THE DEVELOPMENTAL GENETICS OF LUXOID, A NEW SKELETAL VARIATION IN THE HOUSE MOUSE, MUS MUSCULUS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

PAULINUS FREDERICK FORSTHOEFEL, A.B., M.Sc.

The Ohio State University

1953

Approved by:

Earl L. Green

Adviser

TABLE OF CONTENTS

	Page
INTRODUCTION.	1
HISTORICAL REVIEW	6
MATERIALS AND METHODS	16
A. Materials	16
B. Methods	20
RESULTS OF THE BREEDING EXPERIMENTS	27
A. The penetrance of the luxoid gene	27
B. The influence of the luxoid gene on viability . .	28
DESCRIPTION OF THE MORPHOLOGY OF ADULT LUXOID MICE. . .	35
A. The skeleton of the foot.	35
B. The skeleton of the hand.	45
C. The skeleton of the leg and thigh	50
D. The skeleton of the tail.	55
E. Number of presacral vertebrae	58
F. Number of ribs.	58
G. Number of sternebrae.	58
H. The urogenital system	61
EMBRYOLOGICAL DEVELOPMENT OF C57BL/10 NORMAL AND LUXOID MICE	62
A. Development of external characteristics	62
1. The 10½ day stage	62
2. The 11½ day stage	63
3. The 12½ day stage	66
4. The 13½ day stage	67

A 16945

TABLE OF CONTENTS (Continued)

		Page
5.	The 14½ day stage.	69
6.	The 15½ day stage.	70
7.	The 16½ day stage.	71
8.	The 17½ day stage.	73
9.	The 18½ day stage.	73
B.	Development of the internal characteristics. . .	74
1.	The 10½ day stage.	74
2.	The 11½ day stage.	77
	a. The forelimb	77
	b. The hind limb.	78
3.	The 12½ day stage.	80
	a. The forelimb	80
	b. The hind limb.	81
4.	The 13½ day stage.	81
	a. The forelimb	81
	b. The hind limb.	82
5.	The 14½ day stage.	84
	a. The forelimb	84
	b. The hind limb.	86
6.	The 15½ day stage.	88
	a. The forelimb	88
	b. The hind limb.	89
7.	The 16½ day stage.	93
8.	The 17½ day stage.	95

TABLE OF CONTENTS (Continued)

Page

9. The 18½ day stage. 95

10. The newborn stage. 97

11. The week-old stage 97

THE PROBLEM OF THE PRIMARY EFFECT OF THE LUXOID GENE . . 98

DISCUSSION 110

SUMMARY. 125

BIBLIOGRAPHY 127

AUTOBIOGRAPHY 131

ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. Earl L. Green for his guidance and encouragement throughout the work reported in these pages. I am especially grateful for his help in the statistical analysis used.

I wish to thank Dr. Margaret C. Green for generously permitting me to work on the morphological abnormalities and developmental genetics of *luxoid* mice, for supplying a number of cleared *luxoid* homozygotes, for providing me with a foundation stock of *luxoid* animals, and for the use of the information she has gained in her own study of the *luxoid* mice.

I wish to thank Dr. T. C. Carter of the Institute of Animal Genetics, Edinburgh University, for generously providing me with a copy of his own unpublished studies on the embryology of *luxate* mice, which served as a most useful model in the investigations with *luxoid*, and for many practical suggestions on how to proceed in my studies.

I wish to thank Mr. Everett Hrubant for the care he took in taking the photographs used in this dissertation, Mr. Chai Yoon for a number of C57 females used in the embryological studies, and Mr. Edwin P. Les for skillful inking-in of the line drawings and graphs.

The work reported in these pages was done while the author was in receipt of a University Scholarship from The Ohio State University.

INTRODUCTION

In recent years genetics has progressed from the point where investigators were mainly concerned with describing hereditary characteristics and their mode of inheritance to the point where they have attempted to discover the ultimate nature of the hereditary unit, the gene, and how it brings about its effects. Investigators concerned with how genes affect morphogenetic processes in organisms have evolved a new subdivision of genetics to which the name developmental genetics has been given. Workers in this specialized subfield have been forced to use largely morphological methods by which they have attempted to discover what deviations from normal embryonic development give rise to the adult mutant phenotype. They thus seek to find as nearly as possible the primary action or actions of the gene, which, they hope, will shed some light on the ultimate nature of the gene itself. Prominent workers in the field of mouse developmental genetics are Grüneberg, Gluecksohn-Waelsch, and, recently, T. C. Carter.

Developmental geneticists working with mice have found helpful Grüneberg's principle of the unity of gene action (1943). According to this view, a gene has only one primary effect; all other effects are merely direct or indirect effects of the primary effect. There exists therefore no true pleiotropy; all the parts of a genetic syndrome which depend on a single gene should be capable of analysis into

a pedigree of causes tracing back to some primary effect of the gene. The universal truth of this principle has been challenged by various geneticists. However, whether universally valid or not, it serves as a very useful methodological guide and was used as such in the investigations reported in this dissertation.

The present investigations had as their primary object the study of the developmental genetics of luxoid, a new skeletal variation in the house mouse, Mus musculus, which was discovered by Dr. M. C. Green (Green, 1950). This variation depends in its inheritance on a single gene, which behaves as a semi-dominant. (M. C. Green, unpublished). Since the effects of the gene had as yet not been completely analyzed, the adult morphology of mice heterozygous and homozygous for the gene was studied first. The only deviation from the normal phenotype found in heterozygotes was polydactyly of the hind feet. Examination of homozygotes showed an interesting syndrome of effects. The most interesting effect was a reduction, more or less drastic, of the tibia. Of the ankle bones, the talus was most modified, having a shape similar to that of the calcaneus. The toes passed through a stage of an increasing number of digits to a stage where there was a reduction in their number. The innominate bone showed changes only once, involving decrease in size of the obturator foramen caused by hypertrophy of the pubis and ischium. The forelimb was

polydactylous up to a total number of seven fingers. The tail was often kinked. Dr. M. C. Green has investigated the effects on the number of presacral vertebrae, the number of sternebrae, and the number of ribs. She has found that the number of presacral vertebrae tends to be increased from the 26, the normal number in C57, to 27 and in one case to 28. The number of sternebrae anterior to the xiphisternum occasionally is increased from 5, the normal number in C57 to 6. The number of ribs tends to be increased from 13, the normal number in C57, to 14.

The study of the embryology of luxoid showed that the first detectable external difference between luxoid homozygotes and normal C57 embryos, seen in late 11½ day stages, was a marked flaring forward of the footplate of the forelimb. At the 12½ day stage, the footplate of the hind limb of homozygotes could be distinguished in some cases by the fact that they were not round as in the normal embryos but were pointed. In the 13½ day stages, heterozygotes were easily distinguished by a forward projection of the footplates of their hind limbs combined with the presence of normal footplates on their forelimbs. At this stage, the homozygotes were clearly polydactylous in the footplates of their forelimbs and some had a further development of the pointedness of the footplates of their hind limbs. At this stage also the axis of the hind limbs of all homozygotes instead of being at right angles to the main axis of the

embryo inclined forward at a more acute angle. All succeeding stages merely brought into clearer relief the differences just described between the normal, heterozygous, and homozygous luxoid mice.

A possible cause of the tail abnormalities was found in blebs in the tail, containing blood, which were seen as early as the 10½ day stage. These blebs, by pressure on the forming somites, could lead to their malformation. Malformed somites could lead eventually to deformed and fused tail vertebrae, as seen in the adults.

Study of the 10½ day stage showed that there was a caudad shift of the hind limb field in the case of luxoid homozygotes but none was detected in the case of luxoid heterozygotes. The caudad shift in the case of the homozygotes can be used to explain the increase in the number of presacral vertebrae often found in them, and perhaps to explain the changes in the bones of the foot and leg. The absence of the backward shift in the case of the heterozygotes can be used to explain the absence of any increase in number of presacral vertebrae found in them, but this leaves unexplained the frequent occurrence of polydactyly in them. A backward shift of the fore limb field is advanced as a possible explanation of the preaxial polydactyly seen in luxoid homozygotes. Similar caudad shifts of their fields might be used to explain the increase in number of

ribs and in number of sternebrae.

It was tentatively concluded that the primary effect of the luxoid gene is a caudad shift of the fields of the forelimb, the hind limb, the ribs, and sternebrae. All other effects of the luxoid gene were interpreted as secondary to this primary shifting effect.

HISTORICAL REVIEW

The list of inherited abnormalities of the skeleton of the mouse includes such conditions as congenital hydrocepalus, pituitary dwarfism, pseudencepahly, myelencephalic blebs, harelip, otocephaly, etc. (Grüneberg, 1952). Leaving out conditions where the effect on the skeleton is only secondary to an effect on some other system, we have syndactyly (Hertwig, 1942), oligodactyly (Hertwig, 1942), duplication of the hind limbs (Danforth, 1930), postaxial polydactyly of the forelimbs (Strong, 1934), clubfoot (Kobozieff and Pomriaskinsky-Kobozieff, 1946), preaxial polydactyly of the hind limbs (Murray, 1932; Fortuyn, 1939; Grüneberg, 1943; Holt, 1945; Chase, 1946; Kobozieff and Pomriskinsky-Kobozieff, 1946), brachypody (Landauer, 1952), and absence of tibia (Rabaud, 1914; Carter, 1951; Green, 1950).

The latter condition, absence of tibia, was investigated by Rabaud in a stock of mice which he called souris luxées because the legs of the affected mice appeared dislocated. Carter called a similar condition in another stock of mice "luxate" because of the strong phenotypic resemblance between his mice and the souris luxées of Rabaud. Green called her mice "luxoid" because their condition somewhat resembled that of Carter's luxate mice. In the following paragraphs, the first two cases of absence of

tibia will be briefly described. The last case is the subject of the body of this dissertation.

Rabaud (1914, 1917) found that in his stock of mice, absence of tibia behaved as a simple recessive. The expression of the gene was variable, which has led Grüneberg (1952) to suggest that genetic modifiers were involved. Hovelacque (1920) investigated the adult morphology of the condition in considerable detail. He found that the tibia was more or less completely replaced by a ligament, the fibula was bent toward the tibia and increased in size, the toes had ectrodactyly, polydactyly, and syndactyly variously combined, the tarsal bones were often fused and some of them might be missing, anomalies found in the muscles of the affected legs were clearly the results of the modifications of the bony skeleton. Hovelacque remarked that the anomalies in the ankle and foot were not results of modifications in the leg but were anomalies of the same order, parts of a single syndrome. This remark is of interest since in luxoid mice the effect of the gene in heterozygous condition is an induction of polydactyly, but in homozygous condition its effect is a reduction of the tibia and an induction of various other modifications of the skeleton often including polydactyly.

Hovelacque and Noel (1923) investigated the embryology of the condition in Rabaud's mice. They confined their study to the leg and gave a description of the differences

in development found between normal mice and their souris luxées. They found that the chief difference was that while cartilage formed in the fibial blastema, none appeared in the tibial blastema except at the proximal end. The authors did not attempt to explain why in one place in the leg cartilage for the fibula differentiated, while in an adjacent place cartilage for the tibia failed to differentiate.

A new stock of mice with absence of tibia was studied by Carter (1948, 1949, 1951a, 1951b), who found the condition due to a single mutant gene situated in the third linkage group. He called the mutant 'luxate', symbol lx. The gene governing luxate is semi-dominant. Some heterozygotes are entirely normal while others have pre-axial polydactyly of the hind feet. The degree of manifestation of the defects in both heterozygotes and homozygotes varies with the genetic background present with the gene. The following descriptions are based on Carter's published work.

The most obvious manifestation of the gene in luxate homozygotes is a more or less complete reduction of the tibia often accompanied by a bent fibula. As a result, the feet of the animal do not touch the ground, or, at least the plantar surface does not. As a general rule, there is no normal two-jointed hallux, the tibial-sided digits being all triphalangous. There may be added extra pre-axial

digits forming a maximum of seven toes, or, with increasing manifestation of the anomaly, there may be a reduction of toes uutil only three remain, the fibular-sided III, IV, V. Changes in the tarsus include fusion of adjacent elements or losses. In the tridactylous extreme case, all the preaxial elements of the tarsus (tibiale, talus, navicular, cuneiforms I and II) are missing.

Changes in the limb skeleton of luxate homozygotes range from a slight shortening of the tibia to its complete absence. The fibula does not share this progressive reduction but becomes more bowed and simultaneously thicker as the tibia becomes shorter, but when the tibia is completely missing, the fibula becomes straighter and thinner until its final state is rather similar to that of a normal fibula. The femur is shortened. This shortening is most extreme on the right side, with other less obvious modifications accompanying. In certain cases, the femur experiences extreme changes which culminate in its fragmentation. The innominate bone commonly is reduced in size accompanied by some other anomalies as breaking down of the walls of the acetabulum. In extreme cases, fragmentation occurs, and even loss of both pubic bones. Abnormalities of the hind limbs are usually bilateral in the homozygotes, though generally asymmetrical with the right side the more severly affected.

Luxate homozygotes in some stocks showed a strong

tendency toward sacralization of the 26th vertebra, giving 25 as the number of presacral vertebrae. There was some tendency for heterozygotes to show the same phenomenon.

Investigation of the muscles disclosed that all the normal muscles tended to be present and to have their normal origins and insertions, notwithstanding the fact that they might have lost their normal functions as the result of skeletal changes. When the skeletal part on which a muscle normally originates is not ossified, that muscle is absent. If the skeletal part is present but reduced, the muscle is reduced. When the skeletal part on which a muscle normally inserts is absent, that muscle inserts on the nearest appropriate skeletal element so that a flexor muscle retains a flexor function, an extensor muscle an extensor function.

Investigation of the viscera disclosed hydronephrosis (including hydroureter) and horseshoe kidney in one family in which the luxate gene was segregating. By statistical analysis it was shown that these renal anomalies were significantly associated with the homozygosity of the luxate gene.

As a final part of his study on the genetics of luxate mice, Carter has investigated the embryology of the condition. The results of this study are not yet published, but have been very generously made available to the present worker. Carter in his embryological studies used principally

two CBA strains, one the wild type inbred strain and the other a CBA-lx substrain, isogenic with CBA except for the fact that lx had been introduced by backcrossing for six or more generations.

Examination of the external appearance and of serial sections of embryos of the normal CBA strain and of embryos of the CBA-lx substrain in which lx was segregating disclosed no obvious differences before the 11½ day stage. Then examination of a very early 11½ day (4.2 mm) CBA-lx embryo showed that the hind limb buds were narrower, due to a craniad shift of the posterior margin of the bud, the anterior margin being only slightly displaced. The apical ectodermal ridge was shorter than in a like-sized normal CBA embryo. Putatively homozygous embryos of the CBA-lx strain, 4.8 mm long or longer, when still alive showed abnormalities of their hind limb buds, both in shape and in blood distribution. Both buds are narrow and short and blood could not be seen in the base of the right limb bud. On examination of serial sections, it was seen that the lateral course of the umbilical artery had developed on the left side but not on the right side. Normally, the two limb arteries which come off the primitive unbilical arteries experience a "break-through" on either side around the metanephric blastemata and Wolffian ducts forming new lateral channels from the dorsal aorta to the ventral umbilical channels. The results of the failure of the right

umbilical lateral channel to develop in putative homozygotes is that the left lateral umbilical channel becomes greatly enlarged and regression of the left medial root is delayed. These changes in turn cause the left kidney to be impacted in the vascular hoop and delay its normal laterad and cephalad migration. The right kidney has more space than normal and is able to expand in situ without migration. The result of all this is contact and fusion of the kidneys especially at their posterior poles where lateral growth is limited by the lateral umbilical artery roots. The condition is known as horseshoe kidney.

By the 12½ day stage, abnormalities are recognizable in some heterozygotes. In some lx-carrying embryos of about 7.0 mm excessive growth of the footplate is seen near the preaxial end of the apical ectoderm ridge. The marginal sinus (the venous channel running below the apical ectoderm) is ill-defined in this region and in places breaks up into a network. When blastemal condensation starts (at the same time as in normal embryos) excessive condensation occurs in the preaxial part of the footplate forming the blastemata of additional digits.

At the 12½ day and later stages, putative homozygotes are easily recognizable. Their hind limbs are abnormally narrow and the footplates are distorted and pulled over preaxially. Anterior to the second digit, the marginal sinus is ill-defined or broken up into a network.

Blastemata condense at the normal time but are deficient in the preaxial part of the foot-plate. Deficient condensation in the footplate accompanies total or near absence of the tibial blastemata while excessive condensation accompanies tibial blastemata which are abnormally narrow.

At the 13½ day stage, deposition of matrix occurs between the cells of the limb skeleton blastemata at the same time in lx-carrying embryos as in normal embryos. In general, histodifferentiation follows its normal course with the normal time relationships in lx-carrying embryos. Nerves grow into the feet at the normal rate. The apical ectodermal ridge and the marginal sinus disappear as the toes develop, being gone by the 15½ day stage. Ossification starts in the leg at 16½ days. Leg muscle formation begins at 13½ days and is well defined by 14½ days. In most normal CBA embryos, the right umbilical artery becomes larger than the left at about the 15½ day stage. In the luxate homozygotes and in some heterozygotes, the left umbilical artery is the dominant artery, the cause going back to the failure of the right lateral course to develop as described above.

Since lx tends to cause a reduction in the number of lumbar vertebrae, Carter investigated the question whether lx causes a craniad shift of the hind limb region. Using a method which will be described in detail below, he proved that the luxate gene does cause such a shift, most

pronounced when present in homozygous form.

His finding of a craniad shift ascribable to the luxate gene coupled with the discovery by Saunders (1948) that the apical ectoderm of the chick fore limb bud organizes the various parts of the wing out of the mesenchyme lying below, has led Carter to propose a "unitary hypothesis" to account for all the effects found in the luxate syndrome. He proposes that in the mouse embryo the hind limb is formed through an interaction between limb-potent tissue, restricted to a region around a few specified somites, and a limb inductor extending along most but not all of the limb-potent tissue. A slight displacement of the limb inductor relative to the limb-potent tissue can result in the induction of supernumerary digits from normally inactive limb-potent tissue lying immediately anterior to the limb inductor. The action of the luxate gene is a craniad shift of the limb inductor, slight when in single dose, farther when in double dose. This would result therefore in preaxial polydactyly in the heterozygotes, and in loss of tibia in homozygotes. Carter equates the limb inductor, by analogy with Saunder's results with the chick, to the apical ectoderm and the limb-potent tissue to the lateral mesenchyme between, say, the 23rd and 27th intersegmental levels.

At the end of his paper on the embryology of the luxate condition, Carter posed the following question: "Are ectrodactyly and hemimelia in other material associated

with a displacement of the limb buds along the body axis?" When studies by Dr. M. C. Green on a hereditary condition involving absence of tibia found in certain mice derived from the C3H strain showed that the mutation caused an increase in the number of presacral vertebrae, it was apparent that excellent material was available with which to seek an answer to Carter's question.

MATERIALS AND METHODS

A. Materials.

Dr. M. C. Green in July, 1950, reported in the Mouse News Letter, No. 3, that she had found three mice in a litter of seven, offspring of C3H mice, which showed a reduction in size or absence of the tibia in both legs. The variation seemed anatomically like that previously described by Rabaud (1919) in a stock now extinct. Subsequently, Dr. T. C. Carter furnished mice carrying the luxate gene which he was studying at the time and which seemed very similar to Rabaud's mutant. To compare the two mutants, Dr. M. C. Green began to backcross both onto the same genetic background, that of C57BL/10 mice. As this backcrossing progressed, it became increasingly evident that the two mutants were not phenotypically the same. By genetic studies Dr. M. C. Green (unpublished) has shown that the two conditions depend on different genes. Since the condition found at Ohio State University had certain resemblances to the luxate condition studied by Carter, notably polydactyly in heterozygotes and reduction of tibia in homozygotes, Dr. M. C. Green has called the new gene "luxoid" and has assigned the symbol "lu" to it.

Dr. M. C. Green furnished some of the luxoid heterozygotes to the present worker who continued to backcross

the luxoid animals onto the C57BL/10 background so as to secure two strains of animals for comparison, namely, normal C57BL/10 animals and animals isogenic with the normal C57BL/10 animals except for the fact that they carry the luxoid gene. For easy notation, the pure strain C57BL/10 will be referred to hereinafter as C57 and the strain isogenic with C57 except for the presence of the luxoid gene will be known as C57-lu. According to Wright (1921), when one backcrosses one strain repeatedly to another differing in a certain number of genes, the strain being backcrossed approaches isogenicity with the strain into which it is being backcrossed at a rate determined by the fact that at each backcross the amount of anisogenicity existing is halved. This theory does not take into consideration the effects of linkage, but as an approximation to the true situation, it is useful. Table 1 shows the gradual progress to isogenicity brought about by backcrossing of this type. It shows, for instance, that after six backcrosses, the two strains are approximately 97 per cent isogenic. It is of course obvious that a common genetic background is necessary when one desires to study the effects of a mutation like luxoid whose manifold effects vary considerably with the genetic background.

In the study of the morphological effects of luxoid, homozygotes were studied which had been produced by crossing heterozygotes coming from one to seven backcrosses onto the

C57 background. The distinctive characteristics of luxoid on the C57 background appeared already in the progeny of animals derived from one backcross to C57 and showed no great variations from the effects seen in animals derived from a seventh backcross.

In the study of the embryology of luxoid, it would have been desirable to compare homozygote embryos derived from the matings of homozygote animals, heterozygote embryos derived from the matings of normal animals with homozygotes, and normal embryos derived from matings of normal animals only. However, homozygous animals were produced only in small numbers in the course of the breeding program and up to the present no fertile matings between them have been secured. The short semifunctional rear legs of the homozygotes probably handicap them in mating, so that it is probable that fertile matings between them will be sporadic at best. It was decided therefore to use heterozygous animals known to be fertile by previous tests in the matings designed to produce luxoid embryos. Only animals which showed polydactyly and therefore were known heterozygotes were used. Almost all of those used had produced one or more homozygous luxoid young or had produced polydactylous young when crossed with C57 males, so were confirmed carriers of the gene.

In the course of the study, 16 pure strain C57BL/10 females were sacrificed in getting 152 embryos ranging from

Table 1

Rate of increases of isogenicity in repeated backcrosses of one strain to another differing in a certain number of loci. Isogenicity is here taken to mean having the same genes as the other strain at each locus.

Generation	Percentage of increased isogenicity
1	0
2	50
3	75
4	87.5
5	93.75
6	96.875
7	98.4375
8	99.22
9	99.61
10	99.80

9½ to 18½ days in age. Thirty-two C57-lu females were sacrificed in getting 197 embryos ranging from 9½ to 18½ days in age. The total number of embryos dissected out therefore was 349. The luxoid heterozygotes used to produce the embryos were animals derived from 7 to 9 backcrosses to the C57 strain, or were animals produced by mating such backcross animals among themselves.

