea level units we see a weak tendency for the argon and nitrogen animals toward an increased temperature through the experimental period. Individual data show no wide deviations. A typical range is from 37.0 °C to 38.5 °C for all animals in a group throughout the entire period.

Skin temperatures are understandably more variable than core temperatures. The
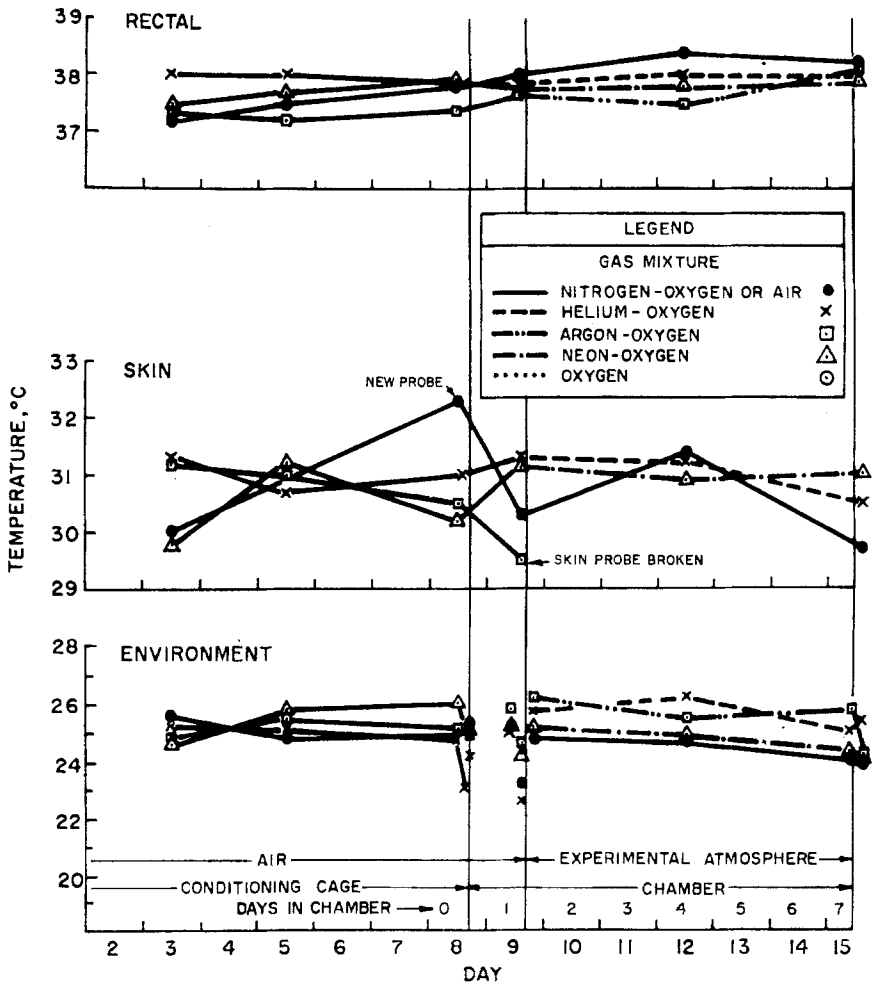
RAT TEMPERATURE SUMMARY
SEA LEVEL EXPOSURES

Fig. 14. Body, skin and environmental temperatures applicable to exposures made at sea level pressure. Environmental temperatures shown are the ones applicable to the location of the animals at the time. Body and skin temperature values represent means of determinations made on 6 animals selected for this purpose.

ranges of values for an individual animal or for any group at a particular time are considerable. Individual data show, for example, that a single set of 6 values (sea level nitrogen unit, day 3) producing an average of 30.0 °C had a range of 28.7 to 32.5 °C, while an individual animal did not usually show this much variation throughout the experiment.

Skin temperature showed changes as the ambient temperature changed during chamber manipulations on days 8 and 9. Likewise, there were occasional problems
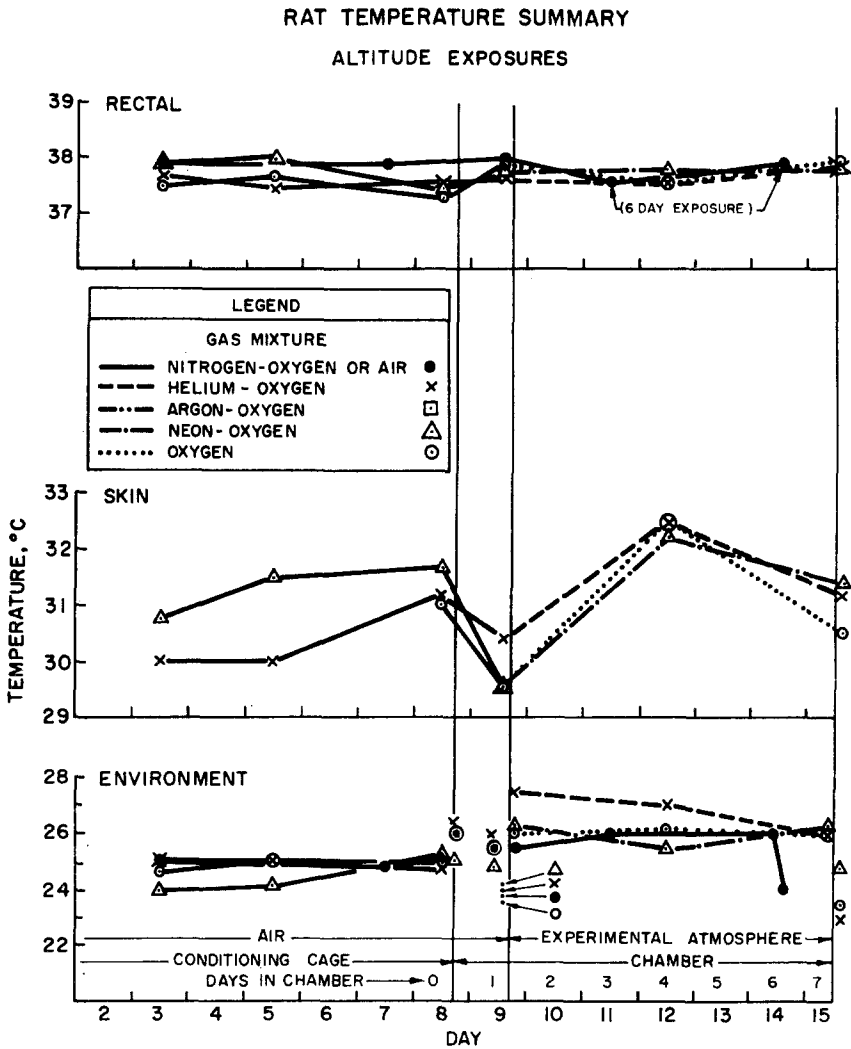
Fig. 15. Body, skin and environmental temperatures applicable to exposures made at reduced pressure (about 480 mm Hg). Environmental temperatures shown are the ones applicable to the location of the animals at the time. Body and skin temperature values represent means of determinations made on 6 animals selected for this purpose.

