Factors Affecting the Fluorine Content of Foodstuffs

by

Mitchell G. Vavich

A Thesis submitted to the faculty of the Department of Nutrition in partial fulfillment of the requirements for the degree of Master of Science in the Graduate College University of Arizona 1940

Approved: [Signature] E. Smith, Major Professor 5/22/40
ACKNOWLEDGMENT

The writer wishes to express his sincere appreciation to Dr. Margaret Cammack Smith for the suggestion of this problem and to both Dr. Margaret Cammack Smith and Professor H. V. Smith for their unceasing encouragement, guidance and constructive criticism throughout the course of this research.

The writer also wishes to thank Mr. A. T. Bartel, Dr. A. H. Finch, Professor I. A. Briggs, Dr. F. V. Sherwood and Dr. W. H. Riddell for their cooperation.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Statement of Problem</td>
<td>1</td>
</tr>
<tr>
<td><strong>HISTORICAL REVIEW</strong></td>
<td>2</td>
</tr>
<tr>
<td>General Distribution of Fluorine</td>
<td>2</td>
</tr>
<tr>
<td>Fluorine in Foodstuffs</td>
<td>4</td>
</tr>
<tr>
<td>Effects of Fluorosis</td>
<td></td>
</tr>
<tr>
<td>On the Osseous System</td>
<td>10</td>
</tr>
<tr>
<td>On Enzymatic Action</td>
<td>14</td>
</tr>
<tr>
<td>On Reproduction</td>
<td>16</td>
</tr>
<tr>
<td>On the Animal Body in General</td>
<td>17</td>
</tr>
<tr>
<td>Amelioration of Fluorosis</td>
<td>20</td>
</tr>
<tr>
<td>Review of Methods for the Quantitative Determination of Fluorine in Foods</td>
<td>23</td>
</tr>
<tr>
<td><strong>EXPERIMENTAL</strong></td>
<td>28</td>
</tr>
<tr>
<td>Fluorine in Plants vs Fluorine in Soil</td>
<td>28</td>
</tr>
<tr>
<td>Method and Materials</td>
<td>28</td>
</tr>
<tr>
<td>Method of Analysis</td>
<td>50</td>
</tr>
<tr>
<td>Results</td>
<td>33</td>
</tr>
<tr>
<td>Fluorine in milk vs Fluorine in Drinking Water</td>
<td>35</td>
</tr>
<tr>
<td>Method and Materials</td>
<td>35</td>
</tr>
<tr>
<td>Results</td>
<td>35</td>
</tr>
<tr>
<td>Fluorine in Cooked Vegetables vs Fluorine in Cooking Water</td>
<td>36</td>
</tr>
<tr>
<td>Method and Materials</td>
<td>37</td>
</tr>
<tr>
<td>Results</td>
<td>37</td>
</tr>
<tr>
<td>Discussion</td>
<td>39</td>
</tr>
<tr>
<td>Summary and Conclusions</td>
<td>44</td>
</tr>
<tr>
<td><strong>BIBLIOGRAPHY</strong></td>
<td>57</td>
</tr>
</tbody>
</table>
INDEX OF FIGURE AND TABLES

Figure I  Multiple-unit Distillation Apparatus  30-a

TABLE I  Fluorine Analyses of Some Foods and Feeds Grown in Soil to Which Calcium Fluoride (CaF₂) Has Been Added.  47

TABLE II  Fluorine Analyses of Some Foodstuffs Grown in Soil to Which Sodium Fluoride (NaF) Has Been Added in Various Concentrations.  48

TABLE III  Table Showing Relation of Level of Fluorine Intake in the Drinking Water of a Guernsey Cow to the Fluorine Content of the Milk and Urine.  49

TABLE IV  Table Showing the Daily Milk Production of a Guernsey Cow While Drinking Water Containing Fluorine in Concentrations of 0.2 to 495 p.p.m.  50

TABLE V  Table Showing Relation of Level of Fluorine Intake in the Drinking Water of a Jersey Cow to the Fluorine Content of the Milk and Urine.  51

TABLE VI  Table Showing the Daily Milk Production of a Jersey Cow While Drinking Water Containing Fluorine in Concentrations of 0.2 to 500 p.p.m.  52

TABLE VII  Analyses of Foods Cooked in Fluorine-containing Water and Distilled Water.  55

TABLE VIII  The Effect of Washing with Distilled Water on the Concentration of Fluorine "taken up" by Vegetables Cooked in Fluorine-containing Water and Analyses of Foods Cooked in Fluorine-containing Water and in Distilled Water.  54

TABLE IX  Analyses of Vegetables Cooked in Fluorine-containing Water and in Distilled Water.  55

TABLE X  Summary of Detailed Measurements Recorded in the Cooking of Foods in Fluorine-containing Water and in Distilled Water.  56
INTRODUCTION

Statement of Problem

Numerous investigators from about 1845 have reported the presence of fluorine in plant materials but scant attention was given such work until 1932 when Smith, Lantz, and Smith (1) announced that fluorine in drinking water was the cause of the condition known as mottled enamel (2). This discovery was the signal for unearthing musty volumes that contained information about the occurrence of fluorine as well as a stimulus for independent study on various phases of what is now termed "the fluorine problem".

Questions arose concerning the distribution and occurrence of fluorine. The strong possibility that plant foods, milk, and foods cooked in fluorine-containing waters may be sources of fluorine was recognized immediately. Only the lack of accurate quantitative methods prevented the immediate study of this problem. The lack of accurate methods is strongly in evidence in the work of many early workers who have reported widely differing analyses for the same substances. Constant efforts to improve analytical methods reached a successful climax in 1938 when a number of workers reported complete recoveries of fluorine by analytical quantitative methods. These reports indicated that this problem might now be settled with some degree of certainty. Research was undertaken therefore to determine some factors which affect the fluorine content of foodstuffs and was planned to obtain specific information as to:

(1) The effect upon the fluorine content of plants of the addition of fluorides in various concentrations to the soil.
(2) The effects upon the fluorine content of milk of the additions of various concentrations of fluorine to the drinking water of cows.

(3) The effect upon the fluorine content of vegetables of cooking them in fluorine-containing water.

HISTORICAL REVIEW.

The discovery at the University of Arizona (1) that fluorine in water causes mottling of the enamel of teeth marks the end of the occasional study of lethal doses of fluorine and their effects upon the animal organism, and the beginning of studies of chronic fluorosis produced by minute traces of the element. This study also marks a change in that investigators now turned mainly to a study of the effect of this element upon the teeth. Heretofore the teeth had been mentioned in a passing way, or not at all, until 1925 when McCollum et al (2) showed that 226 p.p.m. NaF in the rations of rats produced overgrown incisors. It is interesting that McKay in the period from 1918 to 1928 (4) (5) (6) (7) presented circumstantial evidence that there was some connection between the water supply and the endemic occurrence of mottled enamel.

General Distribution of Fluorine

Numerous investigators have pointed out the wide distribution of fluorine in all parts of the world. Rohola (8) writes that fluorspar or fluorite (CaF₂) is found all over the world although the largest and best known deposits are in the U.S.A., England and Germany. Cryolite on the other hand is found only at Ivigtut, Greenland in any large quantity, but is now made synthetically in commercial quantities. These two minerals contain 43.87 and 54.30 per cent fluorine respectively. All the other miner-

...
als containing fluorine contain much smaller percentages. Apatite may contain 3.8 per cent and others such as topaz, tourmaline, lepidolite, etc. still smaller quantities. Of importance in the last forty years has become the use of phosphorite or rock phosphate which has a fluorine content of 3 to 4 per cent depending on the deposit (9). These deposits are obtained in Florida, Tennessee, South Carolina, Kentucky, Arkansas, Idaho, Wyoming, Utah and Montana, in the U.S.A., and in North Africa and some islands in the South Seas as well as some countries in Europe (8).

The reports of various investigators that foods in general contained fluorine was not unexpected in view of the widespread distribution of fluorine throughout the world. De Eds (10) states that fluorine ranks twentieth in quantity in the first mile of the earth's crust. More astounding is the calculation made by De Eds (10) in 1935 that the use of rock phosphate in making super-phosphate fertilizer added 90,000 tons of fluorine annually to the top soil.

The occurrence of fluorine in water supplies was demonstrated by Gautier and Clausmann (11). Churchill (12) found fluorine in the water supplies of Cincinnati, Ohio, Milwaukee, Wis., Birmingham, Ala., Pittsburgh, Pa., Detroit, Mich., Peoria, Ill., Indianapolis, Ind., Buffalo, N.Y., Davenport, Iowa, San Francisco, Calif., Minneapolis, Minn., and Los Angeles, Calif. The work of many others since 1932 has shown fluorine to be present in the drinking water in nearly every country in the world.
Fluorine in Foodstuffs

According to Rehohlm (8), Muller and Blake were the first (1845) to show the presence of fluorine in plant tissue (barley).

The use of phosphorite as a source of phosphorus for plants in the form of fertilizer and for animals as a dietary supplement indicated the detrimental action of the fluorine and created a problem that is studied even at the present time.

The most thorough work done on fluorine up to 1916 was that of Gautier and Clausmann (15). Employing a method of their own (14) they found no plant group to be especially rich in fluorine. Highest of any part of the plant analyzed was the leafy part which showed from 30 to 140 p.p.m. of dry substance. Lowest were the stems with analyses of 5.6 to 17.0 p.p.m., while the seed and fruit meat were in the range between 3.6 and 140 p.p.m. On the dry basis banana pulp showed 3.8 p.p.m. and the skins 51 p.p.m. fluorine.

Three years later these same workers (15) studied the effects of calcium fluoride as a plant stimulant in the soil. A stimulating effect was reported on wheat, oats, carrots, potatoes, peas, beans, cabbage, poppy and hemp. No influence was noted with rye, barley, buckwheat, or mustard, while beets and turnips grew to a smaller extent. About this time Steinkoenig (16) had determined that the average percentage of fluorine in soils was about 500 p.p.m.

By use of pot culture experiments Voelcher (17) found that CaF₂ decreased the yield of wheat by one half, but CaSiF₄, at the time of application, almost doubled the yield from untreated wheat. NaF or KF at the rate of 500 p.p.m. gave yields that were 4½ and 5½ times larger.
than those obtained from untreated wheat. 1,000 p.p.m. fluorine as
NaF produced a caking effect and prevented germination. This same
quantity of fluorine as KF stimulated a yield of 4½ times the un-
treated wheat.

In continuation of their early work on the fluorine problem
Cristiani and Gautier (18) called attention to the damage to the
vegetation around aluminum factories where large quantities of
fluorine-waste was thrown. They were able to show that fluorized for-
age had a toxic effect on herbivora and guinea pigs when it contain-
ed 100 p.p.m. and 1000 p.p.m. fluorine. Mayrhofer et al (8)
found fluorine in most cultivated plants, but not in tomatoes, potatoes,
and tobacco. The quantities found varied from .08 p.p.m. to .48 p.p.m.
of fresh substance. On the fresh basis Gautier and Claussen's (19a)
figures varied between 0.1 and 59 p.p.m. No doubt the difference
in methods of analyses accounts for some of the variation.

Hart et al (20) analyzed plant material from plots which had re-
ceived fluorine-carrying phosphates such as rock phosphate and acid
phosphates for periods of 16-36 years. None of the plant materials
showed consistent or greatly increased fluorine contents over plant
materials grown on plots receiving a low-fluorine-carrying phosphate such
as bone meal. On the other hand lysimeter studies showed considerable
increase in fluorine content of water which had passed through soil
treated with rock phosphate. Soil treated with super-phosphate
contributed fluorine to the drainage water in smaller quantities.

The largest accumulation of fluorine in plant materials was re-
ported by Bartholomew (21) using from 0 to 10 p.p.m. fluorine in a
complete liquid nutrient. When NaF was the source of fluoride ion, as much as 1,086 p.p.m. fluorine was found in the roots and 40 p.p.m. fluorine in the tops of cowpeas. CaF$_2$ proved to be a poorer source of fluorine since 7.72 p.p.m. fluorine in this form gave a maximum of 84.5 p.p.m. fluorine in the roots and from 0.0 to 11.0 p.p.m. fluorine in the tops of cowpeas. Na$_2$SiF$_6$ on the other hand produced a better storage of fluorine in both the roots and tops than either the NaF or CaF$_2$ at the same level of fluorine. 10 p.p.m. fluorine in the nutrient water gave values of 1,116 to 1,970 p.p.m. fluorine in the roots and from 415 to 475 p.p.m. fluorine in the tops. No correlation was found between the phosphorus content and fluorine content of cowpeas.

McClure (22) summarizes the analyses of foods in the literature. An inspection of these tables shows wide variation in the fluorine content of the same type of substance when analyzed by several different methods as well as by the same method, showing that either there is a wide variation in the fluorine content for the same type of substance, or the methods do not agree, or perhaps both.

