INFLUENCE OF DIETARY IRON ON THE DENTAL CARIES INCIDENCE AND GROWTH OF RATS FED AN EXPERIMENTAL DIET[1,2,3]

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SUMMARY

In view of the fact that supplementation with essential nutrients improves the quality of diet NIH 2000 and enhances growth, this study was carried out to determine the effects of supplementation with a specific

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nutrient. Diet 2000 was supplemented with 35 ppm of $FeSO_4$ and fed *ad libitum* for 40 days. Iron supplementation reverses the caries process and reduces its incidence especially in the buccal surfaces which are bathed readily by saliva. In addition, hemoglobin and hematocrit, growth and pigmentation of the incisor teeth improve significantly with supplementation. The results of this experiment suggest that the effect of diet in the caries process involves not only the sucrose component of the diet but also an essential nutrient such as iron.

INTRODUCTION

The effect of iron on tooth decay is not definitive but previous experiments (1) support a role of iron in the dental caries process. Historically, McClure (2) added 250 ppm ferric chloride and ferric citrate to the drinking water of rats maintained on a caries-promoting diet for four months. A 50% reduction in caries development was observed in the group fed iron in the chloride form, and no reduction when fed in the citrate state. A 30% increase was observed in the group fed 500 ppm iron as ferric citrate.

Torell (3) concluded that iron salts affect enamel solubility by establishing layers of ferric precipitates which bind to phosphate irons on the enamel surface, increasing the dissolution of apatite. The same author suggested that for a better understanding of the role of iron in the destruction process, research work is needed dealing with the influence of saliva, the action of iron complex forming agents, and the significance of precipitated ferric hydroxide present in the mouth.

Emilson and Krasse (4) showed that in hamsters, the addition of ferric chloride or ferrous sulphate either to the drinking water or to the diet given *ad libitum*, reduces the level of experimental caries. Topical applications of solutions of these compounds also reduced caries incidence.

Wegner *et al.* (5) published information pertaining to the interrelation of fluoride and iron in anemia. Previous experiments indicated that a reduced fluoride intake by mice has increased the severity of the anemia in pregnancy and infancy. The diet employed had a high content of whole wheat flour (58%) which contains phytic acid, a chelator which interferes with iron absorption.

The purpose of this study was to supplement caries-promoting diet NIH 2000 with iron in order to determine the role of this nutrient in the dental caries process.

MATERIALS AND METHODS

Experimental Design

In order to determine the effects of iron on standard caries-promoting diet NIH 2000, the basal diet was supplemented with 35 mg of ferrous sulphate. In addition fat was added to supply for the observed caloric deficit. The diets were fed at weaning for 40 days. The following five diets were evaluated during the above period:

Group

A MIT 25o/o protein diet[6] (control)
B NIH 2000 basal diet[7]
C NIH 2000 + 35 ppm $FeSO_4$
D NIH 2000 + 1o/o corn oil
E NIH 2000 + 35 ppm $FeSO_4$ + 1o/o corn oil

Sixty 19-day-old males were weighed, measured and randomly assigned to experimental groups (12 per group) and housed in groups of 12 in plastic breeding cages. Food and deionized water were given *ad libitum.*

Animal Husbandry

Sprague Dawley rats[8] of the CD strain were used in these experiments. Animals were housed under controlled humidity (50-55o/o) and temperature (72oF ± 2oF) with equal hours of light and darkness.

They were weighed in a Torbal balance and measured as described by Hughes and Tanner (6) from the day of arrival and throughout the entire period on a twice-a-week basis up to 35 days of age, then weekly thereafter. All animals were observed daily for condition of skin, hair, tail.

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6 Sintes, J. & S. Miller. *Arch. Latinoamer. Nutr.*, *33*(2): 283-307, 1983.
7 Sintes, J. & S. Miller. *Arch. Latinoamer. Nutr.*, *33*(2): 308-321, 1983.
8 Charles River Breeding Laboratories, Wilmington, Mass.

Inoculation Technique

To reduce the experimental period, the animals were challenged on day 19, 20 and 21 with a pure culture of *S. mutans*[9]. The inoculum was also added to the water for the three days. At the end of the inoculation period animals were placed in suspended stainless steel cages, one per cage. The implantation of *S. mutans* was checked by swabbing animals at the end of the first week and the day before sacrifice, and plating on Mitis Salivarius Agar (MSA) plus Streptomycin sulfate.

