The genetics and morphology of two 'luxoid' mutants in the house mouse

BY A. G. SEARLE

*Medical Research Council Radiobiological Research Unit,
Harwell, Berkshire*

1. INTRODUCTION

Members of the luxoid group of mouse mutants are characterized by a twisting of fore- or hind-limbs associated with the reduction or loss of certain long-bones as well as oligo- or polydactyly. These very similar appendicular abnormalities are accompanied by very different pleiotropic effects on the soft parts, which are difficult to relate to the limb-bone changes.

Grüneberg (1963) has described the group as a whole. The present paper is concerned with a spleenless member called dominant hemimelia, *Dh* (described briefly by Searle, 1959), and a sterile one called postaxial hemimelia, *px*. It is felt that studies on the common and contrasting elements in these puzzling pleiotropisms, and on gene interaction between them, should help to reveal the processes underlying development of the mammalian fore- and hind-limbs and of certain internal organs.

2. DOMINANT HEMIMELIA

(i) *Origin and appearance*

This mutant was discovered in 1954 by Dr T. C. Carter at the Institute of Animal Genetics, Edinburgh. The original male was at first thought to be heterozygous for luxate (*lx*). However, some outcross offspring lacked the hallux and had tibial hemimelia with luxation of the hind-legs. Since such severe abnormalities are unknown in *lx*/ + mice, it was realized that this must be a new luxoid mutant, which was named dominant hemimelia (*Dh*) by Dr Carter.

The external abnormalities of *Dh* heterozygotes are confined to the preaxial side of the hind-limbs. Manifestation is very variable; penetrance may be incomplete. The following grades of expression can be seen:

(i) A slight thickening and lengthening of the hallux.
(ii) Triphalangy of the hallux.
(iii) Addition of a digit or part of a digit on the outside of the hallux. Syndactyly may also occur between digits IV and III, III and II, or II and I of the hind-feet.

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(iv) Loss of a digit or part of a digit; sometimes even loss of two digits (Plate I A).

(v) Luxation and reduction in length of one or both hind-limbs, associated with oligodactyly rather than polydactyly (Plate I A).

Thus the range of expression in *Dh* heterozygotes mimics the overall range of expression for both heterozygous and homozygous luxate mice, except that polydactyly seems to be more common and sometimes more extreme in luxate homozygotes, while oligodactyly is less common.

Dh homozygotes usually die in the first few days of life, though about 4% reach weaning age and some may even breed, as discussed later. Their hind-legs are short, twisted (Plate I B) and show oligodactyly, with loss of one to three digits; fore-limbs are normal. In newborn heterozygotes and homozygotes certain visceral abnormalities can be seen by transparency: (i) absence of spleen in both genotypes, (ii) small stomach (iii) hydropic kidneys in the homozygote.

(ii) *Genetics*

The penetrance of *Dh* in heterozygous form seems to be 100% on the basis of spleen classification but about 96% on the basis of careful limb classification (Table 1). Fewer *Dh* homozygotes than expected are recovered from intercrosses, presumably because some are born dead or moribund and eaten. In outcrosses, slightly fewer heterozygotes are found than normals, though the difference is not significant in Table 1. The rate of survival of *Dh*/+ mice to weaning age is about 73% that of non-*Dh*. Analysis of a sample of thirty litters in which deaths had occurred before full classification showed that fifty of these deaths were of *Dh*/+ mice and twenty of +/+ mice, when equal numbers would have been expected. This difference is highly significant ($\chi^2_1 = 12\cdot9$, $P = 0\cdot0003$), confirming the hypothesis of greater post-natal death in *Dh* heterozygotes. These heterozygotes also weight about 15% less than normals on the average at 3–4 weeks.

Table 1. *Results of outcross and intercross* Dh/+ *matings, with classification at birth*

	PHENOTYPE OF OFFSPRING				
· MATING	*Dh/Dh*	*Dh/+*			+/+
		Spleenless, limb defect	Spleenless, normal limb	Total	
Dh/+ × +/+	–	125	2	127	157
Dh/+ × *Dh*/+	110	333	19	352	186

As T. C. Carter first showed, dominant hemimelia is closely linked to leaden (*ln*) in linkage group XIII (Table 2). There were no significant sex differences in *Dh–ln–fz* recombination estimates; combining the data gives an overall recombination frequency of $4\cdot82 \pm 0\cdot38\%$ between *Dh* and *ln* and of $38\cdot18 \pm 0\cdot85\%$ between *ln* and *fz*. In these linkage tests, classification for *Dh* was entirely by limb effects, but there seems little sign of misclassification due to less than 100% penetrance.
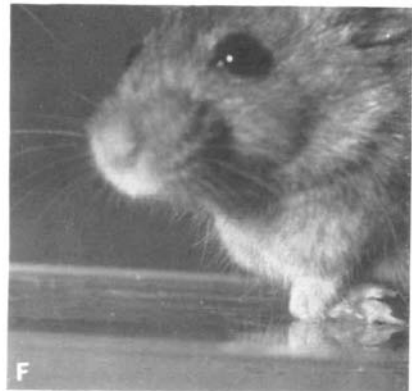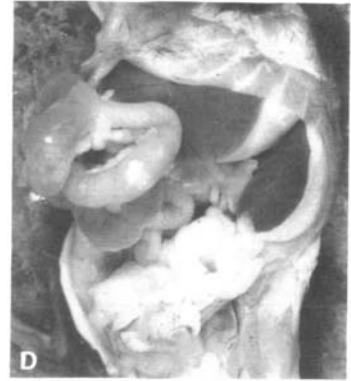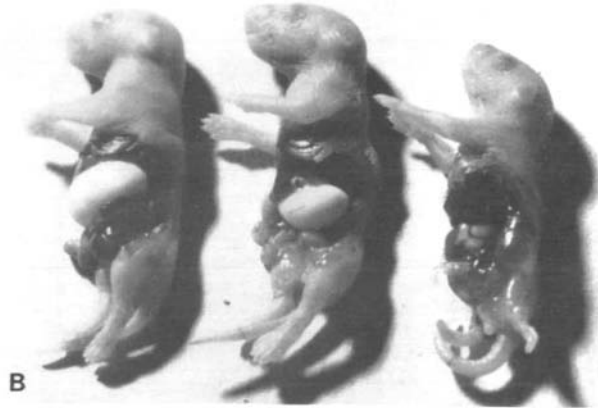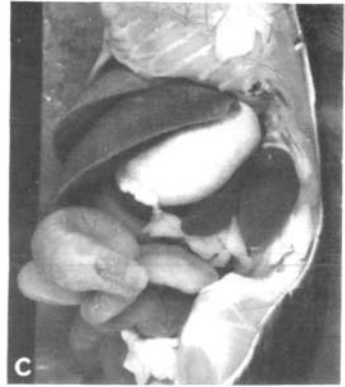
A. Heterozygote for dominant hemimelia, showing reduction and twisting of hind-limb, with loss of two preaxial digits.

B. Dissections of newborn normal (left), $Dh/+$ (centre) and Dh/Dh (right) litter-mates, showing absence of spleen and progressive reduction of stomach in Dh, also with hydro-nephrosis and extreme hind-limb reduction in the lethal homozygote.

C and D. Dissections of normal (C) and $Dh/+$ mice, showing absence of spleen, reduction of stomach and flattening of that part of the kidney abutting on the stomach in dominant hemimelia.

E. Postaxial hemimelic (px/px) mouse, showing loss of digits and abnormal posture of forefeet. Typical black epidermal papillae can be seen on the forefeet and right hind-foot.

F. Postaxial hemimelic mouse, showing abnormal stance associated with high-grade expression of px, involving loss of the ulna.

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Table 2. *Tests for linkage of* Dh *with leaden* ln *and fuzzy* fz *of linkage group XIII. Phenotypes of offspring*

$$\text{from } \frac{Dh + +}{+ \text{ ln fz}} \times \frac{+ \text{ ln fz}}{+ \text{ ln fz}} \text{ crosses}$$

Heterozygous parent				Phenotypes of offspring					
	Dh + +	+ ln fz	Dh + fz	+ ln +	Dh ln fz	+ + +	Dh ln +	+ + fz	Total
father	726	888	410	630	48	74	7	6	2789
mother	106	140	73	106	4	14	1	2	446
									3235

Recombination fraction:

	Heterozygous male:	Heterozygous female:
Between Dh and ln	135/2789 = 4·84±0·41%	21/446 = 4·71±1·00%
Between ln and fz	1053/2789 = 37·76±0·92%	182/446 = 40·81±2·33%

(iii) *Morphology of the heterozygote*

(a) *Skeletal effects*

Classification at birth showed that the range of digital abnormalities went all the way from a triphalangous accessory digit to loss of three digits. Polydactyly was commoner than oligodactyly, while the commonest single abnormality was triphalangy of a thick or normal hallux, as in luxate heterozygotes. Syndactyly (usually of digits I and II) was found in 14% of feet, but was never associated with oligodactyly. An extra digit was associated with a triphalangous rather than a normally diphalangous hallux. Rudimentary extra digits occurred in 7·5% of feet, on the inside.

