INFLUENCE OF SUPPLEMENTATION ON THE DENTAL CARIES INCIDENCE AND GROWTH OF RATS FED TWO MODEL DIETS[1,2,3]

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SUMMARY

In order to improve the quality of caries-promoting diets MIT 200 and NIH 2000, both diets were supplemented with fat. In addition, the NIH 2000 diet was supplemented with minerals and vitamins. Supplementation

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with essential nutrients improves the quality of the diets and enhances growth when compared to control animals. Adding minerals and vitamins to diet NIH 2000 abolishes loss of pigmentation of incisors, improves hemoglobin and hematocrit and reduces caries scores in the buccal surfaces by 50o/o when compared to the non-supplemented diet. It is possible that the cariogenic properties of diet NIH 2000 may be a function in part of a nutrient deficiency.

INTRODUCTION

Previous experiments (1) revealed that under the experimental conditions used, both caries-promoting diets NIH 2000 and MIT 200 did not sustain growth of rats at a rate comparable to those fed control diets. It is possible that the factor restricting rat growth in these experiments was the level of fat in the diet.

In the growing animal, protein accretion is only one of several important metabolic processes. Among others are maintenance of existing tissues and fat deposition. The foremost of these processes is that of maintenance. Storing energy as fat in the tissues is an efficient process. Fat has a very high caloric value, and it is usually deposited with partial replacement of tissue water. At higher intakes, or at a later age when the stimulus to deposit protein as a percentage of daily live weight gain decreases, fat deposition becomes more important. In later stages of growth, energy demands for maintenance increase due to the increasing body weight. The animal's appetite also decreases relative to body weight near maturity. At the same time, fat becomes more and more dominant in the deposited energy stores; near maturity 85o/o–90o/o of the energy is deposited as fat, and only 10-15o/o as protein. It could be said that during growth in man and animals, maintenance metabolism is the most important energy-requiring process (2).

The purpose of this study was to determine if supplementation of caries-promoting diets MIT 200 and NIH 2000 with essential nutrients such as fat (MIT 200) and fat, minerals and vitamins (NIH 2000), improved the growth pattern of animals when compared to controls fed a diet that satisfies the requirements recommended by the National Research Council (NRC).

MATERIALS AND METHODS

Experimental Design

After determining what nutrients were deficient in the NIH 2000 and MIT 200 diets (1), an experiment was designed to determine the effects of specific nutrient supplementation on growth and development of the animals, and its effects on caries experience. The following five diets were evaluated for a 60 day period after weaning:

Group

A MIT 25o/o protein diet[6] (control)
B MIT 200[7]
C MIT 200 + 1o/o cottonseed oil
D NIH 2000[7]
E NIH 2000 + 3o/o corn oil + 3o/o MIT 200 mineral mix + 1o/o MIT 200 vitamin mixture

One hundred and twenty 19-day-old male rats were weighed, measured and randomly assigned to experimental groups (24 per group) and housed in groups of eight in plastic breeding cages. Food and deionized water was 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.

Animals were weighed in a Torbal balance and measured as described by Hughes and Tanner (3) 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 and 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): 322-338, 1983.
8 Charles River Breeding Laboratories, Wilmington, Mass.

Inoculation Technique

In order 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 on those same three days. At the end of the inoculation period animals were placed in suspended stainless steel cages, two per cage. The implantation of *S. mutans* was checked by swabbing the animals at the end of the first and fourth weeks and the day before sacrifice, and plating on Mitis Salivarius Agar (MSA) plus Streptomycin sulfate.

Morphometric Analysis

All animals were sacrificed by guillotine 60 days after weaning. Vital organs, including liver, spleen and heart were weighed in an analytical balance[10] and placed in 10% neutral buffered formalin (NBF) solution for histopathology.

Skulls were fixed in a 10% 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 during the entire experiment either as normal (orange color), partly depigmented, or completely white (4).

Caries Score

Mandibular caries were scored according to the method described by Keyes (5). In order to have a better delineation of the extent of involvement of the carious process, mandibles were stained with murexide (6).

