CHAPTER VI

Some Alterations in the Metabolic Profiles of Growing Chick, *Gallus gallus domesticus* Subjected to Chronic Fluoride Poisoning.

Subacute fluoride poisoning is a form of geographical and occupational pathology. Its significance has increased in recent years with the use of fluorine compounds in industry, agriculture, cosmetics and medicine. In addition, fluoridation of public water supplies for minimizing the incidence of dental caries in some western countries has exposed a large section of the population to fluoride, continuously for prolonged periods (Waldbott *et al.*, 1978). Being a cumulative poison, absorption of relatively small quantities of fluoride causes chronic intoxication. It is readily accumulated in osseous tissues and uptake is directly correlated with environmental concentrations and exposure time (Hemens and Warwick, 1971; Moore, 1971; Hemens *et al.*, 1975). Soft tissues like muscle which has high calcium content are also known to be adversely affected by fluoride when ingested for a longer period of time (Kaul and Susheela, 1974; 1976). Apart from this, chronic fluoride intoxication is also known to hamper the adrenal and the pituitary functions (Das and Susheela, 1991,b). Moreover, it has been documented that blood-brain barrier fails to prevent the entry of fluoride (Geeraerts, 1986) and this could lead to nervous dysfunction. Hence, it is apparent that, fluoride when present as a pollutant impinges severe damage to neuroendocrine system.

Homoeostatic regulation of carbohydrate metabolism in the body is controlled by the nervous and endocrine systems. Metabolic homoeostasis is of prime importance, as any alterations in the plasma metabolite level would influence the working of the central nervous system (CNS) and vice versa (Azam *et al.*, 1990). The CNS, hence manipulates the metabolic activities of other tissues/organs, such as muscle and liver, by regulating endocrine system or autonomic nervous system (ANS) to maintain plasma levels of metabolites.

A pollutant like fluoride which hampers the neuroendocrine tone would in turn lead to deranged metabolic activities. Such a change in energy yielding metabolism at the growing stage of an organism might curtail the growth and development of the animal in question. Sublethal dose of fluoride is known to curb the growth and development of the chicks of

domestic fowl (Chapter 3). Therefore, the present study was designed to unveil, how fluoride affects the metabolic machinery, which is subsequently reflected in the progress of growth and development in the chicks of domestic fowl *Gallus gallus domesticus*.

MATERIAL AND METHODS

Day old female Rhode Island Red chicks of domestic fowl *G. g. domesticus* were purchased from Government Poultry Farm, Baroda and were housed in metal cages of suitable size. They received a constant schedule of 14 h light and 10 h darkness and had *ad libitum* access to food and water. The experimental protocol was same as that described in chapter 5 . Six birds each from control and experimental groups were sacrificed by decapitation on days 1, 5, 10, 20 and 30 following fluoride administration. For initial biochemical values, six birds were sacrificed prior to experimentation. A piece of left lobe of liver and gastrocnemius muscle were quickly excised and used for the determination of principal metabolite quantities and assays of certain related enzymes.

Analytical Methods

Glucose: Blood was drawn as described in chapter 5 and centrifuged to collect the plasma. Glucose was estimated by the o-toluidine method of Winkers and Jacob (1971).

Glycogen: Known amount of liver and muscle were used for the estimation of glycogen (Seifter *et al.*, 1950) by using anthrone reagent.

Lipid: The total lipids of the liver were estimated using the method of Folch *et al.* (1957) by extracting the lipid from a known quantity of dried tissue with chloroform:methanol (2:1) mixture and then measured gravimetrically.

Protein: Total proteins in the liver and muscle were estimated using the method of Lowry *et al.* (1951).

Phosphorylase (EC 2.4.1.1): Total phosphorylase in the liver and muscle were assayed by the modified mehtod of Cori *et al.* (1943) as adopted by Cahill *et al.* (1957). Inorganic phosphate was estimated by the method of Fiske and SubbaRow (1925).

Glycogen synthetase (GS; EC 2.4.1.11): GS activity was measured according to the method of Leloir and Goldemberg (1962).

Succinate dehydrogenase (SDH; EC 1.3.99.1): Known quantity of liver and muscle were used for the estimation of SDH activity (Nachlas *et al.*, 1960).

Lactate dehydrogenase (LDH; EC 1.1.1.27): LDH activity of the liver and muscle were assayed by using the method of King (1959,1965c) as described by Varley *et al.* (1980).

Statistical Analysis

Data were analysed statistically using student's `t' test. Results are expressed as mean $\pm$ SEM. Statistical significance was defined as a p value ≤ 0.05.

RESULTS

Glucose

Glucose level in the blood plasma of control chicks registered a chronological increase till the termination of experiment, except on day 10 where a slight but definite decrease was observed. Although the average glucose level in the treated birds followed a similar course, compared to their controls, an increase in basal glucose value was noticed on day 10 of fluoride administration. The experimental birds than remained in the hyperglycaemic state till

the end of experimental regimen (Table I, Figure 1).

Glycogen

Liver: From figure 2 it is clear that glycogen content in the liver of control birds gradually declined till day 5 of experiment. A sudden rise in glycogen level was observed on day 10, which is followed by a sharp fall on day 20. By the end of experiment liver glycogen value again increased. However, compared to controls the glycogen content in the liver of experimental birds registered a drop on day 10. Further reduction in glycogen content was observed on 20 and 30 days of fluoride treatment.

Muscle: Fluoride administration did not alter the glycogen level in the gastrocnemius muscle of growing chicks till day 5 of experiment. Further fluoride intoxication led to decline in muscle glycogen level (Figure 3).

Glycogen synthetase

Liver: The mean GS activity in the liver of control birds fluctuated at different stages of experiment (Table I). The highest value of GS was observed on day 20 of experiment and the least value, on day old untreated birds. The negative impact of fluoride on GS activity was obvious on day 10 of experiment. Thereafter, compared to controls, the average GS activity in the experimental birds remained at a lower level (Figure 4).

Muscle: In contrast to that of liver, the GS activity in the muscle of growing chicks increased steadily till the termination of experiment (Table 2). This is in concordance with the gradual increase in glycogen content in the muscle of growing chicks. However, compared to control birds the GS activity in the gastrocnemius muscle of experimental birds registered a significant decline on day 10 of fluoride administration. Further treatment with fluoride led to more pronounced reduction in GS activity (Figure 5).

Total phosphorylase

Liver: As compared to control chicks, a significant ($P < 0.05$) increase in liver phosphorylase activity was noticed in chicks which received subacute fluoride for 20 days. The difference in phosphorylase activity was more evident on day 30, where the experimental birds registered a hike of 25% phosphorylase activity compared to that of control birds (Figure 6).

Muscle: Phosphorylase activity in the muscle of fluoride treated growing chicks also showed signs of increase by day 20 of experiment. However, the difference in phosphorylase activity between control and experimental birds was more striking in the last phase of fluoride administration (Figure 7).

Succinate dehydrogenase

Liver: Administration of sublethal dose of fluoride has reduced the activity of SDH, in the liver of growing chicks by day 10 of treatment. Appreciable reduction in SDH activity was recorded in the experimental birds on day 20 and 30 of fluoride administration (Figure 8).

Muscle: In the gastrocnemius muscle of growing chicks (control) the SDH activity remained almost constant throughout the experimental regimen. However, compared to controls the SDH activity in the muscles of experimental birds declined towards the end of the experiment (Figure 9).

Lactate dehydrogenase

Liver: From figure 10 it is evident that the mean LDH activity in the liver of control birds increased till day 5 of experiment, thereafter, a steady decline in LDH activity was noticed till the end of the experiment. The average LDH activity in the fluoride intoxicated birds too,

TABLE I : Effect of NaF on plasma glucose, glycogen, lipid and enzymes related to carbohydrate metabolism in chick liver.

