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ABSTRACT

A protocol is described for the determination of subtle effects resulting from exposure to a marginally stressful environment. The protocol is based upon the bioenergetic state of the organism since life has as its most fundamental characteristic the need for energy. If one can evaluate the energetic state of an organism, one can clearly observe any significant change in that organism.

Utilizing this protocol the metabolic effects of replacing nitrogen with argon, neon, or helium as the diluent gas were investigated. Corroborating the results of others, we have previously reported a change in metabolic rate when nitrogen was replaced by other diluent gases. By measuring metabolic parameters in rats exposed to thermally isoconductive environments, comparing the above listed rare gases with nitrogen under normoxic conditions, we observed changes in metabolic rate which were inversely proportional to (a) the square root of the molecular weight of the diluent gas, (b) the solubility parameter of the diluent gas as defined by Hildebrand, and (c) the molar refraction of the diluent gas which is a measure of clathrate-forming capability. These are the identical parameters which show a relationship to the narcotizing capacity of the same
gases under hyperbaric conditions. The relationships held for neon, nitrogen, and argon. They did not hold well for helium; the reasons for this are discussed.

We conclude that (1) diluent gases have both a direct metabolic effect which relates to the chemical characteristic of the diluent gas molecule and an indirect metabolic effect which relates to the thermal conductivity of the diluent gas as it cools the body surface of the animal, and (2) the direct metabolic effect is significant.

The penultimate studies of the mechanism of marginal oxygen toxicity associated with the 5 psia-100% oxygen environment are described. Using actinomycin D and cycloheximide in separate experiments, it is clear that the hyperoxic environment induces glucose-6-phosphate dehydrogenase. The induction is probably the result of the inhibition of one of the enzymes either in glycolysis or the tricarboxylic acid cycle between glyceraldehyde-3-phosphate dehydrogenase and succinic dehydrogenase. Injection of succinate into the experimental animals partially reduced the induction of glucose-6-phosphate dehydrogenase.

As a part of the mechanism of adaptation to this environment, the animal increased its utilization of the pentose phosphate pathway for the metabolism of carbohydrate. Whereas only one fifth of the carbohydrate
was metabolized via this pathway in the control animals, animals exposed to the 5 psia-100% oxygen environment metabolized approximately 40% of their carbohydrate via the pentose phosphate pathway. Since the hyperoxic animals remained in bioenergetic balance, it is clear that the pyridine nucleotides reduced via this pathway can be utilized as an energy source.

The hyperoxic environment caused the translocation of certain trace elements. Metabolically vital organs including the heart, kidney, lung, and liver tend to concentrate iron, copper, and zinc while the thymus and brain tend to lose these cations. Cation studies of the hemotologic system are consistent with previous reports describing the early demise of older red blood cells. This work is from the thesis of David N. Lankford submitted for the degree of Master of Science to the Graduate School of the University of Oklahoma.

I. INTRODUCTION

Protocol:

Through the experiments conducted under this program over the past several years, a protocol has been developed for the evaluation of the environmental effects on biological systems. The basis of this protocol is bioenergetics and the fact that life has as its most fundamental characteristic the need for energy. If one can evaluate the energetic state of an organism, one can clearly observe any significant change in the living state of that organism.

The protocol involves three levels of investigation:

a. The first level is the initial survey which allows the investigator to determine whether there are any significant changes in the bioenergetic state of the organism based upon the measurement of four different parameters. In an animal these would include:

(1) the measurement of oxygen consumption over an extended period of time (preferably 24 hours).

(2) the measurement of CO₂ production (preferably over a 24 hour period).

(3) observations on the general physiologic state of the animal including his eating habits.

(4) the injection of acetate-1-¹⁴C and the trapping of the subsequent catabolic product, ¹⁴CO₂.
The plot of the natural logarithm of the amount of $^{14}\text{C}$ remaining in the acetate pool versus time will yield a first order reaction, the slope of which is proportional to the rate of metabolism of acetate. From these data, the slope (which is in terms of reciprocal time) will lead to the calculation of the half-life of the acetate pool. This determination appears to be independent of the size of the pool. The determination in this case measures the effect of the environment specifically on the very important metabolic process of catabolizing acetate to $\text{CO}_2$. (If glucose-1-$^{14}\text{C}$ were injected, its rate of conversion to $^{14}\text{CO}_2$ would be approximately proportional to the overall metabolic rate and would be a desirable additional measurement.)

Section II of this report exemplifies the first level type of experiment by investigating the effects of various diluent gases on the bioenergetic state of the animal. Parenthetically, the technique should be usable with any living organism. For a plant, different measurements of energetic status would have to be utilized such as the production of oxygen and the uptake of $^{14}\text{CO}_2$ and its subsequent incorporation into key plant metabolites. The point to be emphasized is that the protocol involves the use of bioenergetic parameters as the key measurements of the overall effect of an environmental stress on an organism. The technique indicated above would be just as adequate for microorganisms as it is for animals.

b. The next level of investigation has as its purpose the bracketing of the site of the physiological manifestation of an environmental insult. An example is the use of glucose-1-$^{14}\text{C}$ and glucose-6-$^{14}\text{C}$ to determine the relative importance of the pentose phosphate pathway compared with glycolysis and its attendant TCA cycle (see Section III). Another example is the study of alterations in trace mineral metabolism in animals exposed to the 5 psia-100% oxygen environments reported in Section IV of this report. Thus, we know at least the sequence of reactions involved in the organism's successful or attempted adaptation.
c. The final step in the protocol is to identify the specific enzyme or limited number of enzymatic reactions and associated hormonal or organ level responses immediately involved in the organism's successful or attempted adaptation. The study on the effects of marginal oxygen toxicity on carbohydrate metabolism described in Section III of this report is an example of this final step in the protocol. Here, work on glucose-6-phosphate dehydrogenase, glycer-aldehyde-3-phosphate dehydrogenase and succinic dehydrogenase represent attempts to pinpoint the metabolic site of action that initiates the adaptation process in rats exposed to the 5 psia-100% oxygen environment.

Focus is placed on the initial evaluation step (step a). It must be simple sensitive, quantifiable, and decisive. The one described fulfills all those criteria.

Program and Personnel:

During the period covered by this report David N. Lankford completed the requirements for the Master of Science Degree at the University of Oklahoma Medical Center using the equipment and based upon the data obtained through this program. His master's thesis is too extensive to be reprinted in full in this report; however, his basic experimental outline was presented in the October, 1969, Status Report and Section IV of this report contains both the summary and conclusions reached in that study. The thesis is now being prepared for submission to the editors of Aerospace Medicine.
Robert R. Gorman has worked full time on his doctoral dissertation which looks promising as a study of the mechanism of adaptation observed in carbohydrate metabolism in animals exposed to the 5 psia-100% oxygen environment, a marginally hyperoxic condition. A progress report on his work is presented in Section III. He expects to complete the requirements for the Ph.D. degree by August, 1970.

