Increased Incidence of Caries in Hamsters with Streptozotocin-Induced Diabetes

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Introduction

Resistance to infection decreases under diabetic conditions\(^{11,38}\). However, this has not been clear concerning the relationship between caries and diabetes. Cohen\(^{10}\) has reported that caries increase in dia-

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betics of various ages, and Wegner has reported that dental caries increase among diabetics. In an animal study, Sweeney et al., Borghelli et al., and Reuterving et al. have reported that dental caries increase in experimental diabetic rats. However Mattson et al., Albrecht et al., and Goteiner et al. have reported that the number of carious teeth is not different between diabetics and non-diabetics.

When we study the relationship between oral disease and diabetes, diabetes is generally induced in the experimental animals by streptozotocin (SZ) or alloxan (AX). In particular, it is important that the direct effects of SZ or AX on salivary glands are examined when we study the relationship between dental caries and diabetes using these animals. Generally, rats or hamsters are used in experiments concerning the incidence of dental caries. However, the functional and histopathological study of the salivary glands of animals with chemically induced diabetes awaits further clarification. Cutler has examined the necrosis of acinar cells in the submandibular gland in rats with SZ-induced diabetes. And Takai et al. have reported that the administration of SZ cause some histopathological change in the submandibular and sublingual gland of rats and a severe decrease in the flow rate of saliva. However, Anderson et al. and Hand et al. have reported that partial histological damage and partial monophological change have little effect on the function of salivary glands.

The purpose of this study was to examine the incidence of dental caries in hamsters with SZ-induced diabetes, the activity of salivary antibacterial factors such as lysozyme (LZ) and salivary peroxidase (SPO), the level of salivary glucose and lactate, and the dissolution of Ca2+ from dental enamel after demineralization in order to clarify the mechanism of the increased incidence of caries in diabetic hamsters.

**Materials and Methods**

**Experimental animals**

Male golden Syrian hamsters (Kyudo, Saga, Japan), aged 21-23 days, with a mean weight of 35 g were used in all the experiments. Under the experimental conditions, the animals had free access to a standard pellet diet and deionized water. Cariogenic diets containing sucrose (see below) were given to the animals in order to examine the incidence of caries.

**Induction of diabetes with SZ**

SZ (lot no. M6R7516; Nakarai Co. Kyoto, Japan) was dissolved at pH 4.5 (0.01 M citrate buffer) and given immediately as a single intraperitoneal administration of 65 mg/kg body weight. Control animals were given an equal volume of the buffer alone. Blood samples for glucose determination were collected in heparin-treated capillary tubes by orbital sinus puncture, and assays were carried out by the glucose oxidase method. Diabetes was considered to be manifest in hamsters with blood glucose levels of 300 mg/dl or above.

**Insulin treatment**

Four units of Lente insulin (Eli-Lilly Co., Indianapolis, Ind., U. S. A.) were given subcutaneously once a day to diabetic hamsters at 2 days after the administration of SZ and continued until the end of experiment.

**Incidence of caries in diabetic hamsters**

The fundamental protocol of the experiment is shown in Fig. 1. The animals received SZ, and at the same time, they were given deionized water containing penicillin G potassium 20,000 IU/l for 2 days. These animals were fed cariogenic diets containing various amounts of sucrose (56%, 28%, 14% and 7%)22). Growing cells of Streptococcus sobrinus strain K 1-R were intra-orally implanted twice on the 4th and 7th days of the experiment. At the 9th day, 2nd week and 8th week, the establishment
Fig. 1 Experimental designs employed to evaluate caries incidence of diabetic hamsters of *S. sobrinus* on the teeth of the animals was confirmed by the method of Keyes\(^{24}\)). Buccal surfaces of the upper molar teeth were swabbed with a sterilized cotton applicator stick. The swabs were placed into 2 ml of 0.15 M NaCl solution, and the suspension was mixed with a Vortex mixer for 1 min and serially diluted with 0.15 M NaCl. Aliquots (0.1 ml) of the dilution as well as aliquots of the original undiluted samples were cultured anaerobically on duplicate plates of Mitis-Salivarius agar (Difco Laboratories, Detroit, Mich., U.S.A.). The colonies were counted and recorded as colony-forming units per ml of the suspension. The experiment lasted for 58 days. The determination of the weight of the animals was made in order to ascertain the systemic condition of the animals. Following the required experimental period, the animals were killed under ether anesthesia, and maxilla and mandible were excised. We then evaluated the incidence of caries according to the method of Keyes\(^{22}\)).