B. Methods.

For the morphological studies, the skeletons of 19 luxoid homozygotes stained with alizarin and cleared in glycerine by the short cut method (M. C. Green, 1952) were furnished by Dr. M. C. Green. These were studied under a binocular dissecting microscope and typical parts drawn with the aid of a camera lucida. These skeletons were supplemented by a few others prepared by the author. The skeletons of 76 heterozygotes prepared by the author by the short cut method were examined over a diffused light source. Camera lucida drawings were made of typical anomalies.

For the embryological studies, several fertile females were placed in a cage together and a fertile male introduced approximately at 5 PM each afternoon. The male was removed at approximately 9 AM the next morning and the

females examined for copulation plugs. As is well known, the male mouse places in the vagina of the female a secretion of his coagulating glands which hardens into a plug closing the lumen of the vagina. The vaginas of the females were opened with blunted forceps and the females with plugs segregated in a special cage. The date of observation of the plug was noted and the date when the female was to be killed to furnish embryos of a desired age was calculated.

Since the embryos were dissected out roughly from 9 AM to 11 AM, and copulation was assumed to have taken place in the first half of the night before the plug was noted, the embryos were given the chronological age of n plus one-half day, the plug having been seen n days before the morning on which the female was to be killed. Thus in an actual case, the plug was noted on May 1st and the female was killed on May 13th. Therefore the plug was noted 12 days before the female was killed and the embryos were assigned the chronological age of 12½ days. After being dissected out, the age of the embryos was always checked by Grüneberg's external landmarks method (Grüneberg, 1943). It was found that quite often the developmental (Grüneberg) age of the embryos was about a day behind their chronological age. This was probably an effect of the inbreeding to which the strains used were subjected.

On the morning of the appropriate day, the female was killed with chloroform, opened up, the uterus and ovaries

removed, pinned out on a block of balsa wood or on cork, and suffused with Ringer's solution for warm-blooded animals. The number of embryos in each horn was noted, then each horn was carefully slit open with a fine scissors, the embryos scraped free from their implantation surface, and placed separately in small glass dishes with Ringer's saline. The ovaries were placed in another dish and the number of corpora lutea counted. Except for one doubtful case, the number of corpora lutea always equaled or occasionally slightly exceeded the number of implantations, including all necrotic or resorbing sites. Using two pairs of Dumont No. 5 watchmaker's forceps and under the lowest power of a binocular microscope, the yolk sac was torn away at its junction with the placenta, but left attached to the embryo by the omphalomesenteric vessels. The same operation was done on the thin amnion and then the embryo was completely exposed, remaining attached to the placenta by the umbilical artery and vein. The embryo would often remain alive for up to two hours while in Ringer's saline and could be carefully observed under the binocular microscope. Gradually, however, the heart beat of the embryo slowed down and then as it was almost dead, the umbilical and omphalomesenteric vessels were cut with a fine scissors. Bouin's picro-formol fixative was added to the Ringer's solution. When fixation was complete which was in a few minutes, the embryos were given a number (if this had not

been done earlier) and were measured with a measuring microscope. An outline sketch of each was made with a camera lucida and then they were transferred each to a separate tube of fresh Bouin's fixative bearing their identification number.

After being left over-night in the Bouin's fixative, the embryos were dehydrated in an alcohol series of 70%, 80%, 95% and absolute alcohol. They were never left over an hour in any one of these reagents, except in the case of the large embryos which were given three half hour periods in the absolute alcohol to complete dehydration. In the case of the smallest embryos (9½ to 11½ days), only a half hour in 80% and 95% alcohol were allowed. This protocol was followed to prevent excessive hardening of the embryos and to allow them to retain the yellow stain of the picric acid of the fixative. After the proper time in absolute alcohol, methyl benzoate was added by a pipette to the bottom of the tube after which the embryo, at first floating at the interface between the absolute alcohol and the methyl benzoate, gradually descended into the pure methyl benzoate. Thus distortion was minimized. The embryo was put in fresh methyl benzoate the next morning and stored in it until the time came for inbedding. Methyl benzoate was used as the clearing agent in preference to xylene because it does not cause any hardening of the embryo.

The embryos were imbedded in 55 degree paraffin wax

tempered with beeswax. The embryos were placed first in a half and half mixture of methyl benzoate and the paraffin for not over one hour, then into pure paraffin for a total of two hours with two changes at 40 minute intervals. Small embryos were rushed through with only a half hour in the mixture of methyl benzoate and paraffin, and a total of one hour in the pure paraffin with two changes at 20 minute intervals. The purpose of the minimal times in the oven (kept slightly over the melting point of the paraffin) was to prevent hardening of the embryos leading to failures in sectioning later.

The embryos were imbedded in little paper boxes whose bottoms were ruled with lines parallel to the longest dimension and with lines perpendicular to these. These lines were drawn with a soft lead pencil before the box was folded (Guyer, 1936), and served a dual purpose. At the time of imbedding, they provided a grid on which to orientate the embryo properly for the desired plane of section. This desired plane was drawn with a rule on the camera lucida sketch of the embryo if possible, and, in any case, the sketch was used to orientate the embryo along the grid. After imbedding, the lines adhered to the bottom of the block when the paper box was peeled off and allowed exact trimming of the block and its correct orientation on the block holder. The yellow picric acid stain of the embryo facilitated orientation of the embryo in imbedding and

also the trimming of the block for sectioning, since the yellow embryo contrasted with the colorless background.

The embryos were sectioned serially at 10 microns, or, in a few cases when it was found difficult to remove wrinkling of the sections on the warming table, at 12 microns. The sections were stained with Delafield's hematoxylin and eosin. Some were stained with Mallory's triple connective tissue stain to detect the early differentiation of cartilage. A total of 104 embryos were serially sectioned, either in whole or in part.

Noback's modification of van Wijhe's method was used to stain the cartilaginous skeleton of some embryos, some newborn young, and some week old mice. This simple and excellent method was used according to the protocol described by Grüneberg (1953). Other late embryos were stained with the alizarin red technique and cleared in glycerine (M. C. Green, 1952) for the detection of bone formation.

The graphical reconstructions referred to later in this dissertation were made with the aid of a 10 mm square grid eye-piece, ruled with squares one millimeter on a side. The graphs were made on Keuffel and Esser graph paper 500 millimeters wide and ruled with lines one millimeter apart. By adjusting the draw-tube on the microscope, each one millimeter on the grid in the ocular was made to cover one-tenth millimeter in linear dimension of the section viewed. Thus the grid covered one square millimeter of the section.

The width of the section covered by the grid, one millimeter, was equated to the width of the graph paper, 500 millimeters. Thus the distance between successive lines on the graph paper, one millimeter, was equivalent to one five-hundreth of a millimeter or to two microns. Since ten microns was the thickness of successive sections, it was easy to plot the longitudinal distance between successive structures seen in the serial sections on the graph paper since the space between six successive lines on the graph paper equaled 10 microns on the scale used. The graphical reconstructions used were projections onto a dorsal plane. For these, transverse sections were cut and an imaginary line through the top of the dorsal nerve tube and the notochord served as a median reference axis. Since in many cases the sections had not been cut exactly transversely to the main axis of the embryo, the angle of error was estimated by counting the number of sections between paired structures (usually the dorsal intersegmental arteries) and the axis of plot offset by this angle. Graphical reconstructions were made of parts of 40 embryos.

RESULTS OF THE BREEDING EXPERIMENTS

A. The penetrance of the luxoid gene.

On beginning the experiments described in this dissertation, it was found necessary to produce many animals heterozygous for the luxoid gene. Thus the first matings made were backcrosses of luxoid heterozygotes obtained from Dr. M. C. Green to pure strain C57 animals. A total of 28 matings of this type produced 52 litters. They were expected to produce animals heterozygous for luxoid and normal wild type animals in equal numbers. Table 2 shows the numbers actually obtained and the numbers expected. Examination of the table shows that of the total number of 327 young produced, 187 were normal in phenotype whereas 140 were polydactylous. If one assumes that all of the normal animals lacked the gene, the deviation of 23.5 animals from the expected number (163.5) of each type is significant at the two per cent level of probability (Chi square is 6.6470 whereas chi square at the two percent level is 5.412 for one degree of freedom). However, Dr. M. C. Green has shown (unpublished) that the animals can carry the gene and yet fail to show its effects when in heterozygous condition. Thus we can assume that the deficiency of heterozygotes is spurious and that actually some of the normal animals carry the gene and are in fact heterozygotes. To

gain a rough idea of the penetrance of the gene, we can divide 140, the number of polydactyls observed, by 163.5, the number expected, and we find that the penetrance of the gene is 0.856 or about 86 per cent.

B. The influence of the luxoid gene on viability

The next step in the breeding program was to make matings of heterozygotes by heterozygotes to produce homozygous luxoid mice. The homozygotes produced would be mated together to produce timed embryos which would all be homozygous for the gene. As mentioned before, no fertile matings were secured from the homozygotes. However, the matings of heterozygotes by heterozygotes served to test the males and females for fertility. Thus when it became necessary to mate heterozygotes to produce embryos of known age, a supply of known fertile animals was at hand.

A total of 73 matings of heterozygotes known to be heterozygous because of polydactyly of the hind feet was made. Of these 31 were sib matings of heterozygous luxoid mice produced by backcrossing to the pure strain C57 animals and 42 were either sib matings of heterozygous animals produced by the above type of mating or were matings of backcross animals which were not sibs. Since by this time the animals used were either themselves products of seven to nine backcrosses to C57 or were the progeny of such animals,

Table 2

Segregation of *luxoid* in animals produced by backcrosses of heterozygous *luxoid* animals to pure strain C57BL/10 animals.

	Observed	Expected
Normal	187	163.5
Polydactyl	140	163.5
Total	327	327

Chi-square = 6.470. $P < 0.02$.

Table 3

Segregation of *luxoid* in animals produced by crosses of heterozygous *luxoid* animals *inter se*. Classification made at birth.

	Observed	Expected
Normal	75	62
Polydactyl	113	124
Luxoid	60	62
Total	248	248

a. Comparing normal plux *luxoid* animals with the polydactyls, chi-square = 1.778; $P > .05$.
b. Comparing normal plus polydactyls with *luxoid* animals, chi-square = 0.0484; $P > .05$.
c. Comparing the observed numbers of normal, polydactyl, and *luxoid* animals with the numbers expected, chi-square = 3.761; $P > .05$.

it was thought that for the purposes of this experiment all the material could be considered isogenic and equated as far as the effect of the residual genotype on the luxoid phenotype was concerned. In other words, any differences in the manifestation of the luxoid gene because of differences in residual genotype could be considered minor as compared with the greater similarities produced in the manifestation of the luxoid phenotype by the sharing of most genes in common.

Pooling all the results from the matings of heterozygous animals, a total of 43 litters was produced. These litters (table 3) consisted of 75 "normal" animals, 113 polydactylous heterozygotes, and 60 luxoid homozygotes, classified as such at birth. Adding the normal animals to the luxoid homozygotes, we get a total of 135 to be compared with the 113 polydactylous animals produced. The expected ratio of normal plus luxoid homozygotes to heterozygotes is one to one. Therefore we expect, of the 248 total, 124 to be heterozygotes and 124 to be normal or luxoid homozygotes. The deviation of the observed from the expected is only 11 and on statistical test we find that the deviation is quite easily explained by chance alone. Chi-square is 1.788 whereas for one degree of freedom, chi-square for 0.05 probability is 3.841. Lack of penetrance is not indicated by these results, since the results are quite probable although we assume that all the normal animals lack the gene.

However since it is known from other breeding tests and from the results noted in the previous section that the gene has not perfect penetrance, we can assume that some of the normal animals did carry the gene. Testing the observed numbers against the 1 : 2 : 1 ratio expected of normal, heterozygous, and homozygous animals (table 3), we find again that the deviations are not significant. No deficiencies in any class are indicated by these results. The general conclusion therefore from this and the preceding section is that the penetrance of the luxoid gene on the C57 background is high.

The question may be asked whether the luxoid gene has any effect on viability. After birth until the animals begin to eat food for themselves, the luxoid homozygotes appear to grow as well as their normal or polydactylous litter-mates. After the young begin to eat for themselves, the luxoid homozygotes are handicapped because they cannot easily reach the food-hopper, nor can they reach the spout of the water bottle. It was found necessary to place more cedar shavings than usual on the bottom of their cages so that they could reach the food-hopper easily and to introduce a ladder which helped them get at the spout on the water bottle. Even so, the homozygotes apparently do not grow as well as their siblings. In one case, one female had to be killed at the age of two and a half months because her upper and lower incisors had grown so long that

they occluded her mouth and prevented her from effective feeding. The ultimate cause of the starvation of this female and the slow growth of the luxoid homozygotes perhaps is that the defect in hind limbs causes locomotion to be difficult and therefore overly energy-consuming and at the same time keeps the animals from having quick and easy access to the food. Thus in the case of the starving female, her teeth apparently were not worn down as fast as in normal mice by attrition against the hard food particles.

However this may be, the question arises whether the luxoid homozygotes are less viable in utero than normal or polydactylous sibs. Adding the number of normal mice to the number of polydactyls gives 188 mice to be compared with the 60 luxoid homozygotes. The expected ratio of normal plus luxoidheterozygotes to the luxoid homozygotes is three to one. Therefore, we expect of the 248 total, 186 to be normal or polydactyls and 62 to be luxoid homozygotes. The deviation of the observed from the expected is only 2 mice. On statistical test, we find that the observed deviation is insignificant (chi-square is 0.0484, whereas chi-square for 1 d.f., .05 probability, is 3.841). Therefore the conclusion is warranted that the luxoid gene in homozygous condition has no effect on intrauterine viability.

The same conclusion is reached when the embryos from females sacrificed at various days of gestation are

classified and their numbers compared. Taking only the embryos dissected out from the 13½ to 18½ days of gestation when classification is certain for all embryos, we find (table 4) that 22 normal animals, 42 polydactylous, and 25 luxoid homozygotes were produced. The expected ratio of these classes is 1 : 2 : 1, which gives expected numbers of 22.25 normal, 44.5 heterozygotes, and 22.25 homozygotes. The observed number of luxoid homozygotes (25) is slightly in excess of the number expected (22.25). We can conclude therefore that so far as this evidence goes, the luxoid gene when in homozygous condition has no effect on the viability of the mouse possessing it.

Table 4

Segregation of *luxoid* in C57BL/10 embryos 13½ to 18½ days old, produced by crosses of heterozygous *luxoid* animals *inter se*.

Age of embryo	Phenotypes and number of embryos					
	Normal	Polydactyl	Luxoid	Total	Dead	Final Total
13½	5	9	7	21	1	22
14½	9	11	5	25	2	27
15½	2	2	3	7	2	9
16½	2	13	6	21	4	25
17½	3	2	1	6	0	6
18½	1	5	3	9	0	9
Total observed	22	42	25	89	9	98
Total expected	22.5	45	22.5	89	-	—

DESCRIPTION OF THE MORPHOLOGY OF ADULT *LUXOID* MICE

No detailed description of the complete normal anatomy of the house mouse, *Mus musculus*, has yet been published. Therefore in the study of the adult skeleton of *luxoid* heterozygotes and homozygotes, help had to be secured from various sources. The description of the anatomy of the Wistar rat by Eunice C. Greene (1935) served as a useful guide. This description was supplemented by Hovelacque's (1920) description of the hind limbs and pelvic bones of the *souris luxées* and Carter's (1951) descriptions of the same elements in his study of the skeletal abnormalities in *luxate* mice.

A. The Skeleton of the Foot.

For brevity, foot will stand for hind foot and hand for forefoot. The bones in the foot of a normal C57BL/10 mouse (fig. 1) are similar to the bones in the foot of the Wistar rat as described by Greene (1935) and are quite like the bones in the feet of the normal mice studied by Hovelacque and Carter. In the proximal row of the tarsus, we see three bones, the lateral calcaneus, the central talus on which the tibia and fibula articulate and the small medial tibiale. Only the calcaneus has a proximal epiphysis. In the distal row of the tarsus, we see the lateral cuboid

which articulates with the calcaneus proximally and with the fourth and fifth metatarsals distally. In most mice, the navicular is a separate bone articulating proximally with the talus and distally with the three cuneiform bones. However, in all C57 mice examined, the navicular bone is fused with the third cuneiform as is seen in figure 1. The three cuneiform bones articulate distally with the first, second, and third metatarsal bones. The first cuneiform is longer than the other two. The first metatarsal has a proximal epiphysis. Each toe has three phalanges except the medial hallux which has only two phalanges and is the shortest of the five toes.

The changes in the feet of luxoid heterozygotes concern only the preaxial elements. In figure 2 is shown a foot in which the changes from normal are slight. The hallux is partly double, the doubling involving the first and second phalanges. The first cuneiform has a distal epiphysis while the proximal epiphysis of the first metatarsal found in normal mice is missing. All of the other bones are in their normal condition. It might be well to remark here that the differences in appearance between normal bones in the various camera lucida drawings is due to different presentations of the parts under the microscope.

In figure 3 is shown a luxoid heterozygote in which further changes are seen. The partly double first phalanx of the hallux articulates medially with two phalanges and

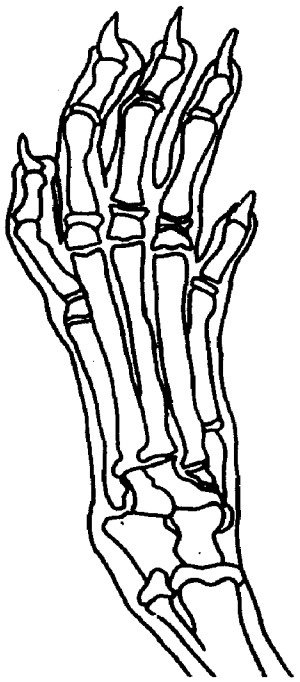

Fig. 1

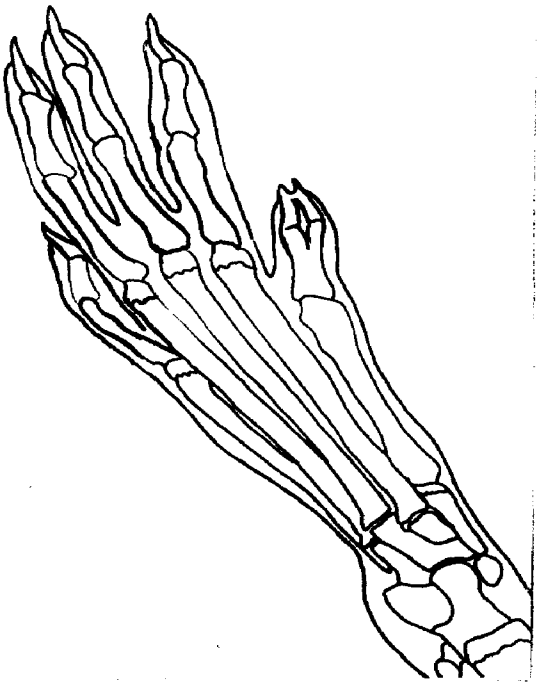

Fig. 2

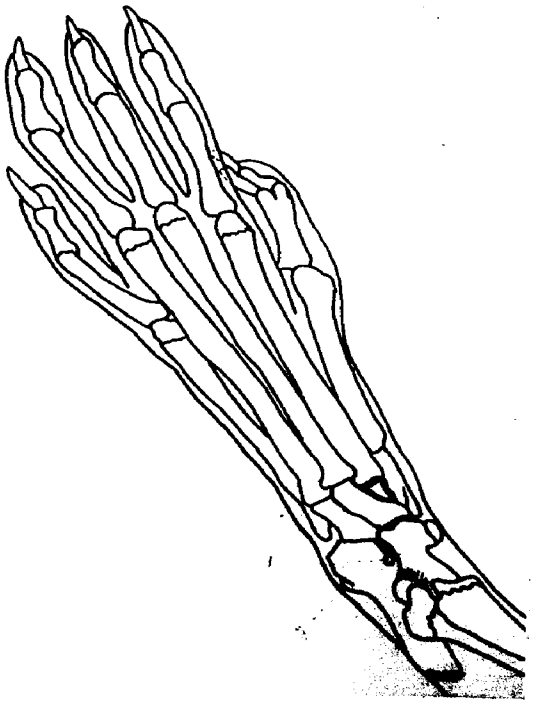

Fig. 3

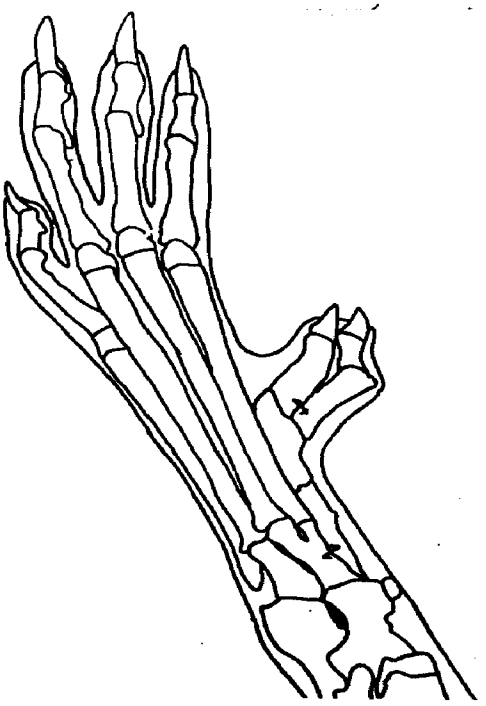

Fig. 4

Fig. 1 Left foot of a normal C57 mouse. Explanation of this and suceeding figures in the text.
Fig. 2, 3, 4. Left feet of C57 <u>luxoid</u> heterozygotes.

laterally with only one phalanx. There is a slight indication of doubling in the proximal portion of the first cuneiform and the calcaneus has a small lateral process.

A further state is seen in figure 4. Here we see a more distinctly six-toed foot. The navicular and the second and third cuneiforms are all one bone and this is fused (indicated here, as in other drawings, by a zigzag line) with the first cuneiform. The first metatarsal articulates medially with a diphalangous and laterally with a triphalangous digit. These digits are syndactylous.

A still further stage is given in figure 5. Here we have more complete doubling of the hallux. The first cuneiform is split distally but the parts are grown together (fused or ankylosed). The first metatarsal is double with a single proximal epiphysis. The two parts of the metatarsal are ankylosed. Distally, each part of the double first metatarsal bears an epiphysis and on these epiphyses, articulate two toes. The more lateral toe is diphalangous while the medial toe ("prehallux") is triphalangous.

