

Position Paper:
The Toxic Effects of Chronic Exposures
to
Low Levels of Carbon Dioxide

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SUMMARY PAGE

PROBLEM

Representatives of various bureaus of the U. S. Navy occasionally consult scientists at NSMRL regarding safe limits of exposure to carbon dioxide during submarine patrols. Of particular concern recently has been the definition of the highest concentration of carbon dioxide which can be safely inhaled during continuous exposures for 30 to 90 days. This concern can be restated in more practical terms: "Are the inspired levels of CO₂ aboard Naval nuclear submarines toxic for humans who are continuously exposed for 90 days?"

FINDINGS

The responses of the human's organ systems were classified into primary and secondary responses to chronic, low-level, carbon dioxide exposures. Neither type of response represented a toxic effect.

APPLICATIONS

The position of this Laboratory is that it is presently unnecessary to conduct further investigations of human tolerance for CO₂ aboard submarines. Future needs of the Navy may, however, require research into the undesirable effects of chronic, low-level CO₂. The financial requirements for supporting such work will be extremely high.

ADMINISTRATIVE INFORMATION

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ABSTRACT

Numerous patrol studies have provided a sizable data base of levels of CO₂ exposure and health problems encountered by Naval nuclear submariners. Humans have been exposed to a variety of concentrations of CO₂ for chronic periods of time aboard submarines, as well as in laboratory environments. Mean data, $P_{iCO_2} = 10$ torr and 40 days, collectively represent this wide variety of CO₂ exposures. >Physiological responses to the CO₂ environment were repeatedly documented, but toxic effects were not apparent.< Human exposures were safely conducted in atmospheres containing up to 5 torr CO₂, for up to 90 days. Such exposures are therefore considered safe at this time.

Animal studies showed that chronic, low-level, CO₂ exposures caused changes of the bone content of CO₂, the bone content of calcium, the soft tissue content of calcium, and histological changes of the lung. These changes were found to be completely reversible upon discontinuance of the animal's exposure to CO₂. >The extrapolation of the animal data to humans is a current problem in the advancement of our understanding of the effects of CO₂ on humans.<

There are considerable technical and financial impediments to conducting fruitful investigations of the effects of chronic exposure to low-levels of CO₂. The accurate maintenance of the required concentrations of CO₂ in the investigated environment is a costly endeavor. Many of the measurements of the organism's responses to CO₂ are difficult and insensitive and therefore currently limit low-level CO₂ research efforts. There are other significant factors which can modify an organism's responses to low-level CO₂. Diet, levels of exercise during confinement, trace contaminants, and time are important modifiers of any organism's response to CO₂. Failure to control these factors may result in false interpretations of the data from the experiments.

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TERMS

Acclimatization to CO₂ - The adaptive changes in response to deviations from normal PaCO₂, which have persisted for days (40).

Adaptation to CO₂ - The time to compensation of respiratory acidosis (4).

Chronic, low-level exposure to carbon dioxide - The continuous exposure to 2% (15 torr) or less of CO₂ for longer than 72 hours (10).

Gas Stores - The gases that may be found in the body (11).

Hypercapnia - Excess of carbon dioxide in the blood.

Hypercarbia - See hypercapnia.

Primary Effects of CO₂ - The changes of CO₂ stores, pulmonary function, blood gases, and acid-base balance during exposures to increased levels of CO₂.

Respiratory Acidosis - A failure of the pulmonary excretion of CO₂ at a rate equal to the rate of production (gain) of CO₂; and with the elevation of arterial PCO₂, elevation of the arterial content of HCO₃⁻, and decline of the arterial pH. The cause can be either inadequate ventilation or CO₂ inhalation (15).

Secondary Effects of CO₂ - Changes of body functions other than those defined under "primary effects of CO₂".

Toxic Gas - a gas which is poisonous to the body (please refer to Chapter 7 for application of this term to CO₂).

ABBREVIATIONS

CO_2 = carbon dioxide

F_{ICO_2} = Fraction of carbon dioxide in the inspired air

$[\text{HCO}_3^-]$ = The concentration of bicarbonate ion

HCO_3^- = The bicarbonate ion

N = Number of crewmembers in the submarine

P_{ACO_2} = The partial pressure of carbon dioxide in alveolar gas

P_{aCO_2} = The arterial carbon dioxide tension

P_{CO_2} = The partial pressure of carbon dioxide

P_{ICO_2} = The partial pressure of inspired carbon dioxide

% CO_2 = Percentage of CO_2 in the submarine's atmosphere

SCFH = Standard cubic feet per hour

t = Time in hours

torr = Unit of pressure

torr-days = The product of inspired partial pressure of carbon dioxide and days spent exposed to carbon dioxide

V = floodable volume of the submarine, in cubic feet

HISTORICAL ASPECTS

Known Toxic Effects

High levels of CO_2 can exert a toxic effect on humans. Indeed, a human death was considered to possibly result from an acute exposure to 2% CO_2 (66) although an overpowering number of reports of similar CO_2 exposures would argue against it (61, 4, 26).

The experimental induction of acute respiratory acidosis results in the accumulation of carbonic acid until the reestablishment of CO_2 equilibrium. The content of HCO_3^- rises in the extracellular fluid. The accumulation of H^+ in the extracellular fluid is partially reduced by exchanges with Na^+ and K^+ from the bone and cells. Plasma proteins and hemoglobin act as buffers, as well as phosphates. The new steady state is characterized by a reduced arterial pH, a reduction of the ratio of $[\text{HCO}_3^-]/\text{PCO}_2$, and presumably a higher cellular PCO_2 . The skeletal muscle pH is acidic (15, 21). Patients with chronic obstructive pulmonary disease have high values for their PaCO_2 (70 to 80 torr) due to inadequacy of their alveolar ventilation. Their chronic respiratory acidosis is not severe and does not require acid-base therapy. Three to 4 milliequivalents of HCO_3^- per liter are reabsorbed by the nephron for each 10 torr increment of PaCO_2 in uncomplicated, chronic, respiratory acidosis. Hence, the change of pH is minimized by buffer supplied from the kidney. Severe deviations of pH can impair the functions of the vital tissues, namely the brain and heart (16). The electroencephalogram (EEG)

undergoes a triphasic response as the fraction of inspired CO_2 rises from below 10% to above 40%. Concentrations up to 10% can depress the excitability of the cerebral cortex. Associated with this are hyperpolarization and accumulation of hydrogen ions within the neurons. The cortex progresses to an excitable state when the fraction of inspired CO_2 progresses toward 40%. The subcortical centers may spontaneously elicit seizures. The neurons are severely acidotic and there is a reduction of the total content of neuronal ions. The level of GABA is inversely correlated with the degree of brain excitability. Hyperexcitability can stimulate the release of catecholamines and cortisol (14). The tremendous decrement of cardiac muscle pH by 0.5 pH units results in 50% decrement of the force of contraction, and bradycardia, of the isolated heart. Inhalation of elevated levels of CO_2 may enhance the cardiac output by enhancement of the venous return or activation of the sympathetic nervous system. The postulated mechanism for enhancement of the venous return is the greater activity of the "respiratory pump" from chemo-stimulation of the respiratory control mechanism. Both the heart rate and arterial blood pressure rise in normal men when their PaCO_2 rises from 40 to 85 torr. Surgical anesthesia may result in transient elevations of PCO_2 up to 140 torr. Such elevations are well tolerated, the apparent danger being hyperventilatory hypotension. Ventricular arrhythmias may occur during hypercarbia or the correction of hypercarbia (17).

In view of the known toxic effects of high levels of CO_2 for brief exposures, a diversity of administrators and scientists have been concerned with the potential for toxic effects from prolonged exposures of humans to low concentrations of carbon dioxide

in the breathing medium. For example, the exploration of space requires that man live in a protective capsule for months while maintaining high standards of task performance and vigilance. Since high concentrations of CO₂ can degrade performance, the space officials need to know the essential requirements for cleansing the capsule of CO₂ in order to design an optimal CO₂-scrubbing apparatus capable of accompanying the astronauts into outer space.

History of CO₂ Research U. S. Navy

The U. S. Navy has had an identical problem with the encapsulated environments of its submarine force. The problem was formally recognized in 1914 when the U.S. Navy began serious study of the possibility of air purification of submarine atmospheres during submergences. For many years thereafter primary supportive effort was devoted to emergency removal of CO₂ because compressed air supplied the necessary oxygen. In 1929, and during the next 25 years, these significant developments were made at the Naval Research Laboratory (NRL):

- (a) Anhydrous lithium hydroxide was developed for the emergency removal of CO₂ during long submergences.
- (b) Self-contained oxygen rebreathers became standard throughout naval vessels. Potassium superoxide (KO₂) released oxygen for breathing and the alkaline residue absorbed CO₂.
- (c) Chlorate candles were developed as a convenient source of oxygen for supplemental or emergency use.

It was recognized in the early 1950's that a whole new dimension in atmosphere life support systems would be necessary to enable nuclear-

powered submarines to reach their potential or prolonged, uninterrupted submergences. Their submergence limitation would not be based on availability of underwater power but by the capability to maintain adequate life support for the crew. The nuclear submarine fleet today is capable of long submerged operations in safety and comfort for the crew.

Analytical data obtained by NRL chemists during early submergences of the first nuclear-powered submarines showed the atmospheres to contain many organic contaminants. Based on sampling and analysis, early nuclear submarine atmospheres contained O₂ 19-21% and CO₂ 0.1-1.3%. As individual contaminants were identified and their concentrations in the atmosphere established, the Navy Bureau of Medicine and Surgery had to consider their potential toxic effects and establish threshold limits for certain compounds. Human exposures are continuous for 24 hours a day for weeks on end in the sealed environment of the submarine. This is in marked contrast to industrial experience where exposure is 8 hours a day, 5 days a week. Therefore, the allowable limits had to be made much lower than those in the more conventional work place. For CO₂, as more was learned about possible effects, the allowable limits have been set lower several times, requiring changes in equipment or its operation.

An absolutely essential requirement in maintaining life under prolonged submergences is continuous removal of CO₂. The standard equipment used for this purpose is the MEA Scrubber. The CO₂ is stripped from the atmosphere by absorption in an aqueous solution of monoethanolamine (MEA) at room temperature. The CO₂ is released from the MEA solution by

passing it continuously through a heated chamber, and the concentrated CO₂ gas is pumped overboard. The atmospheric level of CO₂ is maintained at about 1.0% or less, which is considerably higher than the ambient concentration of a normal atmosphere (0.03%). However, crews have lived continuously with the higher concentrations of CO₂ for long periods without apparent ill effects. This factor has been under nearly constant study and review by the Bureau of Medicine and Surgery with no hard evidence of even minimal toxic effects. The emergency system for removal depends on alkaline absorption in a device in which the submarine atmosphere is blown through canisters of dry LiOH (1).

Respiration accounts for most of the carbon dioxide generated in a submarine. The concentration of the CO₂ varies considerably within permissible limits when the CO₂ scrubbers are working. Should the CO₂ scrubbers be rendered inoperable, intentionally or otherwise, the rise of the % CO₂ in the submarine can be calculated as follows:

$$\% \text{ CO}_2 = 0.03 + \frac{85 \text{ t}}{\text{V/N}}$$

Figure 1 represents the computed rate of rise of CO₂ in the atmosphere and can be used operationally to relate CO₂ scrubber function to safe limits of CO₂ tolerance.

The effects of short-term exposures to CO₂, summarized in figure 2, represents the U. S. Navy's official judgment on the hazards of CO₂ (3). Note there are extremes ranging from irreversible response to apparently absent responses. Apparently, no such chart exists for responses to long-term CO₂ exposures. The following policies have been reached regarding long-time CO₂ effects:

- (a) 0.5 percent to 0.8 percent: There is probably no significant effect on the body.
- (b) 0.8 percent to 3.0 percent: Prolonged exposure could induce impairment of mental functions and slowing of physical activities.
- (c) 3.0 percent and above: Further impairment of mental functions and slowing of physical activities occur.

