GENERAL CONSIDERATIONS

Interest in the biological effects of fluoride started in 1930-31 when it was found that a specific type of abnormal formation of dental enamel is associated with fluoride levels in drinking water above 2-3 ppm fluoride. Interest became even greater in early forties when the first statistics were published which documented comparatively low prevalence of dental caries in populations consuming drinking water containing 1 ppm fluoride (Marthaler, 1987). Since 1940, the caries-preventive action of ingested fluoride and topically applied fluorides have been extensively studied. Preventive measures using fluoride in various forms have been implemented in several industrialised countries. Moreover, fluoride is even suggested as a therapeutic drug for the treatment of osteoporosis (Cohen *et al.*, 1969).

On the other hand, the excessive fluoride ingestion during early ages results in mottled enamel and accumulation of fluoride in the osseous tissue over a period of time, ultimately leads to crippling bone deformities known as `skeletal fluorosis'. The deleterious effects overrules its beneficial effects due to the fact that all around the world people suffer from excessive fluoride effect and is one of the major causes of concern in India.

The main sources of supply or availability of fluoride to man is from water, atmosphere and from food. The effect of fluoride to the health of man stems largely from dissolved fluoride present in many supplies of drinking water. However, suspended particulate fluorides may also have some health importance (Zipkin and Likins, 1957). Natural sources of atmospheric fluorides are volcanoes (Noguchi *et al.*, 1963) and dust storms in areas where soil are high in fluoride (Williamson, 1953). Their environmental effect depends largely on water solubility, which governs their ability to be incorporated into plants and animals. Manmade fluoride emissions are largely from steel, brick, cement, aluminium and fertilizer industries (Allcroft, 1954).

The presence of excessive concentration of fluorine containing compounds in the environment

can be damaging to all forms of life. Fluoride ion is a protoplasmic poison and only a small amount can be tolerated by any living cell (Pack, 1971). Apart from skeletal deformities, fluoride is also known to affect heme metabolism, protein metabolism, carbohydrate metabolism and enzyme activity (Zebrowski *et al.*, 1964; Motoo *et al.*, 1971; Goutschi, 1974; Suketa *et al.*, 1980). Thus, the fluoride seems to have multifarious effects on animal's tissue, structure and function when present as a pollutant in environment.

In recent years analysis of pollutant effects on growth, development and reproduction have been gaining significance in toxicity studies. However, even though fluoride is considered fairly high in the priority list of environmental pollutants, fewer studies with regard to growth and development have been conducted. An organism like chick will serve as an ideal model for such a study, because being a bird with higher metabolic activity, the manifestations of the effect of toxicant will be more prominent in chick. Hence, it was thought desirable to assess the toxicological effect of fluoride under laboratory conditions on post-hatched developing chicks of domestic fowl, *Gallus gallus domesticus* of RIR variety.

Assessment of lethality is of prime importance in any investigation undertaken to evaluate the hazardous effects of a toxicant, since it provides clues for further studies, especially in designing an experimental protocol and to assess the toxicity of repeated exposure to the chemical (Doull, 1986). The lethality of fluoride was assessed according to Finney (1971), by plotting different doses of commercially available sodium fluoride against the probit of mortality of growing chick, *G. g. domesticus*. The value obtained for 50% survival at the end of 24 h (24 h LD_{50}) was 77.62 mg F^-/kg b.w. (fiducial limits, 69.68-86.48 mg F^-/kg b.w.). To evaluate the effect of chronically administered fluoride on the physiological profile of growing chick, a sublethal concentration of 15.4 mg F^-/kg b.w. (1/5 of LD_{50}) was selected.

The newly hatched chicks were provided through intragastric route 15.4 mg F^-/kg b.w. daily

for 30 days and were sacrificed on 1, 5, 10, 20 and 30 days of fluoride treatment to assess fluoride induced alterations in postnatal development.

As a prelude to comprehension of possible mechanism and site of action of pollutant, measurement of body weight as well as weight of target organs become imperative. Hence, after the initial assessment of lethality, rate of body growth and weight of intestine, liver and pancreas have been made at specific intervals following fluoride administration. Growth of the chick was significantly retarded by sublethal dose of NaF. Weight loss in animals subjected to NaF has been documented (Pankhurst, 1980). The probable reason for this might be due to anorexia and reduced food consumption. The lowered gizzard content observed in the treated birds strengthen our notion of lowered food intake and thereby a drop in body weight. Moreover, it has been documented that the body growth in the case of poultry depends largely on the growth of small intestine, liver and pancreas (organs of supply) (Nitsan *et al.*, 1991). In the present study it was also noticed that the growth of small intestine, liver and pancreas has been delayed in the fluoride intoxicated chicks. This could additionally hamper the progress of body growth in fluoride treated birds.

