THE NEONATAL LINE IN HUMAN DECIDUOUS ENAMEL

THE NEONATAL LINE IN HUMAN DECIDUOUS ENAMEL:

A NENONATAL LINE IS A NEONATAL LINE

IS A NEONATAL LINE

By

KRISTINA BLYSCHAK, B.Sc.

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AUTHOR: Kristina Blyschak, B. Sc. (University of Toronto)

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Abstract

Studies of deciduous enamel have concluded that the birth process and perinatal disorders result in the formation of a widened neonatal line and/or neonatal enamel hypoplasia. A widened neonatal line has been associated with a complicated delivery, neonatal difficulties at birth, maternal complications at birth, infant birthweight and short gestation length.

The present study measured the width of the neonatal line in a sample of forty-five primary teeth collected from Canadian children. Birth history information was gathered through in-depth interviews with the mothers of children donating teeth. Statistical testing was carried out to determine if significant differences exist in neonatal line width between groups of children with respect to birth trauma, neonatal health, maternal health and term at birth. A preliminary investigation of the effect of birth duration on the width of the neonatal line was undertaken. Line width differences between tooth classes were also considered. The results of the current study were compared to those of earlier clinical studies of the neonatal line and enamel hypoplasia.

The present data demonstrates that neonatal line width varies within the tooth crown and between tooth classes; a range of widths also exists within individual specimens. The observed relationships between neonatal line width and birth trauma, neonatal health, maternal health, and term at birth were not consistent with those of earlier dental studies. These associations were dependent on the tooth class examined and the location of the width measurements within the tooth crown. No correlation was found between birth duration and the width of the neonatal line.

The most troubling issue to emerge during the present study is the lack of a rigorous methodology in previous investigations of the neonatal line. This lack of procedural rigor may account for the discrepancies between the findings of the present study and those of earlier investigations. At present, the width of the neonatal line does not reflect the severity or the duration of the disturbance that caused it. Its use as a diagnostic tool in physical anthropology and during forensic investigations is not justified.

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Chapter One

Introduction and Statement of Purpose

Introduction

Dental researchers recognize that defects in enamel formation relate to systemic disruptions that occur during tooth growth and development. A disturbance sufficient to alter the regular growth pattern of an individual produces a corresponding disturbance in the metabolic function of all cells and will be recorded in the tissues as a developmental defect. Because enamel does not remodel once it is formed, any disturbance encountered by an individual during active tooth growth will be retained and maintained in the form of a macroscopic and/or microscopic enamel defect.

For more than half a century, dental researchers have accepted that the birth process leaves its mark on human enamel in the form of a distinct line: the neonatal line. The neonatal line is a microscopically visible area of altered growth in the enamel crowns of all primary teeth and first permanent molars. It marks the expected state of development of each tooth at the time of parturition. Since its first description by Rushton (1933 and 1939) and Schour (1936), dental investigators have focused their efforts on identifying those factors surrounding birth that may influence its appearance and its structure. The most common explanation for the development of the neonatal line is that it relates to the physiological changes occurring at birth and/or to the neonate's adjustment to life outside the uterus (Schour, 1936; Kronfeld and Schour, 1939; Schour and Kronfeld, 1938; Massler et al. 1941). Other researchers have attributed its presence and its appearance to birth trauma (Eli et al. 1989), maternal health at birth (Noren et al. 1978b; Noren, 1984), neonatal health (Noren et al. 1978a; Noren, 1983), gestation length and infant birthweight (Noren, 1983; Skinner, 1992; Skinner and Dupras, 1993).

The perinatal and neonatal conditions that have been proposed as causative factors in the formation of the neonatal line are the result of several decades of clinical inquiries of macroscopic aberrations (i.e. neonatal enamel hypoplasia) in human deciduous enamel (Stein, 1936 and 1947; Grahnen and Larsson, 1958; Kreshover et al. 1958; Miller and Forrester, 1959; Grahnen and Edlund, 1967; Grahnen et al. 1974; Funakoshi et al. 1981; Mellander et al. 1982; Noren and Alm, 1982; Johnsen et al. 1984; Pimlott et al. 1985; Seow et al. 1987; Seow et al. 1989; Seow, 1991 and 1992; Brooks et al. 1997). Although these studies recognized that neonatal enamel hypoplasia is related to "an abnormal extreme of the microscopic neonatal line found in most deciduous teeth of normal persons" (Via and Churchill 1959: 705), no attempt was made to measure the width of the neonatal line.

Eli and colleagues (1989) conducted the first study that attempted to correlate the width of the neonatal line to the nature of the birth process. They demonstrated that neonatal line width was significantly wider in children experiencing a complicated delivery and thinner in children undergoing a cesarean section. They suggest that because a neonatal line is apparent in the primary teeth of children experiencing no active birth (i.e. the elective c-section group) the transition from an intrauterine to extrauterine life is important in its formation. They also conclude that the trauma and the severity of the birth process have an impact on the width of the neonatal line. Eli et al. claim that the normal width of the neonatal line is 12 μm and that a line wider than 15 μm signifies complications at birth.

Since the publication of their findings, many researchers have accepted that the study of Eli et al. (1989) is conclusive. Skinner and Goodman (1992: 163) state:

> "It is well known that almost all primary enamel crowns will exhibit internally an accentuated stria known as the neonatal line...In that perinatal physiology and birth trauma are well-documented phenomena, the severity of the neonatal line provides an unappreciated guide to ameloblast sensitivity to stressors of documentable duration and type".

Only very recently have dental researchers briefly commented on the work of Eli et al. (1989). Ranggard et al. (1994 and 1995), whose primary goal was to examine the effect of

neonatal blood calcium levels on the width of the neonatal line, demonstrated that children experiencing optimal perinatal conditions had neonatal lines wider than 15 μm. Furthermore, they found that infants undergoing cesarean sections exhibited both thin and wide neonatal lines. These authors, however, do not suggest the reasons for these discrepancies.

Research into the "normal" width of the neonatal line has also produced conflicting results. Estimates of normal line width range from as small as 5 μm to as large as 40 μm (Weber and Eisenmann, 1971; Jackobsen, 1975; Noren et al. 1978a,b; Whittaker and Richards, 1978; Eli et al. 1989; Ranggard et al. 1994 and 1995). Research into the normal width of the neonatal line and the effect(s) of the birth process on its width is necessary.

A limited number of studies have considered the effects of neonatal and maternal health on the width of the neonatal line. These studies, however, do not provide line width measurements. Noren (1984) and Noren and colleagues (1978b) conducted a microscopic examination of the deciduous teeth of infants of diabetic mothers. They observed a widened neonatal line three times as frequently in children born to diabetic mothers compared to a group of healthy full term infants. Noren et al. (1978a) examined the effects of intra-uterine undernutrition on the neonatal line and found that a widened line was more often observed in infants that were classified "small for date". In a study of primary teeth from low birth weight infants, Noren (1983) found those children born preterm exhibited a widened neonatal line. Jaffe et al. (1985), Judes et al. (1985), and Noren and Gillberg (1987) detected widened neonatal lines in many children with Minimal Brain Dysfunction.

Since the publication of these studies, nearly twenty years ago, no attempts have been made to examine the effects of these, as well as other neonatal and maternal health complications, on the width of the neonatal line.

Statement of Purpose

Despite the relatively large body of published material supporting the assumption that perinatal and neonatal conditions have an effect on the width of the neonatal line and on the

appearance of deciduous enamel, few researchers have challenged these conclusions. No attempts have been made to determine the effect of inter-tooth differences on the width of the neonatal line or to examine whether the duration of the birth event has an impact on line width.

The primary objectives of this thesis are:

- to critically evaluate the study of Eli et al. (1989), to contrast their results with those of the present study and to comment on their conclusions that the birth process has an effect on the width of the neonatal line
- to determine if neonatal health, maternal health and term at birth have an effect on the width of the neonatal line and to compare the results obtained in the current investigation with those from previous neonatal line studies and from clinical investigations of neonatal enamel hypoplasia
- to determine if the duration of the birth process has an effect on the width of the neonatal line
- to determine if inter-tooth differences in neonatal line width exist
- to establish a "normal" neonatal line width
- to advocate the need for procedural rigor during studies of the neonatal line and to promote the creation of a permanent record of all analyses so that observations can be verified and replicated between studies.

To achieve these objectives, this thesis examines the width of the neonatal line in a sample of exfoliated primary teeth collected from forty-five children residing in Oakville and Hamilton, Ontario and in Montreal, Quebec. Using digital imaging and computer software programs, the width of the neonatal line was measured. Statistical testing was undertaken to determine if significant differences exist in neonatal line width between groups of children with respect to birth trauma, neonatal health, maternal health, term at birth. Statistical analyses were

also conducted between line width from different tooth classes and between line width and birth duration.

Contributions to Dental Research and Anthropology

Investigations of the neonatal line are the primary focus of dental researchers. One of the main criticisms, which can be leveled against previous work, is the lack of a rigorous methodology, and the omission of important information, during investigations of the neonatal line. Consequently, it is difficult to replicate earlier studies with confidence and comparison between studies is limited.

With the exception of a very few studies (Eli et al. 1989; Ranggard et al. 1994 and 1995; Rossi et al. 1997) earlier research has failed to include information on how the neonatal line is measured, the position on the tooth crown where measurements are taken, the magnification at which measurements are taken, and the results of statistical testing. Studies that do provide statistical results (i.e. Eli et al. 1989) are highly speculative because of the use of unjustified statistical tests. A careful review of the dental literature also indicates that some, but not all, dental researchers have found a positive relationship between the width of the neonatal line and/or the incidence of neonatal enamel hypoplasia and birth trauma, neonatal health, maternal health, infant birthweight and gestation length. Discrepancies between dental studies may also reflect the lack of agreement among medical experts regarding the effect(s) of birth trauma and neonatal conditions on the health of the neonate.

Skeletal biologists use enamel defects in the permanent and, less frequently, deciduous dentition, as relatively sensitive and nonspecific indicators of infant and childhood morbidity (Rose, 1973; Rose et al. 1978 and 1981; Rudney, 1983; Blakely and Armelagos, 1985; Goodman, 1989; Goodman et al. 1980, 1984 and 1992; Goodman and Rose, 1990; Wright, 1990; Skinner and Goodman, 1992; Duray, 1996; Simpson, 2000). With the exception of very few studies, investigations of the neonatal line in the primary dentition are uncommon (Verner et al. 1998; Rossi et al. 1997). The primary reason for this is the obvious lack of consensus among dental researchers regarding those factors that have an effect on the width of the neonatal line.

It was initially expected that the results of this thesis would provide physical anthropologists with a diagnostic tool for the interpretation of neonatal morbidity and mortality in past populations. Neonatal morbidity and mortality are commonly sought statistics by palaeodemographers. Deaths in the first month are attributed to causes preceding or associated with birth; these deaths reflect the endogenous state of the infant (Saunders et al. 1995; Saunders and Barrans, 2000). Given that the neonatal line forms at/around the time of birth, a widened neonatal line may help to establish whether an infant died because of a difficult birth, neonatal complications, or both. It was also anticipated that forensic investigations involving immature remains could profit from this research if the identification of a child rests on determining whether he/she experienced a natural birth, a complicated birth, or an elective c-section.

The results of the present study do not support the use of the neonatal line as a diagnostic tool during anthropological and/or forensic investigations. The findings also suggest that the width of a microscopic defect (i.e. neonatal line, Wilson band) is not a measure of the severity or the duration of the systemic perturbation that led to its formation. Consequently, deciduous enamel may not be a reliable recorder of prenatal, neonatal and postnatal insults. This thesis also paves the way for future dental and anthropological investigations. The susceptibility of different tooth classes to neonatal line formation, as well as the potential effect(s) of prism morphology, the plane of section, and section thickness on the width of the neonatal line, and other microstructural disturbances, are avenues that require more research than was possible during the present study.

Chapter Two

Tooth Development, Amelogenesis and Enamel Microstructure

A basic review of normal enamel development is necessary for any discussion of the histological defects of tooth enamel and of their use as indicators of infant and childhood morbidity. The material presented in this chapter is drawn from the following sources on dental histology: Raj Bhussry (1980), Yaeger (1980), Osborn (1981), Hillson (1996), Warshawsky (1988), Boyde (1976, 1989 and 1990), Tonge (1989), Aiello and Dean (1990), Avery (1994), Ten Cate (1994), Bath-Balogh and Fehrenbach (1997) and FitzGerald and Rose (2000). For the purposes of this review, development of the primary dentition is emphasized.

Tooth Development

A child's primary (deciduous) teeth develop mainly during the prenatal period. The deciduous dentition consists of twenty teeth, which erupt into the mouth during the first two to three years of life and are eventually shed, beginning around the sixth year of life. They are sequentially replaced by the permanent dentition. The deciduous dentition comprises three tooth types in each dental quadrant: incisors, canines and molars. Each of the deciduous tooth classes begins crown formation and completes crown development during a characteristic age interval (see Smith, 1991 for a review of the standards of tooth formation). Deciduous crown formation begins as early as thirteen weeks in utero with the first incisor and terminates around eleven months after birth, with completion of the second molar crown. The first permanent molar also begins to form in utero, twenty eight to thirty two weeks after fertilization (Hillson 1996: 121-124).

Tooth development is a continuous process; for descriptive purposes, its early development can be divided into a series of stages: the initiation stage, the bud stage, the cap stage, the bell stage, and the apposition stage (Figure 2-1A).

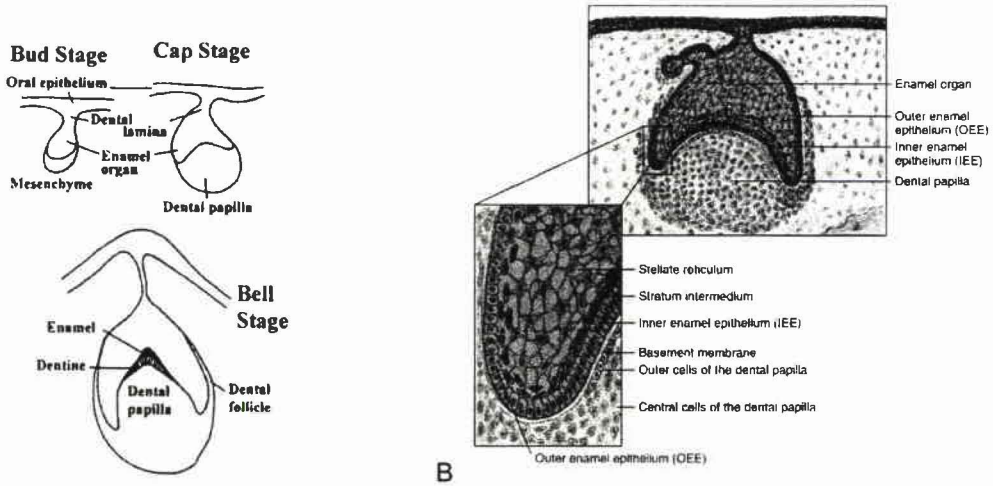

Figure 2-1. A. Stages of early tooth development and B. Four distinct cell types found in the enamel organ during the bell stage (after Hillson, 1996 and Bath-Balogh and Fehrenbach, 1997).

The initiation stage of tooth growth begins around the sixth week of embryonic development and results from an interaction between the oral epithelium and the neural crest derived mesenchyme. The oral epithelium lines both the maxillary and the mandibular arches; the mesenchyme lies deep to the oral epithelium and is separated from it by a basement membrane. Inductive interactions between these components results in the formation of a continuous sheet of epithelial tissue called the primary epithelial band. The epithelial band quickly gives rise to the dental lamina, which, through the subsequent stages of tooth development, produces the enamel organs of the deciduous teeth.

At around the eighth week of embryonic development, extensive proliferation of the dental lamina into the surrounding mesenchyme results in a number of localized epithelial swellings, or buds. This event initiates the bud stage of tooth development. Increased condensation of the mesenchymal cells around the buds also occurs at this time. At the end of this stage, the forming maxillary and mandibular arches will each have ten buds, corresponding to the positions of the future deciduous teeth.

The beginning of the cap stage of tooth development occurs between the ninth and tenth week of prenatal development. This stage is characterized by a shallow invagination on the deep

surface of the bud, leading to the formation of a cap, or the enamel organ. A condensation of mesenchymal cells deep to the bud and within the concavity of the enamel organ also occurs at this time. This mass of condensed mesenchymal cells is called the dental papilla. The remaining mesenchyme surrounding the outside of the enamel organ constitutes the dental sac or follicle. A basement membrane remains between the dental papilla and the enamel organ and is the future site of the dentine-enamel junction (DEJ). Combined, these three structures comprise the tooth germ, or primordium of the tooth. The tooth germ gives rise to the tooth and its supporting structures: the enamel organ to enamel; the dental papilla to the dentine and the pulp tissue, and the dental follicle to the cementum, the periodontal ligament and the alveolar bone.

Continued growth of the tooth germ leads to the succeeding stage of tooth development, the bell stage. This stage occurs during the eleventh and twelfth week of prenatal development. Important developmental changes, which begin late in the cap stage, reach their fullest extent during the bell stage. These changes, termed histodifferentiation, result in the formation of four distinct cell types within the enamel organ (Figure 2-1B): 1) the outer enamel epithelial cells; 2) the inner enamel epithelial; 3) the stratum intermedium; and 4) the stellate reticulum.

The formation of the dental hard tissues occurs during the late bell stage. The differentiation of the hard tissue producing cells is the result of a series of reciprocal inductive interactions between the dental papillae cells and the inner enamel epithelial cells. Inductive influences from the inner enamel epithelial cells cause the outermost layer of the dental papilla to differentiate into odontoblasts (dentine forming cells) and to begin the secretion of dentine on their side of the basement membrane. The basement membrane eventually disintegrates allowing the inner enamel epithelial cells to meet the newly formed dentine. This event induces the epithelial cells to differentiate into enamel cells (ameloblasts) and to begin enamel secretion. In preparation for enamel secretion, the ameloblasts undergo considerable morphological differentiation. The most prominent changes include: 1) elongation of the cell body, 2) the development of polarity, and 3) an increase in the number of organelles, especially those involved in protein synthesis and secretion (Yaeger, 1980; Osborn, 1981; Boyde, 1989; Ten Cate, 1994;

Moss-Salentijn et al. 1997). With the beginning of enamel matrix secretion, mineralization of the disintegrating basement membrane occurs, and the DEJ forms.

The deposition of enamel occurs in successive layers beginning at the cusp tips in the deepest infoldings of the enamel organ. In all anterior teeth, enamel deposition begins in the middle of the incisal edge. In the molars, enamel deposition first occurs in the mesiobuccal cusp (Hillson, 1996). During enamel synthesis, the ameloblasts travel away from the DEJ towards the enamel surface, secreting enamel until its full thickness is achieved. Once enamel formation over the forming dentine is completed, the crown of the tooth continues to increase in length by the differentiation of new ameloblasts and the deposition of enamel along the sides of the tooth towards its cervical margin. Enamel deposition continues until the crown of the tooth reaches its mature dimensions.

Enamel Formation: Amelogenesis

Amelogenesis progresses via a series of chemically and histologically defined stages and results in the creation of the most highly mineralized tissue found in the human body (Robinson et al. 1997). Fully mature enamel consists of approximately 96% (w/w) inorganic material composed primarily of hydroxyapatite crystallites with traces of organic material enveloping each crystal (Ten Cate, 1994). Two stages of amelogenesis are recognized: matrix secretion and maturation.

The secretory stage of enamel development involves two processes: 1) synthesis and secretion of the enamel proteins by ameloblasts to form an extracellular matrix and 2) initial biomineralization of the secreted extracellular matrix with hydroxyapatite crystals (Fincham et al. 1994; Fincham and Simmer, 1997). The onset of initial mineralization occurs extremely close to the secretory front, so that little time elapses (i.e. a matter of minutes) between secretion and the progress of mineralization into newly produced matrix (Boyde, 1989). This initial influx of mineral into the forming enamel may amount to 25%-30% of the eventual total mineral content (Yaeger, 1980). The first organized crystals created during the secretory stage constitute only the initial, central portions of the adult enamel crystals (Cuisinier et al. 1992; Aoba, 1996; Boyde, 1997; Fincham and Simmer, 1997; Risnes, 1998).

Once enamel secretion has begun, a cytoplasmic process, the Tomes process, develops at the secretory end of each ameloblast as it retreats from the dentine surface. The Tomes process does not involve the entire distal surface of the ameloblast; a rim of the original distal surface encircles each process at its base (Risnes, 1998). During active secretion, each Tomes process occupies a depression or pit in the surface of the developing enamel, while the rim abuts the enamel surface proper. In this way, a dual area of enamel matrix secretion becomes operational: 1) from a mutual interameloblastic location at the base of the Tomes process and 2) from the flat aspect of the Tomes process within the pit. Enamel matrix secreted from the first field of growth contains what Boyde (1989) calls inter-pit phase enamel and constitutes an interprism or interrod region. The enamel secreted from the second field of growth is known as pit-phase enamel (Boyde, 1989) and corresponds to a prism or rod region (Figure 2-2).

The dual area of secretion imposed by the Tomes process results in a specific configuration of enamel crystals. Crystal orientation in the interprism region is generally perpendicular to the plane of the developing enamel surface (incremental surface) (Boyde, 1989). Crystals in the prism region, for the most part, are oriented parallel with the prism axis; crystals more distant from the central axis flare laterally as they approach the prism periphery (Risnes, 1998). Enamel in both the prism and interprism regions is of identical composition (Ten Cate, 1994).

The difference in crystal orientation between the interprism and prism regions results in the formation of a discontinuity or a prism boundary. Around approximately three-quarters of this boundary, the crystals of the interprism and prism growth regions meet at sharp angles resulting in the development of a prism sheath. The prism sheath is a zone of slightly more concentrated organic material that develops during enamel maturation (Boyde, 1967). The prism sheath corresponds to the non-secretory surface of the Tomes process, or that aspect facing the pit wall (Sasaki et al. 1997).

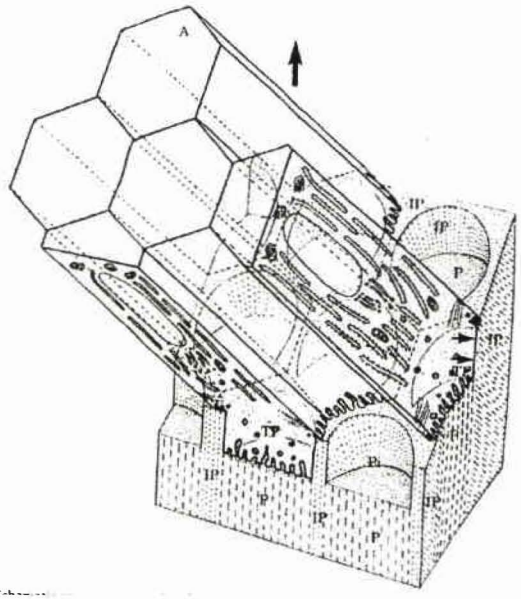

Figure 2-2. Schematic representation of a block of enamel with attached ameloblasts (A). Tome 's processes (TP) occupy pits (Pi) in enamel surface and represent prism growth sites (P). That portion of the ameloblasts abutting the enamel surface proper represents interprism growth sites (IP). The aspect of the Tomes process facing the pit wall is non-secretory (small arrows). Ameloblasts move in the direction of the large arrow. Crystal orientation is indicated by stippling/hatching (after Risnes, 1998).

The portion of the interprism region located directly cervical to a particular prism region is not separated from that prism by a sheath. In this area of enamel, the crystals are confluent between the two regions; the lateral flaring of crystals in the prism region continues uninterrupted into the cervically located interprism region (Ten Cate, 1994). The cross-sectional outline of these two related components constitutes the basic structural unit of enamel, the enamel prism/rod.

The enamel prism/rod is often compared to the shape of a keyhole, which is the predominant prism-packing pattern (pattern 3) found in human enamel (Boyde 1967, 1976, and 1989). The top or "head" of the keyhole is oriented cuspally/incisally and constitutes a prism region. The "tail" is oriented toward the cervix of the tooth and corresponds to an interprism region. Close inspection of this pattern reveals that the formation of the prism "head" results from a single ameloblast, while three ameloblasts contribute to the formation of a prism "tail" (Figure 2-3). Four ameloblasts are therefore responsible for the formation of the enamel prism.

Prisms tend to be maintained in rows arranged circumferentially around the long axis of the tooth (Ten Cate, 1994). They represent the path taken by the ameloblasts during amelogenesis. In their passage from the DEJ to the occlusal surface, prisms deviate from a straight path both transversely (horizontally) and longitudinally (vertically). These deviations, called prism decussation, reflect the changing relationships of neighboring ameloblasts, as well as groups of 10 – 13 ameloblasts, during their movement to the enamel surface (Figure 2-4) (Boyde, 1989). Most authorities claim that decussation is at its greatest in the cuspal area of teeth, where it is called gnarled enamel, and smallest in the cervical region of a tooth, where prisms run predominantly straight (Ten Cate, 1994; Radlanski et al. 1995). There is disagreement on the extent and nature of ameloblast decussation in other areas of the tooth (FitzGerald, 1995). In longitudinal sections, groups of prisms may be predominantly sectioned along their lengths (longitudinally) and some across their ends (transversely) forming an alternating pattern of parazones and diazones, respectively. Under the microscope, these are visible as a series of dark and light bands, the Hunter-Schreger bands.

The appearance of enamel structure at both the DEJ and the tooth surface is markedly different from that just described. In the formation of both the first and last layers of enamel, the ameloblasts lack a Tomes process and the secretory front is relatively flat. All crystals, therefore, have a common perpendicular orientation to the flat secretion surface of the ameloblast and no prism discontinuities exist. The enamel at both the DEJ and the outer surface is often referred to as "aprismatic" because the typical prism structure is either absent or irregular.

When the ameloblasts have completed their principal secretory activities, and the developing enamel has reached its full thickness locally, the secretory stage ends and enamel maturation begins. The process of maturation follows the same path as matrix secretion; it begins at the dentinal end of the prisms and moves out towards the occlusal surface. The sequence of maturing prisms is from cusps or incisal edge toward the cervical end. Maturation of the inner, first formed matrix occurs at the same time as initial mineralization is taking place in the outer recently formed matrix (Figure 2-5) (Yaeger 1980).

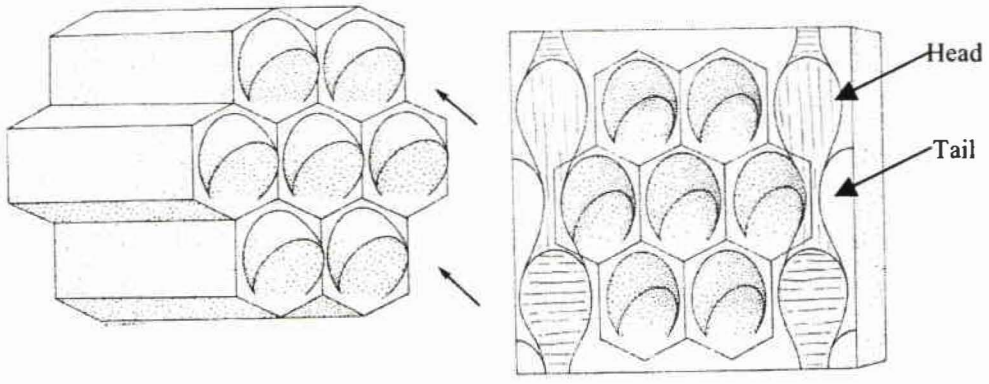

Figure 2-3. A schematic diagram showing on the left, a group of seven ameloblasts with their projecting Tomes processes. On the right is the developing enamel surface with the Tomes process pits into which each ameloblast on the left fits. Pattern 3 prisms are illustrated, indicated by the keyhole-shaped outlines on the right and left of the enamel block with a "head" and "tail" configuration. Each prism is formed by four ameloblasts (after Aiello and Dean, 1990).

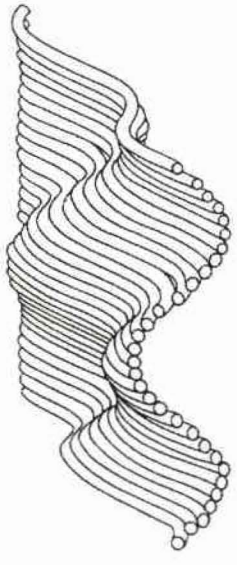

Figure 2-4. Schematic diagram showing prism decussation from the enamel-dentine junction out to the enamel surface (after Aiello and Dean, 1990).

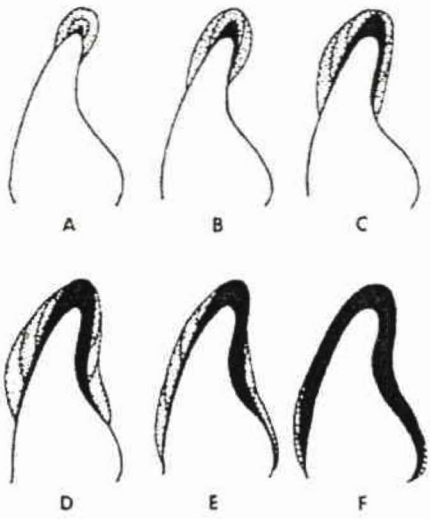

Figure 2-5. Diagram showing pattern of mineralization of incisor tooth. *Stipple zones*: consecutive layers of partially mineralized enamel matrix. *Black areas*: Advance of final mineralization during maturation (after Yaeger, 1980).

The maturation stage of amelogenesis is characterized by significant changes in the enamel extracellular matrix and its mineral content. Although some protein removal does occur during the secretory stage, there is a dramatic increase in protein degradation and a reduction in water content during the maturation stage (Moss-Salentijn et al. 1997). With this reduction in organic matrix and water composition, the mineral content of the maturing enamel increases dramatically (80-90% by volume), as the initially deposited crystals grow in width and thickness.

The manner in which mineral ions, namely calcium, are introduced into the enamel matrix during both the secretory and maturation stages is not completely understood. Currently, it is believed that calcium transport to forming enamel occurs via the transcellular route (Bawden, 1989; Ten Cate, 1994). Studies have demonstrated the presence of calcium adenosine triphosphate (Ca-ATPase) activity in both secretory and maturation ameloblasts, reflecting the existence of an active pump extruding calcium at a constant rate during amelogenesis (Bawden 1989; Ten Cate, 1994; Sasaki et al. 1997).

At the completion of maturation and before tooth eruption, the residual ameloblasts and the cells of the outer enamel epithelium and stratum intermedium, remain together as the reduced

enamel epithelium. This layer serves to protect the newly formed enamel from the overlying connective tissue before its emergence into the oral cavity. With tooth eruption, the reduced enamel epithelium is worn from the tooth surface, eliminating all ameloblasts. The loss of these cells renders enamel a non-vital tissue that subsequently cannot undergo repair when destroyed by any means (i.e. dental attrition or caries) (Ten Cate, 1994).

Enamel Microstructure: Normal and Abnormal Development

Enamel microstructure is a permanent space-time representation of the movements of a tooth's secretory ameloblasts (Moss-Salentijn et al. 1997: 21). Normal development results in the formation of characteristic structures within human enamel. These include cross-striations and striae of Retzius. However, systemic perturbations during enamel formation can produce both microstructural (i.e. Wilson bands, neonatal line) and macroscopic (i.e. hypoplasias) enamel defects (Figure 2-6).

When viewed under polarized light, fine dark bands, approximately 3 – 4 μm wide, can be seen traversing the prisms along their entire length from the DEJ to the enamel surface. These bands, termed cross-striations, occur at regular intervals and represent fluctuations in the rate of matrix production and composition during a 24-hour period (Boyde, 1976 and 1989).

Striae of Retzius are normally occurring incremental lines reflecting the layered apposition of enamel during amelogenesis (Risnes, 1985; Fitzgerald, 1995). They result from a regular rhythmic growth pattern occurring every seven to ten days, which affects all ameloblasts secreting at the time of the perturbation (Weber et al. 1974; Bullion, 1986; FitzGerald, 1998; FitzGerald and Rose, 2000). Striae of Retzius outline the position of the ameloblasts and the stage of enamel growth at the time of the disturbance. Their appearance has been attributed to an altered structure and/or composition of the enamel prisms along the striae (see Sognnaes, 1949; Gustafson, 1959; Gustafson and Gustafson, 1967; Wilson and Schroff, 1970; Osborn, 1971 and 1973; Weber et al. 1974; Weber and Ashrafi, 1979; Risnes 1990).

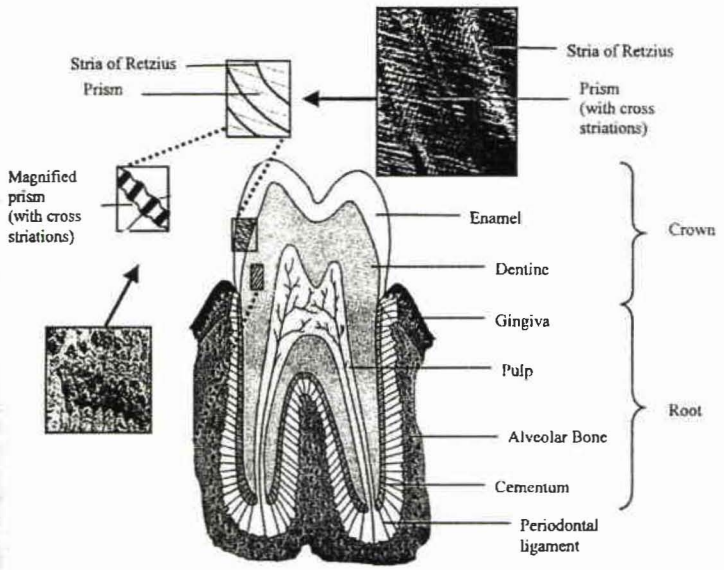

Figure 2-7. Schematic diagram of the various enamel microstructures in a tooth: prisms, cross-striations, lines of Retzius (after FitzGerald and Rose, 2000).

In longitudinal sections viewed under transmitted light, striae of Retzius appear as thin and/or dark bands that run obliquely from the DEJ to the occlusal surface (FitzGerald and Rose, 2000). Striae located around the cusp of the tooth form successive layers around the dentine horn and are called cuspal striae. Those striae located at the lateral and cervical surfaces of the tooth are called imbricational striae (Figure 2-7). In permanent enamel, striae are most visible in both the outer enamel and in the cervical third of the crowns. In deciduous tooth enamel, the striae of Retzius are less prominent in the postnatal enamel (Ten Cate, 1994) and rare in the prenatal enamel (Gustafson, 1959). The most commonly observed striae in longitudinal sections are the "staircase-type" (Figures 4-9 and 4-10, Chapter 4). Risnes (1990) suggests that the morphological changes seen along these striae result from a temporary constriction of the Tomes process during matrix secretion resulting in a decrease in the prismatic region and a concomitant increase in the secretory aspect of the interprismatic region.

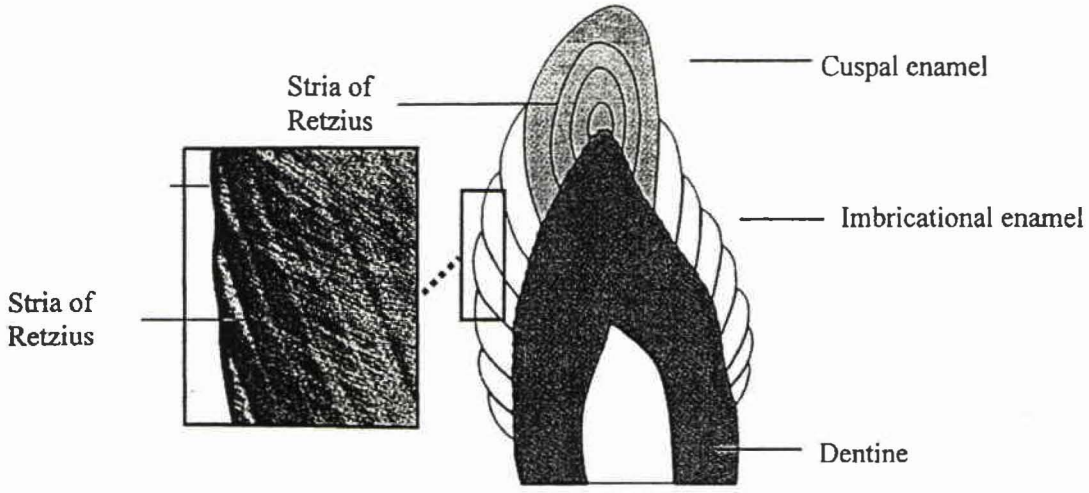

Figure 2-7. Schematic representation of striae of Retzius in longitudinal section, showing cuspal and imbricational striae (after FitzGerald and Rose, 2000).

Many Retzius lines are difficult to integrate in the system of rhythmic lines (Risnes, 1998). Systemic disturbances (i.e. infections, metabolic diseases, exanthematous diseases, nutritional disorders, vitamin deficiencies, pre-, neo- and postnatal conditions) occurring during the secretory stage of enamel formation can disrupt ameloblast metabolism, altering the direction of ameloblast movement, ameloblast morphology (i.e. the Tomes process) and the chemical makeup of the matrix (i.e. protein composition and crystal structure) (Wilson and Schroff, 1970; Rose, 1973, 1977, 1979; Levine et al. 1979; Simpson, 2000). Consequently, the structure of the prisms being formed at this time is affected, resulting in the formation of a "pathological" stria of Retzius, or Wilson band (Figure 2-8 and Figures 4-2a, 4-4a, Materials and Methods) (Gustafson, 1959; Gustafson and Gustafson, 1967; Wilson and Schroff, 1970; Rose, 1977 and 1979; Goodman and Rose, 1990). Wilson bands represent temporary (i.e. acute) and reversible disruptions to the ameloblast cells during amelogenesis (Kreshover, 1940 and 1960; Gruenwald, 1973; Marks, 1988).

Several attempts have been made to classify Wilson bands and to distinguish them from the regular occurring striae of Retzius (Wilson and Schroff, 1970; Weber and Eisenmann, 1971; Whittaker and Richards, 1978; Rose, 1977 and 1979; Marks, 1988). Rose (1977, 1979) was the first to quantify the variability in prism morphology seen along these bands. Using light microscopy, he demonstrated that Wilson bands could be divided into three distinct sub-types: distorted structure bands, black spot bands and structureless bands. In a later study employing scanning electron microscopy, Rose (1979) seriated the Wilson bands in his sample into five sub-types to facilitate comparison with his 1977 light microscope typology. Despite these attempts, disagreement still abounds regarding the precise morphological attributes of "pathological" striae. In general, Wilson bands are discernible from normal striae of Retzius in that they are broader, more accented, exhibit irregular prism structure and tend to be visible along more of their length than regular striae (Wilson and Schroff, 1970; FitzGerald and Rose, 2000). Wilson band morphology may be a result of the plane of section and not a direct measure of susceptibility (Rose, 1979; Marks, 1988 and 1992).

Figure 2-8. Microscopic view of Wilson bands and the neonatal line (after Rossi et al. 1997).

Wilson bands are commonly associated with a second type of enamel defect, enamel hypoplasia. Hypoplasias are macroscopic aberrations in the enamel tooth surface resulting from a deficiency in enamel thickness, which occurs during the secretory stage of enamel formation. These defects are manifest on the tooth surface in the form of pits, linear grooves, or larger areas of missing enamel. Hypoplasias, unlike Wilson bands, represent episodes of severe systemic disruptions (i.e. chronic) that result in complete destruction (necrosis) of the ameloblast cells. Consequently, no further enamel is formed (Kreshover, 1940 and 1960; Gruenwald, 1973; Marks, 1988; Suckling, 1989). Although numerous genetic, environmental, and prenatal, neonatal and postnatal influences have been described for enamel hypoplasias (Sarnat and Schour, 1941 and 1942; Sheldon et al. 1944; Sweeney et al, 1969; Infante and Gillespie, 1974; Small and Murray, 1978; Nikiforuk and Fraser, 1981; Suckling, 1989; Goodman and Rose, 1990; Seow, 1991), the precise physiological mechanism(s) responsible for their formation remains unknown. Studies have demonstrated that susceptibility to enamel hypoplasia varies both within and between tooth types (Goodman and Armelagos, 1985; Condon and Rose, 1992).

A second type of prominent stria is the neonatal line. The neonatal line is essentially the first accentuated striae of Retzius found in the crowns of deciduous teeth and first permanent molars. This line marks the expected state of development of each tooth at the time of birth (Figure 2-9) (Schour, 1936). The neonatal line develops at parturition and is thought to relate to the physiological changes occurring at birth and to the neonate's adjustment to life outside the uterus (Massler et al. 1941; Eli et al. 1989; Hillier and Craig, 1992; Ten Cate, 1994). Other researchers have attributed its presence and appearance to birth trauma, the health of both the mother and child at birth, gestation length and infant birthweight.[1]

The exact time at which the neonatal line forms or period during which it is forming is not known and may vary (Weber and Eisenmann 1971: 377). Schour and Kronfeld (1938) suggested that the neonatal line represents an arrest in growth lasting approximately ten to fourteen days.

[1] Please see Chapter Three for a detailed discussion of clinical studies on enamel defects resulting from perinatal disorders and birth trauma.

Weber and Eisenmann (1971) demonstrated the presence of a neonatal line in a seventy-five day old child who was born after a six-and-one-half month gestation period; no such line was evident in any of the individuals who had lived for up to two and one-half days. Whittaker and Richards (1978) suggested that the neonatal line represents disturbances in physiological activity at birth and the succeeding three to four day period. Levine and co-workers (1979) observed that the majority of primary teeth, from subjects who survive birth by more than a few days, show a neonatal line.

The neonatal line is easily recognised in histological sections because of its characteristic location on the tooth, specific for each tooth class, and because of the difference in appearance between pre- and postnatal enamel (i.e. prenatal calcification being uniformly more homogeneous than postnatal calcification) (Massler et al. 1941; Szpringer-Nodzak, 1984; Skinner, 1992; Skinner and Dupras, 1993). In incisors, the line extends from the DEJ into the cervical part of the crown and out to the surface, leaving only a small portion of postnatally formed enamel. In canines and molars, it is located more towards the incisal/occlusal part of the enamel, with only a small portion of prenatally formed enamel present (Figure 4 -11 to 4 -13, Material and Methods). The neonatal line is occasionally expressed on the tooth surface as an enamel hypoplasia (Kronfeld and Schour, 1939).

The structure of the neonatal line has been the subject of analysis by many investigators. These studies have demonstrated that although the neonatal line is a normal incremental feature of deciduous enamel, its structure conforms to the definition of a Wilson band. Attempts to determine the precise morphological and structural nature of the enamel disturbance using light microscopy and SEM have produced conflicting results.

Rushton (1933, 1939) suggested that the interprismatic substance in the neonatal line was either more or less highly mineralized with respect to the prisms than elsewhere, and that the prisms did not change direction (bend) upon crossing the line. Rushton also found the prism outlines along the line to be "a little irregular" (1933: 170). Schour and Kronfeld (1938: 475) noted that under high magnification the enamel rods (prisms) along the neonatal line could be seen as

"deviating in their course". Sognnaes (1949) described the neonatal line as being comprised of thickenings of the prism sheaths. Gustafson (1959) described an increase in the amount of the interprismatic substance within the line with the consequent narrowing of that portion of the prism passing through the line. Weber and Eisenmann (1971) found that the ultrastructural basis for the neonatal line appeared to be a localized change in the configuration of the enamel prisms along with a possible reduction in crystal concentration. These authors also found that when viewed under light microscopy the neonatal line had a "staircase" configuration built up of a series of prominent dark cross-striations and segments of the prism boundaries (see Figures 4-9 and 4-10, Materials and Methods). Whittaker and Richards (1978) demonstrated that the neonatal line was characterized by changes in prism direction and width and that, structurally, the neonatal line consisted of a sharply defined interruption crossing the prisms at its prenatal side and a wider diffuse zone of reduced crystal density at its postnatal side. Radiographically, the neonatal line has been reported to be relatively radioluscent and therefore hypomineralized (Weber and Eisenmann, 1971).

Summary of Enamel Physiology

A child's primary teeth develop predominantly during the prenatal period. Tooth development begins six weeks after fertilization and involves many complex biological processes that occur during specific developmental stages (bud, cap and bell). Each of the deciduous tooth classes begins crown formation and completes crown development during a characteristic age interval. Crown initiation begins as early as 13 weeks in utero and ends around 11 months after birth.

Histodifferentiation during the bell stage results in the formation of the enamel producing cells (ameloblasts). The formation of enamel is a two-stage process. It involves both matrix secretion and matrix mineralization. Enamel deposition begins at the incisal tip of the future tooth crown and proceeds in a regular manner, moving away from the DEJ and terminating at the cervical region of the tooth. During subsequent mineralization, which follows the same path as matrix secretion, the organic matrix is almost completely replaced by hydroxyapatite crystals. The

secretion rate and the movement of the ameloblasts orients the crystals within the matrix. The specific configuration of these crystals produces the microscopic characteristics of the enamel prisms (i.e. prism boundary, prism region and interprism region). The enamel prisms represent the path taken by the ameloblasts during enamel formation.

Once formed, enamel does not remodel. Consequently, its microstructure is a permanent record of normal and abnormal events that occur during amelogenesis. Normal enamel development results in the formation of characteristic structures, namely prism cross-striations and striae of Retzius. Prism cross-striations represent a 24-hour rhythm in enamel secretion. Striae of Retzius are incremental lines resulting from a regular rhythmic growth disturbance occurring every seven to ten days. They outline the position of the ameloblasts at the time of the disturbance.

Abnormal tooth formation is a non-specific phenomenon that can be related to a variety of systemic disturbances. When such disruptions occur during enamel formation ameloblast metabolism is disrupted, altering the direction of ameloblast movement, ameloblast morphology and the chemical composition of the matrix. If the disturbance is both acute and reversible, a Wilson band is formed. If the systemic perturbation is severe enough and long lasting (i.e. chronic), permanent necrosis of the ameloblasts may occur resulting in enamel hypoplasia.

The neonatal line is a specific type of "pathological" stria. It develops at parturition and is essentially the first accentuated stria of Retzius in deciduous teeth and first permanent molars. The neonatal line is thought to relate to the physiological changes occurring at birth, to the neonate's adjustment to life outside the uterus and to neonatal and/or maternal health at the time of parturition. The neonatal line also conforms to the definition of a Wilson band, because disturbed enamel formation is evident along its length. The precise nature of this disturbance is widely disputed among dental researchers.

Chapter Three

Birth Physiology and Human Deciduous Enamel in Perinatal Disorders

The purpose of this chapter is twofold: 1) to introduce the reader to basic birth physiology, fetal physiology at birth, basic medical interventions at birth and common complications of both the neonate and the mother at the time of delivery; and 2) to review the dental literature concerning the effects of perinatal complications on enamel development. Because of the large number of maternal and fetal difficulties that can arise at the time of delivery, only those complications mentioned in the literature review and encountered during the current study are described. For more information on other birth complications, the reader is directed to the following sources, from which the material presented here is drawn: Crawford (1985), Gabbe et al. (1991), O'Grady (1988), Pauerstein (1987), and Simkin (1989). A section at the end of this chapter is also devoted to anthropological studies of enamel defects and investigations of the neonatal line in archaeological populations.

Part I: Birth Physiology

The Physiology of Labor and its Stages

A. The Physiology of Labor

The function of the human uterus during pregnancy is protection of the fetus during its growth and development. As an organ during labor, its function is to develop sufficient expulsive force (i.e. myometrial contractions) to propel the fetus through the birth canal, against a varying degree of resistance (Fuchs and Fuchs, 1991). Uterine contractions must be intermittent to permit sufficient oxygen to be delivered to the fetus between contractions and their force must progressively increase to enable the soft parts of the birth canal to stretch, allowing passage of the fetus. In preparation for its function during birth, the uterus undergoes several changes. These are described below.

