TITLE *The Influence Of Certain Dietary Factors On The Production Of Dental Caries In A Susceptible Strain Of Rats*

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THE INFLUENCE OF CERTAIN DIETARY FACTORS ON THE PRODUCTION OF DENTAL CARIES IN A SUSCEPTIBLE STRAIN OF RATS

by

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Dedicated to My Wife

INTRODUCTION

It has long been recognized that tooth decay is perhaps the most common disease of mankind. The history of dentistry reveals that the study of its cause and prevention is of long standing, however, progress in the direction of caries prevention has been somewhat more evident during the past 25 years. It has been demonstrated by workers in several fields that dental caries is influenced by a variety of factors of which the most prominent are the bacteriological and the nutritional. However, a more thorough study of caries reveals other factors of which the more important are the physical nature of the diet and heredity.

Each of these four fields has a definite bearing on the content of this thesis, the first three directly and the last, heredity, indirectly. It will be of interest to elaborate further on the matter of heredity. An extensive study carried out by Hunt, Hoppert, and Erwin (1) on the inheritance of caries susceptibility in albino rats resulted in the production of a strain of animals well suited for dental caries investigation. At this writing the susceptible colony is composed of rats in the 16th generation of selective breeding and shows remarkable homozygosity regarding caries of the lower molars.

The cariogenic ration developed by Hoppert, Webber and Canniff (2) was used throughout the inheritance study of Hunt

and his associates. This ration was composed of the following constituents:

Coarsely ground hulled rice.....66%
Whole milk powder.....30%
Alfalfa leaf meal..... 3%
Sodium chloride..... 1%

It was first observed by Hoppert and co-workers (2) that the particle size of the ground rice was important in initiating caries. The rice used in the above ration was ground in such a manner that approximately 70% was retained by a 20 mesh screen (400 holes per square inch). The 9th generation animals showed caries at a mean age of 64.6 ± .7 days when exposed to this ration at the age of 35 days. For nutritional studies on caries prevention, however, this ration was found too drastically cariogenic and caused extensive fracturing. Short term experiments revealed that finely ground rice lengthened the caries time and made for a more typical tooth decay. The above ration containing rice ground to retain 2% on a 20 mesh screen will initiate caries in 35 to 70 days with slight variations in the susceptible strain of animals. This modification will be referred to as the caries producing fine rice ration throughout this thesis and was used as the control diet. This ration is remarkably consistent in producing caries but allows a greater fluctuation between families in initiating time than the coarser rice diets. This may be experimentally compensated for by comparing litter mates within a given family. Under these

conditions animals are rarely found to deviate from their litter mates by more than one week. Variations of two weeks or less are considered insignificant throughout this thesis.

All carious lesions recorded in this work are macroscopic in nature and were observed biweekly in the living animal. An original record of caries initiation and progression was kept by scoring a tooth map and accompanying a particular entry with the date of observation. A positive lesion was recorded as "+" while one in doubt was marked "?". The last observation designated as doubtful "?" was taken as the date of caries initiation. Plate I illustrates the extent of such a lesion. Plates II and III represent moderate and severe caries respectively. This method allows the observation of differences in caries progression as well as initiation. No effort was made to compare the relative number of carious lesions as the progression is so rapid that several small ones terminate in one large involvement in a short period of time.

When studying the tabulated data in some cases blanks appear under the "initial" and"moderate" stages. A blank in the first case means this stage was reached before observations were begun. In cases of "moderate" blanks the animal progressed from the initial to the severe stage between two successive readings.

PLATE I Initial Caries

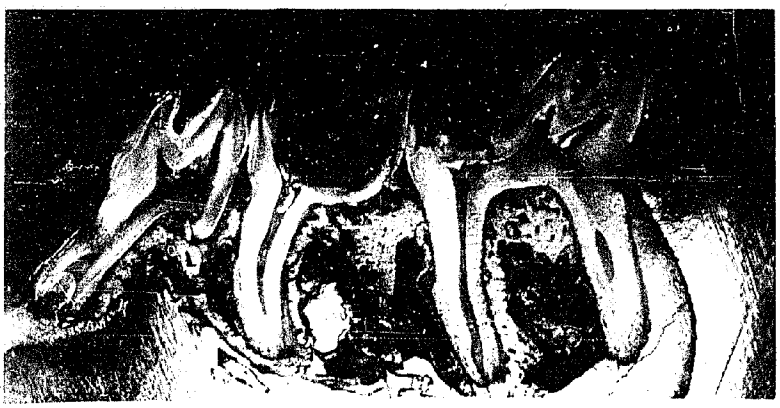


PLATE II Moderate Caries

PLATE III Severe Caries

Plates I, II, and III, represent the approximate degree of caries referred to as initial, moderate, and severe respectively.

GENERAL HISTORICAL

The history of dental caries in rats dates back to 1922 when McCollum, Simmons, Kinney and Grieves (3) recorded positive findings in the albino species. Grieves, (4) using the same data, supplemented the original findings by describing most of the gross features, which were later borne out by other workers. The similarities and differences between human and rat caries were noted with special regard being given to the aspects common to both. The features observed by Grieves were penetration of the enamel, followed by spread in the dentin with subsequent fracturing. These lesions have since been observed by many workers, but seldom in that sequence. He maintained that caries rarely involved fractured surfaces, but that fractures often result from carious-like lesions.

In 1925 Bunting (5) reported caries in 31 of 66 rats fed various diets. Thirty of thirty-eight animals on maize-containing diets developed caries in contrast to only 1 or 2 of 28 on maize free rations. He also observed that after eleven months on a total meat diet animals showed very defective bones but no caries.

Shibata (6) in 1929 fed weenling rats and older animals a ration composed of rice, greens, sucrose, glucose, lactose, and maltose. He reported caries in 70-80% of the animals with some lesions appearing as early as 20 days. The ration was reported less effective in the case of older rats and ineffective for animals started at 300 gms. body wt.

Later reports by the same worker (7) revealed that no caries was produced by polished rice and dextrin. He ascribed caries production solely to the added sugars.

In 1932 Kesel (8) reported a repetition of Shibata's work with negative results, claiming that under similar conditions no caries was produced and the rats showed marked undernourishment.

