RICE FLOUR IN THE DIET, AND AGE AS FACTORS IN THE INCIDENCE OF DENTAL CARIES IN GENETICALLY SUSCEPTIBLE ALBINO RATS (RATTUS NORVEGICUS)

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by

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THESIS

DEDICATION

To my wife, my mother, and my father, who by great personal sacrifice, have made my education possible.

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INTRODUCTION

Dental caries has been concisely defined by Thos. J. Hill[5] as "... a disease causing the disintegration and loss of tooth structure and ... characterized by the formation of acids on tooth surfaces as the result of degradation of carbohydrates by bacterial enzymic action." Although caries is one of the most common afflictions of mankind, its etiology and prevention are only imperfectly understood at present. In the words of Wm. J. Gies,[4] "Many careful researches and numerous competent clinical observations ... have been made ... yet the results have been discordant, the conclusions contradictory, and the outcome confusing." According to Hill[30], "Dental caries has been considered an entity when perhaps it is a syndromic expression of various systemic influences ...."

Tooth decay is a peculiar disease in that the body tissue attacked is non-cellular, in that there is no demonstrable defensive cellular reaction to tissue injury, and in that there is little evidence of an attempt on the part of the body to repair damage to the teeth. For these reasons many investigators insist that caries must not be included in the term "disease". But if we accept a broad definition of the word, that is, a variation of any organ beyond what we consider to be the limits of normalcy, the term is applicable.

The literature of dental caries is far too extensive to permit a review here. Numerous publications exist in almost every language throughout the civilized world. However, by citing occasional contributions from several sources, a generalized

concept of the present stage of man's knowledge of this disease is indicated.

No attempt has been made to cite all references dealing with any one topic. We have referred to the earliest papers on most topics, but because of the tremendous amount of literature, it is possible that some important papers have been overlooked.

Dental caries is known to occur among virtually all the peoples of the world today, but the percentage of persons affected varies considerably from one ethnic group to another. The incidence of caries is high among most of the civilized inhabitants of the world. It is usually less frequently encountered among those whose civilization is comparatively primitive.[19]

In 1938, Russell W. Bunting[65] stated that 85 to 95 percent of public school children in this country were susceptible to tooth decay, and only 5 percent of individuals are caries-free throughout life.

Caries is known to have existed in Europe in Neolithic times, but with a lesser frequency than among present day Europeans, according to Herbert Greth[4] (as translated by R.Kronfeld). H. F. Curtis[4] reports that skulls and mandibles of persons who lived in Egypt about 3400 BC show no caries. Caries was significantly more common among the Egyptians of 1800 years later, and has steadily increased since 1000 A.D. H. Jobe Sedwick[60] reports finding dental caries in 43 of 48 precolumbian Indian skulls from what is now New York State.

The etiology of caries has been both a tantalizing and important problem to man for many centuries. Prior to the latter part of the 19th Century, there were many and varied theories

of its etiology. Two authors [2, 27] remark that in 456 B.C., Hippocrates ascribed this disease to "'the stagnation of depraved juices' in the teeth". Bunting[2] states that Galen, in 131 A.D., believed the process to be similar to inflammation. During the reign of the Roman Emperor Claudius, Scribonium Largus suggested that caries might be initiated by small worms which burrowed into the teeth. There were European adherents to this concept as late as 1824.

As man's knowledge of electricity increased during the 19th Century, there arose two novel hypotheses[2] of the causation of tooth decay. Both have long since been discarded for lack of supporting evidence. In 1861, Bridgeman published a statement that the crowns of teeth were electro-positive, the roots electro-negative, and the mouth secretions electrolytes. Electrolysis supposedly produced acids which in turn decalcified the enamel and dentin. Chase believed in 1880 that when acid saliva bathes a metallic filling, an electric current is produced, and the tooth disintegrates.

In 1883, Willoughby D. Miller, an American student of Koch, the celebrated European bacteriologist, demonstrated that microorganisms are the active factors in the decalcification of enamel. His etiological theory[6,52,53,54,55,56] gained widespread acceptance, and has, with minor restatements and additions by other men, withstood the test of time. Today it is almost universally accepted. Miller demonstrated that some form of acid caused an initial decalcification of the tooth. These acids, he deduced, were made from starch or sugar by the action of a self-reproducing, carbolic acid-sensitive

agent which was contained in the saliva. Bacterial organisms possess these qualities. He described the second stage of dental caries as a digestion of the organic materials of the tooth by organisms capable of liquifying albuminous substances in an acid medium. Miller observed twenty-two kinds of acidogenic and proteolytic oral bacteria, some of which possessed both abilities. He did not believe that any one organism was the specific cause of decay.

Miller's claims have not gone uncontested; several hypotheses somewhat at variance with his have been advanced. For example, Bunting[2] has described the hypothesis of R. Eckerman, of Sweden, who stated in 1910 that Miller's theory held only for the latter stages of caries. He maintained that the tooth acted as an osmotic membrane between the blood (in the pulp cavity) and the saliva. The tooth supposedly deteriorates by an osmotic-pathologic process which proceeds excentrically from the pulp. This is followed by a concentric process similar to that described by Miller. This hypothesis and its subsequent modifications have never been generally accepted by investigators because of the lack of evidence to support them.

Since Miller's fundamental investigations, dental research has progressed considerably. McIntosh, James, and Lazarus-Barlow[48,49] of Great Britain, and Rodriguez[57], a dentist in the United States Army, narrowed the field of study to those organisms which were capable of growing on an acid medium and capable of producing a high concentration of acid. In both instances, it was reported that in cultures

taken from initial dental lesions, Lactobacillus acidophilus was present, and was the predominating organism capable of withstanding a low pH.

C. P. Canby and L. J. Bernier[21] found L. acidophilus in over 90 percent of the superficial dentin cavities which they studied. The (University of) Michigan Dental Caries Research Group [26,65] found a high positive correlation between the quantitative counts of salivary L. acidophilus and the degree of activity of caries. There was also a qualitative difference in strains of the organism from caries-susceptible and caries-resistant individuals. Caries-free persons showed a diminished frequency of localized organisms on the tooth surfaces. Bunting, one of the members of this group[2,65] was also able to induce caries in vivo by placing a culture of the organism on bread (as a medium) under a clasp fastened to one of his own normal teeth. A lesion indistinguishable from a primary carious lesion appeared at the site of the clasp. Likewise, the Michigan Group[18] was able to induce carious destruction in vitro by exposing only a portion of a tooth to salivary bacterial action.

Dental research workers generally agree that the chemical source of acids produced by oral bacteria is carbohydrate in the mouth, as was demonstrated by Miller. Bunting[2], and Hill[5] explain acid production from starches, which may be expressed empirically:

1. $2(C_6H_{10}O_5)_n + n\,H_2O \xrightarrow{\text{ptyalin}} n\,C_{12}H_{22}O_{11}$
(starch) (maltose)

2. $C_{12}H_{22}O_{11} + H_2O \xrightarrow{\text{bacterial enzymes}} 2\,C_6H_{12}O_6$
(maltose) (glucose)

3. $C_6H_{12}O_6 \xrightarrow{\text{bacterial enzymes}} 2\,C_3H_6O_3$
(glucose) (lactic acid)

Thus one would expect a high carbohydrate diet to have a deleterious effect on the teeth by providing a plentiful bacterial substrate. With a group of orphanage children on a low-sugar inadequate diet[42,65] the Michigan Group trebled the low caries rate by administering unrestricted amounts of candy. The saliva of 80 percent of the children showed an increased L. acidophilus count.

Hill and Steggerda[62] observed that Navajo Indians living on a high protein diet, and Maya Indians living on a high carbohydrate diet have similarly low caries rates. In the latter group, the percentage of Indians whose mouths are free from L. acidophilus is greater, and the average counts of the organism are lower than the comparable figures for white people in the United States. However, both tribes eat very little sugar.

Most investigators agree that the form and arrangement of teeth in the mouths of most people provide lodgement places for food particles. Salivary bacteria are thus furnished with suitable media for multiplication and acid production. The

acid produced is in contact with the enamel, and initial caries follows. Hill[5] says, "Any anatomical change in form which contributes to the accumulation of plaque material, contributes also to the occurrence of caries."

The work of C.A. Hoppert, P.A. Webber, and T.L. Canniff[32,33] of the Michigan State College Group, is important in this respect. It will be discussed in greater detail later in relation to the present experiments. Working with rats, they observed that coarsely ground cereal grain in an adequate diet was correlated with earlier caries production than was a more finely ground grain in the same diet. They believe that this phenomenon is somehow related to the manner of impaction of the cereal particles in the irregularities of the teeth.

Hill[30] writes, "It is true that there is a small group of people who remain free from dental caries regardless of where they live, what they eat, or what methods of oral hygiene they practice. There is another small group which has rampant caries in spite of all remedial measures to control it. In between these two groups there is a large group of people close to the borderline who may be thrown across that line by various methods - some by the medicinal treatment of the teeth, some by the maintenance of wholly adequate diets, some by the elimination of sugar from the diet, and perhaps some by better methods of oral hygiene. We must believe that each individual possesses a certain relative resistance or susceptibility to this disease".

