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Structural study of spongiosa tissue in growing sheep

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Summary. A study was made of 160 long bones taken from 40 native Merino sheep of both sexes. These animals, which represented uniform growth (mean growth-curve values), were divided into four groups which were slaughtered consecutively at 0, 45, 105 and 270 days old (0, 6.4, 15 and 38.5 weeks, respectively).

The following bones were studied; humerus, femur, tibia and os coxae. Thin lamellae taken from the metaphyses of the bones obtained were fixed, decalcified and stained with hematoxylin-eosin to assess the development of the various components of growing bony tissue.

The bones studied followed the same maturation pattern; the os coxae proved to be the best histological indicator in differentiating the age of the animals studied.

Key words: Ossification, Trabeculae, Force lines, Osteocytes, Osteoclasts

Introduction

Though researchers broadly agree on the nature of the basic structural components of growing mesenchymatous bone (bone matrix, osteoclasts, osteocytes, trabeculae, blood vessels, etc.), this does not extend to certain specific cases such as the existence of a non-minearlised area of the bone matrix, termed osteoid (Pommer, 1885). McClean and Bloom (1940) used Von Kossa's staining technique for the microscopic identification of bone minerals, reporting that bone matrix can be considered calcifiable as soon as the tissue is recognised as bone tissue. Routine electron microscopic observation has revealed the existence of a thin layer (one micron thick or less) of pre-osseous non-calcified tissue during bone formation, even in animals whose mineral intake is optimal (McClean and Urist, 1968). It should also be borne in mind that any cell capable of depositing a matrix which calcifies without being destroyed in the process is an osteoblast (Trueta, 1968). However, when the cell is undergoing mitosis, it is difficult to decide, for example, whether it is an osteogenic cell or an osteoblast; this doubt can be readily resolved by autoradiography using labelled timidine (Tonna and Cronkite, 1961, 1962; Owen, 1963). Studies on the consolidation of bone fractures and the conditions required for bone transplants have confirmed the existence of blood vessels (Harris and Ham, 1956).

As animals grow and increase in weight, new strains and stresses arise in bones, giving rise to the need for increased support. This support is apparently achieved by transformation of trabecular (or cancellous) bone into compact bone (Amprino, 1965). Transformation starts with remodelling in areas containing osteons together with large numbers of «poorly-situated» osteocytes. The remodelling channels fill with osteoclasts, followed by blood vessels and connective tissues cells (Currey, 1968).

Many papers for various purposes, deal with the surplus or deficiency of given substances in bony tissue; these include studies of iodine, amino-acid and copper deficiency (Smoliar, 1983, 1985, 1989) and the effects of excess saccharose (Smoliar, 1988), biotin (Bain et al., 1989), phosphorus and calcium (Van Kempen et al., 1976) and growth hormones (Neuman, 1986; Bubenik, 1978, 1982).

Other authors have linked changes in bone tissue to a variety of circumstances including overload (Lozupone and Favia, 1981); diet (Theriez et al., 1982) and age (Horn, 1975; García González, 1980, 1981).

The purpose of this study was to analyse the structural development of cancellous bony tissue in growing lambs, and to establish a histological standard for age-differentiation.

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Materials and methods

160 bones were taken from 40 Merino lambs of both sexes, which were salughtered at 0, 45, 105 and 270 days old. The animals selected, which were ideal representatives of uniform growth (mean growth-curve values), were slaughtered by routine commercial methods, and bones were obtained for analysis.

The following bones were selected for histological analysis: humerus, femur, tibia (long) and os coxae (irregular). Bones were cleaned of muscle, fat and adherent matter, and a thin lamella ossea was obtained from the proximal epiphyses of long bones (taking care to include the growing metaphysis) and from specific sites in irregular bones such as os coxae (dorsally to the acetabulum, at the iliosciatic junction). Samples were immediately fixed in 10% formol and decalcified in 5% trichloroacetic acid using routine laboratory techniques. Decalcification took from 15 days (lamb slaughtered at birth) to 3 months (lambs slaughtered at 270 days old). Samples were subsequently processed and stained with hematoxylin-eosin prior to analysis of growing metaphyses.

Results

The term *Haversian canal* is used here to designate those sometimes sinuous canals whose lumina contain blood vessels and mesenchymatous elements surrounded by an osteoid or osseous matrix with osteocytes. The term *force lines* is used to refer to those lines along which osteocytes are distributed; they consist of a linear arrangement of ground substance, the direction being determined by trabecular morphology.

