Histol Histopath (1989) 4: 7-15

Histology and Histopathology

Alveolar bone of BB/W rats: A morphometric and histochemical study

R.B. Johnson[1] and E.C. Carlson[2]

[1]Department of Anatomy, University of Manitoba, Faculty of Dentistry, Winnipeg, Manitoba R3E OW3, Canada and [2]Department of Anatomy, University of North Dakota, School of Medicine, Grand Forks, North Dakota, 58202, USA

Summary. The present study reported histochemical changes in alveolar bone glycosaminoglycans (GAG) (using Safranin O) and in interdental bone height in three groups of BB/W rats: diabetic, diabetes prone, and diabetes resistant. Safranin O staining intensity suggested that total GAG levels were highest in diabetic bone ($p<0.05$ compared to diabetes resistant, $p<0.005$ compared to diabetes prone) but not significantly different between diabetes prone and resistant groups. Following chondroitinase AC and ABC digestion, staining reactions suggested that the highest levels of dermatan sulfate were in the diabetes resistant group ($p<0.001$ compared to diabetic, $p<0.001$ compared to diabetes prone) and the highest levels of chondroitin sulfates were in the diabetes prone group ($p<0.001$). Coincidently the mean height of diabetes prone interdental septum was significantly less than that of diabetes resistant or diabetic groups ($p<0.05$). The study suggested that 1) diabetes and «prediabetes» produce significant changes in levels of chondroitin 4, 6, and dermatan sulfates within alveolar bone, 2) in «prediabetic» animals, interdental bone loss occurs prior to the onset of clinical symptoms and in the absence of local irritating factors, the bone height appears to return to normal levels, and 3) there may be a correlation between alveolar bone height and relative levels of dermatan sulfate.

Key words: Alveolar bone, Glycosaminoglycans, BB/W rat, Histochemistry, Periodontium

Introduction

There is general agreement that diabetes mellitus can result in 1) a generalized osteopenia (Levin et al., 1976; DeLeeuw and Abs, 1977; Rosenbloom et al., 1977; McNair et al., 1978), 2) osteoporosis of bone forming the tooth socket (alveolar bone) (Glickman, 1946; Ramamurthy et al., 1973), 3) increased severity of the inflammatory reaction in periodontal disease (Murrah, 1985) and 4) enhancement of alveolar bone resorption resulting in decreased tooth support (Glickman, 1946; Shklar et al., 1962; Cohen et al., 1961, 1963; Bissada et al., 1966; Borghelli et al., 1967; Murrah, 1985). The effect of this disease is probably indirect, as spontaneous alveolar bone loss is not accelerated by diabetes (Johnson, 1985).

There is no conclusive evidence concerning the etiology of bone alterations coincident to diabetes mellitus. Complications of diabetes are attributed to 1) a decreased rate of bone formation (Shires et al., 1981; Goodman and Hori, 1984), 2) a decreased rate of bone mineralization (Weiss and Reddi, 1980), 3) changes in the concentration and distribution of structural components of bone (Dixit and Stern, 1979; Aufdermaur et al., 1980; Rosholt and Hegarty, 1981; Weiss and Reddi, 1980; Johnson, 1985), and 4) a decreased rate of bone turnover (Shires et al., 1981; Hough et al., 1983; Goodman and Hori, 1984). There is evidence that bone GAG levels decrease coincident to drug-induced diabetes mellitus (Aufdermaur et al., 1980; Weiss et al., 1981; Johnson, 1985), but it is not known whether this can be attributed directly to the disease or to the diabetogenic drug.

There is disagreement concerning a clinical correlation between diabetes mellitus and increased severity of periodontal inflammation and alveolar bone loss. This debate has continued for many years and has been reviewed most recently by Murrah (1985). There is general agreement that local irritating factors are essential to the development of periodontal disease in diabetic animals (Bissada et al., 1966), however, there remains debate concerning whether the periodontal disease and alveolar bone loss often coincident to diabetes is established during a «prediabetic» period

Offprint requests to: Dr. Roger B. Johnson, Department of Anatomy, University of Manitoba, 730 William Avenue, Winnipeg, Manitoba, R3E OW3, Canada

(Borghelli et al., 1967; Johnson, 1985). In a study of pancreatectomized rats, Borghelli et al. (1967) report a thinning and reduction in height of the alveolar cortical plate prior to the onset of clinical symptoms of diabetes. However, they did not quantify the interdental bone loss.

There are relatively few studies of the periodontium of animals genetically predisposed to diabetes mellitus, possibly because of the convenience of its induction by alloxan or streptozotocin. Shklar et al. (1962) and Cohen et al. (1961, 1963) report the development of periodontitis and alveolar bone loss in the Chinese hamster, however, they do not study the «prediabetic» animals or quantify their results. Stahl (1968) and El Geneidy et al. (1974) report conflicting results of the effects of spontaneous diabetes on the periodontium in a strain of mice. Stahl (1968) reports no correlation between diabetes and periodontitis; El Geneidy et al. (1974) report a severe periodontitis, possibly a result of interdental impaction of materials.

The BB Wistar rat exhibits spontaneous diabetes mellitus that has many similarities to human, Type I (insulin-dependent) diabetes (Nakhooda et al., 1977; Seemayer et al., 1980; Marliss et al., 1982, 1983; Like et al., 1982a,b). The onset of clinical symptoms is sudden and is characterized by weight loss, hyperglycemia, glycosuria, polyuria, and insulinopenia (Nakhooda et al., 1977; Seemayer et al., 1980; Like et al., 1982a,b). Both sexes are affected with equal frequency. Genetic and immune factors are involved in the etiology (Like et al., 1982b, 1983, 1984; Butler et al., 1983). Some rats are genetically resistant (diabetes resistant) to the development of diabetes, others are susceptible (diabetes prone) (Marliss et al., 1982, 1983). There have been many studies concerning pathological lesions in the BB/W rat (reviewed by Wright et al., 1983), however, little information is available concerning their periodontium. Such information could be of particular interest because 1) periodontal diseases are thought to be more severe in diabetics and 2) the BB/W syndrome is a) not produced by invasive techniques or by diabetogenic drugs which might be cytotoxic, and b) similar to human, Type I diabetes. Thus, the effects of diabetes on the periodontium can be studied without consideration of drug effects or surgical complications.

