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Two Generational Study on the Effect of Different Levels of Fluoride on Rat Bone and Teeth

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TWO GENERATIONAL STUDY ON THE EFFECT OF DIFFERENT LEVELS OF FLUORIDE ON RAT BONE AND TEETH

by

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Madhav Prasad Upadhyay

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ABSTRACT

Two Generational Study on the Effect of Different Levels of Fluoride on Rat Bone and Teeth

by

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The effects of different levels of fluoride in drinking water on different parameters of femurs and incisors of female rats were studied. Rats and their offspring, before and after weaning, were used for the study. Mother rats received 0, 1 and 5 ppm fluoride in drinking water. After weaning, the offspring were given the following treatments: 0-0, 0-1, 0-5, 1-0, 1-1, 1-5, 5-0, 5-1 and 5-5; the first number indicating fluoride level of mother's water during mating, pregnancy and lactation and the second number indicating the fluoride level of water given to the offspring. Femurs and top and bottom incisors were collected from mother rats, 21 day old pups and 300 g. body weight pups. Femurs were analysed for ash, calcium, phosphorus and fluoride content and breaking strength. Only fluoride analyses were done on incisors. Fluoride ion electrodes (Orion models 94-09 and 96-09) were used for fluoride analysis.

Mother rats that received 1 and 5 ppm of fluoride showed an increase in fluoride content of teeth. There was no increase in the fluoride content of weanling rat teeth suggesting that there was no maternal transfer of fluoride to the offspring.

A significant increase in fluoride content of femur and teeth of all the groups of 300 g. offspring, that received 1 and 5 ppm of fluoride, was observed as compared to the control group (0-0). No significant differences in other femur parameters of 300 g. offspring were observed.

Significant differences in fluoride content of femurs and incisors of 300 g. rat offspring were found due to pre and post-weaning fluoride treatments. Combined pre and post-weaning fluoride administration resulted in higher fluoride content of femurs and incisors.

At the levels used in this study, pre-weaning fluoride administration alone does not appear to affect the fluoride content of bone and teeth of the rat offspring, but fluoride, when given after weaning does contribute to the increased fluoride content of bone and teeth.

(89 pages)

INTRODUCTION

Fluoride (F^-), the most electronegative of all elements, has not only notable chemical qualities but also physiological properties of great interest and importance for the health and well being of animals and man. Fluoride has now been accepted as an essential nutrient by the Food and Nutrition Board of the National Research Council because of its role in the development of teeth that are resistant to caries (33). Fluoride has been known to be always present in bones and teeth since the 19th century. However, there is presently great interest in its effect on skeletal and dental tissues, owing to its considerable beneficial effect on dental health, its potential use in the treatment of bone disease, and its capacity to cause dental and skeletal fluorosis (64).

Fluoride is the most exclusive bone seeking element, because of its great affinity for calcium phosphate (18). It is, therefore, found in all tissues exhibiting either physiological or pathological calcification. Depending on the dosage, fluoride seems to stimulate bone formation, induce partial resorption and cause changes in the degree of mineralization (2). In bones, low fluoride concentrations are reported to be beneficial since they increase the size of apatite crystals (18) and reduce their solubility (18),thus stabilizing the whole skeletal system.

Fluorides have been administered to astronauts to counteract the loss of skeletal calcium due to physical inactivity and weightlessness (2). In optimal

amounts, fluorides are beneficial to teeth and reduce the incidence of dental caries by up to 60 percent (2). In higher doses, however, fluorides can cause fluorosis (2) and mottled enamel (2) in animals and man. Fluoride treatment of osteoporosis and demineralizing bone diseases is still highly experimental, since the therapy simultaneously induces a certain degree of fluorosis.

The purpose of this study is to investigare the effects of different levels of fluoride on the fluoride content of femur bone and teeth of female rats and their offspring. The investigation will also provide a better understanding of the influence of pre and post weaning fluoride administration on bone strength and fluoride retention in rats and their offspring.

REVIEW OF LITERATURE

Although fluoride consistutes only a small percentage (0.065%) of the elements of the earth's crust, its remarkably wide distribution throughout nature suggests some biological role for the element. Fluoride has been found to be essential for optimal growth and general development of rats raised on purified amino acid diets in trace-element-sterile isolators (60). A significant increase in body weight has been reported for female mice after supplementation of 10 ppm of fluoride in drinking water for 3-18 months (60). Fluoride may also be essential for reproduction and prevention of anemia in mice (48). It is also known that fluoride ions may either inhibit or stimulate the activity of certain enzymes, though the processes involved are largely obscure (2).

The extent to which inorganic fluorides are absorbed depends on their solubility. The absorption of soluble fluorides by the gastro-intestinal tract is rapid and nearly complete, whereas less soluble fluorides are more slowly and less completely absorbed (2). Absorption takes place both through the gastric membranes and through the intestinal tract by the normal process of diffusion.

In principle, the extent of the absorption is the same whether the fluorides are ingested in water or in food. But there is one important exception to this principle. If the diet contains high proportions of calcium, phosphate, magnesium or aluminium, complex fluoride ions of low solubility may be formed (2). In these circumstances the fecal excretion of fluoride increases and the absorption

from sodium-mono-fluorophosphate (Na_2Po_3F) is less influenced by the presence of calcium ions, it has been suggested that this compound may be more suitable than sodium fluoride for human supplementation (2). With sodium fluoride, the fluoride absorption may be reduced by the presence of foods rich in calcium (2).

Absorption of fluoride in rats is rapid during the first hour and a half after ingestion (29). Maximum absorption, 85 to 90 percent, is attained during this time period and very little absorption occurs in the following eight or nine hours (29). It is likely that the 10 or 15 percent of the dose which remains in the gastrointestional tract up to 10 hours after administration reflects a state of equilibrium between fluoride leaving and entering the intestinal canal (29).

The rate of disappearance of absorbed fluoride from the blood is greater in younger rats than adult rats. This may depend in part on the greater skeletal uptake of fluoride in the younger animals than adult animals. The skeletal fluoride saturation that can be attained with the administered doses has no decisive influence on the absorption and blood turnover of fluoride (29).

Fluoride and Bone

As about 96 percent of the fluoride in the body is deposited in hard tissues, it is obviously of the utmost importance to know about the function of fluoride ions in the structure, morphology and physiology of bone.

The fluoride ion is unique in that it continues to be deposited in calcified structures after other bone constituents (calcium, phosphorus, carbonate and

citrate) have reached a steady state (2). Even if large amounts of these other constituents are administered, their concentrations, which reached their maximum early in life, remain essentially unchanged (2). Fluorine in bone, on the other hand, increases very rapidly with higher fluoride levels in drinking water (2). However, age is an important factor in the extent to which fluorine is incorporated into the skeleton (2). Adult human skeletons reach equilibrium rapidly as compared to that of children.

Chemical analyses of fluoride rich hard tissues have shown that the incorporation of fluoride slightly alters the chemical composition of bone. The carbonate and citrate contents are lowered and the magnesium levels are increased. The calcium to phosphorus ratio, however, remains unchanged. Fluoride uptake was reduced by raising the pH of the incubation which suggested an analogous competition between fluoride and hydroxide ions for sites in the apatite crystal (72).

Modern analytical techniques have revealed that fluorides are always present in mammalian body fluids and tissues and that relatively large amounts occur in calcified tissues (2). Blood plasma is the most convenient and reliable indicator of fluoride concentrations in body fluids of mammals (2). Plasma fluoride concentration is homeostatically controlled at 0.1 - 0.2 ppm, even if large amounts of fluoride are given (2). Any excess is rapidly deposited in the bone, which acts as the chief depository, or excreted through the urine (2). Normal fluoride levels in teeth and bone of mammals range from 100 to 600 ppm, which can easily reach 1000 to 7000 ppm (72). In the embryo and newborn, vertebrae

and rib contain 50 - 150 ppm of the element. Even in low-fluoride areas, fetal bone ash contains 40 - 47 ppm of fluoride (72). The placenta plays an active role by accumulating fluoride and regulating its transfer to the fetus (72). Fluoride is supplied in rather constant amounts (0.1 - 0.2 ppm of wet weight) by milk (72). The normal fluoride levels in the soft tissues such as liver, heart, kidney and brain are approximately 2 - 5 ppm on a dry weight basis (72). The concentrations are maintained constant, except for the kidney, even if high amounts of fluoride are supplied over extended periods of time (72).

Fluorides are excreted in faeces, urine, sweat, and to a small extent by skin, as it is sloughed off with age. Traces may also be lost in milk, saliva, hair and tears, though it is probably not exhaled in the breath (2). Fecal excretion usually accounts for the loss of some 10 percent of the daily intake (2). Although fluoride is a natural constituent of human milk, the proportion of the daily intake secreted in the milk is negligible (2). It is not known whether this small amount of fluoride in mother's milk is significant in the development of teeth and bones in infants. Under normal circumstances, fluorides are excreted principally in the urine. They appear in urine very rapidly after ingestion (about 20 percent after 3 hours), and generally the level reflects the daily intake (2). Urinary fluoride does not arise only from the fluoride in the feed and the water. It may come also from the release of the element from skeletal sources. Mobilization and excretion of a portion of the fluoride present in the skeleton of animals removed from a high fluoride diet has been observed in rats (29).

The levels of fluoride in bones and teeth increase in proportion to the amount, form, duration and continuity of the fluoride intake and with the age of the animal (54). The relationship is logarithmic rather than linear, so that the rate of deposition falls with increasing fluoride content of the bone (54). The rate also falls with increasing age of the animal, since older animals retain less and deposit less in their bones than do younger animals (54).

In young rats given fluoride, the concentration of fluoride has been found as follows: epiphyses > metaphyses = diaphyses. The higher fluoride concentration in epiphyses than in the other actively growing sites in young rats may be due to higher rates of calcification at this site and for this time (28). In mature rats ingesting fluoride, less fluoride was deposited in all bone sites and the concentration in the metaphyses was nearly twice that deposited in the other sites (28).

Deshmukh et al. (13) found 33 ppm and 6648 ppm fluoride humeri from rats that received 0.2 ppm and 200 ppm of dietary fluoride, respectively. Beary (5) reported that in rats that received 0, 3.4, 10 or 45 ppm of fluoride in the drinking water had, respectively, 100, 300, 700 or 3000 ppm fluoride in their femurs. Fluoride concentration of 297 ppm and 350 ppm were observed in femurs of rats receiving water containing 0.2 ppm and 5.2 ppm fluoride, respectively (72).

Effect and mechanism of action of fluoride on bone

The current theory of bone formation (2) postulates that collagen (the chief protein of bone fibre) forms a matrix for a nucleation process in which calcium

and phosphorus are deposited. This is followed by the formation of the mineral phase called hydroxyapatite ($Ca_{10}(Po_4)_6(OH)_2$). Thus the collagen fibres act as a template for the deposition of the hydroxyapatite crystals. As fluorine ions are of much the same size and shape as the hydroxyl ions, they exchange with the hydroxyl ions to form fluorapatite ($Ca_{10}(Po_4)_6F_2$) which has usually been regarded as a less soluble crystal.

McCann (42) and Neuman et al.(72) as well as other workers cited by Hodge and Smith (33) have clearly demonstrated that at low levels, fluoride reacts with synthetic apatites (42) as well as with bone (48) to form fluorapatite. The reaction can be expressed by the following equation:

$$Ca_{10}(Po_4)_6(OH)_2 + 2F^- \rightleftharpoons Ca_{10}(Po_4)_6F_2 + 2(OH)^-$$

The equation is reversible in that less fluoride was incorporated in ashed bone (47) at high hydroxyl-ion concentrations.

