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"Non-Thermal Metabolic Response of Rats to
He-O₂, N₂-O₂ and Ar-O₂ at One Atmosphere,"
by Christopher L. Schatte, John Patrick Jordan,
Robert W. Phillips, Jack B. Simmons, II, and
David P. Clarkson.

ABSTRACT

The mechanisms by which inert gases influence metabolism were investigated from several viewpoints. Experimental designs centered around the use of environmental conditions which allowed differentiation between the thermal and non-thermal effects of argon, helium, and neon. Groups of rats were exposed at the thermal neutral temperature of the respective mixtures, to normoxic ($P_{AO_2} = 100$ mm Hg) environments with argon, helium or nitrogen as the diluent at a total pressure of one atmosphere. The possible influence of diluent gases on oxygen transport to the cell was examined with hypoxic ($P_{AO_2} = 70$ mm Hg) mixtures of the same diluent gases. Metabolic measurements included food, water, and oxygen consumption, CO_2 production, hematocrit and the rate of $^{14}CO_2$ expiration subsequent to intraperitoneal injection of acetate-1- ^{14}C or glucose-UL- ^{14}C .

Argon-exposed animals showed a consistently decreased metabolic rate while, on the other hand, helium-exposed rats did not significantly alter metabolic rate relative to nitrogen. Certain indices, including acetate and glucose utilization, suggested that helium attenuated the imposed hypoxia at the cellular level while argon facilitated it as compared with nitrogen. These results suggest that metabolic influence of helium is largely thermal in nature while argon has a significant direct metabolic effect and that diluent gases may selectively influence oxygen availability to the interior of the cell.

Identification of the specific mechanisms of the metabolic response to diluent gases will be investigated further from several different approaches. In one approach, a series of rats will be equipped with tracheal cannulas. This will permit the maintenance of the rat in an air environment within its thermal neutral range while allowing the rat to breathe a synthetic gas mixture. Any

observed alterations in metabolism will be primarily due to the inert gas rather than environmental thermal factors. In addition to some of the classical physiological parameters, it is hoped that data can be obtained on the turnover of various labeled substrates in these animals.

A second approach will be directed towards gas-membrane interaction. Examined first will be mitochondrial activity, principally oxidative phosphorylation and utilization of labeled substrates, in rats which have been chronically exposed to nitrogen, argon, or helium. Neuronal function will also be investigated, principally the rate of conduction along nerve fibers and the effects of the various inert gases on membrane potentials.

Still a third approach will involve a comparison of the metabolic response of homeotherms and poikilotherms to artificial environments. These two groups of animals, which possess totally different temperature regulation mechanisms, may or may not differ in their response to inert gas containing artificial breathing atmospheres. In either case valuable information will be gained from this comparison.

Additionally, the metabolic effects of frank narcosis, induced by high diluent pressures, will be investigated. This latter phase will be carried out at the Royal Naval Physiological Laboratory, Alverstoke, Hants, England. The information obtained from these widely differing approaches will collectively constitute a major step towards identification and quantification of the metabolic effects of inert gases at the molecular level.

INTRODUCTION

Previous Status Reports have dealt with the identification of the metabolic effects of artificial environments containing argon, helium, or neon. Research activities are currently directed toward differentiating and quantifying the direct and indirect metabolic effects of these gases.

During the period covered by this report, considerable time was spent in the maintenance and refinement of the Metabolic Control Units (MCU's) and associated animal exposure chambers. These modifications have resulted in more accurate control of environmental conditions within the chambers and more precise monitoring of metabolic parameters.

An initial set of experiments has been completed in which groups of animals were exposed to various diluent-oxygen mixtures at the thermal neutral temperature of the respective mixture. Under these conditions, which were designed to eliminate thermal influences of the diluent, helium exposure resulted in a slight acceleration of metabolic rate relative to nitrogen, whereas a significant depression of metabolic rate was observed in argon. These results suggest that the metabolic influence of helium is largely thermal in nature while argon has a strong direct metabolic effect.

Another series of experiments, at the thermal neutral temperature, is in the planning stage. In these experiments two groups of rats will be acclimatized to a given diluent under normoxic-oxygen tensions at a total pressure of one atmosphere. After acclimatization, oxygen tensions in both chambers will be maintained at normoxic levels. The diluent concentration, on the other hand, will be adjusted in successive experiments to give total pressures of either 292 Torr, 550 Torr or 1000 Torr. In addition to the usual metabolic parameters,

the influence of the diluent gas on both carbohydrate and lipid metabolism will be examined. This series of experiments is expected to be a major step toward the quantification of the thermal and non-thermal effects of argon, helium, and neon, and the effects of pressure on metabolism. They will also be of great significance in the identification of the specific metabolic pathways affected by these gases.

Christopher Schatte has completed the research phase of his doctoral program. The writing of his dissertation is nearly complete and Mr. Schatte's defense is scheduled for late November. While the entire dissertation is too lengthy to be included in this report, a manuscript based on Mr. Schatte's most recent research has been prepared for submission to the editors of the American Journal of Physiology. A preprint of this manuscript entitled, "Non-Thermal Metabolic Response of Rats to He-O₂, N₂-O₂ and Ar-O₂ at One Atmosphere," by Christopher L. Schatte, John Patrick Jordan, Robert W. Phillips, Jack B. Simmons, II, and David P. Clarkson is included as an Appendix to this report.

Mr. Schatte will receive an appointment as a postdoctoral fellow in this laboratory on 1 December 1971. Arrangements are complete for him to carry out collaborative research with Dr. Peter B. Bennett at the Royal Naval Physiological Laboratory, Alverstoke, Hants, England. We estimate that the initial phases of this research will require approximately eight months and begin 1 February 1972. Utilizing Dr. Bennett's unique facilities, Mr. Schatte will investigate the metabolic effects of frank narcosis induced by high diluent gas pressures. This study is expected to illuminate the mechanism by which argon, helium and neon effect the subtle metabolic alterations at pressures of one atmosphere or less. The exchange of scientific expertise through Mr. Schatte will benefit both NASA and Dr. Bennett's laboratory and promote international cooperation in scientific research.

Over the preceding year and one-half, several manuscripts based upon the research program of the Laboratory of Aerospace Biology have been submitted to the editors of various journals. The current status of each of these manuscripts is as follows.

"Mechanisms of Biochemical Adaptation to Marginal Oxygen Toxicity," by R. R. Gorman, J. P. Jordan, J. B. Simmons, II, and D. P. Clarkson has been published in *Biochem J.* 125:439, 1971.

