



**U. S. NAVAL SUBMARINE
MEDICAL CENTER**

Submarine Base, Groton, Conn.

REPORT NUMBER 652

BIOCHEMISTRY OF SUBMARINE AND DIVING STRESS

**I. Lactate-Pyruvate and Redox State Responses of
Blood and Tissue in Chronic Hypercapnia**

by

Michael J. Jacey
and
Karl E. Schaefer

Bureau of Medicine and Surgery, Navy Department
Research Work Unit MF12.524.006-9028BA9K.03

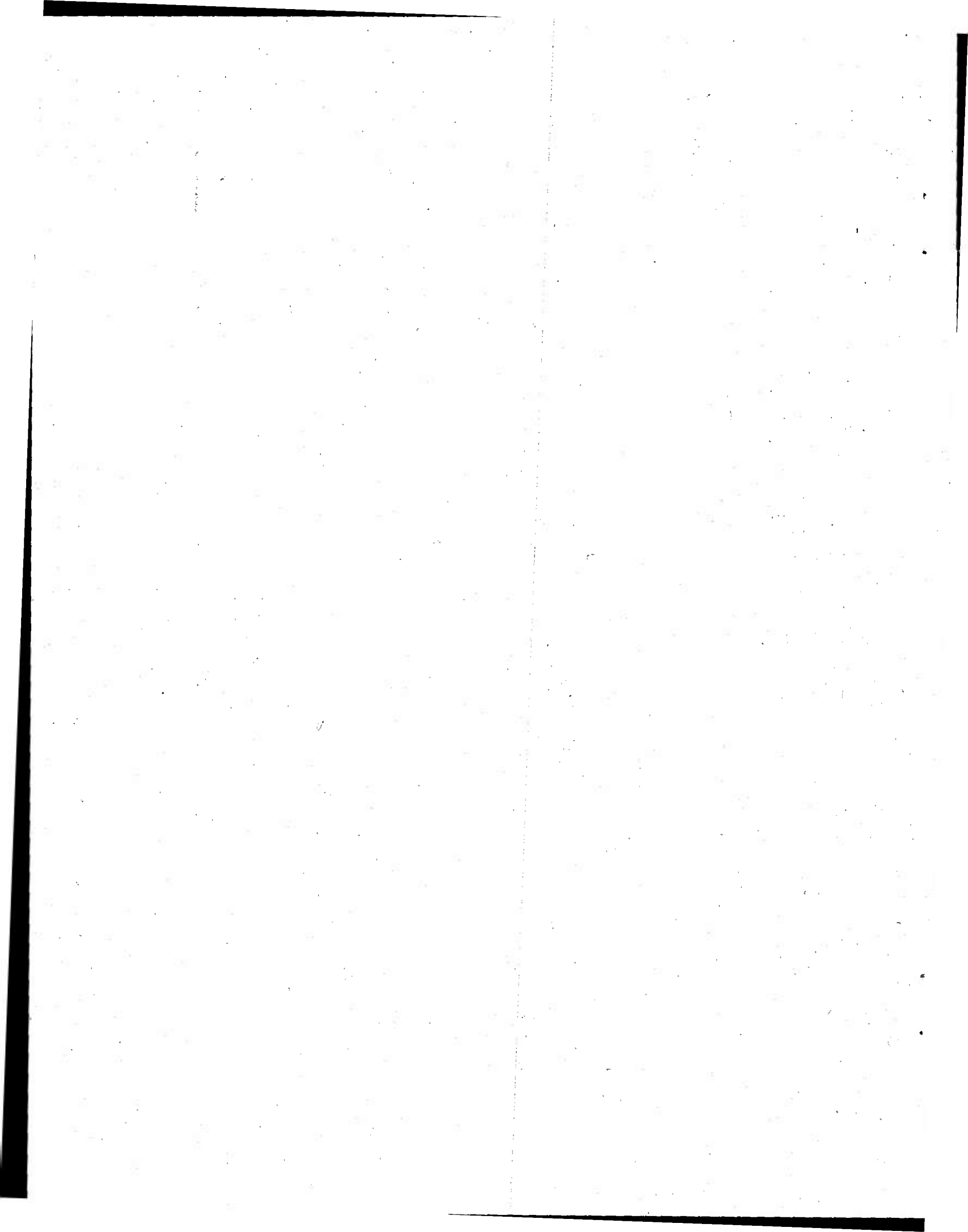
Approved and Released by:

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

22 February 1971



Approved for public release; distribution unlimited.