The study of the metabolic effects of gases has been the main thrust of the laboratory during the period covered by this report. The basic question being asked is whether the change in metabolic rate associated with the changes in diluent gas is due solely to the thermal conductivity factor associated with the specific diluent gas or if it is in part due to a direct metabolic effect. The gases tested include helium, neon, nitrogen, and argon. Christopher Schatte, a Ph.D. candidate in Physiology, has been working on the question of the influence of diluent gases on the oxygen concentration of the blood. His work is summarized in Appendix A which deals with the metabolic effects of diluent gases.

The long term significance of this research lies in the use of diluent gases to control metabolic rate. In prolonged space flights, for example, it may be important to fly the astronaut in a slightly narcotized state, but during critical maneuvers it is important that the astronaut be
able to function at his maximum effectiveness. Data obtained in experiments reported here indicate that both requirements can be satisfied by carefully selecting the diluent gas to be used during various phases of the flight.

II. METABOLIC EFFECTS OF DILUENT GASES

For the last three years we have studied the physiological effects of nitrogen and certain selected rare gases on rats. The importance of these experiments, from a pragmatic point of view, is the determination of the ideal diluent gas used in prolonged space flight. From a basic research point of view it is important to understand the mechanism by which diluent gases including nitrogen produce physiological effects.

Reports from the laboratory of Harold Weiss and his co-workers at Ohio State University (1), William Pepelko at the School of Aerospace Medicine (2), as well as Leon and Cook (3), suggest that metabolic effects seen in the presence of diluent gases are due essentially to the differences in thermal conductivity between the diluent gas being studied and nitrogen. However, most experiments involving diluent gases other than nitrogen have used helium because of its availability and relative inexpensiveness. As shown by Brauer and others (4, 5), helium is the one member of the rare gas family that tends not to follow a predicted pattern seen in the other members.

We have already reported some experiments in which we replaced nitrogen with neon at one atmosphere and exposed rats to this test.
environment for up to four weeks. Animals exposed to the neon-normoxic environment showed an altered rate of gain in body weight, a dramatic increase in food consumption, a 35% increase in CO$_2$ production, and a 25% greater oxygen consumption with a resultant increase in heat production. The respiratory quotient remained essentially unchanged. The neon-normoxic animals experienced a hematocrit change paralleling high altitude exposure. When these animals were injected with acetate-$^{14}$C, they converted that substrate to $^{14}$CO$_2$ at a considerably faster rate than their comparable air control animals. We concluded from these data that the metabolic rate of animals exposed to a neon-normoxic environment at one atmosphere was increased significantly.

We also reported the results of an experiment in which the rats were exposed to a 5 psia neon-normoxic environment in which the thermal conductivity of the environment was only 43% of that associated with air at one atmosphere. Even under these conditions the animals in the low pressure neon-normoxic environment showed an increase in metabolic rate, suggesting that the metabolic rate effect is due not only to the thermal conductivity factor but may also be attributed, at least in part, to a direct metabolic effect. The experiments described in this report are a first step in evaluating whether diluent gases have an effect other than that precipitated by their thermal conductivity.
Some Theoretical Considerations:

Gases have both density and viscosity. Consequently, it is important to consider whether the work of breathing is influenced by the different densities and viscosities of the diluent gas-oxygen mixtures used in the experiments described in this section. Appendix A contains a detailed discussion of the question, but we concluded that the work of breathing the diluent gases studied will not significantly affect the measured metabolic rate under the conditions of our experiment.

The second question is whether the diluent gases selectively influence oxygen transport to the cell. Both from our theoretical calculations and our experimental studies on the dog, neon and argon act like nitrogen and, thus, do not produce an altered effective pO₂ in the blood. Theoretically, helium does produce an effect which should reduce the effective pO₂ below nitrogen at comparable pressures. The detailed calculations on this question are also found in Appendix A.

We are currently doing experiments on the dog to study the extent to which the theoretical effect produces an actual effect, especially in an environment made up of 160 mm Hg of oxygen and less than 100 mm Hg of helium (our 5 psia helium-normoxic environment).
Experimental Design:

Ten male Holtzman rats were placed in each of two chambers with the average weight of 392 grams each. After they were acclimatized to chamber living for two weeks, baseline data were collected for two weeks. These are referred to as "zero time" data. The gaseous environment was made up of 80% nitrogen and 20% oxygen in both chambers during all four weeks. Subsequently, each chamber was adjusted to pressure and gas mixture as indicated in Figure 1. Note that the experiment is essentially duplicated with only the sequence of environments changed to negate residual effects of the preceding environment. The animals were exposed to each test environment for a period of eight days, data being collected during the first seven days. In between each test-gas environment, the animals were brought back to an 80% nitrogen-20% oxygen environment at 760 mm Hg* for a period of four days. After the animals had been exposed to all the test environments they were re-exposed to the air environment at 760 mm Hg for a period of seven days.

Procedures:

Oxygen consumption and CO₂ production were measured daily as outlined in the April, 1967; October, 1967; and April, 1969 Status Reports.

* Hereafter referred to as the "air environment."
Figure 1. Graphic representation of experimental design showing dual experiments with alternating isothermal environments. Ordinate represents pressure. Abscissa represents duration in days.
Body weight measurements were made on a weekly basis and on each day that a group of animals was injected with acetate-1-$^{14}$C. Food consumption was measured on a weekly basis during the first month when the animals were exposed to "air" at one atmosphere, during the first seven days of exposure to each test gas environment, and during the first three days when the animals returned to the air environment between exposures to the various test gas mixtures. Food consumption was also measured over the last seven days of the experiment when the animals were exposed to an air environment at 760 mm Hg. Monitoring of the environmental conditions was accomplished as outlined in the October, 1967, Status Report.

Within each group of ten animals, five of them were injected each time the protocol called for the injection of acetate-1-$^{14}$C (see Figure 2). This was done because it had been observed in earlier experiments that animals injected on a daily basis became hypersensitive to the injection process and data from them were no longer reproducible. Consequently, in each chamber five of the animals were designated as the "green group" and five as the "black group" for purposes of injection. Each group was injected with acetate-1-$^{14}$C every fourth day throughout the series of the experiments beginning with the acclimatization period. Each animal was injected at the rate of 50 $\mu$C/kg of body weight.
Figure 2. Detailed experimental protocol of environment changes with indicated days of acetate-1-\textsuperscript{14}C injection (\textsuperscript{14}C), days of body weight measurement (B.W.), and days of food weight measurement (F.W.). Additionally, O\textsubscript{2} consumption, CO\textsubscript{2} production, water consumption, and environmental pressure and temperature were measured daily.
During exposure to each of the test environments, groups of five animals were injected on the first, third, fifth and seventh days. The same protocol was followed during the final seven days of exposure to air at one atmosphere which was accomplished at the end of the experiment. During the interim periods of exposure to air at 760 mm Hg which occurred between exposure to two successive environments, groups of five animals were injected on the first and third day. The data presented in this report constitute mean values for the replicate experiments. The technique for measurement of the expired $^{14}\text{CO}_2$ is outlined in the 31 October 1967 and 30 April 1969 Status Reports.

