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The Effect of Prenatal Fluoride on Tooth Eruption and Calcification in the Rat Molar

Daniel G. Ellenz
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THE EFFECT OF PRENATAL FLUORIDE
ON TOOTH ERUPTION AND CALCIFICATION
IN THE RAT MOLAR

by

Daniel G. Ellenz

A Thesis submitted to the Faculty of the Graduate School of
Loyola University of Chicago in Partial Fulfillment of the
Requirements for the Degree of Master of Science

September

1980

DEDICATION

To my sons, Mark and Matthew, who could never understand why daddy had to go to school; and to my wife, Cyndi, who made sure I went.

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VITA

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INTRODUCTION

The reduction in dental caries derived from the administration of fluoride has long been recognized. Extensive studies have been carried out concerning the important role played by fluorides in the prevention of tooth decay. These investigations have prompted researchers to examine the effects of prenatal fluoride on the developing tooth.

It is the purpose of this investigation to examine the potential benefits and risks associated with prenatally administered fluoride. The parameters of body weight, total calcium content and calcium concentration per tooth, and tooth eruption will be measured in order to determine the effect of prenatal fluoride on the growing fetus and tooth organ.

REVIEW OF LITERATURE

A. Introduction

Fluoride was brought to the attention of the dental community in an indirect manner. The dentists of the Rocky Mountain area of Colorado were concerned about a peculiar discoloration of children's teeth. This condition was brought to the attention of Black and McKay who initially investigated the problem from 1907 to 1916. Their studies were extensive and the fundamental epidemiological implications of their studies remain unchallenged today; for example, (1) they described the hitherto unknown/unrecognized dental disease -- mottled enamel and (2) they determined that it was a developmental defect.

Some fifteen years after Black and McKay's reports appeared in the literature, their speculation concerning drinking water as the vehicle for the causative agent of mottled enamel was substantiated. Interestingly, although the primary focus of study during this period was on mottled or stained teeth, some authors commented on the status of dental caries in children living in endemic areas. Their observations suggested that the mottled teeth might be less susceptible to disease. This latter effect has proven to be the important one by which fluoride contributes to dental health.

B. Effect of Fluoride on Caries Incidence

Studies of the physiology and toxicology of fluoride received an additional impetus in the late 1930's when it was discovered that fluoride may play a significant role in the reduction of human dental caries and therefore be administered to humans. However, there was concern because the margin of safety of the fluoride supplement that would achieve maximal protection against dental caries with minimal risk of fluorosis of the enamel had not been established.

In 1936, Dean, the Surgeon General of the U. S. Public Health Service, conducted a study in which the fluoride content of a number of cities water supplies were analyzed. On the basis of this work, the Public Health Service established standards for communal water supplies of permissible concentrations of fluoride and later it was determined that the accepted optimum dose they recommended (1.0 ppm F1-) would reduce caries 50-70% without mottling.

In 1946, the Evanston-Oak Park, Illinois study was undertaken by Hill and Blayney. The objective of this investigation was to determine the effect the adjustment of the fluoride content of water, in a range of 1 ppm, would have on the rate of tooth decay observed in school children of fluoridated Evanston, as compared with those living in the fluoride-free, control area of Oak Park. Clinical and radiological examination revealed a reduction in deciduous teeth of the six year old children who were exposed to fluoride during their tooth development. Whereas, the 7 and 8 year old group under investigation did not show a significant reduction in caries.

Hill, Blayney, and Wolf (1952) in their report compared the caries experience rates of the deciduous and permanent teeth of four groups of 6, 7, and 8 year-old Evanston children. The first or baseline group (1946) had no exposure to fluoridated water. The second (1948), third (1950), and fourth (1951) groups were exposed to the fluoridated water from 12 to 22, 35 to 46, and 47 to 58 months, respectively, at the time of examination. As the Evanston water was fluoridated in February, 1947, this study afforded a comparison of dental caries experience rates of children exposed to fluoridated water for various lengths of time. Their results, concerning the deciduous teeth of the 6, 7, and 8 year old children indicated fluctuations of increased and decreased caries experience, but none of any significant difference. The permanent teeth dental caries rate of the 1951 group of 6 to 8 year old children was 46.42 percent lower when compared to the baseline group, thus illustrating a caries inhibitory effect afforded by fluoride to the permanent dentition.

Hayes (1957) in the Grand Rapids, Michigan fluoridation study investigated the post eruptive effects of water fluoridation. Their conclusions based on clinical and bite wing x-ray examination revealed that fluoride has a caries inhibitory effect on first permanent molars that have already erupted when fluoridation was initiated. DMF values were significant at the 0.05 level.

Discontinuation of water fluoridation has also been shown to rapidly lead to an increase caries incidence in erupted teeth. Fluoridation of the water supply of the city of Antigo, Wisconsin, began in June, 1949, and ended in November, 1960. The actions of a militant antifluoridation group brought about its discontinuance. Lemke, et. al., (1970), in an effort to study the effects of water fluoridation

conducted a dental survey of schoolchildren (Kindergarten, second, fourth, and sixth grades) in Antigo shortly before controlled fluoridation was discontinued. DMF rates were comparable to those in other fluoridated areas in the state. Four years after fluoridation was discontinued, and again, just after it was reinstated, additional surveys were made. DMF rates had greatly increased. Results of the 1964 survey indicated children in Kindergarten showed an increase of 92% in the DEF rate, and children in the second and fourth grades showed 183% and 41% increases, respectively, in DMF rates as compared with the 1960 survey. When results were released locally, a resurgence of activity took place among profluoridationists. A referendum was held and fluoride was again added to the public water supply in October, 1965.

These studies, pointing out benefits of certain levels of fluoride ingestion, opened up new areas of interest in fluoride research.

C. Placental Transfer of Fluoride

Following the acceptance of fluoride as a caries reducing agent, and the demonstration that prenatal fluoride was effective, many experimental studies were done concerning the placental transfer of fluoride in the hope of better understanding the manner in which the carious activity is inhibited.

Gardner and others (1952) reported a higher fluoride content of the placenta from women using fluoridated water at the level of 1.0 ppm than from women using fluoride-free water. They did not analyze the tissues of the fetus to determine the fluoride content.

Yudkin, Czerniejewski, and Blayney (1954), examined dental tissues from four stillborn babies whose mothers lived in Evanston, Illinois. The Evanston mothers throughout their gestation used water containing 1.0 ppm of fluoride. They also examined a stillborn baby whose mother lived in a fluoride-free area. Their analysis revealed that the tissues from the Evanston specimens contained four to five times the amount of fluoride found in the one from the fluoride-free area.

In 1955, Feltman and Kosel attempted to correlate the fluoride concentration of fetal blood and placental tissue in a study using fluoride tablets, 2.2 mg sodium fluoride daily during pregnancy. Their results showed the average fetal blood fluoride concentration was 41 $\mu\text{g}/100\text{ ml}$, in the control 17 $\mu\text{g}/100\text{ ml}$. The average placental fluoride concentration in the tablet study group was 111 $\mu\text{g}/100\text{ gm}$; in the control 101 $\mu\text{g}/100\text{ gm}$. There is a marked difference in the amount of fluoride retained in the fluoride group. The average fetal cord blood

fluoride concentration is 250 percent higher than in the control group.

It was also noted that the fluoride was more concentrated at the periphery of the placenta. Two possible reasons were speculated by Feltman and Kosel: (1) Since the calcium content of the placenta is relatively high at the periphery (R. Battin, personal communication to Feltman), the distribution may be merely a chemical manifestation. (2) The placenta may serve as a storehouse and regulator of fluoride. In an attempt to regulate the fluoride from entering the fetal blood supply, the placenta pushes it away from the area of most active maternal-fetal exchange.

The results of this study indicate that the fetal blood level of fluoride can be increased by supplementation. The importance of this statement was difficult to assess because of the unknown optimum concentration of fluoride supplementation.

Feltman and Kosel (1961) following up on their speculations of 1955, presented evidence that prenatal fluoride in tablet form (1.0 mgm CaF_2 , 1.2 mgm NaF, or 0.825 mgm $\text{Na}_2\text{PO}_3\text{F}$) given to pregnant women once daily reaches the fetal tissues metabolically and reduces the caries incidence of their children's teeth. It was noted, in prenatal cases, where the ingestion of dietary fluorides was begun during the first two trimesters of pregnancy, the favorable effect on the incidence of dental caries was quite evident; whereas when it was begun during the last trimester, the effects are not as pronounced.

This may be due to the fact that the element has not been in intimate contact with the forming teeth to become a part of the calcifying structures that form early, and which are in the areas of most susceptibility to caries, normally the occlusal surfaces and contact

points of the deciduous teeth and the beginning of the occlusal surfaces of the first permanent molars.

A delay in the eruption of teeth, in some cases by as much as a year from the accepted eruption dates was noted by Feltman and Kosel in this study but no data was presented to substantiate this finding.

In 1961, results from Gedalia's study in Israel, indicated that pregnant women drinking water with a fluoride concentration of .55 ppm showed more fluoride in the placenta (.15 ppm) than in the cord (.11 ppm) or maternal blood (.09 ppm) thus suggesting an active role in the accumulation and transfer of fluoride to the fetus.

In 1964, the role of the human placenta was further investigated, by Gedalia and others, when the fluoride intake during pregnancy from drinking water containing low amounts of fluoride (0.06 - 0.15 ppm) was compared to high amounts of fluoride (0.6 - 0.9 ppm). Tissue analysis revealed the mean fluoride values for the low intake group to be: placenta (0.121 ppm F), cord blood (0.165 ppm F), and maternal blood (0.150 ppm F); whereas the mean fluoride values for the high intake group for placenta, cord, and maternal blood were 0.228 ppm, 0.175 ppm, and 0.234, respectively. Comparisons between the low and high F- intake groups suggested that when fluoride intake is low, fluoride passes freely through the placenta, but when the fluoride intake is high, the placenta plays a regulatory role and a suggestion is made that it protects the fetus from excess fluoride by its storage.

Buttner and Muhler, 1958, in an animal study with rats receiving varying amounts of sodium fluoride in the drinking water during gestation and lactation observed that the water must contain high levels of fluoride, 10 ppm, before the tissues of the pups contain any appreciable increase

in the fluoride level from the control group. Smith and Smith, (1935) and Lehman and Muhler (1954), show similar transfer of fluoride at only high levels.

The evidence for the passage of fluoride across the placental barrier was reviewed by Zipkin and Babeaux (1965). This review of the literature, on data relating to humans, indicated that over a wide range of fluoride exposure changes in cord blood fluoride reflected those in maternal blood, but at somewhat lower levels, whether the fluoride was administered by drinking water, tablets, or milk.

Armstrong, Singer and Makowski, (1970), in a study concerning the placenta transfer of fluoride and calcium, did not support the theory of a placental fluoride barrier. Blood samples obtained from maternal and umbilical blood vessels at the time of cesarean sections on 16 women demonstrated higher values of calcium and fluoride in fetal circulation than maternal circulation at the time of delivery.

In general, although the calcified tissues of all newborn mammals studied contain some fluoride, the placenta accumulates it; the concentrations in fetal tissue are therefore relatively low. This is in accord with much of the clinical evidence that caries in children is not significantly affected by an increased fluoride intake during pregnancy and that the enamel of deciduous teeth is rarely mottled. Comparison of caries reduction with the timing of the exposure to fluoride shows that more than half the effect depends upon receiving fluoride during enamel formation, or immediately after eruption when the final stage of mineralization occurs from saliva (posteruptive maturation). The remainder of the effect is exerted throughout the life of the dentition, so that to obtain maximum benefit, continuous contact with fluoride is probably necessary.

D. The Effect of Prenatal Fluoride on Caries Incidence

Carlos, et. al., 1962, studied a group of Newburgh children, and reported no evidence that the use of fluoridated water during pregnancy increased the resistance to caries in the deciduous teeth of the children.

In contrast, Blayney, and Hill, 1964, reported that data from the Evanston, Illinois fluoridation study suggested the ingestion of communal water, containing 1 ppm of fluoride, during the prenatal period, afforded additional protection over that provided by only the postnatal use.

In 1966, after reviewing many studies evaluating the effects of prenatal fluoride, the FDA withdrew approval of products labeled as prenatal fluoride. Research showed that prenatal fluoride could only be incorporated into the primary incisors. All other primary teeth remain in the formative stage until birth and only complete crown calcification after birth.

Boller's study (1964), however showed crown tips or incisor edges of the deciduous teeth to be forming by the 12th week of pregnancy, and the cusps and occlusal surfaces of the permanent first molars forming during the eighth and ninth month of pregnancy. His study suggests that tooth initiation occurs earlier than previous studies had indicated, and that the first permanent molar is in reality a primary tooth from a developmental standpoint, because the critical occlusal surface forms well before birth.

Katz and Muhler, 1968, compared the dental caries experience in deciduous teeth of children exposed prenatally to fluoridated water.

The study was designed to investigate whether prenatal ingestion of fluoridated water is effective in reducing dental caries in deciduous teeth or whether this effect was related to the length of exposure of the mother to fluoride before pregnancy. The results indicate a reduction in caries with prenatal communal fluoridation although not a significant reduction. Therefore, it was concluded that the effect of fluoride on deciduous teeth is mainly, if not entirely, postnatal.

Glenn, 1977, reported on four children followed for twelve years; and concluded that sodium fluoride supplementation in tablet form during pregnancy, even when fluoridated water was used, is necessary for maximum caries immunity. Parents with histories of heavy tooth decay were chosen. The mothers were placed on 2.2 mg NaF tablets daily during the third through ninth month of pregnancy. The children's deciduous teeth upon shedding (approximately 5 years of age) were collected for analysis. Fluoride analysis, DFTM values, and tooth morphology indicated a significant uptake of fluoride in the primary teeth receiving fluoride supplementation; 243-571 percent more fluoride than the control group.

In 1979, Glenn compared three groups during pregnancy; (1) control group - mothers receiving only fluoridated water (.7 - 1.0 ppm) (2) vitamin fluoride group - mothers of children in this group ingested a combination tablet containing 1.0 mg of fluoride (3) Sodium-fluoride tablet group - mothers ingested a 2.2 mg tablet daily during pregnancy. Upon comparison, the NaF tablet group children was 96 percent caries free. The vitamin - fluoride group children were 69 percent caries free and the children receiving only fluoridated water via maternal metabolism was 0 percent.

E. Theories of Tooth Eruption

It is the purpose of this literature review to briefly examine the factors concerning the theories of tooth eruption, from the results of clinical and experimental findings, in the hope of effecting a better understanding of the mechanism of tooth eruption.

The term "eruption" is used to designate the process whereby the forming tooth migrates from its intraosseous location in the jaw to its functional position in the oral cavity.

The phenomenon of tooth eruption is not fully understood. The major problem in evaluating changes in eruption is that the identification of forces required for normal tooth eruption is still unknown. Shortly after commencement of root formation, movement of the tooth in an occlusal direction can be detected, and it is the forces responsible for this movement which have been studied with many resulting theories. Because of the intimate relationship between the supporting tissues of the tooth -- ligament, root, fiber attachment to both bone and cementum -- the identification of the forces required for tooth eruption is proving difficult.

In a comprehensive and classical review of the literature prior to 1942, Massler and Schour reported that at least seven theories to explain the mechanisms of tooth eruption had been proposed.

1. Growth of root.
2. Growth of Dentine and Pulpal Constriction.
3. Growth of Periodontal Tissue and Alveolar Bone.
4. Pressure from Muscular Action on the Alveolar Process.

5. Resorption of Alveolar Bone Exposes Tooth.
6. Pressure from Cellular Proliferation.
7. Pressure from Vascular Beds.

The explanations, proponents of, evidence for and conclusions regarding these theories are presented in the following Table I taken from Massler and Schour (1941):

From their exhaustive examination of the literature only to 1940 these authors concluded:

- a. No conclusive proof could be obtained favoring any theory of eruption.
- b. Whatever the ultimate source of the eruptive force, it is probably located in the perapical region of the tooth.
- c. The eruptive force may be related to the vascularity of the tissue which surrounds the tooth, i.e., in the periodontal tissue but not of the pulp.

More recently, Berkovitz (1975) suggested that three theories of eruption merit further consideration when discussing tooth eruption: tissue-fluid pressure, cell proliferation and tension generated by the connective tissue of the periodontal ligament. He indicated that alveolar bone formation appears to have less to offer and need only briefly be mentioned, while root growth appears to be bound up with basal cell proliferation. However, in his review of the literature he concluded that "No one theory appears to have enough experimental evidence to support it as the prime mover in eruption. Neither is there positive evidence to indicate that eruption is a multi-factorial process."

Shulman (1977), in his review of the literature concerning causes and mechanisms of tooth eruption, suggested that of all the theories, the

two most likely are the periodontal ligament "pull" in conjunction with alveolar bone changes or the fluid pressures beneath the erupting tooth.

