invited review

Am J Physiol Renal Physiol 285: F811–F832, 2003;
10.1152/ajprenal.00115.2003.

Bone buffering of acid and base in humans

Jacob Lemann, Jr.,[1] David A. Bushinsky,[2] and L. Lee Hamm[1]

[1]*Nephrology Section, Tulane University School of Medicine, New Orleans, Louisiana 70130-5927; and* [2]*Nephrology Unit, University of Rochester School of Medicine, Rochester, New York 14642*

Lemann, Jacob, Jr., David A. Bushinsky, and L. Lee Hamm. Bone buffering of acid and base in humans. *Am J Physiol Renal Physiol* 285: F811–F832, 2003; 10.1152/ajprenal.00115.2003.—The sources and rates of metabolic acid production in relation to renal net acid excretion and thus acid balance in humans have remained controversial. The techniques and possible errors in these measurements are reviewed, as is the relationship of charge balance to acid balance. The results demonstrate that when acid production is experimentally increased among healthy subjects, renal net acid excretion does not increase as much as acid production so that acid balances become positive. These positive imbalances are accompanied by equivalently negative charge balances that are the result of bone buffering of retained H^+ and loss of bone Ca^{2+} into the urine. The data also demonstrate that when acid production is experimentally reduced during the administration of $KHCO_3$, renal net acid excretion does not decrease as much as the decrease in acid production so that acid balances become negative, or, in opposite terms, there are equivalently positive HCO_3^- balances. Equivalently positive K^+ and Ca^{2+} balances, and thus positive charge balances, accompany these negative acid imbalances. Similarly, positive Na^+ balances, and thus positive charge balances, accompany these negative acid balances during the administration of $NaHCO_3$. These charge balances are likely the result of the adsorption of HCO_3^- onto the crystal surfaces of bone mineral. There do not appear to be significant errors in the measurements.

acid production; acid excretion; acid balance; charge balance; calcium balance; potassium balance; sodium balance; organic anions; sulfate; ammonium

A RECENT EDITORIAL REVIEW of the balance of acid, base, and charge in health and disease (18) suggests that there is continuing controversy regarding the sources of fixed acid or base production relative to the routes and mechanisms for the buffering and excretion of acid or base and thus the maintenance of net external acid balance in health and disease. The quantitative significance of bone in buffering of acid and base has also been questioned (59, 60).

This report reviews the development of techniques of evaluating the sources and rates of fixed acid production in relation to renal net acid excretion, thus allowing assessment of acid balance in humans. The changes from control that occur when acid production is increased or decreased will be used to clarify the responses of both acid production and excretion and of acid balances and mineral balances. We also review the possible sources of error in the measurements of acid production and renal net acid excretion and the relationship of charge balance to acid balance.

Address for reprint requests and other correspondence: J. Lemann, Jr., Nephrology Section, Tulane Univ. School of Medicine, 2601 St. Charles Ave., New Orleans, LA 70130-5927 (E-mail: dr.jack@lemann.net).

The results demonstrate that renal net acid excretion does not increase as much as acid production when acid production is experimentally increased among healthy subjects, so that acid balances become positive. This imbalance is accompanied by equivalently negative charge balances that are the result of bone buffering of retained H^+ and loss of Ca^{2+} from bone into the urine. The data also demonstrate that when acid production is experimentally reduced renal net acid excretion does not decrease as much so that acid balances become negative, or, in opposite terms, there are equivalently positive HCO_3^- balances. This imbalance is accompanied by equivalently positive K^+ and Ca^{2+} balances, and thus positive charge balances during the administration of $KHCO_3$, or positive Na^+ balances and thus positive charge balances during the administration of $NaHCO_3$. These charge balances are likely the result of the adsorption of $KHCO_3$ or of $NaHCO_3$ onto the crystal surfaces of bone mineral.

The evaluation of potential errors in the measurements required to quantitate acid production and net acid excretion shows that the contribution of intestinal absorption of dietary acid or base must be directly evaluated by measuring dietary and fecal composition and cannot be indirectly assessed by measurements of

urinary composition. These assessments also show that there are small errors, both positive and negative, that affect the measurement of urinary organic anions as a component of acid production. There is, however, no evidence for the existence of urinary cations, other than NH_4^+, that contribute to net acid excretion.

EVALUATION OF ACID-BASE BALANCE IN HEALTHY HUMANS

The many biochemical reactions of metabolism result in the production and consumption of acids and bases. In the steady state in health, the state of titration of body buffers and thus the acidity of body fluids and tissues, as reflected by pH or H^+ concentration ($[H^+]$), are closely regulated and stable. Within the readily sampled extracellular fluid (ECF; blood serum or plasma) where the CO_2 /bicarbonate buffer system is quantitatively most important, the plasma HCO_3^- concentration ($[HCO_3^-]$) and P_{CO_2} (proportional to H_2CO_3) concentrations are stable. Thus blood pH or $[H^+]$ is also stable. Because the buffering capacity and acidity of body compartments with which the plasma is in equilibrium are steady in health, normal adults must be in balance (e.g., intake-output = 0) with respect to the daily production and excretion of hydrogen ions (or actual or potential HCO_3^-). The rate of production of the volatile acid H_2CO_3 from neutral fats, carbohydrates, and proteins is matched by an equivalent rate of excretion of CO_2 by the lungs. Similarly, in health there is daily production of nonvolatile or "fixed" acids. When such acids are produced, the protons (H^+) liberated by the dissociation of the acid react immediately with body buffers that can be represented by plasma $[HCO_3^-]$, resulting in the formation of H_2CO_3 that is transported to the lungs and excreted as CO_2. Obviously, ongoing consumption of body buffers by such a mechanism could not continue for any significant time without regeneration of HCO_3^-. Therefore, it can also be assumed that the net rate of fixed acid (e.g., noncarbonic acid) input each day must be matched by an equivalent rate of acid removal so that net external (fixed) acid balance must equal zero. Such a process is analogous to the equivalence of metabolic H_2CO_3 (CO_2) production and pulmonary excretion.

Many studies during the first half of the 20th century led to an understanding that in health the kidneys excrete acid. These processes include the following:

1) The titration of filtered buffers, chiefly the titration of HPO_4^{2-} to $H_2PO_4^-$ (termed titratable acid), that are measured by titration of the urine from urinary pH to blood pH, usually pH 7.4 or estimated by calculation based on the urinary phosphate concentration, urinary pH, and the $pK_{a2} = 6.8$ for the dissociation $H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+$ and on the urinary concentration of creatinine (see below).

2) The production and excretion of ammonium (NH_4^+) by renal tubular cells from neutral precursors to form urinary NH_4^+ (measured as total urinary NH_4^+ plus the negligible amounts of NH_3 when urine pH $\leq$7.5). The protons (H^+) thus added to the urine are, ultimately or in effect, derived from H_2CO_3 and result in the simultaneous addition of equivalent quantities of HCO_3^- to renal venous blood. The excretion of NH_4^+ is quantitatively equal to the net HCO_3^- generated from the metabolism of glutamine to NH_4^+ and HCO_3^- . Thus the NH_4^+ excreted is equivalent to the H^+ excreted and the new HCO_3^- regenerated (2).

3) Additionally, under most conditions, nearly all of the HCO_3^- that appears in the glomerular filtrate is reabsorbed, thus preventing urinary loss of filtered HCO_3^- (base).

Thus during the steady state in health, the daily net rate of acid excretion by the kidneys can be quantitated as urinary titratable acid plus NH_4^+ less any filtered HCO_3^- escaping renal tubular reabsorption and excreted into the urine. This quantity is termed renal net acid excretion. The overall excretion of net acid is now known to be accomplished by multiple transport processes along the nephron, including Na^+/H^+ exchange in both the proximal and distal tubules (7) and by both an H^+-ATPase and an H^+-K^+-ATPase as well as by HCO_3^- secretion in distal nephron segments (14, 27, 57).

Because protons (H^+) are always available throughout body fluids and thus cannot be directly traced, the techniques for identifying and quantifying the sources of fixed acid production are, necessarily, indirect. Measurements of metabolic products (conjugate bases, anions) that, by biochemical reasoning, must have been associated with the production or consumption of H^+ are thus required. Early in the 20th century, several sources of fixed acid production had already been recognized; these can be detected based on urinary constituents:

1) The urinary excretion of organic acid anions reflecting either the ingestion and absorption of nonmetabolizable free organic acids in the diet, the formation of acids as end products of metabolism such as uric and oxalic acids; or the incomplete oxidation to $CO_2 + H_2O$ of acids normally produced during metabolism, such as lactic and citric acids (and acetoacetic and β-hydroxybutyric acids during starvation or diabetic ketoacidosis, etc.), accompanied by the production, retention and buffering of an equivalent quantity of H^+ (73). Urinary organic anions have also been referred to as representing "loss of potential base," but this is correct only with respect to those urinary organic anions such as citrate, lactate, pyruvate, etc. that could have been metabolized to HCO_3^- if retained within the body. Those urinary anions that reflect the formation of acid end products of metabolism such as oxalate, urate, etc. identify acid production.

2) The urinary excretion of SO_4^{2-}, reflecting the oxidation of sulfur contained in the neutral amino acids methionine, cysteine, or cystine of dietary protein or endogenous tissue proteins during starvation (28, 41, 71) that is also accompanied by the generation of an equivalent quantity of H^+. Additionally, it was known that normal foods in the diet contain bases, as actual HCO_3^- or potential HCO_3^- as inorganic cationic salts (principally K-salts) of metabolizable organic anions such as citrate, acetate, etc. (71).

Whether acid or base might be excreted into the feces in health had not been clarified, although large fecal losses of actual or potential HCO_3^- with severe diarrhea as in cholera had been known for a century or more (70). However, techniques had not yet been developed that might permit, among healthy adults in the steady state, both identification of the sources of acid production as well as a quantitative comparison of the daily rate of acid production to the daily rate of renal net acid excretion.

The original impetus for such studies arose from observations among patients with advanced chronic kidney disease who exhibited low, but stable, serum [HCO_3^-] associated with low rates of renal net acid excretion related to the low rate of urinary NH_4^+ excretion that accompanies advanced kidney diseases (67). By contrast, healthy subjects eating comparable diets had normal serum [HCO_3^-] and much higher rates of NH_4^+ and, hence, net acid excretion. These disparate observations led to consideration of two alternative explanations: *1*) the stabilization of serum [HCO_3^-] among the patients with chronic kidney diseases resulted from a reduction in the daily rate of fixed acid production equivalent to the limitation in net acid excretion caused by the kidney disease; or *2*) normal rates of fixed acid production continued, and during acidosis additional routes for acid excretion or disposal (buffering) might become operative, serving to stabilize the low serum [HCO_3^-]. A review of the acid balances and bone among patients with renal failure is presented in a subsequent section of this review.

Assessment of the Sources of Acid Production and Acid Balances Among Healthy Adults Using Liquid-Formula Diets

The original technique of quantitating acid production compared with net acid excretion among healthy adults utilized a liquid-formula diet (65). That diet contained glucose, cornstarch hydrolysate (dextrin), corn oil, and a purified soy protein. Na^+ was provided as NaCl. K^+ was provided as KCl. The soy protein was prepared at its isoelectric point and contained essentially no Na^+, K^+, Ca^{2+}, Mg^{2+}, or Cl^-. However, the protein did contain PO_4 (17 mmol/100 g), presumably mostly as esters of the hydroxylated amino acids serine and threonine. For some of the original studies (65), the metabolism of this esterified PO_4, effectively to H_3PO_4, was considered to quantitatively represent acid production when H_3PO_4 was subsequently present as HPO_4^{2-} and $H_2PO_4^-$ in blood in a ratio of 4:1 at pH = 7.4, at which pH the valence of PO_4 = 1.8. For a few of the original studies (65) and all subsequent studies using soy protein formulas, 0.45 mmol = 0.9 meq of $Ca(OH)_2$ and 0.45 mmol = 0.9 meq $Mg(OH)_2$/mmol PO_4 in the protein were added to the formula, thus neutralizing to pH 7.4 the potential acid from the PO_4 in the protein. Because the other mineral salts in these diets were neutral (NaCl, KCl), the estimated unmeasured anion of these soy formulas was approximately zero, both by calculation and based on analyses of the diets {e.g , Σ ($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$), meq/day $-$ Σ [(Cl^-) + ($1.8 \cdot PO_4$), meq/day] = 0}. Moreover, because the formulas did not contain any fiber, the subjects defecated on average only every 2.5 days, and average fecal weight was only 45 g/day. Consequently, for those studies possible excretion of acid or base into the feces was considered minimal and was neglected.

Utilizing these formula diets, we observed that among healthy subjects, studied when their serum [HCO_3^-] was normal and stable, mean daily net fixed acid production, estimated as urinary SO_4^{2-} + urinary organic acids, was, on average, identical to the mean daily rate of net acid excretion. Thus the net external fixed acid balance was zero, the subjects being in balance with respect to fixed acid production and excretion (65). Additional studies using formula diets were also carried out to assess the effects of experimental alterations in acid production on acid balance achieved by either increasing net fixed acid production among healthy subjects by the administration of NH_4Cl or reducing net fixed acid production by the administration of $NaHCO_3$ (36). Those studies showed that NH_4Cl administration caused systemic acidosis but that the consequent increase in net acid excretion was less than the increase in acid production (= SO_4^{2-} + organic acids + the NH_4Cl load) so that acid balances became positive (36). Moreover, $NaHCO_3$ administration reduced fixed acid production, but the decrease in renal net acid excretion was not as large so that acid balance became negative (i.e., there was positive base balance) (36). Serum [HCO_3^-] was steady during these studies of NH_4Cl or of $NaHCO_3$ loading.

In summary, studies using neutral liquid-formula diets demonstrated that fixed acid production was identified by the urinary excretion of SO_4^{2-} + organic anions, the sum of these two matching the simultaneous rate of renal net acid excretion so that healthy adults with normal and stable serum [HCO_3^-] were in acid balance. Furthermore, the failure of net acid excretion to increase as much as did acid production during NH_4Cl loading or to decrease as much as did acid production during $NaHCO_3$ loading implied the retention of acid or base (HCO_3), respectively, at sites outside the ECF or the apparent space of distribution for HCO_3.

Assessment of the Sources of Acid Production and Acid Balances Among Healthy Adults Using Normal Whole-Food Diets

During the original development of the methods to assess the components of acid production and excretion (41, 65), it again became evident that normal whole-food diets contain base as actual HCO_3^- or potential HCO_3^- as inorganic cationic salts (principally K-salts) of metabolizable organic anions such as citrate, acetate, etc. The precise identity and quantity of such base(s) ingested each day in the diet could not be directly measured. However, that quantity could be indirectly estimated as the difference between the dietary content of inorganic cations (Σ $Na^+ + K^+ +$

Ca^{2+} + Mg^{2+}, meq) and of inorganic anions [Σ Cl^- + (1.8· PO_4), meq] (43). It was also observed that the feces similarly contain actual or potential base, known to be principally acetate, propionate, and butyrate (43). Once again, the precise identity and quantity of such fecal base(s) could not be directly measured. However, the daily fecal excretion of such base could similarly be estimated using the same calculation applied to the analyses of the diet (43). The difference between the dietary intake of unmeasured anion and the fecal excretion of unmeasured anion thus represented the net intestinal absorption of unmeasured anion, generally a positive number, reflecting addition of actual or potential HCO_3^- to the body. During the original studies of this type (43), it was observed that for a group of 16 healthy adults fed various diets that fixed acid production measured in this manner averaged 59 ± 30 (SD) meq/day and net acid excretion averaged 60 ± 21 meq/day. Accordingly, mean acid balance for this group was not different from zero ($P > 0.7$), averaging −1 ± 12 meq/day (43). The individual data for net acid excretion in relation to fixed acid production are compared among these subjects in Fig. 1 (43).

