Changes in Bone Sodium and Carbonate in Metabolic Acidosis and Alkalosis in the Dog

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ABSTRACT Metabolic acidosis and alkalosis were produced in adult dogs over 5- to 10-day periods. Midtibial cortical bone was analyzed for calcium, sodium, phosphorus, and carbonate. In acidosis bone CO_3/Ca decreased 9.5% and bone Na/Ca decreased 6.3%. In alkalosis bone CO_3/Ca increased 3.1% and bone Na/Ca increased 3.0%.

Previous attempts to account for changes in net acid balance by summation of extra- and intracellular acid-base changes have uniformly resulted in about 40–60% of acid gained or lost being "unaccounted for." If it is assumed that changes in tibial cortex reflect changes in the entire skeletal system, changes in bone $CO_3^=$ are sufficiently large to account for the "unaccounted for" acid change without postulating changes in cellular metabolic acid production.

INTRODUCTION

In 1917 Van Slyke noted that the distribution of accumulated acid could not be accounted for by changes in extracellular HCO_3^- plus buffering (1). While initial explanation focused attention on intracellular buffers (1–3), there is a large body of evidence obtained by direct analysis of bone that $CO_3^=$ decreases in acidosis (4–7) and that this is associated with a decrease in both calcium (5–9) and sodium (4, 10, 11). Most of these studies were in growing rats. The two studies of bone in metabolic alkalosis are reports of increased sodium (11) and a suggestion of increased $CO_3^=$ in growing pigs fed large amounts of alkali (9). With the advent of a method of measurement of acid production, acid balance studies confirmed the existence of a tissue mechanism(s) involved in defense against continuing acid and alkali loads (12–14). The introduction of the DMO[1] technique permitted measurement of intracellular pH and the estimation of cellular buffer capacity by CO_2 titration curves (15–19). Reexamination of the distribution of acid gain or loss produced by short-term hemodialysis revealed a large component "unaccounted for" after summation of changes in both extra- and intracellular fluid (19). Acid balance studies in KCl-depleted and Cl-repleted dogs confirmed that a major portion of the net acid changes observed were also "unaccounted for" (20).

This inability to account for all of the added acid or alkali can be explained by alteration in the cellular rate of metabolic acid production (16, 21) or by the existence of changes in bone $CO_3^=$. The present study was undertaken to test the hypothesis that plasma acidity is important in determination of bone $CO_3^=$ and that the magnitude of bone $CO_3^=$ change is adequate to explain the "unaccounted for" acid gain or loss obtained by summation of changes in extra- and intracellular body fluid compartments.

METHODS

Adult mongrel dogs were used. Initial variance from dog to dog, largely a function of age, was so large that it was necessary to use each dog as its own control. After methoxyflurane anesthesia three full thickness mid-tibial bone biopsies were removed from one leg with a 3 mm dental drill. Mean sample weight was about 40 mg. Metabolic acidosis was produced in six dogs (mean weight 10.0 kg) over 5–10 days by daily feeding of NH_4Cl, 15 mEq/kg. Metabolic alkalosis was produced in nine dogs (mean weight 9.0 kg) over 4–6 days by feeding a low Cl diet, plus daily administration of 50 mg of ethacrynic acid, 0.5 mg/kg DOCA, and 18 mEq/kg $NaHCO_3$. The dogs were then sacrificed and mid-tibial bone slices were taken from the other leg by using a high speed dental saw. Both biopsies and slices were frozen in liquid nitrogen (LN_2). For analysis, the frozen samples were powdered in a steel mortar and dried of condensed moisture and CO_2 in a standard vacuum oven at room temperature for 30 min. Triplicate samples of ±25 mg

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[1] Abbreviations used in this paper: DMO, 5,5-dimethyl-2,4-oxazolidinedione; DOCA, deoxycorticosterone acetate.

were weighed into 1.5 ml polyethylene vials and sealed. The contents of each vial were then analyzed for Na and Ca by neutron activation. The same bone powder was then analyzed for CO_2 manometrically. It is recognized that the eluted CO_2 could just as well come from HCO_3^- as from $CO_3^=$. However, since X-ray diffraction studies suggest that most bone CO_2 exists as $CO_3^=$ (22), the term $CO_3^=$ is used throughout. The eluate was then recovered and analyzed chemically for Ca and P.