Parameters studied	Duration of Treatment (Days)										
	0	1		5		10		20		30	
		Con.	Exp.	Con.	Exp.	Con	Exp.	Con	Exp	Con.	Exp
Plasma Glucose (mg/100 ml)	202.50± @ 2 16	242.00± 2 65	242.16± 3.21	298.83± 4.53	295.50± 6.22	276.17± 3 16	**** 311.17± 4.83 ↑	303.83± 3.53	**** 372.50± 3.06 ↑	321.84± 2.36	**** 392.73± 5.74 ↑
Glycogen (mg/100 mg Wet Tissue)	5 34±0.22	5.08± 0.23	4.94± 0.29	3.72± 0.07	3.79± 0.13	5.35± 0.05	*** 4.45± 0.27↓	3.25± 0.06	**** 1.98± 0.05 ↓	4.41± 0.28	*** 2.27± 0 31↓
Lipid (mg/100 mgNFDT)	55 40±1.61	51.50± 1.52	53 10± 2.11	38.11± 1.47	36.32± 1.93	28.37± 0.81	27 13± 1.13	29.13± 0.71	* 32.41± 0.93 ↑	26.40± 1.13	** 33.28± 1.71 ↑
Glycogen Synthetase (μM UDP Formed/mg Pro/ 10 mts)	0 042± 0.001	0 057± 0.001	0.055± 0.001	0 049± 0.0008	0.050± 0 0013	0.062± 0 0008	**** 0.055± 0 0013↓	0.072± 0.0009	**** 0.049 ± 0.0017↓	0.066± 0.0028	**** 0.041 ± 0 0009↓
Phosphorylase (μg P Released/ mg protein/10 mn)	66 27±1 01	56.02± 1.02	55.15± 0 60	52 16± 0.49	51.30± 1.10	50.30± 0.66	50.96± 1.26	56.95± 0.82	* 60.20± 1.10 ↑	47.64± 0.98	*** 52.86± 1.31 ↑
SDH (μg Formazan Formed/ mg Pro/10 mts)	29 33±0.95	21.29± 1.00	20.52± 1 32	25 62± 1 03	25.36± 1 22	38.35± 0.74	**** 23.11± 1 01 ↓	39.83± 0 60	**** 24.39± 1.06 ↓	47 17± 1 01	**** 37.91± 1.03 ↓
LDH (μM Lactate Oxidised/ mgPro/15 mts)	46 45±3.16	54 28± 2 21	54.71± 2.44	76.42± 3.15	78.89± 3.43	72.37± 2.11	71.52± 3.01	55.25± 2.21	* 62.62± 2.68 ↑	36.24± 3.03	**** 53.57± 2.33 ↑

@ Values are expressed as mean ± SEM of six experiments. * $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$; **** $p < 0.001$

TABLE II : Effect of NaF on glycogen content and enzymes related to carbohydrate metabolism in the gastrocnemius muscle of growing chick.

Parameters studied	Duration of Treatment (Days)										
	0	1		5		10		20		30	
		Con	Exp.	Con	Exp	Con	Exp.	Con.	Exp	Con.	Exp
Glycogen (mg/100 mg Wet Tissue)	0.243±[@] 0.013	0.231± 0 012	0.227± 0.012	0 291± 0.013	0 285± 0.013	0.644± 0 013	**** 0.612± 0.013↓	0.636± 0 013	**** 0.423± 0.016↓	1.419± 0 019	**** 0.956± 0.016↓
Glycogen Synthetase (μM UDP Formed/mg Pro/10 Mts)	0.031± 0.001	0.038± 0 001	0 038± 0.002	0.041± 0.001	0.040± 0.002	0.0 64± 0.001	* 0 060± 0.001↓	0.0 66 ± 0.001	**** 0.052± 0.002↓	0.071± 0.002	**** 0.041± 0.002↓
Phosphorylase (μg P Released/mg Pro/10 Mts)	177.67± 4 33	191.87± 4 19	186.65± 5.79	221.31± 6 19	222.17± 6.09	131.63± 4.24	127.87± 5.31	150.71 ± 4.39	* 162.67± 5.59 ↑	143.29± 3 18	*** 160.50± 5.94 ↑
SDH (μg Formazan formed/mg pro/ 15 mts.)	19.31±0.90	18 56± 0 80	18.61± 1.33	22.31± 1.37	21.58± 1.66	18.53± 1.18	18.52± 1.42	24.43± 1.11	* 19.84± 1.51 ↓	23.43± 0.98	**** 17.37± 1.64 ↓
LDH (μM Lactate oxidised/mg Pro/ 15 mts)	47 96±0 93	61.51± 1.20	59.13± 1.45	89 91± 2.72	88.59± 2.56	125.82± 2.70	125.71 3.47	73 34± 2 42	**** 98.97± 3.71 ↑	54.65± 1.89	**** 108.93± 3.12 ↑

@ Values are expressed as mean ± SEM of six experiments. * $p < 0.05$; *** $p < 0.01$; **** $p < 0.001$

