CHAPTER 1

INTRODUCTION
The decisive steps in the formation of urinary calculi as well as pathogenesis of urolithiasis have not yet been elucidated, but it is well established to be a multifactorial disease afflicting mankind. Anderson (1973) introduced a multifold theory of epidemiology of urinary calculi and divided the various factors into Intrinsic factors: (heredity, age and sex) and Extrinsic factors: (climate, diet, water, occupation etc.). Several reports have appeared in the past of the role played by the supersaturation of urine in the formation of urinary stone (Robertson, 1973; Finlayson, 1977; Tiselius, 1982). The supersaturation of urine with respect to calcium oxalate is mainly determined by the urinary volume and by the excretion of oxalate and calcium. A small volume whether caused by low fluid intake or increased fluid loss by either routes has been shown to result in increased stone formation (Robertson et al, 1980) the majority of which are composed of calcium and oxalate (Borghi et al, 1990). Idiopathic hypercalciuria defined as the urinary excretion of more than 300 mg calcium per day in men or more than 250 mg per day in women is observed in about 50 percent of the patients with calcium oxalate/apatite nephrolithiasis and is one of the risk factors for stone formation (Tiselius et al, 1978; Peterson & Hruska, 1979; Joost and Puschendorf, 1983; Jacob & Gray, 1989). However, little is known about the relative importance of hypercalciuria in stone formation and whereas some have found more active disease in patients with high calcium excretion (Ljunghall and
Waern, 1977; Pak et al, 1981; Strauss et al, 1982), others have not (Marshall et al, 1975; Ettinger, 1976). The importance of calcium as a risk factor was also questioned by Robertson and Peacock (1980), who emphasized that the risk of stone formation and degree of crystalluria correlated much better with urinary oxalate. Also the slightly raised oxalate concentration commonly found in urine from CaOx stone formers (Pinto et al, 1974; Hess et al, 1977; Tiselius et al, 1980; Robertson et al, 1986) might affect CaOx supersaturation relatively more than the increased calcium concentration (Robertson and Peacock, 1983, Hallson and Rose, 1989). Nevertheless, both calcium and oxalate are important determinants for reaching the formation product (AP_{CaOx}) and required due attention in the clinical investigation of stone formers (Dik et al, 1990).

Magnesium and citrate by complexing oxalate and calcium respectively are also determinants of the formation product of calcium oxalate (AP_{CaOx}). Both substances probably act as inhibitors in the crystal growth process. The beneficial effect of magnesium citrate and magnesium oxide on the crystallization of calcium salts has been shown by various workers (Gulati et al, 1988; Lindberg et al, 1990). However, urinary citrate was often found reduced in the stone formers (Bach et al, 1980; Menon and Mahle, 1983; Nicar et al, 1983) and this idiopathic hypocitraturic calcium oxalate nephrolithiasis has been
successfully treated with potassium citrate (Pak and Fuller, 1986; Hauser et al, 1990).

A relationship has also been suggested between high urinary urate concentrations and calcium oxalate stone formation (Coe, 1978; Pak et al, 1980). No satisfactory explanation for the role of urate is so far available, although several hypotheses have been put forward. Crystals of uric acid or sodium urate might serve as nuclei for heterogenous calcium oxalate crystallization (Meyer et al, 1977; Coe, 1978; Pak et al, 1979). The inhibiting properties of urine might be reduced by adsorption of inhibitors onto crystals of uric acid or sodium urate (Pak et al, 1979; Fellstrom et al, 1981; Tiselius et al, 1983) or by binding to colloidal urate (Robertson et al, 1976).

