Mark Skinner,[1] *Ph.D. and Tosha Dupras,*[2] *B.A.*

Variation in Birth Timing and Location of the Neonatal Line in Human Enamel

REFERENCE: Skinner, M. and Dupras, T., **"Variation in Birth Timing and Location of the Neonatal Line in Human Enamel,"** *Journal of Forensic Sciences*, JFSCA, Vol. 38, No. 6, November 1993, pp. 1383–1390.

ABSTRACT: The location of the neonatal line in 173 primary teeth from 43 children was investigated and shown to differ significantly among pre-term, term, and post-term births. Approximately 75% of the neonatal lines that lie beyond 2SD of the mean location of the line in term births are from children born outside of 38 to 42 weeks gestation. The duration of pregnancy accounts for about 36% of the variation in location of the neonatal line in non-term births. Based on the small proportion of non-term births whose neonatal line is located beyond 2SD of the mean location of the neonatal line in term births, it is estimated that this technique will provide individualizing information in about 3% of immature skeletonized remains in a forensic context. The relative timing of pre- and postnatal pathological striae in the enamel of primary teeth can be evaluated in terms of the variation, documented here, in the location of the neonatal line due to individual and birth timing differences. Determination of the timing of pathological enamel striae will aid in the identification of both children and adults for whom histological examination of enamel is undertaken.

KEYWORDS: odontology, physical anthropology, enamel, teeth, neonatal line, birth timing, human identification

Both the birth process itself and abnormal events before and after birth can create similar-looking developmental defects in the form of accentuated striae within the enamel of primary teeth. Familiarity with the appearance and location of the "neonatal line" enables forensic anthropologists and odontologists to assess the timing of pathological striae [*1*]. There may be significant medico-legal implications to the demonstration that a child suffered physiological upset in utero or perinatally. In that birth timing is variable, the location of the neonatal line should vary characteristically with gestation length. Departures from normal gestation length as recorded in primary enamel could theoretically contribute to the individualization of unidentified immature remains. Also, the factors behind perinatal morbidity and mortality come under close scrutiny by various branches of the forensic and health sciences. Particularly, viability at birth is significantly influenced by gestation length. For example, about 20% of births in the USA occur outside of term (38 to 42 weeks) but account for almost half of neonatal deaths [*2*]. Clearly, the potential of the neonatal line to disclose birth timing and other perinatal events is contingent upon documentation of variation in the location of the neonatal line among primary teeth, with different formation schedules, as a function of gestation length and other factors.

Received for publication 17 July 1992; revised manuscript received 12 Jan. and 15 March 1993; accepted for publication 13 April 1993.

[1]Associate Professor, Department of Archaeology, Simon Fraser University, Burnaby, British Columbia, Canada.

[2]M.Sc. program, School of Human Biology, University of Guelph, Guelph, Ontario, Canada.

The fact that the timing of birth is preserved in human primary enamel has been acknowledged for almost 60 years [*3,4*]. It is usually termed a birth ring or neonatal line and appears as an accentuated stria in enamel and (occasionally) dentin [*5*]. For practical purposes three varieties of incremental striae in enamel can be distinguished: striae of Retzius, which are normal, regularly spaced, enamel increments; pathological striae (also known as Wilson bands [*6*]), which are abnormally accentuated incremental striae coincident with the former; and the neonatal line, which is a normal accentuated striae, occasioned by birth. The neonatal stria is normal in that it is a predictable consequence of the birth process and occupies a characteristic position within primary dental crowns from children born at term; however high power magnification reveals disturbed enamel formation along the length of the neonatal line conforming to the definition of a Wilson band [*6,7*].

The neonatal line tends to be wider (ignoring differences in tooth size) in children who experienced birth difficulty; an eventuality recognized by the term "perinatal line" [*6,7*]. Except as a guide to the severity of parturition [for example, *8*], diabetes in the mother [*9*], or the timing of birth trauma with clinical implications [for example, *10*], little research has been done on its normal variation in location within the crown as a function of gestation length [*11*] and/or individual variation. Schour initially described the line as occurring at a constant level within the tooth [*4*] but later he and his colleagues concluded that prematurity would likely shift the neonatal line occlusally [*12,13*]. The only test of the hypothesis that the location of the neonatal line varies with gestation length, is by Noren who showed that in low birth weight infants, "the shorter the gestational age, the greater was the tendency for the neonatal line to be positioned towards the incisal parts of the tooth" [*14*]. No quantitative data nor tests of significance are provided.

