NOVEMBER 1978

20.4

RESEARCH REPORT 365 FARM SCIENCE

FROM THE MICHIGAN STATE UNIVERSITY
AGRICULTURAL EXPERIMENT STATION EAST LANSING

Fluorosis from Phosphate Mineral Supplements in Michigan Dairy Cattle

TECHNICAL Information

SUMMARY

During 1975-76 numerous dairymen complained that cattle exhibited inanition, reduced milk production, infertility and high mortality. Classical signs of fluorosis were observed in several herds. Bone ash from eight herds averaged 2,406 ppm F and were within the toxic range of 1,640 to 6,900 ppm F. Blood data collected previously from three of these herds indicated pernicious anemia, depressed serum calcium, cholesterol and thyroxine, and increased eosinophils. Yet, a satisfactory explanation was lacking.

Urine and blood were collected from 72 Holstein cows at uniform stages of lactation in six herds exhibiting minimal to moderate and severe dental and bone fluorotic lesions, indicating previous exposure to high fluoride intake. None were receiving fluoride in excess of the National Research Council standard (30 ppm) at sampling, however. Urine fluoride averaged 5.13 ± 2.84 ppm F, was lowest in herds fed mineral supplements only ad libitum and more variable in fluorosis herds ($P=.003$).

Urine fluoride and several blood parameters were significantly negatively correlated with days-in-lactation. When effects of stage of lactation were removed by least squares analysis of variance, blood serum thyroxine (T_4) and triiodothyronine (T_3) (radioimmunoassay) decreased with increasing urine F ($P=.031$ and $P=.025$, respectively). Eosinophils were increased ($P=.004$) and cholesterol depressed ($P=.061$) by fluoride.

Red blood cells, hemoglobin, hematocrit, serum calcium and bilirubin were highly correlated with thyroid hormones. Cattle afflicted with fluorosis developed anemia, hypocalcemia, hypocholesterolemia, and eosinophilia.

Administration of thyroprotein (iodinated casein) in feed to cows in a fluorosis herd dramatically increased milk production, serum thyroxine, milk iodine and stimulated hemapoiesis of red blood cells, hemoglobin and neutrophils while reducing eosinophils to normal.

Services required per conception and days open post-calving were significantly less in low fluoride herds with minimal fluorosis and increased with increasing severity of the syndrome.

Mineral supplements containing 5,000 to 6,000 ppm F and protein supplements enriched with phosphorus, containing 220 to 1,088 ppm F were the main sources of excess fluoride.

Other dicalcium phosphates and mineral mixtures contained 1,000 to 1,633 ppm F and appeared to produce the fluorosis syndrome at a lower frequency in "normal" herds since fluorotic lesions were common in 25 herds examined. Dental and bone lesions were on calves and yearlings in fluorosis herds. The data indicate that excess fluoride inhibits the thyroids thus depressing metabolism and hemapoiesis over time.

Fluorosis from Phosphate Mineral Supplements in Michigan Dairy Cattle

by DONALD HILLMAN, DAVID BOLENBAUGH and EDWARD M. CONVEY[1]

INTRODUCTION

During 1975 and 1976 more than 75 dairymen complained that cattle failed to produce the normal amount of milk, lost body weight rapidly after calving, and failed to exhibit estrus or rebreed as expected. Death from undetermined causes ranged from 10 to 15% of adult cows and involuntary culling was excessive. Veterinarians reported that cattle responded poorly to therapy. Some calves appeared healthy at birth but failed to grow normally by 2 mo of age and many died from undetermined causes up to a year or more of age. Of the herds reported here, no detectable concentrations of PBB (polybrominated biphenyls) were detected in milk and tissue fat samples analyzed in state and private laboratories.

Low blood serum thyroxine, anemia, hypocalcemia, exopthalmus, periorbital swelling, hypocholesterolemia, rough hair coats and enlarged thyroids from calves, and a dramatic response in milk production in two herds fed iodinated casein (thyroprotein) were observed during initial investigations. Iodide as EDDI (ethylene diaminedihydraiodide), fed at rates exceeding 400 mg per head daily in some herds, was suspected of depressing thyroid function. However, a relationship between iodide intake and thyroid function could not be established in a separate study of farm herds, although blood serum thyroxine concentrations were below normal (8).

Subsequent examination revealed some cows in the six control herds used in the iodide study (8) exhibited moderate to severe dental fluorotic lesions and exostosis of metatarsal bones. Furthermore, on these farms, some mineral supplements contained up to 6,300 ppm fluoride while protein supplements contained up to 1,088 ppm fluoride from soft rock phosphate. Thus, the purpose of this study was to determine whether chronic fluoride toxicity (fluorosis) affects cattle health on some dairy farms.

[1]The authors are, respectively, Professor and Extension Specialist, Graduate Assistant, and Associate Professor, Department of Dairy Science, Michigan State University, East Lansing 48824.

LITERATURE REVIEW

Fluorine (F) is universally present in livestock feedstuffs. Fluorosis involves progressive development of characteristic effects resulting from prolonged ingestion of fluorides. Rock phosphates and phosphatic limestones supply most of the fluoride in livestock feeding. Water in some areas is a significant source of fluoride, and forages contaminated with airborne fluoride from industrial plants or dust from high fluoride soils are other possible sources of excessive fluoride consumed by livestock (18).

Small amounts of fluoride normally ingested by animals are largely excreted in urine and feces and relatively minor amounts are deposited in bones and teeth. When fluoride intake exceeds the safe threshold limit for the particular animal, bone concentrations of fluoride increase and fluorosis gradually develops (18).

The harmful effects of raw rock phosphate containing about 3.5% fluorine and a commercial mineral supplement fed to dairy cattle as 1.5% of the grain ration over a 5-yr period were first reported by Reed and Huffman (59) at the Michigan Agricultural College in 1930. They observed discoloration and excessive wear of the teeth, bone remodeling, poor growth, reduced milk production and high mortality of cattle fed rock phosphate or the commercial mineral supplement. Limestone had no effect and boneflour improved growth of young cattle. When calcium fluosilicate and sodium fluoride were fed to cattle (83) and swine (32, 33), gross effects resembled those from rock phosphate.

Fluorosis from phosphate sources was reported in dairy cattle in Canada in 1954 (10) and England in 1972 and 1977 (30,31). The characteristic skeletal and dental fluorotic lesions described by Michigan workers (59, 83) were confirmed in Wisconsin experiments (53) when increasing amounts of rock phosphate comprising 0.625, 1.25 and 2.5% of the grain mix were fed to cattle from 4 mo of age through 2.5 lactations, and in several experiments where sodium fluoride (NaF) was fed as the source of fluorine (41, 48, 67, 73, 75, 77, 79, 81).

Fluorotic staining and excessive wear of incisor teeth are the first signs of excess fluoride intake. Discoloration of the enamel occurs in unerupted or developing incisors, but may not be seen in cattle exposed to excessive fluoride after permanent teeth are fully developed (about 4½ yr of age). Incisors may be yellowish to dark brown, mottled, or chalky, sometimes in patches.

Bilateral pitting, staining or hypoplasia of the enamel may indicate animal age at the time of excessive intake. Molars become soft and excessively worn; they are more difficult to see in the live animal. Cattle have difficulty masticating feed and are reluctant to drink cold water, apparently because their teeth hurt. The enamel content of teeth decreases with increasing severity of the visual lesions and fluoride content (44).

Osteofluorotic (bone) lesions may occur when the dietary fluoride ingested is above normal for an extended time (45).

Periosteal exostosis (overgrowth and enlargement) of the metatarsal (lower hind leg) and metacarpal (front leg) bones and joints occurs in advanced cases and is more pronounced in young cattle ingesting elevated fluorine levels (20). Palpable and detectable lesions may occur on the mandible and ribs, and the mandible may be notably thickened. In advanced cases there may be spurring and bridging of the joints (65).

Ankylosis (stiffness and/or adhesion) and swelling of the joints occurs in advanced cases resulting in intermittent lameness, frequent shifting of the weight from side to side and an arthritis-like condition of the hip joints (31, 65). Osteofluorotic lesions and calcification of tendons, fascia, cartilage and their insertions generally accompany intermittent lameness (45, 65, 81).

Fluoride concentrations in bones and teeth increase in proportion to the daily intake and exposure duration. Thus, bone and tooth fluoride indicate previous fluoride exposure. Rib bones of adult cattle normally contain less than 1,000 ppm F and averaged 350 to 650 ppm F for control cattle exposed to low levels (3 to 5 ppm) of dietary fluoride for 5 yr (81). Ribs and tail bone normally contain 1.5 to 2 times or more the fluoride found in leg bones.

Rib fluoride increased to 1,700 to 2,200 ppm in cattle fed 20 to 50 ppm F, as sodium fluoride, in the diet for 9 mo, and to 5,500 to 7,000 ppm in cattle fed 50 ppm F for 63 mo in Wisconsin experiments (81). Others reported similar relationships (67).

Excessive fluoride concentrations in bone are generally considered evidence of fluoride toxicity. Greenwood et al. (20) suggested about 1,600 to 2,100 ppm F in bones as borderline toxicity for 2-yr-old cattle; 2,300 to 3,800 ppm F in cows aged 4 to 6 yr. Lameness and osteoarthritis of hip joints was associated with bone fluoride concentrations of 2,000 to 8,000 ppm F in England (3) and Texas (47).

Calcium depletion of bone may cause the osteoplanic lesions associated with severe skeletal fluorosis in dairy cattle. Impaired calcium and phosphorus retention have been shown with rats (12, 35) and cattle (12). Reduced calcium absorption from the gut of calves fed excess fluoride resulted in resorped bone calcium supplying most of the exchangeable calcium pool (56, 57). The ability of fluoride to precipitate calcium may cause hyperexcitability and muscle spasms (37).

Non-skeletal effects of prolonged excessive fluoride intake have been described but their physiological bases have not been adequately established. Inanition (starvation syndrome) characterized by excessive loss of body weight postpartum and a ketosis-like syndrome is common in cattle afflicted with fluorosis (10, 53, 59). Sudden dramatic drops in milk production of some cows coincided with peak fluoride intake when the fluoride was contained in the grain ration (78). Anorexia (loss of appetite) was also associated with fluoride intake ranging from 1.4 to 1.7 mg F/kg body weight as NaF after 2 to 5 yr exposure (67, 70, 73, 77, 78).

The effects of fluoride on milk production are apparently secondary, and coincide with inanition, anorexia and lameness produced by excessive fluoride intake over time (70, 78). Cows produced 93, 82 and 60% as much milk (fat-corrected milk) when fed 28, 55 and 109 ppm fluoride as comparison controls fed 10 ppm F in Utah experiments (70). Similar effects on milk production were reported after development of fluorosis in 1½ to 5 yr depending on rate and duration of fluoride intake (10, 53, 59, 78) while some experiments reported no measurable effect (47, 48).

Prognosis for recovery of seriously afflicted cattle is poor. Cattle previously exposed to 1.2 to 2.0 mg F/kg body weight for 4 yr and afflicted with fluorosis showed no improvement in dental or bone lesions, health, or milk production over a 2-yr period when offered a low fluoride dairy ration, but bone fluoride was reduced 30 to 60% while blood fluoride tended to remain high (55).

The amount of dry matter and energy consumed and apparent protein absorbed from the digestive tract were reduced during lactation with increasing fluoride concentrations in the diet (12 to 109 ppm) when feeding was begun as calves (24). Rumen bacteria were reduced 50% in sheep fed 100 ppm F (NaF) (26). Similarly, growth of actinomycetes microbes in the rumen and biosynthesis of vitamin B_{12} were reduced by feeding fluosilicate (61); folic acid activity in blood was reduced by sodium fluoride (28). The effects of 40 ppm F (NaF) were more severe in underfed than in full-fed cattle (74).

