A STUDY OF THE INFLUENCE OF DIETARY AND OTHER FACTORS ON THE PRODUCTION OF DENTAL CARIES IN A SUSCEPTIBLE STRAIN OF RATS

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By

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INTRODUCTION

The problem of tooth decay is one which has held the attention of workers in many fields for a number of years. It is not a new disease but rather has plagued mankind for centuries. The fact that it dates back to prehistoric times is substantiated by findings in ancient remains of carious human teeth (1). The statement has been made that "there never was an epoch when the human species was not cursed by toothache" (1). It is not easy to attack this problem since it has been demonstrated by workers in various fields that diet, heredity, tooth structure, secretions and micro-organisms all play a very important part in its causation. Therefore, it can be seen that it would be impossible for one person, or a single group of workers, to investigate all of these contributive factors and arrive at an answer to the problem as a whole.

Inasmuch as dental caries has been and is at present one of our chief concerns, it seems desirable to approach the problem from the standpoint of prevention as well as to study causative factors. The availability of a caries susceptible strain of rats developed by Hunt, Hoppert and Erwin (2) greatly facilitates the study of causative and preventive factors. In this study the work was divided into three main parts:

I The Effect of Certain Foods on the Production of Dental Caries.

In this part of the work some foods which are normally a part of man's diet were fed. The foods selected

for special study were two forms of the potato and a cereal breakfast food, wheat flakes.

II The Effect of Ammonia Producing Compounds and of an Ammonium Salt on the Prevention of Dental Caries.

This part of the work sought to determine if urea and urease (alone and in combination) a dibasic ammonium phosphate when incorporated in a cariogenic diet would reduce the incidence of dental caries.

III The Effect of Fluorine on Dental Caries in the Rat When Given in the Drinking Water.

This part of the work was done to determine whether or not drinking water containing fluorine in amounts comparable to and greater than those which have been observed to be caries inhibiting in drinking water for humans would decrease the incidence of tooth decay in this susceptible strain of rats.

HISTORICAL

A. General and Etiology

Dental caries is one of the oldest and commonest diseases of man. Its existence is known to have gone back as far as 2800 B. C. All through the history of mankind one finds quite frequent reference to the fact that dental caries existed, regardless of the degree of civilization, provided that starchy foods were included in their diet. Early attempts to find the basic cause of dental caries met with little success and it has been only in the last thirty years that real progress has been made. Many investigators now think that the basic cause of dental caries is the fermentation with acid production of carbohydrates in the oral cavity (3).

Several groups in this country and abroad began a series of studies on the causation of tooth decay in about 1920. Since that time great strides have been made in the field and much has been done in the way of clarifying the causes and treatment of the disease. One of the foremost groups of this field was Bunting, Jay, et.al.(4) of the University of Michigan. Their contributions have been and are continuing to be most outstanding. Among others in the field are groups headed by Doctors Klein and McCollum at Johns Hopkins University, by Doctor Rosebury and co-workers at Columbia University, and by Doctor Hanke, et.al. in the Chicago Dental Research Club of Chicago, Illinois. Outstanding individual workers include Agnew, Boyd, Bodecker, McBeath, Hunt and Hoppert, McClure,

Mellanby and Armstrong. Each has made his contribution to the complex problem and it is noteworthy that this study has brought together people from the fields of dentistry, physiological chemistry, genetics, bacteriology and nutrition.

The study of tooth decay in animals dates back to 1922 when McCollum, Simmons, Kinney and Greives (5) reported finding lesions in the teeth of the albino rat. The gross features of decay were pointed out by Greives (6) who observed that first a penetration of the enamel occurred which was followed by spread in the dentin with subsequent fracturing. More recently, Klein and McCollum (7) have attributed the cause of macroscopic caries in rats to be (a) fracture of the molar cusps; (b) decay at the bottom of the molar sulci; (c) interproximal caries where food impaction has been frequently noted; and (d) a combination of all three factors. They found that the ingestion of a rachitogenic diet containing coarse corn meal gave carious lesions, whereas the same diet containing finely ground corn showed abscence of caries.

The fact that particle size is an etiological agent in the production of dental caries was first demonstrated by Hoppert, Webber and Caniff (8) in 1931-32. Rats fed rations low in calcium and Vitamin D failed to develop cavities, whereas extensive decay was observed in the control animals fed an adequate stock diet. Further investigation by these workers showed that the stock diet contained coarse corn meal which was directly concerned with the development of caries. Other rations, although nutritionally inferior to

the stock diet, but containing corn meal ground to pass a 60 mesh sieve failed to produce caries.

