Collagenous Layer Covering the Crown Enamel of Unerupted Permanent Human Teeth

Abstract. *A tissue layer, which was continuous with the cementum covering the roots, was found external to the crown enamel of normal, unerupted permanent human teeth. Light microscopy, x-ray diffraction, and amino acid analysis indicated that the major constituent of this tissue was collagen.*

A calcified layer of collagenous tissue covers the crown enamel of unerupted and erupted bovine teeth (*1*). The tissue is continuous with, and has the structural, chemical, and histological characteristics of the cementum covering the root (*1*). Attempts to identify a similar layer either structurally or chemically in human teeth were initially unsuccessful because the extremely tenuous layer removed from the surface of the crown enamel of erupted teeth was heavily contaminated with dental placque, calculus, and other debris. Since the outer collagenous layer of bovine teeth was demonstrated shortly before tooth eruption, attention was focused on unerupted human teeth in a similar stage of development.

Normal, unerupted, permanent human premolar teeth which, for orthodontic reasons, had been surgically removed from male and female patients 9- to 11-years-old were very carefully cleaned of all soft tissue attachments by sharp dissection and rinsed in cold water. The surfaces were scraped with a surgical scalpel to remove any remnants of uncalcified tissues. Removal of the soft tissue was confirmed by observing both the wet and dry tooth under the dissecting microscope. In order to preserve and maintain the structural relationship of the anatomical layers after decalcification, the depulped teeth were first fixed for periods of 4 to 8 weeks in a 2 percent solution of dichloro-*sym*-triazine (Procion Brilliant Red-M-2BS) (*2*), *p*H 6.6, at room temperature; the fixative was changed weekly.

After fixation, the teeth were placed in 0.5*M* EDTA (ethylene diamine tetraacetate), *p*H 8.3, at room temperature for approximately 1 month, with only occasional and gentle stirring to prevent fragmentation of the tissue layers. The decalcifying solution was changed every 4 to 5 days. At the end of the 4-week period, a soft, decalcified outer tissue layer was noted which conformed to the shape of the entire tooth and covered it completely (Fig. 1). By making a longitudinal incision into this layer, the remaining portion of the partially decalcified core of the intact tooth could be removed (Fig. 1). This was subsequently identified histologically as dentin, the relatively mature enamel having been dissolved in EDTA (*3*). Pieces of the outer tissue layer were taken from the apical end of the root, the occlusal region of the crown, and from intermediate regions and were studied by means of x-ray diffraction (*4*), light microscopy, and amino acid analysis.

All specimens after drying gave the characteristic x-ray diffraction pattern of collagen (Fig. 2). The amino acid composition of both root cementum and the tissue covering the crown enamel indicates that the tissue contains about two-thirds collagen (Table 1). These results are consistent with those obtained by x-ray diffraction.

Table 1. The amino acid composition of the decalcified Procion-fixed outer layer of an unerupted, permanent human premolar tooth.

Amino acid	Residues of amino acid per 1000 total residues	
	Root area (cementum)	Crown area
Half-cystine*	4.3	5.5
3-Hydroxyproline	Tr	Tr
4-Hydroxyproline	70	75
Aspartic acid	63	54
Threonine	29	32
Serine	47	47
Glutamic acid	87	81
Proline	122	134
Glycine	261	234
Alanine	99	108
Valine	37	43
Methionine†	7	9
Isoleucine	20	25
Leucine	51	54
Tyrosine	<1	<1
Phenylalanine	14	4
Hydroxylysine	4.6	6.5
Lysine	22	29
Histidine	12	15
Arginine	50	46

* Recovered as cysteic acid and cystine. † Recovered as methionine and methionine sulfoxides.

Histological examination of the Procion-fixed tissue showed it to be a microscopically continuous structure composed of typical collagenous bundles. In the coronal region where the layer is external to the enamel, a cellular layer is observed on the inner or enamel side of the tissue (Fig. 3). These cells, which appeared either columnar or cuboidal, resemble those of reduced dental epithelium.

The foregoing results demonstrate the existence of a collagenous layer external to the enamel and reduced dental epithelium and continuous with the cementum of the root in human, unerupted, but well developed permanent premolar teeth. This layer is tenaciously adherent to the tooth and remains after thorough dissection of all soft tissue remnants. It can be removed after fixation and decalcification as a distinct structure completely covering the surface of the tooth, both root and crown, and after removal conforms to the anatomical shape of the tooth. We therefore feel certain that this structure is distinct from the connective tissue of the periodontal ligament and the connective tissue of the gingiva which surround the unerupted tooth.

