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Original communication

A method of calculating human deciduous crown formation times and of estimating the chronological ages of stressful events occurring during deciduous enamel formation

W. Birch, Ph.D, Anatomist *, M.C. Dean, Ph.D, Professor of Anatomy

Department of Cell and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK

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ABSTRACT

Knowledge of deciduous crown formation times is useful in forensic anthropology and when aging juvenile remains from an archaeological context. Until now, histological techniques for calculating enamel formation times in deciduous teeth have been completely dependent on being able to visualise clear daily incremental markings. In the first part of this study we took twenty deciduous teeth where daily incremental markings were easily visible on both aspects of the crown and used these as the basis for generating regression equations to predict enamel formation times. We were then able to use these regression equations to calculate deciduous crown formation times in a further fifty deciduous teeth where it was not possible to see daily increments. We present here new data for deciduous crown formation times based on these regression equations. In the second part of this study these regression formulae were applied blind to teeth from two individuals with known medical histories. The formulae were able to successfully determine the times of prenatal and postnatal enamel formation relative to the neonatal line and also to correctly estimate the ages at which accentuated 'stress lines' occurred during the period of deciduous crown formation.

1. Introduction

Forensic investigations of the remains of infants and young children often include making estimates of an age at death based on dental development. Age estimation of an individual involves first establishing a biological age and then attempting to correlate it with a chronological age. Biological age may be expressed as either 'skeletal age' or 'dental age' and it is generally recognised that '*the relationship between chronological age and dental age is stronger than that for chronological age and skeletal age*,'.[1:3] The dental age of an individual can be estimated by examining the extent of dental eruption or the state of formation or maturation of the developing tooth germ. Of these two, '*formation of teeth appears to be more robust to environmental influences*'.[2:143] However, in order to estimate the dental age of an individual or an isolated tooth, the specimen in question must be compared to a 'known standard'. Unfortunately by doing this certain incompatibilities are inevitably introduced. For this reason, even though the establishment of age at death of juvenile remains can be considered more accurate than establishing the age of death of adult remains,[1,2] due to the short span of time being considered, the aging of juvenile remains based on dental development is nevertheless always only an '*estimation*'.[1:3] Such age estimations are usually derived from direct comparisons between the stage of dental formation of the deciduous teeth of the individual in question, with a similar stage of dental formation in a child of a known age. Although this is the method most commonly used, age estimations have also been derived from histological methods using counts of the daily incremental markings within enamel.[3–5] Although these histological techniques for estimating an age at death are very labour intensive, potentially they can provide more accurate age estimates.[6]

Previous studies that have attempted to establish a chronology for deciduous crown formation times have struggled to define the prenatal age of initial deciduous tooth mineralisation, the timing of birth during enamel formation and the cessation of enamel formation in the same individuals or specimens. Furthermore, different techniques and methods have been used by different authors using material from a variety of sources to try to identify the timing of these events. Unfortunately this has lead to a degree of confusion in the literature. The identification of initial mineralisation depends on the technique used to observe it.[7–10]

* Corresponding author. Anatomy Laboratory, Rockefeller Building, 21, University Street, University College London, London WC1E 6JJ, UK. Tel.: +44 2076796152.
E-mail address: w.birch@ucl.ac.uk (W. Birch).

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Inconsistencies and inaccuracies incurred in the determination of the actual age of the fetal specimens examined[11,12] and the inclusion of pathological specimens in the sample studied[13–16] have also contributed to the very variable picture of deciduous pre- and postnatal crown formation times. Moreover, in the majority of cases, sample sizes have inevitably been very small, sometimes consisting of only a few individuals,[13–15,17] however a small number of notable more recent studies have used considerably larger sample sizes.[8,12,18]

The main aim of this current study was to develop a simple histological technique which could be used to estimate deciduous crown formation times, without requiring fetal material. We developed a set of regression equations that describe the time taken to form any given thickness of deciduous enamel during crown formation and which can be measured on any longitudinal ground section. These data can then be referred to as 'known standards' and used to help estimate the age of human juvenile remains or isolated deciduous teeth. This work aims to define more clearly the start and finish of enamel matrix secretion in the deciduous crown, in order to improve methods for estimating the age at death of juvenile human remains from forensic, archaeological and even palaeontological contexts.[19] In order to test the usefulness of the equations generated in this study we then blind tested the regression equations on teeth from two individuals with a known medical history of stress events, many of which could be observed as accentuated markings in the enamel. The data presented here are not intended to be taken so much as new improved standards, but rather the methods proposed here offer an example and a solution of how objective data can now be collected from isolated deciduous teeth.

2. Materials and methods

From a sample of approximately 100 longitudinal ground sections of deciduous mandibular teeth sectioned in the true buccolingual axial plane, four of each tooth type were selected that exhibited clearly visible daily cross-striations along the prism paths, between the enamel-dentine junction (EDJ) and the enamel surface ($n = 20$). These sections were made from extracted deciduous teeth collected from dental clinics in the UK and are of multiethnic origins. Any teeth exhibiting pathology or excessive attrition or abrasion were rejected from the sample.