Effects of Base or Acid Administration to Healthy Human Subjects: Results of Paired Studies During Control Periods and During Acid or Base Administration Periods in the Same Subjects

Obviously, multiple analyses of diets, feces, and urine are required for the measurement of acid balance, and multiple analytic errors might significantly bias the results. However, when groups of subjects are studied while eating individually constant diets during both control conditions and during the continuous administration of additional acid or base, the analytic variations should be minimized. Therefore, the changes from control (Δ) in the components of the acid balance should clarify the processes involved in the consequent increases or decreases in acid production, the accompanying increases or decreases in renal net acid excretion, the directional changes in acid balance, and the distribution (buffering) of retained base or acid. Moreover, the resulting changes from control in the balances of other minerals and of charge balance can also be evaluated.

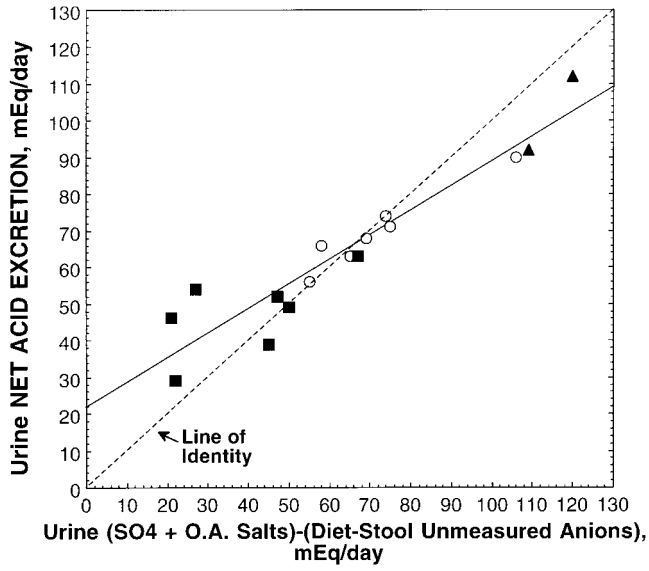


Fig. 1. Daily urinary net acid excretion (titratable acid + NH_4^+ − HCO_3^-) in relation to daily net fixed acid production, urinary (SO_4^{2-} + organic anions) − diet {(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) − [Cl^- + (1.8·PO_4)]} − fecal {(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) − [Cl^- + (1.8·PO_4)]} among 16 healthy adults eating constant whole-food diets (■), soy protein-formula diets (○), or diets containing egg yolk (▲); y = 21.659 + 0.67075x; $r^2 = 0.888$; $P < 0.001$. The slope for the line is not different from 1, and the intercept is not different from 0; $P > 0.8$. Redrawn from the original data in Ref. 43.

Effects of increasing acid production using NH_4Cl. Fourteen healthy adults were studied while eating individually constant diets during control conditions and during the administration of NH_4Cl in varying but individually constant doses ranging from 138 to 384 meq·day^{-1}·$subject^{-1}$ and averaging for the group 231 ± 69 (SD) meq/day (3.23 ± 0.61 meq·kg body wt^{-1}·day^{-1}) (1, 37, 74). The control observations were obtained during a 6-day period after 10 days of adaptation to each subject's constant whole-food diet. The observations during NH_4Cl administration were also obtained during a 6-day period that began 12 days or longer after NH_4Cl was begun. As shown in Table 1, fecal Cl^- excretion did not change from control rates during the administration of NH_4Cl, indicating that all of the administered Cl^- was absorbed. Fecal excretion of unmeasured anion also did not change. Thus on average for the group, the decrease in net intestinal absorption of base (unmeasured anion) was equivalent to the quantity of NH_4Cl administered. The consequent alteration in systemic acid-base balance and renal net acid excretion during NH_4Cl (see below) had no effect on the fecal excretion of actual or potential base (as unmeasured anion). Because the sum of urinary excretion of SO_4^{2-} and organic anion was nearly unchanged during the administration of NH_4Cl, net fixed acid production increased by a quantity equivalent to the increased intestinal absorption of the NH_4Cl administered (in effect reflecting the metabolism of the administered NH_4Cl to urea + HCl). However, renal net acid excretion did not increase to an equivalent degree. Thus the average acid balance for the group became significantly more positive by +24 ± 22 meq/day; P = 0.001. As shown in Fig. 2, these 14 subjects were, on average, in a steady state with respect to body weight, serum [HCO_3^-], and net acid excretion during control and with respect to serum [HCO_3^-] and net acid excretion during NH_4Cl acidosis. However, during induced acidosis these subjects exhibited a decline in body weight, averaging for the group −0.046 kg/day. Accompanying the retention of acid, Ca^{2+} balances (Table 1) became negative by an average of −16 ± 12 meq/day, $P < 0.001$, because of increased excretion of Ca^{2+} into the urine without any detectable change in net intestinal absorption of dietary Ca^{2+}. Additionally, K^+ balances also were slightly, but significantly, more nega-

Table 1. *Changes in body weight and in acid, mineral, and charge balances during NH_4Cl administration among 14 healthy adults eating otherwise constant diets (1, 37, 74)*

	Control	NH_4Cl	Δ	P[a]
Dose of NH_4Cl	0	231 ± 69[b]		Independent
Body wt, kg	71.53 ± 12.07	70.03 ± 12.54	−1.54 ± 1.36	= 0.001
Daily change in body wt, kg	−0.02 ± 0.04	−0.05 ± 0.04	−0.03 ± 0.05	NS
Serum [HCO_3], mmol/l	26.4 ± 1.0	20.2 ± 2.1	−6.2 ± 2.1	<0.001
Diet unmeasured anion, meq/day[c]	75 ± 18	−159 ± 56	−234 ± 68	<0.001
Fecal Cl^-, meq/day	1 ± 1	1 ± 1	0 ± 1	NS
Fecal unmeasured anion, meq/day[c]	33 ± 12	31 ± 11	−2 ± 8	NS
Intestinal absorption of unmeasured anion, meq/day[d]	42 ± 16	−188 ± 62	−230 ± 71	<0.001
Urinary unmeasured anion, meq/day[c]	22 ± 12	−187 ± 46	−209 ± 51	<0.001
Urinary unmeasured anion − measured intestinal absorption of unmeasured anion, meq/day	−20 ± 10	1 ± 23	+21 ± 26	= 0.008
Urinary SO_4, meq/day	44 ± 9	47 ± 9	+3 ± 4	= 0.024
Urinary organic anion, meq/day	45 ± 10	44 ± 11	−1 ± 8	NS
Net fixed acid production, meq/day	47 ± 14	279 ± 67	+232 ± 71	<0.001
Urinary pH	5.94 ± 0.25	5.37 ± 0.09	−0.57 ± 0.25	<0.001
Urinary titratable acid, meq/day	22 ± 5	30 ± 7	+10 ± 4	<0.001
Urinary NH_4, meq/day	43 ± 9	238 ± 56	+195 ± 56	<0.001
Urinary HCO_3, meq/day	3 ± 2	0	−3 ± 2	<0.001
Renal net acid excretion, meq/day[e]	62 ± 14	270 ± 61	+208 ± 59	<0.001
Acid balance, meq/day	−15 ± 10	+9 ± 15	+24 ± 22	= 0.001
Na^+ balance, meq/day	+18 ± 9	+17 ± 11	−1 ± 10	NS
K^+ balance, meq/day	+9 ± 5	+5 ± 4	−4 ± 6	= 0.033
Ca^{2+} balance, meq/day	+4 ± 6	−12 ± 13	−16 ± 12	<0.001
Mg^{2+} balance, meq/day	+2 ± 3	0 ± 4	−2 ± 4	= 0.052
Cl^- balance, meq/day	+7 ± 10	+13 ± 21	+6 ± 22	NS
PO_4 balance, mmol/day	+5 ± 4	−1 ± 4	−6 ± 3	<0.001
PO_4 balance · 1.8, meq/day	+9 ± 7	−2 ± 9	−11 ± 5	<0.001
Charge balance, meq/day[f]	+17 ± 10	−1 ± 23	−18 ± 26	= 0.02

[a]Probability that the mean difference for the group is not different from zero using the *t*-test for paired data. [b]Variances throughout are shown as ±SD. [c]Estimated as ($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day). [d]Difference between diet and fecal unmeasured anion, each estimated as ($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8·$PO_4$, mmol/day). [e]Calculated as (titratable acid + $NH^+ - HCO_3$), meq/day. [f]Estimated as [(diet($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$ meq/day) − (Cl^-, meq/day + 1.8·PO_4, mmol/day)]. [(fecal + urine($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8·$PO_4$, mmol/day)]. NS, not significant.

tive by −4 ± 6 meq/day; $P = 0.033$, and Mg^{2+} balances were marginally more negative by −4 ± 8 meq/day; $P = 0.052$. PO_4 balances were more negative by −6 ± 3 mmol/day; $P < 0.001$. The cumulative quantity of Ca^{2+} lost during the 6-day balance period averaged 96 meq, an amount that could only have been derived from bone, the sole significant body store of Ca^{2+}. In view of the loss in weight accompanying the retention of H^+ during stable NH_4Cl acidosis, it seems reasonable to assume that the K^+ and Mg^{2+} losses and some of the PO_4 losses derived from cells, likely muscle cells but possibly including bone, as a result of the adverse effects of acidosis on protein metabolism (reviewed in Ref. 54), whereas most of the Ca^{2+} was lost from bone mineral together with HCO_3^- /CO_3^{2-}/OH^-/PO_4^{3-} (8, 11–13). Urinary hydroxyproline excretion also increases during NH_4Cl acidosis, providing further evidence for increased bone resorption (33). The effects of metabolic acidosis on bone and bone cells in vitro are well known and reviewed elsewhere (10, 24).

Effects of decreasing acid production using $KHCO_3$. The effects of $KHCO_3$ administration are presented in Table 2 (34). Ten healthy adults were observed during a control 6-day balance period that began after they had eaten their constant diet for 10 days. They were then observed while continuing to eat the same diet during a 6-day experimental balance period that began 6 days after the ongoing administration of $KHCO_3$, 61 ± 1 mmol/day (0.90 ± 0.11 meq·kg body wt^{-1}·day^{-1}) was begun. As shown in Table 2, fecal K excretion did not change from control rates during the administration of $KHCO_3$, and the fecal excretion of unmeasured anion also did not change. Thus on average for the group, all of the administered $KHCO_3$ was absorbed, and the increase in net intestinal absorption of base (unmeasured anion) was equivalent to the quantity of $KHCO_3$ administered. The consequent alteration in systemic acid-base balance and renal net acid excretion had no effect on the fecal excretion of actual or potential base (as unmeasured anion). Because the sum of urinary excretion of SO_4^{2-} and organic anion was nearly unchanged during the administration of $KHCO_3$, net fixed acid production decreased by a quantity equivalent to the increased intestinal absorption of the HCO_3^- administered as $KHCO_3$. However, renal net acid excretion did not decrease to an equivalent degree. Thus the average acid balance for the group became significantly more negative by −13 ± 11 (SD) meq/day; $P = 0.005$. Equivalently, but in opposite terms, HCO_3^- balance became more positive by +13 ± 11 meq/day during $KHCO_3$ administration. Accompanying the retention of HCO_3, K^+ balances became almost equivalently more positive by +11 ± 10 mmol/day; $P = 0.006$. Additionally, Ca^{2+} balances be-

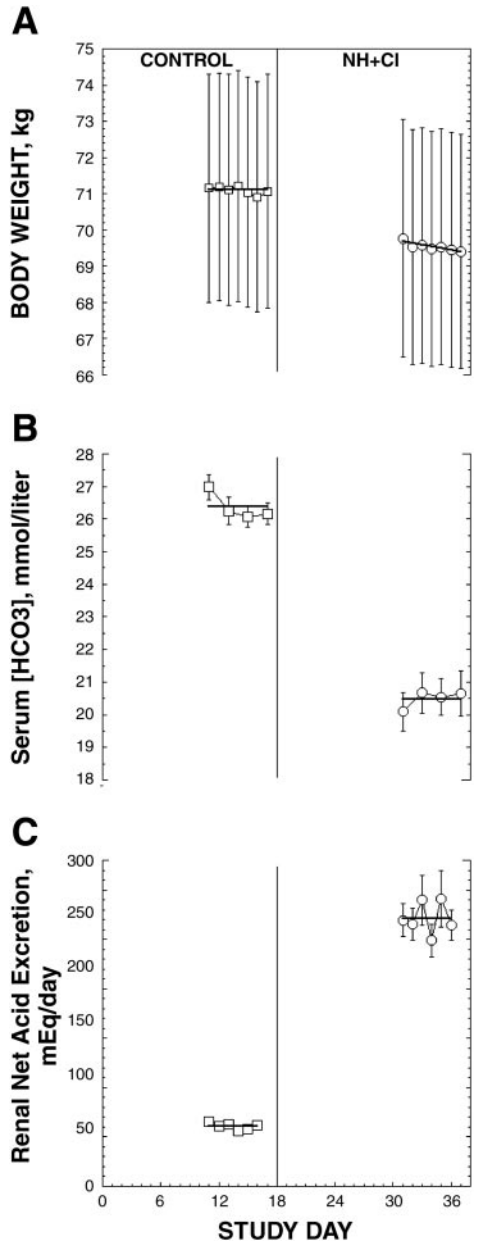


Fig. 2. Mean body weight (*A*), serum HCO_3 concentration ([HCO_3]; *B*), and daily urinary net acid excretion (*C*) among 14 healthy adults studied during control conditions (□) and then during the administration of NH_4Cl (○). Adapted from data in Refs. 1, 37, 74.

came slightly, but significantly, more positive by 1.8 ± 1.6 meq/day; $P = 0.009$. The changes in $K^+ + Ca^{2+}$ balances during $KHCO_3$ administration were matched by an equivalently more positive charge balance or, in other words, a more negative acid balance (equivalent to HCO_3^- retention). Thus it seems reasonable to conclude that $KHCO_3$ was retained. As shown in Fig. 3, these subjects were, on average, in a steady state with respect to body weight, morning fasting serum [HCO_3^-], and daily renal net acid excretion during both control conditions and during $KHCO_3$ administration. Moreover, average body weight for the group did not differ during $KHCO_3$ administration compared with control. Mean fasting serum [HCO_3^-] was slightly, but not significantly, higher during $KHCO_3$ administration in these studies, although during other studies, when 90 mmol of $KHCO_3$ were administered each day, serum [HCO_3^-] was observed to increase significantly when measured 90 min after the ingestion of one-third of the daily $KHCO_3$ dose (30 mmol) (39). Serum K^+ concentrations did not increase (not shown). Body weight was measured to the nearest 0.01 kg daily in each subject and was stable. That observation indicates that intracellular water was not retained in osmotic proportion to the cumulative retention of 66 mmol K^+ over the 6-day balance period because retention of such a quantity of K^+ within cells (with an anion) would have been expected to obligate retention of water, resulting in an increase body weight averaging ~0.5 kg, an amount that should have been detectable. Thus it seems reasonable to propose that the $KHCO_3$ was retained in an osmotically inactive form. Speculatively, $KHCO_3$ was retained as a solid phase adsorbed or secreted onto the vast surfaces of apatite in bone (11, 15).

