Effects of Various Fluorine Compounds on the Albino Rat

Robert Floyd Pevahouse

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I am submitting herewith a thesis written by Robert Floyd Pevahouse entitled "Effects of Various Fluorine Compounds on the Albino Rat." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Charles S. Hobbs, Major Professor

We have read this thesis and recommend its acceptance:

Sam L. Hansard, Marshall C. Hervey, & Ollia E. Goff

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
June 17, 1948

To the Committee on Graduate Study:

I am submitting to you a thesis written by Robert Floyd Pevahouse entitled "Effects of Various Fluorine Compounds on the Albino Rat." I recommend that it be accepted for nine quarter hours credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

[Signature]
Major Professor

We have read this thesis and recommend its acceptance:

[Signatures]

Accepted for the Committee

[Signature]
Dean of the Graduate School
EFFECTS OF VARIOUS FLUORINE COMPOUNDS ON THE ALBINO RAT

A THESIS

Submitted to
The Committee on Graduate Study
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

by
Robert Floyd Pevahouse
August 1948
ACKNOWLEDGMENT

The author would like to acknowledge his indebtedness to Dr. Charles S. Hobbs and Professor Sam L. Hansard for their many helpful and constructive suggestions offered during the course of the work and in the preparation of this paper. He would also like to express his appreciation to Dr. Marshall C. Hervey and Dr. Ollie E. Goff for many constructive suggestions offered as the work progressed.

Due acknowledgment is also expressed to the Department of Home Economics for supplying the experimental animals and to the Chemistry Department of the Agricultural Experiment Station for doing the chemical analysis of the bones.

R.F.P.
FOREWORD

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INTRODUCTION

At the beginning of the 19th century, an Italian chemist demonstrated the presence of fluorine in the teeth of both man and animals. This discovery created considerable interest at that time and can probably be considered the initial step in stimulating research on the problem.

In the light of our present day knowledge, there is evidence that the ingestion of small amounts of fluorine may be distinctly beneficial to livestock. However, the primary problem in animal nutrition is concerned with definitely harmful effects resulting from an excessive intake.

It was only a few years ago that farmers were using raw rock phosphate as a mineral supplement in rations for livestock. Although raw rock phosphate might supply the desired mineral ingredients at a lower cost than they might be obtained from other sources, it was learned that rock phosphate produced abnormal calcification of the teeth and possibly other changes which greatly limited its usage. These changes were the same as could be produced experimentally by using a chemically pure fluorine salt.
Observations in Europe, and in recent years in this country, show that cattle grazing near industrial plants which give off fluorine in the smoke, such as aluminum or superphosphate plants, show excessive wear on the teeth, decrease in the rate of growth, and in some cases cause premature death.

Since Tennessee is one of the largest phosphate producing states, and since industrial plants have started a rather rapid program of expansion, there is a need for more information on the problem of chronic fluorosis.

The experiments reported in this thesis were designed primarily to study the relative toxicity of various fluorine compounds in the albino rat. Experiments have been conducted to study the concentration of sodium fluoride and length of time of feeding necessary to produce fluorosis; to biologically test the fluorine concentration of samples of hay obtained near industrial plants known to give off fluorine substances; and to investigate substances in the ration which might offer promise of alleviating the toxic effects of fluorine in the albino rat.
LITERATURE REVIEW

Fluorine occurs most abundantly in nature in the mineral fluorspar (CaF$_2$) and in cryolite, a double fluoride of aluminum and sodium. Minerals containing small amounts of fluorine as biotite, tourmaline, muscovite, and apatite, distributed throughout the earth's crust, accounts for a part of the fluorine found in soils (24).

Jacobs and Reynolds (18) have investigated the fluorine content of various commercial grades and types of phosphate rock. The amount of fluorine found varied from 2.62 per cent in Tennessee phosphate "run of mine material" to 4.08 per cent for "carefully selected lump rock." A fertilizer grade of the same rock contained 3.72 per cent fluorine, while a high grade washed and ground rock contained 3.89 per cent. These values are similar to those obtained for samples of phosphate rock occurring in North Africa, Idaho, Wyoming, and Florida.

Gautier and Clausman (14), on examination of 63 plant substances found an average of 0.00265 per cent fluorine in the fresh material. Churchill, Royland, and Martin (10) reported the fluorine content of alfalfa hay varied from 0.0015 per cent fluorine in the first cutting to 0.0037 per cent fluorine in the third cutting. A green sample of this third cutting contained 0.0053 per cent
fluorine. Further investigation by the same workers showed alfalfa leaves to contain 0.0046 per cent fluorine while the alfalfa stems contained 0.0013 per cent fluorine. If a forage is exposed to gasses or smoke containing fluorine, the analysis for fluorine will increase greatly according to the work of Meyn and Viehl (26) and Bardelli and Nezani (2).

The fluorine content of so called "normal" bones varies greatly. This may be due partially to the method of analysis. Majumdar, Ray, and Sen (21) observed that the fluorine content of bones and teeth increased with age. This is especially true in the pelvis where the concentration of fluorine per unit weight of bone is proportional to the age of the animal. The pelvis of a 3.5 year old bull contained 27 milligrams of fluorine (0.02 per cent) in a 100 gram sample. A 5.5 year old bull contained 73.9 milligrams fluorine (0.07 per cent) in the same size sample. McClure (24) summarized the results of several different investigators and set the average at from 0.01 to 0.03 per cent fluorine in normal bones. Normal dental enamel contained 0.10 to 0.20 per cent fluorine. McClure (24), in reviewing the work of Trebitsch, suggested that the hardest apatite, namely, fluor-apatite, $3\text{Ca}_3\text{(PO}_4)_2\cdot\text{CaF}_2$ occurs in dental enamel and that the hardness of teeth depends on the presence of crystal masses of apatite.
There is evidence that the fluorine content of bones and teeth, as well as other body organs and tissues, may be increased by an increase in the fluorine intake. Bethke et al. (4) found that the fluorine content of the ash of femurs of pigs varied directly with the amount of fluorine in the ration. When pigs received 2 pounds of limestone or 2 pounds of steam bone meal as a mineral supplement, the fluorine content of the bone ash was 409 and 231 parts per million respectively. However, when 100 grams of sodium fluoride was added to the 2 pounds limestone or when 2 pounds rock phosphate was fed, the bone ash contained 11,077 and 10,934 parts per million of fluorine respectively.

