

CASE REPORT

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Individualization and Enamel Histology: A Case Report in Forensic Anthropology

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ABSTRACT: The cranium of a native Indian child about six years old was found in 1979 near Taseko River, British Columbia, Canada. While the remains matched the report of a child missing for eight years in terms of race, age at death, locale, and elapsed time since death, the cranium and dentition were basically unidentifiable because of the claimed lack of medical or dental history. There was no dental work, and the parents were unknown or dead. We report the presence, in the dental enamel of the primary and secondary dentition, of stress markers, termed striae of Retzius, whose locations correspond well with anecdotal reports and recently discovered medical records which describe the timing of specific episodes of stress. The enhanced probability of personal identification from dental histological stress markers is evaluated.

KEYWORDS: odontology, dentition, dental enamel, enamel histology, stress markers, Native American Indian, individualization

This report describes the authors' somewhat frustrated efforts to show the identity of a child's cranium. Our purpose is to promote the study of stress markers in dental enamel as a fundamental investigative technique in forensic anthropology and odontology. Immature remains are notoriously difficult to identify. Not only do they typically lack dental work and a medical history that describes hard tissue changes, but the sex and race of the child are little expressed in the bones and teeth. One clear advantage, however, afforded by immature remains in comparison with adult skeletonized remains is that the age at death and the timing of physiologically stressful episodes can potentially be determined with some precision. This precision is attributed, first, to the rapidity with which major growth milestones (for example, dental formation) are attained and, second, to the fact that, in some tissues, the moment of birth is recorded. This affords the investigator a baseline from which to gauge the timing of later events.

Incremental brown striae in the enamel of teeth viewed optically in thin section were first described by Anders Retzius in 1837 [1]. The application of enamel histology to the

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problem of human remains identification began with Gustafson's demonstration in 1955 of the similarity in incremental lines between contralateral pairs of teeth from a single individual [2], although they were noted in 1939 by Fujita [3]. From 1963 to the present, Boyde has been the most forceful proponent of the methodology [4-6]. According to Boyde:

the pattern of these lines is the same in all those parts of the enamel that formed at the same time in a given dentition. Thus, the different teeth developing in one individual give the same pattern of incremental lines, which is distinct from that of another individual. The pattern is a kind of fingerprint of enamel development specific to the individual [Ref 6, p. 377].

A clear distinction must be drawn between two forensic science uses of striae of Retzius. The pattern and timing of a series of such incremental lines, as reported in detail here, can be matched to a premortem record of physiological stress experienced by a child reported missing; this procedure's most serious weakness stems simply from our current ignorance of enamel formation rates as they vary from tooth to tooth and child to child. The other forensic application of striae of Retzius, in which the age at death and the timing of specific striae are determined by counting "cross-striations" between major striae, has been historically flawed by the mistaken assumptions (a) that the cross striations are, without question, circadian in rhythmicity and (b) that they can be counted in routine optical microscopy histological sections.

Incremental Growth Markers in Enamel

Enamel is a hard layer of mineralized tissue on the chewing surfaces of teeth. In humans, it is about 1 to 2 mm thick [7]. Many excellent reviews of amelogenesis are available [6,8-10]. The fundamental structural unit of enamel is a prism, typically keyhole-shaped in cross section, which is deposited by four neighboring ameloblasts commencing at the dentinoenamel junction and finishing at the surface of the crown. Enamel is composed of thousands of tightly packed prisms. Mature enamel is 96% mineral by weight. Enamel forms in two basic steps: excretion of an organic matrix, followed immediately by mineralization. The latter is a protracted process that can span years, occurring even post-eruptively [11]. Immature (that is, newly deposited) enamel is formed of tiny crystallites of mineral akin to hydroxyapatite, composed mainly of calcium and phosphorus. Enamel maturation occurs through growth in the size of the crystallites deposited upon initial mineralization, largely replacing the organic matrix. Enamel is deposited in successive layers which extend also at the cervical margin of the forming crown. Under certain conditions of specimen preparation and lighting, the layering is observable within individual prisms, as cross-striations, and between prisms as striae of Retzius. Indeed, it is often claimed that the latter are simply a series of accentuated cross-striations [12].

However, the etiology of both cross-striations and striae of Retzius is not well understood. Boyde has suggested that there is a daily interval of a decreased rate of matrix and mineral secretion, permitting the deposition of more crystallites on the sides of the Tomes' process pit [6]. This produces a localized swelling in the prism which alternates with a narrowing of the prism as the ameloblast's activity speeds up. The result is periodic varicosities along the prism's length which are termed cross-striations. Osborn, in 1973, attributed these to undulations of the prism's surface contour with a periodicity of about 4 to 8 μm ; that is, the prism diameter is constant with kinks which bend the light. It is likely that the cross-striations are less mineralized areas produced by diurnal variation in cellular activity, which results in episodic buckling of the cell as hydrostatic forces recommence after an interval of quiescence [13].

