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Genetical studies on the skeleton of the mouse

XXVII. THE DEVELOPMENT OF OLIGOSYNDACTYLISM

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INTRODUCTION

It has been shown repeatedly in this series of papers that in many 'skeletal' mutants the skeleton is only secondarily involved. Indeed, contrary to what one might expect from the form-giving solidity of the adult skeleton, the membranous skeleton is vulnerable and easily disturbed by anomalies in its vicinity. This is strikingly illustrated by the case of syndactylism (*sm*/*sm*) in the mouse (Grüneberg, 1956, 1960) where fusions between adjacent digits (and occasional tail kinks) can be traced back to a hyperplasia of the epidermis which is present before there is any sign of blastemata in the limb buds. The case of Oligosyndactylism (*Os*/+) to be described in this paper is similar in that the limb skeleton seems to be only secondarily involved. However, the underlying mechanism is quite different as is the anatomical situation in the adult animal.

ADULT ANATOMY

As described in detail previously (Grüneberg, 1956), all four feet of *Os*/+ heterozygotes are regularly affected, the hind limbs more severely than the fore limbs. Syndactylism is usually confined to digits II and III. Hard-tissue fusions between these digits generally start at the basal phalanges and thence spread distally; in more severely affected feet, the metacarpalia or metatarsalia II and III are also more or less completely fused. Loss of a digit may thus arise by fusion between digits II and III. However, digit II is often thinner than normal and may vanish without fusion with digit III. Sometimes blastemal material of digit II seems to become incorporated in digit I which may then be duplicated; the resulting loss of digit II associated with duplication of digit I leads to a pentadactyl though very abnormal limb. Metacarpale V and metatarsale V are nearly always proximally fused with metacarpale IV and metatarsale IV respectively in a state of abduction; this is partly attributable to a reduction of the normal locking mechanism between these elements, and partly to lack of proximal support by the neighbouring carpalia and tarsalia respectively which are reduced in size. Extensive fusions regularly occur in carpus and tarsus. All fusions between metacarpalia and metatarsalia are secondary, i.e. they result from the concrescence of elements at first laid down as separate cartilages. However, cuboideum and cuneiforme III are primarily fused, i.e. they are represented by a single cartilaginous element from the beginning, and the same

applies to some, at least, of the fused phalanges. No other skeletal abnormalities have been discovered.

EXTERNAL FEATURES OF *Os*/+ EMBRYOS

A description from the 14-day stage onwards has been given previously. The hands and feet of 13-day *Os*/+ embryos (Fig. 1) can easily be distinguished from those of their normal litter-mates by a flattening of the preaxial border (on top in

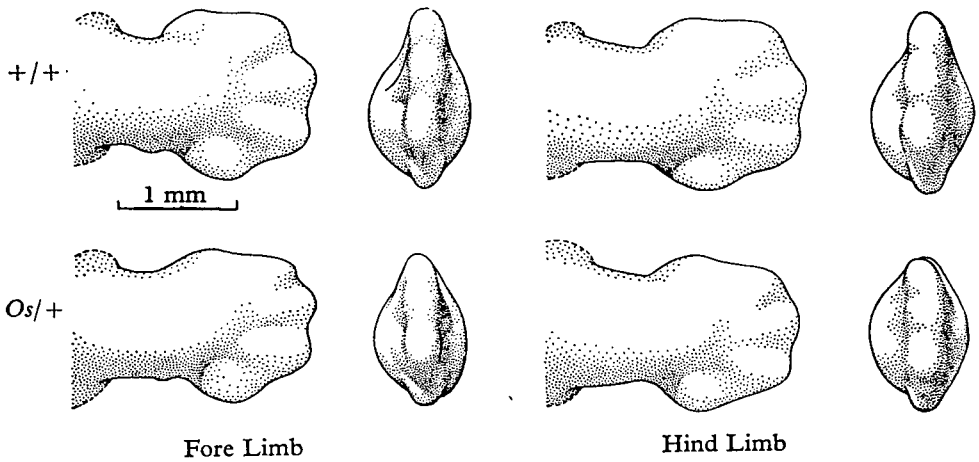

Fig. 1. Right fore and hind limbs of 13-day +/+ and *Os*/+ embryos (litter-mates). Camera lucida drawings.

