1 A Brief History of Cemental Annuli Research, with Emphasis upon Anthropological Applications

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> While cementum, from a purely histological point of view, is the least interesting of the three calcified dental tissues, chiefly on account of the fact that its structure and functions are of a simpler character than those of enamel and dentine, and therefore do not admit of the possibility of so many unsolved problems and so much controversy.
>
> (Hopewell-Smith 1920: 59–60)

Although the authors of this volume would doubtless dispute the notion that cementum is the least interesting of the dental tissues and that there are few unsolved problems concerning its formation, Hopewell-Smith's 1920 statement clearly reflects the storied history of cementum studies prior to the twentieth century. Cementum, due to its relative invisibility, was discovered a century after tooth enamel and dentin. Famous natural philosophers and anatomists debated nomenclature and function, as well as its presence across vertebrate species. Advances in imaging technology led to nineteenth-century debates about the presence of cells and the function of cementum, although texts of the period focused upon dentin and enamel as the primary – if not exclusive – dental structures.

Even without further knowledge of proximate causes, however, during the twentieth century, cementum's apparent age-related thickness and then its regular encircling deposits attracted the attention of wildlife ecologists, followed by archaeologists and forensic scientists. Key in the history of human applications was an article by Stott et al. (1982) that stimulated considerable anthropological interest. These researchers counted light-dark bands or "annuli" in three adults more than fifty years of age and showed close correspondence between annulus count added to tooth-specific eruption and chronological age.

Though interest in Stott's approach developed, it built slowly. Histological preparation methods presented few problems, and many techniques provided satisfactory results. Methods for counting annuli proved challenging, however, leading some workers to consider age estimations using annulus counts intractable, despite mounting evidence to the contrary. A number of validation studies, reviewed here, have proved encouraging, countering concerns about inaccuracy due to factors such as

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periodontal disease, slowed or erratic deposition in old age, and the impact of systemic health conditions, such as diabetes and tuberculosis. Today annulus counts, termed the Tooth Cementum Annulus or TCA method since the early twenty-first century, excite cautious optimism not only for age estimation in mammals, including humans, but also for recording other life-history events relating to gestation, health, and seasonality. Complete tooth or even tooth root destruction is not necessary, and the tooth can be recorded and modeled digitally. While mysteries remain, such as the proximate causes for the annuli, focused research should provide resolution in the near term.

The present volume is, therefore, timely, an interdisciplinary stocktaking of contemporary knowledge and future promise for these long overlooked and underappreciated cementum structures. We begin our historical treatment by focusing first upon the identification of cementum as a tissue distinct from dentin and enamel, followed by considerations of cemental annuli as they became accepted as normal annular dental structures, characteristic of mammals, including humans.

1.1 History of Cementum Discovery and Early Characterizations

Although earlier observers had explored dental structures, it was not until the development of magnifying lenses that cementum was identified. As recounted first by Denton (1941) and then by Foster (2017) in their excellent reviews of the discovery and characterization of cementum from the seventeenth through the nineteenth centuries, technological advances were necessary for this minor but important component of teeth to be recognized and studied (Figure 1.1).

The Greek physician Galen (130–200), writing during the second century AD, set the tone for knowledge of the oral cavity that would only be superseded by Renaissance and Enlightenment anatomists, whose communications benefited from the earlier development of the printing press during the mid-fifteenth century. Galen appreciated that adult humans developed thirty-two teeth, which he divided into incisors, canines, and molars, contrasting the sharp and cutting anterior teeth with those suited for grinding. Comparisons with animals, such as lions, dogs, and cattle, reinforced his discussions of functional associations. He recognized variations in the

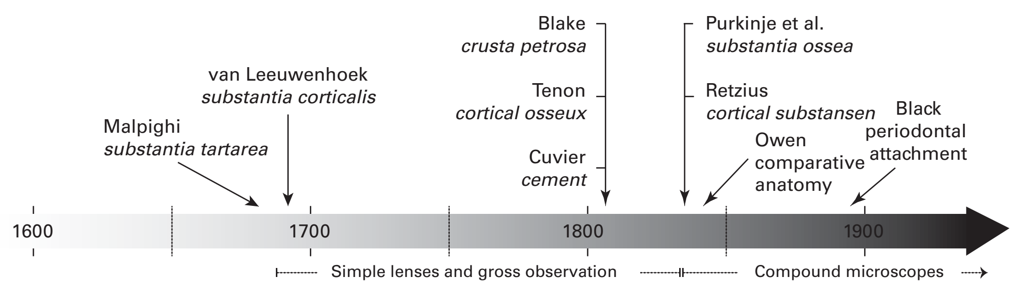

Figure 1.1 Time line of discovery of cementum discussed in detail in the text (based on data from Foster 2017: 2).

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number of tooth roots, while also remarking upon “strong ligaments” that bound human teeth to the alveoli “especially at the roots” (Shklar and Brackett 2009: 26).

It was not until the mid-sixteenth century that anatomists added significant anatomical knowledge of the oral cavity. Among the most well-known was Andreas Vesalius (1514–1564), whose publication in 1543, *De humani corporis fabrica* contained an eleventh chapter of Book One, titled, *De Dentibus, qui etiam ossium numero ascribuntur*, translating to “On the Teeth, Which Are Also Counted as Bones” (Hast and Garrison 1995) or “On the Teeth, which are also included in the number of the bones” (Saunders and O’Mally 1944). Within this essay, he followed Galen’s discussion of tooth numbers and variability in crown shape and numbers of roots (Figure 1.2A), although, in general, *De humani corporis fabrica* was designed to address many of the errors Vesalius perceived in Galen’s work. Vesalius also perpetuated the interpretation of deciduous teeth exfoliating their “appendages,” or crowns, while retaining the roots, upon which the permanent crowns would develop. Comparisons to the structure of bone also anchored his interpretations. Importantly, he described and illustrated the pulp cavity of a tooth, giving lie to the assumption that teeth are solid structures. Vesalius said that this cavity functioned to make the teeth lighter in weight and facilitated the delivery of nourishment (Hast and Garrison 1995).

Vesalius’ contemporary and protagonist, Bartolomeo Eustachio (1520–1574), in his 1563 *Libellus de Dentibus* (Figure 1.2B), clearly illustrated and distinguished enamel and dentin, comparing these two components to the bark of a tree and its softer, more vulnerable inner portion (Bennett 2009; Trenouth 2014). Eustachio also corrected Vesalius’ interpretations of the development of the “milk” dentition. He is credited with the detailed study of the dental pulp cavity and the periodontal ligament (Bennet 2009; Shklar and Chernin 2000). In chapter 4 (of thirty) in his treatise on the dentition, he notes that “there are extremely strong fibers attached to the roots, which provide a firm connection to the socket” (Shklar and Chernin 2000: 28). Thus, careful macroscopic observations had identified enamel, dentin, and the periodontal ligament. Microscopy and then histology would be required to establish the nature of cementum.

Marcello Malpighi (1628–1694) is cited for the first formal recognition of cementum (Denton 1941; Foster 2017). Employing a single-lens microscope, he observed a “substantia tartarea,” covering the human tooth root (Figure 1.2C), distinct from the “substantia filamentosa,” which he said enveloped the upper part of the tooth. His characterization is thought to date to approximately 1667, although published posthumously in 1700. A less clear, but possible seventeenth-century recognition of the cementum layer in a calf was termed “substantia corticalis” by the Dutch draper, Antoni Van Leeuwenhoek (1632–1723), whose observations were also aided by the simple magnifiers that he helped develop (Foster 2017; van Zuylen 1981).

Discoveries of cemental structures in nonhuman mammals have helped stimulate knowledge development of human cementum. For example, more than a century later, physician Robert Blake (1772–1822) identified a “crusta petrosa” covering the roots of

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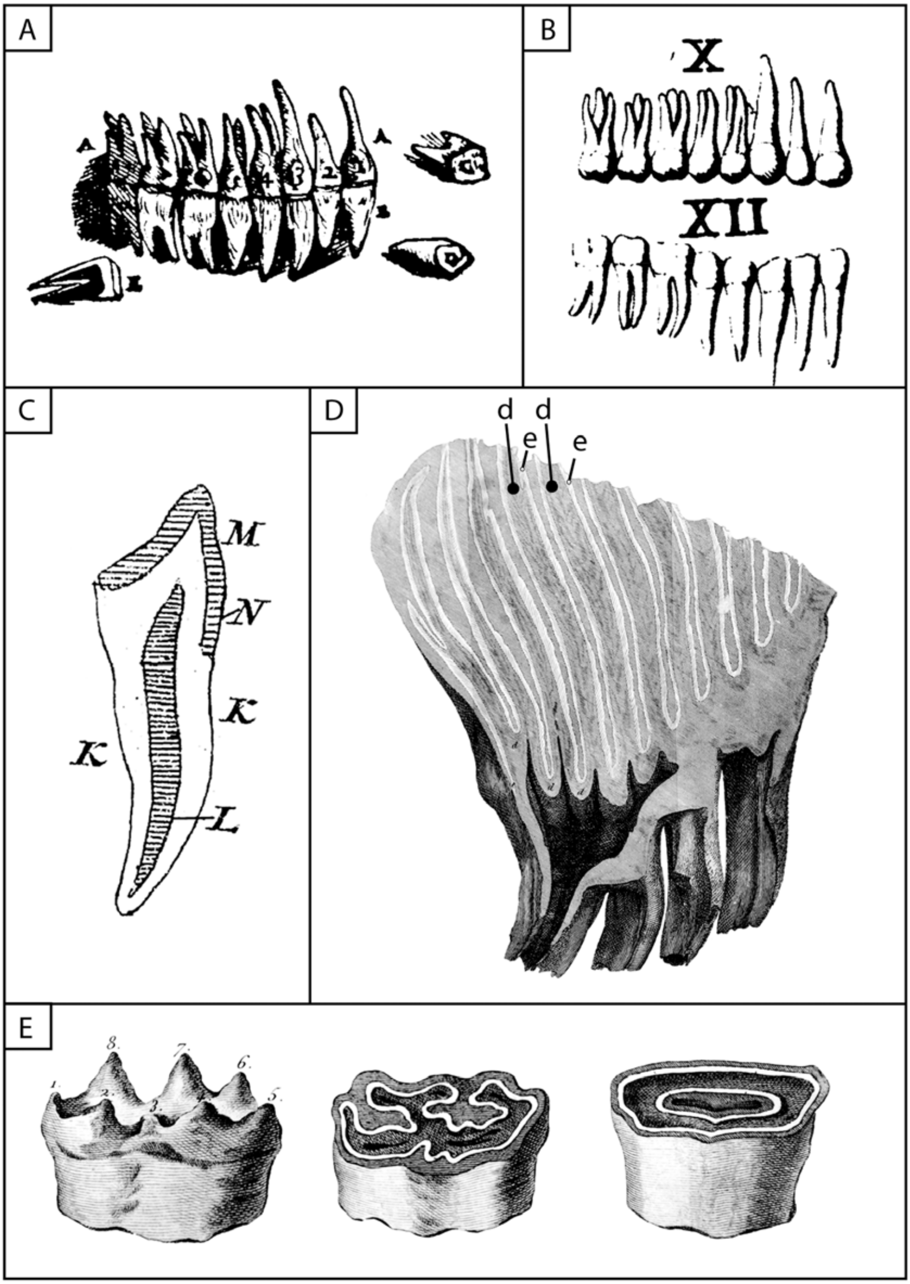

