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Gastrointestinal Response and Plasma and Urine Determinations in Human Subjects Given Erythritol

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This study was undertaken to examine the influence of erythritol on certain plasma and urinary parameters and to assess the gastrointestinal response of humans given erythritol at single oral doses of 0.4 or 0.8 g/kg body wt/day. Three groups of six healthy volunteers each received a midmorning snack containing the equivalent of 0.4 or 0.8 g erythritol/kg body wt or 0.8 g sucrose/kg body wt. A fourth group received no snack and served as a negative control group. Consumption of erythritol did not affect plasma osmolarity, water consumption, or diuresis, and no significant variations in plasma or urine electrolyte balance were observed. Plasma glucose and insulin concentrations also were not affected by erythritol. Gastrointestinal responses to erythritol were comparable to those of sucrose. Plasma and urine erythritol concentrations increased within 2 hr of ingestion in proportion to the amount ingested. Approximately 60% of the erythritol dose was eliminated in the urine within 22 hr. The results of this study demonstrate that ingestion of erythritol at doses of up to 0.8 g/kg body wt does not alter plasma or urine osmolarity or electrolyte balance and is well tolerated by the digestive tract.

INTRODUCTION

Erythritol is a sugar alcohol (polyol) derived from D-glucose of wheat or maize. Its chemical formula is $C_4H_{10}O_4$ and it has a molecular weight of 122. The product is 99.9% pure and is chemically stable in both acidic and alkaline solutions. Erythritol is suitable for use in food and its chemical and physical characteristics make it particularly suitable for use in confectionary as a bulking agent and as a low-calorie sugar substitute (Goossens and Röper, 1994).

Previous studies in humans have shown that erythritol has 60 to 70% of the sweetening power of sucrose (Goossens and Röper, 1994). Its absorption by the small intestine has been reported to range between 60 and 90% (Höber and Höber, 1937; Lauwers *et al.*, 1985; Winne *et al.*, 1985, 1987; Oku and Noda, 1990; Bornet *et al.*, 1992, 1996; Hiele *et al.*, 1993; Noda *et al.*, 1994; Tetzloff *et al.*, 1996). Erythritol is not metabolized and is excreted unchanged in the urine (Oku and Noda, 1990; Hiele *et al.*, 1993), while unabsorbed erythritol may undergo fermentation by colonic microflora (Oku and Noda, 1990; INRA, unpublished report). In a companion study (Bornet *et al.*, 1996), no effect on plasma glucose or insulin levels was observed following ingestion of erythritol.

This study was undertaken to examine the influence of ingested erythritol on certain plasma and urine parameters and to further assess gastrointestinal responses.

MATERIALS AND METHODS

Subjects and Study Design

Twenty-four human volunteers consisting of 12 males and 12 females aged 20 to 46 years were studied. Table 1 shows the mean age, height, weight, body mass index (BMI), and fasting plasma glucose level for each of the study groups. Groups were homogenous with respect to BMI. Subjects included in the study were required to meet the following criteria: fasting plasma glucose level below 5.5 mmol/liter; normal plasma aspartate amino transferase (ASAT), alanine amino transferase (ALAT), alkaline phosphatase, γ-glutamyl transferase (GGT), plasma creatinine, and urea; negative HIV serology; serum cholesterol below 2 g/liter; serum triglycerides below 1 g/liter; and normal complete blood count and sedimentation rate. Subjects were excluded from the study if they were pregnant, had digestive or hepatic abnormalities, or had cardiac or renal abnormalities. The subjects were randomly divided into four groups of six individuals. Three of the groups were administered a snack containing 0.4 g erythritol/kg body wt/day (E4 group), 0.8 g erythritol/kg body wt/day (E8 group), or 0.8 g sucrose/kg body wt/

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TABLE 1
Physical Characteristics of Test Subjects

