CHAPTER I
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The growing population and expanding industrialization are responsible for an increasing and complex range of health hazards in developed and developing countries. In the present day world, people are exposed to a great variety of natural and man made substances. Under certain conditions such exposures cause adverse health effects, ranging from subtle biologic changes to even death. The ever increasing quest of society to identify and present these ill-effects has led to the dramatic evolution of toxicology.

An escalation in the concentration of toxic pollutants in the biosphere and their ultimate entry into the biological system will pose serious problems on human and natural resources and also on the ecological balance. Indiscriminate use of metals in various industrial and agricultural processes also leads to various health hazards in the environment.

One of the most critical environmental issues today is ground water contamination. About 70% of all the water available in our country is polluted. In India, more than 76% of the population who live in several rural settlements of varying populations are dependent on ground water as source of drinking water. Therefore, the preservation, protection and management of the quality of life is dependent on the environmental components, apart from industrial, agricultural and other economic parameters. The extensive use of pesticides in the present decade are also major culprits of environmental pollution. Apart from these, trace elements are often considered to be toxic to man,
despite their active role as cofactors in many metabolic pathways. The trace elements are frequently encountered by human beings in several ways either through drinking water, food or inhalation. The trace elements in their minute concentrations are thought to be beneficial, but pose health hazards at higher concentrations.

Adverse or toxic effects in a biological system are not produced by a chemical agent unless that agent or its biotransformation products reach appropriate sites in the body at a concentration and for a length of time sufficient to produce the toxic effects.

Toxicity can be classified as:

(i) Acute toxicity and (ii) Chronic toxicity

(i) **Acute toxicity effects**: Acute toxicity effects show sudden onset lasting a short time.

(ii) **Chronic toxicity effects**: Chronic exposure typically induces a biological response of relatively slow progress and long duration. The chronic toxicity test is used to study the effects of continuous, long term exposure of a chemical (Gupta and Salunkhe, 1985).

**FLUORINE**

Fluorine is one of the most active elements of the halogen group. It has a relative atomic mass of 19, ranks 13th in periodic table in distribution of the earth’s crust. It is a pale, yellow-green gas, and most electronegative. It is never found free in nature. It is supposed to be an essential element for the formation of caries-resistant dental enamel and for the normal mineralization of bones. About 96 percent of the fluoride in the body is
deposited in hard tissue (WHO, 1984).

Fluorine reacts with most metallic elements to form compounds which are ionic. Most of these fluorides are readily soluble in water. Sodium fluoride is the most important of the alkali fluorides. It is a white, free flowing crystalline powder which is widely used in fluxes and has been proposed for the removal of hydrogen fluoride from exhaust gases. Sodium fluoride was the first fluoride compound used in the fluoridation of drinking water in U.S.A. in 1950 (WHO, 1984).

HALF LIFE

The toxicokinetic studies revealed that the absorbed fluoride is distributed between blood, soft tissues and the skeleton. The half life of fluoride in blood and of tissues has been reported to be few hours, while in skeleton, it has a longer half life of about 8 years (WHO, 1984).

LETHAL DOSE\textsubscript{50} OF FLUORIDE IN ANIMALS

The LD\textsubscript{50} value for male and female rats are 250 mg and 180 mg F/kg body weight respectively and for male mice is 54.41 mg F/kg body weight, while the females have LD\textsubscript{50} value of 51.6 mg F/kg body weight (Pillai et al., 1987; 1988).

OCCURRENCE OF FLUORIDE

At the global level, countries such as Algeria, Argentina, China, Japan, Kenya, Morocco, Senegal, Turkey and Thailand are under the threat of fluorosis. In the USA,
some states like Texas, Utah, North Dakota have high fluoride levels.

In India, occurrence of fluoride bearing water has been reported in Gujarat, Punjab, Haryana, Rajasthan, Uttar Pradesh, Andhra Pradesh, Maharashtra, Tamil Nadu, Kerala, Orissa, Karnataka, Jammu and Kashmir and Delhi. Two other states, Bihar and West Bengal have also been declared endemic for fluorosis.

India's earth crust is rich in fluoride containing minerals. Fluoride is present in ground water in most Indian villages. It has been estimated that fluoride levels in water in India range from 2 to 39 ppm, while the recommended level of fluoride in drinking water is 1 ppm by WHO. Rajiv Gandhi National Drinking Water Mission has been launched by the Government of India to monitor, study and suggest remedial measures, wherever there is problem of excessive fluoride in water, affecting about 62 million people.

SOURCE OF FLUORIDE

Fluoride is found in man's natural environment and under normal conditions is present in our food, water, soil, air and vegetation. The extensive distribution of fluoride in the nature is a direct source for human population resulting in adverse health hazards.

Fluoride in Air

Fluorides emitted into the air exist in both gaseous and particulate forms. Traces of fluoride in the air of rural communities and cities, arise from both natural sources and human activities. The natural dispersal of fluoride into the air has long been recognized
in regions of volcanic activity and in vicinity of industries (US EPA, 1980). Other natural sources of fluoride in the air are the dust from soils, and sea-water droplets, carried up into the atmosphere by winds.

The amount of air borne fluoride increases with increasing urbanization because of the burning of fluoride containing fuels (coal, wood, oil and peat) and due to pollution from industrial sources.

**Fluoride in Rocks and Soil**

Fluorides account for about 0.032% of the earth’s crust. The mean fluoride content of rocks lies between 0.1 and 1.0 g/kg. The main primary fluoride containing minerals are fluorspar (CaF$_2$), Cryolite (3 NaF.AlF$_3$), and apatite (3 Ca$_3$(PO$_4$)$_2$.Ca (F, OH, Cl)$_2$), but in most soils it is associated with micas and other clay minerals. Sodium fluoride and magnesium fluoride are also found as natural minerals.

The mean fluoride content of mineral soils is 0.2-0.3 g/kg (US NAS, 1971), whereas, that of organic soils is usually lower. However, in soils which have developed from fluoride containing minerals it may range from 7 to 38 g/kg (WHO, 1984).

The fluoride content of top soil may be increased by the addition of fluoride containing phosphate fertilizers, pesticides, irrigation water, or by deposition of gaseous and particulate emissions.

**Fluoride in water**

Some fluoride compounds in the earth’s upper crust are fairly soluble in water.
Thus, fluoride is present in both surface and ground water. The natural concentration of fluoride in ground water depends on such factors as the geological, chemical, and physical characteristics of the water-supplying area, the consistency of the soil, the porosity of rocks, the pH and temperature, the complexing action of other elements, and the depth of wells (Worl et al., 1973). Owing to these factors, fluoride concentrations in ground water fluctuate within wide limits e.g., from <1 to 25 mg or more per litre. In some areas of the world, e.g. India, Kenya, and South Africa, the levels can be much higher than 25 mg/litre (WHO, 1970). In surface fresh waters, less influenced by fluoride containing rocks, the fluoride content is usually low, 0.01-0.3 mg/litre (WHO, 1984) than in sea. Air borne fluoride is returned by the way of snow and rainfall, when occurring over land, it eventually reaches the oceans via rivers (WHO, 1984).

