1941

A biochemical study on fluorine: I, Physiological responses to fluorine compounds in the rat; II, Attempts to remove fluoride to the non-toxic level in drinking water

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A BIOCHEMICAL STUDY ON FLUORINE

I. PHYSIOLOGICAL RESPONSES TO FLUORINE COMPOUNDS IN THE RAT.

II. ATTEMPTS TO REMOVE FLUORIDE TO THE NON-TOXIC LEVEL IN DRINKING WATER.

by

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A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Physiological and Nutritional Chemistry

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

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Signature was redacted for privacy.

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Iowa State College

1941

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INTRODUCTION

An over-supply of a trace element, whether or not the element may be classed as an essential one, may be markedly deleterious to the organism. The number of trace elements which by natural means may be supplied to the body in harmful amounts appears to be quite small indeed. Fluorine has been found to belong in this category; even milligram quantities of fluorine, daily gaining access by natural means to the bodies of human beings and other animals, may bring about pathological changes. Some of the changes, such as those occurring in bone, appear to be quite persistent, while other changes, such as those in the enamel of teeth, appear to be permanent.

As it became generally accepted that fluorine in drinking water is the prime etiological factor responsible for the wide-spread hypoplasia of human teeth, known as mottled enamel, numerous investigators began to seek information which might make possible the prevention of this damage to teeth. Some investigators sought information which would lead to a better understanding of the chemical mechanisms through which fluorine causes damage to the teeth. Other workers began to study the toxicity of fluorine toward other tissues of the body since there appeared to be no proof that quantitites of fluorine causing tooth damage could not at the same time damage other

tissues and organs, thus impairing one or more of the vital processes of the body.

The obviously critical nature of the problem of removal of fluorides from both public and private water supplies lent early stimulus to the search for a practical method for removal. Without a workable method for such water treatment, the home or municipality confronted with endemic fluorosis has only one recourse--that of changing water supplies; but the lack of availability of suitable supplies in many communities renders a practical method of removal imperative.

Dangerous sources of fluorine, other than water supplies, were encountered early. These include fruits of a wide variety, which may carry residues of fluorine bearing insecticides, and dusts and gases formed in some mining and manufacturing industries. Fluorine containing dusts and gases can readily enter the lung, from whence the fluorine may be absorbed. Thus the alimentary tract is not the only portal through which fluorine may gain access to the body.

The experimental work of this thesis was planned with the purpose of contributing to the advancement toward two major objectives:

1. Determination of the capacity of ingested fluorine compounds to impair normal functions of the body, and
2. The development of a treatment for removing fluoride from drinking water.

In line with the first of these objectives, answers to the following questions were sought:

1. Are the iron and copper fluorides utilized as sources of iron and copper for hemoglobin formation in the rat?
2. Does sodium fluoride cause a change in hemoglobin in the rat during growth, reproduction and lactation?
3. Do inorganic fluorides differ in toxicity on the basis of the fluorine which they contain?
4. Would it be possible to alleviate the effects of fluorine in the diet by administering a substance that would combine with fluorine and not be absorbed?
5. Are there factors in nutrition that may operate to cause variation in the retention of fluorine in the skeletal structures of the body?
6. Does fluoride impair the regulation of blood sugar in the rat, thus possibly contributing to inanition observed after prolonged periods of fluorine ingestion?
7. Will organic fluorides cause fluorine poisoning?

In pursuit of the second main objective answers to the following questions were sought:

1. Will alum remove fluorine from water in a dependable manner?
2. If so, what is the possibility of devising a continuous process of treatment which might be used on

a large scale for removing fluorine from municipal water supplies?

The albino rat, Mus norvegicus albinus, was used in all of the animal investigations in the work of this thesis.

HISTORICAL

General Aspects of the Literature

The literature pertinent to a study of the physiological effects of fluorine in animals is quite large and has been reviewed in an able manner by several workers interested in the problem. Roholm (83), in his monograph "Fluorine Intoxication", cited 893 references up to the year 1937. D. A. Greenwood (43), in a later review on the same subject, cited 380 references appearing during the seven year period between 1933 and 1939. Other reviews on the subject include those by McClure (63), De Eds (31), and Pierce (79).

Roholm (83) reviews quite completely the early publications dealing with the occurrence of fluorine in biological materials, the distribution of the element in inanimate nature, its effects on biological mechanisms--especially enzyme action, and its general effects in both acute and chronic intoxication of animals.

Articles on fluorine found in the literature before 1920 deal quite generally with the more academic aspects of fluorine, having to do frequently with the chemistry of the element, its quantitative determination, its distribution in the earth's crust and in plant and animal tissues; however, occasional

articles are encountered as far back as 1867 dealing with the toxic properties of fluorine compounds toward animals. A number of sporadic reports of investigations growing out of cases of acute fluorine poisoning are to be found between 1890 and 1920. Between 1910 and 1920 a number of observations were reported which were to have an important influence upon the direction which future studies with fluorine should take. During this period a number of observations of morbid changes involving bones and teeth and general well-being of live-stock in regions surrounding superphosphate and aluminum manufacturing plants in different countries of Europe were reported. In 1916 there appeared an important observation by Black and McKay (6) dealing with the occurrence in Colorado of a tooth defect, mottled enamel, of unknown etiology, but seemingly associated with drinking water in affected areas.

In 1925 Schulz and Lamb (92) and McCollum, Simmonds, Becker and Bunting (65) almost simultaneously published their experimental results of feeding sodium fluoride to rats. The observed effects upon the bones and teeth of rats bore marked resemblance to those observed in domestic animals in the areas surrounding superphosphate and aluminum factories. A marked stimulus to further investigation of physiological effect of fluorine was provided by the discovery by Smith, Lantz and Smith (101) in 1931, that fluorine in water supplies is the etiological factor of prime importance in the production of

mottled enamel.

Because of the existance of a number of good reviews of the extensive literature on fluorine, a complete review of the literature dealing with the physiological effects of fluorine has not been undertaken for this thesis. The historical work of this thesis dealing with the physiological responses to fluorides has been prepared with a number of objectives. These are: first, to show briefly the physiological effects that have been observed in the rat; second, to compare findings in rats with those in other animals; third, to compare findings in animals with those in man. The chronic aspects of fluorine poisoning will be dealt with principally.

Articles dealing with the removal of fluorides from water began to appear only after the discovery of the correlation between the consumption of fluoride bearing waters and the occurrence of mottled enamel.

Some Effects of Chronic Fluorine Poisoning Upon Animals Including Man

Effects upon growth, reproduction, and lactation.

A number of studies have been reported which deal with growth in the rat as influenced by sodium fluoride. Sollman, Schettler and Wetzel (104) appear to be the first to make a systematic study. They found that an intake of 8 mg. of NaF

per kg. of body weight caused no depression of growth or food consumption, while levels equal to or greater than 15 mg. per kg. of body weight caused progressive impairment of growth accompanied with a decrease in food consumption. These investigators likewise found that rats, when given a free choice between sodium fluoride poisoned food and unpoisoned food, did not discriminate between the poisoned and unpoisoned food until the fluoride level corresponded to 0.23 per cent sodium fluoride.

Schulz and Lamb (92) likewise reported impairment of the growth of rats by sodium fluoride. Adding 0.025, 0.05, 0.10, 0.15 and 0.25 per cent sodium fluoride to their basal ration, these workers observed growth impairment at the 0.05 per cent level. Those rats receiving 0.25 per cent of the compound in the ration died between 8 and 14 weeks without reaching a weight of 100 grams. With purified basal rations the effect upon growth was noted at lower levels of NaF. In a much later article, Schulz (91) again stated that the threshold level of sodium fluoride for growth impairment in the rat is 0.05 per cent. Lamb, Phillips, Hart and Bohstedt (54) reported that 20 mgs. per kg. of body weight is the maximum level of fluorine as sodium fluoride that can be tolerated by the rat without a decrease in growth rate.

The question of whether the decreased growth rate in rats is due to the decrease in food consumption alone or to a specific fluoride effect has been attacked by McClure and Mitchell (64)

and by Smith and Leverton (102). The former workers reported that 0.0625 per cent fluorine as sodium fluoride caused a decrease in growth rate greater than would be expected as a result of the reduced food consumption. The latter investigators found that the decreased growth rate of the rats receiving 0.05 per cent or more of sodium fluoride was the result not only of decreased food consumption but also of a decreased efficiency in utilization of feed consumed. Evans and Phillips (37) reported that the degree of bleaching of the rat incisor by cryolite and sodium fluoride was proportional to the depression of growth.

Impairment of growth by fluorine has been observed in other animals as well. Slagsvold (95) found poor lactation in cattle in areas close to a Norwegian aluminum factory. Roholm (83) observed growth inhibition due to feeding fluorides to rats, pigs, and calves. Kick (52_a) observed that 0.029 per cent fluorine as sodium fluoride gave growth in pigs only slightly less than normal whereas levels as high as 0.097 per cent fluorine depressed growth in proportion to the amount of fluorine added. Excessive fluorine feeding resulted in decreased food consumption and caused inefficient utilization. The curtailment of food consumption was noted in suckling sows receiving 0.029 per cent fluorine, excessive loss of weight resulting. With chicks, Kick did not note deleterious effects upon growth or other functions until the level of 0.07 per cent

fluorine was reached.

Controlled experiments upon growth in the human being are totally lacking; but loss of weight has been found by Roholm (83) to be associated with the osteosclerotic symptoms of cryolite workers and, by Shortt (93), with the same symptoms in middle aged residents of mottled enamel areas in India.

The influence of fluoride upon reproduction and lactation in the rat has been studied by Schulz and Lamb (92), Schulz (91), Phillips, Lamb, Hart and Bohstedt (78), Tolle and Maynard (108), Kick (52a), and Smith and Leverton (102). All of these workers found that sodium fluoride would impair reproduction if fed at high enough levels. There is quite good agreement between these workers as to the approximate level at which sodium fluoride causes impairment of reproduction. Schulz and Lamb (92) reported occasional impairment of reproduction at a level of 0.025 per cent sodium fluoride; however, in a later report Schulz (91) presents evidence that the level is much higher and that the threshold level of sodium fluoride for lactation is 0.05 per cent. Phillips, Lamb, Hart and Bohstedt (78) could not detect any specific effect of fluorine upon reproductive functions including fertility, gestation, and parturition; they attributed failure of lactation in the rat to intakes of 30 mg. or more of fluorine per kg. of body weight. A level of 0.045 per cent sodium fluoride was found by them to

be sufficiently high to provide an intake of 30 mg. or more during the lactation period. They considered the resultant effects upon lactation and oestrus to be the direct result of fluorine anorexia and inanition. No cumulative effect of fluorine from generation to generation was observed by these men. Kick (52_a) found no direct effect of fluoride upon the reproduction of rats and considered impairment of lactation to be the result of decreased food consumption. Smith and Leverton (102) found no interference with reproduction by a level of 0.025 per cent sodium fluoride but 0.05 per cent sodium fluoride caused stunting of mothers with delayed pregnancy and poor lactation. Tolle and Maynard (100) reported marked impairment of reproduction by 0.55 per cent sodium fluoride in the ration of rats.

Data dealing with the influence of fluorine with reproduction and lactation in other animals are available. Slagsvold (95) found poor lactation (milk production) in cattle. Kick (52_a) found that levels as high as 0.097 per cent fluorine as sodium fluoride did not impair reproduction in the pig either in terms of the number of young born or in terms of the weight of the young at birth. Decreased lactation as indicated by decreased weaning weights was attributed to poor nutrition rather than to a specific effect of fluorine.

Effects upon bone and calcium and phosphorus metabolism.

The influence of fluorine intoxication upon the bones of rats has been studied by McCollum, Simmonds, Becker and Bunting (65), Ellis and Maynard (35), McClure and Mitchell (64), Hauck, Steenbock and Parsons (46), Kick, et al. (52_a), Schulz (90,91), Smith and Lantz (99), Roholm (83), Evans and Phillips (37) and McClure (63). McCollum and co-workers noted the change in the skull and jaw bones of rats on a sodium fluoride supplemented diet. Ellis and Maynard (35) noted the ability of rat bones to take up increased quantities of fluorine upon the incorporation of quantities of fluoride as small as 8 to 12 parts per million in the ration and considered the bone fluoride a better criterion of fluorine poisoning than the incisor effect. The increased fluorine content of rat bones following fluoride feeding has been noted by a number of workers including Evans and Phillips (37), Kick, et al. (52_a), Roholm (83), McClure (63) and Schulz (90). Evans and Phillips (37) found that 4 parts per million of fluorine in the drinking water of the rat caused measurable storage of the element and they used storage data to measure toxicity.

A large number of workers also have studied the effect of chronic fluorine intoxication upon ash, calcium, phosphorus, magnesium and carbon dioxide contents of bones, and upon the metabolism of calcium and phosphorus. McClure and Mitchell

reported that 0.0106 and 0.0313 per cent levels of fluorine as sodium fluoride did not change the per cent retention of the ingested calcium, whereas a level of 0.0623 per cent fluorine lowered the retention of ingested calcium. Smith and Lantz (99) reported that 0.10 per cent sodium fluoride, in a ration otherwise satisfactory, caused lower values than normal for the ash content of bones of rats. An increase in the calcium to phosphorus ratio of the bone was observed to result from an increase in calcium content accompanied by a decrease in phosphorus content. Later, Lantz and Smith (56) reported that 0.10 per cent of sodium fluoride in the diet of rats caused them to retain much less calcium and less phosphorus than normally. Hauck, Steenbock, and Parsons (46) found that on a diet low or moderate in calcium content the ash content of the bones of rats was decreased absolutely and percentagely by fluorine; but with a high-calcium, rachitogenic diet the ash was definitely increased. In another study, those authors found that the effect of sodium fluoride upon the teeth of rats, when the fluoride was in a low calcium ration, was reduced by the administration of vitamin D. Later, Schulz (91) reported findings which supported those of Hauck, Steenbock, and Parsons (46). Schulz reported evidence that rats on a ration containing a high percentage of calcium and a moderate percentage of phosphorus withstood the effects of large dose of fluorides better than rats fed rations

containing other combinations of calcium and phosphorus. With a high-calcium, moderate phosphorus ration he observed an increase in the amount of calcium in the bones of rats. The inclusion of cod-liver oil in the diet or irradiation of the animals with ultra-violet light appeared to inhibit some of the more severe symptoms of fluorosis due to feeding fluorides in a low calcium diet.

Work dealing with the effect of fluoride feeding upon recovery from rickets in rats has recently appeared. Morgaridge (69) reported that the daily feeding of sodium fluoride by mouth to rats caused a slowing up of the development of rickets if given during the depletion period. If given during the healing period the rate of healing was decreased. The bones were examined by x-ray and by ash determinations. No line tests or fluorine determinations were made upon the bones.

Schulz (91) noted increased magnesium and decreased carbon-dioxide contents in rat bones after feeding 0.025 per cent sodium fluoride in the ration. Kick (52_a) observed the same changes.

In other animals likewise the effects upon bone have been observed. Storage of fluorine has been observed by a number of workers. Gaud, Charnot, and Langlais (40) noted changes in bone density in guinea pigs and a decided increase in fluorine in the total ash at autopsy after feeding sodium fluoride at a level of 10 mgs. per kilogram of body weight

daily. Phillips, Hart, and Bohstedt (77) observed the storage of fluorine in bones of cattle fed fluoride bearing rock phosphate. Roholm (83) noted increased fluoride concentations in bones of pigs, calves, and dogs and observed osteosclerotic symptoms on lower levels of fluorine intake; but, at higher levels of fluorine, symptoms of osteomalacia occurred frequently. Sometimes both osteomalacia and osteosclerosis appeared in the same bones. In work with pigs, Kick and co-workers (52_a) noted increased fluorine content of bones resulting from fluoride feeding. The walls of the femurs were thickened. The mandibles were thickened due to the increase of the medullary cavity. Again, the magnesium content of the bone was observed to increase while the carbon-dioxide decreased, the amount of change being in proportion to the level of fluoride fed. The percentage ash at maturity was unaffected by the fluorine feeding.

Observations on bone changes in the human being have been made in several instances in which the fluoride intoxication seems to be definitely involved. Moller and Gudjonsson (68) noted anomalies in calcification in cryolite workers in whose bodies ligaments and tendons were ossified and the density of the bones markedly altered as revealed by x-ray examination. Roholm (83) noted the same changes with cryolite workers in Denmark. The same changes were noted by Shortt and co-workers (93) in a mottled enamel area in India. The changes noted by Shortt were found in middle-aged persons and were associated

with long, uninterrupted periods of consumption of drinking water containing much fluoride.

Kellner (49) has compared the bone changes in fluoride fed dogs with those previously observed by Roholm (83). Kellner made histological comparisons with normal litter mate controls. The feeding of fluoride to puppies resulted in a disturbance of calcification which in many respects resembled rickets. When older animals were fed fluoride over a longer period of time the changes were sclerotic in character. There was extensive deposition or formation of coarse particles of precipitated calcium salts called "Kalkkörner". These changes were strikingly similar to those described by Roholm (83).

Effects upon teeth.

The reports of Schulz and Lamb (92) and of McCollum (65) and co-workers both describe the peculiar effect of fluorine as NaF upon the incisors of the rat. Their descriptions deal with macroscopic aspects of the changes in pigmentation and in shape of the incisors. Macroscopic and histologic aspects of the tooth defect in the rat were studied by Kick, et al. (52_a), and by Schour and Smith (88). Schour and Smith found that the enamel forming organ was damaged by the fluorine and that single injections of fluoride resulted in a band of subnormally calcified enamel and dentine, light in color, followed by a band of high calcification. Succeeding injections caused a repetition

of the phenomenon. The work of Kick includes a detailed macroscopic and histological study of the incisor which presents the same general picture as does the work of Schour and Smith. Kick describes a "fluting" of the surface of the incisor due to very small quantities of fluorine.

The rat has proven to be a very useful animal in the study of the effect of fluoride upon teeth because the incisors of this animal grow continuously from a persistent pulp. The visible effect of fluoride upon teeth is brought about during the time of deposition of the enamel. Since this deposition of enamel takes place in the incisor throughout the life of the rat the fluoride effect may appear whenever fluoride is fed and disappear when the feeding of the element is discontinued.

The sensitivity of the rat incisor to fluoride has been studied by several investigators. Kick (52_a) detected mottling when as little as 0.0046 per cent sodium fluoride was added to the ration. Schulz (91) detected changes in the incisors with as little as 0.0046 per cent sodium fluoride added to the feed. Dean, Sebrell, Breaux and Elvove (29) found that 2.5 parts per million of sodium fluoride in drinking water caused striations just detectible by the hand-lens. De Eds and Thomas (32) found that 12 parts per million of fluorine as sodium fluoride caused perceptible mottling of the rat incisor. Evans and Phillips (37) found the least noticeable incisor effect to result from the

inclusion of 0.0007 per cent or 7 parts per million of fluorine in the ration. Ellis and Maynard (35) considered the analysis of bone for fluorine a more sensitive test for fluorine intoxication than observations on the incisors because he could detect increments of fluorine in bone when 8 to 12 parts per million were added to the basal diet.

A comparison of the storage in tooth and bone tissue in relation to the degree of tooth defect has been made by McClure (63). The least incisor change visible in enlarged photographs appears to correspond with a fluorine content in bone of about 0.069 per cent fluorine on the dry, fat-free basis and with about 0.040 per cent in the incisor.

The tooth defect is known to take place under controlled conditions with different domestic animals ingesting fluorides in various forms. The changes observed in controlled experiments appear to be identical with those observed in animals in Iceland (83). Taylor (106) and Phillips (77) have reported extensive wearing of molars in cattle. Kick (52_a) has demonstrated the same in pigs.

The tooth defect in the human being has now become widely recognized. Although the defect had been described much earlier, the etiology was not known until 1931 when Smith, Lantz, and Smith (101) gave water from endemic areas to rats and obtained the typical fluoride effect upon the incisors. Since that time the public-health aspects of the problem have

been the object of much vigorous work on the part of public health officials and experiment-station workers in a number of different states and by investigators in other countries. The threshold level for fluorine in drinking water, below which it could be consumed safely, was sought by Smith (95b), who found that quantities larger than 0.9 part per million of fluorine in drinking water were capable of causing the tooth defect. Quantities of fluorine between one and two parts per million caused mild changes in the teeth while three parts per million or more were associated with the more severe types of the defect.

Many of the factors involved in the production of mottled enamel in man have been studied quite extensively by H. T. Dean and co-workers in the U. S. Public Health Service. Dean (25) gives a comprehensive survey of the incidence of the defect in the United States. Dean and Elvove (26, 27) concluded that the critical level for fluoride in water lay at one part per million.

Recently, Dean, et al. (28) have found the incidence of dental caries less in people living in mottled enamel areas where the water contains one part per million or more of fluoride than in areas where the fluoride content is low.

Changes in blood.

The fluoride content of the blood has been investigated

by a small number of workers. Gettler and Ellerbrook (41) found the normal range of the fluoride content of blood to lie between 0.00002 and 0.000064 per cent. In five cases of death from fluoride poisoning the same workers found values ranging between 0.00035 and 0.00155 per cent fluorine in the blood. The fluoride content of the blood of normal dogs was found in the same range as that for normal man. The feeding of 18 to 32 mgs. of sodium fluoride per kilogram of body weight per day caused no accumulation of fluoride in the soft tissues of the dog. Kraft and May (52b) found normal human blood to contain 0.0001 per cent fluorine while with hyperthyroid persons the blood contained between 0.00002 and 0.00008 per cent fluorine.

The level of calcium in the blood of rats was found by Schulz (31) to be uninfluenced by 0.10 per cent NaF in the ration. The feeding of sodium fluoride to dogs at levels which caused typical, severe mottling of the teeth was found by Greenwood, Hewitt, and Nelson (44) not to influence the serum calcium, inorganic phosphorus, clotting time, or hemoglobin concentration of the blood.

The concentrations of a number of clinically important organic constituents of the blood were found by Greenwood, Nelson and Kempf (45) to be unchanged in dogs fed fluorine as compared with the values found in a normal control dog. The fasting level of blood sugar and the total nitrogen, non-

protein nitrogen, protein nitrogen, urea nitrogen, amino-acid nitrogen, creatinine, creatine, and uric acid values of the fluoride fed and the control were within the normal range and almost identical. In the glucose tolerance tests the blood sugar values of two litter-mate dogs, one a control and the other a fluoride-fed animal, receiving 13.57 mg. fluoride per kilo of body weight, differed not more than 7 milligrams per cent during the three-hour test.

Yu (111) found that intraperitoneal injections of sodium fluoride into rabbits caused hyperglycemia which was counteracted by insulin. Mobilization of liver glycogen was considered by Yu to be the cause of the hyperglycemia, since venous blood contained uniformly less sugar than arterial blood.

Roholm (83) found no significant changes in the hemoglobin values of the blood of laborers poisoned by cryolite, but the observations of Shortt (93) indicated a decrease in homoglobin. Anemia was noted in fluoride poisoned animals by Slagsvold (95_a).

Effect on enzyme activity.

In vitro experiments with enzyme-substrate mixtures have shown that fluoride ion has an inhibiting influence upon enzymes catalysing the hydrolysis of phosphatase and sulfate esters and fats. Rothschild (85) set up an expression for the equilibrium constant for the association of fluoride with lipase:

$$k = \frac{(\text{free enzyme})\ (\text{fluoride})}{(\text{enzyme-fluoride complex})} .$$

then:

$$k' = \frac{x \cdot y}{100 - x}$$ when 100 equals the total enzyme concentration, x equals the free enzyme, and y equals the free fluoride.

$$y \text{ then equals } \frac{100 - x}{x} \cdot k'$$

In a series of determinations of enzyme activity Rothschild found that k' varied between 1.53×10^{-4} and 2.5×10^{-4} as the total fluorine concentration ranged between 3.2×10^{-5} and 1×10^{-3} mols per liter. At a concentration of 3.2×10^{-5} mols per liter the fluoride caused twenty-one per cent inhibition while at the concentration of 1×10^{-3} mols per liter an inhibition of eighty per cent was observed. Lipmann (60) also found that the inhibition of lipase followed the mass-action law.

The question of the effect of fluorine ingestion upon the phosphatase concentration of different animal tissues has not yet been definitely determined. Roholm (84) considered the phosphatase values of blood plasma from cryolite-intoxicated persons to be high but still within the range of normal values.

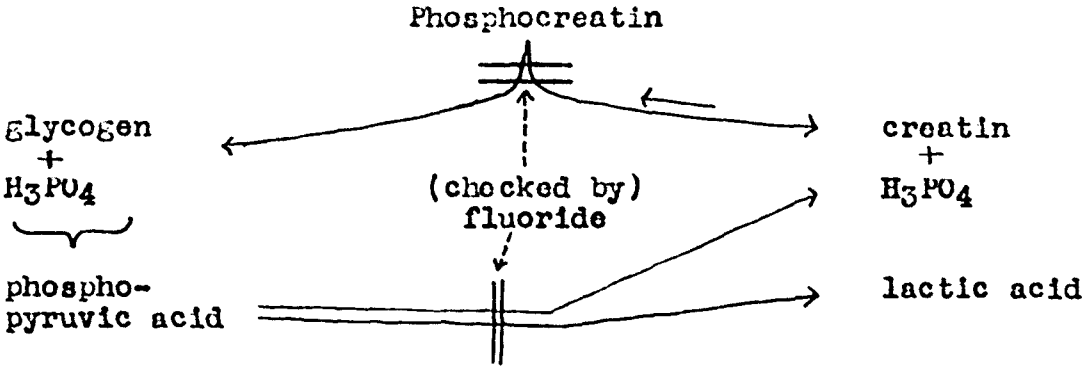
Phillips (75) found abnormally high values in cattle whereas Smith (100) reported no significant deviations from the normal values in the blood, bones and teeth of rats receiving sodium fluoride from the time they were weaned until they were nearly full grown.

Respiratory enzymes and some of the enzymes involved in the dissimilation of glucose have been shown to be affected by the fluoride ion. Lipmann (59) found that the respiration of muscle was noticeably less sensitive to fluoride than was the formation of lactic acid.

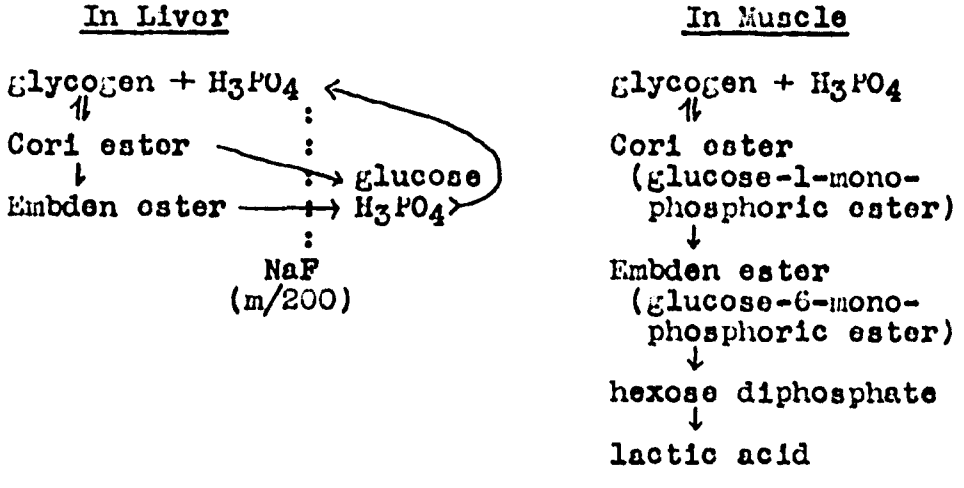
Denticke and Zens (34), using M/16.5 NaF, found that the addition of fluoride to liver hash did not cause accumulation of triosephosphate as it did in muscles. They adhered to the belief that pyruvic acid, rather than lactic acid, is the key substance in carbohydrate degradation in the liver.

