A Comparison of the Effects of Glucose Ingestion and NH_4Cl Acidosis on Urinary Calcium and Magnesium Excretion in Man

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ABSTRACT Both glucose ingestion and NH_4Cl acidosis have been reported to augment urinary calcium ($U_{Ca}V$) and magnesium ($U_{Mg}V$) excretion. Both also cause acidification of the urine and an increase in renal acid excretion. To examine whether a common mechanism of action was involved, the effects of glucose ingestion and NH_4Cl acidosis on $U_{Ca}V$ and $U_{Mg}V$ were tested in the same subjects. Glucose ingestion caused significant increases in both $U_{Ca}V$ and $U_{Mg}V$. During stable NH_4Cl acidosis, $U_{Ca}V$ increased significantly, while $U_{Mg}V$ was unaffected. When a glucose load was given during acidosis, the separate effects of acidosis and glucose on $U_{Ca}V$ were additive, whereas $U_{Mg}V$ increased less than observed during normal acid-base balance. Although renal acid excretion increased and the urine was acidified after glucose in the normal steady state, when glucose was administered during NH_4Cl acidosis urine pH rose and there was no change in renal acid excretion. We concluded that NH_4Cl acidosis and glucose ingestion reduce the renal tubular reabsorption of magnesium and (or) calcium, but they act through separate mechanisms.

INTRODUCTION

The ingestion of food (1) or any of a variety of rapidly metabolizable substrates (2) causes a sharp increase in urinary calcium and magnesium excretion. Because this occurs without detectable changes in the glomerular filtration rate (2, 3) or in the concentrations of calcium and magnesium in serum ultrafiltrates (3), it appears to be due to diminished renal tubular reabsorption of calcium and magnesium. We noted that the urine was sharply acidified following glucose ingestion and renal net acid excretion (titratable acid + ammonium − bicarbonate) increased significantly in parallel with the increases in urinary calcium and magnesium excretion (4). We had observed previously that renal tubular calcium reabsorption was inhibited during stable experimental metabolic acidosis and that the increments in urinary calcium excretion during acidosis were closely correlated with simultaneous increments in urinary ammonium excretion (5). Increases in urinary magnesium excretion have also been found in some (6, 7) but not all (8, 9) studies of experimental metabolic acidosis. We speculated about whether glucose ingestion and chronic metabolic acidosis might inhibit renal tubular divalent cation reabsorption by some common mechanism. To test this, renal clearances of ultrafilterable calcium and magnesium were carried out in the same subjects before and after glucose ingestion and after the induction of stable NH_4Cl acidosis. The effects of glucose ingestion were also examined during induced acidosis to test for saturation of a common mechanism.

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METHODS

We carried out renal clearance studies in seven healthy men before and after inducing metabolic acidosis. All subjects were hospitalized in the Marquette School of Medicine Clinical Research Center. The subjects ate constant diets throughout their hospitalization which provided approximately 25 mmoles of calcium and 15 mmoles of magnesium per day. The diets were well accepted and consumed completely each day. After 4–6 days of adaptation to the diet, 24-hr urine specimens were collected for 4 days. Six of the subjects then had their initial clearance studies performed, after which metabolic acidosis was induced by gradually increasing oral doses of NH_4Cl (3–4 days) and then a constant daily dose (6–7 days). 24-hr urine specimens were collected throughout the period of NH_4Cl loading. In one subject, the first clearance study was performed at the end