Collection of saliva

Whole saliva was collected from animals by stimulation with pilocarpine-hydrochloride (8 mg/kg b. wt.; intramuscular; Wako Pure Chemical Industries Ltd., Osaka, Japan) 1 day before and 1, 3, and 5 weeks after the administration of SZ. After a 6-h fast, the animals were anesthetized intraperitoneally with sodium pentobarbital (40 mg/kg body weight; Abbott Laboratories, North Chicago, IL 60064 U. S. A.) and were fixed in a prone position. A fine plastic tube was inserted into the oral cavity and the outflowing saliva was collected in Pyrex glass tubes. These preparations were kept on ice for 60 min and then centrifuged at 5,000 rpm, 4°C for 15 min. The supernatant was stored at -30°C.

Assay of antibacterial activity

LZ activity was determined by the method of Osserman et al.\(^{33}\) with crystalline egg-white lysozyme (Sigma Chemical Co., St. Louis, Mo., U.S.A.) as a standard and *Micrococcus lysodeikticus* as a substrate (Sigma). SPO activity was determined by the pyrogallol method, essentially by the method of Pruitt et al.\(^{86}\). The activity was calculated from the initial rate of increase in absorbance at 400 nm (ΔA\(_{400}\)) with a model 100-10 spectrophotometer (Hitachi Ltd., Tokyo, Japan) equipped with a recorder and was expressed as ΔA\(_{400}\) per min. per ml of saliva.

Determination of the amount of glucose and lactate

The amount of glucose and lactate was determined by the enzyme electrode method\(^{24}\) with a glucose oxidase- and lactate oxidase-immobilized membrane (YSI Co., Ohio, U.S.A.) applied, using a YSI model 27 analyzer (Nikkaki Co., Tokyo, Japan).

Determination of the amount of Ca\(^{2+}\) dissolving from dental enamel

The upper and lower molars of hamsters with SZ-induced diabetes were excised and covered with nail varnish except the crown. These samples were soaked in 5 ml of the lactate buffer (0.1 M, 2.0 mM and 0.4 mM) for 15 min. After demineralization, the dissolved Ca\(^{2+}\) from the dental enamel was determined with a model 180-60 Polarized Zeeman Atomic Absorption Spectrophotometer (Hitachi Ltd.).

Statistical analysis

The data were expressed as the mean ± standard deviation, and they were statistically analyzed with Student's t-test or with the Chocran-Cox test.
Table 1 Influence of diabetes on caries increment in hamsters

