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What is This?
Antioxidant Inhibition of Experimentally Induced Caries in Hamsters

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Dietary habits have long been under serious consideration to explain the differences in the incidence of dental caries found with one racial stock or one national group versus another. The number of reports concerning this problem are legion. It appeared to us that the widespread ingestion of fats could be fundamental to the low caries incidence of many peoples, especially primitive groups. As a result, studies were undertaken in our laboratories, beginning during February, 1956, which indicated that various types of fat had differing capacities to inhibit or prevent hamster caries induced experimentally by a high-carbohydrate diet. Of the many fats studied, pork fat, cocoa butter, and shortening* proved to be the most effective in reducing hamster caries, producing inhibitions as high as 70 per cent (unpublished experiments).

Several reports appeared previously in the literature that had a bearing on these observations. Rosebury and Karshan¹ have found that corn oil, olive oil, vegetable oil,† shortening,* and lard were effective in reducing the caries index in rats. Paraffin oil was without effect. McCollum² has studied the effect of whale oil in the diet. McClure³ has used various concentrations of vegetable oil and cod-liver oil on rats. King⁴ failed to find caries in 39 rats fed a finely ground maize diet with various additions of olive or cod-liver oil.

Of definite interest was another line of investigation. Seligman⁵ has found that the teeth of children with adiposogenital dystrophy showed a reduced caries susceptibility when compared with those of normal school children. The children studied ranged in age from seven to thirteen. Ninety-five per cent of the normal children demonstrated dental caries, while only 39 per cent of the obese children showed dental caries. Sixty-one per cent of the group had no caries whatsoever. No interproximal caries was noted. This syndrome is typified by an increase in blood lipids and a high sugar tolerance.

Thus the observations of Seligman led us to the belief that the fats consumed by hamsters inhibited dental caries through a systemic effect, in addition to a possible local effect. However, it was also concluded that the ingestion of fats by the hamster may have been incidental to the problem of caries, the fat being little more than a

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* Crisco, Procter and Gamble, Cincinnati, Ohio.
† Wesson Oil, Wesson Sales Co., Fullerton, California.
carrier or vehicle, and the real caries inhibitors or preventatives were "chemotherapeutic" substances that were ingested with the fats, either because they occur naturally in the fat or because they were added to, or produced in, the fat during the processing of foods. The latter idea led to a search for substances, preferably natural in origin, which homogeneously blend with fats, either naturally or man-induced and which would be non-toxic at the concentration levels to be employed. Since antioxidants are known substances that have long been used as additives to preserve foods and vitamins to extend their shelf life prior to human use, a search was made (soon after our fat findings) for compounds that could be put to the test for confirmation or denial of the above rationale.

This report summarizes a group of experiments that were conducted from 1958 through 1960. The effect of three antioxidants—propyl gallate, butylated hydroxyanisole, and nordihydroguaiaretic acid—on the reduction of experimental caries in hamsters is given. In addition, the effect of sodium azide and hydrogen peroxide as caries-inhibiting agents in the hamster is also included.

MATERIALS AND METHODS

Weanling hamsters,* bred in our vivarium, 24–28 days of age, were randomly selected for study from common litters by sex and divided into several groups, each containing approximately 20 males and 20 females. All the hamsters were weighed at the beginning of the study and examined to determine that they were caries-free.

In one experiment over a 50-day period, the first group, serving as the control group, was fed a normal hamster diet.† The second group was fed a caries-inducing diet composed mainly of carbohydrates having the following composition per hundred pounds: 52 per cent corn starch,‡ 10 per cent granulated sugar (sucrose), 10 per cent confectioners' sugar 10×, 1 per cent table salt (NaCl), and 27 per cent skim-milk powder. The third group was fed the identical diet as the second group, except that 0.03 per cent propyl gallate (PG) was added to the drinking water. The fourth group was fed a diet that was identical with that of the second group, except that 0.01 per cent butylated hydroxyanisole (BHA) was added directly to the diet. The fifth group was fed the identical diet as the second group, except that the drinking water contained 0.01 per cent nordihydroguaiaretic acid (NDGA)§ solubilized in alcohol prior to water solution.