We see in figure 6 the right foot of a <u>luxoid</u> heterozygote with seven toes. The cuboid is misshapen and lies partly under the ankylosed navicular and third cuneiform bones. The alterations in the cuboid are probably secondary to the changes induced in the preaxial part of the foot and may be the result of abnormal stresses and strains induced in the tarsus by the extensive preaxial changes.

The second and third cuneiform bones are ankylosed to the navicular proximally but are separate distally. The first cuneiform is partly double and articulates distally with a partly triple first metatarsal. The first metatarsal articulates with three toes, the middle one of which is triphalangous while the two one each side are diphalangous.

No abnormalities are found in the rest of the skeleton of luxoid heterozygotes.

In all the luxoid homozygotes examined, the feet showed some degree of polydactyly or ectrodactyly. The minimum degree of polydactyly observed is illustrated in figure 7, where the hallux has three phalanges and is only slightly shorter than the second toe. In normal mice (fig. 1), the hallux is considerably shorter than the second toe and is the shortest of all the toes. Here we see that it is considerably longer than the fifth toe, ordinarily the second shortest toe. The first metatarsal in normal mice lacks a distal epiphysis but here one is present, while the proximal epiphysis seen in normal mice is absent. The first cuneiform, long and extending into the metatarsus in normal mice, is here apparently reduced to a size similar to the other two, and in fact the three cuneiform bones are one bone with the navicular here. The talus shows changes very characteristic of luxoid homozygotes. It is lengthened backward and has an appearance very similar to that of the calcaneus which remains normal. The similarity is

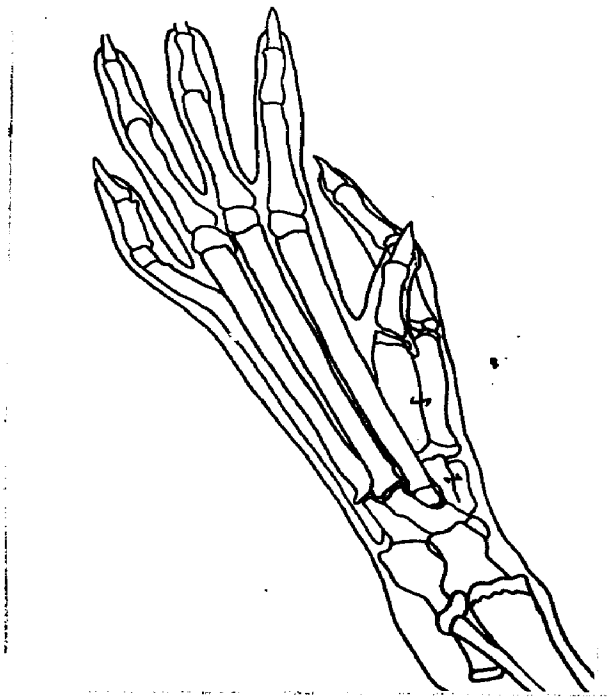

Fig. 5

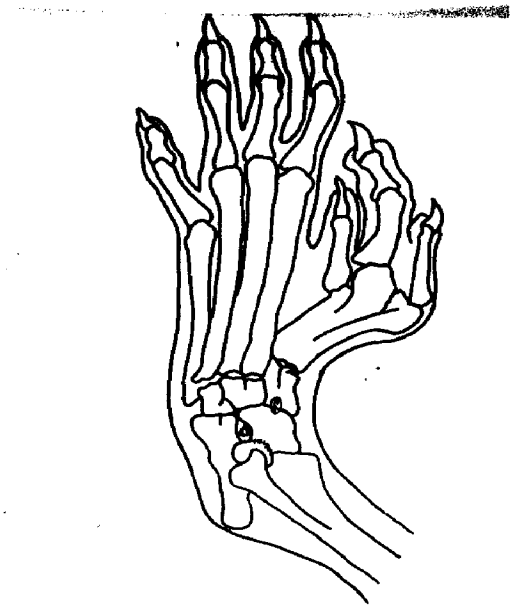

Fig. 6

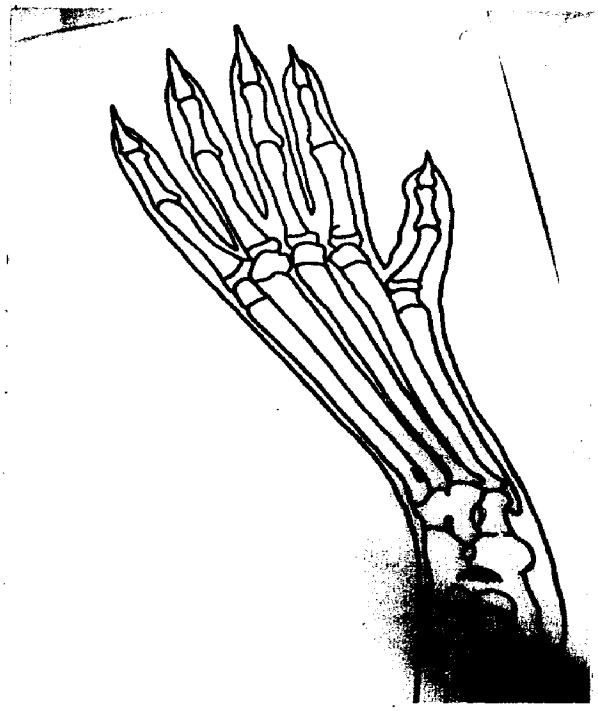

Fig. 7

Fig. 8

Fig. 5, 6 Left feet of luxoid heterozygotes.
Fig. 7, 8 Right feet of luxoid homozygotes.

strengthened by the fact that the talus acquires a proximal epiphysis like that possessed by the calcaneus. In life, the tendon of a muscle inserts on the epiphysis of the talus. The talus is fused to the calcaneus by the articulatory surface. The tibiale is absent.

Figure 8 shows a further degree of polydactyly in a luxoid homozygote. Here we see a bony peg added to the medial side of the first toe and below, another bony peg placed medial to the first metatarsal. These two pieces of bone probably represent the traces of a sixth toe which began to form on the medial side of the first. The remaining five toes are complete with three phalanges and the metatarsals below all have distal epiphyses. The first two complete toes are syndactylous. Two splints of bone lie at the bases of the first and third metatarsals of which the most medial one is free. Possibly they are rudiments of intercalary digits which began to form. The three cuneiform bones are fused whereas the navicular is partly fused to the talus. The cuboid is fused to the calcaneus and the tibiale is absent. The talus again resembles the calcaneus and is ankylosed with it.

We see in figure 9 a different kind of polydactyly. Again there is a suggestion of six toes but his time the beginning of an extra toe is in the middle of the foot. Polydactyly of this nature is not found in luxate homozygotes, according to Carter (personal communication). The middle

toe has a partially double third phalanx, a broader than usual second phalanx, while the first phalanx is partly distorted and is both shorter and broader than usual. This first phalanx articulates with a partly double distal epiphysis of the third metatarsal. The first toe is triphalangous and its metatarsal has a distal epiphysis. A distal epiphysis on the first metatarsal is always found when its toe has three phalanges. Again two bone splints are found at the base of the metatarsals. The first cuneiform is shorter than usual, the second and third cuneiforms are fused to the navicular, and the tibiale is absent. The talus and calcaneus are separate bones but this time the articulatory surface is on the calcaneus. In normal mice, the talus carries the articulatory surface.

Figure 10 shows a more complete vestige of a sixth toe. The proximal fragment of the extra toe may be a vestige of a first phalanx or of a metatarsal. Below, at the medial side of the first complete metatarsal, a nubbin of bone present perhaps represents a vestige of the metatarsal of the extra sixth toe. In this example, the talus was normal, but the tibiale was missing. The three cuneiforms are fused to the navicular.

Another kind of six-toed condition is shown in figure 11. Here the first metatarsal broadens out and carries a wide epiphysis on which articulate two triphalangous digits. The double toe is syndactylous with the third toe. Splints

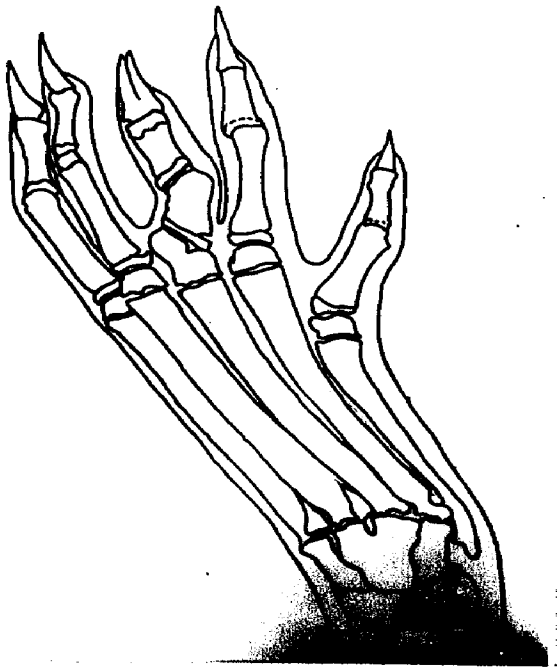

Fig. 9

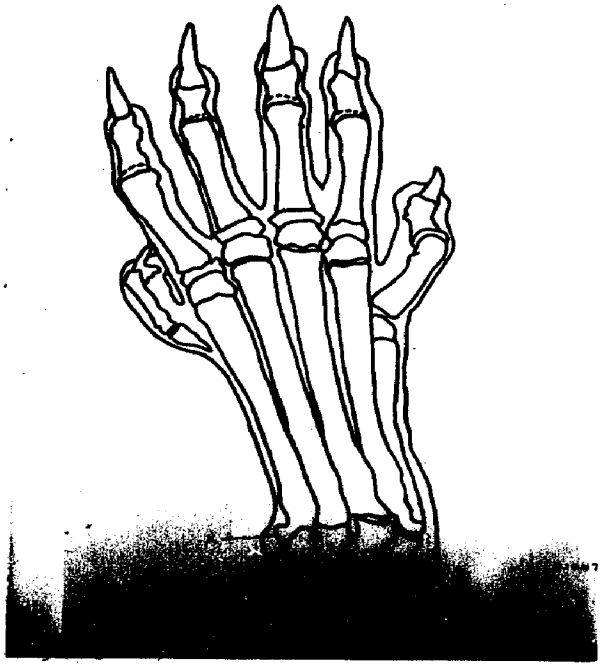

Fig. 10

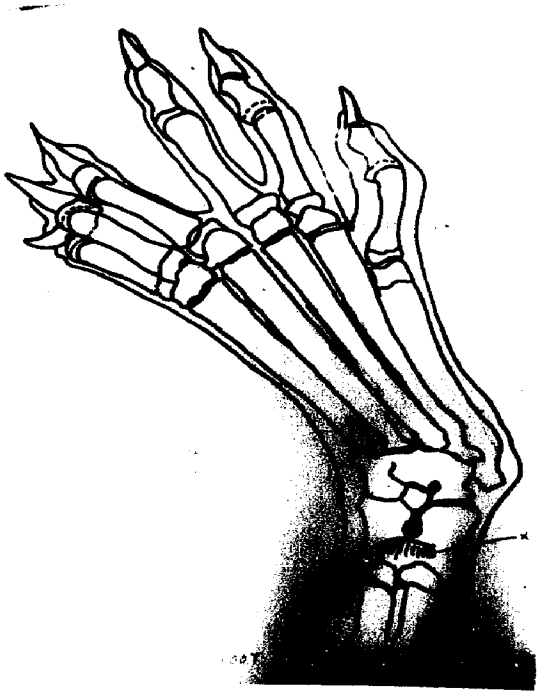

Fig. 11

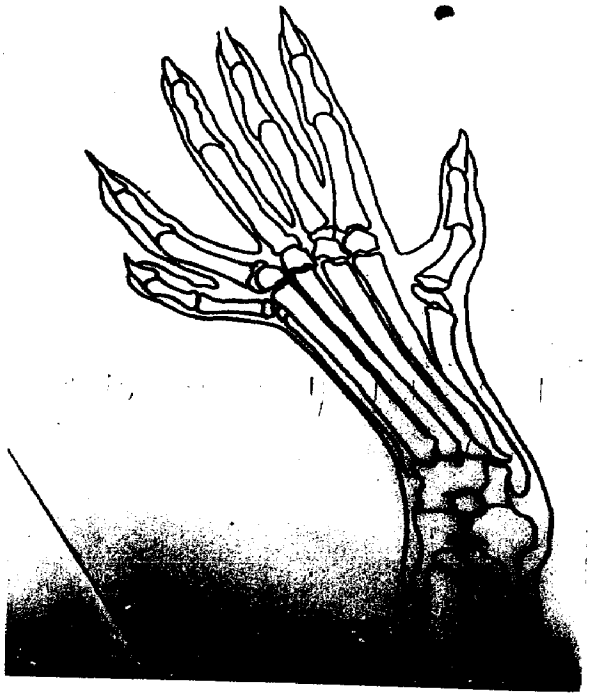

Fig. 12

Fig. 9, 10, 11, 12 Right feet of C57 <u>luxoid</u> homozygotes.

are present, and in this case, the cuboid and the three cuueiform bones are all fused fairly completely. The navicular is free. The talus and calcaneus are fused by articulatory surface common to both.

Figure 12 shows a six-toed condition completely established. All six toes are triphalangous and have metatarsals with distal epiphyses. The first most medial toe is more slender in all its parts than the other toes. The cuneiforms are fused, the tibiale is absent, the navicular and cuboid are free. The talus and calcaneus are joined by their articulatory surface.

An approach to a seven-toed condition is seen in figure 13. The first toe is incomplete and the third and fourth toes share a common first phalanx. The metatarsal of the fourth toe is deficient in the middle, the lower part forming a bony splint similar to another more medial one. The obvious significance of the splint which is a vestige of the fourth metatarsal throws light on the nature of the bony splints found in similar positions in this foot and in others. They most likely represent vestiges of metatarsals of extra digits. The second and third cuneiform bones are fused to the navicular bone, and the talus is fused to the calcaneus.

Figure 14 shows a foot in which ectrodactyly (loss of toes) has taken place. Since the expression of <u>luxoid</u> in the other parts (figure 28 shows the same mouse) is extreme,

we can conclude that ectrodactyly is one of the results when the luxoid gene comes to extreme expression. There are five toes, but the first is very slender and the second and third are fairly completely fused, only the third phalanges being separate. The tarsal bones are all fused as one bone.

B. The skeleton of the hand.

The hand of a normal C57BL/10 mouse (fig. 15) is similar to the hand of the Wistar rat described by Greene (1935). The two most proximal bones of the carpus are the medial navicular-lunate and the lateral triangular. The next row of the carpus, proceeding mesiad from the most lateral bone, is composed of the hamate, capitate, centrale, lesser multangular, and greater multangular. Ventral to these on the sides of the hand are the laterol pisiform with an associated sesamoid bone and the medial falciform. The only peculiarity of the carpus of the normal C57 mouse is the fusion of the lesser multangular with the centrale into one bone. In the figure in which is illustrated the hand of a partly grown C57 mouse, the two bones immediately at the ends of the radius and ulna are the epiphyses of these bones. The metacarpal of the first finger (the pollex) is considerably shorter than that of the other fingers and articulates with only two phalanges. All the other metacarpals articulate with three phalanges. No distinct epiphysis is

found on the first metacarpal.

The hands of a luxoid heterozygote are similar to those of a normal mouse.

Figure 16 shows the right hand of a luxoid homozygote produced by mating sibs backcrossed only once to the C57 strain. Both this hand and the left hand of the mouse are normal. The small medial exostoses on the second and third metacarpals are often found in the hands of normal C57 mice. On the original C3H background in which the luxoid mutation occurred, polydactyly of the hands did not appear regularly nor was it very pronounced (personal communication from Dr. M. C. Green). Since luxoid homozygotes produced by all later backcrosses into the C57 background showed polydactyly of the hands, we can conclude that this mouse did not carry the genes found in the C57 genotype which interact with the luxoid gene in producing the polydactyly of the hands.

In figure 17 we see the lowest grade of polydactyly of the hands found in luxoid homozygotes. The thumb has now three phalanges and its metatarsal has a distal epiphysis. The extreme shortness of the second phalanx in the first and fifth digits is due to a bend of the fingers, and is an optical artifact. The falciform is present but is modified in shape. The greater and lesser multangular are fused.

Figure 18 shows a hand which exhibits a vestige of an extra medial digit. Only the third and a trace of the second phalanx is present. The navicular-lunate is ankylosed

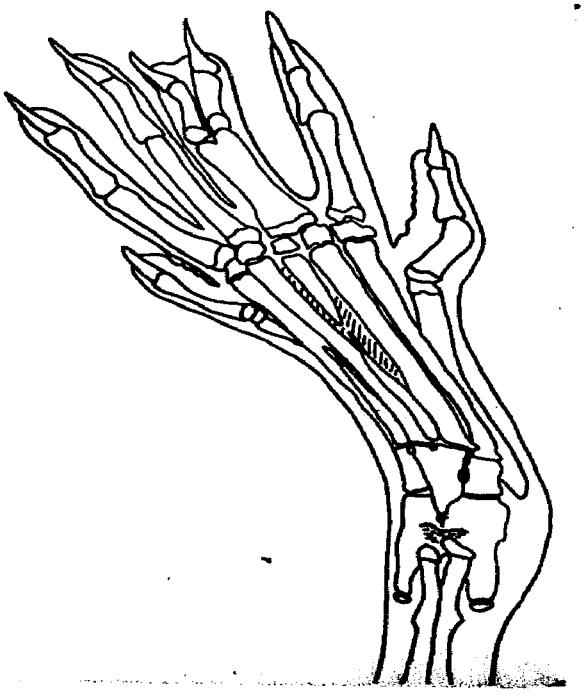

Fig. 13

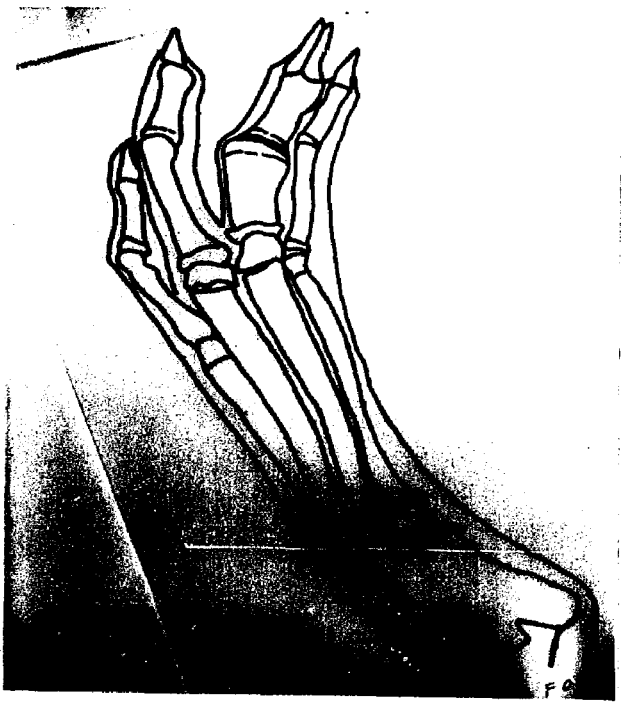

Fig. 14

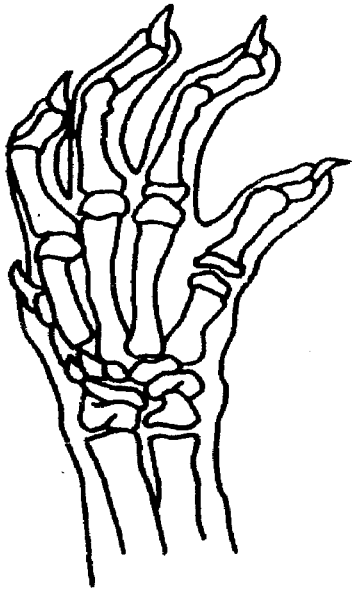

Fig. 15

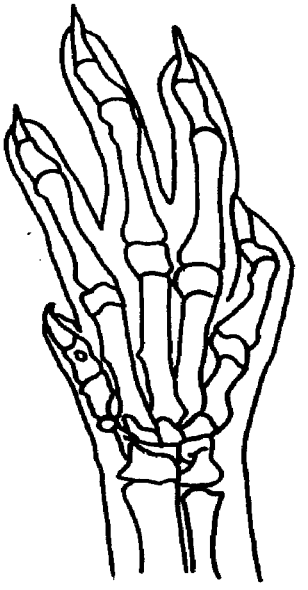

Fig. 16

Fig. 13 Right foot of a C57 luxoid homozygote.
Fig. 14 Left foot of a C57 luxoid homozygote.
Fig. 15 Right hand of a normal C57 mouse.
Fig. 16 Right hand of a C57 luxoid homozygote.

to the triangular and the falciform bone is absent. An extra bone medial to the first metatarsal may be the rudiment of the metacarpal for the extra digit. There are in addition five complete digits with three phalanges and all metacarpals have distal epiphyses.

Figure 19 shows another hand which has a tendency to six fingers. The first digit has a metacarpal with a distal epiphysis. The first phalanx articulating with this metacarpal has two phalanges articulating medially and one laterally. The falciform bone is present and extensive ankyloses have taken place between several bones of the wrist. Thus the navicular-lunate is fused with the triangular, and the lesser multangular, centrale, and hamate are also fused. There is an extra bone present at the side of the first metacarpal, which may represent the metacarpal of the extra digit partly formed above.

A hand with six digits completely formed is shown in figure 20. All have three phalanges and the metacarpals of all have distal epiphyses. The falciform bone is absent and the navicular-lunate is ankylosed with the triangular. Extra bones are present in the wrist on the preaxial side.

