The actual mechanisms underlying the events observed in fluoride poisoning are, at best, partially understood. Present study has demonstrated that fluoride administration irrespective of route of administration, oral or intraperitoneal, caused hyperglycemia at all the doses except at 5 mg F/kg body wt. oral dose. However, persistent and greater degree of hyperglycemia was observed in rats administered fluoride intraperitoneally than in rats administered orally when same dosage was used. Presumably these responses can be related to fluoride concentrations in circulating plasma which are resultant of several processes—absorption from the gastro-intestinal tract, and removal from blood by skeletal system and by the kidneys into the urine. The importance of these processes result in a magnitude of the circulating plasma fluoride elevation is suggested by the observation of Maynard et al.¹ who observed that intraperitoneally injected NaF is approximately twice as toxic for rats weighing 200-300 g as it is for to 100-200 g rats and that of De Lopez et al.² who observed that plasma fluoride concentrations of 8-10 ug/ml or higher were often associated with death and i.p. administration results in greater elevation of plasma fluoride than oral administration. It has been postulated that the concentration of fluoride in plasma and other soft tissues, will increase with time as the ability of the skeleton to retain the
additional fluoride diminishes and that this increase will be associated with an increased severity of fluoride induced effects in the animals.

In the present study fatality was encountered in rats that received 15 and 20 mg F/kg body wt. i.p. and 35 mg F/kg body wt. orally. The death occurred in 50% of the animals at 3 hours with 15 mg F/kg body wt. i.p. and in all the animals by one hour with 20 mg F/kg body wt. and in 60% of the experimental population with oral dose of 35 mg F/kg body wt. Taylor et al.\(^3\) reported death in rats within 3 hours after receiving 45 mg of NaF/kg body wt. orally.

It is also pertinent to note from this study that the symptoms of toxicity also vary with the dose of fluoride. This is obvious that while hyperglycemic response was seen at sublethal doses of fluoride, either oral or i.p., lethal doses of fluoride resulted in hypoglycemia (Tables 1 and 2; Figs. 1-6).

These earlier observations, the degree and persistence of hyperglycemia observed in the present study suggest that the length and persistency of hyperglycemic response may be related to the magnitude and persistency of plasma fluoride concentrations. This data clearly indicate that the degree of toxicity varies with the routes of administration, intraperitoneal being more toxic than oral route.
Studies using adrenalectomized rats and adrenergic blockers\textsuperscript{4,5} indicated that the hyperglycemic action of fluoride is mediated by adrenal catecholamines and this response to fluoride appears to be mediated primarily by splanchnic impulses arising in central nervous system\textsuperscript{6}. Suketa \textit{et al.}\textsuperscript{5} demonstrated an increased serum 17-hydroxy corticosterone prior to elevation of serum glucose and also an increased urinary 17-hydroxy corticosterone excretion by fluoride administration reflecting hyper-adrenalcortical function. Although these studies point to involvement of adrenal function, only partial suppression of fluoride induced hyperglycemia by adrenergic blockers suggests the involvement of other mechanism(s) in the causation of hyperglycemia.

Varying degrees of hyperglycemia are encountered in variety of 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 in blood glucose homeostasis are insulin, glucagon, catecholamines, glucocorticoids, thyroid and growth hormone. While insulin induces hypoglycemia, the other hormones cause hyperglycemia. Thus the blood glucose level is determined not by the absolute levels of any of these hormones but by the relative concentrations of hypoglycemic and hyperglycemic hormones.
The hepatic glycogen stores were likely to be the source of increased blood glucose concentration and in turn these glycogen levels were maintained by the net result-ance of the two processes – glycogenesis and glycogeno-
lysis.

The possibility of liver glycogen being the source of hyperglycemia in fluoride-treated rats was tested. The decreased levels of hepatic glycogen under basal con-
ditions and when challenged with glucose in fluoride-
treated rats suggest the inability of fluoride-treated rats to store glycogen in their livers (Tables 3, 4 and Fig. 7) indicate interference in hepatic glycogen meta-
bolism by fluoride treatment. Studies with [U - $^{14}$C] glu-
cose suggest relative retardation in hepatic glycogen synthesis or increased turnover of liver glycogen by fluoride treatment. (Fig. 10).