with the skin probes and because of this the wide excursions seen, particularly in the sea level nitrogen run, are in question.

One consistent observation is the rise in skin temperature during the altitude runs (Figure 15). All skin temperatures rose on this occasion, showing a uniformity not seen under normal conditions. It must be kept in mind that the day 12 readings taken on the altitude animals involved a temporary sojourn to sea level, and it should also be mentioned that the immediate postexposure measurements probably were not taken soon enough to reflect very accurately the conditions at altitude.

TABLE IX

Summary of rat oxygen consumption and carbon dioxide production: Sea level

Inert gas	Day	Oxygen consumption ml/min/kg	Carbon dioxide production ml/min/kg	R.Q.	Activity (average number of animals moving)
Nitrogen	8 (Air)	13.7			3.2
	9 (Air)	15.7			4.5
	9 (Air)		15.0	1.04	
	9 (Air)	13.3			3.2
	9	15.2			3.7
	10	16.9			4.2
	10		14.4	0.88	
	10	15.8			3.8
	12	18.7			5.5
	12		13.8	0.74	
	12	18.9			5.8
	15[a]	19.8			2.3
	15[a]		15.3	0.79	
	15[a]	18.7			6.2
	Mean[b]	18.1 ± 1.5	14.5 ± 0.7	0.80 ± 0.07	
Helium	8 (Air)	14.6			2.4
	9 (Air)	16.7			6.4
	9 (Air)		16.9	0.99	
	9 (Air)	17.2			5.1
	9	14.9			3.7
	10	16.9			4.1
	10		13.5	0.74	
	10	19.7			4.5
	12	21.1			5.3
	12		14.6	0.75	
	12	18.1			3.2
	15[a]	20.7			2.8
	15[a]		13.8	0.69	
	15[a]	16.5			2.3
	Mean[b]	18.8 ± 2.0	14.0 ± 0.6	0.73 ± 0.03	
Neon	8 (Air)	17.0			3.2
	9 (Air)	18.0			4.7
	9 (Air)		15.7	0.88	
	9 (Air)	17.2			5.5
	9	14.4			3.2
	10	18.3			6.0
	10		14.0	0.81	
	10	16.4			3.7
	12	17.0			3.8
	12		17.7	1.13	
	12	14.1			4.0
	15[a]	18.4			3.0
	15[a]		11.5	0.62	
	15[a]	18.5			3.2
	Mean[b]	17.1 ± 1.7	14.0 ± 3.0	0.9 ± 0.4	

Table IX (continued)

Inert gas	Day	Oxygen consumption ml/min/kg	Carbon dioxide production ml/min/kg	R.Q.	Activity (average number of Animals moving
Argon	8 (Air)	19.0			5.8
	9 (Air)	15.3			2.0
	9 (Air)		14.0	0.95	
	9 (Air)	1.41			1.6
	9	13.9			1.7
	10	16.4			3.4
	10		14.0	0.95	
	10	13.1			2.1
	12	20.8			5.1
	12		13.5	0.78	
	12	13.8			2.2
	15[a]	18.6			2.5
	15[a]		–	–	
	15[a]	18.1			4.0
	Mean[b]	17.0 ± 3.0	13.8	0.86	
Air Mean (all 8 groups)		17.1 ± 1.9	15.3 ± 1.2	0.90 ± 0.09	

[a] Group of 12 animals (6 animals were removed on day 12 for decompression study), all other units consisted of 18 animals.
[b] Mean reflects 6 measurements taken on days 10, 12, and 15; ± 1 std. dev.
R.Q. is respiratory quotient, the ratio of CO_2 production to O_2 consumption.

c. *Oxygen Consumption and Carbon Dioxide Production.* The results of measurements of oxygen consumption and carbon dioxide production in both the sea level and altitude experiments are presented in Tables IX and X and in Figures 16 and 17. These measurements were taken with each unit of animals residing in the chamber breathing the experimental gaseous environment, except for the initial measurements that were carried out under the same conditions but with the animals breathing air.

Graphs showing the average daily oxygen consumption during each chamber exposure are presented in Figure 16. The pooled control (sea level air, pre-exposure) values that apply to each unit of animals are shown on the left, as well as a point that represents all control valves from all groups. The points on the far right are averages covering the experimental exposure.

We felt that the first measurement of oxygen consumption in an experimental gaseous environment reflected an unsteady physiological state of the animals and, therefore, we did not use these values when we determined the chamber averages. Range markers represent plus and minus one standard deviation.

Results of the carbon dioxide production measurements are given in Figure 17, and the manner of presentation is the same. Here, however, only one pre-exposure air control was determined and only the pooled control points are shown.

