Life History Variables Preserved in Dental Cementum Microstructure

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The age and season of death of mammals, as well as other aspects of their life history, can be estimated from seasonal bands in dental cementum that result from variations in microstructure. Scanning electron micrographs of goats fed controlled diets demonstrate that cementum bands preserve variations in the relative orientation of collagen fibers that reflect changes in the magnitude and frequency of occlusal forces from chewing different quality diets. Changes in the rate of tissue growth are also reflected in cementum bands as variations in the degree of mineralization.

Cementum is an avascular, bone-like tissue that anchors tooth roots by mineralizing extrinsic collagen fiber bundles (Sharpey’s fibers) produced in the periodontal ligament (1, 2). Cementum, which is continuously deposited and rarely remodeled or resorbed, grows in incremental bands (Fig. 1) that are visible in cross sections through tooth roots in polarized light or stained sections (3). Correlations between cementum bands and the age and season of death of mammals have been recognized for more than 30 years (4-10), but the underlying causes of the bands have been unknown. In most mammals, opaque acellular bands tend to be deposited during seasons of reduced tissue growth (such as winter), and translucent bands (cellular or acellular) tend to be deposited during seasons of rapid tissue growth (such as spring and summer) (7-10). In this report, I demonstrate that bands in cementum are caused by two different phenomena, occlusal strain and growth rate, that can be used to reconstruct significant life history variables of mammals after death.

Cementum has a complex three-dimensional microstructure that reflects its function to maintain teeth in position for effective occlusion in spite of the high strains caused with the Love wave, can be observed on several seismic records that approach station KIP (Kopuai, Hawaii) from the west.

Recent experiments by N. M. Rice and U. R. Christensen [Ecology (1987), 58(3)] with three-dimensional numerical convection models suggest that plumes do not penetrate the lopolithosphere to a great depth and so may not reset fossil anisotropy. However, large-scale lateral motion in the asthenosphere occurs in their calculations as the plume material spreads at the base of the lithosphere. Lateral gradients in anisotropic properties may exist near the Hawaii plume because quasi-Love waves, in cellular and acellular cementum, tend to be oriented parallel to one another at an oblique angle relative to the dentine-cementum border. Sharpey’s fibers in cementum are therefore aligned so as to counter the tensile forces that tend to depress teeth in their alveoli during occlusion. In addition, the predominant orientation of intrinsic collagen fibers within the cementum matrix is perpendicular to the Sharpey’s fiber bundles (Fig. 2B). These fibers also appear to wrap around Sharpey’s fibers (13). Cementum microstructure is thus analogous to other mineralized tissues, such as bone or tendon, in which collagen fibers are generally aligned at right angles to each other (13-16). However, unlike bone or tendon, cementum microstructure is determined primarily by extrinsic collagen.

I tested the effects of changes in diet on cementum microstructure in a controlled...
experiment on goats that demonstrates that cementum bands can result from variations in either collagen orientation or collagen mineralization. Six goats (Capra hircus) were kept for 12 months under identical conditions with the exception of their diets. Two goats were fed for the entire experiment a control diet of hay and pellets that provided maintenance-level nutrition; two were fed the same food as that of the control group, but the food was ground and softened during the middle 4 months; and the final two were fed food of the same hardness as the control group, but the food was 33% lower in protein and 55% lower in mineral (including calcium and phosphorus) and vitamin content during the middle 4 months. Tissue deposited during different phases was labeled with fluorochrome dyes (oxygenated, calcine, and alizarin).

SEM micrographs of fractured sections of the goats' cementum (Fig. 3) show that Sharpey's fibers became more vertically oriented (relative to the dentine-cementum border) during the process of mineralization under higher and more frequent tensile strains. As shown by measurements made on the lingual surface of the first molars just below the dentine-cementum-enamel junction (Fig. 1A), the two animals fed a softer diet during the middle phase of the experiment had Sharpey's fibers that were oriented less vertically (x = 75.6° ± 1.7°; n = 18) in those 4 months than during the periods when the food was harder (x = 52.3° ± 3.5°; n = 36) (17). The four animals fed food of the same hardness (regardless of nutritional content) all year had no change in Sharpey's fiber orientation (x = 56.0° ± 2.2°; n = 30). In vivo strain-gauge analyses (18) revealed that the strain in the mandible just below the molars while eating the hard food was approximately 1.5 times that while eating the softened food. In addition, the harder food required six times as many masticatory cycles to chew as the softer food. Cementum bands therefore preserve seasonal variations in Sharpey's fiber orientation (Fig. 1C): Acellular cementum bands with different Sharpey's fiber orientations appear alternately opaque or translucent in ground thin sections under transmitted polarized light because regions of cementum with different collagen orientation refract light in different directions (19).