The neonatal line is a normal incremental feature of enamel, corresponding to a stria of Retzius produced at birth, which extends more or less obliquely from the surface to the dentino-enamel junction [*4*]. Several studies confirm that the neonatal line itself is usually less densely mineralized [*15–17*]. It is occasionally expressed on the surface as enamel hypoplasia [*13,18*].[3] All enamel crowns that are forming at birth—that is all primary crowns and the mesio-buccal cusp of about 10% of first permanent molars [*4*] will show the line. Lack of the line in some sectioned teeth (2% according to Sarnat and Schour [*19*], and 7% according to Noren [*9*]) can probably be attributed to vagaries in lighting and sectioning rather than actual absence [*20*]; although Gustafson and Gustafson [*15*] note that the line is sometimes very insignificant. Variation in the optical appearance of the lines may also reflect differential tooth susceptibility as shown for permanent crowns [*21,22*].

Methods and Materials

Primary teeth from 21 males and 22 females were obtained by advertising in local newspapers for "tooth fairies" whose children were born significantly pre- or post-term. In all, 173 teeth were successfully sectioned for examination of the neonatal line.

Crowns were identified from anatomical form and embedded in Fiber-Tek® resin and allowed to cure till hard. Labio/bucco-lingual sections approximately 200 microns thick were obtained with a Buehler Isomet® low speed saw fitted with a three inch (high

[3]Our understanding of the relationship between a pathological stria and surface enamel hypoplasia suggest that deep surface grooves must, perforce, be tall and seemingly of long duration. Tall, shallow linear enamel hyplasia probably reflects longer term, mild stress. While Wilson Bands are of short duration, a component of the height of an episode of linear enamel hypoplasia is attributable to severity of the stress which may also be of short duration. Further research is required on this phenomenon.

concentration) diamond wafering blade. Each longitudinal section was examined under normal and polarized light at 20× magnification for evidence of the neonatal line. Identification of the neonatal line was usually quite easy because of the clear distinction between inner enamel deposited prenatally which has a characteristic homogenous, light color under polarized light and is usually free of even faint striae, separated by a distinctly wide and dark neonatal line, from outer enamel deposited postnatally, which often has faint striae and assumes a different hue under polarized light (criteria introduced by Rushton in 1933 [*3*]). The attribution of a particular stria to birth is supported by McMillan and Kashgarian [*18*] who showed that externally visible enamel hypoplasia invariably coincided with the histologically identified neonatal line. The neonatal line is a histologically distinguishable form of Wilson band, because at high magnification, prism disturbance is discernible. At low power magnification, used in this study, the neonatal line does not possess unique optical properties that would permit discrimination of a neonatal line from a striae due, for example, to severe prenatal stress [*23*]. Such an eventuality will have to be investigated on children with known obstetrical and medical histories using high magnifications, which will allow assessment of prism orientation and integrity. Frankly, pathological striae will prove difficult to distinguish from neonatal lines. However, accurate identification is attainable by the experienced observor who is familiar with the typical location of the latter and its customary optical appearance under controlled conditions of lighting and section thickness. The distance from the neonatal line's intersection with the dentino-enamel junction to the cervix on the labial/buccal surface of the tooth was measured with an eyepiece micrometer, calibrated to a 10 mm objective graticule with 0.1 mm gradations in a Nikon AFM compound microscope. Interobserver error was previously tested and found to amount to 5.2% [*24*].

For the purposes of statistical analysis, gestation length was expressed in days before or after expected term as reported by the mother and also arbitrarily divided into three groups: Pre-term (≤37 weeks), term (38 to 42 weeks), and post-term (≥43 weeks). Statistical analysis was performed on a Macintosh Classic personal computer using StatView 512+®.

