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5.1 SODIUM GLYCOLATE-THE HYPEROXALURIC AGENT AND THE PROPHYLACTIC EFFECT OF TRADITIONAL PLANTS AGAINST HYPEROXALURIA

Glycolic acid, the immediate precursor of oxalate, has been identified as an early product of photosynthesis and is present in significant concentrations in several plant tissues (Wyman and Palmer, 1964; Bravado et al., 1977). It is distributed throughout all the plant tissues but the relative concentrations vary with the plant. Harris and Richardson (1980) analyzed the glycolate content of various foods and showed that leafy vegetables and fruits contain good amount of glycolate. However, meat foods contain lower level of this compound. Although, in the animal, glycolate could be obtained from the diet and may be synthesized endogenously from aminoacids and carbohydrates, the low glycolate content of the tissues is probable because of its metabolism and rapid excretion in the urine. At an estimated average of 33 mg of glycolate consumption daily, nearly 5% or more of the urinary oxalate may be derived from dietary glycolate, which amounts up to 20 mg of urinary oxalate per day (Harris and Richardson, 1980). Talwar et al., (1984) elucidated the mechanism of the intestinal absorption of glycolate and showed the existence of a carrier-mediated glycolate uptake, which was maximum in the ileum and jejunum, followed by duodenum and was lowest in the colon.

Glycolic acid, when fed in the diet, or synthesized in large amounts in the body leads to hyperoxaluria and calcium oxalate stone formation (Richardson, 1965; Murthy et al., 1981) Glycolate feeding either alone (Murthy et al., 1981; Gulati et al.,1988) or in combination with hydroxyproline (Sangeeta et al., 1993) has been used in our laboratory, as a successful model for the induction of hyperoxaluria. In the present study, feeding of sodium glycolate to male rats for 30 days, led to an increase in urinary oxalate excretion(approximately 3-fold) when compared with control rats (Table 1,2). These results are in agreement with those obtained by Murthy et al., (1981). Sodium glycolate induced hyperoxaluria can be explained on the basis that glycolate in the liver is either oxidized to glyoxylate and then to oxalate by
an enzyme glycolate oxidase (GAO) (Murthy et al., 1983; Yanagawa et al., 1990) or directly converted to oxalate without forming glyoxylate as an intermediate by the enzyme glycolate dehydrogenase (GAD) (Fry and Richardson, 1979a), leading to hyperoxaluria. However, lactate dehydrogenase (LDH) and xanthine oxidase (XOD) are also involved in the catalytic conversion of glyoxylate to oxalate (Yanagawa et al., 1990). Various liver and kidney enzymes involved in the oxalate biosynthesis have been estimated and discussed later (Section 5.3).

5.1.1 Traditional Plants And The Urinary Excretion Of Various Lithogenic Substances

Hyperoxaluria plays a critical role in stone formation and the changes in urinary oxalate concentration are fifteen times more potent as changes in calcium concentration in altering the saturation of urine with calcium oxalate (Robertson et al., 1980; Baggio et al., 1983). Oxalate in the urine is constantly present at a supersaturated level, therefore, any increase in its concentration attributable to glycolate feeding, may enhance the chances of formation of calcium oxalate crystals, however, development of effective and safe methods for lowering urinary oxalate and thereby preventing calcium oxalate renal stone disease are needed. Although fluid and dietary modifications (Menon and Koul, 1992), various medical and biochemical therapies like administration of pyridoxine (Yendt and Cohamin, 1985) orthophosphate (Smith, 1989), magnesium (Lindberg et al., 1990) and citrate (Sakhaee et al, 1991) are in constant use for controlling the hyperoxaluria, yet each of these has one or the other limitations like economic constraints, side effects etc. Therefore, in the present study, indigenous plants have been used as the drugs of choice. The efficacies of Achyranthus aspera (Nadkarni, 1976), Crataeva nurvala (Deshpande et al., 1982; Varalakshmi, et al., 1990) and Tribulus terrestris (Santha Kumari and Iyer, 1967; Sangeeta et al., 1993) have been studied in the past. The degree of effectiveness of each plant in lowering of sodium glycolate induced hyperoxaluria is in the order of T. terrestris (50%), C.nurvala (30%) and A. aspera (10%) as compared to hyperoxaluric animals. As T. terrestris
has the maximum oxalate lowering effect, it has been chosen for further studies to evaluate the biochemical basis for its beneficial effects. In the present study, besides oxalate, the urinary excretion of glyoxylate calcium, phosphorus and uric acid has also been studied. In the present study too sodium glycolate feeding had no effect on urinary calcium excretion, similar to earlier report from our laboratory (Gulati et al., 1988). Studies in stone forming patients have shown that majority of these patients excrete either normal or low levels of calcium in their urine (Will and Bijovet, 1979). Baggio et al., (1983) have also observed that only oxalate was found to be significantly higher in stone formers as compared to control subjects. No changes observed in urinary phosphorus and uric acid excretion in the hyperoxaluric rats are in concurrence with the observations of Murthy et al., (1981) and Baggio et al., (1983).

