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THE METABOLISM OF F^{18} IN NORMAL AND CHRONICALLY FLUOROSED RATS

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INTRODUCTION

The manner in which an animal metabolizes small amounts of fluorides is a subject that has received a great deal of attention in recent years. This interest has been stimulated by two major factors; first, the fluoridation of municipal water supplies and the topical application of fluorine compounds to the teeth of children as prophylactic measures to combat dental caries, and second, the possibility of hazards to workers in industries utilizing large quantities of fluorides and to the population and domestic animals residing in areas near these industries.

The work described in this paper was performed with the hope that the use of radioactive tracer techniques might help to clarify some of the controversial problems encountered in the literature on the metabolism and toxicological properties of fluorides. The literature on the subjects of fluorine toxicology and chronic fluorine intoxication is voluminous. However, the major findings have been condensed and reviewed by Roholm[100] who has covered the field from the earliest reports to 1937, by Greenwood[43] whose review covers the period from 1937 to 1940 with some references to the earlier literature and by McClure[79] whose review covers the years 1940 to 1949.

Roholm emphasized the difficulty and uncertainty involved in the chemical analysis of biological material for fluorine. The method devised by Willard and Winters[132] which has been modified by Armstrong[2], although considered to be the most reliable, has several drawbacks: it is time consuming, it involves the use of perchloric acid which may explode with the loss of valuable samples, it must be very carefully standarized and it requires

the use of large amounts of material when the fluorine content of samples is small. It is understandable then that very few studies have been made on the distribution of fluorine in animal tissues other than bone, teeth, blood and pooled excreta; Chang et al[19] in 1934 reported the distribution of fluorine in the tissues of a dairy cow which had been fed raw rock phosphate for a number of years and Gautier and Clasumann[31,32,33] in a series of reports published in 1913 give values for the fluorine content of the pooled tissues of large animals such as sheep and dogs. It is difficult to evaluate the data obtained by different workers on the fluorine content of animal material, especially the older works, because the values obtained vary widely depending on the method of analysis employed. Because of the widespread occurrence of fluorine in soils and water supplies and its appearance as a contaminant of many foods and chemicals McClure[75,76,78], chlorides and bromides in particular, it is extremely difficult to maintain an animal on a diet that is relatively free of fluorides. For this reason it becomes necessary to administer fairly large amounts of fluorine when information is desired on its metabolic pathways and distribution, in an attempt to differentiate between the fluoride administered and that already present in the animal. This situation arises quite often in biological work and can be very nearly overcome by the use of radioactive isotopes.

In the case of fluorine the longest lived isotope available is F^{18} with a half-life of only 112 minutes. The short half-life places serious limitations on its usefulness as a biological tool. This isotope emits positrons which result in annihilation radiation (photons). The reduction of large amounts of mass ordinarily present in biological material in order to increase the efficiency of measurement of the positrons, a time consuming process which involves

ashing or digestion with acid, is out of the question when dealing with a short lived isotope especially one that forms many volatile compounds as does fluorine. The measurement of photons with a Geiger-Muller counter is a very inefficient process, necessitating the use of large amounts of activity. It is therefore understandable that there are only three investigations reported in which radioactive fluorine was used, two of these are by Volker et al[127,128] in 1940 and 1941 and one by Wills[133] in 1940. With the advent of the scintillating crystal gamma counter most of the problems heretofor encountered in the measurement of F^{18} were solved, the counting efficiency for photons is greatly increased and there is no longer any need to be concerned with reducing the mass of the samples, indeed ten to fifteen grams of fresh tissue can be counted with ease and considerable accuracy.

The work to be presented here follows two general lines which are outlined below:

(1) The metabolism of F^{18} in normal rats which includes experiments designed to shed light on some of the following problems.

(a) The excretion of F^{18}, an attempt to obtain quantitative data on urinary excretion and to determine whether or not there is fecal excretion of fluorine.

(b) The cellular permeability of F^{18}.

(c) The maternal transfer of F^{18}, an attempt to determine whether or not small amounts of fluoride are able to cross the placenta of the rat and whether or not there is secretion of fluoride in the milk of the rat.

(d) The extent of absorption of F^{18} when administered orally.

(e) The effect on the distribution of F^{18} when it is given in conjunction with stable fluoride.

(f) The localization of F^{18} in bone by use of the radio-autographic technique.

(g) The differences, if any, in the distribution of F^{18} in young adult animals and older mature animals.

(2) Some aspects of chronic fluoride intoxication in rats.

(a) The metabolism of F^{18} in fluorosed rats, with some references to their growth and general condition.

(b) The possible effect of chronic fluorosis on the function of the thyroid gland using the thyroidal uptake of I^{131} and the cell:plasma ratio of I^{131} as indicators.

(c) The localization of F^{18} in the bones of fluorosed rats with an attempt to correlate these findings with the pathological changes in the bones due to fluorosis.

Some of the more important aspects of the effects of fluorides on biological systems and of chronic fluoride poisoning will be discussed in the following pages.

GENERAL REVIEW OF THE BIOLOGICAL EFFECTS OF FLUORIDES

Effects of Fluorides on Enzymes

Inorganic fluorides are strongly toxic to some enzymes; yeast phosphatase, glucosulfatase, acid phosphatase, hydrogenlyase, urease, carboxylase and enolase Massart[72], while others are little affected: proteolytic enzymes and amylases Sollmann[116].

Certain of the enzymes are activated by bivalent metal ions such as Ca^{++} and Mg^{++}. Massart and Dufait[73] have shown that some of these enzymes are strongly inhibited by fluoride, namely acid and alkaline phosphatase, lipase, cholinesterase, chlorophyllase, carboxylase and enolase. Warburg and Christian[129] attribute this inhibition in the case of enolase to the immobilization of the metallic activator due to the formation of a dissociable inactive complex of magnesium or calcium fluorophosphate with the enzyme protein. They stressed that this formation of an inactive fluorophosphate-metal-enzyme complex was not the only mechanism whereby fluoride could inhibit enzyme activity as in the case of carboxylase, which is inhibited by fluoride regardless of the concentration of phosphate. Borei[15] and Runnström *et al*[102], after a searching investigation of the inhibition of yeast respiration by fluorides, came to the conclusion that the inhibition was due to a competition between F^- and cytochrome oxidase for cytochrome c. Since the cytochrome system and certain of the esterases are fundamental constituents of cells in general and are necessary to the production of energy by means of glycolysis, it is not surprising that Kaplan and Greenberg[61] and Handler and his co-workers[51,52] have found disturbances in the carbohydrate metabolism (accumulation of hexose-6-phosphate and lactic

acid, elevation of blood glucose and depletion of liver and muscle glycogen) of animals acutely poisoned with fluoride. Spira[118a] has shown that animals chronically intoxicated with fluoride are in better physical condition, if they are given sizeable supplements of B vitamins. Since it is common knowledge that certain of the B vitamins are components of coenzymes of oxidative enzyme systems and are therefore intimately linked with the functions of these enzymes, Spira's findings seem quite reasonable.

Acute Fluoride Poisoning

According to Valée[123] distinct symptoms of poisoning have been produced in man from 0.25 gm of sodium fluoride. Recovery has followed up to 9 gm and death has been caused in an adult by 4 gm Roholm[100]. The minimum lethal dose for mammals (dogs, rabbits, rats) averages about 0.05 gm per kilo of body weight by mouth and about 0.03 gm per kilo subcutaneously or by vein Roholm[100]. A slightly larger amount may be administered by vein than the values given above, but only when given in very dilute solution and over a considerable length of time.

Acute fluoride poisoning is not rare and usually results from the accidental ingestion of insecticidal or rodenticidal fluoride salts. Roholm[100] gives a complete record of all reported cases of fluoride poisoning either accidental or intentional together with a compilation of the symptoms and autopsy findings for the period from 1873 to 1935. Goodman and Gilman[40] summarize the symptoms as follows: initially symptoms are referable to the gastro-intestinal tract, salivation, nausea and abdominal pain often accompanied by diarrhea and vomiting. Systemic symptoms are varied and severe. The nervous system is affected and convulsions ofter occur. The blood pressure falls due to both central

vasomotor depression and direct action of fluoride on the heart muscle. The respiratory center is at first stimulated but is eventually depressed and death results from either respiratory or cardiac failure. Soluble calcium salts have been found useful in the treatment of acute fluoride poisoning by precipitating soluble fluorides that have not been absorbed from the digestive tract, and Kochmann[64] reports the successful treatment of acute fluoride poisoning in mice with injections of parathyroid hormone which mobilizes Ca^{++} from the bones.

Absorption, Storage and Excretion of Fluorides

A. Absorption

The absorption of orally ingested fluorine compounds appears to depend mainly on the solubility of the compound McClure *et al*[82]. There is, however, very little correlation between the solubility of the compound and the amount absorbed, when fluorides are ingested at low levels, such as are usually met with in foods, since at this level the solubility products[1] of the compounds are probably not exceeded Lawrenz *et al*[67]. To the extent that the compound is soluble in the fluids of the digestive tract, absorption of fluoride is quite an efficient process. In experiments on one human being with daily doses of 6 mgm of fluorine as NaF, Machle and Largent[70] found that 97 per cent was absorbed.

The form in which fluorides are absorbed and the site of absorption are topics that are still open to question. Although undissociated hydrofluoric acid seems to be able to penetrate the intact epidermis Görlitzer[41], there is no evidence, especially when the amount of fluoride ingested is

1 $K_{sp} = (M_y)^x(N_x)^y$

small, that it is absorbed by the gastric mucosa as undissociated HF formed by the reaction of the fluorine compound with the hydrochloric acid of the stomach, as proposed by Wieland and Kurtzahn[131] in 1923. The formation of HF may be a contributory factor in producing the corrosion of the gastric mucosa found in acute fluorine poisoning. Hauck and her co-workers[53] found no histological changes in the gastric mucosa and minor hemorrhages in the pyloric mucous membrane of rats that had received approximately 50 mgm per kilo of fluoride as NaF daily in their diet for from 1 to 10 months. Until more convincing evidence is presented, it is more reasonable to postulate that when small amounts of fluorine compounds are ingested, especially such simple ones as the alkali fluorides, they are absorbed for the most part as F^- from the small intestine.

B. Excretion

Fluorine seems mostly to be excreted by the kidney, in what form it is not known. McClure et al[82] showed that fluorides were also excreted through the skin in the sweat, which under conditions of elevated intake contained as much as 1.8 p.p.m. of fluorine. There has been considerable doubt as to whether fluorine is actually excreted by the intestinal tract. Since most of the investigators studying this problem administered fluorides to their subjects orally, many of them interpreted the presence of fluorine in the feces as the result of faulty absorption. Faulty absorption undoubtedly plays a part when the solubility of the administered compound is low, however, in 1891 Brandl and Tappeiner[16] proved fairly conclusively that there is fecal excretion of fluorine, when they demonstrated the presence of fluorine in the feces of a dog after a single subcutaneous injection of sodium fluoride. Wills[133] using F^{18} demonstrated that fluorine was secreted

in the saliva of cats after its intravenous administration.

C. Storage

It has been definitely established by many investigators that fluorine is stored to a large extent in the teeth and bones Roholm[100]. There does not appear to be notable storage of fluorine in any of the soft tissues even after long periods of fluorine intoxication. Although Chang et al[19] found that the normal, low fluorine concentration in the soft tissues of fluorosed cows was nearly doubled as was also the fluorine concentration in the blood, their findings do not indicate that fluorine is deposited in the organs to a degree comparable to the fluorine deposition in teeth and bones. The extent to which the skeleton may store fluorine is strikingly demonstrated by the findings of Gaud et al[30] who in 1934 analyzed the skeleton and organs of an ass attacked by darmous and found that the bone ash contained 8.65 per cent of fluorine, an amount greater than the 3.5 per cent, which would be theoretical for pure fluoroapatite.

There has been considerable controversy as to whether or not there is storage of fluorine, when it is ingested at very low levels. McClure et al[77] said, "There was no significant retention of fluorine in the bodies of these young adult men when total daily fluorine ingested did not exceed 4.0 to 5.0 mgm.", thus initiating the widely held view that the animal body tends to come to some sort of an equilibrium state when exposed to low doses of fluorine. Hodge[58] and Glock, Lowater and Murray[35] have shown by analyzing bone samples that the human skeleton accumulates fluorine throughout life even at relatively low levels of fluorine ingestion, where 0.06 p.p.m. of fluorine in the water supply and that present in the food probably do not total more than a daily intake of 0.5 to 1.0 mgm.

Hodge[58] states that the skeletal deposition of fluoride can be considered as a type of detoxification mechanism, that fluorides absorbed into the body fluids are promptly disposed of either by urinary excretion or skeletal deposition, and that this process takes place with great rapidity; in two to three hours it is practically complete. As a rough approximation 50 per cent of all ingested fluorine is excreted in the urine and 50 per cent is stored in the skeleton. That fluorine once reaching the skeleton is quite tightly bound is shown by Savchuck and Armstrong[103]. After withdrawal of the fluoride supplement only 10 to 15 per cent of the skeletal fluoride was excreted.

D. Maternal Transfer

The question of the excretion of fluorine in milk is of greatest importance with respect to the production of "mottled teeth" in children. Phillips, Hart and Bohstedt[94] found that fluorine content of normal cow's milk was 0.05 to 0.25 mgm per liter, averaging 0.14 mgm. They also found that the fluorine content of the milk was not increased significantly when cows were maintained on a fluoride supplement of 1 to 3 grams daily. Gaud et al[30] found no difference in the fluorine content of the milk of normal sheep and sheep attacked by darmous. However, two observations show that fluorine can be secreted in milk in fairly significant amounts. Brinch and Roholm[17] observed "mottled teeth" in children that had been nursed for long periods of time by mothers suffering from chronic fluorine intoxication, and Murray[84] found that the bones of young rats nursed by mothers receiving 0.05 per cent fluorine in the diet contained 30 times more fluorine than the controls. The above investigations seem to indicate a species difference between herbivorous animals and animals that normally feed on a mixed diet. There is only one work

that indicates the secretion of fluorine in the milk when the maternal fluorine intake is very small. Murray[84] found measurable amounts of fluorine (0.0007 per cent of the bone ash) in the bones of the control nurslings whose mothers had been fed a diet with relatively a low fluorine content.

There is a great deal of uncertainty as to whether or not fluorine is able to permeate the placenta, especially when the amounts ingested are small, such as those encountered in the normal diet. Sharpless and McCollum[108] stated that it was doubtful whether rats 16 to 18 days old contained any fluorine at all. However, when the amounts of fluorine ingested by the mother are increased significantly there is considerable evidence for the passage of fluorine across the placenta, although apparently with difficulty Murray[84] and Gaud et al[30].

Chronic Fluoride Intoxication

Fluorides are present in the water supplies in certain areas and in numerous edible foods. The amount in food varies widely with the type as well as the source. McClure[75,76,78] has prepared summaries of the fluoride contents of foods and beverages reported in the literature. The most important foods containing fluorides are phosphate baking powders, bones and bone products, teas from certain areas and insufficiently washed fruits and vegetables that have been sprayed with insecticides containing fluorides. Bartholmew[4] has reported that there are no changes either in growth or composition of plants grown in soil containing high concentrations of fluorides. Gaud et al[30] found that grains grown in fluoride areas (the "phosphate zones") of North Africa contained large amounts of fluoride but that three-fourths of it was on the surface of the grain due to dust.

It is reasonable to assume, therefore, that the chronic fluoride intoxication seen in herbivorous animals in these "phosphate zones" and in the areas where industrial fumes contain fluorides is the result of surface contamination of plants rather than of the direct incorporation of fluorides by the plants themselves. The inhalation of dusts and smokes containing fluorides is the most important factor to be considered when dealing with fluorides as an industrial hazard.

As is well known the most important source of fluorides for both man and animals is in drinking water. Drinking waters containing more than 1 p.p.m. of fluoride seem to be prevalent in the middle western and southwestern portions of the United States. Waters in certain areas of North Africa have been shown to contain considerable quantities of fluorides derived from the trickling of subsurface water through the fluoride laden phosphate rock Velu[124]. Water supplies in other areas of the world have also been found to contain varying amounts of fluoride. Waters may contain anywhere from a few p.p.m. of fluoride resulting in mottling of teeth to several hundred p.p.m., amounts which are considered toxic.

Small amounts of fluorides that produce no apparent effects when administered singly, lead to marked changes when their administration is continued for long periods of time. At the lowest levels of intake in drinking water and in food the only notable effect is "mottled teeth" in children. Spira[118] reports that the appearance of mottled nails is another early sign of chronic fluoride intoxication. Somewhat larger amounts, in experiment, industrial exposure or in areas of high fluoride waters (greater than 10 p.p.m.) may cause bone changes in adults. These bone changes have been summarized by Roholm[100] as follows:

TABLE 1

THE EFFECTS OF THE DAILY INGESTION OF SODIUM FLUORIDE ON RATS

Author	Dose (mgmF/kilo)	Duration of Experiment	Results
Sollmann et al 1921[117]	0.068-3.6	5-24 weeks	None
Bethke et al (1933)[10]	3.6	19 weeks	Incipient dental changes
	7.2-18	19 weeks	Pronounced dental changes
Lamb, Phillips et al (1933), (1934)[65,93a]	18-36	Several generations	Pronounced dental changes. Retarded growth and reproduction, reduced weight of young.
McClure and Mitchell (1931)[81]	36-50	78-95 days	Growth retarded, changes in bone ash, lowered food intake, reduced Ca retention.
Sollmann et al (1921)[117]	36-50	5-24 weeks	Increased mortality
Roholm (1937)[100] Bergara(1927)[7]	Greater than 50		Death in a few days to a few weeks. All of above symptoms highly exaggerated.

(1) Osteosclerosis, an occupational disease found in cryolite[1] workers in Copenhagen and an endemic disease found in natives of the "phosphate zones" of North Africa.

(2) A disease resembling Osteomalacia, endemic among herbivora in the environs of certain factories in Denmark, France, Germany and Switzerland.

(3) Darmous, a dental and mandible disease endemic among man and herbivora in the "phosphate zones" of North Africa.

(4) Gaddur, a dental and bone disease among herbivora in Iceland following volcanic eruptions.

From animal experiments it has been learned that continued ingestion of still larger amounts of fluorides impairs growth and reproduction and if the amount is sufficiently large it will increase mortality. The experimental findings of several investigations on the chronic intoxication of rats with sodium fluoride is summarized in Table I.

1. Calcium and Phosphorous Metabolism

Roholm[100] observed no significant changes in the blood calcium and inorganic phosphorus in cryolite workers with varying degrees of osteosclerosis. Normal values were found by Greenwood et al[44] for blood calcium, inorganic phosphorus and coagulation time in puppies that had been fed fluoride at levels (0.45 to 4.52 mgm/kilo of body weight/day) comparable to and higher than that consumed by man in some of the "mottled teeth" areas. McClure and Mitchell[81] and Lantz and Smith[66] found that growing rats on a diet that contained greater than 0.05 per cent fluoride as NaF retained less calcium and phosphorus than did their controls and that calcification extended over

1 Cryolite is a rare mineral of the composition Na_3AlF_6 found in workable quantities only at Ivigtut, Greenland. Nearly three-fourths of the cryolite quarried in Greenland is shipped to Copenhagen for processing and is used in the manufacture of aluminum.

a long period of time. At this level of fluoride intake they also found that the ratio Ca:P in the bones and in the feces was greater than normal. That chronic fluoride intoxication has little or no effect on the coagulability of the blood seems adequately proved by Roholm et al[101] who found no clinical evidence of increased coagulation time in cryolite workers and by Dyckerhoff[23] who found that fluorides retarded blood coagulation in vitro but not in the animal body.

It was postulated by Chaneles[18] that the effects of chronic fluoride intoxication on calcium metabolism and on the chemical composition and microscopic structure of the teeth and bones were due to the influence of fluoride on the parathyroid glands, however, later work by Hauck et al[53] and by Kick et al[63] failed to show any consistent gross or microscopic changes in the parathyroids of rats or chicks that were fed diets containing toxic amounts of NaF.

2. Gastro-intestinal Tract

Aside from the lack of appetite that accompanies the weight loss, symptoms of chronic fluorine intoxication are sometimes seen which indicate direct action of fluorides on the digestive tract (vomiting and diarrhea) and its associated glands, especially when the fluorine is administered in the form of a solution, or when as solid salts it is not thoroughly mixed with the food Roholm[100].

