CHAPTER 6

FLUORIDE INDUCED ALTERATION IN VARIOUS METABOLITES AND CERTAIN ENZYMES OF GLYCOGEN BODY IN THE POST-HATCHED DEVELOPING CHICKS OF DOMESTIC FOWL, *GALLUS DOMESTICUS*.

Fluoride is known to be beneficial in reducing dental caries and appearance of tooth as a result of early eruption (Marthaler, 1987). It is also used in the treatment of osteoporosis (Cohen *et al.*, 1969). However. fluoride is known to be harmful if ingested in large amounts. It is well known that this compound accumulates in the bones over a period of time utlimately leading to bone deformity known as *skeletal fluorosis*. Fluoride gains entry into an animals body and gets accumulated in a cumulative manner in skeletal as well as soft tissues (Pillai, 1983; Sheikh, 1985). Geerearts *et al.* (1986) have shown that blood-brain-barrier does not exclude the entry of fluoride into the C.N.S. Moreover, many neurological complications have been reported in human subjects residing in endemic fluorotic area (Shashi, 1992). Fluoride, in the form of sodium fluoride (NaF), cryolite and sodium fluorosilicate is used in the poultry industry against the infection of bird lice (Garner, 1986). Though efforts have been directed at understanding the fluoride induced alteration in

carbohydrate metabolism in liver and muscle, such an attempt on nervous system is rarely met with. Homeostatic regulation of carbohydrate metabolism in the body is controlled by the nervous and endocrine system. Metabolic homeostasis is very important, as any change in the plasma metabolite level would adversely affect the working of C.N.S. and *vice versa* (Azam *et al.*, 1990).

A harmful substance like fluoride may bring about derangement in metabolic machinery. Sublethal dose of fluoride is known to retard the growth and development of the chicks of domestic fowl(Suresh, 1994; Fig. I). The present study has been designed to study the fluoride induced effects on the metabolic profile of the glycogen body in the post-hatched developing chicks of domestic fowl, *Gallus domesticus*.

MATERIAL AND METHODS

Day old chicks of domestic fowl, *Gallus domesticus* were purchased from Government Hatchery, Baroda. They were allocated at random into two groups of 60 each and placed in metal cages. The birds were maintained in a constant temperature-humidity maintained animal house for 1 month. During this period the animals were exposed to a 14:10

Fig. 1: The control (C) and experimental (E) chick of domestic fowl (RIR variety) after 20 days of treatment. Note the stunted growth of the experimental bird.

E
C

light-dark lighting regime. The chicks had an access to food and water. The birds in the control group were provided distilled water through intragastric route. The experimental birds were administered orally 1 ml of fluoridated water(15.4 mg F^-/kg body weight) daily for 30 days. Fluoride was administered during early morning hours, after which the food and water were withdrawn for 1 hour. On days 1, 5, 10, 20 and 30 following the commencement of experiment, the chicks were decapitated and bled, glycogen body tissue (from 3-4 chicks) quickly removed and weighed on an electronic Mettler balance and used for various biochemical analysis as follows:

Analytical method :

Glycogen : The anthrone method of Seifter *et al.*, (1950) was used for the estimation of glycogen and the glycogen content of glycogen body expressed as mg/100 mg wet tissue.

Protein : The total protein content of glycogen body was determined using the Folin Phenol method as described by Lowry *et al.*, (1951) using bovine serum albumin as standard and expressed as mg/100 mg wet tissue.

Phosphorylase (EC 2.4.1.1, 1,4-α-D-Glucan : Orthophosphate, α-D-glucosyl transferase): Assay of

phosphorylase was made by the modified method of Cori *et al.* (1943) as adapted by Cahill *et al.*(1957), using dipotassium salt of G-1-P as substrate. The inorganic phosphate liberated was estimated by the method of Fiske and SubbaRow (1925). The absorbance was read at 660nm on a spectrophotometer and the enzyme activity was calculated as µg phosphorous released/mg protein/10 minutes.

Glycogen synthase (EC 2.4.1.11, UDPG α-glucan glucoysl transferase): The enzyme was assayed by employing the method of Leloir and Goldemberg (1962). The activity of glycogen synthase was measured by the amount of UDP formed from UDPG in the presence of glycogen and dipotassium salt of G-6-P. The UDP estimation was carried out by using a preparation of pyruvate kinase which catalyses the transfer of phosphate from phosphopyruvate. The liberated pyruvate was measured at 520 nm and the enzyme expressed as µM of UDP formed/mg protein/10 minutes.

Succinate dehydrogenase [EC 1.3.99.1, Succinate:(acceptor) oxidoreductase]: The activity of this enzyme was calculated by the procedure of Nachlas *et al.* (1960) using INT as electron acceptor and PMS as an intermediate electron carrier between reduced succinate dehydrogenase

and INT. The enzyme activity is expressed as µg formazan/mg protein/15 minutes.

Lactate dehydrogenase (EC 1.1.1.27, L-Lactate: NAD^+ oxidoreductase): Quantitative estimation of LDH was done by employing the colorimetric method of King (1959, 1965c) as described by Varley *et al.* (1980) using sodium lactate and NAD^+ as cofactor. The enzyme activity is expressed as µM lactate oxidized/mg protein/15 minutes.

Acid and Alkaline Phosphatases : The quantitative estimation of acid phosphatase [Orthophosphoric-monoester phosphohydrolase(acid optimum), EC 3.1.3.2] and alkaline phosphatase [Orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1] were done by the method of Lindhardt and Walter (1963). The activities of both the enzymes are measured by the formation of p-nitrophenol and expressed as µMoles of p-nitrophenol released/mg protein/30 minutes.

Statistical Analysis : The results have been expressed as mean ± standard error mean(SEM). The data have been subjected to student's 't' test.

