Report No. IITRI-L6042-12
(Final Report)

OXYGEN TOLERANCE IN ATMOSPHERES SIMULATING UNDERSEA SATURATION DIVES

Biological Sciences Division
Office of Naval Research
Washington, D.C. 20360

Attention: Head, Physiology Branch

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April 1, 1967 through June 30, 1970

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FOREWORD

This is Report No. IITRI-L6042-12 (Final Report) entitled "Oxygen Tolerance in Atmospheres Simulating Undersea Saturation Dives," IITRI Project L6042. The project was conducted for the Biological Sciences Division, Office of Naval Research, Department of the Navy, under Contract No. N0014-67-C-0395, during the period from April 1, 1967 through June 30, 1970.

Mr. James Q. Kissane, Research Biochemist, served as the project leader. Dr. E. J. Hawrylewicz, Assistant Director and Dr. W. H. Riesen, Chief, Biochemistry Section, provided the overall administrative and technical guidance.

The experimental data is recorded in IITRI Logbooks D1525, D1549, D1568, and D1634.

Respectfully submitted,

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ABSTRACT

Studies were conducted to determine the effects of a simulated Sealab environment on selected metabolic systems in rats. The simulated Sealab environment consisted of 98.2% helium and 1.8% oxygen atmosphere maintained at 300 psi at 22°C. After 24 hr exposure and rapid decompression the following observations were made. Liver and muscle mitochondrial P:O and QO_2 were normally functional. Liver LDH activity was slightly decreased. Muscle and liver glycolysis rates were unaffected. Protein synthesis in liver subcellular components measured by ^{14}C-lysine uptake was unaffected while it decreased in muscle. Liver glycogen reserves decreased 8 to 10 fold, while muscle glycogen decreased approximately 50%.

Control experiments in helium atmosphere at ambient pressure and those conducted at submergence pressure at elevated temperature suggested that thermal effects of helium resulted in increased glycogen utilization.

To enable rapid sampling and freezing of tissue at pressure for nucleotide analyses, two biopsy devices were developed and evaluated. The Biopsy Probe, a boring device, was found not to be satisfactory when its performance was measured in terms of liver tissue ATP values. Using the same parameter the Surgical Biopsy Device provided rapid tissue excision and freezing by cryogenic immersion.

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OXYGEN TOLERANCE IN ATMOSPHERES SIMULATING UNDERSEA SATURATION DIVES

I. INTRODUCTION

This report summarizes results of studies concerned with the effects of a simulated SeaLab atmosphere on selected cellular metabolic systems in mammals. An overall summary of the experimental findings is presented in the first section of the report. Experimental details not previously reported are described in the second section.

The experiments were conducted at a pressure of 300 psia equivalent to a sea water submergence of 640* ft, and in an atmosphere consisting of 98.2% helium and 1.8% oxygen. The biological systems investigated during the program included aspects of energy and carbohydrate metabolism, protein synthesis, lipid mobilization, and cell morphology. In order to evaluate the effects of a complete dive cycle, some of the metabolic systems were studied both at a depth of 640 ft and after decompression. In addition experiments were conducted to determine whether changes in energy metabolism due to cooling by helium could be reversed by elevation of the environmental temperature. Inasmuch as during the exposures the experimental animals were completely isolated from direct contact, remote biopsy techniques were developed and evaluated.

*Based on USN conversion: 0.445 psi = 1 foot sea water.

II. SUMMARY OF THE ENTIRE STUDIES

A. Exposure Chambers

The animal exposure chambers were constructed from 3 in. internal diameter, 24 in. long water pipes fitted with gas inlets, internal food and water containers, and gate valve for the biopsy device. Rats used as the experimental animals were exposed to an environment pressurized to 300 psia in a gas mixture consisting of 98.2% helium and 1.8% oxygen. Final oxygen content in the experimental environment was ascertained by analysis of the gas sample.

To remove nitrogen the system was flushed at ground level pressure with pure oxygen and the chamber was adjusted to a 79% helium-21% oxygen atmosphere. Finally pressurization was achieved to 300 psia and oxygen was supplied continuously to maintain normal ambient tension. The oxygen concentration at 300 psia was 1.8%, representing 273 mm Hg pO_2, which is below the threshold of oxygen toxicity. The final flow rate of oxygen provided 2.5 L/hr to each animal. This value was approximately 2.5 times the theoretical requirement. Carbon dioxide and other waste gases were removed from the chamber through the flow-through system. Control rats were housed in similar chambers with room air being driven through by a vacuum pump.

After exposure, the rats were individually decompressed within 30 to 40 sec. Tissue samples were then rapidly taken for all biochemical analyses except for the assay of the highly labile pyridine and adenine nucleotides. For these analyses special biopsy devices were developed to permit rapid freezing of liver tissues while the animals were at pressure. This procedure circumvented decompression artifacts that could be expressed in altered nucleotide levels.

B. Biopsy Techniques

Two different biopsy devices have been developed, constructed, and evaluated for their performance for rapid tissue excision and freezing at elevated pressure.

The Biopsy Probe was a remotely driven stainless steel device that removed tissue samples by a boring action. Tissue was frozen by precooling the device. Due to the slow time to freezing, this probe was not effective in providing tissues suitable for nucleotide analysis.