There is little mention of histological changes in the gastro-intestinal tracts of experimental animals when the fluorine dosages are low, however, Hauck et al[53] found minor hemorrhages in the pyloric mucous membrane of rats fed 0.15 per cent sodium fluoride for many months, and Slavsgold[110] found thickening and hyperaemia of the abomasum (fourth stomach of ruminants) and

the first part of the large intestine of lambs that had been fed hay contaminated with fluorine.

Ogilvie[87] found indications of increased mitotic activity and a greater width of the interlobular and intralobular septa in the pancreases of rats that had received intraperitoneal injections of 7.5 mgm NaF per kilo per day for 100 days.

At the level of fluorine intake mentioned above, Ogilvie[87] found no microscopic changes in the liver, in accord with the findings of Smyth and Smyth[112a]. However, with larger doses of fluorides there seems to be definite evidence of injury to the liver: fatty cell degeneration in the area around the hepatic vein in fluorosed sheep Velu and Zottner[125] and various types of degeneration of the liver parenchyma in chronically fluorosed cattle Phillips, Hart and Bohstedt[95].

Ogilvie[88] found very definite microscopic changes in the parotid and submaxillary glands but none in the sublingual glands of his chronically intoxicated rats. In the parotid gland the major changes were an increase in the mitotic activity of the alveolar cells, an increase in the size of the alveolar nuclei with indications of fatty degeneration. In the submaxillary glands there was vacuolization and unusual staining of the cytoplasm and the "type 2" cells had enlarged nuclei of irregular shape.

The work of Constantini[20] indicates that some of the functions of the gastro-intestinal tract may be inhibited by chronic fluorine intoxication. He made extracts of the pancreas, stomach and intestines of guinea pigs that were poisoned with sodium fluoride to the point of emaciation and found them to be less effective in splitting proteins than extracts made from normal animals.

3. Blood and Bone Marrow, Spleen and Lymphatic Tissue

Although the number of systematic investigations of the effect of chronic fluorine intoxication on the bone marrow and the blood is small, the evidence obtained from them seems to indicate that the ingestion of large amounts of fluorine over a long period of time produces disturbances in the normal activity of the bone marrow and the production of red cells. There is evidence that the white blood cell picture is little affected if at all.

Agate et al[1] performed hematological and radiological examinations of a large number of workers in an aluminum factory in Great Britain and of adults and children living nearby and found that the blood counts and hemoglobin levels were within normal limits, even in those factory workers who showed definite radiological signs of skeletal fluorosis. In a systematic examination of cryolite workers in Greenland and Denmark, Roholm[100] found very slight changes in the blood counts of these workers; a very mild anemia (7.8 to 9.8 per cent below normal) normal hemoglobin levels and an increase in the number of juvenile leukocytes. Pindborg et al[97] found that rats chronically intoxicated with fluoride showed a mild anemia concurrent with a decrease in the iron content of the incisor teeth and the liver and an increased excretion of iron in the feces. Roholm[100] fed two dogs (1) 196 gm of sodium fluoride and (2) 839 gm of cryolite over a period of nearly 20 months and found that both dogs had a severe anemia, an essentially normal white cell picture and a decrease in the number of platelets.

Pande and Lall[89] demonstrated a gelatinous degeneration of the bone marrow in cattle fed 3.0 to 4.0 mgm per kilo of NaF daily for several months. The red marrow in the metaphyses of the long bones and ribs tended to disappear,

hematapoiesis was absent from these parts and the animals were suffering from a hyperchromic macrocytic anemia. Slavsgold[110] found an atrophy in the marrow of the long bones of fluorosed sheep of the same kind as that found in starvation. In an autopsy of a cryolite worker who had severe osteosclerosis, Roholm[100] found that there was red marrow in the diaphyses of the femur and tibia. This hypertrophy may be a compensation for the general reduction in the size of the marrow cavity, however, Roholm feels that neither the marrow changes or the blood changes are secondary to the bone changes, because he was unable to find any correlation between the degree of blood changes and the degree of osteosclerosis.

The only demonstrable change in the spleen is an abnormal deposit of ferrous pigment; Roholm[100] in an autopsy of a man who had been a cryolite worker for 24 years and Leake and Ritchie[68] in dogs that were given 125 mgm of NaF twice weekly for 10 weeks.

Biester et al[11] found marked reaction of the germinative centers in the mesenteric lymph nodes of dogs that had been given 4.5 mgm per kilo per day of fluoride for more than a year.

4. The Kidney

All investigators seem to agree that the kidneys begin to show microscopic changes at a daily level of fluoride intake which produces very few changes in animals other than fluoride storage in the bones and teeth and dental fluorosis in young animals. The lowest level of fluoride intake that has been shown to produce kidney changes in young adult male rats is 7.5 mgm per kilo of NaF (given intraperitoneally for 100 days) Ogilvie[87]. The kidneys of these animals showed an edema in the connective tissue between the tubules of the lower half of the papilla suggesting amyloid degeneration. There was increased vascularity of the glomeruli and of the medulla. At

higher levels of fluoride intake macroscopic alterations appear in the kidneys; Hauck et al[53] found kidneys of rats fed approximately 50 mgm per kilo of fluoride as NaF daily for from 1 to 10 months pale and hob-nailed. Kidney changes ranging from a mild chronic nephritis to fatty degeneration depending on the dose and the length of exposure have also been reported in other species; dogs, Biester et al[11], pigs, Kick et al[62], guinea pigs, Marconi[71] and man, Roholm[100].

That there is interference with the function of the kidneys at fairly high levels of fluorine intake has been shown by Gottlieb and Grant[42], who gave intravenous injections of 5 to 20 mgm per kilo of NaF to dogs. The output of urine was increased as were the urinary outputs of chloride and nitrogen. The urine was distinctly alkaline. The thirst and simultaneous polyuria seen in experiments with pigs Kick et al[62] and McClure and Mitchell[80] may be considered a sign of renal irritation.

5. The Endocrine Glands

(a) The Thyroid Gland and the Basal Metabolic Rate

Some of the earlier investigators claimed that animals on low fluorine supplements for long periods of time showed the following changes in the thyroid gland: (1) a persistent struma-like swelling in the throat of a dog Maumené[74] (1854), (2) a five or six-fold increase in the size of the thyroid glands of white rats that had received 2 to 3 mgm of sodium fluoride in their food for 6 to 8 months Goldemberg[37] (1921), (3) a proliferation of the parenchymatous tissue of the thyroids of guinea pigs which had died of fluoride poisoning Christiani[21] (1930). Goldemberg[39] in 1930 further stated that the basal metabolic rate of white rats on a small fluoride supplement for 6 to 8 months was lowered from 12 to 63 per cent of

normal. These observations prompted him to set forth a theory that mild fluorosis of long duration was the cause of endemic goiter and to suggest the treatment of thyrotoxicosis in humans with NaF. In 1923 he also claimed that the growth inhibition in young rats on fluorine supplements was a form of cretinism and called this condition "cretinism fluorique" Goldemberg[38].

Subsequent investigations have failed to bear out much of the above cited work and Goldemberg's theories on the cause of goiter and cretinism have been fairly well discredited. In 1935 Phillips and his co-workers[92,93] found normal BMR's for rats fed sufficient NaF to produce emaciation in six weeks and instead of being antagonistic to thyroid hormone simultaneous injections of sodium fluoride and dessicated thyroid were found to be more toxic than when either substance was given alone. In work reported in the early 1930's neither Chaneles[18] nor Phillips and Lamb[96] could find any significant microscopic changes in the thyroids of the rats that were fed 15 to 30 mgm per kilo of fluoride for many weeks. They found slight parenchymatous proliferation and occasional signs of fibrosis in about one-half of their experimental animals, but also in 10 to 15 per cent of their controls. At high levels of intake (0.043 per cent of NaF in the diet) they found thyroid changes similar to those found in prolonged starvation. More recently Ogilvie[87] found slight reductions in the size of the nuclei and the amount of cytoplasm in the epithelial cells lining the follicles. Biester _et al_[11] in feeding experiments on dogs found thyroidal changes resembling those of animals in a state of severe malnutrition.

Chang _et al_[19] report that the fluorine content of the thyroid gland in a cow that had been subjected to chronic fluoride intoxication for a long time was increased 24-fold. Evans and Phillips[25] determined

the fluorine and iodine contents of the thyroid glands of about forty patients undergoing thyroid surgery and found no correlation between the fluorine content of the gland and either the basal metabolic rate of the patient or the iodine content of the gland. Murray et al[86] were unable to find any correlation between endemic goiter and fluorosis.

The earlier investigations must be viewed with caution because technical details are scanty, there are no photomicrographs of their histological preparations available for study and they did not seem to have adequate control for their work.

(b) Other Endocrine Glands

Parathyroid-Some mention of the parathyroid has already been made in the section dealing with calcium metabolism. Further emphasis should be made here, however, that no significant changes in the parathyroids have been noted in fluorosed animals and that there is no evidence to support the theory that the effects of fluorosis on the bones and teeth are mediated by the parathyroids.

Hypophysis-Phillips and Lamb[96] found the size, gonad-stimulating function and microscopic structure of the hypophysis normal in all of their animals at all of the dietary fluorine levels tested.

Adrenals-Ogilvie[87] found the adrenals of his animals normal in all respects. At higher dose levels Phillips and Lamb[96] found the adrenals slightly increased in size and a tendency towards hyperemia in the zona reticularis of the medulla.

Testes and Ovaries-A certain tendency towards atrophy of the testes and possibly of the ovaries was seen by Phillips and Lamb[96] in rats receiving 20 to 30 mgm of fluoride per day. Hauck and her co-workers[53]

found considerable atrophy of the germinal tissue of the testes with an almost complete absence of sperm in animals on a diet containing 50 mgm per kilo of fluoride. Phillips[91] showed that there was a reduction of fertility or a cessation of reproduction in fluorosed rats that seemed to be secondary to the general systemic reaction to fluorine, especially when the intoxication interfered seriously with nutrition.

6. Other Tissues and Organs

Biester et al[11] found fatty degeneration of the myelin sheaths of the spinal cord in fluorosed dogs on diets low in Vitamin C. The only other observations of an adverse chronic effect of fluorine intoxication of the central nervous system or on nervous tissue are those of Taylor[122] and Leake and Ritchie[68] who noted a certain irritability in their experimental animals after long periods of ingestion of fluorine compounds.

At moderate levels of fluorine intake, Biester[11] (4.5 mgm/kilo/day in dogs) and Phillips and Lamb[96] (20 to 30 mgm/kilo/day in rats) found no other significantly consistent histological changes in any other tissues or organs than those already covered in these pages.

7. Dental Tissue

The effects of chronic fluorine intoxication on the teeth have been thoroughly studied and there is far reaching agreement among the results of the various investigators. Since this study of fluorine and its physiological effects is not primarily concerned with the changes in the teeth in chronic fluorosis, the subject will be touched upon here very briefly and will be limited to a description of the changes produced in the teeth of rats. The reviews of Roholm[100], Greenwood[43] and McClure[79] as well as the conferences held by the American Association for the Advancement of Science[77,106] cover

the fields of study involving the relationships between the fluorine intake, dental caries incidence and the occurrence of "mottled teeth" in children most adequately.

(a) Gross Changes

The gross changes depend upon the amount of fluorine compound ingested, its nature and the method of administration. At the lowest dosage levels the normal orange pigment of the incisors of the rat disappears. The enamel becomes lighter, loses its lustre and finally becomes a chalky white. Larger doses result in hypoplasia of the enamel which loses its strength and begins to chip off. The sharp edge of the incisors wears off and becomes almost flat; the incisors may become worn down almost to the gums. Often one of the incisors may break and the loss of functional attrition is followed by elongation of the opposite tooth. Position anomalies may also be present Roholm[100].

The lowest levels of intake produce alternating rings of faulty enamel and normal pigmented enamel. Intermittent injections of fluorides will cause the appearance of these alternating bands.

The threshold for producing the alternately pigmented and colorless bands in the rat incisor that are visible with a hand lens is about 0.1 mgm per kilo per day of fluorine. With twice this intake the striations become visible to the naked eye and at an intake of 1 mgm per kilo per day the most marked changes, with chipping of the enamel occur Smith and Leverton[112]. The smallest doses must be given for from two to three weeks before the changes first become recognizable in the rat incisor. In the molars of rats as in animals whose teeth do not grow from a persistent

pulp, dental changes are limited to those parts of the teeth that calcify during the period of fluorine ingestion.

Murray[85] found a significant correlation between the state of nutrition and the severity of dental fluorosis. Using the same water supply in an area of endemic fluorosis in Morocco she found much more severe dental damage in the native Moroccan children on a poor diet with signs of malnutrition than in healthy European children on an adequate diet.

Histological examinations of the teeth of fluorosed animals have been made by Bethke et al[10] and by Schour and Smith[105] among others and in general the descriptions agree quite well. The characteristic features are: (a) calcification, a disturbance in the calcification of the dentin which shows an abnormal interglobular texture and an accentuation of the normal stratification consisting of pairs of light (eosin-staining) and dark (hematoxylin-staining) layers, an accentuation of the normal incremental stratification of the enamel matrix and a tendency for the enamel to persist in the immature acid-resistant stage of calcification. (b) Appositional growth, there is a disturbance in the appositional activity of the ameloblasts as evidenced by rhythmically recurring local arrests in enamel formation. The ameloblasts tend to be short and the epithelial papillae become irregular in both number and arrangement. The dentin shows analogous, but less frequent effects in the presence of vascular inclusions Schour and Smith[106].

(c) Chemical Composition

There is general agreement among the many investigators that chronic fluorine intoxication results in an increase in the fluorine content of both the enamel and dentin. There is considerable doubt as to whether there result any changes in ash content or in the Ca, P, Mg and CO_2 content

of the teeth in fluorosis, although there are indications that the ash content and the ratio Ca:P may be lowered Smith and Lantz[111] and Hauck et al[54]. Phillips et al[95] by studying x-ray spectra found that the crystalline nature of tooth ash is similar to fluorapatite. Enamel and dentin from normal cows in general gave spectra similar to that of cows intoxicated with fluorine.

8. Osseous Tissue

The investigations in this particular field have been complicated by the fact that the various experimental animals do not seem to react in quite the same manner. In view of this, emphasis will be placed on the findings in the rat, with but brief indications on the results with other species.

(a) Gross Changes

When McCollum et al[83] described for the first time the characteristic dental changes in the rat, they also observed certain abnormalities in the cranial bones. The color seemed to be whiter than usual and the quality poorer. The surface was slightly porous and lacked the usual lustre. Bethke et al[10] were unable to show measurable deviations in the dimensions of the cranial bones, although the mandibles seemed to be shorter than normally. In young rats on a diet with 0.1 per cent of NaF Lantz and Smith[66] described a short stunted build and curved legs. In cows Phillips et al[95] saw considerable changes: the bones were softer and thicker than normally and in all of the small joints there was calcification of the cartilage. In man Roholm[100] found calcification of the ligaments and in advanced cases a reduction of mobility of the vertebral column and spine.

(b) The Threshold for Skeletal Changes

It is interesting to note that while skeletal changes are rare in areas in the United States where the fluorine content of the drinking water is high, they are quite common in certain districts in India. The reason for this discrepancy seems to lie in the fact that a certain amount of fluorine in the water (8 p.p.m.) which in itself will produce only dental anomalies, will in combination with either dietary deficiencies (Vitamin C or D) or renal damage produce tangible skeletal injuries Pandit et al[90]. In one case of skeletal fluorosis reported in the United States, there was a chronic pyelonephritis Bishop[12], Bauer et al[5] and in another case the patient was suffering from uremia Linsman and McMurray[69].

The findings in spontaneous and experimental fluorosis are in part contradictory. The bones of human patients are described as sclerotic and on the other hand osteoporosis and "osteomalacia" have been reported in experimental animals. These contradictions have in part been explained by differences in dosage and age. Small doses may be said to produce sclerotic changes in adults, whereas large doses produce "osteomalacia" in young animals Weinmann and Sicher[130].

(c) Histopathology

Dittrich[22] published histological studies of the vertebral columns and long bones of young rats, rabbits and guinea pigs which had been given large amounts of fluorine for about 3 months (120 to 250 mgm/kilo/day of NaF). In the young rats there was disturbance in the ossification of the femur in the epiphyseal line with inhibition of longitudinal growth. The palisade zone lost its columnar arrangement and cancellous bone atrophied under the formation of cavities. In the diaphyses of the long bones the number and

size of the Haversian canals were increased and the lamellary structure around the canals was indistinct. Increasing dissolution and irregularity of the bone substance towards the medullary cavity was observed. In animals killed following a period of increased calcium intake after the fluoride was withdrawn reparative changes were observed. At lower levels of fluoride intake (50 mgm/kilo/day) young rats showed no unusual bony changes after three to five months. After one year or more the fibrils of the matrix showed irregularities and among the fibrils numerous dark-staining granules were noted. Rats that were given 75 mgm NaF per kilo per day developed an abnormal amount of osteoid tissue around the Haversian canals. No osteoclastic activity was noted. Rats on a diet low in calcium died after one to two months administration of fluoride and demonstrated a marked generalized osteoporosis Sutro[119].

Weinmann and Sicher[130] summarize the bone findings as follows: "There is, in all animals, a greatly increased osteoclastic resorption of the premorbid bone. In the shaft of the long bones, the compact layer has either disappeared or has been broken up into an irregular network of trabeculae. The destruction of the bone proceeds eccentrically toward the periphery. At the same time, regeneration and compensatory formation of new bone produces a covering of spongy bone which is immature and coarsefibrillar. The formation of these osteophytes which has evidently started on the periosteal surface of the premorbid bone progresses rapidly in young animals. Evidence of this rapid growth are the numerous and continuously arranged osteoblasts and the wide borders of osteoid tissue on the peripheral trabeculae."

PRODUCTION AND STANDARDIZATION OF F^{18}

The fluorine isotope used in this series of experiments was F^{18} which is radioactive with a half-life of 112 minutes Snell[113] and emits positrons with a maximum energy of 0.64 Mev Blaser et al[13]. F^{18} was produced by the bombardment of fractionally distilled water from the Cutter Laboratories with 28 Mev alpha-particles on the 60" cyclotron at the Crocker Laboratory by the reaction O^{16} (α, pn)F^{18}. The yield from this reaction averaged 750 microcuries per microampere hour bombardment.[1]

Water was used as the target material, because it was easy to handle and there were no other activities induced by alpha-particle bombardment with half-lives of more than a few minutes. The target water was bombarded in a small all-glass cell with a thin window (less than 100 mgm/cm^2). During the bombardment the target cell was cooled by a stream of cold air blown onto the back of the chamber and fitted with a water-jacket condenser to prevent excessive loss of the target material by evaporation. The cyclotron beam intensity was monitored by passing a copper wire through the condenser into the target cell and measuring the current in the water. In order to avoid thermal damage to the thin window of the target cell during the bombardment, the cyclotron beam intensity was limited to less than 6 microamperes. Figure 1 shows the target cell and cyclotron window assembly set up for bombardment.

After a brief interval to allow the short-lived activities produced to decay away, the target material was transferred to a beaker, 350 microliters of 1.5 N NaOH was added and the resulting solution evaporated almost

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1 One microampere hour of alpha-particles equals 3 x 10^{12} incident particles per second for one hour.

