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## EXPERIMENTAL CARIES WITH HUMAN FOODS

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SEVERAL types of diets have been used in the study of dental caries. One of the more common diets<sup>1, 2</sup> used contains ingredients, such as coarse corn meal, which cause fracturing associated with caries production. Another general type of diet<sup>3, 4, 5</sup> is largely synthetic and contains large amounts of sucrose. A third dietary type<sup>6, 7, 8</sup> contains human foods with smaller amounts of sucrose. In this latter group, a limited variety of human foods are used, which are dehydrated, ground in a mill, and fed to the animals. As generally recognized, particle size, moisture content, and physical consistency of the food may affect the amount of caries produced.

The caries-susceptible albino rats used in these studies were descendants of 8 animals obtained from the Naval Medical Research Institute at Bethesda, Maryland.

The diets employed represent the average American diet and modifications thereof and are composed of human foods as customarily prepared and eaten. The foods were thoroughly mixed to make a uniform moist mass of rations which were fed to the animals ad libitum.

### MATERIALS AND METHODS

*Basis of Formulating the Diets.*—The percentage composition of the diet is based on the table of U. S. food supply as computed by Callison and co-workers.<sup>9</sup> This is in close agreement with the findings of Hardinge and Stare<sup>10</sup> who made a study of food consumption in California.

From the basic diet thus computed, the cereals and desserts were removed and were substituted on an equicaloric basis with whole grain cereals (in place of refined cereals), and fruits or fruits and vegetables (in place of desserts). The latter presented the problem of replacing concentrated foods high in calories with foods low in calories but high in fiber and water.

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In the consumption of the individual foods or food groups in both the basic ration and the accessory groups, namely cereals, fruits, vegetables, and deserts, the amount of the individual constituents were proportioned according to the average American per capita consumption.<sup>11</sup> For practical reasons, only representative foods, used in the largest amounts in each food group, were proportioned according to their popularity in this country as follows: meat—beef and pork; dairy products—milk, cheese, eggs; potatoes—white; vegetables other than potatoes—carrots, cabbage; fruits—apple, orange, and banana; cereals—refined wheat and corn, whole wheat and corn; desserts—cake and pie; fats—dairy butter, lard, hydrogenated shortening. The composition of the various foods groups is shown in Table I.

TABLE I  
COMPOSITION OF FOOD GROUPS

GROUP	FOODS	WEIGHT (GM.)	GROUP	FOODS	WEIGHT (GM.)	
A	Basic Ration		C	Whole Grain Cereals		
	Canned beef	61		Whole wheat bread	824	
	Canned pork	51		Unbolted corn meal	176	
	B	Canned potatoes	120	D	Desserts	
		Whole milk	610		Cake, plain white, iced	502
		Canned carrots	83	Pie, lemon meringue	498	
		Raw cabbage	92	E	Fruits	
		Canned kidney beans	100		Apple, raw	394
		Eggs	54		Orange	444
		Raw apple	94	Banana, raw	162	
		Orange	89	F	Fruits and Vegetables	
		Canned peaches	41		Carrots, raw	122
		Butter	23		Cabbage, raw	270
Crisco	19	Apple, raw	240			
Lard	20	Orange	270			
		Banana, raw	98			
B	Refined Cereals					
	White enriched bread	807				
	Refined corn meal	193				

The diets consisted of many of the combinations of these groups, as shown in Table II. The quantity of food mixture from individual groups combined to make up the several diets, was so proportioned as to keep the diets isocaloric, as well as to provide nearly equal intakes of carbohydrate and protein.

TABLE II  
COMPOSITION OF DIETS

DIET	FOOD GROUPS	WEIGHT (GM.)	DIET	FOOD GROUPS	WEIGHT (GM.)
I	A	784	V	A	504
	B	95		C	69
	D	121		E	427
II	A	508	VI	A	459
	B	62		C	63
	E	430		F	478
III	A	462	VII	A	892
	B	56		B	108
	F	482	VIII	A	880
IV	A	774		C	120
	C	106			
	D	120			

For purposes of comparison a modification of the synthetic diet of Sognaes<sup>5</sup> was fed to Group IX as a control. This diet had the following composition: casein, 24; sucrose, 65; salts U.S.P. XIV, 4; dehydrated liver, 4; vitamin mix,<sup>2</sup> 2; choline, 0.2; corn oil, 5.

