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The timing of ossification of the limb bones, and growth rates of various long bones of the fore and hind limbs of the prenatal and early postnatal laboratory mouse

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ABSTRACT

In order to study the pattern of ossification of the skeletal components of the fore and hind limb of the mouse, intact embryos were isolated between days (d) 15 and 19 of pregnancy (the morning of finding a vaginal plug is termed d 1 of pregnancy), and postnatal animals isolated on d 1 (newborns), 7 and 14 after birth. The total number of fore and hind limbs studied for each day of pregnancy or postnatal day for the bone growth study is given in parentheses: d 15 (2), d 16, 17, 18 and 19 of pregnancy (5 specimens for each of these days), d 1 (newborn), wk 1 and 2, postnatal (4 specimens analysed at each of these times), since only the right limbs were studied. For the study involving the time of first appearance of ossification centres, either the right or the left limb of each of these prenatal and postnatal specimens was analysed. All specimens were fixed in 80% ethanol, bulk-stained using alizarin and Alcian blue, in order to stain ossification centres and cartilage, respectively, and cleared. The limbs were then disarticulated from the axial skeleton at the sternoclavicular and sacroiliac joints to facilitate (1) the determination of the sequential pattern of ossification in the various cartilage primordia analysed, and (2) the analysis of the pattern of growth of the humerus, ulna, femur and tibia. The latter values were plotted graphically, and the individual growth rate of each of the long bones studied was then deduced and also plotted graphically. The findings demonstrated that, with the exception of the femur and ulna, all of the long bones studied had significantly different growth patterns. The time of appearance of the various centres of ossification in the skeletal elements studied proceeded in a similar order to that described by previous authors, though there was some discrepancy in the exact time of first appearance of certain ossification centres. Of particular interest was the somewhat unusual pattern of ossification of the first digits of both the fore and hind limb compared with that of the other digits. The data presented here provide useful baseline information on the normal sequential pattern of ossification in the fore and hind limb, and the characteristic growth pattern of the individual long bones of the limbs in this species.

INTRODUCTION

The timing of ossification and the growth rates of different components of the skeleton have long been the objects of study, not least for their importance in determining the age of an individual (albeit indirectly) who is undergoing normal growth, but also for assessing abnormal rates of growth. Studies on the human skeleton have been numerous and detailed (Mall, 1906; Noback & Robertson, 1951; O'Rahilly & Gardner, 1972) and much comparable work has also been carried out in the rat (Strong, 1925; Spark & Dawson, 1928), though relatively few studies have been carried out in the mouse. The principal purpose of this paper therefore is to describe the timing of first appearance of the ossification centres and the rate of long bone growth in the limbs of the prenatal and early postnatal laboratory mouse.

The technique employed in this study involved the bulk staining of specimens with alizarin red S and Alcian blue. The material was then 'cleared'. This double-staining technique allowed the accurate local-

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isation of ossification centres within the cartilage primordia to be made. Previous studies by Meyer & O'Rahilly (1958) revealed that alizarin red S was an extremely efficient means of determining early centres of ossification, being slightly more sensitive than radiography after silver impregnation, and only marginally less sensitive than serial sectioning of comparable-staged material. These authors suggested that the first appearance of alizarin and the silver reactions coincided in time with the laying down of periosteal collars but not with the onset of endochondral ossification. This baseline information provides a basis upon which abnormal growth and development patterns can be compared.

MATERIALS AND METHODS

Ten to 12 wk spontaneously cycling (C57BL × CBA) F1 hybrid female mice were mated with fertile males of the same strain. Male and female mouse embryos were isolated on days (d) 15, 16, 17, 18 and 19 of pregnancy (where the morning of finding a vaginal plug is termed d 1 of pregnancy, and is equivalent to d 14.5, 15.5, 16.5, 17.5 and 18.5 post conception (p.c.), respectively), and newborn animals (termed d 1), and others isolated at d 7 and 14 after birth. The sex of the individuals analysed was not noted before they were cleared and the skeletal elements double-stained, and this was not technically possible after this procedure had been carried out. The total number of fore and hind limb specimens studied for each day of pregnancy or postnatal day for the bone growth study is given in parentheses: d 15 (2), d 16–19 of pregnancy (5), d 1 (newborn), wk 1 and 2, postnatal (4), since only the right limbs were studied. For the study involving the time of first appearance of ossification centres, either the right or the left limb of each of these prenatal and postnatal specimens was analysed.

All prenatal and postnatal specimens were initially killed by deep ether anaesthesia, fixed in 80% ethanol and then bulk stained using alizarin and Alcian blue and then cleared. This technique enables successful demonstration of small and early centres of ossification by the alizarin (i.e. alizarin red S), and when a double-staining technique is used as employed in this study (see Kaufman, 1992), with Alcian blue to stain cartilage, then accurate localisation of the ossification centres may be made. Minimum difference was observed in the time of first appearance of the primary and secondary centres of ossification in the cartilage primordia of the various bones studied between the left and right sides, so that the findings reported are of general applicability. The results obtained are from either the left or the right side of each animal studied, but not from both sides of any animal. Thus the number of specimens indicated in Table 2 indicates the total number of animals studied, as each supplied a single fore or hind limb for analysis.

This study is based upon the analysis of the fore and hind limbs of the mouse, so that these were disarticulated from the axial skeleton at the sternoclavicular and sacroiliac joints, respectively. The cleared and double-stained specimens were viewed under a Wild M5 stereomicroscope and the presence (or otherwise) and length of ossification centres observed at each stage were determined. The total lengths of the cartilage models of the femur, tibia, humerus and ulna from the right side only were recorded using optical methods, as this was relatively easy to achieve and produced satisfactorily sensitive results.

