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THE EFFECT OF CARBOHYDRATES ON EXPERIMENTAL CARIES IN THE RAT

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THE question of the role of sugar in human caries has been so frequently discussed that no attempt will be made to review the literature herewith.‡

In studies of the relationship of carbohydrates to dental caries in man, it has not been possible to recognize influences operating through oral as distinguished from those operating through systemic nutritional channels. Consequently, the interpretations of the effects of carbohydrates on caries in *man* have been controversial.

A feature of special interest which is gained in studies of the *albino rat* is that, at least to some degree, a separation is possible of the forces initiating caries from those forces advancing already established lesions. The albino rat is unique in that it does not have a significant incidence of dental caries when maintained for long periods of time on diets high in carbohydrates,[1-4] but the rat will show caries-like lesions in a relatively short time if maintained on rations containing coarse particles of maize or rice.[5] The nonoccurrence of caries in carbohydrate-fed albino rats indicates that carbohydrates are not potent initiating factors of caries in this species; the occurrence of carious lesions after diets containing identifiable initiating factors shows the albino rat to be susceptible to a tooth injury which our data show develops further like true dental caries.§

Shibata,[6] in 1928-1929, fed to rats diets containing rice and greens mixed (to the extent of 5 per cent) with sucrose, glucose, lactose, and maltose, respectively. He failed to recognize the importance of mechanical factors and ascribed the carious attack to the added carbohydrates. Subsequent to the demonstration of the role in rat caries of coarse corn particles by Hoppert, Webber, and Canniff[5] in 1931, Rosebury, Karshan, and Foley[7] were able to show that a similar causal role could be ascribed the coarse rice particles in the diets used by Shibata.

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‡The reader may find an introduction to the literature in the "General Analysis," p. 254 of Dental Caries, ed. 2, 1941, Lancaster Press, Inc., Lancaster, Pa.

§For the purpose of this discussion, it is suggested that two stages be clearly distinguished: (1) initiation of caries, and (2) promotion, development, acceleration, and cavitation. It is not necessary to assume that the factors involved in either are mutually exclusive or that these factors are ever present alone.

The recent findings by Keyes[9] that a high sugar diet increases the incidence of caries in *hamsters* has opened up the entire field of the relationship of sugar to experimental caries. There is some additional evidence available from studies on *albino rats*[10, 11, 12] and on *cotton rats*.[13, 14, 15]

It is peculiar in the light of the notable attention which has been directed to this subject that no studies have appeared heretofore of the effects on *rats'* teeth of adding excessive amounts* of sugar to the Hoppert-Webber-Caniff coarse corn particle diet.[5] Several such experiments are described in the following pages. However, the data in this report are not offered as conclusive evidence in a long debate, but only to point out a procedure which may be exploited profitably to gain additional knowledge.

The data reported herewith present, *first,* evidence that the maintenance diets containing no coarse corn, regardless of their starch and sugar content, produce caries only rarely when rats are maintained on them for as long as eleven months. *Second,* when rats are placed on a caries-producing diet for a short period and then subsequently maintained on diets containing various carbohydrates in excessive amounts, it was believed that the stage was set for testing the effect of carbohydrates on the incidence and continuation of rat caries. Three experiments have been performed. In the first one, the HWC† diet was just diluted by adding an equal weight of powdered sugar. In the second one, the HWC diet was followed by various diets containing no coarse corn but with high levels of selected carbohydrates. In the third experiment, each HWC ration was prepared using, as nearly as possible, only one carbohydrate, the same carbohydrate that was present at a high level in the diet during the continuation period when no coarse corn particles were present.

THE NONOCCURRENCE OF CARIES IN RATS ON DIETS WHICH DO NOT CONTAIN COARSE PARTICLES

Albino rats at 28 days of age were distributed into three groups and fed the rations shown in Table I. When the rats were 112 days old, they were mated. Some of the offspring were used in the experiments described in the following pages on the promotion of caries after initiation. One, two, and three litters of young, averaging 8.5 rats per litter, were born on these rations, and over two-thirds of the litters were weaned on each of the high carbohydrate diets. Many of the young were raised to maturity and third and fourth generations were obtained. Thus the rations appear to be reasonably complete for growth and reproduction.