Figs. 1–4) whereas the rest of the foot is little, if at all, affected. In cross-section, *Os*/+ limbs are about as thick as those of normal embryos: the defect on the preaxial border is thus not compensated by excess material elsewhere and represents a real reduction in size of hands and feet.

The same situation is already encountered in the 12-day stage (Fig. 2). The

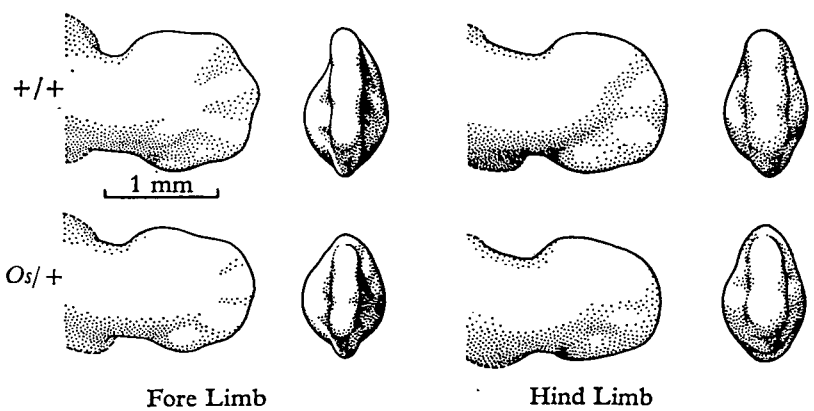

Fig. 2. Right fore and hind limbs of 12-day +/+ and *Os*/+ embryos (litter-mates). Camera lucida drawings.

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preaxial border of hands and feet is flattened to just about the same extent as in the 13-day stage.

In the 11-day stage (Fig. 3), the foot plate of the fore limbs of normal mouse embryos is circular in outline and can just be distinguished from the rest of the

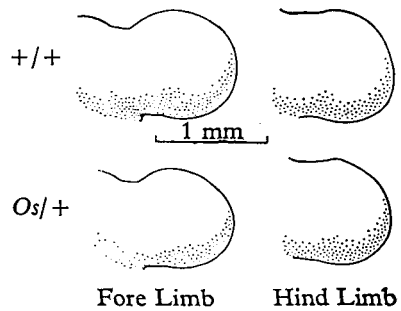

Fig. 3. Right fore and hind limbs of 11-day embryos (litter-mates). Top row putative normal (+/+); bottom row putative *Os*/+ embryo. Camera lucida drawings.

limb; in the hind limbs there is not yet any separation into foot and leg. In segregating litters, *Os*/+ embryos can be distinguished from the rest by a flattening of the preaxial border of the fore limbs so that the outline is ovoid rather than circular; in the hind-limb buds a corresponding difference seems to be just appearing, but, if real, it is still very slight. The difference between putative normal and putative *Os*/+ embryos is more conspicuous when the outlines of the limbs are superimposed on each other (Fig. 4). The incidence of the two types of embryos is in reasonable

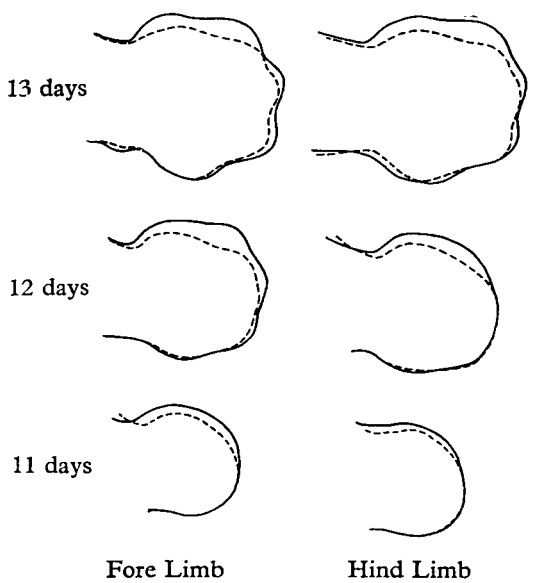

Fig. 4. Outline drawings of the right fore and hind feet of normal (solid lines) and *Os*/+ embryos (broken lines) superimposed on each other. Same specimens as in Figs. 1–3. Note that, throughout, the preaxial area of the *Os*/+ foot plates (on top in drawings) is reduced whereas the remainder of the feet is virtually normal.

