# BIOCHEMISTRY OF SUBMARINE AND DIVING STRESS: II

## The Effect of Chronic Hypercapnia on Blood Phosphofructokinase Activity and the Adenine Nucleotide System

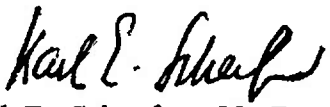
by

Michael J. Jacey
and
Karl E. Schaefer

NAVAL SUBMARINE MEDICAL RESEARCH LABORATORY
NAVAL SUBMARINE MEDICAL CENTER REPORT NO. 659

Bureau of Medicine and Surgery, Navy Department
Research Work Unit MF12.524.006-9028BA9K

Transmitted by:

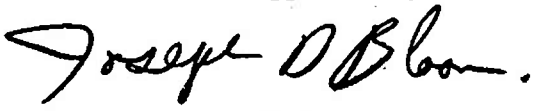


Karl E. Schaefer, M. D.
Chief, Biomedical Sciences Division

Reviewed and Approved by:

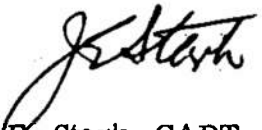
Charles F. Gell, M.D., D.Sc.(Med)
Scientific Director
NavSubMedRschLab

Reviewed and Approved by:



J. D. Bloom, CDR, MC, USN
Officer-in-Charge
NavSubMedRschLab

Approved and Released by:



J. E. Stark, CAPT, MC, USN
COMMANDING OFFICER
Naval Submarine Medical Center

## SUMMARY PAGE

### THE PROBLEM

To investigate and define the role of the rate-limiting enzyme, phosphofructokinase, and the high-energy phosphates in the metabolic adaptation to chronic exposure to increased levels of carbon dioxide as may be potentially found in Navy submarine and diving facilities.

### FINDINGS

Blood phosphofructokinase activity declined during the acute phase of hypercapnia and increased during the chronic phase but never reached control values. These changes paralleled the biphasic response of blood pH. The high-energy phosphates were not affected.

### APPLICATIONS

These findings are of interest to cognizant personnel concerned with toxicological aspects of increased carbon dioxide exposure as found in Navy submarine and diving environments as well as other hazards which might induce an acidosis such as hyperbaric hyperoxia.

## ADMINISTRATIVE INFORMATION

This investigation was conducted as a part of Bureau of Medicine and Surgery Research Work Unit MF12.524.006-9028BA9K - Time-Concentration Exposure Limits of $CO_2$ in Submarine and Navy Diving Facilities. The present report is No. 8 on this Work Unit. It was approved for publication on 9 March 1971 and designated as SubMedRschLab Report No. 659.

PUBLISHED BY THE NAVAL SUBMARINE MEDICAL CENTER

## ABSTRACT

Phosphofructokinase activity and adenine nucleotide levels were determined in blood of guinea pigs exposed to 15% $CO_2$ in 21% $O_2$, balance $N_2$, for varying periods of time up to one week. Acute exposure produced a decrease in enzyme activity while the chronic phase increased activity without attainment of control values. These alterations in blood phosphofructokinase activity strikingly paralleled the biphasic changes in blood pH during acute and chronic hypercapnia. These findings demonstrate that blood phosphofructokinase activity is capable of responding to "in vivo" fluctuations in blood pH. ATP, ADP, AMP, and the energy charge were virtually unchanged during both phases indicating that hypercapnia is characterized by conservation of high-energy phosphates.

# BIOCHEMISTRY OF SUBMARINE AND DIVING STRESS:

## II The Effects of Chronic Hypercapnia on Blood Phosphofructokinase Activity and the Adenine Nucleotide System

The role of phosphofructokinase (ATP:D-fructose-6-phosphate I-phosphotransferase, EC 2.7.I.II) in the regulation of glycolytic metabolism has been the subject of intensive investigation. Many studies have indicated that the enzyme reaction primarily responsible for the Pasteur effect is phosphofructokinase (7, 11, 12). Mansour (8) and Ui (17) has shown that phosphofructokinase activity is pH-dependent, while Murphy (10) has suggested that the inhibition of glycolysis seen "in vitro" by decreasing erythrocyte pH from 7.5 to 7.1 is mediated through the enzyme phosphofructokinase. Furthermore, Lardy and Parks (6) reported that an excess of ATP was inhibitory to the enzyme while Mansour (8) and Passonneau and Lowry (12) demonstrated an activation by ADP and AMP.

