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The fine structure of the proximal growth plate of the avian tibia

C. R. HOWLETT

School of Pathology, University of N.S.W., Kensington 2033, *N.S.W.*

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INTRODUCTION

Endochondral osteogenesis in avian long bones has been well described at the light microscopic level by Fell (1925) and further amplified by Wolbach & Hegsted (1952) and Wise & Jennings (1973). On the other hand, ultrastructural studies on the fine detail of the metaphyseal growth plate of other than embryonic avian long bones has received little attention apart from studies of the differentiation of the various types of chondrocytes (Lutfi, 1974) and the matrical components (Martin, 1954). This paper describes an ultrastructural study of the upper growth plate of the avian tibia in order to establish a basis for an understanding of the pathogenesis of the condition of tibial dyschondroplasia, a condition which produces significant deformity in broiler chickens at marketable age (6–9 weeks).

MATERIAL AND METHODS

Tissues were collected from 7 weeks old chickens (10 normal white leghorns and 10 normal commercial broilers).

The birds were given a pre-anaesthetic dose of 2000 i.u. of heparin intravenously and some 15 minutes later anaesthetized with intravenous sodium pentobarbitone. The femoral artery was then cannulated from the medial aspect and perfused with Tyrode's solution to which had been added sucrose and lignocaine to make a final concentration of 5 % (w/v) and 0·05 % (v/v) respectively. Perfusion was performed at approximately 180 mm Hg employing 25–50 ml until the fluid returning in the femoral vein was clear, at which time the perfusate was immediately changed to either 3 % glutaraldehyde buffered in 0·1 M-sodium cacodylate (pH 7·2) or Karnovsky's (1965) paraformaldehyde/glutaraldehyde (pH 7·2) solution (both solutions with lignocaine added), and perfusion maintained for 10 minutes.

Thin vertical sections were then sliced from the medial aspect of the upper portion of the tibia (while still perfusing) and final trimming of tissue slices was carried out under fixative.

Although most tissue was fixed by perfusion as described above, on occasions fixation by immersion was used, the bird being anaesthetized as previously described and the tibia rapidly exposed and sectioned while the fixative was flooded on to the proximal end of the tibia. For this latter method 3 % glutaraldehyde buffered in 0·03 M PIPES (Piperazine 0 N,N-bis (2-ethane sulphonic acid) sodium salt monohydrate) at pH 7·2 was used (Salema & Brandão, 1973).

Following initial fixation the trimmed tissues were immersed for a further 8–12 hours in fresh fixative, rinsed with buffer several times, and subsequently washed for at least 2 hours in 0·1 M-sodium cacodylate buffer containing 0·04 % ruthenium red.

Tissues were post-fixed with 2 % osmium tetroxide for 4 hours at 4 °C and thence with 4 % aqueous uranyl acetate for 2 hours. Dehydration of the tissue was carried out in ascending concentrations of ethanol and finally in 100 % dry acetone for half an hour. Tissues were then placed into 1 : 1 acetone: Spurr's low viscosity embedding medium for 48 hours and then into low viscosity resin (Spurr, 1969) for 48 hours under a reduced pressure of 200 mm of mercury. Polymerization was achieved by maintaining the embedded blocks at 70 °C for 8 hours. Ultrathin sections showing gold to silver interference (90 nm) were cut on a Huxley microtome with a diamond knife and floated on filtered distilled water saturated with calcium phosphate (Anderson & Parker, 1968). They were settled as quickly as possible on carbon coated Formvar films.

Sections were stained with either 2 % aqueous uranyl acetate for 5 minutes followed by lead citrate (Reynolds, 1963) for 2 minutes, or with 2 % uranyl acetate in 50 % ethanol for 20 minutes at 45 °C and lead citrate (Reynolds, 1963) for 2 minutes.

Some artefactual leaching of the mineralized cartilage took place with the latter staining technique. When tissues were required for studying proteoglycans in the growth plate, washing was carried out for 12 hours in cacodylate buffer with a ruthenium red concentration of 1·5 % (Thyberg, Lohmander & Friberg, 1973).

In the present work, ruthenium red, a compound thoroughly investigated by Luft (1971 *a*, *b*), was only added at the post-fixation wash stage because it was found that ruthenium red added to osmium tetroxide, as recommended by Luft, occasionally caused rapid deterioration of the osmium tetroxide as well as artefactual precipitation. Satisfactory staining of intracellular material with ruthenium red occurred only in extremely thin blocks, which is in keeping with the poor penetrative properties of this chemical (Thyberg *et al.* 1973).

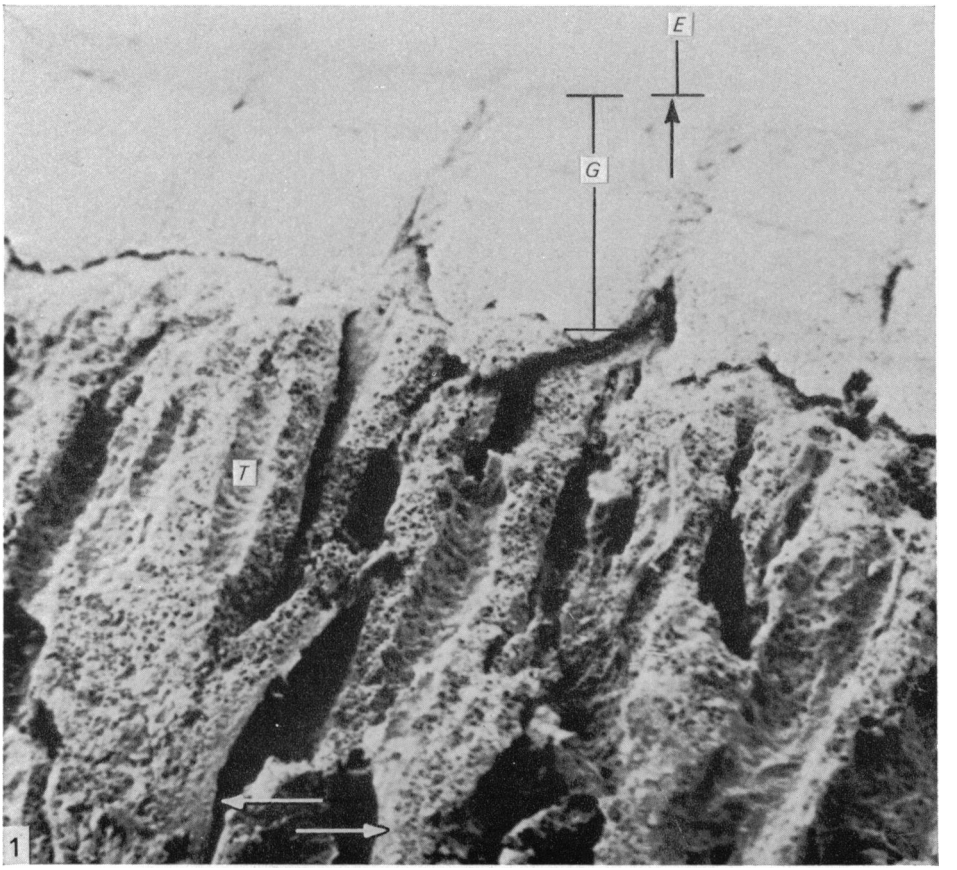
OBSERVATIONS

At the proximal end of the tibia three cartilaginous zones are distinguishable, viz., articular, epiphyseal (or hyaline) and growth plate. The present study is limited to the structure and initial calcification of the growth plate. Macroscopically the growth plate is a translucent grey gelatinous layer immediately proximal to the metaphysis and distal to the white opaque epiphyseal cartilage.

The pattern of vascular tunnelling into metaphyseal portions of growth plates is fairly uniform and is illustrated in Figure 1. The walls of these tunnels are mostly composed of calcified cartilage, covered with bone distally (Fig. 1), but their extreme proximal ends are of uncalcified cartilage (Fig. 2*a*).

The growth plate may be divided into five zones or regions. The division is determined predominantly by the characteristics of the chondrocytes, although distinguishing features of the matrix are also used. The zones are (1) germinal or resting, (2) proliferating, (3) pre-hypertrophic, (4) hypertrophic and finally, (5) degenerating hypertrophic or calcifying (Fig. 2*a*). The five zones (Fig. 2*b*) illustrate an orderly progression of cells through a proliferating stage with a high mitotic rate and high cytoplasmic activity, to pre-hypertrophic and hypertrophic stages with active secretion of matrix components, to a stage of degeneration, death and removal.

Prior to initial metaphyseal vascularization of the growth plate some chondrocytes undergo less extreme degrees of maturation and degeneration, and consequently calcified tongues of cartilage extend deep into the metaphysis, acting as a scaffolding



N.B. All sections shown were stained with uranyl acetate and lead citrate except where specifically mentioned otherwise.

Fig. 1. A stereoscan of a critical point-dried section from the proximal end of the tibia revealing tunnels of calcified cartilage (*T*) which *in vivo* house plexuses of thin walled blood vessels of wide diameter. Distally the calcified cartilage has become covered by plates of bone (arrow). Epiphyseal cartilage, *E*; proximal portion of the growth plate, *G*. Critical point-dried and shadowed with gold palladium. × 60.

for initial bone formation. Osteoblasts secreting osteoid are aligned along tunnels and plates of calcified cartilage as well as along the walls of chondrocytic lacunae.