A 6 wk or more delay in the first estrus period following parturition was associated with inanition and anorexia resulting in poor nutrition and health of cows fed excessive fluoride (53).

Fluorine is a potent enzyme inhibitor (27). Excessive fluoride intake may impair energy metabolism. Excessive fluoride also depresses phosphorylation by the glycolytic system (5, 7, 64); while citrate metabolism decreases (5, 7) because fluoride inhibits the enzyme enolase (64, 87) by forming a magnesium-fluoride-phosphate complex (87).

Fluoride affects the hemapoietic system in some circumstances (28). Feeding excessive fluoride reduces hemoglobin concentrations in blood (73) while fluosilicate feeding reduces red blood cell numbers and cell size (61). The resulting anemia was associated with the inhibitory effect of fluoride on growth of actinomycetes microorganisms in the rumen of cattle, and decreased vitamin B_{12} synthesis.

There is evidence that fluoride affects white blood cells, too. Blood eosinophils increased (11.8% of leukocytes) in cattle fed 100 ppm F (28). Premature death of three (of eight) cows in the high fluoride study group provides basis for speculation as to a decreased resistance to infection, and that the higher eosinophils may be an early manifestation of toxicity (28). A decrease in the percent of multinuclear leukocytes in blood (82) and changes in the myelocytes in bone marrow of cattle fed increasing amounts of fluoride, further suggest (28) that fluoride may affect hemopoiesis.

Some animals given high fluoride develop enlarged thyroids (6, 19, 26, 39). Goldemberg (21) claimed that ingestion or injection of 2 to 3 mg F per day for 6 mo increased thyroid gland weight 5 to 6 times and retarded growth of white rats. He called the effect "cretenism fluorique" and suggested that endemic goiter may be due to fluoride. Phillips et al. (50, 51, 52) could not confirm these results on rats, guinea pigs or chickens, but the thyroids of guinea pigs that died of chronic fluoride poisoning were enlarged (6).

Although protein-bound iodine was not affected in lactating cows fed sodium fluoride (28), fluoride did inhibit thyroid hormone secretion of mice (39), and three-fluorotyrosine inhibits normal thyroid function and has been used therapeutically to treat hyperthyroidism (19). Rantanen et al. (58) noted that the bone changes produced in pigs by fluoride were reduced or reversed by thyroidectomy and the retarding effect of F on cortical bone remodeling did not occur when iodinated casein was given as thyroid replacement therapy. Thus, they suggested that the effect of fluoride may be mediated through thyrocalcitonin produced by the thyroid.

The adrenals exhibited congestion and some hyaline and connective tissue response in cows fed excessive fluoride in Wisconsin experiments (53); and the adrenal glands were enlarged when rats were fed toxic amounts of fluoride (54).

Kidneys of cattle fed 4.88 mg F/kg body weight as rock phosphate contained cysts or abscesses accompanied by inflammation, fatty generation and pressure atrophy (53). The heart was flaccid with fatty and hyaline degeneration; hemorrhagic endocardia was also evident (53). Kidney nephrosis and fibrosis resulted from feeding 1% rock phosphate in pig rations but did not occur in pigs fed similar or higher levels of sodium fluoride, calcium fluoride or phosphatic limestone (32, 33). Thus, fluoride may not be the only toxic substance in rock phosphate. Rock phosphate contains varying concentrations of lead, arsenic, aluminum, titanium, vanadium, silicon, iron and cadmium in addition to calcium, phosphorus and fluorine (29).

Respiratory disorders in humans have been attributed to excessive intake of fluoride from industrial sources (13, 19, 46, 60, 69).

The fluoride content of milk is increased slightly when excess fluoride is fed. Milk normally contains 0.16 ppm F or less and increases to about 0.51 by feeding fluoride (20, 70, 77). The femurs of rats contained 28, 56 and 111 ppm F when fed milk from cows given 0, 30 and 50 ppm F in their diet as NaF (77). Considerable fluoride is bound to the protein in milk (11).

The fluoride content of metacarpal bones from newborn calves increased from 11 ppm in controls to 86, 136, 104 and 140 ppm F when the dam was fed 20, 30, 40 and 50 ppm F as NaF (77), thus, fluoride can be transferred across the placenta. Similar linear increases occurred in pigs (17). The average birth weight of calves born from cows exposed to excessive fluoride from rock phosphate was reduced by more than 12% (53). The significance of increased fetal fluoride on subsequent bone and tooth development or other physiological processes of cattle apparently has not been studied.

The growth rate of gilts and their weanling pigs was reduced by including 150 ppm F in the diet (15). Decreased length, width volume and fresh weight of the humerus in baby pigs from sows fed additional fluoride (NaF) indicated a depression of skeletal growth of the fetus which persisted in the 4-wk-old pigs and reduced collagen formation (16).

The National Research Council (45) recommends that diets for dairy cattle contain less than 30 ppm fluoride based largely on experiments conducted with sodium fluoride and bone fluoride concentrations as evidence of toxicity.

METHODS AND PROCEDURES

There was no satisfactory explanation for the hypothyroidism, anemia, eosinophilia, hypocalcemia and hypocholesterolemia found in an investigation of cows in 26 herds. Herds S1, B2, C4 and V3 were typical problem herds. Initial complaints were similar, i.e. excessive loss of body weight after calving, depressed milk production, a ketosis-like syndrome unresponsive to therapy, delayed estrus and failure to rebreed as expected, a high incidence of uterine infections, lameness and mortality. Subsequently, dental and bone lesions suggestive of fluorosis were observed on most cows in these herds.

To determine whether the blood criteria could be correlated with fluoride concentrations in urine and to provide a basis for comparison with problem herds, six herds varying from minimal (herd W and B) to moderate (MGP) and severe (S1) visual signs of excessive fluoride intake were chosen for study. With the exception of S1, herds were representative of "normal" dairy herds producing more than 7,500 kg milk per lactation. Since Coppock et al. (9) reported that cows limit their intake of dicalcium phosphate when offered only free choice, herds W and P were selected because mineral supplements were fed only free choice and not force-fed in the ration.

Fluoride intake could not be measured accurately under these farm conditions, and the high fluoride supplements had been discontinued in all herds at the time of sampling.

Twelve lactating cows in each herd were selected for study by the herdsman: 2 cows (<30 days), 2 (31-120 days), 4 (120-200 days) and 4 (200-300 days) in lactation. Milk, blood and urine were taken from each cow after the AM milking within a 3-wk period. Teeth, hoofs and bone abnormalities of representative cattle were photographed.

Whole venous blood (containing EDTA) was examined for RBC, Hbg, Hmct, WBC and differential counts.[2] Forty milliliters of venous blood was allowed to clot and cells removed for serum analysis of calcium, phosphorus, glucose, cholesterol, bilirubin, albumin, total protein, uric acid, blood urea nitrogen, alkaline phosphatase enzyme and SGOT (serum glutamic oxalacetic transaminase enzyme) by SMA_{12} auto analyzer[2] according to the manufacturer's procedures.

Blood serum thyroxine (T_4) for herds V3 and B2 was determined in a local medical laboratory[2] by competitive protein binding[3] and for herd C4 by the tetrasorb method described by Convey et al. (8). Serum T_4 and T_3 (triiodothyronine) were determined by radioimmunoassay[4] (RIA) for all specimens to be compared with urine fluoride. The coefficient of variation averaged 4.49% for T_4 and 10.35% for T_3.

Table 1 describes the herds, analysis performed and fluoride sources. Blood and urine data were analyzed for statistical significance by least squares regression and analysis of variance procedures.

[2]Laboratory of Clinical Medicine, Lansing, MI 48910.
[3]Mallinckrodt Chemical Works, 2nd and Mallinckrodt Streets, St. Louis, MO 63160.
[4]Radioassay Systems Laboratory, Inc., 1511 East Del Amo Boulevard, Carson, CA 90746.

Table 1. Herd description and analyses performed

Herd		Signs of fluorosis			Specimens						Milk	Fluoride sources sampled(c)	
					Blood				Urine	Bone			
Ident.	Cows No.	Dental score(a)	Bones(b)	Hoof(b)	Cells	SMA_{12}	T_4	T_3	F	F	cow/day	Mineral	Protein Supp.
			%								kg	Fed., Ref. Table 5	Ref. Table 6
W	56	1	2	2	12	√	√	√	√	0	23.8	ad lib, ½K, ½L	Soybean
B	45	1-2	5	10	12	√	√	√	√	0	25.9	Conc. Z(1977)	Soy
M	120	3-4	15	20	12	√	√	√	√	0	23.0		L
G	80	1-3	5	10	12	√	√	√	√	0	23.1	Conc. 1% D	Soy
P	120	1-4	10	15	12	√	0	0	√	0	22.2	ad lib. ½P, ½S	Soy
S1	152	4-5	25	58	12	√	√	√	√	√	19.7	ad lib. A	A
B2	120	4-5	34	40	13	√	√	0	0	√	7.8	ad lib. B,C,J	B,C,E(b)
C4	80	4-5	33	35	9	√	√	0	0	√	13.1		C(b)
V3	80	4-5	20	25	10	√	√	0	0	√	26.3	Conc. 1½%	C(b)

(a) Dental scores—approximate range as in reference 2.
(b) Percent of cows in each herd showing signs of bone or hoof abnormalities.
(c) Source of supplements had been changed at time of sampling; letter indicates single specimens identified in Tables 5, 6.

Bone specimens and teeth were collected at necropsy or slaughter. Soft tissues were removed from bone by boiling in water. Long bones (metatarsal, metacarpal) were bisected with a band saw into a dorsal and plantar half and the sawdust was collected as described by Suttie and Kolstad (76). Teeth were extracted from the mandible after boiling; and whole coccygeal vertebrae were used for analysis.

Fluoride content of bones and teeth was determined by fluoride ion electrode (22) or the distillation procedure of the Association of Official Analytical Chemists (1). Both methods agreed well with the diffusion technique (68) using spectrometric detection (2). The method proved reliable for teeth, bone, urine and feeds. Recoveries of 20 ug F averaged 96.5% with a coefficient of variation of 6%.

Water samples were analyzed for fluoride content in the Michigan Department of Public Health Laboratories, Lansing. Tissue specimens were examined at necropsy or after slaughter, by veterinary pathologists at the Michigan State University Diagnostic Laboratory.

Thyroprotein (iodinated casein) was administered to 13 lactating cows in one herd (B2) at the initial rate of 19 g per day for 2 wk and reduced to 10 g the third week. Blood specimens were collected as described earlier prior to and after 1 and 3 wk of thyroprotein feeding. Milk from each cow was weighed one day weekly and sampled for iodide analysis. These data were analyzed for statistical significance by analysis of variance using the paired t-test.

RESULTS AND DISCUSSION

Clinical Histories and Observations

The syndrome was similar in afflicted cattle of all herds observed. However, the severity in terms of percentage of cattle presenting specific abnormalities varied among herds. The time and duration of initial exposure to excess fluoride was unknown thus variation in duration of exposure may explain differences in severity. The following syndrome typified herds with the greatest problem: 1) lactating cows lost excessive body weight post-calving (inanition) while offered a diet providing adequate energy and protein; 2) cows failed to attain normal peak levels of milk production; 3) herd average milk production declined 1,000 to 2,000 kg per lactation over a 1- to 2-yr period; 4) most cows appeared healthy before calving and some produced normal amounts of milk for 2 to 4 wk—milk production then dropped dramatically and cows exhibited a ketosis-like syndrome unresponsive to therapy. The post-calving inanition described by Phillips et al. (53) occurring in cattle fed excessive fluoride resembles the displaced abomasum syndrome.