RESULTS

Table I indicates the changes in carbohydrate metabolism of glycogen body of post-hatched developing chicks, *Gallus*

domesticus following exposure to sublethal doses of fluoride. Glycogen content in the glycogen body of the experimental chicks showed no significant alteration from day 1 to day 10 when the same were compared to that of control chicks (Table I, Fig. 1). However, on 20th day, a slight but significant ($p< 0.02$) decrease was observed in glycogen content of the treated chicks. This decrease was more pronounced ($p<0.001$) in birds treated with sodium fluoride for 30 days (Fig. 1).

Succinate dehydrogenase (SDH) activity was seen to increase steadily till the 5th day, followed by a decline on day 10, after which a further increase was recorded in control birds. The SDH activity in the experimental chicks also showed a similar pattern, but the same when compared with controls, however, depicted a significant decline in SDH activity towards the last two phases of experimentation (Table I, Fig. 2).

The activity of lactate dehydrogenase (Table I, Fig. 3) showed no statistically significant difference between the average of control and fluoride birds animals between day 1 and day 10. However, the activity pattern of this enzyme changed and showed significant increases in the birds treated fluoride for 20 and 30 days respectively ($p<0.001$).

Glycogen synthase activity was maintained more or less constant till day 10 in both the control as well as fluoride treated birds. However, there occurred a decrease in the activity of this enzyme by the 20th day in the experimental birds (Table I, Fig.4). On the other hand, though the phosphorylase activity remained steady in the early phases of experimentation following NaF administration, a slight elevation in its activity was noticed in the last phase (Table I, Fig. 5).

Acid phosphatase enzyme showed a lower activity in fluoride treated chicks (20 and 30 days of age) when compared to control birds (Table II, Fig. 6). Similarly alkaline phosphatase activity showed a significant decline in fluoride treated birds by days 20 and 30 respectively (Table II, Fig. 7).

The mean total protein content in the glycogen body of control chicks registered a steady increase till the termination of experiment. Fluoride administration to chicks, however, brought down its protein values in the glycogen body by day 10 of treatment schedule (Table II, Fig. 8).

Table I Effect of sodium fluoride on the levels of glycogen and enzymes related to carbohydrate metabolism in glycogen body of posthatched developing chicks *Gallus domesticus.*

PARAMETER	TREATMENT	DAY				
		1	5	10	20	30
Glycogen (mg/100 mg wet tissue weight)	Control	12.45 ± 0.32 [@]	11.54 ± 0.60	15.64 ± 0.38	17.84 ± 0.95	12.31 0.41
	Experimental	12.30 ± 0.63	11.93 ± 0.33	14.98 ± 0.64	15.18 ± 0.64[a]	08.21 ± 0.25[c]
Lactate Dehydrogenase (μM lactate oxidized/mg protein/15 mts)	Control	190.09 ± 0.91	271.02 ± 1.03	267.11 ± 1.20	141.21 ± 0.90	67.26 ± 1.10
	Experimental	189.18 ± 0.88	276.50 ± 1.09	265.98 ± 1.65	164.15 ± 0.19[c]	121.96 ± 0.95[c]
Succinate Dehydrogenase (μg formazan released/mg protein/ 15 mts)	Control	07.76 ± 0.42	17.21 ± 0.38	10.31 ± 0.24	21.04 ± 0.76	22.85 ± 0.30
	Experimental	07.89 ± 0.52	18.21 ± 0.46	11.75 ± 0.36	18.69 ± 0.48[a]	19.09 ± 0.26[c]
Phosphorylase (μg P released/mg protein/10 mts)	Control	252.68 ± 1.85	331.85 ± 1.80	282.25 ± 2.63	203.05 ± 1.53	142.11 ± 1.21
	Experimental	253.18 ± 2.39	329.75 ± 1.12	279.36 ± 2.21	205.77 ± 1.34	147.66 ± 2.06[a]
Glycogen Synthase (μM UDP formed/mg protein/10 mts)	Control	0.070 ± 0.002	0.061 ± 0.002	0.078 ± 0.003	0.086 ± 0.002	0.073 ± 0.002
	Experimental	0.069 ± 0.002	0.060 ± 0.002	0.079 ± 0.002	0.063 ± 0.002[c]	0.051 ± 0.002[c]

[@] Values are expressed as mean ± SEM of 6 experiments; [a]$p < 0.02$, [c]$p < 0.001$

Table II Effect of sodium fluoride on total protein, acid and alkaline phosphatase enzyme activities in glycogen body of posthatched developing chicks *Gallus domesticus*.

PARAMETER	TREATMENT	DAY				
		1	5	10	20	30
Protein (mg/100 mg wet tissue weight)	Control	4.30 ± 0.05 @	4.70 ± 0.06	5.38 ± 0.04	5.66 ± 0.16	7.18 ± 0.25
	Experimental	4.38 ± 0.09	4.78 ± 0.05	4.98 ± 0.14[a]	4.69 ± 0.21[b]	5.70 ± 0.17[c]
Acid Phosphatase (μM P-Nitrophenol released/mg protein/ 30 mts)	Control	1.17 ± 0.09	0.785 ± 0.01	0.788 ± 0.03	0.773 ± 0.03	0.729 ± 0.02
	Experimental	1.15 ± 0.08	0.804 ± 0.03	0.851 ± 0.02	0.647 ± 0.02[b]	0.602 ± 0.02[c]
Alkaline Phosphatase (μM P-Nitrophenol released/mg protein/ 30 mts)	Control	0.99 ± 0.07	1.30 ± 0.08	1.19 ± 0.03	0.862 ± 0.02	0.793 ± 0.01
	Experimental	0.98 ± 0 05	1.32 ± 0.09	1.20 ± 0.02	0.740 ± 0.02[c]	0.650 ± 0.02[c]

