A STUDY OF FRACTURING IN CARIES SUSCEPTIBLE AND CARIES RESISTANT ALBINO RATS, (RATTUS NORVEGICUS)

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Eugene Carl Nakfoor

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A STUDY OF FRACTURING IN CARIES SUSCEPTIBLE AND CARIES RESISTANT ALBINO RATS, (*RATTUS NORVEGICUS*)

By

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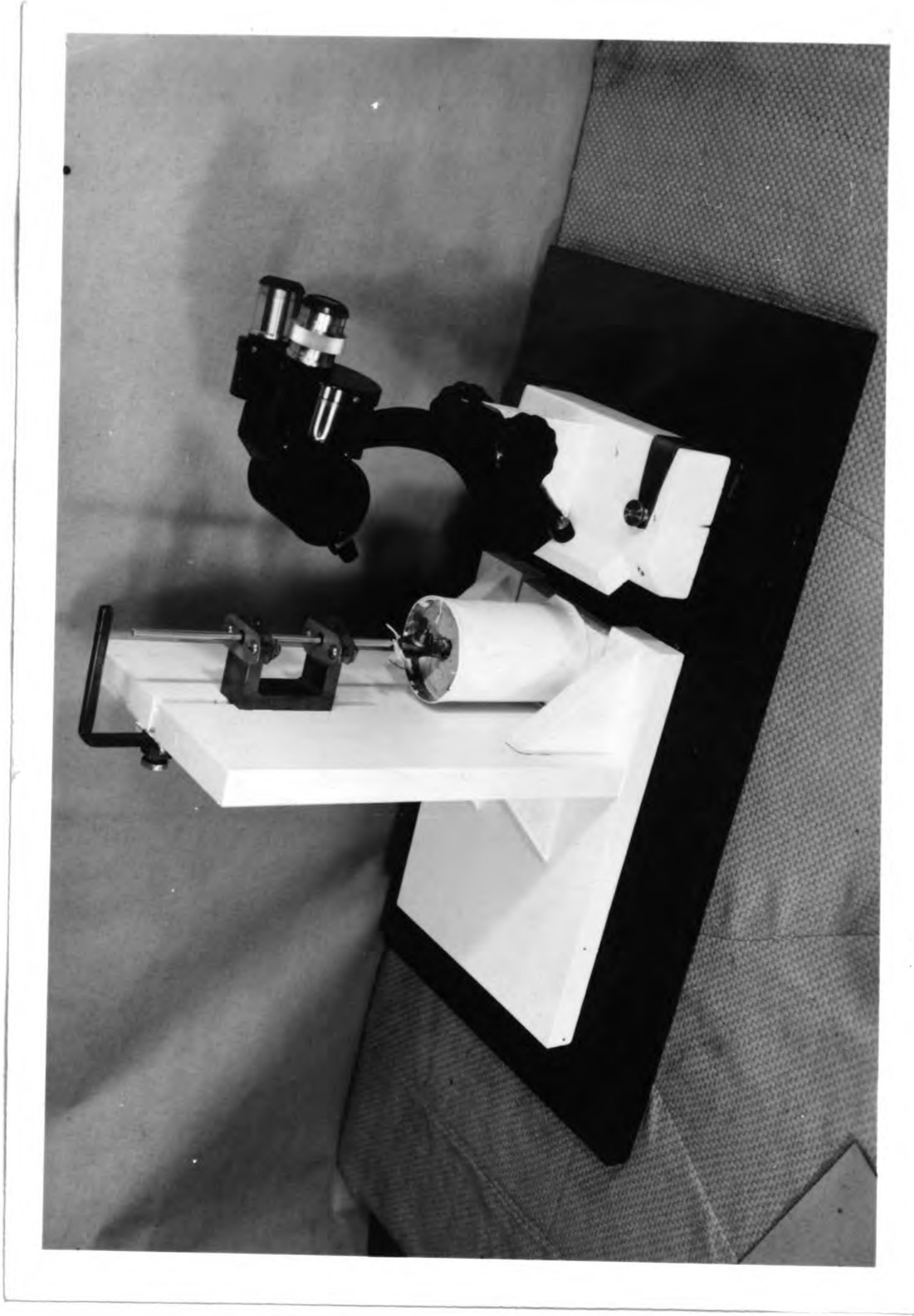

FIGURE 1.

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INTRODUCTION

Dental caries is defined by Bunting[2] as a disintegration of teeth characterized by formation of open lesions in enamel, dentin and cementum. Gottleib[3] adds that the process finally leads to necrosis of the affected tissues. These descriptions are clear and concise but, unfortunately, this is where the simplicity ends. Any explanation of why such a condition appears in the teeth is not so simply stated. People with teeth of poorest quality may not show caries. On the other hand, hard and well formed teeth may be gravely affected by caries[2]. In certain areas of the world children may show a low incidence of tooth decay. In a nearby area the opposite is the case[2]. Carbohydrates are necessary in the diet to produce the pathologic condition[2,3,4]. However, the Maya Indians live on a high carbohydrate diet and have a low caries rate[20]. It is generally agreed that high counts of <u>Bacillus acidophilus</u> indicate high caries susceptibility, but this is not always the case. Low counts are sometimes found in highly susceptible cases and high <u>Bacillus acidophilus</u> counts may be found in immune mouths[3].

In view of these seemingly incongruous illustrations it is no wonder that the problem of dental caries is a complicated one. The fact that investigators have recognized the many complications is an indication of progress. Simple explanations were characteristic of the early theories. Bunting[2] cites three, namely, the inflammation, worm and electrical theories. In a sense each was an expression of the times and each was rejected from the lack of evidence. The inflammation theory persisted longest and was advanced by investigators as far back as Hippocrates and accepted as recently as 1888 by Heitzmann and Boedecker[2].

Inflammationists believed that the carious process began internally in the pulp and proceeded outward to the enamel surface. This was vigorously opposed by the proponents of the acid theory. They believed that the carious lesion began on the surface of the tooth and that it consisted of an acid decalcification of the enamel and dentin. The fact that in many instances the external opening of the cavity was small in proportion to the cavity found in the dentin was used as support of the inflammation theory. However, the hypothesis was dependent upon the supposition that the dentin contained a circulatory system. Later, when no such system was found in its histologic structure, the theory collapsed.