There was no tendency for one foot to be affected more than the other, but there was such a tendency with respect to hind-limb luxation:

		LEFT LIMB		
		Normal	Luxate	Total
RIGHT	Normal	322	79	401
LIMB	Luxate	146	243	389
	Total	468	322	790

Thus 40·8% of left and 49·2% of right hind-limbs showed luxation, a highly significant difference ($\chi_1^2 = 11\cdot48, P = 0\cdot0007$). There is also a clear tendency for the factors causing luxation to act alike on the two sides of the animal.

Luxation of the limb in $Dh +$ mice was particularly associated with oligodactyly. All limbs with complete loss of the hallux also showed luxation, but less than a quarter of those with polydactyly or fairly normal digits. The amount of oligo-dactyly and of luxation varied considerably on different genetic backgrounds.

Alizarin transparencies showed that all the preaxial side of hind-limbs and even pelvic girdle could be affected, but the more proximal the structure the less likely it was to be abnormal (Table 3). The right limb-bones tend to be more affected than the left, explaining why luxation is more frequent on the right. As Fig. 1 shows, the hind-limb abnormalities mimic those found of luxate homozygotes (Carter, 1951) with (i) poly- or oligodactyly, (ii) distoproximal reduction of the tibia or its complete disappearance, with resultant distortion of the fibula, (iii) occasional reduction of the femur and even of the pubis. The effect of Dh on tarsal bones is also very similar to that found by Carter in luxate. The patella and trochlea patellaris tend to be reduced or absent where the limb is severely affected. This seems not to happen in luxate homozygotes, though Forsthoefel (1958) has described patellar loss in luxoid homozygotes.

Table 4 shows that the number of presacral vertebrae in Dh heterozygotes is reduced on the average by about 0·8, this being due both to a tendency for less lumbar and for less thoracic vertebrae. The number of sternebrae was also reduced in $Dh/+$ mice (Table 5). The difference is clearly significant; like the reduction in
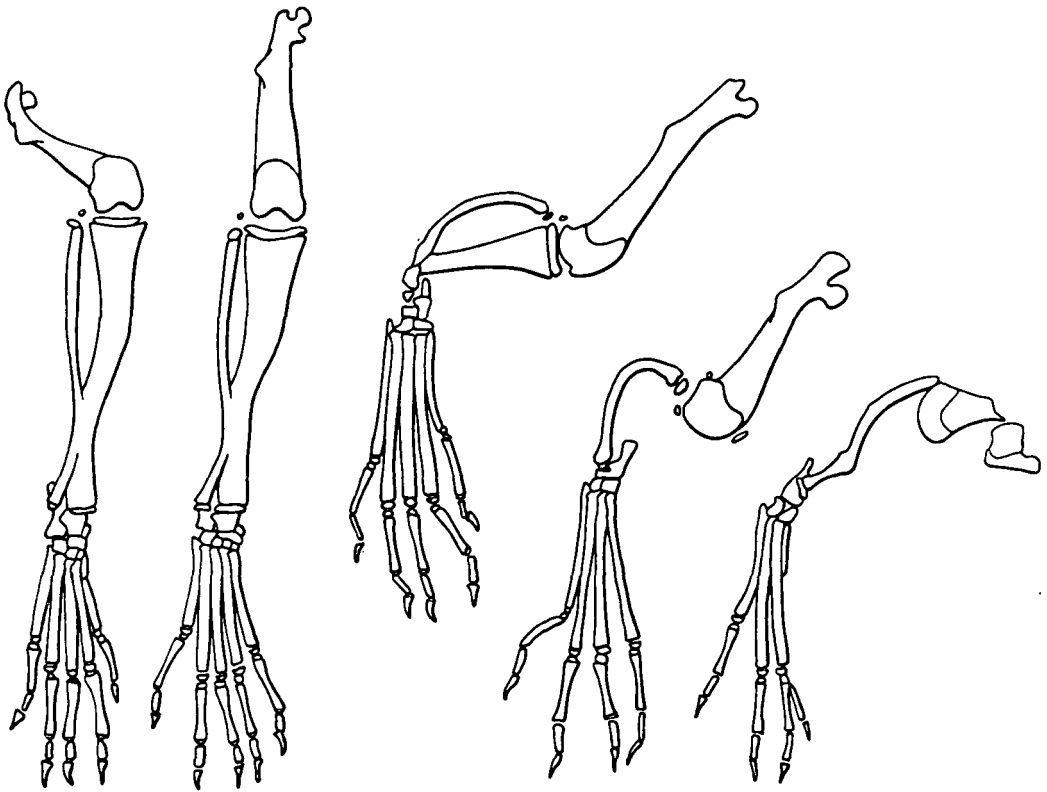
Text-fig. 1. Right hind-limb skeletons of adult normal (left) and *Dh*/+ mice, showing different grades of abnormality in the latter, from preaxial triphalangy to severe oligodactyly, with loss of tibia and fragmentation of femur. Freehand drawings of alizarin clearance preparations.

Table 3. *Comparison of incidence of abnormality in different preaxial structures of the hind-limbs of* 52 Dh *heterozygotes*

	LEFT				RIGHT			
	Normal	Present but abnormal	Absent	% affected	Normal	Present but abnormal	Absent	% affected
Pubis	52	0	0	0	50	2	0	4
Femur	44	8	0	15	41	11	0	21
Tibia	28	13	11	46	18	16	18	65
Hallux	9	27	16	83	8	22	22	85

number of thoracic ribs it is presumably due to an anterior shift in the position of the thoraco-lumbar as well as lumbo-sacral borders.

Table 4. *Comparison of numbers of lumbar vertebrae, thoracic ribs and presacral vertebrae in $+/+$, Dh/$+$ and Dh/Dh mice, based on alizarin and methylene blue preparations (left/right)*

I. Lumbar vertebrae	6/6	6/5	5/6	5/5	5/4	4/5	4/4	Total	Mean
$+/+$	19	2	1	5	0	0	0	27	5·8
Dh/$+$	6	1	1	47	1	1	0	57	5·1
Dh/Dh	0	0	0	6	0	2	2	10	4·7

II. Thoracic ribs	13/13	13/12	12/13	12/12	Total	Mean
$+/+$	27	0	0	0	27	13·0
Dh/$+$	49	1	0	7	57	12·9
Dh/Dh	5	0	0	5	10	12·5

III. Presacral vertebrae	26/26	26/25	25/26	25/25	25/24	24/25	24/24	23/24	Total	Mean
$+/+$	19	2	1	5	0	0	0	0	27	25·8
Dh/$+$	1	0	1	50	2	1	2	0	57	25·0
Dh/Dh	0	0	0	2	0	1	6	1	10	24·2

Table 5. *Comparisons of numbers of sternebrae in $+/+$, Dh/$+$ and Dh/Dh mice*

	5	6	7	Total	Mean
$+/+$	0	24	3	27	6·1
Dh/$+$	29	27	0	56	5·5
Dh/Dh	7	0	0	7	5·0

(b) Visceral abnormalities

Twenty-five adult *Dh/$+$* mice were autopsied and examined for visceral abnormalities, with the following results:

(i) All lacked the spleen completely (Plate I D), but the rest of the lymphatic system seemed unaffected. The splenic artery supplied posterior branches of the pancreas instead.

(ii) Nineteen showed an abnormal flattening of the antero-ventral part of the left kidney (Plate I D) where it would be in close contact with the spleen in the normal mouse; but abutted on the stomach in *Dh*.

(iii) The other six had a hydropic left kidney, with associated hydroureter; none had a hydropic right kidney.

(iv) Seventeen had stomachs which were markedly smaller than normal, chiefly due to a shortening of the cardiac end.

In addition, two were noted as having a reduced caecum, while one female had an imperforate vagina. A higher incidence of hydronephrosis on the left side is also found in *lx/lx* mice (Carter, 1953). The true incidence of hydronephrosis in adult *Dh/$+$* mice is probably somewhat less than 10%; three of the mice in the above

sample were suspected of having a hydropic kidney, being autopsied for that reason. The flattening of the left $Dh/+$ kidney had little if any effect on its volume.

In $Dh/+$ mice the posterior ends of left and right kidneys are sometimes abnormally close to each other, but no examples of horseshoe kidney have been observed. Newborn $Dh/+$ sometimes have umbilical hernia, but the frequency is not accurately known, owing to maternal cannibalism.

The histocompatibility reactions of $Dh/+$ mice when donors and recipients of tail-skin homografts were studied by Dr J. Godfrey and myself, using the method of Bailey & Usama (1960). Breakdown times were within normal limits.

(iv) *Morphology of the homozygote*

(a) *Skeletal effects*

Dh/Dh mice always have oligodactyly and very reduced hind-limbs, with normal fore-limbs. These abnormalities, together with extreme reduction of the stomach and the usual presence of very hydropic kidneys even at birth, permit fairly confident recognition of the homozygote. Table 6 shows that up to four digits may be lost, with associated great reduction of the hind-limb concerned. In 13% of hind-feet there was an incomplete digit (usually III) on the preaxial side. Five per cent of feet showed syndactyly, usually between digits III and IV.