agreement with the Mendelian expectation. Four *Os*/+ × *Os*/+ litters included fourteen putative *Os*/+ and four putative +/+ embryos; one *Os*/+ × +/+ litter included similarly three *Os*/+ and four +/+ embryos. If the identification of the *Os*/+ embryos at the 11-day stage is correct, the flattening of the preaxial border is detectable as soon as the foot plates become distinguishable as separate structures, i.e. before any membranous skeleton can be detected in them by standard histological techniques.

An attempt to identify younger (9½–10-day) *Os*/+ embryos by external inspection has not been successful. Eleven embryos from *Os*/+ × +/+ and twenty-seven from *Os*/+ × *Os*/+ matings showed no obvious differences attributable to segregation in the shape of the limb buds.

SECTIONED *Os*/+ EMBRYOS

The sectioned material is summarized in Table 1. In each case but one, all four limbs were sectioned, so that the total number of limbs examined is 183. Normal and *Os*/+ embryos were litter-mates throughout.

Table 1. *Normal and* Os/+ *embryos sectioned*

Age	Litters	+/+	*Os*/+	Total
15	1	1	2	3
14	3	3	4	7
13	3	5	8	13
12	5	9	10	19
11	1	2	2	4
Total	13	20	26	46

The situation encountered in the limbs of 13- and 12-day-old embryos (Figs. 5–10) is similar in its main features though it somewhat differs in degree. Nearly always, digits II and III are closer together than in normal feet and tend to be parallel to each other, whereas normally these elements include an angle of roughly 20°. The fore limbs (left half of Fig. 5, Fig. 7) tend to deviate less from the normal pattern than the hind limbs (right half of Fig. 5, Fig. 6), as in the adult animal. The angle included by digits III and IV also tends to be rather smaller than normal, but the difference is much less; this again is in agreement with the fact that syndactylism between these digits happens only rarely. Digits IV and V are never webbed and the angle included is about normal. It is obvious that the crowding together of digits II and III (and, to a much lesser extent, of digits III and IV) is the direct consequence of the fact that these digits develop in a foot plate which is reduced in size preaxially. In the 13-day embryo, chondrification has started in metacarpalia II–IV, but not yet in the corresponding metatarsalia; there is no chondrification in the feet of 12-day embryos. As the near-parallel arrangement of digits II and III is clearly present as soon as the blastemata become visible (see particularly Fig. 9), it is obvious that the cartilaginous and indeed the osseous skeleton merely repeats a pattern already present in the membranous skeleton;

and that the membranous skeleton itself is forced to deviate from normality by the pre-existing flattening of the preaxial border of the foot plate: for the shape anomaly