Humerus (Fig. 1)

Birth (Fig. 1A)

Diaphysial and epiphysial trabeculae showed little calcification, and were composed of osteoid tissue (ot), with non-calcified areas and randomly-distributed osteocytes. Trabeculae were surrounded by osteoblastic cuboidal epithelium (oep). No evidence of remodelling was recorded during this period; trabecular structure, however, appeared to be identical to that found in adult animals, though trabeculae were thinner.

45 days (Fig. 1B)

At this stage of growth, trabeculae were partly calcified and osteocytes were orientated along the force lines (fl) which were now starting to appear. Osteoblastic epithelium (oep) was observed to enclose trabeculae, which were somewhat thicker than those of the previous group.

105 days (Fig. 1C)

Trabecular structures revealed an internal structure, with osteocytes (o) arranged between force lines (fl). Haversian canals (Hc) were visible. Some non-calcified areas were observed, though they were apparently remodelling foci. At this stage, some areas continued to contain osteoblastic epithelium (oep) albeit in highly-circumscribed areas.

270 days (Fig. 1D)

Remodelling foci continued to appear, and large numbers of osteoclasts (ocl) were observed in the vicinity of trabeculae. Haversian canals (Hc) were still visible. Trabecular matrix was calcified, and osteocyte orientation (o) between force lines (fl) was notable.

Os Coxae (Fig. 2)

Birth (Fig. 2A)

Trabeculae (t) were only slightly calcified, and intratrabecular lacunae remained uncalcified. Osteoblastic epithelium (oep) was evident.

45 days (Fig. 2B)

Ossification of trabeculae (t) was apparent at this stage of development. Force lines (fl) were clearly distinguishable from Haversian canals, and contained only a small number of osteocytes (o). Osteoblastic (oep) epithelium was visible.

105 and 270 days (Figs. 2C, 2D)

Similar features were observed for these two age-groups, the only difference being in the thickness of the component structures (especially the trabeculae), and the greater degree of development of Haversian canals (Hc). Force lines (fl) and Haversian canals were totally formed. The bone was fully remodelled by this stage.

Femur (Fig. 3)

Birth (Fig. 3A)

Little evidence of trabecular (t) calcification was observed, and it was just possible to make out an osteoblastic epithelium (oep). Some non-calcified intratrabecular lacunae (il) were observed.

45 days (Fig. 3B)

Trabecular calcification was almost complete. Force lines (fl) had started to become distinguishable.

105 days (Fig. 3C)

Remodelling had started and force lines (fl) were clearly delimited. Some non-calcified sinuous lacunae were again visible.