	Negative control group	Sucrose group	E4 group	E8 group
Number/sex	4M/2F	1M/5F	3M/3F	4M/2F
Mean age (years)	28.0 ± 8.3[a]	33.5 ± 8.2	26.7 ± 7.4	27.3 ± 9.2
Mean height (m)	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.84 ± 0.1
Mean weight (kg)	63.3 ± 11.5	57.3 ± 9.8	67.0 ± 13.3	69.0 ± 4.5
Mean BMI[b] (kg/m^2)	22.7 ± 3.1	22.5 ± 2.9	22.5 ± 2.6	22.0 ± 1.9
Mean plasma glucose (mmol/liter)	4.8 ± 0.4	4.7 ± 0.4	4.8 ± 0.4	4.8 ± 0.4

[a] Standard deviation.
[b] BMI, body mass index.

day (sucrose control group). The fourth group received no snack (negative control group). The study protocol was approved by the Comité Consultatif de la Protection des Personnes dans la Recherche Biomédicale, the ethics Committee of Hôtel-Dieu Hospital.

Materials

Erythritol (99.9% purity) was provided by Cerestar, Belgium.

Experimental Regimen

Two meals were served to the subjects on the day of the test: breakfast at 8:00 AM consisting of one croissant, one caffeine-free coffee or tea, and one 9-g sugar cube (meal composition: 2 g protein, 10 g lipids, and 27 g carbohydrates) and lunch at 12:45 PM consisting of one raw vegetable, one 100-g steak, french beans, 30 g cheese, one fruit, and 50 g bread (meal composition: 29 g protein, 33 g lipids, and 55 g carbohydrates). At 10:00 AM, the three test groups were given a milk chocolate snack consisting of 39% cocoa mass, 13% cocoa butter, 0.48% lecithin, vanilla to taste, and 47.5% of either erythritol or sucrose according to the group designation. Subjects in the E8 group received twice as much chocolate as those in the E4 group in order to achieve appropriate exposure to erythritol. Throughout the test day, subjects were given drinking water *ad libitum* and the total volume ingested was recorded.

Prior to the ingestion of the first meal, an indwelling venous catheter was fitted in each subject and left in place until 6:00 PM. Every hour between 8:00 AM and 6:00 PM, blood samples were collected for analysis of glucose, insulin, electrolytes (sodium, potassium, and chloride), creatinine, osmolarity, hematocrit, albumin, and erythritol. Every 2 hr, urine was collected for analysis of creatinine, electrolytes, osmolarity, urea, erythritol, *N*-acetyl glucosamine (NAG), and GGT. Between 6:00 PM and 8:00 AM the following morning, the subjects collected their urine and the above analyses were performed.

Plasma and urine samples were stored at −20°C until analysis. Plasma glucose was measured at Hôtel-Dieu Hospital (Service de diabétologie) immediately following sampling. Plasma insulin also was measured at Hôtel-Dieu Hospital (Service de diabétologie) following a 3-week storage period. Plasma and urine erythritol were measured at Cerestar Vilvoorde Center following 5 weeks of storage. All other plasma and urine parameters were analyzed at Necker Hospital (Department of Biochemistry) following 4 weeks of storage.

Determinations

Plasma glucose levels (expressed in mmol/liter) were measured by a glucose oxidase method (Beckman analyzer II). Plasma insulin levels (expressed in mU/liter) were determined using a radioimmunological assay with dextran–charcoal separation (intraassay variability = 6%). Plasma creatinine levels (expressed in μmol/liter) were measured using a kinetic colorimetric assay (Synchron CX3, Beckman). Plasma sodium, potassium, and chloride levels were measured using a Hitachi 717 automated chemistry analyzer. Plasma bicarbonates were determined using an enzymatic technique (Biomerieux kit). Albumin was measured using a colorimetric technique with bromocresol green. Urea was measured using an enzymatic technique (Urease, Biomerieux kit). Osmolarity was determined by measuring the freezing point depression.