Klein and McCollum (9) in 1931 studied 750 rat skulls and concluded that the minimum time for caries production was 60 days on a ration that would produce caries in 88% of the rats in 81 to 155 days. They observed that rats were usually 100 days old before any appreciable caries could be noted. This is in sharp contrast to Shibata's 20 day period. Klein and McCollum later reported their ration as a rachitogenic one composed of 76% maize, 20% wheat gluten, 1.5% calcium carbonate, and 1% salt. They described and illustrated interproximal caries, but made no mention of frequency relative to the occlusal type.

Agnew, Agnew and Tisdall (10) in 1932 found caries in all of 38 rats on a diet containing 78.4% of "finely ground" maize over a test period rangeing from 2-6 months. They also reported caries production by a casein-starch-fat type ration low in phosphorous. Caries, however, did not occur when this ration was corrected for phosphorous and cane sugar was substituted for starch. From their extensive study of the mechanism of these

carious lesions and their resemblance to those of humans they believed the process in the two species to be analgous. Using a rough quantitative method Johnston, Kaake and Agnew (11) in 1933 studied the oral flora of the rats of Agnew, Agnew, and Tisdall. They reported high counts of L. acidophilus in 27 of 30 rats with caries and low counts in the other 3. Observations made on 153 rats having no macroscopic caries showed high counts in 109 and low counts in the remainder. These workers concluded, therefore, that L. acidophilus is of little significance in the etiology of rat caries. It is important to note, however, that Johnston et. al. did not demonstrate the absence of microscopic caries which detracts from their conclusions.

The same group reported that no caries appeared in 18 rats fed a ration containing 62% cane sugar for a period of many months. The acidophilus count was high in 8 and low in 10 of these animals.

Toverud in 1926 (12) fed 100 rats a ration containing 92% ground wheat and reported no carious lesions. In a group of 4 animals fed bread ad libitum plus 2½ cc of calcium-poor milk daily, however, he observed caries in all cases. The animals were kept on the diet from 3 weeks old to 3½ months and the lesions varied from small fissures to wide openings with pulp involvements.

Knowlton (13) in 1929-30 found no caries in 34 rats maintained on a casein-starch-fat type diet varying in vitamin B. complex and vitamin D. The animals were fed the ration from

weening to 50 days which is probably not long enough for caries to develop in common strains of albino rats.

Krasnow (14) in 1932 studied the role of fluorine in dental caries using rats as the experimental animal. Eight groups of ten animals each were used, but no caries data were reported.

Rosebury and Karshan (15) in 1931 failed to find caries in any one of 60 rats fed rachitogenic, scorbutic, or high carbohydrate rations for from 40 to 60 days. Half of these animals received a gum tragacanth paste containing a human strain of Bacillus acidophilus biweekly. None of the rations contained cereal particles.

Lilly (16) raised rats until 30 days of age on a stock ration of fresh meat, whole milk, bones, bread, and cooked and fresh vegetables. After 30 days they were placed on a ration of:

Starch.....53%
Lard.....25%
Casein.....18%
Osborne & Mendel salt.... 4%

with daily supplements of cabbage and brewers yeast. Variations of this ration were made by substituting sucrose for starch and lettuce for cabbage. Approximately half the animals received a 1 ml. of pure Bacillus acidophilus culture three times weekly throughout the experimental period which was carefully rubbed on the teeth and gums. Sections were prepared of the jaws but no caries was found in 52 rats after a 12 months experimental period.

Lilly also fed the Steenbock rachitogenic diet No. 2965 to a series of ten rats until death or for 12 months. No caries was

found, but no mention was made of the maize particle size. These results are somewhat contradictory to those of other workers and detracts from the significance of Lilly's conclusions regarding the inability of carbohydrate diets to initiate and develop caries. An explanation might lie in the possible inherent resistance to caries of the rats used in his studies.

In 1932 Lilly and Grace (17) reported no caries development in groups of ten rats on high carbohydrate rations for 6½ months. These workers fed glucose, lactose and maltose at a 66% level, the rations being balanced with 20% casein, 10% lard, 4% Osborne and Mendel salt mixture, and viosterol. Two similar groups of rats developed no caries in 5½ months on rations low in fat, namely: 73% starch, 19% casein, 3% salt mixture and cod liver oil or butter fat 5%.

THE DISCOVERY OF COARSE PARTICLE ETIOLOGY

An important contribution to dental caries study was made in 1931-32 by Hoppert, Webber, and Canniff (18) who reported the discovery of cereal particle size as an etiological agent in the formation of caries in rats. Upon failing to find cavities in stock rats fed a ration low in calcium and vitamin D the control animals were examined for comparison. Extensive decay was observed and from subsequent studies they concluded that particle size was a contributing factor. Rations containing coarse corn meal induced caries, those with corn meal that passed a 60 mesh screen produced none.

A criticism of this work was offered by McCollum (9) who suggested that the fine corn meal rations were richer in phosphorus than those containing coarse maize. Hoppert, Webber, and Canniff (2), and Webber (19) replied to this criticism by showing caries production in rats on a corn meal ration high in phosphorous as compared with an oatmeal containing ration which caused no caries. These authors ascribed the origin of caries to the impaction of corn meal particles in the sulci of the rat molars with subsequent fermentation and erosion of enamel by the acid produced.

In 1933 Klein and McCollum (20) agreed to the etiological effects of coarsely ground maize after finding caries in 22 of 34 rats on the Steenbock and Black rachitogenic ration No. 2965, but none in 7 rats on the same ration ground to pass a 60 mesh screen.

The composition of the H-W-C ration is as follows:

Yellow maize meal	60%
Whole milk powder	30%
Lindseed meal	6%
Alfalfa meal	3%
Sodium chloride	1%

The approximate distribution of maize particles in this ration was 45% on a 20 mesh screen, 30% on a 40 mesh, 12% on a 60 mesh, and 13% through a 60 mesh. A simplified formula of the above ration replaces the lindseed meal with the cereal in question. Hoppert, Webber and Canniff in 1932 also reported that the replacement of maize by rice induced caries in rats, but that

oatmeal or hard wheat was ineffective.