At the levels of fluoride found in circulating body fluids, only fluorapatite is found in bone and X-ray diffraction analysis has not revealed the presence of CaF_2 (72). According to McCann (42) no CaF_2 is formed unless the concentration of fluoride in the solution in contact with the solid exceeds about 100 ppm.

The most widely accepted concepts of how fluoride might act on bone (54) include one or more of the following:

A. Concentration of fluoride in bone, with formation of mixed hydroxyfluorapatite; presumably mainly in bone laid down during the time of fluoride administration.

B. Inhibition of osteoclastic bone resorption due to decreased crystal solubility.

C. Secondary hyperthyroidism, presumably to maintain calcium homeostasis.

D. Increase in differentiation of osteoclasts and osteoblasts.

There is a substantial weight of evidence for several of these concepts. There is no doubt at all that fluoride is highly concentrated in bone. Bones of animals treated with fluoride have been found to show a lower resorptive response when exogenous doses of parathyroid hormone were given (54). Furthermore, there is direct and indirect evidence for secondary hyperthyroidism in fluoride treated animals and man.

Although above points must be accounted for in discussing the mechanism of fluoride effect, there are several facts that are difficult to explain in the terms of the concepts stated above. The onset of fluoride effect in bone is far too rapid to be related in any significant way to the bone laid down during the period of fluoride administration (54). This is obvious when one considers the rate of renewal in the adult skeleton. It takes 10 to 20 years or more for half of the mass of human bone to be replaced. In constrast, the effect of fluoride on bone starts to be expressed within 2 - 3 weeks to a month or so after treatment is started. Yates, Doty and Talmage (54) found evidence of hyperthyroidism within days of starting to treat rats with sodium fluoride. Facini and Care (54) have reported that concentration of parathyroid hormone in sheep blood elevated one week after starting treatment. Calcium retention (54) and increased osteoblastic differentiation (54) have been found within 2 to 3 months of

starting to treat human beings with sodium fluoride. All these periods are so short that it appears very unlikely that a significant part of the bone crystals would have been formed as hydroxyfluorapatite. It is, therefore, doubtful that it is necessary or important in the initiation of fluoride effect. Furthermore, reduced crystal solubility probably is not a major factor in maintaining the effect of fluoride on bone. Although additional evidence on the point is needed, there is a general agreement that the bone disease does not progress once exposure to fluoride is stopped.

Rich and Fiest (54) have proposed a hypothesis for the mechanism of action of fluoride in the bone, based on the two most conspicuous aspects of fluoride metabolism in mammals; that it is concentrated at the surfaces of bone and that it is toxic to bone cells. The first assumption is that the fluoride is not distributed evenly throughout bone, but rather, that it is highly concentrated in two specific regions. These are (a) those areas of bone formed during the time of administration, when the blood fluoride concentration is high, and (b) the surface layer of bone immediately bordering upon the osteocyte lacunae and canniculae. The former localization is obvious and well accepted but the latter has not been suggested and deserves comment.

In principle, during administration of fluoride, its concentration in blood and extracellular fluid rises, somewhat proportionately to the dose. The extracellular fluid perfuses the osteocyte lacunae and canniculae, bringing fluoride into contact with these bone surfaces. Presumably, it both is absorbed at high concentration at this interphase and incorporated into the crystal lattice, so

that the concentration of the fluoride in this region may conceivably be quite high. Since it must pass from this region if it is to reach interlacunar bone, it can be concluded that fluoride concentration in this region will be greater than in interlacunar bone. However, it is likely that the fluoride ion would not migrate deeply into interlacunar bone, since crystals in fully calcified bone are so closely packed as to partially exclude fluid and strongly impede diffusion of ions. Accordingly, Rich and Feist (54) have proposed that fluoride would be concentrated at the lacunar and cannicular surfaces, rather than evenly distributed throughout interlacunar bone.

The action of fluoride on bone cells has been proposed to be a toxic one and that the consequence of a high regional fluoride concentration would be inhibition of resorptive function (54). There is ample evidence that fluoride passes into the bone cells and that it inhibits numerous enzyme activities (33) and therefore, the concept that metabolic function might be inhibited when cells are exposed to high fluoride concentration is reasonable. The high local concentration of fluoride presumably would promote formation of hydroxyapatite and reduce crystal solubility in these regions, further inhibiting resorptive efficiency. The probable consequence would be an increase in parathyroid hormone secretion to just that degree necessary to compensate for the inhibition of homeostatic bone resorption, thereby maintaining the blood calcium concentration at or near normal level.

The overall metabolic consequences of fluoride incorporation into bone are illustrated in the following figure (Figure 1).

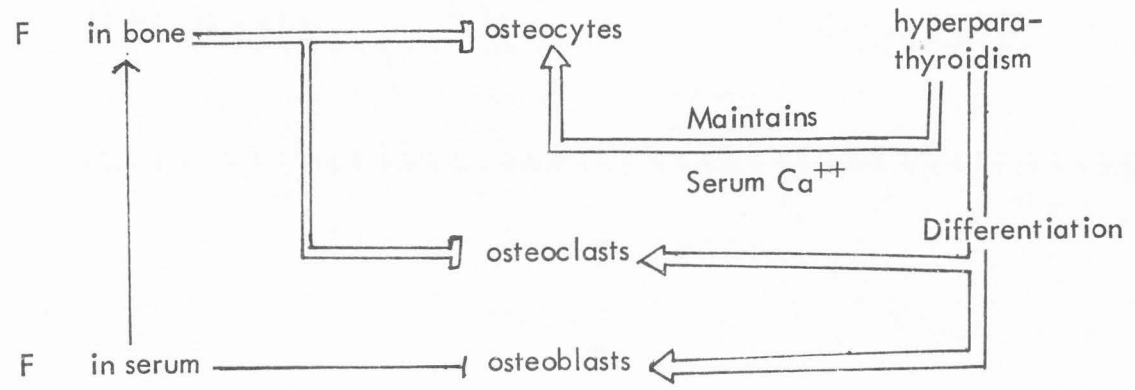


Figure 1. A schematic representation of the effects of fluoride on calcium homeostasis and bone metabolism. The lines ending in a perpendicular bar indicate inhibition; those ending in an arrow indicate stimulation (54).

In this figure, it is assumed that osteocytic resorption has mainly to do with calcium homeostasis and that osteoclastic resorption has mainly to do with bone remodeling. The concept presented is that the degree of hyperparathyroidism exactly compensates for the fluoride induced inhibition of homeostatic bone resorption. It is assumed that parathyroid hormone stimulates osteoblastic as well as osteoclastic differentiation and therefore, that there is a stimulus to osteoblastic differentiation which is proportional to the severity of the F^- inhibition of homeostatic resportion. There is a great deal of indirect evidence (54) to support the concept that parathyroid hormone directly stimulates osteoblastic differtiation, but it remains unproven. Although some direct action of fluoride on osteoblastic differentiation cannot be excluded and certainly may occur (54), it seems less likely than an indirect action based upon secondary hyperthyroidism.

Fluoride and bone strength

Since the fluoride effect on bone is cortical and trabecular thickening, it has been proposed to have favourable effect on the bone strength (18). High levels of fluoride have been claimed clinically to reduce bone pain, restore calcium balance and increase bone density in patients with osteoporosis, Paget's disease and osteogenesis imperfecta (18).

Administration of physiological doses of fluoride to weanling rats (up to a level of 45 ppm fluoride) in drinking water for 16 weeks caused no reduction in femoral bone strength despite the deposition of fluoride to a level of 2000 ppm to 4000 ppm fluoride in the bone (29). Rich and Fiest (54) found a significant correlation between bone strength and fluoride concentration up to the level of 30 ppm in the drinking water.

Saville (59) made compression tests on humeri and femurs from rats given water containing 2, 5, or 20 ppm of fluoride. The calcium content of the axial skeleton and the forelimb was found to a linear function of body weight. There was an increase in calcium content of forelimbs at 20 ppm water fluoride level but not at 2 and 5 ppm level. The breaking strength of the shafts of humeri and femurs was a linear function of the body weight and was unaffected by water fluoridation up to a concentration of 20 ppm.

Beary (5) reported the effects of low (0.1%) and adequate (0.6%) calcium and different levels (3.4, 10.0 or 45.0 ppm) of fluoride in drinking water on the physical properties of rat femur on adequate calcium diet, a significant increase in flexibility in the rat femur was found only at the 45.0 ppm dosage

level. No significant difference in breaking strength was found. On low calcium diet a similar significant increase in flexibility appeared at both 10.0 ppm and 45.0 ppm levels, but a significant decrease in bone strength was observed at the two levels.

The results of mechanical tests carried out on homogenous compact bone (pure cortical bone) of standardized dimensions from rats receiving 50 ppm fluoride in drinking water for eight weeks (54) are not in complete agreement with reports based on studies on whole bone (54, 67). Sodium fluoride decreased the breaking strain and breakup energy absorption capacity in the mobile tibiae of normal rats (50). The tensile strength was not significantly influenced. Sodium monofluorophosphate decreased only the breaking strain in the mobile tibiae of normal rats. The diminished mechanical bone strength of the fluoride treated rats is not consistant with the view of other investigators who administered similar fluoride dosages because their determinations were carried out on whole bone (54). Differences in parameters such as bone weight, cortical thickness and bone diameter could influence the results of measurements of bone resistance to stress (54). It may well be, however, that the results of studies of whole bone are more realistic.

Henrikson et al. (30) studied various doses (0, 25, 85 or 300 ppm in water) of sodium fluoride up to 1 g/Kg of body weight of adult beagles and found no effect of fluoride on mechanical properties of their bone. Riggins et al.(55) also showed that bone strength was unaffected or significantly reduced by the addition of sodium fluoride (50 or 100 ppm) to the drinking water of growing rats.

Increase in bone diameters were observed which were consistant with the increased bone formation recorded with fluoride administration. The addition of sodium fluoride to the diets of growing rats receiving adequate amounts of calcium and Vitamin D did not significantly influence bone strength despite a significant increase in bone diameter in one group (55).

Yamamoto et al. (69) reported that bone microhardness was increased in rats given drinking water containing 300 ppm fluoride.

Whether the strength of intact bone is normal, increased or decreased following fluoride administration must be regarded for the time being as equivocal. For as many investigators that claim fluoride causes a decline in breaking strength, there are equally as many who proposed no significant effect of fluoride on bone strength (Table I).

The increase in the fluoride content of bone has been reported to be accompanied by an increase in its water content and is detrimental to its physical strength as measured by compression of femur shafts (13). Similar observations about breaking strength were reported by Gedalia et al. (27) who observed an increase in bone strength and calcium content of femur with growth.

Chan et al. (11) using Japanese quail showed that in early stages of growth dietary fluoride markedly increases bone calcium retention. They observed 30 percent decrease in bone torsional strength of humeri and tibias of Japanese quail when they were fed a diet containing 75 ppm fluoride (11).

