THE DEPOSITION OF INGESTED STANNOUS FLUORIDE IN THE FEMUR OF THE ALBINO RAT

by

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INTRODUCTION

The role of the fluoride ion in producing mottled tooth enamel has been known for many years. Many workers have reproduced and reported this effect under controlled conditions. Kuhns, in 1888, was first to mention mottled enamel; he was followed by McKay and Black in 1916. However, it was not until 1931 that Smith et al, concurrently with Churchill and Velu, proved that toxic amounts of fluorides in the water caused mottled enamel.

This study was undertaken because of: (1) the natural high fluoride content of the water found in the area of Lubbock, Texas, where the fluoride content varies from 4.0 to 5.8 parts per million, and, (2) the flooding of the market in this area with stannous fluoride dentifrices. The caution "not to use on children under six years of age" is printed on the label in very small type; and this admonition causes one to wonder about the possible harmful cumulative effects of using this product. Since rodents maintain the enamel forming mechanism in their incisor teeth, and because bone and teeth have essentially the same pattern of development, the femur of the rat was chosen as the more accessible part.

The effects of excess fluoride ion in the water supply is revealed in the work of many investigators through their contributions to the literature. Spira (1) states that the fluoride ion is a serious and harmful poison in concentrations as low as one part per million for human beings. He does not document his statements with specific evidence, however, in his book THE DRAMA OF FLUORINE (1942). Spira mentioned in reporting his fluorine work with rats that mites were attracted to the pens and to the animals themselves when the rats were fed on fluorides. Supposedly these mites left when the animals were furnished only water free of fluorides. The observations of Professor R. W. Strandtmann of Texas Technological College and I do not confirm Spira's contentions.

Ockerse (2) (3) in his dissertation Endemic Fluorosis in South Africa has carefully catalogued the sources of water samples and their chemical content with quantitative analysis including fluorides. In addition to a study of the water, he has analyzed the soil, rocks, and vegetation. Ockerse found mottled enamel, increased density of bone followed by calcification of ligamentous fasciae and muscular attachments. He found the water of South Africa to vary from 1.0 ppm to 13.0 ppm in its fluoride ion content. He includes the use of radiographs, photographs and chemical analysis in the presentation of his findings.

K. Roholm (4) in Fluorine Intoxication, London, (1937) reports on the amount and degree of fluoride poisoning caused by the inhalation of fumes from the cryolite industry in Denmark.

Pamela B. Jones (5) reported finding from 0.00 ppm to 2.25 ppm of fluoride in the potable water in New South Wales.

William H. Bauer (6) reported on the experimental fluorine poisoning in dogs. The mothers and pups were fed a high fluoride diet whereas the control groups were fed a balanced diet containing no fluorides. His findings indicated that the animals fed a high fluoride diet from birth to adulthood showed advanced fluoride poisoning with mottled enamel, improper and poor bone formation, whereas animals fed on a high fluoride diet after puppyhood showed no mottled enamel and only slight bone changes. From this he concludes that the most damage is done during the formative and growing years. His work has not been duplicated in human beings.

McCauley and McClure (7) found that exposure to 4.5 ppm of fluorides did not affect the bone fracture experience nor the height and weight of high school boys and young men. He also showed that in 12% of the adults tested in Bartlett, Texas, all of whom were over fifty years of age, the only detectable change was in the bones. These bones had a coarser trabecular structure. This change produced no effective sign or subjective symptom related to the skeletal system and was regarded as having no significance in general health. The Bartlett water system had 8.0 ppm of fluoride concentration.

METHODS

Forty albino rats, Wister strain, were divided into four groups of 10 rats each. These rats were isolated at birth, April, 1956, with their mothers and feeding was instituted by groups until the animals were weaned. After weaning, the mothers were removed and the test feeding continued until January, 1957. Upon the completion of the testing period, the animals were sacrificed and dissected. After dissection, the femurs were dried in the oven at 60° C for two weeks and then X-rayed. The results of the X-ray study is shown in Plate I. (page 13)

Group I was fed a balanced prepared diet containing 1.5 ppm of fluoride ion supplemented by the addition of 5.0 ppm stannous fluoride to the drinking water. This gave a fluoride ion concentration of 6.5 ppm. The water was changed daily. Distilled water was used exclusively for drinking purposes with this group.