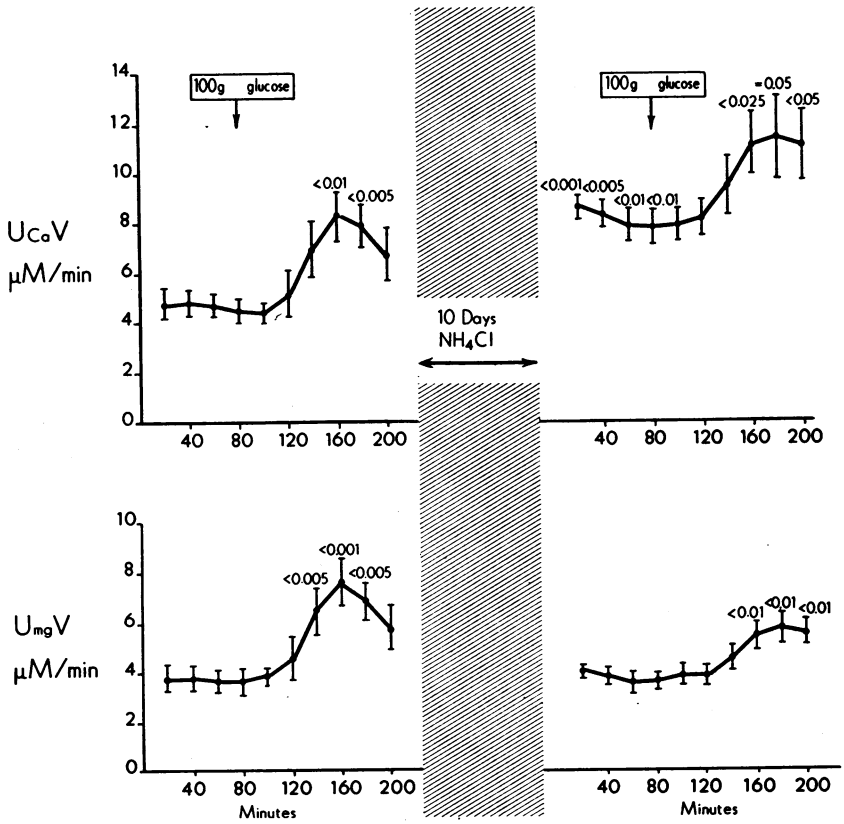

FIGURE 1 Effects of metabolic acidosis and glucose ingestion on urinary calcium ($U_{Ca}V$) and magnesium ($U_{Mg}V$) excretion.

of acid loading and a second clearance study was done 16 days after NH_4Cl administration was stopped. The results in this subject did not differ from those in the other six, indicating that the order of study did not influence the results.

Each clearance study began at 7:00 a.m., after an overnight fast. The subject drank 1 liter of water to initiate a diuresis and drank water every 20 min thereafter to replace urinary losses. After collection of a timed urine specimen and a blood specimen for inulin blank determination, a priming dose of inulin was injected and a sustaining infusion of inulin in isotonic saline was begun at 3 ml/min. After a 45 min equilibration period, four 20 min control periods were obtained.

Glucose (100 g) was then administered orally, and six 20 min experimental periods were observed. The subjects were allowed to sit or stand to void, but remained recumbent at other times during the clearance studies.

The effects of metabolic acidosis on urinary calcium and magnesium excretion were evaluated both by observing changes in the daily urinary content of calcium and magnesium after beginning NH_4Cl loading and by comparing the clearance periods prior to glucose administration in the normal steady state to those during stable metabolic acidosis. The effects of glucose ingestion on blood and urine composition during the normal steady state were compared to those observed during stable metabolic acidosis.

Each subject served as his own control. Estimates of variance throughout the text are presented as the group mean, plus or minus one standard error of the mean.

All other clearance procedures and analytical and statistical techniques employed in our laboratory have been described (5).

RESULTS

Reproducibility of measurements during the control periods were satisfactory. The coefficient of variation for the critical parameters of calcium and magnesium filtration and excretion for the individual subjects averaged: inulin clearance ±3.7%; serum ultrafilterable calcium concentration ±1.1%; serum ultrafilterable magnesium concentration ±1.1%; rate of calcium filtration ±4.2%; rate of magnesium filtration ±3.8%; $U_{Ca}V$ ±6.5%; $U_{Mg}V$ ±7.0%.