Figure 1.2 Discovery of cementum in the seventeenth to eighteenth centuries. (A) Diagram of the human dentition, including teeth in cross-section, by Andreas Vesalius (Vesalius, 1543). (B) Illustration of the human dentition from the *Libellus de Dentibus* (Eustachio, 1563). (C) Diagram of a human tooth by Marcello Malpighi (Malpighi, 1700; table 2, figure 4 from his *Opera Posthuma* – (M) *substantia filamentosa* = crown enamel; (K) *substantia tartarea* = cementum). (D) Elephant molar section by Robert Blake, showing the complex folded crown and root – (d) "bony part of the tooth" = dentin; (e) the cortex striatus = enamel; the *crusta petrosa* (cementum)

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herbivorous mammals such as elephants and horses (Figure 1.2D), being especially thick in an elderly equid. Blake's 1798 thesis in Latin at the University of Edinburgh and its 1801 translation provided an overview of dental structure in humans and other animals (Trenouth 2014). Blake did mention this hardened layer in association with hypercementosis in a single human tooth, but he did not readily generalize his observations from grazers to humans. Cementum in equids was also recognized by Jacques Rene Tenon (1724–1816), a French surgeon and pathologist whose "cortical osseux" was indeed a specialized dental layer (Figure 1.2E). The term "cement," a precursor to "cementum," was coined by the eminent comparative anatomist Georges Cuvier (1769–1832) to reference the substance uniting the plates of elephant teeth. He recognized the presence of cement in many species but erred in arguing that the substance lacked a recognizable structure (Foster 2017).

Although most eighteenth-century researchers failed to identify cementum on human teeth, an exception was the dentistry text of Carl Joseph von Ringelmann (1776–1854), published in 1824. Ringelmann, a practitioner, argued that the "horny substance" previously reported by Blake and others as a pathological condition associated with tooth roots was instead ubiquitous across the human species (Foster 2017).

During the nineteenth century, the creation of compound microscopes facilitated observations of cementum details. The Czech anatomist Jan Purkinje (1787–1869) reported a "substantia ossea" as regularly present on human teeth, represented in the drawings of his student, Meyer Fränkel (1835), as laminated but otherwise unstructured (Figure 1.3). Anders Retzius (1796–1870) described a "cortical substansen" (1837) covering the human tooth root in greater detail, including its greater thickness at the apex and its increased thickness with age.

It was the extensive comparative studies of the British naturalist and paleontologist Richard Owen (1804–1892) that truly established the vertebrate patterning for "ciment," Cuvier's term that Owen embraced. Owen clearly recognized parallels between cementum and bone development, though the relationship between cementum and ligaments attaching the tooth to alveolus was not defined. His detailed volume, *Odontography*, compiled between 1840 and 1845, represented a monumental treatment of teeth across living and fossil vertebrates. In this, he coined the term dentine (Trenouth 2014).

A further contribution by American practitioner and researcher Greene Vardiman (G. V.) Black (1836–1915) established the nature of the periodontium, including

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Caption for Figure 1.2 (cont.)

fills the space between adjacent enamel plates (Blake, 1798). (E) Diagrams of horse molars and incisor by Jacques Tenon, indicating the layers of cortical osseux (cementum; shaded area), enamel (whitish layers), and dentin (cross-hatched layers) (Tenon, 1797). Leftmost image indicates a lower third molar upon eruption, with cementum-covered cusps intact, while the middle image indicates the same tooth after attrition, revealing the complex layers. Right image indicates similar tissue layering in the incisor tooth (based on data from Foster 2017).

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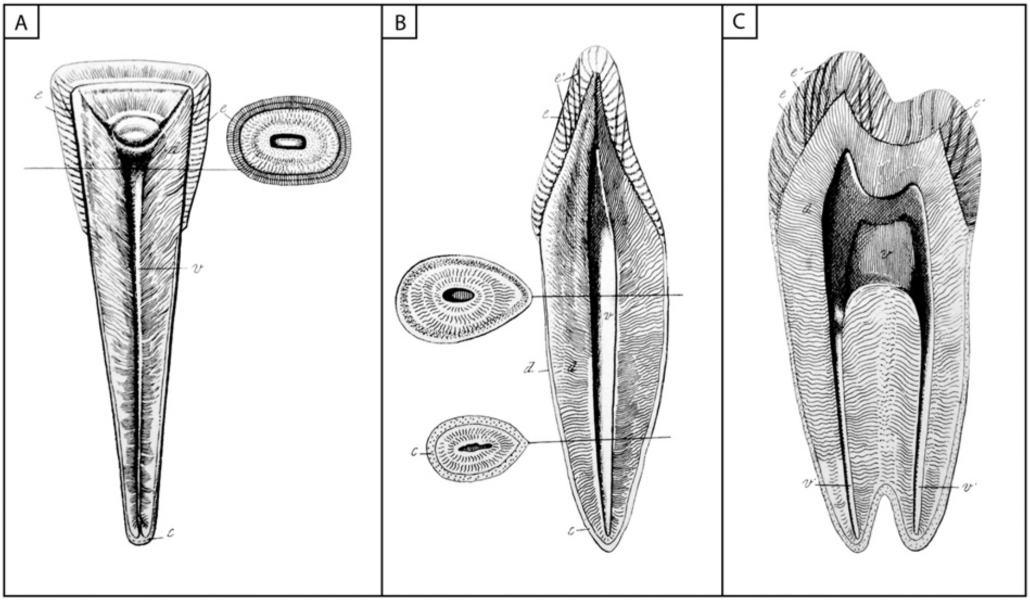

Figure 1.3 Discovery of human cementum in the nineteenth century. (A) Diagram of a longitudinal coronal section of a middle lower incisor by Fränkel (1835) showing dentin (d), enamel (e), and thin *substantia ossea* (acellular cementum) covering the root (c). Transverse cross-section shows the tissues of the crown. (B) Another section of an incisor (with a more sagittal orientation) by Fränkel shows another view of the *substantia ossea* (cementum) distribution, including two cross-sections through the root. (C) Longitudinal section of a lower premolar by Fränkel showing substantia ossea (cementum). Fränkel denotes the presence of *osseous corpuscula* (cementocytes) by dots within the *substantia ossea* (based on data from Foster 2017).

cementum, periodontal ligament, alveolus, gingiva, and supporting vessels and nerves (Garant 1995). The lamellar nature of cementum was established, along with parallels and distinctions between bone-forming and cementum-forming cells (Figure 1.4). Moreover, the alternating deposits of lamellae and incremental lines of the cementum were identified (Black 1887: 105–6).

Thus, by the twentieth century, cementum had been established as a fundamental part of the dental apparatus in vertebrates, with lamellar structure identified in humans and other mammals. The least visible of the three hard dental tissues, it presented cellular and acellular structure, the former more obvious in the thicker apical portion than in the thinned portion near the cervix. It was also intimately linked to the periodontal ligament. Many observations of cementum in humans developed following observations in other species. Most researchers emphasized inter individual variation in the development of cementum within human cohorts, Black's "utmost irregularity" (1887: 106). It would take a considerable part of the twentieth century for this argument to be countered, first by simply measuring cementum thickness across the human life span and also by incorporating comparative mammalian studies into annulus formation models.