Eight animals were sacrificed by guillotine 40 days after weaning. Vital organs including heart, liver and spleen were weighed in an analytical balance[10] and fixed in 10o/o neutral buffered formalin (NBF) solution for histopathology.

Skulls were also fixed in 10o/o NBF, washed thoroughly with running water and placed in the autoclave for 15 minutes at 15 psi. The jaws were removed, cleaned and weighed in an analytical balance.

Pigmentation of the incisor teeth was assessed throughout the entire study either as normal (orange color), partly depigmented or completely white (7).

Caries Scores

Mandibular caries were scored according to the method described by Keyes (8). In order to have a better delineation of the extent of involvement of the carious process, mandibles were stained with Murexide (9).

Determination of Hemoglobin and Hematocrit

Heparinized microhematocrit capillary tubes were used to collect blood samples from the retro-orbital venous plexus of etherized adult rats. Samples were centrifuged for 15 minutes at 2700 rpm and read on an international micro-capillary reader. Hemoglobin concentration was determined by removing 0.02 ml of blood from the retro-orbital venous plexus of etherized adult

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9 Ingbritt 1600, Streptomyces resistant strain, Forsyth Dental Center, Boston, Mass.

10 Mettler Corp., Highstown, N. J.

rats and mixing immediately with 5 ml of Hycel cyanomethemoglobin reagent.

Statistical Analysis

The comparisons of the five groups were analyzed using the F test. If there was a statistically significant F value ($p \leqslant 0.05$), an *a posteriori* test, based on multiple comparisons among means of equal sample sizes was applied (Student-Newman-Keuls test) to determine which groups were statistically different from each other. With both tests, a $p \leqslant 0.05$ was considered to be significant (10).

RESULTS

The purpose of this work was to determine whether iron supplementation of caries-promoting diet NIH 2000 is nutritionally adequate to support growth in the young rat and maintenance in the adult rat when compared to a diet that satisfies the requirements established by the National Research Council (NRC). At the same time, an effort was made to determine the effects of iron supplementation upon the mechanism of caries induction.

Measuring growth by weight gain of the animal revealed that under the experimental conditions used, diet supplementation with iron, fat, or a combination of both, did improve the growth pattern of the animals when compared to the non-supplemented group, but still remained significantly lower when compared to that of the controls (Figure 1). A similar growth pattern is observed when measuring growth by length gained (Figures 2, 3).

Two weeks after inoculation with *S. mutans* I.B. 1600, animals fed diets NIH 2000 basal and NIH 2000 + fat lost the pigmentation of their incisor teeth. Four rats in each group were sacrificed. Hemoglobin and hematocrit values revealed a statistical significant difference between animals without iron supplementation and the other groups (Table 1). Caries scores at the time revealed no significant statistical difference between the NIH 2000 diets (supplemented versus nonsupplemented); only demineralization areas were observed (Tables 2, 3).