The etiological significance of particle size has been confirmed by Klein and McCollum (20), by Shelling and Asher (21), Rosebury, Karshan and Foley (22, 23, 24, 25),Bibby and Sedwick (26), Lilly and Wiley (27), King (28), prior to 1936 and by many others since.

THE RELATION OF FLUORIDES TO DENTAL CARIES

Historical

A survey of dental history reveals that at sometime or other almost every element known to be present in dental tissue has been suspected of bearing a relationship to dental caries. Some attributed the phenomenon to an excess of the element involved whereas others ascribed it to a deficiency.

Relative to fluorine, some of the more pronounced effects were observed and described before the etiological agent was known. In 1916, McKay and Black (29) described the hypoplastic nature of mottled teeth and studied degrees of its severity. Later, by feeding various levels of potassium fluoride, Cheyne (30) produced graded mottling in rat molars. Previous to Cheyne, Smith, Lance and Smith, and Churchill, working independently, showed a similar effect caused during the calcification process of the tooth by the incorporation of excessive amounts of fluorides in drinking water. Cox, Matuschak, Dixon, and Walker (31) reported a milky appearance in rat molars fed various levels of fluorine during the suckling period.

These studies established a relationship between the fluoride intake and the general appearance of dental tissue and suggested a possible influence of fluoride on tooth decay.

In 1933 Armstrong (32) fed rats a ration containing 1000 ppm. sodium fluoride for three weeks and then discontinued the fluoride. During the three weeks following the fluoride-free intake he reported the breaking off of the upper incisors near the gum line. Armstrong and Brekhus (33) in 1938 showed that transverse bands of mottled and normal enamel may be produced and that the previously reported breaking off seems to take place at a band of normal enamel. This information suggests that fluorine acts as a hardening agent when incorporated in dental tissue.

In 1941 Arnold and McClure (34-35) injected sodium fluoride in rats maintained on the H-W-C simplified ration. The level in intake in these animals was approximately equivalent to 40-50 ppm fluorine daily in water. No caries was reported. At the same time no reduction of caries was observed in another series of rats fed 10 ppm fluorine in the drinking water. Twenty-one litters were used in this investigation and analyses of the teeth revealed a four-fold increase of fluorine in the latter group. McClure (36), however, in 1941 reported a marked reduction of caries in rats maintained on 50 and 100 ppm fluorine in water. He stated, "Posteruptive deposits of fluorine may not increase the tooth's caries resistance".

In 1939 Cox, Matuschak, Dixon, Dodds and Walker (37) studied effects of fluorides on pre-eruptive dental tissue by raising control rats on a ration composed of sucrose, casein, crisco and salt mixture, supplemented with yeast, alfalfa and Haliver oil. Experimental rats received 41.2 ppm fluorine as sodium fluoride and their offspring were placed on the H-W-C ration for a standard period of eight weeks after weaning.

Experimental

The purpose of this series of experiments was to establish any caries inhibiting effects of fluoride in a caries susceptible strain of rats and to study such effects on the dental tissues at various stages of the development of the teeth.

The chronological development of rat molars as presented by Cheyne (30) was used as a guide in this study, the periods of primary interest being (1) that previous to dental development or the inutero phase (2) the period of major development or that during lactation, and lastly (3) the final stages of molar growth and maturity where the vital nature of dental enamel is somewhat controversial.

To accomplish certain phases of this study litters were divided at birth into two groups, one group remaining with the mother and the other group being transferred to another lactating female. Elaboration of this technique will follow with specific detail.

PART I

EFFECT OF CONTINUOUS FLUORIDE FEEDING

As a general approach to the overall problem, rats were given a fluoride supplemented diet throughout the experiment. This was accomplished by feeding the mothers a fluoride supplemented ration and continuing the offspring on the same diet. Previous work by the author as well as McClure and Arnold (36), and Miller (38) established an effective fluoride level at approximately 125 ppm of NaF in the ration. For this part of the experiment and for all others in this thesis the fluoride enriched ration consisted of the H-W-C fine rice formula plus 125 p.p.m. NaF. At this level of intake normally growing rats usually showed bleaching of the incissors but no severe fluorosis.

For controls, offspring of litter mates were fed the H-W-C fine rice ration. As previously explained, the homozygous nature of the susceptible strain justifies this procedure. The lower molars were observed at approximately 2 wk. intervals and the caries initiation and progress recorded at three phases of development.

From the accompanying tables (Table I) it will be observed that the controls showed initial caries between 35-60 days of age with moderate or severe involvement following in two weeks. By way of comparison the fluoride animals reveal initial caries between 74 and 140 days of age with moderate caries at 74 to 172 days. In several cases severe cavitation was delayed for 200 days.

TABLE I

EFFECT OF CONTINUOUS FLUORIDE FEEDING

Litter Number	Rat #	Diet	Caries Time in Days* Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
14F(9-20)	1	F†	140	185	172	—	200	200
	2	"	140	140	—	—	172	172
	3	"	120	—	140	75	172	172
	4	"	120	140	140	—	172	172
	5	"	120	120	140	140	172	172
Average of L & R sides			(136)				(176)	
12F(8-12)	6	F	114	114	138	138	160	160
	7	"	114	138	138	—	160	160
	8	"	114	114	138	138	160	160
Average of L & R sides			(118)				(160)	
12F(8-25)	9	F	74	—	—	74	86	86
	10	"	62	—	—	—	86	86
	11	"	74	86	86	—	100	100
	12	"	100	125	125	—	146	146
	13	"	74	74	—	—	86	86
	14	"	74	—	100	—	146	146
	15	"	74	86	86	86	100	100
	16	"	74	—	86	—	125	125
Average of L & R sides			(81)				(109)	
12F(9-27)	17	F	78	78	100	100	114	114
	18	"	136	—	168	168	180	180
	19	"	136	114	168	136	168	168
	20	"	136	—	168	—	168	168
	21	"	100	—	—	128	168	168
	22	"	78	78	100	100	114	114
	23	"	78	78	—	—	100	100
Average of L & R sides			(99)				(145)	
14(8-11)	24	C**	48	60	—	—	60	60
	25	"	35	48	48	—	60	60
	26	"	35	35	—	—	48	48
	27	"	35	—	—	—	48	48
	28	"	35	—	48	48	60	60
	29	"	35	35	—	—	48	48
	30	"	35	—	48	48	60	60
Average of L & R sides			(39)				(55)	

* Days are calculated from birth.

