Proteoglycans of alveolar bone of diabetic and non-diabetic mice: a histochemical study

Roger B. Johnson
Department of Anatomy, University of Manitoba, Faculty of Dentistry, Winnipeg, Manitoba, Canada

Summary. The effect of diabetes mellitus on the interdental alveolar bone has long been debated. The present study reported the distribution of glycosaminoglycans (GAG) in normal and diabetic alveolar bone using histochemical techniques. Animals were rendered diabetic and killed at 2, 4, 6 and 9 weeks after injections. Tissues were stained with Alcian blue 8GX dye (pH 2.5) to demonstrate GAG and the intensity of the staining reactions compared with age-matched controls. During the experiment, weights of control animals did not change significantly; weights of diabetic animals were significantly less than initial weights from 0-6 weeks (p<0.001), but became nearly equal by 9 weeks. Staining intensity of diabetic bone demonstrated initial decrease (0-4 weeks) followed by a marked increase (4-9 weeks) suggesting an early decline in bone GAG levels followed by increased bone GAG levels as compared to age-matched control and initial levels. Bone GAG levels were significantly different between diabetics and age-matched controls at 2 (p<0.005), 4 (p<0.001), 6 (p<0.001) and 9 (p<0.001) weeks after streptozotocin injections. Digestion with chondroitinase AC, ABC and streptomyces hyaluronidase suggested significant differences between control and diabetic bone matrix in the levels of chondroitin 4 and 6 sulfates (p<0.05) and hyaluronic acid (p<0.001) but not dermatan sulfate. In control and diabetic bone, chondroitin sulfates were located within the bone matrix, dermatan sulfate within bone matrix and Sharpey fiber bundles. Thus, bone GAG changes in diabetes mellitus occur predominately within bone matrix and are variable depending on the length of the disease.

Key words: Diabetes mellitus, Alveolar bone, Intrinsic fibers, Extrinsic fibers, Glycosaminoglycans

Introduction

The normal development and structure of bone is reported to be altered in diabetes mellitus. Among other complications, osteolysis of alveolar bone is reported in diabetic human subjects (Benveniste et al., 1967; Nichols et al., 1978) and in diabetic experimental animals (Cohen et al., 1961, 1963; Shklar et al., 1962; Bissada et al., 1966; Borghelli et al., 1967; Murrah, 1985). Osteoporosis of alveolar bone is also reported coincident to this disease (Glickman, 1946; Ramamurthy et al., 1973). Bone changes coincident to diabetes are attributed, in part to: 1) a decreased rate of bone formation (Shires et al., 1981), 2) a decreased rate of bone mineralization (Weiss and Reddi, 1980), 3) changes in the concentration and distribution of bone structural components (Dixit and Stern, 1979; Aufdermaur et al., 1980; Roshoft and Hegarty, 1981; Silberberg et al., 1981; Weiss et al., 1981) and 4) a decreased rate of bone turnover (Shires et al., 1981; Hough et al., 1983). It is generally accepted that there is a decrease in bone glycosaminoglycans (GAG) during experimental diabetes as a result of: 1) increased levels of degradative lysosomal enzymes in bone cells (Silberberg et al., 1981), 2) decreased sulfate incorporation into proteoglycans (Weiss et al., 1981), or 3) decreased total number of proteoglycans and decreased GAG per proteoglycan chain (Weiss et al., 1981). These effects are reversed with insulin, suggesting that they are features of this disease and not toxic effects of streptozotocin.

