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ON OSSIFICATION AND GROWTH OF CERTAIN BONES OF THE RABBIT; WITH A COMPARISON OF THE SKELETAL AGE IN THE RABBIT AND IN MAN

By

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In experimental research work, the need for comparable normal material often necessitates the use of control series which increase the number of animals needed to a disproportionate extent. This could be avoided in many cases if observations made by different investigators could be combined to provide a normal series based on sufficient data. With this idea in mind, I feel justified in publishing the following observations on normal ossification and growth of certain bones of the rabbit extremities. There have been only a few reports on such observations in the literature (*Ring, Ellis* and *Joseph*, among others).

This investigation was chiefly carried out in connection with an experimental study reported elsewhere[1], on imitation of the defect known as congenital aplasia of the radius and treatment of such a defect by transplantation of a growing epiphysis. The present investigation is based upon radiographic examination of a total of 141 rabbits but since these animals were not all examined within the critical age period for estimating the ossification and growth of each of the bones considered in this paper, the number of animals upon which the different parts of the present investigation are based varies from 13 to 120. The series also comprise repeated examinations of the same animal (once before and once after the appearance of the ossification center, for instance). No attention has been paid to the sex of the animal. The observations on the appearance of ossification centers are based on radiograms taken before or in immediate connection with the first operation on the animal, and later observations on growth and fusion of the epiphyseal

[1] Acta Orthop. Scand., Suppl. 39.

lines are based principally on radiograms of intact extremities (there are, however, radiograms of some extremities from which the proximal end of the fibula had been removed and transplanted to another site).

I. TIME OF APPEARANCE OF OSSIFICATION CENTERS

a. *Carpal bones* (102 animals):

TABLE 1
Appearance of Nuclei of the Carpal Bones.

Age (days)	Number of visible nuclei		
	0	1-2	3-5
3	23	2	0
4	37	4	0
5 – 7	23	5	0
11 – 17	0	0	8

The first two carpal bones mostly became visible within the first week and always within *the first ten days of life. The next 1 to 3 nuclei* which were not visible during the first week, appeared during the course of *the next ten days.*

b. *Distal ulnar epiphysis* (102 animals):

TABLE 2
Appearance of Nucleus of the Distal Ulnar Epiphysis.

Age (days)	Nucleus not visible	Nucleus visible
3	16	9
4	27	14
5	7	11
6	1	8
7 – 17	0	9

The nucleus became visible in about one out of three cases within the first three to four days of life and always *within the first week.* Comparison with table 1 shows that this nucleus became visible slightly earlier than the nuclei of the first two carpal bones. *Ring's* observation that the epiphyseal nucleus appears on the fifth day of life agrees well with mine.

c. *Proximal ulnar epiphysis (main nucleus)* (82 animals):

TABLE 3

Appearance of Main Nucleus of the Proximal Ulnar Epiphysis.

Age (days)	Nucleus not visible	Nucleus visible
3	12	10
4	10	18
5	1	15
7 - 17	0	19

The nucleus appeared in slightly more than every second animal within the third to fifth day of life and in all *within the first week.*

d. *Proximal ulnar epiphysis (accessory nucleus)* (21 animals):

TABLE 4

Appearance of Accessory Nucleus of the Proximal Ulnar Epiphysis.

Age (days)	Nucleus not visible	Nucleus visible
30 - 45	4	1
46 - 60	2	6
61 - 90	0	11

The nucleus appeared *between the forty-fifth and the sixtieth days.* The number of animals was too small, however, and the dispersion too wide to allow any exact conclusions to be drawn.

e. *Distal epiphyses of tibia and fibula* (51 animals):

TABLE 5

Appearance of Nucleus of the Distal Epiphyses of the Tibia and Fibula.