The Surgical Biopsy Device, constructed of polystyrene, rapidly exposed and cut sections of liver lobes for freezing by cryogenic fluid immersion. Thus, it provided means for freezing tissue rapidly enough to maintain adenosine-5-triphosphate (ATP) levels comparable to those from tissue removed outside the chamber by rapid surgery. However, due to space limitation, the Surgical Biopsy Device could not be used in our exposure chambers.

C. Biochemical Studies

Male Sprague Dawley rats, weighing between 100 and 200 g, were exposed to the 98.2% helium-1.8% oxygen atmosphere at 300 psia for 24 hr at 22°C. Various changes were observed in the tissues following animal decompression.

1. Histological findings indicated that liver and brain tissue stained with hematoxylin and eosin appeared normal with respect to nuclear and cytoplasmic inclusion.
2. Liver and muscle mitochondria remained highly functional in high-energy phosphate production (P:O ratio) and respiratory quotient (QO_2) with α-ketoglutarate as the substrate.
3. Liver lactic acid dehydrogenase activity was slightly but significantly decreased. The results suggested that the enzyme may have leached from the cells, or was inhibited intracellularly during the exposure, indicating stress to the animal.
4. The rate of anaerobic glycolysis in muscle and liver tissue measured by breakdown of fructose-1,6-diphosphate to lactate was not affected.

5. Liver protein biosynthesis, determined by ^{14}C-lysine incorporation into trichloracetic-precipitable material, did not change significantly in various subcellular fractions (microsomes, mitochondria, nuclei, and whole cytoplasm).

   In muscle tissue, 50% less lysine was incorporated into protein components of nuclear, mitochondrial and microsomal fractions. No change occurred in the amount of incorporation into soluble cytoplasmic protein, and considerably more activity was localized in the non-precipitable cytoplasmic residue. These observations suggested that the protein turnover rate in the cellular organelles was decreased in muscle tissue, or that ^{14}C-lysine transport to muscle was decreased, due possibly to reduced peripheral blood flow.

6. Significant differences were not found in the blood serum concentrations of triglyceride, phospholipid, and free or esterified cholesterol.

7. An 8 to 10 fold reduction in liver glycogen reserves was observed. Control rats maintained in the helium-oxygen mixture at 22°C and at ambient pressure also showed considerable reduction in the liver glycogen levels. However, the liver glycogen was maintained at normal level in rats held at 31° at ambient pressure.

Muscle glycogen levels were also decreased in rats maintained in helium-oxygen mixture at 300 psia and 22°. The reduction was approximately 50% as compared with control animals. This effect was somewhat reversed at 31°.

These experiments suggested that the thermal effects of helium result in increased glycogen utilization to maintain body heat. Increase in ambient temperature reversed this effect.

III. DETAILS OF RECENT EXPERIMENTS

A. Liver and Muscle Glycogen Utilization

In the early experiments (IITRI Report L6042-5) an 8 to 10 fold reduction in liver glycogen reserves was observed in the experimental rats, while muscle glycogen decreased to half that in the control animals. This change in reserve was reflected in greater glycolysis in the same tissues of the experimental rats. It was pointed out, however, that the control rats in these experiments were not exposed in a chamber identical to that used for the experimental animals. Furthermore, the experimental rats fasted during confinement while the control animals had available food and water.

Hence in subsequent experiments (IITRI Report L6042-6) two sets of animal controls were used to determine the effects of confinement in the chamber and to possibly differentiate endocrine or pressure effects. The three groups of rats used were as follows.

1. Experimental group - maintained for 24 hr in helium-oxygen mixture at submergence pressure with fasting.
2. Control chamber group - maintained for 24 hr in normal air atmosphere at ambient pressure with fasting.
3. Room control group - maintained for 24 hr outside the chamber in normal air at ambient pressure and fed and watered ad libitum.

This experimental plan was designed to determine the effects of fasting and chamber confinement in addition to the effects of helium-oxygen atmosphere at elevated pressure. Confinement in ambient air resulted in a precipitous decrease in liver glycogen, and a smaller decrease in muscle glycogen. Liver and muscle glycogen were further decreased in the high-pressure helium group indicating a greater glycogen utilization. All differences between the groups were statistically significant. The decrease in glycogen content following fasting and

confinement was attenuated by imposing the additional stress of exposure to high-pressure helium-oxygen atmosphere. This additional utilization of glycogen could be due to the thermal effect of helium or to effects of compression.

Additional experiments were conducted at two environmental temperatures, namely 22° and 31°C (IITRI Report L6042-10). The experiments were designed if possible to separate the effects due to body heat loss and other metabolic effects. Rats were exposed to air or helium-oxygen mixture for 24 hr using the experimental protocol shown in Table 1.

