IN SEARCH OF CLARITY:

A COMPARISON OF CURRENT AND NOVEL MEASUREMENT TECHNIQUES

INVOLVED IN THE DESCRIPTION OF AGE-RELATED

TRANSPARENT ROOT DENTINE

IN THE HUMAN PERMANENT DENTITION

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ABSTRACT

The primary goal of this thesis is the determination of the most appropriate means of measuring physiological root dentine transparency in the permanent human dentition to estimate age-at-death of skeletal remains. Although a number of internal and external factors may conceivably contribute to the observed discrepancies between predicted values and true ages, the magnitude of the influence exerted by such extraneous factors cannot be quantified until a standardized means of measurement has been agreed upon. To this end, two samples of teeth, one derived from an archaeological context (N=32) and the other a sample of recently-extracted teeth (N=32), have been examined as intact and sectioned specimens. Traditional area and linear measurements of transparency have been made under stereomicroscope. In addition, a novel volumetric measurement of transparent regions has been approximated via micro-CT analysis of densitometric differences within affected root areas. The relative value of each measurement in the prediction of age has been assessed via a description of the association with chronological age for each variable, with unscaled measurements of area displaying the highest correlation with age (r=0.711). Alternative curve fitting techniques have also been explored. The poor correlation with chronological age reported for densitometric measurements of transparent dentine (r=0.142) is likely indicative of local differences in root dentine histology prior to the advancement of transparency. This research has also served to illustrate the amount of variance in measurements of transparency irrespective of the means of measurement applied. Future research should be directed towards the development of age-predictive formulae based on area measurements of transparency made on sectioned specimens. However, until the ultimate causal mechanism underlying the progression of root dentine transparency is uncovered, the strength of the association of this phenomenon with chronological age will continue to fluctuate between teeth in an unpredictable manner. Such error will limit the accuracy of age predictions within both the archaeological and forensic contexts.

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Chapter 1
INTRODUCTION

Accurate age estimation techniques are an imperative in the forensic as well as the bioarcheaological context (Burns and Maples, 1976; Bang, 1989; Maples, 1989; Kemkes-Grottenhaler, 2002; Usher, 2002). For law enforcement officials and the physical anthropologists working in coordination with them, age estimates constitute an essential element of personal identification (Pretty, 2003). Within the bioarchaeological milieu, a major focus of inquiry centers on paleodemography, the reconstruction of the demographic parameters of past populations (Hoppa, 2002). In this realm,

> "paleodemographic reconstructions of past populations depend on accurate determination of age-at-death distributions, sorted by sex, within skeletal samples" (Hoppa and Vaupel, 2002:3).

Accurate age estimates for multiple individuals are therefore an integral aspect of physical anthropological inquiry.

Before the age of twelve, formative or developmental changes can be observed and compared to population standards of growth in order to define an expected age range into which the individual in question should likely fall (Burns and Maples, 1976; Byers, 2002; Hoppa and Vaupel, 2002). However, after adulthood is reached, at approximately the eighteenth chronological year after birth, the majority of skeletal growth indicators will no longer be available for age estimation (Burns and Maples, 1976; Byers, 2002; Hoppa and Vaupel, 2002). An investigator must, therefore, rely upon degenerative changes within the skeleton of an individual in order to estimate the age at death (Burns and Maples, 1976; Byers, 2002).

Yet, following the publication of Bocquet-Appel and Masset's article *Farewell to paleodemography* (1982), the validity of such reconstructions came under question. In particular, the authors questioned the accuracy and reliability of current age estimation techniques which rely upon the observation of morphological changes within the human

skeleton (Bocquet-Appel and Masset, 1982). They argued that all current techniques, regardless of the indicators upon which they are based, are limited by the degree to which skeletal degeneration varies with chronological age (Bocquet-Appel and Masset, 1982). Such a discrepancy marks the distinction between *biological age* (the age an individual appears when morphological criteria are evaluated in comparison to a population standard) and *chronological age* (the amount of time an individual has been alive) (Aykroyd et al., 1999; Hoppa, 2002; Usher, 2002). Bocquet-Appel and Masset (1982) argued that the accuracy of age estimates, whether used to identify an individual or reconstruct a demographic profile for a past population, is limited by the degree to which the rate of change in the biological age of an individual matches the average rate of change for the reference sample upon which the predictive standards have been based. They also lamented the tendency for all current techniques to under-estimate age at the upper end of the age spectrum (Bocquet-Appel and Masset, 1982).

These systematic sources of error do not represent insurmountable impediments to accurate age prediction. Nor have they signaled the end of paleodemography. However, some 25 years later, the need for more accurate and reliable osetological methods of age estimation persists (Hoppa and Vaupel, 2002; Kemkes-Grottenhaler, 2002). The *Rostock Manifesto*, developed in August, 2000 at a meeting of the *Laboratory of Survival and Longevity* at the *Max Planck Institute for Demographic Research* in Rostock, Germany outlines several goals for paleodemographic inquiry in the future (Hoppa and Vaupel, 2002). The first of these is that

> "...osteologists must develop more reliable and more vigorously validated age indicator stages or categories that relate skeletal morphology to known chronological age." (Hoppa and Vaupel, 2002:2).

The research undertaken for this thesis is motivated by such a need. Its focus is on an evaluation of the age-related phenomenon of root dentine transparency as a means of adult age estimation. To date, the value of age-estimation methods based on transparency remains uncertain.

Beginning at around the age of twenty years, following the cessation of primary growth, a macroscopically-visible transparency is noticeable in roots of the adult teeth. This change in the appearance of the root begins at the apex and advances up the length of the root towards the cervical margin (Gustafson, 1950; Vasiliadis et al., 1983a). It has been suggested that such transparency is due to an increase in the mineral content of root dentine, either in absolute or relative terms (Nalbandian et al., 1960; Metzger et al., 1980; Vasiliadis et al., 1983b; Hillson, 1996; Kinney et al., 2005). Although the mechanism by which this process occurs is not yet completely understood, it appears that this phenomenon is related to advancing age, rather than to any pathological impetus (Azaz et al., 1977; Solheim, 1989). Numerous studies have demonstrated a strong correlation between the degree of apical root transparency and chronological age (for example Miles, 1963; Burns and Maples, 1976; Maples, 1978; Solheim, 1989). Current research suggests a positive linear relationship between the amount of transparent root dentine and age (for example Bang and Ramm, 1970; Vasiliadis et al., 1983a; Solheim, 1989; Lamendin et al., 1992).

The method of Bang and Ramm (1970) is currently the most widely used means of age estimation based solely on the empirical measurement of apical transparency. According to this method, measurements of the length of the root affected by transparency are made on both sides of the tooth (Bang and Ramm, 1970). Values obtained through the application of this method are inserted into regression formulae in order to arrive at estimated ages-at-death. The relationship between transparent root area and age has also been explored using single and multivariate regression analyses (Lorentsen and Solheim, 1989; Solheim, 1989). The method of area measurement described by Solheim (1989) is carried out on sectioned specimens under a stereomicroscope using a grid system on a photographic plate (Solheim, 1989).

Measurements of transparency offer several advantages over other means of age estimation. Paramount amongst these are the practical utility of the methods and relative ease with which age estimates may be secured. Transparency appears to be a ubiquitous development in all adult teeth (Gustafson, 1950; Vasiliadis et al., 1983b) and estimates of

age may be rendered based on the observation of a single specimen (Bang and Ramm, 1970; Lamendin et al., 1992). Furthermore, the high concentration of inorganic crystals within tooth tissues means that teeth are often extremely well preserved in both the forensic and archaeological contexts, even in cases where the rest of the skeletal remains are not (Bang and Ramm, 1970; Maples, 1978). Even methods that call for teeth to be sectioned require very little preparation. Estimates may be arrived at within hours (Pretty, 2003).

Yet the accuracy of age estimates based on measurements of transparency lacks consistency between studies. Mean errors as high as 14 years have been reported for the method of Bang and Ramm (1970) and Lamendin et al. (1992) (Solheim and Sundnes, 1980; Lamendin et al., 1992). In general, the error of estimates is exacerbated at both ends of the age spectrum, irrespective of the method applied (Gustafson, 1950; Miles, 1958; Bang and Ramm, 1970; Wegener and Albrecht, 1980; Kashyap and Koteswara Rao, 1990; Drusini, 1991; Lopez-Nicolas and Luna, 1991; Lamendin et al., 1992; Whittaker and Bakri, 1996; Prince and Ubelaker, 2002; Olze et al., 2004). Furthermore, there is currently no standard method of measuring root transparency, a fact which may account for a considerable amount of the error in age estimates between studies.

In light of these difficulties, the primary objective of the current research is to determine the most appropriate means of quantifying transparency in the estimation of age. This issue is examined through a direct comparison of the correlation with chronological age for traditional area and linear measurements of transparency and for a novel densitometric measurement based on micro-CT analysis of affected teeth. Measurements were performed on a subsample of teeth (N=32) extracted from skeletal remains buried in the St. Thomas' Anglican Church cemetery site in Belleville, Ontario between 1821 and 1874 and currently held in the department of Anthropology at McMaster University (Saunders et al., 2002). In addition, a sample of recently-extracted teeth (N=32), collected from the Winnipeg School of Dentistry and from two dentists' offices in the Greater Toronto Area over the summer and fall of 2006 were examined.

The fundamental goals of this research are:

1. *To examine both area measurements and linear measurements of transparency in order to establish the value of each in age estimation formulae based on transparency.* The strength and regularity of the correlation between each type of measurement and the age-at death for each tooth will be compared. The method of measurement displaying the greatest regularity and the closest fit with age will be recommended.
2. *To determine the utility of absolute measures and relative indices of transparency in age-predictive formulae.* All measurements will be expressed both as absolute values and scaled proportions of total root size. The correlation of such measures with chronological age will be compared in order to arrive at this determination.
3. *To explore volumetric measurements of transparency in order to determine their value in future age-predictive formulae based on transparency.* Such measurements will be carried out via micro-CT analysis of intact teeth prior to sectioning. The volume of transparent root dentine will be compared to both linear and area measures of transparency. The potential of three-dimensional measures to improve the accuracy and precision of age estimates will be weighed against the cost and time necessary for such analyses.

In addition, the secondary goals of this research are:

1. *To examine the influence of sex on the observed associations between transparency and age in order to determine the necessity for a consideration of sex in future age-predictive formulae based on the observation of transparency.* This determination will involve the comparison of the observed relationships between area, linear and volumetric measures of transparency

and chronological age with those for which the influence of sex is held constant.

2. *To examine the influence of tooth category on the observed associations between transparency and age in order to determine the value of tooth-specific age-predictive formulae.* This determination will involve the comparison of the observed relationships between area, linear and volumetric measures of transparency and chronological age with those for which the influence of tooth category is held constant.
3. *To explore the degree to which post-mortem interval is a factor that requires consideration when employing age-predictive formulae based on transparency.* The strength of the correlations with age for each of the variables based on the measurement of transparency will be compared for both archaeological and recently-extracted teeth.

The following hypotheses will be tested:

1. Area measurements of transparency better describe the distribution of affected root dentine and therefore display a stronger correlation with age than linear measurements.
2. Relative indices of transparency display a closer correlation with chronological age than do absolute measures since they control for fluctuations in the amount of transparency related to root size.
3. Volumetric measures of root dentine transparency more closely approximate the true extent of hyper-mineralized dentine and therefore exhibit a stronger correlation with age-at-death than either linear or area measures.
4. Regardless of the measurement applied, sex does not significantly influence the degree of observed transparency within a given tooth.
5. Across all forms of measurement, tooth type is a significant factor influencing the observed association between transparency and age.

6. Significant differences will be apparent for measures of transparency made on archaeological specimens when compared to recently-derived samples.

The thesis begins by providing a biological and biochemical description of the affected tooth tissue and a description of the mechanism by which transparency proceeds. Chapter 2 reviews the classification, histology and growth of dentine and describes the process of mineralization underlying root dentine transparency. Following this, the utility of transparency in age estimation is explored. Chapter 3 reviews the alternative means of describing transparency and explores the sources of error that confront researchers who employ them. These difficulties are used to formulate the goals of this research. Chapter 4 provides a description of the tooth samples used in this research and the methods employed in their preparation and examination. In the process, the rationale behind the various methodological decisions and the difficulties in data collection are explored. Chapter 5 presents the results of data collection and the statistical interpretations of these findings. A discussion of the relevance of these findings in relation to methods of age estimation based on root dentine transparency is presented in Chapter 6. The thesis concludes with a discussion of areas that deserve future research inquiry in light of the current findings.

It is hoped that the results of this research will further refine current protocols involved in the description of root dentine transparency. Standardized variables, along with clearly defined measurement procedures would constitute a critical refinement of current methods involved in the quantification of root dentine transparency. It is further hoped that this research will illuminate the degree to which several extraneous variables interfere with the association between transparency and chronological age. This research does not have as its goal the creation of any age-predictive formulae. However, the results of this inquiry have the potential to improve the accuracy and precision of future age-predictive formulae based on the examination of transparent root dentine in the adult human dentition.

Chapter 2
DENTINE AS A TISSUE

2.1 Introduction

Within the literature, there exists a vast lexicon of terminology employed in a description of tooth anatomy – some of which has been associated with standardized definitions and some of which has not. In order that a clear and consistent vocabulary is employed throughout this thesis, this chapter begins with a brief review of the taxonomic and histological particulars of human dentine. Following this, the developmental, structural, chemical and optical properties of human dentine are explored in detail. Such an examination provides the basis for a discussion of the modifications to dentine associated with physiological transparency, thereby laying the foundation for a dialogue concerning its properties and any potential mechanisms underlying its progression.

2.2 Taxonomy of Dentines

The adult tooth is a composite of four tissues: dentine, enamel, cementum and pulp. It is perhaps easiest to conceive of a tooth as being composed primarily of *dentine* and being covered in the crown by *enamel* and at the root by a thin layer of *cementum*. At the innermost aspect of a tooth, dentine surrounds the *pulp cavity*, a highly vascularized region of soft-tissue (see Figure 2.1).

A generalized definition of dentine would describe it as

"...a tissue which is situated superficially in the body and forms the basis of the teeth of vertebrates and the exoskeletons of the elasmobranches and some primitive agnathans. It is of mesodermal origin, develops in a centripetal direction from the dermal dental papilla or dental pulp and in the mature state is usually mineralized" (Bradford, 1967:3).

However, even a relatively broad definition such as this is not universally accepted, as disagreements exist concerning whether to classify dentine based on gross histological appearance or structural organization (Baume, 1980). Further, some researchers choose to classify dentines based on their resemblance to bone, while others elect to stratify the various forms based on their site of initial formation (Baume, 1980). The taxonomy of dentine is therefore a contested subject, complicated by the uncertainty of whether various forms of dentine can be confidently arranged in a hierarchy of evolutionary stages or whether they instead represent "a random pattern of histogenetic diversity based on relatively loose developmental controls" (Smith and Sansom, 2000:66). For a complete review of dentine taxonomy see Orvig (1951), Bradford (1967) and Smith and Sansom (2000).

For the current discussion, the classification set out by Orvig (1951) will be employed. Under this scheme, all dentine within the human tooth is termed *orthodentine*. Such a term describes

> "...a mineralized collagenous tissue surrounding a mesodermal papilla or dental pulp. Its formative cells remain within the dental pulp but, at some stage in the development of the tissue, these cells possess long dentinal processes which penetrate almost the whole thickness of the dentine." (Bradford, 1967:9-10)

Or, according to the uniform World Dental Federation (FDI) definition,

> "Orthodentine is the tubular dentine found in the teeth of all dentate mammals, composed mainly of parallel odontoblastic processes, nonmineralized predentine and mineralized intertubular, peritubular and eventually intratubular matrices." (quoted in Baume, 1980:58).

Both *pallial* (mantle) dentine situated at the peripheral margins of the dentinal tissues and *circumpulpal* dentine which surrounds the outer margin of the pulp cavity are included under such a classification (Smith and Sansom, 2000). Both of these forms of dentine exhibit parallel, branching tubules and a matrix of course organic fibers oriented parallel to the tooth surface in circumpulpal dentine and perpendicular to it in the pallial portion (Smith and Sansom, 2000).

In the following discussion, all human dentine, although properly termed orthodentine, will be referred to simply as dentine. However, salient differences in the formation and histological structure of the two tissues mean that the distinction between mantle and circumpulpal dentine will be retained where appropriate.

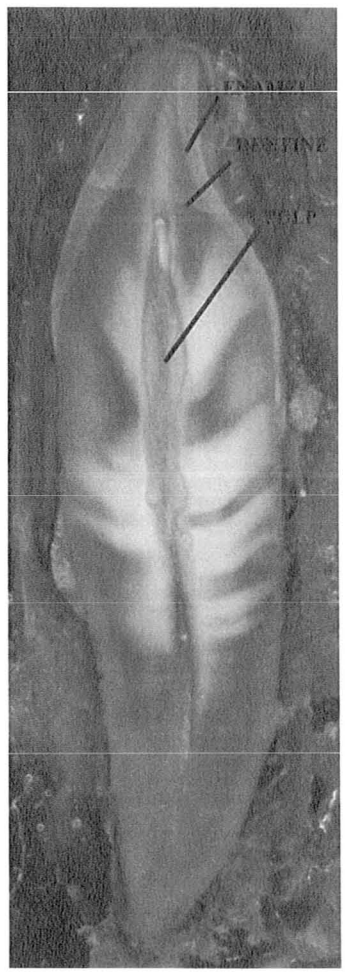

Figure 2.1 Thin section illustrating the distribution of enamel, dentine and pulp. The advanced age of this specimen means that large portions of the dentine that would normally appear opaque are now transparent. This is discussed in detail later.

2.3 Human Orthodentine

In humans, dentine exists as a mineralized connective tissue that is produced via a two-stage process involving the deposition of inorganic mineral within a collagenous organic matrix (Mjor, 1984). By weight, human dentine is composed of roughly 70% inorganic mineral, 18% organic components and 12% water (Mjor, 1984; Torneck, 1989; Dai et al., 1991; Linde and Goldberg, 1993). By volume, it is roughly 47% inorganic material, 32% organic material and 21% water (Armstrong and Brekhus, 1937; LeFevre and Manly, 1938). However, these proportions are variable both with anatomical location and chronological age (Linde and Goldberg, 1993).

Both by weight and volume, dentine constitutes the largest proportion of the mature tooth. It is produced by specialized cells referred to as *odontoblasts* during *dentinogenesis*, a process intimately tied to the microstructure of the tissue in life that has relevance for the later discussion of root dentine transparency (Mjor, 1984).

2.4 Types of Human Dentine

Dentine within the human tooth exists in three morphologically distinct forms, each of which develops in a unique manner. The term *primary dentine* is generally used to refer to all forms of dentine produced prior to the completion of tooth development (Torneck, 1989). It constitutes the majority of human dentine, encompassing the entire pulp chamber of a fully formed tooth and being covered at the crown by enamel and at the root surface by cementum (Hillson, 1996). The FDI definition of primary dentine refers to it as

> "...that portion of regular dentine (orthodentine) produced during the formation period up to near completion of the external shape of the tooth" (quoted in Baume, 1980:61).

Secondary dentine refers to that dentine which is deposited at a much slower rate, on the pulpal aspect of primary dentine following the closure of the root apices - an event

which marks the cessation of initial tooth growth (Torneck, 1989). The FDI definition of secondary dentine describes it as

> "...that circumpulpal portion of regular dentine (orthodentine) in continuity with the primary one produced circumpulpally throughout the later periods of the vital tooth." (quoted in Baume, 1980:61).

Primary and secondary dentine are relatively continuous and the junction between the two is therefore somewhat indistinct; however, the histological structure of secondary dentine is more irregular than that of primary dentine and it is deposited rather unevenly within the pulp cavity (Torneck, 1989).

Tertiary dentine is that dentine which is deposited in response to external stimuli, including caries, attrition or endodontic treatment (Linde and Goldberg, 1993). Although the amount of tertiary dentine often increases with age, its production is the byproduct of a pathological intrusion rather than a regular, physiological process. Both the structure and quantity of tertiary dentine are variable according to the nature and duration of the stimulus (Torneck, 1989). Accordingly, the FDI defines tertiary dentine as

> "the dentine, more or less irregular in structure deposited at sites of the pulpal aspects of primary or secondary dentine, corresponding to areas of external irritations" (quoted in Baume, 1980:61).

2.5 Mantle Dentine

Mantle dentine is the layer of primary dentine deposited during the earliest stage of dentine production, immediately following odontoblast differentiation (Jones and Boyde, 1984; Linde and Goldberg, 1993). Its mineral content is lower than that of circumpulpal dentine and its formation is associated with the presence of large inter-odontoblastic collagen fibers oriented parallel to the course of the tubules which traverse its length (Baume, 1980; Jones and Boyde, 1984).

Within the crown, mantle dentine is the layer of dentine immediately adjacent to the dentino-enamel junction (DEJ) (Jones and Boyde, 1984). Here, its thickness in human teeth is roughly 150 μm (see Figure 2.2) (Torneck, 1989). Within the root, mantle

dentine exists as a layer of variable thickness (15-30 μm) peripheral to the *Granular Layer of Tomes*, a hypomineralized layer of dentine underlying the cemento-dentinal junction (CDJ) (Jones and Boyde, 1984; Hillson, 1996). Root mantle dentine is morphologically similar to crown mantle dentine; however, in contrast to their arrangement in the crown, collagen fibers in root mantle dentine are oriented parallel to the mineralization front and much thicker than those found elsewhere (Jones and Boyde, 1984).

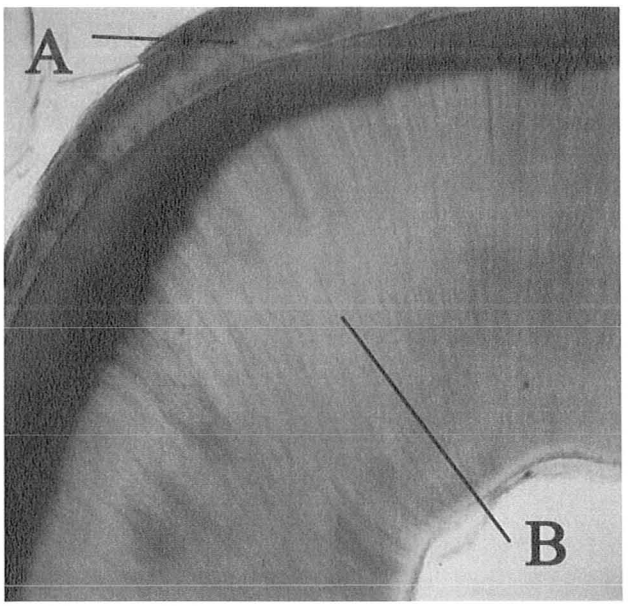

Figure 2.2 Low magnification image showing the dentino-enamel junction (DEJ)(Dozenist, 2005). The line separating enamel (A) from dentine (B) is well defined. The darker dentine immediately below the DEJ is mantle dentine, while the lighter area surrounding the pulp is circumpulpal dentine.

The unique collagen-rich fibers of mantle dentine are often referred to as *von Korff's fibers* in order to denote their morphological and structural distinction from those collagen fibers found within the organic matrix of circumpulpal dentine. Although their presence is a hallmark of mantle dentine, their identity and biochemical structure remain contested. Ten Cate (1989) in particular believes them to be an optical artifact arising from the silver staining of dentinal ground substance. However, a review of the

histochemical and ultrastructural research in the field suggests that von Korff's fibers exist as glycosaminoglycan (GAG) sheathed fibers with a collagenous core oriented parallel to the cytoplasmic extensions of the odontoblasts responsible for dentine production (Baume, 1980).

Much like their existence, the origins of the von Korff's fibers remain a source of controversy. Ten Cate (1978; 1989) maintains that all collagen within human dentine is the product of odontoblastic activity. However, Meyer et al. (1977) and Katchburian and Burgess (1977) have reported the existence of von Korff's fibers between pre-functional odontoblasts concomitant with the dissolving of the basal lamina. Further, the irregular network of collagen fibers that exist between the bipolar subodontoblastic cells of the peripheral pulp, known as *Hohl's cells,* has been shown to be continuous with the von Korff's fibers of mantle dentine (Baume, 1980). Finally, intercellular connections exist between Hohl's cells and odontoblasts (see Frank et al., 1964; Takuma and Eda, 1966; Baume, 1980). Although this does not provide clear evidence of dentine production by cells other than odontoblasts, it does emphasize the continuity of dentine with pulp and the importance of the cells of this region in the metabolic activity of odontoblasts.

2.6 Circumpulpal Dentine

The remaining primary dentine, bounded at its innermost margin by the pulp cavity, is referred to as circumpulpal dentine (see Figure 2.2) (Linde and Goldberg, 1993). The FDI has adopted the following definition for circumpulpal dentine:

> "...the main bulk of orthodentine characterized by fine collagen fibers running approximately at right angles to the long axes of the tubules" (quoted in Baume, 1980:57).

Circumpulpal dentine is a mineralized connective tissue permeated throughout by long canals housing odontoblasts, the cells responsible for its production. During circumpulpal dentine formation odontoblasts move centripetally towards the pulp at the

centre of the tooth (Jones and Boyde, 1984). The two major regions within circumpulpal dentine are designated intertubular and peritubular dentine.

Intertubular Dentine

Intertubular dentine is the mineralized collagenous matrix that exists between the tubules that house the cells responsible for dentine production (see Figure 2.5) (Baume, 1980; Jones and Boyde, 1984). It is the major product of odontoblast production during dentinogenesis (Linde and Goldberg, 1993). It is a composite tissue, constituted by an organic matrix into which an inorganic mineral has been deposited (Linde and Goldberg, 1993). Although the mineral content of intertubular dentine is relatively constant, the amount of intertubular dentine per unit area decreases in a pulpal direction, due to an increase in the relative number of odontoblasts towards the dentine-pulp margin (Mjor, 1984).

Peritubular Dentine

The mineralized cuff of tissue demarcating the outer boundary of dentine tubules (0.5 to 1 μm thick) is referred to as *peritubular dentine* (Linde and Goldberg, 1993; Hillson, 1996). Peritubular dentine is deposited within the tubule following the formation of intertubular dentine (Blake, 1958; Linde and Goldberg, 1993). Although its organic and inorganic components appear identical to that of intertubular dentine, peritubular dentine is more highly mineralized than intertubular dentine, displaying both a higher mineral density and smaller crystallite size (Nalla et al., 2005). Peritubular dentine contains a limited organic matrix for which the few collagen fibers are continuous with those of the intertubular matrix (Blake, 1958; Mjor, 1984). It exists throughout the majority of circumpulpal dentine; however the thickness of peritubular dentine appears to be more irregular in root (radicular) dentine than in crown (coronal) dentine (Blake, 1958). It is not present in unmineralized predentine, nor in anomalous areas in which mineralization has failed to occur (Blake, 1958; Mjor, 1984).

Both intertubular and peritubular dentine contain nearly identical organic and inorganic components. It is the variation in the proportion of each that distinguishes the two. In order to understand the structure of circumpulpal dentine and the process through which it is manufactured, it is necessary to first describe its constituent organic and inorganic components.

2.6.1 The Organic Matrix

Histochemical and microchemical investigations into the organic matrix of circumpulpal dentine reveal that it is primarily composed of collagen (Eastoe, 1967; Johansen, 1967; Hohling, 1989; Wiesmann et al., 2005). Although the exact proportions vary somewhat between studies, it appears that collagen constitutes roughly 90-95% by weight of the organic material contained within dentine, the remaining organic materials consisting of small amounts of mucopolysaccharides and lipids (Johansen, 1967; Hohling, 1989). It is this organic material which is responsible for the genesis, both in terms of precipitation and orientation, of the inorganic crystallites of dentinal mineral (Eastoe, 1967). Collagen is a structural protein found within the vast majority of mammalian connective tissues (Wiesmann et al., 2005). It is the most abundant protein in the human body, and may be described as

> "an insoluble, fibrillar material that is constructed of many highly elongated, thread-like molecules cross-linked together." (Butler, 1984:38)

Numerous varieties of collagen exist, each of which differ according to their structural appearance (Mayne and Brewton, 1993). Dentine collagen is composed almost exclusively of Type I collagen, with a small amount of Type V and Type III collagens present (Wiesmann et al., 2005). All three are banded, fibril-forming proteins (Wiesmann et al., 2005).

At its most basic level each collagen fiber is composed of a variable proportion of 18 different amino acid molecules joined together to form a polypeptide chain (Eastoe, 1967; Butler, 1984). The chemical structure of each amino acid centers around a carbon

atom to which are attached four different chemical groups: an α-amino group, a carboxyl group, a hydrogen atom and an organic side chain of variable length, denoted as the R group (Eastoe, 1967). Biosynthesis of the polypeptide chain involves the joining together of the carboxyl group of one amino acid with the α-amino group of a neighbouring amino acid, forming an amide bond (Eastoe, 1967).

The organization of the constituent amino acids within each chain has important structural and chemical consequences for collagen as a tissue (Eastoe, 1967). For each polypeptide strand of collagen, the sequence of amino acids repeats in a regular pattern, the general form of which is $(\text{-NH} \cdot \text{CHR} \cdot \text{CO-})_n$ where n may be very large (Eastoe, 1967). Within dentine collagen, one in every three amino acid units is glycine, an amino acid without an R group, while one in every ninth position is occupied by alanine with a single methyl group in the R position (Eastoe, 1967; Butler, 1984).

Each collagen macromolecule, or tropocollagen molecule, consists of three polypeptide chains joined together and twisted into a *left-handed minor helix* about a common axis with a pitch of 0.93 nm and three amino acids per turn (Eastoe, 1967; Butler, 1984:38). The tropocollagen molecule itself is twisted into a *right-handed, major helix*, the pitch of which is 2.86 nm, meaning that there are ten amino units per turn (Eastoe, 1967; Butler, 1984:40). Each triple helical molecule is composed of two α1 and one α2 polypeptide chains, so denoted in order to recognize the minute yet important differences in the amino acid composition of each (Eastoe, 1967). These chains are held together laterally by bonds between their amino and carbonyl groups as well as by various covalent bonds (Eastoe, 1967).

Tropocollagen molecules in turn are joined together to form collagen fibrils. Within each fibril, these molecules are arranged in parallel, being held together by bonds between polypeptide chains of adjacent tropocollagen rods (Eastoe, 1967; Butler, 1984). Collagen fibrils may be of indefinite length with a width of approximately 4 nm (Wiesmann et al., 2005). In an electron microscope, these fibrils exhibit a regular pattern of alternating light and dark areas at intervals of 67 nm (Butler, 1984; Wiesmann et al., 2005). This banded appearance arises as a result of the way in which each tropocollagen

rod is arranged with respect to its neighbours, each molecule being staggered a distance equal to roughly one quarter of its length (Butler, 1984). Such an arrangement creates repeating *overlap* and *hole zones* between the constituent molecules of each fibril (Butler, 1984). During mineralization, these hole zones with a depth of 2nm are able to accommodate a greater quantity of inorganic mineral than are the areas of molecular overlap, a phenomenon which produces the regular pattern of optical anisotropy which characterizes Type I collagen fibrils (Hodge and Petruska, 1963). This pattern is referred to as *D*, or the collagen macroperiod (Wiesmann et al., 2005). The length of the overlap zone is equal to $0.4D$ while the hole zone is $0.6D$ in length (Wiesmann et al., 2005).

The highest order of collagen organization is visible under light microscope and involves the parallel arrangement of collagen fibrils into bundles to form fibers (Eastoe, 1967; Butler, 1984). Each fiber is roughly 50-100 nm in width (Wiesmann et al., 2005). Within circumpulpal dentine, their arrangement forms an irregular "feltwork," with the orientation of each fiber being random in two dimensions yet roughly perpendicular to the path of the dentinal tubules (Jones and Boyde, 1984:111; Kinney et al., 2001b; Wiesmann et al., 2005). However, increasing disorganization has been reported at the peripheral margins of dentine nearest the CDJ and the DEJ (Kinney et al., 2001b). Yet, collagen orientation is not entirely random. Recent research has shown that mechanical strain is a regulator of both collagen fiber assembly and the formation of crystals within this matrix (Wiesmann et al., 2005). Collagen fibers oriented with their long axes parallel to the length of the dentinal tubule have also been reported. However, their distribution appears to be limited to the areas immediately adjacent to these structures (Jones and Boyde, 1984; Dai et al., 1991).

The somewhat haphazard arrangement of collagen fibers, along with the low solubility of this tissue and its lack of swelling under acidic conditions, make dentinal collagen distinct from the collagens contained within uncalcified connective tissues (Eastoe, 1967:297). These properties may indicate an additional set of cross-linkages within the collagenous matrix of dentine, designed to offer increased resistance to the forces associated with occlusal loading of the functional tooth (Eastoe, 1967).

Ground Substance

Several non-collagenous proteins are included within the organic matrix of dentine, albeit in much smaller concentrations than collagen. Collectively, these are referred to as the *ground* or *cementing substances*. They are characterized by a relatively high concentration of various mucopolysaccharides and mucoproteins (Eastoe, 1967; Ten Cate, 1968; Ten Cate, 1989). The molecules contained within this portion of the organic matrix include chondritin sulphate, citrate, lactate, and various phospholipids and sialoproteins (Eastoe, 1967; Linde and Goldberg, 1993).

Lipids, including cholesterol, cholesterol esters, triglycerols and various phospholipids, constitute roughly 2% of the organic matrix of dentine (Linde and Goldberg, 1993). *In vitro* deficiencies in dentine production in the absence of essential fatty acids hint at their importance in dentine production (Prout et al., 1973). The exact role of these molecules has yet to be determined. However they are thought to play a vital role in the nucleation of inorganic crystallites within the collagenous matrix of dentine. For a more complete discussion of this subject see the description of predentine below.

2.6.2 The Mineral Phase

An analysis of the inorganic mineral of dentine in human permanent teeth reveals that it is primarily composed of calcium and phosphorous at roughly 35.1% and 16.9% of dry weight respectively (Le Gros, 1991). Magnesium, carbon dioxide and sodium account for the bulk of the remaining inorganic content (for a review of ashing studies of dentine see Rowles, 1967). At an average of 1.61, the molar ratio of calcium to phosphorous in dentine is in general lower and more variable than that reported for enamel (1.63) (Le Gros, 1991).

As in bone and enamel, the preponderance of mineral contained in dentine is found in the form of calcium hydroxyapatite (Johansen, 1967; Le Gros, 1991; Kinney et al., 2001b). The chemical formula for this molecule is $Ca_{10}(PO_4)_6(OH)_2$ (Trautz, 1967).

The three dimensional crystalline structure of hydroxyapatite is characterized by a six fold *c*-axis perpendicular to three equivalent *a*-axes, each at angles of 120° to one another (Trautz, 1967; Le Gros, 1991). The macroscopic structure of the mineral found within dentine arises via the union of many hydroxyapatite molecules or "unit cells" linked together in an appositional manner expanding to indefinite proportions in each of the three directional axes (Trautz, 1967:167).

Mineral size is relatively homogenous in dentine, the majority of crystals being approximately 5 nm in thickness (Balooch et al., 2001; Kinney et al., 2001b). Although chemically identical, the average size of hydroxyapatite in dentine is smaller than in enamel (Trautz, 1967). This difference is likely due to the collagenous matrix into which hydroxyapatite is deposited during dentine growth. Gap zones between collagen fibrils may act to constrain crystal growth, thereby limiting overall crystal size in dentine (Kinney et al., 2001b). Comparatively high concentrations of magnesium and carbonate in dentine may also affect crystallite size, as high quantities of both have been experimentally shown to cause the formation of smaller apatite crystals (Althoff et al., 1982; Le Gros, 1991; Roy and Nishimoto, 2002). Crystal shape is variable by anatomical location within the tooth, changing from needle-like to more plate-like with increasing movement towards the tooth periphery (Kinney et al., 2001b).

Hydroxyapatite crystallites are located both within and between collagen fibrils. Their orientation appears to be dictated by the arrangement of the collagen fibers in which they are embedded (Trautz, 1967). Crystals are organized in a regular manner, each with their long axis (*c*-axis) aligned parallel to the length of a given collagen fibril (Johansen, 1967). Further, mineral density varies regularly with the periodicity of the repeating overlap and hole zones of collagen fibrils, being greatest in the inter-fibrillar areas (Johansen, 1967; Hohling, 1989).

Hydroxyapatite in dentine shares many of the physical and optical properties of other apatite minerals. In particular, it displays uniaxial birefringence, the sign of which is weakly negative (Trautz, 1967). Substitutions of other chemicals within the hydroxyapatite lattice account for the majority of trace inorganic elements associated

with dentine (Rowles, 1967; Le Gros, 1991). Some are incorporated directly into the molecule, while others may be absorbed on the crystal surface (Le Gros, 1991). Among these are carbonate (CO_3^{-2}) and inorganic phosphate (HPO_4^{-2}) as well as fluorine (F^{-1}), magnesium (Mg^{+2}) and chloride (Cl^{-1}) ions (Le Gros, 1991). The addition or substitution of any of these chemicals affects the local chemical, physical and optical properties of dentine (Le Gros, 1991).

Within dentine, the concentration of the mineral phase is highest in the peritubular zone immediately surrounding the tubules (Blake, 1958; Johansen, 1967; Kinney et al., 2005; Porter et al., 2005). Intertubular dentine displays an intermediate mineral content, while the mineral content of mantle dentine is the lowest of the mineralized portions of dentine (Ten Cate, 1989; Kinney et al., 2005; Porter et al., 2005). A mineral phase is not present within predentine (Ten Cate, 1989).

2.6.3 The Odontoblast

Odontoblasts are the cells responsible for the production of all human coronal and radicular tooth dentine (Smith, 2000). They are columnar or cuboidal in shape and have their origin in primitive ectomesenchymal tissue (see Figure 2.3) (Jones and Boyde, 1984; Mjor, 1984). During the *Bell stage* of tooth formation, their division is initiated through growth hormone secretion by the preameloblast cells on the opposite side of the basement membrane separating the epithelial and mesenchymal cell populations (Jones and Boyde, 1984; Linde and Goldberg, 1993; Ruch and Lesot, 2000). In response to this hormonal impetus, undifferentiated mesenchymal cells of the dental papilla undergo mitosis. At the cessation of mitotic division, these cells transform into preodontoblasts, "rounded or oval binucleated cells whose cellular contents include ribosomes, rough endoplasmic reticulum (RER) and a pre-functional Golgi apparatus" (Linde and Goldberg, 1993:686). Mature odontoblasts represent the final stage of preodontoblast maturation, a process that involves both cellular polarization and organelle

reorganization (for a more complete treatment of odontoblast differentiation and maturation see Baume, 1980; Linde and Goldberg, 1993; Ruch and Lesot, 2000).

Within endoplasmic cytoplasm, each odontoblast contains at its proximal end a single nucleus (with two nucleoli) and numerous other organelles including rough endoplasmic reticulum, a Golgi apparatus, several mitochondria, and various vesicles, vacuoles, microtubules and fibrils (Mjor, 1984). However, both the shape of the odontoblast and the frequency of the various organelles contained within it vary according to the secretory activity of the cell (Mjor, 1984; Torneck, 1989).

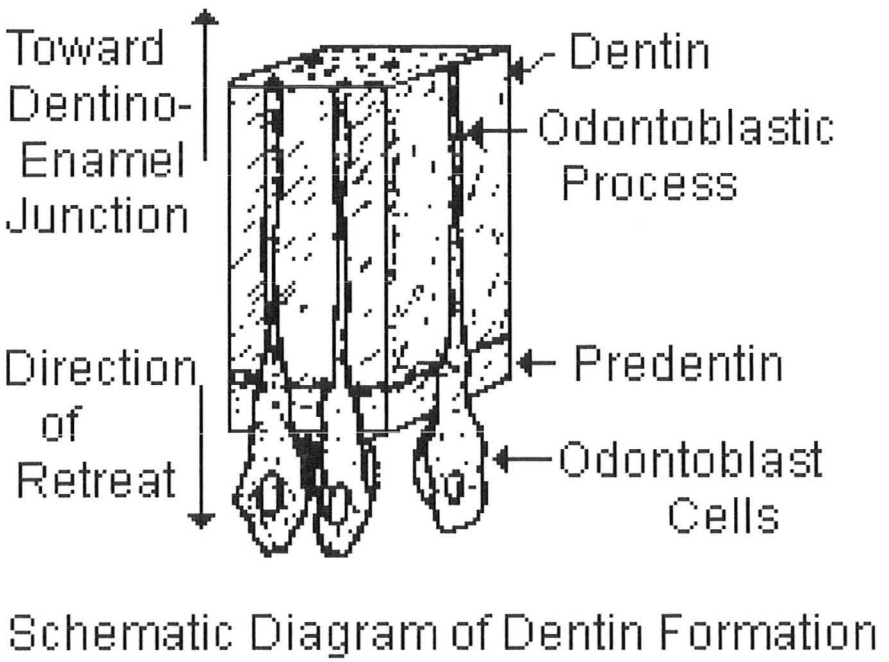

Figure 2.3 Schematic diagram of dentine formation (Johnson, 1998). Each odontoblast is depicted with an elongated odontoblast process (OP) emanating from an oval-shaped cell body. The OP exists within a dentinal tubule which extends throughout the circumpulpal dentine towards the DEJ. The cell bodies face the peripheral margin of the pulp, narrowing the pulp cavity as they deposit dentine during primary dentinogensis.

Odontoblasts are aligned in a single layer at the pulpal border of circumpulpal dentine (see Figure 2.3) (Ten Cate, 1989; Torneck, 1989). They are linked to one another laterally by a series of gap junctions, tight junctions and desmosomes (specialized surface structures of a membrane that serve as zones of adhesion to anchor contiguous cells together) (Baume, 1980; Mjor, 1984). These junctions also form a membranous layer which maintains the separation of predentine and pulp (Baume, 1980; Orchardson and Cadden, 2001). They also allow the odontoblasts to communicate with one another and may facilitate the transport of necessary compounds and elements to the mineralization front (Linde and Goldberg, 1993).

Unlike the ameloblasts responsible for enamel formation, odontoblasts do not expire following tooth completion (Ten Cate, 1989). Instead, they persist at the inner margin of dentine and remain capable of continued dentinogenesis following the cessation of primary dentine production (Smith, 2000). Nevertheless, they are considered a "static, postmitotic cell population," which is incapable of further division (Baume, 1980:71). However, it has been suggested that bipolar cells of the peripheral pulp may constitute a reserve population from which cells may be recruited to replace dead or diseased odontoblasts (Stanley, 1962; Smith, 2000).