Effects of decreasing acid production using $NaHCO_3$. The same 10 healthy adults were also studied, using the same control period and the same individually constant diets, during a 6-day period that began 6 days after the continuous administration of $NaHCO_3$ (60 ± 1 meq/day, 0.88 ± 0.11 meq·kg body wt^{-1}·day^{-1}) was begun (34). As shown in Table 3 and Fig. 3, the effects of $NaHCO_3$ were, for the most part, similar to those of $KHCO_3$ administration in that urinary net acid excretion did not decrease as much as did acid production so that acid balances became more negative by −12 ± 11 meq/day; $P = 0.011$. Accompanying this retention of HCO_3^-, Na^+ balances became almost equivalently more positive by +11 ± 14 mmol/day; $P = 0.033$ without any change in body weight, suggesting the retention of $NaHCO_3$ in an osmotically inactive form as a solid phase adsorbed or secreted onto the surfaces of apatite in bone (11). Unlike the effect of $KHCO_3$ administration, the administration of $NaHCO_3$ was not accompanied by a reduction in urinary Ca^{2+} excretion or more positive Ca^{2+} balances. The failure of $NaHCO_3$ to cause Ca^{2+} retention is the result of the additional Na load, causing extracellular volume expansion and increased urinary Ca^{2+} excretion when the diet also contains NaCl. Increasing dietary NaCl intake is known to increase urinary Ca^{2+} excretion (5, 39, 53, 56). Additionally, the ongoing administration of mineralocorticoid when the dietary intakes of NaCl and Ca^{2+} are constant is accompanied by NaCl retention, weight gain, and increased urinary Ca^{2+} excretion (44). Other studies have also shown that the administration of $NaHCO_3$ when the diet also contains NaCl does not result in a sustained reduction in urinary Ca^{2+} excretion (34, 39). However, when dietary Na intake is kept constant by substituting $NaHCO_3$ for part of the NaCl in the diet, urinary Ca^{2+} excretion does decline (50).

During growth, daily rates of renal net acid excretion among infants have been observed to exceed rates of fixed acid production, so that daily acid balances are negative (30) as during the administration of $KHCO_3$ or $NaHCO_3$

Table 2. *Changes in body weight and in acid, mineral, and charge balances during $KHCO_3$ administration among 10 healthy adults eating otherwise constant diets (34)*

	Control	$KHCO_3$	Δ	P[a]
Dose of $KHCO_3$, mmol/day	0	61 ± 2[b]	+61 ± 2	Independent
Body wt, kg	68.60 ± 8.89	68.86 ± 8.68	+0.26 ± 0.99	NS
Daily change in body wt, kg	−0.03 ± 0.06	0.04 ± 0.08	+0.07 ± 0.09	= 0.039
Serum [HCO_3], mmol/l	27.1 ± 0.8	27.3 ± 1.1	+0.2 ± 0.8	NS
Diet unmeasured anion, meq/day[c]	57 ± 11	118 ± 11	+61 ± 2	<0.001
Fecal K^+, mmol/day	15 ± 3	15 ± 3	0 ± 2	NS
Fecal unmeasured anion, meq/day[c]	28 ± 7	27 ± 5	−1 ± 2	NS
Net intestinal absorption of unmeasured anion, meq/day[d]	29 ± 5	91 ± 7	+62 ± 3	<0.001
Urinary unmeasured anion, meq/day[c]	33 ± 13	85 ± 16	+52 ± 11	<0.001
Urinary unmeasured anion − measured intestinal absorption of unmeasured anion, meq/day	+4 ± 14	−6 ± 14	−10 ± 10	= 0.011
Urinary SO_4, meq/day	39 ± 7	37 ± 6	−2 ± 3	NS
Urinary organic anion, meq/day	35 ± 6	38 ± 7	+2 ± 4	NS
Net fixed acid production, meq/day	45 ± 10	−17 ± 13	−62 ± 6	<0.001
Urinary pH	6.00 ± 0.13	6.68 ± 0.18	+0.68 ± 0.13	<0.001
Urinary titratable acid, meq/day	21 ± 4	10 ± 4	−10 ± 2	<0.001
Urinary NH_4, meq/day	37 ± 5	19 ± 5	−18 ± 4	<0.001
Urinary HCO_3, meq/day	3 ± 2	23 ± 8	+20 ± 6	<0.001
Renal net acid excretion, meq/day[e]	55 ± 9	6 ± 13	−49 ± 9	<0.001
Acid balance, meq/day	−10 ± 10	−23 ± 14	−13 ± 11	= 0.005
Na^+ balance, meq/day	+10 ± 18	+11 ± 16	+1 ± 14	NS
K^+ balance, meq/day	+7 ± 6	+18 ± 14	+11 ± 10	= 0.006
Ca^{2+} balance, meq/day	−2.3 ± 1.9	−1.4 ± 1.9	+0.9 ± 0.8	= 0.009
Mg^{2+} balance, meq/day	+0.5 ± 2.5	+2.0 ± 3.4	+1.5 ± 1.8	= 0.020
Cl^- balance, meq/day	+6 ± 17	+9 ± 17	+3 ± 13	NS
PO_4 balance, mmol/day	+6.1 ± 3.4	+8.4 ± 4.6	+2.3 ± 3.0	= 0.040
PO_4 balance · 1.8, meq/day	+11.0 ± 6.1	+15.1 ± 8.3	+4.1 ± 5.4	= 0.040
Charge balance, meq/day[f]	−4 ± 14	+6 ± 14	+10 ± 10	= 0.011

[a]Probability that the mean difference for the group is not different from zero using the *t*-test for paired data. [b]Variances throughout are shown as ±SD. [c]Estimated as ($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day). [d]Difference between diet and fecal unmeasured anion, each estimated as ($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day). [e]Calculated as (titratable acid + NH_4 − HCO_3), meq/day. [f]Estimated as [(diet($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day)] − [(fecal + urine($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day)].

to adults. That effect necessarily must accompany the deposition of alkaline mineral into the growing skeleton, largely as $Ca_{10}(PO_4)_6(OH)_2$/$Ca_{10}(PO_4)_6CO_3$, a process that may, in opposite terms, be thought of as an additional source of acid production.

Overview of acid production and excretion. Figure 4 summarizes the relationship between the changes from control in daily urinary net acid excretion and the changes in net endogenous fixed acid production for the individual subjects given NH_4Cl (1, 37, 74; summarized in Table 1), the subjects given $KHCO_3$ (34; summarized in Table 2), and among 4 additional subjects in which acid production was increased by increasing dietary protein intake as egg white (1). The relationship is Δnet acid excretion, meq/day = 3.81 [±(SE)2.77] + 0.90667 [±(SE)0.0161]·Δacid production, meq/day. The slope for this relationship is significantly <1, but the intercept is not different from zero. Because Δnet acid excretion did not increase as much as Δacid production when acid production was increased using NH_4Cl or egg white, the data points for those studies lie below the identity line. By contrast, the data points for the subjects given $KHCO_3$, with one exception, lie above the identity line because Δnet acid excretion did not decrease as much as did Δacid production. In other words, the data viewed in this manner also indicate that acid balances become positive when acid production is increased and become negative, reflecting HCO_3^- retention, when acid production is reduced.

To summarize, net fixed acid production among healthy adults eating normal diets can be estimated by the sum of urinary SO_4^{2-} + organic anions less the difference between the sum of inorganic cations and anions in the diet and their sum in the feces. This quantity matches urinary net acid excretion so that healthy adults with normal and stable serum [HCO_3^-] are in acid balance. When acid production is experimentally increased, acid balances become positive and Ca^{2+} balances become negative, reflecting buffering by bone. When acid production is experimentally reduced, acid balances become negative, reflecting equivalent HCO_3^- retention together with K^+ and Ca^{2+} retention during the administration of $KHCO_3$ and of Na^+ retention when dietary Na^+ intake is kept constant during the administration of $NaHCO_3$, either also reflecting buffering of base by bone.

A CONSIDERATION OF POSSIBLE ERRORS IN THE ESTIMATES OF FIXED ACID PRODUCTION AND RENAL NET ACID EXCRETION

Components of Fixed Acid Production

Urinary sulfate. Oxidation of organic sulfur to sulfate, as reflected by the daily urinary excretion rates of

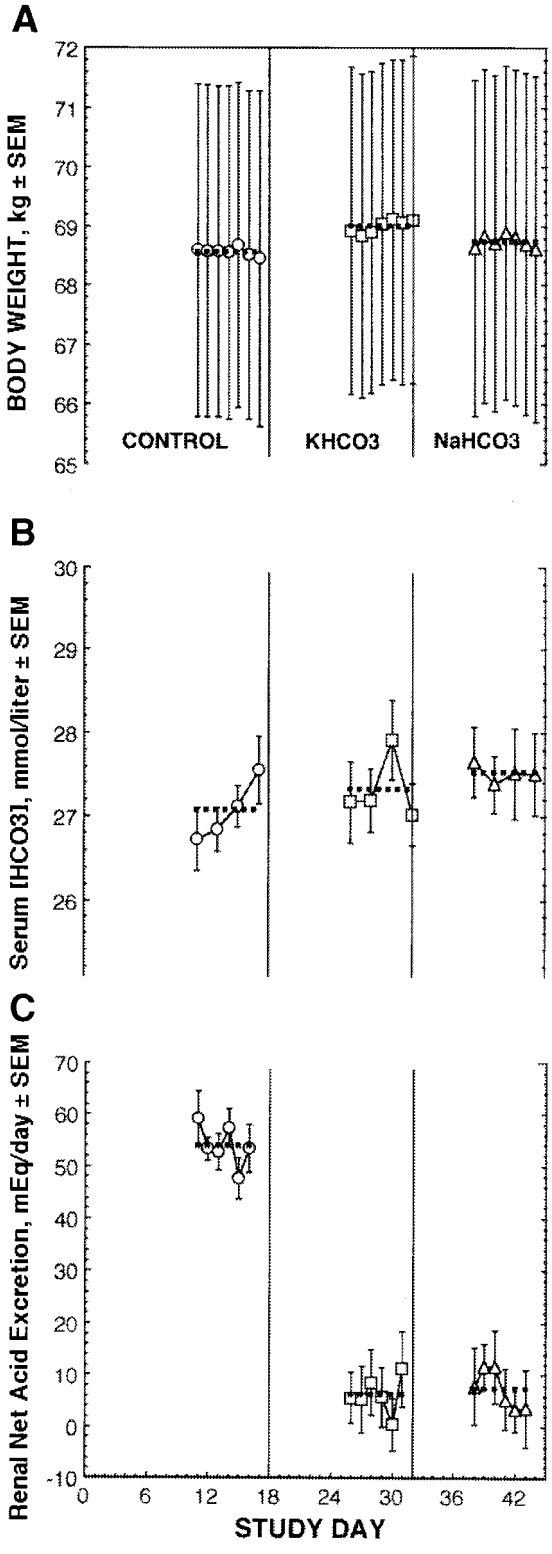


Fig. 3. Mean body weight (*A*), serum [HCO3] (*B*), and daily urinary net acid excretion (*C*) among 10 healthy men studied during control conditions (□) and then during the administration of $KHCO_3$ (○) and $NaHCO_3$ (Δ). Adapted from data in Ref. 34.

SO_4^{2-}, is the first major component of fixed acid production. The measurements of urinary SO_4^{2-} using either gravimetric (63) or turbidometric (4) methods appear to be adequately specific and precise and therefore not a significant source of error. The possibility exists that after its production significant quantities of SO_4^{2-} might be excreted via other routes, thereby escaping detection and leading to an underestimation of fixed acid production. However, even during methionine loading, when SO_4^{2-} production and urinary SO_4^{2-} excretion rates were markedly increased, fecal SO_4^{2-} excretion was negligible (41). Losses of sulfate via the skin would also appear to be negligible even with moderate visible sweating.

Urinary organic anions. Urinary organic anion excretion in humans reflects the second major component of fixed acid production. Presently, various precise methods do not appear to be available that allow individual identification and quantitation of all these organic anions. Thus their estimation remains dependent on an estimate derived from the titration of urine from pH 2.7 to urinary pH, after removal of urinary PO_4 by incubation of the urine with solid $Ca(OH)_2$ and reacidification of the filtrate to pH 2.7, a method devised early in the last century (73).

Studies in rats have shown that the urinary excretion of organic acids falls with acid loading and rises with alkali loading (6, 17, 61). However, as shown in Fig. 5, using the individual data for the subjects whose studies are summarized in Tables 1–3, the daily urinary excretion of total organic acids, as titrated between pH 2.7 and 7.4, did not change significantly as urinary pH or daily net acid excretion varied during the administration of NH_4Cl, $KHCO_3$, or $NaHCO_3$. Thus unlike rats, in which urinary excretion rates of organic anions decrease markedly during acid loading and increase markedly during the administration of HCO_3^- loads (6, 17, 61), humans do not exhibit either an evident decrease in total organic acid excretion in response to acid administration or an increase in response to the administration of base.