Table 6. *Expression of oligodactyly in left and right hind-feet of* Dh/Dh *mice; data from classification of newborn*

| | | DIGITS MISSING ON RIGHT | | | | | | |
		None	I	I+part of II	I+II	I+II+part of III	I+II+III	Total	%
	Part of I	0	1	0	0	0	0	1	20·6
	I	1	14	4	9	0	0	28	
DIGITS	I+part of II	0	6	2	2	0	2	12	62·4
MISSING	I+II	0	15	13	40	1	7	76	
ON	I+II+III	0	1	5	12	0	3	21	14·9
LEFT	I+II+III+IV	0	1	0	1	0	1	3	2·1
	Total	1	38	24	64	1	13	141	
	%	0·7	27·0	62·4		9·9			

Seventy-one per cent of left hind-feet had at least the first and second digits missing, but only 55% of right feet. This difference is significant ($\chi_1^2 = 7\cdot37$, $P = 0\cdot007$), contrasting with the tendency for right hind-limbs to be more severely affected than left in Dh heterozygotes.

At birth, the Dh/Dh femur is shortened, distorted and often fragmented. Both tibiae are usually absent, also the patellae as in many luxoid (lu/lu) mice. Tables

4 and 5 show that numbers of thoracic ribs, sternebrae and presacral vertebrae averaged even less in *Dh* homozygotes than in heterozygotes, the mean number of *Dh/Dh* presacral vertebrae being 1·6 less than in normal members of the same stock.

Abnormalities of the hind-limb cartilaginous skeleton in *Dh/+* and *Dh/Dh* are most clearly seen at about the 14th day of foetal life.

Figure 2 shows that they may include in *Dh/Dh*:

  (i) severe dystrophy of the femur;
 (ii) absence of the tibia;
(iii) absence of all tarsal bones except the os calcaneus and cuboideum;
 (iv) absence of metatarsals I and II with the corresponding phalanges.
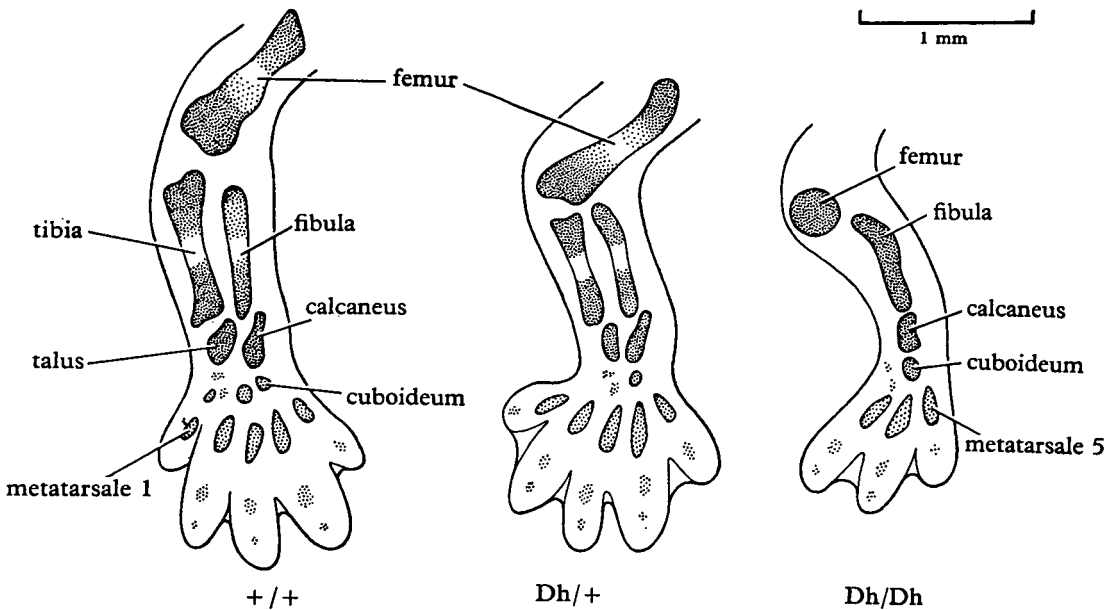
Text-fig. 2. Left hind-limb skeletons of normal, *Dh/+* and *Dh/Dh* 14½-day foetal litter-mates. Note preaxial anomalies present in cartilage. Camera lucida drawing of methylene blue transparency.

In addition, no cartilaginous pubis was seen at this stage in *Dh/Dh* preparations, though present in *Dh/+* and normal litter-mates.

Alizarin transparencies of three surviving *Dh* homozygotes showed similar skeletal defects to those found in cartilage (Fig. 3), although one pelvic girdle and one femur were normal.

(b) Visceral abnormalities

Twenty-five presumed *Dh/Dh* mice (ten females and fifteen males) were examined for visceral abnormalities when newborn or within a few days of birth, with the following results:

  (i) in all the spleen was absent, the stomach greatly reduced and both kidneys and ureters were hydropic (Plate I B);
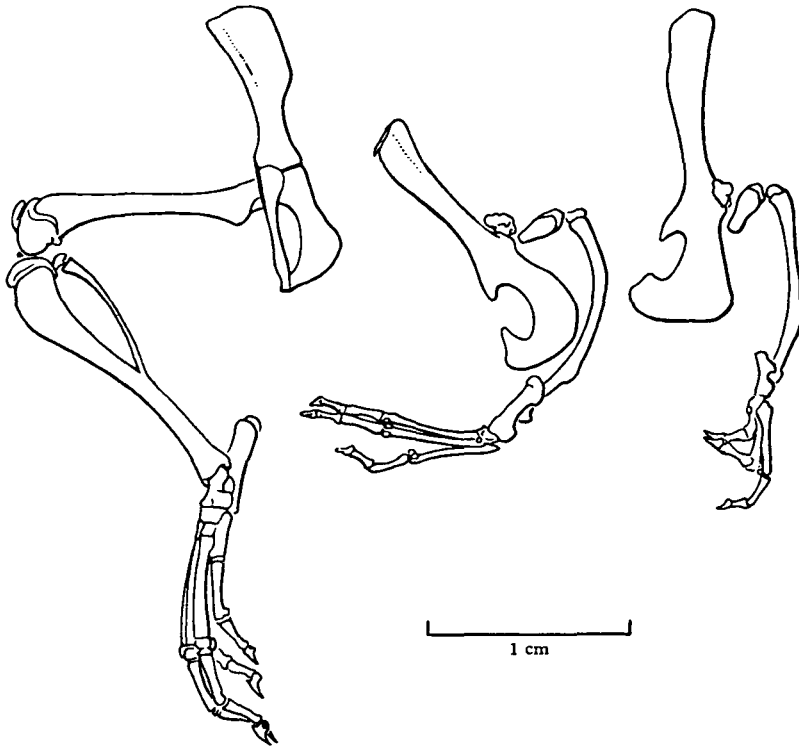
Text-fig. 3. Right hind-limb skeletons of normal (left) and surviving adult *Dh/Dh* mice, with severe preaxial defects in the latter. Freehand drawings of alizarin transparencies.

   (ii) no anus (44%);

  (iii) gap in colon or rectum, or blind ending to rectum (44%);

  (iv) no caecum (28%);

   (v) recto-vaginal fusion in 40% of females, recto-urethral in 7% of males;

  (vi) bladder absent or vestigial (32%);

 (vii) blind endings to ureters (20)%, urethras (20%) or uterine horns (10%);

(viii) gap in vagina (10%);

  (ix) no external genital papilla or opening (4%).

Many of these abnormalities are reminiscent of the uro-recto-caudal syndrome, as discussed later.

(c) *Surviving homozygotes*

A few homozygotes survive the neonatal crisis and become mature. One female (but no males) has even bred, showing that sterility is not a necessary part of the *Dh* syndrome. This female had sixteen classifiable young, all *Dh/+*; thus her presumed genotype was confirmed. Five homozygous survivors (all leaden) were autopsied. In all, the stomach was very small and the spleen absent. Both kidneys were hydropic in one and the left kidney only was hydropic in three. In the fifth

(which successfully bred) the left kidney was vestigial, while the right was hyper-trophied. This female had a greatly enlarged and fluid-filled left uterine horn ending proximally in a solid strand of tissue running to the bladder. However, the right uterine horn looked normal and had clearly functioned satisfactorily.

The caecum was absent in two of the surviving homozygotes and reduced in two others. In two of the three sterile males the testes were very small and the scrotum poorly developed. Spermatozoa were found, but they were reduced in numbers.

These survivors show that hydronephrosis of both kidneys is not an invariable part of the homozygous syndrome and that, as in the heterozygote, the right kidney tends to be less affected. They also give some idea of what abnormalities are compatible with survival under laboratory conditions. It is indeed surprising that a mouse with only one kidney, one functional uterine horn, a very small stomach, no spleen and very reduced almost useless hind-limbs should still manage to live over a year and produce seven litters of live-born young.

In three of these surviving homozygotes it was noted that the preaxial foot-pads were black and apparently necrotic. This was regularly found also in adult $Dh/+$ mice.