Results and Discussion:

With respect to each of the parameters used to measure metabolic activity, the relationship among argon, nitrogen, and neon was both consistent and predictable while that of helium was consistently lower than would have been predicted. The data with respect to oxygen consumption and CO$_2$ production paralleled very nicely the $^{14}\text{CO}_2$ expiration data from the injection of acetate-$1-{^14}\text{C}$. Using the $^{14}\text{CO}_2$ data as the example, Figure 3 shows the plot of the $^{14}\text{C}$ remaining in the acetate pool versus time, a plot which was first presented in the April, 1969, Status Report as a mechanism of determining the metabolic state of an animal with respect
Figure 3. Plot of Log (\(^{14}\)C injected - \(^{14}\)CO\(_2\) expired) vs time post injection of acetate-1-\(^{14}\)C for four isothermal environments. Curves obtained from normalized means of each series of injections.
to a specific metabolic pool. As was previously described, the slope of the curve is proportional to the rate at which that substrate is converted to $^{14}\text{CO}_2$. Table I summarizes the $k$ values for the slopes of each of these curves and the half-life values in each instance.

Unequivocally, animals exposed to an argon environment catabolize acetate at a slower rate than their comparable air control animals (nitrogen as diluent gas) while rats exposed to the neon-normoxic environment catabolize acetate to $^{14}\text{CO}_2$ at a significantly faster rate. Helium did not accelerate metabolic rate as much as would be predicted. If one plots the slope of these curves (the $^{14}\text{CO}_2$ rate constants) versus the square root of the molecular weight of the diluent gas, a straight line relationship is observed for argon, neon, and nitrogen (see Figure 4). Helium does not fall on the curve.

These data clearly show that the theoretical considerations presented earlier in this section regarding the effects of diluent gas on oxygen transport are qualitatively correct; that is to say, argon and neon had little effect on oxygen transport when compared to nitrogen, while helium caused a reduction in the transport of oxygen. Perhaps helium produced a histotoxic hypoxia at the tissue level which was reflected in a decreased metabolic rate over what one would have predicted for helium. In Figures 5 and 6, plots of the $^{14}\text{CO}_2$ rate constants versus molar refraction and the
TABLE I: K VALUES AND HALF-LIFE VALUES FOR FOUR ENVIRONMENTS.

<table>
<thead>
<tr>
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<th>N₂</th>
<th>Ne</th>
<th>He</th>
<th>Ar</th>
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<td>0.00778</td>
<td>0.00863</td>
<td>0.00914</td>
<td>0.00423</td>
</tr>
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<td>t₁/₂</td>
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<td>164</td>
</tr>
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<td>Group A</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>0.00654</td>
<td>0.01070</td>
<td>0.00816</td>
<td>0.00523</td>
</tr>
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<td>65</td>
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<td>133</td>
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<tr>
<td>Group B</td>
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<td></td>
</tr>
<tr>
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<td>0.00931</td>
<td>0.00867</td>
<td>0.00533</td>
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<td>72.5</td>
<td>80.5</td>
<td>148.5</td>
</tr>
</tbody>
</table>

Mean *

* Mean values normalized for length of chamber exposure.
Figure 6
rate constants versus "solubility parameter" give similarly good plots. All three of these plots are reminiscent of plots concerning narcotizing capacity versus the square root of the molecular weight of the diluent gases (6), narcotizing capacity versus lipid solubility (7), and narcotizing capacity versus molar refraction (8) which relates to the ability to form clathrates. With respect to the other three gases, it is impressive that their effects in a thermally isoconductive environment are so predictable. The data present strong evidence that there is indeed a direct metabolic effect associated with the diluent gas.

Hildebrand (9) defines "solubility parameter" as the square root of the cohesive energy density. This is a measure of the attractive energy per unit volume in the liquid and is a measure of the net attractive energy at the minimum of the potential energy curve. It is a measure, also, of the solubility of a gas in various liquids. The difference in solubility parameters of the gas and the liquid determines the gas solubility; the smaller the difference, the greater the solubility. The solubility parameters of the gases (δr) investigated lie in the lipid range; they are considerably lower than the parameter for water. Plots of total CO₂ production and oxygen consumption against solubility parameter (Figure 7) yield similar slopes and confirm the ¹⁴CO₂ data. It is tempting, therefore, to grossly
Figure 7. Oxygen consumption and CO$_2$ production in L/kg/day plotted against "solubility parameter" - $\delta_T$ of Hildebrand.
describe the effect of the diluent gas upon an interaction between the gas molecules and lipids, perhaps in the membranes.

No correlation was observed with molecular size as calculated from (A) Van Der Waal's "b" and (B) gas viscosity data. No correlation was observed with molecular polarizability. This latter function is a measure of the ease of distortion of electrons to form an induced dipole and is a measure of the attractive potential energy between molecules at intermolecular distances considerably greater than the minimum in the potential energy expression.

Data with respect to water consumption, food consumption, and body weight gain are consistent with the active parameters of metabolism, namely, oxygen consumption, CO₂ production, and the rate of conversion of acetate-1-¹⁴C to ¹⁴CO₂.

We conclude that (a) diluent gases have both a direct metabolic effect which relates to the chemical characteristics of the diluent gas molecule and an indirect metabolic effect which relates to thermal conductivity of the diluent gas as it cools the body surface and (b) the direct metabolic effect is significant. Using data from our October, 1969, Status Report, the direct metabolic effect accounts for approximately one-third of the increase in metabolic rate for a neon-normoxic environment at one atmosphere.
In the next experiment it is obvious that we must correct for the effect of the diluent gas on oxygen transport, at least to obtain comparable data for helium. Under those conditions, the partial pressure of oxygen will be in the vicinity of 176 mm Hg. Although corrections for argon and neon will also be made, it is obvious that in reality these gases have little effect on oxygen transport.
III. EFFECTS OF MARGINAL OXYGEN TOXICITY ON CARBOHYDRATE METABOLISM

In the October, 1969, Status Report an induction of glucose-6-phosphate dehydrogenase in rats exposed to a 5 psia-100% oxygen environment was reported. Additionally, it was reported that neither the specific activity of glyceraldehyde-3-phosphate dehydrogenase or isocitrate dehydrogenase were significantly affected by this environment. Prior to the above report, we had been particularly interested in these enzymes for two reasons: (1) as indicators of pathway activities where glyceraldehyde-3-phosphate dehydrogenase represented glycolytic activity and isocitrate dehydrogenase TCA activity; (2) additionally, glyceraldehyde-3-phosphate dehydrogenase is a sulphydryl enzyme and may be prone to oxidation and subsequent inactivation. If glyceraldehyde-3-phosphate dehydrogenase was shown to be inhibited by the 100% oxygen environment, this could have been an explanation for the shift in carbohydrate metabolism toward the pentose phosphate pathway. Since inactivation of these enzymes proved not to be the cause of the increase in shunt activity, we have shifted our attention to enzymes of the TCA cycle.

We do not feel glucose-6-phosphate dehydrogenase is directly affected by oxygen. The induction of glucose-6-phosphate dehydrogenase
is almost certainly a consequence of an inhibition of one of the enzymes of glycolysis or the TCA cycle. Therefore, the increase in glucose-6-phosphate dehydrogenase activity is of adaptive significance, not a direct effect of the oxygen molecule per se. We are approaching the key experiment that should identify the site of blockage. These experiments will involve the injection of different TCA intermediates into the animals. Any injected substrate that is metabolized before the blockage will have no effect on glucose-6-phosphate dehydrogenase, or perhaps even potentiate the induction through negative feedback by TCA intermediates. Substrates metabolized after the blockage will decrease the induction of glucose-6-phosphate dehydrogenase. One experiment of this type involving succinate as the injected substrate has already been completed.

