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Although several studies confirm the role of magnesium in stone formation, the detailed biochemical mechanism underlying the stone formation in magnesium deficiency has not been worked out. The present study was undertaken to evaluate the role of magnesium in calcium and oxalate nephrolithiasis. The effect of magnesium deficiency on oxalate biosynthesizing enzymes in the liver and kidney, absorption and renal handling of calcium and oxalate and erythrocyte handling of oxalate were studied to delineate the underlying mechanisms of hypercalcemia and hyperoxaluria, which leads to stone formation in magnesium-deficiency.

5.1 Effect of magnesium deficiency on body and organ weight

Male weanling rats showed signs of magnesium-deficiency i.e. hyperemia caused by the degranulation of histamine rich mast cells, hyperexcitability, usually observed in this deficiency (Whang and Welt, 1963). A significant (p<0.001) decrease in the body weight after thirty days on magnesium-deficient diet was observed. Despite pair-feeding, magnesium deficient animals lagged behind in growth as compared to pair-fed controls (Fig. 11), thereby suggesting that magnesium is indispensable for growth. This is in confirmation with the observation as reported by several other investigators (Bunce et al., 1974; Mills et al., 1984; El-Hindi and Amer, 1989; Gueux et al.,
It has been postulated that magnesium-deficient rats assimilate less of the administered food or do not utilize as well as the pair-fed controls (Mills et al., 1984). Magnesium deficiency caused a significant (p<0.01) decrease in the liver weight but when results were expressed as liver weight/100g body weight, no change was observed, which suggests a proportional decrease in liver mass related to the body weight loss. The kidney weight showed a significant (p<0.05) increase when the results were expressed as kidney weight/100g body weight. This is in agreement with the report of George and Heaton (1975), who reported an increased water content of kidneys in this deficiency, thereby suggesting that secondary effect of deficiency extend beyond mineral metabolism.

5.2 Assessment of magnesium deficiency

The assessment of magnesium status provides a challenge to clinical laboratory and biochemical technology. Several biochemical parameters are available to assess the magnesium status such as tissue magnesium, physiologic assessment of magnesium and free magnesium.

Gitelman and Welt (1969) defined the magnesium deficiency as "a reduction in total body magnesium content". Unfortunately the 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. Therefore, serum magnesium concentration as the standard marker of magnesium status is generally accepted (Elin, 1988, Fischer and Giroux, 1991).

Plasma and urinary excretion of magnesium was estimated to measure the magnesium status in the deficient animals and was compared to that of control animals. Magnesium deficiency caused a significant (p<0.001) hypomagnesemia and hypomagnesuria in the experimental animals as compared to the pair-fed control animals. This is in agreement with several other investigators (MacIntyre and Davidsson, 1958; Whang and Welt, 1963; Carney et al., 1980; Quamme et al., 1980; Tongyai et al., 1989; Okuno et al., 1990).

Renal magnesium excretion is normally in balance with dietary magnesium intake. Accordingly, tubular magnesium reabsorption has been reported to be increased following dietary magnesium deprivation (Welt, 1964). It has been reported that rats maintained on low magnesium diets excreted only small amounts of magnesium (Carney et al., 1980; Quamme et al., 1980). Parathyroid hormone is also a prime candidate that has been shown to be elevated in magnesium deficient rats (Heaton, 1965; Gitelman et al., 1968). Increased PTH secretion leads to increased magnesium reabsorption in the tubules (Quamme and Dirks, 1986). Recently, Shafik and Quamme (1989) also reported cellular adaptation of magnesium transport in magnesium-deficient rats.
Aggravation of thiamine deficiency by magnesium deficiency has been reported (Dyckner et al., 1985). Several biochemical parameters viz. urinary excretion of thiamine, blood levels of pyruvate, serum lactate - pyruvate ratio after glucose load have been used for assessment of thiamine status. However, these methods are of very limited use due to their low sensitivity and specificity (Buckle, 1965; Dewhurst and Morgan, 1970), and are being replaced by measurement of enzyme transketolase (TK) in leucocytes (Cheng et al., 1969) and in red blood hemolysate (Basu et al., 1974). TPP effect has been used as a reliable index to evaluate the nutritional status of thiamine (Bone et al., 1980). Recently, studies by Takeuchi et al., (1990) showed that TPP effect really reflects the saturation status of transketolase with coenzyme.

Magnesium deficiency is known to have a dual negative influence on thiamine metabolism. Firstly, the conversion of thiamine into its pyrophosphate form is inhibited and secondly, the action of TPP is prevented. A significant decrease (p<0.001) in the specific activity of erythrocyte TK and a 6 - fold increase in the TPP stimulation index in deficient animals was observed (Table 8), thereby suggesting that magnesium deficiency perturbed the thiamine metabolism. The importance of a normal magnesium status for thiamine
metabolism has earlier been demonstrated (Zieve et al., 1968; Zieve, 1969).

5.4 Role of magnesium in glyoxylate and oxalate metabolism

Magnesium deficiency in rats did not alter the total liver magnesium content. The results are in consistent with those of MacIntyre and Davidsson (1958), who found that only bone and muscle magnesium levels fell as a sequela of magnesium deficiency. Similar observations have also been observed by Jaya and Kurup (1987).

Magnesium is a known cofactor for a number of enzymes and enzyme systems. Virtually all enzymes for which phosphate or a phosphate-containing compound is the substrate and those in which, TPP or pyridoxal-5' phosphate is the cofactor require magnesium for activation (Wacker, 1980). A significant decrease in erythrocyte TK and liver alanine transaminase enzyme activities confirmed that magnesium deficiency leads to disturbances in both thiamine and pyridoxine metabolism in rats. A significant decrease in thiamine content of sciatic nerve, liver and kidney has been reported in magnesium deficient rats (Itokawa et al., 1974C). Recently, El-Hindi and Amer (1989) also reported decreased liver thiamine content in magnesium-deficient rats.

