EXPERIMENTS ON THE ACID EROSION OF TEETH IN CARIES-SUSCEPTIBLE AND CARIES-RESISTANT ALBINO RATS, (RATTUS NORVEGICUS)

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H.R. Hunt

Major professor

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EXPERIMENTS ON THE ACID EROSION OF TEETH IN CARIES-SUSCEPTIBLE AND CARIES-RESISTANT ALBINO RATS, (RATTUS NORVEGICUS)

By

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INTRODUCTION

It has been demonstrated (Hunt and Hoppert 27) that resistance to dental caries in albino rats is partly determined by heredity. This hereditary difference was revealed in rats which used the Hoppert diet. Will this hereditary difference appear when a different diet is used? The primary purpose of this research was to determine whether the teeth of Hunt and Hoppert's susceptible strain of rats were affected differently from the teeth of the resistant rats when a non-caries producing diet was used, but both types of rats drink an acidulated fluid.

According to Bunting (4), 85 to 95 per cent of public school children in this country either have, or are susceptible to, dental caries, and only 5 per cent of the population remains free from this affliction throughout life.

Dental caries is recognized as a "disease causing the disintegration and loss of tooth structure" (24). It is unlike other diseases in that none of the body tissue attacked is cellular. There also appears to be evidence of but little defensive reaction on the part of the tooth to the carious process, and no attempt by the body to repair the injured area.

The literature dealing with dental caries is extensive. Our citations are designed to give a clear picture of our past and present understanding and beliefs concerning the etiology of this disease.

HISTORY

The first toothache was suffered long before the dawn of civilizations. In Weinberger's book, "An Introduction to the History of Dentistry", (11) it is shown that the skeletal remains of primitive man prove that he suffered from this disease.

Since the etiology of tooth decay is such a tantalizing and important problem to man, history records many theories which have been presented to explain its occurrence. Lack of mastication, eating foods which form lactic acid during chewing, a diet too rich in proteins, the presence of molds, the nervous strain of modern living and others (2), some fantastic, some probably close to the truth, have been presented as real causes of caries.

The Babylonians believed that cavities were caused by worms gnawing at the tooth. These creatures were supposedly identical to those found in figs. To rid oneself of the animals, beer, oil, the sa-kil-bir plant and prayers were utilized (11). This theory persisted as a medical concept until Fauchard, in his classic "Le Chirurgien Dentiste", written in the eighteenth century, put an end to the ridiculous theory of dental worms (7).

About 1870, when electricity was a novelty and any natural phenemonon was likely to be ascribed to it, an English dentist named Bridgeman suggested a hypothesis which explained "not only the destruction, but the formation of the teeth by electricity" (9). For half a century afterward there were many who believed that caries was caused by a current generated through contact, in the presence of saliva, between the teeth and the metallic fillings in them. In the latter part of the nineteenth century, W. D. Miller and others disproved this idea, and the electrical theory gradually lost its many supporters (2).

Hippocrates in about 55 B. C. suggested that the decomposition of food particles in the mouth caused tooth decay. Galen, a Roman physician in the first century A. D., believed that decay of teeth was initiated by disturbances in the nutrition (3,7). While we have added the idea of microorganisms to the consideration of environmental causes, these two ideas remain essentially unchanged today.

BACTERIA

Among the first of our modern scientists to suggest that acids found in the mouth were produced by microorganisms was a Frenchman named Magitot (16). It had previously been thought by some investigators that caries was a result of inflammation in the capillaries of the dentine. In 1873 John Tomes proved this theory wrong when he demonstrated that there were no blood vessels in this part of the tooth (6).

Koch, Pasteur and their followers, by discovering organisms which were known to cause various diseases, laid a scientific foundation for the concept that bacteria were an important factor in tooth decay.

In 1883 Willoughby B. Miller, a student of Koch, postulated a theory which, with minor changes and additions, is today accepted by most scientists. Miller claimed (6,31) that the first stage in tooth decay was caused by acids dissolving the enamel and subsequently exposing the dentine. These acids were formed, he wrote, by the action of a self-reproducing, acid-sensitive agent found in the saliva. Bacteria met all the qualifications for this agent. Once the enamel was penetrated, organisms then attacked the dentine. Although Miller did not name any specific organism responsible for caries, he did list ten different bacteris found in the oral cavity which were capable of causing the acid fermentation of carbohydrates (16).