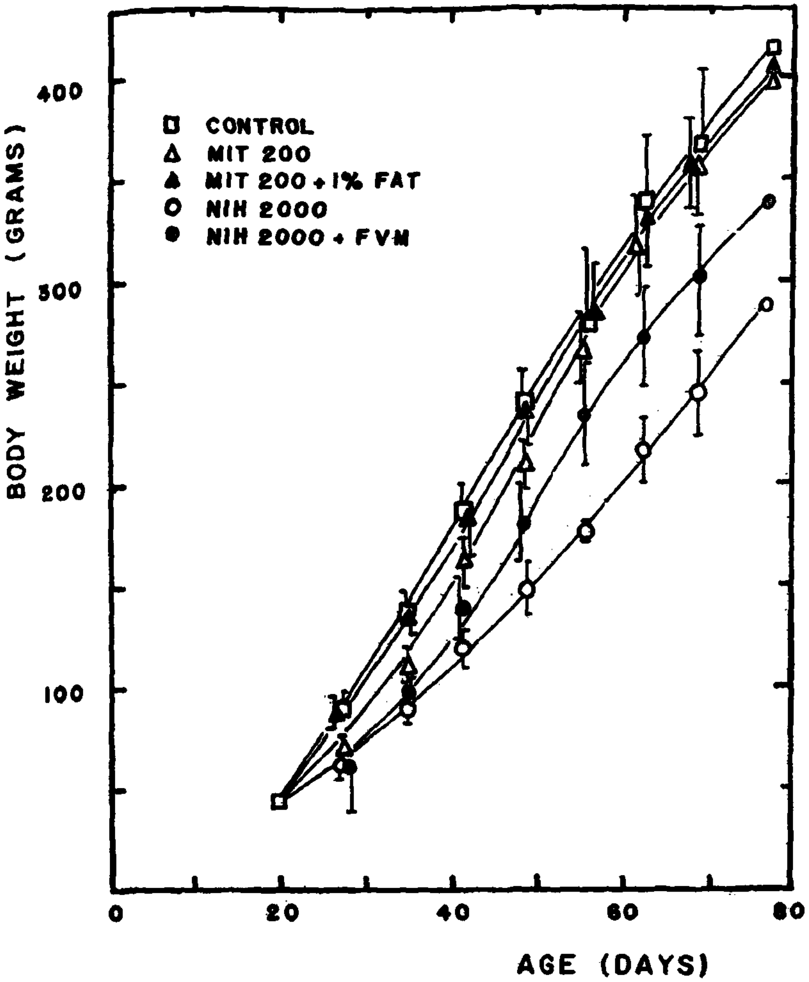

FIGURE 1

Growth curves of male albino rats fed a caries-promoting diet supplemented with iron, fat or both

At the same time, four animals in the NIH 2000 basal diet and in the NIH 2000 +fat were fed NIH 2000 supplemented with 35 ppm of $FeSO_4$, to observe the effects of this treatment on recovery. Four weeks later, animals in all groups were sacrificed.

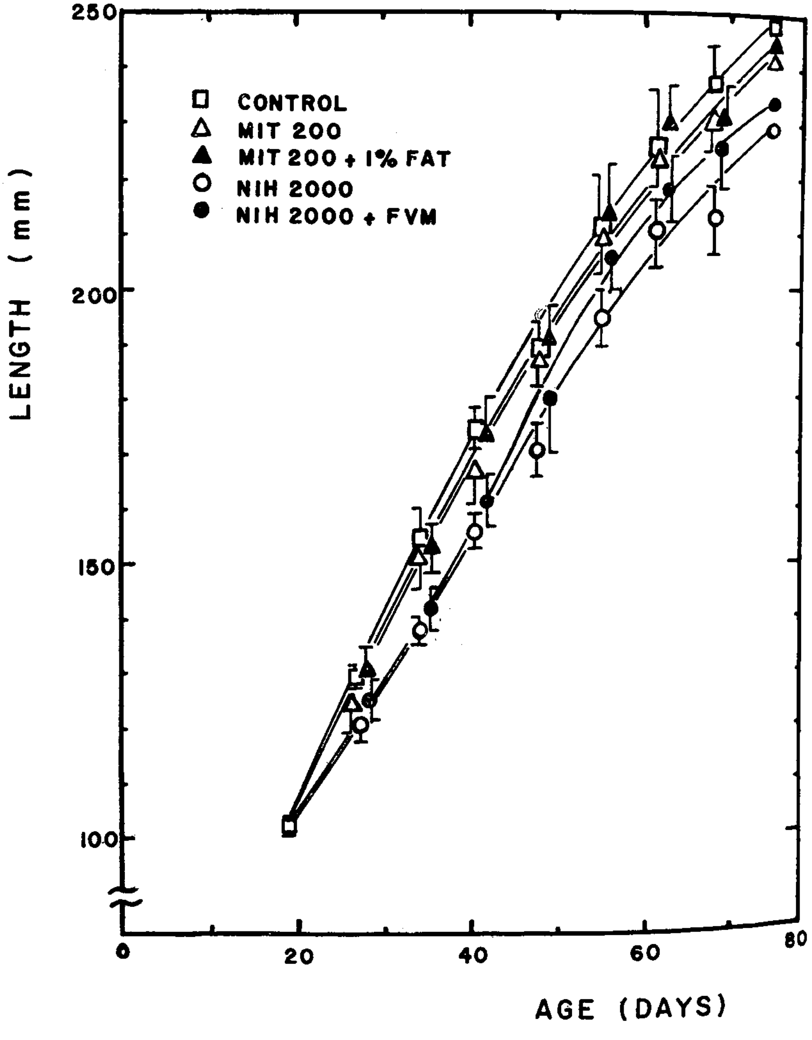

FIGURE 2

Nose-rump length curves of male albino rats fed a caries-promoting diet supplemented with iron, fat or both