Throughout pregnancy, the uterine cervix grows and remodels under the influence of placental hormones (Fuchs and Fuchs, 1991). During the last trimester of pregnancy, biochemical changes in the cervix change the consistency of the cervix (cervical ripening) which thereby becomes soft and distended and its complaisance to stretch increases. As the cervix ripens, both effacement (progressive thinning and shortening of the cervix) and dilatation (gradual opening of the cervix) also begin. Cervical ripening is under the control of several hormones, namely estrogen, the prostaglandins, and relaxin (Fuchs and Fuchs, 1991).

Near term, placental hormones and uterine distension bring about an increase in the concentration of oxytocin receptors in the uterus. Distension also increases gap junction formation between myometrial cells in the myometrium (the muscular layer of the uterus). By mechanisms not yet fully understood, the frequency of oxytocin pulses increases and myometrial contractions are elicited with increasing force (Fuchs and Fuchs, 1991). All oxytocic agents exert their influence by mobilizing calcium, which is vital for the contractile process in myometrial cells and for transmitting the signal of excitation from the cell membrane to the contractile machinery inside the cell (Fuchs and Fuchs 1991: 156). With this increase in oxytocin, prostaglandins (E_2 and F_2) are released from the decidua (tissue layer that separates the placenta from the myometrium). The prostaglandins diffuse into the inner layers of the myometrium, thereby enhancing the action of oxytocin.

The fetus also plays a role in the onset of spontaneous labor by the excretion of substances from various organs (i.e. epidermal growth factor, platelet activating factor) capable of stimulating myometrial contractions. With the progression of intermittent contractions, transient hypoxic episodes occur in the fetus leading to the release of stress hormones through the hypothalamic – pituitary - adrenal axis (i.e. adrenocorticotropic hormone (ACTH), cortisol). These hormones have the ability to reach the decidua from the amnion, increasing the production of prostaglandins and further enhancing the myometrium's response to oxytocin and the progress of labor. During labor, stress hormones also become elevated in the mother (Chestnut and Gibbs, 1991).

B. The Stages of Labor and Abnormal Labor

Labor can be defined as progressive dilatation of the uterine cervix in association with repetitive uterine contractions (O'Brien and Cefalo 1991: 427). Normal labor is a continuous process; for descriptive purposes, it is divided into three stages: the first stage, the second stage and the third stage. The first stage of labor is the interval between the onset of labor and full cervical dilatation. Most of the work performed by the contracting uterus in the first stage is directed toward progressive effacement and dilatation of the cervix. The first stage is further subdivided into three phases: the latent phase (early labor), the active phase (active labor), and the transition phase (transition from the dilatation stage to the birthing stage). The end of the first stage of labor is much easier to define than the onset. This stage typically lasts between two and twenty hours (Simkin, 1989), with an average of six hours for multiparas and eight hours for primigravidas (Pauerstein, 1987).

The second stage of labor, the birthing stage, is the interval between cervical dilatation and the delivery of the infant. During this phase, contractions become stronger and more frequent. The purpose of this phase is to push the fetus through the birth canal until crowning of the fetal head occurs and the child is delivered. The membranes usually rupture during this stage, but may rupture during the first stage. This stage can be timed reasonably precisely. A normal birthing stage may last from fifteen minutes to three hours or more (Simkin, 1989). For the primigravida, the birthing stage is usually completed in less than two hours; for multiparas, in less than one hour (Pauerstein, 1987).

The third stage of labor, the placental stage, begins when the infant is born and ends with delivery of the placenta. This stage has no direct effect on fetal or neonatal health (Chamberlain, 1985).

Abnormal patterns of labor are defined by deviations from the standards for the stages of labor just described (O'Brien and Cefalo, 1991). The most common disorders of labor involve protraction (slowing down) or arrest during the active phase (first stage) and/or during descent (second stage). Consequently, labor is prolonged. A first stage of labor that lasts more than

twelve hours is considered abnormal; more than two hours of labor in the second stage, in the presence of active contractions, is considered atypical (Pauerstein, 1987). The major factors that affect the duration of labor include the strength of the uterine contractions (i.e. uterine inertia), the shape of the mother's pelvis and its size, and the fetus (i.e. cephalo-pelvic disproportion) (Chamberlain, 1985).

The physiological properties of labor can have varying effects on the fetus, depending on its initial status (normal versus abnormal) when labor begins (Pauerstein 1987: 488). A normal infant subjected to an abnormal labor may become distressed; an abnormal fetus (i.e. growth retardation, pre-term) subjected to a normal labor will inevitably become distressed (Chamberlain, 1985). During normal labor, each uterine contraction results in a decrease in blood flow to the placental bed, decreasing the oxygen supply to the fetus and producing a period of hypoxia. With prolonged or abnormal labor, blood flow to the fetus can actually stop, leading to a longer period of hypoxia. In general, with an increasing length of labor, either in the active phase, the birthing phase or both, there is an increased incidence of fetal distress (see below).

Fetal Physiology

A. The Transition to Extrauterine Life

At term, the gastrointestinal tract, the brain, the kidneys, and to a degree, the liver are ready to function in the neonate (Pauerstein, 1987). The transition from intrauterine to extrauterine life involves mostly cardiopulmonary adaptation.

Cardiopulmonary adaptation requires conversion of the fetal lung from a fluid-filled organ to one capable of gas exchange (Rosenberg, 1991). As early as eleven weeks of gestation, the fetus exhibits signs of breathing movements. Fetal respiratory activity is essential to the development of chest wall muscles (i.e. diaphragm) and serves as a regulator of lung fluid volume and lung growth.

The mechanism that initiates the change from episodic breathing in utero to regular, continuous postnatal respiration is not clearly understood. With its first breath, the neonate must overcome several forces resisting lung expansion: 1) the viscosity of fetal lung fluid, 2) the

resistance provided by lung tissue itself, and 3) the forces of surface tension at the air-liquid interface (Rosenberg 1991: 704).

The physiology of the first breath has been studied extensively by several researchers. It is believed that this process begins as the infant passes through the birth canal. The intrathoracic pressure created by the vaginal squeeze initiates extraction of fluid from the lungs and the trachea and allows the passive entry of air into the lungs. Consequently, this aids in initial lung expansion. Normal expansion and aeration of the neonatal lung is also dependent on removal of fetal lung liquid by 1) a marked increase in pulmonary lymph flow and 2) removal via the pulmonary circulation. These two events occur postnatally, within 15 and 30 minutes of birth; removal of liquid from the lungs then continues for several hours after birth. By 30 minutes of age, most infants attain a normal functional residual capacity (FRC) with uniform lung expansion.

B. Signs of Fetal Distress

Fetal distress can be defined as any absence of fetal well-being likely to result in the fetus being born in less than optimal conditions (Steer 1985: 12). The primary stress that the fetus must tolerate during labor is the uterine contraction itself. When uterine activity of any origin or type (i.e. prolonged labor, excessive augmentation of labor, excessive frequency of contractions) exceeds the fetus' tolerance for reduced oxygenation, fetal distress follows. Other possible causes of fetal distress include the use of epidural anesthesia and other drugs during delivery (see below), severe/excessive head compression and molding, and interference with placental diffusion (Steer, 1985).

Obstetricians recognize a number of potential indicators of fetal distress: 1) the presence of meconium (fetal bowel movement) in the amniotic fluid, 2) a decrease in fetal movement, 3) a change in fetal acid-base balance, and 4) changes in fetal heart rate. For the purposes of this review, only indicators 1 and 4 will be described because of their use as criteria of fetal distress in the current study. For a description of all of the methods of intrapartum fetal evaluation, the reader is referred to Petrie (1991), Pauerstein (1987) and Steer (1985).

Meconium staining of the amniotic liquor is widely used to indicate the possible presence of fetal distress. Meconium is passed predominantly in relation to mechanical reflex to distress rather than in response to asphyxia. In patients with meconium, the incidence of fetal and neonatal morbidity is increased when compared to gestations without meconium; some researchers dispute these claims (see Petrie 1991: 459-460). All obstetricians, however, generally accept the combination of meconium staining and an abnormal fetal heart rate pattern as evidence of fetal distress.

Fetal heart rate patterns are assessed through either continuous internal or external monitoring, or both. The mean human fetal heart rate varies between 120 and 160 bpm. Persistent periods of heart rate above 160 bpm (tachycardia) or below 120 bpm (bradycardia) are indicative of fetal distress. Only two mechanisms alter fetal heart rate: 1) a reflex response secondary to the nervous control of the heart by direct nervous innervation or by control of the autonomic nervous system and 2) transient slowing of the heart when fetal myocardial hypoxia is present (Petrie 1991: 469).

Important periodic changes in fetal heart rate have been observed in association with uterine contractions. The four patterns of clinical significance are accelerations (increase in heart rate) and early, variable and late decelerations (slowing of the heart). Early deceleration is due to pressure on the fetal head as it moves down the birth canal. Variable deceleration is generally caused by umbilical cord compression. Late deceleration results from fetal hypoxia. Acceleration usually indicates that a fetus is adequately oxygenated; in some cases, it results from partial cord compression. The most reliable indicator of fetal well-being is the finding of a normal beat-to-beat heart rate variability and an absence of significant repetitive decelerations (Petrie, 1991).

C. Postpartum Assessment and Management of the Newborn

In the first few minutes after birth, the newborn is carefully evaluated and a number of routine procedures are carried out. These include: Apgar scoring, clearing of the airways (nose and mouth), eye care, cord care, administration of vitamin K and blood testing (see Pauerstein 1987: 213 – 214 for a description of these procedures). All other interventions that take place are

done in response to complications that may arise during delivery or after birth; these are described in the next section.

D. Problems of the Newborn

Numerous problems can arise at the time of delivery and/or shortly after. The neonatal complications encountered in the literature review on enamel defects and during the present investigation include: pre and postmaturity, intra-uterine growth retardation (IUGR), breech presentation, breathing complications (i.e. meconium aspiration and ingestion of amniotic fluid), respiratory distress syndrome, jaundice, hypocalcemia, birth injury (i.e. injuries to face and scalp), and perinatal infections (i.e. urinary tract infection, eye infection). It is beyond the scope of this chapter to describe each of these in detail. Instead, this section will describe the neonatal complications that can arise from these conditions.

Prematurity and Postmaturity

Prematurely born children may be defined as those born at or prior to 37 weeks gestation (Seow, 1992: 321; Skinner and Dupras, 1993). A full term infant, defined as one born between 38 and 42 weeks, has an average birthweight of 3333g, although it is generally accepted that a normal birthweight includes a weight greater than or equal to 2500g (Seow, 1992; Skinner and Dupras, 1993). The birthweight of a pre-term infant usually varies with gestational age, the higher the gestational age, the larger the birthweight.

It is well recognized that an infant born before full term is usually poorly equipped for extrauterine life because of the immaturity of organ systems at the time of birth (Seow, 1992). Consequently, pre-term infants may experience numerous complications (see Table 3-1). A major risk factor for the pre-term infant is perinatal asphyxia, possibly resulting in respiratory distress syndrome (see below).

A pregnancy is considered postdate when it exceeds 42 weeks gestation (Pauerstein, 1987). The implications of a postdate pregnancy on the fetus are numerous. These include dysfunctional labor, traumatic delivery, a high incidence of fetal distress (i.e. meconium staining and acidosis), asphyxia, meconium aspiration syndrome, respiratory distress syndrome, and

metabolic disturbances, which include hypoglycemia, hypothermia, hypocalcemia, hyperbilirubinemia and albuminuria (presence of albumin in the urine) (Pauerstein, 1987).

Table 3-1. Common complications of the premature infant (after Pauerstein, 1987).

CEREBRAL IMMATURITY
- Intracranial Hemorrhages
- Bilirubin Encephalopathy

RETINAL IMMATURITY
- Retrolental Fibroplasia

PULMONARY IMMATURITY
- Hyaline Membrane Disease
  - Oxygen therapy → bronchopulmonary dysplasia
  - Respiratory therapy → air leaks
- Patent Ductus Arteriosus
- Cardiovascular Instability (diving reflex)

INTESTINAL IMMATURITY
- Necrotizing Enterocolitis

METABOLIC IMMATURITY
- Hyperglycemia
- Hypoglycemia
- Hypocalcemia
- Hypovitaminosis D → Osteopenia/rickets → fractures
- Hyponatremia

LIVER IMMATURITY
- Cholestasis

RENAL IMMATURITY
- Acute Tubular Necrosis

Intra-Uterine Growth Retardation (IUGR)

Intra-uterine growth retardation (IUGR) broadly refers to suboptimal fetal growth and development occurring secondary to a variety of mechanisms (i.e. maternal undernutrition, drug and alcohol abuse, intra-uterine infections, and congenital syndromes). Infants with IUGR are smaller and lighter at term (i.e. "small for date") than are normal term infants and have an excess of water and a lack of proteins (Pauerstein, 1987). Regardless of the cause of IUGR, these infants are at an increased risk for asphyxia, meconium aspiration, hypoglycemia, hypocalcemia, hypothermia, long-term disability and perinatal mortality (Pauerstein, 1987).

Breech Presentation

Breech presentation refers to the longitudinal lie of the fetus with the buttocks as the presenting part; several varieties of specific breech presentation exist (see Ferguson 1985: 57; Pauerstein 1987: 506 – 507; Seeds 1991: 551 – 562). Some of the major dangers facing the breech infant are intrapartum asphyxia, birth trauma, arrest of the aftercoming head, spinal cord injuries and prematurity (Seeds, 1991). The majority of breech presentations are delivered with the aid of obstetrical instruments (i.e. forceps) or through surgical intervention (i.e. cesarean section) (see below). In some cases, a spontaneous vaginal delivery may occur.

Perinatal Asphyxia, Meconium Aspiration and Respiratory Distress Syndromes

Perinatal asphyxia is the result of some problem during fetal life, labor and/or delivery, which delays the onset of respiration. The problems resulting in asphyxia include maternal diseases such as diabetes and preeclampsia, prematurity, growth retardation, fetal distress, meconium staining, ingestion of amniotic fluid or blood resulting from placenta previa, breech presentation and administration of anesthetics and analgesics during labor and/or delivery (Rosenberg, 1991). Even the normal term infant may experience some asphyxia during the birth process. Perinatal asphyxia usually requires medical interventions including clearing of the airways, ventilation with an endotracheal tube and/or resuscitation and drug administration.

Meconium aspiration occurs in infants who have suffered severe enough asphyxia in utero that they begin to gasp and aspirate large amounts of meconium into their lungs before delivery. The perinatal course of these infants is often marked by fetal distress. The most dire complication of meconium aspiration is severe hypoxia and acidosis (a transient fall in fetal pH levels) resulting in vasoconstriction of the pulmonary arterioles and poor oxygen perfusion (Pauerstein, 1987). In cases of meconium aspiration, it is common procedure to prevent the infant from breathing until the meconium has been cleared from the airway by intubation with an endotracheal tube and suctioning.

The presentation of respiratory distress syndrome is among the most common symptom complexes seen in the newborn (Rosenberg, 1991). Two types of respiratory distress syndrome

are recognized; for this review only Type I (Hyaline Membrane Disease or HMD) will be described. HMD results from a delivery before the mechanisms for synthesis of surfactant are fully developed. Surfactant is a phospholipid that counteracts the physical forces that would otherwise compel the alveoli of the lungs to collapse after expiration. Thus in infants with HMD, the alveoli are unstable, collapse of the expanded lung occurs and a decrease in arterial oxygenation occurs (Pauerstein, 1987). This syndrome usually requires extensive medical interventions similar to those encountered in perinatal asphyxia.

Neonatal Jaundice

Varying degrees of jaundice (hyperbilirubinemia) are extremely common in the first few days of life of normal children. Jaundice occurs when the normal pathways of bilirubin metabolism and excretion are altered (see Rosenberg 1991: 738). This "physiological jaundice" is partly due to the rapid breakdown of red blood cells with a concomitant increase in levels of bilirubin and, in part, to the immaturity of the liver at this period of life. In children suffering from hemolytic disease of the newborn, an excessive breakdown of red blood cells occurs and severe jaundice will result (Miller and Forrester, 1959). A potentially serious condition resulting form very high levels of bilirubin is bilirubin toxicity resulting in bilirubin-induced encephalopathy or kernicterus (the staining of certain areas of the brain by bilirubin). In the event of physiological jaundice, phototherapy (i.e. UV treatment) may be employed.

Neonatal Hypocalcemia

Two forms of neonatal hypocalcemia (NHC) (i.e. low blood calcium levels) are recognized: early neonatal hypocalcemia and "physiological" hypocalcemia. Bergman (1974) demonstrated that infants of diabetic mothers are at a greater risk of developing early NHC because they develop hyperinsulinemia and hypoglycemia, which results in an increase in growth hormone production and a concomitant decrease in blood calcium levels. In this study, pre-term infants also displayed lower values of blood calcium. Physiological hypocalcemia is a result of a temporary drop in neonatal blood calcium levels, which usually occurs in the term infant during the first 48 hours of life (Bergman, 1974).

Birth Injury

Birth injuries are those sustained during labor and delivery. Factors predisposing to birth injury include macrosomia (large infant size), cephalopelvic disproportion, prolonged or difficult labor, breech presentation and instrumental delivery (Rosenberg, 1991). Soft tissue injuries (i.e. lacerations, abrasions and bruising) are the most common birth impairments. Hyperbilirubinemia, particularly in the pre-term infant, is the major neonatal complication related to soft tissue damage.

Perinatal Infections

The newborn sometimes acquires an infection while in the uterus or soon after birth. Depending on the organism causing it, the infection may be very serious and painful interventions may be necessary (Simkin, 1989). These may include spinal taps, bladder taps, and scalp vein intravenous lines to administer antibiotics. Fever is recognized as a manifestation of acute infection. Although the adverse effects of fever on the fetus or newborn are not clear, they can include a decrease in fetal heart rate, fetal hypoxia and acidosis (Pauerstein, 1987).

Medical Intervention(s) and Operative Deliveries: Indications and Effect(s) on the Fetus and the Newborn

A. Induction and Augmentation of Labor

At times, it is necessary to induce (artificially start) or augment (speed up) labor. There are several ways that this can be accomplished: 1) artificial rupture of the membranes; 2) the application of a prostaglandin (E2 or F2) gel; or 3) administration of oxytocin (also called Pitocin). Induction or augmentation of labor is medically indicated when: 1) pregnancy is prolonged (> than 42 weeks); 2) certain medical conditions are present such that continuing the pregnancy may harm the mother and/or the fetus (i.e. preeclampsia/toxemia, diabetes mellitus, high blood pressure); 3) the membranes have ruptured prematurely and labor has not started spontaneously; 4) prelabor is prolonged; and 5) contractions in the active phase slow down and decrease in intensity (Simkin, 1989). For the purposes of this review, infusion with oxytocin will be described.

Induction with oxytocin is accomplished intravenously to stimulate myometrial contractions. The known or presumed benefits of augmenting or inducing labor with oxytocin must be balanced against the potential complications. The most common complications encountered are uterine hyperstimulation (i.e. excessive frequency and strength of contractions) and fetal distress. If the uterus is overstimulated, placental gas exchange may be threatened, leading to fetal acidosis and hypoxia (O'Brien and Cefalo, 1991). An increased incidence of neonatal jaundice has also been reported in infants delivered after oxytocin-stimulated labors (Pauerstien, 1987). It is recognized that the induction process is abnormal in that it attempts to accomplish in hours what may take several days of spontaneous prelabor (O'Brien and Cefalo 1991: 448).

B. Analgesia and Anesthetics at Delivery

The pain of normal labor can be managed through the use of systemic analgesics (drugs that relieve pain, i.e. Phenobarbitol, Demerol, Morphine), regional/local anesthetics (medications that remove feeling in a particular part of the body, i.e. the lumbar epidural block) and general anesthetics/analgesics (medications that remove all awareness and consciousness, i.e. nitrous oxide). It has been demonstrated that pain medications may reduce and sometimes relieve the effects of maternal stress during labor (i.e. decrease the production of stress hormones such as cortisol and ACTH) (Chestnut and Gibbs 1991: 495-7). However, the application of these substances can also have adverse consequences on the fetus and on the newborn (Table 3-2).

C. Instrumental Deliveries: Forceps and Vacuum Extraction

Inability to expel the fetus in the second stage of labor is the classic indication for instrumental delivery. Outlet forceps is the principal instrumental obstetric forceps operation. It is defined as the application of forceps under conditions of full dilatation, with the fetal scalp visible and the head having reached the pelvic floor (O'Grady 1988: 47).[1] A forceps delivery is medically indicated when 1) the second stage of labor is prolonged; 2) there is evidence of fetal distress; 3)

[1] Two other forceps deliveries are recognized: low and mid-forceps. Currently, low-forceps deliveries are considered meddlesome and unnecessary; all mid-forceps deliveries have been condemned.

abnormalities in fetal positioning are present; 4) maternal exhaustion is evident and/or there is a decrease in uterine contractions; and 5) delivery is pre- or post-term (O'Grady, 1988). The indications for vacuum extraction are essentially the same as those for forceps delivery; compared with forceps, vacuum extraction is considered safer when the fetus is positioned higher in the birth canal.

The application of forceps or vacuum extraction can result in different types of fetal damage. Vacuum extraction frequently results in a cephalhematoma, while laceration and bruising are more common with forceps delivery. Neonatal jaundice occurs with greater frequency following vacuum extraction.

Table 3-2. The effect(s) of various pain medications on the fetus and newborn (after Simkin, 1989).

Medication	Effects on Fetus	Effects on Newborn
Systemic Analgesics		
Sedatives (Phenobarbitol)	Heart rate changes	Breathing problems
Narcotics (Demerol, Morphine)	Heart rate changes	Depressed respiration Depression of other reflexes
General Analgesia with Inhaled Gas (nitrous oxide)	none	Possible depression of breathing
General Anesthesia with Inhaled Gas (nitrous oxide)[1]	Fetal distress due to lack of oxygen crossing the placenta	Depression of breathing Poor muscle tone Grogginess at birth Low Apgar score
Regional/Local Anesthesia		
Lumbar epidural block	Temporary heart rate Changes caused by Lower maternal blood Pressure	Subtle changes in reflexes Decreased attentiveness and muscle tone Increased fussiness

1 Used for cesarean sections only.

D. Cesarean Delivery

Sometimes an infant is delivered surgically through an incision in the mother's abdomen; this procedure is called a cesarean delivery. A cesarean birth can either be elective/optional or required, as is often the case with an emergency cesarean delivery. In the former case, the surgery is pre-planned and the mother does not experience active labor. An emergency c-section

often occurs after labor has begun and is medically indicated when: 1) labor is arrested as a result of uterine inertia and/or cephalo-pelvic disproportion; 2) there are problems with the fetus, such as fetal distress, breech presentation, prematurity and postmaturity; and 3) there are problems with the mother including hemorrhage, infection, diabetes mellitus, or preeclampsia (Simkin, 1989). The effects of cesarean delivery on the neonate are addressed in the Discussion.

Maternal Complications at Delivery

Several maternal complications can arise during pregnancy and at the time of delivery. Those encountered during the present investigation include toxemia/preeclampsia, placenta previa and intrapartum hemorrhage, diabetes mellitus, hypothyroidism, iron-deficiency anemia, fever and infection (Group B streptococcus). For this review, the possible effect(s) of these factors on the health of the neonate are considered.

Toxemia/Preeclampsia

Preeclampsia is a form of hypertension that is unique to human pregnancy (Sibai and Anderson, 1991). A diagnosis of preeclampsia, sometimes called toxemia, includes high blood pressure, proteinuria (protein in the urine) and edema (swelling due to fluid retention in connective tissue). Severe preeclampsia can result in convulsions/seizures in the mother (eclampsia) and, as a result, the fetus can suffer oxygen deprivation (Simkin, 1989). In cases of preeclampsia, the mother is given medication to lower her blood pressure (Apresoline) and/or to prevent convulsions (i.e. magnesium sulfate). These are either administered intravenously or by an injection. The birth of the fetus may also be hastened by induction or augmentation of labor, or cesarean delivery (Simkin, 1989).

Placenta Previa and Intrapartum Hemorrhage

Placenta previa is defined as implantation of the placenta low in the uterus (Pauerstien, 1987). When this condition is present, hemorrhage is unavoidable at the time of labor and vaginal delivery. Postpartum hemorrhage is also commonly encountered. Placenta previa has become a much safer condition for the mother because of blood transfusions and cesarean delivery in the majority of cases (Pauerstein, 1987). If a vaginal delivery is attempted, perinatal asphyxia may

occur in the neonate because of the greater risk of ingesting large amounts of maternal blood and fluids (Pauerstein, 1987).

Infants of Diabetic Mothers

Diabetes mellitus complicates 2 to 3 percent of all pregnancies; gestational diabetes constitutes 90 percent of these cases (Pauerstein 1987: 669). Gestational diabetes is a designation restricted to pregnant women whose impaired glucose tolerance is detected during pregnancy. The route of delivery for the infant of a diabetic mother reflects the individual needs of both the fetus and the mother; many infants, however, are delivered early by caesarian section or by induction of premature labor (Grahnen and Edlund, 1967).

Children of diabetic mothers are not underweight at birth; they are frequently overweight for the often relatively short duration of pregnancy. These infants reveal marked metabolic imbalances (i.e. hyperbilirubinemia, hypocalcemia, and hypoglycemia) and a possible deficiency in body proteins (Pauerstein 1987: 217). Studies suggest that alteration in fetal carbohydrate metabolism and hyperinsulinemia may be associated with delayed pulmonary maturation in these children and may contribute to intrauterine fetal asphyxia (Pauerstein, 1987; Landon, 1991). Infants of diabetic mothers also have an increased incidence of respiratory distress syndrome (Pauerstein, 1987). Congenital malformations (i.e. skeletal dysplasia, cardiac anomalies, central nervous system malformations) are more common among these infants (Landon, 1991).

Maternal Hypothyroidism

Hypothyroidism is defined as an absence or decrease in functioning thyroid tissue. This condition is considered rare in pregnancy because women with markedly reduced thyroid gland function are often infertile (Landon, 1991). The fetal hypothalamic-pituitary-thyroid system appears to function independently from that of the mother; fetal levels of thyroid hormone during pregnancy do not correspond to maternal levels (Landon, 1991). The role of maternal thyroid hormone in early fetal development is also unknown. Consequently, the effect of hypothyroidism on pregnancy outcome is widely debated. An increased incidence of preeclampsia, fetal growth

retardation, mental retardation and congenital anomalies have been reported in the setting of maternal hypothyroidism (Landon, 1991).

Anemia During Pregnancy

Approximately 50 percent of pregnant women are anemic (Samuels, 1991); however, 75 percent of anemias that occur during pregnancy are secondary to iron deficiency. If iron deficiency anemia is detected, the primary cause is poor dietary intake of iron. Other causes may include a short interval between pregnancies in the multiparas and if a previous delivery was complicated by hemorrhage. The effects of anemia on the newborn are not described in the obstetrical literature. With respect to tooth development, enamel hypoplasia and aplasia have been demonstrated (Prime et al. 1984).

Fever and Infection

The effect(s) of maternal fever on the fetus are not clearly understood; however, they may involve an increase in fetal heart rate, fetal hypoxia and acidosis. Other complications include teratogenesis (i.e. production of developmental malformations) and premature labor. During delivery, antibiotics may be administered to the mother to combat the infection and/or antipyretics, such as acetaminophen, may be administered (Pauerstein, 1987). The most common source for fever during delivery is an infection of the amniotic cavity (intra-amniotic infections), most commonly after rupture of the fetal membranes.

Group B Streptococcus (*Streptococcus agalactiae*) is a potential perinatal pathogen that may result in premature labor, premature rupture of the membranes and intra-amniotic infection (Isada and Grossman, 1991). If this pathogen is identified from a maternal cervico-vaginal specimen at the time of delivery, antibiotics are administered. Because its isolation from the mother is not predictive of neonatal disease, and because Group B Streptococcus is the most common cause of neonatal sepsis and meningitis in the first week of life, it is also customary to administer antibiotics to the newborn.

Summary of Birth Physiology

The primary stress that the fetus experiences during the birth process is the uterine contraction, which results in a temporary decrease of blood flow and oxygenation. The change from an intra-uterine to extra-uterine environment also requires that the neonate make certain adjustments, the most important one being breathing on its own. Any deviation from a normal birth (i.e. abnormal/prolonged labor, pre/post term delivery, excessive/diminished uterine contractions, breech presentation and other malpositions) can result in fetal distress. In such instances, intervention by the obstetrician may be necessary, requiring induction/augmentation of labor (i.e. oxytocin), instrumental delivery (i.e. forceps, vacuum extraction), or operative delivery (i.e. cesarean section). Such medical interventions can have adverse effects on the fetus and on the newborn.

Neonatal complications at birth (breech presentation, pre/post-maturity, growth retardation, and perinatal infections) and those resulting from the mode of delivery (i.e. soft tissue injuries) can result in numerous complications of the fetus and the newborn. These include breathing complications (i.e. respiratory distress syndrome, meconium aspiration and perinatal asphyxia) and metabolic disturbances (i.e. hypoglycemia, hypothermia, hypocalcemia and hyperbilirubinemia). Neonatal complications usually require extensive postpartum medical interventions (i.e. scalp vein intravenous lines, ventilation with an endotracheal tube and/or resuscitation and drug administration).

Maternal complications at delivery can result in medical interventions at birth (i.e. cesarean section, drug administration, or induced labor). They may also have an effect on fetal outcome, although the impact on the fetus is widely debated. Conditions such as toxemia, placenta previa and diabetes mellitus usually require that the infant be delivered by cesarean section. Infants of diabetic mothers also suffer metabolic disturbances (i.e. hypoglycemia, hypocalcemia). Fetal asphyxia is common in many of these conditions, especially in a vaginal delivery in the case of placenta previa, in cases of diabetes mellitus, and in instances of maternal infection and fever.

The effects of the aforementioned neonatal, perinatal and maternal complications on enamel development are addressed in the following section.

Part II: History of Dental Inquiry of Human Deciduous Enamel in Perinatal Disorders

Rushton (1933) and Schour (1936) initially recognized that the birth process leaves a permanent mark in the human dentition. Rushton investigated the fine contour lines in thin sections of human deciduous enamel and reported the usual occurrence of a well-marked line at the level of the tooth formed at birth. Schour, without knowledge of Rushton's work, conducted a histological examination of human deciduous teeth and first permanent molars and described the presence of a pronounced line of Retzius in the crown of each tooth. Because of its striking constancy and characteristic position in the primary dentition and first permanent molars, Schour argued that the line is a consequence of disturbance in tooth growth and calcification at birth and shortly thereafter. Because of this association, the incremental line originally described by Rushton and Schour is termed the neonatal line/ring, the birth line, or the perinatal line.

Following these first descriptions, Stein (1936), Schour and Kronfeld (1938) and Kronfeld and Schour (1939) reported on the appearance of tooth enamel and its relation to birth. Stein (1936) examined surface enamel defects in deciduous teeth and noted that these defects were frequently located along the line where the prenatal and postnatal portions of the enamel meet. Although he did not establish the exact significance of this dividing line, Stein emphasized that the "shock" nature of the birth process resulted in disturbed calcium metabolism and poorly formed enamel (as cited in Schour and Kronfeld 1938: 472).

In 1938 and later in 1939, Shour and Kronfeld examined the enamel of an infant who suffered an injury of the brain during delivery. They demonstrated a *pathologically accentuated* neonatal line throughout the deciduous dentition. A marked surface neonatal hypoplasia was also present in association with the line; for some of the teeth, enamel formation was permanently arrested at birth. Micrometer measurements of the neonatal line taken from ground tooth sections (no measurements are provided) suggested a neonatal growth arrest of approximately sixty

days.[2] Although a direct relationship between an injury to the brain and the formation of a tooth could not be readily assumed, these authors suggested:

> It is obvious that the greater the disturbance at birth, the more *accentuated* (my emphasis) will be the neonatal ring. The more difficult it is for the organism to make the adjustment from the sheltered intrauterine life to the independent postnatal existence, the *more pronounced* (my emphasis) will be the zone of poorly formed and calcified enamel apposed during this period (Schour and Kronfeld 1938: 473).

Since the first descriptions of the neonatal line in the 1930s, it is no surprise that dental researchers have focused their attention on recognizing those factors surrounding birth that may influence the appearance of human deciduous enamel. Although the neonatal line is the primary focus in this study, an understanding of the dental literature on perinatal and neonatal disorders and their associated disturbances in enamel formation is necessary to appreciate histological investigations of this microscopic feature. A review of the literature demonstrates that the study of microstructural defects was an inevitable outcome of several decades of clinical research into macroscopic aberrations of human deciduous enamel, which resulted from birth trauma, low birthweight, neonatal health and maternal health.

Birth Type and the Width of the Neonatal Line

Numerous studies have attempted to define the normal range of width of the neonatal line and to correlate its width with the nature and the severity of the birth process. No consensus exists; it is not clear what, in fact, constitutes an increased neonatal line width.

Weber and Eisenmann (1971) found that in the longitudinal plane, the width of the neonatal line is between 20 and 40 µm when examined using microradiographs and between 20 and 30 µm when examined using ground thin sections. When successively thinner sections were examined, the width of the line diminished to 10 µm. These results are speculative because most of the teeth examined were from a prematurely delivered child. The observed effects of premature delivery on the neonatal line are examined below.

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[2] Although no measurements of the neonatal line are provided by these authors, it may be hypothesized that if the rate of enamel formation is the same in disturbed enamel as it is in normally formed enamel (3 to 4 µm per day), then the width of the neonatal line observed by Schour and Kronfeld (1938 and 1939) would be between 180 and 240 µm. A

A survey of other histological papers demonstrates the following. Jackobsen (1975), using transmitted light, suggested that the average width of the neonatal line is 10 μm in permanent first molars and that width is highly dependent upon the distance of the section from the cusp tip. Noren et al. (1978a,b), used both light microscopy and microradiography to examine deciduous incisors. They claimed that a width exceeding 10-12 μm in microradiographs is abnormal. Whittaker and Richards (1978) using both light microscopy and scanning electron microscopy (SEM) found the usual width of the line to be 16 μm in all primary teeth. Jaffe et al. (1985) using light microscopy determined the normal width of the line to be 5-8 μm. Eli and colleagues (1989) suggested that the width of the line in primary teeth is 12 μm. Ranggard and colleagues (1994) found that the majority of measured neonatal lines in incisors had a mean width of less than 10 μm.

Several researchers have also examined the relationship between a difficult birth, such as breech presentation, forceps delivery, vacuum extraction, caesarian section, prolonged labor and a multiple pregnancy and the appearance of the neonatal line and/or the occurrence of neonatal enamel hypoplasia.

An early clinical study by Via and Churchill (1959) examined the relationship between enamel defects and abnormal events of gestation and birth. They considered the following pregnancy risk factors: Rh incompatibility, maternal diabetes, toxemia (preeclampsia) of pregnancy, prematurity, breech presentation, twinning, cesarean section, labor in excess of twenty hours, intrapartum hemorrhage and placenta previa and poor respiratory response at birth. Enamel hypoplasia and one or more of the birth complication factors occurred in 93 (30 percent) of the 312 children examined. According to the authors, enamel hypoplasia is related to "an abnormal extreme of the microscopic neonatal line found in most deciduous teeth of normal persons" (p.705). Unfortunately, no conclusions could be reached as to the specific

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review of the literature on estimated neonatal line width reveals that such a width is much larger than that observed in infants undergoing other complications at birth, leading one to question the accuracy of these reports.

mechanism(s) responsible for the enamel aberrations, because in many instances infants suffered several different complications (Via and Churchill, 1959).

Almost thirty years later, Eli and colleagues (1989) conducted a direct analysis of the relationship between the birth process and the neonatal line by comparing its width in children with normal and complicated (operative) birth histories. Primary teeth and birth histories, which included information on the type of delivery and the condition of the newborn, were collected for 147 children. The teeth were divided into one of the following three categories: normal delivery, complicated (operative) delivery and elective cesarean section (Table 3-3). For the purposes of their study, the authors defined normal children as "those with no history of systemic disorders related to pregnancy and/or labor and who had a birth weight no less than 2500g" (p. 221). Complicated (operative) births included deliveries where active outside medical intervention took place, as for instance in breech, forceps, or vacuum delivery.

To consider the effect of the angle of the enamel prisms in different regions of the tooth, Eli and colleagues took three separate measurements of the line at three levels of the tooth crown: incisal, middle and gingival. Measurements were obtained under a light microscope using an eyepiece with an accuracy of 0.1 μm. The neonatal line was observed in all specimens, at at least one crown position. No significant differences in line width (one-way ANOVA) were found between the three crown positions for either normal or operative deliveries. Neonatal line width in children born by normal delivery was found to be between 11.9 and 12.4 μm, depending on the crown level (Table 3-4). In children born by operative delivery, the line was significantly wider at each level (17.4 to 18.6 μm) (Table 3-5), while in those children born by elective cesarean section, the width of the line at the gingival level (the only data acquired for this group) was markedly lower (7.6 +/- 1.5 μm). They further noted that the microscopic appearance of the neonatal line differed among children experiencing normal births and an operative delivery, although this difference was not significant. It was mostly straight in teeth from children born by normal delivery and by operative delivery (85% and 85.7%, respectively), continuous (60% and

78.6%, respectively) or uniformly accentuated (78% and 57.1%, respectively). No images are provided which illustrate why these descriptive terms are used. It is also unclear how these percentages were obtained, since they should total 100 percent for both normal and operative deliveries respectively, yet they do not (normal totals 223% and operative totals 221.4%).

Based on these findings Eli et al. (1989) define the "normal" width of the neonatal line to be 12 μm and suggest that a line wider than 15 μm signifies complications at birth. They conclude:

> The fact that the width of the neonatal line increases in children born by operative delivery and decreases in those who have undergone no active birth process (elective cesarean section), suggests that the change from the intrauterine to extrauterine environment is responsible for only part of the arrest of ameloblast function. The trauma of the birth process itself has a major impact on the newborn's cells, which simultaneously affects all ameloblasts at the different tooth levels, establishing a uniform line (Eli et al. 1989: 222).

Several problems exist with this study, which raise questions about the conclusions. These include: 1) the potential effect of line width differences at different crown positions within tooth classes; 2) the large standard deviations (SD) of all measurements; and 3) the inappropriateness of the statistical tests employed. These problems are examined in detail in the Discussion.

Ameloblasts are extremely sensitive to calcium changes, even of a few hours duration (Seow et al. 1989; Suckling, 1989). Consequently, one of several proposed causative factors of enamel defects is hypocalcemia (Purvis et al. 1973; Levine and Keen, 1974). Earlier microscopic studies have also suggested that there is a relationship between low neonatal blood calcium values and the width of a neonatal line (Noren et al. 1978a,b; Noren, 1983; Noren, 1984).

In a 1994 study, Ranggard and colleagues (1994) examined the relationship between low values of blood-ionized calcium consequent with tooth development and the clinical and histological appearance of enamel of primary teeth. Twenty-five children experiencing optimal perinatal conditions and with known blood values of ionized calcium at birth participated in a dental examination. Thirteen of the 25 children had enamel aberrations. Twenty-four of these children also provided one exfoliated tooth for histological examination.

Table 3-3 Teeth According to Type of Delivery (after Eli et al. 1989)

Tooth	Normal	Delivery Operative[1]	Elective C-section
Incisors	23	2	3
Canines	62	7	1
Molars	40	8	1
Total	125	17	5

1 Includes breech, forceps and vacuum deliveries

Table 3-4 Width of Neonatal Line (µm) in Children Born by Normal Delivery (after Eli et al. 1989)

NNL Location	Incisors n = 23 Mean	SD	Canines n = 62 Mean	SD	Molars n = 40 Mean	SD	Mean*	SD
Apex	n/a	n/a	11.3 n = 4	6.1	12.2 n = 36	5.1	12.1 n = 40	5.1
Middle	11.6 n = 6	6.5	12.1 n = 36	7.0	16.5 n = 4	9.0	12.4 n = 46	7.0
DEJ	13.3 n = 17	4.5	10.9 n = 22	4.9	n/a	n/a	11.9 n = 39	4.8

Total children n = 125
*No difference between groups according to one-way ANOVA.

Table 3-5 Mean Width of Neonatal Line (µm) in Children Born by Normal Delivery Compared to Children Born by Operative delivery (after Eli et al. 1989)

NNL Location	Normal n = 125 Mean	SD	Operative n = 17 Mean	SD	Statistical Sig.*
Apex	12.1 n = 40	5.1	17.4 n = 6	6.4	$p < 0.05$
Mid	12.4 n = 46	7.0	18.3 n = 8	5.7	$p < 0.05$
DEJ	11.9 n = 39	4.8	18.6 n = 3	5.7	$p < 0.05$

*According to t-test

The neonatal line was identified using a polarizing microscope (40x) and measured in the middle of the enamel crown directly from the microscope "between the line's inner and outer limitations" (Ranggard et al. 1994: 255). Five separate measurements were taken of the neonatal line and then averaged. Lines thinner than 5 μm were not measured in detail, but given a set value of 5 μm "because they were not distinct enough to permit more exact measurements" (Ranggard et al 1994: 256). Consequently, the precision of their measurements is questionable.

Ranggard and colleagues (1994) determined that the mean width of the neonatal line ranged from 5 μm to 25.5 μm, with a median value of 5.9 μm (Table 3-6). The majority of children (73 %) had a mean neonatal line width of less than 10 μm. Seven children had neonatal lines wider than the "normal" 12 μm width proposed by Eli et al. (1989). Based on these results, the authors conclude: "it is questionable whether a neonatal line wider than 15 μm can be regarded as a sign of a stressful neonatal situation" (p. 258). Furthermore, the registered findings in enamel morphology could not, in any case, be correlated with the measured values of neonatal blood ionized calcium.

A study in the following year by Ranggard and colleagues (1995) examined the clinical and histological appearance of deciduous enamel from 11 infants subjected to blood exchange transfusions (ET) during the first days of life. These children also had a mean of three consecutive hypocalcemic days. Three children were delivered with a normal vertex presentation and seven by cesarean section. One child experienced postnatal asphyxia (Patient 4, Table 3-6). The neonatal line was examined following the method of Ranggard and colleagues (1994) previously outlined. The mean width of the neonatal lines in these children ranged from 2.7 μm to 19.4 μm, with a median value of 7.2 μm (Table 3-6). Contrary to Eli et al. (1989), infants undergoing cesarean sections exhibited both thin and wide neonatal lines (Ranggard et al. 1995). Unfortunately, Ranggard et al. (1995) do not identify the line measurements of these infants. These authors further conclude: "hypocalcemia caused by ET in the newborn period does not affect the width of the neonatal line or the major enamel morphology to any extent" (p. 123). In

1996, Tievens and colleagues described an *accentuated* neonatal line in an infant delivered by cesarean section; no measurements are provided.

Table 3-6. Mean Neonatal Line Width (µm) and Range Width (µm) After Five Measurements of Each Specimen (after Ranggard et al. 1994 and 1995).

Ranggard et al. (1994)			Ranggard et al. (1995)[‡]	
Patient No.	Mean width	Range width	Patient no.	Mean width
1	5.0	< 5.0	1	19.4
2	5.0	< 5.0		
3	15.4	13.6 - 18.2	2	4.7
4	5.0	< 5.0		
5	15.0	11.8 - 17.6	3	8.3
6	14.4	12.7 - 15.5		
7	5.0	< 5.0	4	16.1
8	5.0	< 5.0		
9	8.7	4.5 - 11.1	5	14.2
10	8.4	7.6 - 9.1		
11	5.0	< 5.0	6	2.7
12	14.1	13.5 - 14.6		
13	5.7	4.6 - 6.5	7	8.0
14	5.0	< 5.0		
15	6.2[†]	11.8 - 17.6[†]	8	5.7
16	5.0[†]	12.7 - 15.5[†]		
17	9.0	8.4 - 9.4	9	7.2
18	5.0	< 5.0		
19	5.0	< 5.0	10	6.8
20	22.0	20.1 - 24.1		
21	25.5	20.7 - 28.5	11	5.6
22	16.0	11.7 - 18.5		
23	8.1	7.1 - 8.9		
24	5.0	< 5.0		

[†] This is the data provided by Ranggard et al. (1994). It is evident that inconsistencies exist with respect to the mean width and the range width provided for these two individuals. The true measurements are not known.
[‡] The range width of the neonatal line measurements is not provided in this study.

Low Birthweight and Enamel Defects

The etiology of low birthweight may be short gestational age (prematurity), intrauterine malnutrition (IUGR), or a combination of these two factors (Noren, 1983). Since the 1930s, the majority of studies comparing low birthweight and enamel defects have shown a higher incidence of disturbances in enamel formation (enamel hypoplasias and/or hypocalcifications[3]) in the perinatal and neonatal periods than that found for normal births (Stein, 1936 and 1947; Grahnen and Larsson, 1958; Kreshover et al. 1958; Miller and Forrester, 1959; Grahnen et al. 1974; Funakoshi et al. 1981; Johnsen et al. 1984; Pimlott et al. 1985; Seow et al. 1987; Brooks et al. 1997). Only a few of these studies (Grahnen and Larsson, 1958; Grahnen et al. 1974; Seow et al. 1987; Brooks et al. 1997) actually incorporated a control group for comparative purposes.

Since the high incidence of enamel defects in low birthweight children was first noted, investigators have attempted to identify perinatal and neonatal factors that may be involved in their pathogenesis. A number of medical complications have been associated with enamel defects in the low birthweight infant (Seow, 1992). These include: hyperbilirubinemia (Grahnen and Larsson, 1958; Grahnen et al. 1974; Funokashi et al. 1981); Type I respiratory distress syndrome (Funokashi et al. 1981; Mellander et al. 1982; Johnsen et al. 1984; Brooks et al. 1997); neonatal asphyxia (Grahnen et al. 1974); hypocalcemia (Purvis et al. 1973; Levine and Keen, 1974; Funakoshi et al. 1981) rickets (Funakoshi et al. 1981; Nikiforuk and Fraser, 1981; Seow et al. 1984); anemia (Funakoshi et al. 1981) hypoparathyroidism (Purvis et al. 1973; Funakoshi et al. 1981), and disturbed vitamin D metabolism (Mellander et al. 1982). Nevertheless, a review of the dental literature demonstrates that little consensus exists regarding the relative importance of these risk factors in affecting tooth development. Seow (1992) states that a lack of agreement between researchers is inevitable because many causative factors co-occur in low birthweight infants, making it almost impossible to isolate their relative importance to enamel growth (Seow et al. 1987; Seow, 1992).

[3] Enamel hypocalcification is visible on dental surfaces as a dull white opacity or a stained area of reduced mineralization and is often associated with disturbances during the maturation stage. Because the timing of enamel

Disagreement among dental researchers also abounds regarding the proposed pathogenic mechanism(s) responsible for enamel defect formation in the low birthweight infant. Since calcium metabolism is directly involved in dental development (i.e. regulating ameloblast function) and matrix secretion is inhibited if calcium concentrations are either too high or too low, conditions that exhibit disturbed calcium metabolism (i.e. hyper/hypoparathyroidism, rickets, hypocalcemia) have been associated with enamel hypoplasia (Purvis et al. 1973; Levine and Keen, 1974; Funokashi et al. 1981; Nikiforuk and Fraser, 1981; Suckling, 1989; Seow, 1991). In contrast, many researchers have not found an association between serum calcium levels and enamel defects, whether macroscopic or microscopic (Pimlott et al. 1985; Seow et al. 1989; Ranggard et al. 1994 and 1995).

Seow et al. (1989) suggest that many of the individual systemic conditions proposed as causative factors in enamel defect formation act through a central mechanism, namely bone mineral deficiency (not reflected in differences in serum calcium levels). Premature infants often suffer serious deficiencies of bone mineral (osteopenia) as a result of metabolic disturbances and inadequate mineral supply (Brooke and Lucas, 1985 as cited in Seow, 1991). Under these conditions, Seow and colleagues (1989) propose that enamel hypoplasia might result directly from calcium being diverted from calcification sites (i.e. tooth enamel) as mineral is conserved for other body functions. This hypothesis was substantiated in a controlled study, which demonstrated that prematurely born children with the greatest bone demineralization suffered the highest incidence of enamel hypoplasia (Seow et al. 1989; Seow, 1991).