An analysis of the various diets is given in Table III.

TABLE III  
FOOD ANALYSIS OF VARIOUS DIETS

DIET	WEIGHT (GM.)	CAL- ORIES	PRO- TEIN (GM.)	FAT (GM.)	CHO (GM.)	Ca (MG.)	P (MG.)	Fe (MG.)	A* (I. U.)	C (MG.)	B <sub>1</sub> (MG.)	B <sub>2</sub> (MG.)	NIA- CIN (MG.)
I ABD	1902	3000	86	108	334	1165	1375	10.8	13,300	125	1.68	2.73	9.58
II ABE	2931	3000	85	93	403	1237	1434	14.7	15,400	444	3.09	2.98	12.6
III ABF	3225	3000	91	93	395	1444	1576	17.0	35,700	584	3.53	4.13	13.8
IV ACD	1925	3000	89	108	339	1232	1758	14.1	13,400	125	3.45	2.86	13.6
V ACE	2954	3000	88	93	408	1304	1814	18.9	13,900	444	3.60	3.10	16.6
VI ACF	3248	3000	94	94	400	1511	1956	20.0	35,700	584	4.05	4.29	17.9
VII AB	2228	3000	101	119	289	1336	1600	14.0	17,200	167	2.17	3.31	12.12
VIII AC	2258	3000	105	120	299	1431	2107	17.2	17,600	167	2.84	3.59	17.6

\*Includes carotene.

The foods comprising each diet were passed through a Hobart food grinder twice and then thoroughly mixed. To limit further the preferential selection of individual foods within a diet, only enough food was given to last the animals 1 day. The foods were prepared fresh 3 times a week and stored in the refrigerator until served to the animals. To make the foods as uniform as possible throughout the 16 weeks, canned foods were purchased by the case.

One hundred seventeen weanling rats, 21 days of age, were divided by litters into 9 groups of 13 animals each and placed on their respective diets. Similar groups and numbers of animals were employed for successive generations. Ten weeks after weaning, 3 females and 1 male, generally littermates, were selected from each of the 9 diet groups for breeding each generation. The animals remained on their respective diets throughout breeding, gestation, and lactation. At the end of 16 weeks, the animals were sacrificed and the teeth examined with a 20 × dissecting microscope by grinding successive planes parallel to occlusal surface. Individual charts which showed the relationship of the enamel, dentin, and the pulp of each molar were used to record the carious process. The lesions were scored by the method of Shaw and co-workers.<sup>3</sup>

The nutritional status of the animals from generation to generation was quantitatively evaluated by noting weaning weights, weekly gain, total weight, length of mandible (measured from the distal surface of the third molar to the tip of the incisor), ability to reproduce, and general physical appearance. Considerable difficulty was encountered in breeding the third generation of animals on Diet VI. Twenty-one days following birth, the young weighed an average of 18 grams. Since this weight was too small for weaning, the animals were left with their mothers for an additional 2 weeks to attain sufficient weight.

FINDINGS

The caries score and nutritional status were determined for each group of animals through successive generations. This information is summarized in Table IV.

