GROWTH, DEVELOPMENT AND DENTAL CARIES IN RATS FED TWO EXPERIMENTAL DIETS[1, 2, 3]

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SUMMARY

In order to determine the nutritional adequacy of diets MIT 200 and NIH 2000 on the growth and development of experimental animals, these two diets were fed to two groups of animals during three periods of develop-

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ment: 1) pregnancy; 2) lactation, and 3) post-weaning. These diets were compared with a diet that satisfied the requirements of the National Research Council for growth and maintenance. It was found that for the two experimental diets, growth was compromised due to a caloric deficit in both diets. In addition, NIH 2000 was deficient in other nutrients such as iron. Analysis of other parameters such as hemoglobin, hematocrit, salivary protein, saliva flow and weight of vital organs upon autopsy revealed a picture of iron deficiency in the NIH 2000 group. Caries formation can be precipitated by a mechanism influenced by a nutrient deficit.

INTRODUCTION

Nutrient supply during development can result in formative changes in teeth and salivary glands which may be irreversible. For example, Navia *et al.* (1) and Shaw and Griffiths (2) showed that changes in the form, morphology and eruption patterns of teeth are attributable to protein deficiency during critical developmental periods. In an extension of these observations, Menaker and Navia (3-5) demonstrated that the offspring of female rats fed a low protein diet grew slowly, were smaller in size, and had significantly increased susceptibility to caries relative to control animals, due to alterations in salivary gland morphology and function.

In addition to the intake of protein, a few experiments have been reported in which the effect of feeding diets low in mineral salts (i.e., calcium and phosphorus) have been studied on rat's teeth (6-9). It was found that the weights of the incisors were lower than in control animals and that the percentages of ash were reduced and of water increased (6-10). An important difference between the behavior of bone and teeth was suggested when a diet with normal salt content was fed to animals which were mineral-deficient. The ash content of the bone returned to normal but that of the teeth formed during the deprivation period remained unchanged (11).

Although iron is a major mineral element, and important for hemoglobin formation and the functional activity of a variety of enzymes, it is not considered normally part of the apatite molecule and bone structure. However, it appears essential for the formation of the orange-brown pigment that is characteristic of the enamel of the rodent incisors (1, 12). The pigmentation of the enamel is characteristic of the rat incisors and does not occur in the rat molars offering another criterion by which to evaluate

experimental effects of nutrient deprivation upon teeth. Smith and Lantz (13) for example, observed an absence or diminution of pigment in the incisors of anemic rats. This is a controversial area, for anemia can be caused by a series of nutritional deficits in addition to iron, for example protein, vitamin E, folic acid, vitamin B_{12} and pyridoxine.

Caries-promoting diets have been successful in achieving their goal, that is, to produce caries in short periods of time. Nevertheless, it seems that the amount of some nutrients in the diet NIH 2000, i.e., iron, 16 ppm versus 35 ppm (National Research Council (NRC) recommendation for growing animals) is marginal for the rat. In addition, the constituents of the diet, i.e., whole wheat flour, have been suggested as a possible metal-binding agent that can interfere with the proper absorption of iron (14).

Implicit in the use of the standard caries-promoting diets, MIT 200 and NIH 2000, is their nutritional adequacy for growth and development of the young animal and maintenance of the mature rat. There is little information in the literature, however, concerning these parameters, particularly in terms of long periods of exposure since caries studies are generally restricted to 50 days of feeding or less. The purpose of this study was to determine the nutritional adequacy of diet MIT 200 and NIH 2000 relative to control diet, and to determine the effect of feeding these diets either pre- or postnatally or both, on the animal's subsequent response to a dental caries challenge.

MATERIALS AND METHODS

Experimental Design

In order to determine the effects of standard caries-promoting diets on the growth and development of the animals, the diets were fed during the following periods of development: 1) pregnancy; 2) lactation; and 3) post weaning. The following three diets were evaluated during each of the above periods:

Group

A MIT 25% protein diet (control), (Table 1)

TABLE 1

25% PROTEIN DIET COMPOSITION

	g/kg
Methionine	7
Casein	250
Dextrose	178
Sucrose	150
Dextrin	183
Corn oil	150
Salt mix (Roger-Harper's)[1]	40
Vitamin mix[2]	10
Choline	4

1 $CaCo_3$, 29.29; $CaHPO_4 \cdot 2H_2O$, 0.43; KH_2PO_4, 34.31; NaCl, 25.06; $MgSO_4 \cdot 7H_2O$, 9.98; $Fe(C_6H_5O_7) \cdot 6H_2O$, 0.623; $CuSO_4$, 0.153; $MnSO_4 \cdot H_2O$, 0.121; $ZnCl_2$, 0.020; KI, 0.0005; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.0025; $Na_2SeO_3 \cdot 5H_2O$, 0.0015.

