GROWTH RATE AND PILOSITY IN CARIES RESISTANT AND CARIES SUSCEPTIBLE ALBINO RATS (RATTUS NORVEGICUS)

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Ronald L. Clise

1952

This is to certify that the

thesis entitled

Growth Rate and Pilosity in Caries Resistant
and Caries Susceptible Albino Rats
(Rattus norvegicus)

presented by

Ronald L. Clise

has been accepted towards fulfillment
of the requirements for

M. S. degree in Zoology

H. R. Hunt
Major professor

Date May 28, 1952.

GROWTH RATE AND PILOSITY IN CARIES RESISTANT AND CARIES SUSCEPTIBLE ALBINO RATS (RATTUS NORVEGICUS)

by

Ronald L. Clise

A THESIS

Submitted to the Graduate School of Michigan
State College of Agriculture and Applied
Science in Partial fulfilment of the
requirements for the degree of

MASTER OF SCIENCE

Department of Zoology

East Lansing, Michigan

1952

DEDICATION

To my wife and parents.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	V
REVIEW OF LITERATURE	1
THE EFFECT OF BACTERIA	2
THE EFFECT OF DIET	7
THE EFFECT OF SALIVA	10
DENTAL CARIES AND BODY MEASUREMENTS	11
DENTAL CARIES AND HEREDITY	11
AIM OF THIS EXPERIMENT	15
METHODS AND MATERIALS	16
ANALYSIS OF DATA	25
CONCLUSIONS AND DISCUSSION	35
DETERMINATION OF HAIR DENSITY	37
METHODS AND MATERIALS	38
ANALYSIS OF DATA	41
CONCLUSIONS AND DISCUSSION	44
SUMMARY	45

TABLES

		Page
Table 1.	Average Weights of Males For All Weighings	19
Table 2.	Average Weights of Females For All Weighings	21
Table 3.	Comparisons of Average Weights of Males at Various Ages.	30
Table 4.	Comparisons of Average Weights of Females at Various Ages. . . .	31
Table 5.	Comparisons of Average Litter Size	33
Table 6.	Comparisons of Hair Density . . .	42
Table 7.	Coefficients of Correlation Between Hair Density and Body Weight	43

ILLUSTRATIONS

Page

Figure 1. Body Weight of Caries Resistant and Caries Susceptible Albino Rats 26

ACKNOWLEDGMENTS

The author wishes to take this opportunity to thank Dr. H. R. Hunt and Dr. C.A. Hoppert for their kindness in supplying the animals used in this experiment. I also wish to thank Dr. Hunt for the many valuable suggestions and criticisms which he made throughout the course of this experiment.

Expressions of appreciation are also due Mr. E. D. Harrison, who instructed the author in the proper methods of handling and caring for the laboratory rat, and Mr. R. F. Keller, who examined the teeth of the animals used in this experiment, and also made many valuable suggestions.

REVIEW OF LITERATURE

At the present time dental caries is one of the most frequent and widespread human diseases. Studies of the more primitive races existing at the present time show that, in a general way, the incidence of dental caries among them is proportional to their contact with white civilization.[1] However, tooth decay is not entirely a product of civilization since anthropological studies give ample evidence that caries afflicted the primitive man of the late Pleistocene period.

Over this long history of the disease theories concerning its cause have been many and varied. One of the earliest and most widely known of these theories originated in Babylonia at approximately 5000 B.C.[12] According to this theory the decay and accompanying pain were caused by an evil spirit in the form of a worm which gnawed at the teeth. As fantastic as it seems now, the worm etiology of tooth decay was accepted by many prominent physicians as late as the eighteenth century. In fact, no serious doubt was cast upon it until Pierre Fauchard,[12] the Founder of Modern Dentistry, did so in the early eighteenth century. The "worm" theory was not the only one put forth by the ancients however. As early as 456 B.C. Hippocrates[2] attributed caries to (...) "the stagnation of depraved juices in the teeth." In 850 A.D. a Persian physician stated that ... "the cause of dental decay and crumbling teeth is an acid moisture ....that comes to the mouth."[12] These two statements, made by men living near the time of Christ, are startlingly similar to the modern concept of the etiology of tooth decay.

More recently (1861) the novel electrical theory was proposed by Bridgeman.[2] He believed that under normal conditions the teeth constituted an electrolytic system in which the crown of each tooth was the positive electrode, the roots the negative electrode, and the saliva the electrolytic fluid. Electric current generated by this system caused the formation of acid which in turn caused tooth decay. In 1880 this theory was modified by Chase[2] who proposed a similar action between metallic fillings and the body of the tooth. Both of these theories were short-lived and eventually discarded for lack of evidence.

THE EFFECT OF BACTERIA

Modern research on the cause of dental caries received great impetus in 1890 when W. D. Miller[2] announced that tooth decay was due to acids formed by bacteria acting on food particles in the mouth. Miller was able to show that bacterial fermentation of carbohydrates resulted in an acid reaction due primarily to the production of lactic acid. He isolated ten different strains of mouth bacteria which would produce acid by fermenting carbohydrates and stated that the action of acid always precedes the invasion of bacteria into the tooth. In its final form Miller's concept of the carious process was that the enamel of the tooth is first dissolved (decalcified) by the acid produced by acidogenic bacteria until the dentino-enamel junction is reached, at which time proteolytic bacteria, which are capable of living in an acid medium and digesting organic material, continue the carious process into the dentin of the tooth. The essentials of this theory are still accepted by the majority of modern researchers in the field of tooth decay.

Miller's theory has not been entirely unopposed however. Perhaps the foremost opponent of Miller's concept of the carious process is Bernhard Gottlieb. (4) Gottlieb believes that the tooth enamel is penetrated by lamellae which provide pathways of organic material from the surface of the enamel to the underlying dentin. Thus, according to Gottlieb, caries is initiated by the invasion of these lamellae by proteolytic bacteria, not by the decalcification of enamel by acid. This theory is not widely accepted since many investigators believe that the "lamellae" are nothing more than artifacts.

However, Kronfeld (9) confirms the existence of lamellae in the enamel and states that (...)

> "the fact that lamellae are basically cracks make them appear to be paths by which the products of micro-organisms could diffuse into the enamel and lead to its disintegration. The distribution of the lamellae is such, however, that they cannot be postulated as always existing in the specifically localized areas where the commonly occurring carious lesions are found."

More recently, Sognnaes (42) has shown that no lamellae-like elements occur in unerupted teeth. He states that lamellae are (...) "apt to be found where functional stresses of one kind or another have caused discontinuity between the enamel prisms," and "they seldom reach the dentin." Bödecker[13] believes that the enamel, particularly in early life, is nourished by the enamel prism sheaths which he believes to be continuous with the dentinal tubules.

When Miller was able to associate bacteria with dental caries, many investigators believed that tooth decay, like so many other diseases, would eventually be traced to a specific micro-organism and a cure found. Unfortunately, however, this has not been the case. Continued research has led to many claims and counter claims. Those who believe that susceptibility to tooth decay is due primarily to the presence or absence of a specific bacterium are still faced with explaining the existence of individuals who have rampant caries without the prescribed bacteria being present. Similarly, investigators who would attribute the presence of tooth decay to improper diet of one form or another must find an explanation for the existence of individuals suffering from severe dietary deficiencies yet posessing excellent teeth. Investigation of some of the existing literature has led the author to the conclusion that tooth decay will eventually be shown to be the expression of a complicated assemblage of causative factors.