Koford et al., (1970) and Koford and Tocci (1973) report decreased levels of GAG in diabetic rat gingiva. However, there is little information concerning GAG distribution in either normal or diabetic alveolar bone, an adjacent tissue with a shared blood supply. Intervertebral alveolar bone, unlike bone at other sites, contains teeth which, because of functional movements, cause a unique pattern of continuous bone remodeling (Johnson, 1986) which occurs very rapidly and may respond more quickly to the effects of metabolic disease.
Effects of diabetes on bone GAG

than bone at other sites. Johnson (1985) reports a decrease in the intensity of Alcian blue staining of diabetic alveolar bone of the STR/N mouse, suggesting decreased concentrations of GAG there. However, the STR/N mouse is an inbred strain exhibiting other pathology. Thus, it seems necessary to study the effects of experimental diabetes on the alveolar bone of a more typical animal model, the Swiss mouse. The periodontium of the molar teeth of this animal is structurally similar to that of the human (Cohn, 1957) and could be representative of the effects of diabetes on human alveolar bone. The present study seeks to determine 1) the normal distribution of GAG in bone, 2) the effects of diabetes on GAG distribution and 3) the effects of duration of diabetes on bone GAG.

Materials and methods

Induction of diabetes

Twenty male Swiss mice, 12 weeks of age, were weighed and then given an intravenous injection of streptozotocin through the tail vein. An appropriate dosage of the drug (200 mg/kg; Rerup and Tarding, 1969) was dissolved in 0.1M citrate buffer at pH 4.5 (Ganda et al., 1976) immediately before injection. Twenty animals were injected with citrate buffer and served as age-matched controls. Mice were housed in cages and fed pelleted laboratory chow ad libitum. Diabetes was confirmed by persistent glycosuria, determined by the use of Clinistix® (Ames).

At 2, 4, 6 and 9 weeks after injections, animals were weighed, (2, 4 and 9 weeks-4 diabetic, 4 control; 4 weeks-8 diabetic, 8 control), blood samples drawn from the orbital sinus, and blood glucose levels determined by the hexokinase/glucose-6-phosphatase method in a DuPont ACA analyzer. Weight and blood glucose data were evaluated by analysis of variance and Duncan’s New Multiple Range Test.

Light microscopy

Following blood sampling, animals were killed, mandibles removed by blunt dissection and immediately fixed by immersion in 4% phosphate-buffered formalin containing 0.5% cetylpyridinium chloride to preserve GAG (Engfeldt and Hjertquist, 1968). Tissues were dehydrated in ethanols, dehydrated in ethanol, embedded in paraffin wax, and serially sectioned at 6 μm in sagittal and coronal planes (Fig. 1). Alternate sections were mounted on slides and stained with Alcian blue 8GX (Polyscience) at pH 2.5 to demonstrate GAG (Luna, 1968). Diabetic and control sections were stained at the same time. Preparations were studied and photographed in an Olympus photomicroscope.

Enzyme digestion

Eight 4 week animals (4 control and 4 diabetic) were prepared for enzyme digestion as previously described. Sections were treated with 5 μl drops of enzyme solution or with buffer alone. Chondroitinase AC (Sigma) (from Arthrobacter aurescens) was prepared at a concentration of 2.0 μg/ml in 0.1M tris-HCl buffer at pH 7.3 (Yamagata et al., 1968; Yamada, 1974). Chondroitinase ABC (Sigma) (from Proteus vulgaris) was prepared at a concentration of 2 μg/ml in 0.1M tris-HCl buffer at pH 8.0 (Yamagata et al., 1968; Yamada, 1974). Chondroitinase AC removes predominately chondroitin 4 and 6 sulfates; chondroitinase ABC additionally removes dermatan sulfate (Yamagata et al., 1968; Yamada, 1974). Streptomyces hyaluronidase (Sigma) (from Streptomyces hyalurolyticus) was prepared at a concentration of 150 turbidity reducing units/ml (Yamada, 1973), in 0.1 M phosphate buffer (pH 5.0). Streptomyces hyaluronidase specifically removes hyaluronic acid from tissue sections (Yamada and Hirano, 1973). Sections were incubated for 4 hours at 37° in 100% relative humidity, then rinsed with distilled water and stained with Alcian blue as previously described. Three types of controls were performed on additional tissue sections from 6 week diabetic and control animals: 1) some sections were kept intact without incubation, 2) some were incubated with heat inactivated enzyme in appropriate buffer, and 3) some were incubated in buffer without enzyme.