Figure 21 illustrates a hand with six digits and also syndactyly. The most medial digit is attenuated and misshapen, but is metacarpal carries a distal epiphysis. The first and second digits are syndactylous. An extra free

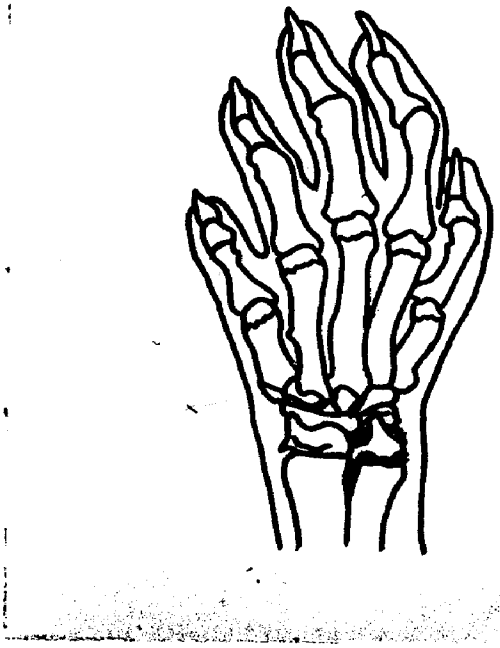

Fig. 17

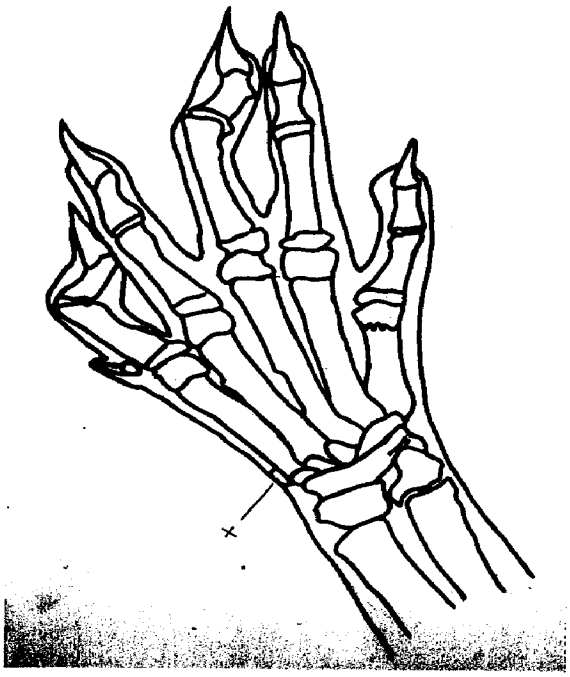

Fig. 18

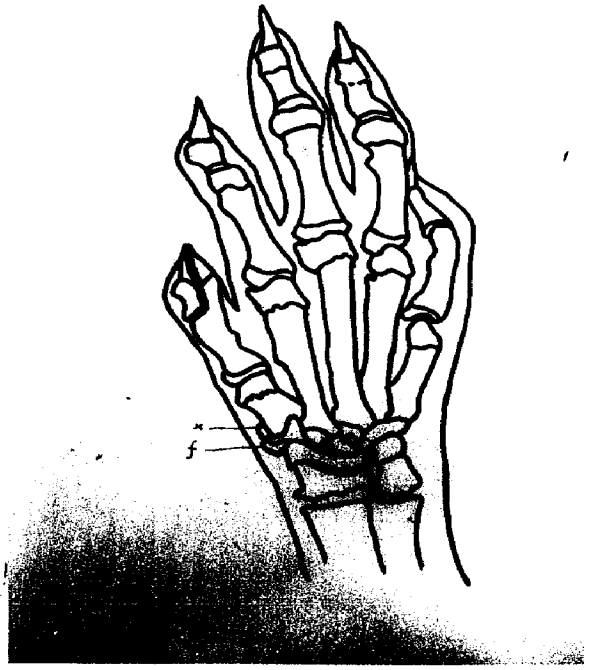

Fig. 19

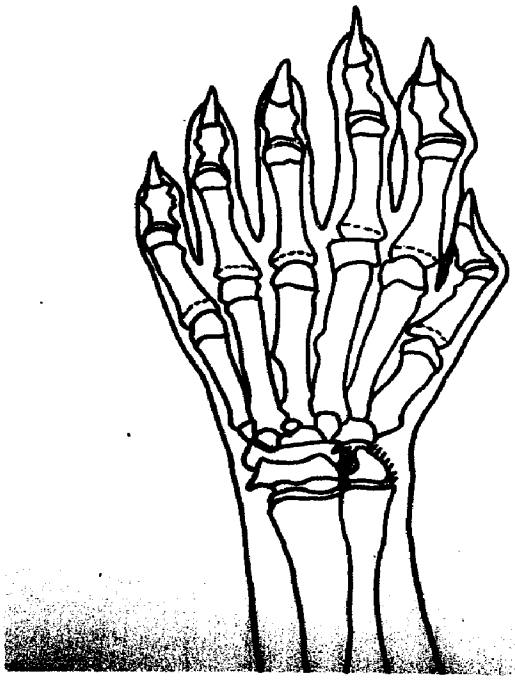

Fig. 20

Fig. 17, 18, 19, 20 Right hands of C57 <u>luxoid</u> homozygotes.

splint lies between the second and third metacarpals.

We have in figure 22 the right hand of a luxoid homozygote where there are six fingers and a vestige of a seventh. All the six fingers have three phalanges and their metacarpals have distal epiphyses. The third finger has a broad second phalanx and two third phalanges articulate with this. The navicular-lunate is fused with the triangular and the falciform is absent, unless the bone below the first metacarpal and fused to it representa a vestige of it.

Although no hands having seven complete digits are figured here, close approaches to such a condition are occasionally seen.

C. The skeleton of the leg and thigh.

Figure 23 illustrates a lateral view of the left leg skeleton of a normal C57 mouse. The medial element is the large and robust tibia. The fibula is the thin bone occupying a position lateral to the tibia but with its upper portion situated somewhat posterior to the tibia. Both are fused together along their distal halves. Both tibia and fibula participate in the ankle articulation with the talus. Both tibia and fibula of the young half-grown mouse figured here have separate proximal and distal epiphyses.

The skeleton of the leg of luxoid heterozygotes is not different from that of normal C57 mice.

In figure 24 is seen a somewhat posterior view of the leg of a luxoid homozygote, whose tibia and fibula are in a condition quite often seen in luxoid homozygotes. The most striking feature is a reduction in the size of the tibia which makes it approach somewhat the size and proportions of the fibula. The tibia and fibula are not fused along their distal parts as in normal mice. The tibia bears a notch which is in close relation to the epiphysis of the calcaneus. Carter has suggested (oral communication) that this notch and its attendant exostosis are very likely caused by movement of the tendon of the tibialis posterior muscle. The point will be cleared up when the muscle anomalies of the luxoid mice are investigated. In the same figure it can be seen that the patella is present over the distal end of the femur but is reduced in size. In the ventral view of the foot afforded by the figure, it is illustrated very well how the talus approximates in shape the calcaneus. Here, which is unusual, the talus exceeds in size the calcaneus. In a pocket between the junction of the talus, calcaneus, and the other ankle bones is seen a new plantar bone. This bone is almost always found in this position when the tibiale is absent from the medial side of the talus and perhaps represents the displaced tibiale.

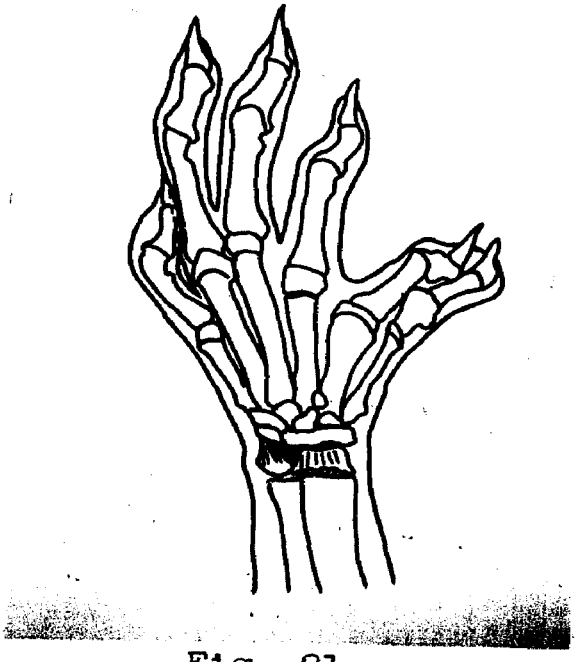

Fig. 21

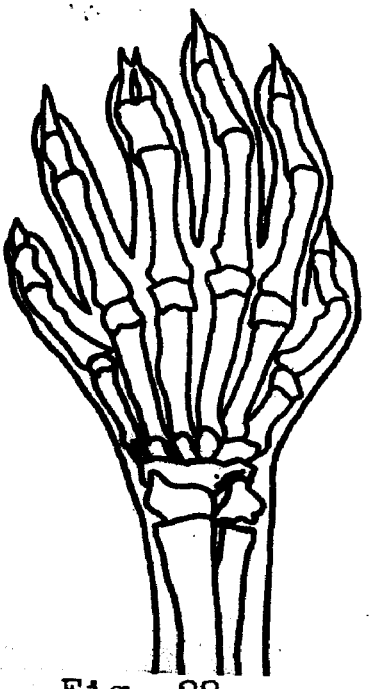

Fig. 22

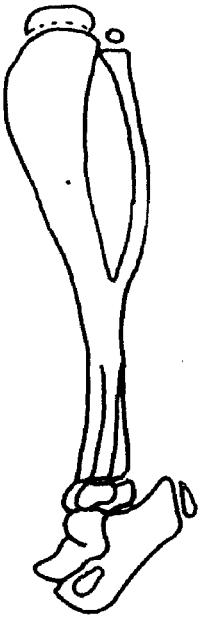

Fig. 23

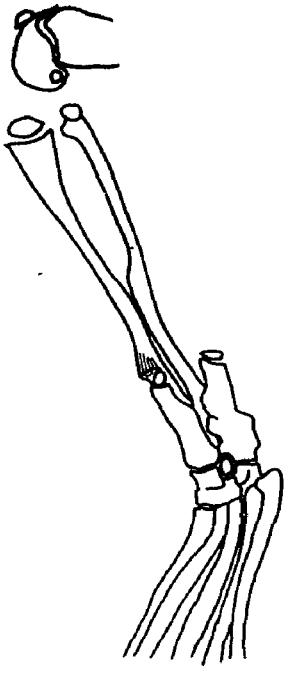

Fig. 24

Fig. 21 Left hand of C57 luxoid homozygote.
Fig. 22 Right hand of C57 luxoid homozygote.
Fig. 23 Left leg of a normal C57 mouse.
Fig. 24 Right leg of C57 luxoid homozygote.

Figure 25 shows a freehand sketch of the skeleton of the right leg and thigh of another luxoid homozygote. The chief novelties here are, first, that the patella is completely absent although the femur is apparently normal in length and shape except for the absence of the condyles. Secondly, the notch this time is on the lateral fibula. Again, this fibular notch is probably caused by action of a tendon passing over this area on the fibula.

Figure 26 is also a freehand sketch of the same parts in another luxoid homozygote. Here the tibia is much shortened and the fibula is bowed, as a result, probably, of the pull of muscles on the feet which would lead to a general shortening of the leg when the opposition of the tibia is reduced. Luxoid homozygotes typically have shorter legs than their normal or polydactylous sibs.

Figure 27, a camera lucida drawing of the right leg skeleton of a luxoid homozygote. Here we see the tibia and fibula both shortened in length and grown together along their lower portions. The fibula is heavier than usual. The notch on the tibia is here rather a bony outgrowth (exostosis). The patella is absent.

Figure 28 illustrates the most extreme condition found in homozygotes. The foot of the same specimen is illustrated in figure 14 and figure 29 is a photograph of the entire specimen. The most striking features are the extreme reduction in the leg skeleton which here is represented by

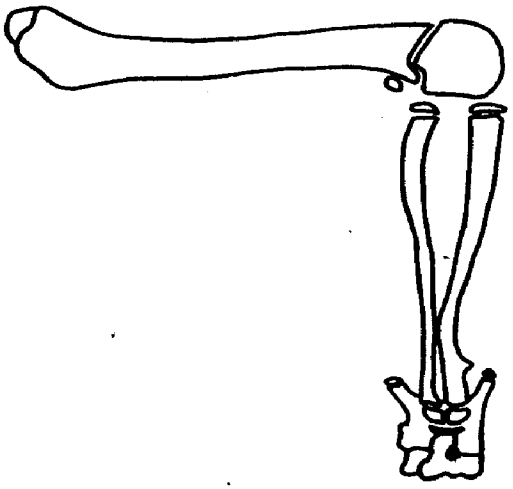

Fig. 25

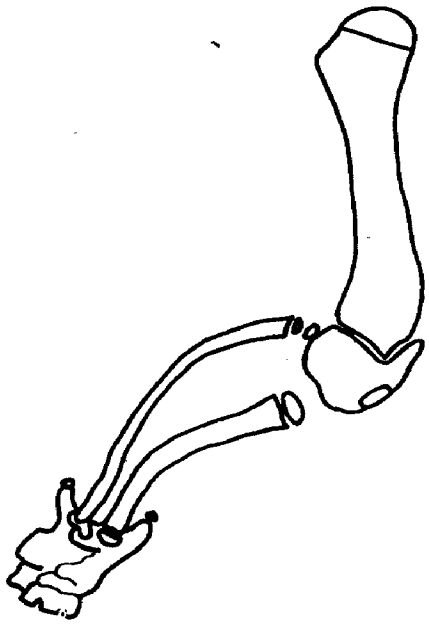

Fig. 26

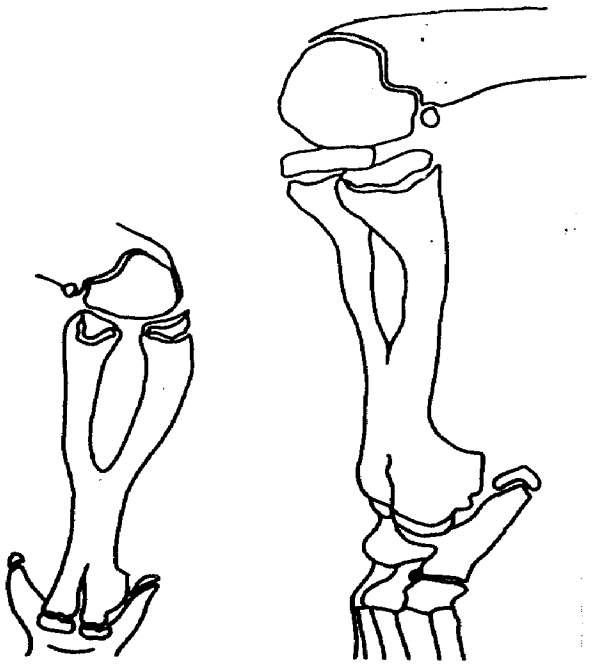

Fig. 27

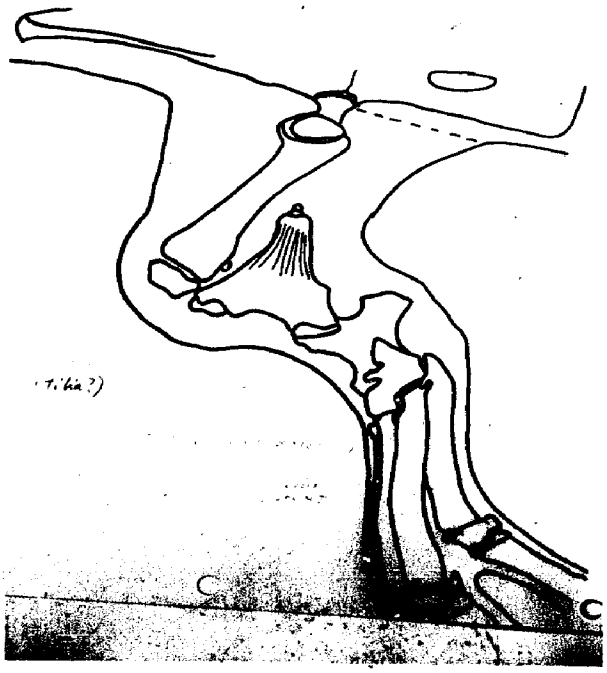

Fig. 28

Fig. 25, 26, 27 Right leg of C57 <u>luxoid</u> homozygotes.
Fig. 28 Left leg of C57 <u>luxoid</u> homozygote.

only one small triangular bone. The tibia seems completely absent and the triangular bone is probably the fibula bowed together to such an extent that its upper and lower portions are fused into one. (A less extreme stage of such bowing is seen in figure 30 of another luxoid homozygote.) No patella is present. The femur is much shortened. The innominate bone is abnormal in that the obturator foramen is much reduced by hypertrophy of the pubis and ischium. The innominate bone on the right side is similar to the one on the left.

Only in this one extreme case has the innominate bone been found affected. The femur, as in the above case, is often shortened and the medial and lateral condyles much reduced.

D. The skeleton of the tail.

The most obvious abnormality of the axial skeleton involves kinking of the tail. Figure 31 and 32 show that such kinking is due to dystrophy and reduction in size of some vertebrae in the skeleton of the tail. Such vertebrae may be fused, as in figure 32. Where no fusions exist, the kinking of the tail may be due to pull by the tendons of the tail which would draw vertebrae on either side of the reduced vertebrae closer together than normal.

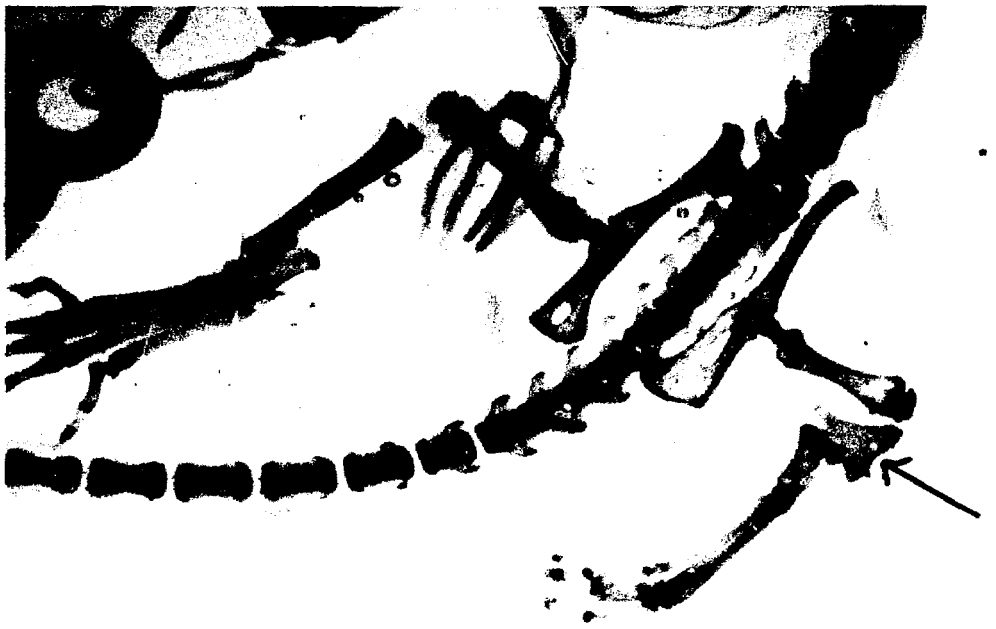

Fig. 29

Fig. 30

Fig. 29 Pelvic region and legs of homozygous luxoid animal. Arrow points to triangular-shaped fibula. Note reduced femur and dystrophy of innominate bones.

Fig. 30 Pelvic region and legs of homozygous luxoid animal. Arrow points to hypertrophied area of fibula. Note four toes.

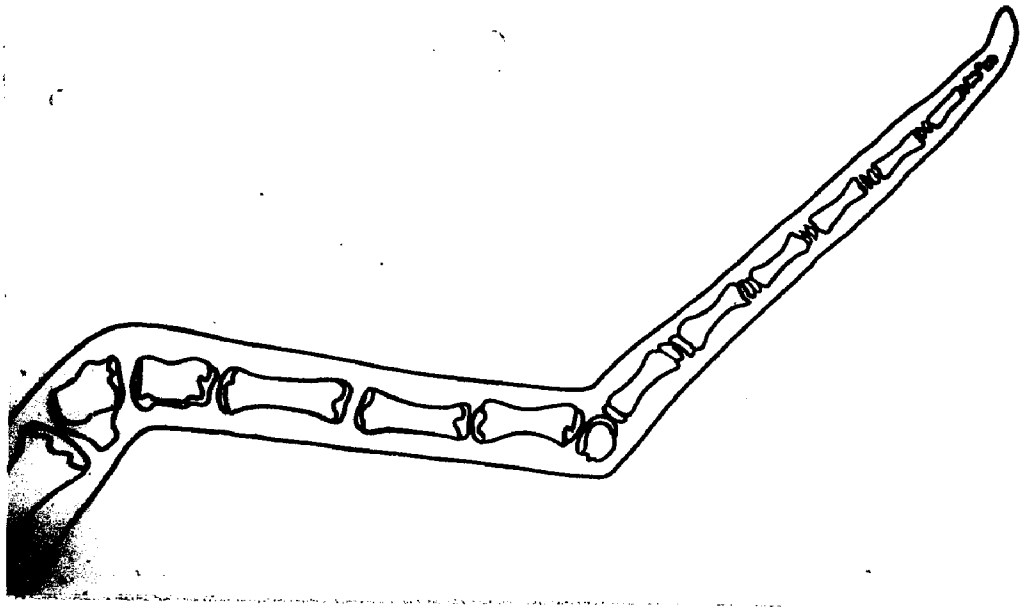

Fig. 31

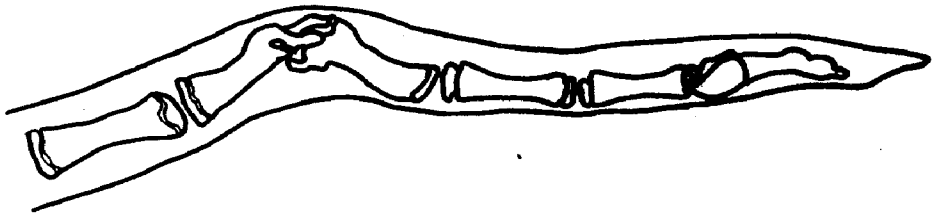

Fig. 32

Fig. 31 Tail of a homozygous luxoid animal showing three kinks.

Fig. 32. Tail of a homozygous luxoidanimal showing two places where vertebrae are fused.

E. Number of presacral vertebrae.

Table 5, based in part on data furnished by Dr. M. C. Green, shows that in luxoid homozygotes the number of presacral vertebrae tends to be increased from 26, the normal number in C57, to 27 and in one case to 28. This effect of luxoid on the number of presacral vertebrae is opposite to the effect of luxate on the same structures. Table 5 shows that the luxate gene introduced on the same C57 background as luxoid causes a reduction in the number of presacral vertebrae to 25. While luxate heterozygotes show some tendency to have a reduced number of presacral vertebrae, no such tendency has been found in luxoid heterozygotes.

F. Number of ribs.

C57 mice normally have 13 pairs of ribs. Table 6, based in part on data furnished by Dr. M. C. Green, shows that luxoid homoxygotes have a strong tendency to have 14 ribs. This effect is not shared by luxoid heterozygotes.

