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The development of centres of ossification of bones forming elbow joints in young swine‡

DENISE M. VISCO, MICHAEL A. HILL*, DAVID C. VAN SICKLE AND STEVEN A. KINCAID†

*Departments of Anatomy and * Veterinary Clinical Science, Purdue University, West Lafayette, Indiana,* 47907 *and the † Department of Anatomy and Histology, Auburn University, Auburn, Alabama,* 36849, *USA*

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INTRODUCTION

The formation and growth of the mammalian skeleton is accomplished by intramembranous and endochondral ossification. The transition of the hyaline cartilaginous *anlage* of long bones to bone during the process of endochondral ossification is initiated in the diaphysis and the nidus of bone is referred to as the diaphyseal or primary centre of ossification. Initially, the cartilaginous *anlage* is avascular but, following calcification of the matrix, a periosteal bud invades the middle of the diaphyseal *anlage* and bone formation ensues at the centre of the model (Jee, 1983). The extremities of the *anlage* are subsequently ossified by a separate focus of bone termed an epiphyseal or secondary centre of ossification (ECO). As the ECO develops, a partition of hyaline cartilage, the growth plate, separates it from the metaphysis and diaphysis. Eventually, as the growth plate closes, the ECO fuses with metaphyseal bone and the trabeculae of the ECO and metaphysis become contiguous.

The initiation, formation and expansion of the ECO and the roles that cartilage canals (C-C) may play in these processes are uncertain. Three hypotheses concerning the role of C-C in the initiation of the ECO have evolved. First, some authors state that endochondral ossification within the ECO begins in an avascular matrix and is similar to the formation of the diaphyseal centre of ossification (Ham & Cormack, 1979; Delmann & Brown, 1981; Jee, 1983; Floyd *et al.* 1987). In a second hypothesis the presence of C-C is acknowledged. However, the second group of researchers considered that the role of the C-C is to provide nourishment for the chondroepiphysis and either do not comment on the relationship of the C-C to the formation of the ECO or state that C-C were not directly related to initiation of endochondral ossification (Haines, 1933; Hurrell, 1934; Levene, 1964; Trueta, 1968). Instead, it was considered that endochondral ossification is initiated in an avascular area located between C-C (Haines, 1933; Hurrell, 1934). A third group of authors hypothesised that the initiation of endochondral ossification occurs in an area rich in C-C or is associated with the glomerular ends of specific C-C (Haraldsson, 1962; Van Sickle, 1966*a*, *b*; Gray & Gardner, 1969; Lutfi, 1970; Wilsman, 1970; Agrawal, Atre & Kulkarno, 1984; Cole & Wezeman, 1985; Van Sickle, 1985).

The present authors were interested in understanding the relationship between cartilage canals and hypertrophied chondrocytes at sites of initiation of centres of ossification and in the postnatal development of the elbow joint in pigs. Because of the

‡ Reprint requests to Dr D. Visco, Miles Inc. – I.A.A., 400 Morgan Lane, West Haven, CT 06516, USA.

conflicting information about the initiation of the ECO in different species, the objective of the present project was to study the development and growth of the ECO in each of the bones forming the elbow joint in pigs.

MATERIALS AND METHODS

Crossbred pigs (n = 39) from 3 farms were divided into 8 groups; 5 pigs each at 1 day, 1 week, 2·5 weeks, 5 weeks, 7·5 weeks, 10 weeks, and 12·5 weeks of age, and 4 pigs at 15 weeks of age.

The perfusion techniques and method of preparation of the tissues for mesoscopical evaluation have been described previously (Visco, Van Sickle, Hill & Kincaid, 1989). Briefly, each pig was heparinised, anaesthetised, exsanguinated and the thoracic limbs were perfused with India ink or a maroon or yellow silicone rubber injection compound. The humerus, ulna, and radius were dissected free of soft tissues, examined, fixed in neutral-buffered 10% formalin or 70% ethyl alcohol and then cleared by the modified Spalteholz technique (Guyer, 1953). While the bones were in either neutral-buffered 10% formalin or ethyl alcohol, a medio-lateral and a cranio-caudal radiograph was made of each bone. All bones were radiographed (22·5 kV, 5·0 mA and 30 seconds) using medical X-ray film and fine screens without a grid (Kodak TM-L Film, Lanex Fine Screens, Eastman Kodak, Rochester, NY). Films were developed in an automatic processor (G. E. MSI 1250, General Electric, Milwaukee, WI).

Following macroscopical or mesoscopical examination of cleared sagittal slabs of cartilage and bone, foci of cartilage that were apparently undergoing calcification or ossification were removed using a razor blade, placed in absolute alcohol and then rehydrated. All specimens were stored in 70% alcohol until they were sectioned. The specimens were mounted in methyl cellulose (O.C.T. compound, Lab-Tek Products, Division of Miles Labs, Inc., Naperville, IL) and were sectioned in a cryostat microtome (IEC CTF Microtome-Cryostat, International Equipment Co., A division of Damon, Needham, MA) at a thickness of 10 μm. A minimum of 60 serial sections was examined. The sections were placed on acid-cleaned, gel-coated slides and stained with haematoxylin and eosin, toluidine blue (Getzy, Malemud, Goldberg & Moskowitz, 1982), alcian blue critical electrolyte concentration technique (AB in 0·4 M-$MgCl_2$ or AB in 0·9 M-$MgCl_2$) (Dorling, 1969), safranin O with fast green (Lillie, 1965), or by the von Kossa technique (Luna, 1968).

RESULTS

Numbers of pigs in which ECOs were observed in radiographs are shown in Table 1. The smallest areas of radio-opacity were approximately 2 mm in their largest dimension. The sites in which ECOs were observed mesoscopically in slabs of bone from pigs of different ages are also shown in Table 1. Tissues from the following sites in pigs at different ages were examined microscopically: the olecranon process from two 1 day old pigs; the medial epicondyle from one 1 week old pig and two 2·5 weeks old pigs; the lateral epicondyle from two 7·5 and 10 weeks old pigs, respectively, and one pig at 12·5 and 15 weeks old, respectively; the medial condyle of one pig at both 1 week old and 2·5 weeks old.