Table 1

GLYCOGEN UTILIZATION STUDY

Experimental Conditions		Number of Rats			
		15 psia		300 psia	
Atmosphere	Housing and Feeding	22°	31°	22°	31°
Air	Cage outside chamber; ad libitum feeding	18	18	--	--
Air	Inside chamber; fasting	12	12	--	--
Helium-oxygen	Inside chamber; fasting	12	12	12	12

Each experimental run consisted of six rats exposed in individual chambers to air or helium-oxygen mixture and three control rats maintained outside the chamber in ambient air. Chamber temperature was regulated by immersion in constant temperature

water baths at either 22° or 31°C. After the 24 hr exposure the rats appeared healthy except those maintained in the helium-oxygen atmosphere at 300 psia and 31°C. These animals were moribund after the rapid decompression. The glycogen values for liver and muscle tissues are presented in Tables 2 and 3, respectively; new data not previously reported are indicated by an asterisk. For comparison the values obtained in the initial group of experiments (IITRI Report L6042-6) at 22° are shown in Table 4.

The effect of the environmental stress on the utilization of glycogen was best typified in the case of the liver tissue. A precipitous decrease in glycogen concentration was observed in fasting rats maintained in ambient air at either 22° or 31°C. The liver glycogen concentrations decreased to a larger extent in rats exposed to the atmosphere consisting of 80% helium and 20% oxygen at 22°C than in the control group of rats kept in ambient air in the chamber at the same temperature. Liver glycogen utilization in rats exposed to the helium-oxygen atmosphere appeared to be independent of the pressure. This was evidenced by the similarity of values obtained in rats maintained at ambient pressure and at 300 psia at 22°C.

Table 2

LIVER GLYCOGEN[a] IN RATS EXPOSED FOR 24 HR TO AIR OR HELIUM:OXYGEN MIXTURES AT VARIOUS TEMPERATURES

	Experimental Conditions								
	15 psia							300 psia	
	Ambient Air Not in Chamber Feed Ad Lib.			Ambient Air Chamber Fasting		80% He-20% O_2 Chamber Fasting		98.2% He-1.8% O_2 Chamber Fasting	
Rat No.	22°	28°	31°	22°	31°	22°	31°	22°	28°
1	106.8	69.4*	157.0	18.3	56.5	2.0	13.1	3.8	1.1*
2	20.4	46.0*	157.7	2.8	12.2	3.4	52.6	2.6	0.9*
3	94.4	59.0*	160.6	8.1	12.2	3.4	14.0	2.5	1.3*
4	108.0		134.3	17.7	66.2		50.8	4.7	1.8*
5	105.0		150.9	34.4	28.2		15.8	3.1	0.9*
6	62.8		140.1	5.7	14.9		11.4	2.6	1.4*
7	58.5		96.4	3.0	27.4		21.0*	1.0*	
8	115.3		81.8	21.5	12.2		30.0*	1.2*	
9	108.5		43.8	9.4	21.9		21.0*	1.0*	
10	109.5		115.7*	2.6	47.6			0.4*	
11	64.4		124.5*	21.9	17.5				
12	106.6								
13*	44.1*								
14*	57.3*								
15*	69.4*								
Average	82.1	58.1	123.9	13.2	26.1	2.9	25.5	2.3	1.2
Std. Dev.	±29.7	±11.7	±37.0	±10.3	±23.7	±0.8	±15.9	±1.4	±0.3

*New data not previously reported.

[a]Micromoles glucose per gram tissue.

Table 3

MUSCLE GLYCOGEN[a] IN RATS EXPOSED FOR 24 HR TO AIR OR HELIUM:OXYGEN MIXTURES AT VARIOUS TEMPERATURES

	Experimental Conditions								
	15 psia							300 psia	
	Ambient Air Not in Chamber Feed Ad Lib.			Ambient Air Chamber Fasting		80% He-20% O_2 Chamber Fasting		98.2% He-1.8% O_2 Chamber Fasting	
Rat No.	22°	28°	31°	22°	31°	22°	31°	22°	28°
1	17.5	18.9*	22.5	19.1	17.6	14.5	11.2	3.9	7.8
2	20.3	16.5*	22.1	7.3	10.7	2.1	7.5	3.3	5.0*
3	26.4	15.3*	36.2	13.8	9.8	8.6	14.2	3.4	9.0*
4	24.7		33.1	11.6	13.7		12.8	2.3	6.1*
5	25.2		32.6	13.8	13.9		10.3	1.5	7.2*
6	15.8		34.6	12.6	10.2		12.4	2.8	5.7*
7	19.5		22.1	13.8	14.8		11.1*	4.7*	
8	14.4		22.4	16.1	8.5		12.0*	2.0*	
9	14.8		16.9	11.8	9.4		13.1*	4.1*	
10	26.9		25.9*	9.3	16.2			2.8*	
11	16.9		25.9*	18.1	8.7				
12	22.8								
13*	15.4*								
14*	13.8*								
15*	18.2*								
Average	19.5	16.9	26.8	13.4	12.3	8.4	11.6	3.1	6.8
Std. Dev.	±4.6	±1.8	±6.4	±3.5	±3.2	±6.2	±1.9	±1.0	±1.5

*New data not previous reported.

[a]Micromoles glucose per gram tissue.

