NUTRITIONAL AND GENETIC FACTORS CAUSING BENT-NOSE IN THE RAT (RATTUS NORVEGICUS)

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INTRODUCTION

An organism is the resultant of a multitude of processes, many of which are initiated by agencies within the fertilized egg, while others are the consequence of external forces which impinge upon the individual during development or later life. The reciprocal relations between these external and internal agents are usually complex and as a rule but little understood. Thus, "inherited" characters may be suppressed, stimulated, or modified by "environmental" forces. The geneticist must always keep in mind the multiple causations of traits; he must remember that light, temperature, food, moisture, etc., as well as the genes create the organism; in short, he must never forget that he is a biologist. Some inherited traits are but little influenced by ordinary fluctuations in the environment, while others are profoundly affected, as the following illustrations prove.

The development of the pattern of the Himalayan rabbit[33] is due not only to the genetic constitution of the rabbit but also to the temperature to which the animal is subjected. Bar-eye[21,34] and vestigial wing[24,29] are classic examples of genetic characters in Drosophila which are influenced by temperature. The character "abnormal abdomen" in Drosophila,[23] although due to a dominant factor, is manifested only in flies which not only have this factor but also have been reared on a moist culture medium. Many other characters could be cited in this connection, some involving environmental factors other than temperature or moisture.

Bent-nose in the rat is another character which is affected not only by genetic factors but by environmental agents as well. The manifestation of bent-nose depends on the proper genetic constitution in conjunction with an imbalance in the calcium-phosphorus ratio in the diet. So essential are both of these types of causative agents that neither could be analyzed without due consideration of the other.

The name "bent-nose" was chosen because it described the character simply and adequately. (See figures 1 and 2). The rostrum was bent either to the right or the left. With the exception of the abnormalities common to rachitic animals, such as the rachitic rosary, pigeon breast etc, no other bone anomalies normally occurred in the bent-nosed animals. Thus, it can be stated that bent-nose is characterized by the bending of the rostrum of the skull.

Preliminary investigations[14] showed that the character did not manifest itself until after birth. The youngest animal to show the trait was thirty-four days of age. No animals have been known to develop the character after they were 120 days old, and in most of the rats the anomaly appeared between the age of sixty and seventy days. The degree of flexure was variable, ranging in a group of sixty-six bent-nosed skulls from 4° to as much as 35°, with 17.5° as the mean grade of flexure. The frequency of the left bends was not significantly different from that of right bends, and there was no statistically significant difference between the mean angles to the left and right. Of the sixty-six bent skulls, thirty-one were bent to the left, and thirty-five to the right. The mean angle of bend to the left was 17.8°, while that to the right was 17.3°. The difference between these two means is $0.5^\circ \pm 1.016$ which is only 0.49 times its probable error.

No description of such a character in rats has been found in the literature. However, Green and Fekete[10] have described two mice which showed a condition identical in external appearances to the bent-nose of the rat. The character might also be compared to "twisted beak", in fowls, reported be Hutt.[17] This is a sub-lethal character which appears to be inherited as a simple recessive.

Figure 1

External appearance of a
bent-nosed animal. Adult
animal with nose bent to
the right. (M.S. thesis)

Figure 2

Skulls showing the bent-nosed condition.
♀ 6 normal. ♀ 7, ♀ 9 and ♀ 11 bent.
The absence of the zygomatic bone
is not an anomaly as in each case
it was lost while the skull was
being cleaned. (M. S. thesis)

HISTORY OF BENT-NOSE

Bent-nose was discovered during 1930, in the Elementary Zoology laboratory at Michigan State College. Some of the animals that were being used for mammalian dissection showed the anomaly. These were rats from the rodent colony of the Zoology Department at Michigan State College.

Immediate investigation of the different stocks in the colony revealed that the character appeared only in the red-eyed-yellow strain derived from Dr. W. E. Castle's stock, and that it was quite prevalent in that strain which was a good indication that the character was inherited. In 1931 and 1932 Mr. L. W. Wiren made some matings of bent x normal from bent stock, and bent x bent, but the data he obtained were inadequate for any definite conclusions.

During 1932, matings were made of the various types: bent x bent, bent x normal, F_1 x F_1, and F_1 x bent. The stock was fed on the colony rat rations consisting in general of a cooked diet of various grains with fruit and meat scraps sometimes added. Occasionally they were given green lettuce, cabbage or alfalfa. Two hundred and four animals were reared, of which 93, or 45.6 per cent, were bent. Then on January 1, 1933, all the rats in the colony were put on the Purina Fox Chow ration as an economy measure. One hundred and ninety-one animals from the same types of matings were reared on this diet, and <u>not one had a bent nose</u>. An inherited trait had disappeared! This was ample proof that food was a factor in the development of this trait.

Knowing that diet was a factor, it was desirable to discover what nutritional agents favored the appearance of bent-nose, and to develop a ration that could be used in studying the genetic factors involved. If genes for bent-nose could produce their effects only under a certain type of diet, then that diet would have to be used throughout the genetic experiments. An appropriate research procedure was accordingly formulated. The nutritional experiments were done under the supervision of Dr. C. A. Hoppert of the Chemistry Department.

NUTRITIONAL FACTORS CAUSING BENT-NOSE

Introduction

To facilitate the production of a bone abnormality, one would normally experiment with the two main bone-forming elements of the diet, namely, calcium and phosphorus. Some of the early works of Steenbock,[1,13,41,42,44] Sherman,[35,36,37] and their colleagues have shown that an imbalanced calcium-phosphorus ratio,[41,42,44] in the absence of vitamin D, gives rise to rickets, a condition often accompanied by various bone defects. Such a rickets-producing ration could be made according to several different formulae. The amount of calcium could be high with the phosphorus low -- the type of ration usually employed; the amount of phosphorus could be high in relation to the calcium; or both could be present in too small amounts. Absence of vitamin D is essential in any case since its presence tends to correct the imbalance in the calcium-phosphorus ratio, or in the case of a deficiency of both of the elements in question, vitamin D permits a more efficient utilization of these elements.

Some of the more recent works have shown that other factors may affect calcification. Sabel, Cohen, and Kramer[30,31] demonstrated that strontium may cause rickets due to the injury it inflicts on the calcifying mechanism. H. V. Smith, M. C. Smith, and others[32,38,39,] have shown that fluorides also may be deleterious to calcification. The effects of fluorides tend to be centered in the enamel of the teeth. Even a lack of vitamin A may cause a change in the calcification of teeth. The work of Smith and Lantz[40] may be cited here.

Geiger, Steenbock, and Persons[8] reported a condition in the rat known as "Lathyrism" in which calcification has been interfered with. Quoting from their paper: "Lathyrism was produced in both young and adult rats by feeding diets which contained Lathyrus odoratus, the flowering sweet pea.

"Growth of the young animals was retarded by sweet peas when they were fed 80%, 50% and 25% of the diet. However, normal growth was obtained for 20 weeks with 12.5% and 5% of sweet peas. Other symptoms of lathyrism noted were lameness, spinal curvature, sternal curvature, enlargement of the costochrondral junctions, and malformation and abnormal red color of the long bones. Calcification was interfered with in young animals."

More stress was laid on the calcium, phosphorus and vitamin D constituents of the diet, in our study of bent-nose, than on the various other elements influencing calcification.

Rations Used.

Seven different rations were used in our experiments on the food factors involved. These included one well-balanced stock ration as a control diet, and six deficient rations. The ration which would produce the highest incidence of bending among progeny of bent x bent matings, and still not be so deleterious that the animals died before they were 120 days of age, was to be the ration later used in the breeding experiments. The following are the formulae for these different rations:

Ration I - Stock ration

Corn meal	-	30 parts	(28.57%)
Oat meal	-	30 parts	(28.57%)
Milk powder	-	30 parts	(28.57%)
Oil meal	-	6 parts	(5.71%)
Alfalfa	-	5 parts	(4.76%)
Yeast	-	3 parts	(2.86%)
NaCl	-	1 part	(0.95%)

Ration II - High calcium ration

Corn meal	-	38%
Oat meal	-	38%
Wheat gluten	-	20%
$Ca\ CO_3$	-	3%
Na Cl	-	1%

Ration III - High calcium ration plus cod liver oil.

This ration was the same as Ration II with 1 gram of cod liver oil added to each 100 grams of Ration II.