The teeth of all rats which died during the course of experiments or which were killed for various reasons have been examined for caries. With one curious

*Rosebury and Karshan[8] added up to 28 per cent sugar to diets in which coarse rice or corn particles were present with inconclusive results.

†The coarse corn meal ration of Hoppert, Webber, and Canniff.[5]

exception,* no caries were found in any rats that had not been on diets containing corn meal. Two rats of the 76 studied showed small cavities. One rat on the glucose diet (Table I) had a small cavity in an upper molar; another rat on the same diet had several questionable cavities. In the other 74 rats that had not been on the corn meal diets, no caries was found. These rats ranged in

TABLE I

PERCENTAGE COMPOSITION OF THE DIETS USED IN THE STUDY OF THE COMPARATIVE EFFECTS OF DIFFERENT CARBOHYDRATES IN THE RATION OF RATS

	STARCH DIET	SUCROSE DIET	GLUCOSE DIET	LOW CARBOHYDRATE DIET*
Corn starch	65.5	–	–	12.0
Sucrose	–	66.0	–	–
Glucose hydrate	–	–	69.3	–
Hydrogenated fat†	5.0	5.0	4.5	26.0
Butter fat	5.0	5.0	4.5	26.0
Casein‡	20.4	20.0	18.1	30.0
Salt mixture§	4.1	4.0	3.6	6.0

*Note that the low carbohydrate diet is a high fat diet. All diets were designed to be isocaloric.

†Crisco.

‡Labco casein, chosen to minimize the presence of lactose. It has a minimum of fluorine.[17]

§Osborne-Mendel[13]

Daily supplement of 0.5 Gm. unirradiated dried brewer's yeast, 0.5 Gm. alfalfa leaf powder, and 0.1 drop of olive oil. About 1 Gm. of fresh beef liver was fed twice a week.

age from 3 months to 11 months, and they had been maintained after weaning on diets as listed in Table II.

TABLE II

DISTRIBUTION OF RATS IN THE VARIOUS DIETS

DIET	NUMBER OF RATS WITH NO CARIES
Starch	19
Sucrose	9
Glucose	6
Low carbohydrate	19
Starch, 2nd generation	4
Sucrose, 2nd generation	7
Low carbohydrate, 2nd generation	5
Sucrose, 3rd generation	4
Low carbohydrate, 3rd generation	1
Total	74

Of the total of 76 rats, 28 were on either the glucose or sucrose diet; these sugars have been shown to promote the development of caries induced by cracked corn meal. As only two rats on the glucose diet showed any trace of tooth decay, it can be concluded that sugars have little if any effect in starting rat caries. This conclusion is in agreement with the findings of Lilly,[1] that rats kept for a year on high sucrose diets do not develop caries.

*The rats of a single litter had a striking deformation of the jaw bones which consisted of an excavation of the bone surrounding the teeth. In some cases, the bone had disappeared or perhaps had failed to form to a level of about the tips of the roots of the teeth. The teeth of this litter showed many cavities but of a distinctly different appearance from that due to corn meal. Because of the probable connection between the deformity of the jaw bones and the decay of the teeth, and because of the evident familial relationship, this litter is not regarded as representative and is omitted from consideration.

ACCELERATION OF THE PROCESS OF CAVITATION BY CARBOHYDRATE

Experiment No. 1.—Plan: Cavities were initiated by maintaining the rats on the HWC diet for six weeks. The effect of sugar was tested by drastically increasing the dietary content of this substance, namely, half HWC diet plus half confectioner's sugar, and by giving access to this diet, to the amount desired, for about nine weeks.

A group of 48 albino, Wistar-strain rats (29 male, nineteen female) were put on the Hoppert-Webber-Canniff diet[5] at weaning (about 4 weeks of age) and continued on this diet for six weeks. In the belief that at this time some of the teeth were suffering early damage from the coarse corn particles of the diet, a group of 13 rats (7 male, 6 female) were placed on a diet prepared by mixing equal weights of the HWC diet with powdered confectioner's sugar (grade marked "XXXX"). Since no extra vitamin or mineral supplements were supplied, it is not surprising that these rats grew somewhat less rapidly than did the control group. For example, the experimental male rats weighed on the average about 266 grams after 104 days on the sugar-rich diet, whereas the control male rats averaged about 336 grams.

