

## Cariogenicity of three foods in rats receiving essential nutrients by gastric intubation

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### Abstract

*The objective of this study was to determine the cariogenicity of 3 foods similar in texture, but varying in their sucrose concentration. The foods were evaluated in rats in which essential dietary nutrients were administered by gastric intubation. A modified Scow diet was used for the intubation procedure, administered twice daily over a 21-day experimental period. The test foods (Kellogg's Corn Flakes, Kellogg's Frosted Flakes, and Kroger's Vanilla Wafers), each were provided ad libitum to 3 groups containing 20 weanling Wistar (Harlan) rats each. The evaluation of sulcal caries was performed according to the methods of Keyes.*

*The vanilla wafers group containing 45% sucrose had significantly fewer carious lesions ( $9.4 \pm 1.4$ ) than did the presweetened corn flakes group ( $14.3 \pm 1.0$ ) containing 35% sucrose ( $p < 0.05$ ). The unsweetened corn flakes group ( $12.7 \pm 0.7$ ) containing 8% sucrose was not significantly different from the other 2 groups in caries scores ( $p > 0.05$ ). With regard to growth, the vanilla wafers group had a significantly higher weight gain than did the other 2 groups during the experimental period.*

Various methods have been used to predict the cariogenicity of human foods. These methods include human clinical studies and investigations of the following: food acid produced in incubated saliva, acid formation in dental plaque, food retention in the mouth, enamel decalcification by foods, in vitro caries, and animal caries. Each of these methods has its inherent strengths and weaknesses.

If practical recommendations are to be made so that consumers can choose foods that are less likely to produce caries, information must be obtained on the caries-producing potential of a great variety of sugar-

or starch-containing foods, especially the new types of presweetened foods which appear almost daily in the food market.<sup>1</sup> A fast, economical method of evaluating cariogenicity of foods is needed.

As a result of the widespread incrimination of sucrose,<sup>2</sup> the opinion sometimes has arisen that other sugar sweeteners such as fructose and high-fructose corn syrups are either noncariogenic or are significantly less cariogenic than sucrose. Recent investigations in primates have shown that mixtures of glucose and fructose are as cariogenic as sucrose and that little benefit would be derived by substituting fructose for sucrose. However, in a normal human diet, sucrose and other sugars per se seldom are consumed on their own and usually are ingested as an ingredient of food or applied directly to food by the user. Consequently, attempts have been made to avoid a total condemnation of sugar-containing products and to assess the cariogenic potential of each product on its own merit.

For ethical and pragmatic reasons, it is impossible to determine the cariogenicity of a wide range of food in humans. Likewise, although primates<sup>3</sup> give caries results which may be directly applicable to man, they are expensive, large, difficult to work with and thus are not appropriate for large-scale screening of the cariogenicity of foods. As a result, the rodent may be the most appropriate animal caries model in view of its small size, relatively low cost, and susceptibility to rapid development of caries.

With regard to the cariogenicity of foods, ready-to-eat breakfast cereals, especially the presweetened forms, have been suggested to have a high cariogenic potential on the basis of high sugar content and their retentive nature in the oral cavity. However, results are inconclusive and sometimes contradictory.<sup>4</sup>

Based on the results of past experience, it appears that the caries-producing capability of food could be evaluated better if it could be consumed as the only food item to pass through the oral cavity. Otherwise, the results of tests might be in response to other dietary factors rather than the specific food tested. Also, it has been recognized for several years that rats which consumed their entire diet by gastric intubation remain caries-free and can be kept in good health.<sup>3</sup>

This study evaluated the caries-producing capability of the following 3 specific food items when they were provided as the sole food to pass through the oral cavity of rats: unsweetened corn flakes cereal,<sup>a</sup> vanilla wafers,<sup>b</sup> and presweetened corn flakes cereal.<sup>c</sup>

## Methods and Materials

Sixty inbred male Wistar<sup>d</sup> rats 40-50 g were distributed randomly into 3 equal groups. All animals in each group were housed in pairs in sanitized stainless steel, raised-wire cages so that all animals of 1 group were together. The rats were maintained on a common commercial diet<sup>e</sup> and deionized drinking water ad libitum until they were 3 days old.

All animals were provided a modified Scow<sup>f</sup> diet administered by gastric intubation at 7 AM and 5 PM daily. The diet tubing apparatus consisted of a 10.0 ml Luer-lok syringe fitted with polyethylene tubing. Water was added to the dry diet daily in a ration of 1.3 g of water/1.0 g of diet.