Group II was fed a balanced diet with 1.5 ppm of fluoride ion. Distilled water only was used for drinking.

Group III was fed a balanced diet containing 1.5 ppm of fluoride ion plus 5.0 ppm of stannous fluoride added to the drinking water which contained 4.0 ppm of fluoride ion occurring naturally for a total fluoride concentration of 10.5 ppm.

Group IV was fed a balanced diet containing 1.5 ppm of fluoride ion plus tap water (containing 4.0 ppm) for a total concentration of 5.5 ppm.

(Balanced diet was a commercial food which, upon analysis, was shown to contain 1.5 ppm of fluoride ion.)

At dissection all animals weighed approximately 250 grams and were in apparent good health.

After the radiographing of the femurs, each femur was ashed at 600°-700° C for an hour, placed in a distilling flask containing 20 ml of perchloric acid and steam-distilled at 137° C until 200 ml of distillate had been collected. To a 100 ml aliquot part of the distillate was added 5 ml of alizarin red S plus 5 ml of zirconium-oxychloride and Scott's acid mixture (8). The resulting color change was obtained by using a Klett-Summerson photoelectric colorimeter with a green filter having an absorbancy band of 500 to 570 millimicrons.

Tables I, II, III, and IV show the scale reading, per cent of transmittance of light, and parts per million of the fluoride. Plates II (page 14) and III (page 15) show graphic scale of known solutions in parts per million. Plate II shows reading (absorbancy) on abcissa with the parts per million on the ordinate. Plate III is graphed on regular graph paper with per cent transmittance on abcissa and the parts per million on the ordinate.

The reading and per cent transmittance in each table is the average of three determinations for each sample and unknown.

TABLE I

TOTAL FLUORIDE DIET 6.5 PARTS PER MILLION*

Sample	Reading	% Transmittance	PPM F^-
1.	50	79	2.6
2.	77	70	1.75
3.	43	82	2.9
4.	30	87	3.4
5.	32	86	3.3
6.	32	86	3.3
7.	31	87	3.4
8.	32	86	3.3
9.	32	86	3.3
10.	34	85.5	3.2
Average	39.3	83.45	3.045

* The figures given are an average of three determinations.

TABLE II

TOTAL FLUORIDE DIET 1.5 PARTS PER MILLION*

Sample	Reading	% Transmittance	PPM F^-
1.	71.6	72	1.9
2.	66.3	74	2.1
3.	32	86	3.3
4.	27.3	88	3.5
5.	69.3	73	2.0
6.	62	75	2.2
7.	82.6	68.5	1.55
8.	134	54	0.1
9.	56.7	78	2.5
10.	20.3	91	3.8
Average	62.21	75.95	2.295

* The figures given are an average of three determinations.

TABLE III

TOTAL FLUORIDE DIET 10.5 PARTS PER MILLION*

Sample	Reading	% Transmittance	PPM F^-
1.	109	60.5	0.9
2.	31	87	3.4
3.	39.33	84	3.1
4.	13	94	4.05
5.	37	84	3.1
6.	15.33	93.5	4.0
7.	14.66	94	4.05
8.	33	86	3.3
9.	12.66	95	4.2
10.	27.33	88.5	3.5
Average	33.26	86.65	3.36

* The figures given are an average of three determinations.

TABLE IV

TOTAL FLUORIDE DIET 5.5 PARTS PER MILLION*

Sample	Reading	% Transmittance	PPM F^-
1.	11	95	4.2
2.	35.66	85	3.2
3.	30	87	3.4
4.	13	95	4.2
5.	14	95	4.2
6.	20	91	3.8
7.	15	93.5	4.0
8.	18	92	3.85
9.	23	90	3.7
10.	24	89.5	3.6
Average	20.2	91.3	3.815

* The figures given are an average of three determinations.

RESULTS

Plate I indicates that the amount of fluoride deposited in the femur has tended to produce a more dense and compact bone structure in ten of the 40 animals, while 24 animals show a normal type bone structure on the radiographs.