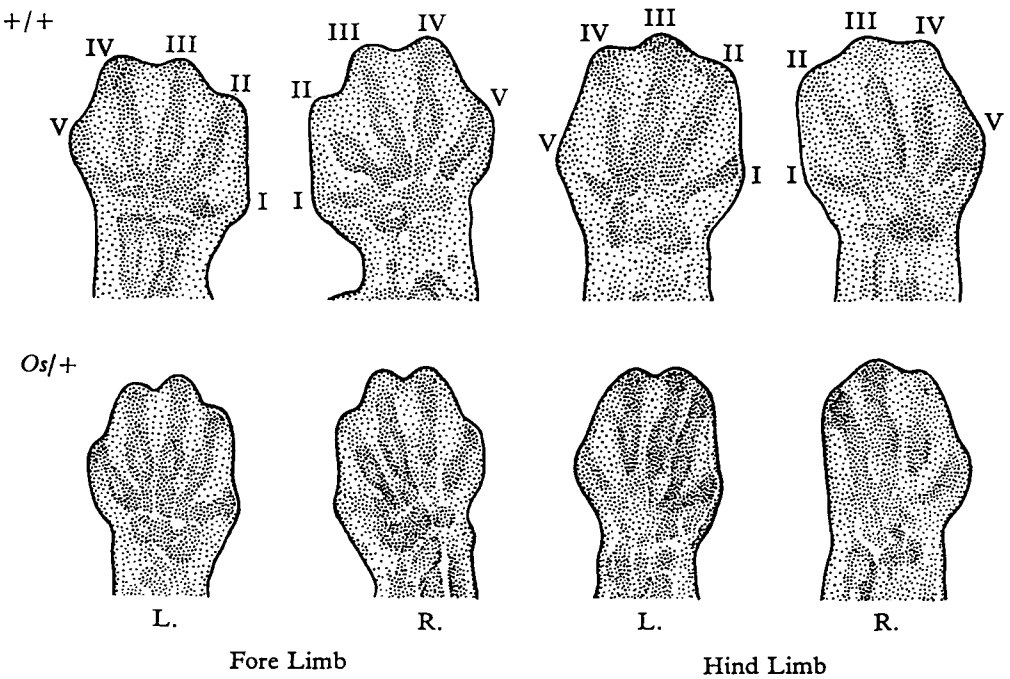

Fig. 5. Sections through the feet of a normal and an *Os*/+ embryo (13-day-old litter-mates, C.R.L. 8·6 and 9·0 mm. respectively). Figs. 5–10 are drawings based on microphotographs. Final magnification × 21.

of the foot plates can be detected in the 11-day stage, i.e. before any condensation of mesenchyme has taken place.

Evidently, a moderate degree of approximation between digits II and III will lead to soft-tissue syndactylism. When the two elements are closer together (such as in the hind limbs, Fig. 5), a common basal phalanx would presumably have been

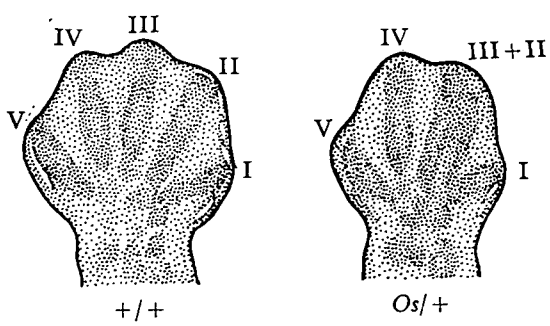

Fig. 6. Sections through the left hind limbs of a normal and an *Os*/+ embryo (13-day-old litter-mates).

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formed. It is also understandable that only in such cases in which all the phalanges are fused will the metacarpals or metatarsals be close enough together to coalesce

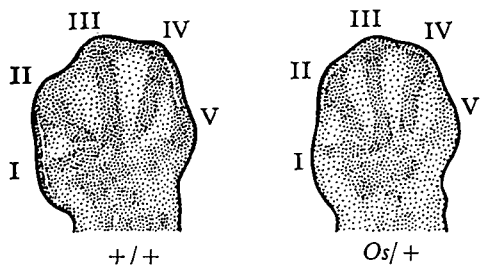

Fig. 7. Sections through the right fore limbs of a normal and an *Os*/+ embryo (12-day-old litter-mates, C.R.L. 7·2 and 7·9 mm. respectively).

with each other. Such a state is apparently reached in the limb shown in Fig. 6 which would almost certainly have become a case of oligodactylism by fusion between digits II and III.

The flattening of the preaxial border of the limbs leads not only to a crowding of

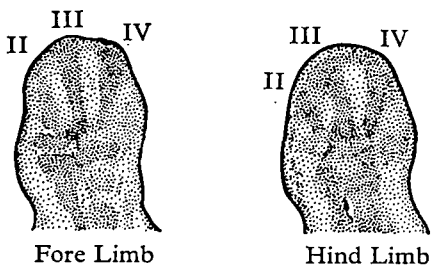

Fig. 8. Sections through the right fore and hind limbs of an *Os*/+ embryo, 12 days old (C.R.L. 7·7 mm.).