Prolonged exposure to elevated levels of CO₂ in the ambient air has been a major topic of study in submarine medicine and physiology for the past 35 years. The first significant studies in chronic CO₂ toxicity in man were done in two U. S. Navy laboratories. These studies were carried out under A. R. Behnke at the Naval Medical Research Institute (NMRI) and C. W. Shilling at the Naval Submarine Medical Research Laboratory (NSMRL). In determining the limits for increased CO₂ and lowered O₂ during emergency conditions aboard a submarine, the group at NMRI found that human subjects could tolerate up to 5% (38 torr) CO₂ and 12-13% (91-99 torr) O₂ for a period of 72 hours. Twenty percent of the men complained about headaches and occasional nausea while demonstrating some impairment in specific motor performance. The conclusion drawn from the experiment was that despite crew discomfort, the safety of a submarine need not be jeopardized. The group at NSMRL also demonstrated that 68 hours of prolonged exposure to 3% (21 torr) CO₂ and 17% (130 torr) O₂ impaired performance. Schaefer found that exposure to 3% (21 torr) CO₂ in air caused

- (a) an attenuation of the ventilatory CO₂ response after 24 hours, and
- (b) a decrease of the slope of CO₂ tolerance curve after 5-6 days. Following an initial drop, the bicarbonate level in the blood sufficiently increased after 3 days

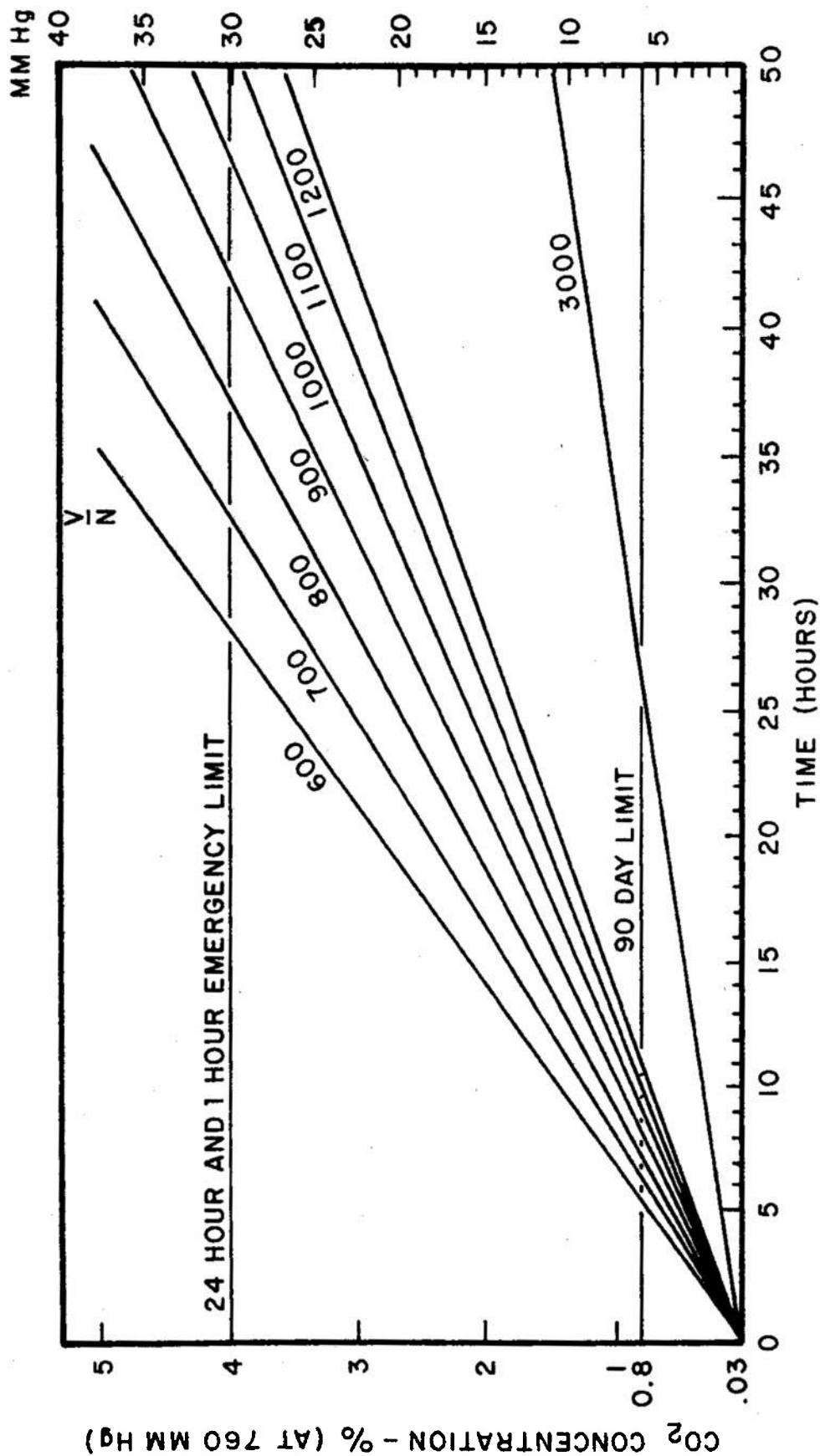


Figure 1

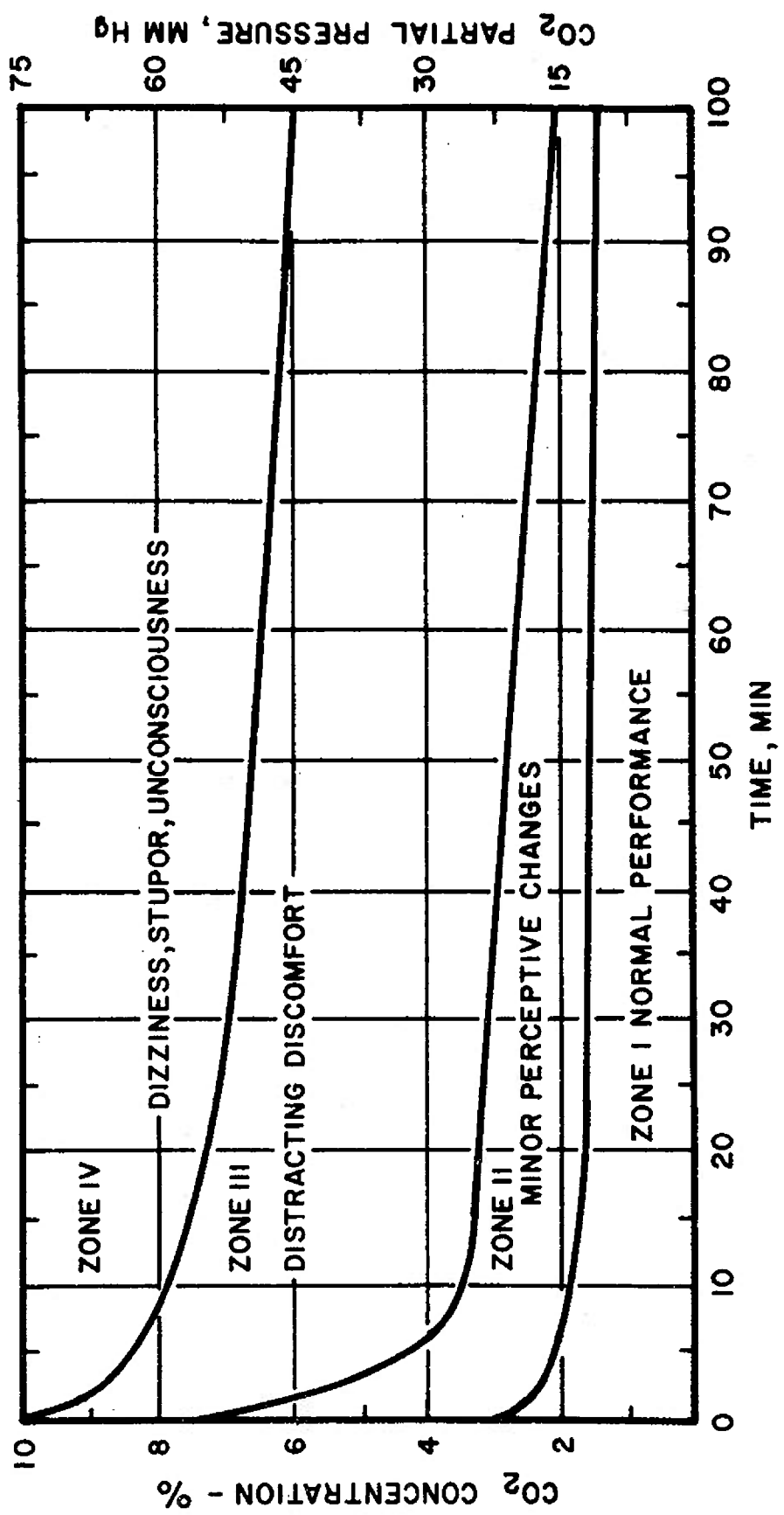


Figure 2

of exposure to 3% (21 torr) CO₂ to bring the pH to a level which was still lower than the control value. This was the first evidence of two forms of respiratory adaptation to CO₂, of which only the second one was related to blood bicarbonate changes caused by increased renal reabsorption of bicarbonate. Later, NSMRL conducted a large-scale study, "Operation Hideout", with 21 human subjects exposed to 1.5% (11 torr) CO₂ for 42 days. Significant adaptive changes in respiration, acid-base balance and calcium-phosphorus metabolism were found.

Upon an evaluation of these studies and a review of the literature on chronic CO₂ exposure of humans, Schaefer proposed a "triple tolerance" approach to chronic CO₂ toxicity. His concept described the action of CO₂ at 3 distinct levels. At Level I, 3% (21 torr) CO₂ and above, human performance deteriorated and alterations in basic physiological functions were readily seen. At Level II, 1.5% (11 torr) CO₂, performance was unaffected; however, slow adaptive processes were observed in electrolyte exchange and acid-base balance regulation which were hypothesized to induce patho-physiologic states on greatly prolonged exposure. Level III, at which no significant physiologic adaptive changes to CO₂ occur, has not yet been fully established. At Level I, the renal regulation of bicarbonate reabsorption is fully active. With lower concentrations the renal compensation becomes less effective and, it may be questioned whether or not the kidney is playing an active compensating role. A concentration of 0.5% (3.8 torr) CO₂ may represent a threshold level for the manifestation effects of chronic hypercapnia. It is a level where pathological indicators in animals are slight, physiological functions

in humans are not altered significantly, and the CO₂ responses are totally reversible when the subject is returned to breathing normal air (2). NSMRL conducted a workshop on Submarine Medicine in 1978. Formal recognition was given to the minimal health effects of Schaefer's "Level III" exposure to CO₂. Proposals were made for focusing future research on the health consequences of the altered life styles which probably exist during submarine patrols. Such studies might lead to more cost-effective safeguards of health than the high costs of further reduction of CO₂ levels aboard submarines (29, 30).

THE PROBLEM

The length of a nuclear-submarine patrol is currently limited by practical matters such as the food supply and the morale of the crew. Ninety-day patrols are realistic capabilities aboard today's nuclear submarines. The U. S. Navy has adapted an average level of inspired CO₂ of 0.8% (6 torr) as its standard for 90-day exposures to CO₂ and permits maximum concentrations of 4% for brief durations (figure 1 (3)). The rationale for this standard probably evolved from several considerations. Schaefer speculated (a) that greatly prolonged exposures to 1.5% CO₂ (11.4 torr) could induce pathophysiological changes (in humans); and (b) that exposures to 0.5 to 0.8% CO₂ (3.8 to 6 torr) probably would not cause significant physiological, or adaptive changes (4). The limited capabilities of the atmospheric control equipment have imposed considerations of cost-effective compromises between health needs of the crew and space limitations for CO₂ scrubbers aboard submarines (6). Unfortunately there is an apparent lack of scientific and administrative agreement concerning the threshold concentration

of CO₂ at which undesired side effects may occur in humans after prolonged exposures of 30 to 90 days(7).

The practical question is, "Are the inspired levels of CO₂ aboard Naval, nuclear submarines toxic for humans who are continuously exposed for 90 days?" There is no body of scientific knowledge which specifically answers this question. A review of data from the literature was therefore performed in order to comment on the following relevant concerns:

- a. Define significant responses to chronic, low-level, CO₂.
- b. Assess the dosage of CO₂ necessary for the significant responses during chronic exposures.
- c. Ascertain whether or not current levels of CO₂ aboard Naval submarines cause harmful effects in man.

NSMRL has accumulated large sets of data from prolonged animal exposures to CO₂. The findings of the animal work will also be presented as a potential model for possible future studies of chronic, low-level, CO₂ exposures.

HUMAN STUDIES

Literature Review

Low-Level CO₂

A detailed literature review was conducted to assess the responses of humans who have been chronically exposed to low levels of CO₂ (41-65). Some reports of high-level CO₂ exposures were included within the review. A reviewer categorized all of the reported responses into "change" or "no-change". "No change" was judged to be the case by one of the following criteria:

a. The author reported a low-level of statistical significance $p > .05$, by comparison with a control.

b. The reviewer judged, from the variability of the measured response to the CO₂ exposure, that the response would have occurred during a non-CO₂ exposure. Such judgment was required when multiple t-tests were used to test statistical significance rather than the appropriate analysis-of-variance.

"Change" was judged to be the case when:

a. The author reported a high level of statistical significance, $p < 0.05$, by comparison with a control.

b. The reviewer judged that the response resulted from an exposure to CO₂.

General Results of Literature Review

The review yielded 22 separate exposures which embodied data collected from 212 adult men (Table 1). Nine studies were performed in the laboratory and 12 were performed during submarine patrols. The mean $PiCO_2$ was 10 torr (S.D. 7 torr) and the mean duration was 40 days (S.D. 23 days). The ranges of exposure were 3.8 to 30 torr, $PiCO_2$, during 1 to 90 days of exposure. There were no reports of health impairments attributed to carbon dioxide.

Primary Responses, Pulmonary Function:

The observed rise of the alveolar ventilation in 1 study raised the possibility that low-level CO₂ might stimulate ventilation (Table 2a).

