A GENETIC STUDY OF DENTAL CARIES IN THE ALBINO RAT (RATTUS NORVEGICUS)

by

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A THESIS

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INTRODUCTION

Dental caries, one of the most common ailments of man, affects from 90 to 95 per cent of all children in this country. This disease has confronted man since the dawn of civilization. According to Lilly[23], the Egyptians three thousand years ago were using a preparation of flint, leaves, and honey to prevent caries, while at the same time the Chinese were using musk and salt for the same purpose. Aristotle was convinced that soft, sweet figs decayed the teeth, and Pliny advised cleaning the teeth to prevent caries. Still later the Romans used tooth picks and dentrifices. In the middle ages it was believed that tooth decay was due to worms in the teeth.

Dental caries is different from other diseases in that it is not accompanied by inflammatory reactions in the tissues involved. First the enamel and then the dentine are destroyed. Thus the factors which initiate the disease must, in the beginning at least, be outside the tooth and within the mouth. Most investigators of today agree that acids produced by bacteria living in the mouth, are the cause of tooth decay. Some of these investigators, however, think that these acids cannot act upon the tooth until it is weakened by innernal changes associated with metabolism. Regardless of what viewpoint is taken, the ecology of the mouth is undoubtedly a very important factor.

The fact that caries are rampant in many mouths, completely absent in others, and present in varying degrees in many

others, is a tantalizing problem. Individual differences in degrees of susceptibility or resistance to the disease are definitely known to exist. The problem that has attracted so many investigators in this field is the underlying cause of these differences. From the time Miller[25] announced his theory that tooth decay results from the activity of acids formed by the fermenting action of bacteria on food particles clinging to the teeth, scientific interest in the cause of caries has markedly increased. Since the turn of the century, especially in the last 15 years, the literature dealing with this subject has become voluminous.

It is desirable at this point to familiarize the reader with a few of the investigations in this field, and to present some of the theories as to the cause of the disease.

Literature on Dental Caries

Generally speaking, there are 3 theories which attempt to explain the cause of caries. Supporters of the first theory insist that diet and metabolism influence and alter the structure of the tooth during the life of the individual, and that these factors therefore are responsible for tooth decay. Advocates of the second theory maintain that changes in the contents of the saliva, or derangements in metabolism that have to do with the proper acid-base balance, constitute the most important causes. The third and most widely accepted theory holds that the primary causal agent is the acid liberated by certain bacteria acting upon food substances adhering to the teeth.

Vitamin deficiency is usually emphasized by most investigators who believe that diet and metabolic alterations are primarily responsible for tooth decay. Mellanby[24] admits that bacterial action is the immediate cause of caries, but she thinks that the practical solution is to be found by developing a resistance in the dental structures. She concludes from her studies that vitamin D, along with calcium and phosphorus, are essential if the tooth structures are to withstand bacterial action. She maintains that dentine may be weakened because of a deficiency of these substances, even after tooth eruption, and therefore tooth decay may ensue.

Contrary to this theory, Lilly[23] was unable to produce caries in rats placed on a rachitogenic diet, although his animals developed rickets and extreme bone and joint deformities. Hess and Abramson[11] studied the relationship of rickets and caries in children. They concluded that there is a lack of parallelism between the occurrence of rickets and the subsequent incidence of caries in deciduous teeth. They are, however, of the opinion that rickets is one of the several factors related to caries, and that this disease results from a systemic disturbance rather than from local factors. These investigators are opposed to the viewpoint that the lack of vitamin C is associated with tooth decay. Rosebury and Karshan[26] report that the addition of cod liver oil as 2 per cent of the basal diet, produced a definite reduction of caries in rats, but did not prevent the disease outright.

Hanke[8] studied the diets of 191 persons and concluded that

the lack of vitamin C may be an important factor in the initiation of caries. His data, however, do not adequately support his conclusion. The almost complete absence of tooth decay among the early Eskimos, as shown by studies of Leigh[22] and Goldstein[7] is difficult to explain on the basis of Hanke's theory. The diet of these early people contained little vitamin C. A study by Collins[6] of Eskimos living today shows that caries has increased among these natives in proportion to their contact with the white man's diet.

Bloch[3] made a study of the relationship of the lack of vitamin A and caries in 64 Danish children, all of whom were suffering from blindness because of xerophthalmia. He concluded that vitamin A has no effect on tooth structure and bears no relation with susceptibility to tooth decay. Boyd, Drain, and Nelson[1,2] studied a number of children under their care. They believe that an adequate amount of all the essential food factors, especially vitamins and minerals, is necessary to prevent caries. It is their opinion that an altered metabolism results in structural changes in the tooth, and that these influence the progress of caries.

Hawkins[9,10] defines dental caries as the disentigration of hard substances of the teeth by acids of fermentation, due to a lack of neutralizing salts in the saliva. He believes that the interprismatic substance is thicker in immune than in susceptible teeth, this thicker substance being due to precipitation of calcium from the saliva, and that this difference in thickness is a factor in immunity. Hawkins also maintains

that a sort of plaque is formed from the gluten of certain cereals. This plaque holds particles of starchy foods to the teeth where fermentation by bacteria takes place. Kesel[18] is rather critical of Hawkins' explaination, emphasizing that such plaques are probably just as imperveous to neutralizing saliva as they are to the confined acid. Klein and McCollum[19] also believe that the condition of the saliva is a factor in caries. They worked with about seven hundred rats and found that caries usually occurred when the calcium of the diet was high and the phosphorus low. When the ratio of these two elements was reversed, caries was not likely to develop. Kesel[18], in discussing the work of Klein and McCollum, points out that most of the rats developing caries consisted of females producing four or five litters of young a year. Male rats, except those on the most severe diets, developed little caries.

The Michigan Research Group reports studies which contradict the views of Hawkins, Klein, and McCollum. Hubbell and Bunting[14] made a study of a number of children, some of which had active caries while others were free from the disease. Certain additions were made to the diet of some of these children. The authors concluded that there is no relation between the calcium and phosphorus content of the saliva and occurrence of caries. Koehne and Bunting[21] report a study of institutional children who had a surprisingly low incidence of dental caries, although almost half of the diet consisted of carbohydrates. They made changes in the diet, increasing the available base 70 per cent, but found that the alkaline reserve was

increased only 8 per cent. They concluded that there was no correlation between dental findings and the carbon dioxide carrying power of the saliva or the calcium-phosphorus or vitamin D intake.

Hill[12], in a recent report, submits evidence of an unknown substance in the saliva which inhibits the growth of Lactobacillus acidophilus. He concludes that caries is associated with the presence of L. acidophilus in the saliva; that the saliva contains some unknown factor which affects the growth, in vitro, of these organisms; that time, increases in temperature, and dialyzation do not destroy this unknown factor; and that this factor can be removed by absorption into bodies of dead L. acidophilus organisms.

Jay, Crowley, Hadley, and Bunting[17] were unable to implant human strains of L. acidophilus in caries-free rats or in the mouths and intestinal tracts of 5 children who were caries free. They succeeded in producing wide fluctuations in the number of lactobacilli in the mouths of highly susceptible individuals by increasing and decreasing the amount of carbohydrate in the diet.

Koehne and Bunting[21] found a direct correlation between dental and bacteriological findings in 21 out of 25 individuals studied. Bunting, Jay, and Hard[5] report a study of caries in 3 orphanages. In 2 of the institutions sugars were eleminated except where absolutely necessary, and the diet was augmented by milk, fruit, and vegetables. No new evidence of caries activity was found in approximately 80 per

cent of the cases. In the third institution no changes were made in the diet. Here the children were given considerable candy. Only about 18 per cent of the children in this institution showed no new evidence of caries at the end of the study. Koehne[20] states that Bunting and collaegues are not as yet prepared to state whether these differences in the activity of L. acidophilus were due to the omission of sweets from the diet, or whether they resulted from the increased consumption of a well-balanced diet.

More recently, however, Jay[16], in summarizing a number of bacteriological studies of the Michigan Research Group, concludes that caries is not related to a nutritional adequacy of the diet, and that the disease is not arrested by supplying the diet with mineral and vitamin preparations. He states that there is a diognostic relationship between oral lactobacilli and dental caries activity, that the number of these lactobacilli is proportional to the amount of carbohydrate in the diet, and that caries can be checked by restricting the amount of carbohydrate eaten.

Hoppert, Webber, and Canniff[13] were successful in producing caries in rats almost at will by the inclusion of coarse particles of grain in the diet. Caries was most conspicuous when the diet contained particles of corn or rice retained in a 20-mesh sieve, and became correspondingly less evident when finer particles were used. When only particles which would pass through a 60-mesh sieve were used, no caries was produced. They found that the addition of liberal amounts

of vitamins A, C, or D, or of calcium and phosphorus, did not appreciably retard tooth decay. They conclude that two conditions are necessary for the decay of teeth. First is the nature and consistency of the food. The particles must be large enough and of the proper consistency to become impacted in the dental grooves. In Humans, they think that retention of food due to plasticity and adhesiveness is probably more important. The second factor is the presence and action of acidogenic bacteria. Such bacteria were found in every carious lesion examined. They believe that impacted particles of food provide an ideal place for the growth of these organisms in contact with the tooth surface, and that the acids produced by these bacteria disentigrate the enamel and dentine.

A History of the Study of Inherited Susceptibility and Resistance to Dental Caries

The foregoing resume of some of the literature on dental caries reveals that very few investigators have recognized the possibility that heredity might play an important role in the susceptibility and resistance to this disease. Bunting[4] suggests that heredity may be a factor which would explain why a small percentage of people are immune to caries.

Hunt and Hoppert are apparently the first to study the effect of heredity on susceptibility and resistance to this disease. Their study is being made possible by grants from The National Research Council Fund, The American College of Dentists, and the American Philosophical Society. Except for a preliminary report[15], their work is as yet unpublished.

They began their investigation with 119 rats from 3 different sources. Individuals which developed caries late were mated to start a caries resistant line, and others which showed caries early were bred to produce a susceptible strain. Before animals were used to continue either of the lines, they were progeny-tested to select the best genotypes for further breeding. The sixth generation of the susceptible line is now almost completed. It is becoming fixed rapidly with respect to this characteristic. The range of the time at which caries developed in this generation was from 11 to 55 days. It is unlikely that the few remaining animals, which are yet to develop caries, will alter these figures appreciably.

The inbreeding for the resistant line has been carried as far as the fourth generation. Although selection is tending to raise the average of the progeny of this line in each succeeding generation, there is still a wide degree of variation among the offspring. In the fourth generation the range in time required for the development of caries was from 44 to 294 days. Data on this generation are also not quite completed. These figures demonstrate a genetic difference in the susceptible and resistant lines of Hunt and Hoppert.

After Hunt and Hoppert had accumulated sufficient data to indicate that the tendency toward susceptibility and resistance to caries is at least partially inherited, the question of the mode of inheritance immediately arose. Why is

there such a wide variability in the time at which caries appears among individual rats of the same litter? Are these differences merely phenotypic, or do they represent genotypic differences? Do two rats having the same phenotypes also have similar genotypes? What would be the results in the F_1 and F_2 generations from P_1 crosses of susceptible X resistant animals? Such questions could not be adequately answered by the data secured by Hunt and Hoppert without making various crosses not directly concerned in developing homozygous susceptible and resistant lines. At least a partial solution of these questions might throw important light on some of their data. After a trait has been demonstrated to be hereditary, it is always desirable to know something about the mode of inheritance. For these reasons the author has attempted this study.

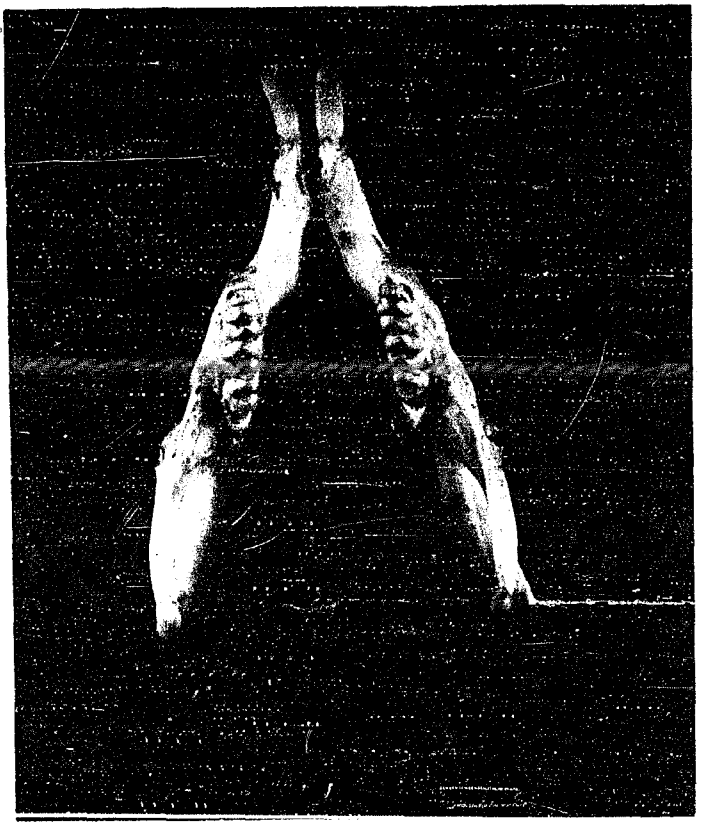

Figure 1

Lower jaw of a rat, showing no caries. Notice the dental grooves between the cusps.