In other studies, Johnsen and colleagues (1984) suggest that the increased incidence of enamel defects in premature children with respiratory distress syndrome and in infants with breathing complications is a function of ameloblast sensitivity to oxygen deprivation. The study of Mellander and colleagues (1982) is the only study that proposes a nutritional factor (i.e. low breast milk intake resulting in low calcium levels) and a winter birth (i.e. reduced sunlight resulting

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hypocalcification cannot be readily inferred and because staining from soil can occur in buried remains, this type of defect has been neglected by dental anthropologists (Skinner and Goodman, 1992).

in a decrease in cord blood levels of vitamin D3 in both the mother and infant) as possible causes of enamel defects in low birthweight infants.

Although the majority of studies on enamel defects in premature infants have focused their attention on the incidence of macroscopic enamel aberrations in the primary dentition, there are few that have focused their efforts on microscopic abnormalities. Stein (1936, 1947) and Schour and Kronfeld (1938) were among the first researchers to propose that the neonatal line would be more *prominent* in the teeth of prematurely born children "because of the relatively greater nutritional difficulties and other disturbances usually encountered by these infants" (Schour and Kronfeld 1938: 473). The studies of Stein (1936, 1947) also provided early information that pre-term births are associated with a high incidence of enamel hypoplasia in the deciduous dentition. Stein further suggested that many of these defects occur at the level of the neonatal line, which itself is *accentuated*. Unfortunately, in both studies, Stein does not provide measurements of the neonatal line.

Almost fifty years later, Noren (1983) was the first researcher to describe the appearance of the neonatal line in 107 teeth (102 incisors, 5 canines) from low birthweight infants and normal full-term infants. Using microradiography, polarizing microscopy and SEM, he found that those children born pre-term exhibited widened neonatal lines, while some also displayed neonatal enamel hypoplasia. Noren suggested that the more marked width of the neonatal line found among low birthweight infants is related to the severity and the duration of the initial weight loss after birth[4], which is also reflected in blood plasma calcium concentrations (Bergman, 1974). Several studies on low birthweight infants have not found any significant correlation between birthweight and the incidence of enamel defects (Johnsen et al. 1984; Pimlott et al. 1985; Brooks et al. 1997). Noren further advocates that because all newborn infants experience physiological hypocalcemia, it is reasonable to assume that this decrease in plasma calcium is responsible for

[4] A neonate born at term loses approximately 5 percent of its birthweight over the first three or four days before starting to gain weight. The nature of this weight loss partly represents a loss of body water and partly a loss of brown fat used for thermoregulation (Pauerstein, 1987; Rosenberg, 1991).

a structural response in the enamel: the neonatal line. A widened neonatal line, on the other hand, and its corresponding hypoplasia, is a structural response to a stressed situation of neonatal hypocalcemia in the low birthweight infant (Tsang et al. 1973; Bergman, 1974). As noted earlier, two studies conducted by Ranggard and colleagues (1994, 1995) on the histologic appearance of the neonatal line in relation to neonatal blood calcium values revealed no correlation between the width of the neonatal line and the measured levels of blood calcium.

In a second study, utilizing histologic and microradiographic techniques, Noren et al. (1978a) found that a widened neonatal line (width greater than 10-12 um on microradiographs) was observed in a higher proportion of incisors from infants classified "small for date" than in control groups. When infants with supposed intra-uterine undernutrition were divided into those who suffered more and for a longer time from undernutrition (i.e. short for gestational age) from those who did not (i.e. normal length for date at birth), the former group harbored a greater number of mineralization disturbances. Although these differences were not significant, quantitative data is not provided. Based on these findings, Noren and colleagues related the higher occurrence of widened neonatal lines in "small for date" children to both perinatal asphyxia and neonatal hypocalcemia. Other studies have questioned these associations (Grahnen et al. 1969; Ranggard et al. 1994 and 1995). Furthermore, Grahnen et al. (1972) found that children who had been classified "small for date" did not display a greater number of macroscopic enamel defects than normal full term infants. Funakoshi et al. (1981), Mellander et al. (1982) and Brook et al. (1997) arrived at similar conclusions.

Several problems exist with the studies of Noren (1983) and colleagues (1978a). Measurements of the neonatal line are not provided and neither is information on infant birthweight. The results of tests of significance are not included. Most importantly, information on how the neonatal line was measured is not provided.

Gestation Length and the Location of the Neonatal Line in Human Enamel

Although abundant research has been carried out on prematurity and its effect(s) on the appearance of deciduous enamel, little research has been done on the location of the neonatal

line within the tooth crown as a function of gestation length. A search of the literature revealed only three such studies (Noren, 1983; Skinner 1992; and Skinner and Dupras, 1993).

In 1983, Noren conducted the first test of the hypothesis that the location of the neonatal line varies with gestation length. He suggested that there is a tendency for the neonatal line to be positioned towards the incisal parts of the tooth in low birthweight infants. However, neither quantitative data nor tests of significance are provided.

Skinner (1992) examined the location of the neonatal line within the crown of 148 primary teeth as a function of gestation length. He examined each longitudinal section under normal and polarized light at 200X magnification for evidence of the neonatal line. Distance from the neonatal line's intersection with the DEJ to the cervix of the tooth was measured with an eyepiece micrometer, calibrated to a 10mm objective graticule with 0.1mm gradations. For the purposes of statistical analyses, gestation length was expressed in days before or after expected term as reported by the mother and arbitrarily divided into three groups: pre-term (> 8 days prior to term), term (0 days +/- 7 days) and post-term (> 8 days after term).

An ANOVA by tooth type and by gestation length demonstrated that the neonatal line was characteristically located in different teeth and that its location usually varied as a function of gestation length (Table 3-7). Significant differences between gestation groups in the location of the neonatal line were found for central and lateral incisors and second molars. No significant differences were found for canines or first molars. Skinner also demonstrated that the duration of the pregnancy affects the location of the neonatal line. For each tooth type and for the sample as a whole, he regressed the distance of the line from the cervix (Y) and the number of days before or after expected term, each child was born (X). Only those regression equations for incisors (especially first incisors) were determined to be reasonably accurate because they included a balance of teeth from pre and post-term births. Linear regression further demonstrated that approximately 46% (r^2 = .462, p = 0.0001, first incisors only) of the variation in the location of the neonatal line (Y) is a function of gestation length (X).

In the following year, Skinner and Dupras (1993) elaborated upon the study of Skinner (1992) by incorporating a larger sample (173 teeth) and defining term as 38 to 42 weeks, in accord with obstetrical and clinical practices. Statistical analyses (one way ANOVA and t-tests) demonstrated that the location of the neonatal line differed significantly between pre-term, term and post-term births for incisors and first molars. No significant differences were found for canines or second molars (Table 3-8). Approximately 75 percent (11/15) of the neonatal lines that lay outside of two standard deviations of the mean location in term births came from non-term births (see Figure 3-1) (Skinner and Dupras, 1993). Linear regression of the distance of the neonatal line to the cervix and gestation length for all primary teeth from non-term births demonstrated that 36 percent (r^2 = 0.36, p = 0.0001) of the variation in location of the line is explained by the duration of pregnancy (Skinner and Dupras, 1993). The remaining variation is attributed to differences in tooth size, individual variation, and errors in estimation of birth timing.

Table 3-7. Mean distance (mm) of neonatal line from cervix: ANOVA by tooth type and gestation length (Skinner, 1992).

		Gestation Interval			
Tooth	ANOVA**	Pre-Term	Term	Post-Term	Significant "between
		0.0109*	0.0001*	0.0001*	Gestation group"
		Mean	Mean	Mean	differences ***
i1	0.0001	1.723	1.019	0.998	1 - 2, 1- 3
		n = 13	n = 17	n = 11	
i2	0.0024	2.264	1.367	1.585	1 - 2, 1 - 3
		n = 8	n = 18	n = 9	
C	0.8401	3.451	3.302	3.253	NS
		n = 2	n = 8	n = 5	
m1	0.0941	-	2.011	1.753	NS
		-	n = 19	n = 11	
m2	0.0101	3.01	2.847	2.441	
		n = 1	n = 17	n = 9	2 – 3

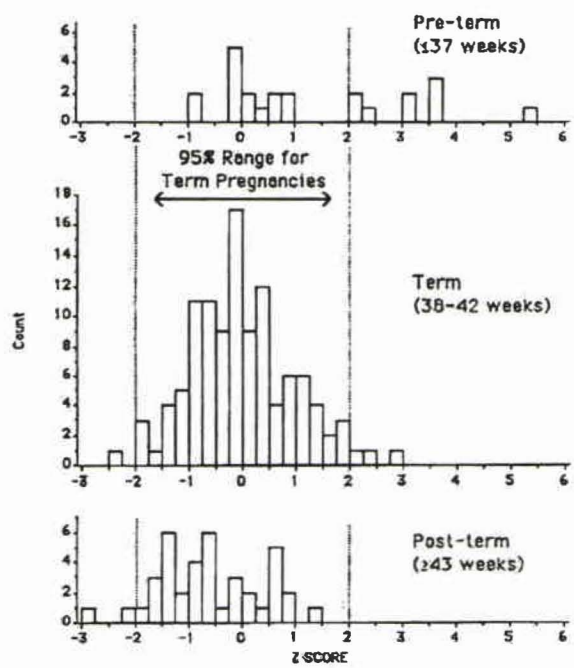
*Statistical significance of ANOVA of difference among tooth types
** Statistical significance of ANOVA of difference among gestation intervals
*** Significant at 0.05 level

Table 3-8. Comparison (ANOVA) of the mean distance (mm) of the neonatal line from the cervix for each tooth type according to gestation interval (Skinner and Dupras, 1993).

	Gestation Interval			
Tooth	Pre-Term Mean	Term Mean	Post-Term Mean	Sig.
i1	1.784	1.207	0.915	0.0001
	n = 10	n = 31	n = 10	
i2	2.178	1.373	1.488	0.0029
	n = 8	n = 25	n = 6	
C	3.443	3.351	3.275	NS
	n = 3	n = 13	n = 6	
m1	1.947	1.983	1.589	0.0136*
	n = 1	n = 22	n = 8	
m2	2.475	2.743	2.461	0.383*
	n = 1	n = 19	n = 9	

* T-test between term and post-term samples

Figure 3-1 Location of the Neonatal Line in all Primary Teeth Expressed as Z Scores from Mean Location of the Line in Teeth from Term Pregnancies (after Skinner and Dupras, 1993).



Newborn Health and Enamel Defects: Jaundice, Minimal Brain Dysfunction and Congenital Hypothyroidism

Studies on newborn health and dental development have focused much of their attention on the health of the premature neonate. Complications can arise for infants born at full term. Existing studies have primarily focused on such disorders as jaundice with or without associated haemolytic disease, Minimal Brain Dysfunction (MBD) and congenital hypothyroidism (CH).

Miller and Forrester (1959) found that jaundice resulting from either haemolytic disease of the full term neonate or prematurity was always associated with severe dental abnormalities and that the severity of the aberrations is related to the severity of the jaundice. Histological examination revealed that in milder forms of jaundice only that portion of the dentine corresponding to the level of neonatal development is affected. In more severe cases (i.e. kernicterus), interruption in both dentine and enamel formation is evident. With respect to enamel, dental disruptions result in either an *accentuated* neonatal line, and, at times, in an arrest in enamel formation (i.e. enamel hypoplasia).[5] Based on these findings these authors conclude, "it appears more than coincidental that increasing levels of jaundice at birth have been associated with increasing degrees of dental upset, ranging from the neonatal line of Rushton to severe enamel hypoplasia" (Miller and Forrester 1959: 104). Unfortunately, no measurements of the neonatal line are provided.

In a later study of full term infants exhibiting physiologic jaundice, Hals and Grahnen (1965) found no associated histological or microradiographic changes in the hard tissues of primary teeth. Although part of their study focused on the neonatal line, no attempts were made to record any difference in the width of the line from one section to another "because the width

[5] Miller and Forrester (1959) suggest that in mild hemolytic jaundice the level of the bilirubin is too low or its presence too transient to affect dental development. As the bilirubin level rises the pigment is incorporated into the dentine, being subsequently oxidized to biliverdin, resulting in a green stained band. In more severe cases of jaundice both dentine and enamel formation is disrupted. Unfortunately, these authors do not explain why increased levels of these substances may lead to accentuated neonatal lines and enamel hypoplasia. A possible explanation may be the high levels of serum albumin (a transporter of bilirubin to the liver) found among jaundiced infants (Rosenberg, 1991). Serum

differs with the relation between the neonatal line and the plane of section" (Hals and Grahnen 1965: 184).

Minimal Brain Dysfunction (MBD) syndrome, which includes deficits in attention, motor control and perception, is commonly associated with such pregnancy risk factors as toxemia, asphyxia and breech delivery (Gillberg et al. 1985). Many researchers have examined the potential use of microscopic dental abnormalities as registers of perinatal and neonatal insults in such children (Jaffe et al. 1985; Judes et al. 1985; Noren and Gillberg, 1987). These authors suggest that because of the common embryonic ectodermal origin and development of the tooth and the nervous tissue, "it would be logical to conclude that an insult to the developing fetal or infant brain of sufficient magnitude to result in brain damage, would likely leave its mark on the tooth enamel" (Judes et al. 1985: 69).

Jaffe et al. (1985) and Judes et al. (1985) found that children with brain damage more frequently exhibited *abnormal* neonatal and perinatal lines than did normal children. According to these authors, their findings support the contention that most brain damage in children originates around birth. However, the specific neonatal and perinatal episodes responsible for these microscopic defects could not be determined. Although Jaffe and colleagues discuss the neonatal line and suggest that a width greater than 15 μm indicates a complicated birth (after Eli et al. 1989), no measurements of the line are provided. Similarly, Judes et al. (1985) note that one infant exhibited a widened neonatal line, but again no measurements are given.

Noren and Gillberg (1987) found that children with MBD more often exhibited a combination of microscopic enamel aberrations in their incisor teeth than did normal children. With respect to the neonatal line, children in the MBD group demonstrated widened lines. There was also a slight tendency towards infants with a wider neonatal line having more perinatal risk factors than those without widening of the line (Noren and Gillberg, 1987). Unfortunately, the perinatal risk factors referred to are not described. The widening of the neonatal line in the infants

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albumin has been implicated as an inhibitor of hydroxyapatite crystal growth and may be a factor in enamel defect formation (ie. disturbed prism structure) (Fincham et al. 2000).

with MBD was explained as a tissue response to a prolonged neonatal hypocalcemia at the time of birth. The possible relationship between MBD and hypocalcemia is not demonstrated by these authors and remains questionable since other studies have failed to find an association between blood serum calcium levels and neonatal line width (Ranggard et al. 1994 and 1995). Neonatal line measurements are not presented.

Noren and Alm (1983) investigated the deciduous enamel of children with congenital hypothyroidism (CH). They found that children with an early onset of hypothyroidism demonstrated hypomineralized areas in both the prenatal and postnatal enamel. Marked microporosity "that progressed far beyond the neonatal line into the postnatal enamel, occupying most of the postnatal enamel" was also noted (Noren and Alm, 1983). They conclude that thyroid hormone influences the maturation of enamel and leaves a large microporous zone in children whose thyroid hormone production is impaired. The effect of CH on the neonatal line is not described.

Maternal Health and Enamel Defects

The majority of studies that have examined the effects of maternal health on the quality of deciduous enamel, have focused their attention on infants born to diabetic mothers. Noren (1984) and colleagues (1978b) conducted numerous histological (polarizing microscopy) and microradiographic studies on primary incisors from infants of diabetic mothers. These authors found that a widened neonatal line could be observed about three times as frequently in infants of diabetic mothers compared to a group of healthy, full term infants. They suggest that this difference is significant, yet neither quantitative data, nor tests of significance are provided. In all three studies the techniques employed in measuring the neonatal line are not stipulated. With the exception of the study of Noren et al. (1978b) (who suggest that a widened line is one that exceeds a width of 10 to 12 μm on microradiographs), the study of Noren (1984) does not identify the measurements of the widened neonatal lines. These authors propose that the development of hypocalcemia in infants of diabetic mothers results in a widened neonatal line. This conclusion is arguable (see pages 45 - 47).

Studies on maternal health and enamel defects have concentrated their efforts on numerous other pregnancy risk factors. Via and Churchill (1959) found that conditions such as preeclampsia, Rh incompatibility, diabetes, intrapartum hemorrhage and placenta previa occurred in those mother's of children displaying enamel hypoplasia. In contrast, Grahnen and Edlund (1967) found that 17 children born to mothers who were toxemic during pregnancy did not have an increased frequency of enamel hypoplasia. Maternal vitamin D deficiency and concomitant secondary hyperparathyroidism have been implicated in the development of a temporary transitory hypoparathyroidism in the newborn, which results in neonatal hypocalcemia (Tsang et al. 1973; Purvis et al. 1973; Hillman and Haddad, 1976). Funakoshi et al. (1981) noted that disorders such as toxemia and anemia figure most prominently in mothers of children with enamel defects. Johnsen et al. (1984) found no correlation between the incidence of enamel defects and such pregnancy risk factors as preeclampsia and intrapartum hemorrhage.

Part III: Bioarchaeological Investigations of the Neonatal Line

Bioarchaeological investigations of enamel defects have focused on the occurrence and prevalence of enamel hypoplasias and Wilson bands in the permanent, and less frequently deciduous, dentition of past populations (Rose, 1973; Rose et al. 1981; Rudney, 1983; Blakely and Armelagos, 1985; Goodman, 1989; Goodman et al. 1980, 1984 and 1992; Goodman and Rose, 1990; Wright, 1990; Skinner and Goodman, 1992; Duray, 1996; Simpson, 2000). These studies have demonstrated that enamel defects are relatively sensitive and non-specific indicators of infant and childhood morbidity (Goodman and Rose, 1990). Many investigations have also found a significant association between the frequency of enamel defects and skeletal evidence of infectious disease, malnutrition (i.e. anemia) and the probability of dying (Boyd, 1978; Cook and Buikstra, 1979; Clarke, 1980 and 1982; Rose 1973; Rose et al. 1978 and 1985). The premise for using disturbed enamel formation as an indicator of infant and childhood morbidity is based on the normal process of enamel development, the body's generalized response to

stress[6], the individual's level of host resistance and threshold of susceptibility (Goodman et al. 1984)[7] and the fact that enamel retains a permanent unaltered chronology of disturbance(s) that occur during amelogenesis.

With the exception of a very few studies (Verner et al. 1998; Rossi et al. 1997), investigations of the neonatal line in the deciduous teeth of archaeological populations are uncommon. Verner and colleagues (1998) using SEM examined the general morphological appearance of enamel in primary second molars and permanent first molars from eleven individuals from archaeological sites in Denmark and Sweden. A neonatal line was identified in all primary teeth and its position was interpreted as indicating a normal full-term gestation. The appearance of incremental lines in the primary enamel is also discussed. In one individual, the neonatal line was "wider than normal", while in two cases incremental lines were found close to the neonatal lines. In one individual two thin prenatal lines were seen, while in eight individuals postnatally located incremental lines were found. Based on these observations, the authors conclude that this sample shows evidence of stress to the mother and child after birth and that the observed incremental lines suggest periods of dietary change, possibly related to periods of illness. Given that only three individuals demonstrate evidence of potential birth complications (one with a widened neonatal line and two with distinct perinatal lines) these conclusions seem unjustified. Furthermore, no measurements are reported in this study and reference to these lines

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[6] Rose (1973) utilized the biochemical processes of Selye's (1973) General Adaptation Syndrome as a unifying concept to explain the etiology of enamel defects. According to this model, physiologic and metabolic disturbance results in increased activation of the sympathetic-adrenal medullary and pituitary-adrenal cortical axes. The activation of these pathways results in an increase in cortisol levels and the concomitant inhibition of protein synthesis throughout the body. A reduction in protein synthesis would decrease or stop the secretion of enamel matrix by the ameloblasts. Changes in ameloblast matrix secretion alter the fluid dynamics of the developing enamel and the ameloblast direction of movement (Osborn, 1970a,b), producing a Wilson band. In more severe physiologic disturbances, cessation of protein synthesis and matrix secretion may result in an enamel hypoplasia.

[7] Goodman and colleagues (1984) have constructed two epidemiological models for enamel defect formation. The first model highlights a chain of events leading to the formation of an enamel defect. Goodman et al. propose that environmentally and culturally induced stressors and constraints combine with an individual's level of host resistance to determine the type, duration and intensity of the systemic physiological perturbation. If ameloblasts are active at the time of the disturbance, their metabolism will be affected. The second model explains the etiology of enamel defects. Goodman et al. suggest that enamel defects result when the magnitude of ameloblastic disruption is pushed over a threshold line due to a combination of forces (etiological/susceptibility/genetic factors, nutrition and illness history). The type of defect is also determined by the functional stage of the active ameloblasts (secretory or maturation) and the severity of the disruption to the ameloblast cells (irreversible or permanent). The size of a defect may reflect the severity and the intensity of the physiologic perturbation that caused it (Suckling, 1989).

as incremental is confusing. Are these authors describing Wilson bands or are they simply remarking on the color/visibility of the striae of Retzius within the enamel?

The Isola Sacra project (Rossi et al. 1997) was initiated in 1992 by the Anthropology Section of the National Prehistoric Ethnographic 'L. Pigorini' Museum of Rome. This project encompasses the study of the human dental and skeletal collections excavated from the Roman Imperial necropolis of Isola Sacra, used intensively by the inhabitants of Portus Romae during the 2nd and 3rd centuries AD. In the course of this study, extensive digital archives (available in the form of CD-ROMS) were compiled, which contain observations on the microstructure (i.e. Wilson bands and neonatal line) of 228 human deciduous teeth from 122 infant and juvenile individuals. Quantitative analyses of the neonatal line (i.e. neonatal line width and estimates of gestation length) were carried out using Digital Image Processing (DIP). To estimate neonatal line thickness, the method proposed by Eli et al. (1989) was used (see page 44). Although quantitative data is provided for each individual specimen, the CD-ROM does not contain detailed discussions of the results. Consideration of the specific or general implications of the findings from a paleoepidemiological perspective is also not included. Similarly, comparative analyses of this material with other available data on the neonatal line are not presented.

Summary of Literature Review

Studies of deciduous enamel defects in perinatal and neonatal disorders demonstrate little consensus of those factors responsible for their development. Some agreement, however, exists between dental researchers. Most agree that complications at birth leave their mark in the dental hard tissues, either in the form of a widened neonatal line and/or neonatal enamel hypoplasia. Dental investigators also propose that insufficient material for matrix formation (i.e. hypocalcemia, mineral deficiency, vitamin D deficiency) and/or disturbed cellular function (calcium metabolism, oxygen deprivation) are potential factors in the pathogenesis of enamel defects.

With the exception of a few studies, published studies of the neonatal line have failed to provide the following: 1) the techniques used in measuring the neonatal line; 2) the location of measurements in the tooth crown; 3) the magnification at which the measurements were taken; 4)

quantitative data (i.e. measurements of the neonatal line, tests of significance) and 5) the results of precision tests (i.e. intra-observer error).

Of those investigations where there is an attempt at detailed description of methodology, problems still exist. These studies do not mention (Eli et al. 1989) or clearly describe that portion of the enamel disturbance being quantified. Descriptions such as "between the lines inner and outer limitations" (Ranggard et al. 1994: 255) and "that portion of the enamel where the prisms were clearly disrupted" (the Isola Sacra Project) are insufficient and fail to recognize the structural variability demonstrated by microstructural investigations of the neonatal line (see Chapter 2). There is a need for a more rigorous methodology for measuring the neonatal line (see Discussion).

Terminology can also be confusing. Many authors use undefined terms such as *"prominent"*, *"accentuated"*, *"pathologically accentuated"*, *"uniformly accentuated"* and *"abnormal"* to describe the neonatal line. The terminology employed in earlier studies seems to refer to color and/or visibility of the neonatal line under the microscope (see Discussion).

With the exception of the studies of Skinner (1992) and Skinner and Dupras (1993), no careful investigation of inter-tooth differences in neonatal line width exists in the dental literature. No investigations have examined the relationship between birth duration and the width of the neonatal line. Dental researchers and medical experts require a better understanding of the effects of the birth process and medical interventions on the health of the neonate (see Discussion).

Chapter Four

Materials and Methods

A fundamental objective of this thesis is to advocate the importance of a rigorous methodology during investigations of the neonatal line. This chapter describes and illustrates in detail (using digital images of neonatal lines) all of the procedures undertaken during this investigation. This research was reviewed and subsequently approved by the McMaster Research Ethics Board (MREB).

The Sample

A. Participant Recruitment and Collection of Specimens

The exfoliated deciduous teeth used in this study were collected from numerous children (n = 45) residing in three Canadian cities: Hamilton and Oakville, Ontario and Montreal, Quebec. Participants from the province of Ontario comprised the majority of the sample (n = 32) and were recruited from four locations: 1) a dental clinic located in Oakville (n = 3), 2) a doctor's clinic located in Hamilton (n = 18), 3) a dental clinic in Hamilton (n = 2); 4) Dearcroft Montessori school in Oakville (n = 4); and 5) friends of the researcher (KB) (n = 5). Participants from Montreal included 13 individuals, who were not selected from any particular location. All participants from Montreal include relatives or friends of the researcher.

Participant recruitment was accomplished through the distribution of a "flyer" to the various clinics and individuals previously mentioned (Appendix A). This flyer briefly outlines the purposes and goals of the current investigation and the nature of participant involvement. All interested participants were required to fill in the necessary information on the flyer (name and telephone number) and to leave a copy with the collaborating institution. Once a sufficient number of subjects was recruited, all flyers were collected from the appropriate institutions and/or individuals.

Initial contact with participants was done through a short telephone conversation, which permitted "Birth History" interviews to be scheduled at the participant's convenience. During the course of participant recruitment, no set criteria were used to either include or exclude individuals or groups of individuals (i.e. multiple births, low birthweight, etc.). The majority of specimens were collected at the time of the interviews. If specimens were not available at this time, they were collected later. In a few cases, participants supplied multiple teeth from each child and these too, if not obtainable at the time of the interviews, were collected shortly after exfoliation. Eighty-five deciduous teeth were collected; however, due to time constraints only forty-five were sectioned for analysis (one tooth per child). The age of the majority of children participating in this study is between 5 and 13 years. In six cases, the specimens used for analysis came from parents who had kept their children's teeth as "souvenirs". All collected teeth were in good condition.

Collected specimens were placed in small Ziploc bags labeled with a participant number (i.e. NNL #), which corresponded to an interview number (i.e. the first participant interviewed was given the number 1, etc.). To insure anonymity, the names of the participants' children were not written on the specimen bags. This procedure also guaranteed that during succeeding phases of this study (i.e. sectioning and analysis), the identity of each individual was undisclosed to the researcher, preventing any possible bias during measurement(s) of the neonatal line. The fact that a hiatus of approximately three months occurred between the conducting of interviews and the analysis of specimens diminished the likelihood that a specific specimen number would be associated with its corresponding interview number and birth data.

B. Collection of Birth History Information

All necessary medical information was gathered through an in-depth and structured "Birth History" interview with the mother of children who had donated a tooth or teeth. Each interviewee was asked the same questions in the same order and manner to ensure continuity between the information gathered from each participant. Forty-nine interviews were conducted; however, due to the loss of four children's teeth during the sectioning process, only forty-five teeth and, consequently, forty-five interviews were available for analysis. A sample of the interview

questions can be found in Appendix A. These questions can be grouped according to the following general categories: 1) obstetrical/medical interventions/procedures prior to and at the time of delivery; 2) the health of the infant at the time of birth and during the first year of life; 3) postnatal care of the infant; 4) the duration of the delivery; 5) the health of the mother during pregnancy and delivery; and 6) demographic profile. Although several questions were initially asked, not all of the gathered information was used in the final investigation. A detailed description of the birth and health variables examined in this study can be found in the *Statistical Procedures* section of this chapter.

Before beginning an interview, all participants were asked to sign a Consent Form (Appendix A). All conversations were tape recorded and later transcribed. Only relevant data was extracted from these interviews and recorded in an Excel spreadsheet.

Specimen Preparation Procedures

A. Measurements

Before sectioning, all teeth were cleaned, identified (i.e. side, maxillary versus mandibular and class), measured and any evident gross pathology recorded (i.e. enamel hypoplasia, caries). Where applicable, crown height, maximum bucco-lingual diameter, and maximum mesio-distal diameter were measured for each tooth following the definitions outlined in Hillson (1996: 70-71). All measurements were attained using Mitutoyo brand digital electronic calipers.

B. Photographs

Due to the destructive nature of the sectioning procedure, digital photographs were taken of selected specimens for documentation and descriptive purposes. Those teeth displaying some form of enamel defect (i.e. pitting of the enamel surface, hypoplasias, severe attrition and carious lesions) were photographed. A representative tooth from each class (incisor, canine and molar) was also photographed. All specimens were photographed at a magnification of 10x using a digital camera (Polaroid) connected to a PC computer and mounted on a Leica brand Stereo Zoom 6 Photo microscope. All images were saved as TIF files and stored on CD-ROMs.

Specimen Thin-Sectioning Procedures

The procedures outlined below incorporate the advice and techniques of Dr. Charles FitzGerald (2000, personal communication).

A. Specimen Preparation

Strict control of specimen identification was essential in this study and extreme care with labeling was taken through the whole sectioning process, up to the point when sections were permanently fixed to an identified microscope slide. Forty-five teeth were sectioned and analyzed: twenty-six incisors, nine canines and ten molars.

Before sectioning, all teeth were soaked in Buehler brand Epo-Kwick epoxy, which filled in surface micro-cracks and provided support to fragile enamel during the sectioning procedure. The preparation process involved mixing four full Samco brand Transfer Pipets of resin and 1 full pipet of hardener in a disposable cup. Once the mixture was thoroughly stirred (stirring duration time: no longer than 1 minute), all specimens were submerged in the epoxy, ensuring maximum infiltration. Next, the cup was placed in a vacuum chamber and evacuated for approximately ten minutes to remove excess air bubbles. Once the evacuation was complete, specimens were removed with forceps and placed on a petri dish coated with Buehler brand release agent. The epoxy was then allowed to harden for a few hours (approximately 3 to 5 hours). Once the epoxy had hardened, all teeth were removed from the dish and sectioned.

B. Tooth Sectioning and Slide Preparation: Cutting, Polishing and Mounting

Researchers have claimed that the width of the neonatal line is "highly dependent upon the distance of the section from the cusp tip" (Jackobsen 1975: 99) and "the plane of sectioning and section thickness" (Weber and Eisenmann, 1971; Eli et al, 1989). Extreme care was therefore taken to section all teeth in an identical manner.

Before cutting, a fine-pointed black permanent pen was used to mark the cutting orientation of each tooth. For anterior teeth, all sections passed through the maximum thickness of the enamel, such that a longitudinal section along the mid-sagittal plane (i.e. labio-lingual axis)

was produced. For posterior teeth, the cutting orientation ran non-obliquely through the mesio-buccal cusp, resulting in a section along the bucco-lingual axis of molar teeth.

All teeth were sectioned on a Buehler brand Isomet low speed peripheral saw fitted with a diamond wafer blade cutting with tap water. Before sectioning, the tooth was precisely oriented on a cutting chuck, such that the "cutting line" ran parallel to the mounting surface of the chuck. The tooth was secured in this position with dental sticky wax applied with a small bladed hot knife. The chuck was then mounted on the Isomet saw and positioned so that the blade cut through the outside edge of the marked line. Once the first cut was made, the chuck was dismounted and an etched (i.e. specimen number and tooth type) microscope slide was attached to the same cut face parallel to the tooth surface using dental sticky wax. The chuck was then remounted on the saw and a second cut was made, resulting in a section measuring between 300 to 400 μm in thickness, using digital calipers. A further section was taken from the tooth in the same manner in the event that one was damaged during the following polishing/grinding procedure. In all cases, the first section taken from each specimen was used during analysis of the neonatal line.

The tooth section, still attached to its slide, was then polished on a Buehler brand Ecomet III polisher/grinder, mounted with a Buehler brand billiard polishing cloth. A three-micron aluminum oxide slurry mixture was prepared and used to remove saw-marks from the exposed surface. Once the exposed surface appeared smooth, the slide and specimen were cleaned in an ultrasonic bath for ten minutes. The slide and section were then removed and dried in a vacuum chamber for fifteen to twenty minutes. Once dry, a small amount of OCON-186 UV resin (acrylic acid and hydroxypropyl methacrylate, obtainable from Logitech Ltd.) was spread over the surface of the section and another slide (etched with the specimen number and tooth type) placed onto it. The two slides were clamped together using forceps and the resin left curing under an UV light for thirty to forty-five seconds. The resultant "slide sandwich" was then placed on a hot plate and the sticky wax attaching the specimen to the first glass slide melted away. The original slide was then

removed and discarded, leaving the polished face of the tooth permanently attached to the glass slide. The slide was also rinsed with absolute ethyl alcohol to remove any unpolymerized resin.

The other face of the section was then polished following the same procedures outlined above. Using digital calipers, the slide was polished to a thickness ranging from 70 to 130 μm. The slide and section were then cleaned in an ultrasonic bath for ten minutes. Once clean, the slide was examined under a light microscope to insure that the section was thin enough and that the neonatal line was visible.

Once a satisfactory section was achieved, the specimen on its slide was then dehydrated in absolute ethyl alcohol and thoroughly dried under vacuum for fifteen to twenty minutes. It was then immersed in xylene and kept under vacuum until air bubbles ceased coming from the specimen. After removal from the xylene, a coverslip was mounted over the specimen using a thin layer of CR-1 Mounting Medium (available form Ward's Natural Science, Rochester, New York). Finished slides were left to cure for several weeks. In the event that air bubbles became trapped beneath the coverslip surface, obscuring enamel microstructure, the slide was soaked in xylene to remove the coverslip and the mounting medium; a new coverslip was then mounted. Once dry, any excess mounting medium was eliminated from the slide using a scalpel and the slide was gently cleaned using a Kim-Wipe dipped in alcohol.

Collecting Data on Neonatal Line Width

A. Observation Equipment and Use of Computer Software Programs

Specimens were observed with an Olympus Model BH-2 microscope equipped with a polarizing attachment and objectives giving magnification powers of 40x, 100x, 200x and 400x. Photomicrographs of tooth thin-sections and of the neonatal line were taken in polarized light using a Polaroid Digital Microscope Camera™ mounted on the microscope.

Adobe Photo Shop 5.5™ was used to create all of the photomontages presented in this thesis. Photomontages of tooth sections (incisors, canines and molars) demonstrating the position of the neonatal line and the sampling location of neonatal line measurements were

recreated using individual photographs taken at a magnification of 100x. Because each image covered only a single portion of the tooth crown section, the final image of the whole crown is the result of between 8 and 12 partial images, depending on individual tooth size and class. Photomontages of complete neonatal lines comprise three or four partial images of the line taken at 200x magnification.

An image measurement software program, Sigma Scan 5.0™, was used to measure neonatal line width (see below).

All photographs presented in this thesis and used for data collection were saved as TIF files and stored on CD-ROMs.

B. Data Collection and Recording Procedures

The following data were collected from each tooth section: 1) the sampling location of neonatal line measurements (DEJ, middle, apex); 2) the neonatal line width at each sampling location; 3) the type of prism disturbance along the neonatal line at each sampling location; and 4) the overall appearance/visibility of the neonatal line in the tooth crown (marked boundaries versus faint boundaries; continuous from the DEJ to the apex or discontinuous).

To begin, each specimen was surveyed under the microscope at the lowest magnification (100x) to determine the location of the neonatal line and the aspect of the crown (labial/buccal versus lingual) where it was most visible. Individual photographs were then taken of the neonatal line at a magnification of 200x for the creation of photomontages and at 400x for observation and measuring purposes.

Collected data were recorded in an Excel spreadsheet. All measurements of neonatal line width were first recorded in a Sigma Scan 5.0™ spreadsheet, which converted measurements to microns, and later transferred into the Excel spreadsheet.

C. Measuring the Neonatal line

The appearance of the neonatal lines in the present investigation is similar to Rose's (1977) "distorted" Wilson band microstructure (see Rose 1977: 441, Figure 1). Consequently, the

descriptions of neonatal line microstructure presented here are in accordance with his terminology.

The most noticeable structural disturbance was a distortion (i.e. widening) of the prism boundaries in the plane of the neonatal line (see Figures 4-1a, 4-2a, 4-3a and 4-4a). In some instances, this disturbance was also accompanied by an amorphous appearance to the prisms between the prism boundaries (see Figures 4-5a, 4-6a, 4-7a, and 4-8a). In only two cases did the line consist of accentuated (i.e. more visible) cross-striations along those prisms within the neonatal line combined with a slight distortion of the prism boundaries (see Figures 4-9a and 4-10a).

All thickness measurements of the neonatal line were taken following the paths of individual prisms, along that portion of the prism where 1) the prism boundaries were clearly distorted (Figures 4-1b, 4-2b, 4-3b and 4-4b), 2) the prism boundaries were distorted and the prisms had an amorphous appearance (Figures 4-5b, 4-6b, 4-7b and 4-8b), or 3) the cross-striations were visibly accentuated (Figures 4-9b and 4–10b). Careful examination of the regular appearance of the prism boundaries on both the prenatal and postnatal sides of the line determined that portion of the prism boundary that was disturbed and that should be measured in each thin section. Before employing this measuring technique, its replicability was tested on a small sample of neonatal lines (see below).

To facilitate data collection on neonatal line width, all measurements of the neonatal line were done from photoimages displayed on a PC high-resolution (1200 pixels by 1600 pixels) 19" screen. Measurements of neonatal line width were accomplished using Sigma Scan 5.0™. Measurements were calibrated using a calibration conversion function provided by the program. Calibration was previously achieved by Dr. Charles FitzGerald (personal communication) by photographing a stage micrometer at each magnification (40x, 100x, 200x and 400x) and then carefully measuring known distances in the resulting photomicrographs using Sigma Scan 5.0™. Sigma Scan measures the known distance in pixels and then converts the number of pixels into

the desired unit of measure, which in this case is a micron. For the purposes of the present investigation, the spatial calibration coefficient at 400x magnification was 0.29 µm for each image pixel.

To estimate neonatal line thickness (after Eli et al. 1989), three images were taken of the line and the associated prism disturbances at 400x magnification at three different crown positions (where applicable, see below): 1) close to the dentine-enamel junction (DEJ); 2) in the middle of the dental crown; and 3) close to the apex (see Figures 4-11 to 4-13)[1]. Neonatal line width was measured on either the labial or the lingual aspect of the tooth crown, depending on where it was most apparent. All measurements of neonatal line thickness were taken to the nearest tenth of a micron.

Measurements of neonatal line width could not always be taken at each sampling location, because of the variability in clarity of the neonatal line throughout the tooth crown. In nine cases, the neonatal line was only measured at two crown locations (DEJ and middle, DEJ and apex, or middle and apex) (see Figure 4-14). In the remaining thirty-six cases, neonatal line width was measured at each sampling location (see Figure 4-15). In some cases the neonatal line was not continuously visible from the DEJ to the apex; measurements at each crown position were still obtainable (see Figure 4-16). For the entire sample of teeth (N = 45), forty-two neonatal lines were measured at the DEJ, forty-three at the middle of the crown and forty-one at the apex (see Table 5-2, Results).

Six to fourteen measurements were taken of neonatal line width along individual prisms at any one-crown location (see Tables 1 – 4, Appendix B). The number of prisms measured at each sampling location within a section is not constant because of the differing visibility of the prism boundaries along the length of the neonatal line. In some cases, where the prism boundaries begin and where they end was not distinct/clear and measurements could not be taken. In such cases a smaller number of prisms and smaller portions of the line were measured

[1] For molars, measurements of neonatal line thickness were not taken in the portion of the enamel crown located above the dentine horn because of the appearance of gnarled enamel (see Chapter 2).

(see Figures 4-2a,b; 4-6a,b; and 4-8a,b). In other instances, prism boundaries were visible along a greater length of the line. Consequently, a greater portion of the line was measured (see Figures 4-3a,b; 4-4a,b; and 4-9 a, b).

D. Repeatability of Measurement Technique

Due to the lack of information in previous research on how the neonatal line is measured, it was necessary to determine if the measuring technique used during the present study to estimate neonatal line width was reliable. To do so, the replicability of this measuring technique was assessed from five randomly selected teeth. Fifty-two prisms were measured at the DEJ from these teeth and the measured prisms were annotated with numbers (using Photo Shop 5.0™). The measurements were recorded in an Excel spreadsheet. A week later, the same prisms were re-measured. The significance of the difference between the mean of the repeated measures and the original measurements was assessed at the 0.05 level using a paired samples t-test. The results of this test are presented in the Results section.

Analysis

A. Birth History Variables

Based on the information gathered from the "Birth History" interviews and in accordance with previous investigations of the neonatal line, the following categories were created and considered: 1) type of delivery; 2) child's health at/around birth; 3) medical intervention(s) at/around birth (child); 4) maternal complication(s) at/around birth; 5) evidence of jaundice; and 6) term of delivery. Each of these birth categories is described below:

- Type of delivery. This category includes four delivery types (modified after Eli et al. 1989): 1) a natural delivery; 2) a delivery with minimal medical intervention (not included in Eli et al. 1989, see Discussion); 3) a complicated delivery and 4) an elective cesarean section delivery. A natural delivery was considered when there was no outside medical intervention such as induction through medication, administration of pain relievers and the use of obstetrical instruments to aid in delivery. In this study, thirteen children were delivered naturally. A delivery with minimal medical intervention was considered when some medical

interference took place (i.e. the use of medication to induce delivery and/or administration of pain relievers and analgesics). Seventeen children were included in this category. A complicated delivery included children delivered through obstetrical means such as forceps, vacuum extraction or emergency cesarean section. Emergency cesarean section deliveries were included in the complicated category because they occur after the birth process has begun and when the infant and/or mother are in distress. The complicated category also includes breech delivery. In all cases, complicated deliveries combine other medical interventions, such as the use of medication(s) to induce delivery and/or the use of pain relievers and analgesics. Eleven children were included in the complicated category. Four children were delivered through an elective cesarean section (see Tables 1-4, Appendix B).

- Child's health at birth. All children in this investigation fell into one of two categories: 1) healthy at birth (n = 20) and 2) not healthy at birth (n = 25). The former category includes children who experienced no systemic/physiological complication(s) at the time of birth and in the two weeks following birth. In the latter category, all children experiencing some form of complication(s) at birth or within two weeks of delivery were considered. This category disregards the type of delivery and only considers the health of the child at birth and shortly after (see Table 5, Appendix B).
- Medical intervention (s) at birth (child). All children fell into one of two categories: 1) no medical intervention(s) at/after birth (n = 32) and 2) medical intervention(s) at/after birth (n = 13). In the former category, all children who did not experience invasive medical procedures after delivery (other than what is considered normal protocol; see page 29) and within the next two weeks after birth were considered. The latter category includes all individuals who underwent such procedures as intubation, blood work, intravenous drug administration, and those infants who were incubated upon delivery (NICU) (see Table 5, Appendix B). This category disregards delivery type and infant health at birth.
- Maternal complication(s) at birth: All children fell into one of two categories: 1) those children whose mother experienced complications at/around the time of delivery (n = 14) and 2) those

children whose mother did not (n = 31). In the former category any child born to a mother who experienced systemic/physiologic complication(s) at delivery (i.e. toxemia/preeclampsia, intrapartum hemorrhage, fever, infection) or who developed complications close to the time of delivery, which were sustained during the delivery (i.e. gestational diabetes, anemia) were considered. The latter category included all children whose mother experienced no such complications (see Table 6, Appendix B).

- Evidence of jaundice. All children fell into one of two categories: 1) jaundiced at birth (n = 12) and 2) not jaundiced at birth (n = 33). Children who experienced physiological jaundice were included in the first category, while those who did not were placed in the second.
- Term at birth. For statistical purposes, children were placed in one of three groups depending upon the number of days/weeks before or after expected term they were born, as reported by the mother (after Skinner and Dupras, 1993). A pre-term birth included children delivered greater than two weeks prior to term (less than 38 weeks gestation) (n = 5). A term birth included children delivered within two weeks of the date expected (between 38 and 42 weeks gestation) (n = 39). A post-term birth included a child delivered greater than two weeks after the date expected (greater than 42 weeks gestation) (n = 1).
- Length of Delivery: For statistical purposes, the length of delivery was grouped into three categories: 1) total birth length; 2) birth length from contractions to delivery; and 3) length of the "pushing stage". The first two categories were calculated in hours, while the third was calculated in minutes. The first category considered the total length of the birth process, from when either the mother's membranes ruptured or contractions began (whichever came first) or both, to when the child was delivered. The second stage includes only the time from when contractions were felt to the time when the child was delivered. In the case of an emergency cesarean section delivery, the length of the surgery was also considered. With an elective cesarean section delivery, the length of the surgery was only considered for the first category; this delivery type could not be included in the other two categories, since women do not experience contractions, nor do they pass through a "pushing stage". The "pushing stage"

category considered the length of the birthing/second stage. In some cases (see Table 5-17, page 130), women did not recall how long they were pushing; these individual cases were not considered. Emergency cesarean sections were not included in the "pushing stage" category.

B. Statistical Procedures

All statistical analyses were carried out using SPSS 10.0 (Statistical Package for the Social Sciences). All statistical calculations were done using individual prism measurements. The mean neonatal line width at a particular crown position (i.e. mean width of ten individual prism measurements at the DEJ) for each individual in a birth category was not used. For example, four hundred and twenty-five individual prisms were measured along the neonatal line in forty-two teeth at the DEJ (Table 5-2, Results). For statistical calculations, the number of measured prisms (425) was used to calculate the descriptive statistics of neonatal line width at this crown position. In this way, the sample size incorporated in this analysis was increased. However, before proceeding with this method of calculation both individual mean neonatal line width and the total sample of prism measurements were used to calculate mean neonatal line width. It was found that the mean widths obtained using either method were very similar.[2] Consequently, the method of calculation for mean neonatal line width using the entire sample of prism measurements was maintained throughout the statistical analysis.

For statistical testing, both non-parametric tests and parametric tests were used. Prior to conducting any tests, the normality of the distribution of the measurements was tested using a Kolmogorov-Smirnov test and a Shapiro-Wilk test (i.e. sample of fifty measurements or less). Homogeneity of variances was also tested using Levene's test. If the data set was not normally distributed every effort was made to normalize the sample of measurements. When this proved impossible, non-parametric tests were employed. In cases where the variances were not homogeneous, non-parametric tests were employed, even if the data were normally distributed.

[2] As an example, mean neonatal line width for a natural delivery at the DEJ and at the middle of the crown using the entire sample of prism measurements (n = 92 and n = 108, respectively) is 7.7 μm; at the apex (n = 100) it is 8.4 μm (see Table 5-3, page 97). Mean neonatal line width for a natural delivery at the DEJ using individual mean widths (n = 9) is 7.7μm, at the middle of the crown (n = 10) it is 7.7μm, and at the apex (n = 9) it is 8.4 μm (see Table 1, Appendix B).

Because non-parametric tests are not as statistically powerful as parametric tests (Madrigal, 1998), parametric tests were conducted when the data permitted. When non-parametric tests were carried out on a data set on which parametric tests could be conducted, similar results were obtained.

All tests of significance computed in this investigation are two-tailed and differences in neonatal line width were considered significant at the 0.05 level. The null hypotheses for all tests are: 1) there are no statistically significant differences between mean neonatal line width at each crown position, independent of tooth class or by tooth class for the sample as a whole, and 2) there are no statistically significant differences between mean neonatal line width for each set of birth variables, independent of tooth class or by tooth class. For a description of the statistical procedures presented in this chapter see Madrigal (1998).

Separate statistics were not calculated by sex. An independent samples t-test for log transformed neonatal line width at each crown position demonstrated no significant differences between the sexes at the 0.05 level (DEJ: $t = -1.108$, $p = 0.268$; middle: $t = -1.304$, $p = 0.193$; apex: $t = -1.433$, $p = 0.153$). In conducting this test, equal variances were assumed.

