PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 704–709 (1974)

The Effects of Argon in the Bioenergetics of the Hamster and the Rat[1] (37879)

GEORGE E. TEMPEL AND X. J. MUSACCHIA

Dalton Research Center and Department of Physiology, University of Missouri, Columbia, Missouri 65201

There has been a growing interest in the use of various inert gases in biomedical research in various mammalian species. Two gases in particular have attracted attention in light of their effects in altering metabolism, viz., helium and argon. Several investigators (1–6) have shown that in the presence of helium (even in a normoxic environment), there is a metabolic challenge to small mammals such as rats, mice, and hamsters. In general, exposure to helium results in increases in metabolism whether measured as increased oxygen consumption or increased rates of respiration (ventilation). These changes are credited, or at least relatable, to increased thermal conductivity. In contrast, Clarkson *et al.* (7) maintained that the metabolic rate of the rat exposed to helium-oxygen (80%, 20%) is reduced from control levels obtained in nitrogen–oxygen (80%, 20%), a depression that could not be explained by thermal factors alone. In their experiment, ambient temperatures were regulated to a thermal neutral temperature, an approach which differs markedly from that taken by others.

Investigations employing argon as a diluent gas have also yielded controversial results; however, this gas does not induce a thermal-related acceleration of metabolic rate to the same extent as helium. Early studies of divers (8) demonstrated a greater narcotic effect for argon at increased pressure (up to 10 atm) than for nitrogen at similar pressure. No effect, however, was observed at atmospheric pressure. Cook (9), in an investigation of the effects of argon on metabolism and metamorphosis, reported that at atmospheric pressure, the substitution of argon for nitrogen accelerated the development of insects. Frankel and Schneiderman (10) in an attempt to confirm and extend these findings, concluded on the contrary that at atmospheric pressure, inert gases were without effect on the development of insects. Galvin *et al.* (11) and Schatte (12) have reported that compared with nitrogen, argon has a depressant effect on the oxygen consumption of rabbits and rats respectively. However, Hamilton *et al.* (13, 14) using a similar approach i.e., normoxic environments, failed to observe such an effect in either species.

These conflicting reports as well as our interest in depressed metabolic states prompted this study of the effect of normoxic mixtures of argon in the golden hamster (*Mesocricetus auratus*). It was hoped that an assessment of both the direction and the significance of the effects of exposure to argon might be made. In order to provide some basis for comparison with other workers, several experiments with Sprague–Dawley rats were also done. The effects of fasting and low ambient temperatures were also studied in order to further elucidate the role of argon, if any, in mammalian bioenergetics.

Materials and Methods. Hamsters, males and females (120–130 g), from our closed colony were used. Sprague–Dawley rats, males and females (130–140 g) purchased

[1] Supported by NASA Grant NGL 26-004-021 S7 and Dalton Research Center funds.

from Carworth Farms were also studied. The hamsters required no additional conditioning to the laboratory as they could be taken directly from the stock supply. Rats, on the other hand, were conditioned for 2 or more weeks in our animal room prior to experimentation. All animals were fed a diet of Wayne Lab-Blox and water *ad lib.* Hamsters were given weekly supplements of fresh lettuce.

Gas mixtures were 80% argon or nitrogen and 20% oxygen. Mixtures are accurate to within 1% and were prepared by Puritan Bennett Corp. The gas cylinders for room-temperature experiments were maintained at room temperature 22° (variation ±2°) and those used in the low-temperature experiments were maintained at 7° (variation ±1°).

Metabolism chambers were made of lucite cylinders with an approximate volume of 1,000 ml. In all experiments conducted, there was only one animal per chamber. The animal was isolated from laboratory disturbances by placing the metabolism chamber in a larger temperature-regulated and sound-insulated cabinet (a converted refrigerator equipped with inlet and outlet ports and a window). Room-temperature (22°) experiments were conducted in this manner while those at 7° carried out by isolating the chamber in a walk-in cold room (Forma Scientific, Inc.). Temperatures within the metabolism chamber, monitored in early experiments with thermocouples, showed approximately a 2° increase above ambient in both the 22 and 7° environments. Oxygen consumption ($\dot{V}_{O_2}$) was determined using a Beckman model G-2 paramagnetic oxygen analyzer which gave full-scale deflection for a 5% oxygen difference. Mean flow ($\dot{V}_I$) through the chamber was 118.9 and 192.4 ml/min for the 22 and 7° determinations, respectively. $\dot{V}_{O_2}$ was calculated according to the following formula:

$$\dot{V}_{O_2} = \dot{V}_I \times 60 \frac{(F_{I_{O_2}} - F_{E_{O_2}})}{100} \times \frac{10^3}{\text{wt}} \text{ ml(kg·hr)}^{-1},$$

where $\dot{V}_I$ is flow in ml/min, $(F_{I_{O_2}} - F_{E_{O_2}})$ is the percent change in oxygen content in the chamber air, and weight is animal weight in grams. Calibration of the instrument was accomplished with room air (20.39% O_2) as the zero gas and Puritan–Bennett primary standard (17.00% O_2) as the span gas. The 0.2% difference in O_2 concentration possible due to differences in dimagnetic properties of the diluent gases was considered negligible due to the fact that the determination involved assessment of Δ oxygen.

In each experiment, the animal served as its own control. A hamster or rat was individually placed in a metabolism chamber, containing cedar shavings which prevented direct contact with the floor. The animal was monitored as it respired a normoxic nitrogen mixture, and then switched to a normoxic argon mixture at comparable flow rates. Those animals which were fasted were deprived of food 16 hr prior to an experimental run. It must be noted, however, that all animals were essentially under conditions of fasting from the time they were placed in their individual metabolism chambers.

The experimental protocol consisted of three stages. During stage 1, which was a period of stabilization, the animal in the metabolism chamber respired a mixture of 80% N_2 and 20% O_2, visual observations were made, and oxygen consumption was monitored. After stabilization, stage 2 was initiated, namely, the continual monitoring of oxygen consumption in the 80% N_2 and 20% O_2 environment. Data were recorded for periods of 4 or more hr. Stage 3 designates the last period, i.e., when the gas mixture was changed from 80% N_2 and 20% O_2 to 80% Ar and 20% O_2. Oxygen consumption was monitored and recorded for an additional 4 or more hr. Overall, oxygen utilization was monitored for periods up to 16 hr. In general, determinations of oxygen consumption of the nitrogen–oxygen environment were made in the morning, while those in argon–oxygen were completed in the afternoon. The morning activity increase between the hours of 10 and 11 reported by Petrasek (15) was

TABLE I. Oxygen Consumption of the Golden Hamster and White Rat in Air and Argon.[a]

Animal	Experimental conditions (1 atm)	Mean oxygen consumption[b] (ml(kg·hr)$^{-1}$)[c]	
		Air	Argon
Hamster	Nonfasted, 22°	1070.2 ± 247.5 (6)	967.4 ± 137.0 (6)
	Fasted 16 hr, 22°	795.2 ± 139.3 (7)	749.1 ± 126.9 (7)
	Fasted 16 hr, 7°	1893.0 ± 291.8 (8)	1548.3 ± 249.2 (8)
Rat	Nonfasted, 22°	1250.6 ± 133.1 (6)	1136.0 ± 225.2 (6)
	Fasted 16 hr, 7°	1499.6 ± 170.5 (7)	1266.6 ± 115.4 (7)

[a] Air = 80% N_2 + 20% O_2, Argon = 80% Ar + 20% O_2.
[b] Volumes are for standard conditions of temperature and pressure.
[c] Data are mean ± SD, number of animals in parentheses.

noted and corrected for by drawing the line of best fit through the data.

Intergroup comparisons of the data were made using the Wilcoxon signed ranks test for populations of small *n*.

Results. The data for both hamsters and rats are summarized in Table I and Fig. 1. At room temperature (T_a 22°), the substitution of argon for nitrogen appears to be without marked effect in unfasted hamsters. Although oxygen consumption is depressed in argon, 967.4 ± 137.0 from 1070.2 ± 247.5 ml (kg·hr)$^{-1}$ in air, the difference is indicative only of a trend and is not statistically significant. A significant ($P < 0.05$) change due to the calorific effect of food may be seen in a comparison of fasted and unfasted hamsters; nonfasted hamster in air utilized oxygen at a mean rate of 1070.2 ± 147.5 ml (kg·hr)$^{-1}$ whereas the 16-hr-fasted animals under similar conditions consumed 795.2 ± 139.3 ml (kg·hr)$^{-1}$. Sixteen-hour-fasted hamsters exposed to 80% Ar, 20% O_2 demonstrated a similar decrease from a nonfasted mean of 967.4 ± 137.0 to 749.1 ± 126.9 ml (kg·hr)$^{-1}$. The oxygen consumption of fasted hamsters exposed to argon indicated a slight (5.8%) decrease from 795.2 ± 129.3 to 749.1 ± 126.9 ml (kg·hr)$^{-1}$ which was statistically significant at the 0.05 level.