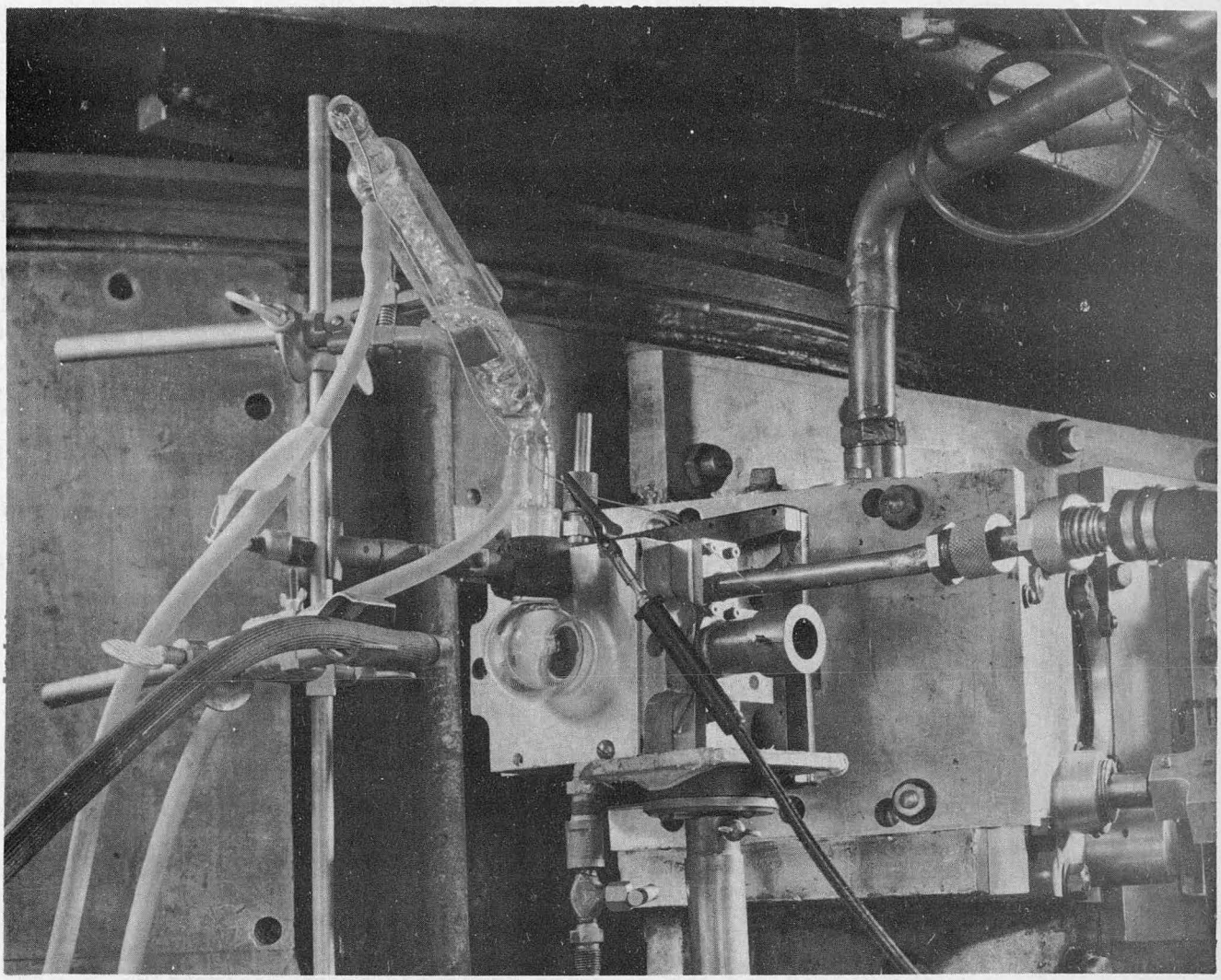

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Fig. 1 Water cell and cyclotron window assembly used for the production of F^{18}.

to dryness. After cooling, an equal amount of 1.5 N HCl was added to bring the solution to pH7. Distilled water was added so that the final volume of the solution was 3.5 ml. The above concentrations of the solutions of NaOH and HCl were chosen so that 100 microliters of each solution when mixed together and diluted with 0.8 ml of distilled water would produce 1.0 ml of neutral isotonic saline suitable for injection. The stock solutions of NaOH and HCl were assayed for fluoride by the firm of Curtis and Tompkins and were found to contain an amount of fluoride such that 1.0 ml of neutral isotonic saline prepared from them contained 0.15 micrograms of stable fluoride. These stock solutions of NaOH and HCl were used in all of the F^{18} preparations in the experiments to be described here, with only one exception which will be mentioned later. Depending on the number of animals used in each experiment the volume of F^{18} solution administered to each animal ranged from 0.5 to 1.0 ml which corresponded to the administration of stable fluoride in amounts ranging from 0.075 to 0.15 micrograms. Despite the presence of the small quantities of stable fluoride present in the F^{18} preparations employed in these experiments, they will for practical purposes be considered essentially carrier-free.[1] For the sake of brevity F^{18} solutions prepared by the method and the reagents described above, containing variable quantities of F^{18} and 0.15 micrograms of stable fluoride per ml, will be henceforth designated simply as F^{18}.

The half-life of all F^{18} preparations was checked with a decay curve for at least eight hours and in the early preparations for 24 hours. At no time was there evidence of the presence of any long-lived contaminants. The positron energy was determined in the early preparations as 0.67 Mev by mass absorption in aluminum, a value that agreed quite well with the

1 A carrier-free preparation of a radio-element is one in which all of the atoms of the element are radioactive.

value of 0.64 Mev determined by Blaser et al[13].

The activity of all samples was determined by taking advantage of the 0.5 Mev photons produced by the annihilation of the positrons. All samples were placed in tin bottle caps and were covered with a 250 milligram aluminum filter to absorb the positrons. The counting device employed was a scintillating sodium iodide crystal gamma counter designed for use with a Tracerlab "Autoscaler" as described by Jenkins[54]. The sensitivity of this instrument for 0.5 Mev photons when the sample is 4 cm from the crystal was such that 2000 c/s represented 1.0 microcurie of F^{18}.

During the assay of samples a standard quantity of F^{18}, 1/100 of the dose administered to the experimental animals, was counted hourly and corrections made for decay.

All samples were counted until at least 512 counts had been accumulated. For a total of 512 accumulated counts and a ratio of total counting rate to background counting rate of 1.5 or less, the error introduced because of the statistical nature of particle counting is greater than 10 per cent, Ghelardi and Brown[34]. This situation arose in the counting of some of the samples in experiments that took long periods of time, approximately five or more half-lives, when decay and excretion had reduced the radioactivity present in some of the soft tissues to very small quantities. It was felt that an error of 10 per cent due to counting alone when added to the error introduced by natural biological variation would raise some doubts as to the validity of conclusions drawn from such assays. When the counting rate of a sample was found to be less than 1.5 times the counter background, the per cent of the administered dose of F^{18} will be shown as less than a certain quantity. This quantity is the per cent of the dose that represents one-half of the counter background. For example; if at any given time the counting

rate of the administered dose was 20,000 c/s and the counter background was 2.00 c/s, one-half of the background was equal to 0.005 per cent of the dose, then all samples for which the total counting rate was less than 3.0 c/s would be shown as containing $<$ 0.005 per cent of the administered dose.

MATERIALS AND METHODS

(a) Experimental Animals

All of the animals used in this series of experiments were female rats of the Long-Evans strain. When the animals were used they averaged 185 grams in weight (range 155 to 210 grams) corresponding to a range in age of from 8 to 13 weeks. Female Long-Evans rats in this age range are considered to be almost mature (young adults), since the age at which rapid weight gain ceases and most of the epiphyses close has been shown to be about 11 to 17 weeks by Simpson et al[109]. In general F^{18} was injected into the external jugular vein while the animals were under light ether anesthesia and the incision was closed with metal wound clips. Following the injections animals were placed in metabolism cages in lots of from three to five and were given water but no food.

(b) Diet

The animals used in experiments (1) through (5) were maintained on tap water and a pelleted stock diet comparable in composition to the "Diet 14" prepared by the University of California Institute of Experimental Biology. The composition of "Diet 14" is given in Appendix I. Both food and water were given ad lib. An analysis of the feed by the firm of Curtis and Tompkins showed it to contain 16 p.p.m. of fluoride. On the basis of a daily intake of 8.5 grams for every 100 grams of body weight this would amount to a daily intake of 1.36 mgm per kilo of fluoride. According to information received from the East Bay Municipal Utility District[24], the Berkeley water supply which is derived from the Mokulmne River drainage contained no greater than 0.03 p.p.m. of fluoride as of June 1, 1952.

TABLE 2

THE NUMBER OF ANIMALS PER GROUP AND THE AMOUNT OF F^{18} ADMINISTERED TO EACH RAT IN MICROCURIES PER GRAM OF BODY WEIGHT.

		1/4 hour		1 hour		4 hours		9 hours	
Exp. No.	Description	No. Rats	μcF^{18}/gm body wt.	No. Rats	μcF^{18}/gm body wt.	No. Rats	μcF^{18}/gm body wt.	No. Rats	μcF^{18}/gm body wt.
(1)	young adults I.V. administration	5	1.25	10	1.20	10	0.69	12	1.45
(2)	G.I. operated			5	0.76				
	ephedrine			5	0.93				
(3)	young adults oral administration			5	0.68	5	3.56	5	2.59
(4)	F^{18} + carrier	5	2.23					3	2.43
(5)	pregnant			2	1.57				
	lactating					3	2.62		
(6)	fluorosed			5	0.49	5	0.22	5	0.63
	mature control			4	0.66	4	0.59	5	0.68

The composition of the semi-synthetic diet fed to the animals in experiment (6) is shown in Appendix II. This diet appeared to be adequate, since the growth curve obtained for the control animals closely followed the curve for the growth of female rats of this strain obtained by the University of California Institute of Experimental Biology using "Diet 14" which is considered adequate to support normal growth and development. An analysis of the purified diet used in these experiments made by Curtis and Tompkins showed that it contained 8 p.p.m. of fluoride. In the case of the control animals this would amount to a daily intake of 0.5 mgm of fluoride per kilo of body weight, assuming that the daily food intake was 15 grams per rat. The occurrence of fluoride in the diet is probably due to small amounts present in the chemicals that were used to prepare the mineral mixture, especially the NaCl and KCl.

The mineral mixture used is a modification of that devised by Hawk and Oser[55]. The most serious modifications were the omission of NaF which they add to the mixture so that the final ration contains 10 p.p.m. of added fluoride and the inclusion of a small amount of $CuSO_4$ to the diet, an addition recommended by Griffiths and Farris[45].

Table 2 summarizes the number of rats in each experimental group, the average body weight for the group and the amount of F^{18} administered to each rat in microcuries.

(c) Calculations (Calculation of the per cent of administered dose of F^{18} in the total blood, muscle, skeleton and balance.)

In the case of muscle and blood, the total F^{18} contents were calculated assuming that these two tissues correspond to 45 per cent and 7 per cent of the total body weight respectively.[1] When red blood cells and plasma were

1 These figures have been found to be reasonably accurate in the experience of this laboratory, Scott and Hamilton[106a].

assayed separately a hematocrit of 50 was assumed. The calculations were made as follows:

per cent F^{18} in muscle = 0.45 x body wt. x per cent F^{18}/gm of wet tissue.

per cent F^{18} in blood = 0.07 x body wt. x per cent F^{18}/ml whole blood.

The F^{18} content of the entire skeleton (including the teeth in the experiments in which they were not removed) was estimated from the calculated wet weight of the skeleton and the per cent of F^{18} per gram of wet bone obtained from the leg bone samples. The wet weight of the skeleton was obtained by ashing the skinned eviscerated carcass in a muffle furnace, removing the soft tissue ash by washing with water and drying and weighing the bone ash. The wet weight was then calculated as follows:

$$\text{wet wt. of skeleton in grams} = \frac{\text{ash wt. of skeleton in grams,}}{0.366}$$

where the ash content of young adult female rat bone from which the fat has not been removed is 36.6 ± 0.71 per cent Ray and Asling[94]. The per cent of administered F^{18} in the skeleton was then calculated from the wet weight of the skeleton:

per cent F^{18} in skeleton = wet wt. skeleton (gms.) x per cent F^{18}/gm wet bone.

The per cent of the administered F^{18} in the muscle, bone, blood and cartilage samples was added to the per cent of dose for the eviscerated, skinned carcass. From this "total carcass" were subtracted the values obtained for total blood, muscle and skeleton. The remainder which varied considerably (from 2.8 to 13.5 per cent of the administered dose) has been designated as "balance". This consists of tissues such as: connective tissue, cartilage, glandular tissue, lymphoid tissue, fat, nervous tissue, urinary bladder and blood vessels.

(1) Studies on the Distribution of Intravenously Administered F^{18} in Young Adult Rats.

In order to determine the rate of disappearance of F^{18} from the blood, 35 rats were each given F^{18} intravenously and were sacrificed in groups of five at the following time intervals: 1, 5, 20, 30, 45 and 60 minutes after injection. Blood was withdrawn by heart puncture and red cells and plasma were separated by centrifugation. One ml of packed red cells and one ml of plasma were assayed for F^{18}.

Large scale tracer studies on the distribution of F^{18} in the rat were set up and two lots of rats were used. The first lot consisted of 21 animals and the second of 16 animals. The animals in the first lot were given F^{18} intravenously and were sacrificed according to the following scheme: five animals at 15 minutes, 1 hour and 4 hours after the injection and two groups of three animals, 9 hours after the injection. At the sacrifice of the animals a blood sample was taken by heart puncture and the pelt was removed. The following tissues and organs were removed and weighed: lung, liver, kidney, spleen, stomach, small intestine, large intestine, cecum, the femur, tibia and fibula of the right hind leg, (hereafter referred to as bone), muscle from the right hind leg, pancreas, brain, cartilage from the xiphoid process, lacrimal glands, cervical lymph nodes, parotid glands, thyroid and adrenals. The skinned eviscerated carcass was ground in a meat grinder and divided into several portions for assay. The contents were removed from the stomach and intestines, and the empty gastro-intestinal segments and their contents were assayed separately. The pelt was weighed and approximately one-fourth was assayed. The extremities and bodies of the bones were counted separately. The small tissues and those for which it was anticipated that the F^{18} content would be low were pooled in order to increase the weighing and counting accuracy; these were pancreas, brain, spleen, salivary

glands, lacrimal glands, lymph nodes, thyroid, adrenal and cartilage.

The second lot of animals were given F^{18} intravenously and sacrificed in groups of five at intervals of 1 and 4 hours after injection and two groups of three animals were sacrificed 9 hours after injection. The procedures followed were the same as those described except that in addition to the above mentioned tissues and organs, the molars, incisors and mandibles were also taken. The results for the two series of experiments were pooled.[1]

(2) Studies on the appearance of F^{18} in the Gastro-intestinal Contents of the Rat Following Intravenous Administration.

This section of the work is an extension of the preceding section and was designed to shed further light on the presence of F^{18} on the gastro-intestinal contents following the intravenous administration of F^{18}.

In the first half of this experiment five animals that had been fasted overnight were given 10 mgm per kilo of ephedrine sulfate intraperitoneally. Forty-five minutes later they were given F^{18} intravenously and were sacrificed one hour after the F^{18} injection. At autopsy the following tissues and organs were taken; blood, stomach, small intestine, cecum, large intestine, bone, muscle and skin. Excreta were collected, urine mainly from the bladder. The contents were removed from the stomach and the small and large intestines.

In the second half of this experiment five animals that had been fasted overnight were put under deep ether anesthesia, a midline incision was made in the abdomen and ligatures were placed around the cardiac sphincter, the pylorus and the bile duct. The operation was performed as rapidly as

1 The per cent of F^{18} in the molars and incisors as determined in the second series of experiments has been subtracted from the per cent of F18 found in the skeletons of the animals in the first series so that the results of the two series might be combined.

possible and on the average took about ten minutes. The gut was handled with utmost care and was kept moist throughout the operation. After suturing the abdominal muscle and the skin, the animals were given F^{18} intravenously. One hour after the operation and injection the animals were sacrificed and the tissues and organs taken were the same as above and in addition included liver and kidney. Saliva samples were obtained from the mouths and throats of three of the animals with cotton swabs. Although there was no appreciable blood loss during the operation, there was seepage of a bloody fluid into the peritoneal cavity afterwards.

(3) The Distribution of Orally Administered F^{18}.

Fifteen rats that had been fasted overnight were given 2.0 cc of F^{18} solution orally by stomach tube followed by 1.0 cc of saline wash. These animals were sacrificed in groups of five at intervals of 1, 4 and 9 hours after the administration of F^{18}. The tissues and organs taken and the sampling and counting techniques employed were the same as in the experiments described in the second paragraph of section (1).

(4) The Effect of Milligram Amounts of Stable Fluoride on the Distribution of F^{18}.

Eight rats whose body weight varied less than 5 grams from each other were given a mixture of F^{18} and stable fluoride as NaF intravenously. The injected solution was F^{18} in isotonic NaF and was given in sufficient amount so that each rat received 10 mgm per kilo of stable fluoride. (These experiments will be referred to later as F^{18} plus carrier). Five of the animals were sacrificed 15 minutes after the injection and the other three, 9 hours after the injection. The tissues and organs sampled were the same

as those listed in the second paragraph of section (1).

(5) The Maternal Transfer of F^{18}

Two pregnant rats were given F^{18} intravenously two days before term. They were sacrificed one hour after the injection and the following tissues and organs were taken for assay: blood, liver, kidney, gastro-intestinal tract, muscle, bone, pelt, fetuses, placenta, uterus and amniotic fluid and excretions. Despite the fact that only two animals were used the results are considered semi-quantitative, since sufficient activity was administered to eliminate serious errors in counting. At the time the samples were counted one per cent of the dose was 370 c/s.

Three rats with 16 day old litters (litters were reduced to six) were given F^{18} orally by stomach tube. Ordinarily animals receiving material by stomach tube were fasted overnight, but fasting the animals was considered unwise in the particular case, since lactation alone places the animals under considerable strain and there was the possibility of the mothers devouring some of their young, if food were withdrawn. Although more control data was available on the distribution of F^{18} administered intravenously, it was considered advisable to give the material in such a fashion that the animals would not be subjected to the trauma of an operation or the after effects of anesthesia, in which case they might not allow their young to nurse. Excretions were not collected, because the animals were allowed to remain in their stock cages where they had made their nests, again to avoid any circumstances which might discourage the mothers from nursing their young. The mothers and their litters were sacrificed four hours after the administration of F^{18} and the following were taken for assay: maternal blood, liver, kidney, gastro-intestinal tract, muscle, bone, skin and

mammary tissue. Gastro-intestinal tracts and samples of leg bone were taken from each nursling and were pooled for each litter. The carcasses of the nurslings were counted separately.

(6) Studies on Thyroid Function and the Distribution of F^{18} in Rats Chronically Intoxicated with Fluoride

Fifty-five Long-Evans female rats approximately nine weeks of age (150 grams in weight) were divided into 11 groups of five and were placed in stock cages. The animals were maintained in two lots, 25 control animals that were given the semi-synthetic ration, (see Appendix II), and 30 experimental animals that were fed this same ration to which NaF was added. Each group of five rats received 75 grams of food daily or an average of 15 grams of food per rat. Tap water was given ad lib.

Sodium fluoride was added to the food of the experimental animals in sufficient quantity so that the average daily intake for each cage of five rats was 20 mgm of fluoride per kilo of body weight. All of the animals were weighed every 7 to 10 days at approximately the same time of day and the NaF content of the ration was adjusted for any weight changes in the experimental animals. Great care was taken to mix the fluoride supplement intimately with the food, since according to Roholm[100], this precaution reduces or prevents damage to the gastro-intestinal tract.

Twenty mgm of fluoride per kilo per day was the daily fluoride supplement chosen, because it has been shown by Lamb et al[65,93a] and McClure and Mitchell[81] that a daily intake of fluoride of greater than 20 mgm per kilo produced serious disturbances in the tissues of the rat other than the bones and teeth. Since the major purposes of the experiments were to determine whether or not there were any disturbances in thyroid function at this level

of fluoride intake or changes in the manner in which a chronically fluorosed rat handled F^{18}, it was considered desirable to give a fairly large amount of fluoride and at the same time to maintain them in a state as close to physiological as possible.

The control animals were maintained on the purified diet and the experimental animals on the purified diet with the fluoride supplement for an average of 18.5 weeks. (Two control animals died in the first weeks of the experiment and one experimental animal escaped from the cage and was lost). Some of the animals were used as early as 17 weeks after the start of the experiment and others were kept for as long as 20.5 weeks. This discrepancy arose because of the difficulties involved in the scheduling of cyclotron bombardments. At the end of the feeding period some of the animals were given I^{131} to study thyroid function and the rest were given F^{18}.

(a) Ten control animals and ten experimental animals were each given 10 microcuries of I^{131} intravenously. Five experimental animals and five controls were sacrificed one day after the I^{131} injection and the five experimental animals and five controls were sacrified two days after the injection. At autopsy the thyroid, blood, pelt, gastro-intestinal tract, carcass and excreta were assayed for I^{131}. The blood was separated by centrifugation and the red cells and plasma were assayed separately. The pelts were divided into four portions all of which were counted. The gamma ray activity of these samples was measured on the same counter as was used for the measurement of F^{18}.

(b) For studies on the distribution of F^{18} in fluorosed animals and their mature controls, the remaining animals were given F^{18} intravenously. Groups of five experimental animals were then sacrificed 1, 4 and 9 hours

after the injection of F^{18} and groups of four control animals were sacrificed 1 and 4 hours after the injection and five control animals were sacrificed at 9 hours. The tissues and organs sampled and the techniques employed in this series of six experiments were essentially the same as those described in section (1) except that the F^{18} contents of pancreas, brain, spleen and lungs were not determined and that there was no differentiation made between the extremities and bodies of the bones sampled. In addition several other measurements were made. The length of the femurs of five fluorosed and five controls were measured from the end of the head to the most prominent portion of the medial condyle. After assaying for F^{18} the bone samples from 10 of the fluorosed and 12 of the control animals were ashed in a muffle furnace at 500°C for at least 24 hours and the ash contents were determined.