Also in this report is evidence that the induction by the 5 psia-100% oxygen environment of glucose-6-phosphate dehydrogenase is a true induction, that is, synthesis of new protein. Both actinomycin D and cycloheximide, two antibiotics recognized as inhibitors of protein synthesis, significantly inhibited the induction of glucose-6-phosphate dehydrogenase. In addition to monitoring the enzymatic activity of glucose-6-phosphate dehydrogenase and succinic dehydrogenase, blood glucose concentration, food consumption, hematocrit levels, $^{14}$CO$_2$ expiration from glucose-$^{1-14}$C.
and glucose-6-\textsuperscript{14}C, O\textsubscript{2} consumption and CO\textsubscript{2} production, and incorporation of glucose-1-\textsuperscript{14}C and glucose-6-\textsuperscript{14}C into glycerol were also measured in these experiments.

**Experimental:**

Five experiments with the same basic experimental design have been completed (see Figure 8); the only variations in the five experiments were the nature of the substrate injected and the enzymes that were assayed.

In addition to glucose-6-phosphate dehydrogenase activity, succinic dehydrogenase activity was also recorded in the glucose-1-\textsuperscript{14}C, the glucose-6-\textsuperscript{14}C, and the succinate injection experiments. In the experiments involving actinomycin D and cycloheximide, glucose-6-phosphate dehydrogenase was the only enzyme that was assayed.

In each experiment 10 male Holtzman rats (220-240 gm) were allowed to acclimate to chamber living at 760 mm Hg for a period of two weeks. At the end of the two-week period, 5 rats were selected at random as controls and the other 5 as experimental animals. The control animals were maintained in air at sea level pressure and the experimentals in 100% oxygen at 5 psia. The appropriate substrate or antibiotic was then injected into both the controls and experimentals. All of the animals were autopsied after 2 days exposure.
<table>
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<tr>
<th>Exp</th>
<th>Substrate or Antibiotic &amp; Concentration Injected</th>
<th>Days of Acclimatization</th>
<th>Days of Exposure</th>
<th>Days Injections Made</th>
<th>Enzymes Assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Actinomycin D 10µg/100g BW</td>
<td>14</td>
<td>2</td>
<td>0, 1, &amp; 2</td>
<td>Glu-6 PDH</td>
</tr>
<tr>
<td>2</td>
<td>Cycloheximide 750µg/kg BW</td>
<td>14</td>
<td>2</td>
<td>0, 1, &amp; 2</td>
<td>Glu-6 PDH</td>
</tr>
<tr>
<td>3</td>
<td>Glucose -1-¹⁴C 150µc/kg BW</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>Succinic DH</td>
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<tr>
<td>4</td>
<td>Glucose -6-¹⁴C 150µc/kg BW</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>Succinic DH</td>
</tr>
<tr>
<td>5</td>
<td>Succinate 5 ml of 0.4 M</td>
<td>14</td>
<td>2</td>
<td>0, 1, &amp; 2</td>
<td>Succinic DH</td>
</tr>
</tbody>
</table>

Figure 8. Protocol for five experiments at 5 psia-100% oxygen showing substrate injected, injection rates, days acclimatization, days exposure, and injection schedule.
At autopsy the animals were guillotined and the blood collected in heparinized beakers. Samples of the blood were placed in hematocrit tubes for centrifugation and subsequent analysis. A two-gram piece of liver was homogenized in cold 0.14 M KCl in a Potter-Elvehjem homogenizer. The homogenate was then centrifuged at 65,000 X g for a period of 30 minutes. The resultant supernatant was used for glucose-6-phosphate dehydrogenase analysis according to the procedure of Szepesi and Freedland (10) using a Beckman DBG recording spectrophotometer at 340 mu.

An additional two-gram piece of liver was removed and homogenized in 0.25 M sucrose and the mitochondria prepared according to Schneider and Hogoboom (11). The succinic dehydrogenase assays were performed on these mitochondria according to the procedure of Green, et al. (12). The remainder of the liver was weighed and frozen in liquid nitrogen and stored for subsequent analysis.

Results:

The data obtained from the actinomycin and cycloheximide experiments clearly show that the increase in glucose-6-phosphate dehydrogenase activity is a true induction. Figure 9 shows that the induction which is usually seen at 24-48 hours is eliminated almost entirely by the administration of either antibiotic. It is apparent that new protein biosynthesis must
Figure 9. Reduction in glucose-6-phosphate dehydrogenase induction by inhibitors of protein synthesis. Oxygen induction represents glucose-6-phosphate dehydrogenase activity normally found in rats exposed to 5 psia-100% oxygen.
occur for the increase in shunt activity to appear. Therefore, new protein biosynthesis is necessary for the adaptation itself.

Two additional experiments involving glucose-1-\(^{14}\)C and glucose-6-\(^{14}\)C as the injected substrates have been completed. From Figure 10 it is evident that both groups of animals expired approximately the same amount of \(^{14}\)CO\(_2\) from glucose-1-\(^{14}\)C. However, the \(^{14}\)CO\(_2\) expiration from glucose-6-\(^{14}\)C was quite depressed in the experimental animals when compared to control animals (Figure 11). The interpretation is that more carbohydrate was metabolized via the pentose phosphate pathway in the experimental animals compared to the air controls. Additional data consisting of \(^{14}\)C incorporate into glycerol has also been collected. Using the incorporation of glucose-1-\(^{14}\)C and glucose-6-\(^{14}\)C into glycerol, we are able to determine the importance of the pentose phosphate pathway to carbohydrate metabolism as follows: Both in vivo and in vitro the activity of liver triosephosphate isomerase is not adequate to equilibrate the triose-phosphates. Indeed, Schambye, et al. (13) have reported that liver glycogen formed from \(^{14}\)C glycerol had more \(^{14}\)C in carbons 1, 2, and 3 than in carbons 4, 5, and 6; and the reverse was observed with pyruvate and \(^{14}\)CO\(_2\). Rose, et al. (14) have shown that such is the result of a slow rate of isomerization via triosephosphate isomerase. Thus, it is apparent that
Figure 10. Cumulative DPM from glucose-1-$^{14}$C indicating approximately equal expiration of $^{14}$CO$_2$ from experimentals and controls.
Figure 11. Cumulative DPM from glucose-6-$^{14}$C indicating depression of $^{14}$CO$_2$ expiration in the experimental animals.
dihydroxyacetone phosphate is a preferential precursor of glycerol while glyceraldehyde-3-phosphate is preferentially incorporated into fatty acids.

