DISCUSSION

Fluorosis is an important problem for many parts of the world and the striking picture of the patients suffering from crippling fluorosis is a challenge calling for the solution of the problem. There is an extensive endemic area in Panjab and the disease is causing serious disability in the people of that region. The suffering due to fluoride intoxication is so great that even if the prevention and cure of bone lesion cannot be accomplished in near future, the reduction in toxicity of fluoride by some external agent may be regarded as a significant contribution to the solution of the problem. In this context, it is important to consider the biochemical changes produced by fluoride. An effort has been made in this work to study some of the factors involved in fluoride intoxication, particularly the role of vitamin C.

Concentration of fluoride in water

The concentration of fluoride in water has been incriminated as a very important factor in the etiology of fluoride intoxication. However, its concentration which may produce deleterious effects is disputed. According to a W.H.O. Report (1958) dental fluorosis appeared when drinking water supplies contained 2 ppm or more of fluoride and when these waters were ingested during first eight years of life. Leone (1960) found no clinically significant adverse physiological or functional effects in persons whose water supplies
contained fluoride in concentration of 8 ppm. In another study, a large group of animals failed to develop osteosclerosis even in periods up to 4 years, when the diet contained as much as 50 ppm fluoride (Hodge and Smith 1954). The very mild mottling of teeth in communities where water fluoride ranged from 1-1.5 ppm was considered to be of no cosmetic significance (W.H.O. 1958). On the other hand, Azar, Nucheo, Bayyuk and Bayyuk (1961) observed skeletal fluorosis in the people of Qatar (Persian gulf) ingesting water containing 0.79 to 3.45 ppm of fluoride. Steyn (1964) has presented data to show that fluoride concentration may even be less than 1 ppm to cause ill effects.

All grades of skeletal and dental fluorosis in persons who were drinking water with fluoride concentration lower than the concentration considered to be normal by some American workers cited above were observed in the present study. This may be due to the fact that the total consumption of water by an individual is variable and amount of fluoride in it may be the main factor which leads to intoxication rather than the concentration of fluoride in water. The variations in this and the above mentioned studies may be due to this factor.

**Amount of fluoride ingestion**

For a particular concentration, the amount of fluoride ingestion will depend on the daily intake of water. The quantity of water ingested is a variable factor and is greatly influenced by muscular activity, atmospheric temperature and humidity. Owing to the high temperature of endemic area (Maximum 116 °F in summer), the consumption of water is considerable.
The amount of water ingested is further accentuated in the farmers engaged in hard manual labour in their fields. It is this group of people which had the highest percentage of skeletal fluorosis as compared to the others, who although drink the same water, but lead a relatively sedentary life, for example, females and shopkeepers. It was estimated that about three litres of drinking water may be consumed by an individual on an average in one day. The ingestion of fluoride from this source may, therefore, range from 3.9 to 32.4 mg per day.

The food may be another source of fluoride ingestion. The normal diet of Panjabis living in rural areas consists mostly of cereals. The amount of fluoride in the uncooked food from endemic area is given in table No. II. It is evident that the amount of fluoride ingested from uncooked food may not be much different from normal. The concentration of fluoride in water may be many times than normal in an endemic area and may, therefore, be of greater significance than other constituents of diet.

In the process of cooking the food in India, a large amount of water is used and this may increase the fluoride concentration of the food considerably. Smith, Smith and Vavich (1945) found increased amount of fluoride in food, cooked in fluoridated water. On an average 2 litres of water may be used in cooking. Thus the total amount of water consumed by the people of endemic area may be about five litres and the amount of fluoride in it may range from 6.5 to 54 mg per day.
Considering the same volume of water consumption by the people of non-endemic area of Panjab, they may be ingesting 1 mg to 2.5 mg of fluoride per day. McClure (1949), Armstrong and Knowlton (1942), Mackie, Scott and Largent (1942) believed that in regions considering to be poor in fluoride, its intake varies from 0.25 to 0.5 mg per day. McClure, Mitchell, Hamilton and Kinser (1945) considered that administration of 3 to 4 mg of fluoride per day to be maximum amount when complete equilibrium of fluoride metabolism can be maintained by humans. According to Glatzel, children up to eight years of age run the risk of chronic poisoning if they ingest 2 mg of fluoride daily (Steyn 1964). Considering the concentration of fluoride in water and its total amount consumed, it is evident that the people in this endemic area of Panjab have a far greater amount of fluoride ingestion, as compared to normal values for non-endemic area and values cited from literature. 

**Increased toxicity of fluoride in India.**

The concentration of fluoride in water which either has no ill effects or produces "benign" osteosclerosis in America may give rise to crippling fluorosis in India. It was reported in some of the surveys carried out in America that osteosclerosis was not detected in residents from areas with less than 4 ppm of fluoride in water supply and, where it occurred, was not associated with physical disability or impairment (Leone et al. 1954; Stevenson and Watson 1960). These findings are in contrast with the present study and some of the other
studies carried out in India (Shortt et al. 1937; Raghvanachari and Venkataramanam 1940; Pandit et al. 1940). Some other factors may, therefore, be involved as far as the severity of the disease is concerned. Nutritional status of the patients may be one of such important factors. Pandit et al. (1940), observed that among the economically higher group of people bone fluorosis was rare as compared to its occurrence among poorer class of the people in an endemic region. A nutritional survey was carried out (table III and IV), and it was found that the diet of the people living in the endemic area was deficient in animal proteins and vitamin C. It is possible that proteins and/or vitamin C deficiency may increase the toxic effects of fluoride.