The search for a specific micro-organism as the cause of dental caries was led by R. W. Bunting[2] [17] [18] and his co-workers at the University of Michigan. Bunting believes

that the organism Lactobacillus acidophilus* is the most important specific etiological factor in tooth decay. In one of his investigations Bunting obtained a correlation of 98.35 between the presense of L. acidophilus and active caries in an examination of 427 individuals. In general, Bunting and the Michigan group have concluded that any factors which tend to create an environment favorable to L. acidophilus are important in the etiology of tooth decay. In this respect he believes that the configuration of the teeth and the presence of carbohydrates in the diet are most important. Bunting believes that inherited tendencies are more important than diet in explaining variations among individuals. Jay[35] states that (...) "the occurrence of dental caries is, in every case, preceded by the appearance of L. acidophilus in the mouth flora." More recently, investigations of the effects of various antibiotics have led indirectly to the support of Bunting's views. For example, McClure and Hewitt[40] demonstrated a reduction of caries in the rat and a corresponding reduction of the incidence of L. acidophilus when penicillin was included in the diet. Cox, Dodds, and Levin[21] found that fermentable carbohydrates in the diet increase caries in the rat. They observed, however, that the incidence of caries

---

*The term Lactobacillus acidophilus is used throughout this report although some of the references cited use the older term, Bacillus acidophilus.

was not so much effected as the extent of caries.

Other investigators do not confirm the findings of Bunting and the Michigan group with regard to the correlation between *L. acidophilus* and dental caries. Hine[11] was unable to find any one type of organism predominant when examining smears from established carious cavities microscopically. He states that (...) "In every cavity studied, a complex mixed flora was present." Fosdick[23] found that acid producing, acid tolerating streptococci and lactobacilli were both intimately associated with dental caries. Johnston, Kaake, and Agnew[36] found *L. acidophilus* equally as abundant in caries-free rats as in those with advanced caries. In an examination of children they found such great variability that no statistical correlation was apparent.

As previously mentioned, one of the most difficult obstacles to overcome in postulating a bacterial etiology for dental caries is the existence of individuals who have rampant caries without the presence of specific bacteria, and of course, the reverse of this situation. An excellent example of the occurence of such individuals is shown in an experiment performed by Collins, Arthur, and Becks.[20] The experiment was designed to determine the effects of diet on tooth decay. Three hundred and sixty-six students of the University of California were chosen in three groups; those who were caries-free, those who had doubtful caries, and those who had positive caries. The saliva of all these individuals was examined for the presence of *L. acidophilus*. Of the 122 caries-free persons 27 had high

L. acidophilus counts, and of the 122 individuals with active caries 15 had no *L. acidophilus* in their saliva!

THE EFFECT OF DIET

Many investigators believe that whether or not a particular individual, or a particular tooth in one individual, succumbs to the carious process is determined by systemic factors of one kind or another. Diet, particularly since the discovery of vitamins, has been the subject of a great deal of investigation as a possible cause of tooth decay. Perhaps the most important pioneer work on the relationship between diet and dental caries was performed by the British scientist, Lady Mellanby,[41((11)] who's investigations were began in 1917 and extended over a period of twenty years. From her experiments on animals and observations of children, she concluded that tooth structure and susceptibility to tooth decay are closely associated and that the structure of teeth is related to diet, particularly during the developmental period. She was able to produce hypoplasisity and caries in dogs maintained on a diet deficient in vitamin D and show that vitamin D influenced the initiation and progress of caries in human children.

However, Bunting,[(17)] who repeated many of Mellanby's experiments, was unable to show any relationship between vitamins D and C or the calcium and phosphorus content of the diet and dental caries. He concluded that the various effects of the diets were due to the amount of carbohydrates present.

Hanke,[5] [24] [25] who also believes that diet is the most important factor in susceptibility to tooth decay, thinks that the effect of vitamin C is more important than that of vitamin D. In an apparently well conducted experiment in which 323 children were observed over a period of three years he states that (...) "the addition of a pint of orange juice and the juice of one lemon to the daily diet has reduced the amount and severity of the disease (dental caries) markedly in all cases; but the incidence of caries is still greatest in those groups that had previously had the most decay."

Howe,[29] [30] in experiments using guinea pigs and monkeys, concludes that vitamin C plus an adequate supply of calcium and phosphorus are beneficial in arresting tooth decay. Boyd and Drain[14] found arrested caries in 28 diebetic children and attributed it to the diabetic diet which was rich in mineral salts and vitamins with fat substituted for carbohydrates as a source of energy. Cohen,[19] in a study of 27 young diebetics who were being treated with insulin, found no decrease in the incidence of caries. Brodsky,[16] like Howe and Hanke, also found that the addition of fruit and fruit juices to the diet had a beneficial effect on tooth decay.

As previously stated, the effect of diet on tooth decay is still a controversial issue. A study made by Mann, Dreizen, Spies, and Hunt,[38] and corroborated by others appears to cast serious doubt on the concept of caries control through diet.

They examined 124 patients who showed such symptoms of malnutrition as pellegra, scurvy, and nutritional macrocytic anemia. The incidence of caries in these patients was compared with that of a randomly selected group of individuals showing no signs of malnutrition. The incidence of caries in the malnourished group was found to be 30.5 percent lower than in the well nourished group.

Many investigators point to the low incidence of tooth decay among primitive races as evidence of the influence of diet on susceptibility to the disease. This conclusion is based upon the fact that wherever studies have been made of primitive races before and after the introduction of the so-called civilized diet, the incidence of caries had risen sharply following the introduction of the civilized diet.

This degenerative effect of modern diet is indicated by Ferguson [22] who studied the children of American Samoa, and by Waugh [43] who studied the American Eskimo, as well as others. W. A. Price [1] [11] who, over a period of years, studied fourteen primitive racial stocks concludes that (...) "the most constant factor in control of caries is diet." Unfortunately, the diets of these primitive people are highly diversified and do not present any readily discernible pattern which would connect them with tooth decay. The diet of the Eskimo consists mainly of meat, whereas the Samoan eats large quanities of starchy tubers and chews sugar cane as a confection.

Similarly, Steggerda and Hill [1] report that the Maya Indian, whose diet is overweighted with carbohydrates, and the

Navajo Indian, who's diet is overweighted with proteins, have equally low incidences of dental caries. These variations have led some investigators to suggest that it may be the physical properties of these primitive diets which influences the development of caries.

THE EFFECT OF SALIVA

There are numerous references throughout the literature concerning the role which saliva plays in preventing tooth decay by cleansing the oral cavity of food particles. In addition, many investigators believe that the ability of saliva to neutralize acids is an important factor in susceptibility to tooth decay. Some of the investigations which have been made in comparatively recent times hold promise of showing that saliva may play a very specific and important role in the etiology of dental caries. Fosdick, Hansen, and Epple (23) have found that any saliva that is allowed to stagnate in the presence of sugar will decalcify teeth. Their studies have also shown that the rate of decalcification is greater when the saliva of a caries susceptible person is used than when the saliva is from a person who is caries resistant.

More recently T.J. Hill (45) has fractionated saliva and obtained an as yet unknown substance which inhibits the growth of _L. acidophilus_ in vitro. Greater quantities of this growth inhibiting fraction have been obtained from the saliva of caries resistant individuals than from the saliva of caries susceptible individuals. When injected into laboratory animals this unknown substance causes prolonged hyperglycemia, which suggests that it may inhibit bacterial growth by interfering with carbohydrate metabolism.

DENTAL CARIES AND BODY MEASUREMENTS

The author has found no reports of experiments designed for the specific purpose of correlating tooth decay with weight and height. However, some attempts at such correlations were found in experiments designed primarily for other purposes. Brodsky,[16] in an investigation of the effect of diet on caries in humans found no correlation between weight or height and the incidence of caries. Similarly, Marshal[39] found no correlation between growth rate and dental caries in rats fed a diet deficient in vitamin D. Hurme,[11] examined 183 first year dental students at Tufts College and observed a relationship between height and tooth decay when he compared the 38 shortest men with the 32 tallest men in the group. On this basis the taller men appeared to have a higher incidence of caries than the shorter men.