"Coenzyme A Aberration in Marginally Hyperoxic Space Capsule Environments," by D. V. Lassiter, J. P. Jordan, R. L. Coleman and J. B. Simmons, II, has been published in *Aerospace Med.* 43:56, 1972.

"Predictability of P_{aO_2} in Different Inert Gas-Oxygen Environments," by C. L. Schatte, J. B. Simmons, II, D. P. Clarkson and J. P. Jordan has been accepted for publication by the editors of *Space Life Sciences*.

"The Thermal Neutral Temperature of Rats in Helium-Oxygen, Argon-Oxygen, and Air," by D. P. Clarkson, C. L. Schatte and J. P. Jordan has been resubmitted with revisions to the editors of the *American Journal of Physiology*.

During the period covered by this Status Report, the principal investigator, Dr. John Patrick Jordan, attended a conference at the Lunar Science Institute on October 11 and 12, 1971, entitled "Conference on Space Biology." Dr. Jordan also attended a coordinating conference at the Wallops Island Station with investigators whose work-focus on regulatory biology is supported by NASA. Dr. Emily Holton assumed monitorship of all these programs July 1, 1971, and co-chaired the conference with Dr. Joseph F. Saunders, Office of Life Sciences, NASA Headquarters. The conference was held October 17 through October 22, 1971, and had as its ultimate goal the development of a cooperative flight experiment in addition to the encouragement for additional in-house collaborative research on regulatory biology.

METABOLIC CHAMBER SYSTEM MODIFICATIONS

During the period covered by this report, modifications of the metabolic chamber system have resulted in a more sophisticated chamber system with regard to both the control of environmental conditions and the acquisition of the metabolic data. No part of the system was left untouched. The performance of each subsystem was reevaluated with respect to reliability and to precision in the case of control devices. The following list is a summary of the major aspects of this effort.

1. Maintenance:

- a. All plumbing was dismantled, cleaned and replaced where necessary. This included Poly-Flo and copper tubing, solenoid valves, needle valves, pressure gauges and switches, NaOH and H₂SO₄ scrubbers, water bubblers and purifil canisters. In many instances, particularly in the scrubbers, brass fittings or valves were replaced by those constructed from stainless steel. An additional precaution against corrosion in this area was the installation of canisters containing activated carbon immediately preceding those filled with purifil.
- b. The entire Metabolic Control Unit console was rewired. The numerous modifications in the MCU's necessitated the routing of more power to the console as well as many changes in the electrical design within the console.

2. Modifications and Improvements:

- a. Five, new, two-cylinder vacuum/compressors have been obtained. These pumps possess several improved design

features compared to their predecessors including teflon-coated interiors and high quality stainless steel valves. These features and other data supplied by the manufacturer, as well as extensive testing in our own laboratory, indicate that these pumps will have a useful lifetime five- to ten-times that of those they replaced.

- b. More reliable maintenance-free fans have been installed in each of the animal exposure chambers. This installation will assure a constant low-velocity test gas circulation within the chamber, thereby minimizing possible temperature and gas composition gradients. This is particularly important in the operation of two test chambers at different temperatures and following the injection of ^{14}C -labeled substrates.
- c. Independent temperature control devices have been installed in each chamber. These devices are quite simple yet highly efficient, consisting of a thermoswitch inside the chamber and a solenoid valve inserted in the chamber coolant line. The solenoid valve is normally closed and is actuated to allow coolant flow when the internal chamber temperature exceeds a preset limit. These systems will permit individual chambers to be operated at different temperatures and will control these temperatures to $\pm 0.5^\circ\text{C}$.
- d. The control circuitry of the automatic food-pellet dispensers (30 April 1971) has been improved considerably. These devices now may be operated in either a master-slave fashion or independently. The use of these devices on a regular

basis has resulted in a much more accurate determination of food consumption than was possible by the previous food tray/weighing method.

- e. The water delivery system, somewhat troublesome in the past, has been rendered considerably more reliable by improved circuitry and new water-level sensors.
- f. An automatic cycle-timer has been included in the chamber lighting system. This simple, albeit important, modification will eliminate the potential problem of the influence of variable LD cycles on metabolic patterns.
- g. An Esterline-Angus event recorder has been installed and interfaced with already existing event counters for oxygen and diluent addition, food and water delivery, and temperature control solenoid systems. This instrument will provide a convenient, permanent record of the frequency and exact times at which the various metabolic parameters are measured. Metabolic patterns can, therefore, be compared for experimental and control animals permitting an improved level of data interpretation.

PHILOSOPHY AND DESIGN FOR CURRENT EXPERIMENTS

Over the past two decades, various laboratories have investigated the effect of a number of the so-called inert or noble gases on various metabolic parameters of organisms ranging in complexity from bread molds to higher mammals such as the rat and man. The majority of these investigations were initiated with the intent to elucidate the causative factors underlying the physiological or metabolic responses elicited by these gases. Although no one investigation has provided us with a complete understanding of how these gases elicit the observed response, there are some marked consistencies among the data. For example, much of the data correlate nicely with certain of the physical characteristics of each of the inert gases used, primarily thermal conductivity, lipid solubility, and molecular weight. Of these three parameters, thermal conductivity is the most difficult to quantify; its effect seems to vary with the complexity of the organism examined. In the lower organisms, e.g., plants and poikilotherms, thermal conductivity obviously is of no particular importance. Helium, for example, which has a high thermal conductivity relative to nitrogen and with which is associated accelerated metabolism, elicits in these organisms augmented metabolic rate independent of environmental temperature. In homeotherms, however, e.g., man and rat, this acceleratory effect can be negated by an adjustment of environmental temperature. Argon, on the other hand, which effects a depression of metabolic activity relative to nitrogen-oxygen mixtures (or air), elicits this response in both poikilotherms and homeotherms. However, in homeotherms the effect seemingly cannot be negated by a reasonable adjustment in environmental temperature such as in helium. It is quite likely that thermal conductivity is a physical parameter

which may have no place in a hypothesis designed to describe the observed metabolic effects of these gases. Fortunately, we do have the other two parameters to look at: that of molecular weight and lipid solubility.

Coupled with the appropriate physical constants, molecular weight indicates the ability of a particular species of gas molecule to interact with water. From this we have Pauling's theory on anesthesia (1) which proposed that the formation of clathrates which are large water cages surrounding the gas molecule may either block access to various regions of the cell membrane or may interfere with various "vital" processes within the cell, e.g., enzyme activity. Another theory was presented by Miller (2) which described the augmented formation of the ice cover on cell membranes. Essentially this is like the clathrate theory in that it involves the structuring of water around the gas molecules, but differs from clathrate formation in that it is an extension of the existing cover of structured water rather than the *de novo* formation of free-water microcrystals.