@ Values are expressed as mean ± SEM of 6 experiments; [a]$p < 0.02$, [b]$p < 0.01$, [c]$p < 0.001$

Figure 1. Effect of sodium fluoride on the levels of glycogen in glycogen body of growing chicks, *Gallus domesticus*

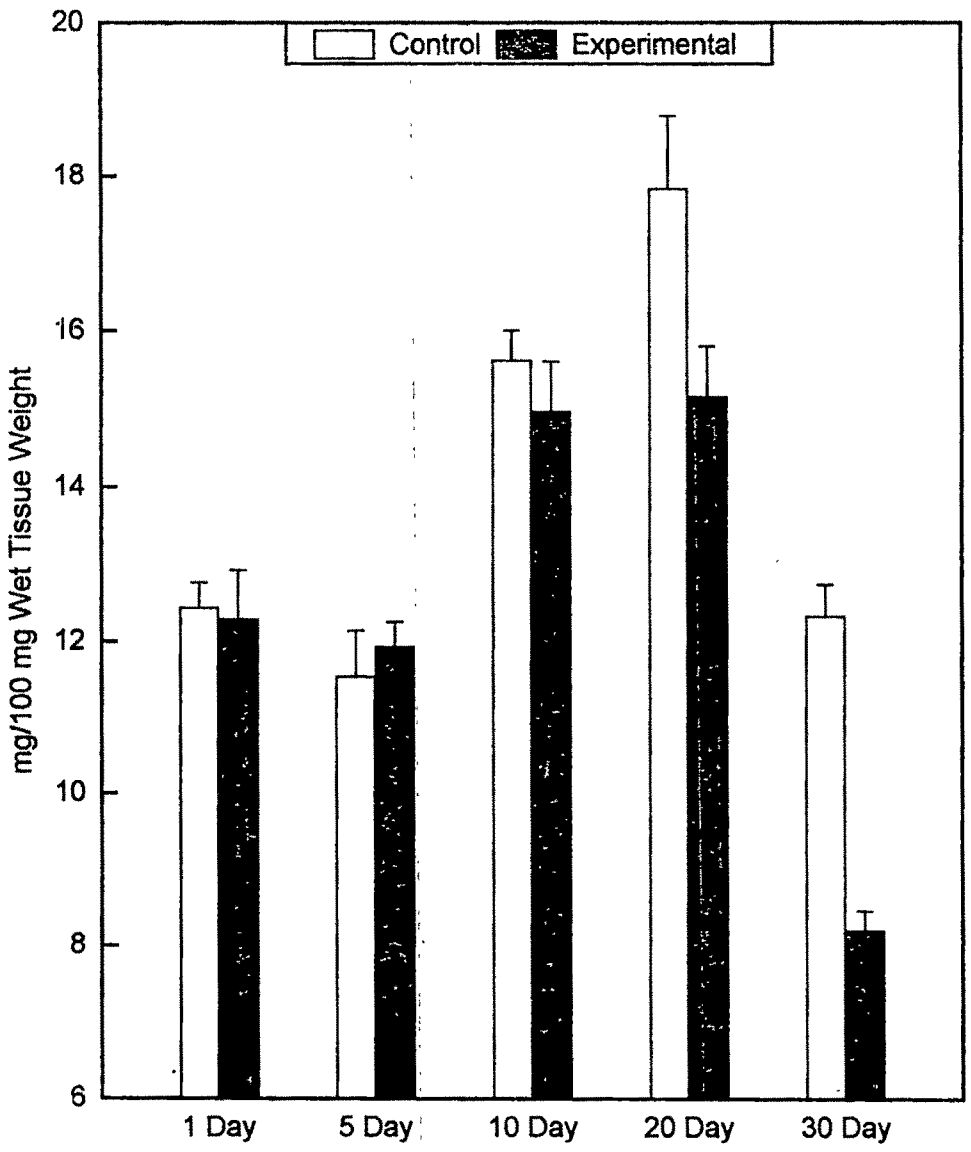


Figure 2. Effect of sodium fluoride on the succinate dehydrogenase activity in glycogen body of growing chicks, *Gallus domesticus*