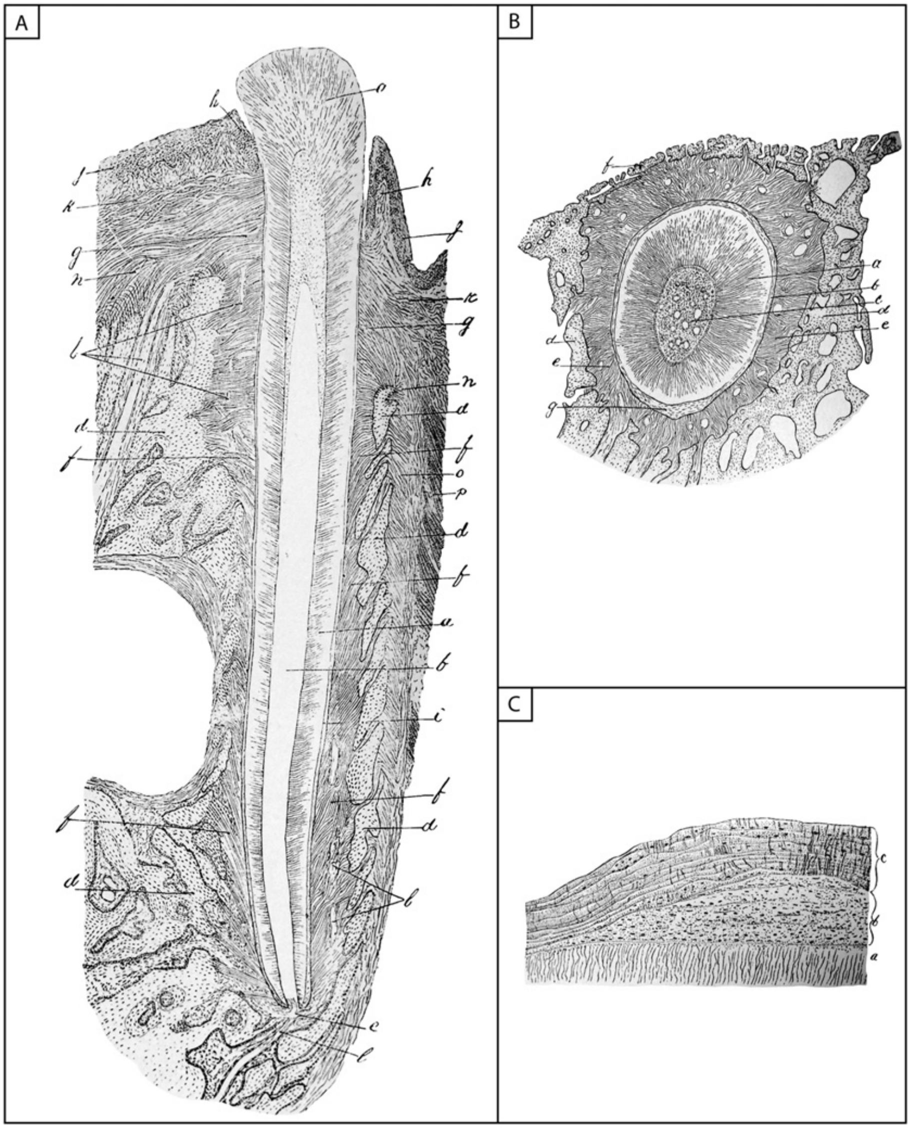

Figure 1.4 Elaboration of the periodontal attachment function. Figures adapted from G. V. Black's *A Study of the Histological Characters of the Periosteum and Peridental Membrane* (1887). (A) Section of a small incisor from a kitten showing the relationship of the tooth to the surrounding periodontia, including detailed depiction of the PDL and alveolar bone. Black noted this tooth was in total only 1/4-inch long. (B) Cross-section of a human incisor tooth illustrating the PDL fibers radiating from the tooth root cementum to the surrounding alveolar bone. (C) Cementum hypertrophy in the cellular cementum of a premolar tooth. Black noted that only the first cementum lamella appeared unusual (labeled by the author as b), while subsequent lamellae (c) presented normal thickness (based on data from Foster 2017).

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1.2 Early Twentieth Century Knowledge of Cementum: 1900–1982

Correlation of Cemental Thickness with Age in Humans

By the beginning of the twentieth century, the basic anatomical structure of the tooth was known. Cementum had been described as cellular and acellular, encasing the tooth root, sometimes overlaying enamel at the cervix, linked to fibers that anchored the tooth to the crown, and more obvious in other mammals than in humans. For our purposes, the early observations made by Magitot (1878) and Black (1887) concerning the increasing thickness of cementum with age in humans were of special significance. This age-related pattern in humans was accepted by many other workers during the final years of the nineteenth and the first half of the twentieth centuries, e.g., Broomell (1898), Gottleib (1943), Kronfeld (1938), and Stillson (1917).

Alternative positions also persisted into the twentieth century, with researchers such as Hopewell-Smith (1920: 64) maintaining that normal cementum was acellular and lacked "histological pattern or design." Even Hopewell-Smith, however, accepted the premise that cementum thickened with age but argued that bounding incremental lines were abnormal and not to be found in "perfectly formed tissue" (Hopewell-Smith 1920: 66).

Skeptics such as Weibusch (1957) persisted in arguing that the deposition of cementum was atypical. In response, Zander and Hürzeler (1958: 1035) developed one of the more rigorous quantifications of the relationship between cementum thickness and age in humans.

Zander and Hürzeler (1958) employed 233 single-rooted healthy teeth from individuals ages eleven to seventy-six. Decalcified and embedded in celloidin, the teeth were sectioned horizontally at eighty microns and stained with hematoxylin and eosin. Sections magnified twenty-five times via an overhead projector microscope were traced on file-folder paper, cut out, weighed, and measured. These observations, facing the methodological issues of characterizing the cementum, used a novel method that facilitated statistical treatment. The authors concluded that their results "showed a straight-line relationship between age and cementum thickness." The thickness of cementum was approximately tripled between the ages of eleven and seventy-six years. This rate was not the same for every area of the root. Cementum accrual was less near the cement-enamel junction and greater in the apical area (Zander and Hürzeler 1958: 1043).

Arguments also developed over the degree to which cementum thickness was a response to biomechanical stressors or associated with oral disease. Azaz and colleagues (1974), by studying cementum thickness in sixty impacted canines and premolars, observed cemental thickening with age in healthy teeth that were not biomechanically stressed, further reinforcing a relationship between cementum thickness and age.

Though it was generally accepted that cementum was thicker in older ages than in youth, the first attempt to explicitly use cementum thickness in estimating age was that of Gustafson (1950), who incorporated a four-stage gradation of cementum thickness

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(normal, slightly greater than normal, great, and heavy) as viewed in section within the apical portion. Noting that grading cementum was especially "difficult" (Gustafson 1950: 48), Gustafson summed across the six variables (attrition, secondary dentin, periodontosis, cementum apposition, root resorption, and root transparency), calibrated in terms of nineteen teeth from individuals of known age. He reported a standard error of 3.63 years. While each of these features has been critically evaluated and reservations expressed (e.g., Dalitz 1962), Gustafson's study remains a landmark effort, upon which other methods of forensic and bioarchaeological age estimation have been based.

Wildlife and Zoo Studies of Cementum Annulation

As in earlier centuries, twentieth-century studies of cementum were informed significantly by comparative studies of other vertebrates, especially mammals. Beginning at mid-century, a number of studies of marine and terrestrial mammals, including various species of pinnipeds, focused upon a hypothesized relationship between age and annulus counts.

Although the earliest studies of seals focused upon root dentine (Scheffer 1950; Laws 1952), cementum layers were soon added to the mix as it became clear that dentine development does not extend into older adult age groups for these marine mammals. Studies focused on large ungulates such as the moose (Sergeant and Pimlott 1959), red deer (Mitchell 1963; Keiss 1969), reindeer (McEwan 1963; Low and Cowan 1963), white-tailed deer (Ransom 1966; Gilbert 1966), mule deer (Erikson Seliger 1969), and bison (Novakowski 1965), all first synthetized by Klevezal' and Kleinenberg (1967, 1969).

Studies were designed to address age-at-death, reproductive status, growth and weight by age, and related issues. Periodicity was assumed to be related to factors such as migration and periods of fasting. Periods of fasting, hibernation, and migration were also hypothesized causes for terrestrial mammal cemental annuli, as well as seasonal rainfall.

By the end of the 1960s, many of these studies experienced validation problems, in that age-at-death or tooth extraction were not known but estimated through other means. Reference collections started to be built along with standardization of analyses (Saxon and Higham 1968; Klevezal' and Kleinenberg 1969; Morris 1972), which also prompted the first fossil implementation on Neolithic samples (Saxon and Higham 1969).

Several researchers who focused upon African animals reported line doubling (two lines per year), which they attributed to bimodal rainfall in the equatorial zone for buffalo, waterbuck, and two species of gazelle (Spinage 1973; Spinage 1976a, b). However, examples of doubling in animals such as greater kudu and bushbuck from unimodal rainfall areas suggested that the simple argument linking line doubling with rainfall patterning lacked explanatory power. Spinage's (1976b) results from a study of twenty-two buffalo first incisors, along with a miscellany of other teeth from areas with unimodal annual rainfall, generally supported the formation of one line per year.

Methodological issues were raised in recognition of doubling and a need for further controlled research endorsed (Spinage 1976b).

The range of species rapidly expanded in the next two decades to maritime mammals (Kasuya 1976, 1977; Kasuya and Matsui 1984); insectivores, such as the hedgehog, the mole, or the bat (Grue and Jensen 1979); and rodents, such as the beaver, ground squirrel, rats, mice, porcupines, rats, rabbits, and voles (summarized in Klevezal' 1996).

This development led to the first archaeological study on prehistoric hunting strategies in France (Spiess 1976). However, the protocol did not include embedding the teeth and was thus not suitable for archaeological remains. Stallibrass (1982) was the first to propose a dedicated method for ancient remains with resin embedding and polarized microscopy.

By the time of Stott et al.'s seminal publication (1982: 814), a range of animals had been studied, including bear, caribou, moose, elk, deer, bison, red fox, coyote, otter, squirrel, and two species of primates – one an Old World macaque and the other a marmoset from the Americas. Additional New World primates representing two species of *Saguinus* were reported by Yoneda (1982), who associated dark bands with possible environmental and endogenous factors, the former including the beginning of the rainy season and the latter being the coincident breeding season. Today, more than seventy-two species of terrestrial and maritime mammals across twenty-one families, and nine orders have been successfully documented.

Uncertainty concerning the proximate cause(s) for line formation has continued into the twenty-first century.

