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The growth of long bones in human embryological and fetal upper limbs and its relationship to other developmental patterns

Renato Bareggi [1], Vittorio Grill [1], Marina Zweyer [1], Maria A. Sandrucci [1], Paola Narducci [1], Antonino Forabosco [2]

[1] Department of Human Morphology, University of Trieste, Via Manzoni 16, I-34138 Trieste, Italy
[2] Chair of Histology, University of Modena, I-41100 Modena, Italy

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Abstract. Measurements were made of the long bones of the upper limbs (humerus, ulna, radius) of 58 aborted embryos and fetuses, developmental age from 8 to 14 weeks, crown-rump length (CRL) between 38 and 116 mm. The specimens were cleared and double-stained, using alcian blue and alizarin red S for a differential detection of cartilage and bone. The values of both the total length (TL) and the ossified part (OL) of each long bone were related to the fetal developmental age previously estimated by freshly measured CRL. The relationship to another developmental pattern, i.e. the number of ossified centres in the vertebral column, suggested that the OL values could be much more significant than TL for the assessment of fetal growth.

Key words: Development – Long bone ossification – Growth patterns – Human fetus

Introduction

Several studies have previously considered the measurement and the degree of ossification of long bones in human fetal limbs and their correlation to the crown-rump length (CRL). Different techniques have been employed, such as the direct measurement of long bones (Kelemen et al. 1984), after clearing and staining (Mall 1906; Moss et al. 1955; Dingerkus and Ühler 1977; Kimmel and Trammel 1981; Kelly and Bryden 1983), by SEM (Chiarasini et al. 1992), as well as by radiographic (Bagnall et al. 1982; Vasconcellos et al. 1992) and ultrasonic (Seeds and Cefalo 1982) methods. In vivo measurements by radiological or ultrasound techniques have been used to evaluate both whole long bones (total length, TL) and their ossified parts (ossified length, OL). However, these observations have provided conflicting results and could only with difficulty be compared to data relating to other fetal parameters.

Correspondence to: V. Grill

Data so obtained may be unreliable due to the variety of fetal positions during measurement. Nevertheless, some authors have proposed the assessment of fetal age on the basis of long bone measurement (Felts 1954; Moss et al. 1955; Gardner and Gray 1969; Mehta and Singh 1972; Kelemen et al. 1984; Kalifa et al. 1989). The appearance of ossified skeletal tracts and their longitudinal measurements could be considered as significant because the skeleton maintains its integrity even in non-vital fetuses persisting in the uterus some days before expulsion.

In our previous study of the developmental pathways of vertebral centra and neural arches in human embryos and fetuses (Bareggi et al. 1993), employing a simple clearing and double-staining method, with alcian blue and alizarin red S, we suggested the possibility of estimating developmental age on the basis of the number of vertebral ossified centres, from the end of the embryonic period, when they first appear until the completion of the whole cervical-thoracic-lumbar-sacral tract. This estimate could be used in conjunction with another commonly used measurement, the CRL.

The aim of this investigation was to measure both the total length (TL) and that of the ossified part (OL) in some long bones of the upper limbs (humerus, ulna, radius) in the hope that a systematic examination of the longitudinal growth of these bones – particularly considering the OL increase – might provide information that is useful for determining developmental age.

Materials and methods

We studied 58 aborted human embryos and fetuses, without any known maternal disease or any detectable malformation.

The specimens were ranged in developmental classes from 8 to 14 weeks on the basis of their freshly measured CRL (mean values), as reported in the common anatomical textbooks.

The specimens were at first eviscerated, then cleared and double-stained with alcian blue 8GX and alizarin red S, according to a previously described technique (Bareggi et al. 1993), so that both cartilage (blue) and calcified tissues (red) were detected.

