Magnesium Transport in the Renal Distal Convoluted Tubule

LONG-JUN DAI, GORDON RITCHIE, DIRK KERSTAN, HYUNG SUB KANG, DAVID E. C. COLE, AND GARY A. QUAMME

Department of Medicine, University of British Columbia, Vancouver, British Columbia; and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada

I. Introduction: Importance of the Distal Convoluted Tubule in Renal Magnesium Balance

II. General Characteristics of Magnesium Absorption in the Distal Tubule

III. Magnesium Uptake in Isolated Distal Cells
   A. Measurement of cellular Mg$^{2+}$ transport
   B. Dependence of cellular Mg$^{2+}$ uptake on membrane voltage
   C. Schematic model of distal cellular Mg$^{2+}$ transport

IV. Load Dependence of Magnesium Absorption in the Distal Tubule

V. Hormonal Control of Magnesium Transport in the Distal Convoluted Tubule
   A. Peptide hormones
   B. Steroid hormones
   C. Prostaglandins
   D. Insulin
   E. Other hormones and factors
   F. Hormonal regulation and renal Mg$^{2+}$ handling

VI. Extracellular Calcium/Magnesium-Sensing Receptors in the Distal Convoluted Tubule
   A. Ca$^{2+}$/Mg$^{2+}$ sensing modulates peptide hormone responses at the receptor level
   B. Ca$^{2+}$/Mg$^{2+}$ sensing modulates steroid hormone responses at the transcriptional level
   C. Ca$^{2+}$/Mg$^{2+}$ sensing modulates other hormone responses

VII. Intrinsic Control of Magnesium Transport in the Distal Convoluted Tubule

VIII. Distal Diuretics That Enhance Magnesium Absorption in the Distal Convoluted Tubule
   A. Amiloride
   B. Chorothiazide

IX. Familial Disorders Affecting Distal Magnesium Transport
   A. Hypomagnesemia associated with abnormal renal NaCl transport
   B. Inherited disorders associated with abnormal extracellular Mg$^{2+}$/Ca$^{2+}$ sensing
   C. Primary inherited disorders of distal Mg$^{2+}$ transport

X. Acquired Disorders That Diminish Distal Magnesium Transport
   A. Potassium depletion
   B. Phosphate depletion
   C. Acid-base changes
   D. Cytotoxic agents

XI. Summary: Future Directions in Research of Magnesium Transport in the Distal Convoluted Tubule

Dai, Long-Jun, Gordon Ritchie, Dirk Kerstan, Hyung Sub Kang, David E. C. Cole, and Gary A. Quamme. Magnesium Transport in the Renal Distal Convoluted Tubule. Physiol Rev 81: 51–84, 2001.—The distal tubule reabsorbs ~10% of the filtered Mg$^{2+}$, but this is 70–80% of that delivered from the loop of Henle. Because there is little Mg$^{2+}$ reabsorption beyond the distal tubule, this segment plays an important role in determining the final urinary excretion. The distal convoluted segment (DCT) is characterized by a negative luminal voltage and high intercellular resistance so that Mg$^{2+}$ reabsorption is transcellular and active. This review discusses recent evidence for selective and sensitive control of Mg$^{2+}$ transport in the DCT and emphasizes the importance of this control in normal and abnormal renal Mg$^{2+}$ conservation. Normally, Mg$^{2+}$ absorption is load dependent in the distal tubule, whether delivery is altered by increasing luminal Mg$^{2+}$ concentration or increasing the flow rate into the DCT. With the use of microfluorescent studies with an established mouse distal convoluted tubule (MDCT) cell line, it was shown that Mg$^{2+}$ uptake was concentration and voltage dependent. Peptide hormones such as parathyroid hormone, calcitonin, glucagon, and arginine vasopressin enhance Mg$^{2+}$ absorption in the distal tubule and stimulate Mg$^{2+}$ uptake into MDCT cells. Prostaglandin E$_2$ and isoproterenol increase Mg$^{2+}$ entry into MDCT cells. The current
evidence indicates that cAMP-dependent protein kinase A, phospholipase C, and protein kinase C signaling pathways are involved in these responses. Steroid hormones have significant effects on distal Mg^2+ transport. Aldosterone does not alter basal Mg^2+ uptake but potentiates hormone-stimulated Mg^2+ entry in MDCT cells by increasing hormone-mediated cAMP formation. 1,25-Dihydroxyvitamin D_3 on the other hand, stimulates basal Mg^2+ uptake. Elevation of plasma Mg^2+ or Ca^2+ inhibits hormone-stimulated cAMP accumulation and Mg^2+ uptake in MDCT cells through activation of extracellular Ca^2+/Mg^2+-sensing mechanisms. Mg^2+ restriction selectively increases Mg^2+ uptake with no effect on Ca^2+ absorption. This intrinsic cellular adaptation provides the sensitive and selective control of distal Mg^2+ transport. The distally acting diuretics amiloride and chlorothiazide stimulate Mg^2+ uptake in MDCT cells acting through changes in membrane voltage. A number of familial and acquired disorders have been described that emphasize the diversity of cellular controls affecting renal Mg^2+ balance. Although it is clear that many influences affect Mg^2+ transport within the DCT, the transport processes have not been identified.

I. INTRODUCTION: IMPORTANCE OF THE DISTAL CONVOLUTED TUBULE IN RENAL MAGNESIUM BALANCE

Control of total body magnesium homeostasis principally resides within the nephron segments of the kidney. The proximal tubule reabsorbs 5–15%, the thick ascending limb of the loop of Henle absorbs 70–80%, and the distal tubule reclaims some 5–10% of the filtered magnesium. Although the distal tubule reabsorbs only 10% of the magnesium filtered through the glomerulus, this amount is significant because it represents 60–70% of the magnesium delivered to this segment from the loop of Henle. Because there is little magnesium reabsorption beyond the distal tubule in the collecting ducts, the tubule segments comprising this portion of the nephron play an important role in determining the final urinary excretion of magnesium. The purpose of this review is threefold: 1) to convince the reader that the distal tubule plays an important role in controlling renal magnesium conservation (as important as the thick ascending limb); 2) to discuss recent observations on how magnesium is reabsorbed and what controls magnesium transport within the distal tubule; and 3) to indicate some of the unresolved issues that require further investigation. Most of the recent studies involve the use of isolated cell lines which, on balance, represent the intact distal convoluted tubule (DCT).

II. GENERAL CHARACTERISTICS OF MAGNESIUM ABSORPTION IN THE DISTAL TUBULE

Most of our early knowledge concerning magnesium transport in the distal tubule has come from micropuncture and microperfusion studies of the superficial nephron (48, 131, 249, 251, 256, 288, 289, 344).1 Micropuncture studies showed that significant amounts of magnesium are absorbed in the distal tubule (19, 92, 93, 248, 252, 254). The mammalian distal tubule, located between the macula densa and the cortical collecting duct (CCD), comprises a short post-macula densa segment of thick ascending limb, the DCT, the connecting tubule (CNT), and the initial collecting tubule (333). The micropuncture studies describing distal magnesium absorption may well have included portions of the superficial CNT as well as the DCT. R. J. M. Bindels failed to detect any apical-to-basolateral (absorption) or basolateral-to-apical (secretion) magnesium movement in a mixture of rabbit CNT and CCD cells (personal communication). However, there are significant functional differences among the species and segments studied. There is a gradual transition between the DCT and CNT in human and rats, whereas there is a sharp transition in the rabbit (216). In the rat and human, chlorothiazide-sensitive NaCl cotransport and parathyroid hormone (PTH)-stimulated calcium transport occurs within DCT segments and PTH-responsive Na^+/Ca^2+ exchange within the CNT (226). This is in contrast to the rabbit, where thiazide-sensitive NaCl cotransport is found in the DCT but PTH-responsive Na^+/Ca^2+ exchange is expressed only by CNT cells (10, 32, 264, 329, 330). The mouse appears to be more like the rat and human because the two transporters are colocalized to DCT cells (108). Again, the species and segmental differences of magnesium transport have not been studied.

Although significant magnesium reabsorption takes place along the distal tubule, this is normally achieved with little change in tubular magnesium concentration. Luminal magnesium concentration may increase along the distal tubule with increased magnesium delivery, or it may decrease with magnesium restriction (92, 131, 248, 298). Normally, distal magnesium absorption is fractionally less than that of sodium or calcium (254). This is to form a collecting duct. The distal tubule of micropuncture literature comprises primarily the distal convoluted tubule and connecting tubule (263). The distal convoluted tubule is restricted to the cells comprising the DCT only.

---

1 The definitions used in this review are those proposed by the Renal Commission of the International Union of Physiological Sciences (170). The distal tubule is used to denote the nephron segment between the region of the macula densa and the confluence with another tubule.
commensurate with the fractional urinary excretion of magnesium, normally \(\sim 3\%\), whereas the fractional excretion of sodium and calcium is \(< 1\%\) (250). The evidence is that net distal magnesium reabsorption is essentially unidirectional because no secretion of magnesium into the lumen has been reported (254). Because the distal tubule is characterized by a negative transepithelial voltage and a high epithelial resistance, it is concluded that magnesium transport is active and transcellular in nature (165, 289). This is unlike magnesium transport within the thick ascending limb that occurs passively through the paracellular pathway (249, 289). Although the micropuncture and microperfusion studies have clearly shown that magnesium is reabsorbed in the superficial distal tubule, little knowledge has been gained concerning the cellular mechanisms of magnesium transport.

### III. Magnesium Uptake in Isolated Distal Cells

In vivo micropuncture and microperfusion approaches do not allow for study of cellular mechanisms, and in vitro microperfusion studies have not been performed on DCT because of the difficulty in isolating intact segments. Accordingly, isolated immortalized cell lines have been used to study segmental responses.

#### A. Measurement of Cellular Mg\(^{2+}\) Transport

We have used an established cell line representing the DCT to study Mg\(^{2+}\) transport. This cell line (designated MDCT for mouse distal convoluted tubule) was originally isolated from mouse distal tubules and immortalized by Pizzonia et al. (238). Friedman and Gesek (111, 115–118) have shown that this cell line exhibits many of the functional properties characteristic of the intact DCT studied in vivo, such as amiloride-inhibitable Na\(^{+}\) transport, chlorothiazide-sensitive NaCl cotransport, and PTH- and calcitonin-stimulated Ca\(^{2+}\) transport. Accordingly, MDCT cells appear to have many of the properties of the convoluted segment of the intact distal tubule.

Electrolyte transport is usually quantitated by isotope flux measurements, but an appropriate isotope for Mg\(^{2+}\) is not available (\(^{28}\)Mg has a half-life of 21 h). Accordingly, we developed a cell model to assess Mg\(^{2+}\) transport using fluorescent determinations of intracellular free Mg\(^{2+}\) concentration (\([\text{Mg}^{2+}]_i\)) (75, 253). Cytosolic free Mg\(^{2+}\) concentration of epithelial cells is on the order of 0.5 mM. This is \(\sim 1\%\) of the total magnesium, the remainder being complexed to various organic and inorganic ligands and chelated within the mitochondria. Using isolated distal cell lines, we have shown that magnesium entry is through specific and regulated magnesium pathways (76, 254). To determine Mg\(^{2+}\) transport, the epithelial cells were first depleted of Mg\(^{2+}\) by incubating in magnesium-free culture media. Subsequently, the cells were placed in solutions containing magnesium and [Mg\(^{2+}\)] \(i\) measured as a function of time (Fig. 1). [Mg\(^{2+}\)] \(i\) increased until it had reached normal levels. The rate of concentration change \(\frac{d[\text{Mg}^{2+}]}{dt}\) is an estimate of Mg\(^{2+}\) uptake rate. Influx of Mg\(^{2+}\) is concentration dependent so that the rate of Mg\(^{2+}\) transport increases with external Mg\(^{2+}\) until saturation is attained (70, 76, 253). Mg\(^{2+}\) influx into Mg\(^{2+}\)-depleted cells was inhibited by Mn\(^{2+}\) and La\(^{3+}\) and by dihydropyridine channel blockers such as nifedipine. Ca\(^{2+}\) neither blocked Mg\(^{2+}\) entry nor was \(^{45}\)Ca uptake changed in the presence of Mg\(^{2+}\) depletion or the Mg\(^{2+}\)-refill process. These observations suggest that the influx pathway is specific for Mg\(^{2+}\) and not shared by Ca\(^{2+}\) (76, 253). We used this approach to characterize the cellular mechanisms of Mg\(^{2+}\) uptake in MDCT cells that may shed light on magnesium transport in distal tubule cells.

Many hormonal and nonhormonal factors influence Mg\(^{2+}\) uptake into MDCT cells. Our observations using MDCT cells closely resemble earlier results using micropuncture and microperfusion techniques in intact rat and dog distal tubules (Table 1). However, little is known about the cellular mechanisms of electrolyte absorption in the DCT from in vivo micropuncture and microperfusion experiments. Accordingly, the use of isolated cells has allowed a greater in-depth study of cellular mecha-

---

**FIG. 1. Intracellular free Mg\(^{2+}\) concentration ([Mg\(^{2+}\)] \(i\)) in normal and Mg\(^{2+}\)-depleted immortalized mouse distal convoluted tubule (MDCT) cells.** Confluent MDCT cells were cultured in either normal (0.6 mM Mg\(^{2+}\)) or Mg\(^{2+}\)-free media (<0.01 mM) for 16 h. Fluorescence studies were performed in buffer solutions in the absence of extracellular Mg\(^{2+}\), and where indicated, MgCl\(_2\) (5.0 mM final concentration) was added to observe changes in [Mg\(^{2+}\)] \(i\). The buffer solutions contained (in mM) 145 NaCl, 4.0 KCl, 0.8 KH\(_2\)PO\(_4\), 0.2 KH\(_2\)PO\(_4\), 1.0 CaCl\(_2\), 5.0 glucose, and 10 HEPES/Tris, pH 7.4, with and without 5.0 mM MgCl\(_2\). The rate of rise in intracellular Mg\(^{2+}\) concentration, \(\frac{d[\text{Mg}^{2+}]}{dt}\), is a reflection of the entry rate of Mg\(^{2+}\) into the cell. Fluorescence was measured at 1 data point/s with 25-point signal averaging, and the tracing was smoothed according to methods previously described. [Data from Dai et al. (76).]
**TABLE 1. Controls of magnesium reabsorption in intact distal tubules compared with MDCT cells**

<table>
<thead>
<tr>
<th>Peptide hormones</th>
<th>Intact Distal Tubules</th>
<th>MDCT Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid hormone</td>
<td>Increase (19)</td>
<td>Increase (68)</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Increase (92, 243)</td>
<td>Increase (UP)</td>
</tr>
<tr>
<td>Glucagon</td>
<td>Increase (19)</td>
<td>Increase (67)</td>
</tr>
<tr>
<td>Arginine vasopressin</td>
<td>Increase (93)</td>
<td>Increase (67)</td>
</tr>
<tr>
<td>β-Adrenergic agonists</td>
<td>?</td>
<td>Increase (UP)</td>
</tr>
<tr>
<td>(isoproterenol)</td>
<td>?</td>
<td>Increase (68)</td>
</tr>
<tr>
<td>Prostaglandins (PGE2)</td>
<td>?</td>
<td>Increase (69)</td>
</tr>
<tr>
<td>Insulin</td>
<td>?</td>
<td>Increase (68)</td>
</tr>
<tr>
<td>Mineralocorticoids (aldosterone)</td>
<td>?</td>
<td>Increase (76)</td>
</tr>
<tr>
<td>Vitamin D 1,25(OH)_2D_3</td>
<td>?</td>
<td>Increase (75)</td>
</tr>
<tr>
<td>Magnesium restriction</td>
<td>Increase (252, 208)</td>
<td>Increase (76)</td>
</tr>
<tr>
<td>Metabolic acidosis</td>
<td>Decrease (208)</td>
<td>Decrease (72)</td>
</tr>
<tr>
<td>Metabolic alkalosis</td>
<td>Increase (298)</td>
<td>Increase (72)</td>
</tr>
<tr>
<td>Hypermagnesemia</td>
<td>Decrease (177, 254)</td>
<td>Decrease (20)</td>
</tr>
<tr>
<td>Hypercalcemia</td>
<td>Decrease (178, 246)</td>
<td>Decrease (20)</td>
</tr>
<tr>
<td>Phosphate depletion</td>
<td>Decrease (347)</td>
<td>Decrease (71)</td>
</tr>
<tr>
<td>Potassium depletion</td>
<td>?</td>
<td>Decrease (70)</td>
</tr>
<tr>
<td>Diuretics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>No effect (254)</td>
<td>No effect (76)</td>
</tr>
<tr>
<td>Amiloride</td>
<td>?</td>
<td>Increase (76)</td>
</tr>
<tr>
<td>Chlorothiazide</td>
<td>Increase (257)</td>
<td>Increase (70)</td>
</tr>
</tbody>
</table>

Results of the intact distal tubule were obtained using micropuncture and microperfusion studies. ?, Not known; UP, unpublished observations; MDCT, mouse distal convoluted tubule cell line; 1,25(OH)_2D_3, 1,25-dihydroxyvitamin D_3.