Perhaps more important is the marked increase in lipid solubility in going from helium to the heavier gases such as argon and xenon. Since the cell membrane contains a great amount of lipid material, a particular gas molecule would be expected to be partitioned between the membrane and the interstitial space according to its lipid solubility. Thus, one would expect to find helium primarily in the intercellular space while the more lipid soluble gases such as argon might be found in greater number in the cell membrane. Consequently, if the gas molecules are of sufficient size and present in sufficient number, normal membrane function might be altered, particularly membrane transport and excitability. It is the latter phenomenon which, I think, warrants considerable scrutiny.

In an attempt to advance our present understanding of indirect and direct metabolic effects of these noble gases, a three-phase approach is being

designed in this laboratory. The principal aim of the research conducted in this laboratory during the past year has been the characterization of the relation between the heat transfer properties of various synthetic gas mixtures and several metabolic parameters. Data from these previous experiments has lead us to an experimental design in which we believe we are looking at only the metabolic effect of the inert gases. There is, however, still some doubt about the effectiveness of the experimental procedures. It is with this in mind that the first phase has been designed.

In Phase One, a series of rats will be equipped with tracheal cannulas. Thus, the rat can externally be maintained in an air environment within the thermal neutral range of this gas, while breathing a synthetic gas mixture. Since the amount of heat loss to the environment through the rat's respiratory system is rather insignificant in the light of the animal's total heat budget, it is felt that the effects observed will be primarily due to the inert gas. In addition to the classical parameters such as oxygen consumption, CO_2 production, and body temperature, it is hoped that data can be obtained on the turnover in these animals of the various labeled substrates; specifically, acetate, glucose, pyruvate, and one or two key TCA intermediates, e.g., succinate. Of necessity, the exposure of the animals to these gases will be acute rather than chronic. However, if the proposed theories relating these gases to aberrations in membrane function are correct, it is felt that any changes in metabolic behavior will be detectable within the period of measurement.

Phase Two will be directed at gas-membrane interaction. One study completed within the last few months in this laboratory (3) suggested that animals exposed to argon-oxygen mixtures may be hypoxic, indicating perhaps that this gas may be interfering in some manner with diffusion of oxygen across either the plasma or mitochondrial membrane. We propose to first look at mitochondrial activity principally oxidative phosphorylation and utilization

of labeled substrates. The mitochondria will be obtained from rats which have been exposed to nitrogen, argon, or helium for varying lengths of time. This will permit a time-response study on the metabolic behavior of animals exposed to these gaseous environments. It is felt, however, that significant changes at this particular level may not necessarily indicate that this is the site of action of the diluent gas. One has only to consider the role of the CSN in regulation of heat production in cold stressed animals. Accordingly alteration in neuronal function will also be investigated, principally the rate of conduction along nerve fibers and across synapses of the lateral sympathetic nerve trunk. Since it is also currently realized that many different types of cells maintain a transmembrane potential and this potential has been shown in some cases to be altered by external factors such as insulin, the effects of the various inert gases on these potentials will also be investigated.

It has been observed that some animals, e.g., amphibians and reptiles, do not respond to the inert gases in the same manner as to homeotherms. Therefore, the third phase of this experiment will be designed to investigate the possible reasons for this. Aside from the more obvious phylogenetic differences between this particular group of animals and homeotherms, there is one outstanding difference; these phylogenetically lower animals are not able to metabolically regulate body temperature. It is interesting to note that in many cases, cell membranes of these particular animals have a much higher proportion of unsaturated fatty acids (4) than do comparable cell membranes in homeotherms, perhaps as a consequence of their inability to chemically regulate body temperature. Thus, one might ask two initial questions: Does the fact that these animals are unable to control body temperature have anything to do with their response to inert gases, or is it more a matter of the amount of unsaturated fatty acids that are found in the cell membranes? It can be seen that a greater amount of unsaturated lipid in the plasma membrane might significantly

increase the polarity of this fraction. If indeed the lipid solubilities of these gases do play a vital role in eliciting the effects observed in the higher animals, then it is entirely possible that an increase in membrane polarity attributable to the increase in unsaturated fatty acids may also affect the partitioning of these gases. A sufficiently large shift may, therefore, alleviate their so-called narcotic effects. It should be pointed out that these are only two possible approaches, and it is quite likely that a more extensive literature search and research in this particular area will suggest other approaches. At the present time, Phase Two and Phase Three are on the drawing board and are prone to modification by the data of Phase One. However, within the next few months during which Phase One will be conducted, the protocol for Phase Two and Phase Three will be established.

COLLABORATIVE RESEARCH WITH THE ROYAL NAVAL PHYSIOLOGICAL LABORATORY

The collaborative research program between the Laboratory of Aerospace Biology (LAB) and the Royal Naval Physiological Laboratory (RNPL) is designed to accomplish four major goals. First, to substantiate recent evidence (5) that metabolic alterations observed in rats exposed to normoxic mixtures of helium, nitrogen, or argon at one atmosphere were caused by a differential histotoxic hypoxia due to inert gas interaction with the cell membrane. Second, to determine whether or not inert gases can also modify enzyme activity directly and thus influence metabolic rate independent of the membrane alterations and ion disbalance associated with narcosis (6). Third, implicit with such an observation would be the suggestion of a protein-related mechanism of narcosis and anesthesia, a theory that has been suggested (1, 2), indicated by some *in vitro* evidence (7, 8, 9) but not demonstrated *in vivo*. Fourth, this study would constitute the beginning of an in-depth, cellular level approach to the metabolic aspects of hyperbaric exposure and gas narcosis in contrast to the cursory urine and blood chemistry analyses reported thus far (10, 11, 12, 13). It is probable that information concerning energy balance under hyperbaric conditions would aid in resolving problems associated with narcosis.

We plan to determine the relative activity of the glycolytic and tri-carboxylic pathways in animals exposed to normoxic mixtures of either helium or nitrogen at both sea level and a narcotic pressure (for nitrogen) between five and ten atmospheres. The latter pressure will be determined using Dr. Peter Bennett's electroencephalographic-evoked response method of measuring sensory narcosis (14). A large animal, such as the goat, which will allow the withdrawal of multiple blood samples, can be readily trained to breathe

through a mask, and which has a reasonably wide thermal neutral temperature range, will be used.