The four remaining fluorosed rats were sacrificed and used for histology and radioautographs.

(7) Radioautography with F^{18}

Radioautographs were made with Eastman Kodak No-Screen x-ray film of the distal ends of the left femurs of three groups of rats one hour after the intravenous injection of F^{18}. The three groups of rats used were as follows: five young animals (150 grams) that had been fed the stock diet, five of the mature controls that had received the purified diet and four fluorosed animals. The femur was disarticulated from the pelvis and tibia, and the patella was removed. The surrounding muscle was removed with care to avoid excessive injury to the periosteum. The bones were then cut in half with a razor blade and the proximal half was discarded. The distal end of the femur was used, because its epiphyses do not close fully until the animals reach an age of over 1000 days Simpson et al[109]. With the posterior

surface uppermost and using the intercondyloid fossa as a guide, the distal end of the femur was cut into nearly equal portions with a jeweler's saw and a fine blade. A new blade was used for each bone, since it was found that bits of bone dust clung to the saw. The pieces of bone were then placed cut surface upward on a piece of sponge rubber in a light tight box. A piece of thin mica (approximately two to five mgm/cm^2) was placed between the film and the cut surface of the bone to prevent the film from sticking to the bone and to avoid chemical fogging of the film or swelling of the emulsion due to moisture seeping from the cut bone surface. A second piece of sponge rubber was placed over the film which was thick enough to fill the box completely when it was closed. A weight was then placed on the lid of the box to assure close contact between the bone surface and the film. The films were exposed for from 5 to 10 minutes depending on the amount of F^{18} injected. The films were then developed for from 4 to 8 minutes in Kodak x-ray developer and fixed in hypo. The pieces of bone from which the radioautographs were made were then fixed in absolute alcohol for 48 hours and stained with silver nitrate according to the method described by McClung-Jones[60].

The right femurs were also removed from the fluorosed animals and four of their mature controls. The distal ends of the femurs were fixed for 3 days in formalin and were cut so that the sections would correspond as far as possible to the surfaces of the left femurs from which the radioautographs had been made.

RESULTS

(1) Tracer Studies on Young Adult Female Rats

The results of the three F^{18} tracer studies on young adult rats described in Section (1) on methods are shown in Table 3.

The plasma of these animals was found to contain very nearly twice as much F^{18} per ml as the red blood cells giving a ratio, cells: plasma of 0.54 ± 0.024. This value is quite similar to those obtained for the other halogens. For chloride and bromide the ratio, red cells: plasma is about 0.51 Everett[26] and for iodide, it is about 0.67 Rall et al.[98]

That intravenously administered F^{18} disappears quite rapidly from the blood is readily seen from Figure 2. This curve appears to be the composite of several individual curves; an initial steep curve which very likely corresponds to the equilibration of the injected F^{18} with the blood and the body fluids, and a relatively flat curve which is probably the resultant of several processes such as equilibration of the extracellular and intracellular fluid, skeletal deposition and urinary excretion.

From Table 3 it is evident that when F^{18} is injected intravenously an equilibrium is rapidly attained between the F^{18} concentration in the blood and the tissues of the body. The F^{18} concentrations, in per cent of administered dose per gram of wet tissue, of the relatively vascular tissues such as liver, spleen and small intestine are very nearly the same as the blood level even at the earliest interval after injection, 15 minutes. The F^{18} concentrations of muscle and skin, tissues that normally have a rather slow circulation, were considerably lower than the blood level at the earliest time interval, although equilibrium was reached by the end of one hour. The F^{18} concentrations of the soft tissues, with a few exceptions

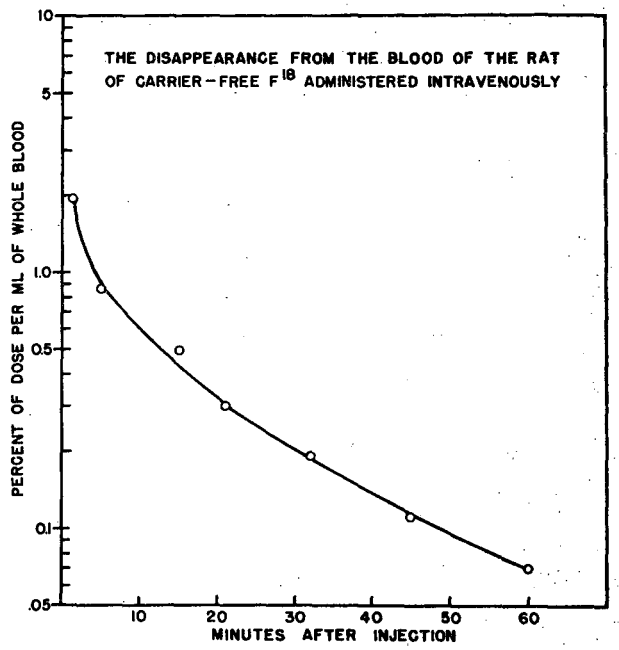
THE DISAPPEARANCE FROM THE BLOOD OF THE RAT
OF CARRIER-FREE F18 ADMINISTERED INTRAVENOUSLY
PERCENT OF DOSE PER ML OF WHOLE BLOOD
10
5
1.0
0.5
0.1
.05
10
20
30
40
50
60
MINUTES AFTER INJECTION

Fig. 2

TABLE 3

THE DISTRIBUTION OF F^{18} IN YOUNG ADULT RATS 1/4, 1, 4 AND 9 HOURS AFTER INTRAVENOUS ADMINISTRATION. VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PER CENT.

	1/4 hour		1 hour		4 hours		9 hours	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Molars	-	-	0.44	3.04	0.41	2.75	0.30	2.50
Incisors	-	-	1.02	3.14	1.13	3.98	1.33	3.61
Skeleton	32.9*	1.76	55.9	3.79	64.0	4.33	56.0	3.31
Muscle	19.4	0.19	6.55	0.08	1.06	0.01	0.55	0.006
Skin	11.2	0.29	2.00	0.07	0.25	0.008	0.17	0.005
Blood	8.05	0.50	0.99	0.08	0.12	0.008	0.06	0.005
Cartilage	-	0.31	-	0.37	-	0.16	-	0.26
Spleen	0.50	0.52	0.06	0.06	0.009	0.009	0.003	0.003
Lung	1.13	0.58	0.19	0.11	0.10	0.04	0.06	0.03
Liver	4.85	0.49	0.49	0.06	0.28	0.03	0.04	0.005
Kidney	1.46	0.84	0.27	0.18	0.04	0.03	0.03	0.02
Stomach	0.27	0.32	0.05	0.07	0.009	0.009	0.004	0.003
Stom.Cont	0.06	-	0.08	-	0.006	-	<0.001	-
Sm.Int.	1.11	0.28	0.24	0.07	0.03	0.009	0.02	0.004
Sm.Int.Cont.	1.73	-	1.26	-	0.14	-	0.03	-
Lg.Int.	0.41	0.35	0.06	0.07	0.03	0.04	0.01	0.006
Lg.Int.Cont.	0.20	-	0.09	-	0.84	-	0.48	-
Cecum and Cont.	0.42	-	0.32	-	1.27	-	0.30	-
Sal.Gland	-	1.23	-	0.08	-	0.01	-	<0.005
Lac.Gland	-	0.37	-	0.04	-	0.01	-	<0.004
Lymph Node	-	0.64	-	0.01	-	0.008	-	<0.004
Brain	-	-	0.02	0.02	0.01	0.009	<0.006	<0.004
Pancreas	-	0.41	-	0.04	-	<0.009	-	<0.002
Thyroid	0.001	-	0.002	-	<0.001	-	<0.004	-
Adrenal	0.04	-	0.004	-	<0.001	-	<0.003	-
Balance	6.09	-	11.6	-	7.20	-	7.54	-
Urine	9.75	-	17.2	-	22.6	-	31.4	-
Feces	0.04	-	0.98	-	0.46	-	1.83	-

* Includes teeth

(kidney, salivary gland) that will be considered separately, remained parallel with the F^{18} level of the blood at all of the time intervals investigated.

The close agreement of the F^{18} concentration in the blood and soft tissues and the above mentioned findings on the red cell: plasma ratio for F^{18} indicate that cells are freely permeable to F^{18}.

It will be noted that 15 minutes after the injection, while the blood level of F^{18} is high, that the concentration in the salivary glands is a little more than twice the blood concentration. In view of the findings of Wills[133] and Volker et al[128] on the presence of F^{18} in the saliva of cats and rats 35 minutes after intraperitoneal injection, this elevated F^{18} concentration in the salivary glands is not surprising. However, since the salivary glands from all of the animals sacrificed at this time were pooled, this value represents only a single determination and cannot be considered quantitative.

It is worthy of note that the thyroid did not accumulate F^{18} to any significant extent at any of the time intervals investigated as might be expected from the work of other investigators on the thyroidal uptake of the other members of the VII group, Baumann and Metzger[6], chloride, bromide and iodide, and Hamilton and Soley[49], astatine (element 85). Assuming that the thyroid of a normal rat weighs approximately 20 mgm (the thyroids were not weighed in these experiments) the F^{18} concentration of the thyroid can be calculated. From such a calculation it can be seen that the F^{18} concentration of the thyroid did not exceed the blood level during the time of any of these experiments.

The kidney was the only soft tissue that consistently showed a higher F^{18} concentration than the blood. The high concentration of F^{18} in the kidney was to be expected, since the excretion of fluorine is for the most part urinary.

That F^{18} is excreted in the feces to a small extent seems fairly well established by these experiments. Although some of the F^{18} that made its way into the contents of the gastrointestinal tract following intravenous injection was apparently reabsorbed, some was also found in the feces. The possibility that the F^{18} in the fecal material is due to urinary contamination seems disproved by the fact that F^{18} is found in the formed feces in the large bowel.

The major site of F^{18} deposition was in the teeth and bones and to a lesser extent in the cartilage. The cartilage samples from each group of animals were pooled for each of the time intervals studied so that the figures for the F^{18} concentration in cartilage should be regarded as only semi-quantitative. In several of the experiments samples of tracheal cartilage were also taken and the F^{18} concentration of the trachea was found to be slightly more than twice that of the xiphoid cartilage.

F^{18} is rapidly accumulated in the skeleton and reached a peak value of 64 per cent of the administered dose at about four hours. Nine hours after its administration the F^{18} content of the skeleton had declined to about 87.5 per cent of the peak value. This peak of F^{18} concentration and total F^{18} content four hours after injection was found in the mandibles as well as in the leg bones as shown in Tables 12 and 13.

When the diaphyses and epiphyses of the leg bones were assayed separately, it was found that 15 minutes after the injection the ratio of the

F^{18} content, diaphyses: epiphyses was 0.45, decreasing to 0.35 for the rest of the time intervals studied. Calculated on the basis of per cent of dose per gram the ratio of the F^{18} concentration, diaphyses: epiphyses was 0.88 at 15 minutes and 0.76 for the remainder of the experiments. The high ratio, diaphyses: epiphyses found 15 minutes after the injection may be due to the presence of F^{18} in fairly large amounts in the marrow at that time, since the blood level of F^{18} is high and the marrow was not removed from the bones.

On the basis of decreasing F^{18} concentration the bony tissues sampled can be arranged in the following order for all of the time intervals studied: mandible, leg bone epiphyses, leg bone diaphyses, which indicates that at short intervals of time after its administration the amount of F^{18} taken up by a bone depends in a large measure on its vascularity, an observation also reported by Volker et al.[128]

With the exception of the F^{18} concentration in the incisors at 9 hours, the concentrations of F^{18} in the teeth were lower than the concentrations in the leg bones at the corresponding time intervals. The incisors followed the same general pattern as the other bony tissues showing a rapid initial F^{18} uptake, a peak concentration 4 hours after the injection and a gradual decrease in the ensuing 5 hours. On the other hand the molars showed a peak F^{18} concentration at one hour and a gradual decline thereafter. The failure of the molars to follow the pattern of the incisors and bone may be tentatively explained as the result of the adsorption of F^{18} from the saliva by the enamel in the first few minutes after the injection. The occurrence of the above mentioned process of adsorption has been fairly well established from experiments by other investigators. Sognnaes and his co-workers[114,115] showed that the P^{32} content of the enamel of the molars

of dogs and cats could be significantly reduced by isolating the teeth from the saliva. Gol'dberg[36] found that although fluorides applied to the tooth enamel were readily adsorbed, a slow leaching process removed a large portion of that which had been adsorbed during the ensuing two to three days. Adsorption of F^{18} from the saliva may play a small part in the incisors as well and would help to explain the observation that the F^{18} concentration of the incisors had decreased only 9.3 per cent from the peak concentration at 9 hours while at the same time the leg bones had lost 23.6 per cent of their peak concentration.

(2) The Appearance of F^{18} in the Digestive Tract Following Intravenous Administration

When injected intravenously to rats, F^{18} appeared quite rapidly in the contents of the intestines and to a lesser extent in the stomach. In order to determine the route or routes of entry of F^{18} into the contents of the alimentary tract the two experiments described in Section (2) on methods were undertaken.

There are several routes by which F^{18} might enter the gastrointestinal contents: secretion in the saliva with subsequent swallowing, secretion by the stomach mucosa, secretion in the bile, in the pancreatic juice, in the intestinal juices and by diffusion into the entire length of the alimentary tract.

In the first experiments, in which animals were given F^{18} intravenously after previous treatment with ephedrine sulfate, a sympathomimetic drug, some or all of the following results might be expected according to Goodman and Gilman:[40] decrease in the motility of the stomach and intestine, contraction of the sphincters and reduction of the blood supply of the abdominal

region as a whole. These are the same effects as would be observed, if an animal were given adrenaline. The effects of ephedrine, although less pronounced than those of adrenaline, are much more prolonged.

The second experiment on animals with ligated bile ducts and isolated stomachs was designed to eliminate saliva from the gastrointestinal tract, to collect the secretions of the isolated stomach and to eliminate the flow of bile into the duodenum.

At autopsy it was noted that the stomach and to a lesser extent, the small intestines of all of the animals of both groups were distended with watery fluid of pH 6 to 8.

The results of both experiments are shown in Table 4, which also includes the results obtained from five control animals that had received no treatment other than an intravenous injection of F^{18}.

On the whole, the results obtained from the animals that had received only the ephedrine injection would seem to be more reliable than those obtained for the operated animals, since the operated animals were more than likely in primary shock as evidenced by the fact that their fur was ruffled, and that they were quite cold to the touch.

In the operated animals, the ligation of the cardiac sphincter essentially eliminated the flow of saliva into the stomach due to swallowing. Although saliva samples were obtained from three of the five rats, only one of the samples contained a large amount of F^{18}, 1.49 per cent of the dose. It must be borne in mind that the salivary gland of the rat contained large amounts of F^{18} only at the earliest time interval studied in the experiments discussed in the previous section (see Table 3), when the blood level of F^{18} was high, and that there was probably considerable loss of saliva into the urine pan, since it could not be swallowed.

TABLE 4

THE DISTRIBUTION OF F^{18} IN YOUNG ADULT RATS TREATED WITH (1) 10 MGM/KILO OF EPHEDRINE SULFATE, (2) LIGATED CARDIAC SPHINCTER, PYLORUS AND BILE DUCT AND (3) CONTROLS ONE HOUR AFTER INTRAVENOUS ADMINISTRATION. VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PER CENT.

	Ephedrine		Operated		Control	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Blood	1.03	0.07	2.43	0.22	0.99	0.08
Liver	-	-	1.41	0.25	0.49	0.06
Kidney	-	-	0.78	0.60	0.27	0.18
Stomach	0.06	0.05	0.23	0.23	0.05	0.07
Stom. Cont.	0.11	-	0.10	-	0.08	-
Sm. Int.	0.19	0.05	0.70	0.22	0.24	0.07
Sm. Int. Cont.	0.58	-	0.50	-	1.26	-
Lg. Int.	0.07	0.05	0.23	0.21	0.06	0.07
Lg. Int. Cont.	0.05	-	0.07	-	0.09	-
Cecum and Cont.	0.23	-	0.32	-	0.32	-
Skeleton*	54.9	3.34	54.0	3.84	57.4	3.79
Muscle	5.11	0.06	9.92	0.14	6.55	0.08
Skin	2.65	0.08	4.02	0.16	2.00	0.07
Balance	15.4	-	19.5	-	11.9	-
Urine	19.6	-	6.11	-	17.2	-
Feces	0.08	-	0.004	-	0.98	-

* Includes teeth

The amount of F^{18} found in the stomach contents did not show any correlation with previous treatment, presence of swallowed saliva or the quantity of fluid present. The results were comparable for all three groups, treated and controls.

There was only one statistically significant difference ($p < 0.05$) between the distribution of F^{18} in the gastrointestinal contents of the control animals and two groups of treated animals in which the distributions were essentially the same. This difference was in the amount of F^{18} present in the contents of the small intestine, which compartment, in the two groups of treated animals contained only about one-half as much F^{18} as in the controls.

The F^{18} concentration in the soft tissues as well as in the empty stomach and intestines of all three groups very closely paralleled the blood level. In Table 3 it can be seen that the same is true of the pancreas.

Since the amount of F^{18} in the intestinal contents of the animals with ligated bile ducts was similar to that found in the animals treated with ephedrine, it would appear that the bile does not contribute greatly to the presence of F^{18} in the intestinal contents.

According to Peters[90a] the gastrointestinal secretions, despite their chemical diversity and with the possible exception of saliva, are isotonic with the blood. The general isotonicity of the gastrointestinal secretions indicates that the digestive glands are unable to perform osmotic work; this does not mean that the secretions are produced simply by diffusion, however. Peters also states that of all of the electrolytes sodium and chloride, seem to play an almost indifferent role in the secretions, serving mainly to maintain osmotic equilibrium when needed and their mode of passage to and from the gut seems closely allied to a process of simple diffusion.

It is noteworthy that the per cent of administered F^{18} found in the four major segments of the gastrointestinal tract was very nearly the same in both treated groups. This similarity between the two groups suggests that there was a common mechanism active in both, despite the radically different treatment they received. One of the major actions of ephedrine is to constrict the peripheral and splanchic blood vessels reducing the blood supply to the skin and abdominal region. This same shunting of the blood from the skin and viscera to the more vital organs is seen in animals in the first stages of surgical shock, which follows a major injury or surgical operation. This blood-vascular pattern arises from nervous and vascular responses to pain and psychic factors, Best and Taylor.[8] Further substantiating the hypothesis that the sympathetic nervous system has been called into play in the operated animals was the observation that the stomach fluids were alkaline, an effect which is observed in experimental sympathetic stimulation, Best and Taylor.[8] The lowered body temperature and the ruffling of the fur also suggest shock in the operated animals and the subsequent intervention of the sympathetic system.

It was found in the experiments on the distribution of F^{18} in normal young adults that the amounts of F^{18} in the contents of the various segments of the gastrointestinal tract were essentially the same one hour after injection as they were 15 minutes after injection. From this it may be assumed that any F^{18} reaching the contents of the gut does so in the first few minutes after injection when the blood level is high.

The following conclusions may then be drawn:

(a) F^{18} appears to be secreted in the saliva, but does not seem to contribute greatly to the presence of F^{18} in the gastrointestinal contents.

(b) The bile does not appear to be a major route of entry of F^{18}

into the small intestine.

(c) In animals displaying the effects of sympathetic stimulation, whether induced by drugs or surgical shock, the only disturbance in the F^{18} distribution in the gastrointestinal contents is the lowering of the amount found in the small intestine, the most vascular and actively secreting portion of the digestive tract, Best and Taylor.[8]

(d) F^{18} has been shown to be as readily able to permeate cell membranes as are the other halides.

Visscher et al[126] have shown that there is a forced flow of water and diffusible ions across the intestinal membrane simultaneously in both directions to and from the blood, the direction and magnitude of the flow depending on the concentrations of the solutes and rates of flow of the two streams. From Visscher's work and from the observations in these experiments it can be tentatively concluded that F^{18} enters the gastrointestinal tract from the blood by some process resembling diffusion and passes into the gastric and intestinal secretions, and that the extent to which it enters these compartments depends upon the level of F^{18} in the blood and the rate of flow of the blood in the secreting organs.

(3) The Distribution of Orally Administered F^{18} in Young Adult Rats.