TABLE 1. Literature Review; Conditions of the Exposure

<u>Reference</u>	<u>PI,CO₂ Torr</u>	<u>Exposure Time, Days</u>	<u>Number of Men</u>	<u>Laboratory Study</u>	<u>Submarine Patrol Study</u>
A	5	90	4	✓	
B	7.6	63	12		✓
C	30	14	4	✓	
D	11.4	42	23	✓	
E	23	5	5	✓	
F	6.1	20	-		✓
F	6.8	20	10		✓
G	22.8	1	1	✓	
H	3.8	13	6	✓	
I	5.3	52	15		✓
J	3.8	40	9	✓	
J	11.4	12	4	✓	
K	6	64	15		✓
L	6.1	56	10		✓
L	7.6	56	-		
M	7.6	56	31		✓
N	6.5	52	20		✓
O	14	30	6	✓	
P	7.6	40	15		✓
Q	9.1	46	10		✓
R	7.6	54	5		✓
S	<u>?</u>	<u>60</u>	<u>7</u>	<u>—</u>	<u>✓</u>
Mean	10	40			
S.D.	7	23			
Total			212	9	12

TABLE 2a. Summary of Changes, Pulmonary Function

<u>Measurements</u>	<u>"No Change"</u>	<u>"Change"</u>	<u>PICO₂</u> Mean & S.D.	<u>References</u>
respiratory minute volume	2	5	9.3±3.3	48, 52, 58
tidal volume	2	4	8.6±3.6	48, 52, 58, 61
respiratory frequency	6	1	8.7±3.3	48, 52, 58, 61, 62
vital capacity	3		8.4±2.7	48, 58
expiratory reserve volume	1			48
inspiratory reserve volume	1			48
physiological dead space		6	8.1±3.3	48, 52, 58, 61
$\dot{V}_A$		1	11.4	48
PACO ₂		3	9.7±5.3	45, 48, 56, 61
Ventilatory response to CO ₂	1	3	9.1±2.7	48, 58

A summary of the pulmonary function changes are given in Table 2a. The respiratory frequency did "not change" while the tidal volume was often found to "change". The physiological dead space "changed" and the respiratory minute volume often changed. The P_{ACO_2} always changed in the few studies reported. There were "no changes" of the vital capacity, inspiratory reserve volume, or expiratory reserve volume. The ventilatory responses to increments of inhaled CO_2 often showed "changes". The data may be presumed to represent changes of the alveolar ventilation by enhancement of the tidal volume.

PRIMARY RESPONSES:
BLOOD GASES AND ACID-BASE:

The arterial PCO_2 always "changed" in the few studies reported (Table 2b).

As shown in Table 2b, the mixed venous PCO_2 tended to "change" in blood and frozen specimens. The venous pH tended to "change" as did the arterial pH and the capillary pH. The content of bicarbonate tended to "change" in the venous plasma, venous blood, arterial plasma, and arterial blood.

The urinary excretion of ammonium probably "changed" during the exposures. Less dramatic were the "possible changes" of urinary pH, CO_2 content of the urine, and gastric acidity. There was some evidence that "no changes" occurred in the urinary excretion of net acid, titratable acidity of urine, salivary content of CO_2 , and urinary excretion of bicarbonate.

Secondary Responses: Electrolytes

The secondary responses to low-level CO_2 were represented by measurements of a wide array of bodily functions (Table 2c). Table 2c summarizes the fluid balance of humans in low level CO_2 . The electrolyte metabolism was examined out of concern for possible responses to alterations of the body's acid-base balance. The few studies suggested that the sodium balance could "change". The urinary excretion, plasma concentration, and red blood cell concentration of sodium tended to "change". Although the plasma and erythrocyte concentration of potassium probably "changed", there was the possibility that "no changes" occurred in potassium's balance, urinary excretion, salivary concentration, and fecal excretion. The chloride concentration of the erythrocyte possibly "changed", but neither its plasma concentration nor its urinary excretion seemed altered. There was possibly "no change" of chloride's salivary concentration or fecal excretion. Sparse data suggested that the calcium balance "changed" and that the magnesium balance did "not change". Aside from "no change" of the salivary concentration, and fecal excretion of calcium, the plasma concentration, urinary excretion, erythrocyte concentration, and fecal excretion of calcium tended to "change". "No changes" tended to occur in magnesium's plasma concentration and fecal excretion. The urinary excretion of magnesium and phosphorus probably "changed". The balance of phosphorus tended to "not change". The phosphorus concentration of plasma and excretion in feces tended to "not change", while its salivary concentration tended

TABLE 2b. SUMMARY OF CHANGES, BLOOD GASES AND ACID BASE				
Measurements	"No Change"	"Change"	PiCO₂ Mean & S.D.	References
PaO ₂		2		57,62
PaCO ₂ , blood or plasma		4	9.8±3.6	50,57,61,62
Pc,CO ₂	1			56
P _v , CO ₂ blood		1	6	57
P _v , CO ₂ frozen plasma	1	2	7.6	41,43,59
PcO ₂ , frozen RBCs		1		43
[HCO ₃ ⁻] a, plasma		2	9.5±2.7	50,62
[HCO ₃ ⁻] v, blood		3	6.7±0.8	43,57,59
[HCO ₃ ⁻] a, blood		1		57
[HCO ₃ ⁻] a, RBC		1		50
[HCO ₃ ⁻] v, plasma	2	3	8.6±4.0	41,43,49,61
[HCO ₃ ⁻] excretion, urine	2	1	7.2±5.89	54,56,61
[CO ₂] urine		1	11.4	50
[CO ₂] saliva	1		9.1	63
pHc, blood		1		56
pHa, blood		3	9.2±4.2	50,57,61,62
pHa, plasma		1		50,57,61,62
pHv, plasma	2	3	7.6±2.4	41,43,49,59
pH urine	1	1	10.8±4.5	59,60
gastric acidity		1	7.6	64
urinary net acid	2	1	7.7±5.5	56,61
titratable acidity	2	2	9.5±6.4	41,61
24 hour urinary "acid-base balance"	1		5.0	42
urinary ammonium excretion		3	6.7±4.9	55,56,61

TABLE 2c. Summary of Changes, Electrolytes			
Measurements	"No Change"	"Change"	References
Na ⁺ balance	1	1	50,56
Na ⁺ excretion	1	4	50,56,57,59
[Na ⁺] plasma	4	4	41,43,44,50,56,57,59,61
[Na ⁺] RBC		3	43,50,59
[Na ⁺] saliva	1		63
K ⁺ balance	2		50,56
K ⁺ excretion, urine	4	2	44,50,56,57,59
K ⁺ excretion, feces	2		56
[K ⁺] plasma	3	4	41,43,50,57,59,61
[K ⁺] RBC		1	43,50,59
[K ⁺] saliva	1		63
Cl ⁻ excretion, urine	3	1	50,56,59,61
Cl ⁻ excretion, feces	1		56
[Cl ⁻] plasma	4	2	41,43,50,56,57,59
[Cl ⁻] saliva	1		63
[Cl ⁻] RBC	1	2	43,50,59
Ca ²⁺ balance	1	1	56
Ca ²⁺ excretion, urine	2	7	
Ca ²⁺ excretion, feces	1	1	56

TABLE 2c. Summary of Changes, Electrolytes, cont.

<u>Measurements</u>	<u>"No Change"</u>	<u>"Change"</u>	<u>References</u>
[Ca ²⁺] plasma	3	5	41,49,55,56,59, 61
[Ca ²⁺] RBC		1	59
[Ca ²⁺] saliva	1		63
Mg ²⁺ balance	2		56
Mg ²⁺ excretion, urine	1	4	44,55,56,59
Mg ²⁺ excretion, feces	2		56
[Mg ²⁺]plasma	3	1	55,56,59
Zn ²⁺ excretion, urine		1	44
Phosphorus balance	2		56
PO ₄ ⁻³ excretion, urine	4	5	44,49,50,55,56, 57,59
PO ₄ ⁻³ excretion, feces	2		56
[PO ₄ ⁻³]plasma	6	2	41,49,55,56,59, 61
PO ₄ ⁻³ saliva		1	63

to "change". The urinary excretion of zinc possibly "changed".

Secondary Effects: Water Balance

There was strong evidence that the 24-hour urinary volume did "not change" (Table 2d).

There was probably "no change" of the plasma water content or the body weight. Despite possible "changes" of the hematocrit and erythrocyte concentration, sparse evidence suggested "no change" of the water balance, fecal excretion of water, water content of the blood, hemoglobin concentration, flow of saliva, creatinine excretion, and creatinine clearance.

Secondary Effects: Bone

The urinary excretion of hydroxyproline, plasma concentration of calcitonin, and plasma concentration of parathormone tended to "not change". Decrements of vitamin D concentration in plasma have been measured (Table 2e).

Secondary Effects: Metabolism

The metabolism tended to "not change" as evidenced by a few studies showing "no change" of the body temperature, daily intake of food, fecal wet-weight, fecal dry-weight, fecal nitrogen excretion, and nitrogen balance (Table 2f).

Possible "changes" were noted in the respiratory exchange ratio, oxygen uptake, ventilatory excretion of carbon dioxide, and the urinary excretion of nitrogen. Possibilities of "no change" were also found for the plasma concentration of cortisone, the reticulocyte count,

and the white blood cell count. Possible "changes" occurred in the polymorphonuclear and eosinophile counts.

Secondary Effects: Cardiovascular and CNS

The blood pressure, heart rate, and psychomotor task performance tended to "no change". Hand steadiness and the electroencephalogram possibly "changed" (Table 2g).

Discussion

A major problem with the conduct of this review was the variety of standards-of-reference for judging significant changes of the measurements. Therefore, the reviewer classified the results into measurements which did or did not change from various reference measurements. The reviewed data collectively represented a continual exposure of men to 1.32% CO₂ (PICO₂= 10 torr) for 40 days.

A preponderance of the changes were in the functionally related responses of the ventilation, blood gases, and acid-base status of the subjects. Equations predicted that the alveolar ventilation (therefore, the respiratory minute volume) will rise when the inspired levels of CO₂ rise for a given state of metabolic activity (32):

$$\dot{V}_A = \frac{V_{O_2} \cdot k}{P_{A,CO_2} - P_{I,CO_2}}$$

The data from this review showed that the respiratory minute volume changed in response to FICO₂'s between 0.5 and 2% (3.8 to 15 torr), implicating a more responsive respiratory center than previously thought (17-20).

TABLE 2d. Summary of Changes, Water Balance			
<u>Measurements</u>	<u>"No Change"</u>	<u>"Change"</u>	<u>References</u>
water balance	1		56
24-hour urine volume	6	1	55,56,59,61
fecal excretion water	2		56
% water blood	1		49
% H ₂ O, plasma	2	1	45,49,57
hematocrit	1	2	60,61
erythrocyte count	1	2	45,60,61
[Hb]	1	1	45,60
salivary flow	1		63
creatinine excretion, urine	2		56
creatinine clearance	1		56

TABLE 2e. Summary of Changes, Bone			
<u>Measurements</u>	<u>"No Change"</u>	<u>"Change"</u>	<u>References</u>
hydroxyproline excretion, urine	2	1	56, 59
[Vitamin D], plasma		2	56, 65
[Calcitonin] plasma	1		59
[Parathormone] plasma	1		62

TABLE 2f. Summary of Changes, Metabolism

<u>Measurements</u>	<u>"No Change"</u>	<u>"Change"</u>	<u>References</u>
body temperature	1		46
body weight	2	1	46,56
N ₂ balance	1		50
daily food intake	1		56
fecal N ₂ excretion	1		50
fecal wet-weight	1		56
fecal dry-weight	1		56
N ₂ excretion, urine		1	50
respiratory exchange ratio	1	1	50,61
$\dot{V}O_2$	1	1	48,61
$\dot{V}CO_2$	1	1	48,61
ventilatory excretion CO ₂		1	45
[Cortisol] plasma	1		62
Reticulocyte count	2		45,60
white blood cell count	1		45
[EOS 's]		1	45
[PMN's]		1	45

TABLE 2g. Summary of Changes, Cardiovascular and CNS

<u>Measurements</u>	<u>"No Change"</u>	<u>"Change"</u>	<u>References</u>
test of performance	2		45,61
blood pressure	1		46
heart rate	1		46
hand steadiness		1	51
EEG		1	51
EEG, sleep		1	45

The stability of the body water balance, nitrogen balance, and body temperature were strongly indicative that chronic, low-level CO₂ (10 torr for 40 days) did not disturb the basic metabolic needs of the body.

Dose Response Trends in Chronic Exposures to Low-Level CO₂

Attempts were made to determine the dosage of CO₂ at which definable responses could be measured (Figures 3 and 4). One type of CO₂-dosage studied was the PICO₂ of the ambient environment in which the men resided for days at a time (Figure 3). Using the "change" and "no change" data from the Literature Review, the alveolar and arterial PCO₂'s were found to "change" at PICO₂ 3.8 torr (Figure 3). The ventilatory responses appeared to be very sensitive to inspired CO₂ levels $\geq$ 6 torr. The venous plasma pH changes were variable, but the arterial pH's "changed" at PICO₂ $\geq$ 6 torr (figure 3b). The venous bicarbonate content "changed" at PICO₂ $>$ 6 torr. There was a "change" of the urinary ammonium excretion above CO₂ levels of 3.8 torr but not necessarily a "change" of the urinary net acid excretion. The responses of the inorganic ions to low-level CO₂ were quite variable (figure 3c). The rates of urinary excretion of calcium and phosphorus "changed" over a wide domain of levels of CO₂ (5 to 30 torr). However, there were occasions when "no change" of the excretion of calcium and phosphorus occurred during exposures to PICO₂ $<$ 12 torr. The plasma concentrations of calcium, sodium, and potassium showed both "changes" and "no changes" at CO₂ levels between 3 and 15 torr. In summary, acid-base balance and the blood gases were very sensitive to low levels of PICO₂. The body fluid and

electrolyte metabolism responded with great variability and showed no clear trend toward a threshold effect.