**Urinary excretion of fluoride.**

The people in the endemic area of Southern Punjab are ingesting comparatively greater amount of fluoride. The urinary excretion of fluoride was, therefore, expected to be above normal.

Largent and Heyroth (1949) observed that the range of fluoride excretion was between 3 to 17 ppm during elevated fluoride intake. They (1953) were of the opinion that urinary fluoride levels in excess of 6 - 8 ppm, when maintained over a sufficiently long period, may lead to slight radiopacity of bone. This observation was confirmed by Smith and Hodge (1959), who noted that industrial workers in America who excrete in their urine 5 mg of fluoride per litre or less do not develop osteosclerosis even after prolonged period of exposure.

In the present study only those cases were included for biochemical investigations in which radiological changes were observed. The people who excreted less than 5 mg of
fluoride in their urine daily (Table V) also showed osteosclerosis in our cases. This observation differs from the findings of Largent and Heyroth (1949) and Smith and Hodge (1959). The radiopacity observed on radiological examination may depend upon the deposition of fluoride or some other changes produced by it rather than its excretion in urine.

Effects of kidney.

Boholm (1937) found incipient changes in kidney at levels of fluoride much below concentration which were required to produce generalised ill effects. Smith and Hodge (1959) reported kidney damage principally in the proximal convoluted tubules and described it as injury and necrosis of columnar cells lining the tubules. Impaired renal function in fluorotic patients was found by Kasliwal and Solomon (1959), Siddiqui (1955), Murthi, Rao and Venkateshwarlu (1953), Shortt, McRobert, Barnard and Nayar (1937).

The blood urea may be considered to be slightly above normal (table XV). The urea clearance test in five cases out of six studied (table XV) showed impairment of kidney function.

The ratio of concentration of inorganic phosphorus in the urine and that in the serum has been found to be nearly 50 in the normal cases. It increases in renal insufficiency (Kleiner and Orten 1958). The ratio has been found to be 67.4 on an average in our cases (table XII) which indicates some amount of renal insufficiency.
In 1890 Hewelke (Roholm 1937) observed a reduction of total nitrogen excretion in the urine of a dog which was given 0.25 gm/kg body weight of sodium fluoride daily by mouth for 19 days. Gottleib and Cerent (1931, 1932) observed an increased excretion of nitrogen in the urine after intravenous injection of 0.005 to 0.02 gm of sodium fluoride per kg body weight in case of dogs. Majumdar and Ray (1946) also noticed greater excretion of nitrogen in urine of fluoride intoxicated experimental animals and considered it to be due to extensive muscular degeneration. In the present study total nitrogen excretion was found to be normal (table XIV). This observation was not in accordance with above reported studies. Perhaps acute intoxication may be the cause of increased nitrogen in studies. The chronic fluoride intoxication considered in the present study may not have produced muscular degeneration as envisaged by Majumdar and Ray (1946).

The amount of amino nitrogen was found to be excreted in the abnormal amounts (table XIV). The true index of the amount of urinary amino acid excretion is the ratio of amino acid nitrogen and total nitrogen. This ratio in the fluorotic patients was found to be raised (table XIV), it is thus obvious that there is amino-aciduria in fluorosis and this observation was confirmed on monkeys given excess of fluoride (table XXV).

Existence of amino-aciduria (Mathur, Singh, Singh and Singh, 1962), slightly higher concentration of blood urea, urea clearance test and high ratio of urinary and serum inorganic
phosphorus show some degree of renal insufficiency in cases of fluorosis. Role of kidney in the removal of ingested fluoride is well recognised. One of the factors in fluoride intoxication may be impaired kidney function.

Haemogram

In experimental animals it was reported that a reduction in haemoglobin level was brought about by giving the animals drinking water containing fluoride (McClure and Kornberg 1947; Majumdar and Ray 1946; Valjavec 1932). This claim was contested by others who had found no effect on haemoglobin concentration (Kniznikov 1958; McClure 1949). From a study of Table IX, it is evident that haemoglobin, R.B.C. value, mean corpuscular haemoglobin (M.C.H.) and colour index (C.I.) were within the normal limits eliminating thereby the existence of anaemia.

In a British survey on industrial fluorosis, with much higher level of fluoride intake, blood count and haemoglobin levels were normal and in fluoride intoxicated livestock, anaemia was not a conspicuous feature (Cannell 1960). In one of the American surveys (Leone 1960) no anaemia was observed in fluorotic endemic area. The non-existence of anaemia in our cases was in accordance with reported findings. The concentration of fluoride in blood (Table V) is not related to its amount of ingestion and excretion. It may be said that fluoride has no direct effect on blood due to very small fluoride concentration.