By contrast, as shown in Fig. 6 (1, 33, 34, 37, 41, 65, 74), the percentage of urinary organic acid anions that are titrated between pH 2.7 and urinary pH and that constitute a component of net fixed acid production increases, in a manner resembling the upper segment of a sigmoid titration curve, from 70 to 80% of total urinary organic acids (titrated from pH 2.7 to 7.4) at a urinary pH of 5 to nearly 100% above a urinary pH of 7. By contrast, the percentage of total urinary organic acids that are excreted as free acids (titrated between urinary pH and 7.4) necessarily decreases from 20 to 30% of the total at pH 5 to only a few percentage points or zero at a urinary pH above 7. These free organic acids do not contribute to fixed acid production. Thus, as shown in Fig. 7, which uses the individual studies that are summarized in Tables 1–3 (1, 34, 37, 74), the daily urinary excretion rates of organic acid anions decrease as urinary pH falls and tend to increase as urinary pH rise, whereas their excretion rates fall as

Table 3. *Changes in body weight and in acid, mineral, and charge balances during $NaHCO_3$ administration among 10 healthy adults eating otherwise constant diets (34)*

	Control	$NaHCO_3$	Δ	P[a]
Dose of $NaHCO_3$, mmol/day	0	60 ± 1[b]	<0.001	Independent
Body wt, kg	68.60 ± 8.89	68.68 ± 9.03	0.09 ± 1.32	NS
Daily change in body wt, kg	−0.03 ± 0.06	−0.01 ± 0.05	+0.02 ± 0.06	NS
Serum [HCO_3], mmol/l	27.1 ± 0.8	27.5 ± 1.3	+0.4 ± 0.9	NS
Diet unmeasured anion, meq/day[c]	57 ± 11	117 ± 11	+60 ± 1	<0.001
Fecal Na, mmol/day	3 ± 2	3 ± 2	0 ± 2	NS
Fecal unmeasured anion, meq/day[c]	28 ± 7	28 ± 9	0 ± 4	NS
Net intestinal absorption of unmeasured anion, meq/day[d]	29 ± 5	89 ± 5	60 ± 4	<0.001
Urinary unmeasured anion, meq/day[c]	33 ± 13	82 ± 18	49 ± 13	<0.001
Urinary unmeasured anion − measured intestinal absorption of unmeasured anion, meq/day	4 ± 14	−7 ± 19	−11 ± 12	= 0.015
Urinary SO_4, meq/day	39 ± 7	37 ± 6	−2 ± 3	NS
Urinary organic anion, meq/day	35 ± 6	38 ± 10	+3 ± 5	NS
Net fixed acid production, meq/day	45 ± 10	−14 ± 13	−59 ± 5	<0.001
Urinary pH	6.00 ± 0.13	6.70 ± 0.20	+0.70 ± 0.15	<0.001
Urinary titratable acid, meq/day	21 ± 4	10 ± 5	−10 ± 2	<0.001
Urinary NH_4, meq/day	37 ± 5	21 ± 6	−17 ± 2	<0.001
Urinary HCO_3, meq/day	3 ± 2	23 ± 7	+20 ± 6	<0.001
Renal net acid excretion, meq/day[e]	55 ± 9	7 ± 14	−47 ± 9	<0.001
Acid balance, meq/day	−9 ± 10	−21 ± 17	−12 ± 11	= 0.011
Na^+ balance, meq/day	+10 ± 18	+21 ± 18	+11 ± 14	= 0.033
K^+ balance, meq/day	+6 ± 6	+7 ± 6	+1 ± 5	NS
Ca^{2+} balance, meq/day	−2.3 ± 1.9	−1.8 ± 3.2	+0.5 ± 1.8	NS
Mg^{2+} balance, meq/day	+0.4 ± 2.6	+1.2 ± 2.7	+0.8 ± 0.9	= 0.024
Cl^- balance, meq/day	+6 ± 17	+5 ± 10	−1 ± 15	NS
PO_4 balance, mmol/day	+6.1 ± 3.4	+7.6 ± 4.1	+1.5 ± 2.6	NS
PO_4 balance · 1.8, meq/day	+11.0 ± 6.1	+13.7 ± 7.4	+2.7 ± 4.7	NS
Charge balance, meq/day[f]	−4 ± 14	+7 ± 19	+11 ± 12	= 0.015

[a]Probability that the mean difference for the group is not different from zero using the *t*-test for paired data. [b]Variances throughout are shown as ±SD. [c]Estimated as ($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day). [d]Difference between diet and fecal unmeasured anion, each estimated as ($Na^+ + K^+ + Ca^{2+} + Mg$, meq/day) − (Cl, meq/day + 1.8 · PO_4, mmol/day). [e]Calculated as (titratable acid + NH_4 − HCO_3), meq/day. [f]Estimated as [(diet($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day)] − [(fecal + urine($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day)].

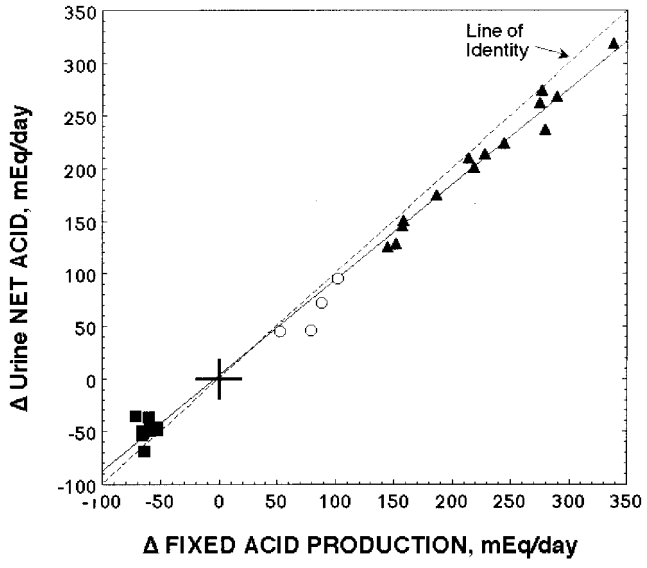


Fig. 4. Changes (Δ) from control in daily urinary net acid excretion in relation to the changes from control in daily endogenous fixed acid production among healthy adults given NH_4Cl (▲), egg white (○), or $KHCO_3$ (■); $y = 3.81 (\pm 2.77) + 0.90667 (\pm 0.0161)x$; $r^2 = 0.992$; $P < 0.0001$. Adapted from data in Refs. 1, 34, 37, 74.

net acid excretion increases and increase as net acid excretion decreases.

The pK_{a2} for the dissociation of $HSO_4^- \leftrightarrow SO_4^{2-} + H^+$ is 1.7. Accordingly, it has been suggested that the titration of organic anions between pH 2.7 and urinary pH will overestimate organic anions because of the titration of some HSO_4^-, theoretically resulting in the titration of about 10% of urinary SO_4^{2-}. A reevaluation of measured mean daily organic anion excretion rates for five subjects observed during control, methionine loading, and recovery (41) demonstrates that organic anion excretion rates do increase slightly above control rates as SO_4^{2-} excretion rates increase during the administration of methionine and on the first recovery day when urinary SO_4 excretion rates remain above control. This relationship is shown in Fig. 8. On average, organic anion increases by ~3.5 meq $\cdot day^{-1} \cdot 100$ meq SO_4^{-1}. Extrapolation of that relationship to the wide normal range of daily rates of urinary SO_4^{2-} excretion, which may range from 15 meq/day during the ingestion of a low-protein diet to 75 meq/day during the ingestion of a high-protein diet, would indicate that the titration could overestimate urinary excretion rates of organic anions by 1–3 meq/day.

Urinary citrate is one of the components of urinary organic anions. Citrate excretion in humans is well

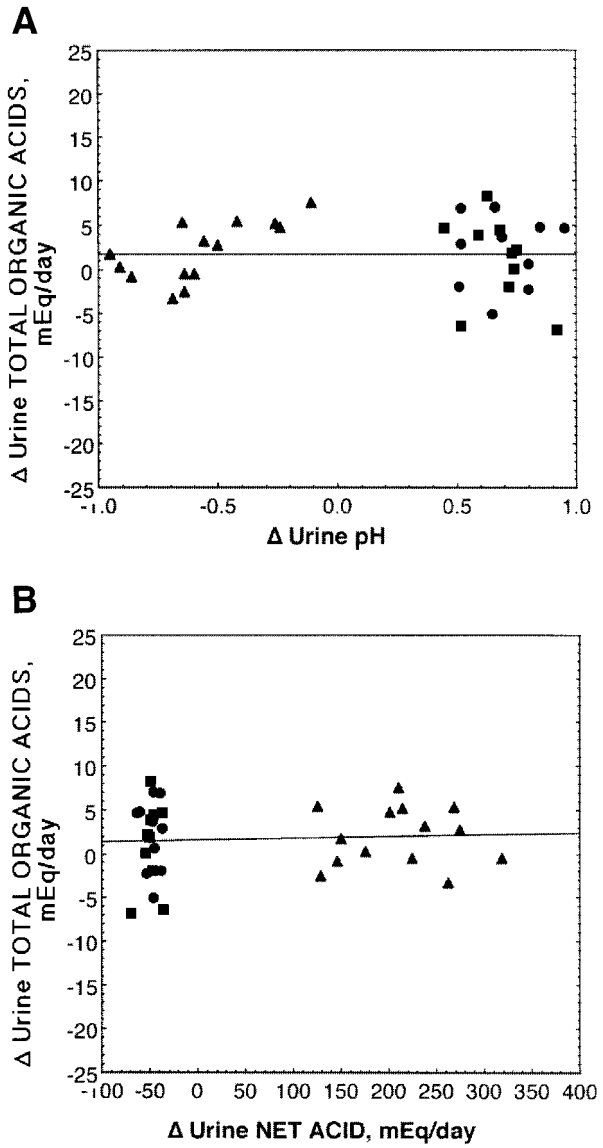


Fig. 5. *A*: changes from control in the daily urinary excretion of total organic acids in relation to the changes from control in urinary pH among healthy adults given NH_4Cl (▲), egg white (○), or $KHCO_3$ (■); $y = 1.7 + 0.045x$; $r^2 = 0.00$, not significant. *B*: changes from control in the daily urinary excretion of total organic acids in relation to the changes from control in daily urinary net acid excretion for healthy adults given NH_4Cl (▲), $KHCO_3$ (■), or $NaHCO_3$ (●); $y = 1.6 + 0.002x$; $r^2 = 0.00$, not significant. Adapted from data in Refs. 1, 34, 37, 74.

known to fall with acid loading and rise with alkali loading. Some data illustrating these effects are presented in Fig. 9 (33, 34, 39). As shown in Fig. 9*A*, daily urinary citrate excretion falls exponentially as net acid excretion rises. Among subjects being given $KHCO_3$ or $NaHCO_3$, citrate excretion averaged 15.3 meq/day when net acid averaged −1 meq/day. Among subjects eating only control diets, citrate excretion averaged 11.2 meq/day when net acid averaged 57 meq/day. Among subjects being given NH_4Cl, citrate excretion averaged only 0.4 meq/day when net acid averaged 264 meq/day. As shown in Fig. 9*B*, evaluation of the changes from control in urinary citrate excretion in relation to the changes from control in net acid excretion among subjects given $KHCO_3$, $NaHCO_3$, or NH_4Cl demonstrates that urinary citrate excretion falls linearly as net acid excretion rises.

It has also been suggested that the treatment of urine with excess solid $Ca(OH)_2$ to precipitate PO_4^{3-} before organic acid titration may cause the loss of citrate, possibly by the precipitation of calcium citrate. To asses this issue, we measured the citrate concentrations in urine specimens from seven healthy subjects before and after that treatment. We found that citrate concentration decreased from 5.02 ± 3.07 to 0.87 ± 0.87 meq/l or by an average of −83%. This methodological error indicates the loss of citrate due to $Ca(OH)_2$ before organic acid titration would result in the underestimation of urinary anion excretion. When acid production exceeds 100 meq/day, urinary citrate falls progressively below 5 meq/day (Fig. 9*A*) so that in such circumstances the loss of citrate as a consequence of the $Ca(OH)_2$ treatment before the determination of organic anions would cause an underestimation of the later value by ~1–4 meq. Urinary citrate excretion rises when acid production decreases (Fig. 9*A*). Urinary citrate excretion was measured before and during the administration of $KHCO_3$ and $NaHCO_3$ in 9 of the 10 subjects given those salts (Tables 2 and 3; Ref. 34) and rose on average 4.8 ± 2.2 meq/day ($P < 0.001$) during the administration of $KHCO_3$ and rose 3.0 ± 3.0 meq/day ($P < 0.025$) during the administration of $NaHCO_3$ (Lemann J, unpublished observations). If 80% of those increments were lost as a consequence of the $Ca(OH)_2$ treatment before determination of organic anions, the resulting decrease in the organic anion estimate would have been only 2–4 meq/day.

Collectively, the overestimation of organic anions resulting from the titration of HSO_4^- and the underestimation of organic anions resulting from the loss of citrate are thus small and nearly quantitatively equal,

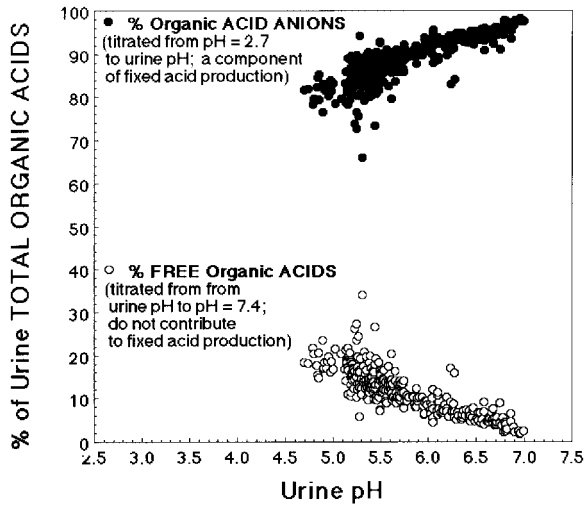


Fig. 6. Percentage of daily total urinary organic acids excreted as organic acid anions (●) and as free acids (○) in 24-h urine collected from adults eating various constant diets. Adapted from data in Refs. 1, 33, 34, 37, 41, 65, 74.

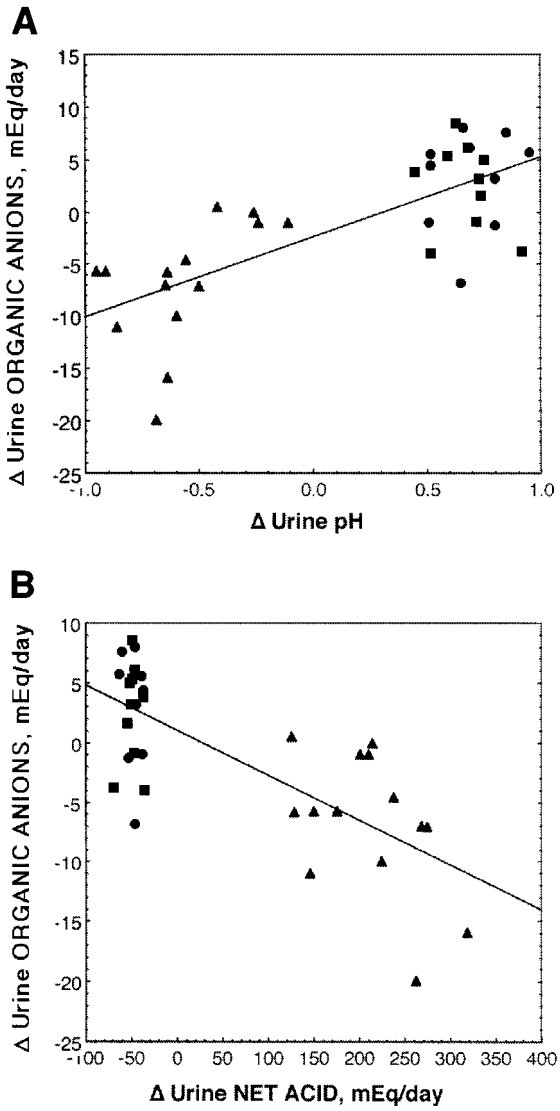


Fig. 7. *A*: changes from control in the daily urinary excretion of organic acid anions in relation to the changes from control in urinary pH for healthy adults given NH_4Cl (▲), $KHCO_3$ (■), or $NaHCO_3$ (●); $y = -2.3943 + 7.6893x$; $r^2 = 0.535$; $P < 0.0001$. *B*: changes from control in the daily urinary excretion of organic acid anions in relation to the changes from control in daily urinary net acid excretion for healthy adults given NH_4Cl (▲), $KHCO_3$ (●), or $NaHCO_3$ (●); $y = 1.0795 - 0.037856x$; $r^2 = 0.543$; $P < 0.0001$. Adapted from data in Refs. 1, 34, 37, 74.

leading to the conclusion that there are no major or significant errors in evaluating by titration the contribution of urinary organic anions to acid production.