DENTAL CARIES AND HEREDITY

There is, as yet, insufficient experimental evidence available to definitely link heredity with dental caries in man. However, many investigators believe that heredity is a factor in susceptibility to tooth decay. As previously mentioned, Bunting believes that inherited tendencies are more important than diet in caries susceptibility. Hanke (5) found great variability in susceptibility among children. When treating 323 children with a diet designed to inhibit caries he found that, although the improved diet greatly reduced the incidence of caries in every case, the relative incidence remained unchanged. He suggested that this consistency may be due to an hereditary factor. Hurme, (11) in an analysis of 108 case histories, found that (...) "most of the better-than-average dental heredities occurred among the men showing the best dental conditions." Klein and Palmer (11) found strong familial resemblences in children as evidenced by the fact that brothers and sisters of caries resistant children had only one-half as much caries as did the brothers and sisters of children who were caries susceptible. It is apparent, however, that these familial resemblances may be due to the tendency for members of the same family to eat the same food rather than to heredity.

Early in 1937 Dr. H. R. Hunt and Dr. C. A. Hoppert, of Michigan State College, began an investigation of the role of heredity in tooth decay, using the albino rat. This study, which is still in progress, is apparently the first genetic experiment concerning susceptibility and resistance to tooth decay.

Hoppert and his associates devised a diet which would

satisfactorily promote health and fertility in the albino rat and also produce caries. This cariogenic diet consisted of 66 percent coarsely ground hulled rice, 30 percent whole milk powder, 3 percent alfalfa leaf meal, and 1 percent sodium chloride by weight. Hunt and Hoppert [34] used this diet, plus a carefully controlled program of selection, progeny testing, and close inbreeding to produce two distinct strains of rats, one caries resistant, the other caries susceptible.

Hoppert, Weber, and Caniff [28] observed that the fineness of the rice particles in the diet influenced the development of caries. Braunschneider [15] showed that when the rice was ground to the fineness of flour the development of caries was delayed significantly in the susceptible strain. In order to control the size of the particles, the rice was ground with a precision grinder and periodically checked by screening. The first fifteen generations of the susceptible strain, and the first twelve generations of the resistant strain were fed a diet in which the rice had been ground so that approximately seventy percent would be retained on a twenty-mesh screen.

Hunt and Hoppert [31] demonstrated that occlusion was a factor in tooth decay. They found that when upper molars were severely injured, or destroyed, caries was delayed in the opposing lower molars. In order to reduce fracturing of the upper molars the cariogenic diet was revised. Beginning with the sixteenth generation of the susceptible strain, and the thirteenth generation of the resistant strain, the rice was ground so that approximately two percent would be retained on a twenty-mesh screen. This greater fineness of the rice particles increased the time necessary for development of caries but did not alter the relative difference between the two strains. The use of this fine rice satisfactorily reduced the injury to the upper molars.

Braunschneider (15) demonstrated that age is a factor in resistance to tooth decay in the rat. The susceptible animals appear to become less susceptible with advancing age.

Keller (37) has shown that the incidence of caries in the upper molars of these animals is significantly less than in the lower molars. He has also shown (unpublished data) that the secretions of the parotid glands are not significant factors in susceptibility to tooth decay.

Hoppert and Shirley (27) found no difference in the rate of deposition and removal of radioactive phosphorus in the teeth of the two strains. In addition, they observed only slight differences between the two strains in the weight, percent of ash, and percent of phosphorus in the molar teeth. The caries resistant rats appear to have lighter teeth containing more ash and phosphorus than the susceptibles.

Hunt and Hoppert (32) have also shown that the sex of the animal does not significantly influence its resistance to tooth decay.

From the foregoing survey of the experiment being conducted by Hunt and Hoppert, it is evident that the incidence of caries in the two strains of rats which they have developed is influenced by several factors both environmental and physiological. It is, therefore, desirable to know as much as possible about these animals in order to achieve the ultimate goal of the experiment which is the determination of the number of genes involved in resistance to tooth decay, and their mode of action.

AIM OF THIS EXPERIMENT

Mr. Earl Harrison, our animal caretaker, believed that there was a difference in the weight and pilosity of the two strains. The caries susceptible animals appeared to weigh less and have less hair than the resistant animals. The question arose as to whether or not there was some fundamental difference in the physiology of these two strains of rats which was expressing itself through body weight and susceptibility to tooth decay.

The possibility of a difference in hair density and skin condition was especially interesting in view of the fact that hair, skin, and teeth have a common embryonic origin, the ectoderm. The question also arose as to whether or not weight, skin, and hair abnormalities might not be symptoms of a thyroid gland dysfunction in one of the strains. These experiments are concerned with determining whether or not these suspected differences in weight and pilosity actually exist.

METHODS AND MATERIALS

The first problem which was undertaken was the determination of growth curves for both the resistant and susceptible strains. The animals in this experiment were produced by the same breeding pairs used by Dr. Hunt to breed his 20th generation susceptibles and 16th generation resistant animals. All but five of the animals used were brothers or sisters of those bred for the main experiment. This close relationship greatly increases the probability that the results obtained are valid for the two strains as a whole as well as for the specific animals used.

The animals used in this experiment lived in the same environment as those which constituted the main breeding lines of the two strains. They were housed in 12"x 14"x 20" cages made of galvanized steel. The floor of each cage was a removable tray containing wood shavings used as litter. The cages were cleaned and the wood shavings renewed every ten days throughout the experiment. Each cage housed, on the average, four animals all of the same sex and strain. All of the animals used in this experiment as well as those of the main experiment were housed in the Zoology Animal House. The temperature in this building was automatically maintained at 78°F., except during the hottest summer months.

The rats were constantly supplied with the revised Hoppert diet[31] consisting of 66 percent coarsely ground hulled rice, 30 percent whole milk powder, 3 percent alfalfa leaf meal, and 1 percent sodium chloride. The rice was precision ground so that approximately 2 percent would be retained on a 20 mesh screen. The animals received water from the college water supply by means of a drip bottle placed in the top of each cage.

Females were removed from the breeding cages upon showing signs of pregnancy. Each was isolated in a cage containing a small wooden house and a handful of paper strips. These materials supplied the animals with shelter and means for making a suitable nest which served to reduce the occurrence of infanticide. Each isolated female was observed daily for the presence of young and the date of the first observation of young was recorded as their birth date. The young were removed from their parents when they were twenty-five days of age. Previous to this time they had weaned themselves and begun to eat the cariogenic diet supplied for the parent.

Careful records were kept of all matings, isolation times, dates of birth and dates of separation of young and parent. Since the first weighing took place when the animals were three weeks of age, it was necessary to mark them prior to this time, usually when they were nineteen days old. They were marked (numbered) by a system of ear notches. Despite the fact that it was necessary to mark the young at an early age and replace them with the parent, no young were lost in the process. Loss of young was feared because the marking process necessitates the shedding of a certain amount of blood and in some instances the presence of blood has been observed to cause the wholesale slaughter of the young by the mother.

All of the methods mentioned above are identical with those of Hunt and Hoppert with the exception of the fact that it was necessary to mark the animals used in this experiment before they were 21 days old. The rats used in the main experiment are not marked until they are approximately 35 days of age.

As previously mentioned, each animal was weighed for the first time when it was three weeks of age. Following this they were weighed each succeeding week on their weekly birthdays until they reached the age of twenty-eight weeks. As each animal reached this age its weighing schedule was shifted to a four week interval and the weighings were continued until the animal was fifty-two weeks old. As an example; if a given litter were born on Sunday then all members of that litter would be weighed on the third Sunday after birth and each Sunday thereafter until they were twenty-eight weeks of age. Following their 28th birthday they would be weighed every fourth Sunday until they were fifty-two weeks old.

