CHAPTER IV

DISCUSSION

PART - I

I. STUDIES ON THE EFFECTS OF FLUORIDE INGESTION TO ADULT MALE ALBINO RATS

During the tenure of the present investigation, the toxic effects of sodium fluoride ingestion were evaluated on the structure and physiology of reproductive organs of adult, male albino rats (Rattus norvegicus) of Charles Foster strain. To the experimental animals, sodium fluoride was administered at a dose of 10 mg/kg body weight per day. The dose used was mainly based on LD50 value, which was 250 mg/kg body weight for rat (Davis and Ohio, 1961). The treatment was given for 50 days, as one complete spermatogenic cycle requires 48 ± 2 days in rat. Since, drinking water is the major source of fluoride to human beings, oral mode of administration was selected.

The various parameters studied at the end of treatment were body weight, testis, cauda epididymis and adrenal gland weights. In addition, some specific parameters in testis viz., cholesterol, 3β and 17β-hydroxysteroid dehydrogenase activities and serum testosterone were investigated. Similarly, in cauda epididymis, certain androgen-dependent parameters like sperm motility, count, sperm acrosomal hyaluronidase, acrosin activities, electrolyte concentration (Na⁺, K⁺), Ca⁺² levels, protein profile and sperm morphology were studied. The concentration of catecholamines in adrenal gland as well as muscle and liver phosphorylase activity were carried out to investigate the alterations in carbohydrate metabolism. Apart from this, the histoarchitecture of testis, cauda
epididymis and adrenal gland together with fertility were also undertaken during the course of investigations.

In a different set of experiments, the treatment was withdrawn after 50 days of NaF ingestion to study the reversible effects if any, upon withdrawal of treatment.

In view of fluoride induced toxic effects, the therapeutic strategy of some agents viz, ascorbic acid and calcium, their ameliorative role was also explored.

The treatment of NaF brought about a reduction in body weight of rats, which could be attributed to low food consumption, protein synthesis inhibition (Chinoy, 1991a,b) and electrolyte imbalance due to adrenal dysfunction (Das and Susheela, 1991). McClendor and Gershon-Cohen (1953) reported retardation in the growth of rats fed a low fluoride diet based on hydroponically grown grains. Schroeder et al. (1968) on the contrary, noticed an increase in body weight and longevity in mice supplemented with fluoride. The earlier observations of Shearer and Suttie (1967) demonstrated a drastic curtailment of food intake in rats fed with 1 ppm fluoride in the diet. In the same experimental animals 600 ppm fluoride fed along with diet did not affect food intake despite several fold elevation in plasma fluoride levels. This discrepancy, they suggested was a sort of adaptation to the high fluoride levels. Further studies of Simon and Suttie (1968) claimed a decline in growth rate in rat by fluoride ingestion due to low food intake as a result of enhanced plasma fluoride levels. On the contrary, Schwarz and Milne (1972) showed an increase of 30% in the daily weight gain of rats, when they maintained the animals on fluoride supplemented diets using a controlled 'isolator system' and highly purified aminoacid diets. However, Saralakumari et al. (1988) demonstrated a reduction in growth rate of rats supplemented 100 ppm fluoride through drinking water for two months. Yu and Hwang (1985) observed a 40% reduction
in body weight upon administration of low-protein fluoride diet to rat. Maheswari et al. (1982) claimed weightlessness in man due to mineral loss after fluoride intake. Recently, Chinoy and Sequeira (1989a) reported loss of body weight in mouse ingested 10 mg/kg body weight fluoride for 30 days. In rabbit also a similar decrease in body weight occurred fed with 40 mg/kg body weight fluoride for 30 days (Chinoy et al., 1991a). A consistent reduction in body weight in mouse by 5.2 mg/kg body weight fluoride for 35 days was also reported by Pillai et al. (1988). Fluoro-silicate, a fluoride derivative was also found to cause loss of appetite in cattle (Egyed and Shlosberg, 1975). The mechanism by which growth rate is inhibited by fluoride could be due to the impairment of action of trophic hormones of the pituitary, besides low food intake (Davis and Ohio, 1961).

The organ weights viz, testis and cauda epididymis were declined, whereas, adrenal gland weight was elevated. The low weights of testis and epididymis might be due to loss of electrolytes (Chinoy, 1991a,b) as well as low protein levels (Chinoy and Sequeira, 1989a) along with low metabolic activity. However, adrenal gland showed weight gain. Similar results were also obtained by Monsour and Kruger (1985), which they attributed to hyperactivity of the gland by fluoride treatment.

TESTICULAR EFFECTS OF FLUORIDE

Several workers have investigated the fluoride-testis interrelationship. Kour and Singh (1980a) reported immature and undifferentiated spermatocytes and loss of spermatogenesis in mice given fluoride 10, 500 and 1000 ppm for a duration of 1 to 3 months. Further studies undertaken by Chinoy and Sequeira (1989b) in mice demonstrated that the spermatogenic cells were sloughed off in the luminal region of the seminiferous tubules leading to disorganization of the seminiferous epithelium resulting in
complete absence of spermatozoa in the lumen following fluoride administration for 30 days at a dose of 10 mg/kg body weight. In support of these results, Shashi (1990) noticed deficient maturation and differentiation of the spermatocytes, an increase in the amount of interstitial tissue and necrosis of seminiferous tubules which led to cessation of spermatogenesis in rabbit by 5, 10, 20 and 50 mg/kg/day for 100 days. Recently, Chinoy et al. (1991b) observed derangement of spermatogenic elements, pyknosis of germinal nuclei, denudation of cells and absence of sperm in the lumen of testis by a single microdose (50 μg/50 μl) vasal injection of sodium fluoride in the rat. The light and scanning electronmicroscopic study of rabbit testis treated with 10 mg/kg body weight fluoride for 29 months revealed disruption and degeneration of spermatogenic cells and the lumen devoid of the spermatozoa, some spermotogenic cells were highly eosinophilic with shrinking cytoplasm and some had deep stained pyknotic nuclei denoting cell death (Susheela and Kumar, 1991). Ridha et al. (1978) also reported testicular damage in mice given 125, 250 and 500 ppm fluoride in feed. Mehdi et al. (1983) showed that 600 ppm fluoride in feed impaired the initiation of spermatozoa. In the present study too, NaF at a dose of 10 mg/kg body weight for 50 days to rats ingested orally resulted in disintegration of seminiferous epithelium, germinal cell pyknosis and absence of sperms in the lumen. Thus, the data clearly evidenced that fluoride ingestion results in arrest of spermatogenesis, which is further confirmed by low sperm count obtained in cauda epididymis. Despite these adverse effects on spermatogenic elements, the Leydig cell morphology and its nuclear diameter were unaffected by fluoride treatment (Chinoy and Sequeira, 1989b; Chinoy et al., 1991b). This discrepancy may be due to blood-testis barrier. Therefore, the mechanism by which fluoride passes through it merits further investigations. In the present study, the testicular 3β and 17β-hydroxysteroid dehydrogenases activities, which are
intermediate enzymes in the androgen pathway were unaltered. In addition, the serum testosterone levels in the current investigation as well as earlier work (Chinoy and Sequeira, 1989a) revealed no change in its concentration. Similarly, testicular cholesterol levels were also within the normal range after fluoride treatment. Therefore, these results, collectively suggest that fluoride does not interfere with cholesterol synthesis and testicular androgenesis. However, the status of gonadal hormones viz., follicle stimulating hormone (FSH) and leutinizing hormone (LH) under fluoride intoxication are not known. Therefore, studies in this direction are called for.