The present study seeks to compare interdental bone height and bone GAG in three groups of BB/W rats: 1) diabetic, 2)diabetes resistant, and 3) diabetes prone. Analysis of the data should provide new information concerning changes in alveolar bone GAG and interdental bone height prior to and coincident to the onset of the clinical symptoms of diabetes mellitus.

Materials and methods

Twelve male BB/W rats, 15 weeks of age, were obtained from the NIH contract colony of BB-Wor. Of this group, 6 animals were diabetic, 3 were diabetes resistant, and 3 were diabetes prone, as determined by tests administered by the NIH colony prior to shipping. Diabetic animals had demonstrated clinical symptoms of diabetes for one month prior to shipping. Animals were weighed and blood and urine glucose tests made upon arrival. Mean weight and mean blood glucose data were compared by Duncan's New Multiple Range Test. Animals were then killed, mandibles removed by blunt dissection and fixed in 10% neutral buffered formalin-acetate containing 0.5% cetylpyridinium chloride to preserve GAG (Engfeldt and Hjertquist, 1968). Tissues were demineralized in 0.2M ethanolic trimethylammonium EDTA (Scott and Kyffin, 1978) to minimize extraction of GAG, dehydrated in ethanols, embedded in paraffin wax, and sectioned (6 μm) in a sagittal plane. Alternate sections were mounted on slides and stained with Safranin O (0.5% w/v), prepared in 0.1M sodium acetate buffer (pH 4.6) for 10 minutes to demonstrate GAG (Kiviranta et al., 1985). Quantitative measurements were made of 1) Safranin O staining reactions and 2) the height of the interdental bone.

Alveolar bone height

Sections demonstrating complete first molar distal and second molar mesial roots and continuous coronal and radicular pulp chambers were chosen for analysis, since these best demonstrated the height of the interdental septum. From these sections, drawings were made of the interdental septum and adjacent teeth using a camera lucida apparatus, the cemento-enamel junction (CEJ) and the crest of the interdental septum were marked and the distance from the CEJ to the alveolar crest was measured using a digitizing tablet and microcomputer. Two measurements were made in each field: 1) from the CEJ of the first molar to the alveolar crest and 2) from the CEJ of the second molar to the alveolar crest (Fig. 1). Measurements were averaged to obtain a mean distance from CEJ to alveolar crest for each field. Mean distances were calculated for each section, animal, and group and compared by analysis of variance and Duncan's New Multiple Range Test.

Measurement of stain intensity

Sections were photographed at 1,000× in an Olympus photoscope for analysis of stain intensity. Three photographs were made of each section: in the crestal, middle and apical thirds. All photographs were made on Panatomic X Film (ASA 32) under identical conditions (i.e., exposure time and light intensity) using a digital photometer attached to the microscope. All three groups of rats were represented on each roll of film. All film was developed for 6 minutes at 22°C in fresh Microdol X developer, washed, and fixed in fresh Kodak Rapid Fixer for 5 minutes. Density of negatives was measured in 12 locations on each negative using a MacBeth TD 502 densitometer with a 0.5 mm aperature. Mean density was calculated for each negative, for each septum, animal and group and were compared by analysis of variance and by Duncan's New Multiple Range Test. Because densitometer readings do not represent a normal distribution, mean readings were transformed to a

percentage of the diabetic group (diabetic = 100% staining reaction), transformed to radians and compared by analysis of variance and Duncan's New Multiple Range Test.

Enzyme digestion

One slide from each animal was chosen for enzyme digestion. Alternate sections were treated with 5µl drops of enzyme solution or with buffer alone. Chondroitinase AC (from *Arthrobacter aurescens)* was prepared at a concentration of 2.0 µg/ml in 0.1 M tris-HCl buffer at pH 7.3 (Yamagata et al., 1968). Chondroitinase ABC (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). Chondroitinase AC removes predominately chondroitin 4 and 6 sulfates; chondroitinase ABC additionally removes dermatan sulfate (Yamagata et al., 1968; Yamada, 1974). Sections were incubated 4 hours at 37°C in 100% relative humidity. Following digestion, sections were rinsed with distilled water and stained with 0.05% Safranin O for 10 minutes as previously described. Stain intensities were calculated using the aforementioned techniques.

Results

Rats were separated into three groups upon arrival: diabetic, diabetes resistant, and diabetes prone. Mean weight of diabetic animals (252.7 ± 3.4g) was significantly less than either diabetes resistant (309.0 ± 1.0g) or diabetes prone (319.7 ± 8.4g) groups ($p<0.001$) (Table 1). Diabetic animals demonstrated glycosuria (+4) as measured with Clinistix®, the other group exhibited no glycosuria. Mean blood glucose of diabetic animals was 353.0 ± 33.9 mg/dl; other groups had blood glucose levels within normal limits.

