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2.1 Scope of the Review

Increased morbidity in renal stone disease has been observed in many industrialized countries during the last century, and epidemiological studies have shown a prevalence in males than females (Abdel - Halim, 1989; Bytyci and Mesaric et al., 1989). Although a significant advancement in the treatment of urinary stones have been made, but the effective ways for the prevention of stone formation still need to be developed. The main reason for this is the obscurity of the mechanisms underlying calcium oxalate urinary stone formation.

Magnesium is of paramount importance in urolithiasis. Studies have shown that deficiency of magnesium in man and animals, or low levels of urinary magnesium in stone formers (Pyrah, 1979; Wangoo et al., 1989) encourage stone formation.

Although several reports confirm the role of magnesium in urolithiasis, however, the full extent of biochemical processes, which occurs due to magnesium depletion has not been properly elucidated. Therefore, the scope of the present work is to investigate the role of magnesium on calcium and oxalate metabolism in relation to urinary stone formation.

2.2 Oxalic acid-Historical aspects

Urolithiasis is one of the ancient diseases afflicting mankind and it continues to pose a universal health problem
even today. The earliest stone to be discovered was found in the mummy of a sixteen year old Egyptian boy, who was buried around 4800 B.C. (Shattock, 1905; Ellis, 1969). Early accounts of urinary tract stone disease have been made by Susruta (from India), Hippocrates (from Greece) and Celsus (from Rome). Gelenus (131-201 AD) related the incidence of stone formation to nutrition, heredity and climate.

Oxalic acid was first discovered by Angelus Sala in the sixteenth century and was found to be associated with urinary stones (Bergman, 1776). The presence of calcium oxalate crystals in the urine and renal stones were demonstrated in the early part of the nineteenth century (Wollaston, 1810). The importance of oxalate-rich foods as a source of oxalate of lime in urine was demonstrated by many workers by the middle of the nineteenth century (Bird, 1853; Neubauer and Vogel, 1863). Earlier workers (Gaglio, 1887; Pohl, 1896) had correctly postulated that "oxalic acid is not destroyed in the animal body," a statement which even today holds true. Several studies on intestinal absorption of oxalate (Dunlop, 1896), the importance of dietary and endogenous metabolism of oxalate (Bunge, 1889; Baldwin, 1900) came into light in the late nineteenth century. Majority of the stones analyzed consist of calcium oxalate as their principal constituent (Thind and Nath, 1989; Borghi et al., 1990), thus emphasizing the need of studying oxalate metabolism.
Several aspects of urolithiasis and oxalate metabolism have been reviewed in the recent years (Nath et al., 1984; Williams and Wandzilak, 1989; Conyers et al., 1990; Williams and Wilson, 1990). Literature on urolithiasis is also being updated every four years at international meets. The sixth of this series was held in July, 1988 in Vancouver, Canada.

2.3 Sources of oxalic acid in the body

The body gets its oxalic acid from exogenous sources i.e., through intestinal absorption of preformed dietary oxalate, as well as by endogenous synthesis from its precursors viz., ascorbic acid, glycine, serine, glycolate, glyoxalate and hydroxyproline, etc.

2.3.1 Dietary oxalate

Oxalic acid is present in most plant tissues in both a soluble and an insoluble form, and the relative proportions vary widely. In most foods - the soluble form - the form most easily absorbed from the gastrointestinal tract predominates (Archer et al., 1957). Therefore, the diets having high oxalate and low calcium are more toxic than those having lower oxalate and higher calcium contents (Hesse et al., 1979). The oxalate contents of many processed and unprocessed foods and beverages have been measured (Suvachittanant et al., 1973; Hodgkinson, 1977). Spinach, rhubarb, peanuts, chocolate, strawberries and tea in the diet has been reported to cause hyperoxaluria in patients
with calcium oxalate nephrolithiasis (Finch et al., 1981; Norden-Vall, 1982). The daily dietary intake of oxalate in man varies from 70 mg to 920 mg on a typically western diet (Archer et al., 1957; Hodgkinson, 1977). However in India, depending on seasonal variation, daily intake of oxalate varies from 78 mg to 2.0 g per day (Singh et al., 1972).

2.3.2 Intestinal absorption of oxalate

Only a small percentage of the ingested oxalate is actually absorbed from the gastrointestinal tract. Jejunum in the small intestine is the major site of oxalate absorption (Madorsky and Finlayson, 1977). Nearly 2 to 5 percent of the ingested dietary oxalate absorption has been reported (Prenen et al., 1984). Part of the reasons for the poor absorption of oxalate is the presence of oxalate-degrading bacteria in the gut (Allison et al., 1986). Increased oxalate excretion in idiopathic stone formers has been related to the hyperabsorption of oxalate in the intestine (Marangella et al., 1982; Lindsjo et al., 1989).

Oxalate absorption in rat and rabbit ileum mucosa has been shown to be linear (10 μM to 2.0 mM) and remains unaffected by ouabain and 2,4 dinitrophenol, suggesting that oxalate is absorbed by a passive, non-saturable, non-energy dependent mechanism (Binder, 1974). The oxalate absorption by the same mechanism has been confirmed by others (Kathpalia et al., 1984; Dobbins, 1985).
However, Hatch et al., (1984), reported that net oxalate absorption occurs in the distal rabbit colon under short-circuited conditions and suggested a carrier-mediated, active process via an anion-exchange system for oxalate transport. Studies by Knickelbein et al., (1986) demonstrated the presence of oxalate: OH and oxalate: Cl exchange systems on the brush border membrane of rabbit ileum. Oxalate uptake was found to be stimulated by formate and oxaloacetate, but not by bicarbonate.

Bile acids and long chain fatty acids are known to increase oxalate absorption in the colon, either by causing a non specific increase in permeability (Kathpalia et al., 1984), or, probably at the tight junction (Freel et al., 1983a). Bile acids and long chain fatty acids block NaCl absorption and induce electrogenic chloride secretion that are transcellular carrier-mediated processes. (Freel et al., 1983b). Thus it is possible that bile acids and long chain fatty acids may have some direct or indirect effects on the oxalate transport in the ileum and colon.

Increased incidence of nephrolithiasis in patients with idiopathic inflammatory bowel disease and in subjects, who have undergone jejunoileal bypass for obesity has also been reported (Dobbins et al., 1985; Lindsjo et al., 1989a). Hyperoxaluria is a common finding in these patients (Robertson and Peacock, 1980). Increased absorption of dietary oxalate has been implicated to be the causative
factor in the genesis of nephrolithiasis in these patients (Bjorneklett et al., 1981; Allison et al., 1986). Combination of reduced urinary volume, pH, citrate, magnesium, sulfate and higher urinary oxalate, ammonia and calcium oxalate relative supersaturation has been shown to enhance the risk of nephrolithiasis in partial ileal bypass surgery patients (Obialo et al., 1991).

2.3.3 Endogenous synthesis of oxalate

Most of the oxalate (80-90%) appearing in the urine is derived from the endogenous source (Hodgkinson, 1977; Arora et al., 1985). The important precursors of endogenous oxalate biosynthesis are summarized in Fig. 1. Ascorbate and glyoxylate are the two main precursors of oxalate, each contributing 30-50% to urinary oxalate (Schmidt et al., 1981). A major portion of the biosynthesis of oxalate occurs in liver. Various precursors other than ascorbate are glucose (Runyan and Gershoff, 1965; Cogoli-Greuter and Christen, 1981), galactose and lactose (Ribaya et al., 1981), fructose (Rofe et al., 1980), sucrose (Thom et al., 1981), xylitol (James et al., 1982), glycine (Crawhall et al., 1959), serine (Runyan and Gershoff, 1965; Noguchi et al., 1978), hydroxyproline (Ribaya and Gershoff, 1965; Tawashi et al., 1980), aromatic amino acids (Gambardella and Richardson, 1977) and ethylene glycol, glycolic acid, glyoxylic acid (Richardson, 1973). Except ascorbate all
FIG. 1 THE PRECURSORS OF URINARY OXALATE
other precursors converge to central glycolate-glyoxylate-oxalate pathway (Fig. 2).

2.4 Pathways of glyoxylate metabolism

In animals or in man, conversion of glyoxylate to oxalate is not the major pathway. Most of the glyoxylate is oxidized to carbon dioxide (King and Wainer, 1968). However, extensive oxalate formation occurs, if the glyoxylate levels are abnormally high. The pathways of glyoxylate metabolism in mammals have been reviewed by several workers (Nath et al., 1984; Williams and Wilson, 1990).

2.4.1 Enzymes of glycolate-glyoxylate-oxalate pathway

Glycolate and glyoxylate are the two major immediate metabolic precursors of oxalate. The enzymes which catalyze the oxidation of glycolate to glyoxylate and then to oxalate include xanthine oxidase, glycolate oxidase, lactate dehydrogenase and glycolate dehydrogenase. However, studies have demonstrated that xanthine oxidase plays a minor role in oxalate production (Gibbs and Watts, 1973). Allopurinol, a xanthine oxidase inhibitor, does not cause a change in oxalate excretion in patients with gout (Gibbs and Watts, 1966).

2.4.1.1 Glycolate oxidase (EC 1.1.3.1)

The enzyme glycolate oxidase (GAO) catalyzing the conversion of glycolate to oxalate via glyoxylate in rat and rabbit liver was first reported by Dohan, (1940). Later Kun et al., (1954) partially purified the enzyme from rat liver
FIG. 2 METABOLIC PATHWAYS OF OXALATE BIOSYNTHESIS IN ANIMALS
and showed it to be a flavoprotein containing flavin mononucleotide and requiring molecular oxygen for its activity. Schumann and Massey, (1971) purified the pig liver GAO and found it to be identical to the rat liver. The pH optima for this enzyme is at pH 8.5. The enzyme has high affinity for glycolate \((K_m = 2.5 \times 10^{-4} \text{ M})\) as compared to glyoxylate \((K_m = 14.1 \times 10^{-4} \text{ M})\). The purification of this enzyme to homogeneity and its characterisation in human livers (Fry and Richardson, 1979 a) showed that this enzyme resembles the rat and pig liver enzyme in its properties. The enzyme is inhibited by p-chloromercuric benzoate, cyanide and copper sulphate. Strong correlation between the increased level of this enzyme in the liver and hyperoxaluria in the rat suggests a major role of this enzyme in oxalate metabolism (Liao and Richardson, 1973).

2.4.1.2. Lactate dehydrogenase (EC 1.1.1.27)

Lactate dehydrogenase (LDH) has been identified as a major enzyme in leucocytes and erythrocytes (Richardson and Liao, 1973) and also in 100,000 g fractions of human liver and heart tissues (Gibbs and Watts, 1973). It has been proposed that, in tissues other than liver, the enzyme accounts totally for the oxalate synthesized from glyoxylate (Smith et al., 1972). The pH optima for the LDH catalyzed oxidation of glyoxylate is 9.3 and for the reduction of glyoxylate to glycolate with NADH is 6.9. Warren (1970)
showed that oxalate is a competitive inhibitor for the oxidation of glyoxylate and non-competitive inhibitor for the reduction of glyoxylate by LDH. Romano and Cerra (1969) showed that the oxidation of glyoxylate to oxalate is stimulated by the addition of pyruvate. Studies by Duncan (1980) on the pH dependent oxidation and reduction of glyoxylate by LDH demonstrated that the LDH-NADH complex cannot dissociate easily at pH 7.0, but can reduce glyoxylate to glycolate. As the pH increases, this dissociation is made easier and binding of LDH with NAD becomes stronger, causing oxidation of glyoxylate at pH 9.6.