During cold exposure (T_a 7°), hamsters increased their oxygen consumption in both air and argon to a level approximately double that of comparable animals at T_a 22°. There is, however, significantly ($P <$

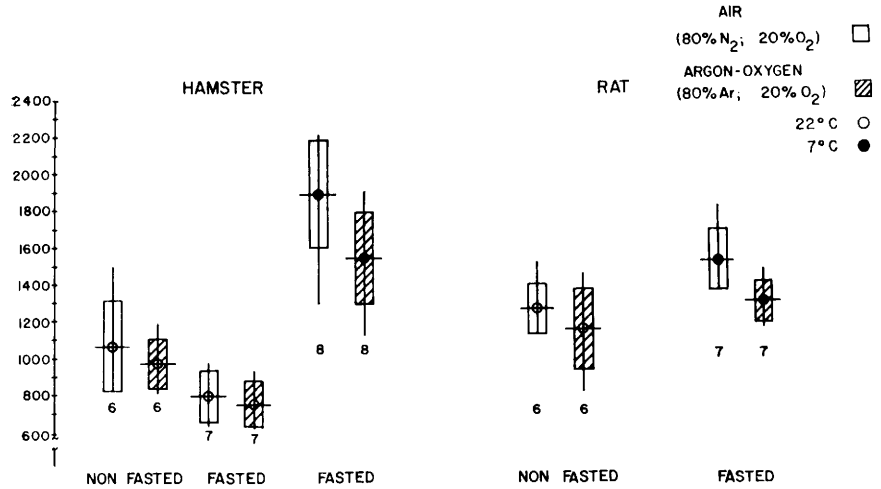

FIG. 1. Comparison of oxygen consumption in air (80% N_2:20% O_2) and in 80% Ar: 20% O_2 of golden hamsters and white rats. (Vertical line indicates range; horizontal line represents mean; rectangles enclose mean ± SD, and number below is sample size.)

0.05) less oxygen consumption in the presence of argon (Table I). This difference, from 1893.0 ± 291.8 to 1548.3 ± 249.2 ml $(kg \cdot hr)^{-1}$, represents a decrease of 18.2%.

The data obtained from rats are in many ways comparable to those from hamsters. At 22° the nonfasted rats had a mean oxygen consumption of 1250.6 ± 133.1 ml $(kg \cdot hr)^{-1}$ in 80% N_2, 20% O_2 environment with no significant change during exposure to the 80% Ar, 20% O_2 environment. Fasted rats exposed to 7° temperature demonstrated an increased oxygen consumption in normoxic nitrogen, but not in normoxic argon. There is, therefore, a depression (15.5%) in the metabolic rate of fasted rats at 7° in the normoxic argon environment. This represents a significant ($P < 0.05$) decrease in oxygen consumption from a mean value of 1499.6 ± 170.5 to 1266.6 ± 115.4 ml $(kg \cdot hr)^{-1}$ in air and argon, respectively.

Discussion. The resting oxygen consumption data from hamsters exposed to air (80% N_2, 20% O_2) are in reasonable agreement with previously reported values. The mean oxygen consumption of hamsters at 22 ± 2° was approximately 1070 ml $(kg \cdot hr)^{-1}$. Hoffman (16) reported a range of oxygen consumption from 930 to 1014 ml $(kg \cdot hr)^{-1}$ for resting hamsters at 28 to 34°. Although the value reported herein is greater than the upper limit of this range, it is likely due to the difference in thermal environments at which the experiments were carried out, or to seasonal differences.

To reduce the variable increase in energy metabolism following the ingestion of food, groups of both hamsters and rats were fasted for 16 hr prior to the experiments. The result suggests that the elimination of the calorific effect of food significantly reduced the hamsters' oxygen consumption in both air and normoxic argon. Individual values (Fig. 1) are suggestive of metabolic depression when the animal finds itself in an 80% argon, 20% oxygen atmosphere. However, the large overlap in values between air and argon exposures suggests the lack of any marked difference between the two conditions.