Earlier workers also reported that the testicular succinate dehydrogenase (SDH) activity was diminished by sodium fluoride treatment (Chinoy and Sequeira, 1989a; Chinoy et al., 1992a). Dousset et al. (1987) also reported a decrease in isocitrate dehydrogenase activity leading to an accumulation of citric acid. It is thus clear that fluoride inhibits oxidative metabolism, since SDH is primarily a mitochondrial oxidative enzyme and its structural damage and metabolic changes would have been the result of low SDH. Therefore, ultrastructural. Studies on mitochondria are needed to be undertaken in future.

EFFECTS ON CAUDA EPIDIDYMIS

It has been well demonstrated that the epididymis is the site for sperm maturation, wherein the sperm acquires both motility and fertilizability (Bedford, 1975; Prasad and Rajalakshmi, 1976; 1977; Chinoy, 1984; 1985; Austin, 1985; Cooper, 1986). Therefore, the internal milieu of the epididymis is necessary for the maintenance of sperm structural and functional integrity. Sodium fluoride treatment at a dose of 10 mg/kg body weight for 50 days revealed damage to secretoryepithelium, pyknotic nuclei,
and the tubules were devoid of sperm in the lumen. Parallel observations have been obtained in mice epididymis treated with sodium fluoride (Chinoy and Sequeira, 1989b). Recently, Chinoy et al (1991b) reported that a single microdose vasal injection of NaF into each vas deferens caused degeneration and confluence of tubules, vacuolation of epithelial cells, pyknosis of their nuclei, increase in the interstitium and hyalinization, which rendered the internal milieu hostile to the spermatozoa and as a consequence the sperm motility was affected. In prepubertal rats also, similar histoarchitectural alterations were obtained by fluoride treatment (Chinoy et al, 1992b,c). Due to histological changes in epididymis its metabolic status was also affected by fluoride. The ATPase activity, an androgen dependent parameter was diminished by NaF treatment (Chinoy and Sequeira, 1989a; Chinoy et al., 1991b). A similar inhibition of ATPase activity in cauda epididymal spermatozoa of NaF treated rabbit was also obtained (Chinoy et al., 1991a). Several observations revealed direct action of fluoride on the motile apparatus of sperm thereby inhibiting the dynein ATPase in cilia (Blum and Hayes, 1984) and restricting the energy supply, which disturbs the intracellular Na⁺/K⁺ balance (Brooks, 1984). A reduction in mitochondrial ATP and ADP by fluoride and low intracellular ATP levels were also reported earlier (Elsair and Khelfat, 1988). Therefore, these alterations would affect the sperm motility.

Sialic acid a sialomucoprotien which is essential for the maintenance of structural integrity of sperm membrane besides maturation, was also reported to be significantly declined by fluoride (Chinoy and Sequire, 1989a). They attributed these alterations due to effect of fluoride on sperm acrosomal membrane.

In the present investigation, the sperm acrosomal membrane integrity and metabolism were evaluated. After 50 days of sodium fluoride treatment to
rat, a significant inhibition of sperm acrosomal membrane bound enzymes, viz., hyaluronidase and acrosin occurred. Hyaluronidase a lysosomal enzyme is essential for the dispersion of cumulus oophorus, the outermost layer of the ovum and the sperm uses it in the penetration (Zaneveld et al., 1973). The low hyaluronidase activity obtained after fluoride treatment may therefore be associated with lower penetrating and fertilizing ability of the sperm. Similarly, the sperm acrosomal acrosin activity, which is believed to be important for gamete fusion, particularly for binding to and penetration of zona pellucida was also affected by NaF treatment. Infact, the results revealed a trend of elevation in procrustean activity and a decrease in free acrosin and acrosin-acrosin inhibitor complexes, which suggests a block in autoactivation of procrustean. Schill et al. (1988) have remarked that activation of procrustean seemed to be associated with capacitation process. The enhanced procrustean levels might be due to alterations in glycosaminoglycans (GAG) concentration as GAGs are known to stimulate the conversion of procrustean into its active forms (Parrish et al., 1980). It is further known that NaF affects glycosaminoglycans in serum (Susheela and Jha, 1982; Jha et al., 1983). Therefore, studies on glycosaminoglycan concentrations are needed to be carried out. Also, in vitro studies by using Hamster oocyte penetration test are mandatory to confirm these results and to focus light on acrosomal membrane functions. The low sperm membrane bound acrosomal enzymes have also been reported to be associated with morphological abnormality or acrosomal damage (Schill, 1974). In correlation to this observation, sodium fluoride caused loss of acrosome, severe deflagellation as well as head and tail anomalies. Earlier observations, in rabbit sperm also revealed disappearance of acrosome, head to head agglutination and deflagellation (Chinoy et al., 1991a). The head to head agglutination process could be due to changes in sperm proteins causing stickiness and the clumping of spermatozoa. As epididymal proteins
are important sperm antigens, it is likely that the NaF treatment caused configurational or qualitative changes in sperm surface proteins/antigens (Chinoy, 1984). Scanning electron microscopic studies also revealed similar head and tail deformities by fluoride treatment which were causative factors for low sperm motility.

Fluoride is also known to inhibit sperm motility, glycolysis and respiration processes. In vitro studies carried out by Schoff and Lardy (1987) demonstrated that sperm treated with 30 mM fluoride became immobile within two minutes and the flagella assumed a linear, rod like configuration. Therefore, in the present study too, the low sperm motility obtained may be due to reduction in metabolic activity of sperm by fluoride. Smith (1985) reported that fluoride even at low concentrations inhibited a number of enzymes and processes including activity of ATPase, which plays an important role in numerous energy processes.

Sodium fluoride treatment also brought about dramatic alterations in cauda epididymal sperm electrolyte levels. This is probably through impairment of androgen supply action, despite the fact that testosterone levels were unaffected by fluoride, for it has been shown that electrolyte and water transport in the rat cauda epididymis are androgen dependent (Wong et al., 1978). This observation is further evidenced by a decline in cauda epididymis weight after treatment. The electrolyte imbalance could also be another factor for low sperm motility, since they are known to maintain the spermatozoa in a motile and viable state (Chinoy, 1984). NaF treatment caused a decline in sperm Na$^+$ and K$^+$, but elevated calcium. In corroboration to these results, Quissell and Suttie (1973) obtained loss of potassium from mouse fibroblasts after 12 hour incubation with fluoride and similarly K$^+$ efflux occurs from intact cells in vitro after fluoride treatment (McIvor et al., 1985). In the present study, enhancement in sperm
Ca+2 levels might be due to affinity for fluoride to form complexes. This increased calcium activates phospho-diesterase activity, which lowers c-AMP and thus sperm motility (Hong et al., 1984). It is also likely that the intracellular calcium levels might have led to potassium depletion from spermatozoa due to activation of calcium-dependent K+ channels as demonstrated by Schwartz and Passow (1983). The possibility that low Na+ levels in sperm might cause inhibition of Na - K+ ATPase after fluoride treatment (Tada et al., 1975) and lead to low sperm motility cannot be ruled out.