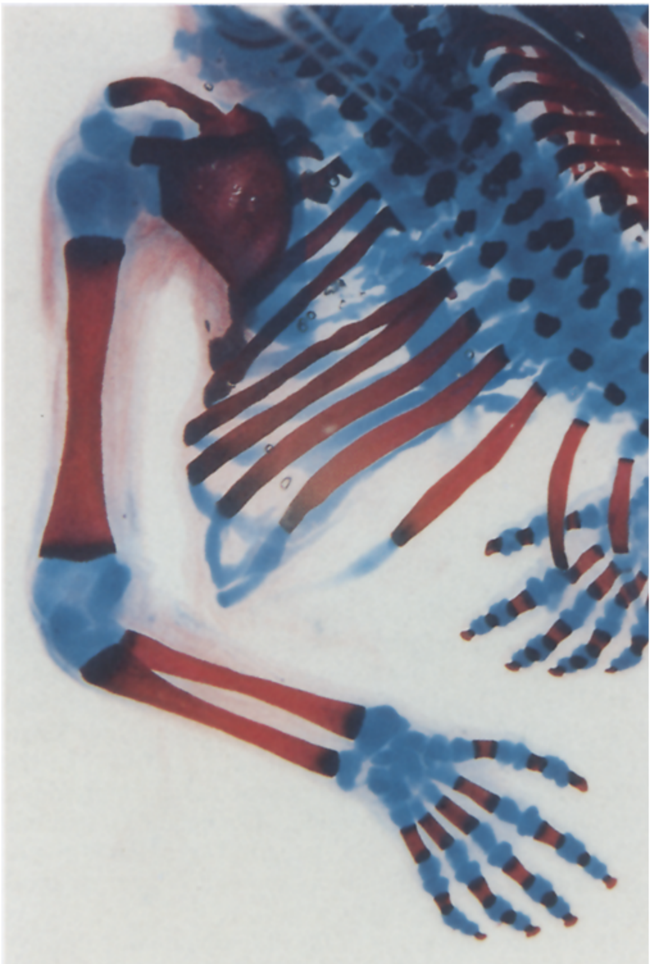

Fig. 1. Upper limb of a fetus (CRL 76 mm) cleared and double-stained with alcian blue and alizarin red S. Ossified skeletal parts appear *red*, cartilaginous *blue*

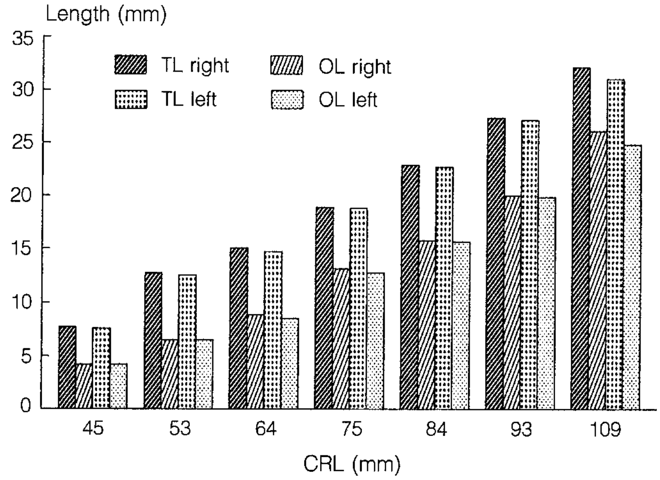

Fig. 2. Graphic presentation of humeral length increase (TL and OL)

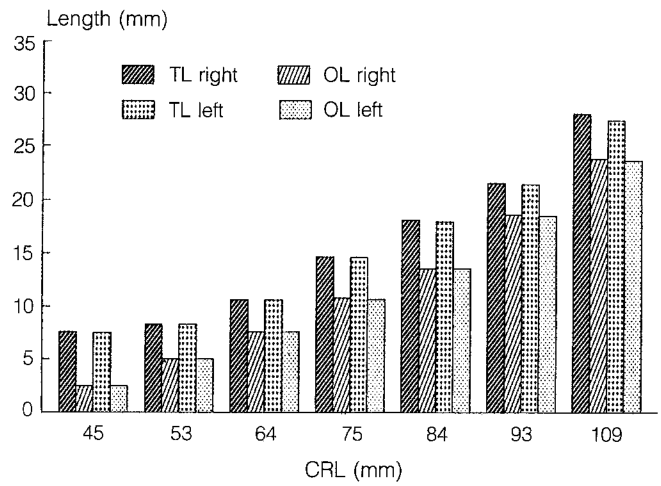

Fig. 3. Graphic presentation of ulnar length increase (TL and OL)

Longitudinal measurements of the upper limbs were taken with a millimetric caliper under a Zeiss stereomicroscope. The total length (TL) of each humerus, ulna and radius, including the cartilaginous extremities, was determined, as well as that of the ossified diaphyseal part (OL). Further measurements of the same parameters (TL and OL) were also made on photographs using an image analyzer Mop Videoplan provided with a software system Kontron.