Alveolar bone height

In all animals the epithelial attachment was at or superior to the CEJ (Fig. 1). There was no evidence of bedding impaction or periodontal pocket formation. There appeared to be a normal number of neutrophils within the gingiva and the transseptal ligament also appeared to be of normal morphology (Fig. 1). Mean distances from the CEJ to the alveolar crest of diabetic (421.1 ± 8.7 µm) and nondiabetic (diabetes prone + diabetes resistant) (457.0 ± 16.8 µm) were not significantly different (Table 2). Within each group, there were no significant differences in this distance between sections or between animals. The mean distance from the CEJ to alveolar crest of diabetes prone animals was 11% greater than diabetic or diabetes resistant animals ($p<0.05$) (Table 3). There were no significant differences in this distance between diabetic and diabetes resistant groups.

Alveolar bone GAG

Safranin O staining reaction of alveolar bone was most intense in the crestal third of the interdental septum of the diabetic group (Figs. 2-4). Staining intensity of

Table 1. Mean weight of BB/W rats at death. Significance levels determined by Duncan's New Multiple Range Test.

GROUP	N (ANIMALS)	MEAN WEIGHT (G+SE)
Diabetic	6	264.67 ±7.48*,**
Diabetes resistant	3	309.0 ±1.0
Diabetes prone	3	319.7 ±8.4

* significantly less than diabetes prone, $p < 0.001$.
** significantly less than diabetes resistant, $p < 0.005$.

Table 2. Comparison of the mean distance from the cemento-enamel junction of the first and second molar teeth to the alveolar crest of diabetic (diabetes prone + diabetes resistant) and non-diabetic BB/W rats. Significance was determined by Duncan's New Multiple Range Test. Numbers in parentheses indicate number of measurements made per group.

GROUP	N (ANIMALS)	MEAN DISTANCE SE (µm)
Nondiabetic (182)	6	457.0 ± 16.8
Diabetic (169)	6	421.1 ± 18.7*

* not significantly different from non-diabetic

Table 3. Comparison of the mean distance from the cemento-enamel junction of the first and second molar teeth to the alveolar crest of diabetes resistant, diabetes prone and diabetic BB/W rats. Significance was determined by Duncan's New Multiple Range Test.

GROUP	N (ANIMALS)	MEAN DISTANCE ± SE (µm)
Diabetes resistant	3	434.8 ± 15.7*
Diabetes prone	3	479.3 ± 20.3
Diabetic	6	421.1 ± 18.7*

* significantly less than diabetes prone, $p < 0.05$

Table 4. Relative differences in Safranin 0 staining intensity between diabetic, diabetes prone, and diabetes resistant BB/W rats following digestion with chondroitinase AC (Diabetic = 100% staining reaction). Significance was determined by Duncan's New Multiple Range Test. Numbers in parentheses indicate percent staining intensity compared to that of "No Enzyme".

GROUP	NO ENZYME	% DIABETIC CHONDROITINASE AC
Diabetes Resistant	98*(100)	103*(64)
Diabetes Prone	88***(100)	80**, ****(10)
Diabetic	100(100)	100(33)

* Significantly different from diabetic, $p < 0.05$.
** Significantly different from diabetic, $p < 0.001$.
*** Significantly different from diabetic, $p < 0.005$.
**** Significantly different from diabetes resistant, $p < 0.001$.

Table 5. Significance levels of comparative Safranin 0 staining reactions in diabetic (D), diabetes prone (P) and diabetes resistant (R) tissues following digestion with chondroitinase AC. Staining intensities after digestion with chondroitinase ABC were not significantly greater than unstained. Significance is determined by Duncan's New Multiple Range Test.

TREATMENT	GROUP COMPARISON	POWER (p)
No enzyme (C)	RC > RU	< 0.001
	DC > DU	< 0.001
	PC > PU	< 0.001
	RC > RAC	< 0.001
	RC > RABC	< 0.001
	PC > PAC	< 0.001
	PC > PABC	< 0.001
	DC > RC	< 0.05
	DC > PC	< 0.005
	DC > DAC	< 0.001
	DC > DABC	< 0.001
	RA > RU	< 0.001
AC	RAC > RABC	< 0.001
	RAC > PAC	< 0.001
	RAC > DAC	< 0.05
	PAC > PU	< 0.001
	PAC > PABC	< 0.001
	DAC > PAC	< 0.001
	DAC > DABC	< 0.001
	DAC > DU	< 0.001

U = unstained
C = Control, no enzyme treatment
AC = chondroitinase AC
ABC = chondroitinase ABC

bone was much less intense than of dentin. Within each group, there were no significant differences in bone staining intensity between sections or between animals, suggesting that sections were of nearly equal thickness. Compared to diabetic animals, staining intensity was significantly less in both the diabetes prone (12% less than diabetic, $p<0.005$) and the diabetes resistant groups (2% less than diabetic, $p<0.05$) (Tables 4,5).

Chondroitinase AC digestion significantly reduced staining intensity 36% in diabetes resistant, 90% in diabetes prone and 67% in diabetic animals ($p<0.001$ as compared to non-enzymatic treated controls). The least intense staining reaction was in the diabetes prone tissues [significantly less (20%) than diabetic, $p<0.001$; significantly less (23%) than diabetes resistant, $p<0.001$] (Tables 4,5; Figs. 5,7). Diabetes resistant tissues demonstrated a significantly greater staining reaction than diabetic tissues (3% greater than diabetic, $p<0.05$) (Figs. 6,7; Tables 4,5). Following chondroitinase ABC digestion, staining intensity was not significantly greater than unstained tissues in any group.