The isoenzymes of LDH catalyze the oxidation of glyoxylate to oxalate with different affinities (Banner and Rosalki, 1967). LDH isoenzyme V (muscle-type) has a Km of 30 mM whereas isoenzyme I (heart type) has a Km of 5 mM for glyoxylate, for the oxidation reaction. All the isoenzymes of rabbit muscle LDH catalyze the oxidation and reduction of glyoxylate (Duncan and Tipton, 1969).

2.4.1.3 Glycolate dehydrogenase

Direct conversion of glycolate to oxalate without involving free glyoxylate as an intermediate has been demonstrated by many investigators. Runyan and Gershoff (1965) found that the rates of conversion of glycolate, ethanolamine and ethylene glycol to oxalate to be about 18, 14 and 10 times higher in Vitamin B₆ deficient rats as compared to controls. Liao and Richardson (1972) observed
that the perfused liver of rats converts about 61% of glycolate and 30% of ethylene glycol to oxalate but only about 28% of glyoxylate is oxidized to oxalate.

All these observations led to the conclusion that glycolate may be directly converted to oxalate without forming glyoxylate as an intermediate. The presence of such an enzyme, glycolate dehydrogenase (GAD), has been reported to occur in rat liver (Richardson and Fry, 1977) and human liver (Fry and Richardson, 1979b). The pH optima for the enzyme is 6.1 and does not require FMN, FAD, NAD or NADP.

On the contrary, recent studies demonstrated that, in rat and human liver the formation of oxalate from glycolate takes place predominantly via glyoxylate. The GAD activity observed in crude enzyme preparation is largely accounted for, if not entirely, by the combined action of GAO and XOD or LDH (Yanagawa et al., 1990).

2.4.2 Transamination of glyoxylate

Glyoxylate can be transaminated in the presence of many aminotransferase enzymes, viz: L-glutamate-glyoxylate aminotransferase (Nakada, 1964), ornithine -glyoxylate aminotransferase (Strecker, 1965), L-alanine-glyoxylate aminotransferase (Thompson and Richardson, 1967) and Serine-glyoxylate aminotransferase (Noguchi et al., 1978). All these reactions are irreversible under physiological conditions, and favour the formation of glycine.
The alanine-glyoxylate aminotransferase is mainly localized in rat liver mitochondria and very little activity is detected in the peroxisomal fraction (Thompson and Richardson, 1967; Noguchi et al., 1978), whereas in kidney, enzyme activity can be detected both in the mitochondrial as well as in peroxisomal fraction (Takada and Noguchi, 1980). On the other hand in human liver, the enzyme is present only in peroxisomes and not in mitochondria. Nucleotide sequence analysis of cDNA clones for alanine: glyoxylate transferase enzyme constructed from primary hyperoxaluria type I patient revealed a point mutation of T to C at position 634, encoding a serine to proline substitution at residue 605 (Nishiyama et al., 1991).

2.4.3 α-ketoglutarate: Glyoxylate carboligase

The enzyme glyoxylate-2-oxoglutarate: Carboligase in the presence of thiamine-pyrophosphate catalyzes the condensation of glyoxylate with 2-oxoglutarate. The enzyme activity in both cytoplasm and mitochondrial fractions of human liver, kidney, spleen and skeletal muscle has been reported (Crawhall and Watts, 1962; Koch et al., 1967). Schlossberg et al., (1968) demonstrated that during the reaction, one of the glyoxylate carbons is liberated as CO₂ and either 5-hydroxy-4-ketovalerate or 2-hydroxy-glutarate are formed. Schlossberg et al., (1970) and Saito et al., (1971) found that this carboligase activity was associated with mitochondrial 2-oxoglutarate dehydrogenase complex.
Localization of glyoxylate carboligase activity in rat liver and its similarity with mitochondrial 2-oxoglutarate dehydrogenase was demonstrated by O'Fallon and Brosemer (1977). This mitochondrial enzyme may thus be involved in a major oxidation pathway of glyoxylate.

2.4.4 Glyoxylate oxidation cycle

In the presence of mitochondrial enzyme, 2-keto-4-hydroxy glutarate aldose, glyoxylate may combine with pyruvate to form 2-keto-4-hydroxy glutarate (KHG). Mitochondrial 2-ketoglutarate dehydrogenase complex can oxidise KHG (Gupta and Dekker, 1980). This reaction has a significant bearing on the oxidative degradation of glyoxylate to CO$_2$. Formation of malyl-CoA instead of succinyl-CoA takes place in this reaction. This pathway of glyoxylate metabolism in mitochondria has been named as glyoxylate oxidation cycle, and is analogous to the tricarboxylic acid cycle (Dekker and Gupta, 1979).

2.5 Hormonal control of oxalate metabolism

The influence of hormones on enzymes involved in oxalate metabolism has not been well examined. Only sex steroids are known to have some direct effect on oxalate-synthesizing enzymes. However, on the reports available so far, an attempt has been made to study the influence of hormones on oxalate metabolism.
2.5.1 Peptide hormones

Hyperoxaluria is often detected in patients of hyperparathyroidism and idiopathic hypercalciuria (Hodgkinson and Zarembski, 1968). This is not directly related to the primary defect in oxalate metabolism. It is due to the associated hypercalciuria which influence intestinal absorption and renal clearance of oxalate.

High incidence of urolithiasis has been observed in insulin-dependent diabetes patients (Maricker et al., 1977). Activity of lactate dehydrogenase involved in oxalate biosynthesis is known to be regulated by insulin (Sabo et al., 1971). Increased peroxisomal β-oxidation of fatty acids in rats due to an increase in the peroxisomal population in the liver cells has been reported in alloxan-diabetes (Horie et al., 1981). Increased proliferation of peroxisomes may increase the activities of all oxalate synthesizing enzymes.

Serine-aminotransferase of liver, which forms hydroxypyruvate, is another enzyme, induced in rats by glucagon administration and wild diabetic conditions (Belivean and Freedland, 1982). Effects of these hormones directly on oxalate biosynthesizing enzymes have not been attempted.

2.5.2 Steroid hormones

Sex steroids testosterone and estradiol have been shown to influence oxalate biosynthetic pathway. Low
incidence of calcium oxalate nephrolithiasis in females as compared to males is well known. Low incidence of urinary calculi has been related to estrogens. Sharma et al., (1981) showed that testosterone induces all the three oxalate synthesizing enzymes, viz., LDH, GAO and GAD in weanling female rat liver, while β-estradiol injection to male weanling rats led to a decrease in the activity of all of these enzymes. Kidney enzymes, however remained unaltered by either hormone treatment.

2.6 Urinary oxalate and renal clearance

In humans, the average urinary excretion of oxalic acid is about 30 mg per day with a range of 15-50 mg. Although the incidence of stone formation is greater in males, as compared to females, no great sex differences in the urinary excretion has been reported (Hodgkinson, 1977a). Hyperoxaluria is a frequent finding in idiopathic calcium oxalate stone formation (Baggio et al., 1983). Diurnal variation in the excretion of oxalate has been reported. This variation is thought to be mainly due to the ingestion of food, since it is abolished in fasting (Zarembski and Hodgkinson, 1969). High excretion of oxalate in mid-morning urines during summer has been postulated in the increased risk of the initiation of calcium oxalate stone formation (Hallson and Rose, 1977). Tiselius and Almgard (1977) suggested that high urinary volumes during the day
time should be maintained in order to reduce the effective urinary oxalate.

Oxalate is freely filtered in the glomerulus. Studies using $^{14}$C-oxalate in normal patients indicate net tubular secretion (Prenen et al., 1982). Micropuncture studies indicate the proximal convoluted tubule as the site for secretory process (Weinmann et al., 1978, 1980). Studies using free flow micropuncture technique demonstrated the presence of two secretory systems in the proximal tubule (Weinmann et al., 1980; Knight et al., 1981).

The simultaneous use of luminal and capillary micropuncture studies revealed a concentration dependent secretory flux with a secretory rate indicating a carrier-mediated secretory system. Sodium cyanide, p-chloromercuric-benzoate, furosemide and p-aminohippurate inhibited the rate of oxalate transport (Knight et al., 1981). The presence of a proximal tubule high-affinity, low-capacity transport system operating at low plasma oxalate concentration and a low-affinity high capacity oxalate transport system operating at high plasma oxalate concentration has been postulated (Knight et al., 1981). Luminal oxalate concentration in the distal tubule remains unaltered (Weinmann et al., 1978). Proximal tubule appears to be the only segment of the nephron, important in oxalate transport. Recently, details of the mechanism of substrate transport in the proximal tubule have been investigated
using plasma membrane vesicles isolated from renal cortex. In rat brush border membrane, oxalate was found to be transported via the Na\(^+\)-SO\(_4\)\(^-\) co-transport system (Bastlein and Burckhardt, 1986). Cl (formate)-oxalate exchange system has also been identified on the brush border membrane of the rabbit proximal tubule cell (Karniski and Aronson, 1987). Kuo and Aronson (1988) demonstrated that oxalate was exchangeable for sulphate or bicarbonate across the basolateral membrane of the rabbit proximal tubule cell. On the contrary, recent studies by Yamakawa and Kawamura (1990) reported the absence of sulphate: bicarbonate exchange system in the rat renal brush border membrane. The authors found that oxalate was transported by the oxalate:OH exchange via an anion transport system and suggested that oxalate:OH exchange, and Cl:formate and oxalate:Cl exchange occur via separate carriers. Renal clearance of oxalate in hyperoxaluric rats has been shown to be higher than that of inulin, thereby indicating that oxalate is secreted by the proximal tubule (Kanazawa, 1990).

2.7 Role of Calcium

More than 90% of the stones are composed of calcium, phosphate besides oxalate. Considerable progress has been made in understanding the homeostatic control mechanism for mineral metabolism specially calcium (Nordin, 1976). Idiopathic hypercalciuria is the most common disorder of stone formation (Insogna et al., 1985). 40% of the recurrent
stone disease can be attributed to idiopathic hypercalciuria (Coe et al., 1973).

Calcium is the most abundant cation in the body. Almost all calcium is in the bone, which averages 1050 g in man. Only one per cent of calcium is present in extracellular fluid and various soft tissues. Calcium ions play a vital role in neuromuscular function, blood coagulation, membrane function and a variety of enzyme reactions (Lyles and Drezner, 1981). Concentration of ionized calcium in various cell compartments plays an important role in regulating many functions. Intracellular calcium concentration is kept comparatively low (10^-7M to 10^-6M), as higher concentrations of free calcium are known to inhibit many important physiological processes. The regulation of cell calcium metabolism is under the influence of various regulators, modulators and controllers. The schematic representation for regulation of cytosolic free calcium is given in Fig. 3.

The serum calcium levels are also precisely regulated within a narrow range. In the normal human adult, net intestinal calcium absorption and renal calcium excretion are similar in a steady state concentration (Wilkinson, 1976).