The mean values (TL and OL) of each class, were finally compared as OL/TL per cent ratio.

In Fig. 1 an example of a cleared and double-stained fetal upper limb (CRL 76 mm) is shown.

Results

Table 1 gives the values of both TL and OL for each long bone for each specimen examined. The order of the specimens corresponds to increasing CRL, since this is the first parameter to which the measurements were to be related.

Humerus

TL ranged from 4.8 mm (CRL 38 mm) to 34.3 mm (CRL 116 mm). Differences were found between paired bones: the right was longer in 39 cases, the left in 4. OL ranged from 2.0 mm (CRL 38 mm) to 28.5 mm (CRL 116 mm); the right exceeded the left in 34 cases, while the left was greater in 4. Humeral length increase is shown graphically in Fig. 2.

Ulna

TL ranged from 5.5 mm (CRL 38 mm) to 31.9 mm (CRL 116 mm). The right ulna appeared longer in 30 cases, the left in 7. OL ranged from 1.8 mm (CRL 38 mm) to 26.2 mm (CRL 116 mm). Higher values were detected in 24 cases on the right and in 10 on the left. Ulnar length increase is graphically represented in Fig. 3.

Radius

TL ranged from 4 mm (CRL 41 mm) to 30.8 mm (CRL 116 mm). Differences between the right and left bone were less evident: the right was longer in 23 cases, the left in 3 cases. OL ranged from 1.8 mm (CRL 38 mm) to

Table 1. Values of TL and OL of each long bone for each examined specimen. CRL, Crown-rump length; TL, total length; OL, length of the ossified part

