Regeneration of Tooth Development In Vitro Following Sodium Fluoride Treatment [1]

MICHAEL A. KERLEY AND EDWARD J. KOLLAR
Department of Oral Biology, University of Connecticut Health Center, Farmington, Connecticut 06032

ABSTRACT Mandibular incisors were dissected from the jaws of 15- and 16-day C57BL/10 mouse embryos and cultured on agar-solidified Eagle's basal medium supplemented with fetal calf serum, an antibiotic, and glutamine. The experimental medium was the same as the control except that fluoride was added such that the final concentrations ranged from 2.0-8.0 mM NaF. Control and experimental explants were recovered after two, four and six days of incubation and studied histologically. After two days of fluoride treatment (3.0 mM NaF), cellular degeneration was observed in the dental papilla mesenchyme while the enamel organ epithelium appeared more resistant. Prolonged treatment or treatment at higher concentrations resulted in destruction of the dental papilla. The enamel organ was still present but was abnormal and reduced. Older tooth germs were less affected overall when incubated at the same fluoride dosage and time of treatment. When explants subjected to limited exposure (2 days) to fluoride were placed on control medium, the suppressed tooth germs recovered. The recovery was enhanced by grafting untreated mesenchyme to the treated explants followed by incubation on control medium. The observations indicate that NaF can suppress the development of tooth germs in vitro and that recovery from the suppresion does occur. The more severe inhibition observed in the mesenchymal component when compared to the response of the epithelial component of the treated explants suggests that fluoride may alter the ultimate morphology of the tooth crown by disrupting the normal epithelial-mesenchymal interaction which occurs during early tooth development.

Although the literature reports numerous studies concerning the effects of fluoride on the postnatal dentition of man and various rodent species, there have been few studies which have dealt with the influence of this ion on prenatal dental development. Alterations in the cell structure of ameloblasts, retardation of enamel matrix formation, and changes in the pulpal blood vessels were reported in mice which were exposed to fluoride as NaF and CaF_2 during gestation by way of the drinking water and injection (Fleming and Greenfield, '54). Paynter and Grainger ('56) described changes in the occlusal morphology and a size decrease in the molars of rats presented fluoride during pre-natal and early post-natal life. Molars characterized by shallow fissures with wide intercuspal angles and decreased enamel and dentin thickness were reported in rats treated with fluoride during the pre-eruptive period of early post-natal life (Kruger, '64, '66). Gray ('73) also found significant changes in the occlusal morphology of molars of rats exposed to fluoride during embryonic development.

Fleming ('53) observed alterations of ameloblasts and retardation of enamel and dentin maturation in embryonic mouse tooth germs which were transplanted to the axillae and brains of mice fed NaF in the drinking water. In addition, he reported that the teeth of neonatal offspring from the treated mice also showed a delay in calcification of the dental hard tissues and structural changes in the ameloblasts.

Morphological changes in teeth ascribed to

Accepted January 20, '77.
[1] Supported by NIH grant 5T22DE0202.

the presence of fluoride during gestation in man have also been reported. Reduced cusps and shallow grooves and fissures were observed in the molars of children from areas in which fluoride was present in the public water supply (Møller, '67). Lovius and Goose ('69) found smaller molar crowns in young boys reared in a fluoridated area. Molar teeth with increased mesio-distal widths, shallow fissures, and wide intercuspal angles were found in children who lived in an area containing fluoride in the public drinking water (Simpson and Castaldi, '69). Increased mesio-distal molar width was also observed in children from a fluoridated area by Wallenius ('59).

These studies indicate that fluoride significantly alters the generally conservative expression of the pattern of tooth morphology when present during early dental development. The developing tooth germ represents an epithelial-mesenchymal interaction in which the mesenchymal component, the dental papilla, acts as an inductive influence on the further differentiation of the epithelial component, the enamel organ. This influence determines whether the enamel organ will assume an incisiform or molariform morphological pattern (Kollar and Baird, '69, '70a,b). The pattern serves as the framework for the eventual deposition of the dental hard tissues, enamel and dentin, which ultimately establishes the anatomical features of the tooth crown. It was the purpose of this study to determine to what extent fluoride can affect the interaction of epithelium and mesenchyme during the development of the embryonic mouse incisor in vitro.

METHODS

Mandibular incisors were dissected aseptically from developing mandibles of 15- or 16-day C57BL/10 mouse embryos in a mixture of fetal calf serum [2] and Tyrode's [3] solution (1:4 v/v). The stage of development was dated from the appearance of a vaginal plug and subsequently verified by the external morphological criteria of Gruneberg ('43). Control explants were cultured for two to six days in a humidified atmosphere of 5% CO_2 in air at 37°C in plastic organ culture dishes containing 1 ml of agar-solidified Eagle's basal medium supplemented with an antibiotic (Gentamicin [4]), 1% glutamine, and 10% fetal calf serum, according to the procedure previously described by Kollar and Baird ('68). The experimental medium was the same as the control except that NaF was added to a final concentration ranging from 2.0 to 8.0 mM. The explants were changed to fresh medium every two days. Some experiments involved growth on the experimental medium for two days followed by four days of incubation on control medium. In one recovery experiment, after two days of fluoride treatment the suppressed explants were positioned adjacent to isolated but otherwise untreated dental mesenchymal tissue at the beginning of the control incubation period. The isolated dental supplements were obtained by separating the enamel organ from the dental papilla of dissected 17-day embryonic mandibular first molars which had been treated for one to two hours at 4°C with 1% trypsin (1:250)[5] in Tyrode's solution (Kollar and Baird, '69). A total of 212 explants were used; 96 control, 66 fluoride-treated, and 50 fluoride-treated followed by a recovery period on control medium. The recovery experiments included five treated explants which were combined with untreated mesenchyme. After two, four and six days of growth, control and experimental explants were fixed in Zenker's-acetic acid fixative. Following fixation, all tooth germs were processed through routine histological procedures for light microscopy, embedded in Paraplast,[6] sectioned at 6 μm, and stained with hematoxylin and Biebrich's scarlet.