McClure (24) in reviewing the work of Sonntag, reported that, whereas normal teeth and bones contain about 0.3 per cent fluorine, the teeth of animals fed sodium fluoride contain 1.73 per cent fluorine and the bones contained 1.29 per cent fluorine. Schultz (30) reported that the chemical analysis of the tibia, femur and humerus of several hundred rats have shown that the bones of rats fed moderate to large doses of fluorine contain increased percentages of fluorine and magnesium and decreased percentages of carbonates (carbon dioxide). While these abnormalities in the chemical composition of the bones of rats are not
strictly proportional to the amounts of fluorine in the rations, they do vary to a definite degree with the percentage of fluorine fed. Kick et al. (20) suggest that an increase in the fluorine content of the ration will cause an increase in the fluorine content of the bones. The increase was greater when sodium fluoride was fed than when calcium fluoride was fed. Meyn and Viehl (26) reported that the bones of cattle with chronic fluorine poisoning contained 7 to 9 times as much fluorine as was found in the bones of sound animals. This figure is based on their findings of 0.48 milligrams fluorine per gram in the lower jaw bone of normal cattle and 4.68 milligrams per gram in infected animals.

The rat is the laboratory animal most commonly used in fluorine investigations. For this reason there is more information available as to the toxic levels of the different fluorine compounds in the rat than in any other animals. Some of the most complete work has been carried on by Schultz (30, 31, 32), Bethke et al. (3, 4), McClure and Mitchell (25), Smith and Leverton (35).

Macroscopic examination of the incisor teeth for abnormal color and growth is the criterion most commonly used to determine fluorosis in rats. Schultz (30) described the teeth of rats fed excessive amounts of fluorine as being
white with an excessive overgrowth of the upper incisors
and excessive wearing or breaking off of the lower incisors.
Different investigators have required different amounts of
fluorine to produce this effect. McClure and Mitchell
(25) found that rats fed 0.0313 per cent fluorine in the
form of sodium fluoride showed the abnormalities. Schultz
(30) obtained a loss of the pink natural color of the teeth
when as little as 0.01 per cent fluorine was fed in the
form of sodium fluoride but did not get an overgrowth of
the incisors until 0.024 per cent was fed. This is in
accord with Kick et al. (20) who reported normal shape of
teeth when fluorine combined as sodium fluoride was fed at
a 0.01 per cent level or 0.05 per cent when combined as
calcium fluoride. However, when the sodium fluoride was
increased to 0.02 per cent, the mandibular incisors showed
considerable wear, while the maxillary incisors became
greatly elongated. Smith and Lantz (33) reported that the
feeding of sodium fluoride to rats at a 0.024, 0.05 and 0.1
per cent of the ration had in each case interfered with the
normal calcification of the teeth to an extent varying with
the concentration. Other workers have reported approximately
the same results.

The amount of sodium fluoride necessary to produce
the characteristic abnormalities may be dependent to some
extent on the length of time the rats are on ration. According to Maynard (23), fluorine is a cumulative poison. At first the fluorine merely accumulates in the bones and teeth without evident harm and considerable time elapses before structural injury becomes evident.

In most of the toxicity studies of the different fluorine compounds, sodium fluoride has been used as a basis for comparison. Growth retardation, as well as calcification of the teeth, has been used to measure these differences. Evans and Phillips (12) did a comparative study with synthetic cryolite and sodium fluoride. A basal ration was supplemented to contain 0.06, 0.03 and 0.015 per cent fluorine from both carriers. The rats were kept on experiment for twelve weeks. The growth rate of the rats receiving 0.03 per cent sodium fluoride was similar to those receiving 0.06 per cent fluorine in the form of cryolite. Similar results were obtained with the 0.015 per cent sodium fluoride and 0.03 per cent cryolite. Apparent normal growth occurred in the lower levels of fluorine regardless of the source. This does not agree with the work of Smith and Leverton (35) who required ten times as much fluorine combined as cryolite to produce the slightly stunted growth caused by 0.0226 per cent sodium fluoride. It should be noted however that Smith and Leverton (35)
used a natural cryolite which is approximately half as soluble as synthetic cryolite.

The work done at the University of Arizona by Smith and Leverton (35) indicated that calcium fluoride was even less toxic than cryolite from the standpoint of growth interference in the rat. It required a concentration of 0.452 per cent fluorine in the form of calcium fluoride to produce the same slight stunting of growth obtained with 0.0226 per cent fluorine in the form of sodium fluoride. This is twice the concentration which interfered with the growth of the animals when the fluorine was in combination as cryolite and twenty times the amount necessary when it was combined as sodium fluoride. They attribute this partially to the fact that sodium fluoride is approximately one thousand times more soluble than calcium fluoride, and at least one hundred times more soluble than cryolite. This comparison is rather high when compared to that of Kick et al. (20), who required 0.05 per cent fluorine in the form of calcium fluoride to produce the same toxic effects as 0.01 per cent as sodium fluoride, and Schultz (31) and McClure and Mitchell (25) who reported calcium fluoride to be approximately half as toxic as sodium fluoride.

Many reports have been published as to the effect the fluorine compounds have on the teeth of rats and
humans when incorporated in the ration at a very low level. Smith et al. (33, 34), Marcovitch (22), DeEds and Thomas (11), and others, have found a concentration of approximately 0.0014 per cent fluorine in the feed will cause a slight striation or motteling of the teeth. Smith and Leverton (35) conclude that the amount of fluorine required to cause initial damage to the rat incisor was so small that difference in solubility of the compounds were not a factor, and no difference in toxicity of fluorine from the various compounds studied could be noted.

Since the various fluosilicates are used as a spray material, most of the work done with them has been in the low level just described. The work of Smith and Leverton stated, however, that there was no difference in the lethal concentration or the effects upon the teeth of the rat when the fluorine was in the form of sodium fluoride, potassium fluoride, barium fluosilicate, or sodium fluosilicate.