Table 1. Effect of fluoride on the breaking strength of bone

Species	Water fluoride level	Duration	Effect on bone breaking strength	Reference
Rats	300 ppm	16 weeks	Increased bone microhardness and mechanical strength in femur noted.	69
Rats	250 ppm	52 weeks	No effect in bending strength.	49
Rats	200 ppm	1 month	17 percent decrease in breaking strength but when correlated with body weight effect was reduced to zero.	13
Rats	200 ppm	2 weeks	Decrease in breakup stress of femurs.	68
Rats	100 ppm	3 months	No effect on torsion in high calcium and fluoride diets. Decrease noted in low calcium and fluoride diets.	55
Rats	50 ppm	Chronic	No effect on machined specimens in tension tests.	50
Rats	50 ppm	6 weeks	Decrease in breaking strength noted.	27
Rats	20 ppm	100 days	No effect on breaking strength	58
Mice	50 ppm 10 ppm	6-25 months 6 months	No influence on breaking strength.	53
Rats	10 ppm	15 1/2 weeks	Low calcium and fluoride brought decrease in breaking strength. Adequate calcium and fluoride showed no effect.	5

Fluoride and Teeth

The most widely and best substantiated physiological role of fluoride is its function in the prevention of dental caries (2). In the regions where the water naturally or artificially contains I ppm fluoride, the incidence of dental caries in children has been reduced by 50 percent.

Fluoride has also been found beneficial in form and appearance of tooth eruption time and alignment in the dental arches and in the reduction of frequency and severity of periodental disease. The only harmful effect of fluoride in teeth is the development of mottled enamel, which is caused by excessive fluoride ingestion during the period of tooth formation (2).

The concentration of fluoride in teeth follows a similar pattern to that in the bone (2). The age of the individual and the fluoride intake in food and water are chief factors. But in dental enamel, which has no cells and no circulation, the uptake of fluoride in man almost ceases after about 30 years of age (2).

It is now well established that, as in bone, the distribution of fluoride within the tooth is not uniform. In human beings, the fluoride levels in the outer layers of enamel are five to ten times higher than those in the inner layers (2). This difference probably arises partly due to the fact that the outer layers are in contact with tissue fluid for a longer time after formation and before eruption than are the inner layers and partly because, after eruption,

the outer enamel is in contact with saliva. The secondary dentine, which forms slowly throughout life and has relatively prolonged contact with the tissue fluid of the pulp, has a higher fluoride concentration than the more rapidly formed primary dentine. The rise in fluoride concentration with age in dentine is due to a rise in the concentration in the primary dentine and partly due to the fact that the proportion of secondary dentine in the whole tooth increases with age (2).

Administration of a high dose of fluoride (250 ppm in drinking water) has been shown to increase the calcium deposition in the growing rat dentine. This effect occurred within hours after fluoride administration. No such effect was observed at a lower level (25 ppm in drinking water) (45).

Ruzicka and Mrklas (55) reported that time required to reach a steady level of fluoride content in rat incisors was found to be inversely proportional to the quantity of fluoride solution (150 ppm) given for 200 days. After the equilibrium state was reached, the fluoride content in the incisors ceased to depend on the amount of fluoride solution given (55). The addition of either 25 or 100 ppm fluoride in the drinking water of rats produced a banded appearance on the incisors but had no significant effect on the impeded or unimpeded eruption rats of the incisors (55).

Fluoride concentrations in molars of rats fed a low fluoride diet (0.5 ppm) or a diet containing 200 ppm fluoride have been reported (46). First rat molars that received the low fluoride diet contained 123 to 142 ppm fluoride

in the first layer and 57 to 73 ppm fluoride in the second layer. The highest fluoride concentrations were found in the third molars. Mean fluoride concentrations in the first layer of third molars in rats receiving the low fluoride diet reached 153 to 241 ppm. The high fluoride diet increased this to about 375 ppm. Fluoride concentration in the second layer was found 249 to 283 ppm in rats fed the fluoridated diet. Fluoride concentrations in teeth within groups fed either the low or the high fluoridated diet do not show a difference related to age (46).

Shinoda (60) studied the effect of long term administration of fluoride on physio-chemical properties of the rat incisor enamel. Rats received 0, 9, 23, 45, 68, or 113 ppm of fluoride in the drinking water for 70 days. The tooth enamel formed during high fluoride exposure showed marked hypocalcification. The microhardness of enamel showed a marked decrease. These changes were not prominent in the outer region of the enamel and were proportional to the concentration of fluoride administered.

Ruzicka et al. (58) found a lower occurance of dental fluorosis in mice fed complex fluorides (sodium monofluorophosphate, sodium difluorophosphate and ammonium hexafluoroaluminate, 150 g in drinking water) as compared to sodium fluoride. Since complex fluorides were found more effective than sodium fluoride, possibility of using complex fluorides as effective agents in caries prevention in human populations was suggested.

Weatherall et al. (66) reported that in normal rats, fluoride concentration in the forming enamel decreased as the tissue matured. The fluoride concentration was higher in partially mineralized than in the more highly mineralized maturing enamel. The fluoride concentration decreased rapidly as the mineral content of the tissue approached that of the mature enamel.

Prenatal Transfer of Fluoride in Rat

Considerable information has appeared in the literature concerning the transfer, or lack of transfer, of fluoride from the pregnant mother to the developing fetus (69, 70). Different experimental approaches have been employed including the measurement of fluoride levels in maternal, fetal and placental circulating fluids, the use of radioisotopes and the determination of the amount of fluoride retained in the fetal hard and soft tissues. In addition, different animal species have been used. Collectively, such broad experiences have resulted in a variety of different conclusions.

Studies concerning the deposition of fluoride in the fetus of rat (63, 26, 69, 41, 8, 9, 10) have suggested that the placenta may act as a barrier and prevent the passage of low levels of fluoride provided during gestation to the fetus until a level of about 8 ppm fluoride in the drinking water is reached. Above this level, fluoride appears to be transferred to the fetus. Such findings suggest that more than optimal levels of fluoride are required in order to provide a physiologically significant amount of fluoride transfer to occur.

Comparable studies designed to evaluate the amount of placental transfer of fluoride, as indicated by the retention of fluoride in the fetus, have been conducted in the dog (12), rabbit (42), guinea pig (35) and cow (6). These studies indicate that the levels of dietary fluoride of 50 ppm or higher result in fluoride transferred to the developing fetus. However, little is known concerning the possibility of a placental barrier existing when low levels of fluoride are ingested in such species, although Knouff and co-workers (37) failed to find any transfer of fluoride in dogs provided drinking water containing 5 ppm fluoride. Ericsson and co-workers (27, 71) using an ^{18}F autoradiographic technique in pregnant mice, located the highest concentration of fluoride in skeleton. The soft tissues were extremely low in fluoride, with the exception of the kidneys and placentas.

Studies regarding the placental transfer of fluoride in humans have been conflicting. The belief that fluoride ingestion during pregnancy will benefit the dental health of a child is based on the knowledge that calcification of deciduous teeth is initiated in utero and that fluoride will pass through the placenta to the fetus. In line with these conclusions are the works of Arnold et al. (4) and Blayney and Hill (4). However, Katz and Muhler (4), Carlos, Gitlelsohn and Haddon (4) and Reiss (4) have shown different results. Placental transfer of ^{18}F has been analysed autoradiographically and quantitatively in the late pregnancy of mice (1-2 days before expected parturition) (27). The fetal skeleton accumulated much less ^{18}F than the maternal skeleton of

these animals, owing to the slow diffusion of fluoride through the placenta and to the great homeostatic capacity for fluoride of the mammalian body (27). A sudden increase in maternal blood fluoride, such as that induced by the intake of fluoride tablets or by injection of ^{18}F in pregnancy, should, therefore, not produce any great rise in the fluoride concentration of the fetal blood.

In animal studies, it is difficult to assess the degree of placental transfer of fluoride in view of the contradictory data available. In addition, fluoride results have not been expressed on a common basis in all studies nor has sufficient information been presented for conversion of the data to a common basis. (Table 2) (71). There are variations in these studies because different regimens for fluoride administration have been used. Differences also exist in the amount of fluoride used in the diet or water.

With the exception of the data of Brzezinski and co-workers (8), markedly more fluoride is deposited in the fetal skeleton of the rat when fluoride is given to the mother in the drinking water than when given in the diet. Brzezinski et al. (8) found only 4 ppm fluoride in ashed fetal bones when as much as 100 ppm fluoride was offered in the drinking water.

Lehman and Muhler (71) and Osborne (71) reported increases of fluoride in the rat fetus when fluoridated water was given to the mother rat, but no data to support the claim was provided.

Table 2. Deposition of fluoride in the fetal skeleton of the rat (71)

Fluoride regimen	Bone	PPM (Ash basis)
25 ppm F added to drinking water during gestation and lactation	skeleton	76[a]
Control	skeleton	50
25 ppm F added to drinking water	femur	160
Control	femur	150
25 ppm F added to drinking water	bone	143
Control	bone	24
50 ppm F added to drinking water	fetus	115
Control	fetus	53
50 ppm F added to drinking water	bone	350
Control	bone	24
50 ppm F added to drinking water	fetus	900
Control	fetus	80
100 ppm F added to drinking water	fetus	1160
Control	fetus	80
100 ppm F added to drinking water	skeleton	3.8
Control	skeleton	0.3
20 ppm F added to diet	fetus	20[b]
Control	fetus	10
50 ppm F added to diet	fetus	0.65
Control	fetus	0.37
90 ppm F added to dietq	fetus	23
Control	fetus	3
100 ppm F added to diet	fetus	1.15
Control	fetus	0.37
200 ppm F added to diet	fetus	4.1[c]
Control	fetus	0.0

Table 2. Continued

Fluoride regimen	Bone	PPM (Ash basis)
200 ppm F added to diet	fetus	3.02
Control	fetus	0.37
225 ppm F added to diet	fetus	5[c]
Control	fetus	1

[a]Dry, fat free basis
[b]Not given
[c]Wet basis

When diets containing 20 - 225 ppm fluoride were ingested by the pregnant rat, (71) about 5 - 25 ppm of fluoride was found in the bone ash and 0 - 10 ppm fluoride was found in the bones from control rats. Lawrenz and Mitchell (71) showed that 9 ppm fluoride given as sodium fluoride (NaF) in the drinking water was 21% more assimilable than an equal amount of fluoride given in the diet. Weddle and Muhler (71) reported 36 percent greater storage of fluoride in the femurs of rats receiving 5 ppm fluoride in the water than in the diet.

Despite the variability of results from different laboratories, it appears that food-borne fluoride is absorbed to a smaller degree by both the adult rat and the fetus than the water-borne ion. It seems possible that food in the gastro-intestinal tract may reduce the rate of absorption of fluoride.

Gedalia et al.(26), using rats given 0, 25 or 50 ppm of fluoride in the drinking water, supported the concept that fluoride is transfered through the placenta. And the fluoride up-take by the skeletal tissues of fetus is enhanced by the elevation of fluoride intake by the mother rat during pregnancy. Administration of fluoride during the 21 days after birth did not change the fluoride content of the bones of the offspring. This result was thought to be due to a greater rate of bone salt deposition than that of fluoride incorporation.

Theuer et al.(64) did not observe the transfer of significant amounts of fluoride from mother rats to their offsprings, when the rats were given 50, 100 or 200 ppm of fluoride in the diet.

In the recent report Messer et al.(47) state that although the neonatal fluoride concentrations were considerably lower than the maternal fluoride

concentrations in the group of rats receiving 50 ppm fluoride, it is apparent that an elevated maternal fluoride intake results in a notable increase in the fluoride content of the bones of offspring. They are of the opinion that a high maternal fluoride intake during lactation does not appear to result in a high fluoride content in milk and the direct provision of fluoride to offspring is necessary to maintain the fluoride concentrations attained by placental transfer.

