STUDIES OF CALCIUM AND INORGANIC PHOSPHORUS LEVELS IN PLASMA AND ERYTHROCYTES DURING ACUTE AND CHRONIC HYPERCAPNIA

by

Elly Heyder

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Transmitted by:

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

Reviewed and Approved by:

Charles F. Gell

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

Reviewed and Approved by:

Joseph D Bloom.

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

Approved and Released by:

J.E. Stark

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

SUMMARY PAGE

THE PROBLEM

To clarify the role of changes in plasma and erythrocyte calcium and inorganic phosphorus levels during acute and chronic exposure to increased environmental carbon dioxide. Studies of the effects of low concentrations of CO_2, such as encountered aboard operational submarines, are pertinent.

FINDINGS

Serum ionized calcium increased linearly with decreasing serum pH in guinea pigs during acute exposure to CO_2 concentrations of 1%-15%. This inverse relationship was not maintained during chronic exposure to CO_2, and the serum ionized calcium remained elevated in spite of partial recovery of the pH.

Total plasma calcium was significantly elevated in guinea pigs exposed to 3% and 15% CO_2. This finding was interpreted as evidence for increased parathyroid function. A decrease in total plasma calcium in guinea pigs exposed for prolonged periods to 1% CO_2 suggests under-function of the parathyroids, possibly related to increased ionized calcium levels. Total plasma calcium levels of submariners exposed to approximately 1% CO_2 on FBM patrol tended to decrease, although not significantly.

Erythrocyte calcium increased in guinea pigs exposed to 3% and 15% CO_2, and in submariners exposed to 1% CO_2. These findings have been interpreted as indicative of metabolic inhibition of active cation transport during 15% CO_2 exposure or increased membrane permeability to elevated serum ionized calcium resulting from 3% or 1% CO_2 exposure.

APPLICATION

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 a part of Bureau of Medicine and Surgery Research Work Unit MR005.01.01-0125B9XX-Exposure to Total Submarine Atmospheric Environment on Physiological Functions. The present report is No. 2 on this work unit. The manuscript was approved for publication on 29 February 1972, and designated as Naval Submarine Medical Research Laboratory Report Number 702.

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This report constitutes a copy of the thesis of Miss Elly Heyder, submitted to and accepted by the University of Rhode Island, Department of Zoology, in partial fulfillment of the requirement for a Master of Science degree in Physiology.

All of the research work was done in the U.S. Naval Submarine Medical Research Laboratory.

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

ABSTRACT

To ascertain the effects of acute and chronic hypercapnia on blood levels of calcium and inorganic phosphorus, guinea pigs were exposed to 1%, 3% and 15% CO_2 for various periods up to seven days. Plasma and erythrocyte calcium and inorganic phosphorus and serum ionized calcium were determined. Blood from submariners on FBM patrol, exposed to up to 1% CO_2 for three weeks and followed by one week recovery, was analyzed for plasma electrolytes and erythrocyte calcium.

The serum ionized calcium of guinea pigs during acute exposure to each concentration of CO_2 shows a pH dependent inverse relationship of a 4.5-5% change per 0.1 unit pH change.

During chronic hypercapnia, elevation of total plasma calcium and depression of inorganic phosphorus in those animals exposed to 3% and 15% CO_2 would suggest increased parathyroid function. In those guinea pigs exposed to 1% CO_2, a depressed total plasma calcium in the presence of increased serum ionized calcium is interpreted as suggesting a possible functional hypoparathyroidism.

In the guinea pigs, increases in erythrocyte calcium and inorganic phosphorus and plasma inorganic phosphorus accompained by a decrease in total plasma calcium, during the acute phase of respiratory acidosis, are interpreted as pH dependent inhibition of active transport and/or increase in membrane permeability.

In the submariners exposed to up to 1% CO_2, both total plasma calcium and inorganic phosphorus tended to decrease although the changes did not become significant.

Erythrocyte calcium, however, increased gradually and became significant after three weeks' exposure. This finding suggests inhibition of active transport and/or increase in red cell permeability to calcium. After one week recovery in air, the erythrocyte calcium had returned to control levels.

STUDIES OF CALCIUM AND INORGANIC PHOSPHORUS LEVELS IN PLASMA AND ERYTHROCYTES DURING ACUTE AND CHRONIC HYPERCAPNIA

INTRODUCTION

The cell membrane has long been recognized as a barrier which maintains the integrity of ionic composition between the interior of the cell and its surrounding medium. Cation exchange between these compartments has generally been considered to occur in three ways: 1. against a concentration or electrochemical gradient by means of active transport; 2. by passive diffusion (leak) down the gradient; 3. by exchange with themselves. That is, sodium will exchange with sodium; potassium with potassium (Ussing,[52] 1960). Net movement of ions therefore can only occur via active or passive transport.

The unequal distribution of sodium and potassium between plasma and erythrocytes appears to be specifically coupled to an active transport (pump) mechanism which compensates for passive diffusion and which derives its energy from the spliting of adenosine triphosphate (ATP) (Dunham and Glynn,[9] 1961; Hoffman,[18] 1966). To effect the splitting of ATP, the membrane-bound enzyme ATPase which is activated by Na^+/K^+ is now generally accepted as the mediator of active sodium-potassium transport (Skou,[48] 1965). In addition, within the erythrocyte, there is evidence for the existence of active calcium transport (Schatzmann and Vincenzi,[46] 1969; Olsen and Cazort,[34] 1969; Lee and Shin,[23] 1969). The maintenance of low intracellular calcium depends upon a calcium-activated enzyme which uses ATP as a source of energy. Cells deprived of metabolic energy take up calcium from Ringer's solution by passive diffusion; whereas cells supplied with metabolites that produce ATP pump calcium out (Romero and Whittam,[37] 1971).

In 1962 Hoffman[17] demonstrated that movement of calcium into the red blood cell resulted in inhibition of Na^+/K^+ ATPase and therefore the sodium-potassium pump. However, addition of extracellular calcium, up to twice the normal plasma levels, affected neither active transport nor passive diffusion. Hoffman[17] (1962) thus concluded, as did Schatzmann[45] (1966), that the action of calcium on the membrane is asymmetrical. Davis and Vincenzi[8] (1971) studied ATPase activities of human red cell membranes as a function of calcium ion concentration. They concluded that both Na^+/K^+-activated ATPase and Ca++-activated ATPase were influenced by calcium ion concentration with calcium acting to inhibit the Na^+/K^+ ATPase. If calcium inhibition of Na^+/K^+ ATPase is related to calcium ion concentration at the inner surface of the red cell membrane, then it might be suggested that increases in red cell calcium, decreases in red cell potassium, and inhibition of active transport may be linked.

In other investigations relating to the metabolic effect of minerals, it has been shown that changes in intracellular cations alter the oxygen affinity of hemoglobin (Sommerkamp[49] et al, 1961;

Waldeck and Zander,[55] 1968). However, the observed linear relationship does not necessarily prove a direct causal relationship, inasmuch as both red cell cation exchange and hemoglobin oxygen affinity are affected by intracellualr levels of 2, 3, -diphosphoglycerate (2, 3-DPG), (Gardos,[10] 1967; Benesch and Benesch,[2,3] 1967, 1968; Lenfant[24] et al, 1968).

When human subjects are exposed to carbon dioxide concentrations up to 1.5% for prolonged periods of time, small increases in sodium and decreases in potassium have been observed in the red blood cells. (Schaefer[40] et al, 1964; Gortner[13] et al, 1971). During the same experimental conditions, Schaefer[39] et al (1963) have found that red blood cell calcium is increased.

It has also been observed during chronic respiratory acidosis, induced by increased environmental carbon dioxide, that increased oxygen affinity of hemoglobin is accompanied by decreases in intracellular cations and 2,3-DPG levels (Schaefer and Messier,[42] 1970; Schaefer[43] et al, 1970; Messier and Schaefer,[27] 1971). Also influencing the intraerythrocytic 2,3-DPG concentration, and hence the hemoglobin oxygen affinity, is the concentration of inorganic phosphorus in the serum (Astrup[1] et al, 1970).

Thus, it is suggested that studies of the changing calcium and inorganic phosphorus levels in plasma and erythrocytes may help to elucidate their role in cation exchange, the changes in hemoglobin oxygen affinity, or the relationships of the cations to membrane phenomena during chronic respiratory acidosis.

II. Materials

The guinea pigs used in these studies were 400 - 500 g. adult males of the Hartley strain obtained from West Jersey Biological Supply Farm, Wenanah, New Jersey. At all times the animals were maintained on Big Red Guinea Pig Diet (Agway) and tap water ad libitum. The animals were allowed to adjust to laboratory conditions for at least four days prior to CO_2 exposure.