5.2 CALCULIUM OXALATE CRYSTALLIZATION AND URINARY INHIBITORS OF STONE FORMATION

Mixed type of stones containing 85-90% oxalate, are most common form of human urinary stones. Even normal human urine is supersaturated with respect to calcium oxalate monohydrate and supersaturation is higher than normal in patients with urolithiasis. Urine supersaturation drives formation and growth of calcium oxalate crystals, which comprise the bulk of human renal stone. Many factors have been advanced in recent years to explain the formation of stones in the urinary tract, of which inhibitors and promoters play an important role.

A calculus begins, when the concentration of crystallizable components in urine exceeds the saturation limit and a crystal nidus is formed. This process is possibly enhanced by organic matrix inducer substances and repressed by organic and inorganic crystallization inhibitors. The newly formed crystal attracts further identical molecules from supersaturated urine to form homogenous enlargement or alternately attracts dissimilar molecules to form organized heterogenous calculus. The energy required for spontaneous
nucleation of calcium oxalate is extremely high. Finlayson (1978) has shown that it corresponds to at least an 80-fold supersaturation, which has never been found in urine or kidney tissue. Crystalluria and stone formation, therefore, seem to be the results of heterogenous nucleation induced by promoters which probably reduce the surface energy required for crystallization. The enlarged crystal becomes trapped in the terminal portion of the nephron, aggregates with other crystals and grows and is finally shed in the urinary stream. Hence stone formation can begin whenever there is an imbalance between promoting and inhibiting factors (Baggio et al., 1983). Thus in brief crystallization process can be divided into nucleation, growth, epitaxy and agglomeration. Spontaneous nucleation and agglomeration occur very rapidly whereas the other processes are time consuming (Baumann, 1990).

Crystallization processes are modulated by chelators, inhibitors and promoters. The state of supersaturation with respect to a stone forming salt is given by the activity product of stone forming ions. Ion activity is reduced by chelators that trap free ions and form soluble complexes. Low molecular weight substances such as citrate and magnesium are chelators as well as inhibitors (Baumann, 1990). Human urine contains inhibitors of calcium oxalate crystal growth and aggregation which fulfill the physiological role of repressing the formation, growth and aggregation of calcium oxalate crystals from supersaturated urine (Ito and Coe, 1977). An inhibitor remains active at very low concentrations at which no chelating effects can be measured. Inhibitors have the following three effects (1) they diminish nucleation and growth rates (2) they increase the metastability of solutions and urine with respect to spontaneous as well as heterogenous nucleation and growth (3) they reduce agglomeration (Baumann, 1990). Inhibitors, however, must be absorbed at the high energy active sites (kink sites) on the crystal surface in order to effectively block growth processes at the surface steps (Nancollas et al., 1989). Among urinary macromolecules, GAGs, RNA and nephrocalcin have important inhibitory effects. Tamm Horsfall protein figures as an inhibitor as well as a promoter. Ryall et al., 1989) have reported evidence that small
molecular weight inhibitor in whole urine preferably influence nucleation and growth, whereas macromolecular compounds inhibit aggregation.