Neutron activation analysis (*NA*). For calcium, the triplicate samples and one standard (15 mg dried $CaCO_3$) were irradiated in a thermal neutron flux of 8×10^{11} neutrons/(cm^2 sec) for 5 min. Counting was done with a Baird Atomic Spectrometer connected to a gamma scintillation detector, 3 × 3 in. NaI (Tl). The discriminator was adjusted to reject scintillations below 2.9 Mev, just below the ^{49}Ca peak of 3.10 Mev ($T_{\frac{1}{2}} = 8.8$ min). Because the ^{24}Na peak is 2.76 Mev ($T_{\frac{1}{2}} = 14.7$ hr), it gave discernible interference so that it was necessary to recount ^{24}Na 3 hr later, when ^{49}Ca had decayed, adjust to zero time, and subtract from the original counts. Coefficient of variation (CV) of the method was 2.58%.

For Na determination the sample vials were placed in cylindrical Micarta holders with a standard (0.3180 mg dried Na_2CO_3) on each layer to monitor spatial flux variations and irradiated for 12 min in a thermal neutron flux of 7×10^{11} neutrons/(cm^2 sec). Counting of the gamma scintillations was delayed for 24–36 hr. At this time only ^{24}Na was left above the discriminator level of 2.2 Mev. Counting was performed by an automatic sample changer and printer using a 3 × 3 in. NaI (Tl) well crystal. As the polyethylene vials contained about 0.5 μg Na, a blank vial was included in each irradiation to serve as the background count. The CV was 1.11%.

Carbonate determination. After neutron activation analysis the sample vial was cut in half and the lower half dropped into the sidearm opening of a reaction tube with a ground glass tip, and a 7 × 2 mm magnetic stirring bar added. A sidearm bulb containing 2 ml of 2 N HCl was attached with the bulb down, the entire unit attached to a manometric system which had been evacuated to less than 0.001 mm Hg. The HCl in the sidearm was frozen with LN_2 and the reaction tube then evacuated to at least 0.005 mm Hg. The flask was sealed off, the HCl thawed, and the sidearm rotated so that the acid would flow over the bone powder. The acid and carbonate were allowed to react with stirring for 3 min. In order to remove H_2O, the CO_2 was passed through an acetone–dry ice dewar before freezing in a LN_2 dewar. This reaction is complete in 2 min and must be timed carefully to avoid the collection of HCl vapor which occurs later. The LN_2 dewar was then removed and the CO_2 thawed and allowed to escape into the manometer, where the displacement caused by the CO_2 was read by a cathetometer. The mmoles of CO_2 were then calculated from the gas law equation $PV = nRT$. The CV was 1.11%. Comparison of biopsy and slice sampling methods and samples from right and left leg, radius and tibia, revealed no significant differences, $P = >0.80$.

Chemical determination of calcium and phosphorus (*AA*). The water captured in the acetone-dry ice dewar was pulled back into the reaction tube by immersing it in LN_2 for 8 min. The tube was disconnected, 2 ml of 6 N HCl was added, and it was placed in a boiling water bath for 30 min to hydrolyze the bone powder completely. The contents were then transferred quantitatively to a volumetric flask and Ca and P determinations run on the Technicon AutoAnalyzer (23, 24). The CV for the Ca method was 1.62% and for the P method 1.54%.

RESULTS

Results in Table I are expressed as milliequivalents per gram wet bone powder. *P* values were calculated by the Student *t* test for paired observations and are considered significant if $P = <0.05$. In Table II and Fig. 1, each individual result is expressed as mEq ratio CO_3/Ca and Na/Ca using neutron activation analysis. In metabolic acidosis the mean decrease in plasma [HCO_3^-] was from 26.9 to 12.1 mEq/liter. Blood pH fell from 7.37 to 7.21. Bone $CO_3^=$ fell from 1.405 mEq/g to 1.268 mEq/g (−9.8%), $P = <0.001$. Bone Na fell from 0.2587 to 0.2413 mEq/g (−6.7%), $P = <0.01$. Changes in Ca and PO_4 were not significant. The mean decrease in CO_3/Ca was from 0.1262 to 0.1142, a decrease of 9.5%, $P = <0.01$. The mean decrease of Na/Ca was from 0.02321 to 0.02175, a decrease of 6.3%, $P = <0.001$.