Once appropriate measurements had been made, the pattern of growth of each bone was shown graphically by plotting bone length against time. It is also possible to show the growth patterns for the individual bones by plotting the percentage of final growth at 14 d after birth against time. The growth rate of each bone at any time can be deduced from these graphs by taking the gradient of the line produced at that time. Mathematically, the growth rate of each bone can be shown as follows, where t is time (in days) p is the proportion of bone length to its final value at 14 d after birth and β_0, β_1, β_2, β_3, are constants. It is known that as time progresses, p approaches 1. It is therefore appropriate to fit a nonlinear curve which has this characteristic. One such curve is the logistic which is defined by $\log(p/1-p)$. It was found that this produced a good fit when regressed against a third-order polynomial. The fitted line, using ordinary least squares is given by:

$$\log\frac{p}{1-p} = \beta_0+\beta_1 t+\beta_2 t^2+\beta_3 t^3.$$

Using standard tests of regression it was found that β_2 and β_3 were equal for all 4 curves. If this equation is differentiated with respect to time, the growth rate can be obtained. This is given by

$$\frac{dp}{dt} = p(1-p)[\beta_1+2\beta_2 t+3\beta_2 t^2].$$

The rate depends on β_1, β_2, β_3. Since β_2 and β_3 are equal, the test for differences (Δ) between each pair is a test for equality of β_1.

Table 1a. *Time of first appearance of primary ossification centres within the forelimb skeleton of the mouse*

Forelimb	Primary centres	
	First seen	Present in all specimens studied
Scapula	NA*	15**
Humerus	NA	15
Ulna	NA	15
Radius	NA	15
Carpus	27	27
Metacarpals		
1	27	27
2	17	17
3	17	17
4	17	17
5	18	18
Proximal phalanges		
1	27	27
2	18	19
3	18	19
4	18	19
5	19	21
Middle phalanges		
2	19	21
3	19	21
4	19	21
5	21	21
Distal phalanges		
1	19	19
2	18	19
3	18	19
4	18	19
5	19	19

* NA, information not available, since centres were already present in all samples of the earliest specimens studied.
** Days of pregnancy extended to include d 1, 7 and 14 after birth as d 21, 27 and 34, respectively.

Table 1b. *Time of first appearance of primary ossification centres within the hindlimb skeleton of the mouse*

Hindlimb	Primary centres	
	First seen	Present in all specimens studied
Ilium	16	16
Ischium	17	17
Pubis	17	17
Femur	15	16
Tibia	16	16
Fibula	16	16
Calcaneus	19	21
Talus	19	21
Tarsus	27	27
Metatarsals		
1	18	19
2	17	17
3	17	17
4	17	17
5	18	18
Proximal phalanges		
1	19	19
2	18	19
3	18	19
4	19	19
5	19	21
Middle phalanges		
2	19	21
3	19	21
4	19	21
5	21	21
Distal phalanges		
1	19	19
2	18	19
3	18	19
4	18	19
5	19	19
Patella	34	34
Fabellae	Not seen	—

RESULTS

Pattern of ossification

Fore limb. The times of first appearance of the primary centres of ossification in the cartilage primordia of the skeletal components of the fore and hind limb of the mouse are summarised in Table 1.

In the forelimb, the scapula has a primary centre visible by d 15 of pregnancy. This centre rapidly extends over the blade and spine. A secondary centre was apparent in the coracoid process in 7 d (postnatal) animals. However, even in 14 d (postnatal) animals, the vertebral border, glenoid fossa and acromion of the scapula have yet to ossify. The humerus has a primary centre evident by d 15 of pregnancy. This centre rapidly extends along the shaft of the bone to include the deltoid tuberosity by d 16 of pregnancy. Secondary centres are evident in the head, greater tuberosity, capitulum and trochlea by 7 d after birth. The radius has a primary centre evident by d 15 of pregnancy and a secondary centre distally by 7 d after birth and proximally by 14 d after birth. The ulna also has a primary centre visible by d 15 of pregnancy, and secondary centres proximally and distally by 7 d after birth. It was, however, noted that in 50% of cases, an additional 'supernumerary' secondary centre was seen in the ulna proximal to the primary centre but distal to the usual secondary centre that invariably develops in the olecranon process by 7 d after birth. The extent of ossification within the cartilage primordia of the scapula and forelimb long bones is illustrated diagrammatically in Figure 1.

The carpus shows no evidence of ossification in any of its components in the d 1 postnatal mice, but by 7 d after birth all have primary centres of ossification present. The metacarpals ossify in the order 3, 4, 2, 5,