Serum protein bound iodide

There is extensive literature on the effect of fluoride on iodine metabolism in experimental animals. However, there is
no report available on one of the most important investigations i.e. levels of serum protein bound iodine in cases of endemic fluorosis. The P.B.I. concentration was found to be normal in this study (table VII), suggestive of absence of hypo or hyper-thyroidism. Phillips, Stare, Velijem (1934) found a high concentration of fluoride in thyroid as compared to other organs. However, in our work on experimental animals, the amount of fluoride in thyroid was not found to be much different from that of other organs (table XXIV). The experimental work, therefore, supports the observations on the patients.

Since the suggestion of Maumenee in 1854 about the goitrogenic nature of fluoride, there has been an active controversy about this aspect of fluoride metabolism (Gordonoff and Minder 1960). Steyn (1955); Steyn and Sunkel (1954); Fiorentini, Galeazzi and Visintin (1947); Wilson (1941); Wespi (1954); observed higher incidence of goitres in regions where fluorosis was in endemic form. On the other hand reports from Morocco and Iceland etc. (Gordonoff and Minder 1960) did not support this observation. Cannell (1960) was of the opinion that only clinical evidence is not sufficiently strong to exclude a local and a coinciding factor of geographical distribution as no direct relationship appears to exist between endemic goitre and fluoride in the causation of the condition.

No evidence of any sign of goitre or other thyroid disorder in the affected population was found during the present study. A part of Panjab is affected by endemic goitre, but this belt extends along its north western border along
the root of Himalaya, while on the contrary fluorosis belt extends along the southern border (Singh, Jolly, Bansal and Mathur 1963). Similarly, not a single case of fluorosis was reported from the region of the endemic goitre.

The normal P.B.I. concentration in our cases was, therefore, in accordance with the clinical findings and excludes the possibility of direct interference in iodine metabolism. The administration of salts of iodine to reduce the toxicity of fluoride may, therefore, be of no significance.

Role of vitamin 'C' in fluorosis.

Malnutrition is often said to be one of the causes of greater toxicity of fluoride in India. The nutritional survey (tables III and IV), indicated the deficiency of animal proteins and vitamin 'C' in the diet. There is a possibility that protein deficiency may be the cause of increased toxicity. To find the validity of this hypothesis, total proteins, albumin and globulin concentrations were estimated in the fluorotic patients, because the concentration of serum proteins is more reliable test to determine its deficiency rather than the intake of proteins. Serum total proteins concentration was found to be within the normal limits (table IX). Kasliwal and Solomon (1959), Azar, Nuchco, Bayyuk and Bayyuk (1961) found no appreciable depression of serum proteins in fluorotic patients. In experimental animals, Majumdar and Ray (1946) reported its normal concentration. Albumin and globulin concentration was within the normal limits in our cases (table IX). The true index of these proteins is their ratio. The ratio of albumin and globulin showed no
abnormality. From these observations it may be assumed that malnutrition with respect to proteins may not be a cause of greater toxicity of fluoride in this endemic area.

The nutrition survey (table III and IV) clearly indicated a vitamin C deficiency. In order to find out whether this dietary deficiency actually produces vitamin C deficiency in the system, a heavy dose of vitamin C (1000 mg) was administered, when healthy subjects have been subsisting on diets containing recommended levels of intake, the administration of test dose is followed in next 24 hours by urinary excretion of 20 to 50 percent of the test dose (Hawk, Oser and Summerson 1954). The twenty-four hours excretion of vitamin C was 106.3 mg (table XXI) on an average with a range of 74.2 to 125.7 mg. This amount was many times the normal excretion but was not related to the amount of ingestion as suggested by Hawk et al. Though such tests are not conclusive yet these are preferred to those in which only vitamin C concentration is estimated in blood or urine. On consideration of the result of this test and dietary level of vitamin C (table III and IV) it may be concluded that there may be vitamin C deficiency in fluorotic patients.

The colour of the urine of fluorotic patients was found to turn dark from top downwards. This gave a resemblance to the urine of the patients of alkaptonuria. Luckily a case of \textit{chronos} with classical clinical picture was available for comparison. Urine of the alkaptonuric patient when made alkaline turned black immediately on account of the conversion of
homogentisic acid to melanin. The colour of the urine from fluorotic patients, however, did not change colour immediately on addition of alkali. This darkening may not be due to homogentisic acid as was thought by Pandit and Rao (1940) who observed the darkening of urine in monkeys administered fluoride. Detailed study of the chromogen responsible for this darkening indicated the absence of blood, urobilin, homogentisic acid, melanogen. The tests for urorosein, and phenolic compounds were positive. In the presence of excess phenols, test for indican was not conclusive.

It was thus considered that the change of colour may be mainly due to presence of excess of phenolic compounds. The change of colour was also observed in the monkeys given fluoride and the result of qualitative tests was the same as in the fluorotic patients. The concentration of phenolic compounds of urine was estimated and table XIII shows that the amount of these was increased. To cross check the results of previous experiments, tyrosine and its metabolites were estimated in urine of fluorotic patients. Table XIII shows that the amount of tyrosine and its metabolites were also increased. From tables XXIII and XXVI, it is clear that these constituents also increased in the urine of guinea pigs and monkeys when fluoride was administered to them. One of the causes for increased amount of tyrosine and its metabolites may be decreased activity of tyrosinase. However, the normal levels of this enzyme (Singh A. 1965) exclude this possibility.