The changes in urinary calcium ($U_{Ca}V$) and magnesium ($U_{Mg}V$) excretion during the clearance studies are shown in Fig. 1. In this and all subsequent figures, the data plotted are the mean values for the seven subjects. The vertical lines through the means indicate plus or minus one standard error of the mean. During the normal steady state (left-hand portion of the figure),

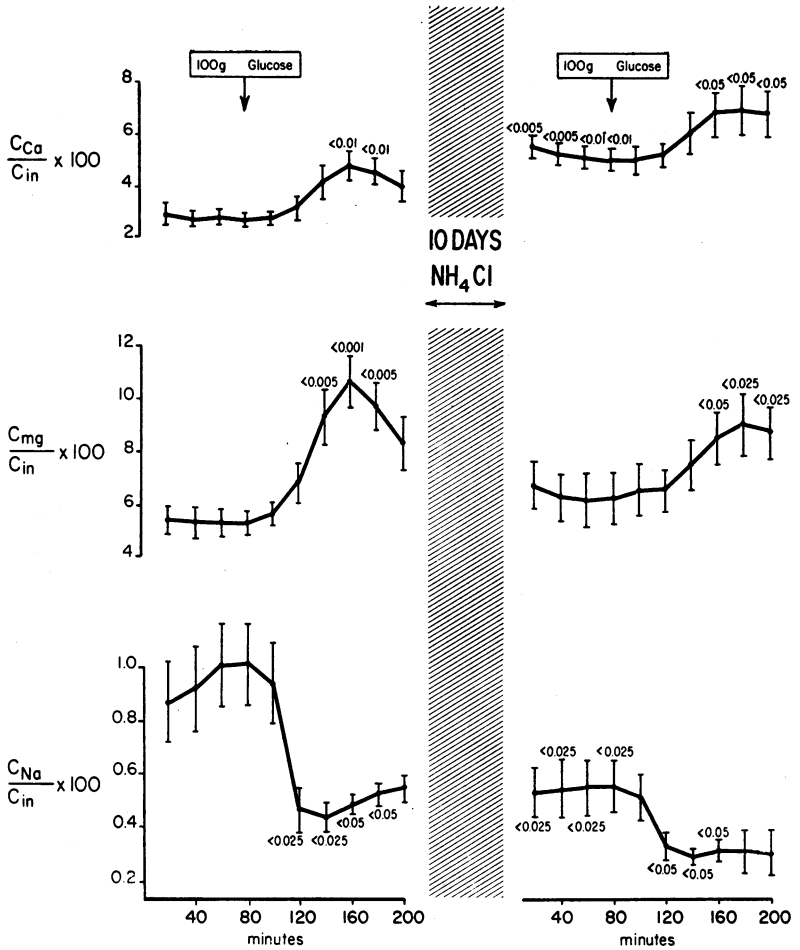

FIGURE 2 Effects of metabolic acidosis and glucose ingestion on fractional calcium, magnesium, and sodium excretion rates. See text.

the subjects' serum bicarbonate concentrations averaged 26.5 ±0.9 mEq/liter. Before glucose ingestion, $U_{Ca}V$ and $U_{Mg}V$ were steady, averaging 4.69 ±0.51 and 3.69 ±0.48 μmoles/min, respectively. After glucose ingestion, both $U_{Ca}V$ and $U_{Mg}V$ increased significantly. The right-hand portion of the figure presents the results of identical clearance studies performed after 10 days of NH_4Cl loading, when the subjects were mildly acidotic (serum bicarbonate concentrations averaging 20.3 ±0.8 mEq/liter). The effects of induced stable metabolic acidosis on $U_{Ca}V$ and $U_{Mg}V$ can be seen by comparing the four base line periods before glucose ingestion during acidosis to the same periods in the normal steady state. As shown, acidosis caused a significant increase in $U_{Ca}V$, averaging + 3.50 ±0.7 μmoles/min, but had no effect on $U_{Mg}V$. Glucose ingestion during acidosis was followed by a further significant increase in $U_{Ca}V$ and a significant increase in $U_{Mg}V$.

For the group, there were no significant changes in inulin clearance rates, UF_{Ca}, UF_{Mg}, or in the calculated rates of filtration of calcium or magnesium after glucose ingestion. During acidosis, inulin clearance rates, UF_{Ca}, and UF_{Mg} tended to fall and there were reductions in the calculated rates of filtration of both calcium (− 4 ±3 μmoles/min), and of magnesium (− 6 ±2 μmoles/min). Since $U_{Mg}V$ did not fall during induced acidosis (Fig. 1), despite a fall in the rate of filtration of magnesium, acidosis may have partially inhibited tubular magnesium reabsorption. Clearly, however, the effect on tubular calcium reabsorption was much greater.