Fluoride has been reported to inhibited protein synthesis (Vesco and Colombo, 1970). In the present investigation, polyacrylamide gel electrophoresis of sperm proteins revealed alterations in their mobility with the disappearance of some proteins in NaF treatment as compared to control. A change in sperm protein would alter their motility and fertilizability, as epididymal proteins are important as sperm antigens and for sperm viability (Chinoy, 1984). The alterations caused by NaF in protein in sperm could also be a contributing factor in the reduction of sperm motility leading to low fertilizability.

Exposure to fluoride in higher concentrations for a prolonged period has been reported to interfere with thyroid functions (Williems et al., 1972, Hillman et al., 1978). After fluoride treatment, the morphological changes including thyroid hyperplasia, colloid goiter, degeneration of follicular epithelium and true glandular hypertrophy occurred (Wadhwani and Ramaswamy, 1953). Further investigations revealed histological changes such as swelling of mitochondria with disintegrated crystae in follicular epithelial cells of thyroid gland in fluoride fed rats (Chongwan and Daijei, 1988). These morphological changes contributed towards alterations in thyroid function as evidenced by low levels of T3 and T4 after fluoride.
treatment for 50 days. Experiments carried out by Kendall-Taylor (1972) and Rantanen et al. (1972) also obtained a similar decrease in T3 and T4 in the thyroid gland of mouse and pig by fluoride intoxication. McLaren (1976) reviewed published literature on the effect of fluoride on the thyroid gland in which he concluded that the evidence was in favour of an increased accumulation of fluoride by the thyroid gland in contrast to the other organs. The studies of Williams et al. (1972) and Bobek et al. (1976) claimed that fluoride interferes with the normal functioning of follicular cells by inhibiting the proteinase responsible for splitting thyroglobulin molecules into thyroxine and triiodothyronine. Their investigations further added that fluoride affects feedback mechanism mediated through the hypothalamus and adenohypophysis which regulates thyroid secretion through thyrotropin (TSH). The low concentration of T3 and T4 obtained in the current study, may alter basal metabolic rate (BMR) as well as implication of goiter problem, especially in endemic population. Earlier workers claimed that higher rates of occurrence of goiters in endemic skeletal fluorosis were reported (Latham and Greely, 1967). The field survey reports of Siddiqui (1955) revealed higher incidence of goiters among fluoride afflicted population. On the contrary, studies carried out by Leone et al. (1964) showed no difference in the basal metabolic rate in population residing in fluoride endemic areas (3.5 ppm) compared with those living in fluoride free (0.9 ppm) region. The studies of Gallettie (1958) reported that fluoride affects iodine concentration and thereby alters thyroid function. In several experimental studies focussed on the subject of fluoride-iodide interaction, variation with regard to animal species used, the nature and dose of fluoride used, route of administration as well as the method of assessment were too large to permit any conclusion to be drawn.

In the present investigation, the adrenal catecholamine (epinephrine and nor-epinephrine) concentrations were elevated significantly after
treatment. The weight of adrenal gland also increased after treatment suggesting its hyperactivity. The histology of adrenal gland revealed pyknosis in some regions of the cortex and hyperactivity of the medullary cells indicating alterations in adrenal function. The increase may be attributed to stress caused by accumulation of fluoride in the body which might have resulted in stimulatory effect on adrenal gland through sympathetic nervous system leading to enhanced release of catecholamines in the manner described by McGown and Suttie (1979). They demonstrated hyperglycemia due to acute fluoride toxicity in the rat, mediated by epinephrine released from the adrenal medulla, which on bilateral splanchnicectomy prevented the elevation in epinephrine levels and manifested normal glucose levels. Similarly, ganglionic blockade diminished the hyperglycemic response. Microinjection of fluoride into the lateral ventricle elicited a rapid hyperglycemia which again was blocked partially by ganglionic blockade. Thus, they concluded that the response to fluoride resulting in elevated catecholamine appears to be mediated primarily by splanchnic impulses arising in the central nervous system. Cheon and Distefano (1973) have reported that fluoride ingestion markedly enhanced the catecholamine concentrations of the heart, kidney and liver and concluded that it interferes with catecholamine biosynthetic mechanism. Caruso et al. (1970) claimed that increase in blood pressure due to continuous fluoride exposure and a direct cardiac stimulatory effect contributed to a high catecholamine release. The experiments with the adrenalectomized rats and the plasma catecholamine assays indicated that the hyperglycemic action of fluoride is mediated by enhanced catecholamines especially epinephrine (Himms-Hagen, 1967). Experiments undertaken by Shaikh and Hiradhar (1985) also revealed that the mudskipper (Boleopthalmus dussumeri) exposed to sublethal concentrations of fluoride i.e. 40 and 80 ppm, the circulating glucose levels were significantly elevated. Chitra and Rao (1981) further
provided evidence of hyperglycemia in Channa punctatus. The studies carried out by Hanke et al. (1983) also elucidated enhanced catecholamine levels in particular epinephrine leading to high glucose concentrations. The increase in adrenal epinephrine and nor-epinephrine in the present study, would directly influence carbohydrate metabolism. Further, it is well known that the enhanced catecholamine especially nor-epinephrine is known to influence gonadotropins mainly LH through hypothalamo-gonadal axis. Therefore, studies in this direction await further detailed investigations.

The extensive studies carried out by Underwood (1977) reviewed that fluoride alters carbohydrate metabolism. Earlier investigations carried out revealed that accumulation of glycogen occurred in vas deferens (Chinoy and Sequeira, 1989a) muscle and liver of mouse and rat (Chinoy, 1991a, b; Chinoy et al., 1992b). Similarly, higher concentrations of glycogen have also been reported in fish exposed to various pollutants (Banerjee et al., 1978). It has been reported that the turnover rate of glycogen was depressed and the levels of glucose-6-phosphate dehydrogenase was diminished (Carlson et al., 1966). In support of these results, Dousset et al. (1987) noticed a decline in glycogen turnover and citrate accumulation in rats fed with NaF, which may lead to decrease in the glycolysis. The enhanced glycogen concentration by fluoride therefore, seems to be inactivation of phosphorylase as obtained in liver and muscle in the present study. In chronic fluorosis decreased activity of phosphorylase occurred in liver and skeletal muscle which would cause accumulation of glycogen (WHO Monograph Series, 1970).

WITHDRAWAL STUDIES ON FLUORIDE INDUCED TOXIC EFFECTS

In view of the above observed fluoride induced toxic effects, in a different group of animals, NaF was fed for 50 days and the treatment was
withdrawn afterwards for 70 days (Group III). During this period, the animals were maintained on standard diet and water given ad libitum. The results revealed that in many of the parameters, recovery occurred from fluoride induced toxicity, but not significant as compared to control. This may be due to delayed sequestration of fluoride from the body as evident by high serum fluoride levels in the present study, even after 70 days withdrawal of treatment. Earlier studies revealed that the fluoride induced effects were completely recovered to normal after withdrawal of treatment (Chinoy and Sequeira 1989 a,b,c.; Chinoy et al., 1991a). This discrepancy may be due to variation in animals species used, the duration of exposure and suggests that recovery in rat is rather slow as compared to mouse. The results revealed that a longer period is preferable for a significant recovery. However, the fluoride induced effects were found to be completely regained to normal by administering ascorbic acid (AA) and/or calcium along with NaF.

BENEFICIAL EFFECTS OF ASCORBIC ACID (AA) ADMINISTERED ALONGWITH NaF

In the course of the study, one group of animals (Group IV) were given combined treatment of ascorbic acid (50 mg/animal/day) with NaF (10 mg/kg body weight) to evaluate the beneficial effects of vitamin C if any. The parameters investigated were same as that of NaF treatment.