5.2.1 Alterations In The Urinary Inhibitory Activity Of Sodium Glycolate And T. terrestris Treated Rats

In the present study, a procedure was established to measure the rate of crystal growth of calcium oxalate monohydrate seed crystals in metastable solution of calcium chloride and sodium oxalate containing traces of $^{14}$C-oxalic acid based on the methodology of Ito and Coe (1977) and Nakagawa et al., (1981). This proved to be a rapid, simple and highly reproducible method, useful to quantitate the inhibitory effect of urine. The inhibitory activity of rat urine was determined in calcium oxalate monohydrate crystal growth system. Strong inhibitory activity was found in the rat urine corroborating with the results of Nakagawa et al., (1983) who have shown a strong inhibitory activity in rat and rabbit urine and their kidney homogenates. Urine contains certain inhibitors like pyrophosphate, citrate, glycoproteins etc. which effect crystal formation and growth by blocking the growing sites whereas aggregation inhibitors e.g. GAGs, change the zeta potentials (Fleisch, 1978). The effects of these multiple urinary components are roughly additive and each contributes partly to solubilization of calcium (Azoury et al., 1984). There is now strong evidence to suggest that urine of idiopathic recurrent calcium oxalate stone formers has a lower inhibitory activity towards calcium oxalate monohydrate crystal growth and aggregation as compared to healthy controls (Teotia and Teotia, 1975; Drach et al., 1984). In the present study, sodium glycolate feeding has produced a significant hyperoxaluria which can lead to urine supersaturation and precipitation of calcium oxalate. Concomitantly, the lower inhibitor activity found in this group of animals as compared to control groups (Table 4) could be due to inactivity of several inhibiting species normally present in urine. Although, present data does not reveal separately the effect of each inhibitor, but the total urinary inhibitory activity of T. terrestris (0-day, gp V) treated animals was found to be increased (1-2 fold) as compared to sodium glycolate fed, hyperoxaluric group (gp IV). The normalization of
inhibitory activity in the urine of *T. terrestris* fed group could probably be due to neutralization of the effect of sodium glycolate on urinary inhibitors of calcium oxalate crystallization by *T. terrestris* extract or *T. terrestris* itself contains some of the various calcium oxalate growth inhibiting substances. The later idea seems less reasonable because when *T. terrestris* extract was given from day-15, it could not result in the enhancement of total urinary inhibitory activity, thereby indicating that *T. terrestris* itself does not contain any inhibitory species, rather it acts as an antagonist to sodium glycolate probably at certain metabolic step.

5.3 EFFECT OF SODIUM GLYCOLATE AND *T. TERRESTRIS* ADMINISTRATION ON OXALATE BIOSYNTHESIZING ENZYMES

The significant role of oxalate in kidney stone formation is well established in as much as 70% of all such stones in man contain calcium oxalate (Nordin and Hodgkinson, 1967). The dietary oxalate accounts for less than 15% of the total urinary oxalate and about 85% of the oxalate found in the urine is formed as the result of endogenous production (Archer *et al.*, 1957). The major precursors of oxalate are L-ascorbic acid, glycine and other amino acids, sugars and glycolate, which, except for L- ascorbic acid and tryptophan, all converge to glycolate- glyoxylate-oxlate pathway (Hodgkinson, 1977a).

Ascorbic acid undergoes non-enzymatic conversion to oxalic acid, accounting for about 40% of the excreted oxalate (Atkins *et al.*, 1964). The remainder of urinary oxalate is formed as a result of glycolate-glyoxylate metabolism (King and Wainer, 1968). Glyoxylate which may be formed in vivo from glycine, glycolate or α-keto-γ-hydroxyglutamate is readily oxidized to oxalate by glycolic acid oxidase (Richardson and Tolbert, 1981). Glyoxylate acid is extremely labile and Williams and Smith (1968) have described 17 different metabolic pathways for its degradation. Gibbs and Watts (1973) has shown that in the presence of nicotinamide adenine dinucleotide (NAD⁺) glyoxylate is converted to oxalate by lactate dehydrogenase (LDH) whereas Liao and
Richardson (1973) have demonstrated that at least in the rat, glycolic acid oxidase (GAO) is the main enzyme catalyzing the oxidation of glyoxylate to oxalate. Studies on human and rat liver by Yanagawa et al., (1990) have shown that xanthine oxidase (XOD) also plays a minor role in the production of oxalate. Besides this, most of the glyoxylate in man and animals is oxidized to CO₂ via the glyoxylate oxidation cycle (Gupta and Dekker, 1980) and by α- ketoglutarate: glyoxylate carboligase enzyme (O'Fallon and Brosemer, 1977).

Glycolic acid, formed in vivo from glycine, ethanolamine or carbohydrates, acts both as a precursor and a product of glyoxylate metabolism in animal systems. Studies in rat and man have shown that glycolate is oxidized to glyoxylate and then to oxalate by a peroxisomal enzyme glycolic acid oxidase (Liao and Richardson, 1973; Fry and Richardson, 1979b). In addition, glycolate is directly converted to oxalate by glycolate dehydrogenase in the isolated perfused rat liver (Gambardella and Richardson, 1978). Fry and Richardson (1979a) have isolated this enzyme from human liver and suggested that it may play an important role in oxalate biosynthesis.