BIOCHEMISTRY OF SUBMARINE AND DIVING STRESS

I. Lactate-Pyruvate and Redox State Responses of
Blood and Tissue in Chronic Hypercapnia

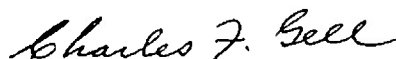
by

Michael J. Jacey
and
Karl E. Schaefer

NAVAL SUBMARINE MEDICAL RESEARCH LABORATORY
NAVAL SUBMARINE MEDICAL CENTER REPORT NO. 652

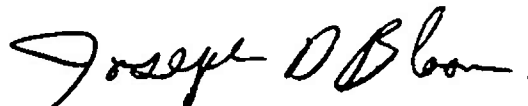
Bureau of Medicine and Surgery, Navy Department
Research Work Unit MF12.524.006-9028BA9K.03

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, Captain, MC, USN
COMMANDING OFFICER
Naval Submarine Medical Center

Approved for public release; distribution unlimited.

SUMMARY PAGE

THE PROBLEM

To clarify and define certain aspects of metabolic adaptation to chronic exposure to increased levels of carbon dioxide in blood and selected tissues. Changes in lactate/pyruvate ratio as well as redox state were used as indices of metabolic alterations.

FINDINGS

Lactate/pyruvate ratios decreased initially in blood, heart, and muscle. Blood and heart ratios normalized, with each following its own time course, while muscle ratio remained depressed for the duration of hypercapnia. Liver ratio was unaffected by carbon dioxide. Redox state increased during the acute phase and gradually declined during the chronic phase.

APPLICATIONS

These findings are of interest to Navy Medical Department personnel who are concerned with toxicological aspects of increased carbon dioxide exposure.