The reports of Blayney and Hill(4), Tank and Storvick (4), Arnold et al.(4) and Feltman and Kosel (4) support the thesis that the combined administration of pre and postnatal fluoride is more effective in inhibiting caries than fluoride administered only postnatally. Only Feltman (4) reported a pronounced cariostatic effect of fluoride when given prenatally alone. No evidence appears to be available that prenatally administered fluoride has any effect on the permanent first molars, the only teeth of permanent dentition showing any evidence of calcification before birth.

Studies in the rat have indicated that fluoride administration up to 25 ppm in the drinking water during gestation and lactation conferred no cariostatic effect on the teeth of the offspring. At a concentration of 40 ppm fluoride, however, some caries reduction was reported.

The enhanced mineralization and reduced solubility of prenatally fluoridated teeth in the rat and the increased fluoride content of deciduous teeth in the human are consistant with the proposition that prenatally administered fluoride in the human may afford some cariostatic effect to the deciduous teeth.

Mammary Transfer of Fluoride in Rat

Fluoride is a natural constituent of human and animal milk (72). The concentration of fluoride in normal human milk ranges from less tha 0.1 ppm to about 0.2 ppm and is little influenced by fluoride supplementation (71). Trace quantities of fluoride in milk are bound to fat and to the albumin-glabulin fraction, whereas casein contains about one-fourth of the total fluoride in the whole milk (72). Fluoride in milk is not completely diffusible. Fluoride administered in milk of rats and humans has been found to be absorbed slightly more slowly than when given in water, but ultimately fluoride in milk is absorbed completely.

Wallace-Durbin (33) found that the mammary tissue of rats contained concentrations of ^{18}F roughly equivalent to those in the blood. In a definitive study, Murrey (71) fed about 225 ppm fluoride in the pregnant rats. Within 24 hours after birth, offspring of these fluoride fed mothers were exchanged with the offspring of mothers receiving fluoride free diet. Then the offspring of fluoride-free fed rats were transferred to the mothers who were exposed to fluoride during pregnancy. Markedly more fluoride in the ashed carcass of the offspring of fluoride-free fed mother was found as compared to appropriate offspring. This study clearly indicated the mammary transfer of fluoride.

The excretion of fluoride in milk has been shown to parallel the blood concentrations of fluoride but the levels are considerably lower than those in

the blood. So little fluoride is excreted naturally into milk that it does not seem to serve as a natural source for proving fluoride in concentrations effective against tooth decay.

Methods of Fluoride Analysis

Analytical methods for the determination of fluoride, such as volumetric (32), conductimetric (39), potentiometric (51), complexometric (40), gravimetric (32), and colorimetric (16), have been reported. The most widely accepted analytical methods for fluoride determination are colorimetric. The greatest difficulty using these methods is the interference by foreign ions (37) which can also form stable complexes with either the metal or the fluoride ions present. When these methods are used for biological materials, fluoride must be separated from other elements to avoid these interactions. Thus the accuracy of such methods can be severely hindered by incomplete separations and there are significant reagent and apparatus blanks. For these reasons, the existing methods are less than desirable.

In recent years, the development of specific ion electrodes has stimulated growth in the field of analytical potentiometry. Specific ion electrodes measure the single ion activity of an ion or a class of ions, in solution, rather than the concentration of the ion. The activity of an ion in solution is a measure of the reactivity of the ion. In dilute solutions, the activity of an ion approaches the concentration. In many cases, the activity is proportional to the concentration, allowing the electrode to be calibrated in terms of concentration. Like the

glass electrode, the specific ion electrode develops an electric potential in response to the activity of the ion for which the electrode is selective. The relationship between the ionic activity and electrode potential is logarithmic as shown by the Nernst equation:

$$E = Ea + 2.3 \frac{RT}{nF} \log A$$

where E equals the measured electrical potential of the system; Ea equals that portion of the total potential due to choice of reference electrode and internal solutions; $2.3 \frac{RT}{nF}$ equals the Nernst Factor (59.16/n mv at 25° C) in which R and F are constants, n is the charge on the ion, and T is the temperature in degrees Kelvin; and A equals the activity of the ion in the sample solution.

When the ionic activity increases, the electrode potential becomes more positive if the electrode is sensing a cation and more negative if the electrode is sensing an anion. For a ten fold change in ionic activity, the electrode potential (at 25°C) changes by 59.2 mv if the ion being measured is monovalent, and 29.6 mv if the ion being measured is divalent (3).

Fluoride ion-selective electrode
Its construction and operation

Frant and Ross (23) announced the invention of a single crystal lanthanum fluoride membrane electrode which is so specific that fluoride ion activity can be measured as easily as pH is measured with a glass electrode.

The fluoride-sensitive membrane of the electrode is a single crystal of Lanthanum fluoride, doped with europium, in the form of a disk about I cm

in diameter and 1 to 2 mm thick, cemented into the end of a polyvinyl chloride plastic tube. The internal solution typically is a mixture of 0.1 M sodium fluoride and 0.1 M sodium chloride, although other concentrations of chloride and fluoride may be employed to adjust the range of operating potential.

The potential of the internal silver-silver chloride electrode is fixed by the chloride ion activity, and the fluoride ion activity controls the potential of the inner surface of the Lanthanum fluoride membrane. When the electrode is immersed in a fluoride solution an electrical potential difference is established across the membrane, and its magnitude depends on the ratio of fluoride ion activities in the inner and outer solutions. The potential of the electrode is measured against a reference electrode (usually a saturated calomel electrode), in a manner exactly analogous to the use of a glass electrode. Unlike the glass electrode, the fluoride electrode requires no conditioning in water before use.

The fluoride ion electrode was described as being highly selective for the fluoride ion and was found to be insensitive to a thousand fold excess of common anions such as chloride, bromide, iodide, carbonate, nitrate, sulphate and phosphate (65). In solutions which contained high concentrations of various electrolytes and non-electrolytes, the observed shift in electrode potential was attributed to changes in the solution's ionic strength. The ionic strength of the test solution was found to be important because the electrode sensed the activity of the fluoride ion and not its concentration. The pH of the test solution had an effect on observed potential. At low pH values the fluoride

would exist as a hydrogen fluoride complex. The pKa of hydrogen fluoride is 3.14. Therefore, it was recommended that the pH of the test solution should be adjusted to at least 5.2 to assure the dissolution of this complex (1). The hydroxide ion also caused interferences on the sensing membrane (23). Significant interference was found when the hydroxide concentration about equalled the fluoride concentration. A ten fold excess of hydroxide would double the apparent fluoride content (23). This did not present a problem as long as the pH was less than 8 at 10^{-6} M fluoride or the pH could be as high as 12 at 0.1 M fluoride (41). Other ions besides hydrogen, which were found to form stable complexes with fluoride in solution, were trivalent aluminium and iron. Fluoride ions were freed from these complexes by the addition of sodium citrate or other chelating agents (15, 16). The temperature of the solution would affect the slope of the electrode response as predicted by the Nernst equation. By controlling the temperature $\pm$ 1° C it was possible to achieve a precision of $\pm$ 1 mv in the electrode potential (1).

Many of the above variables which affected the electrode response could be compensated for by the use of a buffer formulation which when mixed 1:1 with samples and standards would adjust the pH to acceptable limits, binding interfering ions and provide a high background ionic strength. One of these buffers is called total ionic strength adjustment buffer (TISAB) (24) and has been widely used in fluoride analysis.

The sensitivity range of fluoride electrode is from saturated solution to

10^{-6} M solution. Direct measurement of fluoride should be done preferably in the pH range of 5 - 8.

There are two methods of fluoride analysis using the fluoride ion electrode: 1. calibration curve method, and 2. known addition method (3).

In the calibration curve method, a series of standardizing solutions is prepared by serial dilution and their electrode potentials are recorded. A calibration curve is prepared by plotting the electrode potentials against concentration on semi-logarithmic graph paper. A good calibration curve has a straight line relationship between potential and concentration. Potential readings of unknown solutions can be translated into concentrations from the curve. An ionic strength adjustor (ISA)/pH adjustor is used before reading the potentials of standards and unknown solutions.

The known addition technique eliminates the time required to prepare a calibration curve and an use of ISA/pH adjustor is not required. A known amount of the species being measured is added to a known volume of sample and the resulting change in potential observed. The original sample concentration can now be computed using a known addition table (3).

METHODS AND MATERIALS

Twenty-four Sprague-Dawley female rats, weighing 125-132 g. were housed individually in stainless steel wire-mesh cages and were kept in a temperature controlled room (25 C). The rats were divided into three groups of eight rats each and received 0, 1 or 5 ppm of sodium fluoride in their drinking water. Both water and non-pellet Purina Rat Chow were offered at libitum to all the groups. The rats were group housed during mating. The rats received the fluoride water treatments during mating, pregnancy and lactation. Mother rats, after weaning the pups, were killed and their upper and lower incisors and femurs were excised and cleaned.

One pup from each litter was killed at birth. Another pup was killed at 21 days of age for femur and teeth collection. The remaining 21 day old pups were divided into nine treatment groups of eight rats in each group. Approximately one centimeter long section of sciatic nerve in the left leg, at groin level, was removed to produce disuse osteoporosis in one leg of each rat in all groups. The following water fluoride treatments were given to these pup groups: 0-0, 0-1, 0-5, 1-0, 1-1, 1-5, 5-0, 5-1, and 5-5, where the first number indicates the fluoride level (ppm) of the water available to the mother during mating, pregnancy and lactation. The second number indicates the fluoride level (ppm) of water given to the offspring. Offspring received the above treatments until they weighed about 300 g. Then the rats were killed by decapitation and femurs and incisor teeth collected.

Whole carcasses of pups killed at birth were autoclaved and ground separately and then were used for ash, calcium and phosphorus determinations. Femurs and teeth of mothers and 300 g. pups were dried at 100 C overnight and their weights were recorded. Bone strength of femurs of mothers and 300 g. rats was determined. Whole carcasses of weaning pups and femurs of mother rats and 300 g. pups were ashed at 500-600 C overnight in a muffle furnace in acid boiled crucibles.

Calcium Analysis

Ashed samples of carcasses and femurs were solubilized in 5 ml of 6 N hydrochloricacid. Then the crucibles were filled 3/4 full with distilled water and heated to boiling for about five minutes. After cooling, ash solution was brought to 100 g. with deionized water in acid boiled bottles. A 0.5 ml aliquot of this solution was brought to 100 g. with a 10,000 ppm solution of strontium chloride. The samples were analyzed for calcium using appropriate calcium standards on a Varion Techtron AA120 atomic absorption sectrophotometer at 422.7 μm.

Phosphorus Analysis

A 20 g aliquot of ash solutions from the carcasses and femurs were used. Phosphorus determinations were done by the colorimetric method of Gomori (29a).

Fluoride Analysis

For bone fluoride analysis, orion model 94-09 fluoride ion electrode and a calomel sleeve type reference electrode were used with a Corning Model 12

research pH meter. Expanded scale of the pH meter was used to obtain readings proportional to the milivoltage output of the electrode. Fluoride content of bones and teeth were determined using a calibration curve, which was constructed on a semi-logarithonic paper. Electrode potentials of standard solutions were measured and plotted on the linear axis against their concentrations on the log axis. A new standard curve (range 0.1-10 ppm fluoride) was made every day before doing the fluoride analysis. Standard fluoride solutions were made in total ion strength adjusting buffer (TISAB)*. Polypropylene ware was used for all the fluoride work.