It is well known that in vitamin 'C' deficiency, phenolic compounds are excreted in excess which indicates incomplete oxidation of these substances. By administering
guinea pigs, scorbatic diet, the amounts of tyrosine and its metabolites were found to increase (table XIX). The increased amount of phenolic compounds in the urine of fluorotic patients, monkeys and guinea pigs may be due to their incomplete oxidation due to vitamin C deficiency. Administration of excess of vitamin C decreased the excretion of phenolic compounds (table XXIII).

It has been suggested that fluoride may be related to the metabolic activity of vitamin C (Phillips and Change 1934, Phillips and Stare 1934 and Phillips 1933). Phillips (1933) observed scurvy-like symptoms by administration of sodium fluoride. Wadhwani (1952) induced fluorosis in monkeys on normal and scorbatic diets and noted higher mortality in scorbatic animals and further observed that the animal could be protected by vitamin C. Pandit and Rao (1940) also found beneficial effect of vitamin C. Hauck (1934) believed that the inter-relation between fluoride and vitamin C would be in the nature of fluoride interfering with the metabolism of vitamin C. She further suggested that in case fluoride interfered with metabolic function of vitamin C, it did not result in the production of all the features of scurvy. Venkateshwarlu and Narasimharao (1957) were also of the opinion that fluoride interfered with the metabolic function of vitamin C. The increased excretion of phenolic compounds is, therefore, in accordance with the above mentioned suggestions of the interference of fluoride in vitamin C metabolism. There may be vitamin C deficiency in the normal persons but the fluoride aggravates this condition, because the phenolic compounds are not excreted in abnormal amounts by the normal people.
The adult skeleton is maintained by processes of resorption and formation. An abnormal increment or reduction in adult bone mass, suggest some imbalance. The ratio of inorganic to organic substance in the bone was found to be constant by Trotter and Peterson (1962). The increased bone ash contents (table XVII) of fluorotic patients is suggestive of some imbalance in bone metabolism.

It may be that inorganic constituents might have increased at the expense of organic portion to give more ash. There have been no study regarding the major organic constituent of bone i.e. collagen in fluorotic patients. Wadhwani (1954) in the case of experimental animals found decreased nitrogen contents of bone. In the present study decreased amount of collagen was noticed (table XVII). It may be possible that fluoride affects the organic constituents of bone. Histological studies also indicated the effect on organic constituents of bone (Singh, Jolly, Bansal and Mathur 1963).

The decreased amount of collagen may either be due to interference in its biosynthesis or destructive action of fluoride on it. Urinary total hydroxyproline excretion has been considered to be an index of bone collagen metabolism (Ziff, Kibrick, Drener and Gribeatz 1956). Increased amount of urinary hydroxyproline is indicative of increased bone resorption and its decreased amount shows lower metabolic activity of the collagen. The concentration of urinary hydroxyproline in the fluorotic patient was found to be on the lower limits of normal (Table XVIII). It may, therefore, be inferred that
decreased amount of collagen in the bone is not due to the destructive action of fluoride but its inhibitory action on biosynthesis of collagen. It is well known that vitamin C is required for the biosynthesis of collagen. One possibility of the abnormality in bone metabolism in fluorosis may be the interference of fluoride in vitamin C metabolism resulting in imbalance between organic and inorganic constituents of bone.

The excretion of fluoride, calcium, magnesium and phosphorus decreased after administration of heavy dose of vitamin C (table XX). The greater retention of calcium and phosphorus on administration of vitamin C was noted by Lucke and Wolf (cited by Bourne 1956) and Landford (cited by Bourne 1956). The greater retention of fluoride was noted by Muhler (1958). On the consideration of the results of this study and some other studies, it appears that vitamin C not only influences the metabolism of organic component of bone but also its inorganic constituents.

The administration of vitamin C to the patients of crippling fluorosis has been found to have ameliorating effect. This appears to be an anomalous behavior because it also caused increased retention of fluoride. It has been shown in this study that concentration of fluoride in bone is not the index of the disease. The abnormality in calcification may be due to fluoride-induced vitamin C deficiency. The ameliorating effect on increased vitamin C ingestion may be due to the correction of this vitamin C deficiency. The imbalance between organic and inorganic constituents in the form of increase in bone ash was observed in fluoride intoxicated animals (table XXII). In the presence of excess of vitamin C, no imbalance resulted.
Normal calcification consists of formation of matrix and deposition of bone salts. In scurvy both these steps are affected but this is not the case in fluorosis. Fluoride induce hypercalcification. Fluorotic bones may, therefore, have mass more than normal but like scurvy the bones are weak. The fluoride-induced pathological condition may be set right by excess of vitamin C administration by helping the formation of bone collagen. That calcification becomes normal by ingestion of increased amount of vitamin C may be inferred from the observations that bone ash approached normal values (table XXII) thus correcting the imbalance between organic and inorganic portion. Wadhwani (1952) observed that exostosis on the bone of experimental animals disappear after the administration of vitamin C. In America where lesser toxic effects of fluoride on its elevated ingestion are seen, the diet contains higher concentrations of vitamin C as compared to this country. Due to greater amount of vitamin C in the system, the fluoride may not be able to induce vitamin C deficiency and so the bones remain in the healthy state. It may, therefore, be concluded that the vitamin C deficiency is an important factor in fluoride intoxication.