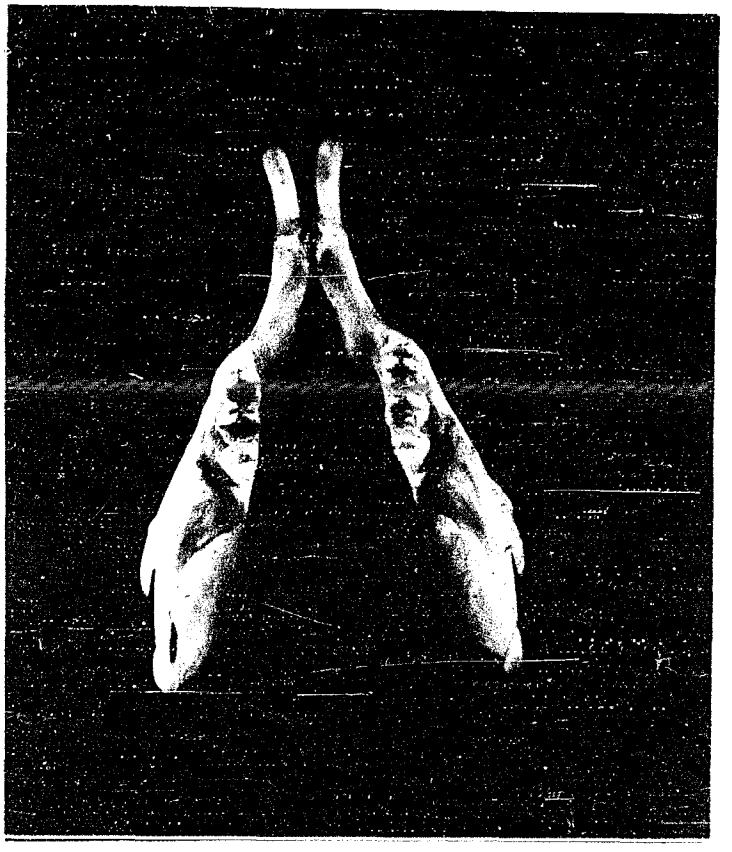

Figure 2

Lower jaw of a rat, showing caries. Two large cavities are seen on the left side in the first and second molars. Caries was just beginning in the first and second molars on the right side.

MATERIALS AND METHODS USED

In this study the albine rat (Rattus norvegicus) was used as the experimental animal. This species is suitable for such a study for a number of reasons. It is relatively small and is not too expensive to raise in large numbers. It produces several young per litter and has a gestation period of only 21 days. It is gentle and easily handled, an important point in a study of this type because the teeth were examined every two weeks. Although molars of the rat are somewhat different from those of humans, dental grooves are present in both. When maturity is reached the growth of the molars stops. They are subject to attrition just as the molars of humans are. One point of difference is the fact that rats do not have deciduous teeth.

This study followed the general procedure used by Hunt and Hoppert in their investigation. All rats were fed the Hoppert diet[13], consisting of 66 per cent rice, 30 per cent whole milk powder, 3 per cent alfalfa leaf meal, and 1 per cent table salt. As previously mentioned, Hoppert, Webber, and Canniff found that with such a diet, caries was produced in rats when the rice was ground coarse enough to be retained in a 20-mesh screen, while no caries resulted when the rice was fine enough to pass through a 60-mesh screen. The diet containing the coarse rice is referred to as the caries diet.

Female breeders were isolated from the breeding cage as soon as pregnancy was evident. They were examined each day thereafter, until young were born. If the number of animals

in a litter was less than 5, the litter was destroyed and the female was placed in a rest cage for 7 days before being returned to the breeding cage. If the litter size exceeded 6, the number was reduced to 6 on the third day after birth. Where possible, 3 males and 3 females were saved in each litter. Thus 6 was the maximum and 5 the minimum number of young suckled by a single female. There were a few instances where a litter of less than 5 animals was produced by a female on the same date that a larger litter was produced by another female. In such cases, one or more of the young from the large litter were marked and placed with the female having the small litter. In this way both the small litter and extra individuals from the large litter could be saved without altering the standards set for the number of young suckled by one female. It is understood, of course, that in such cases the young were recorded as progeny of the female which gave birth to them.

Considerable difficulty was experienced with the loss of young rats from an unidentified disease. This loss usually occurred between the fourth and fifteenth days of life. Ordinarily this disease affected only two or three of the six animals saved, the others being normal and healthy. Animals so afflicted became very poor, and the external nares appeared almost or completely closed by a dried secretion and swollen membranes. Where such a condition occurred in two litters of approximately the same age, the surviving young, not exceeding 6 from both litters, were sometimes combined and placed with one of the females. In a very few instances, where two of a

litter of six animals died late in the suckling period, the remaining were saved without the addition of other young. This was done only in cases where it was considered very desirable to get progeny from a particular female. The small number of progeny secured from some of the females in this study was due to mortality caused by this disease. It was noticed that surviving females from litters suffering from this disease were apt to lose their young in the same manner. Females from litters not so affected produced young which were less prone to have the disease.

The disease affecting the young rats was not identified. A female rat and three of her infected young were autopsied by a member of the Pathology Department. No apparent organic disturbances were found, other than emaciation. Agar cultures were made of the stomach, heart, and lungs of the young rats. Mildly haemolytic streptococci were found in the heart and lungs. After the discovery of these organisms, all of the breeding animals were given sulfanilamide in olive oil, according to directions given by the Department of Bacteriology. These treatments produced no appreciable change in the death rate from the disease.

The young rats were weaned at 25 days of age and kept on the fine diet until 35 days old, at which time they were put on the caries diet. Female breeders were placed in a rest cage for 7 days after their young were weaned, and then returned to the breeding cage. The number of days required for a rat to develop caries was computed from the time the caries

diet was begun, and can be secured by subtracting 35 days from the age of the animal at the time caries first appeared. All of the animals on caries diet were examined every two weeks. In a few cases, it was impossible to examine all of the animals on the caries diet on the same day. The remaining rats were examined not later than one to four days afterwards. This explains why individuals in the same litter sometimes have a variation of four days or less in caries time.

A sketch of the lower molars was made on the record of each rat by means of a rubber stamp. Observations for caries were recorded as negative, questionable, or positive. The exact location and approximate size of the cavity was indicated on the sketch of the molars, along with the date of observation. A questionable observation was considered as indicating the time at which caries was initiated only when it was followed by a positive observation at the same location two weeks later. Thus caries was considered as beginning at the first questionable observation which was followed two weeks later by a positive observation, or, in the absence of questionable observations, at the date of the first positive record. Unless very conspicuous, that is involving a major portion of a tooth, positive observations were verified two weeks later. The rats were held by an assistant while the lower molars were examined with the use of a speculum and a light. The upper molars were not examined, since Hoppert reports that they were rarely affected by the coarse diet.

Animals to be used as breeders were put on the fine diet

as soon as possible after caries was definitely verified. This was done to prevent further development of cavities.

The term "caries time" is used throughout this study to mean the number of days required for the initiation of dental caries, after a rat was placed on the caries diet. Also, the number of days required for a rat to develop caries will often be indicated by placing the number in parenthesis immediately after the identification number of the rat under discussion. For example, rat 470(75) means that the rat whose identification number was 470, developed caries 75 days after being put on the caries diet.

All of the P_1 animals used in this study were from the Hunt and Hoppert susceptible and resistant lines. The identification numbers used for these animals were those originally assigned to them by Hunt and Hoppert. All F_1 and F_2 progeny were raised by the author, and were given numbers beginning with one.

It has already been stated that all of the animals on the caries diet were examined every 14 days. Thus a difference of as much as 14 days in the caries time of two individuals might not be significant. But a difference of 14 days, or even less, in the average caries time of the progenies of two different 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.

PROGENY TESTS

It has already been noted that there was considerable variability in the time required for the development of caries among the individual rats within the Hunt and Hoppert resistant line. This was also true to a lesser degree in the early generations of their susceptible animals.

One of the purposes of this study is to determine to what extent a phenotype corresponds to the genotype within these susceptible and resistant lines. Several female rats, which showed a wide degree of variation in caries time, were selected for progeny tests within each of the two lines.

Susceptible Progeny Tests

Two groups of progeny tests were performed in the susceptible line. One consisted of third generation, and the other of fifth generation animals.

The third generation of the Hunt and Hoppert susceptible line included 147 animals. The range of caries time for these animals was from 19 to 89 days, and the mean was 43 days. Eleven females, having a range of from 25 to 86 days of caries time, were selected from this group of Hunt and Hoppert rats for the purpose of progeny testing with a 25-day male from the same group. The 11 females represent a range in caries time almost equal to the range of the Hunt and Hoppert third generation animals. In their study, Hunt and Hoppert selected only the most promising early animals for breeding within the susceptible line. They have no data on the genetic behavior

of the late caries developers of this line. The present study provides a basis for comparison between the earlly, intermediate, and late animals.

Before comparing the 11 females used in these progeny tests, it is desirable to learn something about the genotype of the male. An examination of Tables I and II reveals that 8 of the 11 females, crossed with male 531, produced progenies with a higher mean caries time than their own. These 8 females ranged from 25 to 59 days in the time they developed caries. The other three females had a caries time near the upper limit of the third generation susceptibles, far above that of the male. These results suggest that this male was genotypically less susceptible than his phenotype showed. An effort was made to determine the approximate genotypic caries time of this male, but no figure could be found which was consistent with the caries time of the dams and the averages of their offspring.

The genotypes of the females used in these third generation susceptible progeny tests may be compared on the basis of the average caries time of the offspring of each, since all were crossed with the same male(No. 531). The average of the progeny of female 499(86) was 50.00 ± 7.27 days, as seen in Table II. This average is significantly less than the 70.18 ± 5.05 day average of the progeny of female 518(72), but is not significantly different from the progeny averages of the other females. The average for the progeny of female 337(29) was significantly less than the average for the progeny

TABLE I

THIRD GENERATION SUSCEPTIBLE PROGENY TESTS

All Females Crossed with ♂ 531(25)

Dams / Progeny

Cross #1	Cross #2	Cross #3	Cross #4	Cross #5	Cross #6	Cross #7	Cross #8	Cross #9	Cross #10	Cross #27
♀ 470(75)	♀ 518(72)	♀ 499(86)	♀ 479(58)	♀ 471(59)	♀ 498(56)	♀ 507(55)	♀ 337(29)	♀ 344(31)	♀ 526(25)	♀ 371(25)
♂ 13(59)	♂ 171(69)	♂ 1(60)	♂ 133(67)	♂ 58(46)	♂ 7(58)	♀ 203(55)	♂ 160(50)	♀ 74(44)	♂ 69(38)	♂ 143(52)
♀ 14(73)	♂ 172(85)	♂ 2(60)	♀ 134(108)	♂ 59(46)	♂ 8(72)	♀ 204(69)	♂ 161(34)	♀ 75(60)	♂ 70(82)	♂ 144(68)
♀ 15(73)	♂ 173(42)	♂ 3(16)	♀ 135(52)	♀ 60(76)	♂ 9(58)	Av. 62.00	♂ 162(50)	♀ 76(60)	♂ 71(68)	♂ 145(33)
♀ 16(87)	♂ 174(55)	♀ 4(60)	♂ 136(69)	♀ 61(76)	♂ 10(58)		♀ 163(50)	♀ 77(60)	♂ 72(68)	♀ 146(52)
♀ 17(73)	♀ 175(68)	♀ 5(44)	♂ 137(69)	♀ 62(76)	♀ 11(72)		♀ 164(34)	♀ 78(74)	♀ 73(52)	♀ 147(68)
	♀ 176(85)	♀ 6(60)		♀ 63(76)	♀ 12(28)		Av. 43.60	♀ 79(44)		♀ 148(52)
♂ 98(63)		Av. 50.00	♂ 266(46)					Av. 57.00	♂ 188(88)	
♂ 99(121)	♂ 250(96)		♂ 267(88)	♂ 155(63)	♀ 271(74)				♀ 189(74)	♂ 217(119)
♂ 100(63)	♂ 251(68)		♂ 268(46)	♂ 156(50)	Av. 60.00				♀ 190(60)	♂ 218(90)
♂ 101(45)	♂ 252(54)		♂ 269(88)	♂ 157(91)					♀ 191(32)	♂ 219(90)
♀ 102(45)	♂ 253(68)		♂ 270(46)	♀ 158(52)					♀ 192(74)	♂ 220(63)
♀ 103(45)	♀ 254(82)		Av. 67.90	♀ 159(52)					♀ 193(46)	♀ 221(63)
	Av. 70.18								Av. 62.00	♀ 222(49)
♂ 322(53)				♀ 281(113)						Av. 66.58
♂ 323(39)				♀ 282(82)						
♂ 324(39)				Av. 69.15						
♂ 325(39)										
♀ 326(39)										
♀ 327(53)										
Av. 59.35										