One factor that must not be overlooked is that of species difference. Perhaps it will explain the report by Shaw, Schweigert, McIntire, Elvehjem and Phillips (9) that the cotton rat is better suited for dental caries studies due to the fact that there is no difference in the response of tooth decay when the particle size of the ration is varied.

Particle size etiology has been confirmed, however, by Klein and McCollum (7), Shelling and Asher (10), Rosebury, Karshan and Foley (11, 12, 13, 14), Lilly and Wiley (15) and King (16).

B. Fluorine

The incorporation of fluorine in the diets and drinking waters of both man and animal has given a new impetus to the prevention of dental caries.

The importance of fluorine was first discovered when it was observed that caries-free individuals were fairly numerous in areas in which the domestic water supply contained an appreciable amount of fluorine. Observations made by Dean (17) and his associates in the United States, by Ockerse (18) in South Africa, by Ainsworth and Weaver (19) in England, and by others in different parts of the world substantiate the statement made by Jay (20) that "the inverse relation between fluoride-bearing domestic water and the prevalence of dental caries in children has been definitely established".

Miller (21) reported in 1938 that the caries-producing potency of the H-W-C coarse rice ration is greatly reduced by the administration of fluorine in the drinking water of rats. He also found that iodoacetic acid is effective in accomplishing the same result.

Armstrong (22) reported in 1943 that caries susceptibility of rat molar teeth was decreased by the use of drinking water containing 20 ppm. fluorine given simultaneously with a caries-producing ration.

Arnold and McClure (23, 24) in 1941 reported that subcutaneous injections of fluorine in rats which was equivalent to 40-50 ppm. fluorine in the drinking water did not show any decrease in dental caries, the animals being maintained on the H-W-C simplified ration. However, they found that the fluorine content of molar enamel was increased. This finding, according to Jay (20), might be interpreted to indicate that the fluorine was adsorbed by the dentinal aspect of the enamel and hence exerted no antienzymatic or antibacterial effect on the oral surface of the teeth. It was suggested that the enamel is capable of adsorbing fluorine (25, 26).

It is of interest to note that the rat does not respond to dosages which are effective in humans. The lowest amount of fluorine reported effective in rats is 20 ppm. (22), whereas a much smaller amount is effective in humans. Ockerse (27) observed in 109 towns representing 86 districts

of South Africa, that the caries initiation rate is high if the fluorine content of drinking water is less than 1 ppm. but that the caries initiation rate is very low if the fluorine content is greater than 1 ppm. It appears that the contact of the teeth with fluorine over a number of years is essential for inhibiting dental caries as Bibby (28) observed that the use of fluorine-containing dentifrices over a period of two years was ineffective.

C. Ammonia-Producing Compounds.

The use of ammonia-producing compounds to inhibit tooth decay is of recent origin. Stephan and Miller (29) reported in 1944 that a 40% solution of urea, when used for about two years as a dentifrice by a small group of young patients, resulted in a marked decrease in the incidence of new lesions and also retarded the progress of decay in lesions already present. A short time later it was observed by Kesel, O'Donnell, and Kirch (30) that caries immune individuals have enzymes in their saliva capable of liberating ammonia from several amino acids although a number of such enzymes were found in the saliva of persons whose teeth were actually decaying, enzyme which is capable of deaminizing glutamic acid was invariably lacking. Keyes (31) reported the use of urea in connection with dental caries in the Syrian hamster. A 50% solution of urea was topically applied by weekly two-minute treatments. The animals were fed a H-W-C-sugar ration. His data showed even a slight increase in the incidence of dental caries over that of the controls. These results are surprising

and may perhaps be attributed to species differences as one would expect a slight decrease in tooth decay.

Kesel, O'Donnell, Kirch, and Wach (32, 33) incorporated dibasic ammonium phosphate in a mouth wash and a dentifrice and used these clinically for approximately five months in persons having active caries lesions. They found that Lactobacillus counts in these subjects were markedly decreased. They also found that the ammonia nitrogen that is developed in human saliva by natural processes has the ability to inhibit the growth of L. acidophillus, that the micro-organism present in the oral flora which seems to be responsible for much of the ammonia production is B. aerogenes, and that the inhibition of the L. acidophillus is not due to any alkalinity developed by the ammonia, since the same alkalinity provided by sodium acetate did not produce the inhibition that ammonium acetate did. In vitro experiments (33) indicate that a combination of 5% dibasic ammonium phosphate and 3% urea is more effective as an antibacterial and antiacidic agent than is either of these substances alone. Kesel suggests (32) that the development of ammonia nitrogen in the oral cavity may be responsible for the abscence of dental caries which some persons naturally exhibit.