CO₂ doses were also examined by expressing them as a combined effect of CO₂-level and duration-of-exposure. The product of PICO₂ and days-of-exposure (torr-days) was computed for those exposures whose responses were just discussed (figure 4). "Changes" occurred in the respiratory minute volume, PACO₂, and PaCO₂ when the CO₂ dose $\geq$ 150 torr-days (figure 4a). CO₂ doses $\geq$ 300 torr-days were accompanied by "changes" of the arterial pH, whereas the venous bicarbonate content and venous pH contained a mixture of "change" and "no change" with increased CO₂ dose (figure 4b). The urinary ammonium excretion "changed" and the urinary net-acid excretion did not necessarily change at CO₂ doses $\geq$ 150 torr-days (figure 5b). No dose-response trends were apparent for the response of the inorganic ions between 125 and 500 torr-days (figure 4c).

The nature of the dose-response trends from chronic, low level CO₂ exposures suggests that the ventilatory and acid-base functions "changed" above a threshold dosage. The respiratory minute volumes transitioned from "no change" to "change" as the CO₂ doses rose above 7 torr and 300 torr-days (figure 3a, 4a). The arterial and alveolar PCO₂'s always showed "change" down to 3.8 torr and 150 torr-days (figure 3a, 4a); and there was no transition of PA₂CO₂ or Pa₂CO₂ into a "no change" status at low doses of CO₂. There was no transition of the arterial pH into a "no change" status below the doses which showed "change", namely $\geq$ 6 torr and $\geq$ 300 torr-days (figures 3b and 4b). The notable feature of the responses of the

Fig. 3 a

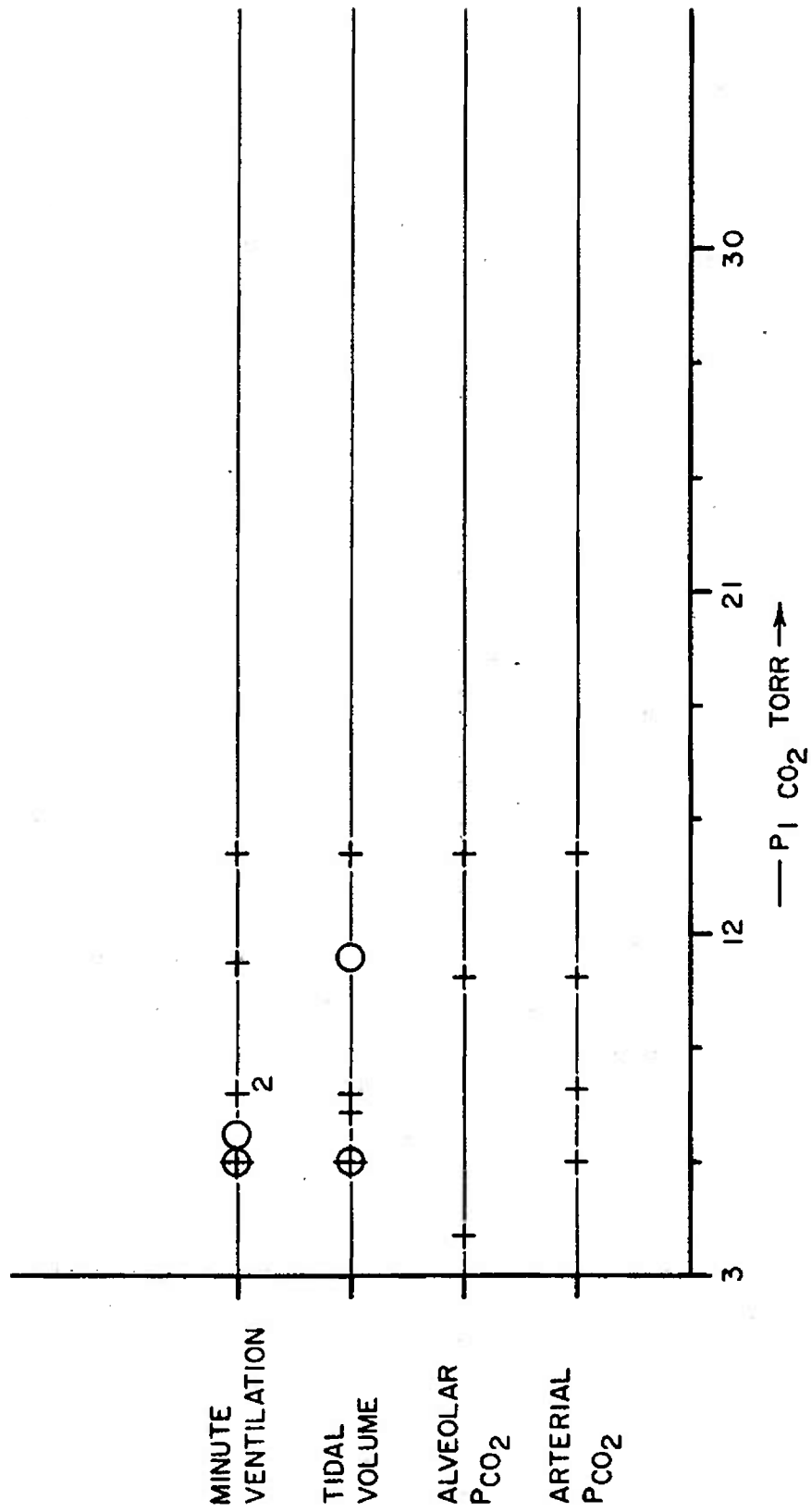


Fig. 3 b

○ "NO CHANGE"
+ "CHANGE"