TABLE X

Summary of rat oxygen consumption and carbon dioxide consumption: Altitude

Inert gas	Day	Oxygen consumption ml/min/kg	Carbon dioxide production ml/min/kg	R.Q.	Activity (average number of animals moving)
Nitrogen	8 (Air)	17.9			9
	9 (Air)	18.8			9
	9 (Air)		–	–	
	9 (Air)	16.8			–
	9	16.0			–
	10	10.1			~ 7
	10		14.5	0.79	
	10	17.5			–
	11	17.1			4.5
	11		14.5	0.77	
	11	20.5			–
	14	18.3			3.0
	14		15.4	0.81	
	14	19.6			–
	Mean[a]	18.7 ± 1.3	14.8 ± 0.5	0.79 ± 0.02	
Helium	8 (Air)	20.0			7
	9 (Air)	19.9			9
	9 (Air)		16.2	0.85	
	9 (Air)	18.7			–
	9	13.5			–
	10	17.6			7.5
	10		15.9	0.89	
	10	18.2			–
	12	18.2			5
	12		–	–	
	12	17.0			2.9
	15	18.5			4
	15		14.0	0.76	
	15	18.2			2.5
	Mean[b]	17.9 ± 0.6	14.9	0.82	
Neon	8 (Air)	15.6			4.1
	9 (Air)	20.3			4.7
	9 (Air)		15.3	0.82	
	9 (Air)	17.0			4.3
	9	15.6			4.2
	10	17.4			4.7
	10		13.3	0.75	
	10	18.2			4.8
	12	18.0			3.7
	12		13.7	0.80	
	12	16.4			6.5
	15	16.8			3.2
	15		12.4	0.70	
	15	18.7			5.1
	Mean[b]	17.6 ± 0.9	13.1 ± 0.7	0.75 ± 0.05	

Tabel X (continued)

Inert gas	Day	Oxygen consumption ml/min/kg	Carbon dioxide production ml/min/kg	R.Q.	Activity (average number of animals moving)
(Oxygen)	8 (Air)	16.9			∼ 4
	9 (Air)	18.2			∼ 4
	9 (Air)		13.8	0.80	
	9 (Air)	16.6			∼ 4
	9	14.4			–
	10	15.6			3.0
	10		14.2	0.94	
	10	14.6			–
	12	16.9			2.5
	12		13.8	0.89	
	12	13.9			–
	15	16.8			2.5
	15		12.9	0.78	
	15	16.0			–
	Mean[b]	15.6 ± 1.2	13.6 ± 0.7	0.87 ± 0.08	
Air Mean (all 8 groups)		17.1 ± 1.9	15.3 ± 1.2	0.90 ± 0.09	

[a] Mean reflects 6 measurements taken on days 10, 11 and 14; ± 1 std. dev.
[b] Mean reflects 6 measurements taken on days 10, 12 and 15; ± 1 std. dev.
R.O. is respiratory quotient, the ratio of CO_2 production to oxygen consumption.

The values of all individual determinations of oxygen consumption and carbon dioxide production are given in Tables IX and X, as well as the calculated respiratory quotients and estimates of animal activity at the time of the oxygen consumption measurements.

A statistical analysis was attempted in order to verify any apparent differences that may exist in these data. All experimental values were compared with the control values for the same animals breathing air before the exposure. The group exposed to nitrogen at sea level showed a significant increase ($p < 0.01$) but the altitude run with helium resulted in an oxygen consumption lower than that of the same animals in air. All test determinations were compared with the pooled control, and here only the sea level helium values appeared to be increased to a significant amount ($p < 0.05$) The apparent decrease in oxygen consumption of the oxygen-exposed rats did not prove to be a real one, statistically speaking ($p < 0.1$).

Because of the small number of determinations made and the lack of obvious trends in the data attributable to the gaseous environment it was not worthwhile to subject the carbon dioxide production data to statistical evaluation.

Although there were considerable variations in the calculated respiratory quotient, no patterns emerge that can be correlated with the gaseous environment. It must be
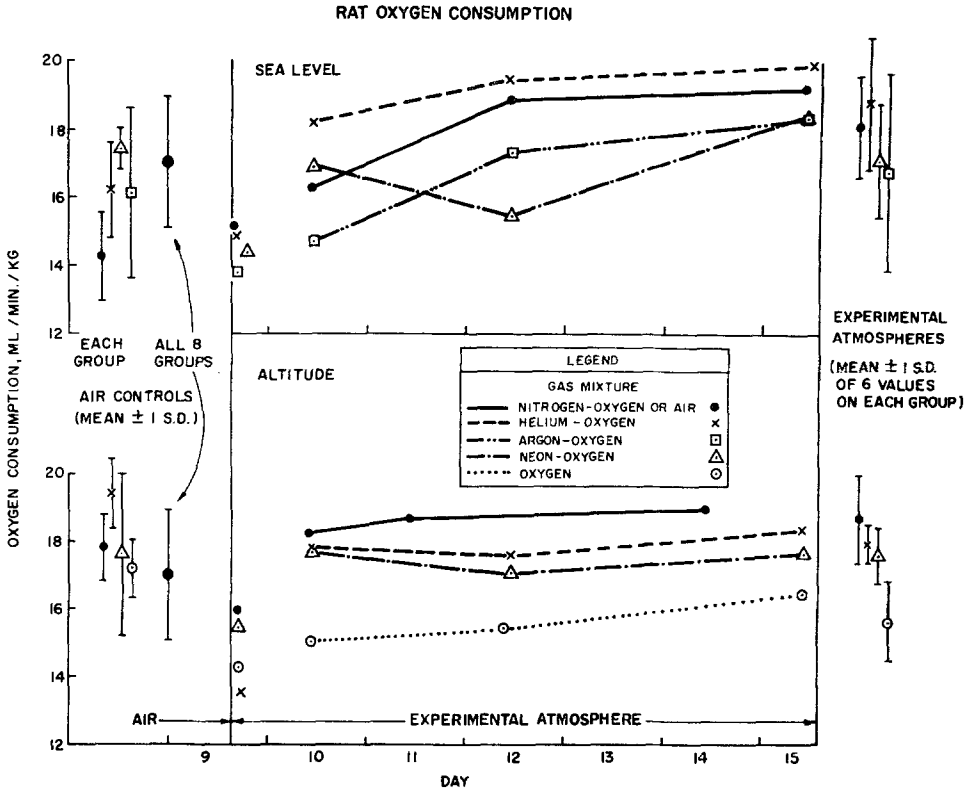
RAT OXYGEN CONSUMPTION

Fig. 16.   Oxygen consumption of all rats in each unit during sea level exposure (top) and at reduced pressure (bottom). In each case the four points at far left are pre-exposure averages (± 1 std. dev.) determined in air. The next point is an average of all pre-exposure control values, including those of both altitude and sea level runs. Points at far right are means of values determined in chamber but excluding the first point, which we felt did not represent a steady state.

remembered that the R.Q. values were only estimates, since oxygen consumption and CO_2 production were determined at different times.

d. *Food and Water Consumption.* Figure 18 presents the mean food (top) and water consumption (bottom) of all rats in each of eight experiments. The rate of food consumption was remarkably uniform during the experimental exposure when compared to the fluctuations seen during the pre-exposure period. Although the nature of our data precludes statistical corroboration it is evident that the rate of food consumption of the oxygen unit decreased with time, while that of both the sea level and the altitude helium units showed a noticeable increase with time.