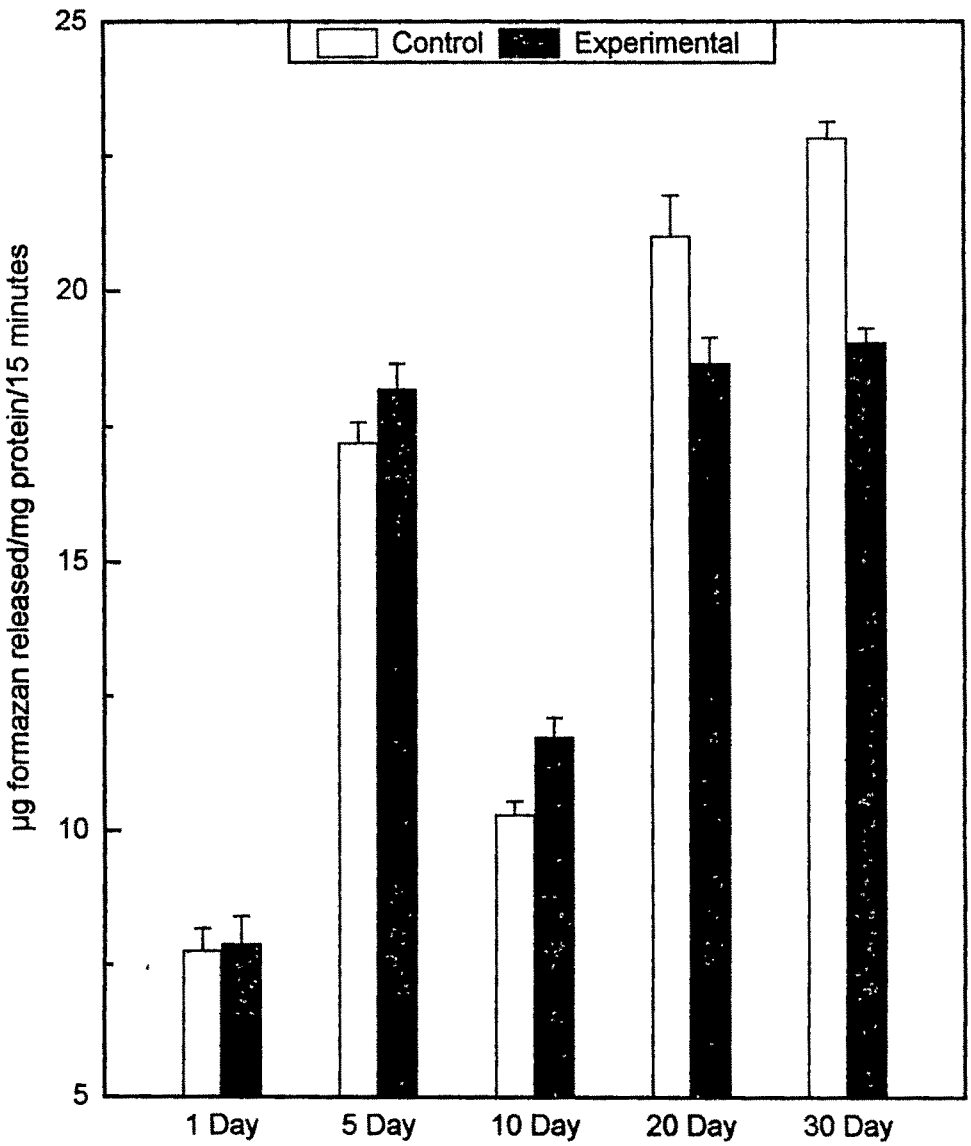


Figure 3. Effect of sodium fluoride on the lactate dehydrogenase activity in glycogen body of growing chicks, *Gallus domesticus*

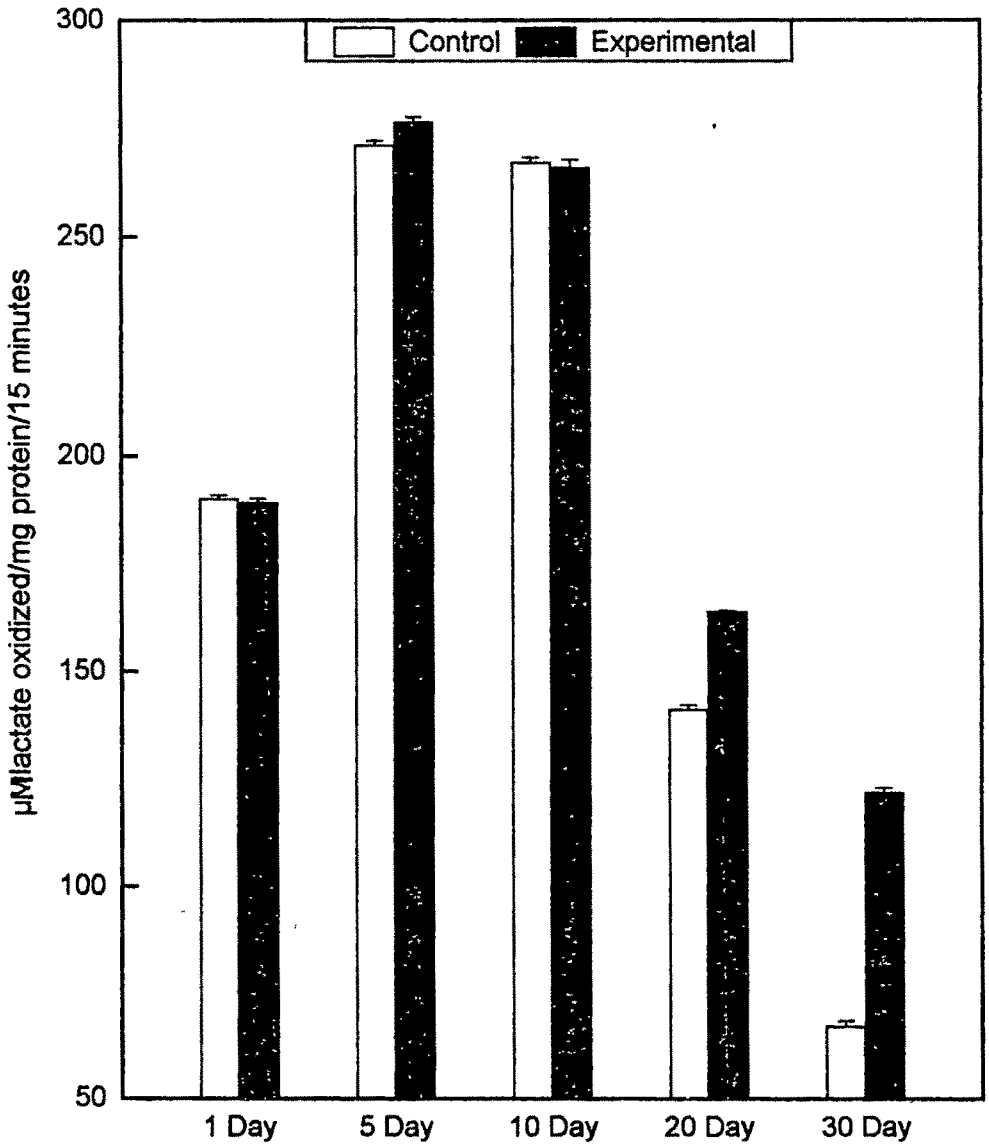


Figure 8. Effect of sodium fluoride on the activity of phosphorylase in glycogen body of growing chicks, *Gallus domesticus*