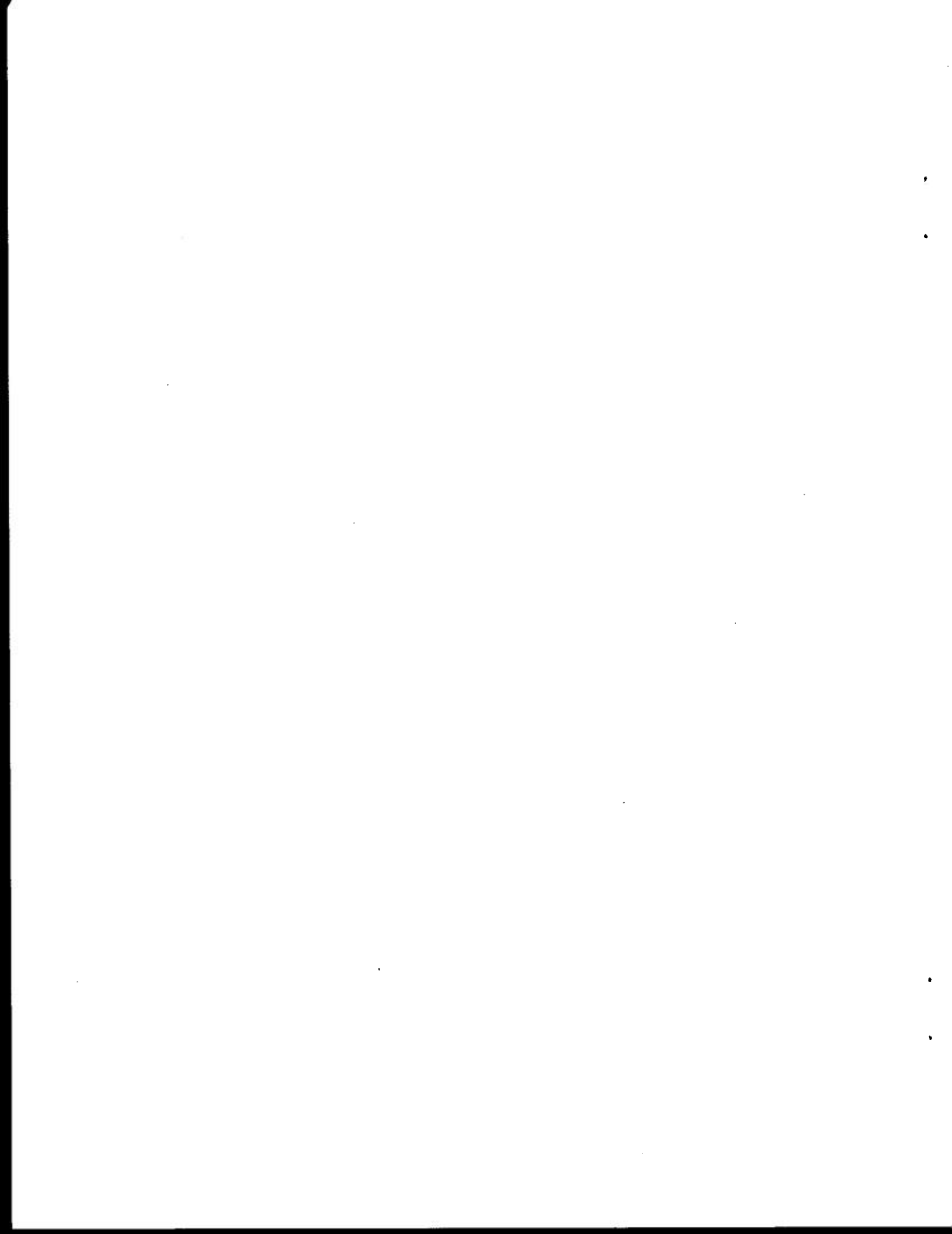
ADMINISTRATIVE INFORMATION

This investigation was conducted as part of Bureau of Medicine and Surgery Research Work Unit MF12.524.006-9028BA9K - Time Concentration Exposure Limits of CO₂ in Navy Submarine and Diving Facilities. The present report is No. 3 on this Work Unit. The manuscript was approved for publication on 22 February 1971 and designated as Naval Submarine Medical Research Laboratory Report Number 652.

PUBLISHED BY NAVAL SUBMARINE MEDICAL CENTER

ABSTRACT

Lactate and pyruvate concentrations were measured in blood and various tissues of guinea pigs exposed to 15% CO₂ in 21% O₂, balance N₂, for varying periods of time up to one week. Acute exposure resulted in reduction of lactate/pyruvate ratios (L/P) of blood, heart, and muscle but not liver. Blood L/P further decreased after one day of exposure and slowly increased during the chronic phase of respiratory acidosis. Heart L/P returned to control values by six hours, while muscle L/P remained depressed for the duration of hypercapnia. Since L/P reflect the redox state of the free cytoplasmic nicotinamide-adenine dinucleotide couplet, the redox state, NAD⁺/NADH was calculated from the L/P and pH of blood for the various periods of hypercapnia. During acute hypercapnia NAD⁺/NADH increased in blood markedly indicating the existence of a more oxidized state during this period. Chronic hypercapnia returned blood NAD⁺/NADH toward initial values suggesting a normalization of glycolytic metabolism. The observed differences in L/P in the various tissues are apparently related to the different CO₂ buffering capacities of tissues.



BIOCHEMISTRY OF SUBMARINE AND DIVING STRESS

I. Lactate-Pyruvate and Redox State Responses of Blood and Tissue in Chronic Hypercapnia

INTRODUCTION

It is a well documented fact that acute hypercapnia has a pronounced effect on the lactate-pyruvate concentrations of some tissues and extracellular fluids. Acute respiratory acidosis causes a decrease of these metabolic acids in blood, cerebrospinal fluid, and brain (5, 13). However, in these studies the duration of hypercapnia did not exceed six hours. In chronic respiratory acidosis lasting 24 hours, Jacey and Schaefer (10) have demonstrated that blood pyruvate levels are significantly elevated. Other than this, little is known of the chronic effects of CO₂ on this vital metabolic system.

The significance of the lactate/pyruvate ratio has taken on added dimensions since it has been shown to be related to the redox state of free cytoplasmic nicotinamide-adenine dinucleotide couplet, NAD⁺/NADH. The ratio of free nucleotides is valuable in the evaluation of the direction of certain reversible reactions in cytoplasmic metabolism (14, 15, 16). The present study was undertaken to investigate and clarify the effects of acute and chronic hypercapnia on the lactate-pyruvate system of blood and tissues and the redox state in blood.

METHODS

Male guinea pigs of the Hartley strain, weighing 400-600 grams, were exposed to 15% CO₂ in 21% O₂, balance N₂, for

varying periods of time, up to a week. Details of exposure and criteria for selection of animals are as described by Schaefer, et al. (22).

At the appropriate time periods, following an intra-peritoneal injection of 40 mg. sodium pentobarbital/Kg of body weight, blood was collected in heparinized syringes from the abdominal aorta, while the animal breathed 15% CO₂ through a filled mask, and an aliquot was immediately precipitated with ice cold .6M perchloric acid. Arterial pH was determined with an Instrumentation Laboratory pH and blood gas analyzer and corrected to body temperature.