Net Intestinal Absorption of Actual or Potential Base or Acid Ingested in the Diet

Net intestinal absorption of dietary actual or potential HCO_3^- is estimated by calculating the difference between measured dietary $[(Na + K + Ca + Mg) - (Cl + 1.8 \cdot PO_4)]$ and measured fecal dietary $[(Na + K + Ca + Mg) - (Cl + 1.8 \cdot PO_4)]$. Some of the dietary anions are ingested as free unionized acids and would not be identified by this calculation but could be a source of acid production. However, if such acids were completely metabolized to $CO_2 + H_2O$, they would not contribute to fixed acid production. Furthermore, if they were absorbed and buffered and not metabolized and excreted into the urine, they would be identified as a source of acid production by their inclusion in the measurement of urinary organic anions and would equivalently increase the urinary excretion of net acid. These effects as well as the effects of other possible substances on the estimation of fixed acid production and net acid excretion are summarized in Table 4.

To avoid the need to analyze diets and to analyze feces collected over periods of sufficient duration to ensure that the fecal collections are accurately timed, it has been proposed that the measurement of urinary $[(Na^+ + K^+ + Ca^{2+} + Mg) - (Cl^- + 1.8 \cdot PO_4)]$ would provide a more convenient estimate of the net intestinal absorption of actual or potential base or acid ingested in the diet (58). Figure 10*A* compares the changes from control in urinary $[(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + 1.8 \cdot PO_4)]$ to the changes from control in the directly measured net intestinal absorption of unmeasured {diet − fecal $[(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + 1.8 \cdot PO_4)]$} for the individual subjects summarized in Tables 1–3 (1, 34, 37, 74). The slope for the relationship shown in Fig. 10*A* is 0.903 ± 0.013, a value that is significantly <1. Thus the estimate based on urine overestimates the changes from control of measured absorption during the administration of NH_4Cl and underestimates the changes from control of measured absorption during $KHCO_3$ and $NaHCO_3$ administration. During NH_4Cl administration, the mean of the changes from control dietary unmeasured anion absorption based on urine overestimates the directly measured mean of the changes from control absorption by an average of 16 ± 15 meq/day ($P = 0.008$), because

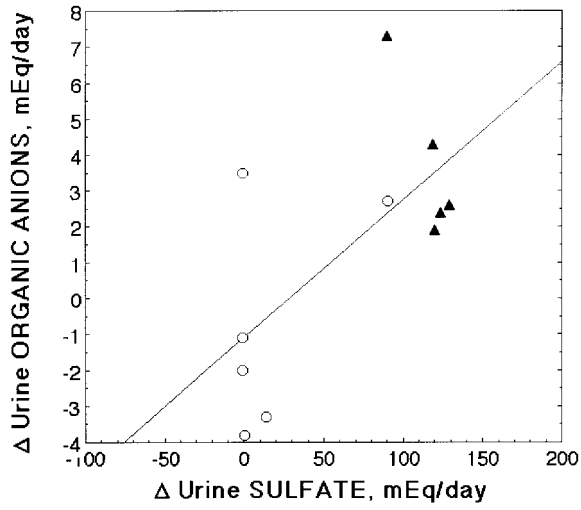


Fig. 8. Group mean changes from control in daily urinary organic acid anion excretion (as titrated from pH = 2.7 to urinary pH and corrected for the titration of creatinine) compared with the group mean changes from control in urinary SO_4^{2-} = excretion for 5 subjects during each of 5 days of methionine loading (▲) and 5 recovery days (○); $y = 1.067 + 0.03471x$; $r^2 = 0.427$; $P = 0.029$. Adapted from data in Ref. 41.

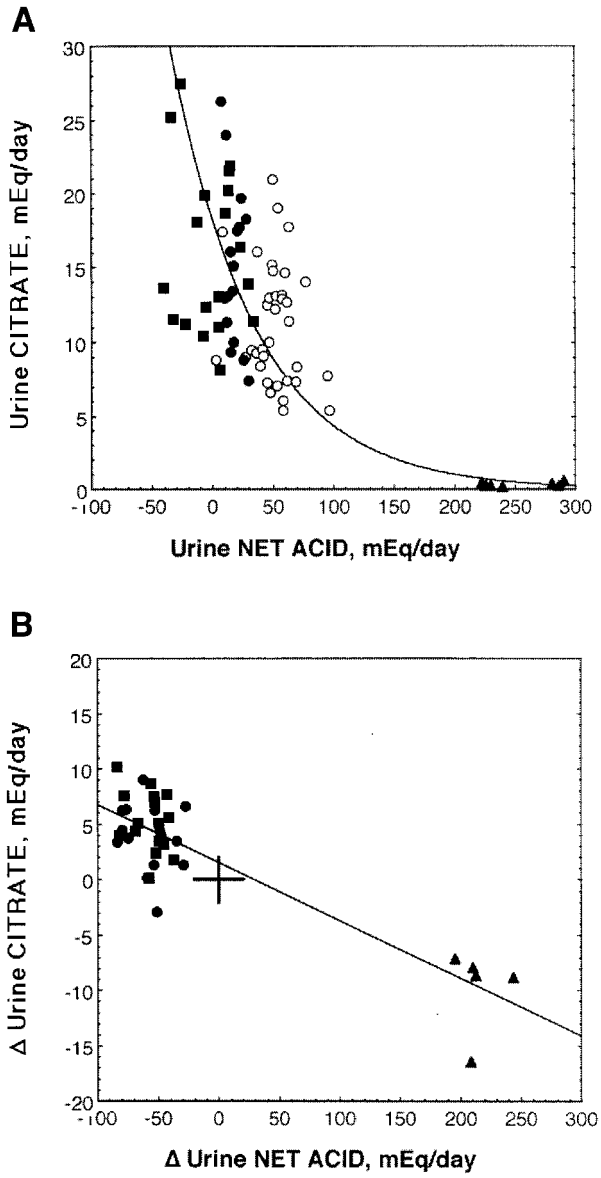


Fig. 9. *A*: urinary citrate excretion (meq/day) in relation to urinary net acid excretion (meq/day) among subjects eating constant diets alone (○) or also taking $KHCO_3$ (■), $NaHCO_3$ (●), or NH_4Cl (▲); $y = 18.2 \cdot 10^{(0.00623x)}$; $r^2 = 0.822$. *B*: changes from control of urinary citrate excretion (meq/day) in relation to the changes from control of net acid excretion among subjects given $KHCO_3$ (■), $NaHCO_3$ (●), or NH_4Cl (▲); $y = 1.52 - 0.0522x$; $r^2 = 0.749$; $P < 0.001$. Adapted from data for some of the subjects included in Refs. 33, 34, 39.

the measurement based on urine does not take into account the ongoing negative Ca^{2+} balances (Table 1). During $KHCO_3$ administration, the mean of the changes from control of dietary unmeasured anion absorption based on urine underestimates the mean of the changes from control of directly measured absorption by an average of -10 ± 10 meq/day ($P = 0.011$), because the urine measurement does not take into account the ongoing positive K and Ca^{2+} balances (Table 2). Similarly, during $NaHCO_3$ administration, the mean of the changes from control absorption based on urine underestimates directly measured mean of the changes from control absorption by an average of -11 ± 12 meq/day ($P = 0.015$), because the urine measurement does not take into account the ongoing positive Na^+ balances (Table 3). Alternatively, the changes from control in charge balances become positive as the changes from control in acid balance become negative during the administration of $KHCO_3$ or $NaHCO_3$, whereas the changes from control in charge balances become negative as the changes from control become positive during the administration of NH_4Cl (see below).

When Oh (58) suggested that the measurement of net intestinal absorption of potential base or acid could be indirectly assessed by measurement of urine $[(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + 1.8 \cdot PO_4)]$, that view was based on a reanalysis of earlier data (36, 39, 43, 45, 65) that are inadequate for such an evaluation. Except for the data for five subjects given NH_4Cl (39), the other data were for studies utilizing liquid-formula diets (36, 43, 63) during which Mg^{2+} was not measured. Moreover, during the latter studies the duration of fecal collections was too short to adequately evaluate daily excretion rates of fecal Na^+, K^+, Ca^{2+}, Cl^-, and PO_4 as well as Mg, had it been measured. Figure 10*B* shows urinary $[(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + 1.8 \cdot PO_4)]$ in relation to directly measured net intestinal absorption of unmeasured anions {diet − fecal $[(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + 1.8^* PO_4)]$} using the absolute measurements during control and during either the administration of NH_4Cl, $KHCO_3$, or $NaHCO_3$ for the studies summarized in Tables 1–3, as proposed by Oh (58). When the data are viewed in this manner, the slope for the relationship is 0.987 ± 0.029, a value that is not different from one. Thus the changes from control (Fig. 10*A*) become undetectable, and the urinary and directly measured estimates of intestinal absorption of cations or anions do not differ. Consequently, during the administration of NH_4Cl, that estimation of intestinal absorption of unmeasured anions based on urinary composition does not, in the absence of Ca^{2+} balance data, reveal that the increase in net acid excretion is less than the amount of potential acid fed, indicating H^+ retention, nor that the increase in urinary Ca^{2+} is derived from body stores (bone), not enhanced intestinal Ca^{2+} absorption (see below). Similarly, the use of the measurement of urinary composition $[(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + 1.8 \cdot PO_4)]$ to estimate intestinal absorption of unmeasured anions during the administration of $KHCO_3$ or $NaHCO_3$ does not, in the absence of K^+ or Na^+ balance data, reveal that the decrease in net acid excretion is less than the amount of base (HCO_3^-) administered nor the retention of K^+, Ca^{2+}, or Na^+, as previously described. Thus, unfortunately, urinary measurements and analyses alone do not adequately reflect important changes in acid-base and electrolyte balance with changes in dietary acid-base content.

In summary, the components of fixed acid production appear to be adequately measurable without significant errors as the sum of urinary SO_4^{2-} + organic anions less the difference between the sum of inorganic

Table 4. *Sources, identification, and fate of organic acids and effects on acid production and net acid excretion*

Source/Type of Acid	Example	Identification	Fate	Effects: Acid production	Effects: Net acid excretion
		From diet			
HX_1 Unmetabolizable organic acid ingested in diet as free acid, H^+ absorbed, X_1^- excreted in feces	? Oxalic acid	↑ Fecal unmeasured anion (↑ cation in feces = H^+ gain or HCO_3^- loss)	Absorbed H^+ titrates body buffers	↑	↑
HX_2 Unmetabolizable organic acid ingested in diet as free acid and completely absorbed from gut (does not affect diet unmeasured anion	Hippuric acid	↑ Urinary organic anion	Absorbed H^+ titrates body buffers	↑	↑
KX_1 K (Na, Ca, or Mg) salt of unmetabolizable organic acid ingested in diet, cation absorbed, and X_1 excreted in feces as free acid HX_1 (unlikely)	?	↑ Diet unmeasured anion (= HCO_3 gain = unmeasured H^+ loss in feces)	Absorbed cation gain + HCO_3 gain as H^+ lost	↓	↓
KX_2 K (or other mineral cation) salt of unmetabolizable organic acid ingested in diet and completely absorbed from gut	K-tartrate	↑ Diet unmeasured anion and ↑ Urinary organic anion	Absorbed anion excreted in urine	↔	↔
HA_1 Metabolizable organic acid ingested as free acid; completely absorbed and completely metabolized	Citric acid	Not identified	$CO_2 + H_2O$	↔	↔
HA_2 Metabolizable organic acid ingested as free acid; completely absorbed but incompletely metabolized; conjugate base (anion) excreted in urine	?	↑ Urinary organic anion	Absorbed H^+ titrates body buffers	↑	↑
KA_1 K (or other mineral cation) salt of metabolizable organic anion ingested in diet, completely absorbed and metabolized	K-citrate	↑ Diet unmeasured anion	HCO_3^- gain	↓	↓
		Endogenously produced			
HA_3 Organic acid end product of metabolism, (same as HX_2 above); H^+ buffered by body buffers and anion excreted into urine	Hippuric acid	↑ Urinary organic anion	Generated H+ titrates body buffers	↑	↑
HA_4 Organic acid, normally produced during metabolism, but either not completely oxidized to $CO_2 + H_2O$ in health or produced at accelerated rate in disease	Citric, lactic, β-OHbutyric, etc.		Generated H+ titrates body buffers	↑	↑

cations and anions in the diet and their sum in the feces.

Components of Urinary Net Acid Excretion

Ammonium. The measurements of urinary ammonium, whether by microdiffusion and titration (19) or an automated method (49), appear to be specific and sufficiently precise and thus not subject to significant error.

The possibility has been suggested that urine may contain other cationic buffer(s) capable of carrying secreted H^+ into the urine in addition to NH_4^+, perhaps histidine or a similar substance (60). On theoretical grounds, histidine is unlikely to serve in this manner. Whereas pK_a for the dissociation of the iminazole group of histidine is 6.0, so that one-half of the histidine present at an average urine pH of 6.0 would buffer a proton, the average urinary excretion of histidine among adults is in the range of 0.5 to 1.0 mmol/day, so that histidine would carry only a very small amount of H^+ secreted along the nephron into the final urine. Further evidence for the absence of significant quantities of unmeasured cation as well as unmeasured anion is summarized in Fig. 11. The mean sum of the directly measured urinary anions for a large number of studies (1, 34, 36, 37, 40, 42, 51; Lemann J, unpublished observations), estimated as $HCO_3^- + Cl^- + HPO_4^{2-} + H_2PO_4^- + SO_4^{2-}$ + organic anions, averaged 298 meq/day. That value was not significantly different from the simultaneously measured sum of the urinary cations, estimated as $Na^+ + K^+ + Ca^{2+} + Mg^{2+} + NH_4^+ +$ creatinine$^+$, that averaged 303 meq/day. Furthermore, when the relationship between the sum of the anions for each subject was evaluated in relation to the sum of

