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Concentration of fluorine in the urine of cattle and sheep

Gerald L. Stow

To the Graduate Council:

I am submitting herewith a thesis written by Gerald L. Stow entitled "Concentration of fluorine in the urine of cattle and sheep." 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 Husbandry.

Charles S. Hobbs, Major Professor

We have read this thesis and recommend its acceptance:

Harold J. Smith, George M. Merriman, O. G. Hall

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

May 18, 1956

To the Graduate Council:

I am submitting herewith a thesis written by Gerald L. Stow entitled "Concentration of Fluorine in the Urine of Cattle and Sheep." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

Charles S. Hobbs
Major Professor

We have read this thesis
and recommend its acceptance:

Harold J. Smith

George M. Merriman

O. Glen Hall

Accepted for the Council:

E. R. Walters
Dean of the Graduate School

CONCENTRATION OF FLUORINE IN THE URINE OF CATTLE AND SHEEP

A THESIS

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

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by

Gerald L. Stow

June 1956

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Gerald L. Stow

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CHAPTER I

INTRODUCTION

The potential problem of fluorosis in cattle and sheep tends to become increasingly important as certain industries using fluorine bearing materials at high temperature locate in farm areas. Several factors adding to the hazard of fluorosis in livestock are: (1) the use of mineral supplements with a high fluorine[1] content; (2) water supplies with a high fluorine content; and (3) pastures contaminated by upsplash of fluorine-bearing soil. The rather large number of sources of fluorine, each capable of causing variable degrees of fluorosis in cattle and sheep make it desirable to constantly improve the present methods of diagnosis.

Present diagnostic measures include a study of teeth changes, determinations of fluorine content of bones and feeds, and general clinical observations. Gross appearance of the teeth of cattle and sheep, supported by a study of the fluorine content of feeds and bones for the period concerned, give an excellent history of an animal's incisor teeth. However, these diagnostic measures do not constitute a definite quantitative measure of acute or chronic fluorine intoxication

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The element fluorine, because of its extreme chemical activity, never occurs in the free state in nature but rather in combination with other elements. Compounds containing fluorine are commonly called fluorides. Therefore, in this thesis the word "fluorine" refers to the fluorine content of the particular fluoride in question.

in cattle and sheep, especially if the animal has developed its permanent incisors prior to the ingestion of increased amounts of fluorine. The fluorine concentration of urine from cattle and sheep has also been used as an aid in diagnosing fluorosis by several investigators; however, more data are needed to demonstrate whether urinary fluorine concentration can be used as a quantitative measure in cases of acute, or chronic fluorosis.

Investigations reported herein were designed primarily to study several aspects of the reliability of urinary fluorine concentrations as a diagnostic measure of fluorosis in cattle and sheep. Specific objectives of the study were:

1. To determine the validity of using the specific gravity as a method of standardizing urinary fluorine concentrations in cattle and sheep;
2. To determine the correlation between urinary fluorine concentrations and dietary fluorine intake in cattle and sheep;
3. To establish, for cattle, the excretion pattern of urinary fluorine concentration at various periods after fluorine ingestion;
4. To study the relationship between the rib fluorine content and the urinary fluorine concentration in cattle;
5. To study the effects of aluminum sulfate and aluminum chloride "fluorine alleviators," upon the fluorine concentration of urine in cattle and sheep.

CHAPTER II

REVIEW OF LITERATURE

Many studies have been conducted to determine the effects of fluorine on man and animals; however, until recent years, many of these studies have been made under field conditions. Excellent reviews of most of these studies have been presented by DeEds and Thomas (1933), McClure (1933), Dean (1936), Roholm (1937), Pierce (1939), Greenwood (1940), Mitchell (1951), Schmidt and Rand (1952), and Mitchell and Edmon (1951-52).

Fluorine studies with animals have been made primarily with cattle, sheep, rats, and swine, with a few reports on dogs, rabbits and horses. These studies deal primarily with the effects of fluorine on the animal body. A limited number of controlled experiments have been conducted on the use of urinary fluorine concentration as a diagnostic aid for fluorosis in cattle and sheep.

Roholm (1937) reported that in 1805 Gay-Lussac suggested that fluorine was probably to be found in urine like other ossifying substances. Two years later, Berselius was successful in demonstrating the presence of fluorine in urine.

Blakemore et al. (1948) found that in advanced clinically detectable fluorosis, urinary fluorine values varied from 16 ppm to 68 ppm (corrected to a specific gravity of 1.040) with a preponderance of the values well over 25 ppm as compared to values for normal cattle of 2 ppm at Weybridge and up to 6 ppm for cattle grazing pastures on fluoride-rich soil at Cambridge. For animals on the edge of the affected belt showing signs of dental fluorosis, urinary fluorine values of the order of 11 ppm were encountered. The fluorine content of urine from young growing stock of other areas has been found as high as 7 ppm without evidence of dental fluorosis. Blakemore concluded that urinary levels of 10 ppm were somewhat critical from the diagnostic point of view. He further reported that one cow, showing a urinary fluorine concentration of 28 ppm, when removed to Cambridge and placed on a normal ration for eight months rapidly improved and was in excellent condition when slaughtered. No gross skeletal lesions were observed at postmortem examination. It was concluded that the urinary excretion of fluorine was not only affected by the current rate of absorption from feed but also by the rate of loss from the skeleton of stored fluorine. Urinary fluorine analysis was demonstrated to be a convenient way to diagnose fluorosis, either from a high present or past consumption of fluorine.

According to E. Kruger (1949), examination of the urine of diseased animals may possibly be employed for early diagnosis of fluorine poisoning from a fluorine-bearing substance. Cattle fed fluorine-bearing fodder containing 815 to 1250 ppm fluorine discharged urine containing 21.3 to 89.6 milligrams fluorine per liter, as compared with normal fluorine content of about 0.4 milligrams per liter.

Studies on the development of fluorosis in dairy cattle by Miller (1955) showed that urinary fluorine concentrations increased in direct proportion to the dietary fluorine intake level. Fluorine was added to the ration from sodium fluoride. Cattle fed the basal ration only, excreted less than 5 ppm of fluorine in the urine (corrected to a specific gravity of 1.040). When 20 ppm of fluorine was added to the ration, the urinary fluorine concentration averaged approximately 14 ppm; when 30 ppm of fluorine was fed, the urinary fluorine concentration averaged 22 ppm. Feeding 40 ppm of fluorine increased the urinary fluorine level to 30 ppm. Fifty ppm of added dietary fluorine resulted in an average urinary fluorine concentration of approximately 38 ppm.

Miller further concluded that urinary fluorine values greater than 20 ppm would indicate adverse effects on the teeth if the fluorine ingestion occurred while the teeth were forming, but would result in no loss in milk production. Urinary fluorine values consistently above 40 ppm for four years would indicate the ingestion of excessive fluorine which could cause a reduction in milk production and possibly severe weight losses.

According to Schmidt et al. (1954) the urinary excretion of fluorine consistently reflected the relative intake of sodium fluoride by an experimental herd of dairy cattle. Urine samples taken eight to twelve hours after the daily dose of sodium fluoride was fed showed a variable increase in fluorine over samples taken twenty-two to twenty-four hours after ingestion of the supplement. They concluded that the measurement of urinary excretion of fluorine indicated the relative current levels of fluorine ingested.

Phillips (1952) stated that, although there was considerable variation in the amount of fluorine eliminated through the kidney, there was a rough correlation between the amount of fluorine voided in the urine and the amount ingested in the ration. Furthermore, there was a greater concentration of fluorine in the urine a few hours after eating as compared to twenty-three hours after eating. Phillips further concluded that a high urinary fluorine measurement as a qualitative measure indicates elevated fluorine ingestion, but single sample analyses were too variable to be quantitative.

Hobbs et al. (1951) stated that the quantitation of the dietary ingestion of fluorine being excreted in the urine was possible only from twenty-four-hour collection periods. These workers (1954) further concluded that the fluorine content of urine should be used as a diagnostic measure only in conjunction with other factors such as: (1) fluorine content of feeds and water consumed; (2) a study of teeth erupted at various periods; (3) fluorine content of bones; (4) general condition of the herd, along with a consideration of the feeding level, management conditions, etc.; and (5) observations on individual animals concerned.

Many field studies with human subjects are also recorded in the literature. Machle et al. (1943), McClure and Kinser (1944), and Largent et al. (1949) stated that fluorine in urine was a good indication of fluorine in the food supply. They further concluded that the correlation of the urinary fluorine with the amount of fluorine ingested was very high.

Certain compounds have been found to partially alleviate some of the effects of increased fluorine ingestion. Marcovitch and Stanley (1942) reported that aluminum sulfate and hydrated lime are capable of saving animals from an otherwise lethal dose of sodium fluoride. Boric acid also had some value as an antidote through the formation of a fluoborate. The aluminum sulfate forms cryolite, which was found to be much less toxic.