Fluoride in Food and Beverages

Fluoride content of foods very considerably. The fluoride content of vegetables cooked in fluoridated water is higher than the content of vegetables cooked in water containing negligible amounts of fluoride (Martin, 1951). Spinach, cabbage, lettuce, and parsley have higher values than other vegetables.

The fluoride content of water used in industrial food production and home cooking affects the fluoride content of ready-to-eat products ranging from 0.60 to 1.0 mg/kg (US EPA, 1980). Mineral waters may contain fluoride levels higher than 1 mg/litre. Infant formulae, infant gruel, syrups, and juices prepared with fluoridated water contain 0.9-1.3 mg fluoride/litre.
Tea leaves are usually very rich in fluoride, and contain levels ranging from 3.2-400 mg/kg dry weight (Canadian Public Health Association, 1979). About 40-90% of the fluoride in tea leaves is eluted by brewing.

TOTAL HUMAN INTAKE OF FLUORIDE

The fluoride contents of air, water and food determine the human intake of fluoride. Bierstekar et al. (1977) estimated that persons living near industrial sources of fluoride could inhale 0.06 mg fluoride during a day of maximal pollution. Occupational exposure may add considerably to the total intake of fluoride viz., the mining and processing of fluorspar, cryolite and apatite (WHO, 1984).

In communities, where the water is fluoridated, people would consume a mean of 2.7 mg fluoride/day as compared with 0.9 mg/day, where the water is not fluoridated (Kumpulainen and Koivistoinen, 1977).

Fluoride also finds its way into the body through fluoridated tooth pastes (WHO, 1984). In India, around 10% of the population are known to use toothpastes.

ABSORPTION OF FLUORIDE

The respiratory tract is major route of absorption of both gaseous and particulate fluoride. Human beings usually absorb at least 45% of the fluoride normally present in the diet (WHO, 1984).
DISTRIBUTION OF FLUORIDE

Fluoride is present in human plasma in a non-bound ionic form and in a bound form associated with albumin. 15-20% of the total fluoride of normal human plasma is absorbed by calcium phosphate. Human plasma contains an average of 0.013 ppm of fluoride.

About 99% of the fluoride retained in the body is localized in the skeleton (WHO, 1984).

EXCRETION OF FLUORIDE

Fluoride is excreted in the urine, sweat and faaces. It occurs in traces in milk, saliva, hair and presumably tears (Underwood, 1977).

URINARY EXCRETION

The principal route of fluoride excretion is via the urine and is influenced by several factors such as total intake, the form of intake into the body, the person’s general health, especially with regard to kidney disease. Urinary excretion of fluoride is very rapid, approximately 20% of the ingested fluoride appears in the urine in about 3 hours.

FAECAL EXCRETION

About 10% of the total daily fluoride excretion takes place through faeces. If the fluoride is ingested as relatively insoluble compounds such as bone meal, cryolite and calcium salts or if such precipitants as aluminium and calcium compounds are present,
larger proportions of the fluoride are unabsorbed in the intestinal tract and appear in the faeces. This may amount for as much as 30% of that ingested (WHO, 1984).

EXCRETION IN SWEAT, MILK AND SALIVA

It is believed that during excessive sweating, up to 50% of the total fluoride excreted may be lost via the perspiration and the rates may sometimes nearly equal those in urine (Underwood, 1977).

Only negligible amounts of the fluoride intake can be accounted for in milk and saliva. The concentrations of fluoride in milk are approximately the same as those in blood. Fluoride concentrations in saliva are low and presently available data do not show a striking correlation with fluoride in drinking water.

IMPORTANCE OF FLUORIDE

A beneficial function of fluoride has been known since the late 1930's, when it was discovered that the fluoride ion can play a significant role in the prevention of human dental caries. It is believed that fluoridation has a beneficial effect on prevention of tooth decay. Nevertheless, the margin between a safe daily dose of fluoride and a potentially harmful one is very narrow. Fluoride has held centre stage in dental research for more than half a century (WHO, 1984). In 1960, evidence was presented which indicates that fluoride is also beneficial for the maintenance of a normal skeleton in the adults. Fluoride may be necessary for normal hematocrit levels, fertility and growth (WHO, 1970).
BIOLOGICAL FUNCTIONS OF FLUORIDE

Fluoride may have a role interrelated with absorption or utilization of some dietary nutrients. There is evidence that fluoride could enhance the intestinal absorption of iron.

EFFECTS OF FLUORIDE

(1) Acute Effects

Acutely toxic doses cause gastroenteritis, muscular weakness, chronic convulsions followed by depression, pulmonary congestion (which may be accompanied by hemorrhage), as well as respiratory and cardiac failure. Enzymes involved in vital processes are inhibited, and severe hyperglycemia has been noted in some cases. Fluoride is a fairly effective inhibitor of cholinesterase, and this characteristic, with the decrease in plasma calcium concentration that has been observed, may be responsible for effects on the nervous system.

The decrease in calcium concentrations have also been postulated to affect blood clotting and membrane permeability as well as an increase in skeletal muscle excitability, hyperactive reflexes and painful spasm (WHO, 1984).

Cell damage and necrosis produce massive impairment in the function of vital organs, and particularly when fluoride is given orally, there are severe local effects on the gastric and intestinal mucosa. The symptoms of acute fluoride poisoning usually include nausea, vomiting, excessive salivation, cramps in the abdomen and diarrhea. In the early stages of acute fluoride poisoning, depending on the prevailing gastric acidity, highly corrosive hydrofluoric acid may be produced in the stomach.
(2) Chronic Effect

Symptoms include mottled teeth, brittle teeth, anorexia, dense bones, loss of weight and strength and pain in back and legs. Sensitive individuals have eczema, atopic dermatitis and urticaria. Prolonged ingestion of water with a high fluorine content causes skeletal fluorosis in adults. There is an extraordinary uniformity in the signs and symptoms of intoxication. The initial symptom noted in India is a recurrent general tingling sensation in the limbs or all over the body. Pain and stiffness next appear, especially in the thoracic and lumbar regions and the cervical spine. Accompanying the spinal disability, there is stiffness of various joints. The bony and cartilaginous skeleton of the thorax is markedly affected. The vertebral column becomes rigid and patient develops a "pokar-back" (WHO, 1984).

FLUOROSIS

The intake of excess fluoride through drinking water, food or inhalation has been found to cause a freak disease 'Fluorosis'. In India, 17 out of 32 states are under the gruesome grip of the disease, wherein, an estimated 62 million people have been affected. The major manifestations of the disease are skeletal and dental deformities.