The similarity of results between the MDCT cell studies and the micropuncture experiments support the use of this cell line in characterizing cellular mechanisms of Mg\(^{2+}\) transport.

### B. Dependence of Cellular Mg\(^{2+}\) Uptake on Membrane Voltage

Our initial studies looked at the changes in Mg\(^{2+}\) entry into MDCT cells following alterations in membrane voltage (76). In these studies, with the same starting [Mg\(^{2+}\)_i] and the same external Mg\(^{2+}\) concentration, the more negative the transmembrane voltage, i.e., more hyperpolarized, the higher was the magnesium influx rate (Fig. 2). Conversely, depolarization of membrane voltage diminishes Mg\(^{2+}\) uptake (70). The dependence of magnesium entry on the driving force induced by the electrochemical gradient indicates that Mg\(^{2+}\) entry may be mediated by an ion channel.

### C. Schematic Model of Distal Cellular Mg\(^{2+}\) Transport

With the above information, we are able to speculate on the mechanisms involved in magnesium absorption within the DCT cell (Fig. 3). Because the transepithelial voltage is normally in the range of 0 to \(-30\) mV lumen negative, magnesium absorption is active in nature. Distal magnesium absorption is entirely transcellular moving across the DCT cell. Magnesium may move passively into the cell across the luminal membrane driven by a favorable transmembrane voltage. The luminal magnesium concentration is on the order of 0.2–0.7 mM depending on the condition studied, and intracellular free Mg\(^{2+}\) is 0.5 mM so that under some circumstances Mg\(^{2+}\) entry is against an appreciable concentration gradient (76, 253). We speculate that Mg\(^{2+}\) entry is through a unique channel, and this transport is dependent on the transmembrane voltage. The active step in transcellular movement is predicted to be at the basolateral membrane where Mg\(^{2+}\) leaves the cell against both electrical and concentration gradients. The means by which Mg\(^{2+}\) actively moves across the basolateral membrane is unknown. Evidence taken from studies using nonepithelial cells suggest that Na\(^+\)/Mg\(^{2+}\) exchange may occur, Na\(^+\) moving back into the cell coupled with Mg\(^{2+}\) exit from the cell into the interstitium (126). Alternatively, a specific energy-dependent Mg\(^{2+}\) pump may be present analogous to that reported for calcium. Intracellular Mg\(^{2+}\) plays an important role in enzyme functions including the transport ATPases (102, 250). The ionic transport pumps such as Na\(^+\)-K\(^+\)-ATPase, Ca\(^{2+}\)-ATPase, and H\(^+\)-ATPase require Mg\(^{2+}\) for activity; accordingly, they are Mg\(^{2+}\)-dependent ATPases.
sensitive Ca\(^{2+}\) channels. Matsunga et al. (206) further claimed that these dihydropyridine-sensitive Ca\(^{2+}\) channels are activated by hyperpolarizing voltages. More recently, Hoenderop and co-workers (149, 150) have identified the gene coding the Ca\(^{2+}\) channel from rabbit CCD and DCT that is activated by hyperpolarizing voltages and inhibited by dihydropyridines (149, 150). These findings concerning Ca\(^{2+}\) entry are in keeping with the earlier ideas of Costanzo (63). On the other side of the cell, two calcium transporters are responsible for Ca\(^{2+}\) efflux: an ATP-dependent calcium pump and, at least in the distal tubule, a Na\(^{+}\)/Ca\(^{2+}\) exchanger (192, 264, 330). These properties of cellular Ca\(^{2+}\) handling are similar to those postulated here for Mg\(^{2+}\) transport, although the presence of a Mg\(^{2+}\) pump and Na\(^{+}\)/Mg\(^{2+}\) exchanger remains to be determined. The regulation of distal magnesium reabsorption may share many of the controls identified for calcium conservation but, as reviewed below, Mg\(^{2+}\) transport may be specifically altered by influences not shared with calcium, thus affecting magnesium homeostasis independent of calcium balance.

IV. LOAD DEPENDENCE OF MAGNESIUM ABSORPTION IN THE DISTAL TUBULE

Distal magnesium absorption is load dependent in that an increase in magnesium delivery to the DCT is associated with an increase in magnesium absorption (19, 71, 248, 254). In one set of studies, rat distal tubules were perfused with solutions containing variable magnesium concentrations, and early and late collections were performed on the same distal tubule. These collection sites were localized in the first 10–60% of the superficial distal tubule and likely represent DCT rather than connecting tubules or initial collecting ducts (254). The first studies involved perfusing DCT with solutions containing zero magnesium. No magnesium was detected in early or late collections when the nephrons were perfused with magnesium-free Ringer solutions in either normal animals or hypermagnesemic rats in which plasma magnesium was elevated to increase the concentration gradient from plasma to lumen. These data suggested that magnesium absorption in the distal nephron is unidirectional, i.e., there is no magnesium secretion in the DCT. Net magnesium absorption was observed when the distal tubules were perfused with solutions containing magnesium. Absolute magnesium absorption increased from 1.35 ± 0.35 to 6.5 ± 0.9 pmol/min/mm\(^{-2}\) as magnesium in the perfusate was increased from 0.5 to 2.5 mM; accordingly, there was about a fivefold increase in magnesium absorption with about a fivefold increase in luminal magnesium concentration (Fig. 4). PTH increased absolute and fractional magnesium absorption independent of delivery. Increased magnesium delivery due to hypermagnesemia or

---

[Diagram showing magnesium absorption in the distal tubule.]
hypercalcemia resulted in an increase in absolute absorption but a decrease in fractional transport (246, 254). Other studies were performed where furosemide was used to inhibit loop magnesium absorption, thereby increasing magnesium delivery to the DCT (248). Absolute magnesium reabsorption increased from 0.6 ± 0.2 to 3.0 ± 1.7 pmol·min⁻¹·mm⁻¹ with increased delivery, whereas fractional absorption remained unchanged in these experiments. The basis for the dependence of magnesium absorption on magnesium delivery and luminal magnesium concentration is also observed at the single-cell level (76). Magnesium reabsorption in the DCT is under the control of a number of hormones and altered by a number of influences. However, the dependence of magnesium absorption with magnesium delivery is maintained in the presence of these factors (246, 254).

V. HORMONAL CONTROL OF MAGNESIUM TRANSPORT IN THE DISTAL CONVOLUTED TUBULE

A. Peptide Hormones

1. PTH

A large number of hormones stimulate magnesium absorption within the distal tubule (Table 1). The first to be described was PTH. Infusion of PTH to thyroparathyroidectomized (TPTXed) animals increased the reabsorption of magnesium and diminished urinary magnesium excretion (204). Micropuncture studies showed that part of this hormonal action occurred within the distal tubule (19, 131, 254). An increase in magnesium conservation was observed even in the face of enhanced magnesium delivery to this segment (248, 252, 254). The largest changes were observed in TPTXed hamsters where the mean tubular fluid-to-ultrafiltrable magnesium ratio (TF/UFMg) at the distal sampling site fell from 0.56 ± 0.08 to 0.33 ± 0.08 after administration of PTH (131). This was associated with a fall in fractional magnesium excretion from ~14 to 3%. De Rouffignac et al. (287) and Bailly and Amiel (16) have shown that PTH and other hormones stimulate magnesium absorption in the rat distal tubule. They used Brattleboro rats with hereditary diabetes insipidus, which lack endogenous ADH, and they infused either glucose or somatostatin to inhibit glucagon secretion. Furthermore, they TPTXed the animals to eliminate circulating PTH and calcitonin. Thus a “hormone-deprived” animal model was created to serve as a basis for evaluating the respective actions of each hormone (287). Micropuncture studies were then performed to determine the effects of hormone administration; importantly, these studies were all performed with physiological hormone concentrations. Infusion of PTH-(1–34) to hormone-deprived rats led to diminished magnesium and calcium delivery to early and late distal tubule sampling sites (Fig. 5). These studies clearly demonstrate that PTH enhances magnesium absorption within the distal tubule. Dai and Quamme (75) showed that PTH also stimulates Mg²⁺ entry in isolated MDCT cells in excess of 30% above control uptake rates. The change in transport was associated with increases in hormone-mediated cAMP formation, suggesting that PTH acts, in part, through protein kinase A signaling pathways (Fig. 6).

2. Calcitonin

Calcitonin infusions have clearly been shown to enhance renal magnesium conservation in the rat (243). Poujeol et al. (243) infused calcitonin to TPTXed rats and observed a fall in fractional magnesium excretion from 4.1 ± 0.4 to 1.0 ± 0.3%, which they attributed to an increase in magnesium reabsorption in the loop of Henle. However, subsequent studies with the hormone-deprived rat showed that calcitonin markedly stimulated fractional magnesium absorption in the superficial distal tubule as well as the loop (81). Fractional absorption in the distal tubule increased by 27 ± 6% in this study (Fig. 5). These micropuncture studies indicate that calcitonin enhances magnesium conservation, in part, by actions within the distal tubule. Calcitonin has been shown to stimulate intracellular cAMP formation and Mg²⁺ entry in MDCT cells, indicating that the DCT segment of the distal tubule is a target for this hormone (Fig. 6). Maximal concentra-
ions of calcitonin increased Mg\(^{2+}\) uptake by 49 ± 5%, which is greater than that observed for PTH. Again, hormone-stimulated Mg\(^{2+}\) entry rates were associated with increases in intracellular cAMP levels.

3. Glucagon

Glucagon is a potent renal magnesium-conserving hormone (16). Bailly and Amiel (16) reported that the acute infusion of pharmacological concentrations of glucagon in parathyroid gland-intact rats leads to a rapid fall in fractional magnesium excretion from 16 ± 1 to 9 ± 2% (16). The response to glucagon is even greater in hormone-deprived animals. Bailly et al. (19) showed that fractional magnesium excretion markedly decreased by ~50% (from 71.5 ± 8.0 to 39.6 ± 5.7 nmol/min) with glucagon administration in rats deficient in endogenous PTH, calcitonin, glucagon, and antidiuretic hormone. This was attributed to a doubling of absolute reabsorption within both the loop of Henle (increase from 6.5 ± 0.7 to 11.7 ± 0.7 nmol/min) and the distal tubule (increase from 0.85 ± 0.1 to 1.75 ± 0.3 nmol/min). Accordingly, glucagon acts within the loop and distal tubule of the rat.

We have performed studies in isolated MDCT cells to determine the cellular mechanisms of hormonal stimulation of magnesium transport (67). Glucagon maximally increased Mg\(^{2+}\) uptake by ~20% (Fig. 6). This stimulation was concentration dependent, associated with intracellular cAMP generation, and inhibited by the channel blocker nifedipine. Clearly, of the segments comprising the distal tubule, the evidence from this study indicates that the convoluted portion is involved with glucagon-induced magnesium conservation.

4. Arginine vasopressin

The actions of arginine vasopressin (AVP) within the DCT are poorly understood (165). AVP has been shown to be an effective magnesium-conserving hormone in anesthetized and conscious hormone-deprived rats (37, 93, 286). Micropuncture studies of these animals have shown that AVP actions occur principally within Henle’s loop (93). Elalouf et al. (93) failed to discern any change in fractional magnesium absorption in the superficial distal tubule after physiological administration of AVP (93). In these studies, Elalouf et al. (93) reported that fractional calcium absorption significantly increased from 42.0 ± 5.8 to 62.8 ± 7.1%, whereas the change in fractional magnesium transport with AVP was not significant although it increased from 45.5 ± 7.8 to 55.3 ± 15.5% (Fig. 5). These changes may have been significant if a greater number of tubules were sampled. Costanzo and Windhager (65) did not observe any change in calcium absorption in the microperfused rat distal tubule with administration of AVP. These animals were TPTXed but not hormone deprived as were those used by Elalouf et al. (93). In both studies, AVP enhanced sodium absorption in the distal tubule.

**Fig. 5.** Fractional reabsorption of magnesium in the superficial distal tubule. Studies were performed in “hormone-deprived” rats lacking vasopressin, PTH, calcitonin, and glucagon. Tubular fluid was collected from the early and late sites of the same distal tubule, and net magnesium reabsorption is expressed as a fraction of that delivered to the segment. The PTH (5 mU/min) and glucagon (5 ng/min) studies were from Bailly et al. (19), and those of calcitonin (1.0 mL/min) and AVP (20 pg/min) were from Elalouf et al. (92) and Rouffignac et al. (286), respectively. Values are means ± SE. *Significance from the respective control values.

**Fig. 6.** Peptide hormones stimulate intracellular cAMP formation and Mg\(^{2+}\) uptake in Mg\(^{2+}\)-depleted mouse distal convoluted tubule (MDCT) cells. Confluent MDCT cells were cultured in Mg\(^{2+}\)-free media (~0.01 mM) for 16–20 h. Measurement of cAMP was performed with radioimmunoassay. Fluorescence studies were performed in buffer solutions in the absence of Mg\(^{2+}\), and where indicated, MgCl\(_2\) (1.5 mM final concentration) was added to observe changes in intracellular Mg\(^{2+}\) concentration. PTH, calcitonin, glucagon, and AVP were added to the buffer solution where indicated. Values are means ± SE. *Changes in significance (P < 0.01) of Mg\(^{2+}\) uptake, and *Significance (P < 0.001) of cAMP release from the respective control values. [Data from Dai et al. (67, 69) and G. Ritchie, L.-j. Dai, D. Kerstan, H. S. Kang, L. Canaff, G. N. Hendy, and G. A. Quamme, unpublished data.]
AVP also stimulates Mg\(^{2+}\) entry into MDCT cells in a concentration-dependent fashion that is sensitive to nifedipine (Fig. 6). These observations suggest that AVP plays a role in control of magnesium conservation within the DCT (67). Many of the hormones that have been shown to increase magnesium reabsorption in the distal tubule have additive effects (91, 285). AVP and glucagon are additive, below maximal concentrations, in stimulating Mg\(^{2+}\) uptake into MDCT cells (67). Accordingly, these hormones, and probably others, together orchestrate magnesium reabsorption in the distal tubule. It would be difficult to predict the effects of one hormone on a background of many. This was the rationale for De Rouffignac (285) using hormone-deprived animals in their studies investigating individual hormone effects. The advantage of using isolated MDCT cells is that hormone-mediated signaling pathways may be investigated, which is difficult or impossible to perform with in vivo studies.