Fig. 1.Plasma Glucose Level

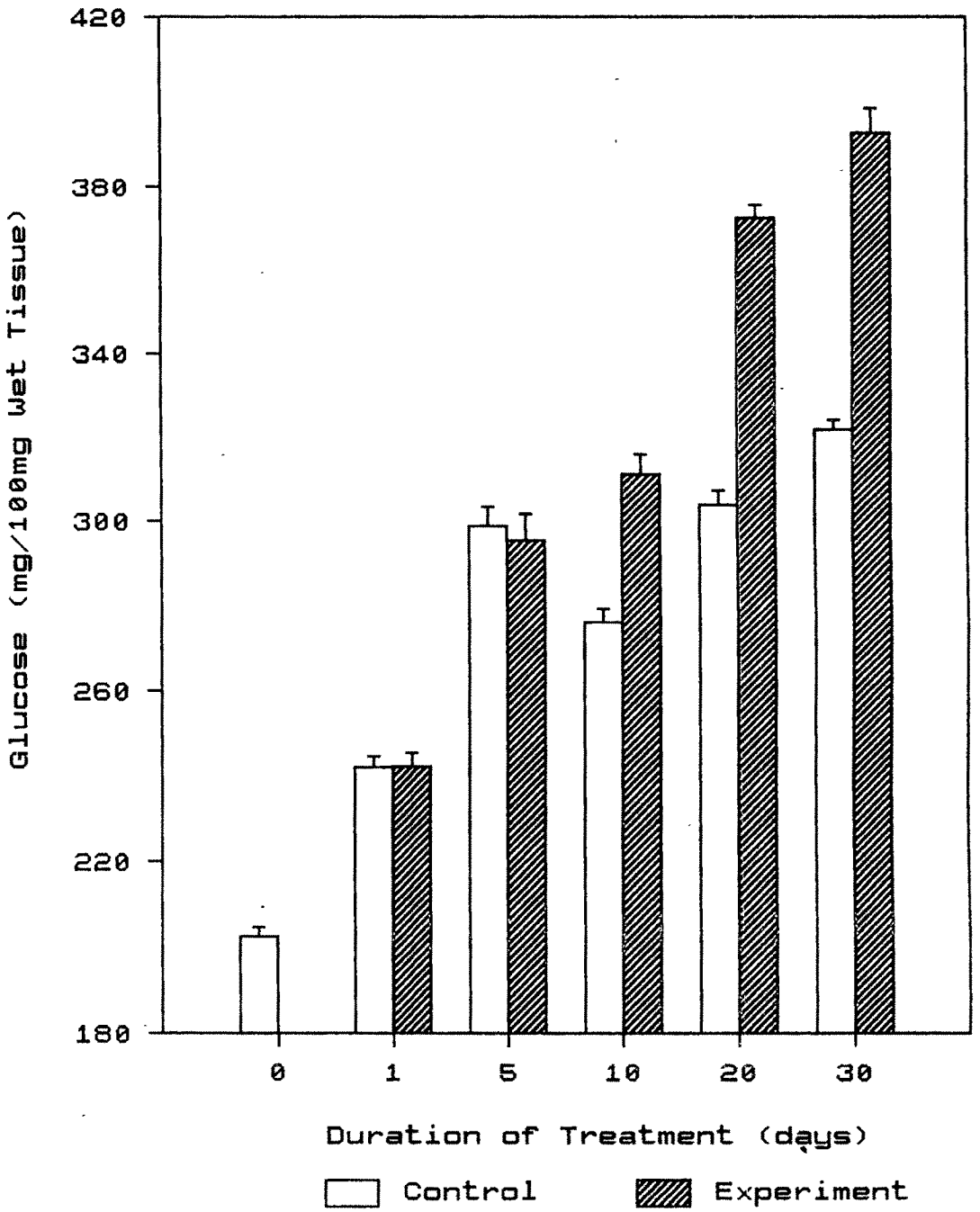


Fig. 2. Glycogen Content in Liver

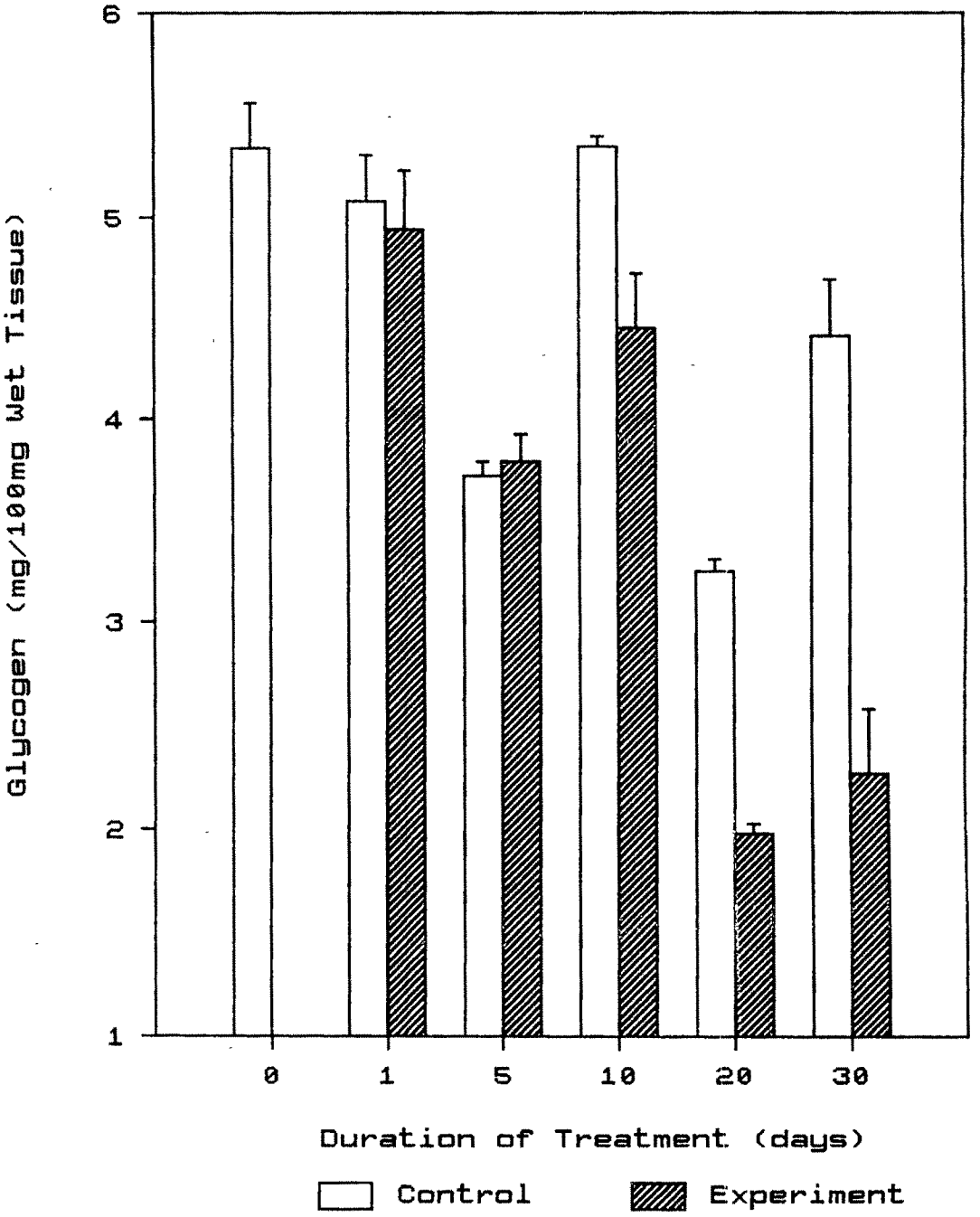


Fig. 3. Glycogen Content in Muscle

