Caries Potentiating Effect of Ethylene Diamine Tetraacetic Acid in the Rat.* (20030)

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Because of its strong metal chelating properties, ethylene diamine tetraacetic acid (EDTA) has received wide application in the study of biochemical reactions requiring metal salts for continued normal activity. Hence, the compound has proved valuable in the study of various calcification and decalcification processes. Studies *in vivo* of decalcifying agents on rat molar tooth surfaces have shown that dilute EDTA solutions adjusted to pH 6.5 have been the most active tried(1).

In the classical studies of W. D. Miller(2) which still serve as a working hypothesis for basic research in the etiology of dental caries, decalcification of dentin and enamel is accorded a prominent role. It was the purpose of this study to investigate the caries potentiating effect of a strong decalcifying agent by adding EDTA to a cariogenic rat diet.†

Experimental and results. Weanling rats of the Holtzman strain were used throughout and the diet and drinking fluids were administered *ad libitum*. At the end of 100 days the rats were sacrificed, the heads were autoclaved, and the molar teeth were charted for caries according to Cox, Dodds, Dixon, and Matuschak(4).

Preliminary study of effect of EDTA in diet and drinking fluid. Eighty rats representing 20 litters were distributed by litter into 2 groups of 40 rats each and housed 10 rats to a cage. Group I (control) received cariogenic diet 585‡ and distilled water. Group II (experimental) received diet 585 containing 0.5% EDTA (diet 585V) and a 0.1% solution of EDTA calculated as the tetrasodium salt adjusted to pH 6.5 as the drinking fluid. The caries scores are presented in Table I. Comparison of the difference of the means for carious areas and caries score in Groups I and II yielded a "t" value of 3.90 and 3.50 respectively ($p < 0.01$). The production of the normal yellow-orange pigment of the incisors was markedly inhibited so that they appeared ivory in color, but unlike the effect produced by fluoride, showed no striations, pitting, or chipping.§

TABLE I. Caries Score of Rats Receiving EDTA in Diet and Drinking Fluid.

Group	I	II
Diet	585	585V
Drinking fluid	H_2O	EDTA
No. of rats	40	34
Rats with caries %	100	100
Carious teeth per carious rat	4	5.1
" areas " " "	7.8	11.8
Caries score " " "	16.3	24.7

Relation between decalcification potential and caries. In an attempt to evaluate the decalcification action of EDTA in caries potentiation, other *in vivo* decalcifying agents(1) were administered in both the diet and drinking fluid to rats receiving diet 585. Litter mates from 20 litters representing 120 rats were divided into 3 groups of 40 rats per group and housed 10 rats per cage. Group I received diet 585 and distilled water; Group II received diet 585 containing an added 1% so-

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† Recently it was reported in abstract(3) that a cariogenic diet containing 0.2% ethylene diamine tetraacetic acid increased the incidence and severity of smooth surface caries in rats.

‡ Formulated by R. M. Stephan of this Institute, and described in(5). Mineral analysis gave the following concentrations based on fresh weight of diet: Ca, .29; Mg, .06; Fe, .006; Mn, .010% respectively.

§ Macroscopic evidence of striations is seen at approximately 10 ppm F, whereas 100-200 ppm F are necessary to produce pitting and chipping.

TABLE II. Effect of Addition of Iron to a Cariogenic Diet Containing EDTA.

Group	I	II	III	IV
Diet	585	585V	585V+Fe	585+Fe
Drinking fluid	H_2O	H_2O	H_2O	H_2O
No. of rats	19	14	20	18
Rats with caries %	100	100	100	100
Carious teeth per carious rat	4.1	4.8	4.6	4.1
" areas " " "	5.4	9.9	7.4	5.6
Caries score " " "	11.6	22.1	15.1	10.7

dium citrate dihydrate (diet 585C) and a solution of 0.25 N sodium citrate dihydrate adjusted to pH 6.5 as the drinking fluid. Group III received diet 585 containing an added 1% of Calgon‖ (diet 585CG) and a solution of 2% Calgon adjusted to pH 6.5 as the drinking fluid. Groups II and III showed only a slight increase in the carious areas and caries score per carious rat. These differences, however, are not significant statistically ($p > 0.05$). No interference with normal incisor pigment could be seen in any of the groups. It appears, therefore, that citrate and calgon did not significantly potentiate caries on diet 585, although extensive decalcification of the molar tooth surfaces was apparent.

