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EXPERIMENTAL ROOT SURFACE CARIES IN HAMSTERS
THE DEVELOPMENT OF THE DISEASE AFTER INOCULATIONS OF TWO TYPES OF CARIOGENIC BACTERIA

BY

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ABSTRACT

The aim of this study was to investigate the development of root surface caries in hamsters fed a high-sucrose diet over a 24-week period after inoculations of two types of cariogenic bacteria. Twenty-one-day old male golden hamsters (n=103) were divided into 5 groups. Four groups were given diet 2000, and one group was given a stock diet CE-2. Of the groups given diet 2000, three groups were infected with *Actinomyces viscosus* ATCC 15987 and *Streptococcus mutans* NTCC 10449 separately (AV and SM groups) or in combination (AVSM group), and one group remained uninfected. A grid method was used to evaluate the plaque accumulation, alveolar bone loss, and root surface caries. After 12 weeks, root surface caries developed mainly on the first mandibular molars in the three infected groups. At 24 weeks, the prevalence of root surface caries was highest in the AV group, but root caries scores were not significantly different among the three infected groups. In the groups SM and AVSM, the molar crowns were extensively destroyed by caries, while in the AV group the crowns were almost intact.

It was concluded that challenge with *Actinomyces viscosus* may be appropriate to study root surface caries in hamsters.

Key words: Root surface caries, Hamster, *A. viscosus*, *S. mutans*

INTRODUCTION

In recent years, the average lifespan of the populations of industrialized countries has been increasing (Nyvad and Fejerskov [1]). In Japan, for example, the elderly population reached fourteen percent in 1994 (Statistics Bureau, Management and Coordination Agency, Japan [2]).

Several studies have reported prevalence rates for populations with root surface caries ranging from less than 10% to over 80% for selected adult populations (Banting [3], Beck [4]). The higher incidences of periodontal diseases and root surface caries in the elderly are the main cause of tooth loss (Jordan and Sumney [5]), which considerably limits their masticatory functions. Therefore, it is important to understand the development of root surface caries to prevent its manifestation in the elderly helping them to retain more sound teeth and mantain good health.

In experiments with animals and humans, it has been demonstrated that dental caries is clearly an infectious disease that is

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dietarily conditioned (Doff et al. [6]; Hix and O'Leary [7]). The complexity of the bacterial flora involved in human coronal and root surface caries has also been demonstrated by a variety of studies (Boue et al. [8], Keltjens et al. [9]). Although animal models differ considerably in many aspects from human models, several animal models have been used to investigate root surface caries during the last two decades (Stookey et al. [10], Bowen and Pearson [11], Rosen et al. [12]). Animal models used for studies on root surface caries have played an important role in the understanding of the disease etiology, as well as in the identification of measures to prevent root surface caries (Stookey [13]).

The purpose of this study was to investigate macroscopically the development of root surface caries in hamsters fed a high-sucrose diet over a 24-week period after oral inoculations with two types of cariogenic bacteria (*A. viscosus* and *S. mutans*), with a view to preventing the disease in the future.

Materials and Methods

Animals and infections One hundred and three newly weaned 21-day-old male golden hamsters were bought from Japan SLC, Inc., weighed, and distributed into cages made of solid plastic walls with a stainless steel bottom and a mesh lid (3–5 animals per cage). All the animals were given distilled water containing a solution of 0.02% streptomycin and were fed a solid stock diet CE-2 (Clea Japan, Inc.) for one day. On the second day, the animals were skin-marked with picric acid and divided into five littermate groups having similar body weights. One group was given a powdered stock diet CE-2 (Clea Japan, Inc.), and the other four groups were given a powdered caries promoting diet 2000 (Keys and Jordan [14]). Among the latter four groups given diet 2000, three groups were each inoculated orally with strains of either *Streptococcus mutans* NCTC 10449, *Actinomyces viscosus* ATCC 15987, or a combination of the two strains, and one group remained uninoculated.

Previously, bacterial strains of *Streptococcus mutans* NCTC 10449 suspended in Brain Heart Infusion (BHI)*[1] broth, and strains of *Actinomyces viscosus* ATCC 15987 suspended in Gam broth "Nissui"*[2] were incubated at 37°C for 18 hours and 48 hours, respectively. After incubation, each broth was centrifuged at 6000 r.p.m. for 5 minutes. The supernatant fluids were discarded, and the sediment solutions were repeatedly gently mixed and washed with sterilized 0.85% saline by means of a pipette, and centrifuged. Final solutions with the respective bacterial strains (10^8 CFU/ml) suspended in saline were obtained and stored at −20°C.

Disposable mini-syringes were used to instill approximately 0.2 ml of the fluids containing the *S. mutans* or *A. viscosus* strains into the mouth and the cheek pouches of the animals when the bacterial strains were inoculated separately, and 0.1 ml of each respective inoculum when the strains were inoculated in combination. In addition, a stick applicator with a cotton pellet on the tip was also used to spread the fluids containing the bacterial strains in the molar region of the animals' mouths. These procedures were repeated daily for 7 consecutive days. Tap water was available *ad libitum* to all the groups, which were maintained at a room temperature of 23±2°C, air humidity of 60%, and light control (lights on from 6:00 a.m. to 8:00 p.m.). Cages were changed twice a week, the animals' weight measured weekly, and the diet and water intake monitored twice

*[1] *DIFCO Laboratories, Detroit, Michigan, USA*
*[2] *Nissui Pharmaceutical Ltd., Tokyo, Japan*

a week.

A period of 10 to 12 days was allowed for recovery of the cariogenic bacteria. Plaque samples were taken by swabbing the molar teeth of the animals with a cotton pellet soaked in 1 ml of an aqueous solution of 1% peptone (pH 7.0). The presence of *S. mutans* and of *A. viscosus* was verified by selective media: respectively, mitis salivarus agar (MS)*[3] added 5% sucrose and the CFAT medium described by Zylber and Jordan [15].

Sacrifices and evaluation methods Animals were killed by chloroform inhalation at 4, 8, 12, 16, 20, and 24 weeks after bacterial challenge. After the animals' jaws were dissected out and slightly washed in tap water, the amount of plaque accumulation on the maxillary and mandibular molar was examined under a stereo-microscope. Afterwards, the jaws were stripped of soft tissues, and the alveolar bone loss and root surface caries were evaluated using a grid method. In preliminary examinations, it was noted that the levels of plaque accumulation and bone resorption were minimal on the maxillary molars, and no root surface carious lesions were detected. Thus, it was decided to assess only the mandibular quadrants for any macroscopical alterations caused by plaque, alveolar bone loss or root surface caries on the three mandibular molars.

The grid method The chart illustrated in Fig. 1 was used to score the development of plaque accumulation, alveolar bone loss, and root surface caries in the animals' mandibular molars. The scoring chart was a modification of the grid method of Rosen et al. [12] for hamsters. The roots of the hamsters' mandibular molars were divided into equal sections as was previously reported (Rosen et al. [12]). To quantitatively evaluate the amount of plaque accumulation, alveolar bone loss, and root surface caries, and to identify which areas of the roots are more affected by these periodontal alterations, the root surfaces of each mandibular molar, including the furcation, were divided into unit areas of about 0.15 mm^2 (0.3 mm×0.5 mm of width). As shown in Fig. 1, the roots of the molars were divided coronoapically into 44, 32, and 32 unit areas, respectively, for the first, second, and third mandibular molars. The roots of the second and third mandibular molars were divided into 32 units because they were shorter than those of the first molars. Therefore, on the root surfaces of the mandibular molars, a total of 216 unit areas could be scored per hamster. In addition, for easy recognition of the unit areas involved, each unit was numbered on the root surface (Fig. 1). The

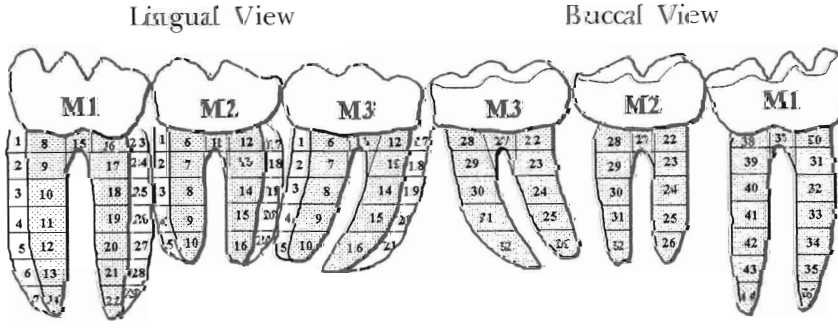


Fig. 1. Chart used for scoring plaque accumulation, alveolar bone loss, and root surface caries on the hamsters' mandibular molars.

