

EFFECT OF MATERNAL ETHANOL INGESTION AT TWO DIETARY LEVELS<sup>1,2</sup>  
OF ZINC ON MOLAR COMPOSITION AND DENTAL CARIES OF RAT OFFSPRING<sup>1,2</sup>

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ABSTRACT

Pregnant rats were fed liquid diets containing either 2 or 10 µg Zn/ml with or without 30% of kcal from ethanol throughout gestation and lactation. At day 20 of lactation, three male rats from each litter were orally inoculated with *Streptococcus mutans* and fed a high sucrose-containing diet for six weeks. Maternal ethanol ingestion coinciding with offspring molar development significantly increased offspring dental caries scores and reduced the zinc content of enamel and dentin. Ethanol did not affect molar content of calcium, magnesium or phosphorus which suggests that ethanol is a specific antagonist of zinc utilization during molar development. Ethanol-antagonized zinc content of enamel may be responsible for the development of faulty enamel which has greater susceptibility to acid attack.

KEY WORDS: Ethanol, Alcohol, Zinc, Caries

INTRODUCTION

In a recently published report (1) we described the effects of feeding liquid diets containing 2 or 10 µg Zn/ml with or without 30% of kcal from ethanol throughout gestation and lactation in rats. Ethanol depressed zinc status of both dams and offspring typical of that produced by the low zinc-containing diet.

In the present study, we describe the effects of this interaction between ethanol and zinc on molar composition and subsequent susceptibility of offspring to dental caries during a post-weaning test period. There are several reasons for considering this study. Ethanol consumption during human pregnancy has been shown to produce offspring with smaller teeth and faulty enamel

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(2). These observations, however, were made under uncontrolled conditions. In the rat, first and second molar development coincides with gestation and lactation (3). At least part of the negative, developmental effect of ethanol on teeth may result from an antagonism of zinc utilization since a zinc deficit has been shown to increase dental caries in rats (4-6).

#### MATERIALS AND METHODS

Pregnant rats were fed liquid diets containing either 2 or 10  $\mu\text{g}$  Zn/ml with or without 30% of kcal from ethanol throughout gestation and lactation as previously described (1). Each of the four treatment groups were represented by four dams and their litter of eight pups. The liquid diet, which has been described in detail elsewhere (7), contained 69.75 g micropulverized casein, 0.5 g DL-methionine, 12.5 g vitamin mixture, 10 g mineral mixture, 10 g cellulose powder, 144 g dextrose, 13 g corn oil, 2 g xanthan gum and sufficient distilled-deionized water to make one liter. Ethanol was isocalorically substituted for dextrose so that all diets provided 1 kcal/ml. Body weights were taken at regular intervals to ensure adequate growth expected of a solid diet and to maintain similar caloric intake on a body weight basis. On day 20 of lactation, three male rats were selected from each of the sixteen litters, group-housed in polycarbonate cages, and fed a caries-promoting diet for six weeks. This diet contained 20% lactalbumin, 5% cellulose powder, 5% vitamin mixture, 3% mineral mixture, 4% corn oil, 10% cornstarch and 53% powdered sucrose. The detailed composition of the vitamin and mineral mixes has been previously published (6). Rats had free access to diet and distilled-deionized water during the caries-test period. The amount of surplus diet per jar however was restricted to 2-3 g over the actual intake, as recorded every two days, to reduce diet spillage. At the start of the caries-test period, each rat had three drops of a 24 hour stock culture of a cariogenic bacteria, *Streptococcus mutans* 6715-15 (American - Type Culture Collection, Rockville, MD) placed into their mouths. A one-hundred fold dilution of this culture was also used as drinking water for two successive days as suggested by Larson et al (8).

At the end of the six-week caries test period, rats were killed by guillotine under carbon dioxide anesthesia. Heads were steam-autoclaved for five minutes to facilitate removal of maxillary and mandibular molars. Enamel and dentin were obtained from maxillary molars, pooled within litters, by the method of Gilda (9). Mandibular molars were stained with murexide for caries score determination on the right and left buccal surface of first and second molars (10). Lesions were evaluated by one of us (FLC) without knowledge of the true sample identity. Our report of caries scores only involves the buccal enamel surface rather than a more rigorous description of all possible surfaces, since the greatest percentage of any treatment differences are expected on this surface (10).