Image analysis

Quantification of the Alcian blue staining intensity was performed using a Cambridge Quantimet Q-10 image analysis system interfaced with a video camera attached to an Olympus photomicroscope. Histological images were digitized to grey values and background corrections made to remove shading effects. Three readings were made over intrinsic fibers and three readings made over extrinsic fibers in the crestal, middle and apical thirds of the septum. Each area of analysis was 100 μm². Intrinsic and extrinsic fiber readings were averaged for each section and means calculated for each animal and for each group. Since densitometer readings do not represent a universal distribution, they were converted to either percentage of age-matched control or percentage of control, time 0, and then transformed to radians prior to statistical analysis. Transformed mean percentages from sections, animals and groups were compared by analysis of variance and Duncan’s New Multiple Range Test.

Results

Streptozotocin was effective in rendering injected animals diabetic. The mean blood glucose for diabetics was very high (657.4±37.2 mg/dl) and was significantly greater than controls (209.1±15.1 mg/dl) (p<0.001) (Table 1). All diabetic animals demonstrated glycosuria as determined by Clinistix® (Ames). Diabetic mice had a
Effects of diabetes on bone GAG

significant loss of body weight compared to age matched controls at 2, 4 and 6 weeks after streptozotocin injections (p<0.001) but not significantly different by 9 weeks (Table 2).

Alveolar bone of mice was composed of extrinsic (Sharpey’s) and intrinsic fibers, the latter demonstrating histochemical changes during diabetes. Sharpey’s fibers were Alcian blue negative (AB-) in both diabetics and controls (Figs. 2, 2-6). Many fibers demonstrated Alcian blue positive (AB+) granules (Figs. 4, 5) which were removed by chondroitinase ABC digestion but not by *Streptomyces* hyaluronidase or chondroitinase AC, suggesting that they were dermatan sulfate.

There were no significant differences in Alcian blue staining of control bone matrix (intrinsic fibers) throughout the experiment; diabetic bone matrix demonstrated significant changes. At two weeks after streptozotocin injection, staining intensity was not significantly different from initial levels (Table 3), however, by 4 weeks, staining intensity had significantly decreased (p<0.001) (Figs. 2, 3, Table 3). By 6 weeks, staining intensity had significantly increased from initial levels (p<0.001) (Figs. 5, 6, Table 3). Analysis of variance revealed significant differences in Alcian blue intensity as a function of treatment and time (p<0.001) (Table 4). There was also a significant interaction between treatment and time (p<0.001) (Table 3).

Digestion of tissue samples with various enzymes yielded differences in Alcian blue staining intensities in control and diabetic bone matrix. Exposure to chondroitinase AC produced a significant reduction in control staining intensity (20%, p<0.001); diabetic tissues demonstrated a slight (1.9%), non-significant reduction (Table 5). Exposure to *Streptomyces* hyaluronidase produced a significant reduction in control staining intensity (30%, p<0.001), a level not significantly different from unstained tissues (Table 5). In diabetic bone, this enzyme produced a significant reduction in staining intensity (8%, p<0.001), which was significantly higher than either unstained tissues or controls (p<0.001) and chondroitinase AC treated diabetic tissues (p<0.005) (Table 5). In both diabetic and control tissues, chondroitinase ABC exposure produced significant reductions (control, 30%; diabetic, 18%) in staining intensity as compared to non-enzyme treated and chondroitinase AC treated tissues (p<0.001) (Table 5). These tissues did not demonstrate a greater staining intensity than unstained ones (p<0.001) (Table 5). Factorial analysis of variance suggested a significant interaction between presence of diabetes and enzyme treatment on reduction of Alcian blue staining intensity of alveolar bone (Table 6).

**Table 1.** Effects of experimental diabetes on blood glucose levels of the mouse. Significance of data is determined by analysis of variance.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>N</th>
<th>MEAN BLOOD GLUCOSE (MG/DL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>209.1±15.12</td>
</tr>
<tr>
<td>Diabetic</td>
<td>20</td>
<td>657.42±37.12*</td>
</tr>
</tbody>
</table>

*significantly greater than control, p < 0.001

**Table 2.** Mean weight of control and diabetic mice. Numbers in parentheses indicate animals per group. Significance of data is determined by Duncan’s New Multiple Range Test.