The acid theory on the other hand gained wider acceptance. When Willoughby D. Miller[2], a student under Koch, established in 1882 the fact that aciduric and acidogenic bacteria and carbohydrates were active factors in the carious process, the theory in its major points, at least, arrived at its present day form. Miller[4] also recognized the presence of peptonizing bacteria in what he described as the second stage of the carious process. Bacteria possessing acidogenic as well as peptonizing qualities could participate in both stages. He felt, therefore, that the process was, initially, decalcification of the enamel by acid. This was followed by an invasion of the organic parts of the enamel by proteolytic bacteria. These views of Miller gained wide acceptance and, except for minor changes, comprise the theory as it stands today.

McIntosh, James, and Lazarus-Barlow, and F.E. Rodriquez[2], a dentist in the United States Army, found "that in cultures taken from initial caries ~~carus~~, Bacillus acidophilus predominated over all other forms of bacteria that were cultivatable in an acid medium and that this organism was present in some form in every case of the disease."

Bunting in examining over 2000 cases found only one instance in which caries was manifestly active and the organism could not be found. He also found that the B. acidophilus was invariably absent from mouths which were free from dental caries or in which the disease had become inactive. He has induced artificial caries in vivo by applying a clasp containing bread and B. acidophilus to the tooth for a period of several weeks[2]. Microphotographs of the organisms in dentin in which they had produced artificial caries were obtained by two Japanese investigators, Okumura and Nakai[2]. These facts led Bunting to believe that dental caries is a specifically infective disease and the Bacillus acidophilus is the active etiologic factor.

Gottlieb[3] opposed the idea that B. acidophilus is the active etioligic factor. Concerning the organism he states, "it is established that all true caries is characterized by the presence of a yellow pigment, but it is also fairly well established that the pigment cannot be the product of B. acidophilus. While making acidophilus counts an exceedingly great number of such cultures have been made, but no one yet has reported that these organisms produce a yellow pigment." What produces the yellow pigment appears to be a problem still, but that a close relationship exists between dental caries and the Bacillus acidophilus can not be denied.

According to the acid theorists, all three of the following are needed for the production of caries:

1. Microorganisms—aciduric and acidogenic and possibly proteolytic.
2. Carbohydrate.
3. Prevention of acid dilution or neutralization by saliva.

The acid formed by the action of acidogenic bacteria on carbohydrates will be quickly washed away by the saliva unless a means is afforded by which the acid will be allowed to remain in contact with the tooth surface. Such protection is provided by the natural fissures of the teeth. The tenacious plaque[2,4,21] was suggested as another means by which this could be accomplished. The adhering substance is a mucinous film which holds the bacteria and food particles in close contact with the enamel. Most investigators support the plaque theory, although Miller questioned the statement that these films are necessary for the production of dental caries. He called attention to the fact that they may be found on teeth which are non-carious as well as on those that are carious. According to Bunting[2], Miller apparently overlooked the fact that the aciduric bacteria may have been present in the carious locations while being absent in the non-carious sites.

Action by acidogenic bacteria on carbohydrates at protected surfaces of the teeth is not the whole story. Dietary and other environmental factors as well as the inheritance agent must also be important to the pathologic process. The incidence of caries among the uncivilized races is from 1 to 20.8%. This rate increases to from 75 to 95% when only the civilized races are considered[2]. Most investigators believe the difference is due to the diet. The minerals available in food and drinking water may also be of consequence. Carl Röse[2] reported that children in certain areas of Germany in which the calcium salts of the water and soil were high tend to have better formed teeth and less caries than children from areas of low calcium content.

Physical properties in addition to the chemical constituents of the diet may also be factors. Working with rats, Hoppert[10,11] and his co-workers, found that the size of food particles was a factor in producing caries. They devised a diet which, though it was nutritionally adequate, produced caries. It consisted of 66% ground cereal, 30% powdered whole milk, 3% alfalfa leaf meal, and 1% sodium chloride. They found that if the cereal was coarsely ground, the caries incidence was high. If the cereal was finely ground, caries incidence was much lower. This result was also confirmed by Braunschneider[7,8], although our own observations failed to support Braunschneider's results. This matter will be discussed in greater detail in the following pages.

The fact that many mouths with deformed, mottled or porous teeth are free from caries, and the innumerable instances[2] of individuals whose oral environments will not allow B. acidophilus to survive, suggests the existance of an inheritance factor in caries production. Racial differences[20] in the incidence of the disease likewise imply genetic involvement. It was these observations that prompted Steggerda of the Carnegie Institution of Washington to suggest to Hunt and Hoppert an investigation of the inheritance of susceptibility to dental caries in rats' teeth. Hunt and Hoppert began the undertaking at Michigan State College in 1937 and the work is continuing. Several papers have been published[12,13,14,15,16,17,18,19]. "There were two objectives at the outset: (1) to determine whether there is an inheritance factor in the development of dental caries in albino rats; and (2) ~~is~~ if there is such a factor, to discover if possible the number of gene pairs involved and the genetic and physiological effects of each gene." [15]

To accomplish the first objective they attempted to produce a caries-susceptible and a caries-resistant line of albino rats. Progeny testing, selection and close inbreeding (brother-sister matings) were the genetic techniques used. The original animals were obtained from various departments on the Michigan State College Campus. Animals from several colonies were used in order to obtain a large assortment of genes for susceptibility and resistance. The diet was essentially the same as the

one Hoppert, Webber and Canniff[10,11] used to produce caries. The diet was comprised of ground polished rice (66%), whole milk powder (30%), alfalfa leaf meal (3%) and Sodium Chloride (1%). The rice was coarsely ground so that about 70% of it was retained on a 20 mesh screen when sifted. The animals were placed on this caries-producing diet at the age of 35 days. The techniques used brought immediate results. From the second generation, the two lines began to differentiate. By the time that the 12th susceptible generation was obtained, the variability in caries had become so low that Hunt and Hoppert[15] believed that the susceptibles had become homozygous for all, or nearly all, the genes responsible for very early caries. On the other hand, variability among resistants has remained high, although they are becoming more resistant with each succeeding generation. The susceptible averages declined from 57 days in the 2nd generation to 24 days in the 12th. The averages for the susceptibles have declined little since the 4th generation which indicates the rapidity with which homozygosity was reached. The resistant averages steadily increased from 116 days in the 2nd generation to 392 days in the 11th generation. Thus, the first objective had been reached.