2.7.1 Intestinal absorption of Calcium

The gastrointestinal tract is the only source for the entry of calcium into the body pool. Transport of calcium
FIG. 3  CONTROL, MODULATION & REGULATION OF CYTOSOLIC FREE CALCIUM

REGULATORS
1. MEMBRANE DEPOLARIZATION
2. HORMONES
3. CYCLIC NUCLEOTIDES
4. CALMODULIN

CONTROLLERS
1. PLASMA MEMBRANE
2. MITOCHONDRIA
3. ENDOPLASMIC RETICULUM

MODULATORS
IONIC COMPOSITION
PHOSPHATE $\text{Na}_0\text{Na}_1$
$\text{PH}_0\text{PH}_1$

CELL FUNCTION
1. CONTRACTION
2. SECRETION
3. METABOLISM
4. Ca TRANSPORT ETC
against the concentration gradient in everted gut sacs of rat duodenum was first demonstrated by Schachter and Rosen (1959). Studies by Schachter et al., (1960) and Wasserman and Taylor (1969), showed that the active transport of calcium occurs predominantly in the duodenum of most of the species, and is least in jejunum and colon. Miller and Bronner (1981) studied the uptake of calcium by brush border membrane vesicles. The process of calcium uptake exhibited saturation kinetics with a pH optimum between 7.5 and 8.0, and was shown to be independent of metabolic energy. Saturation kinetics of calcium uptake in duodenal brush border membrane vesicles from chick and rat intestine has also been demonstrated by other workers (Bikle et al., 1983; Bronner et al., 1986).

2.7.2 Renal handling of Calcium

Studies by many investigators have reported that about 50 to 60% of the filtered calcium is reabsorbed in the proximal convoluted tubule, about 20% is reabsorbed in the thick ascending limb of Henle's loop, another 10-15% is reabsorbed in the distal tubules and a small amount approximately 5% is reabsorbed in the collecting duct (Friedman, 1988; Kumar et al., 1988; Puschett, 1989). Differential handling of calcium by proximal and distal tubule, has been reported (Kumar et al. 1988).
2.7.3 Hormonal regulation of calcium homeostatis

PTH and Vitamin D are known to maintain the calcium balance by acting on target tissues, such as intestine, bone and kidney (Kumar, 1984; Audran et al., 1986). Vitamin D₃ is known to influence the absorption of calcium in the intestine. It has been shown that, idiopathic hypercalciuria patients exhibit an exaggerated calcium excretion following ingestion of standard calcium load (Broadus et al., 1978), reflecting an intestinal calcium absorption resulting from increased production of 1, 25 \((\text{OH})_2\) D₃ (Gray et al., 1977; Broadus et al., 1984). These hormones control the mobilization of calcium into and out of bone, thus both the hormones have a inter-dependent role in the control of this process. PTH has a central role in stimulating renal absorption of calcium and decreasing the urinary calcium excretion. On the other hand, in hypocalcemic, parathyroidectomy or volume expansion state, Vitamin D₃ augments tubular reabsorption of calcium. In normocalcemic subjects, an increase in urinary calcium excretion, occurs, due to enhanced intestinal absorption of calcium by Vitamin D₃ administration. This also causes inhibition of PTH secretion. The differential handling of calcium between the proximal tubule and distal tubule of the nephron is also attributed to the presence of Vitamin D-dependent 28 KDa calcium binding protein (Taylor et al., 1982; Borke et al., 1988). This protein is inducible by \(1,25(\text{OH})_2\)D₃ and is very
likely related to the movement of calcium across the cell (Feher, 1983; Bronner and Stein, 1988). Thus proximal tubule handles calcium in a manner much different from that of distal tubule.

2.8 Role of phosphorus

Phosphate is widely distributed in non-osseous tissues in both inorganic form and as a component of various structural, genomic and functional macromolecules including phospholipids, phosphoproteins, nucleic acids, glycogen and other intermediates of carbohydrate metabolism. The soft-tissue phosphates comprise only about 15 percent in the mineral phase of bone, primarily as hydroxyapatite, but also as more loosely complexed, amorphous forms of bone crystal (Glimcher and Krane, 1968).

Phosphate is absorbed throughout the small intestine in animals and humans (Wasserman and Taylor, 1973). Phosphate transport in isolated brush border membrane vesicles (BBMV) from intestine exhibit biphasic transport kinetics indicating two components; a saturable Na+-dependent uptake and a linear non-saturable Na+-independent passive diffusion (Paterlik, 1978; Lee et al., 1984). Absorption of phosphate is stimulated by 1,25(OH)₂D₃ (Murer and Hildman, 1981). Phosphate transport in BBMV isolated from intestine suggested that Vitamin D affects the Na⁺ dependent active transport and not the passive diffusible component (Hildman et al., 1982). Phosphate absorption is
progressively impaired by increasing dietary calcium exceeding 2g/day (Spencer et al., 1984).

Elements of renal function regarding phosphate excretion are filtration and reabsorption. Under conditions of normal phosphorus intake and intact parathyroid glands, about 20% of the filtrate load is excreted and 80% is reabsorbed by renal tubules by a process thought to be unidirectional (Mizgela and Quamme, 1985). Na⁺ dependent co-transport has been demonstrated in isolated BBMV prepared from several mammalian species including humans (Hruska and Hammerman, 1981; Beliveau and Brunette, 1984). Phosphaturic effect of PTH in normal humans has been demonstrated, at sites that correspond to the known distribution of PTH-sensitive adenylate cyclase i.e., action is mediated by cyclic AMP (Hammerman and Hruska, 1982; Biber et al., 1983). Dietary phosphate supplementation (Ullrich et al., 1977) or deprivation (Caverzasio and Bonjour, 1985; Levine et al., 1991) rapidly invokes a compensatory increase or decrease in renal phosphate reabsorption.

2.8.1 Renal phosphorus leak

Renal tubular phosphate leak as the primary cause of intestinal hyperabsorption of calcium in idiopathic hypercalciuria has been reported (Gray et al., 1977) and dietary deprivation of phosphorus (Portale et al., 1989) produces mild hypophosphatemia. The low serum phosphate levels stimulate the synthesis of 1,25(OH)₂D₃ which augments
the intestinal absorption of calcium. The hypercalciuria originates from increased filtered load of calcium secondary to the intestinal hyperabsorption and from decreased renal tubular calcium reabsorption, as a consequence of decreased serum PTH level due to hypercalcemia. The mechanism of renal phosphate leak is shown in Fig. 4.

2.9 Role of magnesium

Magnesium (Mg\(^{2+}\)) is a ubiquitous element in nature, including the human body. It forms an estimated 2.1% of the earth's crust (Windholz, 1983). In the human body magnesium (atomic number 12 and mass 24.32 Da) is the second most abundant intracellular cation (second only to potassium) and the fourth most abundant total cation in the body. Magnesium has the smallest ionic radius and thus, has the key role in many metabolic functions and is recognised as a cofactor in over 300 enzymatic reactions involving energy metabolism, protein and nucleic acid synthesis.

2.9.1 State of magnesium

In order to understand the magnesium metabolism, it is important to know the state of magnesium. In most of the biologic systems, Mg\(^{2+}\) is present in 3 different states i.e. bound to protein, complexed to anions and free. Only free magnesium has biological activity, whereas protein bound and complexed magnesium is unavailable. Very limited information about the state of magnesium in tissue is available.
PRIMARY RENAL PHOSPHORUS LEAK

MILD HYPOPHOSPHATEMIA

↑ \(1,25(OH)_2\) VITAMIN D₃

↑ INTESTINAL ABSORPTION OF CALCIUM

MILD HYPERCALCEMIA

↓ PTH

↓ TUBULAR REABSORPTION
OF Ca

↑ FILTERED LOAD OF Ca

HYPERCALCIURIA

FIG. 4 RENAL PHOSPHORUS LEAK
2.9.2 Distribution

Several studies have demonstrated that on an average, the human body contains approximately, one mole of magnesium (Wacker, 1980; Aikawa, 1981; Lenter, 1981). About half the magnesium is present in hard tissue and the other half in soft tissue.

The skeleton is the principal store of magnesium in vertebrate animals. While some authors thought that magnesium is contained in the surface-limited pool of bone in the hydroxyapatite crystals (Pointillart and Gueguen, 1978), others consider that in human 30% of bone magnesium content is in the surfacelimited pool and the remaining 70% bone magnesium is integrated into the bone crystals (Heaton, 1981).

About 10% of the total body magnesium is present in serum and interstitial body fluid. Mean serum concentration of magnesium in human is about 0.85 mmole/L (Aikawa, 1981; Lowenstein and Stanton, 1986). Small sex differences for serum magnesium have been reported, with men having higher concentration than women (Lowenstein and Stanton, 1986). In serum, approximately one third is bound by protein. Of the remaining two third of the serum magnesium, that is ultrafiltrable, approximately 8% is complexed and 92% is free (Kroll and Elin, 1985). The free magnesium in soft tissue varies between 0.3 mmole/L and 4.2 mmole/L depending on the study of the tissue (Blatter and McGuigan, 1986;
Corkey et al., 1986). The distribution of magnesium in an adult human is shown in Table 1.

Circadian variations of plasma and/or erythrocyte magnesium have been described in man and in rat (Touitou et al., 1978; Pflug et al., 1979).

2.9.3 Dietary intake

Plants and animals have an absolute requirement for magnesium. Magnesium is widely distributed in foods. It is an essential part of chlorophyll, hence green vegetables are an important source. In addition, drinking water, especially if it is "hard" water, may be a major source of dietary magnesium. An optimal intake of magnesium of 6 to 10 mg/Kg/day has been recommended (Seeling, 1981). Magnesium intake for the average person also depends closely on the consumption of total number of calories (Flink, 1980). Population survey in United States revealed that in majority of adults, the daily intake of magnesium is below the recommended daily allowance (Pao and Mickle, 1981; Marier, 1982). Studies have demonstrated that refining/processing of various food stuffs cause a significant loss of magnesium from them (Marier, 1986; Kazue et al., 1990). Thus, refining and processing of food leads to a significant decrease in daily intake of magnesium and greater vulnerability to magnesium deficiency. The magnesium concentration of selected foods and beverages is shown in Table 2.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration (mmole/Kg wet wt)</th>
<th>Content (mmole)</th>
<th>% of total body magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>3.0</td>
<td>2.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>2.0</td>
<td>5.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>22.7</td>
<td>193.0</td>
<td>19.3</td>
</tr>
<tr>
<td>Muscle</td>
<td>30.0</td>
<td>270.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Bone</td>
<td>12.3</td>
<td>530.1</td>
<td>52.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>70.0</strong></td>
<td><strong>1000.7</strong></td>
<td><strong>100.0</strong></td>
</tr>
<tr>
<td>Nuts (Raw)</td>
<td>Cereals (Dry)</td>
<td>Legumes (Prepared)</td>
<td>Vegetables (Raw)</td>
</tr>
<tr>
<td>------------</td>
<td>---------------</td>
<td>--------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Almonds</td>
<td>296</td>
<td>100% Bran</td>
<td>473</td>
</tr>
<tr>
<td>Cashews</td>
<td>260</td>
<td>Oats</td>
<td>148</td>
</tr>
<tr>
<td>Peanuts</td>
<td>180</td>
<td>Cheenos</td>
<td>138</td>
</tr>
<tr>
<td>Walnuts</td>
<td>169</td>
<td>Wheaties</td>
<td>109</td>
</tr>
<tr>
<td>Pecans</td>
<td>128</td>
<td>Froot loops</td>
<td>25</td>
</tr>
<tr>
<td>Coconut</td>
<td>32</td>
<td>Cornflakes</td>
<td>12</td>
</tr>
<tr>
<td>Dates</td>
<td>35</td>
<td>Turkey</td>
<td>24</td>
</tr>
<tr>
<td>Raisins</td>
<td>33</td>
<td>Pork</td>
<td>22</td>
</tr>
<tr>
<td>Bananas</td>
<td>29</td>
<td>Fish</td>
<td>22</td>
</tr>
<tr>
<td>Figs</td>
<td>17</td>
<td>Chicken</td>
<td>21</td>
</tr>
<tr>
<td>Oranges</td>
<td>10</td>
<td>Beef</td>
<td>18</td>
</tr>
<tr>
<td>Apples</td>
<td>5</td>
<td>Frankfurter</td>
<td>10</td>
</tr>
</tbody>
</table>

(mg/100 g edible portion)
Radioisotope studies using $^{28}\text{Mg}$ has demonstrated that magnesium is absorbed primarily in the small intestine (Engelhardt et al., 1983; Schwartz et al., 1984). In several studies, the major site for magnesium absorption has been shown to be the colon (Chutkow, 1966; Meneely et al., 1982), whereas, others demonstrated that greatest rate of magnesium absorption occurs in duodenum (Urban and Schedl, 1969; Aldor and Moore, 1970). In the normal individual consuming a balanced diet, approximately 40 to 70% of the ingested magnesium appears to be absorbed (Schwartz et al., 1984; Graber and Schulman, 1986). Studies by Graham et al., (1960) reported that absorption of magnesium begins within one hour of ingestion and continues at a steady state uniformly throughout the small intestine for 2 to 8 hours. Intestinal magnesium transport has been reported to occur by diffusion (Ebel and Gunther, 1980), solvent drag (Behar, 1974) and/or a saturable process that may (Meneely et al., 1982) or may not (Roth and Werner, 1979) require metabolic energy. In vitro studies on rat have shown that the mechanism is sodium dependent and in normal conditions magnesium transport is obtained inspite of a net electrochemical gradient (Behar, 1974).

