HISTOPHYSIOLOGICAL AND NEUROENDOCRINOLOGICAL STUDIES ON POST-HATCHED DEVELOPING CHICKS OF DOMESTIC FOWL GALLUS GALLUS DOMESTICUS SUBJECTED TO SODIUM FLUORIDE

[SUMMARY]

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CONCISE SUMMARY

Rapid industrialization is instrumental in progress of a fast growing population. However, attendent problem of pollution often acquires unmanagable proportions which are critical for the very lives whose betterment was intended.

Environmental pollutants impinge a variety of unwelcome influence on animal biota through manipulation of metabolic activities of the organism in question. A large number of studies abound the literature as far as ecotoxicological investigations of an animal exposed to various pollutants, especially heavy metals, pesticides and other industrial effluents are concerned. However, even though fluoride is considered fairly high in the priority list of industrial pollutants, fewer studies in this regard have been conducted. Most of the studies are with reference to effects of fluoride on the skeletal system and occurrence as well as distribution of fluoride in organisms in an ecosystem.

In recent years analysis of pollutant effects on growth, development and reproduction have been gaining significance in toxicity studies.But there is an apparent lack of information regarding the effect of fluoride in this connection. 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. 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. 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). 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 aforementioned 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 have proved that fluoride suppresses DNA synthesis and decreases rate of cell division. Other reports with similar conclusions later confirmed 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. 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. 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. 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. 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 biochemical 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. 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. 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, as well as reduced insulin secretion from the pancreas. Cholinergic dysfunction may also mediate the stimulation of hepatic glucose output by sensitising the liver to basal levels of glucagon and epinephrine. 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. 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. 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. 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 and ACh as well as insulin are known to assist in the uptake of glucose into hepatic cells. However, both insulin and ACh enhanced glucose uptake through a membrane bound mechanism, part of which is coupled to ionic movements. 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. In the present study a decrease in the Na^+-K^+ ATPase activity has been noticed in the tissues of experimental birds. *In vitro* studies proved that NaF activates adenylate cyclase by direct interaction with Gs alpha and it is proved that elevated cAMP concentrations inhibit Na^+-K^+ ATPase activity. 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 break-down 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. 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. 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.

It is hoped that this modest attempt to study the developmental, haematological and metabolic responses of the growing chick to fluoride will further add to our understanding as regards to this pollutant and will open up new avenues of investigation.