Effect of aging on the human pulp

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Nonerupted, young functional, and old noncarious teeth obtained from individuals who varied in age from 15 to 75 years were studied to determine the changes in the collagenous fibers and ground substance during the aging process. It was noted that in the aging process, there is a progressive reduction in the size of the pulpal chamber and a progressive deposition of calcified masses that originate in the root pulp and progress into the coronal pulp. As a result of the calcification of the blood vessels and nerves in the pulp, there is a decrease in the number of blood vessels and nerves in the coronal pulp. However, their connective tissue sheaths persist and give the pulp a fibroticappearance. The stroma of the pulp, regardless of age, consists of fine collagenous fibers and an abundant amount of alcianophilic ground substance.

The literature and the textbooks of oral histology and endodontics have summarized the changes in the pulp due to the aging process as follows: a reduction in the number of fibroblasts and odontoblasts,[1-4] a decrease in the number of demonstrable blood vessels and nerves that supply the pulp,[5,6] a progressive increase in large collagenous fibers that results in fibrosis of the pulp, ■ a concomitant decrease in the ground substance,[8,9] and an increase in deposits of calcified masses throughout the pulp.[10,11] Of these findings, a continual controversy has risen over whether fibrosis is the result of aging.

The point of issue has been whether the fibrosis of old pulps is the result of a continuous formation of collagenous fibers during the aging process, a response to external environmental factors, or a relative increase because of the loss of pulpal area. The purpose of this study, therefore, was to determine the interrelationship between collagen, ground substance, and the vascular and neural structures during the aging process and to attempt to determine the factors that produce fibrosis in old pulps of human teeth.

MATERIALS AND METHODS

One hundred mandibular and maxillary noncarious human teeth were used for this study; they were obtained from both males and females who varied from 15 to 75 years of age. The teeth were extracted either for orthodontic or periodontal involvement or for denture replacement. In addition, impacted molars that showed various stages of development were used.

The teeth were separated into two groups;.- teeth from individuals of both sexes younger than 40 years of age, and teeth from individuals of both sexes older than 40 years of age.

Immediately after they were extracted, the teeth were fixed in an alcoholic Formalin and acetic acid solution (AFA). The teeth were then decalcified and prepared for nitrocellulose embedding in the routine manner. The embedded blocks were sectioned from 15 to 100 micrometers (cm) in thickness. Alternate thin sections (15 tm) were stained with hematoxylin and triosin, periodic acid-Schiff (PAS) and hematoxylin, Verhoeffs iron hematoxylin and picrofuchsin, Alcian Blue (pH 2.5) and PAS, and silver nitrate impregnation. The thick sections (50 to 100 im) were stained with iron hematoxylin to demonstrate the blood vesselsand nerves.

RESULTS

The pulps of nonerupted teeth consisted of stromas of specialized loose connective tissue. The cellfree zone of Weil was demonstrable between the odontoblastic layer and the pulp proper. There were only a few blood vessels and nerves in the pulp (Fig 1A). The pulpal stroma of erupted functional teeth from young individuals still consisted of loose connective tissue. However, there was an increase in the number of blood vessels and nerves that traversed the coronal pulp. These vascular and nerve structures formed an extensive network in the pulp (Fig IB). One of the characteristic changes in the pulp as the result of aging was the decrease in the pulpal area (Fig 1C-F). The reduction in pulpal area was the result of continual deposition of occlusal dentin, calcified masses, and dentinal apposition at and above the area of furcation (Fig 1C-E).

When sections of old teeth were stained with hematoxylin and triosin, the pulps still had a loose appearance. All the pulps from the old teeth that were examined showed root calcification or coronal calcification, or both (Fig 1C-G). The degree of calcification varied from small isolated masses within the root or crown portions to extensive masses that nearly occupied the entire pulpal chamber (Fig 1F,G). An ankylosis between dentin and the calcified masses was noticed in a few teeth (Fig 1G).

Vascular and Nerve Distribution

Only the large blood vessels were evident in the coronal pulp of the nonerupted teeth (Fig 2A). The large nerve bundles were limited to the apical region of the forming roots.

