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Effects of glucose and sucrose variants of the caries-promoting Diet 2000 on the feeding patterns and parotid glands of prematurely weaned rats

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ABSTRACT

Objective: The hypothesis of this study was that feeding glucose instead of sucrose in the cariogenic Diet 2000 to rats weaned at age 18 days would result in greater light/dark differences in feeding activity and secretion and storage of parotid salivary enzymes.
Design of study: Diet 2000 and a stock commercial diet (controls) were prepared in pelleted and powdered forms, as the increased mastication required by pellets has been shown to support circadian rhythms in rats. Food jars were weighed at lights on and just prior to lights off daily. Rats were euthanized at 25 days and their parotid glands removed, weighed, and analyzed for specific activities of the salivary enzymes α-amylase and deoxyribonuclease I.
Results: Light/dark differences in feeding activity were strong in the rats fed the pelleted stock diet and both powdered and pelleted glucose 2000 diets, moderate with the pelleted sucrose 2000 diet, and not significant with the powdered sucrose 2000 and stock diets. Light/dark differences in the parotid salivary enzymes were strong with the powdered glucose 2000 diet and the pelleted forms of the glucose and sucrose 2000 and stock diets, and not significant with the powdered stock and sucrose 2000 diets.
Conclusion: Caries reportedly is higher in sucrose than glucose fed to rats in the standard powdered form of Diet 2000, mainly due to the colonizing advantage *Streptococcus mutans* gains with sucrose. These results suggest that additional factors are more feeding during lights on and less stimulation of parotid salivary secretion with the sucrose powder.
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1. Introduction

Standard caries-promoting diets were introduced to give dietary factors a more precise and reproducible role in caries investigations with laboratory animals.[1–3] Other improvements in the design of caries experiments have included oral inoculation with cariogenic microorganisms,[1,4,5] and beginning the caries challenge in rats as early as 18 days after birth.[5] The rationale for the latter practice is that the time until caries develops to validly measurable proportions is greatly shortened, as compared with starting with rats a week or more older. In most laboratory rats, the first and second molars erupt between 16 and 18 days after birth.[1] It was surmised that the molars are more susceptible to carious attack if the challenge is initiated during or shortly after eruption than after even a short

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period of exposure to saliva, during which additional minerals in the outer shell of enamel are converted from hydroxyapatite to fluorapatite crystals.[6,7] However, such premature weaning may cause long-lasting deviations in metabolism, feeding activity, and serum hormone levels.[8,9]

Much of the caries-promoting potential of the diets is dependent on the amount and kinds of carbohydrate they contain. By substituting different carbohydrates in the formulae and observing the resulting caries activity, it has been found that sucrose generally promotes more caries activity than others, including glucose, fructose and starch.[10–13] However, this differs with diet consistency, addition of sugars to drinking water, and animal and microbial species and strain.[14–16] This observation, together with studies indicating an important caries-promoting role for dietary sucrose in human beings,[17] focused research on the mechanisms involved. It has been amply demonstrated that colonization of teeth by *Streptococcus mutans* results in dental caries, and that in doing this *S. mutans* has an advantage over other bacteria by converting sucrose to polymers of glucose and fructose, forming elements of dental plaque that can be major factors in caries activity.[1,4,5,18–22]

The pelleted stock diets commonly fed to rats have been shown to promote and maintain parotid acinar maturation and the normal circadian cycles of feeding activity and secretion and storage of parotid secretory enzymes. When these and other diets are prepared as a liquid or powder, the light/dark differences in feeding activity and in parotid salivary enzymes are greatly diminished and there is atrophy or truncated maturation of the parotid acini.[23–31] These effects on the parotid gland have been attributed to the greater secretory stimulation associated with the mastication required by the pelleted diets.[32–37]

Experimental caries-promoting diets often are prepared as powders, and it has been shown that differences in feeding frequency and salivation can strongly affect caries activity.[38–40] Therefore, it seemed worthwhile to explore the possibility that different carbohydrate variants of Diet 2000 may vary sufficiently in their effects on circadian rhythms in feeding frequency and parotid secretory stimulation to account for some of the differences in their effects on caries activity when the rats have unrestricted access to food and water. Results of a preliminary study presented at the 62nd annual meeting of the International Association for Dental Research[41] indicated that glucose in Diet 2000 as powder and pellets promoted much stronger light/dark differences in feeding activity and parotid secretory enzymes than did sucrose in Diet 2000. The study was flawed in that accurate gland weights were not obtained and the consistency of the Diet 2000 sucrose pellets was too hard for rats weaned at 18 days, as it was not until age 21 days that they began to ingest this form at a satisfactory rate. Subsequently the study has been repeated with Diet 2000 with the flaws corrected. The preliminary results permitted a Hypothesis that the powdered glucose variant of Diet 2000 would promote stronger light/dark differences in feeding activity and parotid enzymes than would the powdered sucrose variant.

2. Materials and methods

2.1. Diets

Diet 2000[2] was prepared with the original sucrose and with glucose substituted for the sucrose, in two consistencies, the original, uniformly fine powder, and as pellets (Zeigler Brothers, Gardners, PA). The pellets were prepared by compressing the powder into moulds in a moist atmosphere. No ''glue'' or bonding agents were added. A commercial stock diet (Wayne Rodent Blox, Continental Grain Co., Chicago, IL) was fed to control groups in the standard pelleted form or ground into a powder. Thus, there were six dietary groups in all. All of the powdered diets were sifted through a mesh to prepare a uniformly fine powder free of lumps.

The compositions of Diet 2000 and Wayne Rodent Blox are listed in Table 1. The proportions of lactose, protein, fats and fibre in Diet 2000 were calculated from the proportions in the individual ingredients. For example, skim milk solids are 53% carbohydrate, almost all of which is lactose. Thus, 28% × 0.53 = ca. 15% lactose. The manufacturer of Wayne Rodent Blox listed the ingredients, but not their proportions, and the proportions of nutritional categories as 24% protein, 4% fat, and not less than 4% crude fibre). The 68% remainder therefore is carbohydrate, mostly starch from the cereals, essentially in

Table 1 – Composition of the experimental and control diets.

Diet 2000[a]		Stock diet	Food category	Estimated percent in	
Ingredients	Percent	Ingredients		Diet 2000	Stock Diet
Sucrose powder	56	Corn and Wheat Flakes	Sucrose	56	2
Skim milk powder	28	Ground corn	Lactose	15	0
Liver concentrate 1:20	1	Soybean meal	Glucose	0	1
Dry Yeast	4	Fish meal	Fructose	0	1
Sodium chloride	2	Wheat middlings	Starch/Glycogen	6	63
Whole wheat flour	6	Wheat Red Dog	Protein	16	24
Alfalfa meal	3	Dried whey	Fat	2	4
		Soybean oil	Fibre	3	4
		Animal liver meal	Minerals	2	1
		Cane molasses			
		Vitamins, Minerals			

[a] Stock Diet (Wayne Rodent Blox®) ingredients are as listed in the label. Proportions were not provided. Most of the carbohydrate is starch and glycogen. Sugars (from whey and molasses) are rough estimates.[2]