Results

An analysis of variance failed to show statistically significant differences between antimeres, isomeres or between the sexes but confirmed that the neonatal line was differentially located in each tooth type and this varied significantly as a function of gestation length (Table 1). There is considerable overlap in these distributions but clearly the later that birth occurs, the more the tooth crown is completed and thus the neonatal line is closer to the cervix. Figure 1 illustrates this relationship. The effect of later birth on the location of the neonatal line is seen more clearly by combining the observations for all tooth types through expressing the location of the neonatal line as a standardized deviation (z score) from the mean location of the line at term for a particular tooth type (viz., first incisor, second incisor and so on). This is shown in Fig. 2 where location of the neonatal line is regressed against gestation length for the pre- and post-term births. Approximately 36% of the variation in the location of the neonatal line in non-term births is attributable to the duration of pregnancy. The remaining variation can be attributed to differences in tooth size, individual variation, and errors in estimation of birth timing. Regarding the last possibility, Fig. 3 shows an apparent bimodal distribution of the location of the neonatal line in preterm births with a clear separation between those whose neonatal line is clearly shifted occlusally and those whose line coincides with the normal location among term births. Reporting error is suspected to be a factor in the latter cases.

A statistical weakness of this study is that the unit of analysis is the tooth, while the average contribution was four teeth per child; although the modal value was a single

TABLE 1—*Comparison (ANOVA) of the mean distance of the neonatal-line from the cervix for each tooth type according to gestation interval.*

Tooth	Gestation Interval			Sign
	Pre-term Mean	Term Mean	Post-term Mean	
i1	1.784 (10)	1.207 (31)	0.915 (10)	.0001
i2	2.178 (8)	1.373 (25)	1.488 (6)	.0029
c	3.443 (3)	3.351 (13)	3.275 (6)	NS
m1	1.947 (1)	1.983 (22)	1.589 (8)	.0136[a]
m2	2.475 (1)	2.743 (19)	2.461 (9)	0.383[a]

( ) sample size
[a]'t' test between term and post-term samples.

tooth (10/43 individuals). This lends a spurious homogeneity to the data base and reduces variation. A more rigorous, if less realizable, analysis would concentrate on separate analyses of tooth types (for example, male upper central incisor) from individual children.

Discussion

Previous research has shown that the neonatal line shifts cervically on the tooth crown as gestation is prolonged [*24*]. In that study the normal 95% range of variation in location of the neonatal line for births occurring at term (defined narrowly as 39 to 41 weeks), regardless of tooth type was about 1.7 mm. In the present study, based on a larger sample and with term defined as 38 to 42 weeks, in accord with obstetrical and clinical practices, the mean range was 1.5 mm; equivalent to 19 weeks of average crown elongation. This is a sobering statistic for it means that the normal location of the birth line, whether visible internally as a stria or externally as linear enamel hypoplasia, can appear to depart by more than 9 weeks from its average position. Clearly, in a forensic context, we would only consider those neonatal lines that lie beyond 2SD as potentially indicative of non-term birth in a particular individual. In the sample reported here, 11/15 (73%) of those teeth in this extreme range are actually from children born pre- or post-term (Fig. 3). While this is encouraging, we should recognize that extreme locations of the neonatal line are not unequivocal guides to non-term birth. Also, given that non-term newborns account for only 20% of births and that in this study only about 18% (11/62) of neonatal lines from children, not born at term, lie outside of the normal range (Fig. 3), it appears that disclosure of the neonatal line will assist in the identification of only 3 to 4% of unidentified immature remains (with primary teeth).