The action of the periodontal ligament is thought to be such that the fibers of the periodontal ligament are dynamic, and there is a constant turnover of collagen fibers with continued rearrangement and reconnection, which may pull the tooth occlusally. This thought is consistent with Thomas (1964) and Beertsen (1974).

The second theory proposes that the fluid pressure beneath the tooth is activated by an altered vascular permeability in the tissue underlying and/or surrounding the tooth which produces an increase in pressure which may drive the tooth occlusally. This theory is in agreement with Massler and Schour (1941).

Therefore, it can be ascertained that in spite of substantial investigation and observations the question regarding the mechanism of tooth eruption still remains unanswered. Further studies are indeed necessary to shed more light on this topic.

TABLE I

THEORIES OF ERUPTION ¹

THEORIES	EVIDENCE AGAINST	EXPLANATION
Growth of Root	<ul style="list-style-type: none"> a. Eruption occurs in rootless teeth. b. Eruption occurs after root formation is completed. c. Cuspid erupts a distance more than total length of root. d. Eruption continues when odontogenic epithelium is removed and root elongation ceases. e. In hypophysectomy eruption ceases but root elongation continues (with foldings) 	Elongation of root pushes crown into oral cavity.
Growth of dentine and Pulpal Constriction	<ul style="list-style-type: none"> a. Eruption continues in pulpless teeth. b. In hypophysectomy, eruption ceases but growth of dentine and pulpal constriction continue. 	Pressure from growth of dentine and constricting pulp forces tooth into oral cavity.
Growth of and pull by Periodontal Tissue	<ul style="list-style-type: none"> a. Histologic examination of erupting tooth shows that erupting tooth is pulling upon periodontal fibers and through them on the alveolar bone; not vice versa. 	Movement of soft tissues surrounding tooth pulls the latter into oral cavity.
Growth of Alveolar Bone	<ul style="list-style-type: none"> a. Bicuspid erupts rapidly with very little growth of alveolar bone when deciduous molar prematurely extracted. b. Alveolar bone does not grow but resorbs in absence of tooth. c. Teeth in dermoid cysts erupt in absence of bony base. 	Growth of alveolar bone carries or pushes the tooth into the oral cavity.

TABLE I CONTINUED

THEORIES OF ERUPTION¹

THEORIES	EVIDENCE AGAINST	EXPLANATION
Pressure from muscular action upon alveolar process	<ul style="list-style-type: none"> a. Teeth lingual to arch and outside action of cheek musculature erupt at normal rate. b. In cases of unilateral facial paralysis, teeth on affected side erupt at normal rate. 	Pressure from cheek and tongue musculature contracts the alveolar process and squeezes the tooth into the oral cavity.
Resorption of alveolar bone exposes tooth	<ul style="list-style-type: none"> a. Alveolar process increases in size during eruption of teeth. 	Resorption of alveolar crest exposes tooth into oral cavity.
Pressure from cellular proliferation	<ul style="list-style-type: none"> a. Eruption continues after removal of proliferating odontogenic epithelium. b. Number of mitotic figures in periapical tissues not commensurate with relatively tremendous force of eruption. 	Osmotic pressure or tissue tension resulting from (1) proliferation of cells, (2) vascular bed, or (3) both, in the pulp and periapical tissues pushes tooth into oral and cavity, the roof of the bony crypt being resorbed by pressure atrophy.
Pressure from Pulp due to: Cellular Proliferation Vascularity, or Both	<ul style="list-style-type: none"> a. Eruption continues in pulpless teeth. 	Osmotic pressure or tissue tension resulting from (1) proliferation of cells, (2) vascular bed, or (3) both, in the pulp and periapical tissues pushes tooth into oral and cavity, the roof of the bony crypt being resorbed by pressure atrophy.

TABLE I CONTINUED
THEORIES OF ERUPTION¹

THEORIES	EVIDENCE FOR	EXPLANATION
Pressure due to Vascularity of Periapical Tissues	<ul style="list-style-type: none"> a. Submerged teeth erupt under the influence of hypermia induced by mechanical irritation (dentures or finger rubbing) b. Hyperemia in periodontitis causes supra-eruption of tooth. c. In hypopituitarism and hypothyroidism eruption is markedly retarded concomitant with reduced vascularity of periapical tissues. In hyperpituitarism, eruption is accelerated and vascularity of periodontal tissues increased. d. Removal of vasoconstrictor nerve causes accelerated eruption concomitant with increased vascularity of periodontal tissues. 	Osmotic pressure or tissue tension resulting from (1) proliferation of cells, (2) vascular bed, or (3) both, in the pulp and periapical tissues pushes tooth into oral cavity, the roof of the bony crypt being resorbed by pressure atrophy.

¹Modified from Massler and Schour (1941).

F. The Effects of Fluoride on Tooth Eruption

There have been several investigations and much discussion on the possible effects of fluoride on tooth eruption.

Smith (1934) reported that large amounts of fluoride (450 ppm F) administered post-natally into the diet caused the rat incisor eruption rate to fall from approximately 500 microns/day at 28 days to 130 microns/day 70 days later.

Short (1944) reported that the eruption of the permanent teeth is significantly retarded at 2 ppm, in comparison with those where the fluoride level is less than 0.5 ppm. No effect, however, is noticed in tooth eruption by fluoride levels of approximately 1 ppm; although this fluoride concentration imparts to the teeth an almost optimal protection against caries.

Adler (1951) produced evidence that, in low fluoride areas, the permanent premolars exhibit accelerated eruption due to high caries rate of the primary dentition.

A suggestion of tooth delay was mentioned by Baume and Becks (1954), who reported that fluoride is a thyroid inhibitor and that hypothyroidism delays the eruption of teeth.

Feltman and Kosel (1961) noted a delay in the eruption of teeth, in some cases by as much as a year from the accepted eruption dates due to prenatal and post-natal fluoridation. It was speculated that this delay may be a factor in the reduced incidence of tooth decay in fluoridated teeth. The delay in eruption, thus allowing the opportunity for greater tooth maturation prior to becoming exposed to the

forces that trigger caries activity.

Tank and Storwick (1964) observed that the eruption of the deciduous dentition of 1 to 6 year olds is not significantly affected by prenatal and postnatal exposure to water adjusted to 1.0 ppm of fluoride.

Kunzel (1976) noted, in his study of the children in the fluoridated city of Karl-Marx-Stadt (1.0 ppm), that the mean eruption times (permanent premolars) showed a distinct difference compared to those of the fluoride-free cities. However, Kunzel dismissed the suggestion of a direct delay due to fluoridation. Rather, he felt there was "normalization" due primarily to the fact that the deciduous molars receiving fluoride were retained longer because of their reduced caries rate thus slowing the eruption of the permanent premolars.

Ericsson (1977) in his review of the literature stated, "The delayed eruption of the permanent dentition in areas with appreciable levels of fluoride in water, although not confirmed, appears to be valid. By ages 10-12, in areas with optimum or above optimum water F, F accumulation in the jawbone may have reached a level that offers resistance to the resorption necessary for tooth eruption."

G. The Effect of Fluoride of Tooth Morphology

Forrest (1956) in a study concerning caries incidence and enamel defects in areas with different levels of fluoride (0.9 - 5.8 ppm F) in the drinking water stated "The benefit derived from fluoride did not seem to be merely a freedom from caries. The teeth had a better appearance, indicating superior structure, in the fluoride areas, except in West Mersea (5.8 ppm F) where the more severe type of mottling was accompanied by pitting of the enamel. Even the first permanent molars in Burnham, (3.5 ppm F), which showed a great deal of mottling, had well formed rounded cusps and shallow fissures and had a hard glistening appearance. This improved formation may well have contributed to the fact that 75 percent of these teeth were entirely free of caries."

Lovius and Goose (1969) took alginate impressions of 9, 10, and 11 year old boys to compare the effects of water fluoridation on tooth morphology. Their results indicated that those children who had lived all their lives in the zone which had had fluoridation of the water supply at 1 ppm showed smaller measurements of the molar teeth than those in the non-fluoridated area. The incisors appeared to show no difference.

Cooper and Ludwig (1965) studied 100 girls of 7 to 9 years of age in the fluoride area of Hastings, New Zealand (ppm F not given) and compared these with a non-fluoridated city, Palmerston North. The results showed, small but distinct changes in the morphology of the teeth in the Hastings children exposed to fluoridation throughout life. The dimensions of the lower first permanent molars are reduced.

The mean mesio-distal diameter was smaller and significant at the 0.01 level. The results were less marked with the bucco-lingual measurements, although still significant at the 0.05 level. Cooper and Ludwig concluded, "It would seem, therefore, that fluoridation produces a smaller tooth, and one in which cusp height is reduced and the sides of the teeth flattened."

Paynter and Grainger (1956) found that maxillary molars, of rats fed 12 ppm fluoride, were smaller mesiodistally and buccal-lingually than the controls, but comparison of fissure angles and fissure depths revealed no significant differences.

Simpson and Castaldi (1969) compared the crown morphology of first permanent molars of Grade 1 school children living in a city with naturally occurring fluoride in the amount of 1 to 2 ppm and in another city which contained only traces of fluoride. Their analysis revealed that the dimensions of the maxillary and mandibular molars in the optimum fluoride sample are consistently greater than those in the low fluoride sample, however only the mesiodistal diameter of mandibular molars in the optimum fluoride group is significantly greater. (Also it should be noted, there was no difference between the two groups in the state of eruption, as determined by measuring the height of the tip of the mesio-buccal cusp above the gingival margin).

H. Grahnen, et. al., (1974) appraised the mesiodistal widths of teeth in 6 to 11 year old children living in cities with varying amount of fluoridated water (0.1 mg/l to 2 mg/l). No significant differences in mesiodistal widths of individual teeth could be demonstrated.

H. Fluoride Influence on Calcium and Mineralization

Yates, et. al., (1964) described the effects on sodium fluoride on calcium homeostasis. Laboratory rats were prepared to accepted peritoneal lavage through a surgical procedure. The peritoneum was then filled with varying fluoride concentrations (0.036 g/l or 0.072 g/l) for various lengths of time. The amount of calcium released by the body (bone and blood) was then measured. Aliquots of fluid in the peritoneum were gathered and calcium measurements were determined utilizing a Beckman Flame Spectrophotometer. From the data collected, it was strongly suggested that the effect of sodium fluoride on calcium homeostasis results from a decrease in solubility of the bone salt after the incorporation of the fluoride ion and this decrease in solubility effects the equilibrium of calcium ions between blood and bone, and thereby may indirectly stimulate endogenous parathyroid secretion.

Walton and Eisenmann (1974) examined ultra structurally the various stages of amelogenesis -- differentiation, formation, transition, and early maturation -- following IP injections of 2.5% sodium fluoride (17 mg/kg per injection) in young rats. The ability of the cells to recover from the effects of the fluoride ion was also studied.

The fluorotic differentiating and transitioned ameloblasts exhibited no detectable morphological differences from the control cells. However, the formative stage demonstrated a response to fluoride in the morphology of the cells and their products. The ameloblasts contained unusual vacuoles and granules, and the adjacent forming enamel contained paired hypermineralized and hypomineralized zones. During maturation,

groups of ameloblasts often were altered with large intracellular vacuoles; while adjacent enamel appeared to mature normally.

It was suggested that the stages of amelogenesis may be broken down into two distinct categories -- those involving cells with predominantly internal organization, and cells with specialized external activities. As their functions differed, so perhaps their response to fluoride injections also differed. Both differentiating and transitional ameloblasts are involved in internal organization. As observed in this study, these cells have no major external activity. In the experiment neither cell type varied from their counterpart control ameloblast. Both the morphology and the nature of enamel of the ameloblasts in the formative and maturation stages were consistently affected by the fluoride. These results indicate a specificity of action by fluoride which is probably aimed at certain susceptible metabolic processes.

Animals killed after a recovery period (2.5 days) from IP injections of fluoride demonstrated no cellular disturbances, and a layer of normal enamel formed over the fluoride response. Apparently, the ion's effects were temporary and of short duration. Evidently the cells recovered rapidly and were subsequently capable of producing new layers of normal enamel.

Larsen, et. al., (1977) studied the effects of acute intraperitoneal doses of fluoride (10,20, or 40 mg NaF per Kg body weight) on serum fluoride, serum calcium, and forming dental tissues in rats. Their results showed that immediately after injection of fluoride the concentration in the serum increased rapidly and decreased gradually in the following hours. Shortly after the rapid increase in serum fluoride, the

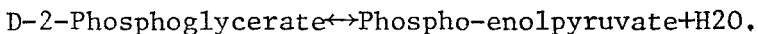
serum calcium concentration decreased and remained low until the fluoride level had returned to normal. There was a direct relationship between the increase in serum fluoride and the decrease in serum calcium.

This study also indicated a change in the forming hard tissues. Following the injection of fluoride, the enamel exhibited a hypermineralized zone which was then followed by a hypomineralized zone. Seemingly, the hypermineralized later in both enamel and dentin correspond to the period of high serum fluoride concentration, whereas the hypomineralized layers formed during the subsequent hypocalcemic period. It was also noted that the changes in the forming enamel and dentin seemed strongly related to the amount of fluoride injected. It was concluded that the hypermineralized zone observed in both enamel and dentin represent formation of a fluoroapatite, whereas the hypomineralized areas are a result of the fluoride induced hypocalcemia.

Gozariu, et. al., (1977) studied calcium release from tooth germs in contact with hormones responsible for the stimulation and inhibition of movements of calcium in living tissues. Their results supported the concept that the calcification of tooth germ is closely dependent on the hormones principally responsible for controlling calcium metabolism in the whole organism. Their results indicate that the addition of parathormone (1 and 5 U per ml) and dibutyryl cyclic AMP (0.8 m μ), to Wistar molar tooth germs in vitro, consistently stimulated resorption or calcium release. Whereas, calcitonin (200 m μ per ml) inhibited the PTH and DBCAMP stimulated calcium release. It was suggested that the stimulation of calcium release by PTH and DBCAMP from tooth germs supposes the existence of a mechanism involving adenyl cyclase to ensure normal classification.

The role of calcitonin in the calcification of the dental matrix was examined by Kline and Thomas (1977). In their animal study, chronic calcitonin deficiency was shown to have several effects on the incisors of young rats. The calcitonin deficient rats exhibited a significantly wider predentin layer ($p < 0.05$), an irregular dentin-predentin border and interglobular dentin. The control teeth were characterized by a regular dentin border and a well defined dentin-predentin junction. From these results, it was suggested, that since the normal rate of calcification of the dentin matrix is approximately equal to the rate of matrix formation, calcification in the calcitonin deficient rats was relatively reduced. It was concluded that calcitonin plays a role in the normal calcification of the dentin matrix.

Several authors have implicated enzymatic inhibition by fluoride. Peters, et. al., (1964) demonstrated that the enzyme enolase (phosphopyruvate hydrolase) is inhibited by fluoride application. Enolase is an important enzyme involved in glycolysis as it catalyzes the following reaction:



Phospho-enol-pyruvate (PEP) is a key intermediate of the glycolytic pathway and it serves as the source of energy for many cells. Fluoride acting at this level may then potentially alter the metabolic process of the cells involved in tooth eruption and calcification.

MATERIALS AND METHODS

A. Breeding and Maintenance Conditions

Albino rats of the Sprague-Dawley stock were used in this investigation. Eleven female rats of approximately the same age and weight were successfully bred in our animal laboratory. Vaginal plug droppings were used as indicators for conception. Following breeding the dams were randomly divided into two groups: Control (5 dams) and Fluoride (6 dams) animals.

The control dams were housed one to a cage and maintained on a standard diet of Purina Rat Chow and distilled water ad libitum during gestation and lactation.

The experimental dams were housed one to a cage and maintained on the same unrestricted standard diet of Purina Rat Chow and distilled water supplemented with 150 ppm sodium fluoride (NaF) during gestation and lactation.

The females of both groups were periodically examined for any abnormal growth variation by physical examination and weighing, throughout their gestation period.

Following parturition, the gestation time period and litter size of each dam was recorded. Birth weights were not recorded, since handling of the animals at this time was limited in an effort to preclude the possibility of mother rejection.

B. Sacrifice

The offspring from both groups were randomly selected, and sacrificed with an overdose of ether at 18, 19, 20, 21, 22 and 23 days postnatally. These periods corresponded to the normal eruption of the first and second molars in the rat.

Only one pup from each litter was selected for sacrifice at each day, i.e., 18-23 days inclusive. This provided a random contribution of each litter to the sacrifice periods. The number of pups in each time period varied due to litter size differences. The weights of the rat pups were recorded at sacrifice.

Following sacrifice, the head of the young rats of both groups were skinned and decapitated with standard dissecting instruments. The upper and lower jaws were removed, bisected and placed in an appropriately labelled bottle containing 95% alcohol for preservation until further study.

C. Photography

In order to compare the stage of eruption of the fluoride and control animals of the same ages, photographs of the jaws were made. A Minolta 35mm camera with a 2X Deesen lens equipped with a field frame allowing a constant frame size of 1/2" x 3/4" was utilized.