Age (days)	Nucleus of tibia		Nucleus of fibula	
	Not visible	Visible	Not visible	Visible
3	1	1	1	1
4	3	3	2	4
5	1	3	1	3
6	1	3	1	3
7 - 17	0	44	0	44

The nuclei appeared in isolated cases at the age of 3 to 4 days and always within *the first week of life.* With one exception both nuclei appeared simultaneously. *Ellis* and *Joseph* found that these nuclei became visible during the first ten days of life. As a rule, the distal epiphyseal nuclei of the tibia and the fibula fused with one another between the thirtieth and forty-fifth days of life, as will be seen from table 6, which is based on the examination of 73 animals.

TABLE 6

Confluence of Distal Epiphyseal Nuclei of Tibia and Fibula.

Age (days)	Nucleus not confluent	Nucleus confluent
13 – 30	64	3
31 – 45	4	17
46 – 60	0	15

f. *Proximal tibial epiphysis* (17 animals):

Two animals were examined during the first, four during the second and eleven during the third day of life. The nucleus was radiologically visible in all these animals but whether it would have been visible in every case during the first day cannot be decided with certainty from this investigation.

g. *Proximal fibular epiphysis* (120 animals):

TABLE 7

Appearance of Nucleus of the Proximal Fibular Epiphysis.

Age (days)	Nucleus not visible	Nucleus visible
3 – 9	50	0
10	19	1
12	0	2
13	4	8
14	6	7
15	6	7
16	2	13
17	2	5
18 – 23	0	53

As a rule, the nucleus appeared *between the tenth and fifteenth days*, but exceptionally not until the seventeenth day of life.

h. *Distal femoral epiphysis* (17 animals):

The nucleus was radiologically visible in all animals, of which two were examined during the first, four during the second, and eleven during the third day of life, but whether it had been visible at birth could not be decided from this investigation.

i. *Head of the femur* (49 animals):

TABLE 8
Appearance of Nucleus of the Femoral Head.

Age (days)	Nucleus not visible	Nucleus visible
0 – 3	17	0
4	3	5
5	1	9
6 – 10	0	43

The nucleus appeared with great regularity during the *fourth to fifth days* of life.

j. *Major trochanter* (90 animals):

TABLE 9
Appearance of Nucleus of the Major Trochanter.

Age (days)	Nucleus not visible	Nucleus visible
0 – 3	17	0
4 – 7	16	6
8 – 11	15	46
12 – 15	0	26

The nucleus appeared in one animal out of four between the fourth and sixth days of life, most frequently *between the eigth and eleventh days*, however.

II. LONGITUDINAL GROWTH

k. *Ulna.*

In connection with the removal of the radius, a piece of stainless steel wire was introduced through a hole drilled into the ulnar diaphysis of 23 rabbits. Measurement of these extremities on 171 radiograms taken at different ages showed that the distance from the greater sigmoid cavity of the ulna to the steel wire label invariably remained the same during the growth of the animal, while the distance from the label to each of the epiphyseal lines of the ulna increased. This tallies with previous observations that longitudinal growth of the long bones takes place exclusively at the epiphyseal lines. It also shows that the growth at an epiphyseal plate can be measured by measuring the in-

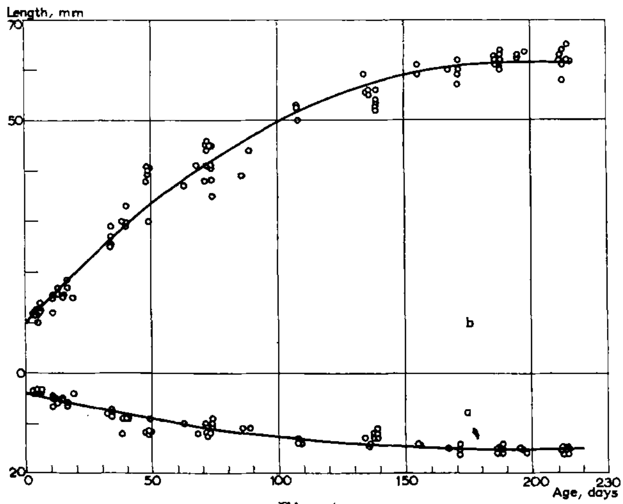

Fig. 1.

Curve showing the longitudinal growth in the proximal (a) and distal (b) growth zones of the ulna drawn on the basis of measurements on 171 radiograms, made at different ages, of the left fore-limbs of 23 rabbits.