It should, therefore, be possible to adjust the amount of $^{14}$CO$_2$ expired as a result of catabolism of glucose-$1^{-14}$C or glucose-$6^{-14}$C to reflect the true picture if one also has measured incorporation of these particular substrates into glycerol, i.e., one should be able to normalize the $^{14}$CO$_2$ output data. We have done this utilizing the ratio:

$$\frac{\text{in glycerol from glucose-$1^{-14}$C}}{\text{in glycerol from glucose-$6^{-14}$C}} = \delta$$

as suggested by Katz, et al. (15). Our further calculations are made on the presumption that glucose-$1^{-14}$C can be catabolized to $^{14}$CO$_2$ via the pentose phosphate pathway as well as the glycolytic pathway and its attendant tricarboxylic acid cycle while labels from glucose-$6^{-14}$C show up in $^{14}$CO$_2$ only as the result of metabolism via the glycolytic pathway + TCA cycle. Therefore, the relative importance of the pentose cycle is:

$$\frac{^{14}\text{CO}_2(G1) - \frac{1}{\delta}^{14}\text{CO}_2(G6)}{^{14}\text{CO}_2(G1)} \times 100 = \% \text{PPP}$$

where $^{14}$CO$_2(G1)$ and $^{14}$CO$_2(G6)$ represent the total amount of $^{14}$CO$_2$ expired over a three-hour period from injected glucose-$1^{-14}$C and glucose-$6^{-14}$C, respectively.
Using this approach, the data (Table II) clearly show a marked increase in the importance of the pentose phosphate pathway in rats exposed to a 5 psia-100% oxygen environment. The experimental animals are metabolizing twice as much carbohydrate through the shunt, and 20% less through glycolysis and the TCA cycle. This is strong support for our hypothesis that a block in metabolism in the glycolytic or TCA pathways is responsible for the increased shunt activity that we see.

**TABLE II. CARBOHYDRATE METABOLIZED VIA GLYCOLYSIS PLUS TCA CYCLES VS. PENTOSE PHOSPHATE PATHWAY.**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>% GLYCOLYSIS and TCA</th>
<th>% PPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air 760 mm Hg</td>
<td>80.43</td>
<td>19.57</td>
</tr>
<tr>
<td>100% oxygen 259 mm Hg</td>
<td>59.44</td>
<td>40.56</td>
</tr>
</tbody>
</table>

As mentioned previously succinic dehydrogenase was monitored in both the glucose-1$^{14}$C, the glucose-6$^{14}$C and the succinate injection experiment. There was strong evidence that succinic dehydrogenase might be susceptible to changes in oxygen partial pressures. Ramasarma, et al. (16) reported an increase in succinic dehydrogenase activity in hypoxic
environments. Therefore, we decided there might be an inhibition of succinic dehydrogenase in a hyperoxic environment. However, there were no significant changes in succinic dehydrogenase activity in any of these experiments indicating that succinic dehydrogenase was not the site of blockage.

The fifth experiment involved the injection of 5 ml of 0.4 M succinate. It has been demonstrated previously that succinate is an effective prophylactic against oxygen toxicity and its injection might reduce the stress we see (17). Figure 12 shows that succinate was successful in reducing the induction of glucose-6-phosphate dehydrogenase in our animals. However, the reduction is not as pronounced as the figure would indicate. The control animals’ specific activity was 30% higher in this experiment when compared to control values in previous experiments. Apparently, this is the result of the succinate injection. Actually, when glucose-6-phosphate dehydrogenase activity in the experimentals in this experiment is compared to previous control levels, the induction is only slightly depressed. This limited ability of succinate to reduce shunt activity indicates that the block in metabolism which results in increased pentose shunt activity is probably before succinic dehydrogenase in the TCA cycle. In order for a complete protection from oxygen toxicity to be achieved, succinate

36
Figure 12. Reduction in glucose-6-phosphate dehydrogenase induction through the administration of cycloheximide or succinate. Oxygen induction represents glucose-6-phosphate dehydrogenase activity normally found in rats exposed to 5 psia-100% oxygen.
would probably have to be continually infused into the animals. Single
injections apparently help but do not entirely restore normal metabolism.

As a result of experiments now in progress, we should be able to
pinpoint the block in metabolism and elucidate the mechanism responsible
for the shift in metabolism from glycolysis and the TCA cycle towards
the pentose phosphate pathway (Figure 13).

The usual depression in hematocrit was noticed in the experimental
animals although over this short period of exposure to 100% oxygen (2 days)
the reduction is slight (Table III).

**TABLE III. EXPERIMENTAL HEMATOCRIT VALUES AS A PERCENT
OF CONTROLS.**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycin D</td>
<td>98</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>92</td>
</tr>
<tr>
<td>Glucose-1-(^{14})C</td>
<td>96</td>
</tr>
<tr>
<td>Glucose-6-(^{14})C</td>
<td>97</td>
</tr>
<tr>
<td>Succinate</td>
<td>93</td>
</tr>
</tbody>
</table>

An interesting observation has been noticed in the food consumption
data (Table IV). The experimental animals eat approximately 7% less food
Figure 13. Glycolytic and TCA pathways indicating likely points of oxygen inhibition. Bars indicate suspect enzymatic steps. Those marked # are points known not to be sites of blockage from previous experiments. The remainder are currently being studied.
TABLE IV. FOOD CONSUMPTION IN GRAMS/DAY/KG. BODY WEIGHT.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>CONTROLS</th>
<th>EXPERIMENTALS</th>
<th>% OF CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycin D</td>
<td>69.67</td>
<td>65.60</td>
<td>94</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>63.97</td>
<td>55.98</td>
<td>88</td>
</tr>
<tr>
<td>Glucose-1-(^14)C</td>
<td>92.82</td>
<td>86.35</td>
<td>93</td>
</tr>
<tr>
<td>Glucose-6-(^14)C</td>
<td>80.70</td>
<td>75.47</td>
<td>94</td>
</tr>
<tr>
<td>Succinate</td>
<td>71.10</td>
<td>65.50</td>
<td>92</td>
</tr>
</tbody>
</table>

per kilogram of body weight when compared to the control rats. This is particularly important when one realizes that it has been established by Tepperman and Tepperman (18) that pentose shunt activity drops as food consumption decreases; however, in our experimental animals pentose phosphate pathway activity is actually increasing, although their food consumption is down.

The experimentals also showed an increased oxygen consumption when compared to control values (Table V). These data are based on a limited number of readings, but the increased oxygen consumption is strikingly parallel in time frame to the induction in glucose-6-phosphate dehydrogenase. Both appear to be maximal at about 48 hours exposure to the 100% oxygen environment.
TABLE V. OXYGEN CONSUMPTION.

<table>
<thead>
<tr>
<th>EXPOSURE TIME HOURS</th>
<th>LITERS/DAY/KG. BODY WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32.00 (19)</td>
</tr>
<tr>
<td>12</td>
<td>34.86</td>
</tr>
<tr>
<td>24</td>
<td>34.20</td>
</tr>
<tr>
<td>36</td>
<td>48.14</td>
</tr>
<tr>
<td>48</td>
<td>40.18</td>
</tr>
</tbody>
</table>

There were no statistical differences in blood glucose concentrations in any of the five experiments. However, in the experiments involving actinomycin D and cycloheximide, the animals had higher blood glucose concentration than in previous experiments (Table VI).