Reversal of effects of EDTA by addition of iron. It has been reported that the incisor pigment is due in large measure to its iron content which may be reduced by as much as 50% in anemia(6). Since the inhibition of incisor pigmentation indicated iron chelation by EDTA, an attempt was made to counteract the effects of EDTA by the addition of iron as the iron and ammonium citrate salt (Mallinckrodt, USP XI, brown scales). Ninety-six rats representing 25 litters were distributed according to litter into 4 groups of 24 rats each, and housed 2 rats per cage. Group I received diet 585. Group II received diet 585V. Group III received diet 585V containing an added 0.6% iron and ammonium citrate, and Group IV received diet 585 supplemented with the same amount of iron salt used by Group III. All rats received distilled water. Hematocrit values were determined on representative rats of each group. The caries data are presented in Table II.

‖ Manufactured by Calgon, Inc. Pittsburgh, Pa., and is presumed to be a hexametaphosphate.

Both the number of carious areas and the caries score per carious rat showed nearly a 2-fold increase when diet 585 contained an added 0.5% EDTA. The addition of iron to this diet (585V) produced a slight but not significant reversal of the effect of EDTA as indicated by the somewhat lowered caries score in Group III as compared with Group II. Adding 0.6% iron and ammonium citrate as a source of Fe to diet 585, had no appreciable effect on either the incidence or severity of caries. It has also been previously reported by McClure(7) that iron appears to have no pronounced effect on induced rat caries, when added to a cariogenic diet.

Supplementation of diet 585V with 0.6% iron and ammonium citrate as a source of iron seemed to completely prevent the hematocrit lowering and the incisor pigmentation inhibiting effect of EDTA. The mean hematocrit values found for Groups I-IV were 43.0, 29.7, 43.2, and 43.0, respectively.

To corroborate the effect of EDTA when added to the diet alone an additional 48 rats representing 24 litters were divided into 2 groups of 24 rats per group and housed 2 per cage. Group I received diet 585 and Group II received diet 585V with distilled water available *ad libitum* to both groups. The mean hematocrit values on representative rats of Groups I and II of 43.1 and 35.1 respectively were significantly different at the 1% level ($p < 0.01$). The carious areas per carious rat were 2.9 and 5.7 respectively, and the caries scores per carious rat were 5.1 and 12.7 respectively. When a 0.1% EDTA solution adjusted to pH 6.5 was administered as the drinking fluid to 24 rats receiving diet 585, no significant caries potentiating effect was apparent when compared to litter mates receiving diet 585 and distilled H_2O. In ad-

TABLE III. Composite Data on the Caries Potentiating Effect of EDTA.

	10 rats/cage		2 rats/cage	
Diet	585	585V	585	585V
No. of rats	78	34	41	32
Rats with caries %	97.4	100	87.8	100
Carious teeth per carious rat	3.5	5.1	3.5	4
" areas " " "	5.9	11.8	4.2	7.5
Caries score " " "	11.8	24.7	8.5	16.8

dition, no interference with normal incisor pigmentation or lowering of the hematocrit value was observed.

The combined studies reported thus far comprise 344 rats. For composite comparisons of the effect of EDTA added to a cariogenic diet the data may be placed in 2 groups, *i.e.*, with rats housed 10 to a cage and with rats housed 2 to a cage. The data are presented in Table III. This separation of data is made since no reports have appeared on the effect of crowding on experimental rat caries.

It appears, therefore, that the carious areas and caries score are increased approximately 2-fold when EDTA is present in the diet. The difference in the means is statistically significant at the 1% level, indicating a marked caries potentiating effect of EDTA when added to cariogenic diet 585.

Discussion. No adequate explanation at present accounts for the apparent caries potentiating effect of EDTA in the rat. A lowered hematocrit value is produced as well as a marked inhibition of incisor pigment formation, and both these effects appear to be reversed by the addition of iron to the diet. The caries potentiating effect of 0.5% added EDTA to the diet does not seem to be associated with its decalcifying ability since other known decalcifying agents when added to the cariogenic diet 585 do not significantly increase the severity of caries. It has been suggested by the work of McClure and Ruzicka (8) that the non-acid decalcifying effect of the citrate anion may possibly play a role in the etiology of dental caries. In the present study, no significant increase in the severity of caries appears to be occasioned by the addition of citrate to the diet and drinking fluid. In addition preliminary studies have indicated that no decalcification, similar to that reported in previous studies(9), is found when EDTA is added to the diet alone.