At the time of exposure the guinea pigs were placed in a Sherer Controlled Environment Chamber, Model CEL 4-4 at a constant temperature of 78° F with alternating 12 hour light/dark periods. Abundant boric acid crystals were placed in the bottom of each cage to absorb excreted ammonia. The exposures to CO_2 were for one hour, six hours, one day, three days, and seven days using 1% CO_2 in air, 3% CO_2 in air or 15% CO_2 in 21% O_2 balance nitrogen. Following exposure, the animals were sacrificed at the same time of day (0900) to eliminate possible effects of circadian rhythms on plasma and erythrocyte calcium and inorganic phosphorus concentrations. Unexposed control animals were taken from the stock supply.

Blood samples were also obtained from human volunteers exposed to CO_2 concentrations of 0.8-1% during submarine patrol. The samples were taken prior to submergence, at seven-day intervals during submergence, and one week after return to the surface.

III. Methods

Total Calcium and Inorganic Phosphorus

At the time of the sampling, the guinea pigs were anesthetized with sodium pentobarbital and blood was withdrawn from the abdominal aorta by heparinized syringe. During the bleeding the animals breathed, through a mask, the same gas mixture to which they had been exposed. The blood was centrifuged at 2000 G. for 20 minutes, and the plasma was then separated from the packed cells.

Using heparinized Vacutainers®,* the blood from the submariners was withdrawn from the anticubital vein, centrifuged, separated, and frozen.** After completion of the patrol, the samples were returned to the laboratory for preparation and analysis as described below.

Using the Technicon# AutoAnalyzer®* Method N-82 I/II, plasma calcium and inorganic phosphorus were measured. This analysis uses modifications of the Kessler and Wolfman[22] (1964) method for the simultaneous determination of calcium and inorganic phosphorus.

Any remaining plasma and the buffy coat were removed from the packed red cells by suction. No washing of the red cells was made to avoid inducing possible electrolyte exchanges between the wash medium and the erythrocytes. A weighed sample of the packed red cells was then hemolyzed in ice cold water. Addition of concentrated perchloric acid was made to the hemolysate to effect a final concentration of 0.6 M perchloric acid. The coagulated protein was removed by centrifugation and the supernatant neutralized with K_2CO_3 crystals to approximately pH 7 using one drop of neutral red as an indicator. Great care was taken not to exceed this pH during the addition of the K_2CO_3 in order to avoid loss of calcium as carbonate and loss of both calcium and phosphorus as calcium phosphate. In preliminary experiments it was determined that the indicator color in no way interfered with the results obtained. After centrifugation to remove the precipitated potassium perchlorate, the supernatant was analyzed for calcium and inorganic phosphorus in the AutoAnalyzer®.*

A diagram of the flow system used in these analyses is presented in Figure 1. The number preceding the reagents at the right of the diagram indicates the nominal inside diameters (I.D.) of the tubing aspirating the reagents. The proportions and thus, the final concentrations, of each reagent are thereby determined.

In analysis for plasma Ca/P, the sample was aspirated through .020 I.D. Tygon tubing, and then mixed with .25 N HCl flowing through a .065 I.D. line. To determine the Ca/P in the processed red cell supernatant, the sample was aspirated through the .065 I.D. line and mixed with 1.1 N HCl flowing through the .020 I.D. tubing. Thus the sample size was increased approximately ten times that of the

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**Registered Trademark*

***The author wishes to thank Dr. William Braithwaite for obtaining the human blood samples.*

#Technicon Corporation, Tarrytown, New York

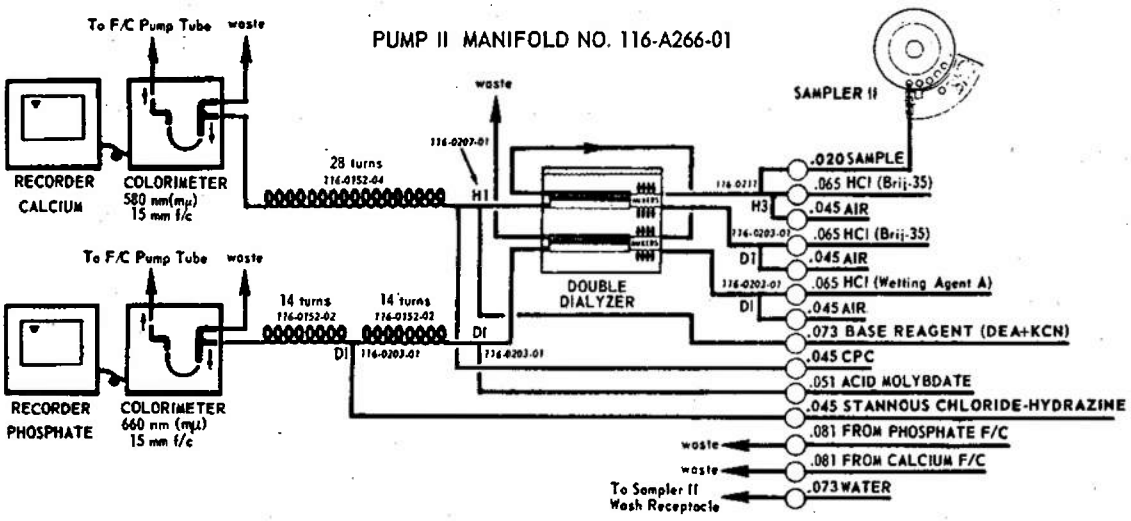

Fig. 1. Flow Diagram of Method N-82 I/II Using the Technicon AutoAnalyzer® for the Simultaneous Determination of Calcium and Inorganic Phosphorus

plasma sample. These alterations were made to obtain adequate recorder responses because of the low Ca/P concentrations in the red cell, as well as to compensate for the dilution introduced during the hemolysis of the cells. The dilution factors resulting from the hemolysis were further corrected by 5% to account for the addition of the perchloric acid. For each of the analyses appropriate standards were employed; standard curves were drawn; and the calcium and inorganic phosphorus values were expressed in mg/100 ml plasma or red blood cells.

Ionized Calcium

Animals were prepared for sampling in the manner described above. However, the blood samples for these studies were withdrawn by syringe and quickly injected to completely fill a Vacutainer® containing no anticoagulant. The blood was allowed to clot at room temperature (25°C) and then centrifuged. Immediately after centrifugation the serum was withdrawn into 1.0 ml disposable tuberculin syringes with care taken to maintain anaerobic conditions. The syringe was attached to an Orion* Model 99-20 Serum Calcium Flow-Thru System connected to an Orion Model 801 Digital pH/mV Meter for determination of serum ionized calcium (Orion,*1969). Ionized calcium values were expressed in mg/100 ml. Immediately prior to ionized calcium determination, serum pH was measured with the use of an Instrumentation Laboratory** Model 113 pH Analyzer.

*Orion Research Inc., Cambridge, Mass.
**Instrumentation Laboratory, Inc., Lexington, Mass.

Standard Recoveries

Known quantities of calcium and phosphorus were added to samples of human plasma and anaerobic serum. The accuracy of the methods described for the determination of total plasma calcium and phosphorus, and serum ionized calcium was measured as the percent of the added amounts recovered.

To evaluate the method used to determine erythrocyte total calcium and phosphorus, blood was obtained from laboratory personnel. The samples were prepared as described above except that aliquots of packed red cells were hemolyzed in two different concentrations of calcium/phosphorus standards as well as in distilled water. In addition, to ascertain the appropriateness of using potassium carbonate crystals to neutralize the solution and to precipitate the perchlorate from the solution, samples from each hemolysate were treated with potassium carbonate crystals, potassium hydroxide solution or potassium carbonate crystals plus potassium hydroxide solution. The resultant supernatants were analyzed for total calcium and phosphorus and the percent recovery determined.

Statistical evaluations of the data employed Student's t test.

IV. Results

Figure 2 is a photograph of a typical tracing as recorded by the Auto-Analyzer® for the simultaneous determination of calcium and inorganic phosphorus. There occurs 1 1/2 peak lag between the calcium response and the corresponding phosphorus response from the same sample. This lag results from the double dialysis necessary to obtain the phosphorus fraction. It can be seen that the baseline for the calcium measurements is stable, and the standard values, particularly at the higher concentration, are reproducible.

On the other hand the phosphorus baseline tends to "staircase" as the standard concentrations increase. However, the baseline stabilizes as samples, whose phosphorus concentrations fluctuate to a relatively small degree, pass through the colorimeter. The standard curves that are used are drawn against the re-established baseline and are reproducible as may be noted by comparing the value for the 5 mg/100 ml standard in the middle of the run with that obtained for the 5 mg/100 ml phosphorus standard at the end of the series.