The Odontoblast Process

Perhaps the most characteristic feature of the odontoblast is the *odontoblast process*, or *Tomes process*, which extends from the cell body towards the periphery of the tooth, passing through both predentine and dentine (see Figure 2.3 and Figure 2.4) (Baume, 1980). Each odontoblast process contains within it a series of microtubules and microfilaments related to the production and secretion of the organic phase of dentine (Mjor, 1984). Branching of the peripheral ends of the odontoblast processes varies according to anatomical location, being more finely divided in the roots (Mjor, 1984; Mjor and Nordahl, 1996). Odontoblast process morphology also varies according to

cellular activity. During early dentine production, branching of the cytoplasmic extension of the odontoblast process is particularly pronounced (Mjor, 1984).

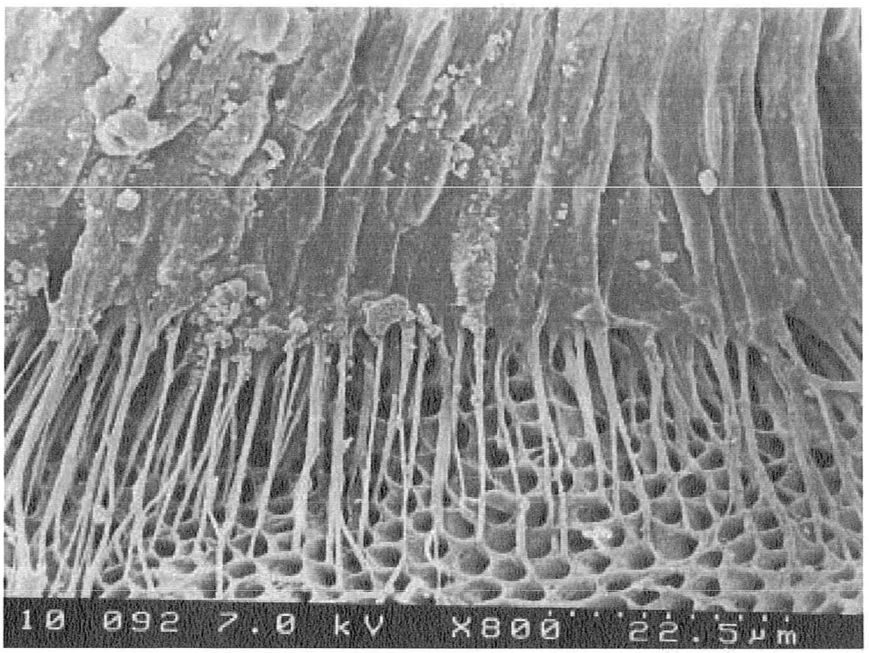

Figure 2.4 Scanning electron microscope image of the pulp-dentine border at 800X magnification (Goracci et al., 1999). Odontoblast cell bodies are visible at the upper portion of the picture. The elongated odontoblast processes can be seen extending towards the bottom of the image where they insert into dentinal tubules (see below).

2.6.4 Dentinal Tubules

The small tunnel-like structures housing the long distal processes of the odontoblasts are referred to as *dentine tubules* (see Figure 2.4 and Figure 2.5). At its outer margin, each tubule is lined with a highly mineralized layer of peritubular dentine of variable thickness (see Figure 2.6) (Blake, 1958). Tubules run the entire length of circumpulpal dentine, however they are not present within predentine, as they only form following apatite deposition (Ten Cate, 1968). These tunnels form around the cytoplasmic processes of odontoblasts as they migrate towards the pulp cavity (Ten Cate, 1968). Although it is unclear how far odontoblast processes extend throughout the length of the fully formed tubule following mineralization, processes have been reported lining

the entire length of tubules, from the pulp margin to the DEJ (Baume, 1980; Arana-Chavez and Massa, 2004).

Within tubules empty periodontoblastic space is scarce (Carda and Peydro, 2006). In addition to the cellular extensions of the odontoblasts, each tubule contains dentinal liquor, a fluid component separating the odontoblast process from the tubule wall (Torneck, 1989). They are also lined by an amorphous material extending from the DEJ to the predentine-dentine junction. Scanning electron microscopic investigation of demineralized dentine indicates that this substance represents a 30 nm thick, organic, sheet-like structure lying free of the odontoblast process and separated from the tubular walls by a distance equal to the peritubular matrix (Thomas and Carella, 1983). This amorphous material is therefore thought to represent the inner hypomineralized layer of the peritubular matrix (Thomas and Carella, 1983). It is referred to as the *lamina limitans* in accordance with the terminology developed for similar tissues within bone. Nerve fibers also lie within the tubules, extending from the pulp towards the peripheral margins of dentine (Orchardson and Cadden, 2001; Carda and Peydro, 2006). These fibers lie in close proximity to the odontoblast processes and in some cases may be entirely encompassed by them (Carda and Peydro, 2006).

The path described by tubules as they run from the outer dentinal margin to the pulp surface is S-shaped. This *primary curvature* is especially pronounced within the crown, becoming less visible towards the cusp tips and in the cervical third of the root dentine where tubules may run in a nearly straight path (see Figure 2.2) (Bradford, 1967). The primary curvature likely arises as an artifact of the crowding of odontoblasts towards the pulpal margin, where their density is known to increase in comparison to peripheral dentine (Ten Cate, 1989; Torneck, 1989). Minute deviations in the primary curvature of each tubule have also been noted and are referred to as *secondary curvatures* (Torneck, 1989). The origin of these smaller oscillations remains unclear.

Lateral branching along the length of tubules occurs roughly every 1 to 2 μm (Torneck, 1989). Branches may be between 1 μm and 25 nm in diameter (Mjor and Nordahl, 1996). In general, the number of tubule branches decreases as the diameter and

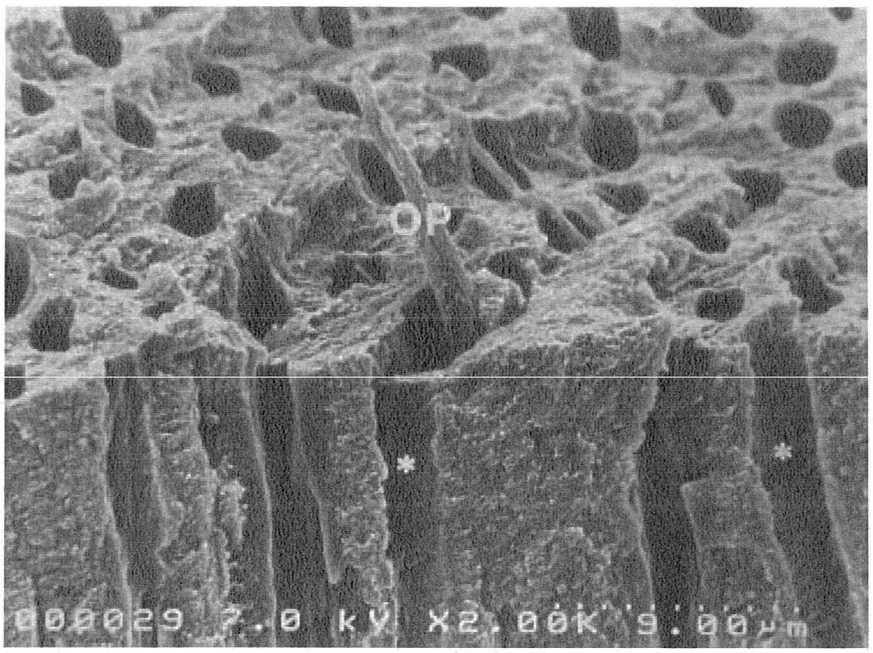

Figure 2.5 Scanning electron microscope image of fractured dentine at 2,000X magnification (Goracci et al., 1999). Odontoblast processes (OP) are visible protruding from tubules (*). The mineral between tubules is intertubular dentine.

density of tubules increases (Mjor and Nordahl, 1996). Terminal branching of dentinal tubules is particularly pronounced in the peripheral portion of root dentine, in which many fine extensions form a network of filamentous tubules extending into the mantle dentine (Torneck, 1989; Mjor and Nordahl, 1996).

Tubule morphology and distribution varies with anatomical location within the tooth. Tubule diameter decreases from 3µm adjacent to the pulp surface to less than 1µm in peripheral dentine (Mjor, 1984; Torneck, 1989; Dourda et al., 1994). Further, the mean number of dentinal tubules per 100 µm at the pulpal aspect of crown dentine is significantly higher than at the periphery (Torneck, 1989; Mjor and Nordahl, 1996). Accordingly, the cross-sectional area occupied by dentinal tubules has been found to increase in a pulpal direction (Marchetti et al., 1992; Dourda et al., 1994; Mjor and Nordahl, 1996; Orchardson and Cadden, 2001).

When root dentine is compared to crown dentine, a significant difference is once again reported, with the density of tubules in the mid portion of crown dentine being

higher than that at a similar depth in radicular dentine (Mjor and Nordahl, 1996). Within root dentine, tubule density increases to a maximum in the cervical third of the root and is lowest at the root apex (Mjor and Nordahl, 1996; Harran et al., 2001). Furthermore, the shape of tubule openings appears to become more irregular towards the apical third of the root (Harran et al., 2001).

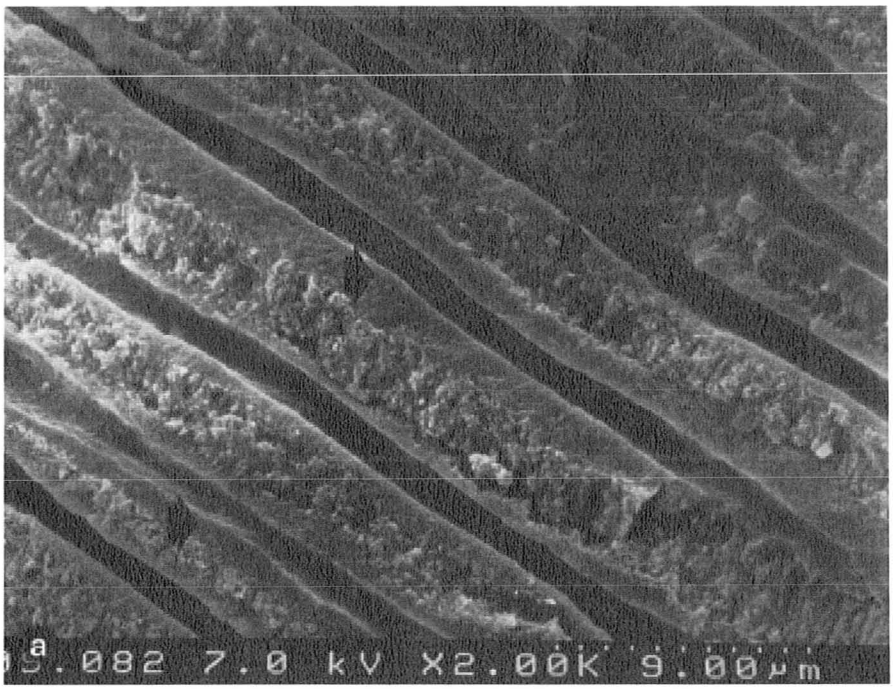

Figure 2.6 Scanning electron microscope image of fractured circumpulpal dentine at 1.0mm from the pulpal surface (Goracci et al., 1999). Tubules are visible as dark canals running from upper left to bottom right. Each highly mineralized cuff of peritubular dentine (arrows) is visible as a light halo separating the tubules from the interstitial intertubular dentine. Odontoblasts have been removed during specimen preparation.

Tubule diameter also varies according to age and pathological changes, due to the deposition of mineral within tubule lumens both in response to pathological intrusion and as part of a poorly understood physiological process (Blake, 1958; Baume, 1980; Traub et al., 1988). The latter process, referred to as root dentine sclerosis underlies the phenomenon of root transparency, the subject of this study. As such, it will be dealt with in greater depth below.

While the heterogeneity of tubule morphology and distribution in cross-sectional studies is agreed upon, a three-dimensional analysis of the spatial distribution of tubules has not yet been undertaken (Kinney et al., 2001a). This is of extreme importance given that tubule orientation varies with anatomical location within the tooth (Vasiliadis et al., 1983b; Kinney et al., 2001a). The spatial arrangement of tubules has major implications for any discussion of alterations in tubule diameter and density, as both may be due to changes in the orientation of tubules with the plane of sectioning at various depths from the tooth surface.

2.7 Dentinogenesis

Dentinogenesis of both circumpulpal and mantle dentine involves two simultaneous processes: the formation of the organic matrix of predentine and the subsequent mineralization of this collagenous template (Linde and Goldberg, 1993).

2.7.1 Organic Matrix Production

Predentine

A layer of unmineralized organic matrix composed primarily of collagen and proteoglycans is referred to as predentine (Linde and Goldberg, 1993). During primary dentinogenesis, predentine exists as a layer preceding the mineralization front (see Figure 2.7) (Linde and Goldberg, 1993). Following primary dentine production, it persists on the inner margin of circumpulpal dentine. Lying between the connective tissue of the pulp and the last layer of mineralized dentine and persisting throughout the life of a tooth, this layer is similar to osteoid in bone (Ten Cate, 1989; Linde and Goldberg, 1993). The thickness of predentine varies between 10 and 47 μm according to the secretory activity of the odontoblasts and is thickest during active dentinogenesis (Ten Cate, 1989; Linde and Goldberg, 1993).

The predentine matrix is primarily composed of Type I collagen, previously discussed. However, the collagenous network within predentine is neither uniform, nor is it identical with that contributing to mature circumpulpal dentine (Beniash et al., 2000). Rather, there is evidence that the collagen fibrils undergo a thickening towards the predentine-dentine junction and that the fibrils within both regions display differences in their banding patterns when compared with one another (Beniash et al., 2000). The biochemical modification of predentine prior to mineralization and the identity of the molecules implicated in this regard are discussed below.

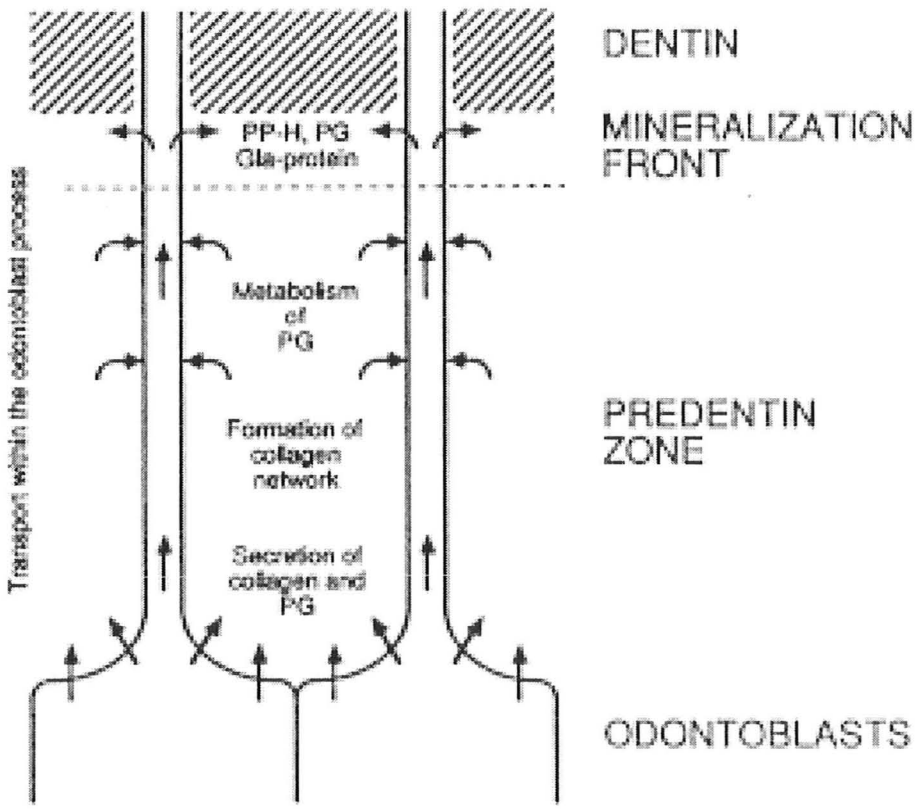

Figure 2.7 Schematic diagram representing the production of predentine and its subsequent mineralization during primary dentinogenesis (Linde, 1989). Collagen and various proteoglycans (PG) are exocytosed into the predentine zone when collagen assembly begins. Towards the mineralization front there is an exchange of un-metabolised PG's and an excretion of phosphorolated phosphoproteins and Gla-proteins, both of which are involved in mineral nucleation. These subjects are addressed below.

Matrix Synthesis

Following differentiation and maturation, odontoblasts begin to produce the organic matrix of dentine (Linde and Goldberg, 1993). The six endoplasmic phases of collagen synthesis and secretion by odontoblasts have been summarized by Weinstock and Leblond (1974). These stages can be simplified into four main steps, beginning with the synthesis of polypeptide chains within the rough endoplasmic reticulum of the odontoblasts (Weinstock and Leblond, 1974). These molecules are then transported to the Golgi apparatus where they assume a parallel organization (Weinstock and Leblond, 1974). The resultant procollagen molecules, immature segments of tropocollagen, are then transported outside of the cell. They exit through lateral branches of the odontoblast process via secretory granules which also contain various phosphoproteins and carbohydrates involved in collagen synthesis and mineral impregnation of the resultant organic matrix (Weinstock and Leblond, 1974). The last stage of collagen synthesis involves the extracellular assembly of tropocollagen molecules into elementary collagen fibrils (Reith, 1968). This process requires the enzymatic modification of the molecules prior to their assembly (Weinstock and Leblond, 1974).

2.7.2 Mineralization

A thorough discussion of the timing and histochemical particulars of dentine mineralization is necessary in order to adequately discuss the processes underlying root dentine transparency. Etiological theories surrounding the progression of root dentine transparency rest on the fundamentals of primary dentine structure and cannot be understood without prior grounding in the processes involved in its formation. Further, knowledge of the character of the mineral phase and its relation to the macroscopic attributes of dentine is imperative, as qualitative differences between areas of transparent and normal dentine arise from modifications and additions to the structure of this portion of dentine.

The Mineralization Front

The mineralization front represents the peripheral mineralizing zone of predentine and marks the transition to mature dentine (see Figure 2.7) (Ten Cate, 1989). It is composed of two distinct areas of mineralization (Fiore-Donno and Baume, 1966). The zone of the mineralization front nearest the odontoblast cell body, the *intertubular mineralization front*, represents the area in which mineralization of the intertubular matrix advances (Baume, 1980). Because intertubular mineralization precedes crystal growth within the peritubular matrix, the other zone of mineralization, the *peritubular mineralization front*, is situated peripheral to this, at a greater distance from the pulp margin (Baume, 1980). It is in this zone where tubules become visible, following the deposition of mineral at the boundary of the tubule lumen (Baume, 1980). The intertubular and peritubular zones of mineralization are separated by a distance which varies according to the speed of organic matrix production, being widest during those times in which deposition rates are at a peak (Baume, 1980).

Mineral Transport

Mineralization of predentine begins following calcium and phosphate precipitation out of solution (Posner and Tannenbaum, 1984). In order for crystal formation to advance, the local concentration of both ions must be sufficiently high (Posner and Tannenbaum, 1984). The role of the odontoblast in the transport and accumulation of the calcium and phosphate necessary for crystal growth is unclear (Irving, 1973; Hohling and Fromme, 1984). However, it appears that Ca^{+2} and P_i (inorganic phosphate) may be moved towards the mineralization front independently of one another via both intracellular and extracellular pathways (Irving, 1973; Hohling and Fromme, 1984). Studies of mineral transport by enterocytes, the polar cells of the small intestine, suggest that both active intracellular and passive intercellular mechanisms of mineral transport and concentration may exist (Murer and Hildmann, 1981). Further, intracellular mechanisms of transport and accumulation of Ca^{+2} and P_i via mitochondria and rough endoplasmic reticulum have been suggested, based on the observation of

similar pathways involved in the calcification of bone (Wuthier, 1977). Despite the obscurity of the mechanisms by which ion transport occurs within and between odontoblasts, it is clear that the concentration of Ca^{+2} and P_i at the mineralization front is considerably greater than that found to be necessary for mineral nucleation in vitro (Hohling and Fromme, 1984; Linde and Goldberg, 1993).

Crystal Nucleation

While elevated local concentrations of mineral ions are a necessary condition for mineralization, they do not constitute a sufficient circumstance for crystal growth. Even within a supersaturated solution, *heterogeneous nucleators* (chemicals which lower the activation energy barrier for precipitation) must be present in order for mineralization to occur (Posner and Tannenbaum, 1984).

In the earliest stages of mineralization, occurring in mantle dentine, *matrix vesicles* - "round or oval-shaped entities with a diameter roughly in the range of 100 nm, containing no collagen and surrounded by a bilaminar membrane" - are thought to serve as sights of apatite nucleation (Wiesmann et al., 2005:132). Located between the collagen fibers of the organic matrix, they act as the earliest sites of calcification in mantle dentine (Bonucci, 1984). Similar entities are found in calcifying bone and cartilage (Ali et al., 1971; Bab et al., 1981). Numerous electron microscopic investigations of calcification in these tissues support the role of matrix vesicles in the onset of calcification (for a full review see Bonucci, 1984; Ohma et al., 2000). The presence of crystals within matrix vesicles prior to mineral formation in the surrounding organic matrix has been documented in bone (Dearden and Espinosa, 1974). In cartilage, the concentration of Ca^{+2} within these bodies has also been shown to exceed that in adjacent chondrocytes by roughly fifty times (Wuthier, 1977). A high concentration of matrix vesicles adjacent to the ameloblast border of mantle dentine prior to calcification is therefore suggestive of their similar role in the initiation of mineral formation in dentine (Takano et al., 2000).

Collagen Mineralization

Matrix vesicles are not present within the predentine of circumpulpal dentine, nor is there evidence of elevated, localized mineral density associated with their prior mineralization in mature circumpulpal dentine (Hohling, 1989; Takano et al., 2000). It therefore appears that matrix vesicles are not required for circumpulpal mineral nucleation (Takano et al., 2000). Instead, once mineralization has begun within mantle dentine, the propagation of crystal formation is maintained by non-collagenous proteins located both between collagen fibrils and attached to their surfaces (Wiesmann et al., 2005).

Predentine contains a number of non-collagenous or extra-collagenous molecules (ECM's) that have been implicated in this regard. Among these are various glycoproteins (including phosphoproteins and proteoglycans) as well as y-carboxylglutamic acid containing proteins (Hohling, 1989). Although their role is still unclear, the results of ongoing research indicate that these macromolecules play a role both in the initiation and regulation of predentine mineralization (see Figure 2.7) (Linde and Goldberg, 1993; Butler et al., 2002; Butler et al., 2003).

Phosphoproteins appear to be excreted by odontoblasts at the mineralization front and are rapidly incorporated into dentine. Their ability to bind calcium suggests that they play an important role in the nucleation of apatite (Hohling, 1989; Wiesmann et al., 2005). Recent research has focused on the role of dentine sialoprotein (DSP), dentine phosphoprotein (DPP) and dentine sialophosphoprotein (DSPP) in dentinogenesis. It appears that DSPP, a highly acidic protein, may act as an inactive precursor molecule that initiates mineralization following cleavage into DSP and DPP (Hunter et al., 1996; Butler et al., 2003). In addition, dentine matrix protein 1 (DMP1) has been shown to initiate apatite precipitation in vitro (He et al., 2003).

Proteoglycans, a group of mobile proteins bound to glycosaminoglycan (GAG) chains, may have a dual function, both inhibiting calcium phosphate nucleation while in solution and binding calcium once they have attached to peripheral branches of the polypeptide chains within the collagen matrix, thereby serving as sites of mineral

nucleation (Hohling, 1989; Goldberg et al., 2003). Small leucine-rich proteoglycans (SLRP) including decorin, biglycan, fibromodulin, lumican, and osteoadherin/ osteomodulin have been implicated in this regard (Goldberg et al., 2003). Further, a highly sulfated group of proteoglycans has been implicated in the assembly of the collagen within predentine (Lormee et al., 1996). Despite these findings, the biochemistry of dentinogenesis is not yet completely understood and the composition of predentine is still the subject of investigation (Ohma et al., 2000).

The histochemical matrix of predentine can be divided into three distinct zones based on varying concentrations of the above organic proteins. The first layer of predentine, extending from the proximal end of the odontoblasts, shows high levels of acidic mucopolysaccharides-containing proteoglycans (AMPS) (Martens, 1968). The second shows lower AMPS concentration and an increasing concentration of glycoproteins. The final layer of predentine, adjacent to the mineralization front, contains high concentrations of zinc and lipids (Martens, 1968). There is also evidence of a similar increase in alkali ion concentration in the direction of the mineralization front (Wiesmann et al., 1998). Sodium, potassium, sulfur and magnesium concentrations are highest at the predentine/dentine border (Wiesmann et al., 2005). These biochemical gradients, along with the relatively uniform thickness of predentine during dentine production, hint at the cellular regulation of these extracellular processes (Ten Cate, 1989; Linde and Goldberg, 1993).

Crystal Growth

Predentine transforms into mature dentine following the deposition of inorganic mineral salts into the organic matrix (Jones and Boyde, 1984). As discussed above, the mineral deposited within predentine takes the form of needle-like or plate-like crystals of hydroxyapatite. The observation that the outer surface of collagen fibers display a higher mineral density than do their inner regions suggests that mineralization begins on the surface of these fibers and progresses towards their interior (Hohling, 1989). Mineralization first occurs at sites where non-collagenous proteins are bound to the

surface of collagen fibers (Wiesmann et al., 2005). Mineral is then deposited within the fibers, in the *microchannels* between parallel collagen fibrils (Wiesmann et al., 2005). In the electron microscope, mineralized collagen fibers display a banding pattern at a periodic distance equal to the collagen macroperiod, *D*, a phenomenon that is suggestive of increased mineral deposition in the hole zones between collagen fibrils (Hohling, 1989). It therefore appears that the orientation of the hydroxyapatite crystals of dentine is highly regulated by the collagen matrix in which they mature (Hohling, 1989; Wiesmann et al., 2005).

Macroscopically visible crystals of hydroxyapatite begin first as nanometer-sized particles, *nanocrystallites*, which grow in size to form larger calcospherites (Schmidt and Keil, 1971; Wiesmann et al., 2005). *In vitro* studies of hydroxyapatite mineralization indicate that the earliest crystal formation begins with the precipitation of amorphous calcium phosphate $Ca_9(PO_4)_6$, an unstable precursor which allows for the nucleation of stable hydroxyapatite (Moore and Araki, 1977). Following this, crystal growth proceeds via epitaxy, the "oriented overgrowth of one crystalline phase on a specific face of another" (Posner and Tannenbaum, 1984:19).

Following nucleation, calcospherites grow in a radial manner until they meet one another laterally, merging to form a relatively homogenous mineralized tissue (Jones and Boyde, 1984; Wiesmann et al., 2005). According to Popoff's laws of simultaneous crystal growth, crystals initiated contemporaneously and having achieved radial contact are limited in shape to parabaloids with their axes oriented in a line from the tooth surface to the pulp (Schmidt and Keil, 1971). Despite their equi-directional growth, mature crystallites therefore appear columnar in tangential section (Schmidt and Keil, 1971). Calcospherites are in general aligned with the long axes of the collagen fibers of dentine; however, they may also be arranged spheritically (in globules) seemingly independent of the organic matrix (Schmidt and Keil, 1971).

Unmineralized regions within circumpulpal dentine are referred to as *interglobular dentine* (Mjor, 1984). These represent areas of disturbance during dentine production in which the crystals of apatite fail to coalesce (Ten Cate, 1989). Neither

intertubular nor peritubular mineral are found in these areas (Blake, 1958). They are particularly common at the junction between mantle and circumpulpal dentine (Mjor, 1984).

Rate of Mineralization

Collagen is deposited at a rate of roughly 4 μm per day, while mineralization occurs approximately twelve hours later, at a rate of 2 μm per day (Ten Cate, 1989). However, the rate of matrix deposition is not uniform over this period (Ten Cate, 1989). Rather, the speed of collagen secretion increases and decreases, resulting in a regular variation in the orientation of the fibers (Ten Cate, 1989). Following mineralization, a record of these oscillations is preserved within dentine and is visible under the light microscope as alternating lines of light and dark (Simmons, 1979).

Lines arising as a result of daily fluctuations in the rates of predentine production are referred to as *lines of von Ebner* or *incremental lines* (Yilmaz et al., 1977). Overlying this daily rhythm is a five-day cycle in which changes in collagen orientation appear more exaggerated (Ten Cate, 1989). These long-period markers, in which more dramatic banding of dentine is evident, are referred to as *Andreson lines* (Ten Cate, 1989). Periods of systemic stress produce similarly exaggerated lines, termed *Owen's contour lines* (Mjor, 1984). For teeth forming at the time of birth, the event is distinguished by a *neonatal line*, a record of the trauma associated with labor (Mjor, 1984).

2.8 Dentine Sclerosis

Tubular Obliteration

Following the cessation of primary dentine production, at the closure of the root apex, the diameters of the tubules of circumpulpal dentine appear to decrease with advancing age (Beust, 1931; Traub et al., 1988; Kinney et al., 2005). Examination of the cross sectional area of tubules in root apices reveals a significant decrease in mean area (Traub et al., 1988). This decrease in tubule diameter appears to be the result of the

gradual infilling of dentine tubules. Sections of dentine made in a plane perpendicular to the path of the tubules reveal that they are progressively filled by a radio-opaque material (Beust, 1931; Nalbandian et al., 1960; Vasiliadis et al., 1983b; Traub et al., 1988). The general process of the infilling of tubules is referred to as *dentine sclerosis* (van Huysen, 1960; Reith, 1968; Ten Cate, 1989; Balooch et al., 2001). Areas of dentine in which tubules have become filled are therefore referred to as *sclerotic*.

Microradiographic and electron microscope investigation suggests that tubule closure occurs in both coronal and radicular dentine (Beust, 1931; Takuma and Eda, 1966). Although tubular closure is observed in the crown, the processes underlying its manifestation are pathological and therefore distinct (although perhaps not independent) from those of root dentine sclerosis (Nalbandian et al., 1960; Mendis and Darling, 1979; Porter et al., 2005). The following will address the age-related phenomenon of tubular sclerosis exclusively within root dentine. This discussion will close, however, with a consideration of the evidence for a physiological origin of radicular sclerosis.

Mineral Composition

The material that fills tubules appears to be a substance with a higher mineral content than that of the surrounding intertubular matrix (Takuma and Eda, 1966). Synchrotron radiation computed tomographic analysis of sclerotic dentine reveals that mineral concentration within affected areas is significantly higher than in normal dentine but that it is variable by location (Hawkinson and Eisenmann, 1983; Kinney et al., 2005). However, areas of highest mineral density always correspond to those areas in which closure of the tubule lumens is visible (Kinney et al., 2005). At all locations, the mineralized material appears to be intimately associated with the peritubular matrix and the two are therefore often indistinguishable (Takuma and Eda, 1966). Electron microscope analysis reveals a similarity in appearance of the two, with the occluding mineral exhibiting a broken, stippled texture (Nalbandian et al., 1960). The results of microradiographic investigation appear to confirm the similarity between peritubular

dentine and the occluding mineral, as both show a similar radio-density (Bergman and Engfeldt, 1954).

The occluding mineral appears to be hydroxyapatite, the same mineral found throughout normal dentine following maturation (Kinney et al., 2005). In completely occluded tubules, crystals appear to be uniformly arranged, having mineralized both the odontoblast process and any collagen fibrils contained within the lumen (Hawkinson and Eisenmann, 1983). Electron micrographs of filled tubules and the adjacent intertubular and peritubular matrices reveal a similar crystal lattice structure within both (Nalbandian et al., 1960). Furthermore, small crystals of hydroxyapatite have been reported within partially filled tubules (Takuma and Eda, 1966; Hawkinson and Eisenmann, 1983; Porter et al., 2005).

Despite chemical similarities, differences exist in the crystalline properties of the mineral and its associated organic matrix (Nalbandian et al., 1960). Decalcification of sclerosed dentine reveals a homogenous organic matrix within tubules (Nalbandian et al., 1960). However, unlike in patent tubules, fibrillar, collagenous structures are not always apparent (Nalbandian et al., 1960; Hawkinson and Eisenmann, 1983). Further, it appears that metallic ion substitution may occur within the apatite filling sclerosed tubules (Balooch et al., 2001). In comparison to surrounding intertubular dentine, the intratubular hydroxyapatite is composed of coarser crystallites that appear darker in the transmission electron microscope (Nalla et al., 2005; Porter et al., 2005). However, despite differences in the crystallite size and structure, the optical properties of the mineral within sclerotic tubules are identical to those of the mineral phase of intertubular dentine (Vasiliadis et al., 1983b).

Pattern of Mineralization

The pattern of mineral deposition within tubules has been the subject of numerous investigations. Amprino and Camanni (1956) conclude that mineral deposition begins on the inner surface of the dentine tubule wall, indicating a centripetal process of deposition. Building on these findings, Takuma and Eda (1966) suggest that lighter areas

visible at the center of partially sclerosed tubules represent areas not yet occluded by intratubular mineral. When combined with the chemical similarities to peritubular mineral, these findings may suggest that the mineral occluding tubules is the product of a continued deposition of the peritubular matrix (Blake, 1958). However, the pattern of mineral accretion within tubules appears to be variable. Some tubules contain mineral in a clumped pattern, partially occluding the lumen (Hawkinson and Eisenmann, 1983). Further, intratubular mineral seems to form more diffusely, without a clear mineralizing front (Takuma and Eda, 1966; Vasiliadis et al., 1983b). To date, the exact sequence of mineral deposition remains obscure (Frank and Voegel, 1980; Kinney et al., 2005).

The role of the odontoblast process and other tubular contents in mineral deposition within the tubules is unclear. In a description of dentine sclerosis, Pindborg (1970) discusses the retraction of the odontoblast process concurrent with tubular obliteration. However, Nalbandian et al. (1960) suggest that the odontoblast may be the essential site of nucleation for intratubular mineral deposition. In an electron microscope investigation of sclerosis, Hawkinson and Eisenmann (1983) report visible odontoblast processes within tubules undergoing obliteration. Further, Frank and Voegel (1980) report that the odontoblast process does not appear to degenerate prior to filling of the tubule. They are also unable to report evidence to suggest its retraction prior to or during mineralization of the periodontoblastic space. In contrast, Vasiliadis et al. (1983b) suggest that the odontoblast is not actively involved in dentine sclerosis and that occlusion of tubules may proceed in the absence of a vital odontoblast, however they postulate that maintenance of a blood supply to the pulp is essential for the development of transparency.

Physical Properties

Dentine sclerosis alters the physical properties of mature dentine. Unlike areas of normal dentine, regions of dentine in which tubules have become filled display an inability to absorb stain (Beust, 1931; Fish, 1948). The microhardness (Vickers hardness) of sclerosed dentine appears to be higher than that of normal dentine, consistent with an

increased calcification of these areas (Grajower et al., 1977; Balooch et al., 2001). The Ca/P ratio of sclerotic areas has been shown to be reduced and the ash content is lower in comparison to areas of normal dentine (Simon and Armstrong, 1941; Manly and Brooks, 1947; Moore and Leaver, 1974). The elastic properties of sclerotic dentine remain unchanged; however, the fracture toughness of sclerotic areas is roughly 20% lower than that of normal dentine and the fatigue life is similarly lowered (Kinney et al., 2005).

2.9 Root Dentine Transparency

A change in the optical properties of sclerosed dentine concurrent with the increasing frequency of tubular closure has been demonstrated. Early studies by Miller (1890), Beust (1931), and Bodecker and Lefkowitz (1937; 1946) point to intratubular mineralization as a causal agent for the progressive optical transparency of sclerosed dentine. In normal light, areas of tubular sclerosis allow more light to pass through, contrasting markedly with areas of normal dentine which appear opaque (see Figure 2.8) (Manly and Brooks, 1947; van Huysen, 1960; Nalbandian et al., 1960; Vasiliadis et al., 1983b; Kinney et al., 2005).

Numerous lines of inquiry confirm the association between dentine sclerosis and root transparency. Microradiographic investigation of transparent areas of root dentine confirms that tubules within these areas are sclerosed (Nalbandian et al., 1960; Weber, 1974; Vasiliadis et al., 1983b). Further, tubules within transparent areas are difficult to distinguish from the surrounding mineralized tissue under light microscope (Nalbandian et al., 1960; Vasiliadis et al., 1983b). Increasing radio-density of dentine is also apparent for areas of dentine appearing transparent to the naked eye (Nalbandian et al., 1960; Weber, 1974). Electron micrographs appear to provide similar results. In contrast to the sieve-like appearance of normal dentine, areas of transparency appear as solid, electron-dense areas devoid of tubule lumens (Nalbandian et al., 1960; Vasiliadis et al., 1983b). X-ray micrograph evidence suggests that the pattern of progression for root transparency is similar to that for dentine sclerosis, both developing from the outer surface of the root

towards the central pulp chamber (Bradford, 1960; van Huysen, 1960; Vasiliadis et al., 1983a).

An explanation for the association between transparency and dentine sclerosis may be found in the fundamentals of optical physics (Simon and Armstrong, 1941). Snell's Law dictates that as light passes from one medium into another, the speed and direction of propagation of its constituent waves are altered in a regular manner (Schmidt and Keil, 1971). Both of these properties vary according to the magnitude of the difference in the *refractive index* (the ratio of the speed of light in air to its velocity in a particular medium) of each of the two media. This difference, referred to as the *strength of birefringence,* determines the degree to which light is altered as it moves between

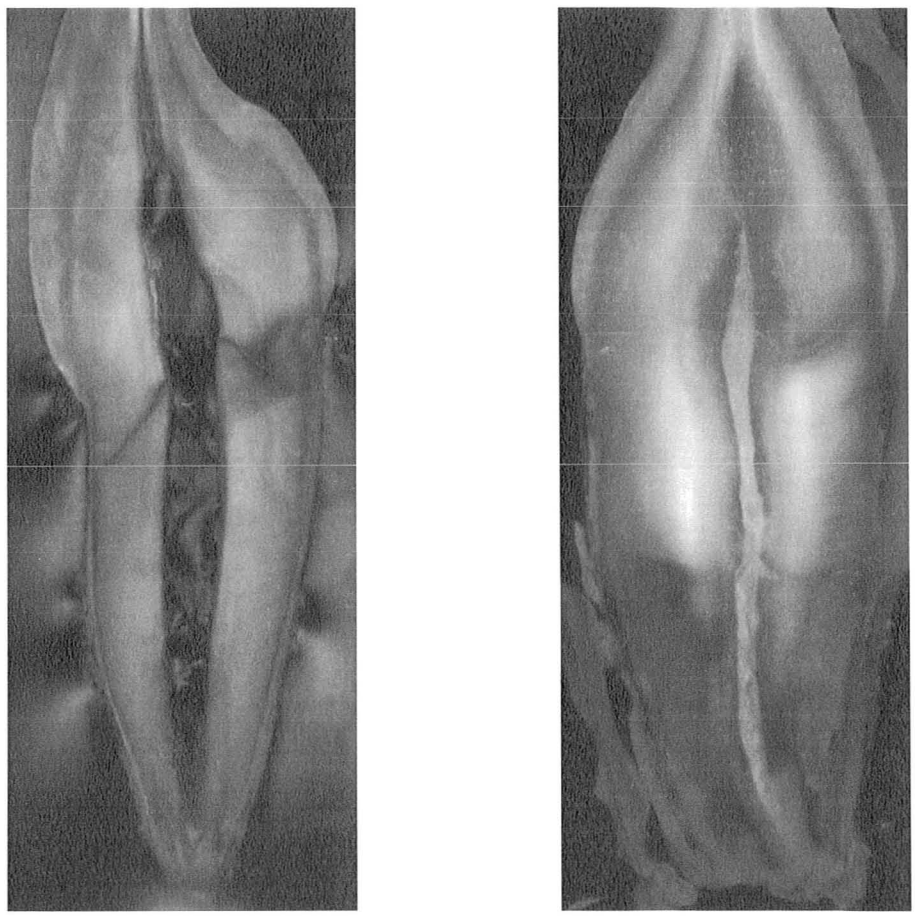

Figure 2.8 Stereomicroscope images of two sectioned tooth specimens photographed under polarized light. At left is a specimen from a younger individual. Note that the root dentine appears opaque throughout. At right is a specimen from an individual of advanced age. Dentine transparency is visible at the apical end of the tooth and towards the CDJ. Note that the line between transparent and normal dentine is somewhat diffuse. It is clear from this image that the pulp canal is not affected by transparency.

materials (Schmidt and Keil, 1971). The speed of light and its direction also vary according to the *angle of incidence* - the angle at which a light wave enters a substance (Schmidt and Keil, 1971). The path of a given wave of light is therefore dependent upon both the optical characteristics of the media through which it is being propagated and the number of transitions between media that it makes. As either increase, so too will the overall scattering of an incident beam (Schmidt and Keil, 1971).

Normal human dentine, free of sclerosis, represents a composite of a number of different materials, each with its own refractive index (Schmidt and Keil, 1971). As light passes through dentine, it is diffracted each time it passes between intertubular mineral and mineral-free tubules. The vast number of tubules within circumpulpal dentine means that it appears opaque, having scattered light in a multitude of directions (Schmidt and Keil, 1971). Sclerosed dentine, on the other hand, represents a more optically homogenous tissue. Since tubules are filled with mineral with a similar refractive index to that of the surrounding intertubular matrix, light does not make as many refractory transitions as it passes through sclerosed dentine (Schmidt and Keil, 1971; Vasiliadis et al., 1983b). The tissue therefore appears less opaque, having allowed more light to pass through unaltered (Vasiliadis et al., 1983a; 1983b).

This explanation for transparency was confirmed by Manley and Brooks (1947), who demonstrated that the immersion of undecalcified sections of normal dentine in a liquid of a similar refractive index to that of intertubular mineral produced transparency in the sections. These findings not only substantiate the association between sclerosis and transparency but also suggest a casual relationship wherein the former begets the latter.

Distribution of Transparency

Based on photomicrographic analysis of serial sections (250 μm thick) of 70 canines displaying transparency, Vasiliadis et al. (1983a) report that transparency advances from the root apex up the length of the root in a coronal direction. However, it appears to progress faster in a mesial and distal direction than it does in the buccal and lingual plane, producing a three-dimensional "butterfly pattern" (Vasiliadis et al., 1983a).