One of the first approaches to the problem of resistance of the tooth to decay was a study of the structure of the teeth.

Miller[55] observed, "... that the enamel of the teeth of different persons, as well as of different teeth of the same person, and of different parts of the same teeth, does show difference in resistance to the action of acids." But the relationship between well-constructed teeth and resistance to decay is not clear cut. L.Schoenthal and R.H. Brodsky[58], and D.H. Shelling and G.M. Anderson[61], working with children, found caries resistance to be similar in both hypoplastic and normal teeth. P.E. Boyle and O.A. Bessey[14], studying guinea pig caries, observed no apparent relationship between carious lesions and hypoplasticity. On the other hand, the experiments of Mellanby[4], of London, seem to indicate that in experimental animals, tooth structure is important in carious decay. She states that while there is not much difference in susceptibility of grossly normal and grossly hypoplastic teeth, still when using her own definitions of hypoplasticity and normalcy, the incidence of caries is much greater in hypoplastic teeth. Kanthak[43] was unable to show any difference in solubility of enamel of different persons, regardless of caries-susceptibility.

The question of whether or not the tooth is subject to change in composition and/or structure during the adult life of the individual, is as yet unsolved. Probably the majority of investigators hold that once fully formed, the tooth maintains its <u>status quo</u> until destroyed or removed. However, there are others, notably C.F. Bödecker[13,64] who maintain that materials may pass between the pulp cavity and the mouth through the tooth. This question is important if one is to explain certain changes in caries susceptibility due to age.

A second approach to an understanding of decay-resistance has been made via nutritional studies. According to Wm. R. Davis[23], "Nowhere in the dental field has there been such a wide divergence of opinion as in the relation of diet and nutrition to dental caries." Deficiency of necessary dietary elements during the period of tooth formation, will, according to Howe[34] and others, have a deleterious influence on deciduous tooth composition and structure. Many workers consider the critical age period to be from an indefinite point in fetal life to the age of 20 years in the human being. However, the work of R. Kronfeld and I. Schour[47,59] seems to indicate that the outer portion of the enamel of the deciduous tooth is not formed until after birth. They narrow the critical period from birth to eight years of age - fourteen if one considers the third molars. The question of prenatal calcification of permanent teeth was investigated by Bunting[2], who studied the teeth through surgical and radiographic examination. He was able to find no instance where calcification of the permanent teeth had begun at birth.

Mellanby and Pattison[50,51] decided that calcification of the teeth of puppies was favorably influenced by the addition of vitamin D to the diet. They also showed that apparently vitamin D inhibited carious destruction of the teeth of children. Other authors have investigated the roles of other vitamins.

Bunting[19,65] and others have observed good caries resistance in human beings on a deficient diet. However, this resistance was maintained only when carbohydrate consumption

was kept low. (See the reports of the Michigan Group, and Steggerda and Hill, on p.6) Brodsky[17], and Schoenthal and Brodsky[58], were able to decrease the incidence of caries in a group of people by balancing their diets.

The saliva has also been studied extensively. L.S. Fosdick, H.L. Hansen, and C.Epple[25] made a preliminary observation that the rate of enamel decalcification by saliva in the presence of sugar after four hours of bacterial action is generally greater with saliva samples from caries-susceptibles than in those from caries-immunes. The rate of bacterial and yeast increase during an eighteen-hour period was usually somewhat greater in saliva samples from caries-susceptible persons. These data might be interpreted by Hill's interesting observation[29] that saliva seems to contain a factor which affects the growth, in vitro, of L. acidophilus. The intensity of this factor varies, and its presence or absence is consistent with the presence or absence of caries.

The Michigan Group[19,46] was able to demonstrate no relation between caries activity and (1) pH of the saliva, (2) content of calcium, phosphorus, total solids, ash, or chlorides, or (3) the carbon dioxide carrying-power of the saliva. Their findings in regard to pH are in agreement with those of Brodsky[16], and Fosdick, Hansen, and Epple[25]. Hanke[28] found that early-morning samples of saliva from caries-resistant persons were better buffered than samples from caries-susceptible persons collected under similar circumstances.

Investigation of the blood with regard to caries etiology has also been undertaken. The Michigan Group[40,41,65] reports

that L. acidophilus agglutinins can usually be demonstrated in the blood sera of caries-free persons. Some of the serological studies are also related to the endocrine system. Broderick[15], of England, holds that the amount of calcium in the blood and saliva is correlated with the absence of tooth decay. He was able to control carious destruction with some success through manipulation of the calcium level by administration of endocrine extracts.

THE EFFECT OF AGE

The question of change in caries-susceptibility as the individual increases in age has also received a measure of attention. Bunting[2] and Hill[5] state from their own experience and from that of their colleagues, that in human beings, caries is much more common before the age of about 18 years than after this time. During adult life, there are times when caries recurs for a period and then abates. Hill writes, "The abrupt changes in caries susceptibility which occur at certain ages are so definite that they doubtless arise from metabolic changes of the body as a whole, concurrent with the age of the individual, through alterations in the oral secretions which favor or hinder the process of caries. It is known that the rise and fall of aciduric organisms in individuals corresponds to the ages of caries susceptibility."

Mary Moore[67], studying a group of young people, reported that the middle and late years of adolescence were the time

when the greatest incidence of dental caries was observed. Broderick[15] attempted to establish a causal relationship between endocrine changes due to age, and caries incidence. Steggerda and Hill[62] mention a correlation between age and caries incidence. Karlström[4] compared the teeth of persons 10 to 19 years old with those of other persons 40 to 49 years old, and found that the specific gravity of the older group was increased slightly. However, Kanthak[43] could demonstrate no change in the solubility of enamel with increasing age.

Hollander[31], using Grenz-rays, detected a continued deposition of calcium salts in human teeth up to the age of 25 or 30 years. Bödecker[13,64] maintains that the permeability of enamel decreases progressively after adolescence.

THE EFFECT OF HEREDITY

The role of heredity in the resistance of the tooth to the processes of decay is only partially understood. Bunting[19], Steggerda and Hill[62], and others, mention that heredity has some role in caries-resistance. The exact manner in which the genes exert their effect is a matter of conjecture. They may partially determine the shape or microscopic structure of the teeth and their arrangement in the dental arch. They may control assimilation of nutritional substances, the composition of saliva, the presence or absence of Hill's activation factor (see p. 10), the presence or absence of antibacterial agglutinins (see p. 10), or the activity of the endocrine glands. It is even possible that the

genes exert an influence on all of these factors, in addition to other factors which are not known today.

Hurme[39], considering his examination of 108 dental students, remarks that most better-than-average dental family histories occurred among the men showing the best dental conditions. Klein[45], and Klein and Palmer[66], who compiled data on almost 4,500 school children of an eastern city, found, "... that siblings of susceptibles have somewhat over twice as much caries in both permanent and deciduous teeth as do siblings of the immunes." However, in interpreting the results of Hurme, and of Klein and Palmer, one must not overlook the similarities in diet and other environmental factors which are usually common to the members of any one family.

Apparently the first actual genetic experiment on susceptibility and resistance to dental decay is that begun in 1937 by H.R. Hunt and C.A. Hoppert at Michigan State College. This experiment is still in progress, but several preliminary reports have already been published.[35,36,37,38] They have succeeded in breeding two strains of rats from a common source, selecting one strain for caries susceptibility and the other for caries resistance. Breeders have been selected from each generation on the basis of the length of time necessary for caries to develop in their teeth when the environmental factors were kept constant. The only pronounced differences in caries etiology between these strains are those governed by the genes. The nature of these gene-governed factors is at present unknown, but the difference between the mean times required in

these two strains for cavity formation is far too great to be accounted for by chance.

Because this present investigation is based on the work of Hunt and Hoppert, a more thorough discussion of their studies is presented here. They have used the "Hoppert Diet", which consists of 66 percent by weight of ground polished rice, 30 percent whole milk powder, 3 percent alfalfa leaf meal, and 1 percent sodium chloride. By "polished" rice is meant that the hulls are removed before the rice is ground. Certain food factors thus lost are replaced through the medium of alfalfa leaf meal. According to present nutritional standards, this diet is wholly adequate.

It was mentioned earlier that Hoppert, Webber, and Canniff[32,33], observed that if the rice of an adequate diet were ground coarsely, caries production was much more rapid than if the rice were finely ground. Utilizing this phenomenon, two variations of the "Hoppert Diet" were compounded, the rice being ground to the degrees of fineness indicated in Table 1.

Hunt and Hoppert have followed the same procedure in managing both the susceptible and resistant strains. Food containing finely ground rice is placed in the dam's cage before the young are able to leave the nest, and the dam is kept on this diet until the young have been weaned. After weaning, the young are kept on "fine" feed until 35 days of age. They are then placed on the "coarse" rice ration, and are examined repeatedly until carious lesions develop in the

lower molars. The term, "caries time" is used to denote the age in days at which the animal develops caries, minus the age in days at which the animal is placed on "coarse" feed.