<table>
<thead>
<tr>
<th>Exp. group</th>
<th>SZ</th>
<th>Sucrose content in the diet (%)</th>
<th>Inoculation</th>
<th>No. of animals</th>
<th>Body weight gain (g)</th>
<th>Blood glucose (mg/dl)</th>
<th>Caries incidence (per animal)</th>
<th>Recovery of strain K1-R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>0</td>
<td>+</td>
<td>6</td>
<td>103±6†</td>
<td>101±6†</td>
<td>4.7±0.3</td>
<td>0.35±0.1</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>9</td>
<td>21±5†</td>
<td>389±18‡</td>
<td>5.0±0.4</td>
<td>0.38±0.1</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>6</td>
<td>105±8‡</td>
<td>98±7‡</td>
<td>4.5±0.3</td>
<td>0.05±0.0</td>
</tr>
<tr>
<td>D</td>
<td>+</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>25±5‡</td>
<td>385±26‡</td>
<td>3.6±0.2</td>
<td>0.04±0.0</td>
</tr>
<tr>
<td>E</td>
<td>-</td>
<td>7</td>
<td>+</td>
<td>6</td>
<td>93±5‡</td>
<td>93±6</td>
<td>10.1±0.1</td>
<td>15.2±1.7</td>
</tr>
<tr>
<td>F</td>
<td>+</td>
<td>7</td>
<td>+</td>
<td>8</td>
<td>19±4‡</td>
<td>396±21‡</td>
<td>10.5±0.2</td>
<td>15.8±1.6</td>
</tr>
<tr>
<td>G</td>
<td>-</td>
<td>14</td>
<td>+</td>
<td>6</td>
<td>85±4‡</td>
<td>103±10</td>
<td>11.8±0.1</td>
<td>27.3±2.5</td>
</tr>
<tr>
<td>H</td>
<td>+</td>
<td>15</td>
<td>+</td>
<td>7</td>
<td>22±6†</td>
<td>411±29‡</td>
<td>12.0±0</td>
<td>29.5±4.3</td>
</tr>
<tr>
<td>I</td>
<td>-</td>
<td>28</td>
<td>+</td>
<td>6</td>
<td>86±3</td>
<td>97±5</td>
<td>12.0±0</td>
<td>69.0±3.3</td>
</tr>
<tr>
<td>J</td>
<td>+</td>
<td>28</td>
<td>+</td>
<td>8</td>
<td>21±3‡</td>
<td>401±42‡</td>
<td>12.0±0</td>
<td>81.6±5.2</td>
</tr>
<tr>
<td>K</td>
<td>-</td>
<td>56</td>
<td>+</td>
<td>6</td>
<td>94±4‡</td>
<td>102±4</td>
<td>12.0±0</td>
<td>83.8±6.3</td>
</tr>
<tr>
<td>L</td>
<td>+</td>
<td>56</td>
<td>+</td>
<td>7</td>
<td>20±3‡</td>
<td>409±34‡</td>
<td>12.0±0</td>
<td>272.5±14.3</td>
</tr>
<tr>
<td>M</td>
<td>-</td>
<td>56</td>
<td>-</td>
<td>6</td>
<td>94±4‡</td>
<td>95±6</td>
<td>6.8±0.3</td>
<td>7.2±0.8</td>
</tr>
<tr>
<td>N</td>
<td>+</td>
<td>56</td>
<td>-</td>
<td>7</td>
<td>20±4‡</td>
<td>408±36‡</td>
<td>7.0±0.6</td>
<td>7.8±0.7</td>
</tr>
</tbody>
</table>

1. Determined by Keyes’ method (Values are expressed as mean±S.D.)
   * p<0.01, ** p<0.001

Results

Diabetic status of hamsters

Hyperglycemia persisted throughout the experimental period following the administration of SZ. In particular, all the animals which developed hyperglycemia at the 2nd after administration of SZ remained hyperglycemic throughout the experimental period. The mean values of glucose levels in blood for diabetic and non-diabetic hamsters were 401±17 and 98±9 mg/dl, respectively. The weight of both diabetic and non-diabetic hamsters increased throughout the experimental period, but diabetic hamsters had significantly lower amounts of weight gain than non-diabetic hamsters.