† Fluoride Ration (Fine rice ration plus 125 p.p.m. NaF).

** Fine rice cariogenic ration (Control)

TABLE I (Cont'd.)

EFFECT OF CONTINUOUS FLUORIDE FEEDING

Litter Number	Rat #	Diet	Caries Time in Days Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
12(8-23)	31	C	62	74	74	—	86	86
	32	"	—	62	62	—	74	74
	33	"	62	62	—	—	74	74
	34	"	62	62	—	—	74	74
	35	"	62	62	—	—	74	74
	36	"	62	62	—	—	74	74
	37	"	—	—	—	—	62	62
Average of L & R sides			(63)				(74)	
12(9-6)	38	C	50	—	—	—	62	62
	39	"	50	—	—	—	62	62
	40	"	50	—	—	—	62	62
	41	"	50	—	—	—	62	62
	42	"	50	—	62	62	74	74
	43	"	62	62	—	—	74	74
	44	"	62	62	—	—	74	74
Average of L & R sides			(55)				(67)	
12(10-9)	45	C	37	—	52	52	76	76
	46	"	52	—	—	—	76	76
	47	"	52	52	—	—	76	76
	48	"	37	—	52	52	76	76
	49	"	37	—	52	52	76	76
	50	"	37	—	52	—	76	76
	51	"	37	—	52	—	76	76
Average of L & R sides			(43)				(76)	
Total Mean		F	(109)				(148)	
" "		C	(50)				(68)	
Mean Difference =			59				80	

PART II

PRENATAL EFFECTS OF FLUORIDES

This part of the experiment was set up to study the effect of limiting fluoride to the prenatal period. The fluoride ration was fed to susceptible breeding stock and the offspring divided into two groups at birth. One group was left with the original mother whereas the other was nursed by a mother maintained on the basal ration. Accordingly the first group received fluoride throughout the experiment, the second, only during embryonic and fetel development. Any differences between these groups would be due therefore, to placental transfer of fluoride.

The seven control animals from the three litters used in the experiment gave responses that were characteristic of the susceptible strain of rats in that the average caries initiation time was 58 days from birth. Severe involvement followed at 77 days in every case.

Data presented in Table II reveal that 6 of the 12 supplemented rats showed no lesions in 200 days, the minimum initiation time being 118 days. In this experiment the study was terminated in 200 days therefore making it impossible to determine average caries initiation time.

TABLE II

PRENATAL EFFECTS OF FLUORIDES

Litter Number	Rat #	Diet	Caries time in days — Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
31F(7-6)	1	C*	60	60	—	—	77	77
	2	F†	Caries free at 200 days					
	3	"	—	132	—	146	—	—
	4	"	118	118	132	118	146	146
	5	"	Caries free at 200 days					
31F(7-13)	6	C	44	44	60	60	77	77
	7	"	60	60	—	—	77	77
	8	F	—	—	104	104	160	160
	9	"	Caries free at 200 days					
	10	"	"	"	"	"	"	
	11	"	"	"	"	"	"	
	12	"	"	"	"	"	"	
31F(7-7)	13	C	60	60	—	—	77	77
	14	C	60	60	—	—	77	77
	15	"	60	60	—	—	77	77
	16	"	60	60	—	—	77	77
	17	F	118	118	160	160	174	174
	18	"	118	118	160	160	174	174
	19	"	Caries free at 200 days					
	20	"	200	200	—	—	—	—
Total Mean		C	(58)				(77)	
		F	Cannot be determined due to the caries-free rats at the termination of the experiment.					

* Fine rice control ration.

† Control ration plus 125 p.p.m. NaF, fed all animals prenatally and to the "F" animals throughout the experiment.

PART III

LACTATION AND POSTWEANING EFFECTS OF FLUORIDES

In order to elaborate further the ineffective nature of fluorides on teeth in the prenatal stage a study of the lactation and postweaning periods was conducted.

Offspring born of susceptible animals fed the basal fine rice ration were divided into two groups at birth. One group remained with the original mothers while the other was transferred to stock mothers receiving the fluoride ration. The first group served as a control whereas the second received the fluoride ration throughout the experiment.

The data recorded in Table III shows that the control animals developed caries in the expected time. The fluoride animals, on the other hand remained caries free for 165 days when the experiment was terminated.

PART IV

POSTWEANING EFFECTS OF FLUORIDE

The final phase in the fluoride experiment concerned a study of withdrawing fluoride from rats which had received fluoride during the prenatal and lactation period. This was done by feeding the fluoride fortified H-W-C fine rice ration to susceptible breeding stock and continuing the offspring on the same diet until the time of weaning. At this point the litters were divided into two groups. One group was continued on the fluoride ration whereas the other was placed on the standard fluorine-free basal

TABLE III

LACTATION AND POSTWEANING EFFECTS OF FLUORIDES

Litter Number	Rat #	Diet	Caries time in days: Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
32(12-1)	1	C*	70	—	82	—	96	96
	2	"	82	82	—	—	96	96
	3	"	82	—	—	—	96	96
	4	F†	Caries free at 165 days.					
42(11-24)	5	C	76	88	88	88	100	100
	6	F	Caries free at 165 days.					
	7	"	"	"	"	"	"	
42(11-28)	8	C	60	60	—	—	73	73
	9	"	73	73	—	—	85	85
	10	"	60	60	—	—	73	73
	11	F	Caries free at 165 days					
	12	"	"	"	"	"	"	
	13	"	"	"	"	"	"	
	14	"	"	"	"	"	"	
32(12-5)	15	C	66	78	78	—	92	92
	16	"	78	78	—	92	92	92
	17	"	78	78	—	—	92	92
	18	"	78	78	—	—	92	92
	19	"	78	78	—	—	92	92
	20	F	Caries free at 165 days					
	21	"	"	"	"	"	"	
	22	"	"	"	"	"	"	
47(12-11)	23	C	60	—	72	—	100	100
	24	"	60	—	72	—	100	100
	25	F	Caries free at 165 days					
Total mean		C	(73)				(93)	
		F	Observations relative to this thesis were stopped at 165 days.					

