# METABOLIC AND HEMATOLOGIC FACTORS IN CHRONIC AIR SATURATION AT 2.5 ATA

by

Michael J. Jacey
and
Donald V. Tappan

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Transmitted by:

Donald V. Tappan

Donald V. Tappan, Ph. D.
Head, Biochemistry Branch

Reviewed and Approved by:

Charles F. Gell

Charles F. Gell, M.D., D.Sc. (Med)
SCIENTIFIC DIRECTOR
NavSubMedRschLab

Approved and Released by:

R. L. Sphar

R. L. Sphar, CDR MC USN
OFFICER IN CHARGE
NavSubMedRschLab

## SUMMARY PAGE

### THE PROBLEM

To evaluate potential metabolic and hematologic responses of animals subjected to chronic air saturation in air at a simulated pressure equivalent to 50 feet of sea water (FSW), 2.5 atmospheres absolute (ATA).

### FINDINGS

Chronic exposure to this compressed air environment produced no alterations in selected metabolic parameters during the two month saturation. However, a steady decline in red cell mass was noted during the first 4-6 weeks of the experiment culminating in a new hematologic steady-state during the rest of the observation period.

### APPLICATION

These findings demonstrate the feasibility of the concept of long-term saturation utilizing compressed air as a breathing mixture. They also serve as a practical model for human saturation diving protocols.

### ADMINISTRATIVE INFORMATION

This investigation was conducted as part of Bureau of Medicine and Surgery Work Unit M4306.02-5003BA9K. The present report is number 4 on this work unit. It was submitted for review on 25 February 1974, approved for publication on 20 March 1974 and designated as Naval Submarine Medical Research Laboratory Report No. 781.

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## ABSTRACT

Mature male rats of the Sprague-Dawley strain were pressurized in air at a simulated pressure equivalent to 50 feet of sea water, 2.5 ATA, for varying times up to 60 days. The internal environment was maintained at: $O_2 = 51\%$, $CO_2 = .42\%$ (sea level equivalent), balance $N_2$. No consistent alterations in lactic and pyruvic acid or adenosine triphosphate (ATP) levels were noted at any time during the two-month experiment. Platelet and platelet aggregate counts were also unaltered. Red cell mass exhibited a steady decline for the first 4-6 weeks attaining and maintaining a new hematologic steady-state for the remainder of the saturation period. Dimunition of red cell mass is consistent with chronic exposure to hyperoxia. Several hyperfibrinogenemic episodes provided evidence of repeated stress.

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## INTRODUCTION

The pioneering experiments of Chouteau and Cousteau[5] demonstrated the feasibility of using compressed air as a breathing medium for shallow saturation dives of one to two weeks' duration. However, the breathing of compressed air at depths is not without its disadvantages. The risks of nitrogen narcosis and oxygen toxicity are ever present. There exists the possibility that adaptation to the narcotic effects of nitrogen may occur during prolonged pressurization[7]. Evidence has been obtained that laboratory animals may tolerate exposures to 60% $O_2$ for long periods of time without apparent deleterious effects[24].

An operational need to define time-depth limits of saturation utilizing pressurized air provided the stimulus for the present work. Since little is known concerning the effects of a prolonged sojourn in compressed air, a study was undertaken to investigate some biochemical and hematological factors in rats exposed to an environment containing air at a pressure equivalent to 50 feet of sea water (FSW), 2.5 atmospheres absolute (ATA).

## METHODS AND MATERIALS

It should be pointed out that the present study was part of a larger multidisciplinary pressure investigation with experimental animals of several investigators being pressurized simultaneously in the same chamber. A comprehensive report documenting logistical, environmental and maintenance aspects has been published[16].

A standard U. S. Navy double-lock pressure chamber, total volume 500 cu ft, was utilized as the saturation vehicle. Mature male rats of the Sprague-Dawley strain averaging 498 $\pm$9 (S.E.M.) g were saturated with compressed air at a pressure corresponding to 50 FSW for varying periods up to 60 days. The average internal atmosphere was maintained at .42% $CO_2$ and 51% $O_2$, sea level equivalent[16].

At the planned time, the animals were anesthetized and sacrificed at the 50 ft. depth. The only exception to this protocol involved those animals exposed for 60 days and decompressed[16]; this group was sacrificed shortly after reaching the surface. Following an intraperitoneal injection of sodium pentobarbital, 40 mg/kg body weight, the abdominal cavity was opened and samples of arterial blood were obtained from the abdominal aortae anaerobically in heparinized glass syringes. An aliquot of blood was immediately precipitated with an equal volume of .6M ice-cold perchloric acid. All blood samples were then brought to the surface at a rate of 8 ft/min. Surface control animals were sacrificed in the same manner at each time interval.