**Dental fluorosis.**

Normally calcified teeth erupt showing a smooth glossy translucent structure, usually of a pale cream white colour. Mottled teeth on the contrary, erupt to show a dull, chalky white appearance, which later on may change to characteristic brown stain, the frequency of brown stain increase with age. In certain
cases, the surface of enamel in addition to mottling are marked by discrete or even confluent pitting. As the enamel-forming cell, the ameloblasts, cease functioning at the time of eruption of a tooth, mottled enamel is a permanent physical disfigurement (Dean 1943). In our survey the incidence of dental fluorosis was found to be inversely proportional to age. It may be speculated that increased fluoride concentration which takes place with age might have bleached the pigment.

It has been suggested (Brudevold, Gardner & Smith 1956) that fluoride deposition in the enamel takes place in three stages viz. (i) during the period of enamel formation, when fluoride is deposited in the enamel matrix, (ii) after calcification is complete but before eruption when more fluoride is taken up by the extreme surfaces of enamel from the tissue fluids and (iii) after eruption and throughout the life span of the tooth, when fluoride from drinking water, food and saliva is taken up by the enamel surface. An important point to be considered in this respect is that mottling is not caused by the actual amount of fluoride deposited in the enamel but rather by an increase in the amount of fluoride during enamel formation. In the present study enamel of teeth of persons in endemic area had an equivalent fluoride content, irrespective of presence or absence of mottling (table XIX). It may, therefore, be concluded that fluoride contents of enamel are not index of mottling.

The maturation of enamel is a process of progressive dehydration and dessication with a formation of more resistant
protein than the enamel of young teeth. According to Leicester (1949) the final transition which is marked by conversion of acid insoluble to acid soluble stage consists in the removal of protein matrix. This loss may be due to proteolytic enzyme secreted by ameloblast. Since in mottling the fluoride is one of the causative factors, it may be possible that this ion reacts in some way with the protein which is present in significant amount during the development of the enamel. The ash content of enamel of fluorotic patients has been found to be slightly raised (table XIX). It indicated lesser amount of organic portion which may be due to interference of fluoride in protein formation. The non-formation of pigment after the eruption of the teeth may be due to lesser availability of organic matrix. Besides a very resistant protein present in dehydrated form after the maturation of the teeth naturally may remain unaffected.

**Pigment of mottled enamel**

The chemical nature of the dark pigment in the enamel is not known. According to Kempf and McKay (1930) it may be manganese. Roholm (1937) considered it to originate not from bleeding or infiltration of serum derivatives. It was observed during the course of this study that this pigment was organic in nature. On calcination it left behind no residue. The pigment of the teeth like the pigment of urine could be extracted by amyl alcohol. It could be bleached with hydrogen peroxide and nitric acid.

The urine of fluorotic patients turned brown on standing. It has been considered elsewhere in this study that
this may be attributed due to the presence of phenolic compounds in the urine. May be by a similar mechanism which causes increased excretion of phenolic compounds, the metabolism of phenolic compounds present in enamel proteins be affected resulting in the formation of similar type of pigment which was present in the urine.

**Effect of fluoride on bone.**

The most characteristic feature of fluoride metabolism in man and animals is its accumulation in skeletal and dental tissue. The calcified tissues by their very nature become extensive depositories of fluoride. The gross changes in skeletal in skeletal fluorosis has been described by Singh, Jolly, Bansal and Mathur (1963). The bones were heavy and irregular. These had dull colour due to irregular deposition of fluoride. The site of muscular and tendinous insertions were rendered abnormally prominent by excessive periosteal reaction with development of multiple exostosis. Irregular bone was laid down along the attachment of muscles and tendons in the extremities as well as joint capsules and interosseous membranes. Maximum changes were detected in the spine particularly in the cervical region. The vertebra showed altered proportions and measurements in all planes, but the striking abnormality was the gross reduction of antero-posterior diameter of spinal canal. The vertebrae were fused at many places which explained the marked limitation of movements and resemblance of the disease to spondylitis ankylopoietica. The irregular bone deposition was obvious clinically in a large percentage of cases as bony excrescences of varying size. Eighty six of our cases had crippling deformities
due partly to mechanical dysfunction and partly to immobilization, necessitated by pain. The exact pathogenesis of skeletal changes is not known and is of great interest. There is certainly an increased amount of fluoride in bone, but it is yet to be determined whether this is the only cause of pathological changes or there are some other factors which lead to this abnormality. The concentration of fluoride in the fluorotic bone (table VI) in this study was many times its concentration in the normal bone, but the concentration which may cause the ill effect is not precisely known. From previous evidence bearing on the relationship between fluoride content and pathological changes in human bone, we must refer to the study of Roholm (1937). In the two autopsy cases studied by him, the bone ash contained 0.76 and 1.319 percent fluoride. Swine bones, according to Kick et al. (cited by McClure, McCann and Leone 1958) retained upwards of 0.3-0.40 percent fluoride before any toxic effects of fluoride were discernible. Mitchell and Edman (1952) found that mild form of exostosis was associated with 0.53 percent fluoride. Heyroth (1942) gave 65 mg of fluoride daily to each of two mate dogs. The intake was maintained for five years when a dog died. During life time no change could be detected radiologically in their bone, although after they were killed the bone ash of the dog given sodium fluoride contained ten times the amount of fluoride found in the control. The bones although chalky in appearance, exhibited no very striking abnormality. On the basis of the evidence that 0.01 to 0.15 percent fluoride is present normally in the bone ash of animals, Pierce (1939) suggested that the concentration of fluoride may increase ten
to fifteen fold in fluoride content without production of any untoward effect. McClure, McCann and Leone (1958) provided additional evidence regarding the threshold level of fluoride which can be tolerated by human skeletal tissue. As much as 0.5-0.6 percent fluoride (about ten times the quantity of fluoride regarded as normal) did not prove to be a physiological hazard. X-ray examination medical and clinical studies made prior to death did not reveal any skeletal abnormality. Smith and Hodge (1959) considered that in human, osteosclerosis would be evident with skeletal concentration of 6000 ppm. According to another study much lower concentration of fluoride are considered to provide toxic effects. According to Manz (cited by Steyn 1964) bone could be considered normal, if it contained 130 ppm and pathologic if it contained 650 ppm fluoride.

