Scanning Microscopy

Volume 1 | Number 3 Article 25

7-22-1987

Ultrastructural Analysis of Enamel Formation During in vitro Development Using Chemically-Defined Medium

P. Bringas Jr.
University of Southern California

M. Nakamura
University of Southern California

E. Nakamura
University of Southern California

J. Evans
University of Southern California

H. C. Slavkin
University of Southern California

Follow this and additional works at: https://digitalcommons.usu.edu/microscopy

Part of the Life Sciences Commons

Recommended Citation

Bringas, P. Jr.; Nakamura, M.; Nakamura, E.; Evans, J.; and Slavkin, H. C. (1987) "Ultrastructural Analysis of Enamel Formation During in vitro Development Using Chemically-Defined Medium," *Scanning Microscopy*: Vol. 1 : No. 3 , Article 25.
Available at: https://digitalcommons.usu.edu/microscopy/vol1/iss3/25

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Scanning Microscopy by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.

Scanning Microscopy, Vol. 1, No. 3, 1987 (Pages 1103-1108)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA
0891-7035/87$3.00+.00

ULTRASTRUCTURAL ANALYSIS OF ENAMEL FORMATION DURING IN VITRO DEVELOPMENT USING CHEMICALLY-DEFINED MEDIUM

P. Bringas, Jr., M. Nakamura, E. Nakamura, J. Evans, H.C. Slavkin

Laboratory for Developmental Biology, Department of Basic Sciences (Biochemistry), School of Dentistry, University of Southern California, Los Angeles, California, 90089-0191.

(Received for publication February 13, 1987, and in revised form July 22, 1987)

Abstract

To test the hypothesis that enamel biomineralization is regulated by sequential expression of an intrinsic genetic program, we designed experiments to determine if a serumless, chemically-defined medium is permissive for position-dependent ameloblast differentiation and subsequent enamel tissue-specific biomineralization in vitro. In the absence of serum or other exogenous growth factors, Swiss Webster strain mouse embryonic (15- and 16-days gestation) mandibular first molar tooth organs (cap stage) developed within 21 days in vitro into well-defined molar tooth organs expressing dentine and enamel biomineralization. Analysis of data obtained from von Kossa histochemistry for calcium salt formation, as well as ultrastructural information obtained from x-ray microanalysis, electron diffraction, transmission electron microscopy and scanning electron microscopy documented tissue-specific patterns of calcium hydroxyapatite formation in the absence of serum within organotypic cultures in vitro. An as yet unknown intrinsic genetic program regulates enamel formation in vitro.

Key Words: Scanning electron microscopy, x-ray microanalysis electron diffraction, enamel biomineralization, embryonic mouse molar tooth organ, serumless in vitro organotypic culture

*Address for Correspondence:
Harold C. Slavkin
Laboratory For Developmental Biology
Gerontology Center, Room 314
University of Southern California,
Los Angeles, California 90089-0191
Phone Number (213) 743-6095

Introduction

To date there have been few studies showing that tissue-specific dentine and enamel can be formed and mineralized in vitro under cellular control and in a pattern comparable to that observed in vivo. Investigators have described organotypic culture systems using chemically-defined medium supplemented with fetal calf sera, horse sera and/or exogenous growth factors in which dentine and enamel biomineralization occurred (Hay, 1961; Wiggelsworth, 1968; Wiggelsworth and Hayward, 1973; Levenson, 1976; Thesleff, 1976; Yamada et al, 1980; Laine and Thesleff, 1986; Sakakura, 1986). Based upon results from these studies, dentine and enamel formation was assumed to require exogenous regulatory factors to facilitate time- and position- specific biomineralization during in vitro tooth formation (Slavkin, 1979; Bronkers, 1983a,b; Bronkers and Woltgens, 1985; Ameloot et al, 1986; Sakakura, 1986; Laine and Thesleff, 1986).