Accumulated evidence has since been collected which proves that acidogenic bacteria are an important factor in caries. Bibby (1), in his paper "Dental Caries" stated it had been demonstrated that cavities

resembling caries could be produced by the action of acids, similar cavitation was produced by bacteria grown in the presence of carbohydrates, organic acids can be found in carious dentine, the chemical changes occurring during the progress of caries seemed no different than those brought about by chemical action, and finally that certain agents which were successful in the prevention of acid formation also prevent dental decay in man.

Most investigators insist that a pH of below 5 is necessary for enamel erosion (1,21). Bacterial research has therefore been concentrated on those microorganisms which are capable of producing this high a degree of acidity.

Although several species of bacteria have been mentioned as the possible causative agents in caries, lactobacilli and streptococci have attracted the most attention. Bunting and Jay of the University of Michigan Dental School have garnered considerable information on the species Lactobacillus acidophilus. They have shown that in about 90 per cent of carious patients, the numbers of this strain are increased considerably above the average (4). These men consider this specific bacterium especially suited for the starting of a carious lesion due to its aciduric and acidogenic properties. Further evidence of the possible importance of L. acidopilus was found in 1925, when Bunting and his associates discovered this strain in only 1 of 184 caries-free patients, whereas this organism was present in 237 out of 243 carious patients (17). From these and other findings, Bunting claims that L. acidophilus is the most important etiologic factor yet found in the production of dental caries.

Other investigators hesitate to be as enthusiastic as he. J. D. Boyd and others (13) have found L. acidophilus in mouths persistently free from dental caries. Bibby (1) is inclined to think that streptococci, which seem more prevelent in mouths, may be more important than at present considered.

DIET

In spite of the fact that tooth decay occurs among virtually all peoples of the world, it does not affect all ethnic groups equally. In the 1880's J. R. Mummery compiled data which H. P. Pickerill used to prove that uncivilized and semicivilized races, living in all kinds of environment and subsisting on many different types of foods, possess a much higher resistance to caries than do the civilized racrs of man (2). Further evidence that the effects of modern civilization may be detrimental was advanced by T. Rosebury (34) in his studies on Eskimos. These people, some of whom are reported to have the best teeth found today, show a caries incidence highest in the settlements in close contact with the white man, and lowest among the more isolated natives.

To explain these and other similar findings, dental investigators suggest that the dietary differences between groups of peoples play the important role in whether these groups have a high or low caries incidence.

It was not until 1917, when Lady Mellanby began her extensive studies, that diet was given the attention it deserved as a possible causative factor in tooth decay. In 1934 Mellanby concluded that "a diet rich in

vitamin D and containing sufficient calcium and phosphorus inhibits the onset and spread of caries" (2). Vitamin C has been subsequently added to the list of supposedly important dietary necessities for caries resistance by M. T. Hanke (22) and others.

The contention that carbohydrates more than any other dietary factor are responsible for tooth decay is widely accepted by dental researchers. T. Rosebury (10) writes, "There is at least one common denominator in the research that claims reduction of dental caries in the human by dietary means and that is the reduction of carbohydrates, especially in the form of refined sugars." Bunting (3) and Hill (8) have shown that lactic acid may be formed in the mouth from starches with the aid of bacterial enzymes.

Hoppert, Webber and Canniff (26) successfully produced caries in rats by including coarse particles of rice or corn in the diet. Inclusion of vitamin A, D, and C, phosphorus and calcium in the diet did not alter the caries rate. They therefore concluded that impaction and subsequent retention of the food particles in the tooth are an important factor in tooth decay.

SALIVA

The role of saliva as a preventive agent in dental caries is imperfectly understood at present. Some investigators postulate that saliva acts as a cleansing agent, while others claim that salivary secretions act as buffers thereby neutralizing acids in the mouth. Whatever

may be the exact influence of saliva, it is probably minor in the over-all picture (23).

TOOTH STRUCTURE

Mellanby states (15), ". . . there is a direct relationship between structure of the teeth and caries: those badly formed having a greater susceptibility." Her view is opposed by many who believe that imperfect structure may alter the rate of caries, but not necessarily induce it. Since caries does not attack the tooth indiscriminately, but only at those sites which favor the lodgement of foods, it is possible that teeth with many deep fissures would be more caries-susceptible than others.