Low inhibitory activity has been observed in urine from stone formers (Coe et al, 1980; Tiselius and Fornander, 1987; Sidhu et al, 1987). This could be the result of reduced excretion of inhibitors or adsorption of inhibitors onto the previously formed crystals (Finlayson, 1982) or the inactivation of these inhibitors by other means (Fellstrom et al, 1981). High pH has also been thought to increase the risk of calcium stone formation (Robertson et al, 1978). Coe et al (1980) showed a higher prevalence of this disease amongst patients with hereditary distal renal tubular acidosis. A high urinary pH was not found in patients forming pure calcium oxalate stones, but only in patients with stones composed of CaOx and CaPO_{4} as a mixture (Robertson et al, 1987). A high pH is certainly

The influence of urinary proteins on the formation and growth of renal lithiasis remains controversial. Specific urinary proteins have been identified in stone forming urine but are absent in non-stone forming urine (Spector et al, 1976; Foye et al, 1982) and the proteins associated with stone matrix have been shown to vary depending on the crystalline composition of the calculus (Yoshika et al, 1989). It is suggested that some selective process is operating in the incorporation of urinary proteins into the supporting stone matrix. However, other studies have indicated that stone matrix forms in a non-specific manner and simply represents adsorption of normally present urinary proteins on the surface of the growing crystal.

Nephrocalcin, a glycopeptide is considered to be a physiological inhibitor of nephrolithiasis (Nakagawa et al, 1983, 1987) and presumably serves as a physiological defence against nephrolithiasis \textit{in vivo} by binding reversibly to calcium oxalate crystals with a dissociation constant of about 0.5 \( \mu \text{M} \) (Hess et al, 1989). The decreased level of amino acid excretion in stone formers and the variation of amino acid patterns according to the type of stone formed is considered to be another factor responsible for the induction of nephrolithiasis (Shaker et al, 1983;
Kohri et al, 1990). Presence of ribonucleic acid or RNA-like material in the human urine as an inhibiting molecule was reported by Ito and Coe (1977). Further, RNA as a citrate complex (Martin et al., 1984), as an adsorption inhibitor (Robertson et al., 1986; Mandel et al., 1987; Brown et al., 1989 and Kleboth et al., 1989) have further strengthened the possible role of RNA in urolithiasis.

Apart from considering the above mentioned factors, it is often helpful to combine different risk factors such as the saturation urinary inhibition index (Robertson et al., 1976), crystal growth factor (Smith, 1976), the relative probability index (Robertson et al., 1978), and the thermodynamic stone formation risk (Brundig et al., 1980), combined discriminant urine analysis, (Coe et al., 1980) and different risk quotients (Tiselius, 1982). Although all these measures help to distinguish stone formers from normal subjects in terms of CaOx crystallization risk, there is considerable overlapping between the two groups. Most of the investigations on composition of urine are performed on 24 h urine collections. Although such studies provide valuable information on the total urinary composition, they are unable to take into account periods with marked risk of crystallization. Diurnal variations in the excretion of risk factors have been demonstrated (Tiselius and Almgard, 1977; Brundig et al., 1980; Vahlensieck et al., 1982; Schulz et al., 1984 and Kinoshita, 1987; Rathore et al., 1989) indicating that the risk of calcium oxalate crystallization fluctuates during the day.
(circadian scale) as well as with the changing climate and photoperiodicity during the year (circannual rhythms). It is therefore very desirable to define these periods and to study how these variations differ in non-stone forming subjects with the help of chronobiochemistry procedures.

Keeping these facts in mind, the present work was undertaken to delineate the chronobiochemical mechanisms involved in the excretion of urinary lithogenic and inhibiting parameters amongst stone formers and normal subjects with the following aims and objectives:

i) The inhibitory activity of urine amongst the idiopathic calcium oxalate stone formers and non-stone formers will be studied.

ii) The quantitative amount of inhibitors (viz. pyrophosphate, citrate and magnesium) and lithogens (viz. calcium, oxalate, phosphate and uric acid) in the urine of stone formers and control subjects will be determined.

iii) To determine the nature of the circadian and circannual rhythms in the urinary inhibitory activity and the excretion of lithogenic, inhibitory and metal ion components amongst stone formers and non-stone formers.

iv) The role of trace metals (Cu, Fe, Zn) as inhibitors of calcium oxalate crystallization in the urine of stone formers and non-stone formers will be evaluated.

v) Isolation and characterization of RNA-like inhibitors from the urine of stone formers and non-stone formers.