2 Vitamin A acetate and vitamin D_2, 1.0; vitamin E (∝-tocopheryl), 40.0; vitamin K (menadione), 0.5; thiamine, 1.0; riboflavin, 2.0; niacin, 5.0; vitamin C, 20.0; pyridoxine, 1.0; PABA, 10.0; biotin, 0.05; Ca pantothenate, 5.0; folic acid, 0.2; inositol, 20.0; vitamin B_{12}, 0.05 and sucrose, 889.25.

B MIT 25% protein diet, with oral inoculation of *S. mutans*[6]
C MIT 200 diet (Table 2)
D MIT 200 diet, with oral inoculation of *S. mutans*
E NIH 2000 diet (Table 2)
F NIH 2000 diet, with oral inoculation of *S. mutans*

1. Influence of caries-promoting diets during pregnancy

Forty pregnant female rats were delivered at the laboratory, three days after conception. Upon their arrival the dams were weighed, randomly assigned to experimental groups, and given the appropriate diet and deionized water *ad libitum*. Upon delivery,

6 Ingbritt 1600, Streptomyces resistant Strain, Forsyth Dental Center, Boston, Massachusetts, USA.

TABLE 2

COMPOSITION OF CARIOGENIC DIETS

Ingredients	NIH 2000[1]	MIT 200[2]
	g/100 g	g/10C g
Sucrose (6_x, pulverized)	56.0	67.0
Skim milk powder	28.0	—
Wheat flour, whole	6.0	—
Yeast (dry)	4.0	—
Alfalfa meal	3.0	—
NaCl	2.0	—
Liver powder (conc.)	1.0	—
Lactalbumin	—	20.0
Salt mixture	—	3.0
Vitamin mixture	—	1.0
Cottonseed oil	—	3.0
Cellulose	—	6.0

1 Keyes and Jordan, *Arch. Oral Biol., 9:* 377-400, 1964.

2 Navia, Lopez and Harris, *J. Nutr., 97,* 133-140, 1969.

litters were weighed, males separated from females, and litters of eight males prepared. The infant pups used in the experiment were all born within an eight to twelve hour time span. Dams were maintained on their respective diets until completion of lactation.

2. Influence of caries-promoting diets during lactation

Thirty-two dams with litters one day old, each containing eight males were utilized. The dams and litters were weighed, randomly assigned to experimental groups, and housed in plastic breeding tubs; food and deionized water were given *ad libitum.*

3. Influence of caries-promoting diets after weaning

One hundred and twenty-eight 19 day old males were weighed, measured, randomly assigned to experimental groups and housed in groups of eight in plastic breeding cages. Food and deionized water were also given *ad libitum.*

Animal Husbandry

Sprague Dawley rats[7] 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 (15) 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.

Microbiological Assays

To reduce the experimental period, animals were inoculated with a pure culture of *S. mutans.* Prior to inoculation, two animals from each experimental group were randomly chosen and sampled by swabbing the animal's mouth with a wood cotton-tipped sterile swab. The swab was placed in a 0.85o/o NaCl solution, mixed, and serially diluted to two appropriate dilutions (10^1 and 10^2). The dilutions were plated on Blood Agar and on selective media [(Mitis Salivarius Agar (MSA) and Mitis Salivarius Agar + Bacitracin (MSB)] for isolation of streptococci.

At the same time, the animals to be challenged were infected with a pure culture of *S. mutans* I.B. 1600 for three consecutive days. The inoculum was also added to the water for the three days. At the end of the inoculation period animals were placed on suspended stainless steel cages, two per cage. The implantation of *S. mutans* was checked by swabbing 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.

Protein Determination of Saliva

Saliva was collected following the technique described by Menaker and Navia (16) and protein was determined according to the modified method of Lowry *et al.* (17).

7 Charles River Breeding Laboratories, Wilmington, Mass., USA.

Morphometric Analysis

All animals were sacrificed by guillotine. Vital organs including brain, lungs, heart, kidneys, adrenals, liver, thymus, spleen, parotid and submandibular glands were weighed in a Mettler H-10 analytical balance[8] and fixed in 10o/o neutral buffered (NBF) formalin solution for histopathology.

Skulls were fixed in a 10o/o NBF washed thoroughly with running water and placed in the autoclave for 15 min at 15 psi. The jaws were removed, cleaned and weighed in an analytical balance. The right half of the mandible was radiographed using an X-Ray machine[9], with the cone approximately 1" from the specimen. The exposed radiographed mandible was used to measure anatomical landmarks following the description of Bunyard (18).

Intact teeth were removed from jaws according to the technique of Navia *et al.* (19), using a ficin solution. The maxillae and mandible were placed in the ficin solution, in individual 10 ml beakers. Upper right first molars were measured directly with an INOX Dial Caliper accurate to 0.1 mm. Both mesiodistal and buccolingual diameters were determined and the length from tip of mesial cusp to the end of the root.

Mandibles with teeth, teeth alone, upper incisor teeth, upper first molars and upper second molars were weighed in an analytical balance. Pigmentation of the incisor teeth was assessed either as normal (orange color), partly depigmented or completely white (18).