* Fine rice control ration.

† Control ration plus 125 p.p.m. NaF fed the "F" animals from birth throughout the experiment.

ration. The rats were observed as usual and the caries progress recorded.

Table IV shows, with very few exceptions, that the control animals developed moderate to severe cavatation in 73 to 98 days. The fluoride supplemented animals, on the other hand, showed minimum initiation at 133 days with moderate to severe involvement between 158 and 277 days. Similar results were obtained with four additional litters from separate families, making a total of 18 controls and 19 supplemented animals.

DISCUSSION OF FLUORIDE RESULTS

A susceptible strain of rats was used to study the effects of fluorides on teeth at various stages of development. A cariogenic fine rice ration fortified with 125 p.p.m. sodium fluoride had a marked inhibiting effect on the caries initiation time when fed to mothers during the lactation period and continued with the weenling rats. Rats discontinued on this ration at weaning time, however, showed early cavatition and rapid progress of caries following initiation. The fluoride ration fed only during the prenatal period transmitted no caries inhibiting effect on the offspring.

The schematic chart reveals that the caries progression time, or the time required for lesions to proceed from the initial to the severe stage, was approximately the same regardless of the initiation time.

TABLE IV

POSTWEANING EFFECTS OF FLUORIDES

Litter Number	Rat #	Diet	Caries time in days: Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
31F(5-12)	1	C*	—	—	73	73	98	98
	2	"	—	—	—	—	98	98
	3	F†	240	240	—	—	252	252
	4	"	158	158	186	186	200	200
31F(5-6)	5	C	—	—	—	—	73	73
	6	"	—	—	73	73	98	98
	7	"	98	98	—	—	114	114
	8	"	—	—	—	—	73	73
	9	F	133	133	—	—	158	158
	10	"	158	158	186	186	200	200
	11	"	158	158	158	158	172	172
31F(5-13)	12	C	73	73	—	—	98	98
	13	"	73	73	—	—	98	98
	14	F	158	158	186	186	died	
31F(5-18)	15	C	—	—	—	—	73	73
	16	"	—	—	—	—	73	73
	17	F	144	144	158	158	227	227
	18	"	200	200	—	—	215	215
	19	"	158	158	186	186	200	200
31F(5-17)	20	C	73	73	—	—	98	98
	21	F	Caries free at 227 days.					
	22	"	186	186	200	200	215	215
	23	"	200	200	215	215	227	227
Total mean		C	(---)				(90)	
		F	(172)				(197)	

* Cariogenic fine rice ration fed to half of the animals after weaning.

† Cariogenic fine rice ration fortified with 125 p.p.m.NaF fed to all animals until weaning time and to half of them throughout the experiment.

In terms of tooth development it would appear that the nearly mature dental tissue derives a great deal of protection from the fluoride fortified ration.

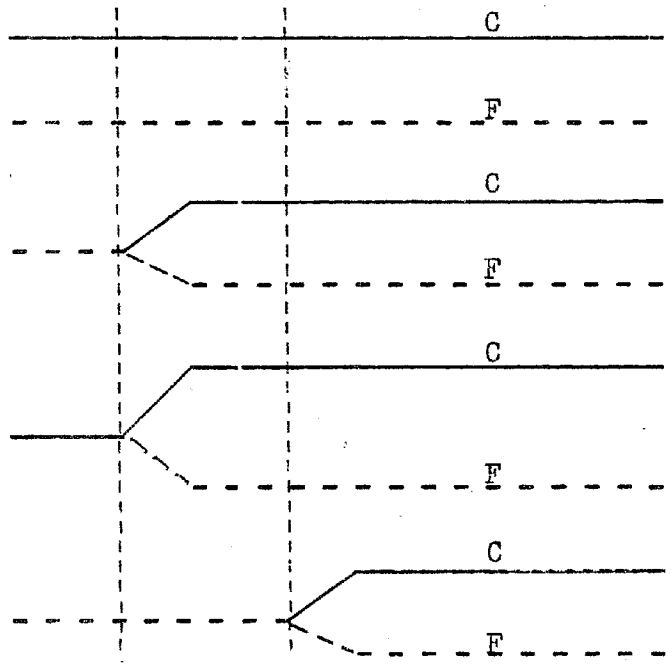
Gerould (39) holds the view that fluorides, when incorporated in the appetite structure of the tooth, exhibit a surface bacteriostatic effect and thus are instrumental in lowering the acid production. He also showed that finely divided dental tissue exposed to fluoride solutions was more resistant to solution by than similar material not treated with fluoride solutions.

If the results observed in this study are the result of systemic transfer of fluorides it is suggestive that dental enamel is composed of vital tissue and that the fluoride turnover is rapid. On this basis it might be expected that the progress of decay to the severe stage would be slower than that in control animals. The data, however, show no consistent or striking differences to support this view.

The caries inhibiting effect of fluorides may also be attributed to the antibacterial or antienzymatic properties of the element. To be effective this mechanism would have to provide a fairly continuous exposure of the enamel surface to fluorides. It also follows that withdrawal of fluorides would again make the teeth vulnerable to decay. The latter explanation finds support in the early development of caries in the rats which were removed from the fluoride supplemented ration after having had the benefit of prenatal and postnatal fluoride supplies up to the time of weaning.

The favorable results that have been obtained from the continuous feeding of fluorides to rats with a high degree of susceptibility to caries indicate that the program of supplying a fluoride enriched source of water for general use in certain communities should be attended by considerable improvement in the conditions of the teeth of the human population.

SCHEMATIC CHART



		Number of Rats	Average Caries time in days Initial	Severe	Progression
I	Effect of Continuous Fluoride Feeding				
	C	28	50	68	18
	F	23	109	148	39
II	Prenatal Effects of Fluoride				
	C	7	58	77	19
	F	13	(50% Caries free at 200 days. (Earliest initiation at 118 days.		
III	Lactation and Post-weaning Effects of Fluorides				
	C	14	73	93	20
	F	12	100% Caries free at 165 days		
IV	Postweaning Effects of Fluoride				
	C	11	--	90	
	F	12	172	197	25

Total number of animals -- 120

CONCLUSIONS

1) Caries susceptible rats receiving fluorides only during embryonic development showed no resistance to dental caries.