TABLE I

*Tibial Bone Cortex Changes in Metabolic Acidosis and Alkalosis**

Bone	Acidosis (n = 6)					Alkalosis (n = 9)				
			Change					Change		
	Pre	Post	mEq/g	%	*P* value	Pre	Post	mEq/g	%	*P* value
CO_3	1.405	1.268	−0.137	−9.75	<0.001	1.583	1.641	+0.058	+3.66	<0.02
Na	0.2587	0.2413	−0.0174	−6.72	<0.01	0.2714	0.2813	+0.0099	+3.64	<0.02
Ca(NA)	11.144	11.098	−0.046	−0.41	>0.50	12.108	12.176	+0.068	+0.56	>0.50
Ca(AA)	10.326	10.393	+0.067	+0.64	>0.50	11.179	11.158	−0.021	−0.18	>0.50
PO_4	10.033	10.024	−0.009	−0.08	>0.50	10.672	10.580	−0.092	−0.86	>0.20
$[HCO_3^-]_e$	26.9	12.1		−55.0		25.1	45.6		+81.7	

(NA) = neutron activation; (AA) = AutoAnalyzer.
* All results are in mEq/g wet bone powder. $[HCO_3^-]_e$ = plasma [HCO_3^-] in mEq/liter. *P* values were performed by Student *t* test.

TABLE II
*Results of Mid-Tibial Bone Cortex Analyses in Metabolic Acidosis and Alkalosis, Using Ca as Base Reference**

Dog	Control			Acidosis			Dog	Control			Alkalosis		
	CO_3/Ca	Na/Ca	$[HCO_3^-]_e$	CO_3/Ca	Na/Ca	$[HCO_3^-]_e$		CO_3/Ca	Na/Ca	$[HCO_3^-]_e$	CO_3/Ca	Na/Ca	$[HCO_3^-]_e$
1	0.1247	0.02404	25	0.1211	0.02243	11	8	0.1308	0.02347	25	0.1377	0.02421	42.5
2	0.1341	0.02362	27	0.1144	0.02197	12	9	0.1340	0.02226	24	0.1401	0.02320	41
3	0.1161	0.02226	28	0.1049	0.02086	11.5	10	0.1365	0.02252	26.5	0.1378	0.02285	48
4	0.1200	0.02269	29	0.1105	0.02174	10.5	11	0.1265	0.02181	23.5	0.1309	0.02242	43.5
5	0.1358	0.02388	29.5	0.1234	0.02182	17.5	12	0.1249	0.02217	21.5	0.1280	0.02306	45
6	0.1262	0.02278	23	0.1110	0.02166	10	13	0.1342	0.02270	21	0.1351	0.02389	52.5
							14	0.1330	0.02184	28	0.1351	0.02261	43
							15	0.1320	0.02262	31	0.1378	0.02284	46.5
							16	0.1240	0.02242	26	0.1302	0.02284	48.5
Mean	0.1262	0.02321	26.9	0.1142	0.02175	12.1		0.1307	0.02242	25.1	0.1347	0.02310	45.6
SD difference				±0.0054	±0.00039						±0.0022	±0.00030	
P value				<0.01	<0.001						<0.001	<0.001	
% change				−9.50	−6.29						+3.06	+3.03	

* All results are in mEq ratios. $[HCO_3^-]_e$ = plasma $[HCO_3^-]$ in mEq/liter. *P* values were performed by Student *t* test.