TABLE II

THIRD GENERATION SUSCEPTIBLE PROGENY TESTS

All Females Mated with ♂ 531(25)

No. of Cross	No. and Caries Time of Females	No. of Progeny Produced	Av. No. Days for Caries in Progeny	♂ of Progeny	No. of Progeny 56 Days or Below			No. of Progeny Above 56 Days		
					o's	o's	Total	o's	o's	Total
1	470(75)	17	59.35±5.25	21.01	5	4	9	4	4	8
2	518(72)	11	70.18±5.05	15.96	3	0	3	5	3	8
3	499(86)	6	50.00±7.27	16.29	1	1	2	2	2	4
4	479(58)	10	67.90±6.76	20.28	3	1	4	5	1	6
5	471(59)	13	69.15±5.56	19.25	3	2	5	2	6	8
6	498(56)	7	60.00±6.01	14.73	0	1	1	4	2	6
7	507(55)	2	62.00	-----	0	1	1	0	1	1
8	337(29)	5	43.60±3.92	7.84	3	2	5	0	0	0
9	344(31)	6	57.00±4.66	10.44	0	2	2	0	4	4
10	526(25)	11	62.00±5.46	17.24	2	3	5	4	2	6
27	371(25)	12	66.58±6.71	22.27	2	3	5	5	2	7
Total		100	62.44±1.93	19.29	22	20	42	31	27	58

The values accompanying the means in this and following tables are standard errors, not probable errors.

of each of the other females, except female 499, the highest phenotype of the group. The number of progeny from females 499 and 337, however, was so small that their genotypes were not adequately progeny tested. If their offspring are omitted from the comparison, no significant differences are found in the mean caries time of the progeny of any two of the remaining females.

The reader will note that 3 of the 4 early females used in these progeny tests produced offspring whose average caries time was comparatively late. The records of Hunt and Hoppert reveal that their third generation susceptible breeders usually produced offspring with a much earlier average. This evidence suggests that genotypes do sometimes differ from phenotypes, even in the moderately early susceptible rats. The average caries time of all the offspring of the third generation progeny tests was 62.4 days as compared to 37.3 days average for the Hunt and Hoppert fourth generation susceptibles. This large difference is undoubtedly due to the fact that the Hunt and Hoppert third generation breeders were all early animals, selected from sibships which were also uniformily early. The rats in this study, on the other hand, were late animals, as well as early ones, and were not selected on the basis of performance of sibs. It is therefore apparent that the phenotypes of these third generation susceptible animals do not accurately represent the true genotypes, for the means of the various sibships were substantially the same in spite of differences among the dams.

TABLE III

A COMPARISON OF SIBSHIP AVERAGES OF THIRD GENERATION SUSCEPTIBLE ANIMALS WITH THEIR PROGENY AVERAGES

P_1 ♂ 531(25) (Sibship average 39.72 days)

No. of Cross	No. and Caries Time of P_1 Females	No. of Progeny Produced	Av. No. Days for Caries in Progeny	Av. No. Days for Caries in Sibship of P_1 ♀'s
8	337(29)	5	43.60	40.89
3	499(86)	6	50.00	44.63
9	344(31)	6	57.00	40.89
1	470(75)	17	59.35	57.94
6	498(56)	7	60.00	44.63
10	526(25)	11	62.00	39.72
27	371(25)	12	66.58	40.00
4	479(58)	10	67.90	39.72
5	471(59)	13	69.15	57.94
2	518(72)	11	70.18	57.94

Table III shows a comparison between the averages for the sibships of the third generation susceptible animals used in progeny tests, with the averages for their progeny. The sibship averages were secured from the data of Hunt and Hoppert. Animals with the same sibship average are from the same sibship, although frequently from different litters. It appears that there is no more relationship between the progeny averages and the sibship averages of these respective third generation susceptible animals than between their progeny averages and their respective phenotypes. This would indicate that the susceptible rats used in these progeny tests were genotypically different from some of their sibs. Such a condition seems to supply additional evidence that the Hunt and Hoppert third generation susceptible animals show some degree of heterozygosity. It should be noted, however, that some of these differences are small, and may not represent genetic differences in every case. It is quite likely that the day, as a unit of caries time, is too small. The 14-day interval may be found to be sufficiently accurate for use as a unit of time.

Referring again to Table II, it will be noted that the progeny of female 470(75) had an average caries time of 59.35 days, while females 526(25) and 371(25) produced progeny with averages of 62.00 and 66.58 days respectively. It seems advisable at this point to suggest possible explainations for such results. It is conceivable that female 470 actually began to develop caries at 25 days, or even earlier. The cavity may have been so small that it was not noticed at first. It

could, nevertheless, have penetrated the enamel, leaving a very small channel. Upon reaching the dentine, an excavation underneath the enamel may have resulted, while the cavity in the enamel remained too small to be observed. The supporting dentine may have thus been removed to such an extent that at 75 days the enamel above caved in. Under these circumstances, this animal would be recorded as a 75 day animal, although caries actually was initiated at about 25 days.

Another possible explaination concerns the question of multiple factors. In crosses 10 and 27 the parents were early caries developers, but a few of the progeny were much later, as shown in Table I. Let it be assumed, for the purpose of illustration, that 3 genes are concerned in the production of susceptibility and resistance to dental caries, and that they are cumulative for resistance. These genes may be represented as A, B, and C. Taking Cross No. 27 for discussion, let it be assumed that the genotype of male 531 was aabbCC, and that of female 371 was aaBbcc. Their progeny would have one of the following genotypes: aaBbCc, or aabbCc. If B and C, when occurring together, produce a greater degree of resistance than the total effect of both when occurring separately, the late progeny of this cross can be accounted for.

A further examination of the third generation susceptible progeny tests reveals that the 11 dams may be divided into 3 groups. This grouping is on the basis of the time at which they developed caries. Table IV shows data on these 3 groups. Group I consists of late animals, group II of intermediates,

TABLE IV

PROGENY TESTS OF THIRD GENERATION SUSCEPTIBLES

Females divided into three groups on the basis of caries time

♂ 531(25)

Group No.	Cross No.	Females, Showing Caries Time	No. of Progeny	Mean Caries Time of Progeny	σ of Progeny
I	1 2 3	♀ 470(75) ♀ 518(72) ♀ 499(86)	34	61.21 ± 3.28	19.82
II	4 5 6 7	♀ 479(58) ♀ 471(59) ♀ 498(56) ♀ 507(55)	32	66.31 ± 3.33	18.53
III	8 9 10 27	♀ 337(29) ♀ 344(31) ♀ 526(25) ♀ 371(25)	34	60.03 ± 3.29	18.90
Total			100	62.44 ± 1.93	19.29

and group III of early caries developers. An analysis of the difference in the mean caries time of the progeny between any two of these three groups shows that the difference is not significant. With decreasing caries time for the mothers, one gets a constant mean caries time for the progeny. It may be said, therefore, that selection of females from this generation on the basis of phenotype alone, would not result in a significant increase in susceptibility in the next generation. Selection, on the basis of phenotype and sibship performance, accompanied by progeny tests, would, however, produce a greater degree of susceptibility in the following generation, as indicated by the experiments of Hunt and Hoppert.

It will be recalled that the Hunt and Hoppert susceptible line is becoming fixed within a relatively narrow range of caries time. Their resistant line, on the other hand, is still highly variable. The late phenotypes often produce some early progeny. Such results suggest that multiple factors are involved in determining susceptibility and resistance to caries. The latter part of this study is concerned with crosses made primarily to secure evidence on the mode of inheritance of caries resistance. But the progeny tests should also shed some light on this question. This is true because many of the animals used in these tests were undoubtedly heterozygous for genes affecting susceptibility and resistance to caries. If multiple factors are involved, a certain amount of segregation would be expected in the F_1 progeny of such heterozygous animals. In order to facilitate an analysis of these

F_1's, it is desirable to have a point of division for the purpose of classifying an animal either as susceptible or resistant. It is readily admitted that any such dividing point is decidedly arbitrary. Nevertheless such a point has been selected, which seems to be as reliable as can be had with the data available. An analysis of the Hunt and Hoppert fifth generation susceptibles reveals that only 4.2 per cent of these animals exceeded 56 days of caries time. This figure, therefore, seems to be near the upper limit of the approximately susceptible line. Animals whose caries time is above 56 days will be classified as resistants, although most of such rats probably represent various genic combinations, and therefore are often heterozygous.

Table II shows all of the offspring of the third generation progeny tests classified on the basis of the 56-day dividing point. Forty-two animals had a caries time of 56 days or less, and 58 were above this figure. As has already been pointed out, the genotypes of many of these animals being progeny tested were not reliably represented by their phenotypes. This undoubtedly means that most of these rats were heterozygous for factors causing susceptibility and resistance to caries. Segregation in the offspring further implies the heterozygous condition of these parents.

The progeny tests of rats from the Hunt and Hoppert fifth generation susceptibles were used to test the genotypes of 3 sibs, 2 males and 1 female. These 3 animals, males 884(76) and 885(76), and female 887(76), came from a

sibship which showed surprising results and demanded further investigation. These three 76-day animals and one 62-day animal, along with two 48-day animals, appeared in the first litter of this sibship. In the second litter there was one 89-day and five 33-day rats. In the third litter there were no late ones, the range for this litter being from 13 to 42 days. The parents for these animals were both 16-day sibs. These parents also were the progeny of 16-day sibs. The range for the Hunt and Hoppert fifth generation susceptibles, not including this exceptional sibship, was from 15 to 49 days, with an average of 30.2 days.

Tables V and VI show data on the progeny of these 76-day animals. Each of the two males was crossed with two early fifth generation susceptible females. One of the females crossed with male 885 proved to be sterile, and the other produced only 5 progeny. The female sib was crossed with an early fifth generation susceptible male. The progenies of the Hunt and Hoppert early fifth generation susceptible animals were consistently early caries developers. For this reason it can probably be assumed that a high average for the progeny of either of these 3 late sibs was influenced to a greater extent by the genotype of the 76-day parent than by that of the early parent. On the other hand, a low progeny average would indicate that the genotype of the 76-day parent was below the phenotype. This would permit the influence of the early parent to express itself.

Male 884 was the sire of 28 progeny with an average

TABLE V

FIFTH GENERATION SUSCEPTIBLE PROGENY TESTS

Sires	♂ 884(76)*		♂ 885(76)*	♂ 873(21)
Dams	Cross #51	Cross #59	Cross #52	Cross #53
	♀ 918(25)	♀ 977(28)	♀ 875(21)	♀ 887(76)*
Progeny	♂ 385(55)	♂ 549(24)	♂ 368(29)	♂ 389(27)
	♂ 386(55)	♂ 550(24)	♂ 369(85)	♂ 390(41)
	♂ 387(55)	♂ 551(24)	♂ 370(29)	♂ 391(41)
	♀ 388(55)	♂ 552(48)	♀ 371(74)	♂ 392(55)
		♀ 553(62)	♀ 372(57)	♂ 393(41)
	♂ 505(25)	♀ 554(48)		♀ 394(41)
	♂ 506(39)		Av. 54.80	
	♂ 507(25)	♂ 617(31)		♂ 499(40)
	♀ 508(39)	♂ 618(31)		♂ 500(40)
	♀ 509(39)	♂ 619(17)		♂ 501(26)
	♀ 510(39)	♀ 620(31)		♂ 502(40)
		♀ 621(45)		♀ 503(26)
	Av. 42.60			♀ 504(40)
		♂ 776(59)		♂ 645(35)
		♂ 777(47)		♂ 646(35)
		♂ 778(59)		♀ 647(24)
		♂ 779(47)		♀ 648(24)
		♂ 780(32)		♀ 649(24)
		♀ 781(48)		♀ 650(24)
		♀ 782(48)		Av. 34.67
		Av. 40.28		

*These are the exceptional animals which were progeny tested.