The right and left quadrants of each jaw from each animal in each group were placed on straight pins and oriented with the occlusal surface horizontal and facing upward toward the camera. They were situated against a black cloth background allowing greater contrast for comparison. The photographs were centered on the interproximal space between the first and second molars. Kodak's tungsten (3200K) high speed Ektrachrome color film was used. Following development and mounting, the slide pictures were labelled according to rat age and group.

D. Photographic Comparison

Photographic comparisons were made utilizing the color slides projected under similar conditions to produce equal magnification at the same time.

The evaluation of the color slides were projected so that the evaluator had no knowledge as to the identity of the quadrants. Each jaw quadrants of the fluoride treated rats were compared to every quadrant of control animals of the same age, 18-23 days inclusive. The evaluator determined if the quadrant in question was accelerated (+), equal (0), or delayed (-) in eruption as compared to each control quadrant. This evaluation process was duplicated in the same manner by a second evaluator.

The criteria for comparing the degree of eruption was based on the number of molar cusps observed and the quantity of tooth surface exposed. When the evaluators were unable to determine a clear difference between the quadrants shown, a designation of equal (0) eruption was recorded.

E. Analysis Preparation

All first and second molars from each rat pup were carefully extracted from the jaws with the aid of an American Optical Stereo-Dissecting Microscope and standard micro-dissecting instruments. Care was taken to minimize handling of the molars to prevent damage to the tooth structure.

Following extraction, the molars were individually transferred to weighed, dessicated weighing boats (6 ml capacity). The weigh boats containing a molar were then placed in a dessication tray in a numbered slot for identification and the tray in turn placed within the dessicated apparatus. Drying was carried out under a vacuum in the presence of Drierite crystals for a period of 24 hours.

Following dessication, the molars were weighed on an Ainsworth Analytical Balance and the weight of the individual molars were recorded in grams.

The weigh boat containers for each molar sample were transferred to correspondingly labelled acid-washed wide mouth 100 ml polyethylene bottles. A 10 ml nitric (double distilled) acid was dispensed by a 10 ml Dispo pipette into each sample bottle. A quantity of 40 ml double-distilled water was measured and dispensed by a 100 ml Dispo Pipette into each sample bottle bringing the sample to a total volume of 50 ml for analysis. This procedure dissolved the tooth, making them ready for analysis.

F. Analysis

The sample bottles were transported to Limnetics, Inc., Milwaukee, Wisconsin, for atomic absorption spectrophotometric measurement of calcium.

Just prior to analysis, potassium chloride (100 mg) and lanthium chloride (100 mg) were added to calcium standards and samples to retard interference from an unrecognized source with the calcium analysis.

For analytical comparison and reference, standards of calcium (10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm) were prepared from commercially supplied stock atomic absorption reference standards. These were analyzed under normal operating conditions to establish charted reference points for comparison. An operational base line for readout on the stripchart was maintained to facilitate analysis.

Analysis was performed on a Perkins-Elmer 305 AA Spectrophotometer equipped with a stripchart recorder. The following conditions were maintained: wavelength (x), 211 nanometers visible, slit width 4, and air-CzHz, and rich yellow flame.

The standards of calcium were run every 25 samples to ensure accurate analysis.

Samples and standards were properly designated numerically on the stripchart readout to facilitate tabulation of raw data for calcium content per tooth.

G. Statistical Analysis

Statistics were applied to the data as appropriate for the particular comparison. Analysis of variance, T test, Chi-square, and correlation statistics were utilized with the aid of computer programming.

Chi square statistics were used on the data concerning eruption. Data from the experimental group was recorded as delayed (-), no difference (0), or accelerated (+) eruption as compared to the control group. Percent of delay within each age group (18-23 days) was determined with chi square to determine whether the observed amount of delayed eruption was statistically different from a 50-50% randomization of accelerated and delayed tooth eruption.

T test statistics were applied to compare the number of rats in the litters of the fluoride treated animals and the control animals. A probability of less than 0,05 was considered statistically significant. ($P < 0,05$). Similar statistics were used to compare the weights at sacrifice, weights of teeth, total tooth calcium, and the mean concentration of calcium per tooth (mg/g) of both the fluoride treated and control animals.

The effect of age, (18-23 days) on the parameters of weight at sacrifice, tooth weight, total calcium per tooth and mean concentration of calcium per tooth (mg/g) as studied for both the fluoride treated and control group, utilizing an analysis of variance statistic. F ratios were determined for each parameter in both fluoride and control animals. Probability levels of less than or equal to 0,05 were considered significant.

Correlation statistics were performed on scatter diagrams concerning tooth weight; tooth calcium content, concentrations of calcium per tooth weight, produced from raw data by computer. The correlation coefficients were determined and goodness of fit expressed as a probability of $<.05$. This level was considered statistically significant.

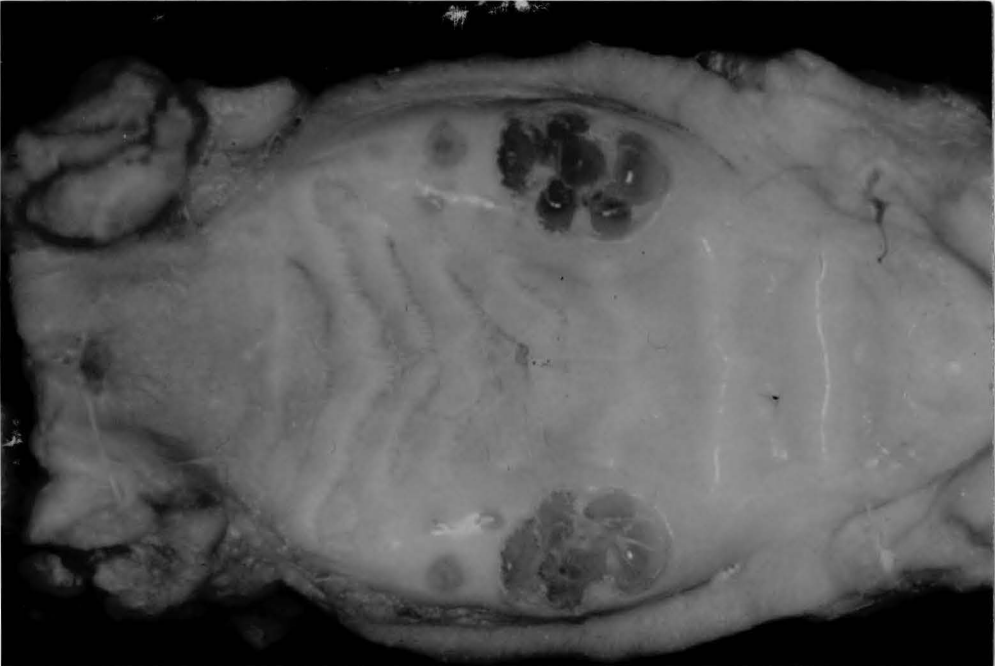


FIGURE 1 FLUORIDE 22 DAY OLD RAT MAXILLAE

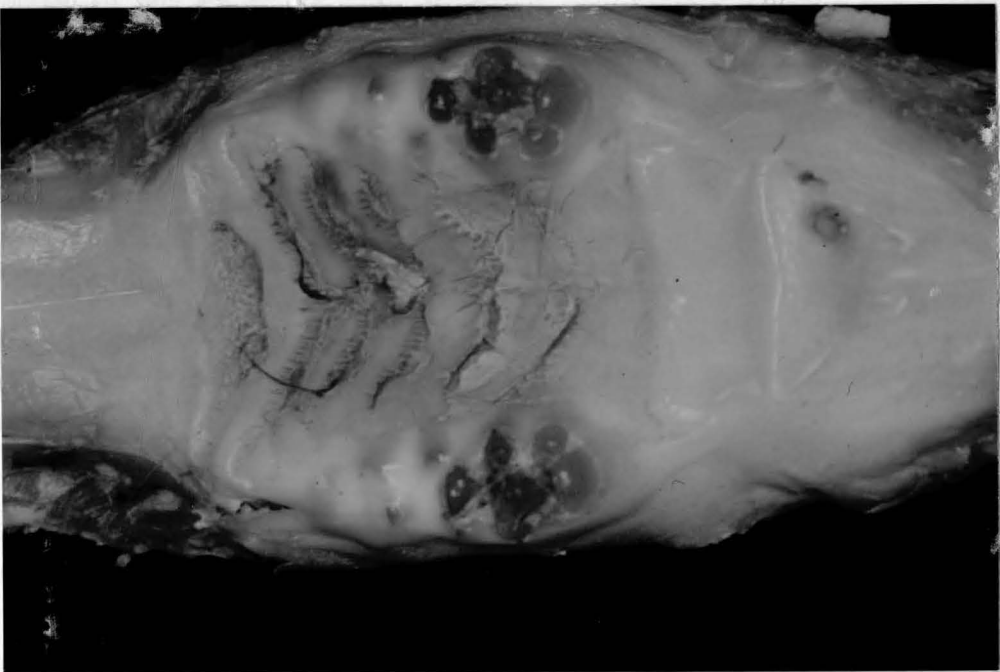


FIGURE 2 CONTROL 20 DAY OLD RAT MAXILLAE

RESULTS

A. Effect of Prenatal Fluoride on Tooth Eruption

A comparison of the eruption rates of the animals in the fluoride-treated group compared to those in the control group is shown in Table II. This comparison is expressed as the percentage of animals in the fluoride group with posterior quadrants (first and second molars) that were ahead (+), about equal (0), or delayed (-) in eruption, as compared to the posterior quadrants of control animals of the same age.

The eruption of the molar teeth in 92 percent of the jaws from the 18 day old fluoride treated animals was delayed in comparison to that in the jaws of the control animals of the same age. At 19 days of age the same effect was observed with the eruption of the posterior teeth from the fluoride treated animals being delayed in 86 percent of the jaws, while the animals in the 20 to 23 day age group still demonstrated a delaying effect of fluoride on eruption. This effect was less pronounced than in the younger animals; the teeth in 55, 76, 64, and 78 percent of the posterior segments from the fluoride treated animals of the 20, 21, 22, and 23 day old pups respectively showing a delay.

It should be noted in Table II that there is a reasonable consistence in the percentages of the accelerated, equal, or delayed categories when comparing the four jaw segments from the same age group of the experimental animals. It is also suggested that the number of jaw segments showing accelerated or equal eruption in the fluoride treated group tended to increase with the age of the animal.

TABLE II

Eruption of Molar Teeth of Fluoride Treated Rats
in Quadrant Units Expressed as Percentage of Control Values

Days of Age	Lt. Mand. Quad.			Rt. Mand. Quad.			Lt. Max. Quad.			Rt. Max. Quad.			Quad. Mean %
	+	0	-	+	0	-	+	0	-	+	0	-	
18	0	.10	.90	0	0	1	0	.10	.90	0	.10	.90	.92
19	0	.10	.90	.17	.10	.73	.03	.06	.91	.06	.03	.91	.86
20	.33	.10	.57	.37	.03	.60	.30	.16	.53	.30	.17	.52	.55
21	.20	.05	.75	.25	0	.75	.15	.05	.80	.25	0	.75	.76
22	.30	.05	.65	.25	.05	.70	.40	0	.60	.40	0	.60	.64
23	.06	.20	.74	0	.31	.68	.06	.09	.86	.06	.11	.83	.78

Key

- + = accelerated eruption in comparison to control quadrants
- 0 = equal eruption in comparison to control quadrants
- = delayed eruption in comparison to control quadrants

Chi-square statistics were applied to the eruption comparisons in Table II, utilizing the raw data obtained in each quadrant for each of the days 18-23. In order to apply chi-square statistics, the tooth eruption data presented as equal (0) has been eliminated allowing the accelerated (+) and delayed (-) comparisons to total 100% of the data. In this manner, Chi-square indicates any statistical variability from a 50-50% theoretical ratio of accelerated to delayed eruption quadrants for a given day.

Table III, demonstrating the Chi-square application, indicates a statistically significant delay in tooth eruption for the 18, 19, 21, and 23 day old fluoride-treated animals with a p value $<.01$. The fluoride-treated 20 and 22 day old animals did not demonstrate a pattern of eruption that was statistically different from those in the control groups of the same age.

Figures 1 and 2 illustrate the maxillae of a fluoride treated 22 day old animal and a control animal of 20 days of age, respectively. The maxillary quadrants (first and second molars) of both animals exhibit an equal amount of tooth eruption. This illustration depicts the typical amount of delay in tooth eruption of approximately 2 days as observed in this investigation for the fluoride treated animals as compared to the control animals.

B. Effect of Prenatal Fluoride on Litter Size

Table IV shows that fluoride had no detectable effect on litter size in this study. Six dams maintained on water containing fluoride during gestation had a mean litter size of 8.67 offspring, whereas those of the control dams showed an average litter size of 9.6 pups. There was no significant difference in litter size between these groups ($p>.05$).

TABLE III

Chi-Square Evaluation of Delayed Eruption of Fluoride
Treated Rat Molars in Quadrant Units Expressed
as Percentage of Control Values*

Days of Age	Lt. Mand. Quad. mean%	Rt. Mand. Quad. mean%	Lt. Max. Quad. mean%	Rt. Max. Quad. mean%	χ^2	P
18	100	100	100	100	37.00	<.01
19	100	81	96	93	41.69	<.01
20	63	62	54	54	3.63	>.05
21	79	75	84	75	12.62	<.01
22	68	74	60	60	4.22	>.05
23	93	100	94	93	46.28	<.01

* The statistical design of chi-square required the elimination of the data for equal (0) tooth eruption. A theoretical 50-50% randomization of accelerated to delayed tooth eruption was established.

TABLE IV

Statistical Summary of the Difference of
Litter Size Between Fluoride - Treated
and Control Dams

<u>FLUORIDE</u>		<u>CONTROL</u>	
<u>Litter Number</u>	<u>Number of Pups</u>	<u>Litter Number</u>	<u>Number of Pups</u>
1	8	1	8
2	14	2	10
3	12	3	10
4	7	4	12
5	8	5	8
6	3		

$$\bar{x} = 8.67$$

$$\text{S.D.} = 3.90$$

$$\bar{x} = 9.60$$

$$\text{S.D.} = 3.0$$

$$t = 0.44$$

$$p > .05$$

Key

\bar{x} = mean

S.D. = standard deviation

p = probability

TABLE V

Statistical Summary of Certain Physical
and Chemical Properties of Fluoride
Treated Rats as Compared to Controls

	Mean Weight at Sacrifice (gm)	Mean Weight of Teeth (gm)	Mean Total Tooth Calcium (mg)	Mean Concentration Tooth Calcium (mg/gm)
Fluoride no. obs.	31.087 280	0.0070 278	0.9667 278	144.607 278
Control no. obs.	39.875 192	0.0080 191	1.1433 191	144.433 191
t value	-16.49	-4.67	-4.47	0.02
p value	<.01	<.01	<.01	>.05

C. Effect of Prenatal Fluoride on Body Weight

The results as shown in Table V reveal that the rat pups in the fluoride-treated group were significantly smaller in weight at sacrifice than the control pups, ($p < .01$). This conclusion is based on the mean weight at sacrifice (18-23 days) utilizing the T test for statistical comparison of the fluoride-treated and control groups. The mean weight at sacrifice for the fluoride-treated group was 31.087 grams for 280 animals and 39.875 grams for 192 control animals.

The effect of animal age on weight at sacrifice is illustrated in Table VI. The mean weight at sacrifice for 18, 19, 20, 21, 22, and 23 days of age are listed for both fluoride-treated and control animals. As one would expect, there is a concomittant increase in weight with age of the growing animal. This fact is borne out in this investigation as the mean weight at sacrifice for both groups studied increased with age (18-23 days) and was statistically significant ($p < .01$). Also it should be noted that day for day comparisons of fluoride to control animal mean sacrificial weight values in Table VI reveal the fluoride-treated animals to be smaller in body weight than their control counterpart. Figure 3 demonstrates this direct relationship between animal age, and animal weight in both the fluoride treated and control animals.

