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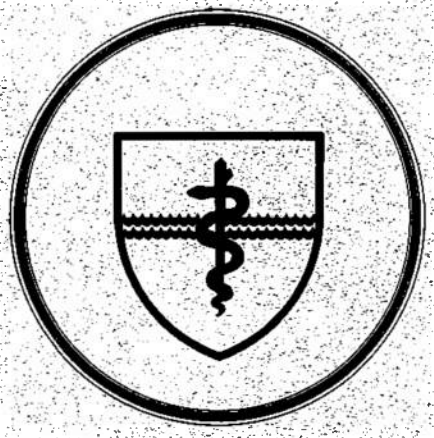

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BONE CO_2-TITRATION CURVES
IN ACUTE HYPERCAPNIA

by

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Bone CO_2-titration curves in acute hypercapnia obtained with a modified titration technique

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PASQUALE, STEPHEN M., ARTHUR A. MESSIER, MICHAEL L. SHEA, AND KARL E. SCHAEFER. *Bone CO_2-titration curves in acute hypercapnia obtained with a modified titration technique.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 48(1): 197–201, 1980.—Bone CO_2-titration curves were obtained in mature rats weighing 500–600 g. Animals were exposed for one hour to 1, 3, 5, 10, or 15% CO_2 in air. Measurements of bone CO_2 were made using a modified titrimetric analysis on fresh and oven-dried samples of paired rat femurs. A manometric method was used for comparison. Arterial blood samples were obtained for measurements of partial pressure of CO_2 in arterial blood (Pa_{CO_2}). Within the range of environmental CO_2 concentrations studied, a linear relationship was observed between the Pa_{CO_2} and the increment in fresh bone CO_2 content. This relationship is defined by the equation: Δfresh bone CO_2 (mmol/kg) = 61.8 + 0.68 Pa_{CO_2}. The CO_2 increment was confined to a heat-labile, presumably soluble pool comprising 10.5% of the total bone CO_2 content. No change in the water content of the bone was observed as a result of acute CO_2 exposure. The results of this study demonstrate the rapid in vivo CO_2 uptake of bone in response to exposure to increased CO_2 levels.

partial pressure of CO_2 in arterial blood; in vivo uptake of CO_2 by bone; heat-labile bone CO_2 pool

BONE IS THE MAJOR STORE for animal carbon dioxide, comprising about 80% of the total CO_2 storage capacity of the body (11). Bone is also known to be a major reservoir for electrolytes such as potassium, sodium, calcium, and phosphorus. It follows that the bone should play an important role in the maintenance of mineral and acid-base homeostasis. However, little information about CO_2 exchange in hypercapnia and other acid-base balance changes is available in the literature. The few studies that were carried out under conditions of hypercapnia, such as those of Freeman and Fenn (4) and Nichols (8), were limited to the measurement of dried bone CO_2 stores, which only represent fixed carbonates. Neumann and Mulryan (7) were able to show that the CO_2 content of synthetic hydroxyapatite crystals was markedly decreased upon heating. Using [^{14}C]bicarbonate in vivo, these authors were able to conclude that 60% of bone CO_2 was fixed in the crystal lattice and thereby unexchangeable, whereas 40% of the CO_2 resided in the hydration layer of the crystals where it could possibly exchange with the surrounding fluids. This finding has important implications for the buffering ability of bone. Using a titration method, Bursaux and Poyart (1) determined the CO_2 content of both fresh and dried paired rat femurs. The loss of CO_2 amounted to 20% of the total CO_2 content and was considered to represent half of the bicarbonate originally present. These authors also demonstrated an increase in bone CO_2 in vivo following mechanical ventilation of eight anesthetized rats for 1 h using 4–6% CO_2 in air. Subsequently, Poyart et al. (9) used a manometric technique to determine bone CO_2 content that provided further evidence for a bone bicarbonate pool. They found that fresh rat bone CO_2 values obtained with the manometric method were higher than those they had previously measured with the titration technique in animals of the same weight.

In our study of the effects of hypercapnia on bone buffering, both the titration and manometric techniques were used to establish a reliable method for the measurement of bone CO_2 content. A modified titration technique produced the best results in our hands.