We describe here a system, adapted from previously reported organotypic cultures (Thesleff, 1976; Yamada et al, 1980), that can be used to study in vitro mechanisms which promote and regulate ameloblast cytodifferentiation, production of the organic enamel extracellular matrix and tissue-specific ultrastructural patterns of enamel-like calcium hydroxyapatite during tooth formation using serumless, chemically-defined medium. We interpret our results to indicate that the enamel tissue-specific patterns of biomineralization are regulated by intrinsic genetic programs independent of humoral factors.

Materials and Methods

Embryonic Mandibular First Molar Dissection and Serumless Chemically-Defined Organotypic Culture System

Mandibular first molar tooth organs were dissected from timed-pregnant Swiss-Webster mice (Simonsen Labs, Gilroy, CA). In vitro studies used 15- and 16-days gestation (Theiler stages 23 and 24) (Theiler, 1972) cap stage tooth organs cultured for 21 days as previously described (Yamada et al, 1980); modified in the present studies by using BGJb medium (Fitton-Jackson's modified BGJ, GIBCO, Staten Island, NY) supplemented with 100 μg/ml L-ascorbic acid and 100 U/ml penicillin/streptomycin. Explanted molars were cultured at 37.5^{o}C with atmospheric conditions

of 95% air and 5% CO_2. Initial pH was adjusted to 7.4; medium was changed every other day.

Von Kossa Assay For Biomineralization

Non-demineralized cultured explants and control 7 day in vivo postnatal molars were processed for von Kossa histocytochemistry to identify the position of calcium salt deposition within dentine and enamel matrix (Thompson and Hunt, 1966).

Transmission Electron Microscopy, X-Ray Microanalysis and Electron Diffraction Analyses

Explants cultured for 21 days, as well as in vivo control 7 day postnatal molar organs were isolated, fixed in ethylene glycol, and then processed for microprobe analyses using the anhydrous preparation method (Landis, 1983). Mandibular first molars from 7-day postnatal stages of development served as control mineralized dentine and enamel. Prepared samples were analyzed for Ca/P ratio in regions of forming enamel by electron diffraction and microprobe analyses using a Philips TEM-420, coupled with Tracor/Northern TN-2000 and the HALL program (Landis, 1983; Landis and Glimcher, 1982).

Electron diffraction was performed on 70 μm thick sections placed on carbon-collodion coated grids. Synthetic calcium hydroxyapatite crystals (OHAP), characterized by x-ray diffraction (J.P. Yeslinowski, personal communication), were mixed in ethylene glycol (10 ug /ml) and sprayed at low angle onto carbon-coated copper grids. Electron diffraction patterns were made on selected areas of enamel using a JEOL 1200EX TEM in the diffraction mode at 80 kV (Watt, 1985). Measurements were made at 90° incident to the sample. Patterns were measured for spot or ring diameter from the direct negatives (Watt, 1985), and the d spacings obtained were compared to those characteristic for OHAP (ASTM, 1966).

Scanning Electron Microscopy

Isolated enamel extracellular matrices were fixed in 2.5% glutaraldehyde (Ted Pella, Inc., Tustin, CA) in 0.1 M sodium cacodylate buffer, ph 7.2, at 4^oC for 2 hours. Specimens were washed in buffer, dehydrated in graded ethanol, and placed in perforated Beem capsules in acetone. They were then dried in a critical-point drying bomb (Sorvall, Newtown, Conn.) using carbon dioxide, mounted on specimen studs, and sputter coated with gold palladium alloy in an argon atmosphere (Hummer V, Technics; pulsed for 7 minutes at 9 volts and 10 milliamps). The topography of the enamel extracellular matrix was viewed with a Cambridge S4-10 scanning electron microscope operating at 10 kV.

Results

Enamel Biomineralization In Serumless, Chemically-defined Medium

Embryonic cap stage molar tooth organs cultured for 21 days in serumless, chemically-defined medium produced overt morphogenesis, cytodifferentiation, dentine and enamel extracellular matrix formation and biomineralization (figure 1). Macroscopic examination of each cultured molar explant (n=70) demonstrated a refractile material (arrows), presumably enamel, covering the outer surfaces of the tooth organs (figure 1A). Figure 1B shows the localization of calcium salt deposition using von Kossa histochemical staining. Both dentine and enamel biomineralization was observed (figure 1C). Dentine and enamel biomineralization was restricted to discrete occlusal regions of the forming tooth organs.