The adsorption of F^{18} from the gastrointestinal tract following oral administration is quite rapid as can be seen from Table 5. Seventy-five per cent of the administered dose was absorbed in the first hour and 90 per cent was absorbed by the end of 9 hours. Comparison of Tables 3 and 5 shows few significant differences between orally administered F^{18} and intravenously administered F^{18}; the larger amounts of F^{18} in the empty stomach and intestines which may be due to the incomplete removal of their contents,

TABLE 5

THE DISTRIBUTION OF F^{18} IN YOUNG ADULT RATS 1, 4 AND 9 HOURS AFTER ORAL ADMINISTRATION. VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PER CENT.

	1 hour		4 hours		9 hours	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Skeleton*	43.6	3.20	59.2	4.53	50.9	3.00
Muscle	4.87	0.07	0.76	0.01	0.56	0.006
Skin	1.71	0.06	0.18	0.008	0.48	0.01
Blood	1.25	0.10	0.14	0.01	0.08	0.005
Cartilage	-	0.43	-	0.46	-	0.29
Spleen	0.06	0.08	0.007	0.01	0.002	0.004
Lung	0.22	0.10	0.08	0.04	0.04	0.02
Liver	0.54	0.10	0.07	0.01	0.04	0.006
Kidney	0.24	0.19	0.03	0.04	0.06	0.04
Stomach	1.18	1.48	0.65	0.76	0.50	0.56
Stom. Cont.	5.48	-	1.06	-	4.64	-
Sm. Int.	1.84	0.72	0.08	0.02	0.05	0.01
Sm. Int. Cont.	21.4	-	0.52	-	0.31	-
Lg. Int.	0.08	0.07	0.04	0.03	0.02	0.02
Lg. Int. Cont.	0.06	-	0.60	-	0.43	-
Cecum and Cont.	0.54	-	12.5	-	6.24	-
Sal. gland	-	0.10	-	0.01	-	-
Lac. gland	-	0.08	-	0.01	-	-
Lymph Node	-	0.10	-	0.01	-	-
Brain	0.02	0.02	0.01	0.008	-	-
Pancreas	-	0.07	-	0.008	-	0.004
Thyroid	0.001	-	<0.001	-	-	-
Adrenal	0.005	-	0.001	-	-	-
Balance	3.81	-	7.57	-	5.86	-
Urine	13.0	-	16.0	-	25.6	-
Feces	0.05	-	0.48	-	4.14	-

* Includes teeth

a somewhat slower bone uptake and less rapid urinary excretion of orally administered F^{18}. If the values obtained for the distribution of orally administered F^{18} are corrected on the basis of the per cent of dose absorbed, the figures thus obtained are comparable to those obtained in the experiments in which F^{18} was given by vein.

It will be noted that there was considerably more F^{18} present in the feces of the animals receiving F^{18} by stomach tube indicating that absorption of minute amounts of fluorine is not quite complete. If F^{18} is absorbed as a simple ion, it may be that the presence of other ions such as sodium and chloride interferes to a certain extent with its absorption.

The possibility of radiation damage due to the administration of large amounts of F^{18} has not been mentioned up to this point. The estimation of the total body radiation dose due to a radioactive element such as F^{18} which is rapidly distributed throughout the body and just as rapidly disposed of by excretion and bone deposition is extremely difficult. It has been estimated that if there were no excretion of F^{18} and if it were evenly distributed throughout the body and remained so, for the entire 9 hours of the longest term experiments, that the total body radiation dose would be in the neighborhood of 5 r.e.p., an amount which can be considered almost negligible. In the experiments where F^{18} was given by stomach tube the estimation of the radiation delivered to the tissues of the digestive tract, tissues that are relatively radiosensitive, is a less difficult problem. Using as an example the animals that received 500 microcuries of F^{18} by stomach tube and were sacrificed 4 hours later, the radiation dose to the stomach and intestines (empty weight 5.3 grams) would be from 35 to 55 r.e.p., if radiation due to the annihilation photons is neglected. Bloom[14] states that microscopically visible changes appear in the stomach and to a greater

extent in the intestinal mucosa within 30 to 60 minutes after exposure to 600 r of x-rays (LD_{50}/30 days) on the stomach of the rat. There is no available data on the early effects of internally administered radiation sources, however, histological changes found several days after the administration of beta and gamma emitters by stomach tube (23 to 33 microcuries/gram of fission products) corresponded fairly well with those found at the same time intervals after exposure to equivalent doses of external x-irradiation Bloom.[14] These latter findings would seem to indicate that there are disturbances in the gastric and intestinal mucosa within a few minutes after exposure to an internal radiation source. However, since the half-life of F^{18} is so short and since absorption from the digestive tract was quite rapid 75 per cent in the first hour and 90 per cent at the end of 9 hours, it would seem that the absorptive processes of the gastro-intestinal tract were not materially impaired by radiation damage in the length of time that the digestive tract was exposed to the positrons emitted by F^{18}.

(4) The Effect of Milligram Amounts of Stable Fluoride on the Distribution of F^{18}.

When 10 mgm per kilo of fluoride as isotonic NaF was given simultaneously with the essentially carrier-free preparation of F^{18} described in the section dealing with the preparation and standardization of F^{18}, the animals received nearly 20,000 times more fluoride than did the animals that received the carrier-free material alone.

Comparing Tables 3 and 6 several notable differences will be observed between the two groups, F^{18} and F^{18} plus carrier, at the 15 minute interval. Nine hours after the injections no significant differences are to be found between the two groups.

TABLE 6

THE DISTRIBUTION OF FLUORIDE IN YOUNG ADULT RATS, USING F^{18} AS A TRACER, 15 MINUTES AND 9 HOURS AFTER INTRAVENOUS ADMINISTRATION. VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PER CENT. EACH RAT RECEIVED 22 MILLIGRAMS OF NaF PER KILO BODY WEIGHT.

	15 minutes		9 hours	
	% per organ	% per gram	% per organ	% per gram
Skeleton*	40.5	2.98	58.0	3.17
Muscle	20.0	0.28	0.42	0.004
Skin	10.2	0.39	0.12	0.003
Blood	7.11	0.65	0.05	0.003
Cartilage	-	0.66	-	0.30
Spleen	0.18	0.39	<0.005	<0.01
Lung	0.60	0.48	0.02	0.01
Liver	2.97	0.43	0.05	0.006
Kidney	3.34	2.45	0.02	0.01
Stomach	0.29	0.30	<0.005	<0.005
Stom. Cont.	0.05	-	0.01	-
Sm. Int.	1.25	0.37	<0.01	<0.002
Sm. Int. Cont.	1.31	-	0.03	-
Lg. Int.	0.46	0.37	0.006	0.004
Lg. Int. Cont.	0.14	-	0.98	-
Cecum and Cont.	0.41	-	0.48	-
Sal. gland	-	0.69	-	<0.01
Lac. gland	-	0.37	-	<0.01
Lymph Node	-	0.36	-	<0.01
Pancreas	-	0.43	-	<0.001
Thyroid	0.006	-	<0.004	-
Adrenal	0.02	-	<0.004	-
Balance	9.82	-	6.84	-
Urine	1.14	-	32.2	-
Feces	0.17	-	0.84	-

* Includes teeth

The major differences between the carrier-free animals and those receiving added stable fluoride 15 minutes after injection can be summarized as follows:

The animals receiving added carrier showed a considerably higher F^{18} concentration in the kidneys and thyroid, slightly higher F^{18} concentrations in the other soft tissues and cartilage, a lower F^{18} concentration in bone and a reduced urinary excretion. All of these differences, noted within minutes after the injection, and the complete similarity noted several hours after injection seem to indicate that (within certain limits[1]) fluorides are eventually metabolized in the same fashion regardless of the size of the dose, but with a little more difficulty when larger doses are given.

(5) The Maternal Transfer of F^{18}

The results of the experiment on the distribution of F^{18} in pregnant rats are shown in Table 7 with the distribution of F^{18} in virgin females of approximately the same age for comparison.

The concentrations of F^{18} in the blood and soft tissues of the pregnant animals were generally higher than those for the corresponding tissues of the control animals. The renal excretion of F^{18} was also greater in the pregnant rats. These observations, high blood and tissue level and more rapid renal excretion along with the relatively lower F^{18} concentration in bone are probably all reflections of the increased circulation rate which accompanies pregnancy and the shunting of the blood supply to the tissues and organs involved in the nourishment of and waste removal from the fetuses, Best and Taylor.[8]

Although the concentrations of F^{18} found in the placenta and fetuses

1 Animals that were given 14 to 17 mgm of fluoride/kilo intravenously in pilot experiments died in 30 to 60 minutes and were obviously in tetany.

TABLE 7

THE DISTRIBUTION OF F^{18} IN 19 DAY PREGNANT RATS ONE HOUR AFTER INTRAVENOUS ADMINISTRATION. VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PER CENT. THE AVERAGE DISTRIBUTION OF F^{18} IN VIRGIN FEMALES ONE HOUR AFTER INTRAVENOUS ADMINISTRATION IS SHOWN FOR COMPARISON.

	Pregnant		Control	
	% per organ	% per gram	% per organ	% per gram
Blood	2.07	0.12	0.99	0.08
Liver	1.47	0.11	0.49	0.06
Kidney	0.40	0.24	0.27	0.18
G.I. & Cont.	3.50	-	2.11	-
Muscle	8.98	0.08	6.55	0.08
Skeleton*	42.3	2.16	57.4	3.79
Skin & Mammary Tissue	3.75	0.08	2.24	0.08
Fetuses	1.76	0.04	-	-
Placenta	0.32	0.06	-	-
Uterus & Amniotic Fluid	0.54	-	-	-
Balance	11.2	-	11.9	-
Urine	23.4	-	17.2	-
Feces	0.28	-	0.98	-

* Includes Teeth

were quite small, less than one-half that of the blood, the ability of F^{18} to pass across the placenta even when present in minute quantities seems fairly well established. In order to ascertain whether or not an equilibrium is established between the maternal and fetal plasma, an experiment of a longer duration is indicated, since according to Flexner and Pohl[29] equilibrium for the transfer of radioactive sodium in the rat requires 6 hours. Flexner and Pohl[28] also found that the difference in placental transfer rates for different species could be correlated with the histological differences between the types of placentas, a finding which may help to clear up some of the controversies on the placental transfer of fluoride.

Table 8 shows the results obtained in the experiment on lactating rats together with the distribution of F^{18} in virgin females of approximately the same age. Values are expressed in per cent of absorbed dose, but are not corrected for recovery, since there was no attempt made to collect excretions.

The mammary tissue showed very nearly the same F^{18} concentration as did the maternal blood. About one per cent of the F^{18} absorbed by the mother was found in the tissues of her litter. As was expected, more than one-half of the activity in the nurslings was found in their gastro-intestinal tracts, and the next greatest amount was found in the infant bone.

Approximately 75 per cent of the administered F^{18} was absorbed by the mothers at the end of 4 hours despite the tremendous amounts of food present in their gastrointestinal tracts. In the control animals about 90 per cent of the orally administered F^{18} was absorbed in 4 hours from gastro-intestinal tracts that were very nearly empty.

Despite the fact that there were only three animals used, the experiment was poorly controlled in so far as determination of recovery was

TABLE 8

THE DISTRIBUTION OF F^{18} IN LACTATING RATS AND THEIR YOUNG, FOUR HOURS AFTER ORAL ADMINISTRATION. VALUES ARE EXPRESSED IN PER CENT OF ABSORBED DOSE. THE AVERAGE DISTRIBUTION OF F^{18} IN VIRGIN FEMALES FOUR HOURS AFTER ADMINISTRATION IS SHOWN FOR COMPARISON.

	Lactating		Control	
	% per organ	% per gram	% per organ	% per gram
Blood	0.44	0.03	0.15	0.015
Liver	0.27	0.02	0.08	0.01
Kidney	0.10	0.06	0.04	0.04
G.I. and Cont.	1.85	0.15	3.63	-
Muscle	2.42	0.02	0.87	0.01
Skeleton*	39.6	2.02	67.4	5.17
Skin	0.72	0.02	0.20	0.01
Mammary Tissue	-	0.04	-	-
Young** Bone	0.28	0.01	-	-
	0.53	-	-	-
	0.18	-	-	-
Excreta	-	-	27.1	-

* Includes teeth.
** Values shown are totals for litter of 6.

concerned and that only 75 per cent of the administered material was absorbed, the results indicate that fluoride present in the maternal blood of the rat even at very low levels can be secreted in the milk. The similarity between the F^{18} concentrations in the mammary tissue and in the maternal blood suggest that the passage of F^{18} into the milk of the rat is not true secretion, but is more likely due to a process resembling diffusion.

(6) Studies on the Thyroid Function and F^{18} Distribution in Fluorosed Rats.

(a) General Findings on Chronically Fluorosed Rats.

Figure 3 shows the growth curves for 29 fluorosed rats and 23 mature control rats for a period of approximately 4.5 months. Comparison of the two curves shows an almost immediate response to the dietary fluoride supplement, the first two points on each of the curves represent the weights obtained three days before and one day before the animals were started on the fluoride supplement. The third points on the two curves were obtained 10 days after the fluoride regimen was started. Although there was some overlapping of the weight ranges of the two groups (the weights of the larger animals of the fluoride group were very nearly the same as the weights of the smaller animals of the control group) comparison of the mean weights of the two groups after the start of the fluoride supplement invariably gave a p value of less than 0.05, indicating that for groups of this size the differences in weight were statistically significant. It must be borne in mind that some of the animals on the fluoride diet received more fluoride and some less fluoride than others, because the amount of fluoride added to the diet was based on the mean weight of all of the experimental animals. The same holds true for the food intake which was an average of 15 grams daily per rat for each cage of five rats.

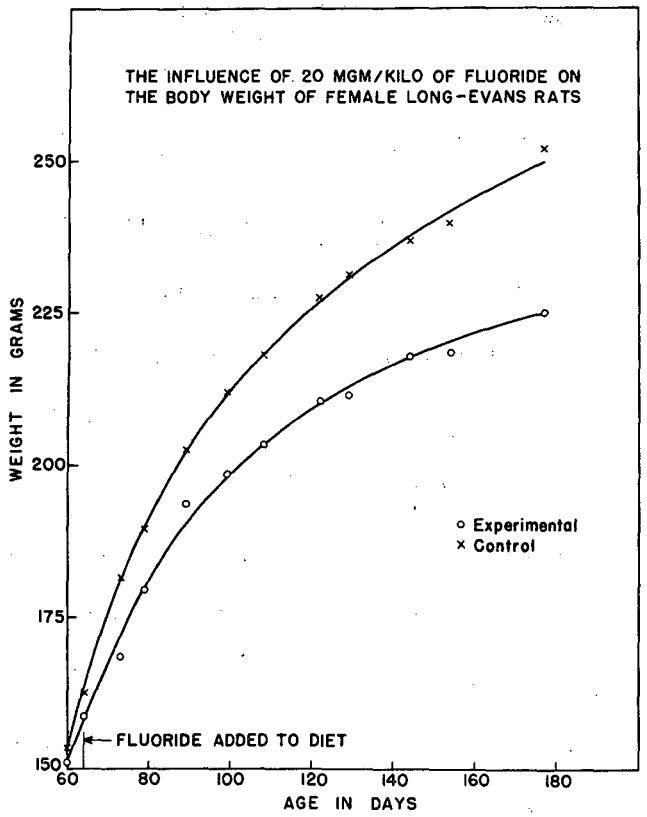
THE INFLUENCE OF 20 MGM/KILO OF FLUORIDE ON
THE BODY WEIGHT OF FEMALE LONG-EVANS RATS
WEIGHT IN GRAMS
250
225
200
175
150
60
80
100
120
140
160
180
AGE IN DAYS
FLUORIDE ADDED TO DIET
o Experimental
x Control

Fig. 3

The possibility that the slower growth rate of the fluorosed animals was due to a lower food intake than the control animals, due either to lack of appetite for their food or difficulty in eating because of elongation of the incisors, seems to be fairly well eliminated by the following: the diet was soft and appeared to be eaten by the fluorosed rats without too much difficulty, the fluorosed animals received per cage of five rats the same amount of food as did the controls, the use of heavy pyramid shaped porcelain feed cups reduced spillage to a minimum, and the feed cups of both groups were almost always emptied during any 24 hour feeding period.

Although most of the animals on the fluoride supplement appeared to be quite normal except for their teeth and slower growth rate, some of them showed other manifestations of intoxication such as poor coat, an untidy appearance and lassitude.

Typical tooth symptoms as described in the discussion of the effects of chronic fluoride intoxication on the teeth of rats were noticeable in these animals within three to four weeks after the start of the fluoride regimen. Figure 4a shows the head of a rat after 4.5 months on a diet of 20 mgm of fluoride per kilo per day and Figure 4b is the head of a normal control animal of the same age after 4.5 months on a diet containing 0.5 mgm of fluoride per kilo per day. The teeth of the fluorosed rat show absence of normal pigmentation. They are opaque and almost chalky in appearance and contain dark spots. Both the maxillary and mandibular incisors are considerably thicker than those of the control animal. Note the flattening of the ends of the maxillary incisors as compared to the almost knife edge sharpness of the corresponding teeth of the control. The spreading, elongation and backward curvature of the maxillary incisors is concurrent with the decrease in length of the mandibular incisors which have either been

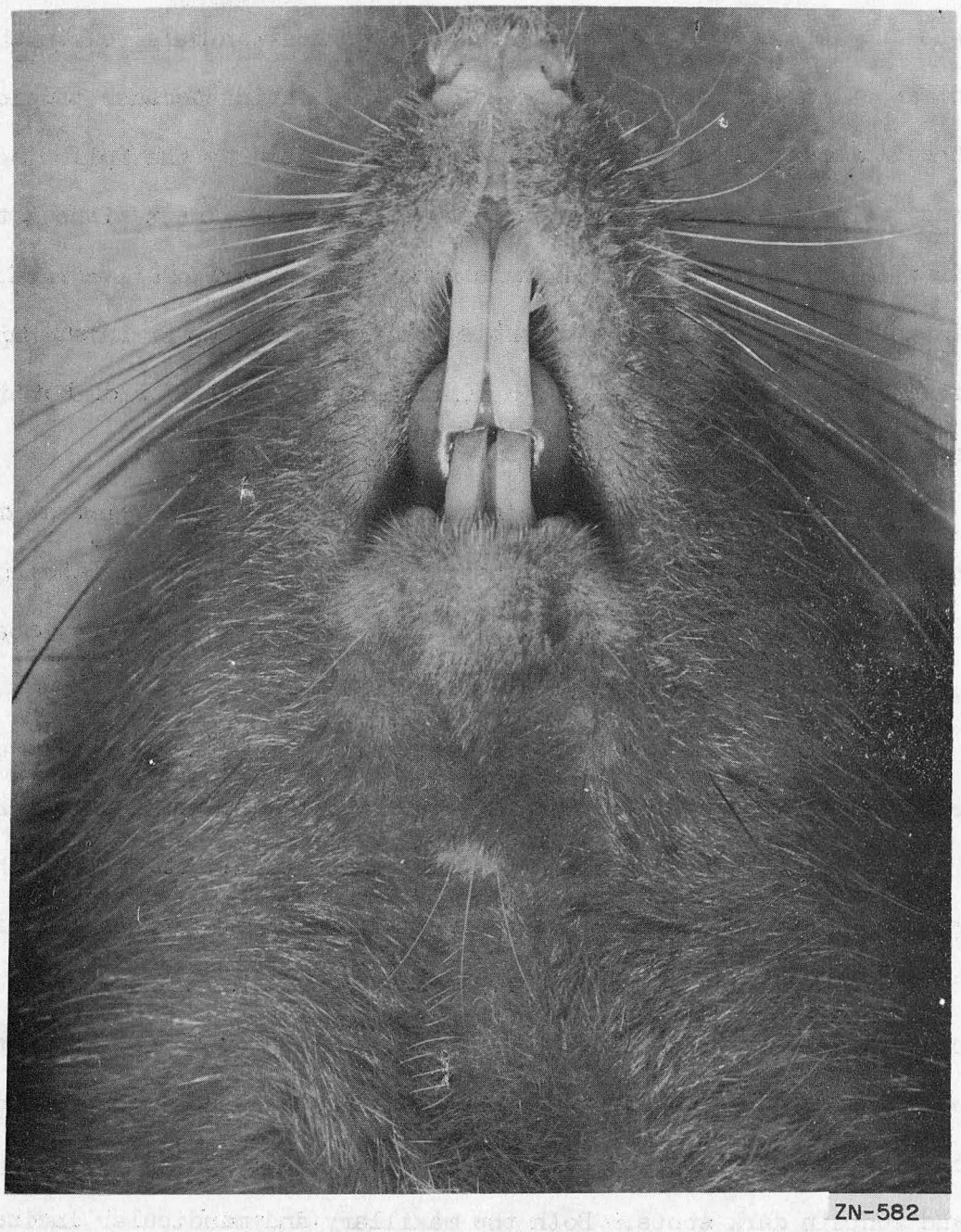

Fig 4a The head of a female Long-Evans rat after 4.5 months on a dietary supplement of 20 mgm of fluoride per kilo per day. (x2).