Boyde's repeated assertions over almost three decades [4-6], based on observations by Asper half a century before [17], that cross-striations with a repeat interval of 4 μm

exist and that these are deposited daily has not gone unchallenged [14–16]. However, there is no evidence that the rate of “banding” is circadian. The latter assumption arose historically through a methodological error in the optical microscopy of sectioned teeth. Only recently has it been realized that, because enamel prisms, which undulate significantly in their course from the dentinoenamel junction to the surface, are so very long and thin, there is virtually no chance that a sectioned surface would parallel a prism throughout its length. In fact, prisms, which are deposited in adjacent rows are, inevitably, sectioned more or less obliquely across their width. This creates the optical illusion of cross-striations, which are in reality the ends of transversely or obliquely sectioned adjacent prisms [14], a conclusion acknowledged by Boyde in 1979 [18]. For this reason, Boyde’s (1963) technique of age at death estimation in an Anglo-Saxon child [4], in which he counted “cross-striations” (equated with days) from the neonatal line to a prominent stria formed just before death on a later forming tooth, is invalid.

Turning now to the major variety of incremental marker, the striae of Retzius, there is general agreement that they are an exaggerated version of the same physicochemical alteration that causes cross-striations; for example, Osborn accounts for striae of Retzius as reflecting hydrostatically mediated changes in secretory rate which result in major changes in prism direction [13]. Several authors distinguish between “pathological” striae (those systemic disturbances described in this case report which act as individualizing “signature” patterns within the enamel [3]) and “rhythmic” or “physiological” striae, whose cause is unknown but which appear evenly spaced, most visibly in the gingival enamel [8,19,20].

A major assumption of this paper is that (nonrhythmic) striae of Retzius reflect physiologic stress. The term “stress” is used here in the Selyean sense described by Goodman et al. [21]: that is, a nonspecific or generalized environmental stressor with physical or psychological components, or both, which disturbs an individual’s physiological homeostasis. That enamel striae form in response to disturbance of the “milieu interieur” is supported by their optical equivalence to the neonatal line, thought to be produced by environmental shock and the birth process itself [1]. While Godt in 1967 failed to find a link between disease and striae, Bouyssou et al. were more successful in 1957 [20]. The brown appearance of striae under transmitted light is caused by “Rayleigh” scattering, which is probably due to a “statistical shift in the balance of crystallite size and intercrystalline space” (Ref. 6, p. 459). Etched enamel sections prepared by Boyde in 1970 showed apparent breaks in adjacent prisms, corresponding to a stria of Retzius, which he attributed to their being less mineralized [22,23]. In that the maturation phase of enamel formation consists of the expansion through accretion of individual crystallites formed at the time of initial matrix secretion, it can be predicted that striae of Retzius are gaps in the enamel [24] occasioned by the lack of minerals. Gaps between prisms where striae of Retzius meet the surface were described by Osborn in 1981 [9]. In this view, striae of Retzius are a problem of mineral availability [10] and are not due to matrix malformation. In summary, we can still agree with Klees and Brabant who wrote in 1962 that “La question de l’interprétation des stries de Retzius est loin d’être entièrement résolue” (Ref 19, p. 100).

Rates of Enamel Prism Elongation

There are few clear observations of the rates of enamel prism elongation, and yet these are essential if we are to infer stress timing from the distance between successive striae. When it was thought that cross-striations were formed daily and were visible in routine histological sections, the repeat interval was reported to be about 4 μm [17]. However, this is the same as the transverse diameter of an enamel prism. What appeared to be striated enamel prisms viewed longitudinally were in actuality rows of aligned prisms

measured in cross section. Shellis and Risnes tried to interpolate prism formation rates by measuring the total cumulative enamel prism lengthening and dividing this by the probable time for the crown to form [25,26]. The latter is only crudely known, however. Their results are contradictory, in that Risnes finds a rate of $2.9 \mu\text{m/day}$ (for maxillary premolars) while Shellis provides generally much higher figures: about $2.6 \pm 0.3 \mu\text{m}$ (inner) to $5.5 \pm 0.8 \mu\text{m}$ (outer) for permanent enamel and $4.5 \pm 0.3 \mu\text{m}$ (inner) and $5.3 \pm 0.8 \mu\text{m}$ (outer) for primary enamel. Only one study provides an estimate of enamel prism elongation rate that was suitable for the present problem, that is, one which did not rely on the assumption of circadian cross-striation periodicity and which included both primary and permanent molars from a single individual. Schour and Poncher in 1937 reported the mean interstriae distance for a primary second molar and a first permanent molar in a terminally ill child given timed injections of a substance which marked the enamel. They observed that primary enamel formed at $3.92 \mu\text{m/day}$ and permanent enamel formed at $2.60 \mu\text{m/day}$ [27]. This, of course, is functional elongation, which does not include undulations in direction.