*Fig I— AU sections, except (b), were stained -with hematoxylin and triosin; original magnification -was *20. (a) Section of nonerupted lower third molar. Note beginning of root formation. Pulpal chamber is wide; zone of Weil is discernible. Pulp consists of loose connective tissue (V, blood vessels; W, zone of Weil), (b) Section of upper molar from young adult. Note wide pulpal chamber, loose appearance of pulp, and abundant nerve and vascular structures ((N, nerve; V, blood vessel) PAS and hematoxylin stain), (c) Section of an old molar. Note narrowing of pulpal chamber, deposition of dentin at region of furcation, loose appearance of pulp, calcification deposits in root, and sparsity of blood vessels and nerves (Ca, calcified mass), (d) Section of old molar. Narrowness of pulp chamber is due to excessive apposition of dentin in area of furcation. Note isolated calcified mass in pulp (Ca. calcified mass), (e) Section of old molar. Note diffuse calcification of root and coronal pulps, (f) Section of old tooth in which calcified masses nearly fill pulpal chamber, (g) Section of old tooth. Note ankylosis between dentin and pulpal calcified mass (Ca, calcified mass; D, dentin).*

There were odontoblastic capillary networks, however. There was an extensive branching of both the vascular and neural structures in young functional teeth. As the pulpal vessels and nerves entered the coronal region, vascular branches coursed toward the lateral and occlusal surfaces of the pulp. As these branches reached the cuspal horns, a rich subodontoblastic plexus of nerves and blood vessels was formed (Fig 2B).

There was an apparent decrease in the number of blood vessels and nerves that supplied the pulps of the older teeth. The sparsity of blood vessels and nerves in an older tooth is illustrated in Figure 2C. In contrast to the extensive patterns in the young functional tooth, only a few large nerves and blood vessels were evident in this pulp. The root pulp of this tooth showed diffuse calcification. Along with progressive deposition of calcified bodies in the pulp, there was a further loss of vessels and nerves (Fig 2D). Only one or two large vessels and nerves were evident.

*Pig 2— All sections were cut at lOOpjn and stajned with iron hematoxylin to demonstrate blood vessels and nerves; original magnification was *20, (a) Section of nonerupted molar. Note few number of blood vessels in pulp. Nerve bundles are restricted to apical region of forming root (V, blood vessels), (b) Section of tooth from young adult. Note rich arborization of blood vessels and nerves in coronal pulp (N, nerves; V, blood vessels), (c) Section of old tooth. In comparison with young tooth, there is a decrease in number of demonstrable blood vessels and nerves, (d) Section of molar from person over 60 years of age. Pulp contains multiple loci of calcification. Note sparsity of blood vessels and nerves in pulp (N, nerves; V, blood vessels).*

Collagenous Elements

When sections of nonerupted teeth were exposed to silver nitrate impregnation or stained with picrofuchsin, the coronal pulp consisted mainly of fine collagenous fibers, the so-called reticular fibers. Not only did the zone of Weil appear cellfree, but there also was an absence of an argyrophilic fiber concentration (Fig 3). In Figure 3A, a high magnification view of the pulp illustrates the concentration of fine collagenous elements that form the fibrous matrix of the pulp. The only large collagen bundles were the adventitial and neural sheaths (Fig 3B).

The pulpal stroma of young functional teeth also consisted of fine collagenous fibers interspersed in the ground substance. The conspicuous vascular and neural connective sheaths were the only large fiber bundles; they followed the pathways of the nerves and blood vessels (Fig 4). Figure 4A, a high magnification view of an area adjacent to the pulpo-odontoblastic border, shows the absence of collagenous elements in the zone of Weil, the coarse fibers of the neural and vascular connective tissue sheaths, and the fine collagenous fibers embedded in the pulpal matrix.

The orientation of the vascular and neural connective tissue sheaths was further emphasized in sections of old pulps that were impregnated with silver nitrate or stained with picrofuchsin. Figure 5 illustrates a silver nitrate impregnated section of an old tooth. It was apparent that the large collagen fiber bundles are directly associated with the blood vessels and nerves. When the sections of old pulps were examined under higher magnification, the pulpal stroma still consisted of fine collagenous fibers, and the only thick argyrophilic fiber bundles were the connective tissue sheaths of both blood vessels and

*Fig 3— Section from nonerupted molar from young adult under 20 years of age was exposed to silver nitrate impregnation. Note that pulp consists mainly of fine collagenous fibers and that zone of Weil appears devoid of collagenous elements (W, zone of Weil), (a) High magnification of area X at pulp shows fine collagenous elements in pulp (orig mag x250). (b) High magnification of area Y of pulp shows that thick fiber bundles are associated with blood vessels and nerves of pulp (orig mag *250).*

Fig 4—Section of adult functional tooth from a 30-year-old person. Neural and vascular connective tissue sheaths are prominent; stroma consists of fine collagenous fibers (silver nitrate impregnation, orig mag X35). (a) High magnification at pulpal-odontoblastic region of pulp. Note that there are only a few fine collagenous fibers at zone of Weil and odontoblastic region. Connective tissue sheaths are prominent with stroma made of fine collagenous fibers (silver nitrate impregnation, orig mag X125).