Thiamine is involved in the metabolism of glyoxylate. TPP is an essential cofactor for the oxidative
decarboxylation of α-ketoacids, non-oxidative decarboxylation of α-ketoacids and formation of α-ketoacids, indicating a key position of thiamine in carbohydrate metabolism. Pyridoxine also plays a significant role in oxalate metabolism (Nath et al., 1984). Several studies have also shown that, whereas deficiency of this vitamin increases endogenous synthesis of oxalate (Sidhu, 1985), its supplementation lowers the oxalate production (Murthy et al., 1982a). Thus deficiency of thiamine leads to disturbances in the carbohydrate metabolism, which leads to mobilization of body fat stores and proteins at a faster rate in deficient animals as compared to the controls. Decrease in plasma protein level has been reported in magnesium - deficient animals (Fahim et al., 1990, Kimura et al., 1990), which may be due to the inhibition of synthesis and/or impaired release of the formed proteins, as suggested by Ko et al., (1962) and Lizaralde et al., (1972, 1974). In the present study no significant change in the liver and kidney protein content was observed in magnesium deficient animals (Table 10,11). Maintenance of protein concentration constant in both liver and kidney in the deficient conditions suggests the reduced muscular protein synthesis as indicated by the lowered body weight gain of the experimental animals. This results in a decreased utilization of dietary protein, resulting in an increased levels of amino acids in the plasma. These amino acids are
then taken up by liver and kidney to maintain their own protein levels. This explanation is in harmony with the views of Mayer et al., (1976) and Rannels and Jefferson (1980), who suggested that any loss of body weight reflected decreased muscular protein synthesis as muscle tissue constitutes 45% of the total body mass.

Amino acids are important precursors of endogenous oxalate and contribute about 50 percent to total urinary oxalate (Hodgkinson, 1977). Glycine may be converted to glyoxylate in the liver and kidney peroxisomes by transamination (Cammarate and Cohen, 1950) and by flavoenzyme glycine oxidase (Ratner et al., 1944) or D-amino acid oxidase (Masters and Holmes, 1977). Increased conversion of glycine -2-^{14}C to ^{14}C-glyoxylate and ^{14}C-CO_2 with a concomitant decreased production of ^{14}C-pyruvate and ^{14}C-oxaloacetate has been reported in thiamine deficiency (Liang, 1976). A significant decrease in the peroxisomal aminotransferase as observed in the present study (Table 10) lead to increased glyoxylate production by D-amino acid oxidase. Increased excretion of the metabolites of aromatic amino acid tryptophan into the urine and a significant decrease in the tryptophan pyrrolase activity has been demonstrated in magnesium - deficient rats (Shibata et al., 1989). The decreased catabolism of tryptophan and other aromatic amino acids such as phenylalanine and tyrosine may also contribute to the glyoxylate pool. A
significant contribution of amino-acid hydroxyproline towards glyoxylate has been reported in thiamine deficiency. A significant decrease of total hydroxyproline content of the skin has been reported in thiamine - deficient rats (Natarajan and Bose, 1965).

Several studies demonstrate the major role of magnesium in insulin action (Sonui and Robin, 1978; Tongyai et al., 1985). Magnesium as a second messenger for insulin action has been suggested (Lostroh and Krahl, 1973). Impaired function of many enzymes utilizing high energy phosphate bonds that are involved in glucose metabolism and requiring magnesium as a cofactor has been shown in magnesium deficiency. Tongyai et al., (1985) suggested that low erythrocyte magnesium content increase the membrane microviscosity, which may impair interaction of insulin with its receptor on plasma membrane. Studies by Moles and McMullen (1982) suggested that hypomagnesemia may contribute to insulin resistance observed during treatment of diabetic ketoacidosis. Durlach and Rayssiguier (1983) reported reduced insulin sensitivity in chronic magnesium deficiency. Inverse relationship between the metabolic control and plasma magnesium in Type I (insulin - dependent) diabetic patients has been demonstrated. Fujii et al., (1982) reported marked depletion of plasma, erythrocyte and urinary magnesium in diabetic patients. Abnormal insulin response following an oral glucose load in 70 percent of idiopathic
calcium oxalate stone formers has also been reported (Rao et al., 1984). High incidence of urolithiasis in insulin-dependent diabetic patients has been demonstrated (Maricker et al., 1977). Studies by Holzer et al., 1962 demonstrated that Fructose-6-phosphate and Xylulose-5'-phosphate (internally derived from xylitol or glucose-6-phosphate) are known to be acted upon by transketolase. TPP bound glycolaldehyde is known to proceed the reaction. Thus in magnesium induced thiamine deficiency, it likely dissociates to release free glycolaldehyde, which is oxidized further to glycolate, glyoxylate and oxalate. Thus decreased catabolism of amino acids and production of glyoxylate from carbohydrates significantly contributes to oxalate production.

Rofe and Edwards (1978) proposed that glyoxylate is a potentially toxic metabolite. Glyoxylate produced within peroxisomes may have a different metabolism as compared to that in the cytosol. As peroxisomal membrane is impermeable to glyoxylate, the enzyme glycolic acid oxidase (GAO) takes care of the increased amount of glyoxylate production. GAO is a flavin mononucleotide (FMN) dependent enzyme, which is localized in liver peroxisomes. In the present study, a significant (p<0.001) increase in the activity of GAO was observed in magnesium deficient rats (Table 10). Increased plasma free cholesterol, incorporation of $^{14}$C-acetate into the cholesterol and HMG-CoA reductase activity has been
demonstrated in magnesium-deficient rats (Jaya and Kurup, 1987). The increased cholesterol pool might be in turn converted to major steroids by adrenals. Testosterone is synthesized both by testes and adrenals and it is possible that in magnesium deficiency, the adrenal testosterone production is elevated due to generalized increase in adrenal steroidogenesis. Studies from our laboratory demonstrated that testosterone increases the activity of GAO as well as GAD in the liver (Sharma, et al., 1981).

Besides peroxisomes, glyoxylate is also generated in the cytoplasm and mitochondria during intermediary metabolism from amino acids and carbohydrates. Mitochondrial membrane is permeable to glyoxylate. Within mitochondria, glyoxylate is converted into CO₂ via glyoxylate oxidation cycle (Dekker and Gupta, 1979) or by α-ketoglutarate:glyoxylate carboligase enzyme (O'Fallon and Brosemer, 1977). In the present study, conversion of ¹⁴C-glyoxylate to ¹⁴C-CO₂ by both liver and kidney mitochondria in magnesium-deficient group was significantly decreased (Table 14). A decrease in pyruvate carboxylase activity in the liver mitochondria has been shown in magnesium-deficient rats (Kimura et al., 1990). Thus, increased pyruvate pool in magnesium deficiency, channelises glyoxylate into this cycle. Glyoxylate is a potent inhibitor of 4-hydroxy-2-ketoglutarate (HKG) - aldolase and at 4mM glyoxylate concentration, almost 70% of the enzyme activity is lost.
Thus, increase in glyoxylate oxidation is not observed due to decrease in this enzyme activity.