While our investigations have been

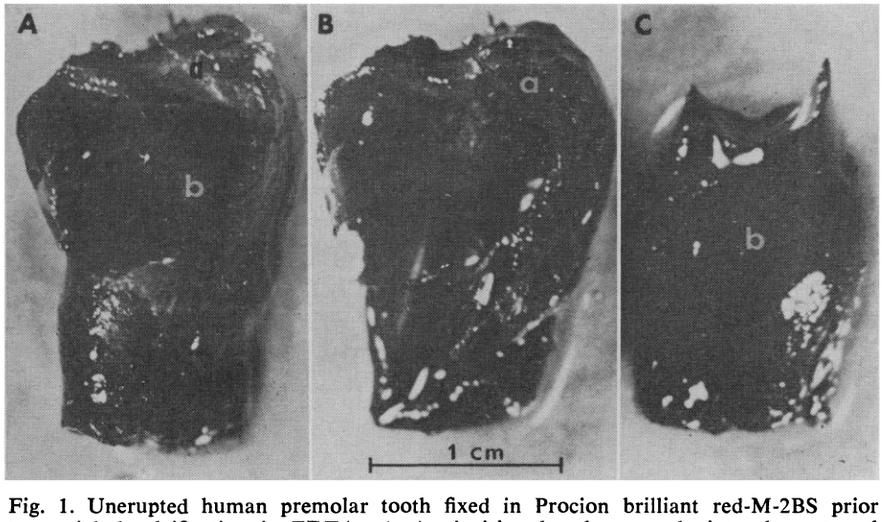

Fig. 1. Unerupted human premolar tooth fixed in Procion brilliant red-M-2BS prior to partial decalcification in EDTA. *A*, An incision has been made into the external tissue layer, *a*, investing the entire tooth and covering the crown enamel to demonstrate the residual dentinal core, *b*. *B*, The outer tissue layer, *a*, after removal of the dentinal core, *b*. The outer tissue layer conforms to the shape of the tooth and is continuous from the root of the tooth to and including the occlusal surface of the crown. *C*, The dentinal core, *b*, after removal of the outer tissue layer, *a*.

limited to unerupted human teeth, Rodriguez has recently demonstrated a clearly delineated region on the external surface of the enamel of fully mature, human erupted teeth, which had the histochemical staining properties of collagen (*5*). He also reported obtaining x-ray diffraction patterns and amino acid analyses characteristic of collagen from the residue remaining after decalcification in EDTA of slabs of enamel which had been carefully dissected free of dentin (*5*). Since the proteins of mature enamel are almost completely dissolved in EDTA (*3, 6*), it seems likely that this collagenous residue was derived from the surface layer external to the enamel which had the histochemical staining properties of collagen. This suggests that this collagenous structure external to the enamel in unerupted human teeth may persist long after eruption.

Our findings together with those of Rodriguez (*5*) may explain why relatively large amounts of hydroxyproline and other amino acids characteristic of collagen have been found in carefully prepared samples of erupted human enamel (*7*). In contrast, when the collagen surface layer was removed from erupted bovine teeth, prior to harvesting enamel, the amino acid composition of the enamel proteins was distinctly different from that of collagen (*3*). A similarly distinctive amino acid composition has also been reported for the EDTA soluble proteins of the enamel proteins of human erupted teeth (*6*). Since the enamel proteins of unerupted embryonic human, porcine, and bovine teeth also differ from collagen in amino acid composition (*8*) and in molecular structure (*4*), it appears likely that collagen does not constitute the structural protein of dental enamel in the species thus far investigated.

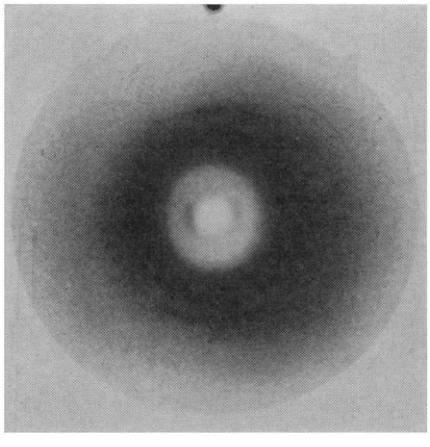

Fig. 2. X-ray diffraction pattern of external tissue layer from the region of the crown showing characteristic reflections of collagen.