Determination of Hemoglobin and Hematocrit

Heparinized microhematocrit capillary tubes were used to collect blood samples from the retro-orbital venous plexus of

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

10 Mettler Corp., Highstown, N. J.

etherized adult rats. Samples were centrifuged for 15 min 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 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 ($p \leq 0.05$), 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 $p \leq 0.05$ was considered to be significant (7).

RESULTS

Supplementation of MIT 200 diet with or without 1o/o fat revealed a growth pattern similar to that of the controls. Both weight and length curves compared with those of the controls that were fed a diet that satisfied the requirements for growth and maintenance (Figures 1, 2, 3). Body weight and organ weights upon autopsy revealed no significant statistical difference when compared to controls (Table 1). There was a significant difference in caries scores particularly with respect to depth of the sulcal lesions. The supplemented group extended far beyond that of the control or the non-supplemented groups (Table 2).

Supplementation of the NIH 2000 diet with 3o/o fat, 3o/o mineral and 1o/o vitamin mixture, revealed a better growth pattern than that of the non-supplemented group but still significantly lower than that of the controls or the supplemented MIT 200 diet (Figures 1, 2, 3). Body weight and liver weight revealed a significant difference when compared to controls and MIT 200. No differences were observed in weight of heart and spleen (Table 1).

Supplementation of diet NIH 2000 also improves hemoglobin and hematocrit. For example, hemoglobin values of 12.2 go/o (NIH 2000), 14.7 go/o (NIH 2000 + supplements) and 15.2 g o/o (controls) (Table 3) were obtained after supplementation.

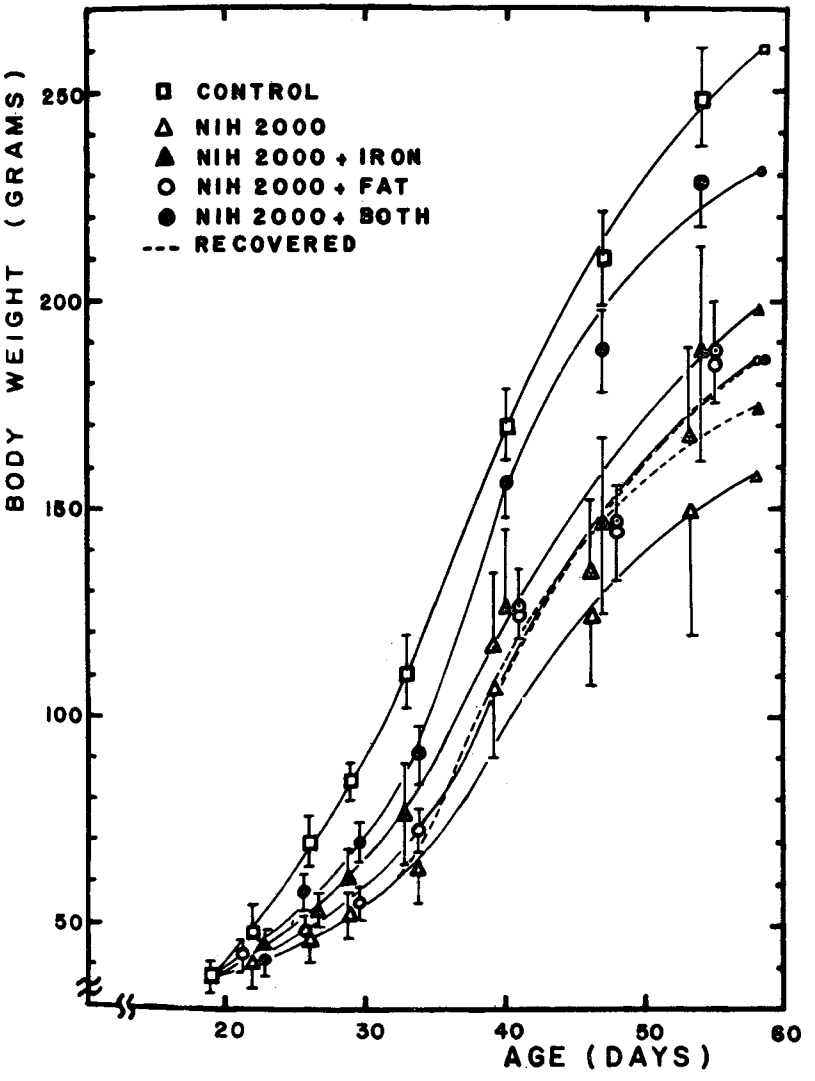

FIGURE 1

Growth curves of male albino rats fed a caries-promoting diet supplemented with essential nutrients

