Physical, chemical, and histologic changes in dentin caries lesions of primary teeth induced by regular use of polyol chewing gums

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Mäkinen KK, Chiego DJ Jr, Allen P, Bennett C, Isotupa KP, Tiekso J, Mäkinen P-L. Physical, chemical, and histologic changes in dentin caries lesions of primary teeth induced by regular use of polyol chewing gums. Acta Odontol Scand 1998;56:148–156. Oslo. ISSN 0001-6357.

A previous clinical trial showed that long-term use of saliva-stimulating polyol (xylitol and sorbitol) chewing gums was associated with arrest of dental caries in young subjects. After a 20–22-month intervention (when the subjects were 8 years old), a total of 23 primary teeth with extensive dentin caries lesions whose surface in clinical examination was found to be totally rehardened (remineralized) could be removed because the teeth were near their physiologic exfoliation time. These teeth were subjected to histologic, microhardness, and electron microscopic tests. The majority of the specimens had been remineralized from the surface by a non-cellular-mediated process within the remaining collapsed, organic extracellular matrix associated with the remaining dentinal surface. Many of the underlying dentinal tubules were filled with a matrix that had been subsequently mineralized. Dental microanalyses showed that the topmost (outer) 20-μm-thick rehardened layer of the lesions exhibited the highest Ca:P ratio, which leveled off at a depth of approximately 150 μm. The rehardened surface layer (normally <0.1 mm in thickness) was significantly ($P < 0.001$) harder than sound dentin and nearly as hard as sound enamel. Although the main source of the mineral present in the rehardened layer was most likely of salivary origin, some extracellular remineralization was probably mediated by odontoblasts. The results complete the clinical diagnoses of the original trial and suggest that regular use of polyol chewing gums may induce changes in dentin caries lesions, which in histologic and physiochemical studies show typical characteristics of rehardening and mineralization. □ *Dental caries; remineralization; sugar alcohols*

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It is well known that, if left untreated, the dental caries process may spread in the dentin and may eventually reach the tooth pulp. It is also known that if the cariogenicity of the tooth's environment can be completely controlled, even large and deep caries lesions may undergo a rehardening process (1–5). A rehardened (arrested, remineralized) caries lesion is normally microbiologically inactive, and its hardness normally approaches that of sound dentin or sound enamel. Recent clinical studies carried out in Belize showed that young Belizean subjects (initially 6 or 10 years of age) have, as a group, a high caries rate. Systematic, long-term use of polyol-containing chewing gums by these subjects was associated with a reduction in caries rate and with rehardening of some dentin caries lesions (6–8). These clinical observations were made possible by the limited restorative care available in Belize at the time of the performance of the clinical trials; most of even the deep dentin caries lesions customarily went unfilled in these subjects. Toward the termination of the trial dealing with preschool-age subjects (8), a total of 23 primary teeth with arrested dentin caries lesions could be retrieved from subjects who used polyol chewing gums, because these teeth were near their physiologic exfoliation time (8). The purpose of this study was to investigate certain physical, chemical, and histologic properties of the rehardened dentin caries lesions of those teeth.

The histology and even the physiochemistry of caries stabilization are relatively well researched (1–5). Consequently, the new hypothesis put forward in this article is that implementation of a systematic chewing gum program can induce favorable histologic and physicochemical changes in carious dentin in young subjects in an environment with a high caries rate and limited access to restorative care. Such favorable reactions were in this study accomplished by habitual use of chewing gums that contained the sugar alcohols (polyols) xylitol and sorbitol, or their mixtures, as the only bulk sweeteners.

Materials and methods

Summary of the clinical trial; collection of teeth

The original clinical program was implemented in the Stann Creek District of Belize in initially 6-year-old subjects who went to area public schools and who were recruited to a chewing gum study involving the consumption of polyol-containing gums 5 times a day for 2 years (8). The subjects were divided into 7 groups, 6 of which received different polyol gums, while in 1 group the subjects did not receive gum as part of the program. The clinical study was double-blind. The masking continued

over the present study; the person operating the hardness tester and those involved in other tests were not aware of the origin of the teeth.