A total of 212 animals were bred for this experiment. However, due to premature deaths and the discarding of some of the late arrivals, all of these animals were not involved in every weighing. There were an average of 43 susceptible and 43 resistant females per weighing, and 46 susceptible and 56 resistant males per weighing. Tables No. 1 & 2 show the number of animals of each sex and strain used to determine the

TABLE NO.1

Average Weights Of Males For All Weighings.

RESISTANT STRAIN			SUSCEPTIBLE STRAIN		
WEEKS OF AGE	NUMBER OF ANIMALS	AVERAGE WEIGHT	WEEKS OF AGE	NUMBER OF ANIMALS	AVERAGE WEIGHTS
3	35	32.8	3	40	34.3
4	40	51.6	4	38	56.9
5	42	72.2	5	37	76.0
6	46	92.4	6	40	93.8
7	53	115.8	7	48	115.5
8	57	138.8	8	48	138.4
9	57	160.6	9	48	160.1
10	57	182.7	10	48	177.9
11	57	200.7	11	48	196.2
12	57	217.1	12	48	214.2
13	57	230.6	13	48	230.2
14	57	246.6	14	48	244.7
15	57	255.7	15	48	257.1
16	57	266.7	16	48	267.5
17	57	276.1	17	48	276.5
18	57	283.8	18	48	284.6
19	57	291.9	19	48	293.0
20	57	299.9	20	48	299.7
21	57	305.8	21	48	302.5
22	57	310.4	22	45	309.4
23	57	317.1	23	44	313.2

TABLE NO. 1 Continued

RESISTANT STRAIN			SUSCEPTIBLE STRAIN		
WEEKS OF AGE	NUMBER OF ANIMALS	AVERAGE WEIGHT	WEEKS OF AGE	NUMBER OF ANIMALS	AVERAGE WEIGHT
24	57	321.9	24	44	315.4
25	57	327.1	25	44	315.4
26	57	332.8	26	47	322.1
27	57	336.5	27	47	320.8
28	57	339.6	28	47	328.3
32	52	352.6	32	47	341.1
36	52	367.3	36	47	354.0
40	52	377.6	40	47	360.4
44	56	386.7	44	47	368.0
48	56	396.3	48	47	371.3
52	55	403.2	52	47	377.7

TABLE NO.2

Average Weights Of Females For All Weighings.

RESISTANT STRAIN			SUSCEPTIBLE STRAIN		
WEEKS OF AGE	NUMBER OF ANIMALS	AVERAGE WEIGHT	WEEKS OF AGE	NUMBER OF ANIMALS	AVERAGE WEIGHT
3	34	32.6	3	40	33.4
4	35	49.8	4	39	52.2
5	43	66.5	5	38	68.8
6	44	81.6	6	46	85.3
7	44	93.6	7	46	97.4
8	44	110.7	8	46	107.7
9	44	121.6	9	46	118.1
10	44	131.3	10	45	127.3
11	44	139.2	11	44	136.5
12	44	147.5	12	44	144.9
13	44	153.2	13	44	150.9
14	44	158.5	14	44	157.3
15	44	161.3	15	42	161.1
16	44	165.6	16	44	166.6
17	44	169.2	17	44	170.6
18	44	173.6	18	44	174.1
19	44	176.7	19	44	177.5
20	44	177.8	20	44	181.0
21	44	181.1	21	42	181.6
22	44	182.3	22	41	184.6
23	44	185.6	23	44	188.6

TABLE NO. 2 Continued

RESISTANT STRAIN			SUSCEPTIBLE STRAIN		
WEEKS OF AGE	NUMBER OF ANIMALS	AVERAGE WEIGHT	WEEKS OF AGE	NUMBER OF ANIMALS	AVERAGE WEIGHT
24	44	188.5	24	44	189.7
25	44	190.1	25	44	192.5
26	44	192.5	26	44	193.5
27	44	196.2	27	44	194.4
28	44	197.6	28	44	196.3
32	44	202.8	32	43	203.8
36	44	210.7	36	43	205.1
40	44	214.1	40	41	206.2
44	44	217.9	44	41	206.6
48	44	221.9	48	40	208.9
52	43	224.7	52	40	209.8

average weight at each age.

A triple beam trip scale was used to weigh the animals. At the beginning of each day's weighing the scale was counterbalanced for the weight of the container into which the rats were put for weighing. Thus the weight of the animal could be read directly from the scale. During each series of weighings the accuracy of the counterbalance was checked repeatedly. A satisfactory container was made from a large cylindrical cereal box with all inner surfaces waterproofed by a generous coating of automobile top dressing.

Errors in weighing due to movements by the animal were kept at a minimum throughout the experiment. The use of an anesthetic was considered impractical due to the frequency with which the rats were weighed. The procedure followed in weighing the animals was as follows:

1. The rat's number was determined with as little disturbance as possible, usually without touching it.
2. The scale was set for the estimated weight of the animal.
3. The animal was placed in the container and the weight determined.

The essential factors for success in this procedure were speed and accuracy. When the animals were suddenly placed in complete darkness in the presence of new and strange odors they almost invariably responded with at least five seconds of absolute quiescense. Young animals remained quiet for considerably greater lengths of time. Thus, by the time any one individual was old enough to conquer fear it had established a growth pattern which enabled the author to estimate its weight accurately (usually within ten grams) so that only the small sliding weight need be moved to balance the scale. The weights were recorded to the one-tenth gram, but under these conditions they were probably not accurate beyond plus or minus one gram.

The animals used in establishing the growth curves were not examined regularly for caries. However, one examination was made to determine whether or not they were typical of the two strains. At the time of this examination the resistants had an average age of approximately 155 days and the susceptibles an average age of approximately 104 days. Among the resistants there were only three doubtful cases of caries, while among the susceptibles only three animals did not have caries. Most of the susceptible rats had badly decayed lower molars, all of the molars on at least one side of the jaw being completely destroyed. This examination established the fact that the animals being used in this experiment were typical of the two strains with respect to tooth decay.