RESULTS

By the fifteenth day of gestation, the mouse mandibular incisor has developed into a structure composed of an invaginated epithelium, the enamel organ, incorporating a mesenchymal condensation, the dental papilla (fig. 1). The enamel organ consists of the inner enamel epithelium which invests the dental papilla as a layer of undifferentiated columnar cells and renders the tooth germ its characteristic spatulate form marked by a depression at the anterior (incisal) end and the cervical loop at the posterior end. The cervical loop represents the point at which the inner enamel epithelium is contiguous with the outer enamel epithelium, the other dominant feature of the enamel organ at this stage of development. The outer enamel epithelium continues anteriorly from the cervical loop

[2] Grand Island Biological Company, Grand Island, New York.
[3] Colorado Serum Company, Denver, Colorado.
[4] Schering Corporation, Port Reading, New Jersey.
[5] Difco Laboratories, Detroit, Michigan.
[6] Sherwood Medical Industries, St. Louis, Missouri.

and connects the developing tooth with the oral epithelium. Even during the early stages the labial aspect of the enamel organ is convex and longer than the concave lingual border. The principle feature of the presumptive dental pulp, the dental papilla, at this stage of development is the peripheral pre-odontoblast cell layer which lies subjacent to the inner enamel epithelium.

After two days of growth in vitro on control medium, the basic structure of the tooth germ remained unchanged (fig. 2). However, in some explants areas of pre-odontoblasts had begun to differentiate on the labial aspect of the incisor as evidenced by the polarization of the nuclei (fig. 3). The pre-odontoblast cell layer was separated from the inner enamel epithelium by a basement membrane. The labial inner enamel epithelium, in many cases, had begun to differentiate in that the nuclei had migrated some distance away from the basement membrane (fig. 3). After six days of culture on control medium, the explants had advanced their development; the labial inner enamel epithelial cells, now clearly identified as ameloblasts, were characterized by a transposition of their nuclei to a position distal to the odontoblast cell layer (figs. 4, 5).

Figure 6 illustrates an excised tooth germ incubated for six days on medium containing NaF at a final concentration of 2.0 mM. The differentiation of the explant was normal; the ameloblasts and odontoblasts had developed to a stage similar to that attained by the controls after six days of growth.

However, when the medium contained 3.0 mM NaF, suppression of development after two days of growth was suggested by cellular degeneration observed in the presumptive pulp and a reduced enamel organ. In a few explants, such as shown in figure 7, the overall size of the tooth germ was not affected but the anterior portion of the mesenchyme was characterized by widely dispersed abnormal cells, many of which were pycnotic (fig. 8). In addition, areas of the enamel organ adjacent to the affected mesenchyme consisted of reduced cells rather than the normal columnar type (fig. 8). On the other hand, the cells of the posterior mesenchyme and enamel organ were normal in appearance. However, there was no evidence of a pre-odontoblast cell layer or basement membrane separating it from the enamel organ (fig. 9).

In some cases, as illustrated in figure 10, the entire enamel organ was substantially reduced. Moreover, degeneration was noted throughout the mesenchyme rather than in a localized area. In most of the treated explants the overall size of the structure was considerably smaller than in controls. Following six days of incubation at this fluoride concentration (3.0 mM NaF), the characteristic curved structure was the predominant morphological feature although much reduced in size (fig. 11). Occasionally, columnar cells could be observed in the enamel organ of a few of the tooth germs. Typically, in most of the explants the entire enamel organ was affected. The dental papilla was severely suppressed although a few viable cells were present in some of the explants.

Older tooth germs were more resistant to the effects of fluoride. A 16-day embryonic incisor cultured for six days on medium containing 3.0 mM NaF is illustrated in figure 12. A few of the explants were not reduced in size although even in these cases abnormal cells and the absence of differentiated odontoblasts were noted in the anterior region (fig. 13). In areas of the enamel organ, the cells were differentiated as indicated by their characteristically polarized nuclei (fig. 13). The stage of development attained by the 16-day experimental explants compared much more favorably with the controls after six days of growth (fig. 14) than the 15-day experimental explants.

At higher concentrations of NaF, such as 4.0 mM, the 15-day explants were affected to an even greater extent (fig. 15). After two days of growth, the enamel organ appeared as a reduced epithelial remnant and the dental papilla was virtually destroyed. After four days of growth in the presence of 4.0 mM NaF the severely suppressed enamel organ was still recognizable as such (fig. 16). The persistence of mitotic figures in the suppressed epithelium emphasized the relative resistance of the dental epithelium to the effects of fluoride when contrasted to the response of the dental mesenchyme (fig. 17). Incubation at concentrations higher than 6.0 mM resulted in such severe cellular degeneration and necrosis that the previous histological organization could only be surmised from a few of the remnants.