Other phases of the problem have been investigated and significant results have been obtained. Braunschneider[7,8], working with the same strains found that age increases resistance to caries. The animals in the study were more resistant to decay at 100 days and at 150 days of age than at 35 days. Hunt and Hoppert[17] reported that sex was not a significant factor. Occlusion (use of the molar) on the other hand does make a difference[18]. They found that caries was retarded in lower molars which were not opposed by the corresponding upper molar. In this connection, they believed that mechanical breakage of upper molars delayed

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the carious process in lower molars of some of the resistant animals, thus, "giving the impression that they are genetically much more resistant than they actually are." Including such animals in their breeding stock, might account for the continuing high variability.

Hunt and Hoppert[19] found that certain areas of the occlusal surface of the rats' lower molars are more susceptible to caries than others. There is also a greater amount of caries on the right side than on the left, and the excess is greater in the susceptible strain. An interesting point of the study is that the susceptible strain shows a distribution pattern different from the resistant.

The study in this paper is actually an outgrowth of the paper by Hunt and Hoppert entitled, "Occlusion as a Factor in Dental Caries."[18] In reviewing this paper, Hamilton B. G. Robinson suggested that the caries observed in the studies of Hunt and Hoppert is initiated <u>by fracturing</u> of the lower molars. The following study resulted from this suggestion.

THE PROBLEM

Thus, if use facilitates the carious process in rats' teeth, then might not mechanical breakage be an important etiologic factor? The problem, simply stated, is to determine the relationship of fracturing to the formation of dental caries in the susceptible and resistant strains of albino rats.

APPARATUS AND METHODS

It was decided that a direct approach to the problem would be to determine the relative breakableness of the molar teeth of the two strains by means of some type of suitable tooth-cracking apparatus. Such an apparatus was developed, of which a detailed description follows (Figure 1). The supports for the working parts were made from hard oak with a thickness of 7/8 inches and a width of 8 inches. The perpendicular support was 10 inches high and the base 12 inches long. Attached to the vertical support was a bar of steel whose ends were bent accurately at right angles so that they extended as arms perpendicular to the support but parallel to the base. Through each arm was bored a hole which provided a bearing surface for a 7 inch aluminum rod, and which allowed the rod to move vertically in much the same manner as a pile-driver. The aluminum plunger was cut from a 3/8 inch rod and weighed 8.55 grams. Its surface was polished with a fine grade of emery cloth. The holes for guiding the rod were 3/8 of an inch in diameter and were polished with emery cloth until the rod moved freely but with a minimum amount of play. The aluminum rod was coated with a fine grade of lubricating oil and then wiped dry before each testing period in order to reduce further the resistance of the bearing surfaces. The lower end of the plunger was tapered to a blunt point which was about 1 millimeter in diameter. A small dentist's screw-type vice was embedded in a metal cylinder filled with concrete. This in turn was placed on the wooden base support beneath the plunger.

Thus, a fresh, lower half-jaw, containing the rats' molars could be placed in the vice, and the aluminum rod allowed to drop from measured distances on specific cusps of the teeth. An L-shaped, steel guide bar

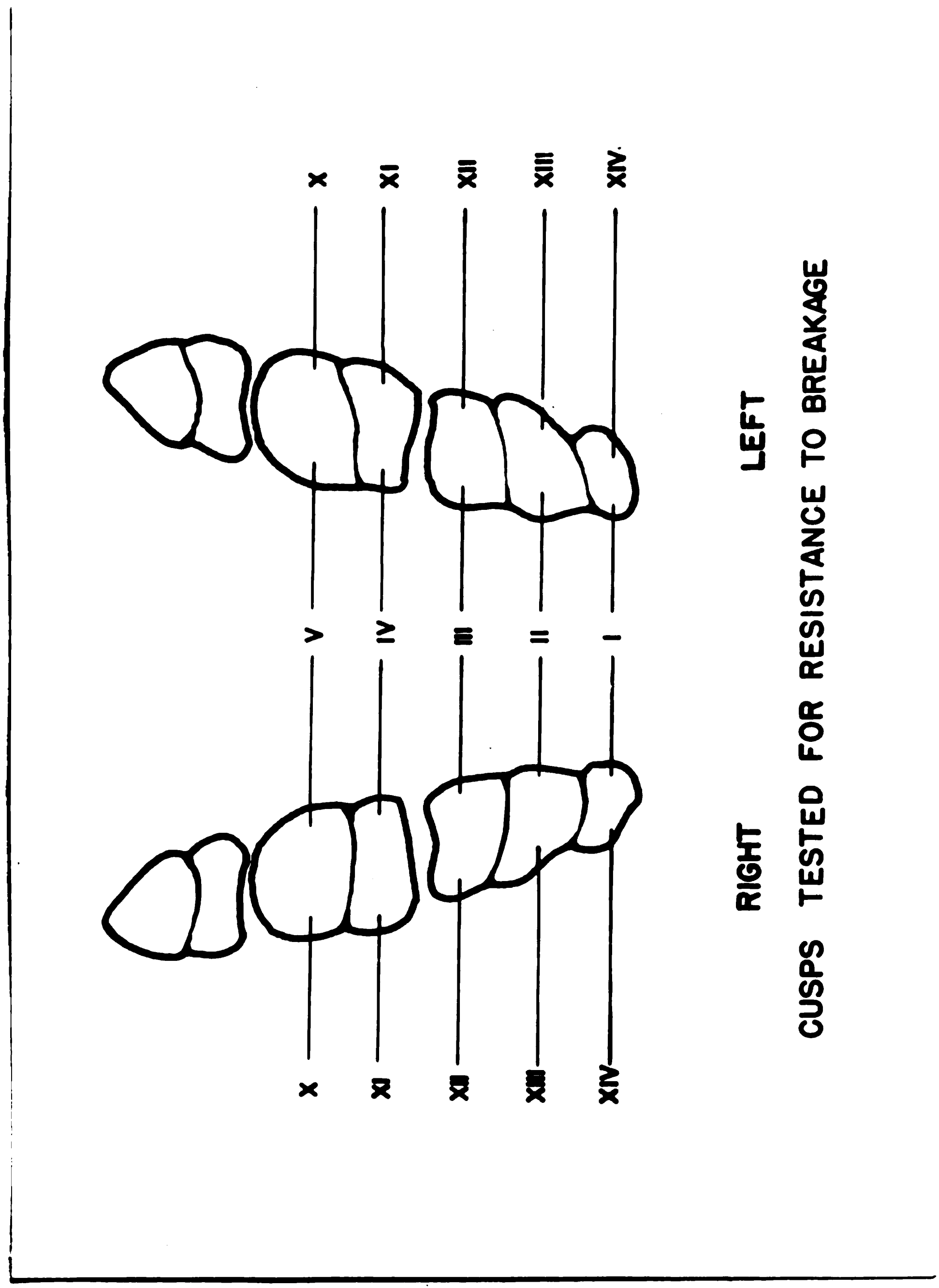
X
XI
XII
XIII
XIV
V
IV
III
II
I
RIGHT
LEFT
CUSPS TESTED FOR RESISTANCE TO BREAKAGE