Adenosine triphosphate (ATP), lactic acid, and pyruvic acid analyses were performed on the perchloric acid extracts utilizing the appropriate

Calbiochem Statpacks. Plasma fibrinogen estimations were made according to a modification of the procedure of Rice and Meusse[20]. Hemoglobin concentrations were measured with the Diagnotest* hemoglobin reagent kit while hematocrit was determined by using a micromethod[18]. Platelets[18] and platelat aggregates[23] were counted according to standard hematological methods.

RESULTS

The responses of lactate, pyruvate, lactate/pyruvate ratio, and ATP in the blood of rats subjected to chronic air saturation at 50 FSW are summarized in Table 1. The data indicate that prolonged exposure to pressurized air had no consistent effects on any of the metabolic indices measured.

Figure 1 portrays graphically the effects of chronic hyperbaric saturation on hematological parameters. Hematocrit values started to decline after one week of pressurization, reached statistical significance and an ultimate low level by the fourth week and maintained this level for the duration of the experiment. Hemoglobin concentration essentially followed the same pattern as that of the hematocrit except that statistical significance occurred by the second week.

Prolonged saturation with compressed air also elicited a trimodal response in plasma fibrinogen levels. Significant increases were observed by the end of the first week, again on the third week and finally near the end of the experiment. Circulating platelets as well as the number of platelet aggregates were unaffected by chronic air saturation at 2.5 atmospheres absolute (ATA). These results are shown in Table 2.

*Dow Chemical Co.

DISCUSSION

Metabolic Factors

Lactic acid is not a true intermediate product, but rather represents a temporary end product of metabolism. The arterial concentration of lactate is influenced by its rate of influx and removal as well as by the significant glycolysis of the erythrocytes. In contrast, pyruvic acid enters into many biochemical pathways in addition to the production of lactic acid. Despite such complicating factors, the lactate-pyruvate ratio has been shown to be a sensitive indicator of the functional state of cellular metabolism through its relationship to the redox state of the free glycolytic $NAD^+$ - NADH couplet[10,22].

Higher than normal concentrations of blood lactate in dogs following breathing of hyperbaric oxygen were reported as early as 1932 by Bean and Haldi[2]. In our experiments, however, lactate and pyruvate levels as well as the lactate-pyruvate ratio were unaffected by chronic exposure to the compressed air environment of the present study. The results from these two series of experiments apparently differ because the threshold level of $pO_2$ for demonstrable effects on glycolysis was not reached in our study.

Timms and Mengel[21] reported increases in the level of the high-energy phosphate, ATP, in the blood of mice acutely exposed to 60 psia of oxygen. Substitution of room air with the same

Table 1. Blood Metabolic Responses to Chronic Air Saturation at a Pressure Equivalent to 50 FSW

| | | DAYS OF SATURATION+ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CONTROL | 1 DAY | 1 WEEK | 2 WEEKS | 3 WEEKS | 4 WEEKS | 6 WEEKS | 60 DAYS (with Decompression) |
| Lactic Acid mg% | $\bar{X}$ | 20.3 | 21.1 | 19.7 | 22.1 | 23.1 | 26.5 | 24.7 | 21.5 |
| | S.E.M | 1.1 | 3.8 | 2.6 | 2.3 | 4.9 | 5.4 | 5.9 | 1.9 |
| | n | 8 | 8 | 8 | 6 | 6 | 6 | 6 | 3 |
| Pyruvic Acid mg% | $\bar{X}$ | .48 | .67 | .44 | .52 | .72 | .71 | .49 | .74 |
| | S.E.M. | .06 | .10 | .03 | .06 | .10 | .17 | .05 | .08 |
| | n | 8 | 8 | 8 | 6 | 6 | 6 | 6 | 3 |
| Lactate/ Pyruvate Ratio | $\bar{X}$ | 45.4 | 30.9* | 32.1 | 44.7 | 44.3 | 31.7 | 49.6 | 29.2 |
| | S.E.M | 4.7 | 2.3 | 3.7 | 3.8 | 4.6 | 2.7 | 8.1 | 1.7 |
| | n | 8 | 8 | 8 | 6 | 6 | 6 | 6 | 3 |
| ATP mg% | $\bar{X}$ | 14.9 | 14.6 | 14.6 | 10.6* | 13.1 | 15.9 | 14.1 | 17.4 |
| | S.E.M. | .8 | 1.6 | 1.2 | 1.7 | 1.5 | 1.6 | .6 | 2.0 |
| | n | 8 | 8 | 8 | 6 | 6 | 6 | 6 | 3 |