The treatment showed fluctuations in serum fluoride concentrations with elevated levels from days 30 to 50, but no further increase upto day 70 of the treatment. This could be attributed to active detoxification of the toxicant as AA has been reported to be having detoxifying properties (Chinoy, 1978). Experiments carried out by Yu and Hwang (1986) elucidated that high retention of fluoride occurred in the bone by fluoride treatment,
but when the animals were maintained on ascorbic acid, the fluoride retention was completely diminished. Earlier investigations undertaken by Wadhwani (1954) emphasized ameliorative effects of vitamin C on fluorosis symptoms in experimental animals. A similar observation of marked reduction in retention of fluoride in bones in fluoride poisoned monkeys (Pandit and Narayana Rao, 1940) was reported. They further reported that green vegetables containing ascorbic acid mitigated effects of fluoride in monkeys and deficiency of vitamin C is a contributing factor in the aggravation of fluoride toxicity. Ingestion of chronic doses of inorganic fluoride were found to enhance vitamin C biosynthesis (Phillips and Chang, 1934). The recent studies carried out in our laboratory also revealed enhanced ascorbic acid levels in adrenal gland and liver (Chinoy, 1991 a, b; Chinoy et al., 1992 b) which might be due to active synthesis in response to overcome the stress and toxicity by detoxification. Although, the detailed mechanism may not be clear, but the workers attributed to active detoxification of fluoride by excess intake of ascorbic acid.

The treatment caused a decline in body and organ weights only after 30 days. Malathi and Ganguly (1974) claimed that reduced food intake led to a specific depletion of ascorbate from tissues. It is well established that fluoride is known to cause low food intake (Saralakumari et al., 1988) resulting in low body weight. However, upon prolonged treatment i.e. 70 days, regain in weight of body and organs occurred, which could be due to reversibility in protein synthesis as well as electrolyte levels. Ascorbic acid is known to bind with macromolecules like proteins, nucleic acids (Chinoy and Buch, 1977) by charge transfer complex formation, which appears to be a very active energy source for biological processes, which seemed to be the probable mechanism occurring for the recovery of body and organ weights.
The cauda epididymal sperm motility decreased especially between 30 and 50 days. The sperm structure, metabolism and respiration were all found to be affected by fluoride in several animals (Blum and Hayes, 1984; Chinoy et al., 1991 a). As mentioned in the earlier part of discussion, NaF treatment caused loss of acrosome, head and tail deformities. These alterations contributed towards diminished activities of acrosomal membrane bound enzymes viz., hyaluronidase and acrosin which were significantly reduced only after 30 days of treatment. However, recovery occured after 70 days of treatment especially in hyaluronidase activity as ascorbic acid is known to activate lysosomal enzymes (Chinoy, 1978). Earlier workers also reported that ascorbic acid caused a significant regain in sperm ATPase, SDH and protein levels (Chinoy et al., 1991a), since AA is known to activate several hydroxyating enzymes and those involved in oxido-reduction reactions in various tissues including reproductive organs (Kutsky, 1973: Chinoy, 1978; 1984). In addition, AA treatment also brought about a significant recovery in electrolyte concentrations viz., \( \text{Na}^+ \), \( \text{K}^+ \) and \( \text{Ca}^{2+} \). This could be due to the action of ascorbic acid as an antioxidant (Chinoy, 1978). Vitamin C is known to provide energy by the formation of its free radical monodehydro-ascorbic acid, which is a powerful reducing agent by virtue of possessing an unpaired electron, which functions as a source of energy for sperm motility and metabolism (Chinoy, 1984). It has also been reported that AA is known to activate adenyl cyclase and further inhibit phosphodiesterase (PDE) resulting in high c-AMP levels, which is an energy source for sperm motility. Therefore, the recovery obtained in the sperm motility in this study could be due to activation of some hydroxylating enzymes leading to high energy. The reversal in sperm motility ultimately led to a significant regain of fertility.

NaF treatment alone caused morpholgical changes in adrenal gland. In vitamin C deficiency rats the morphology of the adrenal gland exhibited
hyperplasia of the glomerulosa with infiltration of the zona fasciculata and a loss of the boundary between the two zones (Blaschke and Hertting, 1971). In addition, they reported areas of cytolysis and disintegration and a morphological stain for 7-hydroxy cholesterol showed a substantial disappearance of this compound. These changes under scorbutic condition have been attributed to intense stimulation with ACTH. They further reported that the diminished steroidogenesis due to low ascorbate levels stimulates the production of ACTH resulting in the cytological changes. Intraperitoneal injection of vitamin C resulted in the correction of cytological changes caused by ascorbate deficiency. Das and Susheela (1991) demonstrated low steroid levels by adrenal in fluoride treated rabbits as well as fluorotic human population. It is known that the synthesis of adrenal steroids requires the conversion of a 3-hydroxy group to a 3-ketogroup. This is accomplished by the enzyme 3-HSD. The activity of this enzyme would be altered in the ascorbate deficient rats as compared to the controls (Blaschke and Hertting, 1971). Brin (1982) claimed that administration of extraneous adrenalin resulted in depressed plasma ascorbate levels. In the present study also, a significant enhancement in adrenal catecholamines were obtained by NaF alone, but reduced to normal upon ascorbic acid administration. Therefore, the results emphasize that ascorbate does play an essential role in the amelioration of fluoride induced toxic effects.

The serum thyroid hormones viz., T3 and T4 levels which were lowered by NaF treatment alone brought back to normal by the administration of ascorbic acid alongwith NaF, which is helpful in maintaining the thyroid functions.

The enzyme phosphorylase, which was diminished by fluoride treatment exhibited its normal activity upon vitamin C ingestion. Chinoy et al. (1992b) reported that ascorbate ingestion caused normal turnover of glycogen in liver and muscle, which was enhanced by NaF alone. Therefore, the
enhancement in the activity of phosphorylase may be due to its activation caused by ascorbic acid maintaining normal carbohydrate metabolism. The data reveals a significant role of AA in fluoride toxicity.

**EFFECTS OF CALCIUM (Ca\(^{+2}\)) TREATMENT ALONGWITH NaF**

In view of known interaction of Ca\(^{+2}\) with fluoride and paucity of information on the role of Cu\(^{+2}\) on soft tissue functions under fluoride intoxication, a separate group (group V) of animals were simultaneously administered Ca\(^{+2}\) (62.5 mg/animal/day) along with NaF (10 mg/kg body weight) for 70 days to study the beneficial effects in fluoride toxicity. The parameters studied were same as in earlier set of experiments.

It is well known that Ca\(^{+2}\) combines with fluoride forming an insoluble compound, calcium fluoride (CaF\(_2\)), thereby reducing its absorption. In the present study also, a significant reduction in serum fluoride levels were obtained by calcium administration, which could be a contributing factor in maintaining body and organ weights by allowing normal food intake, accelerating body metabolism as well as electrolyte concentrations.

The testicular histoarchitecture after simultaneous administration of Ca\(^{+2}\) along with NaF exhibited complete recovery from NaF toxicity, thus maintaining the normal process of spermatogenesis as evident by normal cauda epididymal sperm count.