DENTAL FLUOROSIS

The symptoms of dental fluorosis range from normal, translucent and smooth teeth in the initial stages to a severe form of pitting and chipped off edges in the final stage. At a constant level of fluoride intake, the canines (or fourth incisors) are usually the teeth
most severely affected by fluoride. Dental tissues, like those of skeleton, accumulate fluoride most rapidly during formation and mineralization. During tooth formation, the cells of the dental tissues, particularly the ameloblasts are very sensitive to fluoride. At relatively low doses, e.g. 2 ppm of fluoride in the water, small spots of discoloration may form in the tooth surface. These spots, or mottling, vary in colour from paper-white to dark brown. At higher doses, the cells may be affected and the tooth structure is severely altered, so that the normally smooth surface shows hypoplastic corrugations.

SKELETAL FLUOROSIS

The most toxic effect of fluoride on human beings is skeletal fluorosis wherein stiffness, restriction in movement of joints, flexion deformities at the spine, crippling and neurological complications lead to a bed ridden state. The chemical structure of the bone is adversely affected causing osteomalacia, osteoporosis and osteosclerosis. The single most important feature in osteosclerosis is calcification of interosseous membrane of the forearm. In patients suffering from endemic genu valgum (knock knee), besides osteoporosis, cystic expansion of short and long bones occur (Krishnamachari and Krishnaswamy; 1973). Skeletal fluorosis affects young and old alike. Fluoride ingested by a mother can accumulate in the skeleton of the growing foetus. Other clinical features of fluorosis involve sclerosis of humerus, scapular ribs, radius, ulna and pubic bones together with calcification of muscular attachments. In general, elevated dietary fluoride results in an acceleration of bone mineralization. However, the increase in mineralization is accompanied by a decrease in bone strength.
The disease is slowly reversible with treatment that includes reduction of fluoride intake and improvement in diet.

**TOXIC EFFECTS OF FLUORIDE ON EXPERIMENTAL ANIMALS AND LIVE- STOCK**

There have been reports concerning fluorosis in cattle reared in a polluted area, where the animals were fed on vegetation contaminated by fluoride (Chinoy, 1995). The manifestation of chronic effects of fluoride on cattle have been found to be more or less similar to those found in man i.e. dental and osteofluorosis. The animals exhibited a non-specific and typical lameness or stiffness associated with calcification of periarticular structures and tendon insertions, thickening of bones and mineralization of the tendons (Shupe et al., 1963). Thus, lameness is often found to be transitory in nature and limits feeding or grazing time, thereby impairing performance of the animal (U.S. EPA, 1980).

Perkinson et al. (1955) demonstrated rapid absorption of fluoride by ruminants using radioactive fluoride. The symptoms in teeth were chalkiness and mottling. Udall and Kellers (1952) in their field studies have found poor reproduction, diarrhoea and overgrowth of the hoofs due to high consumption of fluoride in cattle.

There is some information available on the role of fluoride on aquatic animals, especially fishes. Experiments undertaken by Neuhold and Singler (1960) in fish exposed to poisonous amounts of NaF caused weight loss and periods of violent movement. Studies from our laboratory revealed alterations in the activities of some enzymes of muscle of *Channa punctatus* exposed to NaF. Biochemical changes in the liver, kidney
and brain were also observed (Chinoy et al., 1994a).

EFFECTS OF FLUORIDE ON GENERAL BODY METABOLISM

The toxicity of fluoride in aggravated mainly through its adverse effect on general body and tissue metabolism. Therefore the role of fluoride on general body metabolism is presented here.

FLUORIDE AND PROTEIN METABOLISM

Fluoride is known to reduce protein synthesis (Hoerz and McCarty, 1971; Uslu, 1985). Fluoride inhibits growth of cells in vitro due to inhibition of protein and DNA synthesis which are the main targets for the cytotoxic action of fluoride (Holland, 1979; Helgeland, 1976). The protein levels in stomach, duodenum and ileum of fluoride treated rabbits were declined (Shashi et al., 1987). A reduction in protein concentrations have also been observed in several tissues of mice, rats, rabbits and guinea pigs intoxicated with NaF in different doses (Chinoy, 1991a;b; 1992; Chinoy and Sequeira, 1989a; Chinoy et al., 1991a; 1993a; 1994b,c; 1995; 1997a,b; Patel et al.., 1994; Chinoy and Sharma, 1998).

Polyacrylamide gel electrophoresis of proteins of testis and cauda epididymis of NaF treated rats revealed disappearance of some proteins, induction of some new proteins and some were found to be resistant to NaF action (Chinoy et al., 1995; 1997a).
FLUORIDE AND CARBOHYDRATE METABOLISM

Fluoride has been traditionally known as an inhibitor of glycolysis, and to induce dramatic changes in carbohydrate metabolism mainly in the processes of utilization or storage of carbohydrates. In rabbits treated with fluoride, a decline in glycogen concentration in spleen, lens, liver and skeletal muscle occurred (Shashi et al., 1988). On the contrary, glycogen accumulation occurred in fluoride treated fishes (Shaikh and Hiradhar, 1985; Chinoy et al., 1994a) and in liver, muscle, vas deferens and uterus of rats and mice (Chinoy, 1991a,b; 1992; Chinoy and Sequeira, 1989a; Chinoy et al., 1991a: 1992a; 1993b; 1994b; 1995) which could be correlated with the decrease in the activity of phosphorylase in these organs (Chinoy and Sequeira, 1989a; Chinoy et al., 1991a: 1992a; 1994b). The difference in data might be due to the different species, dose and duration of treatment.

A decrease in the isocitrate dehydrogenase and accumulation of citrate was reported by Dousset et al. (1987) in guinea pigs treated with HF. Similarly, Shearer and Suttie (1970) found an elevation of liver citrate concentration of rats fed fluoride (450 to 600 ppm for 3 days). Shearer et al. (1971) also reported that fluoride affects the carbohydrate metabolism mainly through inhibition of the glycolytic pathway rather than the tricarboxylic acid pathway in rats.

FLUORIDE AND LIPID METABOLISM

Interaction of fluoride and lipid metabolism assumes considerable significance since fluoride is involved in arteriosclerosis. Saralakumari et al. (1988) reported that in
rats supplemented with 100 ppm of fluoride resulted in marked reduction in plasma free fatty acids. The liver and serum lipid fractions were also affected and a noticeable increase in total lipids, triglycerides and phospholipids in the serum occurred which points to the formation of a fatty liver. Townsend and Singer (1977) also obtained an increase in serum triglycerides in guinea pigs treated with fluoride. However, according to Leipzig et al. (1967), excess fluoride intake decreased the triglycerides. Similarly, in the liver of rabbits treated with NaF, triglycerides were decreased with a concomitant inhibition of lipase activity (Singh et al., 1985). Treatment with fluoride (5, 10, 20 mg/kg body wt.) in male and female rodents (for 30, 45, 60 days) resulted in increase in cholesterol in testis and ovary concomitant with a decrease in the activities of 3β and 17β HSD and circulating testosterone/estrogen levels (Chinoy, 1992; 1996; Narayana and Chinoy, 1994a; Chinoy and Mehta, 1999a).