(1) *Chondrocytes of the germinal or resting zone*

Cells from the interstitial or germinal zone are fusiform (Fig. 2*b*). The nucleus is centrally placed and appears to occupy about half the volume of the cell. Chromatin is peripherally arranged about the nuclear membrane, with occasional clumps scattered haphazardly throughout the rest of the nucleus. The cytoplasm, with relatively few mitochondria, appears dense because of a plentiful amount of rough endoplasmic reticulum (*RER*). A small Golgi zone exists with a few prominent single membrane vesicles, the contents of which are centrally positioned and appear as a light floccular material from which radiate fine strands to the vesicular membrane. Extending from the plasmalemma to the surrounding matrix are many short blunt filopodia, giving the cellular outline a scalloped appearance. The intercellular matrix

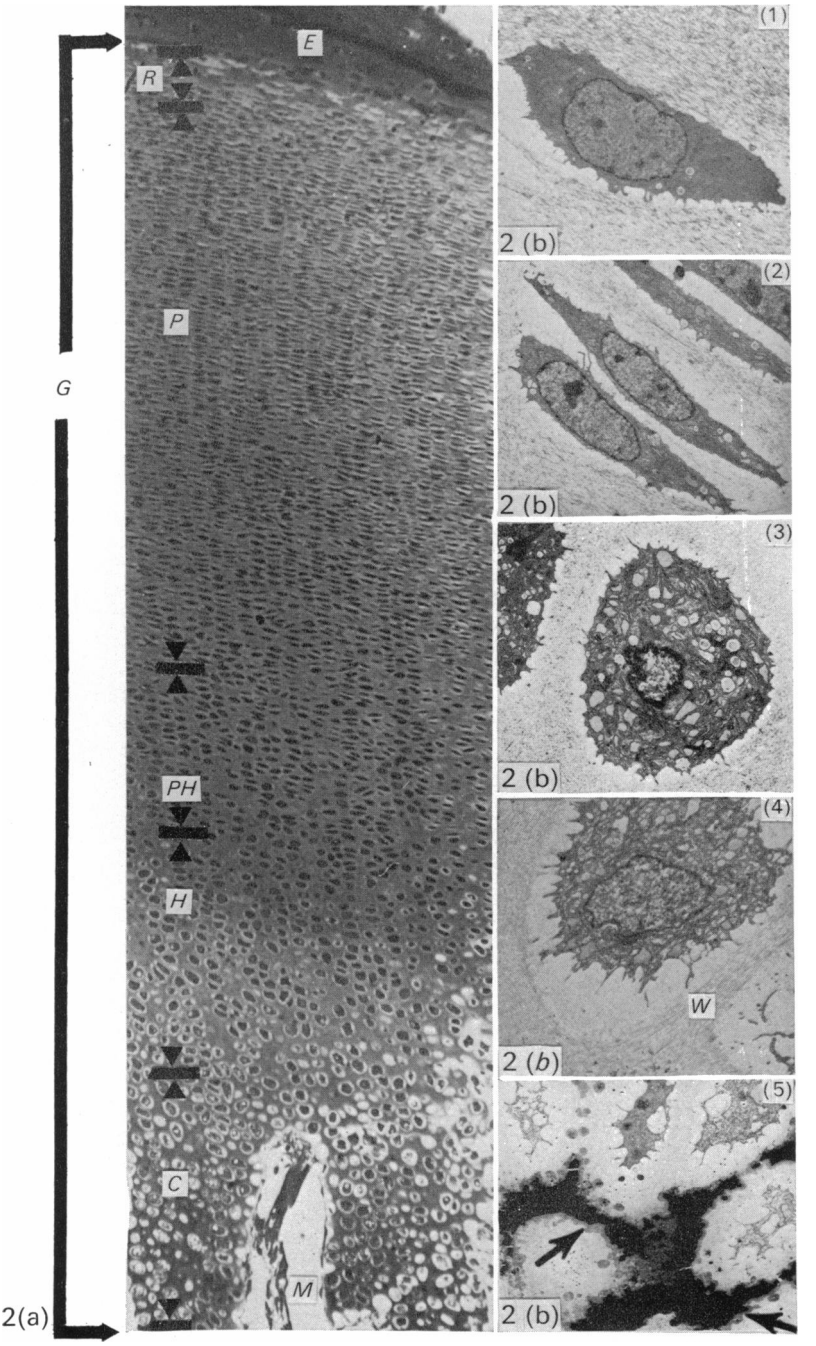


Fig. 2. For legend see opposite.

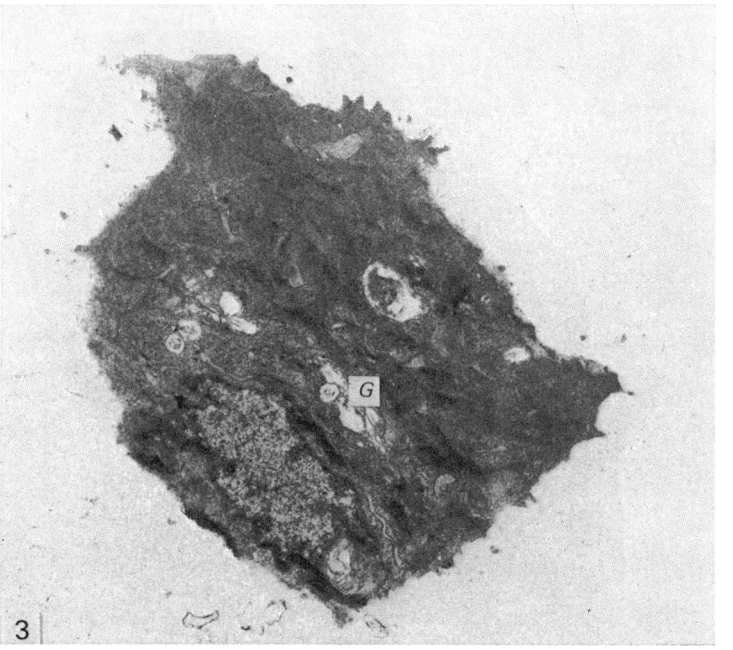


Fig. 3. A transverse section of a proliferating chondrocyte emphasizing its discoid shape and electron-dense cytoplasm. Golgi zone (G). × 10000.

is composed of finely beaded filaments arranged in a tangled criss-cross manner which is packed relatively lightly and in many cases seems to swirl around the cells.

(2) *Chondrocytes of the proliferating zone*

The proliferating chondrocytic layer is approximately four times the thickness of the succeeding (pre-hypertrophic) zone. Proliferating chondrocytes are orientated into columns in proximal portions of this zone, but this relatively orderly arrangement becomes disarranged as the cells enlarge and become separated by larger amounts of matrix.

In longitudinal sections the closely packed proliferating chondrocytes appear crescentic and spindle-shaped (Fig. 2*a*). However, when viewed in transverse

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Fig. 2. (a) The proximal growth plate (*G*) of the avian tibia can be divided into five layers. The division is determined predominantly by the type of chondrocyte, although matrix features are also used. Epiphyseal cartilage, *E*; resting or germinal layer, *R*; proliferating layer, *P*; pre-hypertrophic layer, *PH*; hypertrophic layer, *H*; degenerating hypertrophic or calcifying zone, *C*; metaphyseal blood vessel plexus, *M*. Methylene blue. × 50. (b) Supplements Figure 2*a*, detailing each type of chondrocyte and any distinguishing features of the matrix. (1) Resting or germinal chondrocytes. The collagenous protein of the matrix is tangled, relatively tightly packed, and seems to whorl around the cells. × 3000. (2) Longitudinal presentation of the chondrocytes from the proliferating zone displays them as spindle-shaped cells packed with rough endoplasmic reticulum. Thin collagen fibrils with no apparent orientation are loosely scattered throughout the matrix. × 2000. (3) Pre-hypertrophic zone is characterized by chondrocytes becoming plumper, with their cytoplasm stacked with rough endoplasmic reticulum, enlarged Golgi apparatus and increased vesicular transport system. × 6500. (4) Hypertrophic chondrocytes occupy prominent lacunae, their matrical septal walls (*W*) being quite distinct. × 6000.(5) Chondrocytes of the degenerate hypertrophic or calcifying zone lose their distinctive cytoplasmic features. The matrix is heavily calcified (arrow). × 5000.