TABLE VI

FIFTH GENERATION SUSCEPTIBLE PROGENY TESTS

Exceptional Animals Progeny Tested	No. of Cross	No. and Caries Time of Male Parent	No. and Caries Time of Female Parent	No. of Progeny Produced	Average Caries Time of Progeny	σ of Progeny
♂ 884(76)	51	♂ 884(76)	♀ 918(25)	10	42.6	
	59	" " "	♀ 977(28)	18	40.3	
		Total		28	41.11 ± 2.44	12.69
♂ 885(76)	52	♂ 885(76)	♀ 875(21)	5	54.80±11.44	22.88
♀ 887(76)	53	♂ 873(21)	♀ 887(76)	18	34.67 ± 2.10	8.67
Grand Total				51	40.18 ± 1.98	14.09

caries time of 41.11± 2.44. This progeny average is approximately 15 days above the caries time of the two female parents and 35 days below that of the male. These results suggest that the phenotype of male 884 was somewhat higher than his genotype, in respect to caries time.

The average caries time for the 5 progeny of male 885 was 54.80±11.44 days. Unfortunately this number of offspring is rather small for satisfactory analysis. Nevertheless, certain points are suggestive. These 5 progeny showed a wide degree of variation in caries time, ranging from 29 to 85 days. The 85-day animal exceeded the caries time of its 76-day parent. Segregation may have occured here. On the basis of these results, it appears that male 885 was perhaps genotypically less susceptible than either of his two sibs.

The average caries time of the progeny of female 887 was 34.67± 2.10 days. This average is significantly less than the mean of the progeny of male 884, the t value for the difference being 2.00 This female must have had a genotype which tended toward susceptibility to a greater degree than that of either of the two sibs.

The foregoing analysis of the data on the progeny of these 3 sibs seems to indicate that their genotypes were different, and that male 884 and female 887 were genotypically more susceptible than their phenotypes revealed.

Susceptibility to caries became more definitely fixed in the Hunt and Hoppert fifth generation than in their third generation susceptible line. For this reason, the extent of

segregation should be noticeably less in the fifth generation susceptible progeny tests than in that of the third generation progeny tests. A comparison of the number of offspring above and below 56 days of caries time in these two sets of progeny tests reveals that this is the case. As previously noted, 58 per cent of the 100 progeny of the third generation tests had a caries time of more than 56 days. In the fifth generation tests, only 6, or 11.8 per cent, of the progeny exceeded 56 days. Furthermore, only 2 of these 6 animals exceeded this figure by more than 3 days. This indicates that the fifth generation progeny approach homozygosity for susceptibility.

Resistant Progeny Tests

The animals used in these tests were taken from the second and third generations of the Hunt and Hoppert resistant line. As shown in Table VII, the male and 4 of the 9 females were second generation animals. The remaining 5 females were from the third generation. The Hunt and Hoppert second and and third generation resistants were highly variable. The range in caries time for their second generation was from 35 to 644, with a mean of 115.2 days. The range of the third generation was from 33 to 406 days, and a mean of 128.2 days.

Male 405, which was used in these resistant progeny tests, had a caries time of 173 days. The 9 females with which he was crossed, ranged from 72 to 132 days. The wide distribution of the caries time of the offspring indicates that this

TABLE VII

SECOND AND THIRD GENERATION RESISTANT PROGENY TESTS

All Females Crossed with ♂ 405(173) (2nd Generation)

Dams / Progeny

Cross #11	Cross #12	Cross #13	Cross #14	Cross #16	Cross #30	Cross #31	Cross #32	Cross #33
♀ 397(98)	♀ 386(98)	♀ 444(132)	♀ 402(132)	♀ 488(97)	♀ 712(77)	♀ 713(77)	♀ 820(76)	♀ 719(72)
2nd Gen.	2nd Gen.	2nd Gen.	2nd Gen.	3rd Gen.	3rd Gen.	3rd Gen.	3rd Gen.	3rd Gen.
♂ 48(93)	♂ 36(80)	♂ 30(113)	♂ 53(73)	♂ 80(74)	♀ 244(70)	♂ 261(201)	♂ 240(112)	♂ 255(141)
♀ 49(167)	♂ 37(96)	♀ 31(113)	♂ 54(103)	♂ 81(148)		♂ 262(107)	♀ 241(112)	♂ 256(246)
♀ 50(107)	♀ 38(110)	♀ 32(113)	♀ 55(73)	♂ 82(134)	♂ 283(58)	♀ 263(65)	♀ 242(42)	♂ 257(110)
♀ 51(107)	♀ 39(110)	♀ 33(99)	♀ 56(122)	♀ 84(219)	♂ 284(72)	♀ 264(149)		♂ 258(110)
♀ 52(93)	♀ 40(198+)*	♀ 34(83)	♀ 57(89)	♀ 85(78)	♂ 285(103)	♀ 265(107)	♂ 400(194)	♀ 259(231)
	♀ 41(110)	♀ 35(113)		Av. 130.60	♀ 286(128)		♂ 401(110)	♀ 260(176)
♂ 128(54)			♂ 138(68)		♀ 287(128)	♂ 345(62)	♂ 402(124)	
♂ 129(124)	♂ 122(110)	♂ 104(62)	♂ 139(68)		♀ 288(103)	♀ 346(128)	♀ 403(124)	♂ 328(66)
♂ 130(69)	♂ 123(50)	♂ 105(146)	♂ 140(68)			♀ 347(79)	♀ 404(219)	♂ 329(132)
♀ 131(152)	♂ 124(110)	♂ 106(104)	♀ 141(68)		♂ 433(108)	♀ 348(118)	Av. 129.62	♀ 330(172)
♀ 132(69)	♀ 125(124)	♀ 107(62)	♀ 142(121)		♂ 434(70)	♀ 349(118+)*		♀ 331(66)
	♀ 126(124)	♀ 108(77)			♂ 435(45)	♀ 350(251)		♀ 332(66)
♂ 194(219)	♀ 127(152)	♀ 109(90)	♂ 228(130)		♀ 436(94)			
♂ 195(139)			♂ 230(116)		♀ 437(111)	♂ 477(128)		Av. 137.82
♂ 196(111)	♂ 211(96)	♂ 177(213)	♀ 231(293)			♂ 478(128)		
♀ 197(246)	♂ 212(96)	♀ 178(378)	♀ 232(131)		Av. 90.83	♂ 479(72)		
♀ 198(125)	♂ 213(194)	♀ 179(364)				♀ 480(72)		
♀ 199(139)	♂ 214(96)	♀ 180(91)	Av. 108.79			♀ 481(86)		
♂ 200(125)		♀ 181(148)						
♂ 201(83)	Av. 116.00	♀ 182(364)				Av. 116.94		
♀ 202(125)								
Av. 123.53		Av. 151.83						

*This rat died before caries appeared.

male has heterozygous for resistance. The average caries time of the progeny of each of the females, except number 402, exceeded that of the female parent. This indicates that male 405 was genotypically, as well as phenotypically, more resistant than were the females. It seems unlikely, however, that the genotype of this male was as high in caries time as his phenotype, since the progeny averages of a majority of the dams tend to fall closer to the caries time of the female parents than to that of the male parent. These averages are shown in Table VII.

In each cross, except number 30, the range of caries time of the offspring extended above and below that of the parents. This implies that the females used in these crosses were also heterozygous for resistance. The range of the progeny from most of the crosses does not exceed that which might be expected from segregation of genes from heterozygous parents. Cross 13 has an extreme range of from 62 to 378 days. Since 4 offspring from this cross exceed the caries time for the male parent, it is possible that one of the parents carried one or more genes for resistance which were not present in the other. In such a case, segregation and recombination could have resulted in the production of a few offspring with more factors for resistance than either parent possessed. Such an assumption is all the more plausible when one examines the breeding technique used by Hunt and Hoppert during the early part of their study. Their inbreeding was limited almost entirely to animals of the same families. If the ten-

TABLE VIII

SECOND AND THIRD GENERATION RESISTANT PROGENY TESTS

All Females Mated with ♂ 405(173)

No. of Cross	No. and Caries Time of Females	No. of Progeny Produced	Av. No. Days for Caries in Progeny	σ of Progeny	No. of Progeny 56 Days or Below			No. of Progeny Above 56 Days		
					♂'s	♀'s	Total	♂'s	♀'s	Total
11	397(98)	19	123.53 ± 11.13	47.18	1	0	1	8	10	18
12	386(98)	16	116.00 ± 9.48	36.70	1	0	1	8	7	15
13	444(132)	18	151.83 ± 24.83	102.28	0	0	0	5	13	18
14	402(132)	14	108.79 ± 15.69	56.63	0	0	0	7	7	14
16	488(97)	5	130.60 ± 26.55	53.10	0	0	0	3	2	5
30	712(77)	12	90.83 ± 7.10	23.67	1	0	1	5	6	11
31	713(77)	16	116.94 ± 12.67	49.03	0	0	0	6	10	16
32	820(76)	8	129.62 ± 19.28	51.09	0	1	1	4	3	7
33	719(72)	11	137.82 ± 19.10	60.36	0	0	0	6	5	11
Total		119	122.91 ± 5.67	61.61	3	1	4	52	63	115

dency toward resistance to caries is due to several genetic factors, strict inbreeding and selection for resistance might tend to eliminate one or more pairs of these genes and fix the remaining ones. Crossing of unrelated or distantly related animals, as in the case of the present study, would tend to recombine such genes.

On the other hand, the author is unable to explain why all the extremely late animals from cross No. 13 were in the same litter. These animals were kept in cages with rats from other crosses. These other rats showed no tendency toward late caries development. For this reason it is doubtful if environmental differences could account for these peculiar results. The same condition of heterozygosity, which has just been described in connection with cross No. 13, probably exists in the parents of the other crosses, although to a lesser degree.

Considerable variation is seen in the average caries time of the progeny of the different females, as shown in Table VIII. Significant differences, however, are not as frequent as might be expected, because the the standard error of most of the crosses is large. Female 712, a 77-day animal, had progeny whose aberage was significantly less than the averages for the progeny of females 397(98), 386(98), 444(132), and 719(72). There were no other significant differences between the progeny averages of these crosses. These data show that female 712 was genotypically more susceptible than female 719, a 72-day animal. Thus again it

TABLE IX

PROGENY TESTS OF THE SECOND AND THIRD GENERATION RESISTANTS

Females divided into three groups on the basis of caries time.

♂ 405(173)

Group No.	Cross No.	Females, Showing Caries Time	No. of Progeny	Mean Caries Time of Progeny	σ of Progeny
I	30	712(77)	47	117.32 ± 7.49	50.79
	31	713(77)			
	32	820(76)			
	33	719(72)			
II	11	397(98)	40	121.40 ± 7.12	44.45
	12	386(98)			
	16	488(97)			
III	13	444(132)	32	133.00±15.85	88.32
	14	402(132)			

is demonstrated that the phenotype is a poor indicator of the genotype, with respect to caries time.

Table IX shows these 9 females grouped as early, intermediate, and late animals. As might be expected from the above analysis, there is no significant difference in the average caries time of the progeny of these 3 groups. The data of Table IX would suggest, nevertheless, that selection of late breeders had some effect in producing late caries development in the offspring. The selection of a late animal for breeding purposes, only on the basis of its phenotype, would thus be a slow method of developing a resistant line. Such selections should be accompanied by progeny tests.

As previously mentioned, 4 of the females used in these progeny tests were second generation resistants, and 5 were third generation resistants. Table X shows a comparison of the average caries time of the progeny of these two groups. There is no significant difference in these two averages. This is not surprising for two reasons. These third generation females were all early resistants, as compared to later resistants in the second generation. Even if this were not the case, there would probably still be little difference, since resistance in the Hunt and Hoppert line is being extablished slowly.