That the composition of the tooth is an important etiologic factor in caries resistance has been demonstrated conclusively by experimentation with fluorine. It is widely known today that the presence of minute quantities of flourine in the tooth enamel acts as a caries inhibitor. It has been shown, for example, that children living in areas having one or more parts per million of fluorine in the water supply, not only have 50 to 65 per cent fewer carious permanent teeth, but also have only one-fourth to one-sixth as many lost permanent teeth as children living in areas having fluoride free drinking water (19,20).

HEREDITY

Heredity as a factor in dental caries has been extensively studied at Michigan State College under the direction of Dr. H. R. Hunt and Dr. C. A. Hoppert. They have succeeded in establishing two strains of rats, one of which, the susceptible strain, develops caries in a little over a month on the Hoppert diet. The second strain, which is caries-resistant, does not develop cavities before an average time of 379 days, and some of these caries-resistant animals never have tooth decay.

Since this paper is concerned with the caries-susceptible and caries-resistant rats from Dr. Hunt's stock, more will be said later about experimental findings with these animals.

OTHER FACTORS

In a summation of the results from the University of Michigan Workshop it was concluded that pregnancy, general health, diebetes, endocrine glands, infectious diseases and emotional states appear to be unimportant as factors in tooth decay (5).

MULTIPLE INTERACTING CAUSES

While each possible causative element in tooth decay has its adherents, most dental researchers are now supporting the doctrine of complex interacting causes. This doctrine is exemplified by the following statement after Bunting and co-workers had done extensive research with dental caries

in children. Bunting writes (18), "We cannot trace true caries directly to bacteria, to nutritional deficiencies, poor heredity, or any other simple cause; but all of these things, plus the fundamental characteristics of the tooth and mouth, seem to have some bearing on the matter."

HUNT-HOPPERT EXPERIMENTS AND FINDINGS

While studying American Indians, Negroes and persons of Dutch ancestry, Dr. Morris Steggerda noted differences in resistance to dental caries. He therefore suggested that an investigation be started to ascertain whether an inheritance factor existed in albino rats. In 1937 Dr. Hunt and Dr. Hoppert began such a project and the work is continuing.

By means of standard genetic technique such as progeny testing, brother-sister matings and selection, animals obtained from several departmental colonies were differentiated into two lines, one caries-resistant and another caries-susceptible. All animals were kept under the same environmental conditions. The ration chosen was the one Hoppert and his co-workers used to produce caries. This diet consisted of ground polished rice (66%), whole milk powder (30%), alfalfa leaf meal (3%) and salt (1%). The rice was coarsely ground so that about 70% was retained on a 20 mesh screen when sifted. Rats were placed on this caries-producing ration at the age of 35 days.

Early in the study it was clearly demonstrated that differences in hereditary resistance to dental caries existed. By the time the 12th generation of susceptibles was obtained, Hunt and Hoppert were convinced

that this strain was homozygous with respect to this trait. The resistant rats, on the other hand, which are now in the 19th generation, still remain highly variable.

Having obtained a caries-resistant and caries-susceptible line of rats, it remained to discover the mechanism of inheritance, that is, the number of genes involved and their modes of action. For a complete bibliography of the experimentation to date, see the References.

As previously mentioned it was discovered by Hoppert and his coworkers (26) that coarse particles of food increase the caries rate due to the greater frequency of impaction of these coarse food particles.

Hunt and Hoppert (28) reported that use facilitates dental caries. They found that caries retardation occurred when lower molars were not opposed by the corresponding upper molars. Mechanical breakage of the upper teeth apparently delayed the caries process in the lower molars, thus giving a faulty impression of resistance. Keller, et al. (30) have recently ascertained that some areas on the occlusal surfaces of the lower molar teeth are more susceptible to caries than others. They demonstrated, for example, that the right lower molars develop more cavities than do the left lower molars.

Braunschneider and others (14) have shown that age increases resistance to caries. The susceptible animals in his study were proven to be more resistant to tooth decay at 100 and 150 days than at 35 days.