5. Cellular mechanisms of peptide hormone actions

The cellular mechanisms underlying the hormonal actions on distal magnesium absorption are becoming clearer. Morel and co-workers (17, 215) have shown that PTH, calcitonin, and glucagon stimulate receptor-mediated cAMP release in the DCT (45). They also reported that AVP receptors may be present in the DCT, but there were marked species differences in adenylate cyclase responsiveness (217). There is little AVP-stimulated cAMP release in the DCT of the rabbit and human, intermediate response in the mouse, and greatest in the rat (215). We have shown that PTH, calcitonin, glucagon, and AVP stimulate Mg\(^{2+}\) uptake into MDCT cells (21, 67, 69; Fig. 6). Friedman and Gesek (108, 113, 117) reported that these hormones also stimulate cellular cAMP accumulation. We have demonstrated that an increase in exogenous intracellular cAMP, with 8-bromo-cAMP, or endogenous cAMP formation, with forskolin, increased Mg\(^{2+}\) uptake, whereas inhibition of protein kinase A with \(R_p\)-cAMPS prevented hormone-stimulated uptake (67). Accordingly, receptor-mediated cAMP release and activation of protein kinase A plays a role in hormone-stimulated Mg\(^{2+}\) uptake in MDCT cells. However, it is apparent that other signaling pathways are present for hormone-mediated Mg\(^{2+}\) uptake in MDCT cells. Hormone-stimulated Mg\(^{2+}\) uptake rates do not correlate with the measured intracellular cAMP levels in MDCT cells (38, 107, 138). Furthermore, phospholipase C inhibition with U-73122 and protein kinase C inhibition with Ro31–8220 abolished PTH- and calcitonin-stimulated Mg\(^{2+}\) uptake (Fig. 7). This was true for all of the hormones tested: PTH, calcitonin, glucagon, and AVP (67–69; L.-j. Dai, G. Ritchie, D. Kerstian, H. S. Kang, and G. A. Quamme, unpublished observations). We have previously reported that chelerythrine, a putative protein kinase C inhibitor, did not alter hormone-mediated responses (67, 69). However, this agent has recently been shown to have no inhibitory actions on protein kinase C (176). Our evidence indicates that protein kinase C is involved in hormone signaling responses in MDCT cells; however, the isotype(s) of protein kinase C is not known. These hormones do not elicit receptor-mediated intracellular Ca\(^{2+}\) transients, suggesting that Ca\(^{2+}\) signaling is not involved with the responses (110, 116). It is well known that multiple receptors can converge on a single G protein, and in many cases a single receptor can activate more than one G protein and thereby modulate multiple intracellular signals (128). It is evident that cAMP-dependent protein kinase A, phospholipase C, and protein kinase C pathways are necessary for hormone-stimulated Mg\(^{2+}\) entry into MDCT cells. The details of how peptide hormones act on Mg\(^{2+}\) transport in MDCT cells and intact distal tubules are unknown.

These observations of hormone-stimulated Mg\(^{2+}\) entry are somewhat different from those reported for Ca\(^{2+}\) entry into MDCT cells. Friedman and Gesek (108, 113, 117) showed that PTH and calcitonin increased calcium uptake in the DCT through changes in membrane voltage. They provided evidence that receptor-mediated increases in cAMP activate basolateral Cl\(^-\) channels resulting in cellular efflux of Cl\(^-\), diminished intracellular Cl\(^-\) activity, and hyperpolarization of the apical membrane of the MDCT cell through a decrease in the electrochemical Cl\(^-\) gradient (110). Membrane hyperpolarization activates
Ca\textsuperscript{2+} entry and increases the driving force for Ca\textsuperscript{2+} movement into the cell which, in turn, is removed across the basolateral membrane into the blood. More recent studies indicate that PTH-mediated stimulation of calcium transport is through intermediary pathways involving activation of both protein kinase A and protein kinase C (107, 144). PTH and other hormones may also directly act on Ca\textsuperscript{2+} entry through apical Ca\textsuperscript{2+} channels and Ca\textsuperscript{2+} exit through Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange, again through activation of these kinases (10, 11, 38). Brunette and co-workers (144, 174) have shown that distal luminal vesicles prepared from rabbit tubules pretreated with PTH transport much more calcium than vesicles from untreated tubules, inferring a covalent modification of putative apical Ca\textsuperscript{2+} channels. Accordingly, PTH and possibly other hormones may have direct actions on Ca\textsuperscript{2+} entry in addition to their ability to alter the membrane voltage. Further research is required to determine the cellular mechanisms underlying the action of these hormones on distal divalent cation absorption. Interestingly, Friedman and Gesek (108) failed to demonstrate any effect of glucagon and AVP on Ca\textsuperscript{2+} entry into MDCT cells, although these hormones increased cellular cAMP accumulation (108). It is apparent that hormonal controls of Ca\textsuperscript{2+} entry in MDCT cells are different from those reported for Mg\textsuperscript{2+}. In support of this notion, differential controls of renal Ca\textsuperscript{2+} and Mg\textsuperscript{2+} transport are apparent in the clinical presentation of many familial and acquired diseases (see sects. IX and X).

Finally, a comment should be made concerning the differences between hormone-mediated Ca\textsuperscript{2+} within the CCD and hormone-stimulated Mg\textsuperscript{2+} uptake in MDCT cells because it emphasizes different controls for these two divalent cations in the distal tubule. Hoenderop et al. (151) have extensively characterized hormone-stimulated Ca\textsuperscript{2+} transport in primary rabbit CCD cells. The rabbit CCD does not reabsorb Mg\textsuperscript{2+} even with chronic magnesium depletion (R. J. M. Bindels, personal communication). However, the comparisons of hormonal controls between the two segments are informative. The rate of transepithelial Ca\textsuperscript{2+} reabsorption within the rabbit CCD is determined by an apical 1,25-dihydroxyvitamin D\textsubscript{3} [1,25(OH)\textsubscript{2}D\textsubscript{3}]-responsive calcium channel (149). This Ca\textsuperscript{2+} channel (ECaC) is activated by hyperpolarization but does not transport Mg\textsuperscript{2+}, nor does external Mg\textsuperscript{2+} inhibit Ca\textsuperscript{2+} transport, clearly different from the Mg\textsuperscript{2+} transporter (150). Ca\textsuperscript{2+} reabsorption is stimulated by peptide hormones such as PTH and AVP (32). It has been reported that Ca\textsuperscript{2+} transport is stimulated by hormone-mediated signaling pathways that are independent of cAMP but involve a chelerythrine-inhibitable protein kinase C that is not downregulated after chronic phorbol ester treatment (31, 146, 155, 331). Our studies have shown that both cAMP-protein kinase A and protein kinase C (not sensitive to chelerythrine) are essential in hormonal action within the MDCT cells. Accordingly, hormone signaling within the two segments is qualitatively different. The differential control of calcium reabsorption within the DCT and CCD and magnesium transport in the DCT provides a unique mechanism to differentially regulate renal calcium and magnesium balance.

**B. Steroid Hormones**

1. **Mineralocorticoid hormones**

Mineralocorticoid receptors are present in DCT cells, which are thought to be involved in expression of NaCl cotransport, Na\textsuperscript{+} conductance, and sodium pump activity (57, 283, 333). The effects of aldosterone on distal tubule magnesium absorption have not been studied with micropuncture techniques. Clearance studies have shown that chronic aldosterone administration results in renal magnesium wasting, but this has been explained by extracellular volume expansion leading to diminished NaCl and magnesium reabsorption within the loop (202, 203, 319). We have studied the effects of aldosterone on Mg\textsuperscript{2+} entry into MDCT cells (77). Incubation of aldosterone, for 16 h before determination of Mg\textsuperscript{2+} uptake, failed to have any effect on basal magnesium transport. However, pre-treatment of MDCT cells with aldosterone potentiated hormone-stimulated Mg\textsuperscript{2+} uptake (Fig. 8). This was associated with potentiation of hormone-mediated cAMP release in aldosterone-treated MDCT cells (77). Cycloheximide, an inhibitor of protein synthesis, abolished the potentiation of aldosterone on hormone-stimulated cAMP release and Mg\textsuperscript{2+} uptake (59). Accordingly, aldosterone

![FIG. 8. Aldosterone potentiates hormone-stimulated intracellular cAMP formation and Mg\textsuperscript{2+} entry in MDCT cells. MDCT cells were treated with aldosterone (10\textsuperscript{-7} M) where indicated for 16 h before cAMP determinations and fluorescent measurements. PTH (10\textsuperscript{-7}) was added 5 min before cAMP and immediately with Mg\textsuperscript{2+} uptake determinations. Values are means ± SE. *Significance (P < 0.01) of Mg\textsuperscript{2+} uptake values. **Significance (P < 0.001) of cAMP analysis from control values. [Data from Dai et al. (77).]]
may enhance hormone-stimulated Mg\(^{2+}\) entry by increasing cAMP and its responses. This notion is supported by the observations of others (155). Rajerison et al. (259) demonstrated that adrenalectomy reduced AVP-stimulated adenylate cyclase activity in membrane fractions prepared from rat kidney medulla. Doucet and co-workers (85, 95) have shown that glucagon- and AVP-responsive cAMP generation is diminished in thick ascending limb and collecting tubule segments harvested from adrenalectomized rats compared with animals treated with physiological doses of aldosterone. These investigators postulate that aldosterone induces a protein(s) that stimulates hormone-sensitive adenylate cyclase activity. Studies with kidney membrane fractions and isolated segments in the absence of aldosterone demonstrated an impairment of coupling between hormone receptors and adenylate cyclase catalytic units that was responsible for diminished cAMP generation (95). Steroid hormones have significant effects on expression and posttranslational targeting of heterotrimeric G proteins so that associated channels are covalently modified (218, 281, 293). The mechanism(s) through which steroids control G\(_{\text{q/11}}\) proteins (synthesis and/or degradation vs. activity of each unit) associated with Mg\(^{2+}\) uptake in MDCT cells is not known (85). The role of mineralocorticoids in the physiological maintenance of renal magnesium handling also requires further research.

2. Vitamin D\(_3\) metabolites

Vitamin D\(_3\) metabolites have important effects on mineral metabolism by 1,25(OH)\(_2\)D\(_3\) actions on epithelial transport. Although it is clear that 1,25(OH)\(_2\)D\(_3\) increases calcium and magnesium absorption within the intestine, its actions within the kidney are unclear (172, 332). The 1,25(OH)\(_2\)D\(_3\)-dependent calcium binding proteins, calbindin-D\(_{28k}\) and calbindin-D\(_{25k}\), have long been thought to be involved in facilitating calcium transport across epithelial cells (100, 172, 332). The mechanisms of calcium stimulation are obscure, but because of its close association with the basolateral membrane, it is postulated that they somehow increase the calcium pump (\(\text{Ca}^{2+}\)-\(\text{Mg}^{2+}\)-ATPase) activity (33, 36, 172). Within the kidney, calbindin-D is localized in the distal tubule where a significant portion of calcium and magnesium is reabsorbed (187, 197, 284, 316, 324). The distal tubule, including the convoluted segment, also possesses 1,25(OH)\(_2\)D\(_3\) receptors (186, 187, 284). Accordingly, 1,25(OH)\(_2\)D\(_3\) and perhaps its dependent calbindin-D may have significant actions within the DCT. The effects of 1,25(OH)\(_2\)D\(_3\) and calbindins on magnesium transport are unknown. Calbindin-D\(_{28k}\) has a relatively high affinity for Mg\(^{2+}\) (6, 49, 185), and it is appropriately altered by changes in magnesium balance (137), suggesting a role for these binding proteins in renal magnesium control.

In contrast to calcium, little information is available concerning the effects of vitamin D\(_3\) metabolites on tubular Mg\(^{2+}\) transport. On balance, data from clinical or experimental studies indicate little or no effect of 1,25(OH)\(_2\)D\(_3\) on renal magnesium handling as determined by clearance techniques (51, 129, 130, 184, 213, 219, 270). There have been no micropuncture studies performed to localize the actions, if any, of vitamin D\(_3\) on magnesium transport. The effects of 1,25(OH)\(_2\)D\(_3\) on renal calcium absorption are more substantial but not clear (32, 39, 40, 64, 166). The most convincing data demonstrating that 1,25(OH)\(_2\)D\(_3\) may have some direct effects on Ca\(^{2+}\) transport are those using isolated cells. Bindels and co-workers (32, 330) showed that 1,25(OH)\(_2\)D\(_3\) increased calbindin-D\(_{28k}\) and stimulated transcellular calcium absorption in primary cultures of the rabbit CCD. The maximal response occurred about 48 h posttreatment, suggesting to these investigators that the response involved initiation of transcriptional processes (32). The responses of 1,25(OH)\(_2\)D\(_3\) were independent of PTH and not additive to PTH-stimulated Ca\(^{2+}\) transport. In a more recent report, these authors have shown that 1,25(OH)\(_2\)D\(_3\) increased calbindin-D\(_{28k}\) RNA and protein content without a change in Na\(^+\)/Ca\(^{2+}\) exchanger or Ca\(^{2+}\)-ATPase RNA and protein (32). The second study to clearly show effects of vitamin D\(_3\) metabolites on calcium transport was performed in MDCT cells by Friedman and Gesek (109). These workers reported that 1,25(OH)\(_2\)D\(_3\) did not alter basal Ca\(^{2+}\) uptake but accelerated PTH-dependent calcium entry rates. This response was rapid, concentration dependent, significant at 2 h and maximal by 5 h, and mediated by transcriptional processes because it was inhibited by cycloheximide (109). The reasons for the discrepancies between these two reports are not known; it may be the cell type used in the two separate studies or the techniques by which calcium transport was measured. Nevertheless, it is clear that 1,25(OH)\(_2\)D\(_3\) increases calcium-binding protein in distal tubules and suggests that it may have significant actions on basal or hormone-mediated calcium transport.

Lui et al. (186) have shown that 1,25(OH)\(_2\)D\(_3\) enhances calbindin-D in murine distal cells as it does in other species (8, 59, 66, 330). Friedman and Gesek (110) have reported that the MDCT cell line used here possesses calbindin-D. We have demonstrated that 1,25(OH)\(_2\)D\(_3\) increases Mg\(^{2+}\) entry rates in MDCT cells (Fig. 9). The response is concentration dependent, involves transcriptional processes involving de novo protein synthesis, and does not appear to be related to cAMP-mediated stimulation of Mg\(^{2+}\) uptake (G. Ritchie, L.-j. Dai, D. Kestan, H. S. Kang, L. Canaff, G. N. Hendy, and G. A. Quamme, unpublished observations). Finally, 1,25(OH)\(_2\)D\(_3\)-stimulated Mg\(^{2+}\) transport is additive to PTH-mediated uptake, suggesting that the peptide and steroid hormones regulate magnesium absorption through distinctive intracellular signaling pathways (Fig.
FIG. 9. 1,25(OH)2D3 and PTH stimulate Mg2+ entry into MDCT cells by separate mechanisms. Maximal effective concentrations of 1,25(OH)2D3 (10^{-7} M) and PTH (10^{-7} M) were added where indicated. cAMP was measured by radioimmunoassay and d[Mg^{2+}]/dt by fluorescence. Values are means ± SE for 4 or 5 preparations. *Significance (P < 0.01) of Mg2+ uptake rates. †Significance of cAMP concentrations following PTH or 1,25(OH)2D3 plus PTH compared with the respective control values. ‡Significance (P < 0.01) of Mg2+ uptake of 1,25(OH)2D3 vs. 1,25(OH)2D3 plus PTH. [Data from Dai et al. (68).]