An example may clarify this description. When a dam is observed to be pregnant, she is placed alone in a cage and fed the experimental diet---"coarse" feed if her teeth have not yet shown decay, or "fine" feed if dental caries is present in her teeth. An animal born on January 1, would be left with the dam until January 22 (21 days). During the latter five to ten days of this period, the cage would be supplied only with the diet containing finely ground rice. After the young were removed from the dam's cage on January 22, they would be given "fine" feed. On February 5 (age 35 days), the young would be placed on the diet containing the coarsely ground rice, and be included in the group to be examined every two weeks. If one of these animals were observed to have a carious lesion on March 8 (age 66 days), the "caries time" of that individual would be: 66 days minus 35 days, or 31 days.

At the time when the author's investigation was under way, data were being collected on Hunt and Hoppert's 9th and 10th generations of the susceptible line, and 7th and 8th resistant generations.

Wm. G. Erwin[24], studied the mode of inheritance of susceptibility and resistance to caries in Hunt and Hoppert's rats. He concluded from the great variability in susceptibility among the F_2 animals, that several genes must be

Table 1. Comparison of Degrees of Division of Rice Particles in Two Variations of the Hoppert Diet.

	"Fine" feed	"Coarse" feed
Retained on 20-mesh screen	1%	70%
" " 40- " "	56%	20%
" " 60- " "	21%	10%
" " 80- " "	8%	
" " 100- " "	4%	
Passing through 100-mesh screen	10%	

involved, perhaps four or more. He likewise proved that the phenotype is a poor indicator of the genotype in breeding rats for caries resistance and susceptibility.

AIM OF THIS EXPERIMENT

Thus Hunt and Hoppert's animals were all placed on "coarse" feed at the same age, viz., 35 days. The question was proposed: if the teeth of the genetically susceptible animals could be kept intact to an age much greater than 35 days, and if the animals were then placed on "coarse" feed, would the caries time be changed? In other words, what is the effect of age on the rate of caries development in genetically susceptible animals?

METHODS AND MATERIALS

To answer this question, the following method was devised and used at the outset: Animals were obtained from Hunt and Hoppert's 8th generation of the susceptible line. In Dr. Hunt's opinion, based partially on Dr. Erwin's results, (see p. 15), these animals were probably homozygous for caries susceptibility. They were mated, some in brother-sister combinations, others in out-crossing combinations within the strain, and their offspring were used in making the observations of this study. Toward the end of the investigation, more animals were required, and new matings were made, only

some of which were sibling matings. However, if the animals were nearly homozygous for the genes in question, this probably made little difference in genetic susceptibility. In all, fourteen crosses were made, which produced 124 offspring. The numbers of each type of cross, and the numbers of individuals they produced are described in Table 2.

Eleven crosses involved siblings; three were outcrosses. Seven crosses were composed of members of Hunt and Hoppert's 8th generation. These animals produced 61 offspring, which are comparable to Hunt and Hoppert's 9th susceptible generation. Seven crosses were made from among the 61 offspring of the first crosses. The 63 individuals which they in turn produced might at first seem to be comparable to Hunt and Hoppert's 10th generation, but this is not the case, as there was no selection practiced in choosing the latter breeders. But the point is inconsequential because of the probable homozygosity of the susceptible line (with regard to the genes influencing caries susceptibility only). Considering both groups of matings together, 98 of the animals produced were from sibling matings; 26 were from other combinations.

The rats were kept in galvanized sheet iron cages, closed on all sides except the top and front, which were covered with 1/4 inch galvanized iron mesh. These cages are 12 inches high, 14 inches wide, and 20 inches long. One to five animals (usually four) were kept in each of these cages. Wood shavings were used as litter, and the cages were cleaned and supplied with fresh litter each seven to ten days. Drip bottles supplied

Table 2. The Numbers of Each Type of Cross and the Numbers of Offspring secured therefrom.

	No. of Type X Crosses	No. of Type Y Crosses	Total No. of Crosses	No. off-spring of Type X Crosses	No. off-spring of Type Y Crosses	Total No Offspring
Full-Sibling Crosses	5	6	11	50	48	98
Other Crosses	2	1	3	11	15	26
Totals	7	7	14	61	63	124

Type X: These mated animals were members of Hunt and Hoppert's 8th susceptible generation.

Type Y: These mated animals were offspring of the Type X crosses.

the animals constantly with water from the deep well of the college. Room temperature was maintained automatically at about 77 degrees, except in hot summer weather.

Dams were removed from the breeding cage and isolated as soon as it became evident that they were pregnant. All the young were saved, as is the practice in Hunt and Hoppert's experiment. Complete records were kept on matings, including dates of birth, dates of weaning and parentage of young. The young were left with the dam until between the ages of 21 and 25 days, although they usually wean themselves somewhat earlier than that age. Each animal was given a number, and was marked accordingly by means of a code system of notching of the ears. A record of the dates on which the various rations were given to an individual and of the caries examinations of that individual was kept in a large notebook. One page was devoted to each animal, and all of that individual's records were kept on that one page.

After the age of 35 days was attained, the lower molars of each animal were examined for caries development each 14 days. The examination was not made on the 35th day of life, and each 14 days thereafter, but rather, at the age of 35 days the animal was included in the group to be examined every two weeks. Thus, for example, an individual 34 days old on any given examination day would not receive his first examination until 14 days later, that is, at the age of 48 days. However, an animal 35 days old on an examination day, would receive his first examination that day, at the age of 35 days. The upper

molars were not examined, as Dr. Hoppert states that they are rarely affected by the "coarse" diet. If a negative observation was obtained, this fact was recorded, together with the date. If a carious lesion, a doubtfully carious lesion, a fracture, or any other notable fact was observed, a rubber-stamp sketch of the lower molars was added to the animal's record, and the size, nature, and location of the defect, and the date of its observation were recorded.

The rats were held ventral side up under a strong light by an assistant. The mouth was forced open and the tongue and cheek pushed away from the lower molars with a nasal speculum. Examination was made with the unaided eye. All observations on teeth were made by Dr. Hoppert, rather than by the author. This was deemed advisable because Hunt and Hoppert's experiment was used as a control, and Dr. Hoppert makes almost all of the examinations on that experiment. The personal error of observation should therefore be the same in both groups of rats.

When definite caries was identified in a mouth, no subsequent examinations were made, and the animal, if not to be used as a breeder, was destroyed. Caries was considered to have developed on the date of the first positive examination, unless this observation was preceeded by one or more "doubtful" observations at the same location. In such cases, the doubtful observation immediately preceeding the first positive observation was arbitrarily taken as the date on which the animal developed caries.

Many other dental caries investigations already cited have been carried out by using the Norway Rat. As pointed out by Erwin[24], this species has several advantages for work of this nature. It is small, and the cost of raising large numbers of the animals is comparatively low. The gestation period is relatively short - only 21 days - and several young are born in each litter. Under suitable conditions, the species will continue breeding without seasonal interruption. Although the molars of the rat are very different from those of the human, dental grooves are present in both. When maturity is reached, growth of the molars stops. They are subject to attrition, as are human teeth. A point of difference between the human and the rat species is that the rat does not have deciduous teeth. However, this does not interfere with its use in this type of study.

Some investigators[44], observing that the teeth of this animal show a great tendency to fracture, charge that fracture may be pre-requisite to caries development in the rat, but not in man. However, the work of Applebaum and Adams[9], who studied carious lesions of both species by means of Grenz-rays, indicates that this concept is unfounded. Decalcification, rather than fracture, is the initial lesion in both rats and man.

The young for the present experiment were arbitrarily divided into two groups. It was intended that "Group 1" would be left on "fine" feed until 100 days of age, and

would then be placed on the "coarse" feed. "Group 2" would be left on "fine" feed until 150 days of age, and would then likewise be placed on the "coarse" rice ration.

For these groups, caries time would be calculated as described on page 15, but the number of days to be subtracted from the age at which the animals develop caries is thus 100 or 150, rather than 35.

EARLY DATA AND MODIFICATION OF METHODS

An attempt was made to follow this method. The first eleven individuals were not examined until nearly 100 days of age as it was assumed that they would not develop caries on the "fine" rice ration before that age. However, the examination on that day showed caries to be extensively developed in all eleven animals. These eleven animals are hereinafter referred to as "Group 3-a".

Later, fifteen more animals were left on fine feed and examined every two weeks, beginning at the examination day following the day they were 35 days old. These animals comprise "Group 2-b". The results of the observations on "Group 3", viz., Groups 3-a and 3-b combined, are shown in Table 3. The results from Group 3 indicated that a revision in method was imperative, and the flour feed was devised.

Commercial rice flour was obtained from the Stein-Hall Company of Chicago, Illinois, and was used in place of the ground rice. This company has certified* that their rice flour is made only from polished rice, to which no substance

Table 3. The Age in Days At Development of Dental Caries in Individuals in Group 3.

