DEDICATION

To my mother and father who have helped make my education possible.

THE INCIDENCE OF DENTAL CARIES IN CARIES SUSCEPTIBLE AND CARIES RESISTANT ALBINO RATS, (*RATTUS NORVEGICUS*), WHEN FED DIETS CONTAINING GRANULATED AND POWDERED SUCROSE

By

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INTRODUCTION

Hunt and Hoppert have established the fact that heredity is an important factor in the development of dental caries in albino rats. The diet used in this experiment consisted of 66% coarsely ground hulled rice, 30% powdered whole milk, 3% alfalfa meal, and 1% sodium chloride. The question arises as whether the hereditary differences discovered are specific for the above diet, or whether these differences persist when various other diets are used. This is the problem attacked in the present investigation.

History and Review of the Literature

Many ideas have been presented as to the cause of tooth decay or dental caries. Four general theories have been prominent at various times. Hippocrates in 450 B. C. believed in the inflamation theory, and thought the carious process was due to the stagnation of depraved juices within the tooth.[2] More recent investigators supporting this theory believed the dentine contained capillaries and a circulatory system. Histological work has shown the dentine to be without these tissue constituents.[11,4]

As late as 1824 the worm theory was still accepted as a cause of dental caries. Worms were believed literally to subsist on the enamel.[2] In 1861 Bridgeman presented the electrical theory. He thought of the tooth as a battery and believed differences in electrical potential between the root and the crown caused deterioration of the enamel.

A fourth theory of tooth decay is the acid theory. In 1530 a German investigator, Chr. Egenolff, made the statement

that caries was due to the decomposition of food particles.[2] Robertson in 1835 concurred in Egenolff's views and stated that acid from the decomposition of food caused caries.[2] The first men to introduce the theory that bacteria may be the cause of caries, were Professors Erdl and Ficinus, Dresden physicians.(1843)[2] In 1841 Miles and Underwood working with Kock demonstrated that carious lesions invariably contain micro-organisms.[2] About this time the discovery of aniline dyes made accurate work on the indentification of bacteria possible. Underwood and Miles believed the carious lesions were due to: (1) the action of the acids, (2) the action of germs.[6,2] Although these investigators demonstrated the presence of bacteria in dental caries, they were unable to prove that the bacteria were the causitive agents.

It was left for W. D. Miller to establish the fact that bacteria are active factors in the carious process. In 1891 Miller showed that when calcified tooth substance is incubated in saliva-food mixtures, the enamel is attacked only when the carbohydrate foods are used.[2,9] When the saliva was sterilized with heat, and carbohydrates were added to the mixture the calcified tooth substance remained intact. Miller showed that an acid fermentation takes place which may cause the primary carious lesion in the enamel. He also believed that proteolytic bacteria may be active in the secondary stage of the carious process.[9]

Dietz, who devised an artificial mouth with a tooth in it which could be observed with a microscope noted the following facts:[3]

1. Caries begins on the surface of a tooth.
2. A bacterial plaque forms, sometimes producing acid.
3. Microorganisms in this plaque live on the same food as the animal lives on.
4. The bacterial plaque produces acid and the caries begins where the carbohydrates rest against the enamel.
5. The bacteria are producing acid on a surface which can be dissolved by acid.

The classical Millerian acid hypothesis remains in its present form today.

In 1915 Kligler made the observation that certain types of microorganisms grow frequently on the teeth of individuals with active caries.[37] The most important of these organisms were Lacobacillus acidophilus. Subsequent research work of Dr. Philip Jay and Dr. R. W. Bunting support the findings of Kligler.[35,36,19]

Dental caries is a pathological process.[6] Bunting describes caries as a disintegration of the teeth characterized by the formation of open lesions in the enamel, dentine and cementum.[2]

Bernhard Gottlieb[6], a recent investigator, does not agree with the classical Millerian hypothesis of tooth decay. Gottlieb believes the carious process begins in the organic components of the enamel.[6] By staining teeth with silver nitrate, he has shown lamellae extending from the exterior surface of the enamel to the dentine.[6] The lamellae are thought to be organic constituents between the calcium prisms of the enamel. Many investigators disagree with this hypothesis. However, it may be stated that the formation of dental enamel is among the most controversial

subjects of dental histology and embryology.[1,6,13,14]

Gottlieb points out that naturally caries immune individuals may consume sugar as much as they like and no caries will develop. This would point to another explanation of tooth decay than B. acidophilus[6] in the saliva. Soluble sugar may penetrate the lamellae according to Gottlieb. Fones and Boyle have shown bacteria in carious dentine tubules.[4,1] The enamel lamellae might thus be a possible path along which caries develops. Thus it is evident that the causes of tooth decay have been by no means completely identified, and numerous investigations concerning the histological, embryological, bacteriological, nutritional, chemical and genetical aspects of the decay process need to be carried on. Two main types of research have been followed: (1) Studies of the resistance of the tooth, (2) The natures of the attacking forces.

Although caries has occurred in all stages of man's history, the frequency has increased with the degree of civilization.[1,2] Bunting and others estimate that modern man has a 90-95% incidence of tooth decay.[2] Why is this true? In some populations in which the incidence of caries is low, there is a noticeable lack of refined carbohydrates even on a sub-standard diet.[6,2] Dental caries as we know it is a disease of civilization.[1] An English investigator, Bresse, showed that during the war when sweets were eliminated from the diet, caries was markedly reduced in children.[18] Bibby has also shown by numerous population studies that carbohydrates influence caries susceptibility.[15] Waugh showed that refined sugars increased the B. acidophilus count and caries incidence in Eskimos.[42]

Price made extensive studies of caries in Africa and found an increase in the incidence of caries whenever the tribes were in contact with civilization.[39] Observations of teeth in an isolated mountain village in Switzerland, where sugar and refined carbohydrates were unknown, show a negetive incidence of caries.[1]

These facts suggest that carbohydrates are an active factor in tooth decay. However, all carbohydrates do not produce the same effect.[19] Inhabitants of the Pacific Island, Tristan da Cunha, consume potatoes as a staple in their diet, yet they are free from caries.[19] Sugar appears to be a more important factor in producing caries than starchy foods.[42] Also, to be noted is the important fact that some persons teeth exhibit remarkable resistance to caries even with high sugar consumption.

Mellanby, Rumsey and Rosebury believe that the diet is the controlling factor in the incidence of dental caries.[21] The diet may alter the saliva or the teeth themselves. Bunting noted that B. acidophilus counts are changed by regulating the diet.[19] Fosdick has shown a difference in Ph indices in the salivas of caries immune and caries resistant people.[28]

Fosdick states that acids in the mouth come from carbohydrate materials; however, most oral acids under normal conditions are derived from fermentable sugars.[43] Common sugar or sucrose is a double sugar, each molecule consisting of one glucose molecule and one fructose molecule linked together rather loosely.[43] When sucrose is exposed to acids or certain digestive fluids in the presence of water, sucrose is quickly split into a mixture of glucose and fructose.[43,5] These latter sugars are known as inverted sugars.[43,5] The biochemical equation

for the process is as follows:[43,5]

$C_{12}H_{22}O_{11}$ + H_2O or invertase or acids(HCl) (digestive enzymes)

Sucrose

(disaccharide)
(beet sugar)

↓

$C_6H_{12}O_6$ + $C_6H_{12}O_6$

d- Glucose (dextrose) — d- Fructose (levulose)

(monosaccharides)

The degradation of carbohydrates to lactic acid is thought to be an etiological factor in the process of dental caries.[2,22, 21] The formula for the formation of lactic acid from the glucose molecule is as follows:

$C_6H_{12}O_6$ + enzyme(Bacillus acidophilus)

Glucose

↓

$2\,C_3H_6O_3$

lactic acid[43]

Hockett, in his article on natural and refined sugars states that human saliva is able to convert starch to glucose, but the saliva of rats and dogs does not affect starch. This would support Bunting's statement[19] that different carbohydrates play different roles in the production of dental caries.