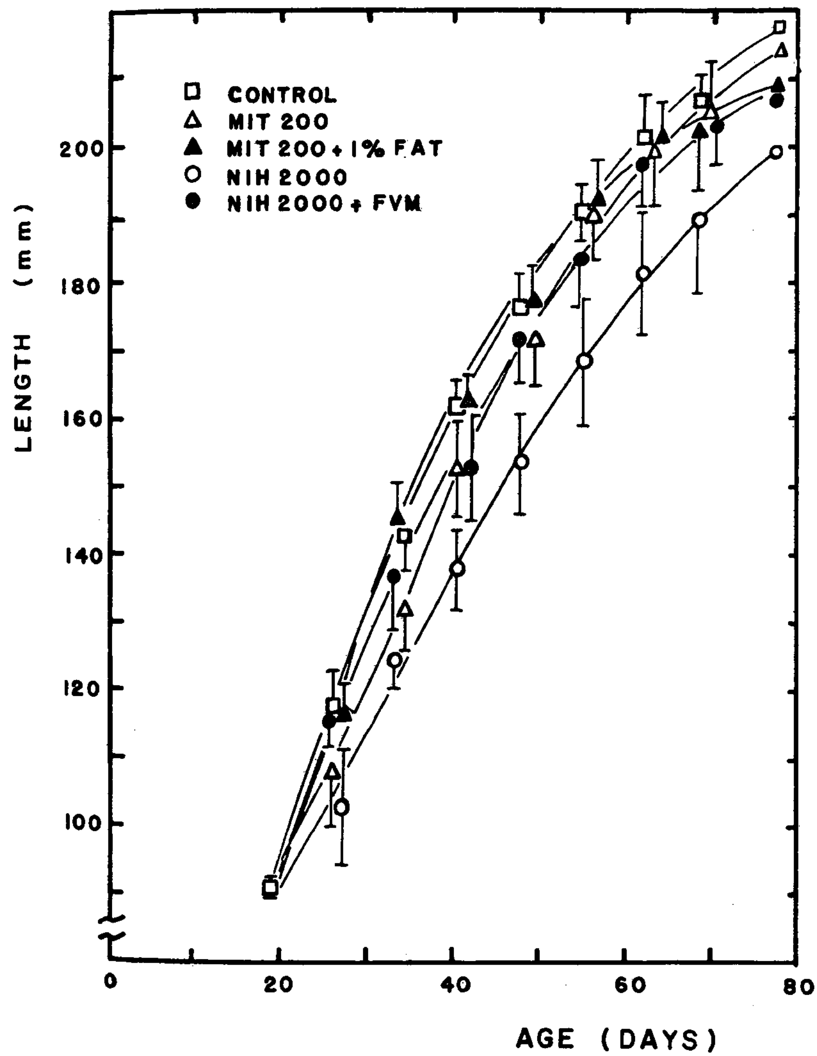

FIGURE 3

Rump-tail length curves of male albino rats fed a caries-promoting diet supplemented with iron, fat or both

TABLE 1

HEMOGLOBINS AND HEMATOCRITS OF MALE ALBINO RATS FED DIET NIH 2000 SUPPLEMENTED[1,2,3]

			NIH 2000			
Control	Iron[1]	Iron, fat[2]	Basal[4]	Fat[5]	Basal	Fat[8]
			Hemoglobin (g%)[6]			
11.3 ±0.9	12.6 ± 0.6	12.5 ± 0.6	–	–	8.6 ± 1.7	8.3 ± 1.1[7]
			Hematocrit (%)[6]			
35.4 ±3.4	35.3 ± 1.4	34.3 ± 1.2	–	–	25.8 ± 2.8	23.8 ±2.0[7]
			Hemoglobin (g%)[8]			
15.6 ±0.4	16.8 ± 0.9	16.8 ± 0.5	14.8 ± 0.4	15.3 ± 0.4	11.0 ± 1.0	10.9 ± 0.5[7]
			Hematocrit (%)[8]			
42.2 ±1.3	42.7 ± 1.1	42.8 ± 1.6	40.8 ± 0.9	40.8 ± 0.8	33.1 ± 3.1	31.6 ± 2.2[7]

The underlines joining the groups denote that these treatments have not been shown to be significant ($p \leqslant 0.05$).