Fluoride is known to stimulate the respiratory burst and the production of superoxide radicals in neutrophils of humans, rabbits and guinea pigs. The high reactivity of superoxide radicals may lead to chemical modification and impairment of proteins, lipids, carbohydrates and nucleotides in living cells (Rzeuski et al., 1998). Recent work has revealed that fluoride administration inhibited the activities of superoxide dismutase, glutathione peroxidase and catalase in the ovary and testis of treated mice which increased lipid peroxidation, thus rendering the tissue susceptible to injury (Chinoy and Patel, 1998a; Chinoy and Sharma, 1998). The most important consequences are the denaturation of proteins and the peroxidation of membrane lipids with an increase in the permeability of the cell membrane (Subramaniam et al., 1994).
NUCLEIC ACID METABOLISM

Fluoride has been reported to cause depression in DNA and RNA synthesis in cultured cells (Strochkova et al., 1984). Sodium fluoride (5 mg/kg body weight) was effective from the 45th day of treatment in causing a significant decline in the DNA and RNA levels of mice ovary and uterus indicating alterations in nucleic acid and protein metabolism in these organs (Patel and Chinoy, 1997). The DNA/RNA ratio declined in the uterus, whereas, it remained unaltered in the ovary. This decrease might be due to a significant decline in RNA concentration. The DNA/protein ratio was also significantly decreased in the ovary and uterus which could be related to the significant decline in protein levels. Thus it is likely that the process of transcription and translation would be affected in NaF treated mice (Patel and Chinoy, 1997).

GENOTOXIC EFFECTS OF FLUORIDE

Conflicting reports are available in the literature regarding the genotoxic effects of fluoride. Information available is very limited on this aspect and the results that have been published are inconclusive (Smith, 1985; Li et al., 1988). The literature review suggests three different observations: (1) Fluoride has no genotoxic effects (Obe and Slacik-Erben (1973) and Thompson et al. (1985) found no fluoride induced increase in the frequencies of chromosomal aberrations or Sister Chromatid Exchanges (SCEs) in human lymphocyte cultures. Sodium fluoride even at maximum tolerance dosage did not cause chromosome damage detectable with micronucleus assay (Li et al., 1987) in mouse bone marrow. Moreover, Martin et al. (1979) showed that life time consumption of 50
ppm fluoride did not cause detectable chromosome damage in bone marrow or testis cells of mice. Leonard et al. (1977) also observed no increase in the chromosome aberrations in the leucocytes of cattle with signs of chronic fluoride poisoning when compared to control animals. (2) The second observation is that fluoride is a mutagenic agent and causes DNA and chromosome damage even at a dose of 0.45 ppm (Mohamed and Chandler, 1982) in mice. Jachimczak and Skotarczak (1978) have reported that sodium fluoride induced chromosome aberrations in cultured human leucocytes. NaF caused chromosome aberrations in cultured ovarian oocyte of mice, ewes and cows (Jagtello and Lin, 1974). Sheth et al. (1994) reported for the first time an increase in the frequency of Sister Chromatid Exchanges in endemic human population of North Gujarat, India, as compared to control. (3) The frequency of micronuclei in peripheral blood lymphocytes of 40 workers chronically exposed to fluoride at a phosphate fertilizer factory in North China was significantly higher than that of controls (Zhang and Meng, 1999).

The incidence of Down's Syndrome with increasing concentrations of fluoride has been reported in human population residing in endemic areas in Sweden (Berglund et al., 1980). Takahashi (1998) has also reported fluoride related incidence of Down's Syndrome births in young mothers in five counties of metropolitan Atlanta, Georgia and in several regions of USA with fluoridated water. Berry (1962), however did not find the difference in the occurrence of Down's Syndrome in populations between areas with low (<0.2 mg/litre) and high (0.8-2.6 mg/litre) fluoride levels.

The above information clearly demonstrates that at present there is no established
opinion regarding the genotoxic effects of fluoride and its potential as a mutagenic agent. It is apparent that further investigations are necessary in order to clarify this important issue and efforts in this direction are underway at present in our laboratory.

TERATOGENIC EFFECTS OF FLUORIDE

Embryo and fetal toxicity from high doses of fluoride have been reported in experimental animals. High doses of fluoride (3 to 12 mg/kg body weight/day) have been found to cause abortions, necrosis of placentae and affect fetal growth in rats (Devoto et al., 1972). Studies carried out by Glenn et al. (1982) suggest that fluoride may also exert effects on human fetal growth. Babies, whose mothers had received fluoride tablets during pregnancy were somewhat heavier and slightly longer at birth, and prematurity was much less frequent as compared to control. Doses of 0.5 to 2 mg fluoride were found to be lethal to chick embryos (Spira, 1956). However, NaF at a dose of 3 mg/kg body weight/day failed to produce still births in mice (Fleming and Greenfield, 1954).

STUDIES ON FLUORIDE AFFLICTION IN MEHSANA DISTRICT OF NORTH GUJARAT

In Gujarat, Mehsana district is endemic to fluorosis, where a large population is under the influence of the disease. Therefore, Chinoy and co-workers (Chinoy et al., 1992a; 1994d; Chinoy and Narayana, 1992; Chinoy, 1996; Mathews et al., 1996) carried out a survey study in over 100 villages. The individuals, about 1000 having skeletal and
dental deformities were studied for the levels of fluoride in urine, blood and their drinking water samples. Similarly, some samples were collected from Ahmedabad population, where fluoride is within the permissible level (1 ppm). The results on fluoride levels as well as several biochemical parameters revealed direct or indirect interrelationship of fluoride and soft tissue functions in the affected individuals.

EFFECTS OF FLUORIDE ON TISSUES AND ORGAN SYSTEMS

A short review of effects of fluoride on some selected tissues and body functions is given below:

BLOOD

The blood acts as a transport medium for fluoride. About 75% of the blood fluoride in present in the plasma, the rest is mainly in or on the red blood cells (Carlson et al., 1960). Macuch et al. (1963) reported decreased haemoglobin, increased erythrocytes and abnormal lymphocyte counts in children living near aluminum plants. Greenberg (1982) has observed morphological abnormalities in cell structure and mitotic figure formation in immature leukocytes of mice given NaF in drinking water. However, no significant changes were obtained in RBC and WBC counts after NaF treatment in mice by Chinoy et al. (1993a), but the fluorotic subjects suffered from mild anaemia (Chinoy et al., 1994d). Erythrocyte membrane abnormality and echinocyte formation were also reported in rabbits and human beings exposed to fluoride (Susheela and Jain, 1986).
SKIN

Several instances of dermatitis attributable to industrial exposures to fluorine, hydrogen fluoride or sodium fluoride have been reported, but detailed information is lacking (WHO, 1984).