The curves have been calculated by Mrs. *S. Asp*, M.A., on the basis of the following regression equations:

Proximal growth zone of the ulna (a):

$$a = 3.82 + 0.1230 \times \text{age} - 0.0003352 \times \text{age}^2 \qquad P = 0.001$$

Distal growth zone of the ulna:

$$b = 9.83 + 0.5367 \times \text{age} - 0.001395 \times \text{age}^2 \qquad P = 0.001$$

Growth in length of the tibia:

$$x_0 = 15.99 + 0.9051 \times \text{age} - 0.002456 \times \text{age}^2 \qquad P = 0.001$$

The curves are parabolic, but since in reality only growth and no shortening takes place, they must be flattened out, after the vertex, into straight horizontal lines.

crease in the distance from this plate to a fixed point on the diaphysis and that as such a fixed point an artificial label or any clearly defined point on the bone will serve, such as, for instance, the distal border of the greater sigmoid cavity.

The intact left fore-limb of 35 rabbits was radiographically examined 98 times on different dates at ages varying between 4 and 214 days and the distance from the distal border of the greater sigmoid cavity to a) the proximal epiphyseal line and b) the distal epiphyseal line, was measured. The values obtained were introduced into a system of coordinates in which the ordinate indicates the distance a (below) and b (above the axis of X) and the abscissa the age of the rabbit (fig. 1). In this way a curve for the average growth of the ulna could be drawn,

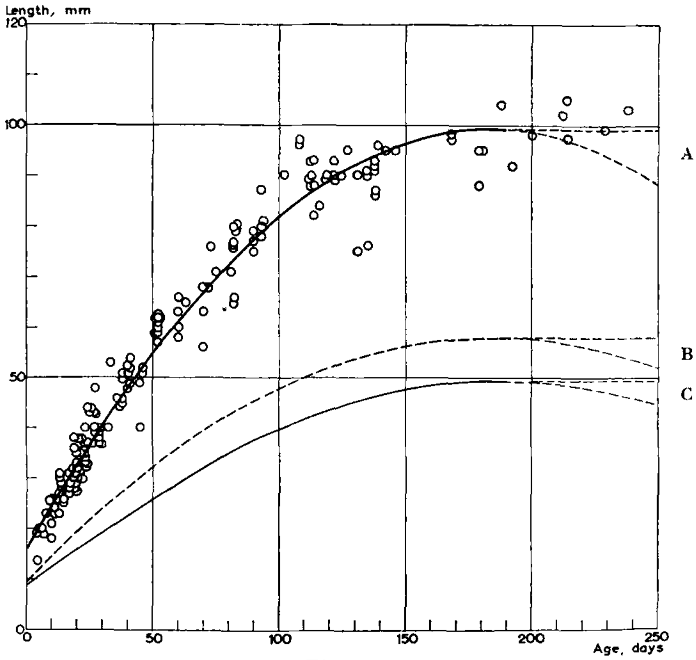

Fig. 2.

Curve showing the longitudinal growth of the tibia (A) drawn on the basis of measurements on 194 radiograms, made at different ages, of the hind-legs of 45 rabbits, and the growth curves, calculated theoretically from curve A, for the proximal growth zone of the tibia (B) and of the fibula (C).

the contribution of each epiphyseal plate to the growth being separately taken into consideration.

It can be read from this curve that *the contribution of the proximal epiphyseal plate to the longitudinal growth was 1/5.6. Ollier* obtained the relation 1/6 and *Silfverskiöld* 1/5.8.