The finding of Kesel, et. al. that an organism, B. aerogenes, may be responsible for the production of ammonia in the oral cavity supports the theory advanced by Schantz and Scrivener (34). They have suggested that the use of mouth

washes and dentifrices may be instrumental in promoting tooth decay, because these products may frequently destroy the delicate organisms that nature has provided as a part of a defensive system against processes destructive to the tooth.

D. Miscellaneous

Some recent findings that are worth mention are those of McClure and Hewitt (35). They placed rats on a corn meal caries-inducing ration containing 75 units of penicillin per gram and drinking water containing 75 units per milliliter. At 125 days the rats receiving penicillin were caries-free whereas control animals had a 50% incidence of lesions. Plate counts of L. acidophillus were negative while those for the controls were greater than 50 colonies per plate. The ingested penicillin did not produce a measurable concentration in the blood. It was, therefore, assumed to have a localized action.

Burrill, Calandra, Tilden and Fosdick (36) administered synthetic Vitamin K (2-methyl-1,4-napthoquinone-sodium bisulphite) to patients in the form of chewing gum. They found the incidence of new lesions were lowered 60-90% by the use of this gum. Gum containing calcium carbonate without the Vitamin K reduced the occurrence of new cavities approximately half as much as the Vitamin K gum.

Turner and Crane (37) found that individuals with extensive cavities produce saliva which hydrolyzes starch very rapidly, whereas those caries-free persons produce saliva

which hydrolyzes starch very slowly. Wach, Kesel, Hine and O'Donnell (38) have reported that the caries activity of a patient may be evaluated by determining the pH and the total titrable acidity.

EXPERIMENTAL

A. General

1. Animals used

The animals which were used in these experiments were of the strain produced by Hunt, Hoppert and Erwin (2). This strain of albino rat was developed by selectively breeding caries susceptible animals and resulted in the production of a strain of rats which develops caries in a very short time and are therefore well adapted for dental caries studies. The animals used represented the 17th generation of selectively bred stock.

2. Care of the animals

The breeding stock was kept in suitable cages supplied with bedding of wood shavings. Each breeding group was composed of animals which were litter mates. The practice of breeding litter mates was suggested by Doctor Hunt and was regularly employed in his genetic studies of tooth decay. It was desirable to have litters containing at least two females and, as a rule, two males were retained for each breeding group. Upon showing signs of pregnancy the females were removed to small isolation cages and were kept on the stock ration until the young had reached an age of two weeks. At this time the mother and litter were transferred to a clean cage and placed on a fermentable carbohydrate-free ration[1] so that caries would not be initiated in the young

1 This ration consisted of casein (40%), alfalfa meal (30%), brewer's yeast (9%), fat (lard or crisco) (20%), and sodium chloride (1%).

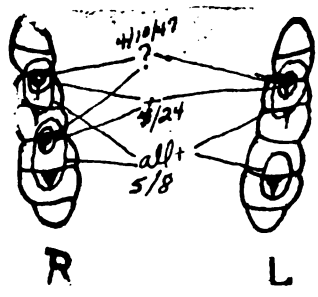
rat before it could be placed on an experimental ration. At twenty-one days the young were weaned, the mother being returned to the breeding cage. The use of the fermentable carbohydrate-free ration was continued until the rats were placed on the experimental ration.

The animals were housed in metal cages supplied with wire mesh bottoms. Food was kept before the animals at all times in glass jar containers and fresh tap water was supplied daily with the exception of one experimental group (Group IX).

3. Observations

Examinations of the lower molars were made at two week intervals. A nasal speculum was found to be very convenient for making the observations. The animals were held by the nape of the neck and the nasal speculum was used to open the mouth, and to expose the lower molars to view. A common desk lamp with a flexible stand equipped with a 100 watt bulb supplied adequate light for the examinations. A record of caries initiation and progression was kept by marking a tooth map similar to the one designed by Doctor Hunt. When there was any doubt as to the existence of a carious lesion in a certain area of a tooth a question mark ("?") was recorded with the date of the observation. When a definite carious lesion was noted a "+" was recorded and the approximate extent of the lesion indicated on the tooth map. In cases in which a number of "doubtfuls" or question marks occurred the date of

the last one recorded previous to the observance of a positive lesion was arbitrarily considered the date of the initiation of the carious lesion. In these studies it was common for a "positive" definite carious lesion to appear two weeks after a "doubtful" was recorded. A sample of the type of records kept is herewith shown.