The blood glucose concentration is dependent on hepatic and renal glucose production on one hand and on the peripheral glucose utilization on the other. Obvi-
ously an increase in glucose production or decrease in the rate of utilization or both could result in hyperglycemia. Fluoride treatment appears to induce a nonsteady state of blood glucose. In depth studies on the changes in glucose turnover by fluoride treatment using both $^{3}$H and $^{14}$C-
labelled compounds of glucose are merited. The lack of availability of $^{3}$H labelled glucose has confined us to draw possible conclusions from the studies using [U - $^{14}$C]-
glucose.

The decline in the blood glucose specific activity by fluoride treatment is obviously due to the elevation of the blood glucose in fluoride-treated rats as the total $^{14}$C-glucose activity in the blood of fluoride-treated rats remained higher compared to the control rats throughout the period of experimentation (Figs. 8 and 9).

The decrease in the specific activity of blood glucose apparently due to an increase in the blood glucose with fluoride treatment observed in this study indicates an increase in the rate of hepatic glucose production due to either enhanced operation of Cori cycle and/or an increased gluconeogenesis and/or a decreased peripheral utilization of glucose.

The enhanced activities of hepatic phosphorylase glucose-6-phosphatase (Tables 29 and 35) point to enhanced glucogenolysis in the livers of fluoride treated rats. Fluoride in high concentrations is a well known stimulator of adenyl cyclase in broken cell preparations. Although no such effect was observed in intact tissues, surprisingly Exton et al. observed a decrease in tissue cyclic AMP in rat livers perfused with 5 mM NaF with increased glucose output associated with deterioration of liver appearance. However, Mc Gown and Suttie did not observe such effect in their studies. While Suketa et al. observed
observed an increased phosphorylase by fluoride treatment, Mc Gown and Suttie\textsuperscript{4} found no effect of fluoride on glucose-caused decrease in hepatic phosphorylase activity. The increased glucose production in the absence of any carbon source by hepatocytes observed by Sahad et al.\textsuperscript{9} lead them to suggest that fluoride induced stimulation results from the activation of glycogen phosphorylase through increased production of c AMP. Stossel et al.\textsuperscript{10} found that 20 mM NaF increased phosphorylase activity and decreased glycogen synthetase activity in polymorphonuclear leucocytes without any change in the level of glycogen. Thus it appears that the fluoride-induced hepatic changes are more complex and appear to be not primarily mediated through cyclic AMP. However, the involvement of catecholamine cannot be ruled out as plasma catecholamine level was reported to be increased in fluoride-infused rats\textsuperscript{4}.

Fluoride inhibits phosphorylase phosphatase and is also used in phosphorylase assays to prevent deactivation of enzyme\textsuperscript{11}. Probably fluoride poisoning enhances hepatic glycogenolysis either directly by inhibiting deactivation of phosphorylase or indirectly by a complex mechanism involving other tissues.

**Glycolysis**

The present study has demonstrated that the inability of the liver and kidney cortex slices from fluoride-
treated rats to utilize glycolytic pathway (Tables 10 and 11) and also inhibition of glycolysis by fluoride \textit{in vitro} in normal fasted rat liver and kidney cortical slices (Tables 12 and 13).

Growth of HeLa cells grown in monolayers or L-cells grown in suspension culture were affected by fluoride (1 mM) in the growth media and that growth ceased almost completely at 2 mM\textsuperscript{12}. Intracellular fluoride concentrations that are associated with the significant decrease in the growth rate of these cells were about 0.5 mM. Steady state levels of some glycolytic intermediates in these cells indicated inhibition of enolase at fluoride levels of 5 and 10 ppm\textsuperscript{13}.

Drescher\textsuperscript{13} reported a simultaneous inhibition of DNA and RNA synthesis to varying degrees in cultured L-cells at 30 ppm F. He considered this as evidence for a primary effect of fluoride on some aspect of cellular activity with energy production a logical target. Glycolytic activity, however, was reduced by only 25% and the cellular ATP levels remained unaffected despite a 50% reduction in cell growth. A 50% reduction in cellular total pyridine nucleotide concentration (NAD and NADH) was also observed.

Enzymes like enolase and SDH, requiring divalent cations as cofactors are inhibited by fluoride and the
inhibition is enhanced by inorganic phosphate\textsuperscript{14,15}. The similarity of fluoride ion to hydroxyl ion in terms of ionic radius and primary hydration number led Nowak and Maurer\textsuperscript{15} to suggest that \(F^-\) serves as a possible analog of \(OH^-\) group involved in the gain or loss of water as part of normal reaction mechanism. Inhibition of enolase occurs by the formation of a tightly bound enzyme-metal-\(F^-\)-\(Pi\) complex in which the \(F^-\) interacts directly with the metal ion in the active site and indirectly with the phosphate binding site\textsuperscript{15}. However, Messer\textsuperscript{16} cautioned in extrapolating results from nonphysiologic \textit{in vitro} studies to \textit{in vivo} conditions.