Ration IV - Yeast, high calcium ration

Corn meal	-	38%
Oat meal	-	38%
Wheat gluten	-	20%
$Ca\ CO_3$	-	2%
Yeast	-	1%
Na Cl	-	1%

Ration V - Irradiated yeast, high calcium ration

Corn meal	-	38%
Oat meal	-	38%
Wheat gluten	-	20%
$Ca\ CO_3$	-	2%
Irradiated yeast	-	1%
Na Cl	-	1%

Ration VI - High phosphorus ration

Corn meal	-	32%
Oat Meal	-	32%
Wheat gluten	-	10%
Oil meal	-	10%
Casein	-	5%
Yeast	-	5%
Milk powder	-	5%
Na Cl	-	1%

Ration VII - High phosphorus ration

Corn meal	-	43%
Ground wheat	-	43%
Wheat gluten	-	10%
Alfalfa	-	3%
Na Cl	-	1%

Ration I, the stock ration, was a well balanced diet affording rapid growth and high fertility. Ration II was quite similar to the Steenbock and Black No. 2965 ration containing a high amount of calcium in relation to the phosphorus present, which in the absence of vitamin D produces severe rickets. In order to determine whether the presence of vitamin D would prevent bent-nose, to each 100 grams of ration II one gram of cod liver oil was added. This constituted ration III. Realizing that 3 per cent of Ca CO_3 might make the ration so severe that the animals could not survive to 120 days, ration IV was formulated with 1 per cent of Ca CO_3 replaced by 1 per cent of yeast. The presence of the yeast would cause more growth chiefly by stimulating the appetite of the rats. Ration V was identical with IV except that the yeast was treated with ultra-violet light, thus adding vitamin D to the diet. The last two rations, VI and VII, were designed to be high in phosphorus in relation to the amount of calcium present, the reverse of the other deficient diets. Since ration VI was quite complex and produced no striking results, it was soon discarded and ration VII substituted.

Procedure

The technique employed was to keep all breeding animals on ration I, the balanced stock ration, to prevent any difficulty in getting them to breed. Pregnant females were isolated in order to get accurate data on the birth of the young. The offspring were weaned at 28 days of age and put on one of the experimental rations. Shavings were used as cage bedding throughout the experiment. Tap water was used, since its mineral content in East Lansing is not high. Rations were kept before the animals at all times. They were fed the experimental ration until at least 120 days of age, after which they were etherized and their skulls examined to determine whether or not they were bent. Some of the bent animals showed the trait markedly before they were 120 days of age, and these were transferred to the stock ration and later used for breeding.

Three strains of rats were used, the bent stock and two control stocks. The animals constituting the bent stock were progeny of bent x bent matings. One control strain was the Wistar experimental strain derived from animals obtained from the Wistar Institute of Anatomy. The second control strain consisted of black-hooded animals obtained from Dr. C. A. Hoppert's chemical laboratory at Michigan State College. It was intended at the outset that at least 50 animals of each stock would be reared on each of the seven rations. Due to a lack of animals, the Hoppert control stock was later limited to ration II, the high calcium ration, and ration VII, the high phosphorus ration.

Each animal used in experiment was given a number by a system of ear markings. Records of matings were kept in order that the ancestry of each animal could be traced. Before any animal was discarded it was etherized and the mid suture of its skull compared to a straightedge for the final check as to whether the rostrum was straight or bent. No final statement on the state of the nose was entered in the record book before this final examination was made.

Presentation and Discussion of Data

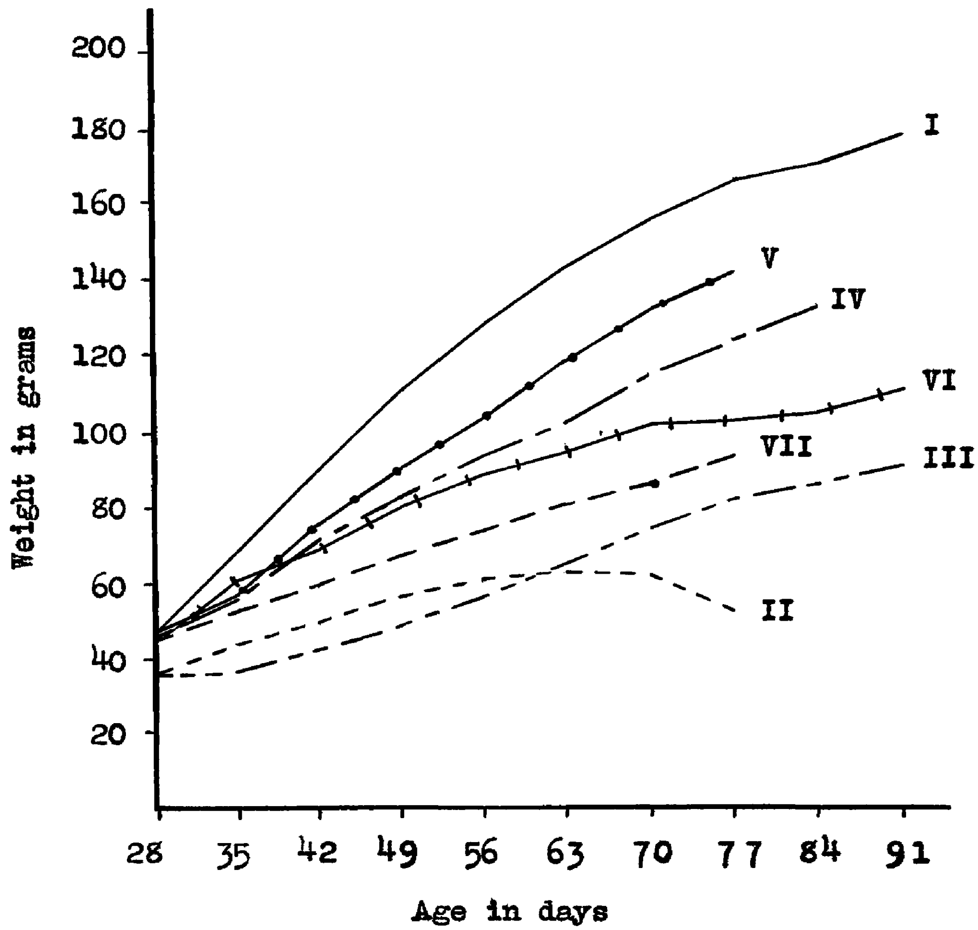
Growth Study.

One of the most effective means of determining the general response of animals to a given diet is by studying their growth curves. A number of animals from the bent stock, subsisting on the various rations, were weighed

Table I

AVERAGE WEEKLY WEIGHTS OF GROUPS OF ANIMALS FED ON THE SEVEN DIFFERENT RATIONS

Ration	No. of animals		Wt. at weaning age	Weights at the end of the week indicated, after weaning. 1st. week	2nd. week	3rd. week	4th. week	5th. week	6th. week	7th. week	8th. week	9th. week
I - stock	28	Ave. weight	47.9	67.3	89.9	110.6	128.9	144.2	157.4	165.9	171.5	178.3
ration		Ave. gain		19.4	22.6	20.7	18.3	15.3	13.2	8.5	5.6	6.8
		Ave. total gain		19.4	42.0	62.7	81.0	96.3	109.5	118.0	123.6	130.4
II - high	16	Ave. weight	36.3	43.9	50.0	56.2	60.2	63.4	62.6	52.3		
calcium		Ave. gain		7.6	6.1	6.2	4.0	3.2	-.8	-10.3		
ration		Ave. total gain		7.6	13.7	19.9	23.9	27.1	26.3	16.0		
III - high	14	Ave. weight	35.4	37.1	43.4	49.0	57.4	64.0	74.8	82.9	86.4	90.7
calcium plus		Ave. gain		1.7	6.3	5.6	8.4	6.6	10.8	8.1	3.5	4.3
cod liver oil		Ave. total gain		1.7	8.0	13.6	22.0	28.6	39.4	47.5	51.0	55.3
IV - yeast,	15	Ave. weight	47.0	56.7	70.8	83.0	94.0	105.6	116.4	123.0	135.5	
high calcium		Ave. gain		9.7	14.1	12.2	11.0	11.6	10.8	6.6	12.5	
ration		Ave. total gain		9.7	23.8	36.0	47.0	58.6	69.4	76.0	88.5	
V - irradiated	15	Ave. weight	47.2	57.7	75.5	91.6	104.1	119.5	132.5	143.9		
yeast, high		Ave. gain		10.5	17.8	16.1	13.5	15.4	13.0	11.4		
calcium ration		Ave. total gain		10.5	28.3	44.4	56.9	72.3	85.3	96.7		
VI - high	28	Ave. weight	45.3	60.5	70.1	81.3	89.6	95.6	103.2	104.6	106.6	112.0
phosphorus		Ave. gain		15.2	10.2	10.6	8.3	6.0	7.6	1.4	2.0	5.4
ration		Ave. total gain		15.2	25.4	36.0	44.3	50.3	57.9	59.3	61.3	66.7
VII - high	17	Ave. weight	40.7	49.2	54.2	62.7	67.8	75.7	82.5	89.7		
phosphorus		Ave. gain		8.6	5.0	8.4	5.1	8.0	6.7	7.3		
ration		Ave. total gain		8.6	13.6	22.0	27.1	35.1	41.8	49.1		



Ration I - stock ration

Ration II - high calcium ration

Ration III - high calcium ration plus cod liver oil

Ration IV - yeast, high calcium ration

Ration V - irradiated yeast, high calcium ration

Ration VI - high phosphorus ration

Ration VII - high phosphorus ration

GROWTH CURVES

Figure 3

at weaning time and later at 7 day intervals. Their growth curves were plotted (Figure 3), and the average weights are given in Table I. The number used from each dietary group ranged from fourteen to twenty-eight, obviously a fair sample of the whole 50 or more animals that were reared on each diet.