2) Susceptible rats continued on a fluoride ration through the lactation period showed no increased resistance to caries.

3) Susceptible rats continued on a fluoride ration after weaning showed marked resistance against tooth decay.

4) From these observations it follows that to be most effective fluoride supplementation must be continuous.

THE RELATION OF CARBOHYDRATES TO TOOTH DECAY

Historical

Some of the earliest observations regarding sugars and starches as etiological agents in dental caries were made by Shibata (6) in 1929 who fed young and old rats rations containing 5 to 10% of sucrose, glucose, lactose and maltose. He reported caries in 70 to 80% of the animals, appearing as early as 20 days and attributed the causal effects to the added sugars.

In 1930 Shibata (7) added further that no caries was produced by, "polished rice or dextrine".

Other investigations, however, have failed to substantiate Shibata's findings. Kesel (8) in 1932 wrote, "I have repeated Shibata's experiments not once but on four different groups of animals. -------Bacillus acidophilus was quite regularly found in their mouths; but not one rat developed one decayed molar". Further discriminating evidence was produced by Rosebury, Karshan and Foley (22) in 1933 who established that the causal factor in Shibata's rations was coarse rice and not the added carbohydrate.

Johnston, Kaake and Agnew (11) in 1933 reported that no caries was developed in a series of 18 rats fed a diet of 62% cane sugar for many months. The acidophilus incidence was high in eight and low in ten of these animals.

Rosebury and Karshan reported no caries in 60 rats fed high CHO rations for from 40 to 60 days. Half of these animals

received an oral inoculation twice weekly of a human strain of Bacillus acidiophilus. They reported no cereal particles in the ration. It is questionable in this work if the term of the experiment was long enough to permit conclusive results. Klein and McCollum, in this connection, studied 750 rat skulls in 1931 and concluded that it was necessary for a rat to be at least 100 days old for any significant carious lesions to appear.

In 1933 Brown and Tisdall (40) reported, "Sugar, so long blamed for caries, has been proved harmless in the rat, to which diets have been given containing as high as 62% carbohydrate but including also adequate minerals and vitamins".

King (28), in 1935 fed rations containing 62.5% carbohydrate to rats for 396 days after weaning. Corn starch, rice starch, and sucrose were the carbohydrates used in these experiments. He reported the rations as being low in Ca, P and vitamins C and D, but that no caries was observed.

In 1934-35, Day, Daggs and Sedwick (41-42) added commercial fudge to fox chow at 20%, 35% and 55% levels. Additional vitamins and minerals were provided for some of the 55% group. 213 animals of varying ages were used and the experimental period ranged from 150 to 213 days. They reported the incidence of 4 carious lesions in the entire group but no relationship could be established to the rations used.

Lilly and associates (16) were unable to find a single lesion in more than 350 rats to which rations had been fed

containing 66% glucose, sucrose, lactose, maltose and corn starch. The rations were fed for periods of 6½ months to a year.

Cox (43) in 1938 indicated that sucrose and glucose do not initiate caries in rats but that they promote caries previously initiated by the H-W-C ration.

PART I

A study of the effect of diets containing finely ground rice, sucrose and no fermentable carbohydrates on the production of dental caries.

Experimental

The majority of investigations in which readily fermentable carbohydrates have been fed to rats lead to the conclusion that sugars do not initiate dental caries. In some cases with stock animals, however, sugars have proved to be caries promoters once the lesions have been initiated by coarse cereal particles. It was thought advisable, however, to re-evaluate this work using the caries susceptible strain of rat as the experimental animal.

Three rations were used in this study, the H-W-C fine rice ration which served as a control diet, a complete ration containing 50% sucrose, and a third ration composed largely of lard, casein, and alfalfa which was considered to be essentially free of fermentable carbohydrates. The composition of these rations is herewith given.

I The H-W-C fine rice ration (previously described)

II The fat ration

casein.....40%
alfalfa.....30%
brewers yeast..... 9%
salt..... 1
fat (lard or crisco)...20%

III The sugar ration

sucrose.....50%
casein.....10%
alfalfa..... 6%
brewers yeast..... 3%
salt..... 1%
powdered whole milk....30%

The mothers were raised on the control ration until the offspring reached 14-15 days of age. At this time the families were transferred to clean cages and placed on the fat ration. At this age the young rats still rely on the mother's milk for nourishment, therefore the chances for exposure to the caries producing ration are negligible. The animals were maintained on the fat ration until several litters had reached the weaning period. Each litter was then divided three ways with sexes as evenly distributed as possible. The three rations described above were fed ad libidum to the respective groups throughout the remaining term of the experiment. Caries initiation and progress were recorded and appear in table V.

Since litter mates were used throughout this experiment the data are recorded in litter groupings to facilitate inter-family comparisons. Severe caries was produced in the control animals between 64 and 110 days. In sharp contrast to this

short period the rats receiving the sucrose containing diet showed severe caries at an average of 210-230 days with the earliest appearing at 124 days.

The rats fed the fat ration remained caries free as long as they were kept on this ration. At 10 months to a year approximately half of these animals were transferred to the H-W-C fine rice ration. Table V indicates that from 130 to 180 days were required to produce severe caries in these animals with 117 days being the minimum time. It is of interest to note that even though the initiation time was longer with the adult animals the progress of caries to the severe state was still rapid. In every case the animals remaining on the fat ration showed no sign of caries during their entire span of life.

In addition to the animals recorded in the table, a litter of three females and three males were maintained on the fat ration throughout their life spans, the purpose being twofold; namely, to obtain further evidence that rats of a caries susceptible strain would develop no caries on this diet and to study the caries susceptibility of the offspring of these rats. Sixteen young from this mating were divided into two groups at weaning. One group remained on the fat diet whereas the other was fed the fine rice ration. Caries developed as usual in the latter group whereas the other, fed the fat ration, remained caries free.