There is some evidence that constitutional differences exist between susceptible and resistant animals. However, it has not been determined whether these differences are directly related to caries. R. L. Clise

found that the growth curves of both susceptible and resistant animals remain substantially the same until 147 days of age. Beyond 147 days the resistant rats maintain a higher weight than do the susceptibles. Clise thinks this weight difference is due to the susceptible animals having none of the lower molars present so that mastication is impared. Clise has also found that the susceptible rats have significantly less hair than do the resistant rats. Whether there is some physiological relation between pilosity and caries-resistance has not been determined. (Unpublished data.)

Eugene C. Nakfoor et al. (33), in testing the caries-susceptible and resistant strains for fracturing, concluded that natural fracturing is not an important factor in the formation of dental caries in the susceptible animals. He did find, however, that at the age of 60 days the susceptible teeth were more susceptible to fracturing when subjected to blows by a tapered aluminum rod than the resistant molars of the same age.

Roger F. Keller, Jr. (data unpublished) severed the parotid ducts on both sides of susceptible and resistant rats and observed the number of days required to produce caries on the stock ration. His results showed that the secretion of the parotid ducts are unimportant in the carious process of these animals.

Keller has also shown that the susceptible rats have thyroid glands of greater weight per 100 grams body weight than the resistant rats. He has further demonstrated that there is a slower turnover of tracer iodine in susceptible strain thyroid glands than in resistant strain thyroids, and that the follicles in the thyroid glands of the susceptibles are larger

than in the resistants. The metabolic rates of both strains, in spite of these thyroid differences are apparently the same. (Unpublished.)

Hunt and Hoppert (29) have found that sex is not important as a factor in tooth decay, the caries time for the two sexes being almost the same.

Workers with the Hunt-Hoppert rats are becoming convinced that the reasons these two strains differ can only be found by investigation of the oral cavity. It appears that either the structure of the teeth, the types of bacteria present in the mouth, or both, are the important immediate factors.

Hoppert and Shirley (25) have made limited studies on the chemical analysis of the teeth of the two strains. They found that the weight of the susceptible teeth is slightly higher than the resistant tooth. There is a higher percentage of ash content of the resistant molars as compared with the susceptible molars. The resistant teeth also show a slightly higher phosphorus content than do the susceptible teeth. Hoppert's judgment is that these chemical differences are not significant.

In a discussion of their findings, Hoppert and Shirley say "Very little chemical work has been done on the teeth of the caries-resistant and caries-susceptible strains of albino rats...." They suggest that further studies be concerned with a comparison of the enamel of these two strains. This paper is an outgrowth of both this suggestion and several erosion studies recently completed (12,32,35), which have attracted my attention.

PROBLEM

Most investigators agree that the first step in caries is the attacking of the enamel by acid. In essence this may be considered erosion in a confined area. This study was initiated in order to ascertain whether the caries-resistant and susceptible teeth show any difference in resistance to acid erosion, thereby possibly indicating physical or chemical differences in the enamel of the two strains.

MATERIALS AND METHODS

It was decided that the best approach to the problem would be to supply caries-resistant and caries-susceptible rats with a phosphorylated drink commencing at 25 days of age. This drink would constitute the only source of liquid available to the animals. As in all of the Hunt-Hoppert experiments, drip bottles supplied the fluid to the rats. The acidulated mixture was prepared daily by adding 3.05 ml. of concentrated phosphoric acid to seven liters of tap water. In order to make the drink more palatable, and since sugar is thought to have erosive qualities, enough sucrose was added to produce a 10% sugar solution. It was found by periodic tests with an electric pH meter that this sweetened acid drink approximated a pH of 2.6 at all times.

It was discovered early in the experiment that our drink was very conducive to the growth of certain molds. Postulating that these unwanted growths might conceivably alter the results of the study, special care

was taken to thoroughly wash all glassware so that the molds were not able to run rampant.

Papers dealing with the caries-susceptible animals of Hunt and Hoppert comment on the rapid and complete destruction of the lower molars, usually before the age of 100 days. Since our experiment was intimately concerned with the erosion of these lower molars, this carious effect had to be either eliminated, or greatly reduced. To do this, Dr. Hoppert formulated a non-carious diet. This ration was composed of powdered whole milk (80%), alfalfa leaf meal (19%), and salt (1%). The milk was run through a sieve so that the ration consisted of no coarse particles or lumps. Susceptible and resistant animals were placed on this diet at the same time they began the acid drink. As will be shown later, this ration proved highly successful in retarding dental caries.