Chang (14) found that fluoride inhibited only the anaerobic activity of the rabbit auricle and attributed it to interference in the formation of phosphopyruvic acid and from phosphoglyceric acid. Sodium fluoride at a concentration of 1 to 3000, M/126, inhibited the transformation of phosphoglyceric acid to phosphopyruvic acid.

Litzka (61) outlined glycolysis in the liver as follows:



Ostern (72) and his co-workers stated that in the presence of M/200 sodium fluoride the phosphorylation of the Cori ester was inhibited and that, under these conditions, the Cori ester from glycogen is converted to the Embden ester. The schemes these workers presented for glycogen breakdown in liver and muscle are as follows:



Innes (48) found that the formation of creatin phosphate took place aerobically at the expense of inorganic phosphate when sodium fluoride was present.

From recent work with cell-free tissue extracts to which traces of succinic acid were added, Colowick, Kalckar and Cori (30) concluded that sodium fluoride inhibits the transformation of phosphoglyceric acid to pyruvic acid. They supported the view that glucose, in order to be stored as glycogen, must first be phosphorylated.

In a study based upon feeding experiments, Phillips and Hart (76) found that the addition of lactate, glycerol or lactic acid to the ration of rats had no ameliorating action upon the effect of fluorine. They concluded therefrom that chronic fluorine poisoning involves more than a mechanism for carbohydrate metabolism. They characterized the mode of action of fluorine as systemic, involving a rather general inhibition of enzyme systems.

The Toxicity of Fluorine in Its Different Forms

The wide variety of fluorides used commercially in such a way as to provide a vehicle for fluoride intoxication has necessitated the study of the comparative toxicity of a variety of fluorine compounds. The previous citations have mentioned the use of a variety of compounds in which the fluorine is in inorganic combination. Smith and Leverton (102) have shown that at high levels of feeding the toxicities of fluorine compounds parallel closely their solubilities, whereas, at low levels of feeding, the levels of fluorine in the different

forms required to cause tooth defects is nearly independent of solubility. Experiments by De Eds and Thomas (32), Kempf, Greenwood, and Nelson (51), and Evans and Phillips (37) indicate that aluminum fluoride may be an exception.

The apparent difference in toxicity of water borne and food borne NaF, mentioned by Lawrenz (57), is not evident when the comparisons are made on the basis of fluorine ingested in milligrams per kilogram of body weight (McClure, 63).

That the naturally occuring fluorine in foods may not in some instances be as toxic as added fluoride is indicated by the study of Lee and Nilson (58) who found that rats receiving as high as 84.47 parts per million in canned mackerel stored only a third as much of the element as did control animals receiving the same amount of fluorine as CaF_2 or NaF added to a basal diet.

The use of organic fluorides as refrigerants and their therapeutic use in the treatment of Basedow's disease (Kraft and May, 52b) raises the question of the toxicity of fluorine in organic molecules. There might also be raised the question as to whether the action upon the thyroid gland might not be due to the small quantities of fluoride liberated.

Removal of Fluoride from Drinking Water

Marked activity in the search for an efficient and practical method of removing fluorides from drinking water has resulted in a large number of publications in the last eight years. The substances that have been studied as removal agents are quite varied and include aluminum compounds, bone-meal, carbon, fused mixtures of salts, lime, magnesium compounds, metallic oxide gels, sand, super phosphate, titanium sulfate, tri-calcium phosphate and zeolites. The substances which have appeared to show the most promise from the practical standpoint are certain forms of calcium phosphate, magnesium compounds, alum and lime. Findings in the field using activated alumina and alum have indicated that the effectiveness of these materials as removal agents may be modified by variations in water composition other than in fluorine concentration.

The use of bone-meal and tricalcium phosphate is meeting with success in removing fluorine from water supplies of schools and homes. Smith and Smith (98) first used the bone filter for removing fluoride from water. The bone for these filters was freed from fat and most of its protein. It was then boiled in 2 N sodium hydroxide until the product became chalky white in color. After washing with water and neutralizing with acid the treated bone was ground to the proper size for filter use. The fineness of the material had an influence upon

its efficiency in removing fluoride since the necessary time of contact increased and the extent of removal decreased as the particle size increased. The 30-100 mesh size was found to remove the fluorine completely from a synthetic water containing 5 p.p.m. of the fluoride ion. Because of the slowness of filtration through the 30-100 mesh material, the smallest size for practical use in filters was the 10-30 mesh material.

Time and rate of flow were found to be factors; but pH had little effect between 2 and 8. Above a pH of 8 the efficiency decreased rapidly as the pH increased. The removal power of bone meal in natural waters was found to be slightly less than in standard fluoride solutions. The mechanism of the removal of fluoride by bone is not yet clear but appeared to Smith and Smith (98) to be one of partial reaction followed by formation of solid solutions.

Bone meal has since been used successfully by Clark and Mann (10) in New Mexico and by Walker, Finley and Harris (100) in Alberta.

Soon after Smith and Smith reported success from the use of bone meal for fluoride removal, additional reports of the use of other calcium phosphates appeared. MacIntire and Hammond (67) studied the removal properties of boiled suspensions of basic phosphates on aqueous solutions of calcium or sodium fluoride. They also studied the properties of baking

powders in combination with $Ca(OH)_2$ and $Mg(OH)_2$. Starting with 5 p.p.m. of fluoride in solution, one gram of tri-calcium phosphate per liter was found to reduce the fluoride content to 0.00 p.p,m. in 30 minutes. Calcined phosphate with baking powder supplemented with calcium hydroxide gave removal to less than one p.p.m. Cold agitated suspensions of basic calcium phosphate proved to be inadequate removal agents.

Adler, Klein, and Lindsay (1) obtained effective removal of fluoride with tri-calcium phosphate in a contact filter column. They concluded that the fluoride content of the water was an insignificant factor in the fluoride capacity of the calcium phosphate filter. The effect of pH was negligible between 6.5 and 8.5. They assumed the mechanism of removal to be an absorption mechanism but also considered the formation of fluorapetite possible.

Regeneration of calcium phosphate or bone filters is necessary. This was accomplished in the earlier work with those materials by treating first with sodium hydroxide solution followed by dilute hydrochloric acid. The acid always dissolved a rather definite fraction of the filter material. Behrman and Gustafson (5) were able to prolong the active life of the absorbent by neutralizing the sodium hydroxide with carbon dioxide. The efficiency of the material appeared not to be reduced by the use of carbon dioxide.

Elvove (36) reported studies dealing with the use of tricalcium phosphate, magnesium hydroxide, and magnesium oxide as a removal agent for fluoride in drinking waters. The fluoride solutions for study were prepared by adding 5 parts per million of fluoride as sodium fluoride to distilled water. The substances to be tested were added as finely divided solids to the fluoride solutions, after which mixing was accomplished by means of a current of air. After one-half hour of mixing the solids were allowed to settle and the clear solution was syphoned off and analysed. Elvove considered all three of the above substances to have good fluoride removing capacities but suggested magnesium oxide as the most feasible because of cost.

Walker, Finlay, and Harris (109), working on the problem of fluoride removal from waters of Alberta, studied the efficiency of a variety of substances as removal agents. For most of their experiments these workers used waters from the taps of the city of Edmonton, the source of the water being the North Saskatchewan River. To this was added sufficient sodium fluoride to give a definite level, usually about 4 parts per million of fluoride. The composition of the water in the fall of 1937 was:

Constituent	p.p.m.
Total solids at 105° C	200.
SO_4	30.
Cl	4.
Bicarbonate Alkalinity	110.
Carbonate Alkalinity	0.
Total hardness	152.
F	0.2
pH	8.2

Only a limited number of naturally occurring waters were used in their studies. The substances tried as removal agents and found to have no value include titanium oxide, titanium hydroxide, zinc oxide, copper hydroxide, manganese dioxide, hydrated manganese dioxide, various silica gels, bentonite, zirconium silicate, beryl, ilmenite, a limy subsoil, copper sulfide, flowers of sulfur, calcium oxalate, calcium oleate, trimagnesium orthophosphate, barium carbonate, barium sulfate, magnesium oxychloride, chromium borate, portland cement, permutite, boracite, and limestone. Low capacities for removal were shown by basic ferric carbonate, magnesium ammonium phosphate, aluminum oxalate, powered aluminum, and various hydrated ferric oxides precipitated on asbestos. They also reported removal of fluorides with MgO, activated alumina and tricalcium phosphate. These workers reported successful removal with aluminum phosphate and with defluorite B, a calcium phosphate used in a filter unit, which was supplied by the National Aluminate Corporation of America. The aluminum phosphate appeared to have somewhat better removal capacity than defluorite B in an experimental run with prepared water. One of the three natural fluoride waters studied contained 4.4 p.p.m of fluoride and gave the following analysis:

Constituent	p.p.m
Total solids at 105° C	1128.
Loss on ignition	136.
SO_4	342.
Cl	65.
Bicarbonate alkalinity	325.
Carbonate alkalinity	56.
Total hardness	22.
PO_4 as P_2O_5	0.5
pH	8.55

These workers state that this water is representative of the high fluoride Alberta waters, the pH being usually above 8.3 and the sodium bicarbonate content being usually quite high. Removal of fluoride from the above water by defluorite B appeared satisfactory since a sample taken after 450 gallons had passed the filter was found to contain 0.2 parts per million of fluoride.

Boruff (8), in 1934, published a report of experiments using aluminum sulfate, bauxite, lime and activated alumina as precipitants or absorbents of fluoride from water. Quantities of aluminum sulfate of from 0.5 grains to 10 grains per gallon were added to quantities of Illinois waters to which various known quantities of fluoride were added to give known final concentrations of fluoride. The alum was added in solid form, mixed for 30 minutes, and then allowed to settle for a period of 18 to 24 hours before filtering off the floc and analysing for fluoride. In a series of experiments using different initial pH values of water to be treated, Boruff found that the optimum initial pH range for alum treatment lay between 6.25 and 7.5 with some slight advantage shown for

a pH of 6.7 when 5 grains per gallon were employed in the treatment. From his experiments Boruff concluded that water containing 5 p.p.m. of fluoride could be treated with 10 grains of alum in a single dose or with 5 and 3 grains respectively in a double dosage treatment. He found that chlorides and sulfates in quantities as high as 1000 p.p.m. did not affect fluoride removal with alum.

The analysis of the water used by Boruff was as follows:

Constituent	p.p.m.
Fe	0.7
SiO_2	18.0
Ca	67.8
Mg	29.6
NH_4	1.5
Na	24.9
SO_4	23.5
NO_3	0.6
Cl	2.7
CO_2	330.

Boruff (8) stated that aluminum forms a soluble but slightly ionized salt with fluorides, and that, in the slightly acid range, the alum floc is largely basic aluminum sulfate and carries in its constitution and adsorbed to it large quantities of other negative ions; however, he believed that it is a question as to whether the fluorides are removed as a basic aluminum fluoride complex or are merely adsorbed on the surface.

Boruff (8) found bauxite to be of little value but found that activated alumina removed the fluoride from an original level of 5 p.p.m. down to a value less than 2 p.p.m. in a contact filter.

Another study on the use of alum was published in 1937 by Doruff, Buswell, and Upton (10). In this study samples of University of Illinois tap water were saturated with CaF_2, MgF_2, and Al_2F_6, respectively, diluted to 4 p.p.m. of fluoride and treated with 0.72 grains $Al_2(SO_4)_3 \cdot 18\ H_2O$. The pH of the water to be treated was adjusted to 6.7. The removals obtained were not sufficient to produce a safe water, i.e., the residual fluoride was considerably greater than one part per million. The reaction was found to affect greatly the completeness of removal; but softening with zeolite was not found by them materially to benefit fluoride removal.

Nichols (71), in 1939, reported successful use of alum as a removal agent. Water containing 2.8 p.p.m. of fluoride was first softened with lime after which it contained 1.3 p.p.m. of fluoride. Alum treatment with 10 grains of alum per gallon reduced the fluoride content to 0.65 parts per million.

Interest in the Laboratory of Physiological Chemistry at Iowa State College in the problem of removal of fluoride from drinking water dates from the discovery by Ostrom, Nelson, Greenwood, and Wilhelm (73) that the prevalence of mottled enamel in the vicinity of Ankeny, Iowa, was due to an unusually high level of fluoride in the municipal water supply of that city and in the waters from many of the deep wells in that community. In 1936, Nelson, Galligan, Greenwood, and Kempf (50) reported experiments using a continuous treatment

pilot plant in which treated waters were obtained with fluoride contents in the range of 1.5 parts per million of fluoride, the original water containing approximately 8 p.p.m. of fluoride.

Kramer (53), in 1934, reported that a contact filter 15 cm. high, made of river sand passing a screen 60 meshes to the inch, to which had been added 2 per cent by weight of powdered aluminum, removed the fluoride from a solution containing 30 parts per million of sodium fluoride. The absence of fluoride in the filtrate was determined by the zirconium-alizarin colorimetric method.

McKee and Johnston (66), in 1934, reported removal of fluoride using activated carbon. The method required such a low pH value that little practical utilization of the method has been made.

The Determination of Fluorine

The analysis of water, foods, and animal tissues for fluorine in amounts as low as one or less parts per million presented new problems in the determination of this element. With the increasing realization of the importance of the problem of fluorine intoxication, numerous analysts undertook to solve these problems. Of the several methods that have been proposed for the above types of materials, the one devised by Willard and Winter (110) in 1933 has been most

widely adopted, though usually in some modified form.

The development and modification of this important method is of interest. In their development of the method, Willard and Winter made excellent use of information already in the literature. Willard and Winter (110) credit Wöhler with the first use of SiF_4 volatilization in liberating fluorine from insoluble compounds. Schrenk and Ode (87), in 1929, volatilized fluorine as BF_3 or HBF_4 from HCl, H_2SO_4, or $HClO_4$ by evaporation. Willard and Winter (110) found it possible quantitatively to distil fluorine as H_2SiF_6 from a wide variety of materials by boiling the sample with aqueous solutions of $HClO_4$ or H_2SO_4. Deladrier (33), in 1903, precipitated thorium as the hydrated thorium fluoride and, after igniting, weighed the thorium in the form of ThO_2. Gooch and Kobayashi (42) described a control titration of fluorine with $Th(NO_3)_4$ in connection with a gravimetric determination of fluorine as ThF_4. Willard and Winter found it possible to use $Th(NO_3)_4$ as a volumetric precipitant in the titration of the fluorine solutions obtained from their H_2SiF_6 distillates. These workers successfully employed as an indicator a zirconium-sodium alizarin sulfonate lake which was suggested to them by the publications of DeBoer (30) and Casares (12).

Soon after the Willard and Winter method appeared a number of modifications were published. Armstrong (4), in the same year, showed that sodium alizarin sulfonate alone is capable

of giving a more distinct end-point, especially with the smaller quantities of fluorine. In the same year Boruff and Abbott (9) adapted the method to the analysis of natural and treated waters. Hoskins and Ferris (47) in 1936, simplified the technique of the titration by the use of a buffer composed of chloroacetic acid half neutralized with sodium hydroxide. Armstrong (3), in 1936, again modified the method by titrating in an aqueous medium, controlling the pH by means of the Hoskins and Ferris buffer. By the use of weaker thorium nitrate solutions and small sized titrating vials, Armstrong was able to determine fluorine in quantities of 5 micrograms or less. Later in 1936 Armstrong (2) published a procedure designed to eliminate the interferences due to chlorides and perchlorates passing into the distillate; both chlorides and perchlorates were found by him to cause positive errors. He removed the chlorides from a first distillate by precipitation with silver perchlorate and then re-distilled to obtain a solution for titration. The disturbing effect of perchlorate was eliminated by adding to the distilling flask a suitable quantity of sodium perchlorate which he found to decrease the volitilization of perchloric acid; this was necessary only when the quantity of fluorine to be determined was less than 5 micrograms, in which case the final evaporation of the distillate to a volume of 5 ml. instead of the usual 10 ml. caused the perchlorate concentration to be excessive.

In contrast with the findings of Churchill, Bridges, and Rowley (18), Armstrong found that phosphates were not distilled in quantities sufficient to interfere.

A series of detailed studies of factors involved in the recovery of fluorine by the Willard and Winter method were published by Dahle and co-workers. In 1936, Dahle (21) described a method for ashing such materials as apples, lettuce, green tomatoes, flour, liver, and eggs in preparation for the determination of fluorine, using $Al(NO_3)_3$ to prevent loss of fluorine during the ashing process. He found ashing temperatures as high as 650 degrees to be safe. Dahle and Wichmann (22), in the same year, found that recovery of fluorine from aluminum solutions of different concentrations is a decreasing logarithmic function of the concentration of the aluminum, regardless of the form in which the aluminum is added. This relation was expressed by them as follows:

$$\log \frac{C}{C_o} = k\,(Al)$$

where (Al) represents the concentration of the aluminum ion, C the amount of fluorine recovered and C_o the amount of fluorine originally present. In 1937 Dahle and Wichmann (23) published a detailed study of the effect of input volume upon the recovery of fluorine using H_2SO_4, $HClO_4$, and H_2PO_4. From this study they concluded: 1. Recovery per ml. of distillate decreases with increasing volume of liquid in the distilling

flask. 2. That distillations carried out at higher temperatures gave increased recovery per ml. of distillate. 3. That these variations in recovery are greater when distillations are made from perchloric or phosphoric acid than when made from sulfuric acid. 4. That the addition of soluble salts of non-volatile acids to the system in the distilling flask causes a greater decrease in the recovery rate than would be expected from the increase in volume caused by the presence of such salts. 5. That the recovery of fluorine using perchloric, phosphoric, and sulfuric acids varies with the amount of distillate collected, and the relationship is logarithmic. In some of their work, Dahle and his co-workers made use of the peroxidized titanium method for the determination of the fluorine in the Willard and Winter distillates.

In 1938, Dahle, Wichmann, and Bonnar (24) published work which indicated that chloride and perchlorate ions, when in the form of their acids, did not interfere in the titration of fluorine in small quantities with thorium nitrate; but when present as their sodium salts they exercised a "salting-out" influence upon the alizarin sodium sulfonate--thus causing high results. They found that the distillation of perchloric acid is efficiently checked by protecting the walls of the distilling flask from super-heating by the use of efficient shields.

Much use has been made of the zirconium-alizarin lake in

colorimetric determination of fluorine in waters. In 1933 Thompson and Taylor (107) applied the De Boer-Casares colorimetric method to the analysis of sea water and found it necessary to equalize the chloride concentration in the standard with that in the unknown. In 1935 Smith and Dutcher (103) applied a similar method to the determination of the element in potable waters. These men modified the above method of Thompson and Taylor by the use of quinalizarin in place of sodium alizarin sulfonate in the preparation of the color lake, a possibility which had been suggested earlier by Willard and Winter in presenting their volumetric method.

A number of workers have investigated the applicability of the Willard and Winter method to the analysis of inorganic materials, of ash from plant and animal tissues, and, in the case of enamel and dentine, of the unashed material. Shuey (94) reported that the perchlorate distillation and subsequent thorium nitrate titration gave quantitative recovery of fluorine from both water-soluble and insoluble fluorides; but, like Willard and Winter, he was unable to get complete recovery from plant ash. Chang, Phillips, Hart, and Bohstedt (13) published work in 1934 which showed that the small quantities of carbon frequently remaining in the ash of animal tissue caused interference with the recovery of fluorine; but when the ash was obtained without carbon--by repeated washing and ignition with Na_2CO_3 at a temperature of 650° C--the

results were described by them as excellent. Reynolds (81), studying the determination of fluorine in phosphate fertilizers, found the Willard and Winter distillation method more accurate than either the SiF_4 volitilization method or the fusion-acid extraction method for determining the element in mineral phosphates, calcined phosphates or phosphate furnace slags.

McClure (63) and Lee and Nilson (58), in critical studies of the metabolism of fluorine, used micro-adaptations of the Willard and Winter method for their analyses of the tissues of rats. Nilson and Lee ashed the tissues in the presence of magnesium acetate to decrease the loss of fluorine in the process. They did not attempt to procure complete ashing. They eliminated the interference of chlorine by distilling in the presence of silver which they added in the form of silver perchlorate. Double distillation was employed by them to eliminate interference from phosphates.

Other methods for fluorine.

Sanchis (86) used a modification of the Thompson and Taylor (107) method for the determination of fluorides in natural waters. Petrey (74) has applied the spectroscope to the determination of fluoride in water. The spectroscopic method was used for the same purpose by Churchill (17) and by Ostrem, Nelson, Greenwood, and Wilhelm (73). Boissvain

and Drea (7) applied spectroscopic methods to the quantitative determination of the fluorine in bones, teeth and other organs in relation to flurode in drinking water. Langer (55) has recently published an amperometric method for titration of fluorine using the dropping mercury electrode.

INVESTIGATION OF PHYSIOLOGICAL RESPONSES

Plan of Investigation

The effect of fluoride upon hemoglobin was to be investigated by studying the availability of CuF_2 and FeF_3 for hemoglobin regeneration in the rat, and by following hemoglobin changes in normal and fluoride-fed rats during growth, reproduction and lactation. Successful regeneration of hemoglobin using CuF_2 and FeF_3 as compared with $CuSO_4$ and $FeCl_3$ would appear to indicate that any formation of CuF_2 and FeF_3 in the process of cooking with fluoride waters would not likely impair hemoglobin regeneration in the human being. The comparison of hemoglobin levels in normal and fluoride fed rats during the periods of growth, reproduction and lactation--periods of high metabolic demands--should give valuable evidence regarding the ability of fluoride to influence hemoglobin formation.

The investigation of the effect of alum upon the toxicity of fluoride was suggested by results of a preliminary experiment in which aluminum fluoride was fed to rats at a high level of fluorine without affecting the incisors or impairing growth or lactation. Plans were therefore made to study the effect of alum on fluoride toxicity by feeding toxic levels

of sodium fluoride, alone and with alum, comparing the influence upon growth, reproduction and lactation with the same processes in the normal rat. It was also planned to compare further the toxicity of aluminum fluoride with some other inorganic fluorides.

Injection experiments were also outlined for the purpose of determining whether or not alum decreased the toxicity of injected sodium fluoride. Experiments were planned for the intraperitoneal injection of lethal doses of sodium fluoride alone and with alum, comparing the killing time. The lethal effect of injected aluminum fluoride was also to be investigated.

Because of the quite definite evidence in the literature not only that bones are altered in macroscopic and microscopic appearance by fluorine, but also that they are major places of storage for fluorine, it appeared important to investigate the interrelationship between calcification of rachitic bones of young rats and the rate of incorporation of fluorine into bone. This study was to be made by feeding fluoride and vitamin D, separately and together to rats rendered rachitic on an experimental ration.

The effects of organic fluorides upon the teeth of rats was to be studied by incorporating different organic fluorides into the ration of the rat and observing the incisors daily to determine whether or not the macroscopic evidence of fluorosis

appeared. Records of growth and reproduction were to be recorded. The organic fluorides to be used in the study included p-fluorobenzoic acid, p-difluorodiphenyl, fluorobenzene, α-fluoronaphthalene, p-fluoroiodobenzene, and p-fluorobromobenzene.

The effect of stomach feeding of fluoride was to be studied by obtaining blood sugar curves on rats, both fasted and unfasted, after administering sodium fluoride alone in varying standardized quantities and with glucose. By means of such curves it was thought that it might be possible to gain information as to the extent to which homeostatic mechanisms for carbohydrate control are interfered with by fluoride. When, after a number of blood sugar curves were obtained, it appeared that hyperglycemia always occurred, with or without accompanying the fluoride with glucose, and with both fasted and unfasted animals, insulin injections were given.

Materials and Methods

Rations.

The basal ration used in the majority of the feeding experiments in this study on fluorine was made up of the following ingredients in parts by volume: ground hulled oats 4 parts, ground yellow corn 4 parts, ground wheat 1 part, alfalfa meal

1 part, tankage 0.5 part, linseed meal 0.5 part, buttermilk powder 0.5 part. To each 100 pounds of the above were added 0.35 pound of bone meal and 0.5 pound of sodium chloride. The ingredients were obtained from a dealer in the city of Ames, Iowa.

This ration was supplemented by different fluorine compounds in a number of the feeding experiments. In the study of the effect of feeding sodium fluoride upon the hemoglobin levels in rats the finely powered sodium fluoride was added as the supplement. In the study of the effect of alum upon the toxicity of sodium fluoride in the ration of the rat sodium fluoride alone and sodium fluoride plus C. P. aluminum sulfate were added. In the comparison of the toxicity of aluminum fluoride with other inorganic fluorides, aluminum fluoride, cupric fluoride, zinc fluoride and calcium silicofluoride were used as supplements. In another set of experiments organic fluorides were added to this ration.

Nutritional anemia in the rat was caused by feeding milk specially collected by the Iowa State College Dairy in such a way as to prevent contamination with copper and iron through contact with metal of the usual milk containers. This milk was fed alone, with copper sulfate and ferric chloride, and with copper and ferric fluorides.

The ration used in the study on recalcification of rachitic tibiae was that of Steenbock and Black (105) consisting

of 76 parts of yellow corn, 20 parts of wheat gluten, 3 parts of powdered calcium carbonate and 1 part of powdered sodium chloride. The supplements used with this diet included sodium fluoride and Squibb's cod-liver oil diluted in corn oil. The oil was administered separately by mouth from a graduated pipette.

The method of incorporating the solid supplements into the stock ration was as follows: The weighed quantity of the finely powdered substance was first mixed thoroughly with approximately 100 grams of the basal ration after which this feed-supplement mixture was added to the remainder of the weighed quantity of the basal ration. The same procedure was used in incorporating the sodium fluoride into the rachitogenic ration.

The iron and copper salts were added to the milk in solution in distilled water.

The rations containing the liquid organic fluorides were mixed every other day because of their high degree of volatility. From the specific gravity of these compounds was calculated the volume of the compound required for the quantity of basal ration desired, and this volume of the liquid was added to the weighed quantity of the basal ration.

With the exception of the animals on the milk rations, all of the rats were supplied with fluorine free distilled water, ad libitum.

Chemicals.

The copper and iron fluorides used in hemoglobin regeneration studies were the C. P. products of Eimer and Amend while the copper sulfate and the ferric chloride used in the same study were prepared from the C. P. quality salts.

The sodium fluoride used for the sodium fluoride supplements was the C. P product of the J. T. Baker Chemical Company. The same product was used in making up standard fluoride solutions.

The chemicals used in the study of the comparative toxicity of aluminum fluoride were Baker's analysed, C. P. aluminum fluoride, Eimer and Amend's C. P. cupric fluoride, zinc fluoride and calcium silico fluoride.

The thorium nitrate used in the titration of fluorine was the reagent grade of the Baker and Adamson Company, and was the dodecahydrate. The sodium alizarin sulfate used as the indicator in the titration was made by Merck and Company. The chloroacetic acid used in preparing the Hoskins-Ferris buffer was the Baker and Adamson product, of reagent quality.