Urinary levels of NAG and GGT were measured using a Boehringer kit and a Roche kit, respectively. The plasma erythritol concentration was determined using a high-pressure liquid chromatograph (HPLC) method with 1,3-butanediol as an internal standard (IS). 1,3-Butanediol has similar chromatographic properties to erythritol and was added to plasma samples before the deproteinization step. A 1/100 dilute solution of IS was originally prepared and the area under the HPLC curve measured under the conditions of the assay.

Plasma samples were centrifuged and analyzed in triplicate. One milliliter of the 1/100 dilution of IS was mixed with 3 ml of centrifuged plasma sample before the deproteinization step. Deproteinization was per-

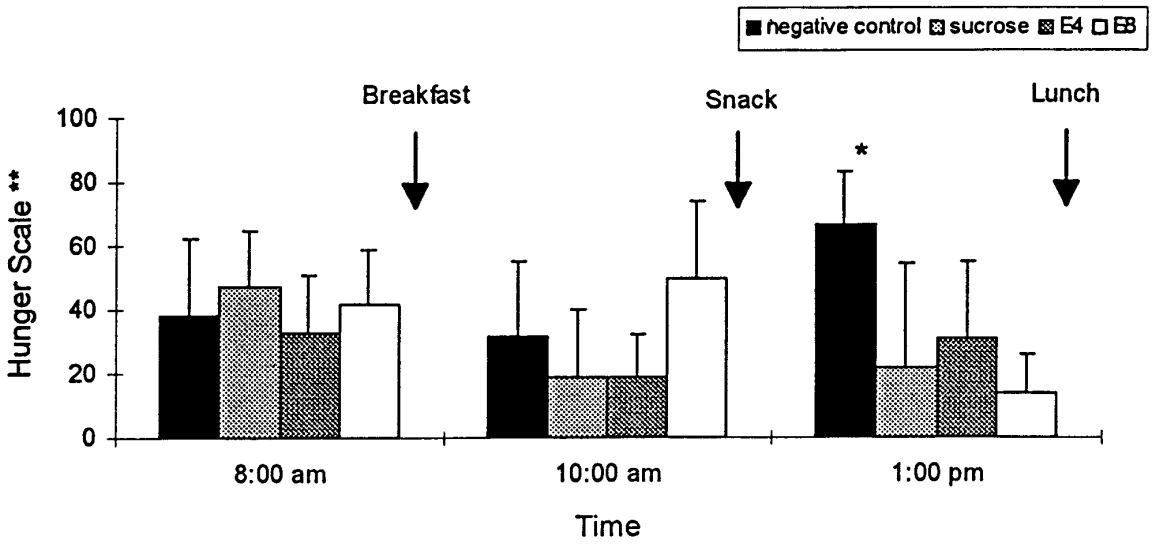

FIG. 1. Estimation of satiety prior to food intake by test subjects. *$P < 0.005$: difference between control and other groups. **An arbitrary scale ranging from 0 (no hunger) to 100 (extreme hunger).

formed by mixing, in a centrifuge tube, 3 ml of ice-cold perchloric acid (0.6 mol/liter) with the IS/plasma sample mixture. After centrifugation, 1 ml of potassium carbonate solution (0.75 mol/liter) was mixed with 3 ml of supernatant and centrifuged after 3 min in an ice bath. The supernatant was immediately frozen at −20°C for HPLC analysis at a later time. After thawing, the samples were centrifuged and the supernatant was decanted and put into HPLC vials. Erythritol was measured by means of HPLC using a Waters HPLC Solvent Delivery System M45 with a Waters HPLC Differential Refractive Index Detector R401 (Millipore Corp., Milford, MA). A Shodex Ionpack Column KC811 with an internal diameter of 8 mm and a length of 300 mm was used. The injected sample volume was 5 μl. The column operating temperature was 75°C and the flow rate was 1 ml/min. HPLC-grade water containing 0.0018 H_2SO_4 was used as the eluent.