In the present study, alterations produced in enzymes of oxalate biosynthesis viz. glycolic acid oxidase (GAO), glycolic acid dehydrogenase (GAD) in liver and lactate dehydrogenase (LDH) in liver and kidneys of sodium glycolate and T. terrestris fed animals have been examined. Glycolate feeding has been shown to accelerate the peroxisomal metabolism. Crane et al., (1980) have observed enhanced β-oxidation of fatty acids in peroxisomes in glycolate fed mice. Stimulated peroxisomal degradation of ethanol in liver has also been reported by glycolate feeding in rats (Cornell et al., 1981). In the present study, glycolate feeding resulted in an increase in the liver GAO activity, which is not in accordance with the earlier observations of Richardson (1965, 67) who showed no changes in this enzyme activity after glycolate treatment. This discrepancy could be due to the difference in the methodology used for the assay of glycolate oxidase. The enhanced liver GAO activity in glycolate fed rats is either due to substrate mediated induction (Murthy et al., 1983) or due to the generalized proliferation of the peroxisomes in
the liver (Crane et al., 1980). Masters and Holmes (1977) also observed that increase in liver peroxisomes was always associated with increased GAO activity. The increased GAO activity by glycolate feeding have also been shown by Varalakshmi et al., (1990) and Kailash and Varalakshmi (1992).

*T. terrestris* extract feeding from 0-day or 15-day along with sodium glycolate has caused a significant lowering of liver GAO activity. Glycolic acid oxidase catalyzes the two step conversion of glycolate to glyoxylate and then into oxalate. The enzyme has a very high affinity for glycolate (Schuman and Massey, 1971) as compared with glyoxylate. Fry and Richardson, (1979b) have also reported that among 13 potential substrates observed, the highest oxidation rate was with glycolate which also had the lowest K_m and the highest V_max. Askar and Davis (1983) have purified GAO from rat liver and showed that it has a K_m of 0.25 μM for glycolate and 2.9 μM for glyoxylate. *T. terrestris* extract administration to sodium glycolate fed rats resulted in a significant decrease in urinary oxalate excretion which is accompanied by increased urinary glyoxylate levels. From these findings, it can be speculated that *T. terrestris* causes the reduction in GAO activity by inhibiting the enzyme at the second oxidation step i.e conversion of glyoxylate to oxalate, thereby resulting in glyoxylate accumulation and its enhanced excretion. *T. terrestris* fed from day 15, i.e. 15 days of sodium glycolate pretreatment also lowered the GAO activity but to a lesser extent, thereby indicating that *T. terrestris* has more prophylactic rather than curative effect.

Experimental evidence from metabolic studies has suggested that an alternate pathway for oxalate synthesis exists, in which glycolate is oxidized directly to oxalate without forming free glyoxylate as an intermediate (Runyan and Gershoff, 1965; Liao and Richardson, 1972). Fry and Richardson (1979a) reported the isolation and characterization of enzyme GAD from human liver. This enzyme catalyzed the direct conversion of glycolate to oxalate and contributes to the biosynthesis of oxalate in man and other mammals. Yanagawa et al., (1990) however, could not detect the presence of GAD under the experimental conditions used and demonstrated that in rat liver, the formation
of oxalate from glycolate appeared to take place predominantly via gloxylate by the action of GAO. In the present data, significant elevation of the enzyme GAD has been found, which is due to substrate mediated induction of this enzyme. Fry and Richardson (1979a) have demonstrated very high specificity of GAD for glycolate with a very low $K_m$ \(6.3 \times 10^{-5} \text{M}\) for glycolate. Moreover, it was also proposed by the same workers, that under optimal conditions, the amount of GAD present in 1g of human liver can convert nearly 1.5u mole of glycolate to oxalate in 24h. Since the average human liver weights about 1500g, this would amount to about 200 mg oxalate/day and as the daily oxalate excretion in the urine is only about 20-40 mg, GAD can account for most of the oxalate synthesis from glycolate in the body, thus supporting the present observation that glycolate load can lead to an elevation of both GAO and GAD.

*T. terrestris* treatment from 0-day along with sodium glycolate (gp V) has significantly suppressed the GAD activity as compared to sodium glycolate fed rats (gp IV). As glyoxylate is a known inhibitor of the enzyme GAD, again the probable cause of suppressed GAD activity in *T. terrestris* fed group is due to the excess glyoxylate produced by the inhibited GAO activity at the secondoxidation step of glycolate to oxalate conversion reaction. Glyoxylate is a potential toxic metabolite and its rapid removal by the body either by further oxidation to $\text{CO}_2$ or to oxalate or by urinary excretion is necessary. The evidence for hyperglyoxylaturia by *T. terrestris* feeding also comes from the earlier work (Sangeeta *et al.*, 1993).