The in vivo relationship between partial pressure of CO_2 in arterial blood (Pa_{CO_2}) and bone CO_2 in acute hypercapnia is reported herein.

MATERIALS AND METHODS

Separate groups of six mature Sprague-Dawley rats weighing 500–600 g were exposed for 1 h in environmental chambers (Sherer-Gilette) to 1, 3, 5, 10, or 15% CO_2 in 21% O_2 with the balance being N_2. The environmental temperature was kept at 25.6 ± 1°C. The gas mixtures were prepared by mixing proportional amounts of CO_2 with air. CO_2 and O_2 were added from high pressure cylinders. The air within the chamber was recirculated 12 times/min. With this fast and large turnover of chamber air, mixing of CO_2 and air was nearly instantaneous. The CO_2 concentration in the chamber was continuously monitored with a Beckman infrared analyzer and the O_2 concentration was sampled intermittently with a Beckman oxygen analyzer. In the 10 and 15% exposure, the CO_2 concentration was kept within ±0.5%. At lower levels, regulation was to within ±0.2%. O_2 was kept at 21 ± 0.5%. Prior to blood sampling, the animals received pentobarbital 40 mg/kg body wt intraperitoneally and were returned to the exposure chamber. The anesthesia was usually effective in approximately 5 min, at which time the animals were taken out of the chamber and immediately placed under a mask through which they breathed the same gas mixtures to which they had been exposed.

Blood samples were drawn from the abdominal aorta. Blood pH and P_{CO_2} were determined with an Instrumentation Laboratory blood gas and pH analyzing system. The femurs of both legs were removed, rapidly cleaned, and stripped free of adhering tissues and bone marrow. Specimens of compact bone between 200 and 300 mg were kept on ice for determination of total CO_2 content. The time between procurement and analysis of the fresh samples did not exceed 2 h. Paired specimens were oven-dried to a constant weight at 150°C for 18 h before analysis.

Bone CO_2, defined as the CO_2 liberated by bone upon complete dissolution in acid medium, was analyzed by indirect titrimetry employing the following modifications of the method of Bursaux and Poyart (1): *1*) the acid medium was heated to 65°C to facilitate complete CO_2 liberation in 6 h rather than 18 h; *2*) a second U tube containing 4 N HCl was added in series to obviate potential carryover of HCl vapors; *3*) $Ba(OH)_2$ was used as the CO_2-trapping medium rather than NaOH. This choice was made based on the observation of Davies (3) that $BaCO_3$ is sparingly soluble and does not precipitate in the pH range of 8–11; *4*) the concentration of CO_2-trapping agent and titrant were decreased by a factor of 50 to achieve greater sensitivity and smaller titration error; and *5*) 2-ml aliquots of $Ba(OH)_2$ were back-titrated with standardized HCl in duplicate to within ±0.005 ml in narrow-mouthed vials over a time period of no more than 30 s. Preliminary experiments were unable to detect any effect of atmospheric CO_2 on the normality of 0.1 N $Ba(OH)_2$ in less than 1.5 min. Figure 1 shows the schematic of the equipment used for bone CO_2 analysis. Seven of these apparatuses were arranged in parallel using the same pump system. Two sets of the apparatuses were employed for the measurement of bone CO_2 in control animals, the others served for analysis of bone CO_2 in experimental animals. Accuracy and precision of the analysis were evaluated by 20 replicate determinations using 20 mg samples of dried $CaCO_3$ that yield an amount of CO_2 approximately equivalent to a 250-mg bone sample. The individual samples contained 0.200 mmol CO_2, of which 0.198 mmol CO_2 (99%) was recovered. The relative standard deviation was 2.5%.

Bone CO_2 content was also measured in fresh and dry cortical bone samples (25–35 mg) with the manometric technique described by Poyart et al. (9). Triplicate analysis was performed on every bone. The water content of bone was determined from the weight loss after drying to a constant weight. Samples were weighed to 0.1 mg on an analytical balance. Group means were compared by Student's *t* test using uncorrelated group design.