Four animals will be carefully selected, trained, and exposed individually to both environments. A typical protocol will consist of a 60 minute test period at one atmosphere and another at the designated pressure, each preceded by a time period suitable for stabilization of the subject to the test conditions. Throughout the entire experiment, radioactive glucose will be infused intravenously and serial blood samples withdrawn at ten-minute intervals. Expired gas will be continuously monitored for oxygen, carbon dioxide, and total flow; expired carbon dioxide will be trapped and analyzed for radioactivity as a measure of glucose catabolic rate. The degree of sensory narcosis will be determined continuously using the above stated method.

At the end of the test period at pressure, the animal will be injected with a cationic detergent such as cetyl trimethyl ammonium bromide or stearylamine, which Dr. Bennett has reported to attenuate sensory narcosis (15). Data from this crucial phase will assist in determining whether or not the locus of metabolic influence is associated with the cell membrane and the ionic disturbance attendant with narcosis, since these drugs rectify membrane permeability changes occurring during narcosis. If the metabolic parameters do not change comparably with the narcosis indices during this phase, a non-membrane, possibly aqueous (protein) locus of activity would be suggested.

Pending the results of these experiments, the animals will be exposed again in a similar manner to at least nitrogen but with radioactive acetate, the "universal" metabolic rate indicator, as the substrate. This facet of the study will gauge the activity of the aerobic tricarboxylic acid cycle relative to the glycolytic pathway under the test conditions.

Blood samples will be analyzed for plasma glucose concentration and specific activity, plasma free fatty acid concentration and specific activity,

hematocrit, total protein, critical ions (6), catecholamines, corticosteroids, and lactate. Coupled with the expired gas and EEG analyses, these parameters should provide insight into the quantitative and qualitative metabolic consequences of exposure to hyperbaric helium and nitrogen, the role played by the "stress" response, the relationship between sensory narcosis and metabolic alterations, and the probable site(s) of gas influence. It is intended that future study, using these experiments as a basis, would include pinpointing specific sites of gas interaction using various metabolic intermediates as substrates in conjunction with specific enzyme assays.

The advantage of collaborative research with the Royal Naval Physiological Laboratory is best explained by the fact that Dr. Bennett is the world's foremost authority on gas narcosis. The necessity of such a visit is that certain of the methods and analyses crucial to the performance of these experiments have been developed in and are unique to that laboratory. The institution has assembled a virtually unequalled array of facilities and experienced personnel, whose assistance with analyses would be available, in the three decades of its existence. Further, Dr. Bennett has made explicit the fact that his association with the laboratories of Dr. A. D. Bangham at Cambridge and Dr. K. W. Miller at Oxford, both recognized workers in the field of gas narcosis, would be used to the benefit of this program.

Finally, Dr. Bennett has expressed an interest in more thoroughly researching the metabolic aspects of hyperbaric physiology, toward which Mr. Schatte's experience with measurement of metabolic parameters in animals exposed to artificial environments would be applied. In turn, his extensive knowledge of both the theoretical and practical aspects of narcosis and depressed organismic function will benefit NASA's long-range research program investigating means of regulating metabolic rate and controlling cellular activity in artificial environments.

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APPENDIX

The following paper entitled "Non-Thermal Metabolic Response of Rats to He-O₂, N₂-O₂, and Ar-O₂ at One Atmosphere," by C. L. Schatte, J. P. Jordan, R. W. Phillips, J. B. Simmons, II, and D. P. Clarkson has been submitted to the editors of the American Journal of Physiology for publication. The referencing and pagination within the manuscript are separate from the remainder of the Status Report.

**NON-THERMAL METABOLIC RESPONSE OF RATS TO He-O₂, N₂-O₂ and
Ar-O₂ AT ONE ATMOSPHERE**

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Metabolic Response to Synthetic Gas Mixtures

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ABSTRACT

Experiments were performed to describe the non-thermal metabolic response of albino rats to normoxic ($P_{AO_2} = 100$ mm Hg) mixtures of helium, nitrogen, or argon at one atmosphere ambient pressure. Ambient temperatures were maintained within the thermal neutral temperature range determined for each gas mixture thus eliminating thermal conductive effects. In order to examine the possible influence of the diluent gases on oxygen availability to the cell, hypoxic mixtures ($P_{AO_2} = 70$ mm Hg) were similarly tested to determine any differences in hypoxic response as a function of the diluent gas. Argon-exposed animals showed consistently lower parametric values relative to nitrogen indicating a reduced metabolic rate. Exposure to helium did not significantly alter metabolic rate relative to nitrogen although biochemical alterations may have occurred. Certain indices suggested that helium attenuated the imposed hypoxia at the cellular level while argon facilitated it as compared with nitrogen. It was postulated that the diluent gases selectively influenced oxygen availability to the cell and possibly interacted directly with key cellular constituents.

helium; argon; metabolic rate; hypoxia; thermal neutrality; O_2 consumption; CO_2 production; hematocrit; glucose kinetics.

INTRODUCTION

It is well known that chemically inert gases of the helium group, when breathed at sufficient partial pressure, can exert a number of biological effects ranging from subtle metabolic alterations to surgical anesthesia. The severity of these effects is dependent on the specific diluent gas and the partial pressure at which it is breathed. Thus, helium, at one extreme, is considered to be essentially non-narcotic at pressures to 261 atmospheres (21), whereas xenon, at the other extreme, has been used as a surgical anesthetic at atmospheric pressure (8). Other gases of the group, i.e., neon, argon and krypton, produce similar effects of intermediate severity (1, 16, 19). Several mechanisms by which these gases exert their influence have been postulated but none has been conclusively demonstrated (3, 22, 23).

With increasing use of artificial environments containing a diluent gas, considerable interest has evolved concerning the metabolic and physiologic consequences of exposure to these gases at ambient pressures of one atmosphere or less. Consequently, it has become necessary to determine whether or not more subtle manifestations of those effects seen at hyperbaric pressures are present at reduced pressures; and further interest in the mechanism of action by which such effects might be exerted has been generated.

Helium has been extensively tested concerning its ability to accelerate the metabolic rate of homeotherms relative to air, Leon and Cook (20) and Rhoades, et al (25) have demonstrated that the relatively high thermal conductivity of helium causes increased loss of body heat which must be replaced by accelerating metabolic heat production. Accordingly, they have reported that upward adjustment of the ambient temperature in helium obviates differences in the metabolic rate of helium-exposed animals relative to those in nitrogen.

The thermal influence of neon, argon, krypton, and xenon does not appear to vary as dramatically as that of helium. With the possible exception of a single report by Galvin, et al (11) concerning argon, no conclusive evidence has been presented that helium, neon, or argon have a significant non-thermal or direct metabolic influence on intact mammals at atmospheric pressure.