In the data tables it may be noticed that, in some cases, blanks appear in the "initial", "moderate", and "severe" stages. A blank in the "initial" column means that moderate or severe caries was found at the initial observation. A blank in the "moderate" column indicates that the lesions progressed from the initial to the severe stage between successive examinations, whereas a blank in the "severe" column indicates that the lesions in that particular animal did not reach the severe stage before examinations were discontinued.

4. Preparation of Diets

The control ration used contained the same ingredients as the cariogenic ration developed by Hoppert, Webber and Caniff (39). However, for nutritional studies on caries prevention

this ration was found to be too rapid in its initiation and production of caries. Therefore, the rice for the control ration used was ground in a Hobart Mill so that only 2% would be retained by a 20 mesh sieve. When rats of this susceptible strain are placed on the control ration, caries of the lower molars will be initiated with 35 to 70 days with slight variations due to genetic differences in the individual animals. The dried potatoes and wheat flakes were ground to the same consistency as the rice. In the case of the dry rations, the constituents were mixed thoroughly by hand in a large pan.

From preliminary experiments it was found desirable to feed whole boiled potatoes along with a supplementary ration to furnish nutrients which are essential for the well being of the animals. This supplementary ration consisted of powdered milk, alfalfa meal, and sodium chloride.[1] The animals relished the boiled potatoes and chose them for the greater part of their daily intake (85%).

The diets containing urease were prepared as follows: the urease powder (Arlco urease) was taken up in a 30% solution of ethanol and then mixed with a portion of the dry feed. This was allowed to dry overnight and then was ground in a small Hobart Mill. If urease alone was to be added to the ration, this "pre-mix" was then incorporated directly with

1 This ration was selected because it was demonstrated previously by Olson to have no caries producing effects.

a calculated amount of the basal ration. When urea was also to be added, it was first finely ground in a mortar and added to the "pre-mix". By this method a more even distribution of the constituents was obtained.

In preparing the rations containing urea only, or those containing dibasic ammonium phosphate, a "pre-mix" was likewise prepared with the finely ground compounds.

5. Composition of Diets

The fine rice ration (Diet I) was used throughout these studies as a control ration. It was also used as the basal ration when ammonia compounds and urease powder were fed. The composition of diets used are listed in the following tables.

Diet I
Fine Rice Ration

Fine rice	66%
Powd. whole milk	30%
Alfalfa leaf meal	3%
Sodium chloride	1%

Diet II
Dried Potato Ration

Ground dried potatoes	79%
Alfalfa leaf meal	10%
Casein	10%
Sodium chloride	1%

Diet III
Boiled Potato Ration

Boiled potatoes	ad libitum
Supplementary ration	"
Powd. whole milk	79%
Alfalfa leaf meal	20%
Sodium chloride	1%

Diet IV
Wheat Flake Ration

Ground Wheat flakes[1]	79%
Alfalfa leaf meal	10%
Casein	10%
Sodium chloride	1%

Diet V
Dibasic Ammonium Phosphate Ration

Fine rice ration	99%
Dibasic amm. phosphate	1%

Diet VI
Urea - Urease Ration

Fine rice ration	98.5%
Urea	1.0%
Urease	0.5%

1 The wheat flakes used were Wheaties, a commercial product of General Mills, Inc.

Diet VII Urea Ration		Diet VIII Urease Ration	
Fine rice ration	99%	Fine rice ration	99.5%
Urea	1%	Urease	0.5%

The rations were kept in covered metal cans and those containing urease, urea or both, were kept in a refrigerator.

B. Procedure

1. Group I

This group consisted of animals which were fed Diet II (dried potato ration). Five animals were included in this group. The litters were divided so that control animals served for both Group I and Group II.

2. Group II

Fourteen animals were included in this group. They received the experimental ration listed as Diet III (boiled potato ration). They represent animals taken from six litters. Boiled potatoes were fed daily and the supplementary ration was eaten ad libitum. Data for Groups I and II are found in Table I.

3. Group III

Diet IV (wheat flake ration) was the ration fed to this group. The group included twenty-three animals from seven litters of which ten animals were fed the control ration (Diet I). Date for this group is found in Table II.

4. Group IV

This group of animals was fed a ration containing 99% of Diet I and 1% of $(NH_4)_2CO_3$. Detailed data are not supplied

for this group because the experiments were discontinued due to reasons to be discussed later.