At the time of sacrifice, the sugar-fed rats were in good coat and condition. Autopsies were performed and weights of each of a number of organs were obtained. The average organ weights of the control and experimental rats are given in Table III. The only abnormality observed in the experimental rats was the presence of striking quantities of nearly pure white fat in the omentum, mesentery, and perirenal regions. These milky fat deposits were not examined chemically. The organs of the peritoneal cavity, stripped of their excessively fatty surrounding tissues, appeared normal and were for the most part of normal average weights. The livers of the experimental rats, both male and female, were somewhat heavier than the livers of the control rats.

TABLE III

AVERAGE ORGAN WEIGHTS OF CONTROL AND EXPERIMENTAL RATS

	BRAIN (GM.)	LIVER (GM.)	SPLEEN (GM.)	KIDNEY (GM.)	LUNGS (GM.)	HEART (GM.)	STOMACH (GM.)
			Male				
Experimental group	1.80	9.89	0.70	1.47	1.60	1.01	0.99
Control group	1.66	9.43	0.78	1.78	1.69	1.15	1.09
			Female				
Experimental group	1.72	7.23	0.58	1.20	1.36	0.74	0.89
Control group	1.71	5.99	0.62	1.14	1.29	0.84	0.92

Blood samples taken from the descending aorta at the time of sacrifice were studied (a) for red blood cell counts and (b) for hematocrit values. The average red blood counts were as follows: control, 8.0 million; and sugar-fed, 8.3 million. Both of these averages are within normal limits for our rats. The average hematocrit readings were as follows: control, 45.6 per cent; and sugar-fed, 44.5 per cent. The latter value is somewhat low, but both values are probably within normal limits.

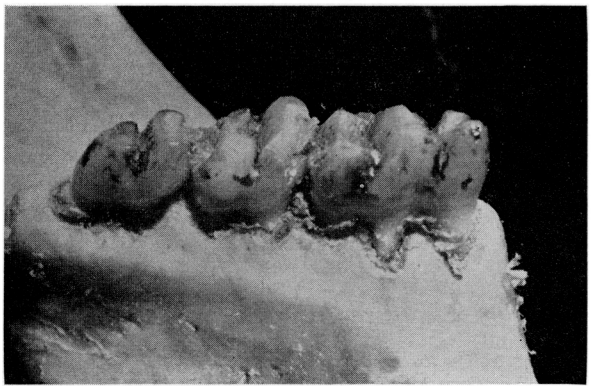

Fig. 1.—(×11). Lower jaw showing slight caries destruction. The size and place of the teeth may be judged from this picture.

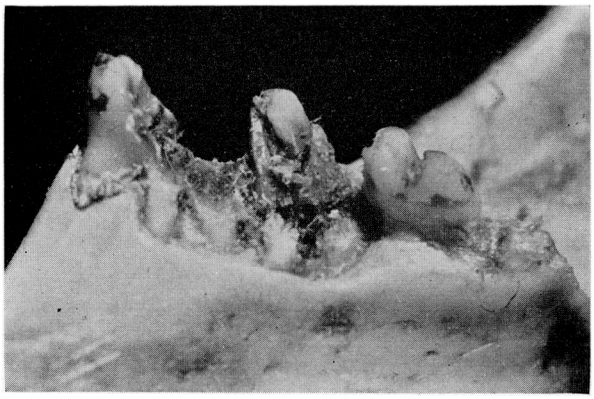

Fig. 2.—(×11). Lower jaw. The major portion of the first molar has been lost and considerably more than half of the second molar is missing.

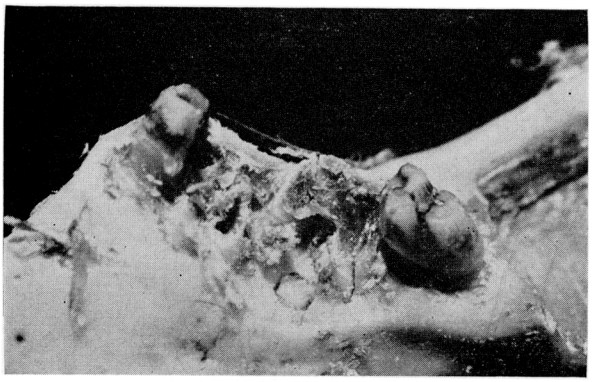

Fig. 3.—(×11). Lower jaw. Advanced destruction. The third molar is intact, and a tiny fraction of the most anterior cusp of the third molar is missing.

