The fluoride content of drinking water is considered to be the cause of fluoride toxicity in human beings (Krishnamachari and Kamala Krishnaswamy, 1974; Krishnamachari and Laxmaiah, 1975; Sivakumar and Krishnamachari, 1976) and in cattle (Elifford, 1947; Sterkowicz and Guminska, 1977; Krook et al., 1979; Crissman et al., 1980; Svezhentsov, 1980) living in endemic flurotic areas. Fluoride toxicity, both chronic and acute, has also been induced in experimental animals either by supplementing drinking water with fluoride or by massive administration of fluoride (Elsair et al., 1976; Herta et al., 1977; Patterson et al., 1977; Suttie and Kolstad, 1977; Holland et al., 1978; Larsen et al., 1978; Bourbon et al., 1979; Makhmi et al., 1979; Rao and Susheela, 1979; Whitford et al., 1979; Crissman et al., 1980; Hoffman 1980). So far the emphasis of the work on fluoride toxicity has been mainly on skeletal and dental tissue, understandably. However, the involvement of soft tissues in fluoride toxicity has not been given due attention. Hence the present study was a beginning to systematically study the toxic effects of fluoride on non-skeletal tissues like liver, kidney and intestines. The present study has shown that in rats given drinking water supplemented with 100 ppm of fluoride for two
months affected the liver and kidney and altered the blood chemistry.

The observed effect of fluoride supplementation of drinking water on the body weight and growth rate of rats points to the interference of toxic amounts of fluoride in the overall metabolism of the animal. The gain in body weight and percent gain in body weight of rats at the end of two months period of experimentation was significantly less in fluoride-treated rats than in controls. Also a marked reduction of growth rate of rats was observed, the effect being seen even within two weeks of fluoride treatment and continued during the rest of the experimental period. Similar to the decrease in the body weight, the liver and kidney weights were also effected. Although the whole weights of these two tissues were reduced by fluoride treatment, the weights when expressed as percent of body weights, were not significantly altered. It reflects the effect of fluoride on the overall growth of tissues and thus affecting the body weight. It may be pertinent to note that in advanced cases of fluorosis the victims exhibited extreme leanness and loss of appetite (Cantarow and Sehepartz, 1961). Although in the present study the daily
food consumption could not be recorded, the experimental rats were found to show aversion to food, probably due to loss of appetite.

The fluoride content in serum, liver and kidney were estimated to understand the fate of extra fluoride given in the drinking water. It was seen that the daily water consumption increased similarly in both the control and experimental groups, during the period of experimentation. However the fluoride intake, calculated on the basis of daily water consumption, was obviously high in fluoride-treated rats due to the supplementation of water with 100 ppm fluoride. The tap water provided for drinking was analysed at weekly intervals and was found to contain 1.05 + 0.075 ppm of fluoride. Thus it could be seen that the control rats had permissible limits of fluoride in their drinking water, unlike the experimental rats. Based on their daily water consumption during the two month period, it was calculated that the total intake of fluoride by control rats was found to be 0.756 mg (increasing from 0.0069 mg per day to 0.0188 mg per day) and by experimental rats 72.756 mg (increasing from 0.6467 mg per day to 1.8189 mg per day at the beginning and ending of two months period,
respectively). The excess amount provided in the drinking water to the experimental rats was found to be absorbed and retained considerably in the liver, kidney and serum. Fluoride is readily absorbed from the intestines and excreted in urine (Cantarow and Schepartz, 1961). A direct correlation between the fluoride intake and excretion was reported (Largent and Heyroth, 1949) and nearly 50 to 75 percent of the ingested fluoride was found to be excreted depending on the intake and the rest retained in the body (Largent and Heyroth, 1949; Cantarow and Schepartz, 1961; Ekstrand et al., 1977). Although the ingested fluoride was reported to be accumulate considerably in the bone (Wilson et al., 1959) it is of interest to note, in agreement with the earlier findings (Armstrong and Singer, 1980; Hangslo et al., 1980), considerable accumulation of fluoride in liver and kidney (Table 4). However the present study has indicated that the kidney retained more fluoride than the liver. Fluoride removal (clearance) through kidneys might be the cause of greater degree of accumulation of fluoride in the kidneys. It is of significance that kidneys were reported to be more affected than liver in fluoride toxicity, Ekstrand et al., (1977) reported some amount of fluoride was also excreted in sweat in addition to urine and accumulated
considerably in body tissues. High levels of fluoride in serum, liver and kidney in fluoride-treated rats, in the present study, these are evidently due to the higher intake of fluoride.

It was noticed in the present study, that fluoride treatment caused hyperglycemia both in fed and fasted states. Varying degrees of hyperglycemia are seen in many conditions due to various causes. The blood glucose level is the result of interplay of various hormones exerting their regulatory role at cellular level. The hormones having considerable role on blood glucose level are insulin, glucagon, catecholamines, corticosteroids and growth hormone. While insulin induces hypoglycemia, the other hormones cause hyperglycemia. Hyperglycemia was shown to result from either increased hepatic glycogenolysis, enhanced hepatic and renal gluconeogenesis, decreased peripheral utilization of glucose, or by increased absorption of glucose from intestines (Cantarow and Schepartz, 1967a).