Significantly, reversal of the effects of fluoride was observed in explants exposed to lower dosages. Figure 18 illustrates a 15-day incisor tooth germ placed on control medium for four days following two days exposure to

3.0 mM NaF. Odontoblasts and ameloblasts appeared in their characteristically palisaded arrangement. The stage of development reached was similar to that of the controls following six days of growth. Recovery from the suppressive effects of fluoride was also observed in explants placed on control medium for four days following two days' exposure to 4.0 mM NaF (fig. 19). However, unlike those cases recovering from the lower fluoride concentration, the enamel organ recovering from 4.0 mM NaF was less well differentiated; none the less, mitotic figures were present in this severely suppressed epithelium. Differentiated odontoblasts were present and presumably with additional time on control medium these more severely inhibited explants would have advanced their development even further.

The developing tooth germ represents an epithelial-mesenchymal interaction. This interaction involves an inductive influence exerted by the mesenchymal component, the dental papilla. In view of this, an attempt was made to further advance the stage of differentiation achieved by the recovering explants exposed for two days to 4.0 mM NaF by incubating the treated tooth germs on control medium with additional mesenchyme obtained from 17-day embryonic molars. The rationale used here was that recovery of the dissimilar dental papilla population would be enhanced by the addition of fresh untreated mesenchyme. Thus, interaction with enamel organ tissue would not be delayed but would begin immediately. The untreated mesenchyme was grafted to the surface of the treated incisor explants and then incubated for four days on control medium. The stage of differentiation attained by both mesenchyme and epithelial components of the treated incisor explant appeared similar to that observed in the recovered explants without grafts (fig. 20). However, differentiated odontoblasts were also observed in the grafted mesenchyme opposite a layer of columnar cells derived from the treated enamel organ surface (fig. 21). This observation not only demonstrated that the capability of response to an inductive influence from mesenchyme had not been lost by the treated enamel organ, but also that the enamel organ surface cells, normally represented by the outer enamel epithelial cell layer, may participate in tooth formation when the appropriate inductive stimulus is present.

DISCUSSION

The morphological pattern of a tooth is determined by genetic influences but structural alterations can be inflicted by traumatizing external factors present during development (Paynter and Grainger, '61). Dental development is dependent upon such basic biological mechanisms as energy transfer and protein synthesis which are required in embryological systems undergoing rapid growth and differentiation. Thus, fluoride, a known enzyme inhibitor of glycolysis and cellular respiration could possibly affect organ morphodifferentiation through an inhibitory influence upon the normal cellular metabolic pathways. Indeed, sodium fluoride administered in a concentration range comparable to that used in this study has been shown to inhibit such embryonic tissues differentiating in vitro as the developing chick feather (Kisher and Hamilton, '63) and heart (Spratt, '50; Duffey and Ebert, '57) and the fusion of the palatal shelves in rats (Myers, '71). Of these systems, chick feather development, like that of the developing tooth germ, results from an interaction between epithelial and mesenchymal tissue (Cairns and Saunders, '54; Rawles, '63). The reduced growth due to fluoride manifested by these structures was thought to be the result of utilization of an alternate glycolytic pathway or a variation of some specific pathway.

The reported minimum fluoride concentration (38-57 ppm F) required for an observable effect upon dental development in this in vitro study is comparable to that (20 ppm F) utilized in the studies in vivo of Fleming and Greenfield ('54), in which molar morphological changes were described in mice fed fluoride by way of the drinking water during pre-natal life. Similarly, this value is consistent with the fluoride concentrations (12-50 ppm F) used in studies in vivo of altered molar morphology reported by Paynter and Grainger ('56) and Gray ('73) in rats administered fluoride during embryonic development. Although these values are somewhat higher than those usually encountered in human clinical studies (1-2 ppm F), the data reported here are probably due to a species difference; the mouse is known to be highly resistant to the toxic effects of this ion (Weber and Reid, '69). Nevertheless, the demonstrated sensitivity of the mesenchyme to fluoride in a system in which differentiation is directed by an epithelial-mesenchymal interaction sug-

gests that the previously described alterations in dental morphology due to the presence of this agent during pre-natal development may be related to an inhibition of the normal influence of the dental papilla upon the enamel organ.

The light microscopic observations reported here do not indicate a specific mechanism of action for fluoride as it affects the cellular metabolism involved in tooth germ development. However, several observations suggest that the ion exerted a differential metabolic inhibition rather than an action of general toxicity. Cellular activity, inferred from the presence of mitotic figures, was never completely arrested in the experimental explants, except in the most severely affected cases, and appeared unaffected by the fluoride in both enamel organ and mesenchyme of tooth germs subjected to limited exposure to the ion. The explants were differentially affected by increasing fluoride dosages, that is, the tooth germs cultured on medium containing 3.0 mM NaF appeared inhibited to an intermediate degree when compared to the normal appearing explants exposed to 2.0 mM NaF and the severely suppressed explants exposed to 4.0 mM NaF. These considerations, as well as the observation that reversal of the suppressive effects of fluoride occurred when the less severely affected explants were transferred to control medium, support the conclusion that fluoride acted as a metabolic inhibitor of development.