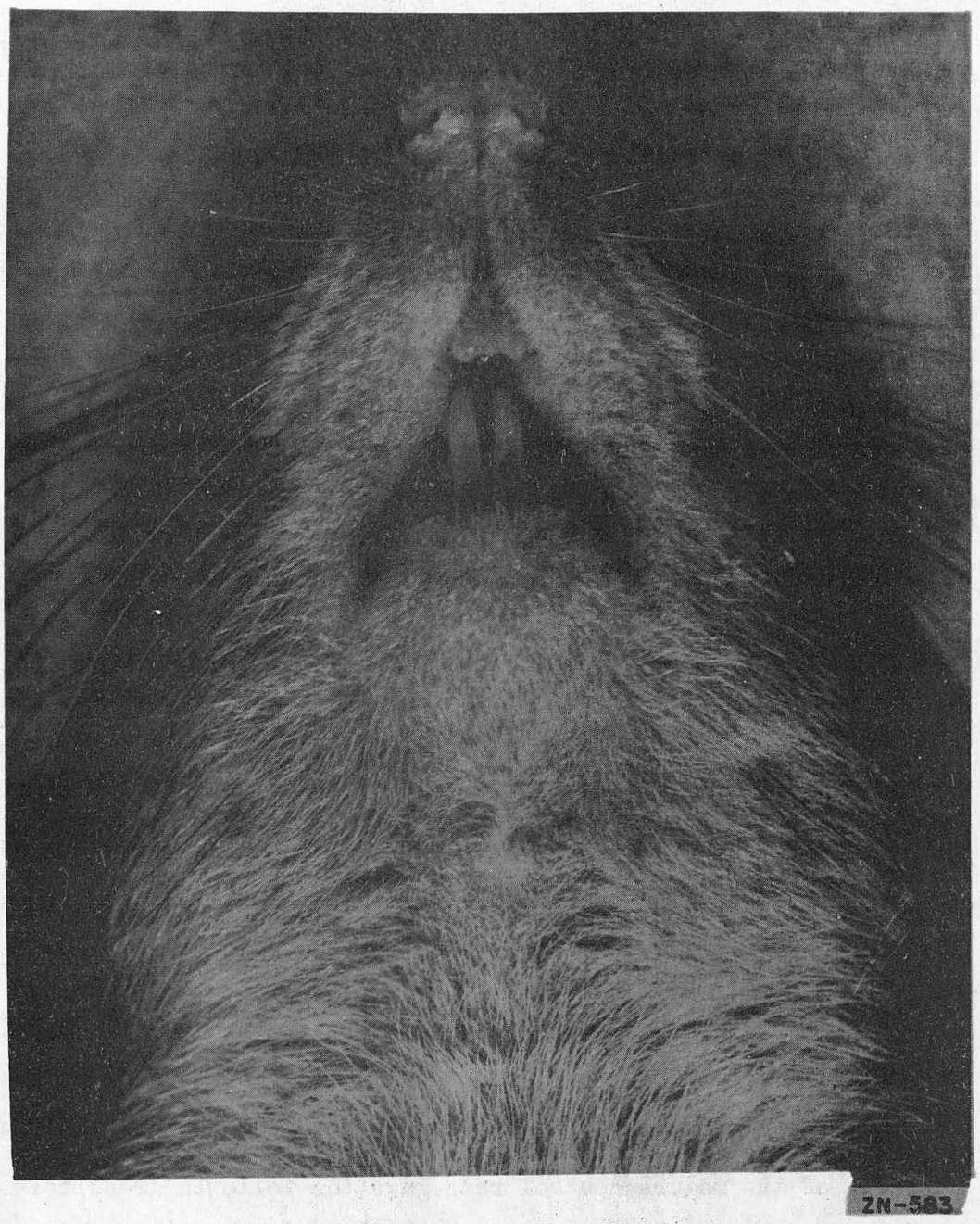

Fig. 4b The head of a normal female rat of the same age and strain as the animal shown in Fig. 4a. (x2).

broken off or worn down excessively.

All of the animals on the fluoride supplement showed the above dental effects to a greater or lesser degree, however, there did not appear to be any correlation between body weight or weight gain and the severity of the tooth symptoms. The average weight gain for the fluorosed animals was 74.8 ± 3.8 grams and for the controls, 97.8 ± 4.9, comparing the two means, $p < 0.01$.

At autopsy when the femurs of the fluorosed rats were cut longitudinally to prepare them for making radioautographs, it was noticed that they seemed to be softer and more easily cut than were the control femurs. The molars of the fluorosed rats were found to be much less difficult to remove than were those of the control animals. These observations are in agreement with the findings on the ash content of the normal and fluorotic leg bones. The ash content of the leg bones of 10 fluorosed rats and 12 control rats gave the following results: control leg bones, 34.2 ± 0.5 per cent ash and fluorosed, 32.5 ± 0.6 per cent ash. The difference between the two means, 1.7 per cent, is significant since for a set of determinations of this size, p is less than 0.05. The above figures for the ash content of the leg bones of these animals were used in the calculation of total skeletal F^{18}.

Measurement of the femur lengths of four of the fluorosed rats and four of the mature control rats gave the following results: control femur, 35.1 ± 0.67 mm and fluorosed femur 32.8 ± 0.62 mm with a p value of less than 0.02 indicating that the difference between the two femur lengths, 2.3 mm, is significant.

(b) Experiments on the Thyroid Function of Fluorosed Rats.

Since the late 1930's when radioactive isotopes of iodine with

suitable half-lives were first produced, a great deal of work has been done on the functional state of the thyroid and the thyroidal uptake of radioactive iodine, pioneered by Hertz and his co-workers[56,57] and by Hamilton and Soley.[47,48,50] More recently Scott et al[107] have devised a method whereby the ratio, red cell: plasma of I^{131} is used as a measure of thyroid function. This latter is essentially a measure of the freely diffusable iodine, and the non-diffusible, protein-bound iodine in the blood at certain time intervals after the administration of I^{131}.

In view of the controversy surrounding the subject of a possible relationship between changes in the morphology and function of the thyroid and an elevated intake of fluoride, it was deemed advisable to make use of these two relatively new methods of measuring thyroid function in fluorosed rats.

Table 9 shows the I^{131} distribution in fluorosed rats and their controls 24 and 48 hours after intravenous administration. The ratios of the per cent I^{131} per ml of red cells to plasma are shown at the bottom of the Table.

No differences in the size of the thyroids of the fluorosed and control animals were observed. The only statistically significant difference in the I^{131} distribution in fluorosed and normal rats was in the per cent uptake of I^{131} by the thyroid at 24 hours, $p<0.05$. The red cell to plasma ratios were comparable for the controls and fluorosed rats at both time intervals studied. The thyroidal uptake of I^{131} varied widely for both the fluorosed and control groups at both of the time intervals studied. This wide spread in individual values is to be expected in view of the work of other investigators who have found that the uptake of I^{131} in animals of the same age and dietary history is quite variable, Hamilton et al[46] and Taurog.[119a] Consequently, results obtained using small groups of animals

TABLE 9

THE DISTRIBUTION OF I^{131} IN NORMAL MATURE RATS AND RATS CHRONICALLY INTOXICATED WITH FLUORIDE 24 AND 48 HOURS AFTER INTRAVENOUS ADMINISTRATION. VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PER CENT. EACH RAT RECEIVED 3.5 μc OF I^{131}

	24 hours				48 hours			
	control		fluorosed		control		fluorosed	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Thyroid	12.0	-	7.50	-	8.62	-	6.65	-
RBC	0.47	0.06	0.48	0.06	0.09	0.01	0.06	0.009
Plasma	0.79	0.09	0.65	0.08	0.36	0.04	0.26	0.04
Skin	5.40	0.13	3.59	0.11	2.48	0.07	2.64	0.08
G.I. Tract	5.04	-	3.57	-	1.98	-	0.80	-
Carcass	8.31	-	9.32	-	3.40	-	2.33	-
Urine	64.9	-	71.2	-	73.3	-	81.4	-
Feces	3.18	-	3.59	-	9.56	-	5.87	-
RBC / Plasma	0.61	-	0.70	-	0.26	-	0.25	-

suitable half-lives were first produced, a great deal of work has been done on the functional state of the thyroid and the thyroidal uptake of radioactive iodine, pioneered by Hertz and his co-workers[56,57] and by Hamilton and Soley.[47,48,50] More recently Scott et al[107] have devised a method whereby the ratio, red cell: plasma of I^{131} is used as a measure of thyroid function. This latter is essentially a measure of the freely diffusable iodine, and the non-diffusible, protein-bound iodine in the blood at certain time intervals after the administration of I^{131}.

In view of the controversy surrounding the subject of a possible relationship between changes in the morphology and function of the thyroid and an elevated intake of fluoride, it was deemed advisable to make use of these two relatively new methods of measuring thyroid function in fluorosed rats.

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	24 hours				48 hours			
	control		fluorosed		control		fluorosed	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Thyroid	12.0	-	7.50	-	8.62	-	6.65	-
RBC	0.47	0.06	0.48	0.06	0.09	0.01	0.06	0.009
Plasma	0.79	0.09	0.65	0.08	0.36	0.04	0.26	0.04
Skin	5.40	0.13	3.59	0.11	2.48	0.07	2.64	0.08
G.I. Tract	5.04	-	3.57	-	1.98	-	0.80	-
Carcass	8.31	-	9.32	-	3.40	-	2.33	-
Urine	64.9	-	71.2	-	73.3	-	81.4	-
Feces	3.18	-	3.59	-	9.56	-	5.87	-
RBC / Plasma	0.61	-	0.70	-	0.26	-	0.25	-

are to be viewed with caution, unless large differences in the thyroid uptake of I^{131} are noted between control and treated animals.

Besides the wide fluctuations in the individual determination, the I^{131} uptakes of all of the rats, both treated and controls, were lower for both of the time intervals studied than the values reported in the literature. Hamilton et al[46] found an uptake on rats on a diet comparable to "Diet 14" (see Appendix I) of about 28 per cent of a tracer dose of I^{131} 24 hours after intravenous administration. Taurog and Chaikoff[121] found that the thyroids of male rats of the Long-Evans strain on a similar diet accumulated 18 per cent at 14 hours and 12 per cent at 50 hours. There seems to be a relationship between the iodine intake in the diet and the ability of the thyroid to accumulate a tracer dose of I^{131}, the greater the daily iodine intake the lower the thyroidal uptake of I^{131} Taurog and Chaikoff.[120] "Diet 14" contains 0.84 micrograms of added iodine per gram of food. The semi-synthetic diet used in the experiments described here on fluorosed rats and their controls (see Appendix II) contained 1.24 micrograms of added iodine per gram of food, or nearly 50 per cent more iodine than "Diet 14", which should explain in part the low I^{131} accumulation by the thyroids of the rats that were fed the semi-synthetic diet.

Taurog and Chaikoff[120] found that while the I^{131} content of the thyroid after the administration of a tracer dose was quite variable, the thyroxine content of these glands was a fairly constant proportion of the total iodine content, usually about 31 per cent of the thyroidal iodine. They also found that the level of protein bound iodine in the plasma depended on the thyroxine content of the thyroid gland. From the above observations, it would seem that the protein-bound iodine of the plasma of which the red

cell: plasma ratio is a measure is actually a more reliable index of thyroid function than the thyroidal uptake of I^{131}.

Since the difference in the uptake of I^{131} by the thyroids of fluorosed and control animals was statistically significant at only one of the time intervals investigated and the red cell: plasma ratios were comparable at both time intervals, it seems safe to say that a prolonged intake of fluoride at the level employed in these experiments did not produce any untoward effects on the function of the thyroid.

(c) The Metabolism of F^{18} in Mature Control Rats and Chronically Fluorosed Rats.

The distribution of F^{18} in fluorotic rats and their normal controls 1, 4 and 9 hours after an intravenous injection of F^{18} is shown in Tables 10 and 11. The F^{18} concentrations of the blood and soft tissues are in general slightly higher in the fluorosed animals than in the controls, a result that might be anticipated from the results obtained 15 minutes after administering F^{18} plus fluoride carrier intravenously (see Table 6).

The urinary excretion of F^{18} by the fluorosed rats was slightly more rapid than the controls, however, since the urine was pooled for each cage of four or five animals, no conclusions can be drawn concerning quantitative differences in urinary excretion between the treated and control groups.

It was anticipated from the work of Savchuck and Armstrong[103] that the F^{18} uptake by the skeletons of the older control animals would be considerably lower than for the young adult animals discussed in Experiment (1). They found that the fluoride retention of mature rats was much lower than that of young animals. The values they obtained were 53 per cent for

TABLE 10

THE DISTRIBUTION OF F^{18} IN RATS CHRONICALLY INTOXICATED WITH FLUORIDE 1, 4 AND 9 HOURS AFTER INTRAVENOUS ADMINISTRATION. VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PER CENT. THE ANIMALS RECEIVED 20 MGM OF FLUORIDE AS NaF PER KILO BODY WEIGHT DAILY FOR AN AVERAGE OF 4 1/2 MONTHS.

	1 hour		4 hours		9 hours	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Molars	0.13	0.94	0.17	1.39	0.16	1.15
Incisors	0.95	1.85	1.23	2.56	1.12	2.00
Skeleton	40.6	2.04	43.4	2.18	48.1	2.22
Muscle	8.30	0.08	1.06	0.01	0.61	0.006
Skin	2.91	0.08	0.45	0.01	0.06	0.02
Blood	1.43	0.09	0.25	0.015	0.12	0.008
Cartilage	-	0.28	-	0.16	-	0.15
Liver	0.89	0.10	0.12	0.01	0.04	0.005
Kidney	0.31	0.19	0.05	0.03	0.03	0.02
Stomach	0.12	0.07	0.02	0.01	0.015	0.009
Stom. Cont.	0.06	-	0.02	-	0.007	-
Sm. Int.	0.27	0.08	0.05	0.01	0.02	0.007
Sm. Int. Cont.	1.81	-	0.28	-	0.04	-
Lg. Int.	0.07	0.06	0.05	0.04	0.01	0.01
Lg. Int. Cont.	0.24	-	1.41	-	1.53	-
Cecum and Cont.	0.48	-	1.16	-	1.09	-
Sal. Gland	-	0.11	-	0.02	-	0.009
Lac. Gland	-	0.09	-	0.01	-	0.007
Lymph Node	-	0.07	-	0.01	-	0.009
Thyroid	0.002	-	<0.001	-	<0.001	-
Adrenal	0.006	-	<0.001	-	<0.001	-
Urine	38.4	-	40.4	-	42.8	-
Feces	0.30	-	1.87	-	0.85	-
Balance	2.83	-	8.04	-	3.02	-

TABLE 11

THE DISTRIBUTION OF F^{18} IN NORMAL MATURE RATS 1, 4 AND 9 HOURS AFTER INTRAVENOUS ADMINISTRATION. VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PER CENT.

	1 hour		4 hours		9 hours	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Molars	0.26	1.68	0.26	1.64	0.18	1.33
Incisors	1.18	2.72	1.40	3.12	1.38	3.01
Skeleton	47.2	2.32	52.8	2.74	48.5	2.46
Muscle	6.81	0.06	0.88	0.008	0.47	0.004
Skin	3.58	0.08	0.62	0.01	0.57	0.01
Blood	1.41	0.08	0.15	0.008	0.08	0.004
Cartilage	-	0.20	-	0.12	-	0.17
Liver	0.48	0.06	0.06	0.008	0.04	0.005
Kidney	0.26	0.15	0.04	0.02	0.02	0.01
Stomach	0.08	0.06	0.02	0.01	0.009	0.008
Stom. Cont.	0.12	-	0.01	-	0.003	-
Sm. Int.	0.17	0.06	0.03	0.008	0.02	0.004
Sm. Int. Cont.	3.26	-	0.22	-	0.04	-
Lg. Int.	0.06	0.04	0.02	0.01	0.02	0.01
Lg. Int. Cont.	0.19	-	0.23	-	1.65	-
Cecum and Cont.	0.42	-	0.94	-	0.95	-
Sal. Gland	-	0.08	-	0.009	-	0.003
Lac. Gland	-	0.07	-	0.01	-	0.003
Lymph Node	-	0.06	-	0.009	-	0.005
Thyroid	0.001	-	<0.001	-	<0.001	-
Adrenal	0.004	-	<0.001	-	<0.001	-
Urine	29.1	-	28.5	-	36.0	-
Feces	0.08	-	0.26	-	0.93	-
Balance	5.04	-	13.5	-	10.6	-

the initial fluoride retention for young animals and 36 per cent retention for older animals. The values found in the experiments reported here at the longest time interval investigated, 9 hours after injection, were 56 per cent retention for the young animals and 48.5 per cent retention for the older rats, indicating that very little of the fluoride depcsited in the skeleton at this time would be subsequently excreted.

The F^{18} contents of the skeletons of the fluorosed rats were quite similar to the control values for the three time invervals studied. The greatest difference, in skeletal F^{18} deposition, 9.4 per cent, occurred 4 hours after the injection. This was also the only time interval at which there was a significant difference in the F^{18} concentrations of the leg bones of the two groups of animals. The control values were higher than the corresponding values for the fluorosed rats. Further, although the skeletal depositions and bone concentrations of the older control animals were lower than the corresponding values found for the young adults (see Tables 12 and 13) the pattern of uptake remained the same.

The skeletons of the older control animals accumulated F^{18} and the F^{18} concentration of the leg bones increased during the first 4 hours after the injection, until a peak bone accumulation and concentration were reached. During the ensuing 5 hours both the F^{18} content of the skeleton and the F^{18} concentration of the leg bones decreased slightly; about 9.2 per cent of the peak values were lost during this 5 hour interval. It will be recalled that this same pattern was seen in the young adult animals, although the peak concentration and skeletal accumulation were higher in the younger rats and the decline of these values in the interval from 4 to 9 hours after injection was greater. On the other hand the leg bones of the

TABLE 12

A COMPARISON OF THE F^{18} CONTENT OF THE MOLARS, INCISORS, MANDIBLES AND LEG BONES OF YOUNG ADULT RATS, FLUOROSED RATS AND THEIR MATURE CONTROLS 1, 4 AND 9 HOURS AFTER INTRAVENOUS ADMINISTRATION. VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE AND ARE CORRECTED FOR DEVIATION OF RECOVERY FROM 100 PER CENT. STANDARD ERROR AND P VALUES ARE CALCULATED AS SHOWN IN APPENDIX III.

Tissue	Young Adult	P*	Mature Control	P**	Fluorosed
1 hour					
Molars	0.44 ± 0.04	<0.01	0.26 ± 0.02	<0.01	0.13 ± 0.02
Incisors	1.02 ± 0.07	>0.10	1.18 ± 0.08	<0.05	0.95 ± 0.04
Mandible	2.56 ± 0.04	<0.01	2.00 ± 0.11	>0.10	1.96 ± 0.05
Leg Bones	5.05 ± 0.12	<0.01	3.78 ± 0.11	<0.05	3.16 ± 0.17
4 hours					
Molars	0.41 ± 0.02	<0.01	0.26 ± 0.008	>0.10	0.17 ± 0.04
Incisors	1.13 ± 0.08	>0.10	1.40 ± 0.11	>0.10	1.23 ± 0.13
Mandible	2.97 ± 0.11	<0.01	2.04 ± 0.12	>0.10	2.11 ± 0.08
Leg Bones	5.87 ± 0.09	<0.01	4.34 ± 0.22	<0.05	3.49 ± 0.21
9 hours					
Molars	0.30 ± 0.03	<0.01	0.18 ± 0.01	>0.10	0.16 ± 0.025
Incisors	1.33 ± 0.07	>0.10	1.38 ± 0.07	>0.10	1.12 ± 0.13
Mandible	2.39 ± 0.12	<0.10	2.10 ± 0.08	>0.10	2.38 ± 0.16
Leg Bones	4.94 ± 0.14	<0.01	3.97 ± 0.13	>0.10	3.81 ± 0.15

* P compares young adults and mature controls.

** P compares mature controls and fluorosed animals.

TABLE 13

THE CONCENTRATION OF F^{18} IN THE MOLARS, INCISORS, MANDIBLES AND LEG BONES OF YOUNG ADULT RATS, FLUOROSED RATS AND MATURE CONTROLS 1, 4 AND 9 HOURS AFTER INTRAVENOUS ADMINISTRATION. VALUES ARE EXPRESSED IN PER CENT OF ADMINISTERED DOSE PER GRAM OF WET TISSUE. STANDARD ERRORS AND P VALUES ARE CALCULATED AS SHOWN IN APPENDIX III.