Sample preparation and HPLC processing were designed to avoid manipulation errors, such as adding the IS before the deproteinization step. Based on the original amount of IS added, the recovery of the IS in the final sample was calculated. Also, the percentage recovery was applied to the analytically determined erythritol levels to compensate for manipulation errors.

A dilution factor of 2.60 was used to account for the dilution of samples which occurred during plasma separation and protein precipitation. Results were expressed as erythritol concentration (g/liter).

After thawing, urine samples were rehomogenized and filtered through a 0.45-μm filter. Samples of 0.5 ml were mixed with 0.25 ml of the IS before being placed in HPLC vials. The HPLC method was the same as described for the plasma erythritol determinations. Urine erythritol concentration was expressed as g/liter and erythritol output as g/min.

Satiety was evaluated before each meal using an arbitrary scale upon which the subjects recorded their sensation of hunger, ranging from no hunger (0) to extreme hunger (100).

The presence of digestive complaints was evaluated on the day following the test using a questionnaire similar to that used by Briet *et al.* (1995). Items on the questionnaire included abdominal pain, nausea, rumblings, bloating, flatulence, decrease in defecation, increase in defecation, soft feces, and hard feces. Each item was graded by the subject on a scale of 0 (no effect) to 3 (severe effect).

Statistical Analysis

Analytical results were analyzed by a variance analysis and the differences between test groups were analyzed by nonpaired test using ANOVA (Macintosh Statview). For the analysis of urinary electrolytes and NAG, if any treatment group-related effects were found to be statistically significant, pairwise comparisons of interest among the treatment groups were performed using the least significant difference. The NAG data were first log-transformed. The results from the questionnaire were analyzed by the nonparametric Kruskall–Wallis test.

RESULTS

Ingestion of erythritol did not affect voluntary intake of water. Over the study period, the mean cumulative water consumptions for the negative control, sucrose, E4, and E8 groups were 1.20 ± 0.37, 1.13 ± 0.15, 1.21

TABLE 2
Results of a Questionnaire on Gastrointestinal Responses[a]

Symptoms	Control	Sucrose	E4 group	E8 group
Abdominal pain	0	0	0	0
Nausea	0	2	2	6
Rumblings	1	1	2	3
Bloating	1	0	2	1
Flatulence	0	3	2	5
Decrease in defecation	0	0	1	0
Increase in defecation	0	0	1	0
Soft feces	2	1	4	1
Hard feces	1	0	3	2

[a] Values represent the sum of individual notations (0, 1, 2, or 3) for each test group. For each symptom, the minimum score could be 0 (0×6 subjects) and the maximum score 18 (3×6 subjects).

$\pm$ 0.33, and 1.11 $\pm$ 0.32 liters, respectively. There was no difference in the perception of hunger among the subjects receiving snacks containing erythritol and those receiving the snack containing sucrose (Fig. 1). The only significant difference ($P < 0.005$) in hunger perception was among groups receiving a snack and those which did not receive a snack (negative control group). Although gastrointestinal effects were reported more frequently in subjects in the E4 and E8 groups than in the subjects in the negative control or sucrose groups, these differences were not statistically significant (Table 2).

Plasma Determinations

Plasma glucose levels remained normal in all the test groups throughout the study period. Plasma insulin levels remained stable in the two erythritol groups, but were significantly increased in the sucrose control group at 1 and 2 hr following ingestion of the chocolate snack, as shown in Fig. 2. Plasma erythritol levels were proportional to the amount of erythritol consumed (Fig. 3). As expected, the plasma erythritol levels were higher in the E8 group ($P < 0.06$) than in the E4 group from 2 hr following ingestion to the end of the study. No significant variations in plasma osmolarity were observed among any of the test groups throughout the study period. Plasma calcium concentration also did not vary significantly among the test groups.