Venkataramanan and Drishnasurany (1948) confirmed the ameliorating effect of aluminum salts in fluorosis induced in albino rats and have shown that the skeletal storage of fluorine was appreciably reduced by the presence of aluminum in the diet.

Hobbs et al. (1954) reported the addition of aluminum sulfate to decrease the fluorine content of bones and to reduce the symptomatic effects of fluorine on developing incisor teeth of cows, ewes, and lambs fed rations containing elevated levels of fluorine, as compared with animals fed similar rations without the added aluminum sulfate. The results of feeding 0.5 per cent of aluminum sulfate in rations containing up to 50 ppm of fluorine for cattle, and aluminum chloride in rations containing 100 ppm of fluorine for wether lambs, indicated that these compounds tied up 20 to 40 per cent of the fluorine in the feces and decreased its output in the urine.

CHAPTER III

EXPERIMENTAL PROCEDURE

A series of fluorine experiments with cattle and sheep has been underway at the University of Tennessee since 1948. These experiments were initiated as follows: Experiment I, 1948; Experiment II, 1950; Experiment III, 1952; Experiment IV, 1952; Experiment V, 1951; Experiment VI, 1953; Experiment X, 1950, and Experiment XI, 1950. Generally, the primary objectives of these experiments were to study the affects of various levels of fluorine upon the criteria listed in Table I. Results reported in this thesis were derived from the cumulative data for cattle in Experiments I, II, IV, V, and sheep in Experiment X.

The results presented herein are concerned primarily with the source and concentration of fluorine in the urine. Other data from these experiments have been discussed by Hobbs <u>et al</u>. (1954) or will be reported at a later date.

Since some phases of this study were initiated using animals from more than one of these experiments, this chapter is divided into two sections. Section I deals primarily with the procedure used for each experimental group, and Section II describes the specific urine phases of each group used. These have been appropriately grouped as Phases 1 - 5.

Section I

<u>Experiment I</u>

Twenty-four grade Hereford heifers were purchased as yearlings

TABLE I

CRITERIA STUDIED IN FLUORINE EXPERIMENTS AT THE UNIVERSITY OF TENNESSEE

Experiment number	Lot or herd no.	Feed consumption	Weights and gains	Reproduction and progeny records	Digestibility of nutrients	Teeth changes	Bone changes	Blood changes	Urine F content
I[a]	1-16	X	X	X	X	X	X	X	X
II[a]	20-26	X	X	X	X	X	X	X	X
III	30-36	X	X	X	-	X	X	X	
IV	40-45	X	X	X	-	X	X	X	X
V	50-56	-	X	X	-	X	X	-	X
VI	A-G	-	X	X	-	X	X	-	-
X[b]	1-9	X	X	-	X	X	X	X	X
XI[b]	1-6	X	X	X	-	X	X	-	-

[a]In Lots 14, 15, and 16 of Experiment I, and in Lots 25 and 26 of Experiment II data were not available on feed consumption, digestibility of nutrients, blood and urine, these being pasture lots.

[b]Experimental animals were sheep.

X indicates criteria used.

in the Mid-west in 1948. The heifers were divided into eight uniform lots of three animals each based on source, weight, type, grade, and condition (Table II). All cattle were hip branded for positive identification.

These cattle were fed a balanced ration consisting of chopped mixed hay *ad libitum* and four pounds of concentrate (three parts corn and one part cottonseed meal) per head per day. These cattle were restricted to a dry-lot for the first five years of the experiment. In the summer of 1953 the animals in this experiment were allowed access to pasture and the concentrate reduced to two pounds per head per day. No hay was fed during the pasture period. These cattle were also allowed access to pasture during the summers of 1954 and 1955.

Fluorine was added to the concentrate mixture from sodium fluoride. The amount of fluoride added to the ration for each group was calculated as parts per million (ppm) on the basis of the total air-dry ration consumed. All animals were individually fed. At the end of each twenty-eight day period feed consumption was checked and the amount of fluorine was adjusted according to the average daily consumption for each lot.

Experiment II

The cattle used in this alleviator experiment were grade Hereford heifers purchased as yearlings in Texas in the summer of 1950. The heifers were allotted on the basis of type, grade, weight, and condition into ten lots of three animals each. All animals were hip branded for positive identification. Fluorine at each level was fed to two comparable lots of cattle, one lot receiving fluorine alone, and the other lot

TABLE II

PLAN OF CATTLE EXPERIMENT I

Lot number	Number of cows	F added to ration ppm	Total F in ration ppm	Approximate range of age[a] (months)	Time on experiment[a] (months)
1	3	-	8	108 - 113	96
2	3	10	18	108 - 113	96
3	1[b]	20	28	108 - 113	96
4	3	30	38	108 - 113	96
5	3	40	48	108 - 113	96
6	3	50	58	108 - 113	96
7	3	70	78	108 - 113	96
8	2[c]	100	108	108 - 113	96

[a]Age of cattle and time on experiment when urine collections were made.

[b]One two-year-old heifer was lost in calving in 1949 and another cow was sacrificed due to systemic mastitis in 1954.

[c]One heifer was lost from this lot due to failure of healing after a rib resection.

receiving fluorine plus 0.5 per cent aluminum sulfate.

These cattle were fed a balanced ration consisting of chopped mixed hay ad libitum and four pounds of concentrate (three parts corn and one part cottonseed meal) per head per day. All animals were restricted to a dry lot for the first two years of the experiment. In the summer of 1953, the animals in this experiment were allowed access to pasture and the concentrate reduced to two pounds per head per day. No hay was fed while the cattle were on pasture. These cattle were also allowed access to pasture during the summers of 1954 and 1955.

The levels of fluorine and aluminum sulfate added are shown in Table III. The procedure for calculating the amount of fluorine added to the ration was the same as that for Experiment I. Aluminum sulfate was calculated as a percentage of the total air-dry ration consumed.

Experiment IV

The cattle used in this experiment were grade Hereford heifers purchased as yearlings in the summer of 1952. All animals were tattooed and hip branded for positive identification.

The heifers were divided into eleven lots on the basis of type, grade, weight, and condition. These animals were individually fed rations once daily containing levels of fluorine ranging from 0 to 600 ppm. The fluorine was added to the ration from either raw rock phosphate or sodium fluoride. The experimental plan for these cattle is shown in Table IV. Fluorine at each level was fed to two comparable lots of cattle, one lot receiving fluorine from sodium fluoride and the companion

TABLE III

PLAN OF CATTLE EXPERIMENT II

Lot number	Number of cows	F added to ration ppm	Total F in ration ppm	$Al_2(SO_4)_3$ added per cent	Approx. range of age[a] (months)		Time on experiment[a] (months)	
					Trial I	Trial II	Trial I	Trial II
20A	3	-	8	-	30-34	66-70	12	48
20B	3	-	8	0.5	30-34	66-70	12	48
21A	3	20	28	-	30-34	66-70	12	48
21B	3	20	28	0.5	30-34	66-70	12	48
22A	3	30	38	-	30-34	66-70	12	48
22B	2[b]	30	38	0.5	30-34	66-70	12	48
23A	3	40	48	-	30-34	66-70	12	48
23B	3	40	48	0.5	30-34	66-70	12	48
24A	3	50	58	-	30-34	66-70	12	48
24B	3	50	58	0.5	30-34	66-70	12	48

[a]Age of cattle and time on experiment when urine collections were made.

[b]One animal in this lot was sacrificed twenty-three months after the experiment began.

TABLE IV

PLAN OF CATTLE EXPERIMENT IV

Lot no.	Number of cows[a]	Total F in ration ppm	F added ppm: Raw rock phosphate	F added ppm: Sodium fluoride	Approximate range of age[b] (months)	Time on experiment[b] (months)
40	3	7	-	-	39 - 45	30
41A	2	57	-	50	39 - 45	30
41B	2	57	50	-	39 - 45	30
42A	1	107	-	100	39 - 45	30
42B	1	107	100	-	39 - 45	30
43A	1	207	-	200	39 - 45	30
43B	1	207	200	-	39 - 45	30
44A	2	307	-	300	39 - 45	30
44B	2	307	300	-	39 - 45	30
45A	1[c]	607	-	600	-	-
45B	1	607	600	-	39 - 45	30

[a]Only one animal per lot was used in some cases as this was a pilot experiment.

[b]Age of cattle and time on experiment when urine collections were made.

[c]This cow died after being on experiment six months.

lot receiving fluorine from raw rock phosphate. These animals were fed a concentrate mixture (three parts corn and one part cottonseed meal) and chopped mixed hay. The method of calculating the amount of sodium fluoride and raw rock phosphate was the same as that for Experiment I. In addition to the experimental ration, these cattle were allowed free access to salt and water and were restricted to a dry lot during the entire experiment.