MUSCLE

Fluoride is known to affect the structure and function of muscle. Kaul and Susheela (1974) had reported fluoride induced degeneration of muscle fibres and defects of plasma membrane in rabbits as evident from the enhanced serum creatine phosphokinase level. Shashi (1989) also reported fluoride induced reduction in muscle fibres, vacuolization and necrosis in rabbits.

Bogin et al. (1976) found a significant decrease in alkaline phosphatase and isocitrate dehydrogenase in the skeletal muscle of mice maintained on water containing 100 ppm NaF. The activities of isocitrate dehydrogenase, cholinesterase, lactate dehydrogenase and alkaline phosphatase in the heart muscle also revealed significant decline. However, Chitra et al. (1983) observed enhanced muscular enzymes in fish exposed to fluoride. Fluoride induced alterations in various enzymes and biochemical parameters of gastrocnemius muscle of mice and rats were also reported by Chinoy et al. (1991a, 1993b).

EFFECTS ON DIGESTIVE SYSTEM

The fluoride enters the body by ingestion and absorption occurs in the
gastrointestinal tract by simple diffusion. Symptoms of vomiting, abdominal pain and diarrhea due to the formation of hydrofluoric acid in the gut were noticed. Fluoride affects cellular protein synthesis in the gastrointestinal organs (Shashi and Singh, 1987). Scanning electron microscopic studies carried out by Susheela et al. (1992) revealed widespread damage to the stomach mucosa viz., loss of microvilli and desquamated epithelium due to fluoride intake. The corrosive nature of hydrogen fluoride possibly leads to inflammation, ulceration and other mucosal abnormalities in the stomach and proximal small intestine.

**INTESTINE**

The intestinal cell lining plays an important role in digestion and absorption. It automatically becomes the most exposed site of contact to fluoride following ingestion. Study has shown significant alterations in the formation of lipid peroxides in rat intestine following oral administration of fluoride (Shayiq et al., 1986). Rastogi et al. (1987) observed that higher fluoride concentrations cause substantial damage to the intestinal brush border membrane.

**LIVER**

Liver being the principal organ for detoxification bears the major brunt of structural insults meted out by toxic substances. Zonal necrosis is the most common symptom in liver of NaF treated rats, mice and mudskippers (Chinoy, 1991a,b; 1992). The hepatic lobules were hyalinized with loss of cells and vacuolization of cytoplasm. The
shape of hepatocyte nuclei was irregular and they were pycnotic. The arrangement of hepatic cord was also disturbed (Kour et al., 1981; Chinoy, 1991a,b). The histology of liver in mudskippers exposed to 40 and 80 ppm of fluoride revealed ruptured cell membrane within 48 to 72 hrs of exposure (Shaikh and Hiradhar, 1987). In many of the hepatocytes, nucleus was pushed to the side while in some nuclear material was extruded out. Therefore, the structural alterations would affect the liver metabolism. The significant increase in the activities of serum transaminases (SGPT and SGOT) indicate alterations in liver function of animals and human fluorotic individuals as these enzymes are specific markers (Chinoy, 1991a,b; 1992; Chinoy et al., 1992a; 1994d). Similar results were also reported by Tsunoda et al. (1985) in goats exposed to air-borne fluoride. A significant decrease in serum protein correlated with the liver damage was observed in rats given a dose of 10 mg NaF/kg body weight for 30 days (Chinoy, 1991a).

Electron microscopic study of rabbit liver revealed fluoride induced alterations in the structure of mitochondria (Chongwan and Daijei, 1988). Many mitochondrial cristae were broken, with their membrane ruptured or disintegrated and RER was reduced in number. Fluoride induced changes in various biochemical parameters of liver were reported by many scientists (Bogin et al., 1976; Chitra et al., 1983; Chinoy et al., 1991b; 1993b).

**EFFECTS ON EXCRETORY SYSTEM**

It is obvious that the acquired fluoride in the body from various sources is actively depleted by kidney through urine. The urinary fluoride excretion is utilised to determine
the degree of danger to which man is being exposed. Therefore, it is considered as a principal route of excretion. High fluoride concentration causes impaired kidney function (Whitford and Taves, 1971) and damage to the kidney tissue with the increasing dose of fluoride has been reported. As the kidney gets damaged, clearance of fluoride is reduced (Kono et al., 1984). The toxic effects of fluoride are also enhanced by the altered renal clearance of other electrolytes, metabolites and wastes. Fluoride is implicated in the etiology of urinary stones.

In mice following the administration of 10, 500 and 1000 ppm NaF caused a cloudy swelling of the kidney tubular cells, marked necrosis and atrophy of the glomeruli which affected its function (Kour and Singh, 1980a). The total lipids, cholesterol, triglycerides and phospholipids were decreased in the kidney of fluorotic rats. The renal and serum Na⁺, K⁺ levels were altered in rats which would affect the electrolyte balance, protein concentration and kidney function (Chinoy, 1991a,b). Bhatnagar and Susheela (1998) reported that chronic fluoride toxicity in glomerulus of the kidney of rabbit treated with 10 mg/kg body weight daily for a period of 25-28 months caused abnormalities in visceral epithelial cells including loss, distortion and fusion of foot processes as well as detachment of the epithelial cell layer in some parts leaving the glomerular basement membrane denuded. Similar ultrastructural changes were observed by Chinoy and Sharma (2000) and Chinoy et al. (2000) in kidney of mouse treated with 10 mg NaF/kg body weight for 30 days.
EFFECTS ON RESPIRATORY SYSTEM

Respiratory system is a potential route of entry of fluoride into the human body. Fluorine and hydrogen fluoride are pulmonary irritants which, in sufficiently high concentrations, can have devastating effects. In mouse, rat and guinea pigs exposed to different concentrations of hydrogen fluoride, irritation of the mucous membranes of the nose and eyes, acute inflammation, focal necrosis of the nasal mucosa and tracheobronchitis were observed (Wohlschlage et al., 1976). In certain species of animals, pulmonary damage due to exposure to reactive gases of fluoride was evident (Morris and Smith, 1982). In acute toxicity, respiratory depression, and coagulation, necrosis and congestion in lung were reported. Kaltreider et al. (1972) reported pneumonia, carcinoma and lung abscess besides the common respiratory obstacles in inhabitants of industrial vicinity. The delicate tissues of the lung got intensely and fatally damaged in industrial workers and bronchial asthma was evident by fluoride. Thus, exposure to fluoride compounds are harmful and damage respiratory tract.