D. Effect of Prenatal Fluoride on Tooth Weight

The first and second molars of the fluoride-treated rats were significantly smaller ($p < .01$), averaging 1 milligram less in weight, than the control rats as indicated in Table V. The mean weight of the fluoride-treated and control teeth were determined to be 0.0070 and 0.0080 grams

TABLE VI

Statistical Summary on the Effect of Age on the Parameters of Sacrifice Weight, Weight of Molars, Total Tooth Calcium, and the Concentration of Tooth Calcium for Fluoride Treated and Control Animals

Days of Age	Wt. at Sacrifice (gm)		FLUORIDE GROUP Tooth Wt. (gm)		Total Tooth Calcium (gm)		Concentration Tooth Cal.	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D. (mg/gm)
18	29.1	2.8	0.0058	0.0026	0.7993	0.5671	158.57	213.72
19	31.2	4.5	0.0068	0.0030	0.8589	0.3409	131.83	32.29
20	29.3	3.7	0.0070	0.0023	0.9554	0.3870	137.33	31.90
21	30.4	2.6	0.0077	0.0027	1.0584	0.4513	136.65	30.78
22	31.3	5.1	0.0070	0.0021	1.0230	0.3321	150.88	34.48
23	36.0	8.3	0.0076	0.0023	1.1365	0.4062	153.89	45.05
F ratio	11.7		3.35		4.046		0.629	
P value	<.01		<.01		<.01		>.05	
CONTROL GROUP								
Days of Age	Wt. at Sacrifice (gm)		Tooth Wt. (gm)		Total Tooth Calcium (gm)		Concentration Tooth Cal.	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D. (mg/gm)
18	34.7	2.3	0.0073	0.0021	1.030	0.3482	145.88	43.3
19	35.0	3.1	0.0078	0.0022	1.062	0.4232	137.71	38.4
20	36.7	3.2	0.0081	0.0018	1.132	0.4196	141.54	47.2
21	44.3	4.4	0.0085	0.0018	1.207	0.3804	143.05	38.5
22	43.7	6.8	0.0083	0.0018	1.215	0.4152	146.49	43.3
23	44.7	5.9	0.0081	0.0020	1.217	0.4048	151.99	39.1
F ratio	36.06		1.628		1.364		0.436	
P value	<.01		>.05		>.05		>.05	

respectively.

The significant decrease in mean tooth weight of the fluoride-treated animals may be related to the smaller mean weight at sacrifice (size) of the same animals as indicated in Figure 4.

Table VI indicates a significant difference ($p < .01$) in the mean tooth weight of the fluoride-treated animals in relation to age of the animals (18-23 days). The relationship however is not the same for the control animals. There is no significant difference ($p > .05$) in the mean tooth weight and age of the control animals studied.

Figure 5 demonstrates the relationship between tooth weight and animal age in both groups studied. It indicates that tooth weight increases with animal age in both groups until 21 days of age; after which time there is no increase in tooth weight with age. The tooth weight in the fluoride group is again seen to be less than the controls.

Figure 6 compares the mean weight of experimental and control teeth and also shows the differences in weight between the first and second molars. The average weight of the control and fluoride treated first molars weigh 0.0097 and 0.0088 grams respectively. The second molars weigh 0.0063 and 0.0052 grams respectively.

The fluoride treated molars showed an average weight which was 13.87% less than the control teeth. These differences are shown statistically in Table V, ($p < .01$). Figure 6 shows the average reduction expressed in weight of each fluoride treated tooth as a percentage in comparison to control molars. This percentage appears above the bar graph of each tooth. Figure 6 also shows that the second molars were always smaller in weight than the first molars for both fluoride and control groups.

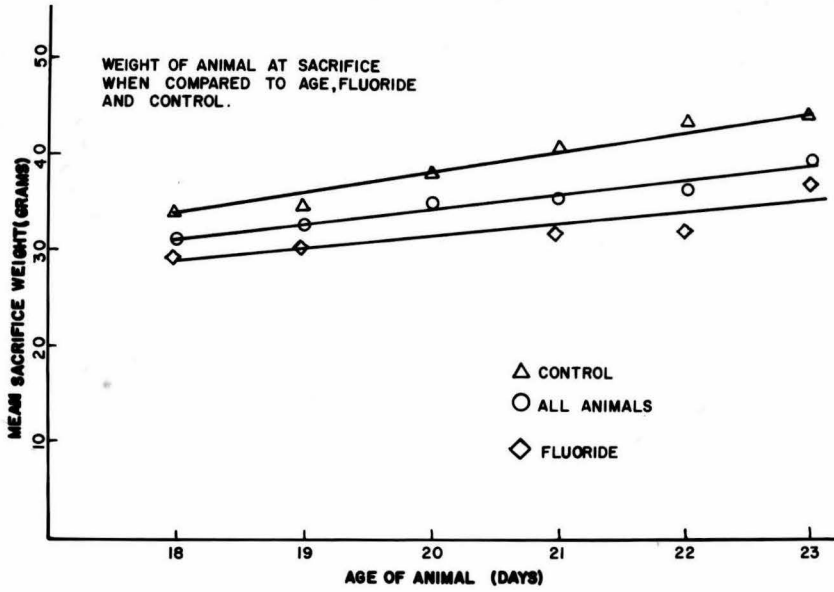


FIGURE 3

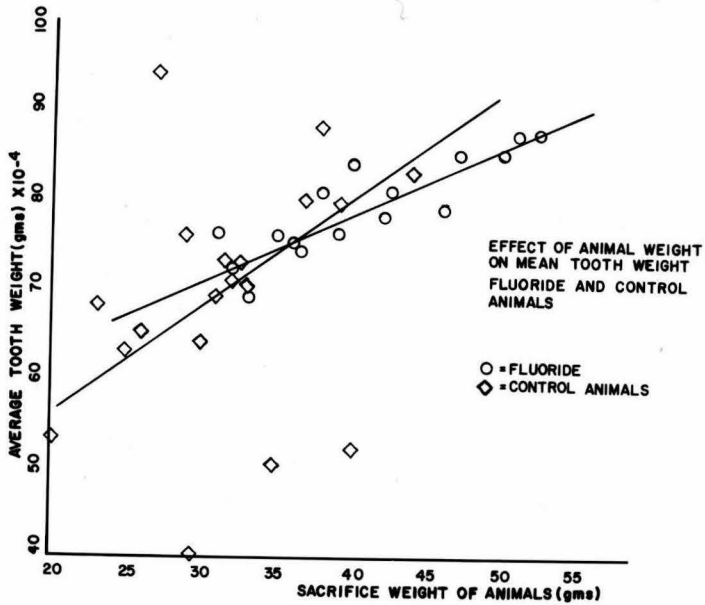


FIGURE 4

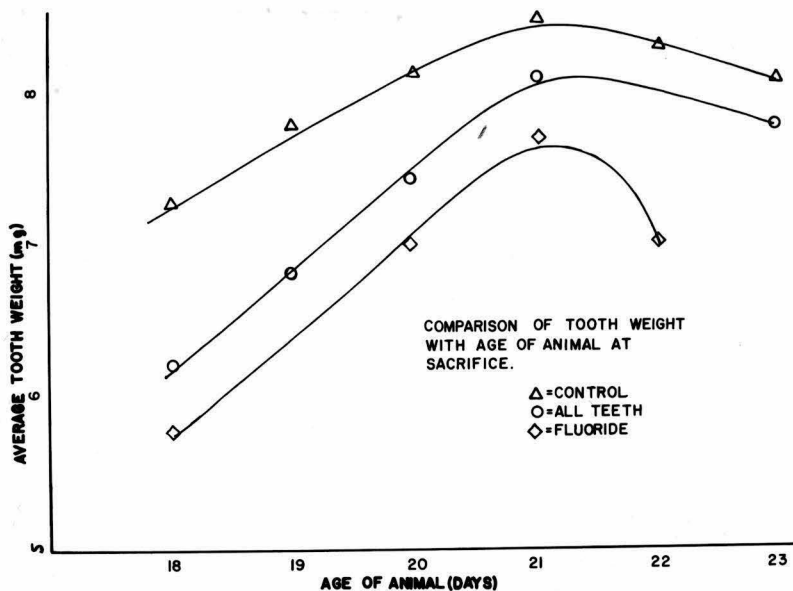


FIGURE 5

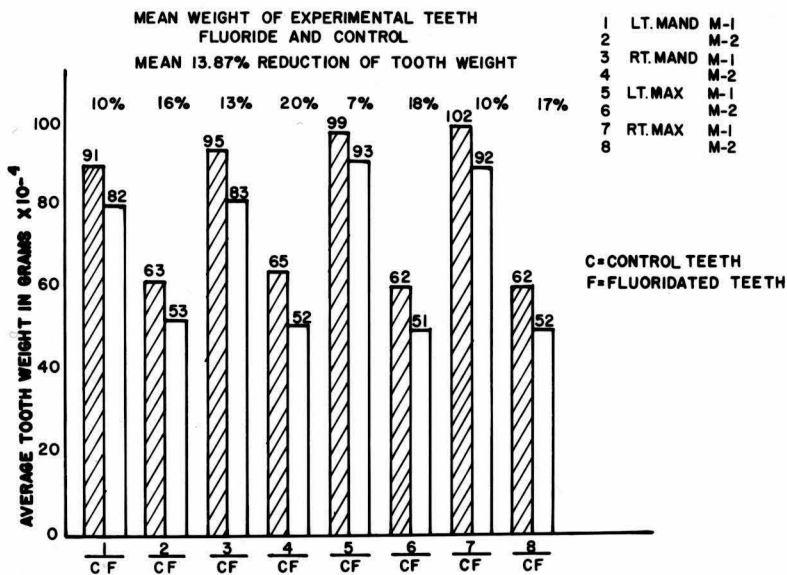


FIGURE 6

E. Effect of Prenatal Fluoride on Tooth Calcium

The significant decrease in the size of the fluoride-treated teeth was also reflected in total calcium content of the teeth; the molars from the rat pups exposed to fluoride averaged 0.1766 mg less calcium per tooth as compared to the control group. However, the mean concentration of calcium in the mineral of the teeth from the fluoride and control groups was very similar. These results are represented in Table V.

The data from the calcium analyses are presented in scattergrams, (Figures 7,8,9) and show the total calcium per tooth plotted against the weight of the tooth for control and fluoride-treated teeth and all teeth combined. They reveal a direct relationship between tooth size (weight) and total calcium per tooth, since the data on each of the three plots may be represented with a straight line which is statistically significant (p value <.01).

Figures 10,11,12 present scattergrams showing the concentration of calcium (mg/g) in the control and fluoride-treated teeth and both of these combined plotted against the weight of each tooth. These graphs illustrate that there is little difference in the concentration of calcium-tooth weight ratio between fluoride and control groups.

The variation of mean total tooth calcium and mean concentration of tooth calcium within the fluoride treated and control group with respect to age (18-23 days) is shown in Table VI. The animals receiving the fluoride supplement show a statistical difference (p<.01) in the total calcium when related to the age of the animals. Whereas the control animals did not show any variation in total tooth calcium with respect

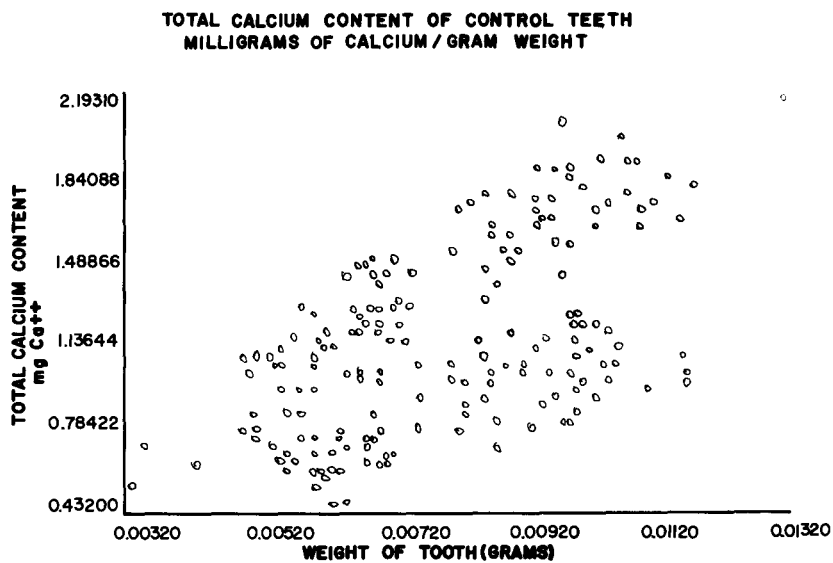


FIGURE 7

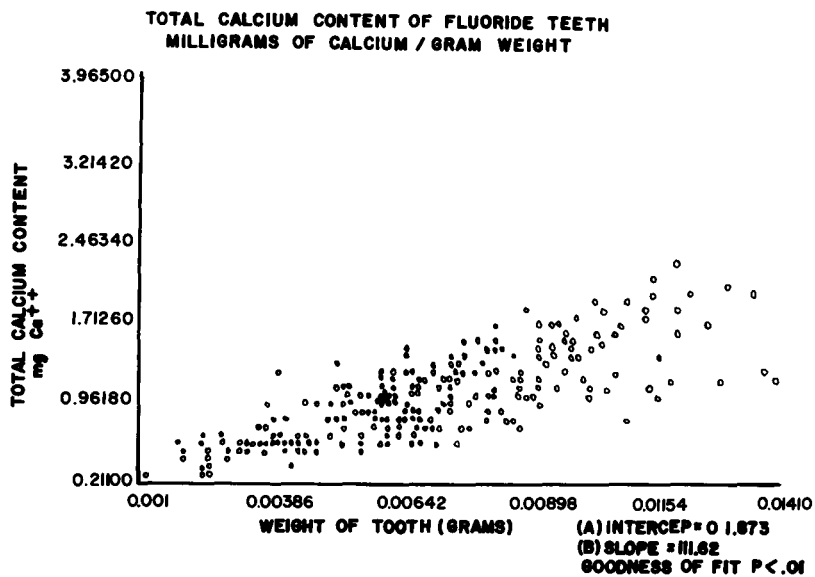


FIGURE 8

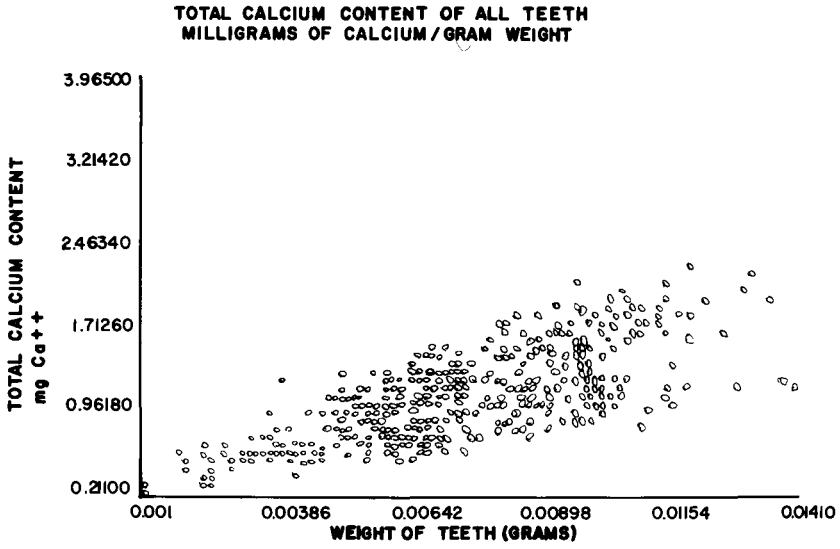


FIGURE 9

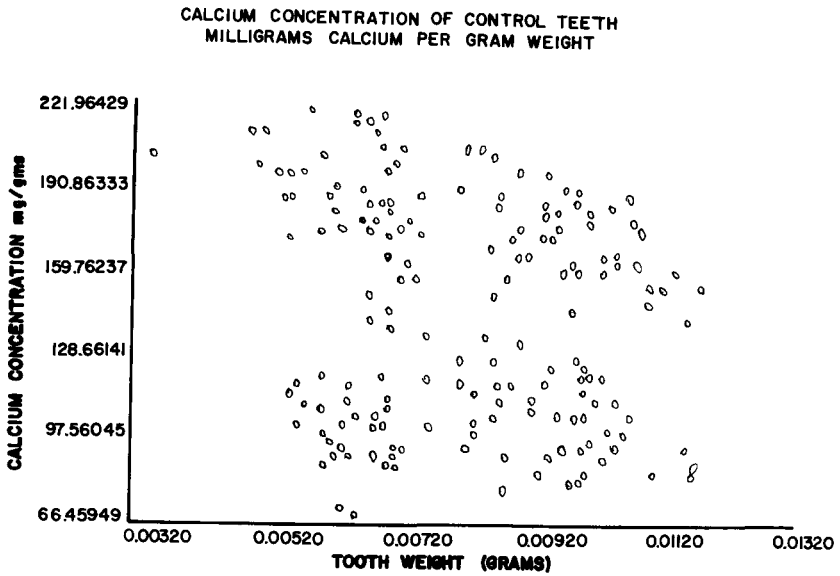


FIGURE 10

to age of the animals. The concentration of tooth calcium for both the fluoride treated and control groups demonstrated no effect when compared to age ($p > .05$). As the concentration of tooth calcium is related to weight of the tooth it is also important to note that no significant variation was observed in mean tooth weight for both groups studied (Table VI) with respect to age. Therefore the results of mean tooth weight and mean tooth calcium appear to be consistent in their relationship to animal age.

Figure 13 illustrates the direct relationship between total calcium content of the teeth and age of the animals. The fluoride treated teeth have statistically significantly less calcium content than the control teeth.

Figure 14 shows the calcium concentration to increase from 19 days of age for all teeth studied; however this increase is not statistically significant ($p > .05$) as shown in Table VI.

Figure 15 compares the relationship between the animal weight and total tooth calcium content of the fluoride treated and control teeth.