The accuracy and precision of the methods used in these studies are presented in Tables 1 and 2. The standard errors of the mean reflect the precision of the methods while the percent recovery of added standard is a measure of the methods' accuracy. When calcium standard is added to a sample of human anaerobic serum and analyzed for ionized calcium 75% of the added calcium is recovered (Table 1). This percentage is the same for the two different concentrations of standard. Since the recoveries are fairly low it is probable that some of the added free calcium becomes bound to the serum proteins of the sample. With regard to both concentration and variability, the ionized calcium measured in serum alone is comparable to the

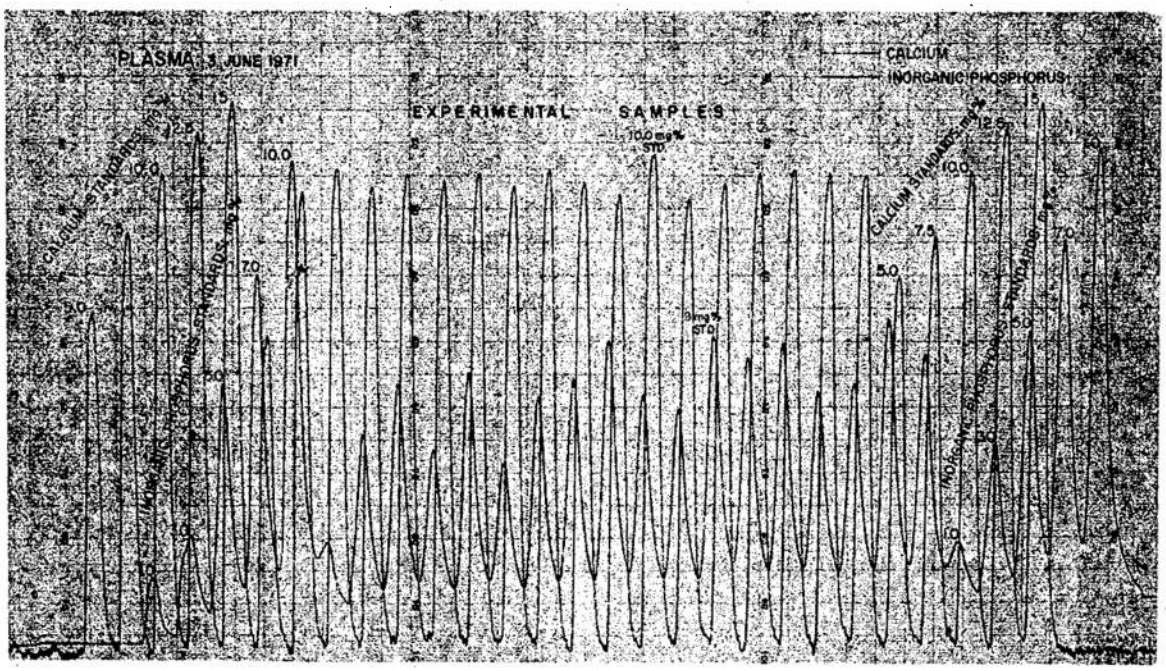

Fig. 2. Recorder Tracing of Calcium and Phosphorus Responses in Standards and Human Plasma. Plasma Concentrations Are Determined by Comparison Against the Appropriate Standard Curve. Red for Calcium, Green for Phosphorus

1.98 ±.03 mEq/1 (3.96 ±.06 mg/100ml) reported by Hattner et al[15] (1970).

With respect to total calcium and inorganic phosphorus in plasma, recoveries of added standard range from 90-96% and 102-104%, respectively.

Table 2 presents the results of recoveries obtained when calcium and phosphorus standards are added to the RBC hemolysates prior to perchloric acid precipitation. To remove the perchlorate and neutralize the protein-free supernatant of the RBC hemolysates there are several advantages to using K_2CO_3 crystals. The potassium is precipitated as the perchlorate; the carbonate is evolved as CO_2 gas from the acid medium; and no additional dilution factor is introduced into the calculation of erythrocyte calcium and phosphorus concentrations. Moreover, the method is quick and easy. However, a disadvantage is the possible loss of calcium, as precipitated calcium carbonate if great care is not taken to avoid exceeding neutrality. Using this method the recovery of calcium is 79-80% in the presence of red blood cells and 93% when standard alone is treated as an hemolysate. The recovery of phosphorus is 93-94% and 85%, respectively.

When KOH is used to effect the perchloric precipitation, the percent

Table 1. Recovery of Added Standards from Serum and Plasma for Determination of Method Accuracy

	Serum Ionized Ca++ mg/100 ml	Total Plasma Ca mg/100 ml	Total Plasma P mg/100 ml
Sample			
$\bar{X}$	3.96	10.00	4.46
SEM	.01	.05	.08
N	8	15	15
	Added	Added	
Standard I	2.00	6.25	3.50
+			
Sample	Recovered	Recovered	
$\bar{X}$	1.49	5.98	3.58
SEM	.02	.07	.05
N	8	10	10
% Recovery	75	96	102
	Added	Added	
Standard II	4.00	7.50	5.00
+			
Sample	Recovered	Recovered	
$\bar{X}$	3.00	6.76	5.20
SEM	.01	.08	.06
N	8	10	10
% Recovery	75	90	104

Table 2. Recovery of Added Standards From Erythrocyte Preparations for Determination of Method Accuracy[a]

	K_2CO_3				KOH				K_2CO_3+ KOH			
	Calcium		Phosphorus		Calcium		Phosphorus		Calcium		Phosphorus	
	T[b]	O[c]	T[b]	O[c]	T[b]	O[c]	T[b]	O[c]	T[b]	O[c]	T[b]	O[c]
RBC												
$\overline{X}$		.204		.558		.191		.613		.184		.582
SEM		.002		.012		.003		.004		.002		.008
N		5		5		5		5		5		5
RBC	.231		.631		.135		.693		.208		.657	
+												
Standard I	.847		.423		.847		.423		.847		.423	
Σ	1.078		1.054		.982		1.116		1.055		1.080	
$\overline{X}$		.866		.978		.904		.995		.838		.958
SEM		.002		.012		.007		.009		.014		.018
N		5		5		5		5		5		5
% Recovery	80		93		92		89		79		89	
RBC	.233		.611		.209		.672		.202		.637	
+												
Standard II	.630		.252		.630		.252		.630		.252	
Σ	.863		.863		.839		.924		.832		.889	
$\overline{X}$		.678		.807		.766		.907		.728		.847
SEM		.006		.004		.001		.007		.003		.016
N		5		5		5		5		5		5
% Recovery	79		94		91		98		88		95	
Standard Only												
$\overline{X}$	.951	.886	.951	.402	.951	.542	.475	.279	.951	.941	.475	.508
SEM		.004		.004		.004		.004		.006		.007
N		5		5		5		5		5		5
% Recovery	93		85		59		59		99		107	

[a] mg/100 ml hemolysate [b] Theoretically present [c] Observed

calcium and phosphorus recovered from standards plus red cells was 91-92% and 89-98%, respectively. The percentage of calcium and phosphorus recovered when standard alone was treated with KOH is presented here to illustrate the ease with which both calcium and phosphorus can be lost if the solution is accidently allowed to become basic. Subjective evaluation of indicator color was used, and no attempt was made to ascertain exact pH.

A combination of both K_2CO_3 crystals for the initial precipitation of the perchlorate and KOH for the final approach to neutrality was also studied and the results are included in Table 2. The recoveries of calcium and phosphorus are comparable to those obtained using potassium carbonate or potassium hydroxide alone.

When guinea pigs are exposed to 15% CO_2 in 21% O_2, balance nitrogen, there occurs within one hour a highly significant decrease in serum pH and total plasma calcium, while at the same time, the serum ionized calcium and red blood cell calcium levels increase very significantly (Table 3, Figure 3). It might be noted here that the pH values used are not so low nor is the magnitude of pH fluctuation so great as would be expected, since no correction is made for body temperature or the alkaline error of .03 resulting from centrifugation at room temperature (Van Slyke et al,[53] 1966). After six hours of exposure, plasma and RBC calcium concentrations return to near control values, the ionized calcium decreases although still remaining significantly elevated, and the serum pH remains significantly depressed.

As exposure to 15% CO_2 continues, the plasma calcium begins to rise and becomes significantly elevated after three days. The rise continues for up to seven days of exposure. The RBC calcium again becomes significantly elevated after one day but gradually returns to the control level after seven days.