Late Twentieth- and Twenty-First Century Knowledge of Cementum Annuli and the Development of Dental Cementum Analysis

At the cusp of the twenty-first century, an authoritative review of contemporary knowledge about cementum was published in *Periodontology* (Bosshardt and Selvig 1997). We refer the reader to Foster et al. (Chapter 2) for a detailed summary of cementum biology and function. Thus, at this point in time, the anatomy of cementum was largely known, although the causes of annulus formation remained speculative. Comparative studies had suggested circannual season cycles of sunlight, nutrition, and related environmental factors (Introduction). Even without knowledge of proximate causes, the ultimate cause of annulus formation appeared securely tied to yearly cycles in humans, as in other mammals.

We now turn our focus to these annuli and their ability to monitor time and life histories in humans, as explored during the final decades of the twentieth century and the early portion of the twenty-first century.

> Countable cemental annulations are present in human teeth. Cross-sections through undecalcified tooth roots can be properly stained and mounted so that cemental annulations can be photographed through a light microscope. Annulations counted from a photograph provide a close estimate of the actual age of the individual from which the tooth was extracted. This technique may be extremely valuable in forensic medicine, forensic dentistry, and anthropology. (Stott et al. 1982: 816)

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The highly influential paper by Stott et al. (1982) stimulated considerable interest in using cemental annulus counts to estimate age-at-death in humans from medico-legal and archaeological contexts. Stott and colleagues, explicitly influenced by the annulus studies of pinnipeds and terrestrial mammals, demonstrated that annulus counts in teeth from three human beings, all in excess of fifty years of age (fifty-seven, sixty-seven, and seventy-six), could be used to estimate age-at-death when counts were added to age at eruption. In a detailed and systematic statement, they described the use of undecalcified, stained, transverse 100–150 μm tooth sections. Thus, they presented readily replicable methods for achieving impressive results for individuals more than fifty years of age, addressing the persistent problem of estimating age-at-death in human remains, whether from archaeological or medico-legal contexts.

Influenced by Stott et al. (1982), as well as the many mammal studies, Naylor and colleagues (1985: 197) presented a method designed to "develop a rapid, controlled technique to enhance and distinguish human cemental annulations with a significant degree of repeatability." Using single-rooted teeth extracted in blocks from cadavers, the authors removed a section 15 to 45 percent of the distance from the root apex to the neck. The most desirable section thickness, they said, was 100 microns, and etched undecalcified sections were preferred for a very practical reason. "Decalcification may require days or weeks depending on the solutions used and if not watched closely may dissolve the entire tooth resulting in complete loss of what may be an irreplaceable specimen" (Naylor et al. 1985: 198, 200). Stains recommended were, first, 0.1 percent cresyl fast (etch) violet in 70 percent alcohol for three minutes, with a second choice being a 5 percent toluidine blue in 70 percent alcohol for forty-five seconds. The authors recommended photomicrographs of the mounted sections, followed by photographic enlargements.

Validation Studies 1986–2001

Naylor's method had presented a refinement of Stott et al.'s protocol, which appeared to anticipate an orderly progression toward acceptance of TCA as an accurate method for estimating age-at-death in human skeletal remains. Further validation tests would be needed, of course, and these followed, with somewhat contradictory results. Table 1.1 summarizes key elements of TCA methodologies and results as they developed during the final decades of the twentieth century and the early portion of the twenty-first century. These tables extend and update earlier versions by Wittwer-Backofen and Buba (2002) and Naji et al. (2016).

Two linked studies directly stimulated by Stott et al. (1982) were explicitly designed to explore methodological best practices and accuracy (Charles et al. 1986; Condon et al. 1986). Our research team collected a relatively large sample of known age teeth from Midwestern US clinical and cadaver contexts. Canines and premolars were chosen from among single-rooted teeth due to availability, with a usable sample from approximately seventy individuals, with numbers varying by test. Both mineralized and demineralized sections were considered. The middle third of the tooth was chosen because of visualization difficulties nearer the crown due to thinned annuli and the obscuring effects of cellular remnants and resorption spaces nearer the apex. This

Table 1.1 List of validation studies. Column abbreviations: (1) S: single-rooted; all*: except M3; I: incisor; C: canine; P: premolar; M: molar; (2) 1. outlier; 2. no visible line; 3. poor quality; 4. fractured; 5. taphonomy; 6. ROI not suitable; (3) T: transverse; L: longitudinal; (4) M: middle 1/3; C: coronal 1/3; A: apical.

Reference		Sample characteristics						
Authors	Year	Teeth[1]	N. Teeth	N. Subjects	N. Eliminated	Reason[2]	Age Range	Pathology
Aggarwal et al.	2008	All	30	20			13–69	Y
Avadhani et al.	2009		25	25	6	6	~16–73	N
Bertrand et al.	2018	C	200	200				N
Blondiaux et al.	2006		112	76			~12–85	
Bojarun et al.	2003		227	178			~11–78	N
Broucker et al.	2016	all	41	18	6	2;4;5	34–78	Y
Caplazi	2004	P	49	49	1	1	16–60	Both
Colard et al.	2015	I,C	9	9			35–76	N
Condon et al.	1986	C,P	73	80	7	1;2	~11–70	Both
Dias et al.	2010		55	42	24	6	17–77	Both
Gowda et al.	2014	P	15	15			20–60	
Grosskopf	1990	all	36	36	4	1	~11–45	Both
Gupta et al.	2014	all	100	100			25–60	Both
Jankauskas et al.	2001	all	51	49	8	1	~10–69	
Joshi et al.	2010		30	30	5	6	20–70	Both
Kagerer & Grupe	2001a,b	all	80	80			Avg = 57.5	N
Kasetty et al.	2010	I,C,P	200	200			17–60	Both
Kaur et al.	2015	all	60	60			Avg = 42.8	Both
Kvaal & Solheim	1995	C,P	95	95			13–89	Both
Lipsinic et al.	1986	P	31	31	1	1	~11–60	N
Lucas & Loh	1986	I,C,P	45	41			~11–80	?
Meinl et al.	2008	S	67	37			20–91	N
Miller et al.	1988	S	100	100	29	3	~10–78	
Padavala and Gheena	2015	all	20	20	4	2	32–72	Both
Pilloud	2004	P	42	24	8	3	21–90	N
Pundir et al.	2009	S	52	40	12	4	22–67	Y
Rao & Rao	1988	all*	15	15			14–56	N
Ristova et al.	2106	S	11	15	4	2;4	55–76	Y
Sousa et al.	1999	all	17	17			23–77	Both
Stein & Corcoran	1994	I,P	52	42			27–84	Y
Stott et al.	1982	all	3	3			57–76	
Shruthi et al.	2015	S	150	150			15–75	N
Swetha et al.	2018	I,C	80	80			22–60	Y
Wittwer-Backofen & Buba	2002	S	42	42			17–81	Both
Wittwer-Backofen et al.	2004	S	433	211	70	3;6	~10–96	Both

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Table 1.1 (cont.)

Thin-section preparation					Microscopy				Results		
Decal-cified	Cut[3]	Region[4]	Width (µm)	Multi. cuts	Light	Zoom	View mode	Multi. obs.	r max.	r min.	Accuracy
No	L	A;M		No	P;BF		Software	Yes	0.95		
No	Both	M		No	BF	×5	Enlarged	Yes	0.95		~2–3
No	T	M	100	Yes	BF	×200–400	Software		0.93		
No	T	all	80–100	Yes	BF	×20	Software	Yes	0.88		Avg = 4.29
No	T		33–35	Yes		×20 –40	Software	Yes	0.95		5.58; 3.71 <50/X/7.86>50
No	T	A;M	100	Yes	BF	×200	Software		0.92	0.62	Avg = 2–3
No	T	A;M	60–100	Yes	Ph	×100–400	Projected		0.89	0.75	
No	T	M	100	Yes	P; BF	×400	Software	Yes	0.96		2.3–6.0
Yes	L		7	No	BF	×400	Projected	No	0.95	0.73	SE = 9.7/7.4; 4.7/9.4
No	T	M	30	No			Software	Yes	0.74	0.06	Avg = 9.7/1.6/22.6
No	T	M		?	Ph	×100	Software		0.97		
No	T	M;C	100	Yes	Ph	×200	Photo		0.84	0.75	Avg = 3.23/2.31
No	L	A;M		No			Software		0.99		
No	T	A	35–100	Yes	BF		Binoculars	Yes	0.88		Avg = 6.46
No	L	M		No			Photo		0.73	0.4	
No	T	C; M	70	Yes			Software	Yes			Avg 5.7/3.9
No	L		100	No	P	×100	Software	Yes	0.42		Predication ±12
No	L	M			P;BF;Ph		Photo		0.99	0.35	
Yes	L	all	5–7	Yes	Fl		Software	Yes	0.84	0.74	
Yes	T	M	5	Yes	BF	×100		Yes	0.93	0.51	SE = 8; 2.2 <30/8.5>30
No	T	M	≤150	No		×100–400		Yes	0.45		
No	T	M	90–100	Yes			Software	No	0.92		Avg = 6.9
No	T	M	350	Yes		×90	Projected	Yes			85% > ±10
No	L	A;M		No	P		Software		-0.015		
No	T	A;M	60–80	Yes	Ph	×100	Software		0.85	0.03	57–90 = 12.7 / 57–77= 8
No	L	A;M	80	Yes	P;BF;Ph		Software	Yes	0.98		
No	L	A;M	4–5	No	BF	×400	Screen	Yes	0.99		14/15 (93% between ±1–2)
No	L	all		No	SEM		SEM	Yes	0.95		±4%
both	T	M	100	Yes			Photo		0.97		
No	L		500	No		×100	Photo		0.98	0.93	
No	T	C;M	100–150	Yes	BF		Photo		0.998		
No	L	A;M	150	No	BF				0.98		3.6
No	L	C;M	80		Ph	×200	Software		0.96		2.6
No	T	M	70–80	Yes	BF	×400–500	Software	Yes	0.94		
No	T	M	70–80	Yes	BF		Software		0.98	0.97	2.5

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Table 1.1 (cont.)