Table 4

GLYCOGEN[a] IN LIVER AND SKELETAL MUSCLE OF RATS EXPOSED FOR 24 HR TO AMBIENT AIR AND A HELIUM-OXYGEN MIXTURE AT 300 PSIA AND 22°C

	Ambient Air Controls		Chamber Controls		Experimental	
Rat No.	Liver	Skeletal Muscle	Liver	Skeletal Muscle	Liver	Skeletal Muscle
1	166	25.1	25.1	15.1	3.0	10.6
2	184	18.2	22.0	17.7	3.0	-
3	157	30.4	18.6	16.2	3.0	10.2
4	195	25.3	10.9	14.0	4.2	15.7
5	182	18.4			2.5	3.8
6	171	32.6			3.2	5.2
7	142	26.8			3.9	13.1
8	102	23.1			3.2	7.1
9	158	26.0			3.9	16.9
10	116	24.8			2.8	7.4
11					3.7	18.9
12					3.7	15.9
13						10.0
Average	157	25.1	19.1	15.7	3.4	11.2
Std. Dev.	±30	±4.5	±6.1	±1.6	±0.5	±4.9

p Value: $He-O_2$ 300 psia versus chamber control: liver-.0002, skeletal muscle .0452. $He-O_2$ 300 psia versus ambient air: liver - < .0001, skeletal muscle < .0001. Chamber control versus ambient air: liver - < .0001, skeletal muscle < .0001.

[a]Micromoles glucose per gram tissue.

Exposures at 31°C appeared to attenuate liver glycogen utilization. Of particular interest was the group of rats exposed to the 80% helium-20% oxygen atmosphere, in which liver glycogen utilization was approximately the same as that in the chamber control group. This was a marked reversal of the effect observed at 22°C. A change of temperature from 31°C to 28°C was found necessary to ensure survival of the rats at 300 psia. Under these experimental conditions attenuation of liver glycogen utilization was not observed. Exposure to ambient air outside the chamber also showed no attenuation at 28°C. However, additional experimental data would be required at 28° for definitive conclusions.

A decrease in muscle glycogen was also observed as the result of 24 hr fasting. However, the effect of temperature and exposure to helium-oxygen atmosphere on utilization of muscle glycogen could not be definitely established. Due to scattering of values the results are obscure in the case of the 15 psia helium-oxygen group at 22°.

In general the results indicated that glycogen utilization in rat liver is temperature dependent. The data suggest that the cooling effect of helium, which promotes hypermetabolism required to maintain body heat, can be reversed by increase in the environmental temperature.

B. Biopsy Techniques

1. Biopsy Probe

The initial experiments on the effects of helium-oxygen atmospheres on the levels of liver adenine and pyridine nucleotides in rats were inconclusive. However, some indication was obtained suggesting a decreased adenosine-5-triphosphate (ATP) and decreased nicotinamide adenine dinucleotide (NAD) concentrations in the liver of rats exposed to the helium-oxygen mixture at both ambient and hyperbaric pressures. Since the nucleotides are sensitive to metabolic changes occurring during hypoxia or ischemia, it was mandatory to develop a technique enabling a rapid and complete arrest of the metabolic processes in the biopsied tissue. This can be accomplished by rapid freezing at cryogenic temperatures (ref. 1,2).

The biopsy probe (IITRI Report L6042-6) was initially designed and constructed to enable rapid removal and freezing of liver from rats kept in the exposure chamber at pressure. The probe was a stainless steel boring device which removed samples 1/2 in diameter corresponding to 100 to 200 mg of liver tissue. The cutting cylinder of the probe was driven by an electric drill through an opening in the exposure chamber with Teflon pressure seal. The cutting cylinder was precooled in liquid freon at -140°C to provide freezing of the tissue core as it is cut, and the biopsy tip retracted to the

cryogenic reservoir within the biopsy apparatus. A restraining board was used to position the rat during confinement to ensure accurate biopsy of liver.

The effectiveness of this biopsy probe was evaluated in terms of the extent of ischemia which might occur before metabolic arrest. Six liver biopsies were performed by surgical excision followed by rapid freezing, and six using the biopsy probe. Analyses for ATP were made according to the procedure of Lowry (ref. 3). The data summarized in Table 5 indicate that the ATP concentration in the biopsy probe samples was approximately 25% lower than the average value obtained in the surgical biopsy samples. The differences were statistically significant and most likely reflect ischemia due to the low rate of tissue freezing.

Table 5

LIVER ATP CONCENTRATIONS OBTAINED BY SURGICAL BIOPSY AND BIOPSY PROBE

	Number of rats	ATP, μM/g tissue[a] Mean	St. Dev.	P
Surgical Biopsy	6	2.60	0.27	--
Biopsy Probe	4[b]	1.97	0.21	0.003

[a]Duplicate determinations were made for each rat.

[b]Insufficient liver tissue was obtained from 2 rats.

2. Surgical Biopsy Device

In order to alleviate the problem of slow freezing rate, the biopsy probe was modified with an accessory device. The modified surgical biopsy device permitted the biopsy of liver tissues itself, excluding the presence of other tissue material which interfere in the rapid freezing of the sample.