TABLE IV  
THE EFFECT OF VARIOUS DIETS ON CARIES SCORE AND NUTRITIONAL STATUS THROUGH SUCCESSIVE GENERATIONS

TYPE OF DIET	NUMBER OF ANIMALS	GENERATION	CARIES SCORE	STANDARD ERROR OF THE MEAN	WEANING WEIGHT	NUTRITIONAL STATUS				LENGTH OF MANDIBLE	GENERAL APPEARANCE
						AVERAGE WEEKLY GAIN	TEN-WEEK WEIGHT	REPETITION OF BLEEDING	REPEATED BLEEDING		
I ABD	13	1	13.6	3.88	56	20.2	228			*	
	13	2	23.0	4.16	43	21.0	236	1		19.51	
	13	3	23.2	2.64	35	17.5	204	2		19.13	
II ABE	13	1	11.8	2.76	53	18.0	201			*	
	13	2	26.5	5.20	26	14.2	152			19.15	
	13	3	13.3	2.36	39	15.4	184			19.48	
III ABF	13	1	7.0	1.42	54	18.0	202			*	
	13	2	8.6	1.51	37	15.6	153			19.37	
	13	3	14.1	2.66	27	11.8	147			19.51	
IV ACD	13	1	1.3	0.84	57	23.0	228			*	Poor
	13	2	16.3	3.54	34	19.0	199			19.23	
	13	3	28.8	5.65	30	18.0	216			19.97	
V ACE	13	1	2.33	0.98	58	20.0	215			*	
	13	2	13.8	2.53	31	15.6	160			18.62	
	13	3	10.5	1.76	39	16.8	195			19.27	
VI ACF	13	1	5.5	1.47	55	15.8	192			*	
	13	2	27.7	4.76	38	13.6	180	2		18.90	
	13	3	37.5	3.58	18	11.0	151	3		18.63	
VII AB	13	1	1.5	0.62	55	29.0	232			*	
	13	2	1.1	0.40	52	23.8	228			19.64	
	13	3	7.7	2.18	48	23.6	254			20.16	
VIII AC	13	1	0.23	0.16	56	26.2	255			*	Good
	13	2	1.2	0.55	38	21.6	204			19.39	
	13	3	2.8	0.99	45	21.6	236			19.79	
IX SYN.	13	1	6.3	2.86	55	26.4	245			*	
	13	2	4.2	1.02	49	30.2	260			19.89	
	13	3	10.5	2.68	64	25.2	256			19.96	

\*First generation mandibles not available.

TABLE V  
TABLE OF CARIES INDEX AND NUTRITIONAL INDEX AS DETERMINED FROM PHYSICAL FINDINGS

GROUP	GENERATION	CARIES INDEX (C)	WEANING WEIGHT	AVERAGE WEEKLY GAIN	10-WEEK WEIGHT	BREEDING	MANDIBLE LENGTH	GENERAL APPEARANCE	NUTRITIONAL INDEX (X)
I	1	64.1	82.6	47.9	71.7	100	*	25	65.4
	2	38.9	54.3	52.1	69.9	67	57.9	25	54.3
	3	38.4	37.0	33.9	50.0	33	33.2	25	35.4
II	1	69.0	76.1	36.5	47.8	100	*	25	57.1
	2	29.5	17.4	16.7	4.4	100	34.4	25	33.0
	3	64.9	45.7	22.9	32.7	100	55.8	25	47.1
III	1	81.8	78.3	36.5	48.7	100	*	25	57.7
	2	77.5	43.3	24.0	5.3	100	48.7	25	40.7
	3	62.8	19.6	4.2	00.0	100	57.8	25	34.5
IV	1	97.1	84.8	62.5	71.7	100	*	25	68.8
	2	56.9	34.8	41.7	46.0	100	39.6	25	47.7
	3	23.3	26.1	36.5	61.1	100	22.7	25	45.3
V	1	94.4	87.0	46.9	60.2	100	*	25	63.8
	2	63.9	28.3	24.0	11.5	100	0.0	25	31.5
	3	72.4	45.7	30.2	42.5	100	42.2	25	47.6
VI	1	85.9	80.4	25.0	39.8	100	*	25	54.0
	2	26.3	43.5	13.5	29.2	33	18.2	25	27.0
	3	00.0	0.0	0.0	3.5	00	0.7	25	4.9
VII	1	96.9	80.4	93.8	75.2	100	*	75	84.9
	2	97.7	73.9	66.7	71.7	100	66.2	75	75.6
	3	80.0	65.2	65.6	94.7	100	100.0	75	83.5
VIII	1	100.0	82.6	79.6	95.6	100	*	75	86.6
	2	97.4	43.5	55.2	50.0	100	50.0	75	62.0
	3	93.1	58.7	55.2	78.8	100	76.0	75	73.9
IX	1	83.7	80.1	80.2	86.7	100	*	75	84.5
	2	89.3	67.4	100	100	100	82.5	75	87.5
	3	72.4	100	74.0	96.5	100	87.0	75	88.8
Mean		68.8							57.1

\*First Generation mandible measurement not available.