Reproduction

In the problem herds, 20 to 30% of cows remained open or were culled as non-breeders. Reproduction data were collected from herds W, B, G, M and S1. Services per conception were 1.4, 1.5, 1.8, 2.1 and 2.9, respectively. Services per conception were lower ($P<.05$) for herds W and B than S1 ($P<.05$), and tended to increase with visual evidence of fluorosis.

Days from calving to conception averaged 79, 85, 88, 96, and 137 for herds W, B, G, M and S1, respectively. The data support farmer complaints of reproductive problems in fluorosis herds.

Other Clinical Problems

Adult cow mortality averaged 15% per year in the four problem herds. Deaths were commonly associated with post-calving uterine infections, mastitis and general infections that normally respond to therapy. An atypical pneumonia with little temperature elevation was involved in some cases. Others were associated with inanition, apparent abomasal displacement, and resultant ketosis syndrome.

Dental Signs of Fluorosis

Normal incisor teeth with lusterous white enamel in control herds W, B, and G are shown in Figs. 1 through 4. Dental lesions were observed on incisors of adult cows in each of the four problem herds (see Figs. 5 through 9).[5] Discoloration of the enamel varied from a mild yellowish to mottled, brown, and black and could not be removed by scraping. Chalky patches (Fig. 5), bilateral discoloration and bilateral pitting of the enamel of some cows indicated they had received excessive fluoride at the time those teeth were developing.

In some cows the surface of teeth was dull or contained vertical rivulets suggesting hypoplasia of the enamel. Excessive wear and mottling of teeth was evident on most cows. Sometimes the eroded teeth were more notable on one side of the mouth.

Moderate to severe dental lesions (Fig. 10)[5] were found on most cows in control herd P, possibly because they had previously consumed considerable fluoride from minerals fed free choice. Attempts to observe molars of live animals were largely unsuccessful. A postmortem specimen exhibiting abnormal wear is shown in Fig. 11. The fourth molar (ash) contained 4,510 ppm fluoride.

---

[5]Figs. 6-10, 12-14 and 16 are color pictures located on the outside back cover.

Fig. 1. Normal incisors of 10-yr old cow, herd W. Teeth show white lustre and little wear.

Fig. 2. Normal incisors of 8-yr cow, herd B.

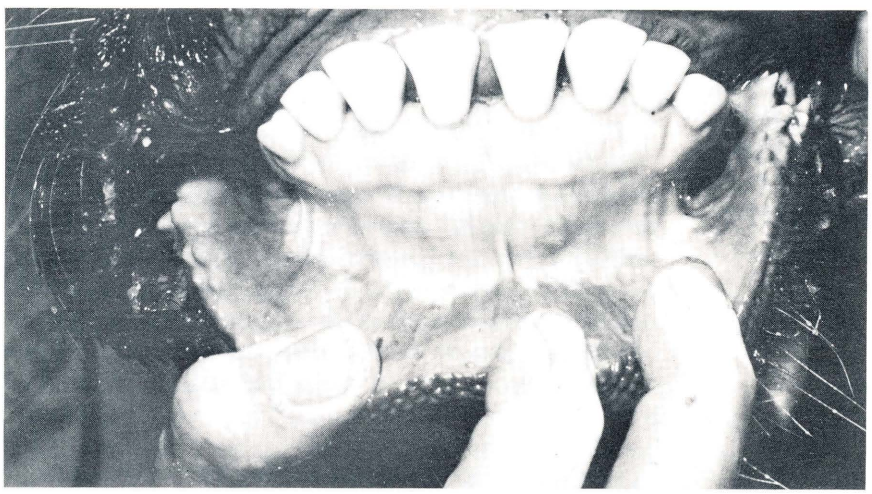

Fig. 3. Normal incisors of 7-yr cow, herd G.

Deciduous and permanent teeth of cattle 1 to 2½ yr old had typical stains and lesions (Figs. 12, 13 and 14),[5] and often were eroded completely to the gums. Teeth from some calves aged 3 to 4 mo were stained and mottled (Fig. 15). These teeth contained 727 ppm fluoride. Black teeth were found in calves less than 6 wk old (Fig. 16).[5] Apparently placental transfer of fluoride from dam to fetus was sufficient to produce these effects on developing teeth in utero. This may explain some problems found with deciduous teeth in cattle at 1 to 2½ yr old.

Enlargement of joints was evident by palpation as bony structure and ranged from 2% of cows in herd W to 20% to 34% in herds S1, B2, C4 and B3 (Figs. 17, 18, 19 and 20). Periosteal exostosis and osteoporosis of metacarpal and/or metarsal bones of cows are shown in Figs. 21 and 22. Numerous cows were culled because of lameness and an arthritis-like condition of hip and shoulder joints.

Stiffness and lameness were generally associated with the exostosis of bone, enlargement of joints and reddened swelling of joints in all herds.

Exostosis of metatarsal bones was evident on 2-yr-old heifers in several herds. In the four problem herds, 20 to 58% of the cows had curled and/or sprawling hoofs (Fig. 23), while only 5 to 15% of cows in control herds had this problem. These findings concur with other reports of fluorosis in farm herds (10, 31).

Fig. 4. Normal incisors of 2-yr-old, 1st calf heifer, herd G.

Fig. 5. Chalky, pitted incisors of 4-yr-old cow, herd S. Teeth contained 3,940 ppm F.

Fluoride Content of Bones

Fluoride (F) concentrations in ashed samples of bones and teeth from adult cows in eight herds ranged from 885 to 6,918 ppm (ash) and averaged 2,406 ppm in 22 specimens (Table 2). Fluoride content of ribs and coccygeal (tail) vertebrae was about twice that in metatarsal and metacarpal bones, which concurs with other reports (67, 75, 81). The fluoride content of specimens from each of the Michigan cattle (Table 2) was above the 350 to 1,000 ppm reported for controls receiving no additional fluoride other than from natural feedstuffs in experiments (53, 67, 75, 81) and comparable to bone fluoride found in cattle afflicted with fluorosis in England (31) and Texas (47).

Fig. 11. Excessive wear on molar teeth, herd S1. Fourth molar contained 4,910 ppm fluoride.

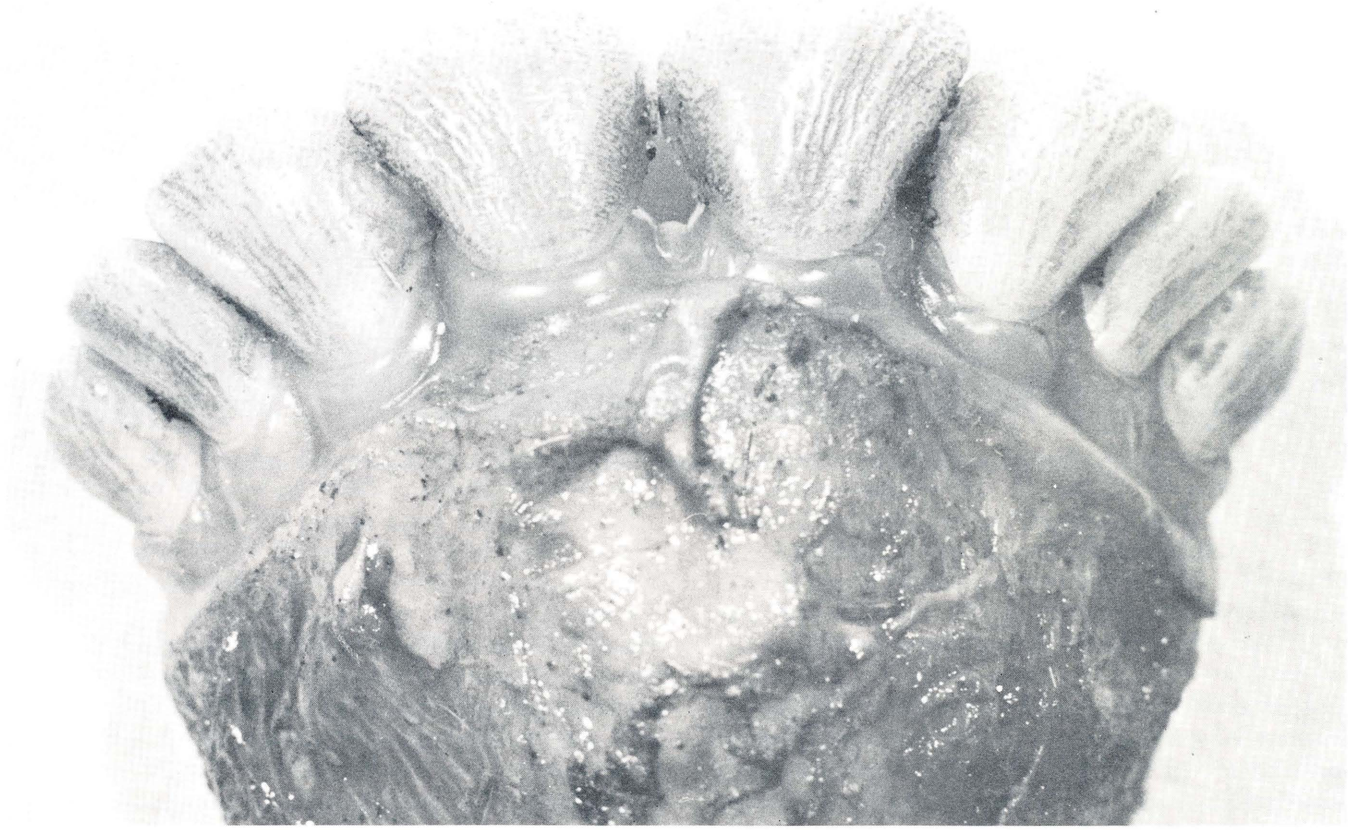

Fig. 15. Stained and mottled teeth of 4-mo-old calf herd S1. Teeth contain 727 ppm fluoride (ash).

Fig. 17. Exostosis of metacarpal bone (S-273). Hoofs are curled, fetlock joints are red and swollen. Cow was lame.

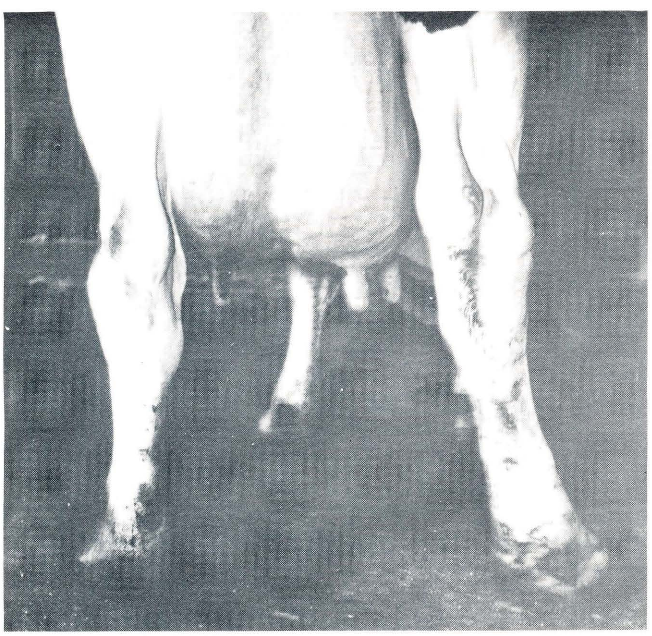

Fig. 18. Exostosis of metatarsal joint, herd V3.

Fig. 19. Exostosis of bone in front of metatarsal joint C-51 (see Fig. 8).

Fig. 20. Classical bone exostosis of steer, herd B2 (see Fig. 14).

Fig. 22. Metatarsal bone from steer in herd B2, showing bone overgrowth (exostosis) and porous structure (osteoporosis of cross-section (center).