Stegerradas study of the Navajo and Mayo Indians showed a marked difference in the amount of tooth decay from the decay in

our present civilization.[41] His study suggested that there may be racial and hereditary factors in tooth decay. Bunting also makes the statement that heredity or individual characteristics may determine caries susceptibility rather than dietary considerations alone.[19]

Thus the four current types of theories concerning tooth decay are:[10,29,30]

1) Chemico-parasitic
   a. Acid production by bacteria.
2) Specific-bacterial
   a. Bacterial degradation of the tooth structure.
3) Metabolic
   a. Dietary and vitamin causes.
4) Heredity
   a. Influence of genes on tooth structure or physiological processes.

It would appear that acid production and an oral environment favorable to acid producing bacteria are important in the causation of dental caries if acid is an etiological factor in that process. We now return to the problem of what causes some teeth to be resistant to sugar diets and others to be highly susceptible?. What factors are involved in the susceptibility and resistance to tooth decay?

The Hunt and Hoppert Experiment

Hoppert, Webber and Canniff in 1932 found that they could

induce caries in laboratary rats which were fed an adequate diet.[24,25] They showed that vitamins, calcium and phosphorus did not appreciably retard the decay of the teeth when added to the caries producing ration. This diet contained cornmeal as the major constituent. When oatmeal was added in place of the cornmeal no caries appeared. The finer the cornmeal was ground the lesser the incidence of caries. In this paper, retention of food by the teeth was thought to be a contributing factor to caries.[25]

Hunt and Hoppert conducted their experiment to determine whether there is an inheritance factor in tooth decay, and if so, how many genes are involved and how do they produce their effects? The diet devised by Hoppert, Webber and Canniff produced caries in the rats and maintained their health, growth and fertility. The composition of the diet was the following:

Coarsely ground hulled rice*	66 %
Whole milk powder	30 %
Alfalfa meal	3%
Sodium chloride	1%
	100 %

* Seventy percent of the rice was retained on a 20 mesh screen when tested for fineness.[30]

Hunt and Hoppert with this diet in 1937 started their inheritance experiment. Phenotypic selection, progeny testing and brother and sister inbreeding have been used to build a caries resistant and caries susceptible strain. Seventeen generations of resistants and twenty-four generations of the susceptibles have been bred and examined.

With this type of mating system the proportion of heterozygosity in the stock steadily decreases. Li has shown that the proportion of heterozygosity in animals practicing brother and sister matings after n generations to be reduced systematically.[7] The generations of animals which I worked with would have the following percentages of heterozygosity:

Resistants $H_{17} = 1.961\%$ Susceptibles $H_{21} = .824\%$

This shows a very small amount of heterozygosity left in the stock at the end of 17 and 21 generations of brother and sister matings.

During the inbreeding experiment the caries time has fluctuated for resistants and susceptibles, but a significant difference is apparent between the two strains.[30] The average caries times for the susceptibles and resistants have been as follows:[29,30] (16th and 22nd generations-Unpublished data)

Gen.	Susceptible Avg. C. T.	Gen.	Resistant Avg. C. T.
2	57 days	2	116 days
15	13 "	15	470 "
16*	43 "	16*	379 "
22	42 "		

*Fine rice added to the diet.

A caries susceptible and caries resistant strain of rats have been produced by selection, progeny testing and inbreeding.[30] Heredity, therefore is a factor in dental caries.

The coarseness of the rice was found to be a determining factor in producing dental caries.[25] Braunschneider,[16,17]

discovered that if the rice component of the ration is in the form of flour, caries in the rats of the susceptible strain is rare until an age of 100 to 150 days.

Braunschneider studied the influence of age on the incidence of dental caries.[16,17] Caries could be delayed in susceptibles until 150 days of age by using the diet which contained rice flour.[17] The composition of the diet was the same as that described on page 8, except the rice flour was substituted for coarse rice. Two groups of animals were raised to 100 and 150 days of age caries free. These animals were then put on a caries producing diet which contained the coarse rice particles. These two groups showed more resistance to caries than the animals at the 35 day growth stage.[17]

Hunt and Hoppert found that sex is not important in influencing dental caries in rats.[32] These investigators studied the distribution of 10,048 gross carious cavities in the lower molars.[34] Some areas of the occlusal surfaces of the rat's lower molar teeth are more susceptible to caries than others.[34] The right lower molars develop a higher percentage of cavities than the left in susceptible and resistant animals.[34] Hunt and Hoppert proved that use is an important factor in developing cavities. It was found that when the opposing upper molar was removed from the mouth it lengthened greatly the caries time in the corresponding lower molar in susceptible rats.[33]

Nakfoor, Hunt and Hoppert studied the resistance to fracturing in resistant and susceptible teeth. The lower molars of resistant rats were found to be more resistant to

fracturing at 60 days of age than susceptible lower molars at the same age.[38] This may have been due to a slight weakening of the structure of the susceptible teeth by the carious processes.[38] About one-half of Nakfoor's susceptible rats unexpectedly produced caries on the rice flour diet. If natural fracturing is a prominent cause of dental caries, then the teeth of these rats should have a considerable amount of the fracturing. But, they did not. Hence natural breakage is probably not important in the carious process.

Keller has severed the parotid gland duct in susceptible and resistant animals and found that the parotid secretion is not of great importance in the carious process of the animals. (Unpublished data) He also found that the resistant strain is practically immune to caries in the upper molars, while there is a 42 percent incidence of caries in the upper molars of susceptibles.(Unpublished data)

R. L. Clise has studied the growth curves of resistant and susceptible rats and found that curves for the two lines are substantially the same until 147 days of age.(Unpublished data) Beyond the 147 days the susceptibles show a slower growth rate than the resistants. He also found that the susceptibles have significantly less hair than the resistants.(Unpublished data)

A. E. Epstein has made a study on the effect of sugar-acid drinks on the erosion of susceptible and resistant teeth. Susceptible animals appear to show more erosion than resistants on this drink.(Unpublished data) He has also delayed caries in susceptible animals until 170-210 days by using a powdered milk diet of the following composition:

Powdered milk--------80 %

Alfalfa meal---------19 %

NaCl----------------- 1 %

100%

THE PROBLEM

Hunt and Hoppert's caries resistant and caries susceptible strains of rats ate a ground rice, powdered milk and alfalfa meal diet. Would this hereditary difference be apparent if other diets are consumed?

Sognnaes has found that a highly concentrated sugar diet would produce a high degree of caries if fed before the teeth erupted.[43,40] With this information it was decided to test the susceptible and resistant strains of rats on a sugar diet similar to the one Sognnaes used.[40,43] Would the resistant animals still be resistant to caries with a high concentration of sucrose, and different from the susceptible rats? In other words, are the hereditary differences discovered by Hunt and Hoppert specific for one diet only?

Procedure

Breeder animals were obtained from Dr. Hunt's 17th and 21st generation resistant and susceptible rats, respectively, in October, 1950. Breeders were selected for their general health, and the matings were between animals of as nearly the same caries time as possible. The same environmental controls which have been maintained for 15 years during the Hunt and Hoppert experiment were used.