Specimen identification no.	CRL (mm)	Humerus (mm)				Ulna (mm)				Radius (mm)			
		TL		OL		TL		OL		TL		OL	
		R	L	R	L	R	L	R	L	R	L	R	L
1	38	4.9	4.8	2.0	2.3	5.5	5.5	1.8	1.9	4.6	4.6	1.8	1.8
2	39	4.9	4.9	2.5	2.5	5.5	5.5	2.2	2.3	4.7	4.8	3.0	3.0
3	41	5.0	4.9	3.0	3.0	5.7	5.8	2.3	2.3	4.0	4.0	2.1	2.1
4	42	7.6	7.4	4.2	4.2	7.1	7.1	2.5	2.5	6.2	6.0	2.9	2.8
5	45	9.2	9.2	5.0	4.7	9.0	8.5	2.5	2.5	7.7	7.5	3.5	3.3
6	46	8.5	8.5	4.8	4.8	8.8	8.9	2.4	2.5	6.7	6.7	2.6	2.7
7	46	8.5	8.4	5.9	5.9	8.2	8.0	2.4	2.4	6.7	6.7	2.7	2.7
8	46	8.8	8.8	4.5	4.5	8.2	8.2	2.5	2.4	7.5	7.5	3.1	3.0
9	49	9.4	9.5	5.0	4.9	8.6	8.6	3.0	3.0	8.6	8.6	3.4	3.4
10	50	8.9	8.7	4.3	4.1	8.4	8.1	2.9	2.8	8.0	7.8	3.9	3.8
11	50	8.7	8.6	5.4	5.4	8.3	8.2	2.9	2.8	8.8	8.6	4.0	4.0
12	50	11.6	11.0	6.3	6.1	8.0	8.0	5.0	5.0	7.5	7.5	5.0	4.5
13	52	14.0	13.5	7.0	6.8	8.5	8.2	5.6	5.6	7.5	7.5	5.0	5.0
14	52	12.5	12.3	6.2	6.2	8.0	8.0	4.9	4.9	7.1	7.1	5.5	5.5
15	53	12.8	12.9	6.3	6.5	8.4	8.5	5.0	5.2	7.6	7.7	5.8	6.0
16	56	13.0	13.0	6.8	6.8	8.3	8.2	5.0	5.0	8.5	8.5	6.6	6.4
17	57	12.5	12.3	6.4	6.4	8.7	8.7	4.7	4.7	8.6	8.6	5.8	5.8
18	61	12.9	12.9	6.8	6.7	8.3	8.1	5.6	5.4	8.0	7.8	5.6	5.5
19	62	13.4	12.9	7.8	7.5	8.0	8.0	5.5	5.5	7.6	7.6	6.6	6.6
20	63	13.8	13.8	7.4	7.4	9.9	10.0	7.4	7.4	8.1	8.1	7.9	7.9
21	63	14.1	13.6	8.5	8.4	11.9	12.0	7.5	7.0	9.0	9.0	7.9	7.8
22	67	17.7	17.5	10.6	10.3	12.5	12.4	8.8	8.8	11.6	11.5	8.0	8.0
23	67	18.0	17.4	11.8	10.9	13.0	13.0	10.5	9.9	12.6	12.6	9.5	9.5
24	69	18.2	18.1	12.6	12.6	13.5	13.6	9.0	9.0	12.3	12.3	9.5	9.5
25	69	18.2	18.0	12.5	12.5	12.7	12.7	10.1	10.0	12.6	12.6	9.5	9.5
26	71	18.0	17.6	12.9	12.6	14.4	14.3	9.2	9.1	12.6	12.6	9.6	9.5
27	74	19.5	19.4	14.1	14.0	15.0	15.0	10.3	10.0	14.2	14.0	10.2	10.1
28	76	20.0	20.0	14.0	13.9	15.2	15.2	10.2	10.0	14.2	14.0	10.0	10.0
29	77	16.8	16.7	11.0	11.0	13.5	13.5	10.0	9.9	11.5	11.4	9.0	9.0
30	78	19.1	18.6	11.9	11.4	15.7	15.3	12.7	12.5	14.4	14.5	10.8	10.8
31	78	18.0	18.0	12.5	12.4	14.9	14.9	12.0	12.0	13.8	13.8	10.5	10.4
32	80	22.1	22.0	15.3	15.0	17.1	17.1	14.1	14.1	17.5	17.5	14.0	14.0
33	80	22.5	22.5	15.8	15.8	17.6	17.6	13.3	13.3	16.4	16.4	14.1	14.1
34	80	22.0	22.0	16.3	16.2	17.0	17.4	12.1	12.4	15.8	15.7	13.2	13.0
35	83	22.6	22.4	15.9	15.7	17.4	17.2	13.8	13.8	17.0	17.0	14.6	14.6
36	85	22.2	22.3	15.6	15.8	18.0	18.0	13.4	13.5	17.5	17.0	15.0	14.8
37	85	20.0	19.6	13.0	12.8	16.7	16.1	11.5	11.4	16.4	16.0	13.0	13.0
38	85	21.8	21.5	14.0	13.9	17.1	16.9	11.4	11.2	16.3	16.2	10.8	10.5
39	86	23.1	23.0	14.1	14.1	17.9	17.9	15.0	15.0	17.3	17.3	15.0	15.5
40	87	25.4	25.4	17.8	17.8	20.6	20.5	15.5	15.5	18.3	18.3	15.5	15.5
41	88	26.0	25.0	19.3	18.1	20.8	20.0	16.4	16.5	19.0	18.9	16.0	16.0
42	89	25.7	25.0	17.6	17.0	20.3	20.2	17.0	16.9	18.2	18.2	17.4	17.4
43	89	26.5	25.5	18.8	18.7	20.8	20.6	17.5	17.1	18.6	18.0	16.5	16.5
44	90	26.0	26.0	18.0	17.8	21.2	21.0	17.0	17.0	18.2	18.2	16.3	16.0
45	90	26.3	26.3	19.0	19.0	20.8	20.8	17.1	17.1	18.1	18.0	16.7	16.7
46	90	26.1	25.9	18.8	18.5	19.5	19.4	17.5	17.4	17.8	17.8	17.1	17.0
47	92	27.1	27.0	21.1	21.2	20.8	20.7	19.1	19.2	19.1	19.1	14.0	14.0
48	95	29.0	29.1	21.5	21.7	22.5	22.6	19.2	19.3	21.8	21.8	16.3	16.3
49	98	29.5	29.2	22.4	22.3	23.6	23.3	21.0	21.0	22.8	22.7	17.3	17.2
50	102	29.9	29.8	22.3	22.3	24.3	24.2	21.6	21.6	22.5	22.5	17.2	17.2
51	102	30.0	28.2	23.9	23.0	24.4	24.0	22.3	22.0	23.2	23.1	19.2	19.1
52	106	31.6	29.8	24.6	23.3	26.0	25.6	22.8	22.7	25.5	25.5	19.6	19.6
53	106	31.0	30.0	25.0	24.2	25.8	25.2	22.6	22.7	24.3	24.0	20.6	20.4
54	109	32.4	32.0	26.2	24.1	27.9	27.2	23.5	23.4	26.6	26.4	20.1	20.1
55	110	32.0	30.6	26.0	24.1	28.8	28.0	24.0	23.0	26.6	26.4	20.0	20.0
56	111	32.9	32.0	27.1	26.5	29.7	29.3	24.7	24.3	28.6	28.3	21.9	21.8
57	113	33.0	32.5	27.0	26.4	29.5	29.0	24.7	24.4	29.0	29.0	22.6	22.6
58	116	34.3	33.1	28.5	27.2	31.9	31.3	26.2	26.0	30.8	30.8	22.4	22.4