Recently, however, Clarkson, et al (6) observed a statistically reliable depression of oxygen consumption in rats exposed to helium or argon at the thermal neutral temperatures for each gas when compared to nitrogen. The authors concluded that at least argon had an appreciable non-thermal metabolic effect at 630 mm Hg ambient pressure. To further examine this possibility, the present study was undertaken to determine whether or not substitution of helium or argon for nitrogen at one atmosphere pressure would change the rate or nature of metabolism independent of thermal influence.

Investigators working with cells both in vivo and in vitro at hyperbaric pressures have implied that diluent gases might cause a selective intracellular hypoxia. Schreiner, et al, who demonstrated that the growth rate of the mold Neurospora was inversely related to the square root of the molecular weight of the diluent gas used (26), later reported that growth depression by the heavier gases could be attenuated by increasing the oxygen tension (27). Stephenson (29), showed that HeLa cells, embryonic lung tissue and certain bacteria survived low oxygen tensions better in helium than in nitrogen. Conversely, these cells exhibited lesser toxicity due to high oxygen tensions in nitrogen than in helium. Chouteau, et al, found that lethargy produced in goats by hyperbaric pressures of helium or nitrogen could be attenuated by increasing the oxygen partial pressure (4, 5). In each of these cases, the authors speculated, or it may be inferred, that oxygen availability to the cell was selectively altered to a degree dependent on the particular diluent gas involved and that the alteration could have been a diffusion limitation of oxygen.

An alternative to a diffusion limitation of oxygen into the cell is histotoxic hypoxia resulting from interaction of a diluent gas with key cellular constituents, perhaps respiratory enzymes. Histotoxic hypoxia has been ruled out as a primary cause of narcosis at high pressures (2), but, nevertheless, may be present at atmospheric pressure on a scale sufficient to account for the apparent cellular hypoxia cited in the examples above. Several investigators have demonstrated that certain anesthetic gas molecules do combine with protein and other aqueous phase cellular constituents (9, 17, 18), and Featherstone et al (9), have suggested several mechanisms by which such interaction could alter the function of the affected cellular component. In this regard, it is noteworthy that South and Cook (28), were able to reduce the effects of cyanide and fluoride on the metabolism

of rat liver under both aerobic and anaerobic conditions by exposure to helium instead of nitrogen; they speculated that helium interacted with glycolytic pathway and respiratory enzymes so as to block the action of the metabolic poisons.

In order to examine the question of diluent gas influence on oxygen availability to the cell, hypoxic ($P_{AO_2} = 70$ mm Hg) mixtures of helium, nitrogen and argon were also tested in tandem with the respective normoxic environment to determine whether or not animals exposed to different diluent gases responded in various degrees to reduced oxygen tension.

METHODS AND MATERIALS

Adult male Holtzman rats weighing between 300 and 400 grams were exposed in groups of eight animals each to either normoxic or hypoxic mixtures of helium, nitrogen, or argon at one atmosphere ambient pressure. The duration of the test exposure was five days preceded by two weeks of acclimatization to chamber conditions in air at one atmosphere ambient pressure. Temperatures were maintained within the thermal neutral temperature range determined in separate experiments (6) for each mixture: helium, 31-33°C; nitrogen, 26-28°C; argon, 27-29°C.

The "normoxic" oxygen tension was calculated, using the alveolar gas equation, to provide a P_{AO_2} of about 100 mm Hg while the "hypoxic" environments produced a P_{AO_2} of about 70 mm Hg (17.2% oxygen, equivalent to 5500 feet altitude). The degree of hypoxia was selected to provide a cellular oxygen reduction severe enough to elicit measurable physiological changes yet mild enough to prevent significant hyperventilation. It was intended that, if diluent gases did differentially alter cellular oxygen availability, the response to the imposed hypoxia would vary with the particular gas tested.

The environmental chamber system used has been described in detail elsewhere (12). It consisted of converted autoclaves in which the rats were continuously exposed to the test environments. Food and water were available ad libitum. Chamber temperature was controlled by passing an ethylene-glycol-water solution through the chamber water jackets and was monitored by thermocouples at opposite ends of the chambers connected to a recorder. Oxygen consumption was continuously measured on the basis

of oxygen added to maintain concentration at a set point on paramagnetic oxygen analyzers (Beckman, Model F-3). Carbon dioxide was trapped in scrubbers containing 10 liters of 2N KOH which could be sampled at intervals for CO₂ content.

Throughout the experiments, readings for oxygen consumption, carbon dioxide production, and ambient temperature were recorded every 12 hours. Absorbant trays to trap animal excreta were changed every 48 hours to minimize chamber odor. On the fourth day of exposure, the rats were removed from the chambers, weighed, injected intraperitoneally with acetate-1-¹⁴C (50 uCi/kg), and resealed in the chambers as rapidly as possible.

The CO₂ scrubbers were then sampled at intervals of 10 minutes for the first hour, 20 minutes for the second, and 30 minutes for the third hour post injection and the samples analyzed for radioactivity. On the last day, this procedure was repeated using glucose-UL-¹⁴C (50 uCi/kg).

The weight change and food consumption for each group of animals was measured as the difference between the pre- and post-experimental values. Oxygen consumption and carbon dioxide production were computed as the mean of the readings throughout the five-day exposure. Hematocrit was calculated as the group mean of duplicate determinations made on each animal at the end of an experiment. Respiratory quotient was computed using the final mean values for O₂ consumption and CO₂ production. Conversion of acetate-1-¹⁴C and glucose-UL-¹⁴C to ¹⁴CO₂ was calculated both as a rate, expressed as a slope constant *k*, and as the percentage of injected label expired during the three-hour collection period. The slope constant *k* is the slope of a line resulting from the logarithmic analysis of the injected label minus the expired label as a function of time; only that period during which the expiration is exponential was analyzed.

Statistical analysis was performed where possible using Student's *t*-test to analyze for difference between group means; differences with chance probabilities of less than 5% were considered significant.

RESULTS

The nitrogen (N₂) data represents the average of two experiments, one using 300-gram rats and the other testing 400-gram animals. This average was considered to represent

the metabolic response to nitrogen of rats weighing 350 grams; on this basis, the nitrogen data were comparable to that in helium (He) and argon (Ar) in which the rats weighed about 350 grams each.

Figure 1a shows the weight change of each experimental group during the five-day exposure. Weight gain among the normoxic groups was relatively similar although the Ar-exposed rats did gain less weight (48.8 g/kg) than did rats exposed to He (55.8 g/kg) or N₂ (58.1 g/kg).