When changes in filtered loads and urinary excretion rates of calcium and magnesium for each subject were compared during all phases of the experiments, some correlation was found for both calcium ($r = +0.51$) and magnesium ($r = +0.45$). However, in over one-half of the experimental periods (58 of 112 for calcium and 64 of 112 for magnesium) a decrease in tubular reabsorption could be inferred, since urinary excretion rates

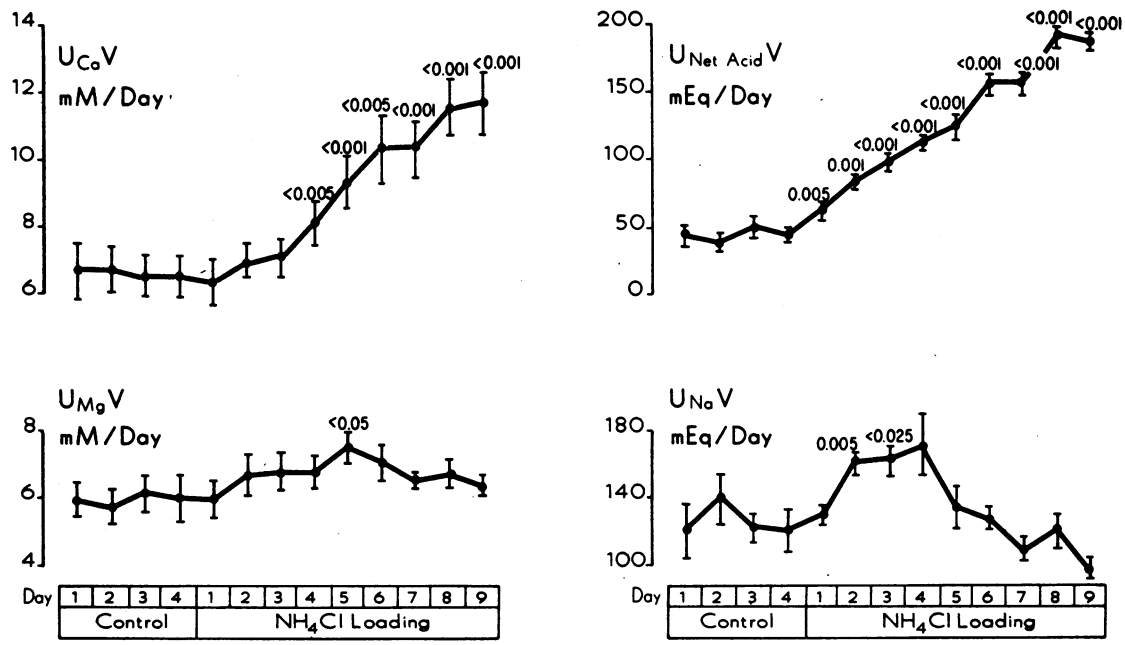

FIGURE 3 The effects of NH_4Cl administration on 24-hr urine contents of calcium, magnesium, net acid, and sodium.

increased more or fell less than did the filtered load, or increased despite a fall in the filtered load.

Fig. 2 indicates that the fractional excretion rates of calcium and magnesium [clearances of calcium (C_{Ca}) or magnesium (C_{Mg}) divided by inulin clearance (C_{In}) × 100] were altered during the experiments in