The surgical biopsy device (Fig. 1,2) consisted of a spring-loaded scalpel blade and two pairs of springs (Warburg flask springs or strong rubber bands) which served as retractors. After incision and retraction of the skin folds, the entire peritoneal contents descended by gravity. A section of the exteriorized liver mass was then cut away by a follower blade and rapidly frozen by immersion in isopentane. The temperature (-140°C) was maintained by external circulation of liquid nitrogen through a coil in the isopentane bath. Due to space limitations in the chamber and volatility of the coolant difficulties were encountered in maintaining the cryogenic reservoir in the chamber. Furthermore, the cryogenic system lower the temperature in the chamber.

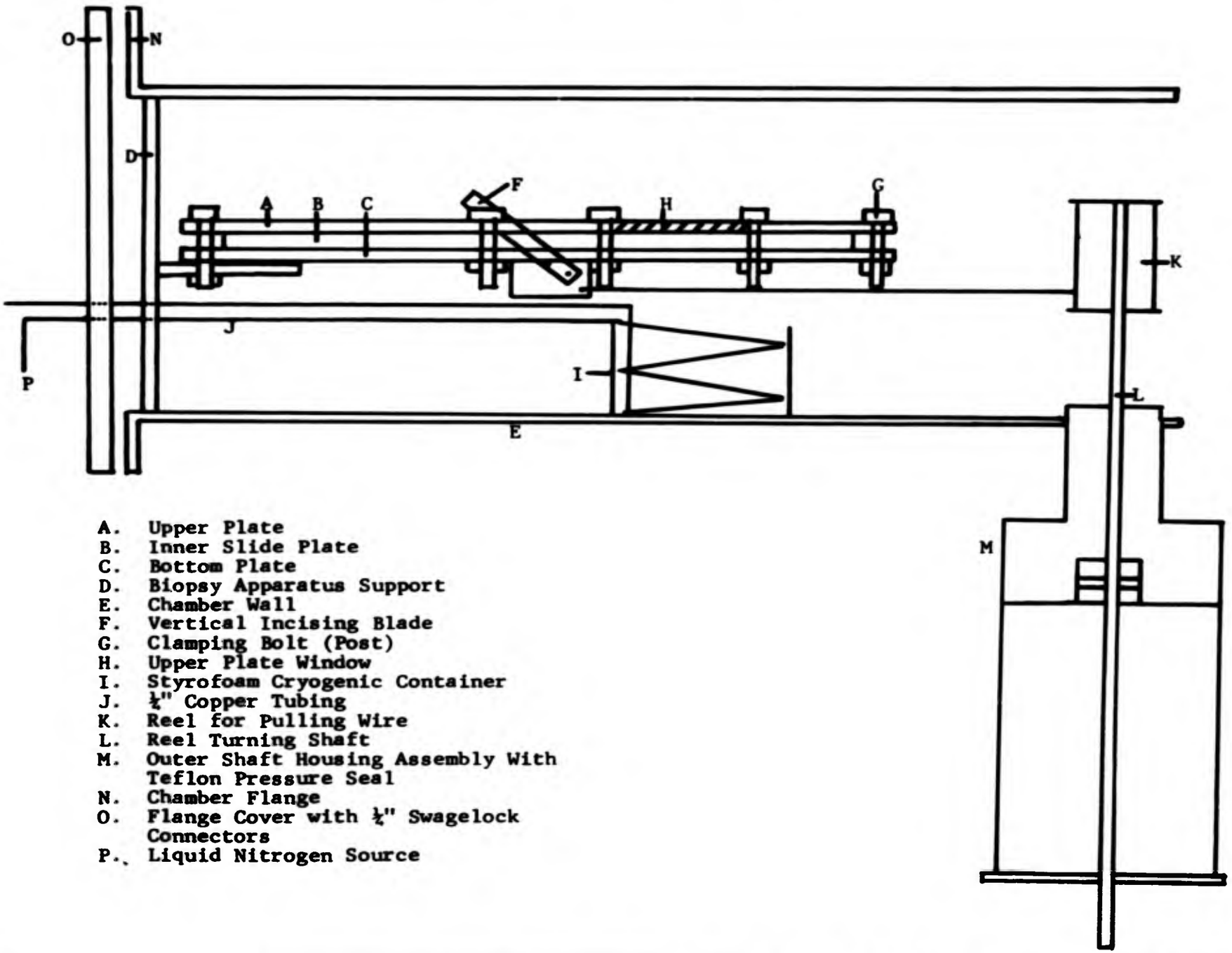
To evaluate the performance of this biopsy device, three rats were biopsied in the chamber in air at ambient pressure and two rats using conventional surgical tissue excision. The ATP concentrations (μM/g) in the livers of the rats were compared and found to be as follows:

Surgical Biopsy: 2.93, 2.75; Avg. 2.85

Biopsy Device: 2.86, 2.87, 2.67; Avg. 2.77

Figure 1

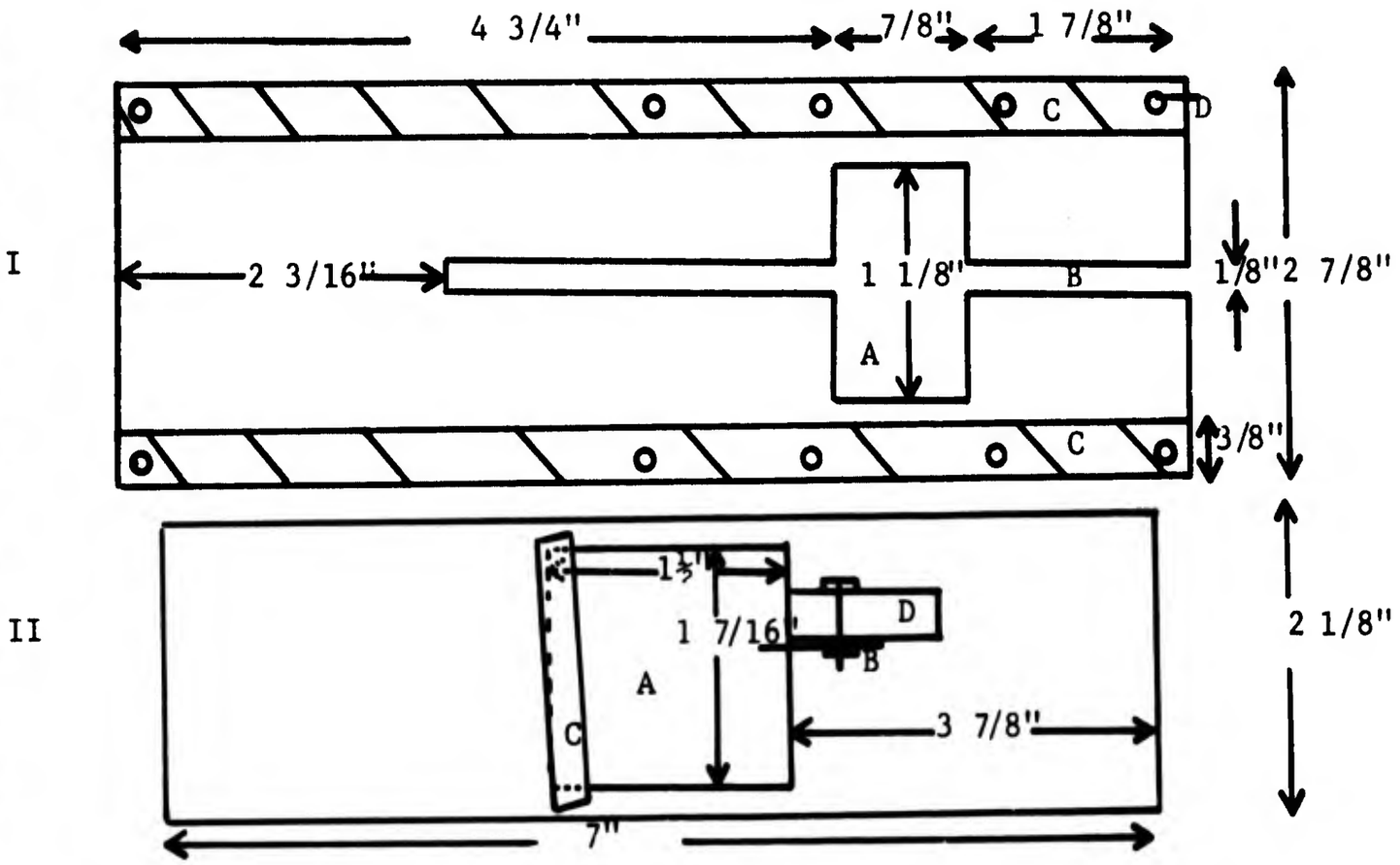
DRAWING OF COMPLETE BIOPSY APPARATUS AND ACCESSORIES
IN PRESSURE CHAMBER SIDE VIEW



A. Upper Plate
B. Inner Slide Plate
C. Bottom Plate
D. Biopsy Apparatus Support
E. Chamber Wall
F. Vertical Incising Blade
G. Clamping Bolt (Post)
H. Upper Plate Window
I. Styrofoam Cryogenic Container
J. ¼" Copper Tubing
K. Reel for Pulling Wire
L. Reel Turning Shaft
M. Outer Shaft Housing Assembly With Teflon Pressure Seal
N. Chamber Flange
O. Flange Cover with ¼" Swagelock Connectors
P. Liquid Nitrogen Source

Figure 2

DRAWING OF BIOPSY APPARATUS COMPONENTS
SURFACE VIEW



I. UPPER PLATE:

A. Window Opening
B. Channel for Incising Blade
C. Track for Inner Slide Plate
D. Opening for Clamping Bolt (Post)

C. Bottom Plate (not shown)

II. INNER SLIDE PLATE

A. Window Opening
B. Vertical Incising Blade
C. Follower Blade
D. Vertical Blade Support

The ATP concentrations obtained compared favorably and are normal for rat liver tissue. Thus the surgical biopsy device provides a rapid remote means of obtaining rat liver tissue from small animals confined in pressure chambers.