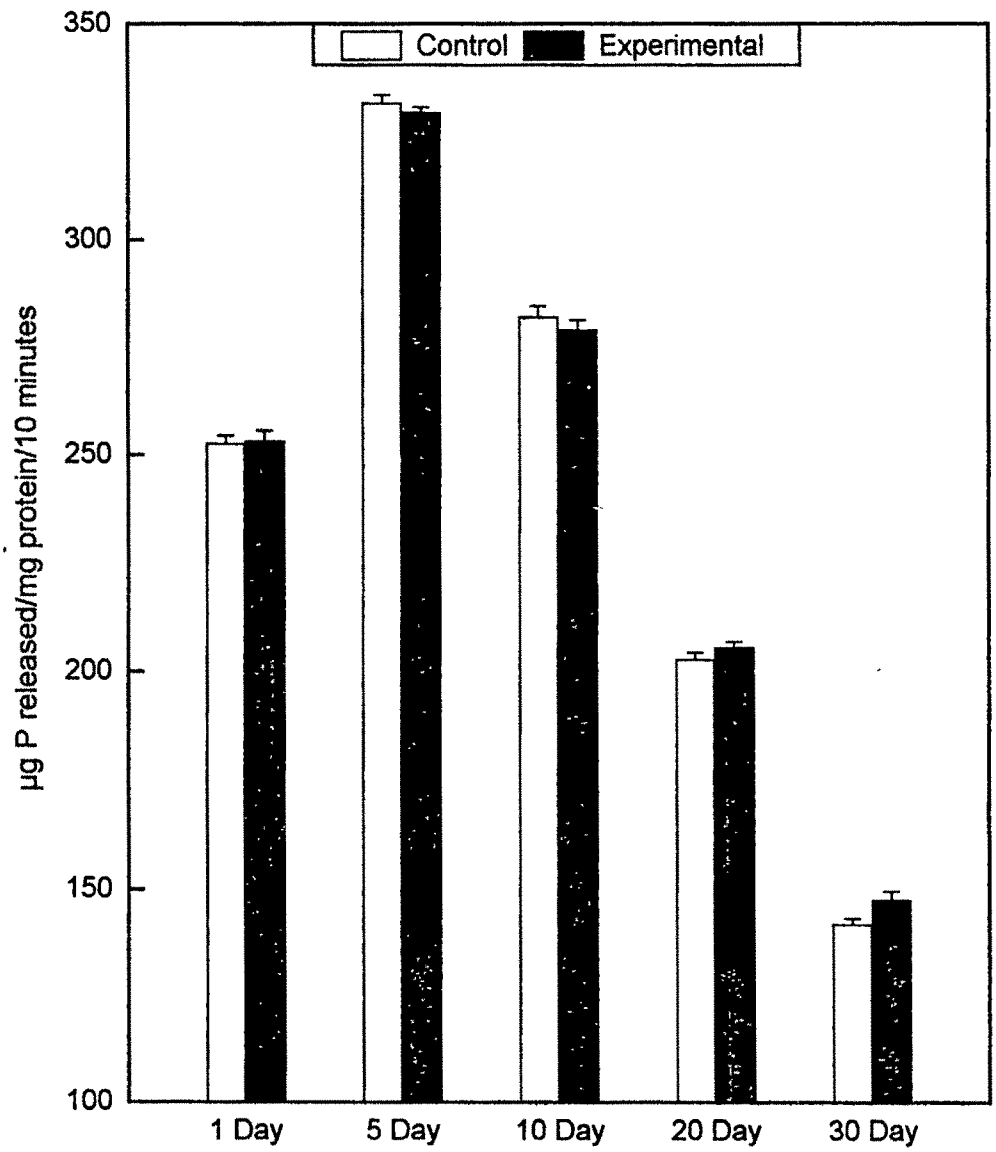


Figure 4. Effect of sodium fluoride on glycogen synthase activity in glycogen body of growing chicks, *Gallus domesticus*