Group 3-a	Age in days at development of dental caries
Male 10	Less than 99 days
Male 11	Less than 99 days
Male 12	Less than 99 days
Female 13	Less than 98 days
Female 14	Less than 99 days
Female 15	Less than 99 days
Female 17	Less than 99 days
Female 18	Less than 99 days
Male 19	Less than 99 days
Male 20	Less than 99 days
Male 21	Less than 99 days

Group 3-b	
Male 22	87 days
Male 23	73 days
Male 24	81 days
Female 25	59 days
Male 26	67 days
Male 27	67 days
Male 28	67 days
Female 29	73 days
Male 30	95 days
Male 31	158 days
Female 32	101 days
Male 33	145 days
Female 34	74 days
Female 35	88 days
Female 36	74 days

is added, as is common practice with wheat flours. No substance, other than the hulls, is removed. Like commercial wheat flours, rice flour is passed through silk bolting cloth before being marketed, to insure a fine degree of division of particles. A sample of this cloth was sent to the author through the kindness of the company. An examination of the cloth reveals that while the sizes of holes and threads vary considerably, the longest diameters of the holes are approximately 200 micra.

Thus the third variation of the "Hoppert Diet" is chemically the same as the first two, and the three variations differ from each other only in the degree of division of the rice particles.

The method of managing Group I was modified so that the animals were fed on the diet containing rice flour until they were 100 days old, and they were then given the "coarse" diet. They were not given "fine" rice feed at any time. The method of managing Group 2 was changed accordingly, and they were given the rice flour ration until 150 days old, whereupon they also were placed on the diet containing the "coarse" feed.

DATA

Group 1

The 49 individuals fed on the rice flour mixture to the age of 100 days developed dental caries at a mean age of

157.6 ± 4.2 days.* The mean caries time for the group was thus 157.6 ± 4.2 days minus 100 days, or 57.6 ± 4.2 days. Figure 1 shows the distribution of caries time for individuals of this group. The standard deviation of the mean caries time was 29.6 ± 3.0 days, and the variance 876.16 .

GROUP 2

This group, composed of 43 rats fed on the diet containing rice flour until 150 days old, developed caries at a mean age of 202.5 ± 8.6 days; or at a mean caries time of 52.5 ± 8.6 days. The standard deviation was 57.0 ± 6.1 days; the variance was 3249.00 . Figure 2 shows the distribution of caries times for individuals of this group.

Group 3-b

This group of 15 animals, fed throughout on the "fine" feed, developed caries at a mean age of 87.3 ± 7.3 days. Although there was no diet change after weaning, we must nevertheless subtract 35 days from their mean age at development of caries in order to obtain a caries time comparable to the control and to the other groups. This is permissible because to the age of 35 days, both the control and Group 3-b were given the "fine" rice diet exclusively. The difference between the methods of management of the two groups is simply that at 35 days, the control was changed to the "coarse" rice

*Throughout this report, the values accompanying means, standard deviations, differences between means, etc., are standard errors, not probable errors.

Figure 1. Distribution of Caries Time of Individuals in Group No. 1

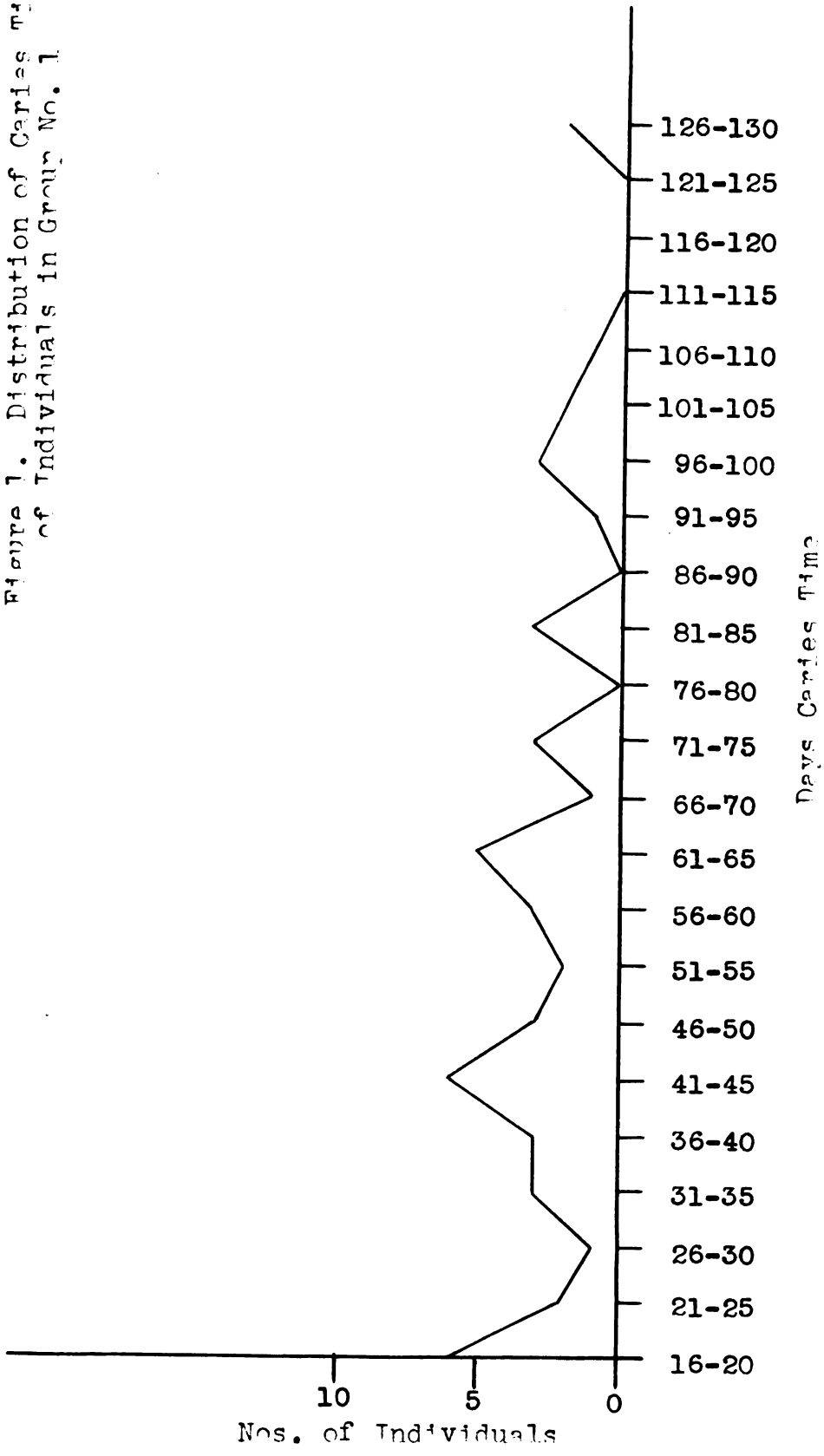

Figure 2. Distribution of Caries Times of Individuals in Group No. 2

diet while the Group 3-b remained on the "fine" diet. Mean caries time for this group, then, was 52.3 ± 7.3 days, with a standard deviation of 28.3 ± 5.1 days. Variance was 800.89 . The distribution of caries times for this group is shown in Figure 3.

Group 4

During the experiment, six animals developed caries on the feed containing rice flour without attaining sufficient age to be placed in the group for which they were intended. The mean age at caries development for this group was 132 days, and the individuals are described in Table 4.

The Control

The 86 rats in the control group developed caries at a mean age of 64.3 ± 1.0 days, that is, with a mean caries time of 29.3 ± 1.0 days. The standard deviation was 9.3 ± 0.7 days; the variance was 86.49 . The distribution curve for the control is given in Figure 4.