Changes in the rate of acellular and cellular cementum deposition also result in bands that are visible in transmitted light because of differences in mineral density. Microradiographs (Fig. 4) indicate that hypermineralized (denser) cementum bands were deposited during periods of reduced cementogenesis. The animals fed a low protein and mineral diet during the middle 4 months of the experiment ceased growing (as measured in body weight); cementum deposited during this period was more mineralized and 70% as thick as the tissue deposited during periods of maintenance-level diet (as measured in the lower first molar (M1)). The control sample and the goats fed a soft food diet exhibited no change in the rate of cementum deposition or mineralization. Variations in the rate of cementogenesis thus affect the rate of formation of the collagen matrix, but the rate of mineralization remains constant. Similar effects of growth rate on mineralization have been documented in cementum (12, 20), bone (21, 22), and dentine (23).

Cementum bands are useful for estimating the age and season of death of goats and other mammals as well as other aspects of their life history (12). The number of bands records the number of seasons since a tooth erupted, and the nature of the outermost band records the season of death. Changes in collagen fiber orientation and relative
mineralization can also be used to reconstruct variations in seasonal diet quality and growth rate over the course of an animal’s life. This technique has many applications. Cementum bands appear in a wide variety of mammals with both grazing and nongrazing diets (7, 8), and aspects of cementum microstructure are visible in many fossils because Sharpey’s fiber orientation is sometimes preserved through either collagen preservation or mineral replacement. In addition, because cementum is rarely remodeled or resorbed, it can be used for longitudinal studies of the effects of growth rate and mechanical stress on the structure of mineralized tissues. These results support the hypothesis that collagen orientation in mineralized tissues can be affected by the orientation and degree of tensile forces (24, 25) and that the rates of cementum matrix production and cement mineralization may be relatively independent (26).

REFERENCES AND NOTES
17. For consistency and comparability, nine measurements were taken on each individual at 10-μm intervals on the lingual surface of MI near the enamel-dentine-cementum junction. Differences are statistically significant (t test = 34.06, P < 0.0001, n = 18). Correlations between diet phases and cementum bands were made with fluorochrome dyes.
27. A fresh tooth was cut out of the mandible with a rotary saw and the periodontal ligament was stripped. The tooth was then immersed in 1 M acetic acid for 10 min, fractured in the meso-distal plane through the tooth root, rinsed with distilled H2O, air-dried, coated with 200 nm of gold, and photographed at 20 kV.
28. Fresh teeth were cut out of the mandible with a rotary saw, and the periodontal ligaments were stripped. Teeth were fixed in 10% formaldehyde for 24 hours, rinsed in distilled H2O for 1 hour, dehydrated in 1 M ethanol, embedded in epoxy, sectioned in the meso-distal plane, ground to a thickness of 50 μm, and polished. Specimens were photographed at 110 kV for 20 min.
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Repair of DNA Methylphosphotriesters Through a Metalloactivated Cysteine Nucleophile

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The Escherichia coli Ada protein repairs methylphosphotriesters in DNA by direct, irreversible methyl transfer to one of its own cysteines. Upon methyl transfer, Ada acquires the ability to bind specific DNA sequences and thereby to induce genes that confer resistance to methylating agents. The amino-terminal domain of Ada, which comprises the methylphosphotriester repair and sequence-specific DNA binding elements, contains a tightly bound zinc ion. Analysis of the zinc binding site by cation-dye 113 nuclear magnetic resonance and site-directed mutagenesis revealed that zinc participates in the autocatalytic activation of the active site cysteine and may also function as a conformational switch.

Methylation of DNA can occur enzymatically and nonenzymatically. Whereas enzymatic methylation fulfills an essential role in many organisms, nonenzymatic methylation insults the genome with a variety of toxic and mutagenic adducts (1, 2). To counter this threat, cells express a variety of proteins that recognize and repair aberrantly methylated DNA. A noteworthy example is Escherichia coli Ada, which repairs the mutagenic adduct 6-O-methylguanine by direct, irreversible methyl transfer to a cysteine residue in the COOH-terminal domain (3). Ada also repairs the 5¢ diastereomer of DNA methylphosphotriesters (MePd) by direct methyl transfer to a second cysteine, Cys69, located in the NH2-