The role of hepatic glycogenolysis in inducing hyperglycemia in fluoride-treated rats was tested by estimating the liver glycogen and the activities of enzymes of glycogenolysis, phosphorylase and glucose-6-phosphatase, in
the liver. The hepatic glycogen level was found to be not altered by fluoride treatment. While the phosphorylase activity showed a significant increase in the livers of fluoride-treated rats compared to controls, the activity of hepatic glucose-6-phosphatase was found to be significantly decreased. Although the increased phosphorylase activity may stimulate the glycogenolysis, because of the decrease in the activity of the glucose-6-phosphatase, a key enzyme both for glycogenolytic and gluconeogenic pathways, it may be concluded that the hepatic glycogenolysis might not be the cause of observed hyperglycemia in fluoride-treated rats. The decrease in the activity of glucose-6-phosphatase in the liver limits the role of liver in inducing hyperglycemia due either to increased glycogenolysis or to gluconeogenesis. However McGown and Suttie (1979) reported that the hyperglycemia which accompanies acute fluoride toxicity in the rats is mediated by epinephrine.

The crossover data of glycolytic and gluconeogenic intermediates obtained by incubating hepatocytes with 10 mM fluoride indicated that some of the enzymes of these pathways, especially enolase and fructose-1,6-diphosphatase, are
partially inhibited by fluoride (Asha, 1979). Thus both

glycogenolysis, gluconeogenesis were found to be inhibited

by fluoride in vitro. However the role of fluoride on the

inhibition of these pathways in vivo require further elu-

cidation. It is known that plasma free fatty acids sti-
mulate gluconeogenesis by activating pyruvate carboxylase

by increased formation of Acetyle CoA (NewSholme and

Gevers, 1967). The observed decrease of plasma FFA in the

present study in fluoride-treated rats also points to lack

of stimulation of gluconeogenesis in the liver and kidney.

The decreased level of plasma FFA in fluoride-
treated rats may be due to decreased lipolysis in the

adipose tissue of these rats. This indicates that fluoride-
treatment may result in the partial inhibition of lipolysis

in the adipose tissue or in the decrease in the fat content

of this tissue. Although the fat content of the adipose
tissue was not estimated in the present study, the lean-
ness and the decrease in the body weight in fluoride-
treated rats indicate a decrease in depot fat. This might

be due to decrease availability and utilization of glucose

for fat synthesis in adipose tissue.
The third possibility for the observed hyperglycemia, that is rapid intestinal absorption of glucose, was considered by studying the intestinal absorption of glucose. Both in vivo and in vitro methods are used for studying the intestinal absorption of glucose. In the in vitro method everted intestinal sacs are used, which show disruption of epithelium after incubation (Levine et al., 1970; Gibaldi and Grundhoffer, 1972; Wolfe et al., 1973). The molecules have to traverse the intestinal musculature in addition to the intestinal epithelium, which was devoid of circulation. In vivo methods where substances are slowly transfused through the intestine or introduced into an intestinal loop in situ (Levine et al., 1955; Schanker et al., 1958) are more physiological due to the presence of intact circulation. The in vivo method of Chakrabarti and Benerjee (1976) was used in the present study, to study the absorption of glucose through the intestines. It was observed in the present study the intestinal absorption of glucose was decreased in fluoride-treated rats. Thus the possibility of enhanced intestinal glucose absorption being the cause of hyperglycemia in fluoride-treated rats could be ruled out. However a detailed study of the mechanism involved in the induction of hyperglycemia in fluoride toxicity is worth attempting.
The decreased level of plasma FFA in fluoride-treated rats in the present study point to the decreased lipolysis in the adipose tissue of these rats. This indicates that either to the partial inhibition of lipolysis or to lesser availability of the fat in the extra-hepatic tissues. The decrease in the plasma FFA in fluoride-treated rats might be the result of hyperglycemia observed in the present study. The inverse relationship between the level of blood glucose and plasma FFA was observed in some conditions (Hales, 1968).

High intake of fluoride also affected some of the lipid fractions in the liver and serum. There was significant increase in the levels of liver total lipids, triglycerides and triglycerides and phospholipids in the serum. No alterations were however observed either in the level of hepatic phospholipids or total cholesterol, or in the serum total cholesterol level. Increase in serum total lipids and cholesterol and in the liver total lipids and triglycerides were reported in rats and guinea pigs receiving high amounts of fluoride for 6 to 10 months (Decamargo and Merler, 1980; Vetassery et al., 1980; Dominiczak et al., 1981). Although similar results were observed in the present
study in total lipid and triglyceride levels in the liver and in triglyceride level in the serum, an increase in the serum phospholipids was also observed in the present study. However, the serum total cholesterol level was not altered in the present study by fluoride treatment, contrary to the observations of Vetassery et al., (1980) in guinea pigs. These differences could have been due to the difference in the species used, the age of the animal and also the mode of fluoride administration and the dosage used.