It is evident from these curves that while ration I, the stock ration, supported the most rapid growth, ration II, the high calcium ration, was the most severe on the animals. On ration II their average weight actually declined as much as 10.3 grams the seventh week, with a total gain of only 16.0 grams, as compared with an average of 118.0 grams gain for the animals on ration I. The average total gains of the animals on the other rations ranged between these two figures, with no other curve showing a loss in weight. The animals on ration III were consistently lighter than those on ration II for the first four weeks. It appears that this was due, in part at least, to the repelling odor of the cod liver oil, causing the animals not to eat so heartily. The death of several of the animals on ration II after the seventh week made it impossible to continue this curve, and consequently some of the others also were discontinued after the seventh week.

Mortality Study

Few deaths occurred except on ration II, the high calcium ration. Table II shows the mortality record of animals raised on this ration. The proportions dying were as follows: 17.86 per cent of 112 rats of the bent stock, 89.09 per cent of the 55 Wistar animals, and 78.13 per cent of the 64 Hoppert rats.

Table II

MORTALITY OF ANIMALS FED ON RATION II - HIGH CALCIUM RATION

Stock	No. of Animals subject to ration II	No. dead before 120 day age limit	Per cent dead
Bent stock	112	20	17.86 ± 2.44
Wistar stock	55	49	89.09 ± 2.83
Hoppert stock	64	50	78.13 ± 3.49

These results tend to substantiate the work of Gowen[9] in which he has shown that the mortality rates differ among different strains of rats fed on a ration deficient in vitamin D and high in calcium content. These three strains of animals differed in their reactions to the high calcium ration as demonstrated by the mortality rates. The mortality in the Wistar stock exceeded that in the bent stock by 71.23 ± 3.74 per cent; this difference was 19.05 times the magnitude of its probable error. The mortality of the Hoppert stock was 60.27 ± 4.26 per cent higher than in the bent stock, the difference being 14.15 times the size of the probable error. Both these differences were decidedly significant in a statistical sense. The Wistar and Hoppert stocks did not differ materially from one another, the former exceeding the latter by only 10.96 ± 4.49 per cent; this difference was only 2.44 times its probable error.

Seven, or 35 per cent, of the animals of the bent stock which died before reaching 120 days of age, were bent-nosed at death while only four, or 0.72 per cent of the Wistar stock, and one animal, or two per cent of the Hoppert stock showed the anomaly. These figures, along with data presented later in the paper, show that the strain which could survive the deficient ration best produced the highest percentage of bent-nosed animals.

Deaths on the various other rations were too few to be of great significance. One rat among the animals of the bent stock on ration III (high calcium plus cod liver oil) died before reaching 120 days of age. This animal displayed a normal nose. The only other death in the bent stock was on ration VI, the high phosphorus ration. This animal also had a normal nose. One bent-nosed and one normal Wistar animal died on ration IV, the yeast high calcium ration, and seven normal animals of the Hoppert stock died on ration VII (high phosphorus ration) before reaching the minimum age limit. No casualities occurred in any of the groups not discussed.

These results justified us in not using ration II (the high calcium diet) in the genetic experiments to follow, because of its deleterious effect upon the animals. The errors introduced by the death of such high percentages of animals would hamper genetic analysis. From this standpoint alone any of the other rations could have been used since the death rate of animals fed on them did not exceed that normally expected for animals on almost any deficient diet.

Study of rations in relation to production of bent.

The cardinal data gathered in the nutritional work are given in Table III. The animals used in computing the per cent bent-nosed on each ration included only those which lived to 120 days of age or more.

Because of so many deaths in both the Wistar and Hoppert stocks on ration II the ultimate numbers were too small to give conclusive data;

Table III

INFLUENCE OF THE SEVEN RATIONS ON THE FREQUENCY OF BENT-NOSE

	Bent stock			Wistar stock			Hoppert stock		
Ration	No. of animals	No. bent	% bent	No. of animals	No. bent	% bent	No. of animals	No. bent	% bent
Ration I Stock ration	115	1	0.87	51	0	0			
Ration II - High Calcium ration	92	46	50.00	6	4	66.66	14	0	0
Ration III - High Calcium ration cod liver oil	48	5	14.17	54	0	0			
Ration IV Yeast, high calcium ration	55	25	45.45	58	22	37.93			
Ration V Irradiated yeast high calcium ration	51	2	3.92	53	0	0			
Ration VI - High phosphorus ration	34	16	47.06	12	8	66.67			
Ration VII - High phosphorus ration	58	37	64.91	68	21	30.88	51	2	3.77

consequently these groups were not included in the discussion. Likewise the twelve Wistar animals on ration VI were omitted because of the paucity of numbers.

A general survey of the different percentages of animals developing bent-nose on the various diets shows that food must be a factor in determining whether or not an animal shall develop the anomaly. In the bent stock the percentages vary from practically no bent-nosed animals in those fed on ration I to as high as 64.91% bent-nosed on ration VII. It was noted that one bent-nosed rat on ration I was an under-sized individual. The progeny of bent x bent matings, comprising the bent stock, probably had about the same genetic constitution as far as the bent-nose character was concerned. Thus, with all environmental factors approximately equal except for the food, one could unquestionably attribute the variation in percentages of bent-nosed animals to the differences in the diets.

The results for the Wistar stock roughly paralleled those for the bent stock and pointed toward diet as a factor, but in no case was the incidence of bent-nose as high as in the corresponding group of the bent stock. On the three rations, I, III and V, producing the lowest percentages of bending in the bent stock, no bent-nosed animals appeared in the Wistar stock. 37.93 per cent of the Wistar strain on ration IV were bent-nosed as compared with 45.45 per cent of the bent stock, while only 30.88 per cent of the Wistar animals on ration VII were bent as compared with 64.91 per cent for the bent stock. A little discrepancy has entered here; in the bent stock ration VII produced a higher frequency of bent than ration IV while the reverse of this was true with the Wistar stock, but as will be shown later the irregularity was hardly statistically significant.

A more thorough analysis of the data revealed that the calcium-phosphorus ratio was the decisive factor in the diet. Ration II, containing 3 per cent Ca CO_3, raised the incidence of bent-nose from less than 1 per cent, as in ration I, to 50 per cent. The addition of vitamin D in cod liver oil (which tends to correct for the imbalance in the calcium-phosphorus ratio) cut the frequency of bent to 14.17 per cent, a decrease of 35.83 ± 4.88 per cent as compared with the frequency on ration II. This difference was 7.35 times its probable error. The addition of yeast to the high calcium diet had little effect on the incidence of bent. When the percentage of bent-nose among the animals on ration IV was compared to that among the animals on ration II, a difference of 4.55 ± 5.78 per cent was found, which was only 0.80 times its probable error. A difference this great might be expected as ration IV had only 2 per cent Ca CO_3 while ration II contained 3 per cent Ca CO_3. A very marked difference in per cent of bent was again observed when vitamin D was added in ration V. This time it was in the form of irradiated yeast. Irradiating the yeast alone reduced the frequency of bent from 45.45 per cent on ration IV to 3.92 per cent on ration V, a difference of 41.53 ± 4.88 per cent which was 8.51 times its probable error.

The percentage of bent obtained on ration VII indicated that reversing the calcium-phosphorus ration, making phosphorus high, tended to increase the frequency of bent-nose. This was not borne out with the results from ration VI. Although rations VI and VII were both high in phosphorus, VI was not as imbalanced as VII, since it was a more varied diet and contained 5 per cent milk powder which would tend to raise the calcium content.

Statistical analysis did not reveal a striking difference between the results from the high calcium rations and those from the high phosphorus rations. Ration VII gave a percentage 14.91 ± 5.49 per cent higher than that of ration II. This difference was only 2.72 times its probable error. Ration VII gave 19.46 ± 6.18 per cent more bents than ration IV, and this difference was 3.15 times its probable error. The two high phosphorus rations differed by 17.85 ± 7.15 per cent, a difference 2.50 times as great as its probable error.

Thus it could be concluded that the calcium-phosphorus ratio was a factor in predisposing bent-nose. On a ration in which these elements were quite well balanced practically no bent animals developed. When young rats were fed a ration in which this balance had been disturbed, the frequency of bending increased. The introduction of vitamin D corrected for the imbalance, and the frequency of bending diminished. These conclusions were likewise borne out by the results from the Wistar animals.

Comparison of growth with incidence of bent-nose.

