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Effect of calcium lactate in erosion and *S. mutans* in rats when added to Coca-Cola®

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Abstract

Thirty-six Sprague Dawley™ rats, 22 days of age, were divided randomly into three groups of 12 each and housed in a programmable feeder. The three experimental groups received either Coca-Cola® (CC), Coca-Cola with calcium lactate (CC-CaL), or distilled water. The programmable feeder was set to deliver 17 equal volumes of fluid per day with each feeding period lasting between 80-90 min. All groups were given Diet MIT 305 in one premeasured amount per 24 hr period (ad libitum). The pH of the CC with calcium lactate was adjusted to match the CC without calcium lactate by the addition of citric and phosphoric acids. The test period lasted five weeks. Each week, the food and fluid consumed and the weight gain were measured. Erosion of the teeth was scored by the method of Restarski et al. (1945).

ANOVA indicated that there was a significant difference in the amount of erosion among groups. A Newman-Keuls analysis showed that the mean erosion score of the CC group was significantly greater ($P < 0.05$) than that of the CC-CaL and distilled water groups (54.2 ± 0.12; 0.0275 ± 0.0213; 0.132 ± 0.070, respectively). There was no significant difference in erosion between the CC-CaL and distilled water groups. There was no difference in the amount of food and fluid consumed among the group of rats. In conclusion, calcium lactate added to CC resulted in significantly reduced tooth erosion in rats.

Presently, the amount of soft drinks consumed annually by individuals around the world is high. In Sweden, the mean intake per capita per day during 1981 was 0.08 L for carbonated beverages (Nordenvall and Wik 1981). In the United Kingdom, sales have risen from 2.8 billion cans of soft drinks in 1970 to 5.3 billion in 1984, with 65% of this increase being consumed by children and adolescents (Rugg-Gunn et al. 1984). Children aged 1 to 8 years increased their average consumption of soft drinks (Ritzcek and Jackson 1980). It also was reported that children 10 years and younger consumed a greater percentage of their soft drinks at meal time (National Soft Drink Association 1982). The second most popular time for consumption was between meals (Morgan et al. 1985). A considerable percentage of the total fluid consumed was sweetened carbonated soft drinks.

In recent years, the tendency among teenagers and children has been to replace milk (which constitutes the greatest single source of calcium) with soft drinks (Greenwood and Richardson 1979; Beal 1980; Marcus 1982). A decrease in milk consumption in conjunction with an increase in consumption of soft drinks and processed foods that are high in phosphorus has been suspected as having adverse effects on calcium absorption. This dietary habit may lead to potential health problems later in life, such as: accelerated bone loss resulting in generalized osteoporosis, alveolar bone loss, and periodontal involvement. Both children and parents are unaware of the harmful effect of acid-containing beverages on the teeth (Rugg-Gunn et al. 1984). Low pH beverages, although cleared rapidly from the oral cavity, cause dissolution and erosion of enamel (Asher and Reed 1987), especially of newly erupted teeth. Many in vitro and in vivo studies have shown that low pH beverages can cause enamel erosion (Ellis and Dwyer 1960; Takota 1971; McDonald et al. 1973; Gedalia et al. 1981). Reussner et al. (1975) and McDonald et al. (1973) have suggested that the addition of calcium phosphate to low pH beverages could suppress enamel demineralization in rats. Other investigators (McClure 1960; Shrestha et al. 1982; Van der Hoeven 1985) indicated that calcium lactate (CaL) is a potential anticariogenic food additive. Several authors have shown CaL to decrease enamel dissolution in vitro (Bibby and Weiss 1976; Mundorff and Bibby 1976). CaL is a tasteless, nontoxic, and fairly soluble compound. Therefore, the purpose of this study was to evaluate if the addition of CaL to Coca-Cola® (CC) (Coca-Cola Classic, regular, pre-mixed, Coca-Cola Company, Columbus, Ohio) in rats would decrease the amount of erosion in rat teeth.