The above mentioned studies involving the body growth and the development of digestive organs, point to a possible inhibition of proliferative and synthetic activities of cells in fluoride intoxicated chicks. Towards this end a study of nucleic acids and protein profiles in experimental birds has been envisaged. The observed reduction in DNA content could be due to hampered mitotic activity of the cells in NaF treated chicks. *In vitro* studies by Proffit and Ackerman (1964), have proved that fluoride suppresses DNA synthesis and decreases rate of cell division. Other reports with similar conclusions were later confirmed by Albright (1964), Hongslo *et al.* (1974) and Holland (1979). However, Imai *et al.*(1983), opined that fluoride induced inhibition of proteins, which are essential for the replication of DNA, could be the reason for reduced DNA turnover. The fluoride poisoned chicks also showed lower level of

total proteins than the controls, indicating depletion and derangement of synthetic machinery. Several studies have confirmed that fluoride inhibits protein synthesis (Helgeland, 1976; Lin *et al.*, 1976; Kathpalia and Susheela, 1978). The experimental birds also registered a hike in RNA/protein ratio indicating derangement in the translatory process. Defective translation by way of dissociation of polyribosome has been recorded in reticulocyte cultures treated with NaF (Marks *et al.*, 1965). Hence it is possible to surmise that the inhibition of protein synthesis by fluoride is probably due to the inhibition of new peptide chain and the dissociation of ribosome.

Since toxicity of a foreign substance is invariably reflected in blood, current study also included an examination of RBC population along with haematocrit, iron and haemoglobin in the fluoride exposed chicks. Reduction of erythrocyte numbers and haematocrit value was a striking feature. This was accompanied by anaemia and low levels of iron. Anoxia and anaemia have been observed in different animals following fluoride intoxication (Kahl *et al.*, 1973; Susheela and Jain, 1983; Karram and Ibrahim, 1992). Susheela and Jain (1983), while explaining similar situation in rabbit subjected to NaF have suggested that hypofuntion of adrenal glands might be the possible reason for anaemia and reduced erythrocyte population. Such a situation in fluoride treated chicks cannot be ruled out. Inadequate nutrition observed in the present study (Chapter 3), could also lead to anaemia. Similar observation made by Suttie (1968) gives additional support for the present findings. However, currently observed decrease in mean corpuscular haemoglobin apparently tells us about defective cells in circulation, which probably have lesser oxygen carrying capacity due to loss of respiratory pigment, whose synthesis seemed to have been affected, as revealed by depletion in the haemoglobin concentration.

Being a toxicant, fluoride provided stress to the developing organism. Such a stress situation is often reflected in the metabolism of the animals in question. Hence, in the present study bio-

chemical analysis of metabolites such as plasma glucose, tissue glycogen and lipids, and enzymes such as lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), glycogen synthetase, phosphorylase (Chapter 6) as well as acid and alkaline phosphatases, Na^+-K^+ ATPase and acetylcholinesterase (AChE) (Chapter 7) in liver and muscle have been carried out.

Glycogen depletion in the tissues along with hyperglycaemia was the major feature of fluoride exposed chicks. Hyperglycaemia has been reported in several animals following fluoride administration (Chitra and Rao, 1981; Shaikh and Hiradhar, 1985; Suresh and Hiradhar, 1990b). This increase in basal glucose level might not be due to stress induced adrenocorticoid hormone(s), as long term fluoride administration has been known to cause adrenal hypofunction (Rao and Susheela, 1979; Li *et al.*, 1990; Das and Susheela, 1991a). The lowered erythrocyte population noticed in the current study also supports this idea of adrenal insufficiency. This, along with the observed decline in AChE activity in different tissues of fluoride treated chicks (Chapter 7), prompted one to think about fluoride induced parasympathetic neuropathy. A disturbance in parasympathetic activity could result in an increased sympathetic tone. Such adrenergic activation may cause a direct stimulation of hepatic glucose output by release of glucagon (Gerich *et al.*, 1976; Miller, 1981), as well as reduced insulin secretion from the pancreas (Robertson *et al.*, 1976; Kuhn *et al.*, 1987). Cholinergic dysfunction may also mediate the stimulation of hepatic glucose output by sensitising the liver to basal levels of glucagon and epinephrine (Cherrington *et al.*, 1976; Wasserman *et al.*, 1984). It could be reasoned therefore, that hyperglycaemia could be an effect due to parasympathetic neuropathy in the fluoride treated growing chicks through inhibiton of acetylcholine (ACh) release, resulting in an impairment in glucose uptake mechanism. This observation is supported by the fact that the activity of glycogen synthetase showed a parallel decrease in both liver and muscle of fluoride treated chicks. Insulin is able to activate glycogen synthetase whereas glucagon and