Table 1. *Radiological evaluation of whole bones for the presence of epiphyseal centres of ossification and sites with an epiphyseal centre of ossification on mesoscopical examination of cleared specimens*

Site	Age of pigs							
	1 day	1 wk	2·5 wk	5 wk	7·5 wk	10 wk	12·5 wk	15 wk
Humerus								
Condyle	10 (10) [10]	10 (10) [10]	10 (10) [10]	(10 (10) [10]	10 (10) [10]	10 (10) [10]	10 (10) [10]	8 (8) [8]
Medial epicondyle	0 (1) [0]	0 (10) [2]	2 (10) [4]	10 (1) [10]	10 (10) [10]	10 (10) [10]	10 (10) [10]	8 (8) [8]
Lateral epicondyle	0 (10) [0]	0 (10) [0]	0 (10) [0]	0 (10) [0]	0 (10) [2]	0 (10) [6]	10 (10) [10]	8 (8) [8]
Ulna								
Olecranon	6 (10) [6]	8 (10) [8]	10 (10) [10]	10 (10) [10]	10 (10) [10]	10 (10) [10]	10 (10) [10]	8 (8) [8]
Radius								
Proximal part	10 (10) [10]	10 (10) [10]	10 (10) [10]	10 (10) [10]	10 (10) [10]	10 (10) [10]	10 (10) [10]	8 (8) [8]

Numbers represent the number of sites with radiographical evidence of centres of ossification.
Numbers in (parentheses) represent the number of sites examined both radiographically and mesoscopically.
Numbers in [brackets] represent the number of sites with mesoscopical evidence of centres of ossification.

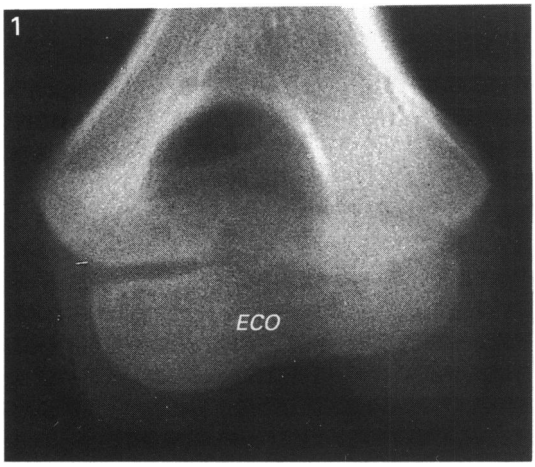
Radiological and macroscopical evaluation of whole bones

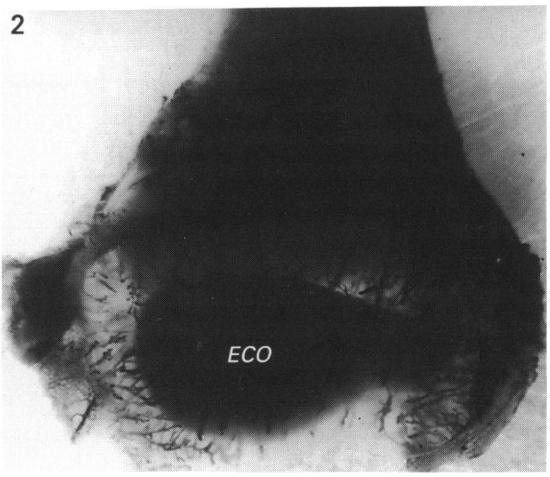
Humerus

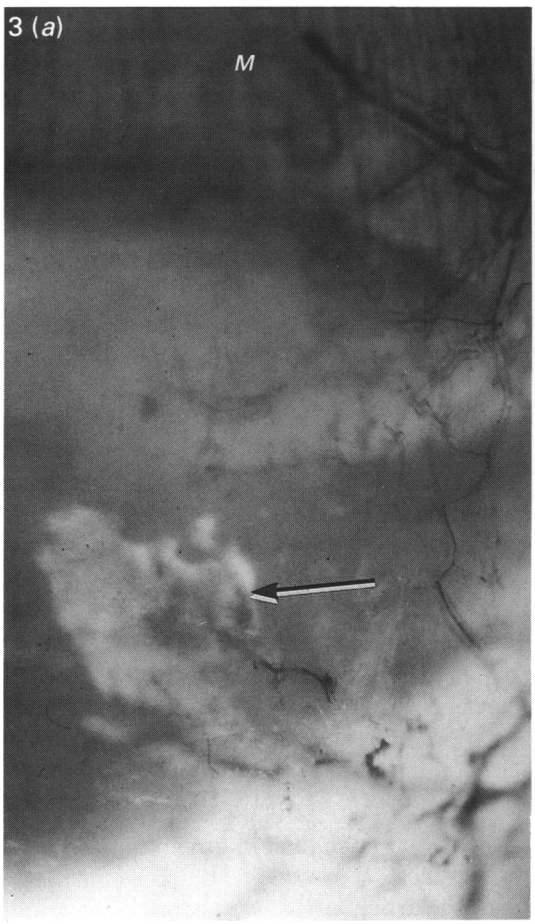
At birth an ECO was present in the humeral condyles of all pigs. In radiographs, initially, the ECO of the humeral condyles was an oval or teardrop-shaped structure. With increasing age the narrow end of the centre expanded until the configuration was that of the outline of a figure eight or an irregular trapezoid (Fig. 1). The ECO of the lateral epicondyle was often incorporated within the image of the ECO of the lateral condyle, whereas, the ECO of the medial epicondyle was a distinct radio-opaque oval structure located proximal and lateral to the medial margin of the medial condyle.

In cleared specimens, the cranial surface of the ECO of the condyles of one day and one week old pigs was ovoid or teardrop-shaped with the base located laterally and the apex medially to the medial sagittal sulcus. The shape and eccentric location of the ECO contributed to the variation in thickness of the articular-epiphyseal cartilage complex across the distal part of the humerus (Fig. 2). The cartilage between the articular surface and the ECO was thicker in the medial condyle than in other regions of the distal part of the humeral condyles. As the pigs increased in age to 15 weeks the ECO enlarged and had a similar contour to that of the articular surface except at the distal corners of the medial and lateral condyles and the proximal corner of the medial condyle which consisted of articular–epiphyseal cartilage complex.