Since hypoxia often causes weight loss or a reduced weight gain (30), changes in animal weight were considered to reflect the relative degree of hypoxia at the cellular level in the different gas mixtures. On that basis, the He-hypoxic rats seemed the least "hypoxic" since their 55.3 g/kg weight gain was nearly identical to that of their normoxic counterparts. The N₂-hypoxic group showed a greatly reduced weight gain (14.7 g/kg) suggesting that they were relatively more hypoxic than those in helium. The Ar-hypoxic animals appeared to suffer the greatest cellular level hypoxia since they lost 69.3 g/kg, the greatest weight change and the only weight loss observed during the entire study.

Substantial weight loss, such as that observed in the Ar-hypoxic group, is generally associated with oxygen tensions much lower than that to which the rats in this experiment were exposed (30). Further, neither these animals nor any rats tested in this study showed signs of disease that might influence any of the parameters. As indicated by weight change, helium appeared to attenuate the imposed hypoxia at the cellular level while argon exacerbated it relative to nitrogen.

Food consumption (Figure 1b) showed a pattern similar to that of weight change. Helium-exposed rats, whether normoxic or hypoxic, ate more food (428 g/kg and 392 g/kg, respectively) than any other group. Nitrogen exposure produced a lower food consumption 356 g/kg in the normoxic animals and 344 g/kg in the hypoxic ones) than in helium but about the same as that in the Ar-normoxic group (347 g/kg) and substantially more than in the Ar-hypoxic rats (281 g/kg). The reduced consumption of food by the latter group supported the postulate of a relatively severe hypoxia in argon since reduced food intake often accompanies exposure to lowered oxygen tension (30).

Further support for a differential response to the imposed hypoxia was evident in the hematocrit results (Figure 1c). Changes in hematocrit usually reflect changes in hematopoiesis but can result from hemoconcentration which often occurs during hypoxia due to abnormal plasma water loss (30). The changes in hematocrit observed in this study were considered to represent hematopoietic changes for the following reasons. Reports concerning hemoconcentration during hypoxic exposure are conflicting; often, it does not occur (30). It is noteworthy that Fryers (10) observed an increase in the plasma volume of rats during the first five days of hypoxia (the exact duration of the present experiments) at a time when weight loss was maximum. It, therefore, seemed possible that the hematocrit changes were not due to hemoconcentration, even in the Ar-hypoxic group which lost weight. Differential water loss from the skin seemed unlikely since chamber humidity was 80-100% in all environments and the animals would have attained an equilibrium after which water loss should have been minimal. Finally, increased respiratory water loss was ruled out because hyperventilation did not occur in the hypoxic environments as evidenced by a check of blood pH.

Regarding the hematocrit values as representing hematopoietic response to cellular oxygen tension, helium (39.8%) and argon (39.9%) significantly ($P < .001$) reduced hematopoiesis relative to nitrogen (41.6%) in the normoxic groups. There was no readily apparent explanation for the lowered hematopoiesis in argon when interpreted in the light of other parameters, which did not suggest cellular oxygenation greater than that in nitrogen. The pertinent facets of the hematocrit data lie in the differences between the respective normoxic and hypoxic groups which should have reflected the degree of cellular hypoxia in each gas mixture. As in the case of weight change, the He-hypoxic rats, with a hematocrit of 39.9%, seemed no more "hypoxic" than their normoxic controls. The N₂-hypoxic group showed some response to a cellular oxygen lack (44.6% versus 41.6% for the normoxic controls) but not as great a response as that seen in argon, in which the hypoxic animals produced the most significant ($P < .0005$) response and the highest hematocrit (45.9%) recorded. Supporting the pattern suggested by weight change and food consumption, the hematocrit data indicated that the imposed hypoxia was obviated at the cellular level in helium and facilitated in argon when compared to nitrogen.

Oxygen consumption, a classic indicator of metabolic rate, did not vary among the environments as much as some other parameters, suggesting that metabolic rate may not

have been grossly different. Figure 1d shows that both helium and nitrogen groups consumed oxygen at about the same rate, ranging from 32.7 l/kg/day to 35.9 l/kg/day, which was not statistically different. The argon groups consumed significantly less ($P < .05$) oxygen, 29.9 l/kg/day and 28.4 l/kg/day, than did any of the helium or nitrogen exposed rats, indicating that metabolic rate was appreciably altered by argon relative to nitrogen.

Differences between respective hypoxic and normoxic groups were not statistically significant although the hypoxic animals consumed less oxygen in every case.

Carbon dioxide production reflected a pattern similar to that of oxygen consumption (Figure 1e). The CO₂ production of the two helium groups (30.3 l/kg/day and 29.4 l/kg/day) did not statistically differ from that in the two nitrogen groups (28.2 l/kg/day and 27.8 l/kg/day). However, CO₂ production in helium and nitrogen was significantly greater ($P < .005$) than in either argon group (22.4 l/kg/day and 22.2 l/kg/day). As with oxygen consumption, hypoxia did not markedly influence CO₂ production as compared with the normoxic groups.

Respiratory quotients were computed using the mean oxygen consumption and carbon dioxide production data in order to gauge the nature of a group's overall metabolism. Using the RQ values as a rough indicator of metabolic substrate utilization, exposure to helium (0.88 and 0.90) apparently caused a greater utilization of carbohydrate than did nitrogen (.78 and .80) or argon (.75 and .78). The latter two gases seemed to induce a higher percentage of fatty acid metabolism than in helium; in view of their weight loss, amino acid catabolism may also have been accelerated in the Ar-hypoxic rats. Hypoxia did not appear to have as great an influence on RQ as did the diluent gas tested since the normoxic and hypoxic values in all cases were quite similar; nevertheless, the value for hypoxic animals was slightly higher than that for the normoxic ones in all cases.

The rate constant k and the percentage of injected radioactive label expired as CO₂ during the three hours after injection of either acetate-1-¹⁴C or glucose-UL-¹⁴C are shown in Table I. The value k reflects the rate of catabolism of an injected substrate to CO₂ and can be used to compare relative catabolic rates of different substrates or the same substrate among different animal groups; the greater the slope, the faster the rate of catabolism. The percentage of injected activity expired as CO₂ indicates how much of the substrate is catabolized to CO₂ as a function of time.