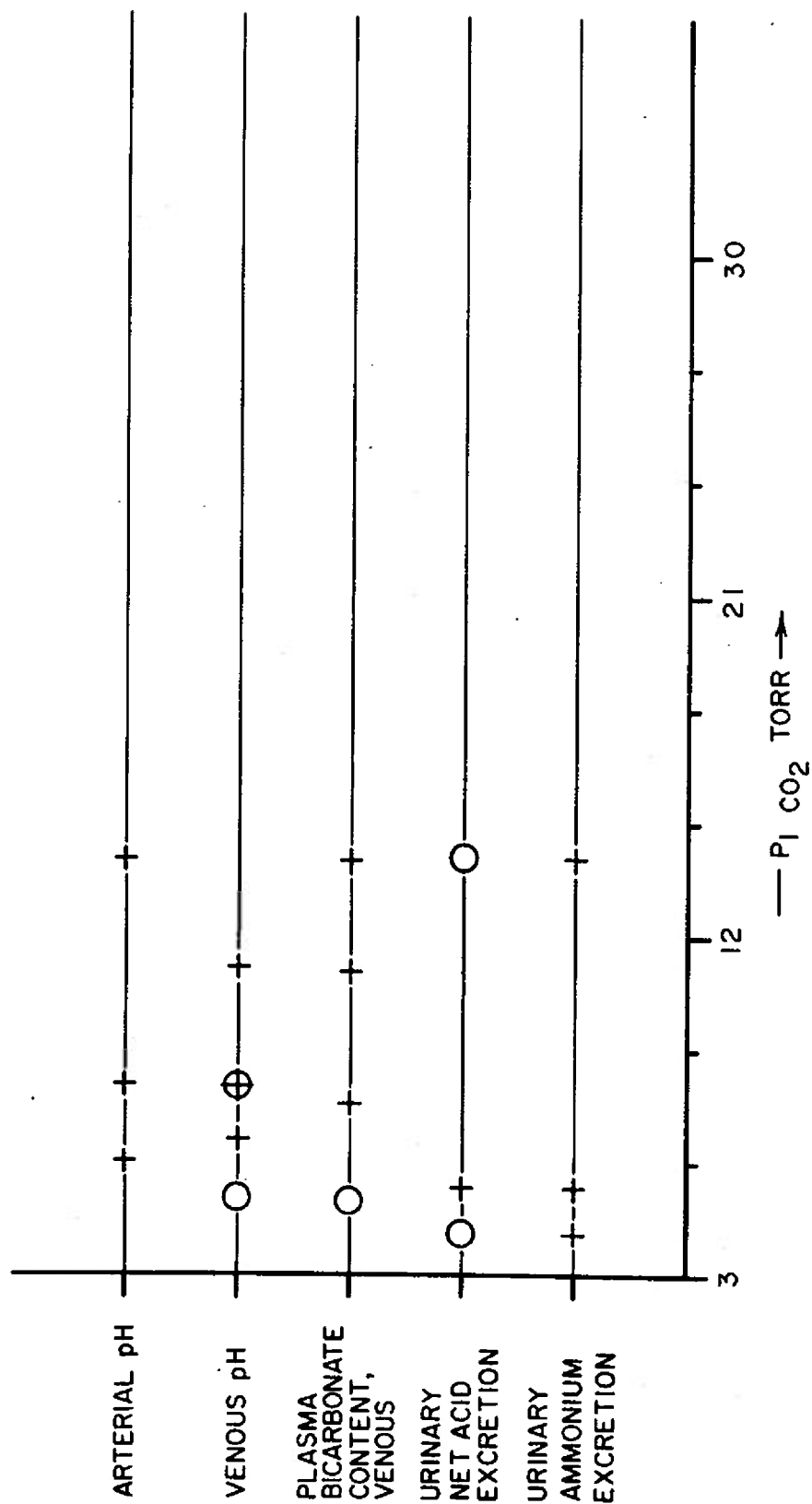


Fig. 3 c

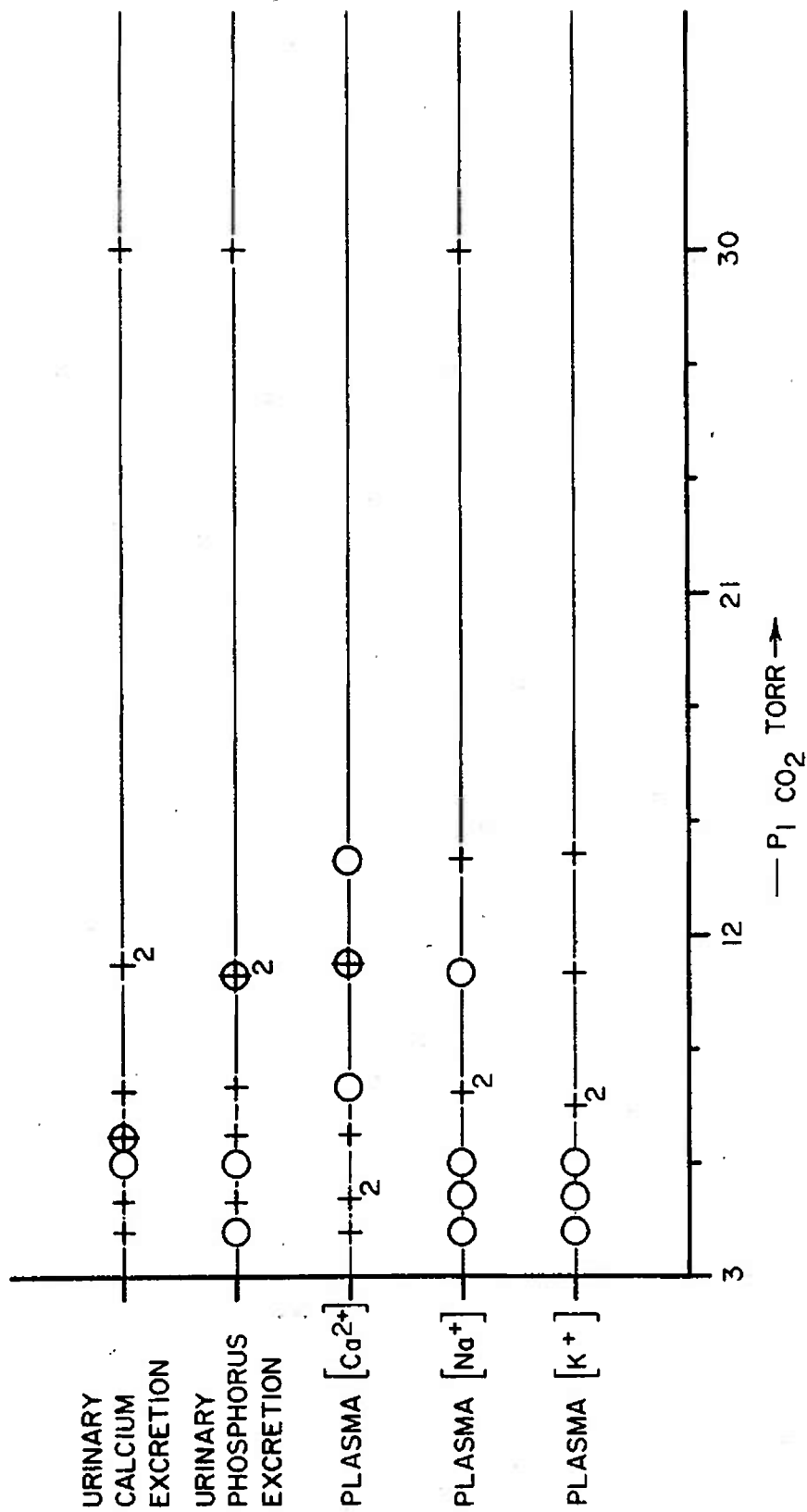


Fig. 4 a

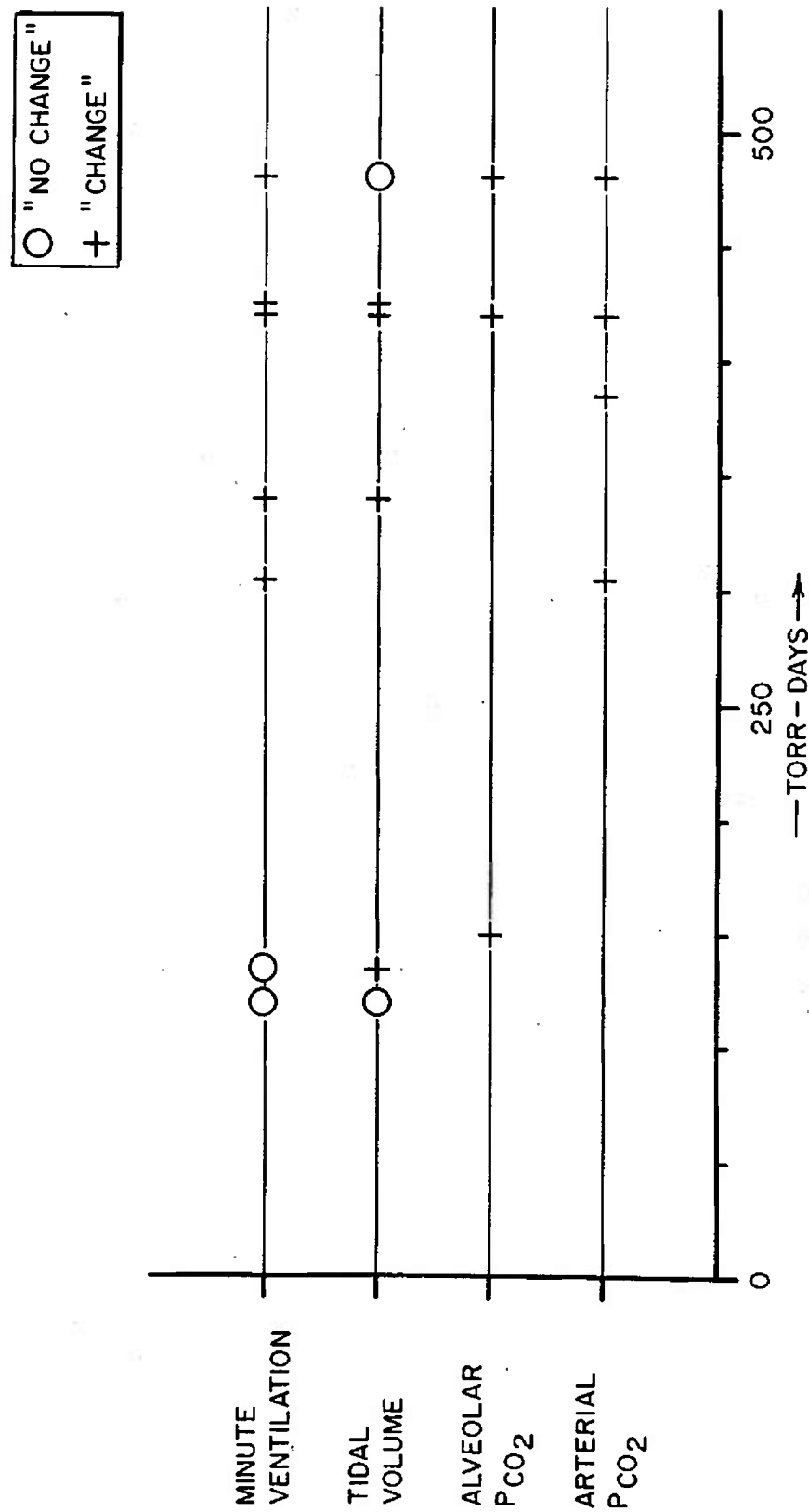


Fig. 4 b

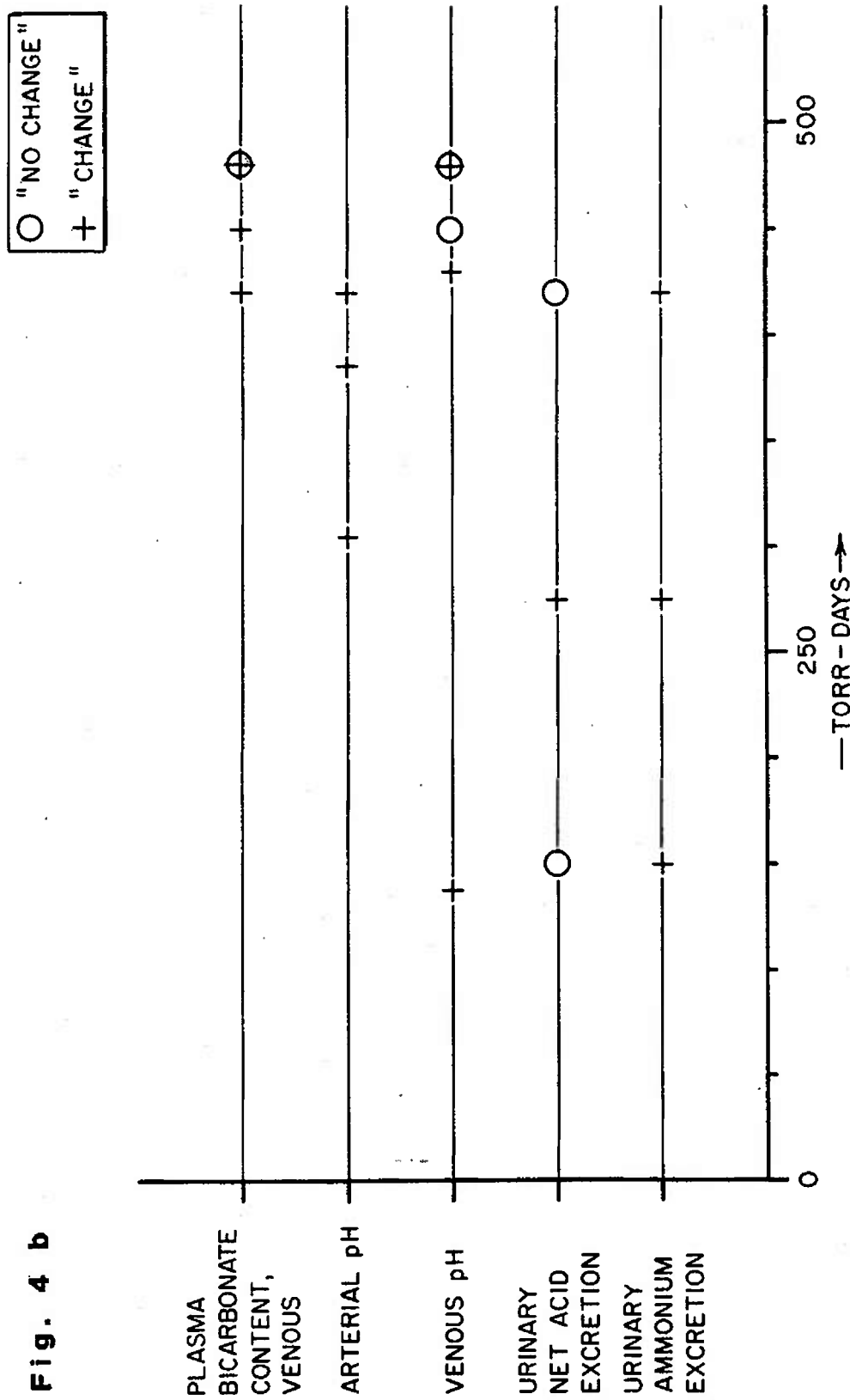
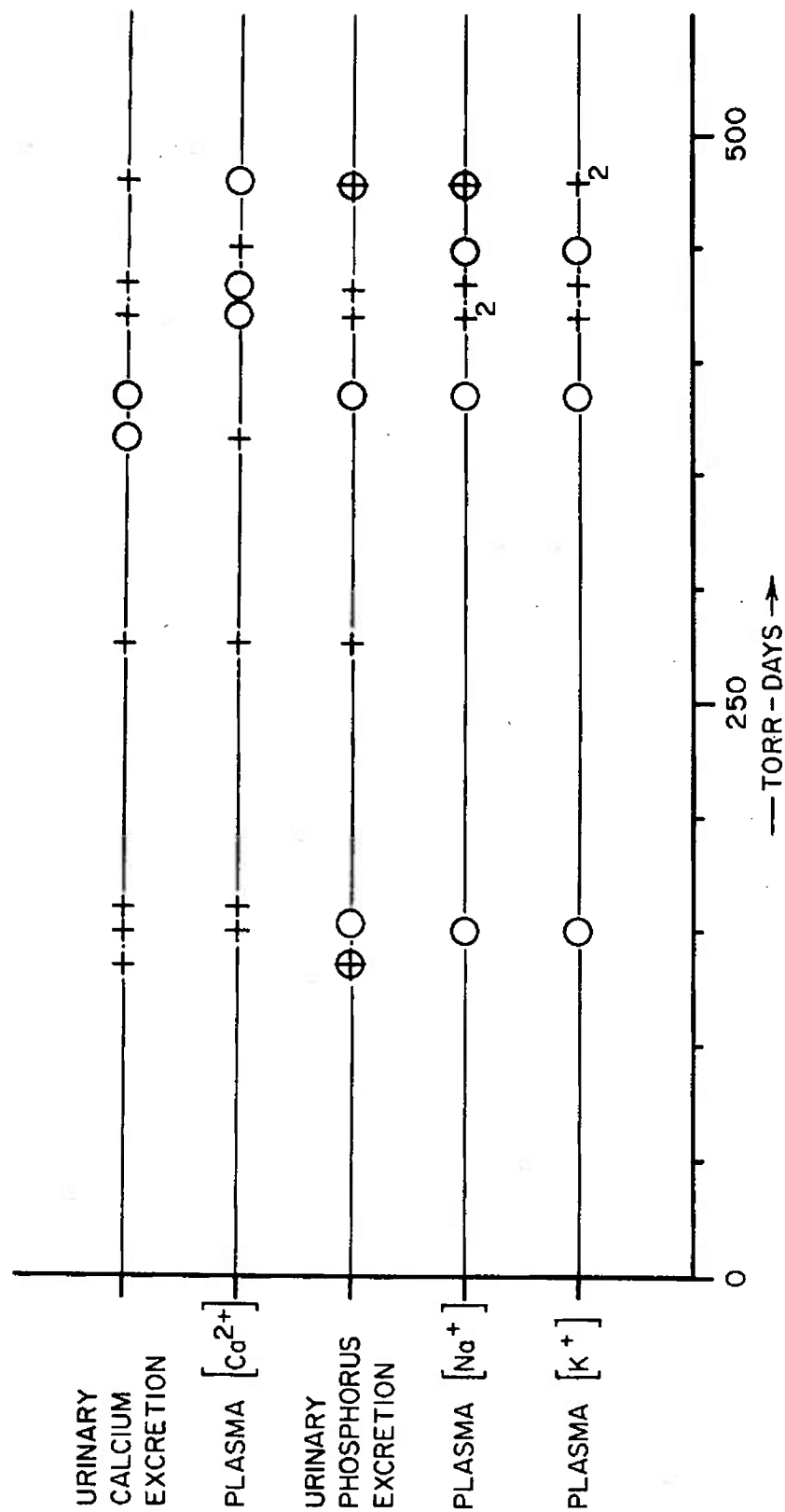


Fig. 4 c



inorganic ions to graded doses of CO_2 was the preponderant lack of a clear transition from "no change" to "change" (figures 3c,4c).

Thus, the threshold dosage of CO_2 below which many physiological functions do not "change" appears at $\text{PICO}_2 < 6$ torr; and at $\text{PICO}_2 \times \text{time} < 150$ torr-days (figures 3,4). Another way to view the primary and secondary responses to doses of chronic, low-level CO_2 is to perceive a continuum of functional responses as the doses become lower. At some point, the secondary responses may occur despite an absence of the primary responses. The reason for this is uncertain at this time.