Fig **5—** *Section of old molar. Thick fiber bundles follow pathways of nerves and vessels (silver nitrate impregnation, orig mag x20). (a) High magnification at pulpal-odontoblastic region. Thick fiber bundles are prominent and related to blood vessels and nerves. Fibrous stroma still consists of fine collagenous elements {(B, bundles of collagenous fibers) silver nitrate impregnation, orig mag XI25).*

Fig 6—Section of old tooth from individual older than 65 years. Apparent fibrosis of pulp is related to persisting connective tissue sheaths of vascular and neural structures (silver nitrate impregnation, orig mag x30).

*Fig 7—(a) Odontoblastic-predentin region from nonerupted molar. Note fine Von Korff fibers emerging from predentin and passing into zone of Weil (silver nitrate impregnation, orig mag *125). (b) In young adult's tooth, note Von Korff fibers are thicker and pass through zone of Weil into pulp (orig mag *125). (c) In contrast to long individual fibers that stream through zone of Weil in young adult's tooth, fibers in old tooth appear to fuse into short thick fiber bundles that terminate in zone of Weil (orig mag *125).*

*Fig 8—All sections were photographed through red filter to emphasize Alcian Blue-positive material (orig mag *125). (a) Section of nonerupted molar stained with Alcian Blue (pH 2.5) and PAS for the presence of acid and neutral mucopolysaccharides. Predentin and pulp stain intensely alcianophilic whereas dentin is Schiff-positive, (b) Section of tooth from young adult. Note that pulp and predentin stain alcianophilic. (c) Section of tooth from individual older than 55 years of age. Pulpal stroma stains alcianophilic whereas calcification mass is Schiff-positive (Ca, calcified mass).*

nerves (Fig 5A). The prominence of the connective tissue sheaths was further accentuated by the progressive apposition of calcified masses as well as by the loss of additional blood vessels and nerves (Fig 6). The fibrosis appears to be related to the pathways of the degenerated vessels or nerves.

Von Korff Fibers

During the aging process, the appearance of the Von Korff fibers emerging from the predentin was noticed. In nonerupted teeth, fine Von Korff fibers emerged from the predentin to pass through the odontoblastic layer into the zone of Weil (Fig 7A). The argyrophilic fibers became more prominent with eruption and function, and extended into the pulpal-odontoblastic border (Fig 7B). The predentin-pulpal border in the old teeth was very irregular in outline. Concomitantly, there was a fusion of the fine Von Korff fibers. These fibers appeared to form short bundles that were limited in their course to the odontoblastic layer (Fig 7C).

Ground Substance

The semiqualitative presence of the protein-acid mucopolysaccharide complex was demonstrated by the Alcian Blue and PAS combination. With this histochemical reaction, the acid mucopolysaccharides were alcianophilic whereas the neutral mucopolysaccharides and sialic acid were Schiff-positive. When sections of nonerupted, young functional, and old teeth respectively were exposed to this histochemical combination, the predentin and the pulp were Alcian Blue-positive whereas the dentin stained pink. In comparing the intensity of alcianophilia between the three age groups, little difference appeared in the staining response of the pulps (Fig 8A-C). Whereas the coronal pulpal tissue was Alcian Blue-positive, the root pulps of the young functional teeth showed a different staining response. The core of the root pulp was Schiff-positive; the lateral regions were alcianophilic. The presence of the calcified masses in the root accentuated the Schiff staining from pink to a hue of deeper red. When there was diffuse calcification in the coronal pulp, the calcified masses stained Schiff-positive in contrast to the surrounding pulpal ground substance that appeared deep blue (Fig *SC).*

DISCUSSION

It is an accepted fact that there is a decrease in the number of cells in the pulp and an increase in the fibrous component with age. A question has been raised as to whether the increased fibrosis in old teeth was the result of a proliferation of collagenous elements during the aging process, or the result of a reorganization of the persisting collagenous elements into larger fiber bundles. Shroff[12] pointed out that the increase in the fibrous elements that occurred in aging could be simply the result of the persistence of fibrous elements of an originally larger pulp. Stanley and Ranney concluded that the amount of collagen did not increase in the coronal pulp tissue after the age of 20 years. They felt that the increased collagen was not a direct result of the aging process, but rather a reflection of previous irritation or stimuli to the pulp.