No obvious trends in the water consumption rates as a function of the nature of the gaseous environment can be discerned from our data.

e. *Relative Activity.* Because of the relative roominess of the cages with only 3 rats in each, the animals were more active than the rabbits and the level of activity was easier to determine. Since the activity of the animals had a drastic effect on the oxygen
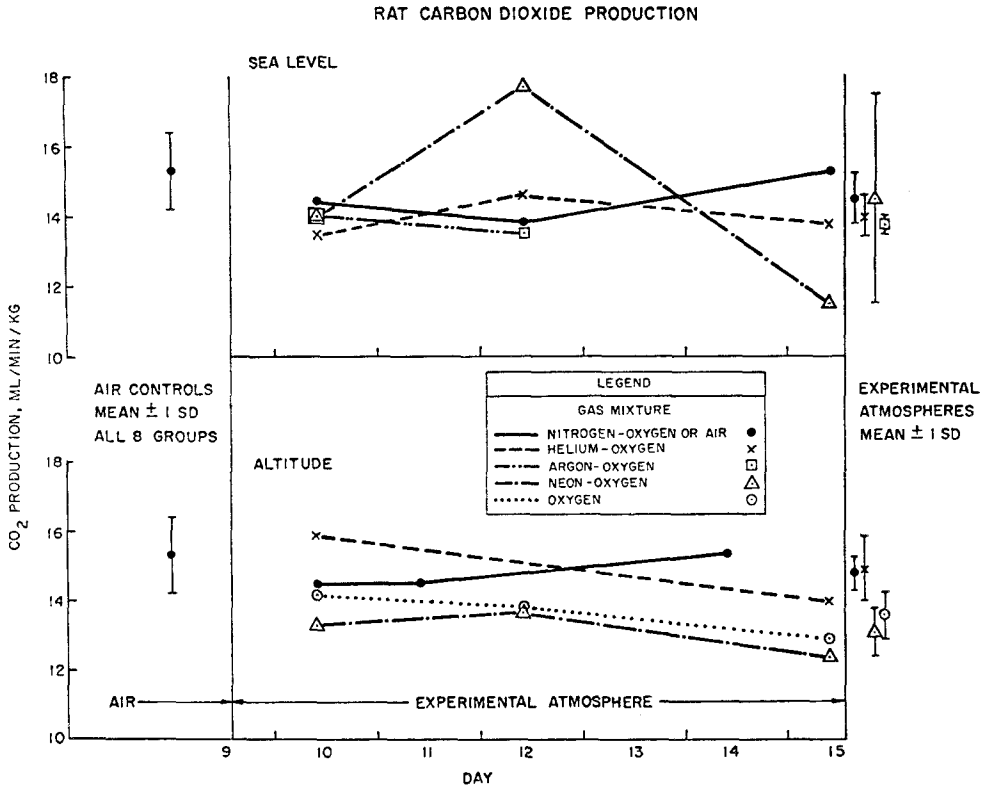
RAT CARBON DIOXIDE PRODUCTION

Fig. 17. Carbon dioxide production of all rats in each unit during sea level exposure (top) and at reduced pressure (bottom). In each case the point at far left represents the mean of a single pre-exposure determination (± 1 std. dev.) on all 8 units in air. Points at far right are means of values determined in chamber.

consumption determinations, we have recorded the average number of animals moving around along with the oxygen consumption data in Table IX and X.

Subjective observations in addition to those listed in these tables revealed initially that there were differences between the different groups of rats during normal conditions; this was considered in evaluating the effects of the test atmospheres.

The most distinctive change in activity was in the low pressure pure oxygen exposure. These animals were indistinguishable from normal for the first day of exposure, but subsequently appeared less active than usual and became somewhat lethargic. However, they were not nearly as lethargic as rabbits during the sea level exposures in the chamber.

Animals in the low-pressure exposure to helium-oxygen were definitely more active than usual, and those exposed to helium at one atmosphere were slightly more active there than in air. None of the groups exposed to nitrogen, argon or neon showed any noteworthy deviations in activity.
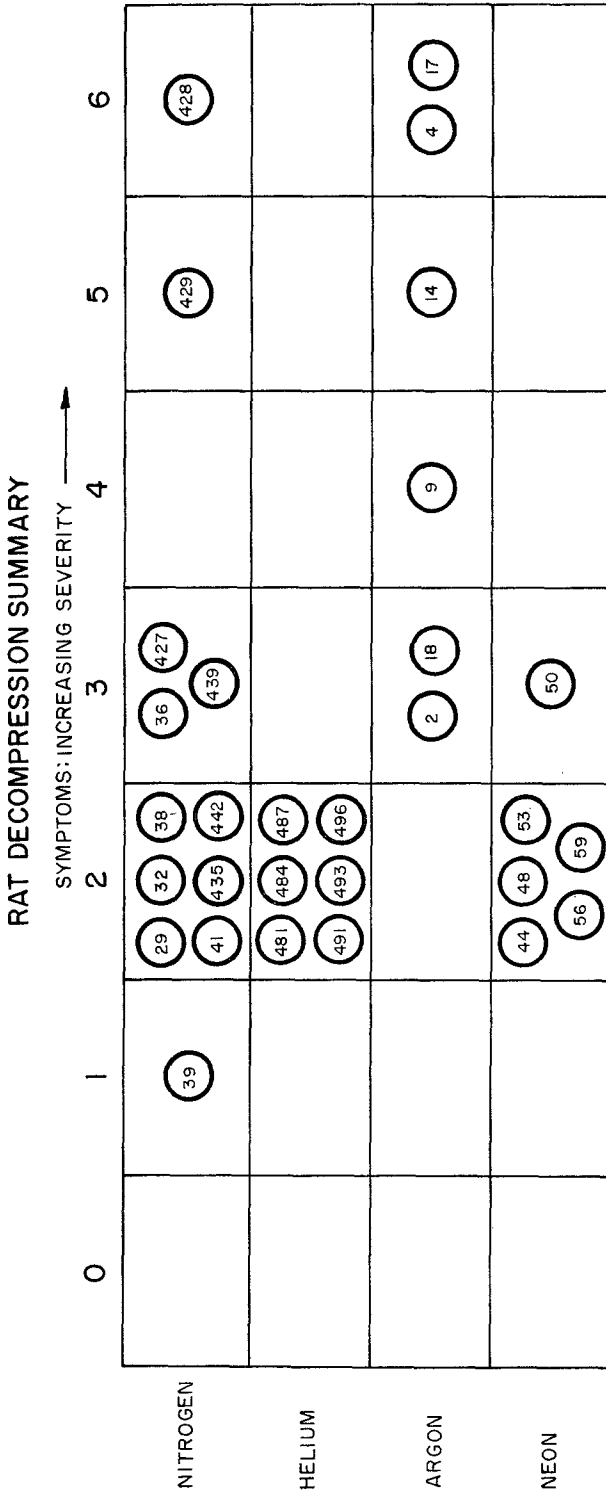
RAT FOOD AND WATER CONSUMPTION