1 Supplemented with 35 ppm of $FeSO_4$.

2 Supplemented with 35 ppm of $FeSO_4$ and 1% fat.

3 Supplemented with 1% fat.

4 Basal recovered.

5 Fat recovered.

6 Two weeks post-weaning.

7 $p \leqslant 0.01$.

8 Forty days post-weaning.

TABLE 2

AVERAGE CARIES SCORES FOR ALL SURFACES OF RIGHT MANDIBLES FOR 14 DAYS POST–WEANING RATS[1,2,3]

Lesion[4]	Basal	NIH 2000 + Iron[1]	+ Fat[2]	+ Iron, Fat[3]
		Buccal		
E	8.5	6.5	8.5	4.5
D_s	1.0	0	1.0	0
D_m	0	0	0	0
		Sulcal		
E	12.8	11.5	12.0	11.5
D_s	0	0	0	3.8
D_m	0	0	0	0
		Proximal		
E	0.5	0	1.0	0.5
D_s	0	0	0	0
D_m	0	0	0	0

1 Supplemented with 35 ppm of $FeSO_4$.

2 Supplemented with 1o/o fat.

3 Supplemented with 35 ppm of $FeSO_4$ and 1o/o fat.

4 E = enamel only; D_s = slight dentinal, and D_m = moderate dentinal.

As Table 4 shows, final body weights and organ weights upon autopsy revealed a statistical difference between those obtained from controls and those fed the supplemented NIH 2000 diet (4).

Hematocrit and hemoglobin values in the supplemented iron and rehabilitated groups were all statistically different from the

TABLE 3

COMBINED AVERAGE CARIES SCORES FOR ALL LESIONS (BUCCAL + SULCAL + PROXIMAL) [1,2,3]

	NIH 2000			
Lesion[4]	Basal	+ Iron[1]	+ Fat[2]	+ Iron, Fat[3]
E	21.8	18.0	21.5	16.5
D_s	1.0	0	1.0	3.8
D_m	0	0	0	0

1 Supplemented with 35 ppm of $FeSO_4$.

2 Supplemented with 1% fat.

3 Supplemented with 35 ppm of $FeSO_4$ and 1% fat.

4 E = enamel only; D_s = slight dentinal, and D_m = moderate dentinal.

nonsupplemented basal diet (Table 1). Caries scores in the iron supplemented groups were reduced from ± 14 buccal lesions (NIH 2000 basal) to ± 3 (NIH 2000 + $FeSO_4$) (Tables 5, 6). There was a significant reduction of caries when iron was introduced into diet NIH 2000.

Depigmentation of incisor teeth was not recovered when animals were placed on a diet supplemented with iron after two weeks on the basal diet or the diet supplemented with fat. However, skin lesions were abolished with iron supplementation, while scaliness of the tail was abolished with fat supplementation. This was interpreted to mean that iron is necessary for the maintenance of the appearance of the fur, and that the essential fatty acids are necessary to maintain a healthy tail.

DISCUSSION

Animals fed diet NIH 2000 supplemented with iron from the beginning of the experiments had less caries scores, especially in the buccal surfaces.

The work of Menaker and Navia (11) demonstrated that marginal protein deficiency can produce increased susceptibility of the tooth to caries in the rat. In our study, there appears to be a close connection between increased caries incidence and iron deficiency,

TABLE 4

INFLUENCE OF SUPPLEMENTATION OF DIET NIH 2000 ON THE FINAL BODY AND ORGAN WEIGHT OF 40 DAYS POST-WEANING RATS

Control	Iron + Fat[1]	Iron[2]	Fat[3]	Basal
		Body Weight		
272.10 ± 10.7	250.50 ± 23.6	217.30 ± 25.8[4]	226.50 ± 11.5[4]	188.80 ± 30.5[5]
			(222.00 ± 15.9)	(200.50 ± 21.1)[4]
		Liver		
14.16 ± 1.2	12.7 ± 0.7	10.1 ± 1.4	9.5 ± 0.9[4]	7.9 ± 1.9[5]
			(10.2 ± 1.5)	(9.4 ± 1.3)[4]
		Heart		
0.96 ± 0.09	1.09 ± 0.08	0.86 ± 0.18	1.12 ± 0.09	0.94 ± 0.14
			(1.04 ± 0.13)	(0.91 ± 0.14)
		Spleen		
0.54 ± 0.07	0.71 ± 0.12	0.45 ± 0.10	0.80 ± 0.23	0.63 ± 0.07
			(0.61 ± 0.19)	(0.58 ± 0.07)

1 Supplemented with 35 ppm of $FeSO_4$ and 1% fat.
2 Supplemented with 35 ppm of $FeSO_4$.
3 Supplemented with 1% fat.
4 $p < 0.05$.
5 $p < 0.01$.
Figures in parentheses are recovered groups.