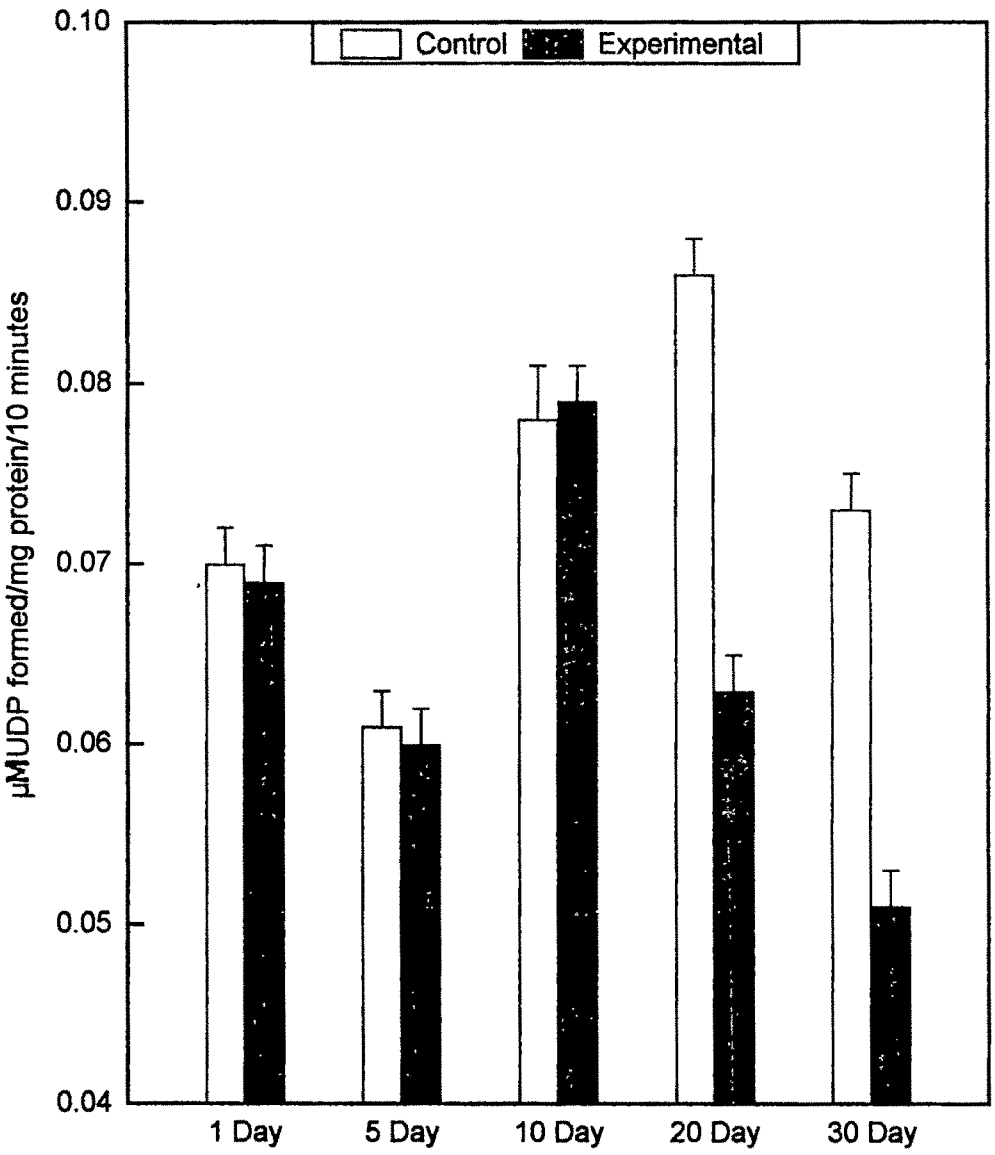


Figure 6. Effect of sodium fluoride on the activity of acid phosphatase in glycogen body of growing chicks, *Gallus domesticus*

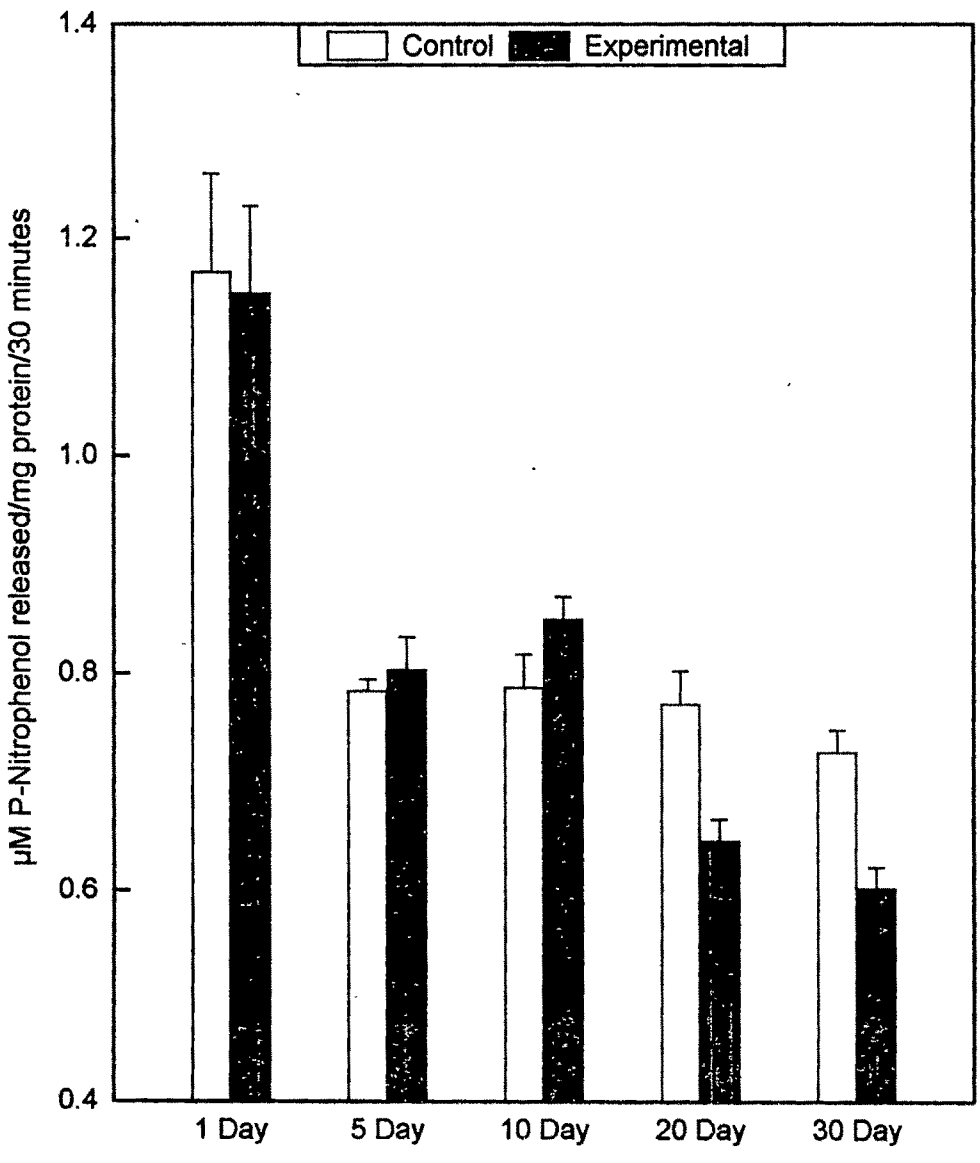


Figure 7. Effect of sodium fluoride on the activity of alkaline phosphatase in glycogen body of growing chicks, *Gallus domesticus*