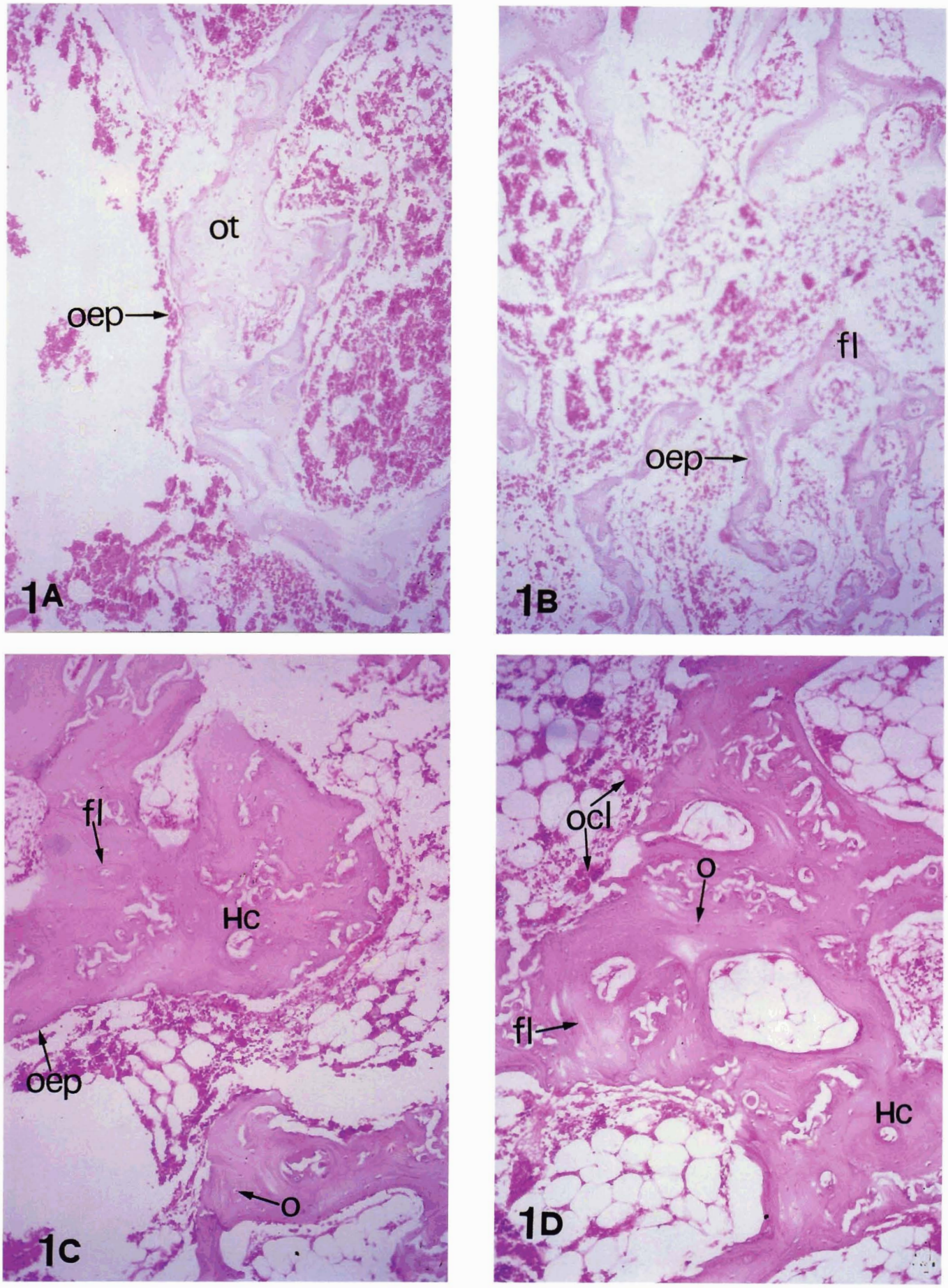

Fig. 1. Histological composition of the humerus. A) Birth; B) 45 days; C) 105 days; D) 270 days.

