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ABSTRACT

In addition to efforts focused towards the identification of rare-gas effects on metabolism, we have begun experiments to identify the nature of "inert gas narcosis." The most prevalent theories regarding the physiological effects of rare gases involve an effect of the gases on biomembranes and the interaction of the gases specifically at the nerve synapse.

With respect to the proposed biomembrane mechanism, a collaborative research program has been undertaken with the Royal Naval Physiological Laboratory, Alverstoke, Hants, England. This study involves the detailed examination of the theory that narcosis results from expansion of the cell membrane under high partial pressures. The research is partially based on the hypothesis that, like oxygen toxicity, the mechanism of metabolic effects of rare gases may be similar at both low and high pressures and are simply more observable at high pressures.

Using adult female goats, the parameters measured include oxygen consumption, CO₂ production, respiration rate, heart rate, rectal and skin temperatures and the analysis of electroencephalograms and evoked response. Additionally, we are measuring the specific activity of plasma glucose subsequent to injection of glucose-UL-¹⁴C, intravenous infusion, specific activity of expired CO₂, unesterified fatty acid levels and
whole blood lactate-to-pyruvate ratios. Also being studied are the effects of acetylsalicylic acid, vitamin E and cationic detergents (which alleviate narcosis) upon metabolic changes induced by high pressure narcosis.

Examination of rare gas effects on the nerve synapse is being investigated using rat brain synaptosomes. Synaptosomes were isolated by discontinuous sucrose centrifugation from rats exposed to normoxic mixtures of argon, helium, and nitrogen and oxygen with all preparative and analytical procedures being performed in glove bags filled with the respective test atmospheres. Oxygen consumption rates for synaptosomes isolated from argon exposed rats were only 70% of the consumption rates for those isolated from nitrogen exposed rats. Acetylcholinesterase activity measurements, on the other hand, are increased by 8% in argon relative to nitrogen. Helium-oxygen experiments are not sufficiently complete to evaluate with confidence. These preliminary results suggest that argon may have the same metabolic effects on synaptosomes that is does on intact organisms.

During the period covered by this report, two manuscripts have been published, two have been accepted for publication and two more are submitted for publication.
INTRODUCTION

In the interim since the last status report, progress toward identification of the metabolic effects of replacing atmospheric nitrogen with argon, helium or neon has continued on several fronts. Research is currently underway on the metabolic effects of these gases at high pressures and the effects of diluent gases on metabolism of nervous tissue at ambient pressures.

Dr. Christopher Schatte received an appointment as post-doctoral fellow in the Laboratory of Aerospace Biology in January, 1972, after successfully completing his doctoral degree program. In February, Dr. Schatte began the collaborative research program in the laboratories of Dr. Peter B. Bennett at the Royal Naval Physiological Laboratory, Alverstoke, Hants, England. This program, now in high gear, is proving extremely beneficial to both the Laboratory of Aerospace Biology (LAB) and Dr. Bennett's group. The current status of this research is described in Section II of this report.

Dr. Schatte will return to the United States approximately 1 September after attending the Fifth Symposium on Underwater Physiology, 21-25 August, Freeport, British Bahamas.

Dr. William W. Martz is conducting a research project designed to ascertain the effects of rare gas-oxygen mixtures on the nerve synapse. The experimental design and preliminary results of this research are included in Section III of this Status Report.
Since the last report, David P. Clarkson has successfully completed both his doctoral preliminary examination and the foreign language examination. The only remaining requirement for the Ph.D. degree is his dissertation research project which is expected to be completed by early 1973. During his association with the Laboratory of Aerospace Biology, Mr. Clarkson has immersed himself in every aspect of the research methodology of the Laboratory. His familiarity with the program is evidenced by his participation in the preparation of several manuscripts as reported here and in previous status reports.

Research on "inert gas narcosis" in the LAB has reached a stage where it would be extremely beneficial to provide a level of automatic control for the Metabolic Chamber System. This chamber system, around which nearly all aspects in the Laboratory are centered, requires a considerable number of man-hours both in conducting experiments and in the processing of data. A proposal for computerization of system control and data acquisition is being sent to NASA's Office of Life Sciences for evaluation. The proposal, included as Appendix A of this report, includes an up-to-date description of the Metabolic Chamber System and a detailed description of two possible systems for automatic control and data processing.

The mathematical method for expressing metabolic rate which has been utilized in this laboratory for several years
has been refined and prepared as a manuscript which has been submitted to the editors of the American Journal of Physiology. The manuscript, entitled "A Rapid Quantitative Measure of Metabolic Activity," by J. P. Jordan, C. L. Schatte, D. P. Clarkson, M. L. Corrin, and W. W. Martz, is included as Appendix B of this status report.

During the period covered by this report, the following papers also have been accepted for publication:

"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. (Due in next issue of Space Life Sciences.)

METABOLIC RESPONSE TO HIGH PRESSURE NARCOSIS

The majority of the recent work from this laboratory has been directed at characterizing the metabolic consequences of exposure to artificial environments encompassing a reduction in ambient pressure, substitution of various diluent gases for nitrogen, or both. The consistent result has been that the so-called "inert or noble gases of the helium group can alter both metabolic rate and the relative importance of certain metabolic pathways in rats. Jordan, et al (1) reported that normoxic mixtures of neon at one atmosphere absolute (ATA) and 0.33 ATA accelerated the metabolic rate of rats when compared to atmospheric air controls; at the end of 4 weeks' exposure, adaptation to the changes had occurred such that metabolic rate was the same in neon as in air. The view was expressed that neon appeared to release a metabolic "brake" which nitrogen normally exerts. Such a "braking" action was consistent with the findings of Lassiter, et al (2) that marginal oxygen toxicity, as manifested by reductions in brain levels of CoA, CoASH, and acetyl CoA, could be attenuated by adding nitrogen as a diluent gas. Clarkson, et al (3), discovered that the oxygen consumption of rats exposed to normoxic mixtures of argon or helium at the thermal neutral temperature of each was significantly different than that in air at ambient pressures of 0.8 ATA. Depression of metabolic rate in argon relative to air was subsequently observed by
Schatte, et al (4); metabolic rate of rats in helium and nitrogen were not significantly different. Of particular interest was the fact that very mildly hyperoxic mixtures of these gases at atmospheric pressure produced a remarkable variation in metabolic response. While the helium-exposed animals were virtually unaffected by the lowered oxygen tension, those in nitrogen showed moderate hypoxic symptoms and those in argon were substantially affected. It was concluded that both intrinsic properties of the diluent gases and an undetermined ability to influence oxygen availability or utilization at the cellular level contributed to the results.

Having determined that different diluent gases exert varying metabolic effects, the fundamental question of "How?" is yet unanswered. The difficulty in answering the question is exemplified by the fact that more than a century of interest of which the last forty years have been particularly intense, has failed to produce an answer. Nevertheless, much progress has been made in the particular areas of high pressure physiology and anesthesia, in which the effects of rare gases are most pronounced and have accordingly been the most extensively studied. There are several good reasons favoring (and none contraindicating) the hypothesis that the effects of diluent gases which we have observed at low pressures may be subtle manifestations of those seen at high pressures or in a state of anesthesia. Accordingly, it is to the field of high pressure physiology that the laboratory has in part turned to
further characterize the metabolic aberrations that have been noted and to perhaps offer a new approach (molecular level metabolism) towards solving the mystery of high pressure inert gas narcosis.

Dr. Christopher Schatte, a 1971 product of this laboratory, has been working since February with Dr. Peter Bennett at the Royal Naval Physiological Laboratory in Alverstoke, England. Dr. Bennett is the world's foremost authority on "inert gas narcosis," and his efforts have resulted in the most plausible mechanism of narcosis offered to date and a program to develop a drug which successfully alleviates the symptoms of narcosis. His pioneering research and vast knowledge of the field coupled with his laboratory's excellent facilities for research, made him the logical choice for collaborative research on a goal common to our two laboratories: definition of the mechanism of narcosis. Additionally, Dr. Bennett is interested in filling a large void in the area of metabolic response to narcosis toward which this laboratory's experience with metabolic measurements can be applied.

During his tenure in England, Dr. Schatte will perform experiments intended to gain insight into the following areas: (1) examination of the theory that narcosis results from an expansion of the cell membrane under high partial pressures of diluent gases, (2) whether the primary locus of activity at the cell membrane is the aqueous or lipoidal phase, (3)
whether there exists a relationship between the degree of narcosis and metabolic changes, (4) whether the drugs which Dr. Bennett has found to alleviate the symptoms of narcosis have any influence on these metabolic changes, and (5) elucidation of the exact biochemical changes which narcosis produces and the ramifications attendant with them.

It is widely hypothesized that the site of action by narcotic gases is the lipid fraction of the cell membrane, an extrapolation of the Meyer-Overton lipid solubility theory (5). Bennett (6), for instance, has observed that an ionic imbalance occurs during narcosis in which plasma K\(^+\) increases while Na\(^+\) decreases, presumably due to exchange between the extra- and intracellular compartments. From this evidence and that of certain in vitro experiments (7, 8), it was inferred that the membrane expands due to increased numbers of gas molecules "packing" into the lipid component under high partial pressures. The membrane thus undergoes, or perhaps cannot undergo, some conformational change which results in altered permeability to these ions. Electrical disturbances in the brain and decrements in nerve conduction are presumed to arise from the altered ionic distribution.

Another theory, championed by Featherstone, et al (9), proposes that all proteins, perhaps enzymes, undergo changes in the polarity of important side chains because of interaction with hydrated gas molecules. Such a phenomenon could
modify enzyme-substrate binding and/or change the net charge of the molecule thereby upsetting electrical balances; either might result in the reduced neuro-electrical activity symptomatic of the narcotic state. Alternatively, at high partial pressures, diluent gases might be forced into the interior of a protein, upsetting its tertiary or quarternary structure to the point that it would become dysfunctional.