The results presented here clearly indicate that the mesenchymal component of the tooth germ is much more sensitive to the effects of fluoride than the epithelial component. In some experiments the enamel organ, although reduced, not only retained the characteristic incisor morphology but also demonstrated cellular activity as indicated by the presence of mitotic figures. In contrast, the mesenchymal area was virtually devoid of viable cells. In other experiments involving limited growth on medium containing fluoride, the more resistant enamel organ was inhibited only in an area adjacent to the affected mesenchyme, indicating that an alteration in the interaction between epithelium and mesenchyme might be involved. Although older tooth germs were more resistant to the effects of fluoride, i.e., either a high fluoride concentration or an increased incubation period was required in experiments utilizing the 16-day incisor in order to obtain the same effects observed in the 15-day incisor, the localized pattern of mesenchyme suppression adjacent to enamel organ inhibition seen in younger explants was also apparent in 16-day incisor explants cultured for longer periods of time at the same fluoride dosage utilized in the 15-day incisor experiments.

The observations are significant when considered with the known concept that the developing tooth germ represents an epithelial-mesenchymal interaction (Koch, '67, Kollar and Baird, '69, and Slavkin, '74). The mesenchymal component, which exerts an inductive and organizing influence upon the differentiation of the epithelial component, determines whether the morphology of the induced enamel organ will eventually be established as molariform or incisiform (Kollar and Baird, '69, '70a). Thus, the previous findings implicating fluoride in alterations of crown morphology as a result of exposure to the ion during the time of early dental development may be explained by the adverse influence of the agent upon the normal mesenchymal control of the further diferentiation of the enamel organ.

The enamel organ, while not affected to the same degree as the dental papilla, on the other hand could be severely suppressed with elevated fluoride concentrations. Recovery from fluoride inhibition was made obvious by the differentiated ameloblasts observed in explants transferred to control medium following treatment. In one recovery experiment additional untreated mesenchyme was grafted to the outer epithelial surface of treated explants. After four days of growth on control medium, the enamel organ consisted of differentiated odontoblasts derived from both the treated and untreated mesenchymal components. These observations suggest that the interior enamel organ cell layer could recover from the fluoride treatment and, moreover, that the surface layer was also capable of responding to inductive mesenchymal signals. Thus, the enamel organ surface layer of these explants, which in situ is termed the outer enamel epithelium, possesses the necessary cellular capabilities for participation in epithelial-mesenchymal interactions leading to tooth formation.

LITERATURE CITED

Cairns, J. M., and J. W. Saunders, Jr. 1954 The influence of embryonic mesoderm on the regional specification of epidermal derivatives in the chick. J. Exp. Zool., *127:* 221-248.

Duffey, L. M., and J. D. Ebert 1957 Metabolic characteristics of the heart-forming areas of the early chick embryo. J. Embryol. exp. Morph., *5:* 324-339.

Fleming, H. S. 1953 Effect of certain concentrations of fluoride on enamel and dentin as formed in transplants of tooth germs and related studies. J. Dent. Res., *32:* 469-485.

Fleming, H. S., and V. S. Greenfield 1954 Changes in the teeth and jaws of neonatal Webster mice after administration of NaF and CaF_2 to the female parent during gestation. J. Dent. Res., *33:* 780-788.

Gray, H. S. 1973 A morphological study of the influence of fluoride on rat molar teeth. Archs. Oral Biol., *18:* 1451-1460.

Gruneberg, H. 1943 The development of some external features in mouse embryos. J. Hered., *34:* 89-92.

Kischer, C. W., and H. L. Hamilton 1963 Effects of respiratory inhibitors on development of the down feather. Am. J. Anat., *113:* 101-115.

Koch, W. E. 1967 *In vitro* differentiation of tooth rudiments of embryonic mice. I. Transfilter interaction of embryonic incisor tissues. J. Exp. Zool., *165:* 155-170.

Kollar, E. J., and G. R. Baird 1968 Effect of beta-2-thienylalanine on developing mouse germs *in vitro*. J. Dent. Res., *47:* 433-443.

——— 1969 The influence of the dental papilla on the development of tooth shape in embryonic mouse tooth germs. J. Embryol. exp. Morph., *21:* 131-148.

——— 1970a Tissue interactions in embryonic mouse tooth germs. I. Reorganization of the dental epithelium during tooth-germ reconstruction. J. Embryol. exp. Morph., *24:* 159-171.

——— 1970b Tissue interactions in embryonic mouse tooth germs. II. The inductive role of the dental papilla. J. Embryol. exp. Morph., *24:* 173-186.

Kruger, B. J. 1964 Pre-eruptive and post-eruptive effect of fluoride on rat molars. Aust. Dent. J., *9:* 90-93.

——— 1966 Interaction of fluoride and molybdenum on dental morphology in the rat. J. Dent. Res., *45:* 714-725.

Lovius, B. B. J., and D. H. Goose 1969 The effect of fluoridation of water on tooth morphology. Brit. dent. J., *127:* 322-324.

Møller, I. J. 1967 Influence of microelements on the morphology of the teeth. J. Dent. Res., *5:* 933-937.

Myers, G. S. 1971 Effect of sodium fluoride and sodium pyruvate on palatal development *in vitro*. Anat. Rec., *171:* 39-52.

Paynter, K. J., and R. M. Grainger 1956 The relation of nutrition to the morphology and size of rat molar teeth. J. Canad. Dent. Assoc., *22:* 519-531.