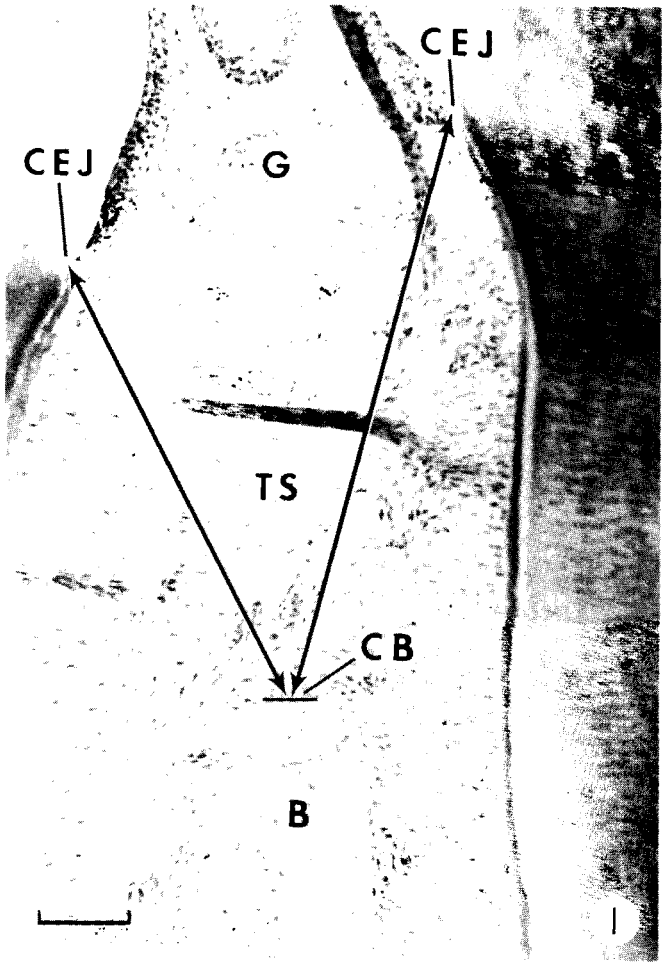
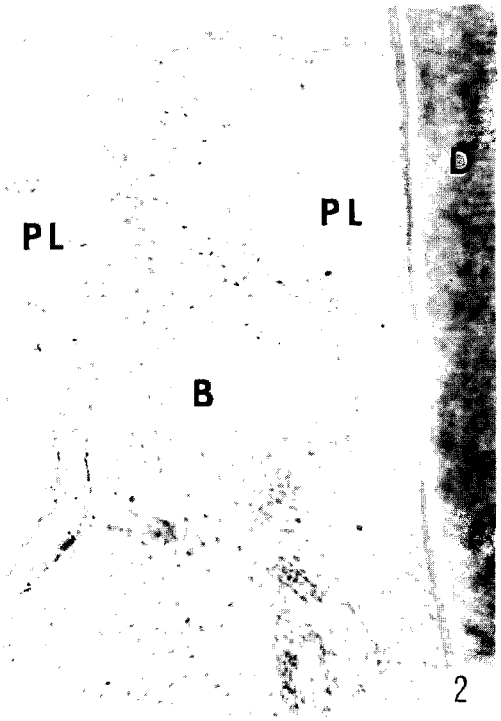
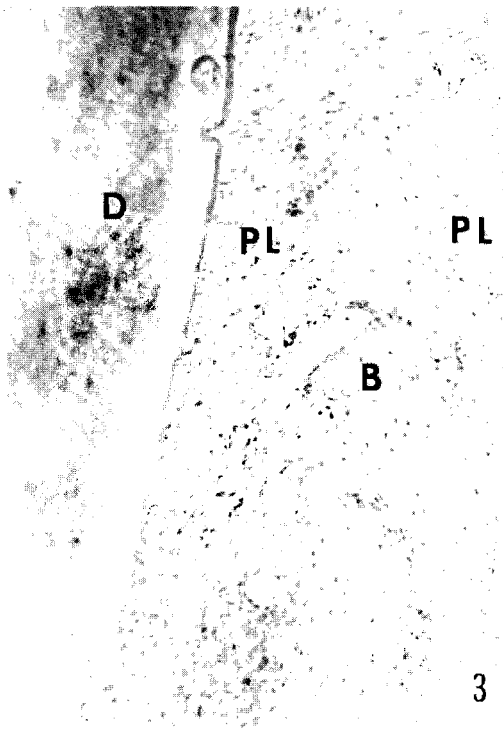
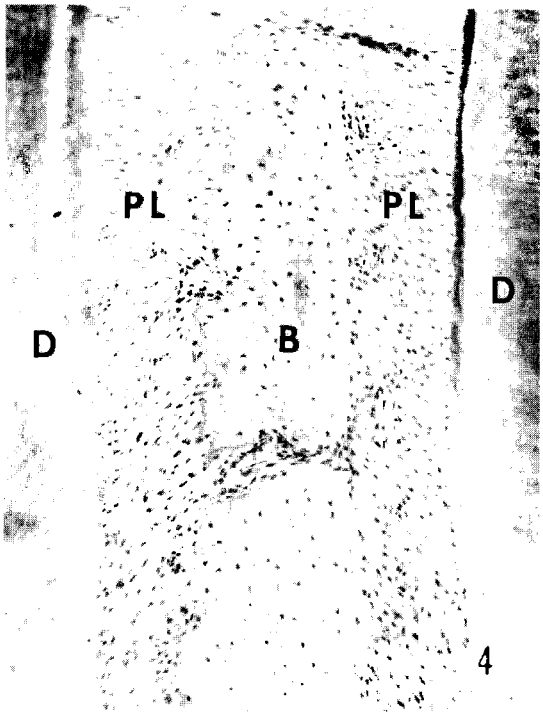