<table>
<thead>
<tr>
<th>TIME (weeks)</th>
<th>GROUP</th>
<th>MEAN WEIGHT (GM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CONTROL</td>
<td>36.12±0.74(4)</td>
</tr>
<tr>
<td>4</td>
<td>CONTROL</td>
<td>37.07±1.15(8)</td>
</tr>
<tr>
<td>6</td>
<td>CONTROL</td>
<td>38.40±1.59(4)</td>
</tr>
<tr>
<td>9</td>
<td>CONTROL</td>
<td>41.42±1.41(4)</td>
</tr>
<tr>
<td>2</td>
<td>DIABETIC</td>
<td>34.79±0.48(40)</td>
</tr>
<tr>
<td>4</td>
<td>DIABETIC</td>
<td>29.48±0.61(4)</td>
</tr>
<tr>
<td>6</td>
<td>DIABETIC</td>
<td>30.98±0.92(8)</td>
</tr>
<tr>
<td>9</td>
<td>DIABETIC</td>
<td>31.35±0.73(4)</td>
</tr>
</tbody>
</table>

*significantly less than control, (p < 0.001) and than initial (p < 0.001).

**Table 3.** Percentage of initial (0 weeks) Alcian blue staining reactions of intrinsic fibers of alveolar bone of control and diabetic mice at various times after induction of diabetes (0 week = 100±0.25% staining reaction). Significance determined by Duncan’s New Multiple Range Test.

<table>
<thead>
<tr>
<th>AGE (weeks)</th>
<th>GROUP</th>
<th>MEAN BLOOD GLUCOSE (MG/DL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CONTROL</td>
<td>99.76±0.29</td>
</tr>
<tr>
<td>4</td>
<td>CONTROL</td>
<td>98.03±0.59</td>
</tr>
<tr>
<td>6</td>
<td>CONTROL</td>
<td>97.38±0.39</td>
</tr>
<tr>
<td>9</td>
<td>CONTROL</td>
<td>99.30±0.19</td>
</tr>
<tr>
<td>2</td>
<td>DIABETIC</td>
<td>99.27±0.76</td>
</tr>
<tr>
<td>4</td>
<td>DIABETIC</td>
<td>85.44±0.36**,+</td>
</tr>
<tr>
<td>6</td>
<td>DIABETIC</td>
<td>116.94±0.19**,+</td>
</tr>
<tr>
<td>9</td>
<td>DIABETIC</td>
<td>130.08±0.48**,+</td>
</tr>
</tbody>
</table>

*significantly different from age-matched control, p < 0.005
**significantly different from age-matched control, p < 0.005
+significantly different from 0 week control and 2 week diabetic, p < 0.001
Effects of diabetes on bone GAG

Table 4. Factorial analysis of variance of percentage of 0 week Alcian blue staining reactions of intrinsic fibers of alveolar bone from control and diabetic mice as a function of treatment (A), time (B) and their interaction (AB). DF, degrees of freedom; SS, sum of squares (variability); MS, mean of squares (variance); F, f (variance) ratio. Percentages were transformed to radians prior to analysis.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>1203.07</td>
<td>1203.07</td>
<td>129.53*</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>2020.89</td>
<td>673.57</td>
<td>72.52*</td>
</tr>
<tr>
<td>AB</td>
<td>3</td>
<td>1388.49</td>
<td>462.83</td>
<td>49.83*</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>4835.17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.001