The organic fluorides were products of the Eastman Kodak Company.

Analytical methods.

Hemoglobin. The hemoglobin determinations were made according to the method of Newcomer (70), making use of a special colorimeter containing a Newcomer plate, and calibrated to read directly the per cent of hemoglobin in the sample. The samples of blood for this determination were taken from the tail of the rat with a special diluting pipette designed for the instrument.

Blood sugar determination. The Folin-Malmros (39) method for blood sugar, requiring 0.10 ml. samples of blood, was modified so that samples of 0.05 ml. of blood could be used. The quantity of dilute tungstic acid, used in the method as a deproteinizing agent for the blood, was reduced from 10 ml. to 5 ml. This was measured into centrifuge tubes of 6 ml. capacity and the deproteinization and centrifugation carried out as usual. Four milliliters of the supernatant solution were pipetted out for analysis. Except for the calculation, the remainder of the method was exactly as described by Folin and Malmros. The formula for the calculation became:

$$\frac{\text{Standard Reading}}{\text{Unknown Reading}} \times (0.04) \times \frac{100}{4/5 \times 5.05} = \text{mg. glucose in 100 ml. Blood}$$

where 0.04 represents the glucose content of the standard glucose solution used in making the colorimetric comparison.

Fluorine in bones. The bones to be used for ash and fluorine determinations were carefully cleaned and then dried in an electric oven for 6 hours at 105° C. These bones were then extracted with alcohol and ether in a Soxhlet extractor for a period of 24 hours for each solvent. After drying to constant weight the bones were placed individually into weighed crucibles and ashed in an electric muffle. The determination of fluorine in this ash was made according to the following procedure based on the method of Willard and Winter (110) as revised by Armstrong (2), Hoskins and Ferris (47), McClure (63), and Lee and Nilson (58).

The entire ash from the tibia was transferred quantitatively to a 50 ml. Claissen flask along with three glass beads, 0.03 gm. powdered silica, 7 ml. of distilled water, 7 ml. of 60 per cent perchloric acid and 0.7 ml. of 20 per cent silver perchlorate. After closing the flask and attaching the condensor, distillation was begun by heating the Claissen flask gradually with a micro-burner until the contents boiled and the temperature rose to 140° C, at which time steam, from an attached generator, was passed into the solution in the Claissen flask in a manner described by Hoskins and Ferris (47). The head of steam and the heating of the Claissen flask were so controlled as to maintain the distillation temperature between 138° C. and 142° C. and to yield 180 ml. of distillate in 70 to 90 minutes. The distillate was collected in a 300 ml.

erlenmeyer containing 5 drops of 5 per cent sodium hydroxide and 1 drop of 1 per cent phenolphthalein solution. More sodium hydroxide was added as required to maintain an alkaline reaction to the phenolphthalein during the distillation. The entire distillate was then concentrated by evaporation to a volume of 10 ml. transferred quantitatively to a 100 ml. platinum dish and evaporated down to dryness over a steam bath. The residue was taken up with water and transferred to a 10 ml. volumetric flask, After rendering the solution just acid to phenolphthalein, the volume was made up to 10 ml. One ml. aliquots, or less if the fluoride concentration were quite high, were transferred into each of six vials 40 mm. deep and having an inside diameter of 11 mm. One drop of 0.05 per cent sodium alizarin sulfonate was then added, followed by 0.1N and 0.01N hydrochloric acid until the light orange transition color of the indicator was obtained. One drop of the Hoskins-Ferris (47) buffer mixture was added and sufficient distilled water introducted to fill the vials to a depth of 25 mm. The fluoride was then titrated with 0.10 N thorium nitrate solution, using a Rehburg micro-burette (Lee and Nilson, 58). A recovery test for the distillation yielded 71 micrograms from a total of 71.25 micrograms added to the Claissen flask as sodium fluoride.

Injection techniques.

A 5 ml. Becton-Dickinson syringe, accurately graduated to one-tenth ml. was used with a 26 gauge needle for the injection. While inserting the needle into the peritoneal cavity the animal was held on its back in the palm of the left hand with the fingers of that hand restraining the hind leg. The head was directed downward and backward between the left wrist and the body of the operator. The needle was then inserted into the lower right side of the abdominal cavity and the injection made. The solutions for intraperitoneal injections were made up with a concentration such that 1 ml. was required per 100 grams of body weight. When the fluoride and alum were injected separately the two solutions were injected from different syringes, the second syringe being filled with the desired amount of second solution so that it could be attached to the needle as soon as the first syringe was emptied.

The insulin solution used in some of the studies on blood sugar changes was injected subcutaneously in the left flank from a Becton-Dickinson tuberculin syringe. The insulin solution was made by diluting 0.33 ml. of U-40 insulin to 50 ml. in 0.9 per cent sodium chloride.

Administrations by stomach tube. The animals to be administered the solutions by stomach tube were first lightly anesthetized with chloroform so that the stomach tube, made

of a small sized rubber catheter, could be introduced into the esophagus without resistance. The tube was then pushed slowly with a rotating motion until it had reached the stomach. The dosing solution was slowly forced into the stomach from a 5 ml. Beckton-Dickinson syringe. The animals were recovering from the light anesthesia before the stomach tube could be removed.

Care of animals.

Feeding. The feed was kept before the animals at all times except when blood sugar determinations were to be performed on fasted animals. The rats fed upon milk rations and upon the rachitogenic diet during the assay period were kept in individual compartments and fed from individual containers. In other instances the animals were fed in groups from a common container.

Housing. The animals on hemoglobin regeneration and those on recalcification experiments were kept in galvanized iron wire cages separated into compartments for a single rat by means of wire screen partitions. The other animals were kept in cages of wire screen, sufficiently large to accommodate six adult animals.

Copper and Iron Fluorides in Hemoglobin Regeneration

Procedure.

Rats 28 days of age were rendered anemic by feeding cow's milk collected in glass to prevent the introduction of small quantities of copper or iron from the container. The milk was fed in earthen-ware mortars which were washed daily and rinsed in copper free distilled water. The hemoglobin values were determined by the Newcomer method.

All rats were first rendered distinctly anemic by the milk ration, after which they were separated into three groups. Group 1 continued to receive the unsupplemented milk diet and served as a negative control, while group 2 received 0.002 mg. of copper as $CuSO_4$ and 0.5 mg. of iron as $FeCl_3$, daily, and served as a positive control. Group 3 received 0.002 mg. of copper as CuF_2 and 0.5 mg. of iron as FeF_3, daily.

Results.

The results of the experiment are presented in three tables in which are shown the times at which the various hemoglobin determinations were made, the ration received, the individual hemoglobin values, and the mean of the values obtained. Table 1 shows the results for the rats receiving milk

only throughout the experiment.

Table 1. Hemoglobin Regeneration. Negative Controls. Milk Only.

Date	Ration	Hemoglobin Values W♀RLS	W♂RLC	W♀LC	W♀R	Mean Hb Value
7-22	Milk	5.1	2.7	4.3	5.1	4.3
7-29	"	3.5	2.7	4.25	3.9	3.6
8-4	"	3.8	3.0	4.9	4.8	4.1
8-15	"	3.75	---	4.9	5.0	4.9
8-21	"	3.75	3.0	5.25	7.5	4.9
8-29	"	3.50	3.50	6.50	7.5	5.2
9-11	"	3.70	3.60	4.00	6.5	4.4
10-8	"	3.20	4.10	5.25	6.1	4.7
11-22	"	3.00	5.00	6.00	7.5	5.4

The results that were obtained with the rats receiving copper sulfate and ferric chloride supplements are shown in table 2.

Table 2. Hemoglobin Regeneration in Positive Controls, Receiving $CuSO_4$ and $FeCl_3$.

Date	Ration	Hemoglobin Values W♂RC	G♀	B♀	BS♀	Mean Hb Values
7-22	Milk	4.1	5.4	4.5	3.6	4.4
7-29	"	4.35	2.8	4.12	4.3	4.25
8-7	0.002* mg. Cu 0.5 mg. Fe daily	5.37	6.1	8.6	9.9	7.50
8-15	"	6.8	5.6	10.0	11.75	8.5
8-21	"	8.7	8.3	11.2	13.4	10.4
8-29	"	7.7	7.3	9.0	15.0	9.8
9-11	Cu & Fe doubled	7.7	8.25	11.0	11.0	9.5
10-8	"	13.5	14.5	13.75	12.0	13.4
11-22	"	14.75	10.5	12.0	12.5	12.4

*Supplement begun 7-30-35

The results with the copper and iron supplements are shown in table 3.

Table 3. Hemoglobin Regeneration in Rats Receiving Copper and Iron Fluorides.

Date	Ration	Hemoglobin Values W♂LC	W♀RV	B♀LC	W♀	Mean Hb Values
7-22	Milk	2.5	3.9	4.7	4.3	3.8
7-29	"	3.5	4.5	3.8	3.1	3.9
8-7	0.002* mg. Cu & 0.5 mg. Fe daily	3.6	4.38	8.0	7.25	5.8
8-15	"	4.75	5.9	8.8	7.00	6.6
8-21	"		8.25	9.6	9.7	9.1
8-29	"		8.7	9.6	9.3	9.2
9-11	Cu and Fe doubled		9.7	9.7	9.7	9.7
10-8	"		11.6	12.7	12.4	12.2
11-22	"		13.5	14.5	15.0	14.2

*Supplement begun 7-30-35

The mean hemoglobin values for the three groups of animals are plotted in figure 1, with the time in days as the abscissa and with per cent hemoglobin in the blood as the ordinate.

Interpretations of results.

An examination of the hemoglobin values shown in the tables reveals that the animals were quite uniformly anemic at the time the copper and iron supplements were begun. The mean hemoglobin values for groups one, two and three were 3.6, 4.25, and 3.9 per cent, respectively, on the day preceding the

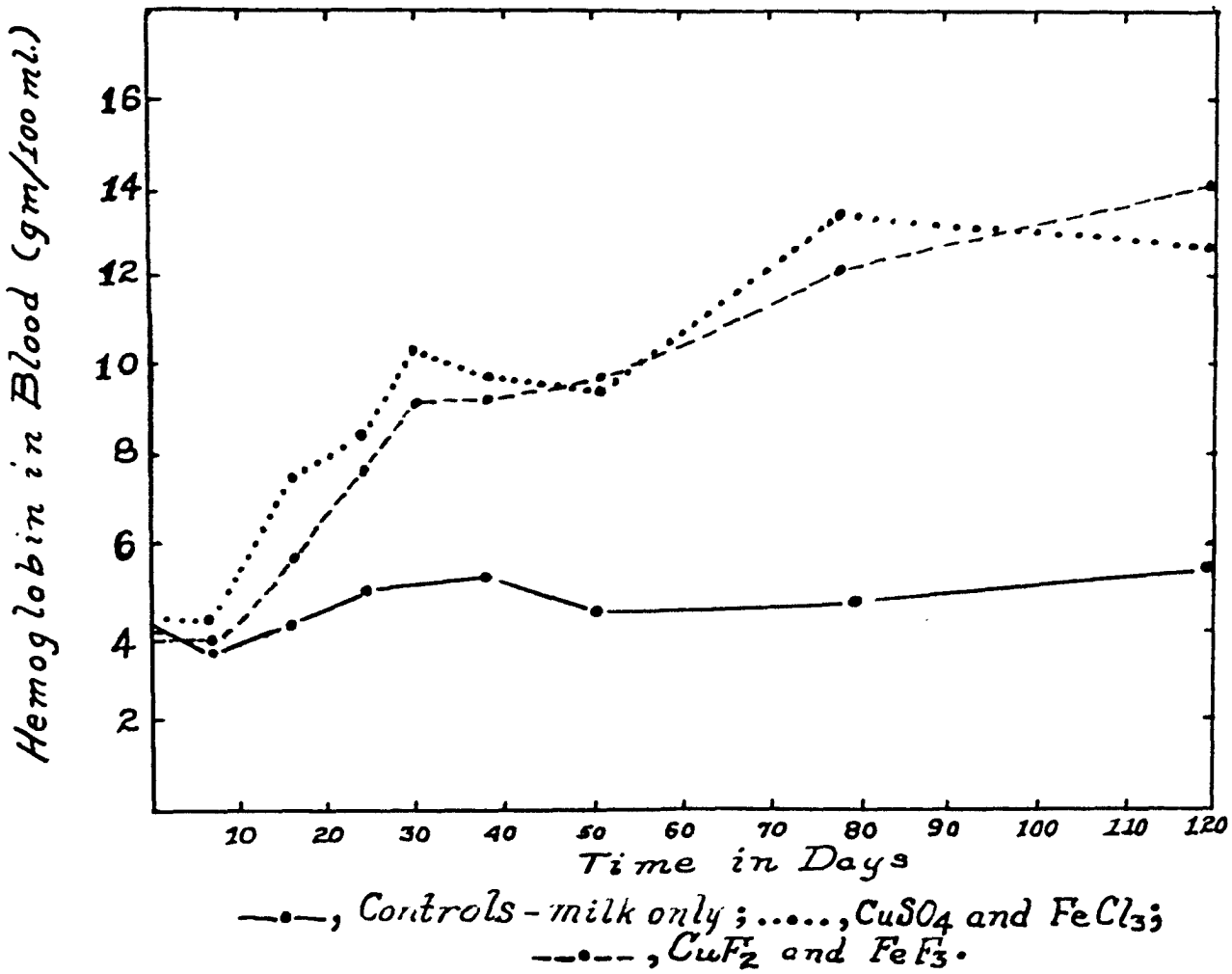


Figure 1. Hemoglobin regeneration in rats fed CuF_2 and FeF_3 as compared to $CuSO_4$ and $FeCl_3$.

beginning of the supplementation. The highest individual hemoglobin value among the animals of all three groups was 4.5 per cent; the lowest value was 2.7 per cent. Since normal values for hemoglobin in rats is approximately 15 per cent, the above values may be considered to be much below normal, thus indicating severe nutritional anemia. The uniformly low hemoglobin values observed in the group which received the unsupplemented milk diet throughout the experimental period indicated that the basal milk diet did not become contaminated with copper or iron.

A comparison of the rates of hemoglobin regeneration in the groups receiving copper and iron supplements revealed no significant differences. This evidence indicated that the rats readily utilized copper and iron when they were supplied as fluorides.

The Influence of Feeding Sodium Fluoride upon Hemoglobin Levels in the Blood of Rats

Procedure.

Forty-eight rats at the weaning age of 28 days were used. They were distributed in eight cages so that each cage contained three males and three females. The animals in four of these cages were fed growing ration throughout the experiment, while those in the remaining cages received the same ration

with 0.05 per cent sodium fluoride. This level of sodium fluoride was expected to cause some impairment of growth and of lactation. Records of growth and of reproduction and lactation were kept along with the records of the hemoglobin values. These latter values were determined at frequent intervals in all females and in one male from each cage until the end of the experiment when the hemoglobin values were determined in all of the males.

Results.

The results obtained in this experiment upon growth, reproduction, lactation and hemoglobin are presented in the form of tables. The mean values for the body weights of the animals are shown in table 4. The values are shown separately for the males and the females.

Table 4. Growth Records for Normal and Fluoride Rats on Hemoglobin Studies.

TIME	NORMAL		0.05% NaF	
	Average body weights (gms) MALES	Average body weights (gms) FEMALES	Average body weights (gms) MALES	Average body weights (gms) FEMALES
1-24	49.6	51.2	51.4	51.3
1-31	71.6	68.6	64.9	59.3
2-14	95.4	83.5	82.0	73.7
2-21	115.2	97.5	97.0	84.8
2-28	121.0	101.8	102.7	91.2
3-19	151.4	120.5	138.0	114.0
4-1	180.4	137.0	157.1	131.9
5-1	209.6	151.2	169.8	146.0

As was expected, the growth of the fluoride fed animals was inferior to that of the normal animals.

The hemoglobin values obtained in the normal and fluoride fed females are shown in table 5, while those for the males are shown in table 6.

The data obtained on reproduction, lactation and hemoglobin are assembed in table 7. In this table are shown the number of females in the control group and in the fluoride group, the number of females that reproduced in each group, the average weights of the females at parturition and at the end of lactation, the sizes of the litters, the weekly average weights of the litters and the hemoglobin values in the females at parturition and at the end of lactation.

Discussion and interpretation of results.

Five of the fluoride fed females reproduced as compared with ten of the normal females that reproduced during the same period of time. The average weights of the fluoride fed females at parturition was 16 grams less than the like average for the normal females. This difference was in accord with the growth shown in table 4 and in figure 2. The average weight of the normal females at the end of the lactation periods was still eight grams greater than the corresponding weights of the fluoride females. This difference is less than at parturition but this reasonably accounted for by the fact that the normal females performed much better in lactation.

Table 5. Hemoglobin Values for Normal Females and for Female Rats Receiving 0.05% Sodium Fluoride.

Normal Rats on Stock Ration					Stock Ration Plus 0.05% NaF				
Rat No.	Hemoglobin Values at 2-2	2-16	3-3	4-6	Rat No.	Hemoglobin Values at 2-2	2-16	3-3	4-6
1	15.6	19.6	17.5	15.4	1	18.1	14.3	16.5	18.4
2	13.5	19.0	15.2	14.4	2	16.4	14.3	16.5	17.5
3	14.2	13.2	14.0	14.5	3	18.3	15.5	15.3	16.5
4	15.4	16.8	13.0	16.5	4	15.9	13.8	15.0	16.4
5	13.7	18.6	15.2	16.5	5	16.1	16.4	15.8	17.2
6	14.6	14.5	13.8	16.4	6	16.2	14.7	14.7	16.2
7	15.7	15.0	16.8	17.0	7	13.4	14.3	15.8	15.9
8	14.8	12.0	14.3	12.6	8	16.4	18.0	12.6	17.4
9	15.2	16.5	16.2	21.0	9	16.2	18.0	13.8	16.8
10	15.4	14.2	17.5	15.6	10	14.0	17.5	16.0	16.6
11	15.3	17.6	15.7	16.8	11	14.7	18.4	16.0	17.4
12	16.7	18.5	15.2	17.5	12	15.5	13.3	15.1	13.6
Mean Values	15.0	16.29	15.36	16.18	Mean Values	16.26	15.70	15.25	16.65

Table 6. Hemoglobin Values for Normal Males and for Male Rats Receiving 0.05% Sodium Fluoride.

Normal Rats on Growing Ration						Stock Ration Plus 0.05% NaF					
Rat No.	Hemoglobin Values at					Rat No.	Hemoglobin Values at				
	2-2	2-16	3-3	4-6	5-11		2-2	2-16	3-3	4-6	5-11
1	13.5	16.8	13.0	14.9	17.8	1	20.0	15.1	16.5	18.4	17.6
2	16.2	17.2	13.2	19.0	16.6	2	18.9	14.3	15.6	17.0	---
3	17.2	15.5	16.1	16.2	17.6	3	14.6	17.5	15.8	16.6	23.0
4	17.5	14.6	16.7	19.7	18.6	4	15.0	15.6	15.6	17.0	16.1
5	---	---	---	---	18.8	5	---	---	---	---	17.6
6	---	---	---	---	16.1	6	---	---	---	---	19.1
7	---	---	---	---	17.5	7	---	---	---	---	17.2
8	---	---	---	---	17.2						
9	---	---	---	---	18.6						
10	---	---	---	---	17.2						
Mean Values	16.2	16.0	14.75	17.3	17.6	Mean Values	17.1	15.6	15.87	17.25	18.43

Table 7. Changes in Hemoglobin During Growth, Pregnancy and Lactation as Influenced by 0.05% NaF.

No. of rats	No. of females reproducing	Wt. of female at reproduction	Wt. of female at end of lactation	No. young in litter	Average Wt. of Young (gm)					Hemoglobin Values of Mothers	
					Birth	weeks: 1st	2nd	3rd	4th	at parturition	end lactation
(Growing ration alone)		153	190	7	6	11	22	36	53	17.8	15.0
		148	166	10	5	12	20	31	40	11.4	14.2
		180	212	7	5.14	13	18	32	47	12.8	---
		202	185	10	5.2	15	20	30	49	11.5	---
		128	150	7	6.28	13	18	22	33	12.6	16.6
		165	180	5	5.4	--	20	26	48	13.0	17.0
		173	169	8	4.25	11	14	(Mother died)		15.2	
		140	150	7	5.7	11	16	23	40	16.5	15.6
		112	114	1	3.0	(Young dead by 1st wk)				17.0	17.0
12	10	148	120	6	5.0	11	19	25	40	16.4	15.4
Mean Growing Ration		155	164	6.8	5.26	11.8	18.5	28.6	49	14.6	15.5
(Growing ration plus 0.05% NaF)		150	158	8	5.5	(All young dead 1st wk)				14.0	14.0
		128	156	9	4.2	4	(young dead 2nd wk)			16.4	17.2
		190	182	9	5.1	--	18	--	26	12.0	11.7
		100	145	8	3.75	3	(young gone 2nd wk)			16.4	15.0
12	5	126	138	7	5.42	8	12	--	20	15.5	14.8
Mean Growing ration 0.05% NaF		139	156	8.2	4.79	5.7	15.8	--	23	14.8	14.5

The processes of ovulation, fertilization, and gestation appeared not to be impaired since the numbers of live young in the litters were good in both groups. The fluoride group produced an average of 8.2 young per litter whereas the normals produced an average of 6.8 young per litter. These are values that may be considered within the range of normal performance. The sizes of the young at birth likewise appeared not to be affected. The lower number of pregnancies in the fluoride group was not expected in view of the findings of Smith (102) that fluorine appeared to delay maturity and thus to delay pregnancy.

The lactation data of the two groups are markedly different. In only two of the five litters receiving the fluoride did the young survive the second week of lactation. Those that did survive attained an average weight of only 33 grams at the age of four weeks. This poor performance is in marked contrast to that of the normal animals. Out of the ten normal litters one litter of six young died before the third week because of the death of the mother rat. Another litter of one young died before the end of the first week. The young in the other normal litters reached a normal weight of 49 grams at the end of the lactation period.

The hemoglobin values of the females of both groups at parturition were within the same general range and the averages of these values from the two groups differed by only 0.2 per

cent hemoglobin. The hemoglobin values obtained at the end of lactation appear for the most part to be normal in both groups of females, one female in the fluoride group had 11.7 per cent hemoglobin, a value which is somewhat low. The other values in this same group appear to fall definitely within the normal range for hemoglobin.

The values for hemoglobin shown in tables 5 and 6 indicate that 0.05 per cent sodium fluoride, although it affects growth perceptibly, does not at the same time influence hemoglobin levels, either in the females or in the males.

The Effect of Alum upon the Toxicity of NaF in the Ration of the Rat

Purpose.

The purpose of this investigation was to learn whether or not the addition of alum to the ration of the rat would alleviate in a measurable degree the toxic effects of sodium fluoride in the same ration.

Procedure.

One group of rats consisting of three males and three females was placed on each of six different rations at the age of 28 days. The six rations were as follows: number 1, growing ration alone; number 2, growing ration plus 0.0113

per cent fluorine as NaF; number 3, growing ration plus 0.0113 per cent fluorine as NaF plus 0.132 per cent $Al_2(SO_4)_3 \cdot 18\ H_2O$; number 4, growing ration plus 0.0113 per cent fluorine as NaF plus 0.3992 per cent $Al_2(SO_4)_3 \cdot 18\ H_2O$; number 5, growing ration plus 0.0226 per cent fluorine as NaF; number 6, growing ration plus 0.0226 per cent fluorine as NaF plus 0.799 per cent $Al_2(SO_4)_3 \cdot 18\ H_2O$.

Records of growth and reproduction were kept. The original weight of each litter was recorded and at the end of the lactation period of 28 days the weight of the litter was again determined. The appearance of incisor teeth was observed regularly and the descriptions of the incisors were recorded.

Second and third generations were continued on the experiment when available.

Results and interpretations.

Growth. Noticeable differences in growth of the rats were obtained on the various experimental diets in this series. The growth of first generation animals is summarized in table 8.

Table 8. Average Weight Gains in Six Months of Rats on Alum-Fluoride Feeding Experiments.

Average 6 month's gain (grams)											
Lot 1.		Lot 2.		Lot 3.		Lot 4.		Lot 5.		Lot 6.	
f.	m.	f.	m.	f.	m.	f.	m.	f.	m.	f.	m.
178	208	141	189	142	185	145	227	104	142	152	217
combined		combined		combined		combined		combined		combined	
192		164		163		185		123		184	

The growth of animals on the basal ration alone averaged 192 grams for the first six months of the experiment; but when 0.0113 per cent fluorine as NaF was incorporated in this diet the average growth in six months was 164 grams or 28 grams less. When 0.132 per cent $Al_2(SO_4)_3.18\ H_2O$ was also incorporated in the ration treated to contain 0.0113 per cent fluorine as NaF, the growth rate was not improved, whereas incorporation of 0.3992 per cent alum into the same ration caused an apparent response as regards growth, increasing the average weight gain to 185 grams over the six months period. When 0.0226 per cent fluorine as administered in the ration in the form of NaF the average gain in weight decreased to 123 grams. However, when the alum was added at the level of 0.799 per cent the growth obtained over a like period of time was 184 grams.

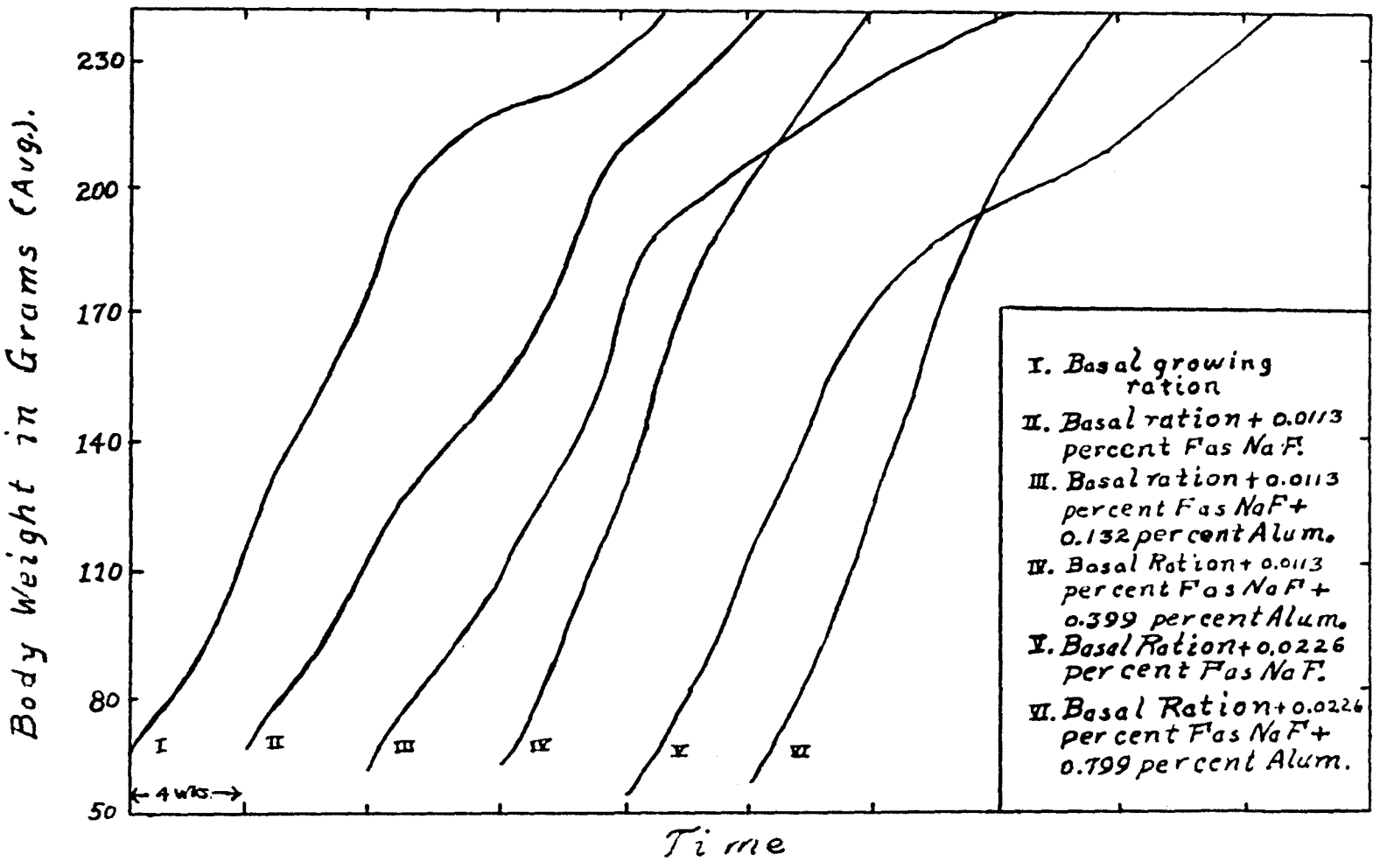


Figure 2. Growth curves of males on alum-fluoride feeding experiments. First generation.