The research reported here is based on variation in the location of the neonatal line, which is readily discernible in the teeth of apparently healthy children whose enamel is otherwise free of accentuated striae at the magnifications employed here. Nevertheless, future efforts could be directed to distinguishing between pathological striae and neonatal lines in children stressed before and after birth; and towards differential primary tooth enamel susceptibility to birth and other stressors [*23*]. The primary canine was least useful in recording gestation-related variation in the location of the neonatal line and yet this tooth has great potential for recording accentuated enamel striae given its long development postnatally. Lower central incisors are less useful since the majority of their

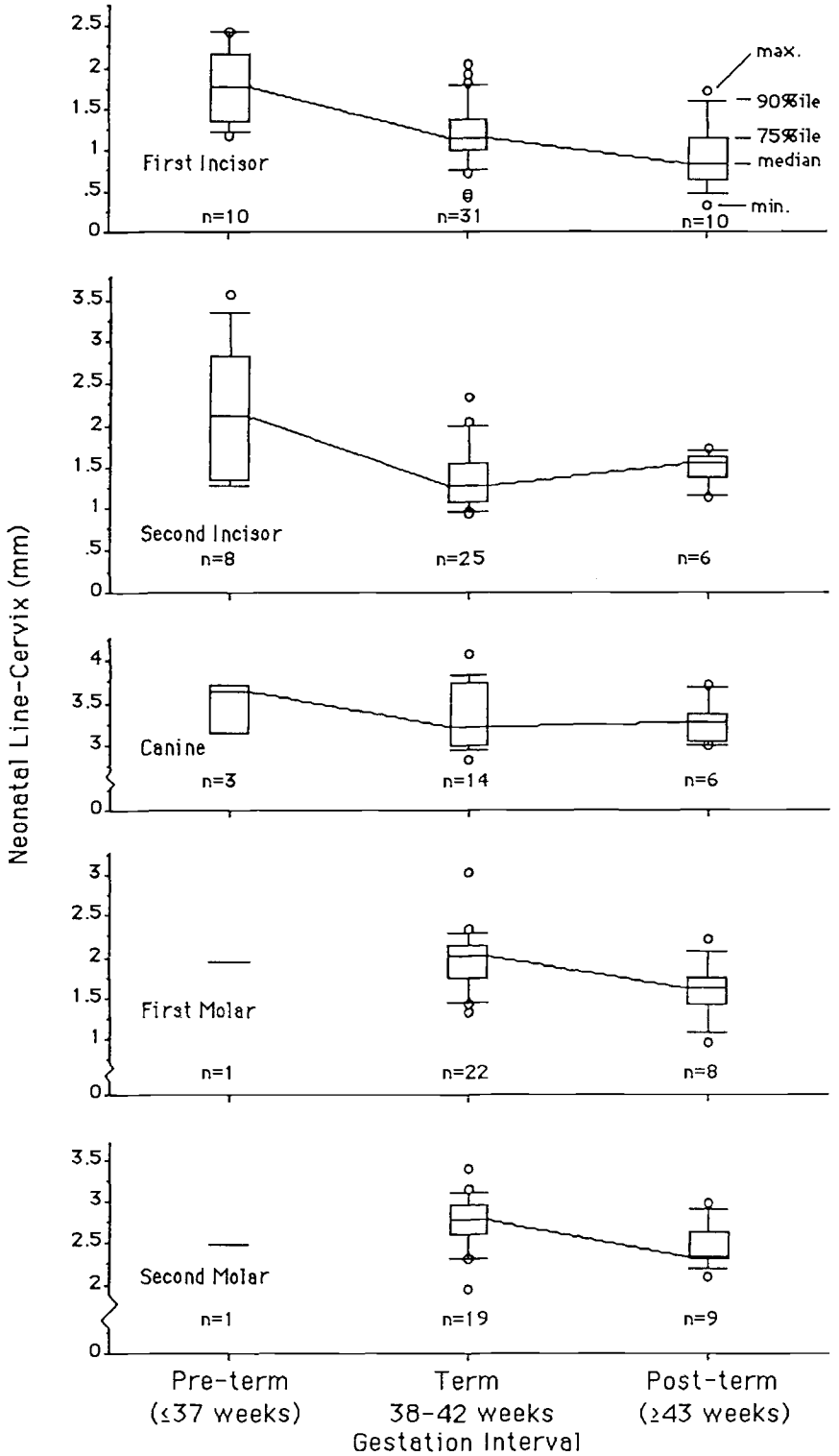

FIG. 1—*Variation in the location of the neonatal line for different gestation intervals for each primary tooth (see text for further details).*