Cattle, both beef and dairy, are affected by excessive intake of fluorine. Reed and Huffman (28) at the Michigan Agricultural Experiment Station, studied the effect of adding rock phosphate to the ration of mature dairy cows. They reported that the animals were thrown off feed when 1.5 per cent of rock phosphate containing 3.5 per cent fluorine was added to the grain ration. Taylor (36)
obtained approximately the same results when rock phosphate containing approximately 3.0 per cent fluorine was fed at the same levels. The animals presented a general unthrifty appearance and their teeth showed an abnormal amount of wear. When calcium fluosilicate was fed at the same levels, similar results were obtained. Phillips, Hart, and Bohstedt (27) required only 0.625 per cent raw rock phosphate in the grain ration to produce typical fluorine toxicosis. This raw rock phosphate contained approximately 3.55 per cent fluorine. More time was required to produce the symptoms of fluorine poisoning at this level of feeding than was required for the higher levels. Huffman and Reed (17) made a comparative feeding test of long duration in which they compared a mineral mixture of bone flour, rock phosphate, limestone, and a complex mineral mixture at a level of 1.5 per cent of the grain ration. They found that the animals receiving rock phosphate which contained 3.5 per cent fluorine made slower gains, presented a more unhealthy appearance, produced less milk and showed more wear on the teeth than did the other animals. When the cattle were approximately 2.5 years old, the teeth were worn to such an extent that they were sensitive to cold water. Some teeth were worn to such an extent that the pulp cavities were exposed. There did not appear to be any effect on reproduction.
Meyn and Viehl (26) described fluorine poisoning in cattle, which was obtained from eating feed produced near an industrial plant which gave off smoke containing fluorine, as follows:

Most of the animals were found to be undernourished to varying degrees. In about a third of the animals, deep hunger hollow, strongly protruding hip and seat bones, and distinctly separated ribs were the sign of severe emaciation. The skin of the animals were dry and hard. In the region of the thorax the skin lay fairly taut over the ribs and it was difficult to make a fold in it. In most animals, the hair was dull and lifeless, in some it was coarse and rough. Many cows stood motionless, with bent heads, in front of filled cribs without any desire to eat. The appearance of some of them was really pitiable. A young milch cow with no udder trouble gave only 1 litter of milk a day. Six cows had pronounced pains in the joints. They disliked getting up and hardly dared to move from one spot. It took energetic driving to get them to move aside, stepping carefully, with shaking limbs, bent backs, and anxious exophthalmic glances. These cows were in a particularly undernourished condition. Almost without exception, these animals had serious tooth damage. The damage consisted mainly of: discoloration, with or without injured spots in the dental enamel; an unnatural, often considerable degree of wear of the incisors.

Forbes and co-workers (13) were among the first to report poor results with swine when rock phosphate was used as the mineral supplement. They found it to be less efficient than limestone, steam bone meal, precipitated bone or calcium carbonate in improving bone formation and calcium and phosphorus retention of growing pigs. Enough
of each supplement was fed to supply 10 grams of calcium per head per day. This amounted to approximately 18 grams of the raw rock phosphate.

The experiment of Bethke, et al. (3) showed that fluorine in the form of sodium fluoride, when added to the ration of growing swine in the amount of 30 grams per 100 pounds of feed, lowered the feed consumption and thereby decreased the daily gains. The decreased feed consumption in lactating sows caused them to loose weight rapidly and they failed to return to normal weight after the pigs were weaned. The teeth of sows kept for two or more years on a ration high in fluorine were frequently worn to the gums and the pulp cavities exposed.

Kick et al. (20) conducted feeding experiments with swine on a long time basis. Varying amounts of sodium fluoride and rock phosphate was added to the mineral supplement. At the conclusion of the experiment, the basal group which had received no fluorine supplement had femurs that presented a normal yellowish color, with smooth exteriors, a dense appearance, and a decided luster. The bones of animals in the other lots had a lusterless appearance, were white in color, had many protuberances, and showed a decided thickening. The only group that showed a significant difference in breaking strength was the high
level of phosphate, which showed a considerable decrease in strength over the control group. Short time experiments with swine by the investigators showed the same conditions but not as marked as over a long time period.

Soft bones occurred in both pigs and rats which had been fed on rations containing rock phosphate at the Arkansas Experiment Station (1). Bohstedt and co-workers (5) found no ill effects on growth and fattening when pigs were fed rock phosphate at a level of 0.4 per cent. In some cases 0.8 per cent of rock phosphate retarded growth, while 1.6 per cent had a harmful effect on both rate and economy of gain. The fluorine content of this particular rock phosphate was not given. However, it was of the kind or grade known as lime phosphate.

The experimental work to determine the toxic level of fluorine in the sheep is rather limited. However, Greenwood (15), in reviewing the work of Pierce, described sheep fed various levels (60-170 micrograms daily) of fluorine in the form of rock phosphate for a period of three years. All of the animals appeared to be in good health for the first year. Thereafter, some of them began to eat less and lose weight. The bones of the animals which received doses greater than 120 micrograms of fluorine daily exhibited the bone changes described by Kick et al. (20). No organs, except the bones and teeth, appeared to be affected by the
treatment.

There seems to be varying opinions as to the ability of the chick to withstand fluorine in the ration. Buckner, Martin, and Insko (7) reported that rock phosphate interfered with the growth of chicks for the first few weeks but did permit normal growth after that time. Kennard and White (19) reported that steam bone meal and rock phosphate appeared to be equally efficient in mineral mixtures fed to pullets or cockerels. No difference was noted on growth or egg production when mixtures consisting of equal parts of limestone, salt, and either steam bone meal or rock phosphate was fed. Buckner, Martin, and Peter (6) did report, however, that mature birds receiving rock phosphate were troubled with diarrhea and egg production was somewhat reduced. Halpin and Lamb (16), after studying various levels of feeding rock phosphate containing 3.52 per cent fluorine, concluded that no harmful effects were indicated at a 1 per cent level; some depression of growth resulted at a 2 per cent level; and seriously harmful effects were observed at a 3 per cent level.