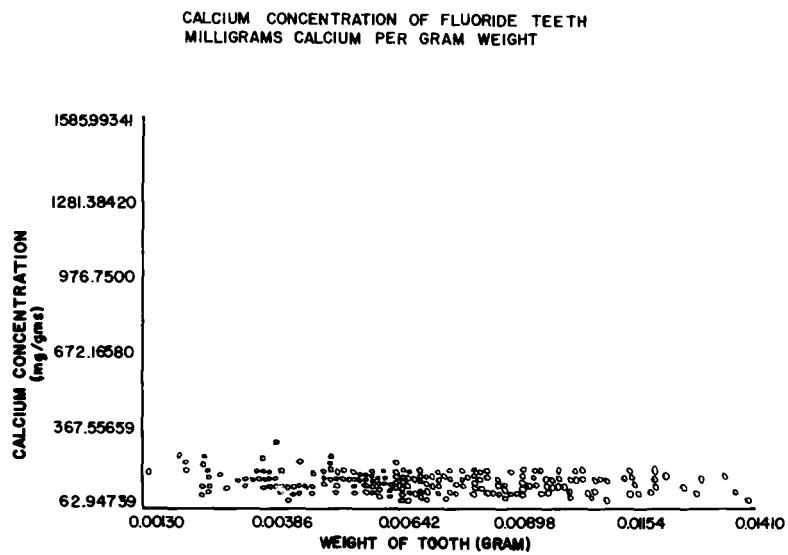


FIGURE 11

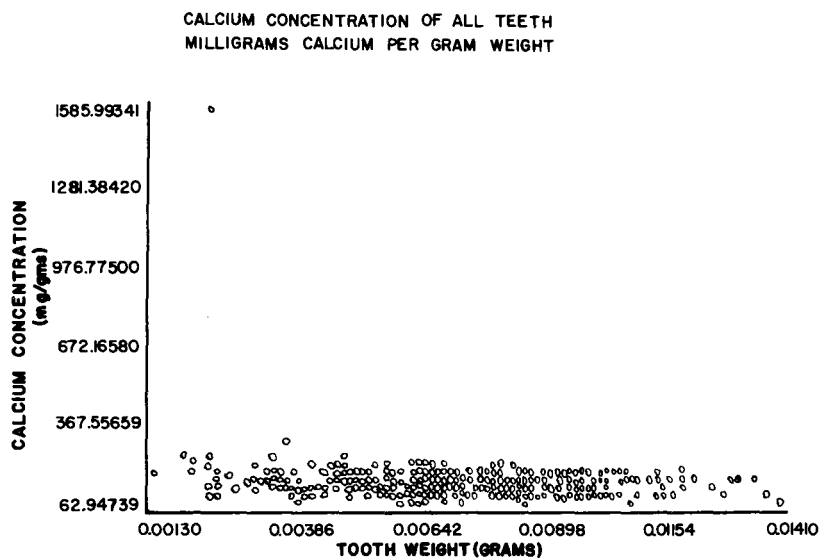


FIGURE 12

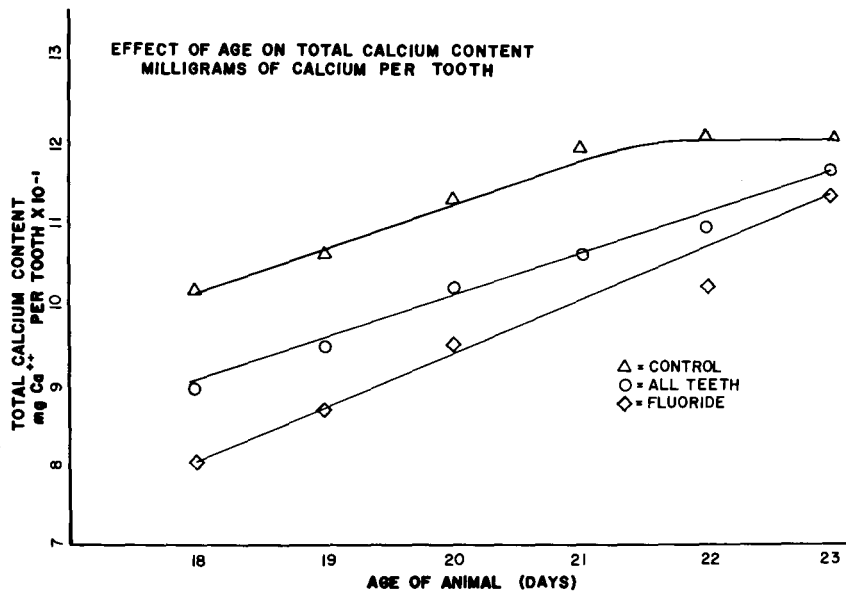


FIGURE 13

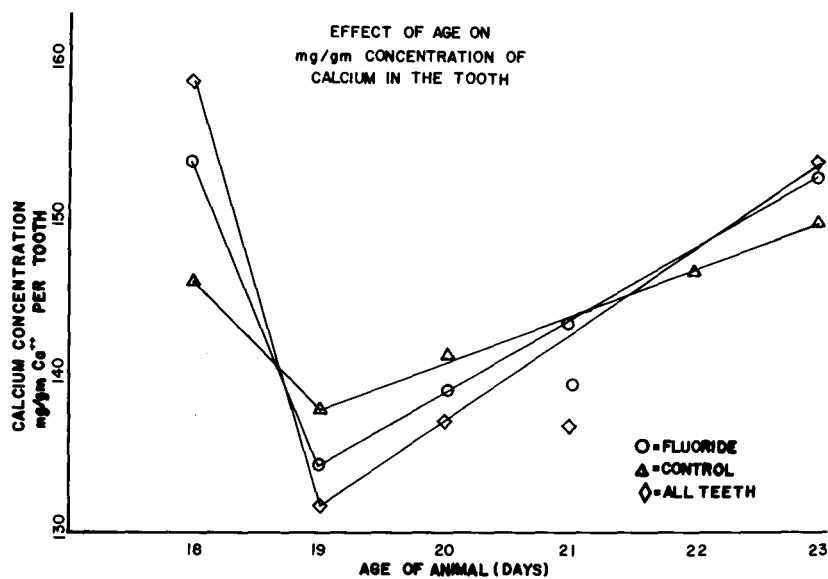


FIGURE 14

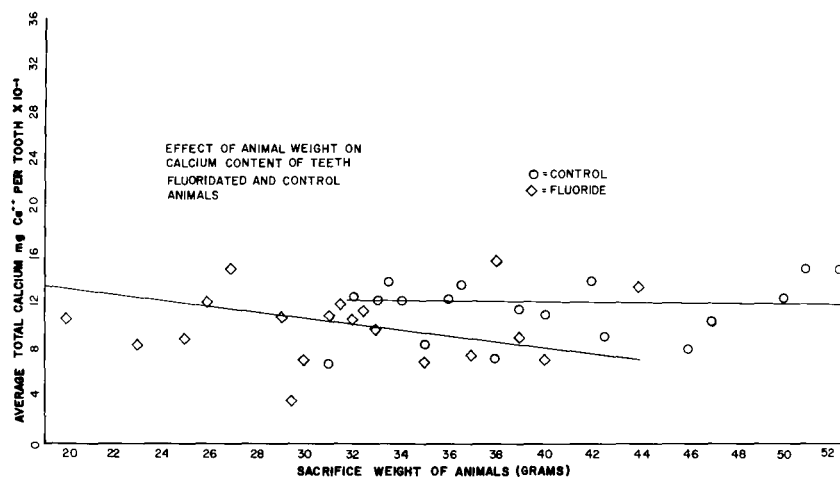


FIGURE 15

DISCUSSION

Although this study is not directly involved with caries incidence rates, the implication regarding the potential of prenatal fluoride is clear. The increased exposure of the developing tooth to fluoride has the potential for reducing caries over and above that of postnatal fluoridation alone. This implication has been demonstrated by Feltman and Kosel, 1961, Blayney and Hill, 1964, Katz and Muhler, 1968, and Glenn 1977 and 1979.

This study utilized the laboratory rat to investigate the effects of prenatal fluoride on tooth eruption and calcification with the desire to exact a better understanding of the action of fluoride on the developing tooth and its surrounding structures. Specifically studied were the parameters of tooth weight, body weight, total calcium content and calcium concentration of the rat molars, and molar development as revealed by eruption.

This study demonstrated that the effects of prenatal fluoride on the number of offspring is negligible. However, rats that were prenatally administered fluoride revealed a statistically significant reduction in body weight, tooth weight, and total calcium content. The calcium concentration in the molars of the fluoride treated animals was shown to be statistically the same as that of the control animals. No other abnormal growth variation was observed in control and fluoride treated dams or their offspring.

From the information gathered, a suggestion is made that the prenatally administered fluoride retarded or diminished the growth of

the fetus and their developing molar teeth. The observed reduction in total calcium content per tooth appears to be due to the reduction in tooth size which in turn is related to the smaller body weight of the fluoride-treated animals. The results of Lovius and Goose, 1969, Cooper and Ludwig, 1965, and Paynter and Grainger, 1956, are compatible with the findings in this investigation with regard to fluoride and reduced tooth size. No previous research is available showing a correlation between prenatal fluoride and body weight or size of the animal. However, Baume and Beck, 1954, speculated that fluoride is a thyroid inhibitor; thereby reducing the action of the growth hormone.

Inspection of the data shows that the total calcium content of the molars of the fluoride treated rats lagged 3-4 days behind the control rats. For example, the mean value of 22 day old fluoride animals (1.023 mg) approximated that of the 18 day old control animals (1.030 mg). The calcium determinations of this study corroborate previous reports by Yates, et. al., 1964, Walton and Eisenmann, 1974, and Gozariu, 1977, that fluoride influences and may interfere with the metabolism of the enamel producing cells. Then fluoride may modify the quality and size of the forming apatite crystals, as mineralization occurs. The period of active mineralization would seem to be the most important phase during which fluoride may induce changes in tooth morphology. It should be emphasized that in this study, the fluoride was administered during this critical period of mineralization of the rat molars.

It is interesting to note that no significant difference was demonstrated with the amount of total calcium per tooth with respect to age (18-23 days) in the control animals. However, the same parameter in

in the fluoride animals revealed a significant effect. It is suggested that the total calcium values in the control animals represent a plateau in growth and mineralization of the molar teeth. Whereas the calcium determinations of the molars in the fluoride treated rats increase with age, perhaps corresponding to an exponential phase prior to this leveling off in mineralization and growth of the molars as observed in the control animals.

Within the scope of this research, it appears that fluoride was responsible for the lagging effect on calcium content of the rat molars as seen in the fluoride-control animal comparison. However, an expanded population in terms of age (i.e. 1-23 days) may produce significantly different calcium content comparison for the control animals. This would lead to a better understanding of the mineralization pattern in rat teeth and the effect of fluoride.

Retardation in eruption was also observed in the fluoride treated rats. This effect of fluoride has been illustrated by Smith, 1934, Short, 1944, Baume and Beck, 1954, Feltman and Kosel, 1961, Kunzel, 1976, and Ericsson 1977. By 18 days postpartum as much as 92% of the fluoride treated rats showed a delay in the eruption of the first and second molars. Significant delays in eruption were also observed in the 19,20,21, 22, and 23 day old fluoride treated animals with a slight decrease in severity of delay with increasing age of the rats.

The explanation of these results may be correlated with available fluoride. Although fluoride determinations in the rat molars were not within the scope of this investigation, it is logical to assume that

following birth, the maternal exchange of fluoride via the placenta is eliminated, and that as the rats increase in age, the fluoride received and stored in the rat pups prior to birth is in turn metabolized resulting in a reduction of available fluoride to the growing rats. Therefore, the observed effect of fluoride is less pronounced in the older animals (23 days) as opposed to the younger animals (18 days).

This thought may be related to Blayney and Hill's, 1964, results that prenatal and postnatal fluoride in conjunction with each other is more beneficial in reducing caries incidence than either of them alone. The suggestion being that at the time the effect of prenatal fluoride is diminishing, a supplementation of postnatal fluoride is being administered resulting in a constant exposure of the developing tooth to the fluoride ion.

A future study involved in fluoride determinations from a time sequence in utero to an appropriate post partum date correlated with tooth eruption should be done to affect a better understanding of prenatal and postnatal fluoride metabolism and its apparent effect on tooth development.

Comparative photographs of the fluoride and control rat jaw quadrants also suggest an approximate delay of two days in the eruption of the first and second molars of the fluoride treated animals with respect to the control group. However, it is not possible to determine from this study whether the significant delay in eruption is attributable to a direct effect of the fluoride ion on the eruptive process, or merely the consequence of an overall inhibition in development of the fetus and postnatal rat. Whatever the mechanism involved in the delayed eruption of fluoride treated rats, there is apparently an overall increase in exposure

of the tooth to the fluoride ion prior to the tooth's entry or eruption into the oral cavity. This factor is of great significance with regard to caries incidence as the tooth is at its most susceptible period.

From a morphological standpoint, gross dissection of the teeth in preparation for analysis revealed an observation consistent with the findings of Forrest, 1956, Paynter and Grainger, 1956, Cooper and Ludwig, 1965, and Lovius and Goose, 1969, with respect to tooth size and quality. The molars of the fluoride treated rats appeared smaller in size with shallower pits and fissures. Inspection of the cusps revealed a more rounded appearance. Although dimensional measurements of tooth size were not recorded, this observation may be corroborated by tooth weight results and photographs of the fluoride treated rat molars.

The observed reduction in body weight, tooth weight, and calcium content per tooth, together with the smaller tooth size in the fluoride group gives credence to the hypothesis that fluoride exerts its biological effect at a systemic level. However, future studies are necessary if we are to fully understand the role played by the fluoride ion on the process of tooth development. Investigations are essential which would provide optimal amounts of fluoride to the prenatal animal while at the same time causing minimal adverse effects. These studies should also determine the concentration of fluoride present in the tissues.

If future studies prove to be conclusive, the benefit afforded by prenatal fluoride may then enhance preventive dentistry and be of unlimited service to mankind.

SUMMARY

The effect of prenatal fluoride on tooth eruption, body weight, tooth weight, total calcium content and calcium concentration of the molars as studied in newborn rats. The experimental dams had received 150 ppm sodium fluoride in their drinking water ad libitum during the period of gestation. The control dams were given water to drink without a fluoride supplement during this same time period.

The young rats studied were 18 to 23 days post partum. Both the first and second molars from the maxillary and mandibular arches were analyzed.

The mean body weight at sacrifice, mean weight of teeth and total calcium content of teeth were all significantly less in the fluoride treated rats compared to the control rat molars. No statistical difference as seen in the mean calcium concentration of the teeth studied.

Significant differences due to age were seen in body weight, tooth weight and total calcium content but not in the calcium concentration of the fluoride treated rats.

The control group showed a significant change in body weight with respect to age but not in tooth weight, total calcium content or calcium concentration per tooth.

A delay in molar eruption was notably observed in the fluoride treated rat pups which was estimated to be two days behind that of the control animals. Morphological differences were also noted in the teeth of the fluoride treated rats during their dissection. The molars of the fluoride group appeared smaller in size, shallower in pits and fissures and reduced in cusp dimension.

It was concluded from this study that prenatal fluoride caused a delay in tooth eruption and molar development. This effect is thought to be one of many on the process of tooth development and eruption as is witnessed by fluorides action on tooth morphology, tooth size, body weight and calcium content per tooth. The mechanism by which fluoride affects these basic biological processes is unknown. It is suggested that the action of prenatal fluoride is exerted largely at a systemic level; resulting in a smaller animal with smaller molars with reduced calcium content. However, these parameters as well as the observed delay in molar eruption after fluoride administration need further study.

The potential of prenatal fluoride is clear. It is accepted that the incorporation of fluoride into the tooth structure results in a tooth more resistant to caries. This additional benefit afforded by prenatal fluoride can only result in a greater reduction in caries especially when administered during the periods most critical in tooth development.