In a study of the effects of acute and chronic respiratory acidosis induced by exposure to 15% $CO_2$, Jacey and Schaefer (5) found an inhibition of blood glycolysis during the acute phase. In view of this, the present investigation was undertaken to identify and define the effects of acute and chronic hypercapnia on phosphofructokinase activity and the adenine nucleotide system in blood.

### METHODS

Male guinea pigs of the Hartley strain weighing 350-450 grams were exposed to 15% $CO_2$ in 21% $O_2$, balance $N_2$ for varying periods of time up to a week. Criteria for selection and details of exposure have been previously described by Schaefer et al (14). After the appropriate exposure period, all experimental animals were anesthetized in the $CO_2$ atmosphere by an intraperitoneal injection of 40 mg sodium pentobarbital/kg body wt. Blood was collected from the abdominal aorta in heparinized syringes while the animals continued to breathe the 15% $CO_2$ mixture through a fitted mask. Immediately thereafter, an aliquot of blood was precipitated with ice cold .6M perchloric acid. The extract was then analyzed for ATP, ADP, and AMP content utilizing the respective Boehringer* test kits. Blood phosphofructokinase activity was estimated according to the method outlined by Boehringer (2) while blood pH was determined with an Instrumentation Laboratory pH and blood gas analyzer.

The energy charge was calculated according to Atkinson and Walton (1) who defined it as (ATP+1/2ADP)/(AMP+ADP+ATP).

### RESULTS

The effects of acute and chronic hypercapnia induced by exposure to 15% $CO_2$ on blood phosphofructokinase ac-

*Boehringer Mannhein Corporation.

tivity and arterial pH are summarized in Table 1.

Acute hypercapnia produced a significant decrease in enzyme activity during the acute phase followed by a rise in activity which never reached control values during the chronic phase. The changes in phosphofructokinase activity mirror those of arterial pH. The correlation coefficient for these two parameters was calculated and found to be .92. This relationship is depicted in Figure 1.

The responses of the blood adenine nucleotide system to acute and chronic hypercapnia are shown in Table 2. A Also depicted are the calculated energy charges for the respective time periods. Acute hypercapnia had no influence upon the ATP, ADP, and AMP levels and therefore no alterations in the energy charge took place. However, during the middle portion of the chronic phase (day 3), a significant but transient increase in ADP and AMP concentration was seen. This occured without change in ATP content but produced a reduction in the energy charge. Values for all adenylate parameters were at control levels by the end of the experiment.

## DISCUSSION

It is a well-known fact that the rate-limiting steps of glycolysis are those catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase. Normally the glycolytic rate is mainly determined by the rates of hexokinase and phosphofructokinase while pyruvate kinase keeps step. Since phosphofructokinase activity has been shown to be pH-dependent, its activity should be affected by hypercapnia with its pro-

TABLE 1. Effects of Prolonged Exposure to 15% $CO_2$ in 21% $O_2$ Balance $N_2$ on Blood Phosphofructokinase Activity and pH

| | Control | 1 Hour | 6 Hours | 1 Day | 3 Days | 7 Days |
|---|---|---|---|---|---|---|
| Blood Phosphofructokinase Activity U/ml | 5.57 ±.31 (8) | 2.47* ±.31 (10) | 2.84* ±.34 (9) | 3.61* ±.26 (12) | 3.56* ±.33 (13) | 3.74* ±.31 (12) |
| Arterial pH | 7.387 ±.022 (8) | 7.039* ±.021 (10) | 7.059* ±.012 (9) | 7.131* ±.017 (12) | 7.190* ±.012 (13) | 7.292* ±.010 (12) |

Values are means ± SE. Number in parentheses equals number of animals.

*Statistically significant at the 5% level or better.