Each aspect (lingual and labial/buccal) of each crown section was then divided into occlusal, lateral and cervical regions (see Fig. 1a). Photomontages were constructed of each of these regions for both aspects. These montages were constructed from a series of overlapping photographic prints taken with an Olympus OM-2N camera loaded with Kodak Gold 200 film attached to a Carl Zeiss Jenamed 2 light microscope with an apochromat 25×/0.65 ∞/0.17-A objective lens. The resulting fieldwidth of a 5 × 7 inch print was 410 μm which is large enough to keep measurement error to a minimum.

Distances of 100 μm, measured along one prism path in each region on each aspect of every crown were indicated on the photomontages (see long broken black line Fig. 1b). A 50 μm zone was included near the surface if the enamel stopped significantly short of a 100 μm measurement.[20] Cumulative daily cross-striation counts were made along each prism path and recorded at every 100 μm or final 50 μm of enamel thickness, from the EDJ to the enamel surface in the occlusal, lateral and cervical regions of each tooth type. This procedure was repeated on both lingual and labial/buccal aspects of each ground section (total prism paths counted $n = 120$).

Linear regression equations were generated from the cumulative cross-striation counts plotted against linear enamel thickness

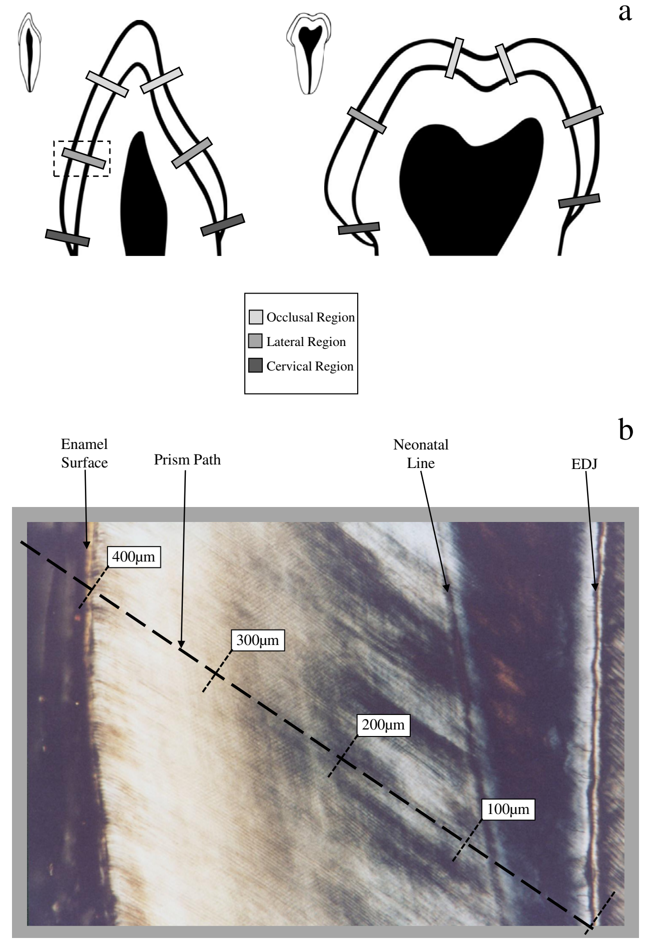

Fig. 1. a. Cervical, lateral and occlusal regions for anterior and posterior teeth as defined in this study. b. Polarised light micrograph of enamel in the lateral region of the labial aspect of a deciduous canine. The long broken black line runs along the prism path from the EDJ to the enamel surface passing through the neonatal line. Counts of enamel cross-striations were made along this prism path at 100 μm intervals (short broken lines) and cumulated.

along a prism path, for each aspect and each region of each tooth type using *Statview* (*Abacus System*™) these were then compared. Independent *t*-tests were performed to determine whether the labial/buccal and lingual aspect of each tooth differed significantly in their number of cross-striations. Three paired *t*-tests were also performed to determine whether there was any significant difference between the three different regions (cervical, lateral and occlusal) on each enamel aspect for each tooth type. Regression equations that combined data from statistically similar regions were then generated.

These combined regression equations were used to produce a reference table of increasing enamel thicknesses for each tooth type with predictions of the average number of days required to produce each thickness from the EDJ to the enamel surface, together with upper and lower 95% confidence limits.

The combined regression equations were next applied to a second sample of ground sections. This second sample consisted of 50 different deciduous ground sections (ten of each tooth type) and was selected from the original collection of 100 ground sections. This sample consisted of sections where the neonatal line and other accentuated striae were visible but where daily incremental markings were not necessarily well preserved.

Photomontages were constructed of the entire enamel crown of each tooth from the second sample. These montages were