

# The early development and ossification of the human clavicle—an embryologic study

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Morphologic studies of the early development of the clavicle were carried out in 46 human embryos and fetuses ranging in age from 6 to 12 weeks. We confirmed that the clavicle is formed by two membranous primary ossification centers appearing by 6 weeks and fusing approximately 1 week later. Cartilage at both ends of the clavicle then develops. In time, the medial cartilaginous mass contributes more to the growth in length of the clavicle than the lateral cartilaginous mass. The spatial orientation of both ossification centers and the development of enchondral bone formation at the ends of the clavicle lead to its characteristic shape. Interestingly, the primary ossification centers contribute little to the growth in length. The junction of the two centers of ossification is situated between the lateral and middle third of the clavicle and, consequently, does not correspond to the site of congenital pseudarthrosis, which is located in the middle part of the clavicle.

The clavicle is the first bone to ossify in the developing embryo. At approximately 5 weeks of gestation, primary ossification of the clavicle proceeds by membranous ossification of two centers with no prior cartilaginous anlage. Both will soon fuse. At about 7 weeks (Streeter's Stages 20 to 21), cartilaginous growth areas develop at the acromial and sternal ends (Gardner 1968). The question of the number of primary ossification centers of the clavicle, be it one (Koch 1960, Gibson and Carroll 1970) or two (Mall 1906, Fawcett 1913, Gardner and Gray 1953, Andersen 1963, Gardner 1968, O'Rahilly and Gardner 1972), remains unsettled. The answer to this question seems important, because congenital pseudarthrosis of the clavicle is thought to result from failure of fusion of the two primary ossification centers in the early embryologic stage. Lloyd-Roberts (1975), on the other hand, believed that the pulsation of the adjacent subclavian artery could lead to a pseudarthrosis.

The purpose of this embryologic study was to establish the number of ossification centers of the human clavicle, to determine how its characteristic shape evolves, and to find out whether there is a morphologic basis for the above-mentioned theories of congenital pseudarthrosis.

## Materials and methods

Serial sections obtained from 11 human embryos and fetuses ranging in crown-rump (CR) length from 14 mm to 65 mm (age of gestation 6 to 11 weeks) formed the basis of this study. Two clavicles from fetuses of 35 and 65 mm in length, respectively, were removed in toto under the dissecting microscope. All the specimens including the two isolated clavicles were decalcified in EDTA after fixation in 10 percent neutral formalin and embedded in paraffin. Serial sections were made in either sagittal, transverse, or frontal planes. During transverse and frontal sectioning, care was taken to orient the plane of sectioning in such a way as to obtain a section of the entire clavicle. The sections were stained with Goldner's trichrome, hematoxyline-phloxine-saffron, or azan.

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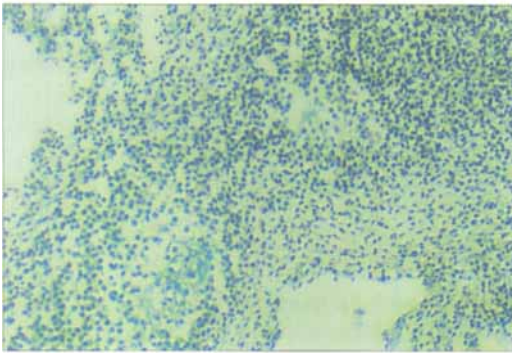


Figure 1. Human embryo, 14-mm crown-rump (CR) length, 6 weeks, Stage 17. Transverse section showing two early ossification centers. In serial sections no bony bridge was found between these two centers. Goldner,  $\times 36$ .

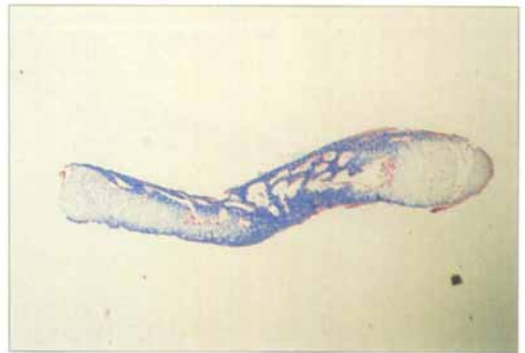


Figure 4. Human embryo, 35-mm CR length, 8.5 weeks. Transverse section showing a bony mass occupying only a small part of the clavicle. The major part of the clavicle is formed by two cartilaginous masses, which form an angle of  $150^\circ$ . Azan,  $\times 6.6$