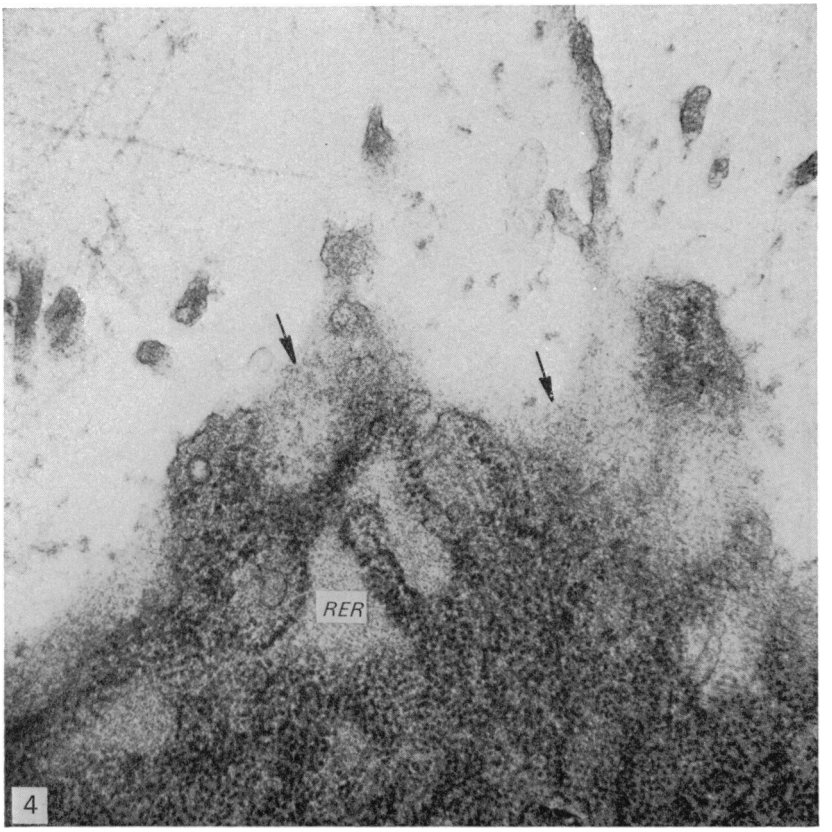

Fig. 4. The plasmalemma of a proliferating chondrocyte is coated by a finely granular material (arrow) similar to that contained within the dilated rough endoplasmic reticulum (*RER*) nearby. Many ribosomes are present in the peripheral cytoplasm. × 40000.

sections, these chondrocytes have a discoid shape (Fig. 3). The cell border is irregular and the plasmalemma is coated by a very finely granular material which seems to be a product of the cell (Fig. 4). Centrally the cytoplasm of this active cell is packed with RER, the cisternae of which are frequently swollen and filled with moderately electron-dense material (Fig. 2*b*), whereas the peripheral cytoplasm exhibits a predominance of densely packed ribosomes (Fig. 4). Fine cellular processes protrude from the cellular membrane and extend into the fibrillary matrix which surrounds each cell.

Stacks of smooth endoplasmic reticulum form a small Golgi apparatus, which is best identified in a transverse section. The Golgi apparatus buds off dominantly smooth-coated vesicles (diameter 30 to 50 nm) which contain granular material. In addition, rough-coated single membrane vesicles are occasionally observed (Fig. 5). Other Golgi-associated vesicles are of much larger calibre. These latter vesicles (diameter 250–450 nm) often have a single scalloped smooth membrane and contain a light flocular material which is mainly concentrated at the central zone, usually comprising a thin rim coating the binding membrane (Fig. 5) and separated from the central core by a lucent zone. Vesicles of similar nature are found at the periphery of the cell, some of which appear to be discharging extracellularly their finely

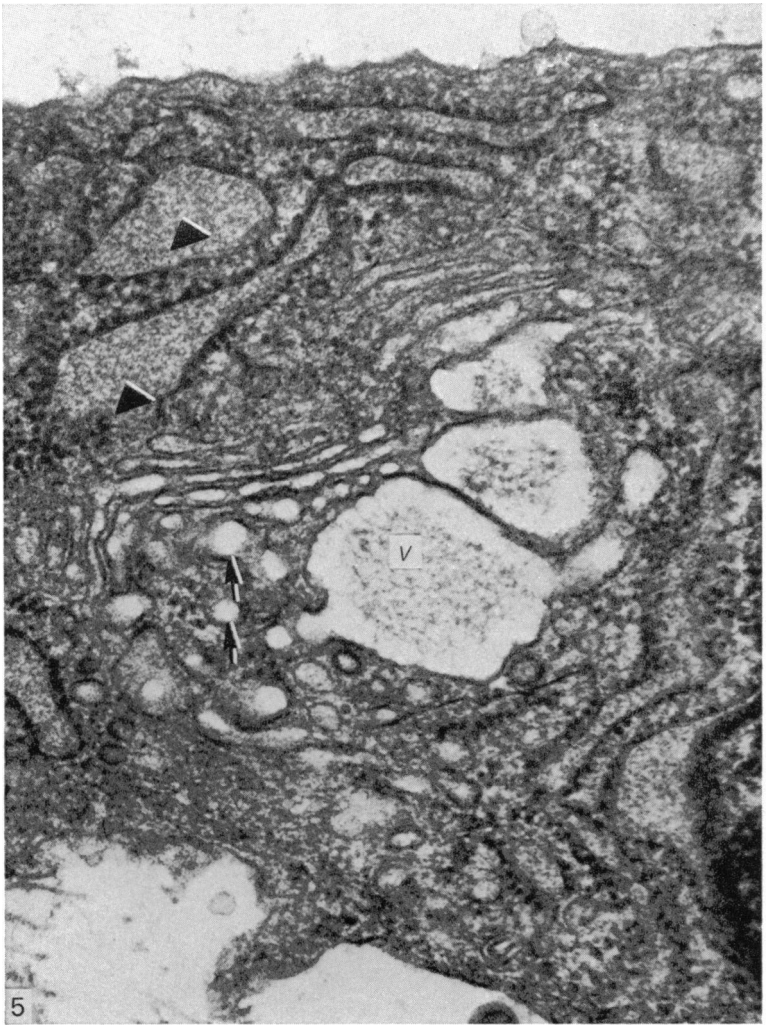

Fig. 5. Associated with the Golgi zone of proliferating chondrocytes are large scalloped-edged vesicles (*V*) and many small smooth-coated vesicles (arrows). Moderately electron-dense material fills the swollen rough endoplasmic reticulum, of which certain portions are quite dilated (arrowheads). × 60000.

granular and partly fibrillar contents (Fig. 6). When the tissue was stained *en bloc* with ruthenium red, a number of vacuoles associated with the Golgi zone contained rounded or polygonal electron-dense granules, which although smaller, are otherwise similar in appearance to those in the pericellular matrix (Fig. 7). Occasional laminated bodies are encountered both within and on, or adjacent to, the plasmalemma of a small percentage of the proliferating chondrocytes. The mitochondria are usually small and elongated and some contain dense particles.

The smooth and oval nucleus occurs in the thickest part of the cellular discs, where frequently it is barely covered by cytoplasm. Small clumps of chromatin are scattered throughout the nucleus and along the nuclear membrane, on which minimal chromatin margination has occurred.

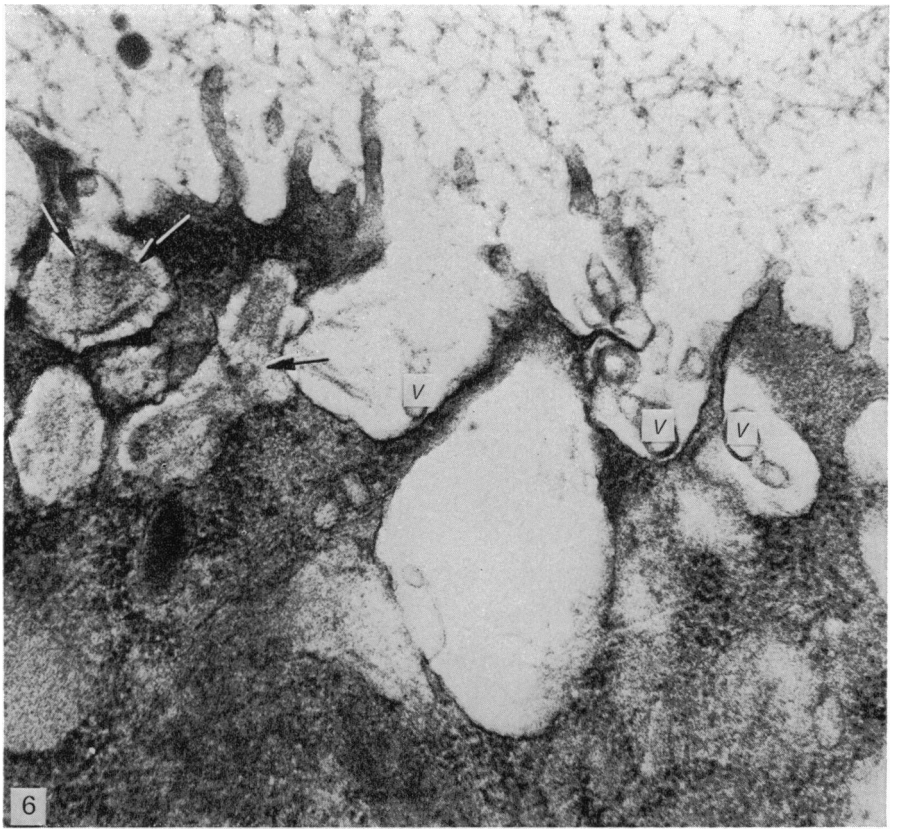

Fig. 6. The fine cytoplasmic processes extending from the plasmalemma of proliferating chondrocytes cause the membrane to be quite irregular. Compounding this irregularity are large vesicles (arrows) which appear to be discharging their contents extracellularly. In addition, small vesicles (*V*) occur both intra- and extracellularly adjacent to the cytoplasmic membrane. × 49 500.

(3) *Chondrocytes of the pre-hypertrophic zone*

The change from 'proliferative' to 'pre-hypertrophic' features in chondrocytes is sometimes a gradual process, but frequently it appears abruptly, so that in a pair of adjacent cells, one exhibits the features of the proliferative zone, and the other the characteristics of the 'hypertrophic regions'.