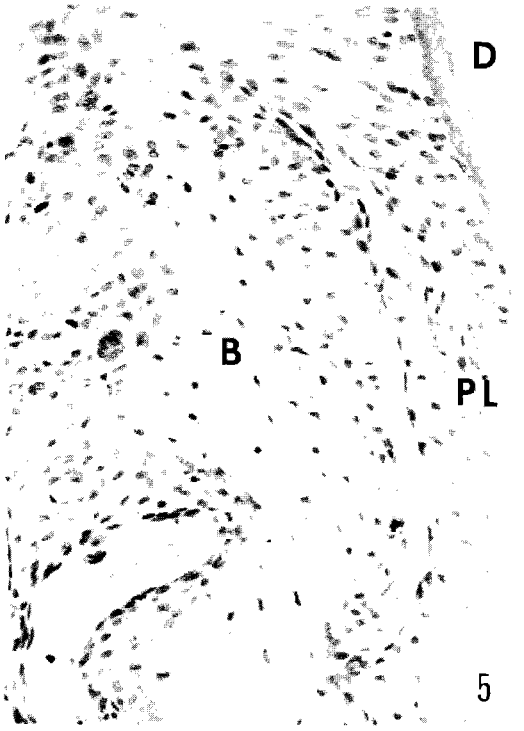
Fig. 1. BB/W rat, diabetes resistant. The distance from the cemento-enamel junction (CEJ) to the crest of the interdental septum (CB) was measured as illustrated (arrows). The interdental septa (B) and surrounding periodontium is also illustrated. The epithelial attachment is at the CEJ in all groups. Gingiva (G) and transseptal fibers (TS) are of usual morphology. Bar = 100 μm. × 130

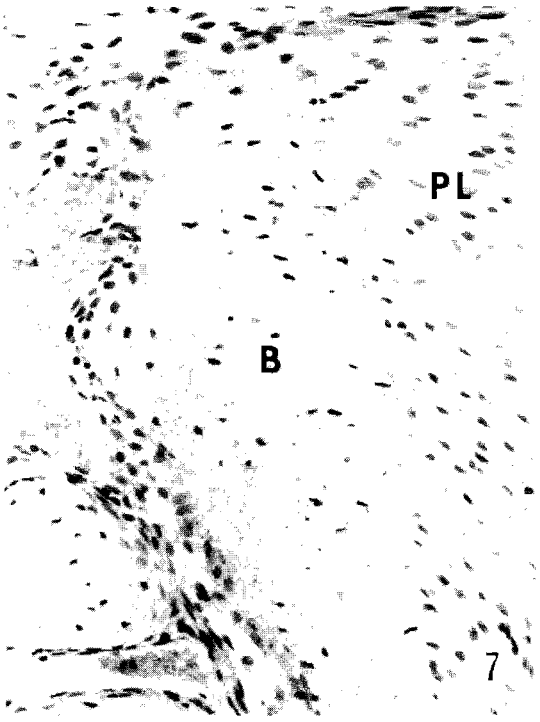


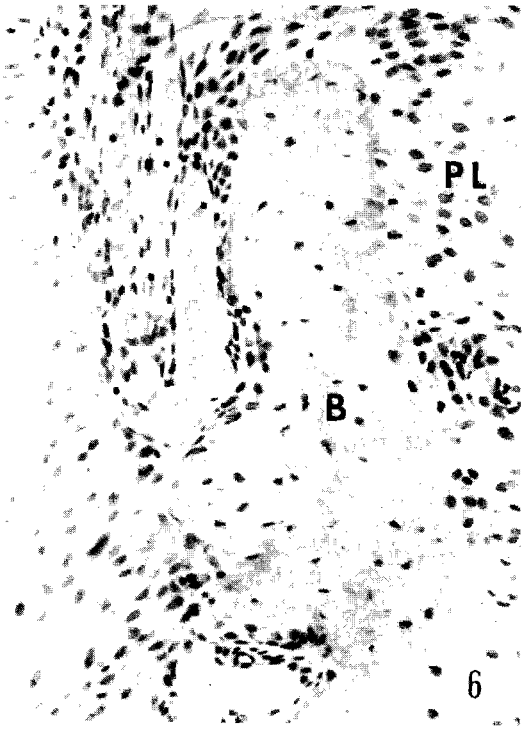




Figs. 2-4. Relative Safranin O staining reactions of alveolar bone of diabetes prone **(Fig. 2)**, diabetes resistant **(Fig. 3)** and diabetic **(Fig. 4)** BB/W rats. Diabetes prone tissues exhibit the least intense staining reaction. **D**, dentin: **B**, alveolar bone; **PL**, periodontal ligament. × 180







Figs. 5-7. Relative Safranin O staining reactions of alveolar bone of BB/W rats following digestion by chondroitinase AC. Diabetes prone tissues **(Fig. 5)** are less intensely stained compared to diabetic **(Fig. 7)**; diabetes resistant tissues **(Fig. 6)** are more intensely stained than diabetic. **D**, dentin; **B**, alveolar bone; **PL**, periodontal ligament. × 180

Discussion

Alveolar bone height

There is little information concerning the periodontium of the BB/W rat, even though this animal has diabetes similar to human, Type I, and, thus could be an excellent model for the study of the effects of diabetes on the periodontium. The BB/W rat demonstrates no significant differences in alveolar bone height between diabetic and non-diabetic animals (diabetes resistant + diabetes prone). Also, because of rigorous criteria for selection of sections for measurement, there were no significant statistical differences between measurements or between animals of a group. Neither group; in the absence of local irritating factors, has periodontal inflammation, pocket formation, or apical migration of the epithelial attachment. These observations confirm some studies utilizing drug-induced and spontaneous models of diabetes mellitus (Bissada et al., 1966, alloxan-rat; Stahl, 1968, mouse mutation) and do not agree with others which report severe periodontal disease and alveolar bone loss in diabetic animals, probably as a result of local irritating factors (calculus and impacted materials) (Glickman, 1946, alloxan-rat; Cohen et al., 1961, 1963, Chinese hamster; Shklar et al., 1962, Chinese hamster; El Geneidy et al., 1974, mutation mouse; Reuterving et al., 1986, alloxan-rat). Thus, the dissimilarity of the periodontium in these studies probably results from local irritating factors to the gingiva. Johnson (1985), in a study of the effects of diabetes mellitus on the STR/N mouse, a model of spontaneous alveolar bone loss, determines that alveolar bone loss is reduced in diabetic STR/N animals suggesting that diabetes may slow the osseous destruction

of a pre-existing periodontal disease. The mechanism of this process is unknown.