the blastemata, but also to a reduction of the mesenchymal material available for skeletal development. It seems that in many cases mesenchyme normally used for the interdigital area between digits II and III is incorporated into digit II which is then of normal or near-normal calibre. In other instances, digit II is clearly thinner

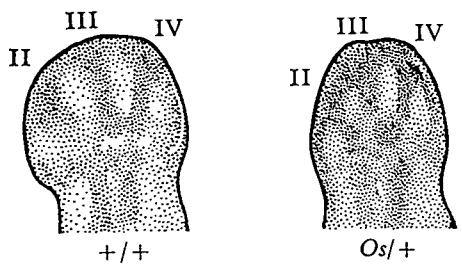

Fig. 9. Sections through the right hind limbs of a normal and an *Os*/+ embryo (12-day-old litter-mates, C.R.L. 7·4 and 7·2 mm. respectively).

than digit III, as in Fig. 6 and in Fig. 10 (see also Figs. 12 and 13, p. 128, in Grüneberg, 1956). Such small elements have a tendency to fuse with their neighbours (as in Fig. 6 in which this process is almost complete). Where small blastemata remain independent, they may fail to chondrify because they fall below a critical threshold of size. Perhaps, in some cases, small blastemata may secondarily disintegrate again by migration of mesenchyme cells towards vigorously-growing larger neighbours (competition between blastemata in the sense of Tschumi, 1954). In either of these two cases, true oligodactylism of digit II is clearly due to the reduction of mesenchymal material in the preaxial area of the foot plates.

In the large majority of cases, digit II is formed closer than normal to digit III. An exception is shown in the right hind limb of Fig. 10 where digit II is abnormally close to digit I. Evidently this is a situation which may lead to oligodactylism of digit II associated with polydactylism of digit I as described previously (Grüneberg,

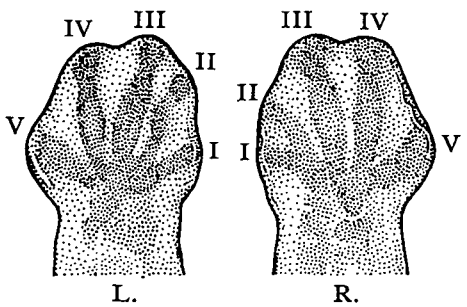

Fig. 10. Sections through the hind limbs of a 13-day-old *Os*/+ embryo (litter-mate to those in Fig. 5; C.R.L. 8·3 mm.).

1956); the material for digit II coming under the 'influence' of digit I and forming an additional hallux rather than a digit II.

Attempts to discover the cause of the preaxial reduction of the foot plates have not been successful. It is probably safe to say that there are no gross pathological lesions which could be held responsible. There is no pathological cell pyknosis, and the vascular system and the apical ectodermal ridge do not appear to be grossly abnormal. On the other hand, the possibility of course remains that a more detailed study, particularly with the aid of more refined (histochemical, etc.) techniques, may be more successful.

DISCUSSION

The first anomaly of *Os*/+ embryos discovered is a reduction of the preaxial margin of the foot plates in the 11-day stage, i.e. before any condensation of mesenchyme has taken place. The whole complex morphology of the adult limb skeleton can be understood as a consequence of this simple initial disturbance.

The preaxial reduction of the foot plates diminishes the space and the material available for the formation of the foot skeleton. Most affected is digit II which is usually shifted towards digit III; depending on the degree of this shift, this leads to webbing of the soft tissues, to cartilaginous and subsequently osseous fusions

between phalanges, and ultimately, when metacarpals and metatarsals are also involved, to oligodactylism by fusion between digits II and III. Sometimes, digit II is deflected towards digit I, and then differentiates into a hallux. Where digit II is reduced in calibre or altogether absent as a hard structure, this is clearly due to a reduction of the mesenchyme available. Presumably the same causes are also responsible for the extensive fusions in carpus and tarsus. Unfortunately, the obscurity which shrouds the early development of the mammalian carpus and tarsus (Holmgren, 1933, 1952; Schmidt-Ehrenberg, 1942; Milaire, 1956) makes direct proof virtually unobtainable.