These phenomena are not mutually exclusive and it is likely that both may occur. Since the mammalian cell and mitochondrial membrane is composed of a lipid base with enzymatic protein embedded in it, expansion of the lipid would be expected to cause changes in the intricate spatial relationships of the membrane-bound proteins. Likewise, the fact that membrane structure is to some extent dependent on maintaining a charged outer surface (particularly due to amino acid chains) juxtaposed to a relatively non-polar interior (lipid hydrocarbons) suggests that the presence of electrical interaction between gas molecules and the outer surface could result in at least a reduced conductive medium and perhaps membrane instability. Thus, a vital molecule such as ATPase might be hindered if its lipid base on the membrane were altered to the point that its proximity to ATP was more distant in addition to the binding site being obscured by a cluster of hydrated gas molecules which might be forced in between it and its ATP substrate; such a condition might prevent its participation in the process of active
transport thereby causing ions to accumulate on the wrong side of the membrane as has been observed.

It is significant that the drugs which Dr. Bennett has observed to attenuate the EEG depression associated with narcosis are primarily cationic detergents which generally associate with non-polar substances (10). Two notable exceptions are the anti-oxidant vitamin E and the anti-inflammatory acetylsalicylic acid (aspirin).

It is at uncertainties such as these that the experiments in England are aimed. Both membrane swelling due to lipid "packing" with gas molecules (11) and protein binding with rare gas molecules (12) have been documented in vitro but the situation in vivo has not been described because of an inability to measure lipid and protein interactions during narcosis. It is here that judicious application of metabolic techniques might prove to be the crucial step. If the lipid portions of membranes do undergo physical alteration, then an alteration in the behavior of enzymes attached to them should result. Since mitochondrial membranes are similar to cell membranes in composition and structure, measurement of tricarboxylic acid (TCA) cycle activity might serve as an indicator of the state of the membrane; one could anticipate that the general nature of any mitochondrial membrane effects could be extrapolated to the cell membrane.
Conversely, if the gases act in the aqueous phase and combine primarily with protein, then membrane-bound enzymes would still be affected albeit in the aqueous phase rather than the non-aqueous. However, measurement of non-membrane-bound enzymes, such as those of the glycolytic pathway, ought to be affected whereas they may not have been in the case of lipid interaction.

Dr. Schatte is proposing to measure the uptake and conversion to CO$_2$ of uniformly-labeled glucose$^{-14}$C as an indication of overall glucose catabolism and particularly the activity of the TCA cycle. Activity of the glycolytic enzymes will be inferred from changes in plasma pyruvate: lactate levels in addition to that of glucose entry rates. These measurements will be made in both a narcotic state induced by exposure to normoxic mixtures of nitrogen at 7 ATA as indicated by the decrement in the cortical evoked response to photic stimuli. Controls will be measured at 1 ATA breathing air. Helium will be similarly tested to assess any influence of the increased pressure alone since it appears to have no narcotic properties and is considered to be a useful gas medium in which to study the influence of pressure alone. Finally, the nitrogen experiments will be repeated except that the animals will have been administered one of the drugs which has been shown to ameliorate the narcotic symptoms; preliminary tests will determine
whether cetyltrimethylammonium bromide or vitamin E is the drug of choice.

Five adult female goats trained to stand restrained while breathing through a fitted mask will each be exposed individually to the nitrogen, helium, and nitrogen plus drug environments. Dietary intake will be uniform to insure similar post-absorptive states. Prior to the actual experiment, EEG and ECG leads, rectal and skin thermistors, and jugular cannulae will be positioned.

A priming dose of the radioactive glucose followed by a continuous infusion will be started one hour prior to the surface control measurements which will be made every 10 minutes for one hour. This will include ECG and EEG traces, frequency analysis of the ECG, and evoked response measurement, body temperatures, and expired gas and blood sample taken for analysis. The surface control period will be followed by a one-hour test period at pressure with a similar sampling sequence. Due to the lengthy decompression involved, a post-pressure control run is not possible.

Physiological analyses will include:

1. oxygen consumption
2. CO₂ production
3. reexpiration rate
4. heart rate
5. rectal and skin temperatures
6. analysis of the EEG and evoked response.
Biochemical parameters will include:

1. specific activity of plasma glucose
2. specific activity of expired CO₂
3. respiratory quotient
4. plasma glucose and free fatty acid levels
5. whole blood levels of pyruvate and lactate.

From the glucose and CO₂ specific activity data, the entry rate and "irreversible" loss of glucose (glucose uptake) can be estimated and the percentage of glucose oxidized by the TCA cycle calculated. Additionally, effects of catecholamine secretion can be derived by scrutiny of those parameters which are known to be influenced by catecholamines, i.e., O₂ consumption, cardiac and respiration rates, plasma glucose and unesterified fatty acid levels.

From these data, any changes in metabolic rate or shifts in the relative importance of various pathways can be monitored. It is anticipated that narcosis will slow metabolic rate which will be reflected in lowered oxygen consumption, CO₂ production, glucose uptake and conversion to CO₂, and plasma pyruvate and lactate levels. Further, it is likely that plasma unesterified fatty acids will rise as part of a general stress response to both the pressure and narcosis. Based on observations that rare gases appear to differentially influence oxygen availability to the cell (4), it is thought that some degree of cellular hypoxia could be manifest during narcosis, perhaps by increased lactate: pyruvate
ratios and elevated fatty acids. It is not felt that the
drugs will have much effect on these metabolic changes since
they appear to rectify only the ionic charge unbalance that
results from narcosis; improvement of the electrical depres-
sion of the brain by the drugs will probably not be accom-
panied by amelioration of the metabolic alterations.

If time permits, experiments may be performed with argon,
to produce a more profound narcosis than nitrogen, or using
different radiosubstrates as might be indicated pending the
results of these experiments. For instance, if glycolytic
pathway enzymes were found to be inhibited by narcosis,
further assessment could be made using glucose-1-$^{14}$C in
conjunction with glucose-6-$^{14}$C to determine shifts in the
importance of the glycolytic pathway versus the pentose phos-
phate pathways. Or, if only mitochondrial-bound enzymes were
affected, additional information could be gained by using
radio-labeled TCA intermediates or acetate; also, other mito-
chondrial systems such as fatty acid synthesis would be tested.
Ultimately, we shall address ourselves to the cell membrane
and the question of the ionic perturbations; these almost
certainly involve inhibition of ATPases or a membrane-bound
factor in active transport. While no in vivo assay of ATPase
activity is currently available, experiments involving hor-
mones known to require cyclic-AMP or involve some membrane
component might serve to further elucidate the nature of the
changes due to narcosis at the cell membrane.
The mechanism of "inert gas narcosis" has eluded identification despite an increased degree of international interest over the past decade. The two most prevalent theories for the metabolic effects of rare gases are described in Section II of this report. While these theories may differ with regard to specific molecular mechanisms involved, they agree that rare gases, in some manner, affect or interfere with normal biomembrane function. Studies on intact animals have not clarified whether the physical effects of rare gases are general or are manifested in specific biomembranes or tissues.

The relative importance of specific tissue interactions versus general membrane effects described in Section II has not yet been quantified. Several reports suggest that important effects of rare gases occur at the nervous system level (13, 14, 15, 16, 17). The majority of investigations were carried out at elevated pressures, often exceeding 20 atm. The studies indicate that "chemically inert" gases can produce depressed nerve excitability (15) and nerve fiber blockade (14); effects which can be reversed upon removal of the "inert gas" or decreasing its partial pressure. Recently it has been demonstrated that gas mixtures of 75% helium-25% oxygen can produce an antiarrhythmic effect in hearts of anesthetized
dogs (17). In those studies, helium-oxygen mixtures reduced baseline heart rate and the concentration of catecholamines. The effects of helium were believed to be the result of changes in sympathetic nervous system activity.

It is not yet known whether rare gas-induced alterations of the electrical properties of the nervous system are the result of changes in transmembrane potentials, nerve impulse transport velocities or are manifested at the nerve synapse. Of these possibilities, nerve synapse function is the most convenient to investigate biochemically.

Examination of the effect of argon, helium or nitrogen on isolated rat brain synapses (synaptosomes) was undertaken. The choice of synaptosomes is based upon their well investigated structural and biochemical properties. Electron microscopic studies indicate that synaptosomes contain a large amount of storage vesicles with fairly small mitochondria. Biochemically, isolated synaptosomes carry out both general metabolic functions as well as those peculiar to the physiological function of nerve endings in vivo. These functions include cellular respiration (18, 19), protein biosynthesis (20, 21) and the synthesis of ATP from inorganic phosphate, as well as the manufacture of the neurohormones acetylcholine (22), norepinephrine (23), 5-hydroxytryptamine (24), dopamine (23), and histamine (26).
Experimental Procedure

Groups of 5 adult male Holtzman rats (340-390 gm) were exposed to test gas mixtures consisting of 79%-21% nitrogen-oxygen, argon-oxygen, or helium-oxygen at ambient pressure (632 mm Hg) and the thermal neutral temperatures of the respective mixture (3). Gas mixtures were prepared in cylinders by partial pressure and final oxygen concentration in the gas mixture measured by a Beckman paramagnetic oxygen analyzer.