FIGURE 2.

I

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was used to limit the drop distance which could be varied by moving the bar. The plunger point could be placed on the tooth at the desired site of impact. The guide bar was moved up or down until the desired distance of drop was secured. The plunger was then raised by hand until it touched the guide bar. The rod was then released, it dropped through the measured distance and struck the cusp. The machine was crude but for the purpose of this experiment, its accuracy was sufficient to produce significant results.

It would have been desirable to know how much kinetic energy was absorbed by the tooth before it was broken. The impact could be easily calculated since the energy expended is provided by the formula, $K.E. = M\, a\, s$,[1] where $\underline{M}$ is the mass, $\underline{a}$ the acceleration and $\underline{s}$ is the distance dropped. The acceleration in this case is the acceleration of a freely falling body which reduces the formula further to $K.E. = W\, s$, which is simply the weight times the distance dropped.

When a certain impact breaks a tooth, how do we know that a lesser blow might not have broken the tooth? It would be illogical to assume that the impact was exactly the amount required to fracture the tooth. On the other hand, if the blow does not break the tooth, then an additional impact would further complicate the problem since we do not know how much was contributed by the preceding blow. Thus, we had to content ourselves with a comparative measure between the two strains of rats of resistance to fracture.

The animals for this experiment were obtained from the caries susceptible and caries resistant stocks of Hunt and Hoppert. Nineteen susceptible breeders, comprising 15 crosses, and 21 resistant breeders, making 16 crosses, were taken from the reserved stocks of Hunt's and

Hoppert's 20th susceptible and 16th resistant generations respectively. The animals were kept in cubical cages which measured 12 inches on a side. This size was satisfactory for this experiment since the animals were kept only until they reached the age of 60 days. The cages were made of galvanized sheet metal and were closed on all sides except for the top which was covered with ¼ inch galvanized iron mesh. Drip bottles supplied the water which came from the deep well of the college. The room temperature was maintained automatically at about 77 degrees, except in hot summer weather. The diet was essentially the same as the one devised by Hoppert, Webber and Canniff[7,8] and described earlier in this paper. Rice flour was substituted for ground rice in order to prevent early dental decay. Contrary to expectations, the rice flour mixture did not prevent caries in all cases. Subsequent to this discovery, diammonium phosphate to the extent of one per cent by weight was added to the feed.

Ten sites were chosen for tests on resistance to breakage. The classification of these sites (cusps) was as indicated in Figure 2. Roman numerals were used to differentiate the various cusps. The numbering began at the anterior median cusp of the first molar and continued posteriorly on the lingual side to the posterior end of the 2nd molar, then proceeded on the labeal side anteriorly to the anterior cusps of the 1st molar. Cusps I, II, III, XII, XIII and XIV were located in the 1st molar. The second molar contained IV, V, X and XI.

It required some experimentation to select the proper sites for testing. During preliminary trials, 3 cusp sites were tested. Later it was decided to test all the cusps in the three molars except the 5 small auxillary cusps which were located either along the outside of the molars or between the molars. But the 3rd molar does not differentiate clearly into cusps, which made it impractical to use this tooth in the experiment so its four cusps were eliminated as testing sites. Thus 10 testing sites remained at which the final breakage trials were made.

In the preliminary trials an unexpected phenomenon occurred. Dental caries appeared in some of the susceptible animals on the rice flour diet at a very early age, in some cases as early as 45 days of age. In a telephone conversation, George N. Streit, Detroit Branch Manager of Stein, Hall and Company, informed me that the rice flour we were using may have been Southern rice flour which was inferior, because of coarseness and foregin matter, to the Western rice flour. Stein, Hall and Company process only Western rice flour, and they kindly consented to send me their flour (processed under the brand name of "Hallmark Rice Flour") in 100 pound shipments. Because of the small size of shipment, this was done at a financial loss to the firm.

I used only Hallmark Rice Flour in my experiment. On this diet, however, some of the animals again showed caries before the age of 60 days. Through further correspondence, I received certification from R. H. Freudenheim of the Industrial Foods Department of Stein, Hall and Company that the rice flour which they are now selling is processed in a similar manner to that sold in 1942 which was when Braunschneider obtained flour from them for his experiment[21]. A sample of the silk bolting cloth, through which the flour was passed, was received from them. In examining the cloth under the microscope, I found that the largest rectangular holes were less than 200 microns on a side, which is essentially what Braunschneider found in a similar examination[21].

At this point in the experiment, diammonium phosphate (1% by weight) was added to the diet in a further attempt to check the occurance of dental caries among the susceptibles. Tooth decay, however, continued to appear in about the same proportion and the following classifications of groups according to diet andappearance of caries resulted:

Group R	Resistant, non-ammoniated diet Resistant, ammoniated diet
Group S	Susceptibles, without caries, non-ammoniated diet Susceptibles, without caries, ammoniated diet
Group Sc	Susceptibles, with caries, non-ammoniated diet Susceptibles, with caries, ammoniated diet

Thus Group R included all resistants, Group S included all susceptibles without caries, and Group Sc included all susceptibles with caries.