The rate and percentage conversion of glucose to CO_2 was similar in helium and nitrogen but markedly lower in argon. Hypoxia did not appear to have a consistent effect on the catabolism of glucose, being very nearly the same as in normoxia. In contrast, helium-exposed rats converted acetate to CO_2 at a rate 3-5 times that in argon such that total conversion after three hours was nearly double the amount converted in argon both in hypoxia and normoxia. The greater conversion of glucose and especially acetate in helium as compared to argon indicated a greater metabolic rate in helium. In conjunction with the RQ data, it may be inferred that the helium-exposed rats utilized carbohydrate to a greater extent than did those in argon. Since the hypoxic and normoxic groups were similar in each environment with regard to acetate and glucose catabolism, it is likely that individual properties of helium and argon accounted for the biochemical differences among the environments.

DISCUSSION

Substitution of argon for nitrogen at one atmosphere caused a statistically significant depression in metabolic rate whereas exposure to helium did not appear to statistically change metabolic rate independent of thermal factors. Additionally, the data indicated that the diluent gases influenced either the degree of cellular hypoxia or the cell's ability to respond to it. It was also postulated that changes in the relative importance of biochemical pathways may have occurred in both argon and helium relative to nitrogen.

The results of the helium exposures were in general agreement with other studies in which thermal influence was considered. Leon and Cook (20), and Rhoades, et al (25) showed that the oxygen consumption of helium-exposed rats did not statistically differ from that in nitrogen when the ambient temperature was raised to 29.7°C and 33°C , respectively. Hamilton, et al (13) exposed three generations of mice for six months to helium-oxygen at atmospheric pressure and observed that, once acclimatized, they consumed no more oxygen than did air controls although they did require significantly more food and water. Clarkson, et al (6) demonstrated that the oxygen consumption of rats in helium fell below that in nitrogen at temperatures above 31°C and concluded that the metabolic influence of helium was mostly thermal in nature although a direct effect

could not be ruled out. Other reports describing an acceleratory effect of helium on the metabolism of intact mammals are complicated by thermal factors (7, 11, 24).

With regard to argon, the present results agreed qualitatively with those of Galvin, et al (7) who observed an unspecified metabolic depression in rabbits exposed to argon at pressures ranging from 0.5 - 15 atmospheres. Clarkson, et al (6) found that the oxygen consumption of rats in argon at one atmosphere was significantly lower than in nitrogen at temperatures ranging from 25-33°C. But Hamilton, et al could detect no significant changes in rabbits or rats exposed to argon from those in air at one atmosphere (14, 15), perhaps due to a limited sensitivity of their measurements.

One mechanism by which argon might have depressed metabolic rate and by which the presumed biochemical changes in helium and argon were induced during the present study could be inferred from the evidence. In view of the apparent influence of the diluent gases on oxygen availability to the cell, it was reasonable to postulate that argon caused an intracellular hypoxia relative to nitrogen such that metabolism was altered. Conversely, perhaps helium facilitated oxygen entry into the cell as compared to nitrogen so that oxygen tensions may have been higher in the cells of helium-exposed rats than in those in nitrogen. Such an elevation in cellular oxygen tension would not have altered metabolic rate, since the latter is not dependent upon oxygen tension except at extremely low levels (30), but could have produced changes in oxidative pathways. Strong support for such a hypothesis came from the results of the hypoxic exposures. The near "normal" performance of the He-hypoxic group suggested that they were not very hypoxic despite the lowered oxygen tension whereas those rats in nitrogen and argon suffered an increasingly severe oxygen lack at the cellular level.

A manner in which the diluent gases might have influenced cellular oxygen tensions was by selectively altering oxygen diffusion through the cell membrane. Such a diffusion limitation could be caused by a diluent gas physically dissolving in the lipid fraction of the membrane and occupying an area of the membrane across which oxygen molecules must pass. The greater the total area of membrane occupied, the less area available for diffusion and the greater the difficulty of transporting oxygen into the cell. The total area of membrane blocked would be dependent on the membrane volume occupied by a diluent gas at a given partial pressure. Helium, a relatively small, lipid-insoluble molecule, would

occupy little space at equilibrium and, therefore, offer minimum resistance to oxygen diffusion. Nitrogen and argon, with increasingly greater molecular sizes and solubilities, would occupy a proportionately larger membrane volume and, thereby, present greater impediment to oxygen diffusion. This hypothesis is consistent with the present data and the results of Schreiner, et al (27), Stephenson (29), and Chouteau, et al (4, 5) all of whom were able to better oxygenate the interior of cells by substituting helium for nitrogen or by increasing the oxygen partial pressure above normal. The latter would provide more oxygen molecules and thus increase the chances of maintaining adequate cellular oxygen tension despite a diluent gas diffusion impediment.

In addition to the intracellular hypoxia, it is possible that direct interaction of the diluent gases with cellular constituents, perhaps enzymes, may have contributed to the results. One discrepancy with the cellular hypoxia theory, suggesting the possibility of direct interaction, is the fact that the Ar-normoxic animals, on the basis of the oxygen diffusion limitation postulate, should have been relatively more hypoxic than the comparable helium or nitrogen groups. But that group's mean hematocrit was the same as that for the He-normoxic rats and actually lower than in nitrogen. Also, despite what was construed as a relatively severe intracellular hypoxia in the Ar-hypoxic rats, those animals were parametrically similar in many instances to their normoxic counterparts. These two observations suggested that the influence of argon at some intracellular locus may have been greater than that of the hypoxia. Accordingly, the large differences in the rate of acetate catabolism between the helium- and argon-exposed animals may have been a function of a greater inactivation by argon of some key enzyme catalyzing acetate conversion. Or, perhaps argon uniquely affected electron transport such that oxygen consumption was lowered.

It is conceivable that the results were a function of more than one mechanism of action at more than one locus of activity. Perhaps the two above described mechanisms both contributed to the changes observed. They may vary in importance with the partial pressure of the diluent gas such that the relative importance of the oxygen diffusion limitation mechanism is greater at one atmosphere whereas direct interaction and inactivation of enzymes may largely account for the narcotic symptoms of inert gases at high partial pressures.

It is apparent that helium, nitrogen and argon can alter metabolic and physiological function at partial pressures less than one atmosphere. The effects of helium, at the thermal neutral temperature are small, indicating that the acceleratory influence of helium on metabolism is largely thermal in nature. Nitrogen and argon are more narcotic than helium and less dependent on thermal influence to exert their metabolic effects. Since the relationships of the diluent gases, with regard to their potency in producing metabolic alterations in this study, are qualitatively the same as those regarding their narcotic potency at hyperbaric pressures, it is likely that the metabolic effects at one atmosphere are subtle manifestations of the more pronounced narcotic symptoms seen at pressure. We feel that thorough description of the modes by which diluent gases alter metabolism at one atmosphere will aid in elucidating the mechanism by which narcosis is produced at high pressures.