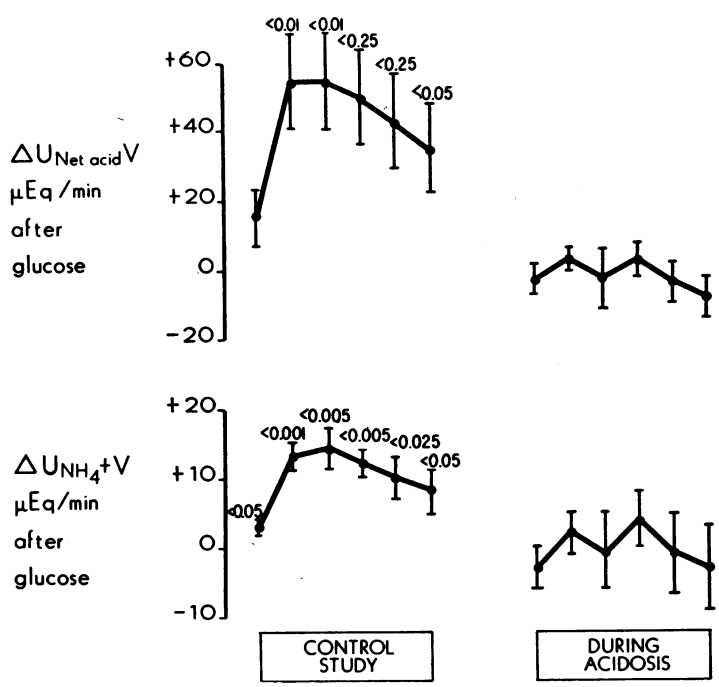

FIGURE 4 The changes from control in renal net acid and ammonium excretion following glucose ingestion in the normal steady state ("control study") and during metabolic acidosis.

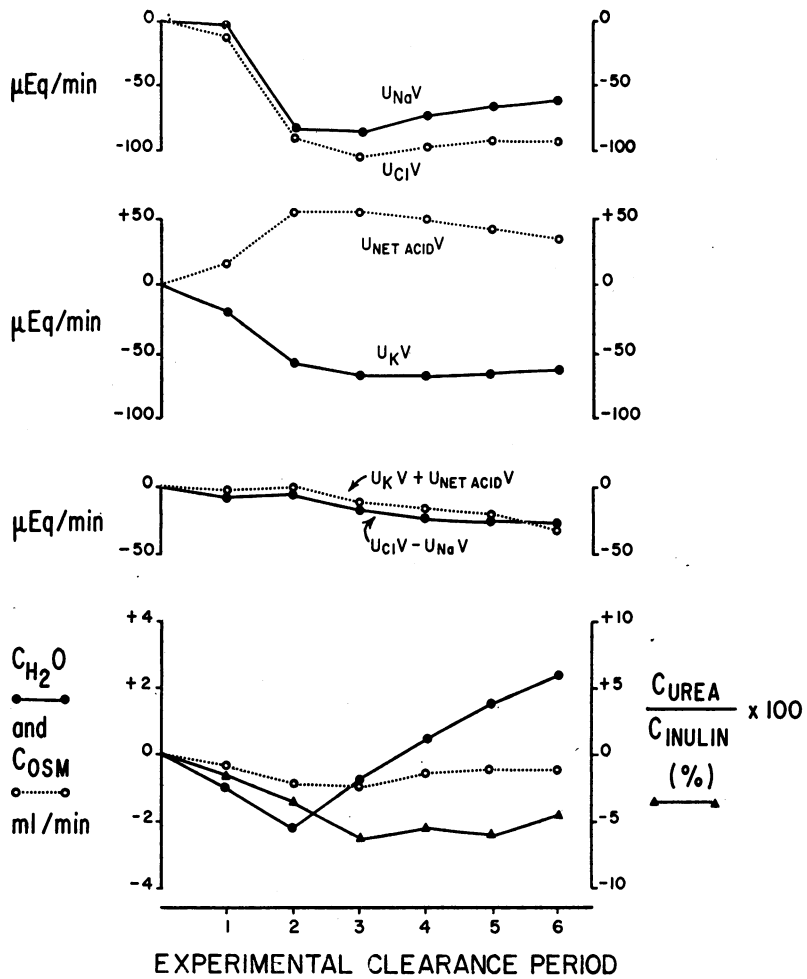

FIGURE 5 Mean changes from control after glucose ingestion in parameters relevant to segmental tubular sodium reabsorption. See text.

the same manner as $U_{Ca}V$ and $U_{Mg}V$. The lower one-third of Fig. 2 shows the simultaneous changes in fractional sodium excretion. Fractional sodium excretion fell significantly both after glucose ingestion and during induced metabolic acidosis. Since there were no significant changes in inulin clearances, in serum sodium concentrations, or in the calculated rates of filtration of sodium throughout the experiments, this must have resulted from an increase in tubular sodium reabsorption.