Fig. 18.   Mean food (top) and water consumption (bottom) of all rats in each of eight experiments. Heavy lines show animals at sea level pressure, light lines and symbols signify reduced pressure. Broken lines signify period of exposure. Food consumption was determined from changes in weights of feeders in each compartment containing three rats. Water consumption obtained by measuring daily depletion of reservoir.

3. Decompression

A summary of the results of decompressing 30 rats is given in Figure 19. The small circled figures are the individual rat ear tag numbers; the location of each figure shows the maximum severity of symptoms experienced by that individual. Table XI lists the weight of these animals.

Two features stand out in Figure 19 – the distinct lack of completely symptom-free animals and the clear separation of effects of different gases.

The fact that all animals (save one) were scored at least 2 in symptom severity is most likely due to the relative hypoxia caused by decompression to 100 mm Hg total pressure. That this is the case was demonstrated by exposing animals on air to a comparable level of alveolar oxygen tension – all such rats showed grade 2 symptoms, but none showed symptoms characteristic of decompression, grade 3 and beyond.

Fig. 19. Sympton scale: 0 – no symptoms; 1 – abnormal actions – scratching, sneezing, etc.; 2 – mild disturbance in warling; 3 – paralysis of hind legs; 4 – hind leg paralysis and partial paralysis of fore legs; 5 – total paralysis – animal slides or tumbles; 6 – death.

TABLE XI

Weights of rats decompressed to 100 mm Hg

Nitrogen		Helium		Argon		Neon	
Ear tag	Weight gm	Ear tag	Weight gm	Ear tag	Weight gm	Ear tag	Weight gm
29	574	481	590	2	572	44	576
33	598	484	566	4	548	48	598
36	556	487	556	9	548	50	584
38	596	491	620	14	566	53	550
39	590	493	570	17	610	56	556
41	572	496	592	18	600	59	562
427	576						
428	598						
429	560						
435	580						
439	564						
442	554						

If it can be assumed that increasing severity of symptoms as classified here indicates increasing risk of altitude decompression sickness as a function of the inert gas, we can list the gases in the order of increasing risk:

$$He, Ne < N_2 < Ar$$
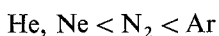
All animals that showed serious symptoms (grade 3 or greater) and about half of those that did not were given a postmortem examination. The 3 animals that died in the chamber contained a very large number of bubbles, while none of the others showed any bubbles at all. This is probably related to the fact that circulation was stopped in the animals that died while they were still at altitude, but continued in those that had lesser symptoms until they were killed by anesthesia several minutes later.

A few animals had atelectatic lung lobes or other evidence of pneumonia, but this did not appear to be related to bends susceptibility. Such pathology might, however, increase the degree of hypoxia. Most of the animals has large fat depots but again this could not be related to either the gas or the decompression symptoms.

D. DISCUSSION

The series of experimental exposures covered in Part II complements Part I by considering a different species, exposed to a wider range of synthetic atmospheres, but studied in less detail. Part II also included a separate type of study in the comparison of the inert gases with respect to their effect on altitude decompression.

With the exception of decompression all the parameters involved in the rat experiments may be classed under the general heading of metabolism.

1. *Metabolic Factors*

As was discussed in Part I, since body temperature is normally controlled rather precisely despite a wide variety of stresses it should not be expected to be a reliable

indicator of subtle differences in the environment. This was certainly true of all but two groups of rats, those exposed to nitrogen and argon at sea level. Without statistical confirmation we interpret Figure 14 as showing a rise in rectal temperature throughout the nitrogen run. This gradual rise began before the exposure and continued to day 12 before receding. The reason for this is not clear, but it seems to be a sufficiently definite trend to offer a possible explanation for the increased levels of oxygen consumption seen in this group. The rise during argon exposure is not distinctive.

A possibly interesting trend is seen in skin temperatures of animals exposed to low pressure. Only one measurement, on day 12, was made while animals were in the test atmosphere, and this one was made after the animals had been temporarily recompressed to sea level. The final reading, though made within half an hour after opening the chamber, most likely is not a valid representation of the test conditions. The consistent rise seen in skin temperature on day 12 may be due to reduced convective heat loss in the lower density atmosphere, or may be an artifact introduced by the decompression. At any rate the rise is consistent in all 3 atmospheres tested (because of a broken probe skin temperature was not measured during the nitrogen exposure) and therefore does not indicate a specific inert gas effect.

The most fundamental index of metabolic function in these experiments is oxygen consumption. There are several problems associated with this parameter. Activity of the animals at the time of measurement has a drastic effect on this measurement. This results in a function having a wide normal variation, and this makes small differences difficult to detect. In our particular experimental arrangement, we have the additional problem of choosing a baseline against which to make other comparisons.