In order to evaluate the nutritional state and the incidence of caries, the various measurements used to define nutritional state were scored on a scale of 1 to 100. An average for the nutritional state of each group was determined. For purposes of convenience this has been called the "nutritional index." Similarly the caries score was rated in order to obtain what has been called the "caries index." These are presented in Table V.

As seen in Table VI the differences in length of mandibles in the third generation of animals are small. The mean length of mandibles of each group was statistically compared with the mean length of the group having the shortest mandibles, namely Group VI. As can be seen from Table VI the variations in mandibular lengths are highly significant.

TABLE VI  
STATISTICAL ANALYSIS OF MANDIBLE MEASUREMENTS\* OF THIRD GENERATION ON VARIOUS DIETS

			<i>Significance</i>
Diet I	X = 19.13 $\sigma$ = 0.6 SE = 0.172	t = 2.31	.04
Diet II	X = 19.48 $\sigma$ = 0.435 SE = 0.125	t = 4.61	.01
Diet III	X = 11.51 $\sigma$ = 0.624 SE = 0.208	t = 3.53	.01
Diet IV	X = 18.97 $\sigma$ = 0.787 SE = 0.227	t = 1.21	>.05
Diet V	X = 19.27 $\sigma$ = 0.485 SE = 0.140	t = 3.33	.01
Diet VI	X = 18.63 $\sigma$ = 0.469 SE = 0.132		
Diet VII	X = 20.16 $\sigma$ = 0.538 SE = 0.156	t = 7.50	.001
Diet VIII	X = 19.79 $\sigma$ = 0.705 SE = 0.213	t = 4.64	.01
Diet IX	X = 19.96 $\sigma$ = 0.608 SE = 0.180	t = 5.96	.01

\*Mean mandible length of respective groups compared with mean mandible length of group with shortest mandible, i.e., Diet VI.

#### DISCUSSION

A detailed analysis of the nutrient content of the various diets used in this study was presented. The long-term effect of these diets is of special significance since minor differences in nutrient content will tend to be either compensated for or magnified through successive generations. An attempt has

been made to compare these differences by means of the nutritional and caries indices. By formulating a caries index "C" and a nutritional index "N," regression lines were derived and the coefficient of correlation calculated for these indices (Fig. 1). The regression of "C" on "N" is 0.965. The coefficient of correlation is  $0.78 \pm 0.05$ . If the nutritional index is known, the most probable