TABLE 5

AVERAGE CARIES SCORES FOR ALL SURFACES OF RIGHT MANDIBLES FOR 40 DAYS POST-WEANING RATS.[1,2,3]

	NIH 2000					
Lesion[4]	Basal	Basal[5]	+ Iron[1]	+ Fat[2]	+ Fat[6]	+ Iron, Fat[3]
			Buccal			
E	6.8	6.5	2.4	7.0	3.0	2.3
D_s	3.5	3.0	0.9	4.0	1.0	0.8
D_m	3.5	2.5	0	2.5	0	0.3
			Sulcal			
E	13.3	12.8	8.1	12.8	11.5	7.1
D_s	12.3	10.5	0.8	12.0	4.5	2.1
D_m	6.0	5.5	0	4.5	0.5	0
			Proximal			
E	1.8	1.5	0	2.0	1.0	0
D_s	1.3	1.0	0	1.3	0	0
D_m	0.5	0.3	0	0.3	0	0

1 Supplemented with 35 ppm of $FeSO_4$.
2 Supplemented with 1% fat.
3 Supplemented with 35 ppm of $FeSO_4$ and 1% fat.
4 E = enamel only; D_s = slight dentinal, and D_m = moderate dentinal.
5 Basal recovered.
6 Fat recovered.

TABLE 6

COMBINED AVERAGE CARIES SCORES FOR ALL SURFACES (BUCCAL + SULCAL + PROXIMAL)[1,2,3]

	NIH 2000					
Lesion[4]	Basal	Basal[5]	+ Iron[1]	+ Fat[2]	+ Fat[6]	+ Iron, Fat[3]
E	21.8	20.8	10.3[7]	21.3	15.5[8]	9.4[7]
D_s	17.0	14.5	1.8[7]	17.3	5.5[7]	2.9[7]
D_m	10.0	8.3	0	7.3	0.5[7]	0.3[7]

1 Supplemented with 35 ppm of $FeSO_4$.
2 Supplemented with 1% fat.
3 Supplemented with 35 ppm of $FeSO_4$ and 1% fat.
4 E = enamel only; D_s = slight dentinal, and D_m = moderate dentinal.
5 Basal recovered.
6 Fat recovered.
7 $p < 0.01$.
8 $p < 0.05$.

as evidenced by results with diet NIH 2000. Supplementation of this diet with $FeSO_4$ reverses the caries process and reduces its incidence, especially in the buccal surfaces which are readily bathed by saliva.

In an attempt to describe the mechanism by which NIH 2000 produces increased caries we must offer a multiple explanation. Such an explanation would include the following:

1. The constituents of the diet, i.e., whole wheat flour, have been suggested as possible metal-binding agents (12).
2. The amount of iron in the diet, 16 ppm versus 45 ppm in the control is marginal for the rat.
3. Pigmentation of rat incisor teeth is dependent on iron and disappears in anemic rats (13).
4. Reduced salivary volume and the amount of protein produced is one reflection of the decreased functional capacity of the salivary gland when stressed with a marginal deficiency such as protein (14).
5. A proline-rich salivary protein has been identified as present

in normal humans. Its amino acid composition is similar to collagen and is cleaved readily by Clostridial collagenase A (15).

Not all of these explanations are mechanistic definitions of iron and caries.

Collagen is dependent on ascorbic acid and iron for its biosynthesis. Ascorbic acid is not a required nutrient in the rat. Therefore, we can hypothesize that one of the marginal deficiencies demonstrated with caries-promoting diet NIH 2000 is due to iron. This diet produces high caries scores especially in the buccal surfaces of molars because it reduces the salivary volume and content of protein. We can speculate that due to the deficiency there is interference with the biosynthesis of salivary proteins dependent on iron such as the proline-rich proteins identified by Oppenheim *et al.* (15). It may also be true that these proteins may be the proteins associated with the remineralization processes which are well known to occur on enamel surfaces *in vivo* as well as *in vitro* (16).