G. Number of sternebrae.

C57 mice normally have 5 sternebrae anterior to the xiphisternum. Table 7, based in part on data furnished by

Dr. M. C. Green, shows that luxoid homozygotes exhibit a slight tendency to have 6 sternebrae anterior to the xiphisternum. This tendency is not shared by luxoid heterozygotes.

Table 5

The effect of the luxate and luxoid genes on the number of presacral vertebrae in C57BL/10 mice.

	25	#a_1	26	#a_2	27	28	Total
*Normal C57	14	28	376	-	-	-	418
**lu/+	-	-	86	-	-	-	86
lu/lu	-	-	10	3	6	1	20
lx/+	4	2	2	-	-	-	8
lx/lx	7	4	1	-	-	-	12

a_1 stands for animals which have one side of the 26th vertebra sacral in form, the other side lumbar in form; a_2 stands for animals which have a similar asymmetrical development on their 27th vertebra.

* Data kindly furnished by Dr. E. L. Green.

** Includes data on 10 heterozygotes furnished by Dr. M. C. Green and data on 76 heterozygotes collected by the author.

Table 6

The effect of the luxate and luxoid genes on the number of ribs in C57BL/10 mice.

	13	14	Total
* Normal C57	417	1	418
** lu/+	86	-	86
lu/lu	9	11	20
lx/+	8	-	8
lx/lx	12	-	12

* Data kindly furnished by Dr. E. L. Green.

** Includes data on 10 heterozygotes furnished by Dr. M. C. Green and data on 76 heterozygotes collected by the author.

Table 7

The effect of the luxate and luxoid genes on the number of sternebrae in C57BL/10 mice.

	4	5	6	Total
Normal C57	7	22	-	29
* lu/+	8	78	-	86
lu/lu	-	16	2	18
lx/+	2	6	-	8
lx/lx	1	11	-	12

* Includes data on 10 heterozygotes furnished by Dr. M. C. Green and data on 76 heterozygotes collected by the author.

H. The Urogenital System.

In one luxoid heterozygous male, extreme hydronephrosis of the left kidney and the bladder was found. Since hydronephrosis is occasionally found in C57 normal animals (oral communication by Dr. M. C. Green), no significance can be attributed to this isolated case. In view however of the fact that luxate has hydronephrosis as one of its effects in one family, the progeny of the exceptional luxoid male will be inspected for further cases.

In eight luxoid heterozygous females, a dorse-ventral vaginal septum was found. The same condition is occasionally found in luxate females (Carter, unpublished; three cases in the Ohio State University mouse colony), and was once found in a C57 female by the author. Very likely the dorso-ventral vaginal septum is an effect of the luxoid gene.

EMBRYOLOGICAL DEVELOPMENT OF C57BL/10 NORMAL AND AND C57BL/10 LUXOID MICE.

In the study of the embryological development of the normal and luxoid C57 mice, Chang's description (1939) of the polydactyly in a strain of mice, Carter's unpublished description of the embryological development of the normal CBA and CBA luxate mice, and Grüneberg's description (1953) of the development of the skeleton of mice of a normal strain and of a congenital hydrocephalus strain were used. In particular, Carter's studies were of very great help.

A. Development of external characteristics.

1. The 10½ day stage.

The normal C57 10½ day embryos ranged in the sample taken from 1.6 to 4.8 mm in length, with a mean of 3.2 mm. All the embryos of this and succeeding stages were measured along their maximum dimension, which thereby gives their crown-rump length. The 10½ day embryo (fig. 33) is coiled in a spiral with the tail curled to the right of the head. Externally, the eye is only a bulge with the lens vesicle visible at the middle. The first and second visceral clefts are open, and the auditory vesicles are visible. The anterior limb buds are merely yet easily visible semicircular projections while the posterior limb buds are but low

swellings of the limb ridge. Somites are easily seen along the length of the embryo from a point slightly anterior to the fore limb buds to a short distance within the tail bud. Figure 33 shows a 10½ day old C57 normal embryo of about 29 somites whose crown-rump length was 4.2 mm.

The C57-lu embryos at the 10½ day stage ranged from 1.8 to 4.7 mm in length with the mean being 3.5 mm. Externally no differences were detectable which would serve to distinguish luxoidhomozygotes or heterozygotes from normal mice of the 10½ day stage.

2. The 11½ day stage.

The normal C57 11½ day embryos ranged from 3.9 to 7.3 mm in length with the mean being 5.9 mm. Externally, the embryo of this stage is less coiled than in the preceding stage. The eye has a ring of pigment, and the nasomaxillary fissure is plainly visible. The forelimb has a round footplate but the posterior limb buds of the early embryos of this stage are only rudders which project rather sharply out from the embryo. In the older embryos (6.5 mm and over) a footplate has begun to differentiate on the posterior limbs. The tail has grown in length.

The C57-lu 11½ day embryos ranged from 4.6 to 7.3 mm with the mean being 5.9 mm. In one of the these embryos, 6.0 mm long, a large bleb of blood was seen at the end of

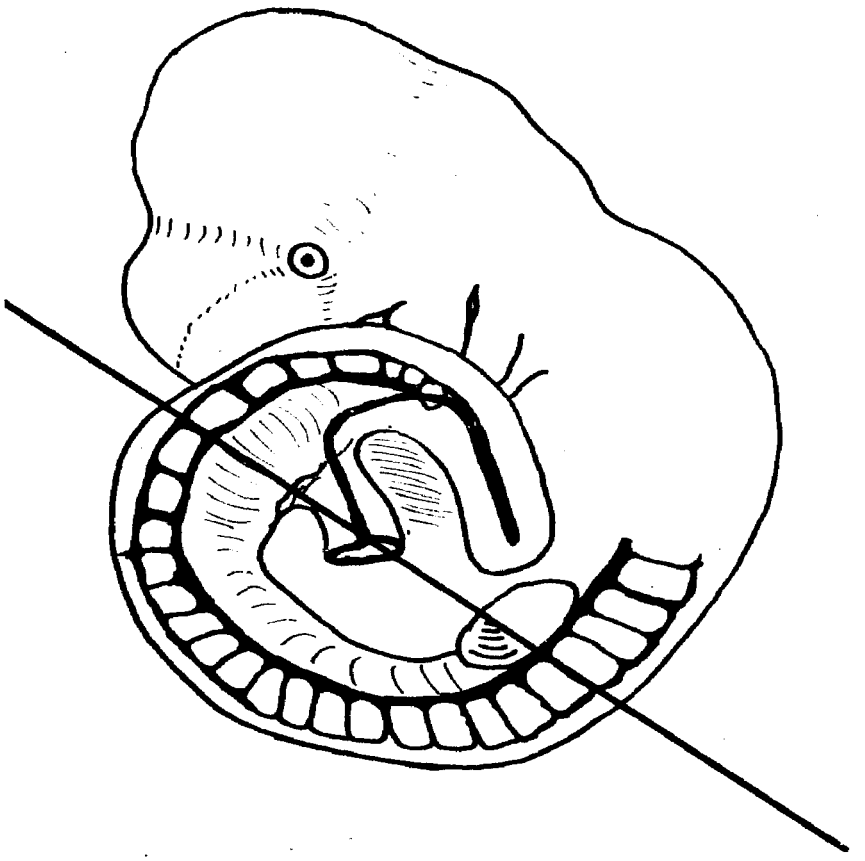

Fig. 33

A 10½ day C57 normal embryo, 4.2 mm in crown-rump length, with about 29 somites. Enlarged about 14X. The line drawn across the hind limb ridge indicates the plane of section employed to secure transverse sections of this region for use in studying the levels of the umbilical roots.

the tail. Since blebs in the tail were found on sectioning in one 10½ day presumptively homozygous luxoid embryo and in an older certainly homozygous luxoid embryo (described below) it is possible that this embryo was a homozygous luxoid animal also. Another embryo (6.6 mm long) had a small bleb of blood in the tip of its tail. In some of the older 11½ day embryos a small projection at the anterior part of the hind limb footplate was seen. These could have been heterozygotes with incipient polydactyly resulting from excessive growth of their foot-plates. They could also have been homozygotes, since homozygotes frequently show polydactyly. In some of the larger 11½ day embryos, one or both of the footplates of the fore limbs had marked anterior projections indicating hypertrophy of the anterior margin. Thus in an embryo of 7.3 mm the right hand was expanded anteriorly. This forward expansion can be taken to prove that the animal is a homozygote, but its absence would not disprove it, since some adult homozygotes have only a slight degree of polydactyly in the hands (figs. 17, 18). No tail abnormalities were seen at this stage except the bleb referred to above. Inspection of umbilical arteries showed them to be equal.

3. The 12½ day stage.

The normal C57 12½ day embryo had a length ranging from 6.6 to 8.4 mm with a mean of 7.4 mm. In the older embryos of this stage, the naso-maxillary fissure is closed, and three rows of whiskers are seen on the upper jaw. The anterior footplate shows the beginnings of fingers while the posterior footplate is circular (fig. 35).

The C57-lu 12½ day embryos ranged from 5.6 mm to 8.0 mm in length with the mean being 6.9 mm. At this stage, embryos were seen with footplates of the forelimbs on one or both sides showing clearly expanded anterior margins or the entire footplate was larger than normal or both features were present simultaneously. The footplates of some of the embryos having these characteristics were not completely round but were rather pointed (fig. 36). One having the enlarged anterior footplate did not have a pointed end to its posterior footplate but instead had a wide footplate with an anterior projection. This embryo probably would have developed into an animal having a foot like that seen in figure 10 or in figure 13. Examination of the umbilical arteries of a putative luxoid homozygote showed that the right umbilical artery was dominant. In the case of a normal-looking embryo, the left umbilical artery was much larger than the right one, while in the case of another normal embryo, both arteries were equal.

4. The 13½ day stage.

The normal C57 13½ day embryos ranged from 9.0 mm to 10.6 mm in length with the mean being at 9.6 mm. At this stage the anterior footplates show obvious indentations but the posterior footplates have only shallow indentations. The embryo has straightened out more and its hind legs now extend out along about a right angle to the body axis and no longer point so markedly at the head as at earlier stages.

The C57-lu 13½ day embryos ranged from 6.4 to 11.3 mm in length with the mean being 9.8 mm. At this stage, it was easy to distinguish homozygous and heterozygous luxoid embryos from their normal sibs. The anterior footplates of homozygotes showed the greater than normal size with anterior projections as in the previous stage, but now one could discern the separate fingers forming and make a count. Thus it was possible to note when the first digit was a s long as the others which indicated a triphalangous thumb forming, as in figure 17. In another case it was possible to see a small preaxial projection over the first digit which digit was equal in length to the succeeding ones. Thus was developing the kind of polydactyly seen in figure 18. In still another case with six fingers, the blastemata of the first two could be seen to be very close together which would lead to syndactyly as seen in figure 21. In another case, six blastemata were seen, but that of the

third finger was much smaller than that of the others.

The feet of the homozygotes showed various anomalies. In one case, the first and second toes were plainly fused, as seen in figure 8. At this stage, the marginal sinus can be easily studied. In the case of the homozygote showing the fusion of the first and second toes, the marginal sinus was everywhere distinct along the edge of the foot. Another homozygote had first toes wider than normal. The marginal sinus in this case was attenuated between the second and third toe on the right foot, and between the second and third, and third and fourth toes on the left foot. In another homozygote, the first and second toes were fused but this time the marginal sinus was a close network rather than an open channel. The feet of this homozygote were pointed rather than rounded, the point occurring at the fourth toe. These feet were a further development of the pointed type noted before in homozygotes of the previous stage. In other homozygotes, the marginal sinus was distinct all around the footplate. At this stage and in succeeding ones, the axis of the hind limbs of <u>luxoid</u> homozygotes instead of being approximately at right angles to the main axis of the embryo is now inclined forward so that the hind feet are clearly closer to the head than in normal mice.

Inspection of the footplates of heterozygotes showed very characteristic forward projections of the footplates. The marginal sinus was faint and in one case broken up into

a network around the projection.

In Carter's study, the right umbilical artery of homozygotes was very much reduced and this reduction was associated with the greater abnormality on the right side of luxate animals bilaterally affected. Therefore the umbilical arteries were examined with particular care in this and succeeding stages. In two homozygotes, the left umbilical artery was dominant, the right being practically absent. In two homozygotes, the right umbilical artery was the dominant artery, the left being practically absent in one. In two homozygotes, both umbilical arteries were equal. In two normal embryos, one had both arteries equal while in the other, the left was larger than the right. In heterozygotes, both umbilical arteries were about equal in size, or the right was larger than the left.

In the case of one homozygote, the tail showed two terminal blebs of blood in the venous system. The tail was sectioned and the results are discussed below.

5. The 14½ day stage.

The normal C57 14½ day embryos ranged from 10.2 to 12.2 mm in length, with the mean being at 11.5 mm. The fingers of embryos at this stage were separate distally but remained webbed proximally. The posterior footplates were deeply indented.

The C57-lu 14½ day embryos ranged from 8.4 to 12.0 mm with the mean being at 10.4 mm. Here the homozygotes and heterozygotes were distinguished from the normals by the more extreme development of the differences previously noted. In the case of one homozygote, seven fingers were noted on one hand. The first and second toes were often represented by one blastema wider than that for one. The heterozygotes showed anterior projections on their feet of varying positions at the anterior margin, indicating various kinds of polydactyly. In one case, the marginal sinus was faint where the extra toe was developing.

Inspection of the umbilical arteries of the homozygotes revealed that in every case the left umbilical artery was reduced relative to the right. In the case of heterozygotes, the umbilical arteries were equal except in three cases where the right was dominant. Normal embryos had equal umbilical arteries.

One homozygote had two kinks in its tail. The tail was sectioned and the results are discussed below.

6. The 15½ day stage.

The normal 15½ day C57 embryos ranged from 11.7 to 13.6 mm in length, with the mean being at 12.6 mm. At this stage, both the fingers and toes are completely separate and diverge widely.

The C57-lu 15½ day embryos ranged from 11.0 to 14.4 mm with the mean being at 13.0 mm. Again diagnosis of the genotypes of the embryos was easy, except for the probable fact that some normal appearing young were in fact heterozygotes. The homozygotes showed clearly the degree of polydactyly in the hands, whether there were only five fingers but with the first as long as the others (putatively triphalangous), or whether there were as many as seven fingers. In one case where there were seven fingers on one hand (the right), the second and third fingers were syndactylous. The forward turning of the hind limbs is more marked with the toes almost touching the fingers. Figure 34 shows for comparison three embryos of 15½ day developmental age, one normal, one heterozygote, and one homozygote. The homozygote shows 6 toes on the right foot, with the first three syndactylous.

Inspection of umbilical arteries of homozygotes showed that the right umbilical artery was the dominant or the only visible artery. In normal and heterozygous embryos the right umbilical artery was dominant.

7. The 16½ day stage.

Embryos in the 16½ and later stages were not all measured, so no measurements will be given here. Among embryos in which luxoid was segregating some had a

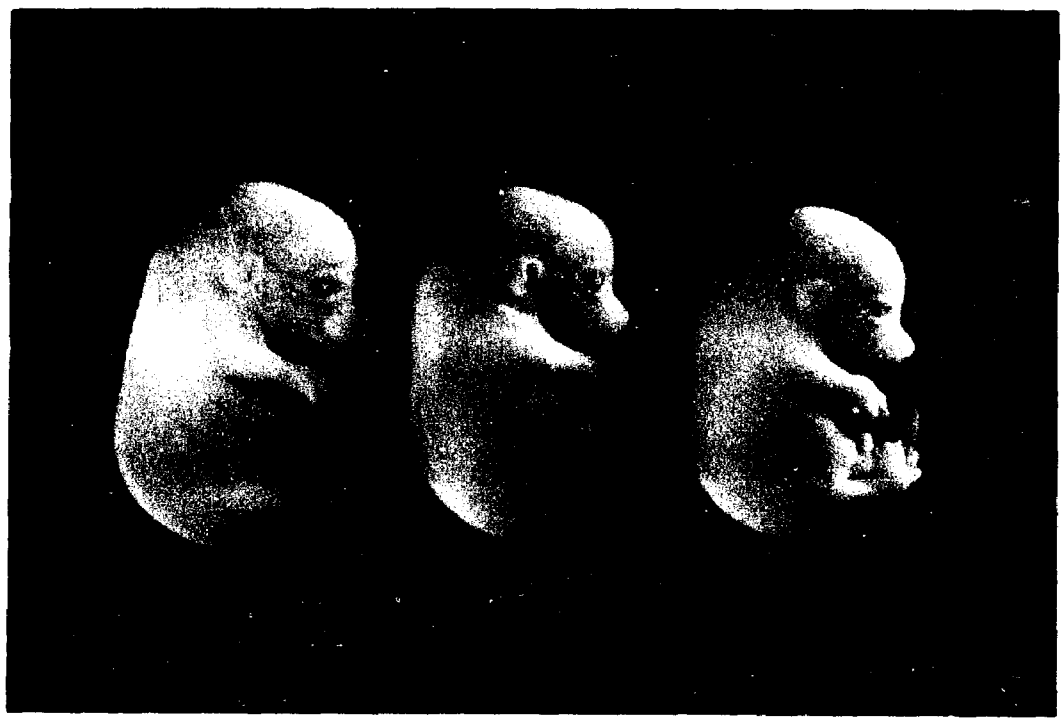

Fig. 34

From left to right, a normal C57, a heterozygous luxoid C57, and a homozygous luxoid C57 embryo. The developmental age of these embryos is 15½ days. Enlarged 3X.

chronological age of 16½ days, but their developmental age, judging by the external criteria suggested by Grüneberg (1943) was really 15½ days. These embryos were capable of motion in the arms when stimulated with a needle. The end of the tail of one was kinked but inspection of the tail when cleared showd no visible abnormality. In general, all the various kinds of polydactyly in the hands and feet were visible. Inspection of the umbilical arteries showed only the left umbilical artery visible or at any rate dominant (larger than the right) in the case of homozygotes. In the case of heterozygotes, the right umbilical artery was dominant in three, the left was dominant in four, while in two the arteries were equal in size. In the case of one normal embryo, the right umbilical artery was the only one visible.

8. The 17½ day stage.

No significant changes were noted among embryos in which luxoid was segregating. No observations on the umbilical arteries were made.

9. The 18½ day stage.

One luxoid homozygote showed only the left umbilical artery. Inspection of heterozygotes showed the right umbilical artery to be dominant in four, while the

left was dominant in a fifth. A normal embryo had the right umbilical artery dominant.

B. Development of the internal characteristics.

Mice of the same chronological age often vary considerably in their degree of development. For this reason, in the following descriptions embryos of the same length as nearly as possible are compared, since length is closely correlated with degree of development. Occasionally embryos are compared with chronological ages a day apart, when of approximately the same length.

1. The 10½ day stage.

A 2.6 mm normal C57 embryo has no somites visible in the tail region. The hind limb bud is merely a ventrolateral swelling of the wall of the body. The mesenchyme in the bud is very dense and the cells show frequent mitoses. The mesenchyme of the bud is covered on the outside with cuboidal ectoderm which has numerous mitoses. No apical ectoderm ridge is visible. There are mitoses in the notochord. No blood spaces are visible in the compact mesenchyme of the limb bud, but a limb artery buds off the umbilical artery on either side and enters the limb bud. The forelimb bud is also composed of dense mesenchyme,

having numerous mitoses. The bud is a pronounced swelling out from the body and has an apical ectoderm ridge present. This ridge is a three to four-layered thickened belt of the ectoderm which runs along the ventro-lateral edge of the bud. Elsewhere the bud is covered by cuboidal ectoderm cells whose nuclei are large and fill up most of the tissue. Mitoses are visible in the apical ectoderm. A large subclavian artery comes off the dorsal aorta and enters the bud.

A 3.1 mm normal C57 embryo shows a few changes from the preceding stage. Somites are not in the tip of the tail but have formed somewhat beyond where the umbilical arteries bud off the dorsal aorta. Little spaces begin to appear in the dense limb mesenchyme which contain blood cells and are blood sinuses. The posterior cardinal veins are now easy to see; they drain the limb swellings. No apical ectoderm ridge has yet formed. As in the preceding stage, the umbilical arteries descending from the dorsal aorta are prominent structures. They pass down on either side of the gut, but are medial to the incipient metanephros and the well-formed Wolffian ducts. About where they descend, the coelom leaves off. The forelimb buds are much as before. The apical ectoderm ridge appears thicker.

A 4.0 mm normal C57 embryo has some changes. The somites are now well into the tail. There are many blood spaces in the mesenchyme. The new lateral umbilical

arteries are beginning to form, since blood spaces are seen along their future course around the Wolffian ducts and the metanephroi. The apical ectodermal ridge is beginning to form. In the forelimb the apical ectoderm ridge is very well established. Mitoses are common in it, as in the dense mesenchyme filling the bud. Nerves from the spinal cord are just outside the bud. Blood enters via the large subclavian artery and is distributed by large branches in the bud. The blood drains by the postcardinal veins.

A 4.8 mm normal C57 embryo has somites well into the tail. The hind limb swellings now are buds which project out from the axis of the embryo. The apical ectoderm of the hind limb buds is very well formed on the ventrolateral aspect of the bud. Abundant mitoses are visible in it, as well as in the compact mesenchyme tissue of the bud. The mesonephric (Wolffian) duct and the metanephros are easily found. The lateral umbilical artery routes are just completed and are still small. The primitive medial routes still carry the main load. No marginal sinuses are established as yet. In the forelimb, abundant mitoses are visible in the mesenchyme of the bud. The apical ectoderm is well established and mitoses are common in it. The nerves are just entering the limb bud and the marginal sinus is formed below part of the apical ectoderm.

Turning now to C57-lu embryos of about the same sizes, we find no great differences except in the case of one embryo of 4.0 mm. Here the arteries in the tail bud are

engorged with solid masses of blood cells; the outline of the wall of the vessels is not clear. From evidence to be presented later on, it is very probable that this embryo was a homozygous luxoid. The condition of the vessels in the tail of this mouse is likely an early stage of the large vascular blebs seen later and detectable externally.

2. The 11½ day stage.

a. The forelimb.

In a normal 6.7 mm C57 embryo, we see a number of condensations of mesenchyme forming. Those of the humerus, radius, ulna, and the second, third, and fourth metacarpals are quite definite. Numerous mitoses are seen in the mesenchyme. The apical ectoderm is present around the hand-plate and the marginal sinus is conspicuous below. The subclavian artery is present between the condensations for the radius and ulna and has numerous branches. Two large nerve trunks pass into the forelimb as far as the distal parts of the radius and ulna condensations.