The foregoing analysis of the progeny of the Hunt and Hoppert second and third generation resistant animals revealed that the phenotype is a poor expression of the genotype. It should also be noted that the offspring of these

TABLE X

A COMPARISON OF THE SECOND AND THIRD GENERATION RESISTANT PROGENY TESTS

All females crossed with second generation ♂ 405(173)

Progeny Test	No. Progeny Produced	Mean caries Time of Progeny	σ of Progeny
2nd generation	67	123.49 ± 8.44	68.52
3rd generation	52	118.59 ± 7.14	51.01

animals had a much wider range of caries time than did the progeny of either the third or fifth generation susceptibles. This fits the theory that inherited susceptibility and resistance to caries results from multiple factors, the factors for resistance being cumulative. If this theory is correct, the progeny of the second and third generation resistant females, taken as a group, should consist of a few susceptible animals, a few highly resistant ones, and a large number of intermediates. Table VII reveals that 4 offspring from these animals had a caries time of 56 days or less, 15 exceeded the 173-day caries time of the male parent, and 100 were between 56 and 173 days.

CROSSES BETWEEN SUSCEPTIBLE AND RESISTANT ANIMALS

These matings were made primarily to determine if sufficient segregation occurs in the F_2 progeny to indicate the presence of multiple factors. The P_1 crosses will also be treated as progeny tests. A second generation resistant male was crossed with 5 third generation early susceptible females. From these 5 crosses 88 F_1 animals were produced. Reciprocal crosses were also made. A third generation early susceptible male was crossed with 5 second generation females. These reciprocal crosses were extremely unsuccessful. One of the females was sterile, and another died before producing young. The disease, which has been previously mentioned, destroyed all the litters produced by another female and 4 of the litters produced by the remaining 2 dams. The total number of litters lost from these crosses was 8. Consequently only 13 F_1 progeny were raised from these matings.

F_1 Progeny

Tables XI and XII show data on the progeny of male 305(185), a second generation resistant, and 5 early females from the third generation susceptible line. The genotypes of these P_1 animals may be analyzed on the basis of the caries time of their F_1 offspring. An examination of Table XI reveals that 13 progeny of male 305 had a caries time between 60 and 70 days, and 5 had a still lower figure. Such early animals

TABLE XI

F_1 PROGENY FROM A SECOND GENERATION RESISTANT MALE

AND THIRD GENERATION SUSCEPTIBLE FEMALES

♂ 305(185)

Dams / Progeny	Cross #18	Cross #19	Cross #20	Cross #21	Cross #22
Dams	♀ 500(30)	♀ 508(29)	♀ 537(35)	♀ 536(35)	♀ 462(36)
	♂ 18(87)	♂ 149(44)	♂ 42(80)	♂ 24(85)	♂ 64(83)
	♂♂ 19(73)	♂ 150(44)	♂ 43(110)	♂ 25(115)	♂♂ 65(69)
	♂♂ 20(87)	♀ 151(90)	♀ 44(110)	♂ 26(101)	♂♂ 66(83)
	♂♂ 21(87)	♀♀ 152(65)	♀♀ 45(80)	♂ 27(85)	♀ 67(69)
	♂ 22(87)	♀♀ 153(65)	♀♀ 46(96)	♀ 28(101)	♀♀ 68(129)
	♀ 23(117)	♀ 154(76)	♀ 47(80)	♀ 29(85)	
Progeny	♂ 86(108)	♂ 223(74)	♂ 116(112)	♂♂ 92(63)	♂ 165(88)
	♂♂ 87(81)	♂ 224(102)	♂♂ 117(52)	♂♂ 93(133)	♂♂ 166(47)
	♂♂ 88(136)	♀ 225(74)	♀♀ 118(57)	♂♂ 94(105)	♂♂ 167(60)
	♂♀♀ 89(405)	♀♀ 226(131)	♀♀♀ 119(73)	♀ 95(133)	♀♀ 168(74)
	♀♀♀ 90(66)	♀♀ 227(60)	♀♀♀ 120(73)	♀♀ 96(119)	♀♀♀ 169(90)
	♀♀ 91(66)	♂♂ 229(88)	♀♀ 121(73)	♀♀ 97(133)	♀♀♀ 170(116)
	♂♂ 245(111)		♀ 205(169)	♂♂ 183(133)	♂♂ 233(115)
	♂♂♂ 246(125)		♀♀ 206(82)	♂♂ 184(133)	♂♂ 234(87)
	♂♂ 247(111)		♀♀ 207(82)	♂♂ 185(161)	♀♀ 235(129)
	♀♀ 248(125)		♀♀♀ 208(96)	♀♀ 186(77)	♀♀♀ 236(101)
	♀♀ 249(111)		♀♀♀ 209(194)	♀♀ 187(133)	♀♀♀ 237(59)
	♂♂ 310(84)		♀♀♀ 210(96)		♀♀♂ 238(73)
	♂♂ 311(67)				♂♂ 239(84)
	♂♂ 312(123)				
	♀♀ 313(67)				
	♀♀ 314(95)				
	♀♀ 315(67)				
	Av. 108.09	Av. 76.08	Av. 95.28	Av. 111.47	Av. 86.44

TABLE XII

F_1 PROGENY FROM HUNT AND HOPPERT SECOND GENERATION RESISTANT MALE AND THIRD GENERATION SUSCEPTIBLE FEMALES

♂ 305(185)

No. of Cross	No. and Caries Time of Females	No. of Progeny Produced	Av. No. Days for Caries in Progeny	σ of Progeny	No. of Progeny Below 56 Days			No. of Progeny Above 56 Days		
					♂'s	♀'s	Total	♂'s	♀'s	Total
18	500(30)	23	108.09 ± 14.26	66.90	0	0	0	14	9	23
19	508(29)	12	76.08 ± 7.00	25.55	2	0	2	3	7	10
20	537(35)	18	95.28 ± 8.41	34.64	1	0	1	3	14	17
21	536(35)	17	111.47 ± 6.42	25.67	0	0	0	10	7	17
22	462(36)	18	86.44 ± 5.34	23.01	1	0	1	8	9	17
Total		88	97.33 ± 4.65	43.40	4	0	4	38	46	84

from a 185-day parent indicate that this male must have been heterozygous for resistance. The mean caries time of all the F_1 progeny from male 305 was 97.33± 4.65 days. This value is almost intermediate between the caries time of the sire and dams. The intermediate figure between the mean caries time of the dams and the caries time of the sire is 109.00 days. Thus these data seem to fit the theory of multiple factors.

The phenotypes of the 5 females used in these crosses were practically identical, as seen in Table XII. Yet an analysis of the mean caries time of their progeny reveals certain significant differences. The average caries time of the progeny of female 508(29) was significantly less than that of the progeny of females 500(30) and 536(35). Also, the mean caries time of the progeny of female 462(36) was significantly less than the average for the offspring of female 536. Thus it is again demonstrated that the phenotype is a poor indicator of the genotype.

Most of the P_1 female parents used in these crosses were undoubtedly not homozygous for susceptibility because individual differences have just been demonstrated. These genetic differences probably represent the presence of one, or a very few, genes for resistance. The male, on the other hand, appears to be heterozygous for several genes for resistance. According to the theory of multiple factors, few offspring from these crosses would be expected to develop caries extremely early. Table XII reveals that only 4 out of 88 progeny, or 4.5 per cent, developed caries at 56 days or less. Also, ac-

cording to this theory, crosses between susceptible and resistant animals should produce F_1 offspring with less variability than crosses between heterozygous resistant animals. Purely for purposes of illustration, let it be assumed that two animals with maximum heterozygosity are crossed as follows: AaBbCc X AaBbCc. It is clearly evident that greater variability would occur in the progeny of such a cross than in the progeny of a cross between aaBbcc and AaBbCc animals.

A comparison of the standard deviations of the progenies from the two sets of crosses, resistant X resistant and resistant X susceptibles, reveals this expected difference in variability. Table XII shows a standard deviation of only 43.40 days for all the progeny from a resistant male crossed with susceptible females. The standard deviation of the progeny of heterozygous resistant parents was 61.61 days, as previously shown in Table VIII.

In cross No. 18, Table XI, it will be noted that female 89, one of the F_1 progeny, had a caries time of 405 days. If it is conceded that the gametes producing this animal received all the genes for resistance in both parents, it is still difficult to explain such a high figure. As will be shown later, the caries time of the few offspring that this F_1 female produced, suggests that her genotype was lower than this value.

Data in Tables VIII and XII suggest that one or more of the factors for susceptibility and resistance to caries might be sex-linked, although the results are not statistically significant. It will be noted in Table VIII that 3 of the 4 offspring, which had a caries time of 56 days or less, were males.

TABLE XIII

F_1 PROGENY FROM HUNT AND HOPPERT THIRD GENERATION SUSCEPTIBLE MALE AND SECOND GENERATION RESISTANT FEMALES

♂ 413(20)

Cross #26	Cross #24
♀ 331(119)	♀ 408(235)
♂ 110(59)	♂ 215(68)
♂ 111(59)	♀ 216(54)
♂ 112(59)	
♀ 113(74)	♂ 295(68)
♀ 114(59)	♂ 296(54)
♀ 115(59)	♂ 297(68)
	♀ 298(68)
	♀ 299(68)
Av. 61.50	Av. 64.00

TABLE XIV

DATA ON F_1 PROGENY FROM HUNT AND HOPPERT THIRD GENERATION SUSCEPTIBLE MALE AND SECOND GENERATION RESISTANT FEMALES

♂ 413(20)

No. of Cross	No. and Caries Time of Females	No. of Progeny Produced	Av. No. Days for Caries in Progeny	σ of Progeny	No. of Progeny 56 Days or Below			No. of Progeny Above 56 Days		
					♂'s:	♀'s:	Total	♂'s:	♀'s:	Total
24	408(235)	7	64.00±2.58	6.32	1	1	2	3	2	5
26	331(119)	6	61.50±2.50	5.59	0	0	0	3	3	6
Total		13	62.85±1.77	6.13	1	1	2	6	5	11

This was true even though the total progeny of these crosses consisted of 64 females as compared to 55 males. The male parent of these progeny was a late resistant animal. Four progeny of the crosses shown in Table XII had a caries time of 56 days or less. All 4 animals were males, even though 46 of the total 88 offspring were females. Here again the male parent of these rats was a fairly late resistant. Such a tendency in both of these sets of crosses suggests that each of the two male parents might have carried one or possibly more genes for resistance on the X-chromosome. In such a case, all of the female progeny would receive this gene. If the female parents carried this possible sex-linked gene, it is unlikely that many were homozygous for it, because most of them were intermediate or early animals. Thus there would be a greater tendency for the female progeny to get the sex-linked factor for resistance than would be the case in the male progeny. As a further argument for this sex-linkage theory, it should be noted that autosomal segregation of the other genes for resistance might tend to mask evidence of sex-linkage in most of the progeny. If homozygous, or approximately homozygous lines are reached in the future, further crosses between resistants and susceptibles should demonstrate the validity or falseness of this sex-linkage theory.

The number of progeny secured from crosses between a susceptible male and resistant females was disappointing. Tables XIII and XIV show data on the 13 F_1 offspring produced. Disease among the young was so extensive and the number of animals so

small that analysis is probably unreliable. The average caries time of these 13 progeny was only 62.85 ± 1.77 days, a much smaller figure than would be expected on the basis of the results from other crosses already discussed. Eleven of the 13 offspring from these 2 crosses had a caries time above 56 days, but the range was small. It is possible that the disease in the young rats lessened the genetic resistance to caries. On the basis of the data shown in Table XIV, there was no genetic difference between females 408(235) and 331(119). Both apparently had phenotypes which exceeded their genotypes in caries time. The number of progeny from these two females, however, is too small for satisfactory analysis.

F_2 Progeny

The F_2 progeny were produced from crosses in which all of the F_1 females from a given P_1 cross were mated with one of their respective male sibs. In each case the F_1 male sib selected most nearly approximated the average caries time of the first litter of the sibship. Tables XV, XVI, XVII, XVIII, and XIX show the caries time of each F_2 animal from the different P_1 crosses.