The 3 groups and food items were as follows:

Group	Test Food Item
A	Corn flakes cereal
B	Vanilla wafers
C	Frosted Flakes sugar-coated cereal

Each group was provided with the test food item and deionized water throughout the study with the food item being removed 30 min before intubation to avoid excess foods and to ensure retention of the food in the stomach. All test foods were ground to the consistency of a powder before providing them to the

<sup>a</sup> Kellogg's Corn Flakes.

<sup>b</sup> Kroger's Vanilla Wafer.

<sup>c</sup> Kellogg's Frosted Flakes.

<sup>d</sup> Harlan Industries: Cumberland, IN.

<sup>e</sup> Wayne Laboratories, Allied Mills, Inc: Chicago, IL.

Component	Amount (%)
Vitamin-free casein	20.0
L-cystine	0.3
Dextrin	15.5
Vegetable oil	8.0
Vitamin diet	2.2
Fortification mixture	0.001

animals and the amount of food consumed daily by each group was determined.

The test period ended on Day 21. Each animal was weighed, sacrificed by chloroform inhalation, and then assigned a randomly selected and coded number.

Carious lesions were scored under a dissecting microscope (40x) by the method of Keyes.<sup>5</sup> Data were analyzed by a Bartlett chi-square test at  $p < 0.10$  to determine if variances were homogeneous. In cases where the variances were homogeneous, a 1-way ANOVA was performed to detect the overall significance of the difference.

In most cases where variances were not homogeneous, the Welch test was used to detect the overall significant differences. In cases where a significant "F" value was found, repeated *t* tests were used to determine the significance of the differences between group means.

## Results

The growth data for this study are summarized in Table 1. Initial mean weights of the rats randomly assigned to the 3 diet groups (unsweetened corn flakes cereal, vanilla wafers, and presweetened corn flakes cereal) ranged from  $47.9 \pm 0.9$  g to  $48.7 \pm 1.1$  g, a difference which is not statistically significant. After the 21-day intubation period, the mean weights ranged from  $74.0 \pm 1.9$  g for the corn flakes group to  $83.5 \pm 1.3$  g for the vanilla wafer group. The weight gain observed in the vanilla wafer group was significantly greater ( $p < 0.05$ ) than that present in the other 2 groups. There was no significant growth difference between the unsweetened and presweetened corn flakes groups (Table 1). Mortality was not a major problem in this study (Table 1). Three animals in the vanilla wafers group and 2 in the unsweetened corn flakes group died during the study. Two of the deaths appeared to be related directly to the intubation procedures, while 3 others were attributed to unknown causes.

The sulcal and proximal caries data from the sectioned jaws are summarized in Table 2. The mean number and severity of lesions were less for the vanilla wafers group. However, significant differences were found only in sulcal lesions and only between the vanilla wafers and presweetened corn flakes diet groups. Both the number and severity of lesions showed significant differences. Buccolingual lesions were not present.

## Discussion

The cariogenic potential of a food item is not related absolutely to the sucrose or total sugar content of that food.<sup>6-9</sup> Other factors present in foods may play an important role. For example, in an extensive

**TABLE 1.** Weight Gain and Percentage Mortality During 21-Day Intubation

Group	Food Product of Animals	Food Product	Mean Initial Wt. (grams)	Mean Weight Gain (grams)	Percentage Mortality
B	17	Vanilla Wafers	47.8 ± 0.8	83.5 ± 1.26	15
A	18	Corn Flakes	48.6 ± 1.13		
C	20	Frosted Flakes	48.7 ± 1.14		

<sup>a</sup> Means within brackets do not differ significantly ( $p > 0.05$ ) as determined by repeated *t*-tests.

**TABLE 2.** Caries Data

Group	Food Product	% Sucrose in Food	Number of Animals	Proximal Caries		Sulcal Caries		Total Caries Score	
				Number	Severity	Number	Severity	Number	Severity
B	Vanilla Wafers	45%	17	0.12 ± 0.12 <sup>a</sup>	0.18 ± 0.18 <sup>a</sup>	9.29 ± 1.40 <sup>a</sup>	18.65 ± 3.23 <sup>a</sup>	9.41 ± 1.4 <sup>a</sup>	18.8 ± 3.3 <sup>a</sup>
A	Corn Flakes	8%	18	0.28 ± 9.28	0.33 ± 0.33	12.44 ± 1.35	25.26 ± 3.64	12.72 ± 0.72	25.89 ± 3.8
C	Frosted Flakes	35%	20	0.25 ± 0.8	0.25 ± 0.2	14.05 ± 0.92	28.25 ± 2.52	14.3 ± 1.02	28.55 ± 2.61

<sup>a</sup> Means within brackets do not differ significantly ( $p > 0.05$ ) as determined by repeated *t*-tests.

in vitro study, Katz et al.<sup>10</sup> found that the cariogenic potential of cereals was not dependent primarily on their sugar content, the amount of sugar retained by teeth, or the amount of plaque formation. Rather, the only parameter that related well with the cariogenic potential of the cereals was their buffering capacity. The greater this buffering capacity, the lesser the cariogenicity potential.