S: single-rooted	1. outlier	T: transverse	M: middle 1/3
all*: except M3	2. no visible line	L: longitudinal	C: coronal 1/3
I: incisor	3. poor quality		A: apical 1/3
C: canine	4. fractured		
P: premolar	5. taphonomy		
M: molar	6. ROI not suitable		

portion of the tooth continues to be preferred in TCA studies today (Naji et al. 2016). The Charles et al. (1986) paper concluded that repeatability was significantly greater than in other macroscopic methods, that demineralized sections of premolars performed best, and that efficiency was increased most by counting multiple sections and then by increasing observers. Demineralized sections, while performing well in validations tests, are generally considered less desirable than mineralized sections, due to the fragile nature of archaeologically recovered remains, as Naylor et al. (1985) had emphasized.

In a companion paper, Condon et al. (1986) explicitly considered accuracy. Using approximately the same sample, the researchers reported an *r* of 0.78 for the total sample ($N = 73$) and 0.86 for fifty-five individuals with no evidence of periodontal disease. Error estimates averaged 6.0 years, with notable sex differences ($M = 9.7$ and $F = 4.7$). Individuals with anomalous doubling (9 percent) and absence of lines (4 percent) were also noted. The authors concluded (Condon et al. 1986: 329) that the cemental annulation method "compares favorably with the summary age technique of Lovejoy et al. (1985) and is superior to any single macroscopic technique reported to date."

Two other studies published the same year, along with Miller et al. (1988), set a more pessimistic tone, however. Lucas and Loh (1986: 386), based upon a Pearson's *r* of 0.45, the difficulty in reading lines, and low inter-observer concordance, reported that the "accuracy of the use of cement lines to estimate age is unconfirmed." They also argued that annual cementum layers should not be anticipated in humans, even when documented in other mammals, as humans were buffered from the environmental stressors associated with seasonal dietary changes and migrations. Lucas and Loh's (1986) figure 1.2 shows that only four estimates fell above expected values for age-at-death in documented individuals, while the remainder is below. Lucas and Loh's (1986) figure 1 indicates that one of their problems involved the obscuring effects of cellular cementum. The systematic undercounting of this brief report from Singapore is therefore not surprising.

Lipsinic and colleague's (1986) research reported a sample of thirty-one, with an *r* of 0.84 after an outlier was removed. The *r* for individuals younger than thirty was 0.93 while, without the outlier, the *r* statistic for the older group was 0.64, again signaling significant undercounting of lines in sections from older individuals. Even with what some might conclude were encouraging results, the authors (Lipsinic et al. 1986: 988) asserted that TCA, due to systematic underprediction, was "not a reliable prediction method for humans."

Although undercounting appears not to have been a problem for Miller and colleagues (1988), the fact that nearly a third of their sample of 100 individuals were eliminated due to unreadable images suggests that a combination of section thickness

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(350 μm) and observer inexperience may have influenced their remarkably inaccurate results, as 85 percent of the observable images produced counts that departed from the known ages by ten years or more.

The authors of subsequent twentieth-century studies (Rao and Rao 1988; Grosskopf 1990; Stein and Corcoran 1994; and Sousa et al. 1999) generally voiced more optimism about the TCA method. Grosskopf (1990) advocated for TCA in both unburned and incinerated teeth. Issues of accuracy in older age groups and diseased teeth were voiced by Kvaal and Solheim (1995), with doubling mentioned by Grosskopf (1990) and Stein and Corcoran (1994). In general, when outliers or individuals without visible lines are removed, these studies report *r* values of 0.84 and 0.99. Writing of research conducted late in the twentieth century, Jankauskas and colleagues (2001) reported an *r* of 0.88 for a clinical sample, also using a largely identified Stalin-era mass grave to evaluate TCA against macroscopic indicators such as Nemeskéri's "combined" method, endocranial suture ossification, and pubic symphysial changes (Garmus 1996). Though Condon and colleagues (1986) had concluded that TCA was as accurate as Lovejoy's summary age method and better than any single morphoscopic method, Jankauskas concluded that Nemeskéri's combined method was the best, the pubic symphysis method the worst, with TCA being intermediate. They further argued that TCA was useful as an independent test of macroscopic techniques and should be the method of choice for fragmentary materials.

Thus, by the turn of the twenty-first century, there was cautious optimism upon the part of a few workers concerning the utility of TCA for archaeological and forensic applications. Most focused on single-rooted teeth, although molars had been utilized. Third molars were difficult, due to frequent root fusion. While both longitudinal and transverse sections provided readable images, the middle portion of the root and to a lesser extent the coronal segment, above the cellular apical portion, were preferred in both sections. Unresolved issues continued to be debated: (1) Were the lines annual events and, if so, why? (2) Can we reliably count lines in older individuals, given knowledge of decreased cementum production in individuals older than approximately fifty years of age (Kvaal and Solheim 1995)? (3) What about the influence of sex? (4) Are lines formed predictably, without resorption, in diseased teeth? and (5) How do we identify "outliers" when dealing with samples of unknown age? These were key issues carried into the twenty-first century. Obviously, while creating readable sections remained challenging, with thicknesses varying between 5 and 500 μm, most agreed that creating undecalcified sections was more facile and less likely to damage fragile teeth. One great problem remained: how best to read the sections? From personal experience with the 1986 project, I can say that it takes training and experience to count annuli. Without multiple observers, multiple observations, and preferably multiple sections, the TCA method is unlikely to achieve its full potential. A further issue with these (and more recent) validation studies is the lack of systematic reporting of key variables that facilitates an evaluation of best practices. Many studies systematically report the number of observers, if not their experience. Such information is vital for a developing methodology.

1.3 Twenty-First Century Teeth Cementum Annulations

The Rostock Paleodemography Workshops (Hoppa and Vaupel 2002) focused attention upon many fundamental matters, including the need for methodologically and statistically rigorous approaches to estimating age-at-death for individual skeletons. Wittwer-Backofen and Buba (2002) presented the TCA method as maybe one of the "best and most reliable" age estimators (Wittwer-Backofen and Buba 2002: 107). In an attempt to review progress and define unresolved issues, Wittwer-Backofen and Buba posed the following questions:

> What methods produce the best results and are time-efficient and cost-effective?
> How can reproducible estimates by image analysis techniques be established?
> How can images be enhanced to improve results?
> How many observers are necessary to produce reliable results?
> How many counts are necessary to produce reliable results?
> Does periodontal reduction influence the TCA and, if so, to what extent?
> Can we calculate missing incremental lines by the amount of periodontal reduction?
> Does tooth type affect TCA age estimation?
> Do all teeth produce the same quality of results?
> Is there intra-individual variability between different teeth?
> How can confidence intervals be calculated properly?
> (Wittwer-Backofen and Buba 2002: 115)

Although the twenty-first century TCA studies summarized in Table 1.1 address many of these issues and, in general, are more supportive of the method, it appears that a clear consensus methodology was not developing. We will return to this issue.

Most of the equivocal twenty-first-century studies (Dias et al. 2010, Kasetty et al. 2010; Padavala and Gheena 2015; Pilloud 2004) do identify subsets, frequently the young and healthy, wherein the TCA works well. While several studies express concern about diseased teeth (Condon et al. 1986; Kagerer and Grupe 2001a; Dias et al. 2010), especially those from individuals suffering from periodontal disease, a number of these recent investigations find that periodontal disease did not significantly affect results (Aggarwal et al. 2008; de Broucker et al. 2016; Pundir et al. 2009; Ristova et al. 2018; Wittwer-Backofen et al. 2004). Age is voiced as a problem in several studies (Bojarun et al. 2003; Dias et al. 2010; Gupta 2014; Meinl et al. 2008; Pilloud 2004; Shruthi et al. 2015) associated with inferences of decreased deposition in older individuals (>50–55). Other studies reported positive results across the age span (Aggarwal et al. 2008; Pundir et al. 2009; Wittwer-Bachofen et al. 2004). Caplazi (2004), as with Condon et al. (1986), reports increased accuracy for females, though Caplazi's results are not statistically significant. Distinctive, enhanced bands perhaps associated with periods of pregnancy in females have been reported (Caplazi 2004; Kagerer and Grupe 2001b; Dean et al. 2018; Chapter 8), which could potentially explain the increased readability of lines in some skeletal series and not others. Until we know the true proximate cause of the line formation, we can report correlations with known life-history events, such as pregnancies, diseases such as tuberculosis (Blondiaux et al. 2006) and diabetes (Dias et al. 2010), and season of death (Klevezal' and Shishlina 2001; see Wedel and Wescott 2016 for an archaeological case study, and Chapter 13), but applications in unknown contexts must remain speculative. Comparative nonhuman mammal studies, such as those by Klevezal'

(1996) and Grue and Jensen (1979), will continue to provide important complementary data for those studying the human condition.

The most impactful methodological advance during the twenty-first century stems from digital technologies in real-time microscopic image recording, enhancement, and software processing (Wittwer-Backofen et al., 2004: 126). As someone who recalls the considerable eye strain of those “former studies,” I can appreciate the manner in which the ability to digitally enlarge and enhance images will increase the ease and accuracy of TCA methods. Researchers have experimented with semiautomated (Klauenberg and Lagona 2007) and automated (Czermak et al. 2011) systems, which, while promising, are not yet beyond experimental stages (Chapters 15 and 16).

Several twenty-first-century authors have attempted to develop a system for estimating line numbers based upon the width of cementum and the measurement of one or a few obvious light-dark bands (Aggarwal et al. 2008; Gowda et al. 2014; Ristova et al. 2018; Struthi et al. 2015) with promising results. Such an adaptation needs to be sensitive to decreasing cementum deposition and, therefore, bandwidth with age. Therefore, the choice of a youthful band or bands will lead to age underestimation for individuals more than approximately fifty to fifty-five years of age. More knowledge of the pattern of decrease is necessary. If, for example, bands begin to decrease gradually to approximately fifty, a sampling of band width in the middle of the post-fifty unit would appear optimal. If there is a clear dichotomy, the sampling anywhere in the post-fifty unit would be satisfactory. If semiautomatic or automatic methods are to be used, then perhaps an inflection point in bandwidth can be identified digitally.