Caries Scores

Mandibular caries were scored according to the method described by Keyes (20). To obtain a better delineation of the extent of involvement of the carious process, mandibles were stained with Murexide (21).

Determination of Hemoglobin and Hematocrit

Because of the low content of iron in the NIH 2000 diet, hemoglobin and hematocrit were assayed. Heparinized micro-

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8 Mettler Corp., Highstown, N.J., USA.

9 Ritter Model G, 65 KVP, 10 ma and 1/10 sec.

hematocrit capillary tubes were used to collect blood samples from the retro-orbital venous plexus of 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 three 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 differed statistically from each other. With both tests, a $p \leqslant 0.05$ was considered to be significant (22).

RESULTS

The principle purpose of this work was to determine whether two frequently used caries-promoting diets were 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 NRC. By analysis, it was determined that the caloric density of diet MIT 200 was lower than that recommended by the NRC standards, due to its lower fat content. In addition to being hypocaloric, NIH 2000 was also deficient in other essential nutrients, particularly iron (Table 3).

Enumeration of organisms obtained by oral swab from animals on the various diets revealed a difference in the counts of bacteria on MSA (4.9×10^3 colonies/ml of saline, NIH 2000; 4.6×10^3, MIT 200; and 4.3×10^3, control). The effect was due probably to the increase in the number of streptococci associated with increased carbohydrate in the diet. A similar difference was also observed on MSB (1.5×10^3 colonies/ml of saline, NIH 2000; 1.3×10^3, MIT 200; and 1.0×10^3, control), a medium favoring the growth of *S. mutans* with increased carbohydrate availability. Therefore, these results suggest that there is less *S. mutans* in animals fed the control diet and more in those fed NIH 2000 (Figure 1).

The inoculation of animals at day 19 with labeled *S. mutans*

TABLE 3

COMPOSITION OF EXPERIMENTAL DIETS

Nutrient	Control	MIT 200	NIH 2000	NRC
Protein	25.0	20.0	14.0	12.0 o/o
CHO	51.9	67.0	78.4	o/o
Fat	15.0	3.0	0.8	5.0 o/o
Ca	4.7	3.42	4.4	0.5 o/o
Cu	24.0	10.5	5.8	5.0 mg/kg
I	0.12	0.87	—	0.15 mg/kg
Fe	45.6	33.0	16.0	35.0 mg/kg
Mg	0.39	0.35	0.09	0.04o/o
Mn	38.0	51.0	54.0	50.0 mg/kg
P	3.12	2.65	3.93	0.4 o/o
K	0.393	0.223	0.568	0.18o/o
Na	0.377	0.260	0.956	0.05o/o
Zn	36.0	41.7	17.2	12.0 mg/kg
Biotin	0.5	0.1	0.13	mg/kg
Choline	4000.0	3000.0	449.0	750.0 mg/kg
Folate	2.0	2.0	1.24	mg/kg
Niacin	50.0	20.0	29.9	15.0 mg/kg
Pantothenic acid	50.0	30.0	14.1	8.0 mg/kg
Pro-vit A	1.0	3.6	3.13	0.6 mg/ret/kg
Piridoxine	10.0	3.0	2.46	7.0 mg/kg
Riboflavin	2.0	6.0	8.37	2.5 mg/kg
Thiamine	1.0	0.8	1.6	1.25 mg/kg
B_{12}	5.0	3.0	1.79	0.005 mg/kg
Vit E	40.0	50.0	27.0	35.0 mg/kg
Vit K	0.5	0.3	—	0.05 mg/kg
Vit D	1000.0	4000.0	1080.0	1000.0 I.U./kg
Vit C	20.0	100.0	20.0	mg/kg

(I.B. 1600, streptomyces resistance strain) did not affect the nutritional status of the animal. This was confirmed by the fact that no difference between inoculated and non-inoculated animals in final weights and weight of organs upon autopsy was detected (Tables 4 and 5). The only observed difference was in the caries scores (Table 6).

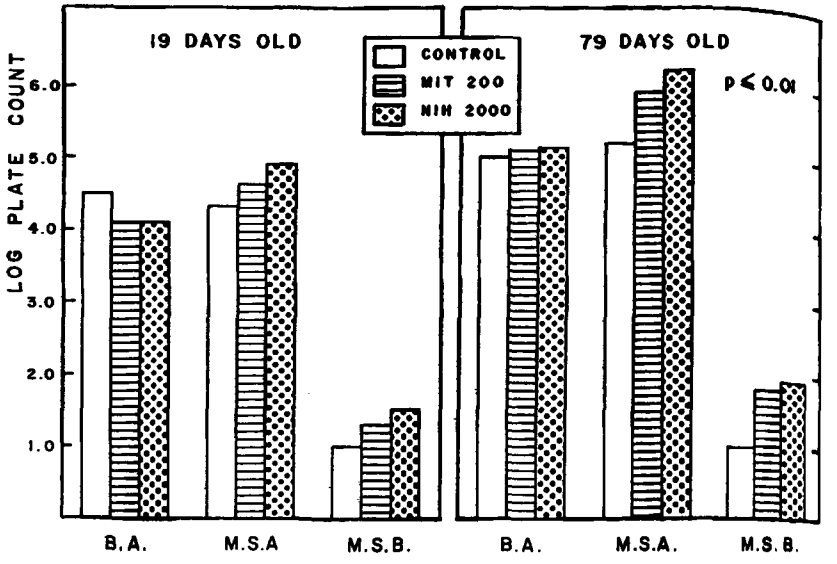

FIGURE 1

Relative counts of bacterial sampling from male albino rats on B.A., M.S.A., and M.S.B. at 19 and 79 days of age