Tissue	Young Adult	P*	Mature Control	P**	Fluorosed
1 hour					
Molars	3.04 ± 0.17	< 0.01	1.68 ± 0.045	< 0.01	0.94 ± 0.096
Incisors	3.14 ± 0.14	> 0.10	2.72 ± 0.18	< 0.01	1.85 ± 0.068
Mandible	5.08 ± 0.33	< 0.01	2.94 ± 0.21	> 0.10	2.94 ± 0.14
Leg Bones	3.79 ± 0.12	< 0.01	2.32 ± 0.19	> 0.10	2.04 ± 0.16
4 hours					
Molars	2.75 ± 0.13	< 0.01	1.64 ± 0.18	> 0.10	1.39 ± 0.37
Incisors	3.98 ± 0.32	< 0.10	3.12 ± 0.22	> 0.10	2.56 ± 0.385
Mandible	5.96 ± 0.19	< 0.01	3.14 ± 0.22	> 0.10	3.04 ± 0.18
Leg Bones	4.33 ± 0.12	< 0.01	2.74 ± 0.225	< 0.10	2.18 ± 0.13
9 hours					
Molars	2.50 ± 0.13	< 0.01	1.33 ± 0.07	> 0.10	1.15 ± 0.19
Incisors	3.61 ± 0.23	< 0.10	3.01 ± 0.185	-	- -
Mandible	4.61 ± 0.42	< 0.05	3.22 ± 0.23	> 0.10	3.54 ± 0.306
Leg Bones	3.31 ± 0.12	< 0.01	2.46 ± 0.18	> 0.10	2.22 ± 0.107

* P compares young adults and mature controls.

** P compares mature controls and fluorosed animals.

fluorosed rats did not show this same pattern, but instead continued to accumulate F^{18} slowly after the first hour so that at 9 hours the values obtained for the fluorosed animals were the same as those obtained for their controls.

Table 12 gives a comparison of the F^{18} uptake of the osseous and dental samples taken from the young adult animals (Experiment 1) and from the fluorosed rats and their mature controls. Table 13 compares the F^{18} concentrations in these samples and Table 14 compares the fresh weights of the same samples. See Appendix III for explanation of the p values given.

It is of interest to note that the mandibles of the fluorosed rats and their mature controls show no differences in F^{18} concentration or total F^{18} uptake at any of the time intervals investigated. The fresh weight of the mandibles of the fluorosed rats was not significantly different from the fresh weight of the control mandibles. This is in sharp contrast to the leg bones for which there were found no differences in fresh weights, but significant differences in F^{18} content and concentration at 1 and 4 hours.

The fresh weight of the molars of the mature control animals was comparable to the molar weight of the young adult animals. It was also found that the weight of the fluorosed molars was less than that of their controls. However, if the molar weights of the fluorosed rats are compared with those of the young adult rats the difference is not statistically significant. Further, the molars of the fluorosed rats were easy to remove and were almost always removed intact, while some of the roots of the mature control molars remained embedded in the mandibles and maxillae. Therefore it is reasonable to assume that the molars of the mature controls actually weighed more than the value recorded in Table 14 would indicate. It was also noted that the root structure of the fluorosed molars was not

TABLE 13

THE MEAN FRESH WEIGHT OF THE TEETH, MANDIBLES AND LEG BONES OF YOUNG ADULT, MATURE AND FLUOROSED RATS. P VALUES ARE CALCULATED AS SHOWN IN APPENDIX III.

	Young Adults	P*	Mature Controls	P**	Fluorosed
Molars	0.14 ± 0.004	>0.10	0.15 ± 0.003	<0.01	0.13 ± 0.004
Incisors (Includes Pulp)	0.33 ± 0.01	<0.01	0.44 ± 0.005	<0.01	0.52 ± 0.02
Mandible	0.51 ± 0.01	<0.01	0.67 ± 0.01	>0.10	0.68 ± 0.02
Leg Bones (Includes Marrow)	1.35 ± 0.04	<0.01	1.64 ± 0.06	>0.10	1.63 ± 0.04

* P compares young adults and mature controls.

** P compares mature controls and fluorosed animals.

as extensive as that of their controls. Other investigators have shown that prolonged fluoride feeding does not affect either the appearance or strength of adult teeth, but that it does retard the growth and eruption of immature teeth Roholm.[100] If the enamel of teeth that do not grow from persistent pulp is laid down during a period of fluoride feeding, it is poorly calcified and wears away with functional attrition relatively easily Slavsgold.[110] The molars of the fluorosed animals and their controls were not fully mature when the fluoride feeding was initiated, since it has been shown that the molars of the rat do not mature until approximately 125 days of age Schour and Massler.[104] Under the influence of the fluoride supplement the final maturation of the molars was very likely retarded and their calcification faulty which would allow them to wear excessively. Excessive wear and retardation of root growth would account for the observed differences in molar weight.

The values obtained for the F^{18} contents and F^{18} concentrations of the molars of both groups of older animals were considerably lower than the corresponding values found for the young adult animals. This difference between older and younger animals in their ability to accumulate F^{18} seems to be a function of the decrease in pulpal activity and of the calcification of the molars with increasing age, Schour and Massler.[104]

The F^{18} contents and concentrations of the molars of the fluorosed rats and their controls were comparable except at the 1 hour interval. The lower F^{18} accumulation by the fluorosed molars at this time may be due in part to the inability of the enamel and exposed dentin of the rat molar to adsorb F^{18} from the saliva efficiently after a prolonged exposure to fluoride in the diet. This explanation is amplified by the findings of others. Volker et al[127] found that enamel and dentin adsorbed radio-fluorine by a

surface reaction according to the Freundlich adsorption isotherm equation, in which case an equilibrium is set up between the material in solution and the material adsorbed on the surface. Further adsorption ceases when the surface of the crystal lattice is covered. Gol'dberg[36] found that tooth surfaces treated with NaF could still adsorb some additional fluoride but that the additional fluoride was very quickly leached.

Since the rat incisor grows from persistent pulp, it was not surprising that the incisors of the mature animals, although heavier than those of the young adults, accumulated F^{18} to the same extent as did the younger animals. The greater weight of the incisors of the mature rats would account for the differences in F^{18} concentration observed.

The F^{18} accumulated by the incisors of the fluorosed and mature rats was comparable at the 4 and 9 hour intervals. There was a significant difference noted at 1 hour after the injection, in which case the fluorosed incisors had not taken up as much F^{18} as had those of the controls. This initial lag in the ability of the fluorosed incisors to accumulate F^{18} is apparently overcome in the next 3 hours, due perhaps to the slightly elevated level of F^{18} in the blood of these animals. The lower F^{18} content of the fluorosed incisors at 1 hour, despite the elevated blood level could be due to the inability of the fluorosed tooth surfaces to adsorb and retain F^{18} from the saliva, as was postulated in the case of the molars.

The greater weight of the incisors of the fluorosed rats, would help to account for the differences observed in the F^{18} concentrations of the fluorosed and mature control incisors.

The results of the tracer studies described here on the distribution of F^{18} in mature adult and fluorosed rats and those discussed in the preceeding

sections on young adult rats have except for the one experiment on lactating rats, been corrected for deviations of recovery of the injected F^{18} from 100 per cent. The actual experimental recoveries for individual animals varied from 76 per cent to 120 per cent of the administered dose due to pooling of excretions and loss of material in the meat grinder used to prepare the carcasses. The average recoveries for each experimental group of three to five rats did not vary quite as much and was generally about 85 per cent to 95 per cent of the administered dose.

(7) Radioautography with F^{18}

Figures 5 ab, 6 ab and 7 ab show the best examples obtained of the cut silvered surfaces of the distal ends of the femurs and the corresponding F^{18} radioautographs prepared from three groups of animals that were sacrificed one hour after the intravenous injection of F^{18}. The groups employed were: (a) young adult females 70 days old, (b) mature females 200 days of age that had been maintained on a semi-synthetic diet and (c) fluorosed females 200 days of age that had been fed the semi-synthetic diet plus 20 mgm of fluoride per kilo of body weight daily.

Unfortunately the relative darkening of the films could not be used to compare quantitatively the radioautographs obtained from the three groups of animals for several reasons: (1) The amount of F^{18} administered to each rat in any given group varied widely due to the variations in F^{18} yields from any given bombardment, (2) There was quite a large variation in the amount of F^{18} accumulated in the bones of the individual animals in any given group and (3) The amount of F^{18} accumulated by the bones of the young animals was considerably greater than for the older animals, either fluorosed or mature controls. An attempt was made to adjust the length of exposure of

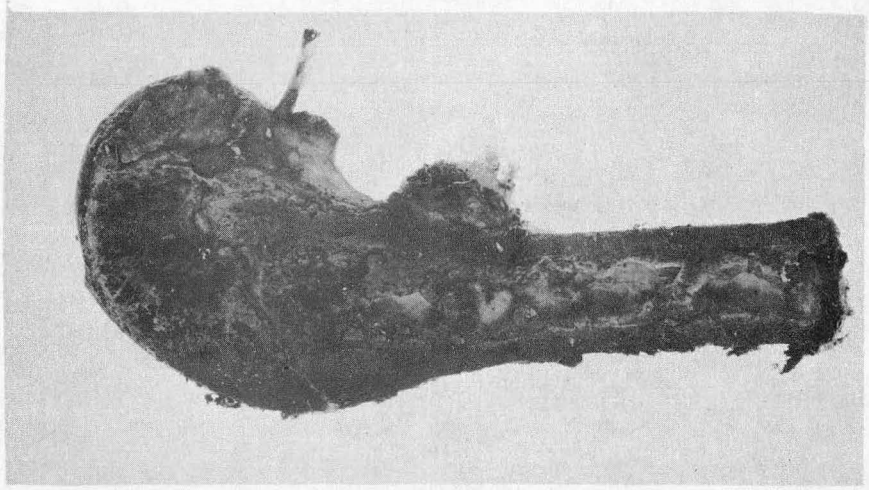

Fig. 5a The silvered surface of the distal end of the right femur of a young adult female rat, 75 days old. (x6)

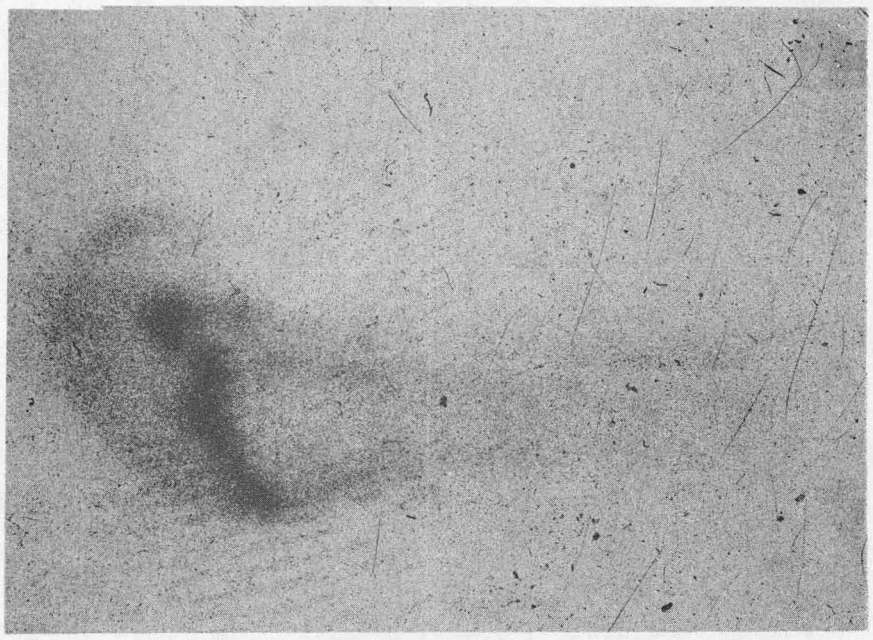

Fig. 5b The F^{18} contact radioautograph of the bone surface shown in Fig. 5a. (x6)

ZN-584

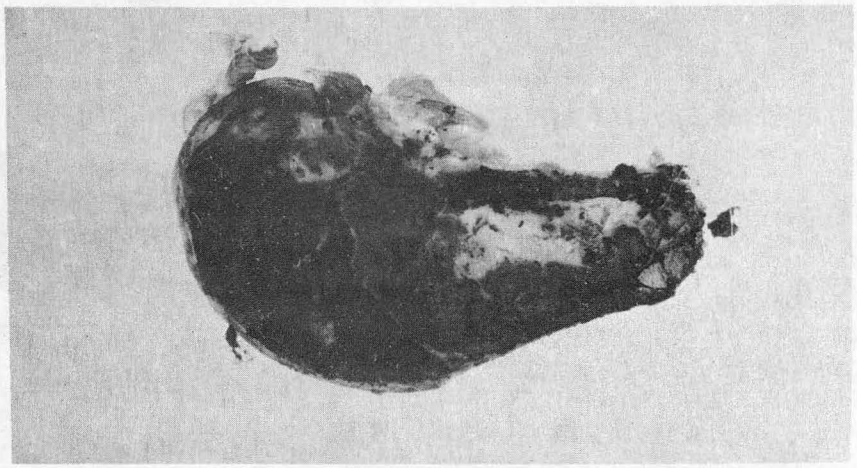

Fig. 6a The silvered surface of the distal end of the right femur of a mature female rat, (Rat No. 50), 200 days old. (x6)

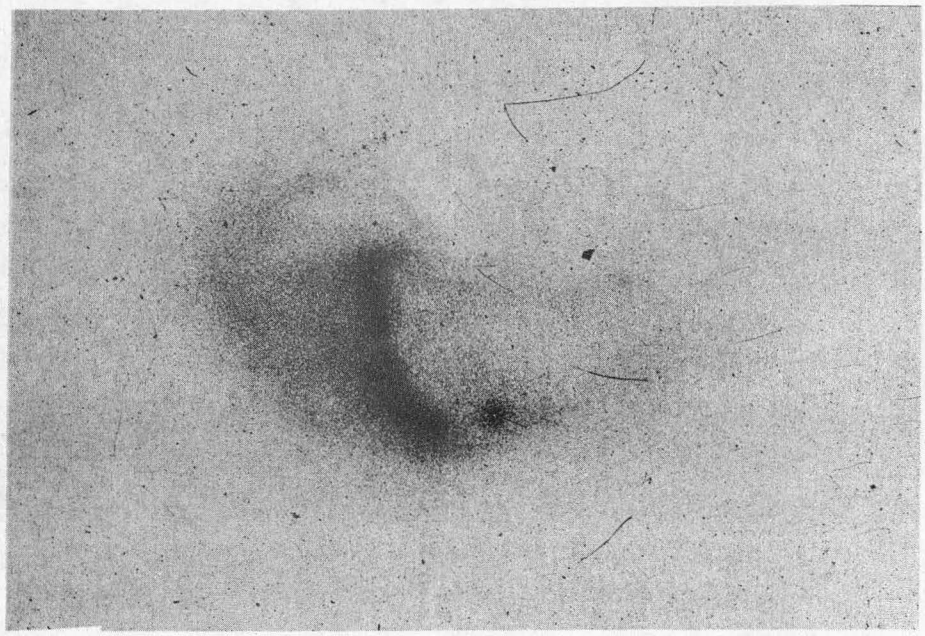

Fig. 6b The F^{18} contact radioautograph of the bone surface shown in Fig. 6a. (x6)

ZN-585

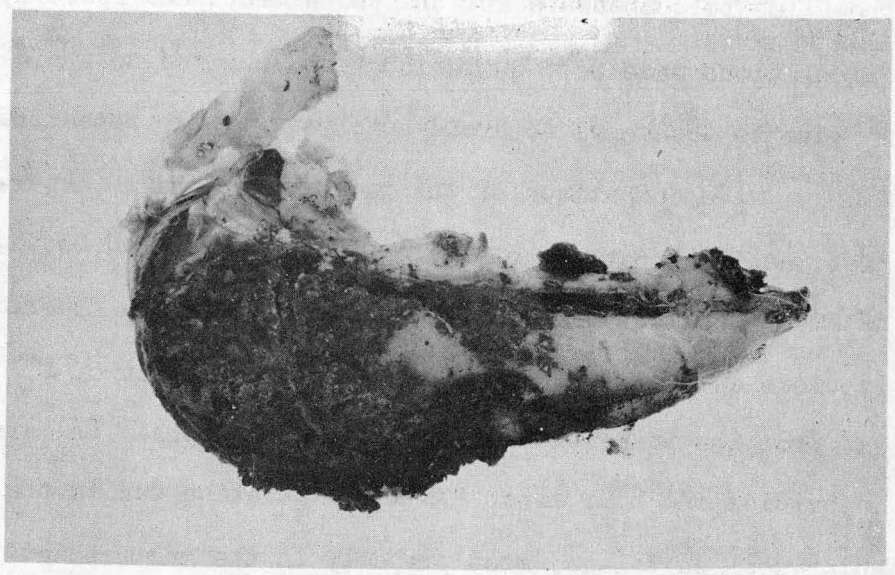

Fig. 7a The silvered surface of the distal end of the right femur of a rat chronically intoxicated with 20 mgm of fluoride per kilo per day for 4.5 months. This animal (Rat No. 30) was the same strain and age as animal No. 50. (x6)

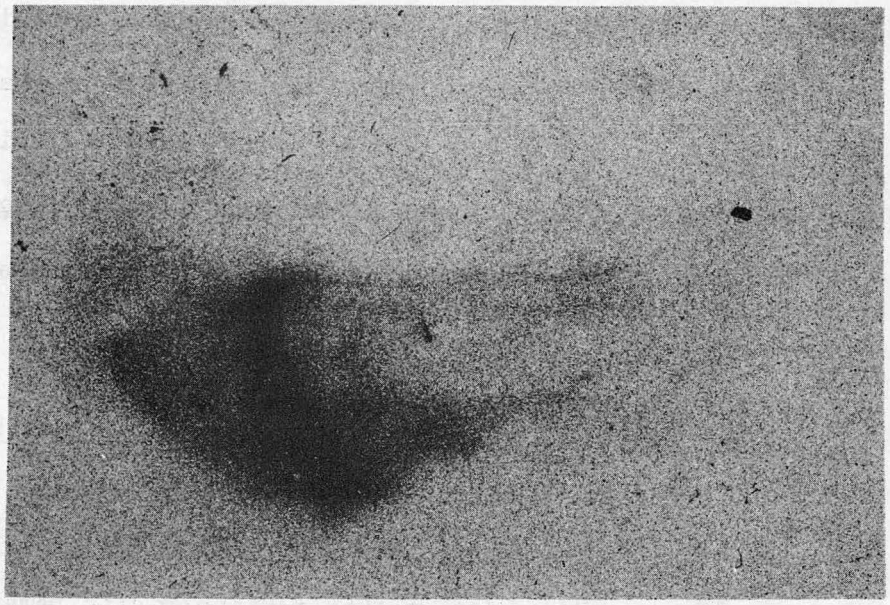

Fig. 7b The F^{18} contact radioautograph of the bone surface shown in Fig. 7a. (x6)

ZN-586

the films to compensate for the above mentioned factors, however, the calculations made were quite rough and hurried to avoid excessive loss of F^{18} due to decay and to avoid autolysis of the bones themselves.

On comparison of the radioautographs no alterations in the general distributional pattern of F^{18} in the bones are to be found either as a result of advancing age or previous exposure to dietary fluoride. Several generalizations can be made, however, on the deposition of F^{18} in the bones of the rat from the inspection of the radioautographs. The area of greatest F^{18} deposition one hour after its administration was in the region of the epiphyseal cartilage, most probably in the most recently calcified bone just below the epiphyseal plate. The spongy bone of the epiphysis and of the upper end of the diaphysis, especially on the front side, were the next most heavily blackened areas. This was not quite as apparent in the radioautographs made from the young bones as it was in the mature bones. Despite the poor resolution obtainable from such relatively crude methods as were used in the preparation of these radioautographs, it can be seen that there appears to be little if any F^{18} in the marrow cavity. In the radioautograph of the fluorosed bone and to a lesser extent that of the young adult bone, there appears to be a small amount of F^{18} in the periosteum and endosteum, but very little in the compact bone.