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Figure 1. Weight change, food consumption, hematocrit, oxygen consumption, carbon dioxide production and respiratory quotient of 8 rats exposed to each environment. Shaded figures represent the hypoxic groups. Hematocrit, oxygen consumption, and carbon dioxide production expressed as the mean \pm standard error.

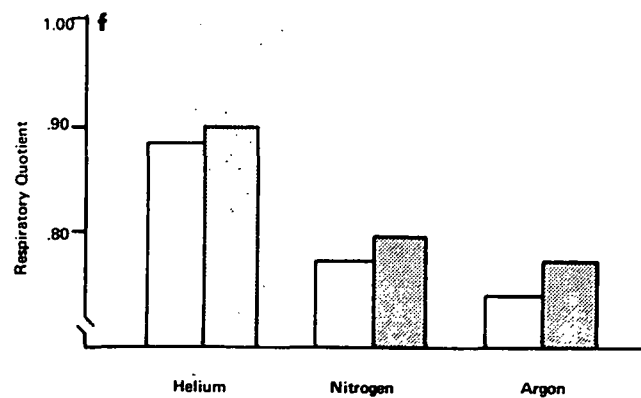
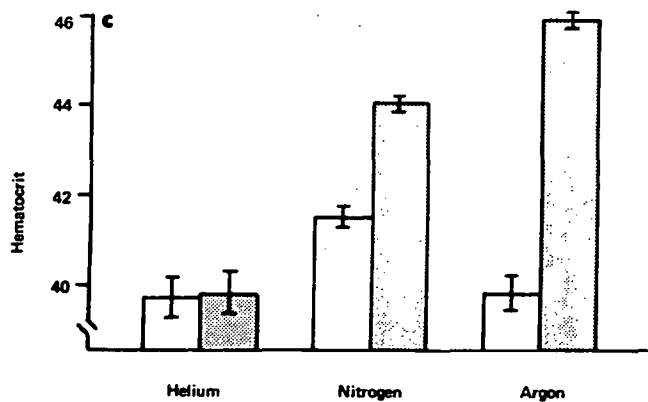
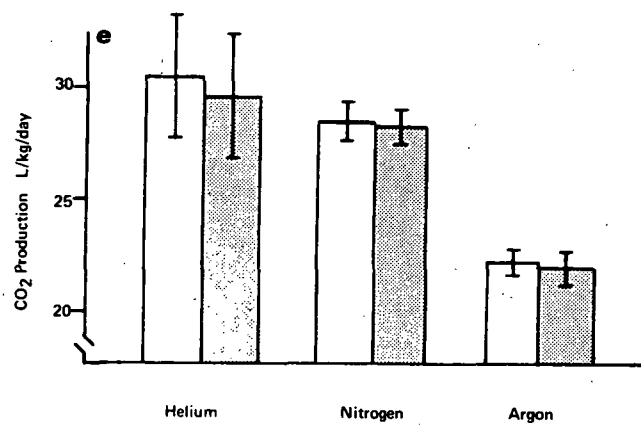
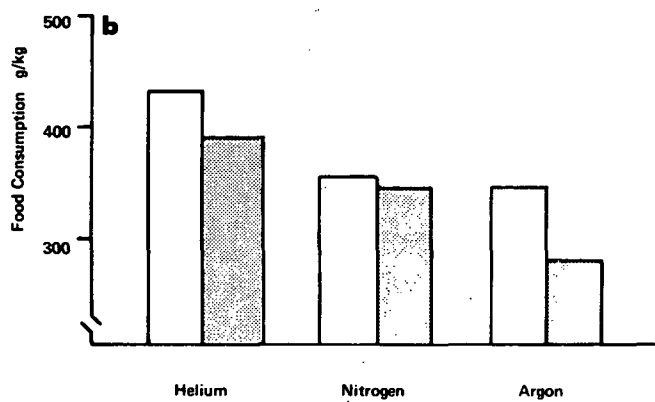
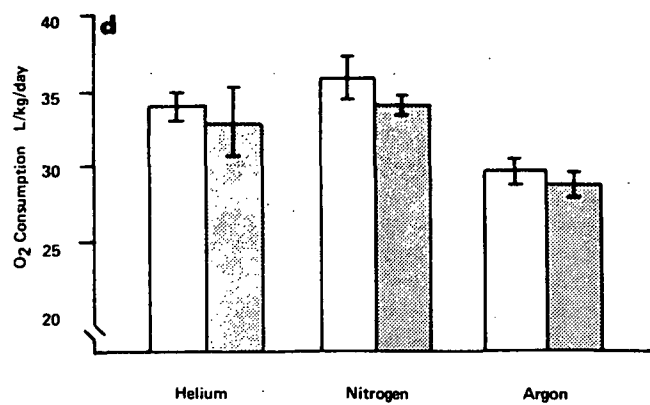
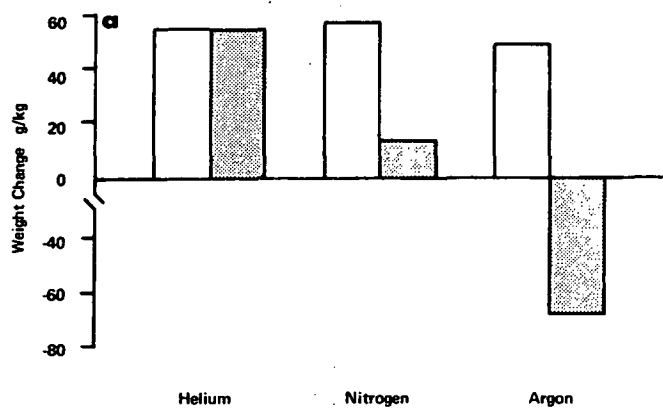


Table 1. Rate constant k and the percent of injected activity expired as CO_2 three hours after injection of acetate-1- ^{14}C or glucose-UL- ^{14}C . The values represent the expiration from a group of 4 rats injected with glucose and eight rats injected with acetate.

Experiment	<u>Glucose</u>		<u>Acetate</u>	
	$k(\text{X}10^3)$	% expired	$k(\text{X}10^3)$	% expired
He-normoxic	2.79	33.1	14.3	81.8**
He-hypoxic	2.35	27.2	19.0	87.2**
N_2 -normoxic	2.73	32.2	*	*
N_2 -hypoxic	2.86	31.5	*	*
Ar-normoxic	2.15	24.0	5.2	44.6
Ar-hypoxic	1.87	24.5	4.2	43.3

* data unavailable

** represent values measured at two hours post injection extrapolated to three hours. The corresponding figures after two hours were 77.0% and 78.4% for the normoxic and hypoxic groups, respectively.