5. Group V

This group represented animals from two complete litters. Sixteen animals were used in this experiment; six of which were placed on the control ration (Diet I), the other ten received Diet V (1% of dibasic ammonium phosphate incorporated in the control ration).

6. Group VI

There were actually two lots of animals included in Group VI. As may be seen in Table IV of the data, animals having numbers 33 through 57 had a much longer record than those listed in the remainder of the table. Originally five animals from four litters were placed on Diet VI (urea plus urease ration). When examinations showed that initiation of caries was not occurring in these animals, an expansion of the group seemed desirable. Therefore, animals from four new litters were then divided into four sub-groups and placed on Diets I, VI, VII, and VIII respectively. Five of these animals were included in the sub-group which received Diet VI and are thus included in this group.

7. Group VII

Seven animals from the four litters mentioned above made up this group. The same animals were used for controls. These animals were fed the experimental ration listed as Diet VII (urea ration).

8. Group VIII

The animals in this group were members of the same four litters as were those of Group VII. Seven animals are included and were fed Diet VIII (urease ration). Control animals are those which also serve for Groups VI and VII.

9. Group IX

This group was comprised of forty-four animals representing nine litters. These animals were divided into four groups. The first consisting of fifteen rats was used as a control group and tap water was supplied for drinking purposes. Fluorine, in the form of sodium fluoride, was added to the drinking water of the remaining three groups. The first of these three groups, containing eleven rats, received water containing two ppm. of fluorine; the second, consisting of eight animals, received five ppm. of fluorine; and the third group, comprised of ten rats, received ten ppm. of fluorine. All of these animals were fed Diet I.

DATA

TABLE I

EFFECT OF POTATO RATIONS ON DENTAL CARIES

Litter No.	Rat No.	Diet	Caries time in Days: Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
41 (12-12)	1	I	55	55	83	-	99	99
	2	II	Caries-free at 281 days					
	3	II	"	"	"	"	"	
	4	III	"	"	"	"	"	
29FCF(12-10)	5	I	55	70	83	-	99	99
	6	II	Caries-free at 281 days					
	7	II	"	"	"	"	"	
	8	III	"	"	"	"	"	
	9	III	"	"	"	"	"	
29FCF(11-27)	10	I	45	70	83	83	99	99
	11	II	Caries-free at 281 days					
	12	III	"	"	"	"	"	
43(12-21)	13	I	49	49	-	-	77	77
	14	I	49	65	-	-	93	93
	15	I	49	-	-	-	77	77
	16	III	Caries-free at 275 days					
	17	III	"	"	"	"	"	
	18	III	"	"	"	"	"	
	19	III	"	"	"	"	"	
43(12-30)	20	I	43	55	-	-	77	77
	21	III	Caries-free at 269 days					
	22	III	"	"	"	"	"	
47(1-7)	23	I	76	76	*	-	110	110
	24	I	46	46	-	-	76	76
	25	III	Caries-free at 244 days					
	26	III	"	"	"	"	"	
	27	III	"	"	"	"	"	
	28	III	"	"	"	"	"	

TABLE II

EFFECT OF WHEAT FLAKE RATION ON DENTAL CARIES

Litter No.	Rat No.	Diet	Caries-time in days: Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
42(1-10)	29	I	46	46	76	76	110	110
	30	I	60	60	76	76	110	110
	31	IV	Caries-free at 343 days					
	32	IV	"	"	"	"	"	
41(1-10)	33	I	76	76	-	-	110	110
	34	I	46	46	76	76	110	110
	35	IV	Caries-free at 343 days					
	36	IV	"	"	"	"	"	
32(1-14)	39	I	-	-	76	76	110	110
	40	I	-	-	76	76	110	110
	41	IV	Caries-free at 343 days					
	42	IV	"	"	"	"	"	
45(1-25)	43	I	-	-	62	62	96	96
	44	IV	Caries-free at 328 days					
	45	IV	"	"	"	"	"	
	46	IV	"	"	"	"	"	
32(1-16)	48	I	46	46	76	76	110	110
	49	IV	Caries-free at 343 days					
47(1-9)	51	I	65	65	-	-	110	110
	52	IV	Caries-free at 343 days					
42(1-25)	54	I	-	-	62	62	96	96
	55	I	-	-	62	62	96	96
	56	IV	Caries-free at 328 days					