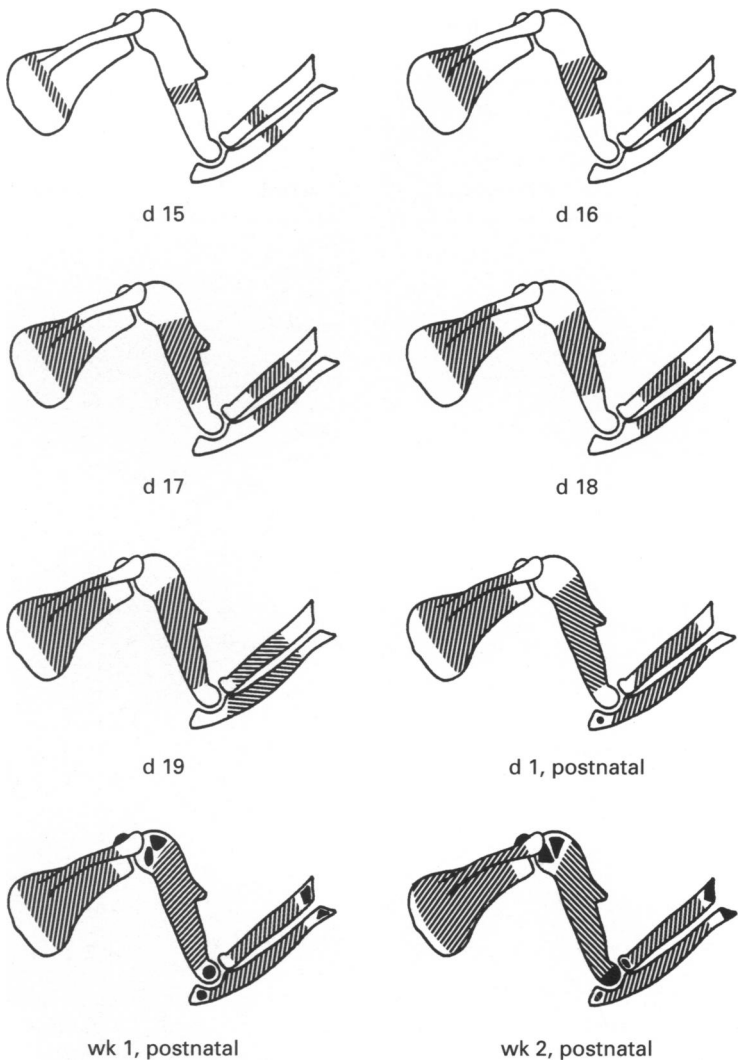

Fig. 1. Diagrammatic representation of the extent of ossification within the cartilage primordia of the scapula and forelimb long bones during the period between d 15 of pregnancy and d 14 postnatally. The shaded areas represent primary centres of ossification, while the small black areas located at the periphery of some of the cartilage primordia represent secondary centres of ossification.

1. The process starts on about d 17 of pregnancy and all are seen to have ossification centres present by d 19 of pregnancy, except for the 1st metacarpal which has only ossified by the end of the first week after birth. Determination of the sequence of ossification in relation to metacarpals 3, 4 and 2, which are all first seen on d 17 of pregnancy, was based on the length of the ossification centres present in the various specimens studied. The centre in metacarpal 3 was invariably found to be longer than that in metacarpal 4, while that in the latter was invariably longer than that in metacarpal 2. This point is alluded to in Figure 2. Secondary centres are visible by 7 d after birth in the distal portions of metacarpals 2, 3, 4 and 5, but no centre is seen in the 1st metacarpal at this time.

The first digit in the forelimb appears to have a bizarre pattern of ossification, with the distal phalanx ossifying at a similar time to the other distal phalanges, while its metacarpal and proximal phalanx do not ossify until between 1 and 7 d after birth. The order of ossification is the same as for the metacarpals, with primary centres being first seen in some embryos at d 18 of pregnancy. The fifth proximal phalanx ossifies by 1 d after birth. The middle phalanges are ossified by 1 d after birth, and secondary centres are visible in the proximal ends of both the proximal and middle phalanges by 7 d after birth. The distal phalanges ossify at d 18–19 of pregnancy. The proximal phalanges of digits 2 to 5 ossify almost in the same order as the distal phalanges. No secondary centres are evident in the distal phalanges. The extent of ossification in the cartilage primordia of the carpal, metacarpal and phalangeal bones is illustrated diagrammatically in Figure 2.