Since both fluoride and iodoacetate inhibit rat caries(10), enzymatic reactions have been implicated in caries etiology. It appears possible that EDTA may have an effect on such enzyme systems linked to the carious process and requiring trace metals. According to our results one of these elements does not seem to be iron. The addition of 0.5% EDTA to cariogenic diet 585 has produced some diarrhea and a depression of weight so that the possibility must also be considered that EDTA *per se* may exert some secondary or indirect caries potentiating effect due to its toxicity. The effects of EDTA described appear to be associated with its presence in the diet, although its effect in the drinking fluid cannot be entirely precluded at this time.

It appears, therefore, that EDTA may be a valuable tool in investigating the possible role of mineral deficiency and cation antagonism in the etiology of experimental dental caries.

Summary. The addition of 0.5% EDTA to a cariogenic diet appeared to potentiate caries to a marked degree in the white rat. Concomitantly, the hematocrit value is significantly lowered, and almost complete inhibition of incisor pigmentation is found. These effects of EDTA do not seem to be associated with its ability to decalcify rat molar teeth *in vivo*. Citrate and calgon added to a cariogenic diet and present in the drinking fluid do not significantly increase the severity of caries.

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Some Observations of Effects of Intravenous Fat Emulsions on Erythrocyte Fragility. (20031)

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After the administration of intravenous fat emulsions, a moderate but self-limited anemia has frequently been reported. Mann *et al.* (1,2) noted the occurrence of moderate anemia in dogs which were given corn and coconut oil emulsions. Anemia also occurred in rats given both coconut and corn oil emulsion, and in 2 human subjects who received coconut oil emulsion. In the latter report, increased osmotic fragility of the red cells was noted. The authors found that the phosphatide used as stabilizer could alone produce changes of the same magnitude. Meng and Freeman(3) observed a slight anemia in a group of dogs given stabilizing agents, but in only one of 5 dogs given complete emulsion. Meng(4) observed no anemia in infused rabbits. Van Itallie *et al.*(5) stated that preliminary studies of red cell fragility failed to show changes in the integrity of the red cell membrane, but no details were given. Lerner, Chaikoff, and Entenman(6) likewise reported the occurrence of anemia in a group of dogs given intravenous fat for prolonged periods as did Dunham and Brunschwig(7) and Collins (8). The latter believed that it was probably not hemolytic since reticulocytosis did not occur. In this laboratory, significant decreases in hemoglobin, red blood cell count and hematocrit were observed in a group of normal dogs after one to 2 weeks of daily intravenous infusion of fat(9). In contrast, Shafiroff *et al.*(10,11) found no hematologic changes after single infusions of fat emulsion in humans and stated that in no case did infusion result in a positive test for urine urobilinogen.

The mechanism of the observed anemia remains unexplained. It could be inferred from the nature of the infused substances that this is a hemolytic anemia. Surface active stabilizers are used in many of the emulsions and these materials might well alter the red cell membrane. The emulsified fat itself might conceivably alter the interfacial relationships between the erythrocyte and its environment. In addition, minute amounts of other surface active agents such as free fatty acids may be present in the emulsion or be liberated after infusion. Some of the hematologic effects of intravenous fat emulsions given to dog and man and the possible significance of these effects are reported here.

Procedure. The fat emulsions used were from 2 sources: Emulsion I is a 10% emulsion of synthetic fats stabilized with Span 20, sodium cholate, soybean phosphatides and Demal—14.* Emulsion II is a 15% olive oil emulsion stabilized with 0.6% soybean phosphatide and 1.0% Demal—14†. The subjects in these experiments were 2 burn patients, one convalescent and the other in the final stages of skin grafting. The dog used was a normal male weighing 22 kg. All subjects were studied in the fasting state. The patients received 500 ml of emulsion and the dogs 300 ml at a rate of approximately 4 ml per minute.

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