After caries scores were recorded, right mandibular molars were ground to the midline from the lingual side with a separating disc (S.S. White, Philadelphia, PA). The disc was held in a straight handpiece (Doriot type, Schein, Inc., Port Washington, NY) which was driven by a dental engine (Teledyne Emesco Model No. 90N, Englewood, NJ). Teeth were acid-etched for ten seconds with 1.5 N hydrochloric acid, rinsed alternately with distilled water and 0.1 M sodium hydroxide several times, and dried with acetone prior to sample preparation for scanning electron microscopy (SEM). For SEM, the etched molars were mounted on aluminum planchets and rotary coated with approximately 100 Å of 60:40 gold/palladium in a Varian VE-10 vacuum evaporator (Varian, Palo Alto, CA). Enamel on the distal side of the middle cusp of the second mandibular molar was examined with an Amray model 1000-A SEM (Amray Inc., Bedford, MA). Both site and instrument parameters were kept constant for all samples. Images

were recorded on Polaroid type 55 film. Teeth were coded during both the acid-etching procedure and SEM preparation so that true sample identity was not known until processing was complete.

Enamel and dentin were analyzed for zinc, calcium, and magnesium by atomic absorption spectrophotometry (Perkin-Elmer Model 403, Norwalk, CT). For calcium determination, the final dilution was made with 0.1% lanthanum to remove interference by phosphorus. Phosphorus was measured by a colorimetric procedure (11).

The experimental design involved two factors, ethanol and zinc, with two levels of each factor. Data were analyzed based upon a 2 x 2 factorial experiment with 4 observations per treatment (12). The three offspring saved from each litter were considered to be a single observation. Effects of ethanol, zinc and the interaction of these factors were considered to be significant at  $P < 0.05$ .

### RESULTS

Offspring Food Intake and Growth. Food intake was remarkably similar for all rat offspring during the caries-test period (19-21 g/rat/d). Diet spillage was not a problem under our conditions although there was some diet loss due to the fact that the sucrose component of the diet was powdered. Offspring body weights were not significantly different at either the beginning or end of the caries-test period (Table 1).

Molar Zinc and Dental Caries Scores. Maternal ethanol ingestion significantly reduced the zinc concentration of offspring enamel and dentin for both first and second molars (Table 1). Low maternal dietary zinc intake significantly reduced zinc concentration of dentin, but not of enamel. An interaction between ethanol and zinc was found for first molar dentin zinc concentration. Dental caries scores were significantly greater for offspring originating from ethanol-fed dams (Table 1). Low maternal dietary zinc intake also increased dental caries scores, but only for dentin.

Scanning Electron Micrographs of Enamel. Scanning electron micrographs of acid-etched sections of offspring second mandibular molar enamel are shown in Figure 1. Each micrograph represents a visual average of a treatment group. Dissolution of enamel prisms and interprismatic substance was observed in all of the rodent enamel. However, there was less dissolution of rodent enamel structure of rat offspring originating from dams fed the high zinc-containing diet without ethanol (photograph A) compared to rats originating from dams fed diets containing either ethanol or low zinc (photographs B, C, D).

Other Mineral Analyses. The concentration of calcium, phosphorus and magnesium of offspring maxillary molars were unaffected by maternal ethanol ingestion. Analyses for these minerals were similar to those previously reported (13).

### DISCUSSION

We conclude that maternal ethanol ingestion by rats is detrimental to dental health of offspring which is in agreement with uncontrolled clinical observations (2). Our rats were only exposed to ethanol during gestation and lactation which coincides with the development of first and second molars (3). Furthermore, our level of maternal ethanol ingestion cannot be considered to be unrealistic in human terms. If an adult consumed 2400 kcal/day and 30% of this energy was derived from ethanol, it would mean that this person consumed about

TABLE 1  
Body Weight, Molar Zinc Concentration and Dental Caries Score of Offspring\*

Measure	Maternal Diet, % kcal from ethanol				Significance Levels		
	10 µg Zn/ml		2 µg Zn/ml		Ethanol	Zn	Ethanol + Zn
	0	30	0	30			
Body weight, g							
weaning	48±3	45±3	44±2	43±2	NS†	NS	NS
6-months Post-weaning	286±28	284±30	271±3	277±12	NS	NS	NS
Enamel Zn, µg/g dry wt							
1st molar	53.0±1.4	45.2±2.0	50.6±3.6	43.5±1.6	P<0.005	NS	NS
2nd molar	65.7±0.8	57.2±6.2	60.5±4.6	55.7±5.7	P<0.025	NS	NS
Dentin Zn, µg/g dry wt.							
1st molar	228±1	204±8	216±3	202±4	P<0.005	P<0.01	P<0.01
2nd molar	222±4	204±4	211±6	197±2	P<0.005	P<0.005	NS
Dental Caries Score							
Enamel	6.8±1.0	11.0±2.6	8.5±1.9	11.3±2.6	P<0.01	NS	NS
Dentin slight	4.8±0.5	8.8±1.0	7.0±0.8	10.5±1.7	P<0.005	P<0.005	NS

\* Values are mean±SD (n=4).