Influence of SZ on the incidence of caries

Table 1 shows the blood glucose levels, the weight gain, and the mean number of carious tooth and caries score at the end of the experiment. The blood glucose levels in diabetic hamsters (groups B, D, F, H, J, L, and N) were significantly higher than in non-diabetic hamsters (groups A, C, E, G, I, K, and M) (p<0.001). The weight gain in diabetic hamsters was significantly lower than in non-diabetic hamsters (p<0.001). We found no significant difference between group A and group B, in the mean number of caries tooth, which was low. Both these groups were fed a standard diet. At the same time we found no significant difference between groups F, H, J, and L and groups E, G, I, and K in the mean number of caries tooth, which was high. However, the mean caries score in the diabetic hamsters (group J) was significantly higher than that in the non-diabetic hamsters (group I) (p<0.001), both of them having been fed a cariogenic diet (sucrose content: 56%). The mean caries score in the diabetic hamsters (group L) was significantly higher than that in the non-diabetic hamsters (group K) (p<0.01), both of them having been fed a cariogenic diet (sucrose content: 28%) (Fig. 2). Thus, a tendency towards severe caries incidence was found in the diabetic hamsters which were fed a high or moderate sucrose diet. On the other hand, we found no significant difference between group N and group M in the mean
Influence of diabetes on caries increment in hamsters

<table>
<thead>
<tr>
<th>Exp. group</th>
<th>SZ</th>
<th>Sucrose content in the diet (%)</th>
<th>Insulin</th>
<th>No. of animals</th>
<th>Body weight gain (g)</th>
<th>Blood glucose (mg/dl)</th>
<th>Caries incidence (per animal)</th>
<th>Recovery of strain K1-R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>−</td>
<td>0</td>
<td>−</td>
<td>6</td>
<td>101±5</td>
<td>98±9</td>
<td>4.8±0.3</td>
<td>0.41±0.06</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>0</td>
<td>−</td>
<td>6</td>
<td>20±2*</td>
<td>406±28*</td>
<td>4.9±0.4</td>
<td>0.44±0.08</td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>6</td>
<td>37±19*</td>
<td>194±87*</td>
<td>4.5±0.4</td>
<td>0.41±0.05</td>
</tr>
<tr>
<td>D</td>
<td>−</td>
<td>56</td>
<td>−</td>
<td>6</td>
<td>81±4</td>
<td>103±8</td>
<td>12.0±0.0</td>
<td>86.6±4.3</td>
</tr>
<tr>
<td>E</td>
<td>+</td>
<td>56</td>
<td>−</td>
<td>5</td>
<td>18±3*</td>
<td>411±32*</td>
<td>12.0±0.0</td>
<td>269.4±5.0</td>
</tr>
<tr>
<td>F</td>
<td>+</td>
<td>56</td>
<td>+</td>
<td>6</td>
<td>40±14*</td>
<td>182±72*</td>
<td>12.0±0.0</td>
<td>170.1±80</td>
</tr>
</tbody>
</table>

1. Determined by Keyes' method (Values are expressed as mean±S.D.)
2. Colony forming units: −: 0, ±: 1~50, 1+: 51~250, 2+: 251~50, 3+: 501~1000, 4+: 1000< * p<0.05, ** p<0.01, ***p<0.001

caries score, neither of which had been inoculated with S. sobrinus. Recovery of S. sobrinus strain K 1-R was not confirmed in the groups A and B, but it more or less confirmed in the groups E through L.

Influence of insulin on the incidence of caries

Table 2 shows the blood glucose levels, the weight gain and the mean number and caries score at the end of the experiment. The blood glucose levels in diabetic hamsters with insulin treatment (groups C and F) were significantly lower than in diabetic hamsters without insulin treatment (groups B and E) (p<0.01). The weight gain in diabetic hamsters with insulin treatment (groups C and F) was significantly higher than in diabetic hamsters without insulin treatment (groups B and E) (p<0.01). We found no significant difference in the mean number of caries lesions among the groups A, B, and C, all of them having been fed a standard diet. Similarly, we found no significant difference in the mean number of caries lesions among groups D, E, and F, all of them having been fed a cariogenic diet (sucrose content: 56%). However, the mean caries score in diabetic hamsters with insulin treatment (group F) was significantly lower than in diabetic hamsters without insulin treatment (group E) (p<0.05). In addition, the mean caries score in non-diabetic hamsters (group D) was significantly lower than in diabetic hamsters without insulin treatment (group E) and diabetic hamsters with insulin treatment (group F) (p<0.01 and p<0.05 respectively). Recovery of S. sobrinus strain K 1-R was confirmed in groups D, E and F. Thus, the tendency towards severe caries incidence decreased in the diabetic hamsters receiving insulin treatment.