RESUMEN

INFLUENCIA DEL HIERRO DIETETICO EN LA CARIES DENTAL Y CRECIMIENTO DE RATAS ALIMENTADAS CON UNA DIETA EXPERIMENTAL

Considerando que la suplementación con nutrientes esenciales mejora la calidad de la dieta NIH 2000 y acelera el crecimiento, se llevó a cabo este estudio con el fin de determinar los efectos de la suplementación con un nutriente específico. Así, la dieta 2000 fue suplementada con 35 ppm de $FeSO_4$, administrándose luego *ad libitum* durante un período de 40 días. Se encontró que la suplementación con hierro revierte el proceso de la caries y reduce su incidencia, sobre todo en las superficies bucales fácilmente humedecidas por la saliva. Además, la hemoglobina y el hematocrito, el crecimiento y la pigmentación de los incisivos también mejora significativamente. Los resultados de este experimento sugieren que el efecto que la dieta en cuestión ejerce en el proceso de las caries, implica no sólo el componente sucrosa de la dieta, sino también la inclusión de un nutriente esencial, tal como el hierro.

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BIBLIOGRAPHY

1. Sintes, J. & S. Miller. Growth, development and dental caries incidence in rats fed two experimental diets. **Arch. Latinoamer. Nutr., 33:** 283-307, 1983.
2. McClure, F. Observations on induced caries in rats. VI. Summary results on various modifications of food and drinking water. **J. Dent. Res., 27:** 34, 1948.
3. Torell, P. The possible caries activating effect of chemical reaction between sucrose and ferric iron. **Odont. Tidsk, 64:** 165, 1956.
4. Emilson, C. & B. Krasse. The effect of iron salts on experimental dental caries in the hamster. **Arch. Oral Biol., 17:** 1439-1443, 1972.
5. Wegner, M., L. Singer, R. Ophaug, & S. Magil. The interrelation of fluoride and iron in anemia. **Proc. Soc. Exp. Biol. Med., 153:** 414-418, 1976.
6. Hughes, P. & J. Tanner. A longitudinal study of the growth of black-hooded rat: methods of measurement and rates of growth for skull, limbs, pelvis, nose-rump and tail lengths. **J. Anat., 2:** 349-370, 1970.
7. Bunyard, M. Effects of high sucrose cariogenic diets with varied protein calorie levels on the bones and teeth of the rat. **Calc. Tiss. Res., 8:** 217-227, 1971.
8. Keyes, P. Dental caries in the molar teeth of rats. II. A method for diagnosing and scoring several types of lesions simultaneously. **J. Dent. Res., 37:** 1088, 1958.
9. Losee, F. Adding color for better visualization of dentinal caries. **Dental Progress, 3:** 280-283, 1963.
10. Sokal, R. & F. Rohlf. Single classification analysis of variance. In: **Biometry.** San Francisco, Calif., W. H. Freeman & Co., 1969, p. 204-252.
11. Menaker, L. & J. Navia. Effect of undernutrition during the perinatal period on caries development in the rat. IV. Effects of differential tooth eruption and exposure to a cariogenic diet on subsequent dental caries development in the rat. **J. Dent. Res., 52:** 692-697, 1973.
12. Ismail-Beigi, F., B. Faraji & J. Reinhold. Binding of zinc and iron to

wheat bread, wheat bran and their components. **Am. J. Clin. Nutr., 30:** 1721-1725, 1977.

13. Smith, M. & E. Lantz. Changes in the incisors of albino rats accompanying a deficiency of vitamin A. **J. Home Econ., 25:** 5, 1933.
14. Menaker, L. & J. Navia. Effect of undernutrition during the perinatal period on caries development in the rat. V. Changes in whole saliva volume and protein content. **J. Dent. Res., 53:** 592-597, 1974.
15. Oppenheim, F., D. Hay & C. Franzblau. Proline-rich proteins from human parotid saliva. I. Isolation and partial characterization. **Biochem., 10:** 4223-4238, 1971.
16. Koulourides, T. Remineralization methods. **Ann. N. Y. Acad. Sci., 153:** 84, 1968.