Urinary Determinations

Urine volume was not significantly different among the groups with the exception of the sucrose control group which showed a statistically significant increase during the period from 8 to 22 hr following snack ingestion when compared to that of the negative controls or E8 groups (Fig. 4). Erythritol appeared in the urine within 2 hr after ingestion of the erythritol-containing snack and was still detectable at 22 hr. The level of erythritol in the urine in the E8 group was approximately twice that of the E4 group ($P = 0.0001$; see Fig. 5). Over the entire study period (22 hr following snack ingestion), 61 and 62% of the ingested amount of erythritol were excreted in the urine in the E4 and E8 groups, respectively. Urinary sodium and chloride contents were significantly higher ($P \leqslant 0.05$) prior to and for up to 6 hr after ingestion of the snack in the erythritol groups and up to 4 hr in the sucrose control group when compared with the negative control group. Relative to the sucrose control group, these parameters were significantly ($P \leqslant 0.05$) increased in the period 2 to 4 hr following ingestion in the E8 group and during the period 4 to 6 hr following ingestion in both erythritol groups; however, over the entire study period these parameters were not significantly different between the sucrose control and erythritol groups. The urinary osmole excretion was significantly higher in the E8 group during the period 2 to 8 hr postsnack ingestion than in the negative control and sucrose control groups, as shown in Fig. 6. The osmoles excreted by the E4 group was significantly increased only in the period 4 to 6 hr postsnack ingestion (104 $\pm$ 26 mosm versus 51 $\pm$ 26 mosm for the sucrose control group). The total mean urinary osmole excretion during the study period was significantly higher ($P = 0.0001$) in the E4 (875 $\pm$ 92 mosm) and E8 (949 $\pm$ 129 mosm) groups than in the negative control group (510 $\pm$ 155 mosm).

Urinary NAG excretion was not significantly increased in either erythritol group during the study period when compared with the sucrose control or the no-snack control groups. Although GGT was measured in this study, the data are not reported, since the storage conditions for urine in this study have been shown to markedly affect the stability of this enzyme (Loeb, 1996).

DISCUSSION

It has been reported that consumption of excessive amounts of polyols and of slowly digested carbohydrates (e.g., lactose) can produce flatulence, abdominal cramping, or laxation as a result of osmotic influences or from the fermentation of unabsorbed carbohydrate in the large intestine (Charney and Bodurtha, 1981; Abraham *et al.*, 1981; Akerblom *et al.*, 1981; Koizumi *et al.*, 1983; Hyams, 1983; Hyams *et al.*, 1988; Corazza *et al.*, 1988; Saavedra and Perman, 1989). A portion of erythritol is incompletely absorbed from the gastrointestinal tract following oral administration and has the potential to elicit similar intestinal changes. In the present study, however, gastrointestinal effects in subjects ingesting erythritol at up to 0.8 g/kg body wt were not different from those of persons ingesting sucrose at similar levels. Similar results have been reported in other studies involving consumption of erythritol at