Ultrastructural Patterns of Enamel Biomineralization

Ultrastructural observations of cultured molar organs processed by the anhydrous fixation technique of Landis (1983) demonstrated highly ordered enamel crystals (figure 2). Observations of non-stained sections provided the opportunity to examine the electron-dense calcium hydroxyapatite mineral crystals under these experimental conditions.

Preliminary SEM observations of cultured tooth organs provided topographical information, potentially

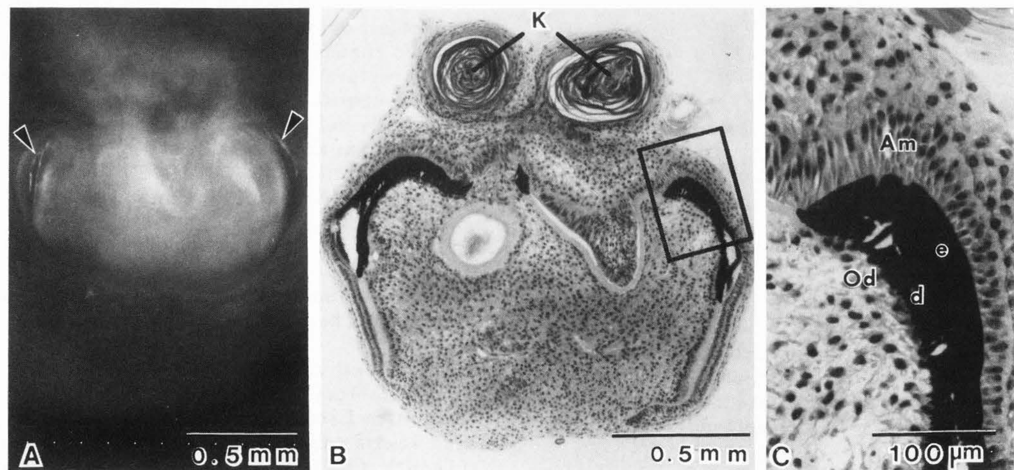

Figure 1. Enamel biomineralization during mandibular first molar tooth organotypic cultures after 21 days in serumless, chemically-defined medium in vitro. (A) Survey of cultured explants; arrows indicate refractile putative enamel close to the surface of tooth organs. (B) Distribution of calcium salt deposits within dentine and enamel using von Kossa histochemical staining. Note oral epithelial keratin pearls (k). Region delineated by rectangle is shown at higher magnification in figure C. (C) Higher magnification demonstrating biomineralization in the occlusal dentine (d) and enamel (e). (Am), ameloblasts; (Od), odontoblasts.

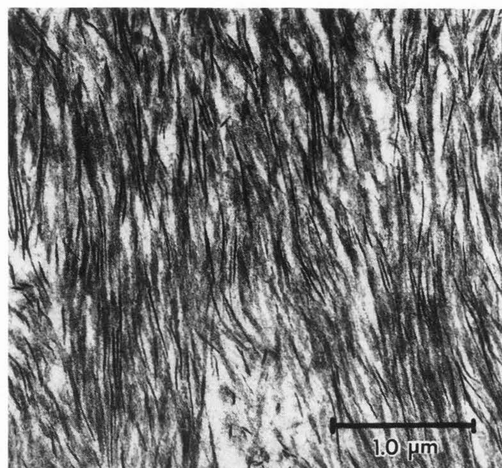

Figure 2. Ultrastructural characteristics of enamel produced in serumless, chemically-defined organotypic cultures. Unstained anhydrous preparation.

useful in evaluation of enamel prism formation. Figure 3A provides a preliminary survey of the major topographical features of molar explants cultured for 21 days in vitro. For these preparations the enamel organ epithelial covering was removed thereby enabling direct observations of the outer enamel surface (figures 3B-C). The interprismatic patterns observed (figure 3C) were comparable to those illustrating the characteristic mouse enamel interprismatic pattern in situ (Greenberg et al, 1983).