Table 2. Developmental age assessment (weeks) of the specimens on the basis of the CRL, the number of vertebral ossified centres and the humeral, ulnar and radial TL and OL. CRL, Crown-rump length; TL, total length; OL, length of the ossified part

Specimen identification no.	CRL means (mm)	Vertebral ossified centres	Humerus (mm)				Ulna (mm)				Radius (mm)				Developmental age (weeks)
			TL		OL		TL		OL		TL		OL		
			R	L	R	L	R	L	R	L	R	L	R	L	
1–11	45	35	7.7	7.6	4.2	4.2	7.6	7.5	2.5	2.5	6.7	6.6	3.0	3.0	8
12–17	53	38	12.7	12.5	6.5	6.5	8.3	8.3	5.0	5.0	7.8	7.8	5.6	5.5	9
18–23	64	44	15.0	14.7	8.8	8.5	10.6	10.6	7.6	7.6	9.5	9.4	7.6	7.6	10
24–32	75	48	18.8	18.7	13.1	12.7	14.7	14.6	10.8	10.7	13.7	13.6	10.3	10.3	11
33–41	84	51	22.8	22.6	15.7	15.6	18.1	18.0	13.6	13.6	17.1	17.0	14.1	14.0	12
42–50	93	54	27.3	27.1	19.9	19.8	21.5	21.4	18.6	18.5	19.7	19.6	16.5	16.5	13
51–58	109	58	32.1	31.0	26.0	24.8	28.0	27.4	23.8	23.6	26.8	26.7	20.8	20.8	14

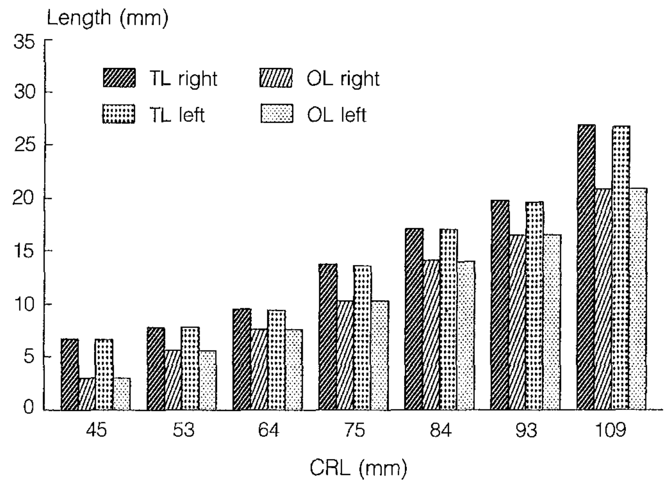

Fig. 4. Graphic presentation of radial length increase (TL and OL)

22.6 mm (CRL 113 mm); the right appeared longer in 20 cases, the left in 3 cases. Radial length increase is shown graphically in Fig. 4.