Many factors in the diet are known to influence the absorption of magnesium. The intake of diet also alters absorption. On a low magnesium diet upto 75% of ingested
magnesium may be absorbed, while on a high magnesium diet as low as 25% of ingested magnesium may be absorbed (Graham et al., 1960). Protein-rich diet (Lindeman, 1980), glucids, more particularly lactose and glucose (Tadayon and Lutnak, 1969) are known to enhance the magnesium absorption. An excess of lipids (Tadayon and Lutnak, 1969; Rayssiguier, 1981), high fibre content (Saito and Suto, 1989; Reinhold et al., 1991) and cellulose (Slavin and Marlett, 1980) are known to decrease magnesium absorption. Fibres are rich in phytates and oxalates and form insoluble complexes in the gastrointestinal tract (Bagheri and Gueguen, 1983). The calcium and phosphorus quantity in the diet also influences the magnesium absorption (Norman et al., 1981; Miyazawa and Yoshida, 1991). A variety of malabsorption syndromes particularly associated with steatorrhea and chronic renal diseases of various severity have also been reported to reduce intestinal absorption of this ion (Wizemann et al., 1982; Spencer, 1986). Recently, it has been reported that, short chain fatty acids derived from fermentation of carbohydrates in the large intestine stimulate magnesium absorption by delivering protons to Mg$^{2+}$/H$^+$ exchanger located in the apical membrane of the epithelium (Scharrer and Lutz, 1990).

2.9.5 Renal handling

The major excretory pathway for the absorbed magnesium is through kidney, which is the main regulator of
serum concentration and total body content of magnesium. The daily renal excretion of magnesium ranges from 100 to 150 mg/24 hours for an individual on a normal diet (Wacker, 1980; Aikawa, 1981). Thus for an individual in magnesium balance, amount of magnesium absorbed from small intestine is similar to the amount excreted in the kidney. Circadian rhythmicity in the excretion of magnesium by the kidney with the maximum excretion at night has been reported (Luce, 1970; Wangoo et al., 1989).

A filtration-reabsorption mechanism for the renal handling of magnesium has been suggested. Approximately 70 to 80% plasma magnesium is ultrafiltrable. Protein bound magnesium does not pass through the glomerular membrane. In a normal individual, about 2 g of magnesium passes from the plasma into the glomerular filtrate each day (Quamme and Dirks, 1986), but only about 6% of the filtered magnesium appears in the urine, due to effective tubular reabsorption of magnesium by the kidney.

Micropuncture studies have indicated that net magnesium absorption is highly dependent on magnesium delivery to the respective segment (Quamme and Dirks, 1986; Roy, 1987). The thick ascending limb of the loop of Henle appears to be the major site of magnesium reabsorption (Carney et al., 1980).

Though the cellular mechanisms involved in the transepithelial transport of magnesium at this site have not
been elucidated, however, it is known to be a major regulatory site for renal homeostasis of magnesium. Increased reabsorption of magnesium by hypomagnesemia, hypocalcemia, parathyroid hormone, calcitonin and increased luminal magnesium concentration have been reported at this site (Beyenbach, 1986; Quamme and Dirks, 1986).

20 to 30% of the filtered magnesium is absorbed along the proximal tubule (Quamme and Dirks, 1986). Approximately 65% of the filtered magnesium is reabsorbed in the loop of Henle, while 5-10% is reabsorbed in distal tubule at nephron. While some investigators (Rios et al., 1977; Sachtjen et al., 1979) have produced evidence for magnesium secretion under certain experimental conditions, others (Alfredson and Walser, 1970; Brunette et al., 1976) have failed to demonstrate magnesium secretion at these sites.

Enhanced renal magnesium excretion on intravenous saline infusion as well as volume expansion due to intravenous fluids or primary aldosteronism have been reported (Massry et al., 1967; Wacker and Parisi, 1968). A number of drugs are known to produce renal wasting of magnesium. Diuretics particularly, the loop diuretics, furosemide and ethacrylic acid may cause significant magnesium diuresis (Duarte, 1968; Martin, 1969). Alcoholism (Martin, 1969), starvation or fasting (Drenick et al., 1975) also cause renal magnesium wasting.
2.9.6 Homeostatic mechanisms

Intestinal absorption, distribution to extracellular, intracellular and skeletal compartments and urinary excretion of magnesium are very accurately regulated, which help in constant plasma levels of magnesium. The homeostatic mechanisms for maintaining the serum concentration within limits are poorly understood. The major factor that appears to regulate magnesium balance are absorption from the gastrointestinal tract and excretion by the kidney. The kidney is the most important organ for controlling the serum magnesium.

Parathyroid hormone (PTH) plays a major role in the regulation of magnesium metabolism (Medalle et al., 1976; Rayssiguier, 1977). Involvement of magnesium in PTH biosynthesis and/or secretion using human (Wagner et al., 1982) and bovine hormone (Habener and Potts, 1976) have been demonstrated. PTH release is inversely proportional to magnesium concentration.

Both the PTH and active form of Vitamin D i.e., $1,25\text{(OH)}_2 \text{D}_3$ promotes the intestinal absorption of magnesium in both humans and rats (Engelhardt, 1983). PTH seems to have the more direct effects on magnesium. The hormone secretion is influenced by plasma magnesium. Studies by Dirks and Quamme (1980) showed that, in rat parathyroidectomy produces a decrease of tubular reabsorption of magnesium, whereas PTH injection induces an
increase in tubular reabsorption. Vitamin D₃ and PTH are also known to increase the bone magnesium content in most species (Pointillart and Gueguen, 1978; Heaton, 1981).

Several authors have described a close relationship between PTH and Vitamin D metabolism, as stimulation of 1-hydroxylase has been reported (Garabedian et al., 1972). 1,25 (OH)₂ D₃ synthesis is increased in some cases of human hyperparathyroidism (Kaplan et al., 1977), while in hypoparathyroidism PTH-extract infusion produces an increase in 25-hydroxy D₃ hydroxylation to 1-α-25-dihydroxy D₃ (Asknes and Aarskog, 1980).

Calcitonin is normally not very active upon magnesemia. Its activity, if any, is a secondary effect to modification in calcium metabolism produced by a disturbance in magnesium metabolism (Bennaceur et al., 1983). Role of calcitonin in the postprandial regulation of magnesium metabolism has been implicated (Barlet et al., 1974).

Estrogens are known to stimulate bone accretion of magnesium by reducing osteoclastic activity and release of minerals (Goldsmith et al., 1970).

2.9.7 Assessment of magnesium status

The assessment of magnesium status provides a challenge to clinical laboratory and biomedical technology. Blood is the tissue of choice for the assessment of most analytes used in clinical medicine. Tests for the assessment of magnesium status, broadly can be divided into three
functional categories: tissue magnesium, physiologic assessment of magnesium and free magnesium.

2.9.7.1 Tissue magnesium

Determination of the concentration or content of magnesium in tissue is the most common tests used today for the assessment of magnesium status in patients. The four tissues used to evaluate magnesium status are mononuclear blood cells (MBC), muscle, red blood cells (RBC) and serum. Measurement of tissue magnesium has limitations, as very little information can be gathered about, what tissue magnesium pools are in equilibrium with other tissue pools and the state of magnesium. Lack of correlation for magnesium between MBCs and serum or RBCs have been reported by investigators (Elin and Hosseini, 1987).

Controversial reports are available in literature regarding the correlation between muscle magnesium and other tissues in humans. Some authors (Lim and Jacob, 1972; Wester and Dyckner, 1980) found no correlation between serum and muscle magnesium. On the contrary, studies in human and experimental animals have shown a correlation of muscle magnesium concentration with MBC magnesium (Dyckner and Wester, 1985; Sjogren et al., 1986), muscle potassium (Alfrey et al., 1974) and glycosylated hemoglobin in RBCs from patients with type 1 diabetic mellitus (Sjogren et al., 1986). Lack of correlation between serum and RBC magnesium
in normal individuals has been reported (Elin and Hasseini, 1985).

Estimation of serum has been determined far more frequently than in any other tissue. In a recent study, Fischer and Giroux (1991) concluded that plasma magnesium concentration is the most useful indicator of magnesium status. Strong correlations were observed between dietary intake and plasma concentration of magnesium, between intake and femur concentration and between plasma and femur concentrations of magnesium.

2.9.7.2 Physiologic assessment of magnesium

Though balance and isotope studies provide useful information regarding the absorption, retention and excretion of magnesium in the body, yet their use is limited to research.

Renal excretion of magnesium provides a valuable assessment for magnesium status, as it is dependent on the absorption of magnesium from small intestine and kidney function.

Retention of magnesium following parenteral or oral administration of magnesium load has been used to assess magnesium status. This test provides a more sensitive index of magnesium deficiency (Danielson et al., 1979).

2.9.7.3 Free magnesium

It is known that only free magnesium has biological activity and magnesium bound to anions or proteins is not
available. Many techniques such as ion-selective electrode, metallochrome dyes, nuclear magnetic resonance spectroscopy and ultrafiltration/equilibrium dialysis have been employed.

Controversial results regarding the concentration of free magnesium in the intracellular compartments using ion-selective electrode (Blatter and McGuigan, 1986) or metallochrome dyes (Durham and Walton, 1983; Heinonen and Akerman, 1987) has been reported.

Nuclear magnetic resonance spectroscopy (NMRS) make use of $^{31}$P and $^{25}$Mg without damaging the specimen (Gupta et al., 1983; Ryzen et al., 1989; London, 1991). Free magnesium may be estimated directly with $^{25}$Mg using this technique (Rose et al., 1980).

Use of ultrafiltration/equilibrium dialysis techniques help in separating free and complexed magnesium fractions from protein-bound fraction, but require very rigid conditions for accuracy such as anaerobic conditions. This technique is also of very limited use to the research field.