(v) *Developmental studies*

Incipient hydronephrosis could be detected in $15\frac{1}{2}$-day presumptive Dh/Dh embryos after dissection, and absence of spleen in $14\frac{1}{2}$-day ones. In $14\frac{1}{2}$-day embryos, umbilical hernia was much more pronounced as a rule in Dh than in $+$ mice, while even at $17\frac{1}{2}$ days two presumed Dh/Dh foetuses had much of the liver and some of the gut still protruding. Differences in stomach size could still clearly be detected at $14\frac{1}{2}$ days, as shown by Fig. 4. A marked reduction of the hind-limb bud and of foot-plate size could be seen in some $11\frac{1}{2}$-day foetuses, but no difference between Dh and $+$ foetuses has yet been detected at $10\frac{1}{2}$ days, when the hind-limb is repre-sented by a ridge rather than a bud.

Serial sections of presumed Dh/Dh and $+/+$ $12\frac{1}{2}$-day embryos were examined. In Dh, the stomach developed on the inner side of the kidney rudiment, the liver and kidney almost completely separating it from the body-wall. The $+$ stomach, however, was on the outer side of the kidney, so that its outer edge was against the body-wall. In this area of juxtaposition the stomach and peritoneum were thickened, with the thickened portion becoming detached posteriorly and continuing backwards as a separate entity, the spleen. In Dh sections the corres-ponding part of the stomach did not become thickened and detached.

At this foetal age the distal limb skeleton is still precartilaginous. Reduction in the number of mesenchymal condensations in the hind-limb bud was clearly seen in the Dh sections, with a maximum of two distal condensations (representing phalangeal *anlagen*) where there were four in $+$ sections. More proximally there was only one condensation (presumably the fibula *anlage*) where there were two in the $+$ embryo.

In the $+$ embryo both umbilical arteries were of about the same size. They ran alongside the dorsal aorta, then turned ventrally and proceeded mesial to the
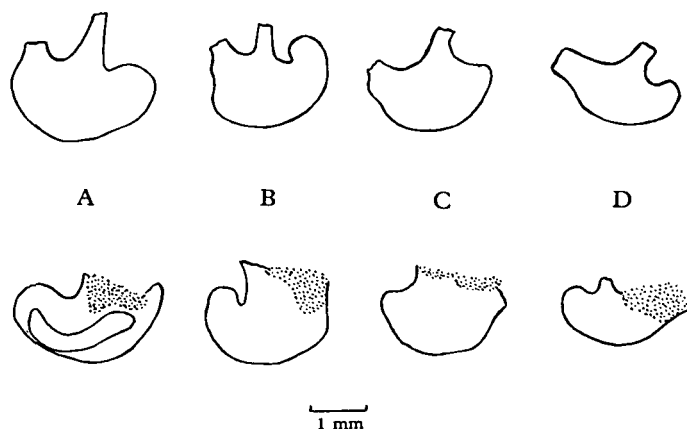
Text-fig. 4. Antero-ventral (above) and postero-dorsal (below) views of stomachs of 14½-day foetal litter-mates, to show smaller stomach and spleen absence in *Dh* mutants. A, normal (with outline of spleen); B, *Dh*/+ with polydactyly; C, *Dh*/+ with oligodactyly: D, *Dh*/*Dh*. Region covered by pancreas is stippled. Camera lucida drawings.

ureters. In the *Dh*/*Dh* embryo, however, the right umbilical artery was much larger than the left, which was extremely small and difficult to follow. The right artery soon left the dorsal aorta, turning ventrally and running on the outside of the ureter.

Thus a preliminary examination has revealed a number of differences between *Dh*/*Dh* and + embryos which need further study.

(vi) *Interaction with luxate*

Alizarin preparations of ten adult *Dh*/+ +/*lx* mice showed complete absence of 19/20 tibiae, while the remaining one was hemimelic: 13/20 femora and 2/20 pubes also showed defects, one pubis being absent. Double heterozygotes were backcrossed to luxate homozygotes. When male heterozygotes were used only one out of eight pairs bred, while two out of three female heterozygotes did so. This sterility was probably due to mating difficulties associated with very reduced hind-legs, as in *Dh*/*Dh* survivors. The fertile matings produced a number of probably *Dh*/ + *lx*/*lx* offspring. These had reduced and twisted hind-legs, high-grade oligodactyly (usually with loss of two preaxial digits) and severe reduction of the stomach.

The very high incidence of oligodactyly and tibial defects in double heterozygotes and the severity of stomach reduction in presumed *Dh*/+ *lx*/*lx* mice suggest a marked interaction between these two genetically independent genes, in which heterozygosity for luxate (which by itself causes mild polydactyly) enhances the oligodactyl and hemimelic tendencies of *Dh* heterozygotes. In addition, homozygosity for luxate apparently tends to enhance the visceral effects of *Dh* heterozygosity.

3. POSTAXIAL HEMIMELIA

(i) *Origin and genetics*

The recessive mutant postaxial hemimelia (*px*) was discovered and named by T. C. Carter at the Institute of Animal Genetics, Edinburgh; it was apparently carried by the first Ragged female (Carter & Phillips, 1954) and may have been induced in her irradiated ancestor.

The most obvious abnormality in homozygotes is laterad deviation of the fore-limb, accompanied by loss of digit V, sometimes also of IV or III + IV (Plate I E). Since homozygotes are sterile the stock must be maintained by breeding from heterozygotes. Carter showed that *px* is closely linked to White (Mi^{wh}) in group XI (Table 7); it has since been maintained by intercrossing $Mi^{wh} + / + px$ mice. In Table 7, recombination fractions have been calculated by the maximum likelihood method described by Mather (1951).

Two other marker genes in group XI are waved-1 (*wa-1*) and Lurcher (*Lc*). Waved-1 shows about 9% recombination with Mi^{wh} (Bunker & Snell, 1948) and lies between the *Mi* and *Lc* loci (Phillips, 1960). Three-point (Mi^{wh}–*px*–*Lc*) and four-point (Mi^{wh}–*px*–*wa-1*–*Lc*) linkage tests showed conclusively (Table 7) that the order is as given below, with the best estimates of map distances using all available data:

$$Mi^{wh} \ldots 2\cdot9 \ldots px \ldots 1\cdot9 \ldots wa\text{-}1 \ldots\ldots 6\cdot4 \ldots\ldots Lc$$

No significant sex differences in recombination frequency were found.

Table 7 I shows that 23·8% of intercross offspring, classified at weaning, were postaxial hemimelics. This does not differ significantly from the 25% expected with undisturbed Mendelian segregation.

(ii) *Morphology of homozygotes*

(a) External appearance

The laterad deflection and reduction of the forefeet in *px* mice, together with a tendency for the forearm to be bent backwards and sometimes inwards, force high-grade postaxial hemimelics to move laboriously on their 'elbows' (Plate I F). Those with less severe defects tend to move with the dorsal or lateral rather than the ventral part of the manus on the ground.

Newborn *px/px* mice were classified for the limb defects, the commonest condition in those affected being loss of digit V on each side (Table 8). 73·7% of right hands lacked at least one digit, but only 50·3% of left hands. As in *Dh*, syndactyly also occurred (in 1–2%) as well as an occasional incomplete digit (in about 2%), usually V.

About 19% of *px* mice showed digital anomalies of the hind-limbs also, usually consisting of reduction or loss of digit V, with occasional postaxial syndactyly. The distribution of these anomalies between sides was as follows:

Both affected	Left only	Right only	Neither	Total
14	48	5	283	350

Table 7. *Linkage tests between* px *and members of linkage group XI*

I. Results of $\dfrac{Mi^{wh} +}{+ px}$ intercrosses

PHENOTYPES OF OFFSPRING

NUMBERS	$Mi^{wh} Mi^{wh} px$	$Mi^{wh} Mi^{wh}$	$Mi^{wh} +$	$Mi^{wh} + px$	$+$	px	TOTAL
	0	892	1839	30	61	842	3664

$$p = 2\cdot61 \pm 0\cdot26\%$$

II. Results of $\dfrac{Mi^{wh} + Lc}{+ px +} \times \dfrac{+ + +}{+ px +}$ crosses

PHENOTYPES OF OFFSPRING

NUMBERS	$Mi^{wh} + Lc$	$+ px +$	$Mi^{wh} + +$	$+ px Lc$	$+ + Lc$	$Mi^{wh} px +$	$+ + +$	$Mi^{wh} px Lc$	TOTAL
	206	84	21	6	26	5	111	0	459

Considering *px* phenotypes, recombination between Mi^{wh} and $px = 5/95$ or 5·26%; between *Lc* and $px = 6/95$ or 6·31% and between Mi^{wh} and $Lc = 11/95$ or 11·58%. Thus order is Mi^{wh}, *px*, *Lc*. The *Lc–px* linkage, when the material is treated as two populations because of shortage of px, $= 6\cdot60 \pm 2\cdot42\%$.