In cauda epididymis also, the morphology was restored to normal providing a conducive internal milieu for maintenance of sperm structural, functional and metabolic integrity. The treatment resulted in recovery in sperm morphology after 70 days of treatment i.e. intact acrosome, head and tail regions as observed by specific acidic alcoholic silver nitrate stain. As a result, the sperm membrane bound enzymes viz., hyaluronidase and
acrosin were regained to normal, after 70 days of treatment. It is known that \( \text{Ca}^{+2} \) activates several enzymes and enzyme systems. Earlier workers demonstrated that calcium ingestion to fluoride intoxicated rabbits brought about a significant regain in the NaF inhibited activity of Atpase, SDH and ACPase enzymes in cauda epididymal sperm (Chinoy et al.,1991a). In the current investigation the electrolyte concentrations such as Na\(^+\) and K\(^+\) which were depleted by NaF treatment also showed reversibility upon Ca\(^{+2}\) treatment. However, Ca\(^{+2}\) levels were still found to be higher which may be due to the extraneous administration of calcium. It has been shown that the action of Ca\(^{+2}\) and c-AMP dependent on each other and c-AMP can control the rate of Ca\(^{+2}\) cycling across the plasma membrane and Ca\(^{+2}\) in turn can regulate the enzymes responsible for the synthesis and destruction of c-AMP (Rasmussen, 1989). In his extensive studies he found that calcium-ion pump is activated by the formation of calcium - calmodulin complex. The stimulation of the calcium - ion pump by Ca\(^{+2}\) calmodulin complex and calcium activated phosphorylation enables calcium efflux to compensate for the increased influx (Rasmussen, 1989). Therefore, the recovery in elevated Ca\(^{+2}\) levels could have been mediated by the calcium calmodulin complex.

Polyacrylamide gel electrophoresis (PAGE) of NAF + Ca\(^{+2}\) treated rat sperm revealed normal mobility of proteins indicating its active synthesis. Rasmussen (1989) reported that Ca\(^{+2}\) and c-AMP exert many of their cellular effects by controlling the activity of a particular class of enzymes called protein kinases, which catalyze the transfer of phosphate groups from ATP to other proteins and maintains sperm motility. Ca\(^{+2}\) has also been reported to be involved in epididymal and vas deferens smooth muscle contraction (Tash and Means, 1982., Chinoy and Chinoy, 1983). Therefore, the recovery in the internal milieu of epididymis and resultant gearup in the metabolic and functional integrity of the spermatozoa contributed towards acquiring normal
sperm motility, ultimately attaining fertility in these animals. Another probable mechanism by which calcium helped in the recovery of sperm motility may be by inhibition of phosphodiesterase (PDE) activity leading to enhanced c-AMP levels as Ca$^{+2}$ is known inhibitor of PDE.

In the present study, a significant recovery in liver and muscle phosphorylase activity was also obtained, causing normal turnover of glycogen. Earlier investigations revealed that following Ca$^{+2}$ administration, the glycogen turnover was maintained in liver and muscle (Chinoy, 1991a,b Chinoy et al, 1992b) Calcium has been found to act on beta cells of Langerhans in the pancreas and control secretion of insulin, which in turn regulates glycogen and glucose levels (Rasmussen, 1989). Therefore, the recovery in glycogen and glucose concentrations may have been maintained by this pathway too, in addition to the recovery in phosphorylase. However, this aspect in relation to insulin - Ca$^{+2}$ interrelationship with reference to fluoride toxicity needs detailed investigation.

The combined treatment (NaF + Ca$^{+2}$) also brought about a significant regain in adrenal catecholamine concentrations. Similarly, the thyroid hormone levels viz., T3 and T4 were also regained to normal state. Although, the mechanism of action of Ca$^{+2}$ is not clearly understood in this situation, it is likely that Ca$^{+2}$ activates several enzymes particularly protein kinase C leading to protein phosphorylation through the formation of calcium-calmodulin complex regulating metabolic processes. The past investigations revealed ameliorative effects of calcium against fluoride. The literature also shows inverse relations of Ca$^{+2}$ levels with fluoride toxicity. Krishnamachari (1978) reported that the cumulative retention of radioactive calcium was high in animals, which received fluoride, but highest in those who received low diets. Cold calcium studies carried out by Srikantia and Siddiqui (1965) also reported high retention of fluoride in fluoride afflicted human population as compared to control. Teotia et al.
(1969) observed that in patients with skeletal fluorosis high Ca\(^{+2}\) retention occurred. Srirangareddy and Narasingarao (1971) induced experimental fluorosis in monkeys. In those animals, Ca\(^{45}\) studies revealed increased retention of Ca\(^{45}\) due to calcium deficiency. High fluoride when fed in low calcium diet caused increased retention of calcium. These observations suggest that in fluoride fed animals avidity for calcium increases; especially when fed low calcium. They also claimed that fluorosis is more frequently seen in population groups who habitually subsist on low calcium diet. High dietary intake of minerals, such as Ca\(^{+2}\), Mg\(^{+2}\) and Al\(^{+3}\) have been shown to reduce fluoride absorption possibly due to formation of more insoluble salts of fluoride (Cremer and Buber, 1970). In a diet survey conducted in the endemic areas revealed very meagre intake of calcium in the population (Krishnamachari and Krishnaswamy, 1974). Therefore, these results clearly indicate that calcium has an essential role to play in fluoride toxicity and it has a therapeutic significance in alleviating the fluoride toxic effects. Thus, calcium is suggested to be a very beneficial agent which could be given to atleast children in fluoride endemic areas as a preventive measure against fluorosis.

EFFECTS OF COMBINED ADMINISTRATION OF VITAMIN C AND CALCIUM (Ca\(^{+2}\)) ALONGWITH NaF

To a different group of animals, (Group VI), vitamin C (AA) and calcium (Ca+2) were administered along with NaF in order to evaluate the faster/better recovery if any over individual treatments.

The combined treatment also contributed in the complete recovery of testicular and cauda epididymal structure. The morphology of the sperm stained with acidic alcoholic siver nitrate stain revealed distinct acrosome and intact head and tail regions. The sperm acrosomal enzymes i.e.
hyaluronidase and acrosin showed a decline only after 40 days of treatment. But, recovery in hyaluronidase, a lysosomal enzyme alongwith acrosin occurred by 70 days of treatment, which is a contributory factor in the regain of fertility.

The sperm electrolyte concentrations i.e. Na⁺ and K⁺ levels were also significantly restored to control. These electrolytes are very essential, since they are known to maintain the spermatozoa in a motile and viable state (Wong et al., 1978; Chinoy, 1984). The maintainence of the normal flagellar movements of the spermatozoa is also regulated by ionic gradients across the tail, where it can retain high K⁺ concentrations. Similarly, the sperm calcium levels were also restored to normal, since their high concentration is known to be detrimental for sperm motility. Therefore, the recovery in electrolyte balance is condusive for sperm motility. In addition, polyacrylamide gel electrophoresis also revealed a significant recovery in the sperm protein profile. It has been reported that the stabilization of chromatin occurs by the formation of disulphide cross links in epididymal spermatozoa (Calvin et al., 1975). The basic nuclear proteins of spermatozoa have a significant role in the shaping of the sperm nucleus (Fawcett et al., 1971). Therefore the recovery in sperm proteins is a contribution for sperm viability. It is known that phosphodiesterase (PDE) catalyzes the conversion of c-AMP to AMP, thus decreasing the levels of c-AMP, which is important for promoting sperm motility. However, both ascorbic acid and calcium are recognised as potent inhibitors of PDE. Thus, it is suggested that the levels of c-AMP increase leading to recovery of sperm motility in AA and Ca⁺² treated rat spermatozoa. Therefore, the recovery in sperm motility finally resulted in significant regain of fertility by the synergistic action of AA and Ca⁺².
The treatment also resulted in a significant recovery in liver and muscle phosphorylase activity. Similarly, the adrenal catecholamine concentrations were also under normal range following the treatment. Recovery in these parameters would result in normal carbohydrate metabolism as glycogen turnover is maintained as also reported by earlier workers (Chinoy et al., 1992b). The serum T3 and T4 also exhibited normal levels thus, helping in normal basal metabolic rate. The findings suggested a significant therapeutic importance of AA and Ca$^{+2}$ against fluoride.