Scoresheets were mimeographed to recordd the testing data. One sheet was used for each half-jaw tested. The drop at which initial, intermediate and final damage was done to the cusp was recorded in the rectangular block alloted to each testing site. The type of damage was also recorded by the letters F, Cr, C and S which symbolized flaking, cracking, chipping and splitting respectively. Flaking referred to the knocking off of minute particles of tooth substance at even the lightest blows. In cracking a distinct fissure was produced, though no part of the tooth was lost. In chipping actual chunks of the cusp were broken off. Often a whole cusp was broken off and remained attached only to the soft tissues. This was defined as splitting. The tooth was tested until either a large portion of the cusp was chipped off or the whole cusp was split off.

The method by which the teeth were tested for fracturing will now be described in detail. When a rat reached the age of about 60 days, it was killed by etherization. The age of the susceptibles tested ranged from

59 to 62 days. The range of the resistants was from 60 to 64 days, except for 4 animals which were 67 days old. The slightly greater age of the resistants resulted from the practice of giving priority to the susceptible animals when both were due for testing. Since the teeth were already well-developed, it was assumed that such a small age differential would not effect the results.

After the animal was etherized, the jaw was immediately removed and washed, and the molars were then scrubbed with a small toothbrush to remove remaining food particles. The half-jaw to be tested was then placed in the vice and examined with a needle probe under the binocular microscope for caries, fractures, etc. This information was also included on the scoresheet. After the testing, the other half of the jaw was examined, labeled with India ink and kept as a control. Alternating the half-jaws, testing the right in one instance, then the left side in the next, eliminated any variation due to the possibility that the right side might be different from the left. This was important since Hunt and Hoppert[19] found that the right side of the lower jaw in both the susceptibles and the resistants showed a greater susceptibility to dental caries than the left lower molars.

The teeth were tested in the fracturing machine immediately after they were microscopically examined. The cusps were subjected to repeated blows by the rod in the following manner. The plunger point was lined up with the testing site and dropped on the tooth from a distance of 5 millimeters. The rod was dropped 5 times on the same cusp from this height, the cusp being examined with a binocular microscope and the damage, if any, being recorded after each blow. The cylinder containing the vice was then moved until the next cusp in order was directly beneath the plunger point, whereupon 5 blows from a height of 5 millimeters were again administered. This proceedure was repeated until all of the cusps were subjected to the prescribed number of blows. The results were continually observed with the binocular microscope. Cusps I, II, III, IV, X, XI, XII, XIII and XIV were tested in that order. After the drops at the 5 millimeter level were completed, the distance was increased to 10 millimeters and from this height all sites were again tested. When a cusp was chipped or split the corresponding dropping distance was recorded as the measure of resistance of the cusp to breakage. The dropping distance was increased by increments of 5 millimeters until all the cusps were chipped or split. Once a cusp had been chipped or split it was not tested further.

DATA AND OBSERVATIONS

Although mechanical breaking of the teeth from any cause could technically be called a natural phenomenon, any fracturing by the aluminum rod will be referred to in this paper as <u>induced</u> fracturing, as opposed to <u>natural</u> fracturing which refers to those fractures present in the teeth before the teeth were tested. Several types of damage to the tooth by the aluminum rod were observed. Minute flakes of tooth substance were seen flying off the crowns with the lowest impacts and this flaking continued with drops from all distances. As the distances increased cracking and breaking off of minute pieces occurred followed, finally, by chipping off of large portions of the crown. In many instances a cusp was actually split off from the molar. In almost all of the cases, chipping was preceeded by flaking. At only one location was flaking not observed. In the susceptible rats, cusp I never exhibited flaking. This phenomenon will be discussed in detail in a succeeding section of the paper.

It was decided, after many possibilities were considered, that the simplest and most direct use of the data obtained from the tests would be to use the distance of the final drop or, stated otherwise, the blow which either broke off a large portion of the cusp or which split off the whole cusp from its molar. For reasons previously mentioned, computing the impact by using the formula K.E. = M a s would provide us, at best, with only a relative measure of the resistance to induced fracturing. Since M is the mass of the aluminum rod and a is the acceleration due to gravity, it is apparent that s, the drop distance, is the only variable. As long as the same pattern of administering the drops, viz., 5 blows at each level, was used throughout the experiment, then the distance of the last blow administered is a relative index of resistance to induced fracturing as between the two strains.

Table I gives the means of the final drop distances computed for each group. The table reveals many interesting relationships. The resistance to induced fracturing in the two resistant groups (Total) is greatest at site XI (28.4) which is closely followed by I (27.8), XIII (27.8), XIV (26.7) and XII (26.9). X (23.1) is next followed by II (20.9). III (14.3), IV (13.8) and V (13.7) show a considerable decrease.

Upon averaging the observations on the two susceptible groups without caries, XIII (27.6) showed the greatest resistance to induced fracture. Following closely were I (26.7) and XIV (25.2). The next in order were II (22.7), XII (22.4), XI (20.8) and X (17.3). Again III (12.3), IV (12.3) and V (11.1) were lowest.

Table II provides comparison between the resistant strain (Group R) and the susceptible strain (Group S) in resistance to induced fracturing.