Table I, Group I reveals that there was a deposition of 1.75 ppm to 3.4 ppm of fluoride ion with an average deposition of 3.045 ppm.

Table II, Group II, which was the control group, shows a deposition of 0.1 ppm to 3.8 ppm, of fluoride ion with an average of 2.295 ppm.

Table III, Group III shows a fluoride deposition of 0.9 ppm to 4.05 ppm of fluoride ion with an average of 3.36 ppm.

Table IV, Group IV shows a fluoride deposition of 3.2 ppm to 4.2 ppm of fluoride ion with an average of 3.815 ppm.

DISCUSSION

Whereas the control group was fed on a diet with a fluoride ion content of 1.5 ppm, the tested groups diets contained 5.5, 6.5, and 10.5 ppm. The average amount of deposition of fluoride ion in the femur of the tested groups was 1.52 ppm more than the control group.

The amount of the fluoride ion fed to the test animals was in amounts considered large enough by some investigators to be toxic (1) (2) (6). However, no visible toxic effects were noted, such as, (1) loss of sheen of the coat, (2) poor weight gain, (3) loss of reproductive power, or (4) early death of the young after birth. The 30 test animals and ten control animals maintained a normal gain in weight and size. All weighed in the vicinity of 250 grams at the time of sacrifice. There was no spotty loss of the fur and it remained glossy throughout the test period. No lack of sex drive was noted and the females produced litters of from six to 10 offspring with each pregnancy during the period. All loss of the newborn was caused by the cannibalistic tendency of the male, which was observed when he was in the same cage at the time of the birth of the young.

The amount of fluoride ion deposited in the bone is not proportional to the amount consumed. The fluoride ion is known to replace the hydroxyl group in the bone structure (9) and does not displace the calcium ion. What happens to the excess fluoride? Perhaps it is taken up by "selective absorption" in the intestines, detoxified and excreted by the kidneys by way of the urine, partially excreted by the sweat glands, or part remains in the blood. The amount stored in the bones may be stored there until it can later be activated and excreted by the body processes; or, it may be retained in the bone to help form a stronger and more dense structure. Further studies determining the amounts of fluoride ion in the blood, urine, tissues, and feces should give more significant information on this subject. The lack of conclusive evidence in my own studies as to the fate of excess fluoride ion would indicate the need for such studies.

SUMMARY

1. Thirty test animals and ten control animals were studied for the amount of fluoride ion deposited in the femur of the albino rat.

2. X-ray studies of the femurs of 40 albino rats revealed only ten cases showing more radiopacity than normal.

3. The average amounts of fluoride ion deposited as determined by ashing, steam-distillation, and photoelectric colorimeter is as follows:

Group I fed a total fluoride diet of 6.5 ppm shows 3.045 ppm.

Group II (Control group) fed a total diet of 1.5 ppm shows 2.295 ppm.

Group III fed a total diet of 10.5 ppm shows 3.360 ppm.

Group IV fed a total fluoride diet 5.5 ppm shows 3.815 ppm.