The present study reports significantly lower alveolar bone height in diabetes prone rats compared to diabetes resistant or diabetics ($p<0.05$). As diabetes prone animals have no clinical signs of diabetes, but are likely to develop the disease, they may be considered «prediabetic». Borghelli et al., (1967) report that cortical plate bone loss occurs during the «prediabetic», and continues into the diabetic state, however, they do not describe interdental bone, which, because of its location in the dental arch, functions differently from the cortical plate. Our study extends that of Borghelli et al., (1967) because we measured the height of the interdental septum and determined that bone loss also occurred there in «prediabetes». Bone height of diabetic animals was not statistically different from that of diabetes resistant, suggesting restoration of bone height following the «prediabetic» period, as these animals must have passed through this stage prior to overt symptoms of diabetes, a situation opposite to that suggested by Borghelli et al., (1967) and possibly reflects regional differences in bone morphology and function. The present study also reports histochemical evidence suggesting lower levels of dermatan sulfate in alveolar bone of diabetes prone compared to diabetic or diabetes resistant animals ($p<0.001$), suggesting biochemical alterations in alveolar bone coincident to bone loss during the «prediabetic» state. Further biochemical study is indicated to clarify these changes.

Thus, it appears from the present data that the periodontium of the BB/W rat, in the absence of local irritating factors, is morphologically similar to those animals in which diabetes has been experimentally induced. However, it exhibits a pattern of alveolar bone loss during «prediabetes» and diabetes not consistent to that reported in other experimental models. Because of the nature of the diabetic syndrome in BB/W animals and its close similarity to human disease, this animal may be the model of choice for the future study of periodontal changes during insulin dependent diabetes mellitus.

Alveolar bone GAG

The present study reports evidence suggesting differences in both types and levels of alveolar bone GAG in various groups of BB/W rats. These changes could be quantitative or qualitative. It is known that GAG undergo more or less glycosylation in a milieu with a high glucose content which may change their electrochemical properties (Rosenberg et al., 1979). Also, changes in chain-length or degree of sulfation may affect Safranin O binding and subsequent staining reactions. GAG levels can be quantified using Safranin O dye (Kiviranta et al., 1985), a cationic dye composed of a mixture of dimethyl phenosafranin and trimethyl phenosafranin (Lillie, 1977). For quantification of GAG, the dye must bind specifically to the substrate in a stoichiometric fashion; that is, one molecule of the dye is bound to each of the negatively charged groups of the GAG. At pH4.6, the pH of the staining solution used herein, virtually all sulfated and carboxyl groups of GAG are ionized and can bind the dye (Kiviranta et al., 1985). Binding results from electrostatic forces between GAG and dye, which is shown to occur in various media; in particular, within sections of fixed tissues (Kiviranta et al., 1985). Rosenberg (1971) and Kiviranta et al., (1985) report that the interaction of Safranin O with GAG is stoichiometric. Thus, we feel that our staining regimen accurately demonstrates all types of GAG present within the tissues. Also, because we used alternate sections for analysis, thicknesses were more uniform and variations in staining intensities between sections and between animals of a group were not statistically significant.

There is evidence that 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 the mineralizing front. With time, the proteoglycan core proteins are degraded, with the single chain form being degraded more slowly than the two chain form. The chondroitin sulfate then persists within the mineral compartment (Fisher and Termine, 1985).

The function of the mineral compartment proteoglycans is currently unknown, although it is postulated that they bind calcium ions and, thus, regulate mineralization. There is little information concerning the effects of «prediabetes» and diabetes mellitus on bone GAG, although there is some indication that levels of bone GAG decrease during experimental diabetes (Weiss and Reddi, 1980; Silberberg et al., 1981; Johnson, 1985) due to several factors: 1) increased levels of degradative lysosomal enzymes in bone cells (Silberberg et al., 1981), 2) decreased sulfate incorporation into proteoglycans (Weiss and Reddi, 1980), or 3) decreased total numbers of proteoglycans and decreased GAG per proteoglycan chain (Weiss and Reddi, 1980). The present study was not consistent with earlier reports, as it documented significantly higher levels of bone GAG in diabetics compared to diabetes prone ($p<0.005$) or diabetes resistant animals ($p<0.05$). These changes in GAG levels probably result from the disease and cannot be a result of the effects of diabetogenic drugs. It is also possible that GAG metabolism is different in drug-induced and spontaneous diabetes. The present data reported no significant differences between GAG levels in diabetes prone and resistant animals, suggesting that there was not a significant change in bone GAG levels in animals genetically prone to development of this disease.