Studies by Ruffo et al., (1967) demonstrated that glyoxylate and its condensation product with oxaloacetate regulate TCA cycle activity, by strongly inhibiting certain key enzymes e.g. aconitase, isocitrate dehydrogenase etc. Glyoxylate even at a concentration of 0.05 mM inhibits the α-ketoglutarate dehydrogenase enzyme activity (Adinolfi et al., 1967). Studies have also provided the evidence of conversion of glyoxylate into malate in rat liver homogenate and that thiamine deficiency leads to decreased conversion of 14C-glyoxylate into malate (Liang and Ou, 1971). Thus magnesium deficiency affects glyoxylate - oxidation cycle, due to the lack of TPP and by inhibiting key enzymes of glyoxylate metabolism.

The α-ketoglutarate: glyoxylate (α-KG:GA) carboligase is a magnesium and TPP dependent enzyme, whose activity is associated with the α-ketoglutarate decarboxylase moiety of the α-ketoglutarate dehydrogenase complex (O'Fallon and Brosemer, 1977). In the present study, a significant decrease in the activity of α-KG:GA carboligase in both the liver and kidney mitochondria prepared from magnesium-deficient rats was observed (Table 14). Itokawa et al., (1972a) reported a significant decrease in thiamine content of liver and kidney in magnesium-deficient rats. A similar decrease in the liver thiamine content has also been
observed recently (EI-Hindi and Amer, 1989). Further studies by Itokawa et al. (1974c) demonstrated that thiamine content in the subcellular fractions of the liver of magnesium deficient rats was most markedly decreased in mitochondrial fractions. Mitochondria has been shown to be the most vulnerable organelle to loose magnesium during prolonged dietary restriction (George and Heaton, 1975). Thus decreased magnesium and thiamine content affects the mitochondrial TPP dependent enzyme e.g. pyruvate and α-ketoglutarate dehydrogenase (Nose et al., 1976), leading to the accumulation of glyoxylate.

Glyoxylate is permeable to mitochondrial membrane enters cytosol. LDH-V isoenzyme (M₄) of hepatic LDH (the predominant form) has a greater affinity towards glyoxylate and efficiently converts it into glycolate, which enters the peroxisomes and gets metabolized into oxalate either directly by enzyme GAD or via glyoxylate as an intermediate. Thus magnesium deficiency leads to enhanced endogenous synthesis of oxalate in rats (Fig. 30).

LDH, also plays an important role in the oxalate synthesis (Gibbs and Watts, 1973). Although liver is a major organ of the endogenous synthesis of oxalate, some of the oxalate producing ability is also present in the kidney, where LDH is known to play a major role. In the present
INCREASED IN MAGNESIUM DEFICIENCY
INHIBITED BY GLYOXYLATE
DECREASED IN MAGNESIUM DEFICIENCY

FIG. 30 EFFECT OF MAGNESIUM DEFICIENCY ON OXALATE & GLYOXYLATE METABOLISM
study, liver and kidney LDH levels remained unaltered (Table 12). Similar observations have also been observed in thiamine and pyridoxine-deficient rats (Sidhu, 1985). However, when kidney LDH was subjected to cellulose acetate electrophoresis, it showed the presence of five LDH isoenzymes, in the kidney (Table 12; Fig. 12). Similar to the pattern observed by Park and Gubler (1969). These LDH isoenzymes were identified by using lactate, NAD\(^+\) and NBT. Magnesium deficiency produced significant alterations in the isoenzyme distribution pattern. The activity of LDH-I fractions was significantly increased in magnesium deficient group (Table 12). Park and Gubler (1969) observed a significant increase in LDH-I fractions in pyrithiamine treated rat kidney. Increased LDH-I and II fractions has also been reported in thiamine-deficient rat adipose and heart tissue (Hirota et al., 1976).

Studies by Everse and Kaplan (1973) suggested that H-LDH is responsible for the conversion of lactate or hydrated glyoxylate to pyruvate or oxalate, and is distributed in tissues with predominantly aerobic metabolism. Whereas, the M-subunit, which preferentially converts pyruvate or unhydrated glyoxylate to lactate or glycolate is prevalent in anaerobic tissue such as kidneys. Studies have shown that the removal of magnesium from normal mitochondria causes increased permeability to monovalent cations (Barnard and Cockrell, 1982). Evidence also suggests an increased
permeability to protons of the inner membrane from mitochondria during magnesium deficiency (Heaton and Elie, 1984). Increased lipid peroxidation of liver mitochondria has also been reported in magnesium-deficient rats (Guenther and Hoellriege, 1989). Thus increased permeability to protons of the inner membrane disrupts ATP production, which implies that magnesium-deficient rats use glycolytic pathway as the primary source of ATP leading to decreased ATP pool. The conversion of pyruvate to lactate and glyoxylate to glycolate is diminished as NADH produced during glycolysis is preferentially used to generate ATP via electron transport chain. Hence M-LDH subunit is no longer required, which results in its decreased biosynthesis suggesting a biological adaptative response. Nance et al., (1963) reported that H and M submits of LDH are synthesized by separate genes. The shift in LDH distribution pattern suggests the repression of M-gene with a concomitant induction of H-gene by pyruvate or glyoxylate. This explains a decrease in LDH-V and a significant increase in LDH-I distribution in magnesium-deficient rat kidney. Similar observations have also been confirmed in thiamine deficient rats (Sidhu, 1985). The mechanism of altered kidney LDH isoenzyme in magnesium deficiency is shown in Fig.31.

Thus kidney, plays a definite role in endogenous synthesis of oxalate, that contributes to the hyperoxaluria observed in magnesium-deficient rats. Studies have
FIG. 31 MECHANISM OF ALTERED KIDNEY LDH ISOENZYME PATTERN
IN MAGNESIUM DEFICIENCY
suggested that kidney peroxisomes contain significant amounts of L-$\alpha$-hydroxy acid oxidase activity, which can act on thiol-glyoxylate adducts and oxidize them to oxalyl-thioesters, that get hydrolyzed to produce free oxalate (Brush and Hamilton, 1981). The magnesium deficiency can be related to a state of primary hyperoxaluria (Type 1), which is due to the absence of cytoplasmic $\alpha$-ketoglutarate: glyoxylate carboligase (Koch et al., 1967), that leads to hyperoxaluria, due to increased glyoxylate pool.