Although absent from all pigs at birth, an ECO in the medial epicondyle was present in radiographs of 2 of 10 humeri from 2·5 weeks old pigs. However, in cleared specimens, there was an ECO in 2 of 10 humeri from one week old pigs and 4 of 10 humeri from 2·5 weeks old pigs (Fig. 3*a*). By the time the pigs were 5 weeks of age, the centre was in all humeri (10 out of 10). Between 1 and 5 weeks of age, the centre







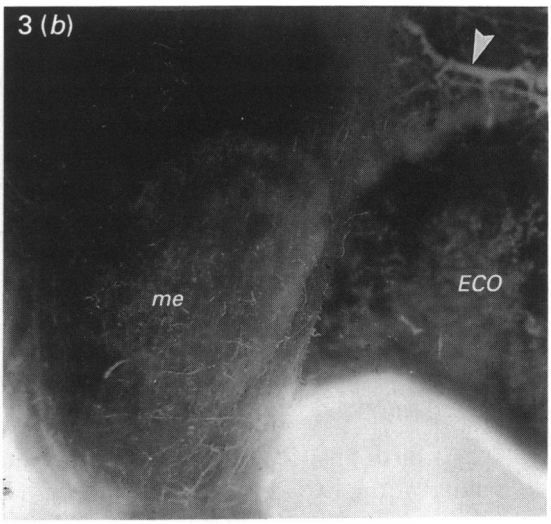


Fig. 1. Radiograph of the distal part of the humerus of a 2·5 weeks old pig. ECO, epiphyseal centre of ossification.

Fig. 2. The cranial surface of the distal portion of the humerus from a one week old pig. Note the teardrop-shaped epiphyseal centre of ossification (ECO) and variations in the thickness of the articular-epiphyseal cartilage complex. India ink perfusion.

Fig. 3(*a–b*). The developing distal portion of the humerus. *M*, metaphysis; ECO, epiphyseal centre of ossification of the condyles. The perichondrium has been removed from the medial epicondyle (*me*). Caudal views. (*a*) From a 2·5 weeks old pig. Note the developing area of calcification (arrow). India ink perfusion. (*b*) From a 12·5 weeks old pig. Note the large, superficial blood vessel (arrowhead) from which cartilage canals originate. Silicone rubber perfusion.

of ossification became oval and it expanded to fill most of the cartilaginous *anlage* of the medial epicondyle by 15 weeks of age (Fig. 3*b*). The proximal part of the cranial surface of the ECO of the medial epicondyle was angled caudally and this ECO occupied an indentation in the caudal surface of the lateral half of the medial condyle. The proximal surface of the ECO of the medial epicondyle was flat and there was a flat growth plate between it and the metaphyseal bone of the crest of the medial epicondyle.

An image of the ECO of the lateral epicondyle was visible in radiographs of humeri from all pigs at 12·5 weeks of age but in none of the radiographs from younger pigs. In cleared specimens, the ECO of the lateral epicondyle was present in 2 of 10 humeri from 7·5 weeks old pigs, 6 of 10 humeri from 10 weeks old pigs, and in all (10 out of 10) 12·5 weeks old pigs. The centre of ossification was located in the proximal one quarter of the caudal aspect of the cartilaginous *anlage* of the lateral epicondyle and originated as one or more foci. By 12·5 weeks of age, the centre of ossification of the lateral epicondyle was a single, small ball-shaped or irregular ovoid structure. In the 15 weeks old pigs, it was larger and occupied most of the lateral aspect of the cartilaginous *anlage* of the lateral epicondyle and approximated to a cube. The centre of ossification of the lateral epicondyle was caudo-lateral to the ECO of the humeral condyles. A discoidal growth plate was present between the metaphyseal bone of the distal part of the humerus and the centre of ossification of the lateral epicondyle. On the caudal surface, the lateral condyle had a similar outline to that observed when the specimen was viewed from the cranial aspect.

Ulna

The ECO of the olecranon process was present in radiographs from six pigs at birth. Initially, in lateral views, the ECO was an oval radio-dense area just caudal to the centre of the cartilaginous *anlage* and with increasing age changed to half of an oval which occupied most of the *anlage*.

In cleared specimens from the four pigs that lacked an established ECO in radiographs of the olecranon process, the epiphyseal cartilage contained numerous C-C (Fig. 4*a*). By one week of age, the centre was in 8 out of 10 ulnae, was ovoid, and was located eccentrically caudad within the olecranon tuberosity. The long axis of the oval ECO was tilted so that the cranial end was more proximal (Fig. 4*b*). The ECO was seen in all 10 ulnae of 2·5 weeks old pigs. It was now larger, forming one-half of an oval located eccentrically within the olecranon tuberosity. The apical end of the long axis was tilted proximally and the midpoint of the long axis of the ECO was caudad to the mid-frontal plane. The base of the ECO was located distally and capped the proximal aspect of the growth plate of the proximal part of the ulna. The cranial edge of the centre was further from the metaphysis than was the caudal edge. The ECO enlarged until it filled most of the cartilaginous *anlage* of the olecranon tuberosity, except for the growth plate and a layer of hyaline cartilage which completely covered the curved proximal surface of the centre (Fig. 4*c*). The growth plate was discoidal in shape and the hyaline cartilage was deeper at the cranial and caudal edges than in the middle portion.

The cranial surface of the bone of the olecranon, proximal to the anconeal process, was flat in the one day and one week old pigs. As the pigs increased in age the bone expanded cranially, especially in the mid-region, and became convex in a cranial direction. The anconeal process developed as a bony prominence and consequently in radiographs the depth of the bony trochlear notch increased as the pigs aged. However, in cleared specimens of one day and one week old pigs, much of the anconeal process was hyaline cartilage (Fig. 4*b*). The cranial surface of the bone of the olecranon, at the