Table 2 shows the mean values of TL and OL for specimens grouped in seven developmental classes (age expressed in weeks). This further classification takes into consideration both the CRL and the number of vertebral ossified centres. Since we have already suggested that the latter could be a significant developmental parameter, we classified 11 specimens (specimen numbers 1–11) as having a CRL between 38 and 50 mm and up to 35 vertebral ossified centres, in the class of 8 weeks; 6 specimens (12–17), CRL from 50 to 57 mm, vertebral ossified centres 37–40 (mean 38), in the class of 9 weeks; 6 specimens (18–23), CRL between 61 and 67 mm, vertebral ossified centres 42–45 (mean 44), in the group of 10 weeks; 9 specimens (24–32), CRL from 69 to 80 mm, vertebral ossified centres 48–49 (mean 48), in the class of 11 weeks; 9 specimens (33–41), CRL between 80 and 88 mm, vertebral ossified centres 50–51 (mean 51), in the group of 12 weeks; 9 specimens (42–50), CRL from 89 to 102 mm, vertebral centres 52–55 (mean 54), in the group of 13 weeks; finally, 8 specimens (51–58), CRL between 102 and 116 mm, vertebral ossified centres 57–58 (mean 58), in the group of 14 weeks.

Discussion

In 1900 Bade had already observed radiographically that the growth in length of the long bones showed no constant relationships between them or to the length of the whole body.

Moss et al. (1955) carried out a more recent and extensive critical study of 119 fetuses, cleared and stained with alizarin red; the CRL of these ranged between 14 and 175 mm. They concluded that the long bones of distal limb segments grow relatively faster than those of the proximal segments, and also relatively faster than CRL.

After a combined radiographic and histological study on the prenatal development of the humerus, Gardner and Gray (1969) stated that its endochondral ossification begins in specimens with a CRL of about 37 mm. The timing and course of both the ossification and remodelling processes are remarkably constant until the end of the third month.

In a study of 50 fetuses (CRL between 65 and 290 mm), Mehta and Singh (1972) suggested that measurements of the ossified regions of humerus and femur could be used to determine the CRL.

Bagnall et al. (1982), in a radiographic study of the growth of ossification centres in limb long bones of human fetuses from 8 to 24 weeks, observed that humeral growth appeared much more marked on the left side of the body, and suggested that some other factors, such as manual dominance, may be related to it. Moreover, they affirmed that the growth rate for all ossified shafts was not constant.

Apart from these findings, few authors have suggested that developmental age could be determined by considering the length of even a single long bone. Mehta and Singh (1972) concluded their investigation by suggesting that once the CRL could be estimated from the length of the ossified shafts of humerus and femur, the fetal age could be estimated with reasonable accuracy. Kelemen

Table 3. Cumulative TL and OL of the three long bone of the upper limb. CRL, Crown-rump length; TL, total length; OL, length of the ossified part

CRL (mm)	Vertebral ossified centres	TL (mm) ±0.5	OL (mm) ±0.5	Developmental/age (weeks)
45	35	44	19	8
53	38	57	34	9
64	44	70	47	10
75	48	94	68	11
84	51	116	86	12
93	54	137	110	13
109	58	172	140	14

Table 4. Per cent ratio OL/TL in relation to the developmental age

Developmental age (weeks)	Ratio (%) OL/TL
8	43
9	60
10	67
11	72
12	74
13	80
14	81