*[3] *See footnote *1, page 84.*

lingual and buccal views of Fig. 1 represent, respectively, the lingual aspect (comprising the mesial, lingual and distal surfaces) and the buccal surfaces of mandibular molars. The evaluation of the unit areas affected was done under a stereo-microscope at magnifications of 10× to 20×. Each unit area was given a score of 1 only if more than half of the unit was involved on the root surface.

Previously, vertical sections of the three mandibular molars had been prepared from hamsters' mandibles. Photographs were taken at magnification of 10× and developed as slide films. Afterwards, images of the hemi-sectioned mandibular quadrants ($M_1M_2M_3$) were projected from a slide projector at 2.8× magnification, and the root contour of each mandibular molar was drawn on a transparent plastic sheet. Each root on the drawing was divided into grids as shown in Fig. 1. Then, a transparent plastic sheet showing the contour of the roots with grids was reduced by photocopying; shaped circularly to the same diameter as the lens inside the eyepiece of a stereo-microscope; and placed in parallel with the lens to serve as a scale. On the root surfaces, all unit areas affected by plaque accumulation, alveolar bone loss, or root surface caries were scored as they appeared through the scale-drawing, which was adjusted to the image of each mandibular molar at the mesio-distal contour of the crowns on the cervical region.

Plaque Accumulation Immediately after each animal was killed, their jaws were dissected out, slightly rinsed in tap water to remove the blood, and stored moistened in sealed plastic jars. Afterwards, the lower jaws were split and each mandibular quadrant stained with a 1% solution of Neutral Red*[4] (Takashima [16]). Then, each stained quadrant was immersed and shaken for 10 seconds in water, dried with absorbent paper, and puffed with compressed air. Both mandibular quadrants were then mounted on blocks of utility wax fixed to glass Petri dishes and examined under a stereo-microscope at magnifications of 10× to 20×.

When gingival recession was present, the number of unit areas affected by plaque accumulation between the cemento-enamel junction (CEJ) line of the scale-drawing and the internal margin of the gingiva receding apically on the root surfaces was recorded. A unit area was given a value of 1 only if more than half of the unit was affected by plaque. Then, each mandibular quadrant was positioned about 1 cm parallel to the stage, and photographs of their lingual and buccal aspects were taken at magnifications of 10× to 15×. Later, slide films were projected on a screen showing the scale-drawing enlarged and the units affected by plaque accumulation were checked.

For each experimental group, the plaque scores (for the lingual aspects or buccal surfaces of a molar's roots) were calculated by dividing the total number of unit areas affected by plaque on the left and right sides of each hamster's mandible by the number of animals killed per group. Student's t-test was used for statistical analysis between groups.

Alveolar bone loss and root surface caries After the plaque accumulation had been scored, the lower jaws were autoclaved and cleaned by removing soft tissues and debris with a small soft toothbrush under tap water. Then, the cleaned jaws were placed into small bottles containing a 0.06% solution of Murexide*[5] (Navia [17]) and stored in a dark room for 24 hours. Later, the murexide-stained samples were washed

*[4] *Wako Pure Chemical Industries, Ltd., Tokyo, Japan*
*[5] *See footnote*4, page 86.*

once in tap water and dried in an oven at 50°C. Afterwards, all samples were examined under a stereo-microscope with a scale-drawing inserted into the eyepiece as described.

Alveolar bone loss was scored by examining the unit areas exposed on the root surfaces between the CEJ and the level of the bone destroyed coronoapically. At the same time, root surface caries was scored only if a unit area on the root exposed by bone loss was stained red and could be penetrated by an explorer (Rosen et al. [12]).

For each experimental group, the bone loss scores (for the lingual aspects or buccal surfaces of a molar's roots) were calculated as the total number of unit areas exposed by bone loss (the distance between the CEJ line and the alveolar bone crest) on the left and right sides of each hamster's mandible divided by the number of animals killed per group. Likewise, for each group, the root caries scores were obtained as the average number of unit areas affected by root surface caries on both sides of each hamster's mandible. The scores for alveolar bone loss and root surface caries were analyzed statistically using the Student's t-test. In addition, to evaluate the development of root surface caries in the animals of each infected group, the chi square test was used to compare the numbers of mandibular quadrants that developed carious lesions on the roots of any of the three mandibular molars.

Results

Animals' growth conditions The mean weight gain and number of animals killed at intervals of 4 weeks after being challenged with cariogenic bacteria are summarized in Table 1. No significant differences in weight gain were found among the groups.

Plaque Accumulation Supragingival plaque affecting the root surfaces was observed only after 12 weeks in the three infected groups. After 4 weeks, however, plaque accumulation was observed on the crowns of the mandibular molars in the groups infected with *S. mutans* (groups SM and AVSM) and minimally on the molars of the other groups.

In the two uninfected groups (D2000 and CE-2), no plaque accumulation was found macroscopically on the root surfaces of the mandibular molars throughout the experimental period. In the three infected groups (AV, SM, and AVSM), plaque accumulation increased from 12 to 24 weeks after the challenge. Plaque was observed affecting the cervical region mainly on the lingual aspects of the first mandibular molars, and to a lesser extent on the second and third molars. From 12 to 20 weeks, the numbers of unit areas affected by plaque accumulation per hamster increased in the three infected groups but showed no significant differences among these three groups.

At 24 weeks, for the total of 216 unit areas on the root surfaces of the three mandibular molars, the mean numbers of unit areas affected by plaque for each group (which are equal to the sum of the plaque scores for the lingual aspects and buccal surfaces of the three molars) were, respectively, 21.8, 15.0, and 22.2 for the infected groups AV, SM, and AVSM. Thus, the rates of plaque accumulation for each group were 10.1%, 6.9%, and 10.3%, respectively. Below the CEJ, in the uninfected groups D2000 and CE-2, no unit area was affected by plaque accumulation. The plaque scores after 24 weeks for each mandibular molar in all the groups are shown in Fig. 2.

After 24 weeks, the plaque scores for the first molars were highest in the AV group (18.6) followed by the AVSM group

Table 1. Weight gain(g) (mean±S.D.) and number of animals killed in each group over a period of 24 weeks.

GROUP		Duration of Experiment						
Diet	Infection	Initial Weight	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks	24 Weeks
CE-2	none	39.8±3.43	*	86.4±28.65 (n=2)	97.0±5.66 (n=4)	110.3±1.10 (n=2)	120.7±14.02 (n=3)	122.5±7.31 (n=3)
2000	none	40.3±4.27	*	68.3±25.10 (n=2)	95.6±11.27 (n=4)	109.8±16.60 (n=2)	112.8±5.75 (n=3)	115.8±10.25 (n=3)
2000	AV	39.9±3.72	63.0±15.69 (n=4)	84.8±15.82 (n=4)	103.9±17.88 (n=5)	111.3±2.08 (n=3)	119.4±6.87 (n=4)	122.3±17.41 (n=5)
2000	SM	40.2±3.46	49.8±14.38 (n=4)	84.3±9.73 (n=4)	87.6±14.33 (n=5)	107.7±8.02 (n=3)	112.1±7.33 (n=4)	114.1±13.52 (n=5)
2000	AVSM	40.4±3.90	61.5±21.91 (n=4)	78.5±17.21 (n=4)	112.0±12.84 (n=5)	122.3±10.25 (n=3)	120.0±7.62 (n=4)	118.7±7.14 (n=5)

Total=103 hamsters

(*)=Not killed after 4 weeks; *SM=Streptococcus mutans; AV=Actinomyces viscosus; AVSM=S. mutans+A. viscosus.*

Table 2. Bone loss scores for the first mandibular molars in each group from 4 to 24 weeks.