The pre-hypertrophic zone is characterized by cells becoming more voluminous and approximately spherical. Prominent cytoplasmic processes, sometimes branching, extend further into the cartilaginous matrix than those of the proliferating cells. The cytoplasm is packed peripherally with swollen RER and centrally shows an extensive Golgi zone and associated vesicles which cause eccentric displacement of the nucleus.

Isolated segments of RER are swollen and filled with moderately electron-dense material (Fig. 8). Vesicles found in the Golgi region are similar in size and morphology to those described for proliferating chondrocytes, but are far more numerous and, in addition, small rough-coated vesicles (diameter 13 to 30 nm) are more frequently encountered (Fig. 8). Fusing of the small with the larger vesicles of this region (Fig. 8) may account for the irregular membrane of the latter structures. Occasional dense bodies with the morphological configuration of primary lysosomes

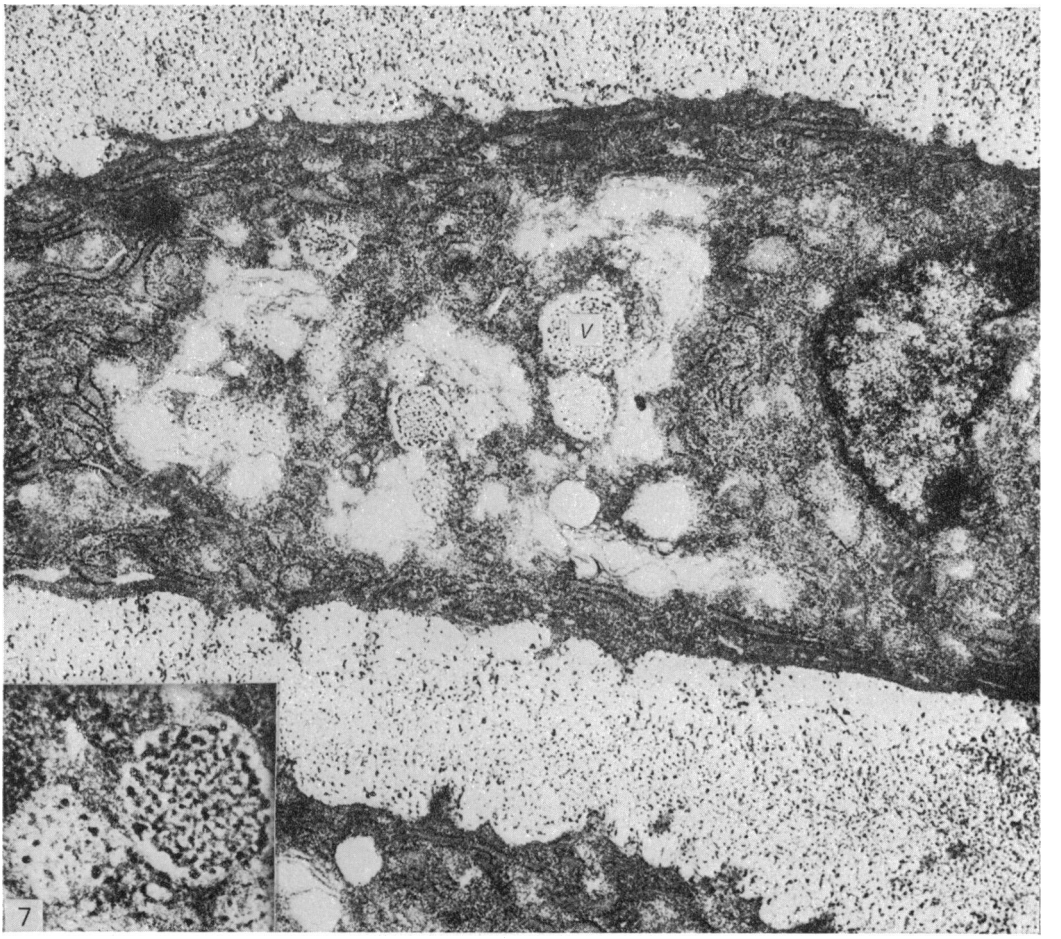

Fig. 7. Prolonged exposure to ruthenium red has stained the granular contents of some of the large Golgi vesicles (*V*). Note the morphological similarity between granules within intracellular vesicles and those of the matrix. ×15000. *Inset*: Golgi vesicles containing granules stained *en bloc* for a long time with 1·5% w/v ruthenium red. ×30000.

occur in most of the chondrocytes in this zone. Scattered oval mitochondrial profiles are observed in similar concentration and distribution to those in proliferating chondrocytes.

The nuclear outline is commonly irregular (Fig. 2*b*) having a margination of fine dense chromatin interspersed with clumps of varying size. Chromatin granules have a relatively even distribution throughout the rest of the nucleus.

Immediately surrounding each pre-hypertrophic chondrocyte is an afibrillar, lightly granular zone. Adjacent to this pericellular zone is the intercellular or interterritorial matrix composed of microfibrils and matrix granules. Often the fibrils and matrix granules are in such close apposition than an irregular beaded appearance is imparted to the indefinite periodicity of the microfibrils (Fig. 9). The interlacing microfibrils commonly have an overall pericellular swirling orientation.

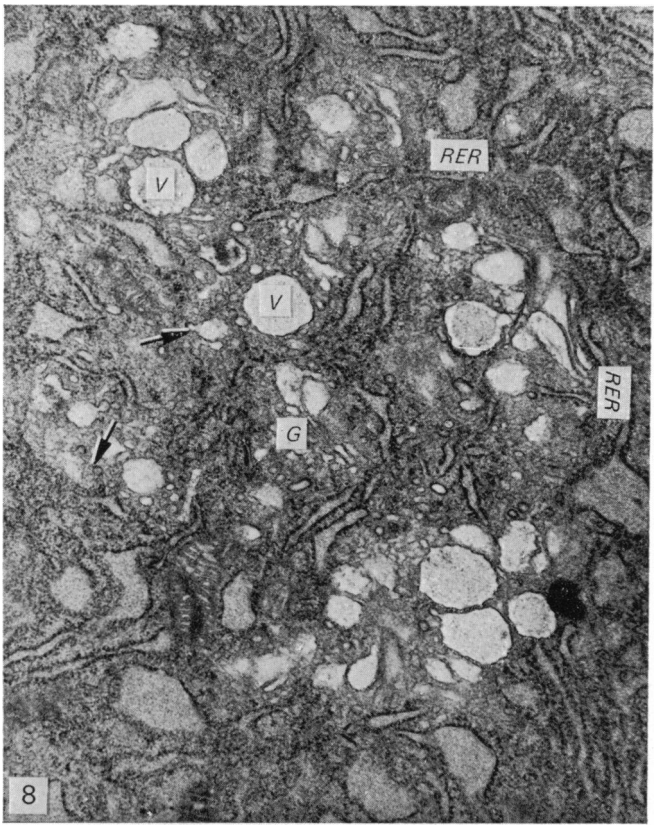

Fig. 8. An extensive Golgi apparatus (*G*) and various associated vesicles, marginated by rough endoplasmic reticulum (*RER*), of which isolated segments are distinctly swollen but filled with moderately electron-dense material, occur in the pre-hypertrophic chondrocytes. Small rough-coated vesicles (arrows) are encountered fairly frequently, as are the large single smooth-scalloped membraned vesicles (*V*). × 24000.

(4) *Chondrocytes of the hypertrophic zone*

Hypertrophic chondrocytes are large round cells up to 50 μm in diameter occupying prominent lacunae; their matrical septal walls are quite distinct (Fig. 2*b*). The plasmalemma has a scalloped appearance accentuated by fine cytoplasmic processes which extend to septal walls, seemingly tethering the cell to the matrix (Fig. 2*b*). Often the eccentrically situated nucleus (although more central than in the pre-hypertrophic zone) has marked indentations of its membrane. In general, the cell is far more electron-lucent than its predecessor. The voluminous cytoplasm contains all organelles recognizable in the pre-hypertrophic cell, but seemingly in reduced amounts. The cisterns of RER are irregularly formed, widely separated, and have isolated swollen segments which are filled with a moderately electron-dense granular material. There is a noticeable reduction in the size of the Golgi zone and in the number of transport vacuoles as compared with pre-hypertrophic cells. Mitochondria occur in about the same numbers as before.