Descriptive statistics (mean, median, standard deviation (S.D.), minimum – maximum, and range) were calculated for neonatal line width at each crown position (DEJ, middle and apex) for the entire sample of prism measurements and for each individual birth variable. To assess the potential effect of inter-tooth differences on neonatal line width, descriptive statistics were also collected by tooth class (incisor, canine and molar) at each crown position for the entire sample of prism measurements and for each individual birth variable. Descriptive statistics calculated for both a natural delivery and for a delivery with minimal medical intervention at each crown position and at each crown position by tooth class do not include neonatal line widths for three individuals from the first category and for four individuals from the second (see below). Descriptive statistics (mean, S.D. and range width) were also collected at each crown position for each individual.

A Kruskal-Wallis rank test was used to compare neonatal line width between crown positions for the total sample of prism measurements (disregarding tooth class) and between

crown positions for each tooth class. This test was also conducted to compare neonatal line width between crown positions, independent of tooth class, for each birth variable. Differences in neonatal line width between crown positions by tooth class for each birth variable were also compared. In the event of a significant difference between neonatal line width by tooth class, a Mann-Whitney U rank test was conducted to determine which tooth's neonatal line width differed significantly.

A Kruskal-Wallis rank test was used to compare neonatal line width between delivery types at each crown position, independent of tooth class, and between delivery types at each crown position by tooth class. In the event of a significant difference between delivery types, a Mann-Whitney U rank test was conducted to determine which delivery type neonatal line width was significantly different. In the analysis by birth type three children from the natural birth category (7, 16 and 24) and four children delivered with minimal medical intervention (4, 9, 15 and 30) were omitted because they exhibited signs of fetal distress at/around the time of delivery. One infant (7) was also not included because of a low birth weight for gestational age (< 2500g) (Table 1, 2 and 5, Appendix B).

An independent samples t-test was used to compare neonatal line width between the two categories for child's health at birth (healthy versus not healthy) at both the DEJ and at the apex. Measurements were transformed using the log function to normalize the data; unequal variances were assumed. Measurements at the middle crown position could not be normalized and a Mann-Whitney U rank test was used to compare neonatal line width between these two categories at this crown position. A Mann-Whitney U rank test was also employed to compare neonatal line width by child's health at birth by tooth class at each crown position. The results were not corrected for ties.

An independent samples t-test was used to compare neonatal line width between the two categories of medical intervention(s) at birth (no medical intervention versus medical intervention) at the DEJ. Measurements were transformed using the log function to normalize the data; equal variances were assumed. Measurements at the middle and at the apex could not be normalized

and a Mann-Whitney U rank test was calculated to compare neonatal line width between these two categories at these crown positions; the results were not corrected for ties.

An independent samples t-test was used to compare neonatal line width by tooth class and by medical intervention(s) at birth at the DEJ (incisors and molars) and at the apex (incisors only). Measurements were only log transformed for incisors; unequal variances were assumed. For molars, the data did not need to be transformed and equal variances were assumed. A Mann-Whitney U rank test was calculated to compare neonatal line width by remaining tooth class and by medical intervention(s) at birth at each crown position (DEJ = canine; middle = incisor, canine and molar; apex = canine and molar). Measurements for these tooth classes at each crown position could not be normalized; the results were not corrected for ties.

An independent samples t-test was used to compare neonatal line width between the two categories of maternal complication(s) at birth (no complications versus complications) at the DEJ and at the apex. All measurements were transformed using the log function to normalize the data; equal variances were assumed. Measurements at the middle crown position could not be normalized and a Mann-Whitney U rank test was conducted to compare neonatal line width between the two categories of maternal complication(s) at birth at this crown position; the results for this test were not corrected for ties.

An independent samples t-test was used to compare neonatal line width by selected tooth class and by maternal complication(s) at birth at each crown position (DEJ and apex = all tooth classes, middle = canines only). Measurements at the DEJ for incisors and canines and at the apex for incisors were log transformed. The remainder of measurements at the DEJ (molars), at the middle of the crown (canines) and at the apex (canines and molars) did not require transforming. Unequal variances were assumed for measurements at the DEJ and at the apex (incisors only); equal variances were assumed for the majority of the data. Data at the middle of the crown for incisors and for molars could not be normalized and a Mann-Whitney U rank test was employed to compare neonatal line width between the two categories of maternal complication(s) at birth; the results for this test were not corrected for ties.

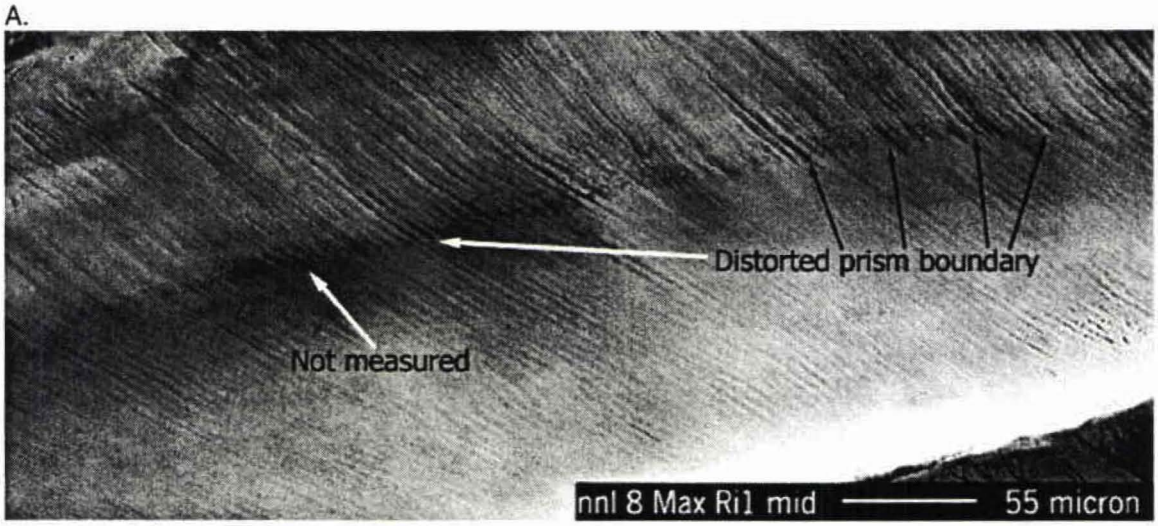
An independent samples t-test was used to compare neonatal line width by jaundiced at birth (jaundiced versus not jaundiced) at the DEJ. All measurements were log transformed and equal variances were assumed. The data at the middle of the crown and at the apex could not be transformed and a Mann-Whitney U rank test was used to compare neonatal line width at these two crown positions; the results for this test were not corrected for ties. A Mann-Whitney U rank test was carried out to compare neonatal line width by tooth class and by jaundiced at birth at each crown position; the results were also not corrected for ties.

An independent samples t-test was used to compare neonatal line width between pre-term and term births at the DEJ. Post-term births were excluded because only one individual fell into this category. All measurements were log transformed and equal variances were assumed. The data at the middle of the crown and at the apex could not be transformed and a Mann-Whitney U rank test was used to compare neonatal line width between pre-term and term births at these two crown positions; the results were not corrected for ties. A Mann-Whitney U rank test was calculated to compare neonatal line width between pre-term and term births for incisors only at each crown position (the sample size for canines was too small in the former category and molars were not available for either category); the results were not corrected for ties.

Non-parametric correlation testing (Spearman's coefficient *r*) was calculated between neonatal line width and the three categories of delivery length at each crown position to determine if a relationship exists between these variables.

Digital Images of the Neonatal Line

A.



B.

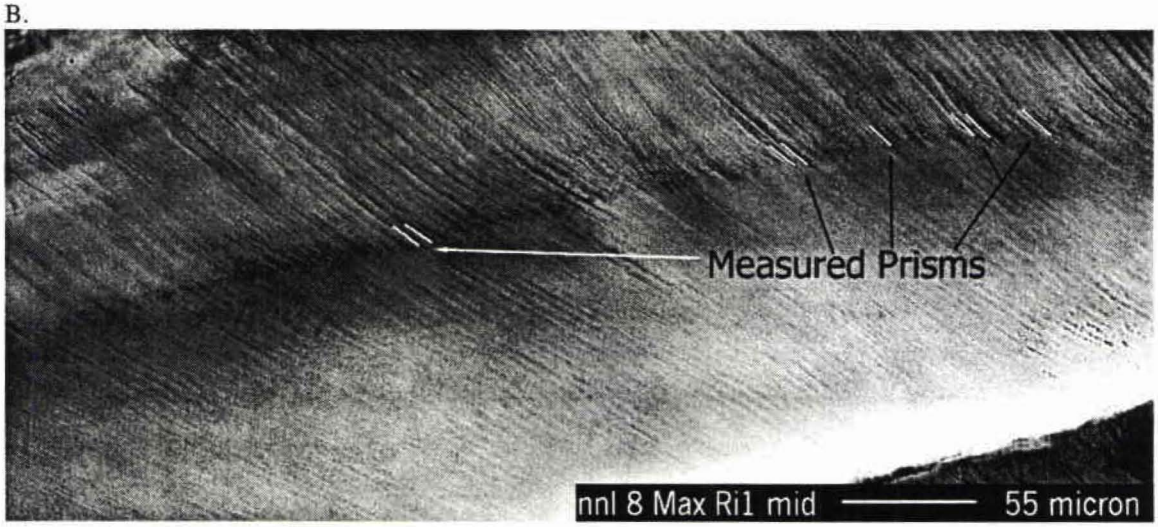


Figure 4-1. a. A digital image of the neonatal line in the middle of the lingual aspect of the enamel crown of a first maxillary right incisor (400x magnification; DEJ is located at the bottom right corner). The structural disturbance consists of distorted/widened prism boundaries. Area of neonatal line to the left was not measured due to the poor visibility of prism boundaries; b. Image of the same line showing those prism boundaries that were measured to estimate neonatal line width. Note that only a small portion of the entire line could be measured. Mean with of the line in this specimen at the middle of the crown is 12.2 microns.

A. B.

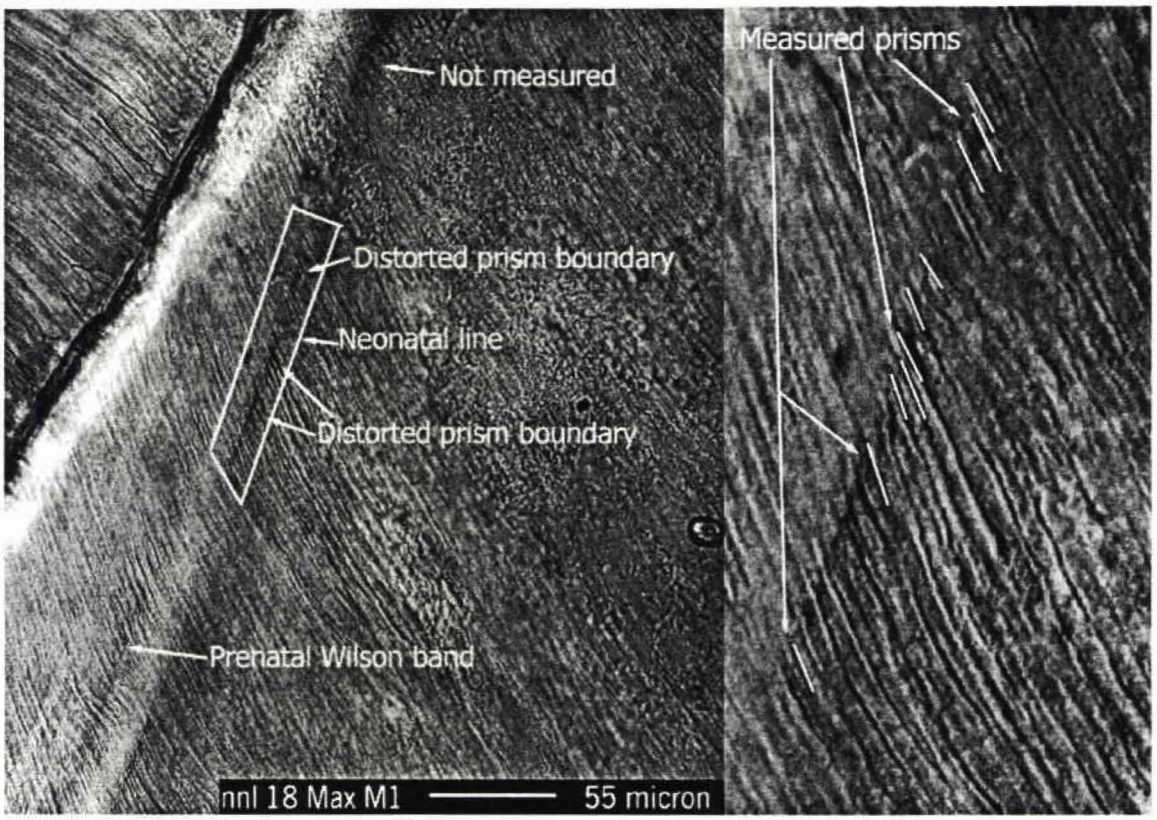
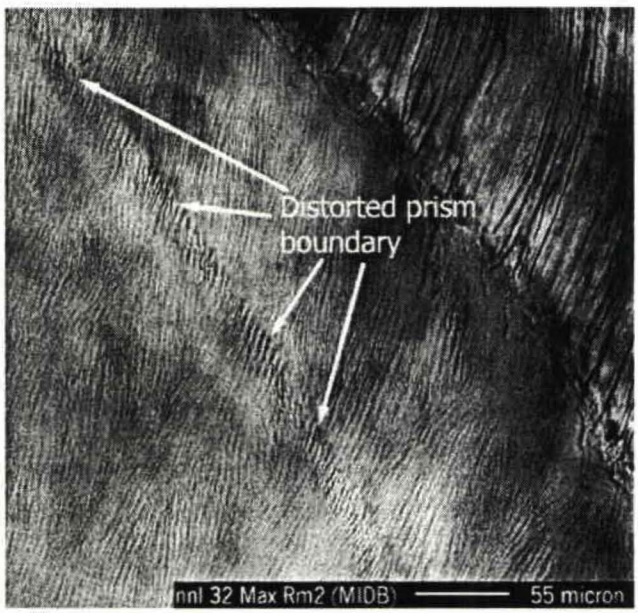


Figure 4-2. a. A digital image of the neonatal line at the DEJ in the enamel crown of a maxillary first molar (side and aspect of tooth not known) (400x magnification; DEJ is located at the upper left corner). The structural disturbance consists of distorted/widened prism boundaries. The area of the neonatal line in the upper portion of the image was not measured due to poor visibility of prism boundaries. Also note the occurrence of a prenatal Wilson band to the left of the neonatal line. b. A close-up image of the portion of the neonatal line (rectangle in 4-2a, not to scale) that was measured to estimate width. Note that only a small portion of the entire line could be measured. Mean width of the line is 8.1 microns.

A.



B.

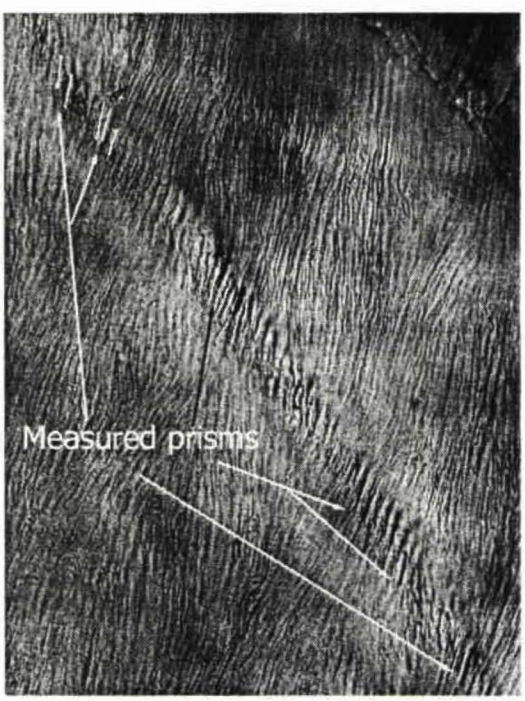
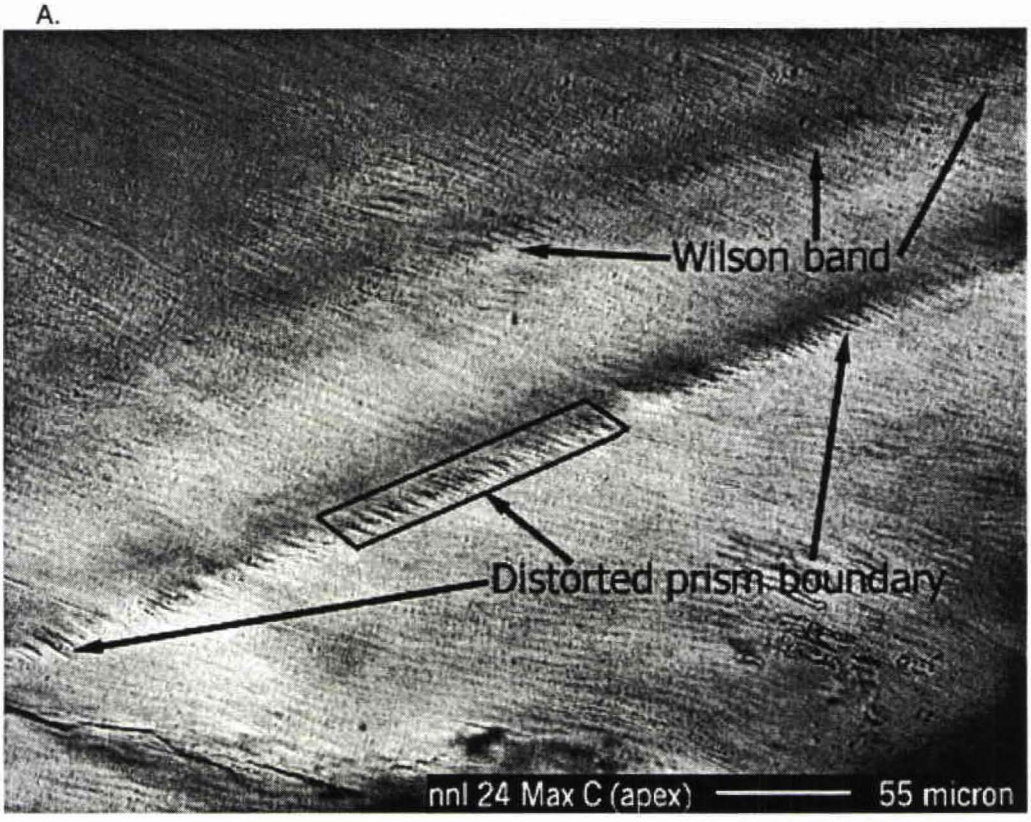


Figure 4-3. a. A digital image of the neonatal line at the middle of the buccal aspect of the enamel crown of a second maxillary right molar (400x magnification; DEJ is located at the upper right corner). The structural disturbance consists of distorted/widened prism boundaries. b. Image of the same line showing those prism boundaries that were measured to estimate neonatal line width. Note that a greater length of the line was measured. Mean width of the line is 11.2 microns.

A.



B.

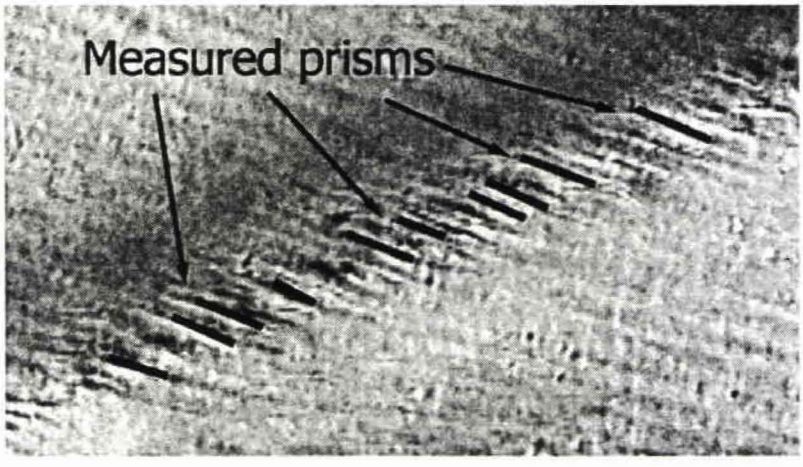


Figure 4-4. a. A digital image of the neonatal line at the apex of the labial aspect of the enamel crown of a maxillary canine (side not known) (400x magnification; DEJ is located at the bottom right corner). The structural disturbance consists of distorted/widened prism boundaries. Note the occurrence of a postnatal Wilson band. b. A magnified image of a portion of the neonatal line (rectangle in 4-4a, not to scale) that was measured to estimate neonatal line width. Mean width of the line is 9.3 microns.

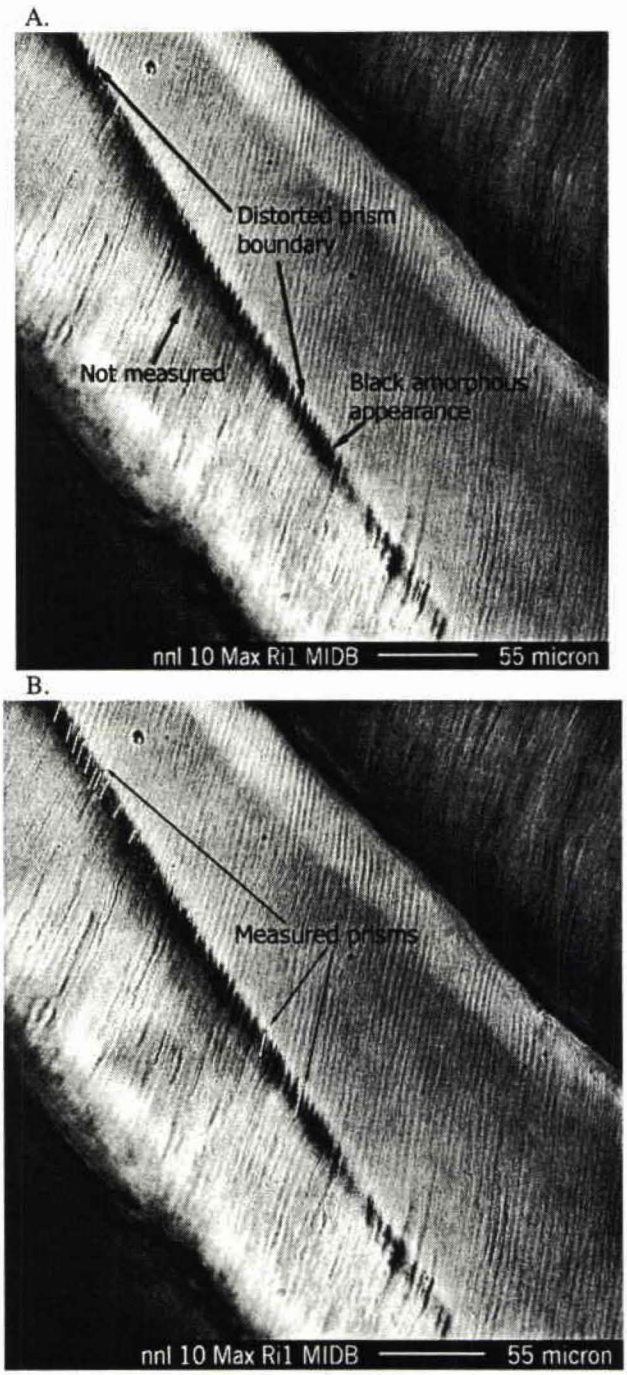
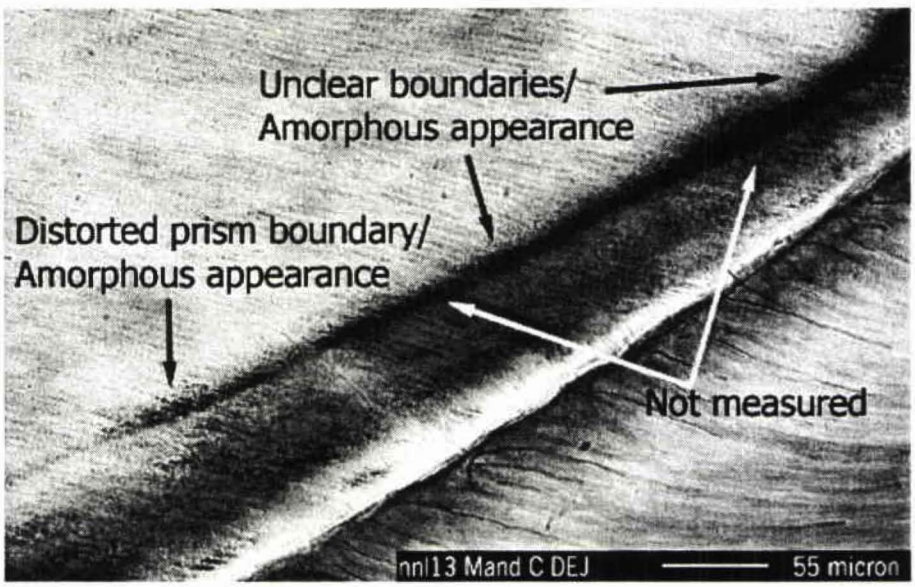


Figure 4-5. a. A digital image of the neonatal line at the middle of the buccal aspect of the enamel crown of a maxillary right central incisor (400x magnification; DEJ is located at the upper right corner). The structural disturbance consists of both distorted prism boundaries and a dark amorphous appearance to the prisms between the boundaries. The area of the neonatal line in the middle of the image was not measured because of unclear prism boundaries on the postnatal side. b. Image of the same line showing those prism boundaries that were measured to estimate neonatal line width. Mean width of the line is 10.7 microns

A.



B.

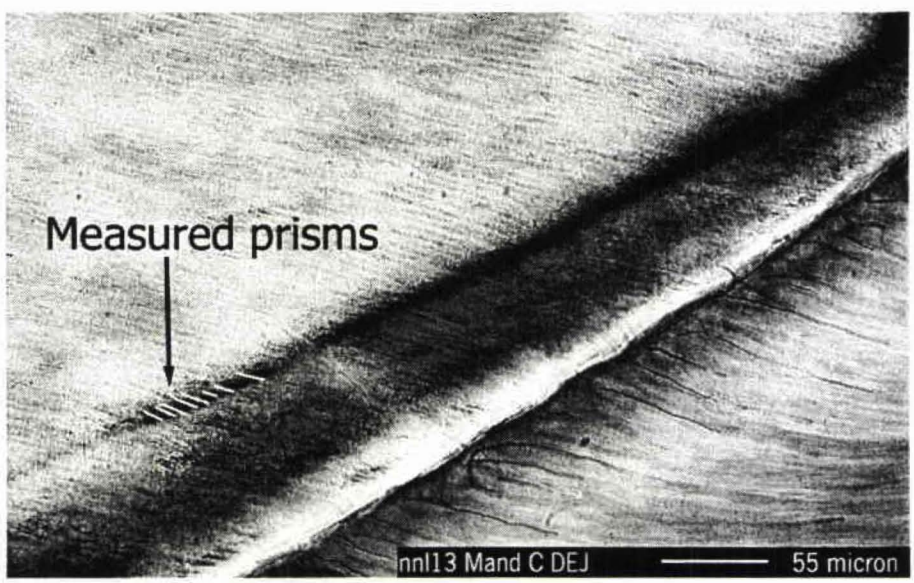


Figure 4-6. a. A digital image of the neonatal line at the DEJ of the lingual aspect of the enamel crown in a mandibular canine (side not known) (400x magnification; DEJ is located at the bottom right corner). The neonatal line close to the DEJ has an amorphous/diffuse appearance with unclear prism boundaries; this portion of the line could not be measured. The portion of the line incisal to the DEJ consists of distorted prism boundaries along with an amorphous appearance along those prisms between the boundaries. b. Image of the same line showing those prism boundaries that were measured to estimate neonatal line width. Note that only a very small portion of the line could be measured close to the DEJ. Mean width of the line is 12.1 microns.

A. B.

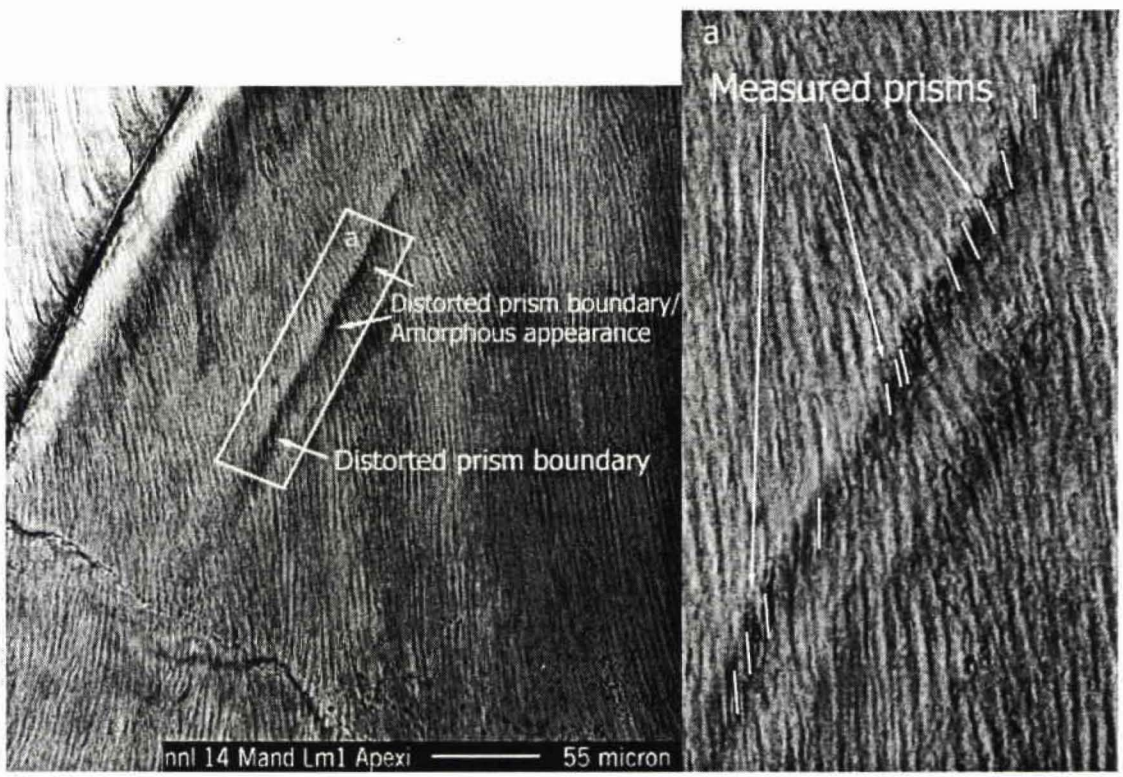


Figure 4-7. a. A digital image of the neonatal line at the apex of the enamel crown in a mandibular left first molar (tooth aspect not known) (400x magnification; DEJ is located at the upper left corner). The structural disturbance consists of both distorted prism boundaries and an amorphous appearance to the prisms between the boundaries. Notice that only a small portion of the line is visible and was measured at the apex of the crown. b. A magnified image of a portion of the neonatal line (rectangle in 4-7a, not to scale) that was measured to estimate neonatal line width. Mean neonatal line width is 7.6 microns.

A. B.

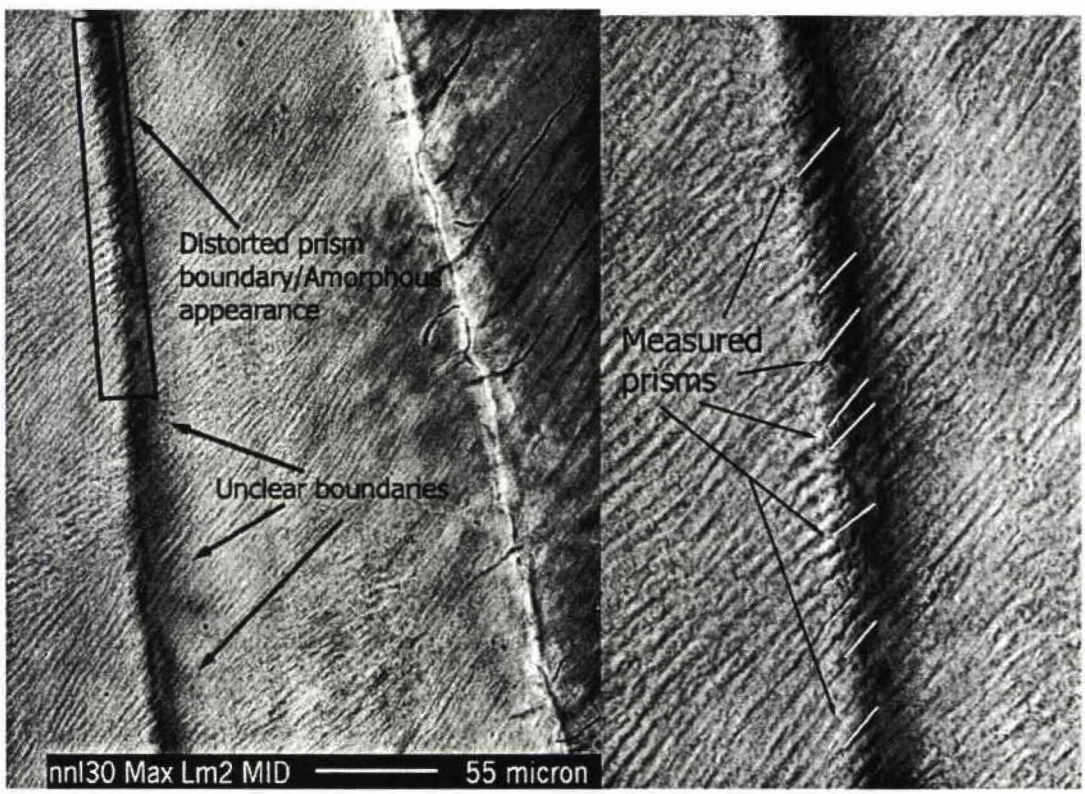


Figure 4-8. A digital image of the neonatal line at the middle of the enamel crown of a maxillary left second molar (400x magnification; DEJ is located to the right of the line). The structural disturbance consists of both distorted prism boundaries and a dark amorphous appearance to the prisms between the boundaries. The area of the neonatal line at the bottom of the image was not measured because of unclear prism boundaries on the prenatal side of the line. b. A magnified image of the line (rectangle in 4-8a, not to scale) showing those prism boundaries that were measured to estimate neonatal line width. Note that only a small portion of the entire visible line was measured. Mean width of the line is 11.4 microns.

A. B.

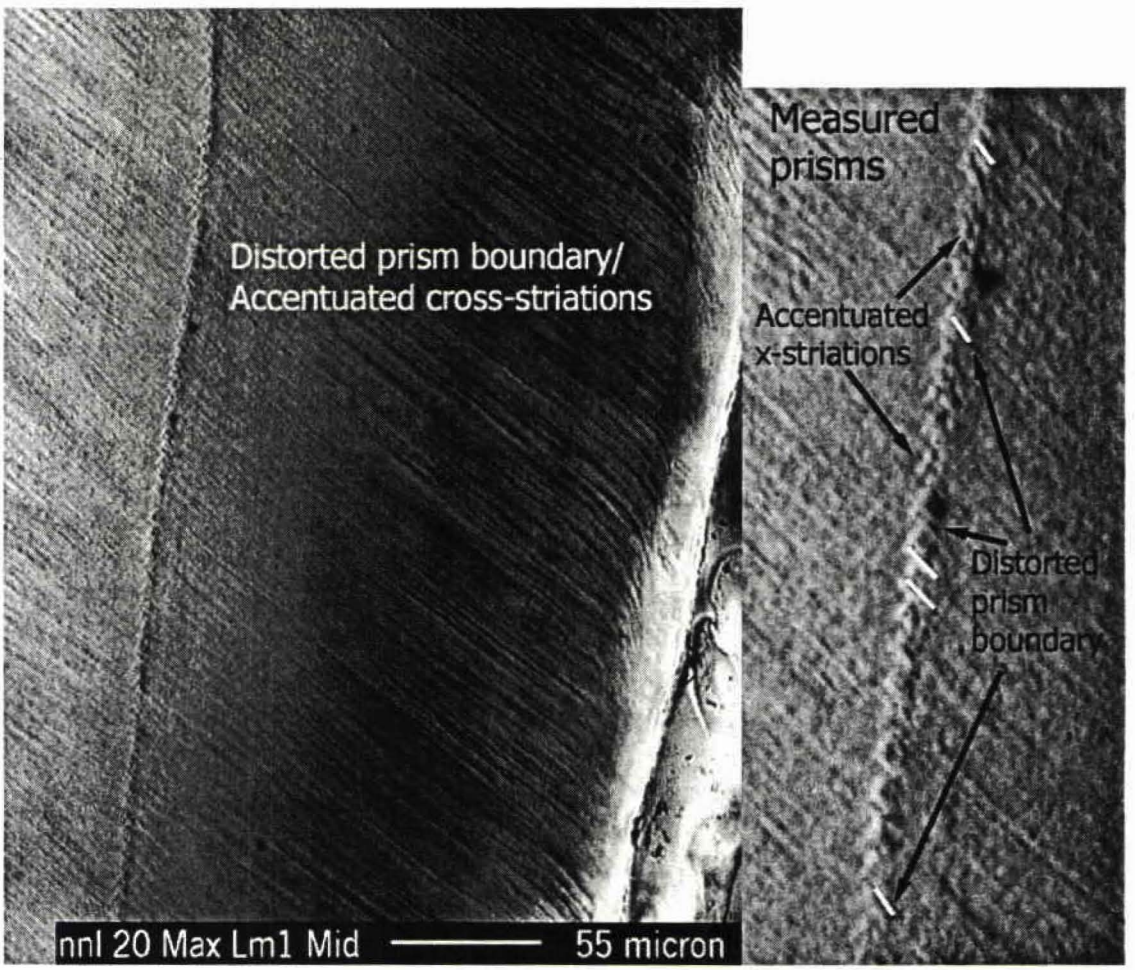
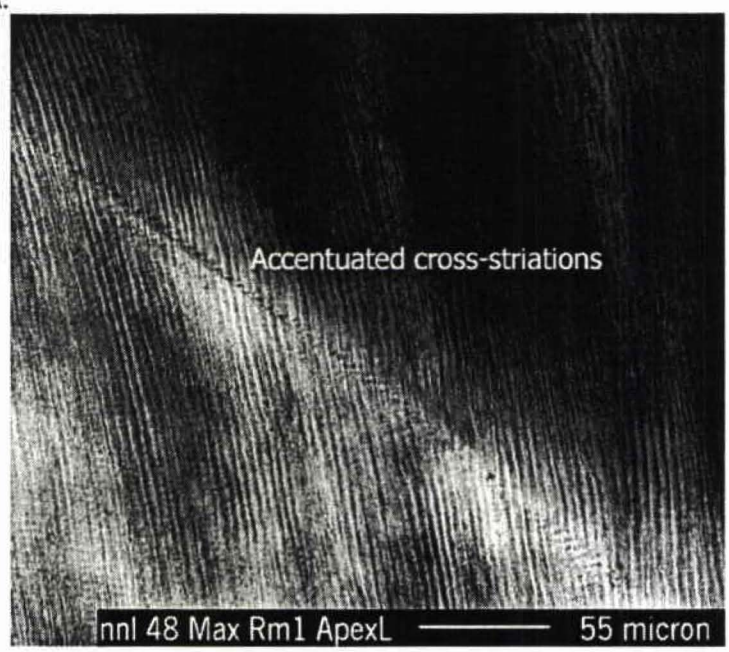


Figure 4-9. a. A digital image of the neonatal line at the middle of the enamel crown in a maxillary left first molar (tooth aspect not known) (400x magnification; crown surface is located at the right of the image). The structural disturbance consists of distorted prism boundaries and accentuated cross-striations. b. A magnified image of a portion of the line in Figure 4-9a showing how neonatal line width was measured and a close-up of the accentuated cross-striations and distorted prism boundaries. Mean neonatal line width is 4.9 microns. This is a good example of a "staircase-type" line of Retzius.

A.



B.

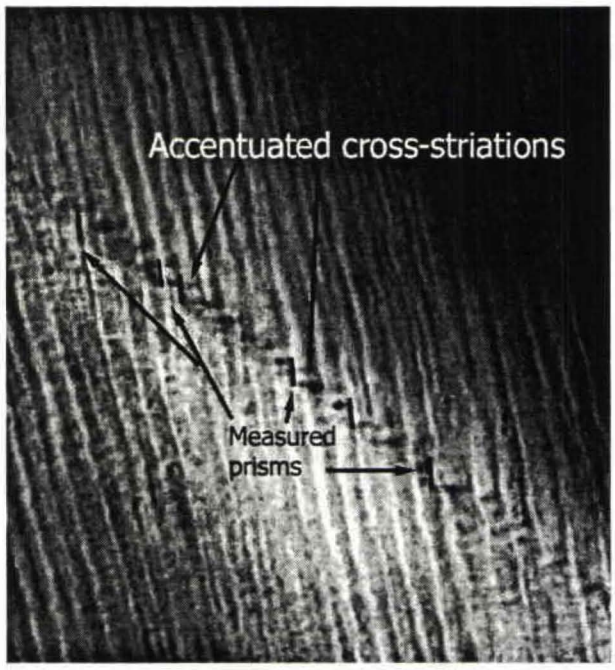


Figure 4-10. A digital image of the neonatal line at the middle of the enamel crown in a maxillary right first molar (tooth aspect not known) (400x magnification). The structural disturbance consists largely of accentuated cross-striations. b. A magnified image of a portion of the line in Figure 4-10a showing how neonatal line width was measured and a close-up of the accentuated cross-striations. Mean neonatal line width of the line is 4.5 microns. This is a good example of a "staircase-type" line of Retzius.

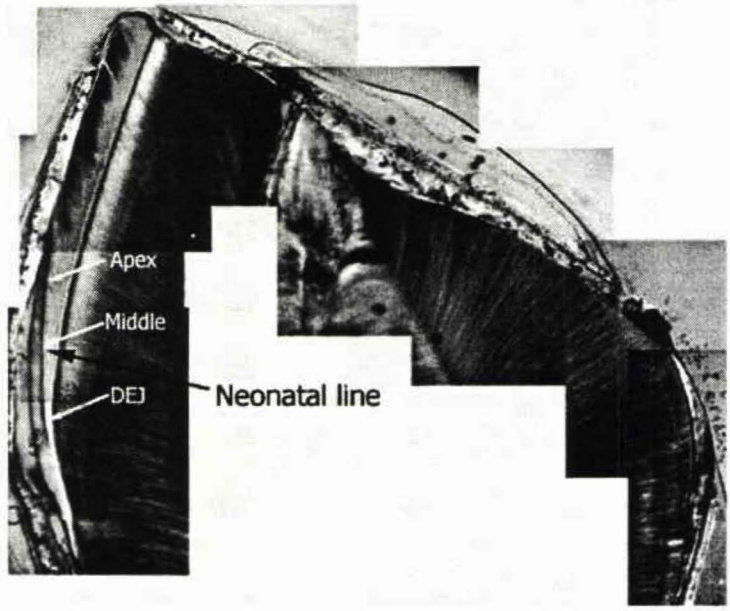


Figure 4-11. Photomontage (100x magnification) of a maxillary left central incisor showing the location of the neonatal line on the labial aspect of the tooth crown and the location of the three images taken at 400x magnification to estimate neonatal line width.

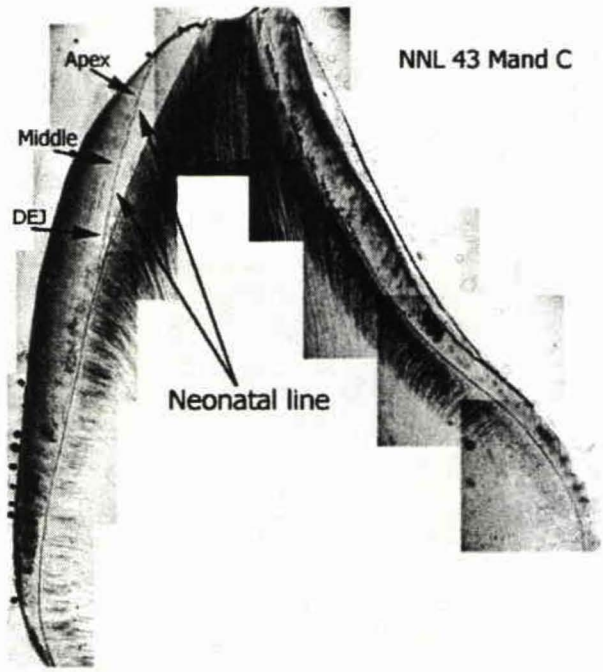


Figure 4-12. Photomontage (100x magnification) of a mandibular canine showing the location of the neonatal line on the labial aspect of the tooth crown and the location of the three images taken at 400x magnification to estimate neonatal line width.

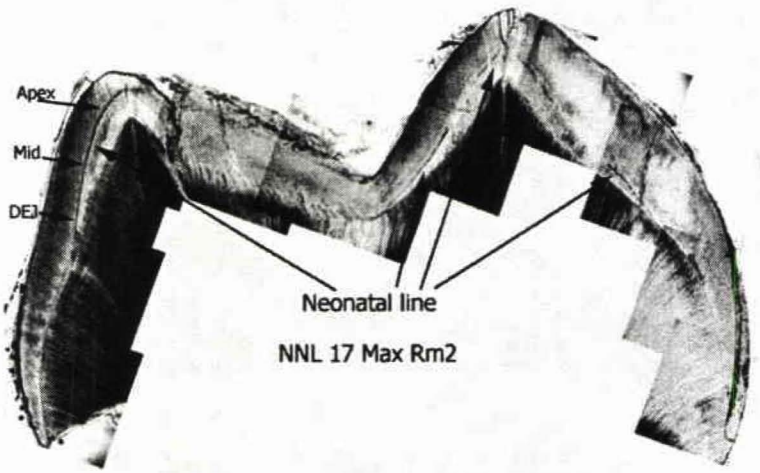


Figure 4-13. Photomontage (100x magnification) of a maxillary right second molar showing the location of the neonatal line on the tooth crown and the location of the three images taken at 400x magnification to estimate neonatal line width.

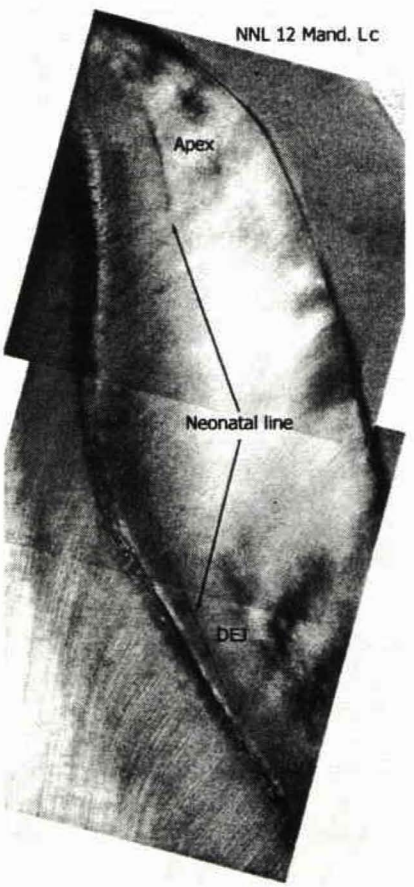


Figure 4-14. Photomontage (200x magnification) of the labial aspect of a mandibular left canine demonstrating the poor visibility of the neonatal line. The line is only visible at the DEJ and the apex. Neonatal line width could not be measured at the middle of the crown.