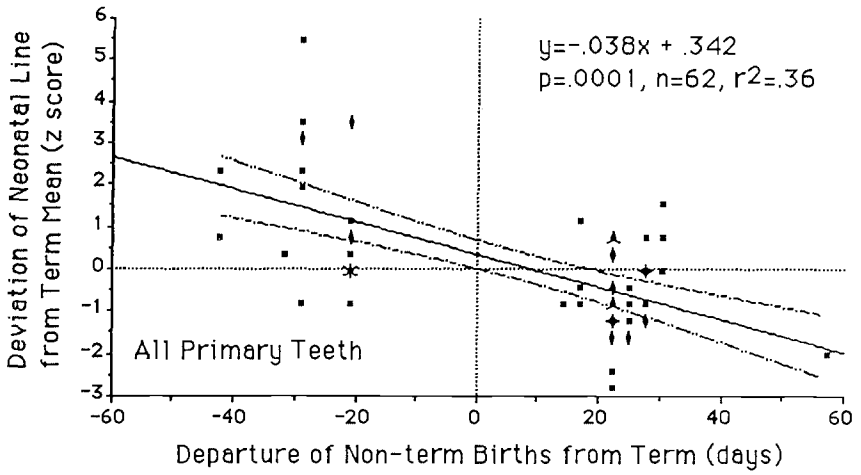

FIG. 2—*Linear regression for all primary teeth from non-term births of the distance from the neonatal line to the cervix (expressed as standardized deviations-z scores) and gestation length expressed as deviation in days from term birth (see text for further details).*

formation occurs prenatally. Primary molars are more often carious and calibration of the neonatal line is made more difficult by differences in which cusps are sectioned. As it stands, the lateral lower and maxillary central incisors are the most easily sectioned and interpreted teeth.

Given the variation in the neonatal line documented here, our ability to match micro and macrodefects of enamel (enamel hypoplasias) to even the best medical records will be problematic [*25*]. The promise of this approach has been demonstrated recently where a reasonably good correspondence was shown, in a particularly disadvantaged child who had spent almost half his life in institutional care, between pathological enamel striae and the timing of physiologically stressful episodes such as illness and surgery as reconstructed from a variety of health records [*1*].

Regardless of the individuality of the location of the neonatal line, its unequivocal identification and relationship with other enamel striae are potentially of great medicolegal utility. This is very much unexplored territory. While there is a large literature on the dental effects of birth hypoxia [for example, *10*], there are few studies of enamel quality before and after birth in affected children. Similarly, to our knowledge, there are no studies of enamel quality in victims of sudden infant death syndrome (SIDS) despite numerous other tests to determine whether the infant's health was compromised prior to death [*26*]. In that a characteristic of SIDS is death at age 2 to 3 months, an attribute shared with remarkably few other diseases [*27*], histological examination of relative enamel quality in utero, perinatally and postnatally, would be of considerable interest.

Further studies along these lines, based on teeth from medically documented sources, are required if the potential of enamel striae in forensic science and medicine is to be realized. Given the permanent nature of this record of physiological events in late gestation and early childhood, we feel the research effort will be warranted.

Acknowledgments

We would like to thank mothers (and one father) from White Rock, south Surrey and Vancouver who showed a keen interest in the scientific significance of their children's teeth; and two anonymous reviewers for constructive criticisms.