5.5 Intestinal absorption of oxalate in magnesium deficient rats

Intestine is very actively involved in the transport of essential nutrients for maintenance of normal metabolism. In the past years, methods have been developed to understand the transport mechanisms of various nutrients with the use of isolated plasma membranes (Schmitz et al., 1973; Kessler et al., 1978). The principal advantage of such systems are that, they are simpler than the whole cells or tissue, lack compartmentation, intracellular metabolism, contamination from basolateral membrane besides having an osmotically active space (Hopfer, 1977).

Oxalate derives its clinical importance from its relative insolubility in urine and consequently its major role in the pathogenesis of stone formation. Besides endogenous synthesis of oxalate, dietary oxalate also contributes to the urinary oxalate. For this purpose,
oxalate uptake studies were performed with BBMV isolated from rat jejunum, as oxalate absorption has been found to be maximum in this part of the intestine (Dulloo et al., 1979).

A 14-fold increase in the specific activity of brush border membrane marker enzyme alkaline phosphatase as compared to the respective homogenate, confirmed the purity of the intestinal and kidney cortical BBMV in both the groups (Table 15 and 18 respectively) which is in agreement with the previous reports (Schmitz et al., 1973; Gupta et al., 1988).

Contamination from basolateral membrane was also checked by measuring, the marker enzyme Na⁺ - K⁺ ATPase in the final membrane preparation. This enzyme has often been used as a distinguishing marker for the plasma membrane (Douglas et al., 1972; Schmitz et al., 1973). In the present study, 90% reduction in the activity of this enzyme in intestinal as well as kidney cortical BBMV confirmed no sizeable contamination of the BBMV preparation by basolateral membrane. This is in agreement with earlier reports (Schmitz et al., 1973) and autoradiographic studies (Stirling, 1972), where no ouabain binding sites on the brush border could be demonstrated.

In the present study, oxalic acid absorption was found to be increased linearly with increasing extravescicular oxalate concentration in pair-fed control rats, suggesting thereby that oxalate is transported across
the microvillus membrane by a simple passive diffusion process (Fig. 18). Similar process of oxalate uptake has also been demonstrated by many other investigators (Binder, 1974; Schwartz et al., 1980; Farooqui et al., 1984; Sidhu et al., 1986). Oxalate is a polar molecule (divalent anion) its translocation across the lipid bilayer is very slow in the absence of specific transport carriers. Therefore, it might be assumed that organic anions are crossing the intestine boundary by way of aqueous channels or passive diffusion.

Passive diffusion across rat intestine for various other organic acids such as hippuric acid, p-aminohippuric acid and sulphanilic acid has been demonstrated (Lanmann et al., 1971). Studies by Gupta (1986) also demonstrated the passive diffusion process of oxalate transport across the rat intestinal BBMV. In the present study, although the mechanism of uptake of oxalate across the rat intestinal BBMV from experimental rats was similar to that in controls, the rate of uptake of oxalate was found to be significantly higher as compared to that in pair-fed controls. Hyperabsorption of oxalate has also been demonstrated by similar mechanisms in experimentally induced vitamin A deficiency in rats (Sharma et al., 1990). In contrast to the above findings, enhanced oxalate uptake has been reported through an induction of an oxalate binding protein in the brush border membrane in rats fed pyridoxine-deficient diet (Nath et al., 1990). The increase in the
slope of the line for oxalate in magnesium-deficient rats suggested that rate of uptake of oxalate is faster than that in control rats. Studies by Strzelecki and Menon (1986) reported a saturated kinetics for oxalate uptake across liver mitochondria, suggesting the different type of mechanisms of oxalate transport in different subcellular organelles.

Interactions between calcium and oxalate has been reported. Increased urinary oxalate excretion with a concomitant increased urinary calcium excretion in recurrent stone formers has been reported (Erickson et al., 1984; Hesse et al., 1984). Lindsjo et al., (1989) reported enhanced calcium and oxalate absorption in recurrent renal stone formers. Restriction of calcium in the diet has been shown to increase the oxalate absorption (Bataille et al., 1985). Studies by Walker et al., (1989) attributed the presence of hyperoxaluria in stone-formers to the low dietary calcium intake, as calcium uptake was found to be only 64% of the recommended daily allowance. Reduction in hyperoxaluria by calcium supplementation has been reported, thereby suggesting the binding of calcium to oxalate in the gut (Earnest et al., 1975; Barilla et al., 1978).

In the present study, hyperabsorption of calcium in the experimental animals was observed (Fig. 15). Thus the increase in calcium leads to reduction of the intraluminal
calcium available to bind oxalate, leaving more of free oxalate to be absorbed from the intestine.

Interactions between magnesium and oxalate has also been demonstrated. Magnesium is known to bind oxalate in the gastrointestinal tract (Berg et al., 1986). Thus it can be speculated that in the presence of diminished magnesium load, more of oxalate will be absorbed as demonstrated (Fig. 18).

5.6 Renal handling of oxalate in magnesium-deficient rats

Organic anions and cations are known to be secreted by the proximal renal tubules (Weiner, 1973). Specific mechanisms underlying the transport of organic ions in the intact tubule are difficult to characterise, because of its complex structure being composed of two distinct membranes i.e. luminal and basolateral membrane. The development of methodology to isolate the brush border membrane vesicles from renal cortex has enabled to study the transport properties of organic anions (Hori et al., 1982; Inui et al., 1984). Very few reports are available on the studies of oxalate handling by the kidneys (Knight et al., 1979). In order to obtain more information, oxalate transport studies were performed by preparing rat renal cortical BBMV. Earlier reports have exhibited maximum oxalate absorption at proximal tubules of cortical origin (Greger et al., 1978), while oxalate permeability to loop of
Henle and distal tubule segments seems to be limited (Hautmann and Osswald, 1977; Weinmann et al., 1978).

Oxalate absorption was measured using vesicular technique. Pair-fed control rat cortical BBMV exhibited both a carrier-mediated (saturable) and a non-carrier mediated (passive) components (Fig. 25). The presence of a proximal tubule high-affinity, low-capacity and low-affinity high capacity, oxalate transport system operating at high plasma oxalate concentration has been reported (Knight et al., 1981). Results of the present study also demonstrate the presence of high-affinity and low-capacity system for oxalate transport across the proximal tubular cells.