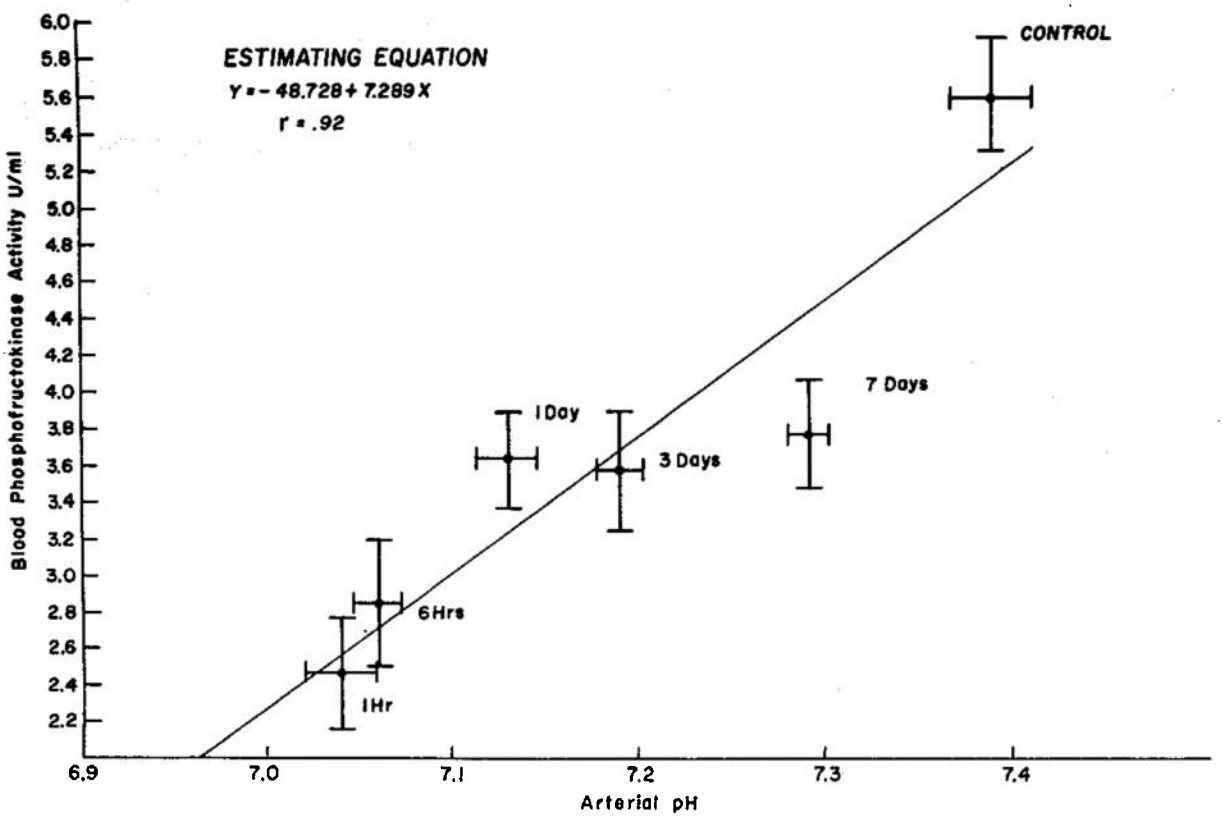



*Fig. 1. Relationship between arterial pH and blood phosphofructokinase activity in acute and chronic hypercapnia, (values are means ± SE).*

found acidosis and accompanying inhibition of glycolysis (5). On the other hand, however, it should be mentioned that according to Hinterberger et al (4) the initial phosphorylation of glucose could be the reaction inhibited by acid pH because glycolysis and hexokinase activity seem to have the same pH-dependence. This possibility, however, is unlikely when the experiments of Minakami and Yoshikawa (9) are considered. When the incubation medium of human erythrocytes was acidified from pH 7.4 to 7.1, these investigators found the glycolytic intermediates before phosphofructokinase increased while those after this step all decreased with a concomitant reduction in lactate production.

The striking correlation between arterial pH and blood phosphofructokinase activity points out the role of the degree of acidosis in regulating this vital enzyme reaction. During the acute phase of hypercapnia when pH change is maximal, blood phosphofructokinase activity is inhibited by 55%. With a trend toward compensation of the respiratory acidosis during the chronic phase,

TABLE 2. Responses of Blood Adenine Nucleotides and Energy Charge to Prolonged Exposure to 15% $CO_2$ in 21% $O_2$, Balance $N_2$

| Condition | | ATP mg% | ADP mg% | AMP mg% | Energy Charge |
|---|---|---|---|---|---|
| Control | Mean | 10.64 | 2.26 | .80 | .85 |
| | SE | 1.16 | .19 | .07 | .01 |
| | N | (18) | (18) | (18) | (18) |
| 1 Hour | Mean | 12.83 | 2.99 | .83 | .84 |
| | SE | 1.42 | .29 | .14 | .01 |
| | N | (7) | (7) | (7) | (7) |
| 6 Hours | Mean | 11.12 | 1.90 | .83 | .86 |
| | SE | 1.39 | .18 | .11 | .02 |
| | N | (8) | (8) | (8) | (8) |
| 1 Day | Mean | 13.73 | 2.67 | .87 | .86 |
| | SE | 1.91 | .35 | .18 | .02 |
| | N | (9) | (9) | (9) | (9) |
| 3 Days | Mean | 11.19 | 4.55* | 1.19* | .78* |
| | SE | 1.02 | .19 | .05 | .01 |
| | N | (9) | (9) | (9) | (9) |
| 7 Days | Mean | 14.58 | 2.90 | 1.00 | .85 |
| | SE | 2.04 | .27 | .03 | .01 |
| | N | (8) | (8) | (8) | (8) |