Further, the line separating transparent and opaque dentine is not always clear and may instead be quite diffuse (Vasiliadis et al., 1983a). Transparent areas may alternate with areas in which the dentine displays normal opacity (Beust, 1931).

With regards to the distribution of sclerosis underlying transparency, Vasiliadis et al. (1983a) report that tubule closure spreads in a similar pattern, progressing from the outer surfaces of the root both in a coronal direction from the root apex and from the CDJ towards the pulp center. However, calcospherites of hydroxyapatite are apparent at the occlusal, lingual and buccal aspects of the pulp surface - a finding which seems at odds with the observation that both transparency and sclerosis spread more quickly in the mesial and distal directions than in the bucco-lingual plane (Vasiliadis et al., 1983a).

Kinney et al. (2005) report a significant elevation in the mineral content of transparent dentine. They also report mineral density to be highest towards the mid pulp, decreasing in an apical direction (Kinney et al., 2005). These results appear to run counter to those expected, given that intratubular mineral deposition associated with sclerosis is believed to underlie root transparency. However, they may be explained if one recalls that both tubule density and diameter increase in a pulpal direction. If transparency results from the deposition within tubules of a material with a similar refractive index to the surrounding intertubular mineral, then in order to achieve a similar level of transparency, a given area of peri-pulpal dentine will have to have been impregnated with a larger amount of intratubular mineral, both in absolute and relative terms. If one assumes that the mechanism of mineral deposition is constant for all tubules and within all areas of a given tubule, then it is not surprising that transparency appears to begin at the root apex and in the periphery of the root, where the number of tubules and their lumen diameter are lower than in the rest of the root.

Etiology of Transparency

As noted above, several authors view intratubular mineralization as a process representing the continued maturation of the peritubular matrix (Bradford, 1960; van Huysen, 1960; Nalbandian et al., 1960). Beust (1931) in particular believes tubular

obliteration to be a result of the continued deposition of the peritubular matrix. Further, he views the mechanism underlying this as one with a physiological origin, related to natural age-related processes. The similarity in texture and mineral content of the peritubular and intratubular matrices seems to support this claim (Vasiliadis et al., 1983b; Kinney et al., 2005). However, Hawkinson and Eisenmann (1983) note that although centripetal accretion of mineral may be observed on the tubule wall, mineral precipitation begins with mineral deposition in the center of the tubule. They therefore view intratubular mineralization as a distinct process from peritubular mineralization - one in which calcification of the tubule wall follows the nucleation of hydroxyapatite on the odontoblast and within intratubular collagen (Hawkinson and Eisenmann, 1983). This conclusion is strengthened by the observation of a clear space between intratubular mineral and the peritubular matrix in human teeth and by the appearance of sclerosis in the dentition of the rat, an animal that does not possess peritubular dentine (Hawkinson and Eisenmann, 1983; Vasiliadis et al., 1983a).

Tubule closure does not occur independently of alterations to the surrounding intertubular matrix. Nalla et al. (2005) report a decrease in the density of intertubular mineral in areas of transparency. Crystallite size of intertubular dentine within areas of sclerosed dentine is also smaller than within normal areas by roughly 19% (Kinney et al., 2005; Porter et al., 2005). Tubular obliteration may therefore arise from the dissolution and re-precipitation of intertubular hydroxyapatite in the tubule lumens (Kinney et al., 2005; Porter et al., 2005).

The association of transparency with the disappearance of the predentine layer on the mesial and distal aspects of the pulp surface is an intriguing observation deserving of further investigation (Vasiliadis et al., 1983a). This may represent an essential stage in the mineralization of dentine tubules, particularly one involving the redistribution of existing dentinal mineral (Vasiliadis et al., 1983a).

Physiological Origin

It has been suggested that, despite its association with age, transparency arises in response to external stimuli (Blake, 1958). In the crown, areas of dentine adjacent to coronal carious lesions display a similar transparency as those areas of root dentine affected by sclerosis (Blake, 1958; Weber, 1974). Further, areas of coronal dentine adjacent to attrited enamel surfaces appear sclerotic (Tronstad, 1973a). Yet, regardless of optical similarities between coronal and radicular transparency, each may have a distinct origin. Based on a microradiographic comparison of tubular sclerosis in root dentine and coronal dentine, Weber (1974) concluded that the two represent similar results of distinct processes, the latter being the result of physiological processes and the former arising from necrosis of the odontoblast which acts as a site of nucleation for aqueous mineral (presumably contained within either the dentinal liquor or perhaps transported actively or passively into the tubules via the pulp). Unlike observations on transparent root dentine, Mendis and Darling (1979) report that tubules of dentine adjacent to carious lesions are filled with a granular material that is not continuous with the peritubular matrix and those subjacent to areas of abrasion remain largely open. Furthermore, unlike intratubular mineral associated with carious lesions, there is no increase in local magnesium concentration in sclerotic root dentine (Porter et al., 2005). In an investigation of the association of root dentine sclerosis, coronal sclerosis and numerous other pathological responses of dentine, Stanley et al. (1983) reported that coronal transparency occurred independently of physiological sclerosis, irrespective of the location or type of carious lesion with which the former was associated. Further, root dentine sclerosis was observed in teeth free of pathology (Stanley et al., 1983).

Perhaps the most convincing evidence of the distinct physiological origin of root dentine transparency is that it appears to develop in a linear manner with age, even in unerupted teeth (Azaz et al., 1977; Stanley et al., 1983). The observation of transparency in teeth that have not yet entered occlusion means that one must exclude both occlusal forces and pathological interference as causal factors in the development of root dentine transparency (Azaz et al., 1977). Transparency has also been observed in non-vital teeth

(Thomas et al., 1994). However, the extent of transparency is significantly elevated when compared to that within age-matched teeth that were vital on extraction, a finding suggesting an inhibitory role for the odontoblast in intratubular calcification (Thomas et al., 1994).

Bradford (1960) suggests that chemicals contained within the diet of an individual may diffuse through the enamel and/or pulp into dentine, slowly accumulating as intratubular mineral. Currently, there is no evidence either to confirm or refute this claim. However, the permeability of enamel and the vascular nature of the pulp suggest that diet and the contents of the oral environment may be an important factor in the alterations of dentine both during primary dentinogenesis and thereafter (Bradford, 1960).

Despite the uncertainty surrounding the impetus for and mechanism of mineral deposition within tubules, root dentine transparency increases in a linear manner with age (Gustafson, 1950; Miles, 1963; Bang and Ramm, 1970; Solheim, 1989; Lamendin et al., 1992). This association has led to the development of a number of age estimation techniques employed in both the forensic and bioarchaeological contexts (Bang and Ramm, 1970; Lorentsen and Solheim, 1989; Drusini et al., 1990; Lamendin et al., 1992). The next chapter will review the various measurement techniques employed to assess transparency and examine the accuracy and validity of these methods with regard to age estimation.

Chapter 3
TRANSPARENCY AND AGE ESTIMATION

3.1 Qualitative Methods of Adult Age Estimation

The earliest methods of age estimation based on the observation of degenerative changes in the dentition are founded on qualitative descriptions of aged teeth. In the application of these methods, chronological age is estimated via the comparison of teeth of unknown age with ordinal degrees of degeneration derived from samples of known age-at-death.

Originally published in 1947 and later translated in 1950, Gustafson's seminal paper on age-related changes in the adult human dentition, *Age determinations on teeth,* presented the first method of adult age estimation using teeth that was grounded in empirically-derived formulae (Gustafson, 1950). According to this method, root transparency is one of six degenerative changes assessed qualitatively in order to arrive at an age estimate (Gustafson, 1950). The other variables include: degree of *attrition* (the loss of tooth tissue due to wear), degree of *periodontosis* (recession of the alveolar bone anchoring the teeth within either the maxilla or mandible), amount of *secondary dentine* (deposition of dentine within the pulp cavity following primary dentine production), *cementum apposition* (deposition of cementum on the root surface of a tooth) and *root resorption* (the loss of tooth roots beginning at the root apex and advancing in a coronal direction) (Gustafson, 1950). Estimates are derived by substituting the sum of the scores for each of the six variables into the following regression formula:

$$y = 11.43 + 4.56x$$

where x is the sum of the scores for each of the variables and y is age in years (Gustafson, 1950).

Although Gustafson reported an extremely high degree of precision (in only one third of the cases did the error exceed 3.6 years), subsequent tests of the method have failed to reproduce this level of accuracy (Dalitz, 1963; Maples and Rice, 1979; Kashyap and Koteswara Rao, 1990). This is in part due to methodological inaccuracies in the original work. Gustafson's research is plagued by statistical errors, the most profound of which is the publication of an incorrect regression equation (Maples and Rice, 1979; Lucy and Pollard, 1995). Further, the small, heterogeneous samples on which the initial predictive formula was based (N=19) and subsequently tested (N=41) make statistical confidence in the accuracy and precision of this method rather low (Maples and Rice, 1979; Solheim, 1993; Lucy and Pollard, 1995).

Numerous modifications to Gustafson's original method have been proposed (for example Dalitz, 1963; Johanson, 1971; Burns and Maples, 1976; Maples, 1978; Pilin, 1981; Kilian and Vlcek, 1989; Xu et al., 1991). In an effort to increase the precision of the method, Dalitz (1963) raised the number of categories for each variable from four to five, reporting a greater accuracy with a refined description of the age-related changes (Dalitz, 1963). Johanson (1971) offered a clearer and more refined protocol for tooth preparation and presented a modified formula based on multiple regression analysis. Solheim (1993) presented an age-predictive formula which incorporates a number of novel variables including dental color and length of root surface area.

3.2 Quantitative Methods of Adult Age Estimation

The reliability of these amended formulae is limited by the replicability of the qualitative assessments upon which the predications are based (Miles, 1963; Burns and Maples, 1976; Solheim, 1993). Although comparison standards are provided for each of the variables under consideration, consistently assigning teeth to a given category is somewhat difficult (Solheim, 1993). For all qualitative methods of age estimation, the

subjective nature of the descriptions represents an unavoidable source of inconsistency, elevating both inter- and intra-observer error (Kashyap and Koteswara Rao, 1990; Solheim, 1993). A re-evaluation of Gustafson's predictive formula by Kashyap and Koteswara Rao (1990) bore this out. The replacement of qualitative measures with index values based on physical measurements of attrition, secondary dentine deposition, cementum apposition and transparency resulted in more accurate and reliable age predications than those generated by Gustafson's qualitative formula (Kashyap and Koteswara Rao, 1990).

Due to the error introduced by the subjectivity of qualitative assessments, current adult age estimation techniques, regardless of the particular morphological criteria they employ, utilize standardized empirical measures in their descriptions (Miles, 1963; Bang and Ramm, 1970; Lamendin et al., 1992; Solheim, 1993; Kvaal and Solheim, 1994).

3.3 Univariate Age Estimates

Although multi-variate estimates of age offer a good degree of accuracy, it appears that not all variables contribute significantly to the accurate estimation of age (Dalitz, 1963; Miles, 1963; Johanson, 1971; Maples, 1978; Kashyap and Koteswara Rao, 1990). Rather, some may actually interfere with the accuracy of estimates of chronological age (Nalbandian et al., 1960; Miles, 1963; Burns and Maples, 1976; Maples, 1978; Kashyap and Koteswara Rao, 1990). Furthermore, inter-correlations between several of Gustafson's variables, suggest that the independence necessary for regression analysis, a condition upon which many multivariate formulae rest, may no longer be reasonably assumed (Johanson, 1971).

Dalitz (1963) and Miles (1963) suggested that root resorption and cementum deposition be discarded as variables for consideration due to low correlations with age. On the other hand, Maples (1978) reported that measures of secondary dentine deposition and root transparency have lower standard errors than do the other variables within Gustafson's formula. These findings are in keeping with those of Johanson (1971) who

has evaluated each variable separately and reported the highest correlation with age for transparency and secondary dentine deposition at 0.84 and 0.63 respectively.

3.4 Measures of Transparency

Of the six age-related variables outlined by Gustafson, measures of root dentine transparency appear to hold an advantage over the others. Numerous lines of research have indicated that root dentine transparency is most strongly correlated with age (Nalbandian et al., 1960; Miles, 1963; Johanson, 1971; Maples, 1978; Metzger et al., 1980; Lopez-Nicolas et al., 1990; Lopez-Nicolas et al., 1993). Above the age of approximately 20 years, transparency increases in a roughly positive linear relationship with chronological age (Miles, 1963; Bang and Ramm, 1970; Drusini et al., 1990; Micheletti Cremasco, 1998). Furthermore, for both intact and sectioned teeth, measures of transparency are easily applied, requiring little experience and no specialized tools (Miles, 1963; Maples, 1978; Pretty, 2003). Therefore, empirical measures of transparency *alone* may be used as accurate indicators of chronological age (Miles, 1963; Bang and Ramm, 1970; Maples, 1978; Lorentsen and Solheim, 1989). Several researchers have focused exclusively on measures of transparency in the formulation of novel age estimation techniques (Miles, 1963; Bang and Ramm, 1970; Drusini et al., 1990; Lopez-Nicolas et al., 1993; Micheletti Cremasco, 1998). For each of these formulae, age estimates are based on comparisons between the observed degree of transparency for a given individual of unknown age and a reference standard for which the relationship between transparency and chronological age has previously been documented.

3.4.1 Linear Measures of Transparency

Bang and Ramm, 1970

The method of Bang and Ramm (1970) is currently the most widely-used means of age estimation based solely on the empirical measurement of root dentine transparency. This technique was based on the examination of 926 teeth collected from both living and deceased individuals (Bang and Ramm, 1970). Data were collected from 158 men and 107 women of known age (Bang and Ramm, 1970). Age estimates were based on the following measurements each of which was made to the nearest 0.5 mm using a sliding caliper in front of a constant light source:

1. Minimal length of root transparency: TL_1 (measured from the apex of the root in coronal section to the borderline between transparent and opaque dentine)
2. Maximal length of root transparency: TL_2 (measured from the apex of the root in coronal section to the borderline between transparent and opaque dentine)
3. Mean length of transparency: TM (the average of the above two variables according to the formula $(TL1 + TL_2)/ 2$)

Measurements were made on both intact and longitudinal ground thin sections of teeth (Bang and Ramm, 1970). All tooth types were used, however, separate coefficients were derived for each (Bang and Ramm, 1970). Although the authors had planned to measure area of sclerosis in addition to length, these measurements were abandoned due to high inter and intra-observer error rates (Bang and Ramm, 1970).

Based on their initial inquiry, the authors derived the following two regression formulae to be used for age estimation.

Equation 1: $A = (B_0 + B_1)X + (B_2)X^2$

Equation 2: $A = B_0 + (B_1)X$

where B_0 is a tooth-specific constant and B_1 and B_2 are tooth-specific regression coefficients and X is equal to the mean length of transparency (TM) measured in millimeters (Bang and Ramm, 1970).

In those cases where transparent length is found to be less than or equal to 9.0 mm, Equation 1 is employed (Bang and Ramm, 1970). Where transparent length exceeds 9.0 mm, Equation 2 is employed. In the derivation of these formulae, the authors examined both absolute and relative measures of the length of transparent root dentine; however, they reported little improvement when measurements were scaled to the overall root dimensions (Bang and Ramm, 1970). The above equations are therefore to be used for absolute measures only.

In the construction of the formulae, reported correlations between transparency and chronological age were high, however they appeared to be variable between tooth types, ranging from 0.62 in upper right cuspids to 0.90 in upper left second incisors (Bang and Ramm, 1970). On average, the highest correlations were reported for anterior teeth (Bang and Ramm, 1970). In a subsequent application of this technique Solheim and Sundnes (1980) reported mean errors of 1.09-14.45 and 0.44-14.11 years for unsectioned and sectioned teeth respectively, with the lowest mean error reported for the 50-60 year age range (Solheim and Sundnes, 1980). Willems et al. (2002) reported mean errors of between 0.5 and 1.8 years for this method when applied to intact specimens. However, significant intra-observer errors were reported between measures, suggesting that the reliability of the method may be influenced by the subjective nature of the assessments (Willems et al., 2002).

The utility of this method within the forensic context appears good (Reppien et al., 2006). Maples (1989) feels this technique has the greatest accuracy with the least amount of complexity. Pretty (2003) has emphasized its value in those cases where identification of human remains is otherwise difficult due to a lack of material evidence. In five separate forensic cases he was able to provide age estimates leading to positive identification of the deceased (Pretty, 2003). This method has been successfully employed in the identification of human remains recovered from mass graves in Croatia (Brkic et al., 2006). The method has also proven to be of value for archaeological remains of considerable antiquity (Bang, 1993; Kvaal et al., 1994; Kvaal and During, 1999). Bang (1993) applied this method to sectioned specimens prepared from teeth dated to the Mesolithic (7950+/-110 yrs BP). Further, Kvaal and During (1999) were able to report age estimates for teeth recovered from the Swedish warship *Vasa* which sank in 1628 AD in Stockholm harbour and lay submerged until its excavation in 1961.

Limitations do however exist in the value of this method. In general, studies have reported a tendency to over-estimate age in younger individuals and under-estimate age in older individuals (Bang and Ramm, 1970; Solheim and Sundnes, 1980). Furthermore, below the age of 20 it has proved impossible to assign age estimates, as sclerosis is not readily apparent (Bang and Ramm, 1970). However, based on similar observations from alternate measures of sclerosis, it has been suggested that these difficulties may in part be the result of the particulars of the phenomenon of root transparency, rather than limitations of the methods employed (Solheim and Sundnes, 1980; Lorentsen and Solheim, 1989; Lamendin et al., 1992; Kvaal et al., 1994). This issue will be discussed in greater depth below in relation to the methodological shortcomings of the statistical methods employed in the derivation of the formulae.

Lamendin et al., 1992

The method of Lamendin et al. (1992) involves the prediction of chronological age via the measurement of both transparency and periodontal recession. For each tooth the length of transparent dentine is measured on the labial root surface from the root apex

to the maximum height (Lamendin et al., 1992). The degree of periodontosis or gingival recession is defined as the maximum distance on the labial surface of the tooth between the CEJ and the line of soft tissue attachment (Lamendin et al., 1992). All measures are made to the nearest millimeter with a sliding caliper in front of a bright light source (Lamendin et al., 1992). Examinations are made on intact teeth, as this method is designed for use on samples for which destruction is inappropriate (Lamendin et al., 1992). The following equation is used for age prediction:

$$A = 0.18P + 0.4T + 25.53$$

where A is age in years, P is an index of periodontosis (periodontosis height x 100)/root height and T is an index of transparency (transparency height x 100)/root height.

Applications of this method have reported reasonable levels of accuracy. Based on an examination of 400 teeth from the Terry Collection, Prince and Ubelaker (2002) reported a mean error of only 8.2 years. In a comparison to six other means of age estimation (including the Suchey-Brooks system based on pubic symphyseal morphology; analysis of the sternal end of the fourth rib; and osteon counting), this method gives the greatest predictive accuracy, with a mean error of only 5.7 years (Baccino et al., 1999). Furthermore, Baccino et al. (1999) reported no significant inter- and intra-observer error for a test of the method on 306 teeth from 208 individuals, despite a lack of formal training for two of three of the observers.

However, in a similar pattern to that found by Bang and Ramm (1970), mean error is elevated at either end of the age spectrum (Lamendin et al., 1992). In the 50-59 year old group, the mean error reported is only 3.3 years, however, in the 30-39 year age group, mean error rises to 13.1 years (Lamendin et al., 1992). In an early test of the method, less than half the individuals under the age of 40 years had an age included within the bounds of the estimate provided (Lamendin et al., 1992). Furthermore, the value of the constant within the original equation precludes its application to individuals

below the age of 25, thus increasing the lower age limit by approximately five years over Bang and Ramm's method (Lamendin et al., 1992).

3.4.2 Measures of Transparent Area

The relationship between transparent root area and age has been explored as an alternative to linear or qualitative descriptions of transparency. However, the value of these measurements remains unclear, as to date no age-predictive formula based on the assessment of the area of root transparency has been formulated.

Lorentsen and Solheim, 1989

Early work by Azaz et al., (1977) indicated that, when expressed as a percentage of total root area, values of transparent dentine area increase in a positive linear manner with age (Azaz et al., 1977). However, as this research was carried out using only 72 canines, these results were regarded as preliminary findings (Azaz et al., 1977).

A more detailed analysis of the correlation between area of transparent dentine and chronological age was undertaken by Lorentsen and Solheim (1989). 500 teeth were sectioned according to the half-tooth technique (Solheim, 1984) and the following measurements were made on enlarged photographs using a planigraph (Lorentsen and Solheim, 1989):

TA: Total area of the sectioned tooth surface excluding enamel
TRA: Total root area of the tooth apical to the cervical margins excluding the pulp
ATD: Total area of transparent dentine

A stepwise multiple regression analysis was performed using chronological age as the dependent variable (Lorentsen and Solheim, 1989). A strong correlation between *ATD* and age was reported, however, correlations were variable by tooth type, ranging from 0.86 for the maxillary canines to 0.64 for the mandibular lateral incisors (Lorentsen

and Solheim, 1989). While the Pearson correlation coefficients for *ATD* are consistently higher than those for the method of Bang and Ramm (1970), the method of Johanson (1971), a revision of Gustafson's qualitative formula, has shown a slightly higher correlation than either of the others (Lorentsen and Solheim, 1989).

Solheim, 1989

Solheim (1989) examined transparent area using a sample of 1000 teeth (100 of each type excluding molars) removed from both living patients and at autopsy. Surface area of transparent dentine and total root area (defined as the area of tooth apical to the cervical margin) were measured directly under stereomicroscope using a grid system on a photographic plate (Solheim, 1989). The length of transparent dentine and the total root were also recorded both on intact and sectioned specimens, all absolute values being expressed in square millimeters (Solheim, 1989). The following indices were calculated:

F1: area of root/transparent area
F2: transparent area/length of transparent zone in sectioned dry teeth
F3: transparent area x length of transparent zone in sectioned dry teeth
F4: transparent area x transparent zone according to scores of Johanson (1971)

Multiple regression analyses were performed for each tooth type, with age as the dependent variable and each of the above variables as the independent variable (Solheim, 1989). In line with the results of previous research, area appears to increase in a linear manner with age, however, Solheim (1989) concluded that area of transparency as measured on sectioned teeth was not so closely correlated with age as is the length of transparent zone measured on unsectioned teeth. This was true for all tooth types (Solheim, 1989). Furthermore, Johanson's (1971) qualitative description of transparency appeared more closely associated with age than either linear or area measures for a number of tooth types (Solheim, 1989). These findings are in agreement with those of

Johnson (1968) who reported a poor correlation (r = 0.36) between age and transparent area for longitudinal thin sections.

3.5 Error of the Methods

Despite the fact that it may be evaluated in situations in which very little material evidence is recovered, transparency is seldom used as a morphological criterion for the description of deceased individuals, either in the forensic or archaeological contexts (Pretty, 2003). This is true despite the fact that transparency is a ubiquitous phenomenon, visible in all teeth of the adult human dentition above the age of approximately twenty years and that it varies in a relatively linear manner with age (Miles, 1963; Bang and Ramm, 1970; Johanson, 1971; Stanley et al., 1983; Drusini et al., 1989; Solheim, 1989; Kashyap and Koteswara Rao, 1990; Ermenc, 1997). Furthermore, deficiencies in the above methods have limited the adoption of any one technique as a standard in the estimation of age-at-death for adult individuals (Solheim and Sundnes, 1980).

Inconsistencies in the accuracy of the age-predictive formulae are perhaps the greatest concern precluding their adoption. Regardless of the way in which transparency has been measured, there appears to be a tendency towards overestimation of age in the young and underestimation of age in the old (Gustafson, 1950; Miles, 1958; Bang and Ramm, 1970; Wegener and Albrecht, 1980; Kashyap and Koteswara Rao, 1990; Drusini, 1991; Lopez-Nicolas and Luna, 1991; Lamendin et al., 1992; Whittaker and Bakri, 1996; Prince and Ubelaker, 2002; Olze et al., 2004). It remains unclear whether this error represents a deficiency in measurement or is instead a by-product of the phenomenon of dentine sclerosis itself. Furthermore, uncertainty surrounds the identity of the extraneous variables that may contribute to the reported inaccuracies in age estimation and the magnitude of their influence on the progression of transparency (Lopez-Nicolas et al., 1993).

3.5.1 Endogenous Sources of Error

Predictive inaccuracies may arise from true differences within the individual from whom the teeth under study were removed. Although a positive correlation between age and transparency has been documented, age is not a causal agent in the progression of transparency. Instead, some salient yet obscure factors influence the amount of transparency within a given tooth. It is conceivable that genetic, behavioral or pathogenic variation may affect the amount of transparent dentine available for measurement. An analysis of the contribution of each of these factors to the observed inaccuracies in age estimation is therefore necessary. Yet such an examination is complicated by the fact that each variable may act alone or in a synergistic manner and that the predictive error introduced by each may be either random or regular.

Sex-Linked Differences

Across a variety of measurement protocols, there exists evidence of sex-linked differences in measures of transparency (Burns and Maples, 1976; Solheim, 1989; Prince and Ubelaker, 2002). A comparison of the methods of Bang and Ramm (1970), Miles (1963), Johanson (1971) and Dalitz (1963) performed on a sample of 100 recently-extracted teeth reported a greater tendency towards overestimation of age amongst males, a result which suggests a faster rate of intratubular mineralization for males (Solheim and Sundnes, 1980). These results were echoed by Lorentsen and Solheim (1989) and Solheim (1989). However, the amount of transparency is not consistently greater for males (Olze et al., 2004). Johanson (1971) indicated a sex-linked difference in which females have a greater amount of transparency than their age-matched male counter-parts.

Age-predictive formulae that take sex into account have been formulated with positive results. Burns and Maples (1976) reported an improved correlation with chronological age for a modified formula based on Gustafson's variables in which sex was included as a variable for consideration. A revision of the method of Lamendin et al.

(1992) in which sex was considered produced a mean error as low as 7.41 years for males and 7.86 years for females (Prince and Ubelaker, 2002).

The observation of sex-linked differences in the rates of transparency makes intuitive sense, given documented differences between males and females in the development of the permanent dentition. Both calcification and root apex closure of female teeth precedes that of males for the majority of tooth types (Garn et al., 1958; Nolla, 1960; Demirjian and Levesque, 1980). Within a given tooth, differences in the rate of root formation are more pronounced than for crown development (Moorrees et al., 1963). The eruption of permanent teeth is also significantly advanced in females (Hagg and Taranger, 1985). Since the sclerosis underlying transparency begins only following primary dentine production (see Chapter 2), it is reasonable to assume that significant differences should be observed between the sexes.

Yet the use of tooth age instead of chronological age does not appear to improve the correlation between age and transparency, regardless of the means of measurement (Lorentsen and Solheim, 1989). Furthermore, sex has not consistently been found to make a significant contribution to the association between transparency and age (Olze et al., 2004). In the original derivation of their formula Bang and Ramm (1970) reported no significant differences between transparency in males and females. A subsequent test of this method in which Drusini (1991) performed a simple linear regression of transparency values against chronological age, reported that neither the slope of the regression lines nor the *y*-intercepts were significantly different between males and females when compared to a pooled sample. Although variance was slightly lower in females, this was not statistically significant at the 0.05 level (Drusini, 1991). Several others studies report similar findings (Nalbandian et al., 1960; Lorentsen and Solheim, 1989; Brkic et al., 2006).

Tooth Type

For an individual of a given age, the amount of transparency is variable according to tooth position (Dalitz, 1963; Bang and Ramm, 1970; Johanson, 1971; Maples, 1978;

Lopez-Nicolas and Luna, 1991; Solheim, 1993). Therefore, when the same predictive formula is tested using several teeth from a single subject, discrepancies are apparent in the values of the estimated ages (Lopez-Nicolas and Luna, 1991). These differences appear to persist across a number of different measurement protocols (Bang and Ramm, 1970; Drusini et al., 1991; Lamendin et al., 1992).

Unfortunately, there is no consensus as to what tooth type is preferable for age estimation. Several authors have reported higher correlations for more distal teeth, including premolars and molars (Bang and Ramm, 1970; Drusini et al., 1991; Solheim, 1993), while others have reported better results based on anterior tooth types (Lamendin et al., 1992). It is generally accepted that contralateral variation in transparency is negligible (Bang and Ramm, 1970; Solheim and Sundnes, 1980; Solheim, 1989). However, given the apparent differences in the extent of transparency within the same dentition, the majority of researchers have limited the scope of their findings to the particular tooth types they have examined (Bang and Ramm, 1970; Johanson, 1971; Drusini et al., 1990; Micheletti Cremasco, 1998). This limits the applicability of age estimation techniques to those particular tooth types from which the formulae were derived. Yet, where tooth type is not accounted for, differences in the degree of transparency both within the same individual and between subjects may contribute to any deviations in the observed association between transparency and age.

Root Size

It is possible that differences in root size may underlie the aforementioned sex and position-related variation (Solheim, 1989). Root size is variable by tooth type and is larger in males than in females (Lahdesmaki and Alvesalo, 2004; 2005; 2006). Yet even after controlling for sex and age, partial correlation analysis of transparent area and total root area have revealed a significant association (Solheim, 1989).

In an effort to mitigate the influence of this association, indices expressing transparency as a fraction of both total root length and area have been constructed (for example Bang and Ramm, 1970; Azaz et al., 1977; Drusini et al., 1989; Drusini et al.,

1991; Sengupta et al., 1998). Drusini et al. (1989; 1990) have followed the method of Lamendin and Cambray (1981) who compute the percentage ratio between the extent of root dentine transparency (mm) and the total root length (mm). However, the value of these relative indices is uncertain, as they do not consistently display a better correlation with age than do absolute measurements (Bang and Ramm, 1970; Solheim, 1989; 1993).

Changes in the Rate of Mineralization

It has been suggested that the systematic overestimation of age in young individuals and underestimation of age in the elderly arises from real changes in the rate of intratubular mineralization underlying transparency (Bang and Ramm, 1970). Such variation may arise either from pathological interference or biologically-controlled changes in the physiological processes underlying sclerosis.

Although the root dentine sclerosis and coronal transparency associated with caries infection are distinct processes (Azaz et al., 1977; Vasiliadis et al., 1983b), it is conceivable that dental disease (periodontal or pulpal) may interfere with the physiological mechanism(s) underlying root dentine transparency. Pilz (1959) reported that the distribution of transparency appeared to be influenced both by the vascular condition of the pulp and by the health of the surrounding periodontium. Johanson (1971) reported that root-filling of teeth appeared to interrupt the progression of transparency, this likely being due to a necrosis of the pulp. Accordingly, Bang and Ramm (1970) reported that several non-vital teeth failed to show transparency while vital teeth from the same individual showed appropriate levels of transparency. These studies seem to implicate pathological interference in observed deviations. However, Solheim (1989) reported that periodontal destruction was not correlated with the magnitude of the transparent area for any tooth type. Furthermore it seems unlikely that random pathological interference would introduce a systematic error in age estimates, particularly in the youngest age categories.

A non-linear relationship between age and the physiologically-controlled processes underlying transparency may also exist. Lorentsen and Solheim (1989)

reported that for several teeth, squared measures of transparent area contributed significantly to a multiple regression, a result they interpreted to indicate a decrease in the rate of mineralization with advanced age. Bang and Ramm (1970) speculated that inaccuracies at the upper end of the age spectrum may be the result of a decrease in the rate of the mineralization underlying transparency. However, regression analyses of age and transparency have consistently demonstrated a positive, linear relationship between the two (Miles, 1963; Bang and Ramm, 1970; Lamendin et al., 1992). Although exploration of alternate curve-fitting models has recently been undertaken, second and third degree polynomial functions appear to offer little, if any, advantage over age estimates generated with linear regression equations (Drusini et al., 1989; 1990; Micheletti Cremasco, 1998).

Differences in the rates of mineral deposition related to genetic factors have also been noted (Whittaker and Bakri, 1996). In a comparison of the extent of transparency in teeth extracted from individuals of varying geographical affinity, Whittaker and Bakri (1996) reported higher correlations between transparency and age for individuals from Britain than they do for teeth collected from Malaysian dental clinics. These results may indicate the contribution of ancestry to inaccuracies in age estimation (1996). However, hygienic and dietary differences are likely more salient factors underlying this variation (Whittaker and Bakri, 1996; Prince and Ubelaker, 2002). At any rate, the magnitude of differences between geographical groups is small (Prince and Ubelaker, 2002). Furthermore, in the generation of an age-predictive formula, selection of a geographically and behaviorally homogenous sample will result in a rather imprecise method in a real world context (Maples and Rice, 1979).

3.5.2 Exogenous Sources of Error

The current lack of information regarding the degree of variation in the rates of sclerosis (due either to pathology or individual variation) and the contribution that this may make to the observed deviations in the linear relationship between transparency and age represents a significant source of error in age estimates based on transparency. However, although the causal factors underlying the advancement of transparency exist within the individual from whom a tooth has been removed, it is likely that a number of factors external to the tooth under observation may affect the amount of transparency observed within a given tooth. Methodological decisions associated with the preparation of specimens and the subsequent description of any observed transparency represent significant sources of error in age estimates based on transparency. Furthermore, the environment to which a tooth has been exposed following extraction may alter the amount of transparency available for measurement. These external factors are of importance when considering sources of error in the derivation and application of age-predictive formulae based on transparency.

Post-mortem Interval

Several authors have reported significant differences between measures of transparency between teeth collected from archaeological contexts and those removed more recently, either in a clinical setting or from cadavers (Vlcek and Mrklas, 1975; Sengupta et al., 1999). Research has been undertaken to better understand the relationship between post-mortem interval and measures of transparency (Vlcek and Mrklas, 1975; Solheim and Sundnes, 1980; Kvaal and During, 1999; Sengupta et al., 1999; Mandojana et al., 2001; Megyesi et al., 2006). Mandojana et al. (2001) reported significantly higher values for measures of transparency, attrition, cementum apposition, secondary dentine and dental color in teeth collected from skeletal remains than from those extracted for clinical reasons. Furthermore, qualitative assessments of archaeologically-derived teeth appeared to be significantly associated with the extent of

transparency, with teeth in a poor state displaying higher measures of transparency (Megyesi et al., 2006).

Varying taphonomic conditions may underlie the observed differences between teeth based on post-mortem interval (Kvaal et al., 1994; Sengupta et al., 1998; Sengupta et al., 1999; Mandojana et al., 2001). Soil staining threatens the ability of researchers to accurately distinguish transparency, especially for intact specimens (Kvaal et al., 1994). Bacteria within soil may also interfere with transparency. In an examination of the dental remains of a subsample of the Spitalfields collection, Sengupta et al. (1999) reported difficulties in accurately outlining areas of transparency due to "chalkiness" of the root dentine. Such chalkiness, characterized by peri-pulpal regions of filiform opacity, confounded the accurate measurement of transparency, thereby altering its linear relationship with age (Sengupta et al., 1999). While the origin of this post-depositional alteration is unclear, it may be related to the demineralization of root dentine (both opaque and transparent) by the acidic byproducts of collagen-consuming micro-organisms (Beeley and Lunt, 1980; Sengupta et al., 1999). The bacteria responsible for these distortions were likely introduced by the surrounding soil, making their way into the dentinal tubules through the pulp (Sengupta et al., 1999). Given that recently-extracted teeth are not exposed to such bacteria, differences in the strength of the association between age and transparency in modern and archaeological samples may be an artifact of the post-extraction environment.

At the same time, root dentine transparency appears elevated with prolonged post-mortem interval, irrespective of taphonomic interference. Although soil conditions appear to exert a significant influence on the degree of transparency, teeth of unburied cadavers also show elevated levels of transparency, a result which has not yet been explained. Solheim (1993) indicated a significant positive correlation between root color and transparency with the latter being elevated in darker teeth. Compared to those extracted from living individuals, those teeth taken from cadavers were darker and displayed more transparency (Solheim, 1988; Solheim, 1993). As these bodies had not yet been interred, taphonomic interference via soil staining seems unlikely.

Sample Preparation

Discrepancies between the preparation methods of various researchers may significantly contribute to the difficulties associated with the adequate visualization of transparent dentine, thereby affecting the association between transparency and age. In particular, the question of whether or not to section teeth remains unresolved. Although intact methods do not require the destruction of teeth, they may reveal less about the individual from whom teeth were removed and about the surrounding archaeological environment. Bang and Ramm (1970) reported that although intact and sectioned methods produce comparable levels of accuracy in age estimation, for some teeth, transparency was only visible following sectioning. Therefore, provided that it is neither prohibited nor inappropriate, several authors feel that sectioning of teeth prior to examination is appropriate (Bang and Ramm, 1970; Soomer et al., 2003).

A number of parameters surrounding the preparation methods for sectioned specimens remain to be standardized. Sections have been prepared to a wide range of thicknesses. The original method of Bang and Ramm (1970) specifies that sections should be prepared to 400 μm. However, this method has not been universally applied. The method of Johanson (1971), involving the manufacture of longitudinal thin sections ground to a thickness of 250 μm, has also enjoyed wide use. This technique has been used for studies employing both the methods of Bang and Ramm (1970) and Solheim (1989) (Soomer et al., 2003). Other authors have produced sections ranging in thickness from 500 μm to 1.0 mm (Kashyap and Koteswara Rao, 1990; Sengupta et al., 1998; Monzavi et al., 2003).

Although thinner sections may allow for a clearer definition of areas of opacity and transparency, they allow more light to pass through undistorted and therefore appear to contain a greater amount of transparent root dentine than they truly do (Johanson, 1971; Metzger et al., 1980). The use of thicker sections has therefore been suggested. Due to artificially high transparency values in sections observed at thicknesses of 250 μm and below, Metzger et al. (1980) advocated the use of 1.0 mm thick ground sections. The

authors noted that thicker sections allow for accurate visualization of the entire pulp chamber and root canal while simultaneously ensuring an even slide thickness (Metzger et al., 1980). Solheim (1984) has developed a half-tooth preparation technique involving the grinding of teeth in the labio-lingual plane from the outer surface to the tooth midline as an expeditious alternative to thin sectioning. This technique has been employed by a number of researchers (Lorentsen and Solheim, 1989; Solheim, 1989; Mandojana et al., 2001). Although the transparency values derived from measures made on samples prepared in this manner are slightly elevated when compared to samples prepared as thin sections, this difference is not statistically significant (Solheim, 1984).

Variation in the orientation of the cutting plane during sectioning will also affect the amount of transparency apparent within a tooth. In general, the standard path of sectioning is oriented in a bucco-lingual plane, passing along the root center and through the apex (Bang and Ramm, 1970; Johanson, 1971; Kashyap and Koteswara Rao, 1990; Sengupta et al., 1998; Monzavi et al., 2003). However, this method of sectioning has rarely been adhered to, as roots are frequently irregular in their orientation, often deviating from the midline and curving distally at their apex (for a more detailed description of root morphology see Kovacs, 1971). For the majority of teeth with irregularly positioned roots, either the entire length of the root will fail to be included in the section or the section will not pass through the root center. It is therefore possible that discrepancies in the orientation of the section may account for inaccuracies in the association of transparency with age.

Yet, even the most carefully made sections will not capture the true extent of transparency. Even for those cases in which it is possible to manufacture a longitudinal thin section that includes the entire length of the root at midline, a single section will never reflect the true extent of transparency (Sengupta et al., 1998). Based on serial sectioning of transparent tooth roots, Vasiliadis et al. (1983b) demonstrated that transparency progresses unevenly in three dimensions, advancing at a faster rate in a mesio-distal plane (Vasiliadis et al., 1983b). The traditional means of sectioning, oriented in a bucco-lingual plane, does not capture the maximum extent of transparency,

regardless of the measurements applied. In recognition of this, it has been suggested that volumetric assessments of transparency be carried out in the future (Sognnaes et al., 1985). However, to date, no three dimensional description of the extent of transparency has been undertaken.

Variables

The supremacy of either linear or area measures of transparency has not been established. Several authors have reported higher correlations with age for measures of transparent length than for area (Solheim, 1989; Whittaker and Bakri, 1996). Yet the area of transparency has in some cases been shown to be strongly correlated with age and is therefore still considered a viable means of measurement (Micheletti Cremasco, 1998). However, given that inaccuracies of age estimates based on root dentine transparency may be related to inadequate descriptions of the age-related changes, novel forms of quantification should be explored (Kvaal and Solheim, 1994). To this end, Kvaal and Solheim (1994) examined the length of transparency on radiographs. However, poor results were reported, likely due to the distortion of tooth roots by X-rays and the superimposition of contralateral teeth.

It has been suggested that volumetric analyses of transparency should be explored, in order to better describe the extent of transparent dentine within a given root (Bang and Ramm, 1970; Bang, 1989; Lorentsen and Solheim, 1989; Lopez-Nicolas and Luna, 1991). Such measures would simultaneously depict the quantity of transparent dentine and offer a description of its distribution within a root. Serial sectioning of affected teeth has revealed that transparency is not distributed evenly throughout root dentine (Vasiliadis et al., 1983a). Therefore, the amount of transparency visible in a thin section can be expected to vary according to the depth and orientation at which it was removed from the root (Vasiliadis et al., 1983b). Since volumetric measurements do not rely on sampling of the root, but rather describe the entirety of the affected regions, a volumetric description of transparency would avoid the artefactual inaccuracies that arise during the sectioning process (Lopez-Nicolas and Luna, 1991). Furthermore, since a

volumetric analysis would be necessarily computer-based, it would also avoid the subjectivity that plagues measurements performed under light microscope. To date, volumetric measures have not been explored. Their cost and the training involved in performing them are deterrents which may ultimately limit the applicability of such methods, regardless of their accuracy.

Measurement

Measurements of transparency and the tools with which they are performed may not adequately describe the true extent of transparency. Inaccuracies in measurement may significantly contribute to errors in age estimation by distorting the true relationship between transparency and age in regression analyses. Due either to limitations in the sensitivity of the equipment or to inconsistencies in the protocols, data recorded for a given section may fluctuate. Where any data are obscured or omitted, the accuracy of any predictive formula based on the observed correlation will be adversely affected. The methods of Bang and Ramm (1970) and of Lamendin et al. (1992) rely upon caliper measurements. Solheim (1989) examined transparent area and length apical to the cemento-enamel junction under a stereomicroscope using a grid system on a photographic plate. Both of these instruments are limited in their ability to resolve differences between transparent and normal dentine. Further, they can only offer one and two-dimensional descriptions of transparency.