——— 1961 Influence of nutrition and genetics on morphology and caries susceptibility. J. Amer. Med. Assoc., *177:* 306-309.

Rawles, M. E. 1963 Tissue interactions in scale and feather development as studied in dermal-epidermal recombinations. J. Embryol. exp. Morph. *11:* 765-789.

Simpson, W. J., and C. R. Castaldi 1969 A study of crown morphology of newly erupted first permanent molars in Wetaskiwin, Alberta (optimum fluoride) and Camrose, Alberta (low fluoride). Odont. Revy, *20:* 1-14.

Slavkin, H. C. 1974 Embryonic tooth formation. A tool for developmental biology. In: Oral Sciences Reviews 4. A. H. Melcher and G. A. Zarb, eds. Munksgaard, Copenhagen, pp. 67-120.

Spratt, N. T. 1950 Nutritional requirements of the early chick embryo. III. The metabolic basis of morphogenesis and differentiation as revealed by the use of inhibitors. Biol. Bull., *99:* 120-135.

Wallenius, B. 1959 The mesiodistal width of the tooth in relation to the content of fluorine in drinking water. Odont. Revy, *10:* 76-83.

Weber, C. W., and B. L. Reid 1969 Fluoride toxicity in the mouse. J. Nutr., *97:* 90-94.

PLATES

Abbreviations

A, Ameloblasts	O, Odontoblasts
BM, Basement membrane	OE, Oral epithelium
CL, Cervical loop	OEE, Outer enamel epithelium
DP, Dental papilla	PA, Pre-ameloblasts
IEE, Inner enamel epithelium	

PLATE 1

EXPLANATION OF FIGURES

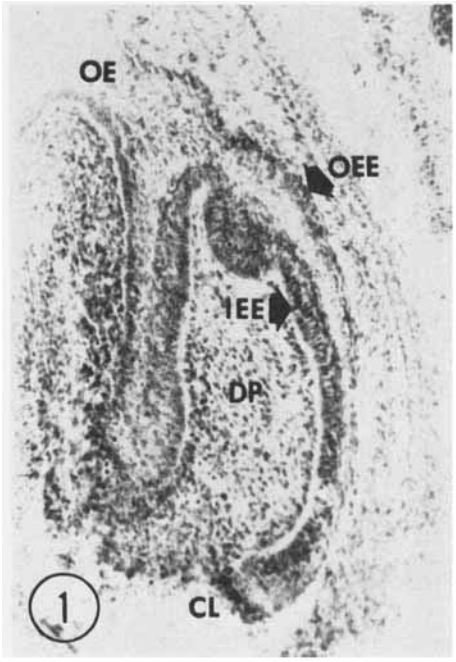
1 Photomicrograph of a longitudinal section of the anterior region of a 15-day embryonic mandible illustrating typical incisor morphology. × 125.

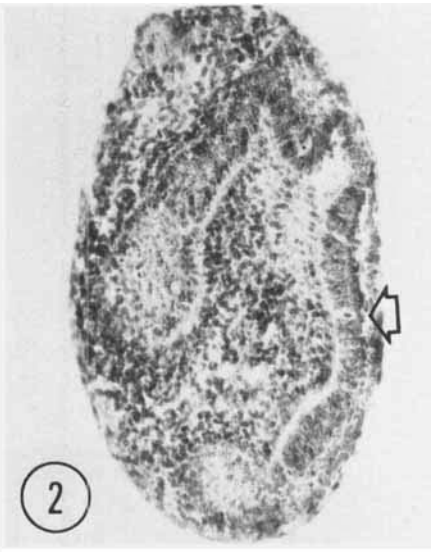
2 Photomicrograph of a section of a 15-day incisor explant cultured for two days on control medium. Note mitotic figure in the labial region of the enamel organ (arrow). × 158.

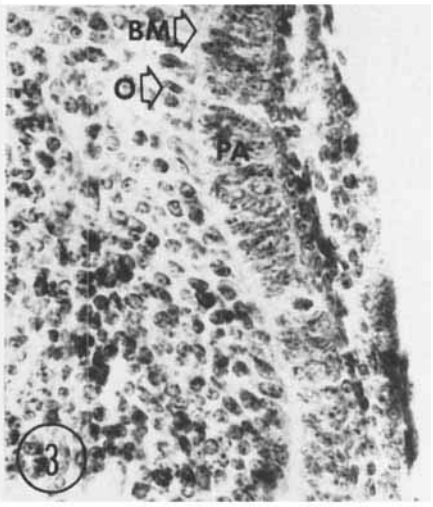
3 Photomicrograph at higher magnification of the region indicated by the arrow in figure 2. The differentiated odontoblasts are separated from the pre-ameloblasts by a basement membrane. × 400.