Materials and Methods

Thirty-six Sprague Dawley™ rats, 22 days of age, were divided randomly into three groups of 12 each. Each animal's initial weight was recorded, and it was placed in an individual cage of a programmable feeding machine according to its assigned group. The animals in each group received one of the three test solutions: Group 1, double distilled water (DDH20); Group 2, Coca-Cola plus 5% calcium lactate (CC-CaL); Group 3, Coca-Cola (CC). All test solutions were analyzed for fluoride ion by using fluoride-specific electrodes. Coca-Cola was regular Coca-Cola and was obtained from the Coca-Cola Company in Columbus, Ohio. Titrable acidity was done for each solution with 0.1N NaOH to a pH of 7.0. The pH was determined using a pH meter connected to an automatic titrator. After adding CaL to CC, the pH was adjusted by adding equal amounts of citric and phosphoric acid to bring pH to 2.4, the same as the original CC pH. All solutions of CC and CC-CaL were made weekly. All animals were innoculated orally with S. mutans 6715 on days one, two, and three of the experiment. All groups were given Diet MIT 305 in one premeasured amount per 24 hr period (ad libitum). Diet MIT 305 contains 5% sucrose. Fluid was delivered in 17 equal volumes over 24 hr. Each drinking session was 80-90 min long. At the end of each 24 hr period, the amount of fluid left in each tube was measured. The difference between the total amount delivered and residual amount was assumed to be the amount consumed in milliliters. The amount of food was measured daily in grams. The animals were examined for signs of ill health.

The rats were sacrificed at the end of five weeks by CO2 inhalation. The hemijaws were removed by conventional procedures and stained with murexide (60 mg/100 ml in 70% ethanol). Tooth erosion was scored by the method of Restarski et al. (1945), and caries was recorded by the method of Keyes (1958). Enamel erosion is distinct from caries, although both processes destroy the teeth. Erosion of the molar teeth produced by ingestion of acidic beverage is characterized by a smooth wearing away of the entire enamel on the lingual surfaces (demonstrated in Fig 1). Caries affects occlusal and buccal areas as well as lingual surfaces. The areas affected are distinct lesions and do not destroy an entire surface in the initial stages. The surface of the tooth at the site of erosion remains relatively hard when compared to the decalcification (caries) caused by bacteria.

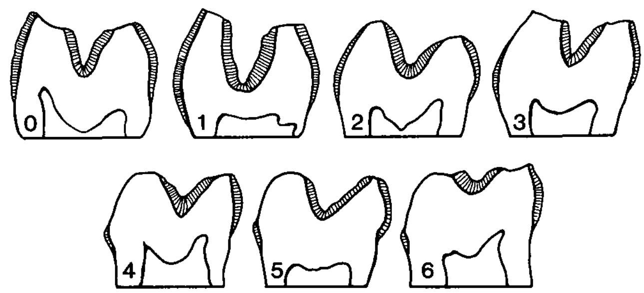

Fig 1. Rat molars showing various stages of destruction by acid beverages.

0. No effect.
1. High polish of lingual enamel.
2. Slight etching of lingual enamel.
3. Mild destruction of lingual enamel, with evidence of slight ridge formation at gingival margin.
4. Moderate destruction of lingual enamel, with ridging more definite and some exposure of dentin.
5. Severe destruction of lingual enamel, with marked ridge at gingival margin, and appreciable exposure of dentin.
6. Almost complete destruction of lingual enamel, with definite evidence of destruction on other surfaces and marked exposure of dentin with some destruction.

One of the mandibular jaws was used to determine the extent of a portion of the microbial population. Bone forceps were used to separate the molar teeth from the rest of the jaw, and the teeth were placed into 2 ml of 0.05% yeast extract. Sonication of the specimens was done for 15 sec on a setting of high and 300 watts (Biosonik IV, Bronwill VWR, San Francisco, California). The tenfold serial dilution of the microorganisms with yeast extract were plated on mitis-salivarius agar (MSA) for all specimens. The plates were incubated in an anaerobic chamber for 24 hr at 37°C, followed by aerobic incubation at 37°C for 24 hr. Total CFUs and CFUs for S. mutans were determined under 20x magnification. Colonial morphology was used to identify *S. mutans. S. mutans* type colonies on MSA are described as having a diameter from 0.5 to 1.0 mm, a highly convex elevation with a smooth or rough surface, undulating edges, light blue color, a finely granular internal structure, and frequently with a glistening drop of polysaccharide on top of the colony (Krasse 1966; Carlson 1967).

The data was analysed by one-way analysis of variance and the Newman-Keuls procedure. In order to stabilize variance, a log transformation was utilized.

Results

Mean weight gain for the groups ranged from 150.1 ± 3.62 to 152.4 ± 2.42 grams, and the mean daily fluid consumption ranged from 33.3 ± 0.86 to 35.0 ± 0.81 ml (Table 1, see next page). There was no significant difference in weight gain or fluid consumed among the

TABLE 1. Weight Gain and Daily Fluid Consumption (Mean ± SEM)

Group	N	*Mean Weight Gain (Grams)*	*Mean Daily Fluid Consumption (ml)*
DDH 0	12	150.1 ± 3.62	33.3 ± 0.86
Coca-Cola + CaL	12	152.4 2.42	35.0 0.81
Coca-Cola	12	150.9 ± 4.34	34.1 ± 0.61

Vertical lines indicate no significant difference ($P < 0.05$).

groups. Mean erosion scores ± standard error ranged from 0.027 ± 0.021 to 5.42 ± 0.127 (Fig 2). There was a significant ($P < 0.05$) reduction in erosion when Ca-CaL was compared to CC. Caries was not observed in any of the animals. Total and per cent recovery of *S. mutans* is shown in Table 2. There was no significant difference among the three groups ($P < 0.05$).