EFFECTS ON CARDIOVASCULAR SYSTEM

There is scant information available on the role of fluoride on cardiovascular functions. Intravenous infusion of fluoride caused a depression of blood pressure, heart and respiratory rate. Caruso et al. (1970) observed a direct vasodilatory effect by fluoride. Vascular changes, characterised by microvascular injury, perivascular disintegration of tissue cells, and vascular proliferation were predominated by fluoride ingestion. It is believed that calcification of arteries is an integral feature of skeletal fluorosis.
HEART

Fluoride is reported to decrease the blood pressure and heart beat. Zhavoronkov (1977) observed chronic myocarditis and dystrophic changes in heart muscle fibres of fluoride treated rats. High doses of fluoride have been reported to cause severe heart damage leading to cardiac irregularities and irregular electrocardiogram in humans (Zhiliang et al., 1987). The aorta is known to accumulate the highest amount of fluoride as compared to other soft tissues (Underwood, 1977). Aortic calcification and degeneration of smooth muscle fibres in the tunica media of the aorta were reported in fluoride intoxicated rabbits (Susheela and Kharb, 1990). In male albino mice, the significantly enhanced levels of sodium, potassium and calcium in ventricle indicates electrolyte imbalance. The protein, DNA and RNA levels in ventricle were significantly decreased while the cholesterol level was significantly increased (Chinoy and Biringwala, unpublished observations) indicating alteration in protein and nucleic and metabolism.

EFFECTS ON CENTRAL NERVOUS SYSTEM (CNS)

Lu et al. (1961) found stimulation of CNS by intraperitoneal injection of NaF to rats. Latency and/or disruption of some of the learned responses were observed by hydrogen fluoride administration.

In humans, the partial and complete paralysis of arms and legs in advanced fluorosis is usually considered to be related to pressure upon the spinal cord by newly formed bone protruding into it and upon nerves at the point of their exit from the spine. However, it has been suggested that the spinal cord lesions and muscular damage in
patients suffering from occupational fluorosis are also the result of a direct action of the fluoride ion on the ganglion and muscle cells (Franke et al., 1975). A neuropathological analysis by Chlubek et al. (1998) revealed marked shrinkage of cerebellar granular and Purkinje cells, perivascular myelin swelling and astroglia reaction, especially in the white matter of brain in NaF treated (60 ppm) rats.

BRAIN

The edible mudskipper, *Boleophthalmus diussunieri* was exposed to sublethal concentrations (viz. 40 and 80 ppm F) of fluoride for 168 hours which caused reduction of telencephalic cytoplasm, nuclear material and Nissl’s substance in the brain (Shaikh and Hiradhar, 1987). Vacuolized appearance around neuronal cell bodies in telencephalic well as mesencephalic compartments was observed. NaF treatment at a dose of 10 mg/kg body weight for 15 and 30 days caused a decrease in protein levels in brain (cerebral hemisphere) (Chinoy and Patel, 2000). This might be due to the alteration in Ca$^{2+}$ ion concentration in brain, which is essential for the release of acetylcholine from synaptic vesicles.

ENDOCRINE SYSTEM

Extensive investigations carried out during the past one decade showed that fluoride toxicity is not confined to the bone and dental tissues alone, but involves more than one endocrine organ and is evident in adult as well as children. Alteration in hormonal profile are now believed to be related to chronic exposure to environmental
fluoride.

**THYROID GLAND**

Thyroid functions are very important in the maintenance of body metabolism. In fluorotic experimental animals the structure of thyroid exhibited swelling of mitochondria with disintegrated cristae in follicular epithelial cells (Chongwan and Daijei, 1988). Fluoride may inhibit the proteinases responsible for splitting thyroglobulin molecule into thyroxine and triodothyroxine (Willems et al., 1972). There could possibly be an effect of fluoride on the feedback mechanism mediated through the hypothalamus and adenohypophysis, which regulates thyroid secretions through TSH. Studies in human population affected by fluorosis revealed low serum thyroid hormones namely $T_3$, $T_4$ as well as TSH (Chinoy, 1992). Siddiqui (1955) reported goitre cases with increase in fluoride content of the environment. Desai et al. (1993) also observed a significant positive correlation between overall prevalence of goitre and dental fluorosis among endemic population of Gujarat.

**PARATHYROID**

The parathyroid gland plays an essential role in endemic fluorosis since its function is to mainly regulate calcium metabolism. Fluoride is known to stimulate parathyroid and thereby enhance circulating parathormone levels. Teotia and Teotia (1973) reported an increase in parathyroid hormone (PTH) levels manifesting secondary hyperparathyroidism in patients with skeletal fluorosis and in children living in endemic
areas. Teotia et al. (1978) opined that the observed changes in man such as osteosclerosis, hypermineralization, osteoclastic resorption of trabeculae and other effects are the attributes of interaction between the changes that occur in the PTH-thyrocalcitonin axis. Community based studies strongly suggest that calcium status is modified by PTH, which in turn is responsible for bone changes observed in fluorosis. Observations on increased hormonal levels were substantiated by Makhni et al. (1980) at autopsy in two fluorosis patients whose parathyroid glands weighed at least four times the normal weight due to the increased size and number of the paranchymal cells which led to hyperactivity of the gland. In some endemic areas of India 'genu valgum' was the manifestation of fluoride toxicity among population groups in whom dietary calcium was low (Krishnamachari and Krishnaswamy, 1973). Genu valgum is a crippling form of fluoride toxicity which occurs in relatively younger children around 8-10 years. It has distinctive epidemiological and clinical characteristics, such as predominantly male involvement, its occurrence in adolescents and evidence of secondary hypothyroidism with elevated levels of circulating immunoreactive parathyroid hormone (Krishnamachari and Krishnaswamy, 1974; Sivakumar and Krishnamachari, 1976).

**THYMUS**

Fluoride is known to injure thymic epithelial cells and thymocytes and affect the growth in mice (Chen et al., 1999). The mitochondria were swollen and their cristae were lost.
ADRENAL

An increase in weight of adrenal gland after fluoride intoxication and a significant increase in plasma epinephrine as well as hyperglycemia was induced by fluoride (McGown and Suttie, 1977). The histology of adrenal gland of rat revealed pycnosis in some regions of the cortical cells and the medulla showed extensive vacuolization and hypertrophy of chromaffin cells, suggesting alterations in adrenal function (Chinoy, 1991a,b). The adrenal ascorbic acid concentration was increased by 10 mg NaF/kg body weight treatment in response to the imposed stress and helps in overcoming it by increased utilization and storage (Chinoy, 1978; 1991a,b).

PANCREAS

Sodium fluoride treatment brought about no alterations in the histology of pancreas as compared to control except that the Islet cells appeared more pyknotic as compared to normal (Chinoy, 1991a,b).

Clinical study on the effect of high fluoride intake revealed that the B-cells of pancreatic islets were damaged (Xie et al., 1999). Hence insulin production may be affected.