STATISTICAL ANALYSIS OF DATA AND CONCLUSIONS

DISCUSSION

Several groups of animals require further discussion before comparison of mean caries times. The first of these is Group 2. This group was fed on the diet containing rice flour to the age of 150 days, and was then placed on the "coarse" rice ration. Figure 2 shows a discontinuous curve for this group with one animal in each of the following

Figure 2. Distribution of Caries Times of Individuals in Group No. 3-b

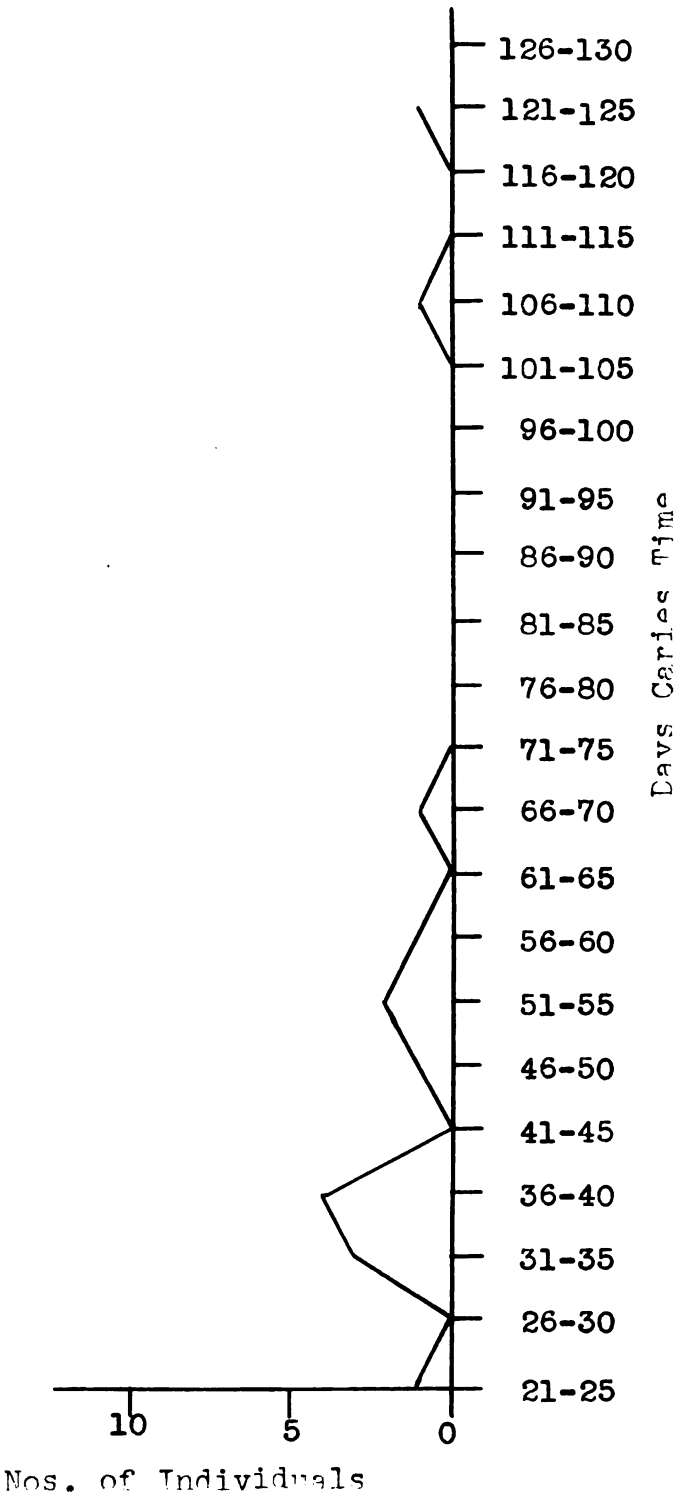

Table 4. The Age in Days at Development of Dental Caries in Individuals in Group 4.

No. of Individual	Age in Days at Development of Caries
Female 73	120 days
Female 74	147 days
Female 79	147 days
Male 87	91 days
Female 105	142 days
Female 113	139 days

Figure 4. Distribution of Caries Times of Individuals in Control Group (Hunt and Hoppert's 9th Susceptible Generation)

caries time classes: 78 days, 103 days, 108 days, 138 days, 163 days, 223 days, and 283 days. The large standard error of the mean caries time of this group, and the large standard deviation are most certainly due in part to this long, irregular "tail" on the curve. This circumstance makes statistical comparison of Group 2 with other groups quite inaccurate.

For the moment, let us consider the two animals with the highest caries times (at the right of the curve). The caries times of these rats are approximately three and four standard deviations, respectively, from the mean caries time of Group 2. They alone increase the mean caries time of the group by 9.7 days.

Now let us consider the four animals with the highest caries times, viz., the two discussed in the preceeding paragraph plus those in the 138-day and 163-day caries time classes. These four animals increase the mean caries time of Group 2 by 15.3 days.

The author is unable to explain with any certainty why these few animals showed such outstanding resistance.*

*This investigation was conducted shortly before and during the early part of World War II, and when the author entered the armed forces, it had to be terminated without even a preliminary analysis of data. The animals were destroyed at that time. Some time later, Hunt and Hoppert observed that the upper molars of some of their animals were broken. They believe there is a tendency of the lower unopposed molars (the lower molars are the only teeth examined in their animals as well as in the author's) to remain caries-free longer than opposed molars. This point is now under investigation by Hunt and Hoppert, but as yet they have published nothing on this topic. There is a chance that the upper molars of these few resistant animals were broken, but this can no longer be investigated. Or some other unrecognized factor may be responsible for this exceptional resistance.

However, it does not seem justified to permit several animals to displace the mean caries time of their group to such an extent, thereby grossly exaggerating or obscuring differences between the various groups.

Dr. Wm. D. Baten, of the Department of Mathematics, has advised eliminating some of the rats showing the greatest number of days caries time, in the interest of accuracy of comparison of groups. The exact number of animals to be eliminated is arbitrary, but should be kept to a minimum. Deletion of the data from the two rats in the 223-day and 283-day categories was chosen as most desirable. These two animals will henceforth be referred to as Group 2-a, and the remaining 41 animals in Group 2 will be called Group 2-b. Group 2-b will be substituted for Group 2 in the following statistical comparisons. Group 2-b showed a mean caries time of 42.8 ± 5.4 days as contrasted with 52.5 ± 8.6 days for Group 2. The corresponding standard deviations are 34.3 ± 3.8 and 57.0 ± 6.1 , while Group 2-b has a variance of 1176.49 as compared to 3249.00 for Group 2.

We should also mention Group 4. These animals cannot be properly compared to any other group, but rather, should be considered as a few selected animals. It will be recalled that they were being prepared to be tested in either Group 1 or Group 2, but did not attain the required age on "flour" feed before developing caries. The majority of susceptible animals had sufficient resistance to attain either 100 or 150 days of age on flour feed without exhibiting dental decay.

This may be interpreted as evidence of a considerable amount of variability among the animals, but in the light of Erwin's observations[24] (which showed that the caries phenotype of an animal is a poor indication of its genotype), it may not be cited as evidence of a lack of homozygosity in the strain.

However, it is true that there has been a certain amount of selection taking place, that is, Group 4 was inadvertently selected from Groups 1 and 2 on the basis of caries susceptibility. This is unfortunate, but the selection is probably not of a sufficient amount to make statistical comparisons grossly inaccurate.

Table 5 gives a complete summary of the data described thus far.

Hunt and Hoppert's 9th generation of the susceptible line was selected as a control for several reasons. First, the animals of this investigation are probably genetically comparable to their 9th generation (see discussion on p. 18). Secondly, from a timing standpoint, work on Hunt and Hoppert's 9th generation was proceeding at the same time as the work reported here.

Thirdly, from the standpoint of experimental error, these animals are an excellent control. The diet of Hunt and Hoppert's animals prior to their receiving the "coarse" rice ration, was the "fine" rice mixture. Groups 1 and 2-b, on the other hand, were fed the rice flour mixture before being given the "coarse" feed. As will be shown later (see p. 40) the rice flour diet does not lend itself to caries production nearly as readily

Table 5. Summary of Data.

	No. of Individuals
Group 1: Developed caries on "coarse" diet after receiving "flour" diet to age of 100 days	49
Mean caries time	57.6 ± 4.2 days
Standard deviation	29.6 ± 3.0 days
Variance	876.16
Group 2: Developed caries on "coarse" diet after receiving "flour" diet to age of 150 days	43
Mean caries time	52.5 ± 8.6 days
Standard deviation	57.0 ± 6.1 days
Variance	3249.00
Group 2-a: Two animals deleted for statistical accuracy	2
Group 2-b: Remainder of Group 2	41
Mean caries time	42.8 ± 5.4 days
Standard deviation	34.3 ± 3.8 days
Variance	1176.49
Group 3: Developed caries on "fine" diet	26
Group 3-a: Caries time not measured (less than 100 days)	11
Group 3-b: Caries time measured	15
Mean caries time	52.3 ± 7.3 days
Standard deviation	28.3 ± 5.1 days
Variance	800.89
Group 4: Developed caries on "flour" diet only	6
Mean age at development of caries	132 days
Total No. of animals participating in experiment, exclusive of control	+ 124
Control: (Hunt and Hoppert's 9th generation of the susceptible line)	86
Mean caries time	29.3 ± 1.0 days
Standard Deviation	9.3 ± 0.7 days
Variance	86.49
Grand Total of animals	+ 210

as does the "fine" feed. It is entirely possible that small foci of decay were already present in the teeth of the control when they were placed on the "coarse" rice diet. Thus there is an experimental error of coarseness of feed, tending to shorten caries time in the control. However, the results of Group 4 indicate that the rice flour diet will not prevent decay indefinitely. Groups 1 and 2-b spent considerably more time on the rice flour ration than did the control on "fine" feed, and it is entirely possible that they, too, had small cavities when placed on the "coarse" rice ration. In this way there is a second source of error in the form of difference between the lengths of time the animals lived prior to being placed on the "coarse" feed, which tends to shorten caries times in Groups 1 and 2-b. (This error is quite obviously greater for Group 2-b than for Group 1, and this will be discussed on p. 40). It is believed, therefore, that the two errors tend to cancel each other and that Hunt and Hoppert's animals make a more accurate control than would a group fed on the rice flour diet to the age of 35 days before receiving the "coarse" feed. In this latter imaginary situation, the error due to time on the flour diet would not be cancelled in any way.