III. Results of $\dfrac{Mi^{wh} + wa\text{-}1 Lc}{+ px + +} \times \dfrac{+ + wa\text{-}1 +}{+ + wa\text{-}1 +}$ crosses

PHENOTYPES OF OFFSPRING

NUMBERS	$Mi^{wh} wa\text{-}1 Lc$	$+ + +$	$Mi^{wh} wa\text{-}1 +$	$+ + Lc$	$Mi^{wh} + +$	$+ wa\text{-}1 Lc$	$Mi^{wh} + Lc$	$+ wa\text{-}1 +$	TOTAL
	173	173	7	15	10	5	1	0	384

Recombination frequency between Mi^{wh} and $wa\text{-}1 = 4\cdot17\%$ and between $wa\text{-}1$ and $Lc = 5\cdot99\%$. Testing 15 crossover progeny to see where recombination had occurred gave 3 Mi^{wh}/px, 4 $px/wa\text{-}1$ and 8 $wa\text{-}1/Lc$ recombinants, confirming that Mi^{wh}–px and px–$wa\text{-}1$ distances were similar and less than $wa\text{-}1$–Lc.

Thus about 18% of left sides were affected but only 5% of right, a highly significant difference, which goes in the opposite direction to the fore-limb asymmetry ($\chi_1^2 = 25\cdot8, P = 10^{-6}$).

There are epidermal as well as skeletal abnormalities of *px* feet, for their upper surfaces show small papillae proximal to the digits and near their base, usually three to each foot. Later they develop dark pigmentation and sometimes a rudi-

Table 8. *Expression of abnormalities of the fore-feet in* px *homozygotes, examined at birth*

|		RIGHT DIGITS				
		V normal or near normal	V absent	V + IV absent	V + IV + III absent	Total
LEFT-DIGITS	V normal or near normal	65	99	10	0	174
	V absent	25	103	17	0	145
	V + IV absent	2	14	12	1	29
	V + IV + III absent	0	0	2	0	2
	Total	92	216	41	1	350

mentary type of claw, so that in the adult 4–6 of these black excrescences can be clearly seen on each foot (Fig. 5 and Plate I E). They consist of a thick cornified layer with a pyramidal core of soft tissue. They were present on hind-feet even when there was no digital abnormality. Claws tend to be malformed, and certain digits of the hind-feet (especially the central ones) show signs of abnormal dorsiflexion in the adult.

(b) Skeletal abnormalities

Fifty-three alizarin and eight papain preparations of the skeleton all showed a large oval *foramen infraspinatum* in the fossa infraspinata of the scapula (Fig. 6) extending from the base of the spine to the posterior edge, which may be incomplete. Sometimes the scapula was shorter than normal, with the spine showing increased curvature and with a particularly large open foramen.

Other fore-limb abnormalities were:

  (i) Distal reduction and distortion of the humerus, associated with absence of the ulna. The trochlea and supratrochlear fossa tend to disappear.
 (ii) Ulnar hemimelia, or (more usually) loss; associated shortening and curvature of the radius.
(iii) Absence of postaxial metacarpals and phalanges; occasional syndactyly due to phalangeal fusion.
(iv) Abnormalities of the carpus (Fig. 7). Postaxial carpal bones tended to be reduced or absent, especially if more than one digit was lost.
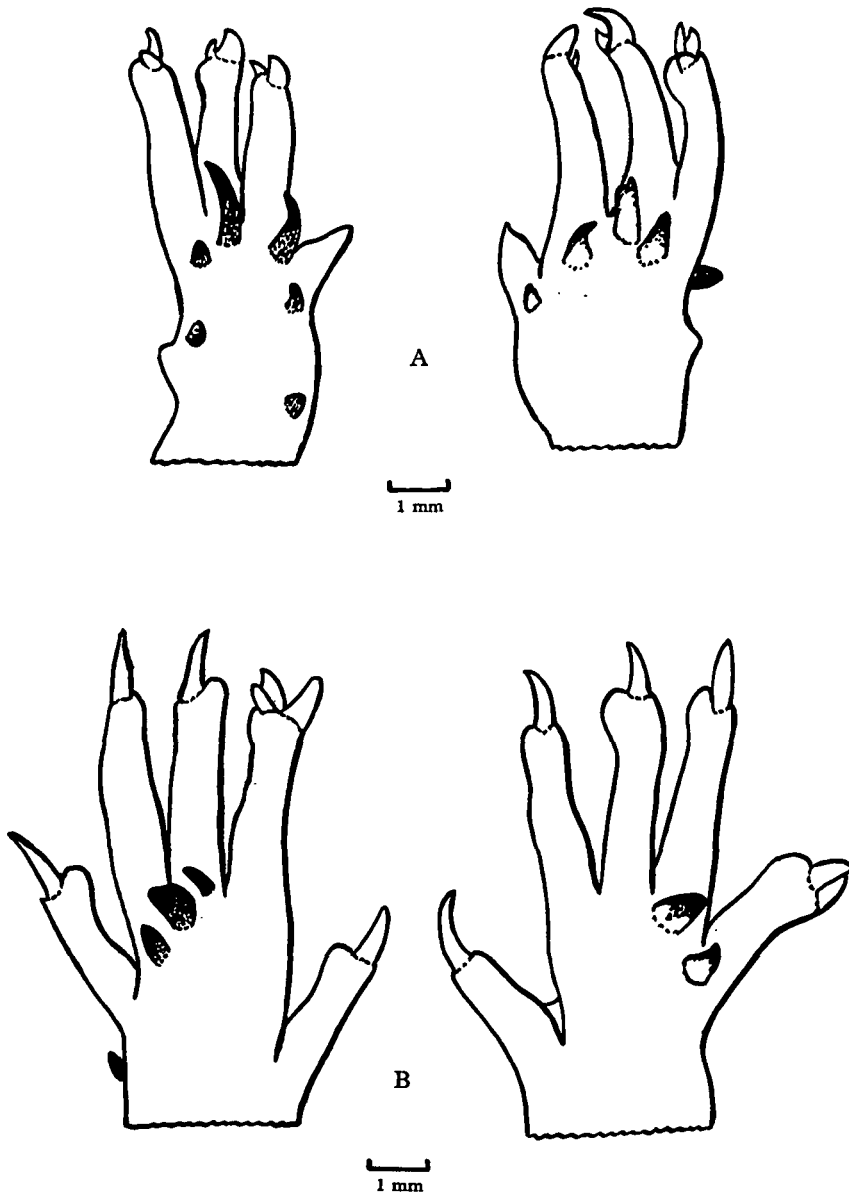
Text-fig. 5. Upper surfaces of left and right forefeet (A) and hind-feet (B) of an adult postaxial hemimelic mouse, showing abnormal black outgrowths and maldevelopment of claws, especially on forefeet. Each manus lacks digit V. Camera lucida drawings.
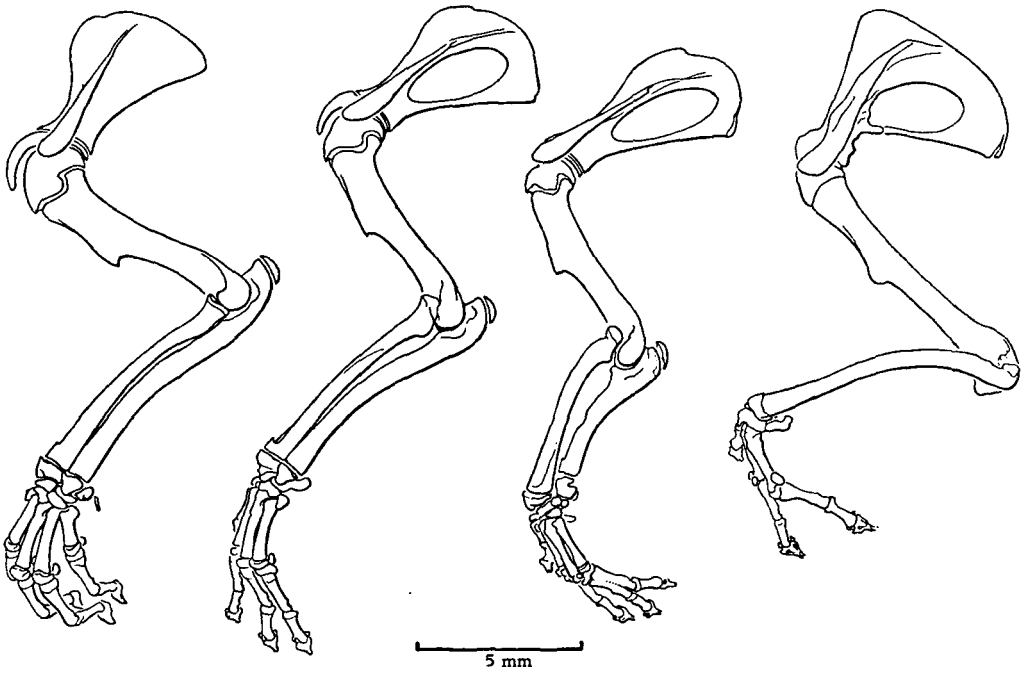
Text-fig. 6. Left fore-limbs of normal (left) and *px/px* mice, to show increasing grades of severity of *px* action, involving reduction and loss of the ulna, distortion of the scapula with presence of a large foramen infraspinatum, also postaxial oligodactyly and syndactyly. Camera lucida drawings.