There were 6 examinations during the 24-month program, and thus 5 periods for which caries rates could be calculated: period 1 (baseline to 4.5 months), period 2 (4.5 to 9.5 months), period 3 (9.5 to 12 months), period 4 (12 to 18 months), and period 5 (18 to 24 months). Because of the presence of a large number of deep dentin caries lesions in these subjects, and because only a few such lesions were filled, the potential of observing arrested, rehardened lesions with different original severity was considered high. Therefore, the examining team agreed initially that if an enamel caries lesion with a physical loss of surface contour and substance (superficial caries, D_s), a dentin caries lesion (moderate caries, D_m), or a deep cavity with probable pulp involvement (severe caries, D_x) did not show the clinical characteristics typical of each type of caries lesion, but had undergone as a whole a clearly discernible rehardening process, the lesion was diagnosed as an arrested or a non-progressive lesion. Caries arrest was observed by probing the lesions with sharp explorers (Hu-Friedy Duraspond Double End EXD 2 or EXD 6, Hu-Friedy, Chicago, Ill., USA (9)). The teeth examined in this study were diagnosed at baseline to have either active D_m or D_x lesions that were soft and sore upon probing and showed no signs of caries arrest.

After 18 months of gum use, it appeared that several carious primary teeth with rehardened D_m or D_x lesions could be removed because the teeth were near their physiologic exfoliation time. Consequently, a total of 23 such teeth were removed after a 20–22-month intervention based on the consent received from the parents or guardians of the subjects. The removal of these teeth was performed under the supervision of the District Dental Officer and the Senior Dental Surgeon of Belize. The teeth were placed in a freezer (−20°C) (for physical studies) or stored at 4°C in 10% formaldehyde (for histologic studies). A total of 10 primary teeth with arrested D_x lesions obtained from 9 subjects were used in microhardness tests, while 13 additional teeth with arrested D_m or D_x lesions were used in other physical and histologic studies. The specimens (Fig. 1) for the microhardness tests were obtained from subjects who used the following gum formulas: 100% xylitol pellet-shaped gum (6 subjects), 100% xylitol stick-shaped gum (2 subjects), 3:2 xylitol–sorbitol gum (1 subject), and sorbitol gum (1 subject) (8). The 13 additional teeth were obtained from the following number of subjects of the above gum groups: 9, 2, 1, and 1, respectively. The entire sample comprised 18 upper or lower first molars, 3 lower second molars, and 2 upper canines. The teeth were normally so thoroughly destructed by caries that most of them had only little or no enamel crescents left fringing the carious cavity. A relatively evenly worn, shallow remnant of the original crown formed the new 'crown' of these teeth. The teeth with arrested lesions were photographed with an intraoral camera before removal. Color photographs of some of these teeth have been published (7). The occlusal surfaces of most of the exfoliated teeth were photographed. The occlusal surfaces of the teeth that were used in microhardness measurements are shown in Fig. 1.

SEM, EDS, and EMPA studies

The preparation of the sections for scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), and electron microbe analysis (EMPA) was carried out as described earlier (10–12). A second identical serial section for each sample was prepared for light microscopy. Photomicrographs (not shown) were taken of the resulting slides and used for general morphology and orientation.

The Ca:P ratios were determined by EDS by means of SEM, taking the spectra from the middle of the caries lesion, starting from the surface and covering a depth of 20 μm. Each subsequent spectrum was moved 20 μm further away from the lesion surface so that a series of spectra representing 20 μm in depth was taken serially up to a final depth of 200 μm from the surface (after this, the spectrum was moved to 250 μm and 300 μm from the surface). Five series were performed on each sample. In these analyses the SEM beam current was adjusted to 0.60 nA by monitoring with a Keithly Model 485 picoammeter. The SEM was set up for 15 kV, and the takeoff angle was determined to be 43° by using the System Four-plus (Princeton Gamma-Tech, Princeton, N.J., USA) EDS takeoff angle program calculation (specimen tilt, 25°; working distance, 25 mm). These parameters were closely monitored and adjusted before the start of each measurement. The spectrum collection time was 120 s. The theoretic $K\alpha_1$ line values for phosphorus (2015 eV) and calcium (3691 eV) were used for the raw counts for each element for every spectrum.

Selected deciduous teeth with deep arrested caries lesions were examined by means of CAMECA electron microprobe analysis (EMPA). These examinations were carried out on the 30-μm sections of teeth prepared as described above. Natural fluoroapatite was used as a standard of comparison, and data were corrected for ZAF effects (those caused by atomic number, absorption, and fluorescence). EMPA data were obtained for the following elements: calcium, phosphorus, iron, magnesium, silicon, molybdenum, and fluorine. These data were also used for the calculation of the molar Ca:P ratios to be compared with those obtained by means of SEM/EDS. The EMPA was carried out on selected sites of the lesions and always in the surrounding sound dentin of the same section.