We tried to correlate activity with oxygen consumption in each particular atmosphere, and though there were some obvious effects, the results of this analysis did not allow us to eliminate this uncontrollable variable. Activity was a particular problem in the rat experiments because the animals had a great deal of room to wander around. It seemed to our observers that they were considerably more active than the rabbits. The activity is not entirely a confounding factor, however, since subjective estimates of this parameter were generally well correlated with the nature of the gas – helium-exposed animals appeared more active than other groups at both sea level and altitude, and the pure oxygen exposure resulted in a reduced activity. The possibility exists that a portion of the increase in oxygen consumption traditionally found in helium is due only to an increase in activity.

On the basis of earlier work we expected to see a rise in oxygen consumption in the animals exposed to helium at one atmosphere. (Cook, 1950; Cook *et al.*, 1951; Leon and Cook, 1960; Rhoades *et al.*, 1965) Helium did indeed cause a 16% increase, but it was not significant statistically ($p<0.1$), except when compared with the pooled control points ($p<0.05$). The latter test is valid, but one would expect that the comparison with control values determined on the same animals would be a more sensitive test. It is interesting that we found a 16% increase at 26°C, since Leon and Cook (1960) found a 22% increase at 25°C and a 5% increase at 29.7°C.

Any increase seen in helium must be considered in the face of the even greater

increase noted in nitrogen (27% increase over controls; $p < 0.01$). It has already been pointed out that rectal temperature was elevated in the nitrogen group. Also, the nitrogen control values are low, and the oxygen consumption during the chamber run on nitrogen does not differ from the pooled control value. If the system were at fault, causing oxygen consumption to be increased during the extended run, this change would probably show up in other gases as well. Since it is not a gas-related phenomenon (the animals were exposed to nitrogen throughout), since it parallels a rise in body temperature and since it is not different from one of the available control values, we are inclined to dismiss the increase in oxygen consumption seen in the animals exposed to a nitrogen-oxygen mixture at sea level. Unfortunately this eliminates one of the baselines that might be used for evaluating other gases.

Incidentally, we were unable to find any information (notes, etc.) in the protocol books that would help to explain this disparity.

The reduction over their own air control values seen in the rats exposed to helium at low pressure is reasonable, and will be discussed in Section 4.

There is a trend toward a reduced oxygen consumption rate in the low pressure, pure oxygen exposure. Though not statistically significant it is in the expected direction based on activity, our experience with rabbits, and the work of Jordan et al. (1966a). It is not clear just exactly what metabolic response should be expected when rats are exposed to pure oxygen with a PO_2 of 180 mm Hg (Johnson et al., 1962; Dines and Hiatt, 1964; Berry and Smythe, 1962; Agadzhanyan et al., 1965), but there seems to be little doubt that the atmosphere is habitable (Felig, 1965; Zeiner, 1966; McHattie and Rahn, 1960).

Since no experiments have been done to verify the theoretical advantages of neon as a space cabin diluent (Roth, 1965; Welch and Robertson, 1965) it is perhaps important to point out that the animals exposed to the neon-oxygen environment showed no important deviations from normal.

One specific case where the neon values deviate from the others is in the case of carbon dioxide production (Figure 17). This deviation is likely to be analytical. The determination of carbon dioxide in the presence of neon as we carried it out in these experiments is less precise than in the presence of other background gases, and we have no other explanation for the observed variation. An estimated R.Q. of 1.13 supports this suspicion.

We have no explanation for the consistent decrease below the control average of carbon dioxide production, seen in all exposures. Except for that single neon point all carbon dioxide production determinations measured during the experimental exposures were equal to or less than the controls. For purposes of comparing inert gas effects this observation can be dismissed, since there were no consistent differences between gases.

A less sensitive index of metabolic function is provided by the estimates of food consumption. These results agree generally with the estimates of oxygen consumption, in that the rats had a tendency to eat more in the helium environment and less in the low-pressure pure oxygen atmosphere.

2. *Decompression*

One of the major criteria for the selection of a suitable inert gas diluent for long duration space travel is the effect that the gas may have on the astronauts ability to resist decompression sickness. The effects of decompression from sea level to the pressure inside a space suit (3.5 psia, or 180 mm Hg) has been studied extensively (Adler, 1964; Degner *et al.*, 1965), but the inert gas history has almost always involved only nitrogen. Experiments of Beard *et al.*, (1966) have shown little difference between helium and nitrogen in the susceptibility to altitude bends in human subjects, while Witherspoon *et al.* (1964) found in mice that helium is more suitable than argon or nitrogen.

The results of our experiments, Figure 19, speak for themselves. We were unable experimentally to separate neon and helium, but found both to be preferable to nitrogen and particularly to argon.

We cannot stress too strongly the preliminary nature of these results; we urge that they be interpreted in the light of the limitations of the experiment. The results are based on only a few animals, the criteria are subjective and susceptible to bias, the animals were in a moderately hypoxic state during decompression, and the rat has inert gas uptake and elimination characteristics only remotely similar to man.

The limitation of the size of the experimental population is illustrated by examining the tag numbers of the nitrogen animals (Figure 19). The most seriously affected animals are all from the 400 series, which are the animals that were in a preliminary experiment which had to be repeated for reasons unrelated to decompression. We do not mean to cast doubt on the justification for including this group, but only to point out the variability seen in otherwise 'identical' experiments of this sort.

The use of the subjective criteria and the struggles to develop an effective decompression test procedure have been mentioned. The role of adipose tissue in decompression sickness has been discussed elsewhere (e.g. Viotti and Walder, 1965), and need not be reviewed here. In future experiments we hope to remove some of the subjectivity and introduce 'blind' grading techniques. But to effect a distinct separation between helium and neon, different species of experimental animals such as dogs, goats, or piglets may be required. These and additional metabolic studies should be carried out before extensive manned exposure to neon is begun.

As in the case of other parts of the over-all experiment, the lack of a distinctive effect of neon is in itself an important finding. Our study, to our knowledge the first altitude decompression to be carried out on subjects equilibrated with neon, provides the first experimental evidence of characteristics of this gas that hitherto have been in the realm of speculation (Roth, 1959).