Other methodological issues include the type of microscope used for viewing images, with experimental evidence that phase contract outperforms polarized and light microscopes (Joshi et al. 2010; Kaur et al. 2015; Pundir et al. 2009). Maat et al. (2006) have argued that sections for observing annuli should be taken parallel to the outline of the root rather than oriented to the main axis of the tooth (Chapter 10).

An issue that has emerged from animal annulus studies is taphonomy as a factor that can complicate counts, a topic treated in Chapters 9 and 11. In previous research on mammalian teeth recovered from the archaeological record, Stutz (2002a) has demonstrated that postdepositional changes in tooth cementum can introduce crystal banding that mimics in vivo events. A method employing polarizing light microscopy coupled with a lambda plate has been proposed for identifying such diagenetic effects (Chapter 11).

As illustrated in columns D and E of Table 1.1, a number of studies eliminated teeth due to a variety of factors, which included breakage, unreadable cementum, cementum without definable lines, and statistical outliers. Although such reductions in sample size are to be expected in experimental work, as they would be in archaeological or forensic applications, only the statistical outliers pose a significant issue, especially in forensic contexts. The outliers are sufficiently rare that they are unlikely to bias paleodemographic inferences, but “doubling” or other perturbations of annual band development could negatively impact forensic casework. The biological profile generated for records search could be artificially elevated and a match unlikely. Therefore, if at all possible, especially in forensic contexts, more traditional gross morphological approaches should be considered along with TCA.

1.4 The Need for a Standard Approach and Why Hasn't It Happened?

After our team completed and published our study (Charles et al. 1986; Condon et al. 1986), I anticipated that the method would soon become a standard method for age-at-death estimation in bioarchaeology and forensic anthropology. I was obviously over-optimistic! Further applications during the 1980s were generally not supportive, and a body of research on humans did not develop. Given that the method was used more commonly in paleozoology and wildlife studies with a manual for Alaskan Brown Bears published by Matson and colleagues in 1993, the case for standard human cementum annuli studies seemed considerably overdue.

With the renewed interest stimulated by the Rostock Manifesto and the development of digital enhancement methods, ongoing resistance to the method is even more surprising. Wittwer-Backofen's scholarly publications surely should have started a groundswell (Wittwer-Backofen and Buba 2002; Wittwer-Backofen et al. 2004), or so I thought at the beginning of the twenty-first century. Yet, we have researchers such as Meinl et al. (2008:104) remarking that "TCA is an expensive, tedious but comparatively precise technique regarding the fact that the measurement depends principally on the absolute count of lines. Unfortunately, no standardized protocol of how to prepare the sections and how to count them exists which may lead to errors in the assessment of the line count." More recently, also publishing in *Forensic Science International,* Cunha and colleagues (2009: 6) in an evaluative overview of various estimators of age-at-death method argues that despite their being affected by taphonomy, the Lamedin dentin translucence method is preferable to TCA because TCA "is more time-consuming, expensive, not user-friendly and less accurate. Recently, its applicability has also been seriously questioned." There is no reference for the "less accurate" assertion, and in fact, Meinl et al.'s study (2008) demonstrates that TCA is more accurate than the Lamedin technique. The "serious questioning" occurred in the course of an article by Renz and Radlanski (2006) that begins with the assumption that the mechanisms for annulus formation in humans should be the same as those for tree rings. While the common nature of cemental annulus formation seems secure across Mammalia and Crocodilia (Introduction), an extension from the Animal Kingdom to the Plant Kingdom, while creative, would seem to be overly bold.

Obviously, the TCA method needs advocacy, which has been building, albeit slowly. This volume is one tangible outcome, as was a recent special issue of the *International Journal of Paleopathology* (2016, vol. 15: 113–63). Wittwer-Backofen and colleagues have advanced the field, including a recently published, detailed methodological protocol (Wittwer-Backofen 2012). As reported by Colard et al. (2015), an international research group (Cementochronology Research Program) has been formed recently (2010), and a protocol has been submitted and approved by the ISO-9000. This protocol (Figure 1.5) specifies equipment, supplies, and procedures that should be readily available in forensic or histology laboratories. As Colard and colleagues note (2015: 7), the cost of a single section would appear to be approximately US$20. Reading sections and counting lines is a learned skill; workshops and other training sessions should solve this problem.

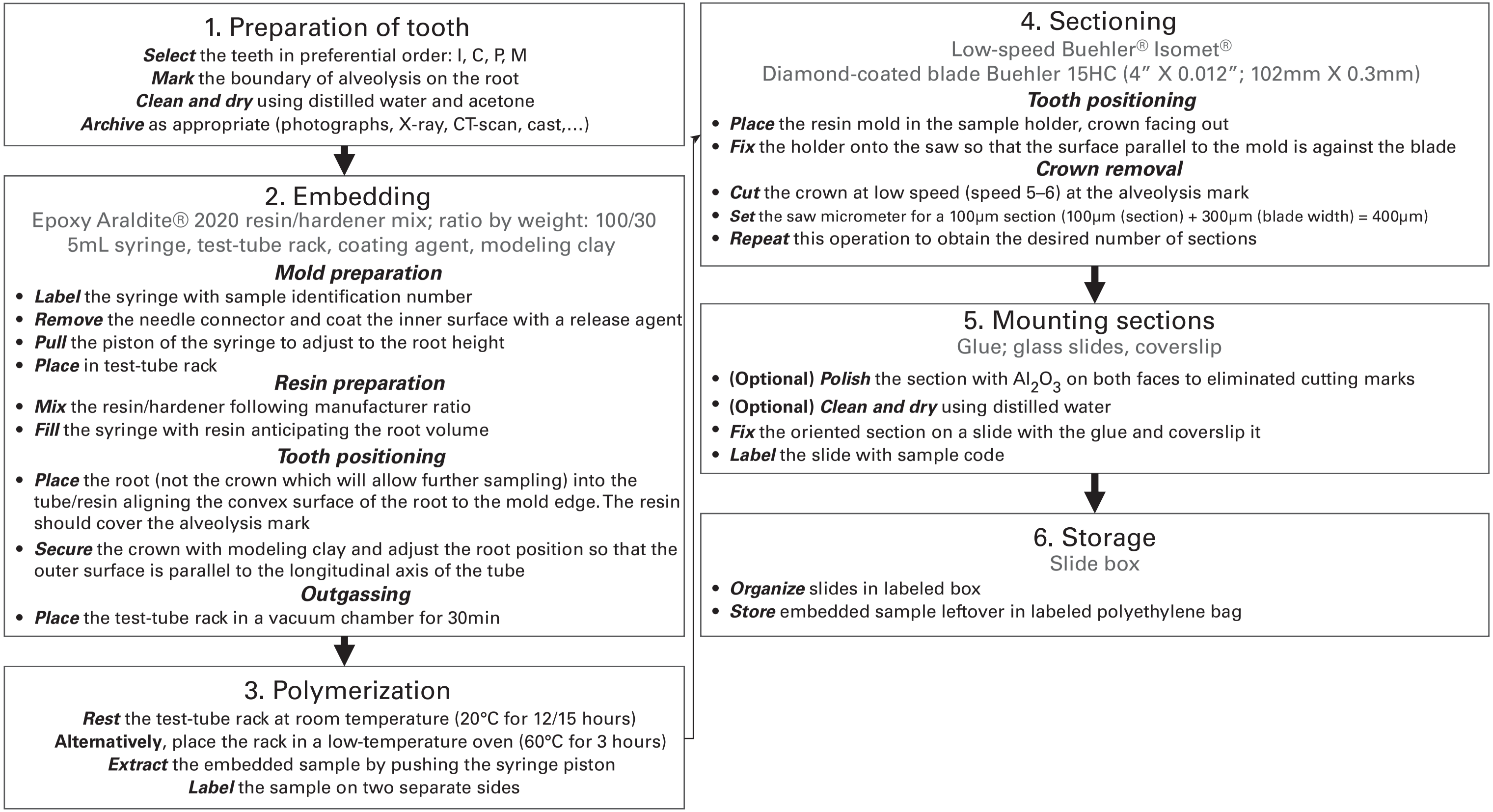

Figure 1.5 Cementochronology procedure for dental preparation, certified according to the ISO-9001 (based on data from Colard et al. 2015).

It is up to forensic anthropologists and bioarchaeologists to overcome their fear of laboratories and accept one of the most accurate methods for estimating age-at-death in human remains.

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References

Aggarwal, P., S. Saxena, and P. Bansal. 2008. Incremental Lines in Root Cementum of Human Teeth: An Approach to Their Role in Age Estimation Using Polarizing Microscopy. *Indian Journal of Dental Research* 19(4): 326–30.

Avadhani, A., J. V. Tupkari, A. Khambaty, and M. Sardar. 2009. Cementum Annulations and Age Determination. *Journal of Forensic Dental Sciences* 1(2): 73.

Azaz, B., M. Ulmansky, R. Moshev, and J. Sela. 1974. Correlation between Age and Thickness of Cementum in Impacted Teeth. *Oral Surgery, Oral Medicine, Oral Pathology* 38(5): 691–4.

Bertrand, B., E. Cunha, and V. Hédouin. 2018. Cementochronology: Too Precise to Be True or Too Precise to Be Accurate? *American Journal of Physical Anthropology* 165 (S66): 27.

Bennett, G. W. 2009. The Root of Dental Anatomy: A Case for Naming Eustachius the "Father of Dental Anatomy." *Journal of the History of Dentistry* 57: 85–8.

Black, G. V. 1887. *A Study of the Histological Characters of the Periostium and Peridental Membrane*. Chicago, IL: W. T. Keener.