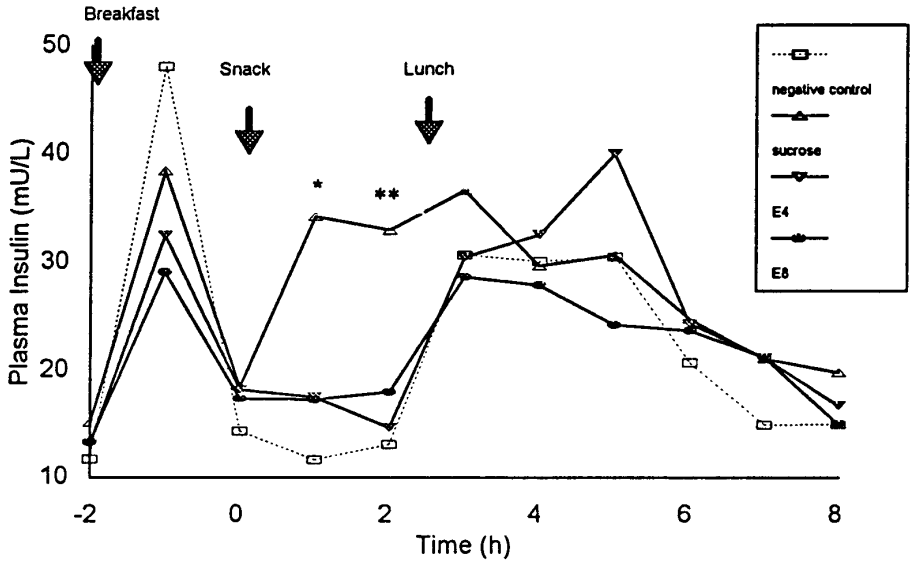

FIG. 2. Mean plasma insulin levels of test subjects. *$P < 0.001$: significant difference between control and sucrose groups. **$P < 0.05$: significant difference between control and sucrose group.

amounts up to 1 g/kg body wt (Hiele *et al.*, 1993; Noda *et al.*, 1994; Bornet *et al.*, 1996).

The systemic absorption of erythritol in this study was approximately 60%, based on urinary excretion, which is comparable to that observed in other studies involving human ingestion (Bornet *et al.*, 1992; Hiele *et al.*, 1993; Noda *et al.*, 1994). At the levels of exposure occurring in this study, no effects on serum insulin or glucose concentrations were observed relative to the negative control group, which also agrees with the results of previous studies in humans (Bornet *et al.*, 1996). The ingestion of sucrose in the present study produced the anticipated increase in serum insulin concentrations. Satiety was comparable between the sucrose and erythritol groups. The high degree of absorption of erythritol and its lack of effects on serum carbohydrate parameters indicate that this sweetener may have applications in foods for persons with diabetes.

This study also examined water and electrolyte balance in response to erythritol consumption. Under the experimental conditions, no changes were seen in the amount of water ingested or urine excreted following erythritol consumption relative to either the sucrose or negative controls. Plasma osmolarity and electrolyte

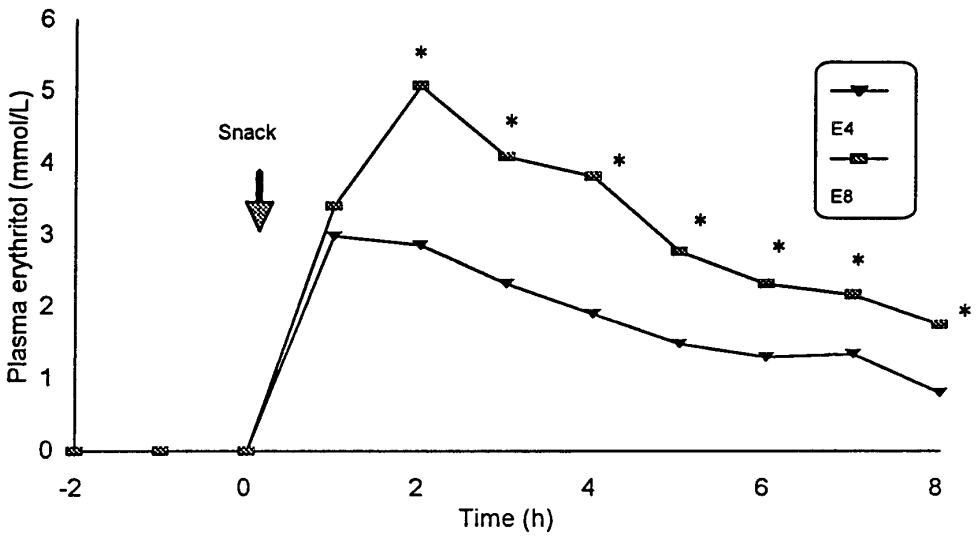

FIG. 3. Mean plasma erythritol levels of test subjects. *$P < 0.05$: significant difference between E4 and E8 groups.