Food retention may be influenced to some degree by the content of sugar or some other ingredient, as well as by the effect of the manufacturing process on physical consistency and retentive nature of the food in the mouth. The possibility also exists that in addition to the ingredients used, the mixing and cooking procedures may modify the cariogenic potential of a food. For example, processing the food removes minerals which may have a protective effect on teeth. These factors were studied in an experiment in which Grenby<sup>11</sup> compared 2 constituents of white bread: starch and gluten (protein). The starch content of bread usually ranges between 55 and 65%, with the gluten ranging from 7 to 10%. Gluten gives bread its cohesive texture and under certain circumstances can form a sticky, pasty mass with water when it is incorporated into the rat's diet. In comparing gluten with cooked wheat starch, Grenby found that gluten was less cariogenic than the starch when used alone.

Along the same line, Bibby et al.<sup>12</sup> demonstrated that laboratory cakes which were prepared with the incorporation of baking soda had a lighter texture than those lacking baking soda. He found that the baking soda reduced food retention. In the present study, the finding that the vanilla wafers gave lower caries scores than the unsweetened or presweetened corn flakes could be attributed to a lower retention value (which might be related to the presence of baking soda as an ingredient). Moreover, its fat content (lecithin-soybean) may reduce retentive ability and help reduce its cariogenicity in spite of the 45% sucrose content.

The importance of physical consistency of the diet with regard to caries-producing ability was recently cited by Stookey.<sup>13</sup> His study showed that the animals provided with a dry breakfast cereal had an average of 7.76 lesions, whereas animals provided with the same diet, but moistened with water were essentially caries free with a mean score of 0.06 lesions. In fact, 34 of the 36 animals provided with the moistened cereal were caries free. Stookey explained that the moistened diet resulted in decreased oral retention. It also is probable that much of the sucrose present in the diet was dissolved in the liquid phase of the moistened diet, thereby increasing the buffering capacity of the mixture.

Relating the animal findings to the human situation is difficult; it is doubtful if anyone eats breakfast cereal dry throughout the day every day of the week. If one were to attempt to apply findings obtained with rats in predicting the cariogenic potential of human foods, a more normal human type of diet should be used (e.g., cereal with milk). These considerations were discussed by Stookey.<sup>13</sup>

Agreement on the cause of a disease does not mean necessarily that a practical method for its prevention is at hand. Indeed, such agreement only points to areas in which further investigation is likely to produce practical results. This is the situation with respect to research concerning foods and their modification to prevent dental caries.

The ultimate practical application is to recommend that patients remove the more destructive foods (foods with refined carbohydrates and highly retentive natures) from their diet and substitute less destructive ones. Such dietary advice would be more likely to be followed by both children and their parents. In this manner, further progress could be made toward developing a practical method for controlling dental caries (such as providing snack foods high in protein and low in carbohydrate). However, a useful step would be taken if foods were listed or classified in some manner according to their caries-producing activity. The results of this study suggest that the method described in this study might be one appropriate means of compiling such a list.

## Summary and Conclusion

This study was designed to determine the relative cariogenicity of 3 different foods when they were the only food items to pass through the oral cavity. This was accomplished by administering an essential nutrient supplement by gastric intubation with only the test foods being provided ad libitum in food cups.

Sixty inbred Wistar male rats were divided randomly into 3 equal groups. All animals were provided a modified Scow diet (the nutrient supplement) twice daily at 7 AM and 5 PM. The 3 test foods were unsweetened corn flakes cereal, vanilla wafer cookies, and presweetened corn flake cereal. Each group of rats was provided the test food item and deionized water ad libitum throughout the study.

The test period ended on Day 21. The animals were sacrificed and mandibular and maxillary quadrants then were dissected free of soft tissues. The carious lesions were scored in each of the 3 test groups.

In evaluating the results of this study, the following conclusions were made:

1. The vanilla wafers group had a significantly higher growth rate than the other 2 groups ( $p < 0.05$ ).
2. Mortality was minimal during the test period.
3. There was a significant difference in caries scores between the vanilla wafers and presweetened corn flakes diet groups (the cereals were the most cariogenic).
4. No direct relationship was observed between the sucrose content of the test foods and the incidence of pit and fissure caries.

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