1. *Tibia.*

On 194 radiograms, taken at ages varying from 4 to 238 days, of the hind-legs of 45 rabbits, the distance between the distal and the proximal epiphyseal line of the tibia was measured. The values obtained were entered in a system of coordinates and the average growth curve of the tibia was calculated. The growth was not calculated separately for the proximal and distal ends, because the tibia lacks a readily identifiable projection such as could be used in measurement, and because no labelling had been done. The angle between the tibial and fibular diaphyses (their distal portions are fused in the rabbit, forming a Y) cannot be used because the increase in thickness of the diaphyses, which takes place simultaneously with the growth in length, probably results in a filling up of the angle between the forks of the Y, the angle being displaced in a proximal direction. Assuming, according to *Silfverskiöld*, that the ratio of growth in the proximal end of the tibia as compared with that in the distal end is 1.4: 1 (according to *Ollier* 1.8: 1), the contribution of the proximal epiphysis to the growth is 1.4/(1.0 + 1.4) of the total growth of the tibia. In fig. 2, curve A is the average growth curve for the tibia and B is the average growth curve for the proximal epiphyseal plate of the tibia, calculated as described above.

m. *Fibula.*

The longitudinal growth of the fibula was not measured but theoretically calculated from the growth curve of the tibia on the basis of the following argumentation: Since there is no interstitial longitudinal growth and the distal portion of the fibula is fused with the tibia, the total growth of the free portion of the fibula takes place in the proximal epiphyseal plate. Since, however, the length of the fibular head (the fibular epiphysis) increases during growth, and its proximal end is attached to the tibial epiphysis close to the epiphyseal plate, the distance between the proximal tibial and the fibular epiphyseal plate increases (actually from about 2 mm. at the age of 6 days to 8.5 mm. at the age of 200 days). The length of the fibula can therefore be calculated by subtracting from the growth in the proximal epiphyseal plate of the tibia the increase in the distance between the proximal epiphyseal line of the tibia and that of the fibula. Curve C in fig. 2 is the curve of the average longitudinal growth of the fibula calculated in this way.

III. FUSION OF THE EPIPHYSEAL LINE

n. ***Distal epiphyseal line of the ulna*** (23 animals):

TABLE 10

Fusion of Epiphyseal Line at Distal End of the Ulna.

Age (days)	Epiphyseal line		
	Open	Almost fused	Fused
121 – 150	6	0	0
151 – 180	6	0	1
181 – 210	7	0	1
211 – 220	3	1	4

Only in two cases did the epiphyseal line fuse before the age of *220 days*. This conforms with the curve for the growth which shows that the longitudinal growth definitely ceased at the age of 6½ to 7½ months, and also with *Ring*'s observations, according to which growth ceased during the fifth month while the epiphyseal line only fused during the eigth month.

o. *Proximal epiphyseal line of the ulna* (28 animals):

TABLE 11

Fusion of Epiphyseal Line at Proximal End of the Ulna.

Age (days)	Epiphyseal line		
	Open	Almost fused	Fused
60 – 120	16	0	0
121 – 150	3	2	2
151 – 180	1	4	2
181 – 210	2	6	2
211 – 220	0	0	6

The epiphyseal line fused at widely varying times *between the ages of 120 and 210 days.*

p. *Distal epiphyseal line of the tibia* (23 animals):

TABLE 12

Fusion of Epiphyseal Line at Distal End of the Tibia.

Age (days)	Epiphyseal line		
	Open	Almost fused	Fused
121 – 150	15	0	0
151 – 180	2	0	5
181 – 210	1	5	5
211 – 225	0	1	3
226 – 240	0	0	1

The epiphyseal line fused in most cases *between the ages of 150 and 225 days. Ellis* and *Joseph* found that this took place between the ages of 133 and 168 days, thus somewhat earlier and with slightly less dispersion than in my series.

q. *Proximal epiphyseal line of the tibia* (34 animals):

TABLE 13
Fusion of Epiphyseal Line at Proximal End of the Tibia.

Age (days)	Epiphyseal line		
	Open	Almost fused	Fused
121 – 150	17	0	0
151 – 180	5	5	1
181 – 210	8	5	0
211 – 225	0	5	4
226 – 240	0	0	3

With some few exceptions, the epiphyseal line fused *between the ages of 150 and 225 days,* i.e. at the same date as the distal epiphyseal line.

r. *Proximal epiphyseal line of the fibula* (13 animals):

TABLE 14
Fusion of Epiphyseal Line at Proximal End of the Fibula.