9). These studies show that vitamin D3 metabolites modulate magnesium transport in the DCT.

Vitamin D3 administration has often been associated with increases in urinary magnesium and calcium excretion. Vitamin D3 metabolites increase intestinal absorption of magnesium and calcium so that a positive divalent cation balance may lead to hypermagnesemia and hypercalcemia and increased divalent cation urinary excretion over time. Vitamin D3 induces hypermagnesemia and hypercalcemia that diminishes magnesium absorption in the loop of Henle (254) and distal tubule (20, 254) through the extracellular Ca2+/Mg2+-sensing receptor (see sect. vi). This may also explain the increase or no change in urinary magnesium excretion after administration of vitamin D3.

The net effect on magnesium balance would thus depend on the relative magnitudes of vitamin D3 actions at the intestinal and renal levels.

C. Prostaglandins

PGE2 is the major arachidonic metabolite synthesized by cyclooxygenase in the mammalian kidney. PGE2 has a number of diverse actions on the kidney in addition to its ability to influence renal hemodynamics. PGE2 inhibits NaCl absorption within the thick ascending limb (313) and modulates sodium and water transport in the CCD (134, 135). On balance, prostaglandins are thought to be natriuretic by way of their actions on the thick ascending limb and CCD (18, 313). Three clearance studies concluded that arachidonic acid metabolites inhibit tubular reabsorption of calcium and magnesium resulting in increased urinary excretion (106, 282, 295). Schneider et al. (295) infused PGE2 into dog renal arteries and showed that calcium and magnesium excretion increased in association with a rise in urinary sodium excretion. Roman et al. (282) and Friedlander and Amiel (106) reported that meclofenamate or indomethacin infusion in rats decreased fractional magnesium excretion by ~40%. Again, the changes in urinary magnesium and calcium were associated with similar changes in sodium excretion. Because PGE2 inhibits NaCl absorption in the thick ascending limb, it may be expected that prostaglandins would increase calcium and magnesium excretion through diminished reabsorption in the loop (342). However, van Baal et al. (330) have shown that PGE2 stimulated calcium reabsorption in the rabbit CCD segment of the distal tubule. They reported that PGE2 stimulated net apical-to-basolateral calcium transport in CCD cells grown to confluence on permeable supports. PGE2 also stimulated cAMP formation in these cells, initially suggesting that protein kinase A-dependent pathways were involved (249). However, in a later preliminary report, these investigators reported that the changes in PGE2-stimulated calcium transport were not directly associated with cAMP formation so that other signaling pathways may be present in rabbit CCD cells (146). Finally, van Baal et al. (329) have shown that primary CCD cells produce endogenous prostaglandins that affect basal calcium transport. Like the CCD, the DCT synthesizes prostaglandins, principally PGE2 (35, 99). The above functions are mediated by four different prostaglandin receptors (EP1, EP2, EP3, and EP4) that are selectively located to the apical and/or basolateral epithelial membranes (42, 61, 135, 291, 317, 329). EP1 and EP3 subtypes mediate intracellular Ca2+ signaling and inhibition of adenylate cyclase, respectively, that result in inhibition of NaCl absorption within the thick ascending limb (313) and CCD (167, 155, 244) and AVP-stimulated water transport in the CCD (134). EP2 and EP4 subtypes are coupled to adenylate cyclase that upon stimulation enhances transepithelial calcium transport in the rabbit CCD (329). Moreover, these receptors may be colocalized to the same cell type but polarized to apical or basolateral membranes (135, 213, 329). Van Baal et al. (329) have shown that apical and basolateral PGE2 stimulate calcium absorption through EP2 and/or EP4 receptors, whereas activation of basolateral EP3 receptors inhibits basal and hormone-stimulated calcium transport. We have shown that PGE2 is a potent stimulator of Mg2+ uptake into MDCT cells (69). These actions are, in part, through cAMP-mediated mechanisms, but we were unable to determine the polarization of receptors because the immortalized MDCT cells used do not form tight junctions and are unlikely to be polarized (110). Accordingly, it is not known if the PGE2 effects in the DCT are due to luminal or basolateral prostaglandin. We infer from these results with MDCT cells that prostaglandins may...
modulate distal tubule magnesium transport and together with peptide and steroid hormones orchestrate renal magnesium conservation.

D. Insulin

Insulin has clearly been shown to have antinatriuretic and antimagnesiuric effects by its actions on the thick ascending limb of the loop of Henle (78, 79, 153, 163, 195). Among the tubular segments studied, insulin receptor binding is highest along the thick ascending limb and DCT so that by inference insulin may affect electrolyte transport within the DCT as well as the loop (52, 101, 105, 221). Insulin also stimulates sodium transport in A6 cells, which are a distal cell line from Xenopus laevis that have properties similar to the mammalian distal nephron (143, 205, 262). Insulin-stimulated sodium transport was partly inhibited by genistein, indicating tyrosine kinase was important but was independent on cAMP levels (262). Insulin did not increase cAMP formation in A6 cells, nor did adenylate cyclase inhibition diminish transport (205, 276). Because genistein did not completely inhibit insulin actions, Rodriguez-Commes et al. (276) suggested that parallel non-tyrosine kinase-dependent pathways were also involved. They showed that insulin actions in A6 cells required intracellular Ca\(^{2+}\) signaling, suggesting to these investigators that protein kinase C is needed for some of the responses. Clearly, the insulin-receptor signaling pathways are different in the various cell types studied. We have recently shown that insulin stimulates Mg\(^{2+}\) uptake in MDCT cells (77). Insulin stimulated Mg\(^{2+}\) entry in a concentration-dependent manner with maximal response of 214 ± 12 nM/s, which represented a 30 ± 5% increase in the mean uptake rate above control values, \(\frac{d([Mg^{2+}])}{dt}\), of 164 ± 5 nM/s. This was associated with a 2.5-fold increase in insulin-mediated cAMP generation, 52 ± 3 pmol-mg protein\(^{-1}\) min\(^{-1}\). Genistein, a tyrosine kinase inhibitor, diminished insulin-stimulated Mg\(^{2+}\) uptake, back to control values, 169 ± 11 nM/s, but did not change insulin-mediated cAMP formation, 47 ± 5 pmol-mg protein\(^{-1}\) min\(^{-1}\). The evidence is that insulin stimulates Mg\(^{2+}\) entry into MDCT cells through genistein-sensitive tyrosine phosphorylation. Additionally, insulin stimulates cAMP formation in MDCT cells and presumably activates protein kinase A (77). Maximal concentrations of PTH plus insulin increased cAMP levels and Mg\(^{2+}\) entry rates to a greater extent than each of the hormones alone (Fig. 10). Mandon et al. (195) have explained this potentiation of insulin with other peptide hormones as interactions at different sites along the established hormone-adenylate cyclase signaling system. The actions of insulin-mediated effects on peptides hormone responses are complicated because aldosterone had no effect on insulin actions but potentiated PTH-stimulated cAMP and Mg\(^{2+}\) uptake (77). Although the intracellular mechanisms are unclear, we infer from these studies that insulin plays a singular role in control of magnesium conservation and modulates hormonal regulation of magnesium transport within the distal tubule.

E. Other Hormones and Factors

1. \(\alpha\) - and \(\beta\)-adrenergic agonists

The nephron is richly innervated along its length from the glomerulus to the collecting tubule (22). Renal nerve stimulation significantly increases NaCl and water reabsorption in the proximal tubule, loop of Henle, and distal tubule (83, 113). Renal nerves also mediate calcium reabsorption through \(\alpha\)-adrenergic receptors (14). Gesek (113) has reported that MDCT cells possess \(\alpha\)-adrenergic receptors, and epinephrine or B-HT 933, an \(\alpha\)-agonist, stimulates Na\(^{+}\) uptake and Na\(^{+}\)-K\(^{+}\)-ATPase activity in this cell line. This response was dependent on protein kinase C activity and was associated with increases in phospholipase C and inositol 1,4,5-trisphosphate and diacylglycerol, but \(\alpha\)-agonists had no effect on basal or hormone-stimulated cAMP accumulation (113, 114). These findings are consistent with a pertussis toxin-insensitive mechanism. Interestingly, Gesek (113) was unable to observe an effect on Ca\(^{2+}\) uptake in MDCT cells. The actions of \(\alpha\)-adrenergic agonists on Mg\(^{2+}\) transport in the DCT have not been studied.
β-Adrenergic agonists also mediate direct effects on tubular transport (22, 211). Although β-adrenergic agents have been shown to increase magnesium absorption in the thick ascending limb, no experiments have been directed at the distal tubule (18). Gesek and White (119) have demonstrated that MDCT cells possess \( \beta_1 \) and \( \beta_2 \) receptor subtypes that upon activation with isoproterenol elicited marked increases in cAMP formation (119). Recently, we tested the effects of β-adrenergic receptor activities on Mg\(^{2+}\) uptake in Mg\(^{2+}\)-depleted MDCT cells. Isoproterenol increased Mg\(^{2+}\) entry by 18 ± 4% and cAMP formation 3.2-fold above control values. Although not tested, β-adrenergic activation may stimulate Mg\(^{2+}\) uptake through cAMP-mediated pathways (119). We infer from these studies with MDCT cells that renal nerves and circulating cateholamines may play a role in control of magnesium transport in the distal tubule.

2. Other endocrine and paracrine factors

In addition to the hormones indicated above, others may be involved in regulation of magnesium reabsorption within the DCT including kinins and growth factors (239). Hoenderop et al. (147) have reported that adenosine increases Ca\(^{2+}\) reabsorption in rabbit CNT and CCD cells. Adenosine responses were through A\(_1\) receptor-mediated pathways involving activation of phospholipase C. Stimulation of Ca\(^{2+}\) transport was independent of cAMP and protein kinase C activity, suggesting that an additional unidentified signaling pathway may be involved in adenosine responses. Based on these observations, we tested whether adenosine stimulates Mg\(^{2+}\) uptake in MDCT cells. Adenosine did not have any effect on Mg\(^{2+}\) increase in MDCT cells but stimulated cAMP formation, indicating the presence of A\(_2\) receptors acting through G\(_s\) coupling. There was no evidence for A\(_1\) receptors in this cell line. Additionally, DCT cells, including MDCT cells, have P\(_2\) purinogenic receptors that modulate distal hormone-sensitive calcium transport (167). Finally, ANP receptors are present in the DCT and MDCT cells, but the effects on magnesium transport have not been studied (Dai et al., unpublished observations). ANP stimulates calcium reabsorption in rabbit CCD through cGMP-dependent protein kinase type III (148).

F. Hormonal Regulation and Renal Mg\(^{2+}\) Handling

Micropuncture and microperfusion studies and experiments with isolated cells have shown that many hormones regulate magnesium absorption in the distal tubule. Interestingly, with the exception of prostaglandins, all of these hormones and factors also increase magnesium transport in the thick ascending limb of the loop of Henle (285). Accordingly, these hormone-mediated responses are serially organized to similarly regulate magnesium absorption in both the loop and distal tubule. This organization may be of benefit because influences acting in either the loop or distal tubule may be modified by changes in the other segment.

The interactions of the various peptide and steroid hormones, prostaglandins, and renal innervations are complex (251, 285). It can be inferred that overall distal magnesium absorption is controlled by all of these influences initiated individually but coming together through shared intracellular signaling pathways. Few studies have been directed at describing these interactions. Clearly, control of renal magnesium handling is important enough to warrant multiple hormonal control.

VI. EXTRACELLULAR CALCIUM/MAGNESIUM-SENSING RECEPTORS IN THE DISTAL CONVOLUTED TUBULE

An extracellular Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor has recently been shown to be expressed along the entire length of the nephron, particularly the loop of Henle, DCT, and inner medullary collecting duct (267, 268, 349). This receptor is very similar to the one expressed in the parathyroid gland (44, 45). It comprises three major domains: 1) a large extracellular amino-terminal domain consisting of 613 amino acids, which is thought to possess cationic binding sites; 2) a 250-amino acid domain with 7 predicted membrane-spanning segments characteristic of the G protein-coupled receptor family; and 3) a carboxy-terminal domain of 222 amino acids that likely resides in the cytoplasm and is involved with intracellular signaling processes (44). The evidence is that elevated plasma Ca\(^{2+}\) or Mg\(^{2+}\) binds to the extracellular domain of the receptor and initiates a number of intracellular signals. Among these is stimulation of \( G_\alpha \) proteins that modulate adenyl cyclase activity and cAMP levels and \( G_\beta \gamma \) proteins that activate phospholipase C releasing inositol 1,4,5-trisphosphate and cytosolic Ca\(^{2+}\) (44). Hebert and co-workers (136, 292, 336) postulate that elevated plasma calcium or magnesium and activation of the Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor leads to diminished salt (sodium, calcium and magnesium) transport within the loop and water absorption within the inner medullary collecting duct. This results in an increase in urinary water flow in addition to calcium and magnesium excretion, minimizing the opportunity of stone formation. Riccardi et al. (266) and Yang et al. (349) have shown that the Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptors are also present on the basolateral membrane of the DCT. Ca\(^{2+}\)/Mg\(^{2+}\)-mediated intracellular signaling may have important effects on distal cellular function including sodium, potassium, and divalent cation transport (136, 228).