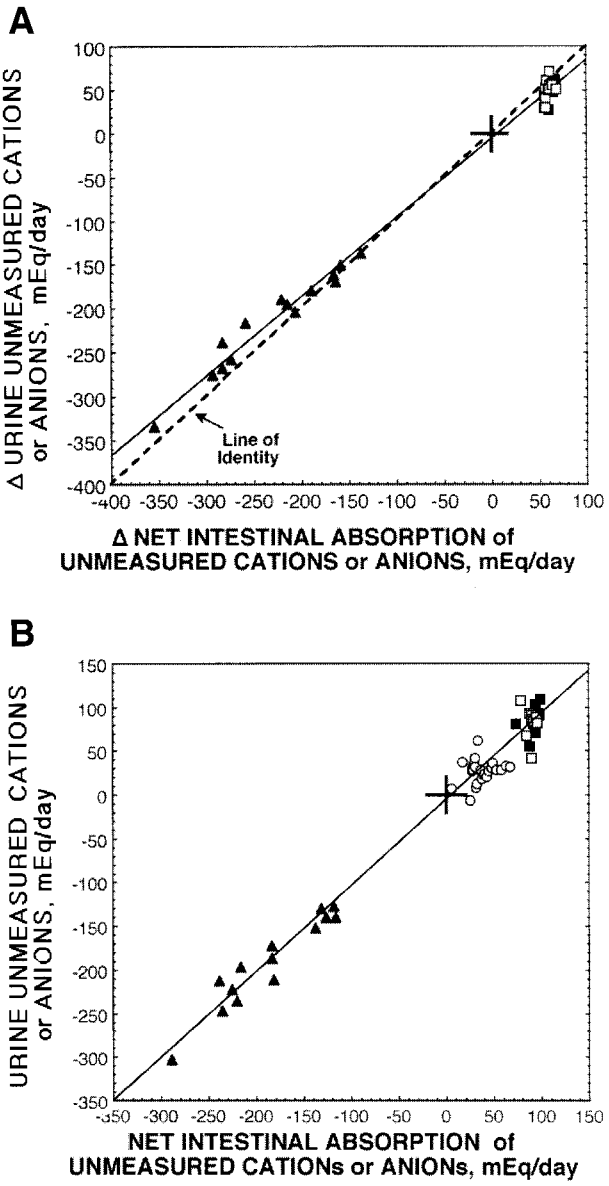


Fig. 10. *A*: changes from control in urine unmeasured anions or cations, estimated as $[(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + 1.8 \cdot PO_4)]$, compared with the changes from control in the measurements of intestinal absorption of unmeasured cations or anions, estimated as dietary − fecal $[(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + 1.8 \cdot PO_4)]$, during the administration of NH_4Cl (▲), $KHCO_3$ (■), or $NaHCO_3$ (●); $y = 5.1$ (± 2.1 SE) + 0.9033 (±0.0132 SE)x; $r^2 = 0.993$; $P < 0.0001$. *B*: estimation of net intestinal absorption of cations or anions based on the analyses of urine $[(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl + 1.8 \cdot PO_4)]$ in relation to the direct measurements of intestinal absorption of unmeasured cations or anions, estimated as dietary minus fecal $[(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + 1.8 \cdot PO_4)]$, during control (●) and the administration of NH_4Cl (▲), $KHCO_3$ (■), or $NaHCO_3$ (●); $y = -3.1$ (±3.2 SE) + 0.987 (±0.0293 SE)x; $r^2 = 0.95$; $P < 0.001$. Adapted from data in Refs. 1, 34, 37, 74.

the cations for each subject, the slope of the relationship was not different from unity and the intercept was not different from zero. Additionally, 76 of these studies were carried out while the subjects ate control diets (mean urinary pH = 6.05 ± 0.44; mean urinary net acid excretio*n* = 49 ± 28 meq/day), 26 studies while the subjects received NH_4Cl (mean urinary pH = 5.40 ± 0.13; mean urine net acid excretion = 272 ± 61 meq/day), and 20 studies while the subjects received $KHCO_3$ or Na HCO_3 (mean urinary pH = 6.67 ± 0.18; mean urinary net acid excretion = 6 ± 13 meq/day). Regardless of variations in fixed acid production and both net acid excretion rate and urinary pH, the data points are similarly distributed above and below the identity line. Thus experimental variation of acid-base balance does not reveal the urinary excretion of presently unrecognized or unidentified urinary cations or anions.

Titratable acid. Historically, titratable acid has most often been directly determined by titration of urine from urinary pH to blood pH, usually pH = 7.40. The titration measures the contribution of the major urinary buffers, phosphate and creatinine, to urinary proton excretion and, in addition, the contribution of urinary organic anions that are, effectively, not ionized at urinary pH. The latter fraction of urinary organic acids, that are titrated between urinary pH and blood pH, does not represent acid excretion because they are excreted as free acids, just as they were produced. Moreover, these free urinary organic acids are not a component of net acid excretion as they do not identify HCO_3^- regeneration. Furthermore, it has been known for almost a century (21), and subsequently confirmed (35), that when urine contains increasingly large concentrations of ammonium, titratable acid, as measured by direct titration, is increasingly overestimated because of the titration of $NH_4^+ \leftrightarrow NH_3 + H^+$ ($pK_a = 9.1$). Additionally, when urine contains increasingly large

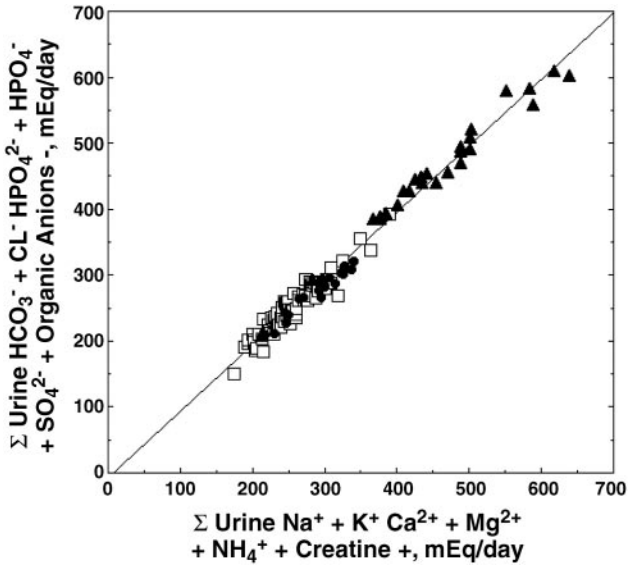


Fig. 11. Comparison of the sums of the mean daily urinary excretion rates of anions, estimated as HCO_3^- + Cl^- + HPO_4^{2-} + $H_2PO_4^-$ + SO_4^{2-} + organic $anion^-$ (meq/day) to the sums of the cations, estimated as $Na^+ + K^+ + Ca^{2+} + Mg^{2+} + NH_4^+$ + $creatinine^+$ (meq/day) during control (○) and during the administration of NH_4Cl (▲) or $KHCO_3$ or $NaHCO_3$ (●). Mean sum of cations = 303 meq/day. Mean sum of anions = 298 meq/day. Mean = 5 ± 14 meq/day; $y = -7.8$ (±4.2) + 1.009 (±013); $r^2 = 0.980$; $P < 0.0001$ Adapted from data in Refs. 1, 34, 36, 37, 40, 42, 51, Lemann J, unpublished observations.

concentrations of Ca^{2+} together with $H_2PO_4^- + HPO_4^{2-}$, there is precipitation of $CaHPO_4/Ca_3(PO_4)_2(OH)_2/Ca_{10}(PO_4)_6CO_3$ from solution when the titration reaches a pH of ≥7.0, resulting in the simultaneous release of H^+ that also causes overestimation of titratable acid. Accordingly, it would appear preferable to estimate titratable acidity by calculation using urinary pH, the urinary contents of PO_4, and a $pK_{a2} = 6.8$ for the reaction $H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+$, and the urinary content of creatinine and a $pK_a = 4.97$ for the reaction creatinine$^+ \leftrightarrow$ creatinine + H^+ (35).

HCO_3^-. The measurement of urinary HCO_3^- excretion requires the collection of urine under a layer of mineral oil or toluene to minimize the loss of dissolved CO_2. The concentration of HCO_3^- is then calculated from measurements of urinary pH and total CO_2 concentration, as employed in the studies reviewed in this report, or of urinary pH and P_{CO_2}. Figure 12 illustrates the exponential relationships between urinary [HCO_3^-] and urinary pH and between daily urinary HCO_3^- excretion rates and pH for a large number of 24-h urinary measurements (34, 39, 40, Lemann J, unpublished observations). When urinary pH is in the range of 6.0–6.4 or less, [HCO_3^-] are mostly ≤5 mmol/l, but daily urinary HCO_3^- excretion rates may reach 10–15 mmol/day, quantities that significantly reduce renal net acid excretion. When urinary pH rises to levels above pH 6.4, urinary [HCO_3^-] and HCO_3^- excretion rates increase progressively. The urinary loss of HCO_3^- becomes negligible when urine pH is ≤6.0.

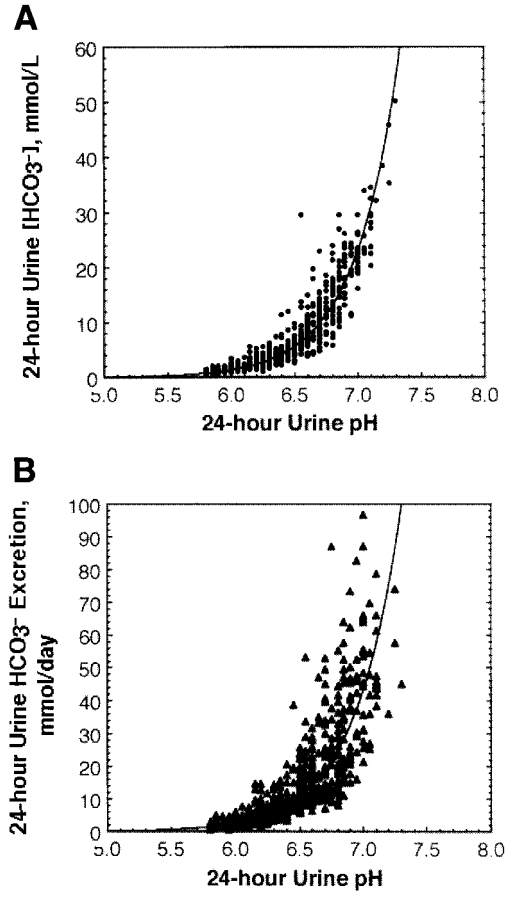


Fig. 12. *A*: urinary [HCO_3^-] (meq/l) calculated from manometric measurements of total CO_2 concentration and pH in relation to urinary pH; $y = 6.5418e - 8 \cdot 10^{(1.2215x)}$; $r^2 = 0.910$. *B*: daily urinary HCO_3^- excretion rates calculated from urinary [HCO_3^-] and urinary volumes in relation to urinary pH. $y = 1.7899e - 7 \cdot 10^{(1.979x)}$; $r^2 = 0.804$. Data are for 631 24-h urine collections from 42 adults studied in Refs. 34, 39, 40, and Lemann J, unpublished observations.

To summarize, renal net acid excretion appears to be accurately measured, without significant errors, by the sum of urinary NH_4^+ plus titratable acid as calculated from the urinary content of PO_4 and creatinine together with urinary pH minus urinary HCO_3.

CHARGE BALANCE IN RELATION TO ACID BALANCE

A fundamental inviolate law of physics and chemistry requires that compounds are electrically neutral. In other terms, the positive charges must always equal the negative charges so that the charges balance. With respect to human biology, charge balance must exist throughout the body, in the foods that are eaten and in the excreta, particularly the urine and the feces as well as in customarily unmeasured losses of sweat and of desquamated skin, hair, and nails. Furthermore, charge balance must prevail whether net external acid, mineral, and nitrogen balances are positive, especially during growth, or negative, either subtly during senescence or slowly advancing chronic diseases, more severely during acute illnesses, or during experimentally induced increases or decreases in acid production. The positive and negative charges must, necessarily, always balance.

Figure 13*A* summarizes the changes from control of charge balances in relation to the changes from control of acid balances for the subjects given NH_4Cl (Table 1), $KHCO_3$ (Table 2), or $NaHCO_3$ (Table 3). As shown in Fig. 13*B*, negative Ca^{2+} balances primarily account for the negative charge balances during the administration of NH_4Cl, whereas positive $K^+ + Ca^{2+}$ or positive Na^+ balances primarily account for the positive charge balances during the administration of $KHCO_3$ or $NaHCO_3$, respectively. Accordingly, as shown in Fig. 13*C*, charge balances become negative during the administration of acid, as Ca^{2+} balances become negative via urinary Ca^{2+} losses as acid balance become positive in relation to bone buffering of H^+. Charge balances become positive during the administration of base as acid balances become more negative due to HCO_3^- retention with $K^+ + Ca^{2+}$ during the administration of $KHCO_3$ or with Na^+ during the administration of $NaHCO_3$.

RELATIONSHIP OF CA BALANCES TO ACID BALANCES IN HEALTHY HUMAN SUBJECTS

Figure 14 shows the relationships between the changes from control of Ca^{2+} balances, net intestinal Ca^{2+} absorption, and urinary Ca^{2+} excretion, each in relation to the changes in acid balances for the individual subjects given NH_4Cl (summarized in Table 1; 1, 37, 74); the subjects given $KHCO_3$ (summarized in

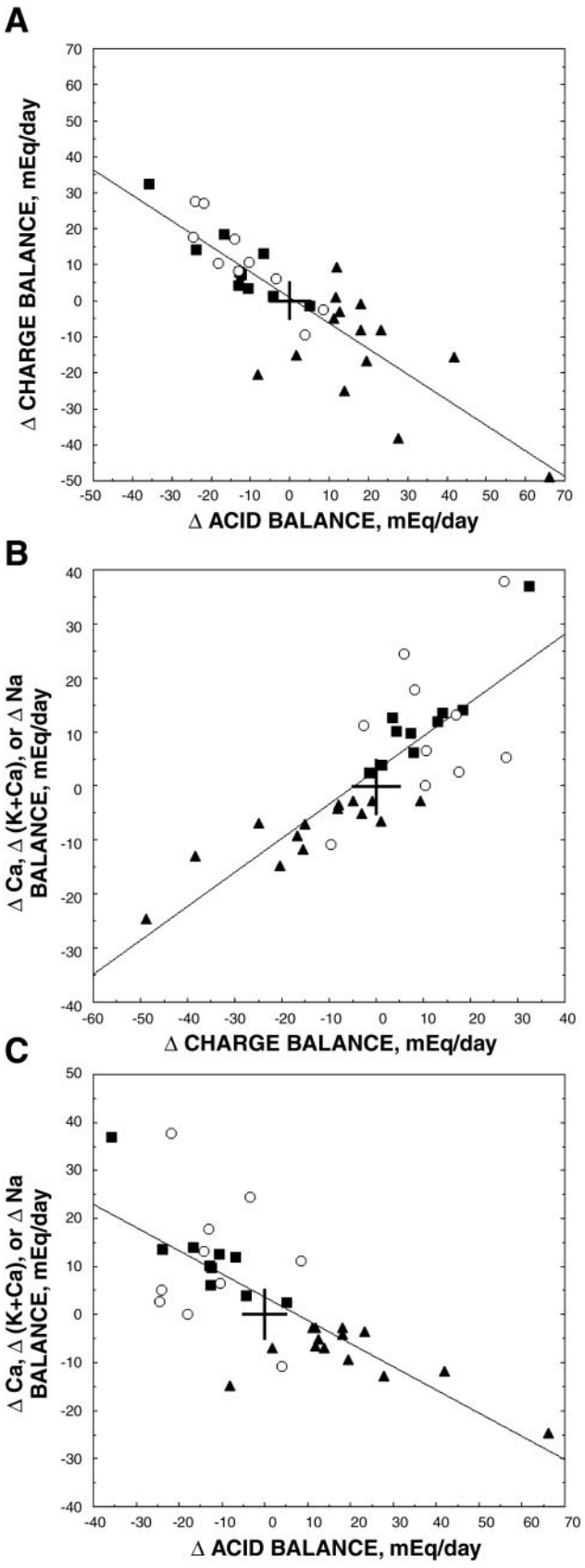
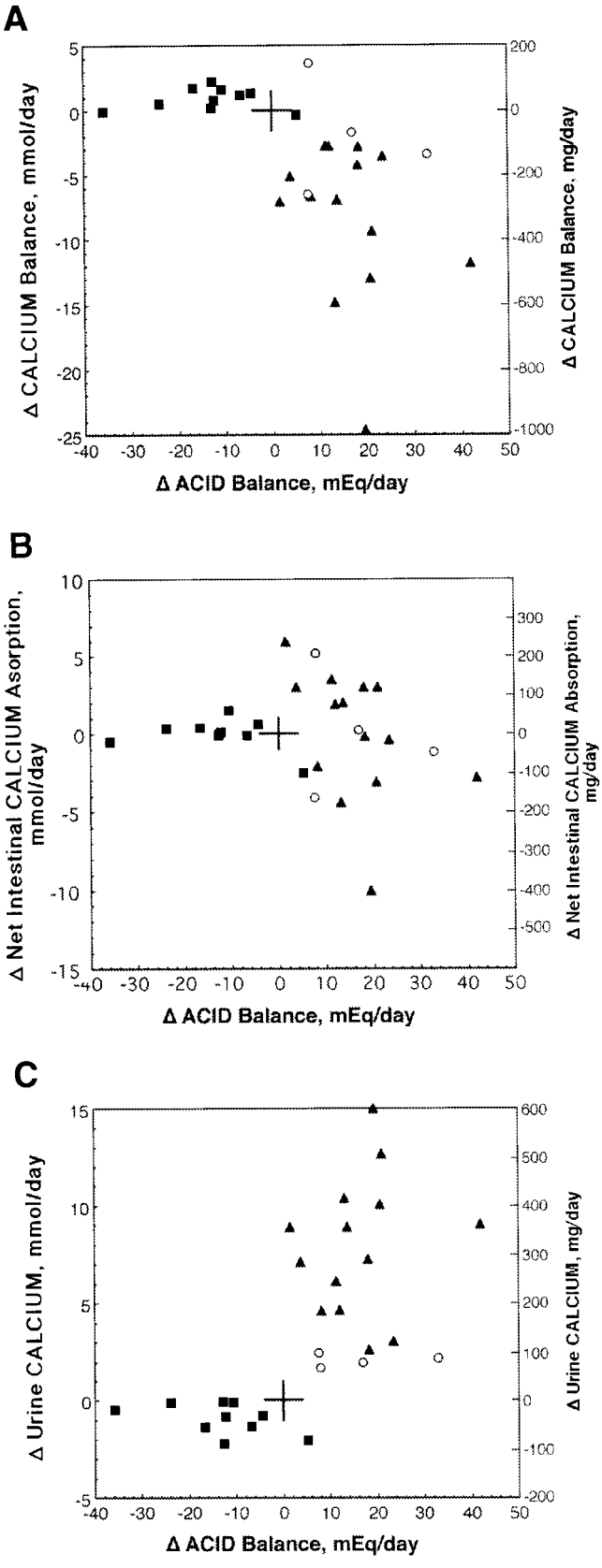