The question of whether to exclude cementum and pulp from area and length measures of transparency has not been settled. The exclusion of both resulted in correlations with age for linear measurements of transparency as high as 0.91 for a recent sample of sectioned teeth (Micheletti Cremasco, 1998). The omission of cementum seems particularly appropriate given that cementum apposition continues throughout life and that there exists a significant correlation between cementum apposition and area measures of transparency (Solheim, 1989; Micheletti Cremasco, 1998). However, several measurement protocols do not address this issue (see Bang and Ramm, 1970; Lorentsen and Solheim, 1989; Solheim, 1989), a situation which may weaken correlations with age

and threaten the consistency of measurements across independent applications of a given method. Even when measuring the same criteria, some researchers may have been measuring the absolute and relative amounts of transparent *dentine*, while those that choose not to exclude pulp and cementum may have been in reality measuring the amount of transparent *root*.

The measurement of transparency is further confounded by a lack of consensus regarding the distribution of physiologically-derived transparent root dentine. There is agreement that transparency begins at the root apex and advances in a coronal direction (Miles, 1963; Bang and Ramm, 1970; Vasiliadis et al., 1983a; Drusini et al., 1991; Lamendin et al., 1992; Micheletti Cremasco, 1998). However, Bang and Ramm (1970) considered the area of transparency at the periphery of the root, adjacent to the CDJ, to be the product of reactionary responses by odontoblasts to attrition of the overlying enamel layer. They therefore disregarded this zone in the measurement of the length, measuring instead from the root apex to the junction between transparent and opaque dentine at a point midway between the pulp cavity and the root surface (Bang and Ramm, 1970). However, this distinction has not been universally recognized, a situation which may have contributed to the differences in the strength of the correlation between age and transparency reported in various studies.

In an effort to standardize the delineation of transparency, the use of automated technologies has been explored (For example Ricco et al., 1984; Sognnaes et al., 1985; Lopez-Nicolas et al., 1990; Drusini et al., 1991; Lopez-Nicolas and Luna, 1991; Lopez-Nicolas et al., 1993). Computer programs developed for use in the medical imaging context have been employed in the description of transparent root dentine. Such programs rely upon differences in the gray level values of the constituent pixels of digital images to distinguish between transparent root areas (which appear lighter due to an increased amount of transmitted light) and areas of opaque normal dentine (which appear darker due to the scattering of incident light) (Sognnaes et al., 1985). Importantly, the threshold values of grayscale intensity can either be controlled by the observer to a desired effect or automatically controlled by the software itself (Sognnaes et al., 1985).

However, the value of automated approaches to measurement remains unclear. Lopez-Nicolas et al. (1990) and Lopez-Nicolas et al. (1993) reported improved precision for measures of transparency using an IBAS-I semiautomatic image analysis system. Micheletti Cremasco (1998) reported a high level of precision, accuracy and ease of application for both linear and area values of transparency based on the application of image analysis software to a mixed sample of teeth. However, Drusini et al. (1991) found a higher correlation for caliper measures of transparency compared to those performed via computer analysis of grayscale digital video camera images.

Observer Error

For a given observer, it appears that experience plays an important role in the consistency of measurements (Willems et al., 2002). Several authors have reported difficulty in consistently outlining areas of transparent dentine, a necessary first step in the measurement of both sclerotic area and length (Miles, 1963; Bang and Ramm, 1970; Lorentsen and Solheim, 1989; Kvaal and During, 1999). Despite low inter-observer error rates, Willems et al. (2002) reported significant intra-observer errors in the application of the intact method of Bang and Ramm (1970) to a sample of 160 anterior teeth.

Imprecision and inaccuracy between repeated measurements of transparency stem largely from the indistinct nature of the margin separating areas of sclerosis from those of normal dentine (Vasiliadis et al., 1983a). Transparency begins at the apex and peripheral margins of the root, advancing toward the pulp cavity with age (Vasiliadis et al., 1983a). Yet, transparent tracts may extend into areas of opaque dentine and the opacity towards the pulp center may be poorly defined (Vasiliadis et al., 1983a). Both for intact and sectioned teeth, the diffuse nature of sclerosis may introduce an element of subjectivity to empirical measurements, as an observer may be required to render a decision regarding the limits of transparency based on personal experience (Miles, 1963; Bang and Ramm, 1970; Bang, 1989; Lorentsen and Solheim, 1989; Kvaal et al., 1994). It has been suggested that this judgment may represent one of the major sources of both

inter and intra-observer error in estimates of age based on transparency (Lorentsen and Solheim, 1989; Kvaal and During, 1999).

3.6 Purpose of This Investigation

Research into the endogenous sources of error described above may reveal the physiological origins of the intratubular mineralization underlying transparency. Yet, the process of distinguishing and weighing the contribution of the physiological sources of variation affecting the progress of root dentine transparency can only proceed after the most appropriate means of description have been discerned. The current research will have as its focus the identification of the most accurate and precise means of describing transparency via the quantification of the variation arising from the alternate empirical measurements of transparency.

The primary goal of this research is to determine the most appropriate means of measuring root dentine transparency in age estimation. While inquiry into the cause of root dentine transparency and the sources of variation in the rate of its progression is certainly called for, the current research will focus only on identifying the most suitable means of quantifying transparency. Sample preparation methods and instrumentation will be standardized. However, aside from screening for obvious evidence of pathology or taphonomic interference, factors affecting individual variation in the amount of transparency will not be controlled for. This is in part due to the obscure identity of the salient variables. However such a decision is also guided by the belief that an adequate age estimation technique must be applicable across a wide range of individual and population variation in the morphological parameter under investigation.

It is hoped that the results of this research will suggest an optimal measurement technique where no firm consensus exists. This would represent a critical refinement of current methods involved in the quantification of root dentine transparency. Such a revision has the potential to improve the accuracy and precision of age estimates based

on the examination of the human adult dentition in both the archaeological and forensic settings.

Chapter 4
MATERIALS AND METHODS

4.1 Introduction

Both archaeologically-derived teeth and teeth recently collected from dental practices following extraction from living patients were included within this study. In total, 64 teeth including 32 archaeological specimens, taken from 7 female and 13 male individuals, and 32 recently extracted specimens, removed from 7 female and 9 male individuals, were examined. Originally, 69 teeth were examined. However, 5 recently-extracted specimens were excluded following measurement, based on a failure to meet the criteria outlined below. The distribution of tooth types and ages within the pooled sample are summarized in Table 4.1 and Table 4.2. A more detailed description of the samples and the populations from which they were drawn is given below.

	Incisor		Canine		Premolar		Total
Age Cohort (years)	Male	Female	Male	Female	Male	Female	
10 – 19	0	0	0	4	0	1	5
20 – 29	2	0	1	1	0	1	5
30 – 39	2	1	0	3	1	1	8
40 – 49	3	0	5	0	4	0	12
50 – 59	5	2	5	1	1	1	15
60 – 69	2	2	2	2	3	0	11
70 -79	0	1	1	1	0	0	3
80 -89	0	0	3	1	0	0	4
90 -	0	0	0	1	0	0	1
Total		20		31		13	64
Mean Age							48.84
Median Age							53.00
Mode							60.00

Table 4.1 Distribution of age and tooth type for the pooled sample.

	Incisor		Canine		Premolar		
	Male	Female	Male	Female	Male	Female	Total
Archaeological Sample	9	3	9	2	6	3	32
Recently-Extracted Sample	5	3	8	12	3	1	32
Total		20		31		13	64

Table 4.2 Age distribution separated according to sample.

4.2 Materials

4.2.1 Archaeological Sample

Measurements were performed on a subsample of teeth extracted from skeletal remains buried in the St. Thomas' Anglican Church cemetery site in Belleville, Ontario dating between 1821 and 1874 (Rogers, 1991; Saunders et al., 2002). These teeth are currently held in the department of Anthropology at McMaster University. The St. Thomas' collection is of great value, as it is one of the largest historic North American skeletal samples and the remains are accompanied by complete parish registers for the period under study (McKillop et al., 1989; Rogers, 1991; Saunders et al., 1995). It consists of the remains of 577 individuals excavated in the summer of 1989 by an archaeological firm under contract to the church (Rogers, 1991; Saunders et al., 1993). Preservation of this sample is excellent, such that for 87% of all adult skeletons, all bones were complete enough to evaluate pathology (Saunders et al., 1995; 2002).

The collection contains individuals of relatively homogenous biological affinity, largely of British and American ancestry (Boyce, 1990; 1991). Belleville was settled in a series of three waves of immigration, the first beginning after the American Revolution, the second after the war of 1812, and the third beginning in 1830 (Hoppa, 1996; Saunders et al., 1997). Overseas immigration flowed largely from Britain, France, Holland and Germany (Mika and Mika, 1986; Rogers, 1991; Boyce, 1991). Following its

incorporation in 1834, the population of Belleville grew from approximately 1,700 to over 11,000 by the late 1870's (Mika and Mika, 1986; Rogers, 1991). High caries rates within the St. Thomas' sample are indicative of individuals of middle to high socio-economic status who consumed large amounts of processed foods including refined flours and pastries (Saunders et al., 1997; Saunders et al., 2002). However, previous research indicating a low to moderate prevalence of enamel hypoplasia is "...consistent with a developing pioneer community, where the quantities of food were generally sufficient and chronic disease levels were relatively low" (Saunders et al., 2002:151). For a more detailed description of the population of Belleville for the period under examination, see Boyce (1991), Rogers (1991), Hoppa (1996) and Saunders et al. (2002).

The St. Thomas' church was completed in 1821 and its cemetery was in use from August of that year until its closure in May of 1874 (Rogers, 1991). Over this period, a total of 1564 individuals were interred in the cemetery. The 577 individuals recovered during excavation constitute roughly 40% of all interments in the cemetery during this interval (Rogers, 1991; Saunders et al., 1995). For the entire sample, age and sex of the remains are known with a high degree of confidence, both through consultation of the church's parish registers and via the application of multiple pelvic and cranial age and sex estimation techniques (Rogers, 1991; Rogers and Saunders, 1994; Saunders et al., 1995). The parish registers represent an extremely valuable resource for this skeletal sample, as they offer age and sex data for all but 125 of the burials.

Teeth used in the current investigation were selected based upon the following criteria:

1. *Age and sex must be known with a high degree of confidence.* Based on this criterion, only teeth collected from 72 personally-identified individuals for whom age-at-death had been previously determined through the comparison of coffin plates to listings of interments in the parish registers were eligible for inclusion (Rogers, 1991; Boyce, 1991; Saunders et al., 1997).

2. *Teeth must be free of obvious taphonomic interference.* Of those that were available for examination, a large proportion of teeth were excluded based on evidence of post-depositional alteration to the root dentine, a process that had obscured the true amount of root transparency. This taphonomic change appeared similar to that noted by Sengupta et al. (1999) in their description of chalky dentine for teeth from the Spitalfields collection. Such alteration likely arose as a result of exposure to the acidic byproducts of soil bacteria (Sengupta et al., 1999).
3. *Thin section preparation should allow for the visualization of the entire length of the root, at a thickness that does not obviously distort the true amount of transparency.* Thin sectioning of the teeth had previously been performed for the purpose of ondonto-chronological research based on growth markers within enamel. Teeth were embedded in epoxy resin and sectioned in a labio-lingual plane. Sectioning was performed to a variety of thicknesses and at varying positions relative to root center. In some cases, sections were too thin to be examined adequately. In others, the plane of the section did not pass through the root apex, resulting in an incomplete root section. In order to ensure that measured areas of transparency were the byproduct of physiological processes rather than artifacts of sample preparation, any teeth for which translucency of the section was elevated due to thinness of the material were not included within the sample under study (Metzger et al., 1980; Solheim, 1984). Similarly, those teeth for which the entire length of the root was not preserved were excluded from analysis.
4. *Teeth must be free of ante-mortem root pathology or root destruction.* Although total percentage prevalence of caries within the St. Thomas' sample is approximately 30%, a value higher than contemporaneous British and American populations (Saunders et al., 1997), no lesions extending below the cervical margin were detected. Pathology did not interfere with measurement of transparency for any of the teeth, either by obscuring transparency or through root

destruction. Furthermore, all of the teeth under examination appeared to have been vital at the time of death.

After excluding those teeth for which the above criteria could not be met, a total of 32 teeth were included for measurement. Of these, 8 were collected from females and 24 from male subjects. In all, 20 individuals (7 females and 13 males) are represented within this sample. Age-at-death within the sample ranges from 17 to 75 years of age. The mean, median and mode ages for the sample are 46.97, 48.50 and 60 years respectively. Therefore, although all 10 year age cohorts between 10 and 80 years are represented within the sample, it is more heavily weighted towards the upper end of the age range. These data are summarized in Figure 4.1 and Table 4.3.

Mandibular single-rooted incisors, canines and first premolars were available for analysis. All teeth had previously been embedded in epoxy resin and sectioned in the labio-lingual plane, such that the section passes both through root center and root apex. Section thickness (as measured by digital calipers) varies between 0.28 and 1.6 mm (see Appendix II). In total, 12 incisors, 11 canines and 9 first premolars were examined. Although the quadrant to which each tooth belongs is uncertain, current research indicates the absence of significant contra-lateral variation in measures of transparency (Bang and Ramm, 1970; Solheim, 1989). The relative frequency of each tooth type is summarized in Table 4.3.

	Incisor		Canine		Premolar		Total
Age Cohort (years)	Male	Female	Male	Female	Male	Female	
10 – 19	0	0	0	0	0	1	1
20 – 29	2	0	1	1	0	1	5
30 – 39	1	0	0	1	1	0	3
40 – 49	3	0	2	0	2	0	7
50 – 59	1	1	3	0	0	1	6
60 – 69	2	1	2	0	3	0	8
70 -79	0	1	1	0	0	0	2
80 -89	0	0	0	0	0	0	0
90 -	0	0	0	0	0	0	0
Total	9	3	9	2	6	3	32
Mean Age							46.97
Median Age							48.50
Mode							60.00

Table 4.3 Distribution of age and tooth types for the St. Thomas' Anglican Church cemetery sample.

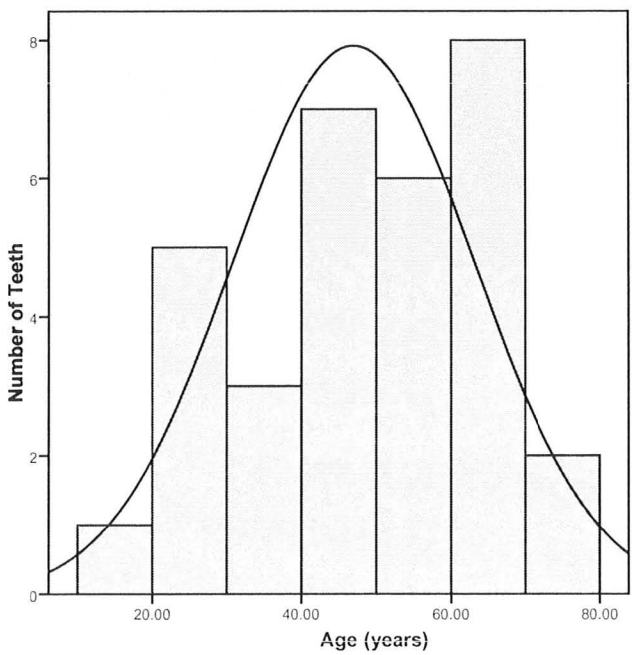

Figure 4.1 Age distribution for the St. Thomas' tooth sample.

4.2.2 Recent Sample

Recently-extracted, intact teeth were obtained from two dentists' offices in the Greater Toronto Area and from the Winnipeg School of Dentistry. Eight teeth were collected at the office of Dr. Vito Gallucci in Oakville, Ontario and 2 teeth were collected from the office of Dr. Alan Zucker at the Ancaster Family Dentistry Center in Ancaster, Ontario. The remaining 26 teeth were supplied by the Winnipeg School of Dentistry. Only vital, permanent, single-rooted teeth free of obvious root pathology were included within this sample. Prior to collection, ethics approval for this research had been obtained, both from McMaster Research Ethics Board and from the Winnipeg School of Dentistry. A copy of the certificate of ethics approval is included in Appendix III. Also included are copies of the participant information sheet and the consent form provided to the patients as well as the labels used to catalogue the teeth after they were collected. Participants were approached to donate their teeth during previously-scheduled extractions for clinical reasons unrelated to this research.

Although data were collected from all 36 samples, four specimens were omitted from statistical analysis because they failed to meet the requisites for inclusion within this study. One specimen had been root-filled and two other specimens had broken roots. A molar was not included for analysis since it had more than one root. In total, 32 recently-extracted teeth (8 incisors, 20 canines and 4 premolars) were included for measurement (see Table 4.4). Following data collection, one specimen was excluded based on observed pathology within the dentine which appeared to have limited the progression of transparency (see Figure 5.17). Although the cause of this remains unclear, it may be related to metabolic deficiencies or some obscure pathology (Sengupta et al., 1999; Megyesi et al., 2006). This specimen was included in the descriptive examination of the sample. However, it was omitted from further analysis. Therefore, in total, 31 recently-extracted teeth (8 incisors, 20 canines and 3 premolars) were included in the subsequent correlation and regression analyses.

For the recent sample the ages range from 15 years to 94 years of age (see Figure 4.2 and Table 4.4). The mean, median and mode ages for the sample are 50.71, 53 and 15 years respectively. The total number of individuals is 18, with 11 males and 7 females represented. The ancestry of the individuals within the sample and their relative socio-economic status and diet are unknown. However, for those teeth collected from dentists' offices, one may assume that the individuals are wealthy enough to afford dental care.

Although all teeth within the sample were vital at the time of extraction, a large number showed signs of dental pathology. For the oldest individuals, periodontal recession was often pronounced. Calculus was observed at the cervical margin of several teeth. The crowns of six teeth had been repaired with both metal and porcelain fillings. Coronal carious lesions were commonly observed throughout the age range, particularly at the cervical margin and at inter-proximal locations. For one individual all four teeth displayed complete crown destruction. It is suggested that this may have been due to extremely advanced caries or to individual behavior which elevated the acidity of the oral environment (i.e. bulimia or drug use). However, without an adequate dental and medical history, the validity of this hypothesis remains uncertain. With the exception of those recent samples omitted from analysis, there was no evidence of root pathology.

	Incisors		Canines		Premolars		Total
Age Cohort (years)	Male	Female	Male	Female	Male	Female	
10 – 19	0	0	0	4	0	0	4
20 – 29	0	0	0	0	0	0	0
30 – 39	1	1	0	2	0	1	5
40 – 49	0	0	3	0	2	0	5
50 – 59	4	1	2	1	1	0	9
60 – 69	0	1	0	2	0	0	3
70 -79	0	0	0	1	0	0	1
80 -89	0	0	3	1	0	0	4
90 -	0	0	0	1	0	0	1
Total	5	3	8	12	3	1	32
Mean Age							50.71
Median Age							53.00
Mode							15.00

Table 4.4 Distribution of age and tooth types for the recently-extracted sample of teeth.

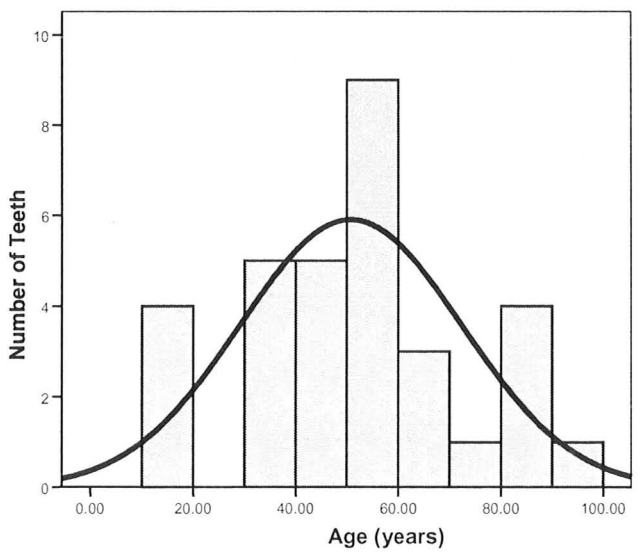

Figure 4.2 Age distribution of the recently extracted sample of teeth

4.3 Methods

4.3.1 Tooth Preparation

Cataloguing

Upon collection of the recently extracted samples, all teeth were immediately soaked in ethyl alcohol for a period of 48 hours. Teeth were then individually catalogued in a database according to the identification number assigned at the time of collection. ID numbers were generated based on the site of collection and the individual tooth's position within the sequence of collection. For example, the third tooth collected by Dr. Vito Gallucci was assigned the ID number GAL003. In addition to the ID number, the following variables were recorded for each tooth: date of extraction; date of birth; age at extraction (measured in years and months to one decimal place); sex; vital at extraction (Y/N); and any evidence of root pathology. These data were been collected at the time of extraction by the attending dentist. Tooth position was recorded based on the reference illustrations of Hillson (1996; 2005). However, since the exact tooth position could not be determined for the majority of the St. Thomas' tooth specimens, each tooth was later assigned to broad tooth categories. All incisors, canines and premolars were grouped together regardless of true position.

Gross Measurement

The crown dimensions of each tooth were then recorded according to the protocol of Hillson et al (2005). The variables recorded for each tooth included: Cervical Bucco-lingual Diameter, Cervical Mesiodistal Diameter, Maximum Bucco-lingual Crown Diameter and Maximum Mesio-Distal Crown Diameter. All measurements were made to the nearest 0.01mm using Hillson-FitzGerald digital calipers. Although the crown was not of interest for this particular study, these data were recorded prior to sectioning in anticipation that they may be of use in the future for alternate lines of research. These

data were not collected for the St. Thomas' thin sections as the epoxy embedding material precluded such measurement.

Sectioning Methods

In the current research, a thickness of 1mm was employed in the preparation of the recently-extracted teeth. In line with the findings of Metzger et al. (1980), section thicknesses below this threshold were found to be of little advantage. Furthermore, below 250 μm the amount of transparency was elevated due to the overall thinness of the specimen. Thicker sections also required less time to prepare and captured a greater proportion of the entire root at midline, especially in those cases wherein roots were irregularly curved at their apices

After micro-Computed Tomography (micro-CT) scanning (for which the protocol is outlined below) was carried out on intact teeth, all teeth within the recent sample were sectioned according to the following protocol, which represents a modification of the FitzGerald - Saunders *Protocol for the Preparation of Undecalcified Ground Tooth Sections* (2006).

1. *Marking the Cutting Line*: Each tooth was marked with a cutting line drawn on the buccal/labial surface. The line was made to pass through the root apex, the lowest point on the cervical margin on the buccal aspect of the tooth and the crown tip. All lines were drawn with an indelible marker, to ensure that they would not fade during the sectioning process
2. *Embedding*: Although the FitzGerald – Saunders (2006) protocol calls for modern tooth samples to be dipped in cyanoacrylate prior to sectioning in order to prevent chipping of the enamel, this step was omitted. Through experimentation it was found that the enamel was of good enough quality that teeth did not fracture. Furthermore, the crown was not of interest in this research. Therefore, the embedding step was deemed unnecessary.

3. *Sectioning*: Intact teeth were mounted to a wafer chuck with dental sticky wax such that the inscribed cutting line was parallel to the surface of the chuck. An initial "zero cut" was made to the right of the cutting line, passing almost, but not quite, through the root apex. A slide was then attached to the cut surface of the tooth with cyanoacrylate. After drying, the tooth was remounted on the saw and a second cut was made approximately 2.0 mm to the left of the zero cut. All sectioning was done using a Buehler IsoMet 1000 Slow Speed Saw set to 100 rpm. The blade was a 4-inch diamond blade mounted to the saw using two 2.5-inch flanges. A counter- weight of 137g was used to increase the pressure applied by the saw to the tooth, thereby controlling the speed at which the saw progressed.
4. *Lapping*: Each tooth was ground to a thickness of 1.0 mm using 400 grit carbide paper attached to the base of a plastic polishing bowl. Water was used for lubrication. All grinding was carried out using a Beuhler MiniMet 100 Grinder-Polisher. A proprietary jig previously manufactured for the McMaster University Department of Anthropology Hard Tissue and Light Microscopy Laboratory was used to grind the teeth while attached to a microscope slide. This jig, combined with the "random polishing action" of the MiniMet polisher eliminated any directional polishing artifacts in the resultant section and produced a specimen of extremely uniform thickness. During the grinding process, measurements of section thickness were monitored and recorded using digital calipers connected directly to an Excel spreadsheet (see Appendix II). To ensure a plano-parallel section, measurements were recorded at six standard points on the tooth surface. Although the amount of time required to reach a thickness of 1.0 mm varied between samples, it rarely exceeded 10 minutes.
5. *Polishing*: No polishing of the sections was carried out, as transparency is a macroscopically apparent phenomenon visible at low magnification. For the same reason, the second tooth surface, which had immediately been fixed to the slide after the first cut was made, was neither lapped nor polished.

4.3.2 Data Collection

Computed Tomography

Following gross measurement, teeth were provided to Dr. Moran, Assistant Professor in the *Department of Medical Physics and Applied Radiation Sciences* at McMaster University, who oversaw imaging of the teeth using a micro-CT imaging system located in the *McMaster Centre for Pre-Clinical and Translational Imaging.* Since the teeth of the St. Thomas' sample consist of sectioned specimens, micro-CT scanning could not be performed on them. Volumetric data were therefore collected only from the recently extracted teeth.

CT imaging involves the visual representation of the density of tissues via measurement of the degree to which they disrupt the path of x-ray photons (Jackson and Thomas, 2004). Although a variety of different scanning arrangements exist, varying primarily with regards to the number, sensitivity and movement of their detector arrays, the principles of x-ray computed tomography remain unchanged (for a complete review of each "generation" of scanner see Rydberg et al., 2000; Siemens AG Medical Solutions, 2003; Ritman, 2004; Kalender, 2006). An object undergoing measurement is placed between an x-ray tube and a radiation detector which converts incoming x-rays to electrical signals (Siemens AG Medical Solutions, 2003; Jackson and Thomas, 2004; Ritman, 2004). The item is then bombarded with x-rays emitted from the x-ray tube which focuses the beam on a concentrated region of the object (Jackson and Thomas, 2004; Ritman, 2004). The intensity of each beam after it has passed through the object is measured by the detector array opposite the x-ray tube (Morgan, 1983).

As an incident x-ray travels through a given medium, the amplitude and intensity of its constituent photons is reduced, a process referred to as *attenuation* (Morgan, 1983). Attenuation of the beam arises due to the absorption and scattering of individual photons by the material under study and is related both to the density and thickness of the material as well as to the energy of the incident beam (Morgan, 1983). The linear

attenuation value of an object is expressed in Hounsfield units (HU), a unit of measure based on a scale of known attenuation values for which the value for water has been made equal to zero (Bushong, 2000; Jackson and Thomas, 2004). For a given thickness, denser materials are characterized by a greater attenuation of x-ray beams and therefore higher HU values (Morgan, 1983). Measurement of the linear attenuation values for various regions within an object therefore allows for the density of that material to be quantified.

During data collection, the angle and position of the x-ray tube in relation to the object under study are manipulated in a systematic manner in order to produce a series of two-dimensional images of the object located within the axial plane (Jackson and Thomas, 2004). Each of these slices is divided into a matrix of elements referred to as *voxels*, small regions whose "cross-sectional area corresponds to that of a pixel and whose depth corresponds to the tissue slice thickness" (Morgan, 1983:28). These elements represent datum points within the scanned object for which HU values have been averaged over a number of readings (Jackson and Thomas, 2004).

The data for an object are recorded and saved on a computer (Morgan, 1983; Bushong, 2000). These data are then processed by a software program which uses a series of mathematical formulae referred to as *reconstruction algorithms, convolution filters* or *reconstruction kernels,* to translate the electrical signals generated by the elements of the detector array into HU values (Bushong, 2000; Siemens AG Medical Solutions, 2003). This pre-processing of the data is designed to both increase the resolution and decrease the "noise" within the resultant images (Bushong, 2000). Following this data filtering, the attenuation value for each voxel is then translated into a visual representation of the density of an object, through a process referred to as *reconstruction* (Bushong, 2000; Siemens AG Medical Solutions, 2003). Densities are represented by a range of grey-scale values for the corresponding pixel within each two-dimensional slice of the CT image (Morgan, 1983). Within each image, the regions of greatest density are displayed towards the whitest end of the spectrum while areas of lower density are displayed as darker regions (Bushong, 2000; Jackson and Thomas,

2004). In this way, external and internal densitometric differences within the object are made discernable based on visual inspection of the resultant image (Jackson and Thomas, 2004). A three dimensional model of the object can then be generated via computer-automated reconstruction of the data from a number of slices (Jackson and Thomas, 2004). As such, reconstructed CT images represent extremely accurate qualitative descriptions of material density, based on quantitative data.

Micro-CT Data Collection

The current research involved the use of micro-CT analysis. This alternate form of CT scanning has become increasingly utilized over standard CT as it employs a finer detector pixel spacing (~50 μm) and a micro-focal x-ray unit which produces a smaller focal point (~10 nm), thereby offering enhanced spatial resolution for reconstructed CT images (Bushong, 2000; Olejniczak and Grine, 2006; Holdsworth and Thornton, 2006).

Images were acquired using a Gamma Medica-Ideas X-SPECT micro-CT scanning system, employing a single panel direct-detector array (approximately 10 x 10 cm) with a cone beam x-ray source. Within this system, the panel and source are fixed within the gantry, directly opposite one another, and rotate around the specimen while acquiring projection data. Measurements were performed at the rate of 1024 projections per 360° rotation of the gantry (i.e. one projection for every 0.351° of rotation). X-rays were emitted at a voltage of 75 kvp and a current of 180 amps. Specimens were placed 225mm from the x-ray source and 164mm from the detector panel in order to achieve optimal magnification of the acquired images. Acquisition time was approximately four minutes per specimen.

Following filtered-back projection reconstruction, the data files were saved with an isotropic pixel size of 0.0586 mm (decreased from 0.115mm during image reconstruction) and a matrix size of 512 x 512 x 512 pixels per slice (with the exception of samples WIN001A and WIN003A for which matrices were 512 x 512 x 640 due to abnormally large root lengths). All slices were contiguous, resulting in serial overlapping two-dimensional sections.

CT Image Analysis

The micro-CT scans provided the volumetric data for each of the recently extracted teeth under study. Volumes, both of transparent and opaque root dentine, were recorded using Amira image analysis software (version 3.1.1). Differences in the tissue density of transparent root areas were exploited in order to extract a volumetric reading from the three-dimensional images. Through the qualitative comparison of CT images and digital images of teeth prepared as thin sections, it proved possible to define a density threshold which corresponded well to areas of the root that appeared transparent under stereomicroscope. At similar locations within the CT images, transparency appeared to correspond to a localized elevation in tissue density. Through experimentation, a range of +4,500 to +6,000 HU was defined as the density values that corresponded best to areas of optically-transparent dentine. These absolute threshold values were subsequently applied to the data from each tooth in order to outline in three dimensions the regions of root dentine that appeared transparent.

For each tooth, the opaque and transparent areas within the root were distinguished by creating two different "materials" for which different densitometric properties were defined. The material *"Trans"* included all voxels within the root with HU values falling within the aforementioned range, while the material *"Root"* included all voxels of the root within the range of +2,500 to +20,000 HU, excluding those that fell within the transparent range of density values. For both materials, voxels of the pulp were excluded from analysis. Tooth crowns were excluded from measurement by creating a third material which included all voxels above the cervical margin. This material was created by manually outlining the crown in each of the serial sections of the tooth while moving along the *x*-axis in a mesio-distal direction. In many cases, this material included not only voxels within the crown, but a number of imaging artifacts that were necessarily excluded from analysis. Interference due to the presence of metallic fillings was eliminated in this way. As such, the material *"Crown"* may be more properly defined as a material layer that included all coronal areas of the tooth under

study as well as any imaging artifacts which were thought to interfere with accurate measurement of opaque and transparent root dentine volume.

For each tooth, the following variables were recorded based on an analysis of the material layers:

1. *Volume of Transparent Root Dentine* (VT) - Defined as the region of the tooth root with attenuation values between +4,500 and +6,000 HU, this was equivalent to the volume of the material layer *Trans*. Values were expressed in mm^3.
2. *Total Root Dentine Volume* (VR) - This is the sum of the material *Trans* and the material *Root* and therefore represents the complement of the transparent and opaque root volumes. Values were expressed in mm^3.

In addition, the following index was calculated for each of the teeth under study:

F4: VT / VR

This ratio represents the proportion of the root dentine that was transparent. It was calculated as a scaling factor to control for the influence that root size and morphology may have on the absolute measures of transparent volume. Figure 4.3 provides a captured image of the working area within Amira 3.1.1 in order to illustrate the data collection process more clearly.

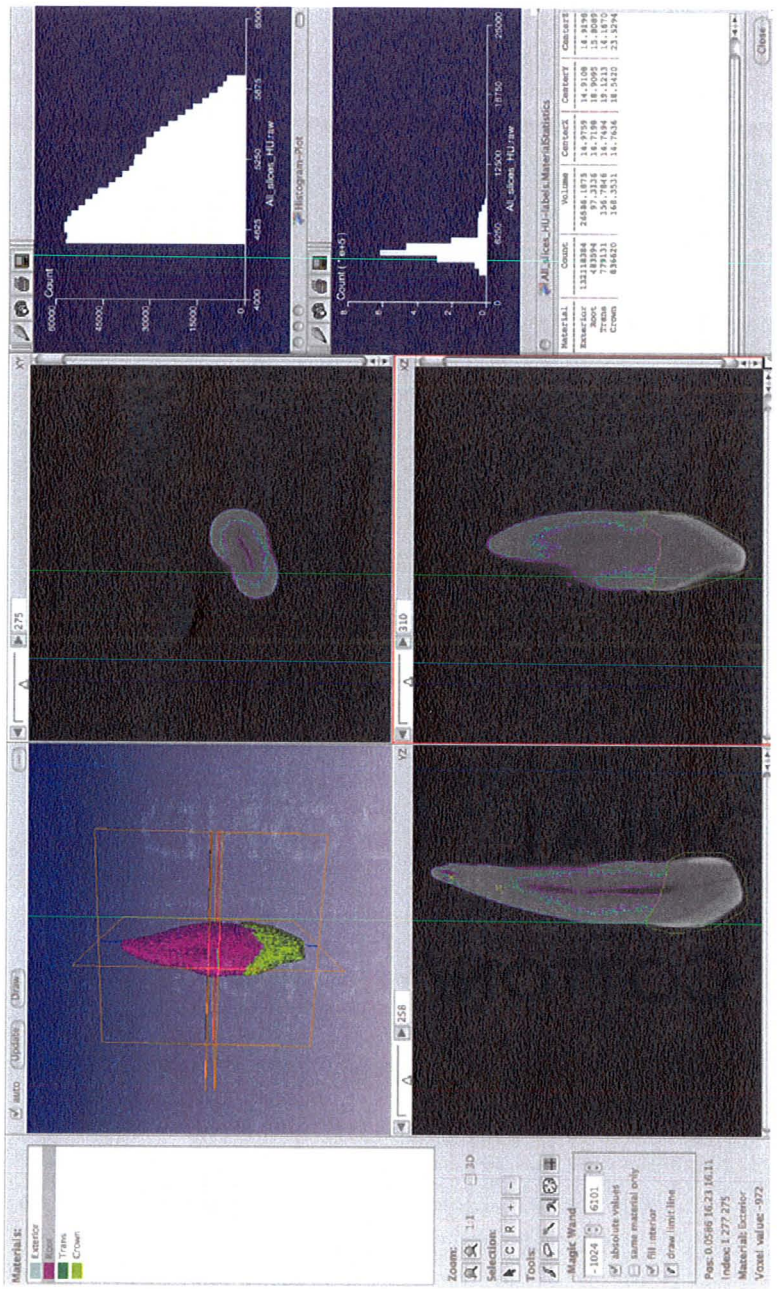

Figure 4.3 Screen shot of the working area in Amira 3.1.1 image analysis software. Tooth sample WIN006A is pictured. A 3D rendering of the tooth is visible in the top left corner of the screen. One can see from the materials list that the purple volume describes the root, while the yellow and blue material layers have been used to define the crown and transparent area within the tooth. In addition to the 3D image, three 2D slices of the tooth, each lying within one of the coordinate planes, are apparent. Once again, each of the material layers is visible within the slices. At the right of the screen are two frequency histograms. The graph towards the upper part of the screen represents the distribution of the various densities within the entire tooth. The lower graph represents the frequencies of the densities within the range of values defined as transparent (+4500 - +6000 HU). A summary of the properties of each of the material layers is provided at the bottom right of Figure 4.3. Included amongst these statistics is the cumulative volume occupied by each of the materials.

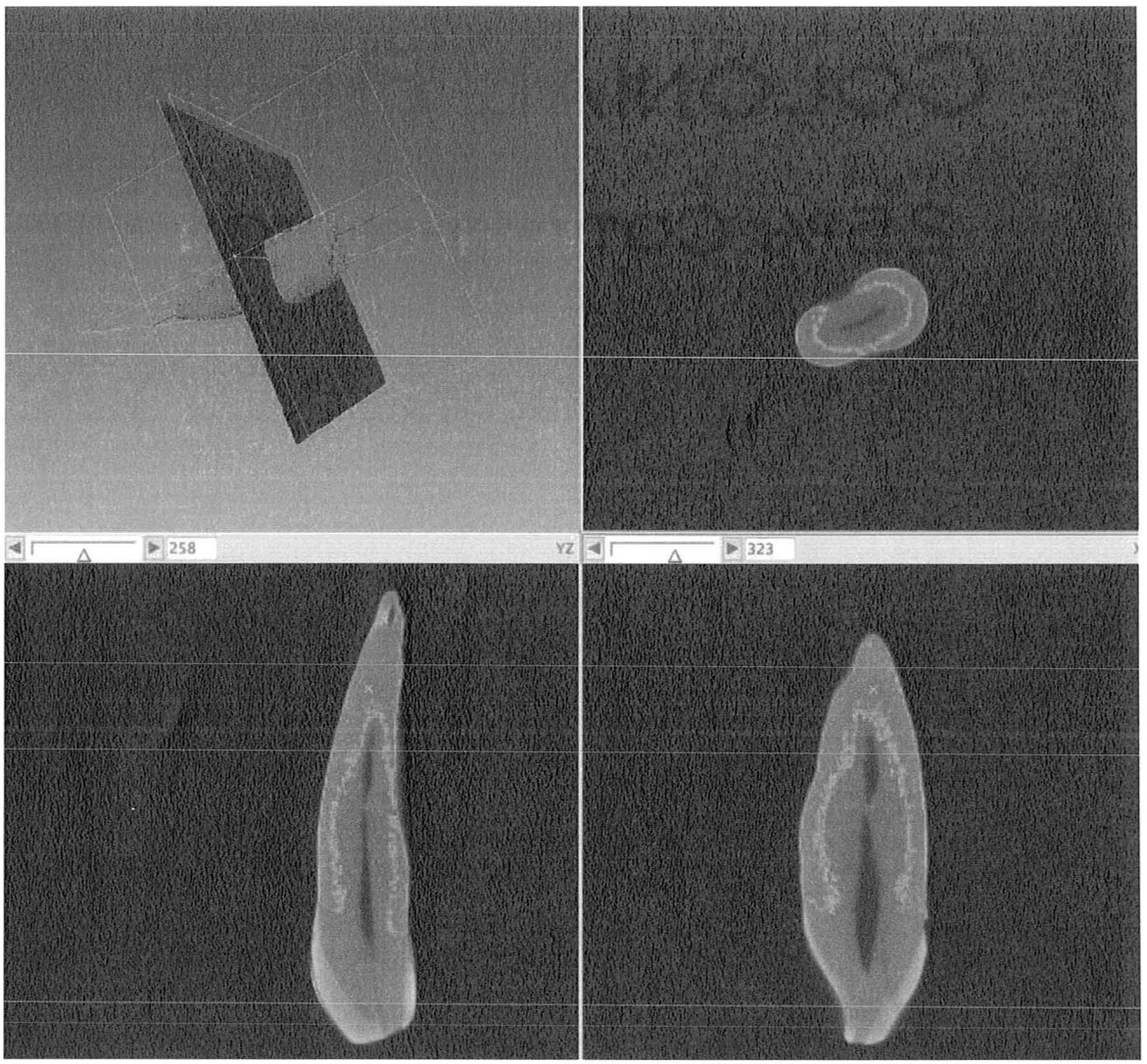

Figure 4.4 Micro-CT images of specimen WIN006A. The area outlined in green corresponds to a region identifed as transparent based on a density of +4500 - +6000 HU. The axial slice at the top right and the 3D reconstruction at top left make it clear that this region exists only at the periphery of the root and towards the apex.

Polarized Light Microscopy

Following micro-CT analysis, recently-extracted teeth were sectioned according to the protocol outlined above. All tooth thin sections were then examined under a Nikon SMZ800 stereomicroscope equipped with two polarizing filters. Ectopic illumination was provided by a Fiber-Lite MI – 150 High Intensity Illuminator set to 100 (maximum). All photographs were captured at *cross polars*, with the polarizer and the analyzer at right angles to one another. Contrary to the findings of Johanson (1971), this was experimentally found to enhance the contrast between areas of transparent and normal dentine.

All teeth were examined at a combined microscope magnification of 5X (where eyepiece magnification = 10X; objective magnification = 0.5X; and zoom magnification = 1X). This level of magnification was chosen because in the majority of cases it allowed for the visualization of the entire length of the tooth root from the apex to the labial and lingual cervical margins. However, for several teeth with larger roots (usually canines), it was not possible to fit the entire extent of the root in the image at this magnification. In these cases, two images of the root were captured: one in which the apex was visible and the other in which both the labial and lingual cervical margins were visible. In order to create a larger montage of the root, both images were imported into Adobe Photoshop CS2 version 9.0 and merged using the automated photo-merge function. For these images, the calibrations associated with each picture were lost during the merging process. Therefore, following the automated photo-merge, the resultant montage was reopened in NIS Elements BR version 2.20 and re-calibrated at the same magnification as those images for which merging was unnecessary.

Images of each tooth section were captured with a Nikon Digital Sight – 5M 5 Mega-pixel camera mounted directly to a Nikon SMZ800 stereomicroscope. The camera was linked to the computer on which data were collected via a Nikon Digital Sight Remote USB control box. Images captured with this camera were controlled and manipulated prior to capture using the NIS – Elements BR version 2.20 image analysis

software. Both linear and area measurement of thin sections were performed using this software.