Although it has been suggested by Moore[30] (1970) that an inverse relationship between serum ionized calcium and pH is one of competition for protein binding sites, these data reveal that as the pH begins to return toward normal levels in compensation of the respiratory acidosis, the ionized calcium no longer maintains the inverse relationship with pH. To visualize these phenomena better, Figure 4 shows the serum pH converted to nM/1 of hydrogen ion concentration plotted against ionized calcium concentration. Further inspection of Table 3 shows that after three days' exposure to 15% CO_2 the ionized calcium increases and maintains a percentage of the total that is equal to that observed in the controls (39%). An ionized to total calcium ratio of 40.3% has been reported in normal human serum (Hattner et al,[15] 1970). The parallel elevations in ionized and total calcium are accompanied by a significant decrease in plasma inorganic phosphorus after three days of 15% CO_2 exposure. Similar results have been reported by Schaefer et al[38] (1961) who suggest that these changes appear to be related to increased parathyroid activity. On the other hand, Hattner[15] et al (1970) have reported similar ionized to total calcium ratios and inorganic phosphorus responses in normal sheep following a

Table 3. Effect of Prolonged Exposure of Guinea Pigs to 15% CO_2 on Serum pH and Ionized Calcium and on Total Calcium and Inorganic Phosphorus of Plasma and Erythrocytes

		Serum		Plasma		RBC	
		Ca++		Ca	P	Ca	P
	pH*	mg/100ml	% of Total	mg/100ml		mg/100ml	
Control							
Mean	7.465	4.37	39	11.07	6.20	1.111	3.51
SEM	.007	.04		.09	.12	.034	.21
N	29	28		34	33	29	32
1 Hour							
Mean	7.264	4.83	49	9.78	10.80	1.336	9.91
SEM	.016	.09		.12	.22	.120	.32
N	10	10		10	10	10	10
p	< .001	< .001		< .001	< .001	< .02	< .001
6 Hours							
Mean	7.266	4.63	42	10.91	5.69	1.159	6.42
SEM	.015	.09		.12	.19	.048	.23
N	10	10		24	33	24	24
p	< .001	< .005			< .05		< .001
1 Day							
Mean	7.250	4.74	44	10.75	6.57	1.342	4.41
SEM	.019	.12		.15	.31	.071	.30
N	9	8		12	19	13	13
p	< .001	< .001				< .005	< .02
3 Days							
Mean	7.313	4.61	40	11.62	5.55	1.289	2.16
SEM	.034	.13		.27	.19	.076	.14
N	9	9		16	19	14	14
p	< .001	< .025		< .02	< .01	< .02	< .001
7 Days							
Mean	7.417	4.69	39	11.96	5.38	1.063	2.16
SEM	.032	.04		.18	.21	.094	.13
N	7	7		17	23	14	14
p	< .05	< .001		< .001	< .001		< .001

*Uncorrected for body temperature and centrifugation

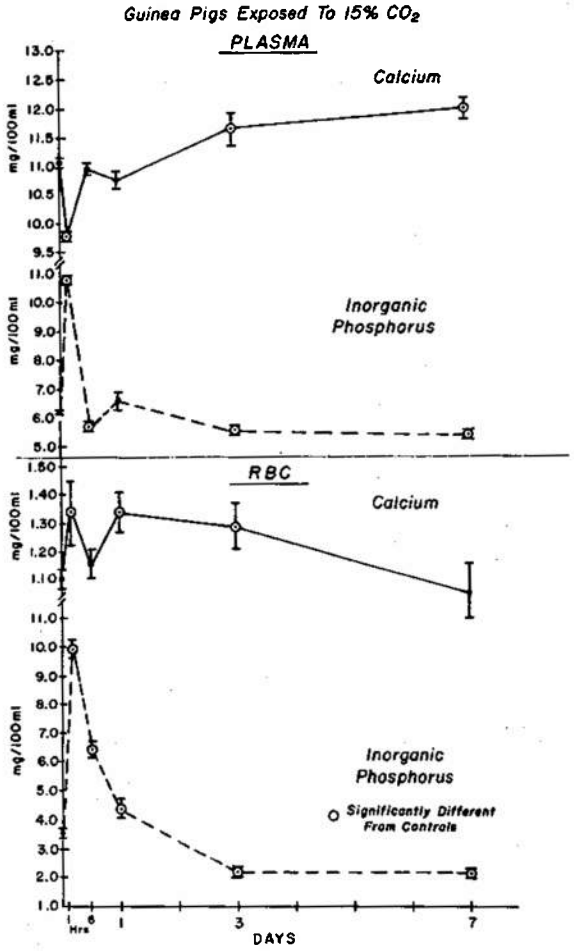

Fig. 3. Effect of Prolonged Exposure of Guinea Pigs to 15% CO_2 on Total Plasma and RBC Calcium and Inorganic Phosphorus. Mean ± SEM. Statistical Significance at the 5% Level or Better.

calcium infusion and suggest that these responses are the result of thyrocalcitonin activity.

The initial response of plasma inorganic phosphorus following one hour exposure to 15% CO_2, however, is a very significant increase. Increase in plasma inorganic phosphorus in response to acute exposure to CO_2 has been reported previously (Brown and Prasad,[5] 1957).

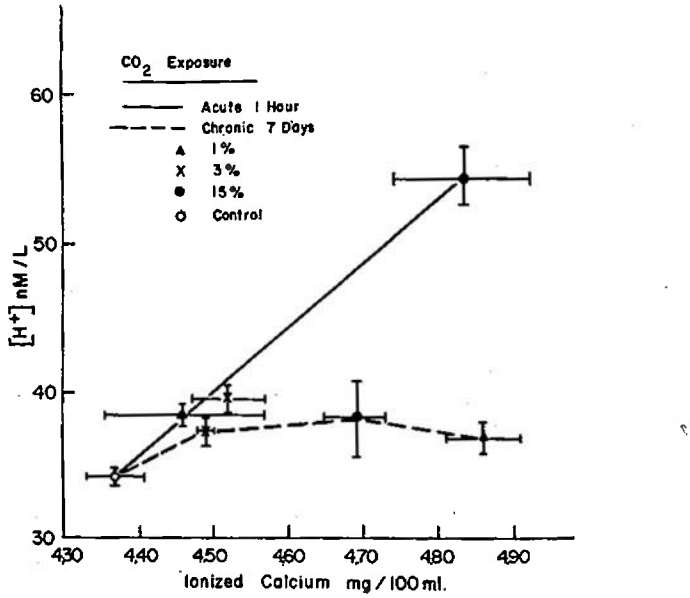

Fig. 4. Effect on Guinea Pigs of Acute and Prolonged Exposure to Different Concentrations of CO_2 on Serum Ionized Calcium and Hydrogen Ion Concentrations. Mean ± SEM

Inorganic phosphorus in the red blood cells triples within one hour of exposure to 15% CO_2; after which time the concentration starts to decrease although it still remains significantly elevated after one day. By three days, the phosphorus concentration falls significantly below that of the controls and remains so through seven days.

When guinea pigs are exposed to 3% CO_2, the serum pH decreases and the ionized calcium increases significantly within one hour. The total plasma calcium concentration, however, does not exhibit an initial sharp decrease after one hour exposure (Table 4, Figure 5). Following six hours' exposure to 3% CO_2, both plasma and erythrocyte

Table 4. Effect of Prolonged Exposure of Guinea Pigs to 3% CO_2 on Serum pH and Ionized Calcium and on Total Calcium and Inorganic Phosphorus of Plasma and Erythrocytes

	Serum			Plasma		RBC	
		Ca++		Ca	P	Ca	P
	pH*	mg/100ml	% of Total	mg/100ml		mg/100ml	
Control							
Mean	7.465	4.37	39	11.07	6.20	1.111	3.51
SEM	.007	.04		.09	.13	.034	.21
N	29	28		34	33	29	32
1 Hour							
Mean	7.405	4.52	41	11.13	6.61	1.153	3.68
SEM	.010	.05		.12	.12	.052	.25
N	12	12		16	16	16	16
p	< .001	< .05			< .05		
6 Hours							
Mean	7.434	4.67	40	11.61	6.06	1.240	3.76
SEM	.007	.05		.15	.12	.043	.35
N	10	10		23	23	23	18
p	< .025	< .001		< .005		< .05	
1 Day							
Mean	7.435	4.72	42	11.37	6.08	1.237	3.58
SEM	.012	.06		.12	.16	.044	.22
N	10	10		23	23	23	18
p	< .05	< .001		< .05		< .05	
3 Days							
Mean	7.424	4.52	40	11.33	5.48	1.198	3.35
SEM	.014	.03		.17	.17	.056	.27
N	8	8		25	25	17	17
p	< .02				< .005		
7 Days							
Mean	7.427	4.49	39	11.42	5.94	1.158	3.51
SEM	.013	.10		.10	.12	.038	.19
N	8	8		23	23	18	18
p	< .02			< .05			

*Uncorrected for body temperature and centrifugation

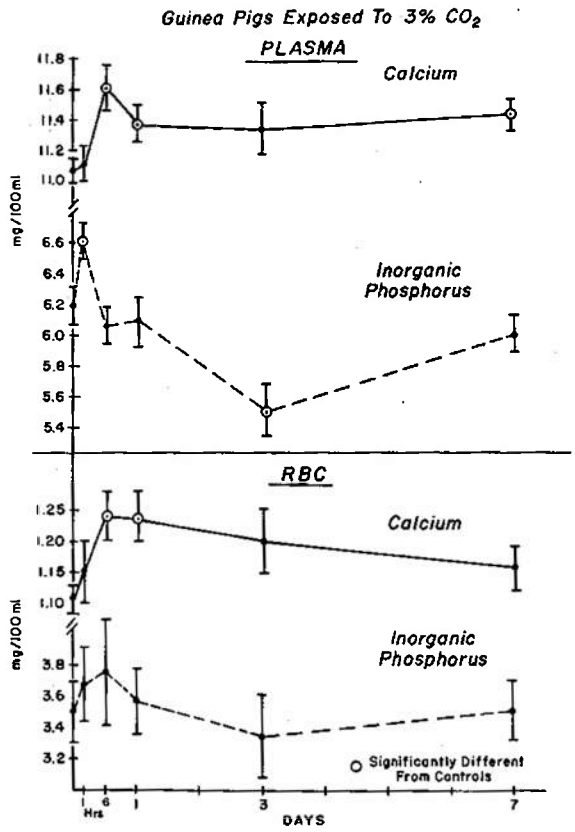

Fig. 5. Effect of Prolonged Exposure of Guinea Pigs to 3% CO_2 on Total Plasma and RBC Calcium and Inorganic Phosphorus. Mean ± SEM. Statistical Significance at the 5% Level or Better

calcium become significantly elevated and gradually return toward control level after three days.

