

Evidence for bicarbonate-dependent magnesium reabsorption

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During ethacrynic acid administration about 50% of the filtered load of magnesium is reabsorbed. To examine whether the remaining component of magnesium reabsorption is bicarbonate-dependent, i.e. varies with factors known to alter passive reabsorption, experiments were performed in anesthetized dogs. During ethacrynic acid administration MgCl_2 infusion raised the plasma concentration of magnesium (P_{Mg}) from 0.64 ± 0.05 to 3.06 ± 0.27 mM and doubled magnesium reabsorption. The infusion of acetazolamide at high P_{Mg} reduced bicarbonate reabsorption by $41 \pm 3\%$ and magnesium reabsorption by $31 \pm 16\%$. When plasma pH was reduced to 7.04 ± 0.02 and increased to 7.83 ± 0.02 by altering P_{CO_2} at a constant plasma bicarbonate concentration of 31.2 ± 0.8 mM, magnesium and bicarbonate reabsorption were correlated ($r=0.82$). The infusion of mannitol, which acts by reducing passive solute transport without affecting bicarbonate reabsorption, halved magnesium reabsorption. By combining mannitol and acetazolamide infusions, only $6 \pm 4\%$ of the filtered magnesium was still reabsorbed. These results indicate that the reabsorption of magnesium remaining after the infusion of ethacrynic acid and after raising P_{Mg} varies with changes in P_{CO_2} and is inhibited by the infusion of acetazolamide and mannitol as expected for bicarbonate-dependent passive reabsorption.

Key words: Acetazolamide, bicarbonate, magnesium, mannitol, P_{CO_2} , plasma pH

Magnesium is poorly reabsorbed in the proximal tubules whereas the more distal nephron segments, notably the thick ascending limb of Henle's loop, reabsorbs magnesium extensively (Quamme & Dirks 1980*b*). Loop diuretics such as furosemide (Eknoyan et al. 1970) and ethacrynic acid (Eknoyan et al. 1970, Peraino et al. 1978) halve the reabsorption of magnesium. The subject of this study is whether the remaining reabsorption of magnesium varies with changes in passive bicarbonate-dependent solute transport.

Studies on the whole kidney of dogs after inhibition of distal NaCl reabsorption by ethacrynic acid support the hypothesis that transcellular transport of NaHCO_3 provides the main osmotic force for the passive paracellular transport of NaCl and water across the tight junction (Mathisen et al. 1976, 1978, 1979). Other electrolytes too, such as magnesium, might be transported passively along the paracellular route. If a passive component of electrolyte transport exists, certain conditions for paracellular

transport must be fulfilled after pharmacological inhibition of distal reabsorption that can be examined in studies on the whole kidney.

In the distal cortical tubules and collecting ducts, the reabsorption of magnesium is close to saturation even before the inhibition of reabsorption in the diluting segment (Quamme & Dirks 1980*b*). In the present study on anesthetized dogs ethacrynic acid was infused to inhibit distal reabsorption of magnesium and to inhibit transcellular NaCl reabsorption without affecting NaHCO_3 reabsorption and associated paracellular NaCl reabsorption (Steen et al. 1981). The inhibition of MgCl_2 and NaCl reabsorption along the diluting segment may not be complete, but the rise in solute concentration of the tubular fluid might saturate the reabsorption of magnesium in the distal cortical tubules and collecting ducts as it does for sodium (Kiil 1978). When the distal reabsorption of magnesium and NaCl is saturated, changes in excretion would at equal glomerular filtration rate (GFR) reflect

Table 1. Effects of magnesium and acetazolamide during ethacrynic acid infusion

Values are mean \pm SE (n=6). GFR=glomerular filtration rate, plasma conc.=plasma concentration, reab.=reabsorption rate, excr.=excretion rate

| | GFR (ml/min) | Urine volume (ml/min) | Magnesium | | | Sodium | |
|-----------------------------------------|-------------------|-----------------------------|-------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | | | Plasma conc. (mM) | Reab. (μ mol/min) | Excr. (μ mol/min) | Reab. (μ mol/min) | Excr. (μ mol/min) |
| 1. Control | 26.5 \pm 3.6 | 8.3 \pm 0.8 | 0.64 \pm 0.05 | 7.9 \pm 2.1 | 5.9 \pm 2.4 | 2 643 \pm 439 | 1 088 \pm 117 |
| 2. Hypermagnesemia | 27.8 \pm 3.0 | 7.8 \pm 0.8 | 3.06 \pm 0.27 | 18.4 \pm 7.0 | 50.4 \pm 6.5 | 2 919 \pm 385 | 935 \pm 105 |
| <i>p</i> (1-2) | NS | NS | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 3. Hypermagnesemia and acetazolamide | 27.2 \pm 3.0 | 14.3 \pm 1.7 | 3.70 \pm 0.15 | 11.9 \pm 3.6 | 68.5 \pm 6.6 | 1 979 \pm 222 | 1 800 \pm 232 |
| <i>p</i> (2-3) | NS | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

changes in proximal tubular reabsorption. Most of the experiments reported in the present study were therefore performed during hypermagnesemia in dogs with expanded extracellular volume.

