THE EFFECT OF THYROXINE AND PARATHYROID EXTRACT ON THE INCIDENCE OF FLEXED TAILS IN A FLEXED TAILED STRAIN OF THE HOUSE MOUSE [MUS MUSCULUS]

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Elden Edward Kline

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THE EFFECT OF THYROXINE AND PARATHYROID EXTRACT ON THE INCIDENCE OF FLEXED TAILS IN A FLEXED TAILED STRAIN OF THE HOUSE MOUSE (MUS MUSCULUS)

by

ELDEN EDWARD KLINE

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Introduction

The purpose of this investigation was to ascertain the effect of thyroxine and parathyroid extract on the flexure in the tail of a flexed tailed strain of mice. It was considered possible that these hormones might affect the incidence of flexed tails in the offspring of parent stock from a flexed tailed strain.

Two problems were studied: (1) the effect of increased metabolism induced by thyroxine administration on the incidence of flexure; and (2) flexure, being partly a bone anomaly, it was desirable to investigate the effect of parathyroid extract on flexure since this hormone is so intimately concerned with mineral metabolism.

This investigation was conducted at the rodent colony of the Department of Zoology at Michigan State College.

History

A flexed-tail strain of the house mouse (Mus musculus) was developed by Dr. H. R. Hunt who discovered the recessive gene mutation in 1927 in a stock of albino mice. The abnormal animals are anemic at birth and develop rigid flexures of the tail. Many individuals also show a white belly spot (Clark, 1934).

Flexed-tail is characterized by stiff, angular bends or spirals, or by rigid segments without flexures. The character is very variable, some tails being extremely bent while a few homozygous individuals can only be distinguished from normals by breeding tests.

Hunt and Permar (1928) found the character was a recessive but gave a ratio of 6.9 normals to 1.0 flexed tail in the F2. Hunt, Minter and Permar (1933) obtained 40.98 ± 1.12 per cent of flexed-tailed mice in a backcross. In an effort to do away with postnatal differential death rate, only undepleted litters of seven or more animals were then used. In these litters the percentage of flexed-tailed mice was 48.30 ± 1.72, which is fairly close to the expected 50 per cent for the backcross. Applying the same method to their F2 data, they increased the percentage of flexed mice from 16.17 ± .68 to 18.66 ± 1.08 per cent (25 per cent expected).

Normal overlap are phenotypically normal individuals which are homozygous for the flexed or belly spot genes. Clark (1934) demonstrated that both the belly spot and flexed tail types had a high percentage of normal overlaps, while anemia behaved as a typical Mendelian recessive without normal overlapping. This normal overlapping of the flexed character has been reported by most investigators. Belly spot was shown to be very closely linked with the flexed-tail-anemia complex.

Kamenoff's classical work (1935) clearly demonstrated the mechanism of tail flexure. The flexures consisted of unilateral fusions of adjacent vertebrae. If fusion were complete, straight stiff segments were produced. The immediate cause of the fusion was failure of early cartilage in part of the invertebral disc to differentiate normally into fibers about the fourteenth day of embryonic life along with an apparent reduction in the numbers of cells in the abnormal region (a retardation of growth). At period of flexure according to Kamenoff the early cartilage peripheral to the nucleus pulposis is differentiating in the normals into fibrous tissue by flattening and elongation of the cells together with a rapid multiplication in the number of cells per unit volume. Where the flexure occurs this differentiation is seen only on one side. A bend in the notochord was often seen but was not the cause of the fusions. Two possibilities were discussed by Kamenoff as to how the gene causes tail flexures and anemia. One is that anemia was produced by the same retardation in growth which produces the flexures. The other is that anemia appears earlier than the flexing and produces the more general retardation evident by the smaller length of the vertebrae. Kamenoff suggested the anemia was caused possibly by a retardation of growth in a haemopoietic center such as the liver. This was supported by the preliminary study of Anderson (1932) and later in his own investigations Kamenoff found anemia was due to a delay in the liver erythrogenesis.

Kamenoff (1935) found the anemia together with the flexed tail condition in the embryo as early as fourteen days after fertilization. This is before the bone marrow assumes a haemopoietic function, which is placed at the sixteenth day by De Aberle (1927).

Mixter and Hunt (1933) demonstrated the transitory nature of the anemia which disappeared during the first few weeks of life. They found the new-born individuals to be, on the average, deficient in the hemoglobin and erythrocytes. Gruneberg (1942a), (1942b) described the anemia as normocytic hypochromic anemia, persisting during the first two weeks after birth, but having ceased at the beginning of the third week. Pathological cells survive in the circulation for at least two, but not more than six weeks after birth. With the disappearance of these surviving cells the red blood picture of flexed mice became normal. Mixter and Hunt (1933), Kamenoff (1935) and Gruneberg (1942) have found that adult flexed mice, following recovery, have blood cell counts at least as high or higher than normal.

In a latter publication, Gruneberg (1942) found adult mice showing an anomoly of red blood cells. This was caused by persistence of some siderocytes into adult life. Otherwise he found the blood picture quite normal. Siderocytes are red cells of anemics containing considerable amounts of free or easily detached iron. These are not confined to anemics. They have been found in normal embryonic rats and mice, in human embryos and new-born babies. These siderocytes, which are haemoglobin deficients, were the basis for the anemia he now calls "siderocyte anemia," which is distinct from all other known anemias. These siderocytes were found 12 days before birth.

F2 generations and backcrosses made by Hunt and Permar (1928), Hunt, Mixter and Permar (1933), Kamenoff (1935) and Clark (1934) produced a significant deficiency of flexed-tailed mice. Anemia has been suggested as the diagnostic character rather than flexed or belly spot. Kamenoff (1935) endeavored to show that the deficiency of the flexed-tail class in the backcross was due to faulty classification rather

than differential prenatal mortality, for only 13 per cent prenatal mortality was found in flexed-tailed mice as compared with 34 per cent by MacDowell (1924) in non-flexed. MacDowell's figure, however, includes all mortality up to weaning time. Clark's (1934) and Gruenberg's (1942) work strongly suggested the anemia is a more constant and dependable expression of the flexed gene than is the flexed tail itself.

Hunt, Mixter and Permar (1933) mated together animals with the same type of flexure to see if the various degrees of flexure were controlled by genetic factors. They were unable to develop pure lines of flexed animals because of sterility due to inbreeding. They found the coefficient of correlation between grades of flexures in flexed parents and their offspring was $+.243 \pm .029$, which is a slight significant correlation indicating that the degree of flexure is inherited to some extent.

Reports of tail mutations in mice similar to the flexed-tailed strain of Hunt's have not been uncommon. Plate (1910) described a kinky tail mutation and concluded that its inheritance did not follow Mendelian laws. An extensive study of the histology of Plate's mutant mice was made by Blank (1916).

Gluecksohn-Schoenheimer (1938) in studying the development of two tailless mutants in the mouse, found the tails became constricted on the proximal end, resulting in necrosis and reabsorption of the tail distal to the constriction. He suggested a disturbance in the early embryonic development. Both Plate's and Gluecksohn's explanations resemble the idea presented by Kamenoff that the abnormality of Hunt's flexed-tailed strain was due to an interference in the early general metabolism of the embryo.

Dunn, Gluecksohn-Schoenheimer and Bryson (1940) reported a tail mutation which was virtually a lethal dominant. In the heterozygous state there were either short tails or no tails at all. Often shortened or crooked spines and a lowered viability after birth were reported in connection with this mutation.

Dabrovolskaia-Zavadskaia (1927a, 1927b, 1928a, 1928b) and Dabrovolskaia-Zavadskaia and Kobozieff (1927a, 1927b, 1928, 1930a, 1930b) described a mutation in the house mouse resulting in partial or complete absence of the tail and in various flexures and constrictions of the tail remnant encountered in X-ray experiments. This trait that Dabrovolskaia-Zavadskaia (1933) proved to be a dominant lethal gene with various flexures modifying genes was isolated by selection into separate strains by Chelsey and Dunn (1936) (1937).

A similar character presented by Caspari and David (1940) consisted of kinky tails and abnormalities in the backbone and ribs. It behaved like an autosomal dominant with high or complete lethality in the homozygous condition.

A short-tailed mutant in the house mouse reported by Chelsey (1935) was lethal in the homozygous state. Evidence was found in an embryological study that the normal development of the neural tubes was, in part, dependent on the presence of a normal notochord.

Reed (1937) described the inheritance and expression of a tail mutation, fused, which responded as a dominant.

In 1931 Crew and Auerback reported a tail abnormality "Pig-tail" in the house mouse. This character closely resembles the flexed-tail strain of Hunt's. "Pig-tail" is due to a recessive gene with modifiers and some intrauterine condition affecting its penetration in the homozygotes.