(8) Histological Findings on Effects of Fluoride on the Rat Femur

Sections were prepared of the distal end of the right femur of four control rats and four rats maintained on a high fluoride diet for 4.5 months. The sections were stained routinely with hematoxylin and eosin and are shown at low power magnification. Since such small groups of animals were used, four control and four fluorosed rats, the results obtained on the

histological preparations described here can be assumed to be only semi-quantitative. Before discussing the findings, it should be stated that of the four fluorosed bones studied, one appeared very nearly normal, one showed complete growth arrest and the other two lay somewhere between the two extremes. The preparations from the latter two animals showed areas of normal bone growth and areas of growth arrest. On the other hand the control bones showed quite a bit of variation in so far as the quality of the marrow was concerned. The marrow of one animal was almost 90 per cent cellular and of another only about one-half cellular, again with the others lying somewhere in between the two extremes. The control animals also showed variation in the number of layers of enlarged, degenerating chrondrocytes. However, when the slides were randomized with the labels face down, it was still possible to align them into two distinct groups, with the position of only one control and one treated animal in doubt.

Measurement of the width of the epiphyseal plate taking the average of four separate determinations on each section gave the following results: control, 182 ± 6.9 microns and fluorosed, 145.4 ± 5.8 microns, with a difference of 37.4 microns between the two groups and a p value of less than 0.01, a surprisingly significant result for the small groups employed. These findings are consistent with the observation mentioned earlier than the femurs of the fluorosed rats were shorter than those of the controls by some 2.3 mm.

The slides chosen for presentation here are those that were considered approximately the midpoints of each group both on the basis of histological evidence and on the basis of the width of the epiphyseal plate and femur length.

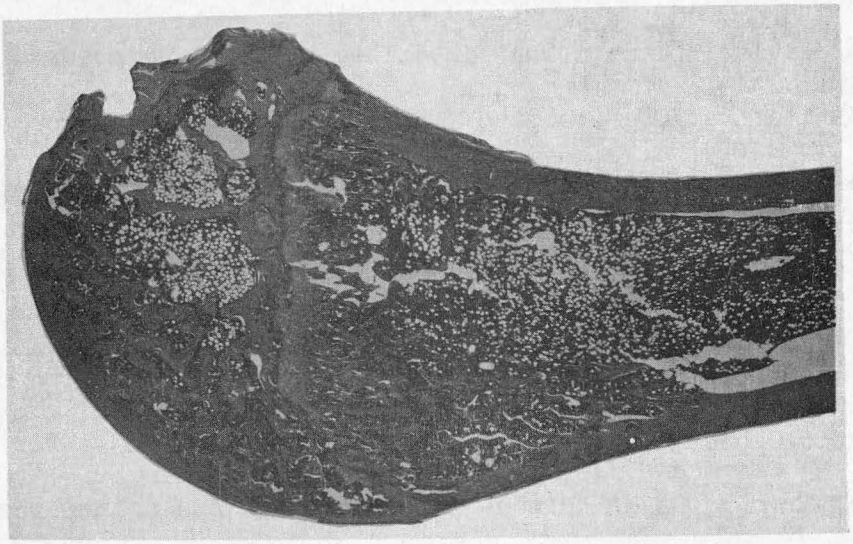

Fig. 8 Histological section of the distal end of the left femur of mature control rat No. 50, stained with hematoxylin and eosin. See text for description. (x12)

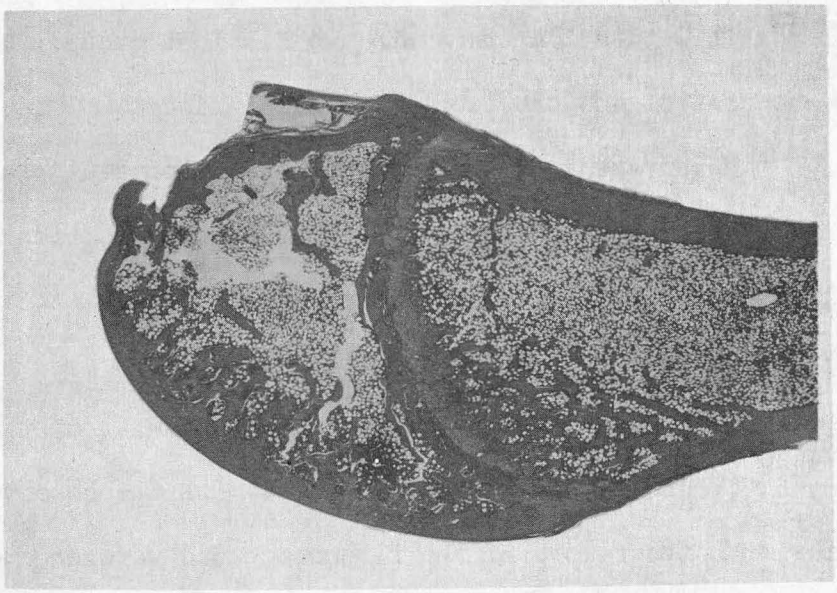

Fig. 9 Histological section of the distal end of the left femur of a fluorosed rat, No. 30, stained with hematoxylin and eosin. See text for description. (x12)

ZN-587

Figure 8, the control, shows short columns of proliferating chondrocytes with one to two layers of slightly vacuolated enlarged chondrocytes. Marrow tufts are abundant and there are quite a few delicate, short primary trabeculae. Secondary trabeculae are less abundant and are located mainly on the front side of the bone. The marrow appears to be about two-thirds cellular and about one-third adipose. There are no signs of growth in the cortical bone. The general impression is that of slow growth and is approximately normal for rats of this strain and age.

Figure 9, the fluorosed animal, shows a few columns of proliferating chondrocytes. However, some chondrocytes are arranged in clumps and in coniform clusters. From the front side of the bone to the center there are some vacuolated cartilage cells, there is some erosion and some formation of a few primary trabeculae. From the center to the back side of the bone there is no evidence of bone formation and no erosion. At this point the marrow lies inactive against the cartilage, although there is no bony seal as was seen in another of the animals. There are no sites of really abundant bone formation at any point. There are a few secondary trabeculae on the front side, however. The marrow is predominantly adipose. The general impression is one of almost complete growth arrest resembling advanced age or hypophysectomy.

Unfortunately, there was no real correlation obtained among any of the following factors: weight gain, severity of tooth symptoms, femur length and extent of marrow affectation.

DISCUSSION

Simple inorganic fluorides administered in microgram quantities appear to be able to permeate cells quite freely as shown by the red cell to plasma ratio which was found to be very nearly the same for F^{18} as for the other halides, by the maintenance of a dynamic equilibrium between the F^{18} in the blood and soft tissues and by the rapidity of urinary excretion of F^{18}. It is reasonable to assume, therefore, that when fluorides are present in the animal body at extremely low concentrations as the result of an ordinary mixed diet and a relatively low fluoride concentration in the water supply, fluorides will behave as simple ions and not as complexes with phosphate, metal ions or proteins.

In the tracer studies in which F^{18} was given in the essentially carrier-free state there was actually sufficient stable fluoride present so that a 200 gram rat received 0.5 micrograms of fluoride per kilo of body weight. The longest time interval investigated in these experiments was 9 hours after injection, however, since by this time F^{18} was being eliminated from the skeleton only gradually, the majority of the F^{18} found in the skeleton at this time can be considered as stored. In McClure's[77] balance studies on young adult men in which he claimed that there was no storage of fluoride when the daily intake was of the order of a few milligrams, the daily intake can be calculated as approximately 53 micrograms per kilo, an amount nearly 100 times greater than was used in the tracer experiments described here. The results of these studies coupled with those of Hodge[58], Savchuck and Armstrong[103] and Glock, Lowater and Murray[55] who found that the skeletal fluoride content increased slowly with age on diets low in fluoride, indicate that there is apparently no lower limit of fluoride intake below which there will be no

storage and complete excretion as was stated by McClure.

It was previously noted that after the total amount of F^{18} in the blood, muscle and skeleton were subtracted from the F^{18} present in the skinned eviscerated carcass there was a remainder, F^{18} which could not be accounted for by the usual means. This remainder which has been designated as balance varied from 2.8 to 13.5 per cent of the administered dose for groups of three to five rats. The size and variability of the balance present a rather perplexing problem, since theoretically the average balance for any given group of rats should only be of the order of a few per cent which could be accounted for by individual variation from the average figures used to calculate the total blood, muscle and skeleton weights.

The size of the balance could not be correlated with the time interval after the injection, the age of the animals or their previous exposure to fluoride. Apparently the size of the balance is not due to any great extent to faulty assumptions concerning blood, muscle or the presence of F^{18} in the marrow of the leg bones, since the balance figures were still high even at the 9 hour interval when total blood and muscle comprised less than one per cent of the administered dose. In view of the findings that the F^{18} concentrations of the soft tissues paralleled the blood level, it is unlikely that the soft tissues present in the carcass other than muscle, such as fat, nervous tissue, connective tissue, lymphatic tissue and blood vessels contained sufficient F^{18} especially at the later time intervals to contribute greatly to the size of the balance.

The use of the fresh leg bones as an index of the F^{18} concentration of bone may introduce some error. Although the leg bones contain both

spongy and compact bone, they may not be present in the same proportions in these bones as they are in the skeleton as a whole. If this is the case then a certain amount of error would be introduced into the calculation of the total F^{18} of the skeleton, since it has been fairly well established by these experiments that the more vascular spongy bone of the mandible and leg bone epiphyses showed a greater F^{18} concentration (in per cent of dose per gram of fresh bone) than the compact bone of the leg bone diaphyses. However, the errors, if any, introduced by the above mentioned discrepancy seem to be minimal, if the following argument is valid. Savchuck and Armstrong[103] found that for rats the fluoride content of the femur, tibia and fibula of one leg constituted 8.8 per cent of the total skeletal fluoride and that this figure did not vary with advancing age or previous exposure to dietary fluoride. The results of the experiments described here were therefore recalculated using the above figure and the per cent of F^{18} found in the leg bones. The skeletal uptake of the individual rats and the corresponding balance values computed on this basis were almost invariably identical to those obtained when the skeletal F^{18} content was calculated using the per cent of dose per gram of wet bone and the skeletal ash weight as described in the section on methods.

There then remain two further possibilities both of which would help to account for the size and variability of the balance, first the incomplete collection of urine from the bladder at the earlier time intervals and second, the F^{18} present in cartilage.

The above mentioned work of Savchuck and Armstrong[103] does not rule out the possibility that F^{18} present in cartilage might be responsible for a sizeable portion of the F^{18} that cannot be accounted for by the usual means. Their animals were skeletonized and ashed to constant weight before the fluoride analyses were made and both of these processes effectively destroy

the essentially organic cartilagenous structures associated with the skeleton as well as the small amounts of cartilage situated elsewhere in the animal body such as in the trachea.

In the study of autopsy specimens obtained from two cryolite workers Roholm[100] found extensive calcification of the ligaments of the vertebral column and to a lesser degree of the costal cartilages. He also found that the fluoride content of some of these ligaments was the same as in the adjacent bone.

No conclusions of a quantitative nature can be drawn concerning the F^{18} in cartilage, due to the difficulties involved in the determination of the amount of cartilage present in the animals and the difficulties encountered in the dissection of cartilagenous structures from the surrounding tissues. Rough measurements indicated that the tracheal cartilage concentrated F^{18} to a greater extent than did the xiphoid cartilage. Considering the findings of Roholm[100] mentioned above, it would not seem to be mere coincidence that the calcification of tracheal cartilage has been found in old rats while the xiphoid cartilage does not appear to be calcifiable under any conditions Asling.[3]

It seems possible then that F^{18} present in cartilage, to an extent apparently involved with its potentialities for calcification, could account for much of the F^{18} in the balance. The presence of F^{18} to such a large extent in the region of the epiphyseal plate as shown by the radioautographs would appear to amplify this line of reasoning.

Evidence obtained from the tracer studies using F^{18} described in these pages and from F^{18} radioautographs of rat femur indicates that the deposition of F^{18} is greater in spongy bone than in compact bone. The radioautographs show further that there is quite a large amount of F^{18} deposited in the trabecular bone and in the region of the epiphyseal plate, a small amount in the regions of the endosteum and periosteum and an almost undiscernable amount in the

compact bone of the femur shaft. It must be remembered, however, that the radioautographs were prepared from animals sacrificed only one hour after the injection of F^{18} and that these were the initial sites of F^{18} deposition. It was also found that the mandible, a membranous bone, concentrated F^{18} to a greater extent than did the leg bone diaphyses or epiphyses especially in the younger animals. Consideration of the above findings allows a general conclusion to be drawn to the effect that the extent to which a bone is able to accumulate F^{18} depends in large measure upon the vascularity of its various bony components. The dependence of the skeletal deposition of F^{18} on the vascularity of the bones provides further evidence for the widely held view that the chemical mechanism responsible for the bony deposition of fluorine when present in body fluids at low concentrations is a surface reaction, Hodge.[58] It is reasonable to assume that the bone salt crystals of the relatively vascular spongy bone which are bathed by a large volume of fluid containing fluoride at a given concentration will take up more fluoride than the bone salt crystals of compact bone which are bathed by a much smaller volume of fluid containing fluoride at the same concentration.

SUMMARY

Radioactive tracer and radioautographic techniques using NaF tagged with F^{18} were employed to study the metabolism of inorganic fluorides in rats. Young adult females, virgin, pregnant and lactating, mature females and chronically fluorosed animals were used. F^{18} was administered orally and by intravenous injection in an essentially carrier-free state and with added fluoride carrier. I^{131} was administered to fluorosed rats and mature controls to investigate the possible effects of chronic fluorosis on thyroid function. Histologic preparations were made of the distal ends of the femurs of fluorosed and control animals to study bone changes due to chronic fluorosis. The results permit the following conclusions:

1. Cell membranes appeared to be freely permeable to F^{18} when it was administered as NaF.
2. There did not seem to be any significant deposition of F^{18} in any of the soft tissues of the rat, with the possible exception of the kidney, when it was given with stable fluoride in amounts ranging from 0.5 micrograms to 10 milligrams per kilo of body weight.
3. Urinary excretion and skeletal deposition of F^{18} were the same regardless of whether the stable fluoride dose was 0.5 micrograms or 10 milligrams per kilo indicating that there was no lower limit of fluoride intake below which all ingested fluoride would be excreted.
4. There appeared to be genuine fecal excretion of F^{18}, although the amount excreted by this route was quite small.
5. Absorption of orally administered F^{18} was not complete 9 hours after its administration. At this time there was about 10 per cent of the administered dose in the cecum, large intestine contents and feces.

6. The distribution of F^{18} in the rat was essentially the same whether it was given orally or by vein.

7. When given with microgram quantities of stable fluoride, F^{18} appeared to be able to pass through the placenta of the rat (2.6 per cent of the dose was found in the fetuses, placenta and uterus), although the work of Flexner and Pohl[28,29] indicated that the time allowed in these experiments was not sufficient to allow for equilibration of F^{18} between the maternal blood and placenta.

8. Small amounts of F^{18} (approximately one per cent of the dose) were found in the nurslings of the lactating rats 4 hours after the mothers were given tracer amounts of F^{18} by stomach tube. The fact that the mammary tissue contained F^{18} in approximately the same concentration as the maternal blood indicates that in this species fluoride passes into the milk by a process resembling diffusion.

9. The extent to which a bone will accumulate F^{18} seemed to depend upon the vascularity of the bone and on its growth activity. Chronic fluorosis did not influence the skeletal uptake of F^{18} except in so far as it affected bone growth.

10. There appeared to be a tendency for cartilage to accumulate F^{18}. The extent to which this occurred could be qualitatively correlated with the potentialities of the particular cartilage for calcification.

11. At the dietary fluoride level studied in these experiments (20 mgm of fluoride per kilo per day for 4.5 months) there was significant retardation of weight gain which was not due to lowered food intake, since the fluorosed rats and their controls were pair fed.

12. Chronic fluoride intoxication instituted when the animals were not yet mature retarded the longitudinal growth of the femur and presumably of the other long bones as was shown by the reduced femur length of the fluorosed rats and the reduction in the width of the epiphyseal cartilage of the distal end of the femur. The ash content of the leg bones of the fluorosed animals was also lower than the ash content of the corresponing bones of the control animals. This observation was in line with the histological evidence of scanty trabecular bone formation in the fluorosed femurs.

13. There did not seem to be any significant impairment of the thyroid function which could be attributed to chronic fluoride intoxication, when the thyroid uptake of I^{131} and the red cell to plasma ratio of I^{131} were used as indices of thyroid function.

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APPENDIX I

COMPOSITION OF "DIET 14" AS PREPARED BY THE UNIVERSITY OF CALIFORNIA INSTITUTE OF EXPERIMENTAL BIOLOGY

Ground whole wheat	68.5 lbs.
Casein	5.0 lbs.
Alfalfa leaf meal	10.0 lbs.
Fish meal	10.0 lbs.
Fish oil	5.0 lbs.
NaCl	1.5 lbs.
	100.00 lbs.*

* 300 ml. of a KI solution (450 mgm. KI/liter) added to every 270 lbs. of feed.

APPENDIX II (a)

COMPOSITION OF SYNTHETIC DIET EMPLOYED IN FLUORIDE FEEDING EXPERIMENTS

Casein	100 grams
Powdered Skim Milk	60 grams
Whey	40 grams
Brewer's Yeast	40 grams
Hydrogenated Vegatable Oil	60 grams
Wheat	660 grams
Mineral Mixture	40 grams
	1000 grams*

* 2 grams of Vitamin A and D supplement "Napco Quadrex" added per kilo.

APPENDIX II (b)

MINERAL MIXTURE FOR SYNTHETIC DIET

$CaCO_3$	68.6 grams
$CaHPO_4$	112.8 grams
$MgCO_3$	35.2 grams
$Ca_3 (C_6H_5O_7)_2 \cdot 4H_2O$	308.3 grams
$MgSO_4$ Anhydr.	38.3 grams
KCl	124.7 grams
NaCl	77.1 grams
$K_2HPO_4 \cdot 3H_2O$	218.8 grams
Trace Mixture	16.2 grams
	1000.0 grams

TRACE SALT MIXTURE

Green Ferric Ammonium Citrate	98.52 grams
KI	0.25 grams
$CuSO_4 \cdot 5H_2O$	0.50 grams
Ammonium Alum	0.59 grams
$MnSO_4 \cdot H_2O$	0.14 grams
	100.00 grams

APPENDIX III

STATISTICAL METHODS

(1) The Standard Error

The standard error of the mean was calculated as follows:

$$\text{S.E. of mean} = \sqrt{\frac{\Sigma d^2}{n(n-1)}} ,$$

where d is the difference between the mean and the individual determination and n is the number of determinations.

(2) Comparison of the Means of Two Groups, The Calculation of t and p.

The composite variance V is calculated as follows:

$$V = \frac{\Sigma d^2 \text{ for group 1} + \Sigma d^2 \text{ for group 2}}{n_1 + n_2 - 2} ,$$

where n_1 and n_2 are the number of determinations in groups (1) and (2) respectively. The standard error of the difference between the two means is then calculated:

$$\text{S.E. of diff.} = \sqrt{\frac{V}{n_1} + \frac{V}{n_2}} ,$$

t is then calculated:

$$t = \frac{\text{Difference between the means}}{\text{S.E. of difference}} ,$$

the probability that the difference between the means is not due to chance, p, is then obtained from a table similar to the one prepared by Fischer.[27] When the p value for two means is greater than 0.10 the significance of the difference is questionable. For a p value of less than 0.01 the difference between the two means must be considered highly significant.

APPENDIX IV

CALCULATION OF INTERNAL RADIATION DOSE

The equation is used to estimate the internal radiation dose in roentgen equivalent physical due to the absorption of the 0.67 Mev positrons emitted by F^{18} (energy due to the annihilation radiation is neglected) in the body or tissues of an animal is as follows:

$$\text{r.e.p.} = \frac{Cn(1-e^{-kt})}{W} ,$$

where n is the number of microcuries in the body or tissue,

W is the weight of the animal or tissue in grams,

k is the decay constant = $0.693/T1/2 = 6.19 \times 10^{-3}/s$,

t is the time during which the tissue is exposed to the radiation in seconds and

C is a constant = 1.61 obtained from the following expression:

$$\underbrace{\frac{(3.7 \times 10^4\ d/s/\mu)}{5.2 \times 10^7\ \text{Mev/r.e.p./gm.}}}_{\text{conversion factors}} \times \underbrace{\frac{(60\ s/min)(112\ min)}{(0.693)}}_{\text{mean life of } F^{18}} \times \underbrace{\frac{(0.67\ \text{Mev/d})}{(3)}}_{\text{average beta energy}} = 1.61$$

$(1-e^{-kt})$ has the following values at:

15 min	0.086
60 min	0.309
240 min	0.775
540 min	0.965