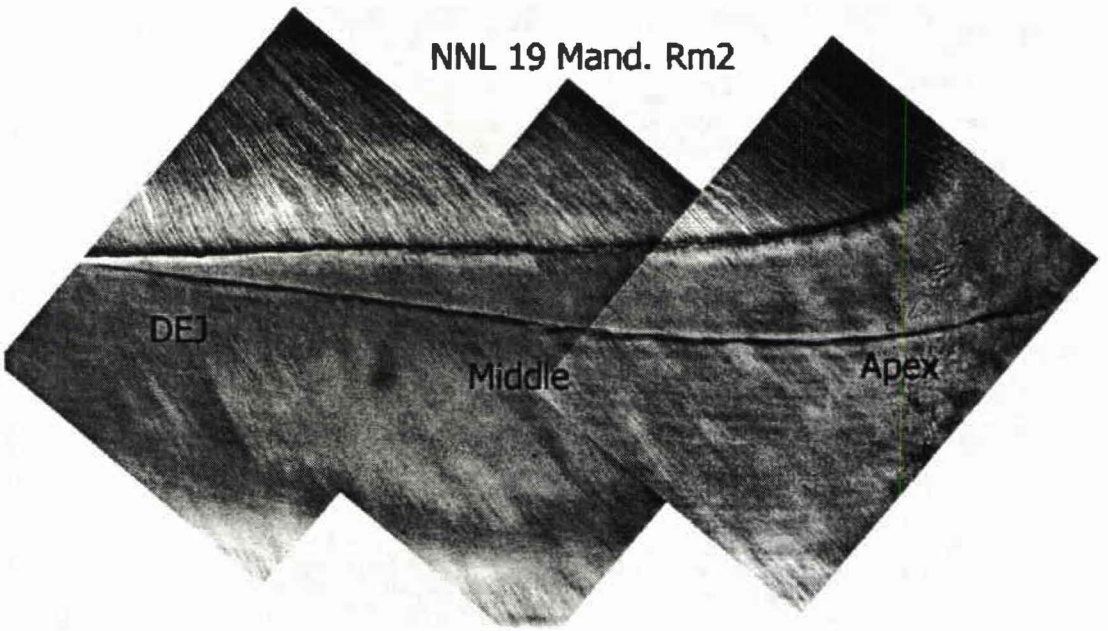


Figure 4-15. Photomontage (200x magnification) of a portion of the enamel crown of a mandibular second right molar demonstrating the continuity of the neonatal line from the DEJ to the apex and its marked visibility at each crown position. Thickness measurements were taken at each location.

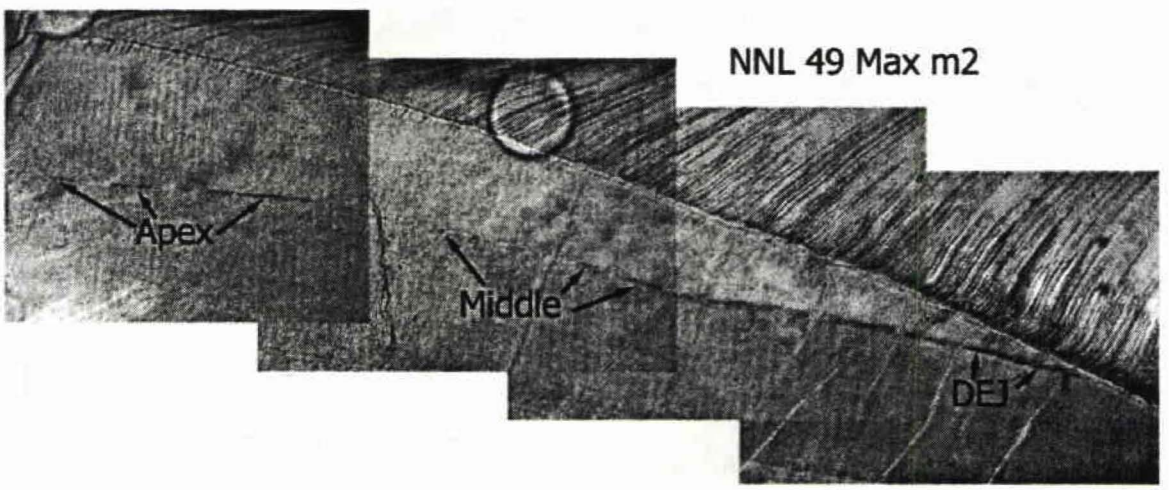


Figure 4-16. Photomontage (200x magnification) of a portion of the enamel crown of a maxillary second molar demonstrating the discontinuity and poor visibility of the neonatal line from the DEJ to the apex. Measurements of neonatal line width were still attainable at all three crown positions.

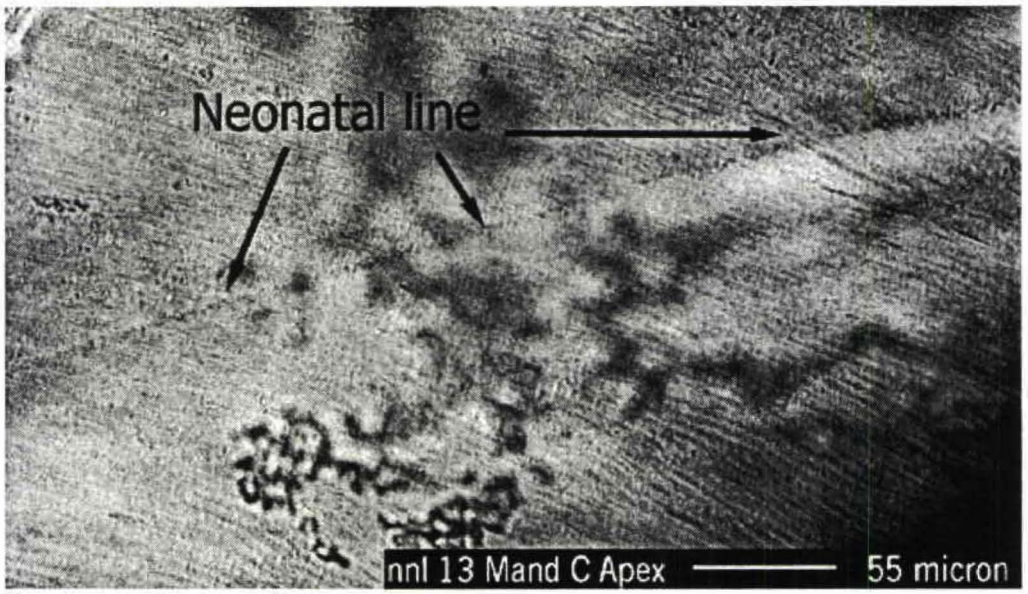


Figure 4-17. Digital image (400x magnification) of the neonatal line at the apex on the lingual aspect of the enamel crown of a mandibular canine. Note how thin the line is when compared with that in Figure 4-6a,b taken at the DEJ of the same specimen. The mean width of the neonatal line is 5.5 microns.

Chapter Five

Results

Repeatability Test

The results of a paired t-test comparing the mean of the original (1st set) and repeated measurements (2nd set) showed no significant difference at $p < 0.05$ level (Table 5-1).

Table 5-1. Paired t-test results for mean neonatal line width (μm) at time of first measurement and at time of second measurement, one week later.

NNL width	Mean	S.D.	N (Pairs)	Corr. (r)	Sig. (P)	Mean diff.	S.D. diff.	S.E diff.	t-value	Sig. (P)
1st set	9.129	2.972	52	0.941	0.0001	0.1058	1.007	0.1397	0.758	n.s. p = 0.452
2nd set	9.023	2.827								

Descriptive Statistics (tooth classes combined)

A summary of the descriptive statistics collected for neonatal line width at each crown position for the entire sample of prism measurements can be found in Table 5-2. The results of a Kruskal-Wallis rank test showed that neonatal line width differed significantly between each crown position ($H = 7.36$, $p = 0.025$), with mean neonatal line width at the middle of the crown significantly greater than that at the DEJ ($p = 0.0001$), but not significantly greater than at the apex ($p = 0.749$) (Figure 5-1).

Descriptive statistics of the neonatal line widths at each crown position for individuals and by type of delivery can be found in Tables 1 – 4 in Appendix B. Descriptive statistics of neonatal line width for the entire sample of prism measurements at each crown position by delivery type can be found in Table 5-3. Descriptive statistics of the neonatal line widths for individuals at each crown position collected for child's health at birth, medical intervention(s) at birth and maternal complication(s) at birth can be found in Tables 5 and 6 in Appendix B. Descriptive statistics of

Table 5-2. Descriptive statistics of neonatal line width (μm) at each crown position.

Crown Position	Mean	Median	S.D.	Min - Max	Range	N[1]
DEJ (N = 42)[2]	7.6	7.2	2.5	3.0 - 17.0	14.0	425
Middle (N = 43)[2]	8.0	7.6	2.7	3.4 - 16.6	13.2	456
Apex (N = 41)[2]	7.8	7.6	2.1	3.9 - 17.0	13.1	432

1 Total number of prisms measured at each crown position
2 Number of individuals

neonatal line widths at each crown position for all birth variables are presented in Table 7, Appendix B.

Mean neonatal line widths for individuals range from 4.2 to 13.6 μm at the DEJ, from 4.5 to 13.8 μm at the middle of the crown and from 4.5 to 12.7 μm at the apex. For individuals, the standard deviation ranges from 0.5 to 2.7μm depending on crown position; the ranges of neonatal line width (i.e. highest – lowest width measurement) are from 1.7 to 8.1 μm at the DEJ, 1.4 to 7.9 μm at the middle of the crown and 1.5 to 7.3 μm at the apex (Tables 1 – 4, Appendix B).

Mean neonatal line width for type of delivery and all other birth categories ranges from 6.8 μm to 13.8 μm, depending on crown position and birth variable, with the majority of mean neonatal line widths falling between 7 μm and 9μm (Table 5-3 and Table 7, Appendix B). For the entire sample of measurements, the standard deviation ranges from 2.1 to 2.7 μm (Table 5-2). For type of delivery, the standard deviation ranges from 1.5 to 3.2 μm depending on crown position and delivery type (Table 5-3). For the remainder of birth variables, the standard deviation for the measurements ranges from 1.6 to 3.3 μm depending on crown position and birth category (Table 7, Appendix B).

For each birth variable at each crown position, the ranges of neonatal line width are quite large. For delivery types, neonatal line width ranges from 7 μm to 13.5 μm, depending on crown position and delivery type (Table 5-3). For all other birth variables, ranges of neonatal line width fall between 4 μm and 14 μm, again depending on crown position and birth variable (Table 7, Appendix B).

Because of the large ranges in neonatal line width at each crown position for all birth variables, there is an overlap in neonatal line width between each birth category at all crown positions (Table 5-3, Table 7 Appendix B, and Figures 5-2 to 5-7).

The distribution of neonatal line width measurements at each crown position for the sample as a whole and for the majority of birth variables is skewed. Consequently, the median value for neonatal line width is generally lower than the mean width at each crown position; there are, however, a few exceptions (Tables 5-2 and 5-3 and Table 7, Appendix B).

Tests of Significance (tooth classes combined)

Results of a Kruskal-Wallis rank test demonstrate that neonatal line width differs significantly between crown positions for those children from a natural delivery ($H = 13.29$, $p = 0.001$), a complicated delivery ($H = 6.02$, $p = 0.049$) and an elective c-section delivery ($H = 23.26$, $p = 0.0001$). There was no significant difference in neonatal line width between crown positions for deliveries with minimal medical intervention ($H = 2.02$, $p = 0.364$). Because of these significant differences, tests of significance between neonatal line width by delivery type were conducted by crown position.

The results of a Kruskal-Wallis rank test demonstrate that neonatal line width at the DEJ does not differ significantly between delivery types. Neonatal line width at both the middle crown position and at the apex does differ significantly between delivery types (Table 5-4). Results of a Mann-Whitney U rank test demonstrate that neonatal line width at the middle of the crown is significantly greater for an elective c-section delivery than it is for a natural delivery ($p = 0.0001$),

Table 5-3. Descriptive statistics of neonatal line width (μm) at each crown position for type of delivery.

Delivery type/ Position	Mean	Median	S.D.	Min - Max	Range	N[1]
Natural (n= 10)[2]						
DEJ	7.7	6.6	3.2	3.5 - 17.0	13.5	92
Middle	7.7	6.9	2.7	3.6 - 13.9	10.3	108
Apex	8.4	8.2	1.8	4.9 - 14.2	9.3	100
Minimal medical Intervention (n = 13)[2]						
DEJ	7.4	7.4	2.4	3.0 - 12.9	9.9	133
Middle	7.7	7.5	2.1	4.1 - 13.6	9.5	142
Apex	7.2	7.4	1.5	3.9 - 11.1	7.2	102
Complicated (n = 11)[2]						
DEJ	8.3	8.0	2.7	4.2 - 16.2	12.0	84
Middle	8.0	6.8	3.2	3.8 - 16.6	12.8	94
Apex	7.3	6.9	2.2	4.3 - 17.0	12.7	108
Elective c-section (n = 4)[2]						
DEJ	7.2	7.0	1.6	4.4 - 11.4	7.0	40
Middle	9.9	10.1	2.2	5.9 - 15.3	9.4	34
Apex	8.6	7.7	3.0	5.0 - 15.9	10.9	42

1 Total number of prisms measured at each crown position
2 Number of individuals

a delivery with minimal medical intervention (p = 0.0001) or a complicated delivery (p = 0.0001) (Table 5-3 and 5-4). Neonatal line width between a natural delivery and an elective c-section delivery is not significantly different at the apex (p = 0.546); however, neonatal line width for both of these delivery types is significantly greater than it is for a delivery with minimal medical intervention (p = 0.0001, p = 0.033 respectively) or a complicated delivery (p = 0.0001, p = 0.012 respectively) (Tables 5-3 and 5-4) (Figure 5-2).

Table 5-4. Results of a Kruskal-Wallis test for neonatal line width (μm) by type of delivery for each crown position.

Position/ Delivery type	N[1]	Mean Rank	*H* statistic	Sig. (p)
DEJ				
Natural	92	162.97	7.01	n.s.
Min. intervention	133	170.55		P = 0.072
Complicated	84	199.81		
Elective C-section	40	165.39		
Middle				
Natural	108	174.94	22.52	0.0001
Min. intervention	142	185.42		
Complicated	94	182.12		
Elective C-section	34	**273.24**		
Apex				
Natural	100	**215.98**	28.76	0.0001
Min. intervention	102	158.55		
Complicated	108	148.07		
Elective C-section	42	**199.19**		

1 Total number of prisms measured at each crown position

Kruskal-Wallis rank tests confirmed that neonatal line width differs significantly between crown positions for the majority of the remaining birth categories, the only exceptions being neonatal line width for children born with health problems and for children born jaundiced. Because of these significant differences, tests of significance between neonatal line width for each birth variable were conducted by crown position.

The results of an independent samples t-test between log transformed neonatal line width by "health at birth" at the DEJ and at the apex can be found in Table 5-5a. The results of a Mann-Whitney U rank test between neonatal line width for the two categories of "health at birth" at the middle crown position are presented in Table 5-5b. A Mann-Whitney U test was used because

the data could not be normalized (pages 76 – 79). All three tests demonstrate that neonatal line width is significantly greater at each crown position in children with health complication(s) at birth (DEJ: 8.2 +/- 2.3μm; middle: 8.5 +/- 2.9μm; apex: 8.1 +/- 2.4μm) than in children without such complication(s) (DEJ: 6.8 +/- 2.6μm; middle: 7.4 +/- 2.2μm; apex: 7.4 +/- 1.6μm (Figure 5-3).

Table 5-5a. Independent samples t-test results for log transformed neonatal line width by "health at birth" at the DEJ and at the apex.

				Levene's Test			
Position/Health	Mean	S.D.	N[1]	F	Sig. (p)	t-value	Sig. (p)
DEJ							
Healthy	0.806	0.1506	199	4.409	0.036	-6.806[2]	0.0001
Not Healthy	**0.898**	0.1244	226				
Apex							
Healthy	0.859	9.6E-02	180	11.388	0.001	-3.214[2]	0.001
Not Healthy	**0.892**	0.12	252				

1 Total number of prisms measured at each crown position
2 Unequal variance test

Table 5-5b. Results of a Mann-Whitney U Test for neonatal line width by "health at birth" at the middle of the crown.

Position/ Health	N[1]	Mean Rank	Sum of Ranks	*U* Statistic	z-score	Sig. (p)[2]
Middle						
Healthy	205	201.11	41228.5	20113.5	-4.011	0.0001
Not Healthy	251	**250.87**	62967.5			

1Total number of prisms measured at each crown position
2 Not corrected for ties

The results of an independent samples t-test for log transformed neonatal line width by "medical intervention(s) at birth" at the DEJ can be found in Table 5-6a. The results of Mann-

Whitney U rank tests between neonatal line width for the two categories of "medical intervention(s) at birth" at the middle crown position and at the apex are given in Table 5-6b. Neonatal line width is significantly greater at the DEJ (yes: 8.2 +/- 2.5µm vs. no: 7.3 +/- 2.5µm) and at the middle crown positions (yes: 9.1 +/- 3.3µm vs. no: 7.7 +/- 2.3µm) in children who experienced medical intervention(s) at birth. In contrast, neonatal line width is significantly greater at the apex in children who did not experience medical intervention(s) at birth (no: 7.9 +/- 2.0µm vs. yes: 7.5 +/-2.2µm) (Figure 5-4).

Table 5-6a. Independent samples t-test results for log transformed neonatal line width by "medical intervention at birth" at the DEJ.

Position/				Levene's Test			
Medical intervention	Mean	S.D.	N[1]	F	Sig. (p)	t-value	Sig. (p)
DEJ							
Yes	**0.896**	0.1304	114	2.362	0.1252	3.558[2]	0.0001
No	0.840	0.1469	311				

1 Total number of prisms measured at each crown position
2 Equal variance test

Table 5-6b. Results of Mann-Whitney U Tests for neonatal line width by "medical intervention at birth" at the middle of the crown and at the apex.

Position/		Mean	Sum of	*U*		
Medical intervention	N[1]	Rank	Ranks	statistic	z-score	Sig. (p)[2]
Middle						
Yes	112	**271.88**	30450.5	14405.5	-4.011	0.0001
No	344	214.38	73745.5			
Apex						
Yes	136	192.92	26237.5	16921.5	-2.661	0.008
No	296	**227.33**	67290.5			

1 Total number of prisms measured at each crown position
2 Not corrected for ties

Table 5-7a gives the results of an independent samples t-test for log transformed neonatal line width by "maternal health at birth" at the DEJ and at the apex. The results of a Mann-Whitney U rank test between neonatal line width by "maternal health at birth" at the middle crown position can be found in Table 5-7b. These tests demonstrate that neonatal line width is significantly greater at both the DEJ (yes: 8.1 +/- 2.6μm; no: 7.4 +/- 2.5μm) and at the middle crown position (yes: 8.8 +/- 2.7μm; no: 7.7 +/- 2.6μm) in children whose mother experienced complication(s) at birth (Figure 5-5). Neonatal line width is not significantly different between these two categories when compared at the apex (yes: 7.7 +/- 2.1μm; no: 7.9 +/- 2.1μm).

Table 5-7a. Independent samples t-test results for log transformed neonatal line width by "maternal health at birth" at the DEJ and at the apex.

Position/				Levene's Test			
Maternal Problems	Mean	S.D.	N[1]	F	Sig. (p)	t-value	Sig. (p)
DEJ							
Yes	**0.885**	0.1379	122	0.001	0.972	2.756[2]	0.006
No	0.843	0.1457	303				
Apex							
Yes	0.8740	0.1072	138	0.466	0.495	-0.558[2]	n.s.
No	0.8810	0.1140	294				P = 0.577

1 Total number of prisms measured at each crown position
2 Equal variance test

Table 5-7b. Results of a Mann-Whitney U Test for neonatal line width by "maternal health at birth" at the middle of the crown.

Position/		Mean	Sum of	*U*		
Maternal problems	N[1]	Rank	Ranks	statistic	z-score	Sig. (p)[2]
Middle						
Yes	120	**267.88**	32145.5	15434.5	3.814	0.0001
No	336	214.44	72050.5			

1 Total number of prisms measured at each crown position
2 Not corrected for ties

The results of an independent samples t-test for log transformed neonatal line width by "jaundiced at birth" at the DEJ are provided in Table 5-8a. The results of Mann-Whitney U rank tests between neonatal line width at the middle of the crown and at the apex in children born jaundiced and children without jaundice are presented in Table 5-8b. All tests demonstrate that neonatal line width is not significantly different between these two groups of children when compared at each crown position (DEJ: yes: 7.5 +/- 2.6μm; no: 7.6 +/- 2.5μm; middle: yes: 7.7 +/- 2.1μm; no: 8.1 +/- 2.8μm; apex: yes: 7.7 +/- 1.8μm; no: 7.9 +/- 2.2μm) (Figure 5-6).

Table 5-8a. Independent samples t-test results for log transformed neonatal line width by "jaundiced at birth" at the DEJ.

Position/ Jaundiced	Mean	S.D.	N[1]	Levene's Test F	Levene's Test Sig. (p)	t-value	Sig. (p)
DEJ							
Yes	0.8520	0.1465	114	0.270	0.604	-0.255[2]	n.s.
No	0.8560	0.1442	311				p = 0.799

1 Total number of prisms measured at each crown position
2 Equal variance test

Table 5-8b. Results of Mann-Whitney U Test for neonatal line width by "jaundiced at birth" at the middle of the crown and at the apex.

Position/ Jaundiced	N[1]	Mean Rank	Sum of Ranks	*U* statistic	z-score	Sig. (p)[2]
Middle						
Yes	120	217.06	26047.5	18787.5	-1.108	n.s.
No	336	232.58	78148.5			P = 0.268
Apex						
Yes	132	213.70	28208.5	19430.5	-0.309	n.s.
No	300	217.73	65319.5			P = 0.757

1 Total number of prisms measured at each crown position
2 Not corrected for ties

Table 5-9a gives the results of an independent samples t-test for log transformed neonatal line width by "term at birth" at the DEJ. The results of Mann-Whitney U rank tests for neonatal line width at the middle crown position and at the apex by "term at birth" can be found in Table 5-9b. Neonatal line width is significantly greater at the DEJ and at the middle crown position in children born pre-term (DEJ: 9.4+/- 3.1μm; middle: 9.0 +/- 2.8μm) than in those born at term (DEJ: 7.3 +/- 2.3μm; middle: 7.8 +/- 2.5μm). In contrast, line width is significantly greater at the apex in children born at term, than in those born before term (preterm: 7.1 +/- 1.8μm; term: 7.8 +/- 2.0μm) (Figure 5-7).

Table 5-9a. Independent samples t-test results for log transformed neonatal line width by "term at birth" at the DEJ.

Position/ Term at birth	Mean	S.D.	N[1]	Levene's Test F	Levene's Test Sig. (p)	t-value	Sig. (p)
DEJ							
Pre-Term	**0.949**	0.1418	38	0.009	0.926	4.608[2]	0.0001
Term	0.840	0.1385	377				

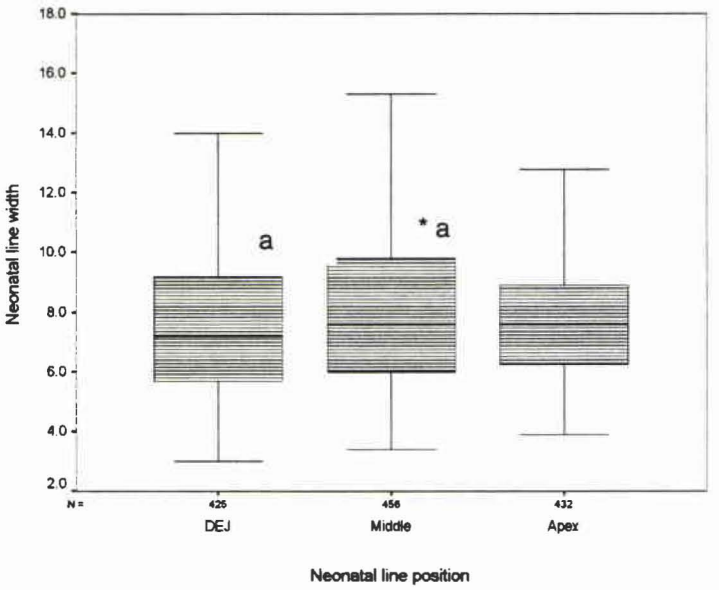
1 Total number of prisms measured at each crown position
2 Equal variance test

Table 5-9b. Results of Mann-Whitney U Tests for neonatal line width by "term at birth" at the middle of the crown and at the apex.

Position/ Term at birth	N[1]	Mean Rank	Sum of Ranks	*U* statistic	z-score	Sig. (p)[2]
Middle						
Pre-Term	40	**276.80**	11072.0	6068.0	-2.677	0.007
Term	408	219.37	89504.0			
Apex						
Pre-Term	42	165.67	6958.0	6055.0	-2.562	0.01
Term	380	**216.57**	82295.0			

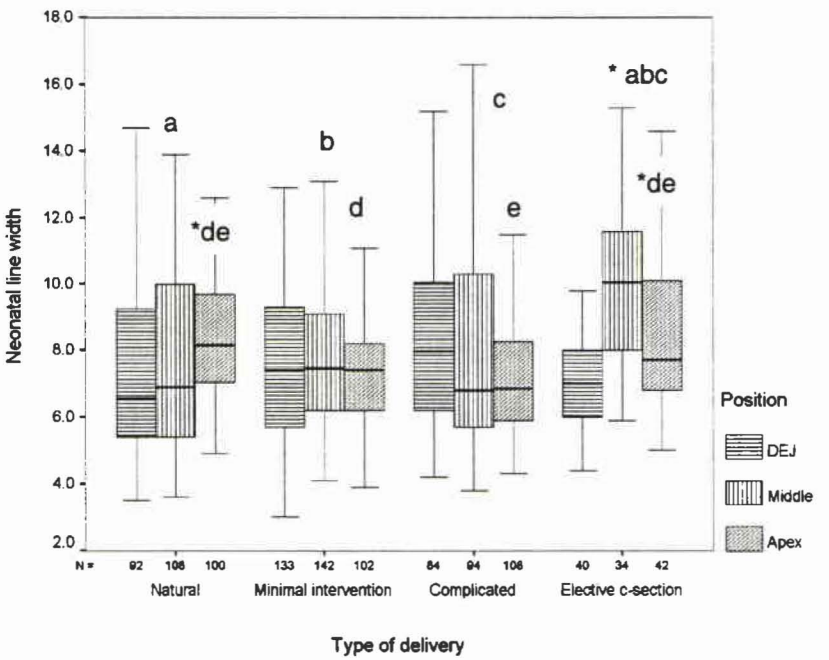
1 Total number of prisms measured at each crown position
2 Not corrected for ties

Figure 5-1. Neonatal line width (µm) at each crown position.



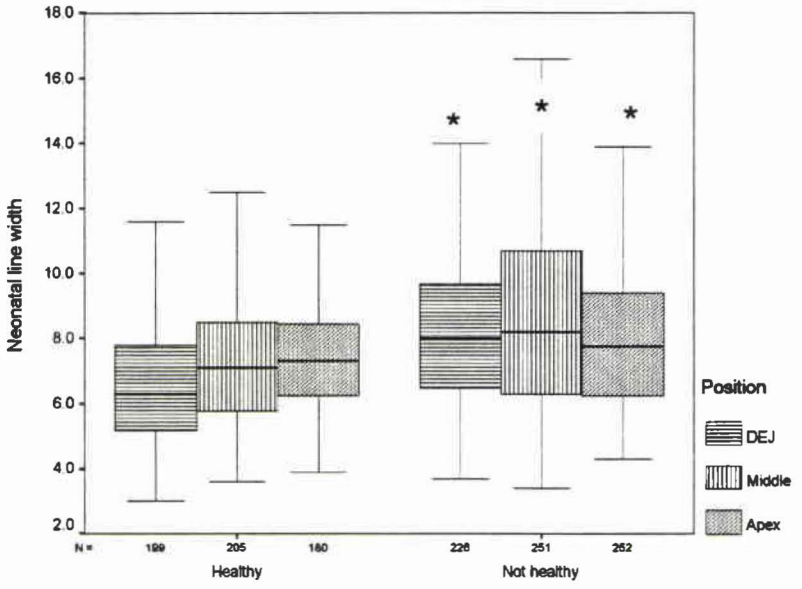
Note: Mean neonatal line width at the middle crown position (*a) is significantly wider than it is at the DEJ (a) (p = 0.0001, Mann-Whitney U Test).

Figure 5-2. Neonatal line width (µm) at each crown position by type of delivery.



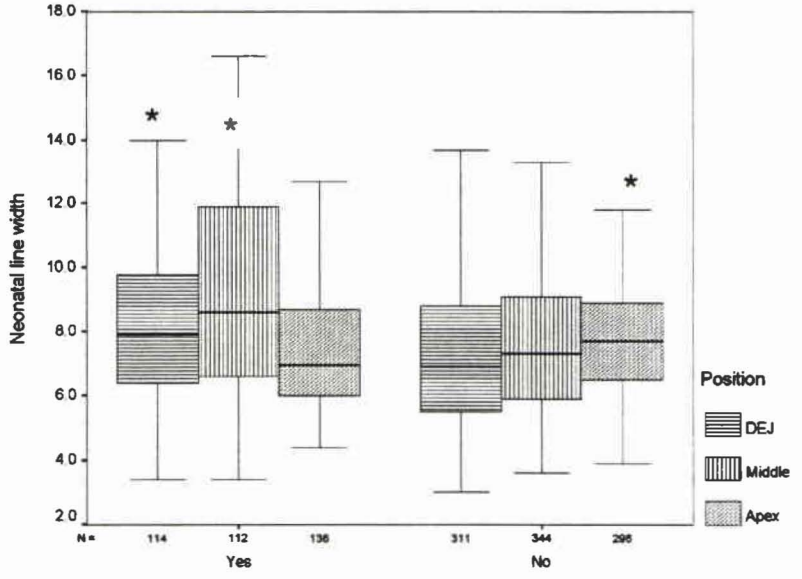
Note (*abc): Mean neonatal line width at the middle crown position for an elective c-section delivery is significantly greater than line width for a natural delivery (a), a delivery with minimal medical intervention (b) or a complicated delivery (c) at the middle crown position.
Note (*de): Mean neonatal line width at the apex for a natural delivery and for an elective c-section delivery is significantly greater than line width for a delivery with minimal medical intervention (d) or a complicated delivery (e) at the apex.

Figure 5-3. Neonatal line width (µm) at each crown position by "health of child" at birth.



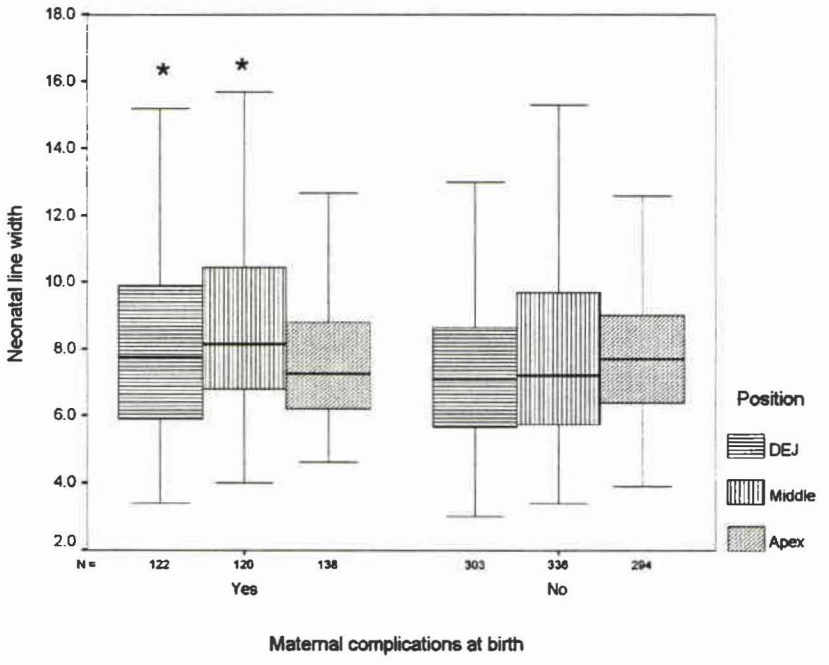
Note (*): Mean neonatal line width is significantly greater for "not healthy at birth" than for "healthy at birth" at each crown position.

Figure 5-4. Neonatal line width (µm) at each crown position by "medical intervention".



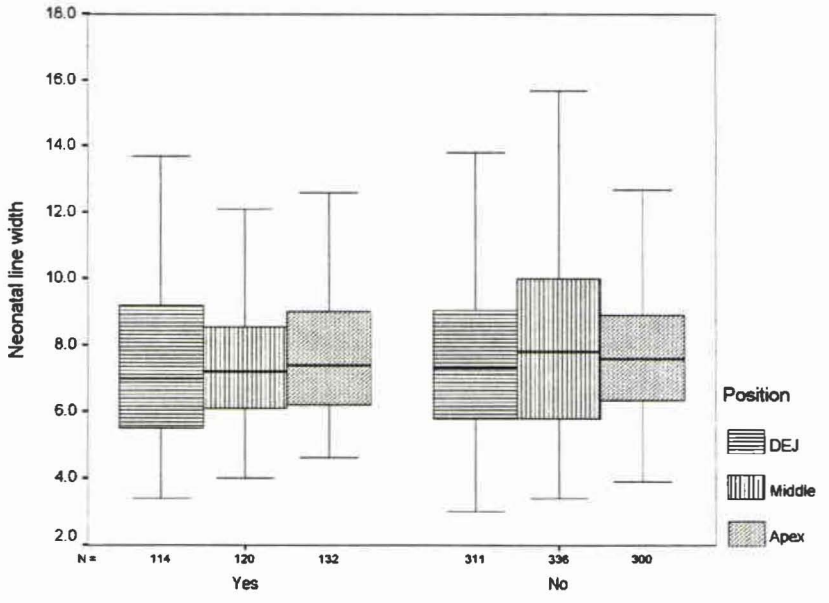
Note (*): Mean neonatal line width is significantly greater at the DEJ and at the middle crown position for children undergoing medical intervention and significantly greater at the apex for those not undergoing medical intervention.

Figure 5-5. Neonatal line width (µm) at each crown position by "maternal complications" at birth.



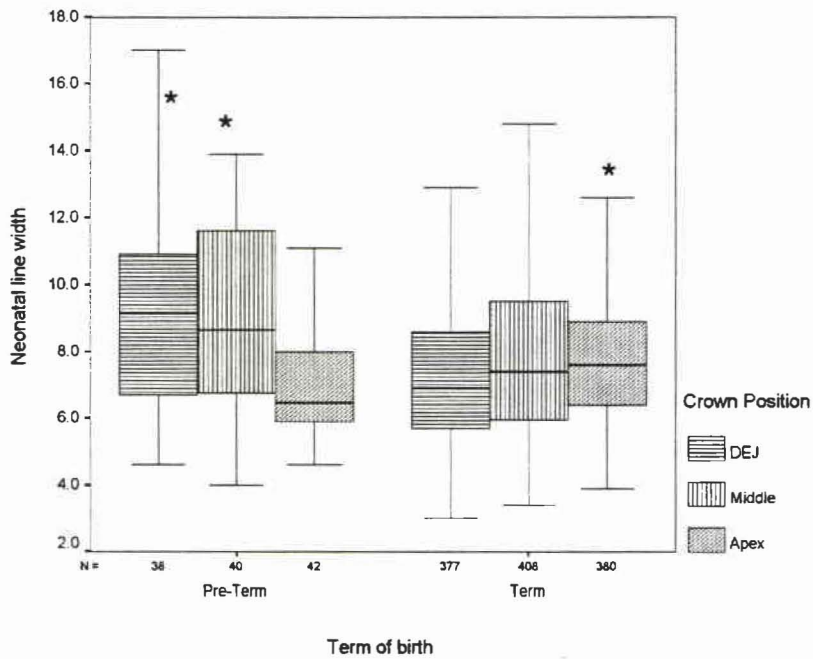
Note (*): Mean neonatal line width is significantly greater at the DEJ and at the middle crown positions for maternal complications at birth.

Figure 5-6. Neonatal line width (µm) at each crown position by "jaundiced" at birth.



Note: Mean neonatal line width is not significantly different between children born jaundiced and those not born jaundiced at any crown position.

Figure 5-7. Neonatal line width (μm) at each crown position by "term at birth".



Note (*): Mean neonatal line width is significantly greater at the DEJ and at the middle of the crown for a pre-term delivery and significantly greater at the apex for a term delivery.

Differences in Neonatal Line Width Between Crown Positions Within Tooth Classes and Between Tooth Classes

A summary of the descriptive statistics for neonatal line width for each crown position by tooth class for the entire sample is presented in Table 5-10. The standard deviations of the measurements range from 1.7 to 3.3 μm, with molars exhibiting larger standard deviations than incisors or canines. The ranges of neonatal line width are between 6.9 and 13.8 μm, with molars having larger ranges overall. With the exception of neonatal line width at the apex in incisors, the median value is similar to or smaller than the mean at each crown position.

Results of a Kruskal-Wallis rank test demonstrate that neonatal line width does not differ significantly between crown positions for incisors ($H = 1.31$, $p = 0.52$) or canines ($H = 0.96$, $p = 0.62$), but it does differ significantly between crown positions for molars ($H = 12.44$, $p = 0.002$). Because of the difference in neonatal line width between crown positions in molars, a Kruskal – Wallis rank test comparing neonatal line width between tooth classes for the entire sample was

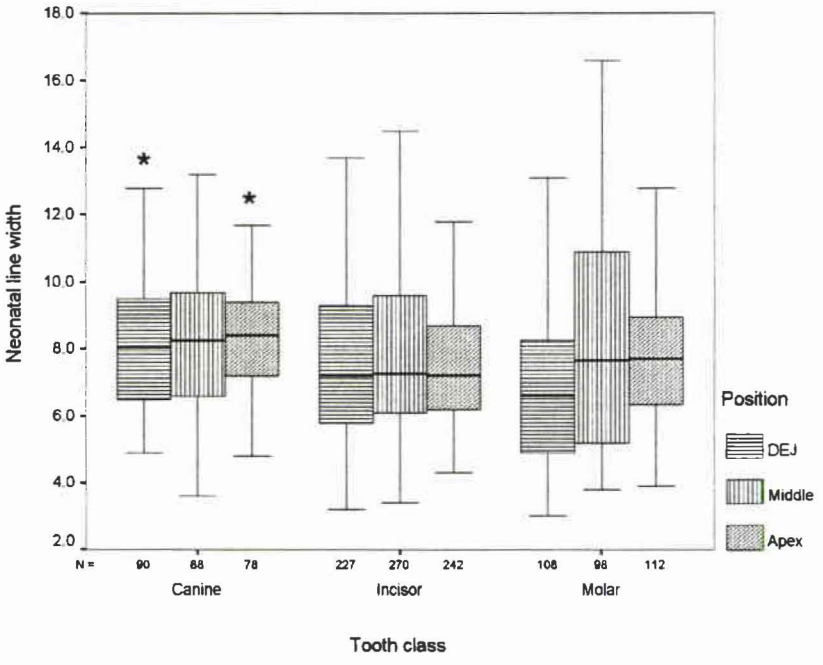
conducted by crown position. The results of this test show that line width differs significantly between all tooth classes at the DEJ ($H = 14.95$, $p = 0.001$) and at the apex ($H = 14.18$, $p = 0.001$), but not at the middle crown position ($H = 2.62$, $p = 0.269$). Results of a Mann-Whitney U rank test established that neonatal line width at the DEJ is significantly greater for canines than it is for incisors ($p = 0.03$) or for molars ($p = 0.0001$). A Mann-Whitney U rank test comparing neonatal line width between tooth classes at the apex showed that neonatal line width is again significantly greater for canines than it is for incisors ($p = 0.0001$) or for molars ($p = 0.028$) (Table 5-10 and Figure 5-8).

Table 5-10. Descriptive statistics of neonatal line width (μm) by tooth class for each crown position.

Tooth/ Position	Mean	Median	S.D.	Min - Max	Range	N[1]
Incisors (n = 26)						
DEJ	7.6	7.2	2.6	3.2 - 17.0	13.8	227
Middle	7.8	7.3	2.4	3.4 - 15.3	11.9	270
Apex	7.6	8.4	1.7	4.8 - 11.7	6.9	242
Canines (n = 9)						
DEJ	8.2	8.1	2.2	4.9 - 16.2	11.3	90
Middle	8.3	8.3	2.5	3.6 - 14.7	11.1	88
Apex	8.3	8.4	1.7	4.8 - 11.7	6.9	78
Molars (n = 10)						
DEJ	6.9	6.6	2.5	3.0 - 14.0	11.0	108
Middle	8.3	7.7	3.3	3.8 - 16.6	12.8	98
Apex	7.9	7.7	2.3	3.9 - 17.0	13.1	112

1 Total number of prisms measured at each crown position

Figure 5-8. Neonatal line width (μm) at each crown position by tooth class.



Note: Mean neonatal line width differs significantly between crown positions in molars.
Note (*): Mean neonatal line width is significantly greater in canines at the DEJ and at the apex.

Descriptive statistics of neonatal line width at each crown position by tooth class for each delivery type can be found in Table 8, Appendix B. Results of a Kruskal-Wallis rank test demonstrate that neonatal line width differs significantly between crown positions for 1) canines ($H = 16.08$, $p = 0.0001$) and molars ($H = 21.17$, $p = 0.0001$) from a natural delivery, 2) incisors ($H = 7.01$, $p = 0.03$) and canines ($H = 30.60$, $p = 0.0001$) from a complicated delivery, and 3) incisors ($H = 23.05$, $p = 0.0001$) from an elective c-section delivery.

Because of these significant differences in neonatal line width between crown positions for tooth classes, a Kruskal-Wallis rank test comparing neonatal line width between tooth classes from a natural delivery and from a complicated delivery was conducted by crown position. No significant difference was found between crown positions for a delivery with minimal medical intervention; consequently, the measurements at each crown position for each tooth class were averaged to yield a mean neonatal line width for that tooth class. This new mean was subsequently used during a Kruskal-Wallis rank test comparing neonatal line width between tooth

classes for a delivery with minimal intervention. A Mann-Whitney U rank test was employed to compare neonatal line width between incisors and molars at each crown position for an elective c-section delivery.

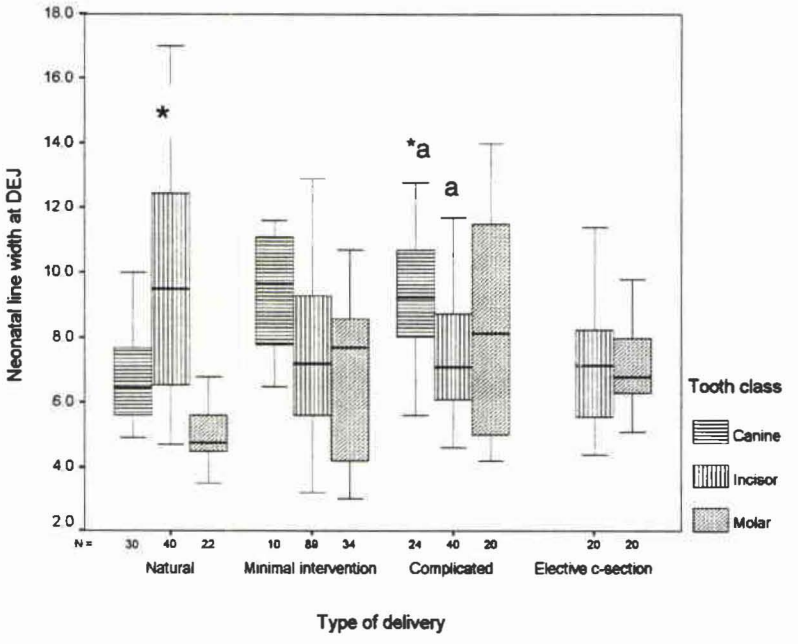
Results of a Kruskal-Wallis rank test demonstrate the following: 1) neonatal line width is significantly different between tooth classes at each crown position for a natural delivery and a complicated delivery (Figures 5-9 to 5-11); and 2) neonatal line width is significantly different between tooth classes for a delivery with minimal medical intervention, with canines and molars having a wider line than incisors ($p = 0.001$, $p = 0.014$ respectively). Results of a Mann-Whitney U rank test at each crown position for an elective c-section delivery show that neonatal line width at the DEJ is not significantly different between incisors and molars ($p = 0.685$, corrected for ties) (Figure 5-9). However, neonatal line width is significantly greater in incisors at both the middle of the crown ($p = 0.0001$, corrected for ties) and at the apex ($p = 0.001$, not corrected for ties) for an elective c-section delivery (Figures 5-10 and 5-11).

Descriptive statistics of neonatal line width at each crown position by tooth class for the remaining birth variables can be found in Table 9, Appendix B. Kruskal-Wallis rank tests confirmed that neonatal line width differs significantly between crown positions for tooth classes for the majority of birth variables. Because of these significant differences, Kruskal-Wallis rank tests comparing neonatal line width between tooth classes were conducted by crown position.

A Kruskal-Wallis rank test shows the following: 1) line width differs significantly between tooth classes at the DEJ ($H = 8.19$, $p = 0.017$) and at the apex ($H = 16.1$, $p = 0.0001$) for children born healthy, while it differs significantly between tooth classes at the middle of the crown ($H = 11.77$, $p = 0.003$) and at the apex ($H = 33.52$; $p = 0.0001$) for children not born healthy (Figures 5-12 to 5-14); 2) neonatal line width differs significantly between all tooth classes at each crown position for both categories of "medical intervention(s) at birth" (all $p = 0.0001$) (Figures 5-15 to 5-17); 3) neonatal line width differs significantly between tooth classes at each crown position for children whose mother experienced complications at birth (DEJ $H = 44.62$, Middle $H = 42.11$; Apex, $H = 37.15$; all $p = 0.0001$), and at the DEJ ($H = 20.38$, $p = 0.0001$) and at the apex ($H =$

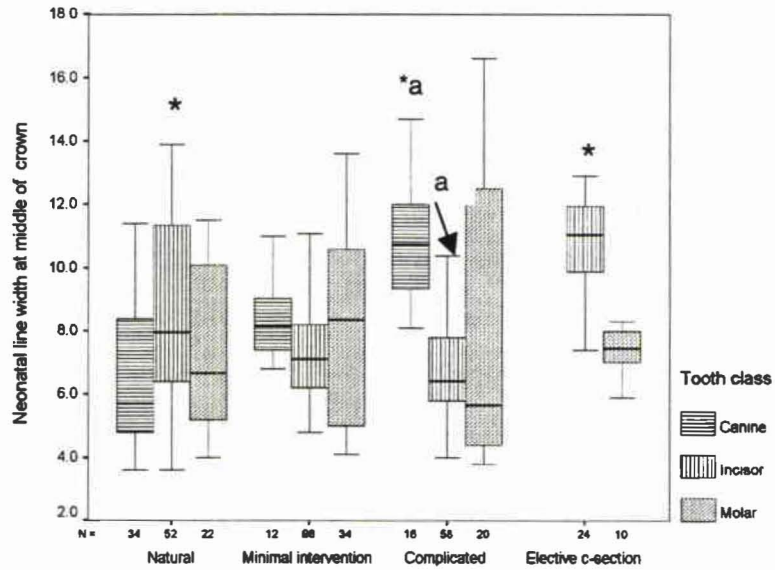
16.50, p = 0.0001) for children whose mother did not (Figures 5-18 to 5-20); and 4) neonatal line width differs significantly between tooth classes at each crown position for children born jaundiced (DEJ: H = 8.29, p = 0.016; Middle: H = 17.58, p = 0.0001; Apex: H = 12.26, p = 0.002), and at the DEJ (H = 26.68, p = 0.0001) and at the apex (H = 8.14, p = 0.017) in those children not born jaundiced (Figures 5-21 to 5-23).

Figure 5-9. Neonatal line width (µm) at the DEJ by tooth class for type of delivery.