The largest fluorine content of any seed or seed product reported was that for wheat grown in a fluorite area. Churchill et al (25) reported wheat from this source to contain 226.0 p.p.m. fluorine, while from a normal area wheat showed 2.0 p.p.m. fluorine. An almost similar difference was noted by these workers in a comparison of barley from a fluorite area and from a normal area. The barley from the fluorite area contained 184.5 p.p.m. fluorine, but only 3.7 p.p.m. from the normal area. In all four cases the fluorine was determined
by the same method, namely the Meyer-Schultz method.

The analyses of teas showed a wide range of fluorine contents depending on the type and source. One sample of "Imported Ceylon" showed the lowest values 8.7-9.5 p.p.m. fluorine. Another sample of tea from a fluorite area showed 1,757.8 p.p.m. fluorine by the same investigator. Reid (24) reported fresh tea from a fluorite area contained 1,757.8 p.p.m. fluorine. Roasted tea contained much less fluorine (15.0-710 p.p.m.) but Reid did not believe that roasting was responsible for the lowered fluorine content. It so happened that the roasted tea came from younger leaves and the difference was explained by Reid on the assumption that fluorine assimilation increased with the age of the leaves.

From the results presented by Churchill et al (25) and others the fluorine content of meats appears to be of minor importance. It is to be expected, no doubt, that animal bones are high in comparison with the meat. The value for bones varied from 95.9 to 367 as reported by Dahle and Wichmann (25).

A significant change in the order of the fluorine content of foods is found in the analyses of Churchill et al (25) who have incorporated many of the recent findings and refinements that make the Willard and Winter method more exact. In almost every case there is a decreased fluorine content when compared with early analyses. In this connection McClure (22a) wrote: "It cannot be said that ordinary foods according to the latest analytical results are showing significant quantities of fluorine present. With the exception of teas and fish foods, the quantities present do not compare with drinking waters which are associated with chronic endemic fluorosis".
Experimental evidence of the deleterious action of fluorine in food was until recently confined to the work of Reid (24) and Gaul et al (26). The latter work was questioned by McClure (22) on the basis that the authors themselves admitted that dust-containing fluorides accounted for as much as 75% of the fluorine in the plant.

Reid (24) observed that 81 to 96% of the fluorine in teas was leached by a 2% infusion. Young rats fed such 1 and 2% infusions containing 398.8 p.p.m. fluorine in the dry substance developed characteristic tooth striations at the end of 4-5 weeks. The daily calculated fluorine intake was 0.07 to 0.14 mg. of fluorine. This was less than the amount reported by McClure (22) that will cause the first discernible effect in seven weeks. Diets containing dry cheap tea produced the same characteristic effects. The intake levels equalled 21.9 and 42.8 p.p.m. of fluorine in the diet and the effects were produced in three weeks (striated teeth).

Reid and Cheng (27) reported appreciable increases in the fluorine content of the young produced when pregnant female rats were fed 1 and 2% infusions of tea. They stated that the increases were similar to the increases produced when NaF at the same level of fluorine was fed to other pregnant female rats. Evans and Phillips (28) substantiate these findings in showing that the small fluorine content of milk is deposited at the rate of 35% of the fluorine ingested in the fetal tissues. Large quantities of fluorine (up to 20 p.p.m. fluorine) showed a larger storage of fluorine in the fetal tissues.

Gaul et al (26) have reported as much as an 800% increase in the fluorine content of guinea pigs fed forage grown in endemic fluoride.
areas. These results are seriously questioned by McClure (22) since dust contamination from these areas was shown to account for as much as 75% of the total fluorine analysis of plant materials.

Laboratory evidence would indicate fluorine in foods is dangerous but direct observations of mottling present contrary although to some degree presumptive evidence that this is not so since endemic regions have always been associated with appreciable quantities of fluorine in the water supply.

In this connection it is important that a number of factors be considered. First of all the effects of mottled enamel are demonstrated mainly in the permanent teeth which are calcified by the 8th year (except for the third molars). Until recently the first few years of life were marked by a diet consisting of almost nothing but milk, which has been shown (29) (30) (31) to be very low in fluorine. In the last few years the dietaries of babies have been supplemented by some commercial foods which have been shown to contain fluorine in appreciable amounts (32). In other words the early years of life may now include appreciable quantities of fluorine in the diet of a child in the years when the teeth are calcifying. It must also be remembered that during this period and even later calcification of the bones is taking place.

Secondly the total quantity of fluorine ingested in an area where mottling is endemic has not been shown to be due solely to the water ingested as drinking water. Smith et al (32) found cases of mottled enamel of the permanent teeth in children who have used 12 p.p.m. fluorine water for cooking purposes but not for drinking.

This raises the question: Is fluorine as induced in foods by cook-
ing as deleterious as fluorine in water? Marcovitch (52), although he does not answer this question, showed that a diet containing beans or rice cooked to dryness in fluorine water increased the fluorine content of the diet. He was able to produce changes in the teeth by feeding this diet.

Studying the metabolism of naturally occurring fluorine in canned Salmon and Mackerel, Lee and Nilson (54) found that roughly it required slightly more than three times as much fluorine in the form of fresh Salmon and Mackerel to produce the same effect as NaF. It was interesting that the percentage of stored fluorine was three times as great for small quantities of fluorine (1.4 p.p.m.) in the form of NaF or CaF₂ as it was for fluorine in fish. A greater difference was noted in the amount of fluorine stored when fed to rats as CaF₂ or NaF than when fed in the form of Salmon or Mackerel although the CaF₂ and NaF were fed at the same level of fluorine, and the Mackerel contained four times as much fluorine per unit weight than did the Salmon (26.87 p.p.m. fluorine for the Mackerel and 5.77 for the Salmon based on fresh weight.)

Effects of Fluorosis on the Osseous System

The effect of chronic fluorosis on the teeth has been thoroughly described as they appear macroscopically, slightly magnified, and histologically by Smith, Lants, and Smith (1) and Smith and Schour (35) (58) (37) (38). Other investigators, too numerous to mention, have shown the effects of fluorosis on the teeth of dairy cattle, pigs, dogs, rats and guinea pigs. A comparison of the results of much of this type of work fails to accomplish its purpose since almost each investigator makes modifications of certain factors to meet his own purpose. Similarly a study
of the effects of fluorine ingestion on the percentage of ash and ash constituents involves so many factors that a comparison of the results of more than two investigators involves an explanation of every modification that appears in each work. Schulz (39) has placed the results of most of these studies in table form but even this fails to provide a good means of arriving at a general conclusion because of the various different ranges in levels fed by different investigators and the different tissues studied. One generalization may be made from the data presented in the table by Schulz. At a level of fluorine at or above 0.046% the ash content of the incisors was decreased.

The finding of large deposits of fluorine in the bones was reported in 1891 (40), but much earlier than this (1805) Gay-Lussac and Berthollet (41) reported fluorine in teeth.

The question of whether or not chronic fluorosis of a degree sufficient to produce mottling will create fluorosis of the bones of inhabitants of endemic areas has received scant study. Roholm (8) has calculated that the osseous system of a deceased cryolite worker contained 1,440 p.p.m. of fluorine (90 mg. fluorine for body wt. of 68 kg.). This man was believed to have ingested cryolite dust at the rate of 0.2 to 0.35 mg. fluorine daily per kilogram of body weight for 24 years. McClure (20) has estimated that a human ingesting 2–3 p.p.m. water will ingest 2.4 to 4.5 mg. of fluorine daily if he drinks between 1,200–1,500 c.c. water daily, assuming that the rate of retention of fluorine is 30–40%. It may be assumed that the retained fluorine will amount to 0.7 to 1.08 mg. per day if in the form of NaF. Over a period of 60 years at this rate 15.3 to 39.4 gm. would be deposited in the body.
In a body weight of 68 kg, this represents a concentration of about 225 to 580 mg fluorine per kg of body weight.

Two factors may modify these figures. On the one hand larger quantities of fluorine may induce a larger retention. On the other hand a period of freedom from fluorine may result in a loss of fluorine from the osseous system with a possibility that the bones may return to normal. The above statement has never been proved but was merely suggested by McClure (20) on the basis that bone calcium has mobile properties.

Wrangelmyer (42) attempted to find if any relation existed between carious teeth and their fluorine content. Sound adult teeth showed 15,600 p.p.m. and carious adult teeth 11,400 p.p.m. fluorine. Sound children's teeth tested 6,500 p.p.m. and carious teeth showed 15,000 p.p.m. fluorine. Dean (43), Dean et al (44) and Cox (45) have suggested a relation between carious teeth and endemic fluorosis.

Others have found larger quantities of fluorine in the osseous system of the old and numerous analyses have been made of the various tissues of the animal body (40) (42) (46) (47) (48) (49). These analyses show beyond a doubt that fluorine storage in the animal body is associated more closely with the osseous system than with any other system.

Fluorine deposition in the teeth has been shown by Brandi® and Tappeiner (40) and Sonntag (49) and later by Chang et al (50).

Carnot (5) demonstrated that fossil bones contained from 4,000 to 19,000 p.p.m. fluorine whereas fresh human, ox, and elephant bones contained from 1,000 to 5,000 p.p.m. He found also that the teeth of elephants and mastodons of Pliocene Ages contained 25,000 to 30,000 p.p.m. fluorine respectively.
In 1916 McKay (1) reported that he had never found mottled enamel on temporary teeth, except "that the molars are affected very slightly", and attributed this to the inability of fluorine to pass through the placenta. Smith (52) in corroboration of this, found that children born in communities where mottling was endemic and where the mothers drank as much as 6 p.p.m. fluorine water, as well as having used it for cooking purposes throughout pregnancy, had normal temporary teeth. It was also found by this worker that female rats receiving 0.05% NaF in the diet produced young that showed no mottling at the time of weaning (three weeks). However, if these young were allowed to eat the mother's diet containing 0.05% NaF changes were noted in the teeth in two weeks.

Somewhat later Smith and Smith (53) found two children, a 2\(\frac{1}{2}\) year old boy, and his 4\(\frac{1}{2}\) year old sister, with mottled temporary teeth. It was interesting that the girl who had been 12 months old at the time this family moved to this area had 8 temporary teeth which were not mottled but all that had erupted (and perhaps calcified in part) since that time were typically mottled. The water from their well analyzed 15.5 p.p.m. fluorine.

Two other children from a different family drinking 12 p.p.m. fluorine water also showed severe mottling of the deciduous teeth. All of these children were artificially fed from birth on and the mother did not drink the fluorine-containing water during pregnancy, but the water was used in making up the milk formula. This indicates, as the authors stated, very strongly that the calcification proceeds so rapidly that only large concentrations of fluorine in the water affect it.
Effects of Fluorosis on Enzymatic Action

The effect of fluorides on enzymatic systems was employed by Phillips (54) in an effort to detect fluorosis in cases where other criteria did not suffice. He found that the plasma phosphatase in cattle, swine, and rats rose in proportion to the fluorine intake and was interpreted as a protective response on the part of the body to maintain and deposit normal skeletal structures even in periods of disturbance such as chronic fluorine poisoning.

Smith and Lantz (55) found that this test was not a sensitive indication of fluorosis in the rat. Fluorine was not found to exert its damage to the teeth of rats through its effect on the enzyme involved in tooth and bone calcification.

A number of interesting inhibitory effects of fluorides on enzymatic action were shown by Amberg and Lovenhart (56) in 1938 demonstrating the ability of one part of NaF in 5,000,003 to cause a 50% inhibition in the hydrolysis of ethyl acetate by the lipase of liver extracts in vitro. Later (1939) Leake et al (57) were able to show that the evidence pointed to an inhibition of the lipase action instead of a destruction of the hepatic enzyme as it was at one time supposed. Another inhibitory action of fluorides was demonstrated by Clifford (58) who showed that clotting of milk by pepsin could be inhibited for as long as 4-6 hours by the addition of small but definite quantities of NaF in solution.

Further attempts to explain the toxic action of fluorides brought forth many more interesting actions of fluorine. Spiro (59) showed
fluorhemoglobin was easily formed. Kobert (60) claimed that the coagulation of blood was prevented by fluorides by the removal of blood calcium when calcium fluoride was formed. This view was contested by both Calugareanu (61) and Sollemann (49). The former believed it was due to a retardation of the elaboration of the fibrin element while the latter believed it had some effect on the thrombin since the addition of thrombin restored coagulation whereas calcium did not. Schwyzser's claim in 1905 (62) that the clotting power of blood was increased by the addition of fluorine apparently was mistaken in view of the claims of the other workers.

In 1924 Embden and Lenge (63) and Embden and Hentschel (64) regarded lactocidogen as a precursor of lactic acid and phosphoric acids. These substances were believed to exist in muscle in a state of dynamic equilibrium. They found that salts of various anions could shift the equilibrium one way or the other and NaF favored the synthesis of lactocidogen. These findings were in agreement with Lipmann's (65) work a few years earlier. Dickens and Simer (66) decided that the fluorine acts on the metabolism of the hexose to the triose stage since they found no effect of NaF on the methyl glyoxal to lactic acid stage.