Histologic procedures

Frozen, intact, remineralized primary teeth (stored in 10% formaldehyde) were fixed in 4% NBF for 24 h at 22°C and demineralized in 0.5 M ethylenediaminetetraacetic acid (EDTA). Tissues were then dehydrated in a series of alcohols and embedded in paraffin. Sections were cut at 5 μm, rehydrated in a graded series of alcohols, and

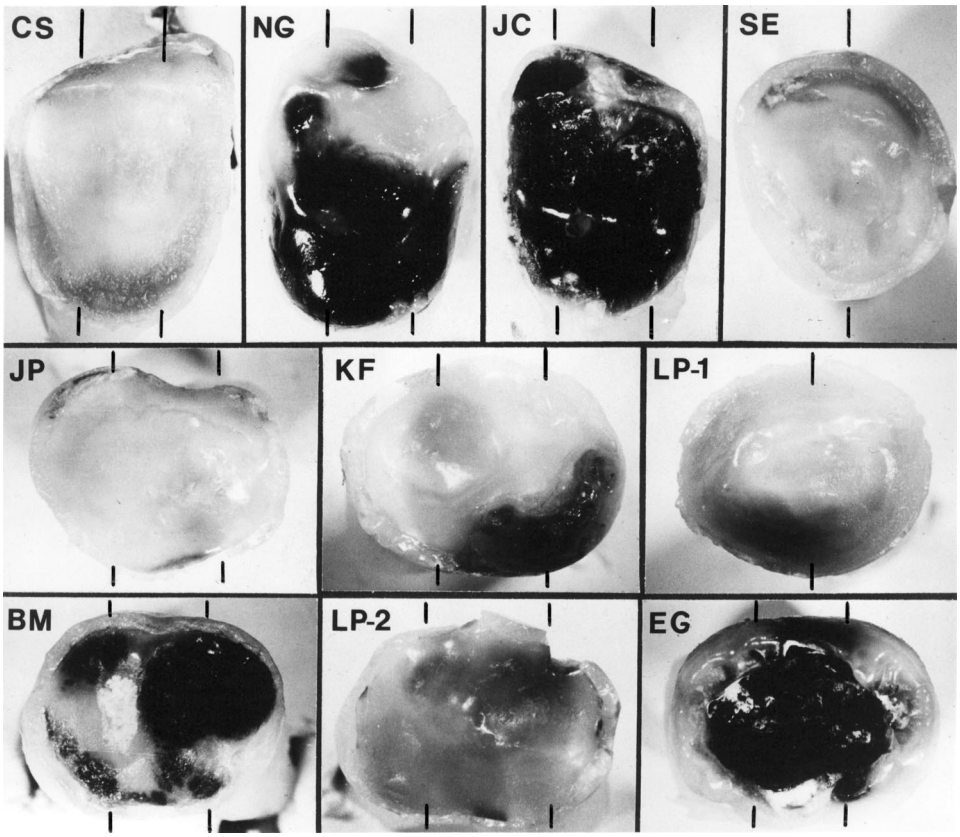

Fig. 1. Top view of the arrested severe caries (D_x) lesions whose microhardness measurements are reported in Fig. 4. The hardness measurements of the flattened occlusal surfaces were carried out from 10 sites that constituted the entire width of the lesions. The concavity of the lesion surface from subject EG prevented its hardness measurement. The thin lines show the segmentation directions.

stained with hematoxylin and eosin or Masson's trichrome for connective tissue. All sections were observed and photographed using a light microscope.

Microhardness measurements

The measurements were performed using a Knoop indenter fitted to a microhardness tester (Duriment, Ernst Leitz GmbH, Wetzlar, Germany) loaded with 200 g for 30 s. The Knoop hardness number (KHN), expressed in kg/mm^2, was determined for each indentation. The aim was to compare the KHN scores of sound dentin with those obtained from the rehardened lesions of the same teeth. The freezing process was not shown to have any measurable effect on the dentin.

The hardness measurements were first carried out at 10 different sites of the carious occlusal surfaces of 9 whole (unsegmented) teeth, which included 2 canines. In 1 tooth the cavity was too concave for surface hardness measurements. The 10 measurement sites were generally slightly elevated areas that rendered a measurement possible, and covered evenly the entire rehardened occlusal lesion. Following the occlusal surface hardness determinations, the teeth were segmented longitudinally in the buccolingual plane (Fig. 1). The canines were cut into 2 and the molars into 3 segments. The cutting was carried out using a cutting–grinding procedure (Exact-Apparatenbau, Hamburg, Germany) (11). The hardness measurements of the lesion bodies were then carried out in the direction of each tooth's longitudinal axis, from the carious occlusal surface toward sound dentin. The 1st measurement was performed at a site 0.1 mm from the rehardened occlusal surface, and the determinations were continued at 0.2-mm intervals until 10 readings had been obtained; the last 2 or 3 readings were always obtained from sound dentin. These measurements of section surfaces were performed at 2 different sites—1 from the buccal and 1 from the lingual side of the pulp—so that from each surface a total of 20 readings were obtained.