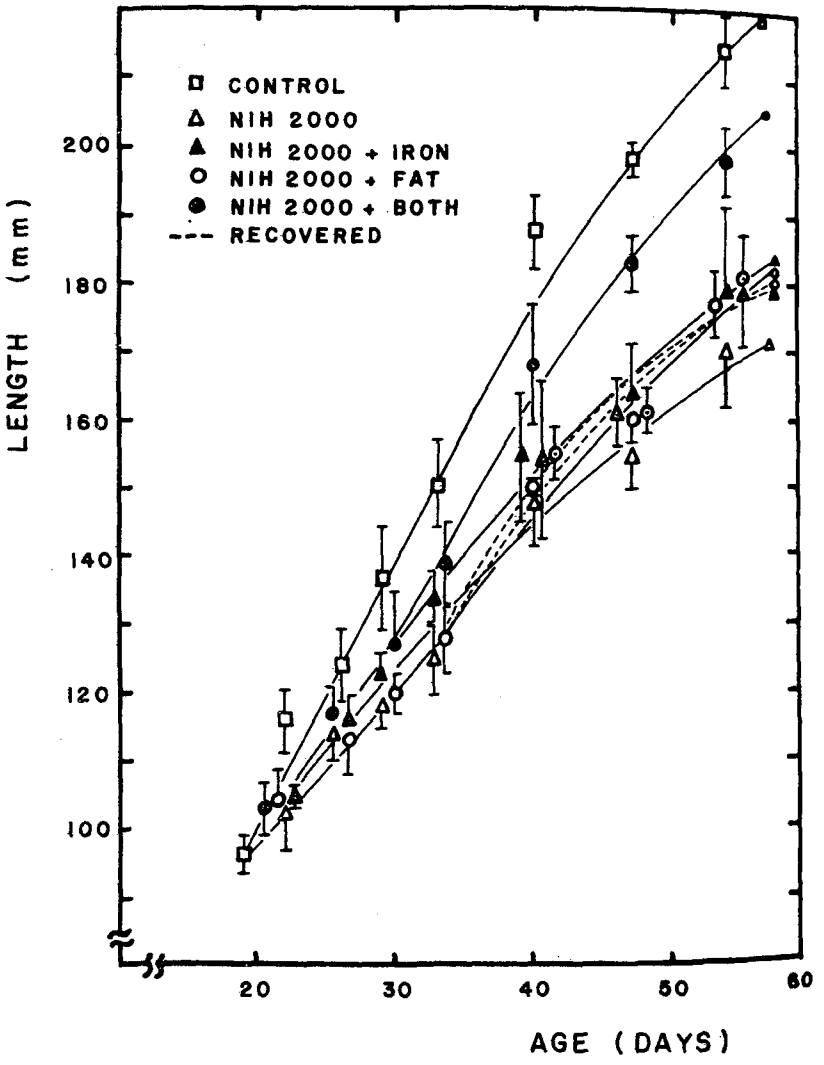

FIGURE 2

Nose-rump length curves of male albino rats fed a caries-promoting diet supplemented with essential nutrients