IV. DISCUSSION

Effects of breathing oxygen atmospheres in which nitrogen has been substituted with helium or other inert gases was reviewed by Roth (ref. 4). The effects were investigated in early studies by Leon and Cook (ref. 5), and subsequently by Schreiner and coworkers (ref. 6,7). Helium substitution in gas mixtures at room temperature results in increased oxygen and food consumption and increased carbon dioxide production. This increased metabolic rate is thought to be the consequence of the greater thermal conductivity of helium at room temperature. The rate of oxygen consumption of experimental animals maintained in helium-oxygen mixtures at 4 and 37°C is not significantly different from that of animals kept in ambient air. Schreiner exposed rabbits and rats at 25-26°C to atmospheres consisting of various inert gases and oxygen at pO_2 of 180 mm Hg. He found that the rate of oxygen consumption correlated well with the relative rate of convective heat transfer from the animals into the various gaseous environments.

Thompson, Nielsen, and Akers (ref. 8) have recently reported that nitrogen and helium potentiate oxygen toxicity at relatively high oxygen pressures. Nitrogen at 13 ata with 2 ata oxygen and helium at 13 ata with 5 ata oxygen significantly decreased the survival times of rats. It should be pointed out that the CNS effects of oxygen toxicity that are predominant at these high partial pressures of oxygen probably differ from lung and other tissue effects that occur at threshold levels of oxygen.

A thermal mechanism for helium effects cannot be readily invoked for tissue slices, cell homogenates, and cell suspensions. Effects that have been described for these biological systems range from depression to stimulation of oxygen consumption. Maio and Neville (ref. 9) reported depression of oxygen consumption in liver slices of rats and postulated that the inert gas decreased the membrane transport of oxygen. South and Cook (ref. 10), however, reported significant increase in oxygen consumption by helium in liver slices and homogenates of mice and ascribed the effect to stimulation of glycolysis. Young and Lundgren (ref. 11) recently reported stimulation by helium of oxygen consumption in kidney and brain slices of rats. Oxygen diffusion through membranes was eliminated as the rate-limiting factor by studies at lower temperature; hence

the effect was ascribed to stimulation of tissue oxidation. It is apparent that the in vitro studies produced variable results and their validity in ascertaining effects of helium in mammals must be questioned. Nevertheless, studies reported by Weiss et al (ref. 12, 13) indicated a possible effect of helium on the whole animal embryo. Chick embryo growth and hatchability was retarded when helium was substituted for nitrogen in the incubation atmosphere.

Helium appears to have the least narcotic potency for man among the helium-group gases and nitrogen. At partial pressures as high as 20 atm performance decrement could not be reliably determined in men exposed to helium for 48 hr (ref. 14, 15). A relative depressive potency at high pressures of helium to nitrogen of 20% has been calculated.

Hamilton et al (ref. 16) conducted a six-month study in which mice from an original and two successive generations were acclimatized to and maintained in either a 80% helium-20% oxygen atmosphere at sea level pressure or in a similar atmosphere in which nitrogen replaced helium. A duplicate chamber provided controlled environmental conditions for mice exposed to helium and nitrogen. The mice adapted to helium showed greater increase in oxygen consumption when subsequently placed in helium-oxygen than did mice raised in air. The mice kept in helium atmosphere consumed more food and water, although growth rates were identical.

No significant differences were seen between the two groups in several biochemical indices, including liver, skeletal, and cardiac muscle LDH, MDH, and G6PDH.

Liver adenine and pyridine nucleotides analyzed at IITRI in the Hamilton experiment reportedly failed to show a significant difference (ref. 16,17). The nucleotides were tablulated as actual levels. It is suggested that the same data, when considered as pertinent ratios may show significance. In the helium environment, ATP increased while ADP and AMP decreased, thus a ratio ATP/(ADP + AMP) should be considered. Similarly, decreased NAD was reflected by an increase in NADH; hence the ratio NAD NADH may show sufficient sensitivity. Since these related nucleotides are interconverted, the respective ratios have a biochemical validity. Any reduced phosphorylation index accompanied by increased reduction of NAD would suggest decreased forward or increased reverse-electron transport flux.

In conclusion, it would appear that breathing of helium atmospheres produces no demonstrable biochemical effects in animals a· pressures simulating the Sealab operations. However, we heed the admonition of Lambertsen (ref. 18) that the multiplicity of atmospheric parameters including inert gas, high pressure, and possible oxygen toxicity, complicate this problem. Oxygen per se is known to have varied effects at many tissue sites. The role of pressure and inert gases upon such oxygen effects in animals remains unknown.

V. SUMMARY AND RECOMMENDATIONS

A. Glycogen Utilization

The data suggest that effects of fasting and confinement in the chamber must be considered in suitable control experiments to properly determine the effects of hyperbaric helium-oxygen atmospheres on glycogen utilization. Due to the high mortality of rats exposed to 300 psi at 31°C the temperature of 28°C was used in limited experiments. However, additional studies must be performed at 28° for all control and experimental groups to properly evaluate this parameter. It is suggested that a comparison be made also of animals exposed to temperatures below 22°C in ambient air to determine if the effect of helium is analogous to that of hypothermia.

B. Biopsy Probe

The Biopsy Probe was inefficient with small laboratory animals such as rats, but should prove to be effective for biopsy in larger animals such as dogs or monkeys which have a larger and firmer tissue mass. The boring device should be manufactured from a thermally conductive material to provide a maximal freezing rate. Freezing would be best accomplished by direct spraying of liquid nitrogen on the biopsy tip. This would require a pressurized delivery system for the liquid nitrogen.