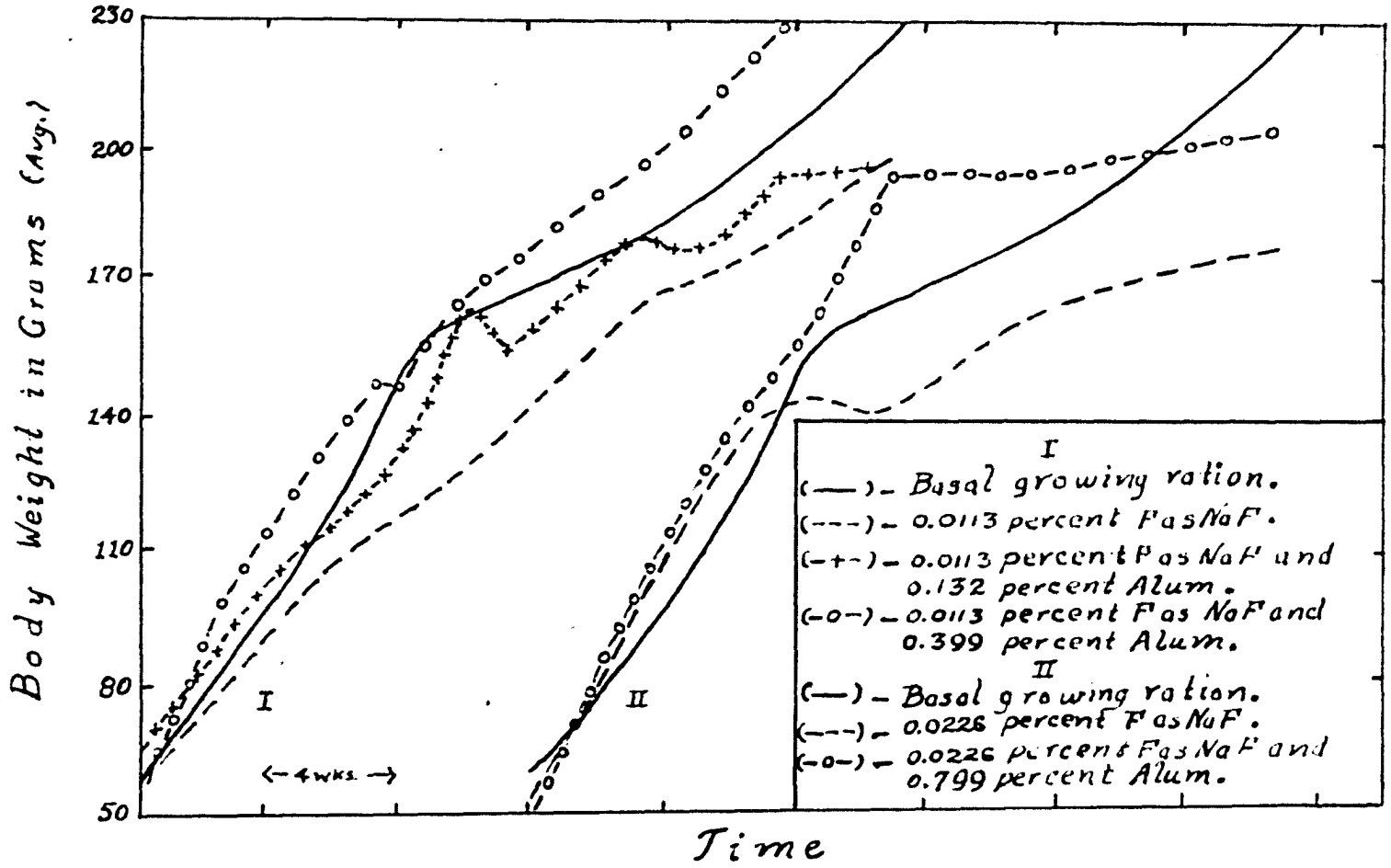


Figure 3. Growth curves of females on alum-fluoride rations. First generation.

The growth curves (figure 2) for the first generation of males of the different groups show that the addition of alum to the rations containing fluoride overcame the effect of fluoride upon growth. The use of 0.132 per cent alum did not noticeably appear to improve growth. But the use of 0.399 per cent alum in conjunction with 0.0113 per cent fluorine had a beneficial effect. When the level of fluorine was doubled the benefit of alum was still noticeable.

The composite growth curves for the first generation females in each lot have been prepared and are shown in figure 3. These curves show in general the same trends as do the curves for the males. Adding alum to the diet containing fluoride raised the position of the growth curves and lengthened the period of more rapid growth. This was true with the females receiving 0.0113 per cent fluorine with both the 0.132 per cent and 0.399 per cent alum supplements and likewise with the females receiving 0.0226 per cent fluorine accompanied by 0.799 per cent of alum. The latter groups of females grew very well. Examination of the curves in figure 4 reveals a rather marked irregularity in the growth of the animals on 0.0113 per cent fluorine and 0.132 per cent alum. This irregularity began at the time that reproduction and lactation began, and possibly was caused by the inability of the females readily to meet the demands of lactation. It should be noted that the animals on this level of fluoride

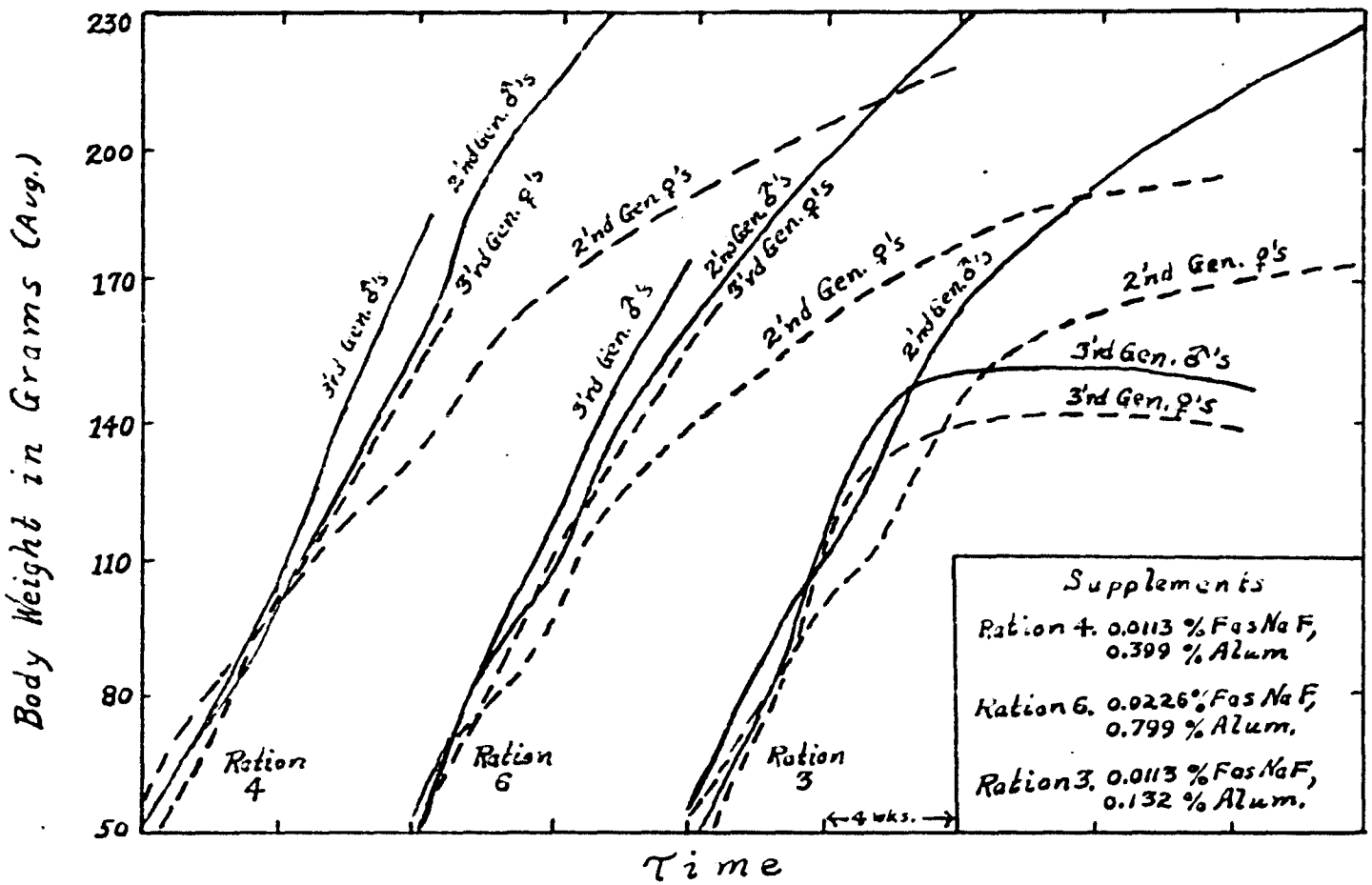


Figure 4. Second and third generation growth curves of animals on alum-fluoride rations.

alone did not show this irregularity. This may be accounted for since these females (on fluorine alone) failed so completely in lactation that no lactation period was sufficiently long to cause a reduction in body weight. The females receiving 0.0113 per cent fluorine as NaF and 0.399 per cent alum maintained body weight well during lactation. This was true in spite of the fact that they reared to weaning age more litters than any of the other groups.

In figure 4 are shown the second and third generation composite growth curves of the animals on alum and fluoride. The first set of curves show the growth record of the animals receiving ration 4, the second set of curves show the growth record on ration 6, while the third set of curves shows the growth record on ration 3. The animals on rations 4 and 6 grew very well in the second and third generations. The animals on ration 3 which received the same amount of fluoride as those on ration 4 did not grow as well as those on ration 4. This was probably due to the different levels of alum incorporated into these two rations, since the level of alum in ration 4 was three times that in ration 3. The nearly equal growth of the animals on ration 6 as compared to the growth of those on ration 4, even though the levels of fluoride and alum are doubled in ration 6, lends further evidence that the alum had in some manner rendered the fluoride less deleterious. The somewhat less favorable growth in the animals of lot 3

Table 9. Reproduction Data from Fluoride-Alum Feeding Experiments.

Generation	Ration Supplement	Number of Pregnancies	Average litter size-gms.	Average birth wt.-gms.	Average weaning wt.-gms.
First	None	5	6.25	5.75	51
"	0.0113% F	3	7.33	5.45	all died before weaning
"	0.0113% F 0.132% Alum	4	5.5	5.36	47
"	0.0113% F 0.399% Alum	4	7.5	5.7	48
"	0.0226% F	5	7.66	3.6	all died before weaning
"	0.0226% F 0.799% Alum	8	6.8	5.56	43.1
Second	0.0113% F 0.132% Alum	5	6.8	5.2	40
"	0.0113% F 0.399% Alum	6	7.4	5.5	40.3
"	0.0226% F 0.399% Alum	6	6.8	4.3	43

cannot be explained upon the basis that alum may have had a deleterious effect since both ration 4 and ration 6 contained more alum than ration 3.

Reproduction and lactation. The data on reproduction and lactation are summarized in table 9 in which the generations, ration numbers, number of pregnancies, average litter size, average birth weights and average weaning weights of the young are shown. In these feeding experiments the addition of fluoride alone to the ration impaired lactation to the extent that no young remained alive until the weaning age. The addition of alum to the ration in all cases resulted in improvement of lactation to the extent that young were weaned successfully. When these young were reared on the same rations, they likewise weaned young which were capable of rapid growth when placed immediately on the same ration. The failure of lactation of the animals receiving 0.0226 per cent fluoride in this experiment was in good agreement with the lactation results obtained at the same fluoride level in the study on hemoglobin.

Tooth changes. Noticeable differences were observed between the teeth of the animals receiving growing ration and of those receiving fluorides and between the teeth of those receiving fluoride alone and of those receiving fluoride plus alum. The animals on the stock ration appeared normal in all respects throughout the duration of the experiment.

The teeth of the stock lot of animals, males and females alike, presented no visible evidence of the influence of fluoride. When the ration was supplemented with 0.0113 per cent fluoride as NaF the general appearance of the animals was not noticeably affected; but this level of fluoride caused visible damage to the teeth. At the end of the third week the evidence of bleaching was unmistakable. By the end of the fifth week bleaching of the teeth in all animals was still more noticeable, although excess wearing or lengthening had not yet begun. At the sixth month there was noticeable wearing or lengthening of the upper incisors with bleaching and wearing of the lower incisors as well. At 16 months the two remaining females had bleached and badly broken incisors with the lower incisors blotched and stained. Both of the remaining males had bleached and striated upper and lower incisors. One male had noticeably lengthened upper incisors while the upper incisors of the other were badly worn.

It was noted in this experiment that during pregnancy the incisors became more severely affected, while partial recovery occurred after these periods. After one pregnancy it was observed that the appearance of the incisors of the female was noticeably more abnormal than those of the male.

When alum at the level of 0.132 per cent was incorporated in the ration containing 0.0113 per cent fluorine the effect of the fluorine upon the teeth was considerably retarded. At

three weeks all animals were free from striation or bleaching while at the five week period only one, a female, showed evidence of striation and bleaching. At nine weeks two females showed slight bleaching while the males and the other female still presented a normal appearance. At six months slight striations in both the upper and lower incisors of one of the males could be detected, but none were noted in the other two males. All females, however, showed striations but no lengthening or wearing at the end of six months. At sixteen months the one male remaining alive showed no apparent abnormalities. Of the females, however, all three showed mottled incisors. Only one of the three females showed a lengthening of the incisors.

When the alum supplement was increased to 0.309 per cent in the ration containing 0.0113 per cent added fluorine, the inhibition of the effects of the fluorine appeared more decided. At the three and five week periods the teeth of all animals, both males and females, appeared normal, while at the ninth week the effect on the teeth of one female was questionable. At the sixth month two females, one pregnant at the time, showed some bleaching of the teeth. The teeth of the remaining female were normal. At fourteen months one of the two remaining males appeared entirely normal while the other appeared normal except that one lower incisor showed slight blackening. This latter animal was emaciated. At this time, one female, in

emaciated condition, showed striation and slight black stain in lower incisors, another showed no abnormalities while the third showed only a few slight striations. The one entirely normal female had not reproduced, whereas the two females showing slight tooth defects were those which had borne two litters of young each and had suckled them quite satisfactorily.

In the animals receiving 0.0226 per cent fluorine - the deleterious effect of fluorine on the teeth became evident very quickly since at three weeks the striations and bleaching in all animals - both males and females - was quite noticeable in both upper and lower incisors. At five weeks complete bleaching with beginning lengthening of the incisors was noted in all animals. At nine weeks noticeable lengthening of the upper incisors accompanied with wearing of the lowers was noted in all animals. At four and one-half months the upper incisors of one female were clipped to prevent their piercing the cheek. Severe lengthening of incisors had occurred in all the other animals as well, while a marked decrease in food consumption accompanied the condition. The lower incisors of one male had to be clipped because they had become so lengthened that they had begun to pierce the nasal cavity. The upper incisors in this same animal had deteriorated to black stumps even with the fleshy surface. At five months all animals appeared very shaggy in appearance and somewhat emaciated. After twelve months only two females and one male

remained alive. These animals all showed chalky, distorted and badly worn incisors. The two females were very emaciated while the remaining male was fairly normal in general appearance. When the ration containing 0.0226 per cent fluorine as NaF was supplemented with 0.799 per cent alum, decided alleviation of the symptoms of fluorosis was observed.

The extent of mottling in this group was decidedly less than in the preceding one. At the fifth week the effects upon the teeth had begun to appear and the lower incisors of all animals were distinctly bleached. In the later weeks of the experiment this condition did not become more noticeable in the males. No lengthening or thickening of the incisors was observed. No noticeable wearing was observed. After 53 weeks one of the three males appeared normal while the other two showed some loss of pigment but no distortion of shape or wearing. Again in the females it was quite apparent that at each pregnancy there began a deterioration of the teeth which progressed through lactation until the young were weaned, after which the condition of the teeth began to improve. Thus one of the females, which weaned a group of 5 young, 9 weeks before, showed at the close of the experiment very nearly normal incisors presenting slight loss of pigment in the left lower incisor but no chalky appearance nor malformation. The other female remaining at the end of 53 weeks showed visible signs of mottling in all incisors.

It seems significant that the divergence of the growth curves of the males receiving fluoride alone and of those receiving fluoride plus alum began at approximately the time when the incisors of the fluoride fed rats approached maximum distortion. Since such gross changes in tooth structure were entirely avoided by alum feeding it seems possible that the inferior growth of the fluoride group may have been due in part to impaired prehension of food.

Comparison of Toxicity of Aluminum Fluoride with Other Inorganic Fluorides.

Purpose of investigation.

Experiments to compare the fluoride toxicity of a number of inorganic fluoride compounds had been planned and several feeding experiments to indicate feeding levels had been started when Smith and Leverton (102) and De Eds and Thomas (32) published their papers dealing with the comparative toxicities of a number of inorganic compounds.

In these preliminary experiments Al_2F_6, CuF_2, $CaSiF_6$ and ZuF_2 were fed at a level equivalent to 0.10 per cent fluorine. It was very soon apparent that the toxicities of these four compounds were of three different orders. Their influences upon growth are shown in table 10.

Table 10. Growth Records of Animals on Preliminary Feeding Trials with Al_2F_6, $CaSiF_6$, ZnF_2, CuF_2.

Time	Al_2F_6*	$CaSiF_6$*	ZnF_2*	CuF_2*
Start	45	45	45	46
1st wk.	76	54	60	58
2nd wk.	99	79	77	77
3rd wk.	107	92	94	85
4th wk.	147	94	99	--
6th wk.	178	Dead at 34 days	Dead at 31 days	--
8th wk.	207			114
10th wk.	214			129
12th wk.	222			133

*at 0.10% F

Observations of the teeth of the animals in these preliminary experiments revealed that calcium fluosilicate, zinc fluoride and cupric fluoride brought about typical fluoride bleaching of the incisors. With $CaSiF_6$ the teeth were markedly damaged in 25 days. With ZnF_2 the bleaching was very severe in 28 days. CuF_2 caused bleaching in 14 days with chipping becoming noticeable by the end of the sixth week. Somewhat surprisingly the animals receiving aluminum fluoride showed no apparent effects. Because these preliminary feeding experiments indicated that aluminum fluoride was of an extremely low order of toxicity more conclusive experiments were planned in which growth, effect upon incisors, and storage of fluorine were to be compared.

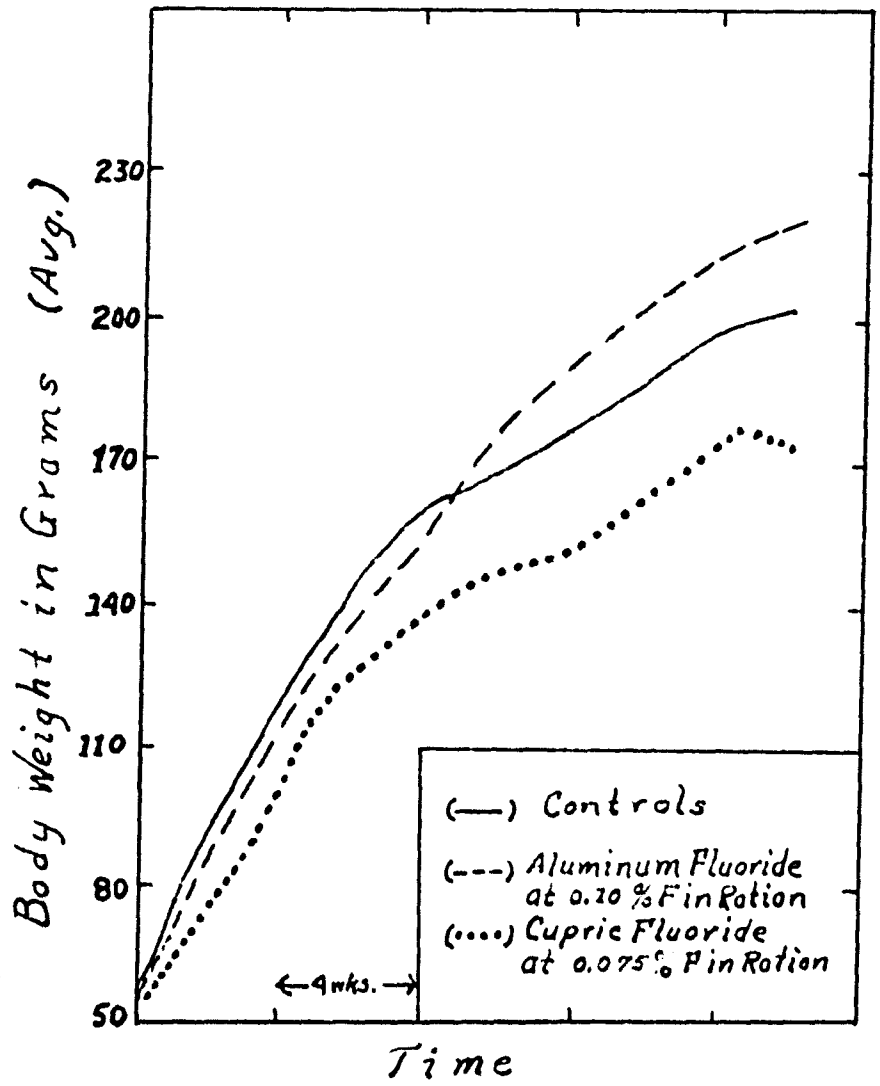


Figure 5. Growth of normal rats and rats receiving Al_2F_6 and CuF_2.

Procedure.

Three lots of 36 rats 28 days of age were placed on experiment. Lot 1 received growing ration alone and served as a control. Lot 2 received 0.10 per cent fluorine as Al_2F_6 while Lot 3 was given 0.075 per cent fluorine as CuF_2. The animals were weighed regularly and examined for incisor defects. At the close of the experiment bones of representative animals of the fluoride fed groups were analysed for fluorine.

Results.

Growth. The growth of the control animals and of the fluorine fed animals is shown in figure 5. The growth of the control rats, shown by the solid line, was quite good, reaching an average body weight of 200 grams in 4 1/2 months. The growth of the aluminum fluoride animals, shown by the broken line, was somewhat better than that of the control animals since they attained an average body weight of approximately 215 grams in the same length of time. Although the CuF_2 rats made rather steady growth, as shown by the dotted line, this growth was inferior to that of both of the other groups. At the close of the experiment these CuF_2 animals averaged 30 grams less than the normals and 45 grams less than the aluminum fluoride group.

Tooth changes. The incisors of the CuF_2 animals showed severe bleaching and lengthening of the teeth whereas the incisors of the control rats and of the aluminum fluoride animals showed no changes.

Storage of fluorine. The fluoride content of the tibiae of six animals from the CuF_2 and Al_2F_6 groups are shown in table 11. In this table are shown the weights of the extracted, dried bones, the weights of ash in the bones, the quantities of fluorine in the bones in micrograms, the per cents of fluorine in the ash and the per cents of fluorine in the bones. The storage of fluorine in the CuF_2 tibiae averaged 2,640 micrograms whereas the storage of fluorine in the Al_2F_6 tibiae averaged only 147 micrograms.

The difference in the per cents of fluorine in the two sets of tibiae were even more marked. The percentages of fluorine in bones of the CuF_2 and Al_2F_6 animals averaged 0.608 and 0.033, respectively. The comparison of the percentages of fluorine in the ash show that the ash of the CuF_2 tibiae contained 1.14 per cent fluorine whereas the ash of the Al_2F_6 tibiae contained only 0.045 per cent fluorine.

Weight of bones. The tibiae of the CuF_2 animals were quite consistently lighter in weight than those of the Al_2F_6 group. The weights of all but one of the Al_2F_6 tibiae exceeded the weight of the heaviest CuF_2 tibia.

Weights and percentages of ash. The differences in

Table 11. Comparison of Storage of Fluorine from CuF_2 as Compared with Al_2F_6

CuF_2 (0.075%F)					Al_2F_6 (0.10%F)				
Wt. of Bone grams	Wt. of Ash grams	Wt. of F in Ash (ug)	Percent F in Ash	Percent F in Bone	Wt. of Bone grams	Wt. of Ash grams	Wt. of F in Ash (ug)	Percent F in Ash	Percent F in Bone
0.2852	0.1822	2,000	1.09	0.52	0.4574	0.2768	152.0	0.055	0.033
0.3479	0.2202	2,510	1.13	0.69	0.4773	0.2843	171.7	0.06	0.035
0.3595	0.2391	2,736	1.13	0.76	0.4234	0.2708	142.0	0.052	0.033
0.4037	0.2621	3,249	1.24	0.80	0.4963	0.2543	134.0	0.052	0.025
0.3761	0.2358	2,660	1.12	0.70	0.3847	0.2243	135.0	0.06	0.035
0.3531	0.2239	2,572	1.15	0.72	0.4270	0.2750	150.0	0.055	0.035
Mean					Mean				
0.3376	0.2272	2,621	1.14	0.698	0.4443	0.2642	147.4	0.056	0.033

quantity of ash did not exactly parallel the differences in the weights of the bones. There was a tendency for the per cent of ash in the CuF_2 bones to exceed that of the Al_2F_6 bones, as shown in table 12.

Table 12. Percentages of Ash in the Tibiae of Rats Receiving CuF_2 and Al_2F_6.

	CuF_2	Al_2F_6
	63.89	60.51
	63.29	59.56
	66.78	66.32
	65.17	51.24
	62.69	58.30
	63.40	64.40
Mean	64.20	60.05

Effects upon hemoglobin. When the experiment was discontinued the hemoglobin values for all of the animals were determined. Table 13 shows the average hemoglobin values for all of the animals at the close of the feeding experiment.