ANALYSIS OF DATA

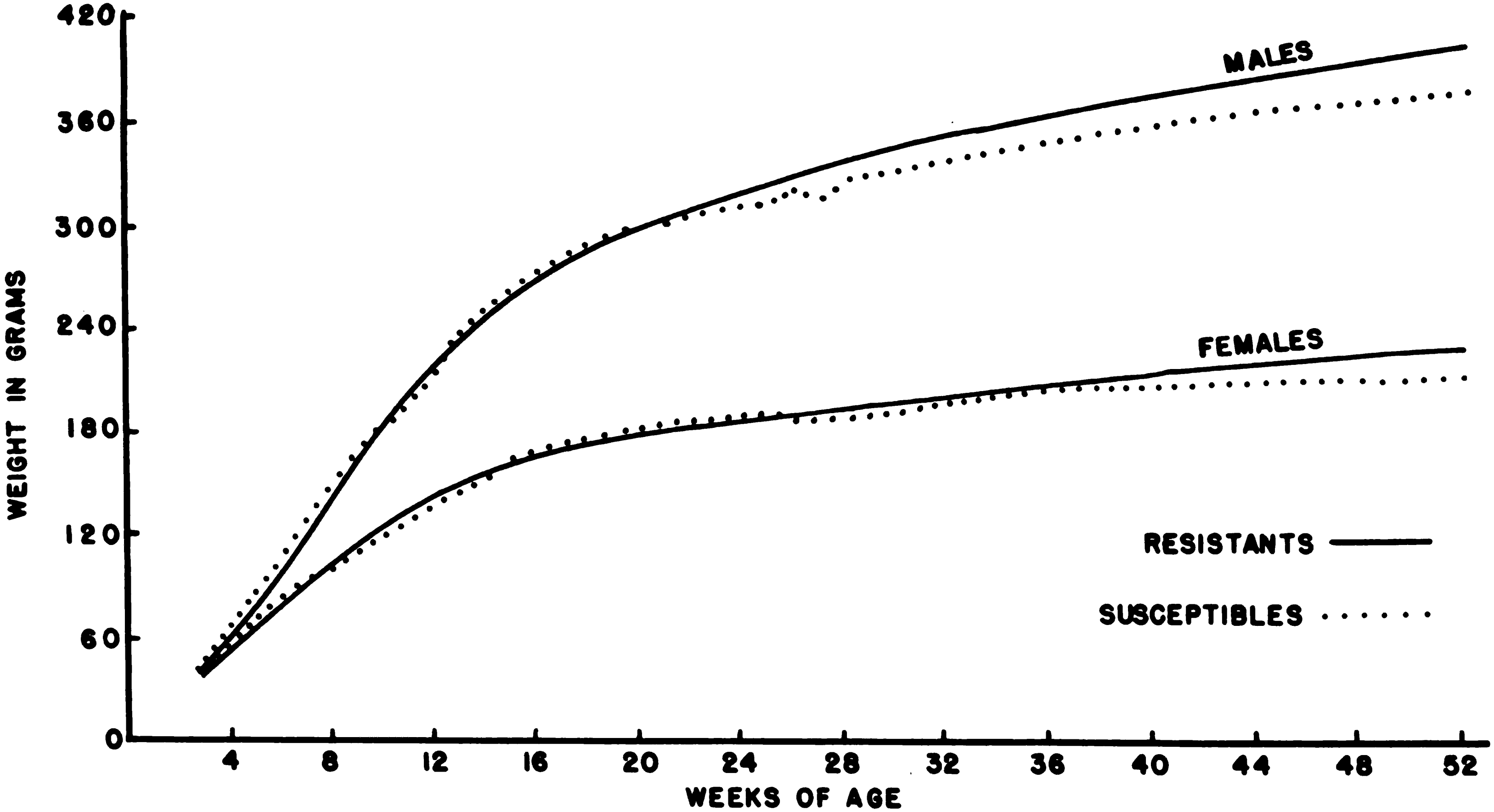
As previously mentioned, Tables No. 1 and 2 show the number of animals and average weight for both sexes of both strains at each weighing. Plate No. 1 is a graphic representation of Tables 1 and 2. The "t" test was arbitrarily chosen as a means for comparing the weights of these two groups of animals. It was advisable to compare males with males and females with females in order to bring out more clearly the effects of the genetic background of the two groups. Thus, with the sexes separate, there is only one criterion of classification, namely strains, and the "F" test if used, would be nothing more than the square of the "t" value[10]. The "t" values were determined according to the following formula[10]:

$$t = (M_x - M_y)\sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{(n_1 + n_2) Sx^2}}$$

where,

$$Sx^2 = \frac{\sum (x - M_x)^2 + \sum (y - M_y)^2}{n_1 + n_2 - 2}$$

The growth curves on page 26 show that during the period of initial growth the weights and rates of growth in the two strains were remarkably similar. The average weights of the males do not differ noticeably until they have attained the age



BODY WEIGHT OF CARIES RESISTANT AND CARIES SUSCEPTIBLE ALBINO RATS of twenty-one weeks. No noticeable difference appears in the females until the age of approximately forty weeks. Thus the curve appeared to consist of two parts, the period of rapid growth during which the strains did not appear to differ in weight, and the period of slow, mature growth, when there was an increasing difference in the average weights of the two strains. In order to check the accuracy of these observations the mean weights were first compared at three weeks, twenty-one weeks, and fifty-two weeks of age.

At three weeks of age the susceptible males had an average weight of 34.3 grams with a standard deviation of 6.01 grams as compared to an average weight of 32.8 grams and a standard deviation of 4.84 grams in the resistant strain. The "t" value was 1.20 which is not significant. At the same age the susceptible females had an average weight of 33.4 grams and a standard deviation of 5.76 grams while the resistant females had an average weight of 32.6 grams and a standard deviation of 3.91. The "t" value was .61 which is not significant.

The weights were next compared at twenty-one weeks of age, the point at which the growth curves of the males diverge, and the period of rapid growth is ended in both sexes. At this age the susceptible males had an average weight of 302.5 grams with a standard deviation of 38.53 as compared to an average weight of 305.8 grams and a standard deviation of 32.36 for the resistants. The "t" value was .151 which is not sighificant. Susceptible females , twenty-one weeks old, had an average weight of 181.6 grams with a standard deviation of 20.4 as compared with an

average weight of 181.1 grams and a standard deviation of 13.1 for the resistant females. The "t" value was .136 which is not significant.

At fifty-two weeks of age the susceptible males had an average weight of 377.7 grams with a standard deviation of 48.2. The resistant males had a mean weight of 403.2 grams with a standard deviation of 44.2 at this age. The "t" value was 2.77 which is significant at the one percent level. Susceptible females of this age had an average weight of 209.8 grams with a standard deviation of 25.94 as compared with an average weight of 224.7 grams and a standard deviation of 15.96 for the resistant females. The "t" value was 3.176 which is significant at the one percent level.

It was felt that further information could be gained by an analysis of the amount of weight gained rather than of the weights themselves. This analysis showed that the susceptible males gained an average of 164.1 grams between eight weeks and twenty-one weeks of age. The standard deviation was 32.18 grams. During the same period the resistant males gained an average of 166.9 grams with a standard deviation of 22.55. The "t" value was .4802 which is not significant. The susceptible females gained an average of 74.9 grams with a standard deviation of 14.9 during this period as compared with an average weight gain of 70.4 grams with a standard deviation of 12.3 for the resistant females. The "t" value was 1.85 which is not significant.

During the period from twenty-one to fifty-two weeks of age, the susceptible males gained an average of 74.1 grams with a standard deviation of 24.8 while the resistant males gained 96.9 grams with a standard deviation of 20.5. The "t" value was 5.089 which is significant at the one percent level.

From the foregoing analysis it is clear that at the end of a year's growth the caries resistant animals were significantly heavier than those that were caries susceptible. It is also clear that the growth characteristics of the two strains do not differ until the animals have reached maturity.

Tables No. 3 and 4 show the complete analysis. From it we may observe a gradual increase in the "t" values until, at the relatively advanced age of forty-four weeks, they finally become statistically significant at the five percent level.

According to Donaldson[3], the rat may be regarded as living approximately thirty times as fast as the human, since such factors as the time necessary to double the birth weight, and to reach the menopause, are approximately thirty times greater in man than in the rat. When this relationship is used to estimate the relative age of the animals used in this experiment, the age at which the first significant difference in their average weights occurs is comparable to that of a man approximately twenty-six years of age. Tables No. 3 and 4 also show the coefficients of variation at various ages.

The coefficient of variation is obtained by dividing the standard deviation by the mean, and is more valuable than the standard deviation as an indication of variation, since it shows

TABLE NO. 3. Comparisons Of Average Weights Of Males At Various Ages.