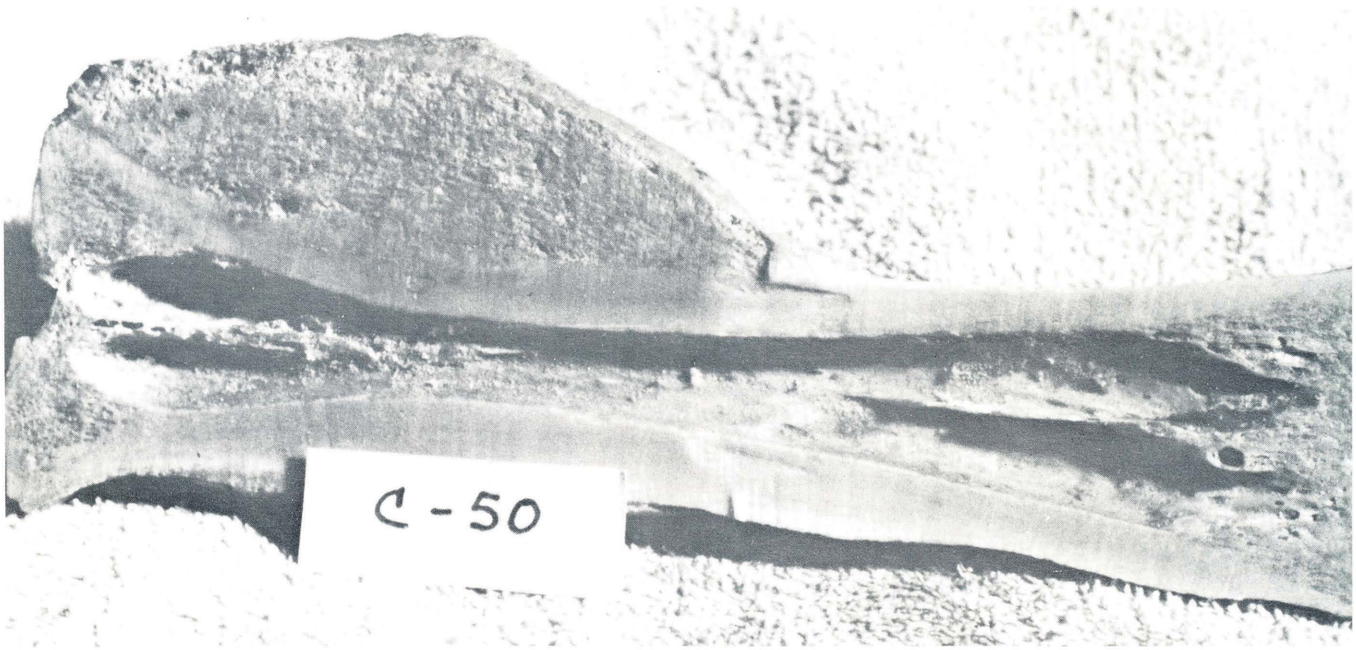

Fig. 21. Metatarsal bone of C-50 showing exostosis contained 2,100 ppm fluoride.

Fig. 23. Typical curled hoofs of S-253. Associated with fluorosis on 58% of cows in herd S1.

Table 2. Fluoride content of bones and teeth of adult cattle from Michigan herds

Herd	Specimen	F (ash) ppm
S1	Cow, 5 yr, tail vertebrae	6918
	metatarsal	3564
	Cow, 4 yr, 2nd molar	4510
	4th molar	3940
	Cow, 2 yr, metatarsal	1680
B2	#1 Cow, 2 yr, rib	2044
	#2 Cow, rib	2024
	#3 Cow femur	2265
	#4 Cow femur	1541
	#5 Cow mandible	1793
	#6 Cow mandible	885
V3	#10 Cow, 4 yr, metatarsal	2299
	metacarpal	1446
C4	Cow, 4 yr, mandible	2103
	tail vertebrae	1640
	metatarsal—whole bone dust	1023 to 1290
	metacarpal—exostosed bone	2405
5(a)	Cow, 4 yr, metatarsal	1740
6	Cow, 7 yr, mandible	2233
	Cow, 5 yr, metatarsal	1323
7	Cow, 5 yr, rib	3100
	metatarsal	1700
8	Cow, 8 yr, mandible	2334
	Cow, 5 yr, metatarsal	1334

(a) Herds 5, 6, 7 and 8 not studied further.

Teeth

The second and fourth permanent molars of a 4-yr-old cow in herd S1 submitted to necropsy contained 4,510 and 3,940 ppm fluoride. The fourth molar was severely eroded into the dentine (Fig. 11).

The deciduous teeth of a calf (aged 4 mo) from herd S1 contained 727 ppm fluoride, and the mandible 991 ppm F (Table 3). The teeth of this calf were notably mottled (Fig. 15).

The tail vertebrae of another calf aged 3 mo contained 2,146 ppm fluoride; while the mandible had 209, first deciduous incisor 92, second molar 78, phalanges 386 and hoof ash 496 ppm F. Data indicate large variations in F content of various skeletal parts of the same animal. Since the incisor teeth of calves are fully erupted at birth and the fluoride of enamel is accumulated in unerupted teeth (44) apparently considerable fluoride was obtained in utero. Calf supplements contained 35 to 107 ppm F, and the grain ration of herd S1 contained 114 ppm F.

Bones and teeth of calves (5-6 mo old) that were born from cows in herd S1 after the high fluoride supplements were discontinued tended to be lower in fluoride content than those in animals born during the period of high fluoride exposure.

Also, the fluoride content of deciduous incisors from cattle 1½ to 2 yr old from herd B2 (Table 4) was similar to that found in comparable teeth from much younger calves in herd S1. The fluoride content of deciduous teeth (2nd pair of incisors and 2nd molar) was more than twice that of the first pair of permanent incisors of these cattle (Table 4). In most cases, the crown (enamel and dentine) of deciduous incisors was so severely eroded that only the stalk remained for analysis. This condition was observed consistently in young cattle of herds where adult cattle exhibited dental lesions.

Data in Tables 3 and 4 and other observations indicate the teeth of calves containing 700 to 1,000 ppm fluoride may erode completely from eating abrasive feeds.

Sources of Fluoride

The fluoride contents of samples of mineral supplements and other feedstuffs containing added phosphate minerals are listed in Tables 5 and 6.

Mineral supplements A and B contained about 6,000 and 5,000 ppm fluoride with acceptable variation in results from three laboratories. The fourth laboratory reported about 17% less fluoride. "Soft rock phosphate" was indicated as an ingredient on the registration tag of supplement A. The other mineral supplements contained various concentrations of fluoride ranging from a low of 17 ppm for monosodium phosphate to 130 to 200 ppm in dicalcium phosphate from processed bone and to a high of 1,633 ppm in other dicalcium phosphates.

Table 3. Fluoride content of bones and teeth from young cattle

Herd	Calf #	Age	Bone Identity	Bone F (ppm)	1st incisor F (ppm)	1st incisor Ash (%)	Molar F (ppm)	Molar Ash (%)
S1	0430	4 mo	Mandible	991	727		727	
S1	5647	3½ mo	Metatarsal	233	183	(68.4)	204	(71.3)
	5627	5½ mo	Metatarsal	226	265	(65.7)	140	(70.6)
S1	5611	6 mo	Metatarsal	368	377	(66.3)	240	(69.9)
S1	2	3 mo	Tail vertebrae	2146	93		78	
			Mandible	209				
			Tarsal	329				
			Metatarsal (dark)	132				
			Metatarsal (white)	241				
			Phalanges	386				
			Hoof (ash)	496				
B2	3901	2 yr	Tail vertebrae	1019				
			Metatarsal	897				
			Metacarpal	520				
B2	3921	2 yr	Tail vertebrae	981				
			Metatarsal	568				
			2nd decid. incisor	840				
			1st perm. incisor	755				

Table 4. Comparison of the fluoride content of deciduous and permanent teeth of heifers under 2 yr of age, herd B2

No.	Age (yr)	Deciduous incisor	Deciduous molar	Permanent incisor
			ppm (ash)	
901	<2	805	880	401
903	<2	954	1032	386
904	<2	955	1057	351
908	<2	1140	1280	637
Avg		963	1062	444

Protein supplements A and B (Table 6) were distributed by the same companies as mineral supplements A and B and appeared to reflect the high fluoride phosphate mineral included in the mixture. Protein supplement C contained fluoride similar to B and was widely distributed in Michigan. The fluoride in grain mixes and pellets corresponded to the concentrations in protein and mineral supplements used as ingredients. Each of the highest fluoride supplements were fed on farms where cattle exhibited classical signs of fluorosis (Table 6).

Feed analyses indicated that several mineral and protein supplements sold in Michigan from 1974 to 1976 contained excessive fluoride. Suttie (72) analyzed 15 samples of 16% protein commercial dairy feeds from Michigan in 1968. They ranged from 3 to 95 ppm fluoride. The fluoride content in feeds may have increased during the phosphorus shortage of 1973 to 1974. Farm grown forages con-

Table 5. Fluoride content of minerals

Supplement	Fluoride
	ppm
A. 15% Ca, 7% P	6235(a)
15% Ca, 7% P	5900(a)
15% Ca, 7% P	6013
15% Ca, 7% P	5020(a)
B. 12% P-mineral mix (1975)	5314(a)
12% P-mineral mix (1975)	4170(a)
C. Dicalcium phosphate .18% F	1633
D. Dicalcium phosphate .21% F	1580
E. Mineral mix (1974)	1490
F. D-14P	1359
G. D-17P	1187
H. C—18P mineral mix	1290
I. C—16P mineral mix	910
J. HI Phosphate (M) mineral mix	1100
K. Dicalcium phosphate	1238
L. CS mineral mix	1063
M. MVS mineral supplement	804
N. CS mineral mix	1080
O. C blend	243
P. CS mineral mix	1164
Q. GRVP mineral mix	1108
R. MVP	676
S. Monosodium phosphate	17.3
T. Hi phosphate mineral	383
U. D-17P (1977)	386
V. FF (1977) mineral mix	615
W. Salt, mineral block	427
X. Dicalcium phosphate, processed bone	130(a)
Dicalcium phosphate, pressed bone	200(a)
Y. V.T. mineral mix	1477
Z. H-mineral mix (1976)	1200
H-mineral mix (1977)	640

(a)Results from other independent laboratories including: Wisconsin Alumni Research Foundation Lab., Madison; Michigan Department of Agriculture Lab., Lansing; and Nutritional Environmental Analytical Lab., Inc., Holt, MI.

tained only trace (2-4 ppm) or nondetectable amounts of fluoride, although one specimen contained 15.7 ppm F.

Water supplies from farms sampled contained 0.1 to 0.8 ppm F. This is an insignificant amount and would only contribute 10 to 100 mg to the daily F intake of high producing dairy cattle. By comparison, mineral and protein supplements A contributed 1,143 mg to the daily intake.

Table 6. Fluoride content of protein supplements and grain mixes or pellets from Michigan farms

Source		F
		(ppm)
A.	38% supplement	1088
B.	38% supplement	230
C.	32% supplement (1976)	220
D.	38% supplement (1977)	120
E.	Dry cow	93
	Calf starter	89
F.	Liquid protein supplement (NPN)	0
G.	Grain pellet (1974) 22% protein	24
H.	Grain mix—10% protein supplement (1976)	22
I.	Farm mix 1% mineral plus SBM (1976)	8
J.	Custom elevator mix (1976)	5.2
K.	Pelleted grain mix (1977)	7.5
L.	Pelleted grain mix (1977)	7.4

Fluoride Content of Milk

Milk from 11 individual cows was analyzed for fluoride during the early stages of this investigation. The fluoride content of milk varied from 0.072 to 0.64 ppm F and was higher in samples ashed prior to analysis than non-ashed samples; these findings concur with those of Dalziel et al. (11) suggesting that fluoride is bound to the protein in milk. The milk values are comparable to those reported by Utah (70) and Wisconsin (77) workers who indicated that milk normally contained less than 0.16 ppm F but is increased to 0.2 to 0.51 ppm F over time by feeding NaF. Further analyses were discontinued because of their limited diagnostic value and difficulty of the analysis.