Since the causitive agents in these experiments were gases, all possible precautions were taken to assure that only the test gas atmosphere came in contact with animals, tissues and solutions. Denitrification of solutions was carried out by heating to 50°C for 30 minutes then placing the solutions in a vacuum desiccator (23 inches Hg vacuum) for one hour. After the degasing period, the desiccator was filled with the test gas mixture under study. Solution containers were then capped and solutions stored at 0-4°C until use. This procedure was followed for the nitrogen-oxygen mixture as well as the rare gas-oxygen mixtures in order to assure uniformity in solution preparation between controls and test-gas evaluation.

Furthermore, all preparative and analytical procedures were carried out in glove bags filled with the test-gas mixture being examined during a given experiment. This included (Figure la) animal sacrifice, brain excision, tissue homogenization and centrifugation preparation steps. Centrifuge tubes were filled, balanced and capped while in the glove bags,
Figure 1. Photographs of glove bag arrangements for (a) tissue homogenization and centrifugation preparations and (b) oxygen consumption measurements.
thereby eliminating the need to flush the centrifuge with test-gas mixtures. Analytical procedures, oxygen consumption (Figure 1b) and acetylcholinesterase activity measurements, were also carried out in glove bags. Glove bag procedures, obviously time consuming and inconvenient, were absolutely required to assure that only the test-gas mixture under investigation came in contact with the synaptosomal preparation.

Animal exposure to test-gas mixtures were carried out in the metabolic chambers using a flow-through system at a gas-flow rate of 8 liters/min. Exposure periods began at 2200 hours one day and terminated at 0600 hours the following morning. The 8-hour exposure period is probably far in excess of that required for animal/test-gas equilibration since Clarkson, et al (3) measured metabolic effects in about one hour under nearly identical experimental conditions. Following the exposure period, animals were quickly transferred to an open-ended glove bag (gas-flow rate = 25 liters/min.) and sacrificed by decapitation. Brains were rapidly excised and placed in a tared, capped centrifuge tube and placed on ice. Each brain was identified and kept separate during the entire isolation and analytical procedure.

Brains were homogenized with a loose-fitting teflon-in-glass homogenizer in 9 volumes of 0.32 M sucrose. Synaptosomes were isolated by discontinuous gradient centrifugation as described by Gray and Whittaker (25) and modified by
Bradford (18). The entire isolation procedure is outlined in the accompanying flow chart (Figure 2). During initial isolation runs, final synaptosomal preparations were examined by electron microscopy (Figure 3) to assure that synaptosomes had indeed been isolated.

The synaptosomal pellet obtained at final centrifugation step was resuspended in 2.0 ml 0.32 M sucrose. The resulting concentrated synaptosome suspension was used for determination of oxygen consumption rates. An aliquot (0.20 ml) of this suspension was diluted to 2.0 ml in 0.32 M sucrose and the resulting dilute synaptosome suspension stored on ice for acetylcholinesterase activity determinations.

Both acetylcholinesterase activity and oxygen consumption determinations were carried out at 37°C. Acetylcholinesterase activity was determined according to Ellman, et al (27). The assay solutions were prepared in the glove bag in Beckman glass-stoppered cuvettes with everything except the substrate (acethlthiocholine--ATC) then sealed with rubber culture-tube stoppers of the appropriate size. These cuvettes were then removed from the glove bag and placed in the Beckman DBG Spectrophotometer. After temperature equilibration, ATC (100 µl) was introduced with a Hamilton syringe to start the assay. The specific activity of acetylcholinesterase is expressed in terms of micromoles of substrate converted per minute per milligram of wet brain tissue.
BRAIN

Weigh and Homogenize in 9 vol. 0.32 M Sucrose

Centrifuge 1000 x g / 10 min.

Precipitate

Wash in 0.32 M Sucrose

Precipitate (discard)

Washings

Centrifuge 10,000 x g / 20 min.

Supernatant (discard)

Pellet

resuspend in 1.0 ml 0.32 M Sucrose

aspirate interface,
slowly add 1 vol.
distilled H₂O, let stand 20 minutes

centrifuge 15,000 x g / 20 min.

resuspend pellet in 2.0 ml.
0.32 M Sucrose

O₂ consumption and acetylcholinesterase assays

Figure 2. Flow diagram of synaptosome preparation methodology.
Figure 3. Electron photomicrograph of synaptosome preparation showing synaptosomes (S), mitochondria (M), synaptic vesicles (SV), and postsynaptic membranes (P). 19500 X. Courtesy of W. J. Banks, Jr. and A. M. Sheppard.
Oxygen consumption rates were determined with an oxygen electrode and meter (Yellow Springs Instrument Model 53). The concentrated synaptosome suspensions were placed (on ice) in a glove bag (Figure 4) along with the water jacketed cell compartment, oxygen electrodes and test solutions. After flushing with test gas (25 liters/min.) the bag was sealed and 3.0 ml of Krebs-phosphate (Krebs-Ringer) medium (pH 7.4) was placed in each of the sample cuvettes and allowed to equilibrate for 5 minutes. An oxygen electrode was then placed in the cuvette, the oxygen meter and chart recorder adjusted to 100%, and a sample (0.20 ml) synaptosome suspension injected into the cuvette with a hypodermic syringe. Oxygen uptake was followed for 10 minutes and quantified in terms of μl of O₂/minute/mg of wet brain tissue.

Results

Preliminary results of these experiments have been obtained for animals exposed to nitrogen-oxygen and argon-oxygen test environments (Table I). These results represent average values for two separate experiments in each test environment. Measurements were run in duplicate for three synaptosomal preparations in each experiment.

Synaptosomes isolated from the argon-oxygen atmosphere consume oxygen at a rate only 70% of that exhibited by synaptosomes from the nitrogen environment. Acetylcholinesterase activities, on the other hand, show an 8% increase for argon relative to nitrogen controls.
Table I. Synaptosomal oxygen consumption (µl O₂/min/mg brain) and acetylcholinesterase activity (µM product/min/mg brain) for rats exposed to normoxic nitrogen-oxygen and argon-oxygen mixtures.

<table>
<thead>
<tr>
<th>ATMOSPHERE</th>
<th>O₂ CONSUMPTION</th>
<th>ACETYLCHOLINESTERASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen-Oxygen</td>
<td>230</td>
<td>321</td>
</tr>
<tr>
<td>Argon-Oxygen</td>
<td>161</td>
<td>349</td>
</tr>
</tbody>
</table>

The argon-produced depression of oxygen consumption is similar to the decrease in metabolic rate which has been demonstrated in vivo for rats exposed to argon-oxygen atmospheres (3, 4). Previous reports by others attempting to correlate in vivo and in vitro effects of rare gases on metabolic rate have utilized entirely in vitro techniques (28, 29). Tissues under examination were exposed to the rare gas under examination only after being placed in the Warburg flask for analysis. Such studies have not been able to provide a clear correlation between in vivo and in vitro effects of the rare gases. One study (28) showed no measurable in vitro metabolic effects for either helium or argon, while another (29) showed slight depressions in metabolic rate for both helium and argon relative to nitrogen controls. It would appear that the ambiguities in in vitro investigations of "inert gas narcosis" would need to be resolved before a more satisfactory understanding of this phenomena is possible.
The increase in acetylcholinesterase activity of nearly 8% for argon relative to nitrogen isolated synaptosomes is difficult to interpret at this stage of the investigation. The results are quite preliminary and additional experiments must be performed before a statistically significant or consistent pattern can be formed. If experiments verify a significant effect of rare gases on acetylcholinesterase activity, subsequent studies will be directed toward identification of the molecular mechanism of this action. Since acetylcholinesterase is a membrane bound enzyme, it would be necessary to perform kinetics studies in which the enzyme was dissociated from the membrane. These studies would permit an interpretation to be made between direct enzyme-rare gas effects and the effect of the gas upon membrane-protein interactions.

Additional experiments are currently in progress in which the test-gas mixture consists of helium and oxygen. An initial experiment with this mixture suggests a small decrease in oxygen consumption with little or no influence of helium on acetylcholinesterase activity.

While evidence suggests that rare gases may indeed associate with cells and cellular components (30, 12), it would appear unlikely that the gases would completely equilibrate in the short time required to start Warburg assay procedures used in previous in vitro investigations (28, 29). Of even more concern may be the fact that tissue homogenates used in these studies probably are not the best system for in vitro
metabolic investigations with rare gases. This is particularly true in light of currently prevalent hypotheses regarding the mechanism of "inert gas narcosis." As explained previously in both this section and Section II, rare gases are thought to act primarily at the biomembrane level; and tissue homogenates contain both intact and disrupted cells. For these reasons, studies such as the one outlined in this report in which intact cells or cell organelles are isolated and assayed entirely in the test environment are expected to render a clearer picture of the cellular and subcellular mechanism of "inert gas narcosis."
REFERENCES


APPENDIX A

The following proposal (Section 2) for compterization of the metabolic chamber system has been submitted to NASA's Office of Life Sciences for evaluation. Pagination and referencing in this appendix are separate from the remainder of the Status Report.
DESCRIPTION OF

METABOLIC CHAMBER SYSTEM

Laboratory of Aerospace Biology
Colorado State University
1972
The investigation of the metabolic effects of artificial environments and breathing atmospheres often requires that experimental animals be held in a variety of test environments for extended periods of time. We wish to describe a metabolic chamber system which will perform this important task as well as facilitate the acquisition of metabolic data.

This chamber system is self-contained except for the addition of O₂ and on rare occasions diluent gases and removal of animal waste products. The system (Figure 1) consists of four major sections. These include (a) the metabolic chambers, (b) the scrubber system for removal of atmospheric contaminants, (c) the atmospheric control unit for maintenance of chamber pressure and atmospheric composition, and (d) the metabolic data monitoring system.