It is true that human skeletal tissue has very high degree of physiological tolerance to the accumulation of fluoride, but there may be a possibility of some other factors besides the fluoride concentration, which may produce pathological changes in bone. The minimum concentration of fluoride which has brought pathological changes in bone in this study is not ten to fifteen times the concentration of fluoride in normal bone (table VI). In case fluoride concentration is the only factor responsible to bring about the pathological changes, the patients having higher concentrations of fluoride ought to be more adversely affected than those having lower concentrations. However, no such effect has been observed. Residents of Bartlett, Texas,
showed no deleterious bone changes when the fluoride concentration in rib, iliac crest and vertebra was 630 mg, 577 mg and 540 mg per 100 gm dry weight respectively (McClure, and Zipkin 1958). In case the concentration of fluoride is the only factor, either the residents of Bartlett ought to have crippling fluorosis or most of the patients of crippling fluorosis of this study should have no deleterious bone changes. It is, therefore, evident that fluoride content of bone may not be the only factor incriminated in the causation of skeletal fluorosis.

Crippling fluorosis is mostly prevalent in old people. It was seldom noticed in people of less than 30 years of age. The age may be an important factor in the causation of the disease. The possible cause for this effect may be due to some difference between the young and old bone.

It is envisaged that the bone free crystal surfaces absorb a surface hydration shell, which is in rapid equilibrium with the surrounding medium and so with the extracellular fluid with which the interior ions of the crystal establish a slow but measurable equilibrium (Fraser and King 1957). Sundvall-Hagland et al. (1959) considered that reaction between bone crystals and fluoride takes place in three stages: (i) Fluoride ion diffuse rapidly into hydration shell. This reaction is reversible and the fluoride is not firmly bound. (ii) Fluoride then reacts with surface of crystals by exchanging positions with surface bound hydroxyl group. The rate of this reaction is slower than
that of the first phase and the attached fluoride is firmly fixed. (iii) Fluoride may penetrate into bone crystals and exchange with interiorly located hydroxyl group. This reaction is exceedingly slow. Due to this slow rate of exchange maximum theoretical concentration i.e. 3.5 percent fluoride in the bone is never reached. Exchanges with newly deposited bone mineral, for example, at metaphysis, are much more rapid than that of older bones. This may be due to decreasing water content with age. With advance in age, the penetration to interior located sites results in more firm binding of fluoride in skeleton. The bone of young people may have, therefore, any concentration of fluoride, but it may be located differently from that of older people. Due to its loose binding on the surface it might not play a significant role in the bone metabolism. In the case of old people besides the increase in concentration of bone fluoride (table VI) the fluoride is firmly bound and may affect the bone metabolism. The old people, therefore, become the victims of this disease.

Previous information on the effect of increased fluoride on the chemical composition of human bones is limited. In one case reported by Wolff and Kerr (1938) excessive fluoride ingestion increased bone ash fluoride up to 1.07% without, however, affecting the calcium, phosphorus or carbon dioxide content of the bones. Gluck, Lowater and Murray (1941) and Call and Greenwood (1958), observed no changes in ash, calcium, phosphorus in human bones containing up to 0.69 and 0.15% fluoride in ash respectively. The former investigators also found no change in magnesium content. McClure, McCann and
Lecne (1958) reported an increase in carbon dioxide, a reduction in magnesium and phosphorus but no change in calcium content of ash of a number of bones of one individual containing up to 0.9% fluoride. Zipkin, McClure and Lee (1960) presented a comprehensive study on the composition of human bone over a wide range of skeletal fluoride concentration. They observed no relationship between fluoride content of the bone ash and its calcium or phosphorus contents. However, a slight increase in magnesium content was observed.