\textbf{Gluconeogenesis}

The maintenance of stable levels of glucose in blood is one of the most finely regulated of all the homeostatic mechanisms, in which the liver, kidney and other tissues and several hormones play a part.

Gluconeogenesis, the synthesis of glucose from amino acids or from other precursors such as lactate, succinate, propionate, glycerol etc. is a process which occurs predominantly in liver and kidney cortex in mammals and whose role in blood glucose homeostasis is well recognized. Hence the present study was extended to assess the contribution, if any, of the liver and kidney cortex to the observed hyperglycemia in fluoride-treated rats.
The rate of gluconeogenic flux depends on the rate of mobilization of gluconeogenic substrates to the gluconeogenic tissues, rate of blood flow and rate of uptake of substrates by the tissues and concentrations of non-gluconeogenic substrates which modulate the rate limiting enzymes.\textsuperscript{17}

Measurement of changes in blood levels of gluconeogenic substrates—lactate, pyruvate and amino acids—by fluoride treatment indicate that increased flow of lactate but not amino acids point to either increased operation of Cori cycle or inability of the gluconeogenic tissues utilize lactate.

The decreased rate of disappearence of blood glucose might be expected to result in decreased peripheral formation of lactate and if lactate accumulates in circulation, in spite of this, it might occur due to greater inhibition of gluconeogenesis.

Studies of Exton et al.\textsuperscript{17} have shown that hepatic gluconeogenesis or ureagenesis can be markedly altered by variations in the levels of amino acids in circulation and the regulation of amino acid release from peripheral tissues may be important in the control of gluconeogenesis. However, marked changes in blood urea level with no alteration in circulating amino acids level suggest renal involvement in the causation of hyperuremia in
fluoride-treated rats. It is noteworthy that NaF in acutely toxic doses produces necrosis of renal tubular cells and dilation of the tubules resulting in derangement of renal function affecting glomerular function also\(^1\)\(^8\),\(^1\)\(^9\).

A marked decrease in plasma FFA level by fluoride treatment reflects decreased lipolysis and mobilization of fatty acids from adipose tissue. Rate of release of FFA is affected by many hormones that influence either the rate of esterification or the rate of lipolysis on which cyclic AMP has a profound effect\(^1\)\(^7\),\(^2\)\(^0\). Increases in tissues adenylate cyclase activity and tissue cyclic AMP are reported in experimental animals given fluoride\(^1\)\(^6\),\(^2\)\(^1\). Increased C-AMP production by whole cells in response to high fluoride concentrations in cultured medium is reported, although not all cell types respond to this\(^1\)\(^6\),\(^2\)\(^2\). Probably, adipose tissue adenylate cyclase may not be sensitive to fluoride.

Stimulation of gluconeogenesis by FFA is suggested to result from an increased concentration of intramitochondrial acetyl-CoA\(^1\)\(^7\),\(^2\)\(^0\) or enhanced transport of pyruvate into mitochondria\(^1\)\(^7\),\(^2\)\(^0\),\(^2\)\(^3\) or increased cytosolic NADH/NAD\(^+\) ratio with the resultant forward shift in the glyceraldehyde-3-phosphate dehydrogenase near equilibrium reaction\(^2\)\(^0\),\(^2\)\(^3\),\(^2\)\(^4\) or decreased flux through pyruvate-Kinase reaction\(^1\)\(^7\),\(^2\)\(^3\),\(^2\)\(^5\),\(^2\)\(^6\). However, such stimulation of
gluconeogenesis might not occur in fluoride-treated rats due to the decreased mobilization of FFA.

Studies on the changes in the overall gluconeogenic rate by fluoride treatment in vivo by providing gluconeogenic substrates and measuring the increments in blood glucose and liver glycogen (Tables 14-17) indicated an inhibition of gluconeogenesis from succinate, pyruvate, alanine and glycerol with lesser inhibition from glycerol. Renal cortical slices from fluoride-treated rats also exhibited decreased capacity to synthesize glucose from glycerol, pyruvate, succinate and glutamate, the inhibition being greater with glutamate (Tables 18-21).