STRAIN	WEEKS OF AGE	AVERAGE WEIGHT	STANDARD DEVIATION	"t"-VALUE	COEFFICIENT OF VARIATION(1)
Resistant	3	32.8	4.84	1.20	14.76
Susceptible	3	34.3	6.01		17.52
Resistant	8	138.85	18.64	.130	13.42
Susceptible	8	138.40	16.32		11.79
Resistant	21	305.8	32.36	.151	10.58
Susceptible	21	302.5	38.53		12.74
Resistant	36	367.3	41.35	1.51	11.26
Susceptible	36	354.0	46.04		13.00
Resistant	40	377.6	41.10	1.96	10.88
Susceptible	40	360.4	46.30		12.85
Resistant	44	386.7	43.04	2.10*	11.13
Susceptible	44	368.0	47.21		12.83
Resistant	52	403.2	44.20	2.77**	10.96
Susceptible	52	377.7	48.2		12.76

* Significant at the five percent level.

** Significant at the one percent level.

(1.) The coefficients of variation are given in percent throughout this report.

TABLE NO. 4. Comparisons Of Average Weights Of Females At Various Ages

STRAIN	WEEKS OF AGE	AVERAGE WEIGHT	STANDARD DEVIATION	"t"-VALUE	COEFFICIENT OF VARIATION
Resistant	3	32.6	3.91	.610	11.99
Susceptible	3	33.4	5.76		17.25
Resistant	8	110.7(1)	11.43	1.59	10.33
Susceptible	8	106.7	11.95		11.99
Resistant	21	181.1	13.10	.136	7.23
Susceptible	21	181.6	20.40		11.23
Resistant	36	210.7	14.1	1.42	6.69
Susceptible	36	205.1	21.9		10.65
Resistant	40	214.1	15.60	1.86	7.29
Susceptible	40	206.2	23.10		11.20
Resistant	44	217.9	17.83	2.52*	8.18
Susceptible	44	206.6	23.27		11.26
Resistant	52	224.7	15.96	3.18**	7.10
Susceptible	52	209.8	25.94		12.36

* Significant at the five percent level.

** Significant at the one percent level.

(1) Females No. 74, 91, and 92 not included in this average.

the <u>relative</u> variability. It is interesting to note that the susceptible animals, both male and female, showed a greater variability than the resistants. Donaldson [3] gives the coefficient of variation for the male albino rat as approximately 14 percent between the ages of 90 and 243 days. During a comparable age period the male rats used in this experiment showed a coefficient of variation of 12.6 percent. One would expect a lower coefficient of variation in these rats since they are the result of a prolonged and intensive program of inbreeding. It is well known that inbreeding tends to reduce variability and H. D. King [8], in her studies on inbreeding, reports a reduction of variability in the weight of the albino rat.

Although it had already been established that no significant difference existed between the average weights of the strains (see Tables 3 and 4) at three weeks of age, further information was sought concerning the possibility that a great difference in size of litters with a high correlation between litter size and growth rate might be factors influencing the weight and growth of these two strains. Investigation of differences in litter size seemed especially pertinent since some difficulty had been experienced in breeding animals of the caries susceptible strain. Table No. 5 shows the results of such analyses. The correlation coefficients were determined according to the following formusa [7] :

TABLE NO. 5

(1) Comparisons Of Average Litter Size.

STRAIN	NO. OF LITTERS	AVERAGE SIZE	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Resistant	12	6.25	1.13	18.1
Susceptible	18	4.94	2.48	50.2

"t" 1.58[1]

(2) Coefficients Of Correlation Between Litter Size And Body Weight At 21 Days Of Age

Resistant Strain............................. -.70±.82

Susceptible Strain........................... -.53±2.10

(1) With 28 degrees of freedom a "t" value of 2.048 is necessary for significance at the five percent level.

$$r_{xy} = \frac{\sum(X-M_x)\ (Y-M_y}{ns_x s_y}$$

The susceptible strain shows an average litter size of 4.94 with standard deviation 2.48 as compared with an average litter size for the resistants of 6.25 with standard deviation 1.13. The coefficients of correlation are $-.53 \pm 2.10$ and $-.70 \pm .80$ for the two strains respectively.

When the mean litter size of the two strains were compared. a "t" value of 1.58 was obtained. This value is not significant. However, the resistant strain had a coefficient of variation of only 18.1 percent as compared to 50.2 percent for the susceptible strain. This suggested that the variances of the two samples were significantly different and, consequently, the results of the "t" test meaningless.

A comparison of the variances resulted in an "F" value of 4.79 which is significant. In view of the fact that the variance was not the same in the two samples, the mean litter sizes were compared according to the method of Cochran and Cox, as given by Snedecor[10]. When the means were compared in this manner a "t" value of 1.84 was obtained. This value is not significant, although it approaches significance more closely than did the previous "t" value of 1.58.

CONCLUSIONS AND DISCUSSION

The growth curves which were obtained for the two strains, and statistical comparisons of the average weights, show that at approximately one year of age the caries susceptible animals are of significantly lighter weight than the resistants. At this age the average weight of the susceptible males is 25.5 grams less than the average weight of the resistant males. At the same age the susceptible females average 14.9 grams less than the resistant females.

The data obtained in this experiment are not sufficient to warrent any real conclusion concerning a possible connection between body weight and tooth decay. However, the evidence seems to indicate that, if the two phenomena are related then lack of weight is an effect of tooth decay rather than a cause. This hypothesis is based on the following facts;

1. The caries susceptible animals used in this experiment were shown to have advanced caries at an average age of 104 days.

2. Brothers and sisters of the animals used in this experiment first developed caries at an average age of 66 days as determined by Hunt and Hoppert.

3. The first noticeable difference in the average weights of the two strains did not occur until the animals were 147 days old.

This sequence of occurrence indicates that the susceptible animals weigh less because they have caries rather than the reverse relationship of caries resulting from whatever factors cause loss of weight.

The lighter weight of the animals of the susceptible strain, and the greater variability in their weight, may be caused by an inability to chew properly due to the destruction of their teeth by caries. Such an hypothesis is weakened, however, by the fact that the diet is ground to such a degree of fineness that chewing may not be necessary.

Statistical analysis has shown that the average litter size of the two strains do not differ significantly in the animals used for this experiment. Analysis has also shown that the variability in the litter size of these animals does differ significantly.

However, a significant difference in the litter size would have had no apparent meaning biologically if it had occurred. Table No.5 shows that the caries susceptible animals are born in smaller litters than the resistants, and that there is a negative correlation between litter size and body weight at three weeks of age. Accordingly, the effect of litter size would be to cause the susceptible animals to weigh more than the resistants rather than less. In addition, the effect of litter size is minimized by the fact that the weight differences, with which we are concerned, do not occur until the animals have reached a relatively advanced age.

The greater variability of the susceptible strain with respect to litter size is of particular interest in view of the fact that Hunt and Hoppert have shown that, with respect to tooth decay, the susceptible strain is less variable than the resistant.

From the foregoing discussion it is clear that, while this experiment has established the existence of a difference in the weight of caries resistant and caries susceptible rats, determination of the causes of this difference must await further experimentation.

DETERMINATION OF HAIR DENSITY

As previously mentioned (page 15), general observation of the animals had led to the suspicion that the caries susceptible rats had less hair than the resistants, particularly at an advanced age. This experiment was undertaken to determine the accuracy of this suspicion, and serve as a guide to indicate whether or not further, more detailed investigation is feasible. This experiment was intended solely as a gross comparison of the hair densities of the two strains and is not a study of fur quality.

The comparisons of density were first made by a visual grading system. Histograms based on data obtained in this manner indicated a difference in the quantity of hair in mature animals of the two strains. When the rats are young there is no observable difference between members of the two strains. Susceptible animals, as well as resistant ones, have full glossy coats typical of the albino rat. Observable differences do not begin to appear until the animals are two hundred or more days old. For this reason I believe the problem is not that of a difference in the number of hair follicles, or of an inherent difference in hair quality, but simply a loss of hair at advanced ages. As the caries susceptible animals grow older, particularly at ages of a year or more, their fur thins out so that the color of the skin is visible. Many animals develop bald spots and the skin appears dry and wrinkled in these areas. Obviously, if these symptoms appeared only among the susceptible animals, the matter would have been settled without experimentation. This was not the case however. The same symptoms appeared in caries resistant animals, but they did not seem to occur as frequently or be as severe. This experiment was designed to determine whether or not there is a greater loss of hair in the susceptible strain than in the resistant strain by comparing the weight of hair per unit area of body surface.