The accumulation of lipids, largely triglycerides, in the liver of fluoride-treated rats as compared to the controls points to the formation of fatty liver. Factors such as decreased oxidation of fatty acids (Dianzani and Marinari, 1961), decreased turnover of phospholipids (Farber, 1967), loss of mitochondrial energy (Christie and Judah, 1954), provision of fatty acids either from the fat depots or by increased hepatic synthesis of fatty acids (Myant, 1968) and defective triglyceride secretory mechanism (Recknagel and Lombardi, 1961) in liver have been adduced as the possible causes of lipid retention in the liver.
The three major factors responsible for the maintenance of serum triglyceride levels are (1) the rate of synthesis of triglycerides, (2) the secretion rate of triglycerides from the liver to the circulation and (3) the removal rate of circulating triglycerides by extrahepatic tissues (Sheorsin et al., 1980).

Although no attempt has been made now to study the possible mechanism(s) for the induction of fatty liver in fluoride-treated rats, certain observations can be made based on the available data from this study. Since the rate of synthesis of triglycerides in the liver depends on the uptake of fatty acids by liver, which in turn was shown to be dependent on the FFA level in the plasma, and thus on the mobilization of fatty acids from extrahepatic tissues (Myant, 1968). The observed decrease in the plasma FFA in the present study suggests that there may be decreased triglyceride synthesis in the liver. There seems to be some impairment in the secretion of hepatic triglycerides into serum because of increased triglyceride level in the liver of fluoride-treated rats. Inspite of this, the observed hypertriglyceridemia in fluoride-treated rats lead to suggest that the removal rate of circulating triglycerides...
by extrahepatic tissues appear to be affected. Unlike in the liver, the total lipid and the lipid fractions namely, triglycerides, total cholesterol and phospholipids and total protein were found to be decreased in the kidney by fluoride treatment. These differences can perhaps be due to the differential responses of the tissues to fluoride toxicity.

It was observed in the present study that the intake of water containing 100 ppm of fluoride for two months did not alter the level of proteins or Pi either in the liver or in the serum. However Kour et al., (1978) reported a significant fall in the total serum protein and also inversion of albumin and globulin ratio in rabbits administered toxic amounts of fluoride. Probably these may be due to differential responses to fluoride toxicity by different species and mode, duration and level of fluoride administration. Pi in the serum or liver or kidney was not found to be altered by fluoride treatment in the present study. Krishnamachari and Kamala Krishnaswamy (1975) also observed normal levels of serum Pi in humans affected by fluoride toxicity.
Phosphatases of the blood and tissues have been broadly classified into two types based on their pH optimal for their activity. The Alkaline phosphatase has a pH optimum of approximately 10, while Acid phosphatase has its optimal activity at a pH of approximately 5. These phosphatases have been shown to occur in various forms. Acid phosphatase has been shown to be present in lysosomes and also used as marker enzyme. The bone have been shown to be rich in Alkaline phosphatase and is related to osteoblastic activity (Davidsohn and Wells, 1963). Liver has been shown to excrete this enzyme through bile (Cantarow and Schepartz, 1967b).

The activity of Alkaline phosphatase in serum was not altered by fluoride treatment. This is in agreement with the observations in humans by Krishnamachari and Kamala Krishnaswamy (1973). However the activity of this enzyme was found to be increased in the liver and decreased in the intestines by fluoride treatment. Increased bone mineralization was reported in chronic fluoride toxicity and is attributed to new bone formation (Narasinga Rao et al., 1979). With the increased bone formation in chronic fluoride toxicity, there is a need for higher alkaline
phosphatase activity in the bone. However this increase is not reflected in the serum levels of this enzymes, probably the liver is able to keep this enzyme level within normal levels by extracting and excreting higher amounts of this enzyme from the serum. This could have been the reason for increase in the activity of this enzyme in the liver.

The suggestion that the stimulation in the glycoprotein synthesis due to tissue injury put forth by Susheela and Sharma (1980) was tested by studying the acid phosphatase activity in liver, intestines and serum. The activity of this enzyme was found to be increased in both tissues and also in serum. It appears that fluoride toxicity might result in injury to tissues/cells resulting in increased synthesis of this lysosomal enzyme, for tissue repair. Thus the increased activity of acid phosphatase in serum may also reflect the damage to the tissues in fluoride toxicity.

The present study has brought to light that high intake of fluoride might result, in addition to skeletal and dental fluorosis, in inducing certain harmful effects
in soft tissues. As this study was aimed at only screening the tissues (liver, kidney and intestines) for toxic effects, it is felt that detailed studies on the basis of these findings would be worth pursuing.