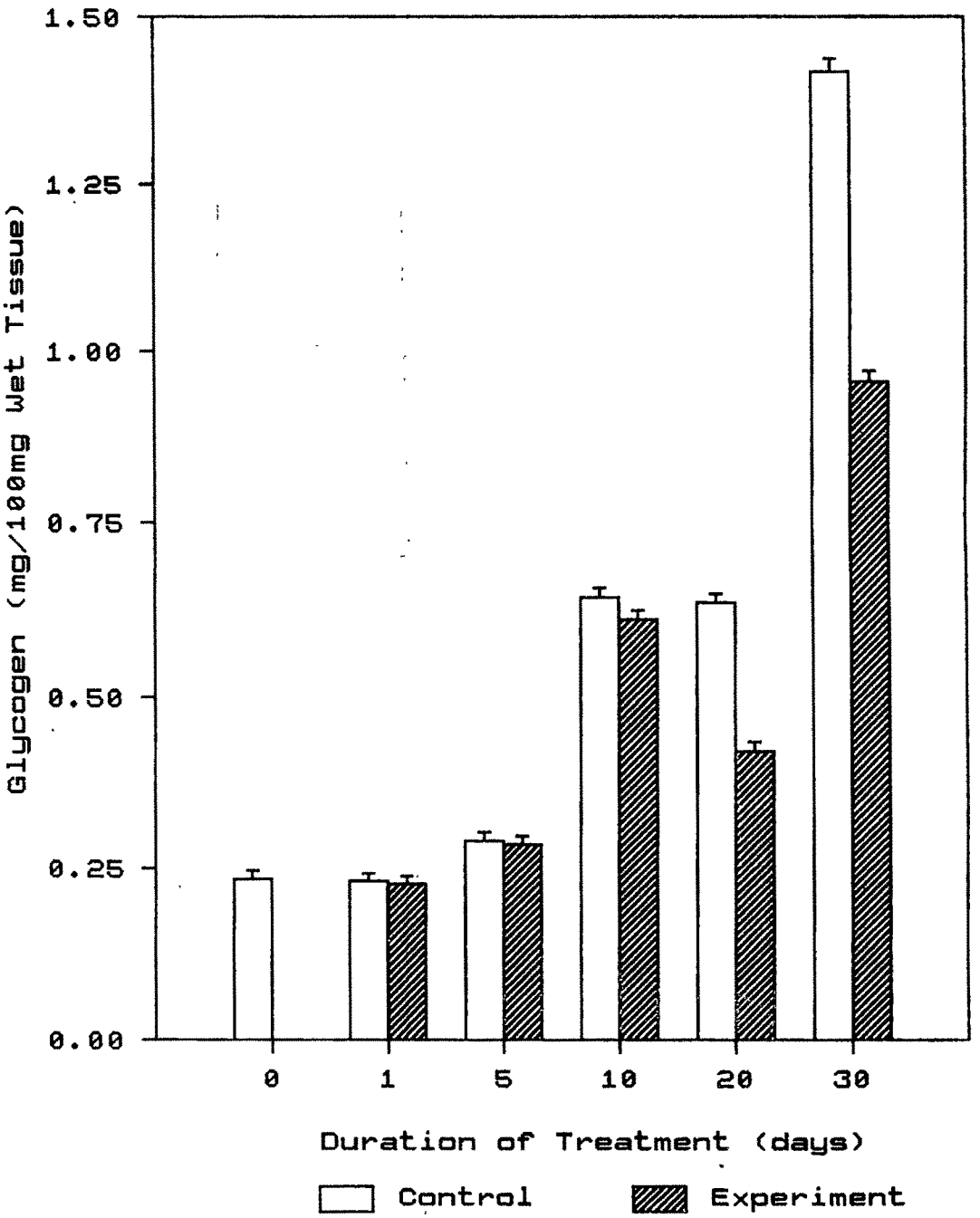


Fig. 4. Glycogen Synthetase Activity in Liver

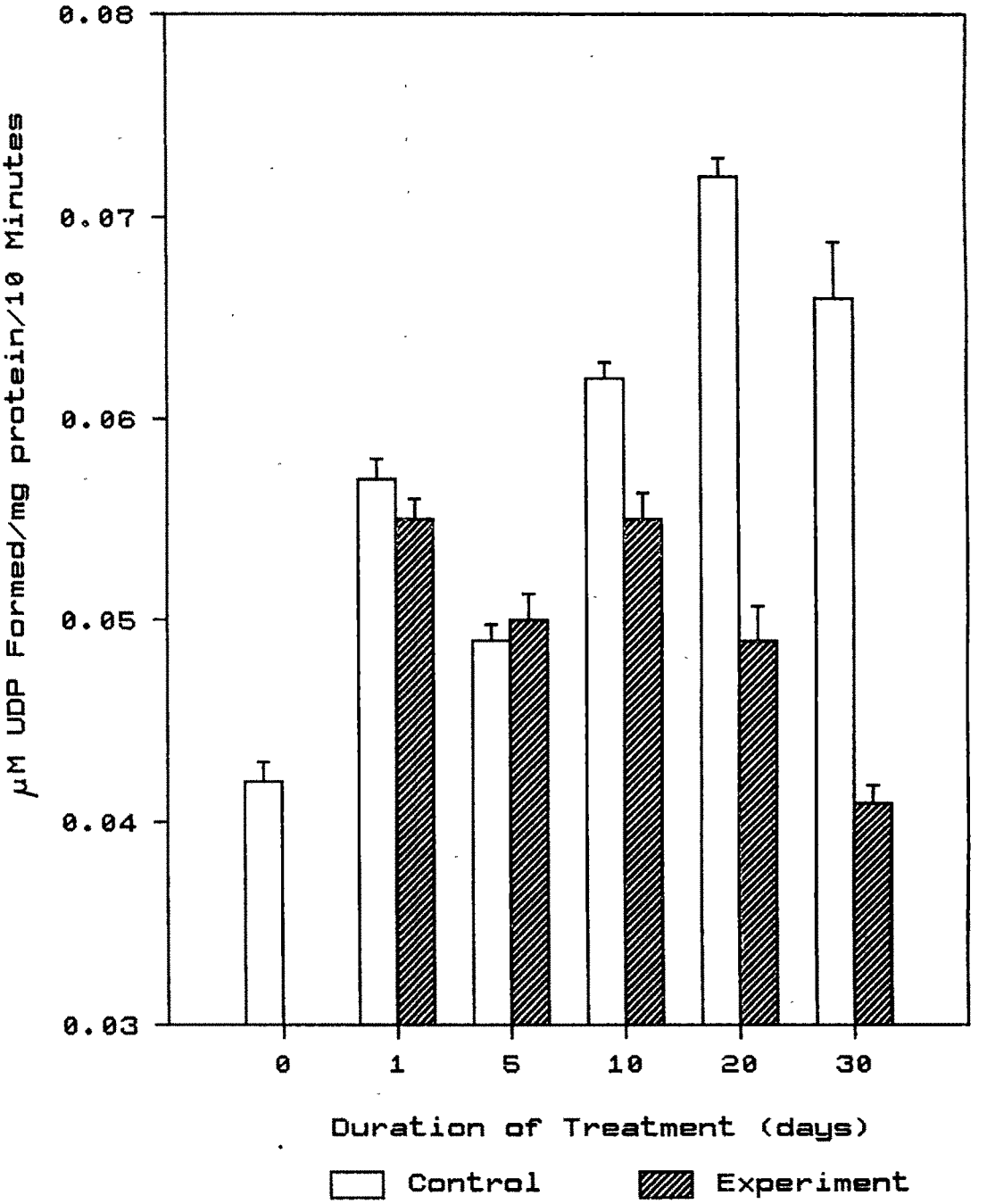


Fig. 5. Glycogen Synthetase Activity in Muscle

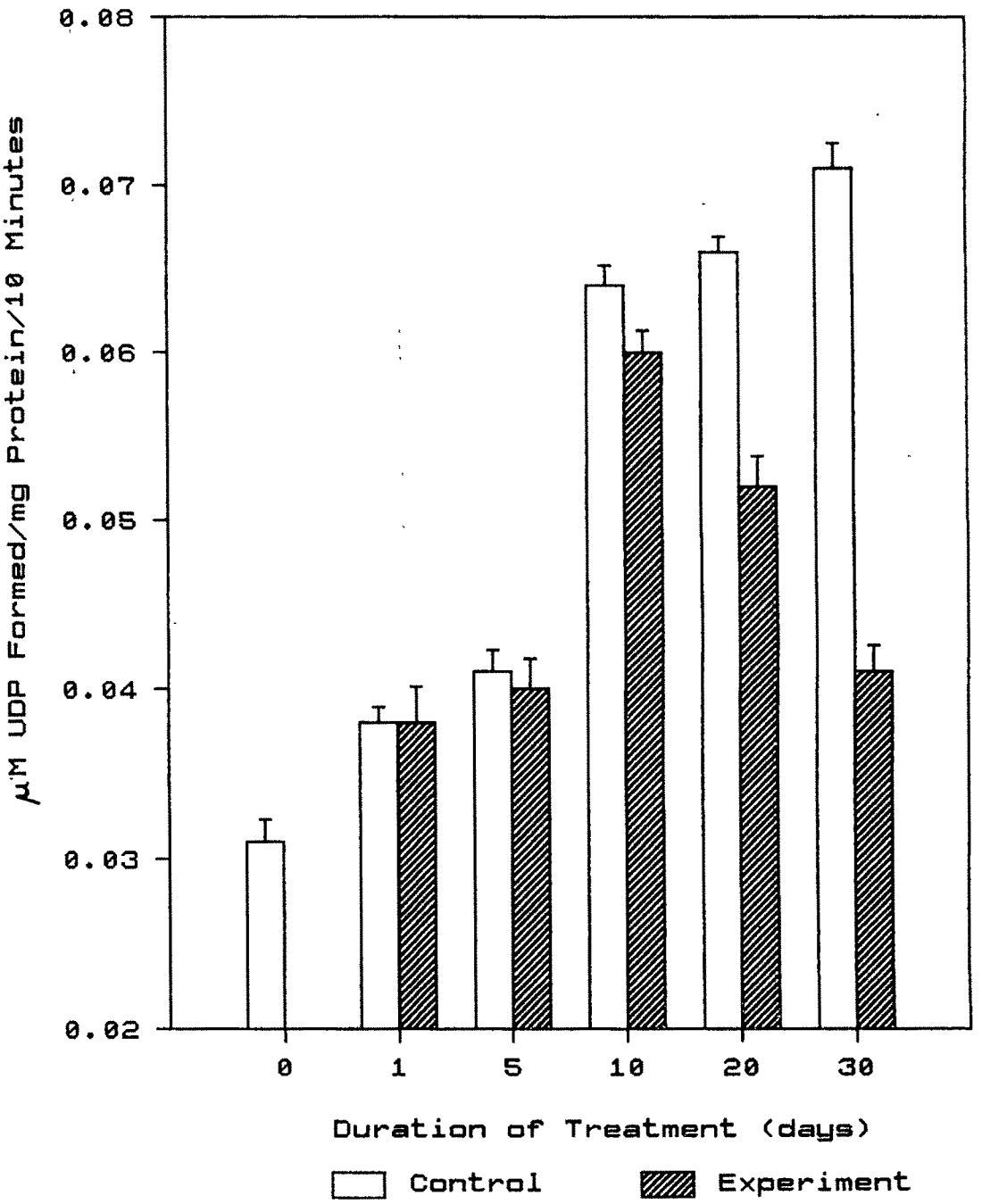