Discussion

The result of this experiment supports previous human and animal studies asserting that acid-containing beverages can cause enamel erosion (Levine 1973; Stookey et al. 1973; Birkhed 1984; Asher et al. 1987; Smith et al. 1988). It also has been suggested that the erosive effect of acid-containing drinks is due to the low pH (2.4 to 2.6) and not the sucrose content (Stookey et al. 1973). This effect is due to the quick clearance time of solutions and their minimum contact time with teeth. The total sugar content of Coca-Cola is about 9-10% (Martin-Villa et al. 1981a, b). A study by Spuller et al. (1988) demonstrated that Coca-Cola was highly cariogenic in rats even when 1 ppm F was added to the Coca-Cola. However, in our study, we found no caries. This discrepancy is probably due to a diet that contained 50% sucrose in Spuller et al. (1988), whereas the diet in our study contained only 5% sucrose. The rationale for using lower sucrose concentration in this study was to see if CaL was anticariogenic or antierosive without masking its effect with the high sugar content characteristic of previous studies (Spuller et al. 1988).

Although the influence of sugar concentration on dental caries remains unclear, animal experiments (Huxley 1977; Hefti and Schmid 1979) have shown that a sucrose concentration in the diet of about 15-20% or more results in high caries activity. While this is true in animals, human clinical studies have demonstrated that as low as 0.1-1% sucrose can cause a critical pH value in approximal dental plaque (Imfeld 1977). It seems that the amount of sugar in the diet is more critical in relation to caries than the amount of sugar in soft drinks. Therefore, if the diet of animals is low in sugar, the soft drinks, even though they contain 10% sugar, will have little effect on dental caries. Instead, they will cause erosion because of low pH (2.4 to 2.6).

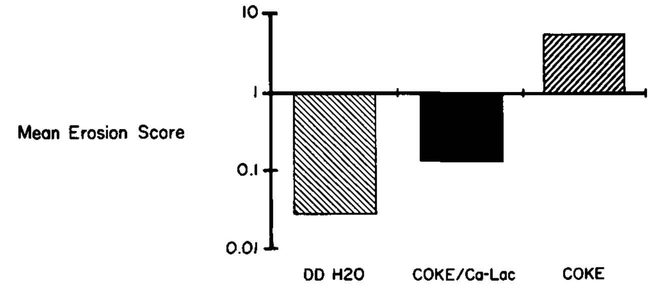

Fig 2. Mean erosion scores of rat molars (Log Scale).

TABLE 2. Mean Streptococcus Mutans Level by Percent of Total and CFU

Group	*% S. Mutans*	*S. Mutans No. × 10^5/Quadrant*
DDH 0	53.7 ± 5.91	4.16 ± 0.62
Coca-Cola	64.7 4.94	3.51 1.25
Coca-Cola + CaL	60.4 ± 3.94	2.97 ± 0.52

Vertical lines indicate no significant difference ($P > 0.05$).

The results of this study demonstrate that the addition of 5% calcium lactate to Coca-Cola significantly decreased erosion in rat molars. Whether this inhibiting action of CaL is due to the calcium ion or lactate, or both, is not clear, since no attempt has been made to determine the mechanism of action in this study. However, it seems likely that the effect was due mainly to reduction of enamel dissolution by calcium ion. After the addition of CaL to CC, the pH was adjusted to 2.4, the same as in CC. Still, erosion was inhibited, which suggests that CaL's action involves a remineralization process. CaL is rather soluble in acidic beverages and does not produce taste problems associated with other compounds. Dietary surveys have indicated that a number of children in the United States are obtaining suboptimal intake of calcium from their diets. Therefore, from a nutritional standpoint, calcium lactate would be more desirable than other compounds such as phosphate as a beverage supplement.

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Dental school enrollments down

First-year dental school enrollments are at their lowest level in two decades, according to a report released by the ADA Division of Educational Measurements, continuing a steady decline for the last 11 years.

First-year students numbered 4195, down 33.4% from the peak year of 1978, when the entering class numbered 6301. Despite overall declines, though, the number of women and minority students entering dental schools is rising, the report showed.

Women in 1988-89 accounted for one-third of the first-year dental school class, an increase of almost 30% over the past decade. Minority students account for 31% of the current entering class. In the past 10 years, the number of minority students has climbed 61%.