The third important enzyme of endogenous oxalate production in liver and extrhepatic tissues is lactate dehydrogenase (Smith *et al.*, 1972; Gibbs and Watts, 1973). In aqueous solutions glyoxylate is largely present as a hydrate and is able to act as a substrate for oxidation by LDH (Warren, 1970). The pH optima for the LDH mediated oxidation of glyoxylate is 9.3 and at PH 6.9 it is reduced to glycolate. The increased LDH activity in liver in glycolate fed group as compared to normal could be due to excess substrate which may potentiate the LDH enzyme protein, an observation which is in
agreement with the earlier report of Murthy et al., (1983). The liver LDH levels remained elevated even with simultaneous feeding of *T. terrestris* either from 0-day or 15-day. This increased LDH in the liver favours the oxidation of accumulated glyoxylate (due to GAO inhibition) to oxalate, in an attempt to lower the glyoxylate content, which is a toxic metabolite. Since urinary excretion of glyoxylate is found to be high in *T. terrestris* fed animals, GAO inhibition seems to exceed the LDH mediated oxidation, resulting in hyperglyoxylaturia. Varalakshmi et al., (1990) have reported no alterations in liver LDH either by glycolic acid feeding or by an ayurvedic extract of *crataeva nurvala* whereby indicating that the role of hepatic LDH contributing to oxalate synthesis is poorly understood.

Although, liver is the major organ for endogenous oxalate synthesis (Richardson, 1973), some oxalate producing ability is certainly present in kidney, since it contains LDH activity. Continued conversion of glyoxylate into oxalate despite complete hepatectomy has also been reported by Farinelli and Richardson (1983). Sodium glycolate feeding led to a significant decrease in the kidney LDH, which could be a metabolic compensatory mechanism against very high renal oxalate levels. Excess oxalate produced by the simultaneous action of both GAO and GAD in the liver, lead to a high body pool of oxalate which has been demonstrated to act as a non-competitive inhibitor for the reduction reaction and as a competitive inhibitor for the oxidation reaction of glyoxylate by LDH (Banner and Rosalki, 1967; Warren, 1970). Inhibition of kidney LDH by oxalate is further confirmed in *T. terrestris* fed group in which the kidney LDH levels were normalized as compared to sodium glycolate fed animals. *T. terrestris* has suppressed the increased activity of liver GAO and GAD, resulting in decreased hepatic synthesis of oxalate and decreased kidney oxalate load, thereby preventing the inhibition of kidney LDH.

Role of kidney LDH in oxalate synthesis was further studied in terms of its isoenzyme pattern. Agar gel electrophoresis has shown the presence of all the five isoenzymes of kidney LDH. The LDH isoenzymes were identified using lactate, NAD$^+$ and NBT and their percent distribution examined in
different groups can be used to evaluate the glyoxylate oxidation by LDH. The % distribution of LDH isoenzymes found in control group (gp I) was 32, 24, 5, 12 and 28 for LDH₁ to LDH₅, confirming the fact that LDH₁ is the most abundant isoenzyme of the kidney. In the present study, LDH₁ isoenzyme has shown the maximum resistance and did not change in any of the groups, thus supporting the view of Nisselbaum et al., (1964) that LDH₁ is inhibited less efficiently by oxalate and is more active in oxalate biosynthesis than LDH₅. The role of LDH₁ in oxalate biosynthesis is also supported by a significant increase in this isoenzyme activity in the kidneys of rats with surgical induction of bladder stones and also in the kidneys of thiamine deficient rats (Thind and Nath, 1977; Sidhu et al., 1985). The isoenzyme distribution of sodium glycolate fed group (V) shows that the % activities of LDH₂ and LDH₄ have significantly decreased at the expense of increased LDH₃ and LDH₅ fractions. T. terrestris administration, on the other hand, either alone or along with sodium glycolate (gp II, III and gp V, VI) showed an opposite effect to that caused by only sodium glycolate (gp IV) i.e. elevation of LDH₅ isoenzyme at the expense of LDH₂ and LDH₃ isoenzymes. LDH₅-isoenzyme which is the most labile isoenzyme (Nisselbaum et al., 1964), in the present study is thus being inhibited by sodium glycolate feeding and elevated by T. terrestris administration. But the isoenzyme, mainly involved in oxalate biosynthesis i.e. LDH₁ remained unaltered. From these observations, it can be concluded that the increase or decrease found in total kidney LDH by sodium glycolate and T. terrestris respectively is due to their antagonistic action which is mainly attributable to LDH₅ fraction.