To be able to adequately determine how much of the tooth's loss of substance was due solely to the erosive action of the acidulated drink, at least one rat from each litter was placed on a control drink of tap water and fed the non-carious diet.

The breeders for this experiment came from Hunt's 21st generation of susceptibles and 17th generation of resistants. Thirty-six susceptible breeders comprising 12 crosses, and 24 resistant breeders comprising 7 crosses, were utilized from the reserve stocks. All matings were between brothers and sisters. Females were isolated from the breeding cage as soon as pregnancy was evident. Daily examinations were then made until the young were born. At the age of 25 days the rats were separated from their mothers, marked, put on the non-carious diet and either the acid or water drink.

Five animals of the same sex and strain were put in each cage. The cages measured 2.5 x 1.5 x 1.0 feet, and were made of galvanized sheet metal on all sides except for the top and front, which were covered with a 1/4 inch galvanized iron mesh.

The phosphoric acid drink produced a high degree of thirst in the rats which subsequently had a high diuretic effect. It was therefore necessary to provide two drip bottles to each cage.

In order to accurately ascertain how effective our non-caries diet was, a control drink of tap water was given to one rat for every four rats put on the acid beverage. These controls were also placed on the non-caries diet plus tap water to determine if any erosion occurred in the absence of the phosphorylated drink.

After having been on the drink fifty days, and at ten day intervals after than until 170 days, at least two resistant rats and one resistant control were killed. The susceptible rats were treated in a similar manner except two rats were also killed after only 30 days on the drink. After the animals were dead, the head was severed from the body and placed in a gallon jug containing 95% alcohol. Within a week after the preceding operation, the lower jaw was removed from each head, cleaned thoroughly with the aid of a sharp scapel and minute sewing needle, marked by attaching a card to it, and then reattached to the head by means of a light cord. The lower jaw was then ready for observations. A binocular microscope was utilized for this purpose.

SCORING AND OBSERVATIONS

To ascertain with a high degree of accuracy the extent of erosion that had occurred, the following plan was used. Erosion had been seen to occur mostly on the lingual side of the teeth, so it was here that the amount of erosion was measured. Following the system of others (35) who had scored erosion by means of an arbitrary system of numbers, it was decided that our specimens would be given ratings ranging from 0 to 5.

Most of the numbers used in a system such as this are self-explanatory. It must be mentioned, however, that a rating of 1/2 was used to identify those teeth which exhibited possible, but not clear-cut, evidence of erosion. A score of 1 was the first number chosen to show positive erosion.

To eliminate error still further in our scoring procedure, the following steps were taken. After careful examination of all the jaws many times, four model jaws were selected as standards in scoring. (See figure one). These models were given grades 1, 3, 4 and 5. No grade 2 model was used, but this grade was between 1 and 3. When scoring, each jaw was compared with these four standards and graded accordingly. The control jaws were studied to find evidence of lingual erosion. Since no control jaw exhibited any lingual erosion, it was assumed that any such erosion present in our experimental jawswas due to the action of the acidulated drink.

All of the jaws, both susceptible and resistant, were rated eight times. On each of these eight occasions, all of the jaws were rated.

I allowed at least four days to elapse between successive ratings, so that any memory of individual teeth would not be retained. The eight repeated scores for any one jaw never differed by more than 1; a difference as high as this occurred only during the first two trials. The eighth session saw only five jaws which differed from the seventh session by a score of 1/2. These five jaws are identified in the tables.

In a rating system with 5 the maximum, a score of 2 1/2 or 50% is indicative of serious erosion.

Table I shows the caries-resistant teeth developed while on the acid drink. It is noted that no resistant teeth receive a rating of 3 or over until on the drink 116 days. It is further evident that these resistant lower molars do not receive a rating of 3 or over with any regularity until on the acid drink 136 days; from which time all scores are above the 3 rating.

Table II is concerned with the susceptible lower molars, their number of days on the acid drink and their subsequent lingual erosion values. Note that these teeth first receive a rating of 3 at 60 days. Furthermore, all but three sets of teeth receive scores of 3 or over on one or both sides after 70 days on the acidulated fluid. The three exceptional animals were killed after using the drinking fluid for 80, 130 and 140 days, respectively.