Thirty miligram of ground femur in 5 ml of 18 percent perchloric acid was digested for one hour. Then 15 ml of TISAB was added to this mixture. The acidity of the mixture was adjusted to be between pH 5 and 6 using 6 mls of 0.5 M NaOH before fluoride determinations were made. Samples were continuously stirred, to reach equilibrium between electrode and mixture fluoride ions faster. Milivolt readings of samples were taken at eight minute intervals. Between readings the electrode was rinsed in TISAB for five minutes. Care was taken to prevent any air bubbles from adhering to the electrode membrane, while the electrode was being positioned into the sample solution.

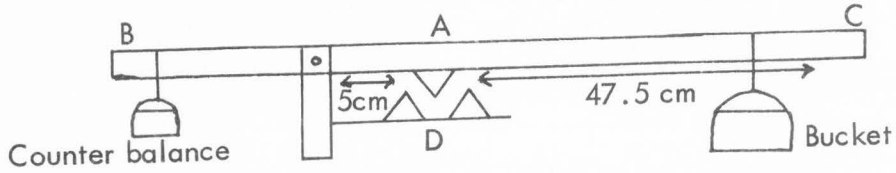
For tooth fluoride analysis, orion model 96-09 combination fluoride ion electrode was used since this model is more sensitive to lower fluoride concentrations as found in teeth. Incisors were grounded in a diamond mortar to a fine powder, which was then quantitatively transferred to a 5 cc polypropylene test

*See appendix for preparation

tube. Between 20-28 mg of ground tooth was digested in 1 ml of 0.5 M perchloric acid for one hour. Acid digestion was stopped by adding 4 ml of 0.5 M trisodium citrate buffer to each sample. Fluoride standards (range 0.019-1.9 ppm) were made fresh in water using orion standard fluoride solution. Milivolt readings were recorded for five minutes after insertion of electrode with continuous stirring. Between the readings the electrode was rinsed with citrate buffer for three minutes.

Bone Strength Determination

Bone strength was determined by using the following device:



Bones were placed upon blades (D) and then the arm (BC) was lowered to put blade (A) over the bone. Sand was slowly poured in the bucket which hung on end (C) of the arm, until the weight became enough that the blade (A) would break the bone. An attempt was made to position the bone in approximately the same direction and location over the blades (D). The breaking strength was calculated from the distance of the arm from the fulcrum (5 cm), the positioning of the weight (47.5cm) at a distance from the fulcrum, and from the amount of weight required to break the bone.

Kg breaking strength = (47.5/5) (Kg to break bone)

The data were statistically processed by analysis of variance, unpaired t test and least significant difference method (63).

RESULTS AND DISCUSSION

Data are presented under four main groups: (A) Mother rats, (B) pups at birth, (C) 21 day old pups, and (D) 300 g. pups. Bone and teeth data are presented and discussed under each group.

Water fluoride levels (0, 1 and 5 ppm) used in this study were lower than normally used. There have been few studies (54, 13, 47) documenting the effect of such low levels of water fluoride on rat bones. Since there is no known study to date on the effect of water fluoride on rat incisors at these levels (0, 1, 5 ppm), no direct comparison of the data of the present study with the published work is possible. Although growing rats adapt themselves to the continuous ingestion of low levels of fluoride by excreting greater and greater proportions of the ingested fluoride in the feces and urine (39a), significant effects on bone and teeth characteristics were observed at the 1 ppm water fluoride level in this study.

A. Mother Rats

There were no significant differences in dry weight, percent ash, calcium, phosphorus or breaking strength of femurs of mother rats due to water fluoride treatments (Table 3). Since these mothers were pregnant and later lactating, the bone mineral was being deposited and used for fetuses and pups, leading to no differences in the above parameters. These results are in agreement with those

Table 3. Effect of fluoride treatment on femur characteristics of mother rat*

Water fluoride (ppm)	Dry weight (g.)	Ash %	Calcium %	Phosphorus %	Bone strength (Kg/g.)
0	$0.59_{(12)}$	$61.2_{(12)}$	$22.6_{(12)}$	10.5	$46.5_{(10)}$
1	$0.49_{(8)}$	$61.6_{(8)}$	$23.8_{(8)}$	10.6	$35.2_{(8)}$
5	$0.53_{(9)}$	$60.1_{(9)}$	$22.6_{(9)}$	10.7	$40.6_{(8)}$

*No significant differences were found. See Table 19 for ANOV
The values in ( ) indicate number of femurs

of Saville (59) and Deshmukh et al. (13). Water fluoride treatments resulted in significant increase in incisor fluoride content of mother rats (Table 4) but incisor weight was not affected. Lawrenz et al. (39a) have also reported increases in bone and teeth fluoride concentration in rats given 3, 6 and 12 ppm fluoride in drinking water.

B. Pups at Birth

Statistical analysis of body dry matter weight, body ash, body phosphorus and body calcium content of pups killed at birth did not show significant differences due to fluoride treatments (Table 4). This is possible because of a general lack of mineralization in the pups. It also appears that the placentae would have controlled the transfer of bone mineral from the mothers resulting in no differences in carcass mineral contents of the pups.

C. 21 Day Old Pups

Fluoride intake had no effect on dry weight and calcium or phosphorus concentrations of the pup femurs (Table 5). Messer et al. (47) also did not find differences in the weight of pups or the ash, calcium and phosphorus contents of the calvaria of weaning pups, whose mothers were given water containing 50 or 100 ppm fluoride. They concluded that a high maternal fluoride intake does not appear to result in a high fluoride content in the milk, and direct provision of fluoride to the offspring is necessary to maintain the fluoride concentration attained by placental transfer. Theuer et al. (64) also did not observe the

Table 4. Effect of fluoride treatment on incisor weight (g.) and fluoride content (mg/g.) from mother rats

Water fluoride (ppm)	Tooth weight (ppm)	Tooth fluoride (mg/g.)
0	0.07 (16)	0.155 (16)
1	0.14 (16)	0.178 (16)
5	0.08 (10)	0.232 (10)
LSD 0.05/0.01	NS	0.057/0.077

The values in ( ) indicate the number of incisors

Table 5. Effect of prenatal fluoride treatment on body composition of rat pups at birth

Water fluoride (ppm)	Body dry matter weight (g.)	Body Ash %	Body Calcium %	Body Phosphorus %
0	1.01 (14)	13.3	1.9	1.3
1	1.02 (14)	13.2	2.1	1.5
5	0.97 (12)	13.4	2.3	1.5

There were no significant differences in the values in above table
The values in ( ) indicate number of pups

transfer of significant amounts of fluoride from mother rats to their offspring, when the rats were given 50, 100 or 200 ppm of fluoride in the diet. Bone data of the present support the findings of Messer et al. (47).

D. 300 g. Pups

Denervated femurs in 0-0, 1-5 and 0-5 treatment groups were found to be heavier than the corresponding intact femurs (Table 6). Significant differences were observed in femur weights among 0-0, 0-5 and 1-5 treatment groups due to post-weaning administration of 0, 1 and 5 ppm levels of fluoride in water (Table 6). Pre-weaning fluoride treatments (0, 1 and 5 ppm) also produced significant weight differences in femurs. Pre-weaning treatment of 1 ppm fluoride had marked effects on bone weight as compared to 0 and 5 ppm fluoride levels (Table 8).

Post-weaning fluoride treatments resulted in significant differences in femur ash content. One ppm fluoride level had marked effects on the ash content (Table 9).

No significant differences in femur calcium and phosphorus percentage were observed due to different water fluoride treatments (Tables 10 and 11). Deshmukh et al. (13) also reported similar findings in growing male rats given diets containing 0.2 and 200 ppm fluoride.

The denervated femurs were found to be stronger than corresponding intact ones in 5-1 and 5-5 treatment groups (Table 12). However, Riggins et al. (55),

Table 6. Effect of preweaning fluoride treatment on femur characteristics of 21 day old rat pups

Water fluoride (ppm)	Dry weight (g.)	Ash %	Calcium %	Phosphorus %
0	0.17 (4)	43.65	14.02	6.72
1	0.21 (7)	45.47	14.01	6.87
5	0.19 (6)	43.60	14.85	7.28

There were no significant differences in the values in above table
The values in ( ) indicate number of pups

Table 7. Effect of fluoride treatment on incisor weight (g.) and fluoride content from 21 day old pups

Water fluoride (ppm)	Tooth weight (ppm)	Tooth fluoride (mg/g.)
0	0.02 (6)	0.034 (6)
1	0.01 (13)	0.021 (13)
5	0.01 (13)	0.033 (13)

There were no significant differences in the values in above table
The values in ( ) indicate the number of incisors

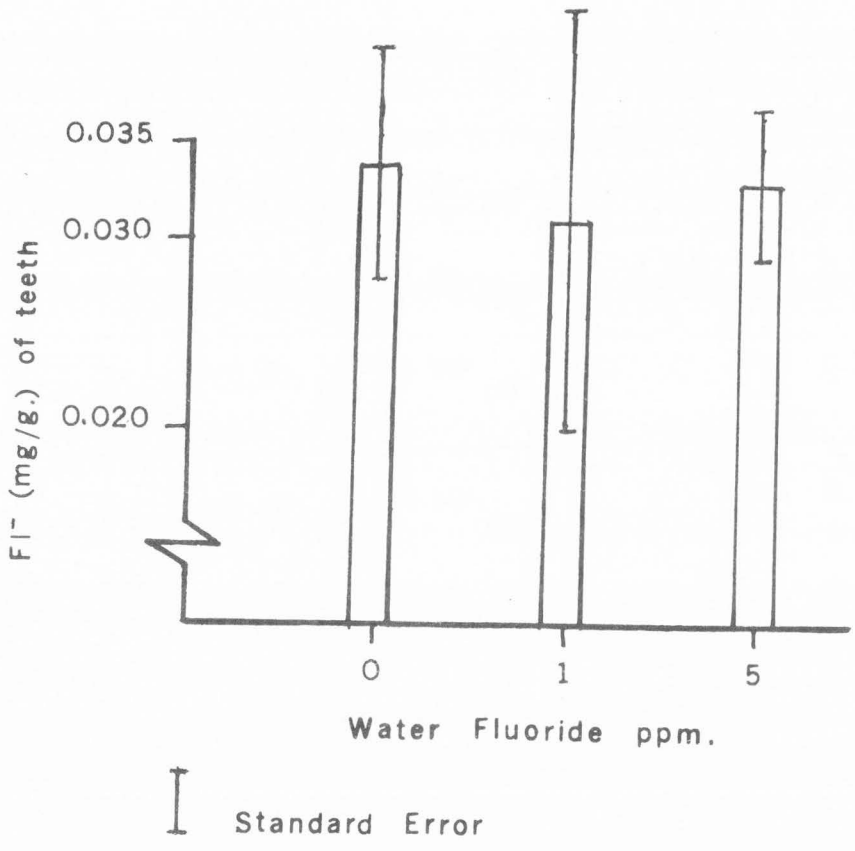


Figure 4. Effect of pre/post weaning water fluoride treatment on the fluoride content of incisors from 21 day old rat pups

Table 8. Effect of pre and post-weaning fluoride treatment on intact and denervated femur dry weight (g.) from 300 g. female rats

Water fluoride (ppm)		Intact	Denervated	Prewean means	Postwean means**
Prewean	Postwean				
0	0	0.65 (3)	0.77* (4)	0.65[b] (20)	0.72[a] (7)
	1	0.54 (4)	0.63 (3)		0.58[b] (7)
	5	0.58 (3)	0.70 (3)		0.65[c] (6)
1	0	0.70 (4)	0.74 (7)	0.72[a] (27)	0.72 (11)
	1	0.71 (2)	0.75 (6)		0.74 (8)
	5	0.65 (3)	0.73* (5)		0.70 (8)
5	0	0.60 (5)	0.68* (5)	0.66[b]	0.64 (10)
	1	0.62 (4)	0.72 (4)		0.67 (8)
	5	0.65 (4)	0.68 (4)		0.67 (8)
Bone means		0.64 (32)	0.72 (41)		