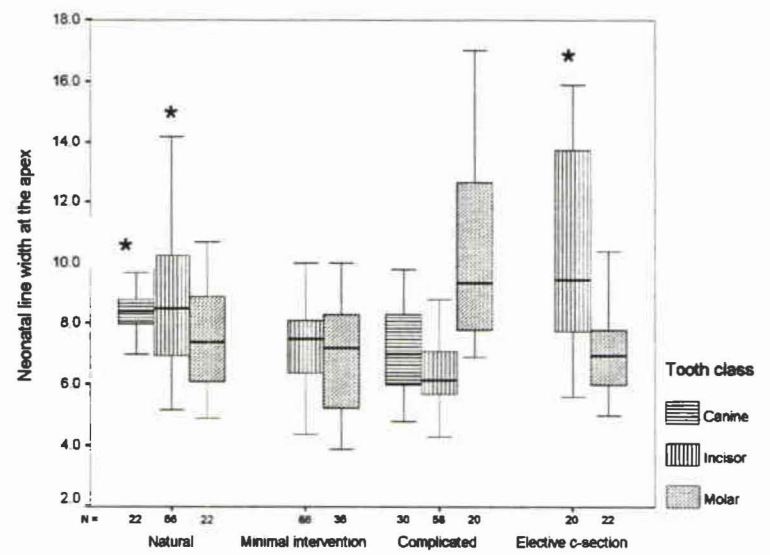
Note (*) Mean neonatal line width is significantly larger for incisors in a natural delivery.
Note (*a): Mean neonatal line width is significantly greater for canines than for incisors (a) for a complicated delivery.

Figure 5-10. Neonatal line width (µm) at the middle crown by tooth class for type of delivery.



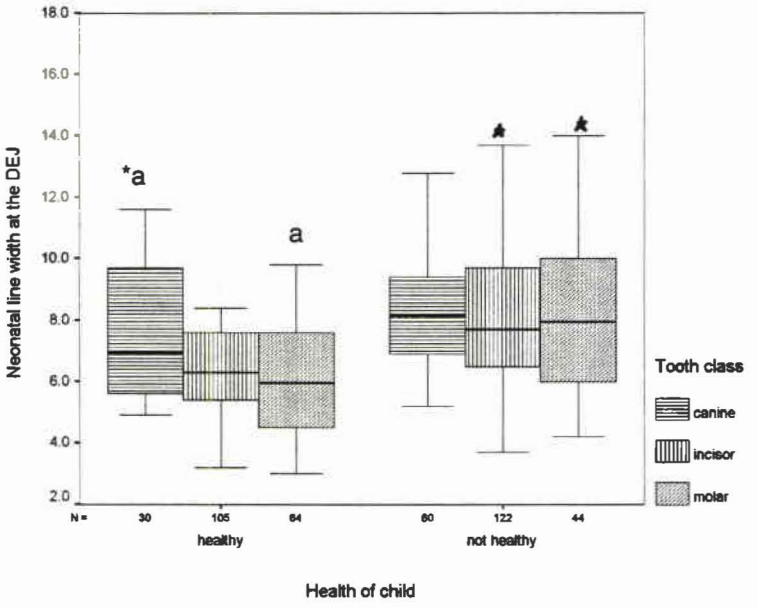
Note (*): Mean neonatal line width is significantly larger for incisors in a natural delivery and in an elective c-section.
Note (*a): Mean neonatal line width is significantly greater for canines than for incisors (a) for a complicated delivery.

Figure 5-11. Neonatal line width (µm) at the apex by tooth class for type of delivery.



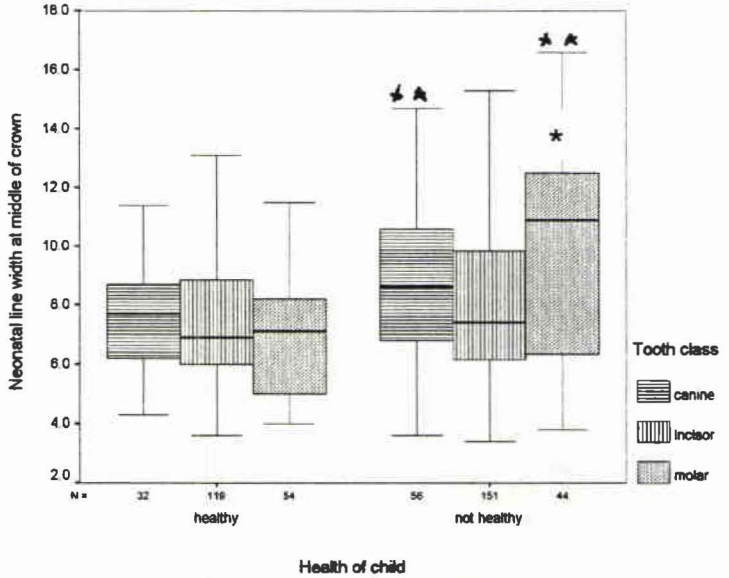
Note: Mean neonatal line width is significantly different between tooth classes for a natural delivery, a complicated delivery and an elective c-section.
Note (*): Mean neonatal line width is significantly larger for incisors and for canines in a natural delivery and for incisors in an elective c-section.

Figure 5-12. Neonatal line width (µm) at the DEJ by tooth class for "health at birth".



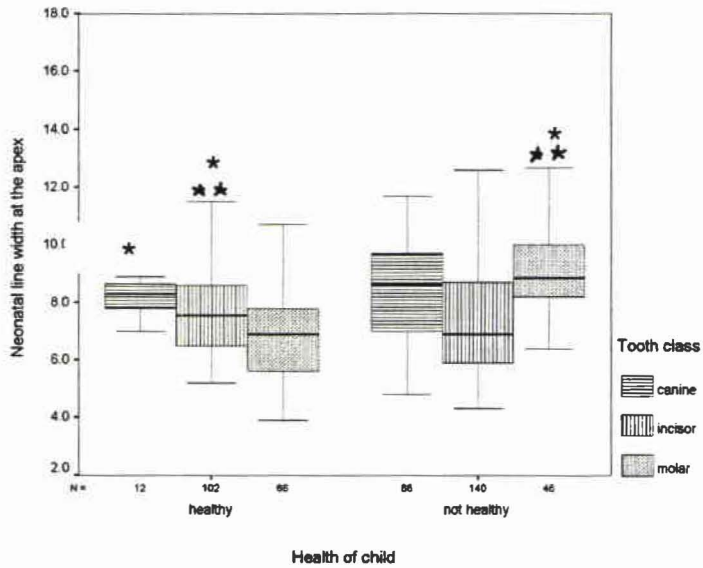
Note (*a): Mean neonatal line width is significantly greater in canines than in molars in healthy children.
Note (*): Mean neonatal line width is significantly larger for "not healthy at birth" when measured in incisors or molars.

Figure 5-13. Neonatal line width (µm) at the middle crown by tooth class for "health at birth".



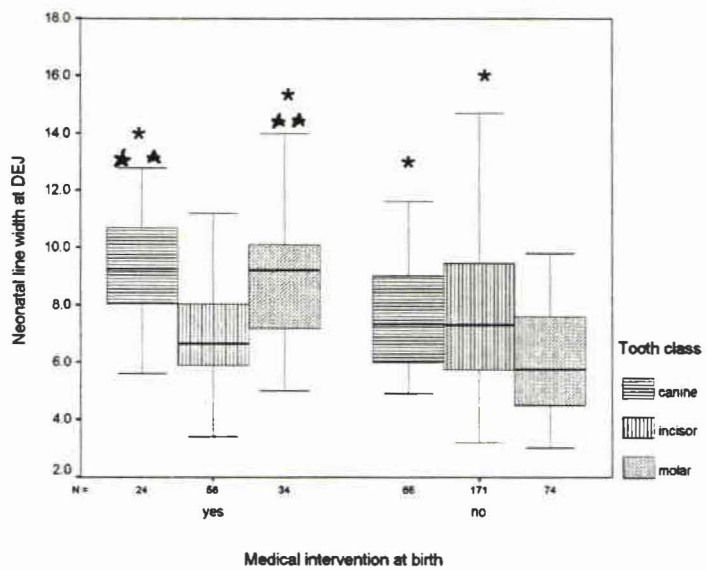
Note (*): Mean neonatal line width is significantly greater in molars than in canines or incisors for "not healthy at birth".
Note (**): Mean neonatal line width is significantly larger for "not healthy at birth" when measured in canines or in molars.

Figure 5-14. Neonatal line width (µm) at the apex by tooth class for "health at birth".



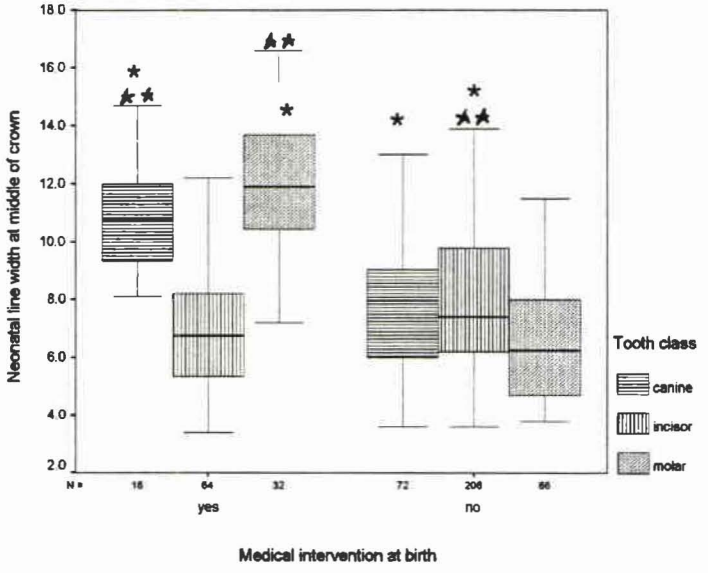
Note (*): Mean neonatal line width is significantly larger for canines and incisors for "healthy at birth" (canine results are tentative due to small sample size) and for molars for "not healthy at birth".
Note (**): Mean neonatal line width is significantly larger for not "healthy at birth" when measured in molars and significantly larger for "healthy at birth" when measured in incisors.

Figure 5-15. Neonatal line width (µm) at DEJ by tooth class for "medical intervention at birth".



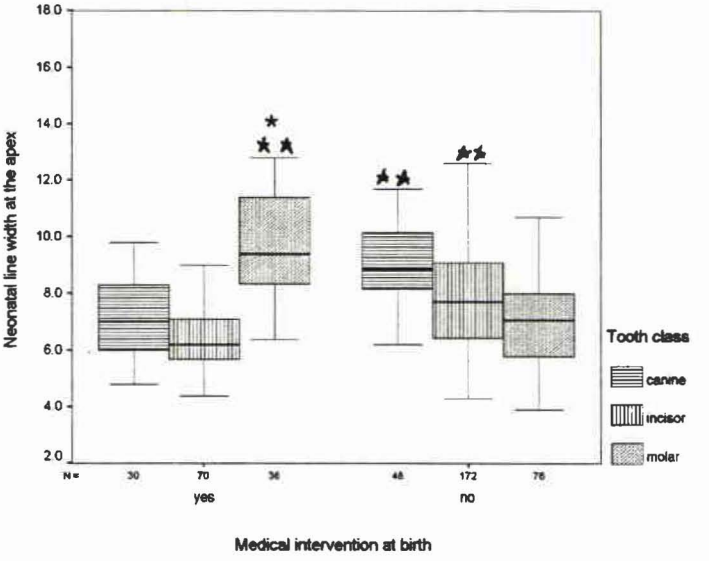
Note (*): Mean neonatal line width is significantly larger for canines and for molars for "medical intervention at birth" and significantly larger for canines and for incisors for "no medical intervention at birth."
Note (**): Mean neonatal line width is significantly greater for "yes" category when measured in canines or in molars.

Figure 5-16. Neonatal line width (µm) at the middle crown by "medical intervention at birth".



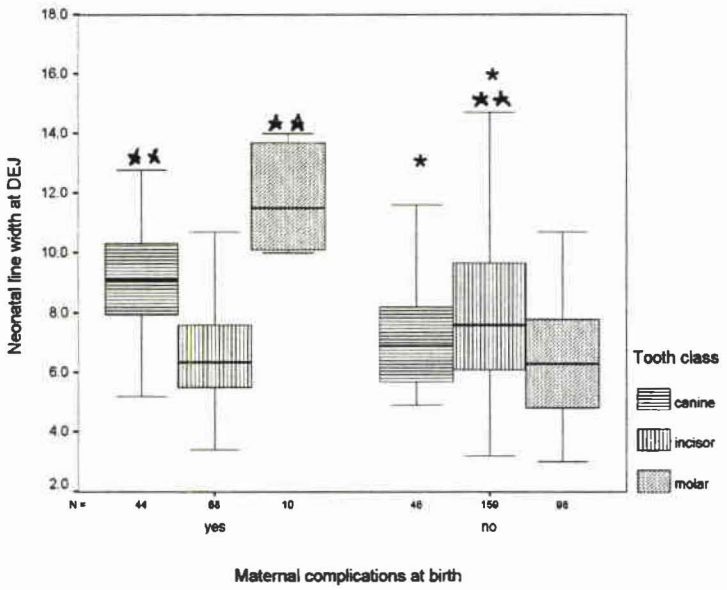
Note (*): Mean neonatal line width is significantly larger for canines and for molars for "medical intervention at birth" and significantly larger for canines and incisors for "no medical intervention".
Note (**): Mean neonatal line width is significantly greater for "yes" category when measured in canines or molars (results for canines are tentative due to small sample size) and significantly larger for "no" category when measured in incisors.

Figure 5-17. Neonatal line width (µm) at the apex by tooth class for medical intervention at birth.



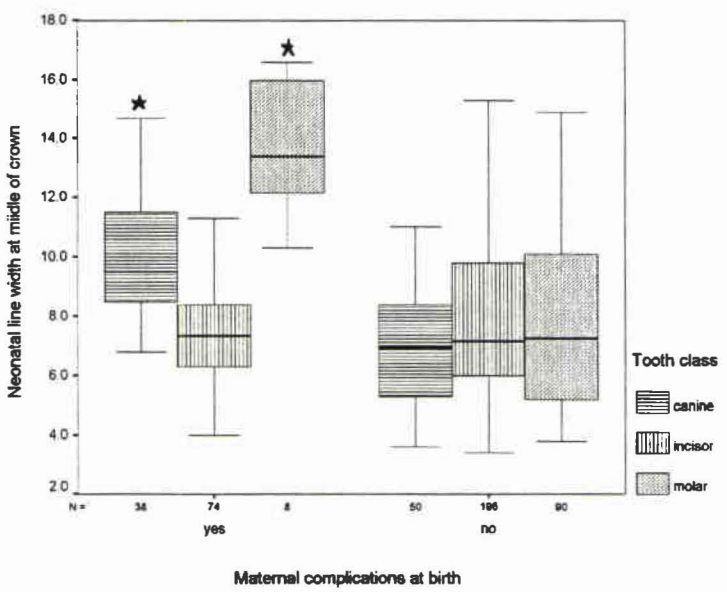
Note (*): Mean neonatal line width is significantly greater in molars than in canines or incisors for "medical intervention at birth".
Note (**): Mean neonatal line width is significantly larger for "no" category when measured in incisors or canines and significantly larger for "yes" category when measured in molars.

Figure 5-18. Neonatal line width (µm) at DEJ by tooth class for "maternal complications at birth"



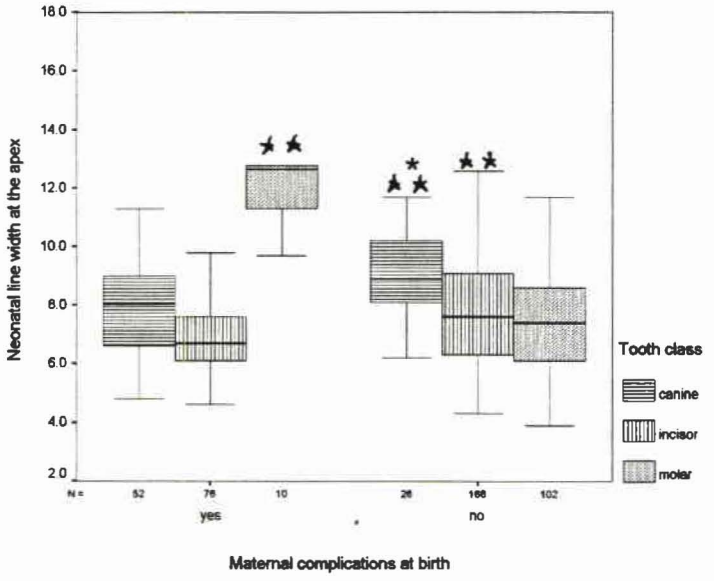
Note (*) Mean neonatal line width is significantly greater in incisors and in canines for "no maternal complications".
Note (**): Mean neonatal line width is significantly greater for the "no" category when measured in incisors, and significantly larger for the "yes" category when measured in canines or molars (results for molars are tentative due to small sample size).

Figure 5-19. Neonatal line width (µm) at the middle crown by tooth class for "maternal complications".



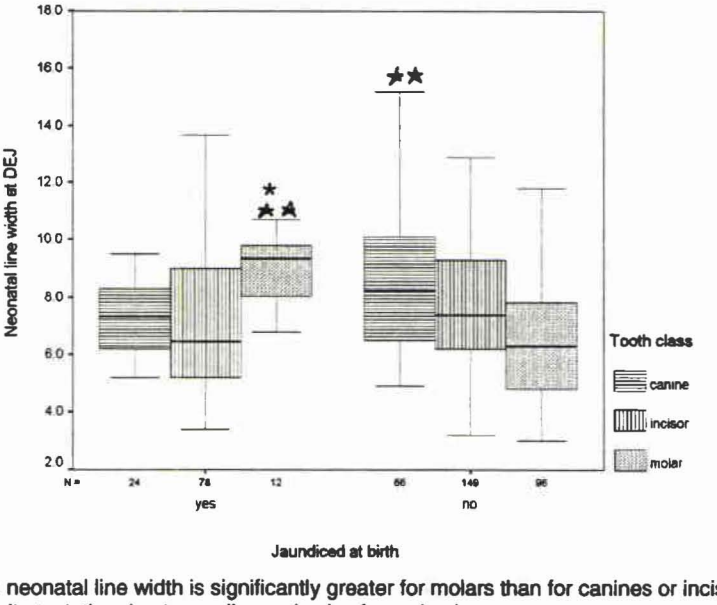
Note (*): Mean neonatal line width is significantly larger for "yes" category when measured in canines or molars (results for molars are tentative due to small sample size).

Figure 5-20. Neonatal line width (µm) at the apex by tooth class for "maternal complications".



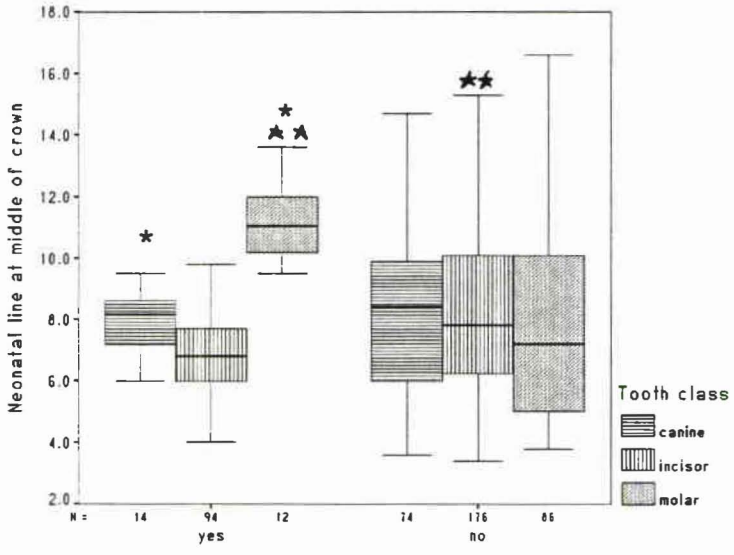
Note (*): Mean neonatal line width is significantly larger in canines than in incisors or in molars for "no maternal complications at birth".
Note (**): Mean neonatal line width is significantly larger for "no" category when measured in incisors or canines and significantly larger for "yes" category when measured in molars (results tentative due to small molar sample size).

Figure 5-21. Neonatal line width (µm) at the DEJ by tooth class for "jaundiced at birth".



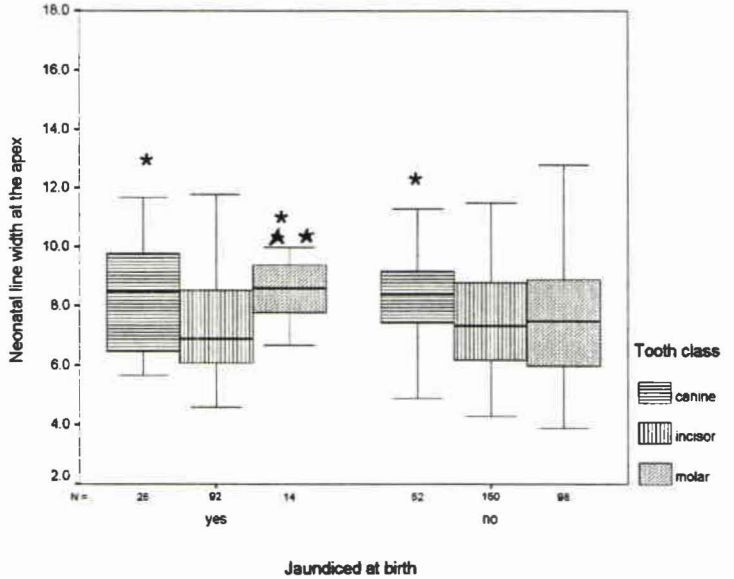
Note (*): Mean neonatal line width is significantly greater for molars than for canines or incisors for children born jaundiced (results tentative due to small sample size for molars).
Note (**): Mean neonatal line width is significantly larger for "no" category when measured in canines and significantly larger for "yes" category when measured in molars (results tentative due to small sample size for molars).

Figure 5-22. Neonatal line width (µm) at the middle crown by tooth class for "jaundiced at birth".



Note (*): Mean neonatal line width is significantly greater in molars and canines than in incisors in children born jaundiced (results tentative due to small sample size for molars and canines).
Note (**): Mean neonatal line width is significantly larger for "no" category when measured in incisors and significantly larger for "yes" category when measured in molars (results tentative due to small molar sample size).

Figure 5-23. Neonatal line width (µm) at the apex by tooth class for "jaundiced at birth".



Note (*) Mean neonatal line width is significantly greater in molars and in canines than in incisors in children born jaundiced (results tentative due to small molar sample size) and significantly greater in canines in children not born jaundiced.
Note (**): Mean neonatal line width is significantly larger for children born jaundiced when measured in molars (results tentative due to small molar sample size).

Tests of Significance Between Birth Categories by Tooth Class

As a result of the significant difference(s) in neonatal line width between crown positions and between tooth classes at each crown position, tests of significance between birth categories were conducted both by tooth class and by crown position.

Results of a Kruskal-Wallis rank test demonstrate that neonatal line width differs significantly between delivery types when compared at each crown position by tooth class. The only exception occurs when the neonatal line is measured at the middle of the crown in molars (Table 5-11). According to a Mann-Whitney U rank test, when neonatal line width is measured at the DEJ in incisors, line width is significantly larger for a natural delivery (i.e. highest rank; mean = 9.8 +/- 3.5μm) than it is for a delivery with minimal medical intervention (7.3 +/- 2.4μm; p = 0.0001), a complicated delivery (7.5 +/- 1.8μm; p = 0.004) or an elective c-section delivery (7.2 +/- 1.9μm; p = 0.007). In contrast, neonatal line width is significantly smaller (i.e. lowest rank; mean = 6.8 +/- 1.5μm) for a natural delivery when measured at the DEJ in canines, than it is for a delivery with minimal medical intervention (9.3 +/- 1.9μm; p = 0.001) or a complicated delivery (9.6 +/- 2.6μm; p = 0.0001). Similar results are obtained when neonatal line width is measured at the DEJ in molars .

According to a Mann-Whitney U rank test, neonatal line width is significantly different between each delivery type when measured at the middle of the crown in incisors, with mean neonatal line width widest for an elective c-section delivery (10.9 +/- 1.6μm) (Table 5-11 and Table 8, Appendix B). Neonatal line width is also significantly different between each delivery type when measured at the middle of the crown in canines with a complicated delivery having the larger mean width (10.8 +/- 1.9μm; Table 5-11 and Table 8, Appendix B).

Table 5-11. Results of a Kruskal-Wallis test for neonatal line width (μm) by tooth class and by delivery type at each crown position.

Tooth class/ Delivery type	N[1]	Mean Rank	*H* statistic	Sig. (p)
DEJ				
Incisor				
Natural	40	**124.21**	14.71	0.002
Minimal intervention	89	86.44		
Complicated	40	90.46		
Elective C-section	20	83.72		
Canine				
Natural	30	**20.92**	21.87	0.0001
Minimal intervention	10	42.55		
Complicated	24	42.79		
Molar				
Natural	22	**26.20**	19.60	0.0001
Minimal intervention	34	51.35		
Complicated	20	60.03		
Elective C-section	20	56.65		
Middle				
Incisor				
Natural	52	130.41	44.54	0.0001
Minimal intervention	96	105.33		
Complicated	58	88.22		
Elective C-section	24	**189.79**		
Canine				
Natural	34	21.79	27.48	0.0001
Minimal intervention	12	33.92		
Complicated	16	**50.31**		
Molar				
Natural	22	38.48	1.70	n.s.
Minimal intervention	34	47.16		p = 0.637
Complicated	20	42.20		
Elective C-section	10	44.70		
Apex				
Incisor				
Natural	56	**129.90**	56.2	0.0001
Minimal intervention	66	96.67		
Complicated	58	60.19		
Elective C-section	20	**147.70**		
Canine[2]				
Natural	22	**34.02**	u = 164.5	0.002
Complicated	30	20.98	z = -3.067	

Table 5-11 (cont'd)

Tooth class/ Delivery type	N[1]	Mean Rank	*H* statistic	Sig. (p)
Molar				
Natural	22	48.14	21.41	0.0001
Minimal intervention	36	42.53		
Complicated	20	**76.93**		
Elective C-section	22	41.89		

1 Total number of prisms measured at each crown position
2 Results presented for canines are for a Mann-Whitney U test (not corrected for ties)

When measured at the apex in incisors, a Mann-Whitney U rank test confirms that neonatal line width is significantly larger for both a natural delivery and an elective c-section delivery (8.7 +/- 2.0μm and 10.4 +/- 3.4μm, respectively) when compared to line width for a delivery with minimal medical intervention (7.3 +/- 1.3μm) or a complicated delivery (6.4 +/- 1.1μm) ($p = 0.0001$, all comparisons). Neonatal line width at the apex in canines is larger for a natural delivery than it is for a complicated one (8.5 +/- 1.0μm vs. 7.1 +/- 1.5μm; $p = 0.002$). When neonatal line width is measured at the apex in molars, line width is significantly larger for a complicated delivery (10.3 +/- 3.0μm) than it is for a natural delivery (7.4 +/- 1.6μm; $p = 0.001$), a delivery with minimal medical intervention (6.9 +/- 1.9μm; $p = 0.0001$) or an elective c-section (7.1 +/- 1.4μm; $p = 0.0001$).

Results of a Mann-Whitney U rank test confirm that neonatal line width is significantly larger for non-healthy infants than for healthy infants when it is measured at the DEJ in incisors (8.1 +/- 2.2μm vs. 7.1 +/- 2.9μm) or molars (8.2 +/- 2.7μm vs. 6.0 +/- 1.8μm) (Figure 5-12), at the middle of the crown in canines (8.7 +/- 2.7μm vs. 7.5 +/- 1.9μm) or molars (10.0 +/- 3.8μm vs. 6.9 +/- 2.1μm) (Figure 5-13), or at the apex in molars (9.5 +/- 2.3μm vs. 6.8 +/- 1.6μm) (Figure 5-14 and Table 5-12). Neonatal line width is significantly larger for healthy children when measured at the apex in incisors (7.7 +/- 1.5μm vs. 7.6 +/- 2.4μm) (Table 5-12). Neonatal line width is not significantly different between these two categories when measured at the DEJ in canines (not healthy: 8.5 +/- 2.2μm; healthy: 7.5 +/- 2.2μm), at the middle of the crown in incisors (not healthy:

8.0 +/-2.5µm; healthy: 7.6 +/- 2.3µm), or at the apex in canines (not healthy: 8.3 +/- 1.8µm; healthy: 8.2 +/- 0.6µm) (Table 5-12).

Table 5-12. Results of a Mann-Whitney U test for neonatal line width by tooth type and by child's health at birth at each crown position.

Tooth class/ Child's health	N[1]	Mean rank	Sum of ranks	*U* Statistic	z-score	Sig. (p)[2]
DEJ						
Incisors						
Healthy	105	93.80	9849.0	4284.0	-4.300	0.0001
Not Healthy	122	**131.39**	16029.0			
Canines						
Healthy	30	37.92	1137.5	672.5	-1.948	n.s.
Not Healthy	60	49.29	2957.5			p = 0.051
Molars						
Healthy	64	43.59	2790.0	710.0	-4.366	0.0001
Not Healthy	44	**70.36**	3096.0			
Middle						
Incisors						
Healthy	119	127.17	15133.5	7993.5	-1.556	n.s
Not Healthy	151	142.06	21451.5			p = 0.12
Canines						
Healthy	32	36.80	1177.5	649.5	-2.139	0.032
Not Healthy	56	**48.90**	2738.5			
Molars						
Healthy	54	38.99	2105.5	620.5	-4.055	0.0001
Not Healthy	44	**62.40**	2745.5			
Apex						
Incisors						
Healthy	102	**132.90**	13556.0	5977.0	-2.163	0.031
Not Healthy	140	113.19	15847.0			
Canines						
Healthy	12	35.83	430.5	352.0	-0.610	n.s.
Not Healthy	66	40.17	2651.5			p = 0.542
Molars						
Healthy	66	40.36	2663.5	452.5	-6.304	0.0001
Not Healthy	46	**79.66**	3664.5			

1 Total number of prisms measured at each crown position
2 Not corrected for ties

Results of an independent samples t-tests comparing neonatal line width between each category of "medical intervention at birth" (yes versus no) at the DEJ in incisors and in molars, and at the apex in incisors can be found in Table 5-13a. Results of a Mann-Whitney U rank test comparing neonatal line width between these same categories at the DEJ for canines, at the middle of the crown for all tooth classes, and at the apex for canines and for molars can be found in Table 5-13b.

Neonatal line width between both categories of "medical intervention at birth" is not significantly different when measured at the DEJ in incisors (yes: 7.0 +/- 1.9μm; no: 7.8 +/- 2.8μm) (Table 5-13a). When line width is measured at the DEJ in canines (yes: 9.6 +/- 2.6μm vs. no: 7.6 +/- 1.8μm) or molars (yes: 9.2 +/- 2.3μm; no: 5.9 +/- 1.7μm) neonatal line width is significantly greater for children who experienced medical intervention(s) at birth (Table 5-13b and Figure 5-15). When measured in canines (yes: 10.8 +/- 1.9μm vs. no: 7.7 +/- 2.2μm) or molars (yes: 11.9 +/- 2.3μm vs. no: 6.5 +/- 2.1μm) at the middle of the crown, neonatal line width is again significantly greater for children who experienced medical intervention(s) at birth. In contrast, neonatal line width at the middle crown position for incisors is significantly larger for children not undergoing medical intervention(s) at birth (yes: 7.2 +/- 2.6μm; no: 8.0 +/- 2.3μm) (Table 5-13b and Figure 5-16). When neonatal line width is measured at the apex in incisors (yes: 6.5 +/- 1.2μm; no: 8.1 +/- 2.2μm) or in canines (yes: 7.1 +/- 1.5μm; no: 9.1 +/- 1.3μm), neonatal line width is significantly larger for children who did not experience medical obstruction. Line width is significantly greater for children undergoing medical interference at birth when measured at the apex in molars (yes: 9.9 +/- 2.4μm vs. no: 6.9 +/- 1.6μm) (Figure 5-17).

Results of an independent samples t-tests comparing neonatal line width by tooth class and by "maternal complications at birth" (yes versus no) at each crown position (except at the middle of the crown in incisors and molars) can be found in Table 5-14a. Results of a Mann-Whitney U test comparing neonatal line width by maternal complications at birth at the middle of

Table 5-13a. Independent t-test results for neonatal line width for incisors and for molars at the DEJ and for incisors at the apex by medical intervention at birth.

Tooth class/ Medical intervention	Mean	S.D.	N[1]	Levene's Test F	Levene's Test Sig. (p)	t-value	Sig. (p)
DEJ							
Incisor[2]							
Yes	0.832	0.119	56	5.876	0.016	-1.817[3]	n.s. p = 0.072
No	0.868	0.152	171				
Molar[5]							
Yes	**9.2**	2.3	34	3.586	n.s. p = 0.061	8.252[4]	0.0001
No	5.9	1.7	74				
Apex							
Incisor[2]							
Yes	0.806	7.67E-02	70	10.177	0.002	-6.979[3]	0.001
No	**0.893**	0.11	172				

1 Total number of prisms measured at each crown position
2 Log transformed
3 Unequal variance test
4 Equal variance test
5 Measurement in microns

the crown in incisors and molars are presented in Table 5-14b.

Neonatal line width is significantly different between these two variables for each tooth class at each crown position; an exception is line width at the middle of the crown in incisors. At the DEJ in incisors, neonatal line width is significantly larger for children born to a mother who did not experience complications at birth (yes: 6.8 +/- 1.9μm; no: 8.0 +/- 2. 8μm). In contrast, when neonatal line width is measured at the DEJ in canines (yes: 9.2 +/- 2.3μm; no: 7.2 +/- 0.8μm) and in molars (yes: 11.9 +/- 1.7μm; no: 6.4 +/- 1.9μm), neonatal line width is significantly larger for children born to women who did experience complications at birth (Figure 5-18). At the middle of the crown in canines (yes: 9.9 +/- 2.0μm; no: 7.0 +/- 2.0μm) and in molars (yes: 13.8 +/- 2.3μm; no: 7.8 +/- 3.0μm), neonatal line width is greater for children whose mother experienced problems at delivery. When neonatal line width is measured at the middle of the crown in incisors, line width is not significantly different between these two birth categories (yes: 7.7 +/- 2.2μm; no: 7.9 +/-

2.5μm) (Figure 5-19). When measured at the apex in both incisors (yes: 6.9 +/- 1.2μm; no 7.9 +/- 2.3μm) and in canines (yes: 7.9 +/- 1.7μm; no: 9.1 +/- 1.4μm), neonatal line width is significantly larger for children born to women who did not experience difficulties at birth. In contrast, when measured at the apex in molars, line width is significantly wider in children whose mother experienced complications (yes: 12.7 +/- 2.3μm; no: 7.4 +/- 1.7μm) (Figure 5-20).

Table 5-13b. Results of a Mann-Whitney U-test for neonatal line width for remaining tooth classes and by medical intervention at birth at each crown position.

Tooth class/ Medical intervention	N[1]	Mean Rank	Sum of ranks	*U* Statistic	z-score	Sig. (p)[2]
DEJ						
Canine						
Yes	24	**60.83**	1460	4240.0	-3.359	0.001
No	66	39.92	2635			
Middle						
Incisor						
Yes	64	115.37	7383.5	5303.5	-2.362	0.018
No	206	**141.75**	29201.5			
Canine						
Yes	16	**70.59**	1129.5	158.5	-4.519	0.0001
No	72	38.70	2786.5			
Molar						
Yes	32	**79.41**	2541.0	99.0	-7.253	0.0001
No	66	35.00	2310.0			
Apex						
Canine						
Yes	30	24.05	721.5	256.5	-4.763	0.0001
No	48	**49.16**	2359.5			
Molar						
Yes	36	**84.57**	3044.5	357.5	-6.298	0.0001
No	76	43.20	3283.5			

1 Total number of prisms measured at each crown position
2 Not corrected for ties

Table 5-14a. Independent t-test results for neonatal line width by tooth class and by maternal complication(s) at birth at each crown position.

Tooth class/ Maternal complication(s)	Mean	S.D.	N[1]	Levene's Test F	Levene's Test Sig. (p)	t-value	Sig. (p)
DEJ							
Incisor[4]							
Yes	0.817	0.119	68	4.757	0.03	-3.244[2]	0.001
No	**0.877**	0.152	159				
Canine[4]							
Yes	**0.950**	0.1048	44	0.042	n.s	4.799[3]	0.0001
No	0.846	0.1002	46		p = 0.837		
Molar[5]							
Yes	**11.9**	1.7	10	0.216	n.s.	8.748[3]	0.0001
No	6.4	1.9	98		p = 0.643		
Middle							
Canine[5]							
Yes	**9.9**	2.0	38	0.105	n.s	6.805[3]	0.0001
No	7.0	2.0	50		p = 0.747		
Apex							
Incisor[4]							
Yes	0.834	7.50E-02	76	16.477	0.0001	-3.911[2]	0.0001
No	**0.883**	0.12	166				
Canine[5]							
Yes	7.9	1.7	52	0.712	n.s.	-3.014[3]	0.004
No	**9.1**	1.4	26		p = 0.401		
Molar[5]							
Yes	**12.7**	2.3	10	0.335	n.s.	8.903[3]	0.0001
No	7.4	1.7	102		p = 0.564		

1 Total number of prisms measured at each crown position
2 Unequal variance test
3 Equal variance test
4 Log transformed
5 Measurement in microns

Table 5 -14b. Mann – Whitney U test results for neonatal line width in incisors and molars for maternal complication(s) at birth at the middle of the crown.

Tooth class/ Maternal complication(s)	N[1]	Mean rank	Sum of ranks	*U* statistic	z-score	Sig. (p)[2]
Middle						
Incisor						
Yes	74	134.28	9936.5	7161.5	-0.158	n.s.
No	196	135.96	26648.5			(0.87)
Molar						
Yes	8	**89.0**	712.0	44.0	-4.102	0.001
No	90	46.0	4139.0			

1 Total number of prisms measured at each crown position
2 Not corrected for ties

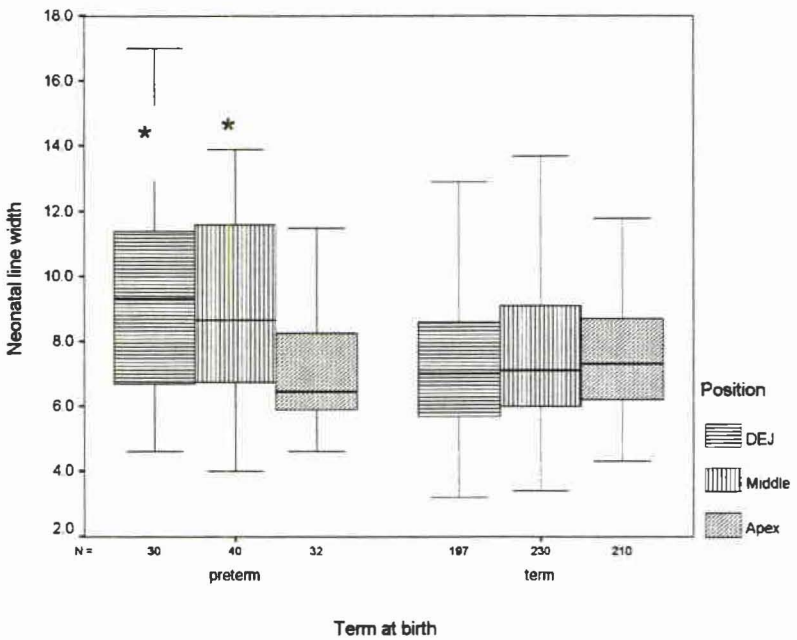
Results of a Mann-Whitney U rank test comparing neonatal line width between pre-term and term births for incisors at each crown position can be found in Table 5-15. At both the DEJ and at the middle of the crown, neonatal line width is significantly greater for a pre-term birth (DEJ: 9.7 +/- 3.4μm; middle: 9.0 +/- 2.8μm) than it is for a term birth (DEJ: 7.3 +/- 2.3μm; middle: 7.6 +/- 2.3μm). When neonatal line width is measured at the apex, neonatal line width is not significantly different between either type of birth (preterm: 7.2 +/- 2.0μm; term: 7.7=/-2.1μm) (Figure 5-24).

Table 5-15. Results of Mann-Whitney U-test by for neonatal line width in incisors by term at birth.

Tooth class/ Term of birth	N[1]	Mean Rank	Sum of ranks	*U* statistic	z-score	Sig. (p)[2]
DEJ						
Incisor						
Pre-term	30	**156.20**	4686.0	1699.0	-3.779	0.0001
Term	197	107.57	21192.0			
Middle						
Incisor						
Pre-term	40	**169.89**	6795.5	3224.5	-3.018	0.003
Term	230	129.52	29789.5			
Apex						
Incisor						
Pre-term	32	101.69	3254.0	2726.0	-1.719	n.s.
Term	210	124.52	26149.0			p = 0.086

1 Total number of prisms measured at each crown position; 2 Not corrected for ties

Figure 5-24. Neonatal line width (μm) for incisors at each crown position for "term at birth".



Note (*) Mean neonatal line width is significantly greater when measured at the DEJ and at the middle crown position in pre-term children.

Results of a Mann-Whitney U rank test comparing neonatal line width by tooth class at each crown position for children born jaundiced (yes) and those not (no) are presented in Table 5-16. When measured at the DEJ in canines, neonatal line width is significantly greater for children not born jaundiced (no: 8.5 +/- 2.4μm; yes: 7.3 +/- 1.4μm); at the DEJ in molars, neonatal line width is significantly greater for children born jaundiced (yes: 9.0 +/- 1.2μm; no: 6.6 +/- 2.5μm) (Figure 5-21). At the middle of the crown in incisors, neonatal line width is significantly larger for children not born jaundiced (yes: 7.2 +/- 1.9μm; no: 8.2 +/- 2.6μm); when measured in molars, it is significantly larger for children born jaundiced (yes: 11.2 +/- 1.3μm; no: 7.9 +/- 3.3μm) (Figure 5-22). At the apex in molars, neonatal line width is significantly larger for children born jaundiced (yes: 8.6 +/- 1.0μm; no: 7.8 +/- 2.5μm) (Figure 5-23). No significant differences in neonatal line width were found between these two birth categories when measured at the DEJ or at the apex in incisors and when measured at the middle of the crown or at the apex in canines (Table 9, Appendix B).

Table 5-16. Results of Mann-Whitney U test for neonatal line width by tooth class and by jaundiced at birth at each crown position.

Tooth class/ Jaundiced	N[1]	Mean Rank	Sum of ranks	*U* statistic	z-score	Sig. (p)[2]
DEJ						
Incisor						
Yes	78	102.26	7976.0	4895.0	-1.95	n.s.
No	149	120.15	17902.0			p = 0.051
Canine						
Yes	24	35.94	862.5	562.5	-2.095	0.036
No	66	**48.98**	3232.5			
Molar						
Yes	12	**85.88**	1030.5	199.5	-3.682	0.0002
No	96	50.58	4855.5			
Middle						
Incisor						
Yes	94	117.23	11019.5	6554.5	-2.81	0.005
No	176	**145.26**	25565.5			
Canine						
Yes	14	42.25	591.5	456.5	-0.389	n.s.
No	74	44.93	3324.5			p = 0.719
Molar						
Yes	12	**76.58**	99.0	191.0	-3.524	0.0004
No	86	45.72	3932.0			
Apex						
Incisor						
Yes	92	115.20	10598.5	6320.5	-1.097	n.s.
No	150	125.36	18804.5			p = 0.273
Canine						
Yes	26	39.96	1039.0	664.0	-0.1277	n.s.
No	52	39.27	2042.0			p = 0.899
Molar						
Yes	14	**74.21**	1039.0	438.0	-2.183	0.029
No	98	53.97	5289.0			

1 Total number of prisms measured at each crown position
2 Not corrected for ties

Correlation Testing

The results of the Spearman's *r* correlation tests between length of delivery and neonatal line width at each crown position can be found in Table 5-17. No significant correlations were found between neonatal line width and delivery length at any crown position.

Table 5-17. Correlation testing of neonatal line width at each crown position and length of delivery.

Delivery length/ Neonatal line width	N	Corr. (r)	Sig. (P)
Total birth length (hours)			
DEJ	42	-0.130	n.s. (p = 0.405)
Middle	43	-0.065	n.s. (p = 0.682)
Apex	41	-0.046	n.s. (p = 0.777)
Contractions to delivery (hours)			
DEJ	37	0.059	n.s. (p = 0.730)
Middle	39	-0.021	n.s. (p = 0.898)
Apex	36	0.077	n.s. (p = 0.655)
Pushing stage (minutes)			
DEJ	32	-0.077	n.s. (p = 0.675)
Middle	32	-0.005	n.s. (p = 0.979)
Apex	29	-0.181	n.s. (p = 0.347)

Summary of Results

The statistical analysis employed in this investigation demonstrates the following:

- For the entire sample of neonatal line measurements, the standard deviation ranges from 2.1 to 2.7 μm depending on crown position; for individuals, the standard deviation ranges from 0.5 to 2.7 μm depending on crown position; for birth categories, the standard deviation ranges from 1.5 to 3.3 μm depending on crown position and birth category.
- The ranges in neonatal line width are quite large for individuals and for each birth variable at each crown position; there is an overlap in neonatal line width between each birth category at all crown positions.

- The distribution of neonatal line width measurements at each crown position for the entire sample and for the majority of birth variables is skewed such that the median value is generally similar to or smaller than the mean width at each crown position.
- Neonatal line width is significantly different between crown positions for the entire sample of measurements (the width is greatest at the middle of the crown) and for the majority of birth variables.
- According to tooth class, neonatal line width differs significantly between crown positions in molars; neonatal line width differs significantly between tooth classes when measured at the DEJ and at the apex, with canines exhibiting the largest width.
- According to tooth class, the standard deviations of the measurements range from 1.7 to 3.3 μm, with molars exhibiting large standard deviations; neonatal line width ranges between 6.9 and 13.8 μm, with molars having large ranges; the median is generally similar to or smaller than the mean at each crown position.
- For the majority of birth variables, neonatal line width differs significantly between crown positions for each tooth class; neonatal line width is also significantly different between tooth classes at each crown position; however, no consistent pattern of differences is evident.
- Neonatal line width is significantly different between delivery types when measured at the middle of the crown and at the apex only; when compared by tooth class, neonatal line width is also significantly different between delivery types at each crown position (except at the middle of the crown in molars); there is no consistent pattern to these differences.
- Significant differences exist in neonatal line width between each pair of birth variables when compared at each crown position (except for the jaundiced category); at both the DEJ and at the middle of the crown these differences are consistent with the relationships identified by earlier dental studies; at the apex, the relationships are either not significant or not consistent with the findings of earlier investigations.

- According to tooth class, significant differences in neonatal line width are evident between each pair of birth variables at each crown position; when line width is measured in incisors, the identified relationships are not consistent with the findings of earlier dental studies; in general, no consistent pattern of differences is evident.
- No significant correlation exists between neonatal line width and the length of the delivery at any crown position.

Chapter Six

Discussion

A Fundamental Shortcoming: Lack of a Rigorous Methodology in Studies of the Neonatal Line

Previous studies of the neonatal line have failed to include important methodological information and relevant data (pages 61-62). This lack of information makes it difficult to replicate earlier investigations with confidence and limits a researcher's ability to compare his/her results with those from earlier studies. The omission of this information does not, however, detract from the results of the present study. The reliability of the measuring technique employed during the present investigation was confirmed by a paired t-test (Table 5-1, page 94), permitting comparison of neonatal line width between individuals and between groups of individuals.

Only three studies attempt to describe their method of measuring the neonatal line: the two studies by Ranggard and colleagues (1994 and 1995) and the Isola Sacra Project. Ranggard et al. (1994 and 1995) measured the neonatal line between its "inner and outer limitations"; this definition is inadequate. Defining the line's boundaries/limits (i.e. where the prism disturbance begins and where it ends) represented the greatest difficulty in calculating line width during the present study. In many cases, the borders of the line were diffuse and visibility of the prisms within the line was poor (see Figures 4-5a, 4-6a and 4-8a, Material and Methods). In such cases, measurements were not attempted (in accordance with the Isola Sacra Project). The visibility of the neonatal line and of the individual prism boundaries was clearest at the middle crown position. Consequently, more prisms were measured at this location (see Table 5-2, page 95). The poor visibility of neonatal lines in other studies makes the accuracy of the measurements doubtful (Figure 6-1A).