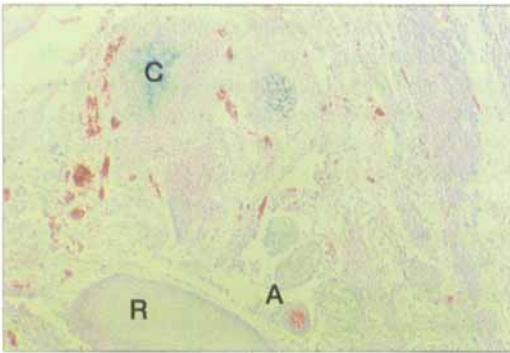


Figure 2. Human embryo, 21-mm CR length, 7 weeks, Stage 29. Sagittal section showing the medial ossification center. Note the subclavian artery (A) and nerves that run near the first rib (R), far from the clavicle (C). Azan,  $\times 16$

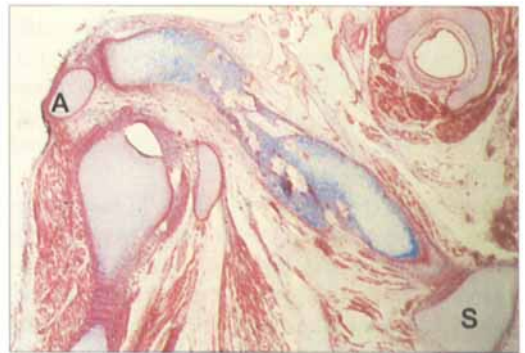


Figure 5. Human fetus, 35-mm CR length, 8.5 weeks. Adjusted transverse section showing the caudal part of the clavicle. At this level the cortical shell is absent. S, Sternum; A, Acromion. Azan,  $\times 6.6$ .

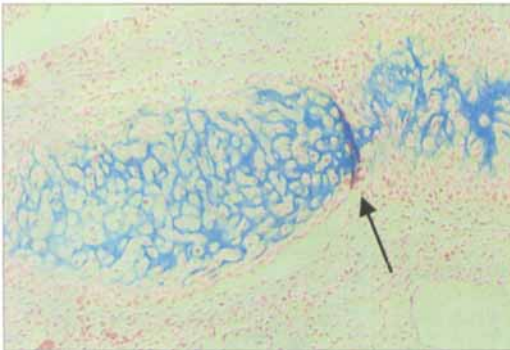


Figure 3. Human embryo, 20-mm CR length, 7.5 weeks, Stage 20. Transverse section showing bony bridge (arrow), which connects the medial (right side) and the lateral ossification centers. Azan,  $\times 33$ .

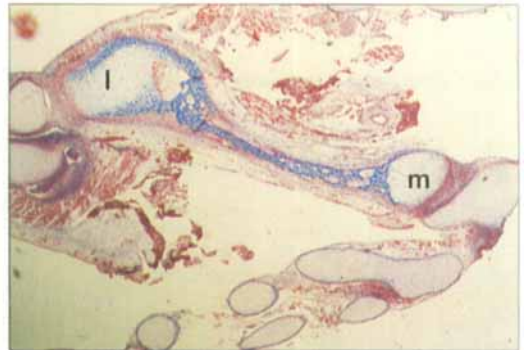


Figure 6. Human fetus, 40-mm CR length, 9 weeks. Transverse section. The bone showing anterior convexity is formed through enchondral ossification. m medial end; l lateral end. Distal to medial end, ribs are seen. Azan,  $\times 6.6$ .



Figure 7. Human fetus, 65-mm CR length, 11 weeks. Transverse section. The shape of the clavicle is similar to that of a clavicle in adults. The lateral cartilaginous mass contributes only to the formation of the shorter lateral part of the clavicle. m medial end; l lateral end. Azan,  $\times 3.3$ .

The distribution as to Streeter's developmental stages or to age was as follows: 14 mm (Stage 17), 18 mm (Stage 18), 20 mm (Stage 20), 21 mm (Stage 19), 24 mm (n 2; Stages 21 and 22), 30 mm (Stage 23), 35 mm (n 2; 8.5 weeks), 40 mm (9 weeks), and 65 mm (11 weeks).