De Eds (10). stated: "The possibility therefore exists that fluorine intoxication, particularly the chronic form, may be due to disturbance not only of calcium metabolism, but also inhibition of enzymatic processes, development of anemia, formation of fluorhemoglobin, and altered muscular metabolism as indicated by decreased lactic acid production. On the other hand, in the absence of evidence to the
contrary, there is a possibility that all these factors are ex-
pressions of a disturbed calcium equilibrium, which would leave the
fundamental mechanism a unity, concerned strictly with the direct
chemical reactivity of calcium and fluorine, as usual".

Effects of Fluorosis on Reproduction

Del Castillo (67) and Chaneles (68) both reported suppression of
the oestrus cycle by chronic fluorine poisoning. Schulz and Lamb (69)
(1925) noticed no unfavorable effect on reproduction until the sodium
fluoride reached a concentration of 0.025% of the ration. Above this
level unfavorable effects were in evidence. Contrary to the evidence
presented by others, Phillips et al (70) wrote that chronic fluorine
poisoning does not inhibit reproduction in the rat. They believed that
any unfavorable effect upon reproduction arose secondarily as a result
of a general systemic reaction to fluorine. It was shown that low
levels of fluorine compatible with normal growth did not affect oestrus.
Larger dosages such as a dosage greater than 20 mgms. fluorine per
kilogram of body weight per day did affect oestrus but as stated prev-
iously inanition and a general systemic reaction were responsible. Smith
and Leverton (94) found no specific damage to reproductive organs when
fluorides were fed to rats. They concluded that fluorosis interfered
with reproduction only when it interfered with growth or caused stunting
of the female.

It is interesting to note that no clear-cut evidence had been
presented to show a cumulative effect upon reproduction. In this con-
nection Phillips et al (70) could find no cumulative effect on reproduction
or other physiological processes from generation to generation over a period as long as five generations of chronic fluorosis. Schuls (59) on the other hand found that NaF at a level of 0.025% tended to inhibit the growth of successive generations. (Phillips' threshold value of 20 mg. per kilogram of body weight per day would amount to .02% in a .044% NaF ration for a rat that weighed 100 gms. and consumed 10 gms. of food per day). At .05% NaF, growth retardation of successive generations was more marked and reproduction was much less certain. At levels of 0.10 and 0.15% NaF, growth and reproduction were quite uncertain but sometimes poor and at times fair. Levels of 0.2 and 0.25% NaF resulted in death in a few weeks.

Effects of Fluorosis on the Animal Body in General

Early records according to De Eds(9) show that Rubuteau (71) in 1887 published the first account of the effects of acute fluorine toxicity. But it was much later (1889) that the effects associated with chronic intoxication were shown experimentally by Schuls (72). By feeding small quantities of sodium fluoride to a dog he found that no effects were apparent for periods as long as 20 days. After this period of time considerable anorexia and emaciation followed. When the dosage of fluorine was increased to 0.2 or 0.4 grams per day death followed in two weeks.

Many of these early accounts of fluorine toxicosis relate the symptoms suffered by victims of accidental poisoning. Baldwin (75) wrote of three people who were poisoned by pancakes made with NaF instead of baking powder. By assuming that the sodium fluoride was evenly distributed throughout the pancake batter Baldwin calculated that
the three victims ingested 9, 6, and 5 gms. of NaF respectively. Severe symptoms of cramps, nausea, and vomiting followed but all recovered.

Reholm (8) states that a review of the literature in regard to the minimal lethal dose of sodium fluoride in an adult shows that in one case as small a quantity as 4 gms. of NaF caused death although as much as 9 gms. has been tolerated. As little as 0.7 to 1.0 gram of sodium fluosilicate has resulted in death.

Baldwin (73) experimenting on himself found that .03 gram of NaF had no effect, .09 gram produced a little salivation and .25 gram brought on nausea in two minutes and increased in severity for 20 minutes. All symptoms disappeared on the second day. Tappeiner (74) listed the symptoms of acute fluorine toxicosis in the following order: salivation, gastroenteritis, dyspnea, muscular weakness, tremors, fall in arterial pressure, and finally stoppage of respiration and heart. He concluded that the lethal dose for mammals was 0.5 gm. NaF per kilogram of body weight.

The first indication of the fate of ingested fluorine was demonstrated by Brandle and Tappeiner (40) in 1891. They fed NaF in sub-lethal doses over a period of 20 months to dogs and found large quantities of fluorine in the bones. De Long (75) reported that Pitotti observed a degeneration of the epithelium of the kidneys and a cloudy swelling of the liver in rabbits and guinea pigs fed lethal doses of sodium fluoride. Another worker in 1901 (76) observed that the intestinal epithelium was destroyed regardless of the channel by which the poison entered the body.

A considerable literature has been built up concerning the effects of fluorine ingestion in growing dogs (77), rabbits (78), rats (3) (79) (80) (81) (82) (83) (84), swine (79) (85) and cattle (50) while very
little is to be found about the effects on humans (8) because of the dangers involved in experimentation. Much of the literature concerning humans is that reported by various physicians who attended persons exposed to fumes and dust of fluorine-bearing minerals used in commercial processes. The study of the toxicity of fluorine with reference to lethal doses for humans is likewise scattered since all records of such cases are medical histories of persons who have accidentally, and in a few cases willfully, swallowed large quantities of fluorides in one form or another (8).

Shortly after Hart et al (8@) had reported improved bone formation when rock phosphate was used to supplement low phosphorus rations for pigs, Forbes et al (87) found that feeding rock phosphate resulted in less dense and weaker bones than when no minerals or minerals practically free from fluorine were fed. These two papers were among the first dealing with the subject of rock phosphate and the effects of its feeding on livestock.

A most interesting picture of dental defects was shown by Hammett (88) (69) to be produced by parathyroidectomy. These defects bear a striking resemblance to that produced by chronic fluorosis. When the thyroids were removed with the parathyroids no dental defects developed. A suggested explanation by the author was that excision of both glands produced a lower level of metabolism as compared with that existing after parathyroidectomy alone. An analysis of the femurs showed that parathyroidectomy alone produced bones low in calcium and high in magnesium and phosphorus. Chaneles (63) found the same
to occur when fluorine was fed. On the other hand Hammett (89) found no evidence of altered calcium, magnesium, or phosphorus ratios when both thyroid and parathyroid glands were removed. Goldemberg (90) reported, in this connection, that the feeding of sodium fluoride caused an increase in the size of the thyroid glands of dogs and rats but Chaneles (91) and Tolle and Maynard (92) could not verify this conclusion.

Amelioration of Fluorosis

The amelioration of fluorosis has been studied from many points of view but nearly all studies were, at least in part, an effort to clarify the action of fluorine on the animal body.

Schlick (92), in 1911, demonstrated that frogs receiving toxic doses of NaF could be protected by treatment with CaCl₂ and that the fall in blood pressure in warm-blooded animals acutely poisoned with fluoride could be antagonized by injections of calcium chloride. Chaneles (68) fed two sets of rats 50 mg. NaF per kilogram per day on a white bread and milk diet. One group that was irradiated with ultra-violet showed better growth and fewer tooth defects than the other group. Neither group, however, compared favorably in growth and tooth development with a set that received only the bread and milk diet.

In comparing effects of rock phosphate with the same level of fluorine fed as NaF, Kick et al (95) found similar symptoms were produced by both except that rock phosphate produced certain pathological changes in the kidney which NaF did not produce. Smith and Leveton
(94) found that fluorine in the form of cryolite and calcium fluoride was much less toxic than the same amount of fluorine in the form of sodium, potassium, or ammonium fluoride. Ten times as much cryolite and 20 times as much calcium fluoride on the basis of their fluorine contents was required to produce the same effect that sodium, potassium, or ammonium fluorides produced. It was shown that 50 times as much fluorine in the form of cryolite as sodium fluoride was required to reduce the growth rate to one-half that of the control-fed litter mates. All the compounds of fluorine, when fed at a level of .0014%, showed the same effects on the teeth. At this level solubility ceased to be a factor and all compounds were equally toxic.

It has been shown in recent years that pyruvic acid arises from phosphoglyceric acid and that pyruvic acid is a normal glycolytic intermediate on the path to lactic acid. Fluorides have been shown to inhibit lactic acid production by specifically inhibiting the conversion of phosphoglyceric acid to pyruvic acid reaction (63) (64) (65) (68). Knowing these facts Phillips et al (95) attempted to cause a shift of metabolites in the body in order to alleviate the toxicity of fluorine. It was assumed that most of the energy metabolites came from a carbohydrate source. For that reason the carbohydrate portion of the diet was kept at a minimum and an excess of metabolites from dietary fat was fed in order to alleviate the effect of NaF. In addition to the shift in metabolites, lactates, lactic acid and glycerol were added to the diet at a level of 3% of the diet but failed to mitigate the toxic effects of sodium fluoride. The authors concluded that fluorine toxicosis in-
tolved more than the mechanism for carbohydrate metabolism and apparently was a generalized systemic action since growth was resumed when fluorine was removed from the diet. The effects noted were not due to a specific action on the digestive tract since fluorine injections produced the same effect.

Smith (96) has shown that dietary improvement does not prevent mottled enamel. Teeth that were defective in formation as a result of lack of calcium, phosphorus, vitamins A or C presented more serious damage but the effect was believed to be additive.

Sebrell et al (97) and Marcovitch et al (98) found evidence that NaF in drinking water produced a relative greater toxicity than the same quantity of NaF (500 p.p.m) in the diet. In explanation of these conclusions Sebrell et al (97) suggested that the differences were probably due to differences in the total quantity of NaF ingested, the more complete absorption, to a more rapid rate of absorption or to other factors not definitely determined. In a similar study, except that the intake of water was recorded in such a manner that the volume consumed was accurately determined, McClure (22) concluded that "the combined evidence is indicative of no variable effects on growth due to food versus water ingestion at levels of 22.6, 45.2, and 90.4 p.p.m. of fluorine in the form of sodium fluoride."

Relating to the toxicity of fluorine compounds is the work of Kempf et al (99) who showed that organic fluorides at levels of 500 and 1,000 p.p.m. were not as effective as inorganic fluorides in producing mottling. Alpha-fluoronaphthalene seemed more effective than p,p' -difluorodiphenyl p-fluorobenzoic acid or fluorobenzene. In this connection it might be
mentioned that Sharpless (100) showed that the presence of aluminum in the diet did not cause as serious an effect on the teeth at comparatively high levels as when fluorine was fed without aluminum. It was also shown that aluminum fluoride was less toxic than the other inorganic forms of fluorine. Evans and Phillips (28) found that 20 p.p.m. of aluminum gave slight protection against storage of fluorine, but none against bleaching. 100 p.p.m. was found to protect over a two-month period. It was interesting that aluminum in a ratio of 40 to 1 of fluorine did not prevent the storage of fluorine in the skeleton when the fluorine was carried in the milk.

Review of Methods for the Quantitative Determination of Fluorine in Foods.

The newer, more accurate and more rapid colorimetric and volumetric methods for the analysis of fluorine have practically done away with the tedious gravimetric methods of earlier days. This review deals only with the colorimetric and volumetric methods most commonly used at this time.

Steiger (101) and Merwin (102) showed that small amounts of fluoride may be estimated by their action on a titanium solution which had been oxidized by hydrogen peroxide. The method is accurate to .002 gram of fluorine and less than half the error of the best gravimetric methods is involved in this method.

Wichmann and Dahle (105) have studied many factors that influence the results. The determination of fluorides in materials containing phosphate was studied by Wagner and Ross (104). Adolph (105) proposed the use of a curve for the specific method in use by the individual laboratory.
Another method used was based on the bleaching of ferric thiocyanate in the presence of fluorides. The color is inversely proportional to the amount of fluorides present but may be used only in the presence of soluble fluorides when no interfering substances are present (106). Foster (107) (108) showed the color change was due to the combining of iron and fluorine. Unfortunately the alteration in color is not a straightline function but the alteration in color is definite and reproducible. It is of utmost importance to correct for all interference (109). The results obtained by this method agree well with the method of alizarin sodium sulfonate and the titration of distilled fluorine as hydrofluosilicic acid (110).

The same principle as used in the preceding method was applied to the use of the color of ferric salts in acetyl acetone (111). This method has been found more accurate than the thiocyanate method since it is less susceptible to sulfate and chloride interference. Its measurement has recently been made with a photoelectric cell (112).

The Sanchis method (113), which is in use at the present, is a modification of the original method (114) for the determination of fluorine in sea water which depends on the reaction of fluorine with sodium alizarin sulfonate and zirconium nitrate in acid solution to produce a series of colors varying from pink to yellow-green depending on the amount of fluorine present. This method as modified by Sanchis (115) eliminates the effects of sulfate and chloride by acidification of the sample with sulfuric and hydrochloric acids. This method is accurate to 0.2 p.p.m. in the absence of organic matter and is suitable for the determination of
fluorides in water but has not been shown conclusively to be applicable
to distillates of the Willard and Winter method (115).