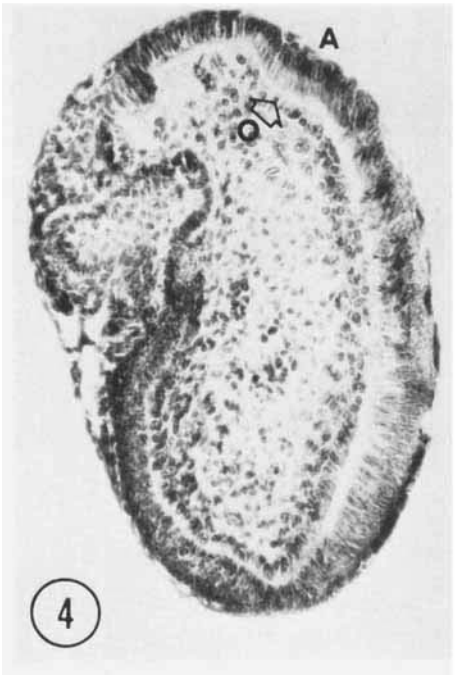
4 Photomicrograph of a section of a 15-day incisor explant cultured for six days on control medium. Note differentiated odontoblasts and labial ameloblasts. × 200.

5 Photomicrograph at higher magnification of the labeled region of figure 4. Differentiated odontoblasts and ameloblasts are separated by a basement membrane. × 400.


OE
OEE
IEE
DP
CL
1


2


BM
O
PA
3


A
O
4

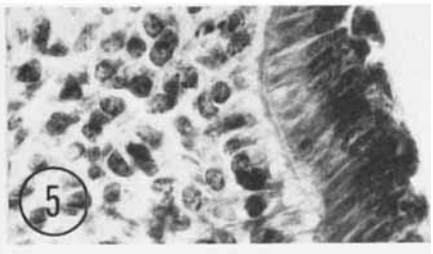

5

PLATE 2

EXPLANATION OF FIGURES

6 Photomicrograph of a section of a 15-day incisor explant cultured for six days on medium containing 2.0 mM NaF. The stage of development attained is similar to that seen in control explants after six days of growth. × 200.

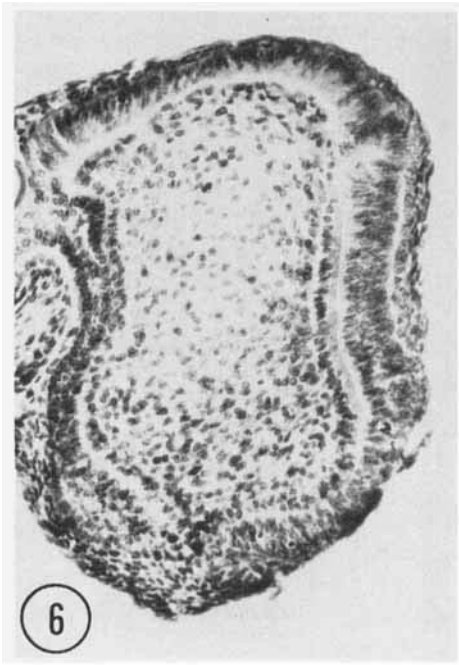
7 Photomicrograph of a section of a 15-day incisor explant cultured for two days on medium containing 3.0 mM NaF. Note the degeneration in the anterior region of the dental papilla and adjacent areas of the enamel organ (lined arrow) and mitotic figure in the posterior labial region of the enamel organ (solid arrow). × 200.

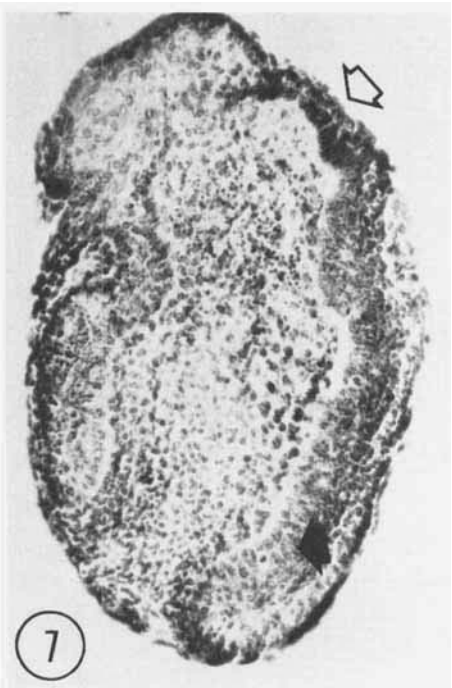
8 Photomicrograph at higher magnification of the region of figure 7 indicated by the lined arrow. Note the abnormal cells of the dental papilla and reduced enamel organ cells. × 400.

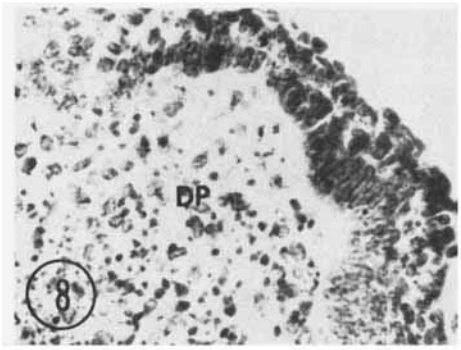
9 Photomicrograph at higher magnification of the region of figure 7 indicated by the solid arrow. The cells of the dental papilla and enamel organ appear normal but an odontoblast layer and basement membrane are not evident. × 400.

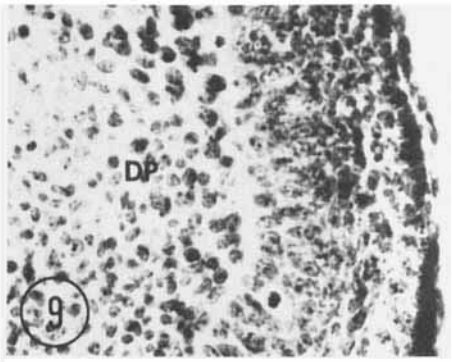
10 Photomicrograph of a section of a 15-day incisor explant cultured for two days on medium containing 3.0 mM NaF showing the reduced size of the dental structure and overall suppression of mesenchyme and enamel organ. × 250.