MECHANISM OF ACTION OF REPRODUCTIVE TOXICANTS

A toxicant, whether a chemical, physical or biological agent, acts by interrupting biological processes, including the transfer of energy and information necessary for normal reproductive function and development.
Following exposure, a toxic chemical compound must be distributed to the target organ (e.g. hypothalamus, pituitary gland, gonad, uterus or epididymis) wherein it interacts with a critical cell or subcellular component, disrupting an event necessary for normal reproductive function. If this interaction goes unrepaired, altered reproductive function will occur.

Metabolism of the chemical by liver or kidney may result in toxicity that is more or less apparent. In some cases, a compound may be metabolised and cleared from the body, and no adverse effect will occur. In other cases, metabolic products may be more toxic or long lived than the original toxin.

Reproductive toxins may act directly: (1) by virtue of structural similarity to an endogenous compound (e.g. hormone or nutrient and/or (2) by altering the structure of or denaturing a protein hormone. Some reproductive toxins may act indirectly, requiring metabolic processing or conversion within the body before exerting a toxic effect. The metabolite formed may then act through one of the direct mechanisms of reproductive toxicity (i.e. structural similarity or chemical reactivity). Other indirectly acting reproductive toxins may exert their effects by producing alterations in the body's physiological control system (i.e. activation or inhibition of enzymes). It is also possible for reproductive toxins to exert adverse effect through multiple mechanisms.

**FLUORIDE AND REPRODUCTIVE SYSTEM**

The studies on the role of fluoride on reproductive system has received inadequate attention and there is paucity of data, while the existing data is controversial. The
interrelationship of fluoride and reproductive functions were first reported by Messer et al. (1973), who found that fluoride plays an important role in reproduction and its deficiency is a cumulative factor for fertility impairment in female mice. They further demonstrated that mice with low fertility improved their reproductive capacity, litter production and breeding performance when maintained on high fluoride diet (Messer et al., 1974). However, these results were contradicted by Tao and Suttie (1976) who claimed that fluoride was not involved in maintenance of reproduction.

TESTES

Degenerative changes, such as atrophy and necrosis of seminiferous tubules, lack of differentiation and maturation of spermatocytes have been shown in the testis of F-treated mice (Kour and Singh, 1980b). These results were supported by the detailed studies carried out by Chinoy and Sequeira (1989b), who reported that fluoride ingestion to mice at a dose of 10 mg/kg body weight for 30 days caused a decrease in the diameter of seminiferous tubules. The germinal epithelium showed denudation and vacuolisation of cells. The tubular lumen was devoid of sperm. Further studies of Chinoy et al. (1991c) revealed that a single microdose vasal injection of NaF to rats also exhibited similar changes in testicular histoarchitecture affecting the spermatogenic process.

The electron microscopic studies in rabbits revealed changes in the structural integrity of testis by fluoride, affecting spermatogenic elements (Susheela and Kumar, 1991). Recent study from our laboratory have revealed that NaF caused disorganisation of mitochondrial cristae and distortion of acrosomal and nuclear membranes (Chinoy and
Sharma, 1999a). Narayana and Chinoy (1994a) have reported that testicular steroidogenesis, Leydig cell functions and serum testosterone levels were altered after fluoride treatment in rats which adversely influenced the structural and functional integrity of target organs.

**CAUDA EPIDIDYMIS**

In cauda epididymis fluoride treatment caused confluence of tubules resulting in larger tubules, decrease in epithelial cell height with denudation of cells in the lumen, which was devoid of sperm. These structural changes contributed towards alterations in cauda epididymal metabolism and function (Chinoy and Sequeira, 1989a,b; Chinoy et al., 1991c, 1992b).

**SPERMATOZOA**

Recent studies on fluoride treated rats and mice have revealed inhibition of sperm acrosomal enzymes, namely, hyaluronidase and acrosin (Narayana and Chinoy, 1994b; Chinoy and Sharma, 2000). Schoff and Lardy (1987) reported that fluoride is a strong inhibitor of glycolysis and respiration process in spermatozoa. The sperm of NaF treated rabbits when stained with silver nitrate (specific for acrosomal integrity) exhibited head to head agglutination, deflagellation and loss of acrosome (Chinoy et al., 1991a). These alterations in sperm structure and metabolism are the result of the hostile internal milieu of epididymis affecting sperm maturation which ultimately led to a decline in sperm count, motility and their fertilizability and subsequently to a significant reduction in
fertility after NaF treatment (Chinoy, 1991a; Chinoy and Sequeira, 1992). Human spermatozoa lost their motility in vitro in the presence at 250 mM NaF within 20 minutes incubation (Chinoy and Narayana, 1994).

**VAS DEFERENS**

The histoarchitecture of the deferens of fluoride treated mice indicated nuclear pycnosis in the epithelial region, clumping of stereocilia, increase in thickness of lamina propria and muscle coat as well as absence of sperm in the lumen (Chinoy and Sequeira, 1989b; Chinoy and Sharma, 1999b). In rabbits treated with NaF (10 mg/kg body weight) for 18 to 29 months, loss of stereocilia on the epithelial cell lining the lumen of the vas deferens with abundant mucus droplets (Susheela and Kumar, 1991) was observed. Alterations in histology of other sex accessory organs were also reported (Chinoy and Sequeira, 1989b; Chinoy et al., 1991c).

**SEMINAL VESICLE**

NaF treatment resulted in lowered fructose concentration in the seminal vesicles. Similarly, the acid phosphatase and protein levels were also affected by NaF which were to a great extent responsible for low sperm motility resulting in reduction of fertility (Chinoy and Sharma, 1998).

**OVARY AND UTERUS**

The histology of ovary of mice after 30 days of NaF treatment showed
vacuolisation of the stromal region and corpora lutea. The follicular cells surrounding the primary and secondary follicles were found to be pyknotic (Chinoy and Patel, 1998b). NaF treatment for 30 days brought about a decrease in the thickness of serosa and myometrium of uterus. Vacuolisation was observed in the serosa with dense pyknosis in the endometrium. Atrophy and confluence of endometrial glands was observed with nuclear pyknosis (Chinoy and Patel, 1998b).

**CYCLICITY AND FERTILITY RATE**

Epidemiological study of gynecological problems in female workers in a superphosphate manufacturing plant offers a direct evidence of fluoride effects in human pregnancies. The female workers were found to suffer more menstrual irregularities, vaginal and uterine inflammation, more frequent toxicosis during pregnancy with hypotension and threatened abortions, a higher percentage of untimely discharge of the amniotic fluid and weakness of labour (Kuznetsova, 1969a,b). In male patients with fluorosis, Tokar and Savechenko (1977) found reduced amount of testosterone but enhanced concentration of follicle stimulating hormone (FSH) and luteinizing hormone (LH).