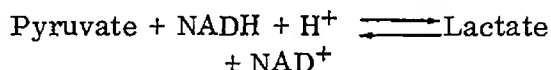
In a parallel series of experiments, but without blood sampling, heart ventricles, the left lateral lobe of liver, and a piece of abdominal muscle were rapidly excised within ten seconds and immediately quick frozen in an acetone-dry ice mixture. Homogenization in the frozen state was accomplished in the presence of ice cold .6M perchloric acid and from this point both blood and tissue extracts were analyzed for lactate and pyruvate content using the respective Calbiochem statpacks.

Calculation of NAD⁺/NADH Ratios.

Hohorst et al. (8) have demonstrated that analysis of certain NAD⁺-linked dehydrogenase systems provide reliable NAD⁺/NADH ratios. Bücher and Rüssmann (2) have further shown that the lactic dehydrogenase system gives

the most reliable values for the cytoplasmic NAD^+/NADH couplet.

Lactic dehydrogenase catalyzes the reaction



with substrates and nucleotides in equilibrium according to the equation:

$$K_e = \frac{[\text{pyruvate}][\text{free - NADH}][\text{H}^+]}{[\text{lactate}][\text{free - NAD}^+]} \quad (1)$$

Since direct measurements of the tissue levels of the nucleotides fail to distinguish between the bound and free fractions, this difficulty may be overcome by direct assay of the concentrations of the lactate and pyruvate concentrations in the tissue. Rearrangement of equation (1) allows calculation of the NAD^+/NADH ratio, equation (2), directly from the substrate content, if K_e , the equilibrium constant, and $[\text{H}^+]$ are known (26):

$$\frac{\text{NAD}^+}{\text{NADH}} = \frac{[\text{pyruvate}]}{[\text{lactate}]} \times \frac{[\text{H}^+]}{K_e} \quad (2)$$

It is apparent that valid numerical values of the NAD^+/NADH ratio depend on accurate knowledge of the following three factors:

1. Tissue concentrations of metabolites:

Because the lactate-pyruvate system is very susceptible to ischemia (7), the tissue samples used in these experiments were quick-frozen within seconds

after being excised and were homogenized in the frozen state.

2. The equilibrium constant:

This constant, determined by Williamson et al (26) in a commercially-obtained rabbit muscle preparation and applied to experiments involving rat liver, was found to be 1.11×10^{-11} mole/l at pH 7.0 and 38°C . Essentially the same value can be calculated from the data of Hakala et al (6) who worked with bovine heart. However, since the K_e is temperature-dependent, fluctuations in body temperature above or below 38°C will affect the calculated NAD^+/NADH ratio.

It is well established that acute exposure to CO_2 produces a hypothermia which is proportional to the concentration of this stressor (23, 24). The data presented by Williamson et al (26) are insufficient for temperature corrections, while those of Kahala et al (6) allow calculation of K_e for any temperature over the range, $16^\circ-40^\circ\text{C}$. In our work, therefore, 6.18×10^{-12} mole/l (6) was taken as the K_e at 38°C and this value was corrected to the body temperature of the animal at the various time points.

3. Hydrogen ion concentration:

In developing the concept of calculating the NAD^+/NADH ratio directly from measurement of the couplet concentrations, Krebs (14) has assumed no change in intracellular pH in his various experimental situations. However, he has pointed out that tissue pH alterations affect the value of NAD^+/NADH . Tissue and blood acidosis induced by exposure to high concentrations of CO_2 has been

demonstrated by many workers and has been extensively reviewed by Waddell and Bates (25). Therefore, hydrogen ion concentration cannot be neglected in calculations involving changes in redox state caused by acute and chronic hypercapnia.

Arterial $[H^+]$ were calculated from the pH values (Table 1) for each of the individual time points. The blood $NAD^+/NADH$ ratio for each time point was then calculated from equation (2).

RESULTS

Data on lactate, pyruvate, and lactate/pyruvate ratio of blood and tissues in acute and chronic hypercapnia are displayed in Figures 1-4. Arterial pH values are summarized in Table 1. The $NAD^+/NADH$ ratios for blood are presented in Table 2.