Statistical procedures

In the microhardness measurements, repeated measures analysis of variance was used in statistical analysis. A

multivariate approach for repeated measures analysis was applied in the planned comparisons between layers.

Results

As detailed elsewhere (7, 8), arrested D_m and D_x lesions began to occur in polyol-using subjects to a more significant extent after the 3rd and 4th periods. Most arrested D_m and D_x lesions after the 4th period and at the endpoint (24 months) were observed in subjects who had used polyol gums (7, 8). After the scheduled clinical examination at 18 months (the time at which the last caries examination was carried out before the removal of the present primary teeth), a total of 662 caries lesions (D_m and D_x) in 325 subjects were diagnosed to have undergone a stabilization process, being hard on probing with moderate pressure. These 662 arrested lesions were observed in the 7 experimental groups as follows: no gum, 37 lesions; xylitol–sorbitol mixture 3:2 (pellet-shaped gum), 118 lesions; xylitol–sorbitol mixture 4:1 (pellet-shaped gum), 82 lesions; 100% sorbitol stick-shaped gum, 108 lesions; 100% sorbitol pellet-shaped gum, 85 lesions; 100% xylitol stick-shaped gum, 138 lesions; and 100% xylitol pellet-shaped gum, 94 lesions. These figures comprise both the error diagnoses and the true rehardenings (7, 8). Significantly more gum-using subjects than non-users exhibited arrest of dentin caries. Upon reexamination of the D_m and D_x lesions of the no-gum group for the present study, none of them were considered to have undergone a rehardening process. Rehardened D_m and D_x lesions were encountered only in the polyol-gum groups. Consequently, no teeth could be obtained for this study from the no-gum subjects.

Histologic observations

These studies did not show any histologic differences between subjects using different polyol gums. Therefore, the following observations concern dentin caries lesions that were affected by long-term use of gums containing xylitol, sorbitol, or the xylitol–sorbitol mixtures. Fig. 2 illustrates histologic observations of users of the 100% xylitol-pellet or the stick-gum formula.

Low-magnification observations of the demineralized tissue sections revealed a heterogeneous surface remaining on the dentin surface (Fig. 2A). Some areas along the dentin surface were relatively smooth, whereas other areas demonstrated a collapsed, acellular, amorphous matrix. It was not possible in this study to determine the morphology of the highly mineralized matrix since it was removed during the process of demineralization, suggesting there was very little remaining organic (cellular) component. This is consistent with the histologic findings showing intermittent areas of remaining organic extracellular matrix interspersed with areas of seemingly smooth dentinal surface.

Higher magnification of dentinal surface demonstrates that many of the underlying dentinal tubules were filled in with a matrix that had been subsequently mineralized (Figs. 2B, 2C, 2E). The remineralized organic extracellular matrix shows some structure suggestive of demineralized dentinal extracellular matrix with the inclusion of dentinal tubules (Fig. 2F). Some areas of the remaining remineralized extracellular matrix project from the surface to a much higher level than that of the surrounding remaining matrix. These areas did not generally show any specific organization, but appeared to be a collapsed, previously intact, dentinal extracellular matrix. The remaining organic matrix also did not appear to be associated with any remaining or previous structures.

Most of the samples examined did not contain viable pulp tissue, and therefore any remineralization was probably not cell-mediated. One specimen did contain viable pulp (Fig. 2D), and reactionary and reparative dentin could be seen under the area where an active lesion was located, suggesting that at least some of the remineralized extracellular matrix was mediated by odontoblasts. Some of the specimens contained dentin that had mineral within the dentinal tubules close to the surface underlying a previously carious lesion. In these specimens remineralization appears to have progressed throughout the area of the lesion in a well-defined area. In summary, the majority of the teeth appeared to have been remineralized from the surface by a non-cellular-mediated process, either within a remaining collapsed, organic extracellular matrix or associated with the remaining dentinal surface.

Dental microanalysis results

The main purpose of these analyses was to study the Ca:P ratio of the arrested dentin caries lesions as a function of lesion depth. The tooth specimens were obtained from subjects who had used the 100% xylitol-pellet formula. Each specimen subjected to EDS by means of SEM showed a higher Ca:P ratio in the topmost layer (extending to a depth of approximately 20 μm) of the lesion compared with the rest of the lesion (Fig. 3). At a depth of approximately 150 μm the Ca:P ratios leveled off. Studies carried out by means of EMPA supported these results. EMPA was subsequently used to test whether the topmost layer of arrested dentin caries lesions showed similarly increased levels of the elements iron, magnesium, silicon, molybdenum, and fluorine. With the exception of fluorine, none of these elements followed such a distribution pattern; their levels were not appreciably different between different layers (not shown). The levels of fluorine closely followed those of calcium.