enzyme activity correspondingly increases, but neither pH nor phosphofructokinase activity reach control values. The biphasic changes in blood phosphofructokinase activity during hypercapnia parallel those seen in blood lactate content previously measured by us under the same conditions of $CO_2$ exposure (5). These results indicate that blood phosphofructokinase activity is capable of responding to "in vivo" fluctuations of blood pH and in turn of affecting the rate of glycolytic metabolism.

The various components of the adenylate system have been shown to influence the activity of phosphofructokinase and must also be considered. ATP is inhibitory to the enzyme (6) while ADP and AMP are activators (8, 12). An increase in erythrocyte ATP levels was

seen concomitant with a decrease in erythrocyte phosphofructokinase activity by Timms and Mengel (16) in rats exposed to hyperoxic conditions. Hypercapnia, acute or chronic, had no discernible effects upon blood levels of ATP, ADP, and AMP with the exception of a transient rise in ADP and AMP at the third day. Similar findings were reported by Granholm and Siesjö (3) in the brain of cats acutely exposed to various concentrations of $CO_2$ up to a level which produced arterial $pCO_2$ values of 100 mm Hg. They observed no changes in tissue ATP and ADP levels or in creatine phosphate content. Moreover, no changes in ATP and ADP concentrations were noted by Minakami and Yoshikawa (9) in erythrocytes in an acidified medium. In the hypercapnic situation inhibition of blood phosphofructokinase activity is apparently not mediated through an excess of ATP since the concentration of this cofactor is unaffected.

The increases in ADP and AMP at three days may be partially explained by the findings of Tappan (15) and Schaefer et al (13) that there are two populations of animals at this time point in chronic hypercapnia. They were able to distinguish between those that are compensating and those that are not. In the present study, no attempt was made to separate the two populations. It is apparent that further investigation is required to clarify this point. However, it must be pointed out that increases in these parameters are consistent with an activation of phosphofructokinase activity. No significant differences are seen when the activity of the enzyme after 3 days of exposure is compared to either the activity at 1 day or at 7 days. The ADP and AMP levels are essentially at control values at these two time points. It must be concluded that the adenylate system plays no major role in regulating phosphofructokinase activity in blood during acute and chronic hypercapnia.

The energy charge of the adenylate system has been proposed by Atkinson and Walton (1) as a fundamental control parameter of metabolism. This concept implies that conservation of ATP is a major feature of metabolic regulation. The constancy of the values for the energy charge during acute and chronic hypercapnia coupled with the observations of Tappan (15) that muscle creatine phosphate levels are virtually unaffected under the same conditions of 15% $CO_2$ exposure would indicate that hypercapnia is characterized by conservation of high-energy phosphates. The exact mechanism of this conservation is unknown but changes in the rates of synthesis as well as the rates of utilization are factors to be considered.

## REFERENCES


1. Atkinson, D. E., and G. M. Walton: Adenosine triphosphate conservation in metabolic regulation: Rat liver citrate cleavage enzyme. J. Biol. Chem. 242:3239-3241, 1967.

2. Boehringer, C. F., and Soehne GmbH. (Germany): Biochemica Catalogue Bochringer-Mannheim Corp., N.Y., 1968.

3. Granholm, L., and B. K. Siesjö: The effects of hypercapnia and

hypocapnia upon tissue levels of NADH, lactate, pyruvate, ATP, ADP, phosphocreatine concentrations of cat brain tissue. Acta physiol. scand. 75:257-266, 1969.

4. Hinterberger, U., E. Ockel, W. Gerishcher - Mothes, and S. Rapoport: Grösse und pH - Abhängigkeit det anaeroben Glykolyse und der hexokinaseaktivität von Erythozyten und Reticulozytem des Kaninchens. Acta Biol. Med. Ger. 7:50-56, 1961.

5. Jacey, M. J., and K. E. Schaefer: Biochemistry of Submarine and Diving Stress: I. Lactate-pyruvate and redox state responses of blood and tissue in chronic hypercapnia. SMRL Report No. 652, 1971.

6. Lardy, H. A., and R. E. Parks, Jr., in O. Garbler (Editor): Enzymes: units of biological structure and function. Academic Press, Inc., New York, 1956 - p. 584.