Enamel Calcium Hydroxyapatite Formation

Microprobe analysis of Ca/P ratio representing enamel biomineralization in serumless cultures and during in vivo 7 day postnatal molar tooth organ development are shown in table 1. The Ca/P ratio observed for enamel produced in serumless medium exceeded unity, a value established as indicative of mineralization (Lee et al, 1986;Landis and Glimcher, 1982).

Selected area diffraction patterns were recorded for enamel produced by cap stage molars cultured for 21 days in serumless medium and for calcium hydroxyapatite crystals (OHAP). For molars cultured in vitro (figure 4), we observed two major reflections in enamel corresponding to the 210 (strong) and 202 (weak) Miller indices of OHAP (ASTM, 1966). The d spacings obtained for these reflections are in good agreement with reported values for OHAP (Lee et al, 1986).

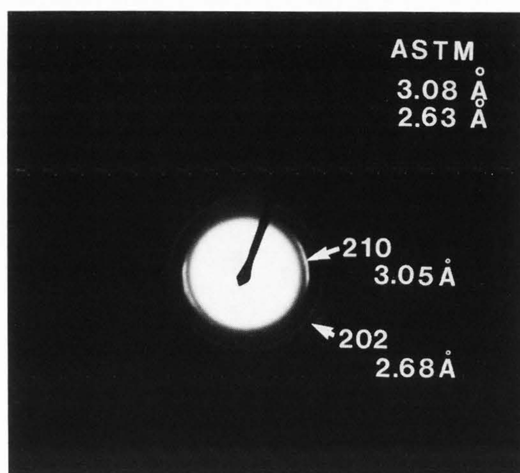

Figure 4. Electron diffraction pattern of enamel formed in tooth organs cultured for 21 days in serumless, chemically-defined medium. Molar explants were processed and embedded according to the anhydrous method (Landis, 1983). Diffraction measurements were performed (90° incidence) in a JEOL 1200EX TEM as described in Materials and Methods. Miller indices are indicated with triangle arrows. The experimentally determined d spacing values are indicated and the ASTM values reported for OHAP are given (ASTM, 1966).

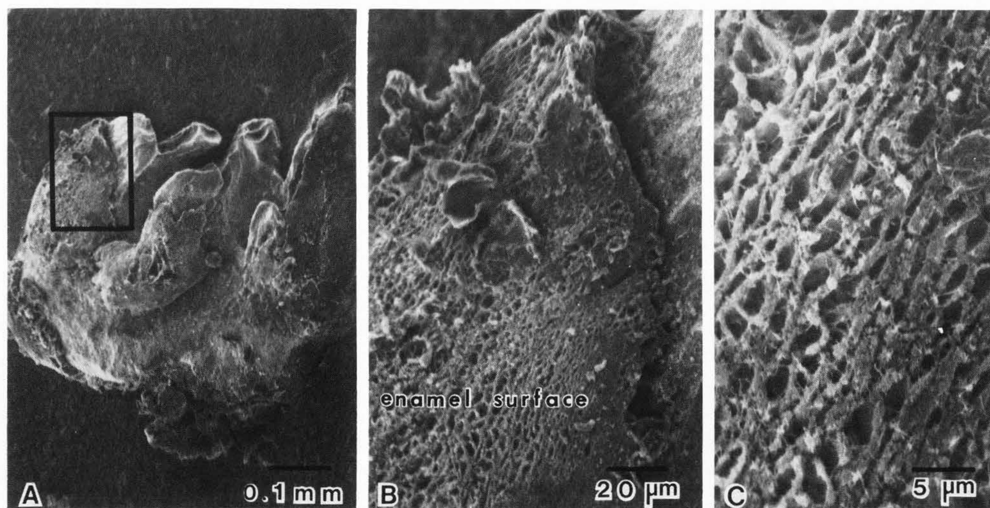

Figure 3. Surface features of molar tooth organs cultured for 21 days in serumless, chemically-defined medium. Specimens were prepared by mechanically removing the enamel organ epithelium. (A) Survey photomicrograph of mandibular first molar tooth organ (B) Occlusal region indicated in rectangle of previous figure showing enamel surface features (C) Mouse enamel prism features.