Little information is available concerning alveolar bone GAG distribution of spontaneously diabetic animals. Studies of the Chinese hamster by Shklar et al., (1962) and by Cohen et al., (1961, 1963) indicate that these animals develop a severe periodontitis with associated alveolar bone loss. In contrast, Stahl (1968) notes no evidence of periodontitis or associated alveolar bone loss in an inbred strain of mice suffering from mutation diabetes. None of

these studies report histochemical changes in the alveolar bone of the animal strains. The present study suggests that bone GAG levels are highest in diabetes animals and lowest in diabetic prone animals ($p<0.005$). These levels likely reflect differences in GAG synthesis, GAG degradation, or bone mineralization. Baylink et al. (1972) report a loss of GAG in mineralizing bone. Osteoid mineralization is reported to occur at a decreased rate in diabetic animals (Goodman and Hori, 1984), which could explain the higher levels of GAG within the alveolar bone of diabetic animals. In the rat, molar teeth undergo continuous, but slow, eruption due to enamel-free areas on the occlusal surfaces, resulting in rapid wearing of the crown (Cohn, 1957). As a result, bone is deposited at the alveolar crest to maintain the periodontal attachment. Thus, it is possible that the higher levels of GAG within the alveolar crest of diabetic animals represent a retarded mineralization of the matrix of this region. Conversely, low levels of GAG, as reported herein in diabetes prone animals, could represent relatively hypermineralized bone.

Chondroitinase AC removes chondroitin-4 and-6 sulfates and chondroitinase ABC additionally removes dermatan sulfate from tissue sections (Yamagata et al., 1968; Yamada, 1974). Dermatan sulfate is not a significant intrinsic component of bone matrix, but is found within Sharpey's fiber bundles of alveolar bone (Johnson, R.B., unpublished observations). From the present data, it is likely that diabetes prone tissues contain proportionally more chondroitin sulfates and less dermatan sulfate than either diabetes resistant or diabetic bone. Diabetes resistant tissues appear to contain proportionally more dermatan sulfate and less chondroitin sulfates than either diabetes prone or diabetic bone. Whether these relative differences in GAG levels affect susceptibility of bone to resorption is unknown. Biochemical analyses of these tissues is necessary to substantiate these preliminary observations.

Acknowledgements. The authors thank Mrs. Rodica Schurath and Dr. J.A. Thliveris for technical assistance, Mr. Roy Simpson for preparing the photographs, and Mrs. Carlyle Andryo for typing the manuscript. Animals were obtained from the contract colony of BB/Wor maintained at the University of Massachusetts under the direction of Dr. Arthur Like. This study was supported in part by PHS Grant EY05106-02 to Dr. E.C. Carlson and in part by Grant MA-8035 from the Medical Research Council of Canada to Dr. R.B. Johnson.

References

Aufdermaur M., Fehr K., Lesker P. and Silberberg R. (1980). Quantitative histochemical changes in intervertebral discs in diabetes. Exp. Cell Biol. 48, 89-94.

Baylink D., Wergedal J. and Thompson E. (1972). Loss of protein polysaccharides at sites where bone mineralization is initiated. J. Histochem. Cytochem. 20, 279-292.

Bissada N.F., Schaffer E.M. and Lazaro A. (1966). Effect of alloxan diabetes and local irritating factors on the periodontal structures of the rat. Periodontics. 4, 233-240.

Borghelli R.F., Devoto F.C.H., Foglia V.G. and Erausquin J. (1967). Periodontal changes and dental caries in experimental prediabetes. Diabetes 16, 804-809.

Butler L., Guberski D.L. and Like A.A. (1983). Genetic analysis of the BB/W diabetic rat. Can. J. Genet. Cytol. 25, 7-15.

Cohen M.M., Shklar G. and Yerganian G. (1961). Periodontal pathology in a strain of Chinese hamster, *Critetulus griseus*, with hereditary diabetes mellitus. Am. J. Med. 31, 864-867.

Cohen M.M., Shklar G. and Yerganian G. (1963). Pulpal and periodontal disease in a strain of Chinese hamsters with hereditary diabetes mellitus. Oral Surg. 16, 104-112.

Cohn S.A. (1957). Development of the molar teeth in the albino mouse. Am. J. Anat. 101, 295-319.

DeLeeuw I. and Abs R. (1977). Bone mass and bone density in maturity-type diabetics measured by the ^{125}I photon-absorption technique. Diabetes 26, 1130-1135.

Dixit P.K. and Stern A.M.K. (1979). Effect of insulin on the incorporation of citrate and calcium into the bones of alloxan-diabetic rats. Calcif. Tissue Int. 27, 227-232.

El Geneidy A.K., Stallard R.E., Fillios L.C. and Goldman H.M. (1974). Periodontal and vascular alterations: their relationship to the changes in tissue glucose and glycogen in diabetic mice. J. Periodontol. 45, 394-401.

Engfeldt B. and Hjertquist S.O. (1968). Studies on the epiphysial growth zone. I. The preservation of acid glycosaminoglycans in tissues in some histochemical procedures for electron microscopy. Virchows Arch. Abt. B Zellpath. 1, 222-229.

Fisher L.W. and Termine J.D. (1985). Noncollagenous proteins influencing the local mechanisms of calcification. Clin. Orthop. Rel. Res. 200, 362-384.

Glickman I. (1946). The periodontal structures in experimental diabetes. N.Y.J. Dent. 16, 226-251.

Goodman W.G. and Hori M.T. (1984). Diminished bone formation in experimental diabetes: relationship to osteoid maturation and mineralization. Diabetes 33, 825-831.

Hough S., Slatopolsky E. and Avioli L.V. (1983). Hormonal alterations in experimental diabetes: role of a primary disturbance in calcium homeostasis. Calcif. Tissue Int. 35, 615-619.

Johnson R.B. (1985). Effects of experimental diabetes mellitus on alveolar bone loss in periodontal disease-susceptible mice. J. Periodont. Res. 20, 307-316.

Kiviranta I., Jurvelin J., Tammi M., Saamamen A.M. and Helminen H.J. (1985). Microspectrophotometric quantitation of glycosaminoglycans in articular cartilage sections stained with Safranin O. Histochemistry 82, 249-255.

Like A.A., Anthony M., Guberski D.L. and Rossini A.A. (1983). Spontaneous diabetes mellitus in the BB/W rat: effects of glucocorticoids, Cyclosporin-A, and antiserum to rat lymphocytes. Diabetes 32, 326-330.