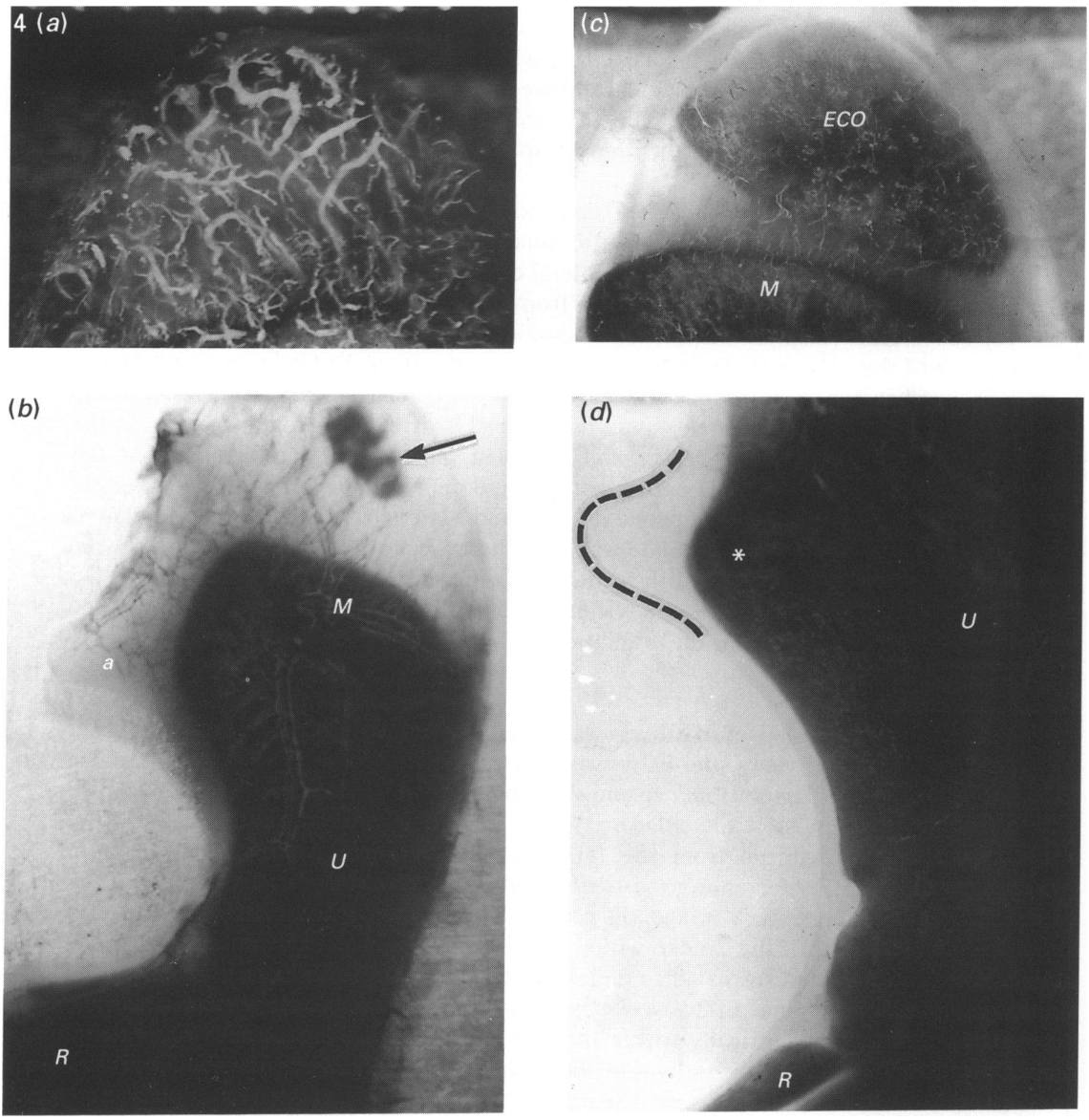


Fig. 4(*a-d*). Developing proximal portions of the ulna (*U*) and radius (*R*). ECO, epiphyseal centre of ossification; *M*, metaphysis; *a*, anconeal process. (*a*) The lateral surface of the olecranon tuberosity of a one week old pig. The perichondrium has been stripped away demonstrating the vascularity of the chondro-epiphysis of the olecranon process. No epiphyseal centre of ossification is present. Silicone rubber perfusion. (*b*) View of the medial surface of the proximal part of the ulna of a one day old pig. Note the rudimentary ECO (arrow). India ink perfusion. (*c*) Epiphyseal centre of ossification from the proximal portion of ulna of a 5 weeks old pig. Silicone rubber perfusion. (*d*) Medial surface of developing bone (*) in the anconeal process in a 12·5 weeks old pig. Dashed line, outline of cartilaginous *anlage* of anconeal process. Silicone rubber perfusion.

base of the cartilaginous anconeal process, was flat in these pigs. By 2·5 weeks of age, bone at the base of the anconeal process protruded slightly cranially. This protuberance continued to increase in size and grew pyramidally, expanding slowly from the base of the anconeal process toward its apex (Fig. 4*d*). In pigs at 15 weeks of age the bone had not completely filled the cartilaginous *anlage* of the anconeal process. At no time was there a separate centre of ossification in the anconeal process.

Radius

An ECO was present at birth in the proximal epiphysis of all radii examined (10 out of 10) and was an oval radio-dense structure with a portion of the proximal surface slightly concave. In cleared specimens, the ECO was round to oval and was located eccentrically with the majority of the centre in the lateral half of the chondro-epiphysis. The proximal surface of the ECO approximated to the contour of the concave articular surface and was smooth except at the outer margins where C-C entered the bone. By 5 weeks of age, the oval-shaped ECO extended into the medial half of the cartilaginous *anlage* of the epiphysis. However, development of the medial side lagged behind that of the lateral side. The ossification centre continued to grow circumferentially as the pigs increased in age. At 15 weeks of age the ECO had replaced the majority of the cartilaginous *anlage*, but it had reached neither the medial nor the lateral margin.