TABLE VI. MEAN BLOOD GLUCOSE CONCENTRATIONS MG/%.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>CONTROLS</th>
<th>EXPERIMENTALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycin D</td>
<td>136.86</td>
<td>158.87</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>126.91</td>
<td>131.53</td>
</tr>
<tr>
<td>Glucose-1(^{14})C</td>
<td>105.24</td>
<td>106.34</td>
</tr>
<tr>
<td>Glucose-1(^{14})C</td>
<td>108.06</td>
<td>108.36</td>
</tr>
<tr>
<td>Succinate</td>
<td>107.71</td>
<td>121.78</td>
</tr>
</tbody>
</table>
However, the increase was probably the usual effect of the antibiotics. Glycogen depletion and blood glucose increases are usual results of treatment with inhibitors of protein synthesis, Bitman, et al. (20).

Discussion:

It is apparent that the induction of glucose-6-phosphate dehydrogenase is a true induction of new protein synthesis. Additionally, this induction is probably the result of an inhibition of one of the enzymes of glycolysis after glyceraldehyde-3-phosphate dehydrogenase, or an enzyme located between pyruvate dehydrogenase and succinic dehydrogenase in the TCA cycle. If the particular enzyme involved in this inhibition is identified, then the mechanism involved here may be basic in overt as well as marginal oxygen toxicity. Regardless of the severity of the oxygen stress, the basis for any change in metabolism could be the same. If the cell were to have a block in the TCA cycle, available ATP stores would be rapidly depleted and convulsions would be the result. In our system the toxicity is mild and the animal is able to adapt through energy transfer from the pentose phosphate pathway. If the stress were more severe, say 5 atm at 100% oxygen, the ATP would be so rapidly and irreversibly depleted that death would certainly occur. Therefore, many of the rapidly appearing symptoms of overt oxygen toxicity may be explained through this simple imbalance in adenylate energy charge within the cell.
IV. TRACE METAL ALTERATIONS INDUCED BY A 5 PSIA-100% OXYGEN ENVIRONMENT

The particulars of this experiment were reported in detail in the 31 October 1969 Status Report and constitute the principal part of the thesis submitted in partial fulfillment of the requirements for the degree of Master of Science by David N. Lankford. The main thrust of this work revolves around the significant role played by cations in enzymatic reactions with particular emphasis on those involved in oxidation reactions, namely iron, copper, and zinc.

After acclimatization to chamber living at one atmosphere in air for a period of two weeks, groups of animals were exposed to the 5 psia-100% oxygen environment for periods of 1, 2, 4, 7, 14, or 28 days. The animals were sacrificed in the test environment; subsequently, the liver, thymus, heart, spleen, kidney, lung, brain, red blood cells, and blood serum were prepared for trace metal analysis. Additionally, an aliquot of each liver was homogenized and fractionated into mitochondria, microsomes, and cytoplasmic fractions for trace metal determinations. The specifics of the technique using the atomic absorption spectrophotometer are outlined in the 31 October 1969 Status Report. The absolute values reported for each fraction in each metal with the statistical treatment of
the data are presented in detail in David Lankford's thesis. However, there appear to be some general conclusions which can be summarized.

The trend of the data is clear; the metabolically vital organs tend to concentrate the three cations in question. The heart showed an increase in concentration of all three metals; the kidneys showed an increase in both copper and zinc with iron essentially remaining unchanged. The liver and the various fractions of the liver generally showed an increase in both iron and zinc throughout the experiment with no bold change in the amount of copper present. The lung consistently showed an impressive increase in iron and zinc with a decrease in copper. The lung results may be related to the lability of cytochrome oxidation in comparison with other cytochrome chain enzymes since this tissue is openly exposed to the hyperoxic environment.

The translocation of cations observed in the four tissues above seem to occur at the expense of organs which are not metabolically as vital. The thymus, for example, showed a decrease in all three metals either on an absolute basis or on a concentration basis; this parallels other data showing the involution of the thymus gland. The brain lost iron and copper while zinc was apparently unaffected. One is tempted to predict that other organs which are not so immediately involved in the homeostatic mechanism of the animal may be additional sources of cations.
With respect to the hematologic system, the spleen shows an increase in iron which paralleled the data by Kaplan (21) which indicate an increase in the destruction of red blood cells involving principally older red blood cells. There were no bold changes with respect to copper or zinc in the spleen. There was a decrease in both iron and copper in red blood cells concomitant with a tendency towards higher values for zinc. This may reflect a deficiency of iron and copper in the bone marrow but also might be interpreted as a part of the homeostatic mechanism reducing the amount of oxygen being carried to the cell by reducing the amount of carrier cations in the blood. These changes in the red blood cell cation content were observable in terms of total concentration of the cations in the red blood cells and on a relative basis, that is, cation concentration per milligram of red blood cells. Consequently, the decrease in concentration of iron and copper was not simply a reflection of the decreased hematocrit value. It is interesting that cation concentrations within the serum were essentially unaffected by this environmental stress.

In preparing this work for publication, interpretation is being approached from the following four points of view: (a) individual metal, (b) individual tissue, (c) key enzymes or enzyme systems, and (d) bioenergetic pathways. Each of these points of view provides an interesting story which will be presented in the next status report.
APPENDIX A

The following is a detailed discussion of the theoretical considerations of the effect of diluent gas on oxygen transport briefly summarized in Section II.
THEORETICAL CONSIDERATIONS OF THE EFFECT OF INERT GASES ON OXYGEN TRANSPORT

In developing the experimental design, certain theoretical considerations should be evaluated. Despite the fact that metabolic rate has been shown to be independent of the oxygen partial pressure (22), the use of different diluent gases as replacement for nitrogen raises the following two questions: Is the work of breathing (and therefore metabolic rate) influenced by the different densities and viscosities of the gas mixtures used? Do the diluent gases selectively influence oxygen transport to the cell?

Since the mixtures to be tested vary both in composition and pressure, it is necessary to ask whether or not their differences in viscosity (\( \eta \)) and density (\( \rho \)) might contribute to metabolic rate by differentially influencing the work of breathing. The following approximation of these effects were, therefore, made.

The intrathoracic pressure, which the respiratory muscles must generate to overcome the resistance to gas flow in the lungs is given by:

\[ P = k_1 \dot{V} + k_2 \dot{V}^2 \]
where: $\dot{V} = \text{time derivative of gas flow}$

- $k_1 = \text{proportionality constant based on the effect of viscosity}$
- $k_2 = \text{proportionality constant based on the effect of density}$.

In general, the $k_1 \dot{V}$ term characterizes the laminar flow component while $k_2 \dot{V}^2$ represents the turbulent flow factor. Breathing, thus, entails both laminar and turbulent gas flow with laminar predominating at rest and during light work, while turbulent flow increases with increasing work load and ventilation rate. Although almost impossible to measure, it is necessary to approximate the degree to which each of these flow components influences gas flow and the intrathoracic pressure at "normal" ventilation rates. The Reynold's Number ($N_R$) approximates this by the equation:

$$N_R = \frac{2 \rho \dot{V}}{\pi r \eta}$$

where:

- $\rho = \text{gas density in g/cm}^3$
- $\dot{V} = \text{ventilation rate in ml/sec}$
- $r = \text{tube radius in cm}$
- $\eta = \text{gas viscosity in poise}$.