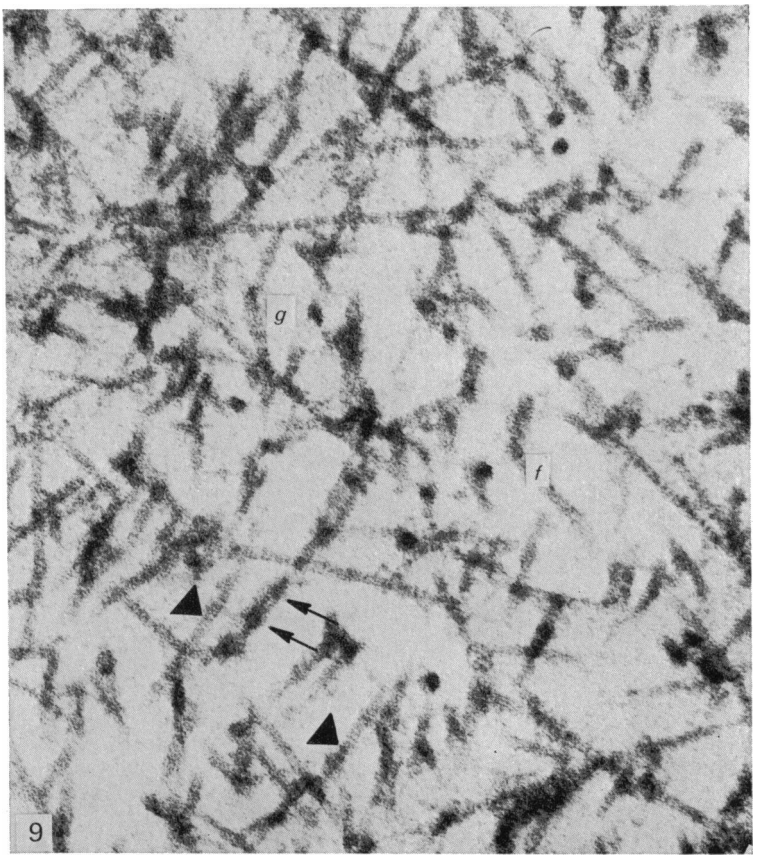

Fig. 9. The matrix of the pre-hypertrophic zones shows granules (*g*) and fibrils (*f*). Relatively frequently the granules are superimposed upon the fibrils, imparting an irregular beaded appearance to the microfibril (arrows). Some microfibrils (arrowheads) have indistinct periodicity. × 105000.

(5) *Chondrocytes of the degenerating hypertrophic zone*

These cells exhibit considerable pleomorphism at electron microscopic levels. Cells from this region lose many of their distinctive cytoplasmic features. Only densely staining mitochondria and isolated ribbons of RER are easily identifiable (Fig. 10). The plasmalemma is a thin tenuous scalloped membrane which is discontinuous in places (Fig. 10). Frequently both cytoplasm and nucleus appear lucent, and very occasionally the nucleus appears to undergo central dissolution. Usually the nuclear outline is very irregular, although the chromatin is uniformly dispersed throughout the nucleus apart from a thin peripheral condensation (Fig. 10).

During the latter stages of chondrocytic maturation, when matrical calcification is prominent, there is a loss of cytoplasmic density as well as fragmentation of RER. In addition, in the distal two zones, an extremely sparse and finely delicate network of microfibrils radiates across chondrocytic lacunae from cellular membrane to septal wall (Fig. 11). This so-called 'afibrillary moat' cannot be discerned when tissues are stained with ruthenium red because the fibrils of the interterritorial region are not apparent, and dense particles adjacent and in contact with cellular membranes as well as those within the walls are highlighted evenly. Fibrillary density is increased

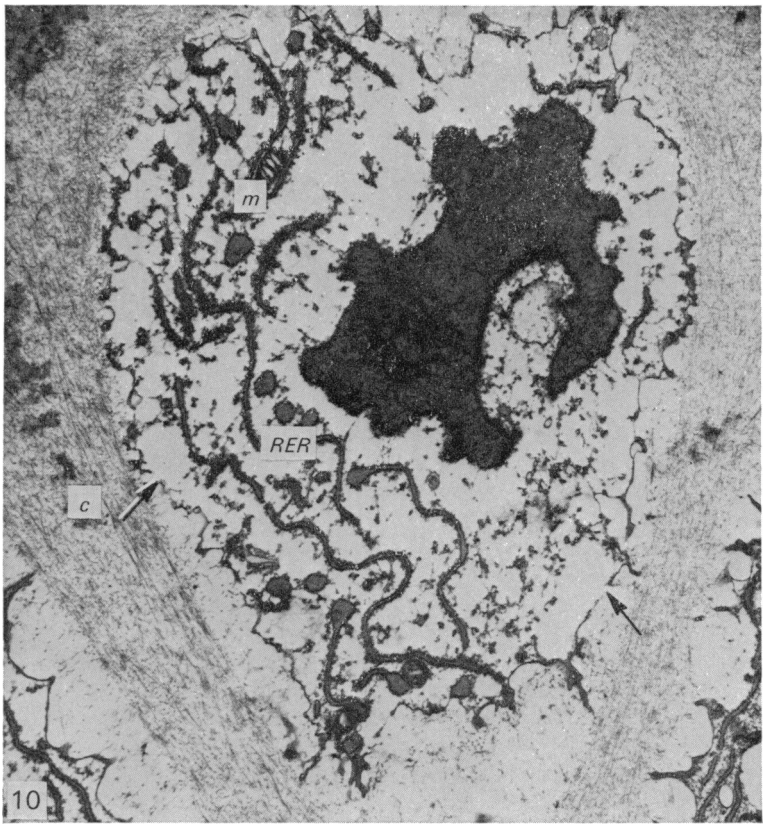

Fig. 10. Degenerating hypertrophic chondrocytes have an electron-lucent cytoplasm in which usually only ribbons of rough endoplasmic reticulum (*RER*) and mitochondria (*M*) are recognizable. The thin scalloped cytoplasmic membrane is discontinuous in places (arrows). Walls of the lacunae, which are composed of numerous microfibrils orientated roughly parallel to the cellular margin, have begun to calcify (*c*). Although the chromatin is evenly dispersed throughout the nucleus the nuclear outline is extremely irregular. × 8000.

in the walls of the lacunae, and the fibrils often have a recognizable periodicity (Fig. 12). Pleomorphic vesicles appear to bud from the plasma membrane (Fig. 13), and contain granular material which closely resembles that in the cytoplasm in nearby chondrocytic processes (Fig. 13). Another matrical structure of note is the 'dense body', which is round or oval and contains finely granular electron-dense material, usually highly osmiophilic. This material is often separated from its surrounding single membrane by an electron-lucent halo; the structure is very similar to a lysosome (Fig. 14). Dense bodies occur more frequently than matrix vesicles, and are present in increasing numbers through the proliferating and hypertrophic zones. Clusters of matrical dense bodies are encountered occasionally, and they are usually arranged with the smaller bodies peripheral and the larger ones central (Fig. 15). These dense bodies appear to be the nidus for development of single (Fig. 14) and small rosettes of mineral crystals (Fig. 16). Occasionally membrane-bound bodies, regular in outline and containing finely granular material, which is not osmiophilic, seem to initiate crystal formation (Fig. 17). In these cases it is difficult to determine whether such bodies are derived from matrical vesicles or

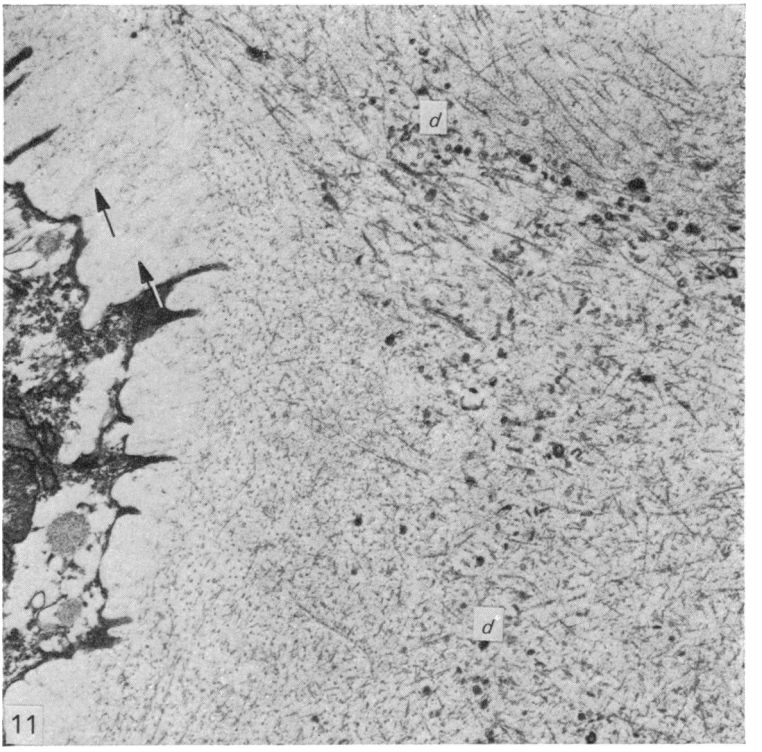

Fig. 11. An extremely sparse, delicate network of apparent microfibrils radiates across the chondrocytic lacuna (arrows). Many dense bodies (*d*) occur in the interterritorial matrix. ×19500.

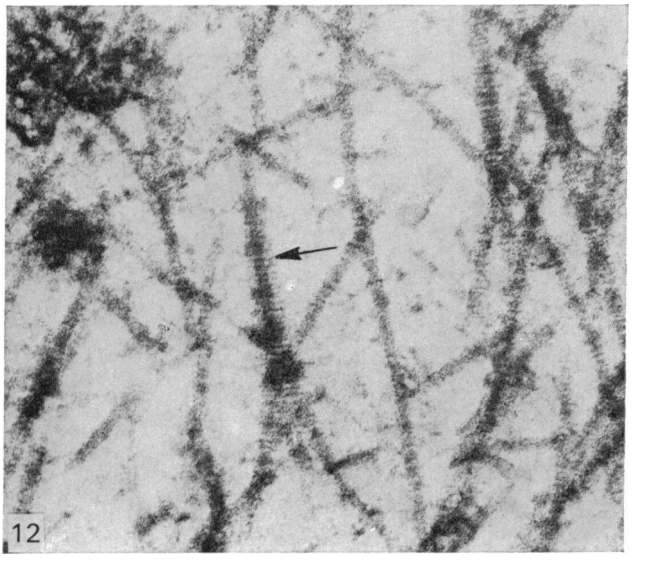

Fig. 12. Some microfibrils in the matrix of degenerating hypertrophic chondrocytic zone have a definite periodicity (arrow). ×97500.