Relationship Between Urinary Fluoride and Blood Criteria

Urinary fluoride concentrations from 72 cows in the six herds studied averaged 5.31±s.d. 2.84 ppm F, and ranged from 1.04 to 15.7 ppm F. Shupe et al. (66) noted considerable variation in urine fluoride and suggested repeated sampling to establish a reliable mean. Most variation was diurnal and related to time of sampling after feeding fluoride. Herds were sampled only once and well after exposure to high dietary fluoride. Urinary F reflects current F intake and is highly related to duration of exposure or total fluoride consumed (66).

Extrapolating from the Utah (66) and Wisconsin data (75, 77), urine F concentrations were equivalent to fluoride intake ranging within 1 to 22 ppm F in the dietary dry matter over an undetermined time period. Herd W averaged 2.92±s.d. 1.79 ppm F in urine—significantly lower than all other herds sampled ($P<.05$). Herd P was second lowest in urine F averaging 3.48±s.d. 1.64 ppm F. Herds W and P were offered phosphate minerals only free choice. Findings support the hypothesis that cattle fed only free choice minerals have lower fluoride intake.

The standard deviations for urinary F in herds S1 (3.8 ppm) and M (3.2 ppm) appeared larger than those in the other four herds (1.5 to 2.6 ppm). Pooled variances of herds S1 and M were greater ($P<.01$) than those in the other four herds. Thus, herds with moderate to severe clinical signs of fluorosis have larger variation in urine fluoride.

The previous sources of elevated fluoride intake from mineral and protein supplements and bone fluoride concentrations within the toxic range were confirmed by analysis for herd S1 but not M. Urinary F was negatively correlated with days-in-lactation. Least squares analysis of variance using age in months (X_1), days in lactation (X_2) and urine F (X_3) as independent variables was used to determine their partial effects on each dependent blood parameter. Blood serum thyroxine (T_4), triiodothyronine (T_3), and blood eosinophils were directly affected by fluoride ($P<.05$) after eliminating partial effects due to age and stage of lactation. Fluoride depressed cholesterol at a lower level of significance ($P = .06$).

Thyroid Hormones

Blood serum thyroxine (T_4) was depressed ($P=.031$) as urinary fluoride increased based on least squares analysis. In addition, T_4 was higher with advanced stages of lactation (days in milk). T_4 was not related to age of the cow.

The regression equation for predicting T_4 was: Y (micrograms T_4/dl) = 4.53 − 0.006 (age, months) + 0.006 (days in lactation) − 0.098 (urine F ppm) (Fig. 24). The effects of stage of lactation and age are relatively small compared to the effects of fluoride. The standard errors of coefficients were: age (.006), days in lactation (.001), and urine F (.044). T_4 concentrations (Table 7) were lower ($P<.05$) for cattle in each of the herds exhibiting classical dental and bone fluorosis (S1, B2, C4, V3) and elevated bone fluoride.

Table 7. Urinary fluoride, thyroxine (T_4), triiodothyronine (T_3), calcium, phosphorus and cholesterol in blood serum of dairy cows with varying severity of fluorosis

Herd	No. obs.	Urine F ppm		T_4 ug/dl		T_3 ng/ml		Calcium mg/dl		Phosphorus mg/dl		Cholesterol mg/dl	
		$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
W	12	2.92	.52	4.60	.34	1.75	.072	10.1	.15	5.4	.28	203	16
B	12	5.37	.43	4.83	.19	1.68	.058	9.5	.11	5.8	.21	162	9
M	12	6.39	.92	5.30	.38	1.77	.084	9.6	.11	5.7	.34	203	17
G	12	6.33	.74	4.82	.28	1.59	.077	9.4	.15	6.1	.37	207	14
P	12	3.47	.47	....	...	....	....	9.3	.12	5.8	.43	174	15
S1(a)	12	6.29	1.08	3.59	.26	1.26	.084	9.1	.17	4.9	.24	189	16
C4	9	....	....	2.21	.54	....	....	9.5	.14	7.2	.60	123	12
V3	10	....	....	3.35	.47	....	....	9.5	.13	5.9	.28	196	11
B2	13	....	....	3.39	.42	....	....	8.9	.12	6.4	.23	95	7
Means ($\bar{x}$) significantly different (P .05)													
		W <all		C4 <all S1, V3, B2 <W,B,M,G		S1 <W,B,M,G		B2 <M,W S1,P,G<W		S1 <B2,C4 W<C4		B2 <all C4<V3,S1,M,W,G	

(a) Dental fluorosis confirmed by elevated bone fluoride in herds S1, C4, V3 and B2. Cows were uniformly distributed throughout lactation in all herds. SE is the standard error of the mean ($\bar{x}$)

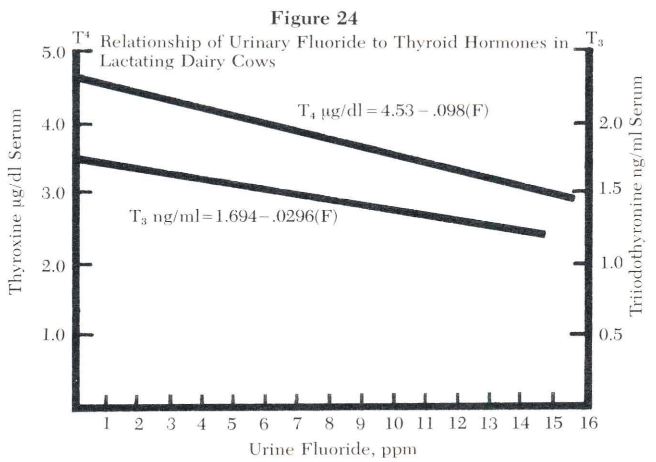

Fig. 24. Thyroid hormones decreased with increasing concentrations of fluoride in the urine.

Average serum T_4 of herd M was the highest of all herds sampled. Five of the 12 cows were sampled 1 yr earlier (8). All were at similar stages of lactation at both samplings. The mean T_4 in 1976 was 2.51 ± 0.48 ug/dl compared to 5.08 ± 1.36 ug/dl when sampled in 1977. The difference was significant by the paired t-test ($P < .01$).

The reason for the change in serum thyroxine over the period of a year is unknown. Four of the five cows had been fed graded levels of iodide (500-1000 mg/d) to measure I secretion in milk just prior to the 1977 sampling. Whether or not these levels of iodide influenced the thyroid activity is unknown. Average thyroxine for herd S1 sampled at the same time as the control herds was low and comparable to other fluorosis herds.

Triiodothyronine (T_3) was depressed with increased urine F ($P = .025$). As number of days-in-lactation increased, T_3 tended to increase ($P \doteq .07$). Age was not significantly correlated with T_3 of adult lactating cows. The regression equation for predicting T_3 was Y (nanograms T_3 per ml) = 1.69373 − .00067 (age, months) + .0007 (days in lactation) − .02958 (urine F ppm). Standard errors of the coefficients were: age (.00163), days in lactation (.0004) and urine F (.01284).

The simple correlations between T_3 and red blood cells ($r = .51$), hemoglobin ($r = .54$), hematocrit ($r = .53$), serum calcium ($r = .52$) and bilirubin were significant ($P < .01$) and larger than the correlation of T_3 on days in lactation ($r = .40$). T_3 was also correlated ($P < .01$) with albumin ($r = .32$), blood urea nitrogen ($r = .34$), cholesterol ($r = .33$), glucose ($r = .37$), serum glutamic oxalacetic transaminase enzyme ($r = -.33$) and T_4 ($r = .74$).

Blood Cell Criteria

Leukocyte (WBC) values for each of the nine herds are in Table 8. The percentage of eosinophils increased with increasing urinary F ($P < .01$) (Fig. 25). Hoogstratten et al. (28) also observed high eosinophils in cattle fed excessive fluoride. Eosiniphils were not related to age, nor days-in-lactation although the latter approached significance ($P = .08$). Eosinophils did not differ significantly among herds suggesting that eosinophilia cows were distributed throughout several herds. The tendency of fluoride to increase percent eosinophils was associated with increased signs of fluorosis on cows in these herds (see herds B2, C4 and G, Table 8).

Figure 25

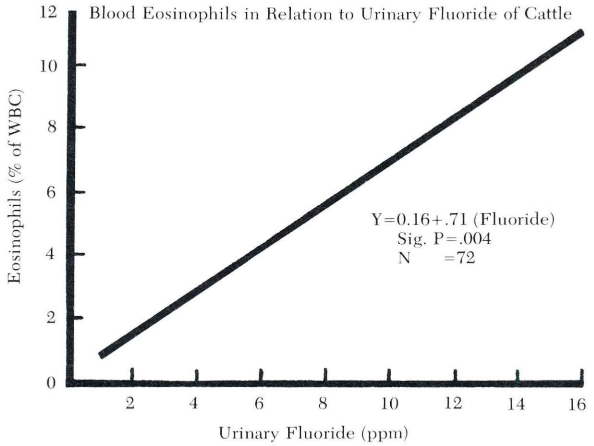

Fig. 25. Eosinophil fraction of white blood cells increased with increasing concentrations of fluoride in urine.

Red blood cell numbers, blood hemoglobin content, hematocrit (packed cell volume), and mean corpuscular volumes were significantly (P<.05) lower in herds exhibiting high incidence of fluorosis (S1, C4, V3 and B2) than in the other herds (Table 9). These results indicate that cattle with lesions on teeth and bones, and elevated fluoride in bones frequently develop anemia. The highest hemoglobin content (12.09+.27 g/dl) was in herd W which also had the lowest urinary fluoride (2.92 ppm).

Development of anemia in response to fluoride concurs with other reports (61, 73), and may be caused by the inhibitory effect of fluoride on vitamin B_{12} and folic acid (28) synthesis in the rumen (61). Morrow (43) observed that blood hemoglobin was negatively correlated with interval to first breeding, days open and services per conception of 200 cows in Michigan dairy herds. Morrow noted

Table 8. Leukocytes (WBC) and percentage of differential leukocytes in blood of cattle with varying degrees of fluorosis

Herd	No. obs.	Total WBC 1000/mm³		Mature neutrophils %		Banded neutrophils %		Lymphocytes %		Eosinophils %		Basophils %	
		x̄	SE	x̄	SE	x̄	SE	x̄	SE	x̄	SE	x̄	SE
W	12	8.23	.42	39.7	1.9	0	..	52.5	1.4	6.1	1.1	.08	.08
B	12	8.23	.69	36.6	3.8	0	..	51.6	3.1	8.0	1.0	.16	.11
M	12	7.27	.57	37.6	2.8	0	..	55.6	2.6	5.3	1.2	0	0
G	12	6.94	.41	34.2	3.0	0	..	52.2	3.3	10.8	2.0	.5	.15
P	12	6.73	.29	47.9	2.2	0	..	43.7	2.8	6.0	1.0	.08	.08
S1(a)	12	7.93	.78	35.1	3.9	0	..	56.6	4.0	6.6	1.0	.08	.08
C4	9	7.91	.55	36.4	6.7	0	..	53.2	5.9	9.6	2.6	0	0
V3	10	7.21	.67	42.0	3.3	0.8	.4	50.2	2.3	5.0	1.5	.8	.24
B2	13	7.80	.45	31.5	1.7	1.4	.9	55.0	2.6	11.5	2.0	.07	.07
		Means (x̄) significantly different (P<.05)											
		N.S.		B2,G,S1<P								V3,G>all	

(a) Dental and bone fluorotic lesions confirmed by analysis for herds S1, C4, V3, B2; visual lesions were apparent on teeth of some cows in herds M, G and P. No evidence of excess fluoride in herd W; minor in B. Cattle were sampled at uniform stages throughout lactation in all herds.