An early method used by Gautier and Clausmann (11) should be
mentioned although it is no longer used. The method is very long and
tedious and involves the use of a specially designed hermetically sealed
apparatus. The fluorine is first precipitated as $\text{BaF}_2$. Next the fluorine
is volatilized as hydrofluoric and hydrofluosilicic acids. These are
condensed and absorbed in a suitable alkaline medium. The same procedure
is repeated only this time the evolved acids are allowed to condense on
lead glass to form lead fluoride. Finally the fluorine is precipitated
as lead sulfide and determined colorimetrically.

Willard and Winter (115) made the greatest contribution to
fluorine analysis in the last decade when they proposed the separation
of fluorine from substances which interfere with its determination by
distillation of hydrofluosilicic acid and its subsequent analysis by
titrination in a 50 per cent alcoholic solution with a standard solution of
thorium nitrate. Armstrong (116) had creditable success in simplifying
the procedure by eliminating the use of an alcoholic solution in favor of
an aqueous solution, and the use of an aqueous solution of sodium alizarin
sulfonate instead of a combined solution of zirconium and sodium alizarin
sulfonate in alcohol. Another modification by Armstrong (117) was his
introduction of a new micro-method for the determination of such small
quantities as 0.5 to 10 gammas of fluorine. At the same time he discovered
that perchloric acid introduced appreciable error when it was present
in the distillate. This was remedied by the addition of the sodium salt
of the acid in order to raise its boiling point. This procedure decreased the amount of perchloric acid that distilled over by 50%. In determining from 2 to 25 gammas of fluorine the average error varied from -0.3 to -2.0%. Since chlorides are volatilised as hydrochloric acid in the acidic distillation the use of silver perchlorate was suggested for the removal of chlorides. Armstrong (117) also used the contributions of Hoskins and Ferris (118) who introduced the use of monochloroacetic acid buffer and of Churchill, Bridges, and Rowley (119) who eliminated the effect of phosphates by a double distillation.

It should be stated that Roeley and Churchill (120) studied and applied the aqueous titration method to the determination of quantities of 1 to 50 mg. of fluorine, and Ebers, Lamb, and Lachele (121) studied the titration in alcoholic solution of quantities of 100 to 150 micrograms of fluorine.

McClure (122) was among the first to give Armstrong's micro-method a trial. He reported that for the best results the actual titration was carried out on at least 5 to 10 micrograms of fluorine requiring from 1.5 to 5.0 cc. of thorium nitrate. He concluded that "these limits are more in keeping with the limits of accuracy found in the actual titration and with the size of the blank titration figure."

In the light of the more recent refinements in methods for the quantitative determination of fluorine it is not surprising that the early workers could not confirm one another's findings.

From the data presented in the literature, at this time, it appears to me that further refinements of the Willard and Winter method (115) will lead to an acceptable method. One chief fault that will always be
found with the method as it now stands and as it is now used in the micro-method perfected by Armstrong (117) is the detection of the color change. In an attempt to overcome this fault Lee and Nilson (54) used a microburette with an accuracy of 0.0001 ml. in order that they could use a more concentrated solution of thorium nitrate to facilitate detection of the color change. In this laboratory we have thought of using a photoelectric colorimeter to detect the difference in color when various amounts of fluorine are added to constant quantities of thorium nitrate, buffer, alizarin red indicator, etc. It was found that a straight line relationship between fluorine and the development of color existed when no interfering ions were present. It is hoped that a study of effects of various ions will produce a method applicable to the distillates of the Willard and Winter method.
EXPERIMENTAL.

Fluorine in Plants vs. Fluorine in Soil

Method and Materials

In order to study the effect upon the fluorine content of plants of the addition of fluorides in various concentrations to soil, two widely separated areas of soil were chosen and prepared for planting. Calcium fluoride was added to one-half of the first area at the rate of 75 pounds per 518 square feet. The other large area was subdivided into four plots and NaF was added at the rate of 12.74 pounds, 25.48 pounds, and 50.98 pounds per 155 square feet to three of the four smaller plots. The remaining plots from the two large areas were designated the controls and were not treated with fluorides.

The source of calcium fluoride was fluorspar that was mined in the Serrita Mountains, southwest of Tucson, by Professor H. V. Smith and the author. This mineral was carefully separated from foreign material and ground to 100 mesh in a ball mill. This fine powder was then spread by hand over a measured area and turned into the soil by machine to a depth of six and two-thirds inches.

The sodium fluoride, on the other hand, was of commercial grade assaying 43.8% fluorine (Mefford Chemical Co., Los Angeles, Calif). This fluoride was mixed into the soil in a manner similar to that described for calcium fluoride except that the mixing was done with a spade. On the basis of calculations the concentrations of fluorine in these three plots was 300, 1600, and 5200 ppm.
Wheat was planted in the calcium fluoride and control plots in March 1959 and harvested four months later. A fluorine analysis of the soil at this time showed 998 p.p.m. in the calcium fluoride plot and 210 p.p.m. in the control plot.

Shortly after the wheat was planted in the calcium fluoride area tomatoes, carrots, beets, alfalfa, and yams were planted in the sodium fluoride area. Six weeks later (May) string beans were planted in the control and medium fluoride plots. After the wheat was harvested the field was cleared and made ready for planting. This time two rows each of soy beans, hegari and corn were planted in the calcium fluoride plot. The soy bean seeds were the variety Charlee and were grown in 1958 on the University of Arizona Farm at Mesa. The hegari seed was 1957 crop bagged head that had been treated with copper carbonate. The corn seed was not of a distinct type. It was grown in Arizona. The ears from which the seed was selected were short.

In harvesting the wheat, border rows and immature plants, which were few, were left standing. The stalk was cut at least six inches above ground level to avoid contamination from irrigation water and soil. The wheat was shocked as it was cut. After thrashing, the grain was re-cleaned twice by machine and once by hand. The growing time was approximately four months, i.e. from March 15 to June 12, 1959.

Hegari, soy beans, and corn were all planted the last week in July 1959. It was necessary to move each head of hegari with a paper bag about the first of October to preserve the grain from birds. On the fifteenth of October the hegari and soy beans were harvested. Three weeks later the
corn was cut. The first cutting of alfalfa was made on June 23, 1959
and subsequent cuttings at intervals of four to six weeks. The carrots,
tomatoes, beets and string beans were harvested during June and July
at the time they appeared best for consumption. Yams had the longest
growing period which lasted from the middle of March to late in October,

Method of Analysis

These foodstuffs were analyzed for fluorine by the method of
Willard and Winter (115) with the refinements introduced by Armstrong and
others, and summarized by Armstrong (117).

In every analysis except for milk and cooked foods, which were
analyzed on a fresh basis, the sample was air-dried and ground to a fine
powder. Usually the maximum size of the sample that was taken was determined
by the largest quantity that could be retained in a 250 cc. silica dish
when the sample was thoroughly wet and mixed with 5 grams calcium hydroxide
(Bakers C.P.). The wet sample, after having been dried first at 100°C, for
about 12 hours then for about 4-6 hours at 135°C., was ashed to a gray ash
in a muffle by a slowly rising temperature that reached a maximum of 800°F.
in about 12 hours. The ashing period required as long as 48 hours. The
ashed sample was transferred to a 250 cc. Claissen flask and 5 glass beads,
0.5 gram acid ignited silica, and 40 ml. 50 per cent boiled sulfuric acid
were added in that order to the flask which was connected to a condenser
and a collecting beaker of 400 cc. capacity containing about 5 ml. of 2
per cent sodium hydroxide. After a quantity of silver sulfate slightly
in excess of that required to combine with chlorides present in the
sample was added to the acidified ash, the flask was stoppered with a two
Fig. 1. Multiple-unit distillation apparatus for the distillation of fluorine by the Willard and Winter method. (Photographed by Dr. R. B. Streets)
hole rubber stopper carrying a thermometer (-5° to 200° C.) and a glass tube
drawn to a fine tip which connected to a liter Florence flask that supplied
steam to the distilling flask.

The heat was turned on simultaneously under the distilling and steam
flasks but steam was not allowed to pass into the distilling flask until
the temperature of the distilling flask reached 150° C. By controlling
the flow of steam entering the distilling flask, and the amount of heat
supplied directly to the flask, the temperature of the flask was kept at
155° ± 2° until 500–550 cc of distillate was collected. The distillate
was kept alkaline to phenolphthalein at all times by the addition of 2% sodium hydroxide solution.

This first distillate was evaporated down to a volume of 25 cc on
a hot plate at a temperature below boiling, and washed into a 250 cc.
Claissen flask. The solution was made acid with 60% perchloric acid and
12 cc. more were added along with 5 glass beads and 0.5 gram silica. The
same procedure was followed for this second distillation as for the first,
except that the final distillate was evaporated to a volume of 75 cc and
made up to 100 ml. in a volumetric flask.

A measured aliquot, the size of which was determined by a preliminary
titration, was placed in a 150 cc beaker and diluted with distilled water
to a volume of 100 cc. and 8 drops of .05% aqueous sodium alizarin sulfonate
were added. The red color was discharged with 1% hydrochloric acid and
1 ml. of monochloroacetic acid-sodium salt buffer was added, and the
solution titrated with standard thorium nitrate to a definite pink color.
The thorium nitrate solution employed gave the best results when the
titration did not exceed 1 ml. This quantity was equivalent to 0.1 milli-
gram of fluorine.

All the analyses were done in groups so that variations in reagents would be minimized. Each group was calculated on the basis of values found for the standard thorium nitrate, reagent blanks, and known quantities of fluorine run through the procedure used in analyzing the organic substances.

This was found to be necessary since 100% recovery of fluorine was not realized.

It was found that a multiple distillation unit (Figure 1) accommodating six distilling flasks as described by Reynolds (125) could be used to a great advantage when many analyses were made. The unit that was used in this work was only slightly different from that described by Reynolds. Instead of a single switch and rheostat to control the heat under all six flasks this unit was wired so that one switch controlled the heat under two flasks thus necessitating three switches. The ideal arrangement would be a switch for controlling the heat under each flask. Another difference was the use of larger flasks that necessitated larger holes over the heating element. The forms over the heating element were shaped from wet asbestos and allowed to bake in a drying oven for several days before moving them. In forming the hole through which the flask would be exposed to the heat from the heating element care was exercised to make the holes uniform in size (diameter of a 50 cent piece). In this way danger of volatilization of dry salts was eliminated.

* Monochloroacetic acid sodium-salt buffer: 9.4487 grams monochloroacetic acid and 2.0000 grams sodium hydroxide in 100 cc. distilled water.
Results.

Table I shows that an increased amount of fluoride in the soil, added in the form of calcium fluoride, increased the content of fluoride of wheat grain, wheat stalks and leaves, soy beans, and hegari grain. No appreciable difference was noted in corn. The largest difference in the grains was found in hegari which contained 0.0 p.p.m. from the control plot and 2.06 p.p.m. from the fluorine plot. Wheat stalks and leaves contained 5.7 p.p.m. from the control plot and nearly twice as much from the fluorine plot (7.2 p.p.m.)

In general, larger concentrations of fluorine and greater differences in fluorine content were found in the foodstuffs grown in soil fertilized with sodium fluoride. It will be noted (Table II.), however, that different foodstuffs were grown in the sodium fluoride-amended soil.

Consistently larger quantities of fluorine were found in alfalfa than in any other substance. Control alfalfa varied from 4.5 to 7 p.p.m. and the low-fluorine alfalfa from 8.9 to 14.0 p.p.m. Medium-fluorine alfalfa ranged from 10.75 to 15.0 p.p.m., and high alfalfa showed 39.5 in the second cutting and 16.1 in the fourth cutting. Comparisons of the fluorine contents of alfalfa grown in the low and medium and the medium and high-fluorine plots, with the exception of the 39.5 p.p.m. analysis, show smaller differences than the fluorine contents of the alfalfa from the low-fluorine and the control plots. It is interesting that in every case a larger concentration of fluorine in the soil produced a larger fluorine content in alfalfa. This statement also holds true for beets but not for carrots, tomatoes, or yams. String beans,
although grown in only two plots, showed no fluorine in either the control or medium-fluorine plots. The variations in fluorine contents of carrots and tomatoes cannot be correlated with the quantities of fluorine in the soil.

A possible explanation for some of the discrepancy in the results observed in yams may be in their unusual type of growth. It was observed that most of the yams in the control and low-fluorine plots were more or less the same size but the medium and high-fluorine plots exhibited numerous sizes and shapes. Several yams in the medium-fluorine plot weighed as much as eight pounds and exhibited large growth-cracks which could be explained by rapid growth in the inner layers that surpassed the rate of growth in the external layers so that the external layers ruptured under the internal stress. The large size would tend to give a larger quantity of substance that might contain less fluorine per unit of weight. If this were true it would explain the discrepancy noted in the analysis of yams from the medium-fluorine plot.

The unusual size of the yams in these plots containing large amounts of sodium fluoride suggested a stimulating growth effect produced by the fluorine. No stimulating effects on growth were observed in any of the other plants grown. However inhibitory effects on germination were noted in the medium and high-fluorine plots on alfalfa, and string beans and to a lesser degree on beets, carrots and tomatoes. After germination was complete no effects on growth were noted.