Measuring growth by weight gain of the animal revealed that both NIH 2000 and MIT 200 caries-promoting diets, under the experimental conditions used, did not sustain growth at a rate comparable to animals fed the control diets.

Weight as a measure of growth of the animal revealed that caries-promoting diet MIT 200 supports growth comparable to that of the control until approximately 5-6 weeks of feeding, when growth of animals fed this diet falls off the growth curve of the control animals. Diet NIH 2000 did not support growth when compared to the control at any time during the study (Figures 2-4). No differences in food intake were observed among the various groups until the sixth week after birth when the animals fed the NIH 2000 started to lose their appetite.

The appearance of the fur was normal for the control group and those fed the MIT 200, but the NIH 2000 group started to

TABLE 4

FINAL BODY AND ORGAN WEIGHTS OF 60 DAYS POST–WEANING MALE ALBINO RATS

	Control	MIT 200	NIH 2000
B. W.	377.88 ± 31.30	325.75 ± 43.78	259.63 ± 26.20[1]
Heart	1.22 ± 0.11	0.98 ± 0.10	1.00 ± 0.11
Lungs	1.75 ± 0.28	1.53 ± 0.32	1.63 ± 0.14
Kidney	1.52 ± 0.13	1.20 ± 0.19[1]	0.95 ± 0.13[2]
Liver	15.93 ± 2.36	12.98 ± 2.82	8.90 ± 1.11[2]
Spleen	0.90 ± 0.37	0.54 ± 0.10	0.55 ± 0.07
Adrenals	0.0512 ± 0.0067	0.0387 ± 0.0082	0.0365 ± 0.0063
Brain	1.88 ± 0.10	1.77 ± 0.10	1.78 ± 0.09
Thymus	0.66 ± 0.13	0.44 ± 0.13	0.49 ± 0.10
Submand. glands	0.60 ± 0.15	0.55 ± 0.04	0.45 ± 0.07
Parotid glands	0.23 ± 0.05	0.18 ± 0.03	0.17 ± 0.02

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

1 $p < 0.05$.

2 $p < 0.01$.

TABLE 5

FINAL BODY AND ORGAN WEIGHTS OF 60 DAYS POST–WEANING MALE ALBINO RATS[1]

	Control	MIT 200	NIH 2000
B. W.	341.63 ± 41.17	344.25 ± 34.42	261.88 ± 34.04[2]
Heart	1.19 ± 0.11	1.14 ± 0.12	1.14 ± 0.11
Lungs	2.06 ± 0.48	1.84 ± 0.24	1.87 ± 0.40
Kidney	1.41 ± 0.24	1.37 ± 0.13	1.01 ± 0.10[2]
Liver	12.17 ± 3.00	12.64 ± 2.46	8.96 ± 1.27
Spleen	0.69 ± 0.13	0.67 ± 0.12	0.54 ± 0.08
Adrenals	0.0420 ± 0.0130	0.0484 ± 0.0127	0.0318 ± 0.0052
Brain	1.89 ± 0.12	1.90 ± 0.07	1.83 ± 0.07
Thymus	0.64 ± 0.12	0.69 ± 0.18	0.52 ± 0.10
Submand. glands	0.55 ± 0.12	0.59 ± 0.08	0.52 ± 0.08
Parotid glands	0.24 ± 0.04	0.23 ± 0.03	0.16 ± 0.05

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

1 Inoculated.

2 $p < 0.01$.

TABLE 6

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

Lesion[2]	Control	MIT 200	NIH 2000
	Weaning		
E	0.1	6.7[3]	11.7[3]
	(3.9)	(10.0)	(16.6)
D_s	0	2.7	6.8[3]
	1.4	5.2[4]	10.8[3]
D_m	0	0.3	1.5[4]
	(0)	(0.3)	(7.4)
	Lactation		
E	1.3	5.4[4]	15.2[3]
D_s	0	1.9	8.6[3]
D_m	0	0	6.7[3]

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

1 The numbers in parentheses are scores for inoculated animals.

2 E= enamel only; D_S= slight dentinal and D_m= moderate dentinal.

3 $p < 0.01$.

4 $p < 0.05$.

show skin lesions with specific areas of alopecia on the dorsal side of the neck and skin of the tail slightly to moderately scaled. This condition was evident in all developmental groups (pregnancy, lactation, and post weaning).