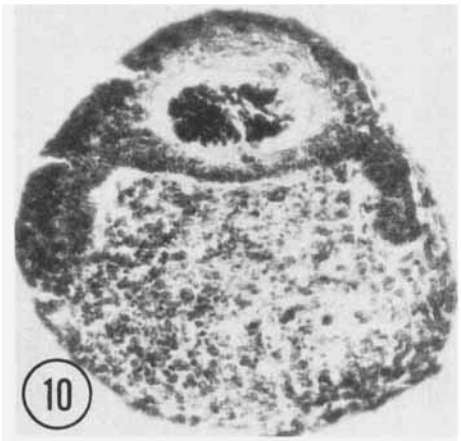
11 Photomicrograph of a section of a 15-day incisor explant cultured for six days on medium containing 3.0 mM NaF. Incisor morphology is recognizable although the explant is much reduced in size. Some viable cells are present in both the mesenchyme and the enamel organ. × 250.


6


7


DP
8


DP
9


10

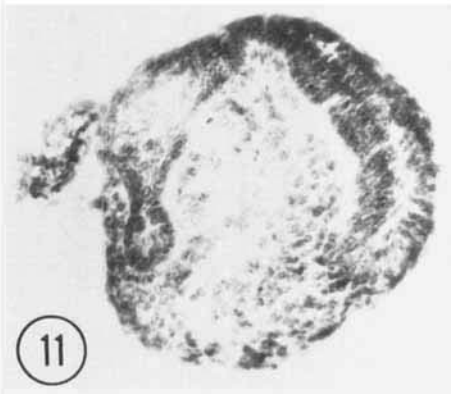

11

PLATE 3

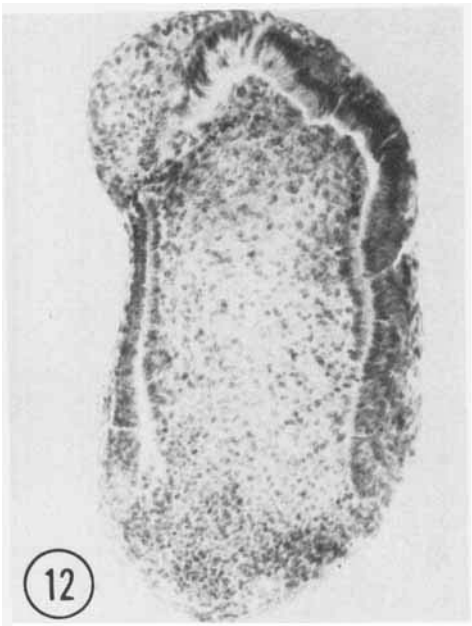
EXPLANATION OF FIGURES

12 Photomicrograph of a section of a 16-day incisor explant cultured for six days on medium containing 3.0 mM NaF. × 158.

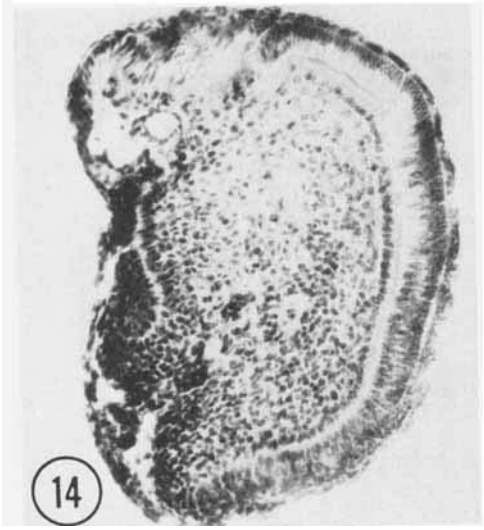
13 Photomicrograph of the anterior region of the explant shown in figure 12. Note the absence of odontoblasts and the abnormal cells of the dental papilla. The cells of the more resistant enamel organ appear normal. × 400.

14 Photomicrograph of a section of a 16-day incisor explant cultured for six days on control medium. × 158.

15 Photomicrograph of a section of a 15-day incisor explant cultured for two days on medium containing 4.0 mM NaF. The mesenchyme and enamel organ are severely inhibited by fluoride. × 200.


12


13


14

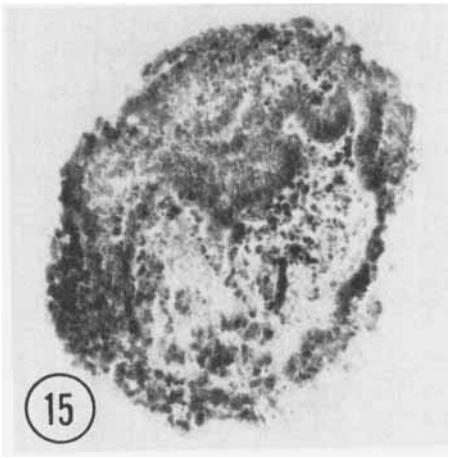

15

PLATE 4

EXPLANATION OF FIGURES

16 Photomicrograph of a section of a 15-day incisor explant cultured for four days on medium containing 4.0 mM NaF. The mesenchyme is essentially destroyed. Note the mitotic figure (arrow) in the more resistant enamel organ. × 250.