Using standard viscosity and density values (Table VII), the relative densities, viscosities, and Reynold's Number of the various mixtures were calculated and are listed in Table VIII.

As a rough guideline, $N_R$ values less than 2000 are considered primarily laminar in nature, those above 4000 primarily turbulent, with intermediate figures representing a combination of both. Since the lining of the

48
Table VII. Standard values for viscosity ($\eta$) and ($\rho$) (23).

<table>
<thead>
<tr>
<th>Gas</th>
<th>$\eta^*$</th>
<th>$\rho^{**}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>189</td>
<td>1.429</td>
</tr>
<tr>
<td>Helium</td>
<td>186</td>
<td>1.178</td>
</tr>
<tr>
<td>Neon</td>
<td>297</td>
<td>0.900</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>168</td>
<td>1.250</td>
</tr>
<tr>
<td>Argon</td>
<td>209</td>
<td>1.784</td>
</tr>
</tbody>
</table>

* in micropoise at 273°K
** in g/L at 273°K

Table VIII. Calculated values of gas mixtures for density, viscosity, and Reynolds's number ($N_R$) at 273°K. All mixtures contain oxygen at 160 mm Hg partial pressure.

<table>
<thead>
<tr>
<th>Mixture</th>
<th>$P_T^*$</th>
<th>$\rho$</th>
<th>$\eta$</th>
<th>$N_R^{**}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argon-oxygen</td>
<td>1000</td>
<td>1.728</td>
<td>205</td>
<td>655</td>
</tr>
<tr>
<td>Nitrogen-oxygen</td>
<td>760</td>
<td>1.287</td>
<td>172</td>
<td>580</td>
</tr>
<tr>
<td>Neon-oxygen</td>
<td>515</td>
<td>1.065</td>
<td>258</td>
<td>320</td>
</tr>
<tr>
<td>Helium-oxygen</td>
<td>259</td>
<td>.901</td>
<td>188</td>
<td>372</td>
</tr>
</tbody>
</table>

* Total pressure in mm Hg.
** Using $\bar{V} = .073$ L/min (24) and $r = .01$ cm (25).

bronchial tree is not smooth, predominantly turbulent flow in the lungs occurs at values around 3000. On this basis, we can see from the calculated $N_R$ for the different mixtures (Table VIII) that resting ventilation rate effects mainly laminar flow and that a five- to ten-fold increase in ventilation would be necessary before turbulent flow would be predominant, if tube diameters do not vary appreciably.
Assuming, then, that we can base our calculations on laminar flow, we see that the pressure-viscosity relationship can be characterized by:

\[ P_1 - P_2 (\Delta P) = 8 \eta l \dot{V}/r^4 \]

where:
- \( \Delta P \) = the pressure gradient between the environment and the lungs during inspiration and expiration
- \( \eta \) = gas viscosity in poise
- \( l \) = tube length in cm
- \( \dot{V} \) = ventilation in ml/sec
- \( r \) = tube radius in cm.

Assuming tube dimensions and ventilation are constant, the equation can be expressed:

\[ P = k \eta \]

It is clear that as viscosity of the gas increases so too must the pressure generated, the latter entailing more work by the respiratory muscles. If the ability to generate a pressure differential becomes maximal, any further increase in viscosity would necessitate a lowered flow rate.

Using the average ventilation (\( \dot{V} = .073 \text{ L/min} \)) and energy output (115 kcal/kg/day) for a 150 gm rat (24), we can calculate the energy used by the rat in respiration over a 24 hour period as:

\[ W = \dot{V} \Delta P = (.073 \text{ L/min}) (\frac{4}{768} \text{ atm}) (1440 \text{ min/day}) \left( \frac{1.98 \text{ cal}}{.082 \text{ L-atm}} \right) = 13.4 \text{ cal/day} \]
Since the efficiency of breathing averages 8-12%, the total energy to be produced would be approximately:

\[(13.4 \text{ cal/day}) \times 10 = 134 \text{ cal/day}\]

As a percentage of the total energy output, this value represents:

\[
\frac{134 \text{ cal/day}}{(.150 \text{ kg}) (115 \text{ kcal/kg/day})} = .8\%
\]

Based on similar calculations for both energy and oxygen consumption in man (1-5%), this value is somewhat low; but it does appear to be in the general range that is usually quoted (26). Since the viscosity values of the different gases (Table VIII) range only 50% greater than that for air, we can assume that the percent of work in breathing would not deviate more than half again as much as the above figure. On that basis, we concluded that the work of breathing the diluent gases studied under all but heavy exercise or emotional conditions will not significantly affect the measured metabolic rate.

This treatment admittedly has been crude, but it seems unlikely that the calculations have not been in error by a factor of more than two or three and that, given the rather great difference between our calculated value and one which would be considered significant (5%), we are confident
that the experimental results can be interpreted without undue concern about the effect of the work of breathing. The second question to be resolved deals with the effect, if any, of the various rare gases upon transport of oxygen and carbon dioxide from the alveoli to the blood and thence by the blood to the tissues. Specifically, we must determine whether or not the animals in one environment might be hypoxic relative to those in another. Hypoxia in a group of animals would lead to increased ventilation and protein turnover both of which influence metabolic rate.

The works of Featherstone, et al. (27) and others (28, 29) have conclusively shown that proteins can undergo structural changes when combined with biologically inert anesthetic gases. Since the membranes through which oxygen must diffuse and hemoglobin itself both contain protein, there is reason to ask whether or not this phenomenon influences oxygen transport. If the "inert" gas bound to the protein were to sterically hinder oxygen passage through membrane or chemically hinder oxygen-heme interaction, one would expect to see an altered oxygen-carrying capacity. More specifically, Overfield, et al. (30) observed a doubling of the alveolar-arterial oxygen tension difference \((A-a \text{ DO}_2)\) in men exposed to a 99.1% helium-0.9% oxygen environment at 31.3 Ata. They concluded that the increased "inert" gas partial pressure was probably the
cause but felt the effect was not biologically significant. Most dramatic is the work of Altland, et al. (31) who demonstrated a distinctly patterned mortality rate in rats exposed to different diluent gas-oxygen mixtures at one atmosphere where the oxygen fraction was varied between 20.93% and 4.9%. The mortality rate increased with increasing hypoxia and the inert gas used as follows:

$$\text{He} < \text{N}_2 < \text{A} < \text{SF}_6.$$  

The site of the apparent diluent gas potentiation of hypoxia was not studied but blood gas transport cannot be overlooked.

The two most probable loci of action are the alveolar-arterial exchange and oxygen binding by hemoglobin. If the former site was affected, one would expect to see a variation in the difference between the alveolar oxygen tension ($P_{A\text{O}_2}$) and that of the blood ($P_{a\text{O}_2}$). There are several factors (exercise, disease, anesthesia) which enlarge this difference, making data interpretation difficult. With a "normal" range of 2-10 mm Hg, we would consider a value on the order of 15-20 mm Hg as significant. However, the (A-a) $DO_2$ would not be meaningful if the $P_{a\text{O}_2}$ of the various animals were approximately equal in the different environments. Since the ability to load hemoglobin with oxygen is dependent on
the $P_aO_2$, a measurement of the $P_aO_2$ versus oxygen saturation (OS) is necessary. If these parameters are dissimilar between the test environments, we would suspect diluent gas interference with oxygen-heme interaction. Specifically, if this were to occur, a higher $P_aO_2$ would be required to produce the same OS as that measured in an environment in which there was interference. Alternatively, one might see a lesser OS at an equivalent $P_aO_2$ in an atmosphere which effects this phenomenon. It would be assumed, then, that oxygen transport to the tissues would be relatively deficient.