5.4 RENAL HANDLING OF OXALATE IN SODIUM GLYCOLATE AND T. TERRESTRIS TREATED RATS

Despite the importance of oxalate, the end product of metabolism, in the pathogenesis of renal stones, the understanding of the renal handling of oxalate is incomplete. Transcellular transport in renal tubules involves the movement of solutes between three compartments, luminal, intracellular and peritubular. They are separated by two barriers, the apical (luminal or
brush border) and the basolateral (contraluminal or serosal) plasma membranes. The two membranes are different in almost every respect: morphology, enzyme content, protein, lipid and carbohydrate composition (Murer and Gmaj, 1986). Differences in enzyme activities provide the criteria for identification of the separated membranes. In recent years, the methodology for the isolation of brush border and basolateral membrane vesicles from renal cortex has been well established and the membrane vesicles have been used for analysis of the transport properties of organic anions (Hori et al., 1982; Inui et al., 1984; Murer and Gmaj, 1986). On the other hand, corresponding studies of oxalate handling by the kidneys are represented by only a few reports.

Oxalate anion appears to be transported by using counter exchange mechanism and the counter exchange anions used by oxalate can be Cl⁻ (Karniski and Aronson, 1987); SO₄^{2-} and HCO₃⁻ (Kuo and Aronson, 1988) or OH⁻ (Yamakawa and Kawamura, 1990). Weinman et al., (1978); and Deetjen et al., (1979) have investigated the oxalate handling in the rat kidney by micropuncture and clearance studies. Such studies have revealed that oxalate is freely filtered from the glomerulus and undergoes net secretion, predominantly in the proximal convoluted tubule. Furthermore, microperfusion and microinjection studies by the same workers have suggested the possibility that more than one oxalate transport system exists in the rat proximal tubule (Knight et al., 1981). Recent studies by several workers support the presence of bidirectional transport of oxalate (Wandzilak and Williams, 1990; Wandzilak et al., 1992; Koul et al., 1992; Ebisuno et al., 1992).

During the last decade, oxalate secretory process in the renal tubules was debatable. The data obtained with rapid intrarenal injection technique reported a ratio of oxalate/inulin excretion of 1.3 to 1.6 (Osswald and Hautmann, 1979). The excretion of a substance into urine in excess of inulin indicates tubular secretion as was demonstrated for p-aminohippurate (Hautmann et al., 1976). The secretory flux in the proximal tubule exhibits all the characteristics of an active, saturable organic anion transport mechanism. This net secretion could be the result of proximal bidirectional transport whereby unidirectional
secretion exceeds the unidirectional reabsorption. However, Gupta (1986) from our laboratory has performed efflux studies with $^{14}$C-oxalate loaded renal BBM vesicles, to distinguish between the reabsorptive and the secretory flux of this dicarboxylic acid, and demonstrated an appreciable reabsorption of oxalate, while ruling out any secretory flux. These results were in agreement with the studies of Zarembski and Hodgkinson (1963, 1969) who have established that glomerular filtration of oxalate is followed by appreciable reabsorption. Although most of the studies on renal handling of oxalate observed till date, demonstrate its bidirectional transport, in the current study, however, only reabsorptive component of oxalate transport has been studied in hyperoxaluric and *T. terrestris* treated rats.

Oxalate absorption was measured using brush border membrane vesicles prepared from renal cortical cells. In the preparation of BBM vesicles, the enrichment of alkaline phosphatase the BBM marker enzyme was about 13-14 fold, whereas activity of Na$^+$, K$^+$-ATPase, the basolateral membrane marker enzyme was decreased, indicating the purity of the vesicle preparation. However, oxalate uptake exhibited a biphasic transport (saturable-carrier mediated as well as nonsaturable-passive diffusion) process, in all the groups. The rate of oxalate uptake was significantly higher in sodium glycolate treated group (IV) as compared to control group (I) at all the concentrations. Since the saturable component follows a Michaelis-Menton equation, the results of the uptake studies (i.e., up to 0.8 mM oxalate) were extended to draw the Lineweaver-Burke plot. Although the $K_m$ in both normal and sodium glycolate fed groups was found to be same (0.240 mM), the $V_{max}$ was higher in sodium glycolate treated group (8.33 nmole/8 min/mg protein), as compared to control group (6.66 nmole/8 min/mg protein), thereby suggesting a greater turnover of transport carrier (increased efficiency of the carrier) or there is an increase in the number of available sites for transport of oxalate in glycolate fed group (capacity effect) *T. terrestris* when fed alone either from 0-day or 15-day (gp II and III) did not show any significant change in oxalate uptake, which is also
evident from the unchanged $K_m$ and $V_{max}$ values for these groups as compared to control group, thereby, showing that the extract itself does not cause any conformational change in the transport carrier.