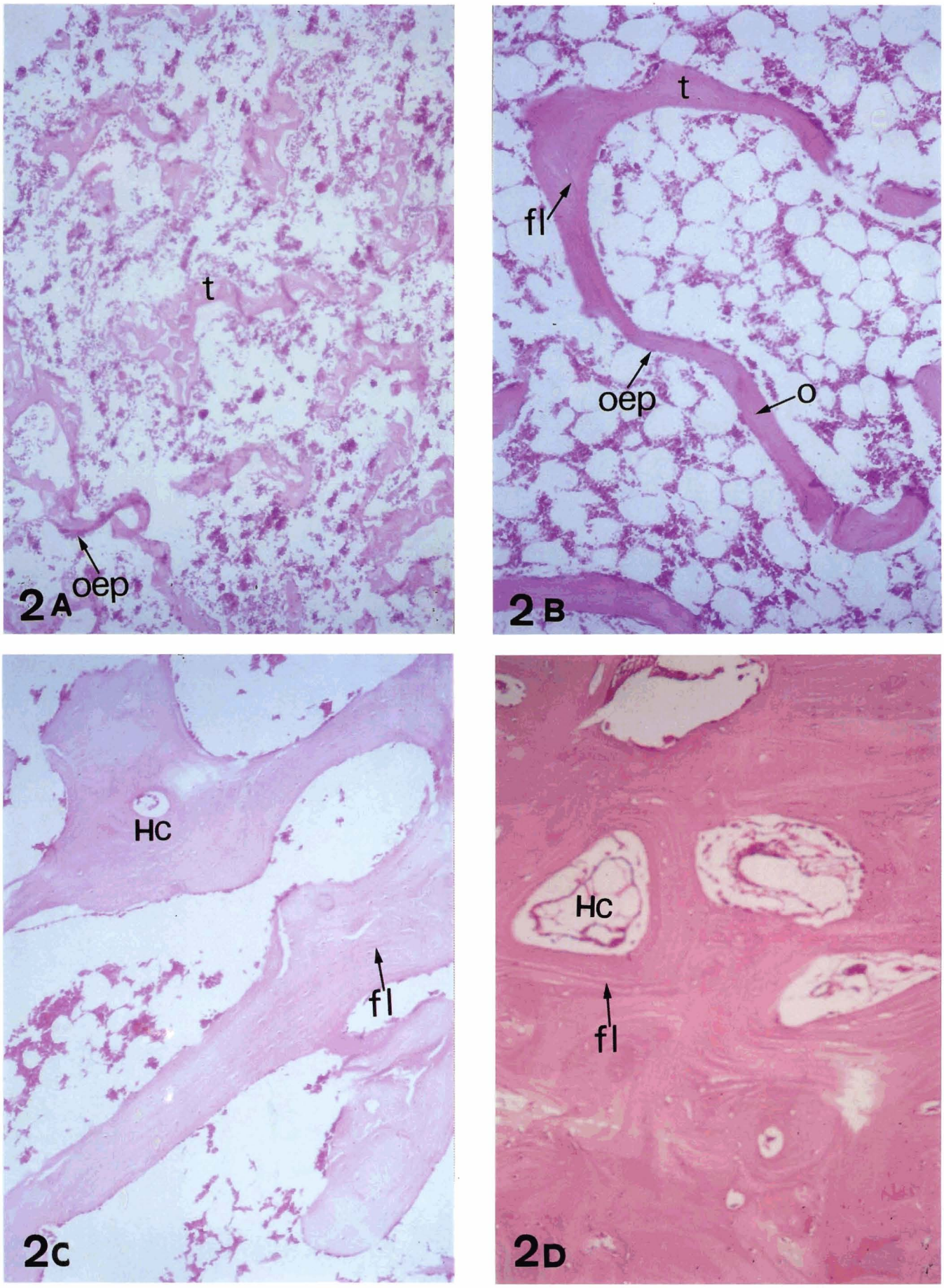

Fig. 2. Histological composition of ox coxae: A) Birth; B) 45 days; C) 105 days; D) 270 days.