2.9.8 Magnesium deficiency

Magnesium is of great importance in biology. Studies by Leroy (1926) for the first time demonstrated the need for magnesium in mammals in order to sustain growth and life. In first of a series of elegant studies, Kruse et al., (1932) described the experimental magnesium deficiency in rats. This study was quickly followed by reports on clinical
symptoms in patients with hypomagnesemia (Hirschfelder, 1934). Since that time a number of studies on experimental animals and humans have clearly defined the physiological consequences of magnesium deficiency (Shils, 1969).

Gitelman and Welt (1969) defined the magnesium deficiency as "a reduction in total body magnesium content". Unfortunately this definition of magnesium deficiency is incompatible with the current technology. Thus, from a practical point of view, magnesium deficiency has been defined as a serum magnesium concentration below the reference interval for the laboratory. Thus serum magnesium concentration as the standard marker of magnesium status is generally accepted (Elin, 1988).

Four general categories of causes of clinical hypomagnesemia have been described (Whang, 1987), which include: gastrointestinal, renal, endocrine disorders with renal wasting and miscellaneous. Gastrointestinal factors include alcoholism, malnutrition, prolonged intravenous therapy (without adequate magnesium replacement), gastric suction, intestinal bypass surgery, diarrhea, laxative abuse, malabsorption etc. Renal losses of magnesium are associated with some diuretics and antibiotics, hypercalcemic states, aldosteronism etc. Endocrine disorders with renal wasting include: diabetic ketoacidosis, hyperparathyroidism, hyperthyroidism and hyperaldosteronism. Excessive lactation, exchange transfusions and acute
intermittent porphyria are included in the miscellaneous category. Symptomatic clinical magnesium deficiency usually develops in a setting of predisposing and complicating disease states, that often is associated with reduced intestinal absorption of magnesium or impaired renal reabsorption.

The biologic effects of magnesium depletion have been studied in many species. The interrelationship between magnesium and calcium homeostasis still remains a matter of dispute. Hypocalcemia is characteristically observed during magnesium deficiency in a number of animals including calves (Smith, 1961), chicks (Reddy et al., 1973), sheep (L'Estrange and Axford, 1964), dogs (Levi et al., 1974), monkeys (Dunn, 1971) and humans (Estep et al., 1969; Muldowney et al., 1970). The rat is the only experimental animal that consistently develops hypercalcemia in response to magnesium depletion (McIntyre and Davidsson, 1958; Whang and Welt, 1963; Gitelman et al., 1968).

2.9.8.1 Mechanism of hypocalcemia in magnesium deficiency

Hypocalcemia has been attributed to the impaired function of the parathyroid gland. Low circulating immunoreactive parathyroid hormone (IPTH) in a number of magnesium-deficient hypocalcemic patients has been described (Rude et al., 1976, 1978; Allgrove et al., 1984). An intravenous injection of magnesium produced a marked increase in IPTH concentration within minutes. Similar
responses were found in magnesium-deficient hypocalcemic patients, in whom basal IPTH levels were normal or elevated. This observation suggests that impaired PTH secretion is a consistent abnormality in these patients (Rude et al., 1978).

End-organ resistance to PTH has also been demonstrated in hypocalcemic magnesium deficiency (Rude et al., 1976; Allgrove et al., 1984). Impaired generation of cAMP as determined by the urinary excretion of cAMP in response to PTH extract has been shown in some, but not all patients with severe magnesium deficiency (Rude et al., 1976).

Skeletal resistance to PTH has also been considered as a factor contributing to the hypocalcemia. A subnormal calcemic response to parathyroid extract has been reported in a number of such patients (Woodard et al., 1972). The concept of skeletal resistance to PTH action is further supported by in vitro studies demonstrating that PTH produced submaximal release of calcium from bones of magnesium deficient rats (MacManus et al., 1971).

The mechanism by which magnesium deficiency impairs PTH secretion has been attributed to adenylate cyclase-cyclic AMP system. The stimulation of PTH secretion by hypocalcemia is mediated by plasma-membrane bound adenylate cyclase (Abe and Sherwood, 1972). It has been demonstrated that the human PTH gland adenylate cyclase is magnesium
dependent (Rodriquez et al., 1978). Reduced urinary cAMP excretion in response to parathyroid extract administration has been related to the depressed activity of renal adenylate cyclase (Rude et al., 1976).

Skeletal resistance to PTH has also been related to reduced adenylate cyclase activity, based on the observation that in isolated perfused bone from magnesium-deficient dogs, there was impaired generation of cyclic AMP (Freitag et al., 1979).

Hypocalcemia of magnesium deficiency is also resistant to the action of Vitamin D (Medalle et al., 1976), which may be due to defect in Vitamin D metabolism. Reversal of PTH end-organ resistance on magnesium therapy returns the serum calcium concentration to normal by enhancing PTH secretion (Allgrove et al., 1984; Cholst et al., 1984).

Resistance to large doses of Vitamin D, 25-hydroxy Vitamin D and dihydroxytachysterol in magnesium depleted patients with rickets (Reddy and SivaKumar, 1974), idiopathic hypoparathyroidism (Rosler and Rabinowitz, 1973), hypocalcemia associated with gastrointestinal disorders (Selby et al., 1984) and variable circulating levels of Vitamin D metabolites (Fuss et al., 1985; Rude et al., 1985) have been reported. Resistance to Vitamin D in magnesium depletion has been related to impaired skeletal response to 1,25(OH)₂ D₃ (Carpenter et al., 1987) or
insufficient production of $1,25(\text{OH})_2\text{D}_3$ (Weaver and Welsh, 1989).

2.9.8.2 Magnesium deficiency in rats

Magnesium deficiency in rats has been shown to result in significant hypomagnesemia, hypophosphatemia, hypercalcemia and hyperphosphaturia (McIntyre and Davidsson, 1958; Whang and Welt, 1963; Gitelman et al., 1968; Tongyai et al., 1989; Okuno et al., 1990). These changes suggest a state of hyperparathyroidism. Magnesium deficiency also induces imbalances of serotonin (Itokawa et al., 1972b, 1974a), catecholamine (Itokawa et al., 1974b; Kimura and Itokawa, 1975), thiamine (Itokawa et al., 1972a; Kimura and Itokawa, 1977; El-Hindi and Amer, 1989). Significant decrease in magnesium levels in other organs such as spinal cord, kidney, testis and bone are also reported (Kimura and Itokawa 1977, 1989). Magnesium deficiency results in significant increased levels of calcium in kidney and decreased levels in the bone of rats. Perturbances in trace element metabolism has also been found (Kimura and Itokawa, 1989).

Metal analysis of erythrocytes from magnesium-deficient rats revealed a significant reduction in magnesium and potassium with a concomitant increase in sodium and calcium (George and Heaton, 1975; Heaton et al., 1989). These changes in metal ion concentration suggest the
increased permeability of the red cell membrane during magnesium deficiency.

Hypokalemia is also a frequent finding in magnesium deficiency (Gitelman et al., 1966; Shils, 1969). It develops secondary to impaired ability of the kidney to reabsorb potassium (Shils, 1969). Renal wasting and decreased intracellular potassium (Whang et al., 1967) are thought to be related to an impaired ability of the Na⁺-K⁺ pump to maintain normal intracellular concentrations of potassium and sodium. The impairment of ion transport is probably explained by the magnesium dependency of adenosine triphosphatase, an enzyme required for normal function of the Na⁺-K⁺ pump (Seller et al., 1970).

Cellular loss of magnesium has been found to occur in severe deficiency (MacIntyre and Davidsson, 1958; Martindale and Heaton, 1964). Cell fractionation studies in magnesium-deficient rat from liver and kidney, suggested that mitochondrial fraction may be the most vulnerable organelle during prolonged restriction of magnesium (Bunce et al., 1974; George and Heaton, 1975).

Nephrocalcinosis has been reported by many investigators studying laboratory rats fed on magnesium-deficient diet (MacIntyre and Davidsson, 1958; Bunce et al., 1974; Al-Modhefer et al., 1986). In nephrocalcinosis, precipitation of calcium phosphate occurs initially in proximal tubule brush border. The crystals subsequently move
down the nephron, enlarging by further mineralization and collection of cellular debris. Eventually blocking of tubule forming characteristic deposits around the kidney occurs (Bunce et al., 1980).

It has been reported that magnesium deficiency does not impair the ability of the kidneys to concentrate urine (Manitius and Epstein, 1963; Grimm et al., 1990). Single nephron function has been found to be altered in nephrocalcinosis, but the whole kidney function remains largely unaffected by the lesion (Al-Modhefer et al., 1986; Grimm et al., 1990). Many studies have demonstrated that magnesium deficiency accelerates renal tubular calcium oxalate deposition in rats fed ethylene glycol (Rushton and Spector, 1982; Ebisuno et al., 1987). Increased excretion of the metabolites of aromatic amino acid tryptophan including kynurenine, xanthurenic acid and anthranilic acid into the urine and a significant decrease in tryptophan pyrrolase activity has been reported in magnesium-deficient rats (Shibata et al., 1989). Kimura et al., (1990) reported a significant decreased manganese concentration in plasma and all tissues except blood. A decrease in pyruvate carboxylase activity in liver crude mitochondria and a positive correlation between enzyme activity and manganese concentration in liver was observed in magnesium-deficient rats. Recently it has been demonstrated that magnesium
deficiency affects bone mineral metabolism and induce loss of bone mineral content (Okuno et al., 1990).

2.9.8.2.1 Hypercalcemia and hyperparathyroidism

Hypercalcemia in magnesium-deficient rats is the result of the stimulation rather than the depression of PTH secretion by the presence of intact parathyroid glands (Heaton, 1965; Gitelman et al., 1968). Hypercalcemia results from an alteration in the equilibrium between the bone mineral and extracellular fluid, increased gastrointestinal absorption of calcium or from enhanced renal tubule reabsorption of calcium or a combination of these factors. Several surveys have been undertaken to determine the incidence of hypercalcemia and hyperparathyroidism (Heath et al., 1980), which has been reported in 2-17% of patients with nephrolithiasis (Broadus, 1980; Galic et al., 1989; Jaeger et al., 1986).

2.9.8.2.2 Gastrointestinal absorption in primary hyperparathyroidism

Increased gastrointestinal absorption of calcium has been reported in patients of hyperparathyroidism (Kaplan et al., 1976, 1977).

Vitamin D is required for intestinal hyperabsorption of calcium (Deluca, 1984) and parathyroid hormone is a known stimulator of the production of 1,25(OH)₂D₃. Increased blood levels of 1,25(OH)₂D₃ in hyperparathyroid patients have been reported. A correlation between 1,25(OH)₂D₃ and the
retention of an oral load of $^{47}$Ca has also been found (Gray et al., 1977; Kaplan et al., 1977). Thus, intestinal absorption of calcium may be increased in primary hyperparathyroidism because of increased production of $1,25(OH)_2D_3$.

2.9.8.2.3 Renal tubular calcium reabsorption in primary hyperparathyroidism

Small contribution of bone in relation to the maintenance of hypercalcemia has been suggested (Parfitt, 1975). Hyperabsorption of calcium from the intestine and increased renal tubular conservation of filtered calcium has been suggested to play a major role (Nordin and Peacock, 1969). These authors suggested that PTH-induced increase in tubule reabsorption of filtered calcium is most important in the maintenance of hypercalcemia, in hyperparathyroidism. Despite the fact that PTH stimulates tubule calcium reabsorption, urinary calcium excretion may be greatly elevated in those patients with primary hyperparathyroidism, who form renal stones even when hypercalcemia is slight (Parks et al., 1980). Elevated levels of circulating $1,25(OH)_2D_3$ have been suggested as a cause of the marked hypercalciuria seen in patients (Broadus, 1980).