Researchers of the Isola Sacra Project measured the neonatal line "where the prisms were clearly disrupted", but they do not specify the nature of the disruption. Microscopic studies of

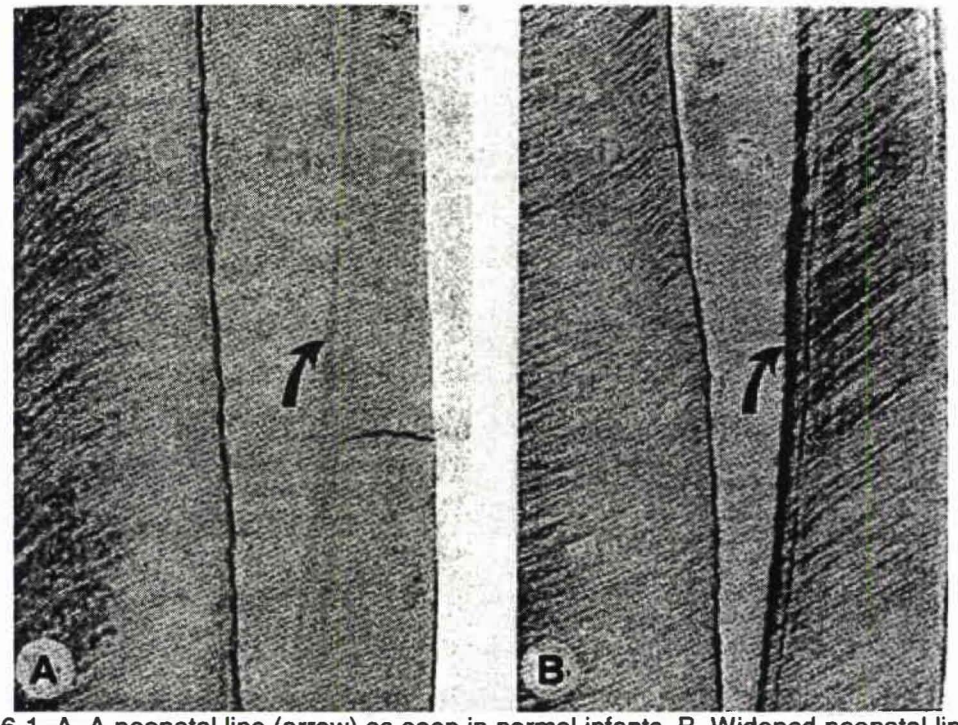


Figure 6-1. A. A neonatal line (arrow) as seen in normal infants. B. Widened neonatal line (arrow) in an infant born to a diabetic mother. Ground section x75 (after Noren, 1984). Measurement of the neonatal line in A is questionable given its very poor visibility. Examination of the line in B shows that it is narrower at both the top and the bottom, and widest at the middle. It is not stipulated at which crown position width measurements were taken.

the neonatal line offer no consensus regarding the structural disturbance responsible for its formation (pages 21-22). Any discussion of how neonatal line width is measured must include a detailed description of the prism morphology visible along its length. Examination of the lines in the present study revealed that the most obvious microstructural disturbance was a distortion/widening of the prism's boundary (compared to prism bending, increase in interprismatic substance, thickening of the prism sheath, or change in prism width, pages 21-22). Other disturbances were also noted (i.e. accentuation of cross striations). It is unclear at this time whether similar structural disturbances were being measured in earlier studies and if comparison between studies is appropriate. One must question whether measuring different types of prism disturbance would have an effect on neonatal line width.

It is not clear whether previous studies considered color changes in their measurements. The appearance of the neonatal line in microradiographs and under the light microscope

suggests that the enamel on either side of the line and within the line is either hypomineralized or hypermineralized, (i.e. hypomineralized = dark, hypermineralized = light) (i.e. Noren, 1983 and colleagues 1978a,b). Scrutiny of the lines in the present study showed that a "gradient" of color exists; the neonatal line is the area of maximum darkness, but its appearance gradually becomes lighter in the enamel surrounding the line. Changes in color, which may reflect changing mineral ion levels (FitzGerald, 1995), were not considered in this thesis (this is also in accordance with the Isola Sacra Project). If other studies did utilize color changes, neonatal line measurements from these studies are not comparable to those of the current investigation; line width may be greater if color changes were included.

Many investigators do not identify the magnification at which neonatal line measurements were taken. It has been shown that the clarity/visibility of striae improves when less magnifying power is used, but the accuracy of the measurement declines (FitzGerald 1995: 183). The majority of studies present images taken at magnifications of 40x, 75x and 100x (Noren, 1983 and 1984; Noren and colleagues 1978a,b and 1987); however, it is uncertain if line width was measured at these magnifications. Ranggard and colleagues and the Isola Sacra Project stipulate a magnification of 400x (the same as this study), while the study of Eli et al. (1989) does not specify a magnification. The results of the current study might be comparable to that of Ranggard et al. (1994 and 1995) and the Isola Sacra Project; but given the limitations already presented, comparisons would be speculative.

The results of this thesis demonstrate that mean neonatal line width differs significantly between crown positions. It is therefore imperative that studies report the location of their measurements (DEJ, middle or apex). The studies of Eli et al. (1989), Ranggard and colleagues (1994 and 1995) and the Isola Sacra Project provide this information; the remaining neonatal line studies do not. Examination of images presented in some studies reveals a visible difference in neonatal line width between crown locations (see Figure 6-1B). In such instances, it is questionable if estimates of neonatal line width represent the mean width of measurements taken at several locations or only at one location (i.e. possibly the widest).

Many studies do not present neonatal line width measurements. Instead, they use descriptive terms like "wider than normal" (Noren, 1983 and 1984; Noren and colleagues 1978a,b and 1987) and others that are not synonymous with a widening of the neonatal line (i.e. accentuated, abnormal, prominent) (Stein 1936 and 1947; Schour and Kronfeld, 1938; Kronfeld and Schour, 1939; Miller and Forrester, 1959; Jaffe et al. 1985; Judes et al. 1985; Eli et al. 1989; Tievens et al. 1996). These descriptive terms are also not defined. By omitting information on normal and abnormal line width and term definitions, the conclusions of these investigations are speculative. These studies seem to suggest that disturbances at birth result in lines that are more visible (Figure 6-2). A discernible neonatal line may simply reflect the magnification at which it is viewed and/or section thickness (see page 157). In the present study, lines that were less visible were, at times, wider than or similar in width to those that were more visible (compare Figure 4-3a and 4-8a, Materials and Methods).

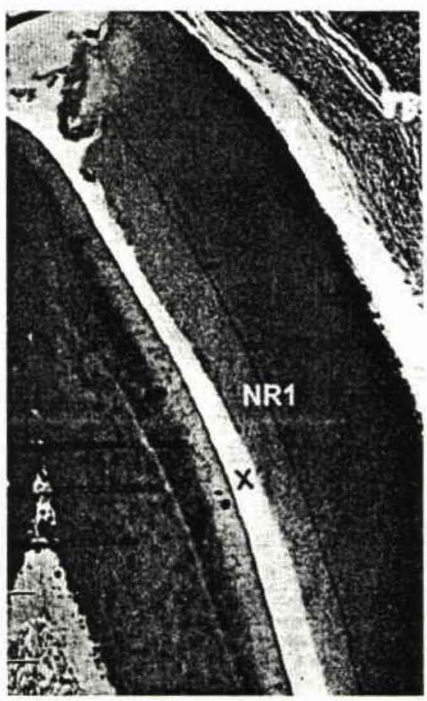


Figure 6-2. A decalcified section of a deciduous incisor from a prematurely born infant (after Kronfeld and Schour, 1939). These authors suggest that the prematurity of this child accounts for the "prominence" of the neonatal line (marked NR1). No measurements are provided of the width of this line. The term prominent is not defined and may indicate that the line is simply very visible. Magnification is not provided.

The significance of the findings of previous studies is disputable because of the exclusion of the results of statistical analyses. More importantly, the lack of precision testing leads one to question the reliability of the measuring techniques and the validity of the published data.

Attention must be paid to the methods used in obtaining neonatal line width. Differing measuring techniques, exclusion of quantitative data and the use of undefined terms produce results that cannot be compared across studies, permitting only general conclusions to be made about inter-group similarities and differences. While many earlier studies collected measurements through a microscope, it is useful to create a permanent record of all analyses using digital imaging. The creation of an archive of measuring procedures during this investigation insured that measurements were taken consistently and that comparisons could be made between specimens. It also allows replication of the present study, and the methods employed, in future investigations of the neonatal line.

Comparison with Other Neonatal Line Studies and With Inquiries into Enamel Defects in Perinatal Disorders

The omission of important methodological information in previous studies of the neonatal line makes it difficult to compare mean neonatal line width in the present study with line width from other investigations. However, it is possible to examine whether the relationships identified by earlier dental studies were observed in the current investigation.

A. Mode of Delivery

Eli and colleagues (1989) propose that "the birth process simultaneously affects all ameloblasts at the different tooth levels establishing a uniform line" (p. 222). The findings of this thesis are not in agreement with this statement. Contrary to the ANOVA results of Eli et al. (see Table 3-4, page 46), the current study demonstrated significant differences in neonatal line width between crown positions for the entire sample of measurements and between crown positions for a natural delivery, a complicated delivery and an elective c-section delivery (Table 5-4, page 98).

These differences confirmed that statistical comparisons between delivery types, and between other birth variables, could only be conducted by crown position.

In the study of Eli et al. (1989), measurements of neonatal line width for an elective c-section delivery are only available at the DEJ. This limited their comparison of all three modes of delivery to one crown level. Comparisons between neonatal line width from a natural delivery and a complicated delivery were still conducted at the remaining two crown positions. In the present study, measurements of neonatal line width were taken at each crown position for the elective c-section group, allowing statistical testing between each delivery type at all crown positions.

Eli et al. (1989) demonstrated a significant difference in neonatal line width between a natural delivery (11.9 +/- 4.8μm) and a complicated delivery (18.6 +/- 5.7μm) at the DEJ and found that neonatal line width for an elective c-section was markedly lower (7.6 +/- 1.5 μm) (Table 3-4, page 46). In the present study, comparison between delivery types at the DEJ revealed no significant difference in neonatal line width (Table 5-4, page 98), even when mean neonatal line width was greatest for a complicated delivery (8.3 +/- 2.7 μm) (Table 5-3, page 97). Comparison between delivery types at the middle of the crown demonstrated that line width was significantly wider for an elective c-section (9.9 +/- 2.2 μm). When measured at the apex, neonatal line width was again significantly different between delivery types, with a natural delivery and an elective c-section demonstrating the largest lines (8.4 +/- 1.8 μm and 8.6 +/- 3.0 μm, respectively) (Tables 5-3 and 5-4, pages 97 and 98).

The discrepancies between the results of this thesis and those presented by Eli et al. (1989) could be explained by three factors: 1) the study conducted by Eli and colleagues is problematic; 2) there are inter-study differences in the grouping of children into birth categories; and 3) there is a lack of consensus among medical experts regarding the effect(s) of mode of delivery on the outcome of the newborn. These are discussed below.

The use of inappropriate analytical procedures can affect the results of a study. Consequently, it is essential to address the methodological problems that are evident in Eli and colleagues' (1989) investigation. These problems render their conclusions doubtful.

Careful examination of the published data of Eli and colleagues reveals that the sample sizes are markedly different between delivery types for the entire sample and at each crown level (Tables 3-3 and 3-5, page 46). The disparity in sample sizes at the DEJ (natural: n = 39, operative: n = 3; elective c-section: n = 5) is consequential given that their published findings of neonatal line width are based upon measurements obtained at this crown location and on analyses conducted between delivery types at this crown level. It is doubtful that the mean neonatal line widths recorded for a complicated delivery and for an elective c-section delivery are representative of the population means for these delivery types. One must question if an increase in sample size would produce similar results.

The statistical analyses employed in the study of Eli et al. are disputable. These authors employed parametric tests (i.e. t-tests) to demonstrate that neonatal line width is significantly different between normal and complicated deliveries at each crown position. They found a significant increase ($p < 0.05$) in neonatal line width in children born by operative delivery at each crown level (Table 3-5, page 46). Given the unequal sample sizes between these two groups at each crown position (natural: DEJ, n = 39; middle, n = 46; apex, n = 40 ; complicated: DEJ, n = 3; middle, n = 8 ; apex, n = 6), the application of a t-test is questionable. In cases of small and unequal sample sizes, non-parametric testing is more appropriate (Madrigal, 1998). It is also not known if the measurements for the complicated sample are normally distributed. Nowhere in their study do Eli et al. (1989) state that their data meet the assumptions required for parametric testing (the assumptions for an unpaired t-test are: 1) random sampling, 2) independence of variates, 3) normality, and 4) homogeneity of variances, see Madrigal 1988: 100).

A further problem with the research of Eli and coworkers is that they <u>only</u> conducted statistical analyses between a natural delivery and a complicated delivery at each crown level. Statistical tests were not employed between all three delivery groups (a natural delivery, a

complicated delivery and an elective c-section) at the DEJ. The authors do not provide their reason(s) for this.

Given the numerous statistical uncertainties of Eli et al.'s study, their conclusions that "the width of the neonatal line increases in children born by operative delivery and decreases in those that have undergone no active birth process (elective c-section)" (p. 222) are speculative.

A second problem with the data of Eli and colleagues is the large standard deviations of their neonatal line measurements at each crown level for both normal and complicated births (see Tables 3-4 and 3-5, page 46). For the normal group, standard deviations range between 4.8 and 7.0 μm, with the middle of the crown exhibiting the largest S.D.; for the operative group, the standard deviations range from 5.7 to 6.4 μm. Consequently, the distribution of measurements between a normal and a complicated birth overlap at each crown position. This overlap is even greater if one looks at measurements of the neonatal line within each tooth class for a normal delivery (i.e. S.D. is largest for molars at the middle of the crown). Given the large S.D.s and the overlap between birth categories, one must question if it would be possible to determine if a child experienced an optimal birth or a complicated one.

In the present study, six to fourteen measurements were taken of neonatal line width along individual prisms at any one-crown location (see Tables 1 – 4, Appendix B). This is in contrast to the method of Eli and colleagues. In their study, only three individual measurements were taken of the neonatal line at each crown location. Because of the range in neonatal line thickness at any given crown position within a specimen (compare Figure 4-17 with Figures 4-6 a,b, Materials and Methods), several measurements are necessary to increase the accuracy of the estimates of neonatal line width. The small number of measurements taken by Eli et al. likely explains their high standard deviations for each delivery type at each crown position. One must question if their mean neonatal line widths are truly representative of the variation in line width within each specimen in each delivery group.

In the present study, measurements of neonatal line width overlap between birth catgeories, even with standard deviations that are much smaller than those observed by Eli et al. Consequently, the statistical results of this thesis are presented as tentative (see Figures 5-3 to 5-7 and Figures 5-9 to 5-24, Results; Tables 7 to 9, Appendix B).

Eli and colleagues do not consider line width differences between crown positions within tooth classes. The figures provided in their study for mean neonatal line width for a normal delivery (Table 3-4, page 46) show that there is a greater difference in neonatal line width between crown positions within tooth classes than there is between crown positions when line width from each tooth class is combined. Also, the neonatal line width measurements used for their ANOVA analysis are biased towards a particular tooth class at each crown position. At the DEJ, mean line width represents line width in both canines (56% of sample) and incisors (44% of sample); at the middle of the crown it primarily reflects line width in canines (canines comprise 78% of the sample at this position); and at the apex it is largely representative of line width in molars (molars comprise 90% of the sample at this position). In addition, line width measurements at each crown position are only available for canines. Because of the unequal representation of tooth types at each crown position, one must question if similar ANOVA results would have been obtained if equal sample sizes for each tooth class were used at each crown position (i.e. if measurements of neonatal line width were included for incisors at the apex and for molars at the DEJ) and if the possibility of intra- and inter-tooth differences was considered. By ignoring these discrepancies, Eli and colleagues compromise the results of their study and leave room for speculation. In addition, these authors do not provide information on intra and inter-tooth differences for the complicated category.

Since the anticipated relationship between the birth process and neonatal line width was not found in the present study, differences in line width between crown positions within tooth classes and between tooth classes, for the entire sample (i.e. disregarding birth variables) and according to delivery type, were examined. Neonatal line width was significantly different between crown positions for molars, with the middle of the crown exhibiting the widest line and the largest

standard deviation (Table 5-10, page 108). The difference in line width between crown positions in molars may have contributed to the statistically significant results of a Kruskal-Wallis test investigating differences between crown positions independent of tooth class (i.e. neonatal line width was significantly wider at the middle of the crown) (Table 5-2, page 95). Line width was also significantly different between tooth classes at the DEJ and at the apex, with canines displaying the largest width at both positions. At the middle of the crown, canines and molars demonstrated slightly larger lines, although these differences were not significant (Table 5-10, page 108; Figure 5-8, page 109).

Differences in neonatal line width between crown positions within tooth classes and between tooth classes were found by type of delivery. There was no consistent pattern to these line width differences. Any tooth class could demonstrate significant differences between crown positions (except within a minimal medical intervention delivery) and any tooth type could demonstrate a significantly wider line depending on the mode of delivery and the crown position examined (Figures 5-9 to 5-11, pages 111 and 112). Because of these differences, statistical testing by tooth class and by delivery type at each crown position revealed inconsistent associations. For comparative purposes, only those tooth classes represented in the Eli et al. study for a natural delivery at each crown position (see Table 3-4, page 46) and for the elective c-section group (see Table 3-3, page 46) are considered. Tooth class composition for the complicated group is not considered because a breakdown by tooth class at each crown position is not provided by Eli and colleagues.

In the current investigation, neonatal line width at the DEJ was found to be significantly larger for a natural delivery when measured in incisors compared to other modes of delivery, but significantly smaller when measured in canines. When measured at the DEJ in incisors, line width for an elective c-section was 7.2 +/- 1.9 μm.

In the natural delivery group of the Eli et al. study, canines show a slightly larger sample size (n = 22) and a smaller line width (10.9 +/- 4.9 μm) when compared to incisors at the DEJ

(13.3 +/- 4.5 μm) (the difference in means is 2.4 μm versus a mean difference of 3.0 μm in the current study). In the elective c-section group, incisors comprise the majority of the sample (n = 3 or 60%); the elective c-section group also demonstrated the smallest line width (7.6 +/- 1.5 μm). In the Eli et al. study, the sample size and mean line width for incisors for the elective c-section group are similar to those of the current study (this study: n = 2; mean = 7.2 +/- 1.9 μm).

In view of the findings of this thesis, it is tempting to suggest that the tooth class composition of the natural delivery group and the elective c-section group at the DEJ in the Eli et al. study affected their statistical testing between all three delivery groups. The larger sample size and the smaller neonatal line width for canines resulted in a small neonatal line width for a natural delivery and the larger incisor sample decreased line width for the elective c-section group. Unfortunately, because a breakdown of neonatal line width by crown position and by tooth class is not provided for their operative group at the DEJ, any conclusions at this time are speculative.

In the present study, neonatal line width at the middle of the crown was wider for an elective c-section when measured in incisors and larger for a complicated delivery when measured in canines. Interestingly, the current investigation revealed no significant difference between a natural and a complicated delivery when neonatal line width was measured in molars (natural: 7.1 +/- 2.5μm; complicated: 8.4 +/- 4.7μm). In the Eli et al. study, both the mean neonatal line width and the standard deviation for a natural delivery at the middle of the crown in molars are large (16.5 +/- 9.0μm). It is doubtful whether a significant difference would be found between a natural delivery and an operative delivery if line width were compared at the middle of the crown in molars (operative delivery: 18.6 +/- 5.7μm). Comparison with the study of Eli and colleagues is tentative at this crown position because the tooth composition for a complicated delivery is not known and because no measurements are available for the elective c-section delivery group.

In the current investigation, neonatal line width at the apex was significantly larger for a natural delivery and an elective c-section when measured in incisors, larger for a natural delivery

than a complicated delivery when measured in canines and wider for a complicated delivery when measured in molars. Again, comparisons with the Eli et al. study are limited due to a lack of tooth class information for their operative and their elective c-section groups.

In the present study no clear pattern of neonatal line width differences emerged between delivery types when examined by tooth class. This is likely a result of the small sample sizes for the majority of tooth classes (especially canines and molars) in all delivery types (Table 8, Appendix B). However, these findings suggest that when a sample is unequally divided by tooth type, expected statistical relationships are possible.[1] When neonatal line width was measured at the DEJ and tooth class was disregarded, no significant difference was found between delivery types. When tooth class was considered, significant differences were achieved. Furthermore, because no consistent pattern emerged between delivery types when examined by tooth class, it is questionable whether a relationship between mode of delivery and neonatal line width truly exists. By disregarding tooth class differences, the results obtained by Eli et al. may simply reflect the tooth class composition of their sample. Further research into inter-tooth differences in neonatal line width is required. Meanwhile, it would be prudent to limit comparisons to the same tooth type.

An obvious discrepancy between the current study and that of Eli and colleagues, is the grouping of individuals according to delivery type. In this thesis, a fourth delivery category was included: delivery by minimal medical intervention. This category was created because medical experts suggest that minimal medical interventions during delivery can have adverse effects on the fetus and on the newborn (Table 3-2, page 36). A similar category was not considered in the Eli et al. study. It is possible that such individuals were accounted for in their natural category given their limited definition for children included in this delivery group: "those with no history of

[1] In the studies of Skinner (1992) and Skinner and Dupras (1993) (pages 53-54), significant differences were found in the position of the neonatal line between pre-term, term and post-term births for some, but not all, tooth classes. Skinner (1992) found significant differences between gestation groups when line position was compared in incisors and second molars. Skinner and Dupras (1993), using the same sample as Skinner (1992), but with additional teeth, found significant differences between gestation groups for incisors and first molars. It is possible that the composition of their sample by tooth class altered their results.

systemic disorders related to pregnancy and/or labor and who had a birth weight no less than 2500g" (p. 221).

In the present study, it was anticipated that line width for a delivery with minimal medical intervention would fall between that of a normal and a complicated delivery. An analysis between delivery types demonstrated that a delivery with minimal medical intervention did not differ significantly from other modes of delivery at the DEJ. At the middle of the crown, line width was the same as for a natural delivery (natural: 7.7 +/- 2.7μm; minimal: 7.7 +/- 2.1μm), while at the apex line width was similar to a complicated birth (minimal: 7.2 +/- 1.5μm; complicated: 7.3 +/- 2.2μm). Given the present data, minimal medical intervention has no apparent effect on neonatal line width.

The current study also included children delivered by an emergency c-section in the complicated delivery group. Eli et al. did not consider children delivered by an emergency c-section in their operative category. They only included breech, forceps and vacuum assisted births. In the present study, these three modes of delivery were considered; however, the majority of children in the complicated group were either delivered by an emergency c-section or forceps. Only one child was delivered by vacuum extraction (Table 3, Appendix B).

Eli et al. claim that children undergoing no active birth process (i.e. an elective c-section delivery) exhibit thin lines. It could be argued that in the present study the inclusion of children delivered by an emergency c-section (where there also is no active birth process) in the operative category decreased the width of the line for this delivery group. Table 3 in Appendix B shows that the neonatal lines in children delivered by an emergency c-section are both thin and wide; this is also the case for infants delivered by forceps. It appears unlikely that inclusion of this delivery type decreased the width of the line for the complicated category.

The research of Eli et al. (1989) must be examined in combination with medical investigations that have considered the effects of the birth process on the outcome of the fetus and the newborn. When birth variables were created for the present study, it appeared that the

grouping of individuals established by Eli et al. was logical. However, a review of the obstetrical literature suggests that had Eli et al. investigated the effects of operative modes of delivery on the health of the neonate, they would not have grouped their children the way they did. They also would not have proposed that infants undergoing an elective c-section should exhibit thin neonatal lines and seemingly less difficult births.

Medical research has demonstrated that infants undergoing an elective cesarean section often have more difficulty with newborn adjustments (i.e. breathing) than do children delivered vaginally (Simkin, 1989; Rosenberg, 1991). The incidence of mild transient respiratory signs in the newborn appears to be higher after a cesarean delivery than after a vaginal delivery (Danforth, 1985). It has also been shown that infants born by cesarean section show increased lung fluid and are less likely to establish a normal FRC (functional residual capacity) with the initial breath and during the first six hours of life (Rosenberg, 1991). The inability to expel lung fluid in infants delivered by an elective c-section results from the absence of the "vaginal squeeze" and from a lack of labor (Bland et al. 1979; Danforth, 1985; Rosenberg, 1991). The importance of labor during birth has been demonstrated by the fact that infants delivered by an emergency c-section after labor behave more like vaginally delivered infants in their breathing response (Bland et al. 1979; Rosenberg, 1991).

There is also disagreement among obstetricians regarding the use of operative modes of delivery and their effect(s) on the outcome of the infant. The literature is too extensive to summarize in a few lines. Generally, most experts advocate that an operative delivery (i.e. forceps, vacuum) is not harmful to the fetus/newborn (even in the presence of fetal distress) if employed judiciously and properly (see Krebs et al. 1982 and O'Grady 1988: 242 - 249 for a review of these studies). Interpretation of fetal heart monitoring is also complex and experts disagree amongst themselves when a child is showing distress and when intervention is necessary. One should not assume that because a child was delivered operatively (i.e. forceps or vacuum), that he/she experienced a complicated birth (i.e. was distressed). It is plausible that a child born naturally experienced a more difficult birth in the event that obstetricians did not detect

fetal distress or that they misinterpreted the fetal heart rate patterns. Fetal heart rate monitoring cannot quantify the metabolic effects of stress on the fetus in any precise way (Steer 1985: 22).

Eli and colleagues propose that "the trauma of the birth process itself has a major impact on the newborn's cells" (p. 222). Given the lack of consensus among the medical community regarding the effects of the mode of delivery on the fetus and on the newborn, it is not surprising that this relationship was not demonstrated in the present study. Classifying a child by mode of delivery is not necessarily representative of the stress (i.e. fetal distress) experienced by the infant at the time of birth. More importantly, given the medical research into the effects of a cesarean section on the newborn, a wide neonatal line (i.e. possibly resulting from amelobalst sensitivity to oxygen deprivation) would be expected for this group, not a thin line as suggested by Eli and colleagues. Similar to the study of Ranggard et al. (1995), the present study found that some children in the elective c-section group exhibited large neonatal lines (individuals 1 and 10 in Table 4, Appendix B), although a widened neonatal line was not consistently found at each crown position. Consequently, it cannot be concluded that this delivery method resulted in the larger lines. A relationship between the birth process and the width of the neonatal line is difficult to identify because of the disagreement among medical experts regarding the effect(s) of the method of delivery on the newborn.

B. Neonatal Health, Maternal Health and Term at Birth

Previous clinical studies of deciduous enamel have demonstrated a higher incidence of enamel hypoplasia in children experiencing perinatal disorders than in children not experiencing these difficulties. Given that the neonatal line forms during the secretory stage of amelogenesis and that pathological striae are recognised as more sensitive indicators of stress (Skinner and Goodman, 1992), it was anticipated that neonatal line width would be larger in children suffering health complications at birth, in children born to sickly mothers, and in children born before term. Although earlier studies have included observations of the neonatal line, few have provided line width measurements. Comparing neonatal line width to these earlier studies is not possible. This

section considers if the results of the current investigation are in accordance with the findings of earlier studies.

The most common health complications experienced by the children in this study were breathing complications (n = 11), followed by signs of fetal distress (i.e. irregular heartbeat and meconium staining) (n = 5), birth injuries (n = 3), infections (n = 3), severe allergic reactions (n = 2), growth retardation (n = 2), stomach complications (n = 1) and a cold (n = 1) (Table 5, Appendix B). With the exception of the last two health problems, all other complications encountered during this investigation have been implicated in enamel defect formation. Among children with difficulties at birth, both thin and wide neonatal lines were observed (range: DEJ: 4.2 to 12.2μm; middle: 4.5 to 13.8μm; apex: 5.4 to 12.7μm) (Table 5, Appendix B). Despite the large range of neonatal line widths in non-healthy children, the present study revealed that at all three crown positions mean neonatal line width in children with health complications was significantly larger than in children who were born healthy (mean difference between 0.7 and 1.4μm, depending on crown position).

Few studies have examined the effects of invasive medical interventions on the newborn. Seow (1992) and Brooks et al. (1997) found that children who were intubated at delivery demonstrated a higher occurrence of enamel hypoplasia. In the current study, infants undergoing medical interventions at delivery and shortly after birth demonstrated significantly wider neonatal lines at the DEJ (mean difference = 0.9 μm) and at the middle of the crown (mean difference = 1.4μm); the opposite relationship was observed when line width was compared at the apex (mean difference = 0.4μm) (Table 7, Appendix B). The most common interventions encountered in the present study were intubation, IV antibiotic administration and incubation (NICU) (Table 5, Appendix B).

Children born to a mother who experienced complications at the time of delivery (toxemia/blood pressure changes: n = 5, bleeding: n = 2 and infection: n = 2) and/or who became ill close to the time of childbirth (gestational diabetes: n = 3, anemia: n = 1, hypothyroidism: n = 2)

exhibited a large range of neonatal line widths (DEJ: 5.1 to 12.1μm; middle: 5.8 to 13.8μm; apex: 5.6 to 12.7μm) (Table 6, Appendix B). As anticipated, statistical analysis demonstrated that mean line width was significantly wider at the DEJ (mean difference = 0.7μm) and at the middle of the crown (mean difference = 1.1μm) in children whose mother was ill at/around the time of delivery. No significant difference was found between mean neonatal line width at the apex in children whose mother experienced complications and those whose mother did not (mean difference = 0.2μm) (Table 7, Appendix B). With the exception of hypothyroidism, all of the maternal complications encountered during this investigation have been implicated by some dental researchers in enamel defect formation.

Dental research has demonstrated conflicting results regarding the effects of hyperbilirubinemia on enamel development. Some studies have found that severe cases of jaundice result in enamel hypoplasia and "accentuated" neonatal lines (Miller and Forrester, 1959). Others have demonstrated no relationship between physiological jaundice and enamel formation (Hals and Grahnen, 1965). In the present study, no significant difference was found in neonatal line width between infants exhibiting physiological jaundice and those not born jaundiced at any of the crown positions. Although the differences were not significant, children not born jaundiced demonstrated slightly larger lines at each crown position (Table 7, Appendix B).

As anticipated, infants born preterm demonstrated significantly wider neonatal lines than children born at term at both the DEJ (mean difference = 2.1μm) and at middle of the crown (mean difference = 1.2μm). At the apex, infants born at term demonstrated wider neonatal lines (mean difference = 0.7μm). These results are tentative given the unequal sample sizes between both groups (Table 7, Appendix B).

When neonatal line width was compared between birth categories at each crown position in molars, the expected relationships were always obtained. Because of the small number of measured prisms in molars in children born to a mother with health complications (n = 8 or 10)

and in children born jaundiced (n = 12 or 14) (Table 9, Appendix B), the relationships observed for these birth categories at each crown position are tentative.

When neonatal line width was compared between birth categories at the DEJ in canines, expected results were obtained for two birth categories: medical interventions at birth and maternal complications at birth. If neonatal line width was compared at the middle of the crown in canines, anticipated associations occurred for the majority of birth categories, with the exception of jaundiced at birth. When neonatal line width was measured at the apex in canines, the associations were either opposite to what was anticipated or not significant. No statistically significant differences were found between both categories of health at birth when neonatal line width was measured at the DEJ or between both categories of jaundiced when line width was contrasted at the middle of the crown. The results for canines are speculative given their small sample size for the sample as a whole (n = 9) and the small number of measured prisms (n < 30) at several of the crown positions for many of the birth categories (Table 9, Appendix B).

The anticipated relationships between birth variables were only observed for infant health at birth when line width was measured at the DEJ in incisors and for term at birth when line width was compared at the DEJ and at the middle of the crown. In all other instances, the relationships between birth categories were either opposite to what was anticipated or not significant when line width was measured in incisors.

The findings presented above are interesting for three reasons: 1) expected statistical differences were always found when neonatal line width was examined at the DEJ and at middle of the crown if tooth class was disregarded, 2) comparison of neonatal line width between birth variables at the apex either resulted in unexpected relationships or in results that were not statistically significant and 3) when line width was compared using incisors, the anticipated relationships between birth categories were rarely observed and, in many instances, non-significant results were obtained (similar results were obtained when line width was measured at the apex in canines). It is difficult at this time to determine if these observations are a reality (i.e. if measuring at the tooth apex or in incisors is not reliable) or whether they are a consequence of

the small sample sizes used during the current analysis. It can further be argued that because incisors comprised the majority of the sample and because anticipated results between birth categories were rarely obtained, neonatal line width has no effect on neonatal line width when measured in incisors. Apart from these observations, no consistent patterns are evident between birth categories when neonatal line width is compared by crown position or by tooth class. Consequently, the results of this thesis suggest that neonatal and perinatal factors may not have an effect on the width of the neonatal line.

C. The Length of the Birth Process

Via and Churchill (1959) are the only researchers who attempted to determine if a prolonged birth (> 20 hours) resulted in a higher occurrence of enamel defects. Although they found that enamel hypoplasia and one or more of the birth complication factors they examined occurred in 30 percent of infants, no conclusions could be reached as to the specific mechanism(s) responsible for the enamel aberrations.

The current study examined the effect of the length of the entire delivery and the length of the second stage of labor (i.e. the pushing stage) on the width of the neonatal line. Based on previous medical research, it was anticipated that children exposed to longer periods of labor would exhibit wider lines (i.e. more uterine contractions would result in more episodes of oxygen deprivation). A Spearman's *r* correlation revealed no significant associations between the length of labor or the "pushing" stage and the width of the line at any of the crown positions (Table 5-17, page 130). Examination of individual line widths from nine children who were exposed to prolonged labor (i.e. greater than 14 hours for the entire process) revealed both thin and wide neonatal lines; four infants endured a second stage of labor in excess of 2 hours (Table 6-1). Based on these results, the width of the neonatal line does not reflect the duration of the birth process.

D. The "Normal" Width of the Neonatal Line

It is difficult to ascertain what in fact constitutes a "normal" width for the neonatal lines in the current investigation. The most conservative approach is to examine neonatal line width for

children in the healthy birth categories as representative of a "normal" line. When examined across birth variables independent of tooth class, neonatal line width ranges between 7 and 9 μm. When examined by tooth class, the normal width falls between 6 and 9 μm depending on tooth class and crown position. Inspection of individual neonatal line widths shows that the majority of neonatal lines fall below 10 μm; 5 individuals show lines exceeding 10 μm at the DEJ, 10 individual neonatal lines are larger than 10 μm at the middle of the crown and 4 individual line widths exceed 10 μm at the apex (Tables 1-4, Appendix B).

Table 6-1. Individual neonatal line widths of children exposed to prolonged labor or a prolonged "pushing stage".

NNL #	Total Birth Length (hrs.)	Pushing Stage (mins.)	Line Width (μm) DEJ	Middle	Apex
2[1]	**36.5**	0	5.2	4.8	8.0
5[2]	**19.0**	-	8.9	9.8	8.4
8	13.25	**> 120**	10.0	12.2	7.8
9	**26.75**	90	9.5	9.8	10.1
12	2.25	**> 120**	8.0	-	6.9
15	**23.0**	60	7.6	8.3	7.5
23	**16.5**	5	10.2	6.5	8.5
24	**24.0**	5	7.0	8.1	9.3
34	6.25	**> 120**	6.2	5.9	6.6
36	14.0	**> 120**	5.6	5.4	-
37	**21.25**	45	9.3	10.7	-
41[2]	**23.5**	-	12.2	11.3	11.1
48	**15.0**	50	3.7	4.6	4.5

1 Emergency c-section; no pushing
2 Mother did not remember how long she was pushing

The range of individual neonatal line widths in the current study is similar to that of Ranggard and colleagues (1994 and 1995) (Table 3-6, page 48). These researchers observed both thin and wide neonatal lines and found that the majority of their lines (73%) fell below 10 μm. The normal line width proposed for this investigation, although tentative, is also similar to that identified by Jaffe et al. (1985) (5 to 8 μm). Wider neonatal lines as proposed by Eli et al. (12 μm), and Noren and colleagues (1978) (10 – 12 μm) were rarely seen. Lines exceeding 16 μm (as proposed by Whittaker and Richards, 1978) were never observed.

Comparison of actual line width between studies is tentative given that the measuring techniques of many investigations are not known, that different observation equipment was employed (microradiography, SEM, light and polarizing microscopy) and that different tooth classes were examined (pages 42-43).

In the present study, there was an evident skewness to the distributions of neonatal line width measurements within specimens and within birth catgeories with a clear dominance of thin neonatal lines. Consequently, the median values were, in the majority of cases, smaller than mean line width. The median value may, in fact, be a better estimate of neonatal line width, not the mean. Ranggard et al. (1994 and 1995) also suggest that the median is more representative of line width because of the variations in line width measurements (i.e. large ranges) within individuals. The only drawback is that statistical testing would not be possible if median values were used.

Criticisms of the Current Investigation

Because the present discussion has focused on criticizing the work of others, it only seems fair to devote a portion of this chapter to a critical review of the methods employed during the current investigation. Two areas will be examined: the statistical procedures employed and the measuring technique used to estimate line width. These are discussed below.

A. Statistical Testing

In an effort to be as conservative as possible during all statistical analyses, non-parametric tests were used in the majority of cases. These tests were employed because 1) they are distribution free (in many instances the data sets were hard to normalize), 2) they are particularly well suited for small and uneven sample sizes (the sample was small overall and there was an unequal representation by tooth classes and by birth categories) and 3) they do not require data to be measured precisely (there is a lack of information in previous studies on how to measure the neonatal line) (see Madrigal 1998: 130). Independent t-tests were only conducted when all of the assumptions required for this test were met (see page 139) and when the sample size was larger than 30. In an effort to increase sample size, individual prism measurements were also used.

Despite these precautions, it is possible that statistically significant results were attained even when neonatal line width was not significantly different between birth categories (i.e. the null hypothesis was rejected when in fact it was true). The probability of such an error (Type I error) is 5% ($\alpha = 0.05$) or that 1 out of 20 results obtained from the study population could have led the researcher (KB) to erroneously reject the null hypothesis. The statistical relationships observed in the present study are presented as tentative because of the large overlap between the distributions of each group at each crown position (i.e. large ranges and S.D.) (Tables 7 and 9 in Appendix B) and the small statistically significant mean differences between many of the birth categories, independent of tooth class and according to tooth class (Tables 7 and 9, Appendix B). This last point is crucial given that the power of a statistical test[2] is higher the more different the means (Madrigal, 1998).

B. The Measuring Approach: Is It Possible to Quantify Disturbances in Enamel Formation?

Some dental researchers have suggested that the width of the neonatal line is dependent on the plane of section. Consequently, these researchers have not attempted to measure the

[2] Power is the ability of a test to reject the null hypothesis when it is false (i.e. the means are significantly different) (Madrigal, 1998).

width of the neonatal line (Hals and Grahnen, 1965; Jackobsen, 1975). In the present study, neonatal line width was measured following the paths of individuals prisms. Since prisms represent the direction of enamel growth, this measuring approach seems reasonable. However, because prisms in a longitudinal plane do not follow a straight course from the DEJ out to the enamel surface, sectioning an entire prism along its length is not possible. By measuring along an "apparent" prism, one may in fact be measuring disturbances along several prisms.

Osborn (1967, 1968a – c, 1981, 1990) has reconstructed prisms in three dimensions. His studies have demonstrated that in a longitudinal section of enamel, what appears as one prism running from the DEJ to the enamel surface, is in fact a composite of several prisms that have been cut along their ends (circular profiles) and/or along their sides (elliptical profiles) (Figure 6-3). Consequently, depending on the angles that the cut prism borders make with the direction of the light, several elliptical prism profiles and round prism profiles can appear as one longitudinally cut prism. Because prisms also move in and out of the plane of section, only a small portion of the cut borders of very tortuous prisms will be seen in longitudinal sections.

In measuring neonatal line width, it is difficult to determine if measurements are being taken along prisms formed by very active ameloblasts (i.e. one that decussated more) or less active ones (i.e. one that decussated less). Consequently, if measurements are taken along a prism whose decussation is great, only small portions of the prism borders are being measured and the entire disturbance along that prism is not being quantified. The issue of prism decussation would not be problematic if all prisms within a tooth and between teeth behaved similarly. However, because variations in prism decussation patterns are evident within a tooth and between teeth (FitzGerald, 1995), it is possible that greater segments of a disturbance are being measured along some prisms and not along others. Comparison of neonatal line width between individuals may not be dependable.

In the present study, the structural morphology seen along the neonatal line was similar to Rose's (1977) description of "distorted" Wilson bands. In 1979, Rose conducted a SEM analysis of his 1977 light microscopic definitions of "pathological" bands, and concluded that the

distorted band could be grouped into ridge bands or trough bands. Ridge bands represented prisms that were cut transversely (i.e. along their ends), while trough bands represented prisms that were cut longitudinally (i.e. along their sides). These results led Rose (1979) and Marks (1988, 1992) to conclude that the prism morphology seen along Wilson bands is not a measure of ameloblast susceptibility to systemic disturbances (i.e. the greater the prism disturbance, the more severe the insult), but merely a result of the plane of section. If, in the present study, some prisms were cut transversely, while some were sectioned longitudinally, measurements taken along the prism borders within the neonatal line may not reflect the severity of the disturbance. In this study, large ranges in line width were evident within individuals and between individuals. Smaller measurements may represent transversely cut prisms while larger measurements may represent longitudinally cut prisms.

The discussion presented in this section is hypothetical; however, this problem warrants more investigation than was possible in this study. When compared to light microscopy, scanning electron microscopy offers a unique opportunity to examine the "structural reality" of the prisms associated with developmental disturbances (Marks, 1992). Only one study has attempted to measure neonatal line width using SEM (Whitakker and Richards, 1978). More studies examining the effect of the plane of section on the width of the neonatal line are required.

Weber and Eisenmann (1971) have argued that section thickness has an effect on the width of the neonatal line. In thicker sections the line is wider and more evident, while in thinner sections of the same tooth, line width is thin and less apparent. In the present study, every effort was made to maintain a uniform section thickness of 100 μm. This thickness is recommended by many investigators for optimum viewing of dental microstructures (FitzGerald, 1995). However, it was not always possible to maintain a consistent size within and between sections using manual preparation techniques. In fact, section thickness ranged between 70 and 130 μm. The effects of section thickness on the width of the neonatal line both within and between specimens were not contemplated. This problem requires further research.

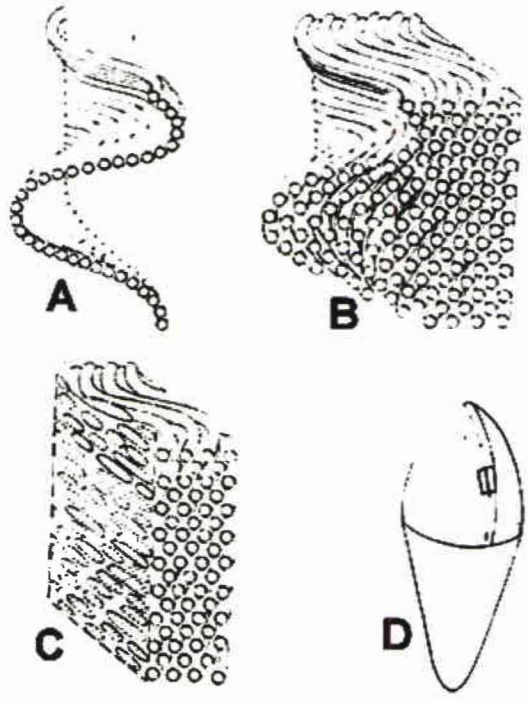


Figure 6-3. The arrangement of enamel prisms in human enamel (after Osborn, 1981). A schematic diagram demonstrating prism decussation of a single vertical row of prisms (A) from the DEJ and of several groups of vertical prisms (B). When sectioned longitudinally, portions of several prisms on the left are removed, resulting in elliptical and circular segments giving the illusion of one prism (C). The orientation of the block of prisms in B is depicted in D.

The Contribution of the Current Study to Anthropology and Other Research

It was anticipated that the neonatal line would prove to be a reliable diagnostic tool for physical anthropologists studying archaeological specimens and during forensic investigations. Presently, the findings of this thesis do not support its use as a measure of neonatal morbidity and birth trauma. The most important drawback is the lack of a standard measuring approach among studies of the neonatal line. If the neonatal line is to be used as a diagnostic tool, consensus must exist among researchers regarding how neonatal line width should be measured.

Statistical analyses demonstrated that significant differences exist between groups of individuals with regard to birth trauma, neonatal health, maternal health and term at birth. However, in many instances, these associations were not in accord with previous dental studies

and the relationships between birth categories were not consistently observed at each crown position, especially when analyses were conducted by tooth class. This is likely a result of 1) the small sample size in the present study, 2) the division of birth categories by crown position and by tooth class and 3) the inclusion of the same individuals in several categories. At present, the lack of a consistent pattern between birth variables suggests that neonatal line width does not reflect the severity of the insult that led to its formation.

The results of this thesis do contribute to the anthropological and the dental literature. It has been suggested that the width of a defect is a reflection of the severity of the insult that caused it (Suckling, 1989; Goodman and Rose, 1990). The findings of this thesis dispute this claim. The lack of an association between defect width and the severity of disruptions might be explained by the fact that primary enamel itself is less susceptible to defect formation because of its fast rate of growth (Goodman and Rose, 1990; Skinner and Goodman, 1992; Skinner and Goodman, 1992). Specifically, an inverse relationship has been proposed between enamel's susceptibility to defect formation and its rate of formation (Skinner and Goodman, 1992). Consequently, deciduous enamel may not be a reliable indicator of infant and childhood morbidity.

The neonatal line is a permanent feature of deciduous enamel. Its presence suggests that ameloblasts are susceptible to systemic disturbances at the time of birth. Although its width may vary within and between individuals, neonatal line width does not represent the severity and/or duration of the birth process, nor does it reflect an individual's susceptibility to complications at birth. A neonatal line is a neonatal line is a neonatal line.

Anthropologists suggest that it is important to distinguish between susceptibility of the individual to insults from susceptibility of the individual's tissue to systemic disturbances (Skinner and Goodman, 1992). Studies have demonstrated that susceptibility to defect formation varies both within teeth and between teeth. Studies of Wilson bands have found that the canine is more susceptible to defect formation; the canine displays more Wilson bands when compared to teeth developing at the same time (i.e. the premolar) (Goodman and Rose, 1990; Condon and Rose,

1992). In the present study, when neonatal line width was examined by tooth class for the sample as a whole, the canine demonstrated a significantly wider line when compared to incisors and molars at both the DEJ and at the apex. Given this finding it is tempting to suggest that the canine is more susceptible to the formation of a wider neonatal line. However, because a significant difference was not found at the middle of the crown and because the sample size for canines was small in the current study, such a conclusion is highly speculative. Further research into the susceptibility of dental tissues to neonatal line formation is required.

The main contribution of this thesis to dental studies is the identification of a lack of rigorous procedures during previous investigations of the neonatal line. The findings of the present study also demonstrate that the conditions once identified as potential causative factors in enamel hypoplasia formation (i.e. jaundice, infant health at birth, maternal health at birth) do not have an effect on the width of the neonatal line. Consequently, the severity of the birth process and the effect(s) of perinatal and neonatal factors on enamel development must be reviewed using stringent techniques. Contrary to earlier dental studies of the neonatal line, the present study examined inter-tooth differences and identified that differences in neonatal line width exist between tooth classes. Future investigations must be done according to tooth type. It is also apparent that dentists and medical experts require a better understanding of the effect(s) of the birth process/mode of delivery on infant health before further research of the neonatal line is attempted.

The physiological pathways of defect formation are current topics in dental research. However, the limited value of enamel defects (i.e. neonatal line, enamel hypoplasias) to dental practitioners (i.e. recognizing that systemic stress of an idiopathic nature has occurred) does not promote the application of methodological rigor during histological analysis and data collection. To the anthropologist, studying how neonatal and childhood conditions impact the demographic profile of an archaeological population is advantageous and important. Collaborative efforts between the dental community and the anthropological community are necessary if enamel defects are to be employed in the study of past populations.