In the calcium fluoride plots no inhibitory or stimulating effects were observed in any case.
Fluorine in Milk vs. fluorine in Drinking Water.

In order to determine the effects upon the fluorine content of milk of additions of various concentrations of fluorine to the drinking water two cows were fed varying concentrations of fluorine-containing water. The milk secreted was analyzed for fluorine.

Method and Materials

A Guernsey cow (No 25. University of Arizona Herd) and a Jersey cow (No 112. University of Arizona Herd) were isolated in individual pens and were fed their usual rations but were not allowed to graze in the pasture. Fresh drinking water containing known quantities of sodium fluoride was made-up three times a week. Each level of fluorine water was given for seven days except that the Guernsey received the 238 and 495 p.p.m. fluorine water for two day periods only. Quart samples of the day's milking were taken the first two and last two days of each week. The milk taken for analysis was measured out in 450 ml. quantities and mixed with 3 gms. of calcium hydroxide in a beaker. This mixture was added to a 250 cc. silica evaporating dish and evaporated to dryness first on a water-bath then in a drying oven at a temperature of 135°C. The rest of the procedure is described in the "Method of Analysis".

The urine was obtained at will by massaging to one side of the vulva in a slow upward motion. This was usually done about the same time of day on each day that the sample was taken. The urine was measured in 50 cc. quantities directly into the distilling flasks. The only further change in analysis was the use of 25 cc. of 50% sulphuric acid
Instead of the 40 cc. commonly used in the first distillation of food ash, the rest of the procedure is similar to that described in "Method of Analysis".

Results.

Tables III, IV, V and VI summarize the data recorded in the experiment on the Guernsey and Jersey cows respectively.

The data shows that as large a concentration of fluorine as 500 p.p.m. did not increase the fluorine content above 0.4 p.p.m. in the Guernsey's milk nor above 0.5 p.p.m. in the Jersey's milk. It is significant however, that an increase in the fluorine content of the water caused a response in the milk. This is shown to be a true response since a decrease in the fluorine content of the drinking water lowered the fluorine content of the milk.

A concentration of fluorine as large as 258 p.p.m. in the form of NaF was found to impart a decidedly salty taste to the water. This was found to be responsible for the decreased water consumption that subsequently led to a lowered feed consumption and decreased milk production at levels of 258 p.p.m. or above in both cows.

It may be concluded that an increase in the fluorine content of the drinking water of a cow causes a definite increase in the fluorine content of the milk but this increase is very small.

Fluorine in Cooked Vegetables vs. Fluorine in Cooking Water.

In order to determine the effect upon the fluorine content of vegetables of cooking them in fluorine-containing water, vegetables, bought in the open market, were cooked in fluorine-containing water and subsequently...
analyzed, after cooking them, for fluorine content.

Method and Materials

Pink beans, potatoes, carrots, and oatmeal were cooked in 24.42 p.p.m. fluorine water as well as in distilled water. Aluminum cooking utensils were used for these foods but glass beakers were employed for all later work in which carrots, cabbage, spinach, cauliflower, Italian squash, Brussel sprouts and beets were cooked in 5 p.p.m. fluorine water. Prior to cooking, the vegetables were prepared in a manner generally employed in preparing vegetables for human consumption. Equal quantities of each vegetable were cooked in fluorine water of known concentration and in distilled water. After cooking the liquid was strained from the vegetable and was analyzed separately.

In an effort to ascertain whether the fluorine that is taken up by the food is present merely as absorbed fluorine water or is adsorbed or held in chemical combination by the food substance, carrots and cabbage were washed with liberal quantities of distilled water after cooking them in 5 p.p.m. fluorine water.

The freshly cooked vegetables and cooking water were analyzed by the method described in "Method of Analysis" after first being weighed and crushed in order to effect a more intimate mixture with the 5 grams of calcium hydroxide that was added as the fluorine-fixative.

Results.

The results presented in Tables VII, VIII and IX show that the fluorine contents of the vegetables tested increased when they were cooked in fluorine-containing water. Pink beans increased from 2.00 p.p.m. to 57.09 p.p.m., potatoes from 0.45 p.p.m. to 9.71 p.p.m., carrots from
0.0 p.p.m. to 20.08 p.p.m. and oatmeal, which took up all the cooking water in which it was cooked, increased from 0.39 p.p.m. to 22.80 p.p.m. when cooked in 24.42 p.p.m. fluorine water.

Of the vegetables cooked in 5 p.p.m. fluorine water cauliflower and cabbage showed the largest increases. When cooked in distilled water cauliflower contained 0.0 p.p.m. as compared with 4.28 p.p.m. when cooked in 5 p.p.m. water. Cabbage likewise contained 0.0 p.p.m. fluorine when cooked in distilled water and as much as 5.74 p.p.m. fluorine when cooked in 5 p.p.m. fluorine water. The smallest difference was found in beets cooked in 5 p.p.m. fluorine water. When cooked in distilled water they were found to contain 0.0 p.p.m. fluorine and from .95 - 1.7 p.p.m. when cooked in 5 p.p.m. fluorine water.

Washing carrots after cooking them in 5 p.p.m. fluorine water reduced the fluorine content from 4.74 p.p.m. to 5.5 p.p.m. The fluorine content of cabbage was reduced from an average value of 5.37 to 3.68 p.p.m., although one analysis showed as much as 5.56 p.p.m.

This would indicate that all the fluorine found in carrots and cabbage after cooking them in 5 p.p.m. fluorine water is not merely retained fluorine water but perhaps fluorine that has been adsorbed or has formed a chemical union with the vegetable.

The "taking up" of appreciable quantities of fluorine by the vegetables cooked in fluorine-containing water indicates the need for a biological study to determine whether or not the fluorine which has accumulated in the vegetable is as effective biologically as the same amount of fluorine in the water.
Discussion

Fluorine in Plants vs. Fluorine in Soil

The work of Gautier and Clausmann (15) showed that the fluorine content of the leaves was the greatest and varied from 5 to 14 p.p.m. per 100 grams of dried substance (or 50 to 140 p.p.m.). The results of this research bear out this general statement but the range in fluorine content is much less than found by these early workers. The greatest amount of fluorine present in any substance was 59.5 p.p.m. in alfalfa grown in a plot of soil to which 3200 p.p.m. of fluorine in the form of NaF had been added. It is interesting to note, in view of the fact that alfalfa is a very leafy plant, that all the analyses of alfalfa were higher in general than for any one of the other substances analyzed.

No mention was found in the literature of the relation of deposition of fluorine in plants to the chemical composition of the plant. However, in the animal body the relation of fluorosis to calcium and phosphorus metabolism was recognized by the effect of fluorosis on the bones and teeth. Is it not logical then that the fluorine is deposited in the part of the plant that is richest in calcium? This theory would hold for an explanation of the deposition of fluorine in the leaves of wheat plants which are relatively high in calcium. It would also explain the low-fluorine content of wheat grain which is low in calcium.

The question arises as to the low-fluorine content of milk, a substance high in calcium. This may be explained, perhaps, on the basis that the animal may have a protective action in the mammary glands.
The observation by Loew (124) that toxicity of NaF to plants is reduced when the soil is rich in calcium and phosphorus may explain why the inhibiting action on germination was observed in the medium and high-fluorine plots, but not in the low fluorine plot to any great extent. The fact that the soil used was highly calcareous fits into this explanation. The absence of these effects in the plot containing added calcium fluoride lends support to this supposition.

An interesting experiment by McIntire et al. (125) at the University of Tennessee throws light upon the fate of fluorides when available calcium is present in the soil or is added to the soil in the form of dolomite or limestone. It may serve to explain why larger quantities of fluorine were not found in the plots to which additions of NaF were made.

They found that a single addition of 1500 pounds of BaSiF₆ had induced an extensive formation of calcium fluoride through action of available calcium in the soil. When limestone was added the formation of calcium fluoride was enhanced and its solubility decreased probably through a common ion effect. However, dolomite allowed a greater amount of fluorine to be leached than could be explained if the fluorine was in the form of calcium fluoride(CaF₂). They concluded that this might be explained by the formation of the more soluble MgF₂ or perhaps even by a reduced common ion effect on the solubility of CaF₂. They also found that the outgo of fluorine through leaching from four equal additions of BaSiF₆ separated by an interval of one year, was proportional to that from a single addition. They concluded that the ultimate fate of fluorine would be the same for any type of fluoride regardless of the composition.

In the light of these findings it is possible that the NaF was
converted to CaF₂, at least in part, thus becoming less available to the plant because of the lower solubility of CaF₂.

This work may also explain the results obtained by Hart, Phillips, and Bohstedt (20) who found that plant materials from plots that had received fluorine-carrying phosphates such as rock phosphate and acid phosphates for periods of 16 to 36 years did not show consistent or greatly increased fluorine contents over plants grown on plots receiving a low-fluorine carrying phosphate such as bone meal.

The results of these experiments, although not strictly comparable with those of Hart et al (20) because of the different source of fluorine, do not agree with the preceding statement since significant differences were found in the fluorine contents of plants grown in soil containing added fluorine. These findings are in accord with those found by Gaud et al (28) in North Africa and Reid (24) in China. A study by Bartholomew (21) using a complete nutrient solution with NaF added in concentrations of 0 to 10 p.p.m. fluorine also tends to bear out these findings. Bartholomew also showed that fluorine in the form of NaF is more available to cow peas than is CaF₂ in a complete nutrient solution.

With the exception of yams no definite stimulating effect of fluorine on the growth of any plant foods was apparent by observation. Studies by others have revealed definite stimulating and retarding effects on growth and yield. Gautier and Clausmann (15) and Voelker (17) have reported effects on various cultivated plants, and Maze (128) has shown that fluorine is essential to growth of corn. Marcovitch (32) has reported that stunting is produced by an unexplained
Bean seedlings were reported to show normal growth in sand cultures containing $\text{KHCO}_3$ in a concentration of 1:500 and in a concentration of 1:2000 of $\text{NaF}$. Yet stunting occurred when the $\text{KHCO}_3$ and $\text{NaF}$ were combined in the same sand culture. In another experiment he showed that the stunting effect could be antagonized to allow normal growth of bean seedlings when dicalcium phosphate was added in a concentration of 1:80. This effect was explained by the supposition that an insoluble apatite removed the fluorine from solution.

**Fluorine in Milk vs Fluorine in Drinking Water**

A discussion of the influence of the level of fluorine in the drinking water of a cow to the fluorine content of the milk secreted has never been reported in the literature. A number of workers have shown that milk contains small quantities of fluorine but only a few cases of mottling of the temporary teeth have been reported (57).

Murray (127) reported that the results of rat experiments lead her to conclude that the small quantities of fluorine ingested by infants in the long human lactation period are significant. A study of her method of analysis for fluorine is disturbing since she used only a single distillation with perchloric acid and glass beads. No mention is made of precautions for removing phosphates or chlorides which would cause serious interference and variable results. In addition the method of Elvove (128) which is usually applied only to the analysis of water was utilized to estimate the fluorine in the distillate.

Phillips (31) studied the influence of fluorine ingestion upon the fluorine content of cow's milk both biologically and chemically and
concluded that by dietary means it was difficult to increase. In this connection it is interesting that work done at the Ohio Agricultural Experiment Station showed that it was exceedingly difficult to increase the iodine content of the milk by dietary means, so Devereaux (129) suggested the addition of iodine directly to the milk.

The findings presented in this study on cow's milk confirm the findings (31) that the fluorine content of milk is very low and is particularly difficult to increase by dietary means. It still remains to be seen whether this statement is true for humans. It is significant however that no previous work has shown that the fluorine content could be increased, however slightly.

In view of the findings of Smith (37) that severe mottling of the temporary teeth of breast-fed infants occurs, it appears that small amounts of fluorine in milk when ingested over a long period of time may, as concluded by Murray (128), be significant. This view is in accord with Evans and Phillips (124) who have shown that small but appreciable amounts of fluorine are transferred to the offspring of rats through milk although the fluorine in milk was less readily metabolized for storage than the same quantity of fluorine in the form of NaF.

Brinch and Roholm (130) reported the mottling of the teeth of two children of a woman who had worked in a cryolite factory. The temporary teeth had not been mottled. They concluded that the placenta had barred the passage of fluorine in utero so that no mottling of the temporary teeth occurred but that mottling of the permanent teeth was due to the fluorine that passed into the milk. This latter conclusion was based on estimations that the severity of mottling in these two cases was proportional
to the periods of lactation of 2 and of 1 1/4 years. In making such a conclusion they must have assumed that the temporary teeth were completely calcified at birth and could not have been affected by the fluorine. This view was shown to be erroneous by Smith (37) who found mottling of the temporary teeth in children who were artificially fed from birth on. The mother did not drink fluorine water during pregnancy but used 12 p.p.m. fluorine water in the milk formula.

On the other hand it is entirely possible that the quantities of fluorine ingested by the children observed by Brinch and Roholm were ineffective in altering the calcification of the rapidly calcifying temporary teeth but effective on the permanent teeth which calcify more slowly.