2.9.8.2.4 Plasma magnesium and hyperparathyroidism

Magnesium is present in serum as ultrafiltrable (including an ionized form and a complexed form) and protein-bound fractions (Ebel and Gunther, 1980).
Parathyroid hormone (PTH) appears to have rather similar regulatory effects on magnesium and calcium (Ebel and Gunther, 1980; Koeger et al., 1983). The hormone increases bone resorption and tubular reabsorption and probably also increases magnesium intake by the digestive tract, resulting in an increase in serum magnesium concentration (Massry and Coburn, 1973; Quamme and Dirks, 1986). However, only little change in serum magnesium as compared to serum calcium concentration has been observed in primary hyperparathyroidism (King and Stanbury, 1970). Since serum magnesium is partly non protein-bound, it can be speculated that its ionized form, possibly the biologically active part regulated by PTH, might be increased in primary hyperparathyroidism.

In a recent study Claeyssens et al., (1990) studied the serum total, ultrafiltrable and protein-bound magnesium and urinary fractional excretion of magnesium in patients with primary hyperparathyroidism. While the total serum magnesium remained unaltered, ultrafiltrable and the protein-bound fractions of the serum magnesium were significantly higher and lower respectively than the control group, suggesting that PTH might induce changes in serum magnesium distribution. A significant correlation was observed between the total and the ultrafiltrable fractions of magnesium, which, suggested that the renal function had
2.9.9 Magnesium and biomembranes

Defective membrane function has been implicated to be the primary lesion underlying the cellular disturbances that occur in magnesium deficiency. Studies have shown that removal of magnesium from normal mitochondria incubated in vitro causes mitochondrial swelling and increase permeability to monovalent cations (Barnard and Cockrell, 1982). Indirect evidence also suggests that an increased permeability to protons of the inner membrane from liver mitochondria during magnesium deficiency in vivo (Heaton and Elie, 1984). Recently, increased lipid peroxidation of liver mitochondria has been demonstrated in magnesium-deficient rats (Guenther and Hoellriege, 1989).

Several studies have shown the effect of dietary magnesium depletion on the properties of the erythrocyte membrane. Increased fluidity of erythrocyte ghosts, reduced osmotic fragility to hypotonic saline and reduced microviscosity has been demonstrated in magnesium-deficient rats (Heaton et al., 1987, 1989; Tongyai et al., 1989). Morphological changes of red blood cells as shown by scanning electron microscopy revealed that the bioconcavity of the cell is lost and cells give flattened appearance with surface irregularities (Tongyai et al., 1989).
The increase in membrane fluidity of red blood cell membrane has been related to the decreased amounts of cholesterol and sphingomyelin, thereby reducing the ratios of cholesterol/phospholipid and sphingomyelin/phosphatidylcholine (Heaton et al., 1989; Tongyai et al., 1989). Thus increased fluidity of erythrocyte membrane occurs due to physicochemical exchange with the plasma. Chemical analysis of membranes suggested that the loss of magnesium from the membrane or the changes in the ratios of lipid constituents are responsible for increased fluidity. Calcium is known to make the membrane more rigid (Shinitzky and Barenholz, 1978), but no detectable loss of calcium from the erythrocyte membrane has been observed, and the concurrent hypercalcemia in the rats makes this unlikely. It is plausible to postulate that magnesium acts by binding to the negatively charged phosphates of the phospholipid headgroups, which stabilizes the bilayer structure and that loss of magnesium has the opposite effect (Tongyai et al., 1989).

Lipid-protein interactions with a biological membrane may modify fluidity and both the lipid-to-protein ratio and the types of protein present may be important (Agar and Board, 1983). However, several observations have indicated that, it is not the proteins that bring about the changes in membrane but anisotrophy in magnesium deficiency, as the total protein content of red blood cell membrane remains
unaltered and structural changes occur in the lipid bilayer region (Tongyai et al., 1989).

Jaya and Kurup (1987) reported an increase in cholesterol and triacylglycerol content in the serum and increased release of lipoproteins in the circulation in magnesium-deficient rats. Studies on the effect of magnesium deficiency on lipid metabolism in Porcine-Kidney Cells (LLK-PK) revealed that magnesium deficiency has adverse effects on cellular membrane properties and functions. Magnesium deficiency perturbs the essential fatty acid metabolism and decrease in the cellular membrane polyunsaturated fatty acid content (Mahfouz et al., 1989a). Phospholipid composition showed a decrease in phosphatidylethanolamine, phosphatidylserine, sphingomyelin, phosphatidylinositol and an increase in phosphatidylcholine, without altering the total phospholipid content of the membrane, as determined by decreased rate of sphingomyelin synthesis (Mahfouz et al., 1989b).

2.9.10 Magnesium and lipid metabolism

In experimental magnesium deficiency, hypertriglyceridemia, hypercholesterolemia with an increase of free cholesterol and decrease of esterified cholesterol has been reported (Rayssiguier, 1985; Jaya and Kurup, 1987). Magnesium-deficient rats also develop dyslipoproteinemia characterized by an increase in very low density lipoproteins and low density lipoproteins and decrease of
Changes in plasma fatty acids were also observed in magnesium-deficient rats and is characterized by a decrease of stearic and arachidonic acids and an increase in oleic and linoleic acids (Rayssiguier et al., 1986). Increased plasma levels of thio-barbituric acid reacting substances used as a measure of lipid peroxidation has also been reported in magnesium-deficient rats (Mahfouz and Kummerow, 1989). Recently, it has been demonstrated that oral supplementation of rabbits fed a high cholesterol diet with the magnesium salt, not only reduces the serum cholesterol and triglycerides but also attenuates the atherosclerotic process markedly. In addition magnesium deficiency augments atherogenesis markedly (Altura et al., 1990; Singh et al., 1991).

2.9.11 Therapeutic aspects of magnesium

Magnesium supplementation has been reported to be beneficial in the prevention of recurrent formation of calcium oxalate renal stones (Tiselius et al., 1980; Gulati et al., 1988; Jarrar et al., 1989). The theoretical support for the therapeutic use of magnesium in the renal stone disease is based on the observations that magnesium bind oxalate in the gastrointestinal tract (Barilla et al., 1978; Berg et al., 1986), increases the solubility of calcium oxalate (Berg et al., 1986) and inhibits the precipitation
of calcium oxalate (Desmars and Tawashi, 1973) and calcium phosphate (Bisaz et al., 1978).

Magnesium has been shown to decrease both the growth and nucleation rates of calcium oxalate crystals (Li et al., 1985; Kohri et al., 1988). Magnesium ions act as a stabilizer of thermodynamically unstable calcium oxalate dihydrate and prevents its conversion to calcium oxalate monohydrate, which is a major constituent of stones (Toshitsugu et al., 1987). Previous studies have shown minimal rise in urinary magnesium following oral administration of either magnesium oxide or magnesium hydroxide. This may be due to its low solubility and absorbability in the intestinal tract. This finding may account for reports of marginal responsiveness and limited popularity of these magnesium salts in prevention of recurrent nephrolithiasis.

Lindberg et al., (1990) found that magnesium citrate was more soluble and absorbable from the intestinal tract than magnesium oxide. Supplementation of magnesium citrate to either hyperoxaluric rats (Ogawa et al., 1990) or to humans (Lindberg et al., 1990a) has been demonstrated to produce much better effects in not only increasing the urinary excretion of magnesium, but also of citrate, an important inhibitor of calcium oxalate crystallization.

Although several reports confirm the clinical efficacy of magnesium salts in calcium oxalate
nephrolithiasis, these salts have a limited citraturic action (Lindberg et al., 1990a) and do not prevent hypokalemia in patients with enteric hyperoxaluria or those treated with thiazide. Recently, it has been reported that a new compound potassium-magnesium citrate gives an optimum citraturic response in addition to providing absorbable potassium and useful to patients of hypercalciuric nephrolithiasis treated with thiazide and to patients suffering from enteric hyperoxaluria or are hypomagnesuric or hypocitraturic or both (Koenig et al., 1991).

2.9.12 Magnesium and Vitamins

Urolithiasis is a multifactorial disease. Nutritional deficiencies, particularly of vitamins, play an important role in the genesis of calcium oxalate nephrolithiasis. Diet deficient in vitamins (fat-and water-soluble) are known to influence the urinary tract stone formation.

2.9.12.1 Vitamin B₁

Thiamine exerts its physiological actions in the body in the form of the coenzyme thiamine-pyrophosphate. The conversion of thiamine into its coenzyme form is accomplished with adenosine triphosphate as a pyrophosphate donor. Magnesium is a known co-factor for many enzymes and enzyme systems. Virtually all enzymes for which phosphate or a phosphate-containing compound is the substrate and those for which thiamine pyrophosphate is the cofactor are dependent on magnesium for activation (Wacker, 1980).
Magnesium deficiency has a dual negative influence on thiamine metabolism; the conversion of thiamine into its pyrophosphate form is inhibited and the action of thiamine pyrophosphate is prevented. The importance of a normal magnesium status for thiamine metabolism has been demonstrated in magnesium-deficient rats (Zieve et al., 1968; Zieve, 1969).

Itokawa et al. (1972a) studied the effect of magnesium deficiency on the distribution of thiamine. Thiamine content of sciatic nerve, liver and kidney was found to be decreased. Subcellular fractions of the liver of magnesium deficient rats showed that the thiamine content was most markedly lowered in the mitochondrial fraction (Itokawa et al., 1974c).

Thiamine deficiency is most commonly diagnosed in alcoholics, but gastrointestinal disorders with prolonged diarrhea may also lead to a successive depletion of thiamine. These two patient categories very often have simultaneous magnesium deficiency (Flink et al., 1954; Flink, 1976). Aggravation of thiamine deficiency by magnesium depletion has been reported (Dyckner et al., 1985).

Thiamine deficiency may also result from inadequate intake of thiamine or consumption of food containing antithiamine factors. The effect of consumption of such foods among Thai population has been demonstrated
Thiamine is thermolabile and is destroyed, when food is cooked and the percent destroyed depends upon a number of factors e.g., pH, temperature reached and presence of reducing sugars (Fox et al., 1983; Doyon and Smyrl, 1983).

Thiamine is involved in the metabolism of glyoxylate. Thiamine pyrophosphate (TPP), is an essential cofactor in the synergistic decarboxylation of glyoxylate via ω-ketoglutarate; glyoxylate carboligase enzyme, a major pathway in the glyoxylate metabolism (Stewart and Quayle, 1967). Thiamine deficiency in rats (Hauschildt et al., 1972; Sidhu, 1985) and man (Buckle, 1963) leads to increased levels of glyoxylate in the tissues and urine. Hyperoxaluria observed in thiamine deficiency has been attributed to increased liver GAO activity, and a significant decrease in the decarboxylation of glyoxylate both via the glyoxylate oxidation cycle and ω-Ketoglutarate: glyoxylate carboligase enzyme in liver and kidney mitochondria of rats (Sidhu et al., 1987a). A significant decreased body weight gain and liver thiamine content with a significant elevation of serum triacylglycerol in magnesium-deficient rats has been reported (El-Hindi and Amer, 1989).