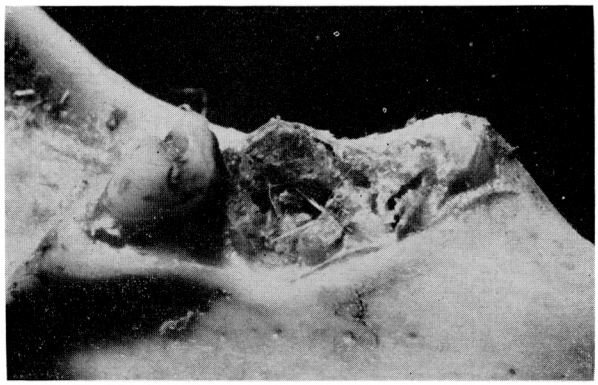

Fig. 4.—(×11). Lower jaw. The third molar is practically intact, and the mesial cusp of the first is intact. The rest of the tooth tissue has been broken.

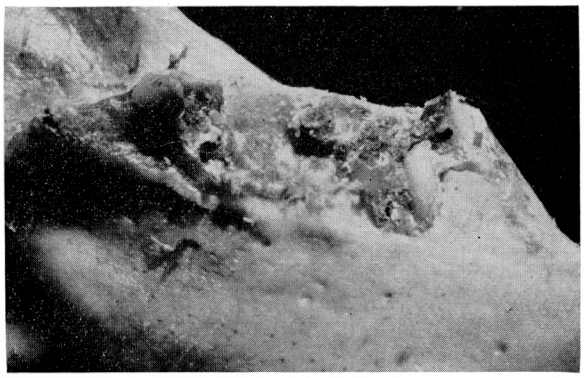

Fig. 5.—(×11). Lower jaw. Only the distal portion of the third molar is present above the gum line. Extremely advanced caries destruction.

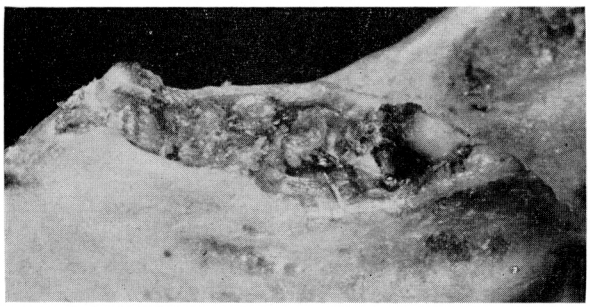

Fig. 6.—(×11). Lower jaw. Practically complete loss of teeth from this jaw. This degree of caries loss was never seen prior to the observation of these rats on high sugar diets.

At death the jaws were freed and the teeth examined under a binocular microscope (15×) according to the technic previously described.[19] No attempt was made to differentiate between missing cusps and carious lesions because of the difficulty in making a clear distinction in the presence of advanced attrition. The data given in Table IV include the average number of teeth having at least one lesion, the average number of teeth totally destroyed, and the average number of cusps missing. In each case the values were calculated per rat.

TABLE IV

DATA ON EXPERIMENTAL CARIES IN RATS FED THE HWC DIET AND IN RATS FED A HWC + SUGAR DIET

DIET	NUMBER OF RATS	AVERAGE NUMBER OF CARIOUS TEETH	AVERAGE NUMBER OF TEETH TOTALLY DESTROYED	AVERAGE NUMBER OF CARIOUS CUSPS
HWC	35	4.9	0.85	12.8
HWC + sugar	13	5.5	1.80	15.0

Comparing the control and experimental groups, there was little difference between the values for the average number of carious teeth (4.9 vs. 5.5) and for the average number of carious cusps (12.8 vs. 15.0) per rat. In sharp contrast to these similarities, there was a 100 per cent difference in the average number of teeth totally destroyed (0.85 vs. 1.80) per rat; this difference was clearly significant by Fisher's "t" test.[20] This finding should be emphasized: twice as many teeth (per rat) were totally destroyed on the diet containing excessive amounts of sugar.