Experiment V

Sixty heifers, approximately fifteen to eighteen months of age, were purchased in Texas in July of 1951 for this experiment. These cattle were divided into four lots of twelve animals each and two lots (Lots 52 and 56) of six animals each on the basis of type, grade, weight, and condition. All animals were hip branded for positive identification.

Pasture for animals in Lot 51 (control) was selected in an area approximately sixteen air miles from an aluminum smelting plant. Based on distance and fluorine analysis of vegetation, this pasture had a normal fluorine content. Pastures for the remaining lots were selected on the basis of chemical analysis to have high fluorine contamination. Pasture samples were taken at periodic intervals of the high fluorine and control pastures. The average fluorine content of these pastures and the plan of experiment are shown in Table V.

At the initiation of the experiment, three animals were sacrificed and analyses were made of bones to obtain initial, or base, fluorine content. After approximately 100 days on high fluorine pastures, Lot 52

TABLE V

PLAN OF CATTLE EXPERIMENT V

Lot number	Number of cows	Pasture I Number of days[a]	Pasture I Av. F analyses ppm	Pasture II Number of days	Pasture II Av. F analyses ppm	Av. initial weight lbs.
51	12	-	-	1080	10	575
52	6	102	427	978	10	558
53	6	209	298	871	10	550
54	12	409	167	671	12	554
55	12	639	126	441	13	558
56	12	828	118	252	14	548

[a]Cattle in all lots except Lot 51 grazed pastures with average fluorine analyses indicated for the length of time specified.

was removed from the high areas, three animals were killed and the remainder of the animals in this lot moved to the control area. At approximately 200, 400, 600, and 800-day intervals, animals from Lots 53, 54, 55, and 56, respectively, were either killed or rib biopsies taken to determine fluorine content of bones. The remaining animals of each lot were then moved to control areas.

Experiment X

Seventy-two crossbred feeder wether lambs, eight to twelve months of age and averaging approximately eighty-eight pounds in weight, were used in this study. These animals were fed for approximately a two-month pre-experimental period in an effort to rid them of internal parasites, get them on feed and accustom them to individual feeding facilities. All animals were docked and treated routinely with phenothiazine. During this period, the daily ration of chopped lespedeza hay and a concentrate mixture (eighty per cent ground yellow corn and twenty per cent wheat bran) was gradually increased until all animals were consuming approximately one pound of hay and one pound of concentrate per head per day. They had free access to salt and water. Individual feed records were obtained for all animals during the last four weeks of this period. The animals were fed in the early morning and late evening, feeding one-half pound of concentrate at each feeding.

Twelve of these lambs, selected at random, were placed in metabolism stalls for a three-week preliminary training period. During this three-week period, final changes and adjustments were made in the metabolism stalls to standardize the balance procedure which was used throughout the experiment.

Following this preliminary period, seven-day collections were made on twelve lambs to determine the digestibility of feeds and mineral balances on representative animals to be used as the control. Representative samples of urine were taken daily from each animal from which an individual composite sample for the seven-day collection period was analyzed for fluorine.

These seventy-two feeder wether lambs were weighed, paint-branded and divided into six lots of ten animals each and three lots of four animals each, using the criteria of weight, previous feed consumption and general appearance. The six lots (Lot I through VI) with ten animals each and the three lots (Lots VII through IX) with four animals each were assigned to treatments at random as shown in Table VI.

Each animal was assigned an individual stanchion which was given a number corresponding to the number on the animal. Each lot of animals had a separate loafing pen with free access to salt and clean, fresh water.

The concentrate mixture, consisting of eighty per cent ground yellow corn and twenty per cent wheat bran, was mixed every two weeks. Fluorine in the form of sodium fluoride was added in parts per million (ppm) to the concentrate mixture as shown in Table VI. The sodium fluoride added to each batch of feed was pre-mixed with a small amount of concentrate previous to mixing with the bulk of the concentrate. After mixing each batch of feed, the mixer was thoroughly cleaned to

TABLE VI

PLAN OF SHEEP EXPERIMENT X

Lot number	Treatment: F added ppm	Treatment: Alleviators per cent	Total F in ration ppm	Number of lambs	Av. initial weight lbs.
I	-	(Control)	6	10	88.4
II	25		31	10	88.3
III	50		56	10	88.1
IV	75		81	10	88.2
V	100		106	10	88.3
VI	200		206	10	88.3
VII	100	0.1 $Al_2(SO_4)_3$	106	4	88.0
VIII	100	0.5 $Al_2(SO_4)_3$	106	4	88.2
IX	100	0.1 $AlCl_3$	106	4	88.0

prevent any contamination. Aluminum sulfate hydrate ($Al_2(SO_4)_3 \cdot 18\ H_2O$) and aluminum chloride hydrate ($AlCl_3 \cdot 6\ H_2O$) were added in the amounts as shown in Table VI. The percentage of alleviator was based on the total ration and mixed with the concentrate as described for sodium fluoride. The concentrate mixture for each lot was then stored in separate galvanized containers.

Section II

Phase 1

The urine samples used in this phase were collected from animals in Experiment I. Daily collections were made for seven days while these cattle were in metabolism racks (Hobbs et al. 1950). Daily water consumption records were maintained. The urine excreted daily was measured for each animal and the specific gravity taken and corrected to 15.5° Centigrade.

All fluorine determinations were made using a slight modification of the method described by Willard and Winter (1933).

Phase 2

Cattle from Experiment I were again used in the first study of this phase. Single voidations of urine and one and seven-day composites of urine were taken for use in this first study.

The second part of this phase was made with cattle from Experiment II. The procedure for the second part was the same as that described above except that collection of three-day composite samples of urine were made and analyzed for fluorine. The procedure used for fluorine determination has been described under Phase 1.

<u>Phase 3</u>

In this study urine samples were collected from nineteen grade Hereford cows (Table VII) at two, six, ten, sixteen and twenty-four hours after ingestion of fluorine either from sodium fluoride or raw rock phosphate. Cattle from both Experiment I and IV were used in an attempt to determine if age influenced the urinary fluorine excretion pattern. These lots, 1, 6, 7, and 8 will hereinafter in this phase be referred to as part of Phase 3.

The cattle were brought into the barn and fed in their usual manner. They were not allowed access to water during the first sixteen hours. However, during the remaining eight hours, they were turned out into a dry-lot and allowed to drink in order to keep the daily routine as nearly the same as possible.

To collect the urine samples, the cattle were locked in individual stanchions and were restrained by use of a side rope and tail hold. The vulva and surrounding areas were cleaned by paper towels and warm water to avoid contamination of the sample. The urine samples were obtained by use of a chrome plated cow catheter one-fourth inch in diameter and nine inches long that had been thoroughly disinfected in a solution of algicide. Attached to the posterior end of the catheter was a two-foot string as a precautionary measure against loss of the catheter. The catheter was held in the right hand while the left index finger was being

TABLE VII

PLAN OF PHASE 3

Lot no.	Number of cows	Total F in ration ppm	F added ppm Raw rock phosphate	Sodium fluoride	Approximate range of age[a] (months)	Time on experiment[a] (months)
1	1	7	-	-	108 - 113	96
6[b]	3	57	-	50	108 - 113	96
7[c]	3	77	-	70	108 - 113	96
8	3	107	-	100	108 - 113	96
40	1	7	-	-	39 - 45	30
41A	2	57	-	50	39 - 45	30
41B	2	57	50	-	39 - 45	30
44A[b]	2	307	-	300	39 - 45	30
44B[c]	2	307	300	-	39 - 45	30

[a]Age of cattle and time on experiment when urine collections were made.

[b]This lot used for first and second trials.

[c]These lots used for second trial.

inserted into the urethera orifice. The catheter was then passed over or under the index finger into the urethera, being careful to avoid the diverticulum. When urine could not be obtained immediately after insertion, the catheter was worked back and forth in order to coax urination. This method of catherization was desirable in obtaining quantities of urine up to 300 milliliters. Whenever possible, urine was collected by normal urination or by palpation of the perineal region with the back of the hand.

Phase 4

Cattle from Experiment V were used in this study. Bone samples taken from these cattle were either by biopsy or autopsy. The urine samples were taken either by catherization as described in Phase 3, or by palpation of the perineal region or normal urination.

The procedure for fluorine determination of urine and bones has been described under Phase 1.

Phase 5

Cattle from Experiment II were used for the first part of this phase. Wether lambs from Experiment X were used in the second part of the study. The method of collection employed has been described by Hobbs *et al*. (1951) and Griffity (1953). Composite samples of urine were taken at the end of a seven-day metabolism trial period for fluorine determination as described under Phase 1.

CHAPTER IV

RESULTS

Phase 1
Validity of Using Specific Gravity as a Method of Standardizing Urinary Fluorine Concentrations

The primary functions of the kidney and the urine are widely known. Numerous investigations have been made concerning the secretion and/or excretion of urine from the body; however, this mechanism is quite complex and is not fully understood.