APPENDIX

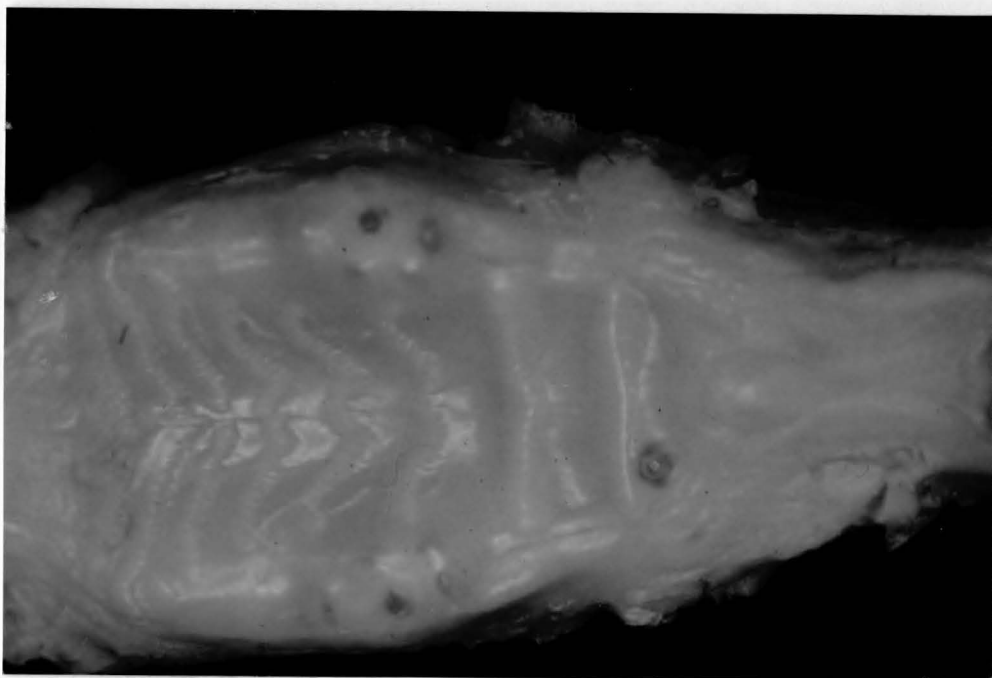


FIGURE 1 FLUORIDE 18 DAY OLD ANIMAL MAXILLAE

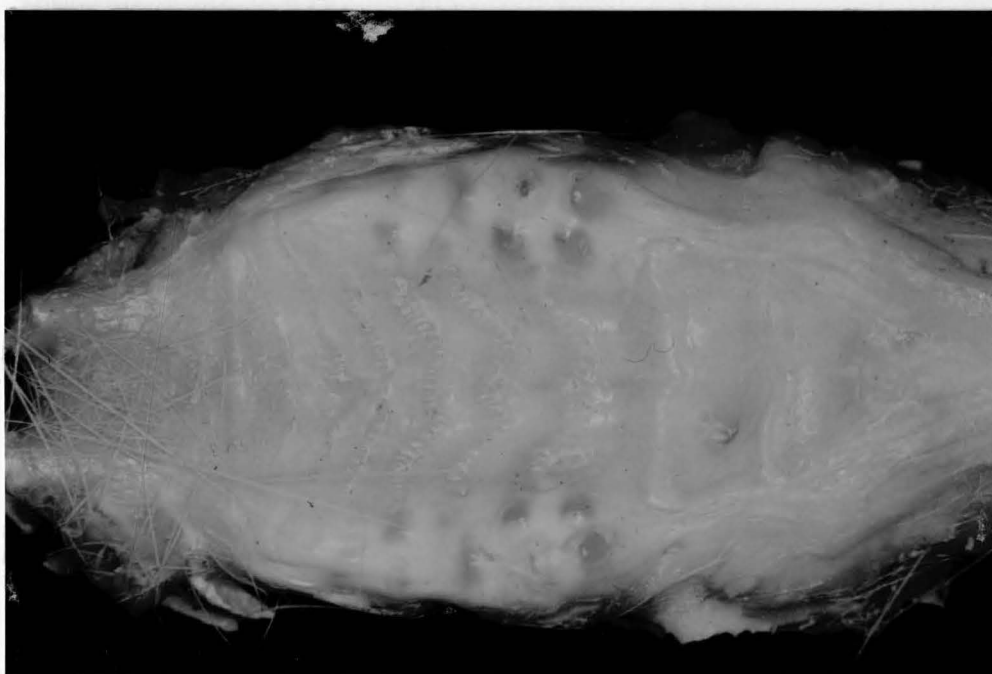


FIGURE 2 CONTROL 18 DAY OLD ANIMAL MAXILLAE

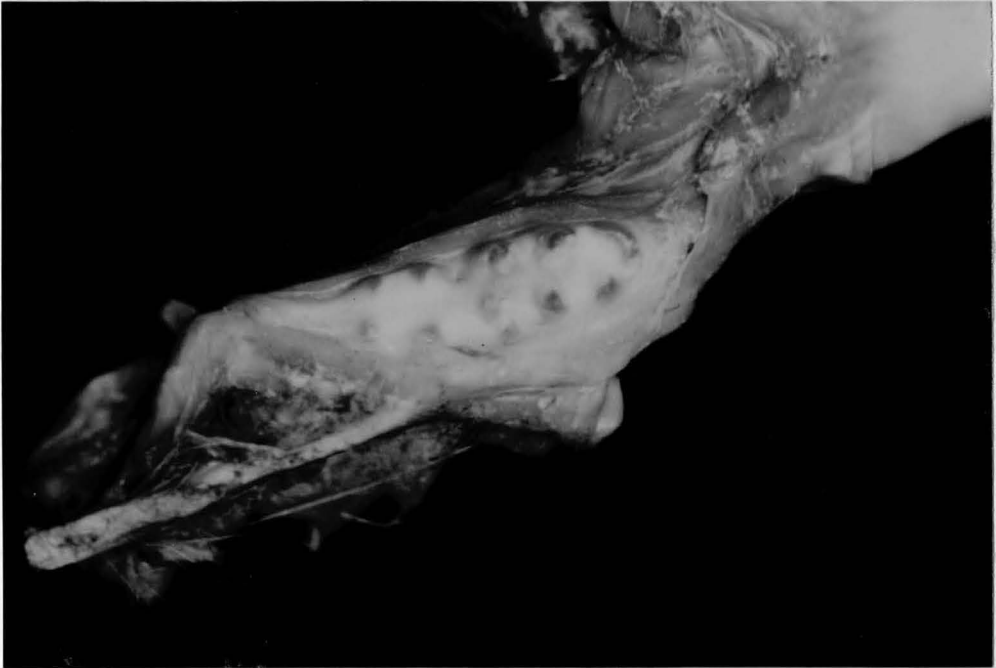


FIGURE 3 FLUORIDE 18 DAY OLD ANIMAL MANDIBLE

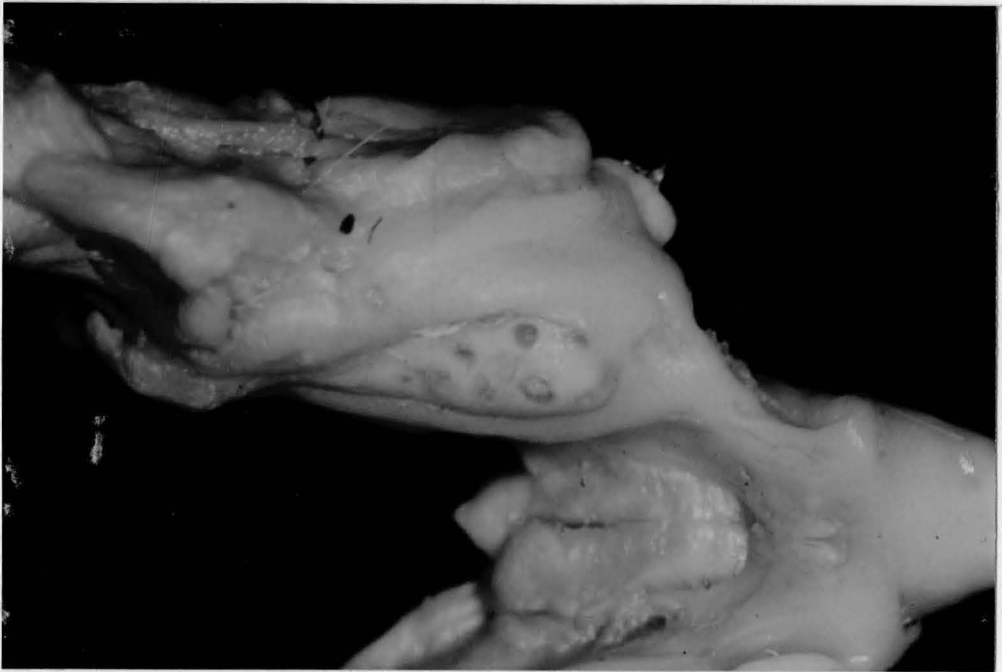


FIGURE 4 CONTROL 18 DAY OLD ANIMAL MANDIBLE

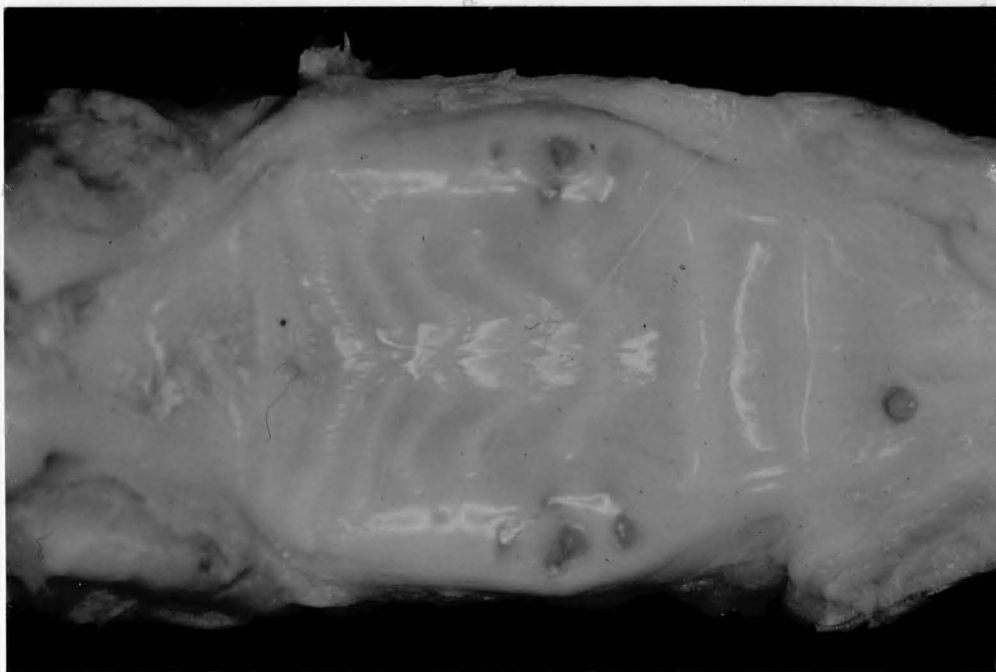


FIGURE 5 FLUORIDE 19 DAY OLD ANIMAL MAXILLAE

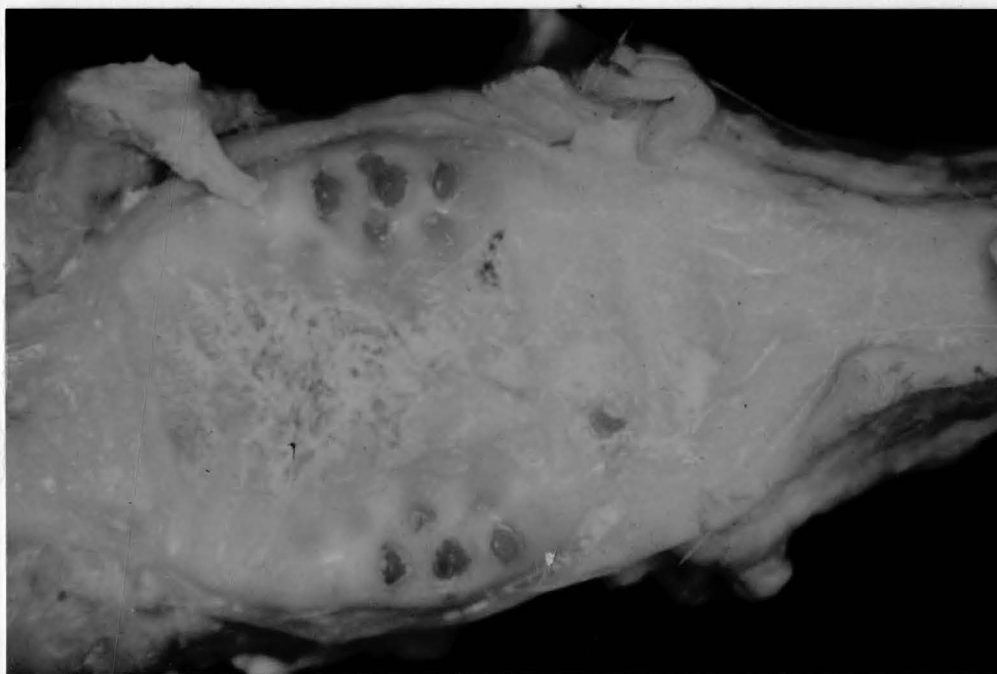


FIGURE 6 CONTROL 19 DAY OLD ANIMAL MAXILLAE

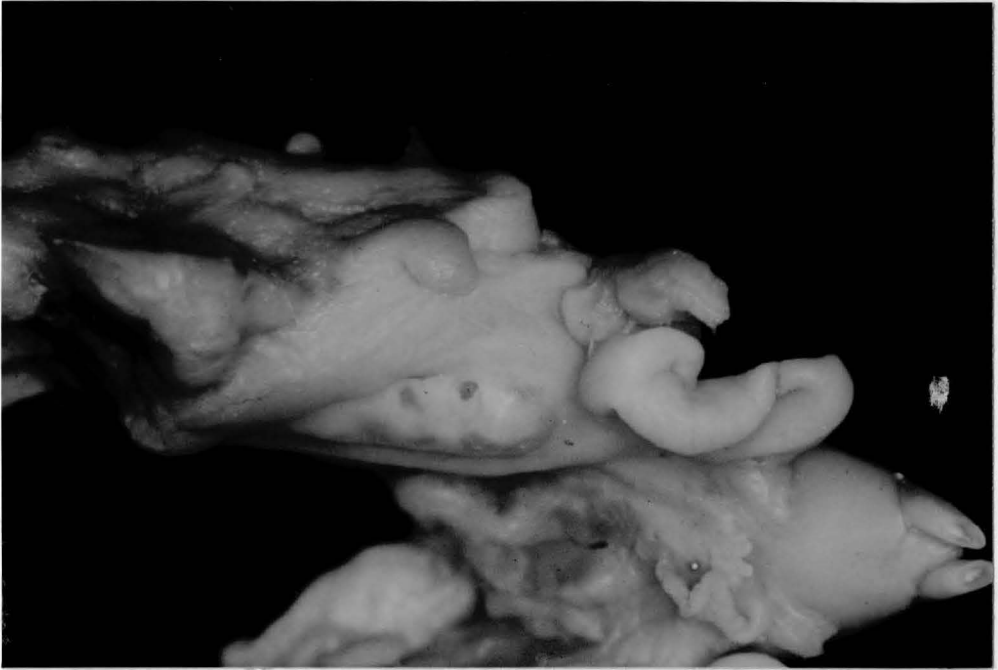


FIGURE 7 FLUORIDE 19 DAY OLD ANIMAL MANDIBLE

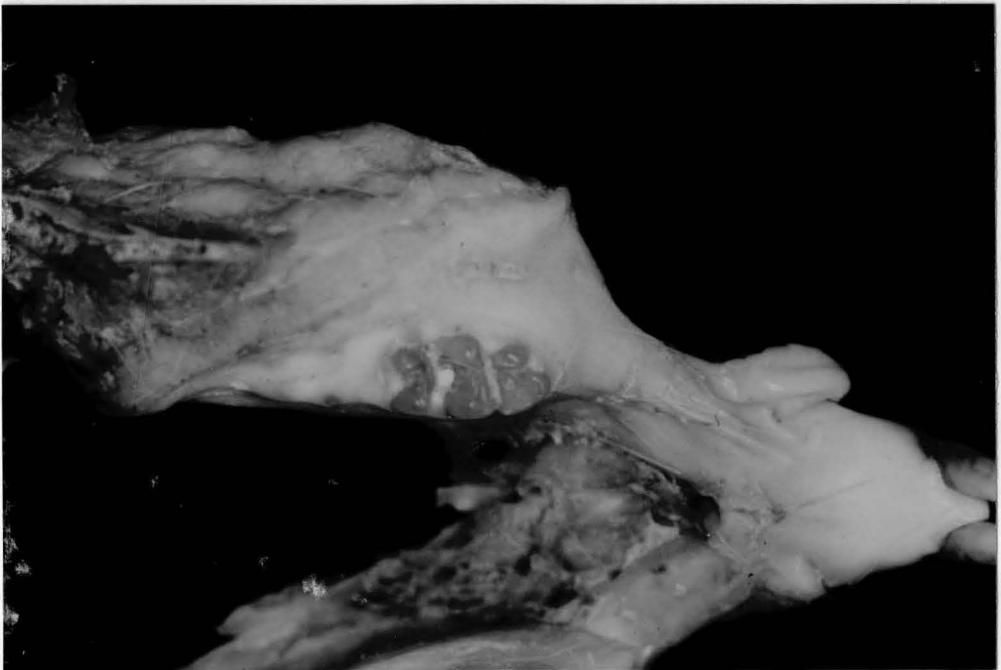


FIGURE 8 CONTROL 19 DAY OLD ANIMAL MANDIBLE

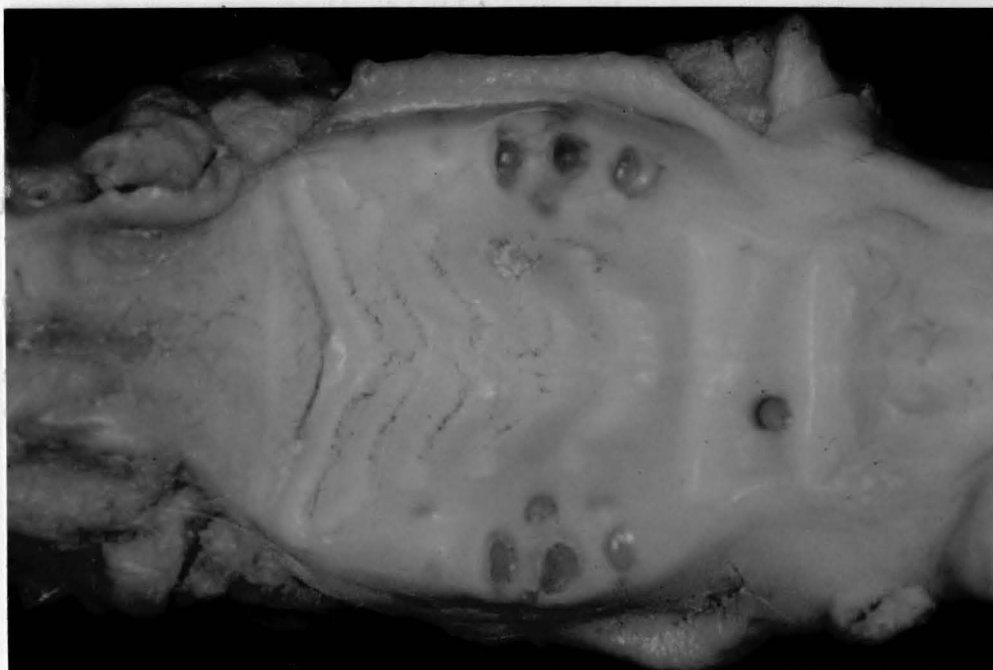


FIGURE 9 FLUORIDE 20 DAY OLD ANIMAL MAXILLAE

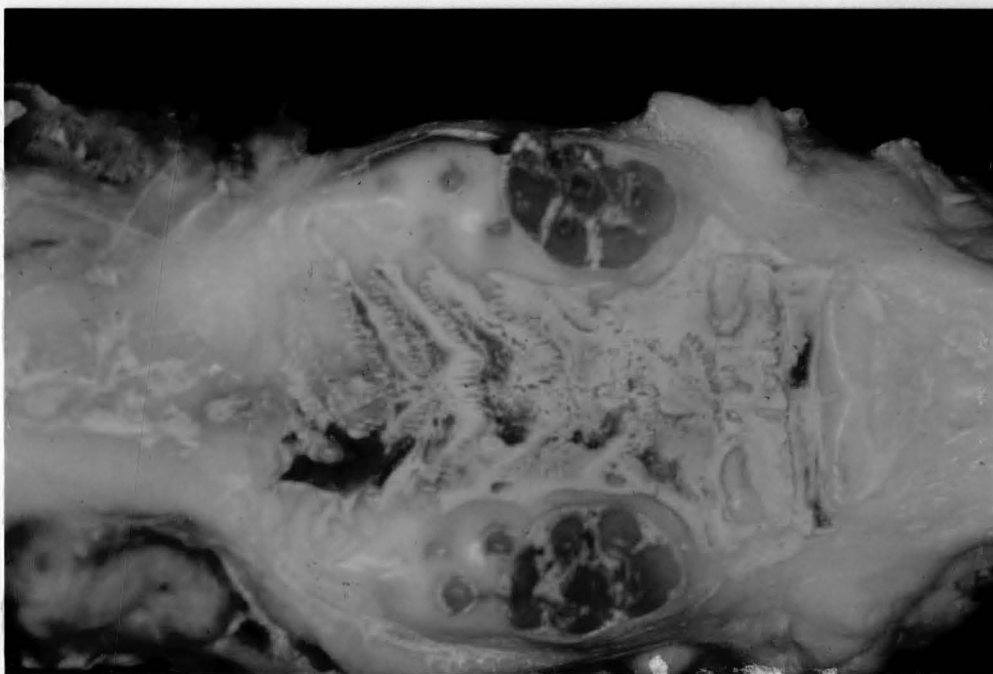


FIGURE 10 CONTROL 20 DAY OLD ANIMAL MAXILLAE