Like A.A., Appel M.C. and Rossini A.A. (1982a). Autoantibodies in the BB/W rat. Diabetes 31, 816-820.

Like A.A., Butler L., Williams R.M., Appel M.C., Weringer E.J. and Rossini A.A. (1982b). Spontaneous autoimmune diabetes mellitus in the BB rat. Diabetes 31, 7-13.

Like A.A., Dirodi V., Thomas S., Guberski D. and Rossini A. (1984). Prevention of diabetes mellitus in the BB/W rat with Cyclosporin-A. Am. J. Path. 117, 92-97.

Lillie R.D. (ed) (1977). H.J. Conn's Biological Stains. 9th Ed. Williams and Wilkins. Baltimore. p. 385-387.

Marliss E.B. (1983). Recommended nomenclature for the spontaneously diabetic syndrome of the BB rat. Metabolism 32 (Suppl.1): 6-7.

Marliss E.B., Nakhooda A.F., Poussier P. and Sima A.A.F. (1982). The diabetic syndrome of the BB Wistar rat: possible relevance to Type I (insulin-dependent) diabetes in man. Diabetologia 22, 225-232.

Marliss E.B., Nakhooda A.F. and Poussier P. (1983). Clinical forms and natural history of the diabetic syndrome and insulin and glucagon secretion in the BB rat. Metabolism 32 (Suppl.1), 11-17.

McNair P., Madsbad S., Christiansen C., Faber O.K., Transbol I. and Binder C. (1978). Osteopenia in insulin treated diabetes mellitus: its relation to age of onset, sex and duration of disease. Diabetologia 15, 87-90.

Murrah V.A. (1985). Diabetes mellitus and associated manifestations: a review. J. Oral Path. 14, 271-281.

Nakhooda A.F., Like A.A., Chappel C.I., Murray F.T. and Marliss E.B. (1977). The spontaneously diabetic Wistar rat. Metabolic and morphologic studies. Diabetes 26, 100-112.

Prince C.W., Rahemtulla F. and Butler W.T. (1983). Metabolism of rat bone proteoglycans in vivo. Biochem. J. 216, 589-596.

Ramamurthy N.S., Zebrowski E.J., Baker C. and Golub L.M. (1973). Alloxan diabetes and reduced bone density in rat mandible. Res. Comm. Chem. Path. Pharm. 5, 614-620.

Reuterving C.O., Hagg E. and Gustafson G.T. (1986). Root surface caries and periodontal disease in long-term alloxan diabetic rats. J. Dent. Res. 65, 689-694.

Rosenberg H., Modrak J.B., Hassing J.M., Walid A.A. and Stohs S.J. (1979). Glycosylated collagen. Biochem. Biophys. Res. Comm. 91, 498-501.

Rosenberg L. (1971). Chemical basis for the histological use of Safranin O in the study of articular cartilage. J. Bone Joint Surg. 53A, 69-82.

Rosenbloom A.L., Lezotte D.C., Weber F.T., Gudat J., Heller D.R., Weber M.L., Klein S. and Kennedy B.B. (1977). Diminution of bone mass in childhood diabetes. Diabetes 26, 1052-1055.

Rosholt M.N. and Hegarty P.V.J. (1981). Mineralization of different bones in streptozotocin-diabetic rats: study on the concentration of eight minerals. Am. J. Clin. Nutr. 34, 1680-1685.

Scott J.E. and Kyffin T.W. (1978). Demineralization in organic solvents by alkylammonium salts of ethylene-diamine tetra-acetic acid. Biochem. J. 169, 697-701.

Seemayer T.A., Oligny L.L., Tannenbaum G.S., Goldman H. and Colle E. (1980). Spontaneous diabetes mellitus in the BB Wistar rat. Am. J. Path. 101, 485-488.

Shires R., Teitelbaum S.L., Bergfeld M.A., Fallon M.D., Slatopolsky E. and Avioli L.V. (1981). The effect of streptozotocin-induced chronic diabetes mellitus on bone and mineral homeostasis in the rat. J. Lab. Clin. Med. 97, 231-240.

Shklar G., Cohen M.M. and Yerganian G. (1962). A histopathologic study of periodontal disease in the Chinese hamster with hereditary diabetes. J. Periodontol. 33, 14-21.

Silberberg R., Scharp D.W. and Lesker P.A. (1981). Biochemical studies of bone from diabetic rats and rats bearing transplanted pancreatic islets. Expl. Cell Biol. 49, 61-67.

Stahl S.S. (1968). The periodontal structures in diabetic mice. J. Oral. Med. 23, 15-17.

Weiss R.E., Gorn A.H. and Nimni M.E. (1981). Abnormalities in the biosynthesis of cartilage and bone proteoglycans in experimental diabetes. Diabetes 30, 670-677.

Weiss R.E. and Reddi A.H. (1980). Influence of experimental diabetes and insulin on matrix-induced cartilage and bone differentiation. Am. J. Physiol. 238, E200-E207.

Wright J.R., Yates A.J., Sharma H.M. and Thibert P. (1983). Pathological lesions in the spontaneously diabetic BB Wistar rat: a comprehensive autopsy study. Metabolism 32 (Suppl. 1), 101-105.

Yamagata T., Saito H., Habuchi O. and Suzuki S. (1968). Purification and properties of bacterial chondroitinases and chondrosulfatases. J. Biol. Chem. 243, 1523-1535.

Yamada K. (1974). The effect of digestion with chondroitinases upon certain histochemical reactions of mucosaccharide-containing tissues. J. Histochem. Cytochem. 22, 266-275.

Accepted June 14, 1988