Table III shows the averages of half jaws rated. This table clearly shows that erosion is greater in the susceptible teeth until 140 days on the drink; from which time erosion appears as serious in the resistant teeth as in the susceptible teeth.

Since serious lingual erosion occurs in the lower molars at approximately 136 days as contrasted with the susceptible lower molars which show such erosion at 70 days, a time difference of over 60 days exists between the two strains.

One plausible explanation as to why erosion precedes faster in the susceptible teeth than in the resistant teeth is that some physical difference exists between the enamels of the resistant and susceptible teeth. Somehow, acid erosion is retarded in the resistant as compared with the susceptible mouth. Whether this difference is due to some element lacking in the susceptible tooth, or whether the resistant tooth contains something in greater amounts than the susceptible molar is open to speculation.

Differences in salivary buffering may also be offered as an explanation for the erosion time differences found. However, as was previously mentioned, Keller found that severing the parotid gland ducts of both strains of rats did not alter the caries time. It therefore seems unlikely that such parotid secretions are important here.

TABLE I

RESISTANTS

Number of Animal	Left Side	Right Side	Days on Drink
88	1/2	1/2	50
89	0	1/2	51
85	1/2	1 1/2	72
84	1/2	1/2	73
73	0	1	79
71	1/2	1/2	79
74	0	0	79
66	0	1/2	81
90	0	0	90
72	1/2	1/2	91
54	1 1/2	0	96
53	0	1/2	96
75	0	0	102
61	2	1 1/2	110
62	2 1/2	2	110
# 2	3 1/2*	5	116
82	1/2*	1 1/2	120
# 51	3	1/2	121
68	0	1	130
56	1/2	2 1/2	130

Those jaws with a rating of three or more.
o Those jaws with severe caries so that scoring is doubtful.
- Those jaws with teeth destroyed by caries.
* Those jaws where scores differed by 1/2 from previous rating.

Table I - Continued

Number of Animal	Left Side	Right Side	Days on Drink
55	1	1	130
# 34	4	4	136
# 26	5	4	140
# 27	4	4	140
# 23	5	5	141
# 3	5	5	142
# 22	5	3 1/2	148
# 63	4	4 1/2	150
# 18	5	5	153
# 17	5	5	153
# 20	5	5	160
# 19	5	5	160
# 6	5	5	160
# 50	5	5	161
# 29	4 1/2	4 1/2	170
# 28	4 1/2	4	170
# 21	5	5	174
# 9	5	5	189

TABLE II

SUSCEPTIBLES

Number of Animal	Left Side	Right Side	Days on Drink
127	1/2	1/2	30
126	1/2	1/2	30
92	1 1/2	1 1/2	50
93	1/2	1/2	50
131	2	2 1/2	50
130	1	1/2	50
# 125	2 1/2	3	60
124	2	2	60
123	1 1/2	2 1/2	60
121	2 1/2	3	70
# 120	3 1/2*	5	70
# 119	3 1/2	3 1/2	70
117	2	2 1/2	80
# 116	3	5	80
# 129	3 1/2	3 1/2	80
# 111	2	3 1/2	90
# 110	-	3 1/2	90
# 109	3 1/2	3 1/2	90
# 114	4 1/2	2	100
# 113	2 1/2	4 1/2	100
# 112	4 1/2	5	100

Table II - Continued

Number of Animal	Left Side	Right Side	Days on Drink
# 106	4 1/2 *	4 1/2	110
# 104	4 1/2	4 1/2	110
# 103	4 1/2	4 1/2	110
# 102	4 1/2	4 1/2	120
# 98	5	5	120
# 14	5	5	120
# 97	5	5	120
# 41	4 1/2	4	130
# 42	4	4	130
86	1	1 1/2	130
# 43	3	2 1/2	130
48	-	-	140
# 49	3	3 1/2	140
47	2 1/2	1 1/2	140
# 15	5	5	140
# 44	4	4	150
45	-	-	150
# 95	5	5	150
# 94	5	5	150
# 77	4 1/2	5	150

Table II - Continued

Number of Animal	Left Side	Right Side	Days on Drink
# 78	5	5	160
# 80	3 1/2	4 1/2	160
# 79	5	3 1/2*	160
# 13	5	5	170
# 11	5	5	170
# 12	5	5	170