Table 9. Red blood cell values suggesting anemia of cows in fluorosis herds S1, C4, V3, B2

Herd	No. obs.	Urine F ppm		Red blood cells per mm³x10⁶		Hemoglobin g/dl		Hematocrit %		Mean corpuscular volume u³	
		x̄	SE	x̄	SE	x̄	SE	x̄	SE	x̄	SE
W	12	2.92	.52	6.46	.16	12.09	.27	33.4	.70	52.1	.69
B	12	5.37	.43	6.46	.20	11.57	.32	32.2	.78	49.9	.84
M	12	6.39	.92(b)	6.05	.17	11.54	.25	31.3	.66	52.0	.96
G	12	6.33	.74	6.22	.16	11.40	.24	31.4	.58	50.9	.66
P	12	3.48	.47	5.96	.12	10.91	.23	30.6	.61	51.4	.41
S1	12	6.29	1.08(b)	5.69	.18	9.93	.27	27.8	.69	48.7	1.10
C4	9	....	....	5.58	.19	9.79	.35	26.2	1.00	46.5	1.00
V3	10	....	....	5.65	.18	9.92	.34	25.8	1.10	44.3	1.00
B2	13	....	....	5.39	.12	9.94	.24	27.6	.60	50.5	.79
		Means significantly different (P<.05)									
		W<all		B2<G,W,B S1,C4,V3<WB		S1,C4,V3,B2 <W,B,M,G		V3,C4<P B2,S1<W,B,M,G		V3<B2,B,S1 C4<W,M,G,P	

(a) Herds S1,C4,V3,B2 visual fluoride lesions confirmed by elevated bone fluoride. Visual F lesions were on some cows in herds M, G, P.
(b) Urinary F variances significantly greater than others (P=.03).

that hemoglobin could be increased 1 to 2 g/dl by feeding additional protein when the protein content of the diet was low, but low hemoglobin did not change by adding dietary protein in herds already fed a ration adequate in protein.

The mean hemoglobin of dairy cows from 87 British herds was 12.0 g/dl±1.1 (s.d.). Whitlock et al. (89) defined anemia in dairy cattle as two standard deviations below the population mean or 9.8 g/dl or less. They found that 10.6% of 7,025 British cows were anemic by that standard. The overall mean of the Michigan cows sampled in this study (104 cows, 9 herds) was 10.8±1.2 g/dl, well below the average in Britain.

The effect of fluoride on total white blood cell numbers (WBC) was not significant, but WBC tended to be lower in herds with evidence of fluorosis (Table 8).

Serum Metabolic Criteria

The effect of fluoride on serum cholesterol approaches statistical significance (P=.06). Cholesterol was depressed to about one-half of normal in herds B2 and C4 (P<.05) (Table 7). Serum cholesterol tended to be lower for herds B, G, P, and S1 but since means lie between the highest and lowest herds they did not differ significantly.

Blood serum calcium (mg/dl) was lower in herds S1, P and G than W, and serum calcium in herd B2 was lower than in W and M. Serum calcium averaged 10.1 mg/dl in herd W. Herd W had the highest average calcium of all herds sampled, and the lowest concentrations of fluoride in urine. Serum calcium was positively correlated (P<.01) with the thyroid hormone T_3.

These results suggest that fluoride may account for the low serum calcium, cholesterol and glucose observed by Mercer et al. (40) in Michigan cattle in 1975. The depressions were evident in the data from several of their control herds and one of their herds had several cows with signs of fluorosis confirmed by elevated bone fluoride in our study.

Serum phosphorus was higher in herds B2 and C4 than S1, and C4 was higher than W (Table 7). Phosphorus mineral supplement was fed only ad libitum to herd W and this may account for the lower phosphorus concentrations in serum since some cows do not eat dicalcium phosphate when given free choice (9). Serum phosphorus fluctuates rapidly (within 3 to 4 days) depending on dietary intake. The low phosphorus values for S1 also reflect low phosphorus intake at the time of sampling. Hewitt (25) noted a relationship between high blood serum phosphorus and dairy cattle infertility in Sweden. The possible relationship of high serum phosphorus and high fluoride intake from phosphate minerals has not been studied.

Total serum protein was not correlated with any blood parameters and differences among herds were not significant.

Protein nutrition via BUN does not explain the anemia and hypothroidism of herds V3 and S1 since their BUN was comparable to herds W, B, G and M, which had higher blood cells and thyroid hormones. The effects of excess fluoride may be more severe when dietary protein intake borders on requirement since reduced feed consumption, reduced apparent protein absorption (24) and depressed rumen microbial population (26, 61) were observed when excessive fluoride was fed to cattle and sheep.

Other blood serum metabolites, e.g. glucose, uric acid, bilirubin, albumin, total protein, alkaline phosphatase and SGOT, were significantly related to one or several blood parameters. These secondary relationships may be important but a direct relationship to fluoride was not found.

Response to Thyroprotein

Feeding 2 kg additional soybean meal daily failed to produce any measurable response in milk production over a 2-wk period. Under most circumstances, milk production responds rapidly (2 to 3 days) when dietary protein is added to a deficient ration.

Preliminary blood samples had indicated anemia, hypocalcemia, hypocholesterolemia and hypothyroidism. Injections of vitamin B_{12}, iron-dextran and selenium-vitamin E by the attending veterinarian 6 wk earlier had no apparent affect on health or milk production.

In herd B2, 13 cows were fed 19 g of thyroprotein[6] per head daily for 2 wk, 10 g during the third week. Blood serum thyroxine increased (P<.01) from 3.4±1.5 ug/dl to 14.1±4.4 by the seventh day (Table 10). Milk production increased (P<.01) dramatically from 8.8±5.1 kg to 14.4±7.6 kg during the first week, peaked (16.0±9.0 kg) during the second week; then declined (P<.01) to 13.9±7.8 kg during the last week when thyroprotein was reduced. Milk production of untreated cows in the herd was unchanged during this period. The milk production response (85 to 100% increase) was similar to that observed by Tennessee workers (42) when hypothyroid cows were fed 8 g thyroprotein, and far greater than the 7% temporary increase reported by Cornell workers (62) when normal cows received 10 or 20 g thyroprotein.

[6]Protamone, Agri-Tech, Inc., Kansas City, MO.

Table 10. Hematology, serum metabolic values, thyroxine and milk production before and after feeding thyroprotein to 13 lactating cows in herd B2

	12-30-75		1-7-76		1-23-76		Sig.
	Mean	S.D.	Mean	S.D.	Mean	S.D.	P<
RBC/mm³, million	5.39	.4			5.83	.64	.05
Hb, g/dl	9.9	.8			10.86	.92	.01
Hct, %	27.7	2.3			27.5	2.77	N.S.
MCV, u³	51.0	2.9			47.0	3.27	.01
MCH, uug	18.4	.5			18.6	1.01	N.S.
MCHC, %	37.0	.9			40.5	1.76	.001
WBC/mm³, thousand	7.8	1.6			7.66	1.35	N.S.
Neutrophils, %	33.0	4.7			44.8	7.94	.001
Lymphocytes, %	55.0	9.6			46.4	8.29	.1
Monocyte	0.5	.9					
Eosinophils, %	12.0	7.3			5.6	4.52	.01
Cholesterol, mg/dl	95.0	27.0	81.0	20.0	85.0	20.0	.05
Calcium, mg/dl	8.9	.4	9.4	.47	9.3	.4	.05
Phosphorus, mg/dl	6.4	.8	6.1	1.04	5.2	.6	.01
Bilirubin			.26	.09			
Albumen, gm/dl	2.5	.2	2.4	.19	2.8	.21	.001
Total protein, gm/dl	7.5	.5	7.6	.53	8.0	.67	.001
Uric acid, mg/dl	1.0	.3			1.25	.3	N.S.
BUN, mg/dl	5.7	1.3	4.0	1.5	4.0	1.2	.01
Glucose, mg/dl	65.0	5.8	69.0	6.14	85.0	10.24	N.S.
Alk.—Phosphatase K-U	8.3	3.3	9.2	3.6	13.7	6.1	.001
SGOT, U	116.0	13.8	112.0	14.0	101.0	15.7	.05
Thyroxine (T_4)ug/dl	3.4	1.5	14.1	4.4	12.01	2.76	.001
Milk iodide, ppm	4.2	.08	2.8	.86	2.7	1.52	.001
Milk, kg/day	7.8	5.1	14.4	7.6	13.9	7.8	.001
Postpartum days, avg	118		125		140		

Milk iodide increased from 0.42±.08 ppm I^- to 2.8±0.86 ppm I^- while feeding thyroprotein for 7 days, and to 2.7±1.52 ppm I^- after reducing thyroprotein to 10 g the third week ($P<.001$). Milk from individual cows ranged from 1.2 to 4.4 ppm I^- while feeding thyroprotein.

Hemapoiesis was stimulated by feeding thyroprotein, as shown by increased red blood cell numbers, hemoglobin, serum cholesterol, calcium, glucose and mean corpuscular hemoglobin content (MCHC) (Table 10).

Total leukocytes (WBC) were unchanged. However, percent neutrophils were increased ($P<.001$). Eosinophils were reduced from 12.0±7.3% to 5.6±4.5% (Sig. $P<.01$) while feeding thyroprotein. Previously, eosinophils ranged from 3 to 28% and the range was reduced notably to 1 to 7% of leukocytes. The effect of thyroid therapy on eosinophils concurs with a previous observation that eosinophils were increased while thyroid hormones were depressed by fluoride.

These findings support the hypothesis that thyroid function was depressed by earlier exposure to high fluoride. Teeth and bone lesions were evident on these cows.

Necropsy and Histopathology Findings

Major organs of five cows (from four herds) with visible signs of fluorosis were examined for microscopic lesions. Four cows had moderate to severe kidney lesions characterized by proliferation of fibrous connective tissue and/or mononuclear cell infiltration (subacute to chronic interstitial nephritis). The pathologist[7] noted that the lesions resembled those described by Phillips et al. (53) in chronic fluorine toxicosis, but also could be attributed to other causes. For example, two cows had kidney stones.

In addition, significant kidney lesions were found in one of three calves in the 3-wk to 3-mo age group from the same farms.

Liver lesions characterized by increased periportal connective tissue and by infiltration of mononuclear cells were observed in three of the five cows.

Thyroid Weights of Calves

Three female calves from herd S1 were submitted for study and tissues were examined for lesions,

[7]Courtesy of Dr. A. L. Dade, Department of Pathology, Michigan State University Veterinary Diagnostic Laboratory.

microbial growth and bone fluoride content at the University Veterinary Diagnostic Laboratory.[8]

All three calves had subacute to chronic pneumonia. There was heavy growth of Pasturella Multocida from lung sections submitted for culture. Thyroids and adrenals were normal microscopically. However, the thyroid weights per kilogram of body weight were 176.37, 303.53 and 523.12 mg/kg. These were about 2, 3 and 5 times the normal weight (38, 49). The weight of adrenal glands was normal (68 to 76 mg/kg live weight). Estimated growth rate was poor for all calves (390 to 460 g/day).

Fluoride concentrations in bone ash (Table 3) ranged from 140 to 337 ppm F. Fluoride data of bones of normal calves of similar age were not found in the literature review. The thyroid glands were enlarged but since the calves were also afflicted with pasturella pneumonia the prior offensive agent could not be determined in the absence of controls.

CONCLUSIONS

Classical signs of chronic fluoride toxicity were observed in more than 25 herds of dairy cattle, i.e. dental fluorotic lesions and exostosis of bones.