Under these experimental conditions some predictions concerning magnesium reabsorption can be tested. First, the passive bicarbonate-dependent reabsorption of magnesium would be expected to increase with plasma magnesium concentration (P_{Mg}). Secondly, if magnesium is reabsorbed across the tight junction along the paracellular route, the administration of acetazolamide would greatly reduce magnesium reabsorption because of a reduction in transcellular $NaHCO_3$ reabsorption (Mathisen et al. 1978, 1980). A previous study showed no effect on fractional magnesium excretion by acetazolamide, but GFR was reduced by more than 30% in that study (Peraino et al. 1978). A tubular effect of acetazolamide might be disclosed by comparing data obtained at similar GFR before and after acetazolamide administration. Because autoregulation is impaired during ethacrynic acid diuresis in volume expanded dogs, GFR can be restored by raising renal arterial perfusion pressure (Raeder et al. 1975).

The third prediction was that magnesium reabsorption, as previously shown for NaCl reabsorption, should vary greatly with variations in plasma CO_2 tension (P_{CO_2}) (Mathisen et al. 1979). The relative changes in reabsorption during hypercapnia and hypocapnia should be as large for magnesium

as for bicarbonate. Previous studies on the effects of hypercapnia on magnesium reabsorption have given conflicting results (Denis et al. 1982, Gray et al. 1973). The reabsorption of bicarbonate is, however, dependent not only on the plasma pH but also on the filtered load of bicarbonate (Langberg et al. 1981). Therefore, we examined the effects of respiratory changes in plasma pH on magnesium reabsorption at constant plasma bicarbonate concentration and constant GFR.

Finally, we examined the prediction that an osmotic agent such as mannitol, which impairs paracellular reabsorption (Mathisen et al. 1981), would reduce magnesium reabsorption without affecting $NaHCO_3$ reabsorption.

METHODS

Experiments were performed in 18 mongrel dogs of both sexes weighing 11–25 kg. They were fasted overnight but had free access to water. Anesthesia was induced with pentobarbital (Nembutal®), 25 mg·kg⁻¹ i.v., and maintained by additional doses of 1–3 mg·kg⁻¹ as needed. They were ventilated via an endotracheal tube with a Harvard respirator (Model 613) and a room air/oxygen mixture to keep arterial P_{O_2} and P_{CO_2} constant. In the second series of experiments, hypercapnia was induced by adding CO_2 to the respirator air, and hypocapnia by hyperventilation. Body temperature was kept normal with external heating pads.

The kidney was exposed through a flank incision. Visible nerves in the renal pedicle were divided, and a snugly fitting flowmeter probe was placed on the renal artery for the measurement of renal blood flow (RBF) by a

| Bicarbonate | | Chloride | |
|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| Reab. ($\mu\text{mol}/\text{min}$) | Excr. ($\mu\text{mol}/\text{min}$) | Reab. ($\mu\text{mol}/\text{min}$) | Excr. ($\mu\text{mol}/\text{min}$) |
| 581 ± 120 | 149 ± 15 | 1 901 ± 274 | 1 062 ± 143 |
| 651 ± 103 | 100 ± 6 | 2 122 ± 234 | 1 034 ± 135 |
| <0.05 | <0.05 | <0.05 | NS |
| 387 ± 67 | 407 ± 48 | 1 511 ± 149 | 1 594 ± 210 |
| <0.05 | <0.05 | <0.05 | <0.05 |

squarewave electromagnetic flowmeter (Nycotron, Drammen, Norway). A nylon snare was placed around the renal artery distal to the flow probe for zero flow determination. A Blalock clamp placed on the aorta above both renal arteries was used to reduce renal perfusion pressure, and an inflatable neck cuff was used to increase arterial pressure via direct carotid sinus compression. Polyethylene catheters of appropriate size were inserted into a femoral artery for blood sampling and pressure determination and into a femoral vein for infusing solutions. A soft polyvinyl catheter was inserted into the ureter for collecting urine. Arterial pressure was measured with an electronic pressure transducer (Statham P23Gb) and recorded with the output of the flowmeter on a Sanborn recorder.