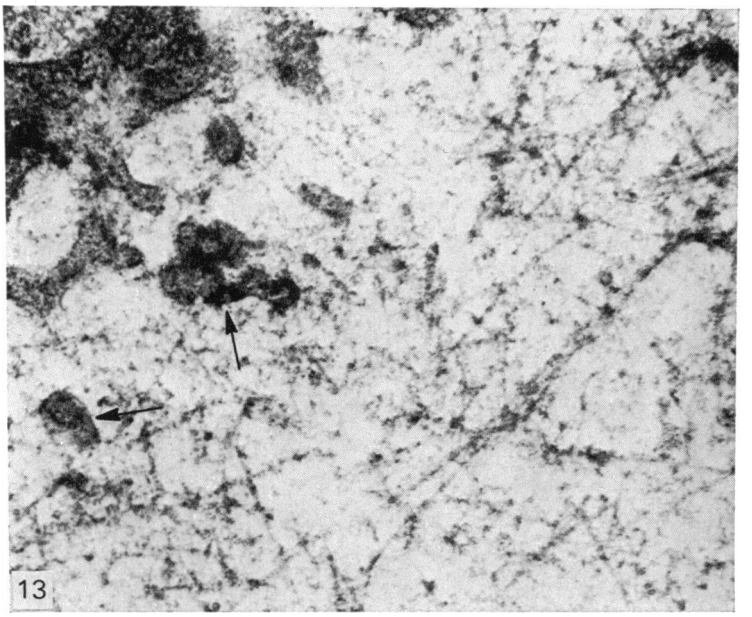

Fig. 13. Pleomorphic vesicles (arrows) seem to be budding from the cytoplasmic membrane of the adjacent chondrocyte. × 58 500.

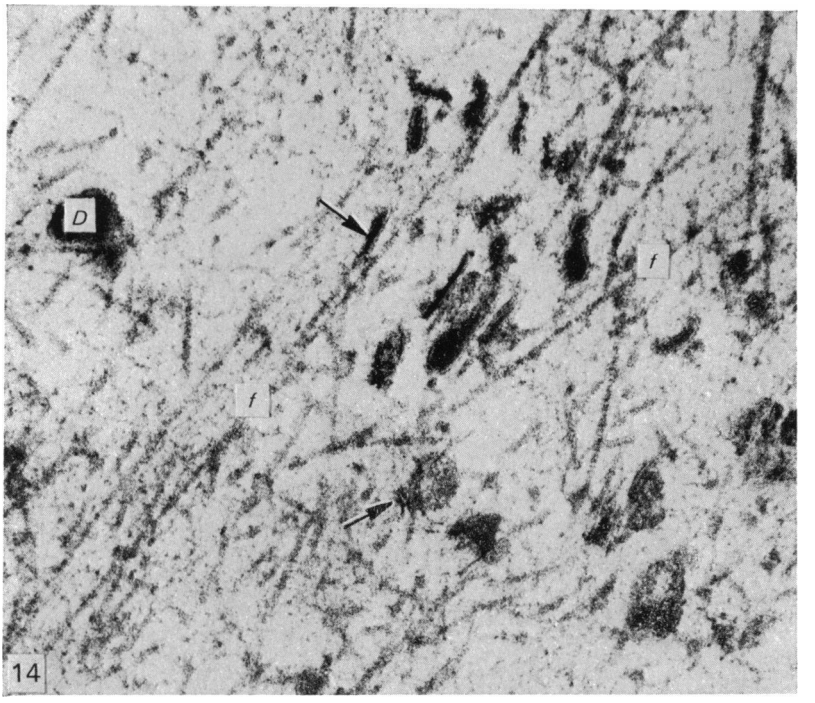

Fig. 14. The matrical dense body (*D*) contains a fine granular, highly osmiophilic material which is separated from the binding membrane by an electron-lucent halo. These dense bodies appear to be the nidus of single mineral crystal deposition (arrows). Microfibrils, *f*. × 82 500.

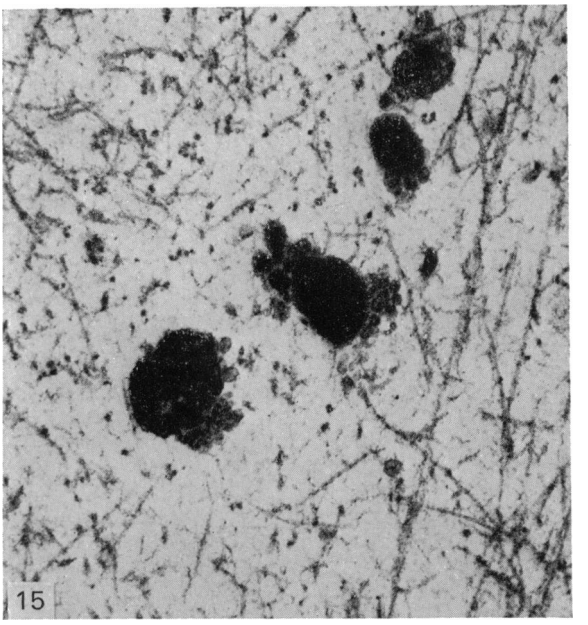

Fig. 15. Clusters of matrical dense bodies occasionally occur, and are generally arranged with the smaller bodies peripheral to the large. × 40000.

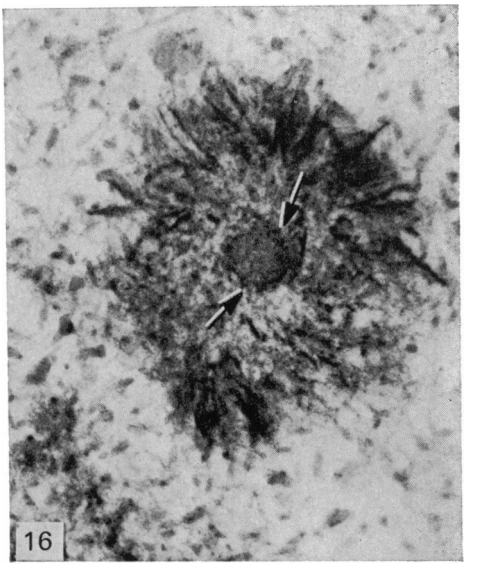

Fig. 16. A presumed dense body (arrows) is centrally positioned within a rosette of radiating mineral crystals. × 60000.

dense bodies. However, the regular shape of bodies and the fact that only a proportion of the contents of some dense bodies is osmiophilic (Fig. 14), suggest strongly that the bodies are derived from the matrical dense body. Deposition of presumed hydroxyapatite crystals initially occurs in these membranous structures of the prehypertrophic chondrocytic zone about 0·1 mm proximal to the tips of the most

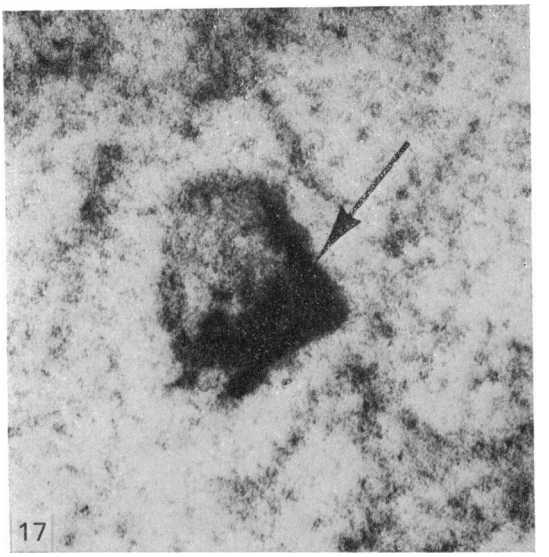

Fig. 17. Smooth membrane-bound bodies whose finely granular contents are not osmiophilic occasionally seem to initiate crystal formation (arrow). × 150000.

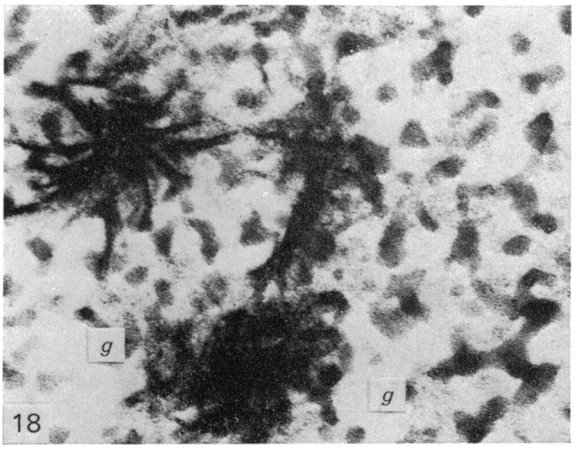

Fig. 18. Lateral to the proximal tips of the ingrowing metaphyseal vascular loops small clusters of crystals are frequently seen within the matrix. Matrical granules (*g*). × 120000.

advanced of the metaphyseal blood vessels (Fig. 14). Lateral to the tips of these blood vessels are small clusters of crystals (Fig. 18), while distally aggregates of crystals become more frequent. Finally, a further 0·1 mm below the most proximal point of the ingrowing vascular loop, many of these aggregates coalesce to calcify a significant proportion of many of the matrical walls of the lacunae (Figs. 19 and 2*b*). Therefore, the distance between the zone of *initial* deposition of hydroxyapatite crystals and the zone of *ready visualization* of matrical mineralization with the light microscope is about 0·2 mm.