GROUP		Unit Areas Affected by Alveolar Bone Loss (mean±S.D.)					
Diet	Infection	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks	24 Weeks
CE-2	none	—	16.0±0.00	16.0±0.00	17.0±0.00	16.3±0.58	16.3±0.58
2000	none	—	16.0±0.00	19.3±4.27	17.0±1.41	16.7±1.15	18.7±2.08
2000	AV	17.0±0.82	17.5±0.58	23.4±3.91	34.0±8.19	28.8±6.24	36.8±10.69
2000	SM	16.5±0.58	17.0±0.82	25.8±6.06	34.7±3.06	22.8±4.35	35.2±10.83
2000	AVSM	17.8±0.50	18.3±1.26	26.6±4.39	33.7±4.16	23.3±2.06	38.8±15.02

SM = Streptococcus mutans; AV = Actinomyces viscosus; AVSM = S. mutans + A. viscosus.

Note: Significant differences between the groups:

*$p < 0.05$

**$p < 0.01$

n.s. (not significant)

Period	12 weeks			16 weeks			20 weeks			24 weeks		
Groups	*AV*	*SM*	*AVSM*	*AV*	*SM*	*AVSM*	*AV*	*SM*	*AVSM*	*AV*	*SM*	*AVSM*
D2000	n.s.	n.s.	*	*	**	*	*	n.s.	**	*	*	*
CE-2	*	*	**	*	**	*	*	n.s.	**	*	*	*

From 12 to 24 weeks n.s. between AV and SM, or AV and AVSM; and n.s. between SM and AVSM.

(14.2), and the SM group (8.0). There was a significant difference between the AV and SM groups ($p<0.05$), but not between the AV and AVSM groups.

In the three infected groups, plaque accumulation was remarkable on the root surfaces of the lingual aspects of the first mandibular molars. The plaque scores for the lingual aspects (Fig. 1, units 1 to 29: a total of 58 units per hamster) of the first molars were, respectively, 16.4, 5.4, and 10.6 for the AV, SM, and AVSM groups.

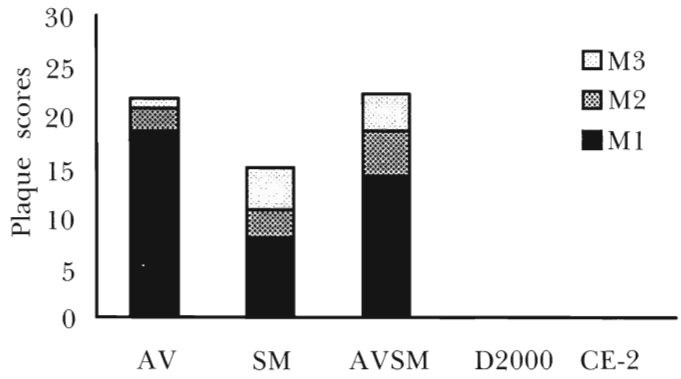


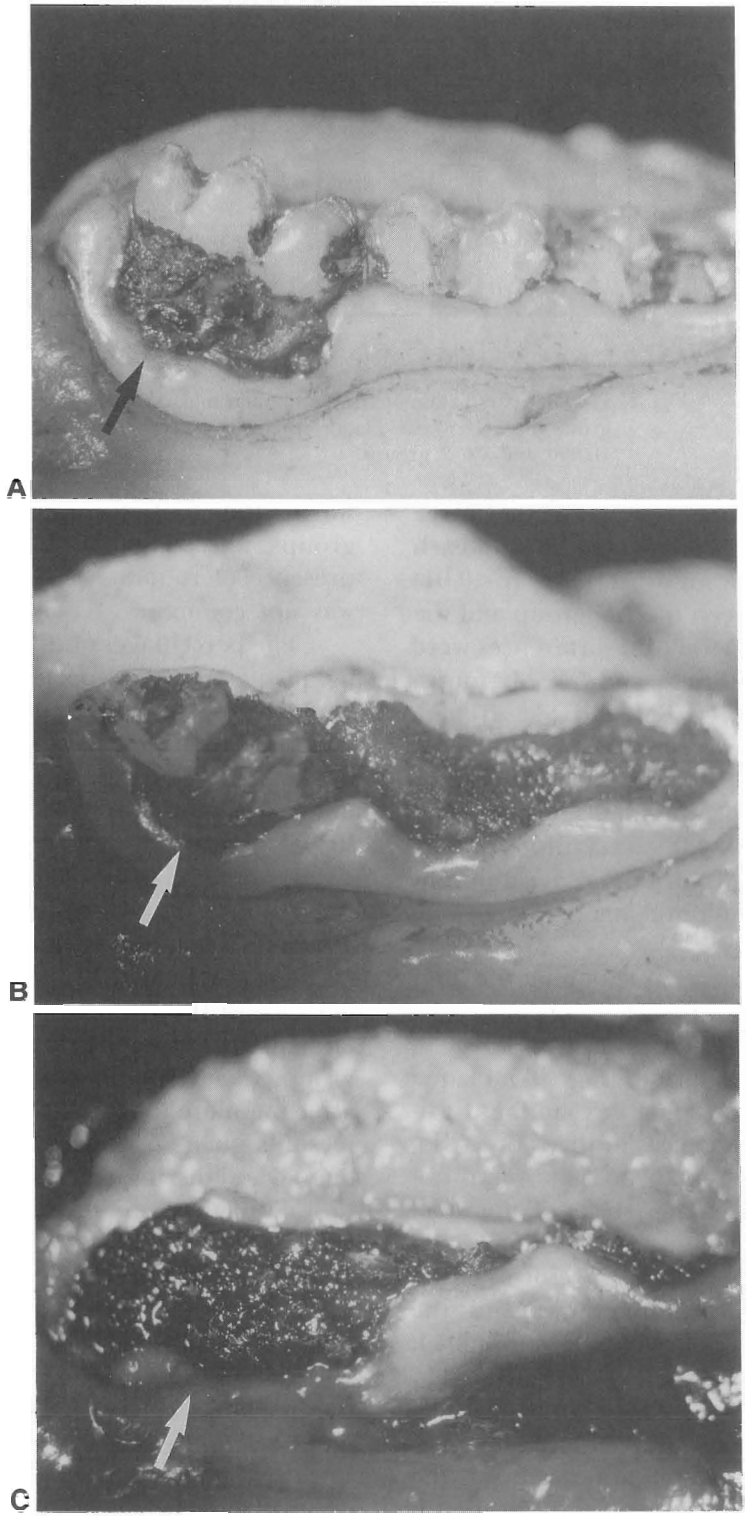
Fig. 2. Plaque accumulation on the mandibular molars in each group after 24 weeks. Plaque scores were zero in the D2000 and CE-2 groups.

When the plaque scores of the three infected groups were compared with each other, a significant difference ($p<0.01$) was found between the AV group and the SM group. No significant differences were found between the AV and AVSM groups, or between the AVSM and SM groups. The plaque scores for the buccal surfaces (Fig. 1, units 30 to 44: a total of 30 units per hamster) of the first molars were, respectively, 2.2, 2.6, and 3.6 for the AV, SM, and AVSM groups. No significant differences were found among these three groups.