Safe 90-Day Exposures

Laboratory Studies: 3.8-5 Torr

The 90-day and 40-day human exposures to 3.8-5 torr resulted in no change of the pulmonary ventilation (41,42,56). The arterialized capillary pH fell by only 0.02 pH-units, which is generally agreed to be a small, clinically insignificant change. The alveolar and mixed venous PCO_2 's rose by 1-1.5 torr whereas the arterialized capillary PCO_2 rose 4 torr. The net-acid excretion by the urine was unchanged, but other urinary excretion rates of bicarbonate and ammonium ions rapidly rose to sustained levels during the CO_2 exposure. The subjects developed a positive sodium balance with a decline of their urinary excretion of Na^+ . Their plasma concentrations of Na^+ , water balance, magnesium balance, phosphorus balance, and potassium balance did not change. The body weights and 24-hour urinary volumes did not change. The calcium balance was transiently negative,

the plasma levels and fecal excretion of calcium were increased, and the urinary excretion rate of hydroxyproline did not rise. The plasma vitamin D levels were decreased (41,42,56).

90 Day Exposures of Primates

At least 2 studies have been conducted in which the responses of primates were measured during continuous exposures to CO_2 for 90 days. Monkeys were exposed to 3% CO_2 and 21% O_2 for 93 days. The CO_2 -exposed monkeys fared as well as controls who were similarly confined in air. The CO_2 exposed monkeys showed no significant changes of their body weight, activity, hematocrit, blood glucose, total leukocyte count, serum chloride, serum calcium, serum phosphorus, erythrocyte sedimentation rate, or serum bilirubin. Autopsied animals showed only the presence of mites in the lung. There was no evidence of adrenal impairment (5). Humans were exposed to 1% CO_2 at 0.7 ATA ($\text{PICO}_2 = 5$ torr) for 90 days. Analyses of frozen, anaerobic venous plasma samples suggested the existence of a mild respiratory acidosis during the middle and latter phases of the exposure to CO_2 . The data from the CO_2 -exposed subjects were statistically analyzed with respect to their values measured during the recovery from the exposure. Aside from a case of Streptococcal infection, the subjects did not develop any significant medical problems during the CO_2 exposure (41,42). The combined information literally suggests that humans may be able to safely tolerate living in 5 torr CO_2 for 90 days without endangerment to their health. Non-injurious phases of respiratory acidosis may be expected to occur (5,26,41,42).

NSMRL ANIMAL CO₂ EXPOSURES

Studies at this laboratory have shown that different animal species exhibit various responses to CO₂. To better understand this phenomenon, guinea pigs and rats were exposed to different carbon dioxide (CO₂) gas mixtures for periods of one hour, one day, and seven days (39). Carbon dioxide titration, absorption, and buffer curves were determined for both species in order to interpret the buffer capacity of the organism as a result of a sudden increase in respired CO₂. Compared to rats, guinea pigs were found to be less tolerant to a given CO₂ load during the initial stages of a CO₂ exposure. As early as one day of an exposure to CO₂, rat blood was better buffered than guinea pig blood. By the seventh day of the exposure both species exhibited similar responses indicating that the guinea pigs had developed a metabolic adjustment to the increased CO₂ load. An explanation for this species difference may be related to the fact that in the guinea pig's kidney the two enzymes glutaminase I and phosphoenolpyruvate carboxykinase occur at a much lesser concentration than in the rat. These enzymes are known to be a factor in the metabolic response of the kidney when the acid-base balance of the organism is altered and in the guinea pigs may result in decreased buffering of an increased amount of CO₂ in the blood. Other factors such as differences in body temperature and an alternate reabsorption of body minerals by the kidney of the guinea pig may also be involved.

Lung changes have also resulted from exposure to CO₂. Douglas, et al reported that 1.0% CO₂ exposure for 4 and 6 weeks produced ultrastructural changes in guinea pig lungs (36). Significant increases

in Type II pneumocyte cell size were observed, as well as increases in the number and size of lamellar bodies in these cells. Recovery was incomplete after 4 weeks. Schaefer, et al demonstrated an increase in kidney calcium content of guinea pigs after only 2 weeks exposure to 1.0% CO₂, persisting until 6 weeks (37). No recovery studies were done in this experiment. There was a persistently elevated plasma calcium in these animals which became significantly elevated only after 6 weeks. Experiments were then carried out at 0.5% in which guinea pigs exposed for 8 weeks showed no significant lung structure changes. There was, however, a significant increase in kidney calcification after 8 weeks, which returned to normal levels after 4 weeks recovery on air. Plasma calcium was reported to be significantly elevated at 8 weeks (38).

To determine a threshold level that elicits kidney and lung alterations, Shea, et al exposed a large number of guinea pigs under the same experimental conditions while varying only the CO₂ concentration (0.1, 0.2, 0.3, and 0.5%) and the time of exposure (3, 6, 9, and 13 weeks) (22). The results of these experiments are as follows: After 6 weeks exposure to 0.1%, 0.2%, 0.3% or 0.5% CO₂, there was no significant change in cell diameter, lamellar bodies (LB) per cell, or LB diameter (Table 3). However, after a 9-week exposure there was an increase in Type II cell diameter in guinea pig lung exposed to 0.3% CO₂ and to a greater extent those exposed to 0.5% CO₂; no changes in LB numbers per cell or LB size were observed. At 13 weeks exposure, cells in 0.2% CO₂ showed a slight increase in lamellar body number. Type II cells exposed to 0.3% and to a much greater extent, 0.5% displayed a marked increase in all

TABLE 3. Changes in Type II Pneumocytes of Guinea Pigs
Exposed to a Range of CO₂ Concentrations

	Cell Diameter (μm)	Lamellar Bodies per Cell (No.)	Lamellar Body Diameter (μm)
(1) 6 Week Controls	9.0±1.5	5.0±1.6	1.0±0.08
6 Week 0.1% CO ₂	9.0±1.4	5.1±1.4	1.2±0.07
6 Week 0.2% CO ₂	9.1±1.3	5.0±1.3	1.3±0.05
6 Week 0.3% CO ₂	9.1±1.8	5.2±1.1	1.0±0.08
6 Week 0.5% CO ₂	9.2±1.6	5.1±0.9	1.1±0.06
(2) 9 Week Controls	9.1±1.2	5.1±1.3	0.9±0.05
9 Week 0.1% CO ₂	9.1±1.1	5.1±1.4	1.1±0.06
9 Week 0.2% CO ₂	9.3±1.08	5.2±1.3	1.2±0.07
9 Week 0.3% CO ₂	9.8±0.93	5.2±1.2	1.1±0.05
9 Week 0.5% CO ₂	11.2±1.2	5.1±1.1	1.1±0.08
(3) 13 Week Controls	9.2±1.4	5.3±1.0	1.3±0.06
13 Week 0.1% CO ₂	9.0±1.3	5.4±1.5	1.4±0.01
13 Week 0.2% CO ₂	9.3±1.9	5.6±1.1	1.5±0.03
13 Week 0.3% CO ₂	10.5±1.1	7.5±1.2	2.1±0.06
13 Week 0.5% CO ₂	15.3±1.9*	9.8±1.9*	3.1±0.07*
(4) 13 Week Controls, Plus 4 Week Recovery	9.3±1.2	5.2±1.2	1.2±0.08
13 Week 0.1% CO ₂ , Plus 4 Week Recovery	9.2±1.1	5.1±0.08	1.2±0.07
13 Week 0.2% CO ₂ , Plus 4 Week Recovery	9.4±1.2	5.2±1.0	1.1±0.03
13 Week 0.3% CO ₂ , Plus 4 Week Recovery	9.2±1.4	5.1±1.1	1.3±0.03
13 Week 0.5% CO ₂ , Plus 4 Week Recovery	10.5±1.4	5.1±1.3	1.5±0.05
(5) 13 Week Controls, Plus 8 Week Recovery	9.2±1.6	5.1±1.2	1.1±0.05
13 Week 0.1% CO ₂ , Plus 8 Week Recovery	9.6±1.2	5.2±1.3	1.2±0.01
13 Week 0.2% CO ₂ , Plus 8 Week Recovery	9.7±1.1	5.4±1.0	1.4±0.02

TABLE 3. Changes in Type II Pneumocytes of Guinea Pigs
Exposed to a Range of CO₂ Concentrations, cont.

	Cell Diameter (μm)	Lamellar Bodies per Cell (No.)	Lamellar Body Diameter (μm)
13 Week 0.3% CO ₂ , Plus 8 Week Recovery	9.3±1.3	5.1±0.08	1.5±0.03
13 Week 0.5% CO ₂ , Plus 8 Week Recovery	9.5±1.0	5.0±0.09	1.1±0.02

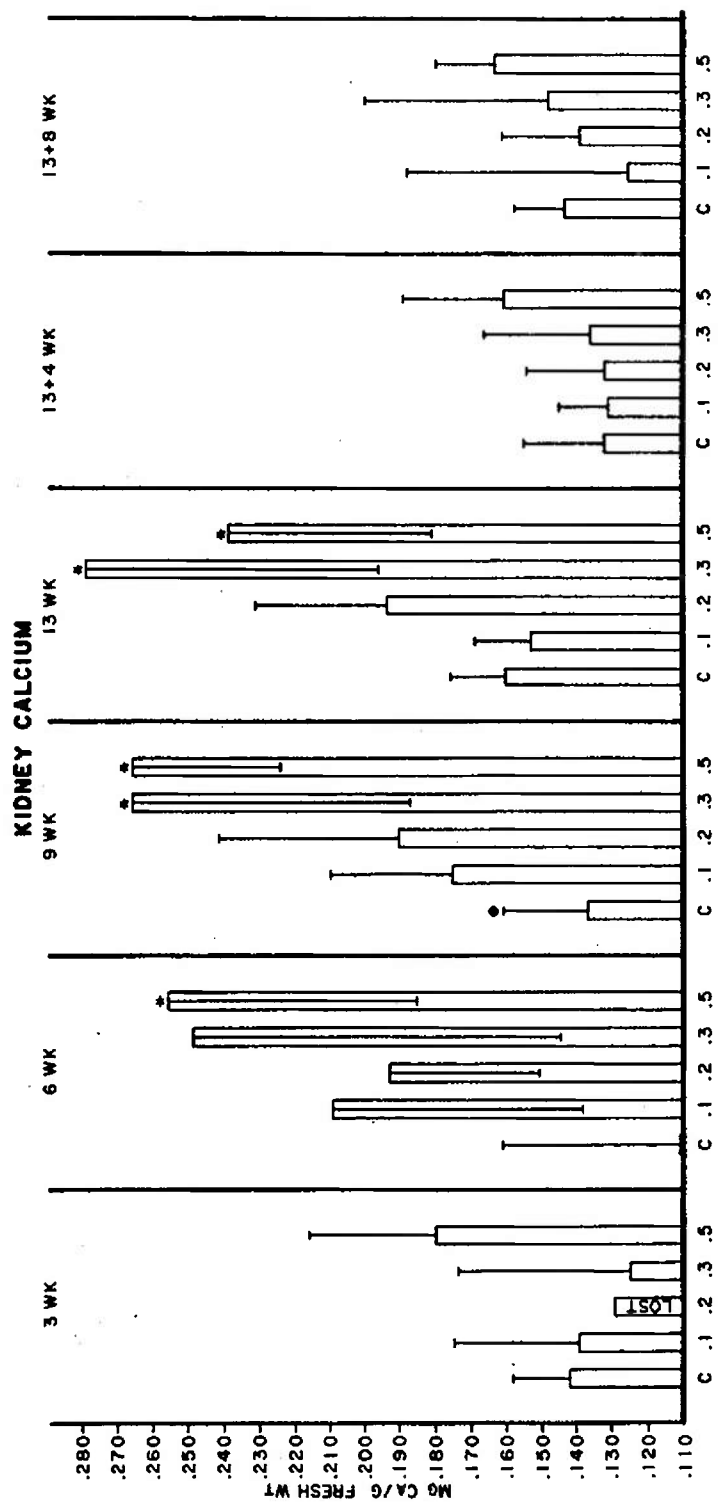
The mean cell diameters of Type II alveolar epithelial cells are measured in micrometers (μm). The mean number of lamellar bodies per Type II alveolar epithelial cell was also calculated. The mean lamellar body diameters are measured in micrometers (μm).

* Statistically Significant

three parameters relative to control values. After exposure for 13 weeks to CO₂ followed by a 4-week recovery period in room air the Type II cells obtained from lungs of animals exposed to 0.1, 0.2, and 0.3% CO₂ remained at, or returned to control values. Type II cells exposed to 0.5% CO₂ for 13 weeks allowed to recover for 4 weeks still retained an increased cellular diameter compared to control values; the number of LB per cell and size of LB, however, did return to control values. These results suggest that the Type II cell is sensitive to CO₂ concentration and that there is a hypertrophic response of the cell cytoplasm after 9 weeks exposure to 0.5% CO₂. Following 13 weeks exposure to 0.3 and 0.5% CO₂ the numbers of LB per cell and LB diameter also increased, suggesting that the synthesis of pulmonary surfactant is stimulated after prolonged exposure to 0.3 - 0.5% CO₂. This change in the Type II cell is not permanent. If the animal is allowed to recover in room air for 4 weeks, LB values

return to control values and the hypertrophic response of the Type II cell cytoplasm also returns toward control values from a significantly elevated state. After 8 weeks of recovery following 13 weeks of exposure to 0.5% CO₂, all lung parameters measured returned to control values. In summary, the Type II cell of the guinea pig responds to increased CO₂ levels first by cellular hypertrophy and later by increased synthesis of pulmonary surfactant (evidenced by increased numbers and size of LB). These parameters return toward control values after recovery in room air (22).