For all thin sectioned teeth, both archaeological and recently-extracted, the camera settings for each of the images were as follows:

Mode: Normal (no binning)
Resolution: 2560 x 1920 pixels
Exposure: Auto exposure with +1.0 EV
Contrast: High
Illumination: Brightfield

Images were captured only after the appropriate manual calibration had been carried out. This calibration was done once and saved so that all subsequent images were captured at this setting. All images were saved as JPEG-2000 (JP2) files, as this is a loss-less form of compression which does not distort the data. Figure 4.6 provides an example of a captured image upon which measurements were later performed.

Linear and Area Data

Images were imported into the NIS – Elements BR version 2.20 image analysis program. After ensuring that the appropriate image calibration had been saved to the file, the following measurements were recorded for each tooth (see Figure 4.5 and Figure 4.7).

1. *Total Area of Root* (ARER) – the total root area apical to the cemento-enamel junctions on both the labial and lingual root surfaces (Solheim, 1989).
2. *Area of Transparent Root* (ATR) –the area of the root (including the pulp) displaying transparency (Lorentsen and Solheim, 1989). Solheim (1989) utilized a similar variable, which he termed Area of Translucent Zone (ARET).
3. *Total Root Length* (RL) – the length of a line drawn perpendicular to a line connecting the labial and lingual cemento-enamel junctions and a line parallel to

this passing through the root apex. Here, the root apex was defined as the last point on the root through which such a line passed when moved in an apical direction.

4. *Minimum Transparent Length* (TL Min) –the distance between two lines parallel to the cervical margin, one passing through the root apex and the other passing through the minimum extension of transparency in a coronal direction at a distance of 1mm from the root surface on the side of the tooth which displays the least extent of transparency (Bang and Ramm, 1970).
5. *Maximum Transparent Length* (TL Max) - the distance between two lines drawn parallel to the cervical margin, one passing through the root apex and the other passing through the maximum extension of transparency in a coronal direction at a distance of 1mm from the root surface on the side of the tooth which displays the greatest extent of transparency. This variable represents a modified version of the variable of the same name outlined by Bang and Ramm (1970).
6. *Mean Transparent Length* (TL) – the average linear extent of transparent dentine in a coronal direction measured from the root apex (Bang and Ramm, 1970). This variable was calculated according to the formula (TLA+TLI)/2 and was measured in mm.

Linear measurements were made in mm, while area measurements were recorded in mm^2. All data were recorded in individual Excel spreadsheets that were later combined such that statistical comparisons could be made. Although an attempt was made to measure the area of the pulp such that it could be eliminated from the measurements of transparent root area, this proved to be impossible for a large number of the specimens, both archaeological and recent. This is because, although sections were carefully ground to root centre, in many cases the root canal was not equidistant from all root surfaces throughout its entire course and could therefore neither be visualized nor measured. Measurements of transparent dentine area were therefore abandoned. Only ATR, F1, TL and F3 were recorded.

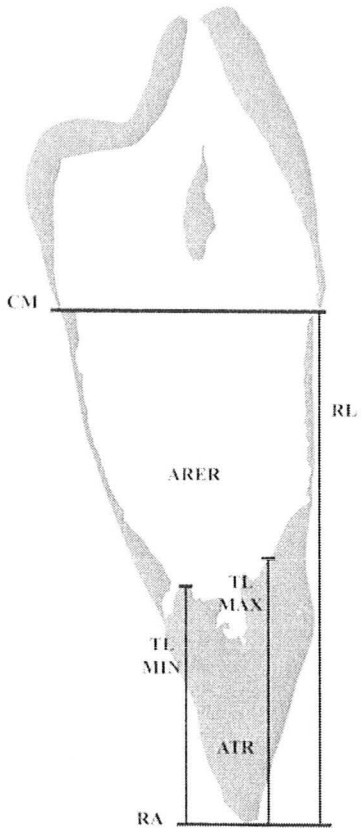

Figure 4.5 Diagram illustrating the area and linear measurements applied to the sectioned tooth specimens. CM and RA denote the lines marking the cervical margin and the root apex respectively. All area and linear measurements are explained above. This diagram was made in Adobe Photoshop CS2 using the magnetic pen tool as per Chan (2007).

As per Bang and Ramm (1970) and Lorentsen and Solheim (1989) the following ratios were then calculated as scaling factors to control for the contribution that varying root dimensions may have on the absolute measures of transparency:

F1: (ATR/ARER) x 100

F3: (TL/RL) x 100

4.3.3 Statistical Analyses

In the following chapter, the results of data collection are summarized. Standard descriptive statistics were calculated for all of the variables measured on each of the teeth within the archaeological and recently-derived samples. Following a standard descriptive analysis, known chronological ages were plotted against the observed values for each of the measurements of transparency. The strength of the association with age for each of these variables was assessed by an examination of the appropriate correlation coefficients. A description of these relationships was also approximated using linear and polynomial regression functions. Multivariate descriptions of the changes in transparency with age were explored as an alternative to simple regression. In each case, the corresponding R^2 values were examined to describe the goodness of fit for the statistical models. Finally, partial correlation coefficients were examined in order to assess the degree of variance in measurements of transparency that could be attributed to sex and tooth category. All statistical analyses were carried out using SPSS™ for Windows (version 15.0).

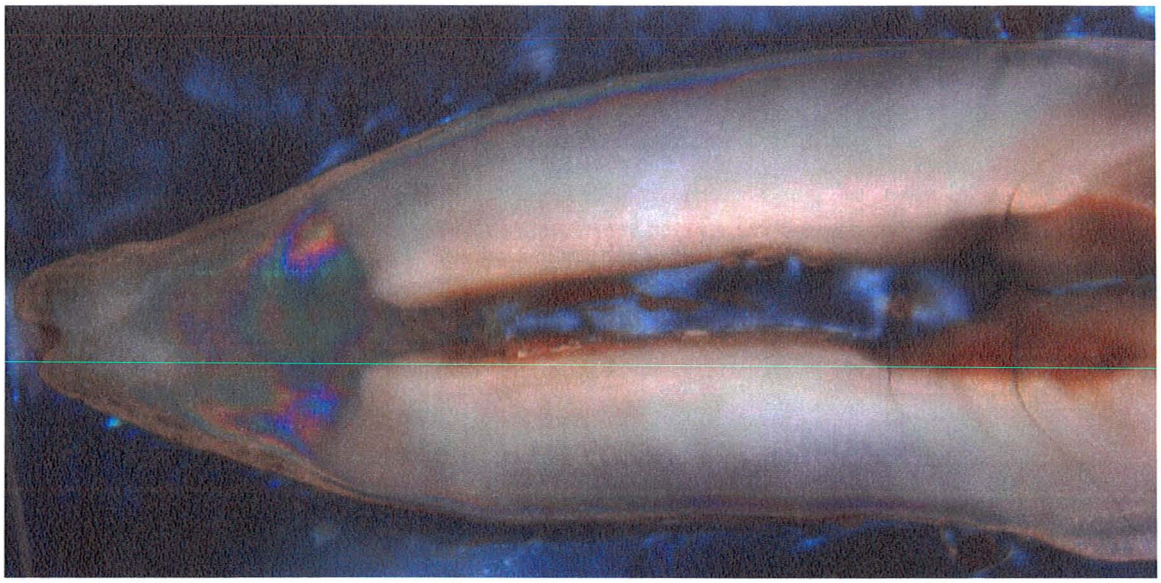

Figure 4.6 Stereomicroscope image of sample 429C captured under polarized light at 5X magnification. Transparency is visible at the apical end of the root.

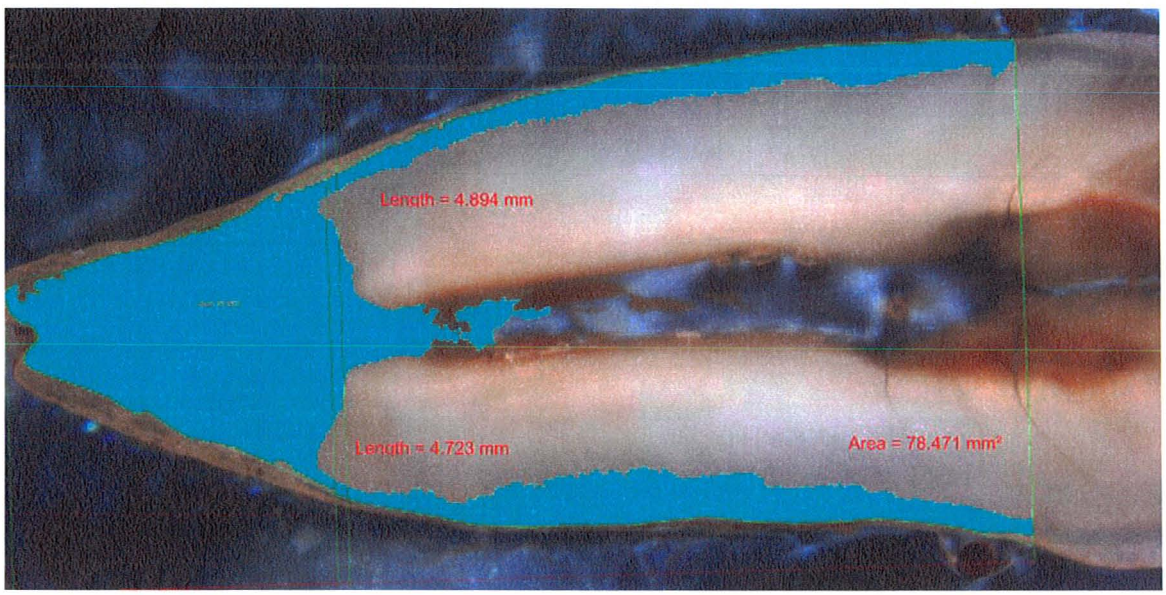

Figure 4.7 Stereomicroscope image of specimen 429C with accompanying measures of transparency recorded using NIS – Elements BR (version 2.20) image analysis software. The area shaded in blue identifies the region of transparency measured via computer analysis. Both area and linear measurements have been burned onto the image.

Chapter 5
RESULTS

5.1 Intra-Observer Error

After an interval of approximately four weeks from the date at which initial measurements were performed, intra-observer error was assessed using a randomly-chosen set of five teeth (three from the St. Thomas' sample and two from the Winnipeg Dental School sample). Each tooth was re-photographed and the area and linear measurements of transparency were re-applied to the captured images according to the protocol outlined in Chapter 4. Wilcoxon signed-rank tests were used to assess the degree of intra-observer error between measurements (see Table 5.1). The results of these tests indicate that the magnitude of the difference between each separate application of the measurements is not significant for any of the variables.

Since the CT data could not be re-collected from the teeth, the exact magnitude of the error between measurements is unknown. However, previous research conducted on live animals using the same micro-CT machine as was employed in this research have demonstrated an error of 5-10% between repeated measurements (Saab, 2007). However, the magnitude of the error would likely be lower for this research, since much of the re-test error reported for previous studies was introduced by the breathing movements of the animals from whom data were being collected (Saab, 2007). Furthermore, with the exception of the delineation of the crown, all data collection made using the Amira software was automated.

In general, previous research indicates that the accuracy of quantitative descriptions based on micro-CT analyses is high. In an investigation of root canal morphology Rhodes et al. (1999) demonstrated a high correlation ($r = 0.94$) between measurements of internal and external surface areas made on video-digitized images of teeth and those made via computer analysis of reconstructed micro-CT images. Kim et al.

(2007) have reported a close correspondence between linear measurements based on scanned images and those made directly on the teeth under study. Similarly, Olejniczak and Grine (2006) reported a mean error of only 3.5% in measurements of enamel thickness derived from micro-CT scans when compared to those made on physical sections. Furthermore, McErlain et al. (2004) have demonstrated that micro-CT may be successfully applied to archaeologically-derived dental materials with a high degree of accuracy and reproducibility.

	ATR - ATRR	F1 - F1R	TL - TLR	F3 - F3R
Z	-0.674	-0.135	-1.214	-0.405
Asymp. Sig. (2-tailed)	0.500	0.893	0.225	0.686

Table 5.1 Wilcoxon paired comparisons of the mean values for re-measurements of the area and linear measurements of transparency for five samples randomly-selected from the pooled sample.

5.2 Methodological Summary

The recent and archaeological samples were analyzed separately and then together, following an assessment of the significance of the difference between the samples. The assumption of equal variances was confirmed by Levene's test, while normality was assessed using the Kolmogorov-Smirnov and the Shapiro-Wilk tests. However, for both the St. Thomas' Anglican Church sample and the recently-extracted sample of teeth, a number of variables were not normally distributed. Attempts to transform the data were unsuccessful; therefore, nonparametric statistics were employed for the entire sample. After a description of the distribution of each of the variables, an examination of the relationship between chronological age and measurements of transparency is provided.

5.3 An Examination of the Data

Recently Extracted Sample

For the sample of recently extracted teeth, the descriptive statistics are summarized in Table 5.2. An examination of the mean values for each of the measurements of transparency indicates that for the majority of teeth (for which the mean age was 50.7 years) transparency was visible. Furthermore, the similarity between the mean scaled proportions within this sample indicates a high degree of agreement between area and linear measurements of transparency. For both F1 and F3, on average roughly ¼ and 1/3 respectively of the root appeared transparent for a given tooth. Since the teeth under examination ranged in age from 15 to 94 years, it is not surprising that the observed range of values appears broad. Values range from 0% to 46% and 56% for the relative indices of transparent area and length respectively. The fact that area and linear measurements of transparency were not reported for teeth extracted from a 15 year old subject, is in agreement with the results of previous research regarding the age at onset of transparency (Gustafson, 1950; Miles, 1963; Bang and Ramm, 1970; Bang, 1989; Lamendin et al., 1992).

An examination of the frequency histograms for each of the variables provides a description of the degree to which the mean values of the measurements are representative of the true variation in measurements (see Figures 5.1 to 5.6). For the absolute and relative measurements of transparent area and length, the standard deviations are quite large, being on average roughly half that of the mean values. Yet, this is not unexpected given the range of ages for which measurements have been made.

It is of considerable interest that the distribution of the volumetric measurements of transparency, VT and F4, appear distinct from the other measurements of transparency. The range of observed values for VT appears quite broad, ranging from 42.71 to 181.03 mm^3. Accordingly, the range of values for the index of this measure, in which the total volume of transparency is expressed as a proportion of total root dentine volume, also

appears large, ranging from 0.24 to 0.82. The maximum proportion of transparent root volume is nearly double that for transparent area (F1) and roughly 30% greater than the index of transparent root length (F3). Furthermore, although both area and length measurements assume zero values the same is never true of either VT or F4.

With the exception of F1, all of the variables within this sample appear normally distributed, an observation which is empirically supported by both the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality. For F1, a skewness value of -0.401 indicates a negatively skewed distribution, suggesting that there are a disproportionate number of observations which fall below the mean ($p < 0.05$). An analysis of the frequency histogram for this variable indicates a strongly bimodal distribution (see Figure 5.2). The scarcity of observations towards the mean may reflect the age distribution of the sample from which measurements were taken or the relatively small sample size. However, the range of variation in overall root dimensions, which are known to vary by tooth type, may also have exerted a significant influence given that such bimodality is not apparent for absolute measurements of the same parameter (ATR) made on the same teeth.

	ATR (mm^2)	F1 (%)	TL (mm)	F3 (%)	VT (mm^3)	F4
Mean	17.61	24.95	4.15	28.74	112.39	0.478
Std. Error of Mean	1.766	2.55	0.46	3.13	6.38	0.03
Median	20.47	30.18	4.17	28.85	109.24	0.48
Mode	0.00	0.00	0.00	0.00	42.71	0.24
Std. Deviation	9.99	14.40	2.63	17.69	36.10	0.14
Variance	99.80	207.39	6.89	312.81	1302.95	0.02
Skewness	-0.45	-0.40	-0.04	-0.191	-0.11	0.33
Std. Error of Skewness	0.41	0.414	0.41	0.414	0.41	0.41
Kurtosis	-0.77	-0.928	-0.83	-1.079	-0.64	-0.17
Std. Error of Kurtosis	0.81	0.809	0.81	0.809	0.81	0.81
Range	34.57	46.73	9.68	56.77	138.32	0.58
Minimum	0.00	0.00	0.00	0.00	42.71	0.24
Maximum	34.57	46.73	9.68	56.77	181.03	0.82

Table 5.2 Descriptive statistics for the recently-extracted tooth sample (N=32).

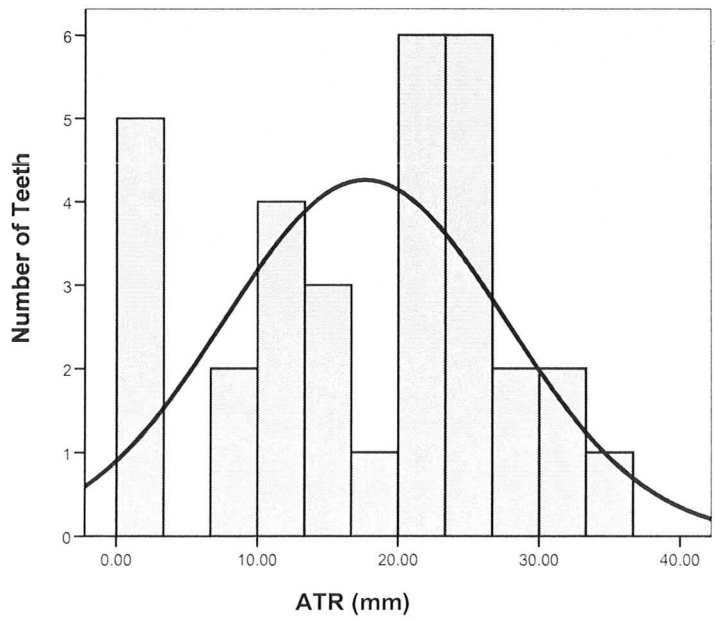

Figure 5.1 Frequency histogram illustrating the distribution of absolute measurements of transparent area (ATR) for the recently-extracted tooth sample.

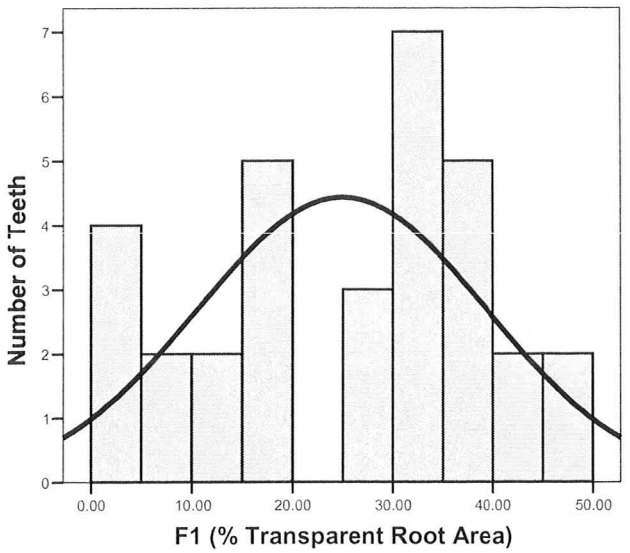

Figure 5.2 Frequency histogram illustrating the distribution of the scaled index of the area of transparent root dentine (F1) for the recently-extracted sample of teeth. Note the distinctly bi-modal distribution

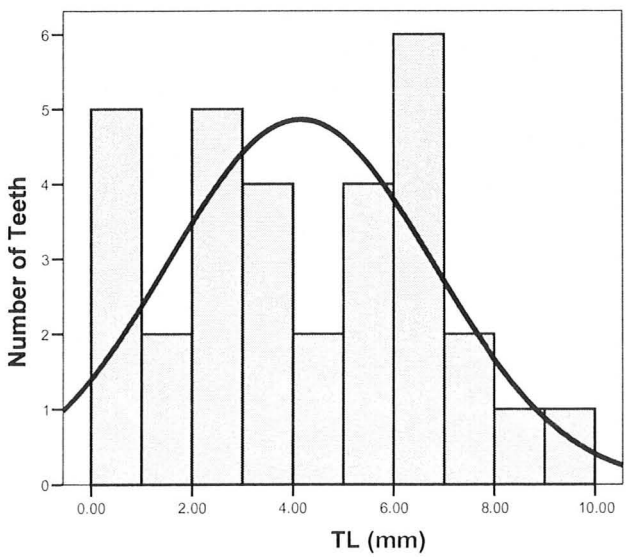

Figure 5.3 Frequency histogram illustrating the distribution of absolute measurements of transparent length (TL) for the recently-collected sample of teeth.

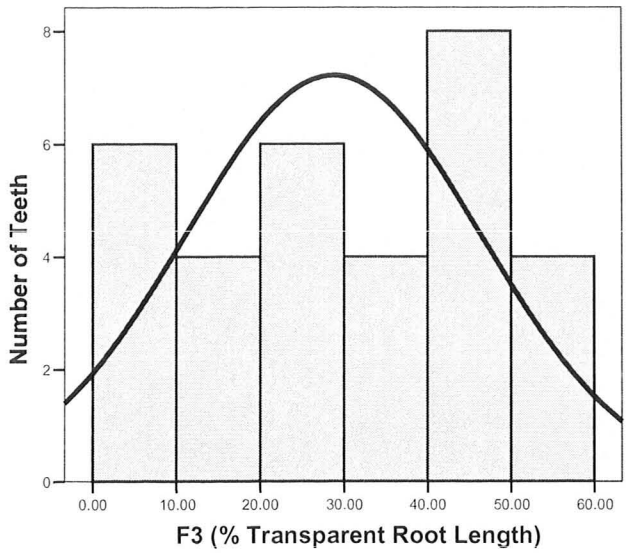

Figure 5.4 Frequency histogram illustrating the distribution of the scaled index of transparent root length (F3) for the recently-collected sample of teeth.

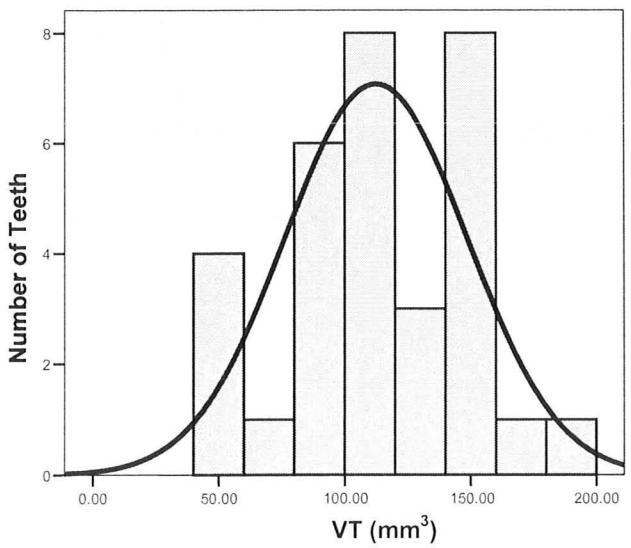

Figure 5.5 Frequency histogram illustrating the distribution of absolute measurements of transparent volume (VT) for teeth of the recently-extracted sample.

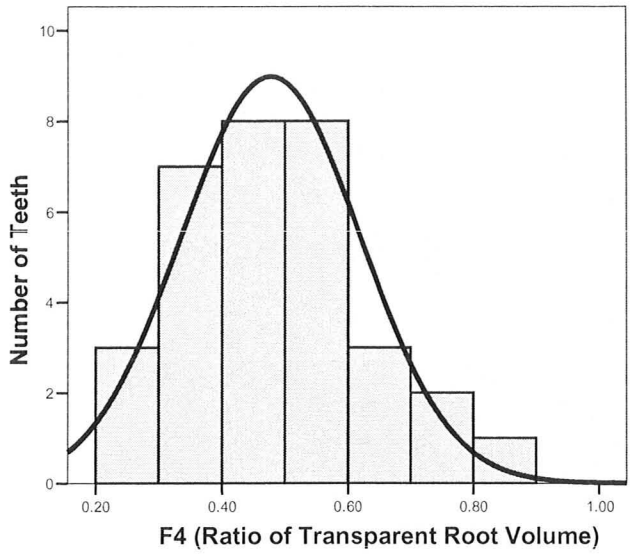

Figure 5.6 Frequency histogram illustrating the distribution of the scaled index of transparent volume (F4) for the recently-extracted tooth sample.

St. Thomas's Sample

The descriptive statistics for this sample are summarized in Table 5.3. In general, the means and observed range of values for the area and length measurements of transparency appear similar to those for the recently-extracted sample. Once again, the standard deviations for all of the variables are extremely large. Yet, as was the case for the recently-extracted sample of teeth, this is likely a reflection of the broad age range of the teeth from which the data were collected.

In contrast to the recent sample, the results of the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality indicate that both absolute and relative measurements of transparent area are normally distributed ($p < 0.05$). However, both TL and F3 display a non-normal distribution, a finding which is not surprising given the skewness values for these variables. An examination of the frequency histograms for TL and F3 (see Figure 5.9 and 5.10) reveals a large number of observations for which the length of transparency falls below the mean. Accordingly, the median values for the linear measurements of

transparency are considerably lower than the mean, indicating the presence of outlier values. The cause of this is unclear; however, the mean age of the sample, 46.97 years, is lower than that for the recent sample.

	Age (yrs)	ATR (mm^2)	F1 (%)	TL (mm)	F3 (%)
Mean	46.97	18.74	30.54	4.25	31.93
Std. Error of Mean	2.85	1.96	3.38	0.53	4.24
Median	48.50	18.47	30.82	3.30	20.02
Mode	60.00	1.88	1.74	0.67	5.81
Std. Deviation	16.12	11.09	19.10	2.98	23.96
Variance	259.77	123.06	364.99	8.87	574.16
Skewness	-0.27	0.14	0.44	0.87	0.92
Std. Error of Skewness	0.41	0.41	0.41	0.41	0.41
Kurtosis	-0.90	-1.11	-0.50	-0.51	-0.35
Std. Error of Kurtosis	0.81	0.81	0.81	0.81	0.81
Range	58.00	37.82	73.12	10.17	80.35
Minimum	17.00	1.88	1.74	0.67	5.81
Maximum	75.00	39.69	74.86	10.85	86.16

Table 5.3 Descriptive statistics for the St. Thomas' Anglican Church cemetery sample (N=32).

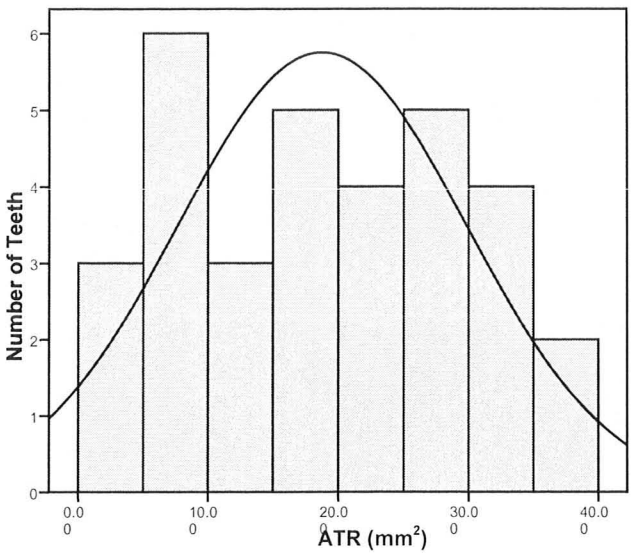

Figure 5.7 Frequency histogram illustrating the distribution of absolute measurements of transparent area (ATR) for the St. Thomas' Anglican Church cemetery sample.

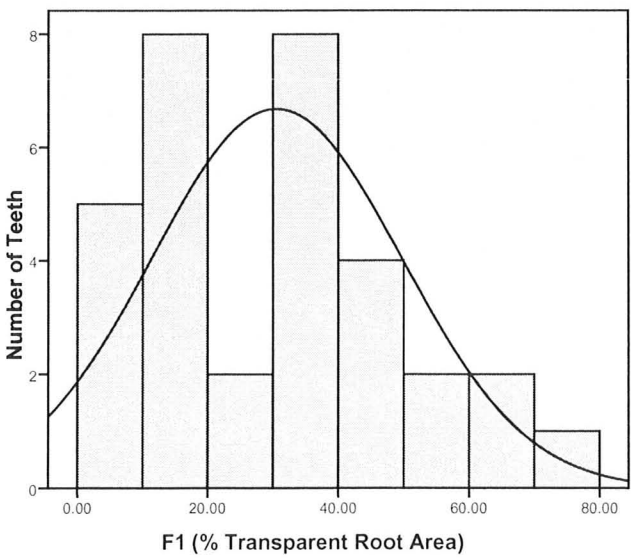

Figure 5.8 Frequency histogram illustrating the distribution of a scaled index of the area of transparent root dentine (F1) for the St. Thomas' Anglican Church cemetery sample.

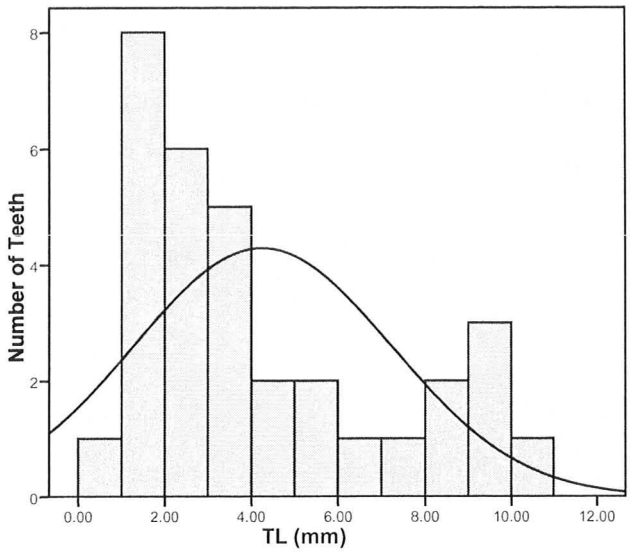

Figure 5.9 Frequency histogram illustrating the distribution of absolute measurements of transparent length (TL) for the St. Thomas' Anglican Church cemetery sample.

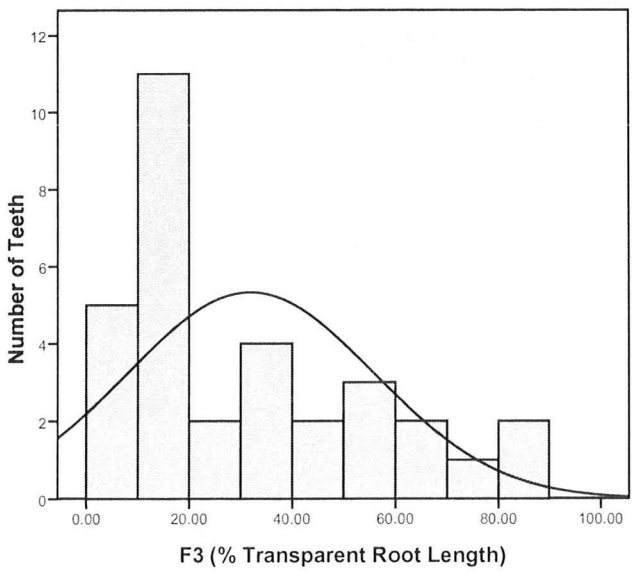

Figure 5.10 Frequency histogram illustrating the distribution of the scaled index of transparent root length (F3) for the St. Thomas' Anglican Church cemetery sample.

5.4 The Association of Transparency with Chronological Age

Recent Sample

Figures 5.11 through 5.16 illustrate the association with chronological age for each of the measurements of transparency for the recently-extracted sample. An examination of these scatterplots reveals that for both area and linear measurements of transparency, there is a positive, roughly linear relationship with age. With advancing age, there appears to be an increase in the amount of transparent root dentine. An obvious exception to this trend is apparent for the volumetric measurements of transparency, for which there appears to be no clear pattern of increase. In fact, the association between the absolute volume of transparency (VT) and age appears to have a slightly negative slope.

Although a positive linear relationship appears to exist between age and measurements of transparent area and length, there is a large amount of variation in the rate of increase. At any given age, there is a broad range of observed transparency values. Most notably, for the relative index of area (F1), at the age of roughly 60 years, the array of corresponding values appears quite broad, ranging from 15 to 45% (see Figure 5.12). Furthermore, for both area and linear measurements of transparency, several outliers are evident. Most prominent is a tooth from an individual of 79 years that appears to have far less transparent area (both in absolute and relative terms) than would be expected based on the pattern displayed by the remaining teeth (see Figure 5.11 and 5.12). An examination of the unmodified image of the particular tooth in question reveals an area of pronounced opacity within the dentine immediately surrounding the pulp cavity (see Figure 5.17). Given the anomalous nature of this tooth and the extremely irregular values of the measurements, it associated data were removed from the correlation and regression analyses. Accordingly, the statistical discussion that follows is based on a reduced sample of 31 specimens.

Table 5.4 presents the results of the nonparametric tests of association for each of the variables under examination. As noted above, not all of the variables are normally distributed. Attempts to transform the data to obtain normality were unsuccessful. It was

therefore necessary to perform ranked correlation analyses rather than parametric tests of association in order that comparisons could be made between all of the variables under examination. The results of these analyses indicate that each of the variables based upon area and linear measurements of transparency displays a significant correlation with age ($p < 0.01$). When all of the variables are compared, the highest correlations are apparent for the area measurements of transparency. The coefficient for ATR and F1 are 0.681 and 0.632 respectively. However, the correlation coefficient for TL is also high ($r = 0.629$). For both area and linear measurements, variables based on unscaled measurements of transparency show higher correlations with age than do their associated indices. In agreement with the visual assessment of their associated scatterplots, there is not a significant correlation between age and transparent volume, either for the absolute or relative measurements. The values of volumetric measurements of transparency do not appear to increase with age.

		Age (years)	ATR (mm^2)	F1 (%)	TL (mm)	F3 (%)	VT (mm^3)	F4
Age	Correlation Coefficient	1.000	0.681**	0.632**	0.629**	.613**	-0.050	0.142
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.788	0.447
ATR	Correlation Coefficient	0.681**	1.000	0.790**	0.852**	0.779**	0.160	0.085
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.391	0.651
F1	Correlation Coefficient	0.632**	0.790**	1.000	0.783**	0.861**	0.085	0.339
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.648	0.062
TL	Correlation Coefficient	0.629**	0.852**	0.783**	1.000	0.962**	0.165	0.091
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.374	0.628
F3	Correlation Coefficient	0.613**	0.779**	0.861**	0.962**	1.000	0.076	0.150
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.684	0.419
VT	Correlation Coefficient	-0.050	0.160	0.085	0.165	0.076	1.000	0.533**
	Sig. (2-tailed)	0.788	0.391	0.648	0.374	0.684	0.000	0.002
F4	Correlation Coefficient	0.142	0.085	0.339	0.091	0.150	0.533**	1.000
	Sig. (2-tailed)	0.447	0.651	0.062	0.628	0.419	0.002	0.000

Table 5.4 Spearman rank correlations for the recently-extracted sample of teeth. Significant correlations have been highlighted. Significant associations (2-tailed) are identified at the 0.05 level (*) and the 0.01 level (**). Note that significant correlations are apparent between a number of the variables, indicating significant inter-correlation of the measures.

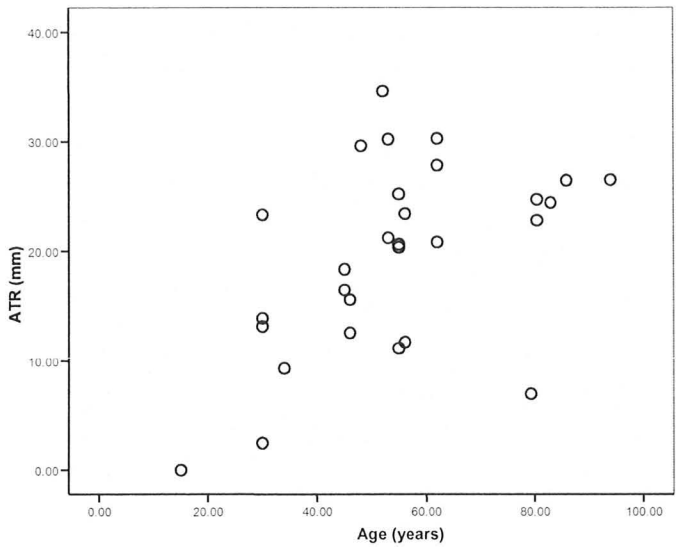

Figure 5.11 Scatterplot illustrating the association between ATR and chronological age for the recently-extracted tooth sample. A roughly positive linear relationship exists between the two variables. Note the persistence of an outlier specimen (age = 79 years) across all area and linear measurements of transparency. This specimen was later removed from the pooled sample.

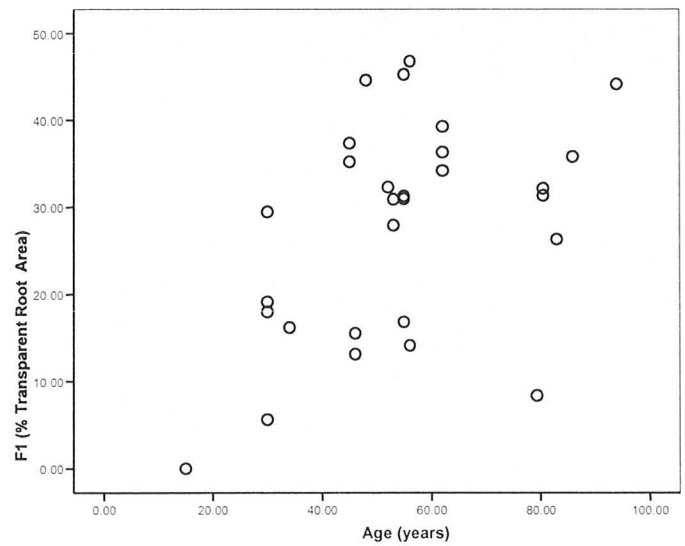

Figure 5.12 Scatterplot illustrating the association between F1 and chronological age for the recently-extracted tooth sample. The strength of the association appears poorer than for ATR.

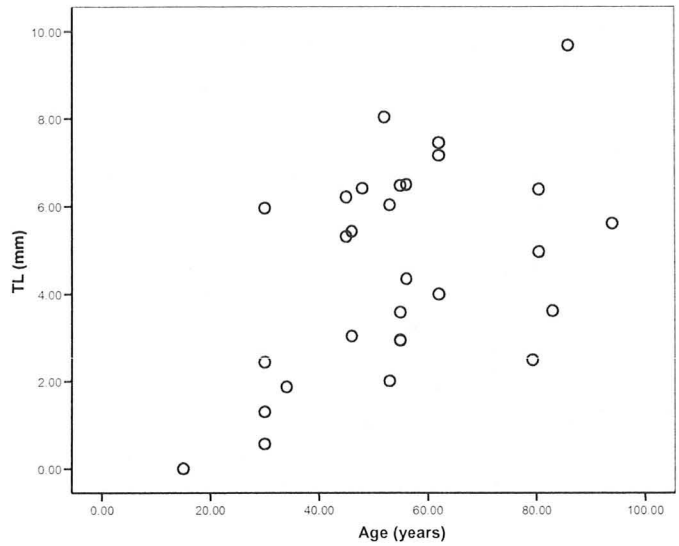

Figure 5.13 Scatterplot illustrating the association between TL and chronological age for the recently-extracted tooth sample. Similar to ATR and F1, a positive linear relationship with age appears to exist. However, there is a greater variance in measurement values with increasing age.

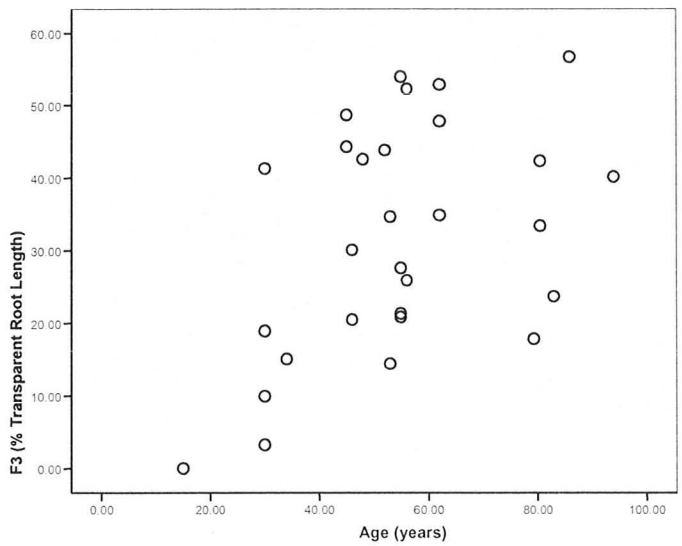

Figure 5.14 Scatterplot illustrating the association between F3 and chronological age for the recently-extracted tooth sample. A positive relationship between the two variables is evident. Yet, just as for the index of transparent root area, a greater variability is evident with increasing age, making the exact nature of the relationship uncertain.

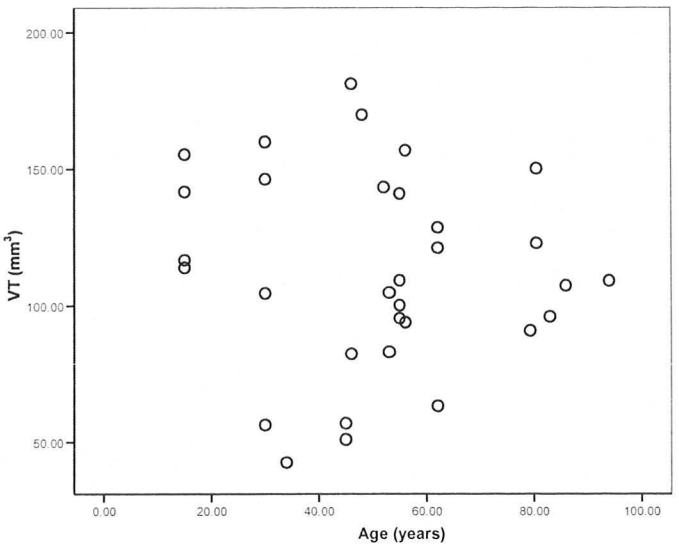

Figure 5.15 Scatterplot illustrating the association between VT and chronological age for the recently-extracted tooth sample. Unlike for the area and linear measurements of transparency, no clear relationship appears to exist. Some of the highest measurements are recorded in the youngest age groups and there is a wide range of observed values for any given age.