RESULTS AND DISCUSSION

Manometric technique. By use of Warburg manometers filled with mercury, the size of the bone sample had to be in the range of 25–35 mg. With larger samples the danger existed that the large volume of CO_2 produced would push the Hg out of the manometer. In carefully controlled tests precise amounts of $CaCO_3$ were substituted for bone samples and CO_2 was generated according to the method of Poyart et al. (9). The results varied greatly up to 10% indicating that the use of Hg in the manometers made it difficult to get an absolutely accurate reading. This problem was compounded by the fact that a small sample was more difficult to handle in a short period of time and would dry out faster increasing the experimental error. The data obtained were generally somewhat higher than those measured with the titration technique in the same samples. We concluded that in our experience the manometric technique was inferior to the modified titration method described above.

Titration method. Results of the experiments in which the titration method was used are presented in Fig. 2. The total CO_2 content of fresh bone (upper graph) and of dried bone (lower graph) are plotted against $P_{a_{CO_2}}$ meas-

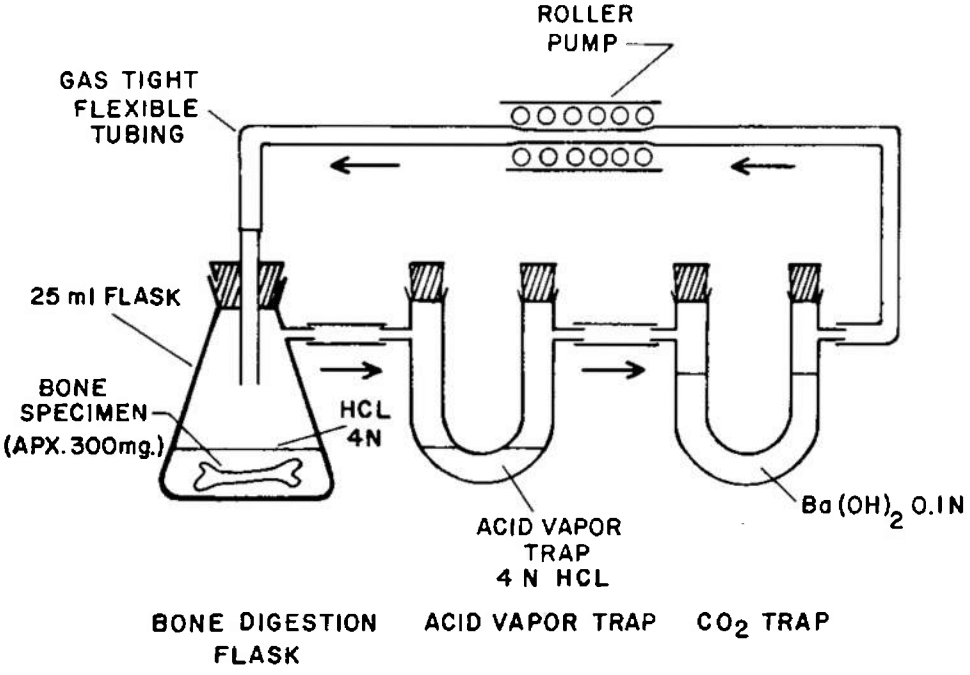

FIG. 1. Equipment used in analysis of bone CO_2 consisting of bone digestion flask, acid vapor trap, and CO_2 trap.