The simplest interpretation to be given these data is that excessive amounts of sugar added to the HWC diet do not initiate experimental caries in the rat; however, existing lesions tend to be extended rapidly and radically. In certain of the jaws of the rats receiving added sugar, not one molar tooth remained. Such a condition had never been observed previously in the scores of rats examined in various experiments in the Rochester laboratory (Figs. 1 to 6).

Summary for Experiment 1.—

1. Forty-eight rats were placed for six weeks on the Hoppert-Webber-Canniff diet, after which thirteen of the rats were given a diet made by adding an equal weight of powdered confectioner's sugar to the HWC diet. These diets were fed for the balance of the experiment of 104 days.

2. Although the experimental rats grew less rapidly than the control rats and laid down excessive amounts of peritoneal fat, there were no gross abnormalities in the organs of either group.

3. On the average, the experimental rats developed the same number of carious teeth and the same number of cusps missing per rat as did the control rats.

4. The experimental rats exhibited twice as many teeth totally destroyed as did the control rats. Apparently, excessive amounts of sugar do not initiate rat caries but existing cavities are rapidly extended.

Experiment No. 2.—Plan: Cavities were initiated by maintaining the rats for eight weeks on HWC diet. Then the colony was divided into groups with various diets as indicated after one week on a cleansing diet. The continuation diets for the next eighteen weeks had excessive amounts of selected carbohydrates.

Rats, at the age of 28 days, were given the HWC diet. Two such diets were used, one containing corn meal as obtained from the market; the other, containing corn meal ground fine, that is, fine enough to be passed through a sixty-mesh sieve. The screen analysis of the original corn meal was: none was retained on a twenty-mesh screen, 84 per cent retained on a forty-mesh screen, 13 per cent retained on a sixty-mesh screen, while 3 per cent passed a sixty-mesh screen. According to Hoppert, Webber, and Canniff,[5] no caries should be expected in the rats given the fine corn meal diet.

The teeth of the rats were prepared for examination by boiling the heads for one-half an hour with a soap solution. The boiled flesh was then easily removed from around the jaw bones. The jaws were removed and flesh brushed away with a tooth brush. The teeth were then cleaned by picking with a fine, sharpened dental broach under a binocular microscope with five power magnification.

After the teeth were dried, an attempt was made to obtain a numerical estimation of the extent of cavities by assigning values to a given size cavity. The values used were: 0 = no visible caries, 1 = a definite cavity, 2 = deep cavity with all cusps intact, 3 = some or all cusps gone, 4 = most of tooth above bone line gone, and 5 = all of crown of tooth gone. It was early found that cavities could not be sharply classified. Therefore intermediate values of ½ were used when desired.

It is obvious that such a numerical scale gives only a rough measure of the degree of caries. Also it is evident that the system does not progress by exact units as "2" is not exactly twice "1", and "4" does not guarantee that the factors have been exactly twice as intense as those which produced caries scored "2." Therefore, averages derived from such numbers must be accepted with caution.

At the end of eight weeks on the corn meal diets, it was found that sixteen of twenty rats on the coarse corn had caries with an average score of 4.8. Contrary to Hoppert, Webber, and Canniff, caries were found in ten of thirteen rats maintained on the fine corn meal diet with an everage score of 2.6.

The entire group of survivors were transferred for one week to a low carbohydrate diet (see Table I). The purpose of this diet was to clean the teeth of the rats of corn meal debris. Two groups of rats were killed at this time and the extent of caries determined (Table V, Initial Phase). These included twenty rats maintained on the coarse ground corn diet and thirteen on the fine ground corn diet. If no cavities were evident, the rats were not considered in the average because continuation of caries was being studied.

The rats were next divided into groups as indicated and allowed free access to the respective diets according to the following schedule: 15 rats were returned to the HWC diet, 12 rats were returned to the fine corn HWC diet, 15 rats were placed on the starch diet (Table I), 16 rats were placed on the sucrose diet (Table I), and 16 rats were placed on the glucose diet (Table I).