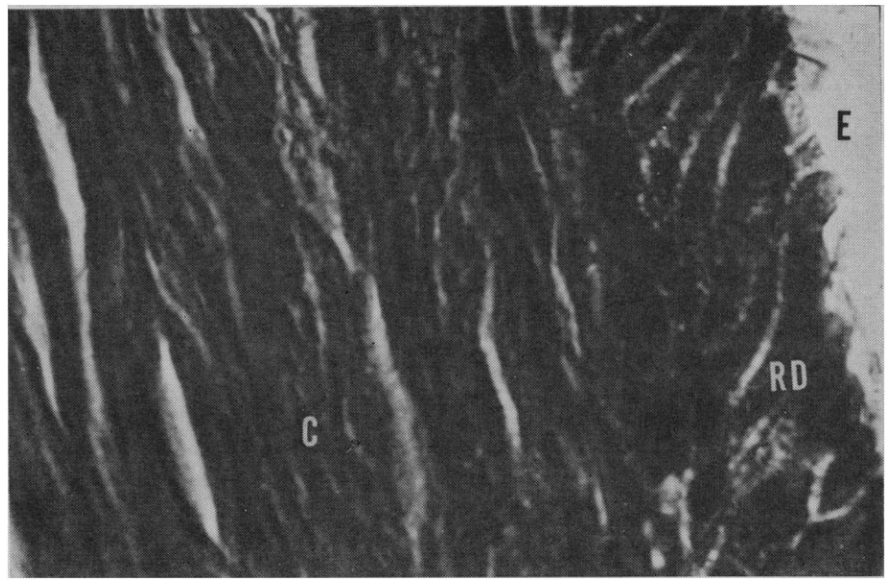

Fig. 3. Longitudinal section of a portion of the outer layer shown in Fig. 1*B*. The section illustrated was taken from the region of the crown, designated *a* in Fig. 1*B*; *E*, enamel space; *RD*, reduced dental epithelium; *C*, collagenous bundles; 1435 ×.

Although the cementum of human teeth is described in modern textbooks as covering only the dentin of the roots and not the crown enamel (*9*), this description may have to be modified in view of our findings that a collagenous layer not only covers the crown enamel, but is continuous with the cementum covering the dentin of the roots and may therefore be analogous to the crown cementum noted in bovine teeth (*1*).

Coronal cementum has been described in human teeth impacted for long periods of time (*10, 11*). Its presence has been attributed to degeneration of the dental epithelial layer and the resulting close apposition of the surrounding connective tissue on the surface of the enamel (*10*). Our results have been obtained with unerupted but normal teeth. In our material, the cells of the dental epithelium are still apparent and separate the connective tissue external to the cells from the enamel.

The presence of coronal cementum on human teeth would be in agreement with the much earlier histological observations made by Owen in 1840 (*12*) that the cementum forms the external layer of the tooth, covering both the crown enamel and the root dentin. He observed this layer of crown cementum in human teeth and in the teeth of a large number of other species as well. He notes that similar observations had been made by others. It is easy to understand how such a layer in human teeth may not have been observed in relatively recent times, since it is easily lost during decalcification unless it is extensively fixed prior to this procedure. Moreover, even in bovine teeth, where the calcified collagenous layer is more prominent, the structure is usually displaced during sectioning or grinding, or both, of undemineralized tissue of the thickness commonly employed at the present time for histological examination.

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Hereditary Deficiency of Serum α_1-Antitrypsin

Abstract. *Deficiency of the serum α_1-antitrypsin appears to be under genetic control. The level of this protein is reduced to less than 10 percent of the norm in individuals homozygous for the trait, who may suffer from pulmonary emphysema. Heterozygous individuals have a concentration of serum α_1-antitrypsin between 50 and 60 percent of normal, but appear to be in good health. The estimated heterozygous frequency of the trait in a small white population in Georgia is 2.1 percent.*

It has become clear since the work of Camus and Glay (*1*) that human serum inhibits the proteolytic activity of trypsin, but only recently was it established that this property resides in two different serum proteins (*2*). Most of the serum antitryptic activity (85 to 90 percent) is associated with an α_1 protein which is designated the α_1-antitrypsin; the remaining 10 to 15 percent resides in the α_2-macroglobulin fraction.

The α_1-antitrypsin, a glycoprotein isolated and characterized by Schultze (*3*), contains 12.5 percent carbohydrate. It has a molecular weight of 60,000 and a sedimentation constant of 3.5*S*. In starch-gel electrophoresis at *p*H 8.5 it migrates as a single band in the post-albumin region.