Amounts of secretion and pH of saliva

The amount of secreted saliva is important for an estimation of the integrity of the function of the
Table 3 Lysozyme (LZ) activity in whole saliva obtained from diabetic hamsters

<table>
<thead>
<tr>
<th>SZ</th>
<th>Number of animals</th>
<th>Blood glucose (mg/dl)</th>
<th>LZ activity (µg/ml)</th>
<th>1w</th>
<th>2w</th>
<th>5w</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>6</td>
<td>101±10*</td>
<td>987±78</td>
<td>974±68*</td>
<td>980±66*</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>6</td>
<td>403±36</td>
<td>937±45</td>
<td>646±125*</td>
<td>487±60*</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. * p<0.001

Table 4 Salivary peroxidase (SPO) activity in whole saliva obtained from diabetic hamsters

<table>
<thead>
<tr>
<th>SZ</th>
<th>Number of animals</th>
<th>Blood glucose (mg/dl)</th>
<th>SPO activity (nA ml⁻¹ min⁻¹)</th>
<th>1w</th>
<th>3w</th>
<th>5w</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>6</td>
<td>101±10*</td>
<td>136±6.1</td>
<td>141±6.8</td>
<td>142±7.8*</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>6</td>
<td>403±36</td>
<td>133±6.2</td>
<td>136±9.2</td>
<td>88±12*</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. * p<0.001

salivary gland. If the salivary gland is exposed to toxic damage by SZ, the amount of saliva would markedly decrease. We found no significant difference between the diabetic hamsters and the non-diabetic ones concerning the amount and pH of saliva secreted throughout the experiment. The mean values for the total amount of saliva collected during 1 h from the diabetic hamsters and from the control hamsters were 1.21±0.31 and 1.24±0.23 ml/h/kg b. wt., respectively.

Activity of salivary lysozyme and peroxidase

The activity of salivary LZ is shown in Table 3. We found no significant difference between diabetic and non-diabetic hamsters in the activity of LZ at 1 week after administration. The activity of LZ in the diabetic hamsters showed a tendency to decrease 3 weeks after the injection of SZ, and levels of 50% lower than those in the controls were noted at 5 weeks (p<0.001).

The activity of SPO is shown in Table 4. We found no significant difference between diabetic and non-diabetic hamsters in the activity of SPO at 3 weeks after administration. But the activity of SPO in the diabetic hamsters showed a marked decrease at 5 weeks after the injection of SZ, and levels 40% lower than those in the non-diabetic hamsters were noted.

Levels of salivary and blood glucose

The levels of salivary and blood glucose are shown in Fig. 3a and Fig. 3b. In the diabetic hamsters, the levels of blood glucose temporarily increased at 2 hours after the injection of SZ, decreasing at 6 hours and gradually increasing again after 6 hours. On the other hand, in the diabetic hamsters, the levels of salivary glucose increased by 120% (p<0.001) and 180% (p<0.001) compared with the non-diabetic hamsters at 12 hours and 24 hours after the injection of SZ, respectively. Thus, the profile of the increase in salivary glucose was different from that of the increase in blood glucose. In other words, the salivary glucose increased after the increase in blood glucose. The marked increase in salivary glucose may influence the severe increase in caries incidence which was seen in the diabetic hamsters due to a great amount of salivary glucose being taken into the dental plaque and utilized as a substrate for acid production.