METABOLIC CHAMBERS

The animal chambers consist of large autoclaves (length = 3.0 ft.; diameter = 1.5 ft., I.D.) from which the distal end has been removed and replaced by a one-inch thick clear plexiglass plate (4). This plate permits visual observation of animals during the course of an experiment and the semi-permanent installation of inlets for electrical leads and chamber service functions. For example, in our particular situation, the plexiglass plate is fitted with Poly-Flo bulkhead fittings for test gas atmosphere inlet and outlet, automatic water delivery system and leads for a barometric pressure altimeter. In addition, a multipin electrical plug is installed to supply power to a demand pellet-delivery food dispensor, thermostwitch and fans. Experimental animals are housed within the chambers in cages constructed of expanded stainless steel. The cage floor area measures 15 x 30 inches and is adequate for 8 to 10 rats in the 300 to 350 gram range.

Four separate animal exposure chambers are housed in a large hypobaric chamber (Figure 2). This large chamber is used as an "elevator" to bring personnel to the same barometric pressure as that of a given animal exposure chamber during the replenishing of the food supply, removal of animal waste products, weighing of animals and subsequent injection of radioactive substrates, and during sacrifice of the animals for tissue analysis.
Figure 1. Schematic diagram of the closed metabolic chamber system.
Figure 2. Cutaway view of eight-man hypobaric chamber used to house animal exposure chambers. Only two animal exposure chambers (C) are shown although the eight-man unit has the capacity to contain four such chambers.
The food dispensers are shown in Figure 3. Food is delivered from the hopper to the cup by a gear motor driven auger. One turn of the auger will deliver approximately 370 ± 20 mg of food pellets (Figure 3 inset). Operation of the feeder is controlled by a photoelectric system as shown in the block diagram (Figure 4a). A cadmium sulfide cell is located in the bottom of the cup and the light source (two Monsanto light emitting diodes, 650 nm) is located directly over the center of the cup approximately 6cm from the photocell. The photocell is connected to a low-gain switching amplifier which in turn actuates a relay connected in series with the drive motor. When the food cup is empty, the motor is turned on. Food pellets dropping into the cup block the light beam deenergizing the relay. The motor continues to operate for at least one complete revolution at which point it is shut off by a microswitch actuated by a cam on the shaft of the auger. Each revolution is tallied by an external electro-mechanical counter. The feeder in each chamber may either be operated independently or in a master-slave fashion. The use of these devices provides a much more accurate determination of food consumption than is possible by the usual food tray weighing method.

Low speed fans and independent chamber temperature control devices are installed in each of the animal exposure chambers. The installation of the fans assures 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 test chambers quantifying the rates of $^{14}$CO$_2$ production following the injection of $^{14}$C-labeled substrates.

The temperature control devices are quite simple yet highly efficient, consisting of a thermostwitch 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 permit individual chambers to be operated at different internal temperatures and will control these temperatures to ± 0.5°C over temperatures 0°C to 100°C.

Water is supplied to the chambers from a common reservoir by means of an air-driven syringe and a system of solenoid valves (which permit the use of a single syringe to deliver water to all the chambers). Animals obtain drinking water from a stainless steel cup positioned at one end of the cage.
Figure 3. Food pellet dispenser for demand-pellet-delivery in pair-fed experiment.
Drive motor and control circuitry located in vertical housing at right, food pellet hopper right center, and pellet receiving cup bottom center.
Insert: Approximately 20 g food pellets.
A temperature compensated thermal conductivity bridge circuit is used to maintain a constant water level in each cup. By means of a stepping relay (Figure 4b), each sensor is sampled at regular intervals (once every 5 minutes). When the water level drops below the level of the cup thermistor, a change in resistance of the sensor due to heating effects an unbalanced bridge circuit. This unbalanced bridge circuit produces a resetting error signal which is fed directly to the gate of a silicon controlled rectifier (SCR 2N5061) which will then conduct the signal. When a conducting SCR is sampled by the relay, the sample and hold circuit is switched on; and, on the next advance of the relay, current is applied to the solenoid supplying air to the syringe activator, to the solenoid connecting the syringe to the appropriate chamber, and to the solenoid connecting the syringe to the reservoir. As a result, 5 ml of water is delivered to the cup. The next two advances of the relay perform the reverse operation: i.e., closing the inlet to the cup, opening the outlet of the reservoir and returning the syringe plunger to its former position (thereby filling it), and resetting the sample and hold circuit. The operation of inlet solenoids is individually tallied, thereby providing an accurate estimate of water consumption.

GAS SCRUBBER SYSTEM

With a closed environmental system, gaseous waste products produced by the animals (not only CO$_2$, but CH$_4$, ammonia, light organics, and volatile acids) must be removed before their concentrations reach toxic levels or even low levels which might affect the metabolism of experimental animals. Humidity control should also be taken into account.

The pump leading to the gas scrubber train (Figure 1) evacuates the chamber and provides approximately a 10 psig head pressure going into the scrubber train. The pump following the gas scrubbers creates the pressure differential over the gas scrubber train to assist the flow and at the same time compress the gas into the pressure-volume tank. Thus, the duplication of pumping capacity is a method of moving gases through high density liquids.
Figure 4. Schematic diagrams of (a) food pellet dispenser with demand-pellet-delivery, (b) watering system for metabolic chambers. Please refer to text for detailed descriptions.
The output of the vacuum pump is fed directly into a 3-gallon bubbler containing 3N KOH where the CO₂ is trapped. The KOH scrubber is fitted with a silicon rubber septum for removal of samples for CO₂ or ¹⁴CO₂ quantification. The output from the KOH bubbler is fed into a concentrated H₂SO₄ bubbler which serves the dual function of removing NH₃ and H₂O vapors. To insure maximum contact of the gas with bubbler liquid, the gas stream is fed into each bubbler through three sintered teflon inlet diffusers.

Canisters containing Purafil odoroxidant (Borg-Warner Corporation) and activated carbon respectively are positioned in that order in the effluent line from the H₂SO₄ scrubber. The installation of the canisters insures total removal of residual H₂SO₄ vapors, extremely important with regard to pumps, solenoid valves, and the health of the animals.

ATMOSPHERIC CONTROL UNIT

The Atmospheric Control Unit is the heart of the metabolic chamber system. The unit possesses three distinctly different sections. These include (a) a constant pressure animal chamber and altitude control system, (b) the negative pressure system which brings the effluent gases from the animal chamber through the scrubber train, and (c) the positive pressure system which reconcentrates the cleaned gases in a pressure-volume reserve tank feeding them into a gas mixing tank in which the composition of the gas is monitored and kept constant by the gas analyzer control system. All portions of the system are controlled from a master console (Figure 5). The constant pressure is maintained within the animal chamber by use of prepositioned electrical contacts on the altimeter (4) which are actuated by changes in chamber pressure opening the appropriate solenoid valves to allow an increase in the inflow or outflow of gas from the chamber.

In order to properly control the addition and mixing of gases, our system employs a positive pressure side (Figure 1) in addition to the altitude controlling negative pressure side. The positive pressure side is necessary for three reasons: (1) to maintain a reserve volume of gas to prevent aberrant flow, (2) to have a base line pressure for oxygen analysis, and (3) to maintain precise control over the addition of
Figure 5. Photograph of console for atmospheric control unit.
gases to be mixed. The negative pressure side provides the pressure differential over the chamber input and output to maintain the preset altitude and flow rate. The effluent of the vacuum pump is compressed on the pressure side thus closing the system. To eliminate the possible interjection of contaminants from the pumps into the gas stream, teflon coated dry-diaphragm aluminum pumps (Thomas Industries) are used.

METABOLIC DATA MONITORING SYSTEM

Several metabolic parameters may be conveniently quantified using this chamber system. Digital counters are connected to electrical leads for the chamber temperature controlling solenoids, water and food delivery systems, and oxygen and diluent systems. These counters tabulate each actuation of the respective systems. A multipen event recorder (Esterline Angus Model A620X), in conjunction with the digital counters, provides a convenient, permanent record of the frequency and exact times at which various metabolic parameters are measured, permitting metabolic patterns to be compared for experimental and control animals. Metabolic parameters which may be obtained are food and water consumption, oxygen consumption carbon dioxide production and the production of $^{14}$CO$_2$ from selected radio-labeled substrates.

The latter parameter has been used extensively in this laboratory to identify specific metabolic pathways affected by test environments. In this procedure, animals are removed from the chamber at predetermined times, weighed and injected intraperitoneally with selected radio-substrates (usually acetate-$^{1-14}$C or glucose-$^{1, -6, or -UL-14}$C at 50 uci/kg body weight) and replaced in the chamber. Rates of $^{14}$CO$_2$ expiration are determined by taking serial samples from the 3N KOH scrubber and quantifying the trapped $^{14}$CO$_2$ as a function of time. Scrubber samples are usually taken at ten-minute intervals for three hours after injection.

DISCUSSION

For a more detailed description of the operation of this system, it is convenient to begin with a unit of gas in the pressure-volume tank. The gas in this tank is the chosen gas mixture depleted of oxygen (due to oxygen consumption by the animals) and cleaned of animal waste by-products. The function of this tank is storage and
compression of the circulating gas, providing the capacity to handle fluctuations in the pressure/volume of the system. Generally, the pressure-volume tank is maintained at 20 psig.

Following the pressure-volume tank is a gas supply tank, whose pressure is maintained by a regulator at 10 psig providing a flow of gas that is constant on a molar basis per unit of time. The automatic analysis and mixing of oxygen and diluent gas is accomplished in this tank.