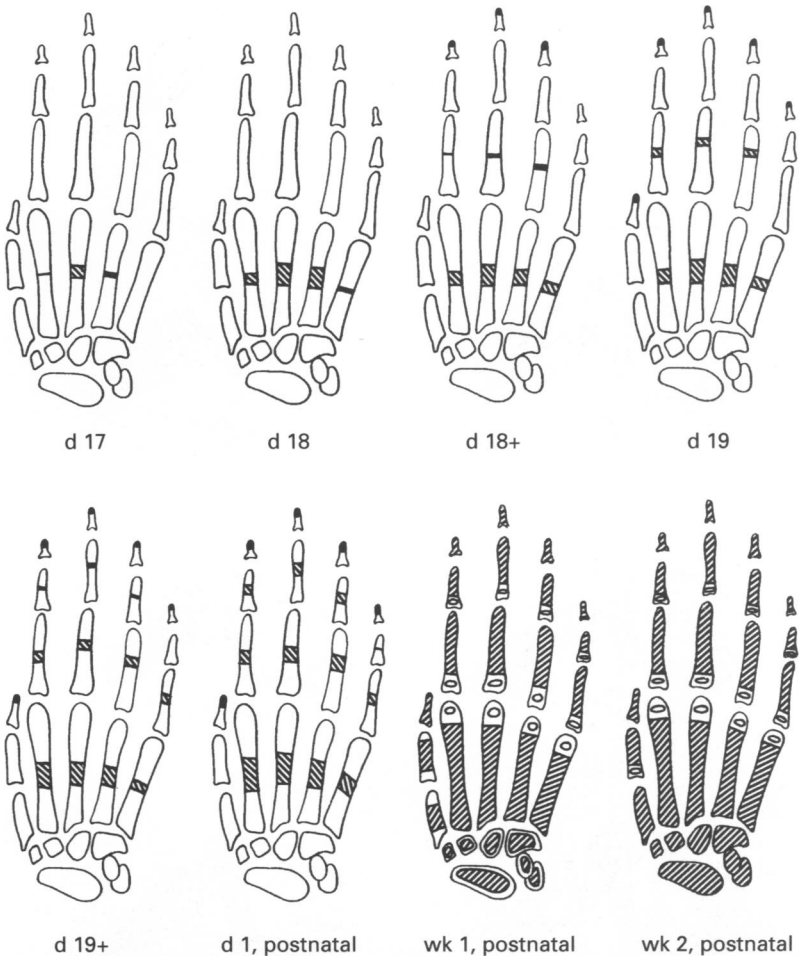

Fig. 2. Diagrammatic representation of the extent of ossification within the cartilage primordia of the carpal, metacarpal and phalangeal bones during the period between d 17 of pregnancy and d 14 postnatally. Some variation was observed in the extent of ossification seen in material isolated on d 18 and 19 of pregnancy. The range observed on these 2 days is illustrated diagrammatically, with the least and greatest degrees of ossification shown in the typical specimens labelled 'd 18' and 'd 19', and 'd 18+' and 'd 19+', respectively. A similar situation is observed for the extent of ossification seen within the cartilage primordia of the tarsal, metatarsal and phalangeal bones of the hindlimb on these 2 days of pregnancy, and the same labelling convention has been used in relation to the comparable components of Figure 4. In this figure, and in Figure 4, the shaded areas represent primary centres of ossification, while the unfilled circles located at the periphery of some of the cartilage primordia represent secondary centres of ossification. Note that the numerous small sesamoid bones located in association principally with the interphalangeal and metacarpophalangeal and metatarsophalangeal regions of the fore and hind limbs, respectively, have been omitted from Figures 2 and 4, as these show no evidence of ossification during the time period studied.

Hind limb. In the pelvis, the ilium is the first bone to show evidence of ossification. This is apparent by d 16 of pregnancy. The ischium ossifies just ahead of the pubis and both are seen to have small centres of ossification by d 17 of pregnancy. These 3 centres gradually grow towards each other, but have yet to meet in the region of the acetabulum, by 14 d after birth. At this time, a large portion of iliac crest and the majority of the inferior pubic ramus remain cartilaginous. Johnson (1933) recorded a secondary centre developing in the iliac crest of the mouse at approximately 14 wk after birth.

A small primary centre of ossification is first seen in the femur by d 15 and is more extensive by d 16 of pregnancy. A secondary centre appears in the distal part of the cartilage primordium by 7 d after birth, and proximally by 14 d after birth. Primary centres are seen by d 16 of pregnancy in the tibia and fibula, with secondary centres visible in the distal regions of the tibia and fibula and proximal part of the tibia by 7 d after birth. The extent of ossification within the cartilage primordia of the pelvic and hindlimb long bones is illustrated diagrammatically in Figure 3.

The talus and calcaneus start to ossify by d 19 of pregnancy, with the rest of the tarsus showing evidence of ossification by 7 d after birth, with the exception of the tibiale mediale which ossifies between 7 and 14 d after birth. An additional centre is first seen in the calcaneal tuberosity at 14 d after birth. While the first evidence of ossification in the patella is seen

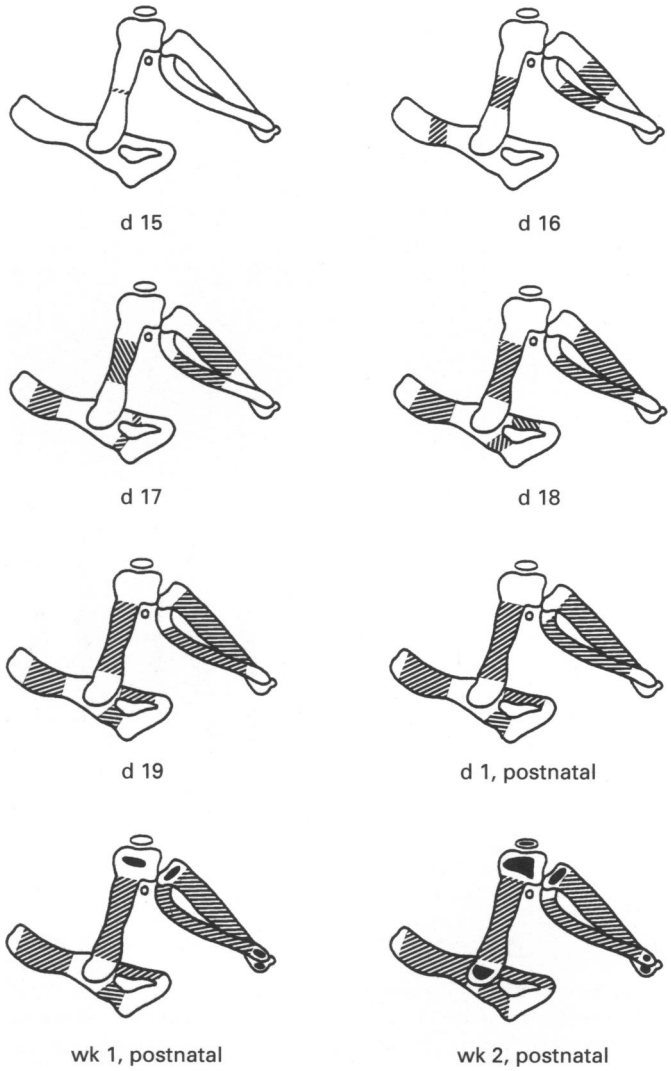

Fig. 3. Diagrammatic representation of the extent of ossification within the cartilage primordia of the pelvic and hindlimb long bones during the period between d 15 of pregnancy and d 14 postnatally. For the labelling convention used to distinguish between primary and secondary centres of ossification, see legend to Figure 1.

between 7 and 14 d after birth, the cartilage primordia of the fabellae show no evidence of ossification by this time.

The pattern of ossification in the digits of the foot is essentially the same as that observed in the forelimb, with the exception of the first digit where ossification of the metatarsal and proximal phalanx is more rapid, and is apparent between d 19 of pregnancy and the time of birth. The extent of ossification in the cartilage primordia of the tarsal, metatarsal and phalangeal bones is illustrated diagrammatically in Figure 4.