Levels of salivary and blood lactate

The levels of salivary and blood lactate are shown in Fig. 4a and Fig. 4b. In the diabetic hamsters, the levels of blood lactate gradually increased after the injection of SZ and the levels of blood lactate...
increased by 50% ($p<0.001$) and 70% ($p<0.001$) compared with non-diabetic hamsters at 12 hours and 24 hours after the injection of SZ. On the other hand, we found no significant difference between the diabetic and non-diabetic hamsters concerning the level of salivary lactate earlier than 24 hours after the injection of SZ, but in the diabetic hamsters, the levels of salivary lactate showed some increase after 24 hours, and at 7 days, the level of salivary lactate had increased by 280% ($p<0.001$) compared with that of the non-diabetic hamsters. The mean values of the level of salivary lactate of the diabetic hamsters and non-diabetic ones were 0.28 ± 0.1 mM and 1.8 ± 0.3 mM, respectively. The pH of the blood from the diabetic and non-diabetic hamsters was almost the same (pH 7.3 ± 0.1).

Ca$^{2+}$ dissolved from dental in diabetic hamsters

The amount of Ca$^{2+}$ dissolved from dental enamel are shown in Table 5. We found no significant differences between the diabetic hamsters and non-diabetic ones concerning the level of Ca$^{2+}$ at various concentrations in the lactate buffer. The higher the concentration of lactate buffer was, the higher the level of Ca$^{2+}$ was.

### Table 5 Influence of diabetes on dissolution of Ca$^{2+}$ (ppm) from dental enamel after demineralization

<table>
<thead>
<tr>
<th>Lactate buffer</th>
<th>0.1M</th>
<th>2.0mM</th>
<th>0.4mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 12</td>
<td>68.8±8.7</td>
<td>3.55±0.42</td>
<td>1.10±0.17</td>
</tr>
<tr>
<td>+ 12</td>
<td>71.3±9.5</td>
<td>3.60±0.51</td>
<td>1.13±0.14</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.D.
Discussion

AX or SZ has been generally used to establish the experimental diabetes models since it has been reported by Jacobs\textsuperscript{18} and Rakieten et al.\textsuperscript{37} both substances induce diabetes in animals. Since then, both have been indicated as excellent diabetogenic agents through histological and biochemical studies, and they have been used in many experiments related to diabetes\textsuperscript15. In particular, SZ has a higher specificity as a β-cytotoxic agent\textsuperscript{6,18,37}, induces Diabetes mellitus, and had a lower general toxicity than AX\textsuperscript{3,47}. Thus, in the diabetic condition induced by this agent there seem to be few complications due to toxicity. In this study, male golden Syrian hamsters, aged 3 weeks, were used in order to examine the influence of diabetes on caries incidence. It has been reported that the older the animal is, the lower the rate of incidence of caries becomes\textsuperscript{32,47}, and at the same time, the lower the establishment of mutans streptococci on the teeth becomes. We therefore used comparatively young animals in this experiment. Concerning the optimal doses and methods of administration of SZ to induce diabetes in the hamster, it has been reported that 6-week-old male golden hamsters receive triple i.p. administrations of SZ (50 mg/kg b.wt.)\textsuperscript{35} and 9-week-old male golden hamsters receive a single i.p. administration of SZ (50 mg/kg b.wt.)\textsuperscript{31}. Differences in species, sex, and age of the animals affect the optimal dose of SZ\textsuperscript{5,8,19,28}.

In particular, it seems that among the same animals, the younger the animals are, the lower the optimal dose of SZ\textsuperscript{28,41} is. In this study, we succeeded in creating by a single i.p. administration of SZ (65 mg/kg b.wt.) a diabetic model of a young hamster which maintained a high level of blood glucose over time.