The temperature of the animal laboratory was held between 77° and 80° Fahrenheit. The water was furnished from the college deep well system. Water was supplied to the experimental animals by drip bottles and these were kept clean by periodic cleaning. Metal cages were used which measured 2 1/2 x 1 1/2 x 1 feet. Five animals or less were kept in a cage. Pine shavings provided litter for the animals. The cages were cleaned about every 10 days. Kerosene was used as an insecticide under the shavings. DDT was added in small amounts to the kerosene.

Breeder females were isolated when they appeared pregnant. The females were observed each day in order that the exact date of birth of the litter could be recorded. When the litters were 25 days old the females were removed and the young animals were put on the experimental diet. The breeder females were put in a rest cage for seven days and then mated again. All males and females in the litters were separated before they matured, usually about 40 days. The animals were all numbered by the regular toe and ear marking system used by Hunt on all of his animals. A description of the experimental diets follows:

The Beet_Sucrose Diet

In order to determine whether the difference in heredity in the Hunt-Hoppert lines is specific for only one diet the following ration was used:

Granulated beet sugar(Michigan beet)	57 %
"Fine" rice	10
Casein	18
Corn oil(Mazola)	2
Brewer's yeast	5
Alfalfa meal	5
Salt mixture	3
	100 %

Description of the Salt Mixture[26]

$CaCO_3$	544.08 gm.
$MgCO_3$	25.00
$MgSO_4$	16.00
NaCl	69.00
KCl	112.00
KH_2PO_4	212.00
$FePO_4 \cdot 4H_2O$	20.50
$MnSO_4$	.35
$Al_2(SO_4)_3 \cdot K_2SO_4$	.17
$CuSO_4$	.90
	1000.00 gm.

Granulated beet sugar is a cuboidal crystal, which dissolves easily and passes through a screen with 20 meshes to the inch. The fine rice in this diet has a 2 percent retention on a 20 mesh screen. Examinations of this diet on a 20 mesh screen showed only oil adhesions and brewers yeast as the major portion of the residue. Some fine rice

and KCl particles from the salt mixture were retained on this screen.

The rats on this sucrose diet liked the food and grew normally in all visible respects. A good coat of fur developed in both the resistant and susceptible animals on the sucrose containing diet. There was, however, a susceptibility to respiratory discharges in the older resistants. This type of discharge was not noticed in the susceptibles as they were usually killed when comparatively young. The blood was of a dark purple color and anemia was improbable. The internal organs appeared healthy on autopsy in both strains.

The control diet used was the same as on page 8 except fine rice is substituted for coarse rice. This diet was known to ~~produce~~ differentiate a caries resistant ~~and~~ from a caries susceptible rat.

During the experiment it was found that the beet sugar containing diet seemed to lengthen the caries time as compared with the effects of the fine rice ration in the susceptible strain. The question then arose as to whether this might be due to a decrease in the percentage of fine rice particles or the presence of sugar crystals. In order to test the effect of a diet having no fine rice particles or sugar crystals the following ration was fed to the susceptible animals:

The Powdered Sugar Diet

Powdered sugar(commercial)----------	67 %
Casein----------	18
Corn oil----------	2

Brewers yeast	5 %
Alfalfa meal	5
Salt mixture	3
	100 %

The salt mixture in this is the same as the mixture used in the beet sugar diet. The powdered sugar contains about 3 per cent starch in order to keep the sugar in a powdery consistency.

This diet was extremely fine. Examination of the coarse particles in this diet based on 75 gram samples showed a 15 percent retention on a 20 mesh screen. The residue was found to be mostly oil adhesions and flakes of brewers yeast. However, there were some hard particles of KCl from the salt mixture. The KCl was ground in a mortar before it was added to the salt mixture; therefore these particles would constitute only a small percentage of the diet. The powdered sugar diet was fed only to the susceptible rats. They grew slower at first than the susceptibles on the beet sugar diet. No comparative weighings were made.

Another observation was that the young susceptible rats did not grow hair in the normal pattern on the powdered sucrose diet. The hair appeared blotchy and thin toward the tail. After this condition cleared the animals grew and had a good coat of fur and appeared normal.

Method of Mixing the Sugar Diets

After measuring the ingredients on an appropriate balance, the diet was thoroughly rubbed together and mixed

in a galvanized iron can. The oil adhesions were thoroughly rubbed into the mixture. The diets were dry and had no tendency to cake unless water came into contact with the mixtures.

Method of Determining Caries

Dr. Hoppert made periodic 14 day inspections of the lower molars of all the susceptible rats. In a few cases the 14 day period was extended. Samples of the resistants were observed at the same intervals until decay was observed, then the resistants were all inspected from that date at fortnightly periods. A nasal speculum was used to pry open the mouths and observe the teeth. The light was furnished by a 100 watt bulb about three feet away. Since Dr. Hoppert observes the teeth for the Hunt and Hoppert experiment, his observations compare with those he made on the Hunt-Hoppert rats. The main point is that one man observes the teeth, and the determination of cavities is constant.

Each animal had a page in a record book. A rubber stamped picture of the lower molars was entered in the book. Observations of the lower molars were entered as no caries, or if caries was present a (/) sign with the date of occurance was entered at the spot on the tooth where the caries was seen. If there was an impaction so that we could not make a positive identification, or if the cavity was at all doubtful a (?) was entered by that tooth with the date and the observations were continued. When a (/) was entered the animal was killed and the head put into 95% alcohol.

The heads were dissected with care so as not to injure the upper and lower molar teeth. These teeth were then observed with a binocular microscope of 20 power magnification and the observations were entered in a table. The caries time in this experiment was calculated as the time the animal was put on the experimental diet to the date of the first (✓) observation.

One or two controls from most litters were put on the fine rice ration used by Hunt and Hoppert. However, Hunt and Hopperts' average for the same generation could have been used for comparison.

A few animals were not treated in accordance with the above experimental procedure. Two resistant litters were put on the experimental sucrose diet at 36 and 32 days of age instead of the prescribed 25 day limit. Magnesium sulfate was substituted for magnesium carbonate in one mixture of 16 kilograms of the beet sugar feed.

DATA AND OBSERVATIONS

Five groups of animals are present in this experiment. They will be designated by the following subscripts:

R_r--- Resistant animals on the fine-rice containing diet.

R_s--- Resistant animals on the beet sugar containing diet.

S_r--- Susceptible animals on the fine-rice diet.

S_s--- Susceptible animals on the beet sugar diet.