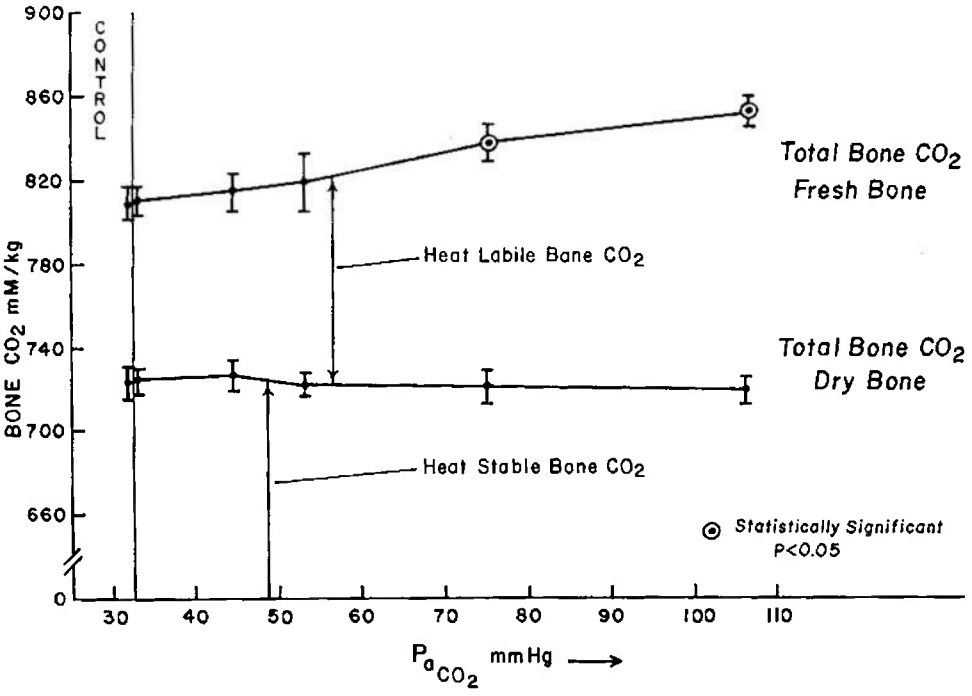

FIG. 2. Relation between bone CO_2 and arterial blood CO_2 tension. Fresh bone CO_2 content (*top curve*); dry bone CO_2 content (*lower curve*). The changes are restricted to fresh bone CO_2 contrast. Difference between fresh bone CO_2 content and dry bone CO_2 content equals heat-labile CO_2 content.

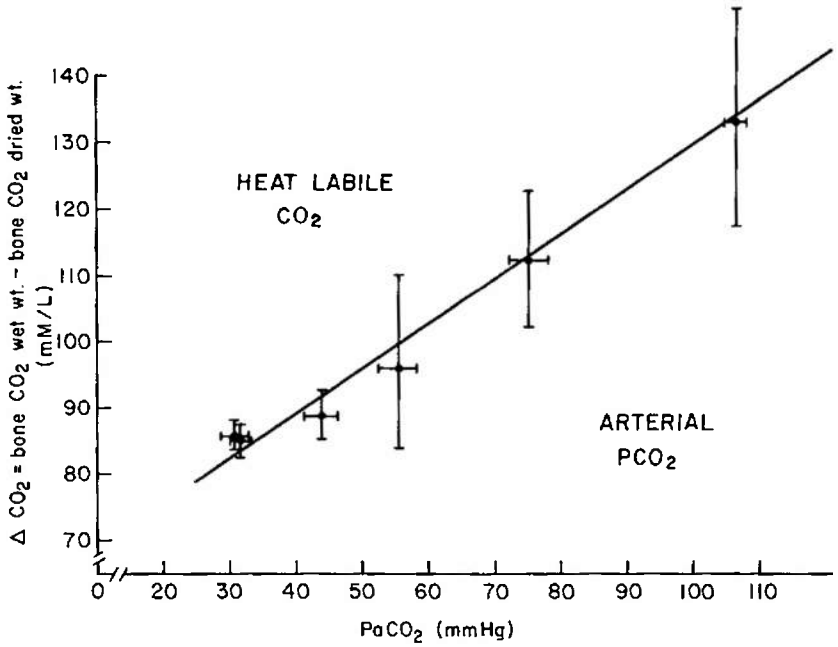

FIG. 3. Increment in heat-labile bone CO_2 as function of arterial CO_2 tension. Each individual point represents mean ± SE of 6 rats exposed to various levels of hypercapnia for 1 h; total no. of animals used was 36. Calculated regression equation: ΔBone CO_2 = 61.8 + 0.68 P_{CO_2} [slope = +0.68 ± 0.05 (SE of estimate); $r = 0.989$; $P < 0.001$; $n = 6$].

ured in the same animals. It should be noted that CO_2 content increased only in the fresh bone samples. In the dried samples, the CO_2 content remained at a constant base-line level, which was 89.5% of the total CO_2 content of fresh control specimens. Based on these data, a heat-labile bone CO_2 pool was defined as the difference between the CO_2 content of fresh and dried bones. It can be seen from Fig. 2 that the increment in bone CO_2 is confined to the heat-labile pool. The absolute change in this pool is therefore equivalent to the absolute change in total bone CO_2 content. Figure 3 shows the regression line for the increment in heat-labile bone CO_2 as a function of Pa_{CO_2}.