*T. terrestris* extract fed from 0-day (gp V) along with sodium glycolate, decreased the oxalate uptake as compared to only sodium glycolate treated group (gp IV). A decrease obtained in both $K_m$ (0.196 mM) and $V_{max}$ (5.71 nmole/8 min/mg protein) in group V as compared to group IV ($K_m$ = 0.240 mM, $V_{max} = 8.33$ nmole/8 min/mg protein) indicates that *T. terrestris* extract inhibits the sodium glycolate mediated elevated oxalate uptake in an uncompetitive manner whereby probably the *T. terrestris* extract itself does not affect the transport carrier, rather it prevents the carrier-oxalate complex on the luminal surface to transport the oxalate anion across the membrane. *T. terrestris* extract when fed from 15-day, along with sodium glycolate (gp VI) lowered the oxalate uptake, but not at all concentrations tested and also to a lesser extent. This could probably happen because 15-day pre-treatment of sodium glycolate has already produced some structural changes in the transport carrier molecule, which results in an increased efficiency of the carrier to transport oxalate molecule. Fifteen day post treatment of *T. terrestris* tries to inhibit the uptake again in an uncompetitive way which is evident from the decreased $K_m$ and $V_{max}$ ($K_m = 0.196$ mM; $V_{max} = 6.25$ nmole/8 min/mg protein) as compared to only sodium glycolate fed group (gp IV) ($K_m = 0.240$ mM, $V_{max} = 8.33$ nmole/8 min/mg protein). However, the degree of inhibition in this group is less because of the higher $V_{max}$ (6.25 nmole/8 min/mg protein) observed than found in group V (5.71 nmole/8 min/mg protein) which was fed with the *T. terrestris* extract from the 0-day along with sodium glycolate, therefore resulting in a lesser lowering down of oxalate uptake.

### 5.5 ALTERATIONS IN THE LIPID COMPOSITION OF RENAL BRUSH BORDER MEMBRANE IN SODIUM GLYCOLATE AND *T. TERRESTRIS* TREATED RATS

Cell membranes are composed of a lipid bilayer consisting primarily of phospholipids and cholesterol. Proteins embedded within this environment
function as enzymes, receptors and transporters. There is considerable evidence that membrane composition plays an important role in the structural and functional properties of the membrane (Melchoir and Steim, 1976; Sandermann, 1978; Spector and Yorek, 1985). Although, proteins are generally the components responsible for translocation of solutes across the membrane, the surrounding lipids have the most important role to keep the proteins in the native or biologically active form (Berdanier, 1988), for example, membrane fluidity (the physical state of membrane lipids) has been shown to influence many enzymes, hormones, various transport processes and membrane permeability to ions like Na\(^+\), K\(^+\) and Ca\(^{+2}\) (Molitoris, 1987). Interactions between proteins and lipids within a membrane may involve protein conformational changes effecting ligand binding. In addition, other properties such as the lateral mobility of proteins or protein-protein interactions within the bilayer may be altered. Thus any change in the chemical architecture of the cell membrane is critical in the control of cellular functions.

The lipid composition of renal brush border membrane of sodium glycolate fed group (IV) showed significant decrease in the cholesterol and a concomitant increase in the total phospholipid content as compared to control group (gp I). Cholesterol is known to increase the packing density of phospholipid bilayers (Van Der Meer et al., 1986). Cholesterol being a rigid wedge-shaped molecule orients perpendicular to the membranes with its hydroxyl group just below the polar head group of phospholipids (Franks, 1976; Worcester and Franks, 1976). It is thus a membrane rigidifying molecule. Similarly, phospholipids the other major component of membrane also contribute to the membrane integrity and physical properties. Changes in the membrane phospholipid head group composition can affect the membrane bound enzymes and the permeability of the membranes to ions (Matsumoto et al., 1981). Cholesterol to phospholipid ratio provides a recognized index for the membrane fluidity (Molitoris and Simon, 1985). A decreased cholesterol content of the membrane observed, thus increases the membrane fluidity of the brush border membrane rendering it to transport more oxalate across the luminal surface. In the T. terrestris treated group (gp V) the BBM phospholipid and cholesterol
The contents were almost equal to those of control rats, indicating the ordered or the rigid form of brush border membrane. Thus *T. terrestris* prevents the increased uptake of oxalate across BBM by maintaining the rigidity of the membrane probably by inhibiting the action of sodium glycolate which has resulted in the loss of cholesterol. However, total lipids, glycolipids and triglyceride contents were found to be unaltered in these groups. Since total phospholipid contents were found to be changed in these groups, the individual phospholipids were estimated as the percentage of total phospholipids. No change was seen in the lysophospholipids (LPL) and phosphatidylethanolamine (PE) components in these groups. In sodium glycolate fed animals (gp IV) phosphatidylcholine (PC) was increased whereas phosphatidylserine + phosphatidylinositol (PS + PI) were decreased. These changes were however not evident in the animals fed *T. terrestris* along with sodium glycolate (gp V).