The early records of the effect of fluorine on the urinary system are few (3). A number of investigators have reported macroscopic changes in the kidneys of dogs (128), rats (127) (129) (130), pigs (130), and humans (6) after the ingestion of various quantities of fluorides.

The findings presented in this paper bear out the work of these various workers who have shown the presence of quantities of fluorine in the urine and who have assumed therefore that the kidneys were able to excrete, at least part, of the fluorine that may accumulate in the bloodstream.

Summary and Conclusions

1. Chemical analyses of food stuffs grown in soil to which CaF₂ in one concentration, and NaF in three different concentrations were added to different plots of soil, showed, by a comparison with the same substances grown in soil without added fluorine, that
(a) A larger concentration of fluorine in the soil tends to cause a larger deposition of fluorine in the plant.

(b) The amount of fluorine deposited in the plant is not proportional to the amount of fluorine present in the soil.

(c) In general, the substances grown in the calcium fluoride plots showed smaller quantities of fluorine deposited.

(d) There is a tendency for the fluorine to be deposited in greater quantities in certain parts of the plant. A possible explanation of this is discussed.

II. A Guernsey and a Jersey cow were fed fluorine-containing water varying in fluorine concentration from 0.2 to 500 p.p.m. fluorine, added in the form of NaF, directly to the usual drinking water. A very small but definite increase in the fluorine content of the milk was noted with an increase in fluorine concentration of the drinking water. The average maximum concentration of fluorine in the milk was 0.4 p.p.m. for the Guernsey's milk, and 0.5 p.p.m. for the Jersey's milk, when the drinking water contained 500 p.p.m. fluorine. The fluorine content of the urine increased significantly with an increase in the level of fluorine in the drinking water, indicating that the kidneys could remove excessive amounts of fluorine from the bloodstream.

III. Cooking foods in water containing fluorine in the form of sodium fluoride in concentrations of 24.42 p.p.m. and 5 p.p.m. fluorine resulted in greatly increased fluorine contents. Beans, carrots, potatoes, and oatmeal cooked in 24.42 p.p.m. fluorine water contained 13 to 25 times more fluorine than the same substance cooked in distilled water. A similar comparison of carrots, beets, cabbage, Brussel
sprouts, Italian squash and spinach cooked in 5 p.p.m. fluorine water showed increases in fluorine contents of 1 to 6 times in most cases and larger increases in several cases. Washing carrots and cabbage with distilled water after cooking them in 5 p.p.m. fluorine water removed only a small part of the fluorine "taken up" by these vegetables. This indicates that the fluorine withheld by cooked carrots and cabbage is adsorbed or forms a chemical union with the food substance. It likewise indicates the need for a biological study to compare the effects of fluorine "taken-up" by cooked foods with the same concentration of fluorine in water.
TABLE I.

Fluorine analyses of some foods and feeds grown in soil to which calcium fluoride ($CaF_2$) has been added.

Results based on dry substance

<table>
<thead>
<tr>
<th>Substance</th>
<th>Control plot</th>
<th>Fluorine plot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>parts per million</td>
<td>parts per million</td>
</tr>
<tr>
<td>Corn</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>Hagari</td>
<td>0.0</td>
<td>2.06</td>
</tr>
<tr>
<td>Soy Beans</td>
<td>0.85</td>
<td>1.25</td>
</tr>
<tr>
<td>Wheat</td>
<td>.69-.82</td>
<td>1.00</td>
</tr>
<tr>
<td>Wheat Stalks and Leaves</td>
<td>5.7</td>
<td>7.2</td>
</tr>
</tbody>
</table>
Fluorine analyses of some foodstuffs grown in soil to which sodium fluoride (NaF) has been added in various concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Control plot</th>
<th>Low fluorine plot</th>
<th>Medium fluorine plot</th>
<th>High fluorine plot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p.p.m.</td>
<td>p.p.m.</td>
<td>p.p.m.</td>
<td>p.p.m.</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>1.0</td>
<td>7.0</td>
<td>14.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Cuttings</td>
<td>2.0</td>
<td>4.5</td>
<td>8.9</td>
<td>10.75</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>5.0</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>6.0</td>
<td>9.4</td>
<td>11.25</td>
</tr>
<tr>
<td>Beets</td>
<td>1.7</td>
<td>6.5</td>
<td></td>
<td>17.7</td>
</tr>
<tr>
<td>Carrots</td>
<td>4.05</td>
<td>3.04</td>
<td>0.45</td>
<td>1.54</td>
</tr>
<tr>
<td>String-beans</td>
<td>0.0</td>
<td></td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Yams</td>
<td>0.0</td>
<td>7.56</td>
<td></td>
<td>8.19</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>0.75</td>
<td>0.53</td>
<td>2.32</td>
<td>0.51-1.22</td>
</tr>
</tbody>
</table>

*Low fluorine plot: 800 p.p.m. fluorine added
Medium fluorine plot: 1600 p.p.m. fluorine added
High fluorine plot: 3200 p.p.m. fluorine added

*Results on alfalfa based on dry weight; all others on fresh weight.
TABLE III

Table showing relation of level of fluorine intake in the drinking water of a Guernsey cow* to the fluorine content of the milk and urine.

<table>
<thead>
<tr>
<th>Fluorine content of drinking water</th>
<th>Water consumption</th>
<th>Fluorine content of milk</th>
<th>Fluorine content of urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>parts per million</td>
<td>liters per day</td>
<td>parts per million</td>
<td>parts per million</td>
</tr>
<tr>
<td>0.2</td>
<td>28</td>
<td>0.0</td>
<td>0.0-0.22</td>
</tr>
<tr>
<td>3.0</td>
<td>28</td>
<td>0.30</td>
<td>3.30</td>
</tr>
<tr>
<td>8.0</td>
<td>28</td>
<td>0.41</td>
<td>5.58</td>
</tr>
<tr>
<td>12.0</td>
<td>26</td>
<td>0.40</td>
<td>14.08</td>
</tr>
<tr>
<td>55.0</td>
<td>26</td>
<td>0.58</td>
<td>50.91</td>
</tr>
<tr>
<td>495.0</td>
<td>7</td>
<td>0.22</td>
<td>45.27</td>
</tr>
<tr>
<td>5.5</td>
<td></td>
<td>0.37</td>
<td>112.8</td>
</tr>
<tr>
<td>29.0</td>
<td>28.0</td>
<td>0.36</td>
<td>108.9</td>
</tr>
<tr>
<td>3.5</td>
<td></td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>45.5</td>
<td>0.39</td>
<td>48.91</td>
</tr>
<tr>
<td></td>
<td>21.1</td>
<td>0.17</td>
<td>54.32</td>
</tr>
</tbody>
</table>

1. No. 23 University of Arizona Herd.
<table>
<thead>
<tr>
<th>Date</th>
<th>Concentration of Fluorine in Drinking Water p.p.m.</th>
<th>Daily Milk Production</th>
<th>Concentration of Fluorine in Drinking Water p.p.m.</th>
<th>Daily Milk Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 5</td>
<td>0.2</td>
<td>11.4</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>0.2</td>
<td>11.2</td>
<td>29</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>0.2</td>
<td>10.7</td>
<td>1 Mar.</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>3.</td>
<td>10.5</td>
<td>2</td>
<td>55</td>
</tr>
<tr>
<td>9</td>
<td>3.</td>
<td>9.8</td>
<td>3</td>
<td>55</td>
</tr>
<tr>
<td>10</td>
<td>3.</td>
<td>9.6</td>
<td>4</td>
<td>55</td>
</tr>
<tr>
<td>11</td>
<td>3.</td>
<td>9.5</td>
<td>5</td>
<td>55</td>
</tr>
<tr>
<td>12</td>
<td>3.</td>
<td>10.2</td>
<td>6</td>
<td>55</td>
</tr>
<tr>
<td>13</td>
<td>3.</td>
<td>10.0</td>
<td>7</td>
<td>55</td>
</tr>
<tr>
<td>14</td>
<td>3.</td>
<td>10.5</td>
<td>8</td>
<td>55</td>
</tr>
<tr>
<td>15</td>
<td>3.</td>
<td>10.4</td>
<td>9</td>
<td>425.0</td>
</tr>
<tr>
<td>16</td>
<td>3.</td>
<td>10.2</td>
<td>10</td>
<td>425.0</td>
</tr>
<tr>
<td>17</td>
<td>8.</td>
<td>10.3</td>
<td>11</td>
<td>235.0</td>
</tr>
<tr>
<td>18</td>
<td>8.</td>
<td>9.9</td>
<td>12</td>
<td>235.0</td>
</tr>
<tr>
<td>19</td>
<td>8.</td>
<td>10.0</td>
<td>13</td>
<td>235.0</td>
</tr>
<tr>
<td>20</td>
<td>8.</td>
<td>9.6</td>
<td>14</td>
<td>0.2</td>
</tr>
<tr>
<td>21</td>
<td>8.</td>
<td>9.6</td>
<td>15</td>
<td>0.2</td>
</tr>
<tr>
<td>22</td>
<td>8.</td>
<td>9.5</td>
<td>16</td>
<td>0.2</td>
</tr>
<tr>
<td>23</td>
<td>8.</td>
<td>8.9</td>
<td>17</td>
<td>0.2</td>
</tr>
<tr>
<td>24</td>
<td>12.</td>
<td>8.7</td>
<td>18</td>
<td>0.2</td>
</tr>
<tr>
<td>25</td>
<td>12.</td>
<td>9.2</td>
<td>19</td>
<td>0.2</td>
</tr>
<tr>
<td>26</td>
<td>12.</td>
<td>9.1</td>
<td>20</td>
<td>0.2</td>
</tr>
<tr>
<td>27</td>
<td>12.</td>
<td>9.0</td>
<td>21</td>
<td>0.2</td>
</tr>
</tbody>
</table>

1. No. 23 University of Arizona Herd
2. Compiled by the Dairy Husbandry Department, University of Arizona.
**TABLE V.**

Table Showing Relation of Level of Fluorine Intake in the Drinking Water of a Jersey Cow to the Fluorine Content of the Milk and Urine.

<table>
<thead>
<tr>
<th>Fluorine content of drinking water</th>
<th>Water Consumption</th>
<th>Fluorine content of milk</th>
<th>Fluorine content of urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>parts per million</td>
<td>liters per day</td>
<td>parts per million</td>
<td>parts per million</td>
</tr>
<tr>
<td>0.2</td>
<td>24.0</td>
<td>0.0-.25</td>
<td>1.54</td>
</tr>
<tr>
<td>50.0</td>
<td>22.0</td>
<td>.27</td>
<td>21.12</td>
</tr>
<tr>
<td>19.0</td>
<td>15.0</td>
<td>.50</td>
<td>220.0</td>
</tr>
<tr>
<td>500.0</td>
<td>9.6</td>
<td>1.6</td>
<td>236.2</td>
</tr>
<tr>
<td>.2</td>
<td>no data</td>
<td>.51</td>
<td>no data</td>
</tr>
</tbody>
</table>

1. No. 112 University of Arizona Herd.
### TABLE VI

Table Showing the Daily Milk Production of a Jersey Cow While Drinking Water Containing Fluorine in Concentrations of 0.2 to 500 p.p.m.

<table>
<thead>
<tr>
<th>Date of Fluorine in the Drinking Water p.p.m.</th>
<th>Concentration of Fluorine in the Drinking Water p.p.m.</th>
<th>Concentration of Fluorine in the Drinking Water p.p.m.</th>
<th>Concentration of Fluorine in the Drinking Water p.p.m.</th>
<th>Concentration of Fluorine in the Drinking Water p.p.m.</th>
<th>Concentration of Fluorine in the Drinking Water p.p.m.</th>
<th>Concentration of Fluorine in the Drinking Water p.p.m.</th>
<th>Daily Production of Milk Pounds</th>
<th>Daily Production of Milk Pounds</th>
<th>Daily Production of Milk Pounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 20</td>
<td>0.2</td>
<td>13.2</td>
<td>Mar. 6</td>
<td>500</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>0.2</td>
<td>15.5</td>
<td></td>
<td>500</td>
<td>3.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>0.2</td>
<td>12.5</td>
<td>3</td>
<td>0.2</td>
<td>4.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>0.2</td>
<td>12.1</td>
<td>9</td>
<td>0.2</td>
<td>5.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>56</td>
<td>11.8</td>
<td>10</td>
<td>0.2</td>
<td>4.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>56</td>
<td>11.7</td>
<td>11</td>
<td>0.2</td>
<td>4.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>56</td>
<td>11.2</td>
<td>12</td>
<td>0.2</td>
<td>4.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>56</td>
<td>11.4</td>
<td>13</td>
<td>0.2</td>
<td>5.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>56</td>
<td>10.8</td>
<td>14</td>
<td>0.2</td>
<td>5.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>56</td>
<td>9.8</td>
<td>15</td>
<td>0.2</td>
<td>7.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar. 1</td>
<td>500</td>
<td>10.4</td>
<td>16</td>
<td>0.2</td>
<td>8.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>10.4</td>
<td>17</td>
<td>0.2</td>
<td>10.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>8.3</td>
<td>19</td>
<td>0.2</td>
<td>10.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>7.5</td>
<td>14</td>
<td>0.2</td>
<td>10.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>6.0</td>
<td>20</td>
<td>0.2</td>
<td>11.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. No. 112 University of Arizona Herd  
2. Compiled by the Dairy Husbandry Department, University of Arizona
### TABLE VII

Analyses of Foods Cooked in Fluorine-containing Water and Distilled Water

<table>
<thead>
<tr>
<th>Food</th>
<th>Fluorine Content of Cooking Water p.p.m.</th>
<th>Fluorine Content of Cooked Food p.p.m.</th>
<th>Fluorine Content of Water After Cooking p.p.m.</th>
<th>Fluorine Recovered per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pink Beans</td>
<td>24.42</td>
<td>37.09</td>
<td>5.87</td>
<td>94.19</td>
</tr>
<tr>
<td>Potatoes</td>
<td>24.42</td>
<td>9.71</td>
<td>15.92</td>
<td>98.97</td>
</tr>
<tr>
<td>Carrots</td>
<td>24.42</td>
<td>20.08</td>
<td>7.88</td>
<td>103.0</td>
</tr>
<tr>
<td>Oatmeal</td>
<td>24.42</td>
<td>22.30</td>
<td></td>
<td>93.77</td>
</tr>
</tbody>
</table>
TABLE VIII

The Effect of Washing with Distilled Water on the Concentration of Fluorine "taken up" by Vegetables Cooked in Fluorine-containing Water and Analyses of Foods Cooked in Fluorine-containing Water and in Distilled Water.