Extensive studies carried out earlier demonstrated that calcium and vitamin C deficiency especially under fluoride toxicity, poor nutrition and hard labour exaggerate the endemic fluorosis (Siddiqui, 1955, Yu and Hwang, 1985). Studies conducted on diet surveys indicated that inadequate calcium and ascorbic acid have been related to severity of endemic fluorosis (Srirangareddy and Srikantia, 1971). Experimental studies on monkeys proved the absolute necessity for Ca$^{+2}$ and vitamin C in the prevention of fluorosis (Srikantia, 1974).

The present study elucidates that calcium and ascorbic acid have significant role in fluoride toxicity and manifest a synergistic effect in recovery of NaF induced alterations. It is well known that due to excessive amounts of fluoride in drinking water and foods, a large parts of the globe have been affected making them crippled. The manifestation of lack of synthesis of Vitamin c and deficiency of calcium are two established situations in fluoride endemic areas. Therefore, it is very necessary to suggest these two agents atleast in children so as to prevent the health hazards.

As mentioned in the earlier part of the discussion, vitamin c due to its active antioxidant as well as detoxification properties, it is a promising and potential agent in suppressing fluoride toxicity. Similarly,
calcium is also known to form complexes with fluoride and reduces its absorption and thereby its action. Besides, the two agents also play a key role in metabolic process. Therefore, combined administration of these two agents should be given top priority in fluoride endemic human population, atleast to children. However, monitoring of dose is necessary since high concentrations of these agents, in particular calcium is harmful.

PART - II

STUDIES ON FLUORIDE AFFLICTED HUMAN POPULATION IN ENDEMIC AREAS OF MEHSANA DISTRICT, NORTH GUJARAT, INDIA

Despite the fact that minute concentrations of fluoride are highly beneficial, but the harmful role of excess fluoride intake in the aetiology of dental mottling and skeletal deformities were obviously known since mid thirties. The pioneer workers established a direct relationship between the amount of fluoride intake and the magnitude of dental and/or skeletal fluorosis (Daver, 1945). In the light of these observations, WHO (1984) has suggested that one ppm fluoride in drinking water is the maximum permissible level. Unfortunately due to the presence of fluoride - bearing rocks in the soil and extensive use of fluorine emitting chemicals, the ground water supplies in India showed that 8,700 villages were having a water supply with fluoride content varying from 1.5 - 30 ppm (Kumaraswamy, 1990). Therefore, the analysis of fluoride is an essential aspect in the environmental field. Gujarat is one among the 15 states in India influenced by high fluoride levels in water, wherein five districts viz., Mehsana, Amreli, Banaskantha, Sabarkantha and Baroda (Chhota Udepur) are under the menace of its contamination and are endemic to fluoride.
Fluoride is a geochemical contaminant and natural sources account for much of the fluoride found in surface and ground waters. It is well established that the natural fluoride level in ground water depends on such factors as geographical location, chemical and physical characteristics, porosity of the rocks present, pH, temperature, complexing action of other elements, depth of wells and consistency of the soil (WHO, 1984). Since endemic fluorosis in Mehsana District of Gujarat state in India has recently been recognised, where numerous people have been affected and the studies on their health status is not known. Therefore, a study survey was carried out in human population from 36 villages of Mehsana District of North Gujarat. Parallel studies were also conducted in Ahmedabad city (non-fluoride endemic area) population and considered as control to compare the alterations occurring due to consumption of high water-borne fluoride in endemic population.

The topography of Mehsana district (fluoride endemic area) revealed a total area of 9,027 sq.km. surrounded by Banaskantha on the North and Ahmedabad on the South while Sabarkantha and Runn of Kutch on Eastern and Western sides respectively.

Analysis of water samples collected from 24 different sites in Ahmedabad city showed a low concentration of 0.56 ppm to a maximum of 1.0 ppm with a mean of 0.9 + 0.02 in the non-industrial area. However, the fluoride content in water in industrial area i.e. Naroda, Leelanganar, Odhav, Vatva and Maninagar, crowded with factories, which emit fluoride residues, contained a minimum of 1.4 ppm and a maximum of 2.5 ppm respectively. Industries as a source of fluoride emission and its implication in industrial fluorosis has been reported (Thompson et al., 1971; Lee et al., 1974; Smith and Hodge, 1979).
In endemic villages of Mehsana District, the source of fluoride to the inhabitants in these villages is mainly through drinking water, although traces of it is acquired through food and vegetables grown in these areas, which are not significant. The water fluoride content in these villages varied from 0.8 - 0.9 ppm, where the source of water is well. In the other 33 villages surveyed, where the water was obtained from borewells, had fluoride content beyond permissible level. It is well known that as the depth increases, the fluoride levels enhance seemingly due to contact with the fluoride bearing rocks (WHO, 1984). Therefore, the individuals living in these rural areas were consuming natural fluoride from drinking water either knowingly due to the only source of water or to some extent, due to lack of awareness. As a consequence, the individuals of all age groups including children were suffering from dental as well as skeletal fluorosis in these villages.

In the present study, fluoride output in urine in fluorotic individuals varied considerably, where a large number of people exhibited extremely high amount of fluoride, while low urine fluoride levels were also obtained in some individuals as compared to control population. This discrepancy may be due to variation in the level of fluoride ingested, duration of ingestion, age of the individual, level of nutrition, meteorological factors and individual metabolic response (Shupe and Alther, 1966; Siddiqui, 1972). It has been reported that fluoride excretion depends on total daily consumption of fluoride, degree of renal efficiency, and interaction of fluoride with other factors such as binders like Mg$^{+2}$, Ca$^{+2}$, Al$^{+3}$ etc. (Krishnamachari, 1986), urinary flow, and pH as well as previous exposure to fluoride (Whitford et al., 1976; Schiffl and Binswanger, 1980). Several workers have established a good correlation between fluoride intake and urinary loss. Therefore, Hodge and Smith (1970) opined that measurement of the urinary fluoride can be regarded as the best indicator of intake level.
of the element. However, this view was ruled out in persons exposed to
fluoride for a prolonged period, which led to a diminution of urinary
fluoride due to inefficiency of glomerular filtration (Schiffl and
Binswanger, 1980). In chronic renal failure, the urinary excretion of
fluoride was reduced resulting in an increase in the bone fluoride content (Parsons et al., 1975). As mentioned earlier, age-dependent alterations in
fluoride excretion, where younger persons excrete less fluoride due to
active absorption as a result of high metabolic activity of bone was
reported (Zipkin et al., 1956). These observation were contradicted by Bagga
et al. (1979), who have reported continuous clearance of fluoride through
kidney for several years even after the source of fluoride was eliminated.
Therefore, these results should be confirmed further through large data
based studies conducted carefully and systematically.