A Beckman F-3 paramagnetic oxygen process analyzer is used to continuously monitor the oxygen concentration. A small portion of the gas mixture in the supply tank is continuously flowed through the analyzer at 350 milliliters per minute and regulated at 4 psig. The gas exiting from the analyzer is returned to the system via the negative pressure side with the exit line back pressure maintained by a needle metering valve. The zero and 100% calibration points are set with diluent gas and pure oxygen, respectively, at the same flow rate and pressure.

The oxygen analyzer readout has both an adjustable low and high set point meter relay to control the addition of oxygen. The analyzer has a 30-second response time; that is to say, with the addition of oxygen to the supply tank, 30 seconds elapse before the analyzer registers a change in the oxygen concentration. This eliminates the problem of over compensation due to lag time in mixing in the gas supply tank.

When the oxygen analyzer registers a demand for oxygen, a 45-second delay timer is started; at the end of this time, a cycle timer opens the oxygen supply valve for a set amount of time. When the timer is cycled, the oxygen supply valve closes and the delay timer starts another 45-second interval. This allows the oxygen analyzer enough time to respond to the new oxygen concentration before further adjustment. This delay and cycling admission of oxygen is continued until the analyzer registers the preset concentration of oxygen when all timers return to the ready position.

Diluent gas concentration is maintained in response to total system pressure. It is added on a similar delay and cycle timer system as the oxygen, so that the oxygen analyzer will be able to respond to the addition of diluent gas (as a reduction in oxygen concentration) and add oxygen to correct the resultant oxygen deficiency. The mixed gas of proper oxygen concentration is fed into the altitude control where its flow rate into the animal chambers is controlled.
The metabolic chamber system will maintain experimental animals in test environments at any desired atmospheric pressure from 200 to 1000 mm. Atmospheric pressure in the chamber will usually not vary more than ± 1% over a 2-day continuous exposure period with even greater precision (± 0.5%) possible during shorter exposure periods. Altitude variations are monitored on both experimental and control chambers by a two-pen Taylor altitude recorder, and the percent of oxygen in a given test atmosphere can be maintained at ± 0.5%. Chamber temperature variations are monitored by a Brown “electronic” 16-point recorder calibrated for use with type T thermocouples which records 2 points of temperature every 30 minutes in each chamber, the circulating water bath temperature, and room temperature.

The efficiency of the unit is checked by using precise gas analyses with test pickoff ports valved into the system for connection to a gas-solid chromatograph (GSC). Separations are conducted on silica gel and molecular sieve 5A columns in series using a thermal conductivity cell as a detector, and helium as the carrier gas as previously described (5).

GSC pickoff points are positioned in the chamber input and output lines to monitor the exact composition of the entrance gas as well as the exhaust gas which contains waste products introduced by the animals. A scrubber efficiency pickoff point has been included immediately after the scrubber system.

Contaminant levels in metabolic chambers are influenced by the number of animals in the chamber and the flow rate of gas through the chamber. Experience has shown that with 8 animals occupying the chamber, a flow rate of 5 liters/minute STP is sufficient to prevent CO₂ and ammonia levels from becoming significant.

With any chamber system, there is the possibility of an equipment malfunction that will accidentally terminate or interrupt an experiment. The likelihood of such a malfunction is perhaps even greater in a system as complex as the one described in this manuscript, operating on a 24 hours a day/7 days a week basis. With this in mind, a malfunction alarm system is installed as an integral part of the metabolic chamber system.

The alarm system is comprised of several pressure- and micro-switches positioned at key points and a dedicated telephone line. In the event that one of the
system parameters (pressure, temperature, %O₂, etc.) varies from preset limits, the alarm is triggered either in the laboratory or, via the dedicated telephone line, in the home of one of the laboratory personnel. Generally, less than 10-15 minutes elapses between the triggering of the alarm and the arrival of laboratory personnel.

This environmental chamber system has certain inconveniences with regard to maintenance and personnel requirements. When running continuously, at least two and preferably three individuals are required to be available at all times for both routine maintenance and emergency repair. System checks, scrubber change, and the taking of CO₂ samples can be performed by a single individual; weighing animals, the changing of drop trays, and the replenishing of food supply requires two people; and animal injection, sacrifice, and obtaining tissue samples usually requires three investigators. Whenever the large chamber is used, one person must be at the controls with another available on the outside for passing items through the air lock and other duties in addition to the individuals inside.

In general, as much as one-third of the total laboratory man hours may be spent on system maintenance. While the diagram of the chamber system in Figure 1 illustrates the scheme of the chamber system, it does not show the myriad of plumbing, solenoid valves, and electrical wiring necessary to render the system functional. The hardware and concomitant maintenance are simply facts of life in chamber systems of this nature.

Apart from the maintenance and manpower requirements, the chamber system possesses several major benefits. It permits metabolic studies to be carried out on groups of unrestrained animals under a wide range of precisely controlled environmental conditions. In addition, in experiments requiring pressures other than ambient, test animals may be weighed, injected, and sacrificed at pressures identical to those used during the exposure periods. This factor is extremely important when attempting to identify and quantify subtle metabolic influences of the test environment.

Using this chamber system, it has been possible to identify the metabolic effects of the marginal oxygen toxicity of the 5 psia-100% oxygen environment used in the United States Manned Space Program (1, 2, 3, 5). Identification of specific
enzymes in key metabolic pathways influenced by this test environment, was possible even though total animal metabolism as measured by O$_2$ consumption did not change (2).

The closed environmental system is proving equally valuable in investigations focused upon identification of the metabolic effects of replacing atmospheric nitrogen with argon, helium, or neon (6). In addition to precise control of environmental conditions, the closed system described in this manuscript has proven even more valuable in these studies particularly in the ease of mixing gases for test environments and economy with regard to the costs of inert gases. One can readily envision how this metabolic chamber system could be applied to the study of a wide range of environmental variables including temperature, the metabolic effects of air pollutants, and smoking.
REFERENCES


The metabolic chamber system described in the previous section is one of tremendous capability. Its ability to detect subtle changes in whole animal metabolic behavior in response to the nature of the gaseous environment has been well documented in this laboratory. However, we find it would be desirable to increase the capacity of this system so that data may be collected at more frequent intervals than is presently possible, thereby enhancing the system's sensitivity, and change the methods of data collection and analysis so that this laboratory's personnel can be more efficiently utilized. Additionally, in this era of cut-backs in research funding, we feel it would be desirable to share this system with other investigators in the NASA Life Science Program having similar interests but unable to assemble such a system due to the expense required. At the present time, it would be difficult to comply with such requests because of the drain on personnel-time imposed by this system's configuration.

To improve on the system's overall efficiency and make it possible to offer these facilities to other NASA investigators, it would be necessary to automate system control, data collection, and data processing. To effect these necessary changes in system configuration, the following additional types of equipment would be desirable: (1) CO$_2$ analyzer, (2) chamber humidity monitor, (3) full servo control of metabolic chamber pressure, (4) real time acquisition of $^{14}$CO$_2$ production data, and (5) computer system capable of data collection, processing, and system control. Figure 1 shows the configuration of the present system. The desired additions to this system are shown in color.

We first considered a terminal to Colorado State University's CDC 6400 computer system, but this was rejected when it was found that this facility would not be available on a 24-hour basis. We subsequently considered several types of mini computers and calculators for the task. Although many were found adequate, our decision to design the proposed system around the Data General Corporation's Nova 1200 system is based on cost, capability, and ability to easily modify the configuration at a later date if desired. The appendix contains equipment lists for two mini computer configurations plus a list of additional equipment necessary for process control and data acquisition.

The first computer system (System I), Figure 2, essentially has full real time capability in that it can be dedicated to several tasks in an apparent simultaneous manner.
Figure 1. configuration of the existing chamber system shown in black. The desired modifications are shown in color. pr = pressure regulator, ms* = mass spectrometer, p = pressure monitoring points.
Figure 2. Configuration of computer system I.

- Position indicator input for multi-sample devices.

- Multi-sample pressure transducer
- Pressure transducer, metabolic control unit 1
- Pressure transducer, metabolic control unit 2
- Thermal mass flowmeter, metabolic control unit 1
- Thermal mass flowmeter, metabolic control unit 2
- Humidity/temperature monitor
- Gas solid chromatograph
- Oxygen analyzer, metabolic control unit 1
- CO2 analyzer/mass spectrometer
- Mass spectrometer
- Mass spectrometer
- Multi-sample pressure transducer sampling control
- Gas solid chromatograph sampling control
- Gas solid chromatograph sample injection control
- CO2 analyzer range control
- Servo control valve selector
- Servo control valve direction selector
- Binary coded decimal input (BCD)
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Figure 3. Configuration of computer system II.

- Position indicator input for multi-sample devices.

- Multi-sample pressure transducer
- Pressure transducer, metabolic control unit 1
- Pressure transducer, metabolic control unit 2
- Thermal mass flowmeter, metabolic control unit 1
- Thermal mass flowmeter, metabolic control unit 2
- Humidity/temperature monitor
- Gas solid chromatograph
- Oxygen analyzer, metabolic control unit 1
- CO2 analyzer/mass spectrometer
- Mass spectrometer
- Mass spectrometer
- Multi-sample pressure transducer sampling control
- Gas solid chromatograph sampling control
- Gas solid chromatograph sample injection control
- CO2 analyzer range control
- Servo control valve selector
- Servo control valve direction selector
- Servo control valve actuation control
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This is made possible by the disk system and large core capacity. The disk system, an absolute requirement for a real time operating system, permits many of the frequently used routines and program languages to be stored for extremely rapid access with a few commands from the terminal or from programs resident in core. It also provides transient storage either for data collected during a sampling routine (which later may be recalled and processed before transferring to tape) or for programs running in core at the time of an interruption effected either by a chamber system service routine or an interruption requested by some peripheral device, e.g., in this case an oxygen addition counter.