<table>
<thead>
<tr>
<th>Food</th>
<th>Conc. of fluorine in cooking water p.p.m.</th>
<th>Conc. of fluorine in water after cooking p.p.m.</th>
<th>Conc. of Fluorine in food p.p.m.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrots</td>
<td>5</td>
<td>2.69 - 3.00</td>
<td>3.50</td>
<td>Washed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.74</td>
<td>Not washed</td>
</tr>
<tr>
<td>Cabbage</td>
<td>0</td>
<td>Not tested</td>
<td>0</td>
<td>Not washed</td>
</tr>
<tr>
<td>Spinach</td>
<td>5</td>
<td>3.94</td>
<td>5.68 - 5.83</td>
<td>Washed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.94</td>
<td>5.00 - 5.74</td>
<td>Not washed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>not tested</td>
<td>4.16 - 4.23</td>
<td>Not washed</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>5</td>
<td>3.99</td>
<td>4.07 - 5.00</td>
<td>Not washed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>Not tested</td>
<td>2.09</td>
</tr>
</tbody>
</table>


TABLE IX
Analyses of Vegetables Cooked in Fluorine-containing Water and in Distilled Water

<table>
<thead>
<tr>
<th>Food</th>
<th>Fluorine content of cooking water p.p.m</th>
<th>Fluorine content of cooked foods p.p.m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italian Squash</td>
<td>5</td>
<td>2.7 - 3.8</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.24</td>
</tr>
<tr>
<td>Carrots</td>
<td>5</td>
<td>3.2 - 4.3</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2.23</td>
</tr>
<tr>
<td>Brussel Sprouts</td>
<td>5</td>
<td>2.9 - 5.5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.90</td>
</tr>
<tr>
<td>Beets</td>
<td>5</td>
<td>3.5 - 1.7</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.90</td>
</tr>
</tbody>
</table>
TABLE I.

Summary of Detailed Measurements
Recorded in the Cooking of Foods in Fluorine-Containing Water and in Distilled Water

<table>
<thead>
<tr>
<th>Food</th>
<th>Concentration of fluor-</th>
<th>Weight of food cooked</th>
<th>Volume of cooking water</th>
<th>Volume after cooking water used in cooking</th>
<th>Water cooking</th>
<th>Cooking time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p.p.m.</td>
<td>grams</td>
<td>c.c.</td>
<td>c.c.</td>
<td>pH</td>
<td>minutes</td>
</tr>
<tr>
<td>Carrots</td>
<td>24.42</td>
<td>657.5</td>
<td>650</td>
<td>396</td>
<td>5.9</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>605.0</td>
<td>800</td>
<td>283</td>
<td>5.9</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>420</td>
<td>500</td>
<td>498</td>
<td>5.5</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>500</td>
<td>408</td>
<td>5.4</td>
<td>25</td>
</tr>
<tr>
<td>Pink Beans</td>
<td>24.42</td>
<td>300</td>
<td>1645</td>
<td>486</td>
<td>——</td>
<td>405</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>2000</td>
<td>955</td>
<td>——</td>
<td>405</td>
</tr>
<tr>
<td>Potatoes</td>
<td>24.42</td>
<td>720</td>
<td>720</td>
<td>358</td>
<td>6.1</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>691</td>
<td>690</td>
<td>320</td>
<td>5.9</td>
<td>35</td>
</tr>
<tr>
<td>Oatmeal</td>
<td>24.42</td>
<td>300</td>
<td>1800</td>
<td>0</td>
<td>——</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>1800</td>
<td>0</td>
<td>——</td>
<td>3</td>
</tr>
<tr>
<td>Italian</td>
<td>5</td>
<td>400</td>
<td>600</td>
<td>499</td>
<td>5.4</td>
<td>25</td>
</tr>
<tr>
<td>Squash</td>
<td>0</td>
<td>400</td>
<td>600</td>
<td>490</td>
<td>5.4</td>
<td>25</td>
</tr>
<tr>
<td>Beets</td>
<td>5</td>
<td>500</td>
<td>1000</td>
<td>580</td>
<td>4.9</td>
<td>60</td>
</tr>
<tr>
<td>(root)</td>
<td>0</td>
<td>500</td>
<td>1000</td>
<td>430</td>
<td>5.1</td>
<td>60</td>
</tr>
<tr>
<td>Brussel</td>
<td>5</td>
<td>170</td>
<td>300</td>
<td>142</td>
<td>5.3</td>
<td>25</td>
</tr>
<tr>
<td>Sprouts</td>
<td>0</td>
<td>170</td>
<td>500</td>
<td>175</td>
<td>5.3</td>
<td>25</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>5</td>
<td>170</td>
<td>450</td>
<td>348</td>
<td>5.3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>170</td>
<td>450</td>
<td>385</td>
<td>5.5</td>
<td>15</td>
</tr>
<tr>
<td>Cabbage</td>
<td>5</td>
<td>550</td>
<td>1250</td>
<td>830</td>
<td>5.5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>550</td>
<td>1250</td>
<td>1080</td>
<td>5.5</td>
<td>30</td>
</tr>
<tr>
<td>Spinach</td>
<td>5</td>
<td>400</td>
<td>1500</td>
<td>1580</td>
<td>5.7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>400</td>
<td>1500</td>
<td>1505</td>
<td>5.7</td>
<td>7</td>
</tr>
</tbody>
</table>
BIBLIOGRAPHY

1. Smith, H.C., Lantz, E.L., and Smith, H.V.  
   The Cause of Mottled Teeth, A Defect in Human Teeth.  

2. Black, G.V. and McKay, F.S.  
   An Endemic Developmental Imperfection of the Enamel of the  
   Teeth heretofore Unknown in the Literature of Dentistry.  

3. McCollum, E.V., Simmonds, N., Becker, J.E. and Bunting, H.W.  
   The Effect of Additions of Fluorine to the Diet of the Rat  
   on the Quality of the Teeth.  
   J. Biol. Chem. 63:553 (1925)

4. McKay, F.S.  
   Progress of the Year in the Investigation of Mottled Enamel  
   with Special Reference to its Association with Artesian Water.  
   J. Am. Dent. Assoc. 5:721 (1918)

5. McKay, F.S.  
   Dental Cosmos 67:847-860 (1925)

6. McKay, F.S.  
   Water Works Engineering 79 (1926)

7. McKay, F.S.  
   Mottled Enamel.  
   J. Dent. Res. 8:553-565 (1928)

8. Roholah, Kaj.  
   Fluorine Intoxication, A Clinical-Hygienic Study.  

9. Johnson, B.L.  
   Phosphate Rock. Part I: General Information.  
   U. S. Bur. of Mines Inf. Circ. No. 6256 (1930)

10. De Eds, Floyd  
    Chronic Fluorine Intoxication.  
    Med. XII (1933)

11. Gautier, A. and Clausmann  
    Le fluor dans les eaux douces.  
    Compt. Rend. Acad. 158:1631 (1914)
12. Churchill, H.V.
   Ind. Eng. Chem. 25:996 (1931)

15. Gautier, A. and Clausmann, P.
   Le fluor dans le regne vegetal.

   Recherche et dosage des plus petites quantites de fluor dans
   les minerais, les eaux et les tissus vivants.
   Compt. Rend. 154:1469-75, 1670-7 (1912)

15. Gautier, A. and Clausmann, P.
   Action des fluorures sur la vegetation.
   Compt. Rend. Acad. Sci. 189:115-22 (1919)

16. Steinkoenig, L.A.
   Relation of Fluorine in Soils, Plants, and Animals.
   Ind. Eng. Chem. 11:465 (1919)

17. Voelcker, J.A.
   Pot Culture Experiments. The Influence of Fluorides on Wheat.
   J. Royal Agr. Soc. 82:292-95 (1921)

18. Cristiani, H. and Gautier, R.
   Etude de l' action des fourrages alteres par les emanations,
   des usines d' aluminium sur les animaux; la cachexie fluo-
   rique du betail.
   Compt. Rend. Soc. Biol. 95:912 (1925)

19. Cristiani H. and Gautier, R.
   Effects of Fluorized Forage on Herbivorous Animals.
   Compt. Rend. Soc. Biol. 92:159 (1925)

19 a. Gautier, N. and Clausmann, P.
   Influence du fluor sur la vegetation.

20. Hart, E.B., Phillips, P.H. and Bohstedt, G.
   Relation of Soil Fertilization with Super Phosphates and Rock
   Phosphate to Fluorine Content of Plants and Drainage Water.

   Fluorine, Its Effect on Plant Growth and Its Relation to the
   Availability to Plants of Phosphorus in Phosphate Rocks.
22. McClure, F.J.
Fluorides in Food and Drinking Water.
Natl. Inst. of Health Bull. No. 172

22a. Ibid. p. 37

23. Churchill, H.V., Bridges, R.W. and Rowley, R.J.
Interference of Phosphorus in the Determination of Fluorine.

24. Reid, E.
The Fluorine Content of Some Chinese Food Materials.
Chin. J. Physiol. 10, No. 2 p. 259 (1956)

25. Dahle, D. and Wichman, H.J.
Further Studies of Fluorine Distillation.

26. Gaud, M., Charnot, A., and Langlais, M.
Le darmous humain.

27. Reid, E. and Chang, R.G.
The Transference of Ingested Fluorine from Parent to Offspring.
Chinese J. Physiol. (1957)

28. Evans, R.J. and Phillips, P.H.
A New Low-Fluorine Diet and its Effect upon the Rat.

29. Boissevain, C.H. and Drea, W.F.
Spectroscopic Determination of Fluorine in Bones, Teeth and
Other Organs, in Relation to Fluorine in Drinking Water.
J. Dent. Res. 15:495 (1953)

30. Stuber, B. and Lang, K.
Small Amounts of Fluorine in Milk.
Biochem. Z. 212:96 (1929)

31. Phillips, P.H., Hart, E.B. and Bohstedt, G.
The Influence of Fluorine Ingestion upon the Nutritional
Qualities of Milk.
J. Biol. Chem. 105:125 (1934)

Cryolite Spray Residues and Human Health.
35. Smith, H.V., Smith, M.C., and Foster, E.O.
Nottled Enamel in the Salt River Valley and the Fluorine Content of the Water Supplies.

34. Lee, C.F. and Nelson, H.W.
Study of the Metabolism of Naturally Occurring Fluorine in Canned Salmon and Mackerel.
Bureau of Fisheries Investigation Report No. 44. (1939)

35. Schour, I. and Smith, M.C.
Injections of Sodium Fluoride on Enamel and Dentin of the Incisors of the Rat.

36. Schour, I. and Smith, M.C.
Nottled Teeth: An Experimental and Histologic Analysis.

37. Smith, M.C. and Smith, H.V.
The Occurrence of Nottled Enamel on the Temporary Teeth.

38. Smith, M.C.
Fluorine Toxicosis, a Public Health Problem.

39. Schulz, J.A.
Fluorine Toxicosis in the Albino Rat.

40. Brandle, J. and Tappeiner, H.
Ueber die Ablagerung von Fluorverbindungen im Organismus nach Futterung mit Fluornatrium.
Ztschr. Bio. 28:518 (1891)

41. Gay-Lussac, De M. and Berthollet
Sur la presence de l'acid fluorique dans les substances animales.
Ann. de Chimie 55(Series 1):258 (1805)

42. Wrampeymeyer, E.
Ueber den Fluorgehalt der Zahne.

43. Dean, H.T.
Endemic Fluorosis and its Relation to Dental Caries.
44. Dean, H.T., Jay, P., Arnold, F.H., McClure, F.J. and Elvove, E.