TABLE III

AVERAGES OF RATINGS OF HALF JAWS

Days on Drink	Susceptibles	Resistants
50	1.25 (8)	.375 (4)
60	2.25 (6)	- (0)
70	3.50 (6)	.75 (4)
80	3.30 (5)	.312 (8)
90	3.12 (4)	.25 (4)
100	3.83 (6)	.33 (6)
110	4.50 (6)	2.00 (4)
120	4.75 (8)	2.33 (6)
130	3.06 (8)	1.00 (6)
140	3.42 (6)	4.50 (10)
150	4.78 (7)	4.75 (8)
160	4.60 (5)	5.00 (8)
170	5.00 (6)	4.58 (6)

Note: Numbers in parenthesis indicate number of half jaws averaged.
Animals under 50 and over 170 days on drink not included.

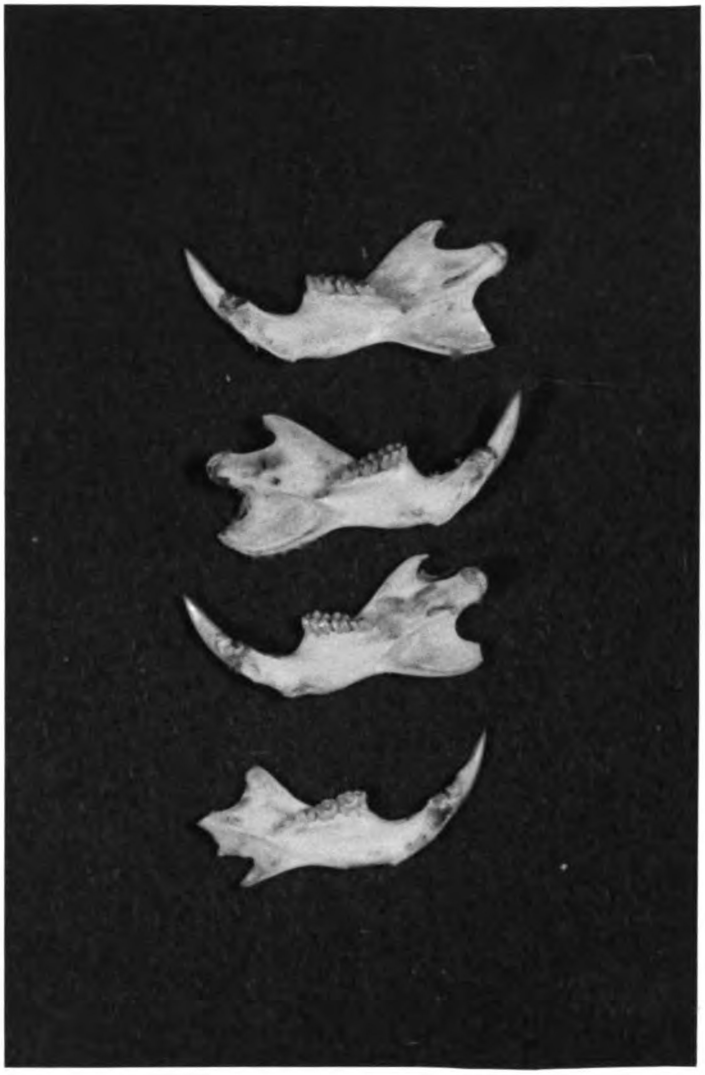

FIGURE 1

At approximately 100 days on the acid beverage, the susceptible teeth first attain a score of 4 1/2 as contrasted with the resistant teeth which earn such a rating at about 140 days. Thus, while there is a difference of over 60 days between the time the susceptible and resistant molars received a score of 3, a rating of 4 1/2 appears with a time difference of 40 days. It is also seen that a rating of 5 occurs with a time difference of only 20 days. It appears, then, that the susceptible lower molars erode on the lingual side faster than do the resistant molars for a time, and then, for some reason, lingual erosion in the resistant teeth "catches up" with the susceptible teeth.

We believe that this phenomonen can be reasonably explained. The drinking habits of the rat, i.e. the use of the tongue in drawing the liquid from the water tube to the gullet, thereby causing the fluid to come into contact with the lingual side of the mouth almost exclusively, helps to explain why erosion in our experiment is mostly lingual.