Table XX shows the distribution of the F_2 progeny from each P_1 cross resistant male X susceptible female. The offspring were grouped into classes having a 14-day width. This class width was used because it represents the interval of time between observations for caries in the rats. Disease killed the progeny from all save one litter of the F_1 females

TABLE XV

F_2 PROGENY FROM P_1 CROSS NO. 18

F_1 ♂ No. 20(87)

Cross #34	Cross #91	Cross #35	Cross #36	Cross #85	Cross #86	Cross #87
♀ 23(117)	♀ 89(405)	♀ 90(66)	♀ 91(66)	♀ 248(125)	♀ 249(111)	♀ 313(67)
♀ 354(48)	♂ 683(59)	♂ 306(100)	♂ 300(92)	♂ 665(62)	♂ 770(78)	♂ 651(46)
♀ 355(48)		♀ 307(58)	♂ 301(50)	♂ 666(87)	♂ 771(53)	♀ 652(61)
♂ 356(48)	♂ 814(127)*	♀ 308(89)	♂ 302(50)	♂ 667(32)	♂ 772(53)	♀ 653(61)
♂ 357(61)	♂ 815(86)	♀ 309(194)	♂ 303(64)	♀ 668(62)	♀ 773(78)	♀ 654(46)
♀ 358(48)	♂ 816(113)		♀ 304(50)	♀ 669(46)	♀ 774(53)	♀ 655(61)
♀ 359(78)	♀ 817(57)	♂ 457(55)	♀ 305(78)	♀ 670(46)	♀ 775(133)	♀ 656(88)
	♀ 818(72)	♀ 458(93)				♀ 657(61)
♂ 482(68)			♀ 567(146)	♂ 808(35)	Av. 74.67	
♀ 484(44)	Av. 85.67	♂ 576(37)	♀ 568(109)	♂ 809(74)		Av. 60.57
♀ 485(137)		♀ 577(51)		♂ 810(35)		
♀ 486(260)*		♀ 578(36)	♂ 675(70)	♂ 811(88)		
		♀ 579(119)	♂ 676(70)	♀ 812(74)		
Av. 84.00			♂ 677(59)	♀ 813(88)		
		♂ 730(93)	♂ 678(96)			
		♂ 731(67)	♂ 679(70)	Av. 60.75		
		♀ 732(52)	♀ 680(138)			
		♀ 733(107)	♀ 681(110)			
		♀ 734(67)	♀ 682(70)			
		♀ 735(79)				
			Av. 82.63			
		Av. 81.06				

*These rats had not developed caries at time of writing. See enclosed data at back of thesis for final results.

TABLE XVI

F_2 PROGENY FROM P_1 CROSS NO. 19

F_1 ♂ No. 223(74)

F_1 Dam		Cross #72
		♀ 153(65)
F_2 Progeny	♂	759(42)
	♂	760(42)
	♂	761(83)
	♀	762(32)
	♀	763(58)
	♀	764(97)
	♀	765(58)
		Av. 58.86

TABLE XVII

F_2 PROGENY FROM P_1 CROSS NO. 20

F_1 ♂ No. 43(110)

Cross #37 ♀ 44(110)	Cross #39 ♀ 46(96)	Cross #40 ♀ 47(80)	Cross #41 ♀ 118(57)	Cross #48 ♀ 119(73)	Cross #50 ♀ 121(73)	Cross #79 ♀ 208(96)	Cross #49 ♀ 120(73)
♂ 417(104)	♂ 272(85)	♂ 360(99)	♂ 405(82)	♂ 333(93)	♂ 339(131)	♂ 569(37)	♂ 351(48)
♂ 418(55)	♂ 273(85)	♂ 361(99)	♂ 406(40)	♂ 334(52)	♂ 340(65)	♂ 570(65)	♀ 352(236)
♂ 419(104)	♂ 274(141)	♂ 362(57)	♀ 407(96)	♂ 335(79)	♂ 341(107)	♀ 571(51)	♀ 353(89)
♀ 420(55)	♀ 275(99)	♀ 363(99)	♀ 408(96)	♂ 336(79)	♀ 342(93)	♀ 572(65)	
♀ 421(187)	♀ 276(85)	♀ 364(99)		♀ 337(65)	♀ 343(131)	♀ 573(52)	♂ 712(98)
♀ 422(66)		♀ 365(74)	♂ 543(49)	♀ 338(65)	♀ 344(82)	♀ 574(93)	
	♂ 423(103)	♂ 366(121)	♂ 544(63)			♀ 575(52)	♂ 834(73)
♂ 592(71)	♀ 424(79)	♀ 367(149)	♂ 545(49)	♂ 519(109)	♂ 465(87)		♀ 835(45)
♂ 593(113)	♀ 425(144)		♀ 546(76)	♂ 520(39)	♂ 466(129)	♂ 719(55)	♀ 836(73)
♂ 594(58)	♀ 426(79)	♂ 487(107)	♀ 547(117)	♂ 521(121)	♂ 467(87)	♂ 720(44)	
♂ 595(58)	♀ 427(54)	♂ 488(149)	♀ 548(62)	♀ 522(217)	♂ 468(49)	♂ 721(44)	Av. 94.57
♀ 596(71)	♀ 428(130)	♂ 489(134)		♀ 523(109)	♀ 469(157)	♂ 722(28)	
♀ 597(113)		♂ 490(42)	♂ 703(142)	♀ 524(43)	♀ 470(210)	♀ 723(123)	
	♂ 580(63)	♀ 491(56)	♂ 704(47)			♀ 724(54)	
♂ 736(63)	♂ 581(49)	♀ 492(175)	♂ 705(74)	♀ 688(55)	♂ 627(56)		
♂ 737(63)	♂ 582(118)		♂ 706(114)	♀ 689(66)	♂ 628(56)	Av. 58.69	
♂ 738(63)	♀ 583(35)	♂ 639(103)	♂ 707(74)	♀ 690(81)	♂ 629(124)		
♂ 739(74)	♀ 584(91)	♂ 640(49)	♂ 708(74)	♀ 691(55)	♀ 630(124)		
♀ 740(48)	♀ 585(101)	♂ 641(159)	♂ 709(47)	♀ 692(66)	♀ 631(43)		
♀ 741(48)		♀ 642(159)	♀ 710(33)	♀ 693(148)	♀ 632(83)		
		♀ 643(66)	♀ 711(100)				
		♀ 644(66)			♂ 790(112)		
					♂ 791(45)		
		♂ 801(103)			♂ 792(70)		
		♂ 802(89)			♂ 793(84)		
		♂ 803(103)			♀ 794(70)		
		♀ 804(89)			♀ 795(56)		
		♀ 805(75)					
		♀ 806(47)					
		♀ 807(61)					
Av. 78.56	Av. 90.65	Av. 97.37	Av. 75.53	Av. 85.67	Av. 93.79		Cross #80 ♀ 210(96)
							♂ 753(58)
							♂ 754(70)
							♂ 755(58)
							♀ 756(59)
							♀ 757(59)
							♀ 758(59)
							Av. 60.50

TABLE XVIII

F_2 PROGENY FROM P_1 CROSS NO. 21

F_1 ♂ No. 26(101)

F_1 Dams

F_2 Progeny

Cross #42 ♀ 28(101)	Cross #43 ♀ 29(85)	Cross #65 ♀ 95(133)	Cross #58 ♀ 96(119)	Cross #64 ♀ 186(77)	Cross #66 ♀ 97(133)
♂ 277(110)	♂ 289(99)	♂ 525(65)	♂ 471(100)	♂ 459(51)	♂ 555(64)
♂ 278(54)	♂ 290(99)	♀ 526(119)	♂ 472(73)	♂ 460(65)	♂ 556(130)
♂ 279(68)	♀ 291(68)	♀ 527(79)	♂ 473(265)*	♂ 461(75)	♀ 557(47)
♀ 280(257)	♀ 292(68)	♀ 528(79)	♂ 474(73)	♂ 462(65)	♀ 558(47)
	♀ 293(68)	♀ 529(107)	♀ 475(100)	♀ 463(75)	
♂ 409(148)	♀ 294(68)	♀ 530(160)	♀ 476(237)	♀ 464(116)	Av. 72.00
♀ 410(175)					
♂ 411(82)	♂ 438(45)	♂ 713(97)	♂ 819(139)*	♂ 633(54)	
♂ 412(201)	♂ 439(70)	♀ 714(56)	♂ 820(139)*	♂ 634(177)	
♀ 413(106)	♀ 440(45)	♀ 715(180)*	♂ 821(139)*	♂ 635(27)	
♀ 414(298)*	♀ 441(111)	♀ 716(72)	♀ 822(111)	♀ 636(54)	Cross #84
♀ 415(82)	♀ 442(245)	♀ 717(152)		♀ 637(81)	♀ 187(133)
♀ 416(92)	♀ 443(136)	♀ 718(111)	Av. 137.60	♀ 638(108)	
					♂ 622(72)
♂ 531(77)	♀ 566(51)	Av. 106.42		♂ 796(150)*	♂ 623(30)
♂ 532(228)*				♀ 797(67)	♂ 624(30)
♀ 533(92)	♂ 745(167)*			♀ 798(53)	♀ 625(125)
♀ 534(134)	♂ 746(139)				♀ 626(30)
♀ 535(134)	♂ 747(17)			Av. 81.20	
♀ 536(106)	♂ 748(43)				Av. 57.40
	♀ 749(126)				
Av.135.78	♀ 750(84)				
	♀ 751(70)				
	♀ 752(43)				
	Av. 88.67				

*These rats had not developed caries at time of writing. See enclosed data at back of thesis for final results.

TABLE XIX

F_2 PROGENY FROM P_1 CROSS NO. 22

F_1 ♂ No. 66(83)

F_1 Dams

F_2 Progeny

Cross #44	Cross #57	Cross #56	Cross #68	Cross #92	Cross #67	Cross #83
♀ 67(69)	♀ 68(129)	♀ 168(74)	♀ 169(90)	♀ 235(129)	♀ 237(59)	♀ 238(73)
♂ 316(39)	♂ 444(224)	♀ 429(130)	♂ 511(38)	♂ 785(113)	♂ 586(32)	♂ 658(61)
♂ 317(39)	♂ 445(104)	♀ 430(79)	♂ 512(38)	♀ 787(71)	♂ 587(73)	♂ 659(128)
♂ 318(53)	♀ 446(129)	♀ 431(187)	♀ 513(199)	♀ 788(85)	♂ 588(32)	♂ 660(61)
♀ 319(270)	♀ 447(143)	♀ 432(267)*	♀ 514(52)	♀ 789(141)*	♀ 589(32)	♀ 661(88)
♀ 320(54)	♀ 448(63)		♀ 515(62)		♀ 590(59)	♀ 662(33)
♀ 321(25)		♂ 559(59)		Av. 102.50	♀ 591(32)	♀ 663(63)
	♂ 598(125)	♂ 560(45)	♂ 671(32)			♀ 664(46)
♂ 455(41)	♂ 599(58)	♂ 561(113)	♀ 672(46)		♂ 742(34)	
♂ 456(55)	♂ 600(71)	♂ 562(72)			♀ 743(85)	♂ 827(63)
	♂ 601(58)	♂ 563(21)	Av. 66.71		♀ 744(32)	♂ 828(119)*
♂ 614(46)	♀ 602(86)	♀ 565(140)				♂ 829(119)*
♂ 615(60)	♀ 603(57)			Cross #82	Av. 45.67	♀ 830(104)
♀ 616(61)		Av. 111.30	Cross #69	♀ 236(101)		♂ 831(77)
	Av. 101.64		♀ 170(116)			♀ 832(91)
♂ 783(83)				♂ 609(48)		♀ 833(104)
♂ 784(128)			♀ 824(63)	♂ 610(61)		
			♀ 825(103)	♀ 611(47)		Av.
Av. 73.38			♀ 826(49)	♀ 612(61)		
				♀ 614(61)		
			Av. 71.67			
				♂ 799(63)		
				♀ 800(22)		
				Av. 51.86		

*These rats had not developed caries at time of writing. See enclosed data at back of thesis for final results.