45. Cox, G.J.
New Knowledge of Fluorine in Relation to Dental Caries.
J. Am. Water Works Assoc. 51:1926-30 (1959)

46. Stanton, J.M. and Kahm, M.
Sodium Fluoride Poisoning.
J.A.M.A. 64:1985 (1915)

47. Trebitsch, F.
Ueber den Fluorgehalt der Zähne.
Biochem. Ztschr. 191:254 (1927)

48. Schulz, J.A.

49. Sonntag, G.
Chem. Abst. II 2691 (1917)

The Effect of Feeding Raw Rock Phosphate on the Fluorine Content
of the Organs and Tissues of Dairy Cows.
J. of Dairy Sci. 17:695-700 (1934)

51. Carnot, A.
Recherche due fluor dans les os modernes et les os fossiles.
Compt. Rend. Acad. Sci. 114:1189 (1892)

52. Smith, N.C.
Fluorine Toxicosis, a Public Health Problem.

53. Smith, N.C. and Smith, H.V.
The Occurrence of Mottled Enamel of the Temporary Teeth.

54. Phillips, P.H.
Plasma Phosphatase in Dairy Cows Suffering from Fluorosis.
Science 76:239 (1932)

55. Smith, N.C., and Lantz, F.M.
The Effect of Fluorine upon the Phosphatase Content of Plasma,
Bones and Teeth of Albino Rats.
J. Biol. Chem. 112:305-11 (1935)
56. Amberg, S. and Loevenhart, A.S.
Further Observations on the Inhibiting Effect of Fluorides on the Action of Lipase, together with a Method for the Detection of Fluorides in Food Products.
J. Biol. Chem. 4:149 (1908)

The Inhibiting Effect of Sodium Fluoride on Hepatic Lipase.
Am. J. Physiol. 90:426 (1929)

58. Clifford, W.M.
Effects of Fluorine on Pepsin Clotting of Milk.
Biochem. J. 22:1128 (1928)

59. Spiro, K.
Einige Ergebnisse über Vorkommen und Wirkung der weniger verbreiteten Elemente.
Ergebnisse Physiol. 24:474 (1925)

60. Kobert
Lehrbuch der Intoxikationen p.200

61. Calugaresanu, D.
Sur le pouvoir anticoagulant de fluorure de sodium.
Arch Internationale de Physiol. 2:12 (1904)

62. Schwyzser, F.
The Pathology of Chronic Fluorine Poisoning.
J. Med. Res. 10:501 (1903)

63. Embden, G. and Lange, H.
Untersuchungen über den Wechsel der Permeabilität von membranartigen Zellgrenzschichten und seine biologische Bedeutung.
Klin. Wschr. 3:129 (1924)

64. Embden, G. and Hentschel, H.
Über die Einwirkung von Fluorionen auf die Arbeitsfähigkeit und den Lactacidogenwechsel des Froschmodkels.
Biochem. Z. 156:343 (1925)

65. Lipmann, F.
Versuche zum Mechanismus der Fluoridwirkung.
Biochem. Z. 196:5 (1928)
Weitere Versuche über den Mechanismus der Fluoridhemmung und die Dissoziationskurve des Fluor-Methemoglobin.
Biochem. Z. 206:171 (1929)

66. Dickens, F. and Simer, F.
Biochem. J. 25:956 (1929)
67. Del Castillo, E.
Action des intoxications par le fluor sur le cycle oestral du rat blanc.
Compt. Rend. Soc. de Biol. 49:1405 (1928)

68. Chaneles, J.
Effets de la fluorose chronique sur les dents des rats blancs et action des rayons ultra violet.

69. Schulz, J.A. and Lamb, A.R.
The Effect of Fluorine as Sodium Fluoride on the Growth and Reproduction of Albino Rats.
Science 61:93 (1925)

70. Phillips, P.H., Lamb, A.R., Hart, E.B., and Bohstedt, G.
Studies on Fluorine in the Nutrition of the Rat. II. Its Influence upon Reproduction.
Am. J. Physiol. 106:356-64 (1935)

71. Rubuteau
Etude experimental sur les effets physical des fluorues, Paris (1867)

72. Schulz, H.
Untersuchungen über die Wirkung des Fluornatriums und der Flussaure.
Arch. Exp. Path. 25:527 (1889)

73. Baldwin, H.B.
The Toxic Action of NaF
J. Am. Chem. Soc. 21:517-21 (1889)

74. Tappeiner, H.
Zur Kenntnis der Wirkung des Fluornatriums.
Archiv. f. Exp. Path. und Path. 97:488-98 (1889)

75. De Long, Dwight, M.
Ohio J. of Science Vol. 54 (1934)

76. Siegfried, A.
Ein Beitrag zur Kenntnis des physiologisch-chemischen und pharmakologischen Verhaltens des kieselsauren Natriums, des Kieselfluornatriums und des Fluornatriums.
Arch. Inter. de Pharm. et de Ther. 9:228-34 (1901)

77. Brandl, J. and Tappeiner, H.
Über die Ablagerung von Fluorverbindungen in Organismus nach Fütterung mit Fluornatrium.
Ztschr. Biol. 28:513 (1892)
78. Machle, W. and Scott, E.M.
   Effects of the Inhalation of Hydrogen Fluoride. II. Fluorine
   Storage Following Exposure to Sub-lethal Concentrations.
   J. Ind. Hyg. 16:280 (1935)

   Record, P.R., Wilder, W., Hill, T.J., and Chase, S.W.
   Fluorine in Animal Nutrition.

80. Smith, H.C. and Lantz, E.M.
   The Effect of the Feeding of Fluorides upon the Chemical
   Composition of the Teeth and Bones of Albino Rats.
   J. Biol. Chem. 101:677-685 (1933)

81. Smith, H.C., and Lantz, E.M.
   The Effect of Fluorine on Calcium and Phosphorus Metabolism
   in Albino Rats.
   Am. J. of Physiol. 109:645-654 (1934)

82. Tule, C. and Maynard, L.A.
   A Study of Phosphatic Limestone as a Mineral Supplement.

83. Ellis, G. and Maynard, L.A.
   Effect of Low Levels of Fluorine Intake on Bones and Teeth.

84. McClure, F.J., and Mitchell, H.H.
   The Effect of Fluorine on the Calcium Metabolism of Albino
   Rats and the Composition of the Bones.
   J. Biol. Chem. 90:237 (1931)

85. McClure, F.J. and Mitchell, H.H.
   The Effect of Calcium Fluoride and Phosphate Rock on the
   Calcium Retention of Young Growing Pigs.

86. Hart, E.B., McCollum, E.V. and Fuller, J.G.
   The Role of Inorganic Phosphorus in the Nutrition of Animals.

   Wells, E.B., Hunt, C.H., Winter, H.R.
   The Utilization of Calcium Compounds in Animal Nutrition.

88. Hammet, F.S.
   Studies of the Thyroid Apparatus.
   Am. J. Physiol. 62:197 (1922)
65

69. Hammet, P.S.
   Studies of the Thyroid Apparatus.
   J. Biol. Chem. 57:285 (1923)

70. Goldemberg, L.
   Action biologique du fluor.

71. Chaneles, J.
   Action de l'iode sur la fluorose chronique.

72. Schlick, A.
   Die Wirkung des Chlorcalciums bei Florumatriumvergiftung nebst
   Versuchen über seine Wirkung bei Morphin- und Chlortalhydrat-
   vergiftung.
   Diss. München (1911)

   Record, P.K., Wilder, W., Hill, T.J., Chase, S.W.
   Fluorine in Animal Nutrition.

74. Smith, W.C. and Leverton, R.M.
   Comparative Toxicity of Fluorine Compounds.
   Ind. Eng. Chem. 26:791 (1934)

75. Phillips, P.H. and Hart, E.B.
   The Effect of Organic Dietary Constituents upon Chronic
   Fluorine Toxicosis in the Rat.

76. Smith, W.C.
   Dietary Factors in Relation to Mottled Enamel.
   J. Dent. Res. 15:281-289 (1936)

77. Sebrell, W.H., Dean, H.T., Elvove, E., and Breaux, R.P.
   Changes in the Teeth of White Rats Given Water from a Mottled
   Enamel Area Compared with Those Produced by Water Containing NaF.
   U.S. Publ. Health Rep. 48:457 (1933)

78. Marcovitch, S. and Stanley, W.W.
   A Comparison of Sodium Fluoride in the Drinking Water with Similar
   Levels of Cryolite in the Diet on the Fluorine Content of the
   Body.
   J. Nut. 16:175-181 (1938)

   Studies Relating to the Toxicity of Fluorine Compounds.
66

100. Sharpless, G.R.
   Limitations of Fluorine Toxicosis in the Rat with Aluminum Chloride.

101. Steiger, G.
   The Estimation of Small Amounts of Fluorine.
   J. Am. Chem. Soc. 30:219 (1908)

102. Merwin, H.E.
   Am. J. Sci. 23:119 (1909)
   Chem. Abstr. 4:2919 (1909)

103. Wichmann, H.J. and Dahle, Dan
   Determinations of Small Quantities of Fluorine.

104. Wagner, G.R. and Ross, W.H.
   J. Ind. Eng. Chem. 9:1116-25 (1917)

105. Adolph, H.M.
   J. Am. Chem. Soc. 37:2500 (1915)

106. Smith, W.K.
   Chem. Trade J. 71:325 (1922)

107. Foster, M.D.
   The Colorimetric Determination of Fluorine Using Ferric Chloride.
   J. Am. Chem. Soc. 54:4464-5 (1932)

108. Foster, M.D.

109. Dahle, D.
   Report on Fluorine in Foods.

110. Smith, H.V.
   The Determination of Fluoride in Drinking Water. Comparison of Several Methods and Establishment of Toxic Concentration by these Methods.

111. Armstrong, W.P.
   Colorimetric Determination of Fluorine.
112. Wilcox, L.V.
Photronic Colorimeter and its Application to the Determination of Fluoride.

113. Sanchis, J. M.
Determination of Fluorides in Natural Waters.

114. Thompson, T.G. and Taylor, H.J.
Determination and Occurrence of Fluorides in Sea Water.

115. Willard, H.H. and Winter, O.B.
Volumetric Method for Determination of Fluorine.

116. Armstrong, W.D.
Modification of the Willard-Winter Method for Fluorine Determination.

117. Armstrong, W.D.
Microdetermination of Fluorine. Elimination of Effect of Chloride.
Ind. Eng. Chem. 8:584 (1936)

118. Hoskins, W.M. and Ferris, C.A.
A Method of Analysis for Fluoride. Application to Determination of Spray Residue on Food Products.

119. Churchill, H.V., Bridges, R.W., and Rowley, R.J.
Interference of Phosphorus in the Determination of Fluorine.

120. Rowley, R.J. and Churchill, H.V.
Titration of Fluorine in Aqueous Solutions.

121. Ebers, W.F., Lemb, F.C. and Lachle, C.E.
Determination of Fluorine Spray Residue on Tomatoes.

122. McClure, F.J.
Microdetermination of Fluorine by Thorium Nitrate Titration.

123. Reynolds, D.S., Kershaw, J.B. and Jacob, K.D.
A Multiple-unit Distilling Apparatus for Determination of Fluorine by the Willard and Winter Method.
124. Loew, O.
   Über die Giftwirkung von Fluornatrium auf Pflanzen.
   Flora 94:330 (1905)

125. MacIntire, W.H., Shaw, W.M., Robinson, B.
   A Barium-Fluorine Study. The Fate of Added Barium Silico-fluoride
   and Its Effect upon Sulfates and other Soil Components, as In-
   fluenced by Limestone and by Dolomite.

126. Hase, P.
   Recherches de Physiologie Vegetale.
   Ann. Inst. Pasteur 28:21 (1914)

127. Murray, H.M.
   Maternal Transference of Fluorine.
   J. Physiol. 87:888-93 (1936)

128. Elvove, E.
   Estimation of Fluorides in Water.

129. Devereaux, E.D.
   Direct Iodizing of Milk is Possible.
   Vol. 11 No. 4 p.p. 135-194 (1929)

130. Brinch, O. and Rohlos, X.
   Zwei Falle von Mottled Enamel nach chronischer Fluorvergiftung
   der Mutter.
   Parodontium 6:1-8 (1934)

131. Herelke, O.
   Beiträge zur Kenntnis des Fluornatriums.
   Dtsch. Med. Wschr. 16:477 (1890)

132. Goldemberg, L.
   Goitre experimentale par le fluor.
   La Semana Med. 28:628 (1921)

133. Smyth, H.F. and Smyth, H.P.
   Relative Toxicity of some Fluorine and Arsenical Insecticides.
   Ind. Eng. Chem. 24:229 (1932)

134. Hauck, H.M., Stoebbeck, R. and Parsons, H.T.
   Is the Effect of Fluorine on Teeth Produced through the Para-
   thyroid Glands?
   Am. J. Physiol. 103:480 (1935)

   Effect of Fluorine on the Nutrition of Swine, with Special
   Reference to Bone and Tooth Composition.
Factors Affecting the Fluorin