+ Denotes time animals spent at 2.5 ATA with the exception of the last group which were exposed for 60 days and then decompressed.[16] (See text).
* Statistically significant

Table 2. Hemostatic Factors in Chronic Air Saturation at 2.5 ATA

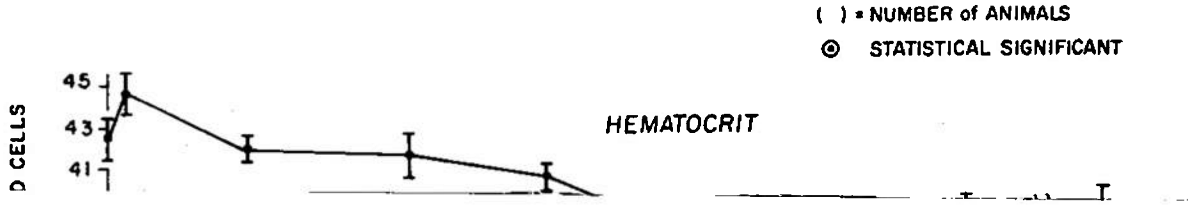
| | | DAYS OF SATURATION* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CONTROL | 1 DAY | 1 WEEK | 2 WEEKS | 3 WEEKS | 4 WEEKS | 6 WEEKS | 60 DAYS (with Decompression) |
| Platelet Count $\frac{\text{cells} \times 10^3}{\text{mm}^3}$ | $\bar{X}$ | 810 | 872 | 869 | 794 | 868 | 881 | 951 | 763 |
| | S.E.M. | .123 | .34 | .33 | 1.06 | .37 | 1.03 | 1.03 | 2.11 |
| | n | 8 | 8 | 8 | 6 | 6 | 6 | 6 | 4 |
| Platelet Clumping Count Clumps per 1/5000 $mm^3$ | $\bar{X}$ | 5.5 | 6.0 | 8.3 | 4.2 | 4.0 | 8.3 | 7.2 | 4.8 |
| | S.E.M. | 1.0 | 1.3 | 2.8 | .5 | 1.1 | 2.8 | 1.6 | 1.3 |
| | n | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |

* Denotes time animals spent at 50 FSW with the exception of the last group which was exposed for 60 days and then decompressed.[16] (See text).



hematocrit values were seen during experiment. However, preliminary ob-

also been shown that adrenalin and stress, as well as non-specific tissue




plasma fibrinogen levels[6]. The fluctuations in fibrinogen levels noted during the 2-month saturation in these studies may very well represent responses to stress factors.

Thrombocytopenic episodes following various compression-decompression regimens are well documented and have recently been reviewed[9]. Associated platelet clumping usually accompanies the decline in circulating platelet population[9]. Moreover Jastrzebski and associates,[13] showed that increased partial pressures of oxygen result in a decreased tendency for platelet adhesiveness. This diminished adhesiveness may be demonstrated after 5-10 minutes of normobaric hyperoxia. It is generally agreed that, in flowing blood, thrombosis is largely dependent on platelet aggregation, and platelet adhesion is the first directly observable step in the reaction sequence leading to thrombus formation[19]. The lack of change in both circulating platelet population and platelet clumping in our experiment would indicate that hemostasis is little affected by chronic air saturation at this depth.

These findings demonstrate the practicality of utilizing compressed air at 2.5 ATA for long-term saturation ventures. It may be postulated that human exposure to this atmosphere would result in little or no change in glycolytic or energy-producing metabolism or hemostasis, but presumably it would profoundly affect the erythropoietic system, through its response to increased partial pressures of oxygen. Very recently, a chronic saturation dive involving two subjects was completed by NavSubMedRschLab. After 2.5 ATA exposure in compressed air for 30 days, the divers were still in a healthy state[1].

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13. ABSTRACT

Mature male rats of the Sprague-Dawley strain were pressurized in air at a pressure equivalent to 50 feet of sea water, 2.5 ATA, for varying times up to 60 days. The internal environment was maintained at: $O_2$ = 51%, $CO_2$ = 42% (sea level equivalent), balance $N_2$. No consistent alterations in lactic and pyruvic acid or ATP levels as well as lactate-pyruvate ratio were noted at any time during the two-month experiment. Platelet and platelet aggregate counts were also unaltered. Red cell mass exhibited a steady decline for the first 4-6 weeks attaining and maintaining a new hematological steady-state for the remainder of the saturation period. Diminution of red cell mass is consistent with chronic exposure to hyperoxia. Several hyperfibrinogenemic episodes provided evidence for repeated stress.

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| Glycolysis | | | | | | |
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