In a 7.3 mm C57-lu embryo diagnosed externally to be a luxoid homozygote by virtue of a flaring forward of the anterior margin of its forelimb footplate, the developmental picture is about the same as above. Nerves appear to about reach three metacarpal condensations. The anterior part of

the footplate is still solid mesenchyme but it is more extensive than in the normal and therfore projects forward. Apical ectoderm is prominent around this forward extension.

b. The hind limb.

In a normal 6.6 mm C57 embryo, the footplate of the hind limb is round (fig. 35). The mesenchyme does not have any condensations, but mitoses are common in it. The central area of the footplate is thinner than the area below the apical ectoderm. The apical ectoderm is present completely around the footplate and the marginal sinus is plain below. A main axial artery is present; its root courses anterad from its source at the rear of the lateral umbilical artery before following the axis of the limb. Nerves are seen in the leg but not in the footplate.

In a 6.8 mm C57-lu embryo diagnosed to be a luxoid homozygote by virtue of its distinctive footplate in the forelimb has 2, perhaps 3, condensations present in the posterior part of the footplate of the hind limb. These represent metatarsals. The condensations of the tibia and fibula are not very distinct. In another luxoid homozygote of the same length, the same three metatarsal condensations are seen, and again the condensations of the tibia and fibula are not very distinct. The marginal sinus is very well developed below the apical ectoderm. The footplate is not so round as in the normal embryos, but is more pointed (fig.

Fig. 35

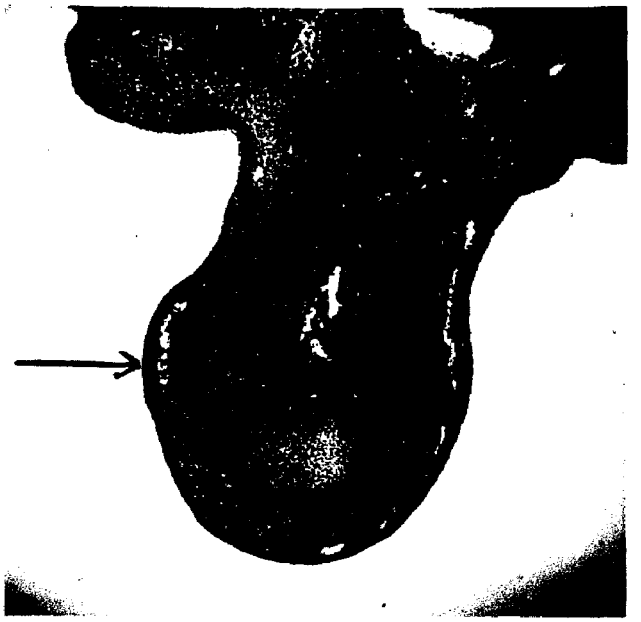

Fig. 36

Fig. 37

Fig. 38

Fig. 35 Left leg of a 6.6 mm normal C57 embryo. Note rounded footplate. 42X.

Fig. 36 Left leg of a 6.8 mm homozygous luxoid C57 embryo. Note pointed footplate. Arrow points to apical ectoderm and marginal sinus below of anterior projection. 42X.

Fig. 37 Left hand of 10.6 mm normal C57 embryo. Arrow points to short blastema of the thumb. 42X.

Fig. 38 Right hand of 9.6 mm homozygous luxoid C57 embryo, showing blastemata for six finger. Arrow points to first finger as long as the others. 42X.

36). There is more of an anterior projection than in normal embryos. This indicates incipient polydactyly.

3. The 12½ day stage.

a. The forelimb.

In a normal 8.4 mm C57 embryo the blastemata of the scapula, radius, and ulna are very clear. There is a stainable matrix already forming about them, the prelude to cartilage formation. The blastemata of the first, second, third, fourth, and fifth metacarpals are plain. The handplate is slightly indented between the digital blastemata. Condensations for some carpal bones seem to be present but are hard to distinguish. The apical ectoderm is present and the marginal sinus lies in a continuous channel below.

In a 8.2 mm C57-lu embryo diagnosed to be a luxoid homozygote by virtue of its distinctive footplate on its forelimb, we see clearly six condensations in the mesenchyme for six metacarpals. There are visible also condensations for the radius and ulna, which have stainable matrix. The anterior margin of the handplate projects forward and indentations are present between the finger condensations. The apical ectoderm is present and the marginal sinus also. The nerves end about at the metacarpals.

b. The hind limb.

In a 8.4 mm normal C57 embryo we see five mesenchymal condensations present in the footplate of the hind limb. These represent the five metatarsals. Condensations for the tibia, fibula, femur, and pelvic bone are present. The marginal sinus is very well developed below the apical ectoderm. Nerves are in the leg but are not present in the footplate.

In a 8.2 mm C57-lu embryo diagnosed as a homozygote by the distinctive footplate of the forelimb, we see condensations for the pelvic bones, the femur, tibia, and fibula. There are five condensations in the footplate for the metatarsals. There is a projection forward on the footplate with a small blastema within it. Nerves have grown up to the footplate but have not entered it.

4. The 13½ day stage.

a. The forelimb.

In a normal 10.6 mm C57 embryo, we find cartilage already formed in the scapula, humerus, and ulna, but only pre-cartilage in the radius. Carpal blastemata are forming, of which that for the hamate bone is most distinct. Blastemata for all the five fingers (metacarpals) are present but

that for the first metacarpal is shorter than those for the others (fig. 37). The indentations are more pronounced between the digits. The marginal sinus is still present below the apical ectoderm. Muscles are forming about the humerus and the radius and ulna. Nerves now reach the metacarpals and blood vessels lie between the digit condensations.

In a 9.6 mm C57-lu embryo diagnosed as a luxoid homozygote, we find six blastemata for six metacarpals present but the first is of the same length and size as the rest, except for the sixth which is smaller (fig. 38). This kind of hand will become the adult type shown in figure 20. Cartilage is present in the humerus, radius, and ulna. The apical ectoderm is still present and the marginal sinus is a good channel below the preaxial digits. Condensations for carpal bones are present but for which ones is not clear. Nerves enter the area occupied by the metacarpals.

b. The hind limb.

In a 9.9 normal C57 embryo, we see five blastemata in the footplate for the five metatarsals (fig. 39). The front end of the footplate is somewhat square, and has no projection. There are indentations between the toes. Blastemata for the tibia and fibula are present, but that for the tibia is much larger than that for the fibula.

There is present stainable matrix in both. Blastemata for the femur, and for various tarsal bones are present. That for the calcaneus appears larger than that for the talus. The marginal sinus is present and is very well developed in the region of the first digit. The apical ectoderm is present and nerves are in the region of the metatarsals.

In a 9.9 mm C57-lu homozygous embryo we see that the condensation for the tibia is shorter and smaller than that for the fibula (fig. 40). There is stainable matrix present in the femur, tibia, and fibula. The first toe is represented by a very short blastema while those of the other four toes are normal in length. This appears to be a form of ectrodactyly. Condensations for the tarsal bones are present. The nerves are in the region of the metatarsals.

In a 9.6 mm C57-lu homozygous embryo the blastemata of the tibia and fibula are about equal, and contain stainable matrix (fig. 41). Such tibial and fibular blastemata would lead to the type of leg seen in many adult luxoid homozygotes (fig. 24). Blastemata with stainable matrix for the five metatarsals are present. Those for the first and second are closer together than the rest, which would lead to the syndactyly of the first two toes, as seen in figure 8. The footplate flares forward; the well defined marginal sinus is an open channel around this flaring anterior margin.

In a 9.5 mm C57-lu heterozygous luxoid embryo we see

in the leg condensations for the tibia, fibula, and femur with stainable matrices present. Tarsal condensations are visible. At the anterior end of the footplate, there is a large forward projection. In this projection there is a long blastema which would become a first toe. Coming off the base of the long blastema laterally is a much shorter blastema. This would become a reduced second toe. Four normal blastemata follow in the rest of the footplate. Here is the beginning of a foot like that possessed by the heterozygote of figure 5. The marginal sinus on the medial side of the projection is broken up into a network.

5. The 14½ day stage.

a. The forelimb.

In a 10.2 mm normal C57 embryo, we find cartilage in the humerus, ulna, and radius. Perichrondium is condensing about the cartilage. There is cartilage in the second, third, fourth, and fifth metacarpals. Blastemata for the first metacarpal, and for the navicular-lunate, triangular, centrale, and hamate are present. That for the first metacarpal has a stainable matrix while the others have cartilage present. Condensations for the first phalanges (those just distal to the metacarpals) are forming. Nerves lie along the phalangeal condensations.

Fig. 39

Fig. 40

Fig. 41

Fig. 42

Fig. 39 Left leg of a 9.9 mm normal C57 embryo. Arrow points to normal-sized tibial blastema. 42X

Fig. 40 Left leg of a 9.9 mm homozygous luxoid C57 embryo. Arrow po nts to much reduced tibial blastema. 42X

Fig. 41 Left leg of a 9.6 mm homozygous luxoid C57 embryo. Arrow points to a tibial blastema somewhat smaller than the fibular blastema. 42X

Fig. 42 Tail of 10.0 mm C57 homozygous luxoid mouse. Arrows point to two connected blebs of blood. 42X

In a 10.0 mm C57-lu homozygous embryo, we find five blastemata present in the hand-plate, but the first one is as long as those of the others. The eventual result might be an adult hand as in figure 17. In this case, the blastemata do not appear to possess stainable matrix in the metacarpals, but in an embryo of the same length and in one shorter (9.7 mm) such is seen. Carpal blastemata are present. Cartilage is forming in the scapula, humerus, ulna, and pre-cartilage is present in the radius. Muscles are forming in the arm and nerves have reached the metacarpals. The apical ectoderm and marginal sinus are still present.

In another homozygous luxoid embryo of 10.0 mm, we find six blastemata in the hand-plate, the first as big as the other large ones. The second and third blastemata (metacarpals 2 and 3) are fused at the base. In the same embryo, two blebs of blood are seen at the end of the tail (fig. 42). They are connected and are in the venous system. There is some distortion of adjacent somites.

b. The hind limb.

In a 11.8 mm normal C57 embryo, we see cartilage well formed in the femur, tibia, and fibula. That for the tibia is much larger than the one for the fibula. Precartilage is forming in the second, third, and fourth metatarsals, also in the first phalanges of these metatarsals.

The blastema for the first metatarsal is distinctly shorter than that for the other toes. The apical ectoderm is still present, and the marginal sinus is regressing. Blastemata for all the tarsal bones are present; those for the calcaneus and talus have cartilage.

In a 10.8 mm C57-lu heterozygous luxoid animal, the first toe is longer than the others. This long first toe would probably become in adult condition triphalangous. The tibia and fibula are in cartilage; the tibia is much larger than the fibula. The marginal sinus is present, and has a fairly clear channel around the long first toe. Precartilage is present in the talus and calcaneus.

In another C57-lu heterozygous embryo of 11.1 mm, there are present six blastemata with dense stainable matrix in the footplate. The first blastema is short while the second is longer than the third. Cartilage is present in the tibia, fibula, talus, and calcaneus. The apical ectoderm is still present.

In a 9.7 mm C57-lu homozygous luxoid animal, cartilage in the femur, tibia, and fibula is found. Cartilage is forming in the second, third, and fourth metatarsals. Dense blastemata are seen in the first and fifth metatarsals. That for the first metatarsal has a medial projection on it. This extra condensation will lead to a vestigial toe. The marginal sinus above this condensation is a network and not a clear channel. Condensations are present for tarsal

bones and cartilage is present in those for talus and calcaneus. These two structures appear about equal in size. The cartilage of the tibia is considerably larger than that of the fibula, but when compared to normal relations, it is not as much greater as it would be if normal.

In a 9.7 mm C57-lu homozygous luxoid embryo, which externally appeared to have kinks in its tail, the somites toward the tip of the tail are seen to be distorted (fig. 46). Three appear to be partly fused at the ends.

6. The 15½ day stage.

a. The forelimb.

In a 12.8 mm normal C57 embryo, there is cartilage in the scapula, humerus, ulna, and radius. The radius is thicker than the ulna. Cartilage is present in carpal bones (triangular, navicular-lunate, hamate, capitate, lesser multangular apparently fused with the centrale, the greater multangular). The falciform is still a mesenchymal blastema. Cartilage is present in all five metacarpals, and in the first phalanges of the last four fingers (fig. 43). The phalanx of the first finger has stainable matrix in its mesenchymal blastema.

In a 13.5 mm C57-lu homozygous luxoid embryo, we see cartilage in the scapula, humerus, ulna, and radius. The triangular and the navicular-lunate share one long blastema in which cartilage cells are beginning to differentiate. Cartilage is just beginning to form in the hamate, capitate, centrale fused with the lesser multangular, greater mulatangular. All five metacarpals are in cartilage. That for the second metacarpal shows doubling at the distal end (fig. 44). On each head of this second metacarpal is seen a phalanx. Cartilage is just forming in the first phalanx of all six fingers. On the first finger is seen a small medial projection containing only mesenchyme with a small condensation (fig. 45). Therefore this hand is a partly seven-fingered hand.

b. The hind limb.

In a 13.6 mm normal C57 embryo we find the femur, tibia, and fibula in cartilage. The anlage of the tibia is much larger than that of the fibula (fig. 47). The calcaneus and talus are in cartilage; the calcaneus is bigger than the talus. Cartilage is just beginning to form in the cuboid, the navicular, and the three cuneiforms. The navicular and the third cuneiform appear separate. The metatarsals of the last four toes are in cartilage, while cartilage is just beginning to form in the first metatarsal. The first

Fig. 43

Fig. 44

Fig. 45

Fig. 46

Fig. 43 Right hand of 12.8 mm normal C57 mouse. Arrow points to short thumb. 42X.

Fig. 44 Right hand of 13.5 mm C57 homozygous luxoid mouse. Arrow points to doubling of second metacarpal. 42X.

Fig. 45 Right hand of same mouse as in fig. 44. Arrow points to a vestigial first finger, making a total of seven fingers. 42X.

Fig. 46 Tail of 9.7 mm C57 homozygous luxoid mouse. Arrow points to distorted somites. 42X.

phalanges of all five metatarsals are beginning to condense in the mesenchyme and appear to have already stainable matrix (fig. 51). Cartilage is just forming in the phalanx of the second metatarsal.

In a 13.8 mm C57-lu heterozygous luxoid embryo, the cartilage anlage of the tibia is much larger than that of the fibula. There are six toes present. The first toe is smaller than the second and is syndactylous with it. The small toe contains only a dense condensation with a stainable matrix. The larger (second) toe has cartilage forming in its metatarsal.

In a 13.4 mm C57-lu homozygous luxoid embryo, we find the femur, tibia, and fibula in cartilage. The cartilage anlage of the tibia is smaller than that of the fibula, the reversal of the normal condition (fig. 48). There is cartilage in the cuboid, cuneiforms, and navicular. The first and second cuneiforms are completely fused while the navicular is partly fused with them. The talus is almost as big as the calcaneus and is similar in shape to it (fig. 49). Both are in cartilage. The first phalanx of all the toes is laid down in dense blastemata with stainable matrix. The first two toes are syndactylous (fig. 52). The muscles are forming, and nerves are running to the phalanges.

In a 13.6 mm C57-lu heterozygous embryo there is cartilage in the femur, tibia, and fibula. The tibia is much larger than the fibula. Cartilage is present in the

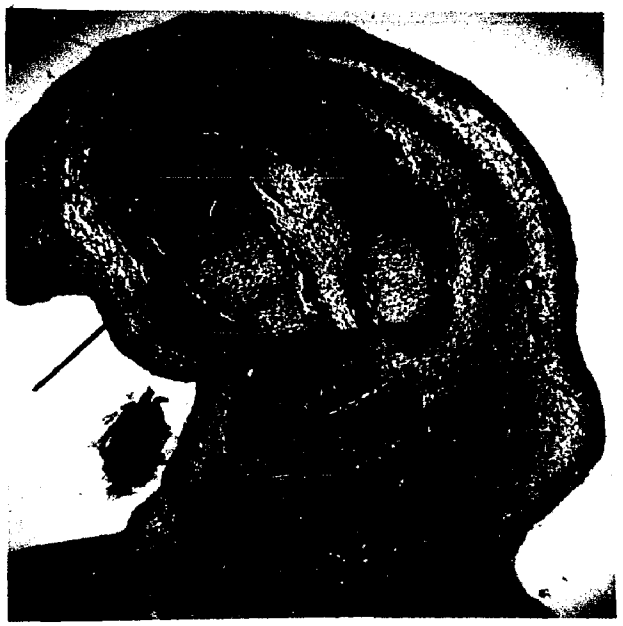

Fig. 47

Fig. 48

Fig. 49

Fig. 50

Fig. 47 Left leg of a 13.6 mm normal C57 embryo. Arrow points to normal-sized tibia. 42X.

Fig. 48 Left leg of a 13.4 mm homozygous luxoid C57 embryo. Arrow points to reduced tibia. 42X.

Fig. 49 Left leg of 13.4 mm homozygous luxoid C57 embryo. Arrow points to talus similar in shape to the calcaneus. 42X.

Fig. 50 Left leg of a 13.6 mm heterozygous luxoid C57 embryo. Arrows point to extra blastemata on first toe. 42X.

talux, calcaneus, navicular, cuboid, and the three cuneiforms. The cartilage anlage of the calcaneus is larger than that of the talus. There is cartilage in the five metatarsals. The first toe has two projections, one on either side (fig. 50). These contain blastemata with precartilage. Muscles are forming in the leg and foot. Nerves are entering the muscles of the foot.

7. The 16½ day stage.

Embryos of this stage were not sectioned but were stained with methylene blue and cleared in methyl salicylate, to show the cartilage. The cartilage appeared stained a deep blue and bone appeared colorless.

In a normal C57 embryo 16½ days old ossification was well advanced in the middle of the long bones (the humerus, radius, ulna, femur, tibia, and fibula). Bone appeared in the middle of the scapula, in the middle of the second, third, and fourth metatarsals.

In a 16½ day C57-lu heterozygous embryo, which developmentally, judging by the external criteria of Grüneberg, was about 15½ days old, bone was just beginning to form in the middle of the long bones. In a similar homozygous luxoid embryo, bone was also just beginning to form in the middle of the long bones. The patella in cartilage was present on both femurs, but the femurs did not show the

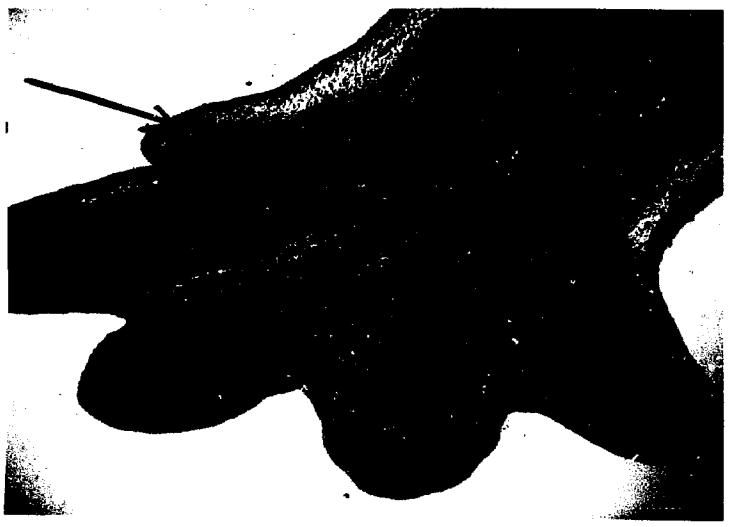

Fig. 51

Fig. 52

Fig. 51 Left leg of a 13.6 mm normal C57 embryo. Arrow points to blastema for short hallux. 42X.

Fig. 52 Left leg of a 13.4 mm homozygous luxoid embryo. Arrow points to syndactylous first and second toes. 42X.

normal condyles for the knee joint. No tibiale was present in the ankle, and the cartilage of the new plantar bone was visible. In another homozygous luxoid embryo, ossification had begun in the middle of the tibia and fibula on the left side, but on the right side where the tibia was much shorter and smaller than the fibula, ossification had not commenced in the tibia but was begun in the fibula. There was no patella present at the right knee, but one was present at the left knee. The femurs on both sides lacked the normal condyles.

8. The 17½ day stage.

The osseus skeleton of this and the next stage were stained with alizarin and cleared in glycerine.

No important changes were noted from the preceding stage, except that in a luxoid homozygote there was not as much bone formed as in its sibs in the long bones, especially in its hind limbs.

9. The 18½ day stage.

In a normal C57 embryo of this stage, the ossification in the long bones had continued. Ossification was now seen in the second to fifth metacarpals and in the first phalanges of the third and fourth fingers. Ossification had

occurred in the second to fifth metatarsals, and in only the first few vertebrae of the tail.

In a heterozygous C57-lu embryo of the same stage, ossification had also progressed in the long bones. In the polydactylous right foot with six toes, all six metatarsals showed good ossification. In the polydactylous left foot with seven toes (the first three syndactylous), only the posterior five metatarsals showd ossification. In the hands, the second to fifth metacarpals showed ossification. Only the first few vertebrae of the tail were ossified in part.

In homozygous luxoid embryos of the same stage, some differences were noted. Ossification had progressed in the long bones. In one case of a six-fingered left hand, the five posterior metacarpals were ossified while all six were ossified in the six fingered right hand of the same specimen. In this same right hand, ossification had begun in the first phalanges of the second to fifth fingers. Ossification had occurred in the second to fifth metatarsals of the feet. The ossification in tibia and fibula was equally advanced. In the six-toed right foot of another specimen, the first, third, fourth, and fifth metatarsals were ossified, whereas that for the second and sixth were still in cartilage.

10. Newly-born stage.

Four luxoid homozygotes which had kinks in their tails were stained with methylene blue and cleared in methyl salicylate. Toward the end of the tail in one specimen, provertebrae not yet staining with the methylene blue showed unequal lengths and were aligned in zigzag fashion. Cartilage a little craniad of this place was forming in a zigzag pattern and one cartilage vertebra was forming a trifle out of line. This vertebra could become the bony vertebra which is dislocated in the adult. Somewhat similar anomalies were found in two of the other specimens.

11. One-week old stage.

In a luxoid homozygote with definite kinks in its tail, stained with methylene blue and cleared in methyl salicylate, the tail had in one region two adjacent vertebrae with their opposing cartilaginous ends reduced. The tail was kinked at this point.

THE PROBLEM OF THE PRIMARY EFFECT OF THE LUXOID GENE.

Carter in his study of luxate mice found a craniad shift of the hind limb region as the earliest detectable effect of the luxate gene. He ascribed the fairly frequent polydactyly in the heterozygotes and the occasional reduction in the number of presacral vertebrae found in them to a craniad shift of the hind limb field of the order of one-third of a somite, which shift gave rise to imperfect morphogenetic control of the anterior end of the hind limb field. He attributed the reduction or loss of the tibia and the frequent reduction in number of presacral vertebrae found in homozygotes to a craniad shift of the hind limb region of the order of about one somite, which gave rise to loss of the anterior end of the hind limb field. Thus the primary effect of the luxate gene would be craniad shift of the hind limb inductor relative to a supposed limited region of limb potency.