After seven days of exposure, the plasma calcium again becomes significantly increased; whereas the RBC calcium continues to decrease toward the control values.

As observed in the study with 15% CO_2 exposure, the serum pH/ionized calcium inverse relationship seems to exist in the initial acute phase (one hour) of 3% CO_2 exposure. As compensation begins and the pH, although still significantly depressed, begins to return toward normal, the competitive relationship no longer exists and alternative mechanisms appear to be at work during the chronic (seven days) exposure phase (Figure 4).

The response of inorganic phosphorus in the plasma of guinea pigs exposed to 3% CO_2 levels seems to mimic that observed following 15% CO_2 treatment. The magnitude of the response, however, is markedly less with the maximum increase of .41 mg/100 ml occurring within one hour of exposure. After three days of 3% CO_2, the plasma inorganic phosphorus falls significantly below that of the controls but returns toward normal by seven days.

The RBC inorganic phosphorus exhibits no significant change from control values although there appears to exist the same pattern of initial increase followed by depression observed in those animals exposed to 15% CO_2. The lack of significance may be due to the rather large variability encountered in the samples obtained from the guinea pigs exposed to the 3% CO_2 (Figure 5).

The blood calcium and inorganic phosphorus responses of guinea pigs exposed to 1% CO_2 appear in Table 5 and Figure 6. The serum pH and ionized calcium values parallel those observed during 3% CO_2 exposure through the first six hours. After one day of exposure to 1% CO_2, however, the ionized calcium decreases somewhat, rises again after three days, and continues to increase through seven days at

Table 5. Effect of Prolonged Exposure of Guinea Pigs to 1% CO_2 on Serum pH and Ionized Calcium and on Total Calcium and Inorganic Phosphorus of Plasma and Erythrocytes

		Serum		Plasma		RBC	
		Ca++		Ca	P	Ca	P
	pH*	mg/100ml	% of Total	mg/100ml		mg/100ml	
Control							
Mean	7.465	4.37	39	11.07	6.20	1.111	3.51
SEM	.007	.04		.09	.12	.034	.21
N	29	28		34	33	29	32
1 Hour							
Mean	7.416	4.46	42	10.56	6.47	1.109	2.92
SEM	.009	.11		.12	.18	.032	.19
N	9	9		20	20	20	20
p	< .005			< .005			
6 Hours							
Mean	7.446	4.66	43	10.82	6.15	1.051	2.74
SEM	.012	.12		.08	.19	.037	.13
N	8	8		20	20	20	20
p		< .01					< .01
1 Day							
Mean	7.442	4.56	43	10.72	5.98	1.039	4.64
SEM	.023	.07		.11	.10	.043	.29
N	5	5		20	20	20	20
p		< .05		< .02			< .005
3 Days							
Mean	7.420	4.59	43	10.77	5.92	1.194	3.94
SEM	.009	.07		.08	.17	.042	.19
N	8	11		20	20	20	20
p	< .005	< .01		< .025			
7 Days							
Mean	7.433	4.86	45	10.77	5.81	1.093	3.00
SEM	.013	.05		.12	.18	.039	.17
N	10	10		18	18	18	18
p	< .05	< .001		< .05			

*Uncorrected for body temperature and centrifugation

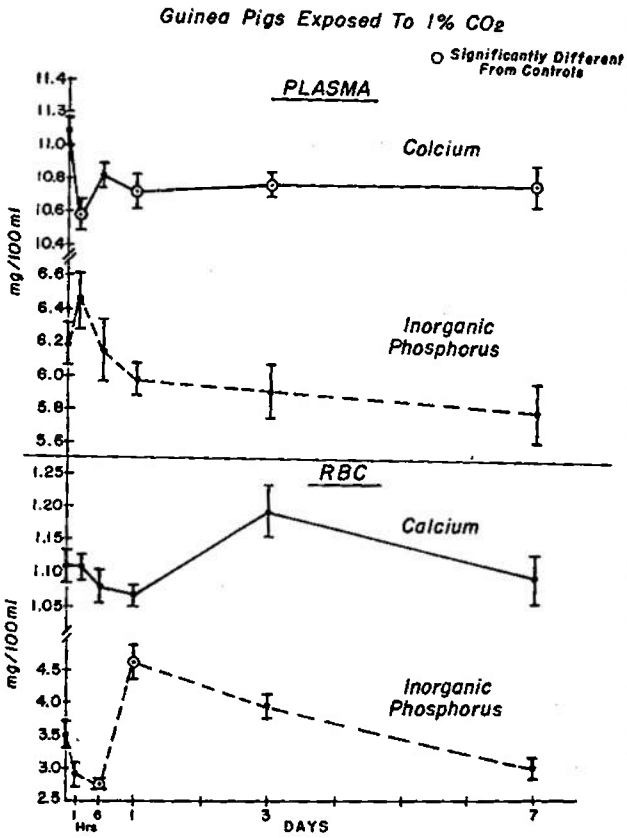

Fig. 6. Effect of Prolonged Exposure of Guinea Pigs to 1% CO_2 on Total Plasma and RBC Calcium and Inorganic Phosphorus. Mean ± SEM. Statistical Significance at the 5% Level or Better

which time it represents 45% of the total plasma calcium.

The total plasma calcium decreases within one hour after exposure to 1% CO_2 and remains significantly depressed throughout the entire exposure period. On the other hand, there are no significant changes observed in the RBC calcium levels. Inspection of the data, however, reveals that, in contrast to those observations made during 15% and 3% CO_2 treatment, there is a slight decline followed by a transient rise in RBC calcium with the values returning to control levels after seven days.

The inorganic phosphorus found in the plasma of guinea pigs exposed to 1% CO_2 does not vary significantly from the controls although an initial transient rise does occur.

The erythrocyte inorganic phosphorus differs from previous observations in that there occurs an initial significant decrease after six hours' exposure followed by a significant rebound increase after one day thence a return toward control levels after three days.

Graphic summaries of the data are presented in Figures 7, 8, 9. Presenting the results of exposure of guinea pigs to 1%, 3%, and 15% CO_2 obtained for each parameter as measured on the same response scale aids in evaluating the relative responses as well as the absolute results of the different regimens.

The calcium and phosphorus concentrations in the plasma and erythrocytes obtained from healthy young men during an operational submarine patrol are presented in Table 6 and Figure 10. In addition, plasma sodium and potassium values are presented in Table 6. No significant changes are observed in the parameters measured in the plasma. However, the plasma calcium does decrease somewhat after one week followed by a gradual return toward normal values after three weeks' exposure. Similar findings have been reported by Schaefer et al (1963) and Gray et al (1969).

In addition Schaefer et al[39] (1963) report that during the recovery period following forty-two days' exposure to 1.5% CO_2 there is a pronounced flood of

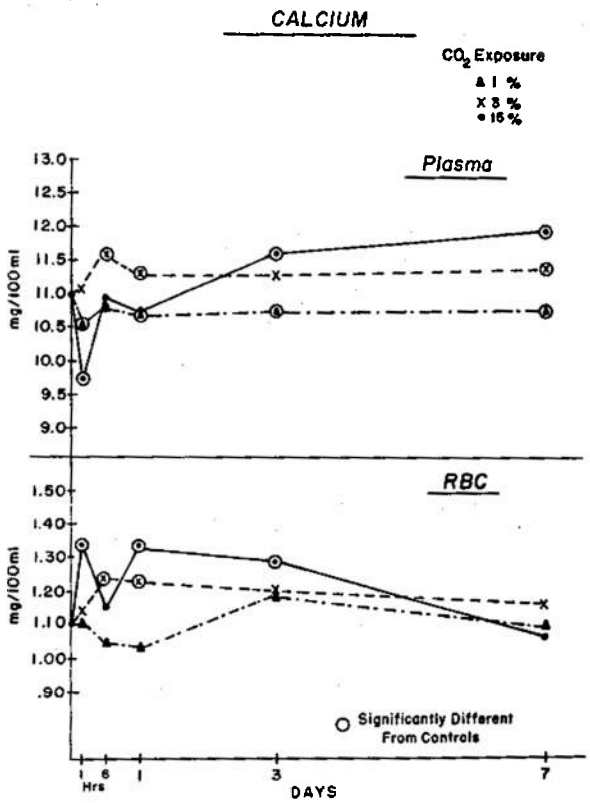

Fig. 7. Summary of Total Plasma and RBC Calcium of Guinea Pigs Exposed to 1%, 3%, and 15% CO_2. Statistical Significance at the 5% Level or Better