We have recently shown that MDCT cells possess a Ca\(^{2+}\)/Mg\(^{2+}\)-sensing mechanism(s) that is equally sensitive.
to extracellular Ca\(^{2+}\) and Mg\(^{2+}\) concentrations normally found in the plasma (21). Figure 11 summarizes the comparative effects of extracellular Mg\(^{2+}\) and Ca\(^{2+}\) on glucagon-mediated intracellular cAMP accumulation. The half-maximal effective concentrations were 0.5 mM Mg\(^{2+}\) and 1.5 mM Ca\(^{2+}\), which are consistent with normal plasma concentrations. This is unlike the observations with other cells where extracellular Ca\(^{2+}\) has been found to be a more potent stimulator of Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor-induced intracellular signaling than external Mg\(^{2+}\). The threshold value for extracellular Ca\(^{2+}\) has been reported to be on the order of 1–5 mM for renal cells, whereas a similar cytosolic Ca\(^{2+}\) response requires as much as 5–20 mM Mg\(^{2+}\) (241, 261). These relative potencies of extracellular Ca\(^{2+}\) and Mg\(^{2+}\) recapitulate their actions in bovine parathyroid cells and in Xenopus oocytes injected with cRNA and HEK 293 cells transfected with DNA of the cloned Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor (43, 55, 222). Thus it was of interest that the polyvalent cation-sensitive mechanism of MDCT cells was apparently as sensitive to extracellular Mg\(^{2+}\) as it was to Ca\(^{2+}\). This is particularly noteworthy because the Mg\(^{2+}\) studies were performed in the presence of normal or elevated Ca\(^{2+}\) (21). The functional consequences of changes in the amino acid sequence of the Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor have been investigated by expressing a variety of mutated receptors in HEK 293 cells (13). Some of the mutations diminish Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor signaling, others enhance the sensitivity to external Ca\(^{2+}\), and still others are completely nonfunctional with no intracellular signaling as determined by changes in intracellular Ca\(^{2+}\) or inositol phosphate (12, 133, 241). Bräuner-Osborne et al. (41) observed that the EC\(_{50}\) for Mg\(^{2+}\) significantly decreased (4.7 ± 0.1 to 2.6 ± 0.4 mM), whereas that for Ca\(^{2+}\) increased (3.2 ± 0.1 to 3.3 ± 0.2 mM) after mutations of Ser\(^{147}\) and Ser\(^{170}\), which are located in the amino-terminal domain and are involved in the agonist binding (41). From these transfection studies of the mutated Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor, it has been suggested that discrete but interrelated cation binding sites may be a feature of this receptor (12). These discrete binding sites remain to be identified, but it is apparent from these studies that changes in the extracellular domain of the Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor may alter its sensitivity to the various ligands (12). Subtle changes in the Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor may significantly affect its selectivity. The present studies with the endogenous polyvalent cation-sensitive mechanism of the MDCT cell show that the intracellular signaling is responsive to both extracellular Mg\(^{2+}\) and Ca\(^{2+}\) and that there appears to be little interaction between these cations. Accordingly, the Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor may function as a Mg\(^{2+}\)-sensing receptor even in the presence of relatively high concentrations of extracellular Ca\(^{2+}\) (20, 21). The sites involved in binding Mg\(^{2+}\) and Ca\(^{2+}\) and the cooperative association in intracellular signaling remain to be determined (261). Alternatively, the results of our studies may indicate the presence of separate receptors for extracellular Ca\(^{2+}\) and Mg\(^{2+}\) in MDCT cells (265). Homologous or heterologous receptor interactions may also modify cation selectivity (14, 15, 258, 229). Again further research is needed to clarify our understanding of the
A. Ca\textsuperscript{2+}/Mg\textsuperscript{2+} Sensing Modulates Peptide Hormone Responses at the Receptor Level

Activation of the MDCT Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-sensing receptor with polyvalent cations, such as neomycin, Mg\textsuperscript{2+}, or Ca\textsuperscript{2+}, abolished PTH-, calcitonin-, glucagon-, and AVP-stimulated cAMP accumulation (Fig. 11). Moreover, activation of Mg\textsuperscript{2+}/Ca\textsuperscript{2+} sensing inhibits hormone-stimulated Mg\textsuperscript{2+} uptake into MDCT cells (20). Figure 12 illustrates the effect of neomycin, a polyvalent cation, on PTH-mediated cAMP formation and Mg\textsuperscript{2+} entry. Accordingly, hormone-mediated Mg\textsuperscript{2+} uptake is dependent on the prevailing extracellular cation concentrations. To test this notion, we determined the magnesium concentration dependence of Mg\textsuperscript{2+} uptake rates with and without glucagon (Fig. 13). Glucagon stimulated Mg\textsuperscript{2+} uptake in MDCT cells at extracellular Mg\textsuperscript{2+} concentrations below ~1.5 mM; concentrations above these levels lead to inhibition of hormone-mediated transport. These recent observations with established cell lines are consistent with earlier micropuncture and microperfusion studies of intact distal tubules. Le Grimellec et al. (177, 178) reported that distal tubules from proximal and distal tubules of hypercalcemic and hypermagnesemic parathyroid gland-intact rats, inferring that magnesium reabsorption is diminished with elevation of extracellular Ca\textsuperscript{2+} and Mg\textsuperscript{2+} concentrations. We perfused superficial distal tubules of TPTXed rats with buffer solutions containing variable calcium and magnesium concentrations. As indicated above, net calcium and magnesium reabsorption was dependent on electrolyte delivery to this segment as the fractional reabsorption remained constant at 59 ± 3 and 94 ± 5\%, respectively (248, 252, 254). However, when these studies were performed on hypermagnesemic (plasma magnesium, 3.6 ± 0.2 mM) and hypercalcemic (plasma calcium, 4.2 ± 0.04 mM) rats, the fractional absorption of magnesium was significantly decreased 6 ± 3 and 14 ± 7\%, respectively (246, 254). Similar findings were observed with calcium absorption in the distal tubule (246, 254). It is apparent from these studies that plasma Mg\textsuperscript{2+} and Ca\textsuperscript{2+} control distal absorption of both calcium and magnesium. Our initial studies also showed that significant elevation of luminal magnesium concentration is also associated with diminished fractional magnesium absorption, 24 ± 2\% (see elevated luminal Mg\textsuperscript{2+} concentrations of Fig. 4; data from Ref. 254). Although immunocytochemistry demonstrates that Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-sensing receptors are located on the basolateral membrane (266), these results suggest that there are Mg\textsuperscript{2+}/Ca\textsuperscript{2+}-sensing receptors on the apical or luminal membrane as well. These recent findings demonstrate an additional receptor-mediated process that is involved with regulation of magnesium reabsorption in the DCT (156).
B. Ca\(^{2+}/Mg^{2+}\) Sensing Modulates Steroid Hormone Responses at the Transcriptional Level

The Ca\(^{2+}/Mg^{2+}\)-sensing receptor modifies steroid hormone responses at the transcriptional level as well as at the level of receptor-coupled signaling. Elevation of extracellular Ca\(^{2+}\) or Mg\(^{2+}\) inhibits aldosterone potentiation of peptide hormone (PTH, calcitonin, glucagon, and AVP)-stimulated intracellular cAMP formation in MDCT cells (20, 21). The acute cellular action of Ca\(^{2+}/Mg^{2+}\) sensing is through stimulation of G\(_i\) proteins, decreased receptor-mediated adenylate cyclase activity, and diminished cAMP levels as reviewed above (21). However, we have evidence that elevated extracellular Ca\(^{2+}\) and Mg\(^{2+}\) may also inhibit adrenocorticoid responses at the transcriptional level. Actinomycin and cycloheximide, inhibitors of protein synthesis, abolish aldosterone potentiation of PTH-stimulated cAMP formation and Mg\(^{2+}\) uptake, providing evidence for transcriptional involvement. The prominent mechanism of steroids, which operate through nuclear receptors, is to control transcriptional regulation, expression, and posttranslational targeting of heterotrimeric G proteins (218). Changes in the levels of expression of G protein subunits in adrenalectomized animals reflect changes in subunit mRNA, suggesting that adrenocorticoids activate genes encoding G\(_{\alpha_s}\), G\(_{\alpha_i}\), G\(_\beta\), G\(_\gamma\), and phospholipase C (PLC)-\(\beta\). Adrenal steroids may also increase adenylate cyclase signaling, in the absence of changes in G\(_{\alpha_s}\) and G\(_{\alpha_i}\), suggesting regulation of the expression of the adenylate cyclase itself (218). In a recent study, we show that incubation of MDCT cells with high extracellular Ca\(^{2+}\) with the aldosterone inhibits aldosterone-potentiated PTH-induced cAMP formation and Mg\(^{2+}\) uptake (Ritchie et al., unpublished observations). The evidence indicates that chronic Ca\(^{2+}/Mg^{2+}\) sensing inhibits transcriptional/translational processes involved in the expression of new G protein subunits. The mechanisms by which the Ca\(^{2+}/Mg^{2+}\)-sensing receptor communicates with the nuclear machinery is not known. These studies strongly support the notion that elevated extracellular divalent cations can modulate expression of mineralocorticoid-dependent G proteins (transcriptional level) involved with magnesium transport as well as directly controlling PTH-mediated (receptor level) magnesium transport.

As reviewed above, vitamin D metabolites enhance Mg\(^{2+}\) uptake in MDCT cells through a genomic mechanism involving transcriptional/translational processes requiring 3–4 h after addition of the hormone (Ritchie et al., unpublished observations). Elevation of extracellular Ca\(^{2+}\) mitigates 1,25(OH)\(_2\)D\(_3\) stimulation, indicating that the Ca\(^{2+}/Mg^{2+}\)-sensing receptor may modify gene expression in MDCT cells (Fig. 14). To determine if elevated extracellular Ca\(^{2+}\) inhibited 1,25(OH)\(_2\)D\(_3\) responses through the Ca\(^{2+}/Mg^{2+}\)-sensing receptor, we preincubated the cells with a specific antibody, ADD (98), to the receptor protein before treating them with high Ca\(^{2+}\) and 1,25(OH)\(_2\)D\(_3\) (Fig. 14). The antibody prevented the effect of Ca\(^{2+}\) on 1,25(OH)\(_2\)D\(_3\)-induced Mg\(^{2+}\) uptake, clearly demonstrating a receptor-mediated response (Ritchie et al., unpublished observations). Accordingly, excess 1,25(OH)\(_2\)D\(_3\) increases distal Ca\(^{2+}\) and Mg\(^{2+}\) transport leading to elevated serum concentrations and activation of Ca\(^{2+}/Mg^{2+}\) sensing that provides a negative feedback on divalent cation reabsorption.

C. Ca\(^{2+}/Mg^{2+}\) Sensing Modulates Other Hormone Responses

The Ca\(^{2+}/Mg^{2+}\)-sensing mechanism has significant effects on the responses of other hormones. Elevation of extracellular Ca\(^{2+}/Mg^{2+}\) also completely inhibited PGE\(_2\) stimulation of Mg\(^{2+}\) uptake in MDCT cells but marginally decreased PGE\(_2\)-mediated cAMP (69). Hartle et al. (132) have reported that polyvalent cations inhibit PGE\(_2\)-stimulated cAMP production in MC3T3-E1 osteoblasts. Accordingly, elevation of extracellular Mg\(^{2+}\) and Ca\(^{2+}\) may have important effects on prostaglandin actions in many cell types including the renal epithelium. To determine if activation of Ca\(^{2+}/Mg^{2+}\) sensing alters insulin actions, we pretreated MDCT cells for 5 min with neomycin before the addition of insulin and MgCl\(_2\). Neomycin did not affect insulin-mediated cAMP formation but diminished insulin-
stimulated Mg\(^{2+}\) uptake (77). The mechanisms by which the Ca\(^{2+}/Mg^{2+}\)-sensing receptor inhibits insulin actions remain undefined. The Ca\(^{2+}/Mg^{2+}\)-sensing receptor is coupled to Go\(_i\) and Go\(_{\alpha}\) proteins that may interact with insulin-mediated signaling pathways (20, 21). It is now clear that Ca\(^{2+}/Mg^{2+}\) sensing plays a significant role in modifying hormone-mediated Mg\(^{2+}\) transport within MDCT cells.

VII. INTRINSIC CONTROL OF MAGNESIUM
TRANSPORT IN THE DISTAL
CONVOLUTED TUBULE

In addition to hormone and Mg\(^{2+}/Ca^{2+}\)-sensing receptor controls described in sections V and VI, the distal tubule is able to regulate Mg\(^{2+}\) uptake in response to diminished extracellular magnesium (298). Dietary magnesium restriction or intestinal magnesium malabsorption is associated with enhanced renal magnesium reabsorption and decreased magnesium excretion (89, 250, 302). Micropuncture studies showed that the increase in magnesium conservation was due to enhanced reabsorption within the distal tubule (252, 298). Distal calcium and sodium reabsorption were not affected. Isolated distal tubule cells, either Madin-Darby canine kidney (MDCK) or MDCT, cultured in magnesium-free media increase their Mg\(^{2+}\) transport rate as determined by microfluorescence. This response is rapid (within 1–2 h) and specific for magnesium because there was no effect on sodium or calcium transport (76, 253). The “adaptation” of magnesium transport rates is intrinsic because there were no hormones in the culture media (Fig. 1). Furthermore, the adaptation was dependent on the concentration of media magnesium and the length of time in the culture media. Pretreatment of the MDCT cells with cycloheximide inhibited this adaptation by ~50\% (unpublished observations). Accordingly, it was concluded that magnesium transport is controlled by gene(s) that somehow respond to extracellular magnesium. Other genetically controlled mechanisms involved in intrinsic regulation of magnesium uptake remain to be identified. PCR-differential display identified one gene (termed the magnesium-responsive gene, MRG) that was upregulated by Mg\(^{2+}\) depletion (271). Upregulation was evident after 30 min and maximum (20-fold increase) after 4 h. Readdition of magnesium to Mg\(^{2+}\)-depleted cells resulted in the return of MRG levels to control levels within 4 h. MRG upregulation was specific to Mg\(^{2+}\) depletion because Ca\(^{2+}\), K\(^{+}\), and phosphate depletion of MDCK cells failed to upregulate the MRG (272). A 15-bp antisense oligonucleotide (ODN) complementary to the MRG inhibited Mg\(^{2+}\) entry into Mg\(^{2+}\)-depleted MDCK cells (48 ± 10 nM/s) compared with cells transfected with randomized noncomplementary ODNs (231 ± 15 nM/s). The results suggest that the gene product of MRG may be important in adaptation of cells to Mg\(^{2+}\) levels. In summary, these studies with isolated distal cells support the notion of intrinsic controls within the cells that adapt their magnesium transport rate appropriately to the environmental magnesium (250). We postulate that this intrinsic adaptation provides the discriminatory control of magnesium transport independent of sodium and calcium. Intrinsic adaptation provides the selective control that hormonal regulation does not have.

VIII. DISTAL DIURETICS THAT ENHANCE
MAGNESIUM ABSORPTION IN THE
DISTAL CONVOLUTED TUBULE

A. Amiloride

A large number of clinical studies have led to the notion that amiloride possesses magnesium-conserving properties in addition to its natriuretic and potassium-sparing effects (48, 251, 340). Despite these observations, very few experimental studies have been published concerning amiloride effects on renal magnesium handling. Devane and Ryan (81) have shown that infusion of amiloride reduced the fractional excretion of magnesium in anesthetized rats, which they attributed to a direct renal action of the drug (81). The nephron segments and cellular mechanisms were not delineated in this study.

We determined the cellular effects of amiloride on Mg\(^{2+}\) uptake in isolated MDCT cells (76). Amiloride stimulated nifedipine-sensitive Mg\(^{2+}\) influx by 41 ± 3\% (Fig.

---

**FIG. 15.** Activation of Mg\(^{2+}/Ca^{2+}\) sensing does not alter amiloride-stimulated Mg\(^{2+}\) uptake. Mg\(^{2+}\) uptake, d([Mg\(^{2+}\)]\(_i\))/dt, was performed in the presence of 1.5 mM MgCl\(_2\) with or without 10 \(\mu\)M amiloride. Neomycin (50 \(\mu\)M) was added where indicated 5 min before the addition of amiloride. Values are means ± SE for 3–6 cells. *Significance (P < 0.01) from control values. There were no significant changes between the values with amiloride and those with neomycin plus amiloride. [Data from Bapty et al. (20).]
Because amiloride does not stimulate Mg$^{2+}$ uptake in the absence of a change in voltage, we conclude that this diuretic acts through hyperpolarization of the membrane voltage (76). As expected, activation of extracellular Mg$^{2+}$/Ca$^{2+}$ sensing does not influence amiloride-stimulated Mg$^{2+}$ uptake (Fig. 15). These findings provide the basis for both clinical and experimental observations that show that amiloride has magnesium-conserving properties (50, 81, 220, 339).