Erwin[24] pointed out that because the animals were examined every 14 days, a difference of as much as 14 days in the caries times of two individuals might not be significant. But a difference of 14 days, or even less, in the average caries times of two different groups of rats might be

significant. This is true because a difference between two individuals is much more likely to be due to chance than is the same difference between the averages of two groups of individuals.

The "t-value" was arbitrarily selected as a means of comparing the mean caries times of the groups. The formula for the "t-value" is given by Baten as follows:

$$t = \frac{(M_x - M_y) - 0}{\sqrt{\frac{(x - M_x)^2 + (y - M_y)^2}{n_1 + n_2 - 2}}} \sqrt{\frac{n_1 \cdot n_2}{n_1 + n_2}}$$

COMPARISONS OF MEANS

In comparing, first of all, Group 1 with the control, we should reaffirm the differences in management between the two groups. The control was raised on the "fine" rice ration after weaning to the age of 35 days, and was then placed on the "coarse" rice diet. Group 1 was raised from weaning to 100 days of age on "flour" feed and then was placed on the diet containing coarsely ground rice. Mean caries time for the control was 29.3 $\pm$ 1.0 days; for Group 1, 57.6 $\pm$ 4.2 days. The difference is 28.3 $\pm$ 4.3 days. The "t-value" is 8.161, which is significant[1] at the 1 percent level. In other words, there is less than one chance in one hundred that this difference is due to sampling. Thus we may state: The teeth of a genetically susceptible rat are more resistant to decay at 100 days of age than at 35 days, the difference

being approximately 28 days of caries time.

The selection of Group 4, in part from Group 1, may have increased the mean caries time of the latter group to a slight extent. However, as Table 4 shows, only one animal from Group 4 could possibly have been selected from Group 1, as the other five rats were obviously being prepared for Group 2. It is inconceivable that the mean caries time of Group 1 would have been reduced sufficiently by this one individual to make the "t-value" so small that the difference between Group 1 and the control would not have been statistically significant.

Group 2-b was fed on "flour" feed to the age of 150 days and was then placed on "coarse" feed. The mean caries time is 42.8 $\pm$ 5.4 days. The difference between this mean caries time and that of the control is 13.5 $\pm$ 5.5 days. However, the real difference between these groups may be somewhat greater than this due to experimental error. This will be discussed presently. The "t-value" is 3.373, which is significant at the 1 percent level. Therefore, there is less than one chance in one hundred that this difference is due to sampling error.

In comparing Group 1 with Group 2-b, the difference between the mean caries times is seen to be 14.8 $\pm$ 6.8 days, with the 100-day group apparently showing more resistance than the latter group. The "t-value" is 2.171, which is significant at the 5 percent level. Or, we conclude that there is less than one chance in twenty that this difference is due to errors in sampling.

But the difference may possibly be explained in another

way. The experimental error due to differences in lengths of time spent on the rice flour mixture before being placed on "coarse" feed was briefly mentioned on p. 35 . This error, uncancelled in the comparison of Groups 1 and 2-b, would tend to place the mean caries time of the latter group at a lower figure than for the former. This hypothesis is strengthened by the "doubtful" observations. "Doubtful" observations and their role in determining caries times are discussed on p. 21 . Of the 49 individuals in Group 1, 2 rats, or 4.1 percent, showed "doubtful" loci before being placed on the "coarse" feed. In contrast with this rate, 19 of the 41 animals in Group 2-b, or 46.3 percent, showed these spots. In other words, the incidence of "doubtful" spots was 11.3 times as great in Group 2-b as in Group 1. The importance of this difference hinges on the exact nature of these defects, and their nature is not known. It is highly probable that at least some of them may have been small primary carious lesions, developed on the rice flour mixture. This is suggested because often, but by no means always, caries later developed in these same locations. If this inference is correct, then many more of the 150-day rats began their time on "coarse" feed with small foci of decay than did the 100-day animals. Thus it is entirely possible that no real difference in caries susceptibility exists between rats 100 days of age and those 150 days old, when all other factors are held constant. The apparent difference may be due solely to the difference in <u>time</u> spent on the rice flour mixture before being placed on the "coarse" feed.

If the mean caries time of Group 2-b is low, due to experimental error of this nature, then it follows that the difference between caries times observed on the foregoing page between Groups 2-b and the control is less than the true difference in susceptibility.

Dr. Hunt has pointed out that in dividing Group 2 into Groups 2-a and 2-b, and using only 2-b for statistical comparisons, we may be exerting a selection against Group 2, that selection being based only on high caries resistance. In an opposing manner, the inadvertent selection of Group 4 from Group 2 was on the basis of low caries resistance. Thus two opposing selective processes may have occurred here. Let us, therefore, combine Groups 2-a, 2-b, and 4 (or Groups 2 and 4), and substitute this newly formed composite group (which we may call Composite A) in place of Group 2-b in our comparisons.

But here we meet a difficulty, because it is not possible to calculate a caries time, in the true sense of the term, for Group 4, that group never having been placed on the "coarse" rice diet. However, we may obtain some sort of <u>rough</u> measure of caries susceptibility in Group 4 by taking the margin by which these six animals failed to attain the age of 150 days without dental decay, assigning a negative value to this figure, and using it in place of a caries time.

An example may clarify this process. From Table 4, we see that Female 73 developed caries at the age of 120 days on the ration containing rice flour. Subtracting 120 days from

150 days, we see that Female 73 failed to reach the age of 150 days by a margin of 30 days. After giving this figure a negative value, we substitute it in place of a caries time in our calculations. Repeating this process for each of the six animals individually, we obtain the values shown in Table 6.

The mean caries time of Composite A was 43.8 ± 8.3 days. The standard deviation was 57.8 ± 5.8 days; the variance, 3340.84 . It will be noticed that the mean of Composite A exceeds the mean of Group 2-b by only 1.0 ± 9.9 days, a difference which is certainly not significant. The standard deviation of Composite A is 23.5 ± 6.9 days greater than that of Group 2-b. Table 7 contrasts Composite A with Group 2-b.

Comparison of the Composite A with the control shows an apparent difference of 14.5 ± 8.4 days, which gives a "t-value" of 2.236, which is significant at the 5 percent level. The difference between 2-b and the control is significant at the 1 percent level. In like manner, the measured difference between Groups 1 and 2-b is significant at the 5 percent level, while the difference between Group 1 and Composite A is not significant.

In short, the substitution of Composite A for Group 2-b serves to make the standard errors of the differences between the groups larger, the "t-values" smaller, and the differences less significant but numerically almost the same. A summary of the statistical analysis of data to this point is given in Table 8.

Table 6. Values to be substituted for Caries Times when Group 4 is Included in Calculations.

No. of Individual	Value to be substituted for caries time
Female 73	-30
Female 74	- 3
Female 79	- 3
Male 87	-59
Female 105	- 8
Female 113	-11

Table 7. Contrast Between Group 2-b and Composite A, Made by Comparing Each With the Control, With Group 1, and With each Other.

Difference in the means between Group 2-b and the Groups indicated:

Group	Difference	t-values	Significance
Control	13.5 ± 5.5	3.373	**
Group 1	-14.8 ± 6.8	2.171	*

Difference in the means between Composite A and the Groups indicated:

Group	Difference	t-values	Significance
Control	14.5 ± 8.4	2.236	*
Group 1	-13.8 ± 9.3	1.485	0

Direct comparison of the means of Group 2-b and Composite A:

Difference	t-value	Significance
-1.0 ± 9.9	0.944	0

0 Not significant.
* Significant at the 5 percent level.
** Significant at the 1 percent level.

Table 8. Summary of Statistical Analysis with Regard to "t-value".

Groups	Compared	Difference	Degrees Freedom	t-values	Signi-ficance
Greater Mean	Lesser Mean				
Group 1	control	28.3 ± 4.3	133	8.161	**
Group 2-b	control	13.5 ± 5.5	125	3.373	**
Group 1	Group 2-b	14.8 ± 6.8	88	2.171	*
Composite A	Group 2-b	1.0 ± 9.9	90	0.944	0
Composite A	control	14.5 ± 8.4	133	2.236	*
Group 3-b	control	23.0 ± 7.4	99	5.998	**
Group 1	Group 3-b	5.3 ± 8.4	62	0.610	0
Group 3-b	Group 2-b	9.5 ± 9.1	54	0.960	0
Group 1	Composite A	13.8 ± 9.3	96	1.485	0

0 Not Significant
* Significant at the 5 percent level
** Significant at the 1 percent level

Probably more reliance can be placed on the values obtained with Group 2-b than on those obtained with the Composite A. This is true, first of all, because of the grave possibility of broken upper molars or some unidentified factor entering into the phenomenal resistance of the two animals in Group 2-a with the excessively high caries time. Secondly, the inclusion of the negative caries values from Group 4, is probably unjustified in obtaining a measure of average caries susceptibility in the 150-day group of rats. Days caries time are based on the consumption of the "coarse" rice diet, while the negative values are based on consumption of the "flour" diet only. Contrasts between the effects of these diets will be discussed later, but the differential effect of the diets is considerable.