† Not significant.

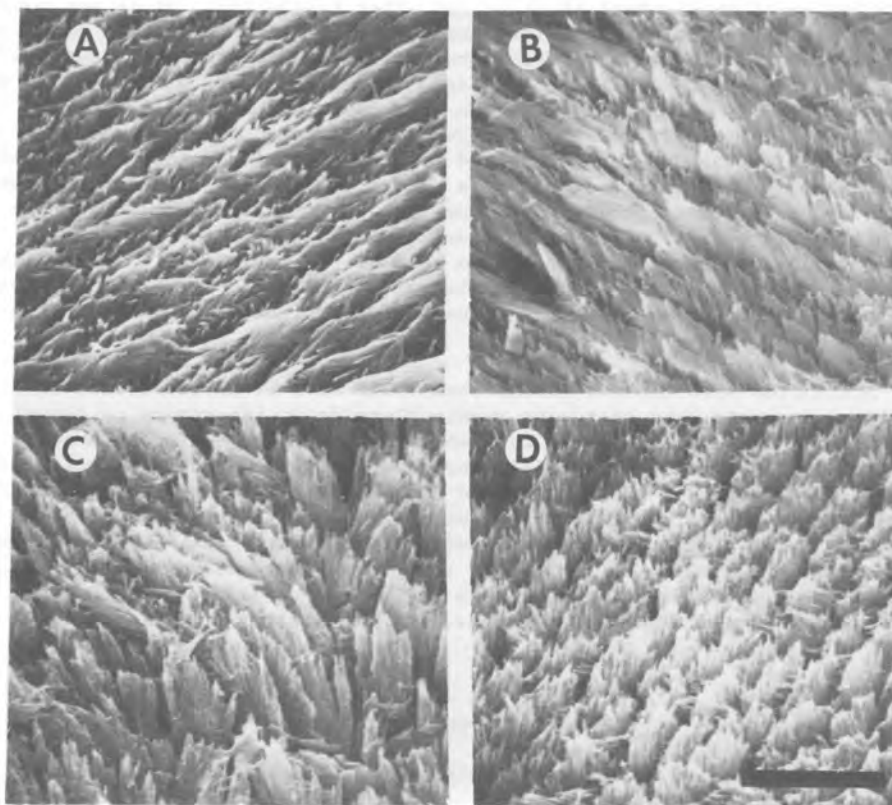


FIG. 1

Scanning electron micrographs of acid-etched offspring second mandibular molar enamel. (A) high dietary zinc without ethanol; (B) high dietary zinc with ethanol; (C) low dietary zinc without ethanol; (D) low dietary zinc with ethanol. Magnification x 3000. Solid bar denotes 10 micron.

3 oz. of absolute ethanol. This level of ethanol intake during human pregnancy constitutes a definite risk to the developing fetus according to the National Institute on Alcohol Abuse and Alcoholism (14).

Our results also demonstrate that ethanol is an antagonist of zinc utilization during molar development as it is for other tissue (1). It is our contention that the simultaneous occurrence of reduced zinc concentration of offspring enamel and dentin, higher caries scores, and an apparent increased susceptibility of offspring molar enamel to acid attack in vitro, as visualized by scanning electron microscopy, is more than just coincidence. Three reports have independently demonstrated that reduced zinc content of rat molars is associated with increased dental caries (4-6). We acknowledge that the dental caries score and SEM procedure results are subjective in nature. Results from our acid-etching procedure, for example, can only be considered as suggestive of a developmental effect of maternal ethanol ingestion on offspring enamel because of variation of prepared enamel as well as the artificial nature of the acid attack. We believe the results of the caries score and SEM procedure have validity however because each determination was made without knowledge of the sample identity.

Our contention that ethanol-antagonized molar zinc is directly related to dental caries does not mean that zinc rivals fluoride in importance to dental health (15). We do however suggest that some of the other 25 trace elements present in enamel, including zinc (16), may have a role in enamel structure or development. Of course it could be argued that ethanol adversely affects enamel matrix formation and subsequently mineralization, with reduced molar zinc concentration as an artifact of that effect. If this were true, we should have observed similar reductions in the enamel and dentin content of major minerals. Our analyses however failed to demonstrate any effect of maternal ethanol ingestion upon the enamel or dentin concentration of calcium, phosphorus or magnesium.

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