In another experiment, over a 55-day period, the first group, control, was fed the normal diet and the second group, the high-carbohydrate, caries-inducing diet. The third and fourth groups also were fed the caries-inducing diet, except that 0.001 M sodium azide (NaN₃) was added to the drinking water of the third group and 3.0 per cent hydrogen peroxide (H₂O₂) in distilled water was substituted for the normal drinking water of the fourth group.

* Golden Nugget line, Golden Nugget Hamstry, Wayland, Massachusetts.
† Purina Laboratory Diet, Ralston Purina Company, St. Louis, Missouri.
‡ Buffalo Brand corn starch, Corn Products Refining Co., Chicago, Illinois.
§ NDGA is β, γ-dimethyl-α, Δ-bis (3, 4-dihydroxyphenol) butane.
Every other day during the course of the experiment, fresh water or experimental drinking solution was supplied to the animals. The animals were permitted to eat and drink ad libitum. They were also weighed every week during the course of the experiment and at the end of the experiment were examined for carious lesions.

RESULTS

Table 1 shows clearly the incidence of dental caries that resulted from the metabolic imbalance produced by the experimental high-carbohydrate diet (Group 2—weight loss) as compared with the normal non-caries-inducing diet (Group 1), and the reduction of caries when PG (Group 3), BHA (Group 4), and NDGA (Group 5) were ingested systemically. Other than the same weight loss seen in Group 2, receiving the experimental high-carbohydrate diet, PG, added to the drinking water, and BHA, added to the caries-inducing diet reduced the caries incidence by approximately 25 per cent in both males and females when calculated by the number of teeth involved. When calculated by the number of tooth surfaces involved, caries was inhibited in the males by 60 per cent for PG and BHA, and in the females by 36 and 44 per cent, respectively. In comparison, NDGA pronouncedly inhibited caries by 73–74 per cent in the males and females, when calculated by the number of teeth involved, and by 80–83 per cent, when calculated by the number of tooth surfaces involved.

Since each of the antioxidants inhibited experimentally induced hamster caries, we suspected that inhibitors of aerobic metabolism might also prove to be efficacious, provided that they were ingested systemically. Thus NaN₃, a known inhibitor of aerobic oxidative metabolism and considered a potent metabolic poison at 0.001 M concentration, and H₂O₂, an aerobic metabolic end-product that inhibits oxidative

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Additive (Per Cent)</th>
<th>Sex</th>
<th>Mean Carious Teeth</th>
<th>Mean Carious Tooth Surfaces</th>
<th>Reduc- tion of Carious Teeth (Per Cent)</th>
<th>Reduc- tion of Carious Tooth Surfaces (Per Cent)</th>
<th>Initial Weight</th>
<th>Final Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1...</td>
<td>Non-caries-inducing</td>
<td>0.00</td>
<td>M</td>
<td>18</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
<td>100.0</td>
<td>M: 48</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>F</td>
<td>19</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
<td>100.0</td>
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<td>2...</td>
<td>Caries-inducing</td>
<td>0.00</td>
<td>M</td>
<td>20</td>
<td>11.5</td>
<td>36.3</td>
<td>0.0</td>
<td>0.0</td>
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<td></td>
<td></td>
<td></td>
<td>F</td>
<td>20</td>
<td>9.1</td>
<td>21.2</td>
<td>0.0</td>
<td>0.0</td>
<td>M: 43</td>
</tr>
<tr>
<td>3...</td>
<td>Caries-inducing</td>
<td>0.03 PG</td>
<td>M</td>
<td>24</td>
<td>8.8</td>
<td>14.0</td>
<td>23.0</td>
<td>60.0</td>
<td>M: 43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>19</td>
<td>6.6</td>
<td>13.4</td>
<td>27.0</td>
<td>36.0</td>
<td>M: 46</td>
</tr>
<tr>
<td>4...</td>
<td>Caries-inducing</td>
<td>0.01 BHA</td>
<td>M</td>
<td>22</td>
<td>8.6</td>
<td>13.8</td>
<td>25.0</td>
<td>61.0</td>
<td>M: 43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>20</td>
<td>6.9</td>
<td>10.8</td>
<td>24.0</td>
<td>44.0</td>
<td>M: 46</td>
</tr>
<tr>
<td>5...</td>
<td>Caries-inducing</td>
<td>0.01 NDGA</td>
<td>M</td>
<td>23</td>
<td>3.1</td>
<td>6.1</td>
<td>73.0</td>
<td>83.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>19</td>
<td>2.3</td>
<td>4.2</td>
<td>74.0</td>
<td>80.0</td>
<td>M: 46</td>
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</table>
metabolism when it accumulates in excess, were added to drinking water of hamsters ingesting the caries-inducing diet.