In the present study no significant difference has been found in calcium and phosphorus content of bone (table XVI). There is a slight increase in magnesium content. It was found during the present study that the mass of the bone increased in fluorosis (Singh, Jolly, Bansal and Mathur 1963). In the determination of the mineral constituents the percentage of various constituents are determined and it is not possible to find total amount of the mineral constituents. It may be said that the proportion of various mineral constituents with respect to each other may not have undergone significant changes in fluorosis. The abnormality may be due to organic portion of bones as considered elsewhere in this study.

One of the important aspects of fluoride metabolism is its effect on calcification. It has been reported that fluoride administration improves calcium retention. Anbar (cited by Steyn 1964) showed that fluoride ion follows calcium in many aspect of its physiological behaviour. Recent studies have suggested that fluoride may be of value in prevention and treatment of some important skeletal diseases like osteoporosis and Paget's disease (Rich, Ensink 1961; 1962;
Hypercalcification is one of the most characteristic features of the fluorosis. The concentration of calcium, phosphorus and alkaline phosphatase was studied to find the effect of fluoride on calcification. The concentration of calcium in blood, urine and bone was found to be normal in the present study (Table VII and XI). In view of this observation, it may be said that fluoride and calcium concentrations are not directly related.

Physiologically, the level of serum calcium is quite closely regulated while serum phosphate concentration may vary considerably (Neuman and Neuman 1958). Serum inorganic phosphorus was found to be slightly less than normal limit but the acid soluble phosphoric ester was found to be raised (table VII). The high ester phosphate concentration may be speculated to be due to inhibition of glycolytic pathway.

It is well recognised that local concentration of phosphorus for calcification is due to action of alkaline phosphatase on phosphoric esters. The concentration of alkaline phosphatase in a large number of the cases was above normal (table VIII). Most of the patients under study were advanced in age and the normal value of alkaline phosphatase in the rest of the cases may be due to decreased osteoblastic activity at that age. The above mentioned conditions are favourable for hypercalcification.

Due to irregular calcification, the narrowing of the spinal canal or intervertebral foramina give rise to radiculo-myelopathic features (Singh, Jolly, Bansal, Mathur 1963). The
neurological complications are the worst feature of the fluorosis. At present no plausible explanation is given for this irregular calcification in this region.

Following observations are to be considered in this respect.

1. The irregular bone deposition was mainly in cervical and lumbo-dorsal region of spine which is subjected to constant motion and not in thoracic region in which the skeletal muscles are comparatively inert.

2. The population in which the crippling fluorosis is common are farmers or labourers who are to do hard manual labour and is rare in sedentary workers.

3. Irregular bone was laid down along the attachment of muscles as well as in joint capsules.

These observations lead to a common conclusion that hypercalcification in a portion of the body or the class of people is associated with increased muscular activity. Since the muscles derive their energy from glycogen, increased muscular activity imply increased glycogenolysis and glycolysis. The idea that calcification depends in some way upon the alkaline phosphatase has been broadened to include the entire glycolytic cycle (McLeans, Budy 1959). The development of exostosis in the region subject to stress and strains and in the people doing hard manual labour may be speculated to be combination of the effect of fluoride and glycogenolysis.

**Bone Citrate**

Fluoride was shown to increase the "crystallinity" of bone apatite of rats (Schraer et al. 1962; Menczel et al. 1962 and 1963) and human being (Zipkin et al. 1962; Posner et al. 1963) with a concomitant decrease in citrate content of bone (Zipkin 1963 and 1960; Zipkin et al. 1962 and 1963).
and Schraer et al. (1962) from their studies on rat femur, suggested that the effective crystal surface area of the bone was reduced as a result of large crystals and/or femur imperfections in the presence of fluoride, thus excluding citric acid from the bone surface. Griffith et al. (1964) were of the opinion that there was possibility of reduced citrate being associated with increased "crystallinity" but they suggested that low citric acid could also result from some other cause.

There is no uniform suggestion regarding the source of bone citrate. Kuyper (1938) reported that it might be due to coprecipitation with bone salts during the process of calcification. According to another view, it has been considered to be formed in the bone tissue itself under the influence of some external source notably the parathyroid glands (Neuman and Neuman 1958).

In the previous studies pertaining to the effect of fluoride on citrate content of bone the first possibility i.e. the non-skeletal origin of bone citrate was only considered and the studies were confined to experimental animals. The present study is the only study in which the concentration of bone citrate in fluorotic patients was determined.

The concentration of bone citrate in fluorotic patients was found to be below normal (table XVII). This observation confirmed the reported studies on experimental animals.
About 90% of total citric acid in the system was reported to be present in bone (Harrow and Mazur 1954) i.e. the concentration in blood is comparatively very small as compared with bone citrate. In case the deposition of citrate in bone from blood was retarded by fluoride (Zipkin et al. 1962 and 1963) it may be of interest to know the change in concentration, if any, produced in blood citrate. The blood citrate concentration was found to be normal (Table XVII). May be that either blood or bone citrate concentrations are not inter-dependent or that bone citrate is not derived from the blood but originate in the tissue itself as reported by Neuman and Neuman (1958).