TABLE III

EFFECT OF DIBASIC AMMONIUM PHOSPHATE ON DENTAL CARIES WHEN INCORPORATED IN THE CONTROL DIET

Litter No.	Rat No.	Diet	Caries time in days: Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
49(7-16)	67	I	-	-	133	133	164	164
	68	I	-	-	-	-	164	164
	69	I	133	-	-	-	164	164
	70	V	Caries-free at 200 days					
	71	V	"		"	"	"	
	72	V	"		"	"	"	
	73	V	"		"	"	"	
54(7-16)	74	I	133	-	-	133	164	164
	75	I	133	-	-	133	164	164
	76	I	133	-	-	133	164	164
	77	V	Caries-free at 200 days					
	78	V	"		"	"	"	
	79	V	"		"	"	"	
	80	V	"		"	"	"	
	81	V	"		"	"	"	
	82	V	"		"	"	"	

TABLE IV

EFFECT OF UREA AND UREASE ON DENTAL CARIES WHEN INCORPORATED IN THE CONTROL DIET

Litter No.	Rat No.	Diet	Caries time in days: Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
41(1-10)	33	I	76	76	-	-	110	110
	34	I	46	46	76	76	110	110
	37	VI	Caries-free at 264 days					
	38	VI	"	"	"	"	"	
32(1-16)	48	I	46	46	76	76	110	110
	50	VI	Caries-free at 343 days					
47(1-9)	51	I	65	65	-	-	110	110
	53	VI	Caries-free at 264 days					
42(1-25)	54	I	-	-	62	62	96	96
	57	VI	Caries-free at 250 days					
54(6-16)	83	I	93	115	115	-	146	146
	84	VI	Caries-free at 162 days					
54(5-28)	85	I	-	-	93	115	146	146
	86	VI	Caries-free at 162 days					
	87	VI	"	"	"	"	"	
44(6-1)	88	I	-	-	93	115	146	146
	89	VI	Caries-free at 162 days					
54(6-19)	90	I	115	-	162	162	-	-
	91	VI	Caries-free at 162 days					

TABLE V

EFFECT OF UREA ON DENTAL CARIES WHEN INCORPORATED IN THE CONTROL DIET

Litter No.	Rat No.	Sex	Diet	Caries time in days: Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
54(6-16)	83	M	I	93	115	115	-	146	146
	92	M	VII	Caries-free at 162 days					
54(5-28)	85	M	I	-	-	93	115	146	146
	93	M	VII	Caries-free at 162 days					
	94	F	VII	"	"	"	"	"	
44(6-1)	88	M	I	-	-	93	115	146	146
	95	M	VII	Caries-free at 162 days					
	96	F	VII	146	146	162	162	-	-
54(6-19)	90	M	I	115	-	162	162	-	-
	97	M	VII	Caries-free at 162 days					
	98	F	VII	146	146	162	162	-	-

TABLE VI

EFFECT OF UREASE ON DENTAL CARIES WHEN INCORPORATED IN THE CONTROL DIET

Litter No.	Rat No.	Sex	Diet	Caries time in days: Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
54(6-16)	83	M	I	93	115	115	-	146	146
	99	M	VIII	Caries-free at 162 days					
	100	F	VIII	115	115	146	146	162	-
54(5-28)	85	M	I	-	-	93	115	146	146
	101	M	VIII	Caries-free at 162 days					
	102	F	VIII	"	"	"	"	"	
44(6-1)	88	M	I	-	-	93	115	146	146
	103	M	VIII	Caries-free at 162 days					
54(6-19)	90	M	I	115	-	162	162	-	-
	104	M	VIII	Caries-free at 162 days					
	105	F	VIII	115	-	162	-	-	-