A   B   C
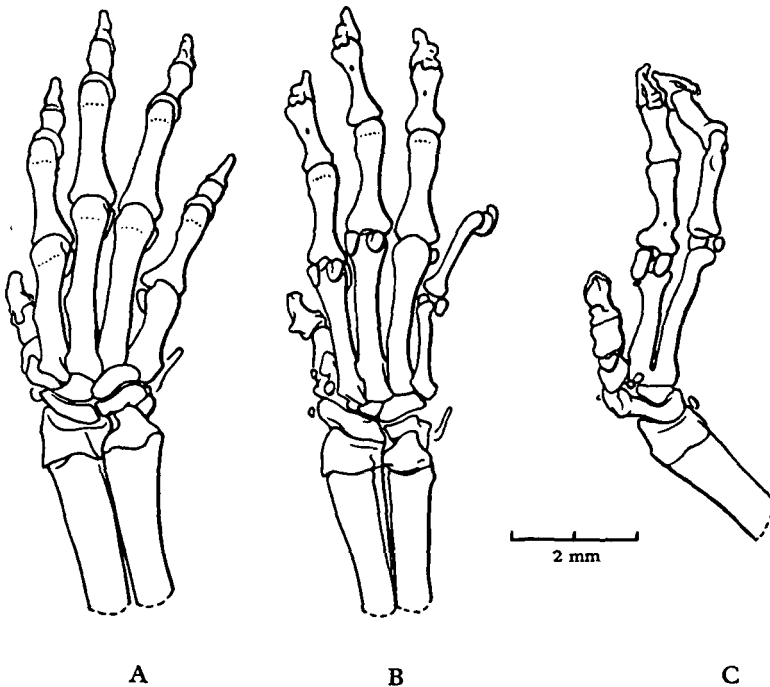
Text-fig. 7. Upper aspects of normal (A) and *px/px* right forefeet. In B, digit V is thin and there are extra pairs of sesamoid bones at the distal ends of metacarpals II and III; in C, digits IV and V are missing, with corresponding carpals, and there are extra sesamoids on II. In B and C small extra projections can also be seen at the base of the distal phalanges. Camera lucida drawings.

Table 9. *Analysis of ulnar abnormalities in alizarin preparations of postaxial hemimelics*

		RIGHT ULNA			
		Normal	Defective	Absent	Total
	Normal	22	1	17	40
LEFT	Defective	0	3	3	6
ULNA	Absent	0	1	5	6
	Total	22	5	25	52

$12 = 23\%$ (for Defective + Absent rows)

$30 = 58\%$ (for Defective + Absent columns)

Table 10. *Analysis of meristic variation (left/right) in axial structures of postaxial hemimelics and normal sibs, based on alizarin preparations. Expression is called heavy when one or both ulnae are missing*

I. *Thoracic rib number.*

Phenotype	Expression	13/13	13/14	14/13	14/14	Total	Mean
+	—	24	0	0	1	25	13·04
px	slight	26	0	0	0	26	13·00
px	heavy	18	0	4	4	26	13·23

II. *Number of lumbar vertebrae*

Phenotype	Expression	6/6	6/5	5/6	5/5	Total	Mean
+	—	17	1	1	6	25	5·72
px	slight	16	1	0	9	26	5·63
px	heavy	1	1	5	19	26	5·15

III. *Number of presacral vertebrae*

Phenotype	Expression	26/26	26/25	25/26	25/25	Total	Mean
+	—	18	1	1	5	25	25·76
px	slight	16	1	0	9	26	25·63
px	heavy	7	3	3	13	26	25·39

(v) Presence of extra sesamoid bones on the extensor surface of the manus (Fig. 7) especially on digits II and III. Extra sesamoid projections on the extensor side of the distal phalanges, with an associated extra foramen, made them dorso-ventrally symmetrical.

Absence of the ulna was usually, but not invariably, associated with high-grade digital defects on the affected side, in particular complete loss of digits IV and V. Table 9 shows that the asymmetrical tendency is even more pronounced with ulnar than with foot abnormalities, 58% of right ulnae being markedly defective or absent, but only 23% of left ones.

As in the manus, accessory sesamoid bones were found on the extensor side of

o

the *px* pes. Again they were commonest on digits II and III. There are also abnormal tendons on the extensor surface of the *px* pes, beneath those of the extensor digitorum longus muscle. They insert on the accessory sesamoids and seem to originate from the base of the second phalanx, running along the upper surface of the first phalanx. No attached muscle was seen; if present it must be very short. The abnormal dorsiflexion of central digits in the hind-feet, mentioned earlier, is presumably due to these abnormal structures.

For analysis of meristic axial variation, postaxial hemimelics were divided into those with one or both ulnae absent (heavy expression of *px*) and those with both ulnae present (light expression). Mean numbers of thoracic, lumbar and presacral vertebrae in mice with light expression of *px* did not differ significantly from normals (Table 10) but severely affected post-axial hemimelics tended to have more thoracic and less lumbar and presacral vertebrae than the other two groups. Numbers of presacral vertebrae showed significant heterogeneity in the three groups ($\chi_1^2 = 11\cdot4$, $P = 0\cdot003$), due mainly to the higher proportion of 25/25 presacral vertebrae in *px* mice with heavy expression.

Mean numbers of sternebrae did not differ significantly in the three groups, being 6·6 in normals, 6·5 in lightly affected and 6·4 in heavily affected hemimelics. Counting was hindered by a tendency for the two sternebrae next to the xiphisternum to fuse; they were considered separate unless the fusion was fairly complete.
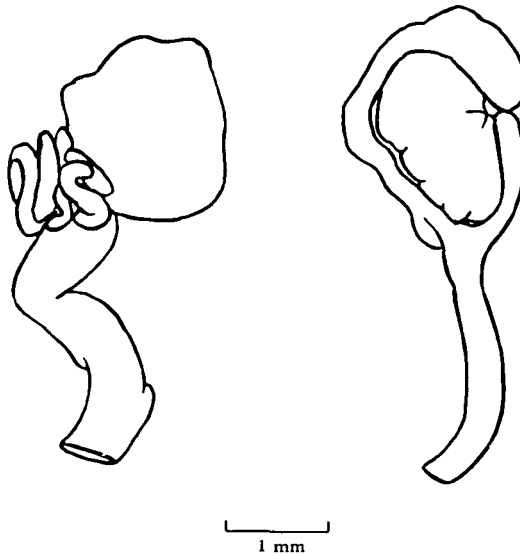
Text-fig. 8. Ventral views of right ovary and oviduct of normal (left) and *px/px* female, showing the absence of coiling in the *px* oviduct and its abnormal course, also lack of the ovarian capsule. Freehand drawing.

(c) *Visceral abnormalities*

Abnormalites of the reproductive system are associated with *px* sterility. In adult females the uterine horns are smaller and less muscular than normal. The
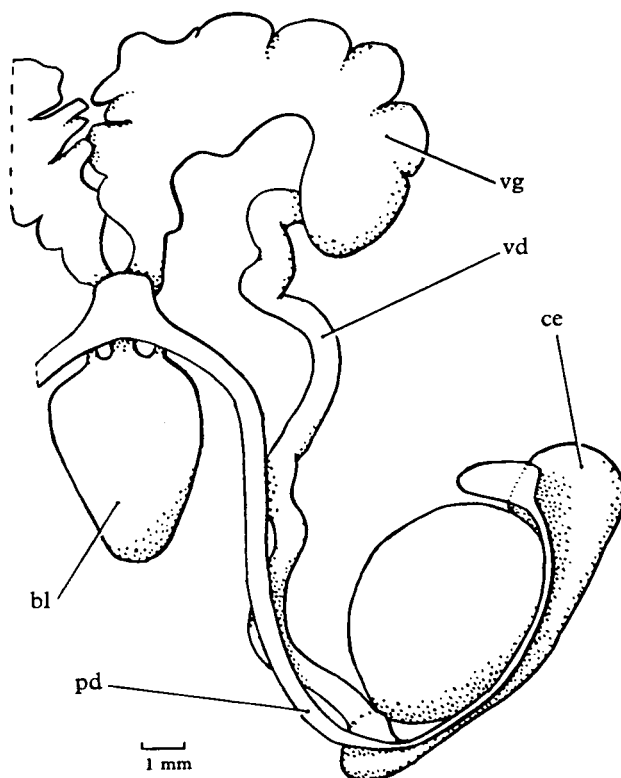
Text-fig. 9. Ventral view of left side of male *px* reproductive system, showing persistence of paramesonephric duct and abnormal course of the vas deferens. Freehand drawing. Key: bl, bladder; ce, caput epididymis; pd, paramesonephric duct; vd, vas deferens; vg, vesicular gland.

uterine (Fallopian) tubes are abnormally short and almost uncoiled (Fig. 8); they seem to end blindly beside or anterior to the ovary. Often they bifurcate before reaching the ovary, while the ovarian capsule may be absent or incomplete. Moreover, dissection of six females revealed that all had complete or partial inner duplication of the vagina. Four of these showed a bridge of tissue across the vaginal portal which extended to form a wall separating the vagina into two halves. Two showed no bridge of tissue on external examination, one half-vagina being imperforate, joining the other by a small opening in the corpus uteri area at the anterior end of the vagina.