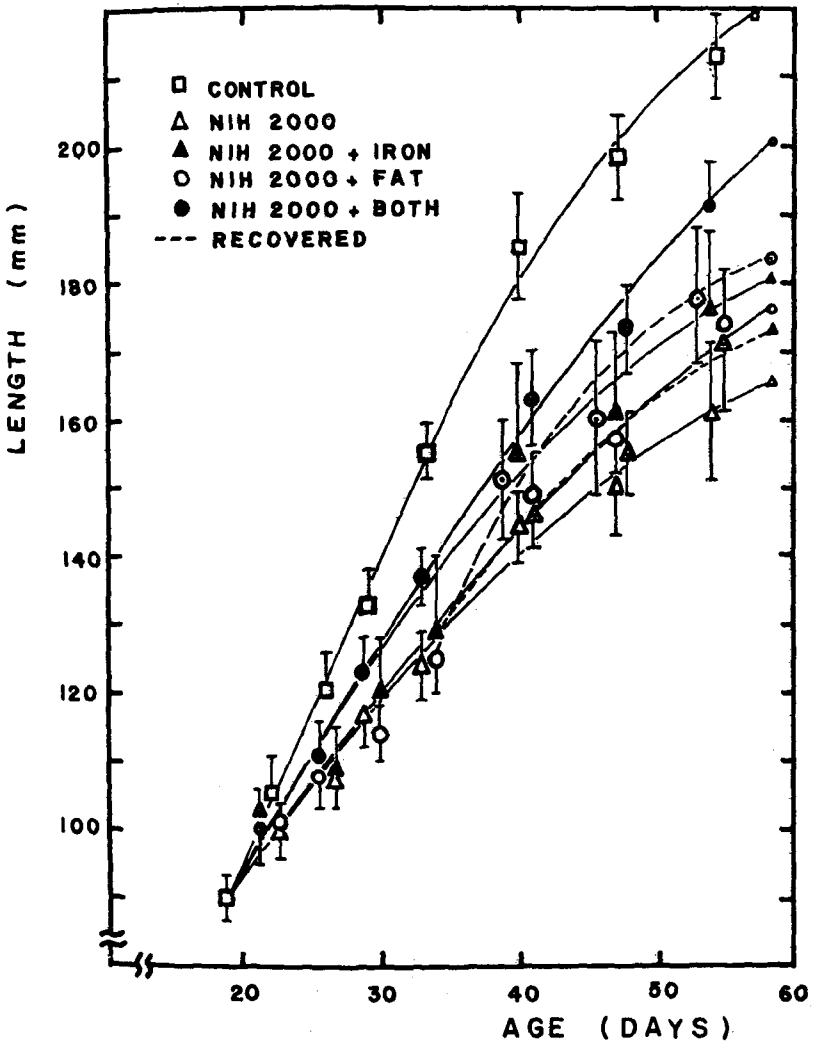

FIGURE 3

Rump-tail length curves of male albino rats fed a caries-promoting diet supplemented with essential nutrients

TABLE 1

INFLUENCE OF SUPPLEMENTATION ON FINAL BODY AND ORGAN WEIGHTS OF POST–WEANING MALE ALBINO RATS[1,2]

Days	Control	MIT 200		NIH 2000	
		Basal	Suppl.[1]	Basal	Suppl.[2]
			Body weight		
40	288.50 ± 20.89	282.40 ± 19.50	300.40 ± 29.77	214.90 ± 18.84[3]	217.00 ± 25.00[3]
60	353.50 ± 24.40	360.35 ± 22.91	355.20 ± 23.18	243.20 ± 25.56[3]	300.90 ± 24.00[3]
			Liver		
40	13.37 ± 1.71	12.90 ± 2.01	14.98 ± 2.98	7.76 ± 1.08[3]	8.46 ± 1.38[3]
60	13.92 ± 1.06	15.73 ± 2.46	14.55 ± 1.50	7.60 ± 2.75[3]	12.20 ± 1.04[3]
			Heart		
40	1.02 ± 0.06	1.04 ± 0.07	1.13 ± 0.05	1.05 ± 0.13	0.95 ± 0.10
60	1.20 ± 0.12	1.21 ± 0.08	1.23 ± 0.11	1.12 ± 0.09	1.14 ± 0.12
			Spleen		
40	0.84 ± 0.28	0.84 ± 0.15	0.96 ± 0.06	0.74 ± 0.13	0.74 ± 0.12
60	0.86 ± 0.21	1.04 ± 0.16	0.75 ± 0.06	0.67 ± 0.13	0.75 ± 0.10

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

1 Supplemented with 1°/o fat.

2 Supplemented with 3°/o fat, 3°/o mineral mix and 1°/o vitamin mix.

3 $p < 0.01$.