Analysis of stimulated salivary volume during a fixed period of time revealed a significant difference ($p \leq 0.01$) between controls and NIH 2000 in post-weaning animals at both ages. For MIT 200, the only significant difference was found in the post-weaning period ($p \leq 0.05$) at 79 days of age. If diets were

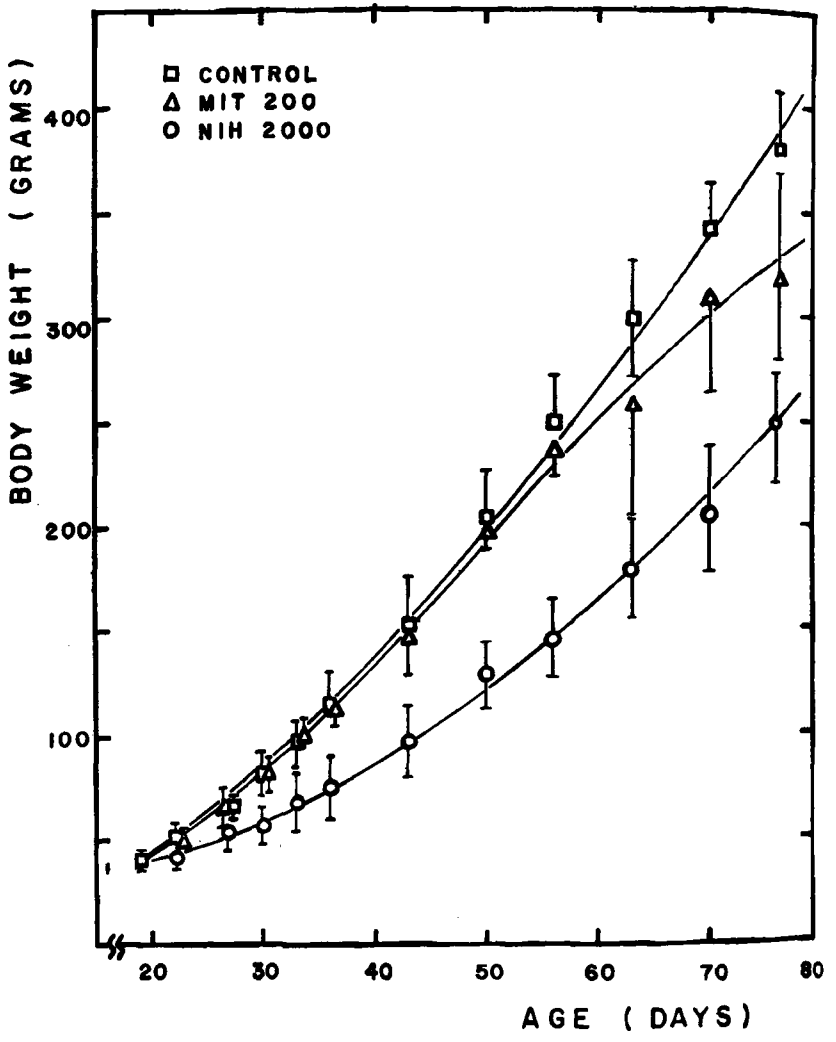

FIGURE 2

Growth of male albino rats fed a caries-promoting diet post-weaning

introduced during the suckling period there was no significant difference at both ages (Table 7). The salivary protein content differed in groups fed either MIT 200 and NIH 2000 when