Table 13. Average Hemoglobin Values of Normal Rats and of Al_2F_6 and CuF_2 Fed Rats.

Normal		Al_2F_6 (0.10% F)		CuF_2 (0.075% F)	
Males	Females	Males	Females	Males	Females
16.16	15.18	15.57	14.68	14.13	14.61
(12 Rats)	(17 Rats)	(15 Rats)	(15 Rats)	(14 Rats)	(10 Rats)

These values did not reveal any differences in hemoglobin levels after a four and one-half months feeding period.

The Effect of Alum upon Toxicity of NaF in Injection Experiments

Procedure.

Healthy young rats between 31 and 33 days of age were employed in all of these experiments. The animals were marked for identification and injected in series, the time of injection being recorded to the nearest half minute. After injection the animals were kept under constant observation for six hours. Those animals which appeared in good condition by the end of six hours were returned to cages for later observation. The approach of death could quite easily be recognized in the animals receiving sodium fluoride alone at the level of 100 mgs.

per kilogram of body weight, since death was always preceded by definite spasms in the limbs. The time of death was taken as the time when the beat of the heart ceased to be detectible by palpation.

When the alum and fluoride were given separately the alum was administered from a second syringe, by replacing the empty fluoride syringe with the alum syringe without removing the needle from the body of the rat.

Results.

Twelve young rats, 31 days of age, equally divided as to sex, were injected with a sodium fluoride solution equivalent to 100 mg. of NaF per kilogram of body weight and the time required to cause death recorded. The results were as follows:

Table 14. Results of Injecting Fluoride Alone at 100 mg. per Kilo. Intraperitoneally.

Sex	Age in days	Wt. in grams	NaF per kilo	Time required to kill	Observations
m	31	52	100 mg.	14 min.	Death occurred quite abruptly. Paralysis of the hind legs was noted soon after injection. Clonic spasms occurred all over body just before death.
m	"	52	"	14 "	
m	"	50	"	16 "	
m	"	38	"	15 "	
m	"	52	"	17 "	
m	"	44	"	15 "	
f	"	46	"	14 "	
f	"	50	"	15 "	
f	"	49	"	15 "	
f	"	55	"	17 "	
f	"	53	"	16 "	
f	"	43	"	15 "	
Mean lapsed time from injection to time of death - 15 min.					

In the next series of experiments alum alone was injected into young normal animals 31 days of age at the levels of 1.598 and 0.799 grams per kilogram of body weight. The results of these experiments are shown in Table 15.

The higher level of alum, when injected alone, brought about death, but only after a mean lapsed time of 132 minutes, whereas the lower level of alum caused little apparent damage to the animals. They appeared normal 48 hours after injection.

In order to observe the effect of alum upon the toxicity of injected fluoride, experiments were performed by following the fluoride injection immediately by alum, by mixing the alum with fluoride and injecting, and by mixing, neutralizing to litmus with sodium bicarbonate, and then injecting. A number of injections were performed in which aluminum fluoride suspension was injected at a level equivalent to 200 mgs. of sodium fluoride. Table 16 contains the results obtained from the first series of injections using both alum and fluoride.

Table 15. Results of Injecting Alum Alone.

Sex	Age in days	Wt. in grams	Alum per kilo	Time required to kill (min)	Observations
m	31	48	1.598 gm.	196 min.	The times of death are correct to 15 minutes. Death occurred in a different fashion than when due to NaF. Indications of paralysis were absent. Animals showed evidence of local pain in the abdomen and noticeable bloating was observed.
m	"	48	"	133 "	
m	"	48	"	139 "	
f	"	48	"	144 "	
f	"	48	"	146 "	
f	"	48	"	158 "	
Mean lapsed time from injection to death:				152 "	
m	33	49	0.799 gm.	did not kill	Animals apparently normal after 48 hours.
m	"	48	"	" " "	
m	"	48	"	" " "	
f	"	50	"	" " "	
f	"	49	"	" " "	
f	"	35	"	" " "	

When NaF was given intraperitoneally at a level of 100 mgs. per kilogram of body weight young rats were killed within an average time of 15 minutes. Upon administration of the same amount of sodium fluoride with alum at a level of 1.599 grams per kilo the time required to kill was increased 13 fold. A 9 fold increase in the killing time was observed when the alum was given at a level one half as large as the above. When the higher levels of alum were injected with the fluoride the clonic spasms did not appear before death. The lower level of

Table 16. Results of Injecting Sodium Fluoride Followed by Alum.

Sex	Age in days	Wt. in grams	NaF per kilo	Alum per kilo	Time required to kill	Observations
m	31	49	100 mg.	1.598 gm.	188 min.	The killing time
m	"	49	"	"	145 "	was increased
m	"	45	"	"	309 "	13.6 times by
f	"	47	"	"	166 "	this level of
f	"	52	"	"	311 "	alum as compared
f	"	48	"	"	129 "	with the same amount of
				Mean:	208 "	fluoride alone.
m	33	56	100 mg.	0.799 gm.	150 min.	Time to kill was
m	"	56	"	"	140 "	increased 9.6
m	"	45	"	"	145 "	times.
f	"	63	"	"	120 "	
f	"	55	"	"	160 "	
f	"	44	"	"	150 "	
				Mean:	147 "	

alum did not cause death when administered alone. When this level of alum was administered with fluoride, death was not prevented. The lapse of time between injection and death, however, was 9 times as great as with sodium fluoride alone. This indicated that alum was in some way decreasing the toxicity of the fluoride.

It seemed possible that mixing the alum and sodium fluoride before injection might alter the killing time still further. An experiment in which the solutions were mixed just before injection gave the following results:

Table 17. Results of Mixing Fluoride and Alum Immediately Before Injection.

Sex	Age in days	Wt. in grams	NaF per kilo	Alum per kilo	Time required to kill	Observations
f	32	62	100 mg.	0.799 gm.	125 min.	Death occurred,
f	"	60	"	"	107 "	preceded by
f	"	60	"	"	153 "	paralysis of
f	"	57	"	"	120 "	extremities. No
f	"	42	"	"	108 "	clonic spasms
f	"	48	"	"	166 "	apparent.
				Mean:	129 "	

Two series of experiments were conducted to learn the effect when the alum-fluoride solution was neutralized to litmus before injection. In one series of trials the alum and fluoride solutions were mixed and allowed to stand 12 hours after which the mixture was neutralized to litmus with sodium bicarbonate.

The mixture after standing for 12 hours was neutralized before injection and gave the following results:

Table 18. Results of Injecting Fluoride and Alum Twelve Hours After Mixing.

Sex	Age in days	Wt. in grams	NaF per kilo	Alum per kilo	Time required to kill	Observations
f	32	54	100 mg.	0.799 gm.	109 min.	The animals were stuporous within a few minutes after the injection and remained so till death. Death occurred with convulsions with two of the animals.
f	"	52	"	"	118 "	
f	"	62	"	"	86 "	
f	"	55	"	"	93 "	
f	"	58	"	"	115 "	
f	"	55	"	"	84 "	
Mean lapsed time between injection and death:					101 min.	

When the alum-fluoride mixture was neutralized 5 minutes after mixing and then injected the following results were obtained:

Table 19. Results of Injecting Fluoride and Alum Five Minutes After Mixing.

Sex	Age in days	Wt. in grams	NaF per kilo	Alum per kilo	Time required to kill	Observations
f	32	54	100 mg.	0.799 gm.	120 min.	The animals became stuporous soon after the injection. Some showed convulsions just before death.
f	"	52	"	"	118 "	
f	"	62	"	"	115 "	
f	"	55	"	"	109 "	
f	"	58	"	"	110 "	
f	"	55	"	"	105 "	
Mean lapsed time between injection and death:					113 min.	

Experiments were next conducted to compare the toxicity of Al_2F_6 with that of NaF. Aluminum fluoride, very finely divided, was injected in the form of a solution-suspension in amounts which, on the basis of the fluorine content, was equivalent to two times the amount of sodium fluoride which caused death in 15 minutes.

Table 20. Results of Injecting Al_2F_6 at a Level Equivalent to 200 mgs. NaF.

Sex	Age in days	Wt. in grams	Al_2F_6 NaF per kilo	Time required to kill	Observations
f	33	83	200 mg.	Did not kill	Behavior of animals apparently normal.
f	"	48	"	" " "	
f	"	67	"	" " "	
f	"	59	"	" " "	
f	"	60	"	" " "	
f	"	65	"	" " "	

When administered at a level equivalent to 200 mgs. NaF per kilogram of body weight the animals suffered no apparent ill-effects.

Interpretation of results.

The results are summarized in table 21.

The higher level of alum was sufficient to cause death in a mean time of 152 minutes which was ten times as long as the time required for 100 mg. of sodium fluoride to kill. The

Table 21. Summarized Data on Alum-Fluoride Injections

Series	No. Rats	Age in Days	Wt. in Grams	NaF per Kilo (mg)	Alum per Kilo (mg)	Mean Death Time (min.)	Notes
1	12	31	48.8	100	none	15.25±1.5	
2	6	31	48.0	none	1.598	152±31.5	
3	6	33	46.5	none	0.799	did not kill	
4	6	31	48.3	100	1.598	208±91	Alum and fluoride injected separately
5	6	33	53	100	0.799	147±30	"
6	6	32	54.8	100	0.799	129±29.5	Mixed before injected
7	6	32	56	100	0.799	101±17	Mixed, neutralized after 12 hours, injected
8	6	32	56	100	0.799	113±7.5	Mixed, neutralized after 5 minutes, injected

lower level of alum did not kill when injected alone. When the larger amount of alum was given with the sodium fluoride the time required to kill was not greatly different from that required by the alum alone. The lower level of alum appeared to reduce the toxicity to a marked degree.

A comparison of the results from series 6, 7, and 8 indicates that the time of death was not different whether or not the alum-fluoride was injected immediately after mixing, 5 minutes after mixing and neutralizing or 12 hours after mixing and neutralizing. These results indicated that the alum reduced the toxicity of the fluoride to a decided degree. If the decrease in toxicity was, as it seemed, due to adsorption of the fluoride or to its chemical combination with alum, it appeared that this combination took place rapidly.

The difference in the toxicity of aluminum fluoride as compared to sodium fluoride when injected, and to cupric fluoride and other fluorides when fed, indicated that the alleviation of the toxic effects of fluorine by alum feeding might have been the result of a chemical combination between the aluminum ion and the fluoride ion. This observation is in accord with the opinion of Evans and Phillips (37) that the toxicity of cryolite (Na_3AlF_6) is due to the NaF moiety, and not to the AlF_3. This observation is also in agreement with the findings of De Eds and Thomas (32) who found mottling of the rat incisor took place at a level of 12 parts per million

of fluorine as sodium fluoride but not at 6 parts per million, whereas with Na_3AlF_6 the mottling effect could be noticed at a level of 24.3 parts per million of fluorine but not at 12 parts per million in the ration.

The Relationship Between Recalcification of Rachitic Tibiae and the Incorporation of Fluorine into These Bones.

Object of study.

It seemed desirable to investigate the effect of fluoride upon the ash content of bones and upon their fluoride content under the super-imposed condition of healing rickets. Although data on the effects of feeding sodium fluoride in a rachitogenic diet had been reported by Hauck, Steenbock, and Parsons (46) and by Schulz (91), no data had been observed which dealt with the changes encountered in the healing process when sodium fluoride is administered or with the changes in the quantities of fluorine incorporated in bone during the process of healing. As this work was nearing completion and the analyses for fluoride were about finished, the work of Morgareidge (69) appeared, dealing with the problem of the effect of fluorine upon the healing process. Morgareidge (69) studied the effect of sodium fluoride upon the appearance of healing bones under x-ray examination and upon the ash content

of the bones. Morgareidge did not use the line test and did not analyse the bones for fluorine.

Experimental procedure.

Because all of the animals had to be killed and the bones taken for analysis on the same day, the experiment was run twice using 16 rats each time.

Rats of weaning age were placed on the Steenbock and Black (105) rachitogenic diet until they were distinctly rachitic at the close of a 21 day depletion period. They were then divided at random into four lots, and their body weights were recorded. The four lots were given the following rations:

Lot 1. Rachitogenic diet plus 0.10 per cent sodium fluoride plus a daily supplement of cod-liver oil as a source of vitamin D sufficient to give approximately 2 healing.

Lot 2. Rachitogenic diet alone.

Lot 3. Rachitogenic diet plus the same vitamin D supplement as Lot 1.

Lot 4. Rachitogenic diet plus 0.1 per cent sodium fluoride.

The feed and fluorine free distilled water were kept before the animals at all times. In the first experiment the animals were sacrificed at the close of the ninth day of the healing period while in the second experiment they were

sacrificed at the beginning of the eleventh day. Body weights were determined just before the animals were sacrificed. Tibiae for analysis were obtained after killing the animals with chloroform.

Results and interpretations.

Examination of the beginning and closing body weights shows that the level of fluoride administered was insufficient to cause a marked loss in weight during the relatively short healing period. Growth retardation due to fluorine did appear and this retardation appeared not to be alleviated by the quantity of vitamin D supplied.

The mean weights of the tibiae were nearly the same in the first three lots of animals. A difference of only 0.3 mg. existed between the mean values for lots 1 and 3, while between lots 2 and 3 a difference of only 1.4 mg. was found.

Slightly more than a ten per cent increase in the mean weight of ash and a corresponding increase in per cent ash were observed when sodium fluoride was added at a 0.10 per cent level to the basal ration supplemented with vitamin D. This increase in ash was not noted when the influence of sodium fluoride alone (lot 4) was compared with that of vitamin D alone (lot 3).

The addition of sodium fluoride alone to the rachitogenic ration (lot 4) increased the fluoride content nearly

Table 22. The Influence of Recalcification of Rach

Lot	Supplement	Body Wts. Start	Body Wts. End	Line Test	Wt. of tibia grams	Wt. of ash grams	Per cent ash	Fluo titr µ
1a	0.1% NaF	60	62	2	0.0971	0.0449	46.26	11.
	C.L.O.	55	54	2	0.1007	0.0456	45.40	11.
	7 days	50	50	2	0.0936	0.0421	45.10	11.
		50	53	2	0.0951	0.0416	43.70	11.
1b	"	70	70	3	0.1107	0.0459	41.46	15.
		64	64	3	0.1127	0.0448	39.63	16.
		70	74	4	0.1321	0.0607	45.95	18.
		54	47	3	0.0976	0.0400	40.98	12.
Mean		59.1	58	2.63	0.1049	0.0456	43.56	--
2a	None	60	62	0	0.0976	0.0391	40.10	1.2
		65	66	0	0.1065	0.0459	43.0	2.2
		46	42	1	0.0950	0.0384	40.44	3.(
		75	82	0	0.0843	0.0330	39.2	1.9
2b	"	65	78	0	0.1280	0.0428	33.43	1.(
		56	74	0	0.1230	0.0506	41.44	1.4
		52	58	0.5	0.1114	0.0370	33.21	4.3
		60	70	0	0.1026	0.0355	34.6	2.4
Mean		59.9	66.5	0.19	0.1060	0.0403	38.17	---
3a	C.L.O.	44	45	1	0.0986	0.0436	44.3	2.(
		58	58	1.5	0.0990	0.0391	39.1	1.(
		57	57	1	0.0882	0.0305	34.6	---
		52	56	1	0.0996	0.0390	39.2	2.2
3b	"	78	82	3	0.1290	0.0552	42.79	2.2
		62	67	2	0.1013	0.0383	37.79	1.(
		55	58	3	---	---	---	---
		78	90	2	0.1154	0.0375	32.49	2.(
Mean		60.5	64.1	1.8	0.1046	0.0404	38.61	--

of Recalcification of Rachitic Tibiae upon the Storage of Fluorine.

Wt. of ash grams	Per cent ash	Fluorine titrated γ gm.	Blank on reagents γ gm.	Fluorine in tibia γ gm.	Per cent fluorine in tibia	Per cent fluorine in tibia ash
0.0449	46.26	11.25	1.026	102.2	0.105	0.227
0.0456	45.40	11.97	1.121	108.5	0.108	0.23
0.0421	45.10	11.096	1.14	99.56	0.105	0.23
0.0416	43.70	11.40	1.14	102.6	0.108	0.247
0.0459	41.46	15.77	1.14	146.3	0.132	0.32
0.0446	39.63	16.00	1.14	148.6	0.131	0.33
0.0607	45.95	18.81	1.14	176.7	0.133	0.29
0.0400	40.98	12.90	1.14	117.6	0.120	0.29
0.0456	43.56	---	---	125.5	0.118	0.276
0.0391	40.10	1.256	1.026	2.40	0.003	0.007
0.0459	43.0	2.206	1.33	8.76	0.008	0.019
0.0384	40.44	3.059	1.14	19.19	0.022	0.05
0.0330	39.2	1.957	1.14	8.17	0.010	0.025
0.0428	33.43	1.026	1.026	0.000	0.000	0.000
0.0506	41.44	1.425	1.14	2.85	0.002	0.006
0.0370	33.21	4.237	1.20	30.37	0.027	0.082
0.0355	34.6	2.483	1.14	13.43	0.013	0.037
0.0403	38.17	---	---	10.63	0.010	0.028
0.0436	44.3	2.60	1.14	14.6	0.015	0.033
0.0391	39.1	1.82	1.14	6.8	0.007	0.017
0.0305	34.6	---	---	---	---	---
0.0390	39.2	2.28	1.14	11.4	0.011	0.029
0.0552	42.79	2.28	1.14	11.4	0.009	0.021
0.0383	37.79	1.61	1.14	4.7	0.005	0.012
---	---	---	---	---	---	---
0.0375	32.49	2.09	1.14	9.5	0.008	0.025
0.0404	38.61	---	---	9.73	0.009	0.022

Table 22. (Continued)

Lot	Supplement	Body Wts. Start	Body Wts. End	Line Test	Wt. of tibia grams	Wt. of ash grams	Per cent ash	Fluor titra µ g
4a	0.1% NaF	62	64	0	0.0844	0.0354	42.0	---
		58	59	3.5	0.0999	0.0391	39.2	0.03
		50	50	0	0.0882	0.0305	34.6	0.04
		52	52	0	0.0996	0.0390	39.2	0.05
4b	"	70	70	0.5	0.1143	0.0452	39.54	0.06
		60	59	0	0.1100	0.0402	36.55	0.06
		50	54	1	0.0869	0.0304	34.98	0.04
		70	72	1	0.1060	0.0462	43.58	0.06
Mean		59.0	60.0	0.75	0.0986	0.0392	38.71	---

f	Per cent ash	Fluorine titrated μ gm.	Blank on reagents μ gm.	Fluorine in tibia μ gm.	Per Cent fluorine in tibia	Per cent fluorine in tibia ash
4	42.0	---	---	---	---	---
1	39.2	0.039	1.14	62.7	0.062	0.16
5	34.6	0.0475	1.14	78.85	0.089	0.26
0	39.2	0.0594	1.14	101.5	0.10	0.28
2	39.54	0.064	1.14	110.2	0.096	0.24
2	36.55	0.0627	1.14	107.73	0.098	0.27
4	34.98	0.0467	1.14	77.33	0.089	0.25
2	43.58	0.063	1.14	108.30	0.10	0.23
2	38.71	---	---	92.36	0.09	0.24

9 fold without appearing to increase the ash content of the tibiae. The administration of vitamin D with 0.10 per cent sodium fluoride augmented the fluoride in proportion with the augmented ash.

The Effect of Sodium Fluoride upon Blood Sugar.

Purpose of investigation.

Blood sugar determinations upon dogs which had received sodium fluoride in their food had shown no marked differences. When the work upon the blood sugar changes in rats was later being considered it seemed desirable to study the influence of large doses of fluoride given by way of stomach tube. It was thought that administration of the fluoride to fasted and unfasted rats, both with and without glucose, might cause changes in blood sugar that would suggest possible changes to be sought in chronic fluoride poisoning.

Procedure.

Normal rats of the male sex, ranging in age between 150 and 173 days and varying in weight between 279 and 378 grams, were used for the blood sugar investigations. In the first experiments the usual glucose tolerance curves were obtained. The rats were fasted 24 hours after which blood samples were taken. They were then very lightly anesthetized with chloroform

so that the stomach tube could be quickly inserted. The calculated volume of glucose solution was given through the tube from an accurately graduated 5 ml. syringe. Following the administration of the glucose, samples of blood in duplicate were obtained at the 15, 30, 90, and 120 minute periods following the administration.

The blood sugar curves were then determined for the animals receiving glucose plus fluoride and for those receiving fluoride alone, using the same technique as that used for the animals receiving glucose alone. When glucose was administered with sodium fluoride the two substances were dissolved together in the calculated quantities in one solution.

After the results were obtained upon fasting rats, determinations were made in which glucose, sodium fluoride and sodium fluoride plus glucose were administered to unfasted rats. Some of the fasted and unfasted rats on fluoride showed symptoms of poisoning. After hyperglycemic tendencies in the fluoride poisoned, non fasted rats became apparent, blood sugar determinations were made on other rats receiving fluoride and fasted for 36 hours. One third of these were normal rats on the growing ration, one third were rats which had been receiving 0.05 per cent sodium fluoride, while the remaining third had been receiving 0.10 per cent sodium fluoride. The fluoride was fed as a part of the growing ration.

Results and interpretations.

The results obtained on fasted rats are shown in table 23. The results obtained from unfasted rats are shown in table 24. Mean curves made by plotting the average values fro blood sugar are shown in figure 6. Sugar concentrations are expressed as mgs. per cent. Curve one shows the normal glucose tolerance curve. The shape of this curve is quite as it should be, attaining a maximum at the thirty-minute period and then falling steadily to a normal value at the end of two hours. This curve indicated that acceptable technique had been used for handling the rats in these and succeeding runs on blood sugar change.

Curve 2 in figure 6 is the composite curve obtained for the animals that received glucose and fluoride at the rate of 68 mg. of fluorine per kilogram of body weight. Curve 3 shows the blood sugar changes that followed the administration of the same amount of fluoride alone. The maxima in these curves appeared at the one-hour period. This was 30 minutes later than the time of the maximum in the normal curve. The maximum blood sugar level of the sodium fluoride series and the fluoride-glucose series was greater than the maximum for glucose alone.

The second series of curves, shown in figure 7, were obtained from experiments planned to study the blood sugar

Table 23. Blood Sugar Changes in Fasted Rats Following the Feeding of Glucose

Run No.	Wt. of rat (gms.)	Age of rat (days)	Sex	Fasting time (hours)	Dosage per kilo by stomach tube	Mg. % Gl			
						Start	15 min.	30 min.	
1	279	155	m	24	1.25 gm. Glucose	129	164	166	1
2	290	153	m	24	" "	130	166	173	1
3	282	153	m	24	" "	125	144	170	1
4	279	160	m	24	" Glucose & 68 mg. F	97	124	154	1
5	377	158	m	24	" Glucose & 68 mg. F	117	147	185	1
6	334	165	m	24	" Glucose & 68 mg. F.	103	125	149	1
7	353	155	m	24	" Glucose & 68 mg. F	105	135	133	1
8	311	165	m	24	" Glucose & 68 mg. F	112	103	129	1
9	281	163	m	26	68 mg. F	100	117	124	1
10	375	163	m	24	"	109	133	139	N
11	304	165	m	24	"	94	103	---	-

*Glucose, glucose plus fluoride and fluoride alone were administered by stomach. Glucose administered at 1.25 gms. per kilo of body weight.

N.D. - No Determination

Feeding of Glucose Only, Sodium Fluoride plus Glucose, and Sodium Fluoride Only.

t	Mg. % Glucose in Blood						Observations and Notes
	15 min.	30 min.	60 min.	90 min.	120 min.	at death	
	164	166	142	132	107		
	166	173	149	135	139		
	144	170	170	142	143		
	124	154	140	131	N.D.	N.D.	(Died in 2 hrs. 21 min. Heart (sample not taken.
	147	185	151	N.D.	N.D.	N.D.	(Died in 1 hr. 26 min. Heart (sample not taken.
	125	149	193	N.D.	N.D.	N.D.	(Died in 1 hr. 44 min.
	135	133	160	150	116	N.D.	(Rat still alive after 48 hrs.
	103	129	168	N.D.	N.D.	139	(Died in 2 hrs. 33 min. Last (sample from heart.
	117	124	153	N.D.	N.D.	110	(Last sample from heart to 2 hrs. (33 min.
	133	139	N.D.	135	N.D.	N.D.	
	103	---	---	---	---	126	(Last sample from heart at 70 min.

istered by stomach tube. Results are plotted in figure 6.

Table 24. Blood Sugar Changes in Unfasted Rats Following Feeding

Run No.	Wt. of rat (gms.)	Age of rat (days)	Sex	Fasting time (hours)	Dosage per kilo by stomach tube	Mg. % G. Start	15 min.	30 min.	
1	294	165	m	0	68 mgs. F	125	142	191	
2	337	168	m	0	"	115	120	145	
3	285	168	m	0	"	135	147	158	
4	311	170	m	0	"	127	139	157	
5	320	170	m	0	"	121	133	173	
6	339	173	m	0	"	123	146	185	
7	363	173	m	0	"	131	136	162	
8	289	160	m	0	1.25 gm.Glucose	129	167	191	
9	300	160	m	0	"	130	171	190	
10	301	160	m	0	Water alone	120	129	129	
11	313	160	m	0	1.25 gm.Glucose & 68 mg. F	128	174	249	
12	363	161	m	0	" Glucose & 68 mg. F	146	203	198	
13	349	150	m	0	" Glucose & 68 mg. F	123	133	170	
14	369	150	m	0	" Glucose & 68 mg. F	128	---	141	
15	399	160	m	0	" Glucose & 34 mg. F	105	159	188	
16	340	160	m	0	" Glucose & 34 mg. F	111	162	207	
17	307	160	m	0	" Glucose & 22.7 gm. F	127	169	169	
18	378	160	m	0	" Glucose & 22.7 gm. F	121	149	165	

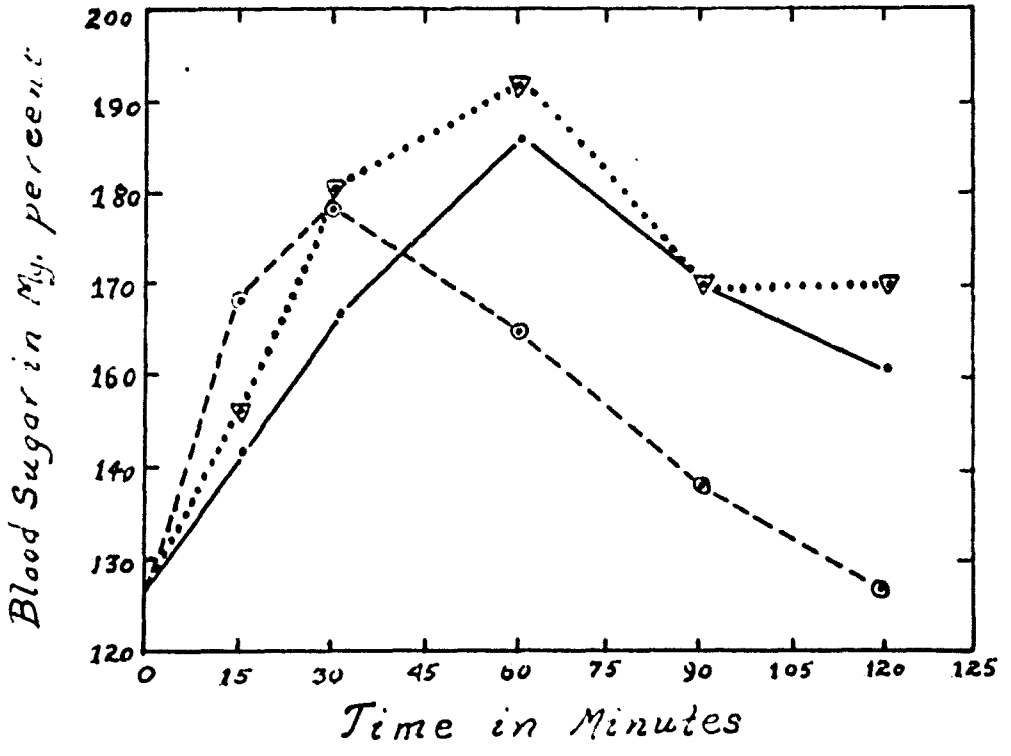
*Glucose, Glucose and fluoride, fluoride alone, and water were administ ered by administered at 1.25 gms. per kilo.