Flexed-tail was described by Hoates and Barto (1936) in Peromyscus maniculatus (deer mouse) as a genetic recessive. The gene expressed itself in a shortening and distortion of the caudal vertebrae with distortion of the invertebral joint.

Loanes and MacDowell (1942) reported a new tail mutation, "Screw-tail" in the house mouse.

"Stub" a mutation, was reported by Dunn and Glueckshn-Schoenheimer (1942). There was a reduction of the tail to a short stiff stub. It acted as a recessive showing a great uniformity of all mutants.

By this survey one can readily see tail mutations are not uncommon, but few have the advantages found in the flexed tail strain of Hunt's where much valuable research has already been accomplished.

Methods

The animals were kept in sheet metal cages, twelve inches square and deep provided with a wire mesh top. Wood shavings were used as litter in the cages. Water was supplied by gravity flow glass bottles and tubes. The diet of the mice was relatively constant, consisting of a ground feed of mixed grains and seeds fed in porcelain saucers, and dog biscuits suspended by a wire from the top of the cage. Food and water were constantly supplied. Sanitation consisted of weekly renewing the wood shaving litter, washing the water-bottles and tubes and replacing clean, dry porcelain dishes for the feed. The cages were thoroughly washed and dried once a month. The temperature was held constant at eighty degrees Fahrenheit by a thermostat regulation of the steam heating system. The building was new, made of cement blocks with concrete floors, a desirable and efficient place to work.

The statistics used to interpret the results obtained in the experiments employed common and standard statistical methods.

The standard error of a percentage was obtained from the following statistical formula where the sum of P and Q is one hundred per cent.

$$S.\ E.\% = \sqrt{\frac{P\,(1-P)}{n}}$$

S. E.% = Standard error of a decimal fraction.

P = The fraction of the mice which are not flexed.

Q = 1-P = The fraction of mice that are flexed.

n = The total number of individuals used in calculating P and Q.

The standard error of a difference was calculated from the formula.

$$S.\ E.\ diff. = \sqrt{(S\ E_1)^2 + (S\ E_2)^2}$$

S. E. diff. = The standard error of the difference between two decimal fractions.

S. E.$_1$ = Standard error of one decimal fraction.

S. E.$_2$ = Standard error of another decimal fraction.

The "t" value was found from the formula:

$$t = \frac{P_1 - P_2}{\text{S. E. of } (P_1 - P_2)}$$

P_1 = The fraction of flexed in one group.

P_2 = The fraction of flexed in another group.

S. E. diff. = Standard error of the difference.

The odds were obtained from the "Tables of Pearson's Type III Function" by Salvora (1930).

Breeding Program

All the mice used as parent stock were numbered serially, allowing no duplicates. The numbering was accomplished by removing toes. The removal of toes caused no noticeable inconvenience.

One male animal was placed in a breeding cage with one to four females. When the females came into advanced pregnancy, they were removed and placed in individual cages, where the young were born. Observations were made daily until parturition. The new-born mice were individually recorded as to number, sex, tail flexure and date of birth. After the first day the new-born mice were observed and recorded weekly for at least three weeks, or longer if the flexure could not be definitely determined.

The female was returned to the same breeding cage and male 21 days after parturition. Each female was always mated to the same male for the control and experimental litters, to keep the control and experimental groups as genetically alike as possible. Homozygous, flexed females were always crossed with homozygous flexed males (flexed x flexed) so that all the progeny were homozygous for flexed.

The first litter from each female was recorded as the control litter; no injections were administered during the gestation. The following litter or litters were experimental litters in that during the gestation period the female received injections of thyroxine or parathyroid extract.

A system of classifying the degree (grade) of flexure was used to designate by a number the amount of tail involvement produced by the expression of the flexed gene. The method used was a modification of Hunt, Mixter and Permar's (1933) classification.

Like these writers the same fundamental characteristics were used; (1) the percentage of the tail length which was rigid and (2) the number and magnitude of the angles. The object was to grade the amount of tail involvement. The scale was as follows:

Grade 0. Non-flexed and cannot be differentiated from a normal tail. By visual inspection and digital examination no fused vertebrae could be detected.

Grade 1. Non-flexed, a straight tail with some fused vertebrae. This grade could not be distinquished from a normal tail by visual examination and sometimes only with difficulty could the fused vertebrae be found by palpation.

Palpation consisted in grasping the base of the tail as close to the body as possible between the thumb and forefinger, then, bending the tail at about a sixty degree angle, the fingers were moved toward the distal end of the tail, allowing the tail to bend and slide over the end of the forefinger. By this method fusion involving only a few vertebrae could be detected.

Grade 2. No, or almost no, visible flexure. A slight enlargement could be discerned in a few. Digital examination easily reveals a fusion incorporating several vertebrae in a straight stiff segment. There may be more than one group of vertebrae involved.

Grade 3. Characterized by one well-defined flexure in the tail. There also may be one or more stiff, straight portions.

Grade 4. Two or more pronounced flexures of the tail.

Grade 5. Flexure assumes the form of a spiral "cork-screw tail" (Hunt). Tail involvement of the severest type, consisting of several bends, extensive vertebral fusion and decided shortening of the total tail length.

In examining the tails for flexure in the control group, doubtful cases were placed in the non-flexed category. Doubtful cases in the treated experimental groups were placed in the flexed group. Errors in classification thus reduced the difference between the two populations. In spite of this procedure real differences between the two groups were obtained.

Material

A flexed-tailed strain of the house mouse, Mus musculus, was obtained from Dr. H. R. Hunt in March, 1940. The mice were direct descendants of Hunt's flexed-tailed strain recovered from the numerous outcrosses performed by Dr. Hunt to insure a vigorous flexed-tailed breeding stock.

In order to obtain sufficient numbers of breeding animals the mice were chosen and bred with no reference to the degree of flexure. Thus, all variations in the degree of flexure were used, but no animals were bred unless they demonstrated a definite tail involvement. Because of the low viability of the flexed-tailed mice, inbreeding was not used. (Hunt, Mixter, and Permar, 1933). The mice were probably highly heterozygous for genes other than flexed, which increased the difficulty in obtaining valid and highly significant results, especially in such a character as the degree of flexure.

TABLE I

NUMBERS OF EXPERIMENTAL MICE

Controls		
Control One	336	
Thyroxine Control	208	
Parathyroid Control	127	
Total Controls	671	671
Thyroxine		
Thyroxine .0015	77	
Thyroxine .0025	98	
Thyroxine .0050	37	
Thyroxine .0050 - 7 days	148	
Total Thyroxine - Experimental (All Dilutions)	360	360
Parathyroid		
Parathyroid 2 units	86	
Parathyroid 5 units	50	
Total Parathyroid Experimental	136	136
Total number of individuals		1167

Controls

A control group designated as "Control One," were offspring whose parents were never used in any experimental capacity except to raise a number of control litters. Three hundred thirty-one offspring were produced of which 5 were non-flexed. This is 1.49 per cent non-flexed and 98.51 ± .6% flexed.

Miss Dorothy Pernar (1928) in a study of the inheritance of flexed-tail, bred fourteen pairs of flexed animals, which produced 127 young, all flexed. Russell Mixter (1930) crossed flexed with flexed, securing 578 flexed (99%) and 6 non-flexed (1% ± 0.41%).

In this work the first litter from each female was a control. Only during the gestation of subsequent litters from the same females were the hormones administered.

The thyroxine control group was composed of the first litter from the females who received thyroxine injections in the following litters. No thyroxine was administered during the gestation of the thyroxine control group.

The thyroxine control numbered 208 individuals of which 0.48 ± .48% were non-flexed.

The parathyroid control group was composed of the first litters from the females who received parathyroid injections in the subsequent litters. The parathyroid control group comprised 127 offspring; of these three were non-flexed, or 2.80 ± 1.46%. This group had a higher percentage of non-flexed than any other control group. The differences between these control groups, in the incidence of flexed tail, were all due to chance, as the following statistical facts show. The "t" value in comparing the parathyroid controls with the "Control One" group was

0.88. If the odds against the chance causation of a difference are small, the odds ~~inform~~ in favor of its being due to chance are large. The odds are 1.64 to 1.00 against this difference being due to anything but chance. The "t" value for the difference between Permar's and the parathyroid control is 1.23. Here the odds are 3.59 to 1.00 against the occurrence of a chance deviation as great as or greater than the one obtained. In comparing Mixter's data with the parathyroid controls, the "t" value was found to be 0.38 and the odds are 2.38 to 1.00 that this was due to chance. The "t" value for the thyroxine controls and parathyroid control is 0.48 and the odds are 1.70 to 1.00.