When a comparison was made between growth rate and the incidence of bending on the various diets among the animals of the bent stock, it was noted that in general those rations producing slight growth caused a high frequency of bending. The contrary was likewise true. Ration I which produced the greatest gain (118 grams) in seven weeks produced less than one per cent bent-nose. In contrast of these figures, animals gained only 16 grams in seven weeks on ration II, while the frequency of bending on this ration was 50 per cent. The ration producing the highest frequency of bent-nose, ration VII, cause the animals to gain on an average only 49.1 grams. Ration IV which produced a rather high frequency of bent-nose (45.45 per cent) produced an

Table IV

THE EFFECT OF EACH RATION ON THE FREQUENCY OF BENT-NOSE AS COMPARED

WITH ITS EFFECT ON GROWTH RATE

Ration	Per cent bent-nosed	Gain in weight during the weeks after weaning
I - stock ration	0.87 per cent	118.0 grams
V - irradicated yeast, high calcium ration	3.92 per cent	96.7 grams
III - high calcium ration plus cod liver oil	14.17 per cent	47.5 grams
IV - yeast, high calcium ration	45.45 per cent	76.0 grams
VI - high phosphorus ration	47.06 per cent	59.3 grams
II - high calcium ration	50.0 per cent	16.0 grams
VII - high phosphorus ration	64.91 per cent	49.1 grams

average increase in weight of 76 grams for the seven weeks. While vitamin D when added to ration IV, forming ration V, decreased the percentage of bent-nose to 3.92 per cent, it increased the average gain in weight to 96.7 grams. Ration III was quite irregular. The frequency of bent-nose among those animals on this ration was 14.17 per cent, but the animals gained on an average only 47.5 grams in the seven weeks. However as before stated it was difficult to get the animals to eat much of a ration containing cod liver oil, and this was probably responsible for this irregularity. Likewise ration II was irregular. However, the total gain in weight of animals fed ration II varied from that in the other groups in that the animals actually lost weight during the last two weeks.

Bone-ash studies

In this investigation it was desirable to make bone ash determinations for two reasons; (1) to determine the severity of the rickets induced by the deficient diets, and (2) to know whether the different strains behaved differently toward the same ration. The percentages of ash in the bones are given in tables V to IX inclusive.

To makes these determinations groups of animals from the three stocks were sacrificed after they had been fed on ration II (high calcium ration) for twenty, forty, and sixty days, and on ration VII (high phosphorus ration) for twenty and forty days. The animals were etherized and their femurs were removed, after which all the surplus tissue was removed by rubbing with cheesecloth. Fat was extracted with alcohol in the conventional manner, and the bones were dried and weighed. After this they were ignited in an electric furnace to destroy the organic matter; the ash was then weighed, and the percentage of bone ash was calculated.

The per cent of inorganic matter in bone has for a long time been the principal criterion for determining the severity of rickets in rats. Femurs of normal rats will vary from the extreme low of 45 to as high as 60 per cent ash, depending somewhat on age, sex, food and various other factors. Females tend to have a higher ash content than do males, and as animals grow older the ash content of their bones normally increases slightly. A proper calcium - phosphorus ratio in the diet, with ample vitamin D, tends to produce a maximum percentage of ash. However, during pregnancy or lactation the percentage of bone ash tends to decrease due to the drain on the inorganic elements of the mother for the formation of the embryo or the production of milk. This percentage of bone ash is markedly lower than normal in animals suffering from rickets. Often it will be as low as 30 per cent, or even lower, depending upon the severity of the condition.

While both rations II and VII produced rickets, the high calcium ration brought about a much more extreme type than the high phosphorus ration. This was probably due to the fact that the calcium-phosphorus ratio is much more imbalanced in the high calcium ration than in the other. The high amount of calcium in ration II was obtained by adding 3 per cent of $Ca\ CO_3$ while the high phosphorus ration was developed merely with a high grain content and no milk powder. For the most part the percentages of bone ash on the high calcium ration ran below 30 per cent with an extreme low of 19.58 per cent. These percentages were probably bordering on the minimum limit compatible with survival. The difference in time beyond the 20 day period which these animals were fed this ration produced no significant difference in percentage of bone ash.

The high phosphorus ration caused the percentage of bone ash in most cases to drop within the rachitic range, i.e. around 40 per cent or lower, although in no case was the percentage extremely low. The Wistar animals which had been on this high phosphorus ration for forty days were on the border line between rachitic and normal animals. All three stocks showed an increase in percentage of bone ash of the 40 day period over the 20 day period. This indicates that the physiological mechanism of these animals had begun to adjust itself to the imbalanced calcium-phosphorus ratio.

In general it can be stated that no stock was more resistant or susceptible to the deficient diets than were the other two stocks in regard to percentages of bone ash. In various groups the average bone ash percentage of one strain may have appeared higher or lower than those of the other two, but with no stock was the percentage consistently high or low throughout.

Seven of the animals fed forty days on ration II were bent-nosed when etherized. It is interesting to note that these animals were neither lower nor higher in bone ash content than other members of their group. This would show that the factors for bent-nose are specific rather than influencing the reaction of the whole body to the deficient diet.

It is interesting to compare the results of the bone-ash determinations with those of the mortality study in regard to the resistance to the deficient diets displayed by each of the three strains. While the death rate showed that the bent stock was superior to either of the other two stocks in resisting the deleterious effects of ration II, the strains did not differ materially in percentage of bone ash.

Table V

PER CENT OF BONE ASH IN THE FEMURS OF ANIMALS

FED 20 DAYS ON RATION II - HIGH CALCIUM RATION

Bent Stock		Wistar Stock		Hoppert Stock	
Animal No.	% Ash	Animal No.	% Ash	Animal No.	% Ash
♂ 3406	24.67	♀ 3327	28.90	♂ 3057	32.41
♂ 3407	30.97	♀ 3328	26.07	♂ 3058	29.99
♂ 3408	26.15	♀ 3329	27.80	♂ 3059	28.39
♂ 3409	27.04	♀ 3330	26.80	♂ 3060	26.74
♂ 3410	29.81	♀ 3331	29.49	♀ 3062	26.77
♂ 3411	29.37	♀ 3332	31.05	♀ 3063	28.96
♂ 3412	27.75	♀ 3333	32.63	♂ 3064	31.79
♀ 3413	28.03	♀ 3334	30.72	♂ 3065	34.43
♀ 3414	30.09	♀ 3343	33.40	♀ 3066	32.69
♀ 3415	29.02	♀ 3344	32.48	♀ 3067	29.50
		♀ 3345	34.19	♀ 3068	29.96
		♀ 3346	31.57	♀ 3069	33.70
		♀ 3347	29.67	♀ 3070	31.55
		♀ 3348	33.79	♀ 3071	28.60
Ave.	28.29	Ave.	30.68	Ave.	30.39

Table VI

PER CENT OF BONE ASH IN THE FEMURS OF ANIMALS

FED 40 DAYS ON RATION II - HIGH CALCIUM RATION

Bent Stock		Wistar Stock		Hoppert Stock	
Animal No.	% Ash	Animal No.	% Ash	Animal No.	% Ash
♂ 2694	25.98	♂ 3311	20.02	♂ 3044	29.43
* ♂ 2695	25.07	* ♂ 3312	21.58	♂ 3045	27.38
♂ 2698	25.87	♂ 3313	22.48	♀ 3047	24.31
♀ 2699	25.31	* ♂ 3314	21.50	♀ 3048	23.90
♀ 3401	24.97	* ♂ 3316	23.19	♀ 3049	27.82
♀ 3402	23.76	♀ 3317	21.19	♂ 3051	25.89
♀ 3403	24.59	* ♀ 3318	21.23	♂ 3052	27.73
♀ 3404	24.29	♀ 3320	21.09	♂ 3053	31.72
♀ 3405	25.05	♂ 3321	21.50	♂ 3054	28.91
		* ♂ 3322	20.98	♀ 3055	24.48
		* ♂ 3323	20.93	♀ 3056	23.36
		♂ 3324	20.78		
Ave.	24.99	Ave.	21.37	Ave.	26.81

*Bent-nosed animals.

Table VII

PER CENT OF BONE ASH IN THE FEMURS OF ANIMALS FED
60 DAYS OF RATION II - HIGH CALCIUM RATION

Bent Stock		Wistar Stock		Hoppert Stock	
Animal No.	% Ash	Animal No.	% Ash	Animal No.	% Ash
♂ 2678	25.13	♀ 3335	26.57	♂ 3039	30.95
♂ 2679	19.58	♀ 3337	23.65	♂ 3041	39.64
♂ 2680	22.66	♀ 3338	25.93	♂ 3038	26.26
♂ 2681	24.30	♀ 3340	22.41	♂ 3040	38.05
♀ 2682	25.46	♀ 3341	20.72	♀ 3042	27.27
♀ 2684	27.00			♀ 3043	33.56
♀ 2686	21.31				
♀ 2687	21.45				
Ave.	23.36	Ave.	23.86	Ave.	32.62

Table VIII

PER CENT OF BONE ASH IN THE FEMURS OF ANIMALS FED
20 DAYS ON RATION VII - HIGH PHOSPHORUS RATION

Bent Stock		Wistar Stock		Hoppert Stock	
Animal No.	% Ash	Animal No.	% Ash	Animal No.	% Ash
♂ 3439	35.16	♂ 35.72	48.85	♂ 3099	39.48
♂ 3440	31.81	♂ 35.73	36.71	♂ 4301	35.63
♂ 3441	31.60	♂ 35.74	41.17	♀ 4302	32.02
♂ 3442	33.33	♂ 35.75	44.17	♀ 4303	38.05
♀ 3443	32.98	♂ 35.76	41.06	♂ 3093	34.45
♀ 3444	37.00	♂ 35.77	42.09	♂ 3094	36.15
		♂ 35.78	41.77	♂ 3095	36.42
		♂ 35.79	38.96	♂ 3096	37.20
		♂ 35.80	41.00	♂ 3097	35.49
		♂ 35.81	36.30	♂ 3098	37.06
		♀ 35.82	41.48		
Ave.	33.66	Ave.	40.96	Ave.	36.40