The plaque accumulation on the lingual aspects of the first mandibular molars in the three infected groups is shown in Fig. 3. In the AV group (Fig. 3A), plaque was found below the CEJ expanding vertically towards the apices of the roots. Also, rounded periodontal pockets were observed on the lingual surfaces of almost all animals in this group. As in the uninfected groups D2000 and CE-2, the crowns of the molars were practically intact and the amounts of coronal plaque were minimal. Conversely, in the SM (Fig. 3B) and AVSM (Fig. 3C) groups, extensive crown destruction by caries was observed in all animals. Supragingival plaque appeared on the cervical region, extending diffusely towards the root surfaces in these two groups. Except for the AVSM group, the presence of rounded periodontal pockets was not common.

The percentages of unit areas affected by plaque on the root surfaces of the lingual aspects of the first mandibular molars in each infected group are shown in Fig. 4. In the three infected groups, the unit areas most affected by plaque are shown in black.

From the CEJ to the root apices, in the AV group (Fig. 4A) plaque was observed affecting 3 (the first 3 units nearest to the CEJ line of a total of 7 units below the CEJ line) out of the 7 unit areas of the mesial, lingual, and distal surfaces. In the SM group (Fig. 4B) plaque was observed affecting more than half of the mesial and mesio-lingual surfaces whereas the disto-lingual and distal surfaces were affected in up to 3 of the 7 unit areas. In the AVSM group (Fig. 4C), the unit areas affected by plaque were disperse. On the lingual surfaces, up to 5 of the 7 unit areas were affected by plaque. However, more than 60% of the units affected by plaque were the first units below the CEJ (Fig. 1, unit numbers 8, 15, and 16) on the lingual surfaces. In the three infected groups, when plaque accumulation was found on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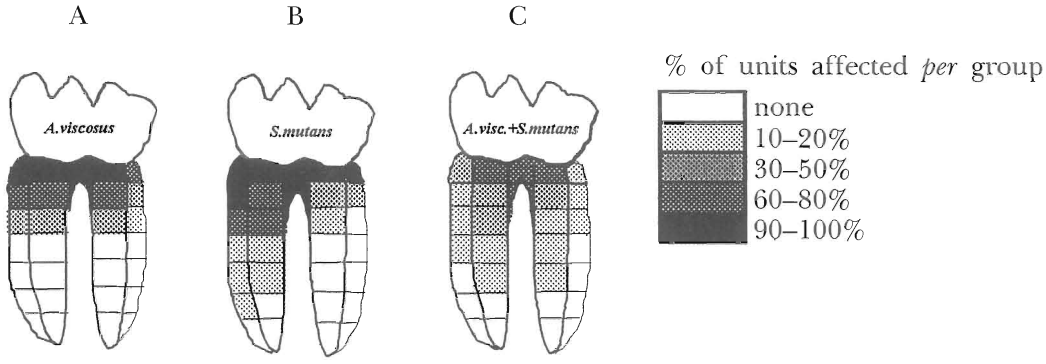


Fig. 4. Plaque accumulation on the lingual aspects of the first mandibular molars (left and right sides) in each infected group after 24 weeks. (A=AV group; B=SM group; C=AVSM group)

the lingual aspects of the first molars, the furcations of the lingual surfaces were always affected by plaque (Fig. 4).

Alveolar bone loss Bone resorption was observed in all animals after 4 weeks. The roots of the first mandibular molars showed more unit areas affected by bone loss, followed in decreasing order by those of the second and third molars.

After 24 weeks, out of 216 unit areas of the three mandibular molars, the mean numbers of unit areas affected by alveolar bone loss in each group (which correspond to the sum of the bone loss scores for the lingual aspects and buccal surfaces of the three molars) were, respectively, 69.2, 69.4, and 71.6 for the AV, SM, and AVSM groups, and 48.7 and 41.7 for the D2000 and CE-2 groups. The respective rates of bone loss were 32.0%, 32.1%, and 33.2% for the groups AV, SM, and AVSM, and 22.5% and 19.3% for the D2000 and CE-2 groups. The bone loss scores after 24 weeks for each mandibular molar in all the groups are shown in Fig. 5.

Throughout the entire experiment, the levels of alveolar bone loss on the roots of the first molars were higher in the three infected groups than those in the uninfected groups. The bone loss scores from 4 to 24 weeks for the affected root surfaces on the first molars are shown in Table 2. From 4 to 24 weeks, there were no significant differences in the bone loss scores among the three infected groups. After 16 and 20 weeks, the scores of each infected group were significantly higher than those of the uninfected groups, except in the SM

Fig. 3. Plaque accumulation on the lingual aspects of the mandibular molars of a hamster in each infected group (AV, SM, and AVSM) after 24 weeks. Orig. magn. 10×.
(A) AV group: Plaque was observed on the cervical region of the first molars extending below the cemento-enamel junction (CEJ) towards the root apices. The crowns were intact and the presence of rounded periodontal pockets on the lingual surfaces of the first molars (arrow) was a feature in this group.
(B) SM group: Plaque was observed on the cervical region of the three mandibular molars surrounding the CEJ (arrow) but not extending towards the root apices. The crowns of the three mandibular molars were destroyed by coronal caries and the severity increased in the second and third molars. Rounded periodontal pockets were uncommon in this group.
(C) AVSM group: Plaque was observed on the cervical region of the first molars and diffusely extending below the CEJ (arrow). Rounded periodontal pockets appeared sometimes affecting only the first molars. The crowns of the three mandibular molars were destroyed by rampant caries.

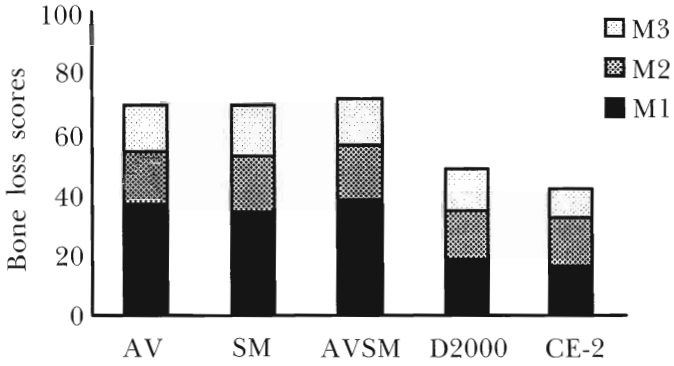


Fig. 5. Alveolar bone loss on the mandibular molars in each group after 24 weeks.

group at 20 weeks.

Alveolar bone loss was remarkable on the lingual aspects (Fig. 1, units 1 to 29: total 58 units per hamster) of the first mandibular molars. After 24 weeks, the bone loss scores for the first molars were the highest in the AVSM group (38.8) followed by the AV group (36.8) and the SM group (35.2). There were significant differences ($p<0.05$) when the scores of each respective infected group were compared with those of the D2000 and CE-2 groups (see note below Table 2). The bone loss scores for the lingual aspects of the first molars were, respectively, 24.6, 20.8, 25.8, 12.7, and 10.3 for the AV, SM, AVSM, D2000, and CE-2 groups. Significant differences ($p<0.01$) were found when the scores of each infected group were compared with those of each uninfected group. For the buccal surfaces (Fig. 1, units 30 to 44: total 30 units per hamster), the bone loss scores were 12.2, 14.4, 13.0, 6.0, and 6.0, respectively. Also, there were significant differences ($p<0.05$) between the scores of each of the three infected groups and those of the uninfected groups.

The alveolar bone loss on the lingual aspects of the first mandibular molars in all groups (except CE-2) is shown in Fig. 6. In the AV (Fig. 6A), SM (Fig. 6B), and AVSM groups (Fig. 6C), the destruction of the bone was extensive exposing more than half of all root surfaces. In the D2000 (Fig. 6D) and CE-2 groups, the alveolar bone loss was minimal and affected only the first units appearing below the CEJ.