The group of elements known as chemically inert or rare gases are characterized by the completeness of their outer electron shell, and consequently, little tendency to gain or lose electrons. This decreased ability to form bonds with biochemical elements of cells and tissues suggests that the effect of these gases on animal metabolism might relate to their physical properties. Some of those characteristics most frequently considered in terms of narcotic potency are listed in Table II. Examination of this table shows that the oil:water solubility ratios of nitrogen and argon are strikingly similar although they differ markedly in thermal conductivity. In order to assess the data in terms of thermal conductivity, it is necessary to consider the animal's other avenues of heat loss: radiation, convection, and evaporation.

Radiant heat loss varies directly with the temperature gradient between the body surface and the average ambient temperature. In both air and argon environments, the ambient temperature and hence heat loss by radiation were the same. Convective heat

TABLE II. Physical Properties of Inert Gases.

Inert gas	Helium	Nitrogen	Neon	Argon	Krypton	Xenon
Atomic number	2	7	10	18	36	54
Atomic weight	4.003	28.016 (N_2)	20.183	39.944	83.80	131.30
Oil:water solubility ratio[a]	1.8	5.2	2.1	5.4	9.6	20.0
Thermal conductivity at 37.8° and 1 atm cal/(sec) (cm^2) (°/cm) × 10^{-6}	368.63	64.06	118.19	44.22	23.56	12.1[b]

[a] Calculated from solubility data of Lawrence *et al.* (19).

[b] Thermal conductivity of He, N_2, Ne, Ar, Kr at 37.8° from Weast (20); Xe thermal conductivity at 0° from Jenkins (21).

transfer likewise depends on a temperature gradient between the body surface and ambient air as seen in the following equation:

$$H_c = K_c A_c (T_s - T_a), \qquad (17)$$

where H_c is heat transfer by convection, K_c is the convection coefficient, A_c the exposed body surface area, and $(T_s - T_a)$ the temperature gradient between body surface and ambient air. In these experiments, both the thermal gradient and exposed body surface area were probably the same in the two environments in that no postural changes were noted and the ambient temperature was not altered. Another influence in the transfer of heat by convection, as seen in the above equation, is the rate at which convective currents bring gas to the body surface to participate in heat exchange (K_c). Use of comparable flow rates in the two environments keeps this factor, and thus convective heat transfer, uniform in air and argon environments.

Finally, heat transfer by evaporation would be the same in both cases since the relative humidity is constant. Humidity, a function of the chamber size, rate of gas flow through the chamber, and evaporative water loss, remains stable as none of the preceeding are varied upon changing the diluent gas from nitrogen to argon.

The effect of alteration of the thermal conductivity of the gaseous environment on heat transfer can be seen in the following equation of Hardy (18):

$$H_D = \frac{KA(T_2 - T_1)}{d} \times t,$$

where heat loss by conduction (H_D) is proportional to the area A, the thermal gradient $(T_2 - T_1)/d$, the time t, and the thermal conductivity K. With other factors constant between the two environments, an increase in the thermal conductivity would cause a proportionate increase in heat loss to the environment under these experimental conditions. Thus, the fact that oxygen consumption observed at T_a 7° is not as great in argon as in nitrogen may be due to the greater thermal conductivity of the latter. The effective cold stress imposed upon the animal might then be greater in the nitrogen atmosphere as a consequence of increased heat loss. Moreover, it is conceivable that the lack of any marked effect of argon at 22 ± 2° is relatable to reduction in the thermal gradient between the hamster's surface temperature and the temperature of the gas.

Although these data do not permit absolute separation of the effects of argon into thermal and nonthermal components, they are consistent with the indirect or physical hypothesis for its effect on metabolism. Thus, at atmospheric pressure, the effects of argon are minimal unless superimposed on cold stress where the reduced thermal conductivity of argon might account for the observed "decrease" in metabolic rate.

Summary. Oxygen consumption was examined in hamsters and rats exposed to normoxic mixtures of argon at 1 atm. In fasted and nonfasted animals, no marked change in O_2 utilization was detectable at 22°. However, at 7° a significant decrease in oxygen consumption was observed where the animals were exposed in argon. The data are interpreted in terms of the greater thermal conductivity of nitrogen.

We thank Jane R. Utsler for technical assistance and Drs. Barbara Horwitz and J. P. Jordan for their suggestions in the preparation of this manuscript.

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Received June 1, 1973. P.S.E.B.M., 1974, Vol. 145.