Table IX

PER CENT OF BONE ASH IN THE FEMURS OF ANIMALS

FED 40 DAYS ON RATION VII - HIGH PHOSPHORUS RATION

Bent Stock		Wistar Stock		Hoppert Stock	
Animal No.	% Ash	Animal No.	% Ash	Animal No.	% Ash
♂ 3432	39.20	♂ 3566	45.11	♂ 3084	43.16
♂ 3433	40.09	♀ 3568	47.21	♂ 3085	46.31
♂ 3434	39.72	♀ 3569	49.09	♂ 3086	45.24
♀ 3435	40.30	♀ 3570	47.36	♂ 3087	42.37
♀ 3436	42.60	♀ 3571	46.42	♂ 3088	43.60
♀ 3437	42.61			♀ 3090	37.30
♀ 3438	43.07			♀ 3091	36.47
				♀ 3092	38.99
Ave.	41.08	Ave.	47.04	Ave.	41.68

Results of subjecting animals to a deficient ration at 16 days of age.

An attempt to increase further the incidence of bending among the progeny of bent x bent matings by putting the animals on the deficient ration at an earlier age proved to be futile. Forty rats were used in this test. At the age of sixteen days the animals with their mothers were put on ration II (high calcium ration). They were weaned at twenty-one days of age, the mother being returned to ration I (stock ration), but the young were continued on the deficient diet.

Such treatment was too severe for the young animals, and they all died long before they reached an age of 120 days. As seen in table X, seven of the animals died at 28 days, and the remainder died at ages ranging from fifty to seventy-eight days. The seven that died early were all of one litter, indicating that they were more susceptible in some way than the others.

It was very evident that this plan of feeding could not be followed in a breeding experiment as most of the animals would be dead before they had reached the age at which the bending normally appeared. However, three of these animals did develop bent-nose, as was discovered on examination of their skulls when they died. One of them was 60 days of age, one sixty-one, and the third seventy-six days old when it died.

Results of inbreeding attempt.

An inbreeding experiment was started to determine whether inbreeding with selection would increase the frequency of bending. If bent-nose was caused by multiple factors or by a simple recessive or simple dominant gene with modifying factors a significant difference should have been observed between the first and second generations of inbreeding, in which only animals with a high degree bending had been used in the matings.

Table X

AGES AT WHICH PROGENY OF BENT X BENT MATINGS DIED WHEN PUT ON RATION II (HIGH CALCIUM RATION) AT SIXTEEN DAYS OF AGE AND WEANED AT TWENTY-ONE DAYS OF AGE.

	No. dying at the given age.
28 days of age	7
50 days of age	2
54 days of age	3
55 days of age	1
56 days of age	2
57 days of age	1
58 days of age	1
59 days of age	1
60 days of age	2
61 days of age	5
62 days of age	1
64 days of age	1
67 days of age	1
69 days of age	2
70 days of age	1
71 days of age	3
73 days of age	1
75 days of age	1
76 days of age	1
77 days of age	2
78 days of age	1
Total no. of animals	40

Fortunately four fertile bent-nosed females and one fertile bent-nosed male, all of the same litter were obtained. These were progeny of a bent x bent mating and had been reared on ration VII (high phosphorus ration). The four bent-nosed females were mated to their sib producing nine litters consituting a total of seventy-six offspring.

At that stage of the experiment, data indicated that ration II - high calcium ration produced the highest frequency of bent-nose. For this reason these inbred animals were subjected to ration II at weaning age -- twenty-eight days. But the inbred animals did not survive the bad effects of this ration as well as animals of the bent-stock had. One hundred and twenty days after birth, or after 92 days on the deficient feed, only 25, or 32.9 per cent of the animals were yet surviving. Of the bent stock 82.14 per cent survived the effects of this ration to the 120 day age limit. This decrease in vitality may have been due in part at least to the inbreeding.

Among the twenty-five animals living 120 days, seven, or 28 per cent were bent. This is a much lower percentage than 50 per cent, that obtained in the bent stock on the same ration mentioned in the previous section. However, twenty-five animals were too few to furnish significant results. Of the fifty-one animals dying before they were 120 days old, sixteen, or 31.37 per cent were bent. These figures would indicate that neither bent nor normal animals tended to die more readily within the 120 day period. The data are given in table XI.

It was impossible to rear a second generation in this phase of the experiment. This was due to the high mortality and low fertility of the animals that lived to 120 days of age. So few fertile animals matured that brother sister matings could not be made.

Table XI

FREQUENCY OF BENT-NOSED ANIMALS AMONG THOSE OF THE FIRST GENERATION OF INBREEDING.

	No. of Animals	No. Normal	No. Bent	Per cent Bent
Animals living 120	25	18	7	28.00
Animals not living 120 days	51	35	16	31.37

However, these animals were not all reared in vain. Each animal was weighed at birth, marked, and again weighed at weaning time. It was desirable to know whether there was any correlation between size at birth or gain in weight during early life, and tendency toward bent-nose. Had it been found that animals which later developed bent-nose were smaller at birth than normals, one would have suspected some prenatal factor as having a causative influence on bent-nose. Moreover, the fact that bent-nosed animals did not gain as rapidly before weaning might have led to the discovery of some factor operating early in life.

Data presented in tables XII and XIII show that there was no significant difference between the mean weights at birth of bent and normal animals. The same is true when the mean weights at weaning age and the gain in weight from birth to weaning are compared. The difference between the means in birth weights was .43 ± .88 grams, or .49 times its probably error. The means of the weight at weaning time differed by 4.25 ± 9.04 grams, a difference of .47 times its probably error. The difference between the means of gain in the two groups of animals was only .42 times its probable error, it being 3.68 ± 8.83 grams. Thus, there is no evidence that the factor or factors causing bent-nose affect body weight either before or after birth.

It has been shown that food affected the incidence of bent-nose and also the growth rate, and that low growth rate tended to be correlated with a high incidence of bending. However, the data just presented indicate that the high frequency of bent-nose was not a result of low growth rate nor was the reverse true. In the animals of the inbred experiment, having approximately the same genetic constitution for bent-nose, practically no difference was observed between the weights and growth rates of the bent animals and those of the normals. This would tend to indicate that the dietary factors causing a high frequency of bent-nose were different from those affecting a low growth rate.

Table XII

COMPARISON OF MEAN WEIGHTS OF BENT-NOSED ANIMALS AND NORMAL ANIMALS OF THE INBREEDING EXPERIMENT

	Normal animals			Bent animals		
	Mean Wt.	σ	P.E.	Mean Wt.	σ	P.E.
Mean wt. at birth	6.40 gms.	.92	.62	5.97 gms.	.94	.63
Mean wt. at weaning	39.33 gms.	9.92	6.69	35.08 gms.	9.02	6.08
Mean gain in weight	32.79 gms.	9.71	6.55	29.11 gms.	8.77	5.92

Table XIII

WEIGHTS OF NORMAL ANIMALS IN
INBREEDING EXPERIMENT

Animal No.	Wt. at birth in grams	Wt. at weaning in grams	Gain in grams
♂ 2802	6.70	33.40	26.70
♀ 2805	7.20	34.52	27.32
♀ 2807	7.14	35.30	28.16
♀ 2827	6.50	17.30	10.80
♂ 2828	6.80	11.30	4.50
♀ 2830	7.00	24.60	17.60
♂ 2831	7.40	25.80	18.40
♀ 2832	6.90	21.00	14.10
♀ 2833	7.40	27.90	20.50
♂ 2810	5.25	32.70	26.45
♂ 2814	5.24	32.00	26.76
♀ 2816	5.14	32.50	26.36
♂ 2835	7.80	36.20	28.40
♀ 2836	6.80	34.40	27.60
♂ 2837	7.70	54.30	46.60
♀ 2838	6.00	31.30	25.30
♀ 2839	7.50	42.60	34.10
♀ 2840	7.00	35.70	28.70
♀ 2841	7.70	46.30	38.60
♂ 2861	6.80	38.10	31.30
♀ 2862	7.60	54.00	46.40

Table XIII (continued)