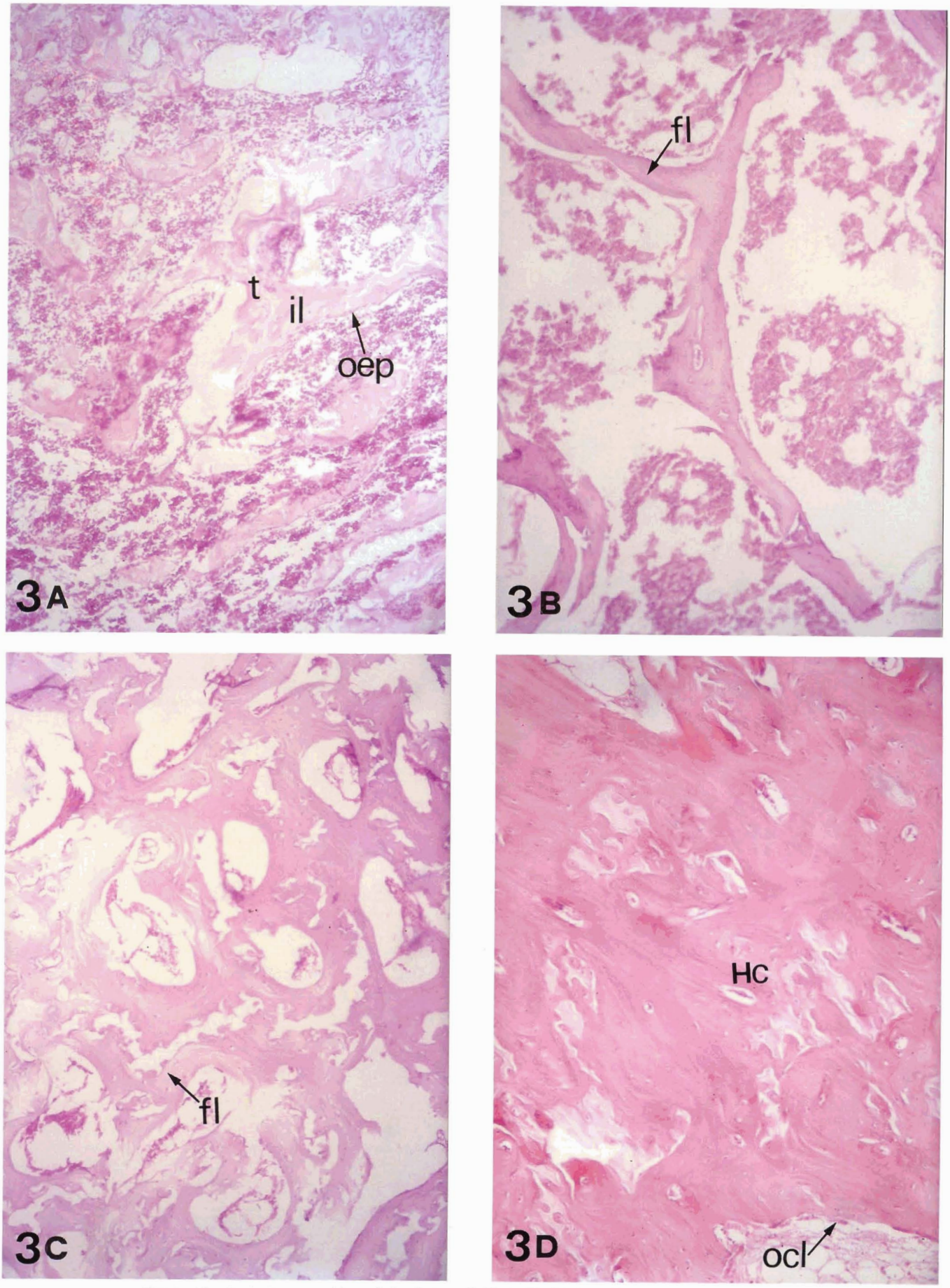

Fig. 3. Histological composition of the femur: A) Birth; B) 45 days; C) 105 days; D) 270 days.