N.D. - No Determination

llowing Feeding of Fluoride, and Glucose and Fluoride.*

Mg. % Glucose in Blood						Observations and Notes
15 min.	30 min.	60 min.	90 min.	120 min.	at death	
142	191	224	209	210	N.D.	Dead 14 hours later
120	145	145	170	195	N.D.	Died
147	158	N.D.	196	N.D.	N.D.	Died at 120 minutes
139	157	196	203	110	N.D.	0.25 units given prior to 90 min. sample.
133	173	212	179	Died next day		0.16 units insulin given just before 60 min. sample.
146	185	247	294	N.D.	N.D.	Last sample from heart.
136	162	190	148	166	166	0.16 units insulin after 40 min.
167	191	150	142	138	N.D.	
171	190	148	135	135		
129	129	119	134	121	N.D.	This run made to determine influence of manipulative procedure
174	249	267	306	323	N.D.	Animal did not die.
203	198	233	214	300	N.D.	Animal did not die.
133	170	236	273	281	N.D.	Found dead 8 hrs. later
---	141	176	181	237	N.D.	Found dead 8 hrs. after last sample
159	188	165	195	205	N.D.	1 wk. later alive & apparently normal.
162	207	175	222	N.D.	N.D.	1 wk. later animal alive and normal
169	169	167	182	N.D.	N.D.	1 wk. later animal alive and normal
149	165	153	160	157	N.D.	1 wk. later animal alive and normal

administ ered by stomach tube. Glucose



1--⊙-- , Normal glucose tolerance - mean curve 3 animals.

2. ··▽·· , Fluoride and Glucose - mean curve - 5 animals.

3. —·— , Fluoride Alone — mean curve 3 animals.

Figure 6. Blood-sugar changes in fasted rats when fed NaF, NaF + glucose, and glucose alone by stomach tube.

changes taking place in unfasted rats when varying amounts of fluoride were administered with a constant level of glucose. When 68 mgs. of fluorine per kilo were administered with glucose the blood sugar concentration was found to rise continuously during the two hour period, reaching a level at two hours of 267 mgs. per cent. When the level of fluorine was reduced to 34 mgs. of fluorine per kilogram the blood sugar rose somewhat more rapidly during the first 30 minutes then fell temporarily to 179 mgs. per cent after which it rose again to 208 mgs. It was still above 200 at the end of two hours. When the fluoride dosage was reduced to 22.7 mgs. of fluorine per kilogram the blood sugar level rose less abruptly, reaching 157 mgs. per cent at 30 minutes and remaining between 148 and 160 mgs. per cent during the remainder of the two hour period. With the water control the blood sugar values were reasonably low with a low value of 113 and a high value of 127 mgs. per cent. The curve for the glucose control showed the expected rise and fell again nearly to the starting level. A comparison of these curves indicates that fluoride in quantities as low as 22.7 mgs. of fluorine per kilo, when administered in one dose, caused a prolonged hyperglycemia. This quantity of fluorine is nearly as large as the 30 mg. quantity of fluorine believed by Phillips, Lamb, Hart, and Bohstedt (78) to be capable of causing anorexia and inanition when administered daily in the ration in the form of NaF.

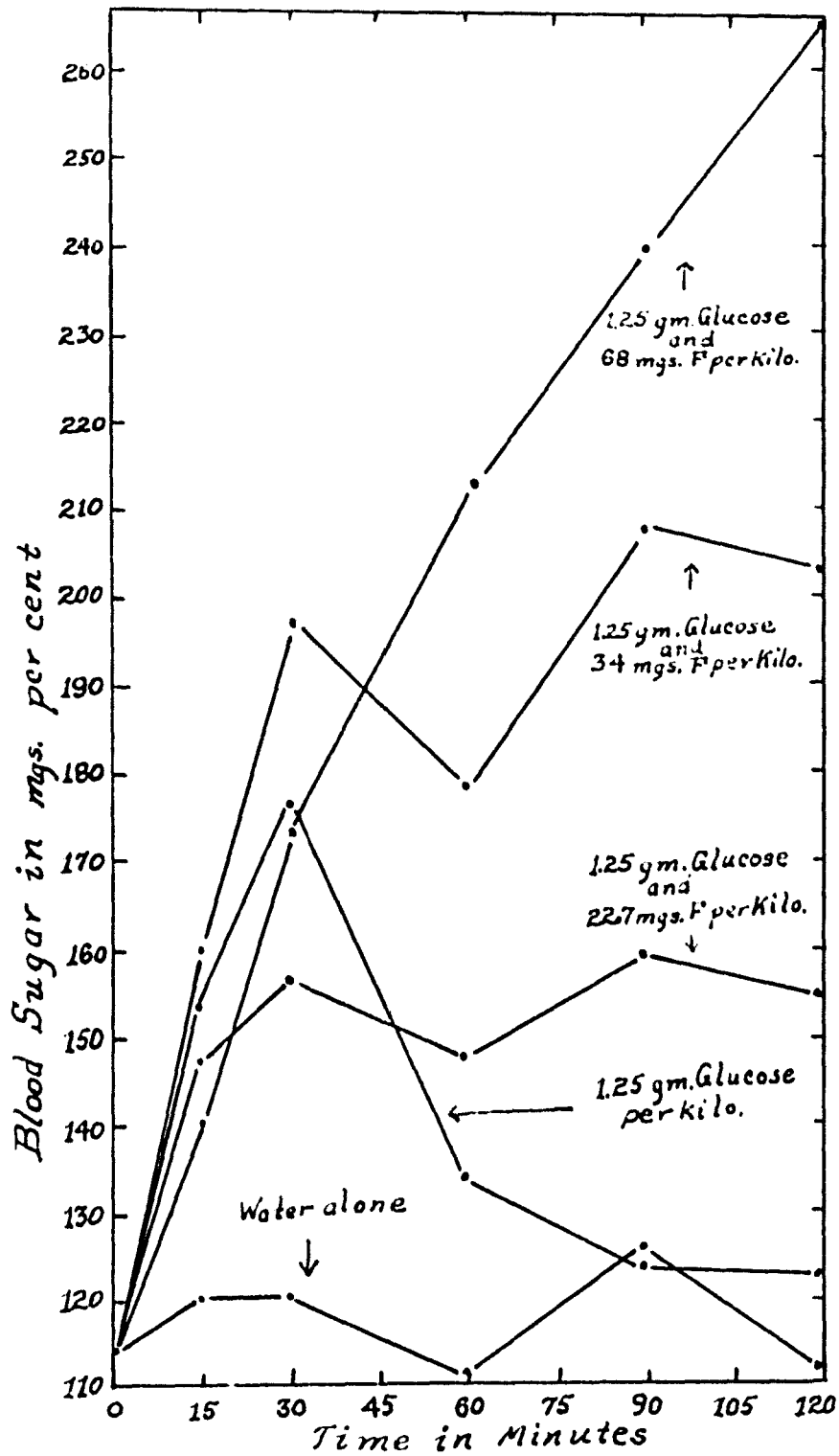


Figure 7. Blood sugar changes in unfasted animals following administration of glucose and sodium fluoride at different levels.

Some experiments were performed to study the nature of the influences that might be causing the hyperglycemia in the unfasted rats. The results are shown in figure 8. A series of determinations was made using unfasted male rats. One group of animals received 68 mgs. of fluorine and 1.25 gms. of glucose per kilogram of body weight. A second group received NaF alone at the level of 68 mgs. of fluorine per kilogram of body weight. When it became apparent that NaF alone was causing marked hyperglycemia, three more animals, treated with NaF at the same level, were injected subcutaneously with insulin after the rise in blood sugar had begun. At the time of injection of insulin the evidence of systemic fluorine poisoning was present with occasional light tremors occurring in the musculature of the back and limbs of the animals. The insulin was injected in order to learn whether or not this hormone would oppose the hyperglycemia caused by NaF. That the hyperglycemia was counteracted by insulin was shown by the distinct decreases in blood sugar concentration following its injection.

A moderate but persistent hyperglycemia was observed in trials with doses of fluorine as low as 22.7 mgs. per kilo of body weight when administered in a single dose. Quantities of fluorine only slightly less than 22.7 mgs. per kilogram are often ingested daily by rats receiving 0.10 per cent sodium fluoride in their diet. It seemed possible, therefore, that the inanition so commonly observed at that level of

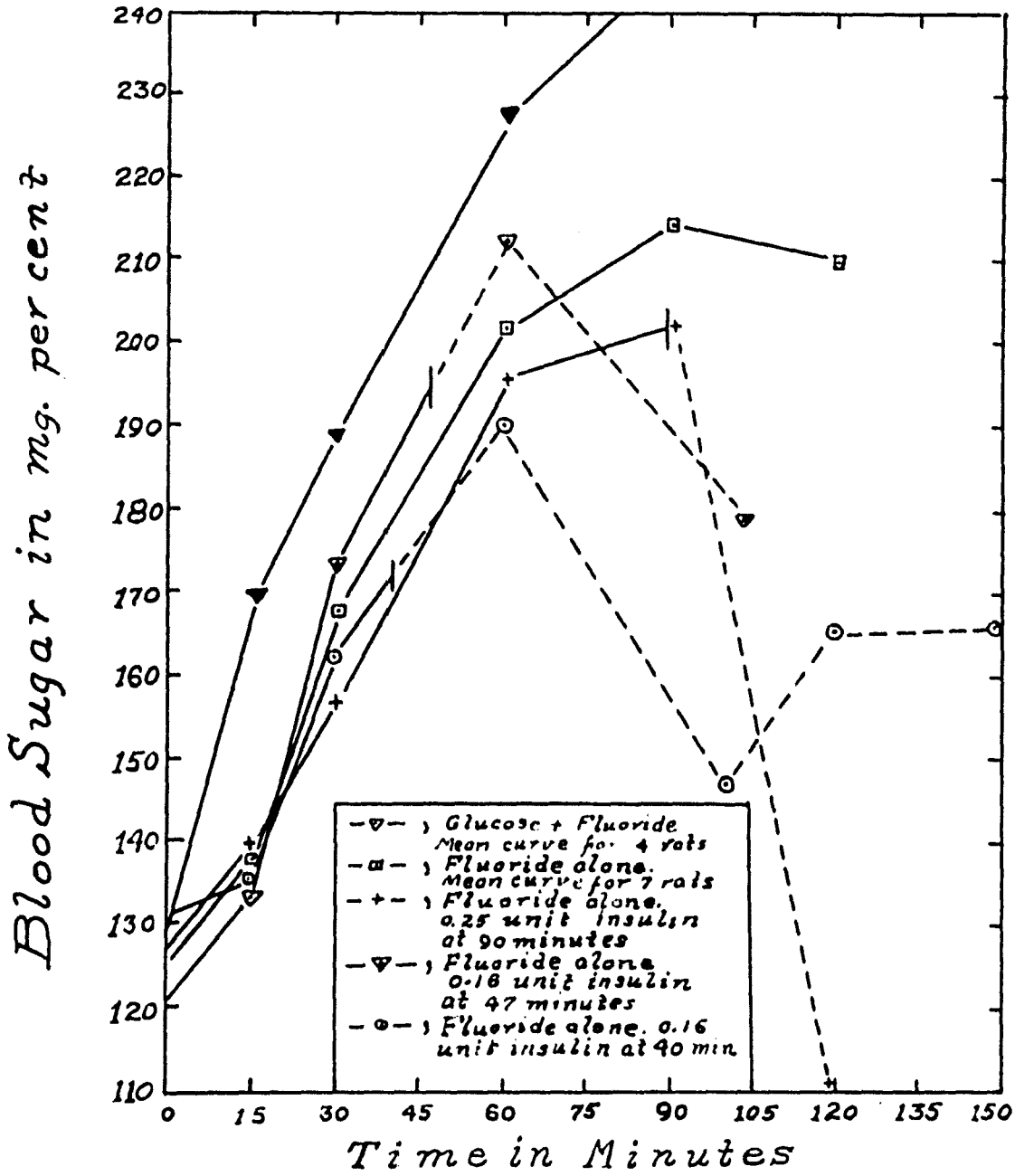


Figure 8. The response of fluoride poisoned rats to insulin.

feeding might be due to impaired ability on the part of the animals to maintain normal stores of carbohydrate. It also appeared that impaired storage might be indicated by a comparison of the blood sugar levels of normal and fluoride fed animals after a rather long period of fasting.

An experiment to compare the fasting levels of blood sugar in normal and fluoride fed rats was therefore made. Rats five months old, that had for four months been on the stock ration containing 0.05 and 0.10 per cent added sodium fluoride were compared with normal animals of the same age on the stock ration without added fluoride. All animals were kept well supplied with their usual ration and water for 48 hours, after which all access to food was prevented for a period of 36 hours. At the close of this fasting period the blood sugar levels were determined in the usual manner. The results obtained were as follows:

Table 25. Blood Sugar Levels in Normal and Fluoride-Fed Rats Following a 36 Hour Fast.

Group	Supplement	Fasting Blood Sugar Levels in mgs. Per Cent				Mean Values
1	none	99	97	97	89	95
2	0.05% NaF	101	86	86	57	82
3	0.10% NaF	71	72	60	56	65

The animals that had been on growing ration alone had fasting blood-sugar levels which may be considered to be within the normal range. The levels found in the 0.05 per cent sodium fluoride group were quite uneven. One value was normal (101), two rather low (86 and 87), while the fourth was 57 mgs. per cent. The values obtained by the animals on 0.10 per cent sodium fluoride were uniformly low, ranging between 56 and 72 mgs. per cent.

The above results appeared to suggest that resistance to decreased blood sugar values following a 36 hour fasting period is decidedly less in animals receiving 0.10 per cent sodium fluoride as compared with animals receiving the growing ration alone. That this decreased fasting level might conceivably be due to an effect of fluoride upon carbohydrate storage is indicated by the studies of blood sugar changes following the administration of sodium fluoride by stomach tube.

It is known that the food consumption is decreased at levels of 0.05 per cent NaF or higher. An indication that this decreased food consumption is the result of impairment of metabolism and appetite was provided by Sollman, Schettler and Wetzel (104) and by Smith and Leverton (102). The former workers found that rats did not distinguish between poisoned and unpoisoned food until a level of 0.23 per cent sodium fluoride was reached, while the latter workers found that the

decreased growth rate of rats receiving 0.05 per cent or more of sodium fluoride in the ration did not result entirely from decreased consumption of food but resulted from a decreased efficiency in the utilization of the feed consumed. Decreased capacity or ability to store carbohydrate, and, as a result, to mobilize carbohydrate from body stores during fast, would appear to be a possible cause for the observations of the above workers.

Some Effects of Organic Fluorides upon the Rat.

The studies on organic fluorides to be described were for the purpose of investigating the possibility that organic fluorine compounds, with fluoride substituted for hydrogen on the benzene ring, might, upon gaining access to the body, bring about fluorosis similar to that induced by inorganic fluorides.

Procedure.

The solid organic compounds were finely pulverized by grinding and mixed in this state with the regular growing ration. The compounds in the liquid state were added by means of a pipette to a weighed quantity of the ration and the moistened portions thoroughly mixed with the unmoistened portion of the feed. The rations containing the solid compounds were mixed in quantities of one kilo, whereas those

containing the more volatile liquids were mixed in quantities that would be sufficient for a two day period.

Of the various liquid fluorine compounds used, fluorobenzene was the most volatile; but feed containing a sufficient quantity to provide 0.10 per cent fluorine at the time of mixing still possessed a distinct odor of the compound after standing four days in the feed cup. There was a detectible decrease in the intensity of the odor in three days. With the other liquid substances, α-fluoronaphthalene, p-fluorobromobenzene, and p-fluoroiodobenzene, no noticeable decrease in odor occurred within two days after mixing.

The animals used in these experiments were young rats of weaning age, taken from the stock colony and weighing between 45 and 55 grams when placed on experiment.

Effects upon growth.

The growth records are shown in figures 9 and 10. In the first experiments p, p'-difluorodiphenyl, α-fluoronaphthalene, p-fluorobenzoic acid, and fluorobenzene were fed at a level sufficient to provide 0.10 per cent fluorine in the ration at the time of mixing. The growth of the animals on fluorobenzene, p-fluorobenzoic acid, and p, p'-difluorodiphenyl are shown in figure 9. The fluorobenzene caused no noticeable effect upon growth. Growth on fluorobenzoic acid was subnormal in rate and irregular. The di-phenyl derivative caused

such severe depression of growth that the feeding level was decreased to 0.05 per cent fluorine to prevent death. Growth on the latter level was still not good, showing marked irregularity over a period of six months. The growth of the animals fed α-fluoronaphthalene is shown in figure 10 by the sets of curves numbered 3 and 6. The set numbered 3 was obtained on 0.05 per cent fluorine as α-fluoronaphthalene. The set of curves numbered 6 was obtained on 0.10 per cent fluorine as α-fluoronaphthalene until the rats appeared to be near death, whereupon the level of this compound was decreased to 0.05 per cent fluorine. Growth was then resumed, but at a subnormal rate. Growth curves for naphthalene, fed at a level equivalent to the α-fluoronaphthalene required to furnish 0.05 per cent fluorine, are shown as set 2 in figure 10. These curves indicate that naphthalene slowed up growth only slightly for about three months, after which it caused loss of weight; it appeared that the effect of the fluorine derivative upon growth might have been in part the result of the naphthalene moiety.

Experiments on p-fluoroiodobenzene and p-fluorobromobenzene gave growths represented in curves 4 and 5 in figure 12.

Effects upon the incisors.

The effects of the organic fluorides upon the incisors

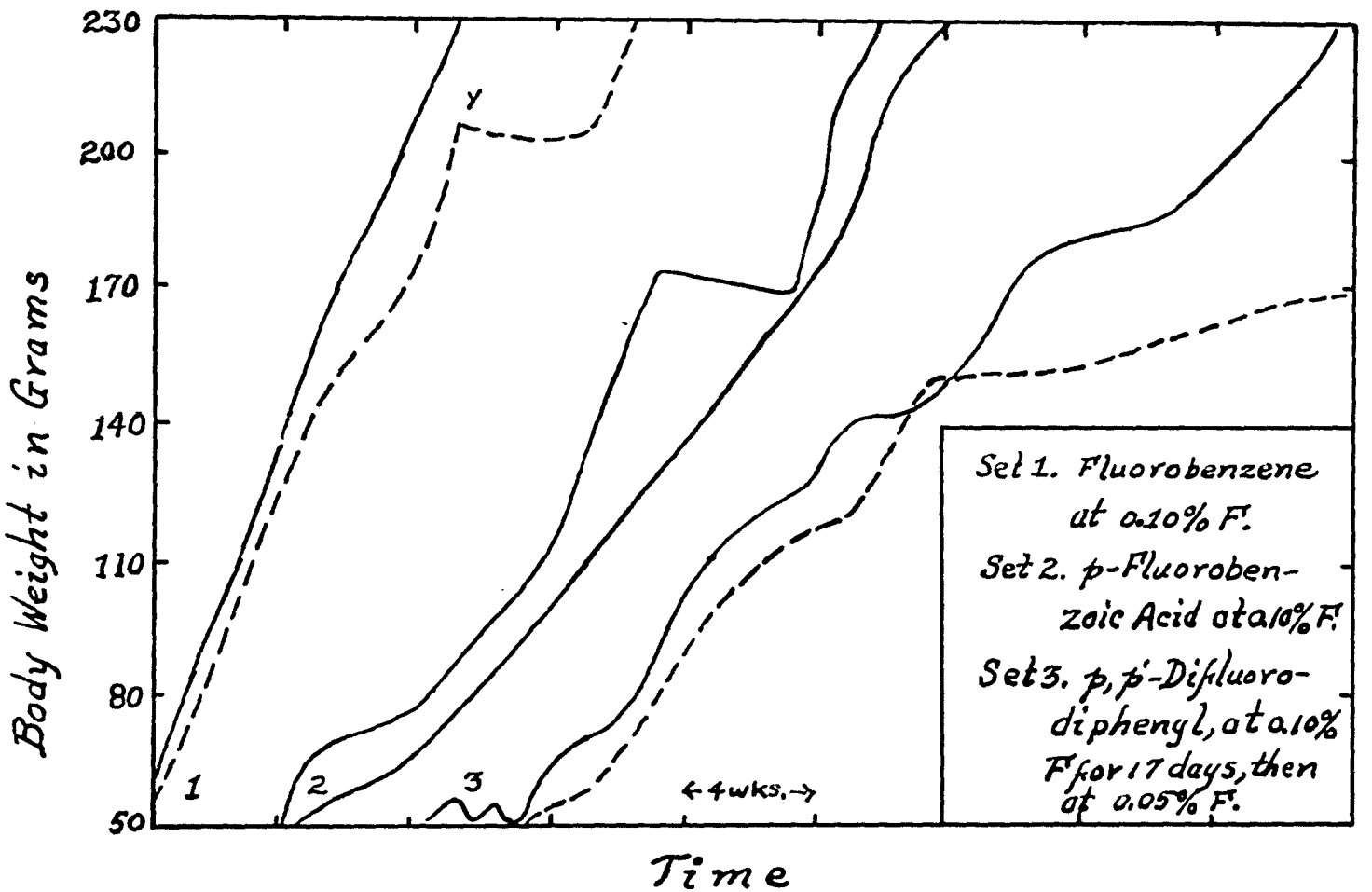


Figure 9. Growth of rats fed fluorobenzene, fluorobenzoic and p, p'-difluorodiphenyl.

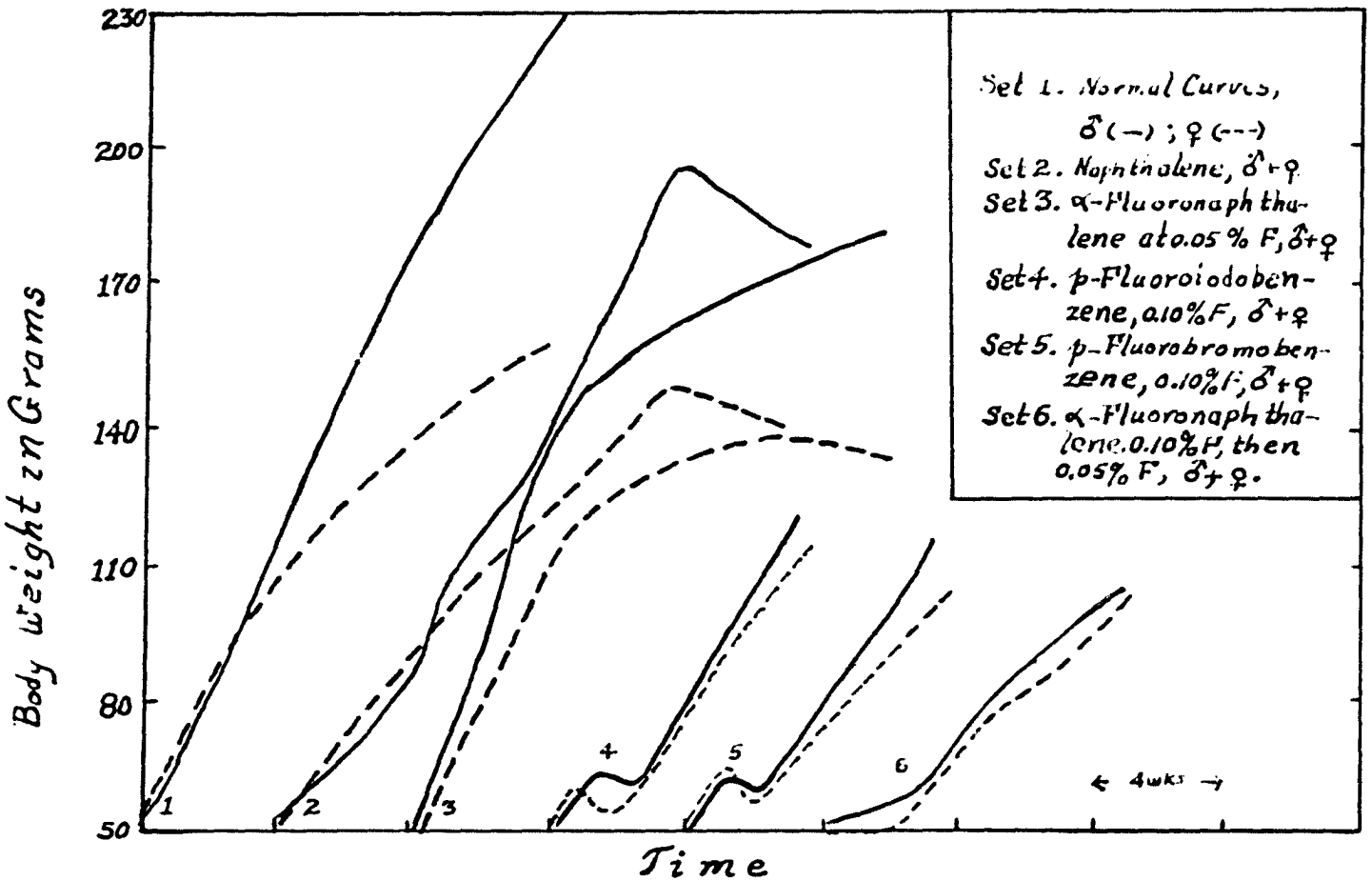


Figure 10. Growth of normal rats and of rats receiving naphthalene, α-fluoronaphthalene, p-fluoriodobenzene, and p-fluorobromobenzene.

are shown in table 26. Both the normal and fluoride animals were examined regularly for incisor changes. The pigmentation of the fluoride fed animals was compared with that of the normals, striations being sought by means of an ordinary hand lens. Alterations in the shapes of the incisors were also noted.

Of the organic fluorides fed in these experiments, only three showed definitely the tendency to cause bleaching and striation. These were the naphthalene derivative, p-fluoroiodobenzene and p-fluorobromobenzene.

Photographs of the incisors of these animals are shown in Plates 1 and 2. In Plate 1 are shown photographs of the normal incisors and the striated incisors of an animal that had received the α-fluoronaphthalene for 49 days. In Plate 2 are shown the incisors of a rat that had been fed the α-fluoronaphthalene ration for seven weeks and then was returned to the growing ration for three weeks. Typical lengthening of the incisors had taken place by this time. In this same plate are the photographs of the incisors of two other rats, one of which had received p-fluoroiodobenzene, whereas the other had received p-fluorobromobenzene. The rats on p-fluoroiodobenzene showed typical fluoride effects upon the incisors. The influence of the p-fluorobromobenzene was much less marked than that of either the p-fluoroiodobenzene or the α-fluoronaphthalene. When the rats that had been on the last two

Table 26. Incisor Effects Due to Different Organic Fluorides.