The magnetic tape system serves as a repository for raw and processed data which can be easily assessed and permanently stored for reference and/or additional data manipulation at some future date. It is also central to the operating system because blocks of data can be retrieved by the central processor under program control or operator request for evaluation of system performance and progress of an on-going experiment. This tape system is also compatible with the University’s computer which will be desirable when manipulation of extremely large amounts of data or very high numerical precision is required.

A CRT terminal has been included to prevent extensive wear and tear on the teletype (which is primarily intended in this configuration for the production of hard copy, paper tape punching, and low-speed paper tape reading) during the development of the routines required for process control, data acquisition, and data processing. The ASR33 teletype selected for this system has a nominal operating life expectancy of approximately 300 hours and is designed as a throw away. Acquisition of a more substantial teletype is in excess of the price of the ASR33 and CRT terminal together. Therefore, the CRT terminal seems justified.

The high-speed paper tape reader is required because all operating systems are supplied by the manufacturer in the form of paper tape, and all routines developed in this laboratory will also be on paper tape. Additionally, the teletype reads at a rate of 10 characters/second as opposed to 300 characters/second for the high-speed reader. Consequently, a very lengthy down time (upwards of one hour) would be required to load the operating systems, e.g., in the event of disk failure, if only the teletype were available.
The second computer system (System II), Figure 3, is a less expensive configuration of System I. Without disk storage, it can be dedicated to only one task at a time, e.g., process control, data acquisition, or data processing. Extensive data processing can only be accomplished after the computer is off-line from process control and data acquisition duties. Further, without magnetic tape, this configuration does not permit review of system performance without human intervention, since the punched paper tape has to be manually loaded into the tape reader. The high-speed tape punch/reader system, however, will permit a fairly rapid input/output of large amounts of data and provide a somewhat convenient means of sorting these data. Because of the manipulation required, it is somewhat less desirable than magnetic tape.

Both systems, however, can be interfaced with analog or digital instruments other than those associated with the chamber system for data acquisition and processing, e.g., pH meters, spectrophotometers, gas chromatographs, liquid scintillation spectrometers, etc. A full description of the Nova Minicomputer system is contained in the publication enclosed with this outline.

The ancillary equipment will permit the chamber system to be operated under computer control. The addition of computerized process control capabilities to this system will not only facilitate its operation but, as a consequence of frequent checks on various chamber system operating parameters, will permit the early detection of system malfunctions. This will effect more prompt corrections of otherwise potentially disastrous situations than is presently possible. In addition, data such as CO₂ production, O₂ consumption, food consumption, and water consumption can be obtained immediately instead of the usual 24-hour turn-around time now experienced with the processing of these data. The addition of equipment to monitor CO₂ production alone will effect approximately a 30-40% reduction in time required to process all experimental data as well as freeing personnel for other pressing tasks. We have elected to delete the request for ¹⁴CO₂ data acquisition equipment at this time because of uncertainties in the performance of the on-line ¹⁴CO₂ monitors presently available.

Two separate systems for the monitoring of CO₂ have been listed. One is based on the Beckman model 315B infrared CO₂ analyzer, the other on the Varian model GD150 mass spectrometer. The primary advantage of the 315B is that it is cheaper than the
alternate monitoring equipment. The 315B, however, does not perform well at CO$_2$
concentrations less than 0.1% or greater than 2-3%. Further, it requires frequent
recalibration (approximately every 8 hours); pretreatment of the gas to be analyzed, e.g.,
removal of water vapor, methane, and ammonia; and remote or manual range switching.
Finally, it can provide an analysis of only one component of the gas stream.

The alternate monitor, the mass spectrometer, is not plagued with any of the
above ailments of the 315B. It requires neither pretreatment of sample gas, unless water
condensation is excessive (this can be gotten around more easily than with the 315B),
nor range switching. In addition to CO$_2$, it can simultaneously analyze the gas sample
for several other gaseous components, e.g., helium, neon, nitrogen, oxygen, and argon.
In addition to providing data for computation of oxygen consumption and CO$_2$
production, it can potentially serve as an initial control device to maintain a given gas com-
position, i.e., oxygen and diluent in the proper proportions. Thus, this instrument would
replace both the presently used gas solid chromatograph and oxygen analyzers and pro-
vide a more complete, accurate and precise method for gas analysis than is possible with
any other type of instrument.

It may be argued that the data acquisition systems outlined in this section are in
excess of that required to actually accomplish the desired tasks. However, it is not an
expression of sound judgment to design a system with a slightly different configuration.
Consequently, a system configured for a given task may no longer be suitable; only through
additional expense and must lost time can it be adapted to the new task. System I would
seem to meet most requirements for adaptability to a large variety of tasks; System II is a
more rigid configuration and such tasks as real time processing can be achieved only
through additional expenditure of time and money. It should be kept in mind, however,
that the two systems presented here represent only two of several possible configurations,
some of which may be equally suitable for the tasks at hand.

It is important to note that prices indicated for the listed equipment are only
approximate; most are as quoted by the various manufacturers in April, May, and June
of 1972, and are subject to change. It is hoped that funding for this project, if approved,
can be distributed over two fiscal years with initial funding in January, 1973, and subse-
quent funding in July, 1973. It would be desirable if the initial funding carried the Nova
1200 system and subsequently the process control equipment.
We appreciate that this is an extremely brief outline of a rather extensive project. For this reason, a more extensive description of the proposed system modification will be supplied as the situation warrants.
In the lists of computer equipment, the numbers in parentheses following the equipment descriptions are type numbers. Those numbers without letter suffixes are subject to a 10% discount; this has been included in the indicated prices.

Except for the mass spectrometer, all listed equipment prices are based on written, catalog, or telephone quotations. The quotation for the mass spectrometer was supplied by a company representative and is approximate only. A written quotation for this instrument is in preparation.

The equipment is grouped by manufacturer (supplier) the address of which follows each group.

NOTE:

It is pertinent to mention here that it will be necessary to negotiate a figure for indirect costs (i.e., overhead) which would be included in the total amount requested for funding.
SYSTEM I COMPONENT LIST:

<table>
<thead>
<tr>
<th>Component</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nova 1200 Series Jumbo Mainframe with 8k Core (8102 + 8174)</td>
<td>$6,435</td>
</tr>
<tr>
<td>Extra 8k Core (8174) 2 ea. @ $3690 ea.</td>
<td>$7,380</td>
</tr>
<tr>
<td>Power Failure/Auto Restart (8106)</td>
<td>$360</td>
</tr>
<tr>
<td>Low Speed I/O Subassembly (4007)</td>
<td>$180</td>
</tr>
<tr>
<td>Real Time Clock (4008)</td>
<td>$360</td>
</tr>
<tr>
<td>Teletype Control (4010)</td>
<td>$150</td>
</tr>
<tr>
<td>ASR33 Teletype (4010A)</td>
<td>$1,250</td>
</tr>
<tr>
<td>Moving Arm Disk Control (4046)</td>
<td>$3,600</td>
</tr>
<tr>
<td>Disk Adaptor and Power Supply (4047)</td>
<td>$1,530</td>
</tr>
<tr>
<td>Diablo Disk, 1247k words (4047A)</td>
<td>$5,000</td>
</tr>
<tr>
<td>Magnetic Tape Control (4030)</td>
<td>$3,600</td>
</tr>
<tr>
<td>Ampex TMX Magnetic Tape Transport, 7 track, 12.5 ips., (4030G)</td>
<td>$4,250</td>
</tr>
<tr>
<td>I/O Interface Subassembly (4014)</td>
<td>$180</td>
</tr>
<tr>
<td>Basic A/D Interface (4032)</td>
<td>$630</td>
</tr>
<tr>
<td>Chassis and Low Capacity Power Supply for A/D (4055A)</td>
<td>$900</td>
</tr>
<tr>
<td>13-BIT A/D Converter (4055F)</td>
<td>$950</td>
</tr>
<tr>
<td>Buffer Amplifier, Single Ended (4055I)</td>
<td>$200</td>
</tr>
<tr>
<td>Multiplex Control (4055K)</td>
<td>$230</td>
</tr>
<tr>
<td>Sample and Hold (4055L)</td>
<td>$300</td>
</tr>
<tr>
<td>16-Channel Multiplexer (4055N)</td>
<td>$300</td>
</tr>
<tr>
<td>General Purpose I/O Interface (4040) with Relays; 16-Channel, 2 ea. @ $1200 ea. (C-Form Contacts)</td>
<td>$2,400</td>
</tr>
<tr>
<td>Back Panel Connector (4045), 2 ea. @ $180 ea.</td>
<td>$360</td>
</tr>
<tr>
<td>Paper Tape Reader Control (4011)</td>
<td>$765</td>
</tr>
<tr>
<td>High Speed Paper Tape Reader (4011B)</td>
<td>$1,800</td>
</tr>
<tr>
<td>Real Time Operating System Source Tapes (3147)</td>
<td>$75</td>
</tr>
<tr>
<td>6-Foot Vertical Rack (1012A)</td>
<td>$700</td>
</tr>
<tr>
<td>Disc Cartridge (4047C)</td>
<td>$200</td>
</tr>
<tr>
<td>CRT Terminal Control (No part number)</td>
<td>$450</td>
</tr>
</tbody>
</table>

SUPPLIER: Data General Corporation
           Southboro, Massachusetts 01772
SYSTEM I (Continued)