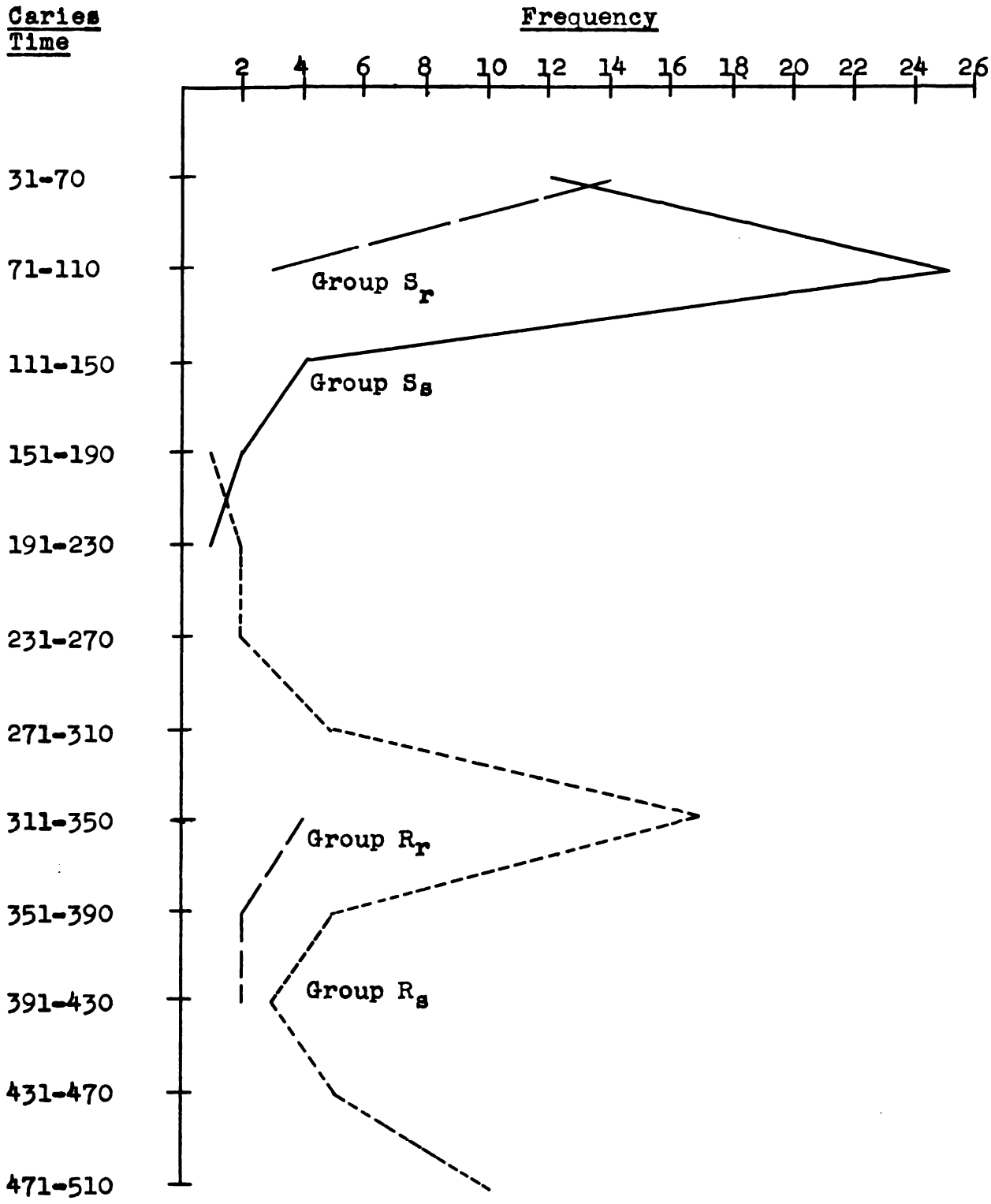
S_x--- Susceptible animals on the powdered sugar containing diet.

The graph(figure 1) of the resistant and susceptible animals on the beet sucrose diet shows the distribution of the two strains on the same diet. The mode for the susceptibles is at the 71-110 day caries time interval, while the mode for the resistants is at the 311-350 day interval. The difference between the two strains on the fine rice ration is also apparent. It is clear that the hereditary difference revealed by the rice containing ration is likewise revealed by the sucrose containg ration. Thus the contrast between susceptibles and resistants is not specific for the rice containing ration.

The data in this graph actually underestimate the difference between the resistant and susceptible strains of rats. It was decided to tabulate these data before all the resistant animals showed caries or died because I had to conform to a terminal date for submitting the thesis. Some of the resistant rats in Hunt's data have reached the age of 784 days without showing caries.[30] Moreover, resistants have died without caries. The high mode of resistants at the 471-510 day interval is occasioned by the animals we completed observations on before they developed caries. Had these animals lived they would have increased the variability of the resistant strain. None of the 8 control resistants on the fine rice diet showed any caries. These 8 rats represented 6 sibships and were a random sample from the resistant rats put on the beet sugar diet.

The average age in caries time of the 16 resistant animals which did not show caries and were included in these data

FIGURE 1, Comparison of Susceptible and Resistant Rats on the Sucrose and Fine Rice Diets



was 453.8 days. If the observation of these animals were continued they would certainly have raised the mean caries time of the resistant strain on the sucrose diet.

All the susceptible animals were observed to have caries. Seventeen susceptibles were randomly picked from each litter and put on the fine rice containing diet. There were 13 sibships represented in the susceptible control group(S_r). The graph, figure 1, seems to indicate that the sucrose diet increased the variability of both the susceptible and resistant rats.

Table 1 shows the means, variances, and standard deviations of the five groups of animals. The first statistical question that presents itself is whether or not the data and mean differences between the five groups; R_s, R_r, S_s, S_r and S_x are significant or are they explained in terms of chance. An analysis of variance test described by Snedecor gives a very good description of this test and its applications.[12]

Source of Variation	D. F.	Sum of Squares	Mean Square
Total	129	3123636.63	
Between Groups	4	2684440.30	671,110.07
Within Groups	125	439,196.33	3513.57

$$F_{4,125} = 671,110.07 / 3513.57 = 191\ **$$

This value of F lies far from the 1 percent probability level given in the tables of F in Snedecor.[12] F needed only to exceed 3.45 to be significant at the 1 percent level. Hence the group means are not different due to chance alone. The hereditary difference is very evident between the resistants

Table I

Groups

	S_s (1)	S_r (2)	S_x (3)	R_s (4)	R_r (5)
Sum of X	4059	991	723	18387	2977
N	44	17	11	50	8
$\bar{X}$	92.25	58.29	65.72	367.75	372.13
Sum of X^2	417405	60769	50435	7139199	1120573
C. T.*	374442.75	57769.47	47520.81	6761635.58	1107816.13
Variance	999.12	187.47	291.41	7705.37	1822.41
Standard Dev.	31.60	13.69	17.07	87.77	42.60
Sum of $(X - \bar{X})^2$	42962.25	2999.53	2914.19	377563.42	12756.87
No. of Litters	18	13 (sampled)	5	11	6 (sampled)
Avg. Size of Litter	3.8		2.8	5.5	

*12

and the susceptibles.

The next calculation is to determine whether there are significant differences between data for pairs of groups. Table II contains F and T tests between all the pairs of groups. F tests the significance of the variances, while T tests the signifiance of the means. Lindquist gives a good description of these tests.[8]

We are mainly interested in the differences between groups 1-4 and 2-5. F and T are highly significant in comparison of the susceptibles and resistants on the sugar containing diet. Groups 2-5 are also significantly different, but we knew this before. Again it is evident that the hereditary differences between the two lines are revealed by the sugar containing diet.

The numbers are small in the other groups which we compared. The meaning of the significance of these group comparisons can only be speculated on. However, one interesting fact emerges. This is the significant F and T test between groups 1-2. Two different diets were fed to a highly inbred strain of susceptible rats. The sugar containing diet increased the variability and mean caries time of these susceptible animals. The comparison between groups 1-2 show that the 57 percent sucrose diet in some way delayed the development of caries as compared with the fine rice containing ration. Both F and T tests confirm the significance of this difference.