Compound fed	Per cent fluorine in diet at mixing	Appearance of incisors
α-fluoro-naphthalene	0.01 *	Died at 20 days without visible changes.
"	0.05	Bleaching unmistakable in lower incisors in 3 weeks. At 25 days the upper incisors were beginning to lengthen.
"	0.01 for 14 days then 0.05	Loss of pigment in 10 days. After this time striations and bleaching became more noticeable. Gross changes in shape developed. (See photograph)
Naphthalene	equivalent to fluoronaphthalene at 0.05% fluorine	No deviations from the normal were noted.
p-fluorobenzoic acid	0.10	Condition of the incisors appeared questionable by the 36th day. No definite abnormalities were visible throughout the remainder of the feeding period.
p-p'-difluoro-diphenyl	0.10 for 17 days 0.05 thereafter	No definite abnormalities were observed during the feeding period of 5 months and 9 days.
fluorobenzene	0.10 *	The teeth appeared normal throughout the feeding period of 6 months and 9 days.
p-fluoroiodo-benzene	0.10 *	Three out of four animals showed faint striations in 10 days. The striations became quite noticeable in all animals in 47 days when they were placed on growing ration. The condition became more marked for a period of several weeks, then improvement took place.

"	0.05	Bleaching unmistakable in lower incisors in 3 weeks. At 25 days the upper incisors were beginning to lengthen.
"	0.01 for 14 days then 0.05	Loss of pigment in 10 days. After this time striations and bleaching became more noticeable. Gross changes in shape developed. (See photograph)
Naphthalene	equivalent to fluoronaphthalene at 0.05% fluorine	No deviations from the normal were noted.
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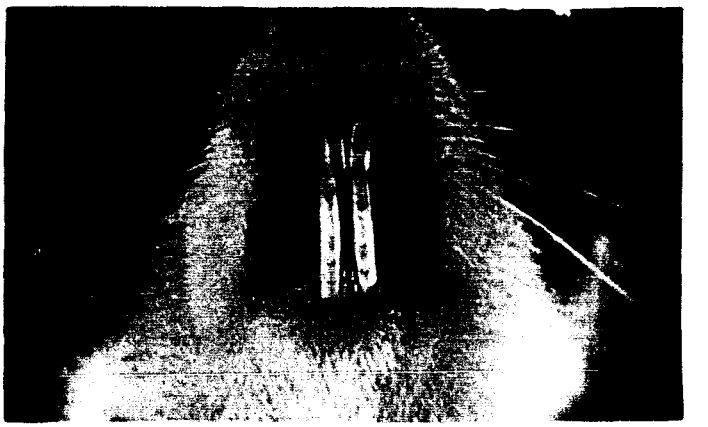
p-p'-difluorodiphenyl	0.10 for 17 days 0.05 thereafter	No definite abnormalities were observed during the feeding period of 5 months and 9 days.
fluorobenzene	0.10 *	The teeth appeared normal throughout the feeding period of 6 months and 9 days.
p-fluoroiodobenzene	0.10 *	Three out of four animals showed faint striations in 10 days. The striations became quite noticeable in all animals in 47 days when they were placed on growing ration. The condition became more marked for a period of several weeks, then improvement took place.
p-fluorobromobenzene	0.10	Loss of pigment occurred in upper and lower incisors in 10 days. Striations were noted in 4 weeks when the rats were changed to growing ration at 7 weeks the condition began promptly to improve.

* The volatility of these compounds being quite high the loss by evaporation from the feed cup must have been considerable. The feed was mixed every other day to minimize this loss.

Plate 1.

The Appearance of the Incisors of a Normal Rat and of the Incisors of a Rat Fed 0.05 per cent Fluorine as α-Fluoronaphthalene in the Basal Ration.



Above: The control animal receiving the basal ration. (49 days on experiment.)

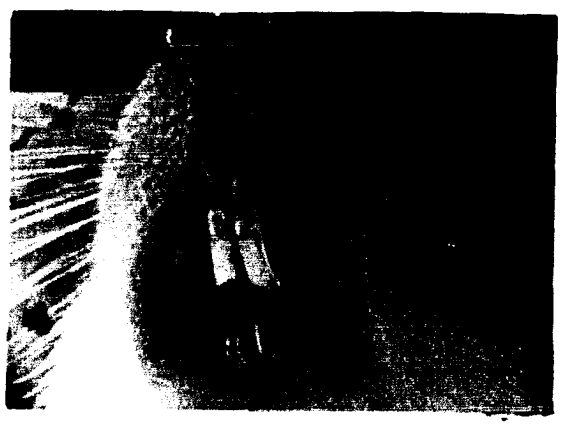
Below: Animal receiving 0.05 per cent fluorine as α-fluoronaphthalene. (49 days on experiment.)

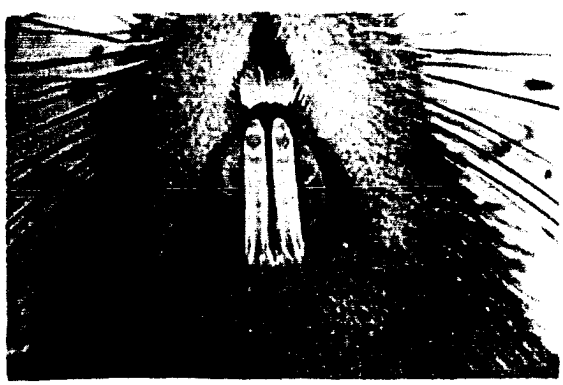
Plate 2.

The Effect of Organic Fluorides upon Teeth.

Left: The appearance of the incisors after 7 weeks feeding of α-fluoronaphthalene at a level of 0.05 per cent fluorine in the ration, followed by 3 weeks on growing ration.

Right: The appearance of the incisors after 7 weeks feeding of p-fluoroiodobenzene followed by 3 weeks on growing ration.





Left: The appearance of the incisors after 7 weeks on p-fluorobromobenzene at 0.10 per cent fluorine in ration, followed by 3 weeks on growing ration.

compounds for seven weeks were placed upon growing ration alone for three weeks, their incisors continued to get worse, whereas, under the same conditions, the incisors of the p-fluorobromobenzene rats showed definite improvement. This improvement is shown by the photograph in Plate 2 in which the upper half of the lower incisor shows mottling, while the lower half of the incisor shows little evidence of the effect of fluoride.

Discussion of results.

When it became evident that α-fluoronaphthalene, p-fluoroiodobenzene and p-fluorobromobenzene were causing changes in the incisors typical of inorganic fluorides it appeared that the effect of these compounds might possibly have been due to traces of water soluble inorganic fluorine compounds as impurities. However, when the compounds were extracted with water the titratable fluorine in the water extracts was very low. In making the extraction one ml. of the organic fluoride was placed in a small erlenmeyer flask of 25 ml. capacity along with 5 mls. of water. The flasks were then stoppered and shaken vigorously at frequent intervals for a period of 12 hours. One ml. aliquots of the water layer were measured out and titrated according to the micro-procedure. The calculated per cents of water extractible fluoride added to the ration in the organic fluorides were exceedingly small, amounting to

5.4×10^{-6} for the fluoronaphthalene, 6×10^{-7} for p-fluorobromobenzene, and none for the p-fluoroiodobenzene.

The lowest percentage of fluoride capable of producing visible changes in the incisors under magnification has been estimated by different investigators to be between 0.0014 and 0.0023 per cent fluorine as sodium fluoride.

A level of 0.0045 per cent fluorine as sodium fluoride has been found necessary to cause the least changes visible to the naked eye. This latter figure is 830 times as great as that accounted for by the fluorine obtained by water extraction of α-fluoronaphthalene. Since the influence of these compounds upon the teeth were easily visible to the naked eye, it appeared that the effects were definitely not due to water soluble impurities in the organic compound.

INVESTIGATION OF FLUORIDE REMOVAL FROM DRINKING WATER

Plan of Investigation

The plan of this part of the study on fluorine included: first, to investigate the use of alum in removing fluoride from the high fluoride water from the municipal water supply of the city of Ankeny, Iowa, by means of small scale laboratory experiments; and second, if removal was accomplished in the preliminary experiments, to investigate the applicability of the treatment to a continuous process, using a pilot plant attached to the water main.

Materials and Methods

Chemicals.

The commercial alum used in these experiments was commercial aluminum sulfate ordinarily used in water treatment. The potassium alum used in an early experiment was the C. P. product of Baker and Adamson. The aluminum sulfate and aluminum chloride used in some of the continuous treatment studies were the C. P. products of Baker and Adamson. The Bentonite used in one set of experiments was obtained from Utah.

The thorium nitrate used in the determination of fluorine was that of Baker and Adamson and was their reagent quality. The sulfuric acid used in the earlier distillations of fluorine and the perchloric acid used in later distillations were tested for fluorine by blank distillations and titrations. The sodium hydroxide and hydrochloric acid solutions were prepared from C. P. products and fluoride free distilled water. The sodium alizarin sulfonate, from which the indicator solution was made, was a product of E. Merck, Darmstadt, Germany. The freedom from all but the most minute traces of fluorine in all reagents used in the determination of fluorine was ascertained by running blank distillations and titrations.

Apparatus.

A student type potentiometer and a quinhydrone electrode were used in determining pH. The apparatus was checked frequently with a standard buffer solution to insure proper working order.

In the small scale experiments in the laboratory a five-liter baloon flask was used as a container for the water being treated. It was equipped with a mechanical stirrer of glass, powered by an electric motor.

The glass percolators into which the mixed treated water was transferred for settling were 46 cm. in depth and had an inside diameter of 10 cm. at the top.

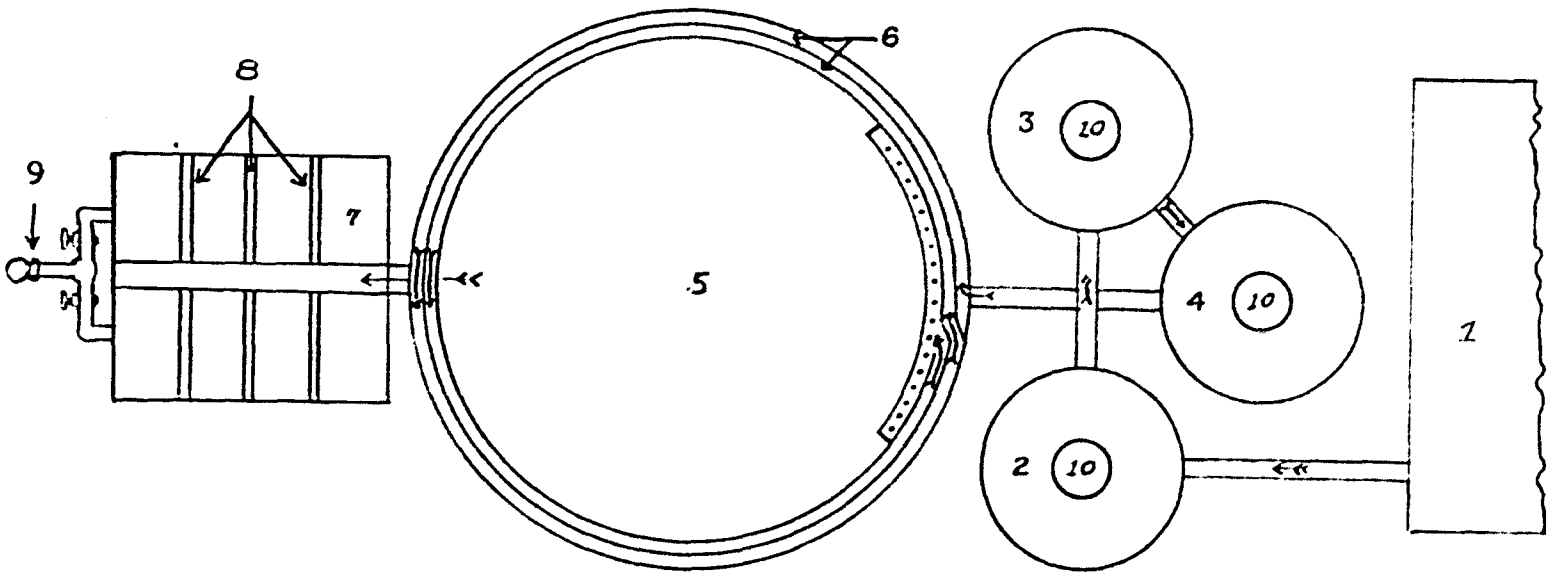
An analytical balance was used in weighing the materials used in the water treatment.

Pilot plant. The pilot plant used in the continuous treatment experiments was designed by Professor W. E. Galligan of the Department of Engineering at Iowa State College and was obtained and installed through the courtesy of Professor W. E. Galligan and the Department of Engineering.

The construction of the pilot plant is shown schematically in figure 11. It consisted of an aeration tank, three mixing tanks of a capacity of ten gallons each, a flat bottomed settling tank with a capacity of sixty gallons, and a sand and gravel filter constructed in two equal parts so that either one or both parts might be used as desired. The stirrers in the mixing tanks were of all-metal construction and were powered with belts from an electric motor.

The dosing apparatus was placed on a platform over the first and second mixing tanks. The container used to hold the alum solution was a battery jar of ten liters capacity. A siphon of glass tubing, floating on cork, was used to deliver the alum solution to the first mixing tank. When added separately from the alum solution, other solutions were introduced by means of an additional siphon from other containers.

The alum dosing solutions were prepared by dissolving the required amount of solid in 40 liters of water in a carboy and stirring by rotation of the carboy until solution and



1. Aeration Tank. 2. First Mixing Tank. 3. Second Mixing Tank. 4. Third Mixing Tank. 5. Settling Tank. 6. Trough to Settling Tank. 7. Sand-gravel Filter. 8. Spreading Troughs. 9. Outlet Valve 10. Pulleys for Mixing Tank Stirrers

Figure 11. The design of the continuous treatment pilot plant shown diagrammatically.

thorough mixing was accomplished. The quantity of alum needed in the dosing solution was dependent upon the rate of flow of the siphon. The float which was attached to the siphon was for this reason fixed so that it could be raised or lowered to give the desired volume of dosing solution per minute. The rate of dosage was checked frequently in all determinations. The rate of flow of the water entering the plant was carefully measured by use of a water meter and an ordinary watch. After the rate of flow of water was controlled, and the dosing siphon was ready and checked for rate of flow, the outlet valve to mixing tank number one was closed. At a rate of flow of approximately one gallon per minute, slightly less than two hours were required to fill the three mixing tanks, the settling tank and the filter. As the level of the water rose to the top of the filter a valve controlled by a float permitted water to flow through the filter.

Water.

The water used in these removal studies was Ankeny City water which contained approximately 8 parts per million of fluorine. The fluorine content of this water is somewhat higher than most of the other fluoride waters in the state of Iowa. It compared quite well in a number of respects with fluoride waters obtained in other parts of the United States.

The composition of the Ankeny City water in the spring

of the years 1933, 1934 and 1935 are shown in table 27. In table 28 are shown the amounts of some of the constituents reported in other fluoride containing waters of North America.

The high fluoride appears to be accompanied quite often by considerable quantities of sulfates and total solids.

Table 27. The Composition of Ankeny City Water.

Constituent	1933	1934	1935
	p.p.m.	p.p.m.	p.p.m.
Total solids	1802.6	1867.2	1856.0
Volatile matter	170.0	183.0	---
SiO_2	19.0	8.33	10.0
R_2O_3	44.5	4.0	7.0
Ca	26.3	26.3	24.0
Mg	4.5	11.5	13.0
Na	550.0	766.0	546.0
SO_4	750.0	880.0	832.0
Cl	40.0	46.3	42.0
Total Alkalinity	420.0	333.0	309.0
Fe	1.0	0.43	0.84
Total hardness	84.0	105.0	113.0

Table 28. The Fluorine Contents and the Total Solid, Sulfate and Chloride Contents of Some Fluorine-Containing Waters.

Observer	Location	Fluorine p.p.m.	Solids p.p.m.	Sulfates p.p.m.	Chlorides p.p.m.
Smith & Smith (97)	Arizona	3.5	1988	350	670
"	"	7.5	2016	500	516
"	"	3.6	3056	700	860
"	"	7.3	1528	595	314
"	"	7.2	1304	500	278
"	"	6.0	696	200	170
"	"	12.0	836	200	290
"	"	5.5	312	75	64
"	"	5.0	4284	800	1540
"	"	5.6	--	550	1200
Walker, Finlay & Harris (109)	Alberta	4.4	1128	342	65
Author	Ankeny, Iowa	8.0	1856	832	42

Determination of fluoride in water.

For these determinations of fluorine the method was essentially that of Willard and Winter as adapted to water analysis by Boruff and Abbott. A 200 ml. quantity of water was measured into a 250 ml. distilling flask, made alkaline with NaOH and concentrated by evaporation to 50 ml. Glass beads were employed to prevent bumping during the concentration. Following this, 50 ml. of 1:1 sulfuric acid were added and the distillation

was carried out--keeping the boiling point during the distillation at 135 ± 5° C. during the entire distillation by adding fluorine free distilled water continually through a dropping funnel attached to a tube which reached below the surface of the boiling mixture. After collecting 200 ml. of distillate it was concentrated by evaporation to a volume of 20 ml. The sodium alizarin sulfonate was then added and 0.1 N HCl was added until the solution was nearly neutral to the alizarin. An equal volume of 95 per cent ethyl alcohol was then added after which 0.01 N HCl was used cautiously to discharge the pink color of the indicator. Two drops of the dilute acid were then added in excess. The solution was then titrated with 0.02 N thorium nitrate to the appearance of a pink color in the solution.

Procedure and Results

Experiments with fluoride removal using potassium alum and aluminum sulfate in the laboratory.

The first experiments were carried out in order to determine whether or not potassium alum, $K_2Al_2(SO_4)_4.24H_2O$, would remove appreciable amounts of fluorine without first adjusting the pH of the water to be treated. A five liter quantity of water was treated with three grams of potassium alum. The mixture was stirred for thirty minutes, after which a percolator was

filled from the contents of the mixing vessel. Analyses of the water for fluoride content were made after the floc had settled. The fluoride content of the water was found to be 2.4 parts per million. When the treatment was repeated with one and one-half and with six gram quantities of the alum, the fluoride contents of the treated waters were found to be 5.98 and 1.5 parts per million, respectively.

The potassium alum was thus found to remove fluoride from the water, but, under the conditions of the experiments, the removal of the element was not proportional directly to the size of the alum dosage. It appeared possible that the variations in efficiency were the result of changes in pH brought about by the acid liberated in the hydrolysis of the alum.

Because of the lower cost of commercial alum it seemed advisable to employ it in experiments to learn whether or not the efficiency of removal would differ when the initial pH was rendered more acid.

Experiments were performed to compare the extent of removal of fluorine employing two different pH values of the water to be treated. The experiments were performed in two series. In one series the initial pH was 7.88. In the other series the water was first adjusted to a pH of 6.95 with HCl. In each of these series three different dosages of alum were employed. The results are summarized in table 29.

Table 29. Fluoride Removal from Ankeny City Water at Two pH Values, Using Aluminum Sulfate.

Dosage in grains/gal of $Al_2(SO_4)_3 \cdot 18\ H_2O$	Original fluorine p.p.m.	Fluorine of treated water after 2.5 hrs. p.p.m.		Fluorine removed p.p.m.	
		pH-7.88	pH-6.95	pH-7.88	pH-6.95
38.7	8.5 0.2	1.19	0.45	7.31	8.05
19.35	8.5 0.2	3.75	3.25	4.75	5.25
11.47	8.5 0.2	6.50	5.85	2.00	2.65

Table 30. Change of Fluoride Content of Water with Length of Time Standing in Contact with the Settling Floc from 38.7 gr./gal. Alum

Time from beginning treatment (hrs.)	Fluorine in water (p.p.m.)	pH of water	Fluorine removed (p.p.m.)	Fluorine removed (per cent)
0	7.5	7.15	---	---
2	1.25	---	6.25	83.3
6	0.85	---	6.65	88.7
12	0.65	---	6.85	91.3
24	0.40	6.78	7.10	94.7

Fluoride removal was better in the experiments in which the water was adjusted to a pH of 6.95 before adding the alum. The total amounts of fluoride removed varied markedly with the size of the dosage of alum, but at neither pH was there a linear relationship between the amounts of fluoride removed and the size of the alum dosage. The ratio of alum used to fluoride removed increased in both series as the dosage of alum was increased.

The influence of the length of standing after treatment was studied. A four liter quantity of water which had an original pH of 8.4 was placed in a baloon flask and the pH adjusted to 7.15 with HCl. Sufficient alum to give a dosage of 38.7 grains per gallon was added. After a 30 minute stirring period the treated water was transferred to the percolator where settling of the floc began promptly. Samples of water for fluorine determinations were siphoned off, filtered through paper and analysed as before. Analyses were made at 2, 6, 12 and 24 hours after the addition of the alum. The results of these experiments are summarized in table 30.

At first there was a rapid decrease in the fluoride content of the treated water; but, after the 2 hour period, further decrease in the fluoride content of the water took place more and more slowly. Eighty per cent of the fluorine had been removed by the end of the 2 hour period but an additional 22 hours were required to remove an additional 11

per cent of the fluoride originally present. After the sixth hour the concentration of fluorine in the water was a logarithmic function of the length of time in contact with the alum floc. The logarithmic period began at approximately the time when the heavy floc had settled out.

Continuous treatments using the pilot plant.

In order to study the problem of removal of fluorine from water on a larger scale a pilot plant was attached to the mains in one of the schools in the city of Ankeny, Iowa. The plant consisted of three mixing tanks, a settling basin and a sand and gravel filter such as is usually used in filtering water supplies. The flow of water into the plant was controlled by an adjustable valve and a regular water meter. The alum was added to the mixing tank in solution by means of a floating siphon delivering at a constant rate. Motor driven stirrers were used in each of the mixing tanks. The velocity of stirring was decreased going from tank one to tank three. The rate of stirring is recorded in terms of the velocity in feet per second of a point two-thirds out on the radius. The initial appearance of the samples of treated water as well as their appearance after 12 hours was recorded. The data from the first five experiments are shown in table 31.

The results from these experiments showed that there was decided removal of fluorine by the continuous treatment with

Table 31. The Effect of Addition of A

Date	Sample	Rate of flow in gal.per min.	Dosage of $Al_2(SO_4)_3$ in grains per gal. of water	pH before	pH after	Rat in 2/3
8/27/35	2 hr.	1	20	8	6.20	2.4
	3 "	1	20	8	6.07	"
	4 "	1	20	8	6.17	"
	5 "	1	20	8	6.32	"
9/14/35	3 "	0.5	20	8	6.20	"
	4 "	0.5	20	8	6.27	"
	5 "	0.5	20	8	6.31	"
	6 "	0.5	20	8	6.36	"
10/12/35	6 "	0.25	20	8	7.32	"
	7 "	0.25	20	8	7.25	"
	7 " 30 min.	0.25	20	8	7.35	"
	7 " 35 "	0.25	20	8	7.25	"
	8 "	0.25	20	8	7.30	"
10/26/35	3 " 13 "	1	20	8	7.40	"
	4 " 13 "	1	20	8	7.35	"
	5 " 13 "	1	20	8	7.35	"
	6 " 30 "	1	20	8	7.40	"
	6 " 13 "	1	20	8	7.40	"
	3 " 13 "	1	20	8	7.40	"
11/30/35	2 " 24 "	1	20	8	7.20	0.8
	3 " 11 "	1	20	8	7.30	"
	3 " 14 "	1	20	8	7.35	"
	3 " 16 "	1	20	8	7.35	"
untreated) Ankeny) City water)				Normal pH 8		

(1) Sediment shaken - analysis on suspension
(2) Sediment not shaken - analysis on supernatant liquid

Addition of Alum to Ankeny City Water

pH after	Rate of stirring in feet per second 2/3 out on radius			Fluorine in p.p.m.		Appearance of treated water	
						initial	after 12 hours
6.20	2.4	1.2	0.6	2.06	(shaken)(1)	cloudy	slight sediment
6.07	"	"	"	2.73	"	"	noticeable "
6.17	"	"	"	2.73	"	"	" "
6.32	"	"	"	2.94	"	"	" "
6.20	"	"	"	2.26	"	clear	slight "
6.27	"	"	"	2.57	"	"	" "
6.31	"	"	"	2.68	"	"	" "
6.36	"	"	"	2.38	"	"	" "
7.32	"	"	"	2.02	"	"	" "
7.25	"	"	"	2.05	"	"	" "
7.35	"	"	"	2.25	"	"	" "
7.25	"	"	"	2.47	"	"	" "
7.30	"	"	"	2.19	"	"	" "
7.40	"	"	"	1.88	(not shaken)(2)	cloudy	much "
7.35	"	"	"	1.88	" "	"	" "
7.35	"	"	"	1.84	" "	"	" "
7.40	"	"	"	1.99	" "	"	" "
7.40	"	"	"	1.93	" "	"	" "
7.40	"	"	"	3.68	(shaken)(1)	"	" "
7.20	0.85	0.425	0.213	1.65	(not shaken)(2)	"	" "
7.30	"	"	"	1.50	" "	"	" "
7.35	"	"	"	1.50	" "	"	" "
7.35	"	"	"	1.60	" "	"	" "

pH 8 — 7 to 10 p.p.m.

alum. Values as low as 2.02 and 2.05 p.p.m. of fluorine were obtained for the clear appearing effluents while some of the cloudy effluents gave, after 12 hours settling, values as low as 1.5 p.p.m. of fluorine. When the sediment was resuspended before analysis the fluorine content was found to be more than twice as high. It appeared that removal to less than one part per million might be accomplished if a sediment free effluent could be obtained. With this in mind, bentonite, a naturally occurring, clay-like material, was used in a series of experiments. Bentonite, when added to water in the pulverized state, forms a heavy floc which settles rapidly. It appeared possible that a bentonite floc in combination with the alum floc might help to give a water more nearly free from traces of the fluorine. Five experiments were conducted on the laboratory scale to test out this possibility. The water used in these experiments was shown by analysis to contain 7 parts per million of fluoride. In the first experiment a four liter quantity of water was treated in a 5 liter baloon flask with 2.648 grams or 38.7 grains per gallon of commercial alum and the same weight of pulverized bentonite without adjusting the pH. Stirring was continued for thirty minutes after which the treated water was transferred to a percolator. Two hours after the beginning of the treatment a sample was taken for analysis. In the second experiment with the bentonite, the alum was added and stirred for twenty-five minutes at which time the bentonite was added and the stirring continued for five minutes before

transferring to the percolator to settle. Other details were as in the previous experiment. In the third experiment only bentonite was added. In the fourth, bentonite was added and stirred for thirty minutes, after which alum was added and the stirring continued for two minutes. The fifth experiment differed from the first one only in that the water was first adjusted to a pH of 7 before starting the flocculation. Table 32 shows the results obtained in this series of experiments. The removal of fluoride in these experiments in the laboratory were so poor that no attempt was made to use bentonite in continuous treatment experiments. The bentonite appeared decidedly to inhibit the removal of fluoride by alum.