Pattern of growth of the long bones of the limbs

The measurements of the length of the entire cartilage models of selected long bones of the forelimb (humerus and ulna) and hindlimb (femur and tibia) at different stages during gestation and during the postnatal period are presented in Table 2, as is the number of specimens analysed in each group. By d 15 of pregnancy the humerus is the longest of the limb long bones, but by 14 d after birth it is the shortest of these bones. The tibia, on the other hand, is initially the shortest but grows to become the longest of the long bones studied. This information is shown graphically in Figure 5. The percentage of maximum length (at 14 d after birth) of the various long bones studied is shown in Table 3.

By taking the gradients of these growth curves, their individual growth rates can be shown plotted against time (Table 4, Fig. 6). The growth pattern of each bone was compared (see Table 5). All the bones studied, with the exception of the femur and ulna, had a significantly different growth pattern.

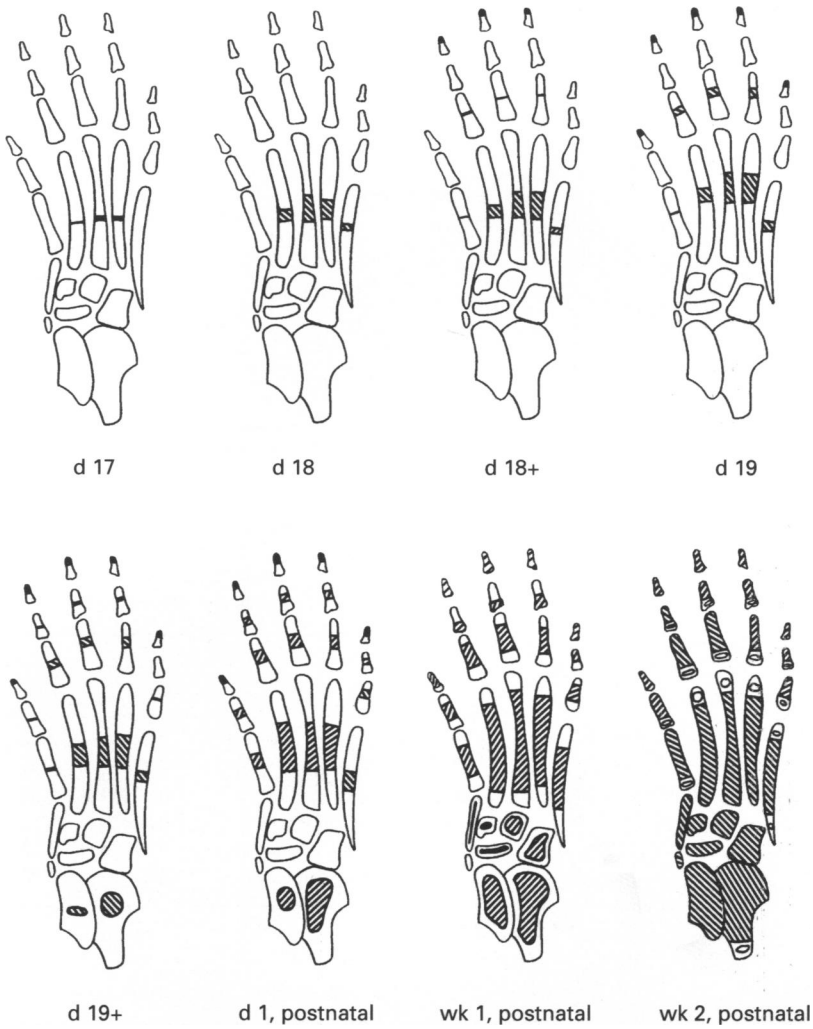

Fig. 4. Diagrammatic representation of the extent of ossification within the cartilage primordia of the tarsal, metatarsal and phalangeal bones during the period between d 17 of pregnancy and d 14 postnatally. Some variation was observed in the extent of ossification seen in material isolated on d 18 and 19 of pregnancy. For labelling convention used to indicate day of isolation of specimen and means of distinguishing between primary and secondary centres of ossification, see legend to Figure 2.

Table 2. *Maximum length of cartilage model (mm) of various upper and lower limb long bones*

		Upper limb		Lower limb	
No. of specimens	Days of pregnancy*	Humerus (Mean ± S.E.M.)	Ulna (Mean ± S.E.M.)	Femur (Mean ± S.E.M.)	Tibia (Mean ± S.E.M.)
2	15	2.25 ± 0.15	2.10 ± 0	1.90 ± 0	1.55 ± 0.21
5	16	3.38 ± 0.12	3.24 ± 0.11	2.58 ± 0.10	2.58 ± 0.05
5	17	4.14 ± 0.07	4.18 ± 0.08	3.40 ± 0.03	3.36 ± 0.06
5	18	4.82 ± 0.10	4.85 ± 0.12	4.32 ± 0.02	4.30 ± 0.09
5	19	5.48 ± 0.08	5.16 ± 0.16	4.84 ± 0.10	4.94 ± 0.07
4	21	6.34 ± 0.07	6.48 ± 0.09	6.00 ± 0.16	6.18 ± 0.23
4	27	8.23 ± 0.10	9.68 ± 0.18	8.43 ± 0.28	10.43 ± 0.12
4	34	10.05 ± 0.29	11.93 ± 0.33	11.25 ± 0.21	13.40 ± 0.23

* For terminology used, see Table 1.