Figure 2 shows the distributions of the susceptible animals on the sucrose, fine rice and powdered sucrose diets. The difference between groups S_s and S_r are clearly shown on this graph

Table II

Values of F and T Tests Between All Combinations of the Five Groups

Groups	F- test	T-test
1-2	5.32**	4.27*
1-3	3.42*	2.68*
1-4	7.71**	19.89*
1-5	1.82	6.91*
2-3	1.55	1.29
2-4	41.10**	14.48*
2-5	9.72**	28.04*
3-4	26.44**	11.35*
3-5	6.25**	21.74*
4-5	4.22*	.137

*significant between 1 and 5%

*(T) beyond 1% significance

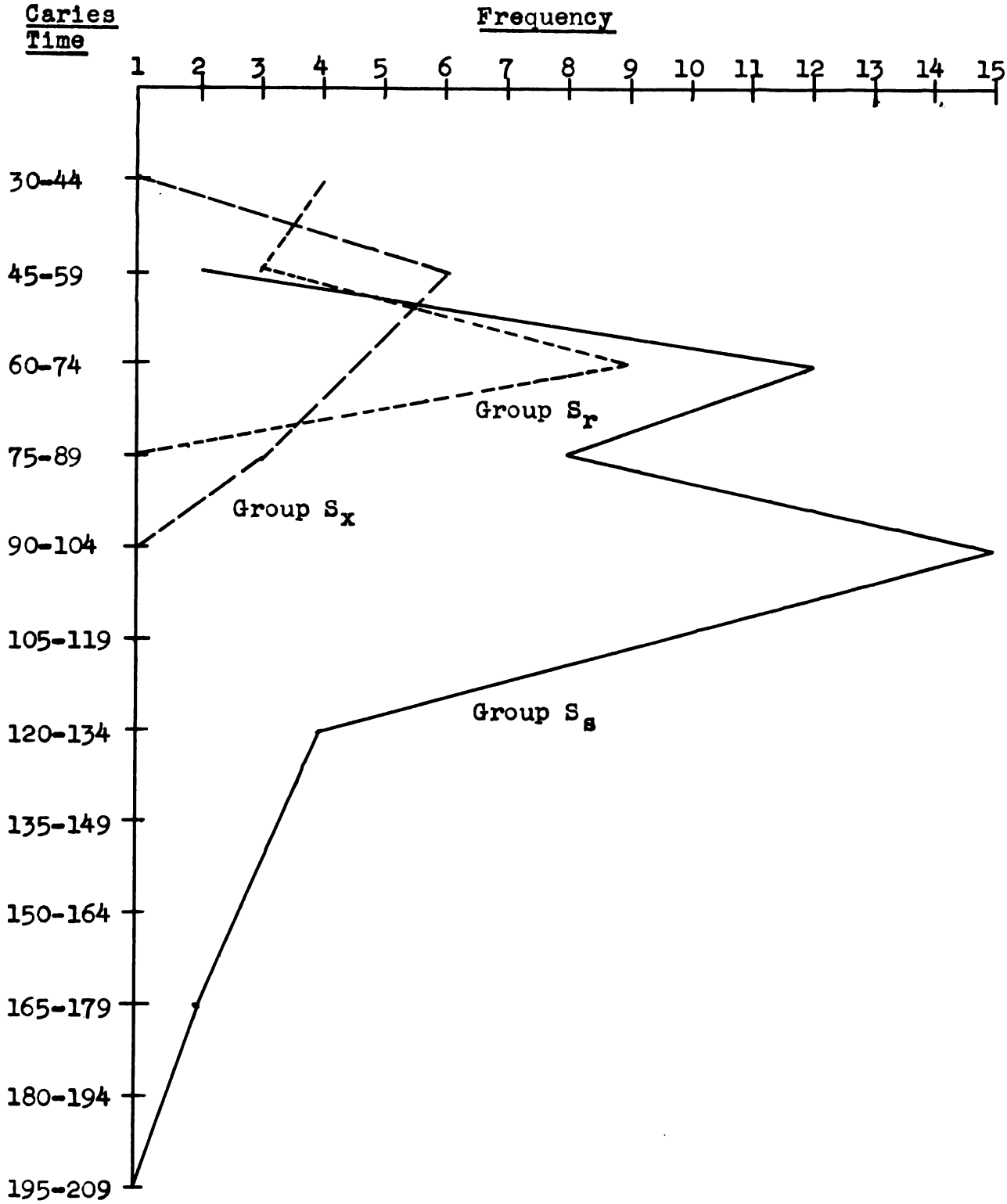
** beyond 1% significance

$$F = \frac{\text{Group with larger variance}}{\text{Group with smaller variance}}$$

$$d'^2 = \text{Sum of } (X - \bar{X})^2$$

$$T = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{d'^2_1 + d'^2_2}{n_1 + n_2 - 2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

FIGURE 2, Distribution of Susceptible Rats on Three Diets



with 14 day intervals. It would appear that the modal caries time is lengthened in the susceptible strain on the sucrose containing diet.

The two diets differed in content; both mineral, fine rice particles, sugar particles and carbohydrates. Which one of these constituents changed the carious effect in the susceptible animals? Did the bacterial or saliva threshold change? Of the 61 susceptibles raised, 17 were randomly picked from the litters and put on the fine rice ration. The two groups were similar in all respects except diet.

Comments on the Powdered Sucrose Containing Diet

The powdered sucrose diet was fed to the susceptibles in order to observe the effect of a diet with no rice particles or sugar crystals. The susceptible animals on the powdered sugar diet behave much the same as the animals on the fine rice ration.(figure 2) However, one susceptible rat has gone 164 days, without caries on the powdered sugar diet. The number of animals on the powdered sugar diet is small, and more research is indicated with this type of ration.

However, the susceptibles still developed caries on a diet consisting of powdered sugar, casein, corn oil, brewers yeast, alfalfa meal, and salt mixture. The number of coarse particles in this diet were less than .3 percent of the total weight.(no fine rice particles at all) These observations emphasize the point that dental caries can develop in the almost complete absence of coarse food particles of any kind. Possibly the bacterial content of the mouths was different for

the different diets; this subject deserves further investigation.

OBSERVATIONS OF THE TEETH

A study was made of the distribution of the cavities in the susceptible and resistant rats. The rats were usually killed when the first (✓) cavity was seen or on the next observation two weeks later. Figure 3 shows how the cavities were scored when decay was observed in each molar. Because of the size of the cavities in the susceptible teeth, only the tooth on which a cavity occurred was recorded. No attention was given to decay in the crevices. The tooth was considered as one area.

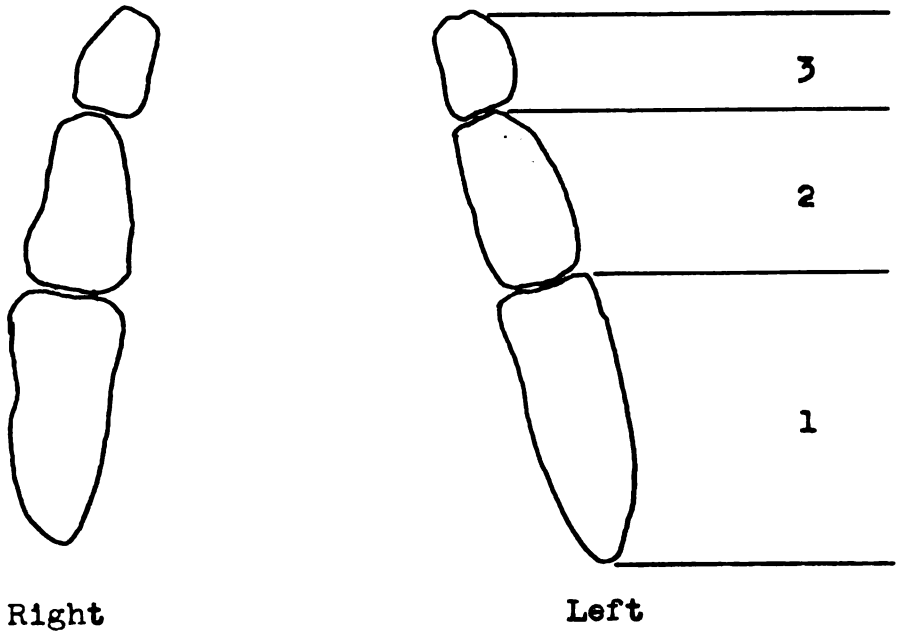
The following groups were investigated and the number of heads observed under the microscope are as follows:

Group	No. Observed
R_s	30
S_s	44
S_r	17
S_x	9

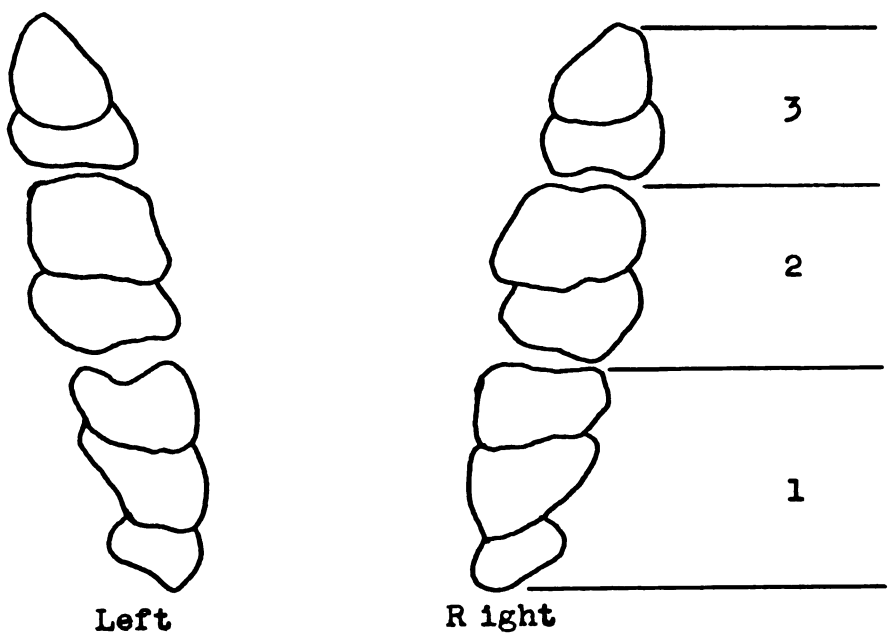
According to figure 3 the teeth were numbered individually as 1st, 2nd and 3rd molars. The teeth were classified in groups; right and left molars, upper and lower molars. When a carious cavity was noted in a tooth it was tabulated as a (✓). These (✓)'s were added together and the results compiled in the form shown in table III. The percentage of carious cavities in each location was calculated by dividing the number recorded for that location by the total number of cavities in that group.

Carious cavities usually have rounded centers and generally

FIGURE 3, Classification of Upper and Lower Molars



UPPER MOLARS



LOWER MOLARS

Table III

Distribution of Caries in the Upper and Lower Molars of the Susceptible and Resistant Groups

	Right			Left			Total
	1	2	3	1	2	3	
(S_s)			Lower Molars				
	44 15.4%	44 15.4%	13 4.56%	43 15.0%	44 15.4%	12 4.21%	
			Upper Molars				
	17 5.96%	14 4.91%	14 4.91%	11 3.85%	14 4.91%	15 5.26%	285
(S_r)			Lower Molars				
	17 14.5%	17 14.5%	15 12.8%	17 14.5%	17 14.5%	14 11.9%	
			Upper Molars				
	3 2.56%	5 4.27%	3 2.56%	3 2.56%	6 5.12%	0 0	117
(S_x)			Lower Molars				
	9 11.6%	9 11.6%	5 6.4%	9 11.6%	9 11.6%	6 7.7%	
			Upper Molars				
	3 3.8%	7 9.09%	7 9.09%	1 1.2%	4 5.19%	8 10.38%	77
(R_s)			Lower Molars				
	25 27.7%	21 23.3%	1 1.1%	19 21.1%	24 26.6%	0 0	
			Upper Molars				
	0 0	0 0	0 0	0 0	0 0	0 0	90
					Grand Total		569

extend into the dentine and even the pulp. The percentage of cavities in the upper molars of the susceptibles are somewhat misleading because table III does not take into consideration the extent of the carious process in each tooth. In some cases the whole lower molar was gone, or only an enamel shell was left. Comparatively speaking, the lower molars had larger cavities than the upper ones. Figures 4,5,6 and 7 are photographs showing cavities in the upper and lower molars of the four groups of rats on the experimental diets.

Sucrose appeared to lengthen the caries time in the susceptible rats; however, this diet produced rampant caries after the initial lesion began. The dentine seemed to disintegrate completely in some cases. I would say that the sucrose containing diet produced a more rampant caries than the fine rice ration.

The data presented in table III suggests that the 3rd lower molars in the susceptibles on sugar (S_s) is more resistant to caries (8.77%) than the 3rd lower molars on the rice ration (S_r), 24.7%. Also, the sucrose diet tends to increase the susceptibility of the upper molars to caries when compared with caries in the uppers of susceptible rats on the fine rice diet.

One unusual finding is that the upper molars of the resistant rats did not show any carious cavities at all on the sucrose diet. However, the susceptible upper molars on the same diet had an incidence of approximately 30% of the total carious sites: The upper molars had impacted food material in their crevices which was very solid. This impaction affords a good place for the bacterial decomposition

of food with resultant acid production. What can be said from the observations tabulated here is that the reistant uppers are more resistant than the susceptible uppers to caries on a high concentrated sucrose diet.

Nine observations were made on the susceptible molars on the powdered sucrose containing diet. These animals were not killed on the (⁄) observation. We thought we might need breeders and they were kept longer on the powdered sugar diet. The high incidence of caries in the upper molars was probably due to the extended time the rats were on the diet. The susceptible teeth on the powdered sucrose diet showed more extensive decay in the lower molars than on the other diets. In some cases only the outermost shell of the enamel was left. Figure 7 shows the decay in the susceptible teeth on the powdered sugar diet.

The 1st and 2nd lower molars still show the highest incidence of caries. This is similar to the findings of Hunt and Hoppert.[34] Inspection of the percentages in table III shows this trend. The number of cavities on the right side appear to be greater than on the left side.

Group	Right Molars	Left Molars
S_s	35.36%	34.61%
S_r	41.8%	40.9%
R_s	52.1%	47.7%

Due to the small numbers of observations too much significance can not be attached to these differences. However, the excess of cavities on the right side does persist.

The caries I observed was usually in the advanced stage and the exact nature of the initial carious lesion is still

unknown. My observations of the heads did not support the fracture hypothesis of caries origin. The smaller cavities were seen usually at the bottom of the sulci in the lower and upper molars. The large carious cavities were all centered around the deep sulci. The early cavities would not originate at the bottom of the crevices if the caries were initiated by fracturing.

Observations of the susceptible teeth showed that there was more impaction in the lower molars on the rice ration, than on the sugar diet. This may have been due to the ability of the rice to pack into the crevices easier than the granulated sucrose, which dissolves quickly.