Disregarding Composite A entirely, and considering the values for Group 2-b as the basis for our conclusions, we may state: The teeth of a genetically susceptible rat are more resistant to decay at 150 days than at 35 days of age; less resistant at 150 days than at 100 days of age; the former difference is approximately 13.5 days, while the latter difference is approximately 14.8 days.

Group 3-b, fed on "fine" rice ration from weaning until caries was observed, had a mean caries time of 52.3 $\pm$ 7.3 days. The difference between this mean and the mean caries time of the control is 23.0 $\pm$ 7.4 days, and the "t-value" is 5.998 . Thus, according to the "t-value" tables, there is less than one chance in one hundred that this difference is

due to sampling error. Thus we may state that the work of Hoppert, Webber, and Canniff[32,33] is confirmed when confined to the study of genetically susceptible animals. They observed that in genetically unselected rats, a finer degree of division of rice particles in chemically similar diets is associated with increased resistance to dental caries.

The "t-value" obtained in comparing Groups 1 and 3-b is 0.610, which indicates that the difference between the means, 5.3 ± 8.4 days is not significant.

Nor is the difference of 9.5 ± 9.1 days between Groups 2-b and 3-b significant, as the "t-value" is 1.908 . Thus it appears that the increased resistance to dental caries associated with increasing age is of the same order as that associated with a moderate reduction in particle size.

The mean age at caries development for Group 3-b has been stated as 87.3 ± 7.3 days. On the other hand, 49 animals fed on "flour" feed attained the age of 100 days (Group 1), and 43 animals on this latter diet attained the age of 150 days (Group 2) without developing caries. Six animals (Group 4), developed caries on the "flour" ration. We are not able, with the present data, to obtain an <u>accurate</u> comparison of the caries producing effects of the "fine" rice feed and the "flour" feed, because the majority of animals fed on the "flour" diet did not develop dental caries on that diet. However, we may obtain a minimum differential effect if we imagine that Group 1 (49 animals)

developed caries on the "flour" ration at 100 days of age, and that Group 2 (43 animals) developed caries on this same diet at 150 days of age. These ages are selected because they are the ages at which the animals were removed from the "flour" feed with no carious lesions as yet developed. We have no way of knowing how much longer the rats could have remained on the "flour" diet without developing dental decay.

Imagining, then, that the 49 animals in Group 1 developed caries on the "flour" diet at 100 days of age, and that the 43 members of Group 2 exhibited dental decay at 150 days of age on the same diet, and including the six animals which developed caries on the "flour" diet at known ages, we form a new composite group (which we may call Composite B). The mean age at "development of caries" is 123.8 $\pm$ 2.5 days; the standard deviation is 24.7 $\pm$ 1.8 days, and the variance is 610.09 .

The difference in mean <u>ages</u> at development of caries in Group 3-b and in Composite B is 36.5 $\pm$ 7.7 days, and the "t-value" is 3.661, which is significant at the 1 percent level. Therefore we conclude that the teeth of genetically susceptible rats are preserved <u>at least</u> 36.5 $\pm$ 7.7 additional days, and probably much longer, by the substitution of the "flour" diet for the "fine" rice ration.

COMPARISONS OF VARIABILITY

A study of Table 5 indicates that there has been a considerable amount of variation in the standard deviations, and therefore in the variability of the different groups. We can further compare the groups through a study of their variance. Variance is defined as the square of the standard deviation of the mean, and the variance of each group is also given in Table 5.

It will be noted that the variance of Group 1 is ten times as large as that of the control. We can compare these variances best by use of the "F-value" , which is given by Baten[1] as:

$$F = \frac{\text{Larger Variance}}{\text{Smaller Variance}}$$

In comparing the variances of these two groups, we obtain an "F-value" of 10.13, which is significant at the 1 percent level. Therefore the increased variability of genetically susceptible rats with regard to caries susceptibility at 100 days, compared to the variability at 35 days of age, is significant to the degree that there is less than one chance in 100 that the difference is due to sampling error.

The "35-day" and the "100-day" rats are genetically very much alike. Therefore the difference between the variance is probably non-genetic. Has there been an accumulation of causes of variability in the "100-day" rats of an environmental (non-genetic) type?

Table 9 compares the variabilities of the groups by means of the "F-value". Thus the variability of caries sus-

Table 9. Comparisons of Variability of the Different Groups by Means of the "F-value".

Greater Variance	Smaller Variance	F-value	Significance
Group 1	control	10.13	**
Group 2-b	control	13.60	**
Group 2-b	Group 1	1.34	0
Group 3-b	control	9.26	**
Composite A	Group 2-b	2.84	**
Group 1	Group 3-b	1.09	0
Group 2-b	Group 3-b	1.47	0

0 Not Significant
* Significant at the 5 percent level
** Significant at the 1 percent level

ceptibility among these animals is greater at 150 days of age than at 35 days of age (control compared with Group 2-b). However, the difference in variability between that at 100 days and that at 150 days is not significant (Group 1 compared with Group 2-b).

It will also be noted that leaving the animals on the "fine" rice diet at 35 days, instead of placing them on the "coarse" rice diet, as was done with the control, results in an increased variability. The increase is significant at the 1 percent level (Group 3-b compared with the control).

The reader will recall that the increased resistance detected by leaving the rats on the "fine" rice diet was not significantly different from the increased resistance obtained by increasing the age from 35 to 100 or 150 days. The same relation is observed in considering variability, viz., variability is increased by leaving the rats on the "fine" ration, and is also increased by advancing the age to 100 or 150 days. The amounts of increase of variability in each instance are not significantly different from each other, but are significantly different from the control. (See comparisons of Groups 1 and 3-b, 2-b and 3-b, and 1 and 2-b).

The discussion on p. 42 , mentions that the substitution of Composite A (Groups 2 and 4 combined) for Group 2-b results in larger standard errors, and therefore smaller "t-values" and less significant differences in the various comparisons. Table 9 further illustrates this point.

The difference in variability between Composite A and Group 2-b is significant at the 1 percent level. Table 7 shows that the difference between mean caries times is very small, being approximately 1 day. Therefore, the only important difference between Group 2-b and Composite A is that Composite A has longer "tails" on the frequency curve, resulting in such a high standard error that differences between Composite A and other groups are obliterated. "Trimming off these tails", that is, substituting Group 2-b for Composite A, results in the differences between the 150-day group and each of the groups with which it is compared becoming more evident.

SUGGESTIONS FOR FUTURE RESEARCH

The effect of age on the production of dental caries in albino rats is not well defined by this experiment. This report compares only three age groups. Studies on groups at intermediate ages and in excess of 150 days, would doubtless reveal some interesting relationships. The present study demonstrates only that there is some sort of change in caries-susceptibility concomitant with increasing age in this genetic line. Much more investigation is needed to produce a graph of this change. However, the present study is sufficient to warn investigators in various phases of the attack on dental caries that the change in caries-susceptibility with increasing age, often

observed in human beings, probably also applies to the experimental rats.

A methodology which would prevent any possible microscopic foci of decay being initiated before the animals reach the desired test ages is urgently needed.

SUMMARY

1. The degree of susceptibility to dental caries in genetically susceptible rats is partially dependent on age:

a. The teeth of the rats are significantly more resistant to decay at 150 days of age than at 35 days; the difference is apparently 13.5 days of caries time, but because of experimental error, may really be somewhat greater.

b. When 100 days old, the genetically susceptible rats have a longer caries time than when 150 days old, the apparent difference being approximately 15 days of caries time. Experimental error is such that this difference may be exaggerated.

2. The work of Hoppert, Webber, and Canniff is confirmed and expanded in several respects:

a. Substitution of the "coarse" rice for the "fine" rice in the Hoppert diet at 35 days of age, results in prompt tooth decay in genetically susceptible rats. The difference is approximately 23 days of caries time. The rats of Hoppert, Webber, and Canniff were genetically unselected.

b. A further increase in caries time was obtained by substituting rice flour for "fine" rice particles in the

Hoppert diet. The difference is at least 36.5 days and probably considerably longer. Hoppert, Webber, and Canniff did not work with rice particles as small as those in rice flour.

3. The increased resistance observed with increasing age is of the same order as the increased resistance observed by substituting "fine" rice particles for "coarse" rice particles in the Hoppert diet.

4. An increase in variability of caries time is observed at 100 days and at 150 days of age when compared to variability at 35 days of age. Variability when 150 days old is not significantly different from that when 100 days of age.

5. The substitution of "fine" rice particles for "coarse" rice particles in the Hoppert diet at the age of 35 days results in increased variability of caries time.

