CHAPTER 6

SUMMARY
The present investigations were conducted to elucidate the risk of stone formation vis-a-vis the urinary excretion profiles of stone forming subjects. Effect of age, and the interrelationship of various types of urinary stones formed by the patients were studied. The excretory pattern of both lithogenic and inhibitory substances in SF were compared to that of non stone forming subjects. Chronometric studies of circadian and circannual rhythms were studied amongst these patients to identify the high risk periods, during the day as well as in the course of the year, in various climatic conditions. Characterization of the various inhibitors and the inhibitory activity of the biological solvent/solute (urine) was also attempted. Isolation and base analysis of the inhibitory RNA, from the urine of SF and NSF was performed and its inhibitory activity was assessed to establish the difference between these 2 groups of subjects. The important aspects of these investigations are summarized as follows:

1) Studies on 120 urinary SF showed an increased urinary excretion of oxalate (p<0.01), uric acid (p<0.01), and calcium. However, decreased levels of phosphate in association with increased Ca/PO$_4$ ratio (1.5) amongst SF suggested an increased availability of free calcium in the urine of these subjects. On the other hand, Ca/Ox ratio found to be lowered amongst SF by a factor of 1.5, suggested a greater association of calcium with oxalate rather than phosphate. Hyperoxaluria and hyperuricosuria were found to coexist amongst SF. A subclinical deficiency of certain
enzymes has been implicated in the induction of such a condition. Ribose-5-phosphate and Fructose-6-phosphate seem to be the common precursors of hyperoxaluria along with hyperuricosuria in these patients.

Inhibitory activity ranging from 0.16 to 3.10 units was observed to be low amongst SF as compared to control subjects. Association of decreased amounts of urinary citrate (p<0.05), pyrophosphate (p<0.01) and magnesium ions (p<0.01), zinc (p<0.01) and iron (p<0.001) amongst SF suggested an additive role of these inhibitors in the prevention of urinary stones. Urinary excretion of copper, electrolytes (Na⁺, K⁺) and Na⁺/K⁺ amongst SF and normal subjects was comparable.

2) The maximum risk group amongst SF was found to be that of 35-45 years followed by the group of 25-35 years of age, thus constituting 67% of all stone episodes. The initiation of the formation of calcium oxalate stone takes place at or before the age of 25 years amongst high risk subjects as these subjects showed an increased urinary excretion of calcium and oxalate and decreased excretion of phosphate ions in those age groups.

3) Sixty six percent of the patients showed the formation of mixed type of stone, with calcium oxalate as a major component, followed by pure uric acid stones (21%) and pure calcium oxalate (13%). Availability of pure uric acid or pure calcium oxalate serves as matrix to such stones. Presence of binding sites on these crystals helps
them to grow and aggregate. Presence of phosphate ions in trace quantities neutralizes these binding sites and hence retards the growth and aggregation of these crystals.

4) Chronometric analysis of urinary lithogenic substances revealed the absence of circadian rhythms amongst SF, in urinary volume, calcium, oxalate and uric acid, with their high mean urinary excretions as compared to control subjects. Analysis of urinary inhibitors on 24 hours scale showed that citrate and overall inhibitory activity of urine, is maximum amongst NSF during the afternoon hours when the excretion of lithogenic substances was also maximum. This phenomenon of protection by the inhibitors of crystallization amongst NSF during high risk period could not be located amongst SF who showed high excretion of lithogenic substances and low inhibitory activity during that period.

Urinary copper and iron excretion showed no significant circadian rhythm. The acrophase of zinc excretion was located at 14^0h amongst SF as compared 02^00 amongst NSF i.e. just opposite to each other. The circadian pattern of electrolytes (Na^- and K^+) revealed no significant variation amongst SF and NSF. The Na^+/K^+ ratio showed an opposite circadian fluctuation with an acrophase at 16^30h for NSF and 05^00h for SF.

5) Circannual rhythmometric analysis showed a maximum excretion of oxalate in July-August amongst SF as compared to NSF in whom the rhythm was found to be absent. Presence of inhibitors (viz. pyrophosphate, citrate and urinary
inhibitory activity) was found to be minimum in June-September amongst SF, suggesting the high risk period of stone formation to be between June and September (hot monsoon season). On the contrary NSF were found to be protected during these months with high urinary inhibitory activity.

6) Centrifugation of the urine did not affect the inhibitory activity of urine whereas dialysis resulted in a decrease in the inhibitory activity of urine of control subjects and showed stimulatory effect (-ve inhibitory activity) in SF, indicating the presence of dynamic equilibrium of inhibitors and promoters in the urine system.

Hyaluronidase digestion resulted in a decrease (p<0.05) in the inhibitory activity of urine and the presence of GAGS molecules (chondroitin sulphate, hyaluronic acid and heparin) inhibited the calcium oxalate crystallization in vitro. Likewise in vitro effect of polyglutamic acid and polyaspartic acid were inhibitory amongst polypeptides; however, polyglutamic acid showed stimulation at higher concentrations.

Enzymatic treatment of urine with ribonuclease decreased the inhibitory activity of urine (p<0.001) and supplementation of urine with RNA showed an increase (p<0.001) in its inhibitory activity. In vitro studies showed that various polynucleotides (RNA, polycytidylic acid, polyadenylic acid and polyguanylic acid) showed
significant inhibitory activity (at the concentration of 50 ug) towards calcium oxalate crystallization.

7) Amount of RNA isolated from the urine of SF was found to be significantly low (p<0.001) as compared to NSF. Urinary RNA from SF showed a decreased inhibitory activity (p<0.001) as compared to non stone formers. Base analysis of this RNA showed a significant decrease (p<0.05) in the purine contents (adenine and guanine) as compared to NSF.

The studies clearly indicate that, both qualitative and quantitative differences exist between the urine of normal subjects and renal stone patients, regarding the biomolecules which can influence the crystallization and decrystallization reactions. The absence of rhythms on circadian and circannual scale revealed that the renal lithiasis occurs on account of endogenous metabolic defect possibly at genetic level. Circannual studies amongst these patients also showed the high risk period during June-September, when maximum amounts of lithogens and minimum amounts of inhibitors were excreted.

The contribution of various inhibitors and RNA or RNA-like material towards the inhibition of calcium oxalate crystallization established this disease to be multifactorial in nature. The decrease in the inhibitory activity of the urinary RNA and its altered base components from SF suggests the defective nature of gene transcription and possibly as one of the causes of the formation of urinary calculi.

The chronometric study of a particular region, and
the detailed structural and compositional studies of the urinary inhibitors with the help of electrophoresis and advanced analytical techniques may lead to a better understanding of the etiology of renal stone disease at a molecular level.