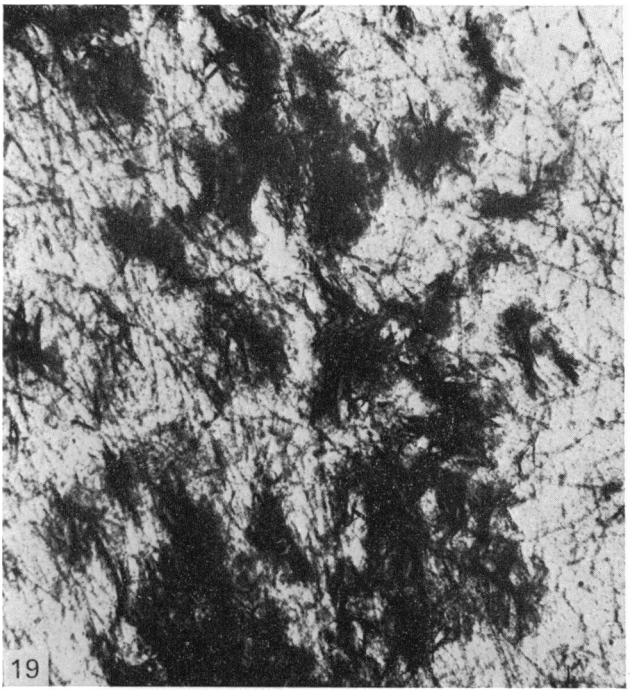

Fig. 19. One tenth of a mm distal to the ingrowing tips of the metaphyseal blood vessels a proportion of the cartilaginous matrix becomes more heavily calcified as the aggregates of crystals coalesce. ×44000.

The matrix

In the chick the growth plate matrix may be classified as 'small fibril cartilage' characterized by its small collagen fibrils with diameters ranging from 9·5 nm in the proximal zones (Fig. 9) to about 19·0 nm in the distal zones (Fig. 20). A major portion of the material in the interfibrillar space of the matrix consists of a syncytium of varying sized, approximately round particles up to 40 nm in diameter. Each particle has a dense staining core surrounded by moderately dense material which not only seems to form an integral portion of each particle but trails from one mucopolysaccharide aggregate to another, resulting in the varied shapes displayed in Figure 21. The overall impression is of a syncytium of mucopolysaccharide aggregates connected by broad to fine threads of moderately dense staining material (Fig. 21). Each lacuna may be divided into an inner afibrillary pericellular region, and an outer region in which a transition to the characteristics of the interterritorial matrix occurs (Fig. 20).

Blood vessels arising from vessels in the epiphysis extend into the growth plate as far as the junction of the proliferating and pre-hypertrophic chondrocytic zones but rarely, if ever, any further. Minor differences are detectable in the chondrocytes immediately adjacent to epiphyseal plexuses of blood vessels.

DISCUSSION

Because of the scarcity of published detail regarding the ultrastructural morphology of the postnatal avian growth plate, much of this discussion will compare

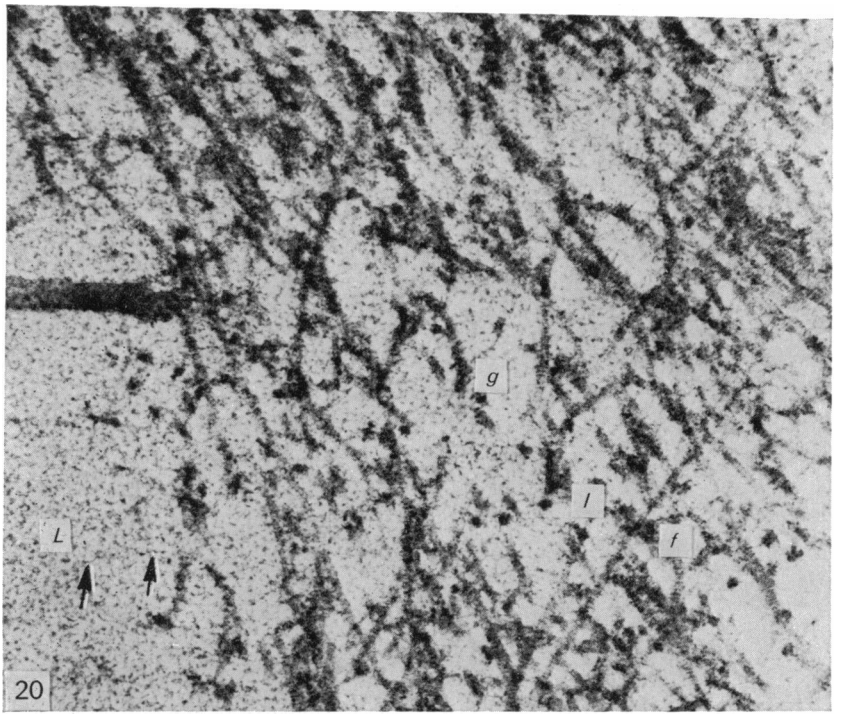

Fig. 20. The differences between an outer portion of a hypertrophic chondrocytic lacuna (*L*) and its adjacent interterritorial matrix (*I*) are displayed. Evenly dispersed throughout the lacuna is a very fine lightly stained granular material (arrows) which is also apparent as a background substance in the interterritorial matrix. Larger mucopolysaccharide granules (*g*) and fibrils (*f*) can only be visualized in the interterritorial matrix. × 60000.

the present observations from the chicken with those previously established for the mammal. In the light of the present reported findings, it can be said at once that avian and mammalian growth plates show many similarities. Certainly, when observed by light microscopy, the cytological sequence of cartilaginous cells from the resting, through proliferating, to maturation zones are similar to those of mammal (Wolbach & Hegsted, 1952; Wise & Jennings, 1973). However, metaphyseal vascular penetrations in the chicken allow a proportion of the chondrocytes to escape the degeneration exhibited eventually by all mammalian growth plate chondrocytes. This is reflected in structural differences between avian and mammalian growth plates which previous authors have recorded by light microscopy (Fell, 1925; Wolbach & Hegsted, 1952; Wise & Jennings, 1973).

The discoid cells of the proliferating region become more spherical towards the metaphysis and the cytoplasm fills with increasing amounts of RER, enlarged Golgi zones and accompanying vesicles, features which have been emphasized for guinea-pig (Thyberg & Friberg, 1971; Thyberg *et al.* 1973), rabbit (Serafini-Fracassini & Smith, 1974), and embryonic avian tibial growth plates (Matukas, Panner & Orbison, 1967).

The extensive Golgi organelle in the distal proliferating and hypertrophic chondrocytes reported here, although containing relatively few stacks of lamellae, shows many small vesicles and larger vacuoles. Lutfi (1974), however, reported that, for the proximal avian growth plate, there was neither an increase in size nor of prominence

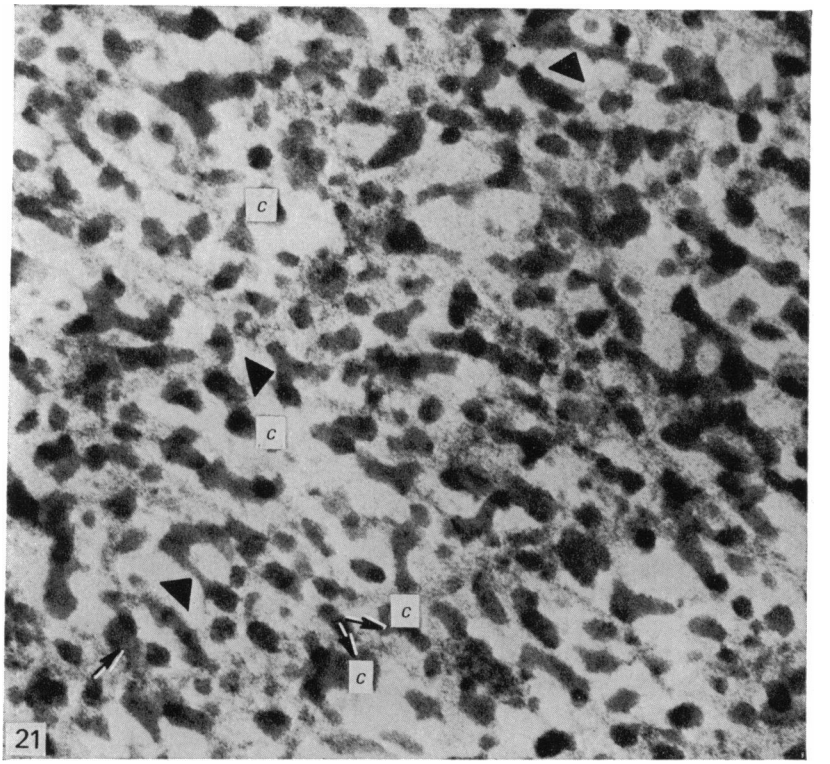

Fig. 21. A high magnification of the interterritorial matrix from the hypertrophic zone. A major portion of the interfibrillary space of this cartilaginous matrix is arranged as a syncytium of mucopolysaccharide aggregates. Each aggregate appears to consist of a dense central core (*c*) surrounded by less electron-dense material (arrows) which frequently trails from one particle to the next (arrowheads). Uranyl acetate and lead citrate plus prolonged *en bloc* staining with 1·5 % w/v ruthenium red. × 120000.