For each group, the percentages of unit areas affected by alveolar bone loss on the lingual aspects of the roots of the first molars are shown in Fig. 7. From the CEJ to the root apices, in the AV group (Fig. 7A), alveolar bone loss was observed affecting, respectively, 6, 5, 4, and 2 of the 7 unit areas of the mesial, mesio-lingual, disto-lingual, and distal surfaces. However, the units most affected were those situated up to 2 of the 7 unit areas below the CEJ line (see Fig. 7A).

In the SM group (Fig. 7B), from the CEJ line to the root apices, bone loss affected, respectively, 6, 5, 3, and 2 of the 7 unit areas of the mesial, mesio-lingual, disto-lingual, and distal surfaces. Coronoapically, the units most affected were the units situated immediately below the CEJ line (first units) and those of the mesial surface situated up to 2 of the 7 unit areas below the CEJ line (second units).

In the AVSM group (Fig. 7C), bone loss was observed affecting 6 of the 7 unit areas of the lingual surfaces and, respectively, 5 and 4 of the 7 units on the mesial

and distal surfaces of the roots. The units most affected were the first units below the CEJ, extending to the second unit of the mesio-lingual surfaces.

In the D2000 (Fig. 7D) and CE-2 (Fig. 7E) groups, all the root surfaces were minimally affected by bone loss. Apically, the units most affected by bone loss were the first units below the CEJ in each respective group, but in the D2000 group the second units of the lingual surfaces were also affected in 50% of the animals. In all groups, the furcations of the lingual surfaces of the first molars were always affected by alveolar bone loss (Fig. 7).

Root surface caries In the animals of the three infected groups, root surface caries was observed only after 12 weeks of challenge. In the D2000 and CE-2 groups, no carious lesions were found on the root surfaces of the mandibular molars throughout the experiment.

The prevalence rates of root surface caries from 12 to 24 weeks, for each infected group are shown in Fig. 8. The numbers of mandibular quadrants that developed carious lesions on the roots of the molars are given in Table 3. When the prevalence rates observed after 12 and 24 weeks were compared, the number of animals with root surface caries were found to have increased in the three infected groups (Fig. 8).

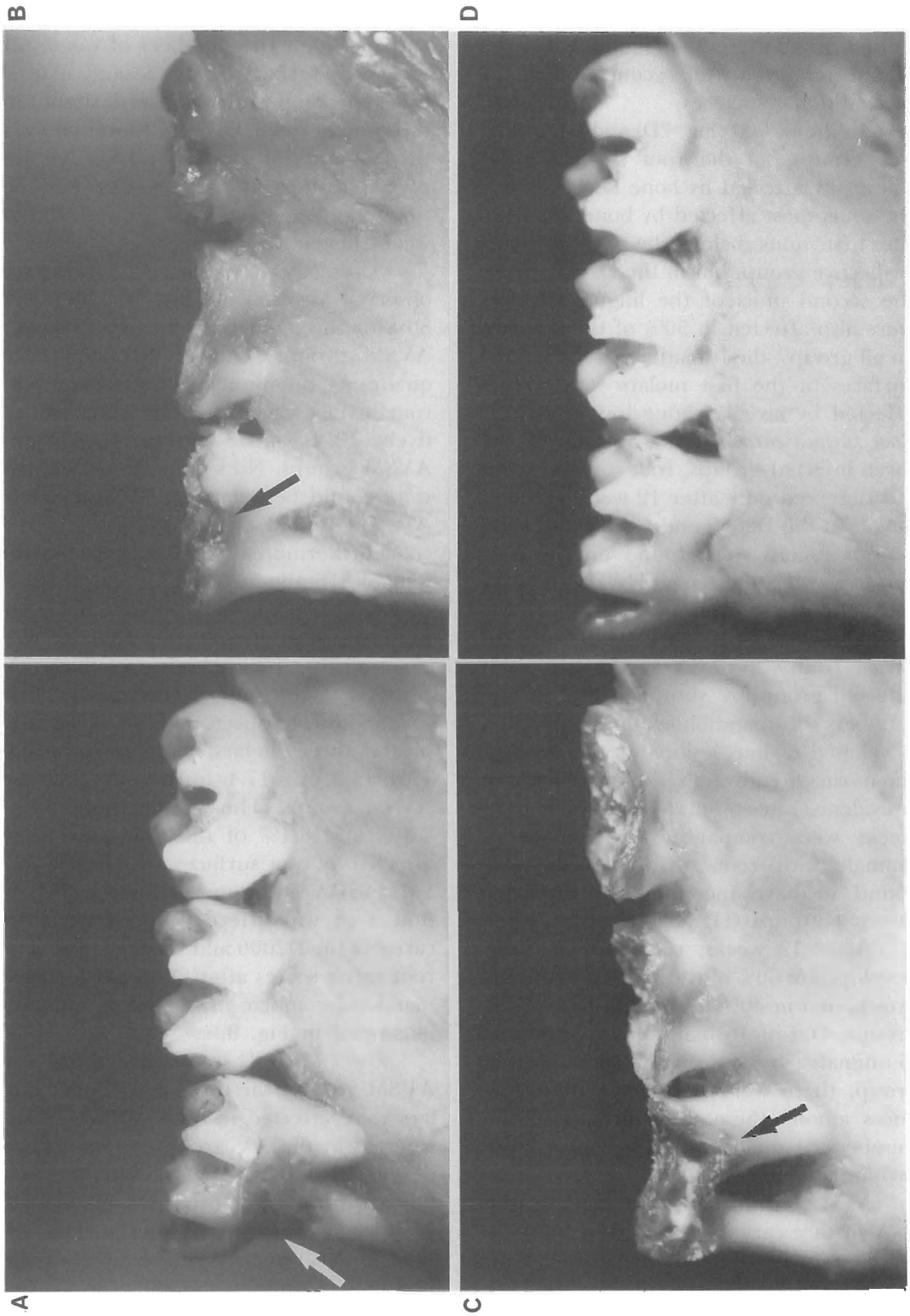
After 12 weeks, root surface caries developed in 60% of the animals in the SM group, and in 40% in the AV and AVSM groups. Out of 10 mandibular quadrants (5 animals per group) from each infected group, there were no significant differences among these three groups in the numbers of quadrants with root surface carious lesions (Table 3).

As shown in Fig. 8, in the AV group root surface caries was observed in 100% of the animals after 16 and 20 weeks. In the SM and AVSM groups, however, the prevalence rates of root surface caries varied. When the numbers of quadrants with root surface carious lesions in each infected group were compared, significant differences ($p<0.05$) were found between the AV and SM groups, and the AV and AVSM groups after 16 weeks, and between the AV and AVSM groups after 20 weeks (Table 3).

After 24 weeks, root surface caries was observed, respectively, in 100%, 80%, and 80% of the animals of the AV, SM, and AVSM groups. Out of 10 mandibular quadrants, the numbers of quadrants with root surface carious lesions were, respectively, 10, 8, and 8 in the AV, SM, and AVSM groups. No significant differences were found between the AV and SM or AV and AVSM groups.

Furthermore, at 24 weeks, for the total of 216 unit areas on the root surfaces of the three mandibular molars, the mean numbers of unit areas affected by root surface caries for each group (which correspond to the sum of root caries scores for the lingual aspects and buccal surfaces of the three molars) were, respectively, 12.6, 15.8, and 17.4, for the AV, SM, and AVSM groups. Thus, respectively, 5.8%, 7.3%, and 8.1% of the unit areas were affected by root surface caries in the AV, SM, and AVSM groups. Below the CEJ, no unit area was affected by root surface caries in the D2000 and CE-2 groups. The root caries scores after 24 weeks for each mandibular molar in all the groups are illustrated in Fig. 9.