Males are also sterile and show reproductive abnormalities. The adult testis looks normal and contains plenty of spermatozoa, with the usual high motility in the vas deferens. But the vas, which normally runs into the urethra joins instead the large vesicular gland ('seminal vesicle') at its distal end (Fig. 9). Free communication between vas and gland was demonstrated in one specimen by the presence of many immobile spermatozoa inside the gland, near to its point of abnormal junction with the vas; normally this gland does not contain sperm, its function being one of secretion rather than sperm storage (Snell, 1941).

Figure 9 also shows a second abnormality. All *px* males have a small rather translucent vessel running alongside each vas deferens. It starts as a swollen transparent sac attached to the caput epididymis and joins its fellow from the opposite side just anterior to the bladder in the neighbourhood of the ampullary glands. It then proceeds dorsally as a single vessel, running into the urethra near
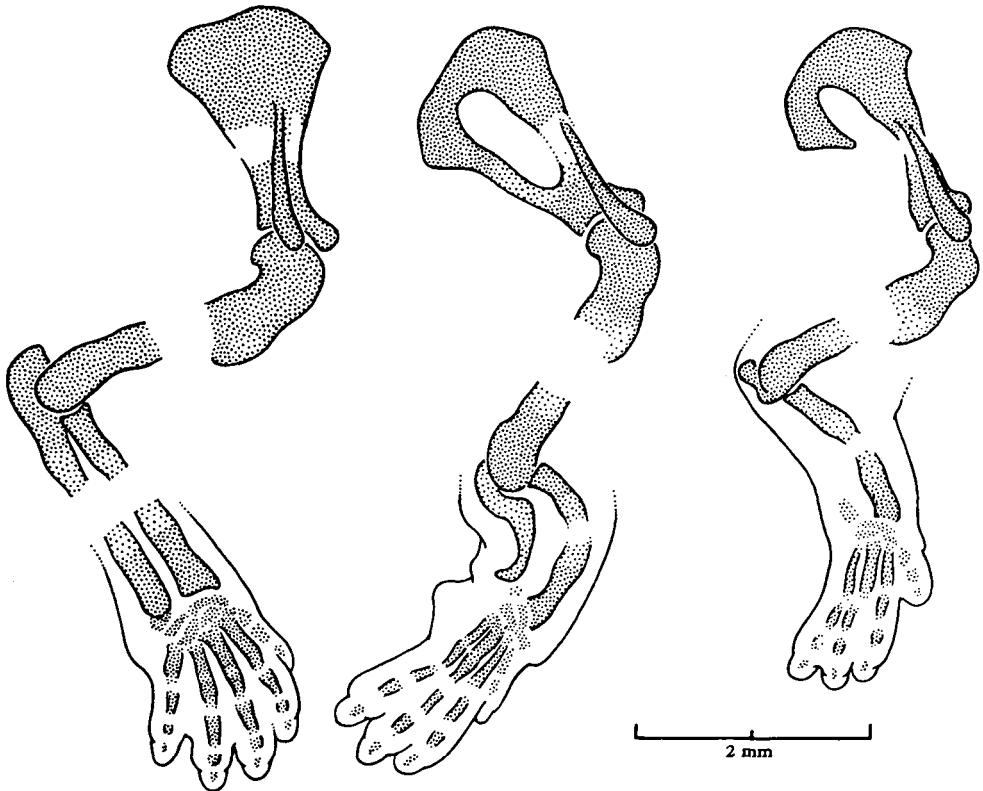
Text-fig. 10. Right fore-limb skeletons of normal (left) and *px/px* 15½-day foetal litter-mates, showing typical postaxial anomalies present in cartilage. Camera lucida drawings.

the entrances of the vesicular and ampullary glands. Sometimes there may be proximal cross-connections between the vas and this accessory vessel. Its position and its resemblance to a vestigial uterus indicate that it is a persistent Müllerian duct.

(d) Development

Examination of litters of embryos segregating for *px* has shown that the action of this gene is clearly discernible at 11½ days of foetal life, when the postaxial quarter of the forefoot-plate was absent in some specimens. In some 10½-day embryos each forefoot-plate only covered 3–4 somites, though it usually covered five. Embryos in the former group were presumably *px/px*. Methylene blue preparations of

$15\frac{1}{2}$-day foetal *px* and normal litter-mates show that the scapular and limb defects are present at the cartilaginous stage (Fig. 10). The state of chondrification in the *px* and normal limbs seem very similar, apart from some retardation in the hemimelic ulna.

(iii) *Interaction with dominant hemimelia*

Crosses between *Dh* and *px* showed no evidence for genic interaction, each gene being able to express itself without affecting the other.

4. DISCUSSION

Both dominant and postaxial hemimelia are similar to other members of the luxoid group in some respects but very different in others. The great similarity between dominant hemimelia and luxate with respect to skeletal effects has already been emphasized; not only is the same region affected, namely the preaxial side of the hind-limbs, but the types and range of abnormality are nearly identical (though *Dh* is somewhat more extreme in homozygous form). Their interaction with respect to skeletal effects provides additional evidence that their normal alleles are both important components of the same epigenetic pathway for full development of the preaxial side of the hind-limb. Sometimes this pathway remains within normal limits even if one normal allele is absent, as shown by occasional $Dh/+$ and $lx/+$ normal overlaps. But the pathway is always abnormal if two normal alleles are missing, as in Dh/Dh, lx/lx or $Dh/+lx/+$ mice.

One other clear resemblance between *Dh* and *lx* is that both tend to cause hydronephrosis. Carter (1953) associated this condition in luxate with lumbar shortening. The fact that in *Dh* homozygotes both hydronephrosis and lumbar shortening are more extreme is additional evidence for this hypothesis. In *Dh*, however, we are confronted with a galaxy of other abnormalities, from invariable absence of spleen and reduction of stomach to many kinds of posterior visceral abnormalities in homozygotes.

The spleen anomaly is unique, in the sense that no other mutant shows the same deficiency, although the spleen is small and deformed in oligodactyl mice (Freye, 1954), this being a luxoid mutant affecting the postaxial side. The spleen in mice is a site of extramedullary erythropoiesis even in the adult, as well as manufacturing lymphocytes, removing waste products from the blood and acting as a blood reservoir. The survival of mice which have been genetically spleenless *ab initio* rather than just splenectomized, shows that these splenic functions do not play a vital part at any stage of development of growth. They may, however, be connected with the slight retardation and extra mortality of *Dh* heterozygotes.

The spleen loss, reduction in stomach size and kidney flattening are undoubtedly closely connected effects. The spleen first arises as a rapidly multiplying mesenchymal mass in a bulge of the dorsal mesentery of the stomach, very close to the stomach itself. The position of the *Dh* stomach seems abnormal in development; if so, this displacement may well be the cause of spleen absence, by preventing a normal inductive process from taking place. The left kidney flattening may be

connected with its close proximity to the stomach in the absence of the spleen between them and with a probable reduction in space available, as suggested by the decreased average number of lumbar vertebrae.

The various rectal and urogenital anomalies found in *Dh* homozygotes, such as confluence of rectum and vagina, and the blind ending of various vessels, are reminiscent of the 'uro-recto-caudal syndrome' (cf. Grüneberg, 1952), apart from the absence of tail anomalies. This syndrome is found in Danforth's short-tail (*Sd*) homozygotes and in urogenital (*ur*) homozygotes, especially when the latter mice carry dominant and recessive alleles at the Brachyury (*T*) locus, e.g. T/t^1. In such mice there is often a failure of separation of the urogenital sinus from the hind-gut, as well as intestinal atresia, hydropic kidneys, missing bladder, imperforate anus, abnormal genital ducts, luxated hind-legs and other anomalies as more or less frequent pleiotropisms. Each of the anomalies sometimes (or always) occurs in *Dh* homozygotes. The uro-recto-caudal syndrome is even found in some homozygotes for postaxial oligodactylism (*o*), which have a short and kinky tail and the same loss of postaxial digits as occasionally occurs in *ur* compounds.

Thus two different genes, *Dh* and *o*, now link the luxoid group with a group of tail mutants *via* the uro-recto-caudal syndrome. The fact that a multiplicity of genotypes can produce a very similar syndrome suggested to Dunn & Gluecksohn-Schoenheimer (1944) that 'the abnormality arises whenever some process in early development is sufficiently deflected from its normal path and that the different mutations affect this process in different degrees'. They considered that this process involved an inductive relationship connecting development of the posterior gut and urogenital system with that of the axial system. In *Dh* homozygotes, however, the axial system is not affected, except meristically.

In its effect on the limb skeleton, postaxial hemimelia resembles oligodactyly rather closely. However, the effect on the hind-limbs is usually more severe in oligodactyly, with more extreme reduction in number of digits and with occasional loss or hemimelia of the fibula. The axial skeleton is also affected, with deformation of tail vertebrae, and the 13th rib tends to be missing. Thus the average number of thoracic vertebrae must be reduced, while it tends to be increased with the more severely affected postaxial hemimelics. In addition, the left side tends to be more reduced in oligodactyl mice than the right side, while in postaxial hemimelics the right side of forefeet and the left side of hind-feet are more heavily affected on the average. This opposite behaviour of fore- and hind-limbs was also found by Deol (1961) in Tail-short mice, the left fore-leg being shorter than the right but the right hind-leg being shorter than the left. It is interesting to note that this mutant (*Ts*) very occasionally shows preaxial triphalangy or oligodactyly of the forefeet, the latter being associated with radial hemimelia. Thus *Ts* could be regarded as a luxoid gene with low penetrance. It also increases the number of thoracic vertebrae.