All these values for "t" are well below values indicating significant differences and this is true of the parathyroid controls as well as others. The control groups are, therefore, essentially alike with respect to the incidence of flexed individuals.

TABLE II

CONTROLS

		Percent Flexed	"t" value when compared with the parathyroid control for the difference	Odds against the occurrence of a chance difference when compared with the parathyroid control as great as or greater than the designated one.
Control One	#336	98.51 ± 00.60	0.88	1.64:1
Permar	127	100.00 ± 00.00	1.23	3.59:1
Mixter	578	99.00 ± 00.414	0.38	2.38:1
Thyroxine cont.	208	99.52 ± 00.48	0.48	1.70:1
Para. cont.	127	97.20 ± 01.46	--	--

PERCENTAGE OF NON-FLEXED IN THE CONTROL GROUPS

1. CONTROL ONE
2. PERMAR (1928)
3. MIXTER (1930)
4. THYROXINE CONTROL
5. PARATHYROID CONTROL

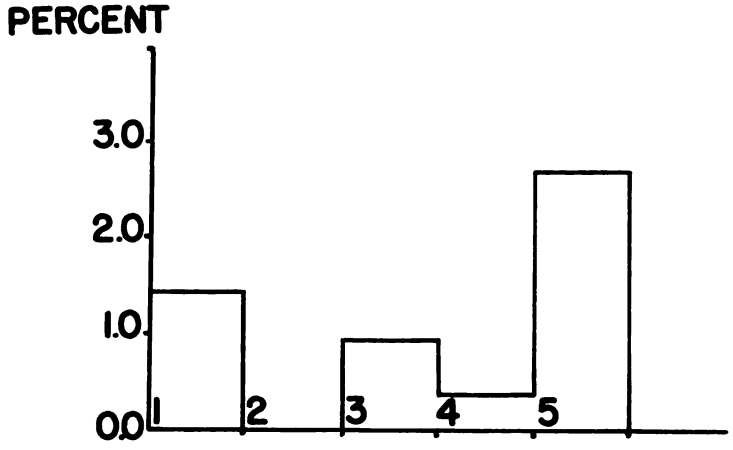


FIGURE 1

Theory and Objective of Thyroxine Administration

Disturbance in the rate of growth can produce abnormalities. Stockard (1921) by stopping the development of Fundulus embryos, produced a greater number of abnormalities than in the controls. It was only at certain critical times that the abnormal embryos were produced. Landauer (1932) in a study of the creeper fowl reported that slowing down of growth affected the development.

Kamenoff (1935) postulates that two things seem necessary for production of abnormalities by modification of the growth rate: first, a slowing down or stoppage of growth which may change the relative rates of growth, and second, a critical point in development which is usually a period of rapid growth. These two conditions he found in the flexed-tailed mouse. First, the slowing of the growth rate had occurred some time before the thirteenth day; second, the vertebral column was still growing rapidly at the thirteenth day.

In a careful embryological investigation of the flexed-tail strain, Kamenoff found that until the thirteenth day the development was apparently normal. On the fourteenth and fifteenth days he observed that the cells on the abnormal side of the developing vertebra did not divide as fast as the normal, indicating a slower cell metabolism. That this was not merely a local manifestation was suggested by the constant shorter length of the proximal caudal, and probably all, vertebrae. This shorter length may be due to reduced rate of mesenchymal cell division of the centra anlage in the early developmental stages. The total number of cells per unit volume was the same in flexed and normal-tailed mice, so the difference was undoubtedly due to the rate of cell division. This difference in size is clearly visible at the thirteenth day.

The foetus was noted to be quite anemic at fourteen days. Whether the anemia is the primary cause of the retarded growth resulting in lack of differentiation of the tail vertebrae, or whether the retardation of growth is a cause of the anemia and the failure of the fibers of the intervertebral discs to differentiate, is undetermined. In either case, the author endeavored to combat the retardation of growth and develop normal tails in the flexed-tail strain. This was done by trying to increase the metabolism of the fetus by injections of the thyroid hormone, thyroxine, into the mother. That the thyroid has the capacity to increase metabolism and growth rate has been shown by many investigators.

A synthetic thyroxine was obtained from Roche-Organon, Inc., Nutley, New Jersey. Three dilutions were administered: 0.0050 mgm.; 0.0025 mgm., and 0.0015 mgms. to each female parent daily. The administration was begun at least five days before the females were placed with the males to be bred, and continued through gestation until parturition. The amount of solution injected was 0.2 cc. subcutaneously for all dilutions to minimize and keep constant any effect due to quantity of the diluting solution.

The amount of thyroxine was arbitrarily determined, the object being to give a sufficient dose. It was found that in the group of mothers receiving the largest amount of thyroxine, five out of ten died and no doubt others would soon have followed if the injections had been continued. The surviving females were subjected to a lower dilution and had normal gestations.

To another group of mothers thyroxine at the level of 0.0050 mgm. daily was administered for a period of seven days only. This period was to begin four days after mating. The injections covered a period

of gestation from the fourth to the twelfth day. The date of mating was obtained by daily examination of each female in the breeding cages for a vaginal plug. When this was found, the female was isolated and thyroxine injections began on the fourth day of isolation.

The object of the deviations from the preceding experiment was to administer an adequate dose of thyroxine for the fetus without killing the mother. The dose of 0.0050 mgms. per day, administered for a period of sixteen days was found in previous experiments to produce severe cachexia, and in some instances death. By the administration of large doses for a short period it was hoped to increase the metabolism of the fetuses at the critical time, but to stop the administration before the mothers became emaciated and failed to produce young.

The injections were not carried beyond the twelfth day of foetal development because Kamenoff's observations suggested that flexure is due to a lowered metabolism antedating this day. It was assumed that the time to prevent retarded growth was before it had begun.

Incidence of Flexure in the Thyroxine Experiments

The thyroxine control litters were considered a composite control group for comparing with litters borne by thyroxine treated mothers in all the experiments, involving the three dilutions of thyroxine. This is justifiable for the first litter of each female was a control litter (thyroxine controls); the second, third or fourth litters were borne after the female had received thyroxine injection. Some of these females had received different doses previous to the birth of different litters. From 33 thyroxine control litters, 208 offspring were obtained. One non-flexed tail was found, constituting 0.48 ± 0.48 per cent of the population.

The "Control One" group is composed of the offspring of those mothers were never used in any experimental capacity except to raise a number of control litters.

The group of females receiving thyroxine at the level of 0.0015 mgms. daily, produced 77 flexed progeny (100 per cent flexed). The mothers receiving the thyroxine dose of 0.0025 mgms. per day had 98 offspring, of which 2 were non-flexed (2.04 ± 1.43 per cent).

The group having thyroxine administered for several days at 0.005, and later at .0025 mgms. per day, had 37 flexed offspring (100 per cent flexed).

The group receiving thyroxine at the level of 0.0050 mgms. for seven days only, produced 148 progeny with 2 non-flexed, or 1.37 ± 0.95 per cent non-flexed.

All the offspring whose mothers received thyroxine in some dilution were combined into one group, termed "thyroxine-all dilutions," which had a total of 360 progeny. Four of these were non-flexed, or 1.11 ± 0.55 per cent.

The percentage of non-flexed was observed to be 0 per cent in the groups receiving .0015 mgms. and .0050 mgms. daily; this is below the control group. The groups receiving thyroxine at the level of 0.0025 mgms. daily and .0050 mgm. for 7 days had respectively 2.04 ± 1.43 per cent and 1.37 ± 0.95 per cent non-flexed. These were both higher than the thyroxine controls. By common statistical analysis these differences were not found significant.

To ascertain whether any of the thyroxine experimental groups had a significant difference the "t" value was computed for the difference between each experimental group and the thyroxine control group. (Table IV) The "t" value for the thyroxine control compared to group "Control One" is 1.24. The odds against this difference being due to anything but chance are 3.67 to 1.00. These are the greatest odds found in the thyroxine experiments.

Thyroxine controls compared to the group thyroxine 0.0015 has a "t" value of 1.00. The odds against this difference being due to chance are 2.16 to 1.00.

The "t" value when computed for the difference between the thyroxine 0.0025 group and the thyroxine control group is 1.04. The odds are 2.35 to 1.00 against the difference being due to chance.

The thyroxine 0.0050 group compared to the thyroxine control group has a "t" value of 2.16 to 1.00 and the odds are 2.16 to 1.00 against the difference being due to chance.