Experimental procedure

All dogs were infused intravenously with a solution of the following electrolyte composition: NaCl (130 mM), KCl (10 mM), CaCl_2 (3 mM), MgCl_2 (2 mM) and KH_2PO_4 (1 mM). To expand the extracellular volume this solution was infused intravenously in a volume of 10% of b. wt. in the course of one hour. Ethacrynic acid was given i.v. in a priming dose of $3 \text{ mg}\cdot\text{kg}^{-1}$ followed by a continuous infusion of $1.5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. A bolus of creatinine, 40 mg/kg, was given i.v. followed by a continuous infusion of $0.6 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. Isotonic sodium bicarbonate was infused separately; to keep plasma HCO_3^- concentration ($P_{\text{HCO}_3^-}$) at about 30 mM, the infusion rate was reduced during hypercapnia and increased during hypocapnia. All infusions were prewarmed, and after initial expansion infusion rates were adjusted to slightly exceed urinary flow rates. During each experimental condition three to five clearance periods were obtained. The clearance periods were 2–6 min in duration depending on urinary flow rate, and arterial blood samples were taken in the middle of each clearance period. Since renal blood flow autoregulation was abolished (Raeder et al. 1975), variations in renal perfusion pressure were accompanied by almost

proportional changes in GFR. This technique permitted a selection of data obtained at similar GFR during the various procedures.

Protocols

1. In six dogs clearance studies were performed 60–90 min after saline expansion at different renal perfusion pressures to cover the same range of GFR in control periods, during hypermagnesemia and after administration of acetazolamide. Hypermagnesemia was induced by the i.v. infusion of MgCl_2 in a priming dose of $500\text{--}750 \mu\text{mol}\cdot\text{kg}^{-1}$ followed by a continuous infusion of $12.5 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. After 30 min of equilibration, clearance examinations were performed once more at different renal perfusion pressures to get measurements at GFR's close to control values. During continuous MgCl_2 infusion, acetazolamide (Diamox®), 100 mg/kg, was given i.v., and after 30 min of equilibration clearance data were again obtained as above.

2. In six dogs hypercapnia was induced by adding 10–15% CO_2 to the respiratory air after volume expansion and MgCl_2 administration. Control P_{CO_2} was reestablished by slight hyperventilation with a room air/oxygen mixture for 20–40 min. Hypocapnia was induced by a 20–40 min period of heavy hyperventilation. Clearance data were obtained at different renal arterial perfusion pressures to permit a selection of comparable GFR's during hypercapnia, normocapnia and hypocapnia.

3. In six dogs one kidney was removed 6 to 11 days prior to the experiment. Clearance measurements were carried out at different perfusion pressures after volume expansion and MgCl_2 administration to provide data at comparable GFR's during control periods and mannitol and acetazolamide infusion periods. Mannitol (15%) was infused i.v. at a rate of $15 \text{ ml}\cdot\text{min}^{-1}$ for 30 min followed by a continuous infusion of $2 \text{ ml}\cdot\text{min}^{-1}$ before samples were taken again, as above. During continuous mannitol infusion, acetazolamide was given, as above, and clearance data obtained.

Analytical procedure

Plasma and urine were analyzed for creatinine according to Bonsnes and Taussky (1945). Creatinine clearance provided the estimate for GFR. Sodium and potassium were determined by a direct flame photometer using lithium as an internal standard (Model 343, Instrumentation Laboratories, Inc.). Chloride was measured by a chloride titrator (Model CM 10, Radiometer) and magnesium by atomic absorption (AA/AE Spectrophotometer 257, Instrumentation Laboratories, Inc.). Mannitol was measured by the method of Smith et al. (1940). Arterial blood and urine samples were collected anaerobically and immediately analyzed for pH, P_{O_2} and P_{CO_2} by a Model 613 pH Blood Gas Analyzer (Instrumentation Laboratories). The concentration of bicarbonate was calculated from the Henderson-Hasselbalch equation for arterial blood and urine, taking the precautions previously reported (Monclair et al. 1978). Filtered loads were corrected by a Donnan factor of 0.95 for sodium and potassium and 1.05 for chloride and bicarbonate; the ultrafilterable fraction of plasma magnesium was estimated as 0.8 (Massry et al. 1969, Quamme & Dirks 1980 b). Since each dog served as

Table 2. *Effects of hypercapnia and hypocapnia during ethacrynic acid infusion and hypermagnesemia*
Abbreviations as in Table 1