TABLE I

	Total No. of Animals	I No. Ave. Drop	II No. Ave. Drop	III No. Ave. Drop	IV No. Ave. Drop	V No. Ave. Drop	X No. Ave. Drop	XI No. Ave. Drop	XII No. Ave. Drop	XIII No. Ave. Drop	XIV No. Ave. Drop
RESISTANTS (GROUP R)											
Non-ammoniated	41	28.2 (17)	21.5 (36)	14.5 (41)	13.9 (40)	13.7 (41)	23.9 (41)	28.0 (40)	28.5 (41)	28.6 (36)	25.9 (23)
Ammoniated	16	27.0 (10)	19.3 (15)	13.8 (16)	13.4 (16)	13.8 (16)	20.9 (16)	29.3 (15)	22.8 (16)	25.9 (16)	28.5 (10)
Total	57	27.8 (27)	20.9 (51)	14.3 (57)	13.8 (56)	13.7 (57)	23.1 (57)	28.4 (55)	26.9 (57)	27.8 (52)	26.7 (33)
SUSCEPTIBLES											
WITH CARIES (GROUP Sc)											
Non-ammoniated	6	27.5 (2)	20.0 (3)	11.7 (6)	10.0 (6)	7.0 (5)	10.0 (3)	15.8 (6)	20.8 (6)	25.0 (5)	30.0 (3)
Ammoniated	19	27.2 (9)	23.5 (10)	12.6 (19)	11.6 (19)	8.7 (19)	9.6 (12)	19.7 (19)	17.1 (19)	24.7 (18)	27.5 (8)
Total	25	27.3 (11)	22.7 (13)	12.4 (25)	11.2 (25)	8.3 (24)	9.8 (15)	18.8 (25)	18.0 (25)	24.8 (23)	28.9 (11)
WITHOUT CARIES (GROUP S)											
Non-ammoniated	7	26.4 (7)	20.0 (7)	11.4 (7)	13.6 (7)	11.4 (7)	19.3 (7)	20.7 (7)	25.0 (6)	29.2 (6)	21.4 (7)
Ammoniated	26	26.8 (19)	23.6 (21)	12.5 (26)	11.9 (26)	11.4 (26)	16.7 (26)	20.8 (24)	21.7 (23)	27.2 (23)	26.7 (18)
Total	33	26.7 (26)	22.7 (28)	12.3 (33)	12.3 (33)	11.1 (33)	17.3 (33)	20.8 (~~2~~31)	22.4 (29)	27.6 (29)	25.2 (25)
ALL SUSCEPTIBLES COMBINED	58	26.9 (37)	22.7 (41)	12.3 (58)	11.8 (58)	9.9 (57)	14.9 (48)	19.9 (56)	20.4 (54)	24.4 (52)	25.8 (36)

Note: The figures in parenthesis are the number of cases.

TABLE II

STANDARD DEVIATION, MEANS, DIFFERENCE BETWEEN THE MEANS, STANDARD ERROR OF THE DIFFERENCE OF THE MEANS AND THE t-VALUES

	σ_R	σ_S	$\overline{M}_R$	$\overline{M}_S$	$\overline{M}_R - \overline{M}_S$	$S.E.^2_R$ $S.E.^2_S$	t - Value*
I	5.3	4.6	27.8	26.7	1.1	1.4	0.8
II	4.8	4.7	20.9	22.7	-1.8	1.1	1.6
III	4.0	3.1	14.3	12.3	2.0	0.8	2.7
IV	3.3	3.3	13.8	12.3	1.5	0.7	2.0
V	3.1	2.1	13.7	11.1	2.6	0.5	4.9
X	5.6	5.4	23.1	17.3	5.8	1.2	4.9
XI	4.9	4.6	28.4	20.8	7.6	1.1	7.2
XII	6.5	4.3	26.9	22.4	4.5	1.2	3.8
XIII	5.2	4.5	27.8	27.6	0.2	1.1	0.2
XIV	5.8	3.9	26.7	25.2	1.5	1.3	1.2

$$* \text{ t - Value} = \frac{M_R - M_S}{\sqrt{S.E._R^2 \; S.E._S^2}}$$

Included are the standard deviations, the means, the difference between the means and the t-values of the final drop distances. The resistants showed greatest variation at XII with a standard deviation of 6.5. The others in order were XIV (5.8), X (5.6), I (5.3), XIII (5.2), XI (4.9), II (4.8), III (4.0), IV (3.3) and V (3.1). In the susceptibles X showed the highest variation with a standard deviation of 5.4. The others in sequence were II (4.7), I (4.6), XI (4.6), XIII (4.5), XII (4.3), IV (3.3), III (3.1), and V (2.1).

Locations XIII, I, XIV and II do not show significant differences between the two strains as indicated by their t-values, 0.2, 0.8, 1.2 and 1.6 in that order. The most significant difference was found at XI whose t-value was 7.2. The other significant t-values were at V (4.9), X (4.9), XII (3.8), III (2.7) and IV (2.0).

Besides the tooth testings, the examinations provided other important information. It was noted throughout the experiment that very little natural fracturing occurred. The author did not find a single instance of fracture which would leave the softer dentin open to attack. In re-examining the controls which had been saved for future reference, only 3 instances of fracture were found among more than 50 susceptible lower half-jaws. Two of the instances were of minor chipping, occurring at the occlusal surfaces of crowns II and IV. The third instance was a sharp edged indentation between cusps V and X.

In the resistants only one instance of natural fracturing was observed, this being minor chipping. This absence of natural fracturing has an important bearing on the experiment and will be discussed in detail later.

As mentioned before, the study was complicated by the unexpected appearance of caries in the susceptible animals. That the occurrance of the caries persisted with the substitution of Western rice flour and the introduction of diammonium phosphate was surprising, especially in view of the fact that Braunschneider in 1942 raised 98 susceptible animals from Hunt's and Hoppert's 8th generation of susceptibles on the same rice flour diet (not ammoniated) and out of the 98, 97 reached 100 days of age without carries.[7] The other one showed caries at 91 days of age. The fact that caries, diagnosed by microscopic examination, are detected earlier than those by the naked eye can account for only a small amount of the difference between my observations and Braunschneider's. Early dental caries in the susceptible rats are of such a rampant nature that a whole molar is often demolished in a week. That the susceptibility of the animals to tooth decay has increased is highly improbable because the strain has shown consistently low variation since the 8th generation. Contamination of the flour fine diet in one of the feed cans by coarse rice particles, similar in size to those found in the regular diet used in the Animal House, was detected. The author is unable to explain how the coarser particles of rice got into the can. A graduate student, Mr. Roger Keller, and Mr. Earl Harrison, the supervisor of the Animal House, occasionaly fed the animals, but both are responsible persons and were well aware of the special diet that was used. The animals on the rice flour diet were kept on a separate rack and each was identified with a colored tag. Of the three animals in the liter which were fed from the polluted feed, one showed dental caries. This was the only instance of contamination detected. Though thorough routine checkes on the feed cans were not made, it is highly improbable that any significant contamination of the diet with coarse rice particles occurred. As shown by Table I, 6 out of 13 of the animals on the non-ammoniated, rice flour diet produced dental caries, while 19 out of 45 of those on the ammoniated diet showed tooth decay. These results would certainly merit further investigation on the effectiveness of the rice flour diet. Four of the resistant control half-jaws showed a slight amount of tooth decay. In each instance the carious area was confined to the small intermolar cusp between the 1st and 2nd molar.