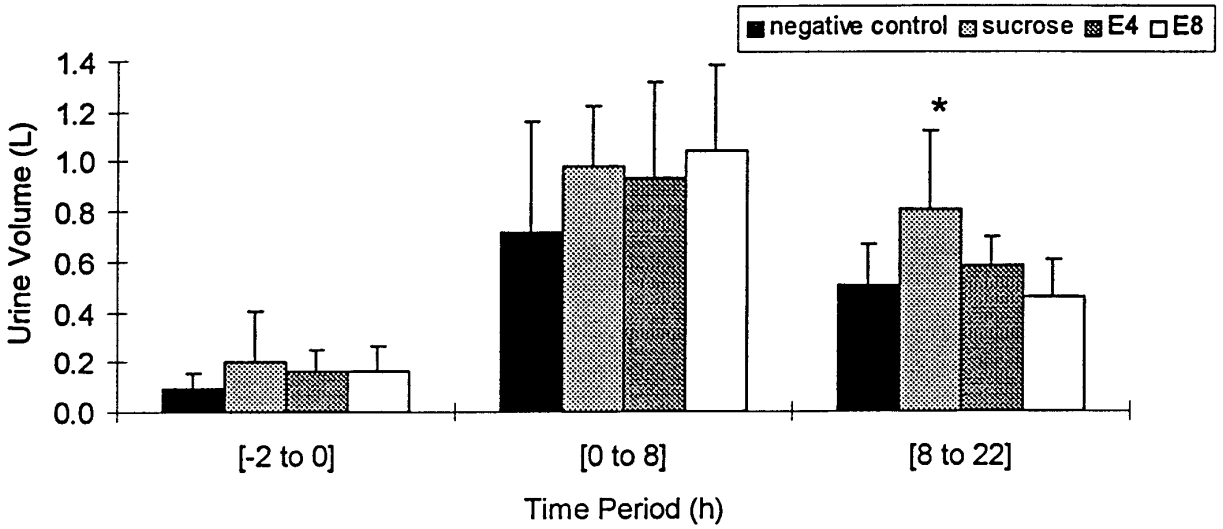

FIG. 4. Mean urine volume of test subjects. *$P < 0.05$: significantly different from control and E8 groups.

concentrations also were not affected by erythritol. Increases in urinary sodium were observed in the erythritol- and sucrose-fed groups relative to the negative control group, but these changes were not considered to be relevant to the safety of erythritol. No changes in urinary potassium or chloride excretion were observed. The slightly higher urine osmolarity observed in the erythritol-fed groups and sucrose-fed groups was considered to be due to the increase in urinary electrolytes. No effect on NAG excretion was observed in the erythritol and sucrose control groups. The lack of an effect on urine parameters in this study is in agreement with another study examining similar parameters (Noda *et al.*, 1994).

CONCLUSIONS

The results of the present study indicate that erythritol does not produce adverse gastrointestinal effects or alter water balance, osmolarity, or electrolyte balance following single oral doses of up to 0.8 mg/kg body wt. Also, erythritol did not affect serum insulin or glucose levels. The favorable systemic response to erythritol ingestion, combined with its lack of effect on carbohy-