Since the levels of fluoride in the effluents obtained in the first five experiments were encouragingly near the accepted level for safety to the teeth, it was thought advisable to perform additional continuous treatment experiments employing C. P. aluminum sulfate and aluminum chloride in addition to commercial alum. It was planned to study the changes in the efficiency of removal when different conditions of pH were caused by adding varying quantities of acid and base in the process of the treatment. The results obtained from these runs are shown in table 33.

In table 33 the experiments are classified under the three kinds of aluminum salts used. The various experiments classified under the different aluminum salts are shown in the order of the increasing pH values of the samples of effluent

Table 32. The Influence of Bentonite upon the Removal of Fluorides by Alum Treatment.

	Treatment	Fluoride Content of water p.p.m.	Appearance of water
	None	7.0	clear
1.	38.7 grains/gallon of Alum and of Bentonite, simultaneously.	4.9	cloudy, some sediment on standing
2.	38.7 grains/gallon of Alum, 25 min. stirring. 38.7 grains/gallon Bentonite, 5 min. stirring.	5.6	cloudy
3.	38.7 grains/gallon of Bentonite, 30 min. stirring	6.3	cloudy
4.	38.7 grains/gallon of Bentonite, 30 min. stirring. 38.7 grains per gallon Alum, 2 min. stirring.	6.9	cloudy
5.	Same as 1 but with initial pH adjusted to 7.	6.6	clear

taken during the course of each experiment. The chemical or chemicals used to modify pH are indicated in a separate column. Under the column showing fluoride content, the values numbered 1 represent the total fluoride content of the effluent (sediment resuspended by shaking) whereas the values under 2 represent the fluoride content of the effluent after settling had taken place.

Table 33. Relation of pH of Effluent to Floc Formation, Filtration, and Fluorine Content of the Effluent.

Alum Compound Used in Treatment	pH of Effluent Water	Grains per Gallon	Acid or Base Added	F Content p.p.m.	Appearance of Sample as collected	Appearance of Sample 12 hours settling	Appearance of Floc
Commercial Alum	6.05 +0.05 -0.03	20 grains	0.0213gm NaOH per gallon	1. 4.27 2. 1.01	moderate cloudy	moderate sediment	Fine, slow settling
"	6.13 +0.19 -0.06	"	0.08ml conc. HCL	1. 2.61 2. ---	"	"	"
"	6.29 +0.06 -0.06	"	"	1. 2.47 2. ---	nearly clear	slight sediment	moderately fine, slow settling
"	6.35 +0.15 -0.10	"	"	1. 1.80 2. 1.47	quite cloudy	much sediment	moderately fine, moderate settling rate
"	6.64 +0.08 -0.06	"	"	1. 2.07 2. 1.73	quite cloudy	much sediment	"
"	6.95 +0.08 -0.06	"	"	1. 1.74 2. 0.92	slightly cloudy	slight sediment	quite heavy, rapid settling
"	7.00 +0.20 -0.14	10 grains	"	1. 4.03 2. 3.00	nearly clear	moderate sediment	quite coarse, rapid settling
"	7.29 +0.06 -0.04	20 grains	"	1. 2.19 2. ---	quite clear	little sediment	quite heavy, coarse, rapid settling
"	7.30 +0.05 -0.10	"	"	1. 1.56 2. ---	quite cloudy	considerable sediment	quite heavy coarse

	-0.06						
"	6.95 +0.08 -0.06	"	"	1. 1.74 2. 0.92	slightly cloudy	slight sediment	quite heavy, rapid settling
"	7.00 +0.20 -0.14	10 grains	"	1. 4.03 2. 3.00	nearly clear	moderate sediment	quite coarse, rapid settling
"	7.29 +0.06 -0.04	20 grains	"	1. 2.19 2. ---	quite clear	little sediment	quite heavy, coarse, rapid settling
"	7.30 +0.05 -0.10	"	"	1. 1.56 2. ---	quite cloudy	considerable sediment	quite heavy coarse
"	7.37 +0.08 -0.13	15 grains	0.46gm NaOH per gallon	1. 0.61 2. ---	trace of cloudiness	trace of sediment	quite heavy
"	7.56 +0.10 -0.10	10 grains	Acid followed by base	1. 1.00 2. ---	no detectible cloudiness	trace of sediment	quite coarse and heavy
"	7.58 +0.20 -0.18	20 grains		1. 1.13 2. 0.95	trace of cloudiness	trace of sediment	moderately coarse
"	8.11 +0.06 -0.16	"	0.92gm NaOH per gallon	1. 4.95 2. 3.85	clear	slight sediment	moderately fine
C. P. Alum	4.57 +0.13 -0.12	40 grains	none	1. 7.8 2. 0.97	very cloudy	much sediment	failed to form
"	5.87 +0.13 -0.11	20 grains	0.08ml conc. HCL per gallon	1. 1.87 2. 1.07	moderately cloudy	moderate sediment	fine, but abundant
"	5.97 +0.06 -0.07	30 grains	0.063gm NaOH per gallon	1. 2.20 2. 1.62	quite cloudy	much sediment	fine, not abundant
"	6.16	"	0.042	1. 4.69	very	very	very fine

	+0.06 -0.16		NaOH per gallon	2. 3.85		sediment	fine
C. P. Alum	4.57 +0.13 -0.12	40 grains	none	1. 7.8 2. 0.97	very cloudy	much sediment	failed to form
"	5.87 +0.13 -0.11	20 grains	0.08ml conc. HCL per gallon	1. 1.87 2. 1.07	moderate-ly cloudy	moderate sediment	fine, but abundant
"	5.97 +0.06 -0.07	30 grains	0.063gm NaOH per gallon	1. 2.20 2. 1.62	quite cloudy	much sediment	fine, not abundant
"	6.16 +0.37 -0.30	"	0.042	1. 4.69 2. 1.08	very cloudy	very heavy	very fine texture, slow settling
"	7.34 +0.15 -0.21	20 grains	0.08ml conc. HCL per gallon	1. 1.50 2. 1.20	nearly clear	slight sediment	quite good, i.e. definite, rapidly settling
Aluminum Chloride	6.73 +0.42 -0.25	equiv-alent to Al in $Al_2(SO_4)_3$ at 20grs	0.92gm per gallon	1. 1.70 2. 1.20	nearly clear	slight sediment	very heavy
"	6.09 +0.13 -0.13	equiv-alent to Al in $Al_2(SO_4)_3$ at 18 grs	0.12gm per gallon	1. 8.00	very cloudy	very heavy sediment	very poor
"	8.13 +0.04 -0.07	equiv-alent to 20 grs.	0.92 NaOH per gallon	1. 4.49 2. 4.43	moderate-ly cloudy	moderate sediment	poor

The results obtained in the series of experiments using a constant dosage of commercial alum are shown graphically in figure 12. The solid line connects points on the graph which show the fluoride contents of the samples when the sediment was resuspended by shaking. The fluoride content is shown on the ordinate and the pH values are shown on the abscissa. The broken line is drawn to connect points representing the fluoride contents and pH values for the effluents from the separate experiments when the fluoride was determined in the settled effluent.

Effluents corresponding to pH values less than 6 almost without exception were markedly cloudy as collected and contained much sediment upon standing. The cause of the cloudiness appeared to be the result of pin-point floc formation and to a failure of the sand filter completely to remove such a floc. After these runs were completed a sharply decreased quantity of floc was noticed in the settling tank.

The effluent from the experiment at a pH of 8.11 was quite clear throughout the course of the experiment and gave very little sediment upon standing. The effluents corresponding to pH values between 6 and 8 pass through a minimum in total fluoride content. The effluents within the region of 2 parts per million of total fluoride content were noticeably less cloudy as received and gave much less sediment upon standing than did the more acid effluents.

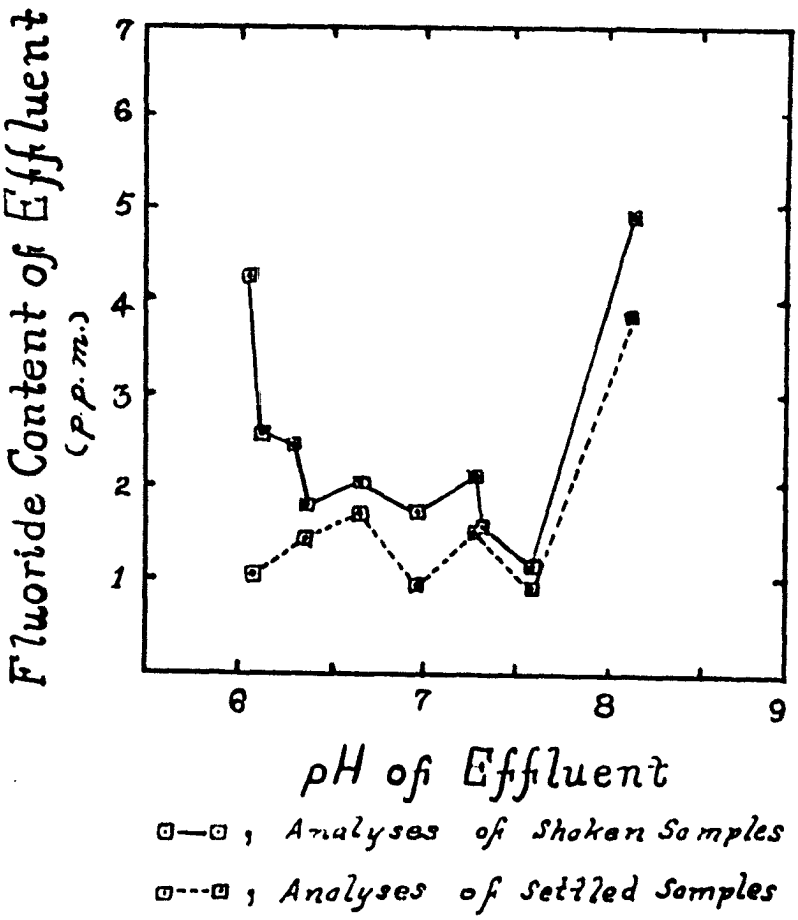


Figure 12. The variation of the fluoride contents of alum effluents with pH.

The values for fluoride contents of the settled samples were usually measurably lower than those of the same samples when shaken. This indicated that fluoride was quite generally present in any floc that settled out of the effluent. The quantity of fluoride in the floc was, however, usually well under one part per million when the pH of the effluent was greater than 6.3.

Figure 13 shows the results obtained with C. P. aluminum chloride and C. P. aluminum sulfate with the data plotted as in the preceding figure. The results shown appeared qualitatively to follow the same trends as the results with commercial alum. Again a measurable amount of fluoride appeared in the sediments, with nearly all of the fluoride present in the original water accounted for in the sediment when C. P. aluminum sulfate was used at the lowest pH value.

Figure 14 shows a pH-fluoride content curve for the sediment that settled out of samples collected from the sand filter in some of the experiments previously described. The values for the fluoride content of the sediment are obtained by difference between the fluoride content when shaken and after settling, and are expressed in parts per million. A rapid rise in the curve as the pH values fall below 6.4 coincides with the rise in the pH-fluoride content curve for shaken samples, shown as the solid line. A remarkably high content of fluoride in the sediment is obtained as the pH approaches 4.5

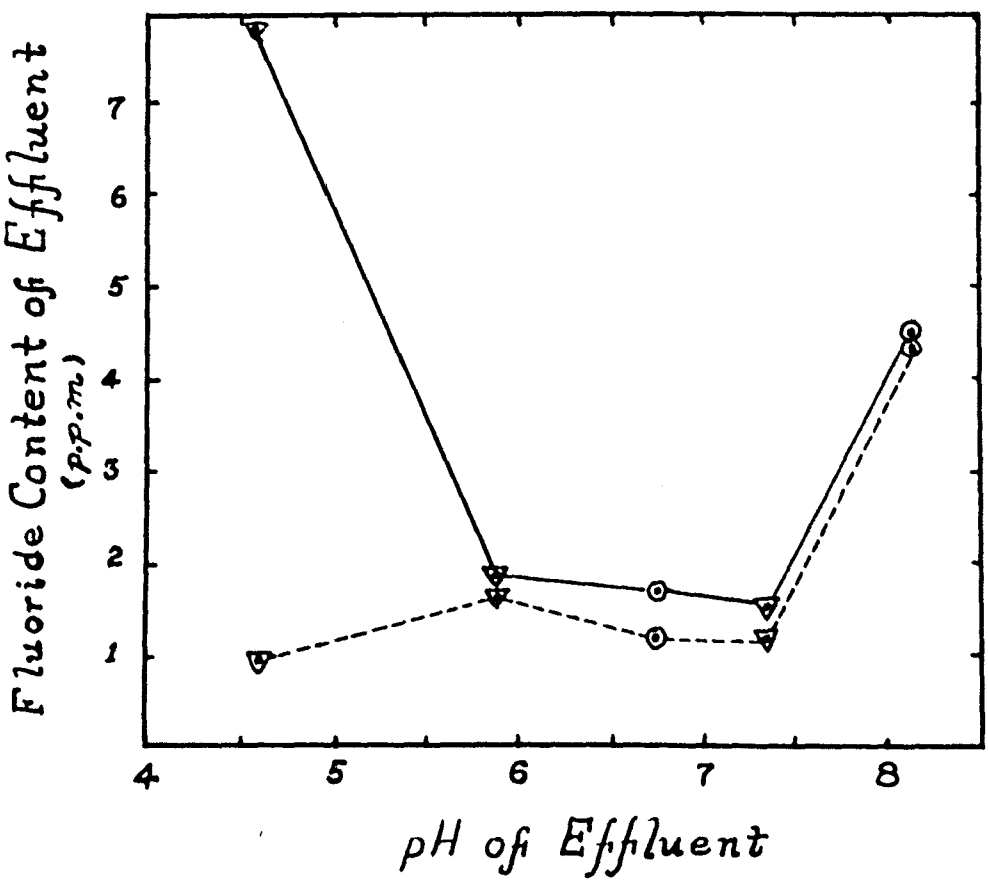
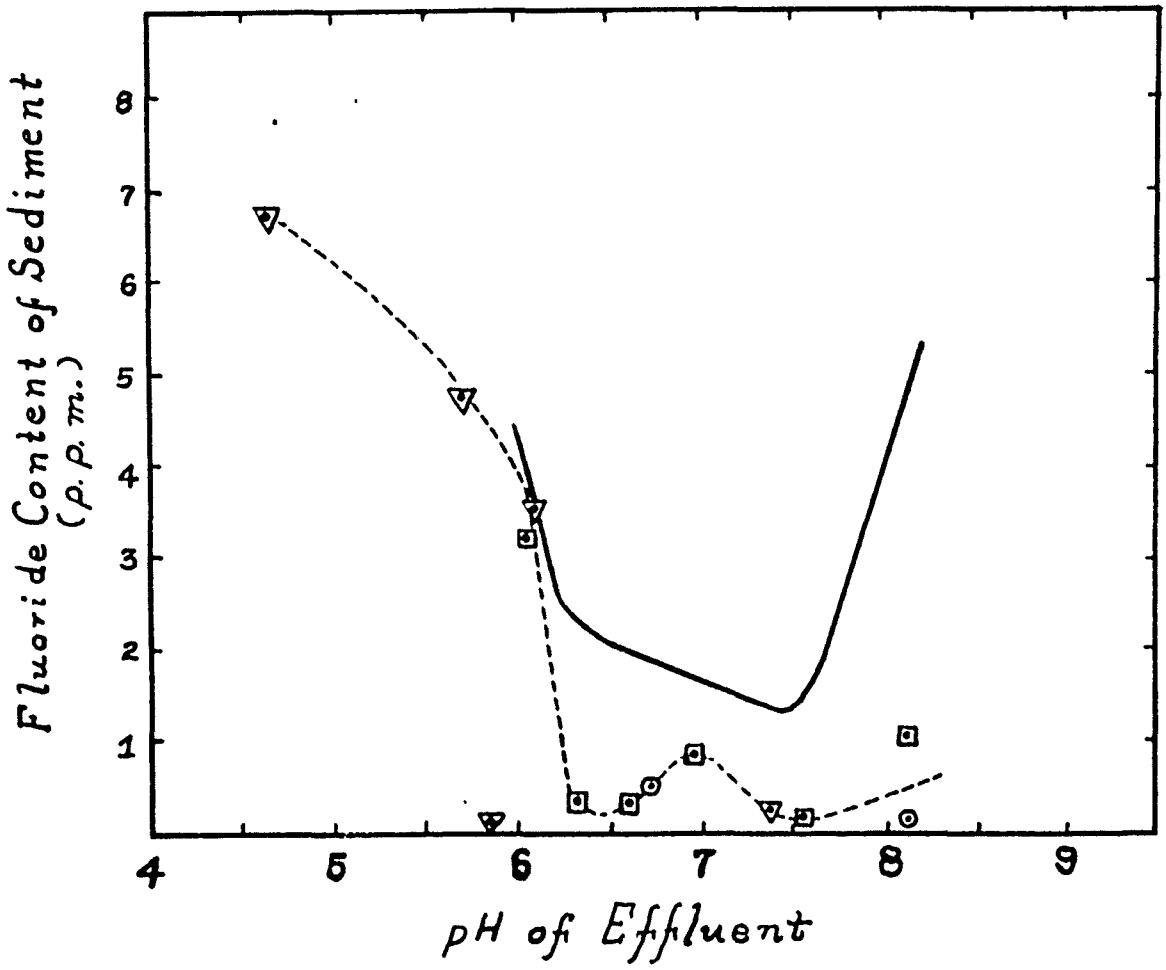


Figure 13. The variation of the fluoride content of effluents with the pH of the effluent, using C.P. aluminum sulfate and aluminum chloride.



⊡, Commercial Alum ; ⊙, C.P. Alum ; △, Aluminum Chloride ; Solid Line, curve for F content of shaken samples ; Broken Line, curve for F content of sediment.

Figure 14. The relation of pH of the effluent to the fluoride content of the sediment in the effluents possessing different pH values.

The fact that all of these waters having a pH of 6 or above tended to show marked cloudiness as received led to the belief that much fluoride may have combined with the alum and that filtration with sand and gravel was not capable of removing the finely divided alum precipitate, thus giving effluents with high fluoride.

The acid pH values apparently were quite favorable to the combination or adsorption of fluoride to the alum; therefore it seemed advisable to experiment with a run conducted in such a way that a pH in the region of 6.0 or less could be obtained in the early stages of mixing at which the fluoride might combine more readily with the alum floc, and, in the later stages of mixing to increase the pH with sodium hydroxide to a value of approximately 7.5 in order to favor the production of a clear effluent.

Three experiments were made according to this plan. One run was made with 20 grains, one with 15 grains, and one with 10 grains per gallon of aluminum sulfate. With the 20 grain dose of alum no additional acid was found necessary to attain the desired acid pH, and sufficient sodium hydroxide was added to the second mixing tank to raise the pH back to 7.5. The fluoride content in the effluent from this run was found slightly greater than 1 part per million. When 15 grains per gallon of alum was used a reduction of fluoride to 0.65 parts per million was obtained. With 10 grains per gallon of alum,

additional acid was added in the dosing solution. Hydrochloric acid was added in a quantity equivalent to 0.09 ml. of concentrated acid per gallon. The effluent obtained from this run contained a total fluoride content of 1.0 part per million. The efficiency of the alum as a removal agent was increased by insuring a distinctly acid pH during the first stage in the treatment and then insuring a pH of approximately 7.5 in the effluent for favorable floc formation by adding sodium hydroxide in the second mixing tank.

SUMMARY

Study of Physiological Responses to Fluorides

The effects of a number of fluorides upon rats have been studied. The availability of copper and iron fluorides for hemoglobin regeneration have been investigated. Young rats were rendered severely anemic on a milk diet after which some of the rats were given a cupric and ferric fluoride supplement, some were given cupric sulfate and ferric chloride, while others were continued on milk alone. The rate of the recovery of rats on the fluorides was compared with the rate of recovery of the animals on cupric sulfate and ferric chloride.

The influence upon hemoglobin of sodium fluoride in the ration of the rat was studied by comparing the hemoglobin values of normal rats during growth, reproduction and lactation with the values for rats receiving a supplement of 0.05 per cent sodium fluoride in the ration.

The effect of feeding alum in the fluoride rations was studied. Rats were fed sodium fluoride at concentrations of 0.025 and 0.050 per cent in the growing ration, whereas others were fed the two different levels of fluorides in rations to which aluminum sulfate had been added. Growth, reproduction,

lactation, and incisor changes of the rats fed fluoride alone were compared with the same changes in the animals receiving the sodium fluoride and aluminum sulfate.

A comparison of the toxicity of aluminum fluoride with that of some other inorganic fluorides was made. Rats were fed zinc fluoride, cupric fluoride, calcium fluosilicate, and aluminum fluoride at the level of 0.10 per cent fluorine as fluoride in the ration. Their effects upon growth and upon the incisors were compared. The storage of fluoride in the tibiae of rats fed cupric fluoride was compared with the storage in the same bones of rats that had received aluminum fluoride.

The toxicity of sodium fluoride when injected alone was compared with the toxicity of the same compound when injected with alum. The effect of injecting aluminum fluoride intraperitoneally was investigated also.

The relationship between recalcification of rachitic tibiae and the incorporation of fluoride into the tibiae was studied. Young rats were rendered rachitic on a standard rachitogenic diet. The rats were then divided into different groups for feeding supplements. One group was given a vitamin D supplement. A second group was given vitamin D plus sodium fluoride at a level of 0.10 per cent in the ration. A third group was fed a supplement of sodium fluoride alone at a level of 0.10 per cent in the ration. The fourth groups was kept on

the rachitogenic ration for a control. At the close of the feeding period the animals were sacrificed and tibiae taken for line tests and for ash and fluoride determinations.

The effect of sodium fluoride upon blood sugar levels in the rat was studied by following the changes in blood sugar concentrations in the rat after the administration of sodium fluoride alone, sodium fluoride and glucose, and glucose alone to fasted and unfasted rats by means of the stomach tube. The influence of insulin upon the sodium fluoride poisoned rats was also investigated.

When it was found that sodium fluoride caused hyperglycemia when administered by stomach tube to unfasted and fasted normal rats, fasting blood sugar levels were determined on normal and fluoride fed rats that had been fasted for 36 hours.

Some effects of organic fluorides upon the rat were studied by incorporating a number of organic fluorides in the ration of the rat and observing the appearance of the incisors and the effects upon growth.

Study of Fluoride Removal from Water

The removal of fluoride from Ankeny City water was tried using potassium alum at different levels. The removal at two initial pH values was studied for three levels of aluminum sulfate dosage. The change in fluoride content of treated water was determined at different intervals of time of standing

in contact with the floc from aluminum sulfate.

Removal with alum was studied by using a continuous treatment pilot plant consisting of three mixing tanks, a settling tank and a filter of sand and gravel. The variation of the fluoride content of the treated water with the pH of the effluent was studied. The pH of the water during treatment was modified by adding hydrochloric acid with the alum solution in the first mixing tank and by adding sodium hydroxide to the second mixing tank, or by adding both the hydrochloric acid and the sodium hydroxide.

CONCLUSIONS

Physiological Responses to Fluorides

1. Both cupric and ferric fluorides were used readily for hemoglobin regeneration in the rat.

2. Sodium fluoride at a level of 0.05 per cent did not affect the hemoglobin levels of the rats during growth, reproduction or lactation.

3. The inclusion of sodium fluoride at a level of 0.0226 per cent fluorine as fluoride did not impair reproduction from the standpoint of the average number of young per litter but the same level impaired reproduction from the standpoint of average birth weights.

4. Sodium fluoride at a level of 0.0226 per cent fluoride impaired lactation so that no young were reared to weaning age out of a total of 38 young born.

5. The feeding of alum with 0.0226 per cent fluorine as fluoride in the ration overcame the effect of the fluoride upon birth weight and restored lactation to a remarkable extent.

6. Both 0.0113 and 0.0226 per cent fluoride caused typical striation, bleaching and lengthening of the incisors of the rats.

7. The feeding of alum along with the sodium fluoride prevented to a remarkable extent the usual changes in teeth due to fluoride feeding.

8. Aluminum fluoride, if toxic at all, possesses an extremely low order of toxicity when fed to rats. This conclusion is supported by growth data, the lack of ability of this compound to damage the incisors and by the fact that the feeding of aluminum fluoride at a level of 0.10 per cent fluorine in the ration of the rat did not cause a marked increase of bone fluorine whereas feeding of cupric fluoride caused a very marked storage of that element. Aluminum fluoride, when injected intraperitoneally, was non-toxic. Sodium fluoride was found less toxic when injected with alum than when injected alone.

9. The administration of sodium fluoride along with vitamin D accelerated recalcification as measured by ash determinations and by line tests.

10. The storage of fluorine in the bones of the rat was increased by the administration of vitamin D.

11. Large quantities of sodium fluoride, when administered by stomach tube, caused marked hyperglycemia whether or not glucose was administered simultaneously.

12. The fluoride-induced hyperglycemia was found to be counteracted in three separate experiments by insulin injections, even though typical fluoride tremors were evident at the time of the insulin injection.

13. The feeding of sodium fluoride at 0.10 per cent in the ration of the rat caused markedly lowered blood sugar levels after 36 hours fast.

14. The inanition of fluoride fed rats may be due in part to impairment of carbohydrate storage.

15. Feeding of some organic fluorine compounds caused typical mottling of the incisors of the rat whereas the feeding of other organic compounds failed to cause visible changes in the incisors.

16. Alphafluoronaphthalene, p-fluorobromobenzene, and p-fluoroiodobenzene caused mottling at levels of 0.10 per cent fluorine in the ration.

17. Mottling was not caused by p-fluorobenzoic acid, p, p'-difluorodiphenyl or fluorobenzene.

Removal Studies

1. The removal of fluoride from water by the use of commercial alum has been demonstrated with Ankeny City water which resembles a number of waters whose compositions have been reported by other investigators.

2. The removal of fluoride from natural water has been accomplished by the use of C.P. alum and C.P. aluminum chloride.

3. The fluoride content of treated water has been found to vary in a definite manner with the pH of the treated water.

4. This variation of fluoride content of treated water with pH is found to occur with alum, both commercial and C. P., and with C. P. aluminum chloride.

5. The optimum pH of the effluent which resulted in maximum combination of fluoride with the floc did not coincide with the optimum pH for rapid foculation and formation of clear filtrates.

6. The optimum pH of the effluent for the formation of clear filtrates which later gave very little sediment was found to be near 7.6; whereas, samples of effluents which gave low fluoride contents if flocs were allowed to settle were obtained at pH values ranging from 4.5 to 7.5.

7. The efficiency of fluoride removal by alum in the continuous treatment experiments was increased markedly by insuring an acid reaction of the water in the first mixing tank to a pH of 6 followed by an alkaline reaction in the second mixing tank corresponding to a pH of 7.5.

8. Removal of fluoride to the levels of 0.65 and 1.00 parts per million was accomplished by the use of 15 and 10 grains per gallon of $Al_2(SO_4)_3$, respectively. The pH in the first mixing tank was 6 and in the second mixing tank was 7.5.

9. The use of bentonite in laboratory experiments, either alone or with alum, proved of no value; on the contrary, it inhibited the removal action of the alum.

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ACKNOWLEDGMENTS

The author is deeply grateful to Professor V. E. Nelson for his assistance in directing the work of this thesis. The generosity of Professor W. E. Galligan and of the Department of Engineering of Iowa State College in permitting the use of the pilot plant in a number of experiments is likewise greatly appreciated.