Uppers may be less susceptible to caries because the crevices are not as wide as those of the lower molars. In picking food particles from the crevices of both lowers and uppers this was a general observation.

Some teeth appeared to be resistant to caries, next to teeth with rampant caries. Regardless of whether the rat was a susceptible or a resistant; pinkish teeth seemed to be associated with resistance. If a tooth was white or cream colored, caries was usually present. A white radiating color change was noted in some small cavities on the enamel of the tooth around the lesion. Gottlieb, in his book on dental caries,[6] goes into detail on color changes.

There was a brownish deposit on the molars. This was especially noticeable in resistant uppers. It flaked off like a dried membrane when the teeth were dry. Further research is indicated in determining what effect color changes and deposits on the teeth have on dental caries.

Some teeth were observed to have erosion under the gum, but no caries was observed. A decay process of the lower dentary bone around the tooth structure was observed in some heads. Figure 4 shows this type of carious process of the bone by a resistant rat's 3rd molar. (Figure 4- resistant male-14). The relationship of caries with this type of periodontoclasia is unknown. Further research should be cognizant of this type of pathological process.

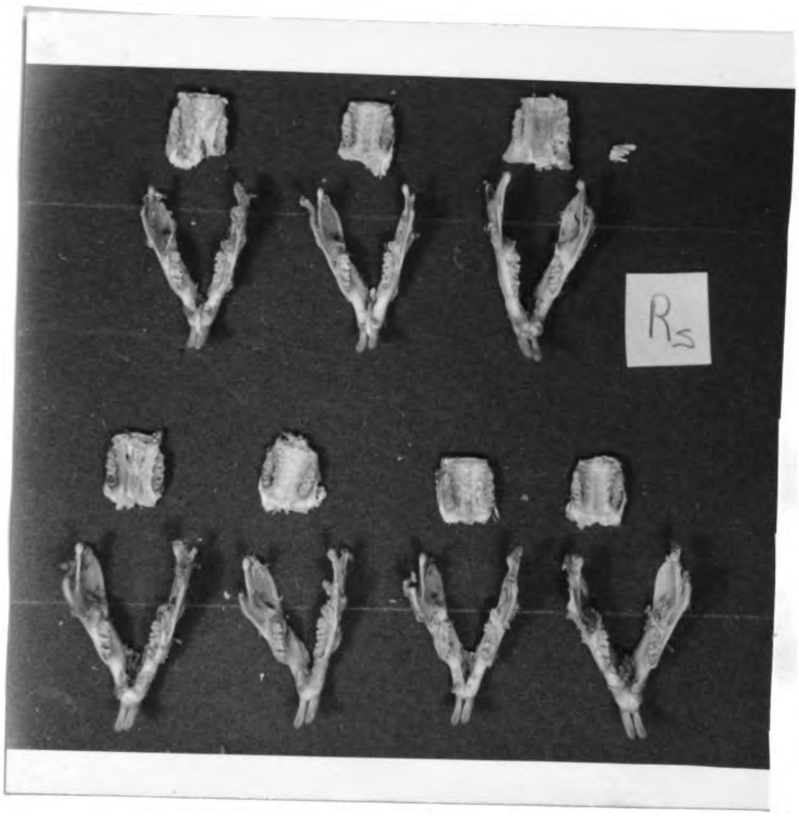
On the following pages, figures 4,5,6 and 7 show the carious cavities in the different groups of rats on the expermental diets. The caries time is given for each set of lower molars under the photograph.

DISCUSSION

Charts and graphs show the distribution in caries time of the susceptible and resistant rats on the granulated sucrose diet. Caries resistant and caries susceptible animals still differ on a diet containing 57 percent sucrose. This fact is shown by a graph (figure 1), analysis of variance test and significant F and T tests. The hereditary difference of the Hunt-Hoppert strain of rats is not specific for only the fine rice diet. A 57 percent sucrose diet still reveals the hereditary difference between the two strains.

A difference in the caries time has been observed in the susceptible animals on the sucrose and fine rice diets. The variances and means of groups S_s and S_r are different in terms of days to the first (/) observation of caries. The number of rats are small for any final conclusion.

FIGURE 4, Resistant Rats Upper and Lower Molars on the Sucrose Diet (Group R_s)



Top Row:

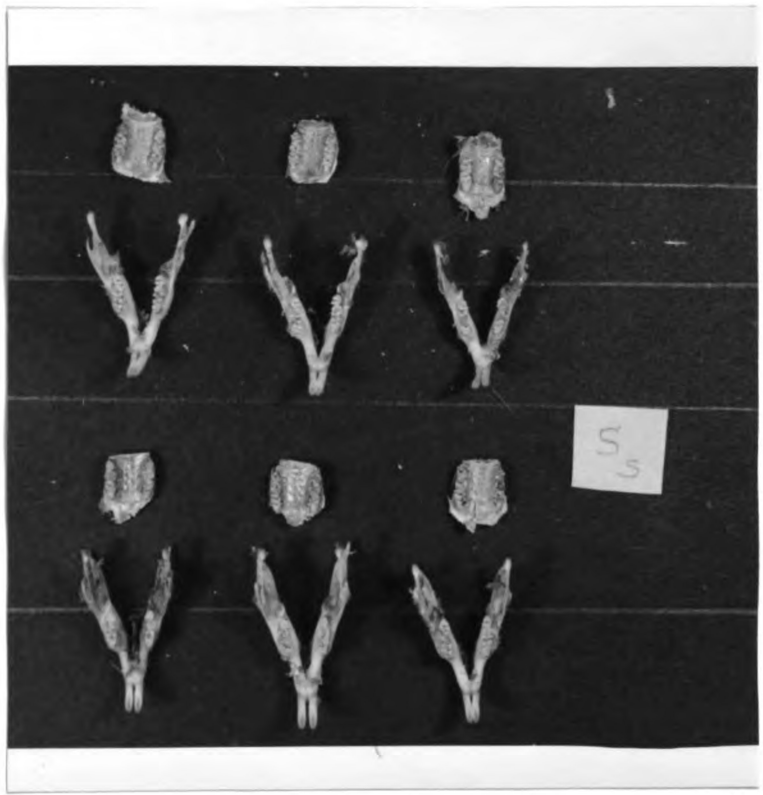
	F- 85	F- 82	M- 14 (bone caries)
Caries Time	213	177	428

Bottom Row:

	M- 58	F- 5	F- 21	M- 27
Caries Time	308	401	313	363

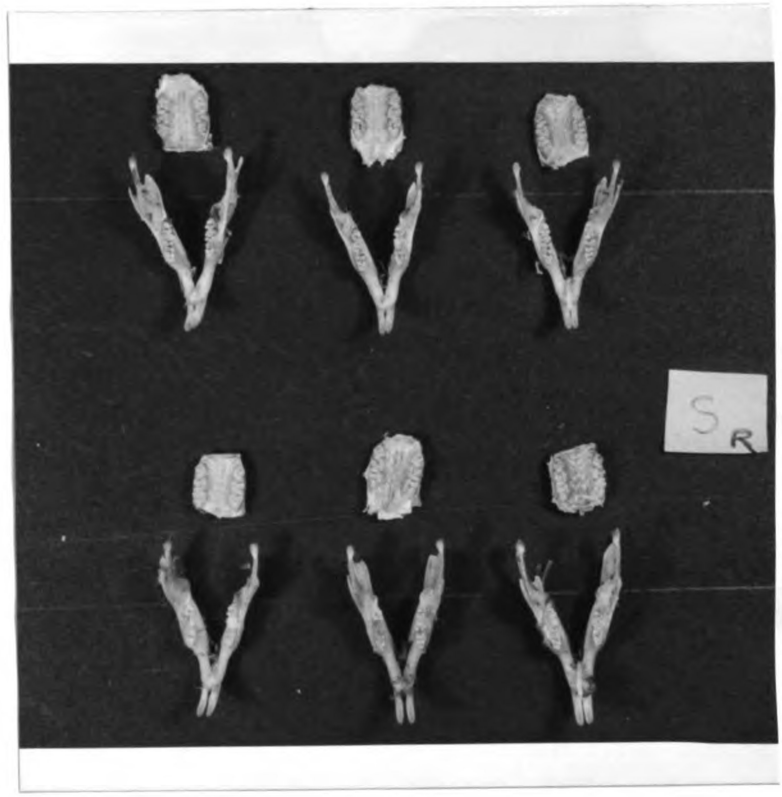
* M- male
F- female
A magnifying glass can be used to advantage in examining these molars.