The rats were killed after eights weeks on these diets and the teeth examined as described. The results are given in Table V. In the table are given the number of rats, the average caries score, the numbers of rats averaged, and the variability. The last column of Table V gives the summed squares of the deviations from the means, values needed for the interpretation of the means and necessary to show dispersion where the complete data are not given.

TABLE V

AVERAGE CARIES COUNTS OF RATS ON VARIOUS DIETS

DIET	NUMBER OF RATS	AVERAGE CARIES	NUMBER OF RATS AVERAGED	ΣD^2
Initial Phase				
Coarse corn (8 weeks)	20	4.8	19	236.7
Fine corn (8 weeks)	13	2.6	12	84.0
Continuation Diets				
Low carbohydrate	15	3.5	12	118.3
Fine corn	12	4.2	11	65.0
Coarse corn	15	7.5	14	224.3
Starch	15	5.0	13	85.5
Sucrose	16	4.8	9	68.6
Glucose	16	6.5	14	146.3

The interpretation of Table V is that diets high in carbohydrates probably promote rat caries more than does a low carbohydrate diet. Statistical analysis has shown that the chances are about 8 to 1 that glucose promotes rat caries more than does sucrose or starch. Since these odds are not sufficient for a conclusive statement that glucose is more provocative of a continuation of rat caries than is sucrose, it was decided to repeat the study with a more rigidly controlled set of conditions.

Summary for Experiment No. 2.—

1. One hundred twenty-two male and female rats were placed on HWC diets for a period of eight weeks. Twenty rats fed coarse corn and thirteen rats fed fine corn were sacrificed at that time; the average caries scores were 4.8 and 2.6, respectively.

2. The remaining rats were divided into groups of twelve to sixteen each, and they were fed diets containing (a) corn ground coarse or fine, and (b) low or high carbohydrate content, but without corn particles.

3. At the end of an additional eight-week period, the rats maintained on coarse corn had an average caries score of 7.5, whereas those on fine corn had an average score of 4.2. In both these groups, continuation on the diets produced increased caries scores.

4. The rats fed high carbohydrate diets in each instance had higher average caries scores than those maintained on the low carbohydrate diet.

5. When the caries scores of the rats fed continuation diets high in starch, sucrose or glucose, respectively, were compared, inconclusive difference were found.

Experiment No. 3.—Plan: Cavities were initiated in an eight-week period on an HWC diet modified to contain only one sugar. Then the rats were given access to a continuation diet (for eight weeks) high in the same sugar but lacking coarse corn particles.

Rats for this experiment were reared in the Pittsburgh laboratory. In order to provide rats as nearly as possible uniform from each breeding group, litters were reduced to eight after three days and to six after a week. The young were taken from their mothers at 21 days of age and placed on the HWC diet made up with a synthetic milk. The basic composition of this caries-producing diet was as follows: coarse, yellow corn meal, 66 per cent; basic mix, 13.75 per cent; butter fat, washed free of lactose, 8.85 per cent; and selected sugar or starch equivalent to 11.4 per cent sucrose. The basic mix of the above diet was casein, vitamin-free (lactose-free), 4.644 Gm.; Osborne and Mendel salt mixture, 1,257 Gm.; sodium chloride, 0.727 Gm.; alfalfa powder, 2,182 Gm.; and unirradiated brewer's yeast, 1.190 Gm.

The selected sugars were sucrose or glucose. Starch, in an equivalent percentage, was used in the diet of a control group. The casein was chosen to be free of lactose so that the HWC diet contained, as nearly as possible, only a single added carbohydrate. The advantages of this experiment over the preceding one were (1) the diet and life history of the parents were known, (2) the lactation conditions of the litters were practically uniform, and (3) a caries-producing diet was used with a selected carbohydrate component.

The rats were maintained on the caries-producing diets for eight weeks and on the caries-extension diets, the starch, sucrose, and glucose diets (Table I), containing no coarse corn particles, for eight weeks more. The rats were then killed and their teeth examined and scored.

Relative Incidence of Caries Among Males and Females.—In sixty-three cases, a male and a female rat from the same litter ate the same diet. In determining whether the male or female had a larger caries count, all pairs were considered except those in which both counts were zero. If only one had a zero count, that pair was included since this discussion is concerned with incidence and continuation of caries.