The parathyroid glands are considered to be one of the factors to regulate the synthesis of bone citrate (Neuman and Neuman 1958). In case the bone citrate is produced in the tissue under the influence of these glands, it may be expected that decreased amount of bone citrate may be due to lesser activity of parathyroid glands. The parathyroid function tests (Singh B, 1963) were found to be normal in this laboratory. This excluded the possibility of interference by fluoride in the synthesis of bone citrate in the tissue under the influence of parathyroid glands.

The presence of all the intermediates of citric acid cycle in bone in metabolic active form has been reported by Lees and Kuypers (1957) contradicting the earlier report (Kuypers 1938) of non-skeletal origin of bone citrate. Neuman and Neuman (1958) have stated that the citrate forming enzyme are in more active form than citrate removing enzymes. These
findings favour the origin of citric acid in bone. Our observation (table XVII) shows no relationship between the blood citrate concentration and bone citrate concentration which also favours the skeletal origin of citric acid.

The defect in the metabolism of citric acid in ascorbic acid deficiency was observed by Takeda and Hara (1955) and Banerjee et al. (1958). The ascorbic acid deficiency was noticed in the fluorotic patients. In the absence of dysfunction of parathyroid glands, it may be speculated that decreased amount of citrate in bone may be due to the ascorbic acid deficiency.

Accumulation of skeletal fluoride has been shown to reduced Ca$^{45}$ turnover in bone and this was attributed to impaired remineralisation and resorption of bone mineral (Dunstone and Payne 1959). Fluoride also decreased artificially induced bone resorption (Hudson 1961). Neuman and Neuman (1958) suggested that inhibited resorptivity could give rise to abnormal changes in bone. This view was substantiated by Storey (1960), who succeeded in altering the balance between deposition and resorption, and demonstrated that a repressed resorptivity could produce various degree of petrification. Cantarow and Trumper (1962) were of the view that citrate may be concerned in resorption of bone. The decreased amount of citrate in bone is suggestive of decreased resorptivity. One of the factor for increased mass of the bone in fluorosis may be this decreased resorptivity and subsequent pathological effects.
Preventive aspect

The real use of any field survey work is to ameliorate the suffering of the people. There is no doubt that drinking water is the cause of fluorosis in the endemic area. Various methods have been suggested to defluorinate the water, i.e., use of columns of ion exchange resin, activated charcoal impregnated with alum, mixture of sand charcoal impregnated with lime. Defluorination by any chemical method has been found to be impracticable due to vast scattered area and lack of technical control.

There is a very intensive network of canals throughout the Punjab and the water analysis at different places show that its fluoride contents are within normal limits. Canal water sample near Bajekhana, a highly affected village, was found to contain 0.44 ppm fluoride. It was suggested that filtered canal water may be used for drinking purposes.

The people in the endemic area mostly consume water from surface wells or hand pumps. On an average the depth of these wells vary from 40 to 60 ft. Raghavachari and Venkataramanam (1940) found no relation between fluoride contents and the depth of the well, the strata in this case was rocky. In America deep sources of water were found to contain more fluoride and these have been abandoned in favour of surface sources of water. In village Papra in endemic area, the fluoride content of a hand pump installed at a depth of 120 ft. was found to be 1.3 ppm as compared to other sources containing 2.5 to 10.2 ppm of fluoride.
This suggested that the fluoride contents may vary with the depth. Deep drilling was affected at 250 ft. and fluoride content of the water was found to be 0.5 ppm. In this region, having sandy soil, the fluoride contents have been found to be proportional to the depth and deep source of water supply is the most effective way to save the people from the toxic effects of fluoride. The Punjab Government has given a practical shape to this observation and tube wells at a depth approximating 250 feet have been installed in village Papra and more are on their way.

The fluoride bearing rock is not uniformly located in a particular area. The fluoride content of water, therefore, differs at different places in the same locality. Due to this survey, the people have become alive to the danger of fluoride intoxication. On an advice from this quarter about the suitability of particular water for drinking purposes, attempts are made by boring at different places to find safe water. The attempts sometimes lead to the discovery of a source having low fluoride concentration.

In the United States, most naturally fluoridated water containing more than 0.5 ppm fluoride are hard waters, in which the "hardness" fluoride ratio may be as high as 500:1 (Dean 1943). The calcium and magnesium present in this "hardness" may provide a protective mechanism reducing fluoride absorption from the digestive tract. 8 ppm of fluoride in Bartlett produced only about 10% fluorosis of mild nature. The water of the endemic area under study is soft. The effect of increasing the hardness is being considered as a preventive measures.
The reduction in toxicity of fluoride by an external agent was considered in the light of biochemical observations. Calcium compounds are often used for such purpose. The concentration of calcium in blood, bone, and urine was normal. The only purpose of extra-dietary calcium may be to decrease the absorption in the alimentary canal because fluoride does not induce calcium deficiency. The same reasoning apply to the proteins and compounds of phosphorus and iodine. No sign of any other deficiency was observed in fluorotic patients except that of vitamin C. On administration of vitamin C, the patients felt better. One such case of paraplegia (B.S.) was completely invalid at the time of admission, is a changed man now. The suffering due to fluoride intoxication may be considerably reduced by the administration of vitamin C.