From 12 to 24 weeks, in the SM and AVSM groups, the three mandibular molars were affected by root surface caries, whereas in the AV group carious lesions developed only on the roots of the first molars. Moreover, in the AV and AVSM groups, the majority of root surface carious lesions remarkably developed on the first molars while in the SM group caries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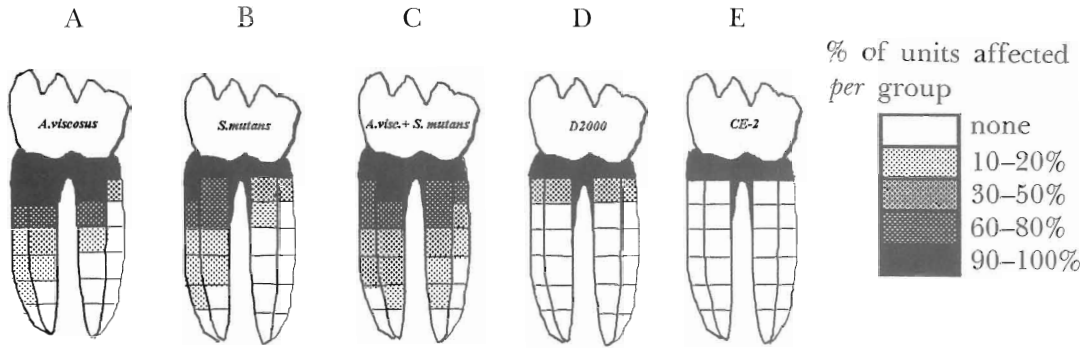

Fig. 7. Alveolar bone loss on the lingual aspects of the first mandibular molars (left and right sides) in each group after 24 weeks. (A=AV group; B=SM group; C=AVSM group; D=D2000 group; E=CE-2 group)

lesions developed mainly on the roots of the first and third molars (Fig. 9).

Table 4 shows the root caries scores for the first molars in all groups from 12 to 24 weeks. When the root caries scores of the three infected groups were compared, the scores obtained for each of these three groups were lowest after 12 weeks and highest after 24 weeks.

After 24 weeks, for all the root surfaces of the first molars, the root caries scores of the AVSM group were the highest (13.8), followed by the scores of the AV(12.6) and SM(6.8) groups. Furthermore, for the AV, SM, and AVSM groups, the respective root caries scores for the lingual aspects of the first molars were 11.4, 4.0, and 9.4, and 1.2, 2.8, and 4.4 for the buccal surfaces. No significant differences were found when the scores for the lingual aspects or buccal surfaces of these three groups were compared (Table 4).

For each infected group, the percen-

Fig. 6. Alveolar bone loss and the development of root surface caries on the lingual aspects of the mandibular molars of hamsters in the infected groups (AV, SM, and AVSM) and the uninfected group D2000 after 24 weeks. Orig. magn. 10×.
(A) AV group: Alveolar bone loss was observed affecting more than half of the mesial and lingual surfaces of the roots of the first molars and minimally affecting the roots of the second and third molars. Carious lesions developed only on the root surfaces of the first molars. The dark areas (stained by murexide solution) show the areas affected by root surface caries (arrow). Note that the crowns of the three mandibular molars are intact.
(B) SM group: Alveolar bone loss was observed affecting about half of the mesial and lingual surfaces of the roots of the first molars and minimally affecting the roots of the second and third molars. The crowns of the three mandibular molars were completely destroyed by coronal caries and carious lesions developed on the root surfaces of the three mandibular molars as an extension of the rampant caries that reached the cervical region of the crowns (arrow).
(C) AVSM group: Alveolar bone loss was observed affecting about half of the mesial and lingual surfaces of the roots of the first molars and to a lesser extent the roots of the second and third molars. The crowns of the three mandibular molars were completely destroyed by coronal caries. Carious lesions developed mainly on the root surfaces of the first molars after the crowns were destroyed by the rampant caries that reached the cervical region (arrow).
(D) D2000 group: Alveolar bone loss was observed minimally on the roots of the three mandibular molars. No carious lesions were observed on the root surfaces of the three mandibular molars. A similar pattern was observed in the **CE-2 group.**

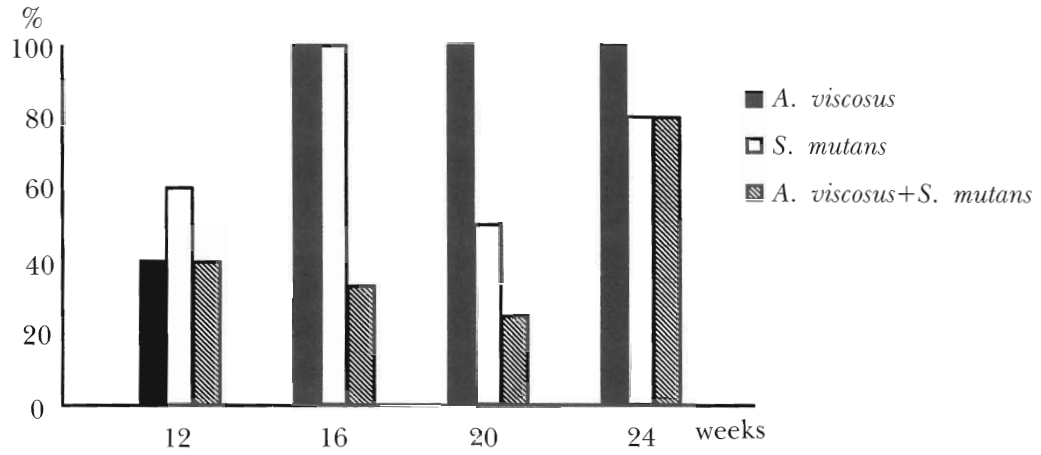

Fig. 8. Prevalence rates of root surface caries on the mandibular molars in each infected group at 12, 16, 20, and 24 weeks after bacterial challenge.

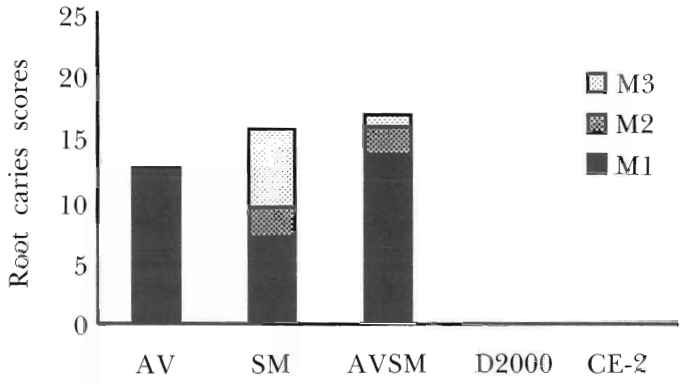

Fig. 9. Root surface caries on the mandibular molars in each group after 24 weeks. Root caries scores were zero in the D2000 and CE-2 groups.

tages of unit areas affected by root surface caries on the lingual aspects of the first molars with the respective percentages of crown destruction by caries are illustrated in Fig. 10. In the AV group (Fig. 10A), mesio-distally and from the CEJ to the root apices, for a total of 7 unit areas per root surface (with the exception of 1 unit for the furcation), the numbers of unit areas affected by root surface caries were, respectively, 3, 3, 3, and 1, for the mesio-lingual, disto-lingual, and distal surfaces of the first molars. Apically, the unit areas most affected by root surface caries (100%) were the units nearest to the CEJ (first units, Fig 1, unit numbers 8, 15, and 16) of the lingual surfaces below the CEJ. Practically no crown destruction by caries was observed in the animals in this group.

In the SM group (Fig. 10B), mesio-distally and apically, for a total of 7 units areas per root surface (except for 1 unit for the furcation), the numbers of unit areas affected by root surface caries were, respectively, 1, 3, 2, and nil, for the mesial, mesio-lingual, disto-lingual, and distal sur-

Table 3. Development of root surface caries on the mandibular quadrants in each infected group from 12 to 24 weeks.