Decompression of animals from ground level to altitude is equivalent in many ways to decompression to sea-level from a saturation exposure at depth. In both instances safe ascent is controlled by the slowest half-time tissue (Schreiner and Kelley, 1966), in which inert gas exchange is limited by diffusion, rather than by perfusion factors. The rate of clearance of inert gas from these slow tissues should, therefore, increase

with decreasing diffusivity of the inert gas. Our results indicate that decompression
from sea level to altitude becomes more hazardous as the relative diffusivity of the
inert gases with which the animals had become saturated decreases. Decompression
of small laboratory animals after exposure to high pressures of inert gas for time
periods sufficient to produce effective saturation in the ascent-controlling tissues has
also revealed this relationship (Schreiner *et al.*, 1966b). Unpublished work by the
Royal Navy (Bennett, personal communication, 1966) provides further substantation
of these observations.

It is gratifying to those who believe that altitude and depth decompression are
governed by the same basic physical principles, that this parallelity in decompression
responses to helium, neon, argon and nitrogen has been shown to exist.

4. Discussion

From the extensive amount of data accumulated during the course of this project we
have selected a few key topics which seem to lead to some reasonable conclusions. To
begin with, the metabolic effects that are a result of animal exposures to various inert
gas atmospheres are summarized in Figure 20. As indicated in the figure, not all of
the observations are distinct and statistically significant, nor is it really clear what the
proper baselines are against which comparisons should be made. We have made some
arbitrary decisions to include in the figure some trends that perhaps lack sound
statistical justification, but feel that it is nevertheless a reasonable representation of
the data.

The chart shows that 'metabolism' is increased in animals living in an atmosphere
largely composed of helium, and that it is decreased in a pure oxygen environment
at one-fourth atmosphere total pressure. No important changes result from exposure
to neon or argon.

Other data indicate that there is some destruction or loss of red blood cells in
rabbits exposed to helium for a week (Table V and Hamilton *et al.*, 1966b).

The experiments were designed with a pragmatic intent, primarily to provide a
generalized evaluation of several possible spacecraft cabin atmospheres (to our
knowledge these are the first quantitative observations on neon as an atmospheric
constituent), but also to approach the basic question of whether there is or is not a
molecular level effect of inert gas at normal and sub-normal pressures. The fact that
chemically 'inert' gases do under certain conditions exert physiological and biochemical
effects is well established (Bennett, 1966; Featherstone and Muehlbaecher, 1963), and
this laboratory is in the forefront of that school (e.g. Schreiner *et al.*, 1962; Rinfret
and Doebbler, 1961). There is evidence that helium acts in a specific biochemical way
in its effect on metabolism (South and Cook, 1953; Cook, 1953; Wright *et al.*, 1966),
but these effects are not seen by all investigators (Maio and Neville, 1966; Rodgers,
1966).

One idea that we felt might provide new insights into the nature of inert gas effects
on the intact mammalian organism was to compare these effects on an animal with

↑↓   Clear cut or statistically significant change

↑ ↓   Trend or change lacking statistical corroboration

—   No change; adequate data

∼   Indifferent results; insufficient or uncertain data

Blank   Not determined in these experiments

	O₂ Cons	Activity	Food Cons	Body Weight	Water Cons	Temp Rectal	Temp Skin	Heart Rate
Rabbits								
N₂	—	↑	—	—	—	↑	↑	—
He	↑	↑	↑	↑	—	—	—	↑
Ar	—	—	—	↓	—	—	—	—
Ne	↑	—	—	—	—	—	↑	—
O₂*	∼	↓	↓	↓	↓	—	↓	↓
Rats, Sea Level								
N₂	↑?	—	—	—	∼	↑	—	
He	↑	↑	↑	—	∼	—	—	
Ar	—	—	—	—	∼	↑		
Ne	—	—	—	—	∼	—	—	
Rats, Altitude								
N₂	—	—	—	—	∼	—		
He	∼	↑	↑	—	∼	—	↑	
Ne	—	—	—	—	∼	—	↑	
O₂	↓	↓	↓	—	∼	—	↑	

* Oxygen rabbits were female, all others male.

Fig. 20. Metabolic summary.

a broad range of thermal neutrality against an animal with a narrow range. 'Thermal neutrality' refers to the temperature at which the metabolic rate is at a minimum, and is 28 to 29 °C for the rat and −5 to 30 °C for the rabbit. On the basis of its high thermal conductivity we would have expected an atmosphere consisting primarily of helium to have little effect on the metabolism of the rabbit and a considerable effect on the rat, with both animals maintained at approximately 26 °C. We found, however, that both species respond to the helium environment in a qualitatively identical manner, with the metabolic response of the rabbit being more pronounced than that of the rat.

It is clear then, that the effect of helium is not fully equivalent to that of cold air, and that we must seek a more sophisticated analysis of the heat transfer situation in the gaseous environments that we have studied.

TABLE XII

Thermal conductivities, viscosities and convective heat conductance ratios of oxygen and mixtures of oxygen with Helium, Neon, Nitrogen and Argon

Gas and mole fraction				Temp. °C.	Total pressure mm Hg	Thermal conductivity $K^a \times 10^5$ cal/cm/sec/°C	$H_2/H_1{}^c$	Viscosity $\eta^b \times 10^6$ g/cm/sec
O_2	0.238	N_2	0.762	24.5	755	6.20	1.00	183
O_2	1.00			30	192	6.442	0.52	207
O_2	0.384	N_2	0.616	26	474	6.24	0.81	188
O_2	0.236	He	0.764	26	755	20.02	1.66	230
O_2	0.380	He	0.620	27	474	15.41	1.18	226
O_2	0.239	Ne	0.761	30	755	10.03	1.16	272
O_2	0.386	Ne	0.614	30	474	9.20	0.89	263
O_2	0.236	Ar	0.764	30	755	5.23	0.84	222

[a] Computed from data for pure components using the Lindsay-Bromley equation (1950).
[b] Computed from data for pure components using the Bromley-Wilke equation (1951).
[c] Computed from above values using equation of Epperson et al. (1966).