Microhardness tests

All measurements were performed twice. Since the two series of tests resulted in virtually identical findings, the results of only the second are presented. The KHN values of rehardened occlusal surfaces were higher than in sound dentin ($P < 0.001$). The highest points of the occlusal

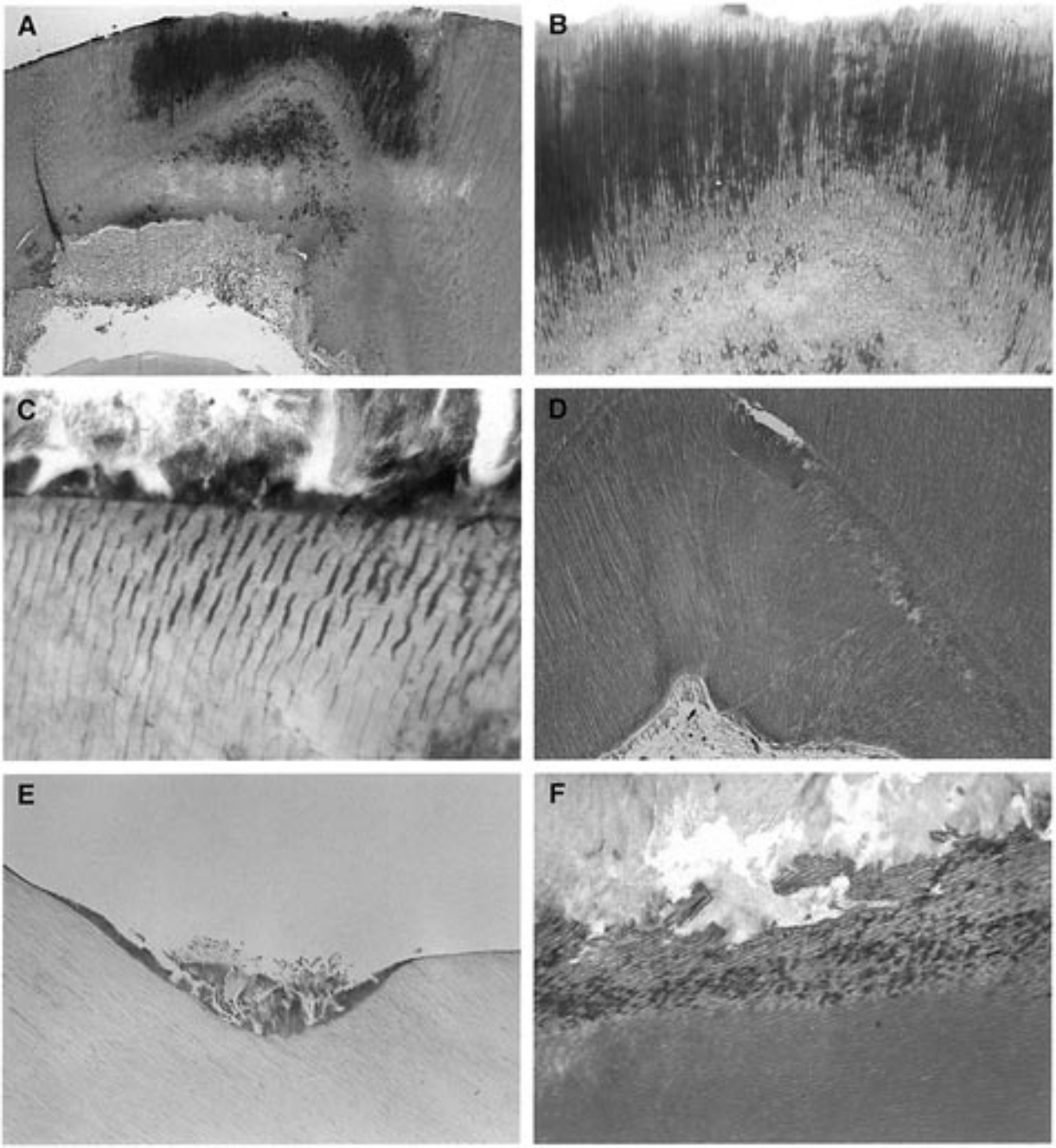

Fig. 2. Histologic observations of primary teeth with deep dentin caries lesions that had undergone a rehardening process during long-term use of polyol chewing gums (either the 100% xylitol-pellet or the stick-gum formula). All specimens in this series of photomicrographs had been completely remineralized, most of them along their entire surface. Fig. 2A. Section from a primary tooth demineralized in 0.5 M ethylenediaminetetraacetic acid. Note the surface staining, indicating the presence of a demineralized organic matrix. The arrested carious lesion is at the top center, and an intense red zone (mineralization) can be seen underlying the lesion. (Original magnification, ×5. Masson's trichrome stain.) Fig. 2B. The pattern of mineralization (red staining) underlying a previously remineralized primary tooth in the dentinal tubules and intertubular dentin. At the surface there is a narrow band of unmineralized matrix, while remineralization (red staining) can be seen in the deeper dentin. (Original magnification, ×40. Masson's