Age (days)	Epiphyseal line		
	Open	Almost fused	Fused
121 – 150	3	0	0
151 – 180	2	1	0
181 – 210	7	2	0
211 – 225	0	6	2
226 – 240	0	0	2

In contrast to *Ellis* and *Joseph*'s observation that this line did not fuse until after the age of 210 days, I found that the proximal epiphyseal line of the fibula fused at about the same time as that of the tibia, i.e. at an age of *between 150 and 225 days.*

s. *Distal epiphyseal line of the radius* (23 animals):

TABLE 15
Fusion of Epiphyseal Line at Distal End of the Radius.

Age (days)	Epiphyseal line		
	Open	Almost fused	Fused
121 – 150	6	0	0
151 – 180	7	0	1
181 – 210	3	5	0
211 – 220	2	2	3

The line did not, as a rule, fuse before the age of *210 days*, thus somewhat later than the distal epiphyseal line of the ulna (the statement is based on the same radiograms).

t. *Proximal epiphyseal line of the radius* (31 animals):

TABLE 16
Fusion of Epiphyseal Line of Proximal End of the Radius.

Age (days)	Epiphyseal line		
	Open	Almost fused	Fused
60 – 90	9	4	0
91 – 120	0	3	1
121 – 150	0	1	8
151 – 180	0	1	4
181 – 195	0	0	9

This epiphyseal line fused earlier than any of the others observed in the present series, *between the 90th and 120th days of life.*

DISCUSSION

My observations tally to a large extent with those published hitherto. There are divergences, however, with regard to the time when the epiphyseal line fuses; these might depend on my material being too limited and on the fact that determination of the time when an epiphyseal line should be considered fused is necessarily to some extent subjective. Since observations at certain ages are lacking, some of the age limits are not quite correct, this being the case particularly with regard to the lower age limit, since only a few animals were examined at an age of less than 3 days.

IV. COMPARISON OF THE SKELETAL AGE IN THE RABBIT AND IN MAN

If a comparison is made between the skeletal age of man and the rabbit, the following points may be established:

1. Both man and the rabbit have at birth or shortly after, a radiologically demonstrable nucleus in the distal femoral epiphysis and the proximal tibial epiphysis, but in no other epiphysis. It may therefore be concluded that *they are both born at almost the same skeletal age.*

2. The last epiphyseal lines in man close at the age of 25, in the rabbit at the age of about 7½ months. *With regard to skeletal develop-*

ment the rabbit thus grows as much older in one day as man does in forty days.

Table 17 shows a comparison between the age limits for the appearance of epiphyseal nuclei in man (compiled from statements in three different textbooks on radiodiagnostics) and in the rabbit, given in days. In the last column the age limits for the rabbit have been multiplied by 40. Table 18 gives a similar comparison between the age limits for fusion of the epiphyseal lines. It will be seen from these tables that *the epiphyseal nuclei almost always appear at an earlier date in the rabbit,* while there is *no marked difference with regard to fusion of the epiphyseal lines.*

TABLE 17

Comparison Between the Times of Appearance of the Epiphyseal Nuclei in Man and Rabbit.

Epiphyseal nucleus	Age limit (days)		
	Man	Rabbit	Rabbit × 40
Distal Femoral	0	0 ?	0
Proximal Tibial	(–30) 0 – 60 (120)	0 ?	0
Capitate + Hamate	(–30) 90 – 120 (365)	–3 – 10–	120 – 400
Distal Tibial	(30) 90 – 365 (915)	–3 – 7	120 – 280
Femoral Head	(180) – 365 – (730)	4 – 5	160 – 200
Distal Fibular	(150) 300 – 540 (1095)	–3 – 7	120 – 280
The three Following Carpal Bones	(180) 670 – 1825 (3650)	–7 – 17–	280 – 680
Proximal Fibular	(545) 730 – 1460 (2370)	10 – 17	400 – 680
Major Trochanter	(545) 1095 – 1640 (2190)	4 – 11	160 – 440
Distal Ulnar	(1460) 2190 – 2555 (3285)	–3 – 7	120 – 280
Proximal Ulnar	(2555) 3285 – 3650 (4480)	–3 – 6	120 – 240

TABLE 18

Comparison Between the Times of Fusion of the Epiphyseal Lines in Man and Rabbit.