Thiamine deprivation has been found to be associated with decreased rate of glucose synthesis via inhibition of activities of glucose-6-phosphatase and fructose 1,6 diphosphatase enzymes in liver and kidney, and also

The TPP effect has been used as a reliable index to evaluate the nutritional status of thiamine. Studies by Takeuchi et al., (1990) indicated that thiamine pyrophosphate effect really reflects the saturation status of transketolase with coenzyme.

Studies by Itokawa et al., (1990) demonstrated that as far as thiamine and magnesium are concerned, high dietary fiber acts counter to sound health. Metabolism, biochemical functions, deficiency signs and various other aspects of thiamine have recently been reviewed (Gubler, 1991).

2.9.12.2 Vitamin B₆

Vitamin B₆ has a stupendous importance for the total health of the human body. Coenzyme of Vitamin B₆ i.e., Pyridoxal-5'-phosphate is essential to some 18 of the 20 amino acids, which make up the structures of countless proteins, enzymes and polypeptide hormones of the entire human body. Studies using radioactive tracers to examine Vitamin B₆ metabolism had been conducted in humans and rats (Coburn et al., 1984). The nutritional status of a person with respect to Vitamin B₆ is a function of the quantity of Vitamin B₆ ingested and its bioavailability. Additional information concerning the bioavailability has been extensively reviewed (Gregory, 1988; Leklem, 1988).
Pyridoxal-5'-phosphate is a cofactor in a number of transaminase reactions with glyoxylate as one of the substrates. There is a strong experimental evidence that Vitamin B₆ deficiency leads to hyperoxaluria and stone formation by increasing the glyoxylate pool in experimental animals (Farooqui et al., 1981; Nath et al., 1984; Sidhu, 1985; Ravichandran and Selvam, 1990a).

Several studies indicate a dietary Vitamin B₆ intake far below recommended daily allowance in large proportions of the elderly population (Guilland, 1984; Lowik et al., 1989). Marginal deficiency of Vitamin B₆ has also been reported in recurrent stone formers (Murthy et al., 1982a). Increased levels of oxalate synthesizing enzymes in liver and kidney (Murthy et al., 1982b), hyperabsorption of oxalate from intestine (Sidhu et al., 1986; Gupta, 1986) and increased reabsorption of oxalate by renal tubular cells (Gupta et al., 1988) has been implicated in the pathogenesis of urinary calculi in the pyridoxine deficiency. Recent study from our laboratory demonstrated that a high affinity oxalate binding protein is induced in the intestine brush border membrane in pyridoxine deficiency, which is responsible for the hyperabsorption of oxalate from the intestine (Nath et al., 1990).

Increased lipid peroxidation in kidneys of Vitamin B₆ deficient rats has been reported (Ravichandran and Selvam, 1990a). A significant increase in calcium and oxalate levels
in nuclear, mitochondrial and microsomal fractions in the 
kidneys of Vitamin B₆-deficient rats has been demonstrated. 
All the three fractions of Vitamin B₆ deficient liver and 
kidney showed increased susceptibility to lipid peroxidation 
in the presence of stimulators such as copper, iron, 
ascorbate and oxalate, thereby showing that accumulation of 
calcium and oxalate in kidney membrane fractions may be the 
site for stone formation through peroxidative damage 
(Ravichandran and Selvam, 1990b).

Studies have shown that Vitamin B₆ is involved in 
lipid metabolism (Abe and Kishino, 1982). Recently, Cho and 
Leklem (1990) demonstrated that Vitamin B₆ is required in 
carnitine biosynthesis in vivo. Studies on Vitamin B₆ uptake 
by rat kidney cells and brush border membrane vesicles 
demonstrated that renal absorption of pyridoxine was sodium-
dependent and might be mediated by sodium-hydrogen exchange 
system (Bowman et al., 1990). In vitro studies have 
demonstrated that pyridoxal-phosphate and not pyridoxal 
forms a coordinated complex with magnesium, and the 
coordination site is related to the primary phosphate and 
aldehyde moiety of pyridoxal phosphate (Boylan and 

A significant decreased (¹⁴C) inulin clearance and 
increased urine oxalate has been reported in Vitamin B₆ 
deficient rats. Histological evaluation also indicated 
increased renal damage in kidneys (Wolfson et al., 1991).
Increased plasma malondialdehyde level, conjugated dienes, lipofuscin like pigments, lipids, calcium, iron and copper has been reported in vitamin B₆ - deficient rats (Ravichandran and Selvam, 1991). Detailed review regarding absorption, bioavailability, interorgan metabolism, nutritional requirements, toxicity etc., has been discussed recently (Leklem, 1991).

2.9.12.3 Vitamin A

Vitamin A exerts its effect on magnesium metabolism by causing perturbances in calcium and oxalate metabolism. Gershoff and McGandy (1981) showed that a Vitamin A deficient, lactose rich-diet leads to hypercalciuria, hyperoxaluria and urolithiasis.

A considerable mitigation of hypercalcemia and nephrocalcinosis at a high dose of Vitamin A to rats has been demonstrated (Baumane et al., 1990). Increased excretion of urinary calcium and oxalate (and formation of calcium oxalate stones in bladder and kidneys) in Vitamin A deficiency has been related to enhanced intestinal absorption of both calcium and oxalate (Kancha and Anasuya, 1989; Sharma et al., 1990). Various aspects of Vitamin A related to its metabolism, biochemistry, functions, deficiency etc., has recently been reviewed (Olson, 1991).

2.9.12.4 Vitamin D

The role of Vitamin D₃ and its active metabolites, as one of the major physiological regulators of mineral
metabolism, has been well established. The metabolically active form of Vitamin D, 1,25 (OH)₂D₃ is known to stimulate active calcium transport in the intestine, bone mineral mobilization and also increased urinary calcium excretion by decreasing tubular renal reabsorption (Deluca, 1984; Kumar, 1984). The resultant hypercalciuria is one of the major risk factors in the genesis of urinary calculi formation. Increased calcium absorption may also influence oxalate absorption (Lindsjo et al., 1989). Various aspects of vitamin D related to its metabolism, biochemistry, deficiency etc., has extensively been reviewed recently (Collins and Norman, 1991).

2.9.12.5 Vitamin K

Vitamin K plays an important role in the synthesis of ψ-carboxyglutamic acid (Lian et al., 1977). Gla is known to chelate calcium, which may form a matrix in the urinary tract for the stone. Gla is excreted in the urine both as the free amino acid and in undegraded Gla-containing proteins (Lian and Gunberg, 1988). Elevated concentrations have been found in the urines of recurrent calcium oxalate stone formers, nephrocalcinosis and primary hyperparathyroidism patients (Joost et al., 1981). Gla has been implicated as a promoter of calcium-oxalate crystallization (Nishio et al., 1990).
2.10 Red cell membrane

The red cell membrane, like all other eukaryotic plasma membranes, is composed of a bilayer of several different classes of lipids along with a variety of protein molecules (Singer and Nicolson, 1972; Branton et al., 1981). About half of the red cell membrane mass is accounted for by lipids (primarily phospholipids and cholesterol) and glycolipids (Ways and Hananan, 1964), and half by proteins and glycoproteins. The membrane is composed of approximately 41% lipids, 52% proteins and 7% carbohydrates.

2.10.1 Membrane lipids

All the lipids in the mature cell are contained within the membrane and are responsible for many of its physical characteristics. Modification of lipid composition of the membrane is known to influence both the passive cation permeability and the mechanical flexibility of the red cell (Cooper et al., 1975). Phospholipid and non-esterified cholesterol account for more than 95 per cent of the total lipid within the membrane. Small amounts of glycolipids, glycerides and free fatty acids are also present (Sweeley and Dawson, 1969). On a molar basis, the phospholipids and cholesterol are present in nearly equal amounts. The major classes of phospholipids in the human erythrocytes are: phosphatidylcholine, 30 per cent; phosphatidylethanolamine, 28 per cent; phosphatidylserine, 14 per cent and sphingomyelin, 25 per cent. Small amounts of
phosphatidic acid, phosphatidylinositolides and polyglycerol phosphatides are also present (Sweeley and Dawson, 1969), but it lacks triglyceride and cholesterol esters (Cooper, 1978). Fatty acid composition of the human erythrocyte membrane revealed the presence of palmitic and stearic acids with their monoethenoic derivatives as minor constituents. No other fatty acids were detected (Zdebska et al., 1989).

Fluorescence digital imaging microscopy used to study the lateral distribution of the lipid components in erythrocyte membrane demonstrated that phospholipids in the membrane are unevenly distributed, thus showing the presence of membrane domains. Similar membrane domains were also present in erythrocyte ghosts. It was concluded that membrane proteins are also involved for creating domains (Rodgers and Glaser, 1991).

2.10.2 Organisation of erythrocyte membrane skeleton

When visualized by various electron microscope techniques, the membrane skeleton appears as a sweater-like lattice directly laminating the inner leaflet of the membrane. The principal protein components remaining in the cytoskeleton after high salt extraction are spectrin, band 4.1, erythrocyte actin and band 4.9 (Sheetz, 1979). The molecular assembly of the individual skeletal proteins can be visualized in membrane skeletal preparations that have been uniformly stretched, so that the individual structural
subunits are clearly visible (Shen et al., 1986; Liu et al., 1987).

The membrane skeleton can be viewed as being constructed around spectrin. Spectrin tetramers are involved in two independent classes of protein association that are both essential for the final structure: a) formation of the two dimensional meshwork underlying the lipid bilayer, by associations with the actin oligomers, band 4.1 and possibly other spectrin molecules; b) linkage of the spectrin/actin meshwork to integral membrane proteins via association with ankyrin and possibly band 4.1. Both spectrin and protein 4.1 band albeit weakly to phosphatidylserine, which is preferentially located at the inner leaflet of the lipid bilayer (Rybicki et al., 1988).

Both computer analysis of the ultrastructural images of the skeleton in situ (Liu et al., 1988) and calculations based on contour dimensions and number of copies of the individual skeletal proteins (Liu et al., 1990) suggest that about 60% of the lipid bilayer is directly supported by the underlying membrane skeleton. The overall model and arrangement of the membrane skeletal protein is shown in Fig. 5.

2.11 Physiology and Biochemistry of small intestine and kidney

Intestine represents a cell, whose special mission in life is transport per se. The variety and complexity of
FIG. 5  ORGANISATION OF THE ERYTHROCYTE MEMBRANE SKELETON
transport processes in the cell is quite staggering. The plasma membrane of this cell possesses the potential for virtually every type of transport mechanism known. Thus intestine provide an absorptive surface for the transport of various nutrients, electrolytes and water.

The kidneys are paired organs that lie behind the peritoneum in the posterior aspect of abdomen, one on each side of the vertebral column. The kidneys in addition to the excretion of nitrogenous wastes, act to preserve the constancy of the extracellular fluid in composition, volume and pH.

2.11.1 The structure of the small intestine

The structure of the small intestine is similar in most of the mammals, and is conventionally divided into three parts namely duodenum, jejunum and ileum. In all the three segments, the wall has some stratified organisation. Three outer strata, the serosa, the muscularis and the submucosa surround the innermost layer, the mucosa, where the process of digestion and absorption is completed. In addition, the membrane of the lining cells appears to be equipped with a diverse array of transport systems. When digestion is complete, these carriers move sugar, amino acids and small peptides into the cytoplasm of the cells. Located over the inner surface of small intestine are literally millions of small villi which project about 0.5 to 1.5 mm from the surface of the mucosa in normal adult rats.
(Fig. 6). Between the villi are depressions called crypts and inside each villus, there is a dense network of blood and lymph vessels. The columnar epithelial cells lining villi are predominantly absorptive cells with few mucus secreting i.e., goblet cells. A fully developed absorptive cell is tall and narrow. The nucleus is situated deep in the cytoplasm, below a broad apical zone richly supplied with mitochondria, endoplasmic reticulum and golgi saccules. The abundance of these organelles indicates intense oxidative and synthetic activity. The most distinctive feature of absorptive cells is brush border, a prominent structure on the luminal surface of the cell. The brush border is made up of rows of minute projections called microvilli, which are about 1 um long and increase the surface area of intestine by a factor of about 20 (Fig.7).