When a large part of the lower jaw has been dissolved on the lingual side, less acid might come into contact with this part of the teeth than formerly, and the tongue would rub with less vigor on this part of the tooth. Thus, after a rating of over 3 is reached, lingual erosion is slowed. Therefore, while the resistant teeth are still being subjected to a considerable amount of acid and rubbing by the tongue, the susceptible teeth are undergoing considerably less erosion than was the case previously. Since lingual erosion approaches a limit, the resistant teeth appear to "catch up" with the susceptible teeth in the amount of erosion accomplished.

The possibility also exists that once the enamel of the resistant teeth is gone and the dentine exposed, the speed of erosion increases.

DISCUSSION

The non-caries diet of powdered whole milk, alfalfa leaf meal and salt proved very successful in delaying caries. Not one resistant lower molar and only three susceptible sets of teeth were so damaged by caries that measurement of lingual erosion was impossible. A few resistant and almost every susceptible set of teeth had cavities ranging from minute to quite large in some of the susceptibles. Whether these cavities were true caries or merely the result of the absorption of acid by the impacted food in the crevices and the subsequent attack of the enamel in contact with this acid soaked food, is not known. The latter is believed to be the case. This assumption is based on the fact that these carious cavities do not resemble the caries seen in rats fed the Hoppert diet, and upon the additional fact that not one of the susceptible or resistant control animals has visible caries.

The susceptible controls were killed at ten day intervals ranging from 50 to 170 days on the diet plus water. Thus 13 sets of teeth showed no caries from a period of 50 to 170 days. One susceptible rat was kept to ascertain when caries would occur. It is now nine months old and has not as yet developed caries.

It is believed that the reason this non-caries diet does not produce caries is the absence of starches and coarse materials. Stewart has

found (unpublished) that rations containing sugar and no coarse particles do produce caries. Why Stewart's diet produces caries, while milk powder which contains milk sugar does not seem to induce caries, is a subject that should be investigated.

It is also interesting to note that L. acidophilus which has been reported to thrive on milk, and which has been cited by many leading investigators as the important etiologic factor in tooth decay, does not seem to cause decay in our animals fed on a diet which consists of 80% powdered whole milk. Part of the answer might lie in the fact that the milk portion of the diet is not readily impacted in the tooth crevices.

Some investigators in the field of dental research have suggested theories which use the color of the teeth as a means of locating caries. I believe that there is something to be said for this concept. While examining the caries-susceptible teeth, it was noticed that around every cavity the enamel appeared white. It also appeared that the susceptible controls, while not exhibiting caries, certainly did not have the same appearance as the resistant controls.

While I believe that there is some value in studying coloration of teeth, I also feel that using color as a device in caries work is very hazardous. I have found, for example, that if resistant animals are killed by ether, the heads severed from the body and put into alcohol, the teeth when examined later will be pinkish if no caries is present. If, however, the resistant rat is killed by a blow on the head, the head then severed from the body and the teeth extracted, the teeth all will appear white. I have further found that by placing a head containing

pinkish teeth on a lamp so that the head become dried, the pink teeth lost their coloration and were white.

There are no indications that either the right or left jaw is the more susceptible to erosion in either strain; nor can any statement be substantiated that one tooth of a specific jaw is affected more by the acid drink than another.

This study has shown the possibility that physical or chemical differences exist in the enamel of the caries-susceptible and caries resistant teeth. Certainly more experimentation is needed either to confirm or disprove this. In vitro studies of the solubility of the enamel of both strains with different acids and acid concentrations should be undertaken. The distinct possibility that sugars somehow aid in enamel erosion other than serving as a food source for bacteria, should also be investigated.

CONCLUSIONS

1. Lingual erosion of a serious nature occurs within a shorter time with caries-susceptible lower molars than with caries-resistant lower molar teeth, when caries-resistant and caries-susceptible rats are fed a non-caries diet in conjunction with a sweetened phosphorylated drink.

2. Caries is delayed in caries-susceptible rats when fed a diet consisting of 80% milk powder, 19% alfalfa leaf meal and 1% salt, and which contains no coarse particles.

3. The method of killing and preserving the rat sometimes affects the color of the teeth, so color would seem to be an unreliable indication of the presence of caries.

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