DISCUSSION

One object of this experiment was to find out whether the susceptible teeth broke more easily than the resistant teeth. But this was only a means for reaching the more important objective which was to determine whether mechanical breakage initiates dental caries in rats. The appearance of caries which complicated the testing of the teeth for fracturing was a phenomenon which had a greater bearing on the experiment's object than the tooth cracking tests themselves, namely, <u>the presence of dental caries in the absence of natural fractures</u>. Had there not been any tooth decay, the results of the fracturing tests might have been interpreted entirely differently. The results might, in the absence of caries, have been used as evidence in support of the theory that fracturing is an important factor in initiating dental caries. In fact the absence of natural fractures would have been considered further evidence since it would then be contended that the rice flour prevented fractures, which in turn prevented tooth decay. Due to the presence of caries in absence of fractures, however, it <u>seems clear that fracturing is not an important factor in causing caries</u>.

Why then did the susceptible teeth break more easily under the aluminum rod than the resistant teeth? There are other possibilities. The susceptible teeth for instance might be comparatively easier to break by our machine than the resistant teeth and yet might be capable of escaping breakage while chewing food as well as the resistant rat's teeth. Thus the fracturing tests might indicate an inherent difference which in reality is never manifested under natural conditions.

It is also altogether likely that the susceptible teeth have been weakened previous to the testing. It is possible, in fact, probable that the carious process had been going on in what appeared to be susceptible teeth without caries. If such were the case, caries would be the cause of mechanical weakness, not its result. In support of this hypothesis the susceptible teeth showed a greater amount of staining which is considered by most investigators as a by-product of the carious process.[1,2] The controls of the tested molars were examined again, this time for staining. Five areas where staining frequently occurs were chosen for study. Out of 54 susceptible half-jaws examined, only 4 showed very little or no staining at the selected sites; the remaining 50 showed staining. Two hundred and seventy sites were examined; of these 211 showed discoloration.

Among the resistants, 39 out of 52 control jaws examined were negative for staining. Only 24 out of the 260 sites were stained.

Another observation which lent support to this hypothesis was the fact that there was no sharp demarcation between susceptible jaws that had caries and those that did not. Even the ones that were selected for Group S in most instances showed staining.

The results of the fracturing tests may also be an indication of a structural difference. As previously mentioned, Cusp I of the susceptibles failed to show flaking. Closer examination of the cusp revealed the reason why it did not. In the resistants the crown of Cusp I has a rather pointed appearance when viewed from the side. This is because the enamel almost completely covers the dentin. In the susceptibles, on the other hand, the enamel fails to rise over the dentin which forms a broad, mound-shaped crown. The aluminum rod struck the pointed enamel in the resistants and the dentin in the susceptibles at this site. This fact makes one wonder whether flaking is characteristic of the brittle enamel but not of the dentin. I did not find the time to pursue this interesting question. At any rate, a minor structural difference was established. It is true that this difference occurred at a site that was unaffected by tooth decay, but the condition, failure of the enamel to cover the dentin, could have a profound effect in other locations.

The thought has often occurred to me while studying the molars under the microscope, that the natural crevices in the susceptible teeth are larger than those in the resistants. While examining the control half-jaws for microscopic caries with a finely tapered needle probe, I became more convinced that this structural difference exists. It was decided to attempt to identify the strain by the depth and width of these crevices. The crevices appeared with the naked eye to be lines, varying from a wavy, thin line to a thick strip with a bulge in the center. Twenty control half-jaws from susceptible rats and 20 from resistant rats were mixed and classified by the sizes of the fissures in the molars, as judged by the naked eye, and not knowing the strain to which each jaw belonged. The results follow:

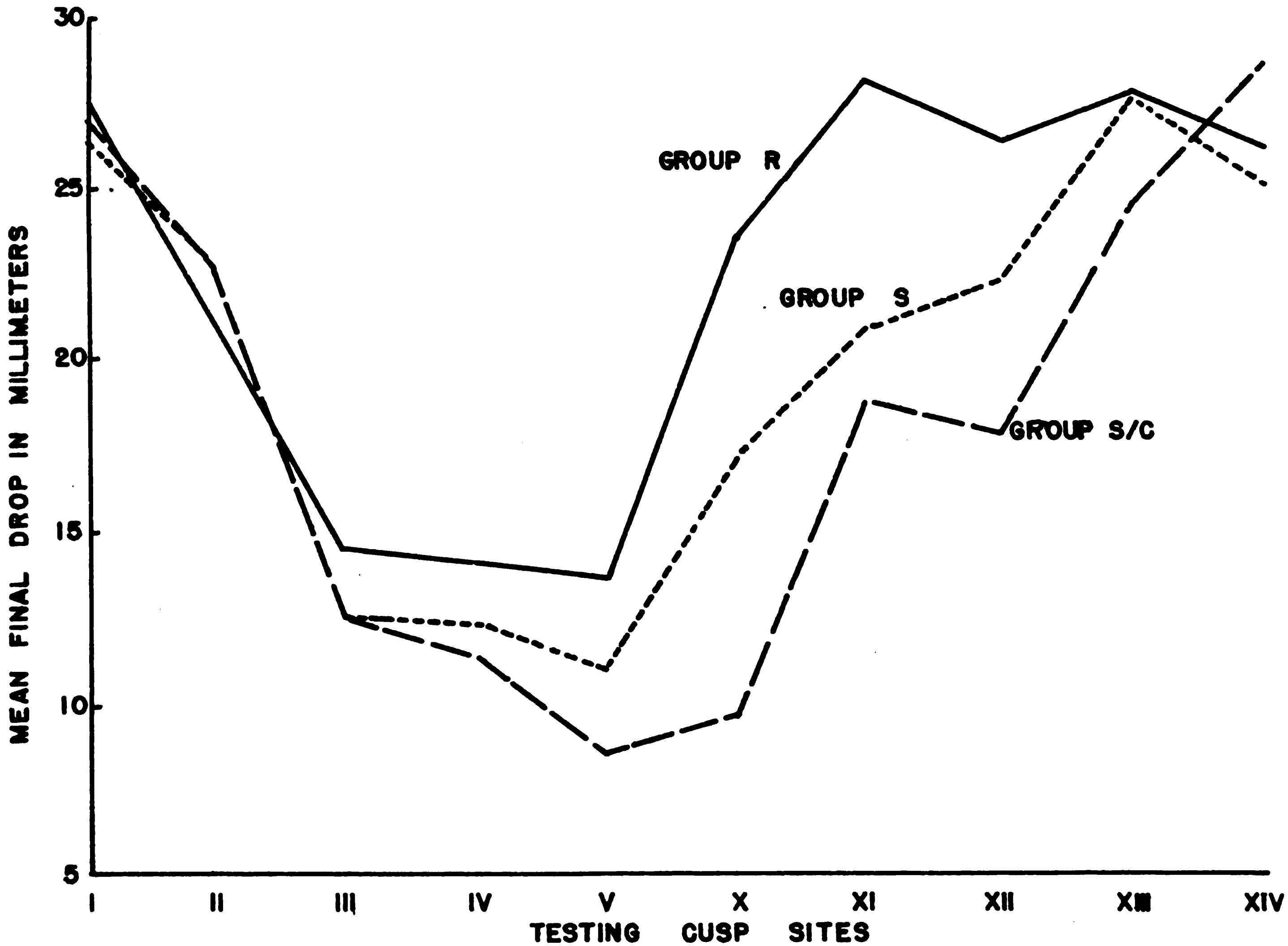
TRIALS	RESISTANTS RIGHT	WRONG	JAW NUMBERS INCORRECTLY IDENTIFIED	SUSCEPTIBLES RIGHT	WRONG	JAW NUMBERS INCORRECTLY IDENTIFIED
1	19	1	24	17	3	60, 131, 59
2	17	3	24, 72, 87	19	1	60
3	18	2	24, 72	18	2	60, 131
4	18	2	24, 72	20	0	
	72	8		74	6	