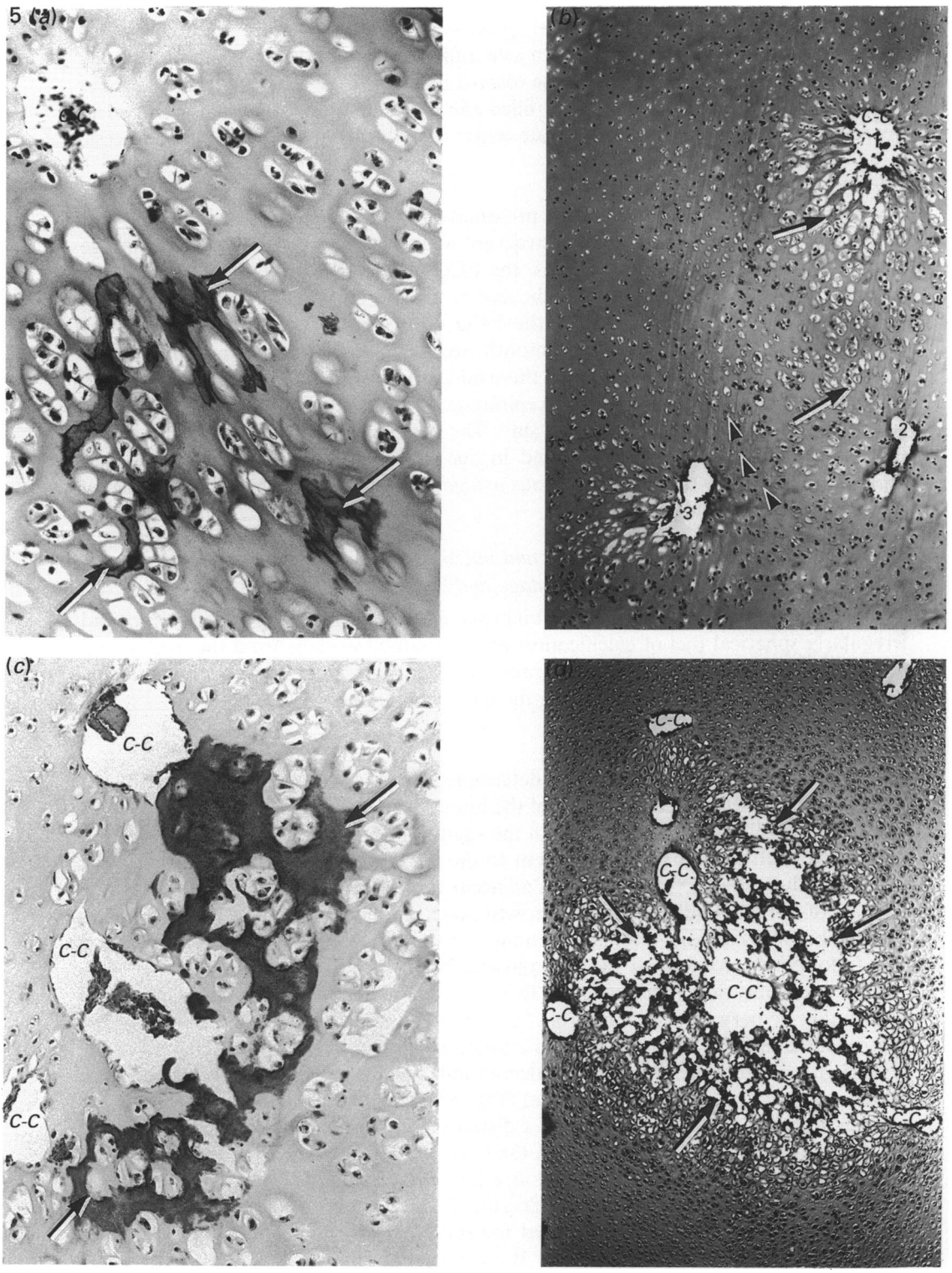
Mesoscopical examination of slabs and microscopical examination of sections of cartilage and bone

Cleared specimens were examined at increasing magnifications up to forty times and irregularly spherical foci of calcification were visualised in slabs from the medial and lateral epicondyles and the olecranon process. At any site, small foci encompassed the ends of a few C-C or were present at the ends of branches of C-C.

Humerus

The shape of the ECO was easily determined by using the slabs. Whereas in the whole bones the ECO of the condyle of the humerus in one day and one week old pigs appeared ovoid or teardrop-shaped, in the sagittal slabs the medial condyle was half of an oval, with the flat surface adjacent to the growth plate. In the slab through the lateral condyle, the ECO was concave adjacent to the growth plate. In older pigs, the portion of the ECO adjacent to the growth plate in the medial sagittal sulcus was V-shaped, but in the medial and lateral condyles the periphery of the ECO was irregular and followed the contour of the metaphyseal bone.

Foci of calcification adjacent to the ECO were examined microscopically and it was found that hypertrophied chondrocytes radiated out from one side of C-C. The matrix surrounding the hypertrophied chondrocytes within these foci was basophilic (Fig. 5*a*), safranophilic (Fig. 5*b*, *d*), alcianophilic, β-metachromatic (toluidine blue), or tan-coloured (von Kossa technique) (Fig. 5*c*). In small areas of calcification, the interterritorial matrix was calcified at a distance equivalent to two or three times the diameter of a chondrocyte away from the C-C. Matrix between chondrocytes within an isogenous group was pink, but the interterritorial matrix between the groups was tan-coloured in larger areas of calcification in sections stained by the von Kossa technique. In sections which contained multiple foci of calcification, hypertrophied chondrocytes were arranged only on the centripetal axis (in the side of the canal towards the centre of a group) of a group of C-C in all but one specimen. The exceptional case had hypertrophied chondrocytes on the opposite side of the canal


5 (a)
(b)
C-C
2
3
(c)
C-C
C-C
C-C
(d)
C-C
C-C
C-C
C-C
C-C

(Fig. 5*b*). The largest foci of calcification surrounded C-C and, in some foci, ossification had begun.

In a slab from the medial condyle of the humerus of a single one week old pig, a focus of calcification was located at the enlarged end of a perforating cartilage canal that originated from the proximal, caudal region of the centre of ossification. After leaving the ECO, the canal curved back toward the ECO (Fig. 6). There was hyaline cartilage between the focus of calcification and the centre of ossification. On microscopical examination, the same focus of calcification contained hypertrophied chondrocytes that were close to the canal and were surrounded by matrix which stained tan-coloured with the von Kossa technique. Between the ECO and the focus of calcification, the chondrocytes were hypertrophied. On the edge of the ECO at the point nearest the developing focus there was an indentation that contained hypertrophied chondrocytes which were surrounded by matrix that was light tan-coloured when stained by the von Kossa technique.