| | GFR (ml/min) | Urine volume (ml/min) | Plasma pH | Magnesium | | | Sodium | |
|----------------|-------------------|-----------------------------|--------------------|-------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| | | | | Plasma conc. (mM) | Reab. ($\mu\text{mol}/\text{min}$) | Excr. ($\mu\text{mol}/\text{min}$) | Reab. ($\mu\text{mol}/\text{min}$) | Excr. ($\mu\text{mol}/\text{min}$) |
| 1. Hypercapnia | 31.0 ± 3.5 | 4.7 ± 0.6 | 7.04 ± 0.02 | 3.13 ± 0.28 | 26.3 ± 5.4 | 53.0 ± 9.2 | 3 870 ± 440 | 420 ± 83 |
| 2. Normocapnia | 31.0 ± 3.9 | 9.9 ± 1.3 | 7.52 ± 0.02 | 3.45 ± 0.26 | 17.0 ± 2.8 | 71.1 ± 15.3 | 3 174 ± 448 | 1 013 ± 162 |
| <i>p</i> (1–2) | NS | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |
| 3. Hypocapnia | 28.8 ± 4.1 | 14.8 ± 1.8 | 7.83 ± 0.02 | 3.60 ± 0.29 | 4.5 ± 1.5 | 83.0 ± 20.8 | 2 149 ± 356 | 1 784 ± 254 |
| <i>p</i> (2–3) | NS | <0.05 | <0.05 | NS | <0.05 | <0.05 | <0.05 | <0.05 |

its own control, Wilcoxon's signed rank test for paired comparison was used, and differences were regarded as significant at $p < 0.05$ (Snedecor & Cochran 1967). Regression lines were calculated by the least square analysis (Brace 1977). The data are presented as arithmetic mean \pm one standard error of the mean.

RESULTS

Effects of hypermagnesemia and acetazolamide administration during ethacrynic acid infusion

During ethacrynic acid infusion about half of the filtered load of magnesium (0.55 ± 0.04) was reabsorbed at a P_{Mg} of 0.64 ± 0.05 mM. Intravenous in-

fusion of MgCl_2 raised P_{Mg} to 3.06 ± 0.27 mM without significantly reducing GFR. In most experiments, however, there was a slight fall in GFR, but this reduction could be counteracted by raising renal arterial pressure in these dogs with impaired autoregulation. The data presented in Table 1 were obtained at control GFR at a P_{HCO_3} of 26.3 ± 0.4 mM. Hypermagnesemia more than doubled magnesium reabsorption. Sodium reabsorption increased during magnesium infusion by $14 \pm 5\%$, chloride reabsorption by $11 \pm 6\%$ and bicarbonate reabsorption by $17 \pm 6\%$.

Acetazolamide infusion during hypermagnesemia reduced GFR by $15 \pm 5\%$ but GFR could be re-

Table 3. *Effects of mannitol and acetazolamide during ethacrynic acid infusion and hypermagnesemia*
Abbreviations as in Table 1

| | GFR (ml/min) | Urine volume (ml/min) | Magnesium | | | Sodium | |
|--------------------------------|-------------------|-----------------------------|-------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| | | | Plasma conc. (mM) | Reab. ($\mu\text{mol}/\text{min}$) | Excr. ($\mu\text{mol}/\text{min}$) | Reab. ($\mu\text{mol}/\text{min}$) | Excr. ($\mu\text{mol}/\text{min}$) |
| 1. Control | 29.0 ± 2.8 | 10.6 ± 1.3 | 2.68 ± 0.25 | 15.7 ± 3.2 | 47.2 ± 8.4 | 2 678 ± 287 | 1 274 ± 172 |
| 2. Mannitol | 29.6 ± 2.6 | 16.3 ± 1.7 | 2.72 ± 0.30 | 8.8 ± 1.9 | 56.5 ± 10.6 | 2 118 ± 199 | 1 799 ± 180 |
| <i>p</i> (1–2) | NS | NS | NS | <0.05 | <0.05 | <0.05 | <0.05 |
| 3. Mannitol + acetazolamide | 27.7 ± 2.3 | 19.0 ± 1.7 | 3.16 ± 0.32 | 3.2 ± 2.4 | 67.7 ± 11.0 | 1 412 ± 157 | 2 357 ± 223 |
| <i>p</i> (2–3) | NS | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 |

| Bicarbonate | | Chloride | |
|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| Reab. ($\mu\text{mol}/\text{min}$) | Excr. ($\mu\text{mol}/\text{min}$) | Reab. ($\mu\text{mol}/\text{min}$) | Excr. ($\mu\text{mol}/\text{min}$) |
| 1 032 ± 141 | 6 ± 3 | 2 890 ± 346 | 724 ± 97 |
| 838 ± 112 | 182 ± 53 | 2 079 ± 298 | 1 253 ± 147 |
| <0.05 | <0.05 | <0.05 | <0.05 |
| 406 ± 81 | 577 ± 103 | 1 407 ± 230 | 1 555 ± 204 |
| <0.05 | <0.05 | <0.05 | <0.05 |

stored by raising the renal arterial perfusion pressure. At control GFR magnesium reabsorption was reduced by $31 \pm 16\%$ and bicarbonate reabsorption by $41 \pm 3\%$. These relative reductions are not significantly different. In agreement with previous studies performed at normal P_{Mg} (Mathisen et al. 1976, 1978), acetazolamide reduced sodium and bicarbonate reabsorption in ratios close to 3:1.