In metabolic alkalosis the mean increase in plasma $[HCO_3^-]$ was from 25.1 to 45.6 mEq/liter. Blood pH rose from 7.37 to 7.54. The mean increase in bone $CO_3^=$ was from 1.583 to 1.641 mEq/g (+3.7%), $P = <0.02$. Bone Na increased from 0.2714 to 0.2813 mEq/g (+3.6%), $P = <0.02$. The mean increase in CO_3/Ca was from 0.1307 to 0.1347, and increase of 3.1%. The mean increase in Na/Ca was from 0.02242 to 0.02310, an increase of 3.0%. Carbonate and Na changes in relation to Ca are significant, $P = <0.001$. In metabolic alkalosis changes in Ca and PO_4 are not significant as related to wet weight, but Ca increase in relation to PO_4, or PO_4 decrease in relation to Ca is significant, $P = <0.01$ (NA) $P = 0.02$ (AA).

DISCUSSION

Although there has been considerable past interest in bone and acid-base metabolism, as described in the introduction, our interest was rekindled by failure to account for all of the acid balance changes by summation of changes in body fluids in studies in dogs (19, 20). The implication was that either bone was more important than generally viewed, or that metabolic cellular events played an important role (16, 21). Since it was postulated that analysis of the kinetics of cellular change argued against the importance of cellular metabolism (19), a direct reexamination of bone in adult animals was indicated.

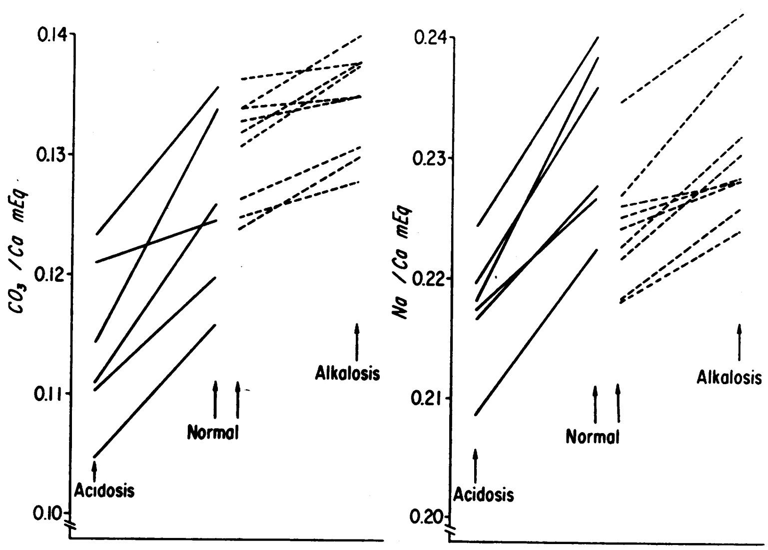

FIGURE 1 Changes in mid-tibial bone CO_3 and Na as related to Ca in metabolic acidosis and alkalosis.

The results of direct analysis of bone in adult dogs demonstrate that bone is a source of HCO_3^- in acidosis and a reservoir for storage of excess HCO_3^- in metabolic alkalosis. From the standpoint of body fluid acid-base chemistry, these changes are large. In 2-hr acidosis experiments, 15 of 35 mEq (43%) of an acid load was calculated to have been buffered by bone $CO_3^=$ (19). In 4-hr alkalosis experiments 29 of 75 mEq (38%) of HCO_3^- transferred from dialysate to dog apparently accumulated in bone. In the metabolic alkalosis accompanying KCl depletion for 5–7 days, 32 mEq of a 67 mEq negative acid balance (48%) and in Cl repletion 65 of 98 mEq of a positive acid balance (65%) were postulated to have entered bone as $CO_3^=$ (20). These data are in general agreement with balance studies in man that a major portion of the buffering is in tissues (3, 12, 13).

In the present experiments acid balances were not performed. However, since the degrees of acidosis and alkalosis produced were of the same order of magnitude as in the previous studies, a comparison is warranted.