WEIGHTS OF NORMAL ANIMALS IN
INBREEDING EXPERIMENT

Animal No.	Wt. at birth in grams	Wt. at weaning in grams	Gain in grams
♀ 2864	6.80	37.05	30.25
♂ 2865	7.80	52.70	44.90
♂ 2866	7.70	51.80	44.10
♀ 2867	7.00	48.80	41.80
♀ 2868	7.20	49.80	42.60
♂ 2869	8.40	55.60	47.20
♂ 2818	5.68	36.00	30.32
♀ 2819	5.20	33.00	27.80
♀ 2821	5.76	47.00	41.24
♂ 2822	6.35	44.00	37.65
♂ 2823	5.87	39.50	33.63
♀ 2824	5.72	47.90	42.18
♂ 2825	5.05	33.50	28.45
♂ 2826	5.75	45.50	39.75
♂ 2852	5.60	41.10	35.50
♀ 2853	5.50	41.80	36.30
♂ 2854	5.10	27.80	22.70
♂ 2857	5.70	27.60	21.90
♀ 2858	5.50	39.00	33.50
♂ 2844	5.70	46.80	41.10
♀ 2846	5.20	40.20	35.00

Table XIII (continued)

WEIGHTS OF NORMAL ANIMALS IN
INBREEDING EXPERIMENT

Animal No.	Wt. at birth in grams	Wt. at weaning in grams	Gain in grams
♂ 2847	5.70	44.40	35.70
♀ 2848	5.22	41.70	35.48
♂ 2849	5.62	42.70	37.08
♀ 2850	5.05	37.20	32.15
♂ 2851	5.60	33.20	27.60
♂ 2870	7.60	54.70	47.10
♀ 2871	6.20	47.60	41.40
♂ 2872	7.30	54.00	46.70
♀ 2873	6.00	45.80	39.80
♀ 2874	6.45	43.00	36.55
♀ 2876	6.50	52.40	45.90

Table XIII (continued)

WEIGHTS OF BENT-NOSED ANIMALS OF INBREEDING EXPERIMENT

Animal No.	Wt. at birth in grams	Wt. at weaning in grams	Gain in grams
♂ 2801	6.75	32.73	25.98
♂ 2803	6.26	30.30	24.04
♀ 2804	6.80	32.85	26.05
♂ 2806	6.35	32.77	26.42
♂ 2808	7.53	34.00	26.47
♂ 2829	7.60	20.00	12.40
♂ 2834	6.90	28.70	21.80
♀ 2809	4.73	32.30	27.57
♂ 2811	5.23	33.20	27.97
♂ 2812	5.55	34.30	28.75
♂ 2813	5.27	31.00	25.73
♀ 2815	5.00	30.40	25.40
♂ 2817	4.60	24.60	20.00
♀ 2842	6.60	47.50	40.90
♀ 2843	7.00	47.20	40.20
♂ 2863	7.00	54.40	47.40
♂ 2820	5.44	43.00	37.56
♀ 2855	4.90	23.10	18.20
♀ 2856	5.20	34.40	29.20
♀ 2859	4.60	26.90	22.30
♀ 2860	5.70	34.40	28.70
♀ 2845	5.60	45.00	39.40
♀ 2875	6.80	54.00	47.20

Summary of Nutritional Studies

1. Diet is a factor influencing the manifestation of bent-nose in the rat. With other factors controlled, different diets produce different frequencies of bent-nose.

2. The calcium-phosphorus balance in the food is a very important factor in determining whether bent-nose develops. That is, if the calcium-phosphorus ratio of the diet is imbalanced, bent-nose occurs. This imbalance may be affected by adding either too much calcium or too much phosphorus.

3. As the introduction of vitamin D ameliorates the deleterious effect of the disturbed calcium-phosphorus ratio, it likewise decreases the frequency of bent-nose.

4. In general, the percentage of bent-nose produced by the various rations varied inversely with the growth rate. That is, the rations most deleterious to growth favored the development of bent-nose. On the same ration, however, significant differences in weight or gain in weight were not found between the bents and their normal siblings.

5. The bent-nosed strain was more resistant to the high-calcium ration as judged by the percentage of mortality than was the Wistar strain or the Hoppert strain.

6. Bone ash determinations failed to demonstrate any difference in bone ash content between the three different strains when fed on a deficient ration. Ration II (high calcium ration) produced a lower percentage of bone ash than did ration VII (high phosphorus ration).

7. The effect of putting the animals on ration II at the age of sixteen days and weaning them at the age of twenty-one days could not be determined, because they were unable to withstand the treatment and died before showing the bent-nose character.

8. An attempt to increase the frequency of bent-nose by inbreeding was unsuccessful, for the progeny reared on the deficient ration showed low fertility and high mortality.

9. We were unable to prove the existence of prenatal or postnatal factors in the production of bent-nose which influenced the birth weights, weights at weaning age, or increase in weight of the bent-nosed rats.

10. Since ration VII (high phosphorus ration) produced the highest percentage of bent-nosed animals and yet did not kill the animals before they were 120 days old, it proved to be the most desirable ration on which to rear the animals in the genetic experiments.

GENETIC FACTORS CAUSING BENT-NOSE

Introduction

Work like Gowen's[9] may prove very enlightening in the study of inheritance of bone abnormalities, since often nutritional factors are involved. He demonstrated that separate lines of rats showed distinct differences in respect to survival on a diet deficient in vitamin D and high in calcium. The author has data tending to substantiate this.

Probably the most satisfactory genetic explanation of a bone abnormality in rodents is that given by Hunt, Mixter and Permar[16,22] who worked on flexed tail in the mouse. This was a condition in which the caudal vertebrae were malformed and fused, usually producing varying degrees of flexure in the tail. Although it has been definitely shown that this trait is inherited as a recessive, modifying factors are involved. The development of this character has been further studied by Kamenoff.[19]

A condition similar to flexed tail in _Mus musculus_ has been reported by Huestis and Barto[15] in _Peromyscus_. It was stated that this character appeared to be due to two recessive genes.

Green and Brown,[12] in making a study of hereditary variations in the skull of the rabbit, showed that profound variations in the form of the skull could be transmitted unaltered from parent to offspring. However, no definite genetic scheme was advanced to explain the inheritance of this variation. A genetic cranometric study of two species of mice by the late C. V. Green[11] has shown that the size of the skull tends to be inherited. However, Green states that a thoroughly satisfactory genetic explanation appears impossible with the data he has gathered. Colton[3] reported work on the "high-brow" rat, but this study failed to demonstrate whether the character was inherited.

Procedure for Studying the Genetic Factors Causing Bent-nose in the Rat.

Before the genetic experiments could be undertaken, a suitable ration had to be found. As mentioned in the preceding section, there were two requisites for this ration: (1) it could not be so severe as to kill the animals before they reached 120 days of age, and (2) it should produce the maximum incidence of bents among progeny of bent x bent matings without causing too high a death rate.

Since it was observed early that ration II (high calcium ration) produced a high incidence of bending, it was first employed in the breeding experiment, but when more data were gathered the high mortality under ration II was revealed, and it was discarded for ration VII (high phosphorus ration). When all the data on the different rations were analyzed, it was found that ration VII filled both of the above requisites.

A strain of rats genetically free from factors for bent-nose was needed for crossing with the bents. The Wistar experimental strain and the Hoppert strain were tested for this purpose. The data given in table III show that the Wistar experimental strain carries factors for bent-nose to a relatively high degree. On ration VII, 30.88 per cent of the Wistar animals were bent as compared to 64.91 per cent bent among those of the bent strain. This reduced the probability of finding a strain genetically free from bent-nose since so many strains of rats were related to the Wistar stock. Dr. W. E. Castle states it was quite probable that our red-eyed-yellow strain formerly obtained from him, in which the anomaly first appeared and from which our bent stock was developed, were related to the Wistar animals. The strain was originally secured in the United States from a fancier in England, but in the course of subsequent experiments has been crossed with a number of strains, among which may have been animals from the Wistar Institute. The Hoppert strain proved to be nearly free from bent factors. On ration VII only 3.77 per cent of this stock was bent. Since the Wistar stock carried factors for bent-nose, it was highly probable that the Hoppert stock was as free from bent-nose as any available strain. Dr. Hoppert's animals were originally obtained from the University of Wisconsin, beyond which their ancestry was unknown. One other alternative would have been to develop a strain free from bent-nose by inbreeding and selection, but this would have extended the duration of the experiment beyond possible bounds. For these reasons the Hoppert stock was chosen for the P_1 matings.

All breeding animals were kept in ration I, the balanced stock ration. Pregnant females were isolated, and the date of the birth of the young was recorded. At twenty-eight days of age the young were weaned and transferred to the deficient ration, the mother being returned to the breeding cage. The deficient ration was kept before the animals constantly. After they were 120 days old they were etherized and the mid sutures of their skulls were carefully compared to a straightedge to determine whether they were bent or normal.

P_1 matings were of bent animals of the bent stock mated to normal animals of the Hoppert stock. Since the deficient diets rendered the animals of little use for breeding, ample F_1's were reared on the stock ration for making the $F_1 \times F_1$ matings. These F_1 animals were also back-crossed to bent-nosed individuals. Normal animals, the progeny of bent x bent, were mated to bents in an attempt to determine whether or not they carried the factors predisposing to bent-nose. Also, brother-sister matings of bents were made in order to study the change in incidence of bending afforded by inbreeding, as reported in the previous section.