TABLE 1: MICROPROBE ANALYSIS OF IN VITRO BIOMINERALIZATION: Ca/P RATIOS IN ENAMEL OF SERUMLESS MOLAR CULTURES.

MOLAR ORGAN	ENAMEL Ca/P RATIO (MEAN +/- SD)
21 Days Serumless	2.03 +/- 0.04
7 Days Postnatal (Control)	2.35 +/- 0.04

Control 7-day postnatal in vivo molars, and 21 day cultured in vitro molars were processed using anhydrous fixation as described in Materials and Methods, and analyzed for calcium and phosphorous content in enamel using microprobe analysis. Five independent regions in enamel in the same molar organ were evaluated in each set of samples. Values represent the mean +/- standard deviation (S.D., n=5). Significance within samples: $p < 0.025$.

Discussion

In the present paper we provide evidence to support the hypothesis that enamel biomineralization in vitro is regulated by sequential expression of an intrinsic genetic program independent of exogenous humoral factors. It has been previously established that cap stage mouse molar tooth organs express morphogenesis and cytodifferentiation with the production of organic constituents of the dentine and enamel tissue-specific extracellular matrices in vitro using serumless, chemically-defined medium (Yamada et al, 1980; Slavkin, et al., 1982; MacDougall et al, 1985; Finkelman and Butler, 1985). However, dentine or enamel biomineralization was not detected without serum supplementation. Many of these previous studies used 17-days gestation mouse mandibular first molar tooth organs cultured in vitro for periods up to 10 days in chemically-defined medium supplemented with and without serum. Serum-supplemented cultures resulted in dentine and enamel biomineralization within 10 days in vitro (see reviews by Slavkin, 1979; Slavkin et al., 1982).

The major modifications of the present study were (i) earlier embryonic tooth organs; (ii) the chemically-defined BGJb medium as modified by Fitton-Jackson; and (iii) extended culture periods of three weeks. These three modifications resulted in tissue-specific enamel biomineralization (table 1) including OHAP formation (figure 4), enamel crystal orientation comparable to that observed for in situ enamel formation (figure 2), and a characteristic rodent enamel interprismatic pattern (figure 3).

Our data supports the interpretation that enamel-specific calcium hydroxyapatite crystals form during in vitro serumless cultures of mandibular first molar tooth organs. These crystals have a Ca/P ratio of 2.03 +/- S.D. 0.04 (table 1), and appear to have a structure comparable to OHAP as seen by electron diffraction (figure 4). We note that the 210 reflection present as a strong, meridional pattern of short arc (figure 4) is indicative of a crystalline structure of OHAP (Lee et al, 1986; Roufosse et al, 1979; Posner et al, 1980; Meyer and Fowler, 1982). The faint reflection at 202, along with the absence of other strong reflections, may be indicative of OHAP crystals which contain substitutions (e.g., carbonates, magnesium, fluoride), or are crystals which are short in length (Brown, 1966; Elliot, 1973; Meyer and Fowler, 1982; Young and Brown, 1982). We cannot rule-out the possibility that other chemical forms of calcium phosphates may exist in this enamel produced during in vitro development in a serumless medium, including octacalcium phosphate and sesquiapatites (Young and Brown, 1982). We interpret our data to indicate that OHAP crystals are formed in the enamel of cultured molar explants.