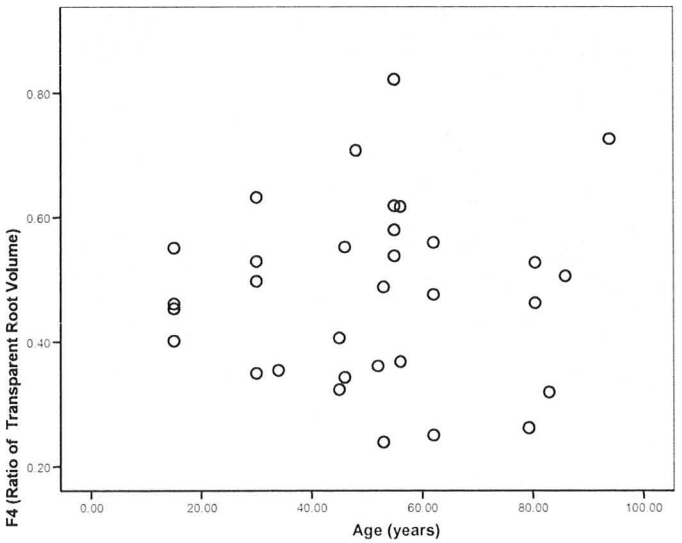

Figure 5.16 Satterplot illustrating the association between F4 and chronological age for the recently-extracted sample of teeth. Scaling for overall root dimensions does not appear to improve the regularity of the association. However, the range of observed values is decreased.

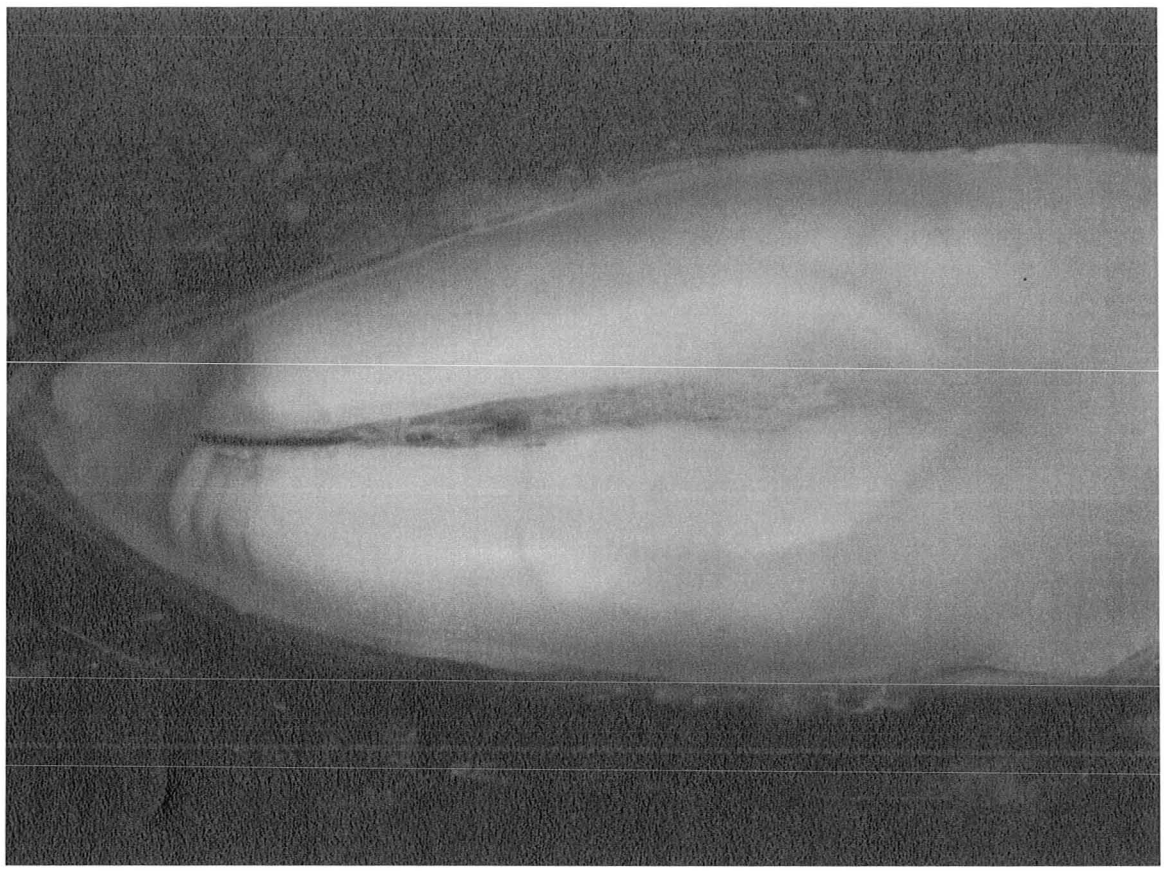

Figure 5.17 Stereomicroscope image of specimen GAL002 captured under polarized light. Note the peri-puplal area of increased opacity within the root dentine.

St. Thomas' Sample

Figures 5.18 through 5.21 illustrate the associated ages and measurements of transparency for the St. Thomas' Anglican Church cemetery sample. As was the case for the sample of teeth derived from living subjects, there appears to be a positive, linear relationship between age and transparency. This is true for both absolute and relative measurements of transparency. Based on an examination of the scatterplots, there appears to be slightly less deviation in the St. Thomas' sample than in the recent sample. The increase in the amount of transparency with advancing age appears more distinctly linear. With the exception of a 60-year-old individual for whom there is considerably less transparency than expected, there do not appear to be any other obvious outliers (see

Figure 5.19). The general shape of the association with age is similar for both area and linear measurements of transparency.

Table 5.5 displays the results of the nonparametric tests of the association between each of the variables under study for the St. Thomas' sample. When the Spearman correlation coefficients are compared, it is evident that there are significant correlations with chronological age for each of the variables ($p < 0.01$). The highest correlation is found for ATR ($r = 0.722$).The lowest correlation with age is found for F3 ($r = 0.636$). Two observations seem important. First, as was observed for the recently-extracted sample of teeth, a closer correlation is evident for measurements of area than for measurements of transparent length. ATR, an unscaled measurement of transparent area, exhibits the closest association with age. Secondly, for both area and linear measurements of transparency, the association with age is weaker for the corresponding scaled indices than for the raw values from which those scaled figures were derived.

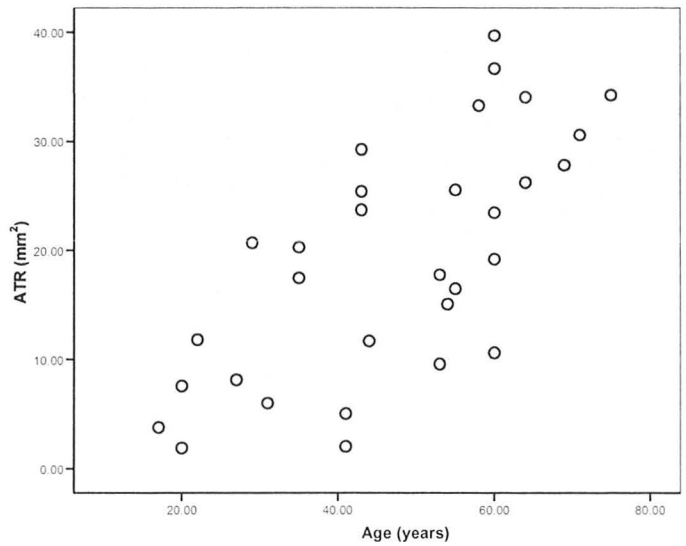

Figure 5.18 Scatterplot illustrating the association between ATR and chronological age for the St. Thomas' Anglican Church cemetery sample. A more distinctly linear relationship with age is evident than for the recent sample. However, there is a wider range of variance in values towards the middle of the age distribution.

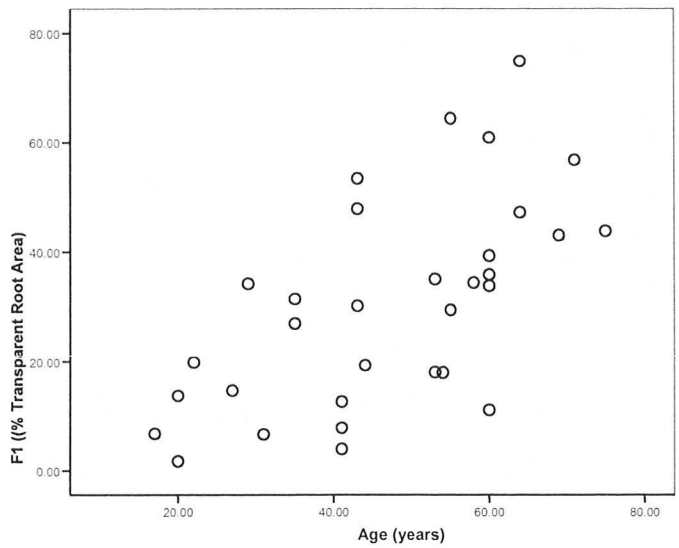

Figure 5.19 Scatterplot illustrating the association between F1 and chronological age for the St. Thomas' Anglican Church cemetery sample. No improvement in the association is evident.

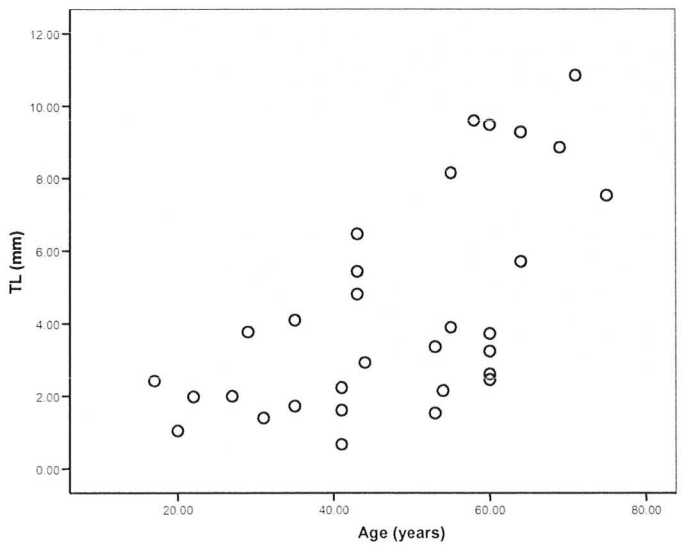

Figure 5.20 Scatterplot illustrating the association between TL and chronological age for the St. Thomas' Anglican Church cemetery sample. A strong positive correlation is evident.

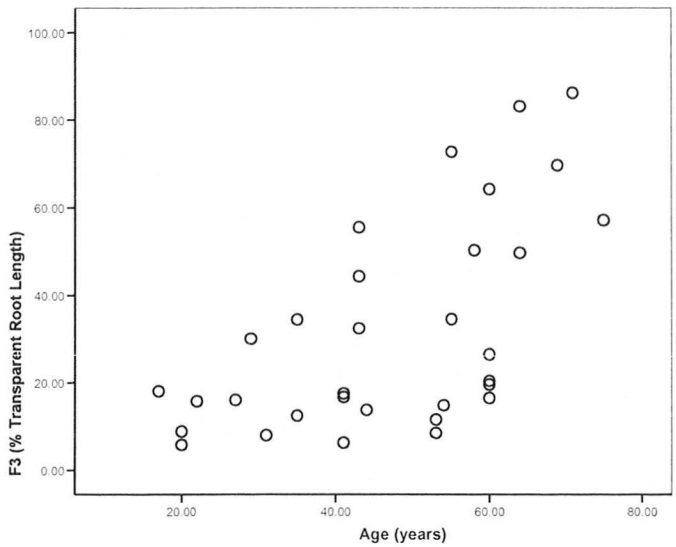

Figure 5.21 Scatterplot of the association between F3 and chronological age for the St. Thomas's Anglican Church cemetery sample.

		Age (years)	ATR (mm2)	F1 (%)	TL (mm)	F3 (%)
Age	Correlation Coefficient	1.000	0.722**	0.673**	0.683**	0.636**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000
ATR	Correlation Coefficient	0.722**	1.000	0.929**	0.860**	0.777**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000
F1	Correlation Coefficient	0.673**	0.929**	1.000	0.857**	0.803**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000
TL	Correlation Coefficient	0.683**	0.860**	0.857**	1.000	0.929**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000
F3	Correlation Coefficient	0.636**	0.777**	0.803**	0.929**	1.000
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000

Table 5.5 Spearman rank correlations for the St. Thomas' sample of teeth. Significant associations (2-tailed) are identified at the 0.01 level (**). As for Table 5.3, significant inter-variable associations are apparent. This is to be expected given that each is measuring the same phenomenon.

5.5 An Exploration of the Pooled Sample

Mann-Whitney and Wilcoxon W tests were performed to compare the means for each of the variables in both of the samples. The results of these non-parametric tests are summarized in Table 5.6. There appears to be no significant difference between the mean values of the two samples for any of the variables. Based on these results, the samples were pooled in order to examine the association between measurements of transparency and chronological age for a larger number of specimens.

The association with age for each of the measurements of transparency is provided in Table 5.7. Once again, ATR has the highest correlation coefficient at 0.711, followed by TL at 0.695. Both of these associations are significant ($p < 0.01$). The degree of variation between the correlations appears relatively small. The lowest correlation with age is reported for F1 ($r = 0.651$). This pattern of association is similar to that seen for both samples when considered separately. Area measurements appear more closely correlated with age than do linear measurements of transparency. For both area and linear measurements, absolute values show a closer correlation with age than do the same values when they are expressed as a proportion of overall root size.

Once again, an inspection of the scatterplots for each of the measurements of transparency plotted against chronological age reveals a positive, roughly linear pattern of increase in transparency with age (see Figures 5.22 to Figure 5.25). This pattern appears to persist across all forms of measurement. However, it is clear that there is a high degree of variation within this relationship, the magnitude of which also increases with age. This increased variance in observed values of transparency with age gives each of the graphs a funnel-shaped appearance.

Figures 5.26 through 5.31 illustrate the simple least-squares regression lines for each of the above scatterplots. Table 5.8 provides both the equation of the line and the associated R^2 value for each of these graphs. R^2 values range from 0.49 for ATR to 0.36 for F3. In keeping with the results of the analysis of correlation, the best fit is approximated for area measurements of transparency. Despite strong correlation

coefficients, these values indicate that less than half of the total variance in the measurements of transparency can be attributed to an increase in age. For VT and F4 the R^2 values approach zero, indicating that there is almost no degree of association with age for either variable (see Figure 5.28 and 5.29). The slopes of the regression lines indicate that there is no increase in either measurement with age. In fact, the regression line for VT appears to have a slightly negative slope.

For all of the regression equations, the standard error of the estimates appear high (see Table 5.8). Although each of the absolute measurements is made in different units, a comparison can be made between them by an examination of the scaled indices. For F1 and F3, the standard errors are +/-13.42 and 16.93% respectively, while the ratio of transparent volume to total root volume has a standard error of +/-0.140

	Age	ATR	F1	TL	F3
Mann-Whitney U	472.000	483.000	431.000	475.000	491.000
Wilcoxon W	1000.000	979.000	927.000	1003.000	1019.000
Z	-0.330	-0.179	-0.894	-0.289	-0.069
Asymp. Sig. (2-tailed)	0.741	0.858	0.371	0.773	0.945

Table 5.6 Mann-Whitney U and Wilcoxon W tests for differences between the recently-extracted and St. Thomas' tooth samples. The results of both tests indicate no significant differences for any of the variables.

		Age (years)	ATR (mm^2)	F1 (%)	TL (mm)	F3 (%)	VT (mm^3)	F4
Age	Correlation Coefficient	1.000	0.711**	0.651**	0.695**	0.663**	-0.050	0.142
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.788	0.447
ATR	Correlation Coefficient	0.711**	1.000	0.881**	0.863**	0.826**	0.160	0.085
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.391	0.651
F1	Correlation Coefficient	0.651**	0.881**	1.000	0.823**	0.842**	0.085	0.339
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.648	0.062
TL	Correlation Coefficient	0.695**	0.863**	0.823**	1.000	0.966**	0.165	0.091
	Sig. (2-tailed)	0.000	0.000	0.000	0.00	0.000	0.374	0.628
F3	Correlation Coefficient	0.663**	0.826**	0.842**	0.966**	1.000	0.076	0.150
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.684	0.419
VT	Correlation Coefficient	-0.050	0.160	0.085	0.165	0.076	1.000	0.533**
	Sig. (2-tailed)	0.788	0.391	0.648	0.374	0.684	0.000	0.002
F4	Correlation Coefficient	0.142	0.085	0.339	0.091	0.150	0.533**	1.000
	Sig. (2-tailed)	0.447	0.651	0.062	0.628	0.419	.0002	0.000

Table 5.7 Spearman rank correlations for the pooled sample of teeth. Significant correlations (2-tailed) are identified for $p<0.01$ (**).

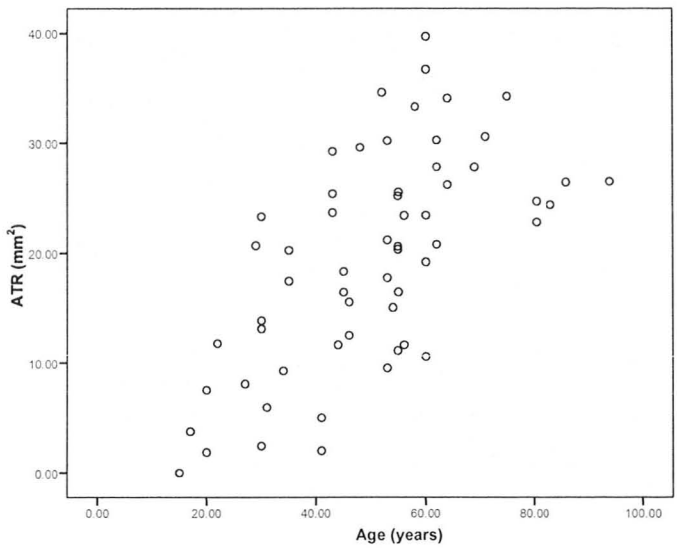

Figure 5.22 Scatterplot illustrating the association between ATR and chronological age for the pooled tooth sample. A distinctly positive association is observable. However, rather than a linear relationship, the increasing variance in measured values with age gives the graph a funnel-shaped appearance.

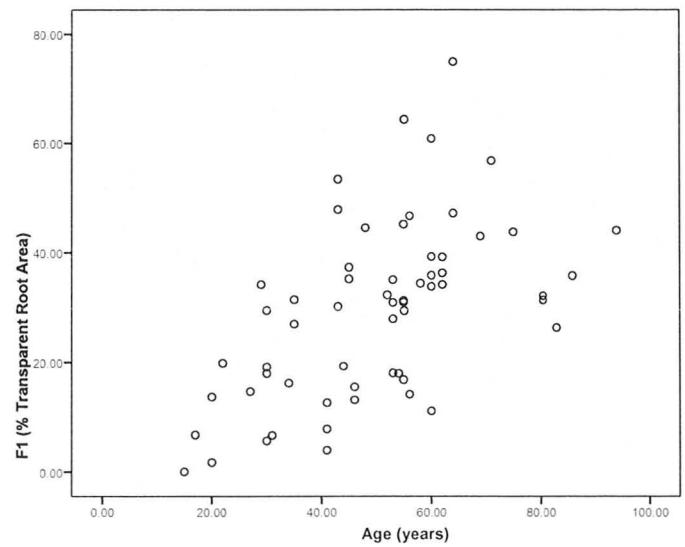

Figure 5.23 Scatterplot illustrating the association between F1 and chronological age for the pooled sample. No marked change in the shape of the graph is observed for the scaled measure of area.

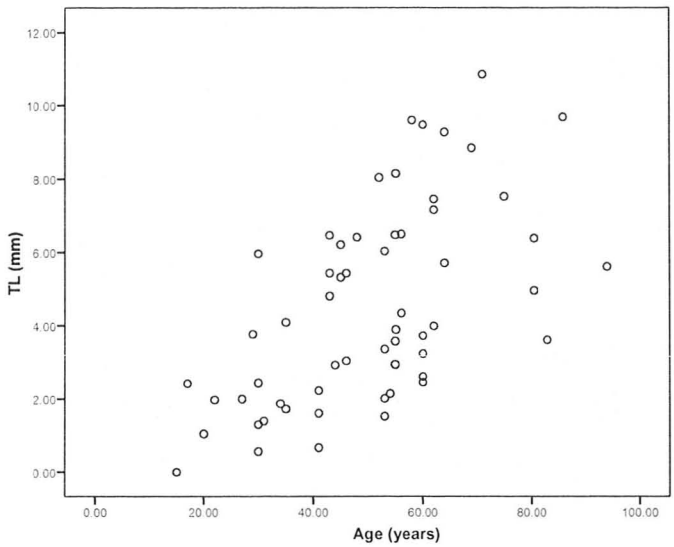

Figure 5.24 Scatterplot illustrating the association between TL and chronological age for the pooled sample. The slope of the graph appears shallower than for both of the area variables. However, the funnel shape is once again apparent.

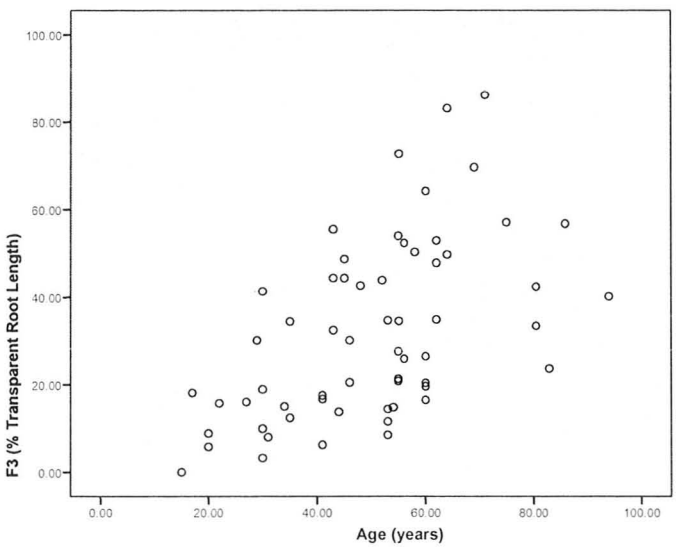

Figure 5.25 Scatterplot illustrating the association between F3 and chronological age for the pooled sample. No improvement is apparent for the scaled index of transparent length.

Variable	Equation of the Line	R	R^2	Adjusted R^2	Std. Error of the Estimate
ATR (mm^2)	-0.485 + 2.654X	0.698	0.487	0.478	7.564
F1 (%)	0.911 + 0.561X	0.620	0.385	0.375	13.424
TL (mm)	-0.477 + 0.097X	0.652	0.425	0.415	2.140
F3 (%)	-2.225 + 0.677X	0.603	0.364	0.354	16.933
VT (mm^3)	117.599 - 0.091X	0.053	0.003	-0.032	37.047
F4	0.445 +7.96E-04X	0.122	0.015	-0.019	0.140

Table 5.8 Model summaries for the linear regressions of measurements of transparency against chronological age for the pooled sample of teeth. Age has been identified as the independent variable.

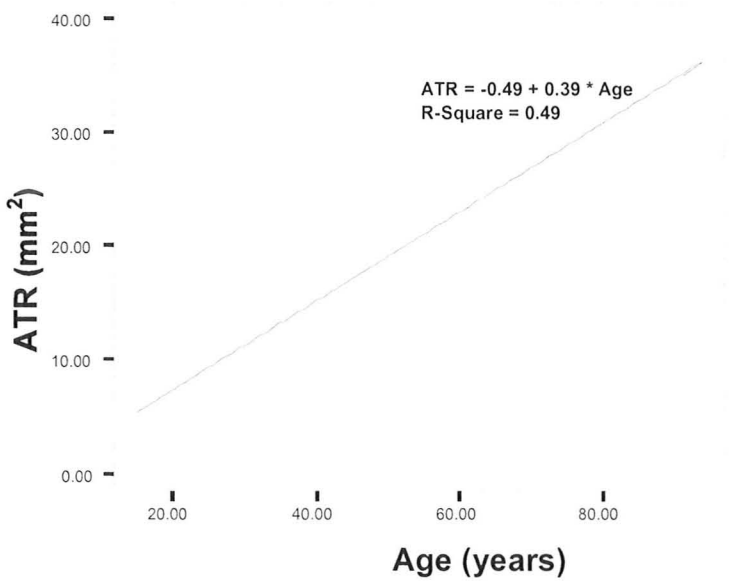

Figure 5.26 Linear regression equation for ATR as a function of age for the pooled sample. This line has the best fit with the observed measurement values for any of the variables.

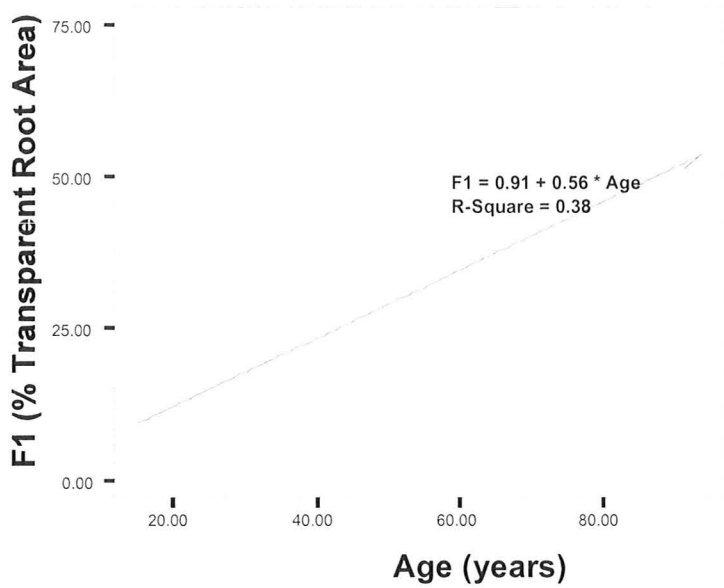

Figure 5.27 Linear regression equation for F1 as a function of age for the pooled sample.

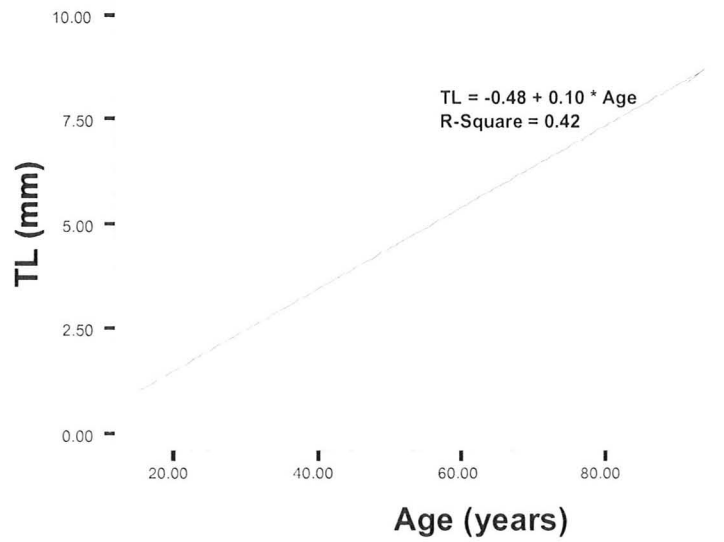

Figure 5.28 Linear regression equation for TL as a function of age for the pooled sample.

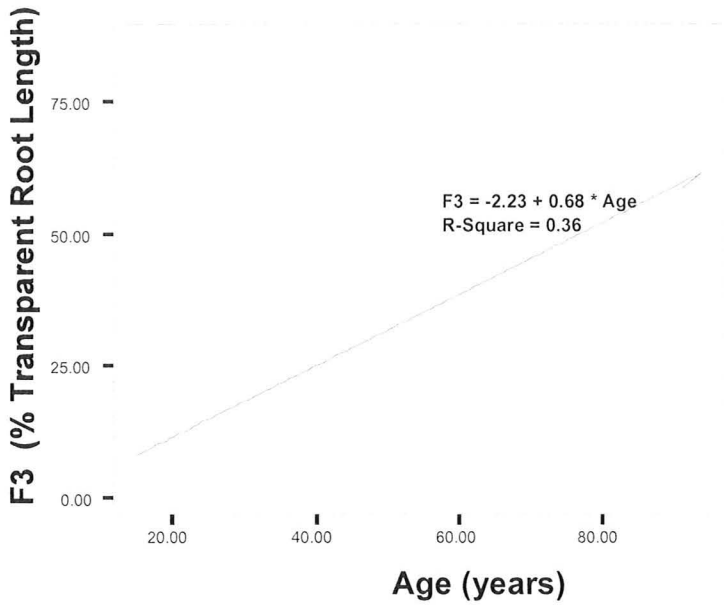

Figure 5.29 Linear regression equation for F3 as a function of age for the pooled sample.

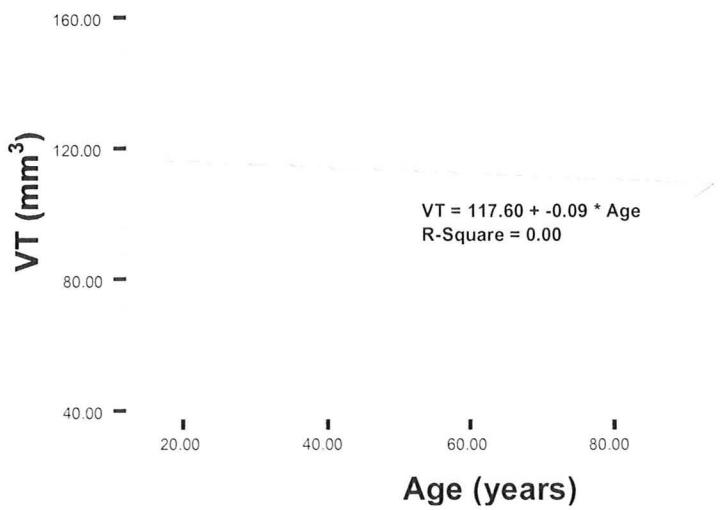

Figure 5.30 Linear regression equation for VT as a function of age for the pooled sample. The strength of the association is extremely poor.

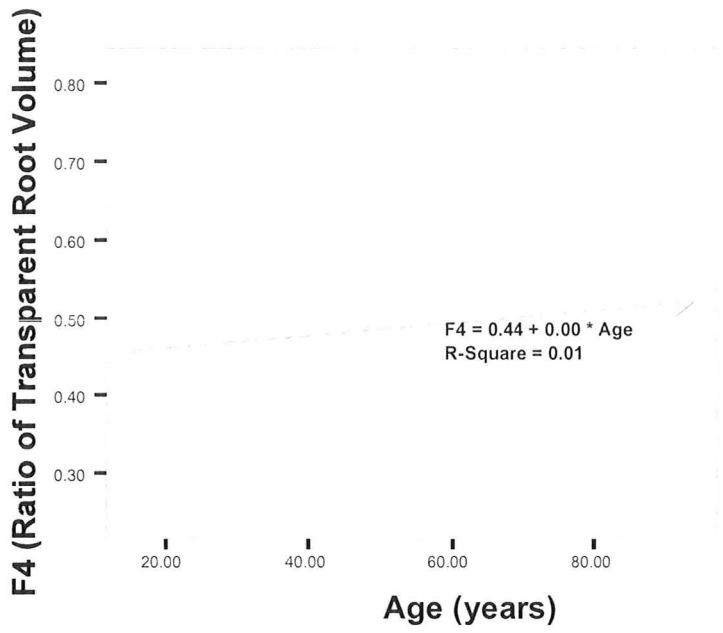

Figure 5.31 Linear regression equation for F4 as a function of age for the pooled sample. The slope of this line is nearly zero, indicating an extremely poor association between the two variables. The associated R^2 value indicates that only 1% of the variance in F4 can be accounted for by changes in age.

5.6 The Influence of Sex and Tooth Category

Partial correlations for which the influence of both sex and tooth category were controlled for were performed in order to assess the influence that each factor has on the observed correlations between age and measurements of transparency. Table 5.9 displays the partial correlations between age and each of the measurements of transparency when sex was held constant. When compared to the original Spearman rank correlations, it is evident that there is a decrease in the observed correlations with chronological age for all of the variables. On average, there is a roughly 10% decrease in the strength of the observed associations. An exception to this is seen for VT and F4, for which the magnitude of the decrease is less. However, note that the degree of decrease in the correlations never threatens the significance of the associations with chronological age,

except for the volumetric variables for which significance was not previously observed ($p < 0.01$).

Table 5.10 provides the partial correlation data for each of the variables when tooth category was held constant. An inspection of this table reveals that the effect on the observed correlations is less than that exerted by sex. On average, there is only a 2-3% decrease in the associations with age. An obvious exception is seen for ATR for which the strength of the correlation with age is improved when the influence of tooth category is controlled for. Once again, the observed associations with age for area and linear measurements of transparency are still significant ($p < 0.01$).

		ATR (mm2)	F1 (%)	TL (mm)	F3 (%)	VT (mm3)	F4	Age (years)
ATR	Correlation	1.000	0.875	0.834	0.789	0.144	0.092	0.686
	Significance (2-tailed)	0.000	0.000	0.000	0.000	0.448	0.629	0.000
F1	Correlation	0.875	1.000	0.808	0.870	0.051	0.334	0.608
	Significance (2-tailed)	0.000	0.000	0.000	0.000	0.791	0.071	0.000
TL	Correlation	0.834	0.808	1.000	0.956	0.134	0.080	0.644
	Significance (2-tailed)	0.000	0.000	0.000	0.000	0.482	0.674	0.000
F3	Correlation	0.789	0.870	0.956	1.000	0.063	0.163	0.596
	Significance (2-tailed)	0.000	0.000	0.000	0.000	0.743	0.391	0.000
VT	Correlation	0.144	0.051	0.134	0.063	1.000	0.496	-0.030
	Significance (2-tailed)	0.448	0.791	0.482	0.743	0.000	0.005	0.873
F4	Correlation	0.092	0.334	0.080	0.163	0.496	1.000	0.118
	Significance (2-tailed)	0.629	0.071	0.674	0.391	0.005	0.000	0.535
Age	Correlation	0.686	0.608	0.644	0.596	-0.030	0.118	1.000
	Significance (2-tailed)	0.000	0.000	0.000	0.000	0.873	0.535	0.000

Table 5.9 Partial correlations for the pooled tooth sample controlling for the influence of sex.

		ATR (mm2)	F1 (%)	TL (mm)	F3 (%)	VT (mm3)	F4	Age (years)
ATR	Correlation	1.000	0.889	0.837	0.794	0.117	0.101	0.701
	Significance (2-tailed)	0.000	0.000	0.000	0.000	0.539	0.594	0.000
F1	Correlation	0.889	1.000	0.817	0.873	0.057	0.313	0.617
	Significance (2-tailed)	0.000	0.000	0.000	0.000	0.767	0.093	0.000
TL	Correlation	0.837	0.817	1.000	0.957	0.122	0.080	0.652
	Significance (2-tailed)	0.000	0.000	0.000	0.000	0.521	0.675	0.000
F3	Correlation	0.794	0.873	0.957	1.000	0.061	0.153	0.601
	Significance (2-tailed)	0.000	0.000	0.000	0.000	0.748	0.419	0.000
VT	Correlation	0.117	0.057	0.122	0.061	1.000	0.553	-0.039
	Significance (2-tailed)	0.539	0.767	0.521	0.748	0.000	0.002	0.838
F4	Correlation	0.101	0.313	0.080	0.153	0.553	1.000	0.106
	Significance (2-tailed)	0.594	0.093	0.675	0.419	0.002	0.000	0.576
Age	Correlation	0.701	0.617	0.652	0.601	-0.039	0.106	1.000
	Significance (2-tailed)	0.000	0.000	0.000	0.000	0.838	0.576	0.000

Table 5.10 Partial correlations for the pooled tooth sample controlling for the influence of tooth type.

5.7 Association of Volume with Other Measurements of Transparency

When qualitative comparisons are made between thin sections and their corresponding Micro-CT images at a similar depth within a given tooth, there is not always a strong similarity between the two. When one compares Figure 5.32 to Figure 5.33 it is clear that for specimen GAL007 there is a good degree of agreement between the micro-CT and stereomicroscope approximations of transparency. However, this does not hold true for the majority of cases. Figure 5.34 is a micro-CT slice for sample WIN001A oriented in the labio-lingual plane at approximately root center. The region identified as transparent is outlined in blue, extending from the root apex and periphery

towards the pulp cavity. Figure 5.35 is a stereomicroscope image of the prepared thin section of the same tooth. No transparency is visible.

The scatterplots illustrating the associations between the volumetric variables and the other measurements of transparency are provided in Figure 5.37 through Figure 5.43. Neither VT nor F4 are linearly related to area or linear measurements of transparency. Increases in absolute and relative measurements of volume are not met with regular increases in either linear or area measurements. The Spearman rank correlations for these associations are provided in Table 5.7. The strength of the correlations between volumetric measurements of transparency and area and linear measurements is not significant for any of the variables.

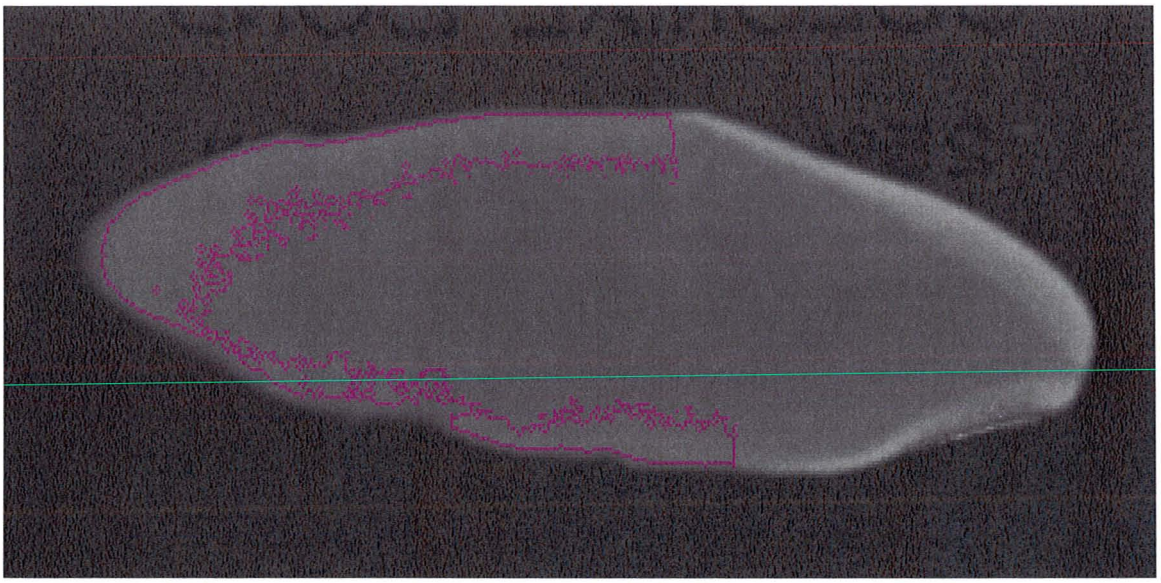

Figure 5.32 Micro-CT image of GAL007 oriented in the labio-lingual plane at mid-root. Transparency is outlined in purple.

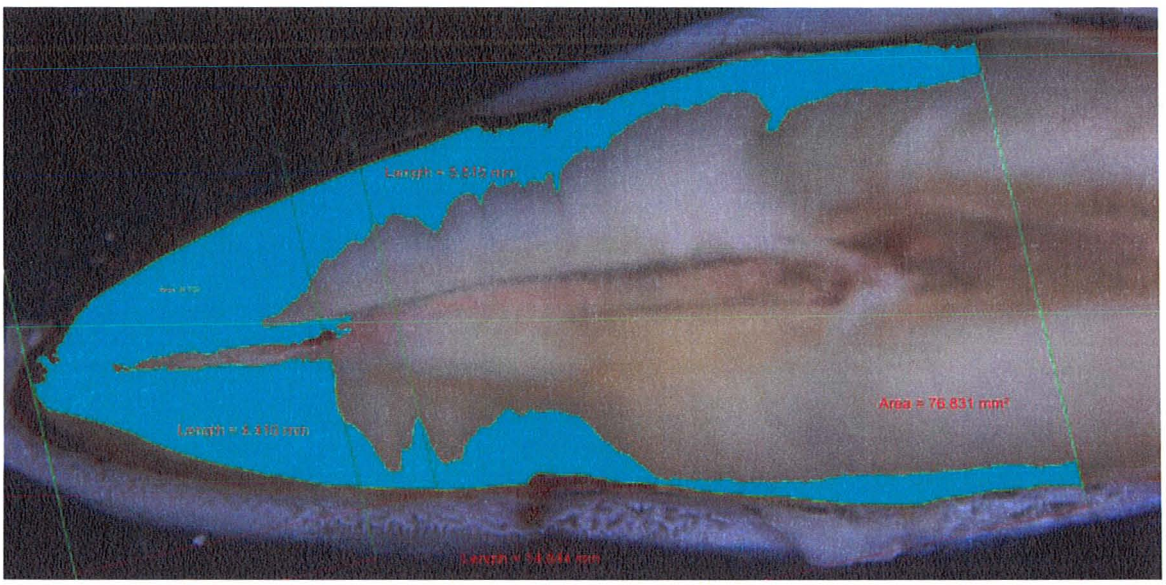

Figure 5.33 Stereomicroscope image of GAL007 illustrating the extent of transparency visible under polarized light.