A condition strikingly similar to *Os*/+ has been obtained by Tschumi (1954), who treated the early limb buds of the clawed toad (*Xenopus laevis*) by means of a mitotic poison; the foot plates which subsequently developed were reduced preaxially, but not postaxially, and showed all stages from webbing of toes to complete fusion, particularly of digits I and II; in addition, toes may be reduced in calibre down to vanishing point; tarsal fusions were also common. Evidently, organisms as different as *Xenopus* and the mouse react to diminution of the preaxial region of the feet in much the same way; i.e. they use space and material at their disposal according to the same rules which obtain in normal development. Tschumi suggests that, in *Xenopus*, a generalized reduction of the limb is secondarily localized on the preaxial side by a process of competition: the digits which differentiate first (III–V) seem to use up about as much mesenchyme as if they developed in a normal limb and thus do not leave enough material for digits I and II.

The same idea has more recently been discussed by Forsthoefel (1959) in relation to the development of the gene for luxoid (*lu*/*lu*) in the mouse. 'The reduction in limb bud size would of itself be expected to result in preferential loss of the preaxial portions of the limb since condensation of blastemata begins in the postaxial portion. Thus the fibula and the lateral digits are first laid down. If no further material is available, the preaxial tibia and the medial digits will be missing. This may explain also why in the mouse anomalies in limb development are almost always preaxial. Excess or defect of material would be expected to affect the elements last laid down.' While Tschumi (1954) may well be right for *Xenopus*, I rather doubt whether Forsthoefel is for the mouse: in the first instance, in the mouse digits II–IV seem to be laid down first (see, e.g., Fig. 9 above), and both marginal rays follow later; hence Forsthoefel's argument could apply to both preaxial and postaxial defects. Moreover, in *Os*/+ at any rate, and probably in most of the entities to be discussed presently, defect or excess material can be detected on the preaxial margin before the onset of blastema formation; hence the anomaly is localized in the foot plate from the start, and not only secondarily as the result of competition for a limited amount of mesenchyme. Postaxial defects, incidentally, are not very rare in the mouse. There is oligodactylism (*ol*/*ol*; Freye, 1954), postaxial polydactylism (*tu*/*tu*; Center, 1955) and postaxial hemimelia (*px*/*px*; T. C. Carter, unpublished).

Inherited preaxial limb anomalies have been described in a variety of animals. Those studied embryologically include the genes for luxate (*lx*/*lx*; Carter, 1954) and for luxoid (*lu*/*lu*; Forsthoefel, 1959) in the mouse in which defects of the zeugo-

podium are associated either with defects or with excess formations (polyphalangy, polydactylism) of preaxial digits. Preaxial polydactylism as part of a complex syndrome has been studied in the guinea-pig (Scott, 1937) and, as a separate entity, in the mouse (Chang, 1939), in the cat (Danforth, 1947) and in the chicken (Zwilling, 1956; Zwilling & Hansborough, 1956; and earlier authors). In all these cases there is defect or excess of limb material before condensations of mesenchyme appear in it. Evidently, the material is used up in much the same way as in a normal limb, no matter whether there is too little or too much of it. As Danforth (1947) put it in the case of polydactylism in the cat, '. . . the only difference between potentially polydactyl and normal specimens is the amount of undifferentiated tissue on the preaxial border of the limb. It seems not impossible that induction of an early excess in the number of cells at this point may be the chief, perhaps only, direct effect of the mutant gene, and that all other associated morphological deviations are merely secondary to this initial volumetric increase.' *Mutatis mutandis*, the same explanation would seem to apply to *Os*/+ in the mouse.

For the present, the cause of the preaxial reduction of the foot plates of *Os*/+ embryos remains obscure. Zwilling & Ames (1958) have suggested that in polydactylism, the excess growth may be due to an anomalous distribution of the hypothetical 'maintenance factor' for the apical ectodermal ridge. Purely formally, a similar concept could account for the present case. But there is so far no supporting evidence for such an hypothesis.