Acute exposure to 15% CO_2 produced a decline in blood lactate and pyruvate levels within one hour (Figure 1), which persisted for six hours. During the chronic phase of exposure, lactate concentration returned to control values by the end of one day and remained at this level for the duration of hypercapnia, while pyruvate content increased two-fold at one day and gradually returned to control values by the seventh day of exposure. Lactate/pyruvate ratio declined slowly during the acute phase of hypercapnia, reaching its lowest point by one day, and increased with time to control values by the seventh day of exposure. Arterial pH declined drastically during the acute phase and exhibited a gradual increase with time during the chronic phase, but never reached control values.

The $NAD^+/NADH$ ratio of blood rose during the acute phase, attained its highest value at one day, and returned to control values after one week of hypercapnia (Table 2).

As shown in Figure 2, muscle lactate concentration fell rapidly within one hour of exposure to CO_2 and remained depressed for the duration of hypercapnia. Muscle pyruvate increased to its highest level by six hours and declined with time to control values by the seventh day. Lactate/pyruvate ratio decreased within one hour and remained lowered for the week of exposure.

Heart lactate fell rapidly within one hour, returned to control value at six hours, and exhibited another transitory decrease at one day, (Figure 3). Pyruvate remained at control levels during the acute phase, dropped to a low point at one day, and returned to control values by the third day. Lactate/pyruvate ratio showed a severe reduction at one hour, followed by an increase to control values at six hours. A second and mild transitory decline occurred at three days.

The lactate-pyruvate couplet of liver as well as the lactate/pyruvate ratio, were unaffected by the stress of both acute and chronic hypercapnia (Figure 4).

DISCUSSION

The production of lactate is not a true intermediate process but rather represents a temporary end product of metabolism. Pyruvate, on the other hand, enters into many other reactions besides the production of lactate, and

These observations indicate the possibility that, under the conditions of chronic hypercapnia, the degree as well as the duration of acidosis in blood are factors to be considered. They further suggest that mechanisms other than a graded response to pH are responsible for the demonstrated alterations in glycolytic metabolism of blood. However, it must be realized that the arterial concentration of lactate is influenced by the rate of influx as well as the rate of removal plus its own significant glycolysis.

The significant and persistent reduction in lactate concentration and lactate/pyruvate ratio of muscle during both phases of hypercapnia indicate that this tissue is extremely susceptible to CO₂.

The transitory response of heart lactate/pyruvate ratio is confined to the early portion of the acute phase of hypercapnia and is due to a drop in lactate content. A similar finding has been reported by Ledingham et al. (17) in the hearts of dogs exposed to about 100 mm Hg CO₂. Myocardial lactate extraction fell after 16 minutes of exposure to CO₂ and reversed itself by the end of an hour.

The profound and persistent reduction in muscle lactate/pyruvate ratio is in sharp contrast to the lack of an effect of CO₂ upon the liver. This becomes more striking when one considers the fact that these two organs figure prominently in the control of blood lactate levels.

Hypoxia would result in an accumulation of lactate and a rise in lactate/pyruvate ratio. Hypothermia, per se,

(1) would also produce an increase in lactate content of blood and tissue. The transient decrease in blood and heart, the persistent decrease in muscle and the steady state in liver demonstrate that in both acute and chronic hypercapnia, hypoxia and hypothermia can be ruled out as contributing factors.

An increase in the redox state, the NAD⁺/NADH ratio, is indicative of a more oxidized state, while a decrease mirrors a more reduced state. It should be re-emphasized that the glycolytic redox state is under discussion and not that of the mitochondria.

The acute period of hypercapnia, associated with a steady rise in blood NAD⁺/NADH ratio which reached its highest value at one day, reflects a shift toward a more oxidized state. The chronic phase produced an almost linear decline in NAD⁺/NADH ratio and is indicative of a return to a normal redox state. The erythrocyte NAD⁺-NADH pair coupled with the enzyme, methemoglobin reductase, are responsible for maintenance of the iron of hemoglobin in the ferrous state. A decrease in reducing power, i. e., an increase in NAD⁺/NADH ratio might result in an accumulation of erythrocyte methemoglobin. Wood and Schaefer (27), investigating the effects of acute and chronic hypercapnia induced by exposure to 15% CO₂ on the hemoglobin-methemoglobin interaction, found the highest level of methemoglobin after one day of exposure. This peak of methemoglobin concentration parallels the time course for the highest value in NAD⁺/NADH ratio.

Reduction of "in vitro" erythrocyte pH from 7.4 to 7.0 by Omachi et al.