TABLE V

THE EFFECT OF DIETS CONTAINING SUCROSE AND NO FERMENTABLE CARBOHYDRATES ON THE PRODUCTION OF DENTAL CARIES

Litter Number	Rat #	Diet	Caries time in days* Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
10(7-8)	1	C	60	60	—	—	74	74
	2	C	60	60	—	—	74	74
	3	S	74	74	110	110	124	124
	4	S	148	148	—	—	170	170
	5	Fat†	130	130	—	—	150	150
6(7-15)	6	C	60	60	—	—	74	74
	7	S	170	170	192	—	210	210
	8	S	170	170	192	192	210	210
	9	Fat	Caries-free for life span					
19(8-29)	10	C	88	88	—	—	110	110
	11	S	177	177	190	190	230	230
	12	Fat	Caries-free for life span					
7(9-9)	13	C	88	88	—	—	110	110
	14	S	190	190	—	—	230	230
	15	Fat	Caries-free for life span					
7(6-14)	16	C	—	—	60	60	90	90
	17	S	178	—	200	—	222	222
	18	Fat†	130	130	—	—	150	150
	19	Fat	Caries-free for life span					
10(6-21)	20	C	—	—	—	—	76	76
	21	C	—	—	60	60	76	76
	22	S	117	—	—	104	139	139
	23	Fat†	130	130	—	—	150	150
	24	Fat	Caries-free for life span					
9(6-5)	25	S	104	104	117	117	139	139
	26	Fat†	144	144	—	—	180	180

Table V Cont'd next page

* Time starts when animals were changed from the fat ration to the experimental ration.

† Changed to the fine rice cariogenic ration after 10-12 months remaining caries-free on the fat ration. Caries time is calculated from the time of this change.

TABLE V (Cont'd)

Litter Number	Rat #	Diet	Caries time in days* Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
10(7-2)	27	C	62	62	--	--	76	76
	28	Fat	Caries-free for life span					
6(9-11)	29	C	88	88	--	--	110	110
	30	C	50	--	64	--	110	110
	31	Fat	Caries-free for life span					
19(9-12)	32	C	64	--	--	--	110	110
	33	S	132	--	164	--	230	230
	34	Fat†	117	117	--	--	130	130
10(9-18)	35	C	64	49	88	64	110	110
	36	Fat	Caries-free for life span					
Mean Average		C	--				92	
		S	146				190	
		Fat†						

* Time starts when animals were changed from the fat ration to the experimental ration.

† Changed to the fine rice cariogenic ration after 10-12 months remaining caries-free on the fat ration. Caries time is calculated from the time of this change.

PART II

The influence of rice on the caries producing effects of Sucrose.

The purpose of this study was to investigate the possibility that the fine rice ration might serve as a caries initiating agent after which the effects of sucrose might be more pronounced than had been observed when rats were placed directly on the sucrose diet after weaning.

Five litters numbering 21 animals were used in this study. The young were born at approximately the same time and were placed on the fat ration at 14 days of age. When the youngest litter had reached the weaning stage all of the litters were divided into two similar groups. One group received the fine rice ration for two weeks and was then changed to the sugar diet whereas the other group remained as a fine rice control. Rat molars exposed to the fine rice ration for only two weeks reveal no macroscopic caries.

Observations for lesions were made and recorded at two week intervals. The control animals showed initiation of caries at 38 to 87 days which is a considerable variation for the susceptible strain. Severe caries developed between 87 and 130 days. When comparisons are made strictly within litters, however, it is evident that caries initiation and development in this experiment were earlier and more rapid than in Part I in which preliminary exposure to fine rice

was lacking. Severe caries with this somewhat less susceptible group fed the sugar ration developed in 162 days as compared with 190 days for the animals in Part I fed only the sucrose ration.

Discussion

Perhaps the most outstanding conclusion to be drawn from this series of experiments is that a sucrose ration in the absence of cereal particles, large or small, can act as a caries initiating as well as promoting agent in rat molars. This fact has been denied by many workers referred to in the historical section. In view of the relatively long period of time required to effect macroscopic caries in the susceptible strain of animals it is not surprising that the factor has been overlooked by workers using ordinary stock albino rats.

The caries promoting character of sucrose following initiation by the H-W-C ration as reported by Cox (43) is clearly supported by Part II of this series. Apparently the diet containing finely ground rice used with a susceptible strain of rats produced microscopic lesions during the two week period of exposure, so that the subsequent effect of sucrose was markedly accelerated.

It is significant also that even though the caries initiation time was markedly longer with the sucrose ration as compared with the fine rice ration, the time required for the lesions to proceed from the initial macroscopic stage to the

TABLE VI

THE INFLUENCE OF RICE ON THE CARIES PRODUCING EFFECTS OF SUCROSE

Litter Number	Rat #	Diet	Caries time in days* Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
16(9-24)	37	C	38	87	87	—	108	108
	38	C	63	—	87	—	108	108
	39	S	63	—	108	108	162	162
	40	S	108	108	130	130	162	162
19(9-25)	41	C	87	87	—	—	108	108
	42	C	38	63	63	87	108	108
	43	C	38	63	63	—	87	87
	44	S	108	108	130	130	162	162
	45	S	63	—	87	87	162	162
	46	S	63	63	87	—	162	162
13(9-20)	47	C	87	—	—	—	108	—
	48	C	63	—	108	—	130	130
	49	S	108	108	—	—	162	162
	50	S	87	87	108	130	162	162
19(9-22)	51	C	63	—	78	78	130	130
	52	S	108	—	130	130	162	162
13(10-6)	53	C	63	63	—	—	87	87
	54	C	63	—	87	87	108	108
	55	C	63	—	87	—	108	108
	56	C	63	108	108	—	162	162
	57	S	87	87	130	130	162	162
Mean Total		C	64				108	
		S	85				162	

* Time was calculated from the beginning of the fine rice feeding. The animals receiving the sucrose diet were fed the fine rice ration for 2 weeks only.

severe stage was practically the same for the two rations. This suggests that the chemical and bacteriological factors involved at the enamel surface are similar in both cases. The difference between the two in initiating caries is probably due to the tendency of the cereal particles to impact in the oclusion surfaces of the teeth thus bringing the acids of fermentation into more intimate contact with the involved surfaces.