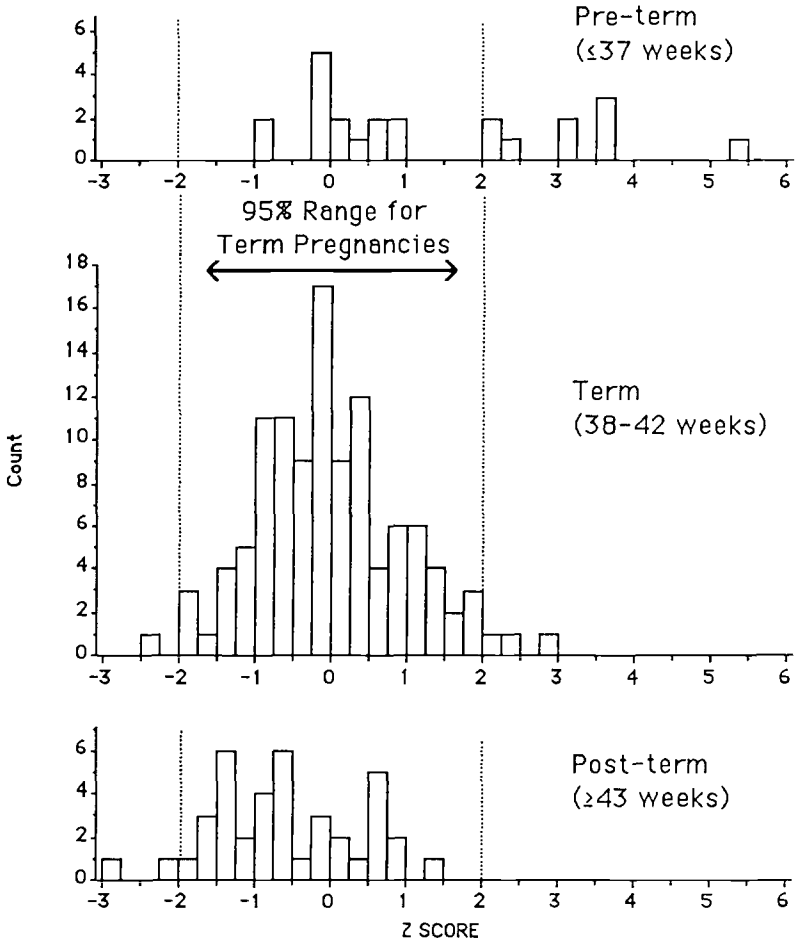

FIG. 3—*Location of the neonatal line in all primary teeth expressed as standardized deviations (z scores) from mean location of the line in teeth from term pregnancies.*

References

[1] Skinner, M. F. and Anderson, G., "Individualization and Enamel Histology: Case Report in Forensic Anthropology," *Journal of Forensic Sciences*, Vol. 36, 1991, pp. 939–948.

[2] Ziegel, E. E. and Cranly, M. S., *Obstetric Nursing (Eighth Edition)*, Toronto, Collier Macmillan, 1984.

[3] Rushton, M. A., "Fine Contour-lines of Enamel of Milk Teeth," *Dental Record*, Vol. 53, 1933, pp. 170–171.

[4] Schour, I., "Neonatal Line in Enamel and Dentin of Human Deciduous Teeth and First Permanent Molar," *Journal of the American Dental Association*, Vol. 23, 1936, pp. 1946–1955.

[5] Schour, I., *Noyes' Oral Histology and Embryology*, Philadelphia: Lea and Febiger, 1953.

[6] Rose, J. C., "Morphological Variations of Enamel Prisms Within Abnormal Striae of Retzius," *Human Biology*, Vol. 51, 1979, pp. 139–151.

[7] Wilson, D. F. and Shroff, F. R., "The Nature of the Striae of Retzius As Seen with the Optical Microscope," *Australian Dental Journal*, Vol. 15, 1970, pp. 162–171.

[8] Eli, I., Sarnat, H., Talmi, E., and Judes, H., "The Width of the Neonatal Line in Primary Enamel and Its Use As a Possible Diagnostic Tool in Developmental Disturbances," *Journal of Dental Research*, Vol. 65, 1986, p. 754.