Effects of altering P_{CO_2} during ethacrynic acid infusion in hypermagnesemic dogs

At a P_{Mg} of 3.45 ± 0.26 mM, hypercapnia ($P_{CO_2} = 15.9 \pm 0.1$ kPa = 20 ± 1 mmHg) had no significant effect on GFR whereas hypocapnia ($P_{CO_2} = 2.7 \pm 0.1$ kPa = 20 ± 1 mmHg) reduced GFR by $30 \pm 5\%$. In these experiments P_{HCO_3} was kept con-

stant at 31.2 ± 0.8 mM by varying the infusion rate of NaHCO_3 . Since autoregulation was impaired, GFR could be restored by raising renal arterial pressure. Data obtained at control GFR are summarized in Table 2. During hypercapnia magnesium reabsorption increased by an average of $59 \pm 21\%$ and bicarbonate reabsorption by $27 \pm 12\%$. During hypocapnia magnesium reabsorption decreased by $74 \pm 9\%$ and bicarbonate reabsorption by $53 \pm 4\%$. This difference is not significant.

Fig. 1 shows that magnesium is correlated to bicarbonate reabsorption ($r=0.82$) (upper panel) and to plasma pH ($r=0.87$) (lower panel). To compare dogs of different size, fractional rather than absolute magnesium reabsorption has been presented.

Effects of mannitol during ethacrynic acid infusion in hypermagnesemic dogs

Mannitol was infused in six dogs to a plasma mannitol concentration averaging 40.3 ± 3.0 mM at a P_{HCO_3} of 26.3 ± 0.6 mM. Table 3 shows data obtained at control GFR at a P_{Mg} concentration averaging 2.68 ± 0.25 mM. Mannitol infusion reduced magnesium reabsorption by $43 \pm 17\%$, sodium reabsorption by $21 \pm 3\%$ and chloride reabsorption by $28 \pm 5\%$ whereas bicarbonate reabsorption was not significantly reduced.

By superimposing acetazolamide, magnesium reabsorption was further reduced. Only $6 \pm 4\%$ of the filtered load of magnesium was still reabsorbed, and in two of the dogs net reabsorption was zero. The reduction in magnesium reabsorption induced by infusing acetazolamide during mannitol infusion averaged $63 \pm 17\%$ and the reduction in bicarbonate reabsorption $53 \pm 3\%$. These relative reductions in reabsorption are not significantly different.

| Bicarbonate | | Chloride | |
|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| Reab. ($\mu\text{mol}/\text{min}$) | Excr. ($\mu\text{mol}/\text{min}$) | Reab. ($\mu\text{mol}/\text{min}$) | Excr. ($\mu\text{mol}/\text{min}$) |
| 641 ± 78 | 163 ± 26 | 1 895 ± 205 | 1 370 ± 171 |
| 598 ± 59 | 227 ± 20 | 1 351 ± 122 | 1 874 ± 216 |
| NS | <0.05 | <0.05 | <0.05 |
| 295 ± 20 | 507 ± 58 | 1 059 ± 130 | 2 114 ± 184 |
| <0.05 | <0.05 | <0.05 | <0.05 |

DISCUSSION

The design of this study in anesthetized dogs permits an examination of the hypothesis that the net magnesium reabsorption remaining after ethacrynic acid administration varies with the bicarbonate-dependent NaCl transport. Transcellular NaHCO_3 reabsorption provides most of the osmotic force for the paracellular passive transport of tubular fluid containing NaCl and other electrolytes to which the tight junction of the proximal tubules is permeable (Mathisen et al. 1979, 1980).

During ethacrynic acid infusion flooding of the

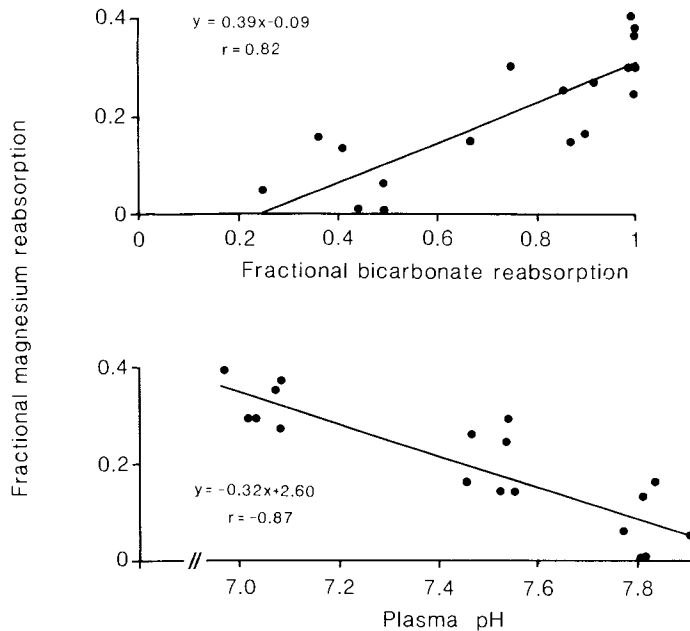


Fig. 1. Fractional magnesium reabsorption plotted against fractional bicarbonate reabsorption (upper panel) and plasma pH (lower panel). Data from experiments in six dogs during hypercapnia and hypocapnia.