In fluorotic human individuals living in endemic areas, the serum
fluoride concentrations fluctuated. The data revealed comparatively low
serum fluoride levels in young age group individuals with those of the old
age group. This difference may be due to maximum absorption by bone in young
individuals, while in old subjects it could be due to release of previously
retained fluoride by Charkes et al. (1978) and Maheswari et al. (1981). The
high serum fluoride in these individuals compared with control population is
obviously due to consumption of high fluoride containing water. Schiffl and
Binswanger (1980) found 9.8 mg/litre concentration of serum fluoride in
individuals drinking water with a fluoride level of 0.06 mg/litre. Similar
results were also obtained earlier by Jardillier and Desmet (1973), who had
reported 27 to 99 µg/litre of blood plasma in individuals drinking water
0.15 mg/litre of fluoride. The retained fluoride in the serum, thus would
affect the general body metabolism in these individuals, probably by
altering soft tissue functions.
In fluoride afflicted human individuals, the serum sialic acid levels were declined significantly in comparison to control human population of Ahmedabad city. In corroboration to these results, Susheela and Jha (1982) and Jha et al. (1983) also reported a decrease in serum sialic acid, but enhanced glycosaminoglycans in fluorotic subjects. Sialic acid a sialomucoprotein maintains equilibrium with its derivatives, the glycosaminoglycans (GAG) in the serum. These GAGs are known to attach themselves to exogenous proteins of the plasma membrane by glycosidic and glucosidic bonds and thus play an important role in the interaction of hormone-membrane bound receptor molecules. Hence, alterations in sialic acid and GAGs might cause disturbance in hormone action at the target cell by fluoride. These workers further suggested, that sialic acid levels are a diagnostic test for detection of fluorosis. However, it is necessary to develop a method for diagnosis of the disease in early stages from easily available material like hair, nail saliva or sweat, since they also retain appreciable amounts of fluoride, which will be more feasible for detection.

Fluoride consumption in endemic population did not affect their serum cholesterol as compared to control population of Ahmedabad city, Wherein the fluoride content in their drinking water ranged from 1 to 2.9 ppm in fluoride endemic villages. Similar results were also obtained in mice and rats fed with 10 mg/kg body weight fluoride for 30 days (Chinoy and Sequeira, 1989a). Saralakumari et al. (1988) also reported unaltered serum cholesterol levels in rats fed with 100 ppm fluoride. Therefore, these data suggest that fluoride might not alter cholesterol metabolism. Also, the possibility of occurrence of hypercholesterolemia \ atherosclerosis could be ruled out. However, in population living in high fluoride endemic areas like Andhra Pradesh, where the fluoride levels range from 25 - 60 ppm, the alterations in cholesterol levels if any are not known. Therefore, studies in chronic cases merit further detailed investigation. In Mehsana district
population, together with cholesterol, the serum testosterone levels were also unaffected. Thus, the circulating androgen levels were unaltered by fluoride. Parallel studies conducted in animal studies also revealed similar results, wherein no androgen deficiency occurred by fluoride intoxication. Yet, the structure and function of target organs were impaired and the androgen dependent parameters were adversely affected. This may be due to reduced target organ response related to impaired hormone-receptor interaction. This lacuna of information requires further attention.

Fluoride has been reported to interfere with normal functions of the liver and cause disruption of hepatocytes (Kour et al., 1981; Lee, 1983). The common manifestation of zonal necrosis was evident following fluoride administration in laboratory animals (Chinoy, 1991a,b; Chinoy et al., 1992b). In the present study also, the serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were also significantly enhanced in fluorotic human individuals. It is known that these enzymes are markers for liver function and in the event of liver cell damage these transaminases would be released (SGOP and SGPT). Therefore, the increased activity of these enzymes would reflect on effect of high consumption of fluoride on liver, and its altered metabolism. In agreement with these results, Chitra et al. (1983) also reported augmented liver GOT and GPT activities by fluoride exposure in fish and a correlation between fluoride and liver damage was established.

Much of the history of fluoride research indicates profound effects on carbohydrate metabolism. Catecholamines are known to regulate carbohydrate utilization and its storage. In fluorotic human cases, the serum, catecholamines were elevated especially epinephrine, which might be due to stress caused by accumulated fluoride in the body. The enhanced catecholamine levels would have a stimulatory action on the sympathetic system.
nervous system and thereby influence the hypothalamo-gonadal axis. Earlier workers (Cheon and Distefano, 1973) had demonstrated that fluoride significantly increased the catecholamine levels of the liver, heart and kidney and concluded that it interfered with biosynthetic mechanism. The marked hyperglycemia which accompanied acute fluoride toxicity has been reported to be mediated by epinephrine released from the adrenal medulla (McGown and Suttie, 1977). Further studies had clearly demonstrated that the effect of fluoride is mediated primarily by splanchnic impulses arising in the central nervous system. Caruso et al. (1970) claimed that the release of catecholamines occurred due to increase in blood pressure resulting in cardiac stimulation of continuous exposure to fluoride. Thus, it is possible that the adrenal response to fluoride is due to splanchnic signals from a region in the central nervous system. The experiments with the adrenalectomized rats and the plasma catecholamine assays indicated that the hyperglycemic action of fluoride is mediated by enhanced catecholamines especially epinephrine (Himms-Hagen, 1967). Therefore, these enhanced catecholamines would alter carbohydrate metabolism as evident by high glucose concentration by fluoride toxicity in experimental animals (Dost et al., 1977).

Although a significant change in serum epinephrine and nor-epinephrine levels occurred, the urinary concentration of these hormones in fluorotic individuals were unaltered.

The thyroid hormones are known to have marked effects on the metabolic rate of the body. In the present study, the human subjects residing in fluoride endemic areas had significantly lower serum thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH) respectively as compared to control population of Ahmedabad (fluoride free region). However, studies confirming true relationship between goitre and fluoride toxicity
are limited. Siddiqui (1955) has reported prevalence of simple goitre cases in high fluoride areas. He observed an increase in the goitre cases with an increase in fluoride content of the environment and a decrease with an increase in iodine content of water. Early epidemiological studies suggested an association between goitre prevalence and high environmental fluoride (WHO, 1970). Higher rates of prevalence of goitre in endemic skeletal fluorosis was also observed by Latham and Greely (1967). Day and Powell-Jackson (1972) have reported a lower prevalence of goitre in Himalayan villages with a low fluoride content (0.1 - 0.19 mg/litre) in the drinking water than in villages with a higher content (0.23 - 0.36 mg/litre). The Royal College of Physicians (1976) did not find any evidence that fluoride was responsible for any disorder of the thyroid. In addition, in a recent German study, no relationship was detected between goitre and the fluoride content of drinking water (Sonneborn and Madlkow, 1981). In studies carried out by Jolly et al. (1976) in 26 patients with skeletal fluorosis and equal number of control subjects, no significant difference in basal metabolic rate, protein-bound iodine (PBI) and cholesterol levels between the two groups were observed. However, Willems et al. (1972) confirmed that fluoride inhibits the proteins responsible for splitting thyroglobulin molecule into thyroxine and triiodothyronine and causes hormonal imbalance. Thus, the low T3 T4 and TSH concentration in fluorotic population in the present study, would suggest alterations in their general body metabolism by altering basal metabolic rate.