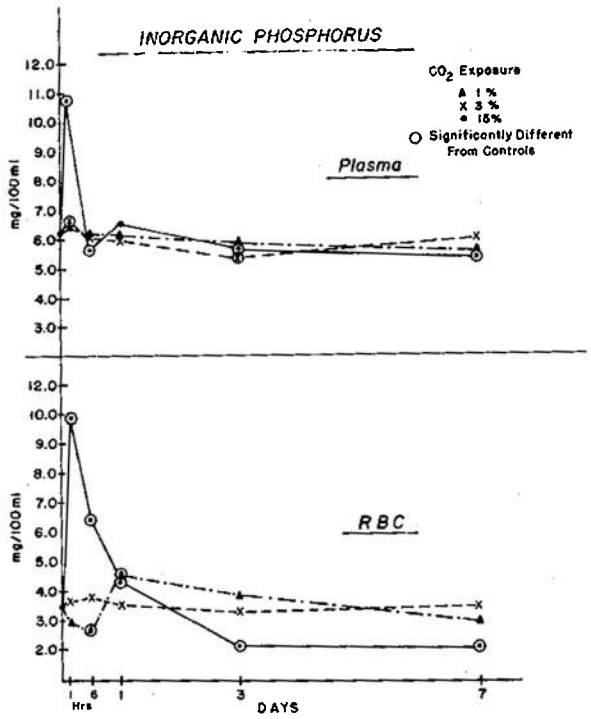

Fig. 8. Summary of Total Plasma and RBC Inorganic Phosphorus of Guinea Pigs Exposed to 1%, 3%, and 15% CO_2. Statistical Significance at the 5% Level or Better

calcium into the plasma. However, the phenomenon was not observed during this study, and may be related to the relatively short exposure to the 1% CO_2 environment.

In view of the reports by Schaefer et al[39] (1963) and Gray et al[14] (1969) and the data from guinea pigs presented above with respect to the response of plasma inorganic phosphorus to increased environmental CO_2 concentrations, the lack of inorganic phosphorus response in the plasma from the men of this study seems surprising.

On the other hand, within one week of CO_2 exposure, there are very pronounced increases in the erythrocyte calcium concentration in the men studied. The calcium concentration continues to rise and becomes significant after three weeks' exposure. One would suspect that significance might have occurred earlier in the exposure period were it not for the rather large standard error of the mean. These large standard errors could be expected since the men stood different watch schedules, blood withdrawal was possible only at irregular hours of the

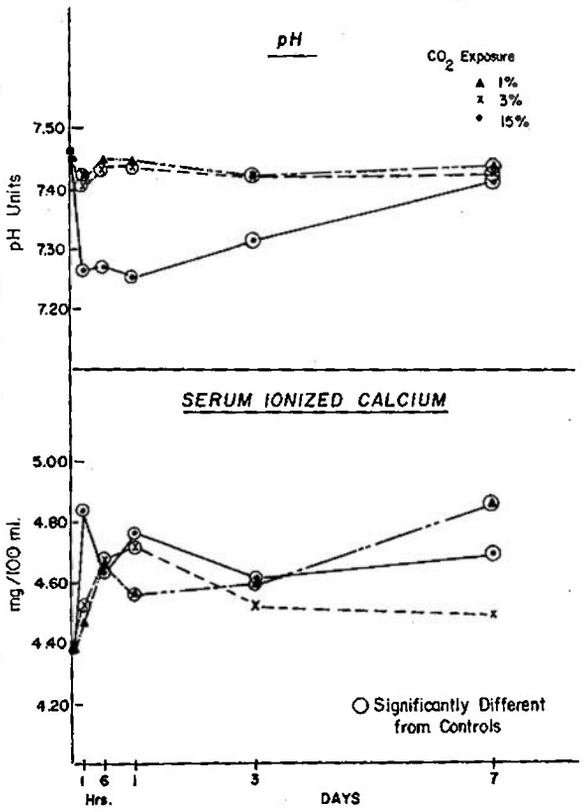

Fig. 9. Summary of Serum Ionized Calcium and pH of Guinea Pigs Exposed to 1%, 3%, and 15% CO_2. Statistical Significance at the 5% Level or Better

day, and no attempt was made to control the diet. Most striking was the return of RBC calcium concentrations to control levels within one week after returning to surface air. These results differ from those reported by Schaefer et al[39] (1963) who indicated a continued rise in RBC calcium values during the recovery phase. However, their results were based on calculated RBC calcium values following a longer (42 days) exposure to CO_2.

Unfortunately no values for RBC inorganic phosphorus are available from this study. It was found in the early phases of this work that unless the erythrocytes were processed immediately as described above or maintained quick frozen under dry ice for no more than one week extremely elevated aberrant results were obtained. This may result from varying degrees of hydrolysis of the high concentration of organophosphates in the erythrocytes.

V. DISCUSSION

Chronic respiratory acidosis, induced by carbon dioxide varies markedly with respect to the initial decrease in pH and is related to the concentration of carbon dioxide used. During seven days of exposure to the two highest carbon dioxide concentrations, 15% and 3% CO_2, the pH does not return to initial levels. Although a full "compensation" of the pH does not occur, one can consider that a "physiological compensation" is acquired after three days of exposure to 15% CO_2 and five days of exposure to 3% CO_2. This "physiological compensation" is based upon the return toward initial values of most physiological parameters affected during the first phase of uncompensated respiratory acidosis. (Schaefer, et al, 1961,[38] 1968,[41] 1970,[42,43] 1971;[44] Messier and Schaefer,[27] 1971). The exposure periods are classified as "acute" during the initial phase of rapid pH decrease and as "chronic" during the subsequent adaptive phase of "physiological compensation" to the respiratory acidosis.

There is another distinction to be made. During exposure to the highest carbon dioxide concentration (15%), red cell metabolism (glycolysis) is inhibited

Table 6. Effect of Exposure of Up to 1% CO_2 in the Total Submarine Environment on Plasma Electrolytes and Erythrocyte Calcium Levels in Human Volunteers (N = 7)

	Plasma				RBC
	Na*	K*	Ca	P	Ca
	mEq/1		mg/100ml		mg/100ml
Control					
Air					
Mean	147.2	4.0	10.10	4.18	1.57
SEM	1.6	.1	.10	.40	.10
One Week					
1% CO_2					
Mean	142.2	3.7	9.53	3.96	1.74
SEM	3.6	.2	.32	.46	.19
Two Weeks					
1% CO_2					
Mean	143.5	3.8	9.70	4.04	1.95
SEM	2.7	.1	.20	.36	.28
Three Weeks					
1% CO_2					
Mean	145.0	3.7	9.94	4.00	1.99**
SEM	1.1	.1	.15	.14	.16
Recovery					
One Week					
Air					
Mean	144.5	3.8	9.83	4.36	1.51
SEM	.9	.1	.11	.25	.18

*Determined by flame photometry

**Statistically significantly different from control values at the 5% level

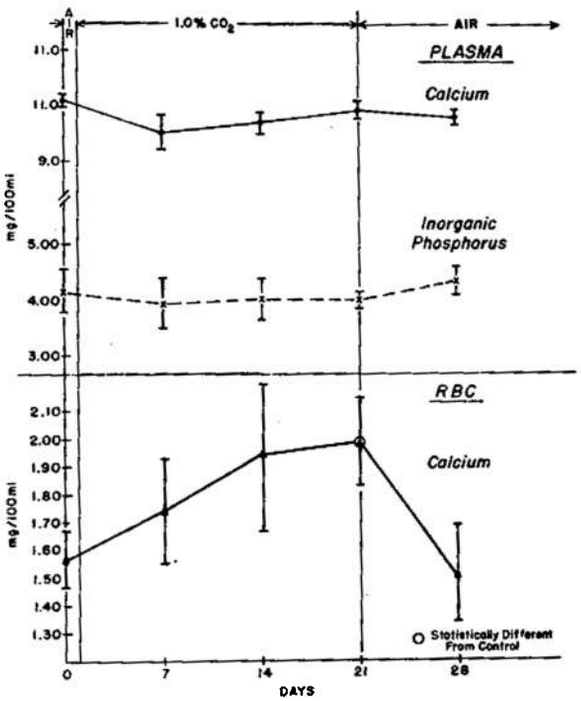

Fig. 10. Effect of Total Submarine Environment on Human Plasma and RBC Calcium and Plasma Inorganic Phosphorus. Pre-dive Control and Seven Days Post-dive Recovery Data Are Included. Mean ± SEM

for the first two days and returns to near normal after three days (Jacey and Schaefer,[19] 1971a). During 3% CO_2 exposure no inhibitory effect on red cell glycolysis is observed (Jacey,[21] et al 1971). Inasmuch as calcium accumulated in the red cells in all three experimental conditions of this study, the results are discussed with regard to (1) pH dependent metabolic inhibition and (2) pH or CO_2 dependent changes in red cell permeability without involvement of glycolysis. In addition, the plasma calcium levels become elevated during prolonged exposure to 15% CO_2 and 3% CO_2, but are decreased during exposure to 1% CO_2. These changes are discussed in connection with parathyroid and thyrocalcitonin function.