Duration	number of animals per group	number of mandibular quadrants	*Actinomyces viscosus*	*Streptococcus mutans*	*S. mutans* + *A. viscosus*
			carious quadrants	carious quadrants	carious quadrants
12 weeks	5	10	2	3	2
16 weeks	3	6	6[a]	2[b]	2[b]
20 weeks	4	8	5[c]	2	1[d]
24 weeks	5	10	10	8	8

Chi test (*) = $p < 0.05$ between a and b; c and d.

Table 4. Root caries for the first mandibular molars in each group from 12 to 24 weeks.

GROUP		Units Affected by Root Surface Caries (mean ± S.D.)			
Diet	Infection	12 Weeks	16 Weeks	20 Weeks	24 Weeks
CE-2	none	0	0	0	0
2000	none	0	0	0	0
2000	AV	1.4±2.19	7.7±8.14	5.0±0.82	12.6±4.22
2000	SM	2.8±3.03	5.0±3.61	0.5±1.00	6.8±9.65
2000	AVSM	2.2±4.38	1.7±2.89	0.3±0.50	13.8±10.59

SM = Streptococcus mutans; AV = Actinomyces viscosus; AVSM = S. mutans + A. viscosus.

faces of the first molars. Of the units affected apically on the lingual surfaces, up to 50% were the first units below the CEJ. Crown destruction by caries was observed in more than 90% of the animals in this group.

In the AVSM group (Fig. 10C), for a total of 7 units areas per root surface (except for 1 unit for the furcation), the numbers of unit areas affected by root surface caries were, respectively, 4, 4, 5, and 1, for the mesial, mesio-lingual, disto-lingual, and distal surfaces of the first molars. However, of the 7 units for each root surface, up to 80% of the units affected apically were the units of the lingual surfaces situated up to 2 units below the CEJ line. Further, crown destruction by caries was observed in more than 60% of the animals. In the three infected groups, the furcations of the lingual surfaces were always affected by root surface caries.

The development of root surface caries on the lingual aspects of the first molars in all groups (except CE-2) is shown in Fig. 6. In the AV group (Fig. 6A), root surface caries developed below the CEJ, and the crowns of the first molars were practically intact. On the other hand, in the SM (Fig. 6B) and AVSM (Fig. 6C) groups, severe and extensive destruction of the crowns was observed in almost all animals. In the groups SM and AVSM, root surface caries developed after the crowns were completely destroyed by caries coronoapically. The first units appearing below the CEJ were the most affected (Fig. 6B, and 6C). In the D2000 (Fig. 6D) and CE-2 groups, no carious lesions were found on the crowns

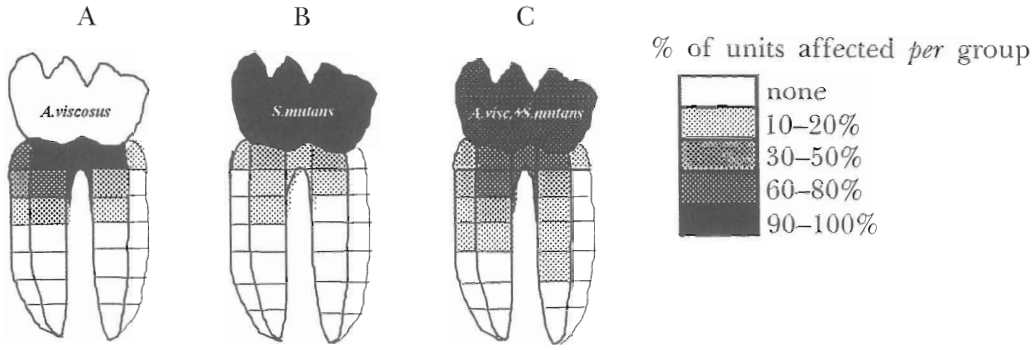

Fig. 10. Root surface caries on the lingual aspects of the first mandibular molars (left and right sides) after 24 weeks. (A=AV group; B=SM group; C=AVSM group)

or below the CEJ of the first molars.

Discussion

In studies on periodontal disease using golden hamsters, some researchers (Tsurumoto et al. [18] and Jordan and Keyes [19]) have reported the occurrence of root surface caries in animals fed a high-sucrose diet without being challenged with cariogenic bacteria. Nonetheless, it has been demonstrated that the presence of indigenous bacteria harboring in the oral environment of the animals after weaning is responsible for the occurrence of root surface caries in those animals (Jordan and Keyes [19]).

The mutans streptococci and sucrose have been strongly implicated in the induction of coronal caries and root surface caries (Tanzer [20]). Inoculation of gnotobiotic rats with mutans streptococci has been reported to induce not only coronal caries but also gingivitis, alveolar bone loss, and root surface caries. Whether hamsters and conventional or SPF rats also develop root surface caries after challenge with mutans streptococci is not yet clear because experiments have been ended before exposure of the root surfaces and the possible inception of root surface carious lesions (Tanzer [20]). Furthermore, *A. viscosus* has been cited, among the bacteria most importantly associated with root surface caries, although lactobacilli have also been implicated (Ellen et al. [21]).

Various studies have isolated *S. mutans* and *A. viscosus* from plaque overlying root surface caries on human teeth (Ellen et al. [22], Keltjens et al. [9]). It has also been demonstrated that strains of *A. viscosus* induce the development of root surface caries and periodontitis in experimental rodents (Stookey et al. [10], Jordan and Keyes [19]), and that the combination of *A. viscosus* and mutans streptococci induces severe coronal caries and root surface caries (Bowen et al. [23], Firestone et al. [24]). In a study on root surface caries in desalivated rats, Sipos et al. [25] reported that inoculation of *S. mutans* combined with *A. viscosus* resulted in greater numbers of root surface caries than did inoculation of *A. viscosus* alone. However, they did not report on the mono-infection of *S. mutans*.

Based on these reports, in order to test the suitability of two cariogenic bacteria associated with root surface caries to be used as a model for root surface caries in hamsters, the present study was conducted to investigate the development of root surface caries in golden hamsters infected with *S. mutans* or *A. viscosus* alone or in combination.

After each animal was killed, it was noted that in the hamsters' maxillary molars the levels of bone resorption were minimal; plaque did not accumulate and root surface carious lesions did not develop. In a study on root surface caries in rats, a similar finding on bone resorption in the maxillary molars of rats fed diet 2000 was reported by Doff et al. [26]. Thus, it was decided to assess only the root surfaces of the mandibular molars for any macroscopical alterations caused by plaque accumulation, alveolar bone loss, and root surface caries.

Root surface caries. In the present study, root surface caries developed in the animals of the three infected groups (AV, SM, and AVSM) in the presence of diet 2000 after 12 weeks. In the groups SM and AVSM, however, root surface carious lesions appeared after the crowns of the molars wee destroyed by coronal caries.

When using an animal model for root surface caries, it is desirable to investigate the occurrences of plaque accumulation, gingival recession, bone resorption resulting in the exposure of the root surfaces and subsequent root surface caries, to experimentally understand the evolution of the periodontal disease and root surface caries in the animals.

In this study, after 16, 20, and 24 weeks, all the animals mono-infected with *A. viscosus* (AV group) remarkably developed root surface caries. In the AV group, the carious lesions developed below the CEJ of the lingual aspects of the hamsters' first mandibular molars, and almost no destruction of the crowns by caries was observed.

The use of hamsters and the potential of these animals for studying root surface caries were reported by Stookey et al. [10]. They compared the effect of time and type of dietary carbohydrate upon the development of root surface caries in hamsters. Golden hamsters were infected with a strain of *A. viscosus* and fed diets rich in glucose or sucrose (diet 2000 and MIT 200 diet, respectively, 56% and 67% of sucrose) over a period of 18 weeks. Root surface caries was scored using a grid method; the results showed that the majority of the animals developed root surface caries after 12 weeks, and that bone loss and root surface caries increased from 12 to 18 weeks. After 18 weeks, all hamsters developed root surface caries. In the present study, the results for the AV group after 16 weeks were in line with those reported by Stookey et al. [10].