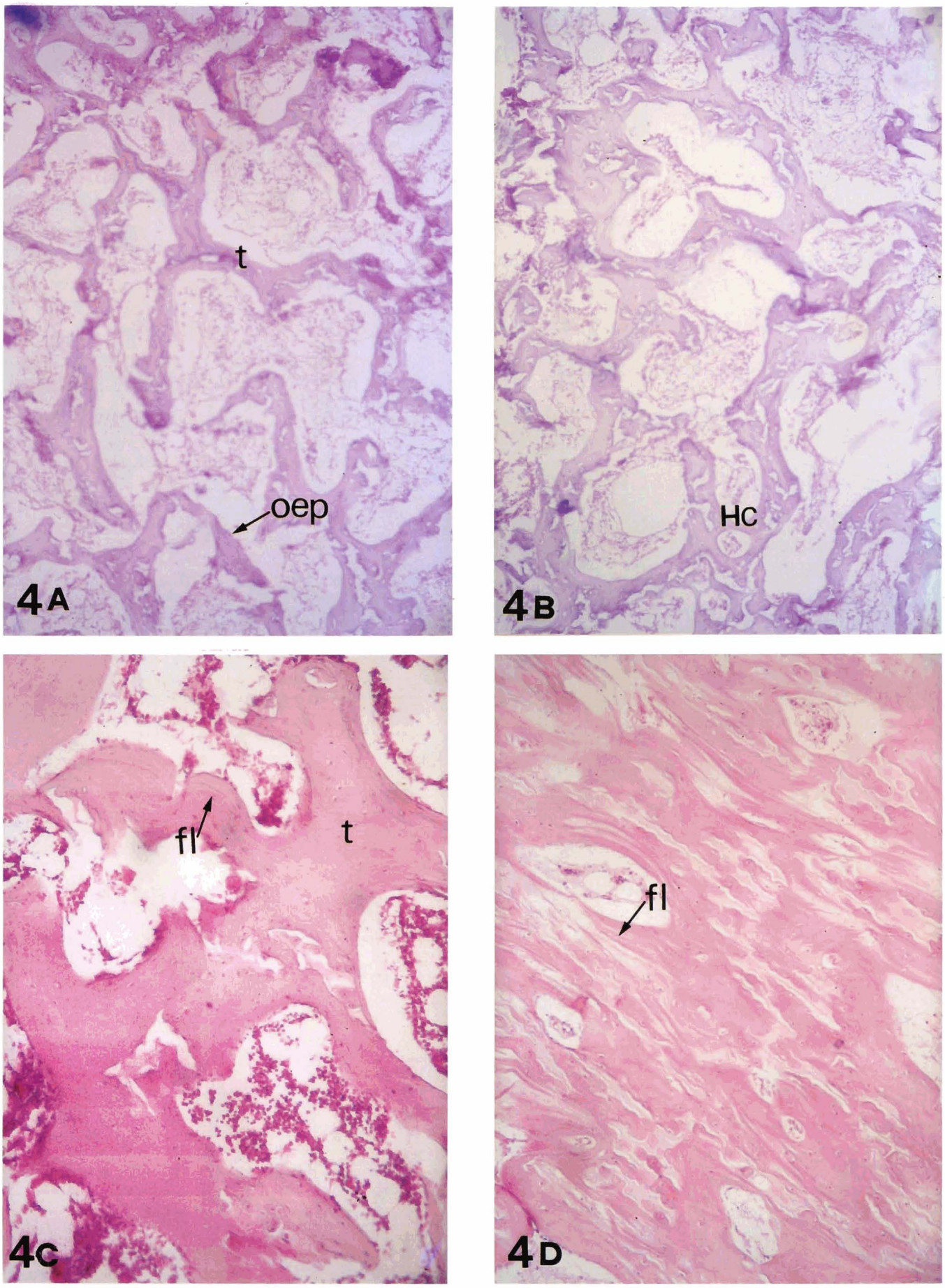

Fig. 4. Histological composition of the tibia: A) Birth; B) 45 days; C) 105 days; D) 270 days.

270 days (Fig. 3D)

Few differences were observed in this age-group with respect to the preceding group, although the former was slightly more developed. The remodelling process was evident, and osteoclasts (ocl) were visible. Clearly-defined Haversian canals (Hc) were observed, and the trabecular matrix was fully calcified.

Tibia (Fig. 4)

Birth (Fig. 4A)

The histological arrangement of trabeculae (t) in this age-group was characterized by thin layers of irregularly-calcified bony tissue surrounded by an osteoblastic epithelium (oep). These lamellae contained sinuous caverns which did not stain, and gave the bone an areolar appearance.

45 days (Fig. 4B)

Trabecular structure was generally similar to that observed in the preceding age-group, although some areas of the trabeculae contained more or less concentratic lamellae, giving the appearance of Haversian canals (Hc).

105 days (Fig. 4C)

Trabeculae (t) showed a greater degree of calcification, and in some cases contained force lines (fl). Haversian canals were also observed.

270 days (Fig. 4D)

Trabeculae were fully calcified, with force lines (fl) arranged concentrically, in some cases, around Haversian canals. These trabeculae can be said no have undergone definitive remodelling.

Discussion

All the data obtained in this study is, as we have pointed out, interrelated and complementary. A characteristic feature of young bone is the presence of an area of non-mineralised matrix termed osteoid tissue; at birth this takes the form of several bone fragments analysed here and previously reported by Pommer in 1885. In growing or completely formed bones (45, 105 and 270 days old), however, osteocytes were found, as reported by Bloom-Fawcett, 1987.

All the bones analysed grow in the form of prolongations or spicules called trabeculae (as also reported by Weiss, 1982), forming force lines.

In the vicinity of the trabeculae, vascular bundles similar in structure to Haversian canals were visible, their function being to provide nutrients to more deeply-situated cells. This agrees in part with the findings reported by Harris and Ham (1956) in bone tissue transplants.

In the more mature age-groups studied (105 and 270 days), once the bone was formed osteoclasts became visible; osteoclasts are responsible for the remodelling of the bone, or for its destruction and non-formation (Kolliker, 1873).

The chronological sequence in which the consolidation of various bone elements (diaphysis, epiphysis and apophysis) takes place, together with the timing of ossification for a given species, provides an acceptable method for age- determination, and also complements other techniques, as reported by García González (1981).

Finally, histological analysis showed that the components of bone structures follow a similar maturation pattern, in decreasing order of significance: os coxae, femur, tibia, humerus.

However, this pattern is not identical for every bone at a given age. Of all the bones analysed, os coxae provides the best histological indicator for age-differentiation in sheep of 0, 45, 105 and 270 days old.

References

Amprino R. (1965). Bone structures and functions. 2nd ed. W. Bargmann (eds). Werkstatt der Anatomen, Georg Thieme Verlag. Stuttgart.

Bain S.D., Newbrey J.M. and Watkins B.A. (1989). Alterations in chick bone growth and bone tissue eicosaonic fatty acids relationship to biotin status pair-feeding and treadmil exercise. Nutr. Res. 9, 1229-1236.

Bloom-Fawcett H. (1987). Tratado de Histología. 11 ed. Ed. Interamericana. Academic Press. London.