Phosphatidylcholine (PC) is a known membrane fluidifier (Pottel et al., 1983). Increased synthesis of PC in the BBM of sodium glycolate treated rats could also be responsible for increasing the membrane fluidity. Mahfouz et al. (1989) have also shown a significant increase in PC with a concomitant decrease in phosphatidylethanolamine, phosphatidylserine, sphingomyelin and phosphatidylinositol in the kidney cell culture grown in magnesium deficient medium. A generalized change in the lipid composition has been shown to alter the permeability of the intestine and kidney in vitamin B<sub>6</sub> deficiency (Mahmood et al., 1985; Gupta, 1986) and in magnesium deficiency (Rattan, 1992). Thus, the changes observed in lipid composition of glycolate fed rats, may be responsible for modifying lipid protein interactions in the kidney brush border membrane, making it more permeable to ion transport, which results in the increased reabsorption of oxalate.

### 5.6 POSSIBLE MECHANISM OF ACTION OF *T. TERRESTRIS* IN LOWERING HYPEROXALURIA

The use of indigenous drugs has gained world-wide importance for the prevention and treatment of various diseases. *T. terrestris* is a drug, acclaimed...
highly for its use in the management of uropathies and has been indicated in the treatment of urinary stones (Nadkarni, 1976). Study of Santha Kumari and Iyer (1967) has shown a generalized beneficial effect of *T. terrestris* extract for urinary disorders through its high potassium content. However, no report is available, so far, on the exact mechanism of action of this drug in the prevention of stone disease. In the present study, therefore, an attempt has been made to delineate the possible biochemical mechanism of action of this drug in lowering hyperoxaluria - a condition predisposposable to stone formation.

Sodium glycolate feeding (Murthy et al., 1981) to male adult rats for 30 days resulted in a significant hyperoxaluria. The hyperoxaluria induced by sodium glycolate feeding can be explained on the basis of increased activities of enzymes GAO, GAD and LDH leading to increased oxalate biosynthesis (Fig. 25). This increased oxalate is then transported to the blood for its excretion through kidneys resulting in hyperoxaluria. Decreased kidney LDH however indicates that kidneys try to play a regulatory role in compensating the increased oxalate load.

Simultaneous administration of *T. terrestris* extracts with sodium glycolate, either from 0-day or 15-day has resulted in the decreased activities of GAO and GAD (Fig. 26). *T. terrestris* treatment has been shown to lower urinary oxalate while it simultaneously enhances glyoxylate excretion (Sangeeta et al., 1993). This observation leads to the speculation that *T. terrestris* extract lowers the glycolic acid oxidase (GAO) levels by inhibiting this enzyme at the second oxidation step of glycolate to oxalate conversion reaction which results in the decreased oxalate and increased glyoxylate formation. The increased glyoxylate in turn suppresses the activity of glycolic acid dehydrogenase (GAD), resulting in less oxalate synthesis. *T. terrestris* treatment thus seems to lower the endogenous oxalate synthesis mainly through GAO and GAD. Lactate dehydrogenase in the liver, which was increased by sodium glycolate feeding remains elevated by *T. terrestris* administration, and tries to lower down the increased glyoxylate formed. Kidney LDH on the other hand decreased by sodium glycolate feeding, due to increased oxalate load, in the presence of
Fig. 25  Sodium glycolate induced hyperoxaluria
*T. terrestris*, remains elevated, because *T. terrestris* has already reduced the hepatic oxalate synthesis and renal oxalate load. Thus both liver and kidney LDH seem to play regulatory roles rather than only participating in oxalate synthesis.

Results of membrane lipid composition shown the increased cholesterol and decreased phospholipid content of renal BBM of *T. terrestris* fed group as compared to sodium glycolate fed group. Cholesterol/phospholipid ratio is an important index of membrane fluidity. As the cholesterol content is decreased, the membrane becomes more fluid and permeable to ion transport, a condition which is evident in sodium glycolate fed group, in which increased reabsorption of oxalate by renal BBM also contributes to hyperoxaluria. However, in the presence of *T. terrestris*, the uptake of oxalate by renal BBM has been found to be low as compared to sodium glycolate fed group. The decreased reabsorption of oxalate in *T. terrestris* fed group is either due to the increased cholesterol/phospholipid ratio and thus decreased permeability to oxalate or due to some interactions between proteins and lipids within the renal BBM, involving protein conformational changes effecting carrier mediated oxalate transport process.
Possible mechanism of action of T. terrestris in lowering hyperoxaluria

Fig. 26