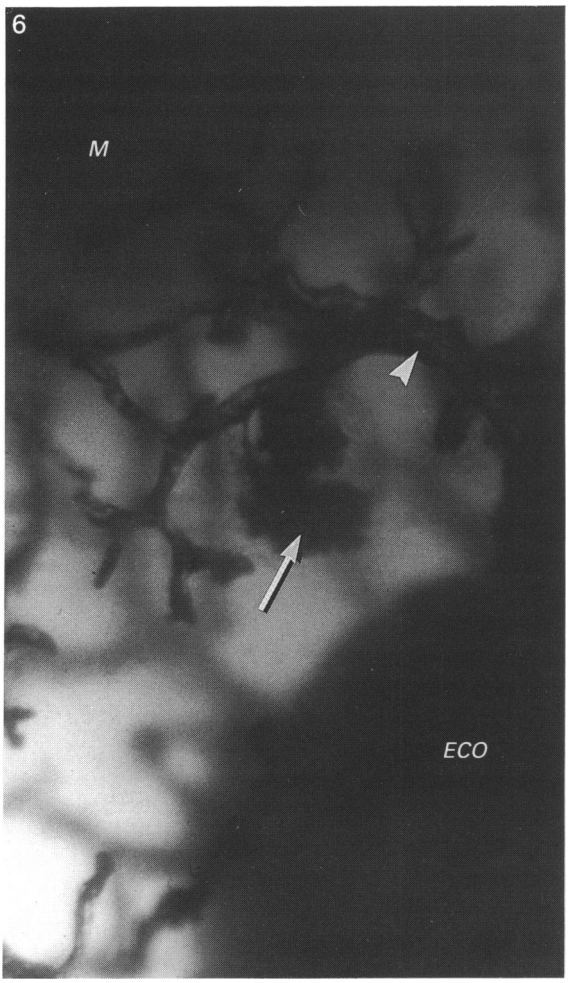
The foci of calcification in the cartilaginous *anlage* of both the medial and lateral epicondyles were located in regions close to the perichondrium. In one 12·5 weeks old pig, the focus of calcification was adjacent to a layer of fibrocartilage which merged with the fibrous connective tissue of the perichondrium. Vessels from the perichondrium entered the focus.

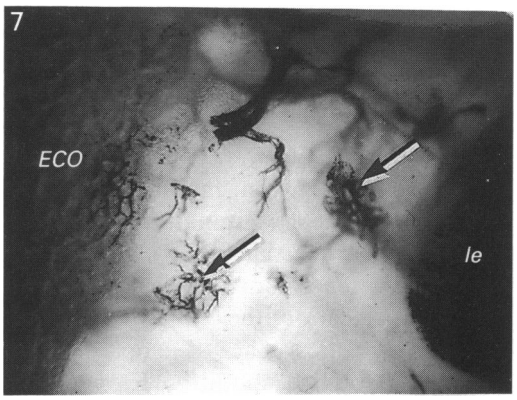
In the medial epicondyle, the ECO was initiated as a single focus or as multiple foci of irregularly-shaped spheres that were located in the proximal and medial portions of the cartilaginous *anlage* of the epicondyle. Small vascularised foci were black, maroon or yellow, depending on the colour of the injection medium used. Foci that were not vascularised were white.

From the time of initiation of the ECO of the medial epicondyle until 15 weeks of age, hyaline cartilage was present between the epicondyle and the ECO of the medial condyle. Cartilage canals were present in this cartilage and in some cases they linked the vasculature of one centre to that of the other.

In the lateral epicondyle, each focus of calcification surrounded the end of a canal. In one specimen three C-C gave origin to four foci of calcification since two separate branches from one canal each supplied a focus. In the cartilaginous *anlage* of the lateral epicondyle of two 15 weeks old pigs additional foci of calcification that were observed mesoscopically were located between the ECO of the lateral epicondyle and the ECO of the lateral condyle (Fig. 7). Microscopically, the foci were tan-coloured with the von Kossa technique. The chondrocytes which were between the ECO of the lateral condyle and the developing foci of calcification were not hypertrophied. One focus of calcification was developing adjacent to three branches of a C-C (Fig. 5*a*).

Fig. 5(*a–d*). Photomicrographs documenting the initiation of centres of ossification. C-C, cartilage canals. (*a*) A focus of calcification is developing adjacent to a canal near the caudal surface of the lateral epicondyle from a 7·5 weeks old pig. Interterritorial matrix (arrows) between the hypertrophied chondrocytes is basophilic. Matrix between the C-C and the groups of hypertrophied chondrocytes is not mineralised. H & E. × 100. (*b*) Three foci of calcification are developing adjacent to C-C near the caudal surface of the lateral epicondyle from a 7.5 weeks old pig. The hypertrophied chondrocytes adjacent to C-C 1 and 2 are radiating centripetally (arrows), whereas those adjacent to canal 3 are on the opposite side. There are small chondrocytes (arrowheads) between the hypertrophied chondrocytes and canal 3. Safranin O. × 38. (*c*) An accessory focus of calcification is developing adjacent to 3 branches from a C-C in the lateral epicondyle of a 15 weeks old pig. Note the calcified matrix (arrows). VK. × 118. (*d*) A well-developed focus of calcification (delineated by arrows) from near the caudal surface of the olecranon process of a one day old pig contains C-C. Safranin O. × 36.





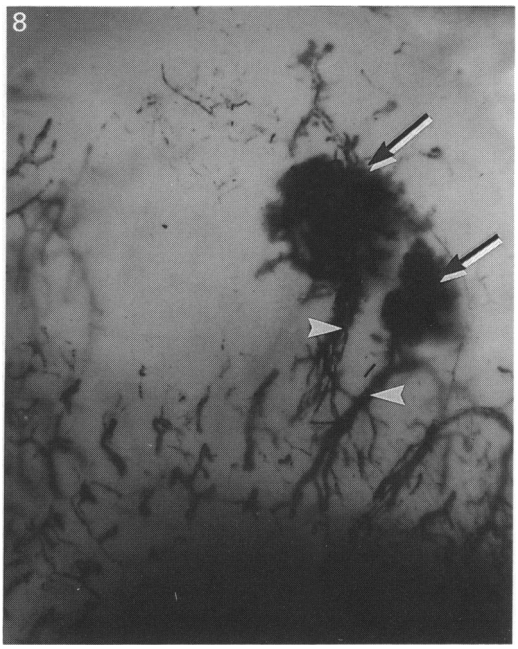


Fig. 6. The proximal, caudal edge of a slab cut in a sagittal plane through the epiphyseal centre of ossification of the medial condyle (ECO) of a one week old pig. Note the focus of calcification (arrow) around the enlarged end of a perforating cartilage canal (arrowhead) that is looping cranially. *M*, metaphysis. India ink perfusion.