Hazeltine CRT Terminal (2000) $ 2,486

SUPPLIER: Hazeltine Corporation
Greenlawn, New York 11740

Magnetic Tape, 8½ inch Reel, 1200 Feet ea., 36 ea. @ $8.25 ea. $ 297

SUPPLIER: Memorex Corporation, Denver District
Denver, Colorado

MISCELLANEOUS: Paper Tape, Paper Rolls, Instrumentation Cable and Connectors not Supplied by Manufacturers $ 1,200

SUPPLIER: Various

TOTAL $48,518
SYSTEM II COMPONENT LIST:

<table>
<thead>
<tr>
<th>Description</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nova 1200 Series Jumbo Mainframe with 8k Core (8102 + 8174)</td>
<td>$6,435</td>
</tr>
<tr>
<td>Extra 8k Core (8174)</td>
<td>$3,690</td>
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<tr>
<td>Power Failure/Auto Restart (8106)</td>
<td>$360</td>
</tr>
<tr>
<td>Low Speed I/O Subassembly (4007)</td>
<td>$180</td>
</tr>
<tr>
<td>Real Time Clock (4008)</td>
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<td>$950</td>
</tr>
<tr>
<td>Buffer Amplifier, Single Ended (4055I)</td>
<td>$200</td>
</tr>
<tr>
<td>Multiplex Control (4055K)</td>
<td>$230</td>
</tr>
<tr>
<td>Sample and Hold (4055L)</td>
<td>$300</td>
</tr>
<tr>
<td>16-Channel Multiplexer (4055N)</td>
<td>$300</td>
</tr>
<tr>
<td>General Purpose I/O Interface (4040) with Relays; 16-Channel, 2 ea. @ $1200 ea.</td>
<td>$2,400</td>
</tr>
<tr>
<td>Back Panel Connector (4045), 2 ea. @ $180 ea.</td>
<td>$360</td>
</tr>
<tr>
<td>Paper Tape Punch Control (4012)</td>
<td>$630</td>
</tr>
<tr>
<td>Remote Control for Paper Tape Punch (4013)</td>
<td>$270</td>
</tr>
<tr>
<td>High Speed Paper Tape Punch (4012A)</td>
<td>$1,500</td>
</tr>
<tr>
<td>Paper Tape Reader Control (4011)</td>
<td>$765</td>
</tr>
<tr>
<td>High Speed Paper Tape Reader (4011B)</td>
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</tr>
<tr>
<td>6-Foot Vertical Rack (1012A)</td>
<td>$700</td>
</tr>
</tbody>
</table>

SUPPLIER: Data General Corporation
Southboro, Massachusetts 01772

MISCELLANEOUS: Paper Tape, Paper Rolls, Instrumentation Cable and Connectors not Supplied by Manufacturer $1,200

SUPPLIER: Various

TOTAL $25,815
PROCESS CONTROL AND DATA ACQUISITION EQUIPMENT:

Operational Amplifier, Model 118K, with Mating Socket $ 24
SUPPLIER: Analog Devices, Inc.
Norwood, Massachusetts 02062

Humidity and Temperature Monitor, 4-Channel with Remote Switching $ 1,285
SUPPLIER: Atkins Technical, Inc.
Gainsville, Florida 32601

Infrared CO₂ Analyzer, Model 315B, with Remote Range Switching, 2 ea. @ $3759 ea. $ 7,518
SUPPLIER: Beckman Instruments, Inc.
Fullerton, California 92634

Thermal Mass Flowmeter, Type 5810, 0-15 Liters/Minute Max., 4 ea @ $373 ea. $ 1,492
Thermal Mass Flowmeter, Type 5810 with Type 5820 Indicator, 0-10 Standard Cubic Centimeters/Minute Max. $ 550

Blind-end Power Supply, Type 5810, for Thermal Mass Flowmeters $ 160

Purge Flowmeter, E/C 1500 Series, 2 ea. @ $25 ea. $ 50
SUPPLIER: Brooks Instruments Division
Hatfield, Pennsylvania 19440

Pressure Controller, Series 213, with 303 Stainless Steel Tubulations, 2 ea. @ $470 ea. $ 940
SUPPLIER: Granville-Phillips Company
Boulder, Colorado 80303

Fluid Switch Wafer, Type W1261/1P-12T, 3 ea. @ $245 ea. $ 735
12-Step Solenoid for Fluid Switches, Type WS6-12, 24VDC, 3 ea. @ $55 ea. $ 165
SUPPLIER: Scanivalve, Inc.
San Diego, California 92120

Pressure Transducer, Type DP7, 0-75 psi $ 250
Pressure Transducer, Type AP10, 1-45 psia, 3 ea. @ $325 ea. $ 975
Carrier Demodulator, Type CD16, for Pressure Transducers, 4 ea. @ $250 ea. $ 1,000
SUPPLIER: Validyne Engineering Corporation
Northridge, California 91324
PROCESS CONTROL AND DATA ACQUISITION EQUIPMENT (Continued)

Solenoid Sample Valve, Type 57-000064-00 $ 260
Mass Spectrometer, Type GD150 $23,000
SUPPLIER: Varian Aerograph
Walnut Creek, California 94598

Miscellaneous Hardware:
This material will consist of service parts for the various equipment listed as well as tubing and connectors for the fluid switches, pressure transducers, IR CO₂ analyzers/mass spectrometer, and pressure valves.
SUPPLIER: Various

TOTAL with Mass Spectrometer $31,886
TOTAL with IR CO₂ Analyzer $16,404

SYSTEM I with Mass Spectrometer $80,404
SYSTEM I with IR CO₂ Analyzer $64,922
SYSTEM II with Mass Spectrometer $57,701
SYSTEM II with IR CO₂ Analyzer $42,219
APPENDIX B

The following paper entitled, "A Rapid Quantitative Measure of Metabolic Activity," by J. P. Jordan, C. L. Schatte, D. P. Clarkson, M. L. Corrin, and W. W. Martz 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.
A Rapid Quantitative Measure of Metabolic Activity

by

John Patrick Jordan, C. L. Schatte, D. P. Clarkson, M. L. Corrin and W. W. Martz. Laboratory of Aerospace Biology
Department of Biochemistry
Colorado State University
Fort Collins, Colorado 80521
A quick, simple measure of metabolic activity by measuring the conversion of a $^{14}$C-labeled substrate ($C_0$) to $^{14}$CO$_2$ (P) is described. A plot of $\ln (C_0-P)$ versus time provides a readily quantified parameter sensitive to both changes in metabolic rate and the nature of the substrate oxidized. Although acetate-1-$^{14}$C was the substrate most frequently used, labeled glucose and pyruvate have also been used. The procedure involves only an initial injection of labeled substrate followed by collection of expired $^{14}$CO$_2$ for approximately 60 minutes. The animal is not anesthetized, restrained, or starved. Possible interpretations are discussed.
INTRODUCTION

Because of the definitive nature of mathematics, biologists have long sought to use it as a tool for quantifying changes in animal metabolism. While classical metabolic parameters such as oxygen consumption and carbon dioxide production are used as whole body metabolic rate indicators, it is advantageous to measure substrate level metabolic activity in order to increase the sensitivity of measurements of metabolic aberrations in the whole animal. Such a parameter must be quickly and easily measured, readily quantified for comparative purposes, and cause minimum disturbance to the animal. The development of such a parameter has been crucial to work in this laboratory which is concerned with long-term biochemical adaptation of rats to artificial environments. The need to maintain as unperturbed a state as is possible while performing repeated measurements over periods of several weeks has prompted us to search for a cellular level indicator that can be repeatedly and reproducibly used while leaving the rats physiologically unaffected.

We have found that the injection of acetate-1-$^{14}$C and treatment of the resulting $^{14}$CO$_2$ expiration curve as a simple exponential function provides us with an accurate measure of tricarboxylic acid (TCA) cycle activity (and, therefore, a major part of cellular oxidative activity) which is readily
quantified for comparisons between different groups of animals. The sampling time can be as little as 60 minutes, radioactive analysis requires no chemical treatment, and reproducibility can be as good as 5%. Except for an initial intraperitoneal injection, the animals are unrestrained, unstarved, uncatheterized, and essentially undisturbed in the environment to which they are exposed. When interpreted in conjunction with other metabolic parameters, the procedure provides an additional measure of metabolic rate plus insight into possible biochemical changes which may occur even in the absence of a change in metabolic rate.

METHODS

In a series of twenty-five experiments, groups of rats were exposed to artificial environments for periods of a few days to thirteen weeks. At intervals of 1-7 days throughout the exposure, groups of 4-12 rats were injected intraperitoneally with 25-150 μCi/kg of sodium acetate-1-14C in 0.3-0.5 ml distilled water. The expired gas was bubbled through a carbon dioxide scrubber containing 10 liters of 3N KOH. The contents of the scrubber was sampled for periods of 1-24 hours at intervals of 10 minutes for the first hour post-injection, 20 minutes during the second hour, and 20, 30 or 60-minute intervals thereafter. Activity of the samples was
determined by pipetting 0.1 ml of the KOH solution into 15 ml of p-dioxane-PPO-POPOP-napthalene-ethanol-Cab-O-Sil* and counting in a liquid scintillation spectrometer for three 5-minute counts each.