One factor involved is the large amount of individual variation in daily water consumption. Dukes (1955) stated that while there is a large variation in water intake, there is a direct correlation in the amount of water consumed and the quantity of urine excreted.

This wide variation in water consumption was apparently observed by Blakemore _et al_. (1948) in their investigations of fluorosis in cattle in England. These workers used the urinary fluorine concentration from these cattle as an aid in the diagnosis of fluorosis. They further proposed that variations in specific gravity of different urine samples made it difficult to compare the fluorine concentration of these samples. It was stated that the variations in specific gravity were due to variations of water intake among cattle. These workers suggested correcting for such apparent variations by standardizing all values to an arbritrary specific gravity of 1.040. This correction is simply made by multiplying the observed fluorine value by the last two figures of the arbitrary standard specific gravity of the urine and dividing by the last two figures of the observed specific gravity.

This formula has been accepted and used by many workers (Phillips, 1952; Hobbs et al. 1951, 1954; and Schmidt et al. 1954). However, a search of the literature has not revealed the original basis of such a formula. The wide variation in water consumption among individual animals does not in itself justify the use of this formula. Its justification could only be possible by assuming that all solid material, other than fluorine, remained constant, and that any change in specific gravity is due to fluctuation in water intake by the animal.

The data presented in this phase were collected from animals in Experiment I, Phase 1, that were being used in digestibility studies previously described in Chapter III. The current rate of intake, as well as the dietary fluorine histories of these cattle, were known.

The average daily water consumed, average daily urinary output, and average specific gravity of urine for cattle in Phase 1 are shown in Table VIII. In general, these data showed a correlation of 0.3438 between water intake and urinary output. This correlation was not significant at the 0.5 level of confidence. Individual animal comparisons indicated a large amount of variation. The correlation between the amount of urine voided and the specific gravity of this urine was 0.1324. This correlation was not statistically significant. It was observed in some cases that animals refusing to drink either in the morning or afternoon would excrete as much urine as for a previous day. This would indicate that when the water intake is greatly varied there is a draw on

TABLE VIII

AVERAGE DAILY WATER CONSUMPTION, URINARY EXCRETION AND SPECIFIC GRAVITY OF URINE FROM CATTLE IN EXPERIMENT I

Lot number	Animal number	F added in ration ppm	Number of samples	Water consumed (c.c.)	Urine excreted (c.c.)	Specific gravity of urine
1	42	-	7	24,995	6041	1.0384
	13	-	7	27,426	5360	1.0401
	24	-	7	20,915	4630	1.0414
	Average			24,445	5344	1.0400
2	46	10	7	23,649	5941	1.0391
	16	10	7	26,943	5020	1.0383
	11	10	7	23,891	5838	1.0401
	Average			24,828	5600	1.0392
3	47	20	7	23,795	5314	1.0408
	Average			23,795	5314	1.0408
4	31	30	7	26,163	5940	1.0348
	48	30	7	25,092	4763	1.0416
	30	30	7	24,280	5966	1.0361
	Average			25,178	5556	1.0375

TABLE VIII

AVERAGE DAILY WATER CONSUMPTION, URINARY EXCRETION AND SPECIFIC GRAVITY OF URINE FROM CATTLE IN EXPERIMENT I (Continued)

Lot number	Animal number	F added in ration ppm	Number of samples	Water consumed (c.c.)	Urine excreted (c.c.)	Specific gravity of urine
	9	40	7	24,638	4718	1.0401
5	23	40	7	20,438	4574	1.0364
	49	40	7	25,667	6094	1.0381
	Average			23,581	5129	1.0382
	1	50	7	27,922	5246	1.0410
6	6	50	7	18,965	4566	1.0364
	50	50	7	21,906	5010	1.0371
	Average			22,931	4941	1.0382
	2	70	7	25,862	6218	1.0318
7	58	70	7	26,543	5700	1.0387
	21	70	7	24,430	5131	1.0334
	Average			25,611	5683	1.0346
	29	100	7	22,036	4607	1.0400
8	61	100	7	18,225	5908	1.0318
	Average			20,131	5258	1.0359

body stores or an increased diuretic action brought about in the animal.

The results of average observed analyses and average corrected analyses of fluorine content in single voidation, one-day composite, and seven-day composite urine samples are presented in Table IX. There was more variability within the corrected analyses than among the observed fluorine analyses for each type of sampling.

These data would further indicate that the correction formula proposed by Blakemore <u>et al</u>. (1948) would not be justified under the conditions of these experiments at the Tennessee station. A method similar to the one proposed by these workers would definitely be advantageous. Perhaps in future years, with a more complete knowledge of kidney and urine functions in relation to fluorine excretion, such a formula can be derived. These data (Table IX) indicate the observed analyses to be more accurate than the corrected analyses. Additional information on this subject is presented in Phase 2 of this thesis.

Phase 2
Comparison of The Fluorine Concentration of Single Voidations and One, Three, and Seven-Day Composite Voidations of Urine From Cattle

The urine samples used in this study were collected from cattle in Experiments I and II that were being used in digestibility studies previously described. The current rate of fluorine intake, as well as the dietary fluorine history of these cattle were known. Samples for analyses were taken from single voidations, and from composites of one, three and seven-day voidations.

TABLE IX

COMPARISON OF AVERAGE OBSERVED AND CORRECTED FLUORINE ANALYSES OF URINE COLLECTED AS SINGLE VOIDATIONS, ONE-DAY AND SEVEN-DAY COMPOSITE SAMPLES FROM COWS ON DIFFERENT DIETARY LEVELS OF FLUORINE

Lot number	F added in ration ppm	Single voidation: Observed analyses av. ppm F[a]	Single voidation: Corrected analyses av. ppm F[b]	One-day: Observed analyses av. ppm F[a]	One-day: Corrected analyses av. ppm F[b]	Seven-day: Observed analyses av. ppm F[a]	Seven-day: Corrected analyses av. ppm F[b]
1	-	4.6(13)	3.7(9)[c]	6.8(4)[c]	5.7(4)	3.8(8)	2.9(6)[c]
2	10	9.2(13)	8.6(9)	12.0(1)	10.4(1)	10.9(7)	9.5(6)
3	20	14.5(7)	13.9(5)	-	-	19.2(4)	17.8(3)
4	30	19.9(13)	20.1(9)	24.0(1)	21.8(1)	23.8(6)	19.5(6)
5	40	21.0(10)	25.8(9)	28.7(3)	26.3(3)	26.7(5)	24.5(4)
6	50	30.8(10)	30.3(8)[d]	-	-	34.6(5)[d]	27.5(5)
7	70	31.2(11)	33.5(9)	38.8(4)	37.7(4)	40.4(6)	36.6(6)
8	100	36.6(14)	41.6(9)	37.5(2)	31.7(2)	40.0(4)	39.0(4)
	Average	21.0 ± .952	22.6 ± 1.0946	25.3 ± 1.447	23.2 ± 1.435	23.0 ± 0.988	21.4 ± 1.314

[a]Average analyses of tests run at twenty-four and thirty-six months after initiation of experiment.
[b]Corrected to standard specific gravity of 1.040.
[c]Significant at .05 level. Single voidation and seven-day corrected analyses for Lot 1 was significant at the .05 level to the one-day observed analyses.
[d]Significant at .01 level. Single voidation corrected analyses for Lot 6 was significant at the .01 level to the seven-day observed analyses.

Since many workers have used the method described by Blakemore (1948) for presenting fluorine content of urine, all data in this thesis will be presented by both methods, corrected for a specific gravity of 1.040, and the observed analysis, unless otherwise specified. The term "corrected" as used in this thesis means that the value or values have been calculated to a standard specific gravity of 1.040. The term "observed" fluorine analysis means the values observed by the analyst.

The results of average observed analyses and average corrected analyses of fluorine content in single voidation, one-day composite, and seven-day composite urine samples are presented in Table IX. The results are the average urinary fluorine analyses determined from two metabolism trials, the first conducted when the cattle had been on test twenty-four months and the second when they had been on test thirty-six months. In general, there were no significant differences among urinary fluorine concentrations for either corrected or observed for the three methods of collection. However, statistical differences, depending upon method of collection and whether or not the analyses were reported as observed or as corrected to a specific gravity of 1.040, were noted in two of the eight lots. In Lot 1 (control) there was a significant difference between the corrected analyses for the single voidation samples and the observed analyses for the one-day samples. There was also a significant difference between the corrected analyses of one-day and seven-day samples. Furthermore, there was a significant difference between the single voidation corrected analyses and the seven-day observed analyses for Lot 6.