In the same experiment by Shea, et al a significant increase in kidney calcium was obtained for 6, 9, and 13 weeks of 0.5% CO₂, and for 9 and 13 weeks at 0.3% (Figure 5). As in the lung tissue, kidney calcium values return back to normal when the animals are allowed to recover on air after a 13-week exposure.



* Statistically Significant
 Control Data For 9 Week
 Exposure Were Lost.
 Value Depicted is an Average
 of All Other Control Data.

Figure 5

In addition to the above parameters, blood gases, ionized plasma calcium, total plasma calcium, bone, heart, muscle, and lung calcium and tissue water content were analyzed. These parameters, as well as intracellular pH, show no significant changes from control for any exposure. It is surprising that the plasma calcium did not show positive results since it did in previously published 0.5% CO₂ experiments (22).

Thus in a sensitive animal model, there appears to be a definite physiologically indicated threshold to CO₂ exposure and most importantly, alterations in structure seem to be fully reversible upon return to ambient air.

CHRONIC LOW-LEVEL CO₂ TOXICITY?

The U. S. Navy continues to search for more feasible methods of further reducing the levels of CO₂ aboard its nuclear submarines. Such an expensive practice stems from a concern for toxic effects from prolonged exposures to low levels of CO₂. These toxic effects, if they exist, have not been defined! Reasoning suggests that CO₂ could become toxic when the body can no longer maintain tolerable levels of CO₂ content. One of the following effects theoretically represents a toxic response of humans to chronic, low-level CO₂:

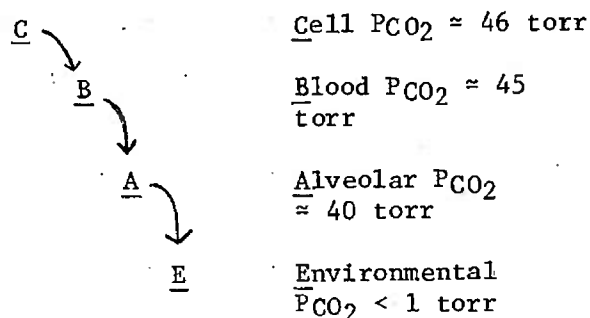
- a. Cellular injury.
- b. A lowering of the human's defenses against stress.
- c. A reduction of human performance.
- d. A change of human behavior.
- e. A shortening of life expectancy
- f. Oncogenesis or genetic mutations.

However, a universal finding of all the articles and data reviewed in this manuscript was the lack of documented toxic effects from the exposures (41-65). The lack of incriminating evidence would permit a reasonable presumption that chronic, low-level CO₂ exposures are non-toxic!

The body's responses to inhalation of low and moderate concentrations of CO₂ may be physiological and reversible rather than toxic and damaging. This view was shared by Lambertsen, who suggested that lifetime exposures to $\leq 1\%$ CO₂ could be tolerated without detectable limitations (26). The extent to which the atmospheric conditions aboard submarines affect the health of the crewmembers appears to be minimal (25,29). According to a recent review of the health effects of the nuclear submarine atmosphere, it was found that the levels of CO₂ in Naval submarines dropped below 1% after 1967. Headaches, eye-ear-nose-throat symptoms, injuries, and infections could not be attributed to atmospheric causes in either the " $\geq 1\%$ FICO₂" or the " $< 1\%$ FICO₂" submarine environments (25).

The long-term effect of the submarine atmosphere on submariners' health is currently being investigated by NSMRL and the Department of Epidemiology at Yale University. Morbidity and mortality data from active duty and retired submariners will allow the most critical assessment to date of the health effects of exposure to Naval nuclear submarines (27). The results of the study may allow an estimation of the effect of the total environment aboard submarines on the life expectancy and stress tolerance of submariners.

Perhaps the proper measurements of chronic, low-level CO₂ toxicity were not measured in the studies reviewed. It is not too difficult to postulate a mechanism for the development of chronic, low-level CO₂ toxicity. Consider, briefly, the analogy of hyperthermia. Sudden exposures of humans to heat stress cause remarkable physiological adjustments to occur. These adjustments may prevent the rise of body temperature, and therefore the net gain of body heat. On the other hand, the condition of hyperthermia is always accompanied by a gain of the body's heat content and a rise of the core temperature. From this analogy, it is suggested that the sine qua non for any form of CO₂ toxicity is the forced expansion of the tissue content of CO₂. The tissue CO₂ content is controlled by a cascade of PCO₂'s which drive the transport of CO₂ from the cells to the environment;



The cellular CO₂ diffuses quite rapidly into the blood. The net transport of CO₂ out of the tissue is limited by the tissue blood flow; therefore, the cellular PCO₂ would rise if the customary tissue blood flow were to be reduced. After entering the venous blood, the CO₂ from the mixed-venous blood diffuses rapidly into the alveoli (8,9). An important implication of the PCO₂ cascade is that elevation of the environmental load of CO₂ results in elevation of the blood and cellular PCO₂ (21).

The experimental determination of those chronic exposures to low-level CO₂ which are toxic to humans rests on a basic assumption that there are safe tolerance limits for the net gains or losses from the body's CO₂ stores. Safe limits for acute changes of the body content of CO₂ have been suggested. Tetany may develop upon the loss of 3.5 liters of CO₂ per m² body surface area. Narcosis can develop in a 70-kilogram man when he gains 3 liters of CO₂ at a PCO₂ of 70 torr. The range of soft-tissue stores of CO₂ permitting accustomed performance in men was estimated to be 14 to 23 liters (11,12). Safe limits for chronic changes of the body content of CO₂ have not been suggested. The tissue stores of CO₂ have apparently not been studied in men exposed to chronic, low level CO₂. The largest soft tissue storage site is muscle, while bone represents an even larger repository for CO₂ (8,11,12). Animal studies have shown that bone is capable of accumulating larger quantities of CO₂ with multi-week exposures to 0.3 to 1.0% CO₂. Of particular interest was the increased content of calcium within the kidney and decreased content of calcium in the bone (22, 28). Animals are not humans, however, so that it remains to show whether or not the hard or soft tissues of humans store CO₂ during residence in such comparably low levels of CO₂. Recent evidence suggested that submarine patrols do not alter the calcium content of bones in submariners (23). If submariners experience increases of their bone CO₂ stores they may do so without mobilizing calcium from their bone.

The difficulties with documenting chronic, low-level CO₂ toxicity in the laboratory are the tremendous costs of performing such studies and the insensitivity of the laboratory

measurements. There is a certain amount of "noise" inherent within the various biophysical measurements of CO₂ toxicity. For example, there is an error in accuracy of 1-2% with usage of the PCO₂ electrode (24), so that small increments of the PICO₂ may not cause measurable changes of the arterial PCO₂. Sampling error can also distort the PCO₂ measurement in blood samples. Many physiological and environmental factors can alter the PCO₂ and pH of the blood and tissues. The level of muscular activity, diet, and ambient temperature are renowned modifiers of the actual CO₂ levels within a host. These are, in turn, influenced by prolonged confinement in crowded living conditions. The combined effects of confinement and atmosphere contaminants other than CO₂ (25) make it imperative to conduct well-controlled experiments permitting discrimination of CO₂ effects from all other effects.

POSITION OF NSMRL REGARDING EXPOSURE TO LOW-LEVEL CO₂

NSMRL has been actively involved with studying the responses of humans and animals to chronic, low-level, CO₂ exposures since the advent of the nuclear-powered submarine. Numerous patrol studies have provided a sizable data base of levels of CO₂ exposure and health problems encountered by Naval nuclear submariners (25,29). Humans have been exposed to a variety of concentrations of CO₂ for chronic periods of time aboard submarines, as well as in laboratory environments. Mean data, PICO₂ = 10 torr and 40 days, collectively represent this wide variety of CO₂ exposures. Physiological responses to the CO₂ environment were repeatedly documented, but toxic effects were not apparent. Human exposures have been safely conducted

in atmospheres containing up to 5 torr CO₂, for up to 90 days. Such exposures are therefore considered safe at this time.

The animal studies showed that chronic, low level, CO₂ exposures caused changes of the bone content of CO₂, the bone content of calcium, the soft tissue content of calcium, and histological changes of the lung (22,28). These changes were found to be completely reversible upon discontinuance of the animal's exposure to CO₂ (22,31). The extrapolation of the animal data to humans is a current problem in the advancement of our understanding of the effects of CO₂ on humans.

There are considerable technical and financial impediments to conducting fruitful investigations of the effects of chronic exposures to low-levels of CO₂. The accurate maintenance of the required concentrations of CO₂ in the investigated environment is a costly endeavor. Likewise, the proper maintenance of a control environment (which would be in every way identical to the CO₂-environment except for the addition of CO₂) is costly. Many of the measurements of the organism's responses to the CO₂ are difficult and insensitive and therefore currently limit low-level CO₂ research efforts. There are also significant factors which can modify an organism's responses to low-level CO₂. Failure to control these factors may result in false interpretations of the data from the experiments.

Future, unforeseen developments may compel the U. S. Navy to continue its medical research into the health effects of CO₂. High-level CO₂ research would not be fraught

with as many technical limitations to the gathering of meaningful information as would research in low-level, CO₂ exposures. Given the appropriate facilities, manpower, and funding for research, the following categories represent potentially useful endeavors with regard to learning more about chronic, low-level CO₂:

- a) Epidemiological studies of submariners should probe the possible toxic effects of CO₂;
- b) Field studies of naval submariners should examine the effects of 90-day exposures (or longer) to CO₂. Attempts could be made to document the existence of increased stores of CO₂ in the body immediately following submarine patrols;
- c) More sensitive tests of biochemical, bio-physical, and behavioral functions should be performed on man, and appropriate animals, during studies of the effects of residence in CO₂, and
- d) Continued emphasis should be placed on the proper "controls" for CO₂ experiments. The goal is to discriminate the effects of CO₂ from all other factors which can modify the organism's CO₂ stores and general physiological function.

ADDENDA

Leitch and Smith wrote an excellent review of the literature on chronic human exposures to CO₂. Of particular relevance was their excellent discussion of the primary effects of CO₂ as related to sub-

mariners (33). The Undersea Medical Society published proceedings from a workshop on CO₂ contamination of the atmosphere (34). The emphasis of the conference was on the physiological and health effects of CO₂ in submarines. The information which was presented reinforced the position of this paper.

Other reports of human exposures to CO₂ were reviewed after the preparation of this manuscript (67-73). Of particular interest was the reported deterioration of health and work capacity at the beginning of the third month of human exposure to 0.8 to 1.8% CO₂. The report was in Russian, so that details await translation of the manuscript (67). The remaining references (68-73) confirmed the safety of low-level CO₂ exposures aboard Naval nuclear submarines.

REFERENCES

1. Carhart, H. W. and J. E. Johnson (1980). A history of the Naval Research Laboratory Contributions to Submarine Life Support Systems. Presented at the Intersociety Environmental Systems Conference, American Society of Mechanical Engineers, San Diego.
2. Shea, M. L., A. A. Messier, and K. R. Bondi (1980). Carbon dioxide effects on submariners. Presented at the Intersociety Environmental Systems Conference, American Society of Mechanical Engineers, San Diego.
3. U. S. Navy's standards for carbon dioxide as of 15 May 1981 (unclassified).
4. Schaefer, K. E. (1961). A concept of triple tolerance limits based on chronic carbon dioxide toxicity studies. *Aerospace Medicine* 32:197-204.

5. Stein, S. N., H. E. Lee, J. H. Annegers, et al. 1959. The effects of prolonged inhalation of hypernormal amounts of carbon dioxide. I. Physiological effects of 3 percent CO₂ for 93 days upon monkeys. Research Report NM 240100.01.01. Naval Medical Research Institute, Bethesda.
6. Margulies, R. A. 1980. MPC 90 days for carbon dioxide - U. S. Navy. p 85 in E. J. Jolly and J. R. Harrison, eds. Proceedings of a Submarine Medicine Conference, Alverstone.
7. Jolly, E. J. and J. R. Harrison (1980). Proceedings of a Submarine Medicine Conference 20-22 May 1980. Institute of Naval Medicine, Alverstone (U.K.)
8. Farhi, L. E. and H. Rahn (1960). Dynamics of changes in carbon dioxide stores. Anesthesiology 21:604-614.
9. Guyton, A. C. 1966. Textbook of Medical Physiology, 3rd ed. W. B. Saunders Company, Philadelphia.
10. Thomas, S. C. and C. W. Shilling (1980). Carbon Dioxide Effect on Mammalian Tissue; An Annotated Bibliography. Undersea Medical Society, Inc., Bethesda.
11. Farhi, L. E. 1964. Gas stores of the body. pp 873-879 in W. O. Fenn and H. Rahn, eds. Handbook of Physiology. Section 3: Respiration. American Physiological Society, Washington.
12. Rahn, H. 1962. p 297 in Man's Dependence on the Earthly Atmosphere ed by K.E. Schaefer, The MacMillan Company, New York.
13. Cherniack, R. M. (1964) Work of breathing and the ventilatory response to CO₂. pp 1459-1474 in Handbook of Physiology. Section 3: Respiration Volume II ed by W. O. Fenn and H. Rahn, American Physiological Society, Washington.
14. Woodbury, D. M. and R. Karler. 1960. The role of carbon dioxide in the nervous system. Anesthesiology 21: 686.
15. Kennedy, T. J. 1960. The effect of carbon dioxide on the kidney. Anesthesiology 21: 704-716.
16. Kassirer, J. P. 1964. Serious Acid-Base Disorders. New England Journal of Medicine 291: 773-776.
17. Price, H. L. 1960. Effects of carbon dioxide on the cardiovascular system. Anesthesiology 21: 652.
18. Bellville, J. W. and J. C. Seed. 1960. The effect of drugs on the respiratory response to carbon dioxide. Anesthesiology 21: 727.
19. Comroe, J. H., Jr. 1964. The peripheral chemoreceptors. p 557 in W. O. Fenn and H. Rahn, eds. Handbook of Physiology Section 3: Respiration. American Physiological Society, Washington.
20. Kellogg, R. H. 1964. Central chemical regulation of respiration. p 513 in W. O. Fenn and H. Rahn, eds. Handbook of Physiology Section 3 Respiration. American Physiological Society, Washington.