The fundamental role of fermentable carbohydrates in contributing to the production of caries finds further support in the failure to develop caries at all with the fat ration. These results possibly justify the practice of certain dentists in prescribing diets low in sugars and starches to patients with rampant caries. It is therefore inconceivable that dental caries could develop in the absence of some source of fermentable carbohydrate in the food supply.

CONCLUSIONS

1) Caries susceptible rats fed an essentially fermentable carbohydrate-free ration remained caries-free throughout their life span.

2) Animals of the caries susceptible strain consistently developed carious lesions when fed a diet containing 50% sucrose.

3) Caries was slow to become initiated on a 50% sucrose ration but proceeded to a severe stage rapidly.

4) Caries susceptible rats fed a sucrose ration developed caries more rapidly if previously fed a fine rice ration for two weeks.

5) Rats kept caries-free for a year by feeding a fat ration were slow to initiate caries when fed a fine rice cariogenic ration. The progress of decay from the initial to the severe stage, however, was rapid.

THE INFLUENCE OF POWDERED WHOLE MILK, EVAPORATED MILK, AND EVAPORATED MILK PLUS SUCROSE ON THE PRODUCTION OF DENTAL CARIES

In view of the wide spread use of milk in the human diet it was of interest to study its effects on dental caries production in the susceptible strain of rats. Two types of milk products were used; namely, powdered whole milk and evaporated milk. Evaporated milk was used in preference to fresh whole milk because of its higher dry matter content. The evaporated milk was limited to popular brands and was fed as the only source of nourishment. The powdered milk was used in a ration consisting of 79% powdered whole milk, 20% alfalfa meal, and 1% sodium chloride.

In the case of the powdered milk study 3 litters comprising 14 susceptible animals were raised until 14-15 days of age and placed on the fat ration as previously described. When all were weaned they were then divided into two groups. One group received the powdered milk ration whereas the other was fed the fine rice control ration. The caries time recorded in Table VIII reveals the response of the control animals to be characteristic of the strain. In contrast all of the animals fed the powdered milk ration remained caries-free during the entire period of observation of 160 days.

Twenty-nine susceptible rats from six litter matings were treated in a similar manner to those above except they were divided into three groups. One group served as a control whereas the other two were fed evaporated milk and evaporated

TABLE VIII

THE EFFECT OF POWDERED WHOLE MILK ON THE PRODUCTION OF DENTAL CARIES

Litter Number	Rat #	Diet	Caries time in days* Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
43(11-19)	1	C	54	54	—	—	66	66
	2	C	54	54	—	—	66	66
	3	P.M.	Caries-free at 160 days					
43(11-7)	4	C	54	54	66	66	82	82
	5	C	54	54	—	—	82	82
	6	C	54	54	—	—	82	82
	7	P.M.	Caries-free for 160 days					
	8	P.M.	"	"	"	"	"	
	9	P.M.	"	"	"	"	"	
41(11-19)	10	C	66	66	—	—	82	82
	11	C	66	66	—	—	82	82
	12	C	66	66	—	—	82	82
	13	P.M.	Caries-free for 160 days					
	14	P.M.	"	"	"	"	"	
Mean total		C	58				78	
		P.M.	All animals remained caries-free for 160 days.					

* Caries time calculated from the beginning of the experimental ration.

milk plus 10% sucrose. Table IX shows that the animals fed condensed milk, either with or without sucrose, produced no caries throughout the entire 190 day experiment. Most of the animals, however, became emaciated and developed symptoms of anemia during the latter part of the study.

Discussion

The results of the feeding tests with evaporated milk and powdered whole milk indicate that milk products contribute little or nothing to the development of caries in a highly susceptible strain of rats. This is of particular interest because of the importance of milk in the human diet especially during the early period of life when caries in humans is particularly evident. It is quite likely that milk which has an inherent tendency to undergo an acid fermentation under favorable conditions of temperature and the presence of acidogenic bacteria does not remain in contact with any of the dental surfaces long enough to cause decay. Whether milk might contribute to the extension of cavities has not been proven by these experiments. This should be one of the first tasks in further studies of the general problem of tooth decay.

TABLE IX

THE EFFECT OF SWEETENED AND UNSWEETENED CONDENSED MILK ON THE PRODUCTION OF DENTAL CARIES

Litter Number	Rat #	Diet	Caries time in days* Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
37(8-25	15	C	74	74	90	90	120	120
	16	S.M.†	Caries-free for 190 days					
	17	U.S.M.	"	"	"	"		
	18	"	"	"	"	"		
39(8-25)	19	C	80	80	—	95	120	120
	20	S.M.	Caries-free for 190 days					
38(8-28)	21	C	95	95	—	—	120	120
	22	S.M.	Caries-free for 190 days					
	23	S.M.	"	"	"	"		
	24	U.S.M.	"	"	"	"		
34(9-74)	25	C	—	—	90	90	120	120
	26	C	—	—	78	78	90	90
	27	S.M.	Caries-free for 190 days					
	28	S.M.	"	"	"	"		
	29	U.S.M.	"	"	"	"		
	30	U.S.M.	"	"	"	"		
32(10-11)	31	C	—	—	—	—	68	68
	32	C	68	68	—	—	80	80
	33	S.M.	Caries-free for 190 days					
	34	"	"	"	"	"		
	35	U.S.M.	"	"	"	"		
	36	"	"	"	"	"		
32(10-14)	37	C	—	—	68	68	94	94
	38	C	94	94	—	—	106	106
	39	C	80	80	94	94	106	106
	40	S.M.	Caries-free for 190 days					
	41	"	"	"	"	"		
	42	U.S.M.	"	"	"	"		
	43	"	"	"	"	"		
	44	"	"	"	"	"		
Mean total		C	81				102	
		S.M.	Remained caries-free for the 109 day experiment					
		U.S.M.	"	"	"	"	"	"

*Caries time calculated from the beginning of the experimental rations.

† S.M. — sweetened milk
U.S.M. — unsweetened milk

CONCLUSIONS

1) Caries susceptible rats fed condensed milk with or without sucrose added show no tendency toward caries development over a period of 190 days even though signs of malnutrition are evident.
2) Susceptible strain rats fed a ration containing 79% whole powdered milk remained caries-free for 160 days.

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