Materials and Methods

Histological enamel structures from a cranium found 20 Oct. 1979 in the bush near Taseko River in north central British Columbia are described. No other bones or artifacts were found. The cranium was extremely bleached, showing exfoliation of the outer vault table due to prolonged exposure to the elements. A remnant of cartilage from within the nose was recovered, enabling an estimate of elapsed time since death of 5 to 10 years. The unerupted maxillary incisors were discernible in their crypts as being shovel-shaped; consequently, given the geographic location of the find, the remains were judged to be most likely native Indian [28] (not precluding admixture). The maxillary primary molars were erupted and retained, while the permanent first molars were erupting. Radiological determination of dental formation yielded an age at death estimate of 5.6 ± 0.75 years [29,30]. This original estimate can now be augmented by recourse to Trodden's combined sex standard of tooth formation in Indian children [31], which provides an average of 5.36 ± 0.87 years.

The remains were consistent with being those of Stanley S., a native boy who was lost in the woods 8 years earlier when he was 5 years, 7 months old. The senior author was advised in 1980 and 1985 that medical records did not exist. Apart from a noticeably small palate and a counterclockwise rotated left central incisor, there were no individualizing traits. We thought of trying to match occlusal cusp details with Stanley S.'s parents but were advised that the father was unknown and the mother was dead. A relative, acting on behalf of the Nemiah Valley Indian Band, declined to accept the remains until more conclusive evidence was found. However, a death certificate was issued and the remains were retained at the Forensic Anthropology Laboratory at Simon Fraser University.

Recently, we returned to this case on the presumption that, had Stanley's mother predeceased him, this event would have been sufficiently traumatic to leave a stress marker in the teeth whose timing could be determined with some precision from thin sections of the enamel. At first it proved difficult to obtain a death certificate for his mother, as her first name was not recorded in the police file. The band office directed us to the Alexis Creek Health Centre, through which we obtained, to our great surprise and satisfaction, a very detailed medical history for Stanley S. However, we performed the histological analysis prior to obtaining these.

Due to the brittleness of the teeth and the need to obtain a continuous, overlapping record of enamel formation from birth to death, almost all of the primary and (unerupted) permanent teeth were processed. Overlying alveolar bone was removed with a motorized

Dremel burr. Most of the crowns had already split into two or more fragments. Reconstructed crowns were embedded in Crystal Clear polyester casting resin with five drops of Fiber-Tek catalyst, allowed to cure, and longitudinally sectioned at approximately 180 to 200 μm with a Buehler Isomet low-speed saw with a diamond wafering blade. The sections were not ground. Mounted sections were examined and photographed at $\times 20$ magnification with ordinary and polarized light with a Nikon AFM microscope.

In order to homologize striae between teeth, composite photographs showing the entire labial/buccal enamel were created (Fig. 1). Matching striae is difficult. It requires a knowledge of approximate crown formation spans and a familiarity with the variation in interstriae spacing in primary as opposed to secondary teeth and earlier as opposed to later-forming crowns. It is a labor-intensive process, and several dozen hours were spent on this task alone before the major striae were identified with confidence in all the teeth.

Results

Striae were recorded in detail on three maxillary teeth (Table 1): 5-5 (right second primary molar), 1-6 (right first permanent molar), and 1-3 (right permanent canine). Stria No. 1, the neonatal line, was present only on the primary tooth. On this tooth, a triplet of lines (Nos. 5 through 7) spanned 137.7 μm (measured along the prisms). At a formation rate of 3.92 $\mu\text{m}/\text{day}$ [27], this triplet lasted 35.1 days, finishing at age 0.51 years. A homologous triplet on the first molar spanned only 63.4 μm , yielding a prism elongation rate of 1.81 $\mu\text{m}/\text{day}$ (somewhat less than the 2.60 $\mu\text{m}/\text{day}$ reported by Schour and Poncher for the same tooth).

A similar procedure was used to homologize Striae 8 and 9, between the first molar and the canine. This resulted in a prism elongation rate of 1.59 $\mu\text{m}/\text{day}$ for the latter tooth. The estimated age for each significant stria and the probable matching stress event, derived from the newly discovered health records, are shown in Fig. 2.