Table 2 shows clearly the marked reduction of caries when NaN₃ or H₂O₂ were added to the diet. The NaN₃ inhibited caries by 99 per cent when quantitated on the basis of the total number of involved teeth or involved surfaces. The weight gain of the NaN₃ animals was similar to that of the animals receiving the caries-inducing diet alone. The fact that drinking a 0.001 M NaN₃ solution over a period of 55 days did not kill or hamper the growth of the hamsters indicates that the azide essentially interfered with the aerobic oxidative mechanisms involved in caries production at the local level only, the azide apparently having been detoxified at the systemic level. Where 3 per cent H₂O₂ was added the normal drinking water, the reduction of caries again was marked, yielding 100 per cent inhibition of the caries incidence. In addition, the animals fed H₂O₂ grew slightly better than the hamsters that were maintained on the high-carbohydrate, caries-inducing diet and water. We believe that the large peroxide concentration prevented caries production by overwhelmingly inhibiting key oxidative metabolic systems at the local level.

**DISCUSSION**

Our findings, compared with those of others,⁶-⁸ indicated that hamsters are metabolically different from Holtzman strain rats in their ability to handle H₂O₂;⁶ from Webster strain mice in their ability to handle PG;⁷ and from Sprague-Dawley rats in their ability to handle BHA and possibly NDGA.⁸ Shapiro, Brat, and Ershoff⁸ have found that rats on a “non-cariogenic” diet, but drinking a 1.0 or 1.5 per cent solution of H₂O₂, developed extensive caries lesions after 56 days. This is in contrast to our findings with hamsters using 3 per cent H₂O₂ in the drinking water for 55 days. Shapiro, Galpern, and Ershoff⁷ have found that PG enhanced destruction (caries) of the exposed dentin at the end of the molar cusps in mice receiving a normally non-

**TABLE 2**

**INHIBITION OF HAMSTER CARIES BY SODIUM AZIDE AND HYDROGEN PEROXIDE**

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Additive</th>
<th>Sex</th>
<th>No.</th>
<th>Mean Carious Teeth</th>
<th>Mean Carious Tooth Surfaces</th>
<th>Reduction of Carious Teeth (Per Cent)</th>
<th>Reduction of Carious Tooth Surfaces (Per Cent)</th>
<th>Initial Weight Range (R)</th>
<th>Final Weight Range (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Non-caries-inducing</td>
<td>0</td>
<td>M</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>R: 38-52</td>
<td>R: 92-121</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>M: 45</td>
<td>M: 116</td>
</tr>
<tr>
<td>2</td>
<td>Caries-inducing</td>
<td>0</td>
<td>M</td>
<td>20</td>
<td>8.90</td>
<td>19.60</td>
<td>0</td>
<td>0</td>
<td>R: 35-54</td>
<td>R: 67-90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>17</td>
<td>7.40</td>
<td>15.30</td>
<td>0</td>
<td>0</td>
<td>M: 41</td>
<td>M: 77</td>
</tr>
<tr>
<td>3</td>
<td>Caries-inducing</td>
<td>NaN₃</td>
<td>M</td>
<td>19</td>
<td>0.03</td>
<td>0.02</td>
<td>99</td>
<td>99</td>
<td>R: 44-56</td>
<td>R: 48-104</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>18</td>
<td>0.04</td>
<td>0.02</td>
<td>99</td>
<td>99</td>
<td>M: 50</td>
<td>M: 75</td>
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<tr>
<td>4</td>
<td>Caries-inducing</td>
<td>3% H₂O₂</td>
<td>M</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>R: 31-56</td>
<td>R: 66-112</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>M: 43</td>
<td>M: 86</td>
</tr>
</tbody>
</table>
cariogenic diet. Their work is in contrast to that of Jordan, Bowler, and Berger, who have observed that PG reduced rat caries initiated in the molar sulci and is in contrast to our findings, which showed that PG reduced the caries incidence in the teeth of hamsters maintained on a cariogenic diet. In addition, our findings that BHA partially reduced and NDGA markedly reduced hamster caries were in contrast to the negative results that Jordan et al. obtained for high concentrations of the antioxidants (0.5 per cent) in rats. The notable lack of response for NDGA observed by Jordan et al., in addition to possible species differences, may be explained by the fact that they added NDGA in the dry state to the diets of their experimental animals.