TABLE 2

COMBINED AVERAGE CARIES SCORES FOR ALL LESIONS

(Buccal + sulcal + proximal)[1, 2]

Lesión[3]	Control	MIT 200		NIH 2000	
		Basal	Suppl.[1]	Suppl.[2]	Basal
E	2.4	14.3	14.3	16.3	20.1[4]
D_s	0.4	1.9	9.4	9.7[5]	16.8[4]
D_m	0	0.8	3.9	2.9	10.0[5]

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

1 Supplemented with 1% fat.

2 Supplemented wit 3% fat, 3% mineral mix and 1% vitamin mix.

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

4 $p < 0.01$.

5 $p < 0.05$.

TABLE 3

HEMOGLOBIN AND HEMATOCRIT OF MALE ALBINO RATS FED SUPPLEMENTED DIETS[1,2]

	Control	MIT 200		NIH 2000	
		Basal	Suppl.[1]	Suppl.[2]	Basal
			59 days		
Hemoglobin (g%)	15.2 ± 1.1	14.7 ± 1.4	15.0 ± 1.1	14.7 ± 1.7	12.2 ± 1.3[3]
Hematocrit (%)	40.5 ± 4.1	38.1 ± 3.8	37.3 ± 3.2	38.3 ± 5.4	31.8 ± 3.4[3]
			79 days		
Hemoglobin (g%)	16.8 ± 0.8	16.8 ± 1.0	16.8 ± 0.7	16.4 ± 0.7	13.0 ± 1.0[3]
Hematocrit (%)	42.5 ± 1.8	41.6 ± 1.4	41.8 ± 1.2	41.4 ± 1.3	34.1 ± 2.9[3]

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

1 Supplemented with 1% fat.

2 Supplemented with 3% fat, 3% mineral mix and 1% vitamin mix.

3 $p < 0.01$.

Pigmentation of the incisor teeth does not disappear when the supplemented diet is fed.

Finally, there is a 50% reduction in buccal caries lesions (Table 2) in the supplemented NIH 2000 diet when compared to the non-supplemented basal diet.

DISCUSSION

The data obtained indicate that while both caries-promoting diets are inadequate when compared to a diet that satisfies the requirements for growth (1), under the conditions of this experiment diet MIT 200, compares with the standard National Research Council (NRC) diet. In order to obtain better growth of animals fed diet MIT 200 the investigator had to offer the experimental diet in excess of the daily requirement so as to satisfy the caloric deficit caused by the reduced fat with increased intake of food. When this is accomplished we then conclude that MIT 200 is significantly better than NIH 2000.

Supplementation of diet NIH 2000 with fat, minerals and vitamins improves growth of the animal and brings parameters of iron nutriture to the normal levels of controls. Due to the fact that we are dealing with a so-called "natural diet" because its ingredients are added without considering amounts of nutrients supplied, supplementation with a vitamin mixture and a mineral mixture probably increases the requirement of other nutrients. This observation is based on the improved growth yet not quite reaching levels of the controls.

The interesting finding in the supplementation of diet NIH 2000 is the fact that caries scores are reduced 50% in the buccal lesions. This in turn supports the hypothesis that there is a mechanism mediated by a mineral and/or a vitamin which interacts with caries formation in buccal areas. This could be mediated through saliva which is readily available to bathe these surfaces. In another publication (8), supplementation with a specific nutrient (iron) clarifies this mechanism.

RESUMEN

INFLUENCIA DE LA SUPLEMENTACION EN LA CARIES DENTAL Y CRECIMIENTO DE RATAS ALIMENTADAS CON DOS DIETAS MODELO

Con el fin de mejorar la calidad de las dietas MIT 200 y NIH 2000, ambas promotoras de caries, éstas fueron suplementadas con grasa. Además, la dieta NIH 2000 se suplementó con minerales y vitaminas. Se encontró que la suplementación con nutrientes esenciales mejora la calidad de ambas, y refuerza el crecimiento al compararse con el de animales testigo. Asimismo, la adición de minerales y vitaminas a la dieta NIH 2000 abolió la pérdida de pigmentación de los incisivos, mejoró la hemoglobina y el hematocrito, y redujo los puntajes de caries en las superficies bucales en 50%, en contraste con la constatada en las ratas que recibieron la dieta no suplementada. Es posible que las propiedades cariogénicas de la dieta NIH 2000 se deban en parte a la deficiencia de un nutriente dado.

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