Since a normal $P_aO_2$ determination is difficult to make in animals, alveolar oxygen pressure was estimated using the alveolar gas equation (32):

$$P_{A\text{O}_2} = F_{\text{I}\text{O}_2}(P_T - P_{H_2O}) - P_{A\text{CO}_2}\left(F_{\text{I}\text{O}_2} + \frac{1 - F_{\text{I}\text{O}_2}}{R}\right)$$

where: $F_{\text{I}\text{O}_2}$ = fraction of inspired oxygen
$P_T$ = total barometric pressure
$P_{H_2O}$ = vapor pressure of water at 37°C
$P_{A\text{CO}_2}$ = partial pressure of carbon dioxide in the alveoli
$R = \text{gas exchange ratio}, \ CO_2/O_2$.

The calculated values are given in Table IX.
Table IX. Calculated values for $P_A O_2$ in the various environments.

<table>
<thead>
<tr>
<th>Mixture</th>
<th>$F_1O_2$</th>
<th>$P_A O_2$*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argon-oxygen</td>
<td>.16</td>
<td>106.6</td>
</tr>
<tr>
<td>Nitrogen-oxygen</td>
<td>.21</td>
<td>103.4</td>
</tr>
<tr>
<td>Neon-oxygen</td>
<td>.31</td>
<td>100.2</td>
</tr>
<tr>
<td>Helium-oxygen</td>
<td>.62</td>
<td>88.4</td>
</tr>
</tbody>
</table>

assumes: $P_A CO_2 = 40$ mm Hg

$P_{H_2O} = 47$ mm Hg

$R = 0.85$

To test the theoretical calculations, we sampled arterial blood from the aorta of mongrel dogs for the $P_a O_2$ and OS determinations. Initially, anesthetized dogs were tested using constant respiratory conditions. After the animal was anesthetized and intubated to deliver the breathing mixture, the left common carotid artery was exposed and a teflon catheter was passed through it into the lumen of the aortic arch. After the entire preparation and technician were taken to the experimental pressure, a pneumothorax was performed, and the dog was artificially respired using the test gas mixture delivered by a Bennett respirator at constant rate and pressure of delivery. The animal was respired 15-20 minutes with the test mixture to assure saturation of the blood with the gas, after which samples were drawn under anaerobic conditions. The preparation was then taken to the next experimental pressure and the procedure repeated.
Additionally, we have used alert dogs comfortably restrained in a sling with a chronically implanted catheter and breathing through a fitted mask. With adequate training, the animal can be induced into a resting state for prolonged periods of time such that it can be tested successively in all the experimental environments with little or no perturbation.

All blood samples were chilled to 0°C until analysis which was carried out in duplicate on a Radiometer blood-gas apparatus equipped with microelectrodes and an American Optical Oximeter at ambient pressure (632 mm Hg). The results are shown in Tables X.

Table X. Averages for arterial blood-gas parameters for anesthetized and alert dogs.

| Mixture  | n  | pH  | $P_a$CO$_2$ | $P_a$O$_2$ | OS  | (A-a)DO$_2$ *
|---------|----|-----|-------------|------------|-----|-----------
| Ar-O$_2$ | 10 | 7.46| 30.1        | 74.2       | 94.4| ----      
| N$_2$-O$_2$ | 12 | 7.44| 32.7        | 72.6       | 93.2| ----      
| Ar-O$_2$ | 6  | 7.41| 26.0        | 93.8       | 97.3| 28.9      
| N$_2$-O$_2$ | 10 | 7.41| 30.2        | 95.5       | 96.6| 20.1      

* mm Hg
** based on calculated $P_a$CO$_2$ using actual $P_a$CO$_2$ measured

Interpretation of the data for anesthetized dogs must be made on a relative basis since the effects of anesthesia cannot be quantified. Despite
controlled respiration, the animals had a fairly wide $P_a CO_2$ range (25-45 mm Hg) which also makes interpretation difficult. However, it can be seen that the $P_{aO_2}$ and OS compared favorably in air and argon-oxygen; and we feel that any differences are accounted for by experimental error.

The preliminary results for alert dogs are very encouraging since the values are almost identical with those reported for air (33), and the range of values was quite narrow indicating good reproducibility. Here too, differences in $P_{aO_2}$ and OS are probably due only to experimental error. The rather large $(A-a)DO_2$ values are almost certainly due to the somewhat hyperventilated state of the animals, a problem which we are presently working to resolve. While the apparent difference in $(A-a)DO_2$ between air and argon-oxygen may be real, it has little biological significance since the end result ($P_{aO_2}$) seems to be nearly identical in both cases.

We intend to expand the study with alert dogs using argon-oxygen, helium-oxygen, and air with the goal of obtaining accurate and statistically valid data for these blood parameters in these atmospheres. Neon-oxygen will probably not be tested due to its prohibitive cost; but it is reasonable to assume that, since it falls within a range of expected values bounded by argon-oxygen and helium-oxygen, it will not be significant with regard to
hemoglobin binding of oxygen. It will be tested, though, if necessary. The results of this study will then be used in the design of a complementary experiment.
APPENDIX B

The following is the abstract of a paper entitled, "Aberrational Effects of Space Capsule Atmospheres on Rat Brain Coenzyme A Content," which was presented at the American Federation for Clinical Research meeting held in Oklahoma City, Oklahoma, on 7 April 1970.
ABERRATIONAL EFFECTS OF SPACE CAPSULE ATMOSPHERES ON RAT BRAIN COENZYME A CONTENT*

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Coenzyme A (CoA) and acetyl coenzyme A (AcCoA) concentrations were determined in the brains of male Holtzman rats exposed to proposed space capsule breathing mixtures including:

a) 5 psia-100% Oxygen \((\text{PO}_2 - 235 \text{ mm Hg})\)
b) 5 psia-Oxygen-Neon \((\text{PO}_2 - 160 \text{ mm Hg})\)
c) 5 psia-Oxygen-Nitrogen \((\text{PO}_2 - 160 \text{ mm Hg})\)
d) One atmosphere-Oxygen \((\text{PO}_2 - 235 \text{ mm Hg})\)

A dramatic decrease which occurred at the end of the two weeks in the AcCoA content of the brains of animals exposed to the 100% Oxygen-5 psia atmosphere was not observed in the brains of animals exposed to any of the other three atmospheres.

It was concluded that the observed decrease in the AcCoA content of the brains of the animals was precipitated by the increased \(\text{PO}_2\) while the absence of any such aberration on the brains of the animals exposed to the hyperoxic-Nitrogen atmosphere was most probably due to a "masking" effect of the Nitrogen on Oxygen.
VI. REFERENCES


33. Donald W. DeYoung, Colorado State University, personal communication.