Firestone et al. [27], in a study on methods for assessing root surface caries in hamsters, divided the lingual surfaces of the roots of the first mandibular molars into 14 units. In the present study, the lingual and buccal surfaces of the roots of the first molars were divided into 15 units as described, including the furcation. Below the CEJ, it was observed that the furcations were affected by bone loss in all animals. The grid method used in this study was useful to identify which unit areas are more affected by root surface caries.

The results of the present study indicated that in the groups challenged with *S. mutans* (SM group) or a combination of *S. mutans* and *A. viscosus* (AVSM group) the crowns of the molars were severely destroyed by caries, and it was apparent that root surface caries developed only after the destruction of the crowns reached the CEJ and subsequently affected the roots. On the other hand, in the animals mono-infected with *A. viscosus* (AV group), only the root surfaces decayed and practically no destruction of the crowns by caries was observed.

It is known that *A. viscosus* are facultative anaerobes which colonize the hamster's periodontal tissues inducing peri-

odontitis and root surface caries (Irving et al. [28], Jordan and Keyes [19]). In presence of sucrose or other carbohydrates, *A. viscosus* has the ability to adhere and colonize the periodontal tissues (Syed et al. [29], Behbehani and Jordan [30]). These organisms appear to invade the gingival sulcus forming large amounts of plaque and to extend themselves on the root surface. After forming the plaque, they produce acids which attack the root surfaces (Jordan and Keyes [19], Behbehani and Jordan [30], Page and Schroeder [31]). Evidence suggests that root surface caries may differ in microbial etiology from caries in enamel; particularly, *A. viscosus* and *A. naeslundii* have been considered as the potential pathogens for the development of root surface caries (Syed et al. [29], Behbehani and Jordan [30]). No reports exist on potent coronal caries induction in rodents in response to *A. viscosus* infection alone (Tanzer [20]).

For root surface caries studies, the use of an animal model is important for understanding the patterns of development of root surface caries caused by specific pathogens. However, the infectants capable of provoking root surface caries without causing severe destruction of the crowns by coronal caries should also be considered. From the findings of the present study, it may be accepted that challenge with *A. viscosus* alone was sufficient and appropriate to induce root surface caries experimentally in golden hamsters.

Plaque accumulation. This study clearly demonstrated that plaque did not accumulate on the roots of the mandibular molars of animals in the uninfected groups D2000 and CE-2. Consequently, root surface caries did not develop on the molars of the animals in these two groups, either.

In the three infected groups (AV, SM, and AVSM), plaque accumulation was observed on the roots of the mandibular molars after 12 weeks. Furthermore, after 12 weeks, gingival recession with deposition of plaque was remarkable on the lingual aspects of the first mandibular molars.

Several reports on experimental periodontal disease using golden hamsters have shown that the responses to plaque vary in these animals when they are fed a high-sucrose diet (Miller and Ripley [32]). In a study on periodontal disease in golden hamsters, Takizawa [33] found marked deposition of plaque associated with gingival recession on the mesio-lingual aspects of the first mandibular molars after 15 weeks. After 50–52 weeks, the author reported that plaque deposition was much more increased and involved all mandibular molars. As in the present study, plaque deposition was remarkable on the mandibular molars rather than on the maxillary molars.

In the hamster, as well as in other rodents, morphological and physiological changes occur over time in their oral cavity, and there is an age-related epithelial downgrowth, which is more extensive on the lingual than on the buccal side of the mandibular molars (Page and Schroeder [31]). It has been demonstrated that a high-sucrose diet induces the rapid proliferation and overgrowth of particular bacteria, and it is the subsequent plaque accumulation that initiates and monitors the periodontal disease in rodents. After bacteria have covered the supragingival tooth surfaces, they invade the gingival sulcus by growing in an apical direction and end up reaching the junctional epithelium. As a consequence, the gingival tissue is displaced from the buccal and lingual surfaces as well as from the mesial surface of the first molars and the distal surface of the third molars (Page and Schroeder [31]). Furthermore, because a large diaste-

ma exists between the incisives and the first molars of the hamster, plaque accumulation resulting from food debris is likely to be increased in the mesial and lingual regions of the first mandibular molars.

Miller and Ripley [32] studied the periodontal disease in hamsters fed diet 2000 for six months, and periodically flushed the animals' oral cavity with broth cultures of *Actinomyces viscosus* during the experimental period. At five and six months, plaque accumulation was marked, and the presence of rounded pockets was very apparent, as shown in Fig. 3A of the present study.

An attempt was made to measure the amount of plaque accumulation (and coronal caries) on the molar crowns by means of a planimetric method (Takashima [15]). However, after 8 weeks, in the groups infected with *S. mutans*, it was impossible to define each surface of the molar crowns, because of extensive crown destruction by rampant caries. Thus, it was decided to evaluate only the plaque accumulated below the CEJ, as previously described in the present study.

No method to evaluate plaque accumulation on the root surfaces of rodents has been found in the literature. The grid method used in this study proved to be useful to evaluate plaque accumulation in the hamster model.

Alveolar bone loss. In the present study, it was found that alveolar bone loss occurred in all groups after 4 weeks and increased until 24 weeks. The levels of bone resorption did not differ significantly in the three infected groups during the entire experimental period. In the uninfected groups D2000 and CE-2, the levels of bone loss were less extensive than those of the infected groups. This finding is in accord with other reports (Crawford et al. [34], Coleman et al. [35]).

In a study on bone loss in gnotobiotic rats, Crawford et al. [34] reported that after about 60 days of challenge with either *S. mutans* or *A. viscosus*, or *A. naeslundii*, the infected animals generally showed patterns of bone loss similar to those seen in germfree animals, although the extent of loss was more marked in the former. They also reported that the major increases in bone loss (both horizontal and vertical) were observed in the period between 60 and 90 days.

In a study on periodontal disease in hamsters fed a high-sucrose from age 2–8 months, Saffar and Baron [36] reported that bone resorption in the hamster occurs with a different intensity at different sites of the alveolar bone. They concluded that the lingual region of the hamster mandibular molars are more strongly affected by bone loss than the buccal region. It should also be considered that greater amounts of plaque accumulation will contribute to an increase of alveolar bone loss. Thus, because of the increased plaque accumulation in the lingual region of the first mandibular molars (Page and Schroeder [31], Takizawa [33]), alveolar bone loss will possibly be increased in that region.

In this study, the lingual aspects of the roots of the first mandibular molars showed higher levels of alveolar bone loss than did the buccal surfaces. This finding is in accord with those of Crawford et al. [34]. Furthermore, the presence of *A. viscosus* caused extensive destruction of the alveolar bone in the AV and AVSM groups. A similar result was reported by Kavanagh et al. [37] for rats inoculated with *A. viscosus*. However, the factors involved in the alveolar bone loss observed in rodents are not completely understood (Crawford et al. [34]).

It was concluded that root surface caries developed after 12 weeks in the three infected groups (AV, SM, and

AVSM), and the lingual aspects (Fig. 1., mesial, lingual and distal surfaces) of the first mandibular molars were the most highly affected areas of the mandibular quadrants.

Apparently, when *S. mutans* was challenged alone (SM group) or in association with *A. viscosus* (AVSM group), the development of carious lesions on the roots was observed following severe destruction of the crowns by coronal caries. Therefore, it might be said that the real meaning of root surface caries did not apply in the presence of *S. mutans* (groups SM and AVSM). Moreover, if the purpose is to provoke root surface caries experimentally in golden hamsters, the appropriateness of challenge with *S. mutans* may be questioned.

On the other hand, when monoinfection with *A. viscosus* occurred (AV group), the plaque accumulation and alveolar bone loss were remarkable, and the percentages of root surface caries were the highest on the lingual surfaces below the CEJ of the first mandibular molars. Therefore, *A. viscosus* may be the inoculant of choice for root surface caries studies in the hamster.

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