6. The increased variability observed in 4 (above) is not significantly different from that observed in 5 (above).

7. Several genetically susceptible rats exhibited outstanding resistance to dental caries when placed on the "coarse" rice diet at 150 days of age. No comparable animals were observed at either 35 or 100 days of age. The reason for this can only be speculated upon.

8. Six animals exhibited extreme susceptibility to dental caries, so that they were unable to attain the age of 150 days on the "flour" ration without developing carious lesions.

9. Suggestions for future research on this topic are included.

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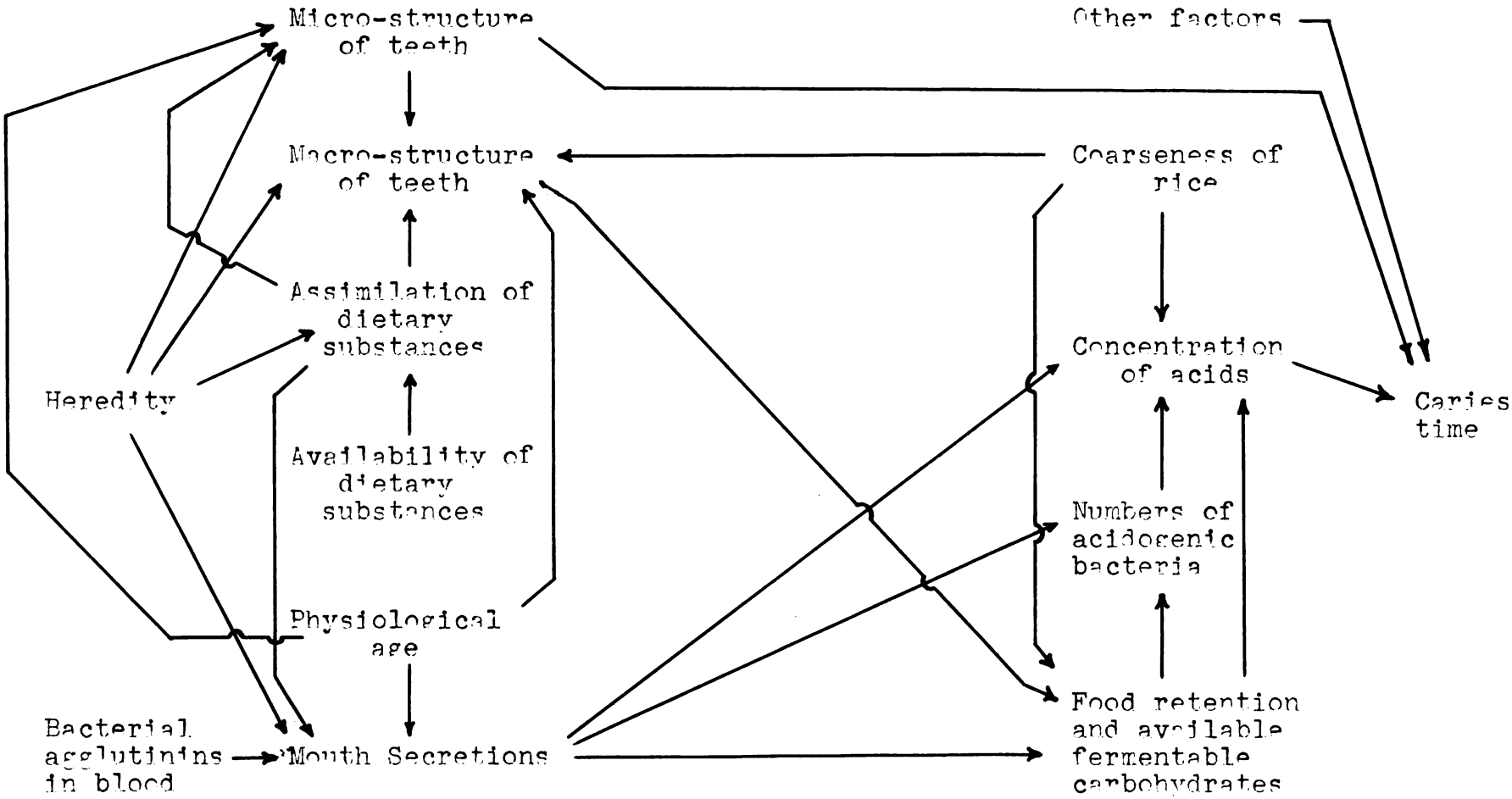
ADDENDUM

The diagram on the following page is presented only as a temporary working hypothesis of some of the causes of variability in caries time in laboratory rats. The system used by Sewall Wright* has been followed insofar as possible at the present time. When a change in any variable is correlated with a change in another variable, the two are connected by an arrow in the probable direction of causation. Accordingly, the reader is cautioned against misinterpreting the diagram as an exposition of the causes of dental caries. Rather, it is an explanation of the causes of variability in susceptibility to dental caries.

To make the difference between these two interpretations clear, let us take an example from the diagram. "Physiological age" is connected by an arrow to "Mouth secretions". Quite obviously, physiological age cannot be a cause of mouth secretions. But it is entirely reasonable that variations in physiological age are accompanied by, and may cause, variations in the chemical and physical properties of the mouth secretions.

Many of the relationships here presented have not been proven to exist in the laboratory rat, but have been shown to exist in human beings. Other relationships have not been demonstrated in either species, but are only reasonable. Most probably, future research will demand drastic changes in this diagram, but it is believed to have a temporary value.

*Wright, Sewall, "Correlation and Causation", Journal of Agricultural Research, vol. 20, p. 557, 1921.



A diagram illustrating a temporary hypothesis of some of the causes of variability of caries time in laboratory rats.

Wright's systems of path coefficients require the use of coefficients of correlation. These are not available for most of the paths here shown, and therefore, a path coefficient system may not be set up at this time.

Probably a discussion of the reasons for placing some of the arrows is appropriate. Some ways in which variation in age is correlated with variation in the micro-structure of the teeth are described in Karlström's[44], Hollander's[31], and Bödecker's[13], [64] works (see discussion on p. 12). That the macro-structure of the teeth may be altered by attrition as age advances, is generally agreed upon. It is reasonable to suppose that mouth secretions vary with physiological age.

Quite obviously, the assimilation of dietary substances is partially dependent on their presence or absence in the diet. Dietary influence on the micro-structure of the teeth is indicated by the reports of Howe[34], and Mellanby and Pattison[50,51] (see p. 9). It is only assumed that variations in the assimilation of dietary substances may affect the macro-structure of the teeth. Reason suggests the liklihood that the gross structure of a tooth is partially dependent on the micro-structure of the same tooth.

It is likewise reasonable to form the hypothesis that assimilation of dietary substances affects mouth secreations by making available supplies of "raw materials" to the salivary glands.

The work of Hunt and Hoppert[35, 36, 37, 38] (see p. 13) makes it clear that the genes do affect caries time in rats. Statements by Bunting[19], Steggerda and Hill[62], Hurme[39], Klein[45],

and Klein and Palmer[66] (see p. 6 and pp. 12-13) lend credence to the opinion that human genes exert a comparable effect on caries susceptibility. One may logically expect that this effect is by way of the micro-structure of the teeth, or their macro-structure, or assimilation of dietary substances, or the mouth secretions; or combinations of these factors, or even all of them may be concerned. Much further research is required to clarify the proper path of the influence of variations in the gene complex.

That the micro-structure of the teeth may have a direct effect on caries time is indicated by the work of Mellanby and Pattison[50,51]. The influence of the macro-structure of the teeth on food retention is generally agreed upon by investigators.

The reports of Hill[29] and of Hanson, Fosdick and Epple[25] are the basis for the arrow between "Mouth secretions" and "Numbers of acidogenic bacteria". Variation in the viscosity of the saliva may affect food retention according to several authors.

The observations of several investigators in regard to the buffering action of saliva are indicated by the arrow from "Mouth secretions" to "Concentration of acids". A possible influence of blood bacterial agglutinins on the mouth secretions is drawn in the diagram because of the work of the University of Michigan Group[40, 41, 65]. (see pp. 10-11)

The numbers of acidogenic bacteria are necessarily limited by the amount of available substrate, and thus the arrow from food retention to acidogenic bacteria is justified. The arrows from acid-forming organisms to concentration of acids, and from

this latter factor to caries time, are explained by W.D. Miller's[6,52,53,54,55,56] observations (see p.3).

Hoppert, Webber, and Canniff[32,33], (see p. 7) showed that the coarseness of the rice particles are a factor in the variability of caries susceptibility, and the present report confirms their observations. The path, once again, is unknown, but reason alone indicates that it may be via an influence on the gross structure of the teeth through attrition, fracture, or fracture of an opposing molar. Or the path may be by way of food retention through the packing of material into crevices of the teeth. Or both paths may be involved. Again, further research is required to establish the proper paths.

The reader is again respectfully reminded that this diagram is only hypothetical, and is probably incorrect, at least in part. It's value lies in stressing our lack of knowledge of the causes of variation in caries susceptibility, in indicating foci for further research, and in providing a temporary hypothesis of the causes of variation in susceptibility to dental caries.