In the sixty-three pairs, thirty-nine males exceeded the females in the caries count; twenty-four females exceeded the males. The term exceeded here does not take into account the somewhat dubious averages which made the usual statistical methods applicable only with caution. The data, however, can be treated by the binomial distribution analysis. The mean expected of 63 pairs is 31.5. The distribution found was 24 and 9 or a deviation from the mean of 7.5. The standard deviation, σ, is $63 \times \frac{1}{2} \times \frac{1}{2} = 3.97$. The probability associated

with this deviation is 0.0588 corresponding to chance of 16 to 1. The data, therefore, show that the odds are only 16 to 1 that young male rats are more likely to have caries and more extensive decay than young female rats.

The Progress of Caries.—In order to determine the relative rates of progress of caries on the starch, sucrose, and glucose diets, respectively, the same method given above was used to consider the relative state of decay in pairs from the same litter. However, because of the chance that male rats may have a greater incidence of corn meal caries than females, no pairs consisting of male and female are used in this analysis. This treatment entails loss of data from only a few litters. As progress of caries is being determined, pairs in which either or both show a zero count are omitted from the analysis.

Of the pairs of rats of the same sex and from the same litter but on the starch and sucrose diets, respectively, eight pairs showed more decay on the starch diet and twenty-one on the sucrose diet. The probability associated with this deviation from the expected mean is 0.0155 or the chances are 63 to 1 that caries in young rats progresses faster on a diet high in sucrose than on one high in uncooked corn starch.

Thirty pairs of rats are available on the starch and glucose diets; of these ten show higher caries count on starch and twenty on glucose. The probability of this distribution, if due to chance alone, is 0.0688, or the odds that glucose promotes the caries to a greater extent than uncooked corn starch is 14 to 1.

If the preceding results are combined as starch versus soluble food carbohydrates, then eighteen pairs of rats show the starch-fed animals to have more tooth decay compared with forty-one pairs on the sucrose and glucose diets. The probability of this distribution is only 0.0027, or the chances are 370 to 1 that uncooked corn starch is less provocative of caries than are the soluble food carbohydrates.

Of the pairs of rats fed sucrose and glucose, respectively, twenty-one showed more decay on the sucrose diet and fourteen more on the glucose. The probability in this case is 0.238, or the chances are 3 to 1 that sucrose is more likely to cause continuation of caries than is glucose. Such a probability is, however, well within the limits of a chance distribution.

In Table VI is given the analysis of the decay on the three diets based upon the comparison of specific teeth. For example, in Line 1 of Table VI are given the results of a study of all the pairs of males eating the starch and sucrose diets, respectively. The same teeth in each pair were compared to determine which was more decayed. In a number of pairs the counts were the same for both teeth. In these cases a comparative re-examination of the teeth was made to determine if one was slightly more decayed than the other. Only cases in which decay occurred in both teeth were considered in this analysis.

Of the pairs of teeth in the male rats fed starch and sucrose, respectively, twenty-one showed more decay on starch and twenty-eight more on sucrose. The odds that sucrose in this case is not more provocative of caries are 2 to 1, showing the distribution to be mere chance. In contrast, the chances against sucrose in the case of the female pairs are 100,000 to 1 (Line 4, Table VI).

TABLE VI

ANALYSIS OF THE DATA OF COMPARATIVE DECAY OF INDIVIDUAL TEETH OF PAIRS OF RATS ON STARCH, SUCROSE, AND GLUCOSE

PAIRS	NUMBER OF PAIRS OF TEETH	DECAY GREATEST ON				PROBABILITY	CHANCES
		STARCH	SUCROSE	GLUCOSE	SUCROSE VS. GLUCOSE		
1. Starch vs. sucrose (males)	49	21	28			0.32	2:1
2. Starch vs. glucose (males)	55	27		28			1:1
3. Sucrose vs. glucose (males)	66		37	29		0.32	2:1
4. Starch vs. sucrose (females)	49	9	40			0.00001	100,000:1
5. Starch vs. glucose (females)	38	9		29		0.0014	700:1
6. Sucrose vs. glucose (females)	57		32	25		0.36	2:1
7. Starch vs. sucrose (males and females)	98	30	68			0.0001	10,000:1
8. Starch vs. glucose (males and females)	93	36		57		0.029	35:1
9. Sucrose vs. glucose (males and females)	123		69	54		0.18	5:1
10. Starch vs. sucrose vs. glucose (males and females)	191	66			125	0.00005	50,000:1