TABLE VII

THE EFFECT OF ADDING FLUORINE TO THE DRINKING WATER

Litter No.	Rat No.	ppm. of F*	Caries time in days: Initial R	Initial L	Moderate R	Moderate L	Severe R	Severe L
43(4-15)	106	None	-	-	97	97	124	124
	107	None	124	124	-	-	-	-
	108	2	97	97	-	-	124	124
	109	2	97	97	-	-	-	-
	110	5	124	124	-	-	-	-
41(5-17)	111	None	124	124	-	-	-	-
	112	"	97	97	-	-	124	124
	113	5	-	-	-	-	97	97
	114	5	97	97	-	-	124	124
49(5-2)	115	None	124	124	-	-	-	-
	116	"	-	-	97	97	124	124
	117	"	-	-	97	97	124	124
	118	2	Caries-free for 124 days					
	119	2	-	-	97	97	124	124
	120	5	-	-	97	97	124	124
	121	5	96	-	124	-	-	-
52(5-8)	122	None	-	-	97	97	124	124
	123	2	-	-	97	97	124	124
	124	2	-	-	-	-	97	97
	125	5	97	-	97	-	124	124
47(5-10)	126	None	-	-	97	97	124	124
	127	2	Caries-free for 124 days					
	128	5	124	-	-	-	-	-
49(5-25)	129	None	117	-	-	-	-	-
	130	2	117	-	-	117	-	-
	131	5	-	-	117	117	-	-
	132	10	Caries-free for 117 days					
43(5-25)	133	None	90	90	-	-	117	117
	134	"	-	-	117	117	-	-
	135	10	-	-	117	117	-	-
	136	10	90	-	117	117	-	-
	137	10	-	-	-	117	-	-
	138	10	90	90	-	-	117	117
41(5-21)	139	None	117	-	-	-	-	-
	140	2	-	-	117	117	-	-
	141	10	Caries-free for 117 days					
	142	10	117	-	-	117	-	-
	143	10	Caries-free for 117 days					
	144	10	117	-	-	-	-	-
42(5-23)	145	None	117	-	-	-	-	-
	146	"	117	-	-	-	-	-
	147	10	-	-	90	90	117	117
	148	2	-	-	117	117	-	-
	149	2	-	-	117	117	-	-

*All animals were fed Diet I

DISCUSSION

1. Groups I and II

Potatoes (White or Irish) were selected for special study because of their prominence in human diets and because it was felt that their non-glutenous character might cause them to be non-cariogenic. They were therefore fed in two forms, rats in Group I receiving a diet containing dried, finely ground potatoes and those in Group II receiving boiled potatoes supplemented with a non-cariogenic complete food mixture.

As may be seen in Table I, the control animals (those on Diet I) showed tooth decay characteristic of the susceptible strain of rats used in these experiments, whereas the animals on both Diets II and III were caries-free when last examined, 281 days from the start of the experiment.

The results of these tests may have far-reaching practical application although it must be recognized that the experimental procedure employed here was arbitrary and that no claims can be made that potatoes would be non-cariogenic in humans. However, the striking results with the highly susceptible strain of rats would make it seem very probable that similar results might be obtained with humans. It would certainly seem logical on the basis of these results to permit individuals having rampant tooth decay and restricted by their dentists in the use of carbohydrate foods, to make liberal use of potatoes. In this connection it is fortuitous that the potato lends itself to preparation in so many different ways.

2. Group III

The animals included in this group were fed Diet IV (wheat flake ration). The control rats were fed the fine rice cariogenic ration (Diet I).

All of the animals fed Diet IV remained caries-free for the duration of the experiment, namely 343 days, whereas the control rats developed caries in a fairly short time (see Table II). It is likely that the former would have continued to remain caries-free indefinitely. That the method of using the wheat flakes as part of a complete diet was arbitrary must be admitted. Certainly there is no similarity to the conventional use of breakfast foods by humans. Nevertheless, it is significant that a highly susceptible strain of rats should remain caries-free on a diet containing 79% of a ground commercial breakfast food.

3. Group IV

Eleven young rats from the caries-susceptible strain were divided into two groups, one of which received the cariogenic diet containing 1% ammonium carbonate and the control group the unsupplemented diet. No detailed data is given because there was no significant difference in the caries-time of the two groups. However, it had been expected that ammonium salts would inhibit tooth decay so it was apparent that possibly the volatile nature of ammonium carbonate had been ignored. Although the ration had been stored in the refrigerator, subsequent analysis employing the Van Slyke and Cullen method

for ammonia disclosed that only traces of ammonia could be detected in the ration. This accounts for the apparent failure of ammonium carbonate to inhibit tooth decay. A positive inhibition was strikingly demonstrated when dibasic ammonium phosphate was substituted for ammonium carbonate. The results of this test are presented in the next section.

4. Group V

The animals comprising the group were fed Diet V, containing 1% dibasic ammonium phosphate. The results shown in Table III indicate that dibasic ammonium phosphate, when incorporated in a cariogenic ration, tends to inhibit tooth decay in a highly susceptible strain of rats. The animals were caries-free at 200 days, whereas the control rats showed initial caries at 130 days.

It is apparent that the initiation time of caries in the control animals was longer than previously reported to be characteristic of the susceptible strain of rats. This may be due to the fact that the rats were somewhat older than those previously used and had been kept on a fermentable carbohydrate-free ration until the start of the experiment. Nevertheless, the results clearly demonstrat that dibasic ammonium phosphate definitely inhibited the development of caries and are in substantial agreement with those reported with humans by Kesel, et. al. (32,33).