These figures are not statistically significant. A "t" value to be statistically significant has a value of 2.5 or above. The odds against any number below 21 is usually not considered statistically significant.

In comparing the thyroxine control group to the thyroxine 0.0050 for 7 days the "t" value is 0.83. When the control group is compared

to the composite group, thyroxine--all dilutions, the "t" value is 0.87. For both these two latter groups the odds are 1.46 to 1.00 and 1.60 to 1.00 than any difference would be due to anything but chance.

Added evidence that these were not significant differences was observed by noting the percentage of non-flexed in other control litters. Group "Control One's" percentage of non-flexed individuals is 1.49 ± 0.66, which is about the same as the 1.37 ± 0.95 per cent of non-flexed in the progeny of the thyroxine group which received 0.0050 mgms. for seven days. The parathyroid control group possess 3 non-flexed. This is 2.80 ± 1.42 per cent, which was higher than any thyroxine group, either control or experimental.

The degree (grade) of tail flexure was shown by Hunt (1933) and others to be inherited to some extent. In our experiments no effort was made to mate mice with the same degree of tail flexure. The parents' average degrees of flexure were mostly in classes three and four, but individuals in all classes were used except Class 0. (Figure VIII)

The distributions of degrees of flexure in the offspring were much the same as in the parents. (chart III) The offspring in general differed only from the parents in that the extremes, Class 0 and Class 5 contained higher percentages of individuals among the offspring, which is due to the segregation of genes modifying the degree of flexure.

It is justifiable to conclude that there was no effect on the incidence of flexed tail in the progeny from homozygous matings of Hunt's flexed-tailed strain by the administration of thyroxine to the pregnant females under the conditions of these experiments.

TABLE III

THYROXINE

Tails

Total	Number flexed	Number non-flexed	Percent non-flexed	Percent flexed	
208	207	1	00.48 ± 00.48	99.52	Thyroxine Control
336	331	5	01.49 ± 00.60	98.51	Control One
77	77	0	00.00 ± 00.00	100.00	Thyroxine .0015
98	96	2	02.04 ± 01.43	97.96	Thyroxine .0025
37	37	0	00.00 ± 00.00	100.00	Thyroxine .0050
148	146	2	01.37 ± 00.95	98.63	Thyroxine .0050 - 7 days
360	356	4	01.11 ± 00.55	98.89	Thyroxine All Dilutions

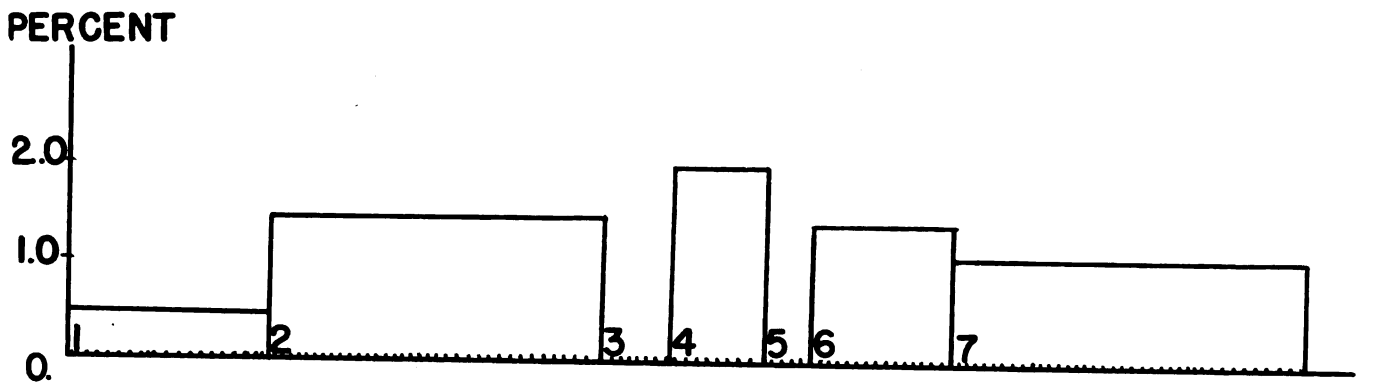
TABLE IV

THYROXINE

	Standard Error of the difference	"t" value	Odds against the occurrence of a deviation as great or greater than the designated one being due to anything but chance
Thy. Cont. vs. Cont. One	0.816	1.24	3.67:1
Thy. Cont. vs. Thy. .0015	0.00479	1.00	2.16:1
Thy. Cont. vs. Thy. .0025	0.0150	1.04	2.35:1
Thy. Cont. vs. Thy. .0050	0.00479	1.00	2.16:1
Thy. Cont. vs. Thy. (7 days) .0050	1.067	0.83	1.46:1
Thy. Cont. vs. Thy. all dilutions	0.7211	0.87	1.60:1

THYROXINE
PERCENT NON-FLEXED

1. THYROXINE CONTROL
2. CONTROL ONE
3. THYROXINE .0050
4. THYROXINE .0025
5. THYROXINE .0015
6. THYROXINE .0050 (7 DAYS)
7. THYROXINE ALL DILUTIONS



EACH GRADUATION EQUALS TEN ANIMALS

FIGURE 11

THYROXINE
DEGREE OF FLEXURE IN THE OFFSPRING

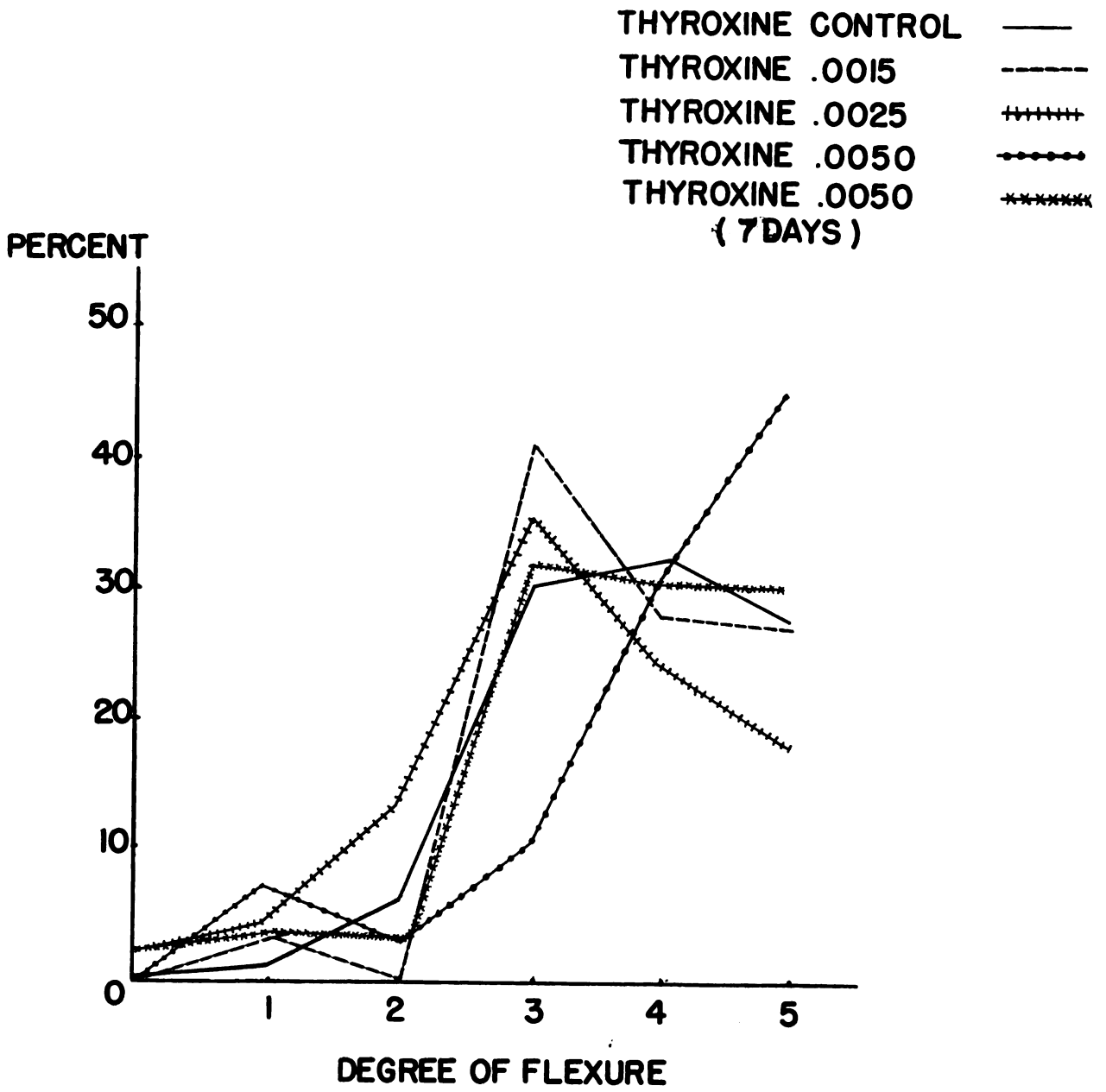


FIGURE III

Theory and Objective of the Parathyroid Experiment

The hormone of the parathyroid gland "parathamone" regulates calcium and phosphate metabolism in the body. A deficiency lowers the calcium level of the plasma and tissues. If the level falls low enough, tetany results. Injections of the hormone result in muscular and nervous sedation. Parathyroid hormone lowers the phosphate level and withdraws calcium from trabecular bone.