Discussion

Estimation of the age of occurrence of the striae was done without prior knowledge of the medical history. Nevertheless, matching the two records is a subjective process. In our opinion the correspondences, shown in Fig. 2, are close. Discrepancies can be explained primarily as reflecting our relative ignorance of the enamel prism elongation rates. The apparent rates for the permanent teeth, reconstructed in this case at about 1.6 to 1.8 $\mu\text{m}/\text{day}$, seem very low compared with values in the literature (compare this with 2.9 $\mu\text{m}/\text{day}$ reported by Risnes in 1986 and 2.6 to 5.5 $\mu\text{m}/\text{day}$ by Shellis in 1984). Numerous factors could explain this discrepancy, but there is virtually no research available on enamel prism elongation rates which will clarify the situation.

Perhaps the most convincing aspect of Fig. 2 is the presence in the enamel of two lengthy intervals of homogenous enamel: the first includes Striae 8 through 10, which correspond to the period when Stanley S. was in a foster home close to public health care and during which time he experienced surgery and became ill (equated with Stria 9); the second corresponds with the period after his mother died when Stanley returned to foster care for at least a year and includes the striae-free enamel between Nos. 11 and 12. Also significant in our opinion is the major stria (No. 8), marking an abrupt change in most of the teeth from white to brown enamel, which probably marks Stanley's hospitalization with the combined stressors of food poisoning, pneumonia, and asthma.

Although this study does not make the claim that the pattern and timing of brown striae in enamel are sufficient for identification, the likelihood is, in our opinion, much increased. However, any doubts as to the identity of the cranium were dispelled when the recently discovered medical records reported surgery for cleft of the soft palate. The

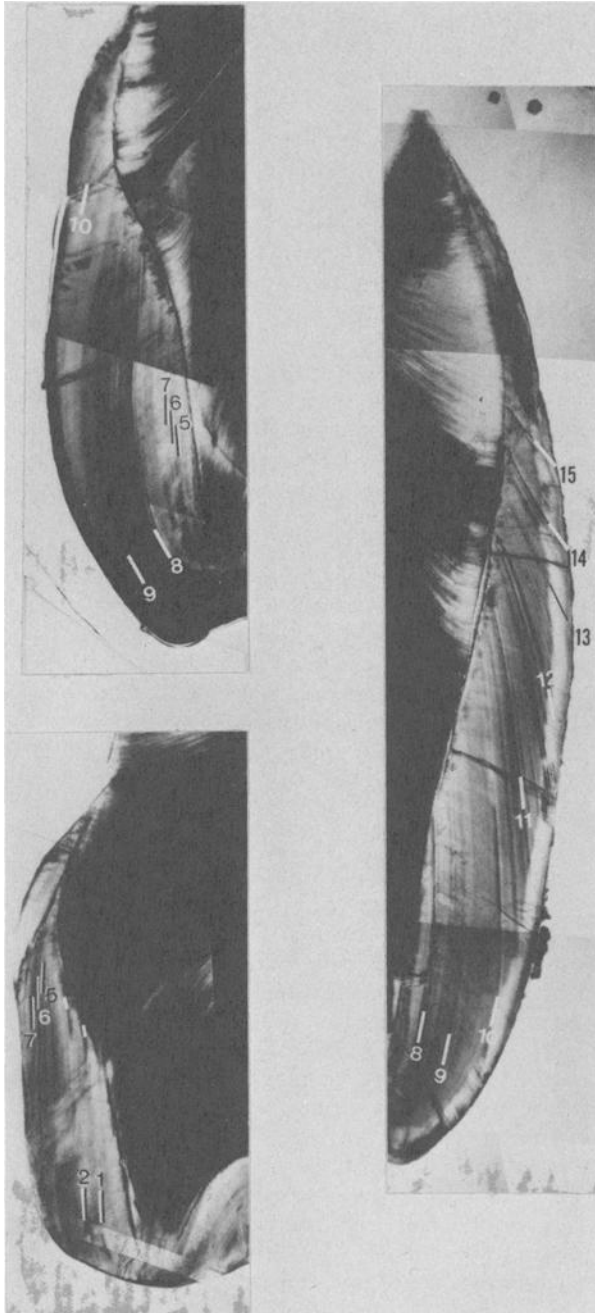


FIG. 1—Longitudinal thin sections of dental enamel from subject Stanley S., showing the location of corresponding striae in three developing maxillary teeth (FDI designation): (bottom left) 5-5, right second primary molar; (top left) 1-6, right first permanent molar; (right) 1-3, right permanent canine. Stria No. 1 on Tooth 5-5 is the neonatal line; Striae Nos. 5 through 7 are present in both Tooth 5-5 and Tooth 1-6; Stria No. 8 indicates the so-called "major stressor" (see text and Fig. 2 for further details).