In fluoride afflicted human population, the serum calcium levels exhibited fluctuations. In many of the individuals, the serum calcium levels were lowered, while some individuals had high serum calcium content as compared to control population. This discrepancy may be due to several factors including the relative intake of fluoride amount of calcium ingestion, hormonal levels and urinary loss of the minerals (Krishnamachari,
Cold calcium kinetic studies carried out by Srikantia and Siddiqui (1965) revealed that administration of 800 mg calcium to both fluoride endemic population as well as to the control human individuals resulted in high bone retention of calcium in the former group. This observation was substantiated either as an avidity for calcium or the patients were at a lower intake of calcium. In another study undertaken by Narasinga Rao et al. (1968) reported 53% retention on a 800 mg daily intake of calcium in fluorosis patients, thus corroborating the earlier observations of Srikantia and Siddiqui (1965). Radioactive calcium balance studies (Narasinga Rao et al., 1979) revealed enhanced bone mineralization and low resorption. Earlier studies of Sriranga Reddy and Narasinga Rao (1970) in fluorosis induced monkeys, using Ca$^{45}$ demonstrated that animals maintained on low protein diet retained less Ca$^{45}$ and those animals maintained on high protein diet had increased Ca$^{45}$ retention. These studies also revealed that calcium deficiency seemed to cause higher retention of calcium. Similarly, high fluoride when fed on a low calcium diet caused increased retention of fluoride. Thus, this observation suggests that in fluoride ingested animals, avidity for calcium increases, particularly when fed low calcium. An analogous situation has been observed in population living in endemic fluorosis areas. Therefore, the low calcium levels obtained in the present study may be either due to low resorption of bone or soft tissue calcification as reported in fluoride intoxicated rabbit aorta (Susheela and Kharb, 1990). Previous studies of Suketa and Kanamoto (1983) also reported calcium accumulation in kidney, thereby aiding the calcification process. In agreement with these results, several workers reported a rare to common occurrence of renal calculi in fluorotic human individuals (Herman, 1958). Although parathyroid hormone levels are known to be enhanced by fluoride, it is necessary to estimate both calcitonin and parathyroid hormone in this particular population inorder to establish specific effects.
The urinary output of calcium also showed individual to individual variation, similar to serum calcium levels. However, the mean values for calcium in these fluorotic individuals showed no significant difference with those of control human subjects. Rao et al. (1978) investigated clinically confirmed patients of skeletal fluorosis, who were maintained on 800 mg of calcium, 400 mg of phosphorous and 150 mg of magnessium per day. In these patients, the urine output of calcium was low. The study confirms the view that even among osteosclerotic type of fluorosis patients, avidity for calcium exists. The need for calcium for the new bone formation which is the characteristic feature of the disease may perhaps be the immediate factor responsible for the observed avidity for calcium.

In human population influenced by fluoride affliction, the electrolyte balance was altered. The sodium and potassium levels in serum were enhanced with a concomitant increase in their output through urine. The toxic effects of fluoride were aggravated by the altered clearance of electrolytes. The rise in Na⁺ and K⁺ levels in urine and serum could be attributed to changes in electrolyte balances in inter and intracellular fluids. This in turn may influence the movement of water in and out of the cellular matrix. The differential distribution of these two cations is essential in many membrane systems, where energy requiring active transport is functional. It has been reported that fluoride is known to cause potassium efflux (McIvor et al., 1985). In agreement with these results, Suketa and Terui (1980) also reported disturbances in Na⁺ and K⁺ levels in urine and serum of fluoride intoxicated rats. Which they attributed to changes in adrenal functions. Recently, Das and Susheela (1991) confirmed these results in fluoride human population as well as experimental animals. In their study, they obtained low corticosteroid levels in adrenal causing adrenal hypofunction. Therefore, the altered electrolyte balance may be due to adrenal hypofunction. Studies carried out by Suketa and Mikami (1977) described that
diminished mitochondrial ATPase activity in the kidney has been found to be apparently responsible for urinary sodium loss. Therefore, the altered ionic concentrations might result in dysfunction of aldosterone action at selective resorption sites in kidney. The loss of electrolytes can thus bring about a decrease in body weight due to loss of water along with the salts, as these individuals apparently look very thin and weak. In addition, the serum protein pattern by PAGE revealed a significantly low number of proteins, which suggest that its synthesis was affected by fluoride. Therefore, the low protein concentration along with excess of electrolytes would affect the body weight and growth especially in children.

In view of high fluoride intake through drinking water and to some extent food stuffs, the health problems have been aggravated enormously in many parts of the globe. The preventive aspects in endemic fluorosis are highly essential, since once the onset of bone disease occurs, there is no specific treatment.

Many antidotes are known to reduce fluoride toxicity. Jowsey and Riggs (1978) in their short-term studies in experimental animals as well as human population in endemic areas suggested calcium as calcium gluconate to be most logical antidote to fluoride, which mainly helps in the reduction in absorption of fluoride. However, this effort was rejected in chronic fluorosis cases, as serum calcium levels were inconsistent and administration of Calcium may further enhance the osteosclerotic changes.

Several efforts strongly support aluminium as an effective antidote to fluoride due to its binding capacity. Therefore, in India and elsewhere deflouridation technique is established, where in by using activated alumina, fluoride level is brought down to 0.5 mg/litre which is the permissible level according to WHO and is indeed optimal requirement for health. This chemical acts as an ion exchanger and has high selectivity for
fluoride. However, it is unsatisfactory as minor quantity is transferred into the defluoridated water, which on long-term consumption proved to be harmful especially to growing children. Also these is disadvantage of involvement of recurring costs of chemicals, maintenance and operations in defluoridation technique. Therefore, it is a difficult task to establish such technique on a large scale.

During the mid 1970s, Rao et al. (1975) observed that serpentine, a naturally occurring metasilicate of magnesium is capable of combining with large quantities of fluoride. However, it does not singly appear to induce a significant change in urinary or fecal excretion of fluoride. Therefore, in search of an effective antidote to fluoride, a team of East German Scientists suggested boron and iron to reduce fluoride toxicity. Judging by several pathological and biochemical findings, some benefit although observed by boron, but iron failed to counter the fluoride toxicity.

Recently, an electrocondensation technique, which has been applied in the Soviet Union and Japan for sterilizing potable water and decreasing its turbidity has been used to eliminate excessive fluoride with an aluminium anode. This method not only successfully reduces fluoride content from 5 ppm to 1 ppm or below, but it also has the advantage of killing germs and bacteria and lessening turbidity. Unfortunately, the technique involves high expenditure and less output of defluoridated water. In the present situation, the approach "prevention is better than cure" is seemed to be more logical and essential. Therefore, as described in the previous discussion part as reported by Chinoy and associates (1991a) ascorbic acid and calcium are likely to be promising agents which can effectively prevent fluoride toxicity. Therefore, these agents must be administered atleast to children who are on their way to be affected as a preventive measure and to the safety of economically backward communities in India as well as other
parts of the world.

Due to lack of synthesis of Vitamin C in human beings, its deficiency in endemic population is an aggravating factor in exaggerating fluoride toxic effects. The extensive studies carried out earlier (Chinoy, 1978) revealed its active metabolism in all tissues in rodents, potentiates its protection against drug as well as chemical toxicity. Similarly, calcium due to its affinity for fluoride forms an insoluble compound, CaF2 thereby suppresses fluoride action. Therefore, the combined administration of these two agents act synergistically against fluoride and act as ameliorative agents in the prevention of fluoride affliction at least in earlier stages to children, to be considered as therapeutic agents, which is the outcome of the efforts of the present investigations.