In the present study of luxoid mice, the possibility of a caudad shift of the hind limb field was investigated. Such a caudad shift might be used to explain the increase in number of presacral vertebrae found in the homozygotes and possibly the limb defects in both homozygotes and heterozygotes.

The method adopted was essentially that used by Carter in his study of the similar problem in luxate mice. In

mice of 10½ days from each branch of the dorsal aorta (double in the hind limb and tail region) a root of the umbilical artery can be traced downward. The right and left roots pass around the gut but are medial to the Wolffian ducts and the metanephroi. By 11½ days these primitive medial roots are supplanted by roots running laterally to the Wolffian ducts and metanephroi, but the medial roots remain, though much reduced, and mark the posterior limit of the coelom. When the hind limb buds are well developed, they occupy a constant relation with the posterior limit of the coelom. Therefore we have this set of relationships: the posterior limit of the coelom marks the position of the hind limbs, and the primitive medial roots of the umbilical arteries mark the posterior limit of the coelom. Therefore we can use the position of the primitive medial roots to serve as an indirect marker of the position of the hind limbs. This fact is of value when we study a possible shift in the level of the hind limb region in the 10½ day embryos where the hind limbs are represented only by rather indefinite swellings of the limb ridge (fig. 53).

On graph paper, the position of each umbilical root was plotted with respect to the segmental distance (fig. 53). For the position of each umbilical artery root, the most posterior point on its anterior wall was taken. It was next necessary to observe in which segment the particular roots lay. Along the dorsal aorta small paired intersegmental arteries arise and enter the somites. These

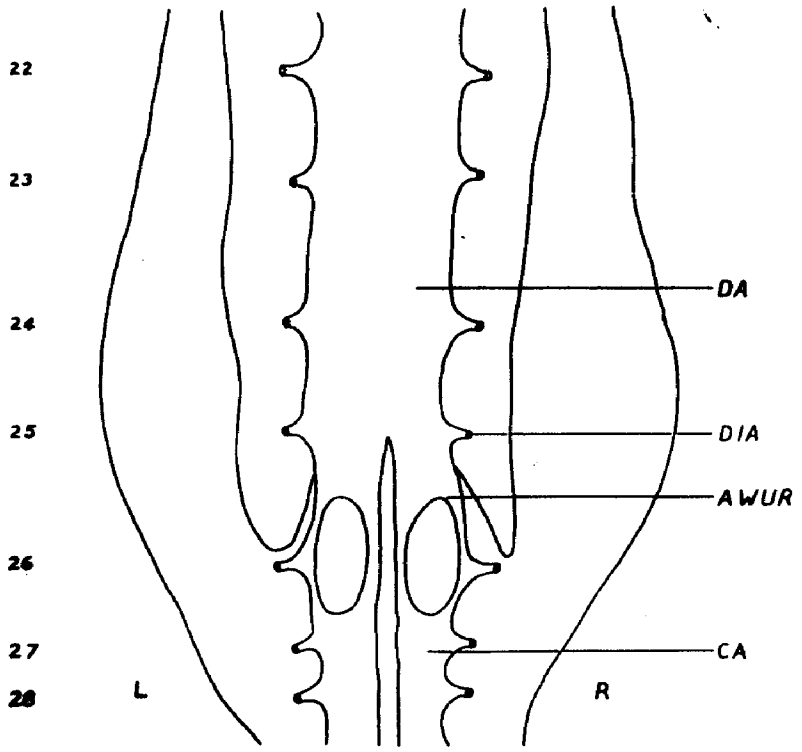

Fig. 53

The hind limb region of a 10½ day 3.4 mm normal C57 embryo, based on a dorsal reconstruction. Enlarged 110X.

DA, the dorsal aorta.

DIA, a dorsal intersegmental artery.

AWUR, the most posterior point on the anterior wall of the right umbilical artery root.

CA, right branch of the caudal artery.

therefore can be used to define levels in the embryo if one knows the serial location of any one pair. The intersegmental arteries associated with the large subclavian artery (fig. 54) were assigned the number seven, since in man (Hamilton, Boyd, and Mossman, 1945) the seventh cervical pair of intersegmental arteries is associated with the subclavian artery. Counting back from this seventh intersegmental artery in the serially sectioned embryos, it was fairly easy to find between what intersegmental arteries the primitive medial umbilical roots descended. Thus in a particular case (fig. 53), the umbilical roots were found between the 25th and 26th intersegmental arteries.

By this method, the amount of variation in level of the umbilical roots was found in a sample of 20 10½ day C57 embryos. Since the sample included embryos ranging from 3.0 to 4.4 mm with varying segmental lengths, all the segmental lengths were equated and the relative levels of the umbilical roots calculated for each side of the embryo. Table 8 contains the data for the 20 C57 embryos, with the mean umbilical level calculated for each embryo and also the grand mean for all the embryos.

Next the levels of the right and left umbilical roots were plotted for each embryo in the sample on graph paper with axes determined by the successive segmental levels. The left diagram of figure 55 gives the results. One point is seen for each embryo; its position on the graph is

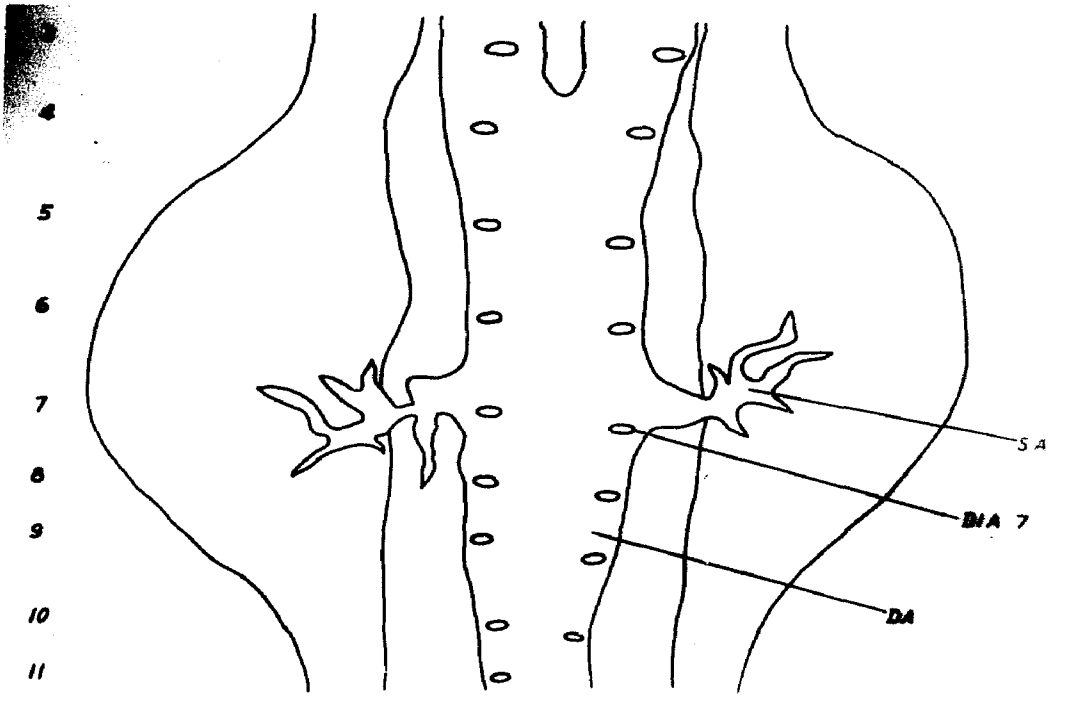

Fig. 54

The forelimb region of a 10½ day 3.4 mm normal C57 embryo, based on a dorsal reconstruction. Enlarged 110X.

SA, subclavian artery.
DIA 7, the seventh dorsal intersegmental artery.
DA, the dorsal aorta.

Table 8

The levels of right and left umbilical roots with respect to the segmental levels in a sample of 20 C57BL/10 embryos. Each embryo is identified by its serial number. The mean level for each embryo is given in the column at the extreme right.

Embryo number	Right	Left	Average
5-2	25.935	25.525	25.730
5-3	25.235	25.708	25.472
5-6	24.867	24.747	24.807
11-1	25.900	25.700	25.800
11-2	24.812	24.139	24.476
11-3	25.512	25.512	25.512
11-5	25.292	25.143	25.218
11-6	25.686	25.537	25.612
11-7	25.364	25.364	25.364
11-8	25.800	25.729	25.765
12-1	25.750	25.250	25.500
12-2	24.907	24.562	24.735
12-4	25.651	25.465	25.558
12-8	25.081	25.095	25.088
39-1	25.472	25.304	25.388
39-2	24.348	24.596	24.472
39-4	25.532	25.481	25.507
39-5	25.753	25.753	25.753
39-6	24.585	24.610	24.596
39-7	24.826	24.872	24.849
Grand mean			25.260

Table 9

The levels of right and left umbilical roots with respect to the segmental levels in a sample of 20 C57BL/10-lu embryos. Each embryo is identified by its serial number. The mean level for each embryo is given in the column at the extreme right.

Embryo number	Right	Left	Average
25-2	24.195	24.471	24.333
25-3	24.756	24.744	24.750
25-5	25.288	25.346	25.317
25-7	24.914	24.671	24.793
25-8	24.780	24.735	24.758
25-9	25.607	25.656	25.632
32-2	26.771	26.531	26.651
32-3	25.176	25.848	25.512
32-4	26.381	26.103	26.242
32-5	25.471	25.507	25.489
32-6	26.771	26.531	26.651
32-7	24.627	24.494	24.561
32-8	24.493	24.356	24.425
36-3	27.060	27.175	27.118
36-6	25.838	25.705	25.772
36-8	25.582	25.629	25.606
37-3	25.611	25.468	25.540
37-5	25.854	25.740	25.797
37-6	25.566	25.845	25.706
27-7	27.308	27.357	27.333
Grand mean			25.613

determined by the level of the right and left umbilical roots. The distance of the point along the heavy vertical line gives the mean umbilical level of the embryo. The gap in the line shows the range in the entire distribution of levels.

The same method was applied to a sample of 20 C57-lu embryos, offspring of matings between luxoid heterozygotes. Table 9 gives the levels of their right and left umbilical roots, the mean umbilical level of each embryo, and the grand mean umbilical level for all the embryos. The right diagram of figure 55 is a copy of the levels of the roots as plotted on graph paper. Again, distance along the heavy vertical line gives the mean umbilical level of any particular embryo and the range of the distribution is shown by the gap in the line.

Examination of tables 8, 9, and figure 55 shows that the grand mean umbilical level of the C57-lu embryos, 25.613 segments, is 0.353 segments farther caudad than the grand mean umbilical level of the normal C57 embryos, 25.260 segments. This caudad shift of the grand mean umbilical level is entirely due to the five C57-lu embryos whose mean umbilical levels lie caudad to those of all the 20 C57 embryos. This is seen when these extreme deviants are removed from the C57-lu distribution and the grand mean umbilical level calculated for the remaining fifteen embryos. The mean of the truncated C57-lu distribution turns out to be 25.199,

even a little less than the grand mean umbilical level for the 20 C57 embryos, 25.260.

Before going any further with the examination of the data, the question must be posed as to whether the two distributions, that of the C57 and that of the C57-lu embryos, are in reality different or whether they are in fact homogeneous distributions from the same population and the five extreme deviants found in the C57-lu sample reflect only sampling error and are explicable by chance alone. To answer this question precisely, Fisher's exact method for double dichotomies is available (Fisher, 1944). Table 10 shows the data arranged for the use of the method. The data are divided first as to whether they are from C57 or from C57-lu embryos. They are further divided according to whether the particular mean umbilical level is cranial to the 26th intersegmental level or caudad to this point. The probability of getting the observed split in the sample distributions when in fact they are from the same population is given by the expression:

$$P = \frac{20!\ 20!\ 5!\ 35!}{5!\ 15!\ 0!\ 20!\ 40!}$$

This probability is equal to 0.0236. Therefore the probability of getting the observed split in the sample distributions when in fact they are from the same population is only about one in fifty. We can conclude that the two

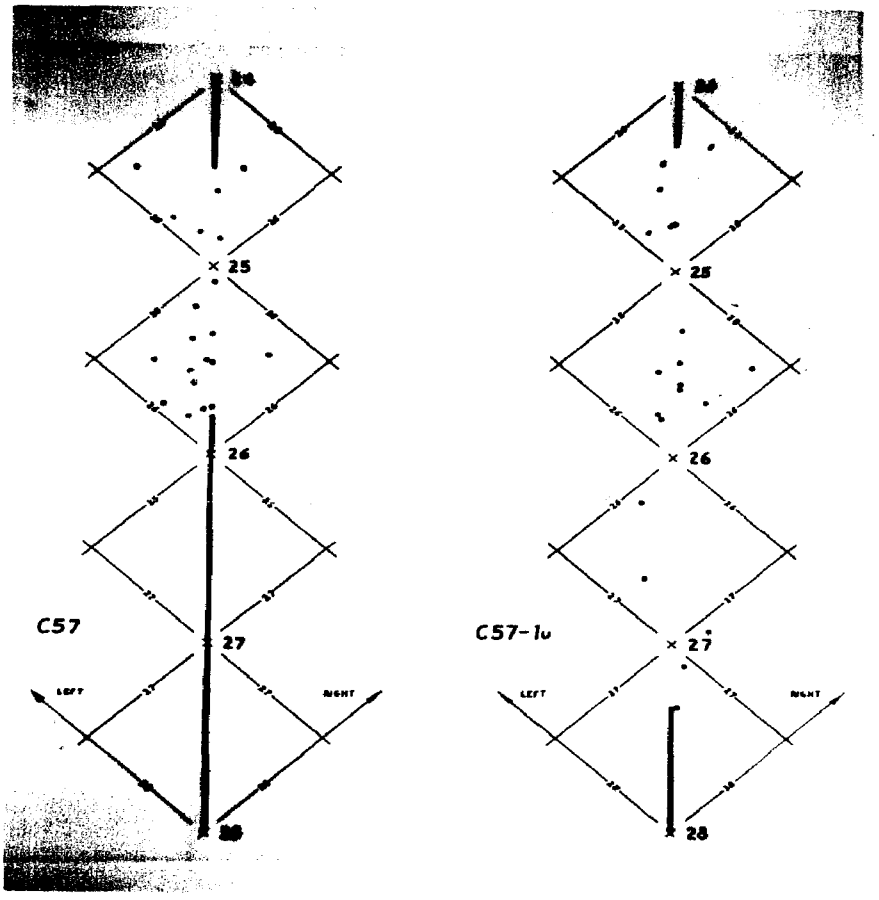

Fig. 55

Correlation diagrams of the left and right umbilical arterial levels measured with respect to the neighboring intersegmental levels of twenty 10½-day C57 embryos (left) and 20 C57-lu embryos (right). Distance along the heavy vertical line measures the mean umbilical level for each embryo. The gaps in the lines mark the ranges of each distribution. See text for full explanation.

sample distributions, that of the 20 normal C57 embryos and that of the 20 C57-lu embryos, are in fact from two different populations, and that the caudad shift of the mean umbilical level in the case of C57-lu population is real. Since the only known difference between the two populations is that in one the luxoid gene is present and in the other it is absent, we can conclude that the caudad shift of the mean umbilical level is an effect of the luxoid gene.

In a sample of 20 embryos, offspring of luxoid heterozygotes mated inter se we expect on the average five to be homozygous luxoids. In the sample of 20 C57-lu embryos, only five were found whose mean umbilical levels were caudad to all twenty in the C57 sample. It seems reasonable to conclude that these five are really homozygous luxoid animals.

Table 10

The distribution of mean umbilical levels in the samples of C57BL/10 and C57BL/10-lu embryos according as the level for a particular embryo fell caudal to the 26th intersegmental level or craniad to it.

	Caudal	Cranial	Totals
C57BL/10	0	20	20
C57BL/10-lu	5	15	20
Totals	5	35	40

DISCUSSION

The conclusion from the breeding results is that on the C57BL/10 genetic background the luxoid gene in homozygous condition has no effect on prenatal viability and that in heterozygous condition it has a high degree of penetrance expressed as polydactyly.

A review of the morphological abnormalities shows that the anomalies affecting the forelimbs and the hind limbs are all preaxial in nature. Thus the frequent absence of the preaxial falciform bone or its modification in the polydactylous hand of homozygotes is coupled with no effect on the postaxial pisiform bone which is always present. Any modification of the postaxial skeleton elements appears to be secondary. Thus bowing and thickening of the fibula is associated with reduction in length of the tibia.

Most of the modifications of the feet due to luxoid are not novel, being similar to those found in luxate animals. Thus no significant differences between the feet of luxoid and luxate heterozygotes exist. There are some differences between the feet of luxoid and luxate hompzygotes. The most striking difference is the proximal elongation of the talus and the development of an epiphysis on it making it micmic the calcaneus lateral to it. Such a talus is never found in luxate homozygotes. Another difference is seen in that toes in the middle of the foot of luxoids

sometimes show doubling which phenomenon does not occur in luxates (Carter, oral communication).

The modifications of the legs of luxoid animals when compared to those induced by the luxate gene on the same C57 background are quite different. The luxate gene here has effects very similar to those described by Carter, namely more or less complete reduction of the tibia, beginning at its distal end. The luxoid gene also leads to reduction of the tibia in homozygotes, but the reduction is usually much less drastic, leading to a slender tibia quite similar in appearance to the fibula. In most cases, the effect on the tibia is the same for both right and left legs (13 out of 19 cases). The right leg was most affected in three cases and the left leg in another three cases. In luxate, usually the effect on both legs is the same but in cases of asymmetry in expression, it is invariably the right leg which is most affected.

The luxoid gene differs radically from the luxate gene in that it induces polydactyly of the forelimbs in homozygotes. Polydactyly in the forelimbs is quite rare in mice. Hovelacque (1920) found six fingers on each hand on two mice out of 242 souris luxees. Holt (1945) found two cases of polydactyly of the forefeet in a sample of over 400 mice having polydactyly of the hind feet. Strong (1934) found a very low grade of postaxial polydactyly in several hundred individuals of the SK inbred stock. Here only a rudiment of a claw was present on the outside of the hand.

The effect by the luxoid gene of increasing the number of sternebrae and the number of ribs is not found in luxate.

Luxoid differs strikingly from luxate (table 5) in its opposite effect on the number of presacral vertebrae, tending to increase the number while luxate tends to decrease it. In this effect of increasing the number of presacral vertebrae, luxoid is like screw tail (MacDowell, Potter, Laanes, and Ward, 1942). This mutation moves the pelvis approximately one vertebra nearer the tail. This caudal shift of the whole pelvis causes corresponding modifications in the 27th and 28th vertebrae. The 27th vertebra which in the normal mouse is the first sacral has many of the characteristics of a lumbar vertebra. The 28th vertebra, normally the second sacral, now has the characteristics of the first sacral. Examination of the pelvis region of a luxoid homozygote showed a similar backward shift of the pelvis. There were 27 presacral vertebrae but the first sacral occupied a position anterior to normal and its left process was lumbar in form. The first caudal vertebra occupied a position anterior to normal; its side processes were in line with the heads of the femur.

Study of the embryology of C57 normal and C57 luxoid mice showed that the earliest external differences between them were excessive growth of the anterior margin of the footplate of the hind limb and a similar excessive growth of the anterior margin of the footplate of the forelimb,

shown by late 11½ day embryos. The latter character served for the diagnosis of homozygous luxoid animals, while the former character could mean that the animals were either homozygous or heterozygous luxoid animals. Later, in the 12½ day stage, some homozygotes had footplates that were pointed. This pointed appearance was perhaps due to excessive forward growth of the anterior margin which would lead to the polydactyly and occasional syndactyly seen in the adults. At the 13½ day and later stages (fig. 34) the axis of the hind limbs of luxoid homozygotes was inclined forward. This could be due to the caudad shift of the hind limb field which would increase the length of the animal between its forelimbs and hind limbs, perhaps leading to a slight "buckling" which would cause the hind limbs to point slightly more anterad than normal.

Examination of the serial sections of the normal and luxoid C57 embryos showed that the chief differences consisted in extra blastemata condensing in the case of polydactyly of the forelimb and hind limb, this arising from excessive growth of the preaxial portions of the footplates. In the case of reduction of the tibia, the first sign was the reduction in size of the tibial blastema. This was followed by a smaller cartilage differentiating in the site of the tibia. Histodifferentiation in general seemed normal and followed the normal time sequences. Thus the ultimate cause of the luxoid anomalies in the hands, legs, and feet

could be traced to altered morphogenesis. A possible cause of the altered morphogenesis will be discussed below.

The results of the study of 10½ day embryos showed that the first detectable effect of the luxoid gene is a caudad shift of the hind limb field. These results are similar but opposite in direction to those of Carter who found that the first detectable effect of luxate is to cause a craniad shift.

Carter, on examination of the distribution of levels of the CBA-lx embryos found that if he assigned the five most craniad points to luxate homozygotes, the truncated distribution still had a craniad shift of grand mean umbilical level of about one-third of a somite. He attributed this shift to an effect of the luxate gene in the heterozygotes. This is plausible, since the luxate heterozygotes occasionally show a reduction in number of presacral vertebrae. Further, Carter attributed the polydactyly found in 36% of the CBA-lx heterozygotes to this slight craniad shift of the hind limb field, or, more specifically, to a slight craniad shift of the hind limb inductor relative to a limited region of limb-potent tissue in the neighborhood of a few somites. This craniad shift of the limb inductor might result in imperfect morphogenetic control of the anterior part of the limb field and result in extra digits being formed from normally inactive but limb-potent tissue.

In the present case, no evidence for a caudad shift of the hind limb field in the case of heterozygote luxoid animals is furnished by the data secured. As mentioned before, when the C57-lu distribution is truncated by the removal of the five most extreme variants, the remaining fifteen, of which ten would be expected to be luxoid heterozygotes, have a distribution entirely similar to that of the twenty normal C57 mice. The conclusion from the data is that no observable caudad shift is caused by the luxoid gene in heterozygous condition. This conclusion seems supported by the fact that, in all the heterozygous luxoid animals examined, there was no increase in number of presacral vertebrae, whereas if the luxoid gene in heterozygous condition caused a shift, one would expect some heterozygotes to show the increase in number of presacral vertebrae.