Fluorine analyses of both single-voidation and seven-day composite samples were closely correlated with the amounts of ingested fluorine (Table X). Analyses for this study were made after the cows in Experiment I had been on test thirty-six months. Observed fluorine analyses showed a close relationship (r = .966 for single-voidation and r = .960 for seven-day composites) to fluorine intake than analyses corrected (r = .896 for single voidation and r = .954 for seven-day composites) to a standard specific gravity, but both were highly significant.

A further study of the validity of fluorine analyses for single voidation samples only was made. In Table XI are given the urinary fluorine values of single voidation samples from cows in Experiment I after twenty-four months on test and again after thirty-six months on test. If both the corrected and observed analyses are considered, there would be apparent differences in the data shown in the table for Lots 5, 6, 7, and 8. There were, however, no statistically significant differences found in this comparison, probably due to the variations in analyses within lots.

Studies were made of the correlation between total milligrams of fluorine excreted in urine and the concentration of fluorine (ppm) in urine for single voidation and seven-day composite samples. The data shown in Table XII, were obtained after cattle in Experiment I had been on test thirty-six months. Statistical analyses of these data indicate that there was a high correlation (r = .949, .907, .854, and .910) between the fluorine analyzed for both observed and corrected values and total milligrams excreted. This was true for both the single

TABLE X

CORRELATION OF AVERAGE OBSERVED AND CORRECTED FLUORINE ANALYSES OF URINE COLLECTED AS SINGLE VOIDATIONS AND SEVEN-DAY COMPOSITE SAMPLES WITH THE AVERAGE DAILY FLUORINE INTAKE OF COWS ON DIFFERENT DIETARY LEVELS OF FLUORINE

		Single voidation			Seven-day	
Lot number	F added to ration ppm	Observed analyses[a] av. ppm F	Corrected analyses[b] av. ppm F	Av. daily milligrams F consumed	Observed analyses[c] av. ppm F	Corrected analyses[d] av. ppm F
1	-	4.88	3.06	45.56	3.33	2.78
2	10	9.66	8.04	155.80	11.67	9.68
3	20	13.22	15.07	252.44	21.00	16.47
4	30	19.00	20.29	377.16	22.33	18.12
5	40	15.50	28.59	459.42	24.00	19.80
6	50	24.06	21.48	552.50	26.00	20.88
7	70	24.86	25.82	758.35	37.33	31.74
8	100	35.39	30.33	898.26	36.67	29.04

[a] r - 0.966

[b] r - 0.896

[c] r - 0.960

[d] r - 0.954

TABLE XI

COMPARISON OF AVERAGE OBSERVED AND CORRECTED VOIDATIONS OF URINE AFTER TWENTY-FOUR MONTHS ON TEST WITH FLUORINE ANALYSES OF SINGLE VOIDATIONS AFTER THIRTY-SIX MONTHS ON TEST FROM COWS ON DIFFERENT DIETARY LEVELS OF FLUORINE

		Twenty-four months			Thirty-six months			Average		
Lot no.	F added to ration ppm	Number of samples	Observed analyses av. ppm F	Corrected analyses[a] av. ppm F	Number of samples	Observed analyses av. ppm F	Corrected analyses av. ppm F	Number of samples	Observed analyses av. ppm F	Corrected analyses av. ppm F
1	-	6	4.32	3.97	7	4.88	3.06(3)	13	4.62	3.67(9)
2	10	6	8.63	8.86	7	9.66	8.04(3)	13	9.18	8.59(9)
3	20	4	15.40	13.66	3	13.22	15.07(1)	7	14.47	13.94(5)
4	30	6	20.87	19.94	7	19.00	20.29(3)	13	19.87	20.06(9)
5	40	6	24.72	24.45	4	15.50	28.59(3)	10	21.03	25.83(9)
6	50	6	35.28	33.22	4	24.06	21.48(2)	10	30.79	30.28(8)
7	70	6	36.51	37.36	5	24.86	25.82(3)	11	31.22	33.52(9)
8	100	6	38.27	47.26	8	35.39	30.33(3)	14	36.62	41.62(9)

[a]All values corrected to specific gravity of 1.040.

( ) Numbers in parentheses represent number of samples.

TABLE XII

CORRELATION OF AVERAGE OBSERVED AND CORRECTED FLUORINE ANALYSES OF SINGLE VOIDATIONS AND SEVEN-DAY COMPOSITE VOIDATIONS OF URINE WITH TOTAL MILLIGRAMS OF FLUORINE IN THE URINE FROM COWS ON DIFFERENT DIETARY LEVELS OF FLUORINE

		Single voidation			Total	Seven-day		
Lot number	F added to ration ppm	Number of samples	Observed analyses[b] av. ppm F	Corrected analyses[a,c] av. ppm F	Mg. F excreted in urine	Number of samples	Observed analyses[d] av. ppm F	Corrected analyses[a,e] av. ppm F
1	-	7	4.88	3.06(3)	23.5	3	3.33	2.78
2	10	7	9.66	8.04(3)	86.0	3	11.67	9.68
3	20	3	13.22	15.07(1)	94.9	1	21.00	16.47
4	30	7	19.00	20.29(3)	159.8	3	22.33	18.12
5	40	4	15.50	28.59(3)	160.6	2	24.00	19.80
6	50	4	24.06	21.48(2)	155.1	3	26.00	20.88
7	70	5	24.86	25.82(3)	171.2	3	37.33	31.74
8	100	8	35.39	30.33(3)	179.3	3	36.67	29.04

[a]Values corrected to specific gravity of 1.040

[b]r - 0.949

[c]r - 0.907

[d]r - 0.854

[e]r - 0.910

and seven-day voidations. Both correlations were significant at the .01 per cent level.

Since the total milligrams of fluorine excreted presents an excellent baseline for comparison, it would seem that the observed values should be as good to use as the corrected values.

A comparison of average observed fluorine analyses and average fluorine analyses corrected to the standard specific gravity was made for single voidation, one-day, three-day, and seven-day composite urine samples from cows in Experiment II (Table XIII). As was the case with data in Table XI, there were cases of apparent differences within lots, but these differences were not statistically significant. There were several cases of variations between corrected and observed fluorine analyses for each type of collection.

Comparison of treatments showed highly significant differences in the urinary fluorine content among cattle receiving no fluorine, 20, 30, 40, and 50 ppm fluorine as sodium fluoride. Also, there was a statistically significant difference in the urinary fluorine concentrations between the lots of cattle receiving no alleviators as compared to corresponding lots receiving 0.5 per cent aluminum sulfate in their ration.

Phase 3
Daily Excretion Pattern of Urinary Fluorine in Cattle

This phase was initiated primarily to determine the fluorine content of cattle urine at various intervals following fluorine ingestion.

TABLE XIII

COMPARISON OF AVERAGE OBSERVED AND CORRECTED FLUORINE ANALYSES FROM SINGLE VOIDATION, ONE-DAY, THREE-DAY AND SEVEN-DAY COMPOSITE SAMPLES OF URINE FROM COWS ON DIFFERENT DIETARY LEVELS OF FLUORINE WITH AND WITHOUT ALUMINUM SULFATE

	Treatment		Single voidation		One-day		Three-day		Seven-day	
Lot no.	ppm F added	Per cent $Al_2(SO_4)_3$ added	Observed analyses av. ppm F	Corrected analyses av. ppm F	Observed analyses av. ppm F	Corrected analyses av. ppm F	Observed analyses av. ppm F	Corrected analyses av. ppm F	Observed analyses av. ppm F	Corrected analyses av. ppm F
20A	-	-	2.3(3)	10.2(2)	4.2(1)	4.4(1)	3.8(1)	3.8(1)	3.1(2)	3.0(2)
20B	-	0.5	2.0(3)	3.3(3)	-	-	-	-	2.6(2)	2.0(2)
21A	20	-	8.1(4)	18.0(2)	11.3(2)	10.5(2)	11.1(2)	10.3(2)	11.7(3)	9.9(3)
21B	20	0.5	4.9(3)	5.8(2)	6.1(1)	6.1(1)	5.4(1)	5.2(1)	6.5(2)	5.6(2)
22A	30	-	4.7(3)	10.8(2)	-	-	-	-	42.0(1)	35.0(1)
22B	30	0.5	4.7(4)	6.0(2)	6.3(1)	6.0(1)	7.2(1)	5.8(1)	9.1(3)	7.1(3)
23A	40	-	8.6(4)	25.6(3)	7.8(1)	8.4(1)	8.9(1)	10.2(1)	16.6(3)	14.2(3)
23B	40	0.5	3.4(3)	-	-	-	-	-	11.4(2)	10.5(2)
24A	50	-	12.2(4)	34.5(3)	27.2(1)	20.9(1)	26.7(1)	20.5(1)	26.4(1)	-
24B	50	0.5	7.2(4)	11.6(3)	8.9(1)	8.9(1)	10.3(1)	9.8(1)	13.6(3)	11.7(3)

( ) Number in parentheses shows number of samples.