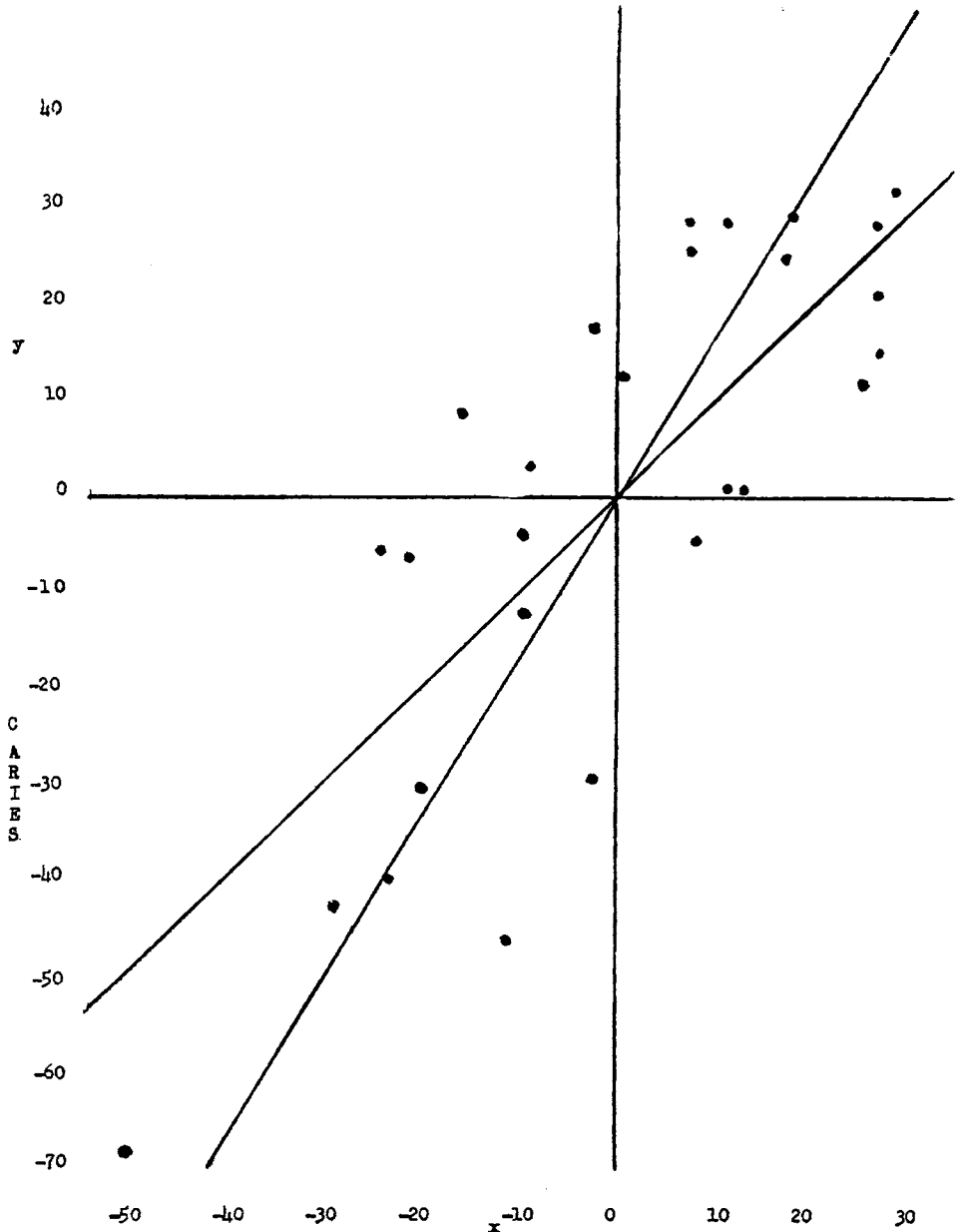


Fig. 1.

caries index may be calculated from the following formula:

$$\begin{aligned} I_c &= \text{Mean Caries Index} + \text{most probable deviation from the mean} \pm \\ &\quad \text{the probable error of the Index} \\ &= 68.8 - .965 (I_n - 57.1) \pm 11.5 \\ &= .965 I_n + 13.7 \pm 11.5. \end{aligned}$$

The statistical evidence indicates a high inverse correlation between general nutritive status and caries incidence.

The nutritional state of all animals at the beginning of the first generation was uniformly high. It was not until the beginning of the second generation that noticeable differences in weaning weights occurred. Groups I and VI did not reproduce as well as did the other groups.

In Groups I, III, IV, and VI there was a steady decline in general nutritional status in the second and third generations. This was most noticeable in Group VI. This physical deterioration was associated with a concomitant increase in caries.

The nutritional well-being of Groups II and V degenerated between the first to the second generation with a corresponding increase in caries. It would appear that these two groups adapted to their dietaries between the second and third generations with an improvement in their nutritional state associated with a decline in caries incidence.

The general physical well-being of Groups VII and VIII remained high and did not significantly vary during the 3 generations studied. Throughout this period, the caries incidence varied but little and remained low. In Group IX, where the standard cariogenic diet was employed for comparative purposes, a constant and relatively high state of nutrition was maintained through successive generations. Again this constancy in physical well-being was accompanied by only minor fluctuations in caries incidence.

#### CONCLUSION

A critical analysis of the data obtained from 3 generations of rats fed on 8 different combinations of human foods is presented. The nutritional status and physical well-being of all animals showed an inverse correlation with caries incidence. It would appear that with these diets, good nutritional status is associated with a low caries incidence while nutritional deterioration is accompanied by an increase in caries.

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