Fluoride in concentrations over 60 ppm in the feed of dairy cows interfered with breeding efficiency (Hobbs et al., 1954). A dose of 150 to 300 mg fluoride per kg body weight over 0-72 hour interval blocked gonadotropin stimulation of the rabbit ovary (Guercio and Cazzola, 1941). Similarly, fluoride at a dose of 10 mg/kg in a 21 day period blocked follicular development in guinea pigs (Sanfilippo, 1946) Studies on
histopathological changes in rabbit ovary during experimental fluorosis revealed atrophy of follicles along with oocyte distintegration and marked necrosis of cells (Shashi, 1990).

REVERSAL OF FLUORIDE TOXICITY

Fluoride is a potent health hazard and affects virtually every phase of body metabolism. In view of the millions of people afflicted with fluoride and a variety of pathological manifestations in soft tissues of both animals and human beings, necessitates the investigation of therapeutic agents which are easily available, are cheap and have promising results in mitigation of fluoride induced health hazards in endemic populations.

FLUORIDE AND MINERALS

The extent of fluoride toxicity in a cell or tissue is influenced by many minerals, due to its strong affinity to form complexes with minerals and elements. Some divalent ions like Ca\textsuperscript{2+}, Mg\textsuperscript{2+} etc., form strong complexes and thus reduce fluoride action.

Extensive studies on calcium kinetics showed that the cumulative retention of radioactive calcium was enhanced in animals fed with fluoride, higher retention of fluoride was observed in those receiving low calcium diets (Krishnamachari, 1978). Urinary calcium excretion, calcium deposition into bone and mass of exchangeable calcium was not affected by fluoride. However, fecal excretion of fluoride was low suggesting that this was not a direct effect of fluoride (Ramberg et al., 1970).

Administration of 30, 50, 150 or 450 ppm additional fluoride to pigs increased the bone fluoride. High dietary calcium-phosphorus, however, lowered bone fluoride
concentration in swine (Forsyth et al., 1972a). The increase in fluoride level was still higher when they were maintained on low calcium phosphorus diet (Forsyth et al., 1972b). Therefore $\text{Ca}^{2+}$-phosphorus interaction with fluoride has been reported to be highly effective in lowering fluoride action.

**FLUORIDE AND VITAMINS**

Wadhwani (1952) induced fluorosis in monkeys on normal and scorbutic diets and noted higher mortality in scorbutic animals. He observed that stiffness of limbs and restricted movements disappeared and death postponed when each animal was given 20 mg vitamin C. Studies carried out by Chinoy and co-workers (Chinoy et al., 1991a, 1993b, 1994b,c; 1995; 1997a,b; Narayana and Chinoy 1994b; Patel and Chinoy, 1997; Chinoy and Sharma, 1998; Mehta and Chinoy, 2000) in rodent models further revealed the therapeutic role of ascorbic acid or calcium as they brought about significant recovery in fluoride induced toxicity. However, recovery was most pronounced in animals given both ascorbic acid and calcium due to their synergistic/additive effect. According to Muhler and Hine (1959), elevated levels of vitamin A, C and D, mitigated the symptoms of fluorosis.

Marks (1975) found that vitamin E reduced reproduction abnormalities in rats. Vitamin E has a powerful anti-oxidant effect within the animal body particularly for lipids. In rats, the main symptoms of vitamin E deficiency are degeneration of testis, regression in the ovary, changes in ovulation and abnormalities of gestation. Various adverse health affects of vitamin E deficiency in vertebrates are well documented,
including disorders of the reproductive organs (Nelson, 1980). Chinoy and Sharraa (1998) reported that ingestion of vitamin E to fluorotic male mice brought about a significant recovery in NaF induced reproductive failure.

Vitamin D acts as a regulator of calcium and phosphate metabolisms. It is used in treating conditions such as reduced renal functions, calcium malabsorption and osteoporosis. The rodent data (Chinoy and Sharma, 1998; Chinoy and Patel, 1998) revealed that ingestion of vitamin E and/or vitamin D to fluorotic mice manifested a significant recovery in all NaF induced effects in the tissues studies.

**FLUORIDE AND AMINO ACIDS/PROTEIN SUPPLEMENTATION**

Amino acids are required as the building blocks for the synthesis of proteins of blood and tissue. In addition, many of the amino acids are utilized in the formation of hormones and enzymes. Amino acids are rapidly taken up by the tissues particularly the liver, intestine and kidney. Free amino acids apparently are not stored as such in the tissue to any great extent but are metabolised by incorporation into protein or by transamination and deamination and further oxidation.

Certain amino acids found in protein must be supplied preformed in the diet. These are termed the "nutritionally essential" amino acids. Other amino acids which are synthesized in the tissue from what are termed "amphibolic" intermediates (which occur in glycolysis and citric acid cycle), are termed as "non-essential" amino acids. Amino acids may be grouped as (I) glycogenic and (II) ketogenic amino acids. Glycogenic amino acids when fed to experimental animals give rise to glucose or glycogen, while ketogenic
amino acids give rise to acetoacetic acid and other ketone bodies.

Glycine can be synthesized by many animals. The recovery obtained by glycine feeding to fluorotic mice (Chinoy and Patel, 1996) might be due to its role in various important physiological functions.

Glutamine is needed for the growth of mammalian cells in tissue culture in concentrations considerably higher than the other amino acids (Meister, 1965). Glutamate and glutamine systems serve as source of ketoglutarate and may aid in regulating the concentration of metabolite entering the citric acid cycle. This amino acid is glycogenic (Harper, 1965).

Suttie et al. (1974) reported that addition of various amino acids to the growth media in concentrations in excess to those present in the media enhanced the growth of fluoride treated L-cells wherein glycine and glutamine were found to be the most potent. Other reports (Chinoy and Patel, 1996; Patel and Chinoy, 1998; Chinoy and Mehta, 1999a) have revealed that supplementation of amino acids, glycine and glutamine alone or in combination was beneficial in mitigation of fluoride induced effects in female and male mice.

Sriranga Reddy and Srikantia (1971) have reported that in experimentally produced fluorotic monkeys, administration of a low protein diet appeared to accelerate the development of reflection of bones and a higher incidence of rarefaction was observed in these animals. This could be the result of fluoride treatment and the lack of protein in the diet. It is evident that a protein supplemented diet could ameliorate the toxic effects.
of fluoride (Chinoy and Mehta, 1999b). The basis for the protective effect might involve recovery in enzyme activities and thereby influence metabolic reactions.

The work incorporated in the present thesis highlights the interrelationship of fluoride and reproductive functions as well as those of some non-reproductive tissues in male mice. The work embodied in the thesis also emphasizes the role of some therapeutic agents administered alone or in combination as well as the effects of ingestion of a protein-deficient and protein supplemented diet for prophylaxis of fluoride toxicity.