**B. Chlorothiazide**

A number of experimental studies have suggested that acute administration of chlorothiazide decreases urinary magnesium excretion relative to the diuretic effects of increased sodium excretion. Duarte (86) acutely administered chlorothiazide to parathyroid-intact dogs undergoing a diuresis. Fractional excretion of sodium (FE$_{Na}$) markedly increased from 1.3 to 7.9%, but the fractional excretion of magnesium (FE$_{Mg}$) remained unchanged, 7.3%, after chlorothiazide administration. In these studies, fractional calcium excretion (FE$_{Ca}$) increased from 1.5 to 3.1%. Relative to the FE$_{Na}$, magnesium excretion fell sixfold from 5.6 to 0.9, and calcium excretion decreased from 1.2 to 0.4 after chlorothiazide administration. Eknoyan et al. (90) confirmed these studies and attributed the observation of marked natriuresis and modest magnesuria and calciumuria to the differential reabsorption of these cations in the loop and distal tubule. These studies suggest that chlorothiazide may increase tubular reabsorption of magnesium relative to the natriuretic effects. We performed clearance and micropuncture studies in TPTXed dogs (257). The acute administration of chlorothiazide resulted in a relatively greater increase in FE$_{Na}$ from 0.5 to 5.6% than calcium (FE$_{Ca}$/FE$_{Na}$ increased from 1.3 to 1.9) or magnesium (FE$_{Mg}$/FE$_{Na}$ fell from 1.4 to 1.3). Similar results were also observed in TPTXed hamsters, where FE$_{Na}$ increased from 1.0 to 3.4% while FE$_{Ca}$/FE$_{Na}$ decreased from 22.7 to 5.0 and FE$_{Mg}$/FE$_{Na}$ fell from 19.3 to 5.0 (347). Micropuncture experiments demonstrated that this action occurred along the distal tubule. Micropuncture studies of the hamster have also shown that chlorothiazide increases magnesium reabsorption relative to sodium in the distal tubule (345). Accordingly, the acute administration of chlorothiazide produces a significant natriuresis together with little or no change in urinary calcium or magnesium excretion. The results of these studies clearly demonstrate a divergence of sodium absorption with calcium and magnesium transport within the DCT.

Studies with isolated MDCT cells have shown that chlorothiazide increases Mg$^{2+}$ uptake in a dose-dependent fashion. Maximal concentrations ($10^{-4}$ M) of chlorothiazide increased Mg$^{2+}$ transport by 58% (70). This was associated with hyperpolarization of the plasma membrane voltage from $-65 \pm 5$ to $-80 \pm 5$ mV. Inhibition of Na$^+$/Cl$^-$ cotransport leading to diminished intracellular sodium and chloride concentration results in hyperpolarization of the apical membrane of the DCT (67, 316, 329) and MDCT cells (115). An increase in the membrane voltage enhances Mg$^{2+}$ uptake into MDCT cells (Fig. 2). Accordingly, chlorothiazide appears to stimulate Mg$^{2+}$ transport through changes in the membrane voltage similar to that seen with amiloride. These studies with MDCT cells demonstrate that chlorothiazide enhances Mg$^{2+}$ entry in DCT cells that may be translated into an increase in magnesium reabsorption and diminished urinary magnesium excretion.

**IX. FAMILIAL DISORDERS AFFECTING DISTAL MAGNESIUM TRANSPORT**

Familial diseases, resulting from single gene mutations, are interesting because they demonstrate the phenotypic diversity of transport physiology (Table 2). Moreover, the variation of genetic diseases suggests ways of magnesium transport that have not been heretofore envisioned. An example of the application of genetic studies to the clarification of our understanding of renal magnesium handling is the recent investigations of the familial disease “hypomagnesemia associated with hypercalciuria and nephrocalcinosis” (HHN). This disease is an autosomal recessive disorder that presents early in childhood. It is characterized by severe renal magnesium wasting resulting in persistent hypomagnesemia and marked hypercalciuria leading to nephrocalcinosis (198, 212, 223, 224, 269, 325, 328). Through linkage studies, Simon et al. (308) have identified the gene responsible for this disease. This gene termed “paracellin-1” or “claudin 16” encodes a protein of 305 amino acids that is a member of the claudin family comprising proteins such as occludins that form TIGHT JUNCTIONS.

<table>
<thead>
<tr>
<th>TABLE 2. Familial disorders affecting distal magnesium transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypomagnesemia associated with abnormal renal NaCl transport</td>
</tr>
<tr>
<td>Gitelman syndrome</td>
</tr>
<tr>
<td>Inherited disorders associated with abnormal extracellular Mg$^{2+}$/Ca$^{2+}$ sensing</td>
</tr>
<tr>
<td>Autosomal dominant hypoparathyroidism</td>
</tr>
<tr>
<td>Familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism</td>
</tr>
<tr>
<td>Primary inherited disorders of distal magnesium transport</td>
</tr>
<tr>
<td>Hypomagnesemia with secondary hypercalcemia</td>
</tr>
<tr>
<td>Infantile primary hypomagnesemia with autosomal dominant inheritance</td>
</tr>
<tr>
<td>Infantile primary hypomagnesemia with autosomal recessive inheritance</td>
</tr>
</tbody>
</table>
the tight junctions of epithelial paracellular pathways. Claudin 16 is found in the paracellular regions of medullary and cortical segments of the thick ascending limb and the DCT. Simon et al. (308) propose that claudin 16 is the protein involved in controlling magnesium and calcium permeability of the cortical thick ascending limb. Mutations in this gene presumably result in abnormal permeability of the paracellular pathway leading to decreased magnesium and calcium reabsorption. These studies explain the earlier microperfusion data of Mandon et al. (197), who demonstrated that magnesium absorption within the cortical thick ascending limb was passive and paracellular in nature so that it is influenced by transepithelial voltage and the permeability of the paracellular pathway. The permeability of the paracellular pathway is presumably determined by electrostatic charges of proteins comprising this route (255). De Rouffignac and co-workers (84, 285) have postulated that these paracellular charge complexes may be influenced by hormones that regulate magnesium absorption. Accordingly, genetic diseases involving defective claudin 16 expression are associated with diminished magnesium absorption. The recent work of Simon et al. (308) is a good example of the use of genetic studies in addressing constitutive and congenital disturbances of magnesium metabolism to further our understanding of the physiology of cellular magnesium transport. These investigations may also lead to other avenues of research. Claudin 16 comprises the paracellular pathway of the medullary thick ascending limb and the DCT, but no magnesium is absorbed through the paracellular pathways in these segments. It remains to be determined what physiological role, if any, this protein plays in these segments. Finally, these observations led to speculation of other proteins that may play a role in paracellular magnesium absorption.

A. Hypomagnesemia Associated With Abnormal Renal NaCl Transport

Gitelman syndrome and Bartter syndrome are two autosomal recessive disorders of renal electrolyte transport that have been associated with hypokalemia due to renal potassium loss, chloride-resistant metabolic alkalosis, and elevated plasma renin and aldosterone levels but normal blood pressure (24, 124). The cellular basis for Gitelman and Bartter syndromes is diminished NaCl absorption in the distal tubule and Henle’s loop, respectively (307). Renal magnesium wasting and hypomagnesemia is a distinctive characteristic of Gitelman syndrome, whereas it is not a common feature of Bartter syndrome (30, 124, 278).

Bartter syndrome can be distinguished from Gitelman syndrome on the basis of the clinical presentation and biochemical profile (28, 29, 30, 160, 290, 311, 320, 326–328). Patients with infantile Bartter syndrome characteristically present in infancy with a urinary concentrating defect, polyhydramnios, failure to thrive, and fasting hypercalciuria leading to medullary nephrocalcinosis (80, 280, 338). Those with classic Bartter syndrome present later in childhood with features of water and salt depletion, including polydipsia, polyuria, and episodes of dehydration. Bartter syndrome patients fail to respond normally to furosemide, suggesting to investigators that this disease was due to defective loop function (123, 164, 166, 280, 297). Using family linkage studies, Simon and co-workers (304–306) delineated three genetic defects that form the basis for distinguishing three distinct physiological phenotypes in these patients (Table 3). Type I Bartter syndrome is due to defective Na⁺-K⁺-Cl⁻ cotransport (NKCC2 gene) and characterized by severe hypokalemia in addition to the above (305). Type II Bartter syndrome is associated with mutations in a potassium channel (ROMK gene, 30-pS K⁺ channel) activity (305). Because this K⁺ channel is involved in potassium secretion in the CCD, hypokalemia is less severe in this form of the disease. The type III Bartter syndrome is based on a mutation of the basolateral membrane chloride channel (CICNKB) (306). Like type I patients, type III patients have severe hypokalemia, but unlike type I and type II phenotypes, type III patients usually have normal calcium excretion, and nephrocalcinosis is not observed. It has been suggested that up to 30% of Bartter patients may have hypomagnesemia due to renal magnesium wasting (173, 297). However, it is clear that some of these cases represent a form of Gitelman syndrome, and others may be another variant of hypokalemic metabolic alkalosis. In addition, hypomagnesemia does not reliably segregate to any of the three types of Bartter syndrome defined by molecular studies, suggesting that other circumstances may affect renal magnesium absorption. Supporting this notion is evidence that magnesium balance is normal in a Na⁺-K⁺-Cl⁻ cotransport knockout mouse mimicking type I Bartter syndrome (323). Chronic usage of furosemide is sometimes associated with hypomagnesemia due to excessive urinary magnesium excretion, but it is not universal (251). Thus it is not apparent why most individuals with Na⁺-K⁺-Cl⁻ cotransport defects should be comparatively free of renal magnesium wasting. It may be that the loop and distal tubule adapts to conserve magnesium in this disorder as it does for NaCl (156, 157, 190, 285). Bartter syndrome typifies the serial control of magnesium conservation in the loop and distal tubule. Distal tubular function compensates for diminished loop absorption even though the hormonal controls are similar in the two nephron segments.
1. Gitelman syndrome

Patients with Gitelman syndrome are not polyuric or polydipsic but have hypocalciuria and usually show renal magnesium wasting (236). They often present in late childhood with a hypokalemic metabolic alkalosis and low serum magnesium, which may be asymptomatic or may be severe enough to cause hypomagnesemic tetany (27, 158, 173). Patients with Gitelman syndrome fail to respond to chlorothiazide, leading to the prediction that the renal defect is in the DCT (26, 62, 214, 320). Simon et al. (309) have shown that Gitelman syndrome families are genetically linked to a locus at 16q13 and identified causative mutations in the chlorothiazide-sensitive NaCl cotransporter expressed in the DCT (Fig. 16) (309). There have now been over 82 distinct mutations that are associated with Gitelman syndrome. Nearly all of these mutations are nonconservative and affect amino acids that have been conserved throughout evolution. Schulteis et al. (296) developed a mouse model of Gitelman syndrome by deleting the gene coding for the apical NaCl cotransporter expressed in the DCT (26, 62, 214, 320). Simon et al. (309) have shown that Gitelman syndrome families are genetically linked to a locus at 16q13 and identified causative mutations in the chlorothiazide-sensitive NaCl cotransporter expressed in the DCT (Fig. 16) (309). There have now been over 82 distinct mutations that are associated with Gitelman syndrome. Nearly all of these mutations are nonconservative and affect amino acids that have been conserved throughout evolution. Schulteis et al. (296) developed a mouse model of Gitelman syndrome by deleting the gene coding for the apical NaCl cotransporter of the DCT. These mice show all the cardinal features of Gitelman syndrome including renal calcium conservation and diminished serum magnesium concentrations presumably due to urinary magnesium wasting, although the latter was not directly determined in this study. Because chlorothiazide enhances calcium reabsorption in the DCT, the hypocalciuria of Gitelman syndrome is readily explained (278). The reasons for renal magnesium wasting are unknown (251).

Some of the features of Gitelman syndrome may be observed in patients chronically receiving thiazide diuretics raising the possibility that loss in \( \text{Na}^{+}\text{-Cl}^{-} \) cotransport could result in this disease. Although renal magnesium wasting and hypomagnesemia in Gitelman patients remain to be explained, a number of mechanisms may be invoked. We have shown that cellular potassium depletion diminishes \( \text{Mg}^{2+} \) uptake in MDCT cells (70, see below). However, potassium deficiency is not always associated

---

**TABLE 3. Inherited disorders of renal magnesium handling**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Chromosome</th>
<th>Selectivity</th>
<th>OMIM No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gitelman</td>
<td>Autosomal</td>
<td>SLC12A3</td>
<td>16q13</td>
<td>Mg( ^{2+} )</td>
<td>263800</td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td></td>
<td></td>
<td>Mg( ^{2+} )</td>
<td>600968</td>
</tr>
<tr>
<td>Bartter1</td>
<td>Autosomal</td>
<td>NKCC2</td>
<td>15q15-21</td>
<td>Ca( ^{2+} )/Mg( ^{2+} )</td>
<td>241200</td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td></td>
<td></td>
<td>Ca( ^{2+} )/Mg( ^{2+} )</td>
<td>600830</td>
</tr>
<tr>
<td>Bartter2</td>
<td>Autosomal</td>
<td>ROMK1</td>
<td>11q24</td>
<td>Mg( ^{2+} )</td>
<td>600350</td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td></td>
<td></td>
<td>Mg( ^{2+} )</td>
<td>600178</td>
</tr>
<tr>
<td>Bartter3</td>
<td>Autosomal</td>
<td>CICNKB</td>
<td>1p36</td>
<td>Mg( ^{2+} )</td>
<td>602023</td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td></td>
<td></td>
<td>Mg( ^{2+} )</td>
<td>603050</td>
</tr>
<tr>
<td>Hypomagnesemia, hypercalciuria, and nephrocalcinosis</td>
<td>Autosomal</td>
<td>PCLN1</td>
<td>2q27</td>
<td>Ca( ^{2+} )/Mg( ^{2+} )</td>
<td>603050</td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td>CLDN16</td>
<td></td>
<td>Ca( ^{2+} )/Mg( ^{2+} )</td>
<td>603050</td>
</tr>
<tr>
<td>Inactivating Ca( ^{2+} )/Mg( ^{2+} )-sensing “FHH/NSHPT”</td>
<td>Autosomal</td>
<td>( \text{Ca}^{2+}/\text{Mg}^{2+} )-SR</td>
<td>3q13.3-21</td>
<td>Ca( ^{2+} )/Mg( ^{2+} )</td>
<td>601198</td>
</tr>
<tr>
<td></td>
<td>Dominant</td>
<td></td>
<td></td>
<td>Ca( ^{2+} )/Mg( ^{2+} )</td>
<td>601198</td>
</tr>
<tr>
<td>Activating Ca( ^{2+} )/Mg( ^{2+} )-sensing “ADH”</td>
<td>Autosomal</td>
<td>( \text{Ca}^{2+}/\text{Mg}^{2+} )-SR</td>
<td>11q23</td>
<td>Mg( ^{2+} )</td>
<td>601190</td>
</tr>
<tr>
<td></td>
<td>Dominant</td>
<td></td>
<td></td>
<td>Mg( ^{2+} )</td>
<td>601190</td>
</tr>
<tr>
<td>Late-onset, isolated hypomagnesemia</td>
<td>Autosomal</td>
<td>?</td>
<td>9q12-22.2</td>
<td>Ca( ^{2+} )/Mg( ^{2+} )</td>
<td>600950</td>
</tr>
<tr>
<td></td>
<td>Dominant</td>
<td></td>
<td></td>
<td>Ca( ^{2+} )/Mg( ^{2+} )</td>
<td>600950</td>
</tr>
<tr>
<td>Late-onset, isolated hypomagnesemia</td>
<td>Autosomal</td>
<td>?</td>
<td>Mg( ^{2+} )</td>
<td>154020</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recessive</td>
<td></td>
<td></td>
<td>Mg( ^{2+} )</td>
<td>154020</td>
</tr>
</tbody>
</table>