trichrome stain.) Fig. 2C. Residual organic matrix at the surface of a previously remineralized tooth. Mineralization extends into the dentinal tubules with some intertubular mineralization seen at the lower right. (Original magnification, ×63. Masson's trichrome stain.) Fig. 2D. Reparative dentin formation in a pulp horn underlying a carious lesion in a remineralized primary tooth. Areas of the reparative dentin contain dentinal tubules, while other areas are atubular. (Original magnification, ×40. Masson's trichrome stain.) Fig. 2E. Residual organic matrix at the surface of a previously remineralized tooth. (Original magnification, ×40. Hematoxylin-eosin staining.) Fig. 2F. Residual organic matrix along the surface of a previously remineralized tooth. The dentin at the surface does not appear to be well mineralized, although areas in the deeper dentin are well mineralized. (Original magnification, ×63. Masson's trichrome stain.)

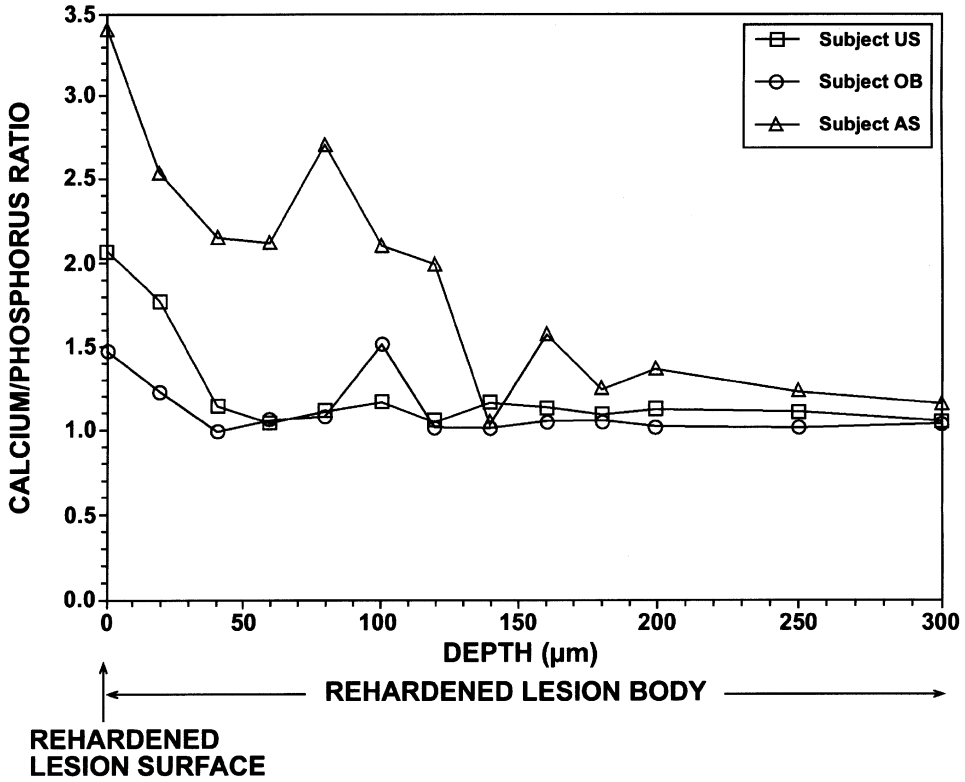

Fig. 3. Ratio of calcium to phosphorus (Ca:P) of successive layers of arrested severe caries (D_x) lesions of three exfoliated primary teeth. The subjects had systematically used a xylitol gum (the 100% xylitol-pellet formula) for 20 months. The measurements of Ca and P were begun from the outer rehardened surface of the lesions and were carried out by means of scanning electron microscopy.