Inhibition of gluconeogenesis was also observed from pyruvate by the addition of fluoride to normal fasted rat kidney cortex slices at concentrations ranging from $2.5 \times 10^{-3} - 10^{-2}$ M (Table 22).

Glucose formation from glutamate and pyruvate require the uptake and entry of glutamate and pyruvate into mitochondria where they are converted to oxaloacetate leading to generation of ATP. Mitochondrial membrane is impermeable to oxaloacetate. Since the next step is gluconeogenesis, the PEPCK reaction, is extra-mitochondrial in rat, the carbon skeleton of oxaloacetate has to cross the mitochondrial membrane as another C-4 intermediate. This is facilitated by transporting the C-4 skeleton as aspartate after transamination or as
malate after reduction, which results in net NADH efflux from mitochondria. The NADH will be transported back to the mitochondria via the glycerol-phosphate shuttle. Alterations in the permeability of the membranes, and in membrane bound enzymes and alterations in the formation of lipid peroxides have been reported under the influence of fluoride.

Treatment of animals with low doses of fluoride was reported to cause changes in hepatic metabolites demonstrating an inhibition of enolase in the whole animal. The fluoride concentration in the livers from these fluoride injected rats were comparable to those used in vitro inhibition of enolase.

A decrease in renal glucose synthesis by kidney cortex slices might be due to decreased availability of energy for glucose synthesis or to interference in the oxidation of free fatty acids and amino acids (glutamate) or decrease in the activity of malate shuttle resulting from changes in the membrane permeability or decrease in the activity of some of the enzymes of the pathway.

Although fluoride treatment did result in the enhancement of some of the enzymes of gluconeogenic pathway in the liver and kidney cortex, the decrease in the activity of PEPCK suggests the blockage of gluconeogenesis at this step. The measurable activity of an enzyme is
resultant effect of many factors including turnover rate (which depends upon the balance between synthesis and degradation of the enzyme) and the presence of activators and inhibitors which have a direct action on the enzyme activity. The half-life of rat liver PEPCK is reported to be 4 hours.\(^{32}\)

An enzyme may be inhibited by fluoride either directly\(^{33}\) or through a change in the concentration of such bivalent cations as Ca, Mg, Mn etc. in the media.\(^{34}\) Results concerning the effect of fluoride on the metabolism of these ions in animals are inconclusive. Leone et al.\(^{35}\) found that large dose of NaF given intravenously to dogs slightly reduced the calcium level in blood. Simpson et al.\(^{36}\) reported severe hypocalcemia in a patient following ingestion of fatal dose of fluoride.

Recent evidences indicate the involvement of calcium ions in the hormonal regulation of carbohydrate metabolism.\(^{37}\) Calcium ions were observed to stimulate partially purified pyruvate dehydrogenase phosphatase.\(^{38}\) These are also evidences to suggest regulation of gluconeogenesis by calcium ions through interactions at pyruvate kinase step\(^ {39}\) and activation of PEPCK by mitochondrial release of Fe\(^ {2+}\).\(^ {40}\) The prominent feature of acute fluoride poisoning is a profound hypocalcemia which has been often ascribed to the complexing of calcium by fluoride in rats. The hypocalcemic action of fluoride requires
the presence of thyroid gland implying a role for calcitonin in the hypocalcemia. So the inhibition of renal gluconeogenesis by fluoride in vitro could be attributed to the complexation of calcium and thus making free Ca\(^{2+}\), nonavailable for gluconeogenesis. However, the importance and the resultance of this complexing in vivo in the inhibition of gluconeogenesis requires further indepth study.

Since the actual mechanism(s) underlying the events resulting in hyperglycemia during the acute fluoride poisoning are, at best, partially understood, the data obtained from the present study focusses the role of hepatic and renal gluconeogenesis in the causation of hyperglycemia in acute fluoride poisoning and few conclusions seem to be warranted. The rise in blood glucose in fluoride poisoning appears to be due to an accelerated glycogenolysis in liver and decreased peripheral glucose utilization. With deranged hepatic and renal gluconeogenesis the animal is unable to sustain the hyperglycemia due to the exhaustion of hepatic glycogen stores which led to terminal hypoglycemia resulting in death at higher doses of fluoride. Further studies are required to throw light on the involvement of membrane processes, operation of shuttles, energy availability and intracellular calcium pools to explain the actual mechanism of fluoride induced inhibition of gluconeogenesis.
LITERATURE CITED