*Significantly higher value than corresponding value ($p < 0.05$)
The values in ( ) indicate number of femurs
Means having different superscripts differ significantly ($p < 0.05$)
**Within prewean fluoride treatment

Table 9. Effect of pre and post-weaning fluoride treatment on intact and denervated femur ash percent from 300 g. female rats

Water fluoride (ppm)		Intact	Denervated	Prewean mean	Postwean mean**
Prewean	Postwean				
	0	34.2 (3)	33.7 (4)		33.9[b] (7)
0	1	35.0 (4)	37.9 (3)	34.8 (20)	36.2[a] (7)
	5	34.4 (3)	34.1 (3)		34.3[b] (6)
	0	34.6 (4)	34.6 (7)		34.5 (11)
1	1	34.1 (2)	36.0 (6)	34.6 (27)	35.5 (8)
	5	33.8 (3)	34.1 (5)		34.0 (8)
	0	34.0 (5)	34.7 (5)		34.3 (10)
5	1	35.0 (4)	34.9 (4)	34.8 (26)	34.9 (8)
	5	36.5 (4)	34.1 (4)		35.3 (8)
Bone means		34.7 (32)	34.8 (41)		

The values in ( ) indicate number of femurs
Means having different superscripts differ significantly ($p < 0.05$)
**Within prewean fluoride treatment

Table 10. Effect of pre and post-weaning fluoride treatment on intact and denervated femur calcium percent from 300 g. female rats

Water fluoride (ppm) Prewean	Postwean	Intact	Denervated	Prewean means	Postwean means**
0	0	30.5 (3)	27.5 (4)	26.8 (20)	28.8 (7)
	1	25.6 (4)	25.0 (3)		25.4 (7)
	5	25.6 (3)	26.9 (3)		26.2 (6)
1	0	24.9 (4)	26.9 (7)	26.2 (27)	26.2 (11)
	1	27.5 (2)	26.4 (6)		26.7 (8)
	5	27.6 (3)	24.7 (5)		25.8 (8)
5	0	26.3 (5)	26.4 (5)	25.3 (26)	26.9 (10)
	1	24.5 (4)	24.0 (4)		24.3 (8)
	5	23.4 (4)	25.2 (4)		24.3 (8)
Bone means		26.0 (32)	26.0 (41)		

The values in the above tables are not significant
The values in ( ) indicate number of femurs
**Within prewean fluoride treatment

Table II. Effect of pre and post-weaning fluoride treatment on intact and denervated femur Phosphorus percent from 300 g. female rats

Water fluoride (ppm) Prewean	Postwean	Intact	Denervated	Prewean means	Postwean means**
	0	12.2 (3)	12.0 (4)		12.1 (7)
0	1	11.7 (4)	12.1 (3)	12.0 (20)	11.9 (7)
	5	12.4 (3)	11.4 (3)		11.9 (6)
	0	12.0 (4)	11.5 (7)		11.7 (7)
1	1	12.5 (2)	11.7 (6)	11.7 (27)	11.9 (8)
	5	11.13 (3)	12.2 (5)		11.7 (8)
	0	12.38 (5)	12.0 (5)		12.2 (10)
5	1	11.47 (4)	12.2 (4)	12.0 (26)	11.8 (8)
	5	11.82 (4)	12.4 (4)		12.1 (8)
Bone means		11.9 (32)	14.1 (41)		

The values in the above tables are not significant
The values in ( ) indicate number of femurs
**Within prewean fluoride treatment

Table 12. Effect of pre and post weaning fluoride treatment on intact and denervated femur breaking strength (Kg/g.) from 300 g. female rats

Water fluoride (ppm)		Intact	Denervated	Prewean means	Postwean means**
Prewean	Postwean				
0	0	24.3 (3)	24.8 (4)	24.8 (20)	24.6 (7)
	1	24.2 (4)	24.6 (3)		24.3 (7)
	5	25.5 (3)	25.9 (3)		25.7 (6)
1	0	21.9 (4)	23.5 (7)	23.6 (27)	22.9 (11)
	1	23.5 (2)	24.5 (6)		24.2 (8)
	5	23.5 (3)	24.3 (5)		24.0 (8)
5	0	24.3 (5)	22.9 (5)	24.3 (26)	23.6 (10)
	1	23.6 (4)	26.5* (4)		25.1 (8)
	5	23.2 (4)	25.4* (4)		24.3 (8)
Bone means		23.8 (32)	24.5 (41)		

*Significantly higher value than corresponding value ($p < 0.05$)
The values in ( ) indicate number of femurs
**Within prewean fluoride treatment

Nordenberg et al. (50), Deshmukh et al. (13) and Saville (59) did not find any effect of 50, 100, 2, 5, and 20 ppm of fluoride on bone strength of rats.

Pre-weaning fluoride intake at 1 and 5 ppm levels was found to increase the fluoride content in femurs. Highly significant effects were observed in fluoride concentration at 5 ppm fluoride intake (Table 13).

Post-weaning water fluoride in 1-0, 1-1 and 1-5 treatment groups also resulted in higher fluoride content, but the effect was more prominent in 1-5 treatment group (Table 13).

Disuse atrophy of bone is characterized by a reduction of the mass of bone tissue accompanied by hypercalcemia and increased excretion of calcium in the urine (25). Gedalia et al. (25) found a reduction in specific gravity, the ash content calculated on volume basis and calcium content in disuse atrophy of the rat femur. In this report, young growing rats were given no fluoride and 25 ppm fluoride in drinking water and disuse atrophy was induced by sectioning the sciatic and femoral nerves. These workers also reported the beneficial effects of fluoride in mitigating the development of disuse atrophy in rats, based on specific gravity and ash content of bones. However, Menczel et al. (49a) did not find any effect of fluoride or calcium on ash, calcium and phosphorus contents of femur of rats given 50 ppm fluoride in water for 320 days, maintained on both low calcium (0.1 percent) and adequate calcium (0.8 percent) diets. Deshmukh et al. (13) also did not observe any changes in ash, calcium and phosphorus content of femurs of rats, given 200 ppm fluoride in diet containing adequate calcium.

Table 13. Effect of pre and post-weaning fluoride treatment on intact and denervated femur fluoride content (mg/g.) from 300 g. female rats

Water fluoride (ppm)				Prewean means	Postwean means**
Prewean	Postwean	Intact	Denervated		
0	0	25.6 (3)	30.3 (4)	32.2[a] (20)	28.2 (7)
	1	33.2 (4)	34.3 (3)		37.7 (7)
	5	34.3 (3)	36.1 (3)		35.2 (6)
1	0	32.9 (4)	34.4 (7)	37.4[b] (27)	34.8[a] (11)
	1	38.1 (2)	34.4 (6)		35.3[a] (8)
	5	39.3 (3)	45.2 (5)		43.0[b] (8)
5	0	38.9 (5)	28.8 (5)	40.2 (13)	33.9 (10)
	1	39.9 (4)	43.7 (4)		41.8 (8)
	5	46.8 (4)	46.2 (4)		46.6 (8)
Cumulative	0				32.8 (29)
	1				37.1 (23)
	5				42.2 (22)
Bone means		36.9 (32)	37.0 (41)		

Means having different superscripts differ significantly ($p \leq 0.05$)
The values in ( ) indicate number of femurs
**Within prewean fluoride treatment

When data of the present study are compared with the one reported by Gedalia et al. (25), it was obvious that disuse atrophy was not induced in denervated femurs. There was an increase (instead of decrease) in the bone mass of the femurs in 0-0, 1-5 and 5-5 treatment groups. Similarly these denervated femurs had higher (instead of lower) bone strengths than the corresponding intact femurs in 5-1 and 5-5 treatment groups (Table 12). No differences in ash and calcium content of intact and denervated femurs were found, while Gedalia et al. (25) have reported a decrease in ash and calcium content in disuse antrophic femurs of rats.

These contrasting findings may have been caused either by incomplete denervation or the left femurs were still in use. Rats were observed putting their weight on the distal end of femur while standing. Increase in weight of denervated femurs could have resulted from induced hypertrophy of the femur. In these denervated rats the whole weight of the hind body parts was put on the distal end of the femur, while the femurs from the intact rats had the advantage of more uniform weight distribution on the bones due to a leverage effect, i.e. their weight was evenly distributed on femurs, tibia, fibula and metatarsals, instead of on the femur alone. Gedalia et al. (25) severed both sciatic and femoral nerves to induce disuse atrophy in rats while only the sciatic nerve was removed for the present study.

Then it was decided to pool the bone data assuming that both left and right femurs were intact and the statistical analysis was done on different bone parameters (Table 14).

Table 14. Effect of pre and post-weaning fluoride treatment on characteristics of femur (assuming both intact) from 300 g. female rats

Water Pre-wean	Fluoride post-wean	Numbers of femurs	Dry bone wt. (g.) *	Ash %	Calcium %	Phosphorus %	Bone strength g./kg	Fluoride mg/g. *
	0	7	0.72	33.9	28.8	12.1	24.6	28.2
0	.1	7	0.58	36.1	25.4	11.9	24.3	33.7
	5	6	0.64	34.3	26.3	11.9	25.7	35.2
	0	11	0.72	34.6	26.2	11.7	22.9	34.8
1	1	8	0.74	35.5	26.7	11.9	24.3	35.3
	5	8	0.70	34.0	25.8	11.7	24.0	43.0
	0	10	0.64	34.4	26.4	12.2	23.6	33.9
5	1	8	0.67	35.0	24.3	11.8	25.1	41.8
	5	8	0.67	35.3	24.3	12.1	24.3	46.6

*Significant differences among treatment groups

Significant differences were found in weights of the femurs among all the nine treatment groups. However, when comparisons were made within treatment groups, differences were observed in 0-0, 0-1 and 0-5 groups only. Differences in bone weight due to pre-weaning water fluoride treatment were observed among all the groups, but no significant differences were found among post-weaning treatment groups within the preweaning treatment groups (Tables 15 and 16). Pre-weaning water fluoride at 1 ppm level had a marked effect greater than the 5 ppm fluoride level.

Pre and post-weaning water fluoride treatments did not affect the ash, calcium and phosphorus contents and bone strength; however, marked differences were observed in fluoride content among the nine treatment groups. Highest fluoride concentration in 5-5 treatment group, followed by 1-5, 5-1, 1-1, 0-5, 5-0, 0-1 and 0-0 groups (Tables 15 and 16). Pre and post-weaning fluoride treatments resulted in significant differences in femur fluoride contents in that 5 ppm fluoride level caused a higher fluoride concentration as compared to the 1 ppm level.

Combined effect of both pre and post-weaning administration of fluoride resulted in higher fluoride content as compared to only pre-weaning effect indicating the beneficial effects of postnatal administration of fluoride. Lawrenz et al. (39a) have reported that by increasing the fluoride concentration in the diet (3, 6 and 12 ppm) in growing rats, the percent retention of fluoride was also increased. Observations in the present study follow the same trend (Tables 15 and 16, Figure 2).