Bubenik G.A. and Bubenik A.B. (1978). The role of the sex hormones in the growth of antler bone tissue influence of an antiestrogen therapy. Saeugetierkd mitt. 26, 284-291.

Bubenik G.A., Bubenik A.B., Stevens E.D. and Binnington A.G. (1982). The role of the sex hormones in the growth of entler bone tissue. I. Endocrine and metabolic effects of antiandrogen therapy. J. Exp. Zool. 194, 349-358.

Currey J.D. (1968). Biology of hard tissue. National Aeronantics and Space Administration. SP-161.

García González R. (1980). Crecimiento del esqueleto en corderos de raza Rasa Aragonesa ecotipo ansotano. I. craneo; II. esqueleto apendicular y III. esqueleto axial: crecimiento alométrico, variación y anomalías vertebrales. P. Cent. Pir. Biol. Exp. 12, 101-123.

García González R. (1981). Estudio de la osificación postnatal en ovinos de raza Rasa Aragonesa. Sociedad de Ciencias Aranzadi. San Sebastian. Año 33. No 3-4.

Harris W.R. and Ham A.W. (1956). The mechanism of nutrition in bone and how it affects its structure repair and fate on transplantation in G.E.W. Wolstenholme and C.M.O'Connor (eds). Bone structure and metabolism. J&A. Curchill Ltd., London.

Horn V., Sauman Z., Messner P. and Dufek P. (1975). The observed growth of bone tissue in people of different age groups. Acta Fac. Med. Univ. Brun. 52, 47-51.

Kölliker A. (1873). Die normale resorption des Knochengewebes und Ihre bedetung fur die entsechung der typischen knochenformen. Vogel, Leipizig.

Lozupone E. and Favia A. (1981). Bone tissues linear appositional growth rate in long bones submitted to mechanical overload. Boll. Soc. Ital. Biol. Sper. 57, 1383-1388.

McClean F.C. and Bloom W. (1940). Calcification and ossification: calcification in normal growing bone. Anat. Rec., 78, 333.

McClean F.C. and Urist M.R. (1968). Bone. Fundamentals of the physiology of skeletal tissue. 3d ed. The University of Chicago Press, Chicago.

Neumann J. (1986). Changes in bone tissue of broilers treated with growth promoters. Acta vet. Brno. 55, 285-292.

Owen M. (1963). Cell population kinetics of an osteogenic tissue. J. Cell Biol. 19, 19.

Pommer G. (1885). Untersucchunger über Osteomalacie und Rachitis, nebst Apposition in Verschiedenen Altersperioden und der durchbohrenden gefässe. Vogel. Leizpig.

Smoliar V.I. (1983). Effect of iodine deficiency on the growth and formation of the bone tissue. Vopr. Pitan. 2, 38-42.

Smoliar V.I. (1985). Effect of amino acid. Deficiency on the growth and formation of the bone tissue. Vopr. Pitan, 3, 38-42.

Smoliar V.I., Biniashevskii E.V. (1988). The effect of saccharose of growth and bone tissue formation. Vopr. Pitan. 1, 52-56.

Smoliar V.I. and Biniashevskii E.V. (1989). Effect of copper deficiency on growth and bone tissue formation. Vopr. Pitan, 6, 28-32.

Theriez M., Villete Y. and Castrillo C. (1982). Influence of metabolized energy content of the diet and of feeding level on lamb performances. I Growthg and body composition. Livest. Prod. Sci., 9, 471-485.

Tonna E.A. and Cronkite E.P. (1961). Autoradiographic studies of cell proliferation in the periosteum of intact and fracture femora of mice utilizing DNA labeling with H3-thymidine. Proc. Soc. Exp. Biol. Med., 107, 719.

Tonna E.A. and Cronkite E.P. (1962). An autoradiographic study of periosteal cell proliferation with tritiated thymidine. Lab. Invest. 11, 455.

Trueta J. (1968). Studies of the development and decay of human frame. Philadelphia. W.B. Saunders Company.

Van Kempen G.J.M., Van de Kerk P. and Grimbergen A.H.M. (1976). Influence of phosphorus and calcium content of feeds on growth feed conversion and slaughter quality and on the chemical mechanical and histological parameters of the bone tissue of pigs. Neth. J. Agric. Sci. 24, 120-139.

Weis H. (1982). Histología. 4ed. Ateneo S.A. (ed).

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