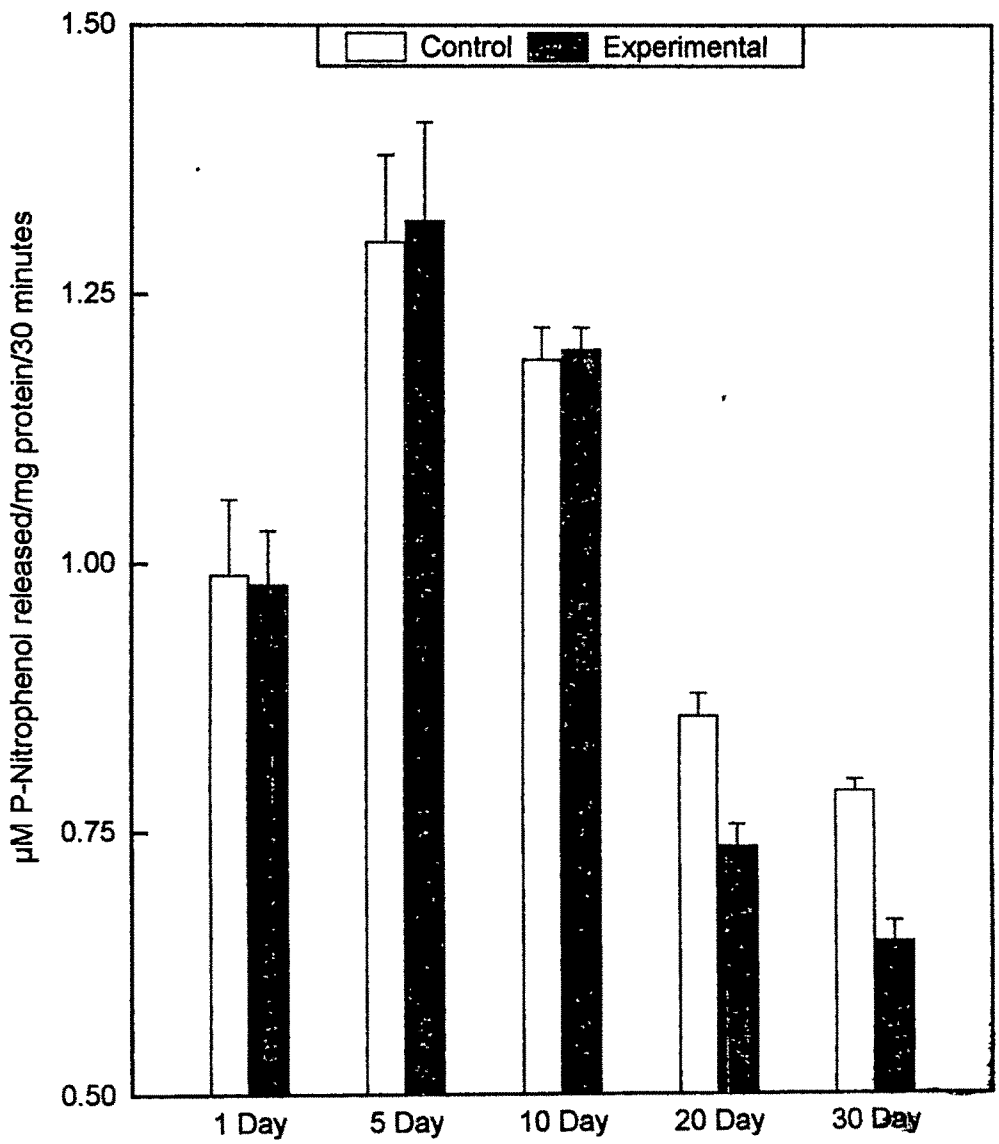
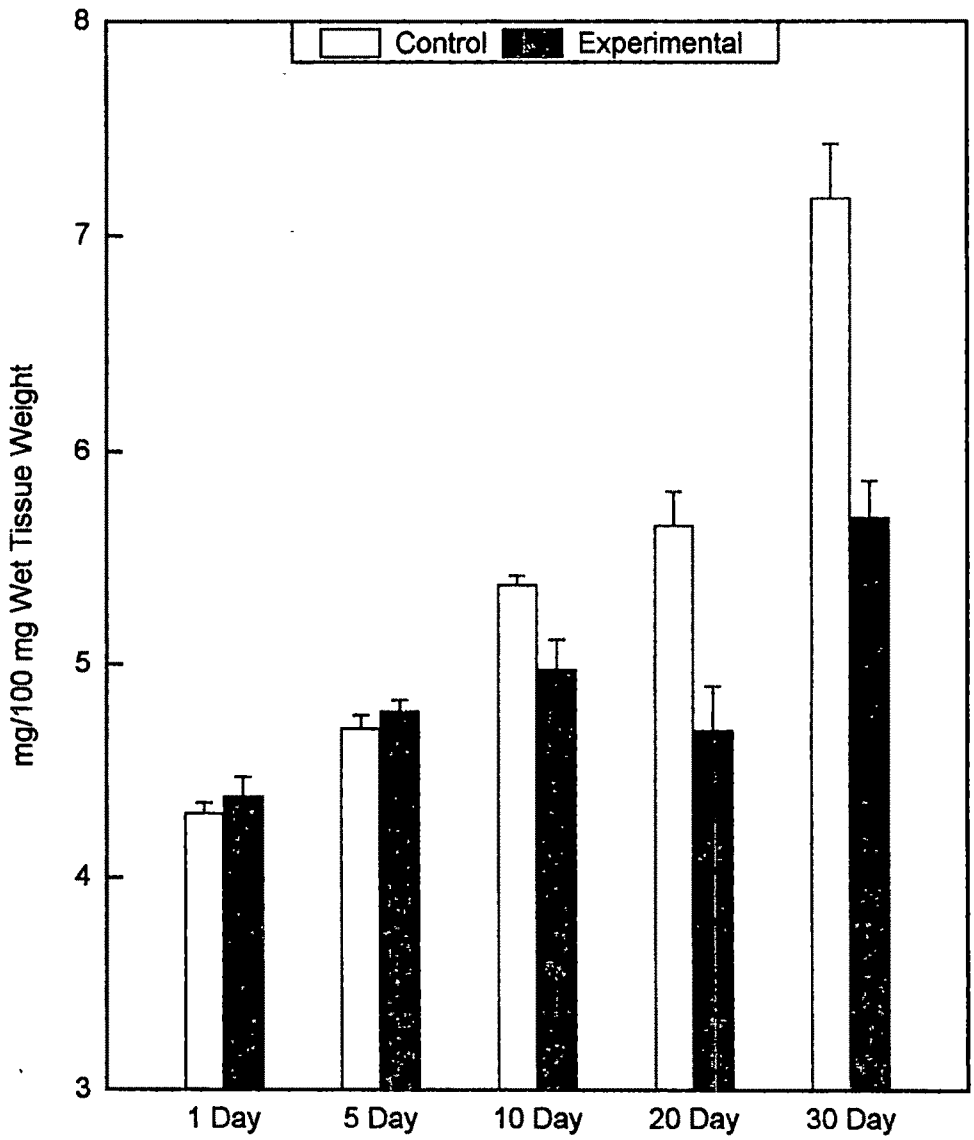