In all observations relating to the aging pulp, little attention was given to the connective tissue sheaths of both the blood vessels and the nerves that supply the pulp. In the current study, the fibrous components of the pulp and the vascular and neural connective tissue sheaths were observed in teeth during their formative nonerupt ive, functional, and aging states. In the preemptive stage, the pulp consisted of loose connective tissue rich in fine collagenous fibers and ground substance. There were only a few blood vessels and nerves, and the only large fiber bundles were directly associated with the vascular and neural structures. The increase in large collagenous fibers in the pulps of young functional teeth was mainly related to the additional number of vessels and nerves that supply the coronal pulp. The intercellular stroma still consisted of fine collagenous elements as well as a mucopolysaccharide-protein ground substance.

During the aging process, there was not only a decrease in the size of the pulpal chamber but also a loss of blood vessels and nerves. At the same time, the coronal pulp showed an increase in the number of collagenous bundles. These fibrous bundles were associated with the connective tissue sheaths of the blood vessels and nerves. The stroma of the pulp still consisted of fine collagenous fibers. Changes as the result of aging in collagen in the skin and elsewhere have been summarized by Verzar,[14] Gross,[15] and Milch.[10] They agree that there is an increase in the thickness of the collagen fibers and changes in the chemical and physical properties of the collagen during the aging process. The pulpal stroma, in contrast to the dermis of the skin, consists mainly of fine collagenous fibers interspersed in the abundant ground substance. At no time during the life of the tooth were thick collagen fibers seen independent of the connective tissue sheaths. The data of the current study indicate that the connective tissue of the pulp is specialized in nature; no comparison can be made between the aging effect of the connective tissue of the pulp and other areas rich in connective tissue, such as skin or even gingiva.

A common finding in the pulps of the old teeth was the presence of diffuse calcification. Milch[10] postulated that the apparently increased cross-linkage of collagen with advancing age enhances its tendency for mineralization. It was noticed in this study that the collagen bundles of the vascular and neural sheaths of the old pulps were the loci for calcification. The calcified masses in the root and coronal pulp were directly associated with the collagen bundles. In the young pulps, the collagen bundles of the sheaths stained blue with the Alcian Blue and PAS combination whereas in the old pulps they appeared Schiff-positive. The Schiff-positive fibers were the sites of calcification.

Since the pulp was of loose connective tissue in nature, it was rich in ground substance; it stained intensely blue with Alcian Blue. The staining response of the pulp of old teeth to the Alcian Blue and PAS combination did not change drastically from that in the young pulps. The only structures that reacted differently with this histochemical combination were the vascular and neural connective tissue sheaths. In contrast to the alcianophilia of these collagen bundles in young pulps, the old fibrous bundles were Schiff-positive. Bhussery[8] and Zerlotti[*9] reported a decrease in mucopolysaccharides in aging pulps, and Mancini and associates,[17] Wentz and associates,[18] and Lorincz[10] agreed that the amount of amorphous ground substances decreases as the number of collagen fibrils increases in the skin and elsewhere. Since biochemical analysis was not carried out in the current study, a definite conclusion cannot be made concerning the amount and nature of the mucopolysaccharide-protein complex in old pulps. However, the semiqualitative histochemical data from the current study would indicate that any comparison regarding the loss of ground substance for skin and other connective tissue areas is not valid because the nature and amount of mucopolysaccharides differ. In addition, it may be assumed that the decrease in ground substance previously reported for old pulps may partly be due to the decrease in the pulpal area and not due to the nature of the carbohydrate moiety.

Our findings agree with the conclusions of Shroff[12] and Stanley and Ranney[13] that pulpal fibrosis in old teeth is not the result of a continual formation and reorientation of collagen fibers during the aging process. The prominence of fiber bundles in old pulps may be, in part, attributed to the persistence of the connective tissue sheaths in a narrowed pulpal chamber after the vascular and neural structures were diminished.

SUMMARY

Nonerupted, young functional, and old noncarious teeth obtained from individuals who varied in age from 15 to 75 years were studied to determine the changes in the collagenous fibers and ground substance during the aging process. It was noted that in the aging process, there is a progressive reduction in the size of the pulpal chamber and a progressive deposition of calcified masses that originate in the root pulp and progress into the coronal pulp. As a result of the calcification of the blood vessels and nerves in the pulp, there is a decrease in the number of blood vessels and nerves in the coronal pulp. However, their connective tissue sheaths persist and give the pulp a fibrotic appearance. The stroma of the pulp, regardless of age, consists of fine collagenous fibers and an abundant amount of alcianophilic ground substance.

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