Rat kidney cortical BBMV from magnesium-deficient rats also exhibited a saturable and non-saturable component (Fig 23). However, the rate of uptake of oxalate was significantly higher (p<0.01) at all the concentrations (0.1 - 1.0 mM). Since the saturable component follows a Michaelis-Menten equation, the results of the uptake studies (i.e. upto 0.8 mM concentration) were extended to draw the Lineweaver-Burk plot. Although the Km was found to be similar in both the groups (0.35 mM), the Vmax was found to be higher in magnesium-deficient group (7.69 nmol/8min/mg protein) as compared to the pair-fed control group (5.55 nmol/8 min/mg protein), thereby, suggesting a greater turnover of transport carriers in the deficient group (Fig. 26).
Further oxalate handling investigated by the use of micropuncture and clearance studies (Greger et al., 1978; Weinmann et al., 1978), suggest that oxalate is freely filtered from the glomerulus and undergoes net secretion predominantly in the proximal convulated tubule (Weinmann et al., 1978, 1980). Studies using free-flow micropuncture technique has demonstrated the presence of two secretory systems in the proximal tubule (Weinmann et al., 1980; Knight et al., 1981). Renal clearance of oxalate in hyperoxaluric rats has been shown to be higher than inulin, thereby suggesting that oxalate is secreted by the proximal tubule (Kanazawa, 1990). Thus the hyperoxaluria observed in the present study originates from the secretory processes present in the proximal tubule. The study also suggests the increased risk of stone formation in magnesium-deficient rats due to increased retention time of oxalate as compared to the pair-fed controls.

5.7 Mechanism of hypercalcemia in magnesium-deficient rats

More than 90% of the stones are composed of calcium, phosphate besides oxalate. Considerable progress has been made in understanding the homeostatic control mechanism of mineral metabolism specially calcium (Nordin, 1976). Under normal conditions, calcium homeostasis is maintained by the concerted action of PTH, calcitonin and 1,25 (OH)₂ D₃. PTH plays a major role in the regulation of magnesium
metabolism. Involvement of magnesium metabolism in PTH biosynthesis and/or secretion has been well demonstrated (Wagner et al., 1972).

Hypocalcemia is characteristically observed during magnesium deficiency in a number of animals including man (Estep et al., 1969; Muldowney et al., 1970). This has been attributed to low circulating immunoreactive PTH, end organ resistance (Rude et al., 1976; Allgrove et al., 1984), skeletal resistance (Woodard et al., 1972) and resistance to the action of vitamin D (Medalle et al., 1976). The rat is the only experimental model, that consistently develops hypercalcemia in response to magnesium deficiency (MacIntyre and Davidsson, 1958; Tongyai et al., 1989; Okuno et al., 1990).

In the present study, a significant (p<0.001) hypercalcemia in magnesium-deficient rats was observed (Table 7) which is in agreement with the other investigators. Several studies has attributed the hypercalcemia to stimulation rather than depression of PTH secretion by the presence of intact parathyroid glands (Heaton, 1965; Gitelman et al., 1968). Thus changes in calcium metabolism in magnesium deficient rats suggests a state of hyperparathyroidism, which has been diagnosed in 2-17% of patients with nephrolithiasis (Jaegar et al., 1986; Galic et al., 1989).
5.7.1 Intestinal absorption and renal handling of calcium in magnesium-deficient rats

Calcium absorption in the intestine in magnesium-deficient rats has been reported to be increased (Alcock and Maclntyre, 1960, 1962; Kessner and Epstein, 1966; Morehead and kessner, 1969), unaffected (Lifshitz et al., 1967; Krawitt, 1972), and decreased (Walling et al., 1975). The decreased rate of intestinal calcium absorption in magnesium-deficient rats was due primarily to the reduction in the rate of passive diffusion (Chow et al., 1978). One of the possible factors that makes it difficult to compare the results of different laboratories, is the technique employed to determine the calcium absorption, with the exception of the studies by Alcock and Maclntyre (1960, 1962), in which net calcium absorption was measured by the balance method. Other investigators had employed either a ussing apparatus, ligated loop technique, intestinal rings or in situ perfusion. In order to obtain information, somewhat more applicable, while avoiding the inaccuracies of the above mentioned techniques, brush border membrane vesicles (BBMV) were prepared, characterized by the purity of the marker enzymes to study the calcium absorption in the intestine and kidneys. Studies have shown that any nutrient transport can be best studied by means of the solute flux across subcellular membrane vesicles (Menard and cousins, 1983; Kragh - Hansen et al., 1984). The duodenal BBMV were
prepared as the absorption of calcium in this part of the intestine has been shown to be predominant (Bronner et al., 1986).

Calcium uptake in the brush border membrane (intestinal and kidney cortical) has been demonstrated to be passive at lower concentration and saturable as the concentration of calcium in the extravascular medium increased gradually (Miller and Bronner, 1981). Studies by other investigators have also shown the presence of both a saturable and a non-saturable component of calcium uptake across the luminal border membrane (Pansu et al., 1981; Hardwick et al., 1990). In the present study, intestinal calcium uptake in the BBMV demonstrated a positive relationship between the uptake and the extravascular calcium concentration (0.1 - 1.0 mM), thereby suggesting that calcium is transported across the intestinal microvillus membrane by a simple passive process (Fig. 15). However calcium uptake across the kidney cortical BBMV demonstrated a saturable kinetic, thereby showing the involvement of a transport carrier in the translocation process. Since the uptake showed a saturable kinetics, it follows a Michaelis–Menten equation of an enzyme reaction with Vmax equal to 16.66 nmole/6min/mg protein for both the groups, whereas, km for deficient group was 2.08 mM as compared to that of pair-fed control group, where it was 5.0 mM. The renal transport of calcium was significantly
increased in the deficient group. Further lowering of Km in the deficient group signifies greater affinity of calcium towards its transport carrier, leading to its higher absorption luminal calcium by magnesium - deficient rat kidneys (Fig. 22).