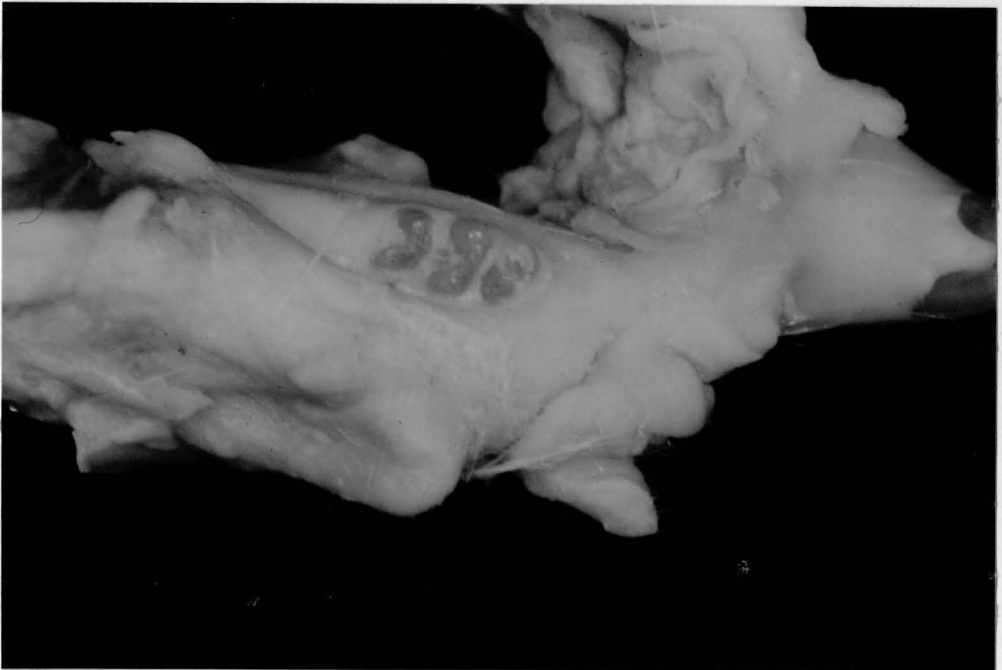


FIGURE 11 FLUORIDE 20 DAY OLD ANIMAL MANDIBLE

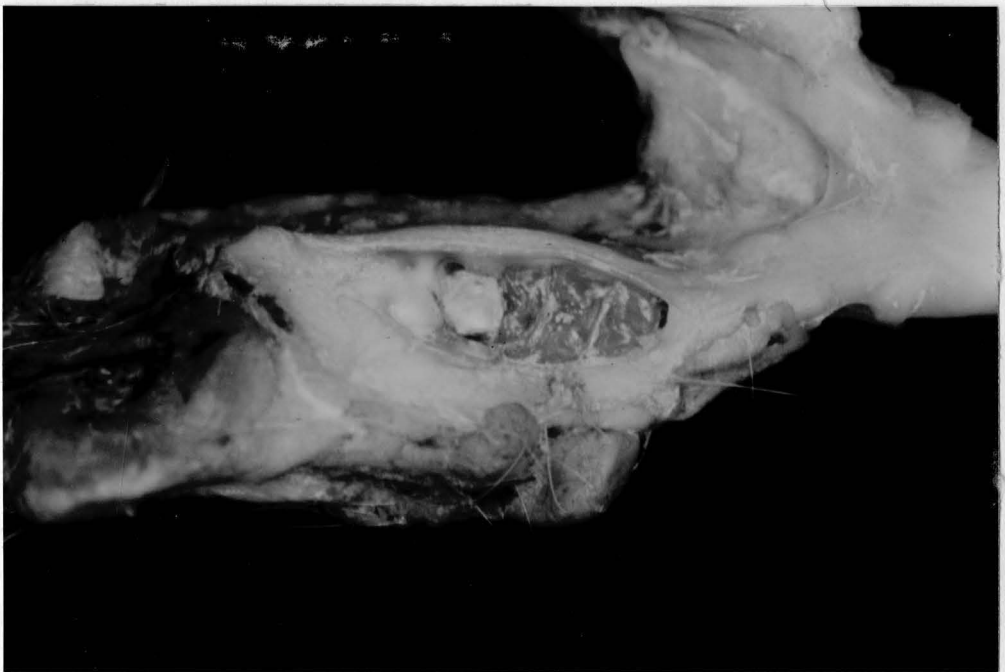


FIGURE 12 CONTROL 20 DAY OLD ANIMAL MANDIBLE

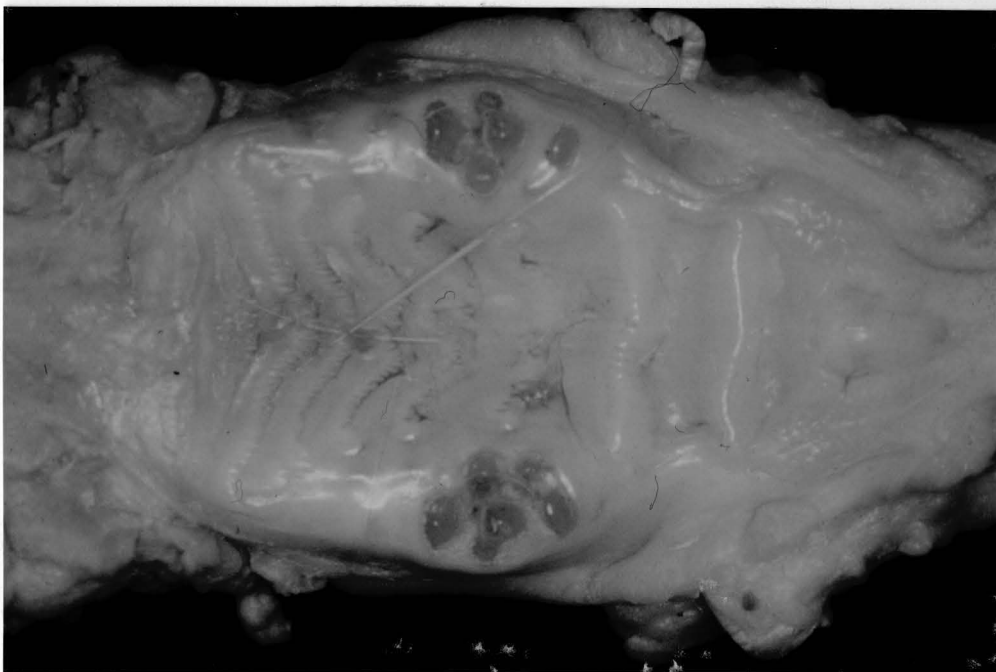


FIGURE 13 FLUORIDE 21 DAY OLD ANIMAL MAXILLAE

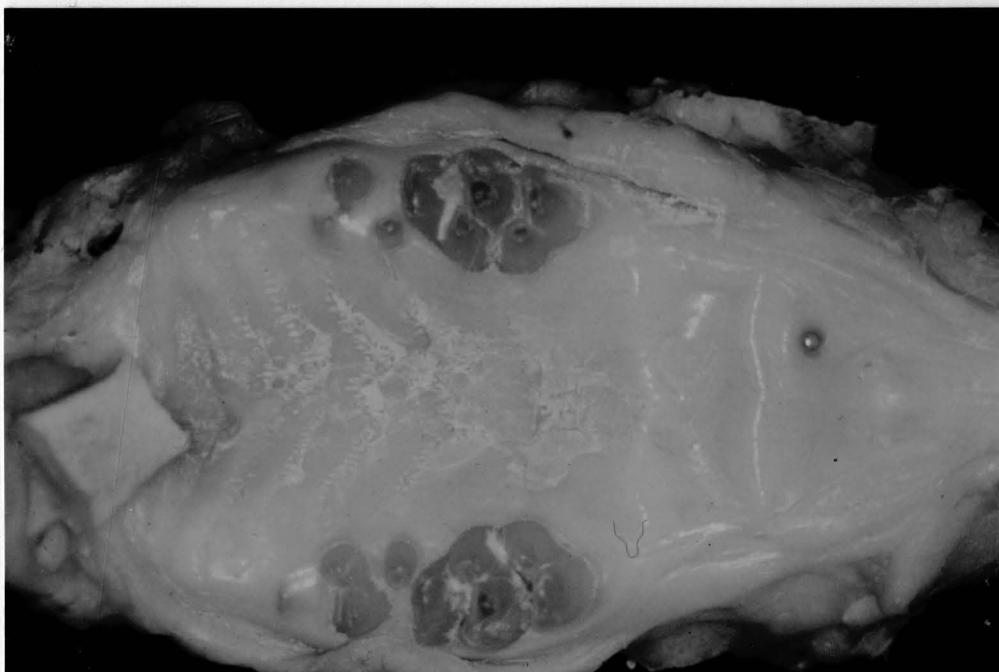


FIGURE 14 CONTROL 21 DAY OLD ANIMAL MAXILLAE

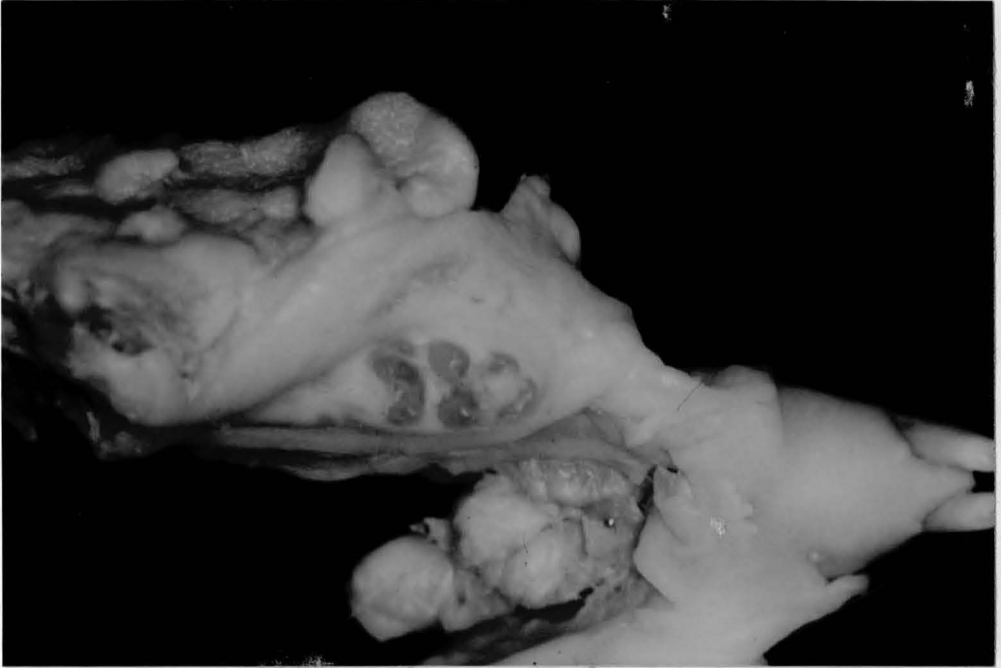


FIGURE 15 FLUORIDE 21 DAY OLD ANIMAL MANDIBLE



FIGURE 16 CONTROL 21 DAY OLD ANIMAL MANDIBLE

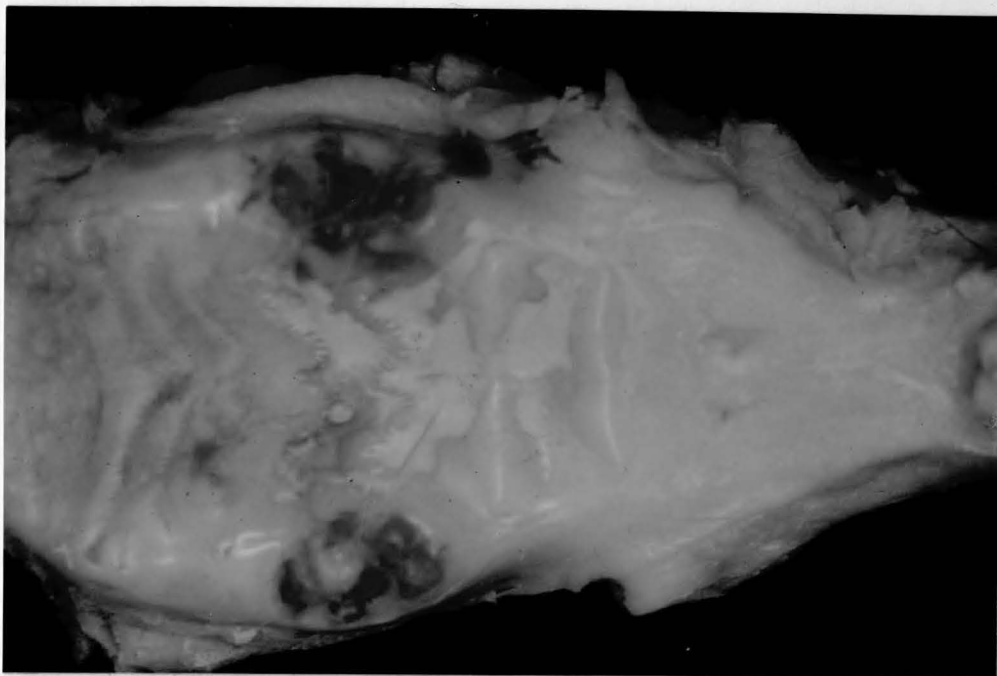


FIGURE 17 FLUORIDE 22 DAY OLD ANIMAL MAXILLAE

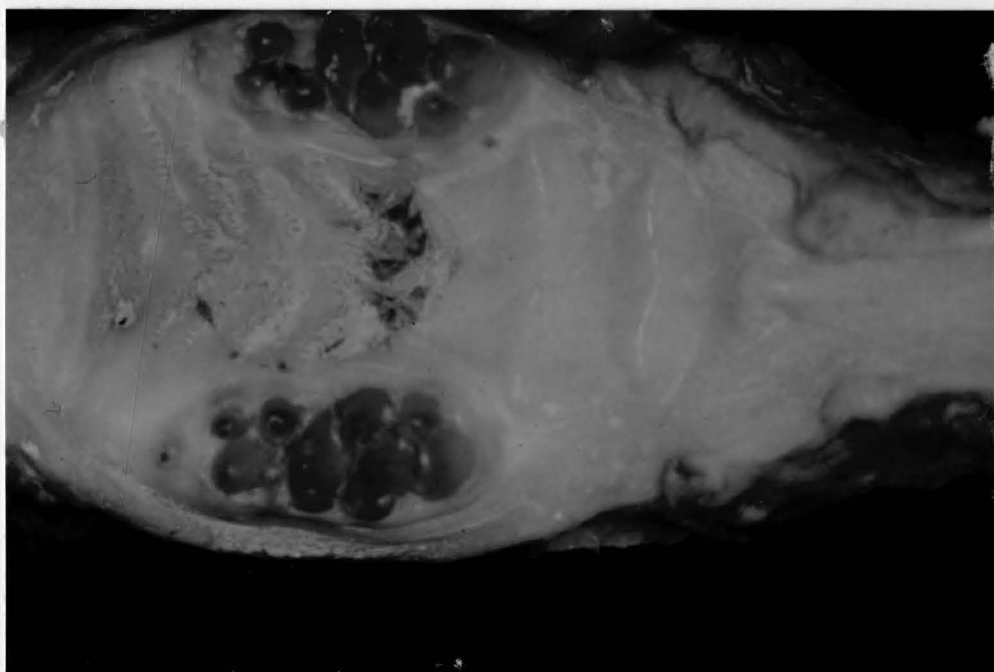


FIGURE 18 CONTROL 22 DAY OLD ANIMAL MAXILLAE

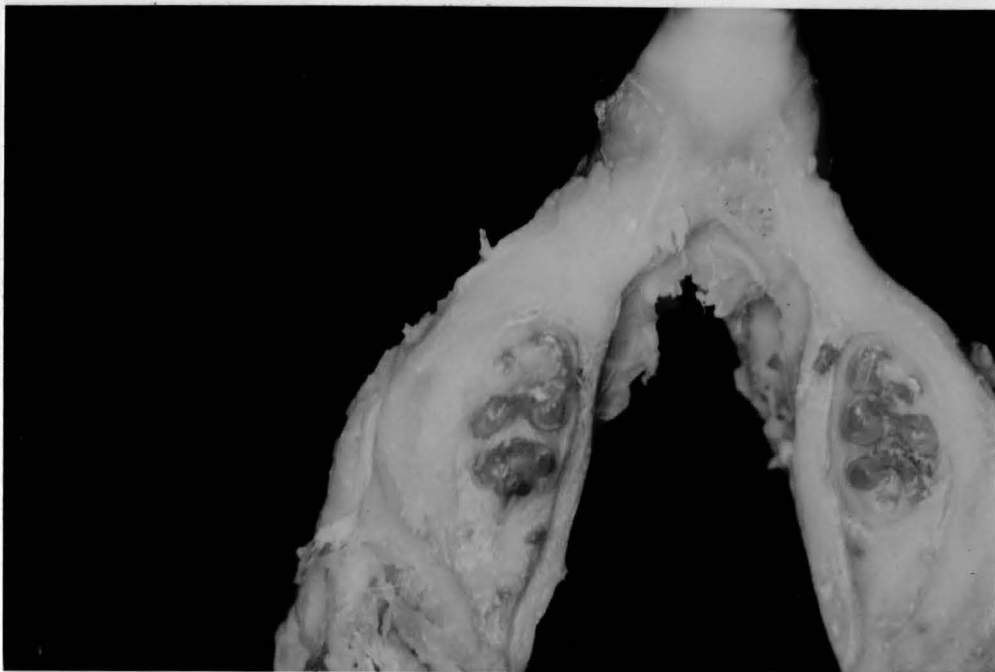


FIGURE 19 FLUORIDE 22 DAY OLD ANIMAL MANDIBLE

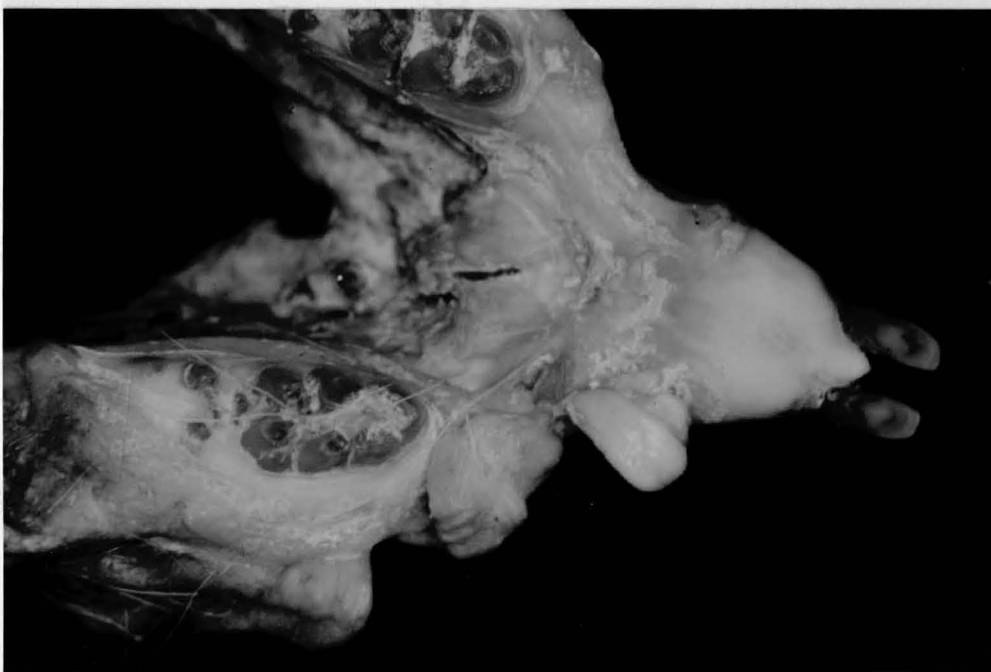


FIGURE 20 CONTROL 22 DAY OLD ANIMAL MANDIBLE

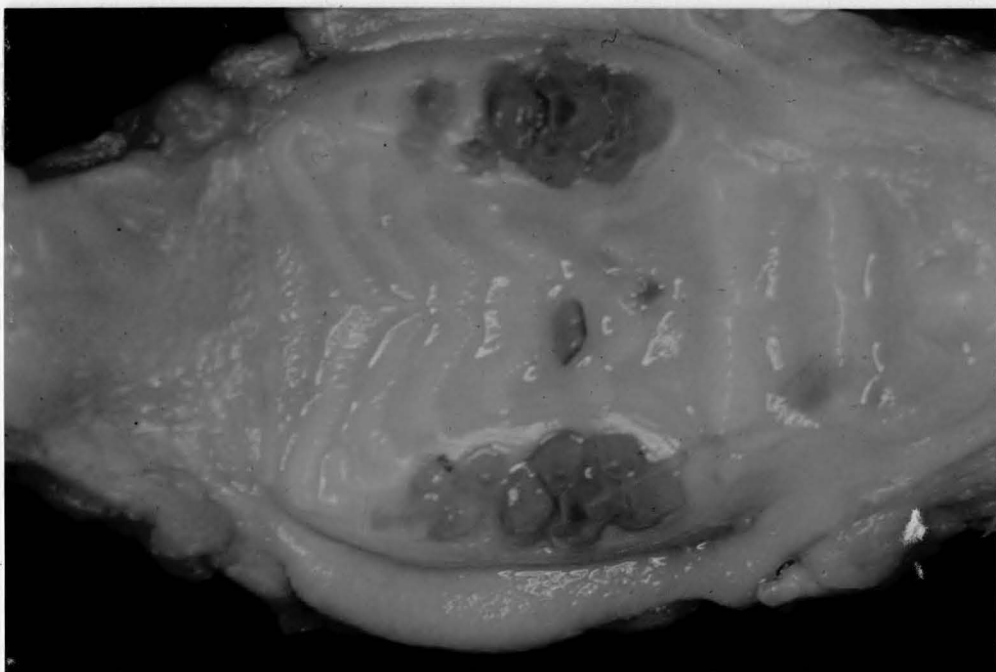