Table 5. Percentage of 4 week, non-enzyme treated (NE) Alcian blue staining reactions of 4 week control and 4 week diabetic intrinsic fibers of alveolar bone of mice following digestion by either chondroitinase AC (AC), chondroitinase ABC (ABC), or Streptomyces hyaluronidase (H) (Non-enzyme treated control = 100%). Significance is determined by Duncan's New Multiple Range Test. Percentages were transformed to radians prior to analysis.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ENZYME</th>
<th>CONTROL (%NE control)</th>
<th>DIABETIC (%NE control)</th>
<th>DIABETIC (%NE DIABETIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td></td>
<td>100.00 ± 1.06</td>
<td>85.44 ± 0.86†</td>
<td>100.00 ± 1.00</td>
</tr>
<tr>
<td>AC</td>
<td></td>
<td>81.99 ± 0.35***</td>
<td>71.58 ± 0.34***</td>
<td>98.11 ± 0.43</td>
</tr>
<tr>
<td>ABC</td>
<td></td>
<td>71.78 ± 0.31***</td>
<td>79.57 ± 0.77***</td>
<td>63.77 ± 0.40***</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td>71.87 ± 0.85***</td>
<td>93.99 ± 0.36***</td>
<td>99.99 ± 0.30***</td>
</tr>
</tbody>
</table>

* significantly different from control, p < 0.001
** significantly different from control, p < 0.05
*** significantly different from non-enzyme treated, p < 0.001
**** significantly different from AC, p < 0.001
† not significantly different from unstained
tt significantly different from AC, p < 0.005

Table 6. Factorial analysis of variance of the percentage of Alcian blue staining intensity of control (4 week, non-enzyme treated) intrinsic fibers of alveolar bone of 4 week control and 4 week diabetic mice following digestion with either chondroitinase AC, chondroitinase ABC, or hyaluronidase as a function of disease (A), enzyme treatment (B) or their interaction (AB). DF, degrees of freedom; SS, sum of squares (variability); MS, mean of squares (variance); F, f (variance) ratio. Percentages were transformed to radians prior to analysis.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>3.61</td>
<td>3.61</td>
<td>1.86</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>1813.87</td>
<td>604.62</td>
<td>311.49*</td>
</tr>
<tr>
<td>AB</td>
<td>3</td>
<td>413.88</td>
<td>137.96</td>
<td>71.07*</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>2277.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.001

Discussion

The present study demonstrated significant differences in the intensity of Alcian blue staining reactions of diabetic and control alveolar bone, suggesting biochemical differences in these tissues. There is biochemical data describing GAG levels in alveolar bone (Shimizu, 1965). However, localization of alveolar bone GAG is not reported, possibly because of the difficulty of separating intrinsic and extrinsic fibers for assay. Thus, histochemistry, despite its inherent technical problems, remains a preferred methodology for localization of various bone GAG. In the present study, use of a standardized time for demineralization and alternate sections yielded nearly consistent staining reactions between tissue samples in each animal. Analysis of variance indicated no significant differences in staining intensity between sections made from an animal, suggesting that sections were of nearly equal thickness. The present study extends previous biochemical

Fig. 1. Diagram of the interdental septum between the first and second molar teeth of the mouse. Fibers of the periodontal ligament (PL) attach the root cementum (C) to alveolar bone (B) becoming embedded in its matrix as Sharpey's fibers (S). Many Sharpey's fibers are transalveolar; that is, they pass through the septum without interruption. Tissue sections are made in either a sagittal plane, as illustrated, or in a coronal plane (dashed line), D, dentin.
Effects of diabetes on bone GAG

Fig. 2. Interdental septum, sagittal section, 4 week control, Alcian blue (pH 2.5). Intrinsic fibers of alveolar bone (B), osteocyte lacunar walls (W) and resting lines (R) are AB+; Sharpey's fibers (S) are AB−. PL, periodontal ligament. × 180

Fig. 3. Interdental septum, sagittal section, 4 week diabetic, Alcian blue (pH 2.5). Intrinsic fibers of alveolar bone (B), osteocyte lacunar walls (W) and resting lines (R) are not as intensely stained as controls; Sharpey's fibers (S) are AB−. PL, periodontal ligament. × 170

Fig. 4. Interdental septum, coronal section, 9 week diabetic, Alcian blue (pH 2.5). Intrinsic fibers of alveolar bone (B) stain intensely. AB+ granules (G) are evident within Sharpey's fibers (S), which are AB−. × 800

Fig. 5. Interdental septum, coronal section, 9 week control, Alcian blue (pH 2.5). Intrinsic fibers of alveolar bone (B) are less intensely stained than diabetics, Sharpey's fibers are mostly AB− but contain AB+ granules (G). × 800
Effects of diabetes on bone GAG

Fig. 6. Interdental septum, sagittal section, 9 week diabetic. Alcian blue (pH 2.5). Intrinsic fibers of alveolar bone (B) resting lines (R) and osteocyte lacunar walls (W) are intensely AB+. Sharpey's fibers (S) are AB-. PL, periodontal ligament. × 140

Studies by localizing GAG within alveolar bone and reporting alterations in diabetes.