Blondiaux, J., N. Gabart, A. Alduc-Le Bagousse, C. Niel, and E. Tyler. 2006. Relevance of Cement Annulations to Paleopathology. *Paleopathology Newsletter* 135: 4–13.

Bojarun, R., A. Garmus, and R. Jankauskas. 2003. Microstructure of Dental Cementum and Individual Biological Age Estimation. *Medicina (Kaunas)* 39(10): 960–4.

Bosshardt, D. D., and K. A. Selvig. 1997. Dental Cementum: The Dynamic Tissue Covering of the Root. *Periodontology* 13(1): 41–75.

Broomell, I. N. 1898. The Histology of Cementum. *Dent Cosmos* 40(9): 697–723.

Broucker, A. de, T. Colard, G. Penel, J. Blondiaux, and S. Naji. 2016. The Impact of Periodontal Disease on Cementochronology Age Estimation. *International Journal of Paleopathology* 15 (December): 128–33.

Caplazi, G. 2004. Eine Untersuchung über die Auswirkungen von Tuberkulose auf Anlagerungsfrequenz und Beschaffenheit der Zementringe desmenschlichen Zahnes. *Bulletin de la Société Suisse d'Anthropologie* 10(1): 35–83.

Charles, D. K., K. Condon, J. M. Cheverud, and J. E. Buikstra. 1986. Cementum Annulation and Age Determination in *Homo Sapiens*. 1. Tooth Variability and Observer Error. *American Journal of Physical Anthropology* 71: 311–20.

https://doi.org/10.1017/9781108569507.003 Published online by Cambridge University Press

Colard, T., B. Bertrand, S. Naji, Y. Delannoy, and A. Bécart. 2015. Toward the Adoption of Cementochronology in Forensic Context. *International Journal of Legal Medicine* 129: 1–8.

Colard, T., G. Falgayrac, B. Bertrand, S. Naji, O. Devos, C. Balsack, Y. Delannoy, and G. Penel. 2016. New Insights on the Composition and the Structure of the Acellular Extrinsic Fiber Cementum by Raman Analysis. *PLOS ONE* 11(12): e0167316.

Condon, K., D. K. Charles, J. M. Cheverud, and J. E. Buikstra. 1986. Cementum Annulation and Age Determination in *Homo Sapiens*. II. Estimates and Accuracy. *American Journal of Physical Anthropology* 71(3): 321–30.

Cunha, E., E. Baccino, L. Martrille, F. Ramsthaler, J. Prieto, Y. Schuliar, N. Lynnerup, and C. Cattaneo. 2009. The Problem of Aging Human Remains and Living Individuals: A Review. *Forensic Science International* 193: 1–13.

Czermak, A., A. Czermak, H. Ernst, and G. Grupe. 2011. Age at Death Evaluation by Tooth Cementum Annulation (TCA) – A Software for an Automated Incremental Line Counting. Poster presented at the 80th annual meeting of the American Association of Physical Anthropologists, Minneapolis, MN.

Dalitz, G. D. 1962. *Age Determination of Adult Human Remains by Teeth Examination*. PhD thesis, University of Melbourne: Australia.

Dean, C., A. Le Cabec, K. Spiers, Y. Zhang, and J. Garrevoet. 2018. Incremental Distribution of Strontium and Zinc in Great Ape and Fossil Hominin Cementum Using Synchrotron X-Ray Fluorescence Mapping. *Journal of the Royal Society, Interface* 15 (138).

Denton, G. B. 1941. *The Discovery of Cementum*. Chicago: Northwestern University.

Dias, P. E. M., T. L. Beaini, and R. F. H. Melani. 2010. Age Estimation from Dental Cementum Incremental Lines and Periodontal Disease. *Journal of Forensic Odontostomatology* 28(1): 13–21.

Erickson, J. A., and W. G. Seliger. 1969.Efficient Sectioning of Incisors for Estimating Age of Mule Deer. *Journal of Wildlife Management* 33(2): 384–88.

Foster, B. L. 2017. On the Discovery of Cementum. *Journal of Periodontal Research* 52(4): 666–85.

Garant, P. R. 1995. G. V. Black's Contribution to the Structural Biology of the Periodontium. *Periodontal Clinical Investigation* 17: 16–20.

Garmus, A. 1996. *Lithuanian Forensic Osteology*. Vilnus, Lithuania: Baltic Medico-Legal Association.

Gilbert, F. F. 1966. Aging White-Tailed Deer by Annuli in the Cementum of the First Incisor. *Journal of Wildlife Management* 30(1): 200–2.

Gottlieb, B. 1943. Continuous Deposition of Cementum. *Journal of American Dental Association* 30: 842–7.

Gowda, C. B. K., P. Srinivasa Reddy, G. Kokila, and L. Pradeep. 2014. Cemental Annulation and Phase Contrast Microscope: Tool for Age Estimation. *Journal of South India Medicolegal Association* 6(1): 9–13.

Grosskopf, B. 1990. Individual Age Determination Using Growth Rings in the Cementum of Buried Human Teeth. *Zeitschrift für Rechtsmedizin* 103(5): 351–59.

Grue, H., and B. Jensen. 1979. Review of the Formation of Incremental Lines in Tooth Cementum of Terrestrial Mammals. *Danish Review of Game Biology* 11: 1–48.

Gupta, P. 2014. Human Age Estimation from Tooth Cementum and Dentin. *Journal of Clinical and Diagnostic Research* 8(4): 7–10.

Gustafson, G. 1950. Age Determination of Teeth. *Journal of American Dental Association* 41: 45–54.

Hast, M. H., and D. H. Garrison. 1995. Andreas Vesalius on the Teeth: An Annotated Translation from *De Humani Corporis Fabrica* 1543. *Clinical Anatomy* 8: 134–138.
Hopewell-Smith, A. 1920. *Concerning Human Cementum*. Philadelphia: Evans Dental Institute, University of Pennsylvania.
Hoppa, R. D., and J. W. Vaupel, eds. 2002. *Paleodemography: Age Distributions from Skeletal Samples*. Cambridge: Cambridge University Press.
Jankauskas, R., S. Barakauskas, and R. Bojarun. 2001. Incremental Lines of Dental Cementum in Biological Age Estimation. *HOMO – Journal of Comparative Human Biology* 52(1): 59–71.
Joshi, P. S., M. S. Chougule, and G. P. Agrawal. 2010. Comparison of Polarizing & Phase Contrast Microscopy for Estimation of Age Based on Cemental Annulations. *Indian Journal of Forensic Odontology* 3(3): 17–25.
Kagerer, P., and G. Grupe. 2001a. On the Validity of Individual Age-at-Death Diagnosis by Incremental Line Counts in Human Dental Cementum. Technical Considerations. *Anthropologischer Anzeiger* 59(4): 331–42.
2001b. Age-at-Death Diagnosis and Determination of Life-History Parameters by Incremental Lines in Human Dental Cementum as an Identification Aid. *Forensic Science International* 118(1): 75–82.
Kasetty, S., M. Rammanohar, and T. R. Ragavendra. 2010. Dental Cementum in Age Estimation: A Polarized Light and Stereomicroscopic Study. *Journal of Forensic Sciences* 55(3): 779–83.
Kasuya, T. 1976. Reconsideration of Life History Parameters of the Spotted and Striped Dolphins Based on Cemental Layers. *Scientific Reports of the Whales Research Institute* 28: 73–106.
Kasuya, T. 1977. Age Determination and Growth of the Baird's Beaked Whale with a Comment of the Fetal Growth Rate. *Scientific Reports of the Whales Research Institute* 29: 1–20.
Kasuya, T., and S. Matsui. 1984. Age Determination and Growth of the Short-Finned Pilot Whale off the Pacific Coast of Japan. *Scientific Reports of the Whales Research Institute* 35: 57–91.
Kaur, P., M. Astekar, J. Singh, K. S. Arora, and G. Bhalla. 2015. Estimation of Age Based on Tooth Cementum Annulations: A Comparative Study Using Light, Polarized, and Phase Contrast Microscopy. *Journal of Forensic Dental Sciences* 7(3): 215–21.
Keiss, R. E. 1969. Comparison of Eruption-Wear Patterns and Cementum Annuli as Age Criteria in Elk. *Journal of Wildlife Management* 3(1): 175–80.
Klauenberg, K., and F. Lagona. 2007. Hidden Markov Random Field Models for TCA Image Analysis. *Computational Statistics & Data Analysis* 52(2): 855–68.
Klevezal', G. A. 1996. *Recording Structures of Mammals: Determination of Age and Reconstruction of Life History*. Rotterdam: A. A. Balkema Series.
Klevezal', G. A., and S. E. Kleinenberg. 1969. Age Determination of Mammals from Annual Layers in Teeth and Bones. Akademiya Nauk S.S.S.R, 1967. Translated 1969 from Russian by Israel Progr. Sci. Transl. Jerusalem.
Klevezal', G.A., and N. I. Shishlina. 2001. Assessment of the Season of Death of Ancient Human from Cementum Annual Layers. *Journal of Archaeological Science* 28(5): 481–6.
Kronfeld, R. 1938. The Biology of Cementum. *Journal of the American Dental Association* 25: 1451–61.
Kvaal, S. I., and T. Solheim. 1995. Incremental Lines in Human Dental Cementum in Relation to Age. *European Journal of Oral Sciences* 103(4): 225–30.

https://doi.org/10.1017/9781108569507.003 Published online by Cambridge University Press

Laws, R. M. 1952. A New Method of Age Determination for Mammals. *Nature* 169: 972–3.

Lipsinic, F. E., D. G. Paunovich, D. G. Houston, and S. F. Robinson. 1986. Correlation of Age and Incremental Lines in the Cementum of Human Teeth. *Journal of Forensic Sciences* 31: 982–9.

Lovejoy, C. O., R. S. Meindl, R. P. Mensforth, and T. J. Barton. 1985. Multifactorial Determination of Skeletal Age at Death: A Method and Blind Tests of Its Accuracy. *American Journal of Physical Anthropology* 68: 1–14.