1. Red Cell Calcium/Phosphorus Levels Related to pH Dependent Metabolic Inhibition (15% CO_2)

The initial trigger during the acute phase of this exposure appears to be the precipitous fall in blood pH resulting from elevation in the CO_2 content of the blood. It has been shown in vitro that decrease in pH per se can cause decrease in total glucose utilization within the red blood cell (Murphy,[31] 1960; Minakami and Yoshikawa,[29] 1966). These authors found that incubation at low pH resulted in decreases in lactic acid formation and nonhydrolyzable organic phosphates, with an accumulation of inorganic phosphorus; a reaction which does not occur in the presence of inosine. They conclude that inhibition of glucose metabolism occurs in the Embden-Meyerhof pathway at the level of the conversion of fructose-6-PO_4 to fructose-1,6-PO_4 by phosphofructokinase.

In vivo it has been found that decrease in pH during acute respiratory acidosis also results in inhibition of red cell glycolysis (Jacey and Schaefer,[19] 1971a). In addition these authors report that phosphofructokinase activity correlates with fluctuations in pH under the same conditions (Jacey and Schaefer,[20] 1971b). Interestingly, they found no change in the energy charge during acute respiratory acidosis. Conservation of high energy phosphate as a characteristic of hypercapnia is suggested by these

observations and those of Tappan[51] (1971) who found, under the same conditions, an influx of creatinephosphokinase into the plasma without a concomitant decrease in muscle creatine phosphate.

On the other hand, the enormous increase in erythrocyte inorganic phosphorus and inhibition of active transport, as seen by increased levels of intracellular calcium, would suggest an energy-depleted state during the acute hypercapnia of the present study (one hour exposure to 15% CO_2). Moreover, these findings appear to be the corollary of those reported by Murphy[32] (1963) who found that a 50% reduction in glucose utilization and inhibition of energy production in red blood cell preparations at reduced pH was associated with inhibition of active transport. This inhibition in turn resulted in intracellular loss of potassium and gain of sodium.

Since it has been shown that phosphofructokinase activity is inhibited at low pH (Jacey and Schaefer,[20] 1971b), the most likely source of the elevated inorganic phosphorus levels observed in the present study would be the reduced formation of 1,3-diphosphoglycerate (1,3-DPG), inasmuch as it is the only site below phosphofructokinase in which inorganic phosphorus is utilized in red cell glycolysis. Reduced levels of 1,3-DPG in turn, limit the activity of phosphoglycerate mutase and hence the formation of 2,3-DPG. This notion is supported by the findings of Mills[28] (1969) who observed that a decrease in 2,3-DPG is accompanied by a rise in inorganic phosphorus in erythrocytes incubated at pH 6.8-7.0. In addition to the effect of low pH on the overall glycolysis, thereby reducing the glycolytic flux and hence the formation of 2,3-DPG, low pH has also been shown to increase the binding of 2,3-DPG to hemoglobin (Caldwell, et al,[6] 1971). Therefore, if only small amounts of 2,3-DPG are formed and what little is produced becomes bound to hemoglobin, subsequent hydrolysis of 2,3-DPG is probably not a source of the observed increase in inorganic phosphorus. During acute hypercapnia, the maximum inhibition of erythrocyte glycolysis was observed after one hour (Jacey and Schaefer,[19] 1971a) and coincides with the maximum increase in inorganic phosphorus observed in the present studies. The time lag between these phenomena and the maximum 2,3-DPG depletion observed at one day (Messier and Schaefer,[27] 1971) is most likely related to different time courses in pH dependent enzymatic shifts.

The second, or "physiologically compensated", phase of respiratory acidosis has been considered to exist after three days of chronic exposure to 15% CO_2 based on extensive previous studies of lung function, stress parameters, and hemoglobin oxygen affinity (Schaefer, et al, 1964,[40] 1968,[41] 1970[42,43]). Although pH is still depressed during this time, there are very marked reversals of calcium and inorganic phosphorus levels in the blood. The decreases in intracellular calcium and inorganic phosphorus suggest renewed red cell glycolytic activity. As incorporation of inorganic phosphorus into organo-phosphates occurs, energy becomes available for reactivation of the active transport pumps. Moreover, it has been shown

that during the compensated phase of chronic respiratory acidosis, other indicators of erythrocyte glycolysis, i.e., phosphofructokinase activity, lactate levels, lactate/pyruvate and NAD+/NADH ratios and 2,3-DPG levels, return toward normal (Jacey and Schaefer,[19,20] 1971a, 1971b; Messier and Schaefer,[27] 1971).

2. Red Cell Calcium/Phosphorus Levels Related to CO_2 Effects on Membrane Permeability

Interpretation of the movements of calcium and inorganic phosphorus in the blood of guinea pigs during the acute exposure to 3% CO_2 is less clear cut and somewhat equivocal. Although the responses tend to be similar, their magnitude is markedly less than those observed during the acute exposure to 15% CO_2. Jacey et al[21] (1971) have indicated that the decrease in blood pH in guinea pigs under these conditions is insufficient to inhibit red cell glycolysis. Consequently, the apparent inhibition of active transport of intracellular calcium and increases in intracellular inorganic phosphorus in the animals exposed to 3% CO_2, as well as the alterations observed in those animals exposed to 1% CO_2 must be the result of other mechanisms.

In addition to being transported across membranes, calcium is also a key component of the membrane. Calcium binds to phospholipid in the membrane and, if displaced, the geometry of the membrane is changed. Thus alterations in calcium binding increase the membrane's permeability to water and other ions (Manery,[25] 1966). The binding appears to be an ion exchange type that is not affected by metabolic processes. (Bolingbroke and Maizels,[4] 1959; Gent,[11] et al. 1964). Moreover, ion displacement experiments show that sodium, potassium, and hydrogen compete with calcium for the negative binding sites in monolayers of phosphatidylserine, a large component of erythrocyte membranes (Rojas and Tobias,[36] 1965).

The gradual increase observed in red blood cell calcium during the three-week period of exposure to 1% CO_2 during an FBM submarine patrol agrees with previous findings in human subjects exposed to 1.5% CO_2 (Schaefer, et al,[39] 1963). If we interpret intracellular calcium concentration as a measure of active transport then inhibition of red cell glycolysis may occur in men exposed to low concentrations of carbon dioxide for prolonged periods of time. On the other hand, alterations in calcium binding at the membrane, under the influence of CO_2 or the decreased pH of acidosis, may be sufficient to permit calcium to leak into the cells faster than it can be pumped out.

3. Effects of CO_2 on Parathyroid Function

a. Total Plasma Calcium/Phosphorus Changes

Plasma calcium increases and plasma inorganic phosphorus decreases during the chronic (physiologically compensated) phase of respiratory acidosis following exposure to both 15% CO_2 and 3% CO_2. In previous studies of exposure to 15% CO_2 similar observations have been made in guinea pigs (Schaefer, et al,[38] 1961) and rats

(Stanmeyer, et al,[50] 1962). These authors suggest that the plasma calcium and inorganic phosphorus responses are characteristic of chronic respiratory acidosis and reflect an increased parathyroid activity. In addition, Wachman and Bernstein[54] (1970) have found that during metabolic acidosis parathyroid activity is increased, as measured by increased urinary hydroxyproline, increased plasma immunoreactive parathyroid hormone and increased clearance of phosphate.

When guinea pigs are exposed to 15% CO_2 a metabolic acidosis becomes superimposed upon the respiratory acidosis. This superimposed metabolic acidosis occurs between six and twenty-four hours of exposure (Schaefer,[44] 1971) and could be responsible for parathyroid stimulation. The increase in parathyroid hormone activity becomes pronounced after the third day if increases in plasma calcium and decreases in inorganic phosphorus are considered as a measure of parathyroid activity.

In addition, an increase in urinary hydroxypoline excretion was found in a human subject during intermittent exposure to 3% CO_2 for nine hours per day. This finding is also interpreted as an increase in parathyroid activity (Schaefer, unpublished observations).

The decrease in plasma calcium in guinea pigs exposed to 1% CO_2 is similar to that reported in men exposed to 1% CO_2 for twenty days aboard a submarine (Gray, et al,[14] 1969) and in human volunteers exposed to 1.5% CO_2 (Schaefer, et al,[39] 1963). The former investigators consider the possibility that some metabolic consequences of the environment mimic a state of mild parathyroid insufficiency and that the causative factor could be the 1% CO_2 breathed through the twenty-day period. Schaefer, et al[39] (1963) conclude that decreases in plasma calcium during the first twenty-four days of exposure to 1.5% CO_2 coincide with an uncompensated phase of respiratory acidosis and result from storage of calcium and carbon dioxide in bone.