TABLE 1—Method for estimating stress timing.

Tooth (FDI) ^a	Stria No.	Interstriae Distance, μm	Rate, μm	Formation Time, days	Age at Stress, years
5-5	1(birth) to 7	727.6	3.92 ^b	185.6	0.51
5-5	5 to 7	137.7	3.92	35.1	...
1-6	5 to 7	63.4	1.81 (inferred)	35.1 (equated)	...
1-6	7 to 8	373.9	1.81	206.6	1.07
1-6	8 to 9	407.7	1.81	225.3	...
1-3	8 to 9	358.3	1.59 (inferred)	225.3 (equated)	1.69
1-3	9 to 10	523.3	1.59	329.1	2.59
1-3	10 to 11	325.3	1.59	204.6	3.15
1-3	11 to 12	477.9	1.59	300.6	3.98
1-3	12 to 14	687.1	1.59	432.2	4.48
1-3	14 to 15	398.2	1.59	250.5	5.17

^aFDI = Federation Dentaire International.

^bSchour and Poncher, 1937. (See text for details of calculations).

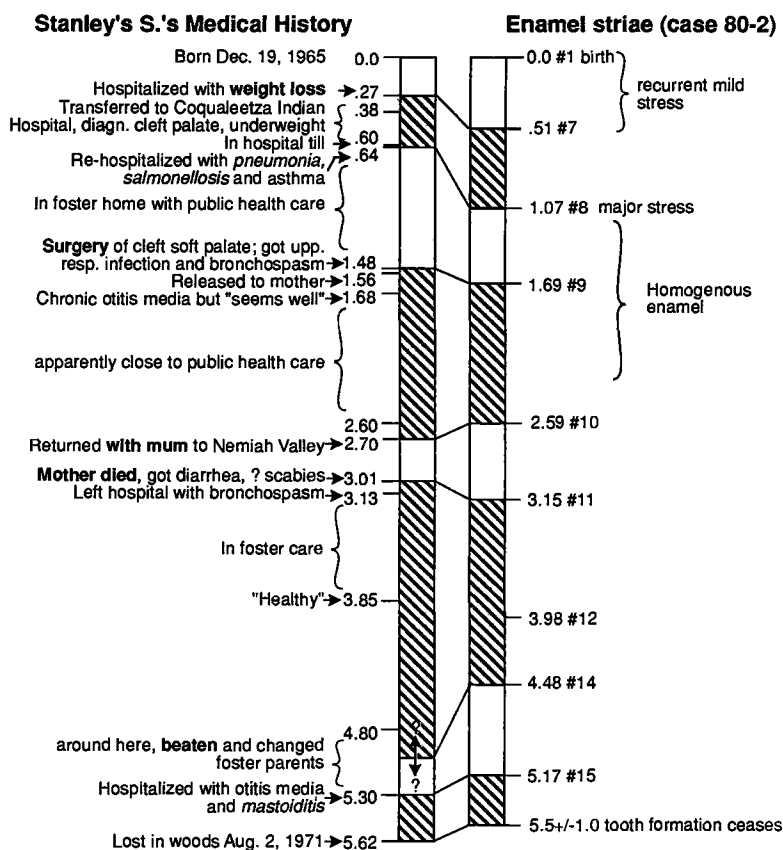


FIG. 2—Proposed correspondence of the actual timing of stress events with estimated time of formation of enamel striae (see text for details).



FIG. 3—Superimposition of dental cast on the cranium. The cast was prepared before extraction of teeth for histological study and before the following individualizing peculiarities of the palate were appreciated: deviation of both intermaxillary and palatine sutures, pocketing of the palatal surface, and a microcleft on the posterior margin of the hard palate (sequelae of surgical correction of a cleft of the soft palate).

posterior margin of the hard palate on the cranium shows a microcleft, while the intermaxillary suture meanders unusually between palatal processes that are markedly irregular in surface contour (Fig. 3). These changes, not previously recognized by us, are clear, if subtle, expressions of restoration of palatal clefting.³

Conclusions

This case shows that enamel striae have the potential for making the identification of immature, and otherwise unremarkable, skeletons much more probable, although not absolutely so. Clearly, there is a need for research on variation in enamel prism formation rates and the factors affecting the expression of striae. Lastly, the potential contribution of enamel histological structures such as striae of Retzius to human remains identification will require controlled studies of replicability and observer error.

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