4. The amount of fluoride ion deposited is not proportional to the amounts ingested.

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APPENDIX

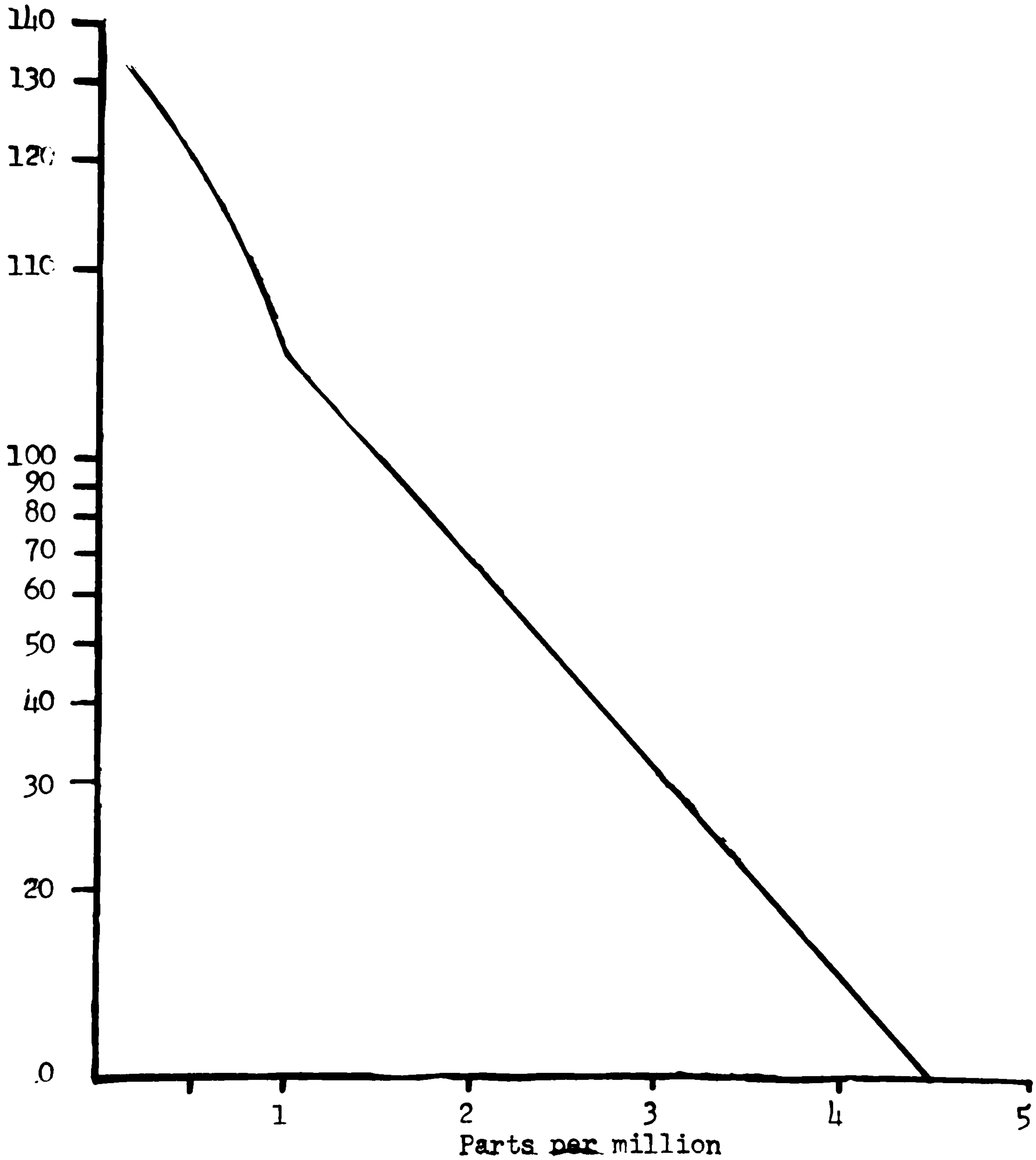
PLATE I

X-RAY COMPARISON OF FEMURS OF RATS FED ON FLUORIDES

	GROUP I	GROUP II	GROUP III	GROUP IV
1.	Cortical plate is thickened with narrowing of medullary spaces	Normal	Thickening of cortical plate with some thickening in epiphyseal area	Same as Group III
2.	Narrowing of medullary space	Normal	Normal	Irregular thickening of cortical plate
3.	Irregular narrowing of medullary space	Normal	Slight thickening of cortical plate with narrowing of medullary space	Normal
4.	Heavy thickening of cortical plate with narrowing of medullary space	Normal	Slight thickening of cortical plate	Normal
5.	Normal	Thickening of cortical plate and elongation of femur	Normal	Normal
6.	Normal	Normal	Normal	Normal
7.	Normal	Normal	Normal	Normal
8.	Narrowing of medullary space	Normal	Thickening of cortical plate	Normal
9.	Normal	Normal	Thinning of cortical plate	Thinning of cortical plate
10.	Normal	Normal	Radiolucency of upper portion of femur	Normal

PLATE II

STANDARD CURVE FOR FLUORIDE SOLUTIONS



(Semi-logarithmic 2 cycles X 10 to the inch)