It was hoped that this information might make urinary fluorine analyses more applicable and accurate under field conditions. Age, variation in levels of fluorine intake, and two dietary sources of fluorine were considered in this study. Mature cows from Experiment I that were approximately 109 months old and cows from Experiment IV that were approximately forty-two months old were used. These cows received fluorine from either raw rock phosphate or sodium fluoride at levels ranging from control to 300 ppm fluorine in the total air-dry ration.

The urine samples were collected, either by catherization, palpation of the perineal region, or during normal urination at intervals of two, six, ten, sixteen and twenty-four hours after ingestion of fluorine. During the first sixteen hours the cows were kept in the barn without water. During the remainder of the twenty-four hour period they were given access to water and the run of a dry lot in replication of their daily routine.

From the reports found in the literature concerning the peak of concentration of fluorine in the urine, Phillips (1952) stated that the peak is from two to six hours after ingestion and that a marked decrease occurs twenty-two to twenty-four hours afterward. Data presented in Table XIV apparently support this hypothesis; however, there are some variations and the data are too limited to be conclusive. Schmidt (1954) reported similar findings but his data indicated an increase in urinary fluorine content up to eight hours and a marked decrease in twenty-two to twenty-four hours.

The excretion data presented in Table XIV do indicate, however,

TABLE XIV

THE OBSERVED FLUORINE CONCENTRATIONS OF URINE AT INTERVALS AFTER FLUORINE INGESTION

Lot no.	Animal number	Age[a] (months)	F added ppm NaF	F added ppm RRP	Number of samples per interval	Hours after ingestion 2	6	10	16	24	Av.
						Av. F content (ppm)					
1	24	108-113	-	-	1	4	4	4	3	4	3.8
40	40-1	39-45	-	-	1	4	4	5	4	4	4.2
6	1	108-113	50	-	2	26	30	28	20	36[b]	27.1
6	6	108-113	50	-	2	32	34	36	32	31	33.2
6	50	108-113	50	-	2	22	22	24	24	18	22.0
41A	41-1	39-45	50	-	1	28	24	18	18	21	21.8
41A	41-2	39-45	50	-	1	26	20	23	20	5	18.8
41B	41-4	39-45	-	50	1	13	17	16	20	6	14.4
41B	41-5	39-45	-	50	1	23	12	13	10	3	12.2
7	2	108-113	70	-	1	60	58	53	25	23	43.8
7	21	108-113	70	-	1	38	36	-[c]	22	48	36.0
7	58	108-113	70	-	1	31	54	53	28	26	38.4
8	61	108-113	100	-	1	34	34	27	25	17	27.4
44A	44-1	39-45	300	-	2	58	62	50	42	34	49.3
44A	44-2	39-45	300	-	2	66	64	54	47	33	54.8
44B	44-4	39-45	-	300	1	18	31	31	26	8	22.8
44B	44-5	39-45	-	300	1	25	38	31	22	24	28.0

[a]Cattle 108 to 113 months old had been on experiment ninety months and cattle 39 to 45 months old had been on experiment thirty months.

[b]One sample.

[c]No sample.

that while there are many variations in the urinary concentration of fluorine, the mean level of fluorine concentration for each lot tested occurred between eight to twelve hours after ingestion. Furthermore, the peak for all lots, with the exception of Lots 6 and 7, was between two to six hours. The observed fluorine analyses used in this phase were not corrected to a standard specific gravity.

The comparison of urinary fluorine concentrations from two age groups of cattle receiving fifty parts per million of fluorine from sodium fluoride in their ration is presented in Table XV. While there is much individual variation, these data indicate a trend toward a lesser concentration of urinary fluorine from the younger animals when compared to the older cattle. This may have been due to the fact that the bones in the older cattle had reached a point of saturation and therefore, excreted a higher concentration of fluorine in the urine.

Hobbs et al. (1955) have shown that there may be up to 35 to 40 per cent less fluorine stored in the bones of animals receiving raw rock phosphate as compared to animals receiving fluorine from sodium fluoride. Comparisons of urinary fluorine concentrations from two sources of fluorine at two different levels are given in Tables XVI and XVII. There was a very marked decrease in fluorine concentration in the urine of Lots 41B and 44B as compared to Lots 41A and 44A. This reduction of fluorine in both bone and urine content is thought to be due to the difference in the solubility and resulting availability of the two products. No data on the fluorine content of the feces were available to substantiate this hypothesis.

TABLE XV

COMPARISON OF OBSERVED URINARY FLUORINE CONCENTRATIONS IN CATTLE RECEIVING FIFTY PPM FLUORINE FROM NAF ON EXPERIMENT FOR THIRTY AND NINETY MONTHS, RESPECTIVELY

Age of animal	109 months				42 months		
Animal no.	1	6	50		41-1	41-2	
Hours after ingestion	Fluorine in urine ppm						
				Av.			Av.
2	30.5	29.6	23.6	27.9	28.2	26.0	27.1
6	30.4	31.1	25.4	29.0	23.8	20.0	21.9
10	28.0	28.8	21.7	24.5	18.1	22.6	20.4
16	22.3	25.5	10.0	19.3	18.2	19.9	19.0
24	36.4	29.6	26.0	30.7	20.6	4.8[a]	12.7

[a]This cow had urinated twice prior to catherization.

TABLE XVI

COMPARISON OF OBSERVED URINARY FLUORINE CONCENTRATIONS FROM CATTLE RECEIVING 50 PPM FLUORINE FROM SODIUM FLUORIDE AND 50 PPM FLUORINE FROM RAW ROCK PHOSPHATE

Level and source of F	50 ppm NaF			50 ppm RRP				
Animal no.	41-1	41-2		41-4	41-5			
Hours after ingestion	Fluorine in urine ppm						Reduction[a]	
							F ppm	Per cent
			Av.			Av.		
2	28.2	26.0	27.1	12.6	22.6	17.6	9.5[c]	35.0
6	23.8	20.0	21.9	17.0	11.5	14.2	7.7	35.0
10	18.1	22.6	20.4	16.5	12.8	14.6	5.8	28.0
16	18.2	19.9	19.0	19.9	9.9	14.9	4.1	22.0
24	20.6	4.8[b]	12.7	6.0	2.9	4.4	8.3[c]	65.0

[a]Calculated reduction in fluorine attributed to the relative insolubility of RRP and resulting in the unavailability of fluorine.

[b]This cow had urinated twice prior to catherization.

[c]Statistically significant at .05 level.

TABLE XVII

COMPARISON OF OBSERVED URINARY FLUORINE CONCENTRATIONS FROM CATTLE RECEIVING 300 PPM FLUORINE FROM SODIUM FLUORIDE AND 300 PPM FLUORINE FROM RAW ROCK PHOSPHATE

Level and source of F	300 ppm NaF[a]			300 ppm RRP				
Animal no.	44-1	44-2		44-4	44-5			
Hours after ingestion	Fluorine in urine ppm						Reduction[b] F ppm	Reduction[b] Per cent
			Av.			Av.		
2	48	61	54.5	18	25	21.5	33	61
6	66	64	65.0	31	38	34.5	30	47
10	48	56	52.0	31	31	31.0	21	40
16	29	58	43.5	26	22	24.0	20	45
24	42	22	32.0	8	24	16.0	16	50

[a]Average of two trials.

[b]Calculated reduction in fluorine attributed to the relative insolubility of RRP, and resulting in the unavailability of fluorine.

The fluorine concentrations of urine from animals in the raw rock phosphate-sodium fluoride comparison studies indicate that the concentrations of fluorine in the urine may vary considerably with age of the animal, and the source and level of fluorine intake. The exact length of time after ingestion that is best for obtaining representative urine samples cannot be determined. These data would indicate, however, that a range of eight to sixteen hours after fluorine ingestion for these mature cattle under the conditions of this experiment.

Phase 4
Influence of Rib Fluorine Content Upon Urinary Fluorine Concentration After Cessation of High Fluorine Intake

Cattle in Experiment V were used in this study. General observations on the response of the fluorine content of ribs to fluorine consumption, and to cessation of fluorine consumption should be outlined here prior to presenting the data on urinary fluorine concentration of these animals. Rib samples were recovered from representative members of the different lots at the beginning of the test and at intervals of approximately 100, 200, 400, 600, and 800 days. It should be pointed out that all cattle grazed pasture containing 427 ppm fluorine for part of their experimental period. However, the amount of fluorine decreased over the experimental period of 828 days due to the installation of a recovery system in the aluminum smelting plant.