Presentation and Discussion of Data

Data obtained in nutritional studies

Before making an analysis of the percentages of bent-nose among the F_1, F_2, and back-cross generations it was well to analyze genetically the data obtained in the nutritional studies. These data are given in table III.

The figures secured in that study furnish evidence that bent-nose is influenced by genetic factors. When a group of animals were fed on one particular ration with other environmental factors constant, only a part of these developed the bent-nose condition. For instance, of the animals of the bent stock fed on ration VII, only 64.91 per cent were bent-nosed. Thus, there must have been some innate factors acting toward the manifestation of bent-nose in these animals which were absent, or in some way deficient, in the remaining 35.09 per cent. If not, one would expect all of these animals to be bent-nosed or all normal. The same reasoning holds wherever both bent and normal animals appeared in a group fed on any of the diets.

However, all the groups of the bent stock on the various rations probably had approximately the same genotypes for bent-nose. Yet the different rations induced different percentages of bents. Thus, certain genetic constitutions react differently to different diets. An animal possessing the genes which would cause it to appear bent on the high phosphorus ration might be normal if fed one of the other diets.

The fact that the three different stocks, when fed on the same ration with other environmental factors nearly constant, produced different percentages of bent-nosed animals is evidence that the character is genetic. The percentage of bent-nosed animals of the Wistar stock on ration VII was 30.88 per cent as compared with 64.91 per cent for the bent stock on the same diet. The difference between these percentages was 34.03 ± 5.66 per cent, a difference which was 6.01 times its probable error. In the Hoppert stock on the same ration 3.77 per cent were bent-nosed. This was 61.14 ± 4.58 per cent less than that of the bent stock, a difference 13.35 times its probable error, and 27.11 ± 4.18 per cent less than that of the Wistar stock. The latter difference was 6.49 times its probable error. Thus, it was evident that there was a difference in the innate constitution of these different stocks as to the production of bent-nose. Factors for bent-nose must have been rare in the Hoppert stock, more prevalent in the Wistar stock, and still more numerous in the bent stock.

Only normal-nosed animals were used for matings in the Wistar stock, and for this reason one would expect a greater variation in the accumulation of factors for bent-nose in the Wistar stock than in the bent stock in which all matings were bent x bent. Since they were all normal, it was not known which of the normal Wistar animals used for breeding carried genetic factors for bent. This probably explains why, while ration IV produced a lower percentage of bent-nose than did ration VII in the bent stock, a higher percentage of bent-nose appeared on ration IV than on ration VII in the Wistar stock. The parents of those Wistar animals on ration IV may have carried more genes for bent-nose than did the parents of those on the other ration.

Data obtained in genetic studies

The data secured from the various crosses (P_1, F_1, back crosses, etc.) do not reveal any simple mode of inheritance for bent-nose. Although several theories might have been advanced as to the factorial situation for bent-nose, none were found that satisfactorily fits all the ratios obtained. However, the data are quite enlightening. See table XIV.

One of the characteristics of the data given in table XIV which first appears to the observer is the decline in percentage of bent-nose with a decrease of bent-nosed ancestry. Progeny of bent x bent matings were 64.91 per cent bent-nosed, while progeny of bent x normal (from bent x bent) matings were only 29.07 per cent bent-nosed. Farther down the scale bent x normal (Hoppert stock) matings gave progeny of which only 3.36 per cent were bent-nosed. With all environmental factors constant, such data afford evidence that the character is genetic.

Unquestionably there is evidence of segregation. There were only 3.36 per cent bent-nosed among the F_1 animals from bent x normals (of the Hoppert stock), and of the F_2's from the matings F_1 x F_1, the F_1's derived from matings bent x Hoppert normal, 10.71 per cent were bent. Although this was not a high percentage of bent-nosed animals in the F_2 generation, it was significantly higher than that obtained in the F_1 generation. The difference was 7.35 ± 1.96 per cent, which was 3.75 times its probable error.

Likewise, there was a significant difference between the percentage of bents among the F_1's and that among the backcross (F_1 x bent) progeny. This difference was 7.89 ± 2.63 per cent which was exactly three times its probable error. The percentage of bent among the backcross progeny was low, and constituted the greatest barrier in formulating any genetic interpretation.

The data indicate that bent-nose is recessive to normal. The percentage of bent among the F_1 animals was very much lower than that expected if dominant factors were responsible for the trait. If the factors were dominant one would expect between 50 and 100 per cent of the F_1's to be genetically bent and there are no good reasons for supposing that the action of ration VII would have lowered the phenotypic percentage to 3.36 per cent. The more logical explanation is that the factors are recessive and that the Hoppert stock carried some of these factors for bent-nose. Bent-nose genes were present in the Hoppert stock as indicated by the two bent nosed Hoppert animals which appeared in the nutritional experiments.

It appears that more than one pair of genetic factors are responsible for bent-nose. However, as with Reed's investigation on harelip in the house mouse,[26,27,28] it may be that one gene is necessary for the production of the character and the others are merely modifiers. If the character were controlled by two recessive genes one would expect 6.25 per cent of bents in the F_2 generation. This is not beyond the bounds of probability when compared to the 10.71 per cent actually obtained, the difference being 3.46 ± 1.61 per cent, which is only 2.15 times its probable error. However, such a genetic scheme would not give a backcross percentage comparable to that obtained. One would expect 25 per cent bent-nosed in the progeny of the backcross, and actually 11.25 per cent was all that appeared. Three

Table XIV

FREQUENCIES OF BENT-NOSE AMONG ANIMALS FROM THE

VARIOUS CROSSES, ALL REARED ON RATION VII

Type of mating	Offspring: Total offspring	Number bent-nosed	Per cent bent-nosed
Bent x bent	58	37	64.91 ± 4.22
Normal (from bent x bent) x bent	86	25	29.07 ± 3.37
F_1 (bent x normal)	119	4	3.36 ± 1.11
F_1 (F_1♂ x F_1♀)	168	18	10.71 ± 1.61
Backcross (F_1 x bent)	80	9	11.25 ± 2.38

pairs of recessive factors would give 12.5 per cent bent-nosed among the backcross progeny, which closely approximates the percentage obtained. However, among the F_2 animals, a percentage of 1.6 would be expected. Thus, the actual percentage of bent-nosed animals in the F_2 generation is too high to justify this theory.

Another type of mating, the results of which are well worth further consideration, is that of normal animals (from bent x bent matings) mated to bent animals. Such matings were desirable in order to test whether or not such normal animals carried factors for bent-nose. The results showed that these normals did carry such factors. Of the eighty-six progeny reared, twenty-five or 29.07 per cent were bent. This was significantly different from the 3.36 per cent obtained when bent-nosed animals were outcrossed to the normal Hoppert stock. The difference was 25.71 ± 3.55 per cent, which was 7.24 times its probable error.

However, the normal animals obtained from bent x bent matings were not of the same genetic constitution as the bent-nosed animals. Progeny tests showed this. The 64.91 per cent bent-nosed, the percentage obtained among the progeny of bent x bent matings, was significantly greater than the 29.07 per cent bent among the animals one of whose parents was a normal from a bent x bent mating. The difference was 35.84 ± 5.40 per cent, which was 6.64 times its probable error.

A part of the F_1 and F_2 animals, i.e. those born at the beginning of the genetic experiments, were put on ration II, the high calcium ration. The F_1 animals were kept on ration II throughout the experiment, while the F_2's were later changed to ration VII, the high phosphorus ration.

(These data are not included in table XIV.) Of the forty-eight F_1 animals which were put on ration II, nineteen, one of which was bent, lived to 120 days of age. Also, one of the twenty-nine which died at an earlier age was bent. The eighty-one F_2's which were put on ration II were transferred to ration VII early enough so that they all survived to the 120 day age limit. Sixteen, or 19.75 per cent, of these F_2's were bent. This is a higher value than that obtained among the F_2's reared from weaning on ration VII only, which is not exactly consistent with the results of the nutritional experiment which showed that ration II produced fewer bent-nosed than ration VII.

The fact that a higher percentage of bents was obtained in these F_2's whose diet was changed from high calcium to high phosphorus than in those fed the high phosphorus ration throughout is worth noting although the difference between the two percentages was not quite great enough to be significant in a statistical sense. The difference was 9.04 ± 3.39 per cent, which was 2.67 times its probable error. However, the difference is great enough to indicate that here is a possible approach to the solution of the problem. The alteration made in the feeding increased the percentage of bents in the F_2's to 19.75 per cent which is not so far below 25 per cent, that expected if the trait were controlled by a single pair of recessive factors. Future work on the problem could profitably be directed in search of some possible dietary changes and the proper time for making these changes which would increase the frequency of bent-nose. Thus, ratios might be obtained which would fit some genetic explanation.

Summary of Inheritance Studies

1. Bent-nose in the rat is influenced by genetic factors. Different types of matings produce different frequencies of bent-nose with environmental factors constant.

2. There is definite evidence of segregation of factors. The frequency of bent-nose in the F_2 generation is significantly greater than that in the F_1 generation.

3. It is evident that in general the factors for bent-nose are recessive to those for normal. The percentage of bent animals in the F_1 generation is too small to indicate any dominant factors for bent.