(21) caused a decrease in cell NADH content. Under the same conditions, lactate production fell (19, 20). Direct measurements by Granholm et al. (4) of brain NADH content during an acute respiratory acidosis resulted in a reduction in tissue nucleotide concentration. Under the same conditions, lactate and pyruvate levels decreased. However, it was not clear if the NADH measurement was cytoplasmic or mitochondrial. Jöbsis and Stainsby (12), who measured NADH in skeletal muscle with the same technique, concluded that they were assaying mitochondrial rather than cytoplasmic NADH. In any case, in the experiments of Granholm et al. (3), NADH was oxidized by hypercapnia.

The higher blood redox states in our work, associated with the acute and early chronic phases of hypercapnia are indicative of an oxidation of free glycolytic NADH. The decreases of muscle and heart lactate/pyruvate ratio during hypercapnia may well be accompanied by increases in NAD^+/NADH ratio.

The observed differences in the response and time course of changes in the lactate/pyruvate ratio and redox status suggest that they may be related to the CO_2 buffering capacities of the various tissues. A series of studies by Clancy and Brown (3) and Martin et al. (18) lend support to this concept. They noted the CO_2 buffering capacity of several tissues and concluded that skeletal muscle and extracellular fluid are buffered roughly to the same degree, heart is better buffered than muscle, and liver is particularly well buffered. These results parallel the magnitude of changes in the lactate/pyruvate ratios

during hypercapnia. Muscle and blood had the greatest change with time, heart had a change at one hour, and liver was unaffected.

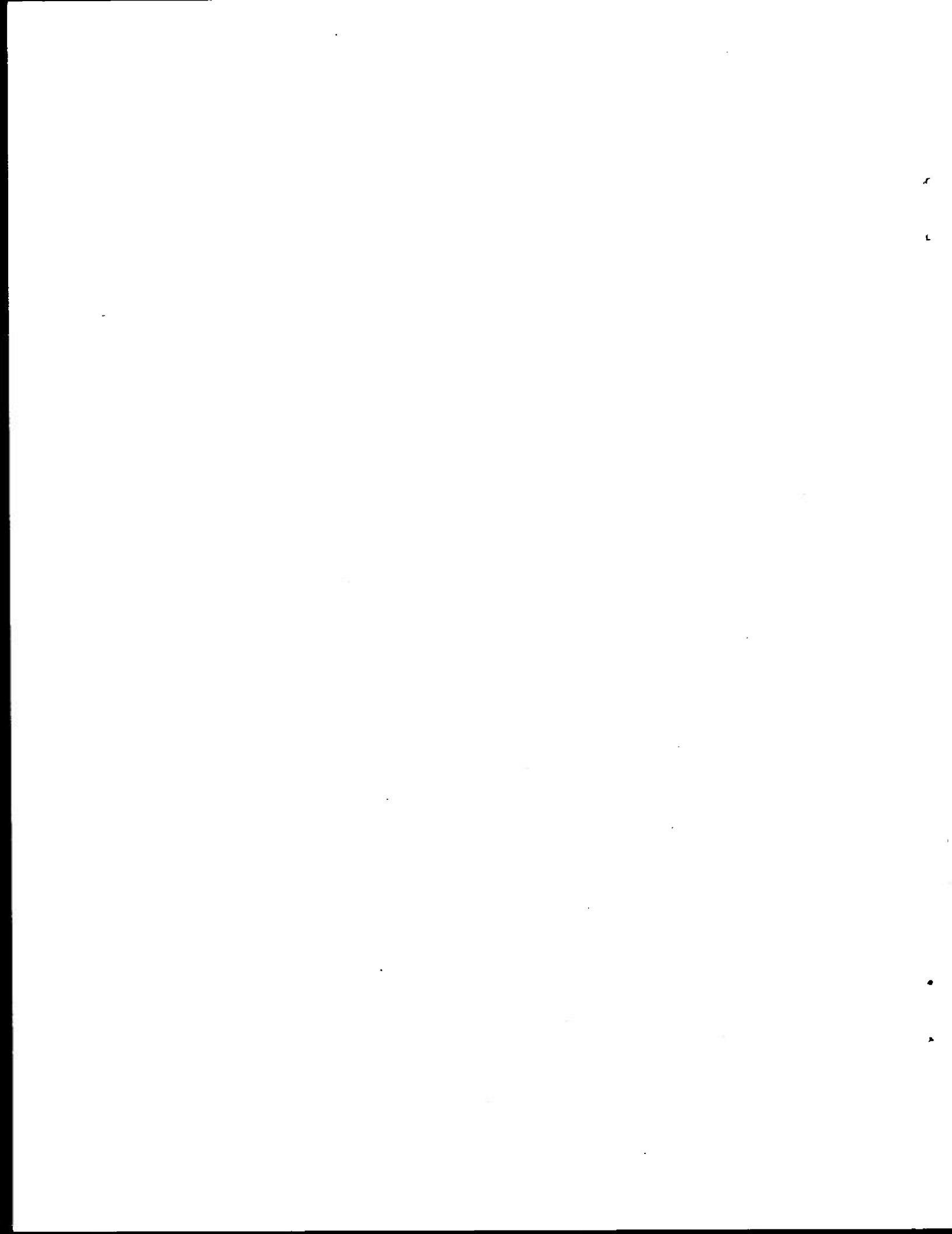
Since glycolysis is mediated through the pH-dependent enzyme, phosphofructokinase (11), the CO_2 buffering capacity of blood and tissues may play a vital role in regulating metabolism during acute and chronic hypercapnia.