It is tempting to postulate a caudad shift even in heterozygotes because a slight caudad shift of the hind limb field, by analogy with Carter's explanation, might be used to explain the presence of polydactyly in the heterozygotes. Taking the penetrance of the luxoid gene as 86%, we would expect about 9 of the 10 heterozygotes expected in the sample taken of 10½ day C57-lu embryos to be potential polydactyls. Thus 9 of the 10 would be expected to show a caudad shift of their hind limb fields. As has been seen, no evidence for this is available. There remains the possibility that the shift involved is slight and not detectable

by the means employed. That there is actually a real tendency in luxoid heterozygotes to exhibit a caudad shift is supported by a critical examination of the data in table 5. About 10% of the sample of 418 normal C57 mice show some sign of reduction of the number of presacral vertebrae from 26 to 25, whereas not one of the sample of 86 luxoid heterozygotes show any sign of a reduction.

In the case of the five extreme variants, the caudad shift is of the order of 0.44 to 1.5 segmental lengths, the average being 1.05. Carter found that the most extreme craniad shift caused by the luxate gene in his sample was about three-fourths of a segmental length. Thus luxoid causes a more decided caudad shift than the luxate gene causes craniad. That this may be a real difference is supported by the fact that one luxoid homozygote (table 5) had 28 presacral vertebrae, instead of the 26 usual in the C57 strain.

In seeking a possible connection between the caudad shift of the hind limb field and the limb defects seen in luxoid homozygotes, Saunder's (1948) findings with the chick embryo give valuable clues. This worker employing carbon-marking and extirpation experiments showed that the apical ectoderm on the chick's forelimb bud played the role of inductor of the various skeletal elements in the chick's wing. Thus if part of the apical ectoderm ridge was removed at a certain early stage of development, a part of the limb ordinarily found below this part of the ridge would be

missing later. Zwilling (1949) showed that the apical ectoderm ridge is partially or completely absent from chick embryos having the wingless condition.

Carter has supposed that in the mouse the apical ectoderm ridge on the hind limb represents the hind limb inductor and that the mesenchyme adjacent along a few somites and extending somewhat beyond the ridge cranially and caudally represents the limb-competent tissue. Using this idea (fig. 56), we can suppose that the luxoid gene acts to displace the limb-competent tissue caudad, thus removing the competent tissue from the influence of the preaxial part of the inductor. The normally preaxial part of the field would now be under the influence of the postaxial part of the inductor. The result would be reduction of the tibia, modification of the talus, and various degrees of polydactyly of the foot. The most extreme caudad shift would withdraw the preaxial part of the hind limb field completely from the influence of the preaxial part of the inductor and cause absence of tibia, reduction of the tarsus, and loss of preaxial toes. Support for the idea that a moderate caudad shift of the limb-competent tissue brings the preaxial part of the leg and foot under the influence of the postaxial part of the inductor can be seen in the facts that the most common effect of luxoid on the tibia is to slenderize it and make it and the fibula appear quite similar, while a very common effect on the talus, a preaxial bone, is to make it resemble the calcaneus, a postaxial bone.

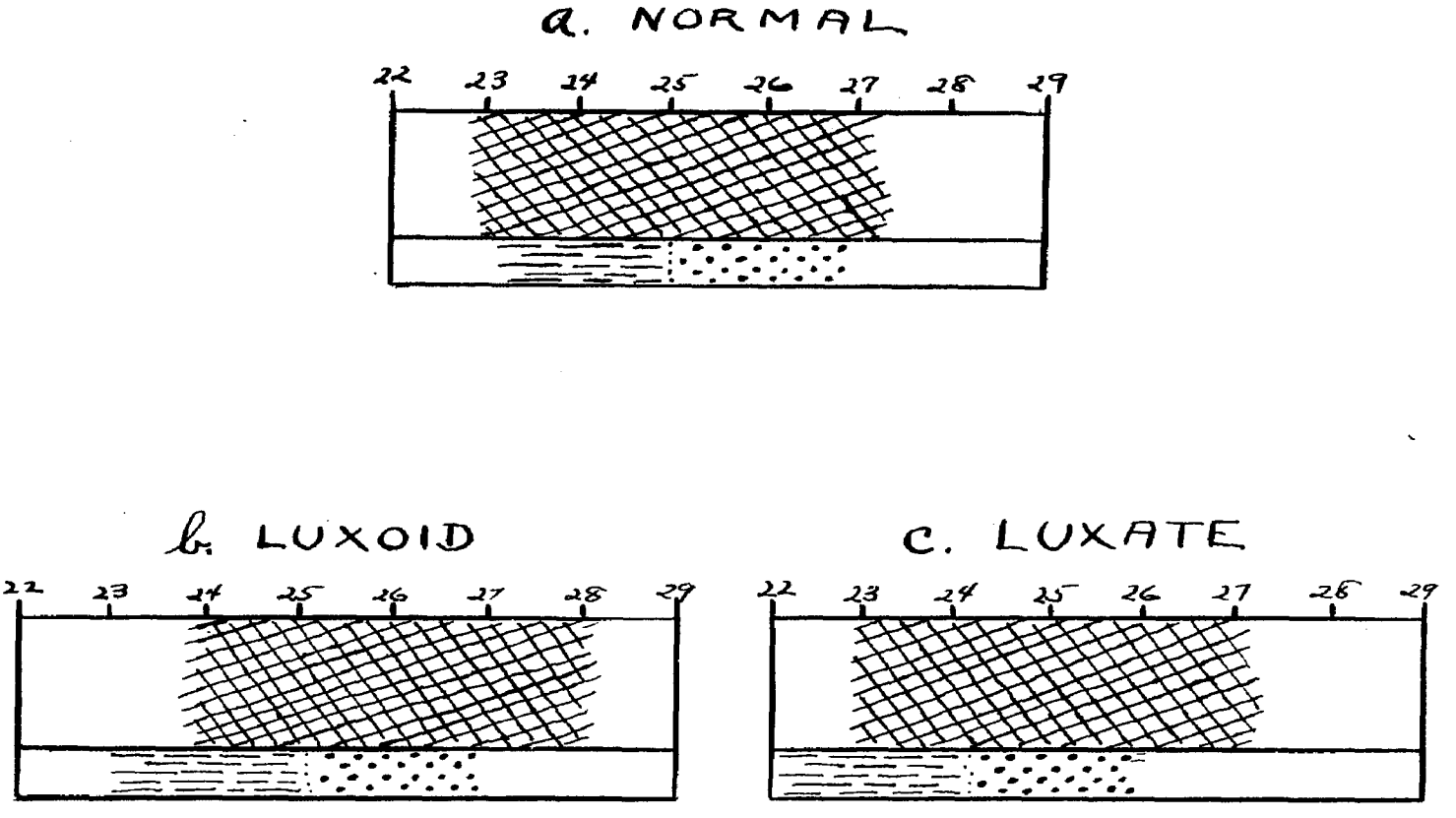

Fig. 56

Diagrams to represent the hypothetical relations between competent tissue for the hind limb and the limb inductor.

a. The situation in normal C57 embryos. The cross-hatched area represents the extent along the segments of the limb-competent tissue. The lined area represents the preaxial part of the limb inductor, while the dotted area represents it postaxial part.

b. The situation in homozygous luxoid embryos. The competent tissue has shifted back about a segmental length, bringing part of the preaxial part of the competent tissue under the influence of the postaxial part of the inductor.

c. The situation in homozygous luxate embryos. The inductor has shifted forward about a segmental length, bringing part of the preaxial part of the competent tissue under the influence of the postaxial part of the inductor. In both a. and b. the preaxial part of the inductor has a reduced amount of competent tissue to influence, leading to tibial reduction.

The question might be raised why caudad shift of the hind limb inductor is not postulated instead of a caudad shift of the limb-competent tissue. The reason is that a caudad shift of the hind limb inductor (the limb-competent tissue remaining fixed) could not cause preaxial polydactyly and reduction or absence of the preaxial tibia, the changes actually observed. Such a shift would induce postaxial changes and reductions, as of the fibula, as a primary effect. The actual changes in the fibula and other postaxial structures are secondary to the changes in the preaxial structures.

The dystrophy of caudal vertebrae, including reduction and fusion of adjacent vertebrae, could be caused by the caudad shift of the hind-limb-competent tissue. If we suppose that the ectodermal envelope of the rear portion of the body remains unchanged from normal in its growth rate while the somites and attendant vascular structures of the tail are impacted in their ectodermal envelope because of the caudad shift of the hind-limb-competent tissue, we can suppose that various distortions of these structures would result because they are growing in a space kept somewhat restricted by dis-proportional growth of their ectodermal covering.

As a matter of fact, in one of the two luxoid homozygotes of 10½ days showing an extreme degree of caudad shift of the hind limb field, large vascular blebs

continuous with the dorsal aorta were found in the tail bud. Apparently abnormal expansion of the dorsal aorta had occurred in this case leading to large blebs, but the situation might be explained according to the previous idea by supposing that the blebs resulted from growth of the aorta in a restricted space. In a 13½ day homozygous embryo, the tail had two large blebs connected together and apparently this time in the venous system (fig. 42). In a still later stage (14½ day), when a kink was externally observed in the tail of a homozygous embryo, sectioning (fig. 46) showed that the somites were distorted at the place having the kink. The vascular system here showed no blebs but it could be supposed that preceding blebs brought pressure onto adjacent somites and caused their dystrophy. The next stage at which tail kinks were observed was in homozygotes newly born. Here examination showed that the provertebrae in the ends of kinky tails showed various distortions. These could be a direct result of preceding dystrophy of the somites. Finally, in tails of homozygotes one week old, the cartilaginous vertebrae showed reduction at adjacent ends and sometimes only one piece remained which was out of line with the rest. Ossification of such vertebrae would result in misshapen and possibly fused vertebrae.

Connection of the polydactyly found in the hands of luxoid homozygotes with the caudad shift of the hind-limb-competent tissue must at this stage be purely hypothetical.

Possibly, there is a slight caudad shift of the forelimb-competent tissue which would remove the anterior part of the competent tissue away from perfect control by the forelimb inductor (the apical ectoderm ridge of the forelimb bud). A slight craniad shift of the limb inductor could explain the polydactyly in an analogous way. However, the increase in the number of ribs and in the number of sternebrae lends support to the idea that shifts in the thoracic region are caudad and that therefore this shift of the forelimb field most likely involves the limb-competent tissue, If we postulate a caudad shift of the fore-limb competent tissue, it seems reasonable also to postulate a similar caudad shift of the competent tissue for the ribs and sternebrae.

Examination of the data as to which umbilical artery was dominant in the C57-lu embryos shows a picture far from clear. Thus for example, at the 14½ and 15½ day stages, all the luxoid homozygotes examined had the right umbilical artery as dominant, which seems to be the tendency uniformly in heterozygous and normal embryos. However at the 16½ and 18½ day stages, all the homozygotes examined had the left umbilical artery as dominant. Since in the adults, in cases of asymmetry of expression of luxoid in the legs, both right and left sides were equally likely to be the most severely affected, it may be that the ultimate cause why a particular side is most severely affected is associated with the regression of the umbilical artery on that side.

Against this view is the fact that while in most cases noted, one or the other umbilical artery was dominant in the homozygotes, the expression of luxoid is generally symmetrical. In other words, frequent asymmetry of umbilical artery development in the embryos should result in frequent asymmetry of expression in the adults. Yet this is not found. The conclusion seems reasonable that regression of the umbilical artery has no great influence on the development of luxoid on that side.

As for the influence of the development of the marginal sinus on the polydactyly observed, changes from its normal open channel to a network seen in some polydactylous feet are most likely secondary to the way the supernumerary digits form and are not the cause of the polydactyly. This view is supported by the fact that normal open channels are found sometimes associated with supernumerary digits.

At best, at this stage, a unitary hypothesis to trace all the effects of the luxoid gene back to one primary gene effect is purely hypothetical and provisional. However, the following tentative hypothesis is proposed.

1. The primary action of the luxoid gene is to shift certain competent tissues caudad. Thus it shifts the tissue competent for the forelimbs, ribs, and sternebrae slightly caudad and the tissue competent for the hind limbs very slightly caudad in the case of heterozygotes and more extremely caudad in the case of homozygotes.

2. The slight caudad shift of the forelimb-competent tissue brings on imperfect morphogenetic control of the forelimb field's anterior portion. This is expressed as various degrees of polydactyly. The shifts of the rib-competent tissue and sternebrae-competent tissue also bring on imperfect morphogenetic control of those regions. These are expressed as an increase in the number of their elements.

3. The very slight caudad shift of the hind-limb-competent tissue (not detected in the above experiments) leads to the polydactyly seen in heterozygotes.

4. The decided caudad shift of the hind-limb-competent tissue found in homozygotes leads to partial or complete loss of the anterior part of the hind limb field expressed as reduction or loss of the tibia and polydactyly or ectrodactyly in the hind foot. The frequent micmicking by the tibia of the fibula and by the talus of the calcaneus results from the fact that the anterior part of the competent tissue is now under the influence of the postaxial part of the limb inductor. The caudad shift leads to occasional defects of the vascular system in the tail which cause vascular blebs. These blebs induce deformation of somites, which in turn leads successively to deformations in provertebrae, cartilaginous vertebrae, and finally to misshapen or fused vertebrae in the adult.

It might be asked whether others have concluded that imperfect morphogenetic control is expressable as polydactyly or increase in the number of elements as ribs or sternebrae.

Scott (1937) ascribed preaxial polydactyly in guinea-pigs carrying the gene Px to a general increase of growth at a particular stage of development which led to abnormal morphological relationships. Gabriel (1946) ascribed it to partial duplication of the limb field, an incomplete mirror-image field arising anterior to the normal field. Danforth (1947) thought that the main effect of the polydactyly gene in cats is to increase the number of cells in the preaxial border of the limb. Landauer (1948) thought that polydactyly in poultry is due to a disturbance of the proper organization of the limb field of unknown nature. He felt that the mirror-image theory could not explain the ectrodactyly occasionally seen in polydactylous poultry stocks. It seems that Landauer's idea is most reasonable since polydactyly is not merely a quantitative effect but a qualitative effect. His idea is about the same as saying that imperfect morphogenetic control leads to polydactyly.

Certain experimental evidence lends weight to this view. L. B. Russel (1950) induced polydactyly in mouse embryos by X-irradiation of pregnant females at $8\frac{1}{2}$ and $9\frac{1}{2}$ days of gestation when as yet no hind limb bud has formed. This observation leads one to conclude that polydactyly must be traced to early disturbances of the hind limb field which bring on imperfect morphogenetic control. Russel observed that irradiation at later stages when the footplate is actually forming led to damage and loss of toes and not to polydactyl

SUMMARY

A study was made of the developmental genetic of luxoid, a new skeletal variation in the house mouse. The material used for comparison was two strains of mice, normal C57BL/10 mice and a substrain made approximately isogenic with the normal C57 mice by repeated backcrosses but carrying the luxoid gene.

Study of the breeding experiments showed that on the C57 background, luxoid has high penetrance in the heterozygotes (86%) and that it has no effect on prenatal viability of the homozygotes.

Study of the adult morphology showed that the only deviation from the normal phenotype found in luxoid heterozygotes was frequent preaxial polydactyly of the hind feet. Examination of homozygotes showed several effects: rare dystrophy of the innominate bone, occasional shortening of the femur, more or less reduction of the tibia, altered shape of the talus, polydactyly or ectrodactyly of the hind feet, frequent kinking of the tail, polydactyly of the forefeet, a tendency to increase of the number of presacral vertebrae, of number of ribs, and of number of sternebrae. It was concluded that all the changes affecting the limbs were primarily preaxial.

Study of the embryology showed that the earliest obvious difference between normal and luxoid embryos, seen in

the late 11½ day stage, involved excessive growth of the anterior portions of the footplates of the forefeet and hind feet, the excessive growth in the forefeet being found only in homozygotes. Soon the luxoid embryos showed extra blastemata in the footplates of their feet and the presumptive homozygotes showed deficient tibial blastemata. Histodifferentiation proceeded as in normal embryos. Origin of the kinky tail in homozygotes was tentatively traced to vascular blebs at early stages causing deformation of somites, which in turn could ultimately lead to the abnormal bony vertebrae seen in the adult.

Search for the possible ultimate cause of the increase in number of presacral vertebrae and the limb anomalies seen in the luxoid animals revealed a caudad shift of the hind limb field in the case of the homozygotes of the order of one segmental length on the average.

As a tentative unitary hypothesis, it was suggested that the primary effect of the luxoid gene is to shift the competent tissue of the forelimbs, the ribs, the sternebrae, and the hind limbs caudad. All other effects of the luxoid gene were interpreted as secondary to this primary shifting effect.

BIBLIOGRAPHY

Carter, T. C. 1948 A new strain of luxate mice. Heredity, 2: 405-406 (Abstr.)

---------- 1949 The development of tibial hemimelia in mice, Heredity, 3: 378-379 (Abstr.)

---------- 1951a The genetics of luxate mice. I. Morphological abnormalities of heterozygotes and homozygotes. J. Genetics, 50: 277-299.

---------- 1951b The genetics of luxate mice. II. Linkage and independence. J. Genetics, 50: 300-306.

---------- The genetics of luxate mice. III. Horseshoe kidney, hydronephrosis and lumbar reduction. J. Genetics, 51: 441-457.

---------- The genetics of luxate mice. IV. Embryology (unpublished).

Chang, Tso-Kan 1939 The development of polydactylism in a special strain of Mus musculus. Peking Nat. Hist. Bull., 14: 119-134.

Chase, H. B. 1946 A sex-linked recessive in the mouse. Genetics, 31: 214 (Abstr.)

Danforth, C. H. 1930 Developmental anomalies in a special strain of mice. Amer. J. Anat., 45: 275-288.

---------- 1947 Morphology of the feet in polydactyl cats. Amer. J. Anat., 80: 143-171.

Fisher, R. A. 1944 Statistical methods for research workers. Oliver and Boyd, Ltd., London and Edinburgh. 356 pp.

Fortuyn, A. B. D. 1939 A polydactylous strain of mice. Genetica, 21: 97-108.

Gabriel, M. L. 1946 The effect of local applications of colchicine on leghorn and polydadtylous chick embryos. J. Exp. Zool., 101: 339-350.

Green, M. C. 1950 Mouse News Letter, 3: 9.

---------- 1952 A rapid method for clearing and staining specimens for the demonstration of bone. Ohio J. Science, 52: 31-33.

Greene, E. C. 1935 Anatomy of the rat. Trans. Amer. Phil. Soc., 27: 1-370.

Grüneberg, H. 1943a Congenital hydrocephalus in the mouse, a case of spurious pleiotropism. J. Genetics, 45: 1-21.

---------- 1943b Two new mutant genes in the house mouse. J. Genetics, 45: 22-28.

---------- 1943c The development of some external features in mouse embryos. J. Heredity, 34: 89-92.

---------- 1952 The genetics of the mouse. Second edition. Martinus Nijhoff, The Hague. 650 pages.

---------- 1953 Genetical studies on the skeleton of the mouse. VII. Congenital hydrocephalus. J. Genetics, 51: 327-358.

Guyer, M. F. 1936 Animal micrology. 4th edition. Univ. of Chicago Press, Chicago. 331 p.

Hamilton, W. J., J. D.Boyd, and H. W. Mossman 1945 Human embryology. The Williams and Wilkins Co., Baltimore. 366 p.

Hertwig, P. 1942 Neue Mutationen und Koppelungsgruppen bei der Hausmaus, Z. indukt. Abstamm.- u. Vererbungs Lehre, 80: 220-246.

Hovelacque, A. 1920 Anatomie et morphogenie d'une anomalie hereditaire des membres abdominaux (Absence congenitale du tibia). Bull. Biol. de France et Belg., Suppl. 3: 1-156.

Hovelacque, A., et R. Noel 1923 Etude embryogenique de l'absence congenitale du tibia chez des souris ectromeles. Bull. Biol. de France et Belg., 57: 133-142.

Holt, S. B. 1945 A polydactyly gene in mice capable of nearly regular manifestation. Ann. Eugen., 12: 220-249.

Kobozieff, N., et N. A. Pomriaskinsky-Kobozieff 1946 Sur les anomalies congenitales et hereditaires des membres chez les souris. C. R. Soc. Biol, Paris, 140: 200-202.

Landauer, W. 1948 The phenotypic modification of hereditary polydactylism of fowl by selection and by insulin. Genetics, 33: 133-157.

---------- 1952 Brachypodism: a recessive mutation of housemice. J. Hered., 43: 293-298.

MacDowell, E. C., J. S. Potter, T. Laanes, and E. N. Ward. 1942 The manifold effects of the screw tail mouse mutation. J. Hered., 33: 439-449.

Murray, J. M. 1932 Polydactylism in mice. Science, 75: 312.

Rabaud, E. 1914 Sur une anomalie hereditaire des membres posterieurs chez la souris. C. R. Soc. Biol., Paris, 77: 411-412.

---------- 1917. Domiance et recessivite chez les souris "luxées." Bull. Zool. France, 42: 87-97.

Russel, L. B. 1950 X-ray induced developmental abnormalities in the mouse and their use in the analysis of embryological patterns. J. Exp. Zool., 114: 545-602.

Saunders, Jr. J. W. 1948 The proximo-distal sequence of origin of the parts of the chick wing and the role of the ectoderm. J. Exp. Zool., 109: 363-403.

Scott, J. P. 1937 The embryology of the guinea pig. III. The development of the polydactylous monster. J. Exp. Zool., 77: 123-157.

Strong, L. C. 1934 Two new structural characters in mice. Anat. Rec., 58: 88 (Abstr.)

Wright, S. 1921 Systems of mating. I. The effects of inbreeding on the genetic composition of a population. Genetics, 6: 124-143.

Zwilling, E. 1949 The role of epithelial components in the developmental origin of the "wingless" syndrome of chick embryos. J. Exp. Zool., 111: 175-188.

AUTOBIOGRAPHY

I, Paulinus Frederick Forsthoefel, was born in St. Sebastian, Ohio, April 5, 1915.. I received my secondary school education in the parochial schools of the city of Celina, Ohio. My undergraduate training was received at St. John's College, Toledo, Ohio; Xavier University, Cincinnati, Ohio and Loyola University, Chicago, Illinois, from which I received the degree of Bachelor of Arts in 1939. During the summers of 1939-1943, I took courses in biology at the University of Detroit, Detroit, Michigan, and in the summer of 1944 at St. Louis University, St. Louis, Missouri. In the summer quarter of 1949, I entered the Graduate School of the Ohio State University where I specialized in genetics in the Department of Zoology and Entomology. I took courses in biology at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the summers of 1950 and 1951. I received the degree Master of Science in the autumn quarter of 1951 from the Ohio State University. I was a University Scholar from September 1952 to June, 1953.