7. Lonberg - Holm, K. K.: A direct study of intracellular glycolysis in Ehrlich's ascites tumor. Biochem. Biophys. Acta, 35:464-472, 1959.

8. Mansour, T. E.: Studies on heart phosphofructokinase: Purification, inhibition, and activation. J. Biol. Chem., 238:2285-2292, 1963.

9. Minakami, S., and H. Yoshikawa: Studies on Erythrocyte Glycolysis III. The effects of active cation transport, pH and inorganic phosphate concentration on erythrocyte glycolysis. J. Biochem., 59:145-150, 1966.

10. Murphy, J. R.: Erythrocyte metabolism III. Glucose metabolism and pathways. J. Lab and Clin. Med., 55:286-302, 1960.

11. Newsholme, E. A., and Randle, P. J.: Regulation of glucose uptake by muscle 5. Effects of anoxia, insulin, adrenaline and prolonged starving on concentrations of hexose phosphates in isolated rat diaphragm and perfused isolated rat heart. Biochem. J., 80:655-662, 1961.

12. Passonneau, J. V., and O. H. Lowry: Phosphofructokinase and the Pasteur effect. Biochem., Biophys. Res. Comm., 7:10-15, 1962.

13. Schaefer, K. E., E. Heyder, and A. A. Messier: Species difference and tolerance to $CO_2$ in enzyme activity. SMRL Report No. 656, 1971.

14. Schaefer, K. E., N. McCabe, and J. Withers: Stress response in chronic hypercapnia. Am. J. Physiol., 214:543-548, 1968.

15. Tappan, D. V.: Biochemistry of Submarine and Diving Stress: III. Plasma creatine, creatine phosphate and creatine phosphokinase responses to carbon dioxide stress. SMRL Report No. 661, 1971.

16. Timms, R., and C. E. Mengel: Effects of in vivo hyperoxia on

erythrocytes: VII Inhibition of RBC phosphofructokinase. Aerospace Med., 39:71-73, 1968.

17. Ui, M.: A role of phosphofructokinase in pH-dependent regulation of glycolysis. Biochem., Biophys. Acta. 124:310-322, 1966.

UNCLASSIFIED
Security Classification

**DOCUMENT CONTROL DATA - R & D**

*(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)*

1. ORIGINATING ACTIVITY *(Corporate author)*
Naval Submarine Medical Research Laboratory
NAVAL SUBMARINE MEDICAL CENTER

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
THE EFFECT OF CHRONIC HYPERCAPNIA ON BLOOD PHOSPHOFRUCTOKINASE ACTIVITY AND THE ADENINE NUCLEOTIDE SYSTEM

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*
Interim Report

5. AUTHOR(S) *(First name, middle initial, last name)*
Michael J. JACEY and Karl E. SCHAEFER

| 6. REPORT DATE | 7a. TOTAL NO. OF PAGES | 7b. NO. OF REFS |
|---|---|---|
| 9 March 1971 | 7 | 17 |

| 8a. CONTRACT OR GRANT NO. | 9a. ORIGINATOR'S REPORT NUMBER(S) |
|---|---|
| b. PROJECT NO. MF12.524.006-9028BA9K.08 | NSMRL Report Number 659 |
| c. | 9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)* |
| d. | |

10. DISTRIBUTION STATEMENT
Approved for public release; distribution unlimited.

| 11. SUPPLEMENTARY NOTES | 12. SPONSORING MILITARY ACTIVITY |
|---|---|
| | Naval Submarine Medical Center<br>Box 600 Naval Submarine Base<br>Groton, Connecticut 06340 |

13. ABSTRACT

Phosphofructokinase activity and adenine nucleotide levels were determined in blood of guinea pigs exposed to 15% $CO_2$ in 21% $O_2$, balance $N_2$ for varying periods of time up to one week. Acute exposure produced a decrease in enzyme activity while the chronic phase increased activity without attainment of control values. These alterations in blood phosphofructokinase activity strikingly paralleled the biphasic changes in blood pH during acute and chronic hypercapnia. These findings demonstrate that blood phosphofructokinase activity is capable of responding to "in vivo" fluctuations in blood pH. ATP, ADP, AMP, and the energy charge were virtually unchanged during both phases indicating that hypercapnia is characterized by conservation of high-energy phosphates.

| 14. KEY WORDS | LINK A | | LINK B | | LINK C | |
|---|---|---|---|---|---|---|
| | ROLE | WT | ROLE | WT | ROLE | WT |
| Phosphofructokinase | | | | | | |
| Adenine nucleotides | | | | | | |
| Hypercapnia | | | | | | |