Fig. 7. Slab cut in a sagittal plane through the epiphyseal centre of ossification of the lateral condyle (ECO) and the lateral epicondyle (*le*) of a 15 weeks old pig. Note the foci of calcification (arrows) around the C-C. India ink perfusion.

Fig. 8. View from the medial surface of partially injected olecranon tuberosity from a one week old pig. The perichondrium has been stripped away exposing the developing centres of ossification (arrows) and some of the vessels that enter the centres (arrowheads). India ink perfusion.

The cartilage between the ECO of the lateral epicondyle and the ECO of the lateral condyle in another 15 weeks old pig contained foci of calcification around the ends of several C-C which originated directly from the perichondrium. Although there were C-C in the cartilage between the ECO of the lateral condyle and the ECO of the lateral epicondyle, the vasculature from the two centres did not anastomose.

Ulna

One or more foci of calcification were present in the cartilaginous *anlage* of the olecranon of 6 out of 10 pigs at birth and 8 out of 10 pigs at one week old (Fig. 8). In the slabs from older pigs, the ECO of the olecranon was triangular and was orientated caudally within the olecranon. The cranial edge was tilted proximally and the depth of the growth plate between the ECO and the metaphyseal bone of the olecranon tuberosity was greatest at this edge. This pattern was present from the time of development until between 10 and 12·5 weeks of age, at which time the middle portion of the centre was closest to the metaphysis. The cranial edge of the growth plate remained deeper than the caudal edge.

Radius

If examined from the intact proximal surface, the ECO of the radius was seen to be a flat oval structure. However, when the sagittal slabs were examined, the centre was triangular, the caudal aspect being wider than the cranial aspect. This was consistent from one day to 15 weeks of age. Microscopically, the development of foci of calcification and ossification in ulnae and radii appeared to be similar to that in humeri.

DISCUSSION

There was variation in the time of appearance of the ECO of the medial epicondyle, the lateral epicondyle and the olecranon. For example, the ECO of the olecranon was well-developed in eight ulnae from one week old pigs but was completely absent in the other two ulnae. Although it was possible that body weight rather than chronological age correlated with maturation of the skeleton, body weights were not obtained.

Developing centres of ossification were observed at an earlier age using mesoscopical examination of cleared specimens when compared with examination of radiographs taken at corresponding ages. In support of mesoscopical findings, the centres were examined microscopically using the von Kossa technique which indirectly indicates the presence of calcium in tissues (Thompson, 1966; Meloan & Puchtler, 1985). Because silver phosphate was yellow or brown in the study of Meloan & Puchtler (1985), it appeared that the tan pigment obtained in the present study was a more accurate representation of the distribution of the calcium salts than were pigments in other studies quoted by Meloan & Puchtler, in which tissues became black or dark brown. All foci that were examined contained calcium and it is possible that smaller foci of calcification and ossification were too small or insufficiently calcified to have been detected radiographically. Likewise, it is possible that some of the smallest foci may have been missed on mesoscopical examination.

Endochondral ossification of ECOs has been described as beginning with multiple foci of calcification (Waugh, 1958; Van Sickle, 1966*a*; Caffey, 1967; Wilsman, 1970; Wilsman & Van Sickle, 1970; Van Sickle, 1985). Several different findings from research on human fetuses and children have been reported. Haraldsson (1962) found that the ECO of the distal portion of the humerus developed in an area rich in C-C. Caffey (1967) stated that ECOs appeared as several fine radio-dense foci which later fused into a single larger bony centre. Gray & Gardner (1969) reported that the margins of certain C-C became calcified and that bone formation took place within these C-C. Agrawal *et al.* (1984) demonstrated that a layer of osteogenic tissue and a thin layer of osteoid lined the walls of C-C within the ECO. In dogs, the ECO

originated as multiple foci in the anconeal process (Van Sickle, 1966*a*) and the head of the humerus (Wilsman, 1970; Wilsman & Van Sickle, 1970). Whereas Waugh (1958) suggested that in children the foci of calcification developed around separate, but adjacent C-C, Wilsman (1970) and Van Sickle (1966*a*) demonstrated that the foci of calcification occurred around the ends of specific C-C in dogs. Wilsman (1970) and Wilsman & Van Sickle (1970) described a focus of calcification as a sphere which had as its centre the glomerular end of vessels in C-C. Floyd *et al.* (1987), who examined femoral condyles of CD1 mice, considered that chondrocytes underwent hypertrophy, vascular invasion occurred and subsequently the ECO formed. In broilers, ECOs developed in areas that were penetrated by many C-C and the number of C-C increased as initiation of an ECO occurred (Thorpe, 1988*a*).

Hurrell (1934) observed that, in the human carpus and tarsus, zones of swollen chondrocytes were separated from the margins of the C-C by a distance equivalent to 20 to 30 times the diameter of a chondrocyte. Although Lutfi (1970) stated that, in the foci of calcification from chickens, the first hypertrophied chondrocytes appeared close to the C-C, his illustrations seemingly had hypertrophied chondrocytes midway between canals. The present authors concurred with Lutfi but, in the present study, hypertrophied chondrocytes were much more closely associated with the C-C. However, even with a closer proximity, the C-C usually did not abut the plasma membrane of the hypertrophied chondrocytes. Instead, they were separated from them by a narrow band of matrix.

In the present study, except in one case, the ECO developed as multiple foci. Mesoscopically and microscopically, the foci of calcification were adjacent to the ends of C-C and branches from C-C, or the focus surrounded the ends of several C-C. Mesoscopically, many foci of calcification were white. However, none of the C-C were calcified at their margins. Because blood vessels within C-C contained coloured dyes, the fact that the foci were white correlated well with the observations made microscopically, i.e. that initially the C-C were adjacent to, and not within, the foci of calcification. Unlike the observations concerning cartilage from dogs (Wilsman, 1970), the present study showed that chondrocytes radiated from only small portions of C-C and were not centrifugally arranged until well-developed centres were present.