C. Surgical Biopsy Device

The Surgical Biopsy Device appears most suitable in application to small laboratory animals. By modification of the design, other organs could be biopsied and quickly frozen. Freezing could be accomplished by either spraying a cryogenic coolant over the isolated organ or immersion in the coolant. For immersion the coolant should be isolated from the experimental animal and the exposure chamber. A suitable air lock chamber located below the region of biopsy would provide the most effective means of carrying out immersion freezing. Prior to the biopsy the cryogen could be cooled to freezing temperature (-140°C) by means of a liquid nitrogen cooling coil. At the time of biopsy the air lock door could be opened and the biopsy sample recovered.

REFERENCES

1. Burch, H. B., Lowry, O. H., and Dippe, van P., J. Biol. Chem. 238, 2838, 1963.

2. Chance, B., Eisenhardt, R. H., Gibson, Q. H., and Lonberg-Holm, K. K., ed., "Rapid Mixing and Sampling Techniques in Biochemistry," VII. Tissue Freeze Quenching," pp. 229, 255; Academic Press, New York, 1964.

3. Lowry, O. H., Passonneau, J. K., Hasselberger, F. X., and Schulz, D. W., J. Biol. Chem. 239, 18, 1964.

4. Roth, E. M., "Space Cabin Atmospheres. III. Physiological Factors of Inert Gases," Report NASr-115, Washington, D.C., National Aeronautics and Space Administration, 1965.

5. Leon, H. A. and Cook, S. F., Am. J. Physiol. 199, 243, 1960.

6. Schreiner, H. R., "The Physiological Effects of Argon, Helium, and the Rare Gases," Office of Naval Research Technical Report No. NR:102-597, prepared by Union Carbide Corp., Linde Div., Tonawanda Research Laboratory, Tonawanda, N. Y., July 31, 1966.

7. Schreiner, H. R., Fed. Proc. 27, 872, 1968.

8. Thompson, R. E., Nielsen, T. W., and Akers, T. K., Aerospace Medical Association Annual Scientific Meeting, April 27-30, 1970, Preprints p. 102.

9. Maio, D. A. and Neville, J. R., Aerospace Med. 38, 1049, 1967.

10. South, F. E. and Cook, S. F., J. Gen. Physiol. 37, 335, 1954.

11. Young, H. L. and Lundgren, P. R., Aerospace Medical Association Annual Scientific Meeting, April 27-30, 1970, Preprints, p. 190.

12. Weiss, H. S. and Wright, R. A., J. Appl. Physiol. 24, 330, 1968.

13. Grimard, M. and Weiss, H. S., Aerospace Medical Association Annual Scientific Meeting, April 27-30, 1970, Preprints, p. 188.

14. Hamilton, R. W., Jr., MacInnis, J. B., Trovato, L. A., and Schreiner, H. R., Aerospace Med. 37, 281, 1966.

15. MacInnis, J. B., Trovato, L. A., Hamilton, R. W., Jr., and Schreiner, H. R., Aerospace Med. 37, 289, 1966.

16. Hamilton, R. W., Jr., Cohen, J. D., Doebbler, G. F., Exposito, L. F., King, J. M., Smith, K. H., and Schreiner, H. R., "Biochemical and Metabolic Effects of a Six-Month Exposure of Small Animals to a Helium-Oxygen Atmosphere," National Aeronautics and Space Administration, Contract NAS 2-3900, Final Report, prepared by Union Carbide Corp., Linde Div., Tonawanda Research Laboratory, Tonawanda, N. Y., October 30, 1967.

17. Kissane, J. Q. and Hawrylewicz, E. J., Final Report No. IITRI-L6045-1 prepared by IIT Research Institute, Chicago, Ill. for National Aeronautics and Space Administration, Ames Research Center, August 1967.

18. Lambertsen, C. J., ed., "Underwater Physiology," Proceedings of the Third Symposium on Underwater Physiology, Williams and Wilkins Company, Baltimore, 1967, p. 474.

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13. ABSTRACT

Studies were conducted to determine the effects of a simulated Sealab environment on selected metabolic systems in rats. The simulated Sealab environment consisted of 98.2% helium and 1.8% oxygen atmosphere maintained at 300 psi at 22°C. After 24 hr exposure and rapid decompression the following observations were made. Liver and muscle mitochondrial P:O and QO_2 were normally functional. Liver LDH activity was slightly decreased. Muscle and liver glycolysis rates were unaffected. Protein synthesis in liver subcellular components measured by ^{14}C-lysine uptake was unaffected while it decreased in muscle. Liver glycogen reserves decreased 8 to 10 fold, while muscle glycogen decreased approximately 50%. Control experiments in helium atmosphere at ambient pressure and those conducted at submergence pressure at elevated temperature suggested that thermal effects of helium resulted in increased glycogen utilization. To enable rapid sampling and freezing of tissue at pressure for nucleotide analyses, two biopsy devices were developed and evaluated. The Biopsy Probe, a boring device, was found not to be satisfactory when its performance was measured in terms of liver tissue ATP values. Using the same parameter the Surgical Biopsy Device provided rapid tissue excision and freezing by cryogenic immersion.

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