[9] Noren, J. G., "Microscopic Study of Enamel Defects in Deciduous Teeth of Infants of Diabetic Mothers," *Acta Odontologica Scandinavia*, Vol. 42, 1984, pp. 153–156.
[10] Herman, S. C. and McDonald, R. E., "Enamel Hypoplasia in Cerebral Palsied Children," *Journal of Dentistry for Children*, Vol. 30, 1963, pp. 46–49.
[11] Massler, M., Schour, I., and Poncher, H. G., "Developmental Pattern of the Child as Reflected in the Calcification Pattern of the Teeth," *American Journal of Diseases of Children*, Vol. 62, 1941, pp. 33–67.
[12] Schour, I. and Kronfeld, R., "Tooth Ring Analysis: IV. Neonatal Dental Hypoplasia: Analysis of the Teeth of an Infant with Injury of the Brain at Birth," *Archives of Pathology*, Vol. 26, 1938, pp. 471–490.
[13] Kronfeld, R. and Schour, I., "Neonatal Dental Hypoplasia," *Journal of American Dental Association*, Vol. 26, 1939, pp. 18–20.
[14] Noren, J. G., "Enamel Structure in Deciduous Teeth from Low Birthweight Infants," *Human Deciduous Enamel in Perinatal Disorders*, J. G. Noren, Ed., Goteborg: Department of Pedodontics, 1983, pp. 1–15.
[15] Gustafson, G. and Gustafson, A.-G., "Microanatomy and Histochemistry of Enamel," A. E. W. Miles, Ed., *Structure and Chemical Organization of Teeth*, Vol. 7, New York, Academic Press, 1967, pp. 75–133.
[16] Weber, D. F. and Eisenmann, D. R., "Microscopy of the Neonatal Line in Developing Human Enamel," *American Journal of Anatomy*, Vol. 132, 1971, pp. 375–392.
[17] Noren, J. G., "Neonatal Lines and Enamel Hypoplasia in Deciduous Teeth of Infants of Diabetic Mothers," *Human Deciduous Enamel in Perinatal Disorders*, J. G. Noren, Ed., Goteborg: Department of Pedodontics, 1983, pp. 1–5.
[18] McMillan, R. S. and Kashgarian, M., "Relation of Human Abnormalities of Structure and Function to Abnormalities of the Dentition. I. Relation of Hypoplasia of Enamel to Cerebral and Ocular Disorders," *Journal of the American Dental Association*, Vol. 63, 1961, pp. 38–47.
[19] Sarnat, B. G. and Schour, I., "Enamel Hypoplasia (Chronological Enamel Aplasia) in Relation to Systemic Disease: A Chronologic, Morphologic and Etiologic Classification," *Journal of American Dental Association*, Vol. 29, 1942, pp. 67–75.
[20] Eli, I., Sarnat, H., and Talmi, E., "Effect of the Birth Process on the Neonatal Line in Primary Tooth Enamel," *Pediatric Dentistry*, Vol. 11, 1989, pp. 220–224.
[21] Condon, K. W., Correspondence of Developmental Enamel Defects Between the Mandibular Canine and First Premolar. MA thesis, Department of Anthropology, University of Arkansas, Fayetteville, 1981.
[22] Goodman, A. H. and Armelagos, G. J., "The Chronological Distribution of Enamel Hypoplasia in Human Permanent Incisor and Canine Teeth," *Archives of Oral Biology*, Vol. 30, 1985, pp. 503–507.
[23] Donat, L. D. and Rose, J. C., "Enamel Hypoplasia: A Morphological Classification," *American Journal of Physical Anthropology Supplement*, Vol. 12, 1991, p. 69.
[24] Skinner, M. F., "Gestation Length and Location of the Neonatal Line in Human Enamel," *Journal of Paleopathology* (Monographic Publications), Vol. 2, 1992, pp. 41–50.
[25] Goodman, A. H. and Rose, J. C., "Assessment of Systemic Physiological Perturbations from Dental Enamel Hypoplasias and Associated Histological Structures," *Yearbook of Physical Anthropology*, Vol. 33, 1991, pp. 59–110.
[26] Peterson, D. R., "Epidemiology of the Sudden Infant Death Syndrome: Problems, Progress, Prospects. A Review," *Sudden Infant Death Syndrome*, J. T. Tildon, L. M. Roeder, and A. Steinschneider, Eds., Toronto: Academic Press, 1983, pp. 89–97.
[27] Peterson, D. R., "The Epidemiology of Sudden Infant Death Syndrome," *Sudden Infant Death Syndrome: Medical Aspects and Psychological Management*, J. L. Culbertson, H. F. Krous, and R. D. Bendell, Eds., Baltimore, Johns Hopkins, 1988, pp. 3–17.

Address requests for reprints or additional information to
Mark Skinner, Ph.D.
Dept. of Archaeology
Simon Fraser University
Burnaby, BC V5A 1S6
Canada