Increases in calcium but not magnesium excretion were also found in the 24-hr urine collections during NH_4Cl loading, as shown in Fig. 3. During the four control days, $U_{Ca}V$ averaged 6.40 ±0.58 mmoles/day and $U_{Mg}V$ averaged 5.98 ±0.48 mmoles/day. When acid loading was begun, $U_{Ca}V$ rose promptly and progressively, while $U_{Mg}V$ was increased significantly only on the 5th day of NH_4Cl administration. The upper right-hand portion of Fig. 3 shows that net renal acid excretion ($U_{Net\ Acid}V$) increased *pari passu* with the increases in $U_{Ca}V$. Sodium excretion ($U_{Na}V$) rose transiently when NH_4Cl loading was begun, but thereafter fell progressively to or below control rates.

Fig. 4 shows the changes in renal acid and ammonium excretion that followed glucose ingestion in the clearance studies. In the normal steady state ("control study"), both $U_{Net\ Acid}V$ and $U_{NH_4}V$ increased significantly after glucose and (not shown) urine pH fell significantly. During induced acidosis, however, glucose ingestion had no effect on the already high rates of $U_{Net\ Acid}V$ and $U_{NH_4}V$ and (not shown) urine pH actually rose.

Other changes in blood and urine composition occurring after glucose ingestion or the induction of NH_4Cl acidosis have been described previously (3, 4) and were confirmed in the present studies.

Fig. 5 presents some additional data regarding the changes in $U_{Na}V$ which followed glucose ingestion dur-

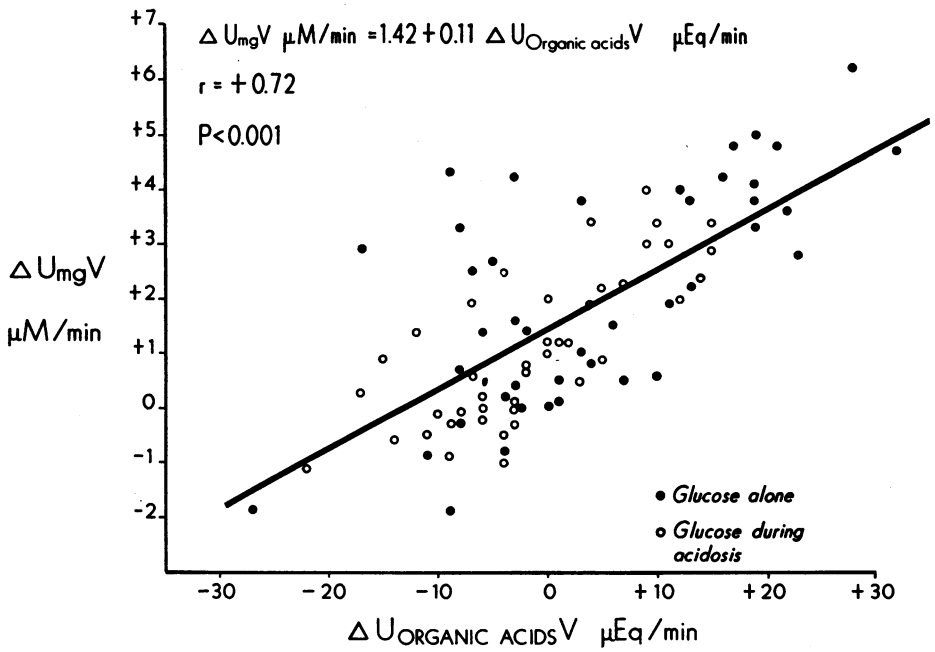

FIGURE 6 The correlation between increments above control in urinary magnesium and total organic acid excretion rates after glucose ingestion.