DISCUSSION

The process of ossification of the majority of the mouse skeleton takes place over a relatively short time period, especially when compared with that of the human or even that of the rat. The growth of the skeleton even within that restricted time period is also very rapid. From d 15 of pregnancy to 14 d after

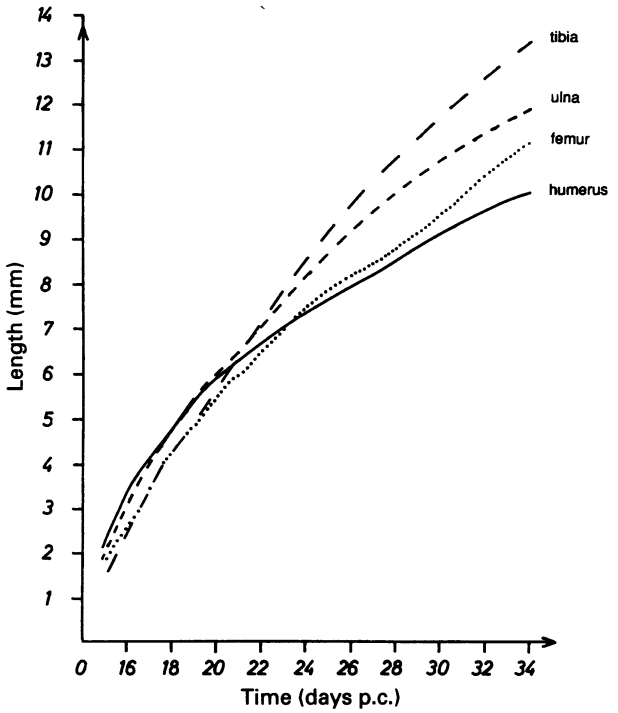

Fig. 5. Growth curves of right humerus, ulna, femur and tibia.

Table 3. *Percentage of maximum length at 2 wk after birth of cartilage models of various long bones*

Days of pregnancy*	Humerus	Ulna	Femur	Tibia
15	22.4	17.6	16.9	11.6
16	33.6	27.2	22.9	19.3
17	41.0	35.1	30.2	25.1
18	48.0	40.7	38.4	32.1
19	54.5	43.2	43.0	36.9
21	63.1	54.3	53.3	46.1
27	81.8	81.2	74.9	77.8
34	100.0	100.0	100.0	100.0

* For terminology used, see Table 1.

Table 4. *Rates of growth of various long bones at different times during pregnancy and the first 14 d after birth*

Days of pregnancy*	Humerus	Ulna	Femur	Tibia
15	0.0826	0.0698	0.0642	0.0531
16	0.0825	0.0750	0.0704	0.0621
17	0.0752	0.0732	0.0700	0.0658
18	0.0645	0.0667	0.0650	0.0647
19	0.0535	0.0583	0.0577	0.0606
21	0.0372	0.0437	0.0444	0.0504
27	0.0340	0.0400	0.0430	0.0485
34	0.0072	0.0074	0.0083	0.0077

* For terminology used, see Table 1.

birth, the tibia increases in length in absolute terms over eightfold.

The process of ossification was found to progress in a generally proximal to distal manner, with the forelimb being initially in advance of the hindlimb. However, by the time of birth, the ossification of the various components of the hindlimb was seen to be at a similar stage to that of the forelimb.

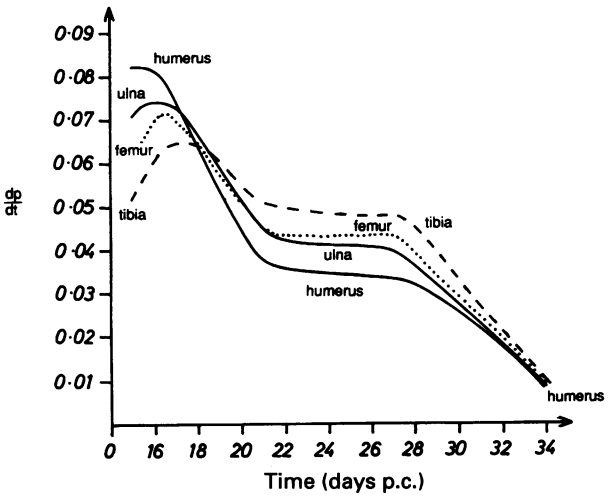

Fig. 6. Growth rate curves of right humerus, ulna, femur and tibia.

Table 5. *Tests for differences and growth rate*

	Δ	t value (22 D.F.)	*P* value
Tibia–humerus	0.04339	6.79	< 0.01
Tibia–ulna	0.02436	3.81	< 0.01
Tibia–femur	0.02339	3.66	< 0.01
Femur–ulna	0.00097	0.15	Not significant
Femur–humerus	0.02000	3.13	< 0.01
Ulna–humerus	0.01903	2.98	< 0.01

The growth of all the long bones proceeds in a similar fashion. Initially, the proximal upper limb long bone shows the highest growth rate, followed by the distal upper limb bones and then the proximal and distal hindlimb bones, respectively. However, after birth the order is reversed, so that the distal hindlimb long bones have the highest growth rate. It may be surprising therefore that the ossification process proceeds in such an ordered manner, with all the bones ossifying at a relatively predictable time and in a predictable order. What may be even more surprising is that the elongation of all the long bones in the specimens studied seems to follow such a rigid and characteristic pattern of growth that is peculiar to each bone.

Ossification

In the literature, some variation exists in the timing of the first appearance of ossification centres during

prenatal and postnatal mouse development. When a detailed comparison is made between the timing of first appearance of specific primary and secondary ossification centres as reported in the present study, and those reported by others (Johnson, 1933; Wirtschafter, 1960; Hoshino, 1967), it will be apparent that slight variations exist. This may be attributable to the fact that in each of these studies, different strains of mice were analysed.