Interrelationship between calcium and magnesium has been reported. Studies have demonstrated that injection of calcium salts increased the renal excretion of magnesium (Mendell and Benedict, 1909) and that administration of magnesium salts increased calcium excretion via urine (Mendell and Benedict, 1909; Clark, 1968; Blacklock et al., 1985). Schachter and Rosen (1959) demonstrated that calcium transport across the intestinal wall in rats was depressed by the presence of magnesium ions in the medium. Studies by Drach et al., (1985) reported that magnesium and calcium absorption tend to parallel each other in a saturable, competitive transport mechanism and animal studies imply that some competition between calcium and magnesium transport exists in gut as well as in renal tubules. This leads to increase in calcium absorption as magnesium absorption decreases.

All these observations support the postulate that magnesium and calcium compete for a common reabsorptive system in the intestine as well as in the renal tubules. The results of present study also strongly support the above finding. Hyperabsorption of calcium in the intestine as well
as in the renal tubules, in the presence of diminished magnesium load thus, substantially contributes to the hypercalcemia in the deficient animals as compared to the pair-fed control group.

The effect of vitamin D and PTH on intestinal absorption and renal handling of calcium cannot be ignored. PTH release is inversely proportional to magnesium concentration. Magnesium deficiency leads to enhanced secretion of PTH in the experimental animals (Anast and Forte, 1983; Welsh and Weaver, 1988). The presence of hypercalcemia and hyperphosphaturia in the present study also confirms the state of hyperparathyroidism in the experimental rats. Vitamin D is required for intestinal absorption of calcium (Deluca, 1984) and PTH is a known stimulator of production of $1,25\,(\text{OH})_2\,\text{D}_3$. Increased blood levels of $1,25\,(\text{OH})_2\,\text{D}_3$ in hyperparathyroid patients have been reported (Broadus, 1980). Studies by Nordin and Peacock (1969) suggested that hyperabsorption of calcium from the intestine and increased renal tubular conservation of filtered calcium play a major role in maintenance of hypercalcemia in hyperparathyroidism patients. Only small contribution of bone in relation to the maintenance of hypercalcemia has also been reported (Parfitt, 1975).

The active form of vitamin D i.e. $1,25(\text{OH})_2\,\text{D}_3$ increases intestinal absorption both via genomic as well as non-genomic effects. Once the calcium enters into the cell,
it is followed by transcytotic diffusion and finally extrusion across the basolateral membrane (Roche et al., 1986). The genomic effects include increase levels of cytosolic calcium-binding protein (Bronner, 1989), a protein proposed to function in the transcytotic diffusion step (Roche et al., 1986). However, the exact functional role of the intestinal calcium-binding protein has not been clearly established. Liponomic control hypothesis has been proposed to explain the non-genomic effects of this secosteroid. 1,25(OH)$_2$D$_3$ increases brush border membrane fluidity by altering the lipid composition. Administration of vitamin D to animals has been reported to cause an increase in phosphatidylcholine of its lipids and an increase in the degree of unsaturation (Max et al., 1978).

Recently, a novel mechanism for the transport of calcium across the BBM has been proposed (Bikle, 1989), which suggests the role of receptor (105 KDa protein) for calmodulin in the luminal membrane as a calcium channel. Once calcium enters through this channel it binds to calmodulin and the complex diffuses to a coated pit area at the base of the microvilli, where internalization by endocytosis takes place. Studies have demonstrated the endocytotic vesicles as carriers of calcium from the plasmalemma to the basolateral membrane via lysosomes (Nemere et al., 1986; Favus et al., 1989). However, the ferrying role of calcium binding protein has also been
suggested (Nemere et al., 1986; Favus et al., 1989). Recent studies by Takito et al., (1990) also demonstrated that the initiation of calcium absorption by vitamin D₃ appears to be due to increase in the rate of calcium efflux at basolateral membrane rather than BBM. Thus the effects of vitamin D₃ may involve transient increase efflux of calcium at BLM, which in turn activate exocytosis of calcium-containing vesicles as well as efflux by calcium pump, and Na⁺-Ca²⁺ exchangers, resulting in a net increase in calcium transport (DeBoland et al., 1990). Recently, the action of secosteroid hormone in stimulating phosphoinositide hydrolysis and causing translocation and activation of protein kinase C has also been reported in rat colonic epithelium (Wall et al., 1990). Such changes bring about a rapid rise in cytosolic calcium partly due to calcium influx. Some of the present concepts involving transcellular calcium transport and the action of vitamin D is shown in Fig.32.

5.8 Magnesium deficiency and altered urine composition

Calcium and oxalate are the major constituents of urinary tract stone formation (Thind et al., 1989). Hyperoxaluria rather than hypercalciuria has been suggested to be an important causative factor of calcium oxalate nephrolithiasis (Robertson et al., 1981). Oxalate is a metabolic end product of metabolism, and is excreted unchanged in urine. Several studies have demonstrated net
FIG. 32 EFFECT OF 1,25-DIHYDROXY VITAMIN D3 ON TRANSCELLULAR TRANSPORT OF CALCIUM
tubular secretion of oxalate (Weinmann et al., 1978; Knight et al., 1981; Kanazawa, 1990). Micropuncture studies have demonstrated that oxalate is freely filtered in the glomerulus and undergoes net secretion in the proximal portion of the nephron (Hauntmann and Osswald, 1978, 1979). In the nutritional stress conditions of magnesium deficiency, significant hyperoxaluria (p<0.001) was observed in the experimental animals, which can be attributed to increased endogenous synthesis of oxalate and also increased intestinal absorption as compared to pair-fed controls (Fig.33). Hyperoxaluria has also been observed in other nutritional stress conditions such as that of thiamine deficiency (Sidhu, 1985), pyridoxine deficiency (Farooqui et al., 1981; Murthy et al., 1982b) and Vitamin A deficiency (Sharma et al., 1990).

Magnesium deficiency in rats did not cause any alterations in the creatinine excretion, suggesting thereby that whole kidney function is not altered in this nutritional deficiency. This is in agreement with earlier reports (Al-Modhfer et al., 1986; Grimm et al., 1990). Studies by Manitius and Epstein (1963) and Grimm et al., (1990) has also demonstrated that magnesium deficiency does not alter the ability of the kidneys to concentrate the urine.