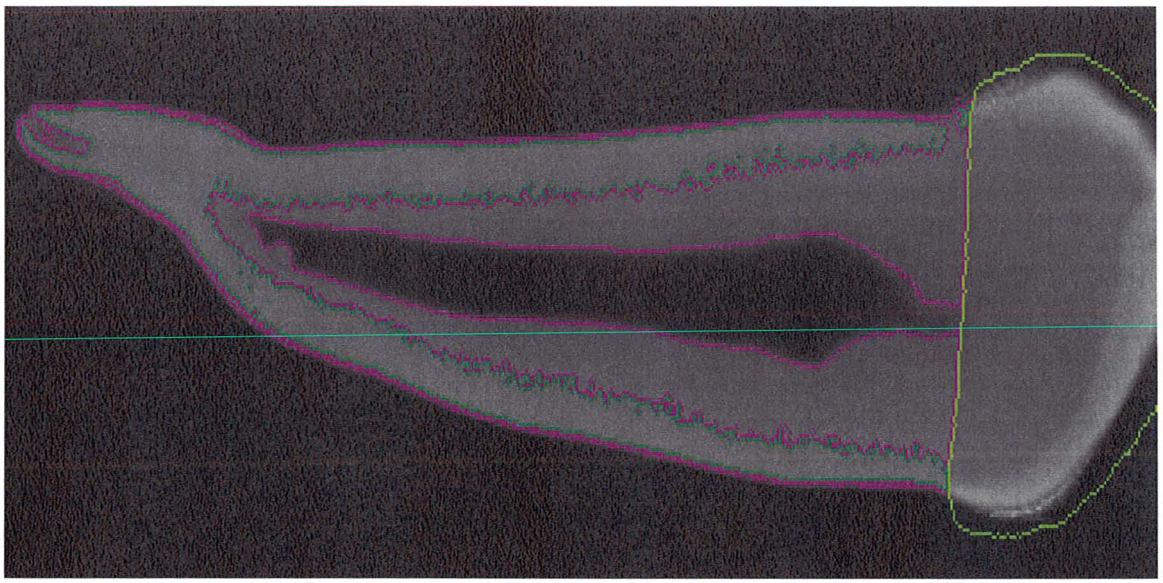

Figure 5.34 Micro-CT image of WIN001A oriented in the labio-lingual plane at approximately root center. The root is outlined in purple, while the area identified as transparent is outlined in green.

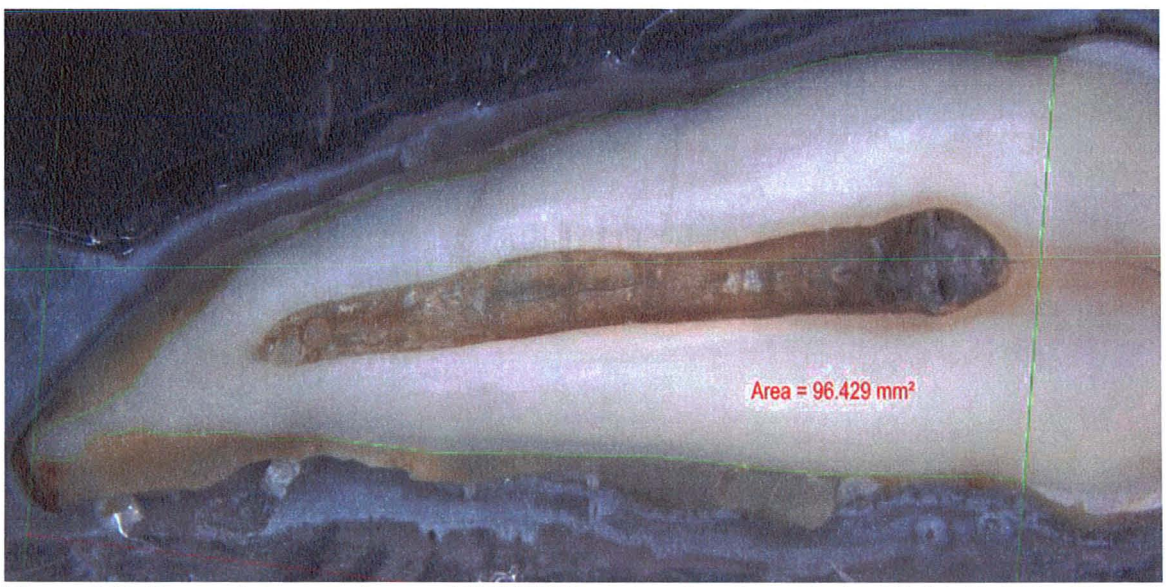

Figure 5.35 Stereomicroscope image of WIN001A captured under polarized light. Total length and root area have been measured. However, no root dentine transparency is visible for measurement.

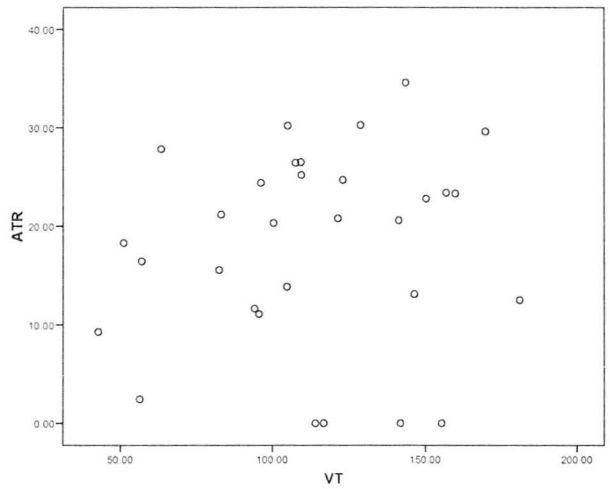

Figure 5.36 Scatterplot of ATR against VT for the recent sample

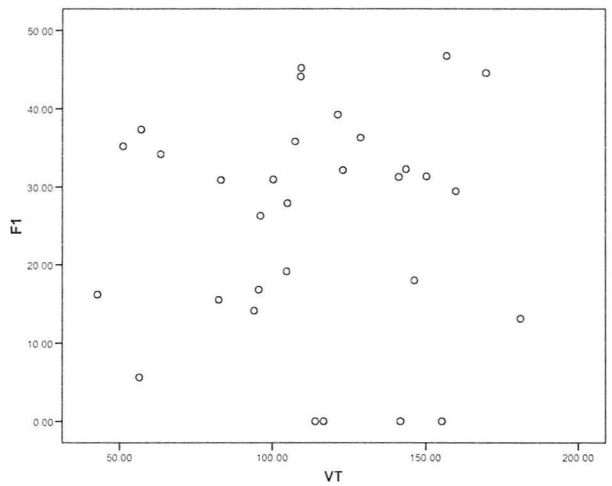

Figure 5.37 Scatterplot of F1 against VT for the recent sample.

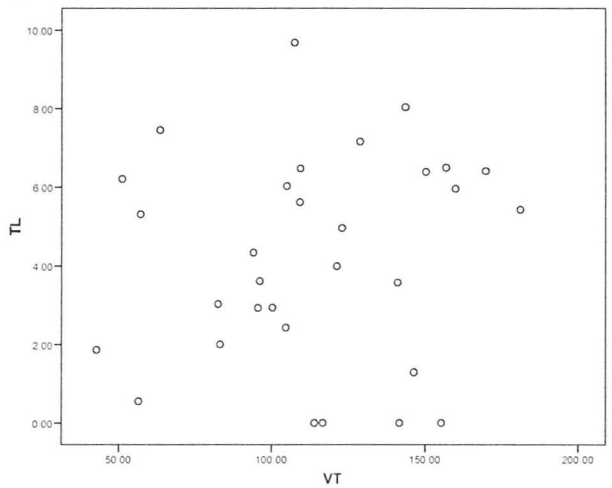

Figure 5.38 Scatterplot of TL against VT for the recent sample.

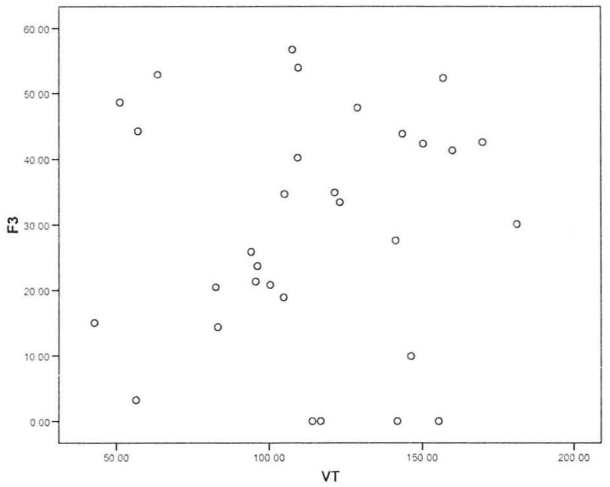

Figure 5.39 Scatterplot of F3 against VT for the recent sample.

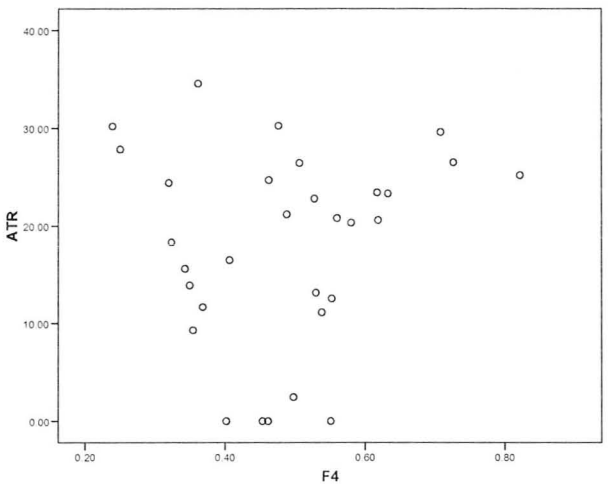

Figure 5.40 Scatterplot of ATR against F4 for the recent sample.

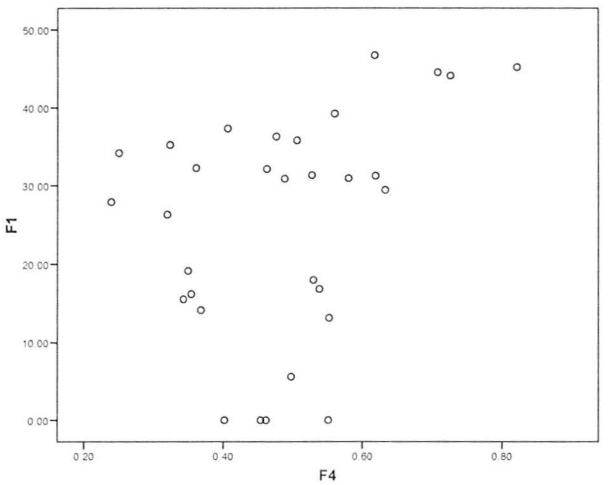

Figure 5.41 Scatterplot of F1 against F4 for the recent sample.

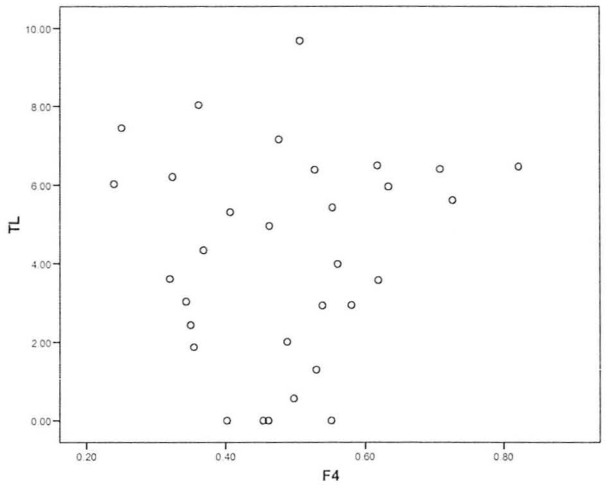

Figure 5.42 Scatterplot of TL against F4 for the recent sample.

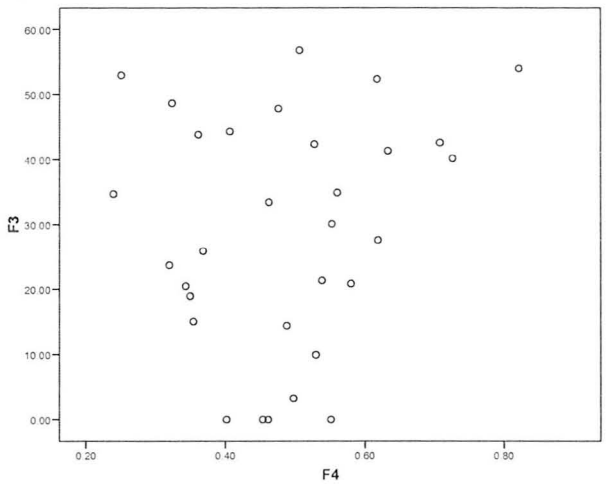

Figure 5.43 Scatterplot of F3 against F4 for the recent sample. Absolute and scaled measurements of transparent volume do not appear to be correlated with either area or linear measurements of transparency

5.8 Alternate Curve-Fitting

Given that the assumption of linearity in the relationship between chronological age and transparency has been questioned by others (Bang and Ramm, 1970; Drusini et al., 1989; Lorentsen and Solheim, 1989; Solheim, 1989; Drusini et al., 1990; Micheletti Cremasco, 1998), alternative curve fitting procedures were applied to each of the plotted associations between age and measurements of transparency. The equations for each of these models are provided in Table 5.11. For all measurements of transparency, the R^2 values for the quadratic and cubic functions appear to be higher than for the linear regression equations, indicating a closer fit between the observed values of transparency and those predicted by the polynomial functions. For each of the variables, the best fit is provided by a third degree polynomial equation. The magnitude of the improvement based on a cubic model of association is not large; however, for the area and linear variables a cubic model of association accounts for an additional 3-5% of the variance in transparency due to age. The R^2 values remain extremely low for the volumetric measurements of transparency, regardless of the descriptive model employed.

Variable	Model	Equation of the line	R^2	F Value	Sig.
ATR	**Linear**	$Y= -0.485 +0.39x$	0.487	57.866	0.000
	Quadratic	$Y= -11.068 +0.896x -0.005x^2$	0.535	34.579	0.000
	Cubic	$Y= -5.766 + 0.479x + 0.004x^2 - 5.87E\text{-}5x^3$	0.538	22.907	0.000
F1	**Linear**	$Y= 0.911 +0.561x$	0.385	43.963	0.000
	Quadratic	$Y= -17.855 +1.459x -0.009x^2$	0.443	23.845	0.000
	Cubic	$Y= -11.93 + 0.993x + 0.001x^2 - 6.56E\text{-}5x^3$	0.444	15.71	0.000
TL	**Linear**	$Y= -0.477 +0.097x$	0.425	45.053	0.000
	Quadratic	$Y= -2.310 +0.185x -0.001x^2$	0.445	24.077	0.000
	Cubic	$Y= 0.100 - 0.005x + 0.003x^2 - 2.67E\text{-}5x^3$	0.453	16.271	0.000
F3	**Linear**	$Y= -2.225 +0.677x$	0.364	34.917	0.000
	Quadratic	$Y= -17.424 +1.45x -0.007x^2$	0.389	19.087	0.000
	Cubic	$Y= 4.079 - 0.287x + 0.030x^2 - 0.000x^3$	0.399	13.079	0.000
VT	**Linear**	$Y= 117.599 - 0.091x$	0.003	0.082	0.777
	Quadratic	$Y= 138.277 - 1.069x + 0.010x^2$	0.024	0.341	0.714
	Cubic	$Y= 201.331 - 6.351x + 0.127x^2 - 0.001x^3$	0.064	0.616	0.616
F4	**Linear**	$Y= 0.445 + 0.01x$	0.015	0.441	0.512
	Quadratic	$Y= 0.493 - 0.002x + 2.26E\text{-}5x^2$	0.023	0.329	0.722
	Cubic	$Y= 0.326 + 0.013x + 0.000x^2 + 2.00E\text{-}6x^3$	0.043	0.401	0.754

Table 5.11 Summary of alternative curve fitting models for the pooled sample of teeth

5.9 Multiple Regression Analysis

In order to assess the degree to which each of the variables contribute to an accurate age prediction, a series of multiple regression analyses was performed, resulting in six alternate formulae. In these analyses, age was identified as the dependent variable and each of the independent measurement variables was entered into the equation via hierarchical forced entry. The order in which they were entered was determined based on the strength of the Spearman rank coefficients provided in Table 5.7. A summary of the multiple regression equations and their associated contribution to chronological age is provided in Table 5.12 and Table 5.13.

The standardized Beta coefficients contained in Table 5.12 provide a measure of the contribution of each variable within each equation to the observed variance in age. Note that for several of the equations, some variables display negative coefficients, indicating that they actually detract from the accuracy of prediction. Table 5.13 reveals that the highest R^2 is reported for the last equation, for which all of the area, linear and volumetric measurements of transparency have been included. However, the standard errors of the estimates for these equations are not improved, being +/-13.83 years on average. Furthermore, note that the F change is significant only for the first model in which ATR is the lone predictor of age (see Table 5.13). For models 2 though 5, the 95% confidence intervals for the coefficient values cross zero, indicating that the sign of the contribution for each of these variables is not constant (Field, 2000). The collinearity statistics provided in Table 5.12 indicate that for equations 3 through 6, the variance inflation factors (VIF) for the majority of variables are above 10 and the tolerance values lie below 0.2. Both results indicate significant inter-correlation amongst variables, a result which is corroborated by the significant correlations provided in Table 5.7 (Field, 2000). Figure 5.44 provides a scatterplot of the standardized residuals against the standardized predicted values. The funnel shaped pattern indicates a violation of the assumption of heteroscedasticity.

Model		Coefficients		95% Confidence Interval for B		Collinearity	
		B	Std. Error	Upper Bound	Lower Bound	Tolerance	VIF
1	Constant	25.42	5.01	15.17	35.68		
	ATR	1.25	0.24	0.76	1.73	1.00	1.00
2	Constant	25.24	5.03	14.93	35.53		
	ATR	0.91	0.44	0.02	1.80	0.30	3.33
	TL	1.52	1.63	-1.82	4.86	0.30	3.33
3	Constant	25.33	5.15	14.76	35.90		
	ATR	0.97	0.57	-0.20	2.13	0.18	5.46
	TL	1.61	1.74	-1.96	5.17	0.27	3.64
	F1	-0.06	0.33	-0.73	0.62	0.21	4.81
4	Constant	25.17	5.25	14.38	35.95		
	ATR	0.78	0.73	-0.72	2.29	0.11	8.81
	TL	3.30	4.50	-5.94	12.54	0.04	23.68
	F1	0.10	0.51	-0.95	1.15	0.09	11.26
	F3	-0.26	0.64	-1.58	1.06	0.04	27.41
5	Constant	33.83	9.33	14.62	53.04		
	ATR	0.81	0.73	-0.70	2.31	0.11	8.82
	TL	4.08	4.53	-5.25	13.40	0.04	24.25
	F1	0.09	0.51	-0.95	1.14	0.09	11.26
	F3	-0.36	0.65	-1.69	0.97	0.04	27.90
	VT	-0.08	0.07	-0.23	0.07	0.94	1.06
6	Constant	22.29	10.85	-0.11	44.68		
	ATR	1.62	0.82	-0.08	3.31	0.08	12.26
	TL	3.81	4.32	-5.11	12.73	0.04	24.28
	F1	-0.68	0.64	-2.00	0.64	0.05	19.54
	F3	-0.15	0.63	-1.44	1.14	0.03	28.83
	VT	-0.21	0.10	-0.40	-0.01	0.49	2.04
	F4	56.15	30.20	-6.17	118.47	0.34	2.91

Table 5.12 Description of the multiple regression analyses for the pooled sample of teeth with chronological age as the dependent variable. Models have been created via hierarchical forced entry.

Model	R	R^2	Adjusted R^2	Std. Error	Change Stats		
					R^2 Change	F Change	Sig. F Change
1	0.70	0.49	0.47	13.67	0.49	27.51	0.00
2	0.71	0.50	0.47	13.70	0.02	0.87	0.36
3	0.71	0.50	0.45	13.94	0.00	0.03	0.87
4	0.71	0.51	0.43	14.16	0.00	0.17	0.69
5	0.73	0.53	0.44	14.09	0.02	1.26	0.27
6	0.77	0.59	0.49	13.45	0.06	3.46	0.08

Table 5.13 Model summaries for the multiple regression models for which age is the dependent variable.

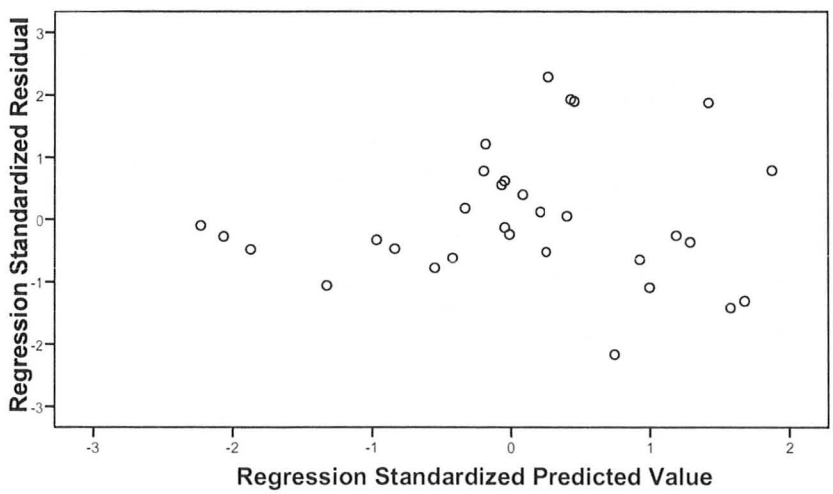

Figure 5.44 Scatterplot of the Regression Standardized Residual against the Regression Standardized Predicted Value for the multiple regression analyses of the pooled sample. The distinctly funnel-shaped distribution of the graph indicates unequal variance.

Chapter 6
DISCUSSION AND CONCLUSIONS

6.1 Introduction

The principle goal of this investigation has been to determine the most appropriate means of quantifying root dentine transparency. To this end, traditional area and linear measurements have been applied to tooth thin sections for which chronological age-at-extraction or age-at-death was known with certainty. In addition, for a sample of recently-extracted teeth, micro-CT densitometric measurements of transparency have been made in order to approximate a volumetric measure of transparency. In this chapter, the results of these investigations are compared in a discussion of the relative value of each measurement. Differences in the observed amounts of transparency due to sex, tooth category and antiquity of the specimens are examined. The chapter concludes with a summary of the findings and a series of suggestions for future research in light of the current findings.

6.2 Area and Linear Measures of Transparency

The most widely-used method of age estimation based on empirical measurements of transparency is the method of Bang and Ramm (1970) which involves a description of the length of transparency measured in a coronal direction from the root apex to the most apical extension of normal dentine. This method can be employed both for intact and sectioned tooth specimens. Several alternate techniques of measurement have also been explored based on the assessment of the area occupied by transparent root dentine measured on sectioned teeth (Azaz et al., 1977; Lorentsen and Solheim, 1989; Solheim, 1989). In general, the strength of the observed correlation with age for each of these forms of measurement is variable (Solheim, 1989; Whittaker and Bakri, 1996;

Micheletti Cremasco, 1998). So too is the accuracy of age-predictive formulae based on these parameters. Furthermore, direct comparisons of the relative value of each type of measurement of transparency are scarce (Lorentsen and Solheim, 1989). As such, there exists no consensus as to which measurement(s) should be employed in the estimation of age based on root dentine transparency.

In the current research, both area and linear measures of transparency have been applied to 1mm longitudinal tooth thin sections prepared in the labio-lingual plane. Measurements were made on digital images of the specimens, captured with a digital camera attached to a stereomicroscope equipped with a polarizing filter. These measurements have been recorded both as absolute values and relative indices of overall root dimensions.

When the correlations with age for area and linear measurements of transparency are compared, it appears that area measurements are more closely correlated with chronological age than are linear measurements. This is true both for the St. Thomas' sample of archaeologically derived teeth and for the recently-extracted tooth specimens collected from local dentists' offices and from the Winnipeg School of Dentistry (see Table 5.4, Table 5.5 and Table 5.7). Although the magnitude of the difference for each of the variables is not large, ATR, an unscaled measure of the root area occupied by transparent dentine, consistently displays the highest correlation with chronological age. For the pooled tooth sample ATR is the only variable with a Spearman rank correlation value above 0.7 ($p < 0.01$). This finding is in line with the values reported by Lorentsen and Solheim (1989). However, it runs counter to several studies that indicate a stronger correlation with age for linear measures of transparency (Solheim, 1989; Whittaker and Bakri, 1996).

In agreement with the results of the Spearman rank tests of correlation, the R^2 value for the least-square regression lines for ATR plotted against chronological age is higher than for any of the other measurements (see Table 5.8). Therefore, the residuals for the predicted values of ATR based on the regression line are less than for linear

measures of transparency. This implies that a greater proportion of the observed variance in transparent area can be accounted for by chronological age.

The consistently higher correlation with age for area measurements of transparency makes sense given that area measurements offer a more complete picture of the irregular distribution of transparency. Linear measurements of transparency represent a one-dimensional description of a phenomenon which progresses in a three-dimensional tissue. In this regard, it is therefore reasonable to assume that descriptions of area may offer more accuracy given that they offer two-dimensional descriptions of the affected regions.

However, these findings may in part arise from the increased precision involved in the computer-aided methodology employed in the measurement of transparency. In previous inquiries, the process of consistently outlining transparent area has presented difficulties given that the separation between transparent and opaque dentine is often diffuse (Miles, 1963; Bang and Ramm, 1970; Bang, 1989; Lorentsen and Solheim, 1989; Kvaal et al., 1994). In this regard, the current results may indicate the value of automated approaches to quantifying transparency in which the subjective nature of area measurements is reduced by the use of a computer algorithm designed to identify all similar pixels once a given threshold of values has been provided.

Yet, previous research that employed automated technologies to measure dentine transparency has produced mixed results (Lopez-Nicolas et al., 1990; Drusini et al., 1991; Lopez-Nicolas and Luna, 1991; Micheletti Cremasco, 1998). In particular, Drusini et al. (1991) were unable to demonstrate an improvement in the precision of measurements of transparency when automated methods were compared to caliper measures of the same specimens. Furthermore, the threshold function within the NIS Elements software used in the current research does not operate without guidance from the researcher. In order to perform measurements of transparent regions, one must manually identify areas of interest. Once this is done, it is then possible to adjust the threshold values to expand or limit the measured area. The current approach can therefore more accurately be described as a semi-automated protocol in which the

researcher guides the automated functions of a software program. It therefore seems unlikely that the entirety of the improvement in the observed correlation between transparent area and chronological age is attributable to the novel instruments employed in its measurement.

Given the consistently higher correlation with chronological age for unscaled measurements of transparent area within both tooth samples and the higher R^2 values for the associated regression equations, it is suggested that future research be directed towards the construction of age-predictive formulae based on measurements of transparent area made on sectioned tooth specimens, since none currently exist.

6.3 Absolute and Relative Measurements

Absolute measurements of transparency are more strongly correlated with chronological age than are the indices for which these values are considered as scaled proportions of overall root size (see Table 5.7). For both area and linear measurements of transparency, the R^2 values indicate a better fit for the associated linear regression lines for unscaled values (see Table 5.8). These findings apply to each of the samples separately and to the pooled sample. It is not immediately evident why this is so; however, these results are in agreement with those of other researchers who report a more regular, linear relationship with age for un-scaled measurements of transparency (Bang and Ramm, 1970; Solheim, 1989; Solheim, 1993).

One explanation for the strength of the observed association for absolute measures may lie in the inconsistent manner in which scaling was performed. The original measurement protocol called for transparent area and length to be expressed both as proportions of total root area (including pulp) and total root dentine area (excluding pulp). However, the latter measurements had to be abandoned since the irregularity of the positioning of the root canal meant that, for the majority of thin sections, the entirety of the canal was not visible under microscope. Since both pulp tissue and root canals were included within measures of transparent area and length, despite the fact that they do not

undergo sclerosis, it is conceivable that differences between teeth in the relative size and appearance of the pulp cavity may have disrupted the uniformity of the scaling process. Furthermore, for several of the recently-extracted teeth, the cervical margins used to define the coronal-most extent of the root were indistinct due to the presence of carious lesions.

Regardless of the cause, it is evident that absolute measurements of transparent area consistently appear to correlate better with age than do their scaled indices. Given that they are easier to calculate and require less time, it is suggested that absolute measures of transparency be performed in the quantification of transparency. However, the magnitude of these differences in the associations is not overwhelming. Therefore, a similar investigation should be performed on a larger, more homogeneous sample of teeth prior to making any recommendation to exclude relative measures of transparency. Until measurements are performed on a larger sample of teeth for which tooth type is carefully controlled, neither method of describing transparency should be abandoned.

6.4 Volumetric Measures of Transparency

Correlation with Age

Previous research in which serial sectioning of transparent tooth roots has been performed has revealed that transparency is not evenly distributed throughout root dentine (Vasiliadis et al., 1983a). Rather, it appears to progress in a "butterfly" pattern, advancing more quickly in the mesio-distal plane (Vasiliadis et al., 1983a). Since traditional area and linear measures do not describe the entirety of the affected dentine, volumetric measures of transparency have been suggested (Sognnaes et al., 1985; Lopez-Nicolas and Luna, 1991).

In the current study, micro computed tomography (micro-CT) analysis of the recently-extracted sample of teeth was undertaken on intact specimens prior to thin sectioning. Volumetric measures of transparent root dentine were approximated via the measurement of densitometric differences in the affected tissue. Based on a preliminary

analysis of four teeth for which age-at-extraction was unknown, density thresholds between +4500 and +6000 HU were used to define these areas. All subsequent measurements were performed using Amira 3.1.1 CT analysis software.

Counter to the expected outcome of the theoretical hypothesis outlined in Chapter 3, volumetric measures of transparency displayed the lowest correlation with chronological age of all the measures applied. The correlation coefficients for VT and F4 were -0.050 and 0.142 respectively, while the R^2 for the corresponding regression lines were 0.003 and 0.015 respectively (see Table 5.7 and Table 5.8). These results indicate that less than 2% of the variance in volumetric measures of transparency can be attributed to changes in age. While the results are improved for scaled indices of volume, in no case is there a statistically significant association with age.

Explanation of the Observed Association

The micro-CT instruments employed in this research cannot measure transparency directly. Rather, densitometric changes in root dentine have been differentiated based on differences in the attenuation of x-ray beams (Jackson and Thomas, 2004). As such, changes in tissue structure have been used as a proxy for a volumetric measurement of optical transparency. This methodological decision was based on current research indicating that transparency arises as the result of the deposition of hydroxyapatite within the tubules of normal radicular dentine (Bradford, 1960; van Huysen, 1960; Nalbandian et al., 1960; Weber, 1974; Vasiliadis et al., 1983a; Vasiliadis et al., 1983b).

The poor correlation between volumetric measurements of transparency and chronological age may be due to three distinct possibilities. First, it is conceivable that significant changes in the density of root dentine may exist, yet may be too small to be detected with the current micro-CT technology. Although the *contrast resolution* of current reconstructed CT images is increased by a factor of 10 over radiographic images, a number of factors may diminish their ability to accurately discern changes in tissue density (Bushberg et al., 2002). Included amongst these are: the *dose*, the number of x-

ray photons used per scan; the *gantry rotation speed*, the speed at which the housing containing the x-ray tube and/or the detector array circles the object under study; and the *pixel size*, the dimensions of the two-dimensional units of data acquisition (Bushberg et al., 2002). In addition, the resolving power of the scanned images, termed the *spatial resolution*, is limited by a number of factors (Kim et al., 2007). Included amongst these are: *detector pitch*, the spacing of the detector elements within the detector array; *focal spot size*, the size of the focal point of the x-ray beam used to measure object density; *object magnification*, the amplification of the data collected from the detectors which is enhanced by increasing the distance between the object and the array; and the *slice thickness*, the depth along the z-axis of a given voxel, largely determined by the detector aperture in the cranio-caudal plane (Bushberg et al., 2002). As any of these factors increases, so too will the magnitude of the distortion of the reconstructed micro-CT images in relation to the true dimensions of the object under investigation (Bushberg et al., 2002).

Second, it is possible that the accuracy of volumetric measures based on micro-CT analysis may also be flawed. Although the accuracy of linear measurements based on micro-CT analyses has been demonstrated, significant underestimation of tooth tissue volumes when compared to corresponding three-dimensional surface scanning measurements has been reported (Kim et al., 2007). When isolating individual tissues for measurement, their dimensions are ultimately defined by manipulation of the density threshold values by which each of the tissues are distinguished (Olejniczak and Grine, 2006; Kim et al., 2007). Variation in the threshold may therefore significantly alter the proportions of these tissues and ultimately change the values derived from their measurement (Olejniczak and Grine, 2006; Kim et al., 2007).

In the current research, the threshold values applied to the micro-CT images appeared to correspond well to the distribution of transparency for the initial test specimens. However, for the recent sample of teeth there was not a significant correlation with density for the area and linear measures of transparency made on the sectioned specimens (see Figure 5.36 through Figure 5.43). It is possible that the threshold of

density values applied to these teeth may have been too broad to adequately distinguish transparency. Furthermore, the pattern of elevated density in the periphery of the roots may indicate a pre-existing structural distinction in the root dentine which exists regardless of the advancement of sclerosis. The appearance of this distribution of density values in teeth for which no optical transparency was visible (see Figure 5.34 and 5.35) may indicate that the observed density distinctions are artefacts of a greater proportion of intertubular dentine in these areas which exists regardless of the advancement of sclerosis with chronological age. It is therefore possible that an alternate set of density values may better describe optically transparent root dentine.

The final and perhaps most plausible explanation for the poor correlation between micro-CT measurements of transparency and age, is that no fixed correlation between density and transparency exists. Transparency is an optical phenomenon which arises from the filling of tubules. Previous research indicates that there is a change in the mineral content within affected areas, however the magnitude of this difference is extremely minute (Brinkmann and Hartmann, 1980; Hawkinson and Eisenmann, 1983; Kinney et al., 2005). Yet, if the rate of sclerotic mineralization is constant, then areas of dentine in which there are fewer tubules or tubule diameters are reduced (for example at the periphery and apex of the root) will appear optically transparent in advance of those regions of dentine where the number and diameter of tubules is greater (closer to the pulp canal) (Mjor, 1984; Ten Cate, 1989; Marchetti et al., 1992; Dourda et al., 1994; Mjor and Nordahl, 1996; Orchardson and Cadden, 2001). Therefore, differences in the basic structure of radicular dentine between and within teeth mean that equivalent amounts of intra-tubular mineral deposition will produce varying levels of transparency. As such, there may be no universal, absolute density value that corresponds to areas of transparency. Indeed, the lack of a significant association between densitometric volumes and area and linear measurements of transparency (see Figure 5.37 through Figure 5.43) indicates that there is not a linear association between optical transparency and densitometric changes in radicular dentine. Yet, while unexpected, the poor correlations between tissue density and age are also of importance since they stress the influence of

the pre-existing distribution of tubules in the development of transparency and illustrate the necessity for further research into the relationship between tubule density and the threshold at which optical transparency becomes manifest.

6.5 Modeling the Rate of Increase in Transparency

The results of the least-squares regression of area and linear measurements of transparency against chronological age indicate a relatively linear increase in transparency with age. These findings are in agreement with those of other researchers (Miles, 1963; Bang and Ramm, 1970; Solheim, 1989; Lamendin et al., 1992). Yet for all measurements, examination of the scatterplots reveals a large amount of variation in the values of transparency. For all variables the R^2 values for the regression equations were below 0.7, the generally-accepted lower limit for age-predictive models of association (see Table 5.8). In addition, the high standard errors for the linear regression lines for area and linear measurements of transparency imply that changes in age are not an adequate predictor of the observed variance in transparency. Therefore, much of the observed variability in measurements of transparency could not be accounted for by differences in age.

For all measures of transparency, a better fit with age was approximated by polynomial functions than by a linear regression line (see Table 5.11). The R^2 values for the cubic and quadratic equations were higher than those for the linear functions. This implies that for these equations, changes in age are a better predictor of variance in transparency. These results may indicate that the relationship between transparency and age is not linear, that it is not constant throughout life, but rather that it decreases with age. Given that there is a limited number of tubules in which mineral may be deposited, it makes sense that the rate of mineral deposition would slow with advancing age (Bang and Ramm, 1970). Yet, despite the improved fit for these models, the associated R^2 values are still below 0.7, the highest being 0.538 for a third degree polynomial function of ATR. As such, the improvement for the polynomial functions over linear equations is

not pronounced and may instead be a reflection of the large amount of variance in the amount of transparency towards the upper end of the age spectrum.

Multiple regression analyses were performed with age as the dependent variable and each of the measures of transparency as the independent variables. Variables were entered in a hierarchical manner based on the observed correlations with chronological age. Model summaries for each of the six equations are provided in Tables 5.12 and 5.13. Based on the results of these analyses, the value of multivariate measures appears low. While the R^2 values are improved, the F change is significant only for the first equation which is identical to the original linear regression for ATR. Furthermore, the standard error of these models is still large, being on average +/-13.83 years.

In addition to the predictive inadequacy of these models, the assumptions underlying them have not been met. The collinearity statistics indicate significant inter-correlation amongst variables (see Table 5.12). Figure 5.44 illustrates that for these models, the assumption of heteroscedasticity (equal variance) cannot be met either. These results call into question the validity of any age-predictive model based on multiple measures of transparency, regardless of any observed improvement in the age prediction for the sample from which the data were drawn.

6.6 The Influence of Sex on Transparency

The strength of the partial correlations between each of the measurements of transparency and chronological age decreased by roughly 0.10 when the influence of sex was held constant (see Table 5.9). Therefore, it appears that sex exerts an influence on the expected amount of transparency, irrespective of chronological age. These observations are in keeping with those of other researchers who have reported significant sex-linked differences in the amount of transparency within adult tooth roots (Burns and Maples, 1976; Solheim and Sundnes, 1980; Solheim, 1989; Prince and Ubelaker, 2002). Such differences make sense given that male roots are on average larger than those of female teeth (see Lahdesmaki and Alvesalo, 2004; 2005; 2006) and that developmental

timing of permanent root formation and eruption is significantly different for males and females (Garn et al., 1958; Nolla, 1960; Demirjian and Levesque, 1980; Hagg and Taranger, 1985).

However, for the pooled sample Spearman rank correlations for sex were not significant for any of the measures of transparency ($p < 0.01$) (see Table 5.9). Furthermore, not all researchers are in agreement regarding the importance of sex in the quantification of transparency (Bang and Ramm, 1970; Lorentsen and Solheim, 1989; Drusini, 1991; Olze et al., 2004). More research is therefore necessary in order to understand the influence of sex on the development of root dentine transparency. It is suggested that in order to adequately address the issue of sex-linked differences in transparency a large sample of age-matched male and female teeth be compared.

6.7 The Influence of Tooth Type on Transparency

When teeth were grouped according to the individuals from whom they were extracted, the amount of transparency was not constant between teeth. Furthermore, the partial correlations with age for the area and linear measures of transparency were lower when the influence of tooth type was held constant (see Table 5.10). However, the magnitude of the influence of tooth type on transparency was only approximately 2%, much smaller than that reported for sex. This finding is interesting considering that many authors consider tooth position to exert a significant influence on the observed amount of transparency (Dalitz, 1963; Bang and Ramm, 1970; Johanson, 1971; Maples, 1978; Lopez-Nicolas and Luna, 1991; Solheim, 1993). Bang and Ramm (1970) reported stronger correlations for anterior tooth types. Lopez-Nicolas and Luna (1991) indicated that the majority of error in age estimates based on transparency arises from intra-individual variation by tooth type.

In the current research, it is possible that the manner in which teeth were catalogued may have distorted true difference between tooth types. Tooth categories were created by pooling all tooth positions within a general tooth type. For example, all

incisors, regardless of position, were assigned a single identifying number. While this was necessary given the small sample size and the lack of accompanying information for the St. Thomas' sample, this may have obscured important differences related to exact tooth position. Therefore, in order to adequately address the importance of variation related to tooth type, a much larger sample of teeth collected from age-matched individuals for which tooth types can be analyzed separately should be examined. In the interim, definitive conclusions regarding variation in transparency by tooth type cannot be reached.

6.8 The Influence of Antiquity on Transparency

When the mean values for each of the measures of transparency were compared, the results of the Mann-Whitney tests indicated no significant differences between the St. Thomas' and recently-extracted sample of teeth (see Table 5.6). The two samples were therefore pooled in order to increase the power of the statistical analysis of the correlations between transparency and chronological age.

However, the lack of significant differences for the non-parametric comparisons of the means does not rule out the practical importance of a consideration of antiquity for measurements of transparency. Prior to analysis, teeth within the St. Thomas' sample were carefully selected to be free of post-depositional damage. Many teeth from the wider sample were excluded based on evidence of taphonomic effects related to the presence of soil bacteria which had drastically altered the appearance of the radicular dentine. For these teeth, areas of pronounced opacity that obscured areas of transparency were evident. Had they been included for analysis, the degree to which this interference would have altered the correlation between transparency and chronological age is uncertain; however, Sengupta et al. (1999) reported that the presence of similar regions of "chalky" dentine within teeth collected from the Spitalfields sample significantly disrupted the expected association between age and transparency. In this regard, the lack of any significant difference between the St. Thomas' sample and the recently-extracted

teeth may reflect the diligence with which the "chalky" teeth were removed from analysis.

The evidence of taphonomic interference with the visibility of transparency for teeth within the St. Thomas's sample suggests that, whenever possible, future attempts to quantify transparency in teeth of considerable antiquity should be made on sectioned teeth, rather than on intact specimens. While diagenetic forces may radically alter teeth, obscuring transparency in the process, these effects may remain undetected for intact specimens. A recent application of the Lamendin method to the Spitalfields collection revealed a high percentage of teeth for which transparency could not be assessed (Megyesi et al., 2006). For 35% of the sample transparency was absent, despite the advanced chronological age of the specimens under study (Megyesi et al., 2006). While examination of the internal aspect of these teeth was not carried out, it seems likely that these specimens represent teeth affected by the organisms producing "chalky" dentine (Megyesi et al., 2006). Furthermore, similar changes may have distorted the transparency values derived from teeth for which measurements were possible. Yet, without an examination of the internal aspect of the roots of these teeth, the degree of error associated with taphonomic interference remains unknown. Such difficulties emphasize the advantages of sectioned methods over those performed on intact teeth, particularly in archaeological contexts in which the degree of taphonomic interference is uncertain.

Given the difficulties associated with measurements of transparency in the archaeological context, it has been suggested that the evaluation of transparency in historical teeth be abandoned (Vlcek and Mrklas, 1975). However, while acidic environments may interfere with measurements of transparency, the use of sectioned methods would guard against this, allowing for the exclusion of those teeth that have been so affected. Nonetheless, a controlled exploration of the affects of varying taphonomic conditions on transparent and opaque dentine seems appropriate.

6.9 Future Research Considerations

This study has demonstrated the value of un-scaled area measurements of transparency over other means of measurement. Yet, in so doing, this inquiry has unearthed a number of other concerns. This research has suggested the influence of pathology on the rate at which transparency develops. The exploration of the density of regions of transparent dentine has also revealed an important area of research concerning the mechanics of intra-tubular mineralization. Furthermore, a review of the current literature has revealed the necessity for caution in the application of regression-based statistical methods to the development of formulae for use in age estimation. A discussion of the requirement for research in each of these areas follows.