Fig. 6. Phosphorylase Activity in Liver

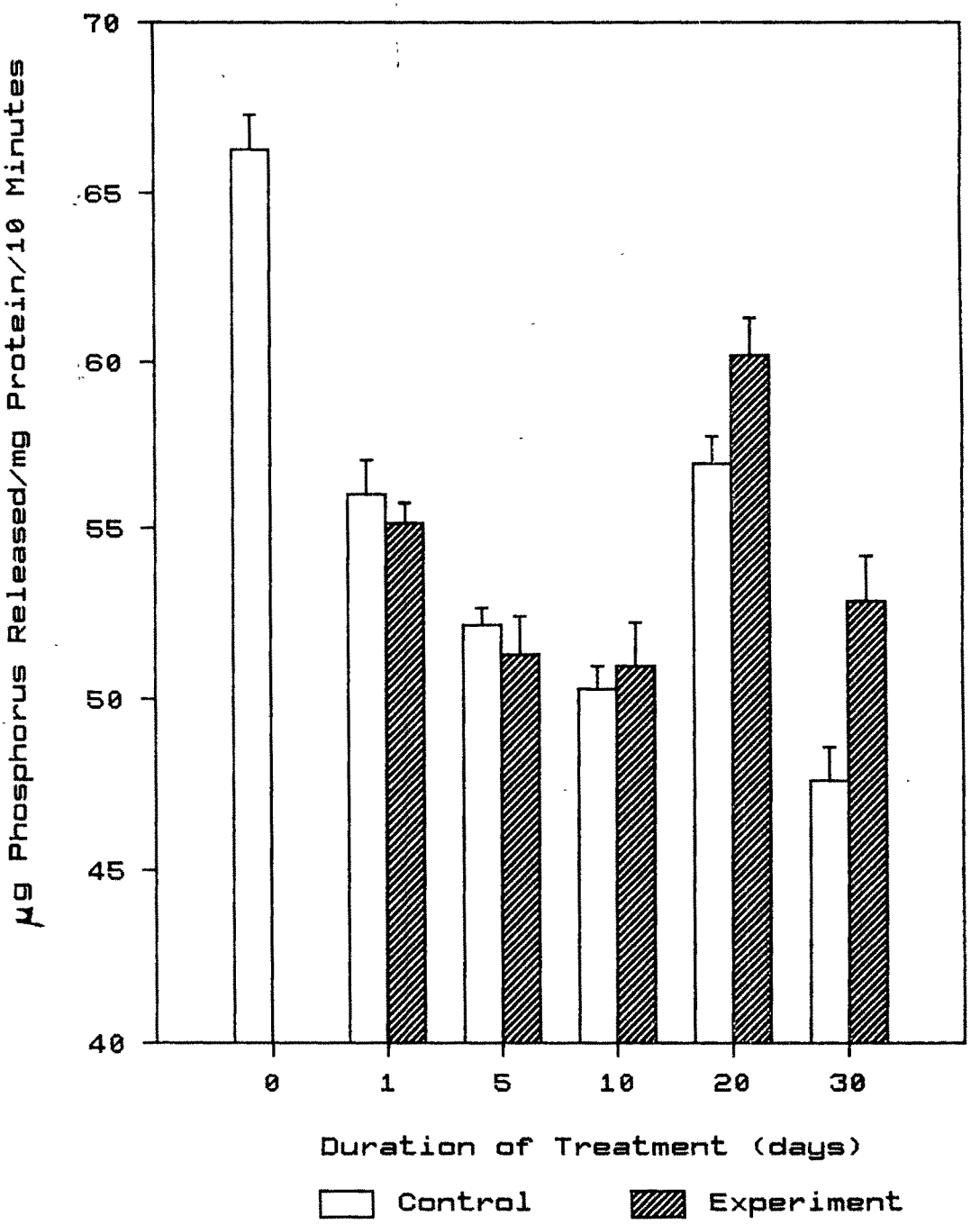


Fig. 7. Phosphorylase Activity in Muscle

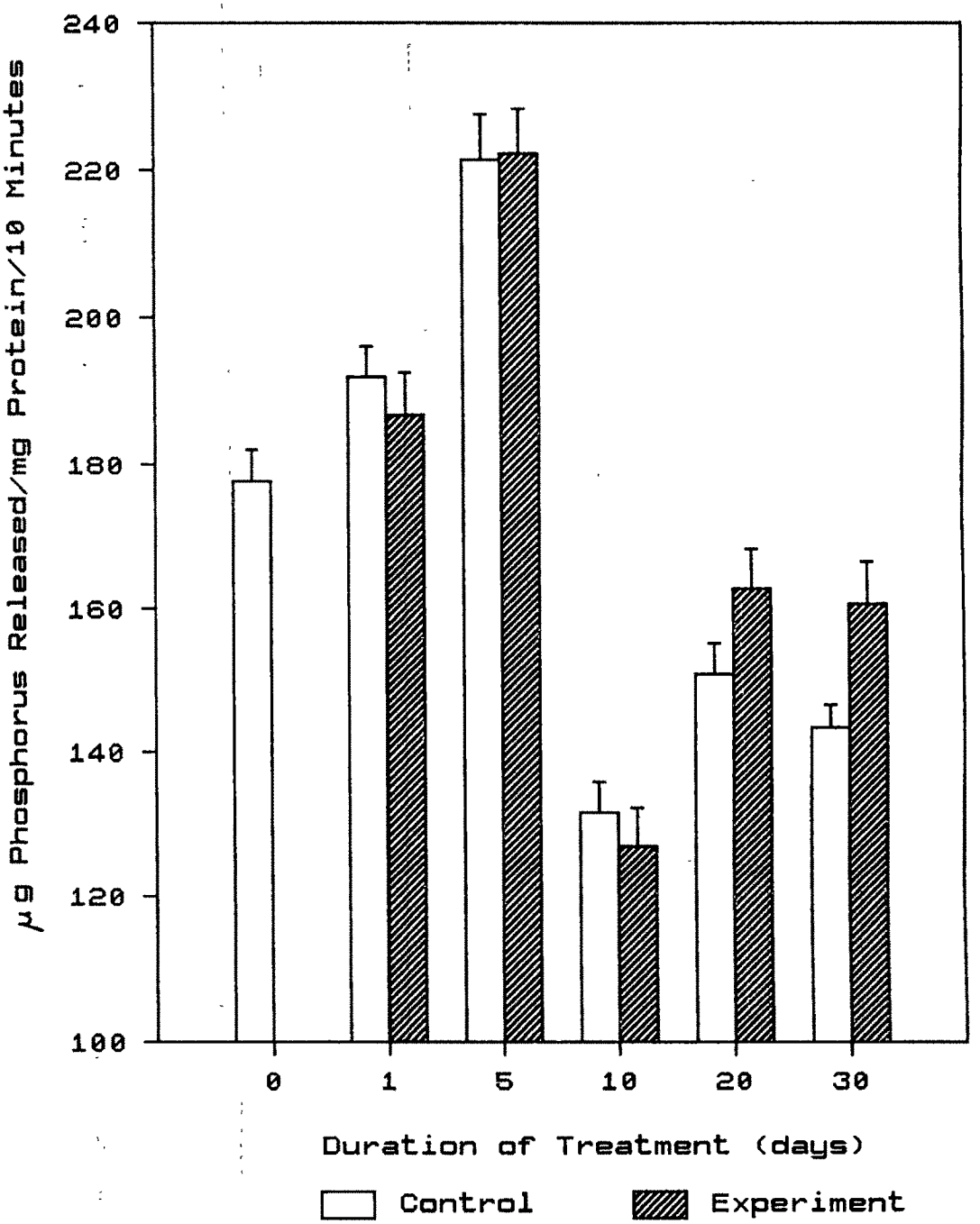