The solubility of some of the more important fluorine compounds in water have been reported by Carter (8, 9) and Seidell (29) as follows:
<table>
<thead>
<tr>
<th>Compound</th>
<th>Grams/100 cc water</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium Fluoride</td>
<td>48.70</td>
<td>$20^\circ$ C.</td>
</tr>
<tr>
<td>Magnesium Fluosilicate</td>
<td>23.53</td>
<td>$20^\circ$ C.</td>
</tr>
<tr>
<td>Calcium Fluosilicate</td>
<td>10.58</td>
<td>$20^\circ$ C.</td>
</tr>
<tr>
<td>Sodium Fluoride</td>
<td>4.05</td>
<td>$25^\circ$ C.</td>
</tr>
<tr>
<td>Sodium Fluosilicate</td>
<td>0.65</td>
<td>$17.5^\circ$ C.</td>
</tr>
<tr>
<td>Potassium Fluosilicate</td>
<td>0.12</td>
<td>$17.5^\circ$ C.</td>
</tr>
<tr>
<td>Synthetic Cryolite</td>
<td>0.06</td>
<td>$25^\circ$ C.</td>
</tr>
<tr>
<td>Natural Cryolite</td>
<td>0.04</td>
<td>$25^\circ$ C.</td>
</tr>
<tr>
<td>Calcium Fluoride</td>
<td>0.004</td>
<td>$25^\circ$ C.</td>
</tr>
</tbody>
</table>
EXPERIMENTAL

The general plan of the experiment was to feed growing albino rats different fluorine compounds, or in some cases varying levels of the same compound, over a period of time necessary to determine the level that would produce chronic fluorine poisoning. The rats were killed and the long bones and lower jaw bone removed and analyzed for fluorine content.

Rats ranging in age from 22 to 30 days were obtained from the stock colony in the Department of Home Economics. Three rats were placed in each cage. They were sexed to the extent that each cage contained one male and one female, the third being selected as the supply on hand permitted.

The rats had access to a stock diet before weaning, and some were maintained on this diet up to seven days after weaning. This diet was very low in fluorine.

All experimental animals were confined in round wire cages approximately nine inches in diameter and ten inches high with an elevated bottom. The feces and urine were collected on paper placed under each cage. This paper was changed daily. The feed jars and water bottles were cleaned as often as considered necessary for sanitary purposes. The animals received distilled water ad lib.
The laboratory was heated with a thermostatically controlled electric heater and a temperature of 75° to 80°F. maintained at all times.

At the conclusion of each experiment the rats were killed by etherization and the meat removed from the long bones and lower jaw bone by gross dissection followed by boiling 1.5 hours in three gallons of water to which had been added 100cc of soap powder. The bones were allowed to stand over night to dry and then delivered to chemistry department for fluorine analysis.

**Feeding Data**

A synthetic basal ration was used which consisted of the following ingredients:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>42.0</td>
</tr>
<tr>
<td>Dehydrated Alfalfa Meal</td>
<td>30.0</td>
</tr>
<tr>
<td>Casein</td>
<td>15.0</td>
</tr>
<tr>
<td>Fat (Snowdrift)</td>
<td>7.0</td>
</tr>
<tr>
<td>Cod Liver Oil (5 USP units of D per gram feed)</td>
<td></td>
</tr>
<tr>
<td>G.B.I. Salt Mixture No. 2</td>
<td>3.0</td>
</tr>
<tr>
<td>NaCl (Iodized)</td>
<td>1.0</td>
</tr>
<tr>
<td>Brewers Yeast</td>
<td>2.0</td>
</tr>
</tbody>
</table>

This basal ration was supplemented with the different fluorine compounds to make up the potency calculated for each ration. This was computed on the molecular concentration of each fluorine carrier used.
A general analysis of the basal ration showed the following results:

<table>
<thead>
<tr>
<th>Component</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>18.22</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>10.18</td>
</tr>
<tr>
<td>Protein</td>
<td>6.53</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0015</td>
</tr>
<tr>
<td>Fluorine</td>
<td>0.581</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.445</td>
</tr>
</tbody>
</table>

The ration was not changed throughout the feeding period. The quantity of feed given was regulated according to the demands of the animals.

In cases where a hay was to be studied for its fluorine content, a finely ground portion of this hay was substituted for the dehydrated alfalfa leaf meal in the basal ration.

The different fluorine compounds fed, along with the concentration and length of time on feed, are shown in Table I.

**Weight Records**

The rats used in this experiment were placed in their respective cages and given a lot number. Each rat was individually marked within each lot. All animals were weighed for three consecutive days and the average of these weights used as the initial weight. The second day of the three day series was used as the starting date of the
## Table I

**Concentration of Different Fluorine Compounds Used, Length of Time on Ration and Fluorine Analysis of Bones**

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Fluorine Compound Used as Supplement</th>
<th>Calculated Concentration</th>
<th>Fluorine Analysis of Bones</th>
<th>Number of Rats</th>
<th>Length of Time on Ration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>0.0015</td>
<td>0.026</td>
<td>18</td>
<td>34</td>
</tr>
<tr>
<td>2 a</td>
<td>Sodium Fluoride</td>
<td>0.0150</td>
<td>0.463</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td>2 b</td>
<td>Sodium Fluoride</td>
<td>0.0300</td>
<td>0.706</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td>3 a</td>
<td>Potassium Fluoride</td>
<td>0.0150</td>
<td>0.416</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>3 b</td>
<td>Potassium Fluoride</td>
<td>0.0300</td>
<td>0.735</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>4 a</td>
<td>Calcium Fluoride</td>
<td>0.0300</td>
<td>0.202</td>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>4 b</td>
<td>Calcium Fluoride</td>
<td>0.0600</td>
<td>0.222</td>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>5 a</td>
<td>Rock Phosphate</td>
<td>0.0150</td>
<td>0.343</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td>5 b</td>
<td>Rock Phosphate</td>
<td>0.0300</td>
<td>0.614</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td>5 c</td>
<td>Rock Phosphate</td>
<td>0.0600</td>
<td>0.716</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td>6 a</td>
<td>Sodium Fluosilicate</td>
<td>0.0150</td>
<td>0.662</td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>6 b</td>
<td>Sodium Fluosilicate</td>
<td>0.0300</td>
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<td>Fluorine Analysis of Bones Per cent</td>
<td>Number of Rats</td>
<td>Length of Time on Ration Days</td>
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<td>Fluorine Analysis of Bones</td>
<td>Number of Rats</td>
<td>Length of Time on Ration</td>
</tr>
<tr>
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<td>-------------------------------------</td>
<td>--------------------------</td>
<td>--------------------------</td>
<td>----------------</td>
<td>-------------------------</td>
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<td>0.507</td>
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TABLE I (Continued)