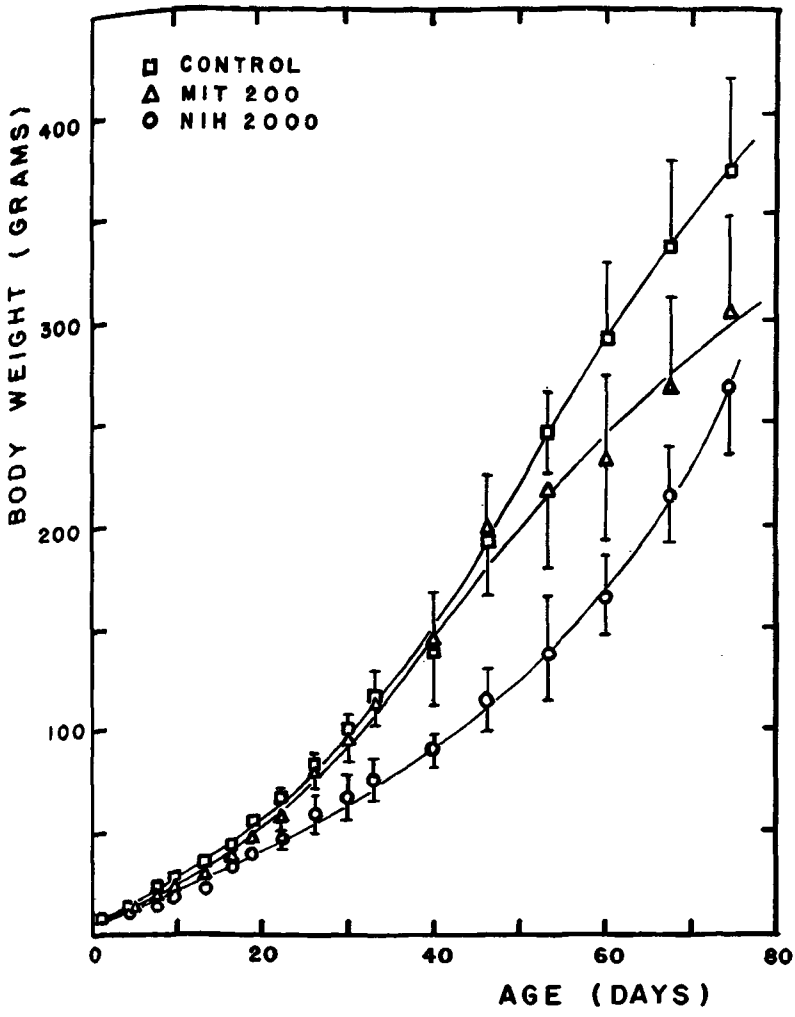

FIGURE 3

Growth curves of male albino rats fed a caries-promoting diet during lactation and weaning

compared to controls, especially when diets were introduced during the suckling period (Table 8).

Upon autopsy the weight of several organs was compared to those of the control group (Table 4). In the animals fed the NIH

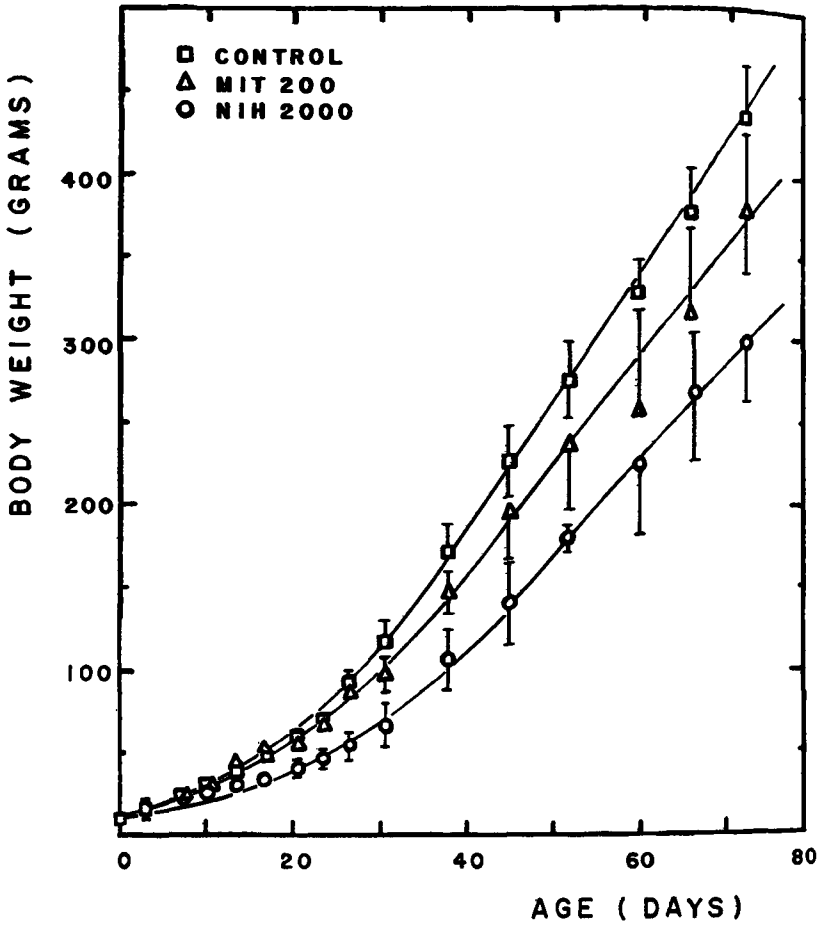

FIGURE 4

Growth curves of male albino rats fed a caries-promoting diet pre-natally, and during lactation and weaning

2000 diet, the weight of the heart, expressed as per cent body weight, increased (Table 9). Examination of the heart revealed hypertrophy of the left ventricle and dilation mainly of the right side. This pathology was present in animals as young as 59 days old (post-weaning group). Upon examination under the light microscope, no morphological changes between the control diet

TABLE 7

STIMULATED SALIVARY VOLUME PER RAT DURING A FIFTEEN MINUTE PERIOD

Age (days)	Control (ml/rat)	MIT 200 (ml/rat)	NIH 2000 (ml/rat)
	Weaning		
59	0.75 ± 0.08	0.71 ± 0.11	0.40 ± 0.09[1]
79	1.39 ± 0.09	1.12 ± 0.10[2]	0.88 ± 0.12[1]
	Lactation and Weaning		
59	0.89 ± 0.11	0.88 ± 0.16	0.85 ± 0.23
79	1.16 ± 0.04	1.10 ± 0.04	1.11 ± 0.03

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

1 $p < 0.01$.