The results of these rib fluorine analyses for the various intervals are presented in Figure 1. These data support the following

UPTAKE AND DEPLETION OF FLUORINE IN RIBS
OF CATTLE ON PASTURE

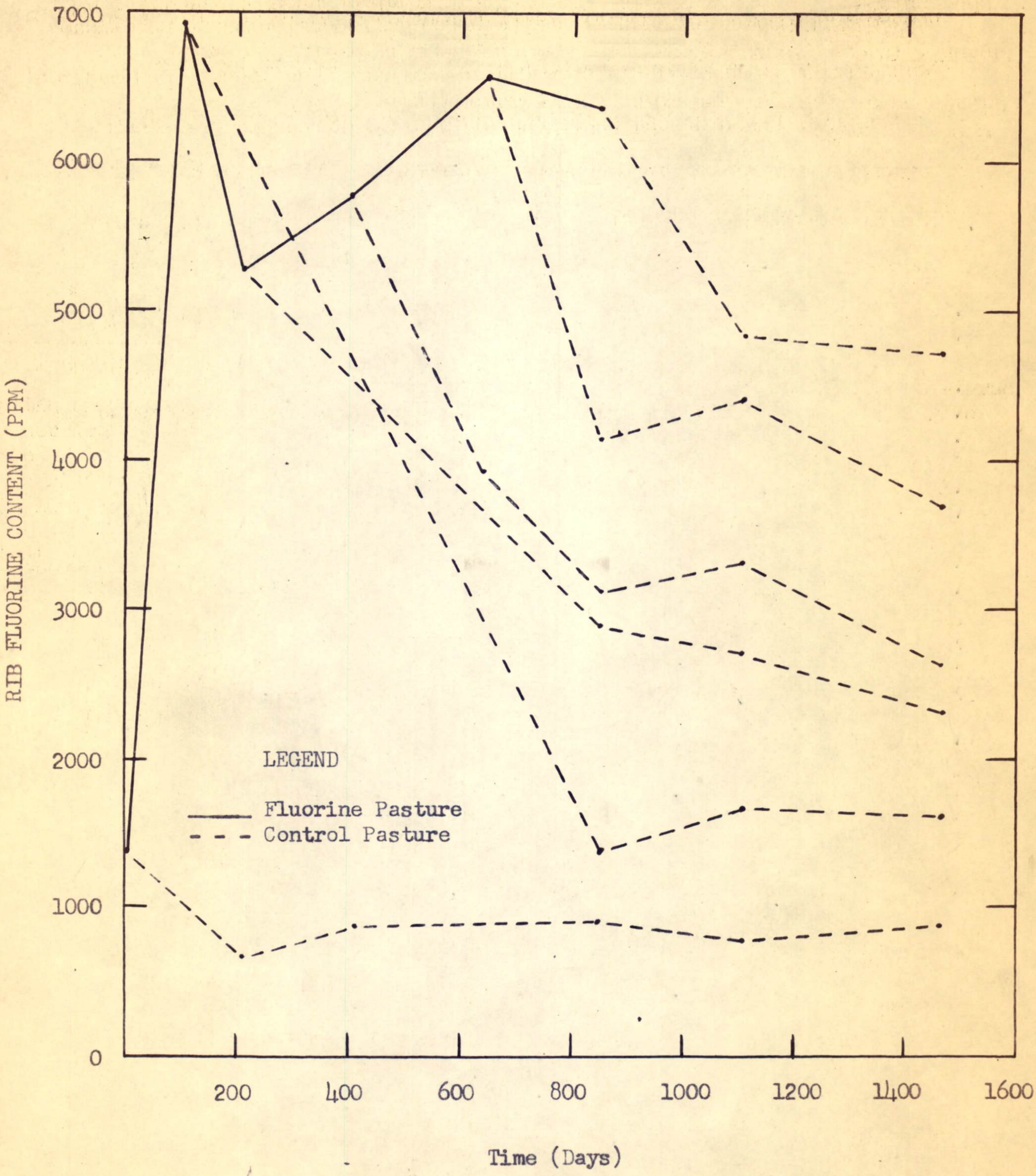

Figure 1.

observations: (1) Fluorine content of ribs increased rapidly during the period the animals grazed pastures of high fluorine content; (2) When the animals were removed to control (uncontaminated) pastures, the fluorine content of the ribs was significantly reduced; (3) The degree of reduction of rib fluorine content varied inversly in these cases, with the length of time the cattle had grazed high fluorine pastures; (4) The rate of reduction of rib fluorine content was greatest immediately after removal of the animals from high fluorine pasture and leveled off after approximately 200 to 700 days.

The urine samples from these animals were obtained by catherization in one day. On that date, cattle which had been on the high fluorine pasture had been removed from that pasture for the following number of days: Lot 52, 978 days; Lot 53, 871 days; Lot 54, 671 days; Lot 55, 441 days; and Lot 56, 252 days.

The data on fluorine concentration in these urine samples are presented in Table XVIII. No significant differences in the urinary fluorine content was observed among the different lots, even though there were differences in rib fluorine concentration and in length of time the cattle had grazed on high fluorine pasture. In these cases, urinary fluorine did not indicate the amount stored in the rib but did reflect the dietary intake.

Phase 5
Influence of Fluorine Alleviators Upon the Urinary Fluorine Concentration of Cattle and Sheep

Cattle from Experiment II were used in the first part of this

TABLE XVIII

URINARY FLUORINE CONCENTRATION AND RIB FLUORINE CONTENT OF CATTLE IN EXPERIMENT V

Lot number	Pasture I: Number of days	Pasture I: Av. F analyses ppm	No. of urine samples	Average F content (ppm): Urine[a] Observed	Average F content (ppm): Urine[a] Corrected[e]	Average F content (ppm): Rib[b]
51	-	10(control)	7	1	4	800
52	102	427	3	1	5	1775[c]
53	209	298	3	2	9	2900
54	409	167	4	2	5	3400
55	639	126	6	2	5	4450[d]
56	828	118	8	2	6	4900

[a]Samples taken on July 6, 1954.

[b]Based on average analyses of the ninth and tenth ribs of one animal from each lot taken July 9, 1954 or July 12, 1954.

[c]Average of three animals.

[d]Average of two animals.

[e]Urine analyses corrected to a standard specific gravity of 1.040.

alleviator study. (The urine collections were made at the end of twelve months on test and again at the end of forty-eight months on test.) The influence of the added aluminum sulfate (0.5 per cent of the total air-dry ration) and of time on the observed urinary fluorine concentration is shown in Table XIX. These data indicate a marked decrease in the fluorine concentration of the urine in the lots receiving the alleviator as compared to their companion lot receiving no alleviator. These data are in close agreement with those reported by Hobbs et al. (1954) on the bones of these same cattle. These workers demonstrated a 20 to 40 per cent decrease in fluorine stored in the bones of the alleviator lots as compared to their companion lot.

Further study of the data in Table XIX shows that the average fluorine concentration in the urine was greater at the end of forty-eight months on test than at the end of twelve months on test. This was true in the lots receiving aluminum sulfate as well as in the lots not receiving aluminum sulfate. The percentages of reduction in urinary fluorine concentrations were approximately the same for each period. It should also be noted that the observed fluorine analyses for Lot 22A (one sample) at the end of twelve months on test was higher than the analyses of urine from cows consuming larger concentration of fluorine.

The second part of this study was conducted using sheep from Experiment X. The effects of alleviators upon the fluorine content of the urine is shown in Table XX. There was a highly significant decrease between all lots receiving 100 ppm fluorine plus alleviators and Lot V receiving 100 ppm fluorine from sodium fluoride in the ration. There was

TABLE XIX

THE INFLUENCE OF ALUMINUM SULFATE AND TIME UPON THE OBSERVED URINARY FLUORINE CONCENTRATION OF CATTLE ON DIFFERENT DIETARY LEVELS OF FLUORINE

Lot no.	Treatment: F added ppm	Treatment: $Al_2(SO_4)_3$ added per cent	Observed analyses[c]: 12 Months[a] Av. ppm F	Observed analyses[c]: 48 Months[a] Av. ppm F	Reduction[b]: 12 Months F ppm	Reduction[b]: 12 Months Per cent	Reduction[b]: 48 Months F ppm	Reduction[b]: 48 Months Per cent
20A	-	-	3.1(2)	5.5(2)	0.5	16.2	1.5	27.3
20B	-	0.5	2.6(2)	4.0(2)				
21A	20	-	11.7(3)	16.0(2)	5.2	44.5	4.7	29.4
21B	20	0.5	6.5(2)	11.3(3)				
22A	30	-	42.0(1)	25.7(3)	32.9	78.4	11.7	45.5
22B	30	0.5	9.1(3)	14.0(2)				
23A	40	-	16.6(3)	27.0(2)	5.2	31.4	11.5	42.6
23B	40	0.5	11.4(2)	15.5(2)				
24A	50	-	26.4(1)	41.5(2)	12.8	48.5	19.8	47.7
24B	50	0.5	13.6(3)	21.7(2)				

[a]At the time these collections were made cattle had been on test twelve and forty-eight months, respectively.