CONCENTRATION OF DIFFERENT FLUORINE COMPOUNDS USED, LENGTH OF TIME ON RATION AND FLUORINE ANALYSIS OF BONES
<table>
<thead>
<tr>
<th>Group Number</th>
<th>Fluorine Compound Used as Supplement</th>
<th>Calculated Concentration Per cent</th>
<th>Fluorine Analysis of Bones Per cent</th>
<th>Number of Rats</th>
<th>Length of Time on Ration Days</th>
</tr>
</thead>
<tbody>
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<td>14 a</td>
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<td>0.0021</td>
<td>0.079</td>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>2 e</td>
<td>Sodium Fluoride</td>
<td>0.0025</td>
<td>0.072</td>
<td>3</td>
<td>34</td>
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<tr>
<td>15 a</td>
<td>High Blount Hay plus Sodium Fluoride</td>
<td>0.0050</td>
<td>0.114</td>
<td>6</td>
<td>34</td>
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<tr>
<td></td>
<td>Defluorinated Rock Phosphate (2%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>None</td>
<td>0.0015</td>
<td>**</td>
<td>3*</td>
<td>34</td>
</tr>
<tr>
<td>16 a</td>
<td>Sodium Fluoride</td>
<td>0.0025</td>
<td>3*</td>
<td>3*</td>
<td>34</td>
</tr>
</tbody>
</table>

*All groups from 16 through 16 g contained 3 rats for first 2 weeks and 2 each until the end of the experiment.

**Bone analysis for groups 16 through 16 g reported in Table III.
experiment. All animals were fed a stock diet on the first
day and placed on the experimental diet the second day.

The experimental animals were individually weighed
at weekly intervals.

Table II gives the average gain for each individual
group together with the lot total gain throughout the ex-
periment.

**Bone Analysis**

The bones were analyzed by the method of Willard and
Winter (37) with modifications as used by the Aluminum
Company of America and the University of Tennessee.

The bones from each rat used in the experiment were
individually analyzed, but only the average for each group
are reported in this paper.

The average fluorine content of the bones analyzed
in each group together with the length of time on the ration
and number of rats per group, are reported on Table I.
<table>
<thead>
<tr>
<th>Group Number</th>
<th>Fluorine Supplement</th>
<th>Calculated Fluorine Content of Ration</th>
<th>Number of Rats</th>
<th>Average Weekly Gains per Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1st Week</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd Week</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3rd Week</td>
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<td>4th Week</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Total Gain</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>Per cent 0.0015</td>
<td>18</td>
<td>26 29 26 28 109</td>
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<tr>
<td>2 a</td>
<td>Sodium Fluoride</td>
<td>0.0150</td>
<td>6</td>
<td>23 21 19 21 84</td>
</tr>
<tr>
<td>2 b</td>
<td>Sodium Fluoride</td>
<td>0.0300</td>
<td>6</td>
<td>15 20 14 19 68</td>
</tr>
<tr>
<td>3 a</td>
<td>Potassium Fluoride</td>
<td>0.0150</td>
<td>3</td>
<td>25 22 25 23 95</td>
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<tr>
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<td>Potassium Fluoride</td>
<td>0.0300</td>
<td>3</td>
<td>22 20 20 21 83</td>
</tr>
<tr>
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<td>Calcium Fluoride</td>
<td>0.0300</td>
<td>6</td>
<td>18 21 21 28 88</td>
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<tr>
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<td>6</td>
<td>17 21 18 25 81</td>
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<tr>
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<td>0.0150</td>
<td>6</td>
<td>20 25 21 26 92</td>
</tr>
<tr>
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<td>Rock Phosphate</td>
<td>0.0300</td>
<td>6</td>
<td>19 22 17 24 82</td>
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<td>5 c</td>
<td>Rock Phosphate</td>
<td>0.0600</td>
<td>6</td>
<td>20 18 18 26 82</td>
</tr>
<tr>
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<td>Sodium Fluosilicate</td>
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<td>3</td>
<td>24 20 21 34 99</td>
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<td>0.0300</td>
<td>3</td>
<td>19 17 13 27 76</td>
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<td>14 15 2 15 46</td>
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<td>0.0150</td>
<td>3</td>
<td>18 23 17 35 93</td>
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TABLE II (Continued)

AVERAGE GROUP WEEKLY GAINS AND TOTAL GAINS FOR FOUR WEEK PERIOD

<table>
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<tr>
<th>Group Number</th>
<th>Fluorine Supplement</th>
<th>Calculated Fluorine Content of Ration</th>
<th>Number of Rats</th>
<th>Average Weekly Gains per Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1st Week</td>
</tr>
<tr>
<td>7 b</td>
<td>Potassium Fluosilicate</td>
<td>0.0300</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>7 c</td>
<td>Potassium Fluosilicate</td>
<td>0.0600</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>8 a</td>
<td>Natural Cryolite</td>
<td>0.0150</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>8 b</td>
<td>Natural Cryolite</td>
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<td>19</td>
</tr>
<tr>
<td>8 c</td>
<td>Natural Cryolite</td>
<td>0.0600</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>9 a</td>
<td>Magnesium Fluosilicate</td>
<td>0.0150</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>9 b</td>
<td>Magnesium Fluosilicate</td>
<td>0.0300</td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>9 c</td>
<td>Magnesium Fluosilicate</td>
<td>0.0600</td>
<td>3</td>
<td>16</td>
</tr>
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<td>10 a</td>
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<td>0.0300</td>
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<td>0.0600</td>
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<td>33</td>
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<td>Average Weekly Gains per Group</td>
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<tr>
<td></td>
<td></td>
<td>Per cent</td>
<td></td>
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</tr>
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<td>High Blount Hay</td>
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<td>High Blount Hay</td>
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<td>and Sodium Fluoride</td>
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<td>and Sodium Fluoride</td>
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</tr>
<tr>
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<td>0.0150</td>
<td>3</td>
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</tr>
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<td>27</td>
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<tr>
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<td>Phosphate (2%)</td>
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TABLE II (Continued)