A significant hypocalciuria, despite the presence of hypercalcemia was observed in the experimental animals,
FIG. 33 MECHANISM OF HYPEROXALURIA IN MAGNESIUM DEFICIENCY

ENDOGENOUS BIOSYNTHESIS

GLYCOLATE → GAO → GLYOXYLATE → GAO → OXALATE

LIVER

KIDNEY

GLYOXYLATE → LDH-I → OXALATE

INTESTINE

DIETARY OXALATE ABSORPTION

MAGNESIUM DEFICIENCY

HYPEROXALURIA
suggesting that calcium and magnesium share a common transport mechanism both at the intestinal as well as renal tubular level. Magnesium deficiency in rats is known to increase the secretion of parathyroid hormone, which is known to increase the renal tubular reabsorption of calcium. Increased calcium content of kidneys has been reported in magnesium–deficient rats (Kimura and Itokawa, 1989).

Magnesium deficiency caused a significant hyperphosphaturia (p<0.01) in the experimental rats as compared to the pair-fed controls. PTH is known to decrease the renal tubular reabsorption of phosphorus. This is in agreement with earlier reports (MacIntyre and Davidsson, 1956; Whang and Welt, 1963; Gitelman et al., 1968). The presence of hypercalcemia as well as hyperphosphaturia also suggests a state of hyperparathyroidism. Hyperphosphaturia leads to hypophosphatemia, which is known to cause increased synthesis of 1,25(OH)₂D₃ resulting in hyperabsorption of calcium from the intestine which substantially contributes to the presence of hypercalcemia (Table 9).

No change in the excretion of uric acid was observed in the present study, which suggests that magnesium deficiency did not alter the purine metabolism in rats, (table 9).
Magnesium deficiency led to a significant (p<0.001) decreased excretion of citric acid in the experimental animals as compared to the pair-fed controls (Table 9). It has been speculated that because of the molecular similarity between glyoxylate and acetate, glyoxylate accumulation (as in the present study) lead to diminution of citrate synthesis, by competing with acetate in the citrate synthetase reaction (Ruffo et al., 1962). Studies have also demonstrated that oxalate even at physiological concentration lowers the citrate flux through tricarboxylic acid cycle by about 48% and reduces the steady state level of oxaloacetate, citrate and maleate. Thus magnesium deficiency, which caused increased oxalate production by increasing the endogenous synthesis could significantly lower the intracellular citrate synthesis, thus causing hypocitraturia. Recently decreased excretion of citric acid in primary hyperparathyroid patients has also been reported (Alvarez - Arroyo et al., 1992), suggesting that PTH could be involved in citric acid metabolism, by causing increase in citric acid renal tubular reabsorption.

Thus magnesium deficiency in rats lead to hyperoxaluria, hypocitraturia, hypomagnesuria, which are known to be an important causative factor of stone formation.
Magnesium deficiency and altered lipid composition of intestinal and renal brush border membrane

Studies have demonstrated that composition and physical state of the membrane lipids greatly influence the functional properties of the biological membrane (Metchoir and Steim, 1976; Sandermann, 1976). The lipid bilayer primarily consists of phospholipids and cholesterol which play an important role in the membrane function (Demel and de Kriyff, 1976). Any change in the chemical architecture of cell membrane, thus is critical in the control of cellular functions (Asatoor et al., 1972). Accordingly, the present study was carried out, to examine the role of brush border membrane lipids in relation to the changes observed in intestinal and renal handling of calcium and oxalate, in magnesium - deficient rats.

A relation between lipid metabolism and magnesium deficiency has been reported. Magnesium deficiency in rats leads to hypertriglyceridemia and hypercholesterolemia with increase of free cholesterol and decrease of esterified cholesterol (Rayssiguier 1985; Jaya and Kurup, 1987). Magnesium deficient rats also develop dyslipoproteinemia characterized by increase in very low density and low density lipoproteins with concomitant decrease in the high density lipoproteins. (Gueux et al., 1991). Increased plasma levels of thio-barbituric acid reacting substances used as a
measure of lipid peroxidation in magnesium-deficient rats has been reported (Mahfouz and Kummerow, 1989).

Major changes observed in the lipid composition of intestinal (Table 21) and renal (Table 22) brush border membrane of magnesium-deficient rats showed significant decrease of cholesterol and an increase of total fatty acid content with no change in total phospholipid content.

Cholesterol is known to increase the packing density of phospholipid bilayers (Pottel et al., 1983; Van Der Meer et al., 1986). Cholesterol being a rigid wedge-shaped molecule, orients perpendicular to the membrane with its hydroxyl group just below the polar head group of phospholipids (Franks, 1976), thus is a membrane rigidifying molecule. Similarly, phospholipid are other major compounds of membrane, which, provide it with its structural integrity and physical properties. Changes in the membrane phospholipid head group composition can affect the membrane-bound enzymes (Malkiewicz-Wasowicz et al., 1977) and the permeability of the membrane to ions (Matsumoto et al., 1981). Cholesterol to phospholipid ratio provides recognised indices for the fluidity of the membrane (Molitoris and Simon, 1985). A decreased cholesterol content of the membrane observed, thus increases the membrane fluidity of the brush border membrane.

Studies by Mahfouz et al., (1989b) on porcine kidney cell (LLC-PK) grown in magnesium-deficient medium
demonstrated that although, the total phospholipid content of membrane remained unaltered, but a significant increase in phosphatidylcholine with a concomitant significant decrease in phosphatidylethanolamine, phosphatidylserine, sphingomyelin, and phosphatidylinositol was observed. Phosphatidylcholine is a known fluidifier. Thus increased synthesis of phosphatidylcholine in the brush border membrane could also be responsible for increasing the membrane fluidity. Moreover, magnesium is known to bind to phosphates of the phospholipid headgroups. Thus a decrease in magnesium content of the membrane could also add to the increased membrane fluidity, as has been demonstrated in magnesium-deficient erythrocyte membrane (Tongyai et al., 1989).

A significant increase in triglycerides in the intestinal and renal brush border membrane could be related to the defect in the removal of triglycerides-rich lipoproteins from the circulation in magnesium-deficient rats (Rayssiguier and Gueux, 1983).

Fatty acid modification of the cellular membrane lipids is also known to influence the degree of ordering and motion in the hydrocarbon core of the membrane lipid bilayer i.e. the membrane fluidity (Spector and Yorek, 1985). Studies have also demonstrated that unsaturation of fatty acids also influence many cellular functions such as carrier-mediated transport, membrane bound enzymes and receptor
properties (Spector and Yorek, 1985). A significant increase in the total lipid content of the brush border membrane was observed in the experimental rats. Magnesium deficiency in rats has been shown to cause perturbances in the essential fatty acid metabolism, by decreasing the poly-unsaturated fatty acid content of the membrane in porcine kidney cell line (Mahfouz et al., 1989a). Studies by Rayssiguier et al., (1986) also demonstrated decreased plasma levels of stearic acid and arachidonic acid.