distal nephron with tubular fluid of high NaCl concentration would saturate transcellular NaCl reabsorption in the distal nephron (Monclair et al. 1978). A change in the delivery of NaCl from the proximal tubules would therefore be quantitatively reflected in the urine. Ethacrynic acid in the doses administered does not interfere with NaHCO_3 reabsorption (Steen et al. 1981) but by keeping P_{HCO_3} high, a large fraction of the filtered load was excreted except during hypercapnia. Hence, the increase in proximal tubular reabsorption of bicarbonate during hypercapnia may be underestimated whereas bicarbonate reabsorption was close to saturation during normo- and hypocapnia. Because distal magnesium reabsorption is close to saturation at normal P_{Mg} (Quamme and Dirks 1980b), distal magnesium reabsorption would be completely saturated after inducing hypermagnesemia and inhibiting magnesium reabsorption along the diluting segment with ethacrynic acid. Therefore, during hypermagnesemia a change in proximal tubular magnesium reabsorption would be quantitatively reflected in the changes in urinary excretion.

The results of the present study consistently show that magnesium reabsorption is linked to bi-

carbonate-dependent reabsorption. More specifically, our study favors the hypothesis that magnesium is reabsorbed across the tight junction of the proximal tubules with NaCl and probably other permeant solutes of the tubular fluid.

Bicarbonate-dependent magnesium reabsorption

One condition for passive magnesium transport is proportionality between plasma magnesium concentration and reabsorption. In our study magnesium reabsorption more than doubled during the infusion of MgCl_2 , but fractional reabsorption fell because of remaining distal reabsorption which at the low control P_{Mg} may account for a rather large fraction of total magnesium reabsorption. Micropuncture studies in both rats and dogs show that the fractional reabsorption of magnesium remains constant in the proximal tubules when hypermagnesemia is induced (Quamme and Dirks 1980a, Wong et al. 1979a).

In this as in other studies (Mathisen et al. 1976, 1978) acetazolamide reduced NaHCO_3 and NaCl reabsorption in ratios close to 1:2, indicating that the transcellular reabsorption of one mol NaHCO_3 provides the osmotic force for the paracellular

transport of water containing about 2 mol of NaCl. In our study about 40% of the reabsorption of magnesium and chloride was inhibited by acetazolamide. By comparing data before and after acetazolamide infusion it can be calculated that the reabsorbate/filtrate concentration ratio is 0.25 ± 0.11 for magnesium and 0.74 ± 0.04 for chloride, indicating a higher reflection coefficient for magnesium than for chloride. The same conclusion is reached by comparing the changes in magnesium and chloride reabsorption as P_{CO_2} was altered.

A previous study has shown that bicarbonate reabsorption varies with plasma pH (Langberg et al. 1981). We therefore predicted that magnesium reabsorption would vary in proportion to bicarbonate reabsorption during variations in P_{CO_2} . We found that relative variations were even greater for magnesium than for bicarbonate reabsorption. As shown in Table 2, bicarbonate excretion approached zero at the plasma pH of 7.0, indicating that bicarbonate reabsorption in the distal nephron was not saturated. Therefore, the absolute change in bicarbonate reabsorption would be an underestimate of the absolute change in bicarbonate reabsorption in the proximal tubules. Since distal reabsorption is not associated with paracellular transport (Mathisen et al. 1976), the relative variations in reabsorption would be greater for magnesium than for bicarbonate.

The third requirement for a paracellular transport of magnesium is inhibition of magnesium reabsorption by osmotic diuretics. In agreement with previous studies from this laboratory (Mathisen et al. 1981), mannitol infusion greatly reduced NaCl reabsorption without affecting bicarbonate reabsorption. This finding suggests that NaCl reabsorption in the proximal tubules proceeds by the paracellular route and that mannitol acts by reducing the osmotic force across the tight junction of the proximal tubules. The great reduction in magnesium reabsorption might, therefore, be secondary to the reduction in osmotic force.

A rise in magnesium excretion during mannitol infusion was observed by Wesson (1962) and attributed to a proximal tubular inhibitory effect. In contrast, Wong et al. (1979b) claimed that most of the effect of osmotic diuretics is localized in the diluting segment. They found that mannitol infusion increased fractional magnesium reabsorption in the proximal tubules of dogs. Since in our study mannitol was infused after inhibiting electrolyte transport

in the diluting segment with ethacrynic acid, the inhibitory effect of mannitol infusion is probably predominantly localized in the proximal tubules. Any effect of mannitol on magnesium reabsorption in the diluting segment would be minimized by ethacrynic acid administration.