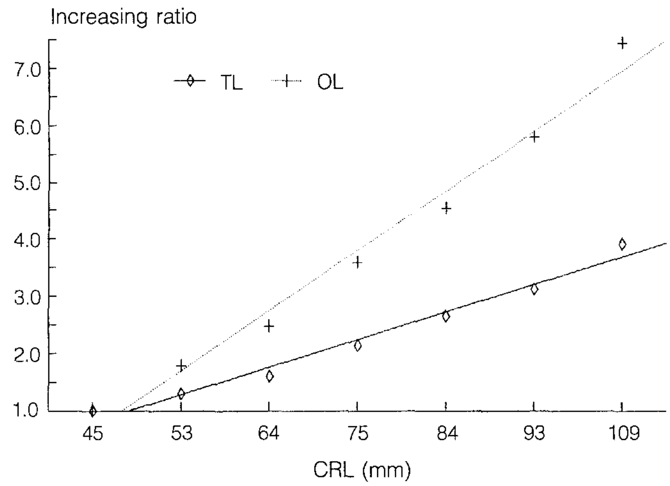

Fig. 5. Graphic presentation of the trend of the increasing ratio in TL and OL

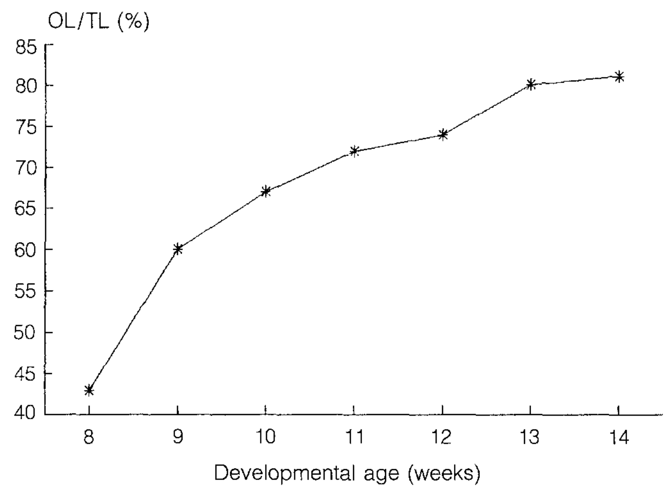

Fig. 6. Graphic presentation of the developmental pattern of the OL/TL per cent ratio

et al. (1984) measured the length of 491 long bones derived from 193 human fetuses (developmental age from 7 to 22 weeks) and suggested a linear correlation between the fetal age (assessed by measuring the CRL) and the long bone length. Seeds and Cefalo (1982) studied fetuses aged from 12 to 33 weeks and concluded that the length of both femur and humerus is a reliable parameter in the ultrasonic assessment of the gestational age.

In the present work, we observed that the count of the vertebral ossified centres enabled us to assign a progressive ratio between the CRL and the developmental age. Only a few specimens with the same CRL were assigned, on the basis of the number of vertebral ossified centres, in different developmental classes, while the values of the progressive longitudinal growth of long bones did not appear to be a reliable parameter.

Moreover, it would be hard to find any statistical significance in the differences between right and left sides; we can only observe that in two classes the TL mean values of the humerus were the same on the right as on the left, or the differences were lower than 0.1 mm, whereas the OL-mean was the same, or with differences lower than 0.1 mm in four developmental classes among seven. Differences of the ulnar TL greater than 0.1 mm were found in one class, whereas those of OL were found in two. In the radius, no differences greater than 0.1 mm between the right and the left side were detected either for TL or OL (Table 2).

A further consideration was related to the analysis of the TL and OL data. We observed that in 4 cases the humerus of a developmental class presented a TL exceeding the minimum value of the immediately successive upper class, the ulna in 15 and the radial in 12 cases. As regards OL, a finding analogous to the above was reported in 7 cases for the humerus, in 5 for the ulna and in 10 for the radius. Thus, these linear measurements showed a different pattern of increase when compared either with the CRL or with the number of ossified vertebral centres. In our opinion this could explain the difficulties if the measurement of long bones is used for the determination of developmental age.

On the other hand, conflicting findings may be due to the different methods employed. We thought it remarkable that we obtained higher values in almost all the cases we examined than those reported by other authors. We attributed this result to our clearing and double-staining technique that traced a better outline of the cartilaginous epiphyses, and consequently allowed a more accurate TL measurement, mainly in the earlier developmental classes. Moreover, since the technique we employed also allowed the evaluation and comparison of both TL and OL, we decided to investigate whether the OL values could provide a more significant assessment of fetal growth.

Table 3 shows the cumulative measurements of TL and OL of the three long bones of both the right and left upper limb. We grouped these values without consideration of any differences between the right and the left limb, in order to focus our attention on the relationship between TL and OL on the one hand, and CRL and the number of vertebral ossified centres, on the other, in each developmental class. By examining the reported values, CRL means ranged from 45 to 109 mm (ratio 1:2.4), TL means increased from 44 to 172 mm (1:3.9), and much more significantly OL means from 19 to 140 mm (1:7.4) (Fig. 5).

Table 4 and Fig. 6 show the per cent ratio between OL and TL in each developmental class and emphasize a further conclusion: the quantitative calcified contribution occurs much more rapidly in the earlier developmental phases (the OL/TL percentage is 43% at 8 weeks and 60% at 9 weeks) rather than the later.

In conclusion, we think that the TL evaluation may provide only debatable information relevant to an assessment of fetal age, whereas, in our opinion, the OL may be considered as an important parameter related to the degree of skeletal ossification. These data indicate an important developmental pattern, at least in the period examined in this work, and may further supply a useful background for the detection of fetal developmental diseases.

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