REFERENCES

1. Beaton, J. R., and T. Orme. Phosphorus and lactic acid metabolism in the hypothermic rat. Can. J. Biochem. Physiol. 39:1267-1275, 1961.
2. Bücher, Th., and W. Rüssmann. Gleichgewicht und Ungleichgewicht in System der Glykolyse. Angew. Chem. 75:881-893, 1963.
3. Clancy, R. L., and E. B. Brown, Jr. In vivo CO_2 buffer curves of skeletal and cardiac muscle. Am. J. Physiol. 211:1309-1313, 1966.
4. Granholm, L., L. Lukjanova, and B. K. Siesjö. The effect of marked hyperventilation upon tissue levels of NADH, lactate, pyruvate, phosphocreatine, and adenosine phosphates of rat brain. Acta physiol. scand. 77:179-190, 1969.
5. Granholm, L., and B. K. Siesjö. The effects of hypercapnia and hypocapnia upon the cerebrospinal fluid lactate and pyruvate concentrations and upon the lactate,

- pyruvate, ATP, ADP, phospho-
 creatine and creatine concentra-
 tions of cat brain tissue. Acta
physiol. scand. 75:257-266, 1969.
6. Hakala, M. T., A. J. Glaid, and
 G. W. Schwert. Lactic dehydro-
 genase II. Variation of kinetic and
 equilibrium constants with temper-
 ature. J. Biol. Chem. 221:191-
 209, 1956.
 7. Hohorst, H. J., P. Adrese, H.
 Bartels, D. Stratmann and H.
 Talke. L (+) lactic acid and the
 steady state of cellular red/ox sys-
 tems. Ann. N. Y. Acad. Sci.
 119:974-992, 1965.
 8. Hohorst, H. J., F. H. Dreutz, and
 Th. Bücher. Über metabolit-
 gehalte und metabolit-konzentra-
 tionen in der liber der ratte.
Biochem. Z. 332:18-46, 1959.
 9. Jacey, M. J., A. A. Messier, and
 K. E. Schaefer. Responses of
 blood lactate-pyruvate and redox
 state to chronic exposure to 3%
 CO₂. SMRL Report No. 662, 1971.
 10. Jacey, M. J., and K. E. Schaefer.
 Regulation of plasma lactic de-
 hydrogenases in chronic respiratory
 acidosis. The Physiologist.
 7:165, 1964.
 11. Jacey, M. J., and K. E. Schaefer.
 The effects of chronic hypercapnia
 on blood phosphofructokinase
 activity and the adenine nucleotide
 system. SMRL Report No. 659,
 1971.
 12. Jöbsis, F. F. and W. N. Stainsby.
 Oxidation of NADH during contrac-
 tions of circulated mammalian
 skeletal muscle. Respir. Physiol.
 4:292-300, 1963.
 13. Kazemi, H., L. M. Valenca and
 D. C. Shannon. Brain and cerebro-
 spinal fluid lactate concentration in
 respiratory acidosis and alkalosis.
Respir. Physiol. 6:178-186, 1969.
 14. Krebs, H. A. The redox state of
 nicotinamide adenine dinucleotide
 in the cytoplasm and mitochondria
 of rat liver. Advances in Enzyme
Regulation. 5:409-434, 1967.
 15. Krebs, H. A. Role of the redox
 state of nicotinamide adenine
 dinucleotides in the regulation of
 metabolic processes. Nat.
Cancer Inst. Monogr. 27:331-
 343, 1967.
 16. Krebs, H. A. and R. L. Veech.
 Equilibrium relations between
 pyridine nucleotides and adenine
 nucleotides and their roles in the
 regulation of metabolic processes.
Advances in Enzyme Regulation.
 7:397-413, 1969.
 17. Ledingham, I. M., T. I. McBride,
 J. R. Parrate, and J. P. Vance.
 The effect of hypercapnia on
 myocardial blood flow and metab-
 olism. J. Physiol. 210:87-105,
 1970.
 18. Martin, E. D., F. L. Scamman,
 B. A. Atterbery, and E. B.
 Brown, Jr. Time-related