By combining the various diuretic agents during hypermagnesemia, only a small fraction of magnesium reabsorption remained, showing that the proximal bicarbonate-dependent reabsorption of magnesium can be effectively inhibited.

Stimulation of sodium reabsorption by hypermagnesemia

An unexpected finding was that hypermagnesemia raised the reabsorption of sodium, bicarbonate and chloride by about 15%. It is well known that magnesium is an important co-factor in several enzyme systems, including Na,K-ATPase. The intracellular magnesium concentration is not known and optimal Na,K-ATPase activity might not be reached except at supranormal plasma values of magnesium. Although there is considerable evidence that Na,K-ATPase is the only energy-requiring sodium pump, both in the diluting segment and in the proximal tubules (Mathisen et al. 1980, Sejersted et al. 1982), ouabain, in doses which reduces renal Na,K-ATPase activity by 80%, greatly reduces transcellular NaCl reabsorption without affecting $NaHCO_3$ reabsorption. Thus, an even more complete inhibition of Na,K-ATPase activity would be needed to reduce proximal tubular reabsorption (Kiil 1978). Conversely, because of the surplus of Na,K-ATPase, a stimulatory effect of magnesium infusion may not be detectable in the proximal tubules. On the other hand, considerable active transcellular sodium reabsorption by Na,K-ATPase may remain in the distal nephron during ethacrynic acid infusion (Sejersted et al. 1982). Since in this segment the Na,K-ATPase turnover seems to be the limiting step for transcellular transport of NaCl a stimulatory effect of magnesium infusion would increase the remaining distal reabsorption of sodium in the distal nephron.

In contrast to the stimulatory effect on total magnesium reabsorption, an inhibitory effect of magnesium infusion has been reported in micropuncture studies on the proximal tubules. In the rat an inhibitory effect on proximal tubular sodium reabsorption by hypermagnesemia has consistently been found (DiBona 1971, 1972, 1974, Pløth et al. 1976).

In the dog, however, micropuncture studies on the proximal tubules have given conflicting results since in one study an inhibitory effect of magnesium on proximal tubular reabsorption was reported (Brunette et al. 1969) and in another no change was found (Wen et al. 1970). Thus, it is likely that the increased reabsorption of sodium, chloride and bicarbonate in our study occurred in segments beyond the proximal tubule.

CONCLUSIONS

Three lines of evidence indicate that magnesium reabsorption in the proximal tubules is bicarbonate-dependent. The inhibition of magnesium reabsorption by acetazolamide, hypocapnia and mannitol and a stimulation during hypercapnia are observations compatible with a paracellular rather than a transcellular reabsorption of magnesium in the proximal tubules.