Fig. 8. Succinate Dehydrogenase Activity in Liver

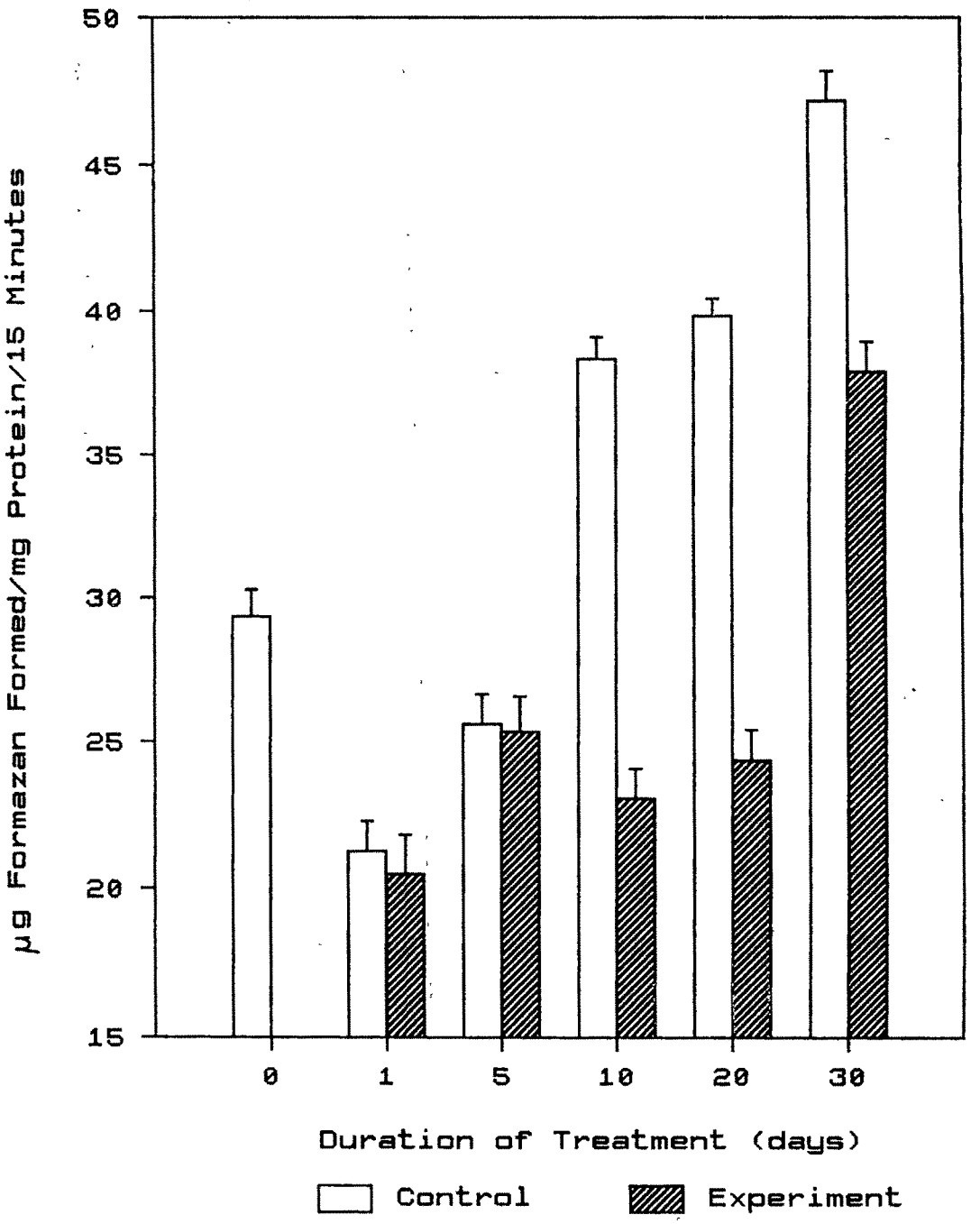


Fig. 9. Succinate Dehydrogenase Activity in Muscle

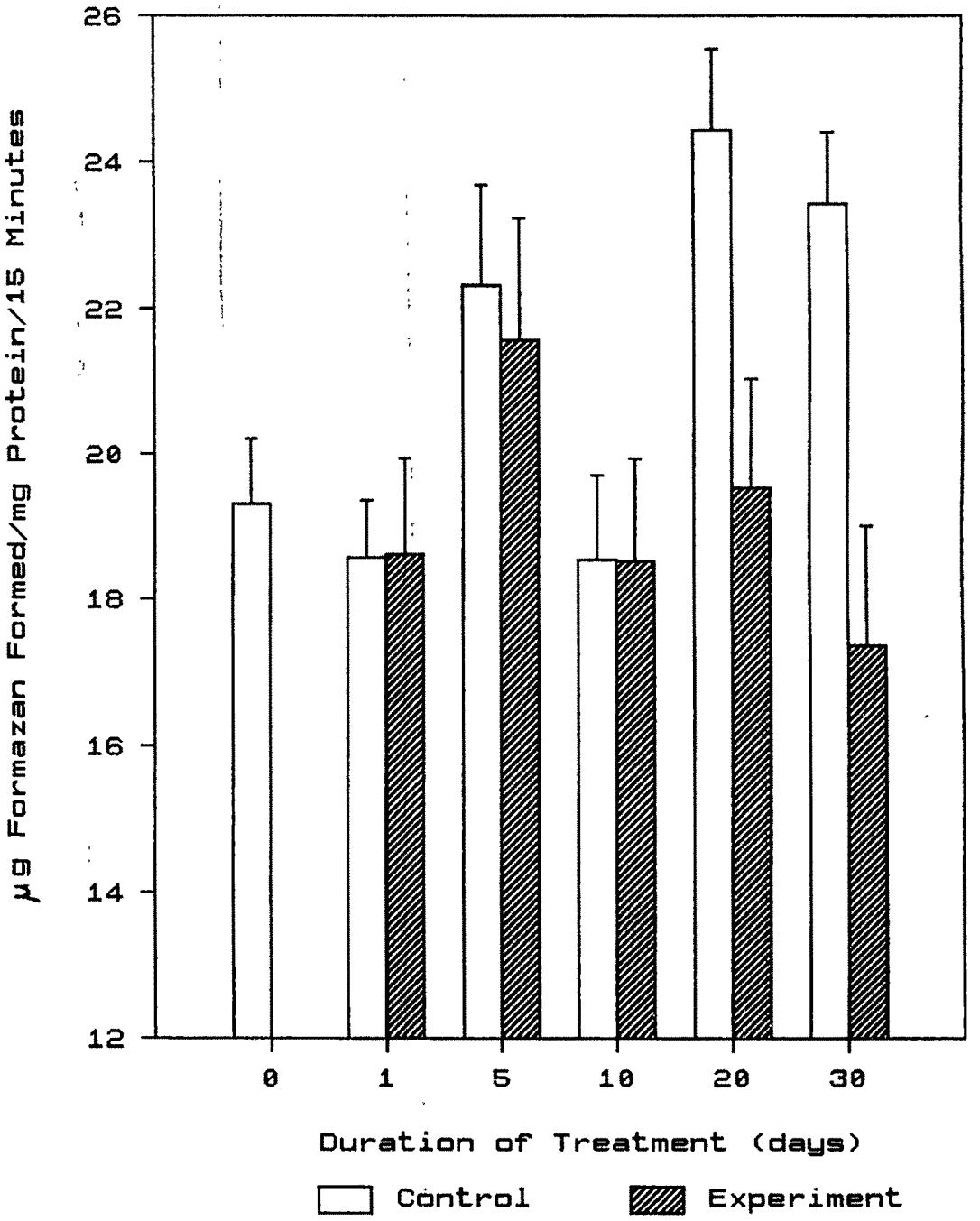


Fig. 10. Lactate Dehydrogenase Activity in Liver