AVERAGE GROUP WEEKLY GAINS AND TOTAL GAINS FOR FOUR WEEK PERIOD

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<th>Group Number</th>
<th>Fluorine Supplement</th>
<th>Calculated Fluorine Content of Ration</th>
<th>Number of Rats</th>
<th>Average Weekly Gains per Group</th>
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<td>1st Week</td>
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<td>13 b</td>
<td>Sodium Fluoride</td>
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<td></td>
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<tr>
<td></td>
<td>Defluorinated Rock</td>
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<td>Phosphate (4%)</td>
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<td>Defluorinated Rock</td>
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<tr>
<td></td>
<td>Phosphate (2%)</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>0.0050</td>
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<tr>
<td>16</td>
<td>*None</td>
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*All groups from 16 through 16 g contained 3 rats for first 2 weeks and 2 each until the end of the experiment.
<table>
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<th>Group Number</th>
<th>Fluorine Supplement</th>
<th>Calculated Fluorine Content of Ration</th>
<th>Number of Rats</th>
<th>Average Weekly Gains per Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per cent</td>
<td></td>
<td>1st Week</td>
</tr>
<tr>
<td>16a</td>
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<td>3*</td>
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</tr>
<tr>
<td>16b</td>
<td>Sodium Fluoride</td>
<td>0.0050</td>
<td>3*</td>
<td>23</td>
</tr>
<tr>
<td>16c</td>
<td>Sodium Fluoride</td>
<td>0.0075</td>
<td>3*</td>
<td>21</td>
</tr>
<tr>
<td>16d-1</td>
<td>Sodium Fluoride</td>
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<td>3*</td>
<td>24</td>
</tr>
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<td>Sodium Fluoride</td>
<td>0.0100</td>
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*All groups from 16 through 16g contained 3 rats for first 2 weeks and 2 each until the end of the experiment.
TABLE III

EFFECT OF TIME ON ACCUMULATION AND DEPLETION OF FLUORINE IN BONES OF RATS FED GRADUATED LEVELS OF SODIUM FLUORINE

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Number of Rats</th>
<th>Calculated Fluorine Content of Ration</th>
<th>Fluorine Content After 15 Days on Ration</th>
<th>Fluorine Content After 34 Days on Ration</th>
<th>Fluorine Content After 34 Days on Ration and Then Returned to Basal for 15 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>3*</td>
<td>0.0015</td>
<td>0.017</td>
<td>0.010</td>
<td>0.016</td>
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<td>16 a</td>
<td>3*</td>
<td>0.0025</td>
<td>0.123</td>
<td>0.095</td>
<td>0.052</td>
</tr>
<tr>
<td>16 b</td>
<td>3*</td>
<td>0.0075</td>
<td>0.246</td>
<td>0.197</td>
<td>0.032</td>
</tr>
<tr>
<td>16 c</td>
<td>3*</td>
<td>0.0100</td>
<td>0.258</td>
<td>0.341</td>
<td>0.144</td>
</tr>
<tr>
<td>16 d-1</td>
<td>3*</td>
<td>0.0100</td>
<td>0.316</td>
<td>0.320</td>
<td>0.250</td>
</tr>
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<td>3*</td>
<td>0.0125</td>
<td>0.384</td>
<td>0.335</td>
<td>0.320</td>
</tr>
<tr>
<td>16 e</td>
<td>3*</td>
<td>0.0150</td>
<td>0.406</td>
<td>0.414</td>
<td>0.080</td>
</tr>
<tr>
<td>16 f</td>
<td>3*</td>
<td>0.0200</td>
<td>0.513</td>
<td>0.578</td>
<td>0.416</td>
</tr>
</tbody>
</table>

*One rat from each group was killed at each of the times indicated.
DISCUSSION OF RESULTS

This investigation was made primarily to study the relative toxicity of the different fluorine compounds when fed in the ration of the albino rat. The criteria used for study were: calcification of the teeth; total weight gain; and fluorine analysis of the bones.

All rats that were maintained on the rations supplemented with fluorine throughout the feeding period showed signs of bleaching of the teeth. Some of the groups that were maintained on a low fluorine diet showed only a slight change in teeth color and no irregularities as to shape and form of teeth. Other groups which were maintained on relatively high fluorine diets, showed not only a complete bleaching of both the mandibal and maxillary incisors but a great wearing or shortening of the lower incisors accompanied by an elongation of the uppers. This is in accord with the findings of Schultz (30) who reported a loss of the pink natural color of the teeth when 0.01 per cent fluorine was fed in the form of sodium fluorine but did not get an overgrowth of the incisors until 0.024 per cent was fed. In comparing groups of rats fed a ration with equal fluorine concentration of at least 0.03 per cent fluorine, calcium fluoride produced less change in the teeth than any other group studied, and sodium fluosilicate
produced the greatest change. This is in accord with Kick et al. (20), Schultz (31), McClure and Mitchell (25), and Smith and Leverton (35), who reported calcium fluoride to have $1/2$ to $1/5$ the toxicity of sodium fluoride. Smith and Leverton (35) considered the effects on the teeth to be approximately the same when the fluorine was combined as sodium fluoride or sodium fluosilicate.

On the average the first change in the color of the teeth was observed after approximately 14 days on our experimental ration. There were variations from this figure, depending upon the compound used as a supplement. White striations were first observed near the base of the tooth, gradually spreading with a constant whitening effect toward the end of the tooth until it was completely bleached. The upper incisors showed little signs of change until after the bottom incisors were completely bleached. None of the rats fed calcium fluoride showed any changes greater than those just described. Rats receiving the lower levels of calcium fluoride, 0.015 and 0.03 per cent fluorine, relatively few individuals showed complete bleaching of the upper teeth. In the case of the rats fed more soluble compounds, such as sodium fluosilicate, the lower teeth started to wear and the uppers showed excessive growth about the time or even before they were completely bleached. These teeth obtained a dull chalky white color and often showed spots of erosion.
in the enamel. This is the same condition as described in the literature by Schultz (31), Kick et al. (20) and many others.