Chapter Seven

Summary and Conclusions

Summary

The objectives of this thesis were a) to critically examine the study of Eli and colleagues (1989) and to compare the results of the present study with their findings, b) to test statistically the effect of birth trauma, neonatal health, maternal health, term at birth and birth duration on the width of the neonatal line and to compare the results of this thesis to the expected associations demonstrated by earlier studies of deciduous enamel in perinatal disorders, c) to examine line width differences within tooth classes and between tooth classes, d) to determine a "normal" neonatal line width, and e) to achieve the preceding using objectively credible methodological rigor. The intention of this thesis was that the results would either validate the use of the neonatal line as a diagnostic tool in physical anthropology and during forensic investigations or provide evidence to strongly question its use.

The overall conclusion from this study is that the results of previous studies of the neonatal line are problematic and that the width of the neonatal line does not reflect the severity of the disturbance that caused it. Although this study demonstrated significant results between birth categories, these findings are not definitive because of the small sample size of the present study and the even smaller sample sizes when associations were examined by crown position and by tooth class. Unfortunately, divisions of each birth category by tooth class and by crown position were necessary. Future investigations of the neonatal line must include a larger sample size as well as an equal representation of each tooth class. Despite these limitations, several important conclusions can be drawn based on the findings of this thesis.

Main Conclusions

- The lack of a rigorous methodology and the omission of important information in previous studies of the neonatal line make it difficult to replicate earlier studies with confidence and only permit general conclusions to be made about inter-group similarities and differences. Future dental investigations must include detailed methodological information. The creation of a permanent record using digital images is advocated.
- Neonatal line width is significantly different between crown positions within an individual tooth, indicating that ameloblast sensitivity to disturbances is not uniform within the enamel crown (in contrast to Eli et al. 1989) and that comparisons between individual neonatal line widths must be conducted by crown position.
- Neonatal line width is significantly different between tooth classes, with canines exhibiting the largest line width at the DEJ and at the apex. Comparisons between neonatal line width should be conducted according to tooth class. Susceptibility of different teeth to neonatal line formation requires further research.
- There is a large overlap in neonatal line width estimates between individuals and between birth categories. Although neonatal line width differences were detected, the differences in mean neonatal line width between birth categories are small. These observations render the statistical associations speculative.
- There is an evident skewness to the distributions of neonatal line width measurements within specimens and within birth categories with a clear dominance of thin neonatal lines. The median value may be a better estimate of neonatal line width.
- Because of the variation in line width measurements within a specimen, numerous measurements (i.e. more than three as proposed by Eli et al. 1989) must be taken to better approximate neonatal line width.
- The range in neonatal line width estimates within individuals and between individuals may reflect the measuring approach used during this investigation and/or the plane of section.

Research is required regarding the effect of prism morphology, the plane of the section and section thickness on the width of the neonatal line.

- The conclusions of Eli et al. (1989) that neonatal line width is significantly wider for a complicated birth and significantly smaller in children undergoing a cesarean section were not observed in this investigation. In fact, no consistent pattern exists between delivery types; the significant associations change depending on crown position and tooth class. Lines greater than 15 μm (indicative of a complicated birth, after Eli et al. 1989) were never observed.
- The discrepancies between the present study and that of Eli et al. (1989) are attributed to the numerous problems associated with their choice of statistical procedures, their sample size and its constitution, and their classification of children according to mode of delivery as reflecting the severity of the birth process. Grouping children by mode of delivery is not justified given the lack of understanding among medical experts regarding the effect(s) of the mode of delivery on the health of the neonate and when medical intervention is necessary.
- Comparison of neonatal line width with the findings of previous studies on perinatal disorders and neonatal enamel hypoplasia demonstrate inconsistent findings. At times, the associations are as anticipated, while at other times they are not. The significant associations change depending on crown position and tooth class.
- No correlation exists between the duration of the birth event and the width of the neonatal line. In the present study, both thin and wide neonatal lines were observed, regardless of the duration of the delivery.
- Because of the lack of methodological rigor in earlier studies, it is difficult to determine the "normal" width of the neonatal line. In the present study, the majority of neonatal lines fell below 10 μm with a clear dominance of thin lines. This finding is comparable to the results of Ranggard and colleagues (1994 and 1995).
- The findings of this thesis do not support the use of the neonatal line as a diagnostic tool in physical anthropology or during forensic investigations.

Appendix A: Participant Recruitment Flyer and Consent Form and "Birth History" Interview

The Tooth Fairy Needs Your Help!

My name is Kristina Blyschak. I am a Masters student in McMaster University's Department of Anthropology. I am currently investigating the relationship between early tooth development and birth history in a sample of Canadian children. I am looking for parents and children who would be willing to help me in my research. From the **children, between the ages of 5 and 12, I would require any shed baby teeth (both front and back) that have been lost in the past or that will be lost in the near future (within the next year).** From the mother, I would ask that you donate half an hour of your time to participate in a short interview, answering key questions about the birth of your child. Please note that confidentiality with respect to your responses, and yours and your child's identity will strictly be maintained. In return, both you and your child will have the opportunity to learn about scientific research, especially as it relates to tooth development, and to visit the labs at the university. Your child will also receive a personalized colored image of their tooth as viewed under the microscope!!

If you are interested in participating in this study, please leave your name and a number where I can contact you, and return this notice to _____. Please be advised that scheduling of the interview, as well as collection of teeth will be done at your convenience. If you require further information, you can contact me at (905) 525-9140, ext. 24423, or at home at (905) 524-9862.

Sincerely,
Kristina Blyschak

Name: _____

Number (work/home): _____

CONSENT TO PARTICIPATE IN RESEARCH

Dear Participant,

My name is Kristina Blyschak. I am a Masters student in McMaster University's Department of Anthropology. I am currently investigating the relationship between the neonatal line, a microscopically visible line of altered growth found in the enamel crowns of all baby teeth, and birth history in a sample of Canadian children. I am exploring this topic because few studies have examined how the birthing history of both the mother and child affect early dental development. Studying the effects of health and the circumstances surrounding birth on the neonatal line will also allow inferences to be made about fetal growth and the birth process in past human populations. To conduct this research I ask that you donate at least one of your child's baby teeth for microscopic analysis and that you take part in a short interview at your convenience. This interview will consist of key questions regarding your health during pregnancy, the type of pregnancy you experienced, and any complications that you and your child may have experienced during both the pregnancy and delivery.

This interview takes approximately half an hour to 45 minutes to complete. There are no right or wrong answers. You are free to decline answering any questions that make you feel uncomfortable and may withdraw from the interview at any time. With your permission, the interview will be tape-recorded. If at any time you feel uncomfortable with the tape recording of the interview, please let me know and I will turn off the tape recorder and take notes instead. If you wish, I will provide you with a written copy of your interview and a report of my study findings. I promise to keep your identity, your child's identity and all information gathered from this study confidential. In return for your participation, both you and your child will have the opportunity to learn about scientific research, especially as it relates to tooth development, and to visit the labs at the university. Your child will also receive a personalized digital image of their tooth as seen under the microscope.

I sincerely appreciate your participation. If you require more information about the study you can reach me at McMaster University at (905) 525-9140, ext. 24423, or you can contact my supervisor, Dr. Shelley Saunders, at (905) 525-9140, ext. 23903. This study has been reviewed and received ethics clearance through the McMaster Research Ethics Board (MREB).

Thank you,

Kristina Blyschak

I understand the information provided for this study as described herein. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

Participant's Name _____
Participant's Signature _____
Date: _____

In my judgement, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent to participate in this research study.

Investigator's Signature: _____

Birth History Interview

Code: _____
Date: _____
Participant's Name: _____
Address: _____
_____
Phone Number: _____
Child's Name: _____
Number of teeth: _____
Location of Interview: _____
Interview Start Time: _____
Interview End Time: _____

Interview Questions

1. Can you please describe the birth of _____ (child's name).
2. Where was your child born?
3. What is the date of birth of the child for whom teeth have been donated for this study?
4. What is your date of birth?
5. Can you tell me the approximate weight of your child at birth?

   a. Was your child considered "small for date" at the time of the delivery?
6. Was your child born around the date expected for a normal full term birth (38-42 weeks)?

   YES

   NO - Was he/she born before or after the date expected for a normal full term birth?

   How much earlier or later was he/she born?
7. What type of birth did you experience? Did you experience a:

   a. normal birth (no medical intervention)

   b. complicated birth (includes breech birth, forceps, vacuum extraction, Cesarean section)

   c. elective c-section

   d. home delivery
8. If you experienced a complicated birth, can you describe/name the type(s) of medical intervention you received?

induction (starting) and augmentation (speeding up) of labor i.e. stretch and sweep, artificial rupture of membranes (breaking of water), prostaglandin gel;

forceps: two spoonlike instruments are inserted into the vagina and applied to each side of the baby's head;

Vacuum extraction: a cup device is applied to the baby's head and attached to a rubber tube that connects to a vacuum pump;

Cesarean section (emergency/elective);

Other: _____

9. Did you receive any medications during your delivery?

   NO

   YES – Can you tell me what medications you received?

   oxytocin/syntocinon: medication given in intravenous by a pump to stimulate contractions;

   narcotic analgesics (i.e. demerol, morphine): given in muscle or vein using a needle; reduces pain and increases relaxation;

   narcotic antagonists: given to mother during labor or after birth to reverse the effects of narcotics (such as Demerol);

   Gravol: given in muscle or vein using a needle; decreases nausea; most often given with demerol/morphine;

   sedative and hypnotic: pill taken by mouth; promotes sleep and relaxation and decreases anxiety;

   local anesthetic: most often used when incision is made; effective only in the area where medicine is injected (used only for episiomtomy);

   pudendal block: given to relieve discomfort during delivery, when forceps or vacuum extraction are needed, episiotomy and repair; needle inserted through vagina;

   epidural: regional anesthesia given in active labor or for forceps, vacuum delivery, Cesarean section;

   general anesthetic: may be used for Cesarean section; complete loss of sensation and consciousness;

   nitrous oxide: sometimes referred to as "laughing gas";

   blood transfusion

10. How long was your delivery? (To answer this question, the beginning of delivery begins when the cervix is fully dilated, followed by the pushing stage and ends when the baby is born)

11. Did you experience any illnesses during your pregnancy?

    NO

    YES – What illnesses did you experience?

    gestational diabetes

    high blood pressure

    toxemia/eclampsia

    infections (viral, parasitic, bacterial)

    anemia (iron deficiency)

    cardiovascular disease

    hormonal disorders (i.e. thyroids and parathyroids)

    disorders of the adrenal and pituitary glands

    renal (kidney) disorders

    liver disorders

    pulmonary disease (i.e. asthma)

    gastrointestinal complications (persistent nausea and vomiting i.e. morning sickness)

    vitamin and nutrient deficiency/overefficiency (i.e. vitamin C, D B, folic acid, calcium, phosphorous)

    genetic disorders, what kind(s)?

    Other: _____

How did this/these illnesses affect your health during pregnancy? What symptoms did you experience?

12. If you did experience illnesses during your pregnancy, did you receive/take any medications?

    NO

    YES – Can you name/describe the medications you received?

13. How would you describe your eating habits during your pregnancy?

    a. Did you make an effort to eat properly?

    b. Did you make an effort to eat foods from all four food groups (milk and dairy products, fruits and vegetables, protein rich foods, breads and cereals)?

c. Did you take any vitamin and/or nutrient supplements?

d. Did you experience any vitamin and/or mineral deficiencies/oversufficiencies?

e. Did you experience gastrointestinal complications (i.e. morning sickness)?

f. Approximately how much weight did you gain during your pregnancy?

14. Did your child experience any illnesses/complications during and/or shortly after birth (2 weeks after birth)?

NO

YES - What illnesses/complications did he/she experience?

irregular heartbeat

breathing complications – coughing/wheezing/asphyxia

fever

prolonged irritability and crying (colic)

skin changes – rash/ yellow colour of skin (jaundice)

cranial/scalp injuries (may result from use of forceps)

spinal cord injuries

fractures due to birth process (i.e. collar bone)

Other: _____

15. Did your child receive/require any medical interventions after delivery?

NO

YES – Can you name/describe the type(s) of medical intervention(s) that he/she received?

Intensive Care (incubation)

Blood transfusion

Orotracheal tube/laryngoscope/ventilator

Intravenous fluids/alimentation

Jaundice treatment

Other: _____

16. Can you tell me if your child experienced any illnesses/complications during his/her first year of life?

    NO

    YES – Do you recall at what age he/she experienced these complications and what the nature of the illness/complication was?

17. How soon after delivery did you begin to breastfeed your child?

    a. How long did you breastfeed?

18. Did you take part in prenatal classes during your pregnancy?

    a. If yes, did you use breathing relaxation techniques?

19. Is there anything else you would like to add regarding your birth experience and yours and your child's overall health during your pregnancy and delivery?

Demographics

20. What is your ethnic background?

21. What is your husband/the father of your child ethnic background (if not current husband)?

22. Can you tell me which of the following categories best describes the total household income from all sources?

    less than 10000$
    10000 – less than 20000$
    20000 – less than 30000$
    30000 – less than 50000$
    More than 50000$

Appendix B: Descriptive Statistics

Table 1. Individual neonatal line width (μm) at each crown position for children undergoing a natural delivery.

NNL #	Tooth Type	Sex	DEJ Mean (SD)	Range width	N	MID Mean (SD)	Range width	N	APEX Mean (SD)	Range width	N
7†	I	F	6.5 (0.8)	5.4 - 8.2	10	9.4 (1.8)	6.7 - 11.9	8	7.5 (1.6)	4.8 - 9.8	10
11	I	M	13.6 (2.2)	10.9 - 17.0	10	12.2 (1.1)	10.3 - 13.9	10	9.7 (1.6)	7.0 - 11.5	10
16†	I	F	7.5 (1.0)	6.2 - 9.7	10	4.5 (1.2)	3.4 - 7.1	10	7.5 (1.4)	6.1 - 10.4	10
19	M	F	5.4 (1.0)	4.2 - 7.6	12	8.9 (2.0)	6.4 - 11.5	12	8.2 (1.4)	5.6 -10.7	12
20	M	F	4.4 (0.7)	3.5 - 6.0	10	4.9 (0.6)	4.0 - 5.7	10	6.4 (1.3)	4.9 - 9.3	10
21	I	F	7.1 (1.3)	4.7 - 8.4	10	6.2 (1.2)	4.2 - 8.0	10	7.4 (1.3)	5.8 - 10.5	10
24†	C	M	7.0 (1.2)	5.2 - 9.2	16	8.1 (1.3)	6.0 - 10.9	14	9.3 (1.5)	6.2 - 11.7	16
26	C	F	7.2 (0.9)	6.0 - 8.7	10	5.9 (2.4)	3.6 - 10.7	14	8.8 (1.3)	7.1 - 11.3	10
34	I	M	6.2 (0.8)	4.8 - 7.3	10	5.9 (0.9)	3.6 - 6.6	10	6.6 (0.8)	5.2 - 8.2	14
35	I	M	-	-	0	7.9 (1.3)	5.4 - 9.8	12	9.4 (0.8)	7.6 - 10.7	12
36	C	M	5.6 (0.6)	4.9 - 7.2	10	5.4 (0.8)	4.3 - 6.7	10	-	-	0
41	I	F	12.2 (1.0)	10.6 - 13.7	10	11.3 (1.3)	8.2 - 13.0	10	11.1 (1.7)	8.0 - 14.2	10
43	C	F	7.8 (1.8)	5.2 - 10.0	10	8.7 (1.3)	6.9 - 11.4	10	8.2 (0.6)	7.0 - 8.9	12

† These children were not included in the analysis by delivery type (see pages 72 and 73)

Table 2. Individual neonatal line width (μm) at each crown position for children undergoing a delivery with minimal medical intervention.

NNL #	Tooth Type	Sex	Medical Intervention	DEJ Mean (SD)	Range width	N	MID Mean (SD)	Range width	N	APEX Mean (SD)	Range width	N
4†	I	F	nitrous oxide	4.2 (0.6)	3.7 - 5.1	8	6.3 (0.6)	5.4 - 7.4	14	7.0 (1.6)	5.0 - 9.4	14
9†	C	F	oxytocin epidural	9.5 (1.0)	7.6 - 10.8	10	9.8 (2.1)	6.8 - 13.0	12	10.1 (1.0)	8.8 - 11.3	10
15†	I	M	demerol (2x)	7.6 (2.1)	4.7 - 11.1	10	8.3 (1.0)	7.2 - 10.3	8	7.5 (1.4)	6.1 - 10.4	8
22	I	M	oxytocin epidural	6.2 (1.3)	4.0 - 8.4	11	7.6 (1.2)	5.9 - 9.5	10	7.0 (1.0)	5.4 - 8.3	10
23	I	M	oxytocin	10.2 (2.4)	6.5 - 12.9	12	6.5 (1.1)	5.2 - 9.0	12	8.5 (1.3)	6.5 - 11.1	12
25	I	F	epidural	4.7 (1.34)	3.2 - 7.3	12	6.4 (1.2)	5.2 - 9.3	13	-	-	0
29	I	M	oxytocin epidural	7.5 (0.8)	6.3 - 8.8	10	9.0 (1.3)	6.2 - 10.0	10	7.7 (0.5)	7.2 - 8.7	6
30†	M	M	oxytocin epidural	7.1 (1.2)	5.0 - 9.2	12	11.4 (2.7)	7.2 - 14.9	12	9.1 (1.7)	6.4 - 12.6	12
31	I	M	oxytocin epidural	6.9 (1.4)	5.7 - 9.5	6	6.0 (1.0)	4.8 - 7.4	8	5.5 (0.8)	4.4 - 6.7	8
32	M	M	oxytocin	9.0 (1.2)	6.8 - 10.7	12	11.2 (1.3)	9.5 - 13.6	12	8.6 (1.0)	6.7 - 10.0	14
33	I	M	epidural	5.8 (0.9)	4.7 - 7.2	8	7.3 (1.1)	6.0 - 9.8	10	7.3 (0.9)	5.7 - 9.1	10
37	I	M	oxytocin epidural	9.3 (0.5)	8.5 - 10.2	10	10.7 (1.2)	8.8 - 12.1	10	-	-	0
38	I	F	epidural episiotomy	5.1 (1.2)	3.4 - 7.2	10	6.6 (1.0)	5.0 - 7.8	10	6.5 (0.7)	5.7 - 8.1	10
40	C	M	epidural episiotomy	9.3 (1.9)	6.5 - 11.6	10	8.4 (1.2)	6.8 - 11.0	12	-	-	0
44	I	F	oxytocin epidural (3X)	10.0 (0.7)	8.8 - 11.1	10	7.4 (0.8)	6.3 - 8.6	13	8.4 (0.7)	7.6 - 10.0	10
48	M	M	demerol entonox (gas)	3.7 (0.6)	3.0 - 5.1	10	4.6 (0.5)	4.1 - 5.5	10	4.5 (0.6)	3.9 - 5.7	10
49	M	F	oxytocin	7.6 (0.7)	6.3 - 8.6	12	8.1 (1.2)	5.5 - 10.0	12	7.0 (0.8)	5.5 - 8.2	12

† These children were not included in an analysis by delivery type (see pages 72 and 73).

Table 3. Individual neonatal line width (μm) for children undergoing a complicated delivery.

NNL #	Tooth Type	Sex	Delivery Type	Other Medical Intervention(s)	DEJ Mean (SD)	Range width	N	MID Mean (SD)	Range width	N	APEX Mean (SD)	Range width	N
2	M	M	emergency C-section	oxytocin general anasthetic	5.2 (0.8)	4.2 - 6.3	10	4.8 (0.8)	3.8 - 6.6	12	8.0 (0.8)	6.9 - 9.0	10
5	C	F	forceps	Demerol epidural	8.9 (1.4)	6.9 - 10.9	8	9.8 (1.3)	8.1 - 11.9	10	8.4 (1.3)	6.7 - 9.8	12
6	I	F	emergency C-section	oxytocin (2x) epidural (2x) nitrous oxide (2x)	-	-	0	6.6 (1.1)	5.4 - 8.5	10	6.4 (1.0)	4.3 - 7.7	10
8	I	M	forceps	epidural; IV meds for high blood pressure	10.0 (1.1)	8.1- 11.7	10	12.2 (1.6)	9.8 - 14.5	8	7.8 (1.0)	6.2 - 9.5	10
12	C	M	breech forceps	episiotomy	8.0 (1.4)	5.6 - 9.5	8	-	-	0	6.9 (1.1)	5.7 - 9.0	10
13	C	F	emergency C-section	general anasthetic	12.1 (2.7)	8.1 - 16.2	8	12.5 (1.3)	11.0 - 14.7	6	5.6 (0.7)	4.8 - 6.8	8
17	M	F	emergency C-section	general anasthetic Phenobarb	11.9 (1.7)	10.0 - 14.0	10	13.8 (2.3)	10.3 - 16.6	8	12.7 (2.3)	9.7 - 17.0	10
27	I	F	vacuum extraction	oxytocin epidural local anasthetic episiotomy	6.2 (0.8)	5.4 - 7.5	10	6.0 (1.1)	4.5 - 7.7	10	7.0 (1.3)	5.4 - 8.6	6
28	I	M	forceps	oxytocin epidural	7.3 (1.0)	5.9 - 9.0	10	5.9 (0.7)	5.0 - 7.3	10	5.4 (0.6)	4.4 - 6.3	10
39	I	F	emergency C-section	oxytocin general anathetic morphine drip magnesium sulfate	-	-	0	5.8 (1.1)	4.0 - 7.4	10	5.8 (0.7)	4.6 - 6.8	12
42	I	F	forceps	oxytocin epidural oxygen Antibiotics	6.3 (1.0)	4.6 - 8.0	10	7.4 (0.9)	5.9 - 8.5	10	6.4 (0.6)	5.7 - 7.3	10

Table 4. Individual neonatal line (μm) width for children undergoing an elective c-section

NNL #	Tooth Type	Sex	Medication	DEJ Mean (SD)	Range width	N	MID Mean (SD)	Range width	N	APEX Mean (SD)	Range width	N
1	I	F	general anasthetic	6.1 (1.1)	4.4 - 7.8	12	11.2 (1.1)	9.5 - 12.9	12	7.2 (1.2)	5.6 - 8.6	8
10†	I	M	local anasthetic	8.9 (1.4)	7.2 - 11.4	8	10.7 (2.1)	7.4 - 15.3	12	12.5 (2.5)	8.8 - 15.9	12
14	M	M	epidural	6.3 (0.7)	5.1 - 7.7	10	7.4 (0.8)	5.9 - 8.3	10	7.6 (1.4)	5.1 - 10.4	12
18	M	F	epidural	8.1 (1.1)	6.4 - 9.8	10	-	-	0	6.4 (1.1)	5.0 - 8.4	10

† breech

Table 5. Individual neonatal line width (μm) in children experiencing complication(s) at birth and/or medical intervention(s) at birth.

NNL #	Tooth Type	Sex	Complication	Medical Intervention	DEJ Mean (SD)	Range width	MID Mean (SD)	Range width	APEX Mean (SD)	Range width
2	M	M	allergic reaction shortly after birth	none	5.2 (0.8)	4.2 - 6.3	4.8 (0.8)	3.8 - 6.6	8.0 (0.8)	6.9 - 9.0
4	I	F	cord around neck	none	4.2 (0.6)	3.7 - 5.1	6.3 (0.6)	5.4 - 7.4	7.0 (1.6)	5.0 - 9.4
5	C	F	Asphyxiated at birth	NICU	8.9 (1.4)	6.9 - 10.9	9.8 (1.3)	8.1 - 11.9	8.4 (1.3)	6.7 - 9.8
6	I	F	Irregular heartbeat (distress)	none	-	-	6.6 (1.1)	5.4 - 8.5	6.4 (1.0)	4.3 - 7.7
7	I	F	low birth weight (< 2500 g); fUGR	NICU	6.5 (0.8)	5.4 - 8.2	9.4 (1.8)	6.7 - 11.9	-	-
8	I	M	irregular heartbeat (distress) bruising of face and scalp from forceps use	given oxygen during delivery NICU	10.0 (1.1)	8.1- 11.7	12.2 (1.6)	9.8 - 14.5	7.8 (1.0)	6.2 - 9.5
9	C	F	Irregular heartbeat (distress)	none	9.5 (1.0)	7.6 - 10.8	9.8 (2.1)	6.8 - 13.0	10.1 (1.0)	8.8 - 11.3
10	I	M	Breathing comps.	none	8.9 (1.4)	7.2 - 11.4	10.7 (2.1)	7.4 - 15.3	12.5 (2.5)	8.8 - 15.9
12	C	M	Breech	blood test 1 week after delivery	8.0 (1.4)	5.6 - 9.5	-	-	6.9 (1.1)	5.7 - 9.0
13	C	F	breathing problems and vomiting: ingested amniotic fluid	intubated to extract ingested fluid	12.1 (2.7)	8.1 - 16.2	12.5 (1.3)	11.0 - 14.7	5.6 (0.7)	4.8 - 6.8

Table 5 (cont'd)

NNL #	Tooth Type	Sex	Complication	Medical Intervention	DEJ Mean (SD)	Range width	MID Mean (SD)	Range width	APEX Mean (SD)	Range width
15	I	M	Irregular heartbeat (distress)	none	7.6 (2.1)	4.7 - 11.1	8.3 (1.0)	7.2 - 10.3	7.5 (1.4)	6.1 - 10.4
16	I	F	Breathing complications: swallowed meconium Eye infection within hours of delivery	suctioning of meconium	7.5 (1.0)	6.2 - 9.7	4.5 (1.2)	3.4 - 7.1	6.0 (0.6)	5.1 - 6.9
17	M	F	Oxygen deprivation	NICU	11.9 (1.7)	10.0 - 14.0	13.8 (2.3)	10.3 - 16.6	12.7 (2.3)	9.7 - 17.0
24	C	M	Breathing complications: cord around neck bruising of scalp	none	7.0 (1.2)	5.2 - 9.2	8.1 (1.3)	6.0 - 10.9	9.3 (1.5)	6.2 - 11.7
26	C	F	Breathing comps.	none	7.2 (0.9)	6.0 - 8.7	5.9 (2.4)	3.6 - 10.7	8.8 (1.3)	7.1 - 11.3
28	I	M	injuries to face from forceps use	none	7.3 (1.0)	5.9 - 9.0	5.9 (0.7)	5.0 - 7.3	5.4 (0.6)	4.4 - 6.3
29	I	M	allergic reaction to cow's milk shortly after birth	none	7.5 (0.8)	6.3 - 8.8	9.0 (1.3)	6.2 - 10.0	7.7 (0.5)	7.2 - 8.7

Table 5(cont'd)

NNL #	Tooth Type	Sex	Complication	Medical Intervention	DEJ Mean (SD)	Range width	MID Mean (SD)	Range width	APEX Mean (SD)	Range width
30	M	M	Breathing complications: cord around neck swallowed fluid	NICU	7.1 (1.2)	5.0 - 9.2	11.4 (2.7)	7.2 - 14.9	9.1 (1.7)	6.4 - 12.6
31	I	M	urinary tract infection 1 week after birth	IV antibiotics	6.9 (1.4)	5.7 - 9.5	6.0 (1.0)	4.8 - 7.4	5.5 (0.8)	4.4 - 6.7
32	M	M	Breathing comps.	UV treatment for jaundice	9.0 (1.2)	6.8 - 10.7	11.2 (1.3)	9.5 - 13.6	8.6 (1.0)	6.7 - 10.0
37	I	M	obstructed stomach muscle; projectile vomiting	none	9.3 (0.5)	8.5 - 10.2	10.7 (1.2)	8.8 - 12.1	-	-
38	I	F	None	IV antibiotics	5.1 (1.2)	3.4 - 7.2	6.6 (1.0)	5.0 - 7.8	6.5 (0.7)	5.7 - 8.1
39	I	F	growth delayed (premature)	NICU for 28 days			5.8 (1.1)	4.0 - 7.4	5.8 (0.7)	4.6 - 6.8
41	I	F	Breathing problems: swollen adenoids	none	12.2 (1.0)	10.6 - 13.7	11.3 (1.3)	8.2 - 13.0	11.1 (1.7)	8.0 - 14.2
42	I	F	Irregular heartbeat (distress) Fever of unknown etiology	IV antibiotics lumbar puncture UV treatment for jaundice	6.3 (1.0)	4.6 - 8.0	7.4 (0.9)	5.9 - 8.5	6.4 (0.6)	5.7 - 7.3
44	I	F	cold at 1-2 weeks	none	10.0 (0.7)	8.8 - 11.1	7.4 (0.8)	6.3 - 8.6	8.4 (0.7)	7.6 - 10.0

Table 6. Individual neonatal line width (μm) in children whose mothers' experienced complication(s) at birth.

NNL #	Maternal Complication(s)	Medication(s)	DEJ Mean (SD)	Range width	MID Mean (SD)	Range width	APEX Mean (SD)	Range width
5	High blood pressure Intrapartum haemmorhage	Demerol	8.9 (1.4)	6.9 - 10.9	9.8 (1.3)	8.1 - 11.9	8.4 (1.3)	6.7 - 9.8
7	Hypothyroidism	Eltroxin/Levothyroxine	6.5 (0.8)	5.4 - 8.2	9.4 (1.8)	6.7 - 11.9	-	-
8	high blood pressure	IV meds. for blood pressure	10.0 (1.1)	8.1- 11.7	12.2 (1.6)	9.8 - 14.5	7.8 (1.0)	6.2 - 9.5
9	low blood pressure	IV meds. for blood pressure	9.5 (1.0)	7.6 - 10.8	9.8 (2.1)	6.8 - 13.0	10.1 (1.0)	8.8 - 11.3
12	Developed gestational diabetes at 30 weeks, remained throughout pregnancy	none	8.0 (1.4)	5.6 - 9.5	-	-	6.9 (1.1)	5.7 - 9.0
13	Intrapartum haemmorhage	None	12.1 (2.7)	8.1 - 16.2	12.5 (1.3)	11.0 - 14.7	5.6 (0.7)	4.8 - 6.8
15	Gestational diabetes last 2 weeks of pregnancy Hypothyroidism	Levothyroxine	7.6 (2.1)	4.7 - 11.1	8.3 (1.0)	7.2 - 10.3	7.5 (1.4)	6.1 - 10.4
17	High blood pressure Toxemia last month of pregnancy Seizures at delivery	Phenobarb IV meds. for blood pressure	11.9 (1.7)	10.0 - 14.0	13.8 (2.3)	10.3 - 16.6	12.7 (2.3)	9.7 - 17.0
27	Anemic	none	6.2 (0.8)	5.4 - 7.5	6.0 (1.1)	4.5 - 7.7	7.0 (1.3)	5.4 - 8.6
33	Developed gestational diabetes during second trimester, remained throughout pregnancy	none	5.8 (0.9)	4.7 - 7.2	7.3 (1.1)	6.0 - 9.8	7.3 (0.9)	5.7 - 9.1

Table 6
(cont'd)

NNL #	Maternal Complication(s)	Medication(s)	DEJ Mean (SD)	Range width	MID Mean (SD)	Range Width	APEX Mean (SD)	Range width
38	Group B strep	Antibiotics	5.1 (1.2)	3.4 - 7.2	6.6 (1.0)	5.0 - 7.8	6.5 (0.7)	5.7 - 8.1
39	High blood pressure Toxemia; proteinuria	Morphine; blood pressure meds; magnesium sulfate	-	-	5.8 (1.1)	4.0 - 7.4	5.8 (0.7)	4.6 - 6.8
42	Fever during delivery; cause unknown	oxygen antibiotics	6.3 (1.0)	4.6 - 8.0	7.4 (0.9)	5.9 - 8.5	6.4 (0.6)	5.7 - 7.3
43	High risk pregnancy Endometriosis	Provera	7.8 (1.8)	5.2 - 10.0	8.7 (1.3)	6.9 - 11.4	8.2 (0.6)	7.0 - 8.9

Table 7. Descriptive statistics of neonatal line width (μm) at each crown position for a. child's health at birth, b. medical intervention(s) at birth, c. maternal complication(s) at birth, d. jaundiced at birth and e. term at birth.

Birth Variables	Mean	Median	SD	Min.-Max.	Range	N[1]
a. Child's health						
Healthy (n = 20)[2]						
DEJ	6.8	6.3	2.6	3.0 - 17.0	14.0	199
Middle	7.4	7.1	2.2	3.6 - 13.9	10.3	205
Apex	7.4	7.3	1.6	3.9 - 11.5	7.6	180
Not healthy (n = 25)[2]						
DEJ	8.2	8.0	2.3	3.7 - 16.2	12.5	226
Middle	8.5	8.2	2.9	3.4 - 16.6	13.2	251
Apex	8.1	7.8	2.4	4.3 - 17.0	12.7	252
b. Medical intervention(s)						
Yes (n = 13)[2]						
DEJ	8.2	7.9	2.5	3.4 - 16.2	12.8	114
Middle	9.1	8.6	3.3	3.4 - 16.6	13.2	112
Apex	7.5	7.0	2.2	4.4 - 17.0	12.6	136
No (n = 32)[2]						
DEJ	7.3	6.9	2.5	3.0 - 17.0	14.0	311
Middle	7.7	7.3	2.3	3.6 - 15.3	11.7	344
Apex	7.9	7.7	2.0	3.9 - 15.9	12.0	296
c. Maternal complication(s)						
Yes (n = 14)[2]						
DEJ	8.1	7.8	2.6	3.4 - 16.2	12.8	122
Middle	8.8	8.5	2.7	4.0 - 16.6	12.6	120
Apex	7.7	7.3	2.1	4.6 - 17.0	12.4	138
No (n = 31)[2]						
DEJ	7.4	7.1	2.5	3.0 - 17.0	14.0	303
Middle	7.7	7.2	2.6	3.4 - 15.3	11.9	336
Apex	7.9	7.7	2.1	3.9 - 15.9	12.0	294
d. Jaundiced						
Yes (n = 12)[2]						
DEJ	7.5	7.0	2.6	3.4 - 13.7	10.3	114
Middle	7.7	7.2	2.1	4.0 - 13.6	9.6	120
Apex	7.7	7.4	1.8	4.6 - 14.2	9.6	132
No (n = 33)[2]						
DEJ	7.6	7.3	2.5	3.0 - 17.0	14.0	311
Middle	8.1	7.8	2.8	3.4 - 16.6	13.2	336
Apex	7.9	7.6	2.2	3.9 - 17.0	13.1	300

Table 7(cont'd)

Birth Variables	Mean	Median	SD	Min.-Max.	Range	N[1]
e. Birth						
Pre-Term (n = 5)[2]						
DEJ	9.4	9.2	3.1	4.6 - 17.0	12.4	38
Middle	9.0	8.7	2.8	4.0 - 13.9	9.9	40
Apex	7.1	6.5	1.8	4.6 - 11.5	6.9	42
Term (n = 39)[2]						
DEJ	7.3	6.9	2.3	3.0 - 16.2	13.2	377
Middle	7.8	7.4	2.5	3.4 - 15.3	11.9	408
Apex	7.8	7.6	2.0	3.9 - 15.9	12.0	380
Post-Term (n = 1)[2]						
DEJ	11.9	11.5	1.7	10.0 - 14.0	4.0	10
Middle	13.8	13.4	2.3	10.3 - 16.6	6.3	8
Apex	12.7	12.7	2.0	9.7 - 17.0	7.3	10

1 Total number of prisms measured at each crown position
2 Number of individuals

Table 8. Descriptive statistics of neonatal line width (μm) at each crown position by tooth class for type of delivery.

Delivery type/ Tooth class	Mean	Median	S.D.	Min - Max	Range	N[1]
Natural						
Incisors (n = 5)[2]						
DEJ	9.8	9.5	3.5	4.7 - 17.0	12.3	40
Middle	8.7	8.0	2.8	3.6 - 13.9	10.3	52
Apex	8.7	8.5	2.0	5.2 - 14.2	9.0	56
Canines (n = 3)[2]						
DEJ	6.8	6.5	1.5	4.9 - 10.0	5.1	30
Middle	6.5	5.7	2.2	3.6 - 11.4	7.8	34
Apex	8.5	8.4	1.0	7.0 - 11.3	4.3	22
Molars (n = 2)[2]						
DEJ	5.0	4.8	1.0	3.5 - 7.6	4.1	22
Middle	7.1	6.7	2.5	4.0 - 11.5	7.5	22
Apex	7.4	7.4	1.6	4.9 - 10.7	5.8	22
Minimal medical Intervention						
Incisors (n = 9)[2]						
DEJ	7.3	7.2	2.4	3.2 - 12.9	9.7	89
Middle	7.5	7.1	1.7	4.8 - 12.1	7.3	96
Apex	7.3	7.5	1.3	4.4 - 11.1	6.7	66
Canines (n = 1)[2]						
DEJ	9.3	9.7	1.9	6.5 - 11.6	5.1	10
Middle	8.4	8.2	1.2	6.8 - 11.0	4.2	12
Apex	-	-	-	-	-	-
Molars (n = 3)[2]						
DEJ	6.9	7.7	2.4	3.0 - 10.7	7.7	34
Middle	8.2	8.4	2.9	4.1 - 13.6	9.5	34
Apex	6.9	7.2	1.9	3.9 - 10.0	6.1	36

Table 8(cont'd)

Delivery type/ Tooth class	Mean	Median	S.D.	Min - Max	Range	N[1]
Complicated						
Incisors (n = 6)[2]						
DEJ	7.5	7.1	1.8	4.6 - 11.7	7.1	40
Middle	7.1	6.4	2.3	4.0 - 14.5	10.5	58
Apex	6.4	6.2	1.1	4.3 - 9.5	5.2	58
Canines (n = 3)[2]						
DEJ	9.6	9.3	2.6	5.6 - 16.2	10.6	24
Middle	10.8	10.8	1.9	8.1 - 14.7	6.6	16
Apex	7.1	7.0	1.5	4.8 - 9.8	5.0	30
Molars (n = 2)[2]						
DEJ	8.5	8.2	3.7	4.2 - 14.0	9.8	20
Middle	8.4	5.7	4.7	3.8 - 16.6	12.8	20
Apex	10.3	9.4	3.0	6.9 - 17.0	10.1	20
Elective C-section						
Incisors (n = 2)[2]						
DEJ	7.2	7.2	1.9	4.4 - 11.4	7.0	20
Middle	10.9	11.1	1.6	7.4 - 15.3	7.9	24
Apex	10.4	9.5	3.4	5.6 - 15.9	10.3	20
Molars (n = 2)[2]						
DEJ	7.2	6.8	1.3	5.1 - 9.8	4.7	20
Middle	7.4	7.5	0.8	5.9 - 8.3	2.4	10
Apex	7.1	7.0	1.4	5.0 - 10.4	5.4	22

1 Total number of prisms measured at each crown position
2 Number of individual teeth

Table 9. Descriptive statistics of neonatal line width (μm) at each crown position by tooth class for a. child's health at birth, b. medical intervention(s) at birth, c. maternal complications at birth, d. jaundiced at birth and e. term at birth.

Birth variable/ Tooth class	Mean	Median	S.D.	Min - Max	Range	N[1]
a. Child's health						
Healthy						
Incisors (n = 11)[2]						
DEJ	7.1	6.3	2.9	3.2 - 17.0	13.8	105
Middle	7.6	6.9	2.3	3.6 - 13.9	10.3	119
Apex	7.7	7.6	1.5	5.1 - 11.5	6.4	102
Canines (n = 3)[2]						
DEJ	7.5	7.0	2.2	4.9 - 11.6	6.7	30
Middle	7.5	7.7	1.9	4.3 - 11.4	7.1	32
Apex	8.2	8.3	0.6	7.0 - 8.9	1.9	12
Molars (n = 6)[2]						
DEJ	6.0	6.0	1.8	3.0 - 9.8	6.8	64
Middle	6.9	7.1	2.1	4.0 - 11.5	7.5	54
Apex	6.8	6.9	1.6	3.9 - 10.7	6.8	66
Not healthy						
Incisors (n = 15)[2]						
DEJ	8.1	7.7	2.2	3.7 - 13.7	10.0	122
Middle	8.0	7.4	2.5	3.4 - 15.3	11.9	151
Apex	7.6	6.9	2.4	4.3 - 15.9	11.6	140
Canines (n = 6)[2]						
DEJ	8.5	8.2	2.2	5.2 - 16.2	11.0	60
Middle	8.7	8.6	2.7	3.6 - 14.7	11.1	56
Apex	8.3	8.6	1.8	4.8 - 11.7	6.9	66
Molars (n = 4)[2]						
DEJ	8.2	8.0	2.7	4.2 - 14.0	9.8	44
Middle	10.0	10.9	3.8	3.8 - 16.6	12.8	44
Apex	9.5	8.9	2.3	6.4 - 17.0	10.6	46

1 Total number of prisms measured at each crown position
2 Number of individual teeth

Table 9 (cont'd).

Birth variable/ Tooth class	Mean	Median	S.D.	Min - Max	Range	N[1]
b. Medical intervention						
Yes						
Incisors (n = 7)[2]						
DEJ	7.0	6.7	1.9	3.4 - 11.7	8.3	56
Middle	7.2	6.8	2.6	3.4 - 14.5	11.1	64
Apex	6.5	6.2	1.2	4.4 - 9.8	5.4	70
Canines (n = 3)[2]						
DEJ	9.6	9.3	2.6	5.6 - 16.2	10.6	24
Middle	10.8	10.8	1.9	8.1 - 14.7	6.6	16
Apex	7.1	7.0	1.5	4.8 - 9.8	5.0	30
Molars (n = 3)[2]						
DEJ	9.2	9.2	2.3	5.0 - 14.0	9.0	34
Middle	11.9	11.9	2.3	7.2 - 16.6	9.4	32
Apex	9.9	9.4	2.4	6.4 - 17.0	10.6	36
No						
Incisors (n = 19)[2]						
DEJ	7.8	7.3	2.8	3.2 - 17.1	13.9	171
Middle	8.0	7.4	2.3	3.6 - 15.3	11.7	206
Apex	8.1	7.7	2.2	4.3 - 15.9	11.6	172
Canines (n = 6)[2]						
DEJ	7.6	7.4	1.8	4.9 – 11.6	6.7	66
Middle	7.7	8.0	2.2	3.6 – 13.0	9.4	72
Apex	9.1	8.9	1.3	6.2 – 11.7	5.5	48
Molars (n = 7)[2]						
DEJ	5.9	5.8	1.7	3.0 – 9.8	6.8	74
Middle	6.5	6.3	2.1	3.8 – 11.5	7.7	66
Apex	6.9	7.1	1.6	3.9 – 10.7	6.8	76
c. Maternal Complications						
Yes						
Incisors (n = 8)[2]						
DEJ	6.8	6.4	1.9	3.4 - 11.7	8.3	68
Middle	7.7	7.4	2.2	4.0 - 14.5	10.5	74
Apex	6.9	6.7	1.2	4.6 - 10.4	5.8	76

1 Total number of prisms measured at each crown position
2 Number of individual teeth

Table 9 (cont'd).

Birth variable/ Tooth class	Mean	Median	S.D.	Min - Max	Range	N[1]
Canines (n = 5)[2]						
DEJ	9.2	9.1	2.3	5.2 - 16.2	11.0	44
Middle	9.9	9.5	2.0	6.8 - 14.7	7.9	38
Apex	7.9	8.1	1.7	4.8 - 11.3	6.5	52
Molars (n = 1)[2]						
DEJ	11.9	11.5	1.7	10.0 – 14.0	4.0	10
Middle	13.8	13.4	2.3	10.3 - 16.6	6.3	8
Apex	12.7	12.7	2.3	9.7 – 17.0	7.3	10
No						
Incisors (n = 18)[2]						
DEJ	8.0	7.6	2.8	3.2 - 17.0	13.8	159
Middle	7.9	7.2	2.5	3.4 - 15.3	11.9	196
Apex	7.9	7.6	2.3	4.3 - 15.9	11.6	166
Canines (n = 4)[2]						
DEJ	7.2	6.9	0.8	4.9 - 11.6	6.7	46
Middle	7.0	7.0	2.0	3.6 - 11.0	7.4	50
Apex	9.1	8.9	1.4	6.2 - 11.7	5.5	26
Molars (n = 9)[2]						
DEJ	6.4	6.3	1.9	3.0 – 10.7	7.7	98
Middle	7.8	7.3	3.0	3.8 – 14.9	11.1	90
Apex	7.4	7.4	1.7	3.9 – 12.6	8.7	102
d. Jaundiced						
Yes						
Incisors (n = 9)[2]						
DEJ	7.4	6.5	2.9	3.4 - 13.7	10.3	78
Middle	7.2	6.8	1.9	4.0 - 13.0	9.0	94
Apex	7.4	6.9	1.9	4.6 - 14.2	9.6	92
Canines (n = 2)[2]						
DEJ	7.3	7.4	1.4	5.2 - 9.5	4.3	24
Middle	8.1	8.2	1.3	6.0 - 10.9	4.9	14
Apex	8.4	8.5	1.8	5.7 - 11.7	6.0	26

1 Total number of prisms measured at each crown position
2 Number of individual teeth

Table 9 (cont'd).

Birth variable/ Tooth class	Mean	Median	S.D.	Min - Max	Range	N[1]
Molars (n = 1)[2]						
DEJ	9.0	9.4	1.2	6.8 - 10.7	3.9	12
Middle	11.2	11.1	1.3	9.5 - 13.6	4.1	12
Apex	8.6	7.0	1.0	6.7 - 10.0	3.3	14
No						
Incisors (n = 17)[2]						
DEJ	7.8	7.4	2.5	3.2 - 17.0	13.8	149
Middle	8.2	7.8	2.6	3.4 - 15.3	11.9	176
Apex	7.7	7.4	2.2	4.3 - 15.9	11.6	150
Canines (n = 7)[2]						
DEJ	8.5	8.3	2.4	4.9 - 16.2	11.3	66
Middle	8.3	8.4	2.6	3.6 - 14.7	11.1	74
Apex	8.3	8.4	1.6	4.8 - 11.3	6.5	52
Molars (n = 9)[2]						
DEJ	6.6	6.3	2.5	3.0 - 14.0	11.0	96
Middle	7.9	7.2	3.3	3.8 - 16.6	12.8	86
Apex	7.8	7.5	2.5	3.9 - 17.0	13.1	98
e. Birth						
Pre-Term						
Incisors (n = 4)[2]						
DEJ	9.7	9.3	3.4	4.6 - 17.0	12.4	30
Middle	9.0	8.7	2.8	4.0 - 13.9	9.9	40
Apex	7.2	6.5	2.0	4.6 - 11.5	6.9	32
Canines (n = 1)[2]						
DEJ	8.0	8.0	1.4	5.6 - 9.5	3.9	8
Middle	-	-	-	-	-	-
Apex	6.9	6.4	1.1	5.7 - 9.0	3.3	10

1 Total number of prisms measured at each crown position
2 Number of individual teeth

Table 9 (cont'd).

Birth variable/ Tooth class	Mean	Median	S.D.	Min - Max	Range	N[1]
Term						
Incisors (n= 22)[2]						
DEJ	7.3	7.0	2.3	3.2 - 13.7	10.5	197
Middle	7.6	7.1	2.3	3.4 - 15.3	11.9	230
Apex	7.7	7.3	2.1	4.3 - 15.9	11.6	210
Canines (n = 8)[2]						
DEJ	8.2	8.1	2.3	4.9 - 16.2	11.3	82
Middle	8.3	8.3	2.5	3.6 - 14.7	11.1	88
Apex	8.5	8.6	1.6	4.8 - 11.7	6.9	68
Molars (n = 9)[2]						
DEJ	6.4	6.3	1.9	3.0 - 10.7	7.7	98
Middle	7.8	7.3	3.0	3.8 - 14.9	11.1	90
Apex	7.4	7.4	1.7	3.9 - 12.6	8.7	102

1 Total number of prisms measured at each crown position
2 Number of individual teeth

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