The mechanisms that relate carbon dioxide, parathyroid hormone activity, and calcium and inorganic phosphorus metabolism to alterations in calcification can only be speculated upon at this time. However, Neuman et al,[33] (1968) have developed a model for a cycling concept of ion exchange in bone defined by three variables; rate of bone perfusion, concentration of plasma ion, and concentration of the ion in bone. They conclude that blood circulation in the bone is the most important aspect in determining early bone deposition. This is an important consideration with respect to carbon dioxide exposure since it has been shown that carbon dioxide increases blood flow in bone (Cumming,[7] 1962; Shim and Patterson,[47] 1967).

The finding of decreased total plasma calcium in guinea pigs and man exposed to 1% CO_2 could be interpreted as either parathyroid underfunction or a response to thyrocalcitonin, inasmuch as thyrocalcitonin is known to decrease plasma calcium and inorganic phosphorus (Hirsch,[16] et al, 1964).

b. Ionized Calcium

So far in our discussion, the references to calcium, both intra and extracellular, have been in terms of the total calcium determined. Total calcium, in fact, equals the sum total of three existing forms: protein bound (non-diffusible) calcium, diffusible complexes and chelates of calcium, and ionized calcium. However, it has been clearly shown that the physiologically active species is the ionized calcium. It is the level of "calcium activity" and not the total calcium concentration per se that is physiologically important (McLean and Hastings,[26] 1935). Moreover, little apparent correlation has been found between ionized calcium and total calcium concentrations. Under normal physiological conditions, ionized calcium varies little and alterations in total serum calcium levels have been taken as an indirect measure of serum albumin (Moore,[30] 1970).

With alteration in serum pH, however, Moore[30] (1970) found a linear relationship to serum ionized calcium; a 0.1 pH unit increase resulted in an instantaneous and reversible 4% decrease in ionized calcium. This is presumably a result of competition between calcium ion and hydrogen ion for negative binding sites on the serum proteins and perhaps on the erythrocyte membranes as well. Our observed 4.5-5.0% increase in serum ionized calcium per 0.1 pH unit decrease during the acute phase of respiratory acidosis corroborates Moore's[30] (1970) finding. That observed relationship holds true for each exposure regimen (1%, 3%, and 15% CO_2--Figure 4) during the initial stages of hypercapnia.

As the respiratory acidosis becomes compensated, the pH begins to rise and the ionized calcium levels to fall as expected. It is interesting to note, however, that in those animals chronically exposed to 15% and 3% CO_2 the ionized calcium and the total calcium remain elevated and the ionized to total calcium ratio returns to normal. The increased levels of both total and ionized calcium are interpreted as an indication of increased parathyroid activity.

In contrast to these responses to 15% and 3% CO_2, total plasma calcium is consistently depressed throughout exposure to 1% CO_2, while serum ionized calcium becomes markedly elevated. This situation results in an increased ionized to total calcium ratio. The etiology of this completely unsuspected response to a low concentration of 1% CO_2 is unknown and cannot be explained on the basis of the available data. However, one may speculate on the possibility of a functional hypoparathyroidism due to a continuous elevation of ionized calcium. This idea would be the corollary of that suggested by George,[12] et al (1964), namely, that a diminished ionization of serum calcium could account for the increase in total calcium that results from the parathyroid stimulation of hyperventilation (respiratory alkalosis).

On the other hand, the decrease in total plasma calcium and inorganic phosphorus during 1% CO_2 exposure could also be explained by an increase in thyrocalcitonin activity since this

hormone is known to decrease both total calcium and inorganic phosphorus in the plasma (Hirsch,[16] et al 1964). In order to clarify the mechanisms involved, assays of parathyroid hormone and thyrocalcitonin during chronic low level hypercapnia must be made.

4. Relationship of Calcium/Phosphorus Changes to Erythrocyte Sodium/Potassium Exchange and Oxygen Affinity

Normally erythrocytes maintain a very low calcium concentration within the cell. It has been recognized in recent years that the intracellular concentration of calcium is regulated by an ATP-driven calcium pump which extrudes the calcium that enters down a concentration gradient. (Schatzmann and Vincenzi,[46] 1969; Romero and Whittam,[37] 1971). When internal calcium is raised, the permeability of the red cell membrane to sodium and potassium is increased (Romero and Whittam,[37] 1971). The interesting feature of their experiments is the demonstration that metabolic inhibitors have little effect on red cell permeability if calcium is absent. Moreover, the effects of metabolic poisons depend on the entrance of calcium into the cell. In addition, calcium alone can cause changes in permeability of erythrocytes.

During exposure to high concentrations of CO_2 (15%), it was found that calcium accumulation in erythrocytes coincides with a metabolic inhibition of glycolysis (Jacey and Schaefer,[19] 1971a), a large loss of potassium associated with a slight increase in sodium (Schaefer,[43] et al 1970), and a decrease in 2,3-DPG (Messier and Schaefer,[27] 1971). The changes in sodium and potassium, as well as the changes in 2,3-DPG concentrations have been considered responsible for the increase in hemoglobin oxygen affinity (decrease in P_{50}), found under the same conditions (Messier and Schaefer,[27] 1971).

During exposure to 3% CO_2, calcium accumulation in the erythrocyte still occurs. However, this accumulation is found in the absence of metabolic inhibition (Jacey, et al[21] 1971), and in the absence of changes in 2,3-DPG levels and hemoglobin oxygen affinity (Messier, unpublished observations). This would suggest that hemoglobin oxygen affinity is controlled more by the 2,3-DPG concentration than by cation shifts. This interpretation raises arguments against the suggestion of Benesch and Benesch[3] (1968) that relates 2,3-DPG changes to alterations in erythrocyte permeability to potassium.

5. Submariners Responses to Low Concentrations of CO_2

In an attempt to relate the observations made in the experimental laboratory with the responses in blood calcium and inorganic phosphorus of men exposed to low levels of carbon dioxide, studies were made of submariners during an operational Fleet Ballistic Missile Submarine patrol. The problems with studying submariners exposed to levels of up to 1% CO_2 are manifest. Many physiological responses are influenced by synergistic or antagonistic interactions of environmental conditions, i.e., diet, watch schedule, exercise, gaseous environment, noise, etc.

One of the important considerations in evaluating the calcium and phosphorus results obtained in these personnel is the roll of circadian rhythms. As was noted, the men were on rotating watch-standing schedules; their wake/sleep periods were altered, and no control of blood sampling time was possible. Thus the standard deviations are large and may therefore mask otherwise significant alterations in plasma calcium and phosphorus.

In spite of these circumstances, a significant increase in erythrocyte calcium was observed after three weeks' exposure to the submarine environment. This finding acquires additional importance in view of the subsequent return to initial values following one week's recovery in air. The calcium accumulation in the red cells, produced by prolonged exposure to low concentrations of CO_2, compliments the findings of Gortner et al[13] (1971) who observed a loss of red cell potassium and a gain of red cell sodium under the same conditions. This presents an in vivo demonstration in man, that calcium movements across the red cell membrane affect sodium and potassium exchange. In addition, these observations corroborate the in vitro findings of Davis and Vincenzi[8] (1971) and Romero and Whittam[37] (1971) showing that calcium accumulation in the erythrocyte controls sodium/potassium exchange through an increase in membrane permeability that does not necessarily involve metabolic inhibition.

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13. ABSTRACT

To ascertain the effects of acute and chronic hypercapnia on blood levels of calcium (Ca) and inorganic phosphorus (P_i), guinea pigs were exposed to 1%, 3%, and 15% CO_2 for various periods up to seven days. Plasma and RBC Ca and P_i and serum ionized Ca were determined. Blood from submariners exposed to up to 1% CO_2 for three weeks, followed by one-week recovery, was analyzed for plasma electrolytes and RBC Ca. The serum ionized Ca of guinea pigs during acute exposure to each concentration of CO_2 shows a pH dependent inverse relationship. During chronic hypercapnia, increase in total plasma Ca and decrease in P_i in those animals exposed to 3% and 15% CO_2 suggests increased parathyroid function. In those guinea pigs exposed to 1% CO_2, a depressed total plasma Ca in the presence of increased serum ionized Ca is interpreted as a possible functional hypoparathyroidism. In the guinea pigs, increases in RBC Ca and P_i, and plasma P_i accompanied by a decrease in total plasma Ca, during the acute phase of respiratory acidosis, are interpreted as pH-dependent inhibition of active transport and/or increase in membrane permeability. In the submariners exposed to up to 1% CO_2, both total plasma Ca and P_i tended to decrease although the changes did not become significant. RBC Ca, however, increased gradually and became significant after three weeks' exposure. This finding suggests inhibition of active transport and/or increase in RBC permeability to Ca. After one-week recovery in air, the RBC Ca had returned to control levels.

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