Table 15. Effect of pre-weaning fluoride treatment on bone weight and fluoride content of femurs from 300 g. female rats

Water fluoride (ppm)	Bone weight (g.)	Bone fluoride (mg/g.)
0	0.64^{a} (100)	32.2^{a} (100)
1	0.72^{b} (112)	37.4^{b} (116)
5	0.66^{a} (103)	40.2^{c} (124)

Values having different superscripts differ significantly ($p < 0.05$)
Values in ( ) indicate effects relative to control group (0-0)

Table 16. Effect of post-weaning fluoride treatment on bone weight and fluoride content of femurs from 300 g. female rat

Water fluoride (ppm)	Bone weight (g.)	Bone fluoride (mg/g.)
0	0.70 (100)	32.8^{a} (100)
1	0.67 (116)	37.1^{b} (113)
5	0.67 (124)	42.2^{c} (128)

Values having different superscripts differ significantly ($p < 0.05$)
Values in ( ) indicate effects relative to control group (0-0)

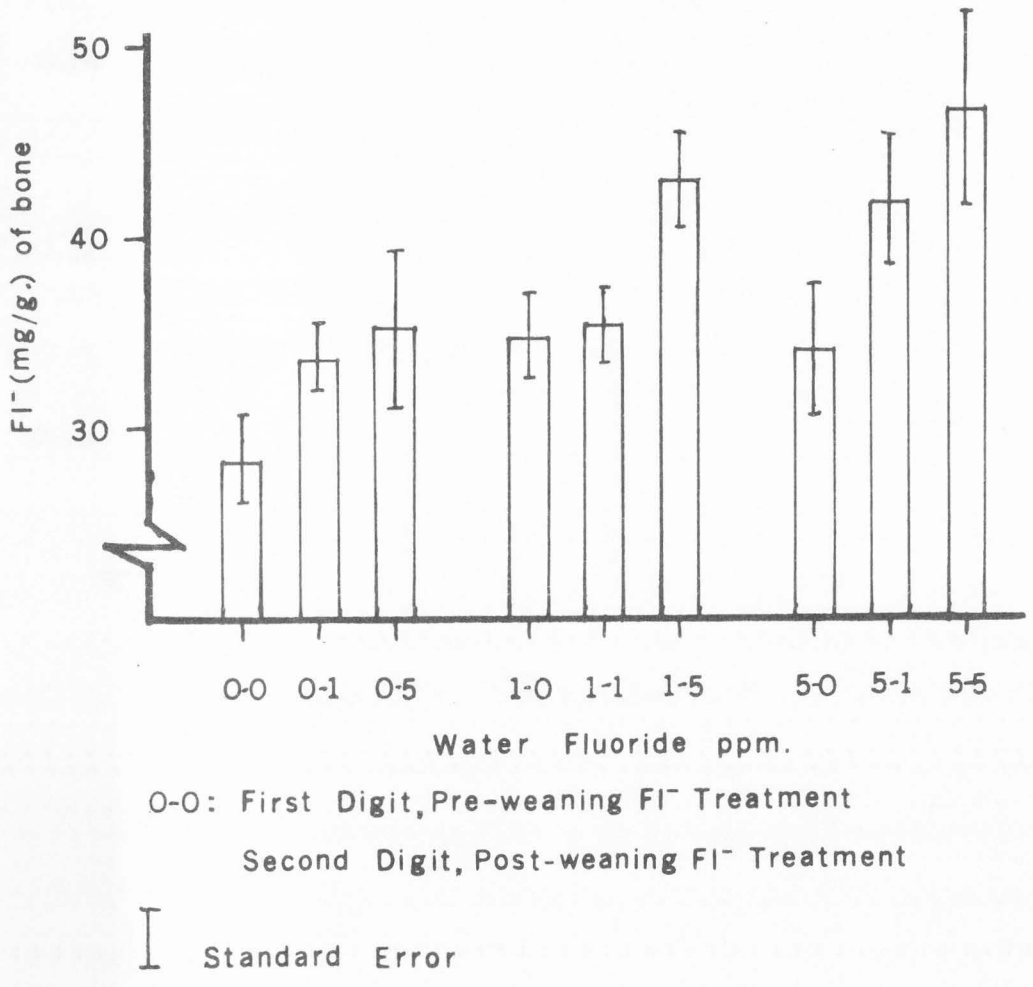


Figure 2. Effect of pre/post weaning water fluoride treatment on the fluoride content of femurs from 300 g. rats

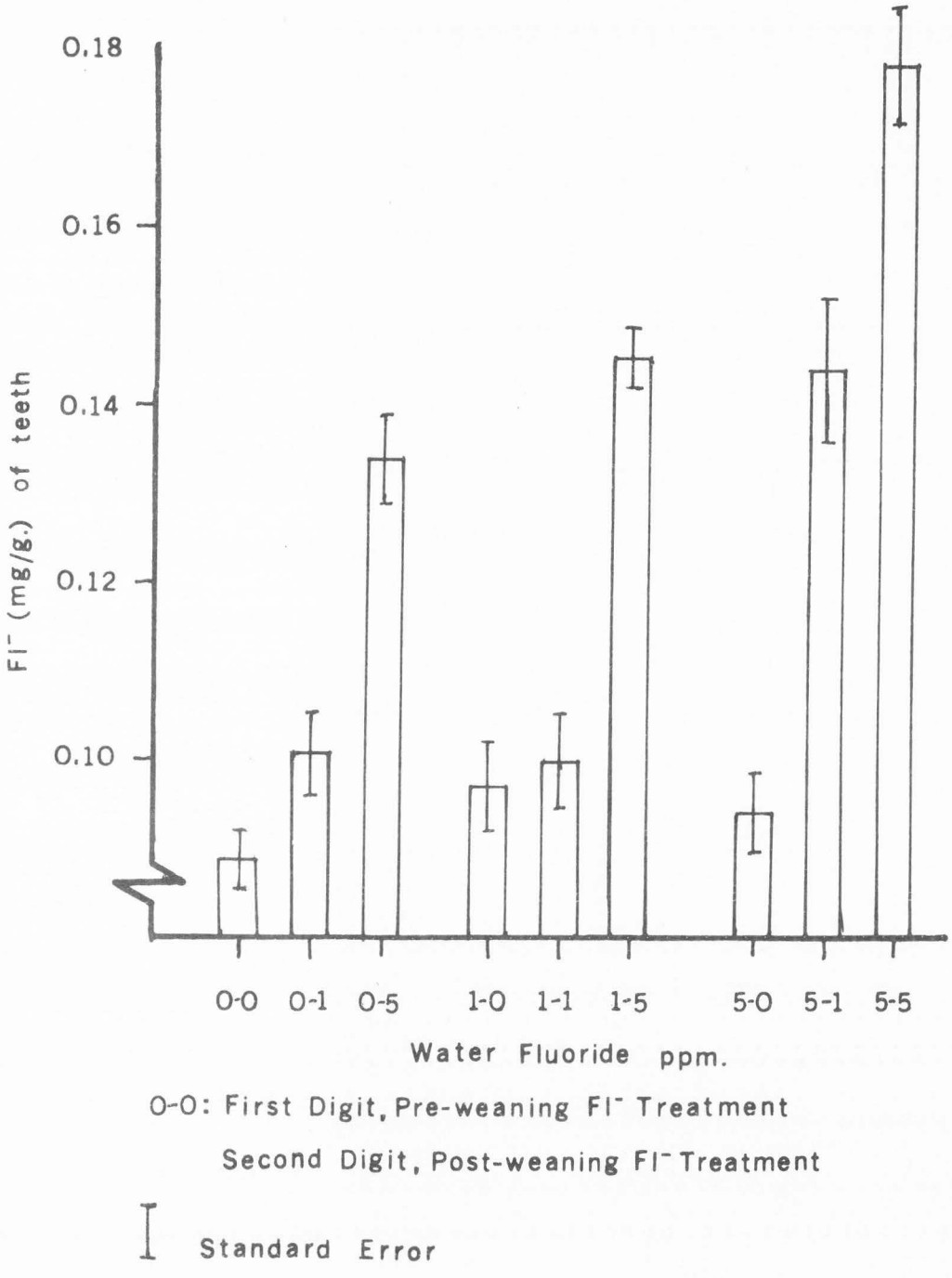


Figure 5. Effect of pre/post weaning water fluoride treatment on the fluoride content of incisors from 300 g. rats

Significantly higher fluoride content in incisors from mother rats receiving 1 and 5 ppm water fluoride were found as compared to control rats (0 ppm) (Table 15, Figure 3). These data indicated that higher fluoride intake may increase the amount of fluoride in mother teeth, but no significant differences were observed in fluoride content of weaning rat incisors (Figure 4). The above data suggest that there was no maternal transfer of fluoride to the offspring. It is also possible that enough fluoride could not be deposited in embryonic teeth because the growth rate of the skeleton of the pups was higher.

Femur fluoride and incisor fluoride data presented in this study support the proposition that pre-weaning fluoride administration alone does not affect the fluoride contents of bone and teeth of rat offspring. It seems that placental transfer of fluoride was ineffective due to low fluoride levels (1 and 5 ppm) used in this study. It has been reported (8, 26) that fluoride intake of over 10 to 15 ppm in food or water by pregnant rats affects the total fluoride content of the pups.

A highly positive correlation (r-0.93) was found between fluoride content of femurs and incisors of 300 g. female rats.

It appears from the bone and teeth fluoride data (Tables 15, 16 and 18, Figures 2 and 5) that increased amounts of water fluoride brought about increases in fluoride contents of femurs and incisors of female rats. Amount of increasing fluoride content of incisors was greater than in the bones. The teeth were more readily affected than the bones, since at the 1 ppm fluoride

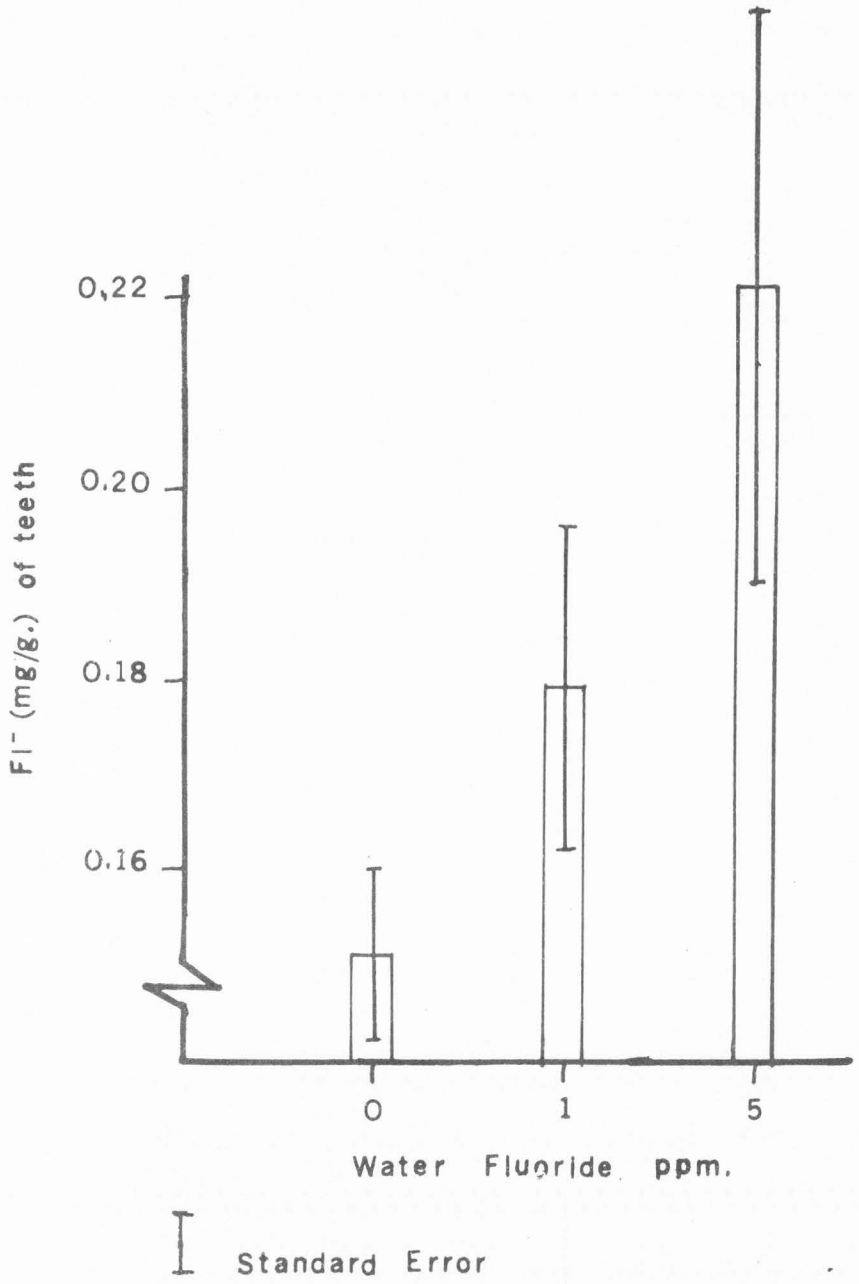


Figure 3. Effect of pre/post weaning water fluoride treatment on the fluoride content of incisors from mother rats