Low, W. A., and I. McT. Cowan. 1963. Age Determination of Deer by Annular Structure of Dental Cementum. *Journal of Wildlife Management* 27(3): 466–71.

Lucas, P. W., and H. S. Loh. 1986. Are the Incremental Lines in Human Cementum Laid Down Annually? *Annals of the Academy of Medicine, Singapore* 15(3): 384–6.

Maat, G. J. R., R. R. R. Gerretsen, and M. J. Aarents. 2006. Improving the Visibility of Tooth Cementum Annulations by Adjustment of the Cutting Angle of Microscopic Sections. *Forensic Science International* 159, Supplement (0): S95–99.

Magitot, E. 1878. *Experimental and Therapeutic Investigations*. Boston: Houghton, Osgood, and Company.

Matson, G., L. Van Daele, E. Goodwin, L. Aumiller, H. Reynolds, and H. Hristienko. 1993. *A Laboratory Manual for Cementum Age Determination of Alaska Brown Bear First Premolar Teeth*. Milltown, MT: Alaska Department of Fish and Game. Division of Wildlife Conservation and The Matson Laboratory.

McEwan, E. H. 1963. Seasonal Annuli in the Cementum of the Teeth of Barren Ground Caribou. *Canadian Journal of Zoology* 41: 111–13.

Meinl, A., C. D. Huber, S. Tangl, G. M. Gruber, M. Teschler-Nicola, and G. Watzek. 2008. Comparison of the Validity of Three Dental Methods for the Estimation of Age at Death. *Forensic Science International* 178(2–3): 96–105.

Miller, C. F., S. B. Dove, and J. A. Cottone. 1988. Failure of Use of Cemental Annulations in Teeth to Determine the Age of Humans. *Journal of Forensic Sciences* 33: 137–43.

Mitchell, B. 1963. Growth Layers in Dental Cement for Determining the Age of Red Seer (*Cervus elaphus L.*). *Journal of Animal Ecology* 36(2): 279–93.

Morris, P. A. 1972. A Review of Mammalian Age Determination Methods. *Mammal Review* 2 (3): 69–104.

Naji, S., T. Colard, J. Blondiaux, B. Bertrand, E. d'Incau, and J.-P. Bocquet-Appel. 2016. Cementochronology, to Cut or Not to Cut? *International Journal of Paleopathology* 15 (December): 113–9.

Naylor, J. W., W. G. Miller, G. N. Stokes, and G. G. Stow. 1985. Cemental Annulation Enhancement: A Technique for Age Determination in Man. *American Journal of Physical Anthropology* 68: 197–200.

Novakowski, N. S. 1965. Cemental Deposition as an Age Criterion in Bison, and the Relation of Incisor Wear, Eye-Lens Weight, and Dressed Bison Carcass Weight to Age. *Canadian Journal of Zoology* 43(1): 173–8.

Padavala, S., and S. Gheena. 2015. Estimation of Age Using Cementum Annulations. *Journal of Pharmaceutical Sciences & Research* 7(7): 461–3.

Pilloud, S. 2004. Läßt sich mittels der Altersbestimmung anhand Zahnzementes auch bei älteren Individuen ein signifikanter Zusammenhang zwischen histologischem und reellem finden? *Anthropologischer Anzeiger* 62(2): 231–9.

Pundir, S., S. Saxena, and P. Aggrawal. 2009. Estimation of Age Based on Tooth Cementum Annulations Using Three Different Microscopic Methods. *Journal of Forensic Dental Sciences* 1(2): 82.

Ransom, A. B. 1966. Determining Age of White-Tailed Deer from Layers in Cementum of Molars. *Journal of Wildlife Management* 30(1): 197–9.
Rao, N. G., and N. N. Rao. 1998. CCTV Study of Cemental Annulations in Determining the Age from a Single Tooth. *Indian Journal of Dental Research* 9: 41–5.
Renz, H., and R. J. Radlanski. 2006. Incremental Lines in Root Cementum of Human Teeth – A Reliable Age Marker? *HOMO – Journal of Comparative Human Biology* 57(1): 29–50.
Ristova, M., M. Talevska, and Z. Stojanovska. 2018. Accurate Age Estimations from Dental Cementum and a Childbirth Indicator – A Pilot Study. *Journal of Forensic Science & Criminology* 6: 1–12.
Saunders, J. B., and C. D. O'Malley. 1944. A Reading from the *De Humane Corporals Fabric* of Andreas Vesalius. *Journal of the American College of Dentists* 11: 211–18.
Saxon, A., and C. Higham. 1968. Identification and Interpretation of Growth Rings in the Secondary Dental Cementum of *Ovis aries*. *Nature* 219: 634–5.
1969. A New Research Method for Economic Prehistorians. *American Antiquity* 34(3): 303–11.
Scheffer, V. B. 1950. Growth Layer on the Teeth of Pinnipedia as an Indication of Age. *Science* 112: 309–11.
Sergeant, D. E., and D. H. Pimlott. 1959. Age Determination in Moose from Sectioned Incisor Teeth. *Journal of Wildlife Management* 23(3): 315–21.
Shklar, G., and C. A. Brackett. 2009. Galen on Oral Anatomy. *Journal of the History of Dentition* 57: 24–8.
Shklar, G., and D. Chernin. 2000. Eustachio *Libellus de Dentibus* the First Book Devoted to the Structure and Function of the Teeth. *Journal of the History of Dentition* 48: 25–30.
Shruthi, B. S., M. Donoghue, M. Selvamani, and P. V. Kumar. 2015. Comparison of the Validity of Two Dental Age Estimation Methods: A Study on South Indian Population. *Journal of Forensic Dental Sciences* 7(3): 189–94.
Sousa, E. M., G. G. Stott, and J. B. Alves. 1999. Determination of Age from Cemental Incremental Lines for Forensic Dentistry. *Biotechnic & Histochemistry: Official Publication of the Biological Stain Commission* 74(4): 185–93.
Spiess, A. 1976. Determining Season of Death of Archaeological Fauna by Analysis of Teeth. *Arctic* 29(1): 53–5.
Spinage, C. A. 1976a. Age Determination of the Female Grant's Gazelle. *African Journal of Ecology* 14(2): 121–34.
1976b. Incremental Cementum Lines in the Teeth of Tropical African Mammals. *Journal of the Zoological Society of London* 178: 117–31.
Spinage, C. A. 1973. A Review of the Age Determination of Mammals by Means of Teeth, with Special Reference to Africa. *East Africa Wildlife Journal* 11: 165–87.
Stallibrass, S. 1982. The Use of Cement Layers for Absolute Ageing of Mammalian Teeth: A Selective Review of the Literature, with Suggestions for Further Studies and Alternative Applications. In *Ageing and Sexing Animal Bones from Archaeological Sites*. B. Wilson, C. Grigson, and S. Payne, eds. BAR International Series 109. Oxford: British Archaeological Report, 109–26.
Stein, T. J., and J. F. Corcoran. 1994. Pararadicular Cementum Deposition as a Criterion for Age Estimation in Human Beings. *Oral Surgery, Oral Medicine, Oral Pathology* 77(3): 266–70.
Stillson, W. C. 1917. A Study of Cementum. *Dental Summary* 37: 30.
Stock, S. R., L. A. Finney, A. Telser, E. Maxey, S. Vogt, and J. S. Okasinski. 2017. Cementum Structure in Beluga Whale Teeth. *Acta Biomaterialia* 48 (January): 289–99.

Stott, G. G., R. F. Sis, and B. M. Levy. 1982. Cemental Annulation as an Age Criterion in Forensic Dentistry. *Journal of Dental Research* 61(6): 814–17.

Stutz, A. J. 2002a. Polarizing Microscopy Identification of Chemical Diagenesis in Archaeological Cementum. *Journal of Archaeological Science* 29(11): 1327–47.

Swetha, G., K. K. Kattappagari, C. S. Poosarla, L. P. Chandra, S. R. Gontu, and V. R. R. Badam. 2018. Quantitative Analysis of Dental Age Estimation by Incremental Line of Cementum. *Journal of Oral and Maxillofacial Pathology* 22(1): 138.

Trenouth, M. J. 2014. The Origin of the Terms Enamel, Dentine, and Cementum. *Faculty Dental Journal* 5(1): 26–31.

van Zuylen, J. 1981. The Microscopes of Antoni van Leeuwenhoek. *Journal of Microscopy* 121: 309–28.

Wedel, V. L., and D. J. Wescott. 2016. Using Dental Cementum Increment Analysis to Estimate Age and Season of Death in African Americans from an Historical Cemetery in Missouri. *International Journal of Paleopathology* 15 (December): 134–9.

Wiebusch, F. B. 1957. Periodontal Research: Problems Related to Practice. *Journal of the American Dental Association* 55: 612–16.

Wittwer-Backofen, U. 2012. Age Estimation Using Tooth Cementum Annulation. In *Forensic Microscopy for Skeletal Tissues: Methods and Protocols*. L. S. Bell, ed., Chapter 8. *Methods in Molecular Biology*, 915: 129–43.

Wittwer-Backofen, U., and H. Buba. 2002. Age Estimation by Tooth Cementum Annulation: Perspective of a New Validation Study. In *Paleodemography, Age Distributions from Skeletal Samples*. R .D. Hoppa and J. W. Vaupel, eds. Cambridge: Cambridge University Press, 107–28.

Wittwer-Backofen, U., J. Gampe, and J. W. Vaupel. 2004. Tooth Cementum Annulation for Age Estimation: Results from a Large Known-Age Validation Study. *American Journal of Physical Anthropology* 123(2): 119–29.

Yoneda, M. 1982. Growth Layers in Dental Cementum of Saguinus Monkeys in South America. *Primates* 23(3): 460–64.

Zander, H. A., and B. Hürzeler. 1958. Continuous Cementum Apposition. *Journal of Dental Research* 6: 1035–44.