Evidence of excessive fluoride intake was confirmed with bone ash ranging from 1,640 to 6,900 ppm fluoride and averaging 2,406 ppm in 22 specimens from adult cows in eight herds. Mineral supplements containing 5,000 to 6,000 ppm F and protein supplements containing 220 to 1,088 ppm F were the principal sources of fluoride in herds severely affected.

Cattle afflicted with fluorosis developed hypothyroidism. Blood serum thyroxine (T_4) and triiodothyronine (T_3) were depressed in relation to increasing fluoride concentrations in urine.

Eosinophils, as a percentage of white blood cells, were increased with urine fluoride (P=.004) and may be an early manifestation of toxicity and reduced resistance to disease.

Cattle afflicted with fluorosis also developed anemia. The thyroid hormones (T_3,T_4) were positively correlated with red blood cell numbers, hemoglobin, serum cholesterol, calcium, glucose and albumen. Urinary fluoride ranged from 1 to 16 ppm and averaged 5.13 ppm F. The herd with the lowest average urinary fluoride (2.9 ppm) had the highest hemoglobin and calcium concentrations in blood.

Herds fed minerals only free choice, not force-fed in the ration, had the lowest urine fluoride indicating less consumption of minerals and lower fluoride intake.

Administration of thyroprotein (iodinated casein) to hypothyroid cows afflicted with fluorosis resulted in dramatic increases in thyroxine, milk production, hemapoiesis, serum albumin and calcium while reducing eosinophils to normal, further supporting the relationship of thyroid activity to animal health and performance.

A depressing effect of fluoride on serum cholesterol approached significance (P=.061) suggesting a possible interference with blood antigen sensitivity and an important precursor of steroid hormones.

Milk production was depressed in herds severely afflicted with fluorosis. Reproductive inefficiency due to delayed estrus, uterine infections that failed to respond to treatment and failure to conceive after repeated inseminations, resulted in 20 to 30% of cows open or culled as non-breeders. Services per conception and days open were higher in fluorosis herds than in herds with minimal signs of fluorosis.

Curled and abnormal hoofs were associated with the fluorosis syndrome.

Deciduous teeth of newborn calves were yellowish to brown in fluorosis herds suggesting considerable placental transfer of fluoride to the developing fetus. Deciduous teeth of yearling cattle were mottled and severely eroded indicating that the teeth were soft and prevented consuming sufficient feed to maintain normal growth when fed only hay.

Thyroids of calves were enlarged 2 to 5 times normal weight but since calves were afflicted with pneumonia the prior offensive agent could not be determined.

Nephrosis and fibrosis were found in the kidneys of cows afflicted with fluorosis.

Moderate to severe fluorotic lesions were found on teeth and bones of cows in 25 herds fed mineral supplements containing 1,000 to 1,633 ppm fluoride and/or corresponding amounts of fluoride in protein supplements enriched with calcium-phosphate minerals from the same sources. These supplements may have an adverse effect on the health and performance of some cows in "normal" dairy herds over time but at a lower frequency than higher levels of fluoride. The effects of fluoride are insidious and since the maximum safe level over time is disputable, fluoride intake should be as low as possible. The 30 ppm maximum dietary fluoride recommended by the National Research Council may be too high for high producing dairy cattle fed phosphate sources of fluoride.

[8]Cooperation of Dr. K. K. Keahey, Department of Pathology, Michigan State University, East Lansing, MI 48824.

ACKNOWLEDGEMENTS

Funds for blood analysis of specimens were provided through a North Central Regional Research project grant to Michigan State University for research in Large Herd Management. Funds totaling $649 were allocated to this project through the courtesy of Dr. John Speicher. All other analyses were performed through the cooperation of university and state laboratory personnel. In some cases, the cost of bone assays was paid by the cooperating farmers.

The authors are grateful to: Dr. Frank Carter, DVM, Michigan Department of Agriculture and others for assistance in collecting specimens; Dr. John Suttie, Department of Biochemistry, University of Wisconsin, through whose courtesy the first 27 specimens were analyzed; Mr. Percy Hamming, Wyeth Laboratories, Mason, Michigan, for analysis of preliminary milk and urine samples; Michigan Department of Public Health for analysis of water; Drs. Kenneth Keahey, A. W. Dade, G. L. Waxler and A. L. Trapp, Department of Pathology, Michigan State University, for council and tissue examination; Dr. David Morrow, Department of Large Animal Surgery and Medicine, who collected the reproduction data; Dr. R. S. Emery, Department of Dairy Science, Michigan State University, for helpful suggestions with the manuscript; Dr. John Gill, Departmental Statistical Consultant; Dr. Roger Neitzel for processing of the data; and to the numerous dairy farmers whose cooperation and encouragement made the study possible.

REFERENCES

1. Association of Official Analytical Chemists (1975). Fluorine Sec. 25.046-25.050, 12th Edition.
2. Belcher, R., and T. S. West (1961). A comparative study of some lanthanan chelates of alizarin complexan as reagents for fluoride. Talanta 8:863.
3. Belyea, R. L.; C. E. Coppock and G. B. Lake (1976). Effects of low calcium diet on feed intake, milk production and response to blood calcium challenge in lactating Holstein cows. J. Dairy Sci. 59:1068.
4. Bolenbaugh, David L. (1977). The effects of various dietary levels of potassium on subsequent parturition and lactation in the dairy cow. Thesis for Degree of M. S., Mich. State Univ., East Lansing.
5. Busch, Harris, and Van R. Potter (1952). Localization of enzymatic block in kidneys of rats treated with fluoroacetate. Proc. Soc. Expt. Biol. Med. 81:172.
6. Christiani, H. (1930). Compt. rend. Soc. Biol. CIII, 544.
7. Clowes, G. H. A., and A. K. Keltch (1952). Action of fluoride and other reagents on phosphorylation in malignant and certain normal tissues. Proc. Soc. Expt. Biol. Med. 81:356.
8. Convey, E. M.; L. Chajsen, J. S. Kesner, D. Hillman and A. R. Curtis (1977). Serum thyrotropin and thyroxine after thyrotropin releasing hormone in dairy cows fed varying amounts of iodine. J. Dairy Sci. 60:975.
9. Coppock, C. E.; R. W. Everett and W. G. Merrill (1976). Effect of ration on free choice consumption of calcium-phosphorus mineral supplements by dairy cattle. J. Dairy Sci. 55:245.
10. Dale, D. G., and E. W. Crampton (1955). Observations on chronic fluorosis in dairy cattle. Canadian J. Comp. Med. XIX:6.
11. Dalziel, J. A., and A. V. Dangi (1972). The determination of free and total fluoride in milk. Proc. Soc. Analyt. Chem. 9:197.
12. DeToit, P. J.; D. B. Smuts and A. I. Malan (1937). Studies in mineral metabolism. XXXVI. Fluorine metabolism in rats and bovines. Onderstepoort J. Vet. Sci. and Animal Indust. 8:359.
13. Evang, K. (1938). Nord. Hyg. tisdkr. 19:117. (In Greenwood, reference 19).
14. Faccini, J. M. (1969). Fluoride and bone. Calcified Tissue Res. 3:1.
15. Forsythe, D. M.; W. G. Pond and L. Krook (1972). Effect of dietary calcium and fluoride levels on growth and reproduction in swine. Nutr. Rept. Int. 5:313.
16. Forsythe, D. M.; W. G. Pond, R. A. Wasserman, L. Krook (1972). Dietary calcium and fluoride interactions in swine. Effects on physical and chemical bone characteristics, calcium binding protein and histology of adults. J. Nutr. 102:1623.
17. Forsythe, D. M.; W. G. Pond and L. Krook (1972). Dietary calcium and fluoride interactions in swine: In utero and neonatal effects. J. Nutr. 102:1639.
18. Greenwood, D. A.; P. H. Phillips, C. S. Hobbs and C. F. Huffman (1955). The fluorosis problem in livestock production. Nat. Acad. Sci., National Research Council, Pub. 381.
19. Greenwood, D. A. (1940). Fluoride intoxication. Physiol. Rev. 20:582.
20. Greenwood, D. A.; J. L. Shupe, G. E. Stoddard, L. E. Harris, H. M. Nielsen and L. E. Olson (1964). Fluorosis in cattle. Agr. Exp. Sta. Spec. Rep. 17, Utah State Univ., Logan.
21. Goldemberg, L. (1927). J. de Physiol. et de Path. Gen. XXV 65.
22. Hall, R. J. (1960). The determination of microgram amounts of fluorine by diffusion. Analyst 85:560.
23. Hamming, Percy (1974). Determination of fluoride in SMA products and raw material. Wyeth Laboratories, Mason, Mi (Internal Mimeo 691).
24. Harris, L. E.; R. J. Raleigh, G. E. Stoddard, D. A. Greenwood, J. L. Shupe and H. M. Nielsen (1964). Effects of fluorine on dairy cattle. III. Digestion and metabolism trials. J. Anim. Sci. 23:537.
25. Hewett, Christopher (1974). On the causes and effects of variations in the blood profile of Swedish dairy cattle. ACTA Vet. Scand. Supp. 50:126.
26. Hobbs, C. S.; R. P. Moorman, Jr., J. M. Griffith, J. L. West, G. M. Merriman, S. L. Hansard and C. C. Chamberlain (1954). Fluorosis in cattle and sheep. Bul. 235, Univ. Tennessee Agr. Exp. Sta.
27. Hodge, H. C., and F. A. Smith (1965). *Fluorine Chemistry*. Vol. 4, ed. J. H. Simons, Academic Press, NY.
28. Hoogstratten, B.; N. C. Leone, J. L. Shupe, D. A. Greenwood and J. Lieberman (1965). Effects of fluorides on the hemapoietic system, liver and