Figure 8. Effect of sodium fluoride on the levels of protein in glycogen body of growing chicks, *Gallus domesticus*



DISCUSSION

Detectable traces of fluoride occur in almost every substance, however, excessive fluoride concentration in the natural environment can be harmful to all forms of life. Fluoride when consumed in excess can cause several ailments besides skeletal and dental fluorosis. It damages skeletal muscles, red blood cells, gastrointestinal system and ligaments in patients with fluorosis (Susheela, 1991).

Fluoride-ion being a protoplasmic poison can only be tolerated in trace amounts by living cells (Pack, 1971). In the present study, fluoride administration caused a significant decrease in glycogen level in glycogen body of post-hatched developing chicks. An important feature of fluroide action is its capacity to activate enzyme system. Fluoride does not have a binding site on the outer side of plasma membrane of living cells (Strochkova and Zhavoronkov, 1983). Fluoride ion is known to interact with many trace metal ions within the cell thus bringing about disturbance in the overall metabolism of the cell (Prasad, 1978). Adenylate cyclase activity has been reported to increase following sodium fluoride administration (Rodriguez-Pena *et al.*, 1991; Boyd, 1992). Due to scarcity of experimental data concerning the effects of fluoride on the nervous tissue, it is difficult to arrive

at conclusion. However, it seems that ingestion of low doses of fluoride stimulate enzyme system by influencing its action on adenylate cyclase (Susheela and Singh, 1982; Strochkova and Zhavoronkov, 1983). Thus with the resultant fluoride induced phosphorylation there would occur an increase in phosphorylase enzyme activity and a decrease in glycogen synthase activity leading to decreased levels of glycogen. Pillai (1983) noted a similar decrease in glycogen level in the fry of a fresh water carp exposed to various concentrations of fluoride. Zebrowski and Suttie(1966) and Tadashi(1958) have shown glycogen content of liver to decrease in fluoride fed rats and rabbits respectively. Chitra and Rao (1980) observed hyperglycemia in *Channa punctatus* following NaF treatment. Similarly, McGown and Suttie (1979) observed marked hyperglycemia following injection of fluoride into the ventricles of the rat brain.

Succinate dehydrogenase activity in fluoride treated chicks decreases significantly. Similar results have been obtained in liver of fluoride exposed mudskipper(Shaikh and Hiradhar, 1986), growing pigs (Seffner *et al.*, 1990) and *ex-ovo* developing chicks (Suresh, 1994). They have attributed this to the disturbance in oxidative metabolism of hepatocytes and their mitochondrial

membranes. Fluoride is known to affect the metabolism of heme. In fishes, mammals and birds decrease in iron content and anemia following fluoride treatment has been reported (Messer *et al.*, 1973; Pillai, 1983; Shaikh and Hiradhar, 1985; and Suresh, 1994). Reduction in glycogen could also be due to an altered heme metabolism as a result of which aerobic oxidation of glycogen cannot occur. This can be evidenced by an increased lactate dehydrogenase activity.

Intake of excessive amounts of fluoride is known to cause changes in serum proteins and serum enzymes in both rats (Riekstneice *et al.*, 1965) and growing chickens (Yu *et al.*, 1980). The changes in serum proteins and enzyme concerntation results from an altered membrane permeability induced by Fluoride-ion. Takagi and Shiraki (1982) showed that acid phosphatase is among the highly impaired enzymes in the early stages of acute toxic NaF nephropathy. Alkaline phosphatase activity is seen to decrease in liver of a number of animal models (Singh, 1984; Kessabi *et al.*, 1985; Sheikh and Hiradhar, 1988; Suresh, 1994) following NaF administration. A similar trend of decreased alkaline phosphatase activity has been noted in the present work. Alkaline phosphatase is known to play an important role in synthetic and transport activities of metabolites across the cell membrane

(Brandes *et al.*, 1962 and Breecher and Tanaka, 1965). In the context of this and other works quoted above it appears that a derangement in transport culminates into depletion in glycogen stores. Since the oxidative metabolism is curbed as evidenced by the decreased SDH activity, it is pertinent that the cells of glycogen body might deviate its metabolic course, possibly towards an accelerated glycolytic pathway. The escalated LDH activity observed in the glycogen body of experimental birds support the present notion of a possible shift in the metabolic machinery towards anaerobic side.