FIGURE 5, Susceptible Rats Upper and Lower Molars on the Sucrose Diet (Group S_s)



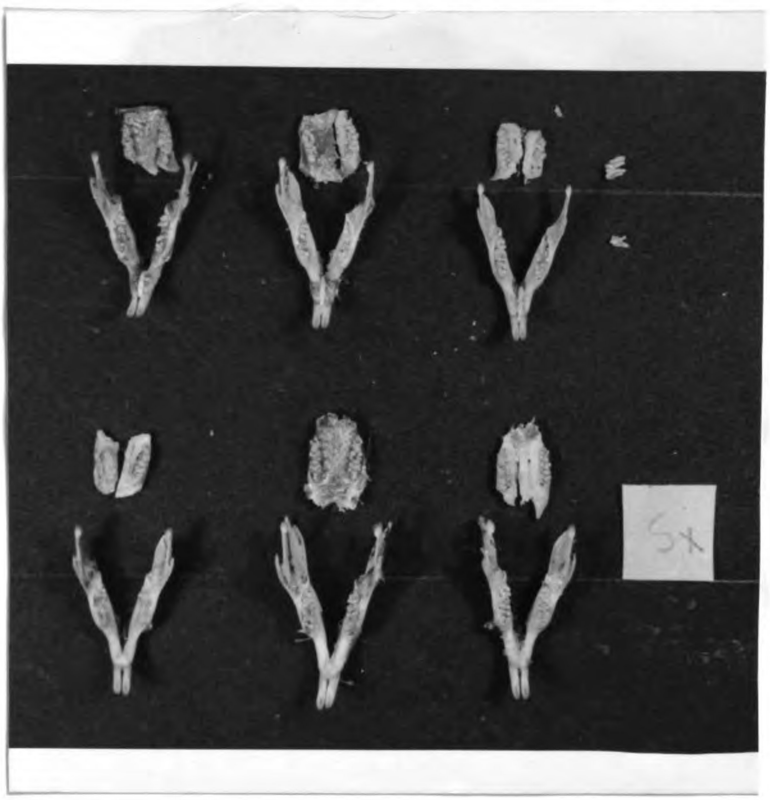
Top Row:	M- 96	M- 98	F- 71
Caries Time	69	55	49
Bottom Row:	F- 114	M- 105	F- 111
Caries Time	73	203	80

FIGURE 6, Susceptible Rats Upper and Lower Molars on the Fine Rice Diet (Group S_r)



Top Row:	M- 108 (upper molars reversed)	M- 103	M- 119
Caries Time	66	41	37
Bottom Row:	F- 42	M- 60	M- 79
Caries Time	64	63	84

FIGURE 7, Susceptible Rats Upper and Lower Molars on the Powdered Sucrose Diet (Group S_x)



Top Row:	F- 132	F- 123	F- 124
Caries Time	43	87	37 (57 days on diet)
Bottom Row:	F- 122	M-121	M- 131
Caries Time	78 (139 days on diet)	92	85 (85 days on diet)

However, what factors caused this fluctuation in a highly inbred strain of caries susceptible rats?

This difference may have been due to the impactibility of food, number of coarse particles or some quality of the carbohydrate degradation in the mouths of the susceptible rats. Examinations of the teeth in the susceptible rats on the fine rice and sucrose diets showed the following facts, (Groups S_s and S_r):

1. More food impaction in the lower molars on the rice diet.
2. The 3rd lower molar may be more resistant to caries on the sugar diet than on the fine rice diet.
3. The sucrose diet increased the susceptibility of the upper molars to caries in the susceptible rats.

A difference in the effect of these diets has been noted. Further research is indicated in analyzing this effect.

Analysis of the granulated sucrose diet and changing the constituents in it would be valuable in determining what substance is important in the causation of tooth decay. Would an increase in the sucrose level over 57% increase the susceptibility to caries? Would a decrease lower the incidence?

Resistant rats have reached over 500 days of age without tooth decay and may die without showing any carious effect on the granulated sucrose diet. The granulated sucrose produced initially slower caries in the susceptible animals, but produced rampant caries after the initial lesion. All the susceptibles showed caries at an average of 92.25 days on the granulated sucrose diet.

Again the hereditary difference is apparent between the caries resistant and caries susceptible strain of rats on the granulated sucrose diet.

A very fine powdered sucrose diet has produced caries in susceptible rats at an early age. Fracturing by coarse particles does not appear to be an initiator of caries. Examination of small cavities deep in the sulci supports this statement. No resistant rats were fed the powdered sucrose diet, research is needed in testing its effect on these animals.

The variabilities of the different groups of rats on the different diets shows that the caries process is not a simple cause and effect relationship. There are many interacting factors present in the process. Experimental error may also be a cause of variability, however, the animals were controlled in all experimental procedures.

Upper molars have been shown to be more resistant to decay than the lowers. No decay was observed in resistant upper molars on the granulated sucrose diet. Caries was observed in the susceptible uppers on this same diet. Uppers in the resistant rats are more resistant to caries than uppers in susceptible rats.

Pinkish teeth seem to show more resistance to caries than white ones. The cause of the initial carious lesion can only be speculated on. Is the decay process due to the basic nature of the tooth as shown by the color difference or is the action of bacteria on the enamel through the decomposition of the food the primary cause? More experimentation is suggested to determine this cause and effect relationship.

A finer date for the determination of the initial carious

lesion would be productive when working with diets producing rampant caries. Only gross carious lesions are seen. An early investigation in-vivo of the carious process in the lower molars may show some pertinent facts concerning the initial carious lesion. However, the observations as carried out in this experiment showed the hereditary difference in the caries resistant and caries susceptible rats very well on the granulated sucrose diet.

CONCLUSIONS

1. The hereditary difference is still apparent for the caries resistant and caries susceptible strain of rats when fed a diet containing a high percentage of granulated sucrose. The resistance and susceptibility is not specific for only the fine rice containing diet.
2. The granulated sucrose diet increased the variability of the incidence of caries in the susceptible animals, when compared to the control, fine rice diet.
3. A very fine powdered sucrose diet produces early caries in the susceptible animals. This conflicts with other data on the effect of fine diets on the incidence of caries.
4. Some areas of the resistant and susceptible lower and upper molars are more resistant to caries than other areas on the sucrose diet. Namely, these are the 3rd lower molars, and the upper molars generally.

5. Resistant upper molars are more resistant to dental caries than susceptible uppers on the granulated sucrose diet.
6. Sucrose diets produce rampant caries.

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