FIGURE 21 FLUORIDE 23 DAY OLD ANIMAL MAXILLAE

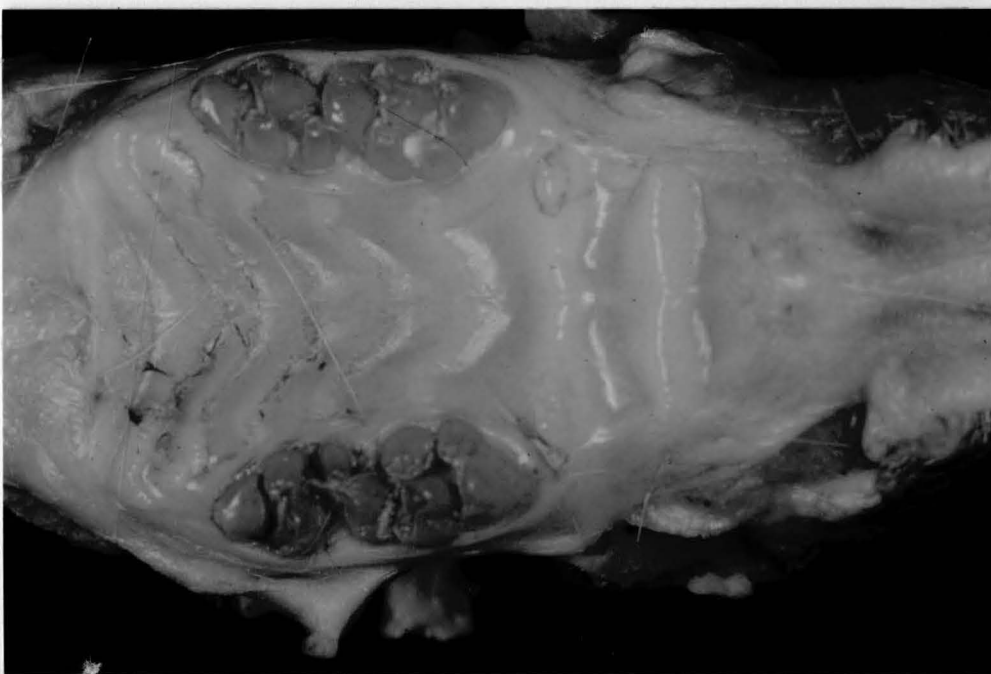


FIGURE 22 CONTROL 23 DAY OLD ANIMAL MAXILLAE

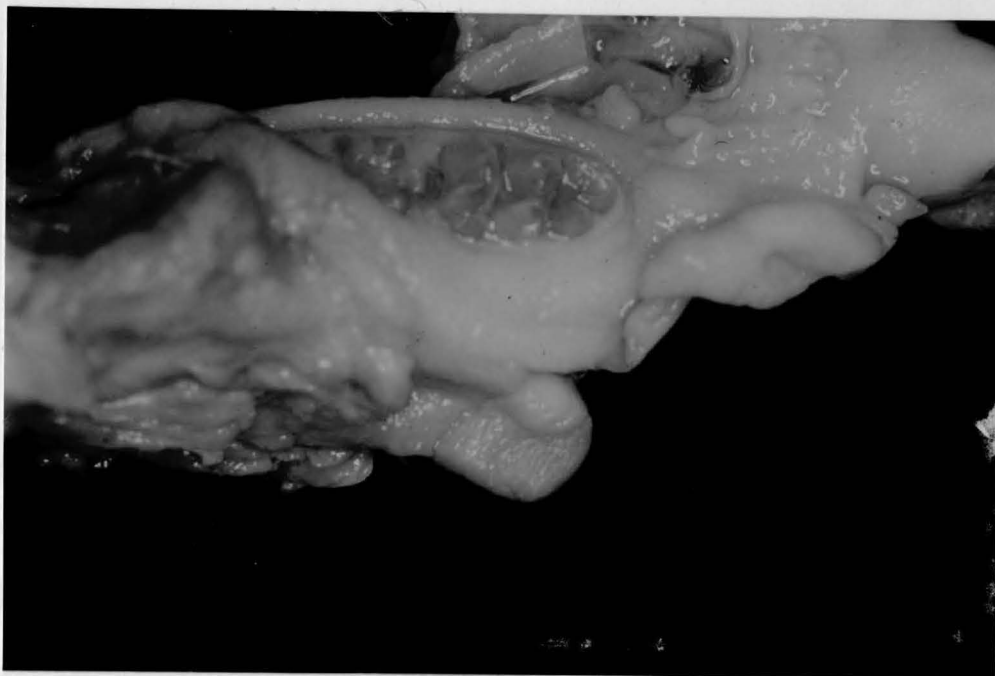


FIGURE 23 FLUORIDE 23 DAY OLD ANIMAL MANDIBLE

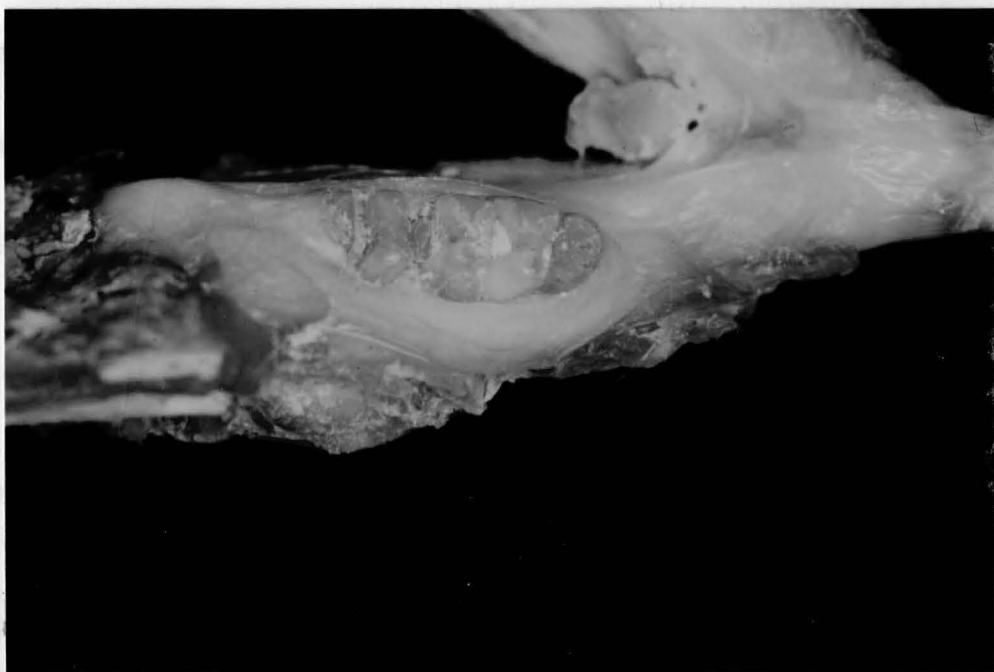


FIGURE 24 CONTROL 23 DAY OLD ANIMAL MANDIBLE

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APPROVAL SHEET

The thesis submitted by Mr. Daniel G. Ellenz has been read and approved by the following committee:

Dr. Michael L. Kiely, Chairman of Thesis Committee,
Associate Professor, Anatomy, Loyola

Dr. Ioannis S. Scarpa,
Assistant Professor, Biochemistry, Loyola

The final copies have been examined by the Chairman of the thesis committee and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form. The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science, Oral Biology.

November 26, 1980
Date

Michael L. Kiely Ph.D.
Chairman's Signature