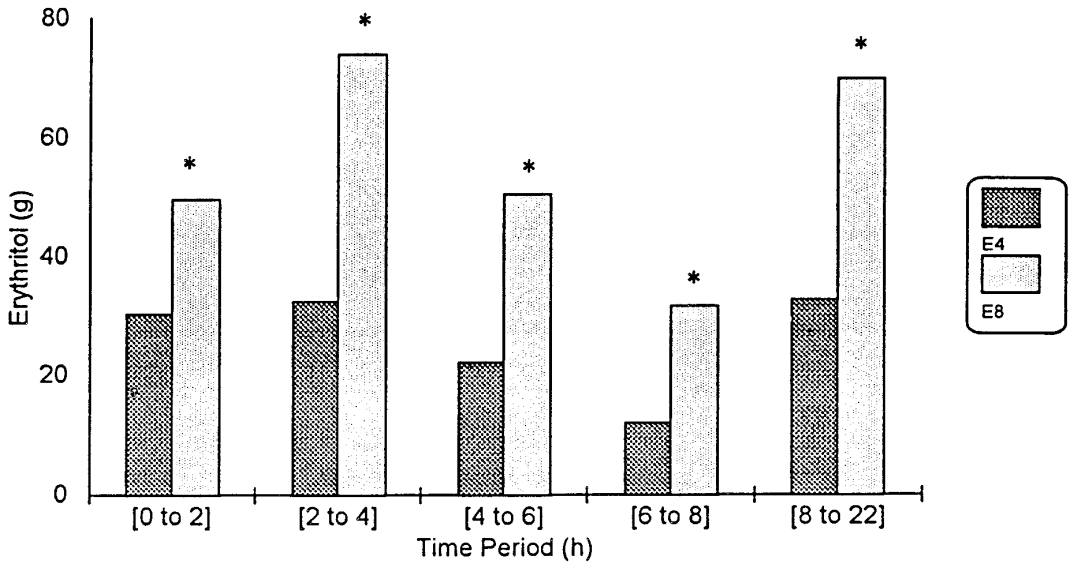

FIG. 5. Mean amount of erythritol excreted in the urine of test subjects. *$P < 0.0001$: significant difference between E4 and E8 groups.

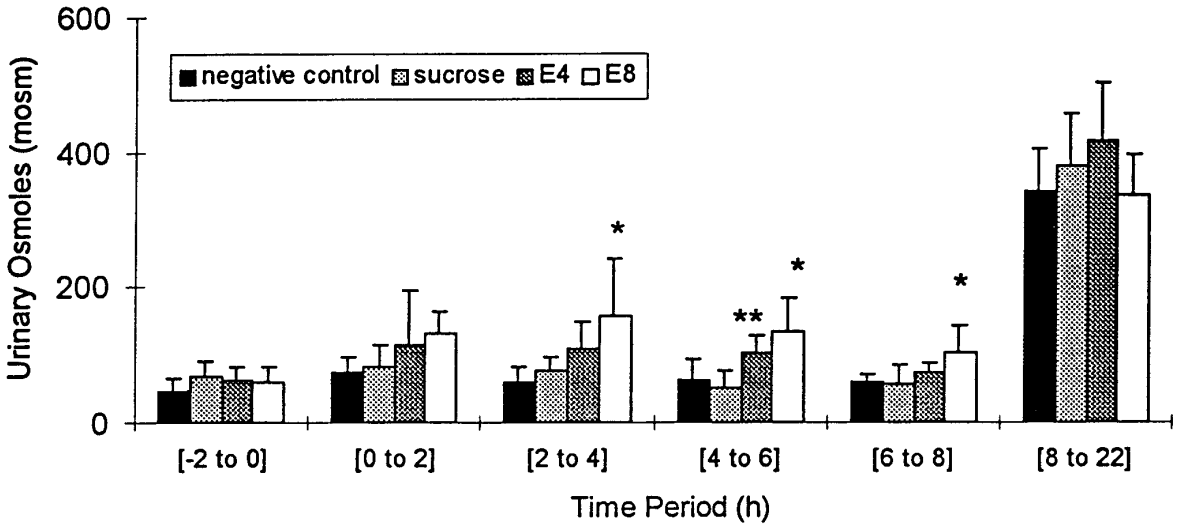

FIG. 6. Mean urinary osmole excretion of test subjects. *$P < 0.005$: significantly different from control and sucrose groups. **$P < 0.005$: significantly different from sucrose group.

drate metabolism, indicates that this sweetener could have applications in reduced calorie and diabetic foods.

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