21. Nichols, G. Jr. 1958. Serial changes in tissue carbon dioxide content during acute respiratory acidosis. *Journal Clinical Investigation* 37: 1111.
22. Shea, M. L., et al. 1981. Effect of prolonged exposure to low level CO₂ concentrations of kidney calcium and lung ultrastructure. *Federation Proceedings* 40(3): 675. abstr # 2548.
23. Messier, A. A., et al. 1981. Assessment of bone and body composition in recently retired and active-duty submariners. *Federation Proceedings* 40(3): 920. abstr # 3914.
24. Winters, R. W., K. Engel, and R. B. Dell. 1967. Acid Base Physiology in Medicine. The London Company, Cleveland.
25. Weybrew, B. B., C. R. Bryan, and E. M. Noddin. 1978. Time Course of Morbidity Patterns During 564 SSBN Patrols. Naval Submarine Medical Research Laboratory Report No. 842, Groton.
26. Lambertsen, C. J. 1971. Carbon Dioxide Tolerance and Toxicity. Institute for Environmental Medicine, Philadelphia.
27. Notice of Research Project. U.S. Dept. of Defense DN777382. Hunter, W. L. and A. M. Ostfeld. Health and Longevity in Relation to Length of Occupational Exposure to the Naval Submarine Environment.
28. Schaefer, K.E., et al. 1980. Phasic changes in bone CO₂ fractions, calcium, and phosphorus during chronic hypercapnia. *Journal Applied Physiology: Respiratory, Environmental and Exercise Physiology* 48(5): 802-811.
29. Tansey, W.A., J.M. Wilson, and K.E. Schaefer (1979). Analysis of health data from 10 years of Polaris submarine patrols. *Undersea Biomedical Research Submarine Supplement* 6: S217-S246.
30. Schaefer, K.E. (1978). Comments in Submarine Medicine Conference. Naval Submarine Medical Research Laboratory, Groton, CT.
31. Bondi, K.R. (1981). Presentation of findings at Tri-Partite Conference on Submarine Medicine, June 24-26, CERTSM, Toulon.
32. Rahn, H. and W.O. Fenn. 1955. A Graphical Analysis of the Respiratory Gas Exchange. The O₂-CO₂ Diagram. The American Physiological Society, Washington.
33. Leitch, D.R. and D.J. Smith. 1978. A Review of the Literature on Investigations into Man's Respiratory and Acid-Base Response to Exogenous Chronic Hypercapnia. Report No. 32/78, Institute of Naval Medicine, Alverstoke.
34. In Press, 1981.
35. 1981. Tripartite Conference on Submarine Medicine: France, Great Britain, and the United States. CERTSM, 24-26 June, Toulon.
36. Douglas, W.H.J., K.E. Schaefer, A.A. Messier, and S.M. Pasquale. 1979. Proliferation of pneumocyte II cells in prolonged exposure to 1% CO₂. Undersea

Biomedical Research Submarine
Supplement: S135-S142.

Research Laboratory Report No.
692, Groton.

37. Schaefer, K.E., S.M. Pasquale, A. A. Messier, and H. Niemoller. 1979. CO₂-induced kidney calcification. Undersea Biomedical Research Submarine Supplement: S143-S153.
38. Schaefer, K.E., W.H.J. Douglas, A. A. Messier, M. L. Shea, and P. A. Gohman. 1979. Effect of prolonged exposure to 0.5% CO₂ on kidney calcification and ultrastructure of lungs. Undersea Biomedical Research Submarine Supplement: S155-S166.
39. Messier, A. A. and K.E. Schaefer. Species differences in blood buffer indices of guinea pigs and rats exposed to acute and chronic hypercapnia. Unpublished.
40. Kellogg, R.H. 1960. Acclimatization to carbon dioxide. *Anesthesiology*, 21:634-641.
41. Messier, A.A., E. Heyder, and K.E. Schaefer (1971). Effect of 90-day exposure to 1% CO₂ on acid-base status of blood. U.S. Naval Submarine Medical Research Laboratory Report Number 655. Groton.
42. Jackson, J. K. et al. 1972. Program Operational Summary: Operational 90-day Manned Test of a Regenerative Life Support. McDonnell-Douglas Astronautics Co., NASA CR-1835, Washington.
43. Gortner, D.A., A.A. Messier, E. Heyder, and K.E. Schaefer. (1971) The effects of elevated atmospheric CO₂ acid-base balance and red-cell electrolytes of FBM crew members. Naval Submarine Medical Research Laboratory Report No. 692, Groton.
44. Gionnetta, C. L. and H. B. Castleberry. 1974. Influence of bedrest and hypercapnia upon urinary mineral excretion in man. *Aerospace Medicine* 45: 750-754.
45. Faucett, R. E. and P. P. Newman. 1953. Operation Hideout. Preliminary Report. U.S. Naval Medical Research Laboratory Report No. 228, Groton.
46. Schaefer, K. E. 1963. Acclimatization to low concentration of carbon dioxide. *Industrial Medicine and Surgery* 32:11-13.
47. Schaefer, K. E. 1963. Respiratory adaptation to chronic hypercapnia. *Annals of the New York Academy of Sciences*. 109: 772-780.
48. Schaefer, K. E., et al. 1963. Respiratory acclimatization to carbon dioxide. *Journal of Applied Physiology* 18:1071-1078.
49. Schaefer, K. E., et al. 1963. Calcium phosphorus metabolism in man during acclimatization to carbon dioxide. *Journal of Applied Physiology* 18:1079-1084.
50. Schaefer, K. E., et al. 1964. Acid-base balance and blood and urine electrolytes of man during acclimatization to CO₂. *Journal of Applied Physiology* 19:48-58.
51. Schaefer, K. E. 1951. Studies of Carbon Dioxide Toxicity (1) Chronic CO₂-Toxicity in Submarine Medicine. Naval Submarine Medical Research Laboratory Report No. 181, Groton.

52. Gude, J. K. and K. E. Schaefer. 1969. The effect of respiratory dead space of prolonged exposure to a submarine environment. U.S. Naval Submarine Medical Research Laboratory Report No. 587, Groton.
53. Schaefer, K. E. et al. 1970. The effect of intermittent exposure to 3% CO₂ on acid-base balance and electrolyte excretion. U.S. Naval Submarine Medical Center, Report No. 635, Groton.
54. Davies, D. M., J. E. W. Morris, et al. 1978. Mineral metabolism and circadian patterns of renal excretion in man in a closed environment study involving hypercapnia and abnormal physical activity. Institute of Naval Medicine Report No. 25/78, Alverstoke.
55. Gray, S.P., J.E.W. Morris, and C.J. Brooks. 1973. Renal handling of calcium, magnesium, inorganic phosphate and hydrogen ions during prolonged exposure to elevated carbon dioxide concentrations. *Clinical Science and Molecular Medicine* 45:751-764.
56. Davies, D. M. 1976. Calcium Metabolism, Chronic Hypercapnia, and Closed Environments. MD Thesis, University of Wales.
57. Peck, A. S. 1971. The time course of acid-base balance while on FBM submarine patrol. U. S. Naval Submarine Medical Research Laboratory Report No. 675, Groton.
58. Covington data, cited in Schaefer, K. E. 1979. Physiological stresses related to hypercapnia during patrols on submarines. Undersea Biomedical Research, Submarine Supplement 1979: S20-S22.
59. Messier, A. A., et al. 1979. Calcium, magnesium, and phosphorus metabolism, and parathyroid - calcitonin function during prolonged exposure to elevated CO₂ concentrations on submarines. Undersea Biomedical Research, Submarine Supplement 1979: S57-S70.
60. Wilson, A. J. and K. E. Schaefer, 1979. Effect of prolonged exposure to elevated carbon monoxide and carbon dioxide levels on red blood cell parameters during submarine patrols. Undersea Biomedical Research, Submarine Supplement 1979: S49-S56.
61. Guillerm, R. and E. Radziszewski. 1979. Effects on man of 30-day exposure to P_iCO₂ of 14 torr (2%): application to exposure limits. Undersea Biomedical Research, Submarine Supplement 1979: S91-S114.
62. Pingree, B. J. W. 1977. Acid-base and respiratory changes after prolonged exposure to 1% carbon dioxide. *Clinical Science and Molecular Medicine* 52:67-74.
63. Hughes, M. F. 1966. Salivary CO₂ and electrolytes secretion during exposure to an elevated CO₂ atmosphere. Submarine Medical Qualification Thesis, U. S. Naval Submarine Medical Center, Groton.
64. Foster, E. D. 1968. Effects on Gastric Secretion by Hypercarbia. Submarine

- Medical Qualification Thesis.
U. S. Naval Submarine Medical
Center, Groton.
65. Preece, M. A., et al. 1975.
Studies of vitamin D deficiency
in man. Quarterly Journal of
Medicine 44: 575-89.
 66. Zink, P. and G. Reinhardt. 1974.
Die protrahierte CO₂ - Vergiftung.
Beitraege Gerichtlichen Medizin
33: 211-213.
 67. Apanasenko, G. L., V. S. Schego-
lev and V. I. Kuleshov. 1978.
Tolerance to controlled exercises
of man exposed to chronic hyper-
capnia. Kosmicheskaya Biologiya
Aviakosmicheskaya Meditsina 12
(2): 49-52.
 68. Edmonstone, W. M. 1978. The
effect of hypercapnia on venti-
lation in nuclear submariners.
Journal Royal Naval Medical
Service 64(2):125-129.
 69. Gale, R., Redner-Carmi, R. and
Gale J. 1977. Accumulation of
carbon dioxide in oxygen hoods,
infant cots, and incubators.
Pediatrics 60:453-6.
 70. Gray, S.P., R. J. W. Lambert and
J. E. Morris. 1969. Calcium,
magnesium and phosphate meta-
bolism during prolonged exposure
to carbon dioxide. Journal Royal
Naval Medical Service 55:238-243.
 71. Leitch, D. R. 1977. Aspects of
chronic hypercapnia. South-
ampton, United Kingdom, Univ.
Southampton, Thesis, 377 p
(abstract).
 72. Radziszewski, E., R. Guillermin,
R. Badre and C. Abran. 1976.
Kinetics of the compensation of
respiratory acidosis induced by
experimental chronic hypercapnia
in man. Bull Eur Physiopathol
Resp 12:87-100.
 73. Schaefer, K. E. 1974. Physio-
logical stresses during patrols
of polaris submarines. In:
Abstracts of papers presented
at Undersea Medical Society
Annual Scientific Meeting, May
10-11, Washington, DC.
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Numerous patrol studies have provided a sizable data base of levels of CO₂ exposure and health problems encountered by Naval nuclear submariners. Humans have been exposed to a variety of concentrations of CO₂ for chronic periods of time aboard submarines, as well as in laboratory environments. Mean data, PICO₂ = 10 torr and 40 days, collectively represent this wide variety of CO₂ exposures. >Physiological responses to the CO₂ environment were repeatedly documented, but toxic effects were not apparent.< Human exposures were safely		

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conducted in atmospheres containing up to 5 torr CO₂, for up to 90 days. Such exposures are therefore considered safe at this time.

Animal studies showed that chronic, low-level, CO₂ exposures caused changes of the bone content of CO₂, the bone content of calcium, the soft tissue content of calcium, and histological changes of the lung. These changes were found to be completely reversible upon discontinuance of the animal's exposure to CO₂. >The extrapolation of the animal data to humans is a current problem in the advancement of our understanding of the effects of CO₂ on humans.<

There are considerable technical and financial impediments to conducting fruitful investigations of the effects of chronic exposure to low-levels of CO₂. The accurate maintenance of the required concentrations of CO₂ in the investigated environment is a costly endeavor. Many of the measurements of the organism's responses to CO₂ are difficult and insensitive and therefore currently limit low-level CO₂ research efforts. There are other significant factors which can modify an organism's responses to low-level CO₂. Diet, levels of exercise during confinement, trace contaminants, and time are important modifiers of any organism's response to CO₂. Failure to control these factors may result in false interpretations of the data from the experiments.

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