The other compounds that were studied varied in their effects on the teeth between these two extremes, when fed at the same concentration in the ration. Potassium fluosilicate produced a little earlier and more severe effects than did calcium fluosilicate, magnesium fluosilicate, potassium fluoride, or sodium fluoride, these latter compounds appearing to have approximately the same effect. This is not entirely in agreement with Smith and Leverton (35), who reported the effect on the teeth of the albino rat to be approximately the same when fluorine was combined as sodium fluoride, potassium fluoride, barium fluosilicate and sodium fluosilicate. The effects on the teeth were approximately the same when the supplement was in the form of synthetic cryolite or rock phosphate. Both of these produced more pronounced abnormalities than did natural cryolite fed at the same level. This work parallels to some extent the study by Evans and Phillips (12) where the feeding of twice as much synthetic cryolite was required as when sodium fluoride was fed to produce the same abnormalities in the teeth. Smith and Leverton (35) required 10 times as much natural cryolite as was required of sodium fluoride to produce the same tooth changes when the natural
cryolite was fed at 0.0226 per cent of the ration.

A study was made with a graduated level of sodium fluoride (groups 16 through 16 g) to determine the concentration necessary to produce recognizable signs of fluorosis in the teeth in a 15 day period. At the end of this period a slight striation could be observed, when the teeth were studied with the aid of a hand lens, in the group that received 0.0025 per cent fluorine in the ration. The group that received 0.0050 per cent showed definite striations and a slight bleaching effect at the end of 15 days. The visible symptoms of fluorosis increased with the concentration of the fluorine carrier for the rest of the groups in this study. Two groups were fed a ration containing 0.010 per cent fluorine. The only difference being that one contained plain salt (NaCl) and the other iodized salt. The iodized salt seemed to retard the bleaching effect of the fluorine for 3 to 5 days, but after that time no difference could be observed.

In the study of the animals that were fed to study the toxic effects of hay that was obtained near an aluminum plant, the symptoms, as shown by the teeth, appear to be approximately the same as those obtained by feeding the same level of sodium fluoride. A little more wear was observed, however, in these animals than in those receiving
sodium fluoride at the same fluorine level. This wear resembled greatly that obtained by the feeding of the fluosilicates, but, probably due to the low concentration in the ration, was not as severe.

The addition of 2 per cent and 4 per cent defluorinated phosphate (group 13 a and 13 b) to an adequate ration containing 0.015 per cent fluorine in the form of sodium fluoride, appeared to produce more normal tooth developments than did their control group (2d). The action of the fluorine on the teeth was not completely averted but appeared greatly retarded. The teeth of the animals in group 2d were completely bleached on both the upper and lower incisors and showed some wear at the end of the feeding period. Group 13b had complete bleaching of the bottom teeth but the top incisors were a normal pink color and showed no visible wear. This was probably caused by the increased calcium and phosphorus content of the ration. However, McClure and Mitchell (25) reported that the calcium balance of rats was not affected by sodium fluoride at levels of 0.0105 per cent and 0.0313 per cent fluorine in the ration. They did report however, that a level of 0.0623 per cent fluorine in the form of sodium fluoride lowered the per cent of calcium that would have otherwise been retained by the experimental animals.
No record was maintained of the feed consumed by each group. It was noted however, that in the higher levels, above 0.015 per cent fluorine, the feed wasted increased with the fluorine concentration in the ration.

In most of the compounds studied, an increased concentration of fluorine in the ration caused a decrease in the total gain as shown in Table II. The most noticeable exception to this was with the groups fed magnesium fluosilicate. The week to week decrease in gain within one group was not consistent. It must be remembered however, that the weekly gains were taken only over a four week interval, a period of time considerably shorter than most previous workers have used. As an example of the time other investigators have continued their work, Smith and Leverton (35) fed their animals for a 6 weeks period, while Schultz (31) continued his investigation for approximately 17 weeks. This could probably account, in part at least, for the almost equal gains made when some of our groups were fed different levels of the same compound.

Some of the groups had hay which was known to contain fluorine substituted in the basal ration for the dehydrated alfalfa leaf meal. When studying the decreased total gains made by animals receiving this ration, consideration should be given to the fact that it probably contained more fiber and lower T.D.N. than the meal which
it replaced.

The study made using graduated levels of sodium fluoride does not show a proportional decrease in total gain with an increase of fluorine in the ration as presented in Table II. The highest level fed was probably significantly lower than the control group, but because of the small number of experimental animals and the length of time on feed, more study is needed before definite conclusions are drawn. This is in accord with Smith and Leverton (35) who required a ration containing 0.0226 per cent sodium fluoride to produce a slight stunting of growth.

The fluorine analysis of the bones of the experimental animals as presented in Table I are in line with the findings of earlier workers. McClure (24) reported the average of fluorine content of normal bones to be between 0.01 and 0.03 per cent. This range, according to Majumdar et al. (21), will vary with the age of the animal. The fluorine content of the bones from all the control animals in this experiment was 0.026 per cent.

McClure (24), Schultz (30), Kick et al. (20), and others have reported an increase in the fluorine content of the bones when this element was added to the ration. The findings from this work are in complete agreement with the findings of these investigators.
The experimental animals whose rations were supplemented with fluorine combined as calcium fluoride stored less fluorine in the bones than any other group receiving the same concentration in the ration, as is presented in Table I. The groups showing the greatest storage of fluorine were the ones fed fluorine in the form of potassium fluosilicate and sodium fluosilicate. More fluorine was stored in the bones of the animals when sodium fluosilicate was fed at a level of 0.0150 per cent than when potassium fluosilicate was fed at the same level. However, at the 0.030 and 0.060 per cent level of feeding, more was stored in the bones when combined as potassium fluosilicate.

In all groups fed compounds containing fluorine at the higher levels of feeding—0.015 to 0.06 per cent fluorine—there was one group which received a ration containing 0.030 per cent fluorine. Therefore, using this level of fluorine as a basis for comparison, the compounds resulting in the greatest storage of fluorine in the bones were in this order: potassium fluosilicate, sodium fluosilicate, potassium fluoride, sodium fluoride, rock phosphate, natural cryolite, synthetic cryolite, calcium fluosilicate, magnesium fluosilicate, and calcium fluoride. There is some variation from this, however, when considering the fluorine compounds combined in the concentrations of 0.015 and 0.06 per cent of the ration as shown in Table I.
Each of these groups were composed of three rats, and one bone sample was tested for each of these rats, and these individual averages compiled in Table I. Consequently, a slight error in analysis or individual differences of the bones analyzed could cause a distorted view of the whole group, because of the limited number of animals used in each group.