Kugler, Tomlin, Wagstaff & Ward (1979) and Floyd *et al.* (1987) hypothesised that C-C grew toward an area of hypertrophied chondrocytes. However, in specimens from the present study, despite the fact that chondrocytes were not hypertrophied, many C-C were in areas of the cartilaginous *anlage* in which an ECO would develop. Although most foci of hypertrophied chondrocytes and calcification were initiated at the ends of the C-C, sometimes they encompassed the end of branches of C-C and the parent canals continued deeper into the uncalcified cartilage. If the hypothesis that hypertrophied chondrocytes directed the growth of C-C were correct, it was unlikely that C-C would have been attracted to the focus of hypertrophied chondrocytes only to pass by it to areas in which chondrocytes were not hypertrophied. Therefore, the present authors hypothesised that the chondrocytes hypertrophied near the ends of a limited number of C-C. It was noteworthy that, initially, the hypertrophied chondrocytes were adjacent to only one side of the C-C.

As was reported by Kincaid & Lidvall (1983), in the distal part of the humerus ossification of the medial condyle lagged behind that of the lateral condyle. The articular-epiphyseal cartilage complex adjacent to the ECO of the medial condyle was thick and the ossification front of the ECO was not exactly parallel with the contour of the articular surface. However, in poultry, Thorp (1988*c*) demonstrated that growth of the medial condyle outstripped that of the lateral condyle.

Typical of the incomplete descriptions of development of ECO in veterinary literature, a standard textbook of anatomy (Getty, 1975) does not indicate adequately either the morphogenesis or the chronology of ossification of the lateral epicondyle of the humerus of the pig. In a radiographical study of the initiation of ossification centres in the pig, Wenham, Fowler & McDonald (1973) indicated that the centres in the epicondyles of the humerus appear at approximately three weeks postnatally. However, the periphery of the epicondyle was not clearly defined by these authors. In the present study, mesoscopical and radiographical evidence for the development of centres of ossification in the medial epicondyle of some one week and 2·5 weeks old pigs and in all 5 weeks old pigs supported an interpretation of the work of Wenham *et al.* (1973) who seemed to be referring only to the ossification centre of the medial epicondyle. The ECO of the lateral epicondyle was not observed in the pigs from the present study until 7·5 weeks of age, indicating initiation of the centre between 5 and 7·5 weeks. This further supports the inference of the present authors who considered that Wenham *et al.* (1973) were describing osteogenesis of the medial epicondyle, rather than both epicondyles.

In the distal part of the humerus, ECOs of the epicondyles were separate from the larger principal centre of ossification of the condyles. The initial focus (or foci) of ossification of the lateral epicondyle was located caudal and lateral to the principal ECO, whereas the medial epicondyle developed caudal to the medial edge of the medial condyle. However, the location and size of the lateral epicondyle was such that it was overshadowed by the lateral condyle and was easily overlooked radiographically. Therefore, there was a discrepancy between the times of appearance of the ECO on the basis of radiographical or mesoscopical examination.

The medial and lateral epicondyles respectively are the sites of attachment of the tendons of origin of flexor and extensor muscles of the distal portion of the thoracic limb. Location of the centres of ossification of the medial and lateral epicondyles may help to explain why the ossification of the medial epicondyle was initiated between 5 and 7·5 weeks before that of the lateral epicondyle. The centre of ossification of the medial epicondyle was located caudal and ventral to the edge of the medial condyle, whereas the lateral epicondyle was located caudal and lateral to the lateral condyle. Under physiological loads, and in comparable situations, cartilage has been assumed to grow faster under compression than under tension (Frost, 1979). Therefore, it was possible that the ECO of the medial epicondyle developed earlier because it was subjected to compressive as well as tensile forces, whereas the lateral epicondyle was only under tension. In broilers, Thorp (1988*c*) demonstrated an accessory centre of ossification below the lateral collateral ligament near its site of attachment, further supporting the hypothesis that tensile forces influence the development of additional centres of ossification. However, in the absence of information on the biomechanics of the elbow, these postulates are speculative for ECOs in pigs.

Unlike the developmental changes in the foal (Brown & MacCallum, 1974) or the German Shepherd and Greyhound pup (Van Sickle, 1966*a*, *b*, 1975), ossification of the anconeal process of the ulna in swine occurred as a pyramidal extension of the metaphyseal bone at the base of the anconeal process. As with the findings of Kincaid & Lidvall (1981), a separate centre of ossification was not observed in the present study. Kincaid & Lidvall (1981) described the ossification of the anconeal process as an extension of the proximal ulnar physis. However, in the present study the anconeal process was located on the cranial surface of the ulna and a minimum of 50% of its surface was covered by articular cartilage. The remainder of the surface was covered

by perichondrium. Therefore, in the accepted sense, this area was not a simple physis. Instead, the distal portion of the anconeal process could be classified as an articular–physeal cartilage complex.

SUMMARY

Epiphyseal centres of ossification in the bones forming the elbow joints of pigs between one day and 15 weeks of age were examined radiographically, macroscopically, mesoscopically and microscopically. Thoracic limbs from 39 pigs were perfused with India ink or silicone rubber injection compound and the bones were dissected free of soft tissues. The humerus, ulna and radius were fixed in formalin or ethyl alcohol and then cleared by the modified Spalteholz technique. Bones were radiographed, examined grossly, and then cut into slabs for mesoscopical evaluation. Foci considered to be calcifying within cartilaginous *anlage* were selected for microscopical examination.

It was concluded that the epiphyseal centre of ossification develops at different times in different sites in the bones forming the elbow joint. Centres of ossification are initiated when foci of chondrocytes adjacent to one side of a cartilage canal undergo hypertrophy and the interterritorial matrix becomes calcified. Osteogenesis then proceeds in the calcified focus, presumably with osteoprogenitor cells that originate within the cartilage canals. Subsequently, each epiphyseal centre of ossification enlarges by one of two methods. Firstly, the layer of cartilage adjacent to the centre undergoes endochondral ossification, thus allowing for the circumferential growth of the epiphyseal centre of ossification. Secondly, foci of calcification develop adjacent to the ends of cartilage canals near the epiphyseal centre of ossification and eventually the focus of calcification coalesces with the developing epiphyseal centre of ossification, thus establishing a new ossification front. Endochondral ossification continues at the periphery of the mass of bone. Mesoscopical examination is more useful than radiographical evaluation for identifying small foci of calcification which precede epiphyseal centres of ossification.

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