thyroid gland in cattle. J. Amer. Med. Assoc. 192:26032.
29. Jacob, K. D.; W. L. Hill, H. L. Marshall and D. S. Reynolds (1933). The composition and distribution of phosphate rock with special reference to the United States. Tech. Bul. 364, USDA, Washington, D.C.
30. Jones, W. Griffith (1972). Fluorosis in a dairy herd. Vet. Rec. 90:503.
31. Jones, W. Griffith (1977). Fluorosis in dairy cattle.Vet. Rec. 100:84.
32. Kick, C. H.; R. M. Bethke and B. H. Edgington (1933). Effect of fluorine on the nutrition of swine with special reference to bone and tooth composition. J. Agr. Res. 46:1023.
33. Kick, C. H.; R. M. Bethke, B. H. Edgington, O. H. M. Wilder, P. C. Record, W. Wilder, T. J. Hill and S. W. Chase (1935). Fluorine in animal nutrition. Bul. 558, Ohio Agr. Exp. Sta.
34. Kritchevsky, David (1958). *Cholesterol*. John Wiley and Sons, Inc.
35. Lantz, E. M., and M. C. Smith (1934). Amer. J. Physiol. 109:654.
36. Lipman, F. (1928). Biochem. Leitschr CCVI:171. From: McClure, F. J. A review of fluorine and its physiological effects. Physiol. Rev. XIII (3).
37. Loeb, J. (1901). Amer. J. Physiol. 362.
38. McGuffey, R. Kenneth (1975). The effect of thyrotropin releasing hormone and 3-methyl thyrotropin releasing hormone on growth and hormonal release in the growing calf. Thesis for degree of Ph.D., Mich. State Univ., East Lansing.
39. McLaren, J. R. (1976). Possible effects of fluoride on the thyroid. Quart. Rep. Intern. Soc. Fluoride Res. Fluoride 9:105.
40. Mercer, H. D.; R. H. Teske, R. J. Condon, A. Furr, G. Meerdink, W. J. Buck and G. F. Fries (1976). Herd health studies of animals exposed to polybrominated biphenyls (PBB). J. Tox. Environ. Health 2:335.
41. Merriman, G. M., and C. S. Hobbs (1962). Bovine fluorosis from soil and water sources. Bul. 347, Agr. Exp. Sta., Univ. of Tennessee.
42. Miller, J. K., and E. W. Swanson (1967). Performance and iodine metabolism of dairy cattle with $iodine^{131}$ irradiation injury. J. Dairy Sci. 50:90.
43. Morrow, David A. (1975-77). Relationships between blood hemoglobin and reproduction in Michigan dairy cattle. Mich. State Univ., East Lansing. Unpublished data.
44. Mortenson, F. N.; L. G. Transtrum, W. P. Peterson and W. S. Winters (1964). Dental changes as related to fluorine content of teeth and bones of cattle. J. Dairy Sci. 48:186.
45. National Academy of Sciences (1974). Effects of fluorides in animals. Natl. Res. Coun.
46. National Academy of Sciences (1971). Fluorides: biological effects of atmospheric pollutants. Natl. Res. Coun.
47. Neeley, K. L., and F. G. Harbough (1954). Effects of fluoride ingestion on a herd of dairy cattle in the Lubbock, Texas area. J. Amer. Vet. Med. Assoc. 124:344.
48. Newell, G. W., and H. J. Schmidt (1958). The effects of feeding fluorine as sodium fluoride to dairy cattle—a six-year study. Amer. J. Vet. Res. 19:363.
49. Newton, G. L.; E. R. Barrick, R. W. Harvey and M. B. Wise (1974). Iodine toxicity. Physiological effects of elevated dietary iodine on calves. J. Anim. Sci. 38:449.
50. Phillips, P. H. (1937). Amer. J. Physiol. 117:155.
51. Phillips, P. H., E. H. English and E. B. Hart (1935). Amer. J. Physiol. 113:441.
52. Phillips, P. H., E. H. English and E. B. Hart (1935). J. Nutr. 10:399.
53. Phillips, P. H., E. B. Hart and G. Bohstedt (1934). Chronic toxicosis in dairy cows due to the ingestion of fluorine. Res. Bul. 123, Agr. Exp. Sta., Univ. Wisconsin.
54. Phillips, P. H.; A .R. Lamb, E. B. Hart and G. Bohstedt (1933). Studies on fluorine in the nutrition of the rat. II. Its influence upon reproduction. Amer. J. Physiol. 106:356.
55. Phillips, P. H.; J. W. Suttie and E. J. Zebrowski (1963). Effects of dietary sodium fluoride on dairy cows. VII. Recovery from fluoride ingestion. J. Dairy Sci. XLVI:513.
56. Ramberg, C. F., Jr., and S. E. Olson (1970). Fluoride effects on bone morphology and calcium kinetics. Univ. Penn., Kennett Square, Third Annual Conference of ISFR.
57. Ramberg, C. F.; Jr., J. M. Phang, G. P. Mayer, A. I. Norberg and D. S. Kronfeld (1970). Inhibition of calcium absorption and elevation of calcium removal rate from bone in fluoride treated calves. J. Nutr. 100:981.
58. Rantanen, N. W.; J. E. Alexander and G. R. Spencer (1972). Interaction of fluoride, calcium, phosphorus and thyroidectomy on porcine bone. Amer. J. Vet. Res. 33:1347.
59. Reed, O. E., and C. F. Huffman (1930). The results of a five-year mineral feeding investigation with dairy cattle. Tech. Bul. 105, Mich. Agr. Exp. Sta.
60. Roholm, K. (1939). Ergeb. inn. Med. 57:822 (see reference 19).
61. Sahashi, Y.; K. Iwamoto, M. Mikata, A. Nakayama, H. Sakai, J. Takahasi, J. Hayaski, N. Seno, T. Akatsuka, T. Miki, K. Harashima and R. Matsumoto (1952). Biosynthesis of vitamin B_{12} in various organisms. J. Biochem. (Japan) 40:227.
62. Schmidt, G. H.; R. G. Warner, H. F. Tyrrell and William Hansel (1971). Effect of thyroprotein feeding on dairy cows. J. Dairy Sci. 54:481.
63. Shaw, G. H.; E. M. Convey, H. A. Tucker, E. P. Reineke, J. W. Thomas and J. J. Byrne (1975). Bovine serum thyroxine, prolactin, growth hormone, glucocorticoid and thyroxine binding globulin response to thyroprotein. J. Dairy Sci. 58:703.
64. Shearer, T. R., and J. W. Suttie (1970). Effect of fluoride on glycolytic and citric acid cycle metabolites in rat liver. J. Nutr. 100:749.
65. Shupe, James L.; Arland E. Olson and R. P. Sharma (1972). Fluoride toxicity in domestic and wild animals. Clin. Tox. 5:195.
66. Shupe, J. L.; L. E. Harris, D. A. Greenwood, J. E. Butcher and H. M. Nielsen (1963). The effect of fluorine on dairy cattle. V. Fluorine in the urine as an estimator of fluorine intake. Amer. J. Vet. Res. 24:300.
67. Shupe, J. L.; M. L. Miner, L. E. Harris and D. A. Greenwood (1962). Relative effects of feeding hay atmospherically contaminated by fluoride residue, normal hay plus calcium fluoride and normal hay plus sodium fluoride to dairy heifers. Amer. J. Vet. Res. 23:777.

68. Singer, L., and W. D. Armstrong (1954). Determination of fluoride: procedures based upon diffusion of hydrogen fluoride. Anal. Chem. 26:904.
69. Spira, Leo (1962). Bronchotetany presenting as bronchial asthma in a case of chronic fluorine poisoning. Annals of Allergy 20:115.
70. Stoddard, G. E.; G. Q Bateman, L. E. Harris, J. L. Shupe and D. A. Greenwood (1963). Effects of fluorine on dairy cattle. IV. Milk production. J. Dairy Sci.
71. Stoddard, G. E., L. E. Harris, G. Q. Bateman, J. L. Shupe and D. A. Greenwood (1963). Effects of fluorine on dairy cattle. I. Growth and feed consumption. J. Dairy Sci. XLVI:1094.
72. Suttie, J. W. (1969). Fluoride content of commercial dairy concentrates and alfalfa forage. Agr. Food Chem. 17:1350.
73. Suttie, J. W.; J. R. Carlson and E. C. Faltin (1972). Effects of alternating period of high- and low-fluorine ingestion on dairy cattle. J. Dairy Sci. 55:790.
74. Suttie, J. W., and E. C. Faltin (1973). Effects of sodium fluoride on dairy cattle: Influence of nutritional state. Amer. J. Vet. Res. 34:479.
75. Suttie, J. W.; Ray Gesteland and P. H. Phillips (1961). Effects of dietary sodium fluoride on dairy cows. VI. In young heifers. J. Dairy Sci. XLIV:2250.
76. Suttie, J. W., and D. L. Kolstad (1974). Sampling of bones for fluoride analysis. Amer. J. Vet. Res. 35:1375.
77. Suttie, J. W.; R. F. Miller and P. H. Phillips (1975). Studies of the effects of dietary NaF on dairy cows. I. The physiological effects and the developmental symptoms of fluorosis. J. Nutr. 63:211.
78. Suttie, J. W., R. F. Miller and P. H. Phillips (1957). Effects of dietary NaF on dairy cows. II. Effects on milk production. J. Dairy Sci. XL:1485.
79. Suttie, J. W., and P. H. Phillips (1960). Studies of the effects of dietary NaF on dairy cows. IV. Dental changes as the result of long term fluorine ingestion. J. Nutr. 71:109.
80. Suttie, J., and P. H. Phillips (1959). Studies of the effects of dietary sodium fluoride on dairy cows. V. A three-year study on mature animals. J. Dairy Sci. 42:1063.
81. Suttie, J. W.; P. H. Phillips and R. F. Miller (1958). Studies of the effects of dietary sodium fluoride on dairy cows. III. Skeletal and soft tissue fluorine deposition and fluorine toxicosis. J. Nutr. 65:293.
82. Swyzer, F. (1913). Biochem. Zeitschr. LX:32 (see reference 19).
83. Taylor, G. E. T. (1929). Effect of fluorine in dairy cattle ration. Mich. Agr. Exp. Sta. Quart. Bul. 2:101.
84. Torma, Laszlo, and Burton E. Ginther (1968). Determination of fluorine in feeds by the fluoride ion electrode. J. Assoc. Off. Anal. Chem. 51:1181.
85. Tusl, Jan (1972). Fluoride ion activity electrode as a suitable means for exact, direct determination of urinary fluorine. Anal. Chem. 44:1693.
86. Udall, D. H., and K. P. Keller (1952). A report on fluorosis in cattle in the Columbia River Valley. Cornell Vet. 42:159.
87. Warburg, O., and W. Christiani (1941). Chemical mechanism of fluoride inhibition of yeast. Naturwissenschaften 29:590.
88. Willard, H. H., and O. B. Winter (1933). Volumetric methods for determination of fluorine. Ind. Eng. Chem. Anal. Ed. 5:7.
89. Whitlock, R. H.; W. Little and G. J. Rowlands (1974). The incidence of anemia in dairy cows in relation to season, milk yield and age. Res. Vet. Sci. 16:122 (England).

Fig. 6. Incisors of 6-yr-old cow showing fluoride stain, herd V3.

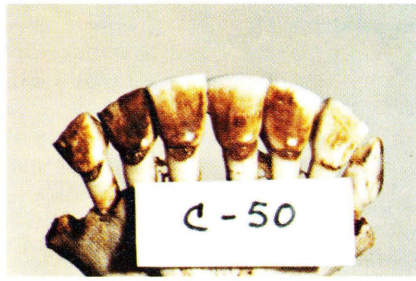

Fig. 7. Incisors of 5-yr-old cow C-50 showing brown fluoride stain and excessive wear.

Fig. 8. Inside view of incisors of C-50 showing excessive wear. Teeth were impacted with feed.

Fig. 9. Severely eroded incisors cause difficulty in eating and drinking cold water.

Fig. 10. Fluoride stains on incisors of 4-yr-old cow indicating excessive fluoride intake continuously while teeth were developing. Herd P fed commercial mineral supplement only free choice. About one-half of cows in herd were similar indicating some cows consumed more fluoride than others.

Fig. 12. Erosion of deciduous incisor teeth. Yearling heifer, herd B2. Cattle could not eat enough hay to grow normally. Deciduous teeth ranged from 800 to 1,200 ppm fluoride.

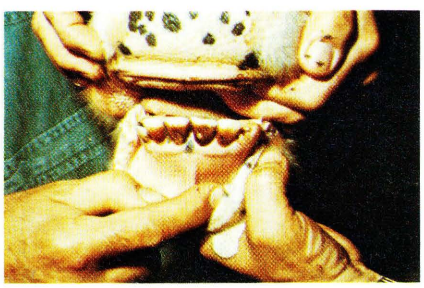

Fig. 13. Fluoride stains on 2½-yr-old steer, herd B2. See exostosis of metatarsal bone, Fig. 22.

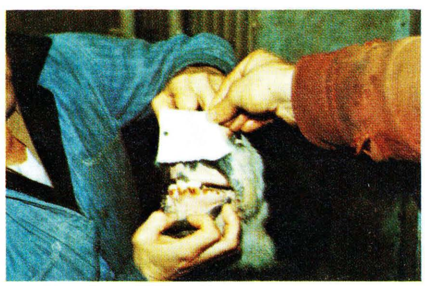

Fig. 14. Fluoride stains on permanent incisors of 2-yr-old heifer C-282. Note discoloration and erosion of deciduous teeth. Herd C4.

Fig. 16. Teeth of newborn calf showing fluoride discoloration in fluorosis herd.

10-78-5M