2 $p < 0.05$.

and experimental diet NIH 2000 were observed in the following tissues and organs: bone, muscle, liver, heart and spleen.

The mandibular X-rays were examined and measured for anatomical landmarks. Differences were observed in all parameters between control and the NIH 2000, except for the combined mesio-distal length of first and second molars.

The size of the first upper molar of the animals fed NIH 2000 for the post-weaning group did not differ from that of the controls. This was expected, for these teeth were already formed when the animals were exposed to the diets.

There was no difference in weight of mandible, the extracted teeth of the mandible, the upper incisors, upper first molars and upper second molars at 40 days in the post-weaning group, but there was a significant difference at 60 days for all parameters measured.

Inspection of the color of the teeth revealed that the normal

TABLE 8

SALIVARY PROTEIN CONTENT IN A FIFTEEN MINUTE PERIOD

Age (days)	Control (mg o/o)	MIT 200 (mg o/o)	NIH 2000 (mg o/o)
	Weaning		
59	426.0 ± 6.1	445.0 ± 5.6[1]	522.0 ± 3.7[2]
79	490.0 ± 9.4	499.0 ± 9.2	491.0 ± 4.5
	Lactation and Weaning		
59	599.0 ± 7.9	445.0 ± 5.4[2]	481.0 ± 8.0[2]
79	551.0 ± 4.2	477.0 ± 2.2[2]	472.0 ± 3.2[2]

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

1 $p < 0.05$.

2 $p < 0.01$.

orange pigmentation of the incisors was present at the end of both periods in all developmental stages and in all experimental groups with the exception of teeth from animals fed NIH 2000. In these, the pigment was absent at all stages; thus, the teeth were totally white or partially white.

The caries scores were lower in animals fed the control diet when compared to those fed NIH 2000. It is interesting to note that there were more smooth caries lesions in animals fed the NIH 2000 diet than in the other groups. This area was still immature when the molars erupted and, at that point, the animals were stressed not only with weaning but also with super inoculation as well as exposure to the caries-promoting diets. As stated previously, there was also a difference between the caries scores of the non-inoculated and the inoculated groups (Table 6).

Because of the low content of iron in the NIH 2000 diet (16 mg/kg of diet), hemoglobin and hematocrit were assayed. There was a decrease in the hemoglobin and the hematocrit of the NIH 2000 as compared to the control in all experimental periods (Table 10).

TABLE 9

FINAL WEIGHT OF ORGANS PER 100 g OF BODY WEIGHT OF 40 DAYS POST–WEANING MALE ALBINO RATS

	Control	MIT 200	NIH 2000
B. W.	252.25 ± 18.58	238.25 ± 16.94	163.00 ± 16.37[1]
Heart	0.38 ± 0.03	0.33 ± 0.04[2]	0.54 ± 0.18
Lungs	0.57 ± 0.13	0.53 ± 0.04	0.70 ± 0.19
Kidney	0.48 ± 0.02	0.41 ± 0.04	0.42 ± 0.04
Liver	4.86 ± 0.41	4.87 ± 1.08	0.30 ± 0.61
Spleen	0.32 ± 0.07	0.24 ± 0.06	0.30 ± 0.10
Adrenals	0.015 ± 0.003	0.013 ± 0.002	0.018 ± 0.006
Brain	0.73 ± 0.06	0.75 ± 0.05	1.04 ± 0.09[1]
Thymus	0.29 ± 0.08	0.26 ± 0.08	0.22 ± 0.05
Submand. glands	0.10 ± 0.02	0.11 ± 0.03	0.12 ± 0.01
Parotid glands	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01

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

1 $p < 0.01$.

2 $p < 0.05$.

TABLE 10

HEMOGLOBIN AND HEMATOCRIT OF MALE ALBINO RATS EXPOSED TO DIETS DURING DIFFERENT DEVELOPMENTAL PERIODS[1,2]

		Control	MIT 200	NIH 2000
		40 days		
Hematocrit	W	45.5 ± 2.9	42.7 ± 4.0	39.8 ± 2.9[3]
(o/o)	L	44.0 ± 2.6	42.2 ± 2.6	39.5 ± 1.7[3]
	P	51.7 ± 2.9	48.2 ± 0.8	36.9 ± 2.2[4]
Hemoglobin	W	14.3 ± 2.1	13.5 ± 0.5	10.8 ± 1.4[3]
(g o/o)	L	14.4 ± 1.4	13.4 ± 2.1	11.9 ± 0.6[3]
	P	15.9 ± 1.0	14.6 ± 1.0	10.7 ± 0.4[4]
		60 days		
Hematocrit	W	48.2 ± 1.0	48.0 ± 0.0	40.0 ± 3.0[4]
(o/o)	L	50.1 ± 1.9	47.0 ± 0.6	41.2 ± 1.7[4]
	P	51.8 ± 1.7	50.0 ± 1.9	40.5 ± 3.0[4]
Hemoglobin	W	14.4 ± 1.0	14.3 ± 0.6	11.0 ± 2.9[4]
(g o/o)	L	15.6 ± 1.0	14.5 ± 0.6	12.4 ± 0.6[4]
	P	16.5 ± 0.8	15.8 ± 1.0	12.5 ± 0.4[4]

1 W = post-weaning; L = lactation; P = pre-natally.

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

3 $p < 0.05$.