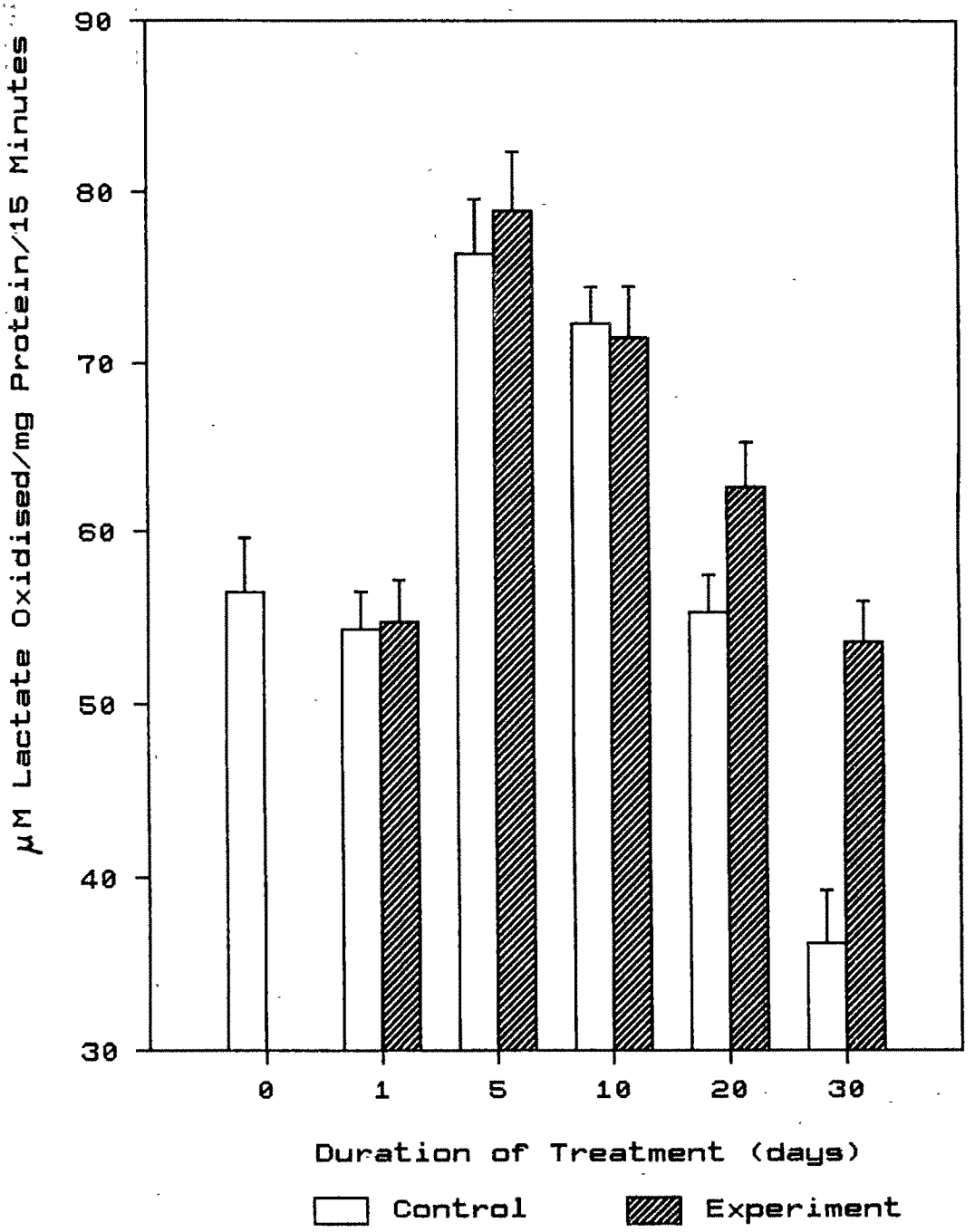


Fig. 11. Lactate Dehydrogenase Activity in Muscle

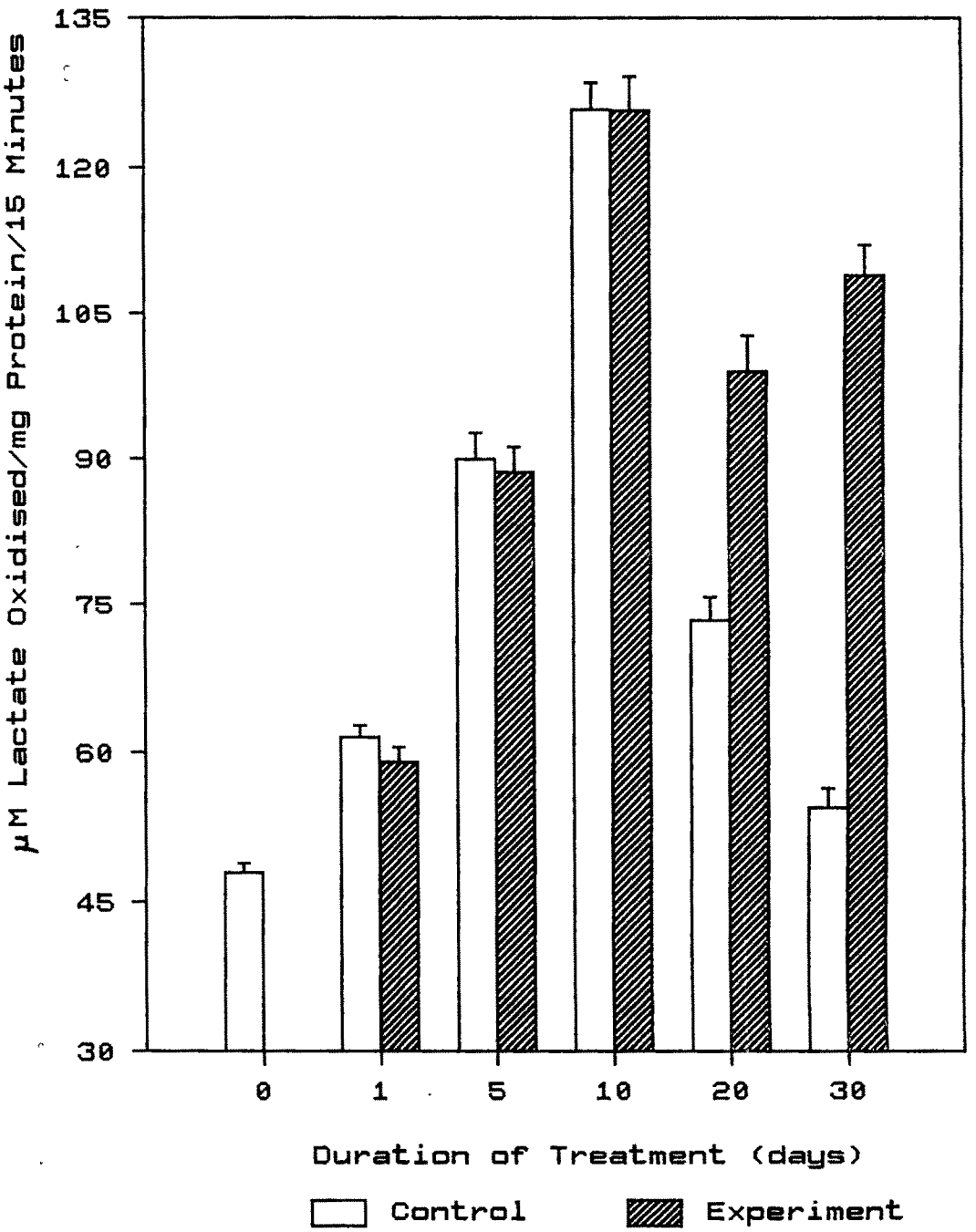
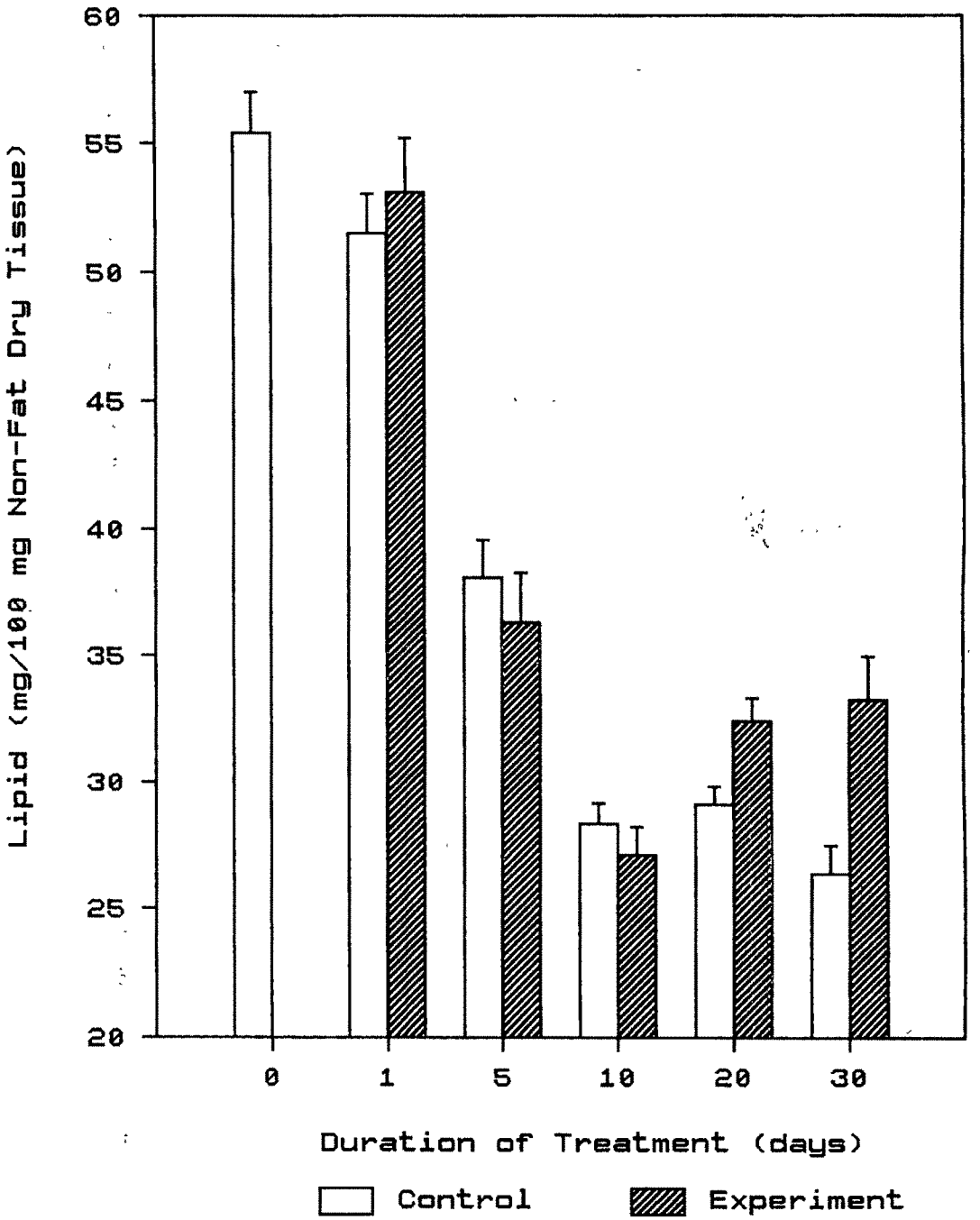