The mechanisms by which specific organisms regulate tissue-specific biomineralization are not known. Our study supports the thesis that sequential epithelial-mesenchymal interactions regulate (somehow?) morphogenesis, cytodifferentiation, tissue-specific extracellular matrix formation and subsequent biomineralization. It is also evident from the present studies that serum-associated factors are not required for in vitro dentine and enamel biomineralization. We argue that reciprocal epithelial-mesenchymal interactions induce and modulate the intrinsic expression of factors required for tissue-specific biomineralization. Moreover, we suggest that these factors are encoded within the intrinsic developmental programs for dentine and enamel biomineralization. The present in vitro system provides an experimental approach to pursue the identification and characterization of regulatory molecules required for tissue-specific biomineralization.

Acknowledgements

We are extremely grateful to Mr. Valantino Santos for his untiring technical assistance. We thank Mr. Jack Warall (Department of Material Sciences, School of Engineering, University of Southern California) for his technical assistance in the microprobe analyses. We appreciate the assistance of Mr. Dwain Lewis in the preparation of this manuscript. This research was supported in part by United States Public Health Service grant DE-06425.

References

1. Ameloot PC, Coomans D, Smeyers-Verbeke J, Boute P. (1986). Characteristics of Mineralization of Rat Molar Tooth Germs in Organ Culture. J Biol Buccale. 14:25-37.

2. American Society For Testing Materials (1966). X-ray Powder Diffraction Data File, 1916 Race St., Philadelphia, PA.

3. Bronkers ALJJ. (1983a). A Histological and Biochemical Study of the Effect of Vitamin C-Deficiency on Induction of Amelogenesis in Hamster Molars In Vitro. Arch Oral Biol. 28(8):681-692.

4. Bronkers ALJJ. (1983b). Effect of Oxygen Tension on Matrix Formation and Mineralization in Hamster Molars During Development. In Vitro J Biol Buccale. 11:195-207.

5. Bronkers ALJJ, Woltgens JHM. (1985). Short Term Effects of Fluoride on Biosynthesis of Enamel-Matrix Proteins and Dentine Collagens and on Mineralization During Hamster Tooth-Germ Development in Organ Culture. Arch Oral Biol. 30(2):181-190.

6. Brown WE. (1966). Crystal Growth of Bone Mineral. Clin Ortho Rel Res. 44: 205-220.

7. Elliot JC. (1973). The Problems of the Composition and Structure of the Mineral Components of the Hard Tissues. Clin Ortho Rel Res. 93: 313-345.

8. Evans J, Bringas P, Nakamura M, Nakamura E, Santos V, Slavkin H. (1987). Metabolic Expression of Intrinsic Developmental Programs for Dentine and Enamel Biomineralization in Serumless, Chemically-Defined, Organotypic Culture. Calcif Tissue Int. (in press).

9. Finkelman RD, Butler WT. (1985). Appearance of Dentin Gamma-Carboxyglutamic Acid Containing Factors in Developing Rat Molars In Vitro. J Dent Res. 64(7): 1008-1015.

10. Greenberg G, Bringas P, Slavkin HC. (1983). The Epithelial Genotype Controls the Pattern of Extracellular Enamel Prism Formation. Differentiation 25: 32-40.

11. Hay MF. (1961). The Development In Vivo and In Vitro of the Lower Incisors and Molars of the Mouse. Arch Oral Biol. 3:86-109.

12. JEOL 1200EX Operating Manual (1981). JEOL LTD., Tokyo, Japan, pp. 134-255.

13. Laine M, Thesleff I. (1986). Development of Mouse Embryonic Molars In Vitro: An Attempt to Design Defined Culture Conditions Allowing Mineralization. J Biol Buccale. 14:15-23.

14. Landis WJ. (1983). Special Techniques for Problem Tissues, In "Electron Microscopy" (Ed). B.R. Jones Academic Press, New York. pp. 459-476.

15. Landis WJ, Glimcher MJ. (1982). Electron Optical and Analytical Observations of Rat Growth Plate Cartilage Prepared by Ultracryomicrotomy: The Failure to Detect a Mineral Phase in Matrix Vesicles and the Identification of Heterodisperse Particles as the Initial Solid Phase of Calcium Phosphate Deposited in the Extracellular Matrix. J Ultra Res 1982; 78:227-268.