Fig. 13. *A*: changes from control of charge balances in relation to the changes from control of acid balances among the subjects given NH_4Cl (▲, Table 1); $KHCO_3$ (■, Table 2); and $NaHCO_3$ (○, Table 2); $y = 1.0277 - 0.70891x$; $r^2 = 0.716$; $P < 0.0001$. *B*: changes from control of Ca^{2+} balances among the subjects given NH_4Cl (▲, Table 1); $K^+ + Ca^{2+}$ balances among the subjects given $KHCO_3$ (■, Table 2); and Na balances among the subjects given $NaHCO_3$ (○, Table 3) in relation to the changes from control in charge balances; $y = 3.02 + 0.63048x$; $r^2 = 0.662$; $P < 0.0001$. *C*: changes from control of Ca^{2+} balances among the subjects given NH_4Cl (▲, Table 1), $K^+ + Ca^{2+}$ balance among the subjects given $KHCO_3$ (■, Table 2); and Na^+ balances among the subjects given $NaHCO_3$ (○, Table 3) in relation to the changes from control of acid balances; $y = 3.69 - 0.48378x$; $r^2 = 0.554$; $P < 0.0001$. Adapted from data in Refs. 1, 34, 37, 74.

Table 2; 34); and among the four additional subjects in whom acid production was increased by increasing dietary protein intake as egg white (1). As depicted in Fig. 14*A*, the changes from control of Ca^{2+} balances became progressively more negative as the changes from control of acid balances became more positive. By contrast, the changes from control of Ca^{2+} balances became positive when the changes from control of acid balances became negative, although in this circumstance the increase was not progressive; rather, the changes of Ca^{2+} balances appear to have reached a modestly positive but stable level. No significant changes from control in net intestinal Ca^{2+} absorption were detected as the changes from control of acid balances varied, as shown in Fig. 14*B*. As a consequence, the more negative Ca^{2+} balances that appeared as acid balances became more positive were only the result of the increases from control in urinary Ca^{2+} excretion, as shown in Fig. 14*C*. When the changes from control in acid balances were negative during $KHCO_3$ administration, urinary Ca^{2+} excretion decreased but did not fall progressively.

Among healthy adults eating a wide range of diets of different composition, the type and quantity of dietary protein are determinants of acid production related to the dietary content of sulfur-containing amino acids and their oxidation to sulfate. Some data illustrating these effects are summarized in Fig. 15. As shown in Fig. 15*A*, daily urinary SO_4 excretion rates increase as dietary protein intake increases. Additionally, the magnitude of the increase in SO_4 excretion varies with the type of dietary protein. The increase in SO_4 excretion is greatest when dietary protein is provided as egg white, which contains large amounts of sulfur-containing amino acids; is less when a mixture of normal foods including meat, milk, and cereal are the dietary protein sources; and is least when a soy protein, which is deficient in methionine, is used (1, 20, 36, 37, 41, 45, 52, 65, 74, unpublished observations). As illustrated in Fig. 15*B*, when acid production is increased by the administration of wheat gluten and lactalbumin (66), egg white (1) or methionine (41), the resulting increases from control in urinary net acid excretion are less than the increases in acid production, as estimated by the increases in urinary SO_4 excretion, the slope of the regression line being significantly <1. Thus renal compensation, in terms of increased net acid excretion,



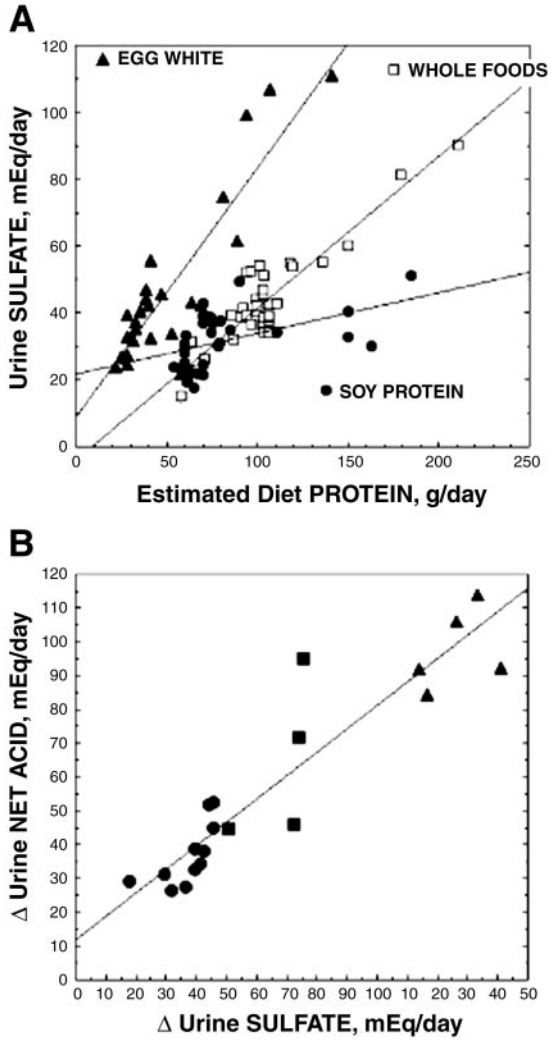


Fig. 15. *A*: relationships between urinary SO_4^{2-} and dietary intake of protein among subjects fed diets providing protein as egg white ($y = 8.7 + 0.744x$; $r^2 = 0.788$); as mixed proteins contained in meat, milk, and cereals of normal whole-food diets ($y = -3.7 + 0.45x$; $r^2 = 0.839$); and as soy protein ($y = 21.9 + 0.12x$; $r^2 = 0.238$). Adapted from data in Refs. 1, 20, 36, 37, 41, 45, 52, 65, 74, and unpublished observations. *B*: relationship between the changes from control in urinary net acid excretion rate and the changes from control in urinary SO_4^{2-} excretion rate when dietary sulfur-containing amino acid intake is increased by methionine (▲), egg white (■), or wheat gluten and lactalbumin (●); $y = 11.95(\pm 5.62\ \text{SE}) + 0.6932(\pm 0.0.0661\ \text{SE})x$; $r^2 = 0.859$; $P < 0.0001$. Adapted from data in Refs. 1, 41, 66.

in response to acid loads derived from protein is incomplete as it is for NH_4Cl loads.

The other dietary factor affecting acid production is the intake in fruits and vegetables of actual $KHCO_3$ or potential $KHCO_3$, as K-salts of organic acids (citrate, lactate, etc.), that are metabolized to HCO_3^-. That

Fig. 14. Individual changes from control of Ca^{2+} balances (*A*), net intestinal Ca^{2+} absorption (*B*), and urinary Ca^{2+} excretion (*C*) in relation to the changes from control of acid balances among 14 subjects given NH_4Cl, summarized in Table 1 (▲), 10 subjects given $KHCO_3$, summarized in Table 2 (■), together with additional data for 4 subjects given egg white (○). Adapted from data in Refs. 1, 34, 37, 74.

process, by contributing base, reduces acid production. There apparently are no studies presently available that evaluate the separate effects of increased intakes of individual fruits or vegetables on K^+ and net acid excretion. However, the effects of adding or removing $KHCO_3$ from the diet have been assessed. The results of such studies (34, 39, 40) are shown in Fig. 16. When $KHCO_3$ is added to the diets of subjects fed otherwise constant diets, the changes from control in daily urinary net acid excretion falls in inverse proportion to the increments from control of urinary K^+ excretion. The opposite sequence occurs when $KHCO_3$ is removed from the diet. Additionally, the administration of $KHCO_3$ to postmenopausal women is accompanied by significantly less negative Ca^{2+} balances, a decrease in urinary hydroxyproline excretion, and an increase in serum osteocalcin concentrations, all reflecting a decrease in bone resorption (69). The interaction of the dietary intakes of protein and of K^+ to determine net acid excretion is reviewed in detail elsewhere (23, 31, 55, 68).

Measurements of urinary net acid excretion are more readily made and are more precise than are measurements of rates of acid production rates as well as acid balances. Moreover, net acid excretion rates vary directly with acid production, and urinary Ca^{2+} excretion rates vary inversely with acid balances and directly with net acid excretion rates.

Thus it is informative to evaluate the changes from control of urinary Ca^{2+} excretion in relation to the changes in net acid excretion from control. Figure 17 illustrates such data for urinary Ca^{2+} excretion among healthy adults subjected to several differing alterations in diet composition that change net acid excretion (1, 33, 34, 36, 37, 39–42, 74). The changes from control of urinary Ca^{2+} excretion vary exponentially with the changes from control of net acid excretion. As the changes from control in net acid excretion become >0, the changes from control of urinary Ca^{2+} excretion increase progressively, reflecting loss of Ca^{2+} from bone as retained H^+ is buffered. As the changes from control in net acid excretion become negative, the changes from control of urinary Ca^{2+} excretion fall to a minimum, approaching a plateau. Speculatively, such maximally low rates of urinary Ca^{2+} excretion reflect a lower limit of renal Ca^{2+} conservation.

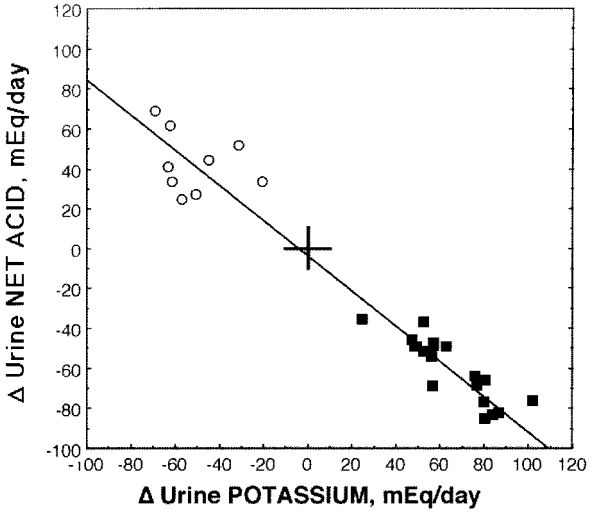


Fig. 16. Changes from control in urinary net acid excretion rates in relation to the changes from control in urinary K^+ excretion rate among healthy adults when $KHCO_3$ is added to (●) or removed from (○) the diet; $y = -3.36 + 0.8806x$; $r^2 = 0.941$. Adapted from data in Refs. 34, 39, 40.

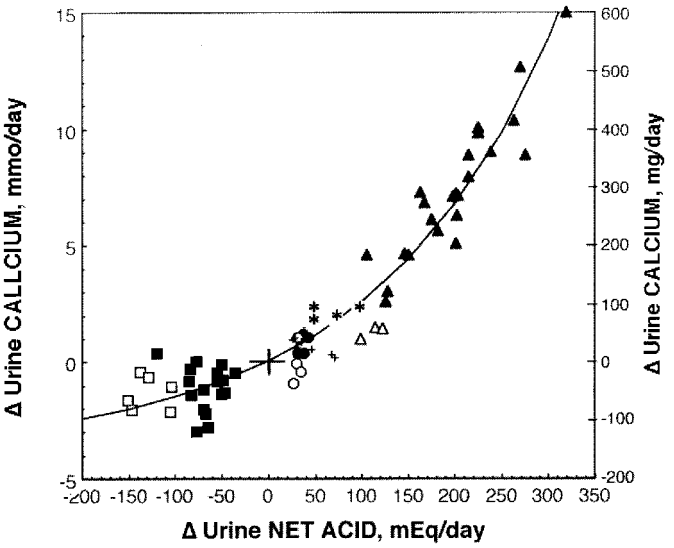


Fig. 17. Individual changes from control of urinary Ca^{2+} excretion in relation to the changes from control in net acid excretion among healthy adults given NH_4Cl (▲), methionine (△), egg white (*), beef (●), soy protein (○), deprived of $KHCO_3$ (+), given $KHCO_3$ (■), or given $NaHCO_3$ by replacing some of the dietary NaCl and maintaining Na^+ intake constant (□). Urinary Ca^{2+}, mmol/day = 0.05 + 10[(0.002217·Net Acid, meq/day) + (0.5814)] r^2 = 0.933. Adapted from data in Refs. 1, 33, 34, 36, 37, 39, 40–42, 74.

ACID-BASE BALANCE IN PATIENTS WITH THE ACIDOSIS OF CHRONIC KIDNEY DISEASES

Chronic metabolic acidosis is well known to develop among most patients with chronic kidney diseases as glomerular filtration falls, primarily as a result of a reduced capacity of the kidneys to excrete NH_4^+ (reviewed in Ref. 25). Similarly, metabolic acidosis is the cardinal feature of renal tubular disorders that impair either reabsorption of filtered HCO_3^-, as in proximal RTA, or H^+ excretion, as in classical distal RTA.