The amount of fluorine deposited in the bones from the different dietary compounds were not always in the same order as expected, using the wear and bleaching of the teeth as the criterion for judgment. Sodium fluosilicate and potassium fluosilicate on this basis would have been expected to store the greatest per cent of fluorine, and calcium fluoride would have been expected to have stored the smaller percentage. This was true in all levels tested when the fluorine made up more than 0.015 per cent of the ration.

Again, judging from the changes observed in the teeth, one would have expected calcium fluosilicate, magnesium fluosilicate, potassium fluoride, and sodium fluoride to have deposited approximately the same amount of fluorine in the bones. Sodium fluoride and potassium fluoride did deposit approximately the same amount of fluorine in the bones when fed at a concentration of both
0.015 and 0.03 per cent of the ration. The amount deposited was approximately 60 per cent to 70 per cent of the amount deposited by sodium fluosilicate and potassium fluosilicate. However, the chemical analysis of the bones showed less fluorine stored when the ration was supplemented with calcium fluosilicate and magnesium fluosilicate at 0.015 and 0.03 per cent of the ration than was found when the same concentration of natural cryolite, rock phosphate, or synthetic cryolite was fed. Both of these compounds resulted in a greater retention of fluorine when fed at 0.06 per cent of the ration than did synthetic cryolite fed at the same concentration. Magnesium fluosilicate showed a greater storage of fluorine in the bones than did rock phosphate at this high level of feeding as shown in Table I.

The feeding of natural cryolite resulted in a higher fluorine content of the bones than rock phosphate when both were fed in such an amount to supply 0.015 per cent fluorine in the ration. However, when the ration contained 0.03 per cent fluorine, the ration supplemented with rock phosphate caused a greater retention of fluorine in the bones than did either natural cryolite or synthetic cryolite. There was little difference in fluorine content of the bones of rats when the basal ration was supplemented
with either rock phosphate or synthetic cryolite to make the total fluorine content up to 0.6 per cent. Natural cryolite caused a slightly higher storage at this level, however, than either rock phosphate or synthetic cryolite at the same levels. This is not in agreement with earlier workers, Smith and Leverton (35), who have reported synthetic cryolite to be more soluable than natural cryolite and, probably for this reason, more toxic.

When the same compound was fed at different levels, the amount of fluorine stored in the bones was, in most cases, proportional to the concentration in the ration. There are some exceptions to this however, as observed in Table I. This is in agreement with the work of Schultz (30) who reports that the feeding of fluorine to the albino rat will cause an increase in the fluorine content of the bones, but not always strictly in proportion to the amount in the ration.

The study of the animals that received hay which was known to contain a high per cent fluorine, substituted into the basal ration for the dehydrated alfalfa leaf meal, presents an interesting picture. Group 12b, which received a ration containing this hay and enough sodium fluoride to make a total fluorine content of 0.015 per cent of the ration, showed a greater storage of fluorine in the bones than did similar animals receiving only sodium fluoride at
the same per cent of the ration (Group 2a). This would suggest that the fluorine present in the hay was combined in some form which was more toxic than sodium fluoride. The same condition was presented in group 2c and 12c as shown in Table I. The ration fed both groups was calculated to contain the same amount of fluorine, but the group receiving the high fluorine hay showed a greater storage of fluorine in the bones. Group 14a received a ration containing no fluorine supplement added and was calculated to contain 0.0021 per cent fluorine. Group 2e received a ration to which sodium fluoride had been added to make the entire fluorine content of the ration 0.0025 per cent. Group 14a showed a greater storage of fluorine than did group 2e even though the total fluorine content of the ration was less. This would strengthen the previous statement that the fluorine in the hay was apparently combined in a form more toxic than sodium fluoride.

When defluorinated rock phosphate was added to the ration containing fluorine, the amount of fluorine deposited in the bones appeared to be slightly retarded in most cases. Groups 2d, 13a, and 13b received a ration

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1The hay that was substituted into the basal ration in the amount of 30 per cent contained 0.0070 per cent fluorine.
containing 0.0150 per cent fluorine in the form of sodium fluoride. This ration was supplemented with defluorinated rock phosphate as shown in Table I. At this level of feeding it required 4 per cent of the phosphate to bring about a slight reduction in fluorine storage in the bone. However, when the fluorine content of the ration was only 0.005 per cent, as is the case in groups 2c and 15a, 2 per cent defluorinated rock phosphate caused a slight decrease in the amount of fluorine stored in the bones. This would suggest that the higher concentration of fluorine in the feed will require a relatively higher per cent of mineral in the form of defluorinated rock phosphate to partially alleviate the toxic effects.

Because of the small number of experimental animals involved, there was very little definite information obtained from the study of fluorine analysis of the bones of rats maintained for different lengths of time on the graduated level of sodium fluoride as presented in Table III. These animals showed considerable variance so that the difference between lots is not graduated in direct proportion to the per cent of fluorine in the ration. However, the per cent of fluorine in the bones did decrease in all cases when the animals were taken off the supplemented ration and returned to the basal ration for a 15 day period.
SUMMARY AND CONCLUSIONS

The previously reported work of many investigators that the supplementation of a ration with a fluorine compound would cause abnormal calcification of the teeth, retarded growth and an increase in the fluorine stored in the bones was supported by the evidence presented in this study.

Evidence was presented to show that there was a difference in the toxic effects of fluorine, depending upon the substances with which it is combined. This toxic effect does not always vary directly with the solubility of the compound.

Evidence of fluorine poisoning was observed in the teeth of young growing albino rats in 15 days when as little as 0.0050 per cent fluorine was present in the ration in the form of sodium fluoride.

It was indicated that the fluorine present in legume hay grown near an aluminum plant was more toxic than sodium fluoride.

Experimental evidence indicated that the addition of an excess amount of a mineral, such as defluorinated rock phosphate, would retard to some extent the toxicity of fluorine in the albino rat.
Bibliography