Additionally, nonserial sections of 35 human embryos or fetuses ranging in CR length from 15 mm to 78 mm (age of gestation 6 to 12 weeks) were reviewed. These specimens were processed and stained in a similar manner. The cutting plane for the transverse or frontal sections, however, was not adjusted. The distribution of Streeter's developmental stages or age was as follows: Stage 17, Stage 18, Stage 19 (n 4), Stage 20 (n 4), Stage 21 (n 4), Stage 23 (n 2), 8.5 weeks (n 2), 9 weeks (n 8), 10 weeks (n 5), 11 weeks (n 2), 12 weeks (n 2).

## Results

No differences between right and left clavicles were found. Therefore, in order to facilitate the description of the observations, only the right clavicles were chosen for reproduction.

### Embryonic period

Membranous bone formation was present at 6 weeks (Stage 17) and the ossification localized in two distinctly separate centers (Table 1). The medial center was larger than the lateral one. Serial transverse sections showed that no bony bridge existed between the two centers (Figure 1). A specimen of 6.5 weeks (Stage 18) showed only mesenchymal cell condensation of the primordial clavicle. The other nonserially cut specimen showed early ossification. The number of ossification centers, however, could not be determined. In the 7-week-old (Stage 19) sagittally cut specimen, early ossification at two independent sites could be observed. In certain sections the subclavian artery could be found; it was surrounded by nerve bundles of the brachial plexus and coursed near the first rib, but remote from the clavicle (Figure 2). On reviewing transversely cut serial sections of a 7-week-old (Stage 20) cranially to caudally, two centers of ossification were united by a narrow bridge (Figure 3). The medial ossification center was larger than the lateral one. Both were lying in a straight line.

In the frontally sectioned clavicle of a 7.5-week-old (Stage 21) embryo, the two ossification centers of the clavicle were united by a bridge. The lateral center consisted of rather coarse trabecular bone

Table 1. Embryology of the clavicle

Age (wk)	Stage	Section	Ossification numbers	Centers size	Angle between ossification centers	Capping of acromial and sternal ends
6	17	S	two, separate	150 $\mu$ m med	too early	none
6.5	18	N	none present	—	too early	none
6.5	18	N	ossif. center, number ?	—	too early	none
7	19	S	two, separate	350 x 250 $\mu$ m med 250 x 200 $\mu$ m lat	180°	undiff. mesench. cells
7	20	S	two, united	550 x 250 $\mu$ m med 450 x 350 $\mu$ m lat	180°	undiff. mesench. cells
7.5	21	S	two, united	1100 x 380 $\mu$ m med 900 x 170 $\mu$ m lat	180°	mesenchymal cell condensation
7.5	21	N	two, united	750 x 500 $\mu$ m med 450 x 200 $\mu$ m lat	170°	mesenchymal cell condensation
7.5	22	S	two, united	1400 x 500 $\mu$ m med 1100 x 300 $\mu$ m lat	150°	cartilage

S serial section. N nonserial section.

with a cortical shell resembling a honeycomb. The cross section of a nonserially cut specimen of the same age showed that the lateral part was small and flat, whereas the medial part was large and oval. At 7.5 weeks (Stage 22), the clavicle consisted of two well-defined bony masses united by a bridge, under which early bone marrow formation was found. Sites of cartilage formation were found at the sternal and the acromial ends. In the sagittally cut clavicle of an 8-week-old embryo (Stage 23), the bony part was invaded by vascular channels that entered from the posterior aspect. Hematopoietic cells and osteoclasts were also present.

### Fetal period

The sinusoidal shape of the isolated clavicle of an 8.5-week fetus (CR length 35 mm) could be seen well under the dissecting microscope. The lateral part, which was shorter than the medial one, appeared to be oriented posteroinferiorly. The clavicle measured 4.2 mm in length, of which bone occupied only 0.6 mm; it was located more laterally. The lateral cartilaginous mass was shorter than the medial one. The major axes of cartilaginous masses formed an angle of 150° (Figure 4). Enchondral ossification was observed between bone and the cartilaginous masses. In another specimen of the same age, the bone was also found more laterally. Both cartilaginous masses were elliptical in shape and the lateral one was shorter than the medial one. In more caudal sections, the bone lacked its cortical shell and consisted of clusters of small bony masses (Figure 5). The S-shape of the clavicle was also evident at 9 weeks (CR length 40 mm); it was mainly due to the anterior convexity of the medial bony portion. The overall length of this clavicle was 3.7 mm, of which the bone occupied 2 mm; it was mostly formed through enchondral ossification (Figure 6).