METHODS AND MATERIALS

The rats used in determining variation in hair density were chosen from among those used for determining the growth rates. Since age appeared to be a factor in causing loss of hair the animals used were chosen as near to one age as possible. Twenty-five animals of each of the two sexes and strains were selected. The average age of the caries susceptible animals was 388 days as compared to an average age of 391 days for the resistants. The age of 84 per cent of the one hundred animals used was between 386 and 390 days. This grouping eliminated age as a factor in the comparisons.

The method used in removing the hair sample from each was essentially the same as used by Wentze[44] in her studies on fur quality in mink.

The procedure was as follows:

1. The animal was etherized and placed on its left side.

2. The hair on the exposed right side was moistened and brushed.

3. High-speed electric hair clippers were used to clip the hair from an area approximately four centimeters long between the shoulder and the flank.

4. The mass of hair was put into a small manila envelope and the area was brushed to obtain any other hair which had not clung to the larger mass as it curled up from the clippers.

5. The two parallel sides of the clipped area were carefully measured and the measurements along with the animal's number, strain, sex, date of birth, and date of clipping were recorded on the sealed envelope.

Throughout the experiment the cutting edge of the clipper was set for the closest cut. The clipper was moved very slowly over the area and always in the direction from the shoulder toward the flank. Nothing but a hardly visible fuzz remained on the area after the first run of the clippers. This uncut hair, less than one sixteenth of an inch in length, was regarded as a constant quantity due to the construction of the clippers. For this reason, and in order not to distort the shape of the area or inadvertently clip one animal more than another, each area was clipped only once.

The problem of measuring the dimensions of the area was simplified by the fact that the animals were etherized and did not

contract their skin at the touch of the rule used in measuring. All measurements were made while the animal's skin was in a relaxed state. The success of this method of determination of hair density depends to a large extent upon accuracy in calculating the area from which the hair was clipped. Calculation of this area was based on the premise that the width would be a constant quantity equal to the width of the cutting edge of the clipper, which was four centimeters. Thus two sides would be parallel and the area could be calculated by the formula for the area of a trapozoid which is,(6)

$$A = \frac{1}{2}(a + b)h$$

where,

a and b are the length of the parallel sides and h the altitude.

After the samples were obtained they were first thoroughly dried and then cleaned by removing all foreign matter by means of tweezers. Each sample was weighed immediately after it had been cleaned. The weight of the sample was then divided by the area from which it had been taken so that comparisons could be made on the basis of milligrams of hair per square centimeter of body area.

A minimum number of steps is essential in an experiment of this nature. At any time when the hair samples were out of the sealed envelopes they were subject to dispersal by uncontrollable air currents, and the opening of a door or an accidental cough or sneeze would have sufficed to terminate the experiment.

ANALYSIS OF DATA

A series of "t" tests were again used for comparing means, although the "F" test would have been more concise perhaps. However, for the sake of clarity, Table No. 6 shows the comparisons between the two sexes as well as between the two strains. The "t" values obtained when the sexes were compared showed no significance between sexes in either strain. The "t" values resulting from comparison of the two strains were significant. The susceptible males had an average hair density of 6.30 mg/sq. cm. with a standard deviation of 1.47 as compared with an average hair density of 8.18 mg/sq. cm. and standard deviation of 2.12 in the resistant males. The "t" value was 3.62 which is significant at the one percent level. The female resistants had an average hair density of 9.26 mg/sq.cm. and standard deviation of 2.92 as compared to an average of 6.36 mg/sq.cm. with a standard deviation of 1.50 for the susceptibles. The "t" value was 4.43 which is significant at the one percent level. As is indicated by the coefficients of variation shown in Table No. 7, the relative variation in the two strains is more uniform in the case of hair density than it was in the case of body weight. These results clearly indicate that at an advanced age caries susceptible rats have less hair than the caries resistant animals.

Since the susceptible strain does weigh less and have less hair than the resistant it was hoped that these two phenomena could be shown to be highly correlated. It seemed logical to

TABLE NO. 6. Comparisons Of Hair Density

(1) Comparison of males and females of the same strain.

	NO. OF ANIMALS	HAIR DENSITY	STANDARD DEVIATION	"t"-VALUE	COEFFICIENT OF VARIATION
Resistant Males	25	8.18	2.12	1.51	25.9
Resistant Females	25	9.26	2.92		31.5
Susceptible Males	25	6.30	1.45	.124	23.0
Susceptible Females	25	6.36	1.50		23.5

(2) Comparison of resistant and susceptible animals of the same sex.

	NO. OF ANIMALS	HAIR DENSITY	STANDARD DEVIATION	"t"-VALUE	COEFFICIENT OF VARIATION
Resistant Males	25	8.18	2.12	3.62**	25.9
Susceptible Males	25	6.30	1.45		23.0
Resistant Females	25	9.26	2.92	4.43**	31.5
Susceptible Females	25	6.36	1.50		23.5

** Significant at the one percent level.

All values shown for hair density are in milligrams of hair per square centimeter of body area.

TABLE NO. 7

Coefficients Of Correlation Between Hair Density and Body Weight.

(1) Correlation between hair density and body weight at the age of three weeks.

Resistant Males....................	$.028 \pm 2.10$
Resistant Females..................	$.262 \pm 2.83$
Susceptible Males..................	$.110 \pm 1.42$
Susceptible Females................	$.152 \pm 1.48$

(2) Correlation between hair density and body weight at 364 days of age.

Resistant Males....................	$-.022 \pm 2.10$
Resistant Females..................	$-.038 \pm 2.90$
Susceptible Males..................	$-.092 \pm 1.43$
Susceptible Females................	$.035 \pm 1.48$

suppose that an animal which suffered greatly from loss of weight would also suffer greatly from loss of hair. However, when coeffecients of correlation between hair density and body weight were obtained, they were very small and afforded no statistical evidence to support the hypothesis that hair weight and body weight are related in these animals. Table No. 7 shows the values obtained in correlating hair density with body weight at twenty-one days and at three hundred and sixty-four days of age. The largest value obtained was $.262 \pm 2.83$ for the resistant females. None of the other values exceeded .20.

CONCLUSIONS AND DISCUSSION

Analysis of the average hair density in the two strains shows that the caries susceptible animals have less hair per unit area of body area than the resistants at the age of a year or more.

It has been shown previously that these same animals also weigh less than those of the resistant strain but statistical analysis indicates that the two phenomena are not related. A biological relationship may exist however, and further experimentation is necessary to determine whether or not lack of hair and failure to gain weight are in any way related with each other or with susceptibility to tooth decay.

SUMMARY

1. Caries susceptible rats have been shown to weigh significantly less than caries resistant rats at the age of approximately one year.

2. The weight of hair per unit of body area has been shown to be significantly less in caries susceptible rats than in caries resistant rats at ages of a year or more.

3. The variations in the weight of the caries susceptible rats have been shown to be greater than weight variations in the caries resistant rats.

4. The existence of differences in litter size and in variability of litter size have been indicated.

5. No significant correlation was found between hair density and body weight in either strain.

6. Evidence has been presented to support the hypothesis that loss of weight and lack of hair are, if at all related, results of dental caries rather than causes.

7. Suggestions have been made concerning a possible method by which tooth decay may cause loss of weight in these animals.