This intimate connection with calcium metabolism suggested its use for this experiment.

The parathyroid preparation was obtained from Parke, Davis and Company, Detroit, Michigan. The trade name was "Paroidin." Each cubic centimeter contained one hundred units. The amounts injected into the mice were two and five units, diluted with sterile water so that each subcutaneous injection totaled 0.2 cc. The solution was tested for pH with "Nitrazine paper" (E. R. Squibb and Sons, New York) and brought to pH 7.0 with sodium carbonate. When the pH was not adjusted, sterile subcutaneous abscesses formed at the point of injection. When adjusted there were no difficulties.

The first litter recorded from a female served as a control litter. The mothers of the succeeding litters received the extract. The injections of the extract began two days after the females were put into the breeding cages with the male and continued until the birth of the offspring. The females were isolated from the males as soon as they were observed to be pregnant.

The dosage was arbitrarily based on the suggested dose for the dog calculated by the animal's weight.

Incidence of Flexure in the Parathyroid Experiments

The offspring in the parathyroid control group numbered 127, of which 3 were non-flexed or 2.80 ± 1.46 per cent. This was a higher percentage than in any other control group. The offspring whose parents received two units of parathyroid extract per day numbered 86; of these 11 were non-flexed or 12.79 ± 3.60 per cent. Non-flexed offspring appeared 4.57 more times in these litters than in the litters of the parathyroid control group, and 8.58 times more often than in the litters of group Control One.

As the parathyroid control group had the highest percentage of non-flexed individuals of any control group and was the logical control, the "t" values were derived by common statistical methods from comparison of the data of the parathyroid controls with the data from those offspring whose parents received parathyroid extract.

The "t" value of the parathyroid group receiving two units daily when compared with the parathyroid control group was 2.57. This is a significant difference.

Among the 50 offspring whose mothers received five units of parathyroid extract daily, there were four non-flexed, or 8.00 ± 3.80 per cent. The non-flexed individuals were 5.37 times more prevalent than in the group, "Control One," and 2.86 times more numerous than in the parathyroid control group. The "t" value for this group was found to be 1.27. This group is then not significantly different from the parathyroid controls.

The parathyroid groups of five units and two units per day were compared statistically. As there was no significant difference between the two groups the combination was valid. The "t" value was 0.91. The combined data of progeny whose parents received either two or five units

of parathyroid daily, total 136 offspring. Fifteen were non-flexed, representing 11.03 per cent. The "t" value of this combined group as against the parathyroid controls was 2.69. This is significant. The odds against this happening again by chance were 141.85 to 1.00. The odds against the occurrence of a deviation as great as or greater than the disignated are 99.00 to 1.00 for the parathyroid group of two units and 3.90 to 1.00 for the parathyroid group of five units daily.

Statistically, the effect of parathyroid extract on the incidence of flexure in the flexed-tailed strain of Hunt's was significant.

The degrees (grades) of flexure in the parathyroid combined group, two and five units, had a higher representation in the grades zero and one and an apparent reduction in the other extremes, grades four and five. This deviation from the parathyroid controls, as seen in Table VI, was also observed when plotted with other groups in Figure VI. This difference was again shown in Figure VII where the average degree of flexure of the parathyroid combined group, two and five units, was clearly shown below that of its controls and any other group. The degree of flexure of the offspring when compared to the parents of the parathyroid groups is show in Figure IX. For the offspring grade zero is possibly higher while grade 5 may be lower than one would expect in random bred populations. No conclusion can be drawn concerning the effect of parathyroid extract on the grade of flexure because of the small number available for this study.

In observing Tables V and VI a discrepancy is found in the percentage of non-flexed individuals in the parathyroid combined groups. The percentage (15 per cent) on Table VI is greater than the percentage (11.03 per cent) on Table V. The data from which the percentages were

obtained in Table V represent a fraction of the data in Table VI. This discrepancy is due to the fact that not all the individuals which were observed for being flexed or non-flexed were recorded as to the degree (grade) of flexure. While one can determine whether a tail is flexed at an early age classification of the degree of flexure was left until the animals were of weaning age (three weeks) or longer if necessary. In certain cases entire litters died from dysentery before they were of weaning age. These litters were recorded for the presence of a flexure but not for the degree of flexure.

No data were incorporated in this thesis from any animal that died before the presence or absence of a flexure was definitely determined.

It is strongly indicated that there was a decrease in the incidence of flexed-tail in the offspring from homozygous matings of Hunt's flexed tailed strain by the administration of the parathyroid hormone to the pregnant female under the conditions of these experiments.

TABLE V

PARATHYROID

Tails Total	Number Flexed	Number Non-flexed	Per Cent Non-flexed	Per Cent Flexed	
336	331	5	1.49	98.51 ± .006	Control One
127	124	3	2.80	97.20 ± .0146	Parathyroid Control
86	75	11	12.79	87.21 ± .036	Parathyroid 2 units
50	46	4	8.00	92.00 ± .038	Parathyroid 5 units
136	121	15	11.03	88.97 ± .0268	Parathyroid 2 and 5 units

TABLE VI

PARATHYROID AND CONTROL POPULATIONS

DEGREES OF FLEXURE - EXPRESSED IN PERCENTAGES

Offspring

Degree of Flexure	0	1	2	3	4	5
Control One	1.56	8.59	7.81	37.11	31.25	13.67
Parathyroid Control	2.80	2.80	11.21	30.84	33.64	18.69
Parathyroid Combined (2 and 5)	15.00	15.00	12.00	36.00	14.00	8.00
Parents						
Parathyroid	0.00	9.09	3.03	48.48	36.36	3.03

TABLE VII

PARATHYROID

	Percent Non-flexed	Number of non-flexed individuals found in the parathyroid group for everyone found in 1. Parathyroid control 2. Control one	
		Parathyroid Control	Control One
Parathyroid 2 units	12.79%	4.57	8.58
Parathyroid 5 units	8.00%	2.86	5.37
Parathyroid (2 and 5 units) combined	11.03%	3.94	7.40

TABLE VIII

PARATHYROID

	Standard error of the difference	"t" value	Odds against the occurrence of a deviation as great as or greater than the designated one.
Parathyroid Control vs. Control One	01.50	0.87	1.60:1
Parathyroid Control vs. Parathyroid 2 units	03.88	2.57	99.00:1
Parathyroid Control vs. Parathyroid 5 units	04.11	1.27	3.90:1
Parathyroid 2 units vs. Parathyroid 5 units	05.26	0.91	1.75:1
Parathyroid Controls vs. Parathyroid 2 and 5 units	03.05	2.69	141.85:1