Epiphyseal line	Age limit (days)		
	Man	Rabbit	Rabbit × 40
Proximal Radial	5840 – 6935	90 – 120	3600 – 4800
Distal Tibial	6205 – 6935	150 – 225	6000 – 9000
Proximal Tibial	6205 – 8760	150 – 225	6000 – 9000
Proximal Fibular	6205 – 8760	150 – 225	6000 – 9000
Proximal Ulnar	6530 – 7660	150 – 220	6000 – 8800
Distal Ulnar	6530 – 9125	120 – 210	4800 – 8400
Distal Radial	6530 – 9125	150 – 225	6000 – 9000

SUMMARY

By means of radiographic examinations of a total of 141 rabbits performed in connection with an experimental investigation, the time of appearance of the epiphyseal nuclei in certain bones of the extremities and fusion of their epiphyseal lines were investigated. With regard to skeletal development, the rabbit grows as much older in one day as does man in 40 days. The skeletal ages of the rabbit and man are compared (tables 17 and 18). On the basis of measurements of length, average growth curves for the two epiphyseal plates of the rabbit ulna (fig. 1) and for tibia (fig. 2) were drawn and the growth curve for the proximal epiphyseal plate of the tibia and for the epiphysis of the fibula were calculated theoretically (fig. 2).

RESUME

Au moyen d'examens radiographiques de 141 lapins au total pratiqués en relation avec une enquête expérimentale, on a examiné le moment où apparaît le noyau épiphysaire à l'extrémité de certains os et la fusion de leurs lignes épiphysaires. En ce qui concerne le développement des os, le lapin devient beaucoup plus âgé que l'homme en 40 jours. L'âge squelettique chez le lapin et l'homme sont comparés (tableaux 17 et 18). Sur la base des mesures de longueur, des courbes de la croissance moyenne des deux plaques épiphysaires de l'ulna du lapin (fig. 1) et du tibia (fig. 2) ont été tracées et la courbe de croissance pour la plaque épiphysaire proximale du tibia et de l'épiphyse du fibula ont été calculées théoriquement (fig. 2).

ZUSAMMENFASSUNG

Mittels röntgenologischer Untersuchungen von insgesamt 141 Kaninchen, die im Zusammenhang mit einer experimentellen Forschung vorgenommen worden waren, wurde der Zeitpunkt des Auftretens der Epiphysenkerne in gewissen Knochen der Extremiteten und der Verschmelzung ihrer Epiphysenlinien untersucht. Hinsichtlich der Skelettentwicklung ältert das Kaninchen in einem Tage gleich viel wie der Mensch im Verlaufe von 40 Tagen. Das Alter des Skelettes von Kaninchen und Mensch werden vergleichen (Tabelle 17 und 18). Auf Grund von Messungen der Längen wurden durchschnittliche Wachstumskurven für die beiden Epiphysenplatten der Ulna (Fig. 1) und der Tibia (Fig. 2) gezeichnet und die Wachstumskurve für die proximale Epi-

physenplatte der Tibia und der Epiphyse der Fibula wurden theoretisch errechnet. (Fig. 2).

REFERENCES

1. *Ellis, F. G. & Joseph, J.:* Time of Appearance of the Centres of Ossification of the Fibular Epiphyses. J. Anatomy. 1954: 88: 533.
2. *Ollier:* (quoted by *Silfverskiöld*).
3. *Ring, P. A.:* Ossification and Growth of the Distal Ulnar Epiphysis of the Rabbit. J. Anatomy. 1955: 89: 457.
4. – Transplantation of Epiphyseal Cartilage. J. Bone and Jt Surg. 1955: 37-B: 642.
5. *Silfverskiöld, N.:* Über Längenwachstum der Knochen und Tranplantation von Epiphysenscheiben. Acta Chirurg. Scandinav. 1934: 75: 77.