16. Lee DD, Landis WJ, Glimcher MJ. (1986). The Solid Calcium-Phosphate Mineral Phases in Embryonic Chick Bone Characterized by High Voltage Electron Diffraction. J Bone Min Res. 1(5):425-432.

17. Levenson, GE. (1976). Effects of Ascorbic Acid Deficiency in Mouse Second Molar Tooth Germs Cultivated In Vitro. J. Embryol. Exp. Morph. 36:73-85.

18. MacDougall M, Zeichner-David M, Slavkin HC. (1985). Production and Characterization of Antibodies Against Murine Dentine Phosphoprotein. Biochem J. 232:493-500.

19. Meyer JL, Fowler BO. (1982). Lattice Defects in Nonstoichiometric Calcium Hydroxyapatites: A Chemical Approach. Inorg Chem. 21(3):3029-3055.

20. Posner AS, Betts F, Blumenthal NC. (1980). Formation and Structure of Synthetic and Bone Hydroxyapatites. Prog Crystal Growth Charact. 3:49-64.

21. Roufosse AH, Landis WJ, Sabine WK, Glimcher MJ. (1979). Identification of brushite in newly deposited bone mineral from embryonic chicks. J. Ultractruct Res. 68:235-245.

22. Sakakura Y. (1986). A New Culture Method Assuring the Three -Dimensional Development of the Mouse Embryonic Molar Tooth In Vitro. Calcif Tissue Int. 39:271-278.

23. Slavkin HC. (1979). Amelogenesis in vitro. J Dent Res. 58(B):735-739.

24. Slavkin HC, Honig LS, Bringas P. (1982). Experimental Dissection of Avian and Murine Tissue Interactions Using Organ Culture in a Serumless Medium Free from Exogenous (Non-Defined) Factors. In "Factors and Mechanisms Influencing Bone Growth" (Eds) B. Sarnat, D. Dixon Alan R. Liss, Inc. New York. pp. 217-228.

25. Theiler K. (1972). The House Mouse. Springer-Verlag Press, New York.

26. Thesleff I. (1976). Differentiation of Odontogenic Tissues in Organ Culture. Scand J Dent Res. 84:353-356.

27. Thompson SW, Hune R.D. (1966). Histochemical Procedures: Von Kossa's Technic For Calcium. In: Selected Histochemical and Histopathological Methods. C. Thomas Publishers, Springfield, IL. pp. 581-584.

28. Watt IM, (1985). The Principles and Practice of Electron Microscopy. Cambridge University Press, England. pp 117-195.

29. Wiggelsworth DJ (1968). Formation and mineralization of enamel and dentine by rat tooth germs in vitro. Exp Cell Res. 49:211-215.

30. Wiggelsworth DJ, Hayward AF. (1973). The ultrastructure of dentinogenesis and amelogenesis in rat molar tooth germs grown as organ cultures in vitro. Z Zellforsch. 138:171-186.

31. Yamada M, Bringas P, Grodin M, MacDougall M, Cummings E, Grimmett J, Weliky B, Slavkin HC. (1980). Chemically Defined Organ Culture of Embryonic Mouse Tooth Organs: Morphogenesis, Dentinogenesis, and Amelogenesis. Jour Biol Buccale. 8:127-139.

32. Young RA, Brown WE. (1982). Structures of Biological Minerals. In: Biological Mineralization and Demineralization (Ed). G.H. Nancollas. Springer-Verlag Press, New York. pp 101-142.

Discussion with Reviewers

D. R. Eisenmann: What is the ultrastructural appearance of the ameloblasts in association with forming enamel in this culture system? Authors: We observe that the inner enamel epithelial sequence of differentiation into ameloblasts during in vitro serumless organotypic culture is very similar to the Kallenbach description of differentiation zones I-VI for in situ amelogenesis in the rodent incisor tooth organ (J. Ultrastructure Res. 35, 508-531, 1971). Kallenbach differentiation zones I-VI, secretory ameloblasts and post-secretory ameloblasts were observed. However, the post-secretory ameloblasts in our culture system did not produce a basal lamina upon the enamel during enamel maturation.