5. Group VI

The animals in this group received Diet VI containing urea and urease. To date, all of these animals have remained caries-free. Part of the group was fed Diet VI for 343 days and the remainder of the group, which was started later, for 162 days. As may be seen in Table IV, the control animals showed characteristic susceptibility to tooth decay, although some variations in initiation time were observed.

The exact mechanism of the inhibition by urea and urease is not completely understood, although we can be certain of the liberation of ammonia by the action of urease on urea when the food is moistened by saliva. That the ammonia acts merely as a neutralizing agent is questionable since Kesel, et.al. (32,33) have observed that the L. acidophillus is not inhibited by a degree of alkalinity supplied by sodium acetate equivalent to that resulting from the production of ammonia in the oral cavity of humans. Apparently the ammonium ion supplied either by ammonium salts or by the action of urease on urea will directly inhibit acidogenic bacteria.

6. Group VII

When the animals fed Diet VI showed an absence of caries after eating the ration for 180 days, it was decided to determine whether urea or urease alone would exert a caries-inhibiting action. Accordingly, Groups VII and VIII were set up receiving the urea and urease supplemented diets VII and VIII respectively. The data for Group VII is recorded in Table V.

Most of the animals fed Diet VII were caries-free at 162 days whereas two showed an initiation-time of 146 days. The fact that the two positive reactors were females as were two positive reactors in Group VIII indicates that sex may be a factor in caries susceptibility. Litter-mate males were caries-free at the time the last examinations were made. At any rate the inclusion of 1% urea in an otherwise cariogenic ration effected a definite delay in the development of caries.

Although Keyes (31) has shown that the topical application of urea to the molars of the Syrian hampster does not inhibit dental caries in this species, Stephan (40) found that a mouth wash containing urea did demonstrably inhibit tooth decay in humans. Stephan attributed this effect to the denaturation of bacterial enzymes by the urea solution, although he presented no direct proof for this view.

It has also been suggested that ammonia might be produced from urea by bacterial action. If the latter is true, bacterial stasis would be caused by the production of ammonia by the bacteria themselves. Until definite proof is supplied the explanation for the inhibiting effect of urea will remain a matter of speculation.

7. Group VIII

The animals in this group were fed Diet VIII containing urease. They were taken from the same litters as those of Group VII.

Here, again, we find sex differences. Two females in this group showed little evidence of caries inhibition. As may be seen in Table VI. The rest of the group remained caries-free.

It would seem, therefore, that urea and urease alone may exhibit a caries-inhibiting effect when incorporated in a cariogenic diet. However, the effect is not as striking as when both urea and urease are present. In the latter case the production of ammonia is inevitable, whereas the formation of ammonia is less certain in the presence of urea or urease alone. Whether other modes of inhibition are operative for urea or urease has not been demonstrated.

8. Group IX

The animals used in this experiment were divided into four groups. All were fed the fine rice cariogenic ration. The first was used as a control and was supplied with tap water. The other three groups received 2 p.p.m., 5 p.p.m., and 10 p.p.m. of fluorine in their drinking water.

In view of the fact that only two animals receiving 2 p.p.m. of fluorine in the drinking water and three animals receiving 10 p.p.m. of fluorine remained caries-free at 124 days, the experiment was discontinued. The inhibiting effect of fluoride at these levels was insignificant inasmuch as the average initiation-time in the control animals was 113 days.

It is quite apparent that levels of fluorine in the drinking water up to 10 p.p.m. do not inhibit tooth decay in this

susceptible strain of rats. These results agree with those of McClure (23,24) who reported that no reduction of caries occurred in albino rats fed 10 p.p.m. of fluorine in the drinking water. However, McClure did find levels of 40-50 p.p.m. to be effective. This represents a much higher concentration than that found effective in humans viz. 1-1.5 p.p.m.

CONCLUSIONS

The following conclusions may be drawn from the studies carried out with a highly susceptible strain of rats:

1. Potatoes fed separately as boiled potatoes or as finely ground after cooking and drying proved to be non-cariogenic.
2. A diet containing 79% of ground commercial wheat flakes was found to be non-cariogenic.
3. The addition of 1% of dibasic ammonium phosphate to a cariogenic diet completely inhibited dental caries.
4. The addition of 1% of urea and .5% of urease (Arlco Urease) to a cariogenic ration also completely inhibited dental caries.
5. Additions of 1% urea or .5% urease were partially effective in preventing tooth decay.
6. The supplying of drinking water containing up to 10 p.p.m. of fluorine was ineffective in preventing tooth decay in a caries-susceptible strain of rats.

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