A third method of analysis of the data is to determine the average amount of decay per cavity. In all the rats fed the caries-producing and caries-continuation diets containing starch, 174 cavities were produced with a total count of 168. The average count per cavity is thus 0.97. For the rats fed sucrose-containing diets, the data are 206 cavities with a total count of 258 and average of 1.25. The glucose-fed rats had 233 cavities with a count of 250 and average of 1.07. These data on count per cavity are obtainable because of the nature of the values, that is, the caries counts, from which the averages are taken. However, the conclusions substantiate those drawn from the two previously described analyses and are included here as additional evidence.

The data therefore show that sucrose and glucose promote the development of corn meal caries in rats. This experiment does not show whether uncooked corn starch promotes rat caries; that is not essential to the solution of the problem of any caries promoting action of fermentable sugars.

Summary of Experiment 3.—

1. One hundred eighty rats were placed on a specially modified HWC diet in which a synthetic milk replaced the powdered milk usually included. The synthetic milk contained a single sugar, sucrose or glucose; in a control group, uncooked starch was added in an equivalent amount.

2. After eight weeks on these diets, the rats were given new diets in which no corn meal was present, but in which large amounts (about 65 per cent) of each diet was the same sugar or starch which had been in the initial diets.

3. After eight additional weeks on these continuation diets, on the basis of either whole mouth or tooth-by-tooth comparison, it was shown that rats fed uncooked corn starch had less progress of cavitation than rats fed fermentable sugars.

DISCUSSION

The question may be posed: why does sugar provoke the rapid and profound advance of caries in the rat molar? In answer, a few possible factors may be suggested: (a) the solvent action of sucrose for calcium compounds is well established;[21] (b) the sugars may provide a favorable medium for the growth or oral bacteria which are inimical;[22] and (c) sugar may be metabolized by organisms in the mouth to produce sufficient local acidity to decalcify tooth-hard tissues.[23] Experimental rat caries may well prove to be a testing ground for some of the hypotheses which attempt to describe the mechanisms of dental caries.

It seems reasonable to assume that diet may exert an influence on tooth health in ways which may be divided into three phases: (1) dietary factors in the development and calcification of teeth (pre-eruptive); (2) dietary factors in the erupted teeth but prior to the appearance of carious lesions (initiating factors); and (3) dietary factors in the progress of caries (cavitation).[10]

1. Pre-eruptive Factors. No attempt has been made in these experiments to control the pre-eruptive factors. In the third experiment, the maternal diets were considered to be part of the experiment. However, the only importance to the question as to the effects of starch versus fermantable sugars in the diets of the offspring is the clearly established fact that the young rats were susceptible to caries.

2. Initiating Factors. The initating factors were not studied in these experiments; all rats were placed on HWC diets in each of the three tests. A review of the literature indicates that the fermentable carbohydrates would not initiate caries except through a possible coarse particle effect. Thus, McClure[12] found a significant incidence of caries in rats fed granulated sugar as compared with rats fed finely ground sugar. In the experiments reported herewith, coarse corn particles were used as the initiating agents in all cases. In the third test, in order to control the influence of the carbohydrate as far as possible, the same sugar was added to the HWC diets as was used in the continuation diets. The effect of this procedure was only to add the specific promoting effect (if any) of the carbohydrate during the initiating period.

3. Extension Factors. Only one of the many possible factors involved in the destruction of tooth tissue has been studied, that is, the importance of the fermentable carbohydrates. The fermentable carbohydrates, sucrose and glucose, permit more rapid extension of existing cavities than does uncooked corn starch. Whether the initiating agents in experimental rat caries and in human caries are comparable, and whether the mechanisms for the enlargement of cavities in rat molars and in human molars are similar, are problems beyond the scope of this experiment. However, if an application of these findings to human caries prevention can be made, it would seem to stress the importance of insuring the perfection of tooth structure, because once the caries process is initiated, modern civilized diets would accelerate it.

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