4. Possibly more than one pair of genetic factors is necessary for the manifestation of bent-nose. Whether some of these factors are merely modifiers has not been determined, but the ratios do not indicate merely a single pair of factors.

LITERATURE CITED

1. Bethke, R. M., H. Steenbock, and Mariana T. Nelson. 1923.
Fat-soluble Vitamins. XV Calcium and Phosphorus Relations to Growth and Composition of Blood and Bone with Varying Vitamin Intake.
Jour. Biol. Chem. 58: 71-103.

2. Bittner, John J. 1936.
Differences Observed in an Inbred Albino Strain of Mice Following a Change in Diet.
I. Litter Strain.
Roscoe B. Jackson Memorial Laboratory Nutritional Bulletin No. 1.
II. Mortality.
Roscoe B. Jackson Memorial Laboratory Nutritional Bulletin No. 2.

3. Colton, H. S. 1929.
"High brow" Albino Rats.
Jour. Hered. 20: 225-227.

4. Dawson, Alden B. 1927.
Further Studies on the Epiphyses of the Albino Rat Skeleton, with Special Reference to the Vertebral Column, Ribs, Sternum, and Girdles.
Anat. Record. 34: 351-363.

LITERATURE CITED Continued

5. Dawson, Alden B. 1934.

Further Studies on Epiphyseal Union in the Skeleton of the Rat.

Anat. Record. 60: 83-86.

6. ____________. 1934.

Additional Evidence of the Failure of Epiphyseal Union in the Skeleton of the Rat. Studies on Wild and Captive Gray Norway Rats.

Anat. Record. 60: 501-511.

7. Dodds, G. S. and Hazel C. Cameron. 1934.

Studies on Experimental Rickets in Rats.

Am. Jour. Anat. 55: 135-165.

8. Geiger, Beatrice J., H. Steenbock, and Helen T. Parsons. 1933.

Lathyrism in the Rat.

Jour. of Nutrition. 6: 427-442.

9. Gowen, John W. 1936.

Inheritance as it Affects Survival of Rats Fed on a Diet Deficient in Vitamin D.

Genetics. 21: 1-23.

10. Green, Charles Velmar, and Elizabeth Fekete. 1932.

Some Cranial Anomalies in Mice.

Anat. Record. 53: 187-191.

LITERATURE CITED Continued

11. Green, C. V. 1932.

A Genetic Craniometric Study of Two Species of Mice and their Hybrids.

Jour. Exp. Zool. 63: 533-551.

12. Green, Harry S. N., and Wade H. Brown. 1932.

Hereditary Variations in the Skull of the Rabbit.

Science. 76: 421-422.

13. Hart, E. B., H. Steenbock, and C. A. Hoppert. 1921.

Dietary Factors Influencing Calcium Assimilation. I. The Comparative Influence of Green and Dried Plant Tissue, Cabbage, Orange Juice, and Cod Liver Oil on Calcium Assimilation.

Jour. Biol. Chem. 48: 33-50.

14. Heston, W. E. 1934.

Inheritance of Bent-nose in the Rat, (Rattus norvegicus), with Observations on the Effect of Nutrition.

Thesis for the Degree of M. S., Michigan State College.

15. Huestis, R. R., and Elizabeth Barto. 1936.

Flexed-tailed Peromyscus.

Jour. Hered. 27: 73-75.

LITERATURE CITED Continued

16. Hunt, Harrison R., Russell Mixter, and Dorothy Permar. 1933.
Flexed Tail in the Mouse, Mus musculus.
Genetics; 18: 335-366.

17. Hutt, F. B. 1932.
Eight New Mutations in the Domestic Fowl.
Proc. 6th. Internat. Cong. Gen. 2: 96-97.

18. Hydak, Mykola H. 1935.
Pigmentation in Black-haired Rats.
Science. 82: 107-108.

19. Kamenoff, R. S. 1935.
Development of the Flexed Tailed Mouse.
Jour. Morph. 58: 117-155.

20. King, Helen Dean. 1931.
Studies on the Inheritance of Structural Abnormalities in the Rat.
Am. Jour. Anat. 48: 231-259.

21. Krafka, Joseph. 1920.
The Effect of Temperature upon Facet Number in the Bar-eye Mutant of Drosophila.
Jour. Gen. Physiology. 2: 409-464.

22. Mixter, Russell, and Harrison R. Hunt. 1933.
Anemia in the Flexed Tailed Mouse, Mus musculus.
Genetics. 18: 367-387.

LITERATURE CITED Continued

23. Morgan, T. H. 1915.

The Role of Environment in the Realization of a Sex-linked Mendelian Character in Drosophila.

Am. Nat. 49: 385-429.

24. Nadler, J. E. 1926.

Effect of Temperature on Length of Vestigial Wing in Drosophila virilis.

Genetics; 11: 584-589.

25. Orent, Elsa R., H. D. Kruse, and E. V. McCollum. 1934.

Studies on Magnesium Deficiency in Animals.

VI. Chemical Changes in the Bone, with Associated Blood Changes, Resulting from Magnesium Deprivation.

Jour. Biol. Chem. 106: 573-593.

26. Reed, Sheldon C., and George D. Snell. 1931.

Harelip, a New Mutation in the House Mouse.

Anat. Record. 51: 43-50.

27. Reed, Sheldon C. 1936.

Harelip in the House Mouse.

I. Effects of the External and Internal Environments.

Genetics. 21: 339-360.

LITERATURE CITED Continued

28. Reed, Sheldon C. 1936.

Harelip in the House Mouse.

II. Mendelian Units Concerned with Harelip and

Application of the Data to the Human Harelip Problem.

Genetics. 21: 361-374.

29. Roberts, E. J. 1918.

Fluctuation in a Recessive Mendelian Character.

Jour. Exp. Zool. 27: 157.

30. Sabel, Albert Edward, Julius Cohen, and Benjamin Kramer. 1935.

The Nature of the Injury to the Calcifying Mechanism

in Rickets Due to Strontium.

Biochemical. Jour. 29: 2640-2645.

31. ____________. 1935.

Phosphatase Activity and Calcification in Strontium Rickets.

Biochem. Jour. 29: 2646-2650.

32. Schour, Isaac, and Margaret Cammack Smith. 1934.

The Histological Changes in the Enamel and Dentin of the

Rat Incisor in Acute and Chronic Experimental Fluorosis.

Univ. of Ariz. Agri. Exp. Sta. Tech. Bul. No. 52.

33. Schultz, W. 1930.

Kälteschwärzung Weisser Haare und Roter Albinoaugen.

Schriften Phys. Kon. Gesell. Königsberg. 68.

Cited from Sinnott and Dunn. Principles of Genetics.

Second Edition. McGraw-Hill Book Company, Inc.

New York and London. p. 346.

LITERATURE CITED Continued

34. Seyster, E. W. 1919.

Eye Facet Number as Influenced by Temperature in the Bar-eye Mutant of _Drosophila melanogaster_ (ampelophila). Biol. Bul. 37: 168-181.

35. Sherman, H. C., and A. M. Pappenheimer, 1921.

Experimental Rickets in Rats.

Jour. Exp. Med. 34: 189-198.

36. Sherman, H. C., and H. K. Stiebeling. 1929.

Quantitative Studies of Responses to Different Intakes of Vitamin D.

Jour. Biol. Chem. 83: 497-504.

37. __________. 1930.

The Relation of Vitamin D to Deposition of Calcium in the Bone.

Proc. Soc. Exp. Biol. Med. 27: 663-665.

38. Smith, H. V., and Margaret Cammack Smith. 1932.

Mottled Enamel in Arizona and its Correlation with the Concentration of Fluorides in Water Supplies.

Univ. of Ariz. Agri. Exp. Sta. Tech. Bul. No. 43.

39. Smith, Margaret Cammack, and Edith M. Lantz. 1933.

Experimental Production of Mottled Enamel.

Univ. of Ariz. Agri. Exp. Sta. Tech. Bul. No. 45.

LITERATURE CITED Continued

40. Smith, Margaret Cammack, and Edith M. Lantz. 1933.
Changes in the Incisors of Albino Rats Accompanying a Deficiency of Vitamin A.
Jour. of Home Economics. 25: 411-415.

41. Steenbock, H. and A. Black. 1924.
Fat Soluble Vitamins.
XVII. The Induction of Growth-promoting and Calcifying Properties in a Ration by Exposure of Ultra-violet Light.
Jour. Biol. Chem. 61: 405-422.

42. Steenbock, H., E. B. Hart, J. H. Jones, and A. Black. 1923.
Fat-soluble Vitamins.
XIV. The Inorganic Phosphorus and Calcium of the Blood Used as Criteria in the Demonstration of the Existence of a Specific Antirachitic Vitamin.
Jour. Biol. Chem. 58: 59-70.

43. Steenbock, H. and M. T. Nelson. 1924.
Fat-soluble Vitamins.
XIX. The Induction of Calcifying Properties in a Rickets-producing Ration by Radiant Energy.
Jour. Biol. Chem. 62: 209-216.