Ca\( ^{2+} \)/Mg\( ^{2+} \)-SR, Ca\( ^{2+} \)/Mg\( ^{2+} \)-sensing receptor; FHH, familial hypocalciuric hypercalcemia; NSHPT, neonatal severe hyperparathyroidism; ADH, autosomal dominant hypoparathyroidism. OMIM no. refers to the Online Mendelian Inheritance in Man database reference number (URL = http://www3.ncbi.nlm.nih.gov/omim/).
associated with magnesium problems. This is evident in patients with Bartter's syndrome. Although all patients with Bartter's syndrome have hypokalemia, few have abnormal serum magnesium concentrations. Clearly, hypokalemia does not consistently lead to renal magnesium wasting. How then do chronic inhibition of Na\(^+\)-Cl\(^-\) cotransport with thiazides or point mutations in Na\(^+\)-Cl\(^-\) cotransport within the DCT, as observed in Gitelman's patients, lead to renal magnesium wasting? We propose the following possible explanations. Inhibition of Na\(^+\)-Cl\(^-\)cotransport results in diminished intracellular Cl\(^-\), apical membrane hyperpolarization, enhanced Mg\(^{2+}\) entry (63, 115, 303, 312), and elevated cellular Mg\(^{2+}\) concentration (54). Elevated intracellular Mg\(^{2+}\) may be perceived by the DCT cell, resulting in downregulation of distal magnesium transport leading to urinary magnesium excretion inappropriate for the plasma magnesium concentration (see sect. VII). Reilly and Ellison (263) have postulated another way to explain magnesium wasting of Gitelman's patients (263). They suggest that the absence of claudin 16 expression may somehow allow magnesium secretion via the paracellular pathway. The notion is that Gitelman syndrome converts some DCT cells that are predominantly electroneutral cells to cells that reabsorb Na\(^+\) in an electrogenic manner. As discussed by these authors, the cells are also responsive to the actions of aldosterone. Accordingly, the combination of the dominance of electrogenic ion transport pathways, the stimulation by aldosterone, and the increased Na\(^+\) concentration all favor electrogenic Na\(^+\) reabsorption. This greatly increases the magnitude of the transepithelial voltage that drives magnesium secretion. We do not favor this explanation because our earlier micropuncture studies failed to detect any secretion of Mg\(^{2+}\) in microperfused distal tubules (246, 254). Furthermore, perfusion of superficial distal tubules of hypermagnesemia rats with solutions containing sodium sulfate to elevate the transepithelial voltage failed to elicit luminal Mg\(^{2+}\) entry (247). Finally, claudin 16 is thought to be involved with enhancing Mg\(^{2+}\) and Ca\(^{2+}\) permeability in the thick ascending limb; accordingly, it is not evident that the aberrant or absent protein expression would increase permeability, thereby increasing Mg\(^{2+}\) back-flux or secretion. As an alternative explanation, Kaisssing et al. (156) have reported that complete block of NaCl entry with thiazide treatment results in apoptosis of rat DCT (156). The Na\(^+\)-Cl\(^-\) cotransporter knockout mice of Schultheis et al. (296) also demonstrated a decrease in number, height, and basolateral infolding of DCT cells. The mitochondria was less well developed in the homozygous null mice compared with the heterozygotes. Additionally, acute interstitial nephritis has been observed in human subjects chronically using thiazide diuretics (193). It is postulated that inhibition of Na\(^+\) entry leads to increased intracellular Ca\(^{2+}\) and impaired volume regulation with a decrease in DNA synthesis leading to apoptosis and tubule atrophy (188, 189). Whether diminished cell surface is sufficient to jeopardize magnesium reabsorption within this segment is unknown, but this is a testable tenant in isolated DCT cells.

B. Inherited Disorders Associated With Abnormal Extracellular Mg\(^{2+}\)/Ca\(^{2+}\) Sensing

The Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor plays an important role in controlling calcium and magnesium transport in both the loop and the distal tubule (20, 136). Both inactivating and activating mutations of the Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor have been described that are now well characterized (241). These abnormalities of Ca\(^{2+}\)/Mg\(^{2+}\) sensing are not selective for magnesium because calcium is also similarly affected (Table 3).

1. Familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism

Familial hypocalciuric hypercalcemia (FHH) (9, 169, 175, 201) and neonatal severe hyperparathyroidism (NSHP) are conditions resulting from inactivating heterozygous and homozygous mutations, respectively (240–242). Renal excretion of calcium and magnesium is reduced leading to hypercalcemia and sometimes hypermagnesemia (9, 169, 175, 201). Defective extracellular Ca\(^{2+}\)/Mg\(^{2+}\) sensing likely leads to inappropriate absorption of calcium and magnesium in both the thick ascending limb (136, 336) and magnesium transport in the distal tubule (15, 16). Ho et al. (145) made a Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor knockout mouse that displays many of the characteristics of FHH and NSHPT patients. Homozygous mutant mice had elevated serum calcium and PTH concentrations but modest elevations of serum magnesium. The urinary concentration of calcium was inappropriately low given the marked elevations in serum calcium levels; urinary magnesium was not reported. Careful clearance studies are warranted to establish renal magnesium wasting because these animals are dehydrated and underweight. The homozygous mice also had severe skeletal abnormalities compared with the heterozygous or control animals (145).

2. Autosomal dominant hypoparathyroidism

Activating mutations termed autosomal dominant hypoparathyroidism (ADH) are dominant and present clinically as isolated hypocalcemic hypoparathyroidism. Hypomagnesemia may be observed in up to 50% of the patients (227, 234). The mutant parathyroid and kidney Ca\(^{2+}\)/Mg\(^{2+}\)-sensing receptor has a lower set point for plasma Ca\(^{2+}\) and Mg\(^{2+}\) concentrations so that for any given divalent cation concentration PTH secretion and
renal calcium transport are suppressed. The hypomagnesemia is usually asymptomatic, but significant deficiencies have been reported (227). Some of this variability is due to the heterogeneity of the activating mutations and a corresponding variability in the set-point detection limit for serum Ca\(^{2+}\) and Mg\(^{2+}\) concentrations.

C. Primary Inherited Disorders of Distal Mg\(^{2+}\) Transport

1. Hypomagnesemia with secondary hypocalcemia

Hypomagnesemia with secondary hypocalcemia (HSH) is an autosomal recessive disorder that manifests in the newborn period and is characterized by very low serum magnesium and low calcium concentrations (1, 58, 127, 233, 244, 279). Patients usually present before 6 mo of age with neurological symptoms of hypomagnesemia and hypocalcemia, including tetany, muscle spasms, and seizures (54, 88). In older children with inadequate control, clouded sensorium and disturbed speech are often seen, and choreoathetoid movements have been described. The hypocalcemia is secondary to parathyroid failure (7, 318) and peripheral PTH resistance as a result of magnesium deficiency (314). Hypokalemia is occasionally present that is only corrected with normalization of plasma magnesium (314). The disease is primarily due to defective intestinal magnesium absorption and may be fatal unless treated with high oral intakes (43, 225, 232, 245, 300, 310, 315). Walder et al. (335) reported that HSH is an autosomal recessive disease and showed by genetic linkage studies that the gene segregates to chromosomes 9 (9q12–9q22.2). They suggest that the candidate gene codes a receptor or an ion channel involved in active intestinal magnesium absorption (335). Because passive intestinal transport is normal, the disease can be treated by high oral magnesium supplements. Renal magnesium conservation has been reported to be normal in most studies, suggesting that the kidney responds appropriately to low circulating magnesium levels by reabsorbing fractionally greater amounts of filtered magnesium. In some cases, however, a renal leak may also be present that manifests primarily when oral supplements are insufficient to normalize the serum magnesium (207). We speculate that the renal leak may be due to altered Mg\(^{2+}\) entry into DCT cells. Whether this condition is genetically heterogeneous remains to be seen, but further studies to address renal tubular magnesium absorption as a function of plasma concentration and filtered magnesium in patients with well-delineated intestinal defects are clearly warranted.

2. Infantile isolated renal magnesium loss

Hypomagnesemia due to late-onset isolated renal magnesium loss is an autosomal dominant condition associated with few symptoms other than chondrocalcinosis (121). Patients always have hypocalciuria and variable, but usually mild, hypomagnesemic symptoms. Meij et al. (210) have reported that the disorder maps to chromosome 11q23 in two large Dutch families. Database searches of the linkage region have so far failed to identify candidate genes, but Meij et al. (210) speculate from our experimental studies (251) that the mutation may lie in the distal tubule. Additionally, there is evidence for a variant form hypomagnesemia due to infantile isolated renal magnesium loss that is more consistent with autosomal recessive inheritance. Meij et al. (personal communication) excluded linkage to any of the known loci previously reported indicating a novel disease. The patients also have variable symptoms, but they usually have normal urinary calcium excretion (104, 120). Because the epithelial transporters of magnesium have not been delineated, it is unclear at what level the tubule magnesium absorption is affected.

Clinical observations suggesting both dominant and recessive inheritance argue for a number of familial renal wasting diseases. As the distal tubule reabsorbs 10–15% of the filtered magnesium (80–95% of that delivered to it), one would expect that if the primary reabsorptive channel were affected, then renal magnesium conservation would be severely compromised leading to marked hypomagnesemia. Alternatively, there may be separate magnesium transporters within the distal tubule under separate genetic control. This is a fertile ground for further genetic studies.

3. Experimental genetic studies

In addition to clinical investigations, experimental studies are being used to define the genetic basis of magnesium homeostasis. Henrotte and colleagues (138–141) established inbred lines of mice by selecting for high and low plasma magnesium levels. Mice of the hypomagnesemic line have inappropriately high urinary magnesium excretion relative to serum levels (142). The hypomagnesemic, hypermagnesiuric mice have normal calcium homeostasis, suggesting a selective tubular defect of magnesium reabsorption. Genetic analysis of these mice indicated that both histocompatibility (H2) related and H2 unrelated to loci were significant determinants of extracellular and intracellular magnesium content in the mice (142). These mice are being used by Andre Mazur, Unite Maladies Metaboliques et Micronutriments, Theix, France, to genotype quantitative traits associated with renal magnesium wasting (personal communication). Studies such as these will be useful in explaining the clinical observations.
X. ACQUIRED DISORDERS THAT DIMINISH DISTAL MAGNESIUM TRANSPORT

A. Potassium Depletion

The relationship of cellular potassium and magnesium metabolism is complex and far from understood. Potassium depletion sometimes results in increased urinary magnesium and calcium excretion (87, 154, 179, 348). The increase in urinary excretion of divalent cations may be explained by the known effects of potassium depletion on the thick ascending limb of the loop. Chloride conservation is impaired in potassium-depleted rats, which may be related to altered basolateral transport resulting in diminished NaCl transport (187). To date, there is no direct evidence for changes in magnesium absorption in the thick ascending limb with potassium depletion. Because magnesium and calcium are absorbed by passive mechanisms, dependent on NaCl transport, it is probable that impaired NaCl transport may lead to diminished divalent cation absorption in this segment (249, 289). Our studies, using isolated MDCT cells, however, suggest that potassium depletion may have important effects on magnesium transport within the DCT (Fig. 17). MDCT cells were cultured in low potassium, 2.5 mM, for 16 h before determination of Mg$^{2+}$ uptake (70). Cellular potassium depletion (89 ± 5 mM vs. control 126 ± 6 mM) resulted in diminished Mg$^{2+}$ uptake as determined by microfluorescence. The cellular mechanisms for the decrease in Mg$^{2+}$ entry with potassium depletion are not known.

![Distal Tubule](Image)

FIG. 17. Schematic model of inherited disorders of magnesium absorption in the distal convoluted tubule. The cellular mechanisms of phosphate depletion, potassium depletion, cyclosporin, and cisplatin are not known, but the evidence suggests that they affect intracellular regulatory processes rather than direct effects on Mg$^{2+}$ channels or the Ca$^{2+}$/Mg$^{2+}$ sensing.

The interrelationships of plasma potassium and magnesium have clinically important ramifications but remain unexplained. As reviewed by Agus (2), hypokalemia is commonly associated with hypomagnesemia. The hypokalemia in these patients is difficult to correct with potassium supplementation alone but rapidly responds following correction of the magnesium deficit (341). Intracellular Mg$^{2+}$ activates or rectifies many K$^+$ channels leading to the speculation that magnesium depletion may alter potassium reabsorption or potassium secretion within the distal nephron. It is unlikely that cellular Mg$^{2+}$ would fall sufficiently to diminish Mg-ATP levels so that luminal ATP-regulated K$^+$ channels are probably not involved (2). Again, this notion would appear to be testable in isolated distal tubule cells.

B. Phosphate Depletion

One of the hallmarks of phosphate depletion is the striking increase in urinary excretion of calcium and magnesium (202). Magnesium excretion may be sufficiently large to lead to overt hypomagnesemia. The increase in divalent ion excretion in both human and experimental animals occurs within hours after initiation of dietary phosphate restriction. It is evident from clearance experiments that the urinary excretion of divalent cations of phosphate-depleted subjects is inappropriate for the plasma concentration, supporting the notion of defective tubular transport (60). Using micropuncture, we have demonstrated that defective magnesium absorption occurred in the loop of Henle and the distal tubule of phosphate-depleted dogs (347).

We have also shown that cellular phosphate depletion leads to diminished Mg$^{2+}$ uptake in MDCT cells (71). This observation supports the notion that the DCT may be involved, in part, in decreased magnesium absorption and increased magnesium excretion observed with phosphate depletion. The effects of phosphate depletion on Mg$^{2+}$ uptake in MDCT cells are reminiscent of those observed in the intact kidney. Removal of phosphate from the media rapidly leads to diminished Mg$^{2+}$ transport, which is dependent on the degree of phosphate depletion. Mg$^{2+}$ uptake is inhibited by 50% when cultured in ~0.3 mM phosphate. These actions are fully reversible with the return of phosphate to the media. The induction of defective transport that is associated with phosphate depletion must reside within the cell either to prevent the normal upregulation of Mg$^{2+}$ transport with Mg$^{2+}$ deficiency or to inhibit Mg$^{2+}$ uptake through actions on transport processes. To determine if phosphate depletion acts through posttranslational mechanisms, MDCT cells were first Mg$^{2+}$ depleted for 16 h to maximally upregulate Mg$^{2+}$ transport. The cells were then phosphate depleted for various time periods, and Mg$^{2+}$ uptake was assessed by microfluorescence (Fig. 18). Phosphate deple-
tion resulted in diminished Mg\textsuperscript{2+} uptake in preadapted cells, which suggests it affects transport through actions on pre-formed pathways rather than through transcriptional or translational mechanisms. Further studies are necessary to define these posttranslational events. Interestingly, phosphate depletion is associated with diminished expression of the MRG described above (258). Again, the role of MRG in magnesium reabsorption requires further work to clarify our understanding of control of cellular transport. It is evident from these studies with isolated MDCT cells that magnesium wasting commonly observed in hypophosphatemia and phosphate depletion could be due, in part, to diminished Mg\textsuperscript{2+} uptake in the DCT.