Evaluation of Acid Balances Among Patients With Chronic Renal Kidney Failure or Distal RTA Using Formula Diets

The liquid-formula diets, devised originally to assess net fixed acid balances among healthy adults, were similarly used to evaluate rates of net fixed acid production among seven patients with acidosis associated with chronic kidney failure ($C_{creatinine}$ = 18 ± 8 ml/min) together with one patient with distal RTA ($C_{creatinine}$ = 96 ml/min) (26). For this group, acid production averaged 50 ± 13 meq/day or 1.02 meq·kg body wt^{-1}·day^{-1}. This rate is comparable to that observed

among healthy subjects fed the same diets that averaged 0.93 meq·kg^{-1}·day^{-1}. However, among those patients, rates of renal net acid excretion were less than normal, averaging only 31 ± 16 meq/day when their rates of acid production averaged 50 ± 13 meq/day. Thus their acid balances were positive, averaging +19 ± 7 meq/day, despite low but stable serum [HCO_3^-] that averaged 16.1 ± 3.3 mmol/l. Six of these subjects, including the patient with distal RTA, were restudied during the ongoing administration of $NaHCO_3$ given in individually constant doses sufficient to maintain normal serum [HCO_3^-] that averaged 25.7 ± 1.6 mmol/l. With correction of the acidosis, acid balances became less positive in each patient, averaging for −4 ± 10 meq/day for the group, a mean not different from zero, as observed in healthy subjects. Accompanying the less positive acid balances, urinary Ca^{2+} excretion decreased among the five azotemic patients from an average of 2.77 ± 1.88 to 1.09 ± 0.93 meq/day ($P = 0.045$) and from 8.85 to 3.21 meq/day in the patient with distal RTA. These studies showed that stabilization of serum [HCO_3^-] levels among patients with renal acidosis was not the result of a fall in fixed acid production in proportion to their reduced capacity to excrete net acid into their urine. Because correction of acidosis by $NaHCO_3$ administration restored net fixed acid balance to values not different from zero, it appeared that acidosis initiated an extrarenal mechanism for buffering or excretion (disposal) of the acid not excreted into urine.

Evaluation of the Acidosis of Chronic Azotemic Kidney Diseases Using Normal Whole-Food Diets

Eight paired studies during spontaneous stable acidosis and during ongoing correction of the acidosis by the administration of $NaHCO_3$ were performed among patients with chronic renal failure ($C_{creatinine}$ = 12 ± 7 ml/min) fed constant whole-food diets (47). The balance periods in each phase lasted 18 or 24 days and began after each patient adapted to their constant diet for 6–10 days. The results of these studies are summarized in Table 5.

When the mean data only for the periods of stable spontaneous acidosis among patients with chronic kidney disease are considered (Table 5), it can be seen that although acid balances were positive, averaging +10 ± 13 meq/day, that figure is not different from zero ($P > 0.1$), as described in the original report of those data (47). Moreover, urinary Ca^{2+} excretion averaged only 1.68 ± 0.48 meq/day among the eight patients with advanced kidney failure when their serum [HCO_3^-] averaged 18.7 ± 3.2 mmol/l (Table 5). Nevertheless, the HCO_3^-/CO_3^{2-} content of bone obtained postmortem

Table 5. *Changes in acid, mineral, and charge balances during $NaHCO_3$ administration to 8 patients with the spontaneous acidosis of chronic renal failure (47)*

	Acidosis	$NaHCO_3$	Δ	P[a]
Dose of $NaHCO_3$	6 ± 11	107 ± 37[b]	+101 ± 36	Independent
Body wt, kg	56.38 ± 14.15	56.36 ± 14.21	−0.02 ± 1.61	NS
Daily change in body wt, kg	+0.06 ± 0.05	+0.02 ± 0.05	−0.04 ± 0.04	= 0.046
Serum [HCO_3], mmol/l	18.7 ± 3.2	27.4 ± 2.1	+8.7 ± 3.5	<0.001
Basic diet unmeasured anion, meq/day[c]	54 ± 10	56 ± 12	+2 ± 7	NS
Fecal Na, meq/day	2 ± 3	2 ± 2	0 ± 1	NS
Fecal unmeasured anion, meq/day[c]	29 ± 8	28 ± 6	−1 ± 4	NS
Intestinal absorption of unmeasured anion, meq/day[d]	31 ± 10	135 ± 35	+104 ± 33	<0.001
Urine unmeasured anion, meq/day[c]	32 ± 17	124 ± 33	+96 ± 30	<0.001
Urine unmeasured anion − measured intestinal absorption of unmeasured anion, meq/day	−1 ± 10	11 ± 14	−12 ± 11	= 0.045
Urinary SO_4, meq/day	25 ± 10	23 ± 9	−2 ± 2	NS
Urinary organic anion, meq/day	36 ± 7	44 ± 9	+8 ± 7	= 0.013
Net fixed acid production, meq/day	30 ± 20	−68 ± 40	−98 ± 34	<0.001
Urinary pH	5.93 ± 0.41	7.39 ± 0.24	+1.46 ± 0.35	<0.001
Urinary titratable acid, meq/day	10 ± 4	1 ± 2	−10 ± 4	<0.001
Urinary NH_4, meq/day	12 ± 6	4 ± 3	−8 ± 4	<0.001
Urinary HCO_3, meq/day	2 ± 2	71 ± 28	+69 ± 28	<0.001
Renal net acid excretion, meq/day[e]	20 ± 11	−66 ± 32	−86 ± 30	<0.001
Acid balance, meq/day	+10 ± 13	−2 ± 15	−12 ± 9	= 0.002
Na^+ balance, meq/day	+17 ± 15	+12 ± 15	−5 ± 18	NS
K^+ balance, meq/day	+3 ± 6	+3 ± 4	0 ± 3	NS
Ca^{2+} balance, meq/day	−5 ± 4	−1 ± 4	+4 ± 4	= 0.021
Mg^{2+} balance, meq/day	+1 ± 4	+1 ± 2	0 ± 4	NS
Cl^- balance, meq/day	+13 ± 15	+2 ± 5	−11 ± 14	NS
PO_4 balance, mmol/day	+2 ± 2	+1 ± 3	−1 ± 2	NS
PO_4 balance · 1.8, meq/day	+4 ± 4	+2 ± 5	−2 ± 4	NS
Charge balance, meq/day[f]	−1 ± 10	+11 ± 9	+12 ± 11	= 0.048

[a]Probability that the mean difference for the group is not different from zero using the t-test for paired data. [b]Variances throughout are shown as ±SD. [c]Estimated as ($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day). [d]Difference between diet and fecal unmeasured anion, each estimated as ($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day). [e]Calculated as (titratable acid + NH_4 − HCO_3), meq/day. [f]Estimated as [(diet($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day)] − [(fecal + urine($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$, meq/day) − ($Cl^-$, meq/day + 1.8 · PO_4, mmol/day)].

from patients dying of uremia in the predialysis era was found to be low compared with bone from patients without kidney failure (62). Thus positive acid balances with bone buffering of retained H^+ and loss of bone CO_3^{2-} must have occurred earlier during the progression of chronic renal failure.

By contrast, when the mean data only for the periods of experimental stable NH_4Cl acidosis among 14 healthy adults are considered (Table 1), it is seen that the absolute acid balances were positive, averaging $+9 \pm 15$ meq/day, a figure that is significantly >0 ($P < 0.05$). Furthermore, the mean Ca^{2+} balances of -12 ± 13 meq/day were significantly <0 ($P < 0.005$) as a consequence of increased urinary Ca^{2+} excretion that averaged 24.7 ± 8.5 meq/day when the serum [HCO_3^-] averaged 20.2 ± 2.1 mmol/l (Table 1). Thus there must have been H^+ retention and bone buffering, resulting in the loss of Ca^{2+} into the urine, the skeleton providing the only body stores of Ca^{2+}.

The effects of correcting the acidosis among the patients with kidney failure (Table 5) were, in most respects, similar to the effects of administering $NaHCO_3$ to healthy subjects (Table 3). Daily fecal Na excretion during spontaneous acidosis among the patients with kidney failure averaged 2.0 meq/day and did not change during the administration of $NaHCO_3$, averaging 1.8 meq/day. Moreover, estimated rates of excretion of fecal unmeasured anion also did not differ, averaging 29 ± 8 meq/day during spontaneous acidosis and 28 ± 6 meq/day during ongoing $NaHCO_3$ administration. Thus as shown in Table 5, net intestinal absorption of unmeasured anion increased on average from 31 to 135 meq/day or by an average of 104 meq/day, an amount nearly identical to the quantity of $NaHCO_3$ that was administered that averaged 101 meq/day. Urinary SO_4 excretion decreased insignificantly by -2 meq/day during $NaHCO_3$ administration, whereas the urinary excretion of organic anion increased, on average, by 8 meq/day, because urine became significantly more alkaline. Net fixed acid production thus decreased during $NaHCO_3$ administration by an average of -98 meq/day, an amount also nearly equivalent to the average increment in $NaHCO_3$ administered of 101 meq/day. However, renal net acid excretion did not decrease equivalently, the average decrement being only -86 meq/day. Thus average acid balances became significantly less positive by -12 meq/day or, in equivalent but opposite terms, HCO_3^- balance became more positive by $+12$ meq/day. Additionally, average daily charge balance became more positive by $+12$ meq/day. The direction and magnitude of these changes in acid and in charge balance during the administration of $NaHCO_3$ to these patients with renal acidosis are comparable to those observed during the administration of either $KHCO_3$ (Table 2) or $NaHCO_3$ (Table 3) to healthy subjects. However, more negative Cl^- and PO_4 balances relative to $Na^+ + K^+ + Ca^{2+} + Mg^{2+}$ balances appear to have accounted for the more positive charge balances among the patients with renal acidosis given $NaHCO_3$ while positive $K^+ + Ca^{2+}$ or Na^+ balances, respectively, accounted for the positive charge balances during the administration of $KHCO_3$ or $NaHCO_3$ to healthy subjects. The basis for this difference is not apparent because serum concentrations of both Cl and PO_4 were stable during both spontaneous acidosis and during the administration of $NaHCO_3$ to the patients, although, as expected, absolute Cl^- concentrations were lower during $NaHCO_3$ administration. Additionally, as previously noted, the patients with chronic kidney diseases did not exhibit hypercalciuria when acidotic, consistent with long-known observations that urinary Ca^{2+} excretion falls early during the progressive decline in glomerular filtration rate in chronic kidney diseases (46).

Additionally, six of the patients summarized in Table 5 (47) were given a measured amount of stable strontium, a bone-seeking element (22), during both the acidosis- and $NaHCO_3$-treatment phases of their studies. When acidosis was corrected with $NaHCO_3$, the cumulative strontium excretion into urine and feces over 6 days was less and the serum strontium concentrations on the *day 6* were lower than when the subjects were acidotic (47). Thus when acidosis was corrected, a greater quantity of strontium was retained at sites outside the ECF, presumably bone, providing further evidence that net bone resorption had been inhibited by ongoing $NaHCO_3$ treatment.

Despite hypocalciuria in the presence of chronic renal acidosis, Ca^{2+} balances became less negative when the acidosis was corrected by the ongoing administration of $NaHCO_3$ (Table 5). A minor fraction of this improvement in Ca^{2+} balances was contributed by a very slight and insignificant decline in the already low urinary Ca^{2+} excretion of -0.16 ± 0.52 meq/day. Most of the improvement was due to decreased fecal loses of Ca^{2+}, net intestinal absorption of dietary Ca^{2+} improving from -3.8 ± 3.2 to -0.6 ± 4.6 meq/day ($+3.2 \pm 3.4$ meq/day; $P = 0.033$). However, despite the correction of acidosis by $NaHCO_3$ treatment, absolute Ca^{2+} balances remained slightly, but not significantly, negative at -1 ± 4 meq/day (Table 5).

The acidosis of chronic kidney diseases is clearly different from experimental NH_4Cl acidosis among healthy adults. Acidosis develops among patients with chronic kidney failure because of the inability of the damaged kidneys to excrete acid (25), not because of increased rates of acid production. Furthermore, while urinary Ca^{2+} excretion rates were very low, as discussed previously, Ca^{2+} balances, nevertheless averaged -5 ± 4 meq/day, a value significantly <0 ($P < 0.01$), also as originally described (47). Thus the magnitudes of both H^+ retention and Ca^{2+} loss are far less than those observed during experimental NH_4Cl acidosis in healthy subjects. Among these acidotic patients, fecal Ca^{2+} excretion rates exceeded dietary Ca^{2+} intakes so that net intestinal Ca^{2+} absorption was, on average, <0 at -2 ± 2 meq/day, reflecting the long-known impairment of Ca^{2+} absorption among patients with advanced kidney diseases (48) that is now known to be due to the failure of calcitriol production in advanced kidney diseases (72). That effect appears to be of overriding significance in determining Ca^{2+} balances among patient with ad-

vanced kidney failure. Speculatively, acidosis enhances Ca^{2+} secretion into the intestine that then cannot be normally reabsorbed when calcitriol levels are very low, thus increasing fecal Ca^{2+} losses. Correction of acidosis may then minimize such losses. Such effects could be directly assessed by evaluating the disposition of different Ca^{2+} isotopes given orally and intravenously before and after correction of acidosis. At the same time, PO_4 retention could, speculatively, protect bone against the effects of acidosis by independently inhibiting bone resorption (3, 29, 64).

As noted previously, acid balance was found to be positive in a patient with distal RTA, and correction of acidosis by the ongoing administration of $NaHCO_3$ restored acid balance to zero and reduced urinary Ca^{2+} excretion (26). Other studies have demonstrated that treatment of distal RTA with $NaHCO_3$ is accompanied by more positive Ca^{2+} balance as a result of reductions in both urinary and fecal Ca^{2+} excretion (16).

When kidney function is normal, increased rates of acid production that are not matched by an equivalent increase in the rate of renal net acid excretion must lead to bone buffering together with increased urinary Ca^{2+} excretion rates that appear to be the result of inhibition of renal tubular reabsorption of filtered Ca^{2+} (38). Such effects, which also can be caused by high dietary intakes of protein and low dietary intakes of fruits and vegetables, may be contributory to hypercalciuria in the pathogenesis of Ca^{2+}-containing kidney stones (31) and to the development of osteoporosis (55, 68).

However, chronic metabolic acidosis alone is not sufficient to cause either positive acid balances or negative Ca^{2+} balances. Patients with isolated familial proximal RTA have been observed to exhibit normal rates of both acid production and net acid excretion and thus are in acid balance. Moreover, they also exhibit normal rates of urinary and fecal Ca^{2+} excretion relative to their Ca^{2+} intakes and are in Ca^{2+} balance (32).

Thus it appears that both positive acid balances and increased Ca^{2+} excretion rates are necessary for the development of negative Ca^{2+} balances in the presence of metabolic acidosis. The routes of Ca^{2+} loss appear to be hypercalciuria when acidosis occurs among subjects with normal kidney function and greater fecal losses of Ca^{2+} when active transcellular Ca^{2+} absorption is already markedly reduced among patients with advanced kidney disease because of the failure of renal calcitriol synthesis.

DISCLOSURES

This work was supported in part by National Institutes of Health Grants DK-15089 and RR-00059 (to J. Lemann, Jr), AR-46289, DK-57716, and DK-56788 (to D. A. Bushinsky); DK-54952, and a grant from the Department of Veterans Affairs (to L. L. Hamm).

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