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REFERENCES

- BONSNES, R. W. & TAUSKY, H. H. 1945. On the colorimetric determination of creatinine by the Jaffe reaction. *J Biol Chem* 158: 581-591.
- BRACE, R. A. 1977. Fitting straight lines to experimental data. *Am J Physiol* 233: R94-R99.
- BRUNETTE, M., WEN, S. F., EVANSON, R. L. & DIRKS, J. H. 1969. Micropuncture study of magnesium reabsorption in the proximal tubule of the dog. *Am J Physiol* 216: 1510-1516.
- DENIS, R. R., BLOUCH, K. L. & JAMISON, R. L. 1982. Effects of acute acid-base disturbances on renal tubule reabsorption of magnesium in the rat. *Am J Physiol* 243: F197-F203.
- DIBONA, G. F. 1971. Effect of hypermagnesemia on renal tubular sodium handling in the rat. *Am J Physiol* 221: 53-57.
- DIBONA, G. F. 1972. Effect of magnesium on water-permeability of the rat nephron. *Am J Physiol* 223: 1324-1326.
- DIBONA, G. F. 1974. Effect of magnesium on unidirectional and net sodium fluxes in microperfused rat proximal tubules. *Am J Physiol* 226: 470-474.
- EKNOYAN, G., SUKI, W. N. & MARTINEZ-MALDONADO, M. 1970. Effects of diuresis on urinary excretion of phosphate, calcium and magnesium in thyroparathyroidectomized dogs. *J Lab Clin Med* 76: 257-266.
- GRAY, S. P., MORRIS, J. E. W. & BROOKS, C. J. 1973. Renal handling of calcium, magnesium, inorganic phosphate and hydrogen ions during prolonged exposure to elevated carbon dioxide concentrations. *Clin Sci Mol Med* 45: 751-764.
- KIIL, F. 1978. Principles of active sodium reabsorption in the kidney. *Scand J Clin Lab Invest* 38: 597-602.
- LANGBERG, H., MATHISEN, Ø., HOLDAAS, H. & KIIL, F. 1981. Filtered bicarbonate and plasma pH as determinants of tubular bicarbonate reabsorption. *Kidney Int* 20: 780-788.
- MASSRY, S. G., COBURN, J. W. & KLEEMAN, C. R. 1969. Renal handling of magnesium in the dog. *Am J Physiol* 216: 1460-1467.
- MATHISEN, Ø., MONCLAIR, T. & KIIL, F. 1980. Oxygen requirement of bicarbonate-dependent sodium reabsorption in the dog kidney. *Am J Physiol* 238: F175-F180.
- MATHISEN, Ø., RAEDER, M. & KIIL, F. 1981. Mechanism of osmotic diuresis. *Kidney Int* 19: 431-437.
- MATHISEN, Ø., MONCLAIR, T., HOLDAAS, H. & KIIL, F. 1978. Bicarbonate as mediator of proximal tubular NaCl reabsorption and glomerulotubular balance. *Scand J Clin Lab Invest* 38: 7-17.
- MATHISEN, Ø., MONCLAIR, T., RAEDER, M. & KIIL, F. 1979. Coupling of NaHCO₃ and NaCl reabsorption in dog kidneys during changes in plasma P_{CO₂}. *Am J Physiol* 236: F232-F239.
- MATHISEN, Ø., RAEDER, M., SEJERSTED, O. M. & KIIL, F. 1976. Effect of acetazolamide on glomerulotubular balance and renal metabolic rate. *Scand J Clin Lab Invest* 36: 617-625.
- MONCLAIR, T., MATHISEN, Ø. & KIIL, F. 1978. Characteristics of transcellular NaCl reabsorption in the kidney. *Scand J Clin Lab Invest* 38: 615-626.
- PERAINO, R. A., STINEBAUGH, B. J. & SUKI, W. N. 1978. Renal excretion of calcium and magnesium during acetazolamide or parathyroid hormone administration in dogs with distal blockade. *Renal Physiol* 1: 19-27.
- PLOTH, D. W., SAWIN, L. L. & DIBONA, G. F. 1976. Effect of magnesium on rat nephron sodium reabsorption: A segmental analysis. *Am J Physiol* 230: 398-402.
- QUAMME, G. A. & DIRKS, J. H. 1980a. Intraluminal and contraluminal magnesium on magnesium and calcium transfer in the rat nephron. *Am J Physiol* 238: F187-F198.
- QUAMME, G. A. & DIRKS, J. H. 1980b. Magnesium transport in the nephron. *Am J Physiol* 239: F393-F401.
- RAEDER, M., OMVIK, P. & KIIL, F. 1975. Renal autoregulation. Evidence for the transmural pressure hypothesis. *Am J Physiol* 228: 1840-1846.
- SEJERSTED, O. M., STEEN, P. A. & KIIL, F. 1982.

- Energetics of tubular sodium reabsorption sensitive to ethacrynic acid and ouabain. *Am J Physiol* 242: F254–F260.
- SMITH, W. W., FINKELSTEIN, N. & SMITH, H. W. 1940. Renal excretion of hexitols (sorbitol, mannitol and dulcitol) and their derivatives (sorbitan, isomannide and sorbide) and of endogenous creatinine-like chromogen in dog and man. *J Biol Chem* 135: 231–250.
- SNEDECOR, G. W. & COCHRAN, W. G. 1967. *Statistical methods*, 6th ed. Iowa State University Press, Ames, IA.
- STEEN, P. A., HARTMANN, A. & KIIL, F. 1981. Ethacrynic acid inhibits transcellular NaCl reabsorption in dog kidneys in doses of 1 to 10 mg·kg⁻¹ and proximal bicarbonate-dependent reabsorption at higher doses. *J Pharmacol Exp Ther* 219: 505–509.
- WEN, S. G., EVANSON, R. L. & DIRKS, J. H. 1970. Micropuncture study of renal magnesium transport in proximal and distal tubule of the dog. *Am J Physiol* 219: 570–576.
- WESSON, L. G. 1962. Magnesium, calcium and phosphate excretion during osmotic diuresis in the dog. *J Lab Clin Med* 60: 422–432.
- WONG, N. L. M., QUAMME, G. A. & DIRKS, J. H. 1979*a*. Tubular maximum reabsorptive capacity for magnesium in the dog. *Kidney Int* 16: 845.
- WONG, N. L. M., QUAMME, G. A., SUTTON, R. A. L. & DIRKS, J. H. 1979*b*. Effects of mannitol on water and electrolyte transport in the dog kidney. *J Lab Clin Med* 94: 683–692.