PARATHYROID
PERCENT NON-FLEXED

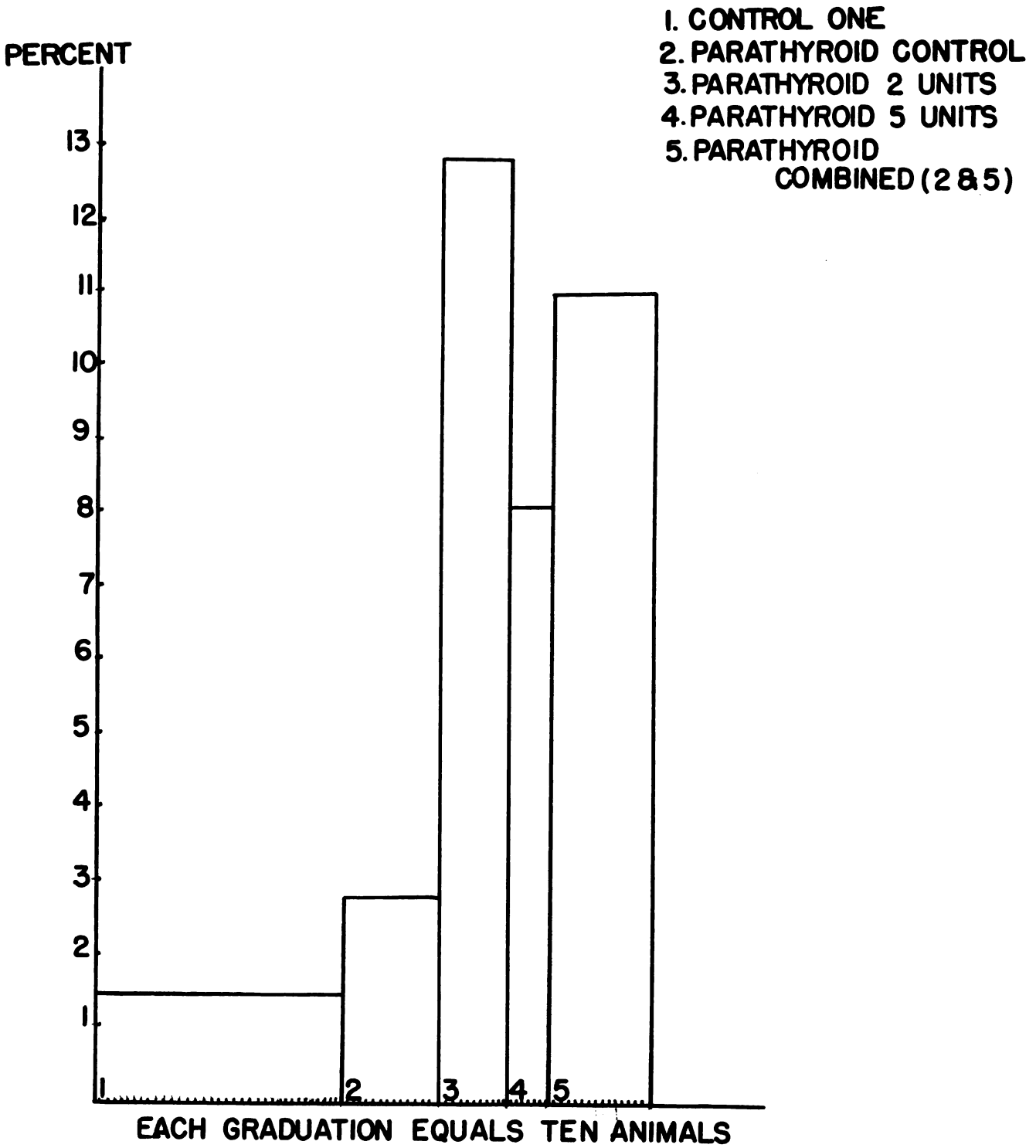


FIGURE IV

PARATHYROID
DEGREE OF FLEXURE IN THE OFFSPRING

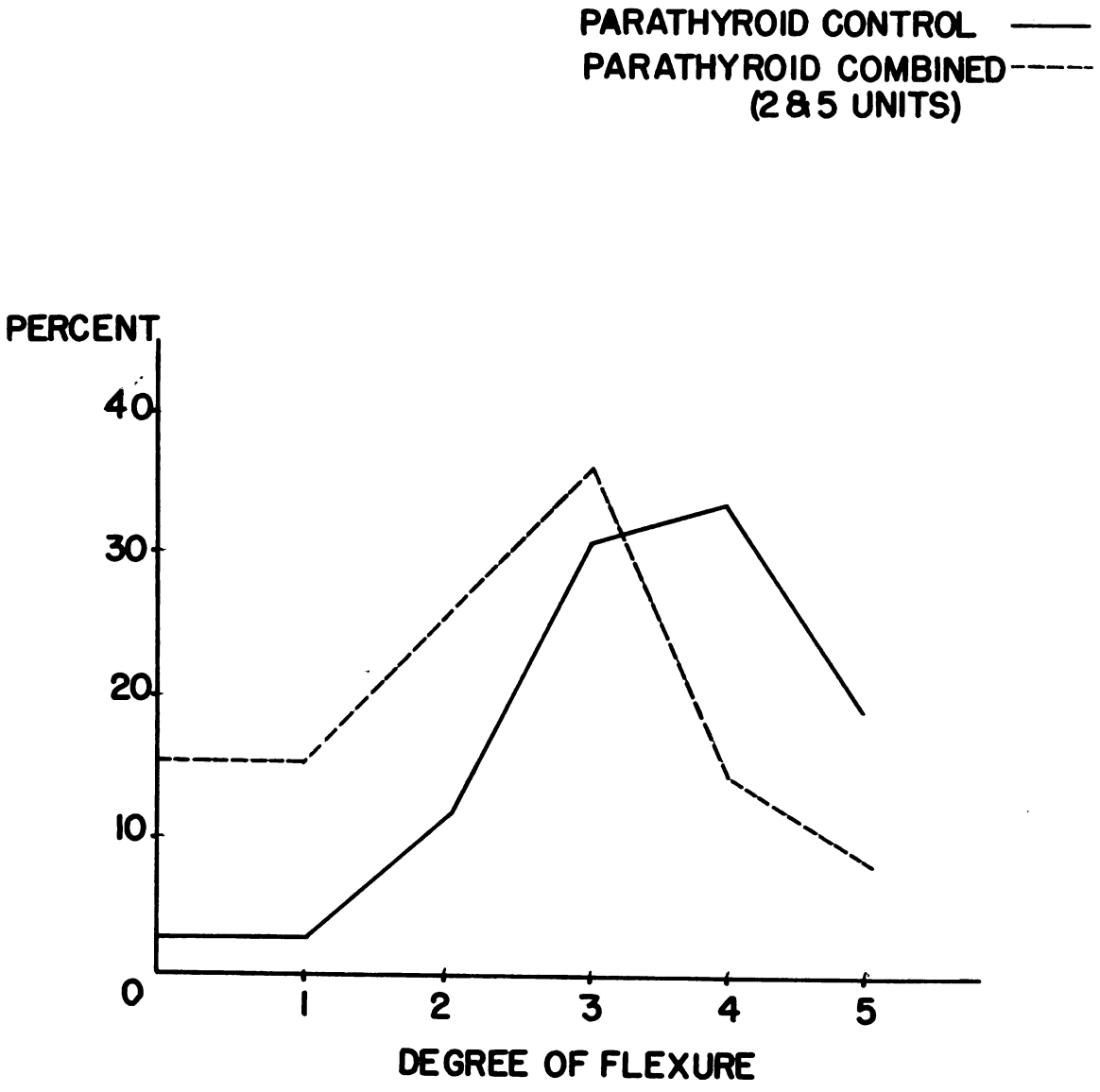


FIGURE V

PARATHYROID
DEGREE OF FLEXURE IN
THE OFFSPRING

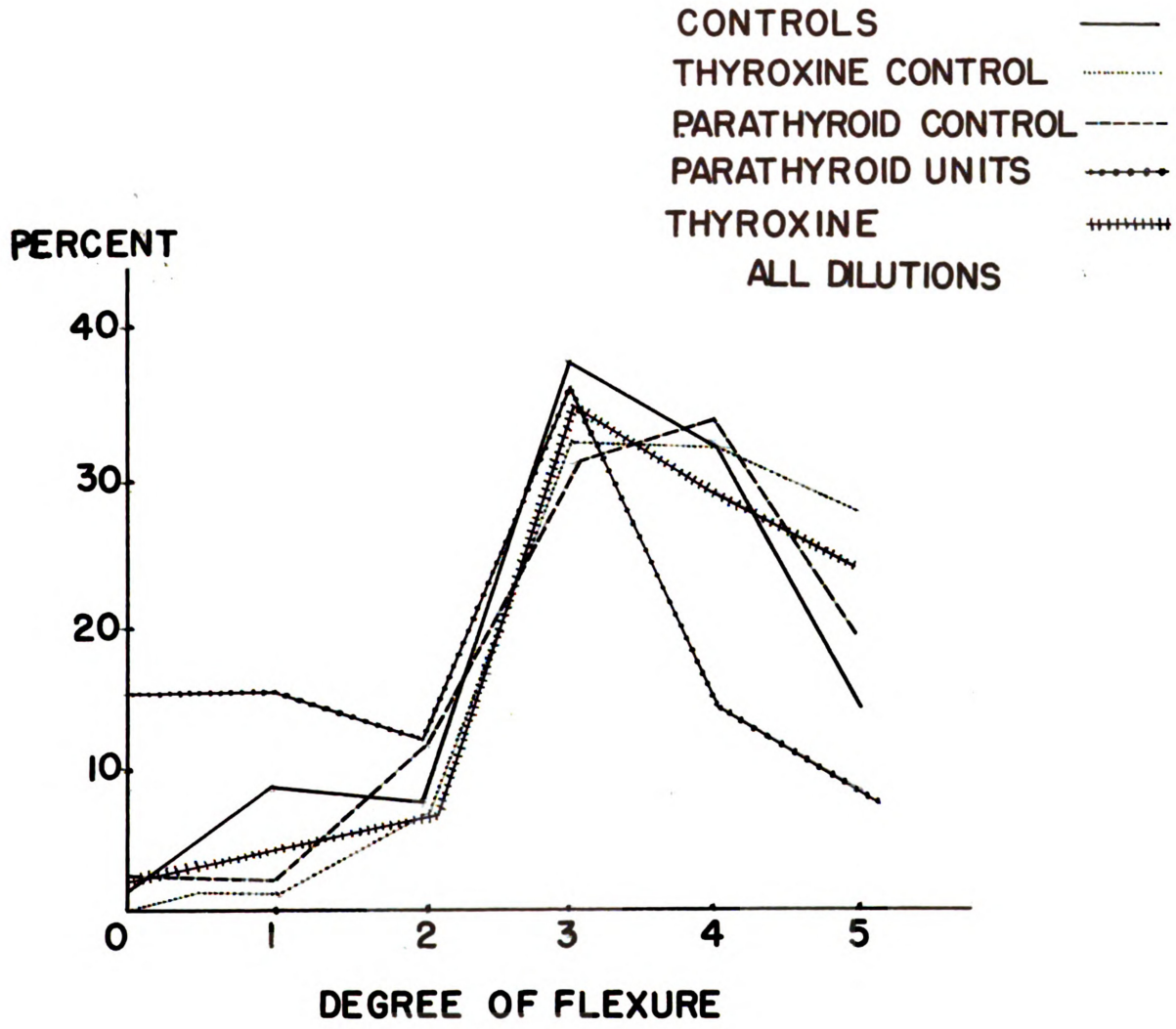


FIGURE VI

AVERAGE DEGREE OF FLEXURES

PARENTS
1. CONTROL ONE
2. THYROXINE
3. PARATHYROID

OFFSPRING
4. CONTROL ONE
5. THYROXINE CONTROL
6. PARATHYROID CONTROL
7. THYROXINE ALL DILUTIONS
8. PARATHYROID 2/5 UNITS

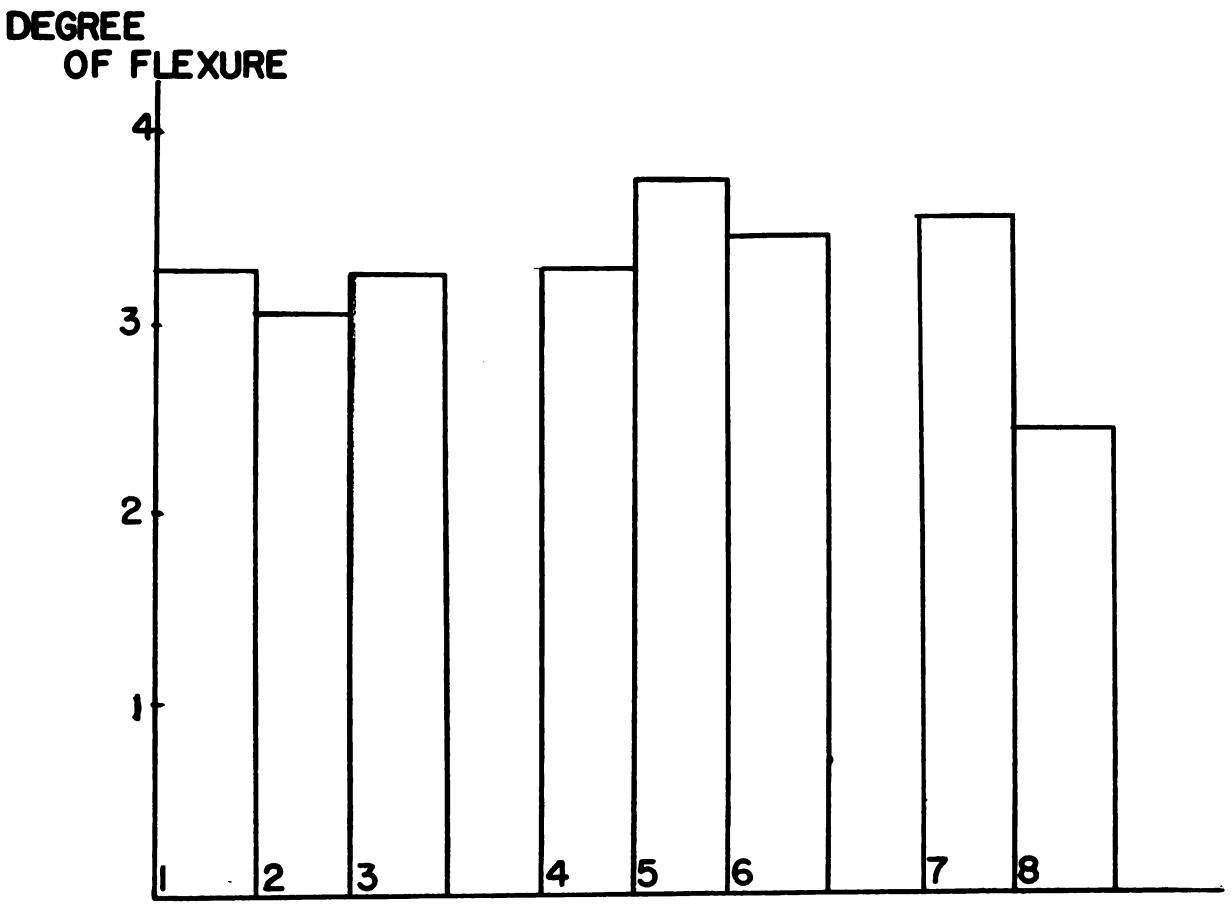


FIGURE VII

DEGREE OF FLEXURE OF THE PARENTS

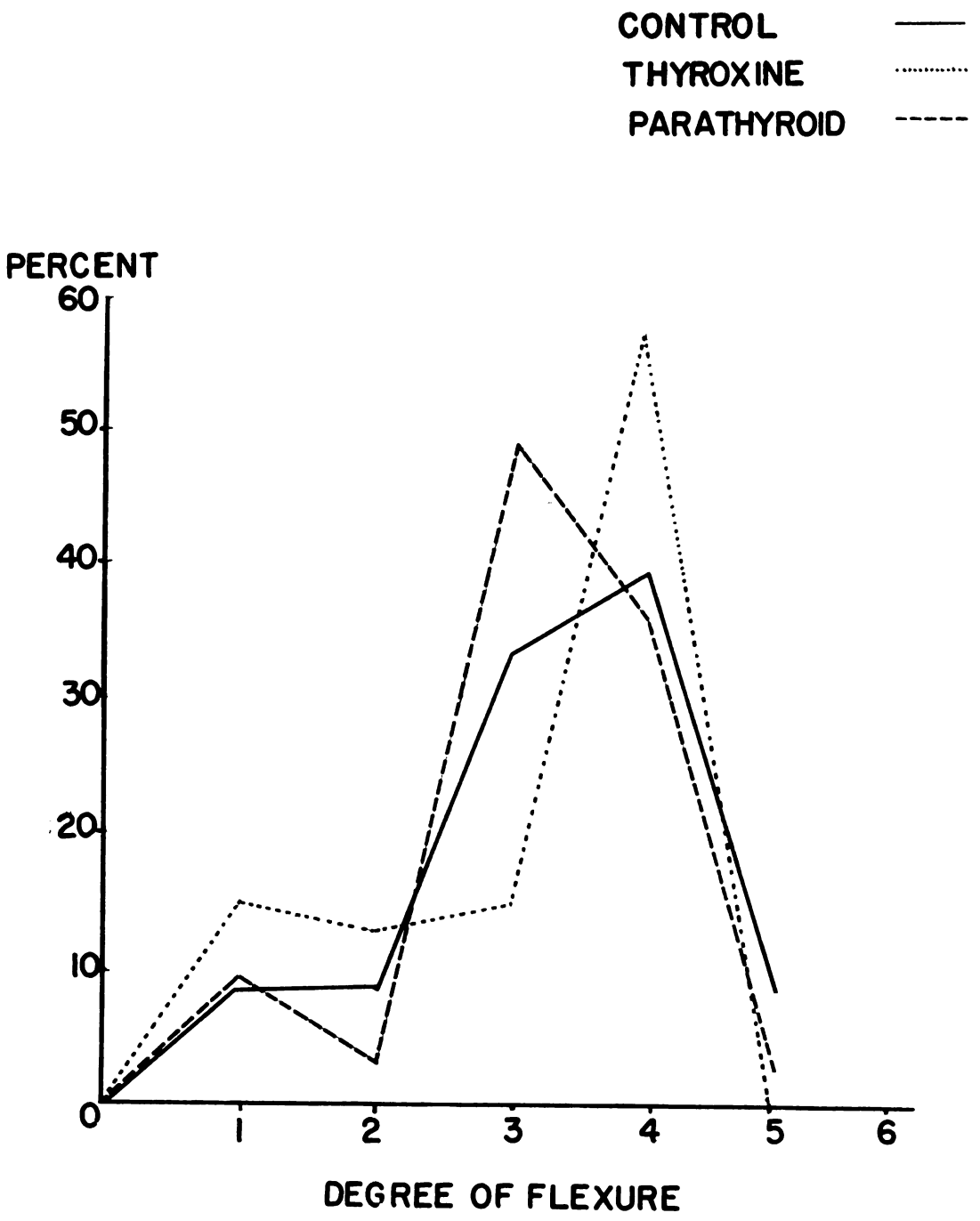


FIGURE VIII

DEGREE OF FLEXURE OF PARATHYROID PARENTS AND OFFSPRING OF PARATHYROID 2&5 UNITS

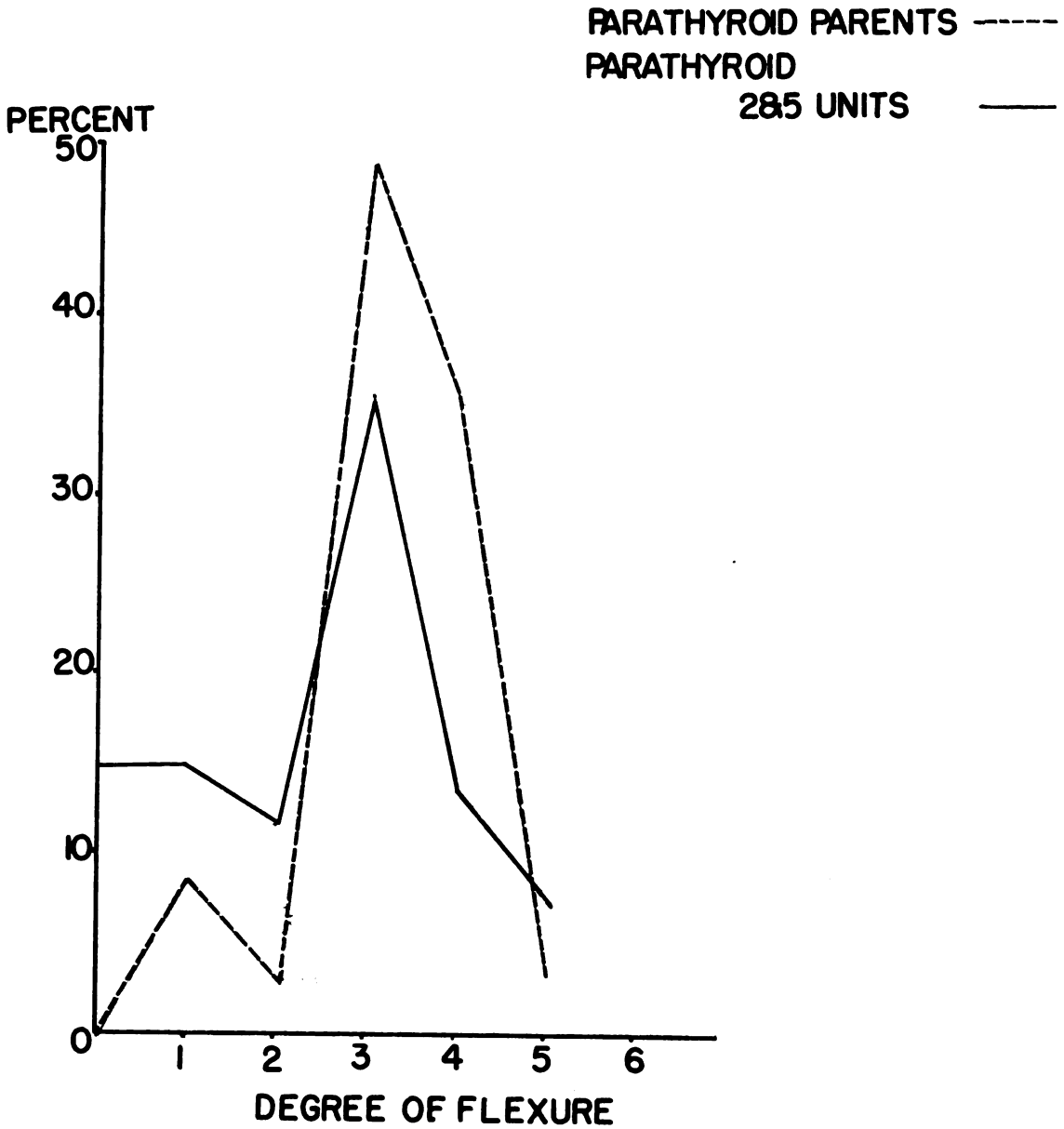


FIGURE IX

Discussion

The discussion concerning the relative importance of heredity and environment was a major conflict at one time. In this experiment, as in many others dealing with heredity, the two factors work together. The common concept of a gene is that the gene is an entity influencing a process of life rather than dictating it. The environment modifies the effects of gene action. In this experiment the only manifestation of the flexed gene used was the more obvious one, the tail flexure. Anemia, the belly-spot, and the lowered metabolism were not investigated. The thyroid injections into the pregnant females of this experiment had no effect on the incidence of flexure in the offspring. Injections of the parathyroid hormone into pregnant females produced a significant decrease in the percentage of flexed offspring. The investigation did not prove that the parathyroid extract acted directly on the fetus. The effect may have been indirect, through some modification in the metabolism of the pregnant mother.