epinephrine inhibit the enzyme activity (Apkan *et al.*, 1974; Witters *et al.*, 1978). Thus cholinergic dysfunction and the resultant insulin deficiency, set in due to fluoride administration, could be expected to enhance the stimulatiuon of glycogenolysis by glucagon and catecholamines. This fact is further strengthened by the present finding that glycogenolytic hormones while inactivating glycogen synthetase, caused the activation of glycogen phosphorylase. It is well known that this enzyme is activated by Ca^{2+} mobilizing hormones or cyclic adenosine-3', 5'-monophosphate (cAMP) producing hormones like glucagon. Fluoride is known to promote adenylate cyclase (enzyme that catalyses the conversion of adenosine triphosphate (ATP) to cAMP) activity (Rodriguez-pena *et al.*, 1991). This gives additional support to the present notion. Current study revealed a lowered oxygen carrying capacity in terms of reduced haemoglobin and inhibition of SDH activity apparently led to accumulation of lipids. Accelerated LDH activity observed in the experimental birds also supports the above contention about adaptive alteration to hypoxia seemingly induced by fluoride.

Glucose uptake by tissues can occur by one or more of transport mechanisms. In the case of birds, insulin dependent flow coupled and ACh facilitated sugar transport mechanisms form the predominant ones (Wilbrandt, 1975). Chronic fluoride poisoning reduced the vagal influence in both the liver and the muscle of postnatal developing chick. This was evident from the fact that AChE activity was very much reduced in both the tissues of the experimental birds. The level of activity of AChE is an indicator of quantity of ACh secreated by the nerve endings (Pilo *et al.*, 1976) and ACh as well as insulin are known to assist in the uptake of glucose into hepatic cells (Mondon and Burton, 1971; Pilo and Mehan, 1988). However, both insulin and ACh enhanced glucose uptake through a membrane bound mechanism, part of which is coupled to ionic movements (Pilo and Mehan, 1986; 1988). Na^+ and K^+ along with Ca^{2+} play a major role in membrane polarization and permeability by their differntial distribution on either side of the cells. The cation concentration of the cell is regulated by the transport enzyme Na^+-K^+

ATPase. This membrane bound enzyme is involved in the active transport of Na^+ and K^+ ions as well as essential metabolites like glucose and amino acids (Ganong, 1989). In the present study a decrease in the Na^+-K^+ ATPase activity has been noticed in the tissues of experimental birds. According to Luly *et al.* (1972) elevated cAMP concentrations might inhibit Na^+-K^+ ATPase activity. *In vitro* studies proved that NaF activates adenylate cyclase by direct interaction with Gs alpha (Boyd *et al.*, 1992). An alternate explanation is that in case of hampered vagal tone (due to fluoride poisoning), sympathetic tone, which secretes catecholamines at their nerve endings, expresses in full and this could lead to increased formation of cAMP. Hence it could be concluded that fluoride might have exerted its inhibitory effect on Na^+-K^+ ATPase through elevated cAMP level.

Enzymic inhibition by fluoride has been reported, which could additionally hamper the normal metabolic pathways and even force the cell to resort to alternative pathways as a part of stress induced response mechanism.

The lysosomal enzymes are well known to possess strong hydrolytic action leading to breakdown of macromolecules such as proteins. Currently examined hydrolases *viz.* acid and alkaline phosphatases are not only lysosomal, but are known to distributed in the soluble fraction of the cytoplasm as well. Such localization endows these enzymes with broad spectral functioning of not only lytic nature but also in other metabolic reactions (Cori and Cori, 1952; Rosenthal *et al.*, 1960; Barka, 1963; Palade and Forquliar, 1965). There was a decrease in the phosphatase activity which may be construed to contribute towards histopathological events. Such lytic activities were also accompanied by depletion in level of protein. Pollutant induced decrease in activities of acid and alkaline phosphatases have been reported in tissues of several animals (Smith *et al.*, 1950; Riekstniece *et al.*, 1965). This reaffirms the fluoride inhibition of enzyme activity.

These results showed that fluoride, when present as a pollutant suppresses the growth and development of postnatal chicks. The growth inhibitory effect of fluoride is achieved: 1. directly by suppressing the proliferative and synthetic activity of cells and 2. indirectly through stress induced deranged carbohydrate metabolism. Present study also threw light on fluoride induced neural dysfunction.