The organic matrix (intrinsic fibers) of alveolar bone is approximately 50% of its dry weight and contains approximately 90% Type I collagen and approximately 5% non-collagenous glycoproteins, sialoproteins and GAG (sulfated and carboxylated), the latter demonstrable with Alcian blue at pH 2.5 (Lev and Spicer, 1964). Type III, IV and VI collagens are also matrix components (Becker et al., 1986). Sharpey's (extrinsic) fibers contain both Type I and III collagen (Wang et al., 1980) and possibly GAG (Johnson and Low, 1983). Unlike bone matrix, they are not uniformly mineralized, but contain unmineralized cores, possible sites of GAG (Johnson, 1983). The three-dimensional arrangement of collagen fibrils in connective tissues and the amounts and proportions of matrix GAG are good indicators to its mechanical properties. There is a significant inverse correlation between GAG levels, rate of collagen fibril turnover and fibril diameter (Scott et al., 1981; Parry et al., 1982; Svoboda et al., 1983; Flint et al., 1984; Merrilees et al., 1987). Dense, parallel bundles of large collagen fibrils (<150 nm) and low amounts of GAG (dermatan sulfate) are characteristic of tissues designed to cope with tensional forces (e.g., tendon) (Anderson, 1975; Scott et al., 1981; Parry et al., 1982; Flint et al., 1984). There is substantial information concerning this correlation in studies of rat tail tendon. However, there is not information about periodontal ligament, a modified tendon. Tissues with a dense network of fine collagen fibrils (60-150 nm) and chondroitin sulfate are designed to withstand compressive forces. Alveolar bone matrix is an example of this type of tissue. There is also evidence that high chondroitin sulfate levels inhibit collagen fibril growth (Merrilees et al., 1987).

Bone proteoglycans contain mostly chondroitin-4-sulfate with lesser amounts of chondroitin-6-sulfate and iduronic acid containing polymers (Prince et al., 1983). Recent studies report the isolation of two types of bone proteoglycans containing either: 1) one chondroitin-sulfate chain attached to a glu-gln-rich core protein, or 2) two chondroitin sulfate chains attached to a leu-rich core (Fisher and Termine, 1985). Both types are components of osteoid at the mineralizing front. GAG levels are highest in osteoid, decline rapidly during mineralization (Baylink et al., 1972; Engfeldt and Hjerpe, 1976) and residual levels may represent the degree of bone mineralization (Engfeldt and Hjerpe, 1976). With mineralization, proteoglycan cores are degraded; the one-chain form more slowly than the two-chain form. The chondroitin sulfate then persists within the mineral compartment (Fisher and Termine, 1985). The function of mineral compartment GAG is unknown, although it is postulated that they bind calcium ions and, thus, regulate mineralization (Engfeldt and Hjerpe, 1976).