4 $p < 0.01$.

DISCUSSION

The caloric density for both experimental diets was found to be lower than that recommended by the NRC standards for the

rat. This was due to the low fat content of the caries-promoting diets. Final examination of weight gains revealed that under the experimental conditions used, both caries-promoting diets did not sustain growth of rats at a rate comparable to those fed the control diets. It is possible that the factor restricting rat growth in these experiments was the level of dietary fat. This is critical, for it is at this time that the animal is reaching maturity, and suggests that the higher caloric need of this age is not met by the diets studied.

Menaker and Navia (4) have shown that caries susceptibility in the rat can be increased by inflicting marginal protein malnutrition during tooth development. One mechanism suggested was based on changes in salivary gland development and function. Malnutrition imposed on rats during the suckling period produced diminished protein synthetic and secretory capacity of the salivary glands. Under these conditions, rats challenged after weaning with a caries-promoting diet have higher caries scores than rats fed adequately during this period.

The reduced salivary volume in both age groups fed the NIH 2000 diet is evidence that changes which occurred in these glands may be the result of a marginal nutrient deficiency.

Iron deficiency is probably the most frequent single nutritional deficit encountered in both industrialized and underdeveloped countries, while iron deficiency anemia is the most common type of anemia observed. The incidence of this anemia may be as high as 30o/o in certain high risk groups such as children and women of child-bearing age. In addition, recent findings indicate that iron deficiency may be more common than is generally recognized in apparently healthy young men (23).

The principal causes of iron depletion are related to chronic blood loss, increased physiologic demands of iron, or intestinal malabsorption syndromes. Ultimately, most of these cases are the result of, or complicated by, diets containing inadequate iron. When body iron stores are depleted, anemia develops. Since it occurs as a late manifestation of iron deficit, the incidence of latent iron deficiency is undoubtedly quite high throughout the world.

Iron deficiency is associated with differences in weight and, consequently, blood volume of the animals. These differences may result from the adverse effects of the deficiency on metabolism (24). Iron is required by every cell; thus, presumably any cell may suffer as a consequence of iron deficiency. Dermal

changes are easy to observe in some patients with severe iron deficiency as well as blood losses (25).

Since hemoglobin deficiency *per se* does not appear to be a reasonable candidate for the role of biochemical mediator in functional disturbances, enzymes and metabolic pathways known to require iron in some form for normal function have also been identified. It has been possible to demonstrate deficiencies in some of these enzymes or pathways in iron-deficient animals and in man, i.e., proto-collagen proline hydroxylase (26). This enzyme is reduced in activity in rat periodontal membrane and in other rat tissues during iron deficiency (27). Moreover, buccal mucosa, with an extremely rapid turnover, is both more sensitive to depletion and more quickly responsive to repletion than a tissue such as skeletal muscle.

Further support for a role for iron deficiency in the effects observed when diet 2000 was fed, is found when the changes in tooth pigmentation are considered. Pigmentation on rat enamel is generally believed to be a function of iron availability (28-31).

The studies of Menaker and Navia (5) demonstrated that marginal protein deficiency can produce increased susceptibility of the tooth to caries in the rat. There appears to be a close connection between increased caries incidence and iron deficiency as evidenced by results with diet NIH 2000.

Final evidence for iron deficiency playing a role in cariogenesis on diet NIH 2000 is obtained from the refeeding experiments which are discussed in another publication. Animals fed diet NIH 2000 supplemented with iron from the beginning of the experiments had lower caries scores especially in the bucal surfaces.

RESUMEN

CRECIMIENTO, DESARROLLO Y CARIES DENTAL EN RATAS ALIMENTADAS CON DOS DIETAS EXPERIMENTALES

Se proporcionaron las dietas MIT 200 y NIH 2000 a dos grupos de animales experimentales con el fin de determinar su adecuación nutricional en relación al crecimiento y desarrollo de las ratas. Estas dietas se les administraron a dos grupos de animales durante tres períodos de desarrollo: 1) preñez; 2) lactancia, y 3) post-destete. Luego se compararon con otra dieta que se ajustaba a los requerimientos para crecimiento y mantenimiento

establecidos por el Consejo Nacional de Investigaciones de los Estados Unidos. Se encontró en ambos casos, que debido a su déficit calórico el crecimiento era afectado por las dos dietas experimentales. Además, la dieta NIH 2000 era deficiente en otros nutrientes, hierro, por ejemplo. El análisis de otros parámetros tales como hemoglobina, hematocrito, proteína salivar, flujo salivar y peso de los órganos vitales al momento de la autopsia, reveló un cuadro de deficiencia de hierro en el grupo alimentado con NIH 2000. La formación de caries puede ser precipitada mediante un mecanismo que es influenciado por el déficit de un nutriente.

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