surfaces were hard. The shallow depressions were softer, although even their hardness was comparable to, or higher than, that of sound dentin located deeper in the same tooth. The hard layer responsible for the high occlusal KHN scores was obviously relatively thin, because the hardness measurements of the lesion's body generally revealed softer tissue at a depth of just 0.1 mm (Fig. 4). This softer layer was generally less than 0.5 mm in thickness. The difference in KHN scores between the surface and the 2 layers below the surface (0.1 and 0.3 mm) was statistically significant ($P < 0.001$). If the measurements were begun from the bottom of a shallow pit, the softer layer could reach a thickness of 1 mm (1.3 mm in 1 case, subject LP). However, if the determinations were begun from a shallow elevation in the occlusal surface, the softer layer could begin at a depth of 0.3 mm, could be relatively thin, or could be indiscernible. The difference in KHN scores between the surface and the 2 deepest sections was also statistically significant ($P = 0.004$). Overall, the hard surface layer resulted in KHN scores ranging between 900 and 1000, while the underlying softer tissue and the sound dentin gave average KHN scores of about 540 and 640, respectively. The specimens representing the different polyol chewing gum groups behaved similarly.

Discussion

This study showed that regular long-term use of polyol-containing chewing gum can be associated with histologic and physiochemical changes in dentin caries lesions, which are typical of arrested (rehardened) lesions. Owing to the limited number of near-exfoliation-time teeth that could be obtained from the gum-using subjects, the purpose of this study was not to relate lesion-rehardening to the use of a particular polyol gum. Overall, the present series of studies and especially the original clinical trial (7, 8) unequivocally demonstrated, however, that lesion-rehardening took place to a significant extent only in subjects who used polyol gums, and that arrested D_m and D_x lesions could not be nearly as extensively observed in non-users. The present results thus complete the clinical diagnoses performed on the same subjects and on the same teeth (7, 8). The chemical reasons leading to caries arrest can be envisaged to be associated with significant oral biologic changes that had taken place during the prevention program. Such changes included alterations in the subjects' dietary regimen and perhaps increased awareness among the families of the importance of oral hygiene (because of the attention the school-based program understandably evoked). However, a decisive new variable was

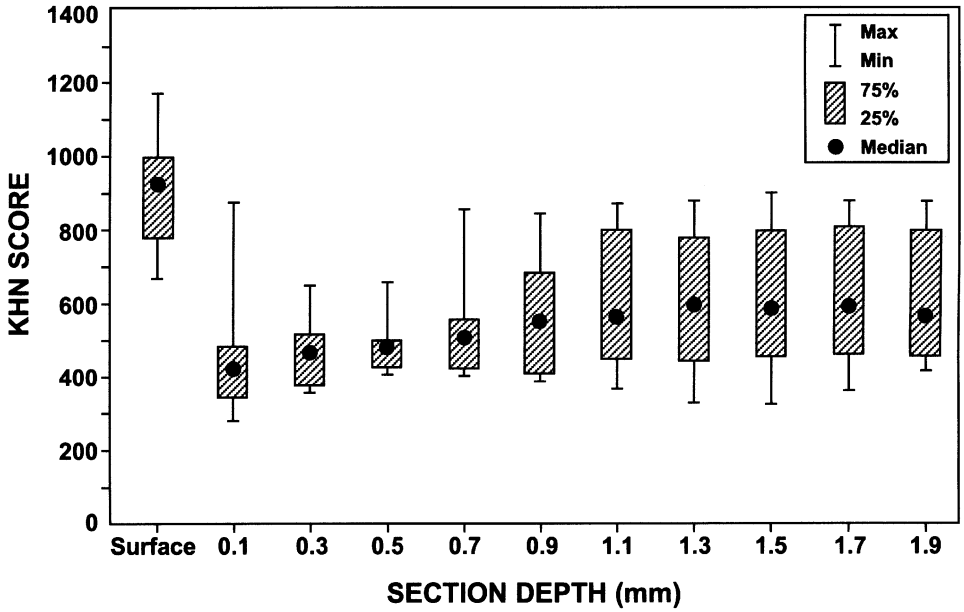

Fig. 4. Box plot of the Knoop hardness number (KHN) scores of the bodies of severe caries (D_x) lesions, showing the maximum and minimum values and the medians. The measurements were carried out on diamond-cut surfaces of nine extracted primary teeth obtained from nine subjects who used different polyol gums. These lesion-body KHN scores were compared with scores obtained from rehardened occlusal surfaces of the same teeth.

the relatively intense gum usage; subjects in the no-gum group, who were similar to the gum-chewing subjects with regard to all relevant measures, had as a group a significantly smaller number of arrested dentin caries lesions than other subjects (7). The polyol gum program induced oral biologic changes that led to the arrest of dentin caries. The new and hard, although thin, surface layer of the occlusal surface enclosed the advancing caries process. The propagation of the process requires that the microorganisms that have invaded the tissue continue to receive nutrients. The availability of nutrients, however, is effectively impaired by the formation of a hard barrier and by the washing action of saliva.