- adjustments in acid-base status of extra-cellular and intracellular fluid in chronic respiratory acidosis. SAM-TR-67-116, USAF School of Aerospace Medicine. Brooks Air Force Base, Texas, December, 1967.
19. 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.
 20. Murphy, J. R. Erythrocyte metabolism II. Glucose metabolism and pathways. J. Lab. & Clin. Med. 55:286-302, 1960.
 21. Omachi, A., C. B. Scott, and T. E. Parry. Influence of glycolysis on NADH content in human erythrocytes. Am. J. Physiol. 216: 527-530, 1969.
 22. Schaefer, K. E., N. McCabe and J. Withers. Stress response in chronic hypercapnia. Am. J. Physiol. 214:543-548, 1968.
 23. Stupfel, M. Action due gaz carbonique sur la thermorégulation du rat blanc. I. Effets de différentes concentrations de CO₂ à diverses températures. J. Physiol. Paris 52:575-606, 1960.
 24. Szegvári, G., I. Várnai, and S. Donhoffer. The effect of environmental temperature, hypoxia, and hypercapnia on total heat production and the electrical activity of muscle in the rat. Acta Physiol. Acad. Sci. Hung. 23:49-63, 1963.
 25. Waddell, W. J. and R. G. Bates. Intracellular pH. Physiol. Reviews. 49:285-538, 1969.
 26. Williamson, D. H., P. Lund and H. A. Krebs. The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Biochem. J. 103:514-527, 1967.
 27. Wood, S., and K. E. Schaefer. Methemoglobin formation and red cell metabolism of guinea pigs during chronic hypercapnia. SMRL Report No. 665, 1971.



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 CENTER, Naval Submarine Medical Research Laboratory		2a. REPORT SECURITY CLASSIFICATION Unclassified	
		2b. GROUP	
3. REPORT TITLE BIOCHEMISTRY OF SUBMARINE AND DIVING STRESS: I. Lactate-Pyruvate and Redox State Responses of Blood and Tissue in Chronic Hypercapnia			
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 22 February 1971		7a. TOTAL NO. OF PAGES 11	7b. NO. OF REFS 27
8a. CONTRACT OR GRANT NO. b. PROJECT NO. MF12.524.006-9028BA9K c. d.		9a. ORIGINATOR'S REPORT NUMBER(S) NSMRL Report Number 652 9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
10. DISTRIBUTION STATEMENT Approved for public release; distribution unlimited.			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY Naval Submarine Medical Center Box 600 Naval Submarine Base Groton, Connecticut 06340	
13. ABSTRACT <p>Lactate and pyruvate concentrations were measured in blood and various tissues of guinea pigs exposed to 15% CO₂ in 21% O₂, balance N₂, for varying periods of time up to one week. Acute exposure resulted in reduction of lactate/pyruvate ratios (L/P) of blood, heart, and muscle but not liver. Blood L/P further decreased after one day of exposure and slowly increased during the chronic phase of respiratory acidosis. Heart L/P returned to control values by six hours while muscle L/P remained depressed for the duration of hypercapnia. Since L/P reflect the redox state of the free glycolytic nicotinamide-adenine dinucleotide couplet, the redox state, NAD⁺/NADH was calculated from the L/P and pH of blood for the various periods of hypercapnia. During acute hypercapnia NAD⁺/NADH increased in blood markedly indicating the existence of a more oxidized state during this period. Chronic hypercapnia returned blood NAD⁺/NADH toward initial values suggesting a normalization of glycolytic metabolism. The observed differences in L/P in the various tissues are apparently related to the different CO₂ buffering capacities of tissues.</p>			

UNCLASSIFIED

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Hypercapnia						
Lactate						
Pyruvate						
Redox state						

UNCLASSIFIED

Security Classification