[b]Calculated reduction in observed fluorine concentration due to the addition of aluminum sulfate.

[c]Analyses are based on seven-day composite samples.

TABLE XX

OBSERVED URINARY FLUORINE CONCENTRATION FROM LAMBS FED KNOWN LEVELS OF FLUORINE AND ALLEVIATORS FOR DIFFERENT TIME INTERVALS

Lot no.	Treatment: Added F ppm	Treatment: Alleviator per cent	Number of animals[a]	Fluorine concentrations from seven-day composite samples (ppm): 21 Days	54 Days	83 Days	112 Days	143 Days	Av. F in urine ppm	Av. F in urine/day (mgs.)
I	-	(control)[b]	2	3.0	11.0	7.0	3.5	7.5	6.4	3.8
II	25		2	36.5	22.5	17.0	11.5	34.0	24.3	9.1
III	50		2	43.0	34.0	35.0	10.0	49.0	32.6	10.4
IV	75		2	68.5	58.0	58.0	24.5	60.0	53.8	11.8
V	100		2	94.5	77.5	62.0	39.5	41.0	65.3	22.1
VI	200		2	91.5	53.0	48.5	37.0	72.0	60.4	18.6
VII	100	+ 0.1 $Al_2(SO_4)_3$	2	-	65.5[c]	6.7[d]	33.5[e]	50.0[f,g]	37.3	10.9
VIII	100	+ 0.5 $Al_2(SO_4)_3$	2	-	66.5[c]	11.7[d]	37.0[e]	65.0[f,g]	42.2	11.3
IX	100	+ 0.1 $AlCl_3$	2	-	56.5[c]	10.5[d]	29.5[e]	54.0[f,g]	35.3	11.3

[a]Each animal on metabolism trial for 7 days during each period.

[b]Urine from 12 pre-experimental animals averaged 4.4 ppm F.

[c]41 days from beginning of experiment.

[d]69 days from beginning of experiment.

[e]103 days from beginning of experiment.

[f]133 days from beginning of experiment.

[g]One animal.

no significant differences in the urinary fluorine concentrations among the lots receiving alleviators. These data are in close agreement with the bone data subsequently collected from these animals.

The average observed parts per million urinary fluorine, overall average observed fluorine concentration, and the average daily milligrams of fluorine excreted from lambs fed known levels of fluorine and alleviators for different intervals of time are shown in Table XXI. The variation in the fluorine concentration from one period to another should be pointed out.

In general, the urinary fluorine concentration of the last period was higher than that of other periods. Furthermore, there was a highly significant correlation of 0.778 between the amount of fluorine ingested and the amount analyzed in the urine. This correlation between urinary fluorine and dietary fluorine was significant at the .01 level of confidence.

TABLE XXI

EFFECTS OF ALLEVIATORS UPON OBSERVED URINARY FLUORINE CONCENTRATION

Lot number	Treatment: Added F ppm	Treatment: Alleviator per cent	Days on test: 41	69	103	133
			(Av. total mgs. of F in urine for 7 days)			
V	100	-	143.86[a]	174.37[b]	135.70[c]	209.25[d,e]
VII	100	+ 0.1 $Al_2(SO_4)_3$	107.55	17.77	75.86	132.20[e]
VIII	100	+ 0.5 $Al_2(SO_4)_3$	116.70	14.38	69.54	153.99[e]
IX	100	+ 0.1 $AlCl_3$	100.72	23.17	88.57	130.15[e]

[a] 54 days from beginning of experiment.

[b] 83 days from beginning of experiment.

[c] 112 days from beginning of experiment.

[d] 143 days from beginning of experiment.

[e] One animal

CHAPTER V

GENERAL DISCUSSION

The reliability and adaptability of urinary fluorine analyses as one diagnostic measure of fluorosis in cattle and sheep were the primary objectives of this study. An attempt was made to evaluate for the field worker: (1) the validity of "standardizing" urinary fluorine values to a given specific gravity; (2) the method of urine sampling that would be practical and yield accurate results; (3) the best time to collect urine samples for fluorine analysis in relation to the time fluorine-bearing feed was ingested: (4) the effect of fluorine previously stored in the body might have upon the accuracy of urinary fluorine analyses, and (5) the effect of fluorine "alleviators" upon the concentration of fluorine in the urine. Consideration was also given to the effects that the age of an animal and total period of fluorine intake would have on urinary fluorine concentrations.

Investigations of the validity of standardizing urinary fluorine values to a given specific gravity were made in Phases 1 and 2. These studies did not reveal justification for correcting the observed fluorine concentration of a urine sample to conform to a given specific gravity. Such a correction may double or halve the observed urinary fluorine value and thus mask the diagnosis. Such adjustments of the observed urinary fluorine value should, on the basis of this study, be used with caution if at all.

The collection of composite urine samples for one, three, or seven days is impractical under field conditions. Work in Phase 2 indicates that the urinary fluorine values of single voidations, where an adequate number of samples were taken, are on the average as valid as the values from the composite samples. At the same time, it was shown that there are occasional wide variations in urinary fluorine values for any type of sampling. Regardless of the type of urine collections repeated, sampling is necessary to overcome the error caused by these variations.

In Phase 3, it was demonstrated, as has been done by other workers, that urinary fluorine values tend to drop sharply twenty-two to twenty-four hours after ingestion of fluorine-bearing feeds. Work with a limited number of animals indicated that urine samples collected eight to sixteen hours after cattle have ingested fluorine-bearing feeds would give fluorine analyses approaching the average for those animals.

The results in Phase 3 indicated that urinary fluorine values tend to be slightly lower in young animals than in older animals ingesting comparable levels of fluorine. It was also demonstrated that urinary fluorine levels from cattle consuming fluorine from raw rock phosphate were 22 to 61 per cent lower than urinary fluorine levels from cattle consuming comparable amounts of fluorine from sodium fluoride.

Urinary fluorine values have been reported by Blakemore (1948) as reflecting stores of fluorine in the skeleton. In Phase 4, it was found that high fluorine concentration in the ribs 250 days after cessation of high fluorine intake had no influence on urinary fluorine values. Data from periods close to the cessation of fluorine intake were not available.

In some industrial and natural situations of fluorine contamination, it may be impossible to completely control the contamination. Maintenance of cattle and sheep in these areas may depend upon the use of aluminum sulfate or other fluorine alleviator in the diet of these animals. In Phase 5, data were presented on the urinary fluorine values from cattle and sheep receiving these alleviators. In general, the alleviators reduced urinary fluorine concentration 27 to 47 per cent in these species.

Other general information obtained in this study would show that urinary fluorine values from cattle receiving up to 8 ppm fluorine in the ration (control) ranged from 1 to 7 ppm.

From all phases of this study, certain general information on urinary fluorine concentrations was derived. There was a close correlation, under controlled conditions, between urinary fluorine values and levels of ingested fluorine. Data on values of urinary fluorine from all phases indicate that there is occasional wide variation between urinary fluorine values from cows of the same age and receiving the same levels of fluorine from a given source. This further shows that fluorine analyses of urine samples should function as a diagnostic aid of fluorine only when supported by fluorine analyses of feed and bones, appropriate studies of teeth, and general observations of the farm and animals in question.

CHAPTER VI

SUMMARY

The urinary fluorine concentrations were studied on one hundred twenty-three cattle and seventy-two sheep. These animals were fed various levels of fluorine either from sodium fluoride, raw rock phosphate, or pasture contaminated with fluorine. Some of the experimental animals also received aluminum sulfate and aluminum chloride (fluorine alleviators) in their rations. The major results of these studies may be summarized as follows:

1. Observed urinary fluorine analyses were found to be less variable and more valid than values corrected to a standard or constant specific gravity.
2. The excretion pattern of urinary fluorine concentrations indicated that for most reliable results single voidation samples should be taken eight to sixteen hours after fluorine ingestion. Where sufficient samples were taken, urinary fluorine analyses of single voidation samples were, on the average, as valid as the values from one, three, and seven day composite samples.
3. Urinary fluorine concentrations from cattle receiving up to 8 ppm fluorine in their ration (control) ranged from 1 to 7 ppm. A urinary fluorine concentration of 3 to 11 ppm was found for sheep receiving up to 6 ppm fluorine in their ration (control).

4. Urinary fluorine concentrations were not influenced by high fluorine concentration in the ribs 250 days after cessation of high fluorine intake.
5. Urinary fluorine concentrations of cattle and sheep were reduced by the addition to the ration of 0.1 and 0.5 per cent aluminum sulfate and 0.1 per cent aluminum chloride.
6. The urinary fluorine concentration of cattle and sheep should function as a diagnostic aid of fluorine only when supported by fluorine analyses of feed and bones, appropriate studies of teeth, and general observations of the farm and animals in question.

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