The histologic observations suggest that the majority of the specimens showed remineralization—as a result of caries arrest—from the surface of the dentin caries lesions by a mechanism that was mediated mainly by non-cellular factors. Remineralization appeared to have taken place within the remaining collapsed, organic extracellular matrix of the lesions and/or was associated with the remaining dentinal surface. The non-cellular factors involved were most likely present in saliva and were of glandular origin. Typical contributing factors may include the normally high Ca^{++} content of saliva and perhaps the complex formation between Ca^{++} and the polyols present in the chewing gums (13–16). Histologic observations also suggested that remineralization sometimes penetrated the dentinal tubules to a depth of 0.5 mm, although normally to a lesser depth. In some areas there was an organic matrix that had been remineralized, while in other areas there was no remaining organic matrix, although during gross examination these areas were also remineralized. In conclusion, the histologic studies supported the idea that the examined primary teeth had large dentin caries lesions that had undergone a rehardening process. A discernible pulpal response to both the caries process and the rehardening process was possibly also involved.

The SEM/EDS and EMPA data suggested that the topmost layer of the arrested dentin caries lesions displayed a high calcium content. This observation is in congruence with the possibility that the increased hardness of the arrested lesions could be attributable to the precipitation of calcium phosphate salts in the topmost zone of the lesion, forming a certain type of 'sealant' that affected the transfer of bacterial nutrients from saliva to the lesion body. Other studies have shown that the saliva precipitates formed in the presence of xylitol go through a substantial crystalline phase involving the crystal structure of apatite, the individual crystallites being extremely small (<1 μm), with a Ca:P ratio of 1.46 (16).

The EMPA analysis of certain elements (such as silicon, fluorine, and molybdenum) were carried out to elucidate the possible role of those elements in caries arrest in Belize. The Belizean diet contains relatively large amounts of rice (which may contribute to a higher silicon intake) and marine products (which may increase the intake of fluorine). The fluoride levels of drinking water are extremely small throughout the country, 0.05–0.1 parts

per million, or less (6). The EMPA data indicate that the rehardening of the dentin caries lesions in this study cannot be attributed to the above dietary patterns; the no-gum group was similar to the treated groups in these regards, and it did not show as high a degree of caries arrest as the treated groups did (7, 8).

The microhardness tests supported the idea of significant rehardening of the lesions. Other, even more sensitive techniques to measure remineralization are available (17–21), although such procedures have not been used in the study of arrested human caries lesions. The iodide penetrability method and other chemical methods were not used because various chemical studies had been planned for the specimens, and quantitative microradiography, a preferred procedure in remineralization studies, was not available. The present microhardness measurements suffer from the lack of an analysis of reading error. Furthermore, the KHN scores obtained from the surface of a tooth cannot be compared with scores obtained from the body of the lesion or from sound dentin of the same tooth because the different orientation of the collagenous matrix at these locations may lead to different behavior of the indentations following removal of the load. However, the reading error was estimated to be small, as the rater had become fully acquainted with the procedure. In spite of the weakness of the microhardness measurements, the results obtained were in agreement with histologic, chemical, and clinical observations.

Although the original clinical data (7, 8) showed that all lesions tested were diagnosed as arrested, a hardened surface of a lesion may not allow a definite classification of such lesions as arrested. Such a classification needs the examination of both the surface and the lesion proper by radiographs and/or by transmission electron microscopy. Therefore, the lesions examined in this study were judged to be arrested on the basis of clinical diagnosis only.

In summary, the results showed that long-term use of polyol gums was associated with a rehardening process that extended throughout the dentin caries lesion, and that the histologic, physical, and chemical studies correlated with the clinical diagnoses (6–8). This type of caries arrest is not uniquely associated with chewing polyol gums, but must be regarded as a general, saliva-associated repair mechanism triggered by dramatic changes in the oral environment. In this particular instance, the triggering factor was the long-term, relatively intense, and mostly supervised use of saliva-stimulating polyol gum. The present results demonstrate the ability of a simple modification of diet to induce significant oral health effects in a situation where the subjects had very limited access to restorative dental care and where comprehensive preventive strategies could not be systematically implemented. These results are in agreement with enamel microhardness studies made with partly decalcified bovine enamel slabs—Scheinin et al. (22) associated the use of xylitol with increased microhardness of enamel. The present results are also in agreement with those of human clinical trials reviewed by Mäkinen (23) and Birkhed (24). Collectively, these data suggest that rehardening of artificial or natural caries lesions as a result of intense use of chewing gum should not be considered uncommon provided that the gum does not contain readily fermentable carbohydrates that produce large amounts of acids.

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Received for publication 8 August 1997
Accepted 28 January 1998