Laurell and Erikson (*4*) described several individuals in whose serums the concentrations of α_1-antitrypsin were greatly decreased; in all instances the detectable amount was less than 10 percent of the norm. Subsequently Erikson reported a family whose serum antitrypsin was either normal, moderately decreased (40 percent normal), or greatly decreased (less than 10 percent normal), according to the individual; he suggested that the concentration of serum α_1-antitrypsin was under genetic control (*5*). Moderate deficiency of α_1-antitrypsin was apparently compatible with normal health, but several individuals in whom only very small quantities were detectable suffered from pulmonary emphysema (*5*).

Since the association of deficiency of serum α_1-antitrypsin with pulmonary emphysema appeared more prevalent than could be expected by chance, a pilot study was undertaken on 99 random patients attending the emphysema clinic at Bellevue Hospital. Serum α_1-antitrypsin was examined by starch-gel electrophoresis in a barbital buffer at *p*H 8.5 (*6*) and by fibrin-agar electrophoresis (*7*); its activity was quantitatively determined by observing its inhibitory effect on tryptic digestion of the chromogenic substrate *N*, α-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (*8*). Inhibitory activity of the serum α_2-macroglobulin was estimated from the fibrin-agar electrophoresis.

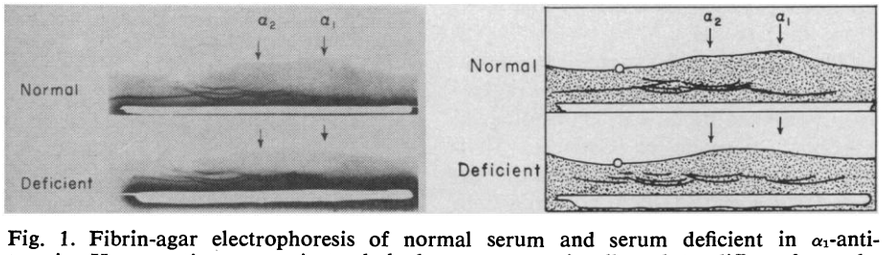

Fig. 1. Fibrin-agar electrophoresis of normal serum and serum deficient in α_1-antitrypsin. Horse antiserum against whole human serum is allowed to diffuse from the antibody trough to identify the serum proteins. A solution of trypsin diffuses from the upper trough, digesting the fibrin in the agar. Inhibition of fibrin digestion in the α_1 and α_2 regions in normal serum is demonstrated by the two broad peaks on the upper edge of the shaded area, whereas there is very little inhibition in the α_1 region of the serum deficient in α_1-antitrypsin. The important features of the photograph are shown in the drawing (right).

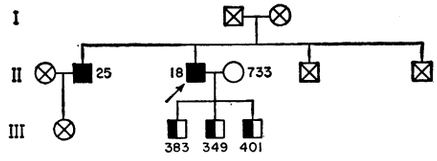

Fig. 2. Pedigree. The numbers are the numbers of micrograms of trypsin inhibited per milliliter of serum. Solid square, presumptive homozygote; half-solid square, presumptive heterozygote; open circle, normal; crossed circle, not examined.

In all but one of the patients (A.B.) the serum antitrypsin was approximately normal. Fibrin-agar electrophoresis of this one serum deficient in α_1-antitrypsin resulted in a markedly decreased peak of inhibition in the α_1 region; the α_2-macroglobulin peak of inhibition appeared normal (Fig. 1). Quantitative analysis confirmed that the serum antitrypsin activity was greatly decreased; the α_1-antitrypsin in 1 ml of normal serum inhibits approximately 745 μg trypsin, compared to 18 μg trypsin for A.B.'s serum. A.B. was a 53-year-old Caucasian male with clinical and x-ray findings consistent with the diagnosis of pulmonary emphysema. His arterial oxygen saturation was normal (94 percent) at rest but abnormally low (83 percent) during exercise. He was unusual among those whose emphysema is severe enough to disable them, in having a relatively well-preserved maximum breathing capacity (84 liters/min), a large vital capacity (5.6 liters), and an unusually large total lung capacity (12 liters). His older brother had similarly decreased serum α_1-antitrypsin activity (25 μg of trypsin inhibited by 1 ml of serum); although not examined, he was in apparent good health. A.B.'s three sons had α_1-antitrypsin activities approximately 50 percent of normal (383, 349, and 401 μg of trypsin inhibited by 1 ml of serum, respectively) (Fig. 2); they were healthy and showed no evidence of pulmonary emphysema. The wife of A.B. was healthy, and the α_1-antitrypsin activity of her serum was normal.

These findings, in conjunction with those of Erikson (*5*), suggest that the deficiency is inherited as a single-gene effect. Individuals with a serum α_1-antitrypsin concentration less than 10 percent of the norm would be presumed homozygotes, whereas those whose concentration was 50 to 60 per-