Vitamin D is also known for its liponomic effect on the brush border membrane. Administration of Vitamin D to animals has been shown to increase the membrane fluidity of BBM by causing an increase in the phosphatidylcholine content and increasing the linoleic acid content of intestinal (Max et al., 1978) as well as renal (Tsutsumi et al., 1985) brush border membrane. Both these changes influence the fluidity of the membrane.

Increased membrane fluidity of the erythrocyte and hepatocyte plasma membrane has also been reported in magnesium-deficient rats (Rayssiguier et al., 1989). Thus increased membrane fluidity of intestinal and renal cortical BBM is partly responsible for the increased translocation of both calcium and oxalate across.
It has been hypothesized that defective membrane function could be the primary lesion underlying the cellular disturbances that occur in magnesium deficiency. Increased uptake of both calcium and oxalate across the intestinal and renal cortical brush border membrane leading to hyperoxaluria suggested the defective cellular oxalate transport. Hyperabsorption of oxalate in idiopathic calcium oxalate nephrolithiasis has been reported, which also supports the defective oxalate transport. To further test this hypothesis, transmembrane oxalate flux studies were carried out in intact erythrocytes of magnesium-deficient and the pair-fed control rats.

Magnesium deficiency caused a significant increase (p<0.001) in transmembrane oxalate flux in intact erythrocytes of experimental animals as compared to the pair-fed controls (Fig. 29). Several studies have shown the effect of magnesium depletion in the erythrocyte membrane. Metal analysis of erythrocytes from magnesium-deficient rats revealed a significant reduction in magnesium and potassium and an increase of sodium and calcium showing the increased membrane permeability of the red cell membrane (George and Heaton, 1975; Heaton et al., 1989). Increased fluidity of erythrocyte ghosts, decreased osmotic fragility to hypotonic saline and decreased microviscosity has been
reported in magnesium-deficient rats (Heaton et al., 1987, 1989). Studies have also revealed increased membrane fluidity of the magnesium-deficient rat red blood cell membrane due to decreased cholesterol/phospholipid and sphingomyelin/phosphatidylcholine ratio (Heaton et al., 1989; Tongyai et al., 1989), suggesting that it occurs due to physicochemical exchange within plasma. Cholesterol/phospholipid and sphingomyelin/phosphatidylcholine ratios are the indices of membrane fluidity (Shinitzky and Barenholz, 1978). Calcium is known to make the membrane more rigid (Schinitzky and Barenholz, 1978), but studies have revealed no detectable loss of calcium from the erythrocyte membrane of magnesium-deficient rats (Tongyai et al., 1989). It is the loss of magnesium from the membrane, that brings about destabilization of the lipid bilayer, due to its decreased binding to negatively charged phosphates of the phospholipid headgroups (Tongyai et al., 1989).

Increased transmembrane oxalate flux in red cells of idiopathic calcium oxalate stone patients has also been reported (Baggio et al., 1984, 1986a), which has been attributed to the hyperphosphorylated anion transporter band-3 protein in the erythrocyte membrane (Baggio et al., 1986 b).

Thus increased membrane fluidity of the membrane brings about enhanced oxalate flux in the intact
erythrocytes of magnesium - deficient rats, thereby further supporting the defective membrane function in magnesium - deficiency.

5.11 Mechanism of altered calcium metabolism in magnesium deficiency

The present study was carried out to elucidate the biochemical mechanism of hypercalcemia by investigating the intestinal absorption and renal tubular reabsorption of calcium by preparing brush border membrane vesicles. The results demonstrated that enhanced uptake of calcium by the intestine (Fig. 15) and kidney (Fig. 21) substantially contributes to the hypercalcemia in rats. PTH induced mobilization from the bone also contributes to the increased calcium concentration in the plasma. The overall view of the mechanism of altered calcium metabolism is shown in Fig. 34.

5.12 Mechanism of altered oxalate metabolism in magnesium deficiency

The effect of magnesium deficiency on oxalate metabolism was investigated by studying oxalate synthesizing enzymes in the liver and kidney. Uptake of oxalate by the intestine and kidney was also studied by preparing BBMV from these tissues. The study demonstrated that magnesium deficiency in rats produces hyperoxaluria by not only increasing the endogenous synthesis of oxalate in liver as well as kidney (Table 10, 11) but also by enhancing absorption and tubular reabsorption by the intestine (Fig.
Fig. 34 MECHANISM OF ALTERED CALCIUM METABOLISM IN MAGNESIUM DEFICIENCY

- Increased reabsorption of calcium
- Decreased excretion of calcium
- Calcium release
- Increased contribution to body pool

INCREASED CALCIUM RELEASE

Blood

Mucosal membrane
Serosal membrane
Transporter

Intestinal mucosal cell

decreased excretion of calcium
Increased reabsorption of calcium
18) and kidney (Fig. 25) respectively. The overall view of the biochemical mechanism of altered oxalate metabolism is shown in Fig. 35.

5.13 Risk of stone formation in magnesium deficiency

Hypomagnesuria, hypocitraturia and hyperoxaluria are common findings amongst stone formers and have been described as major risk factors for stone formation. Magnesium deficiency in rats also produces these changes, thus making the magnesium-deficient rats more prone to risk of stone formation as compared to control rats.

The highlights of the present study are as follows:-

1. Hyperabsorption of calcium also contributes to the hypercalcemia.

2. Enhanced endogenous synthesis and hyperabsorption of oxalate substantially contribute to hyperoxaluria in magnesium-deficient rats.

3. Altered transmembrane oxalate flux in magnesium-deficient rats.

4. Altered lipid composition of the BBM responsible for enhancing the uptake of calcium and oxalate in the intestine as well as kidney further supports the hypothesis that defective membrane function could be the primary lesion underlying the cellular disturbances that occur in magnesium deficiency.
Increased Reabsorption of Oxalate
Increased excretion of oxalate
Increased secretion of oxalate

Glycolate
Glyoxylate
GAO
LDH
GAD

LUMEN
Mucosal membrane
Serosal membrane
Transporter
Oxalate

Intestinal mucosal cell

BLOOD

Glyoxylate
LDH-I
Oxalate

LIVER

Oxalate
Increased contribution to body pool

Hyperoxaluria