The clavicle of an 11-week-old fetus (CR length 63 mm) showed a shape similar to that of the mature clavicle. Its entire length was 8 mm, of which 6.2 mm was occupied by bone. Primary trabeculae, formed through enchondral ossification, were found 1.5 mm from the lateral end and 2.3 mm from the medial end. The direction of growth was medial at the medial end and anterolateral at the lateral end. Periosteal bone formation and resorption contributed to growth in width and to modeling. Large amounts of periosteal bone were found at the posterior aspect of the lateral part and at the anterior aspect of the medial part of the clavicle (Figure 7).

### Discussion

Only serially cut specimens allow an exact observation of the process of early ossification, in particular the determination of the exact number of ossification centers. We showed that the clavicle is formed from two membranous ossification centers that appear at Stages 17 to 19 and fuse by Stage 20. Gibson and Carroll's (1970) 18-mm specimen showing one center corresponds to Stage 19 or 20 at a moment when fusion has already occurred. At Stage 21 the medial part of the clavicle resembles a honeycomb, corresponding to the description by Koch (1960). Chondrocytes appear at both ends of the clavicle at Stage 22. Our findings of the development of the clavicle in staged embryos are similar to those of Gardner and Gray (1953), of Gardner (1968), and of Andersen (1963). However, they stated that the lateral center was more advanced than the medial one. Our findings revealed that the medial ossification center is larger and thicker than the lateral one.

Frost (1983) postulated that mechanical factors initiate the change from a straight fetal clavicle into the S-shaped adult configuration during the postnatal period. However, Gardner (1968) noted that the clavicle is already angulated at 7 to 8 weeks, an observation confirmed by us. At Stage 20 (7 weeks) the clavicle consists of two identifiable bony masses; a flat lateral mass and a cylindrical medial mass. At Stage 21 (7.5 weeks) the bony centers start to form an angulation. At Stage 22 (7.5 weeks), cartilaginous areas appear at both ends of the clavicle; thereafter, the clavicle grows like other long bones, with enchondral bone formation starting at 8.5 weeks. At 9 weeks the S-shape of the clavicle can be seen. The two cartilaginous masses, the medial in particular, contribute to growth in length and to the curved configuration. Growth in width and modeling occur by periosteal bone formation and resorption.

Some textbooks (Warwick and Williams 1973, Kamina 1984) located the site of primary ossification in the middle of the clavicle, which signifies that both cartilaginous growth areas contribute equally to the growth in length. Ogden (1979) studied postnatal development of the clavicle and found that the longitudinal growth of the clavicle depends mainly on the growth of the medial end. He assumed that the transition point of the two curves of the clavicle coincides with the original primary ossification center. We documented that the medial part contributes more to growth than the lateral part, even *in utero*. Further, our results strongly suggest that the anterior convexity of the medial part is the

result of enchondral bone formation of the medial cartilaginous tissue, whereas this process contributes only to formation of the lateral, flattened part of the clavicle. These findings indicate that in the mature clavicle the junction of the two parts of the clavicle, which is located one fourth to one third distance from the lateral end, corresponds to the site of the fused ossification centers. In none of our specimens was the subclavian artery close to the clavicle.

Our observation of the normal intrauterine development of the clavicle contradicts the theory of the pathogenesis of the congenital pseudarthrosis of the clavicle suggested by Fawcett (1913) and by Alldred (1963). Whereas congenital pseudarthroses develop in the middle part of the clavicle, the site of the fused ossification centers is located between the lateral and middle one third of the clavicle. In addition, we could not observe a single instance of nonfusion after Stage 20. In addition, our findings fail to lend support to the theory of Lloyd-Roberts et al. (1975), because the distance between the clavicle and the subclavian artery would prevent any effect of pulsation on bone formation.

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