6.9.1 Pathology

The majority of research seems to indicate that the mechanism underlying root dentine transparency is a distinct process from that which produces coronal dentine transparency (Weber, 1974; Azaz et al., 1977; Mendis and Darling, 1979; Stanley et al., 1983; Porter et al., 2005). The latter is characterized as a reactive response to the destruction of tooth tissues due to external stimuli, while the former occurs as a physiological process, even in the absence of pathological conditions within the oral environment. Root dentine transparency has been observed for impacted teeth which have not yet come into occlusion (Azaz et al., 1977). Furthermore, in the current study, four teeth collected from a 15-year-old female for which complete crown destruction was recorded, were found to be free of transparency (see Figure 5.35). This observation seems to strengthen the assertion that physiological processes related to aging rather than pathology may be the impetus for the onset of root dentine transparency.

However, it is conceivable that pathology may interfere with the rate at which transparency advances. Olze et al. (2004) reported an error of over 12 years when applying the intact method of Bang and Ramm (1970). This error was attributed to a

history of drug use and diabetes, both of which may have altered the metabolism of the individual from whom the teeth were collected (Olze et al., 2004). Ziller (1996) has discussed the difficulties in assessing root transparency for teeth extracted from drug-addicted individuals, noting that transparency is substantially advanced in such cases, perhaps due to a premature aging of the pulp tissue. Kuhl (1984) and Reinwarth et al. (1987) have asserted that diabetes mellitus accelerates the advancement of transparency.

In the current study, one recently-extracted tooth, collected from a 79-year-old female, was removed from statistical analysis due to abnormally low levels of transparency. Across all measures, transparency appeared dramatically lower than expected. No external trauma or pathology was observed prior to sectioning. However, the dentine immediately surrounding the pulp appeared far more opaque than expected. This region formed a halo of dense, white dentine several millimeters distance from the pulp canal (see Figure 5.35). While the medical history of the person from whom this particular specimen was extracted is uncertain, these observations may suggest the importance of metabolism in the progression of transparency. It would be interesting to have a more complete data set regarding any illness during childhood or adult life that may have affected the advancement of sclerosis in this case.

Systemic disturbances including diabetes mellitus, HIV, osteoarthritis and rheumatoid arthritis affect the oral health of individuals (Rhodus, 2005). So too do the medications prescribed to treat these diseases (Rhodus, 2005). The affects of a negative calcium balance on skeletal health are also well documented (Nordin, 1996). Yet, the effects of osteoporosis on the progression of transparency have not been explored, nor have the affects of calcium and vitamin D intake. Given that transparency appears to arise from the deposition of hydroxyapatite within tubules of normal dentine, any behavioral or pathological factors which affect the levels of circulating blood calcium directly or alter the homeostatic balance of calcium within the body may conceivably alter the rate of intratubular mineral deposition. Therefore, chronic systemic abnormalities may affect the deposition of intratubular materials by limiting local levels of the mineral associated with sclerosis. Future research should therefore examine the

degree to which transparency is affected by metabolic disturbances through a controlled comparison of teeth extracted from ill and healthy individuals.

6.9.2 Chemical Analysis

Although micro-CT analysis is capable of identifying differences in the density of tissues, it cannot identify the constituents of the dentinal mineral under study. Thus far, changes in the chemical contents of dentine with age have not been explored in great depth. This is unfortunate, given that the chemical content of dentine is dynamic, changing over the course of an individual's life. For instance, dentinal water content appears to decrease with age (Pilz, 1959) while fluoride and magnesium concentrations appear to increase (Nakagaki et al., 1987; Schram, 2002).

Chemical analysis of transparent dentine has previously been carried out, yet more detail is still needed. Brinkmann and Hartmann (1980) measured the mineral content of 101 teeth via photon beam absorption. Seven measurements were made for each tooth at roughly 2mm intervals and mineral content within each zone was calculated in g/cm^2. In agreement with the results of ashing studies (Simon and Armstrong, 1941; Moore and Leaver, 1974), mineral content was found to decrease in an apical direction. However, teeth that displayed high levels of transparency according to the sectioned method of Bang and Ramm (1970) did not consistently display an elevated mineral content. Yet, the measurement values recorded in the apical end of the root lay within the lower range of sensitivity for the instruments employed (Brinkmann and Hartmann, 1980). Furthermore, the proportion of mineral to organic components within affected areas of the root was not detailed, nor was the exact identity of the contributing minerals revealed.

Since transparency arises from an equalization of the refractive index of dentine as tubules are filled with inorganic material, an analysis of the change in relative or absolute quantities of the chemical constituents of root dentine may offer a more viable means of age estimation. Despite the uncertainty surrounding the origin of sclerosis, it is

clear that the relative inorganic contents of affected root dentine are in flux (Vasiliadis et al., 1983b; Kinney et al., 2005; Porter et al., 2005). Given that TEM analysis indicates that tubules within regions of transparent dentine are filled with hyrdoxyapatite, it may be worth pursuing an analysis of the chemical content of these areas (Takuma and Eda, 1966; Hawkinson and Eisenmann, 1983; Porter et al., 2005). A more comprehensive chemical analysis in which the entirety of the root is analyzed and the proportions of the organic and inorganic components of dentine within both transparent and normal regions are quantified may yield useful distinctions between transparent and opaque dentine. Such an exploration might reveal an alternative means of quantifying changes in root dentine based not on transparency but on the chemical changes underlying it. If firm rates of intra-tubular mineralization could be established, a more objective and precise method of age estimation based on changes in root dentine might be established.

6.9.3 Statistical Inference

The current research has dealt exclusively with the description of the association between measurements of transparency and chronological age. However, the ultimate motivation for any research into root dentine transparency in the anthropological context is related to the construction of age-predictive formulae. Unfortunately, the inferential statistical methods currently employed to extend observed associations within a sample to predict ages for teeth collected from other populations may amplify the error associated with these age estimates. Furthermore, both the age distribution of the reference sample and the range of observed values for the samples used to construct the predictive formula(e) will affect the accuracy and precision of future age predictions.

The following discussion of the error associated with the various inferential statistical methods employed to date is provided as an illustration of the error introduced by traditional approaches to age prediction based on the observation of continuous morphological indicators in the human skeleton. It is hoped that the following discussion will clarify the necessity for a Bayesian approach to age estimation based on

transparency. It is also hoped that future research will be directed towards an exploration of the value of such an approach for other skeletal indicators of age.

Linear Regression

The vast majority of age-predictive formulae based on root dentine transparency have been formulated using conventional *least-squares regression* (Gustafson, 1950; Miles, 1963; Bang and Ramm, 1970; Azaz et al., 1977; Lorentsen and Solheim, 1989; Solheim, 1989; Lamendin et al., 1992). Such an analysis involves establishing a generalized relationship between related variables (although it may be applied to more than two) by determining the mathematical equation which best describes the observed variation in the two based on repeated sampling (Aykroyd et al., 1997).

In the case of transparency, the resultant linear equation takes the form:

$$y = a + bx + e$$

where, based on repeated observations of transparent teeth of known age, x is the observed amount of transparency; y is the corresponding age; a is the age below which there is no observed transparency; b is the rate of change in age with increasing transparency; and e is the random error associated with an age prediction (Aykroyd et al., 1997).

A linear regression equation such as the one above represents an approximation of the relationship between the two variables and is therefore a line of best fit for the observed data set (Aykroyd et al., 1997). Only two variables exist and there is no change in the relation between the two (in this case, the rate of change in transparency remains constant with age) (Aykroyd et al., 1997). An equation is derived by determining the equation of a line for which the magnitude of the mean differences between the observed values (represented by known data points plotted on a Cartesian grid) and their corresponding points on the regression line, referred to as a *residual*, is minimized

(Konigsberg and Frankenberg, 1994; Aykroyd et al., 1997). Hence, the values of *a* and *b* are estimated based on an analysis of the data used to derive the formula. Regression formulae derived in this manner are then used to predict the unknown value of chronological age for individuals based on the observation (measurement) of transparency.

The error of a prediction, equal to the magnitude of the difference between true age and predicted age, can be tested using a holdback sample of known age through a process referred to as *inverse calibration* (Aykroyd et al., 1997; Aykroyd et al., 1999). When the errors of such a test are plotted against the known age, a systematic bias emerges in the error of the age predictions, wherein the magnitude of predictive error is most pronounced at either end of the regression line and reduced towards the center (Aykroyd et al., 1997; Aykroyd et al., 1999). The sign of the errors varies in a regular manner, being negative in the lower half of the regression equation and positive at the upper end of the age spectrum (Aykroyd et al., 1997). This guarantees that younger individuals will appear older than their true age and that older individuals will appear younger based on observations of given morphological criteria (in this case transparency) (Aykroyd et al., 1997; Aykroyd et al., 1999). Importantly, *this systematic bias will persist regardless of the data set on which the regression equation is applied* (Aykroyd et al., 1997; Aykroyd et al., 1999).

It appears likely that the systematic bias emerging from the way in which the relationship between age and transparency is described significantly contributes to the observed error of the methods applied (Konigsberg and Frankenberg, 1994; Lucy and Pollard, 1995; Lucy et al., 1996; Aykroyd et al., 1997; Aykroyd et al., 1999). As noted earlier, across a number of different methods age estimation based on the observation of root dentine transparency, numerous authors have reported a tendency towards over-estimation of true age below 40 years and an underestimation of true age above 60 years (Gustafson, 1950; Miles, 1958; Bang and Ramm, 1970; Kashyap and Koteswara Rao, 1990; Drusini, 1991; Lopez-Nicolas and Luna, 1991; Lamendin et al., 1992; Whittaker and Bakri, 1996; Prince and Ubelaker, 2002).

Classical Calibration

It has been suggested that a novel statistical approach to the description of the relationship between transparency and age will remedy this error. In particular, the value of *classical calibration* over linear regression has been emphasized (Aykroyd et al., 1997). Such an approach is similar to regression except that *x* and *y* are reversed, *y* being the observed value of transparency and *x* being the age of an individual (Aykroyd et al., 1997). This is a subtle yet important distinction, as it recognizes that age is not truly the dependent variable in the observed relationship with transparency (as is assumed by a traditional regression analysis) (Konigsberg and Frankenberg, 1994). Instead, in a classical calibration the amount of transparency is designated as the dependent variable, *y*, which increases with age, *x*. Since age is designated as the independent variable, its value is "exactly determined," and therefore free of error (Aykroyd et al., 1997). Furthermore, since *x* is fixed and *y* varies about *x*, the systematic bias associated with estimates of age is eliminated, the error of any estimate being equal, irrespective of the predicted age (Aykroyd et al., 1999).

Such a regression of *y* on *x* produces the following equation:

$$x_i = [(y_i - a)/ b] + e$$

where y_i is the observed transparency; x_i is the age in years; *a* is the age below which there is no observed transparency; *b* is the rate of change in age with increasing transparency; and *e* is the random error associated with an age prediction (Aykroyd et al., 1997).

The utility of this statistical approach has been explored, albeit to a very limited extent. Classical calibration was employed by Micheletti Cremasco (1998) in a computer-aided analysis of transparent area made on longitudinal thin sections of premolars. Although the equations were not tested on a holdback sample, the reported

correlations between age and transparency were high at 0.91 and 0.88 for recent and pooled samples respectively (Micheletti Cremasco, 1998).

Yet, the age predictions based on classical calibration are not free of error; rather the resultant equations are still an approximation of the relationship between age and transparency based on a finite number of observations. The uncertainty for any point estimate is described by a parabolic distribution about the calibration line, centered on a given estimate (Aykroyd et al., 1997). This is because the predictive error arises from two sources: the variability in the ages at which a given amount of transparency is visible; and the uncertainty in the values of a and b, both of which increase with distance from the mean of x and y (Aykroyd et al., 1997). Both are byproducts of individual differences in the rate at which degenerative changes accumulate within the skeletal system. Such a discrepancy marks the distinction between biological age (the age an individual appears when morphological criteria are evaluated in comparison to a populations standard) and chronological age (the amount of time an individual has been alive) (Aykroyd et al., 1999; Hoppa, 2002; Usher, 2002). Since very few people display an average rate of age-related changes, this distinction is an important one, which represents an inescapable source of error in age estimation (Aykroyd et al., 1999; Hoppa, 2002).

Another drawback of a classical calibration is that, while the distribution of the error is flattened, its magnitude for any given age estimate actually increases (Aykroyd et al., 1997). At a given level of confidence, the associated interval of values is wider than that of a similar regression-based estimate (Aykroyd et al., 1997). The problem of systematic bias is addressed; however this solution comes at the expense of predictive precision (Aykroyd et al., 1999). Further research involving a direct comparison between regression and calibration-based equations derived from the same data is necessary in order to determine the degree to which this difficulty detracts from the increase in predictive accuracy associated with the smoothing of the error.

A Bayesian Approach

It has been argued that the systematic error associated with age estimates based on morphological criteria (including transparency) is in part an artifact of the age distribution of the sample population upon which such a predictive equation is based (Bocquet-Appel and Masset, 1982; Aiello and Molleson, 1993; Hoppa and Vaupel, 2002). The reasoning behind this argument is most easily illustrated by dividing a sample population into two separate sub-samples. The regression equations derived from each of the two will be markedly different and what's more, neither will exactly correspond to the regression derived from the sample as a whole (Bocquet-Appel and Masset, 1982). Since one will never have access to the entire population one is sampling and age distributions vary across populations, such a bias is inevitable.

This difficulty is further compounded by the issue of how representative a given sample is of the variation within the total population, that is, the degree to which the corresponding proportions and ranges of variation within a population are represented within the sample drawn from that population (Aykroyd et al., 1999; Hoppa, 2002). In the case of age estimation based on transparency, the accuracy of a universal method is limited by the size and heterogeneity of the sample upon which it is based (Hoppa, 1996; Aykroyd et al., 1999).

Aykroyd et al. (1997) acknowledge the artefactual influence of a sample population on the accuracy of subsequent applications of the method. They suggest that when calibration expressions are employed over traditional regression, the influence of the age distribution of the reference population on the magnitude of the error of subsequent age predictions is reduced (Aykroyd et al., 1997). Yet this approach does not entirely eliminate inaccuracies based on the demographic profile of a sample or the reference population from which it was drawn.

An alternative statistical approach that accommodates inter-population variation and incorporates any available knowledge of the population under study has been suggested. This method, based on a theory of probability known as *Bayes' theorem*, is universally applicable, and is adaptable to formulate both individual age estimates and

population profiles (Aykroyd et al., 1999; Hoppa and Vaupel, 2002). Three concepts are central to Bayesian age prediction:

> *prior probability* (the probability of a particular hypothesis being true prior to a consideration of any experimental data); *likelihood* (the conditional probability of the information being true, given a set of experimental observations); and the *posterior probability* (the conditional probability of a hypothesis being true, given a particular points score). (Aykroyd et al., 1999:65)

For age predictions based on transparency, the prior probability, denoted $P(A_i)$, is the likelihood of an individual being of a given age, given no information regarding the amount of transparency in their teeth (Aykroyd et al., 1999). This term is variable between populations and its inclusion allows for population-specific modifications to be made to the formula based on any prior knowledge of the age structure of that population (Aykroyd et al., 1999). Likelihood, denoted $P(I \mid A_i)$, is equal to the probability of an individual being a given age based on the observed amount of transparency (Aykroyd et al., 1999). The posterior probability, denoted $P(A_i \mid I)$ is equal to the product of the prior probability and the likelihood (Aykroyd et al., 1999). It is this term which represents the age estimate generated by a Bayesian approach. For a given individual, age predictions are to be made according to the following formula:

$$P(A_i \mid I) = [\, P(A_i) \times P(I \mid A_i) \,] / [\, \sum P(A_j) \times P(I \mid A_j) \,]$$

where j refers to all age values within the population, and i refers to the specific age category of interest (Lucy et al., 1996). For those cases in which multiple pieces of information are available for a given individual based on repeated sampling (i.e. where several teeth are collected from the same individual), each of the observations in the above equations are made according to the following formula:

$$P(I_n \mid A_i) = n(I_n, A_i) / n(A_i)$$

where n (I_n, A_i) is the number of individuals in the reference sample with the indicator variable I_n and age i and n (A_i) is the total number of individuals within the reference sample of age i (Lucy et al., 1996). The original formula is modified to read

$$P(A_i \mid I_1, I_2, \ldots, I_n) = \{[\, n(I_1, A_i) / n(A_i) \,] \times [\, n(I_2, A_i) / n(A_i) \,] \ldots [\, n(I_n, A_i) / n(A_i) \,] \times [\, n(A_i) / N \,]\} \div \sum P(I_1 \mid A_j) \times P(I_2 \mid A_j) \ldots P(I_n \mid A_j) \times P(A_j)$$

The advantage of this approach is that it allows for the introduction of prior knowledge regarding a specific population of interest. In this way, a Bayesian approach offers a universal set of procedures that may be tailored to a specific population. The resultant age estimates (posterior probabilities) are expressed as probability distributions about a given mean estimate (Lucy et al., 1996; Aykroyd et al., 1999).

Another advantage to a Bayesian approach is that the estimates generated offer much more information than do those generated via regression or calibration. Age estimates are expressed as probability functions, centered on a given mean estimate (Lucy et al., 1996). Since Bayesian approaches to age estimation do not make any simplifying assumptions regarding the distribution of probability about estimates, each prediction is described by a unique probability distribution which varies according to the prior probability of an estimate for a particular population (Lucy et al., 1996). Estimate functions may be quite irregular (Lucy et al., 1996). For those cases in which extraneous variables have influenced the age prediction, the probability distribution will appear bi-modal (Lucy et al., 1996). Such cases may easily be examined and situated within a broader discussion of the reference population itself (Aykroyd et al., 1999).

The accuracy and precision is also improved for Bayesian estimates. The *Mean Absolute Deviation*, the difference between the predicted age and true age for a given series of individuals, is lower for a Bayesian approach than for regression-based methods (Aykroyd et al., 1999). Except for those cases in which the strength of the correlation exceeds 0.9, Bayesian predictions show a higher degree of precision, with narrower 95

percent confidence intervals than those of regression-based predictions (Aykroyd et al., 1999).

There are, however, limitations to a Bayesian approach. In particular, the accuracy of the estimates is dependent upon the sample size (Lucy et al., 1996; Hoppa, 1996; Kemkes-Grottenhaler, 2002). The best predictions are generated when the specimens under examination are drawn from a large reference sample of known age, spanning the entire age range (Aykroyd et al., 1999). Further, although the magnitude of the error is on average reduced over regression-based methods, the error of Bayesian posterior probabilities may display a small systematic bias (Aykroyd et al., 1999). However, the relative novelty of this technique means that the reality of this bias has not been conclusively proven, nor have its origin and magnitude been explored (Aykroyd et al., 1999).

Application of a Bayesian Approach

In the future, the St. Thomas' sample may serve as an ideal collection of teeth on which to assess any improvement in age estimation based on a Bayesian statistical approach. Parish registers recording all death during the period for which the cemetery was in use are available and can be used to provide an estimation of the prior probability for subsequent age estimates. They will provide an approximation of the likelihood of a given tooth belonging to an individual of a given age in the absence of any information regarding transparency. The parish registers have previously been inspected for completeness and their accuracy evaluated according to the methodology of Drake (1974) (Boyce, 1990; Rogers, 1991). They indicate that 1564 individuals were buried in the cemetery between 1821 and 1874, of which 1439 represent individuals of known age and sex (Boyce, 1990; Saunders et al., 1995). Although there were several month-long gaps in the registry entries and several entries for which age-at-death was not included, the St. Thomas' register appears to satisfy all of Drake's criteria (Drake, 1974; Boyce, 1990; De Vito, 1994). Although one can never be sure that all the reported ages are not

approximations, it may be said that in general "a sex and age profile created from the register data should be unbiased and reliable" (Rogers, 1991:30; De Vito, 1994).

6.10 Conclusions

1. *Area measurements are more strongly correlated with chronological age than are linear measurements of transparency.* For both archaeological and recently-derived tooth samples, area measurements of transparency made on sectioned specimens should be employed in future age-predictive formulae based on the observation of transparency. Measurements are easily made on 1mm thick tooth sections for which the line between transparent and opaque dentine is easiest to distinguish.
2. *Absolute measurements of transparency are more strongly correlated with chronological age than are scaled indices which account for variation in overall root dimensions.* It is unclear why this is so. However, since scaled measurements appear to offer no advantage, despite an increase in time and effort on behalf of the researcher, absolute measurements of transparency appear to offer a distinct advantage.
3. *Three dimensional approximations of the volume of transparent regions of root dentine do not offer an advantage over either area or linear measures of transparency made on sectioned specimens.* The poor associations for both absolute values and scaled indices may be due to a systematic error of the micro-CT methods involved in data collection or may indicate a non-linear relationship between tissue density and transparency. Yet, regardless of the cause, alternative methods of quantifying transparency, based on the optical properties of the affected tissue, should be explored.
4. *Sex exerts an influence on the amount of transparent root dentine.* Yet, future research should be directed towards examining the expected degree of sex-linked variation for a large sample of age-matched teeth of known age and sex.
5. *Tooth category exerts a small influence on the amount of transparency, the magnitude of which was not large for any of the measures of transparency.* However, given the small sample sizes employed in this research and the broad

descriptive categories used in tooth identification, more research is required to determine the true nature of variation related to tooth position.

6. *Archaeological and recently-extracted teeth do not differ significantly in the amount of transparency.* However, taphonomic interference may limit the degree to which age-predictive formulae based on transparency can be applied to teeth collected from the archaeological context. In order to adequately address the degree to which post-mortem interval may influence the extent of transparency, larger samples of varying antiquity for which age and sex have been matched should be compared.

6.11 Summary: The Value of Transparency in Age Estimation

The practical value of age estimates based on the observation of root dentine transparency remains unclear. Reasonable predictive accuracy has been achieved in both the forensic and archaeological contexts (Bang, 1993; Kvaal et al., 1994; Kvaal and During, 1999; Pretty, 2003). Furthermore, measures of transparency are easily applied, even on sectioned specimens, for which a minimal amount of preparation time is required (Pretty, 2003).

Yet, the accuracy of age predictions has been variable. Mean errors as high as 14 years have been reported both for the method of Bang and Ramm (1970) and that of Lamendin et al (1992) (Solheim and Sundnes, 1980; Lamendin et al., 1992). Furthermore, for teeth of recent and advanced antiquity, transparency is not always measurable (Megyesi et al., 2006). In the current research, evidence of taphonomic interference meant that a large number of eligible teeth had to be excluded from examination. Such cases have been noted by other researchers (Bang and Ramm, 1970; Sengupta et al., 1999; Megyesi et al., 2006). The magnitude of influence exerted by pathology during life is also unclear. For the recently-extracted sample of teeth, one specimen was excluded due to abnormally low levels of transparency, perhaps due to an obscure pathological disturbance.

In general, descriptions of the increase in transparency with age are characterized by a wide degree of variance, regardless of the measurements applied (Gustafson, 1950; Miles, 1958; Bang and Ramm, 1970; Wegener and Albrecht, 1980; Kashyap and Koteswara Rao, 1990; Drusini et al., 1991; Lopez-Nicolas et al., 1993; Whittaker and Bakri, 1996; Zadzinska et al., 2000; Prince and Ubelaker, 2002; Olze et al., 2004).This observed variation in the amount of transparency is likely not random, but rather due to a number of salient variables which influence the rate of mineralization underlying transparency. The identity of these variables, (which may include sex, diet, pathology and taphonomy) and the magnitude of their influence should be investigated. Furthermore, it is imperative to remember that despite the strength of the observed association, age is not a causal mechanism in the progression of transparency. Rather, the amount of root dentine transparency increases with age due to the influence of an unknown causal mechanism that acts over time. Observations of transparency have allowed age prediction in cases where true age is unknown through the comparison of individual samples to predictive formulae. Yet, the exact mechanism driving the development of transparency remains obscure. Accordingly, researchers are at a loss to explain, predict or guard against errors in age estimates based on transparency.

Further research into the nature of the sclerosis underlying transparency and the causal mechanism controlling intra-tubular mineralization would likely increase the accuracy and precision of age estimates based on the observation of root dentine transparency. The causal mechanisms underlying transparency must be understood before full confidence can be placed in methods of age prediction based on its observation. In the interim, the value of the methods of Bang and Ramm (1970) and Lamendin et al. (Lamendin et al., 1992) remains uncertain. It is recommended that neither method be used alone, unless out of necessity. Wherever possible, these methods should be used in conjunction with alternative means of age estimation based on both dental and skeletal indicators of age. When used alone, researchers should be mindful of the potential sources of error related to tooth preparation, pathology, taphonomic interference, sex and tooth position.

Appendix I

Raw Measurements for the Archaeological and Recent Samples

ID Number	Tooth Pos.	Sex	Age (years)	ATR (mm2)	F1	TL (mm)	F3	VT (mm3)	F4
071C	2	1	20	1.88	1.74	1.05	5.81		
071I	1	1	20	7.55	13.71	1.04	8.86		
115I	1	1	27	8.12	14.68	2.00	16.06		
133C	2	1	41	5.03	7.82	2.23	17.57		
133I	1	1	41	5.03	12.62	1.61	16.74		
133PM	3	1	41	2.01	3.95	0.67	6.30		
156C	2	1	55	16.46	29.43	3.89	34.58		
156I	1	1	55	25.54	64.35	8.15	72.71		
297C	2	1	75	34.24	43.81	7.53	57.12		
303C	2	1	58	33.28	34.42	9.59	50.29		
317C	2	2	35	20.27	31.44	4.09	34.46		
374I	1	1	64	34.05	74.86	9.27	83.08		
374PM	3	1	64	26.22	47.24	5.71	49.70		
375C1	2	1	60	10.59	11.08	3.24	20.42		
375C2	2	1	60	36.67	39.30	2.61	16.52		
375I1	1	1	60	19.18	33.85	2.45	19.62		
375PM1	3	1	60	23.44	35.86	3.72	26.48		
375PM2	3	1	60	39.69	60.87	9.47	64.22		
385I	1	1	35	17.46	26.96	1.73	12.45		
400PM	3	2	29	20.68	34.21	3.77	30.11		
429C	2	1	43	23.69	30.19	4.81	32.44		
429I	1	1	43	25.40	53.43	6.47	55.51		
429PM	3	1	43	29.25	47.90	5.44	44.34		
443I	1	1	44	11.67	19.28	2.92	13.80		
465C	2	2	22	11.79	19.84	1.98	15.78		
467C	2	1	54	15.06	17.95	2.15	14.83		
470I	1	2	53	17.76	35.07	3.36	8.51		
470PM	3	2	53	9.56	18.04	1.53	11.60		
514PM	3	2	17	3.76	6.74	2.42	18.10		
516I	1	2	69	27.81	43.05	8.85	69.65		
527APM	3	1	31	5.97	6.66	1.40	7.99		
544I	1	2	71	30.57	56.81	10.85	86.16		
LOR001	2	2	94	26.51	44.11	5.62	40.19	109.18	0.73
WIN001A	2	2	15	0.00	0.00	0.00	0.00	141.67	0.45
WIN001B	2	2	15	0.00	0.00	0.00	0.00	155.29	0.46

WIN001C	2	2	15	0.00	0.00	0.00	0.00	113.99	0.40
WIN001D	2	2	15	0.00	0.00	0.00	0.00	116.69	0.55
WIN002	2	2	83	24.41	26.32	3.62	23.71	96.08	0.32
WIN003A	2	1	52	34.57	32.28	8.04	43.84	143.33	0.36
WIN004	2	1	48	29.60	44.56	6.41	42.58	169.75	0.71
WIN005	1	1	34	9.31	16.19	1.87	15.06	42.71	0.35
WIN006A	1	2	56	23.41	46.73	6.50	52.36	156.78	0.62
WIN006B	2	2	56	11.67	14.14	4.34	25.90	93.99	0.37
WIN007A	2	1	45	16.46	37.34	5.31	44.30	56.97	0.41
WIN007C	3	1	45	18.33	35.22	6.21	48.67	51.03	0.32
WIN008A	2	2	62	27.83	34.20	7.45	52.94	63.39	0.25
WIN008B	1	2	62	20.80	39.24	3.99	34.91	121.24	0.56
WIN008C	2	2	62	30.26	36.30	7.16	47.83	128.66	0.48
WIN009A	1	1	53	21.20	30.89	2.01	14.40	83.11	0.49
WIN009B	2	1	53	30.19	27.92	6.03	34.69	104.86	0.24
WIN010A	1	2	30	2.46	5.64	0.57	3.25	56.38	0.50
WIN010B	2	2	30	13.88	19.13	2.44	18.94	104.60	0.35
WIN010C	2	2	30	13.13	17.98	1.30	9.95	146.24	0.53
WIN010D	3	2	30	23.32	29.46	5.96	41.32	159.85	0.63
WIN012A	2	1	46	12.53	13.13	5.43	30.10	181.03	0.55
WIN012B	3	1	46	15.57	15.52	3.03	20.48	82.46	0.34
GAL001	2	1	85.95	26.44	35.81	9.68	56.77	107.38	0.51
GAL002	2	2	79.32	6.96	8.39	2.49	17.85	90.89	0.26
GAL003	1	1	54.89	11.12	16.82	2.94	21.35	95.48	0.54
GAL004	1	1	54.89	20.34	30.97	2.95	20.85	100.23	0.58
GAL005	1	1	54.89	25.20	45.21	6.47	54.00	109.31	0.82
GAL008	3	1	54.89	20.61	31.28	3.58	27.60	141.03	0.62
GAL006	2	1	80.47	22.81	31.35	6.39	42.34	150.14	0.53
GAL007	2	1	80.47	24.71	32.15	4.96	33.43	122.89	0.46

Appendix II

Slide Thicknesses for the Sectioned Specimens

Number	Left (mm)	Centre (mm)	Right (mm)	Av	Left (mm)	Centre (mm)	Right (mm)	Av
071_I	0.51	0.52	0.57	0.53	0.42	0.44	0.47	0.44
071_C	1.26	1.26	1.31	1.28	1.54	1.60	1.70	1.61
115_I	0.51	0.51	0.50	0.51	0.46	0.47	0.47	0.47
133_C	0.66	0.62	0.56	0.61	0.51	0.48	0.45	0.48
133_I	0.46	0.45	0.46	0.46	0.40	0.40	0.40	0.40
133_PM	0.46	0.47	0.45	0.46	0.44	0.44	0.43	0.44
156_C	0.48	0.49	0.50	0.49	0.44	0.45	0.46	0.45
156_I	0.52	0.49	0.52	0.51	0.43	0.41	0.46	0.43
297_C	0.31	0.32	0.44	0.36	0.27	0.26	0.29	0.27
303_C	0.27	0.40	0.51	0.39	0.44	0.47	0.53	0.48
317C	1.01	1.01	1.01	1.01	0.98	0.97	0.98	0.98
374_I	0.40	0.39	0.37	0.39	0.34	0.32	0.31	0.32
374_PM	0.34	0.34	0.36	0.35	0.31	0.33	0.32	0.32
375_C1	0.46	0.48	0.51	0.48	0.39	0.40	0.42	0.40
375_C2	0.39	0.40	0.41	0.40	0.38	0.38	0.41	0.39
375_I	0.55	0.56	0.56	0.56	0.49	0.51	0.50	0.50
375_PM1	0.36	0.41	0.54	0.44	0.34	0.41	0.54	0.43
375_PM2	0.50	0.50	0.50	0.50	0.42	0.43	0.42	0.42
385_I	0.42	0.43	0.42	0.42	0.41	0.43	0.42	0.42
400_PM	0.61	0.61	0.63	0.62	0.46	0.47	0.50	0.48
429_C	0.35	0.38	0.44	0.39	0.25	0.26	0.32	0.28
429_I	0.41	0.40	0.37	0.39	0.38	0.38	0.37	0.38
429_PM	0.50	0.51	0.51	0.51	0.47	0.48	0.47	0.47
443_I	0.69	0.75	0.80	0.75	0.55	0.62	0.68	0.62
465_C	0.29	0.30	0.33	0.31	0.26	0.28	0.30	0.28
467_C	0.57	0.58	0.58	0.58	0.53	0.54	0.56	0.54
470_I	0.70	0.71	0.71	0.71	0.56	0.56	0.54	0.55
470_PM	0.50	0.49	0.53	0.51	0.44	0.44	0.45	0.44
514_PM	0.55	0.56	0.57	0.56	0.54	0.55	0.56	0.55
516_I	0.37	0.40	0.51	0.43	0.36	0.39	0.45	0.40
527A_PM	0.53	0.56	0.58	0.56	0.48	0.48	0.49	0.48
544_I	0.29	0.30	0.31	0.30	0.32	0.32	0.32	0.32
GAL 001	1.01	1.01	1.01	1.01	1.00	1.00	1.00	1.00
GAL 002	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03
GAL 003	1.01	1.02	1.03	1.02	1.00	1.02	1.03	1.02
GAL 004	1.03	1.03	1.01	1.02	1.03	1.03	1.01	1.02
GAL 005	1.00	1.01	1.01	1.01	1.00	1.01	1.01	1.01
GAL 006	1.03	1.03	1.01	1.02	1.03	1.03	1.01	1.02

GAL 007	1.00	1.00	0.99	1.00	1.00	1.04	1.01	1.02
GAL 008	1.05	1.02	1.02	1.03	1.00	0.99	1.00	1.00
LOR 001	0.96	1.01	1.06	1.01	1.00	0.98	1.01	1.00
LOR 010	1.04	1.04	1.05	1.04	1.03	1.05	1.05	1.04
WIN 001a	1.03	1.06	1.03	1.04	1.02	1.11	1.02	1.05
WIN 001b	1.02	1.01	1.02	1.02	1.02	1.01	1.02	1.02
WIN 001c	1.00	1.01	1.01	1.01	0.99	1.01	1.01	1.00
WIN 001d	0.98	0.98	0.99	0.98	0.98	0.98	0.99	0.98
WIN 002	1.02	1.01	1.02	1.02	1.02	1.01	1.02	1.02
WIN 003a	1.01	1.02	1.01	1.01	1.02	1.01	1.01	1.01
WIN 003bm	1.02	1.03	1.02	1.02	1.02	1.03	1.02	1.02
WIN 004	1.00	0.98	0.98	0.99	1.00	1.01	0.99	1.00
WIN 005	1.02	1.02	1.02	1.02	1.03	1.03	1.02	1.03
WIN 006a	1.03	1.03	1.04	1.03	1.04	1.04	1.05	1.04
WIN 006b	1.04	1.05	1.04	1.04	1.05	1.05	1.05	1.05
WIN 007a	1.06	1.03	1.01	1.03	1.05	1.04	1.02	1.04
WIN 007b	1.03	1.04	1.06	1.04	1.00	1.03	1.03	1.02
WIN 003bd	1.02	0.99	1.00	1.00	1.03	1.03	1.01	1.02
WIN 007c	1.01	1.01	1.00	1.01	1.03	1.03	1.04	1.03
WIN 008a	1.00	0.99	1.01	1.00	1.00	0.99	1.00	1.00
WIN 008b	1.02	1.01	1.02	1.02	1.02	1.01	1.01	1.01
WIN 008c	1.00	0.99	1.01	1.00	1.01	1.03	1.03	1.02
Win 009a	1.05	1.00	0.99	1.01	1.06	0.99	1.01	1.02
WIN 009b	1.03	1.04	1.04	1.04	1.04	1.05	1.04	1.04
WIN 010a	0.73	0.71	0.84	0.76	0.74	0.75	0.78	0.76
WIN 010b	1.01	1.01	1.04	1.02	1.04	1.05	1.04	1.04
WIN 010c	1.05	1.04	1.05	1.05	1.04	1.05	1.04	1.04
WIN 010d	1.04	1.04	1.01	1.03	1.01	1.02	0.99	1.01
WIN 011	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
WIN 012a	1.01	1.01	1.01	1.01	1.01	1.02	1.01	1.01
WIN 012b	1.01	1.02	1.01	1.01	1.00	1.01	1.02	1.01

Appendix III

Certificates of Ethics Approval and Consent Forms Provided to Participants

McMaster University Research Ethics Board (MREB)
c/o Office of Research Services, MREB Secretariat, GH-305, e-mail: ethicsoffice@mcmaster.ca

CERTIFICATE OF ETHICS CLEARANCE TO INVOLVE HUMAN PARTICIPANTS IN RESEARCH

Application Status: New ☑ Addendum ☐ Renewal ☐ Project Number 2006 078

TITLE OF RESEARCH PROJECT:

A Comparison of Current and Novel Measurement Techniques involved in the Description of Transparent Root Dentine in Human Teeth

Name(s)	Dept./Address	Phone	E-Mail
Faculty Investigator(s)/ Supervisor(s)			
S. Saunders	Anthropology	24069	saunders@mcmaster.ca
Student Investigator(s)			
H. Kluge	Anthropology	905-521-8814	klugehf@mcmaster.ca

The application in support of the above research project has been reviewed by the MREB to ensure compliance with the Tri-Council Policy Statement and the McMaster University Policies and Guidelines for Research Involving Human Participants. The following ethics certification is provided by the MREB:

☐ The application protocol is approved as presented without questions or requests for modification.

☐ The application protocol is approved as revised without questions or requests for modification.

☒ The application protocol is approved subject to clarification and/or modification as appended or identified below:

COMMENTS AND CONDITIONS:

See attached.

Reporting Frequency:	Annual:	Other:

Date: May 31, 2006 Dr. D. Maurer, Chair, MREB: Daphne Maurer

UNIVERSITY OF MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. R. Hoppa

Protocol Reference Number: H2002:194
Date of Approval: February 21, 2007
Date of Expiry: November 25, 2007 (new anniversary date)

Protocol Title: "Tooth Cementum Annulation (TCA): A Validation Study"

The following is/are approved for use:

- Annual Approval

The above was approved by Dr. John Arnett, Ph.D., C. Psych., Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated February 13, 2007. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations*.

This approval is valid until the expiry date only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval must be sought from the relevant institution, if required.

Sincerely yours,

John Arnett, PhD., C. Psych.
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary. **Telephone:** (204) 789-3255 / Fax: (204) 789-3414

www.umanitoba.ca/faculties/health/research_ethics

PARTICIPANT INFORMATION SHEET

Title of Study: An Examination of Age-Related Changes in Tooth Tissue

Locally Responsible Investigator and Principle Investigator: Hagen F N Kluge
Title, Institution: Master's Student, McMaster University Department of Anthropology
Supervising Investigator: Dr. Shelley R Saunders
Title, Institution: Professor of Anthropology, McMaster University Department of Anthropology
Sponsor: Social Sciences and Humanities Research Council of Canada

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You are being invited to participate in a research study conducted by Hagen Kluge, a Master's student at McMaster University, because you have recently had a tooth removed by extraction. In order to decide whether or not you want to be a part of this research study, you should understand what is involved and the potential risks and benefits. Please take your time to make your decision.

WHAT IS THE PURPOSE OF THIS STUDY?

The research in which you have been invited to participate will measure age-related changes in teeth with more advanced instruments. This research has the potential to help law enforcement personnel quickly and accurately identify human remains.

WHAT ARE MY RESPONSIBILITIES IF I TAKE PART IN THIS STUDY?

It is important for you to know that *you do not have to donate your tooth for use in this study*. If you volunteer to participate in this study you will be asked to donate your previously extracted tooth for analysis. You will also be asked to provide your date of birth and sex. However, *no records, which identify you by name, will be kept*. If you donate your tooth for this research *you have the option of removing both your tooth and the information you have provided from this study*. However, any data generated from research involving your tooth will remain the property of the researcher. At the completion of this study, both the tooth and the information you have provided will be kept in the research collections of the Department of Anthropology at McMaster University.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

Your decision to participate in *this study will not expose you to any additional physical or psychological harm*. This research protocol has been reviewed and approved by the McMaster Ethics Review Board.

IF I HAVE ANY QUESTIONS OR PROBLEMS, WHOM CAN I CONTACT?

If you have any questions about the research now or later, please contact Hagen Kluge at klugehf@mcmaster.ca or Dr. Saunders at saunders@mcmaster.ca If you have any questions regarding your rights as a research participant, you may contact the McMaster Ethics Review Board at 905-525- 9140 ext. 23142 or by email at ethicsoffice@mcmaster.ca

CONSENT STATEMENT

SIGNATURE OF RESEARCH PARTICIPANT:

I have read the preceding information thoroughly. I have had the opportunity to ask questions, and all of my questions have been answered to my satisfaction. I agree to participate in this study. I understand that I will receive a signed copy of this form.

_____ _____

Name of Participant Date

Specimen Number__________________
Date of Extraction (Y/M/D)___________
Date of Birth (Y/M/D)________________
Sex: Male____ Female____
Tooth Vital on Extraction? Y___N___
Evidence of Root Pathology? Y___N___

Specimen Number__________________
Date of Extraction (Y/M/D)___________
Date of Birth (Y/M/D)________________
Sex: Male____ Female____
Tooth Vital on Extraction? Y___N___
Evidence of Root Pathology? Y___N___

Specimen Number__________________
Date of Extraction (Y/M/D)___________
Date of Birth (Y/M/D)________________
Sex: Male____ Female____
Tooth Vital on Extraction? Y___N___
Evidence of Root Pathology? Y___N___

Specimen Number__________________
Date of Extraction (Y/M/D)___________
Date of Birth (Y/M/D)________________
Sex: Male____ Female____
Tooth Vital on Extraction? Y___N___
Evidence of Root Pathology? Y___N___

Specimen Number__________________
Date of Extraction (Y/M/D)___________
Date of Birth (Y/M/D)________________
Sex: Male____ Female____
Tooth Vital on Extraction? Y___N___
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Specimen Number__________________
Date of Extraction (Y/M/D)___________
Date of Birth (Y/M/D)________________
Sex: Male____ Female____
Tooth Vital on Extraction? Y___N___
Evidence of Root Pathology? Y___N___

Specimen Number__________________
Date of Extraction (Y/M/D)___________
Date of Birth (Y/M/D)________________
Sex: Male____ Female____
Tooth Vital on Extraction? Y___N___
Evidence of Root Pathology? Y___N___

Specimen Number__________________
Date of Extraction (Y/M/D)___________
Date of Birth (Y/M/D)________________
Sex: Male____ Female____
Tooth Vital on Extraction? Y___N___
Evidence of Root Pathology? Y___N___

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