Many authors have noticed that under pathological conditions, cartilaginous elements have a tendency to fuse with each other. The reason for this seems to be obscure. Where a cartilage of reduced size fuses with an adjacent element, the explanation may be found in Tschumi's (1954) suggestion that a vigorously growing blastema may compete with a smaller neighbour for mesenchyme cells; if such cells migrating across from one to the other are overtaken by the process of chondrification, fusion between the two elements will result. Sometimes cartilages of about normal dimensions fuse with each other when they are located abnormally close to each other. In such cases, the blastemata, instead of attracting mesenchyme cells from the intervening area, seem mutually to attract cells from each other, with a similar result. Fusions between elements which have arisen in a continuous blastema (such as carpals and tarsals) are presumably due to a different mechanism.

SUMMARY

The complex anatomy of the adult limb skeleton of *Os*/+ mice is attributable to a reduction of the preaxial margin of the foot plates. This is detectable, in the fore limbs, in the 11-day stage, i.e. before condensations of mesenchyme have taken place. The involvement of the skeleton is thus secondary to an earlier defect in the foot plates. The cause of the latter has not been discovered.

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REFERENCES

CARTER, T. C. (1954). The genetics of luxate mice. IV. Embryology. *J. Genet.* **52**, 1–35.

CENTER, E. M. (1955). Postaxial polydactyly in the mouse. *J. Hered.* **46**, 144–148.

CHANG, TSO-KAN (1939). The development of polydactylism in a special strain of *Mus musculus*. *Peking nat. Hist. Bull.* **14**, 119–132.

DANFORTH, C. H. (1947). Morphology of the feet in polydactyl cats. *Amer. J. Anat.* **80**, 143–172.

FORSTHOEFEL, P. F. (1959). The embryological development of the skeletal effects of the luxoid gene in the mouse, including its interactions with the luxate gene. *J. Morph.* **104**, 89–142.

FREYE, H. (1954). Anatomische und entwicklungsgeschichtliche Untersuchungen am Skelett normaler und oligodactyler Mäuse. *Wiss. Z. Univ. Halle, Math.-Nat.*, **3**, 801–824.

GRÜNEBERG, H. (1956). Genetical studies on the skeleton of the mouse. XVIII. Three genes for syndactylism. (With an appendix by D. S. Falconer.) *J. Genet.* **54**, 113–145.

GRÜNEBERG, H. (1960). Genetical studies on the skeleton of the mouse. XXV. The development of syndactylism. *Genet. Res.* **1**, 196–213.

HOLMGREN, N. (1933). On the origin of the tetrapod limb. *Acta Zool., Stockh.*, **14**, 185–295.

HOLMGREN, N. (1952). An embryological analysis of the mammalian carpus and its bearing upon the question of the origin of the tetrapod limb. *Acta Zool., Stockh.*, **33**, 1–115.

MILAIRE, J. (1956). Contribution a l'étude morphologique et cytochimique des bourgeons de membres chez le Rat. *Arch. Biol., Paris*, **67**, 297–391.

SCHMIDT-EHRENBERG, E. C. (1942). Die Embryogenese des Extremitätenskelettes der Säugetiere. Ein Beitrag zur Frage der Entwicklung der Tetrapodengliedmassen. *Rev. suisse Zool.* **49**, 33–131.

SCOTT, J. P. (1937). The embryology of the guinea pig. III. The development of the polydactylous monster. A case of growth accelerated at a particular period by a semi-dominant lethal gene. *J. exp. Zool.* **77**, 123–157.

TSCHUMI, P. (1954). Konkurrenzbedingte Rückbildungen der Hinterextremität von *Xenopus* nach Behandlung mit einem Chloraethylamin. *Rev. suisse Zool.* **61**, 177–270.

ZWILLING, E. (1956). Interaction between limb bud ectoderm and mesoderm in the chick embryo. II. Experimental limb duplication. *J. exp. Zool.* **132**, 173–187.

ZWILLING, E. & AMES, J. F. (1958). Polydactyly, related defects and axial shifts—a critique. *Amer. Nat.* **92**, 257–266.

ZWILLING, E. & HANSBOROUGH, L. A. (1956). Interaction between limb bud ectoderm and mesoderm in the chick embryo. III. Experiments with polydactylous limbs. *J. exp. Zool.* **132**, 219–239.