Fig.12. Lipid Content in Liver



followed the same pattern. However, compared to controls LDH activity in the liver of experimental birds registered a hike on both 20 and 30 days of fluoride intoxication. .pa

Muscle: Chronic fluoride poisoning led to accelerated LDH activity in the muscles of growing chicks. Significant increase in LDH activity was observed in the experimental birds on day 20 and 30 (Figure 11).

Total lipids

Chicks subjected to sublethal dose of sodium fluoride started accumulating lipids in the liver by day 20 of experiment. Further increase in the lipid content was observed in the liver of experimental birds with prolonged fluoride treatment (Figure 12).

DISCUSSION

The present findings are amenable to previous observations that chronic fluoride administration leads to hyperglycaemia (Chitra and Rao, 1981; Shaikh and Hiradhar, 1985; Suresh and Hiradhar, 1990b). In addition, glycogen content in the liver and muscle of experimental birds showed a concomitant decrease. The observed hike in plasma glucose level is most unlikely due to stress induced adrenocorticoid hormone(s). Hyperactivity of adrenal cortex is usually followed by a concomitant increase in erythrocyte population. However, it is clear from chapter 5 that fluoride administration led to erythropenia. Moreover, long-term fluoride administration has been known to cause adrenal insufficiency (Rao and Susheela, 1979; Li *et al.*, 1990; Das and Susheela, 1991,a). A secondary adrenal hypofunction due to suppressed ACTH release system, has also been reported in rabbits subjected to sublethal dose of sodium fluoride (Das and Susheela, 1991b). This along with the observed decline in AChE activity in different tissues of fluoride intoxicated chicks (Chapter 7) prompted one to think about fluoride induced parasympathetic neuropathy. Parasympathetic nervous system

(PNS), a branch of ANS, is known to be involved in several direct and indirect glucoregulatory processes, such as, glycogen deposition in tissues, promotion of insulin secretion from B cells and inhibition of A cells of pancreatic islets as well as signalling the glycaemic state of the liver to hypothalamus (Lautt, 1980; 1983; Shimazu, 1983; Rohner-Jeanrenaud *et al.*, 1983). A disturbance in parasympathetic activity could result in an increased sympathetic tone. Such adrenergic activation may cause a direct stimulation of hepatic glucose output, release of glucagon (Gerich *et al.*, 1976; Miller, 1981), as well as reduced insulin secretion from the pancreas (Robertson *et al.*, 1976; Fujimoto *et al.*, 1981; Broadstone *et al.*, 1987; Kuhn *et al.*, 1987), and restrained peripheral glucose utilization (Ahlborg *et al.*, 1974; Galbo *et al.*, 1977; Hoelzer *et al.*, 1986; Ahren *et al.*, 1987). Parasympathetic neuropathy with attendant reduction in insulin level may also help to mediate the stimulation of hepatic glucose output, in part, by sensitizing the liver to basal levels of glucagon and epinephrine (Felig and Wahren, 1971; 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 inhibition of acetylcholine release, resulting in an impairment in glucose uptake mechanism.

This observation is supported by the fact that the activity of glycogen synthetase, the rate limiting enzyme involved in converting glucose-6-phosphate to glycogen showed a parallel decrease in both liver and muscle of fluoride treated chicks. Insulin is able to activate glycogen synthetase in rat hepatocytes (Apkan *et al.*, 1974; Witters *et al.*, 1978), whereas glucagon and epinephrine inhibit the enzyme activity. This inactivation is the result of hormone-induced multiple phosphorylation of the enzyme (Arino *et al.*, 1984; Ciudad *et al.*, 1984; Akatsuka *et al.*, 1985). Thus cholinergic dysfunction and resultant insulin deficiency, set in due to fluoride administration, could be expected to enhance the stimulation 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. This enzyme is activated by Ca^{2+} mobilizing hormones (α_1-adrenergic catecholamines, vasopressin etc.) or cyclic-3'5'-adenosine monophosphate (cAMP) producing hormones like glucagon (see review by Van de Werve and Jeanrenaud, 1987). Fluoride is known to elevate catecholamines level (Chinoy and Narayana, 1992) and stimulates adenyl cyclase activity (Rodriguez-pena *et al.*, 1991; Boyd *et al.*, 1992). These observations give additional support to the present notion.

In the present study on post-hatched developing chicks, it was also observed that there are appreciable changes in the liver lipid metabolism. Phillips and Hart (1935) reported that fluoride has the capacity to alter lipid metabolism in rat. The present observation is similar to the disorder known as `lipid storage diseases'. Lipidosis is a disorder of lipid metabolism leading to abnormal fat accumulation in body tissues particularly in the liver and brain (Brady, 1970). Fluoride is known to inhibit many enzymes involved in lipid metabolism (eg lipases, phospholipases) which are capable of hydrolysing the fatty acids from phospholipids (Cannell, 1960). The inhibition of these enzymes could result in elevated levels of stored lipids in tissues. Similar observations were also made by Shashi (1992) in the brain of rabbits subjected to subacute sodium fluoride.

The accumulation of lipids points to a possible reduction in oxidative metabolism. The curb in SDH activity observed in the liver and muscle of fluoride treated birds strengthed this view of hampered oxidative metabolism. Similar reduction in SDH activity has been reported in mudskippers (Shaikh and Hiradhar, 1985) and in growing pigs (Seffner *et al.*, 1990) following fluoride administration. Seffner *et al.* (1990) have opined that the reduced SDH activity might be due to disturbance in respiratory metabolism of hepatocytes or their

mitochondrial membrane. Hochachka and Somero (1973) described an accumulation of succinate in response to anoxic stress in some invertebrates, gold fish and carps. Current study also reveals lowered oxygen carrying capacity in terms of reduced haemoglobin (Chapter 5). This also supports the above contention about adaptive alteration to hypoxia seemingly induced by fluoride. The enhanced LDH activity observed in the present study too indicates a possible shift in the energy metabolism towards anaerobic pathways. Enzymic inhibiton by fluoride has been reported (Wiseman, 1970) which could additionally hamper the normal metabolic pathways and even force the cells to resort to alternate pathways as a part of stress induced response mechanism.