BIBLIOGRAPHY

Books

1. Brekhus, P. J., Your Teeth, Their Past, Present, and Probable Future, University of Minnesota Press, 1941

2. Bunting, R. W., Oral Pathology, Lea and Febiger, Philadelphia, 1929

3. Donaldson, H. H., The Rat, Memoirs of The Wistar Institute of Anatomy and Biology, No. 6, Philadelphia, 1924

4. Gottlieb, B., Dental Caries, Lea and Febiger, Philadelphia, 1947

5. Hanke, M. T., Diet and Dental Health, University of Chicago Press, 1933

6. Hodgman, C. D. (Compiled by), Mathematical Tables From the Handbook of Chemistry and Physics, 8th ed., Chemical Rubber Publishing co., Cleveland, Ohio, 1947

7. Hoel, P. J., Introduction to Mathematical Statistics, J. Wiley And Sons, Inc., New York, 1948

8. King, H. D., Studies on Inbreeding, The Wistar Institute Of Anatomy and Biology, Philadelphia, 1919

9. Kronfeld, R., Histopathology of The Teeth, Lea and Febiger, Philadelphia, 1949

10. Snedecor, G. W., Statistical Methods, 4th ed., Iowa State College Press, Ames Iowa, 1950

11. The American Dental Association, Dental Caries, Lancaster Press, Lancaster, Pa., 1939

12. Weinberger, B. W., An Introduction to The History of Dentistry, vol. 1, C. V. Mosby Co., St. Louis, 1948

Periodicals and Theses

13. Bodecker, C. F., "Permeability of the Enamel in Relation to Stains," Journal of the American Dental Association, vol. 10: p. 60, 1923

14. Boyd, J. D., and C. L. Drain, "The Arrest of Dental Caries in Childhood", Journal of the American Medical Association, vol. 90: p. 1867, June 1928

15. Braunschneider, G. E., H. R. Hunt, and C. A. Hoppert, "The Influence of Age in the Development of Dental Caries in the Rat", Journal of Dental Research, vol. 27: p. 154, 1948

16. Brodsky, R. H., "Factors in the Etiology and Arrest of Dental Caries", Journal of the American Dental Association, vol. 20: p. 1440, 1933

17. Bunting, R. W., "Diet and Dental Caries", Journal of the American Dental Association, vol. 22: p.114, 1935

18. Bunting, R. W., G. Nickerson, and D. G. Hard, "Further Studies of Bacillus Acidophilus in its Relation to Dental Caries", Journal of the American Dental Association, vol. 14: p. 1927

19. Cohen, M. M., " Clinical Studies of Dental Caries Susceptibility in Young Diabetics", Journal of the American Dental Association, vo;. 34: p. 239, 1947

20. Collins, R. O., L. J. Arthur, and H. Becks, "Study of Caries Free Individuals. II. Is an Optimum Diet or a Reduced Carbohydrate Intake Required to Arrest Dental Caries?", Journal of the American Dental Association, vol. 29: p.1169, 1942

21. Cox, G. J., M. M. Levin, and H. C. Hodge, "The Effect of Carbohydrates on Experimental Caries in the Rat", Journal of Dental Research, vol. 27: p. 562, 1948

22. Ferguson, R. A., "A Dental Survey of the School Children of American Samoa", Journal of the American Dental Association, vol. 21: p. 534, 1934

23. Fosdick, L. S., H. L. Hansen, and C. Epple, "Enamel Decalcification by Mouth Organisms and Dental Caries", Journal of the American Dental Association, vol. 24: p. 1275, 1937

24. Hanke, M. T., " The Relation of Diet to Caries and Other Dental Disorders", Journal of the American Dental Association, vol. 16: p. 2263, 1929

25. Hanke, M. T., "Relation of Diet to General Health and Particularly to Inflammation of the Oral Tissues and Dental Caries", Journal of the American Dental Association, vol. 17: p, 957, 1930

26. Harrison, R. W., "Lactobacilli Versus Streptococci in the Etiology of Dental Caries", Journal of the American Dental Association, vol. 37: p. 391, 1948

27. Hoppert, C. A., and R. L. Shirley, "The Use of Radioactive Phosphorus in the Study of the Teeth of Caries Resistant and Caries Susceptible Strains of Albino Rats", Journal of Dental Research, vol. 29: p. 29,1950

28. Hoppert, C. A., P. A. Webber, and T. L. Canniff, "The Production of Dental Caries in Rats Fed on an Adequate Diet", Journal of Dental Research, vol. 12: p. 161, 1932

29. Howe, P. R., "Diet as Related to the Prevention of Mouth Diseases", Journal of the American Dental Association, vol. 10: p. 755, 1923

30. Howe, P. R., "Further Studies of the Effect of Diet Upon The Teeth and Bones", Journal of the American Dental Association, vol. 10: p. 201, 1923

31. Hunt, H. R., and C. A. Hoppert, "Occlusion as a Factor in Dental Caries of Albino Rats", Journal of Dental Research, vol. 27: p. 553, 1948

32. Hunt, H. R., and C. A. Hoppert, "Sex and Dental Caries in Albino Rats", Journal of Dental Research, vol. 27: p. 486, 1948

33. Hunt, H. R., C. A. Hoppert, and W. G. Erwin, "Inheritance of Susceptibility to Caries in Albino Rats", Journal of Dental Research, vol. 23: p. 385, 1944

34. Hunt, H. R., and C. A. Hoppert, "Inheritance in Rat Caries", Genetics, vol. 24: p. 76, 1939

35. Jay, P., "Bacillus Acidophilus and Dental Caries", Journal of the American Dental Association, vol. 16: p. 231, 1929

36. Johnston, M. M., M. J. Kaake, and M. C. Agnew, "The Relationship of Lactobacillus Acidophilus to Dental Caries in Experimental Animals and in Human Beings", Journal of the American Dental Association, vol. 20: p. 1777 1933

37. Keller, R. F., H. R. Hunt, and C. A. Hoppert, "The Relative Incidence of Caries in Upper and Lower Molar Teeth of Albino Rats", Journal of Dental Research, vol 30: p. 382, 1951

38. Mann, A. W., S. Dreizen, T. D. Spies, and F. M. Hunt, " A Comparison of Dental Caries Activity in Malnourished and Well Nourished Patients", Journal of the American Dental Association, vol. 34 p. 244, 1947

39. Marshal, J. A., "Dental Caries and Pulp Sequelae Resulting From Experimental Diets", Journal of the American Dental Association, vol. 14: p. 3, 1927

40. McClure, E. J., and W. L. Hewitt, "The Relationship of Penicillin to Induced Rat Dental Caries and Oral L. Acidophilus," Journal of Dental Research, vol.25: p.441, 1946

41. Mellanby, M., "Experiments on Dogs, Rabbits, and Rats, and Investigations on Man, Which Indicate the Power of Certain Foods to Prevent and Control Dental Diseases", Journal of the American Dental Association, vo. 17: p. 1456, 1930

42. Sognnaes, R. F., "The Gross Morphology and the Histological Relationship of the Lamellae to the Organic Framework of the Enamal", Journal of Dental Research, vol. 29: p. 260, 1950

43. Waugh, L. M., Influences of Diet on the Jaws and Face of the American Eskimo", Journal of the American Dental Association, vol. 24: p. 1640, 1937

44. Wentz, P. E., "The Measurement of Quality in the Fur of Mink", Thesis for the M. S. Degree, Michigan State College, 1950

45. Hill, T. J., B. White, M. Matt, and S. Pearlman, " A Biologically Active Salivary Fraction Possibly Related to Caries Susceptibility", Journal of the American Dental Association, vol. 38: p. 656, 1949