H. Warshawsky: I question the need for using "biomineralization," when mineralization conveys the same meaning. Also, "tissue-specific" seems unnecessary when dealing with dentine and enamel. The Discussion opens by stating that evidence is provided to support a hypothesis that enamel mineralization in vitro is "regulated by sequential expression of an intrinsic genetic program..." I find no evidence for "regulation" or "sequence." Enamel mineralizes if the environmental conditions are appropriate. This does not imply regulation or sequence. Finally, I would like to pose the following question. Although the authors have shown that dentine and enamel can mineralize in a chemically defined medium, and offer several reasons for why other systems failed, they never discuss the affect of serum factors on bringing about the mineralization that, in these systems, would not occur in the absence of serum. Could the authors speculate on why addition of serum permits mineralization that would not occur without it?
Authors: In response to the first point raised by the reviewer, we have adopted the term "biomineralization"

as originally defined by Professor Heinz Lowenstam (Science 211, 1126, 1981) used to distinguish cell-mediated and cell/matrix mediated from non-biological models of mineralization. We use the term biomineralization to abbreviate mineralization in biological systems. With regard to the second query, we employ the expression "tissue-specific" to critically describe the specificity of the supramolecular organization of the particular extracellular matrix produced in these organ culture studies. The literature is replete with papers citing enamel or dentine production but lacking in the criteria used to define "tissue-specific" enamel or dentine. We have attempted to provide several different assays which confirm the specificity of the extracellular matrix produced under these unorthodox in vitro conditions. In contrast to the reviewer's summation, we are approaching the interpretation of these studies from a different perspective. We know that reciprocal epithelial-mesenchymal interactions operate during tooth organogenesis, in situ and in vitro, and we have observed a sequence of tooth development which is comparable to the sequence documented for in situ tooth development. We also realize that the products of these epithelial-mesenchymal interactions are likely autocrine and paracrine factors which regulate growth, differentiation and possibly biomineralization. We do not envision the problem as one of the macroenvironment, but rather a problem of close-range molecular interactions mediated by factors intrinsic to the tissues and not derived from the external environment. Finally, we have not reported comparisons between serum-supplemented versus non-serum supplemented media in the present communication. However, a preliminary report of such a comparison is presently in press (Evans J, Bringas P, Nakamura M, Nakamura E, Santos V, Slavkin H. (1987). Metabolic Expression of Intrinsic Developmental Programs for Dentine and Enamel Biomineralization in Serumless, Chemically-Defined, Organotypic Culture. Calcif Tissue Int. (in press)). In responding to the last question we note that all fetal calf sera (FCS) do not support biomineralization of bone, cartilage and dental tissues. It is not as yet established what constituents of FCS are rate-limiting to biomineralization. It is also not clear as to the net effects of up- and down- regulating molecules together serving as constituents of FCS.

J. H. M. Woltgens: This paper contains very interesting results and is clearly written. Additional information is requested regarding the following: (a) the success rate of the cultures of the tooth germs; (b) the developmental stage of the tooth germ at the explanation time; and (c) comparison of cultured tooth germs with tooth germs of the same developmental stage but cultured in serum containing medium is the early developmental stage used responsible for the observed mineralization?).

Authors: We have observed 100% success in our cultures using serumless conditions. All explants showed biomineralization. No necrosis was observed. We have found that early cap stage mouse mandibular first molars (circa 15/16-days gestation based on time zero=time of observing the vaginal plug) were ideal for these studies. Late cap and early bell stages were limited, unreliable and gave equivocal results. We assume that starting earlier in development and culturing longer gave the best results. Previous studies in the literature, including our own previous studies, used late cap stage or early bell stage tooth organs cultured for 10 days in vitro with or without sera supplementation. We have recently completed a study comparing several parameters of tooth organogenesis during serum-supplemented versus serumless culture (Evans et al, Calcified Tissue International, in press).