Epperson *et al.* (1966) have suggested a method of comparing convective heat transfer in different gas mixtures, a more realistic evaluation of thermal properties of gaseous atmospheres than a mere comparison of thermal conductivities. Using Epperson's equations and more traditional methods for handling thermodynamic properties of gas mixtures we have assembled the data showed in Table XII. This table lists calculated properties of the various mixtures and pressures used in these experiments, including the forced convective heat exchange ratios, H_2/H_1, compared with our most air-like mixture (23.8% O_2, 76.2% N_2).

When the oxygen consumption data from all experiments is plotted against the ratio H_2/H_1 (calculated values) the surprising correlations seen in Figure 21 are obtained. To the extent that these oxygen consumption figures are valid, there is a striking dependence of oxygen consumption on relative convective heat loss regardless of the nature of the gaseous atmosphere.
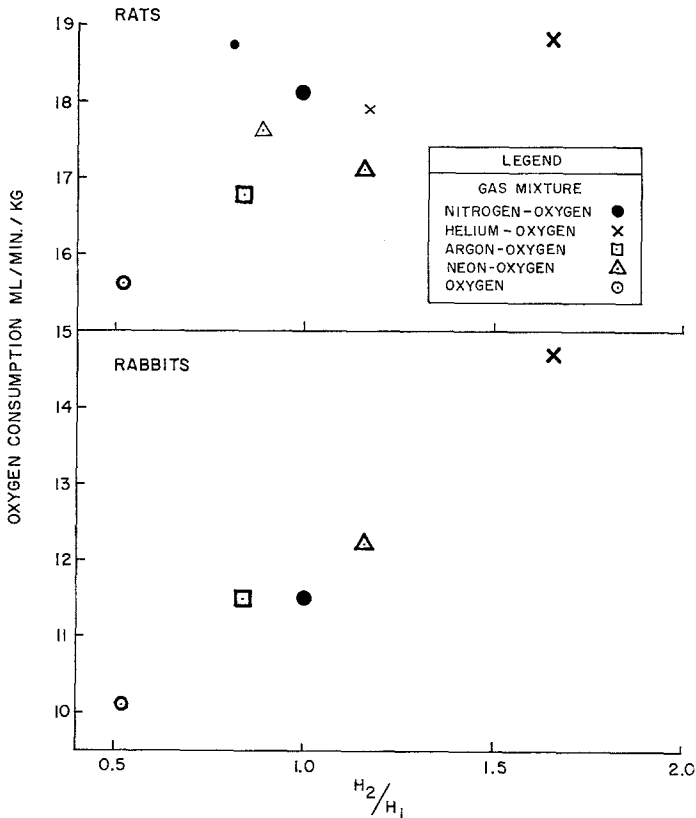
Fig. 21.   Effect of forced convective heat loss on oxygen consumption. H_2/H_1 represents heat loss relative to the one atmosphere nitrogen-oxygen atmosphere, as calculated by Epperson's equation (1966). Helium, neon and nitrogen are each shown twice on the upper graph; the point of each pair having the lower H_2/H_1 value is the one determined at reduced pressure. Lower curve (rabbits) includes data on pure oxygen atmosphere at 200 mm Hg total pressure; this point was determined using female rabbits.

It is interesting to speculate on the meaning of the slope of the lines defined by the 2 sets of points. The fact that the relative convective heat loss had slightly less effect on rats may be due to their higher initial metabolic rate or higher level of animal activity, or it may mean that these rats lost a slightly lower fraction of their body heat by the convective route.

The thermal interpretation of the helium effect is in agreement with many other experiments and speculations (Young and Cook, 1953; Dianov and Kuznetsov, 1964; Welch and Robertson, 1965). Experiments on humans in which the atmosphere is kept at a comfortable temperature do not show this effect (Bowers, et al., 1966; Hamilton, 1966), or show it to a reduced extent when the total pressure (and hence H_2/H_1) is reduced (Robertson et al., 1966).

A thermal interpretation may also be applicable to the question of oxygen consumption at normal PO_2 but at reduced pressure. Our animals definitely consumed less oxygen when the inspired oxygen tension was 180 mm Hg with no inert gas present, than they did when breathing air. Similar results have been seen in man (Kuznetsov et al., 1964) but the question still remains open (Johnson et al., 1962; Pichotka and Luthard, 1959; Froese, 1960).

As was mentioned in Part I, the loss of red blood cells from the rabbits in helium may itself be due to cold stress (Szkutnik, 1965), but Horvath (1964), mentions just the opposite effect, a transient elevation of hematocrit during the first 2 to 5 days of cold adaptation. Cold and hypoxia adaptations are to a slight extent antagonistic (Fregly, 1954), possibly because cold causes a reduction in red cells.

The findings of Rhoades et al. (1965) as well as our own observations (Schreiner et al., 1965) that rats, chicks, and mice experience a decrease in metabolism on returning to air from a helium-oxygen environment may lend itself to explanation on a thermal basis. Likewise, the decreased germination of helium-stored seeds (Latterell, 1966) is possibly due to increases in the rate of heat transfer to the gaseous environment.

Conclusions

The results of our experiments in the light of other available experimental evidence lead us to the following conclusions:

(1) The metabolic acceleration in a helium atmosphere is due primarily to the thermal properties of that gas, mediated to some extent by increases in spontaneous activity. Helium may cause a transient decrease in red cell indexes, and this too is likely to be due to the thermal stress.

(2) Oxygen consumption is slightly reduced on exposure to a low pressure, pure oxygen atmosphere that provides a normal alveolar oxygen tension. This effect is intimately interrelated with changes in evaporative water loss and activity and may be caused by the thermodynamic properties of this gaseous environment.

(3) A neon-oxygen atmosphere shows no immediately obvious biological hazard for exposures of up to one week, and along with helium this gas appears to offer some protective advantage in altitude decompression. A suitable neon-oxygen atmosphere

at half an atmosphere total pressure would have thermal properties akin to air and experimentally seems indeed to possess some of the theoretical advantages proposed for it (Bond, 1963; Roth, 1966).

(4) The question of whether light inert gases such as helium and nitrogen exert molecular effects at atmospheric and subatmospheric pressures is still unresolved as is the enigma of an absolute 'metabolic' requirement of mammals for gaseous nitrogen.

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