ing the normal steady state. The upper portion of Fig. 5 shows that, for the group, $U_{Na}V$ and $U_{Cl}V$ fell together during the first two experimental periods. Thereafter, $U_{Cl}V$ remained low, while $U_{Na}V$ tended to rise slightly back toward control rates. $U_{Net\ Acid}V$ rose, while U_KV fell. As shown in the center of the figure, the increasing discrepancy between $U_{Cl}V$ and $U_{Na}V$ appeared to be due to diminishing sodium reabsorption at the distal cation exchange site, since the sum of $U_{Net\ Acid}V$ and U_KV fell progressively. In the lowest portion of the Figure, changes in free water clearance (C_{H_2O}) and osmolar clearance (C_{osm}) are presented along with changes in fractional excretion of urea ($C_{Urea}/C_{In} \times 100$).

The subjects had been in brisk water diuresis (urine osmolalities averaging 80 ±6 mOsm/liter) for at least 2 hr before ingesting glucose, and "urea exhaltation" (10) had subsided to stable values. Urine osmolalities remained at these low levels after glucose ingestion except for a transient increase (to levels of 102 and 137 mOsm/liter, respectively) in two subjects during the second experimental period. We have thus assumed that the subjects were in near maximal water diuresis and that the ingestion of the hypertonic glucose solution did not cause any major or sustained increases in the secretion of antidiuretic hormone. Under these circumstances, it has been suggested that back diffusion of urea and of water in distal tubular segments is minimal and that changes in fractional urea excretion (11) and in the fraction of filtered water excreted ($V/GFR \times 100$) (12) reflect primarily fluid delivery from proximal, relatively water-impermeable tubular segments. As shown in Fig. 5, C_{osm} fell slightly after glucose ingestion, while C_{H_2O} fell initially and then rose above control rates. The fractional excretion of urea fell in five of the seven studies and, on the average, remained below control rates throughout the studies. Considering all experimental periods in all subjects, this change was significant, averaging $-3.8 \pm 1.1\%$ ($P < 0.025$). Not shown in the figure, $V/GFR \times 100$ also fell after glucose ingestion, but to a lesser degree, averaging, for all periods, $-1.1 \pm 0.3\%$ ($P < 0.05$).

As in earlier studies of glucose-induced augmentation of $U_{Ca}V$ and $U_{Mg}V$ (2, 3) no significant change in urinary organic acid excretion were found. However, an analysis of the present data indicated that while urinary organic acid excretion varied in a completely random manner after induction of acidosis, it tended to increase consistently but to a variable degree after glucose ingestion. Moreover, the magnitude of the increases in $U_{Ca}V$, $U_{Mg}V$, and $U_{Organic\ Acids}V$ after glucose ingestion appeared to be related. Fig. 6 presents a plot of the increments in $U_{Mg}V$ after glucose ingestion as a function of the simultaneous increments in $U_{Mg}V$ after glucose ingestion as a function of the simultaneous increments in $U_{Organic\ Acids}V$ for each of the subjects. Good correlation was found ($r = +0.72$; $P < 0.001$). Equally good correlation was found between changes in $U_{Ca}V$ and $U_{Organic\ Acids}V$ after glucose ($\Delta U_{Ca}V$ μmoles/

min = 1.53 + 0.17 $\Delta U_{Organic\ Acids}V$ μEq/min; $r = +0.75$; $P < 0.001$).

DISCUSSION

The mechanism by which glucose ingestion augments urinary calcium and magnesium excretion has not been established. Previous investigators have concluded that this is the result of diminished net tubular reabsorption of calcium and magnesium (2, 3). More recently, this has been verified by demonstrating that glucose ingestion augmented $U_{Ca}V$ and $U_{Mg}V$ significantly, even when their rates of filtration were significantly reduced by having normal subjects stand after ingesting a glucose load (13). A distal site of action has been suggested because of an associated fall in urinary potassium excretion (2). Because urinary net acid excretion increases and the urine is acidified after glucose ingestion (4), we have drawn an analogy to the impaired net tubular calcium reabsorption previously demonstrated during stable NH_4Cl acidosis (5) and speculated that a common mechanism might be involved. The present studies appear to refute that possibility.