Noback & Robertson (1951) also noted that there was some degree of variation in the timing of ossification in human embryos. Factors that have been proposed that might account for this variation between embryos are the sex of the individual (Spark & Dawson, 1928; Pyle & Sontag, 1943), its health (Todd, 1938; Francis, 1939) and nutritional status (Francis, 1940), as well as genetic (Pryor, 1939; Sontag & Lipford, 1943) and endocrine factors (Means, 1937; Talbot, 1939; Turner et al. 1941; Scow & Simpson, 1945; Becks et al. 1948; Noback et al. 1949).

Within all species, there is likely to be some degree of variation, even within a normal group of individuals (Todd, 1938; Pyle & Sontag, 1943). Asymmetry of appearance of ossification centres has also been noted, both in humans and rodents, but the minimum was detected in this study (human: Mall, 1906; Noback & Robertson, 1951; rodents: Johnson, 1933). Ossification was seen to proceed in a similar order to that described by previous authors. We are aware that the long gaps between the postnatal sampling times precluded the establishment of any detailed sequence of ossification of, for example, the carpal bones, where none displayed centres of ossification by d 1, postnatal, while by d 7, postnatal, all had centres of ossification present. Clearly, a shorter interval between samples might well have allowed us to establish this information. There is some discrepancy, however, as to the exact timing of first appearance of certain ossification centres, especially with regard to the ossification of the patella, which was seen to ossify somewhat earlier in our study than had previously been reported. While we first noted the presence of an ossification centre in the patella at 14 d after birth, this centre was first seen at 18 d after birth by Johnson (1933).

Of interest is the late ossification of the cartilage primordium of the first metacarpal bone. In this study it was noted that a primary ossification centre was present 7 d after birth. This is in general agreement with Johnson (1933), who also noted that no ossification centre was present in the first metacarpal until about 5 d after birth. This is therefore approximately 8–10 d after ossification is first seen in the other metacarpals. The first metatarsal does not show such delayed ossification, however, having a primary centre by about d 19 of pregnancy. The presence of a 'supernumerary' secondary centre of ossification in 50% of the ulnas examined at 7 d after birth, located proximal to the primary centre but distal to the secondary centre that invariably develops in the olecranon process by this time, has not previously been noted in the literature. Its significance in those animals that possess it is, however, unclear.

Comparison with ossification of the human metacarpals and metatarsals is of interest, for in the human the first metacarpal/metatarsal ossifies last but proportionately considerably sooner after ossification is first seen in the other metacarpals/metatarsals. The data presented by Noback & Robertson (1951) are helpful in this regard. These authors indicated that the smallest specimens with ossification centres present were as follows, where the crown–rump length is indicated in parentheses: metacarpal 1 (45 mm), metacarpals 2 and 3 (37 mm), metacarpals 4 and 5 (38 mm). Similar findings were reported for the metatarsal bones, where the smallest specimens with ossification centres present were as follows: metatarsal 1 (49 mm), metatarsals 2 and 3 (38 mm), metatarsal 4 (40 mm) and metatarsal 5 (45 mm). These findings are in general agreement with those of Mall (1906), though in this study there was less certainty as to the gestational age of the embryos/fetuses studied, as the age of the material is referred to as 'probable gestational age, in days'.

Growth

Bone growth (as determined by measurement of the total length of the cartilage primordia at the different stages studied), appears to display a characteristic pattern for each bone. The humerus (the proximal forelimb bone) has the highest growth rate at any one time, but this declines more rapidly over time than that of the other long bones studied. Conversely, the tibia has a smaller peak growth rate, but since its growth is sustained over a much longer period this enables the tibia to 'overtake' and outgrow all the other long bones. Interestingly, the growth curves of the femur and ulna were not significantly different. Why this should be the case is at present unclear, though it should be noted that very consistent findings were obtained in this study. Right-sided limbs were measured in all cases to avoid possible differences between sides, as has previously been noted by others (Hamilton et al. 1971; Bagnall et al. 1982). Gender

has also been proposed as a source of variation and therefore error (Pryor, 1923, 1933; Menees & Holly, 1932; Dunham et al. 1939; Hill, 1939; Bagnall et al. 1982), and Bagnall et al. (1982) noted that female human embryos had longer primary ossification centres than those present in the male, though these findings were, however, not found to be statistically significantly different. Gender was not taken into account in the present study.

It is hoped that the data presented here will provide useful baseline information which may provide a guide to the normal growth and development of the limb bones during the latter part of pregnancy and during the first 2 postnatal weeks in this species. The possibility exists that the variation in the rate of growth of the different limb bones may also provide a useful means of detecting abnormalities of growth of individual long bones. Unfortunately the number of specimens studied was not adequate to allow the absolute rates of bone growth of each of the various bones studied to be determined. In order to establish this value, considerably more specimens would have had to be examined. However, the small standard errors noted here, and the ease by which the curves were fitted to the data, should provide an indication of abnormal bone growth when measurements of individual long bones are compared with those shown in the growth curves presented here.

It is of interest to note that a number of ultrasonic studies have been carried out to establish the length of the long bones of the human fetus in vivo from about 12 wk of gestation to term. In an early article on this topic, Seeds & Cefalo (1982) compared the lengths of the humerus and femur. While similar in length from 13 wk up to about 24 wk of gestation, the femur at birth was found to be just over 10% longer than the humerus. Similar findings in relation to the lengths of all the long bones of the upper (i.e. humerus, ulna and radius) and lower (i.e. femur, tibia and fibula) limbs, have also been reported by Jeanty & Romero (1984) on a week-by-week basis, in sufficient detail to allow the prenatal growth rates of each of these bones to be compared.