During the exposure to 15% CO_2, the increase in total bone CO_2 content amounted to 5.4% of the control level. The heat-labile pool, however, demonstrated a 56.5% increase.

The percent by weight of bone water showed no significant change as a result of CO_2 exposure. These results are summarized in Table 1.

The data on CO_2 content of dry and fresh bone obtained in these experiments before and during exposure to hypercapnic conditions are similar to those reported in the literature. Most of the published values of bone CO_2 content of rats are based only on the analysis of dry bone.

Considerable differences exist in data on CO_2 content of dry rat bone published in the literature. This variation appears to be due to at least three major factors: *1*) the age of the animals, *2*) the anatomic nature of the bone

TABLE 1. *Effects of 1-h exposures to various CO_2 concentrations*

%CO_2 in air	n	Pa_{CO_2}, Torr	Bone CO_2, mmol/kg wet wt		Heat-Labile CO_2		%Bone H_2O
			Total (*A*)	Dried (*B*)	(*A* − *B*)	% Increase	
Control	10	33.1	890	724	85		14.4
		±1.9	±9.2	±7.6	±4.9		±0.14
1	6	32.1	810	724	86		13.9
		±2.4	±5.7	±3.6	±5.3	1.2	±0.20
3	6	44.5	815	727	88		14.8
		±2.6	±8.7	±5.8	±7.4	3.5	±0.15
5	6	53.1	819	723	96		14.5
		±2.2	±14.2	±2.4	±14.0	12.9	±0.15
10	6	75.2	839*	722	117*		14.7
		±3.0	±3.1	±7.6	±5.3	37.6	±0.27
15	6	106.4	853*	720	133*		14.0
		±0.9	±6.6	±15.8	±16.0	56.5	±0.19

Values are means ± SE. * Statistically different, $P < 0.05$.

specimen, and 3) the treatment of the bone prior to analysis.

Kramer and Shear (5) showed that the CO_2 content of dry rat bones increases steadily from birth and is not stabilized until approximately 120 days of age. Poyart et al. (9) also observed an age/wt-related increase in CO_2 content of fresh bone. Composition varied in different parts of the same bone. CO_2 and mineral analyses of whole femurs (including both cortical and epiphyses) yielded lower concentrations than those reported for cortical bone alone. Freeman and Fenn (4) reported a value of 582 mmol/kg for animals 86–92 days old (weighing approx 150 g). Nichols (8) employed the method of Danielson and Hastings (2) and found 870 mmol/kg CO_2 for whole ashed femurs of mautre rats (290–400 g). Using Warburg nanometers, Poyart et al. (9) measured an average CO_2 content of 751 mmol/kg dry bone in rats weighing between 300 and 352 g. In our studies with the titration technique, the dried bone CO_2 content of mature rats (500–600 g) was found to be 724 mmol/kg. These values agree closely with those of Poyart et al. (9). Larsen et al. (6) using an adaptation of a standard vacuum-line technique measured 500–900 mmol CO_2/kg bone of dogs that is similar to values reported for dry rat bones.

Fresh bone CO_2 content has only recently been studied in conjunction with dry bone CO_2 content. Poyart et al. (9) determined an average of 893 mmol CO_2/kg fresh bone in rats weighing 300–352 g as compared to 809 mmol CO_2/kg fresh bone in rats weighing 500–600 g in our studies. The differences in absolute values of fresh bone CO_2 content obtained with the manometric and titration techniques were much larger than those observed for dry bone. This is most likely related to the small sample size employed with the manometric technique that is approximately 1/10 of that used in the titration technique. It is our experience that handling of such small bone samples (20–35 mg) using the Warburg manometers makes some loss of water practically inevitable which results in a higher measured bone CO_2 content.