C. Acid-Base Changes

It has long been known that systemic acidosis is associated with renal magnesium wasting (34, 180, 200). Acute metabolic acidosis produced by infusion of NH\textsubscript{4}Cl or HCl leads to significant increases in urinary magnesium excretion (179, 200). Chronic acidosis also leads to urinary magnesium wasting which, as with acute acidosis, may be partially corrected by the administration of bicarbonate (181, 235). In contrast to metabolic acidosis, acute and chronic metabolic alkalosis consistently leads to a fall in urinary magnesium excretion (346).

Although it has long been known that metabolic acidosis and alkalosis alter renal magnesium handling, relatively little information is available regarding the tubular segments involved. Wong et al. (344) showed that metabolic alkalosis resulted in increased magnesium reabsorption in the distal tubule of the dog. Magnesium reabsorption was closely associated with bicarbonate delivery to the distal tubule in this study. We have shown that acute bicarbonate infusions into chronic acidotic rats lead to a marked increase in magnesium reabsorption in the loop of Henle and distal tubule (301). Thus, on balance, the evidence is that metabolic acidosis and alkalosis act on the distal tubule to change renal magnesium conservation.

We have used the MDCT cell line to determine the direct effects of proton changes on cellular Mg\textsuperscript{2+} uptake (72). Unlike previous reports, this approach has the advantages of isolating the direct cellular effects of H\textsuperscript{+} on Mg\textsuperscript{2+} transport in a controlled fashion that is independent of extrarenal influences. The results of these experiments show that elevation of pH markedly enhances Mg\textsuperscript{2+} uptake, whereas acidosis significantly diminishes transport (72). The studies with isolated MDCT cells, where we could carefully control pH values and bicarbonate concentrations, clearly show that bicarbonate does not directly alter Mg\textsuperscript{2+} uptake (72). This information indicates that protons affect Mg\textsuperscript{2+} entry through changes in intracellular pH or directly affects the Mg\textsuperscript{2+} transport pathway. A change in extracellular pH has significant effects on many kinds of ion channels. For example, Chen et al. (56) postulated that protonation of glutamates within the Ca\textsuperscript{2+} pore of L-type Ca\textsuperscript{2+} channels blocks the permeation pathway. We envision that protonation of the Mg\textsuperscript{2+} pathway may alter Mg\textsuperscript{2+} influx leading to diminished transport. This would have significant effects on distal magnesium absorption, leading to renal magnesium wasting with metabolic acidosis.

Poorly controlled diabetes mellitus is often associated with metabolic acidosis. Hypomagnesemia has been reported in ~25% of diabetic patients, and renal magnesium wasting has been associated with both type I and type II diabetes mellitus (152, 162). Because insulin stimulates magnesium conservation in the loop (195) and distal tubule (68), insulin deficiency could explain the increase in urinary magnesium excretion. A number of indirect influences commonly present in diabetes mellitus may also explain an increase in magnesium excretion. First, uncontrolled hyperglycemia and hyperglycuria may increase excretion through osmotic diuresis (251). Second, metabolic acidosis commonly observed in diabetes may increase magnesium excretion by its actions within the distal tubule (251). Finally, hypophosphatemia and hypokalemia are often associated with diabetes; both may decrease distal magnesium reabsorption (152). Acidosis with any one of these entities may underlie the diminished magnesium reabsorption and hypomagnesemia commonly observed in diabetes mellitus.

D. Cytotoxic Agents

The use of a number of antibiotics, tuberculostatics, and antiviral drugs (3, 158, 299) may be associated with
renal magnesium wasting (Table 4). The cellular basis by which some of these agents lead to diminished magnesium transport has recently become clear, but others remain unexplained. Many are associated with general cellular toxicity and associated electrolyte abnormalities, but it is of interest that some are relatively selective for magnesium.

1. Cisplatin

Treatment with the cancer antimetabolite cisplatin commonly results in renal magnesium wasting and hypomagnesemia (3, 158, 299). The incidence of magnesium deficiency is >30% but increases to >70% with longer cisplatin usage and greater accumulated doses. Interestingly, cisplatin-induced magnesium wasting is relatively selective (25). Hypocalcemia and hypokalemia may be observed but only with prolonged and severe magnesium deficiency (125, 191, 198, 209, 321). The influence of magnesium deficiency on PTH secretion and end-organ resistance is the likely explanation for enhanced urinary calcium excretion and diminished mobilization resulting in low plasma calcium concentrations (7, 96). The effects on potassium balance are more difficult to explain. The hypokalemia observed with magnesium deficiency is refractory to potassium supplementation (275). Cisplatin results in proximal tubular damage, but the evidence from both clinical and experimental studies indicates that the drug acts on distal tubular magnesium transport (194, 209, 322). Because there is no magnesium reabsorption within the CCD, it is likely that actions within the DCT are responsible for the renal magnesium leak. Using micropuncture, Mavichak et al. (209) showed that magnesium reabsorption was diminished in the distal tubule of rats receiving cisplatin. The cellular mechanisms for the apparent selective effects on magnesium remain undefined. It would be of interest to determine if amiloride retains its magnesium-conserving actions in these patients (251). The effects of cisplatin may persist months or years later, long after the inorganic platinum has disappeared from the renal tissue (199, 334). Whatever cellular mechanisms are involved, it must include genetic alteration of magnesium transport. The fact that cisplatin exerts its therapeutic effects by binding cellular DNA may be relevant (294).

2. Aminoglycosides

Aminoglycosides, such as gentamicin, cause hypermagnesuria and hypomagnesemia (Table 4). As many as 25% of patients receiving gentamicin will present with hypomagnesemia (299). The hypermagnesiuric response occurs soon after the onset of therapy; it is dose dependent and readily reversible upon withdrawal (94, 122, 230, 237). Hypokalemia is frequently observed with the magnesium deficiency. Magnesium wasting is associated with hypercalciumia that may lead to diminished plasma calcium concentrations (94, 161). This would suggest that aminoglycosides affect renal magnesium and calcium transport in the tubular segments where both are reabsorbed, namely, the thick ascending limb and the DCT. Experimental studies with animals support this notion (4, 103, 112, 231). The cellular mechanisms are unknown, but hypermagnesiuric and hypercalciumia are observed in the absence of histopathological changes (337). Gentamicin is a polyvalent cation so that it may have its effects on the Ca\(^{2+}/Mg^{2+}\)-sensing receptor. Activation of this receptor by polyvalent cations inhibits passive absorption of magnesium and calcium in the loop and active hormone-mediated transport in the DCT (20, 21, 136). In support of this notion, we have shown that gentamicin inhibits PTH-mediated cAMP formation and PTH-stimulated Mg\(^{2+}\) uptake in MDCT cells (159). The inhibition was concentration dependent and reversed by the application of high concentrations of 8-bromo-cAMP or forskolin. We infer from these studies that gentamicin inhibits hormone-stimulated Mg\(^{2+}\) absorption in the DCT that contributes to renal magnesium wasting, which is frequently observed with the clinical use of aminoglycosides.

3. Cyclosporin and FK506

The immunosuppressants, cyclosporin and FK506, commonly lead to renal magnesium wasting and hypomagnesemia (23, 171, 273). Unlike the other agents, these drugs usually cause a significant reduction in glomerular filtration rate (GFR) (up to 60%) and modest hypercalcemia and hypokalemia (273). The hypomagnesemic effect is probably attenuated by the fall in GFR and reduction in filtered magnesium, but this defect appears to be specific for magnesium (260, 274, 343). The distal tubule is probably the site of the tubular magnesium leak. It is not known whether these drugs act through calcineurin, which is the intracellular receptor for these agents.
XI. SUMMARY: FUTURE DIRECTIONS IN RESEARCH OF MAGNESIUM TRANSPORT IN THE DISTAL CONVOLUTED TUBULE

Although the majority of filtered magnesium is reabsorbed in the thick ascending of the loop of Henle, it is apparent that the distal tubule also participates in the sensitive and selective control of renal magnesium handling. It also serves an important site for familial and acquired magnesium wasting disorders. As with other cations, such as sodium, potassium, and calcium, the distal tubule plays a significant role in control of daily electrolyte balance and perhaps more importantly in homeostasis over a long period of time. The DCT may reabsorb a minor fraction of filtered salt, but it provides an additional regulation that is serially posed to act in concert with the other nephron segments to affect electrolyte homeostasis. The aim of this review has been to review our current understanding of magnesium handling by the distal tubule and to integrate these observations into the overall renal control of magnesium. Like other cations, the reabsorptive rate of magnesium in the distal tubule is load dependent; that is, absolute magnesium absorption increases with enhanced delivery to this segment. This association is valid whether magnesium delivery is altered either by an increase in tubule fluid magnesium concentration or by an increase in fluid flow rate issuing from the loop of Henle. Transepithelial magnesium absorption is active in the distal tubule as transport is against luminal-to-interstitial electrical and concentration gradients. The evidence is that Mg\(^{2+}\) moves across the apical membrane dependent on the transmembrane concentration and down a favorable electrical gradient. Accordingly, this transport is passive and likely through a selective Mg\(^{2+}\) channel. The mechanism whereby Mg\(^{2+}\) exits across the basolateral membrane into the interstitium is unknown but must be active. The rate-limiting step and the site of regulation appears to occur with the entry of Mg\(^{2+}\) into the DCT cell. The peptide hormones, PTH, calcitonin, glucagon, and AVP, increase Mg\(^{2+}\) transport in the distal tubule through cAMP-, phospholipase C-, and protein kinase C-mediated signaling pathways. PGE\(_2\) and isoproterenol increase Mg\(^{2+}\) entry into MDCT cells by unknown means. Aldosterone potentiates hormone-stimulated cAMP release and Mg\(^{2+}\) uptake in MDCT cells. 1,25(OH)\(_2\)D\(_3\) enhances Mg\(^{2+}\) uptake via intracellular pathways that are independent of cAMP-protein kinase A and additive to the actions of peptide hormones such as PTH. Insulin stimulates Mg\(^{2+}\) uptake, but its role in renal magnesium balance is not fully understood. Further studies are needed to clarify our understanding of the cellular events involved with magnesium transport and to describe the integrated actions of these important peptide and steroid hormonal controls. Hypercalcemia and hypomagnesemia inhibit hormone-stimulated cAMP release and Mg\(^{2+}\) uptake through activation of an extracellular Ca\(^{2+}\)/Mg\(^{2+}\)-sensing mechanism. Selective control of Mg\(^{2+}\) uptake and absorption appears to occur after magnesium restriction. Our evidence suggests that this is through an increase in selective Mg\(^{2+}\) transport, in part, by transcriptional-translational processes involving de novo protein synthesis. Adaptation to magnesium restriction is observed at both the organ and cellular levels. The mechanisms used by the DCT cell to sense the extracellular Mg\(^{2+}\) concentration and appropriately adapt the transport rates are fertile areas for future research. Mg\(^{2+}\) uptake may also be modulated by a number of posttranslational events. Amiloride and chlorothiazide are magnesium-conserving diuretics by virtue of their ability to stimulate voltage-sensitive Mg\(^{2+}\) entry in DCT cells. A large number of acquired disorders affect magnesium absorption in the DCT. These include those associated with abnormal NaCl absorption (Gitelman syndrome), extracellular Ca\(^{2+}\)/Mg\(^{2+}\) sensing (FHH and NSHPT), and primary magnesium wasting most likely affecting selective cellular Mg\(^{2+}\) transport. Genetic studies are interesting because they describe nature’s experiments in control of magnesium transport and lead to here-to-unforeseen transport possibilities. A large number of acquired disorders affect magnesium absorption in the DCT. Cellular potassium depletion diminishes Mg\(^{2+}\) transport through undefined ways. As marked increases in Mg\(^{2+}\) concentration or hyperpolarization do not normalize transport, it is probable that the effect of potassium depletion is on the magnesium transporter. Similarly, cellular phosphate depletion decreases Mg\(^{2+}\) uptake in MDCT cells. Again, the mechanisms are not known but must be posttranslational because removal of phosphate rapidly leads to diminished Mg\(^{2+}\) uptake in cells that have previously been fully adapted. Both of these conditions, potassium and phosphate depletion, lead not only to diminished Mg\(^{2+}\) uptake in the DCT but to renal magnesium wasting, supporting the notion that the defect in DCT transport provides the basis for these diseases. Our studies suggest that cellular potassium and phosphate depletion act through different mechanisms. Acid-base changes alter cellular Mg\(^{2+}\) uptake and renal magnesium reabsorption. These changes are due to direct effects of protons on Mg\(^{2+}\) transport and are independent of alterations in membrane voltage and modulations due to potassium and phosphate depletion. Again, metabolic alkalosis or acidosis may play a significant role in dictating the control of renal magnesium handling. Magnesium wasting and hypomagnesemia are common observations in metabolic acidicotic patients. Because these three entities, potassium depletion, hypophosphatemia, and metabolic acidosis, act at different sites along the Mg\(^{2+}\) transport pathway, they may exacerbate renal magnesium wasting when present in combination. Finally, various drugs including antibiotics, antitumor agents, and immunosuppressants may affect magnesium handling out of proportion to other electrolytes. Areas for future research include electrophysiological definition of Mg\(^{2+}\) entry into epithelial cells, molecular identifi-
cation of Mg$^{2+}$ transport pathways, and description of the controlling mechanisms regulating Mg$^{2+}$ transport such as transcriptional processes involving the magnesium response element. Finally, cellular magnesium handling remains to be studied in distal segments other than the DCT. Mechanisms and controls of Mg$^{2+}$ uptake in connecting tubule and the initial collecting tubule may differ from those described for the DCT.

We acknowledge Lucie Canaff and Dr. Geoffrey Hendy for their collaboration on the Ca$^{2+}$-sensing receptor studies. We thank Dr. Peter Friedman for the MDCT cells. We appreciate Drs. Michele Gagnan-Brunette and Iwan C. Meij for fruitful discussions and critical reading of parts of this manuscript.

This work was supported by Medical Research Council of Canada Research Grant MT-5793 and by grants from the Kidney Foundation of Canada (to G. A. Quamme), the National Sciences and Engineering Research Council, and the Dairy Farmers of Canada (to D. E. C. Cole).

Address for reprint requests and other correspondence: G. A. Quamme, Dept. of Medicine, University Hospital, Koerner Pavilion, 2211 Wesbrook Mall, Vancouver, BC, Canada V6T 1Z3 (E-mail: quamme@interchange.ubc.ca).

REFERENCES


33. Blumberg D, Bonetti A, Jacomella V, Capello S, Truittmann AC, Luthy CM, Colombo JP, and Bianchetti MG. Free circulating mag-


81. DiVANE J and RYAN MP. Evidence for a magnesium-sparing action by amiloride diuretic renal clearance studies in rats. Br J Pharma-


82. DE WEER P. Cellular sodium-potassium transport. In: The Kidney Physiology and Pathophysiology, edited by Seldin DW and Gib-


83. DiBONA GP AND SAWIN LL. Effect of renal nerve stimulation on NaCl and water transport in Henle’s loop of the rat. Am J Phys-


111. GIFFEIN WB, MONTENS LA, WILLEMS HL, BULS WC, AND TEEBAAR BG. Renal magnesium wasting in two families with autosomal domi-