External to the microvillus membrane proper is the fuzzy coat or glycocalyx. This 'fuzzy coat' is rich in sialic acid and glycoproteinaceous fibrillar material (Groniowski et al., 1969). The glycocalyx performs some vital functions such as adhesion, recognition, intercellular interactions, defence and separation of different types of molecules (Ugolev et al., 1979). However, the ideas on this mechanism remained uncertain. The membrane covering the microvillus is the actual site of admittance of nutrients into the body. Purified microvillus membranes have revealed that an array of digestive enzymes viz; disaccharides,
Wall of the intestine; showing four concentric layers. Three outer strata - the serosa, the muscularis and the submucosa surrounding the inner mucosa.

Villi extending into the interior space from the folds and the surrounding surface.

Dense net work of blood and lymph vessels inside each villus.
FIG. 7 STRUCTURE OF THE INTESTINAL ABSORPTIVE CELL
peptidases, alkaline phosphatase etc., and transport systems are incorporated into the membranes (Both and Kenny, 1976; Kenny and Maroux, 1987). The epithelium of the intestine comprises a single layer of cell surrounding the lumen. The thickness of the epithelial cell membranes varies between 75\,\text{Å} and 105\,\text{Å}.

2.11.2 The structure of the kidney

The functional unit of the kidney is the nephron, an expression first coined by Brauss (1929). There are approximately 30,000 to 34,000 nephrons in each adult rat kidney.

Each nephron is a thin tube approximately 20-50 um wide and 50 mm long with one end closed and the other opening into a collecting duct. The upper end of each nephron lies in the cortex, invaginated and expanded by a cluster of capillaries (the glomerulus). Next to the glomerulus, the proximal convoluted tubule, about 14 mm long, is continuous with the relatively straight descending limb of the loop of Henle, which passes into the medulla. The tube then forms a U-bend and returns to the cortex as the ascending limb of the loop and on return forms the distal convoluted tubule. This last portion terminates as the junctional tubule by joining with a collecting tubule, which opens into one of the calyces of the ureter (Fig.8). The filtering membrane of the corpuscle consists of three
FIG. 8  DIAGRAM OF THE STRUCTURAL ORGANIZATION OF THE KIDNEY.
layers: (1) an endothelial layer, (2) a basement membrane and (3) an epithelial layer.

The cells of the proximal tubules are large columnar type cells with a well marked brush border. Each cell has about 150 brush border extensions per square micron, which enormously increase the surface area of the cells in contact with the fluid in the lumen of the tubule. The cells of the loop of Henle also have microvilli but these are too small and few in number to produce a brush. The cells of the distal convoluted tubule have microvilli and contain a large number of microvesicles. The mitochondria are smaller than in the proximal tubule, and there are few particles of the ribonucleoprotein.

In their excretory function, the kidneys exercise a fine selective discrimination. The production of urine begins with the filtration through the glomerular capillaries of a fluid that resembles plasma. The glomerular filtrate passes down the tubule and its volume is reduced and its composition altered by processes of tubular reabsorption and tubular secretion. The former accounts for the removal of water and solutes and the latter for the addition or exchange of solutes.

2.11.3 Chemical composition of brush border membranes

The brush border membrane (BBM) is the outer lining of the microvilli, which separates the luminal environment
from the epithelial tissue and is invested with a number of peculiar permeability properties.

The brush border membrane is considered to be a mosaic of structural and functional proteins intercalated in lipid bilayer with some carbohydrates. The most important carbohydrate content of membrane is sialic acid, which is a group name given to acylated neuraminic acid derived from the pyranose ring. The intestine and kidney possess similar transepithelial transport systems by which solutes common to the intestinal and renal tubular lumen cross the cell via luminal brush border membrane.

A number of investigators have been able to successfully isolate and characterize intestinal brush border membrane (Schmitz et al., 1973; Kessler et al., 1978; Hopfer et al., 1983) and renal cortical brush border membrane (Fukuhara and Turner, 1983; Takano et al., 1984) A detailed analysis of the brush border chemical structure has been elucidated by various investigators (Schmitz et al., 1973; Bode et al., 1976), but there is too much variation in the results to make an average value of various constituents. In general, the purified intestinal brush border membrane preparations contain about 60 per cent carbohydrates as expressed on dry weight basis (Kenny and Booth, 1978; Christiansen and Carlson, 1981). The membrane contains neutral lipids, phospholipids, neutral glycolipids and gangliosides in the ratio of 18:50:29:2 per cent based
on weight percentage. The structure of the intestinal brush border membrane revealed regional differences in the lipid fluidity and composition of the rat enterocyte microvillus (Luminal) membrane (Brasitus and Schachter, 1980) and in basolateral membrane (Brasitus and Schachter, 1984).

In the renal brush border membranes, several enzymes show characteristically large and comparable increase in specific activities relatively to that in the cortical homogenate. These include trehalase and maltose, alkaline phosphatase, 5'-nucleotidase and two amino-peptidases (Sactor, 1968). These enzymes have also been reported in intestinal brush border membranes. In addition, other enzymes, whose principal functions seem to be directly related to digestive processes have been localised in the intestinal membrane (Crane, 1975).

It is well established that membrane phospholipid and cholesterol can modulate the activity of certain membrane bound enzymes (Brasitus and Schachter, 1980). The changes in the lipid composition of the plasma membrane influences the reactions associated with this membrane through alterations in the protein-lipid interactions (Sandermann, 1978). Physical manipulation of the lipid environment has been reported to affect activities including Na⁺-K⁺ ATPase (Szamel and Resch, 1981), Ca²⁺-Mg²⁺ ATPase (Galo et al., 1981), adenylate and guanylate cyclase (Chambaz et al., 1983).
2.11.4 Transport of nutrients across brush border membrane vesicles isolated from mammalian intestine and kidney

The epithelium of the small intestine and the renal proximal tubules, which mediates translocation of solutes and fluid, is characterized by cells with determined polarity. This asymmetry is evident ultrastructurally by differentiation of the plasma membrane into two distinct components, the apical brush border and the basolateral membranes.

In the last two decades, lot of interest has been generated in isolating the main absorptive site, i.e., the brush border membrane from the basolateral membrane. The vesicle system has a number of advantages. The major advantages are the absence of metabolism for many compounds and the control over the composition of solution on both sides of the membrane. In transport with the isolated membranes, direct uptake measurements have become feasible for metabolizable solutes.

Studies using intestinal brush border membrane vesicles (BBMV) has revealed that sugar and amino acid transport is energized by the chemical and potential gradient due to the Na⁺ ions difference across the brush border membrane (Hopfer, 1978; Semenza et al., 1984). Intestinal BBMV have been widely used for the influx of amino acids (Hayashi and Kawaski, 1982; Munck, 1984), glucose (Dorando and Crane, 1984), zinc (Menard and Cousins,
1983), iron (Simpson and Peters, 1986), tri-carboxylic acids (Wolffram et al., 1990), propionate (Harig et al., 1991), and biotin (Said and Derweesh, 1991).

Similarly, reabsorptive transport of organic solutes across the luminal membrane of the mammalian renal tubules has been characterized. Renal cortical BBMV have been used for the influx of neutral amino acids (Lynch and McGivan, 1987), Taurine (Park et al., 1991), glucose (Berteloot et al., 1991), nucleoside (Williams and Jarvis, 1991; Sayos et al., 1991) and urate (Dan and Koga, 1991).

In the present study, intestinal BBMV were prepared by the combined procedures of Schmitz et al., 1973 and Kessler et al., (1978) and renal BBMV by the method of Malathi et al., (1979). The uptake of calcium and oxalate in brush border membrane prepared from intestine and kidney cortex was studied.

2.11.5 Mechanism of translocation of nutrients

Nutrients are translocated across the gastrointestinal epithelial membranes and reabsorbed or secreted by the renal tubular epithelium in response to both electrochemical gradients (diffusion) and energy dependent mechanisms (active transport). Mainly three important pathways are considered for the translocation of metabolites across the biomembranes.
2.11.5.1 Passive diffusion

Passive diffusion is undoubtedly an important pathway for absorption of substances like water soluble vitamins, nucleic acid derivatives and many lipid soluble substances across the membranes. The main features of this process are: (a) movement is not across the concentration gradient; (b) its absorption rate is not inhibited by metabolic or competitive inhibitors; (c) a linear relationship between absorption rate and concentration gradient is observed.

2.11.5.2 Facilitative diffusion

There are many substances which could not be included within the simple passive diffusion. For these substances, permeation was much faster, than the simple model would have predicted. Permeation was not a linear function of substrate concentration, and was often inhabitable by analogous of the substrate or by reagents capable of attacking proteins. For all such molecules or ions, one might suggest, that there exist membrane components, probably proteins, which mediate the movement across the membrane of these substances. Such mediated transport may be a net movement down its electrochemical gradient, in which case the process is often termed facilitated diffusion. Sugars enter red cells by carrier-mediated facilitated transfer (Berteloot et al., 1991; Joseph et al., 1991). Several sugars enter the intestinal epithelia through this process.
2.11.5.3 Active transport

The special function of the small intestine and kidney is the rapid and efficient absorption of many nutrients required by all the organs of the body. By this process, the essential substances are rapidly and efficiently transferred across the cell membrane against the barrier of concentration gradient. Active transport process are specifically requiring energy, saturable and inhibited at low temperature. There are a number of hypothesis proposed to explain the mechanism of an active absorption phenomenon and the most important is the sodium-gradient hypothesis.

2.11.5.4 Sodium gradient hypothesis

Sodium ions are associated with many processes, which actively absorb the substances. Crane (1965) proposed a sodium gradient hypothesis to explain the uphill transport of nutrients in intestine. According to this hypothesis, the entry of sugars and amino acids against their electrochemical gradient requires the presence of Na⁺ on the mucosal surface. The ternary complex (Na⁺-carrier substrate) translocates across the mucosal membrane and because of low concentration of Na⁺ inside the cell, it dissociates to release Na⁺ and the substrate. The difference in Na⁺ concentration across the brush border provides the necessary driving force for the uphill movement of the substrate. The excess of Na⁺ then leaves cell from basolateral side via an
ouabain sensitive Na\textsuperscript{+}-K\textsuperscript{+} ATPase system (Fig. 9). Studies using isolated epithelial cells and membrane vesicles prepared from purified brush border also revealed that sugar and amino acid transport is energised by chemical and potential gradient due to Na\textsuperscript{+} difference across the brush border membrane (Hopfer, 1977, 1978). In a review of Na\textsuperscript{+}-dependent D-glucose transport, Semenza et al., (1984) have demonstrated that Na\textsuperscript{+}-D-glucose cotransporter is structurally and functionally asymmetric, thus ruling out diffusive and rotative modes of operation in accordance with the gated or pore mechanism.
FIG. 9 SODIUM GRADIENT HYPOTHESIS FOR SODIUM AND ORGANIC SOLUTE TRANSPORT