The differences are apparently significant. There are many hazards in a subjective type test such as this. Some other visual cue may have been the unrecognized key to the identification. Also the staining, which was found on most of the susceptibles' crevice surfaces, may have made the fissures appear larger. The results of this type of test, of course, are useless as scientific data. Direct measurements would be necessary to determine whether such a difference exists.

The information gained from this research, indicates, I think, that probably two factors were mainly responsible for the difference in resistance to induced fracturing. One of the possible factors is that there were cavities already weakening the susceptible teeth before the testings took place. The greater amount of staining in the susceptibles indicates the presence of a decay process already active but otherwise not visible. No sharp demarcation in amount of staining and size of carious cavities between the susceptibles with and without observed caries also lends support to this hypothesis. Some cavities were exceedingly small.

A graphic representation of the average final drop distances of the three groups, R, S and Sc, makes an interesting study (Figure 2) in this connection. If Group S was considered carious, as well as Group Sc, but

FIGURE 2, COMPARISON OF AVERAGE FINAL DROPS IN ALL THREE GROUPS



only in a lesser degree, then the graph would indicate that susceptibility to fracture and extent of carious damage went hand in hand. Thus, Group S is less carious, therefore more resistant to fracture than Group Sc, and Group R unaffected by caries is more resistant to fracture than Group S. The fact that there were no significant differences in resistance to fracture in the non-carious locations, viz., I, II, XIII and XIV between the two strains also supports this view. In other words breaking may have been the result of caries, not the cause.

The other factor which may easily affect the resistance to induced fracturing might be a structural difference. That a minor structural difference exists has been established previously in the paper for Cusp I. A more far-reaching difference is indicated by the tests in which I was able to identify the two strains by the appearance of their fissures. If the crevices are larger in the susceptibles then it could account for the fact that these teeth are more easily broken by the plunger, than are those of the resistants. In fact, from general impression I would say that the resistant cusps look thicker and better formed. They also seem to have a better lustre. They might come in the classification of what Pickerill[2] calls "Sclerotic" teeth. "Malacotic," on the other hand, might include the susceptible teeth which appear to have a duller finish and thinner cusps. Malacotic teeth were described among other things as more permeable, which also may have been a factor. At any rate the crevices appear to be important factors both in caries and in induced fracturing. The cusps which showed a significant difference to induced fracturing were those which surrounded the two crevices in question, namely, the 2nd crevice in the 1st molar and the only crevice in the 2nd molar.

The distribution of caries in the two strains as described in the paper by Hunt and Hoppert[19] is interesting in this connection. The two sulci that appeared larger to me in the susceptibles, according to Hunt's and Hoppert's study, were the loci for 60.4% of the caries in the susceptible and for only 15.7% of the decay in the resistants. This would seem to support further the hypothesis that in the susceptibles the crevices are larger, therefore allowing excellent lodgment for bacteria and food particles and resulting in quick disintegration of surrounding tooth surfaces. Perhaps caries and breakableness have a common cause— size of crevice. No proof is offered in this paper that such a structural difference exists but the facts thus far uncovered would seem to demand a further investigation into this matter.

Besides measuring the sulci of the teeth, other suggestions for further research have become apparent from this experiment. The conflict of the results of this experiment with those of Braunschneider's certainly suggests an experiment in which the animals are placed on the rice flour diet described previously and the caries time determined.

A histologic study of the teeth would be revealing. Carefully prepared ground sections might disclose important structural contrasts in the enamel and dentin between the two strains. Penetration of graphite and silver nitrate in this connection might also be feasible.

CONCLUSIONS

1. The molars of resistant rats at 60 days of age showed a greater resistance to fracturing when subjected to blows by a tapered aluminum rod than the molars of susceptible rats.
2. Unexpected dental caries appeared in the susceptible rats on the rice flour diet before the age of 60 days. This is in contradiction

to the observations of Braunschneider in 1942.

3. Fracturing is not an important factor in initiating early dental caries. Almost no fractures were observed in the teeth of 60-day-old susceptible rats, although dental caries did appear in nearly half of these animals. The caries were formed without the assistance of fractures.

4. A minor structural difference was established between the two strains. The first lingual cusp of the susceptibles was mound-shaped with the dentin rising above the enamel. The corresponding crown of the resistants was more pointed and the enamel covered the dentin. Flaking occurred with the lightest impact in the resistants whereas no flaking occurred at this site in the susceptibles. There was also an indication that other structural differences existed.

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