Postaxial hemimelia and oligodactyly are completely different in their visceral effects, those of *px* being confined to the reproductive tract. In both sexes there seems to be a tendency for the paramesonephric (Müllerian) ducts to persist in a quasi-embryonic and at the same time a primitive condition. For these ducts are

laid down in the embryo as paired structures separate throughout their length. In female monotremes and marsupials (Didelphia) this paired condition persists, but not in eutherian mammals. In male mammals, apart from monotremes, the paramesonephric ducts wholly or partly disappear, persisting in some as the appendix testis close to the caput epididymis and the glandular or sac-like utriculus prostaticus (uterus masculinus) near the urethra. This is normally absent in the mouse, as Eckstein & Zuckerman (1956) point out in their discussion of these vestigial structures. In the postaxial hemimelic male, however, the paramesonephric duct persists throughout its length, ending distally in a translucent sac-like structure exactly where the appendix testis is found in those mammals possessing it. The mesonephric duct forms the vas deferens, but this opens into the distal end of the vesicular gland instead of separately into the urethra. The sterility of the male, despite the presence of an abundance of motile sperm in the vas deferens, is probably due to this diversion in the course of the vas, resulting in loss of sperm motility.

The production of abnormal sesamoids, tendons and epidermal outgrowths on the extensor surfaces of *px* feet suggest that this mutation leads to a partial epigenetic inability to distinguish extensor from flexor sides of each foot. For upper outgrowths resemble lower foot-pads (differences being explicable by the different environment), while upper sesamoids are mirror images of lower ones.

Table 11 compares the morphological effects of luxoid genes in the mouse, the luxoid mutant of Kobozieff and Pomriaskinsky-Kobozieff (1953) being omitted, as it is very similar to luxoid (*lu*) and has not yet been tested for genetic identity with it. It can be seen that all the genotypes considered have an effect on the hindlimbs (sometimes with normal overlaps) but only about half affect the fore-limbs; similarly preaxial defects are much commoner than postaxial ones, the two types being mutually exclusive. Preaxial polydactyly is particularly common, while postaxial polydactyly is not found at all. In other mammals also polydactyly tends to be preaxial rather than postaxial.

In three of the luxoid mutants there is an association between polydactyly and oligodactyly, the latter tending to appear (or be more pronounced) when the mutant gene dosage is increased. Oligodactyly is nearly always associated with hemimelia of the corresponding long-bone, but hemimelia is always associated with polydactyly in *lst/lst* mice and frequently associated with polydactyly in *lx/lx* and *lu/lu* mice. These associations have been discussed by Forsthoefel and others. Forsthoefel (1958, 1962) and Green (1955) have shown interaction between *lu*, *lx* and *lst* with respect to skeletal effects on the limbs, with cumulative action in double heterozygotes. *Dh* and *lx* also interact, as shown in this paper; so it can be assumed that these four genes have a close epigenetic relationship.

In *lx*, *lu*, *Dh* and *px* genotypes the mean number of presacral vertebrae is changed, probably in *o* also; the pelvic shift tends to be greater when the limb is most affected. There is no doubt that the number of presacral vertebrae can be altered by a mutant gene which has no effect on the limbs (Grüneberg, 1955) but this does not exclude the possibility that some of the ways in which such a change could arise in development would also lead to limb abnormalities of the luxoid type. Carter

Table 11. *Comparison of the pleiotropic effects of six luxoid genes in the mouse. $+++$ = heavily affected, $++$ = affected, $(+)$ = effect slight or very occasional. With presacral vertebrae, d = number decreased and i = number increased*

Structure affected or nature of abnormality	Luxate		Luxoid		Strong's luxoid		Oligodactyly	Dominant hemimelia		Postaxial hemimelia
	$lx/+$	lx/lx	$lu/+$	lu/lu	$lst/+$	lst/lst	o/o	$Dh/+$	Dh/Dh	px/px
Fore-limbs	−	−	−	+	(+)	+	+	−	−	+
Hind-limbs	+	+	−	+	+	+	+	+	+	+
Preaxial side	+	+	+	+	+	+	−	+	+	−
Postaxial side	−	−	−	−	−	−	+	−	−	+
Polydactyly	+	+	+	+	+	++	−	+	−	−
Oligodactyly	−	+	−	+	−	−	+	+	++	+
Hemimelia	−	+	−	+	−	+	+	+	++	+
Limb-bone duplication	−	−	−	−	−	+	−	−	−	−
Extra sesamoids	−	−	−	−	−	−	−	−	−	+
Tail-kinks	−	−	−	+	−	−	+	−	−	−
Presacral vertebrae	d	d	i	i	−	−	d?	d	d	d
Skull	−	−	−	−	−	+	−	−	−	−
Stomach	−	−	−	−	−	−	−	+	++	−
Spleen	−	−	−	−	−	−	−	++	++	−
Kidney	(+)	+	−	−	−	−	+	+	++	−
Reproductive system	−	−	−	−	−	+	−	+	+	+
Sterility in male	−	−	−	−	−	+	+	−	+	+
Sterility in female	−	−	−	−	−	−	+	−	+*	+
Uro-rectal defects	−	−	−	−	−	−	+	−	+	−
Umbilical shift	−	−	−	−	−	++	−	−	−	−
Epidermal defects	−	−	−	−	−	++	−	−	−	+
Anaemia	−	−	−	−	−	+	−	−	−	−
Usual lethality	−	−	−	−	−	−	+	−	+	−

* With one exception.

(1954) used the craniad shift of the hind-limb girdle in luxate as the basis for his unitary hypothesis to explain the luxate syndrome. This hypothesis was criticized by Zwilling & Ames (1958) as a result of experimental embryological studies on the chick, but no satisfactory alternative theory has yet taken its place.

As a contrast to the similarity in skeletal effects, luxoid genes show a remarkable diversity in visceral and other effects, with renal abnormalities and male sterility as the commonest components. This diversity shows what a complex network of gene interactions must underlie developmental pathways, as discussed by Waddington (1957). But the task of disentangling the cross-connections is only just beginning.

SUMMARY

1. Dominant hemimelia (*Dh*) and postaxial hemimelia (*px*) both belong to the luxoid group of mouse mutants, tending to cause luxation of limb-bones associated with hemimelia and polydactyly or oligodactyly.

2. *Dh* is about 4·8 units from leaden in linkage group XIII, while *px* is about 2·9 units from the microphthalmia locus in linkage group XI.

3. The nature and range of skeletal anomalies found in *Dh* heterozygotes closely mimic those found in luxate heterozygotes and homozygotes. As in luxate, only the preaxial side of the hind-limb is affected, with (i) polydactyly or oligodactyly (sometimes also syndactyly), (ii) tibial hemimelia, (iii) reduction and fragmentation of the femur, (iv) reduction of the pubis. The more proximal the anomaly the less frequently does it occur; about 4 % of *Dh*/+ mice show no limb anomaly. The defects in *Dh* homozygotes are similar but usually more extreme, with severe oligodactyly, loss of tibia, fragmentation of femur and reduction of pubis.

4. All mice carrying *Dh* lack the spleen. Stomach size is reduced slightly in *Dh*/+ and greatly in *Dh*/*Dh* mice. In *Dh*/+ mice, the left kidney is either flattened antero-ventrally or (less frequently) hydropic; the right seems normal. *Dh*/*Dh* mice nearly always have severe hydronephrosis, as well as posterior visceral defects similar to those of the 'uro-recto-caudal syndrome'. They usually die before weaning, but a few survive to maturity and a female has even bred.

5. Preliminary developmental studies show that *Dh*/*Dh* skeletal defects can be traced back to the precartilaginous stage.

6. When homozygous, *px* affects the postaxial side of the fore-limb and sometimes of the hind-limb also; a large 'foramen infraspinatum' is always present in the scapula. There may also be (i) oligodactyly and occasional syndactyly, (ii) ulnar hemimelia, (iii) distal reduction and distortion of the femur. Fore-limbs tend to be more severely affected on the right, but hind-limbs on the left. Extra sesamoid bones occur on the extensor side of digits in all four feet and are associated with extra tendons.

7. Both sexes are sterile and show anomalies of the paramesonephric (Müllerian) ducts. In the female, the vagina is wholly or partly double and the uterine tubes uncoiled, with an abnormal relationship to the ovary. In the male the paramesonephric duct persists in the adult, while the vas deferens runs into the distal end of

the vesicular gland instead of into the urethra. The epidermis of manus and pes shows abnormal dark papillae on the extensor side.

8. The mean number of presacral vertebrae is reduced by 0·8 in $Dh/+$ and 1·6 in Dh/Dh mice. It is also reduced in high-grade postaxial hemimelics.

9. The relationships of these two genes to other luxoid mutants are discussed.

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