The parathyroid hormone may reduce the degree of flexure or inhibit it altogether. The total inhibition of flexure was found in a limited, but statistically significant, number of animals. In general the average degree of flexure was less as a consequence of the injections. The reduction effect of the degree of flexure was suggested by the fact that in the parathyroid groups the grades zero, one and two had a higher representation than in the controls, and the grades four and five were lower. The character, flexed-tail, is highly variable and there is only a slight significant correlation in the inheritance of the degree of flexure, thus larger numbers of offspring are needed before any conclusion can be drawn as to any reducing effect the parathyroid injections may have had on the grade of flexure.

One must consider whether or not the hormone can pass through the placenta from mother to fetus. One cannot assume such a passage. That the thyroid hormone passes through the placenta from the foetal to the maternal circulation was given indirect support by the observations of Spielman, Petersen and Fitch (1944) on the bovine. Windle (1940) suggested an apparent lack of transmission of the thyroid hormone in the bovine. In the human it seems probable that the thyroxine of the mother is available to the foetus, because it can traverse the placental barrier. The parathyroid hormone is apparently unable to pass the placenta in dogs (Windle, 1940).

In studies concerning the placenta one must keep foremost in mind that the physiologic characteristics of the placenta are not constant throughout embryonic and fetal development, nor are they the same in all species of mammals. This is shown by the density and number of tissue layers separating maternal and foetal blood. The type found in rodents consists only of a layer of endothelium between the maternal and foetal circulations. With only a few cells between the foetal and maternal circulations it would seem that if a substance were to pass through the placenta, this type would be advantageous. If the hormone can pass through from the foetal to placental circulation only after a certain stage in the development of the placenta, it may have proved a barrier to the action of the hormone when it may have been able to produce its greatest influence on the gene action in the developing embryo. The flexing may have begun much earlier than the fourteenth day and be due to an earlier lowered rate of metabolism. If this factor is the vital one, early interference with the process may be desirable.

With the methods used in this experiment early interferences with the developing foetus may not have been generally accomplished with the

exception of the successful cases where non-flexed individuals were obtained by parathyroid injections. On parathyroid administration the phosphate level of the blood is reduced and calcium is withdrawn from the trabecular bone, the blood plasma value of calcium is high. The calcium level of the blood and the mechanism of the nervous system are intimately associated. These changes may affect the foetus indirectly by the change in the metabolism of the mother or directly by placental transmission of a change in the composition of the maternal blood.

The parathyroid hormone has a statistically significant effect on the expression of the flexed-tailed gene. The fact that the hormone did not produce all normal tails leaves much to be investigated, but suggests that the parathyroid could be an important factor in the expression of the flexed-tailed gene.

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