A typical plot of accumulative disintegrations per minute versus time is shown in Figure 1. The shape of the curve and its mathematical analysis have been rigorously treated by others (1, 2, 3) as conforming to the general equation:

$$A = A_1 e^{k_1t} + A_2 e^{k_2t} + A_3 e^{k_3t}$$

where:

- $A$ = activity in the expired CO$_2$ at a given time, $t$
- $A_n$ = activity in the expired CO$_2$ at time, $t$ as a function of rate, $k_n$
- $k_n$ = rate of increase of activity in the expired CO$_2$ for a particular length of time

With the intent of using the parameter for comparisons between groups of animals rather than on an absolute basis, we have chosen to approach analysis of the data as a first order exponential function. Further, since it was found that factors likely to influence the shape and time course of the expired $^{14}$CO$_2$ curve do not vary appreciably in our experiments

* PPO=2,5-Diphenyloxazole
POPOP=1,4 bis (2 (4-methyl-5-phenyloxazolyl) )-Benzene
(i.e., bicarbonate pool size, cellular uptake of CO₂, exchange with bone), the detailed three-compartment analysis has proved unnecessary. A satisfactory interpretation has resulted by expressing the data as:

\[
\ln (^{14}\text{C injected/kg} - ^{14}\text{C expired/kg}) = kt + C_0
\]

where \( k \) is the rate of decrease in total body activity per kilogram of rat as a function of time \( t \) and \( C_0 \) is the total activity in the pool at zero time. This represents a combination of the \( A_1 \) and \( A_2 \) components while disregarding \( A_3 \) of the three-compartment analysis. The initial \( (A_1) \) component coincides with the dead time of our system, about 10 minutes, and, therefore, cannot be accurately measured. The dead time consists of the events between injection of the substrate and its appearance as CO₂ in the scrubbers. These include absorption into the blood, distribution to the cells, metabolic conversion, transport to the lungs and expiration as CO₂, equilibration with the animal chamber gas, and transport to and equilibration with the scrubber contents.

We have consistently obtained a good fit of the data during the time \( (A_2) \) in which the kinetics were first order; the non-first-order component \( (A_3) \) did not consistently lend itself to logarithmic analysis. Fortunately, the curves usually reflected first order kinetics for about 90 minutes.
Thus, a sufficient sampling period was available over the linear portion of the curve (10-90 minutes) to calculate the slope of the first-order curve. Additionally, both our data and the data of others (1, 4) have indicated that a substantial fraction of injected acetate is expired during a period of one or, at most, two hours post injection. Subsequent elimination of label from the body does not appear to significantly affect the results.

The slope of the logarithmic plot was determined by best fit of a least squares regression analysis (Figure 2). The resulting value, k or rate constant, was used for quantitative comparisons among different experimentally-treated groups of animals.

RESULTS

As shown in Figure 3, the value of k will vary in magnitude as a function of the catabolic rate of the injected substance; the greater the rate, the greater the value of k. This particular set of curves represents the conversion of acetate-\(^{-14}C\) to \(^{14}CO_2\) in rats exposed to neon-oxygen for 4 weeks as compared with air controls and was calculated from data previously published (5). In this case, acetate oxidation to \(CO_2\) mirrored both total metabolic rate and the activity of the tricarboxylic acid cycle; on that basis, the results graphically illustrate metabolic adaptation to the neon environment for the four-week period.
The half-life of the metabolic pool can be determined by extrapolation of the slope to the abscissa. This represents a graphical computation of the half-life of the substrate as expressed by:

\[ t_{1/2} = \frac{\ln 2}{k} \]

If the injected substrate mixes with the appropriate substrate pool(s) of the body within about 10 minutes post injection, turnover rate of the substrate's metabolic pool can be calculated in the absence of significant changes in pool size during the mixing and measuring period.

Of further importance is the fact that the percentage of injected substrate converted to CO₂ can be predicted with good accuracy from the rate constant, k. Figure 4 shows the regression line of slope versus percentage of injected label expired for periods of 2-3 hours post injection. The points represent determinations with acetate-1-¹⁴C injected into rats whose age and weight varied from 90-240 days and 200-500 grams, respectively. The correlation coefficient of 0.958 indicates that the slope, determined during the first hour post injection, can serve to predict the conversion of labeled acetate for up to three hours. It is likely that little loss of accuracy would occur for longer collection periods since a substantial amount of the injected compound
is expired during the first hour after injection. With acetate-\textsuperscript{1-14}C, we have consistently recovered 40-50\% and as much as 75\% of the injected label during the first 60 minutes after injection. These values agree with the data of Gould, et al (4) who recovered 50\% of acetate-\textsuperscript{1-14}C from rats in 42 minutes.

We have found the slope values to be repeatable within \(\pm 5\%\) when at least 4 animals are used. With groups of 6-12 animals, the sensitivity is such that changes in catabolic rate due to time of day, prandial state, and reaction to stress of handling are readily apparent. It is, however, necessary to maintain a minimum level of total activity injected into the animals. A group of 3 or 4 animals require 100-150 \(\mu\)Ci/kg to insure sufficient expired activity. Groups of 6-8 rats were customarily injected with 50 \(\mu\)Ci/kg while a 12-rat group needed only 25 \(\mu\)Ci/kg for sufficient activity in the expired CO\textsubscript{2}.

DISCUSSION

Requisite with the use of this analysis as an indicator of metabolic rate are three assumptions. First, changes in the pool size must be known or assumed to not occur under the experimental conditions. Without plasma acetate specific activity measurements, the size of the pool diluted by the tracer cannot be known; if pool size does change, then an
alteration in metabolic rate might be suggested when none, in fact, occurred.

Second, changes in the fraction of the total acetate pool entering the TCA cycle must be known or assumed to not occur. If changes in the amount of label entering the TCA cycle do occur, an alteration in metabolic rate would be implied when none had occurred. Such a situation is readily detected by measuring CO₂ specific activity. Since lipid biosynthesis is the only major alternative for acetate utilization, changes in the rate of lipid synthesis can be measured by incorporation studies. In the present circumstances, we have not found appreciable changes in the fraction of total acetate entering the TCA cycle and have, thus, been able to dispense with expired CO₂ specific activity measurements.

Third, it must be assumed that the appearance of $^{14}$CO₂ in the expired gas is proportional to its actual rate of oxidation; the experimental conditions should not differentially alter those factors influencing the correlation between actual oxidation rate and appearance as CO₂ in the expired gas. These factors include CO₂ diffusion constants, blood supply to the tissues, exchange of $^{14}$CO₂ with total body CO₂ "stores," and ventilation rates. Gould, et al (4) pointed out that the rate of catabolism and expiration of $^{14}$CO₂ can vary with the substrate. They found the two rates did not differ in the case of acetate or bicarbonate but that succinate oxidation was faster than its appearance as CO₂ in the expired gas.
A further consideration, although untested as yet, is that the pathway by which exogenous acetate enters the cell may be influenced by the experimental conditions while endogenous acetate is not. The latter enters an acetate pool primarily as a result of either carbohydrate or fatty acid catabolism, whereas injected acetate must cross both the cell and mitochondrial membranes. It is possible that the two routes of entry might be differentially affected by an experimental condition such that the injected substrate would not reflect the true endogenous situation. We feel that under our experimental conditions of mild physiological stress, neither the number of acetate pools which may exist nor the route of entry into the mitochondria complicate interpretation of the results.

The use of acetate as the test substrate minimizes these problems while offering an optimal indicator for aerobic oxidation. Since the TCA cycle is dependent on acetate as a substrate its activity must be sensitively reflected in acetate pool flux. An additional advantage is that acetate uptake by the cycle must be proportional to oxygen uptake by the cell and CO₂ produced from it since the major amount of oxygen consumption and CO₂ production occurs via the TCA cycle. In our hands, the labeled substrate method is significantly more sensitive than the measurement of oxygen consumption or total CO₂ production.
Unlike many other metabolic substrates, acetate is used principally for fatty acid synthesis, steroid synthesis and TCA cycle oxidation. With inputs into the acetate pool mainly from pyruvate and fatty acid oxidation, and outflow into the TCA cycle and lipid synthesis pathways, the variables affecting pool size and turnover rate are limited. It is unlikely that minor pathways such as ketone metabolism significantly influence acetate pool characteristics in all but exceptional circumstances or specific tissues. Thus, if in a given experiment there is some doubt about the validity of the assumption that the fraction of the total acetate pool entering the TCA cycle has not occurred, excellent challenge data can be obtained by simply measuring the uptake of the acetate label into the total body lipid as a function of time.

We have used labeled glucose in a similar manner with good results. Since its pool fluctuates more drastically, correlation of its rate constant with metabolic rate indicated by other parameters has not been as good as with acetate. Using glucose-1-\textsuperscript{14}C and glucose-6-\textsuperscript{14}C and a modification of the mathematical procedure of Katz, Landau and Bartsch (6), we have been able to quantify both the aerobic and anaerobic catabolism of glucose in rats (7). Rats in a marginally hyperoxic environment were found to have "normal" total CO\textsubscript{2} production despite partial inhibition of the pyruvate and succinate dehydrogenase systems, which caused description of the TCA cycle as indicated by gas-solid chromatography of TCA
cycle intermediates. It was found that in the hyperoxic animals, twice as much glucose was catabolized via the pentose phosphate pathway at the expense of the glycolytic pathway. This accounted for the normal total CO$_2$ production despite a slowing of the TCA cycle.

Pyruvate-2-$^{14}$C has also been used in limited studies. It should be emphasized that the method allows the animals to remain in as normal a state as is possible under the conditions. This is particularly important when subtle metabolic changes are being examined since starvation, restraint, repeated withdrawal of blood, or removal to a foreign testing apparatus often changes either the rate or the nature of metabolism so as to obscure the effects of the test variable. Apart from the initial injection, our rats were tested in the same environment and physiological state to which they had become accustomed; the fact that the slope constants predictably varied with the often marginal stressors to which the animals were exposed is consistent with the concept that the results are uncomplicated appreciably by other factors.

Of great importance, too, is the fact that the analytical procedure is quick and simple. We have adapted a computer key punch to a liquid scintillation counter which allows the counts to be prepared directly for computer processing on the same day as the samples are counted.
LITERATURE CITED