The analysis of these data alone, however, is of limited value, principally because they represent only a relatively short component of the total duration of human long bone growth. To gain a more complete picture, it is essential that a series of postnatal time points is included in this analysis in order to provide meaningful estimates of long bone growth rates. This exercise was therefore undertaken using both the prenatal data indicated above and the findings for long bone lengths of children and adolescents (Maresh, 1955). The findings from this analysis were disappointing, however, in that no obvious relationship was observed between the growth rates of the various human long bones analysed, as might have been expected from the situation seen in the mouse.

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REFERENCES

Bagnall KM, Harris PF, Jones PRM (1982) A radiographic study of the longitudinal growth of primary ossification centers in limb long bones of the human fetus. *Anatomical Record* **203**, 293–299.

Becks H, Simpson ME, Scow RO, Asling CW, Evans HM (1948) Skeletal changes in rats thyroidectomized on the day of birth and the effects of growth hormone in such animals. Tibia, metacarpal and caudal vertebrae. *Anatomical Record* **100**, 561–576.

Dunham EC, Jenss RM, Christie A (1939) Consideration of race and sex in relation to growth and development of infants. *Journal of Pediatrics* **14**, 156–160.

Francis CC (1939) Factors influencing appearance of centers of ossification during early childhood. *American Journal of Diseases of Childhood* **57**, 817–830.

Francis CC (1940) Factors influencing appearance of centers of ossification during early childhood. II. A comparative study of degree of epiphyseal ossification in infancy under varying conditions of diet and health. *American Journal of Diseases of Childhood* **59**, 1006–1012.

Hamilton WJ, Simon G, Hamilton SGI (1971) *Surface and Radiological Anatomy for Students and General Practitioners*, 5th edn. London: Macmillan.

Hill AH (1939) Fetal age assessment by centers of ossification. *American Journal of Physical Anthropology* **24**, 251–272.

Hoshino K (1967) Comparative study on the skeletal development in the fetus of rat and mouse. *Congenital Anomalies (Japan)* **7**, 32–38.

Jeanty P, Romero R. (1984) *Obstetrical Ultrasound.* New York: McGraw-Hill.

Johnson ML (1933) The time and order of appearance of ossification centres in the albino mouse. *American Journal of Anatomy* **52**, 241–271.

Kaufman MH (1992) *The Atlas of Mouse Development.* London: Academic Press.

Mall FP (1906) On ossification centers in human embryos less than one hundred days old. *American Journal of Anatomy* **5**, 433–458.

Maresh MM (1955) Linear growth of long bones of extremities from infancy through adolescence. *American Journal of Diseases of Children* **89**, 725–742.

Means JH (1937) *The Thyroid and its Diseases.* Philadelphia: J. B. Lippincott.

Menees TO, Holly LE (1932) The ossification in the extremities of the new-born. *American Journal of Roentgenology and Radium Therapy* **28**, 389–390.

Meyer DB, O'Rahilly R (1958) Multiple techniques in the study of the onset of prenatal ossification. *Anatomical Record* **132**, 181–193.

Noback CR, Barnett JC, Kupperman HS (1949) The time of

appearance of ossification centers in the rat as influenced by injections of thyroxin, thiouracil, estradiol and testosterone propionate. *Anatomical Record* **103**, 49–68.

NOBACK CR, ROBERTSON GG (1951) Sequences of appearance of ossification centers in the human skeleton during the first five prenatal months. *American Journal of Anatomy* **89**, 1–28.

O'RAHILLY R, GARDNER E (1972) The initial appearance of ossification in staged human embryos. *American Journal of Anatomy* **134**, 291–308.

PRYOR JW (1923) Differences in the time and development of centers of ossification in the male and female skeleton. *Anatomical Record* **25**, 257–274.

PRYOR JW (1933) Roentgenographic investigation of the time element in ossification. *American Journal of Roentgenology and Radium Therapy* **29**, 798–804.

PRYOR JW (1939) Normal variations in the ossification of bones due to genetic factors. *Journal of Heredity* **30**, 249–255.

PYLE I, SONTAG LW (1943) Variability in onset of ossification in epiphyses and short bones of the extremities. *American Journal of Roentgenology and Radium Therapy* **49**, 795–798.

SCOW RO, SIMPSON ME (1945) Thyroidectomy in the newborn rat. *Anatomical Record* **91**, 209–226.

SEEDS JW, CEFALO RC (1982) Relationship of fetal limb lengths to both biparietal diameter and gestational age. *Obstetrics and Gynecology* **60**, 680–685.

SONTAG LW, LIPFORD J (1943) Effect of illness and other factors on appearance pattern of skeletal epiphyses. *Journal of Pediatrics* **23**, 391–409.

SPARK C, DAWSON AB (1928) The order and time of appearance of centers of ossification in the fore and hind limbs of the albino rat, with special reference to the possible influence of the sex factor. *American Journal of Anatomy* **41**, 411–445.

STRONG RM (1925) The order, time, and rate of ossification of the albino rat (*Mus norvegicus albinus*) skeleton. *American Journal of Anatomy* **36**, 313–356.

TALBOT NB (1939) The effect of estrogen on the skeletal age of immature rats. *Endocrinology* **25**, 325–327.

TODD TW (1938) *Atlas of Skeletal Maturation. 1. The Hand.* St Louis: C. V. Mosby.

TURNER HH, LACHMANN E, HELLBAUM AA (1941) Effect of testosterone propionate on bone growth and skeletal maturation of normal and castrated male rats. *Endocrinology* **29**, 425–429.

WIRTSCHAFTER ZT (1960) *The Genesis of the Mouse Skeleton: A Laboratory Atlas.* Springfield, Illinois: C. C. Thomas.