We have recognized the selective destruction of pancreatic beta cells of the SZ-induced diabetic hamsters from histopathological observation\textsuperscript{30}. These changes are almost the same as those seen in other diabetic animals after injections of SZ\textsuperscript{19,28}. Generally, the rat and the hamster are used in experimental dental caries models, but a severe reduction in the secretion of saliva is observed in diabetic rats after injections of SZ\textsuperscript{40,42}, and the cause of the phenomenon is said to be the direct influence of SZ on the salivary glands of rats with SZ-induced diabetes\textsuperscript{49}. In our previous study, no histopathological findings have been observed in submaxillary and sublingual glands except for a slight enlargement of the parotid gland in diabetic hamsters after injection of SZ\textsuperscript{30}. In diabetics, a swelling of the parotid gland is often observed and it is said to be an enlargement without inflammatory change. Recently, Anderson\textsuperscript2 and Hand et al.\textsuperscript{17} reported that morphological changes of the parotid gland in diabetic rats arise from wide intra-cellular accumulation of lipids, but the changes do not influence the function of the salivary gland. Namely, if there are changes in the function of saliva in diabetic animals after the administration of a diabetogenic agent, they are not caused by the agent itself but they are by the characteristics of diabetes.

Moreover, the results from the experiment using hamsters seem to be more valid in a discussion of the mechanism behind the relationship between diabetes and caries incidence, because the molar of the hamster more closely resembles that of the human than does that of the rat, and there is hardly loss of enamel in the molar of the hamster.

Concerning dental caries, the three major factors such as microflora, host and teeth, and substrate are important, especially saliva is very important as host. We found no significant differences between the diabetic hamsters and non-diabetic ones concerning the amount of Ca\textsuperscript{2+} dissolving from dental enamel.

Concerning microflora, we could not record an exact plaque score for the diabetic hamsters due to severe destruction of tooth crown, but using a stereomicroscope we observed that there was a large quantity of plaque in the carious lesion of the diabetic hamsters (data not shown). It is not clear whether the hyperphagic tendency of diabetic hamsters is related to the increased incidence of caries seen in our
Concerning saliva, we observed a marked decrease of LZ and SPO activity in diabetic hamsters. LZ has bacteriolytic, bacteriocidal and bacterial agglutinative action and LZ is an important antibacterial enzyme in saliva. SPO catalyzes oxidation of thiocyanate (SCN⁻) by hydrogen peroxide into hypothiocyanate (OSCN⁻), which oxidizes bacterial enzymes containing the sensitive thiol groups. The SPO system has an inhibitory effect on the growth and acid production of a variety of microorganisms, including streptococci, fungi, and enteric bacteria, and may have a depressing effect on plaque formation in addition to its caries-reducing effect. The cause of an increase in incidence of caries in diabetic hamsters seems to be related to the decrease in LZ and SPO activity.

Concerning the level of salivary glucose, the results suggest that when the level of blood glucose exceeds the threshold, glucose above the normal range appears in the saliva, similar to the relationship between blood glucose and urinary glucose in the kidney. It is almost the same in the case of diabetic rats. The increase in the level of lactic acid in the blood is often found in diabetics who are under inadequate control of their blood glucose, and when serious diabetic metabolic acidosis, such as there have been no reports about the changes of salivary lactate level in diabetes, this is difficult from some reports concerning the effect of beverages on teeth, there seems to be strong demineralization of teeth. In our study, we found no difference between diabetic hamsters and non-diabetic hamsters concerning the pH of saliva in spite of the increase in the level of salivary lactate. It is perhaps due to the buffering capacity of saliva. Therefore, the increase in the level of salivary lactate might not affect directly the incidence of caries.

However, the influence of salivary lactate on microflora in plaque remains obscure. The results of our study may indicate which direction should be taken in future researchers to clarify the mechanisms of an increase in the incidence of caries in diabetic patients.

References

13) Fulop, M., Hoberman, H. D., Rascoff, J. H., Bonheim, N. A., Dreyer, N. P. and Tannenbaum, H.: Lactic acidosis in dia-