Table 17. Effect of pre and post weaning fluoride treatment on the incisor weight (g.) from 300 g. female rats

Water fluoride (ppm)		Tooth weight (g.)		Prewean means		Postwean means	
Prewean	Postwean						
0	0	0.025	(20)	0.024	(52)	0.023	(80)
	1	0.025	(18)				
	5	0.022	(14)				
1	0	0.027	(40)	0.023	(100)	0.023	(82)
	1	0.023	(36)				
	5	0.034	(24)				
5	0	0.023	(20)	0.023	(63)	0.023	(53)
	1	0.023	(28)				
	5	0.023	(15)				
LSD 0.05/0.01		0.0018/0.0024					

Means having different superscripts differ significantly
The values in ( ) indicate the number of incisors

Table 18. Effect of pre and post weaning fluoride treatment on the fluoride content of teeth (mg/g.) from 300 g. female rats

Water fluoride (ppm)		Tooth fluoride (mg/g.)		Prewean means		Postwean means	
Prewean	Postwean						
	0	0.085	(20)				
0	1	0.102	(18)	0.104^{a}	(52)	0.93^{a}	(80)
	5	0.135	(14)				
	0	0.097	(40)				
1	1	0.099	(36)	0.111^{b}	(100)	0.115^{b}	(82)
	5	0.155	(24)				
	0	0.094	(20)				
5	1	0.144	(28)	0.121^{c}	(63)	0.115^{b}	(53)
	5	0.178	(15)				
LSD 0.05/0.01		0.017/0.022					

Means having different superscripts differ significantly
The values in ( ) indicate the number of incisors

level, femur fluoride content was higher than the teeth, while at the 5 ppm level it decreased. Similar findings have been reported by Lawrenz et al. (39a).

Findings of the present study support the thesis that combined administration of pre and post-weaning fluoride results in increased amounts of fluoride in bone and teeth. Fluoride supplementation before birth, during pregnancy and lactation may act as a primer for the post-weaning effects of fluoride. This effect was most obvious from the teeth data of the present study.

SUMMARY

The effects of fluoride on the femurs and incisors of growing female rats and their offspring were studied. Fluoride levels of 0, 1 or 5 ppm were given in the drinking water. No effect of fluoride intake on bone weight, ash, phosphorus and breaking strength of the mother femurs was observed. Similarly fluoride had no effect on carcass mineral content of pups at birth and bone mineral content of 21 days old pups.

Fluoride at 1 and 5 ppm levels was found to increase the fluoride concentrations of femur and teeth in 300 g. pups. A fluoride level of 5 ppm resulted in a higher fluoride concentration as compared to 1 ppm level. Pre and post-weaning water fluoride treatment did not affect the ash, calcium and phosphorus content and breaking strength of femurs from 300 g. pups.

Combined effect of both the pre and post-weaning fluoride intake resulted in a higher fluoride content of femurs and teeth as compared to only pre-weaning intake indicating that preweaning intake was beneficial.

Fluoride content of mother incisors was significantly higher at 1 and 5 ppm levels but no differences in the fluoride content of weaning rats were found indicating that there was no maternal transfer. It can be concluded that pre-weaning intake of water fluoride by itself is not beneficial, however, pre and post-weaning fluoride treatment would result in higher fluoride concentrations in bone and teeth.

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APPENDIX

Total Ion Strength Adjusting Buffer (TISAB)

To prepare 4 liters of total ion strength adjusting buffer, following chemicals were added in 2 liters of distilled water:

1. 76 g. of Ethylenedinitrilotetraacetic acid (EDTA)
2. 58 g. of Sodium Chloride (NaCl)
3. 57 cc of glacial Acetic acid

Dissolve the ingredients by warming on a hot plate for 15-20 minutes, but do not boil the contents.

Adjust the pH of the mixture to 5.5 with 5M Sodium Hydroxide (NaOH).

Bring the final volume of the mixture to 4 liters by adding distilled water.

Table 19. Analysis of variance for femur dry weight data of mother rats

Source	df	SS	MS	F
Total	28	0.333		
Treatment	2	0.051	0.025	2.39
Error	26	0.281	0.010	

Conclusion: No significant differences in dry weight were found ($p < 0.05$).

Table 20. Analysis of variance for femur ash percentage data of mother rats

Source	df	SS	MS	F
Total	28	255.597		
Treatment	2	9.971	4.985	0.52
Error	26	245.626	9.440	

Conclusion: No significant differences in ash percentage were found ($p < 0.05$).

Table 21. Analysis of variance for femur calcium percentage data of mother rats

Source	df	SS	MS	F
Total	28	143.093		
Treatment	2	7.968	3.984	0.76
Error	26	135.124	5.197	

Conclusion: No significant differences in calcium percentage were found ($p<0.05$).

Table 22. Analysis of variance for phosphorus percentage data of mother rats

Source	df	SS	MS	F
Total	2			
Treatment	2	0.130	0.060	0.16
Error	26	10.590	0.400	

Conclusion: No significant differences in phosphorus percentage were found ($p < 0.05$).

Table 23. Analysis of variance for body weight data of rat pups at birth

Source	df	SS	MS	F
Total	39	3.296		
Treatment	2	0.058	0.029	0.33
Error	37	3.238	0.087	

Conclusion: No significant differences in body weight were found ($p < 0.05$).

Table 24. Analysis of variance for body ash data of rat pups at birth

Source	df	SS	MS	F
Total	40	135.661		
Treatment	2	0.370	0.185	0.052
Error	38	135.291	3.560	

Conclusion: No significant difference in body ash were found ($p < 0.05$).

Table 25. Analysis of variance for body calcium data of rat pups at birth

Soo rce	df	SS	MS	F
Total	39	7.156		
Treatment	2	1.029	0.514	3.10
Error	37	6.127	0.165	

Conclusion: No significant differences in body calcium were found (p<'..0.05)

Table 26. Analysis of variance for body phosphorus data or rat pups at birth

Source	df	SS	MS	F
Total	39	16.726		
Treatment	2	0.376	0.188	0.42
Error	37	16.349	0.441	

Conclusion: No significant differences in body phosphorus were found (p < 0.05).

Table 27. Analysis of variance for femur dry weight data of 21 day old pups

Source	df	SS	MS	F
Total	16	0.108		
Treatment	2	0.005	0.002	0.34
Error	14	0.103	0.007	

Conclusion: No significant differences in femur dry weight were found (p 0.05).

Table 28. Analysis of variance for femur ash percentage data of 21 day old pups

Source	df	SS	MS	F
Total	16	806.084		
Treatment	2	14.12	7.06	0.12
Error	14	791.96	56.56	

Conclusion: No significant differences in femur ash percentage were found ($p < 0.05$).

Table 29. Analysis of variance for femur calcium percentage data of 21 day old pups

Source	df	SS	MS	F
Total	16	24.617		
Treatment	2	2.686	1.343	0.85
Error	14	21.931	1.566	

Conclusion: No significant differences in femur calcium percentage were found ($p < 0.05$).

Table 30. Analysis of variance for femur phosphorus percentage data of 21 day old pups

Source	df	SS	MS	F
Total	16	12.40		
Treatment	2	0.890	0.440	0.54
Error	14	11.510	0.820	

Conclusion: No significant differences were found ($p < 0.05$).

Table 31. Analysis of variance for intact and denervated femur dry weight data from 300 g. rat

Source	df	SS	MS	F
Total	31	0.267		
Treatment	8	0.090	0.011	1.46
Error	23	0.177	0.007	

Conclusion: No significant differences were found in femur weights ($p < 0.05$).

Table 32. Analysis of variance for intact and denervated femur ash percentage data from 300 g. rat

Source	df	SS	MS	F
Total	31	179.210		
Treatment	8	20.316	2.540	0.367
Error	23	158.90	6.90	

Conclusion: No significant differences were found in ash percentage ($p < 0.05$).

Table 33. Analysis of variance for intact and denervated femur calcium percentage data from 300 g. rat

Source	df	SS	MS	F
Total	31	322.00		
Treatment	8	126.517	15.814	1.86
Error	23	195.482	8.50	

Conclusion: No differences were found in calcium percentage ($p < 0.05$).

Table 34. Analysis of variance for intact and denervated femur phosphorus percentage data from 300 g. rat

Source	df	SS	MS	F
Total	31	19.980		
Treatment	8	5.968	0.746	1.22
Error	23	14.011	0.609	

Conclusion: No significant differences were found ($p < 0.05$).

Table 35. Analysis of variance for intact and denervated femur breaking strength data from 300 g. rat

Source	df	SS	MS	F
Total	31	120.660		
Treatment	8	28.557	3.570	0.89
Error	23	92.106	4.004	

Conclusion: No significant differences were found ($p < 0.05$).

Table 36. Analysis of variance for intact and denervated femur fluoride content data from 300 g. rat

Source	df	SS	MS	F
Total	31	4235.000		
Treatment	8	987.862	123.482	0.874
Error	23	3247.580	141.200	

Conclusion: No significant differences were found ($p < 0.05$).

Table 37. Analysis of variance for femur characteristics data of 300 g. rat

Source	df	SS	MS	F
Total	71	0.567		
Treatment	8	0.170	0.0213	3.37
Error	63	0.397	0.006	

Conclusion: Significantly different at $p < 0.05$ level.

Table 38. Analysis of variance for incisor weight data of 300 g. rat

Source	df	SS	MS	F
Total	214	0.0023		
Treatment	8	0.0001	0.00002	2.09
Error	206	0.0021	0.00001	

Conclusion: Significantly different at $p < 0.05$ level.

Table 39. Analysis of variance for incisor fluoride content data of 300 g. rat

Source	df	SS	MS	F
Total	214	381249.84		
Treatment	8	182387.13	22798.4	24.98
Error	206	1881862.70		

Conclusion: Significantly different at $p < 0.05$ and 0.01 level.

VITA

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Master of Science

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