The previous studies involved rather gross estimates of acid balance; nonetheless, the per cent unaccounted for by summation of body fluid changes varied from 38% to 65% of the net acid balance change. In metabolic acidosis produced by hemodialysis, 43% of HCO_3^- removed from the dog apparently came from bone (19). In the longer-term studies of metabolic acidosis herein reported there was a 9.5% decrease in CO_3/Ca. If it is assumed that changes in tibial cortex reflect changes throughout the skeleton and that a dog has 3400 mEq Ca/kg body weight (25, 26), then mEq Ca/kg $\times$ kg $\times$ CO_3/Ca change (mEq) = total $CO_3^=$ change (mEq). This becomes $3400 \times 10.0 \times (0.1262 \times 0.095) = -408$ mEq $CO_3^=$. Even though the net acid balance associated with the decrease of 408 mEq in bone $CO_3^=$ is unknown, this amount in general terms appears consistent with the indirect estimate of 43% of a 38 mEq load in 2 hr, and with large amounts of acid buffered by tissues during NH_4Cl loading (13, 14).

Comparison of acid balance data and direct bone data from our laboratory is possible in metabolic alkalosis. In balance studies of metabolic alkalosis ($HCO_3^- = 42$ mEq/liter) induced by KCl depletion and repair by Cl repletion (20), the calculated bone change per 15.6 kg dog was 48 mEq. In the present studies [$HCO_3^- = 45$ mEq/liter) the directly measured bone $CO_3^=$ change in 9-kg dogs was 120 mEq ($3400 \times 9.0 \times 0.1307 \times 0.03$). While these are gross comparisons, it appears that the measured bone changes are sufficient to explain the "unaccounted for" negative acid balance without having to implicate changes in cellular metabolic acid production or buffer capacity.

Bone crystals have been described as nonhomogeneous with isomorphic substitutions producing variable composition from part to part of the crystal. Bone has been conceptualized as an equilibrium with moment to moment interchanges of ions between crystal surface and extracellular fluid (*27*). The observation that the composition of bone crystals from uremic humans parallels the high [$PO_4^=$] and low [HCO_3^-] of the bathing media confirms this idea (28). The present observations provide further extension of such a concept, namely, that the bone crystal $CO_3^=$ content is in equilibrium with the [HCO_3^-] content of extracellular fluid. However, there is nothing in the present experiments which differentiates changes in pH from change in [HCO_3^-].

In both acidosis and alkalosis the mEq ratio of CO_3/Na mobilized or deposited in the bone is 6–8:1, the same as in normal bone. The ratio is constant, whether one uses wet weight, Ca, or PO_4 as a reference base. This result requires that another cation moves with sodium or that there is anionic exchange. Both magnesium and citrate exist in such small amounts that they seem unlikely to play an important role. Unfortunately, the question of Ca movement and(or) $PO_4/CO_3^=$ exchange must remain unresolved. Even though analytic methods permit identification of changes near 1–2% for Ca and P, the over-all amounts are so large that significant changes would be unidentifiable. Nonetheless, statistical analysis of our data suggests either Ca increases in alkalosis in reference to PO_4 or PO_4 decreases in reference to Ca ($P = 0.02$). Some skepticism should exist, however, because it would be expected that opposite changes would be observed in acidosis. The bone $CO_3^=$ changes in acidosis were greater and yet changes in Ca/P were not identified ($P = >0.20$).

In order to reconcile the data herein reported with prior knowledge, it appears that one must postulate in metabolic acidosis two separate phases of bone buffering. The appearance within 2–3 hr of large amounts of HCO_3^- (1–3, 19) suggests that the first phase would be a rapid, dynamic equilibrium (hours) between extracellular fluid [HCO_3^-] or [H^+] and bone $CO_3^=$. The $CO_3^=$ changes may well be a surface phenomenon and are not dependent upon Ca mobilization because during the short time period no significant mobilization of Ca or P takes place. The second phase would be the long-term (days) mobilization of $CO_3^=$ from bone seen in more chronic metabolic acidosis (4–7, 12–14). This requires both lowering of extracellular [HCO_3^-] and mobilization of Ca and P (5–9, 12, 14).

In metabolic alkalosis, evidence of two phases is less clear. Short- (19) and long-term balance studies (13, 20) and the direct bone data herein reported suggest $CO_3^=$ deposition in bone. However, there is not yet clear separation of short- and long-term effects and our direct studies suggest that the $CO_3^=$ deposition is in excess of Ca and may involve CO_3/P exchange rather than new bone formation.

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