TABLE XX

DISTRIBUTION OF F_2 ANIMALS FROM P_1 CROSSES

RESISTANT MALE X SUSCEPTIBLE FEMALES

No. Days of Caries Time	No. F_2 Progeny					
	P_1 Cross No. 18	P_1 Cross No. 19	P_1 Cross No. 20	P_1 Cross No. 21	P_1 Cross No. 22	Total
14 - 28				2	3	5
28 - 42	5	1	6	3	13	28
42 - 56	18	2	28	12	11	71
56 - 70	19	2	30	11	18	80
70 - 84	8	1	22	14	7	52
84 - 98	10	1	15	4	5	35
98 - 112	4		18	12	4	38
112 - 126	2		11	3	5	21
126 - 140	4		5	9	4	22
140 - 154	1		6	3	3	13
154 - 168			3	2		5
168 - 182			1	3		4
182 - 196	1		1		1	3
196 - 210				1	1	2
210 - 224			2			2
224 - 238			1	2	1	4
238 - 252				1		1
252 - 266	1			2		3
266 - 280					2	2
294 - 308				1		1
Total	73	7	149	85	78	392

from P_1 cross No. 19. Ample numbers of F_2's were produced from the other P_1 crosses. At the time of writing 18 F_2 animals from P_1 crosses 18, 21, and 22 had not developed caries. All, however, had been on the caries diet over 100 days, and 5 over 200 days. Consequently, when these animals have developed caries, the means and standard deviations of the F_2 progeny from these 3 P_1 crosses will be larger than the values shown in tables of data on these matings.

Table XXI was constructed to compare the data of the F_1 and F_2 animals. It is interesting to note that the mean caries time of the F_2 progeny is less than the mean of the respective F_1 offspring. This tendency will be modified to some extent when the records of all of the F_2's are complete. In P_1 cross No. 20, however, the records are already complete.

The possible reasons for the mean of the F_2 progeny being lower than that of the F_1's should be analyzed. It is probable that the P_1 females carried one, or possibly two, genes for resistance in the heterozygous state. The P_1 male may have carried the alleles of these particular genes. For instance, let it be assumed that one of the P_1 females had the genetic constitution of Aabbcc, and the P_1 male aaBBCC. Some of the F_1's would thus receive A, B, and C. If A and B, or A and C have a total effect greater than the sum of their separate effects, resistance in the F_1 progeny would be increased above that which otherwise might be expected. In the F_2 generation, however, segregation would tend to again break apart some of these genic combinations and re-

TABLE XXI

DATA ON F_1 AND F_2 PROGENY FROM P_1 CROSS RESISTANT MALE X SUSCEPTIBLE FEMALES

P_1 ♂ 305(185)

No. of P_1 Cross	No. and Caries Time of P_1 Females	No. of F_1 Progeny	Mean Caries Time for F_1 Progeny	σ of F_1 Progeny	No. of F_2 Progeny	Mean Caries Time for F_2 Progeny	σ of F_2 Progeny	Coef. of Variation of F_1's	Coef of Variation of F_2's
18	500(30)	23* 22	108.09±14.26* 94.59 ± 4.83	66.90* 22.13	73	76.23±4.36	36.68	61.89* 23.40	48.12
19	508(29)	12	76.08 ± 7.00	25.55	7	58.86±8.90	21.81	33.58	37.54
20	537(35)	18	95.28 ± 8.41	34.64	149	85.83±3.12	37.94	36.36	44.20
21	536(35)	17	111.47 ± 6.42	25.67	85	102.36±6.21	56.98	23.03	55.67
22	462(36)	18	86.44 ± 5.34	23.01	78	81.31±5.91	51.94	26.62	62.65
Total		88* 87	97.33 ± 4.65* 93.79 ± 3.06	43.40* 28.36	392	86.29±2.34	46.34	44.59* 30.24	53.70

*These data include F_1 female 89(405) from P_1 cross 18. Data immediately beneath do not include her.

duce the total progeny average by reducing the resistance of the intermediate animals. There seems to be evidence that this is actually true. Figure 3 shows graphs of the distribution of F_1 and F_2 progeny. It will be noted that the mode of the F_2's is located further toward the susceptible side than that of the F_1's. The region of the mode includes the more or less intermediate animals. If the number of pairs of genes involved is greater than three, such combinations and segregations would be still more likely.

In Table XXI two values are given for the means, standard deviations, and coefficients of variation of the F_1 progeny in P_1 cross No. 18, and for the column of totals. One F_1 female, No. 89, shown in Table XI, did not develop caries until 405 days. None of her sibs exceeded 125 days. Thus it would seem that some unknown factors, possibly not entirely genetic, were operating to produce this single extremely late caries developer. Since there is such a wide gap between this animal and her sibs, two sets of data are shown. The first includes this female and the second omits her. In the analysis of these data, the values secured by omitting this rat will be used for comparison with the F_2 data.

The degree of segregation in the F_2's as compared to that in the F_1's should indicate whether or not multiple factors are involved in the production of dental caries. The relative size of the standard deviation indicates the degree of segregation. Table XXI shows a comparison between the standard deviations, as well as the coefficients of variation,

of F_1 and F_2 animals from P_1 crosses resistant male X susceptible females. The number of F_2 progeny from P_1 cross No. 19 is so small that this cross will not be comsidered in these comparisons.

The standard deviations of the F_2 progeny from P_1 crosses 18, 21, and 22, and totals for all the crosses combined, are significantly greater than those of the respective F_1 offspring. These differences could not have been due to chance in one case out of a hundred. In P_1 cross No. 20, the standard deviations of the F_1 and F_2 progeny are not significantly different, although that of the F_2's is greater than that of the F_1's. The fact that the degree of segregation in the F_2 progeny is significantly greater than that in the F_1's strongly suggests that multiple factors are involved in susceptibility and resistance to caries.

The same difficulty with the loss of young was experienced among the F_2's from the reciprocal crosses susceptible male X resistant females, as was mentioned previously in the case of the F_1's from the same crosses. Consequently the number of F_2 progeny produced is too small to be of much value. Tables XXII and XXIII show the caries time of the F_2's from P_1 crosses 24 and 26, and 4 back-cross progeny. The back-cross animals came from a F_1 male from P_1 cross 26, and a Hunt and Hoppert fifth generation susceptible female. As suggested in the case of the F_1's from these two P_1 crosses, it appears that the prevalence of disease among the F_2's may have lessened their resistance to caries. There is, neverthe-

TABLE XXII

F_2 PROGENY FROM P_1 CROSS NO. 24

F_1 ♂ No. 297(68)

Cross #89	Cross #90
♀ 299(68)	♀ 216(54)
♂ 673(32)	♂ 725(42)
♀ 674(46)	♂ 726(26)
	♀ 727(40)
	♀ 728(26)
	♀ 729(40)
Av. 39.00	Av. 34.80

TABLE XXIII

F_2 PROGENY FROM P_1 CROSS NO. 26

F_1 ♂ No. 112(59)

Cross #47
♀ 115(59)
♂ 395(62)
♂ 396(62)
♀ 398(87)
♀ 399(62)
♂ 516(45)
♂ 517(45)
♂ 518(45)
♂ 684(57)
♂ 685(82)
♀ 686(40)
♀ 687(55)
Av. 58.36

BACK-CROSS PROGENY FROM F_1 MALE 112(59) (FROM P_1 CROSS 26)

AND H&H FIFTH GENERATION SUSCEPTIBLE FEMAL 966(28)

Cross #61
♀ 966(28)
♂ 694(54)
♀ 695(38)
♀ 696(54)
♀ 823(49)
Av. 48.75

less, some evidence of segregation among the offspring from cross No. 47, Table XXIII.

Back-cross Progeny

This discussion will be concerned with back-crosses made by mating F_1 males (from P_1 crosses resistant male X susceptible females) with Hunt and Hoppert early fifth generation susceptible females. It would have been more desirable if these F_1 males had been back-crossed to their respective P_1 female parents. This was not considered feasible, however, because these dams were approaching the age at which sterility usually occurs.

Progeny were secured from 4 of the 5 crosses made. Table XXIV shows the results of these matings. In cross No. 63 it will be noted that one of the back-cross progeny, female 454(235) was an extremely late animal. Like the late F_1 female 89(405), it seems improbable that this high degree of resistance was due entirely to heredity. Table XXV shows the mean and standard deviation of these animals. Data are shown which both include and exclude female 454. It should be noted that the mean of these back-cross animals is significantly less than the means of both the F_1's and F_2's shown in Table XXI.

If resistance results from multiple factors, the percentage of F_1 progeny having a caries time exceeding 56 days should be greater than that of the F_2's, and that of the F_2's

TABLE XXIV

BACK-CROSS PROGENY FROM F_1 MALES AND HUNT AND HOPPERT FIFTH GENERATION SUSCEPTIBLE FEMALES

Sires	F_1 ♂ 20(87) from P_1 Cross No. 18	F_1 ♂ 43(110) from P_1 Cross No. 20	F_1 ♂ 26(101) from P_1 Cross No. 21	F_1 ♂ 66(83) from P_1 Cross No. 22
	Cross #62	Cross #54	Cross #55	Cross #63
Dams	♀ 941(33) H&H 5th Gen. Sus.	♀ 917(39) H&H 5th Gen. Sus.	♀ 926(39) H&H 5th Gen. Sus.	♀ 942(33) H&H 5th Gen. Sus.
Progeny	♂ 537(26)	♂ 373(85)	♂ 379(42)	♂ 449(35)
	♂ 538(26)	♂ 374(57)	♂ 380(42)	♂ 450(84)
	♂ 539(64)	♂ 375(99)	♂ 381(63)	♂ 451(125)
	♂ 540(26)	♀ 376(74)	♀ 382(56)	♂ 452(46)
	♀ 541(119)	♀ 377(85)	♀ 383(98)	♀ 453(84)
	♀ 542(26)	♀ 378(43)	♀ 384(70)	♀ 454(235)
	♂ 697(63)	Av. 73.83	♂ 493(54)	♂ 604(56)
	♂ 698(22)		♂ 494(40)	♂ 605(42)
	♂ 699(36)		♀ 495(40)	♀ 606(69)
	♀ 700(35)		♀ 496(40)	♀ 607(69)
	♀ 701(50)		♀ 497(54)	♀ 608(69)
	♀ 702(50)		♀ 498(78)	♂ 766(29)
	Av. 45.25		Av. 56.42	♀ 767(55)
				♀ 768(55)
				♀ 769(39)
				Av. 72.80

TABLE XXV

DATA ON BACK-CROSS PROGENY FROM F_1 MALES (FROM P_1 CROSSES RESISTANT MALES X SUSCEPTIBLE FEMALES) AND FIFTH GENERATION SUSCEPTIBLE FEMALES

No. of Cross	No. of Progeny	Mean Caries Time of Progeny	σ of Progeny
62	12	45.25 ± 8.02	26.63
54	6	73.83 ± 8.38	18.78
55	12	56.42 ± 5.25	17.44
63	15* 14	72.80 ± 13.16* 61.21 ± 6.71	49.27* 24.22
Total	45* 44	63.31 ± 5.57* 59.50 ± 4.14	36.96* 27.16

*These data include ♀ 454(235). Those just beneath omit her.

TABLE XXVI

A COMPARISON OF THE SEGREGATION OF F_1, F_2, AND BACK-CROSS PROGENY ABOVE AND BELOW 56 DAYS OF CARIES TIME

No. of P_1 Cross	No. of F_1 Progeny	F_1 Progeny		No. of F_2 Progeny	F_2 Progeny		No. of Back-cross Progeny	Back-cross Progeny	
		% 56 Days or below	% above 56 Days		% 56 Days or below	% above 56 Days		% 56 Days or below	% above 56 Days
18	23	0.00	100.00	73	31.51	68.49	12	75.00	25.00
19	12	16.67	83.33	7	42.86	57.14	--	-----	-----
20	18	5.88	94.12	148	25.00	75.00	6	16.67	83.33
21	17	0.00	100.00	85	21.12	78.82	12	66.67	33.33
22	18	5.88	94.12	78	35.44	64.56	15	53.33	46.67
Total	88	4.56	95.44	392	27.81	72.19	45	57.77	42.23

greater than that of the back-cross animals. Table XXVI reveals that this is the case in every instance except one. In this case there were only 6 animals in the back-cross generation. The totals shown in this table include a sufficient number of animals to give reliable results. These totals reveal that 95.44 per cent of the F_1's, 72.19 per cent of the F_2's, and 42.23 per cent of the back-cross animals had a caries time which exceeded 56 days.

The distribution of F_1 , F_2, and back-cross progeny is probably best shown graphically. Figures 3 and 4 show graphs of each of these three sets of animals. Figure 3 shows the number and Figure 4 the percentage of animals in each generation. There are as many back-cross animals in the first class, 14-28 days, as there are F_2's, although there is a total of almost nime times as many of the latter as of the former. With the exception of the extremely late animal, female 454, the back-cross generation did not extend beyond the 126-day class. The F_2's, however, extended to the 308-day class, with one or more animals in every group except one.