The effects of glucose ingestion and stable NH_4Cl acidosis were compared in the same subjects. Glucose ingestion caused significant and equivalent increases in $U_{Ca}V$ and $U_{Mg}V$. During stable NH_4Cl acidosis, $U_{Ca}V$ was significantly increased, but $U_{Mg}V$ was unaffected. Both glucose ingestion and metabolic acidosis appeared to act by diminishing the net tubular reabsorption of magnesium and (or) calcium. Although some correlation between changes in rates of filtration and excretion was evident throughout the studies, in over one-half of the individual clearance periods the observed changes in $U_{Ca}V$ and $U_{Mg}V$ could not be explained by measured changes in their filtered loads. Some impairment of net tubular magnesium reabsorption during induced acidosis was implied by unaltered rates of $U_{Mg}V$ despite a fall in the filtered load of magnesium. However, the rate of filtration of calcium fell comparably, despite which $U_{Ca}V$ increased significantly.

When a glucose load was administered during induced acidosis, the individual effects of glucose and acidosis on $U_{Ca}V$ were additive. No evidence to suggest competition for or saturation of a common mechanism was found. By contrast, glucose ingestion during acidosis had a slighter but significantly lesser effect on $U_{Mg}V$ than that found in the normal steady state. Finally, glucose ingestion during acidosis augmented $U_{Ca}V$ and $U_{Mg}V$ without altering the already high rates of renal acid excretion and despite increases in urine pH.

The close correlation between increments in urinary sodium, calcium, and magnesium excretion which are found during osmotic diuresis (14–18) or after the administration of drugs which inhibit tubular sodium reabsorption (19–21) have led to the suggestion that these cations might share a common tubular transport mechanism (22). Walser (23) has also pointed out that a nonspecific relationship between sodium reabsorption and calcium and magnesium reabsorption in proximal, freely water-permeable tubular segments would be expected, since sodium (with water) reabsorption would tend to increase calcium and magnesium concentrations in tubular fluid, facilitating their reabsorption. After glucose ingestion, however, net tubular sodium reabsorption increased. The simultaneous reductions in $U_{Na}V$, $U_{Cl}V$, C_{osm}, C_{H_2O}, fractional urea excretion, and $V/GFR \times 100$ after glucose suggest increased sodium and water reabsorption in the proximal tubules. Hoffman, Martino, Wahl, and Arky (12) have carried out similar experiments and found evidence suggesting increased proximal tubular reabsorption of sodium and water when subjects ingested sucrose after a 3 day fast. In contrast to our studies, they did not find changes in fractional proximal sodium and water reabsorption when sucrose was ingested after a shorter (overnight) period of fasting. Distal sodium reabsorption, as estimated by changes in the sum of K^+ and H^+ excretion, fell after glucose ingestion. Although studies of the acute effects of parathyroid extract (24) and of adrenal mineralocorticoids (25) suggest strongly that specific and separate mechanisms are present in the distal tubule for sodium reabsorption and for calcium and magnesium reabsorption, a nonspecific change in distal tubular energy metabolism after glucose ingestion could account for our findings.

The correlation between increments in $U_{Ca}V$ or $U_{Mg}V$ and the simultaneous increments in urinary organic excretion after glucose ingestion is intriguing. We did not measure serum organic acid concentrations. The modified Palmer-Van Slyke titration (5) used to determine total urinary organic acids is crude and provides no information about the identity of the organic acids titrated. Thus, we cannot state whether $U_{Organic\ Acids}V$ increased because of increase in serum levels and filtered loads or because of changes in tubular reabsorption and (or) secretion. Although an increase in urinary organic acid content could decrease calcium and magnesium reabsorption by complex formation, this seems unlikely because of the high urine flow rates and very dilute urines present throughout the experiments (26). If the increased quantities of organic acids excreted after glucose arose within the tubule cells, then a change in tubular cell energy metabolism could account for the reduced reabsorption of calcium and magnesium. Additional studies will be required to support or eliminate these possibilities.

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