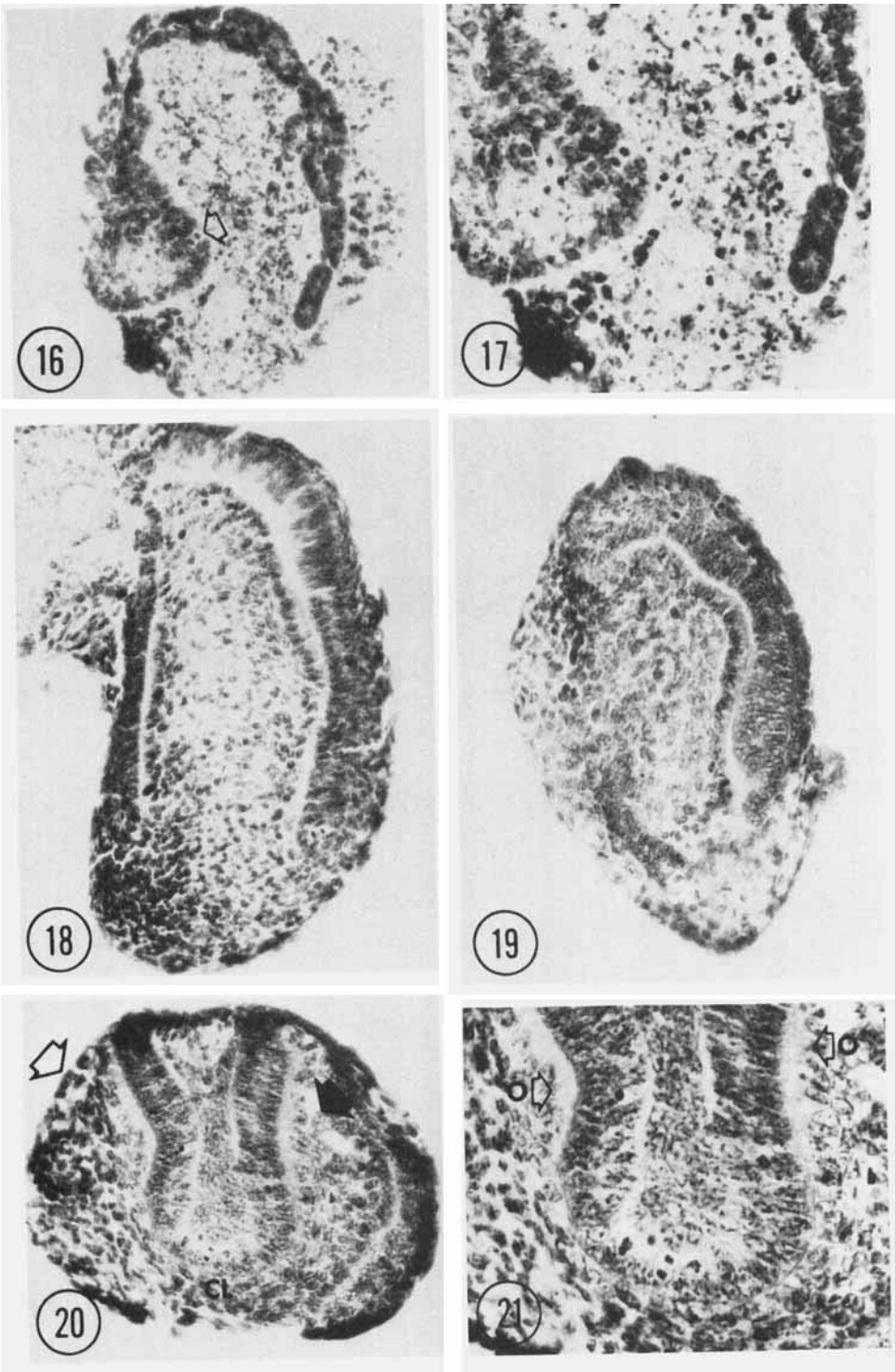
17 Photomicrograph at higher magnification of the area of the mitotic figure seen in the posterior enamel organ and adjacent mesenchyme of figure 16. × 400.

18 Photomicrograph of a section of a 15-day incisor explant cultured for two days on medium containing 3.0 mM NaF, then transferred to control medium for four days. Differentiated ameloblasts and odontoblasts are recognized by the presence of polarized nuclei. × 200.

19 Photomicrograph of a section of a 15-day incisor explant cultured for two days on medium containing 4.0 mM NaF, then transferred to control medium for four days. The stage of differentiation attained is slightly behind that of recovering explants exposed to lower fluoride concentrations (fig. 18). × 250.

20 Photomicrograph of a section of a 15-day incisor explant cultured for two days on medium containing 4.0 mM NaF, then transferred to control medium and positioned adjacent to isolated dental mesenchyme obtained from 17-day embryonic mandibular molars. After four days of growth, the enamel organ consists of columnar cells contiguous with a cervical loop, adjacent to treated mesenchyme (solid arrow) and untreated mesenchyme (lined arrow). × 250.

21 Photomicrograph at higher magnification of figure 20. Note the odontoblasts from treated mesenchyme (right) and untreated mesenchyme (left). × 400.


16
17
18
19
20
21