As shown in Figure 3, the P_1 females fall in the class 28-42 days of caries time. The P_1 male is located in the class 182-196 days. One F_1 rat is in the same class with the P_1 male, and another F_1, female 89, had an extreme caries time of 405 days. All other F_1's were distributed between the P_1's. The F_2's exceed both extremes of the P_1's.

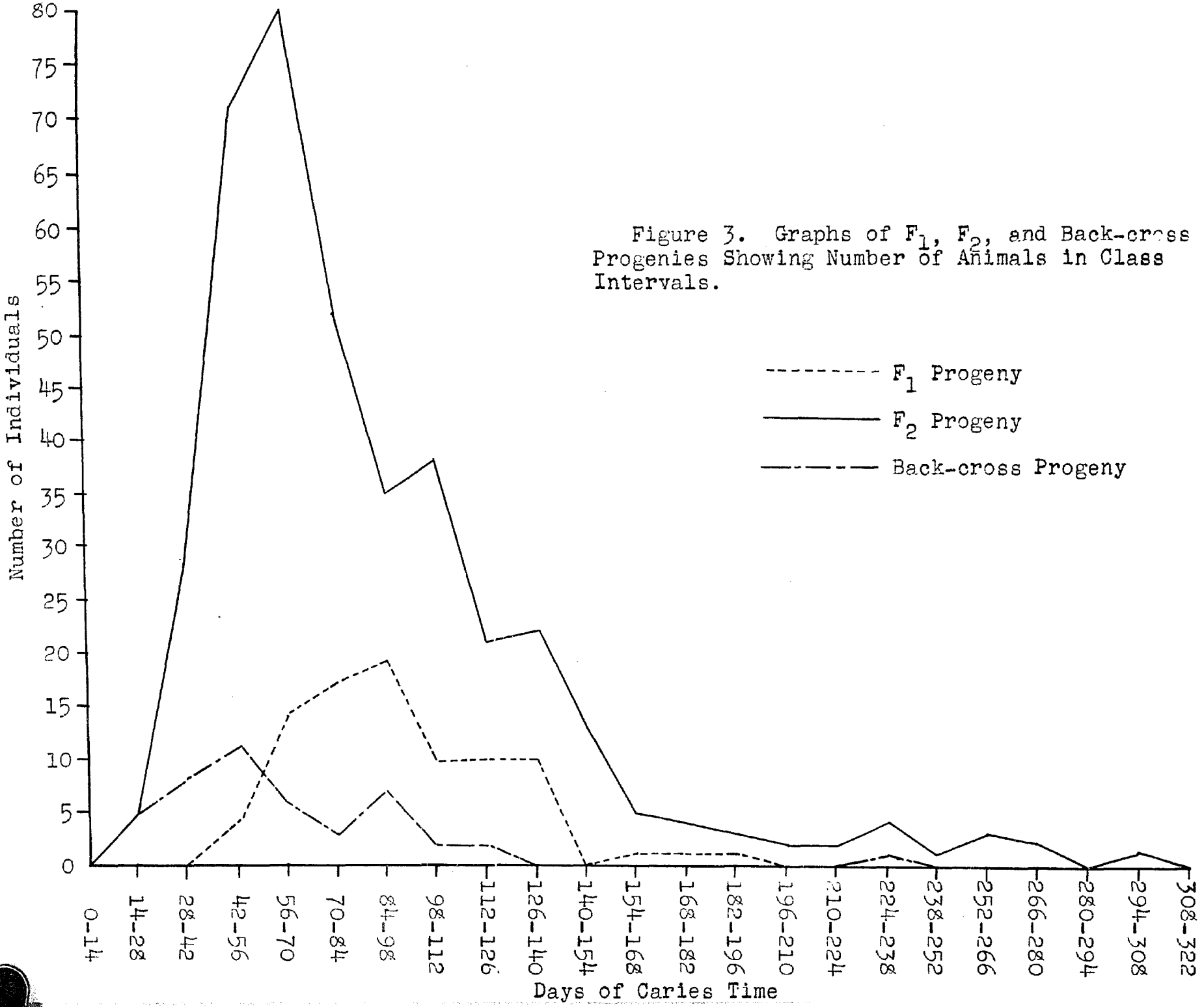

Figure 3. Graphs of F_1, F_2, and Back-cross Progenies Showing Number of Animals in Class Intervals.

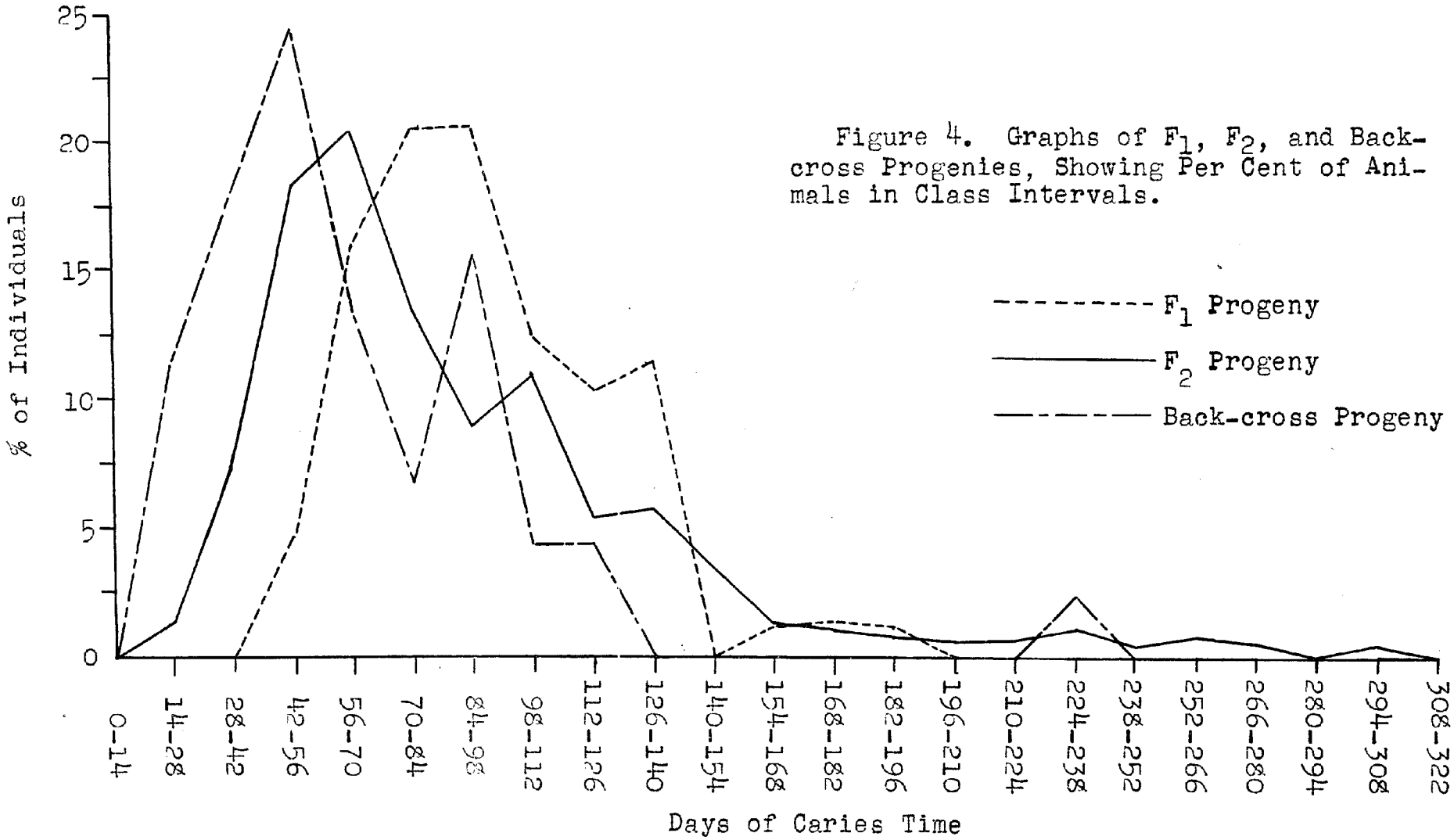

Figure 4. Graphs of F_1, F_2, and Back-cross Progenies, Showing Per Cent of Animals in Class Intervals.

This would occur if some of the P_1 females carried one or two genes for resistance which were not present in the P_1 male. This is probable, as has been previously shown.

Both the mode and the mean of the distribution of the F_1 and F_2 progeny are slightly skewed toward the susceptible side of the graph. If the P_1 females had been homozygous for susceptibility, and the P_1 male homozygous for resistance, the mode and mean of their F_1 and F_2 offspring would be expected to fall at a point intermediate between the parents. It has been shown, however, that these P_1's were apparently heterozygous. It is probable that the P_1 susceptible females carried only one, or a very few, genes for resistance. The P_1 resistant male, however, probably carried several genes for susceptibility, as indicated by the distribution of his offspring. This would mean that the total genes for susceptibility carried by both P_1 parents exceeded the total number of genes for resistance. If such were the case, the distribution of F_1's and F_2's would be skewed toward the susceptible side of the graph.

The mode for the back-cross progeny is further toward susceptibility than that of the F_1 and F_2 offspring. The same is true for the mean. The mode is located in the class which represents the lower limit of the F_1 distribution. The dispersion of back-cross animals is less than that of both F_1's and F_2's, if the single 235-day animal shown in the graph is omitted.

In general the distribution of F_1's and F_2's, and the

back-cross animals fits the theory of multiple factors. The difficulty with which resistance is being established in the Hunt and Hoppert resistant line, and the wide degree of variation in this line, suggests that the number of genes involved is not small. On the other hand, the ease with which a nearly homozygous susceptible line has been created, suggests that the number of pairs of genes is not large.

This tendency for susceptibility to become fixed rapidly may have been due, however, to a possible homozygous condition of one, or a few, genes for this trait in many of the animals used by Hoppert and Hunt in starting their experiment. This is indeed suggested by a graph of their first generation, from which the resistant and susceptible lines were produced. This graph is decidedly skewed toward susceptibility. A few animals showed considerable resistance. Such a condition, coupled with the results in succeeding generations of their lines and the data presented in this study, strongly suggest that most of the early caries developers of this first generation were homozygous for some of the genes for susceptibility. The late animals, which were used to start the resistant line, were undoubtedly much more heterozygous, carrying several genes for resistance. If such were the case, perhaps 4 or more genes are involved in the production of resistance and susceptibility to caries.

DISCUSSION AND CONCLUSIONS

The data presented in this study clearly indicate that susceptibility and resistance to dental caries are hereditary in the rat. This substantiates the findings of Hunt and Hoppert.

The evidence shown indicates that the phenotype of a rat is not a reliable indicator of the genotype, with respect to caries time. Animals which have late phenotypes may often produce some progeny with a much earlier caries time, and visa versa. Such a situation must frequently be due to heterozygosity. This is probably not always the case, however. Animals may have sometimes been recorded as late because of failure to recognize caries at its first appearance. On the other hand, accidental fractures of the teeth may have sometimes caused the initiation of caries at an earlier date than it would otherwise have appeared.

There is apparently a variation in the degree of resistance to the activity of caries after it is initiated. It was noted that rats, which had gone a considerable length of time without showing evidence of the disease, often developed only a single cavity, when caries did set in. Such a cavity frequently increased in size very slowly, so that after two or three observations the diameter was not much larger than when first noted, although there was no doubt as to its presence. Such a tendency was rarely noted in the more susceptible animals. On the contrary, the teeth of these rats frequently disentigrated very rapidly. Often two, three, or even

four cavities were present in the same mouth. An observation which was recorded as questionable when first detected, frequently had extended to include the major portion of the tooth two weeks later. Thus it is evident that susceptible animals not only develop caries earlier than resistants, but also are apt to have the disease more extensively.

A lesser degree of genetic resistance might conceivably be sufficient to prevent the appearance of caries in an older rat during a given period of time, than is the case in a young rat. The caries diet was begun when the rats were 35 days old. The teeth at that age have not reached their mature size. They may, therefore, be more susceptible to decay at this stage than when maturity is reached.

The distribution of offspring from the different progeny tests suggests that multiple factors are involved in the production of susceptibility and resistance to caries. The results from the F_1, F_2, and back-cross progenies indicate that this is the case. Genes for resistance apparently are cumulative. The fact that the caries time of a few offspring sometimes exceeds the total caries time for both parents combined, suggests that possibly two or more different genes for resistance have a greater effect when present together than the sum of their separate effects.

The number of factors involved is not known. The wide distribution among the F_2 progeny suggests that there are several, perhaps four or more.

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