The strain of hamsters bred in our colony is from a line used in these laboratories for several years.* This strain seems to be sufficiently inbred to have produced a caries-susceptible animal. There have been occasions when some litters have been less susceptible. When this was found to be true, fecal transfer from highly susceptible animals was employed, and the caries-lesion production was enhanced as shown in the shorter time and increased involvement (45 days and more surfaces affected). In contrast, the caries-resistant animals obtained from other sources did not react to the fecal transfer. Shaw and Griffiths have shown with Harvard caries-susceptible and caries-resistant strains of rats that these attributes are genetically inherited characteristics. Keyes has indicated that transmissibility of certain bacterial strains, particularly those found in the susceptible animal, can cause caries in the so-called non-susceptible experimental animal. Our studies have indicated that the latter appears to be true only when the animals have some degree of known susceptibility response.

It is our opinion that the basic metabolism within the human oral cavity is a result of the interaction of bacterial and animal cellular components (cellular and tissue response) , causing dental breakdown among other possible, as yet unknown, systemic defects. Expressing this idea in terms of the hamster experiments reported herein, a metabolic defect with respect to the tooth is induced within hamsters by the high-carbohydrate diet, and this defect is partially corrected in the presence of PG and BHA, is almost completely corrected by NDGA, and is completely corrected by NaN₃ and H₂O₂. The question still to be resolved revolves around the similarity of the experimental animal and the human with respect to their disease response (dental caries). It is our opinion that the hamster responds quite similarly to humans.

The specific metabolic mechanisms that are affected by the antioxidants, especially NDGA, are a prime matter of concern. Some aspects dealing with the effect of NDGA upon human saliva metabolism and other aspects of its action, as determined by us, have been provided and referred to recently in a published report by Burk and Woods. In their studies they effectively established, in agreement with our human saliva metabolism findings, that NDGA is a potent metabolic inhibitor of the anaerobic and aerobic carbohydrate metabolism at animal cancer cells.

SUMMARY

Experimental caries induced in male and female hamsters maintained ad libitum on a high-carbohydrate diet were inhibited when 0.03 per cent propyl gallate (PG), 0.01 per cent nordihydroguaiaretic acid (NDGA), 0.001 M sodium azide (NaN₃),

* Golden Nugget line, Golden Nugget Hamstry, Wayland, Massachusetts.
and 3.0 per cent hydrogen peroxide (H$_2$O$_2$) were ingested ad libitum in the drinking water. 0.01 per cent butylated hydroxyanisole (BHA) added directly to the high-carbohydrate diet also inhibited caries. When calculated by the number of teeth involved, PG and BHA inhibited caries by 25 per cent, NDGA by 74 per cent, NaN$_3$ by 99 per cent, and H$_2$O$_2$ by 100 per cent in both males and females. When calculated by the number of tooth surfaces involved, PG and BHA inhibited caries by 60 per cent in the males and by 36 and 44 per cent, respectively, in the females; NDGA by 83 per cent in the males and 80 per cent in the females; NaN$_3$ and H$_2$O$_2$ by 99 per cent and 100 per cent, respectively, in both males and females. It is concluded that a metabolic defect with respect to the tooth is induced within hamsters maintained on the high-carbohydrate diet and that this defect is partially corrected in the presence of PG and BHA, is almost completely corrected by NDGA, and is completely corrected by NaN$_3$ and H$_2$O$_2$ at the concentrations and mode of administration employed for these reagents.

REFERENCES