The results of the study of bone CO_2 uptake in acute hypercapnia demonstrated a linear relationship between the arterial P_{CO_2} and the increment in fresh bone CO_2 content. The slope of this relationship was found to be 0.68 mmol CO_2/kg per Torr. Since the increment of bone CO_2 during acute exposure to different CO_2 concentrations for 1 h was limited to the heat-labile CO_2 pool; further discussion of the nature of this pool is in order.

The chemical nature and location of CO_2 in the bone are at present incompletely defined. Experiments by Neumann and Mulryan (7) have tentatively established at least two CO_2 pools in bone. Based on studies of synthetic hydroxyapatite crystals as well as in vivo labeling experiments with $^{14}CO_2$, these authors concluded that approximately 60% of bone CO_2 was associated with the crystal lattice of bone mineral. Over a 3-wk period, no CO_2 exchange was observed in this pool that was presumed to represent fixed carbonates. The remaining 40% of bone CO_2 was rapidly exchangeable and is presumed to represent HCO_3^-. They also found that the heating of bone results in a loss of CO_2. Approximately 47% of the $^{14}CO_2$ of the rapidly exchangeable HCO_3^- pool was lost upon heating bones from young animals.

Similar findings were obtained by Poyart et al. (1). They produced a steady $^{14}CO_2$ specific activity in the blood after a constant infusion lasting 30 min and found that bone samples heated to constant weight lost more than 50% of the $^{14}CO_2$ activity.

Bursaux and Poyart (1) attributed the CO_2 loss in bone upon heating to the hydration of HCO_3^- according to the following reactions: $2HCO_3^- \rightarrow CO_3^{-3} \downarrow + CO_2 \uparrow + H_2O$. From this reaction as a model, bone HCO_3^- could be calculated as twice the CO_2 lost upon heating. The present study established that the CO_2 increment during acute hypercapnia is confined to what is operationally defined as a heat-labile pool. The anatomic nature and chemical composition of this CO_2 space are not definitely known; however, the effect of heat on this pool suggests that it is largely a soluble pool. If it is assumed that this pool is limited to the aqueous phase of bone, and if the model of Bursaux and Poyart cited above for the dehydration of HCO_3^- is valid, then using our data for heat labile CO_2 and bone H_2O, the HCO_3^- concentration in bone water is calculated to be 1.2 M in control animals with an increase to 1.8 M (33%) after breathing 15% CO_2 in air for 1 h. These concentrations are quite high and suggest that the heat-labile pool may in fact be much larger than the aqueous phase alone.

In conclusion, the present study demonstrates the rapid in vivo uptake of CO_2 by bone. The acute increment in CO_2 is shown to be a linear function of Pa_{CO_2} and to be limited to a heat-labile pool.

This paper is designated Bureau of Medicine and Surgery, Navy Department Research Work Unit MF51.524.014-9024. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as officially reflecting the view of the Navy Department, the Naval Submarine Medical Research Laboratory, or the Naval Service at large.

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Bone CO_2 titration curves were obtained in mature rats weighing between 500-600 grams. Animals were exposed for one hour to 1%, 3%, 5%, 10%, or 15% carbon dioxide in air. Measurements of bone CO_2 were made, using a modified titrimetric analysis, on fresh and oven-dried samples of paired rat femurs. Arterial blood samples were obtained for measurements of blood P_aCO_2. Within the range of environmental carbon dioxide concentration studies, a linear relationship was observed between the arterial P_{CO_2} and the increment in fresh bone CO_2 content. This relationship is defined by the

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equation: Δ fresh bone CO_2 (mM Kg^{-1}) = $-23.7 + 0.69\ P_aCO_2$. The CO_2 increment was confined to a heat labile, presumably soluble pool comprising 10.5% of the total bone CO_2 content. No change in the water content of the bone was observed as a result of carbon dioxide exposure. The results of this study demonstrate the rapid in vivo CO_2 uptake of bone in response to exposure to increased carbon dioxide levels.