The present histochemical data, based on enzyme digestion by chondroitinase AC, suggests that chondroitin sulfate is a major GAG component of alveolar bone matrix but is not present in significant quantities within Sharpey's fibers. Chondroitinase AC is reported to digest chondroitin 4 and 6 sulfates (Yamagata et al., 1968). Chondroitin sulfate levels appear to be highest within osteocyte lacunar walls and resting and reversal lines. There may be dermatan sulfate present within bone matrix, based upon digestion by chondroitinase ABC an enzyme capable of removal of chondroitin 4 and 6 sulfates and dermatan sulfate (Yamagata et al., 1968). Dermatan sulfate is a component of Sharpey's fibers, as AB+ granules are removed by chondroitinase ABC but not by chondroitinase AC or Streptomyces hyaluronidase, a situation similar to tendon (Anderson, 1975; Scott et al., 1981). Levels of hyaluronic acid within alveolar bone is more difficult to interpret as chondroitinase AC from Athrobacter aurescens, as used herein, may also digest hyaluronic acid (Hiyama and Okada, 1975). Alcian blue staining intensities following chondroitinase ABC and Streptomyces hyaluronidase digestion are nearly equal, suggesting two interpretations: 1) the former enzyme digested bone hyaluronic acid or 2) the latter enzyme broke linkages between chondroitin sulfates and core proteins causing removal from tissue sections (Yamada, 1982). Following digestion by chondroitinase ABC or Streptomyces hyaluronidase, Alcian blue staining reactions of bone matrix were not significantly different from unstained tissues, suggesting that other types of GAG are not significant components of this tissue.
There is evidence of a decline in bone GAG coincident to diabetes mellitus (Silberberg et al., 1981; Weiss et al., 1981; Johnson, 1985). This situation is postulated to be a result of 1) increased GAG degradation (Silberberg et al., 1981) or 2) decreased GAG synthesis (Weiss et al., 1981). Weiss et al. (1981) also report alterations in the structure of proteoglycans of diabetic bone; that is, a lower percentage of them form aggregates. GAG abnormalities coincident to diabetes are reversed by insulin administration (Kofoed et al., 1970; Weiss et al., 1981). The present study extends previous ones by reporting the effects of time on the loss of bone GAG. In early stages of diabetes (0-4 weeks), Alcian blue staining intensity was reduced in bone matrix, suggesting decreased levels of GAG and possible hypermineralization. Staining intensity of Sharpey's fibers was not affected. Enzymatic digestion studies utilizing chondroitinase AC, as reported herein, suggest that decreased levels of chondroitinase 4 and 6 sulfates might account for the decrease in total GAG levels of diabetic bone matrix. This situation also occurs in diabetic gingiva (Kofoed et al., 1970; Kofoed and Tocci, 1973). Initial hypermineralization of alveolar bone coincident to diabetes is reported in a scanning electron microscopic study by Johnson (1985), which corroborates the present histochemical data. Later in the course of diabetes (6-9 weeks), Alcian blue staining intensities of bone matrix increased, suggesting higher levels of GAG and possible hypomineralization. Staining reactions of Sharpey's fibers were unaltered. Hypomineralized bone coincident to diabetes is reported (Ramamurthy et al., 1973; Dixit and Stern, 1979; Weiss and Reddi, 1980). Bone calcification defects produced by diabetes are readily reversed by insulin administration (Weiss and Reddi, 1980; Dixit and Stern, 1979). It is possible that hypomineralized bone matrix could allow more rapid alveolar bone resorption coincident to periodontal inflammation, a common situation in diabetic patients (Cheraskin and Ringsdorf, 1970). Increased GAG levels after 6 weeks of diabetes are not consistent with reports by Silberberg et al. (1981) or Johnson (1985), possibly due to species or bone remodeling differences (Silberberg et al. studied rat tibia and femur; Johnson studied alveolar bone of STR/N mice, an inbred strain demonstrating kidney and gastrointestinal complications). The relatively higher turnover rate of bone alveolar bone collagenous proteins and the relatively lower turnover of alveolar bone mineral (Murray et al., 1982) as compared to femur and tibia could explain the different response of bones to diabetes.

The present study provides new histochemical evidence for the effect of diabetes on alveolar bone and suggests that alterations in diabetic bone GAG occurs in bone matrix and is variable during the early course of the disease.

Acknowledgements: The author thanks Mrs. Rodica Schurath for technical assistance, Mr. Tom McEwen and Dr. A.A.F. Sima for use of the microdensitometer, Mr. Roy Simpson for preparing the photographs, and Mrs. Carlyle Andryo for typing the manuscript. This study was supported by the University of Manitoba Research Board, the Dean's MRC Fund and the Medical Research Council of Canada, Grant MA-8035.

References


Effects of diabetes on bone GAG


Accepted March 4, 1988