of the Golgi apparatus even though the intraplasmic RER and vesicles were increased, during the course of differentiation. Some of these vacuoles have relatively electron-lucent contents with fine fibrils, while others contain granules with an electron density greater than the fibrils: such granules stain intensely with ruthenium red. The nature of the material within the vacuoles which stains with ruthenium red is obscure. Thyberg and colleagues (1973) suggested that this material may represent proteoglylcans, a suggestion based upon the fact that the main tissue component staining with ruthenium red is a glycosaminoglycan (Luft, 1971*a*, *b*). This is supported by the fact that similarly structured matrical granules from embryological avian cartilage (Matukas *et al.* 1967) and the growth plate of guinea-pigs (Thyberg *et al.* 1973) contained proteoglycans. Moreover, Thyberg *et al.* (1973) and Matukas *et al.* (1967) established that a major portion of these extracellular granular proteoglycans is chondroitin sulphate, although this is probably not a true reflection of the native state but rather a dehydrated fixation precipitated form of the molecule (Anderson & Sajdera, 1971; Matukas *et al.* 1967; Serafini-Fracassini & Smith, 1974). In the present study, specific staining by ruthenium red highlighted extracellular matrical granules and their presumed precursors in some Golgi vesicles. Thyberg *et al.* (1973) reported a similar finding for the guinea-pig growth plate, and concluded that matrix granules contained proteoglycans and assumed, because of the

morphological similarity between intra- and extracellular granules, that the granules within vacuoles associated with the Golgi apparatus are at least in part intracellular proteoglycans. Less certainty surrounds the fibrillary component of intracellular vesicles, although Serafini-Fracassini & Smith (1974) concluded that available evidence pointed to it being a matt of monomeric collagen, as was first suggested in 1963 by Revel & Hay.

Cytoplasmic dense bodies were observed in all zones of the avian growth plate, more particularly in the latter portion of the proliferating and the pre-hypertrophic chondrocytic zone, a similar pattern to that described for mammalian growth plates (Bonucci, 1970; Thyberg & Friberg, 1970, 1971; Serafini-Fracassini & Smith, 1974). Indeed, these cellular organelles are considered to be the probable source of mammalian matrical vesicles 1 (Thyberg & Friberg, 1970) or dense bodies of the matrix (Serafini-Fracassini & Smith, 1974), a supposition based upon similar morphology and the fact that both intra- and extracellular structures contain acid phosphatase (Thyberg & Friberg, 1970; Serafini-Fracassini & Smith, 1974) and therefore might well be lysosomes (Novikoff, 1963). In addition this structure appears to be the nidus for early mineralization of the mammalian growth cartilage (Anderson, 1969; Bonucci, 1970; Thyberg & Friberg, 1970, 1971; Serafini-Fracassini & Smith, 1974); certainly in the present study crystals occur either in or in close proximity to similarly shaped bodies. Indeed the initial crystal often seems to distort the confining membrane, while, later, rosettes of crystals form around these structures. It has also been suggested that these dense bodies might be responsible for disintegration of the avian cartilaginous matrix (Matukas & Krikos, 1968). From the data reviewed by Tappel (1969) it appears that lysosomal enzymes can degrade glycosaminoglycans and the protein to which they are bound. However, it does seem unlikely that the same population of matrical dense bodies can initiate the formation of hydroxyapatite crystallites (Anderson, 1969; Bonnucci, 1971) as well as engage in lysosomal-line destruction of the growth plate (Thyberg & Friberg 1970) particularly in view of the fact that the former process is considered to occur at neutral to slightly alkaline pH (Anderson *et al.* 1970), while the latter occurs at an acid pH. Therefore it is probable that there are *two* functionally distinct populations of matrical bodies which morphologically are indistinguishable.

The other type of vesicle that occurs in the avian growth plate is most frequently located pericellularly, particularly in the hypertrophic zones. Although smaller, this second type of vesicle seems to have a counterpart in the mammalian growth plate matrix. In the mammal, various workers (Anderson, 1969; Bonucci, 1970; Thyberg & Friberg, 1970, 1971; Serafini-Fracassini & Smith, 1974) considered that this type of vesicle may arise from, or be part of, nearby chondrocytic cellular processes: this opinion is based on the similar morphology of vesicles and cytoplasmic processes as well as their pericellular distribution. However, serial sections demonstrated that these vesicles were distinct and separate from the cellular villi (Bonucci, 1970; Serafini-Fracassini & Smith, 1974). Matrix vesicles were not observed in the cartilaginous matrix of the avian embryo (Matukas *et al.* 1967), even though specifically sought (Olson & Low, 1971). More recently, however, Crissman & Low (1974), examining embryological avian vertebrae, found that mineralization occurred within cartilaginous matrical vesicles, bodies which morphologically resemble those described in the present study. Crissman & Low (1974) made no mention of the pericellularly sited vesicles.

A significant proportion of the avian growth plate matrix is composed of colla-

genous microfibrils between which are many extracellular granules with material projecting from, and often linking, adjacent granules. Such material projecting from extracellular matrical granules has been described previously (Matukas *et al.* 1967; Anderson & Sajdera, 1971; Serafini-Fracassini & Smith, 1974). Its nature has yet to be decided; it has been variously suggested that this filamentous material is collagen (Matukas *et al.* 1967), non-collagenous protein (Serafini-Fracassini & Smith, 1972), and glycoprotein necessary for the formation of aggregates of proteoglycans (Anderson & Sajdera, 1971).

Results from the present study show that the extracellular cartilaginous fibrils not only marginally increase in diameter from proliferating to degenerating hypertrophic zone, but more of them are distinctly banded in the latter zone, giving some fibrils a distinct periodicity. The measured fibrillary diameter of 9·5–19 nm classes them as microfibrils according to the criteria of Bloom & Fawcett (1968). The diameter of collagen fibrils from avian embryological limbs ranges from 5–25 nm (Fitton-Jackson, 1960), increasing in diameter from 5 up to 20 nm during embryological development (Olson & Low, 1971). In seeming contrast, Matukas and colleagues (1967) state that fibrils of avian embryological growth cartilage have an approximate diameter of 20 nm with a periodicity, when apparent, of about 21 nm. Small fibrillary size together with lack of axial periodicity in the growth plate cartilage has been attributed to additional α_1 chains (Miller & Matukas, 1969). Apparently the rate of collagen fibrillogenesis is enhanced by the mucopolysaccharide substrate of cartilage, while transverse growth of individual collagen fibrils is limited (Keech, 1961; Lowther, 1963; Jackson & Bentley, 1968).

The interdigitating and haphazard arrangement of microfibrils in the avian growth plate is best described as an interlacing feltwork (Martin, 1954). In most mammalian species microfibrils have been reported to range from 5–40 nm in diameter (Anderson & Parker, 1968), with demonstrable periodic banding of 8–10 nm for the rat (Cameron, 1963) and 22 nm for the mouse (Takuma, 1960). More recently, fibrillary diameter in the hare was recorded as 8–20 nm (Serafini-Fracassini & Smith, 1974) while that for guinea-pigs was reported as 20 nm (Thyberg & Friberg, 1970), and perceptible cross-banding was occasionally encountered.

The present study has not only amplified earlier observations on avian embryological growth plate cartilage, but has detailed the fine structure of the proximal growth plate of the tibia in 7 weeks old white leghorn and broiler chickens. It was noted that there was little ultrastructural difference between the growth plates from the two groups of birds. In addition, although recognizing the existence of microscopic structural differences between avian and mammalian growth plates, this study has concentrated on the ultrastructural similarities between the chicken and young mammal, and it is concluded that similar mechanisms govern the growing regions of long bones in these two vertebrate classes.

SUMMARY

The ultrastructure of the proximal tibial growth plate of the 7 weeks old chicken has been described. Little ultrastructural difference could be ascertained between growth plates examined from normal white leghorn and commercial broiler chickens. The growth plate may be divided into five zones: interstitial, proliferating, prehypertrophic, hypertrophic, and degenerating hypertrophic. These zones reflect a maturation of chondrocytes, beginning with a stage of high mitotic and cytoplasmic

activity passing through a stage of active secretion of matrical components (pre-hypertrophic and hypertrophic) and ending with degeneration of the cells and calcification of the matrix.

Mineralization of the matrix appears to be initiated within matrical dense bodies, as in the mammal. Single hydroxyapatite crystals are first encountered about 0·1 mm proximal (i.e. towards the knee) to the limit of metaphyseal blood vessel ingrowth, while dense calcification is observed 0·1 mm distal to the tips of these metaphyseal vessels. The diameter of microfibrillary collagen in the growth plate matrix ranges from approximately 9 nm in the proximal zones to 19 nm in the distal zones. Many of the fibrils in the distal zones have a more or less distinct periodicity. Other major elements of the growth plate matrix are the ruthenium red-stained syncytial aggregates of mucopolysaccharides which are probably derived from the granules within the large intracellular Golgi vesicles.

These findings have led the author to conclude that, while light microscopy indicates that avian and mammalian growth plates have very different structures, electron microscopy finds many similarities, suggesting that the physiological control mechanisms in these two vertebrate classes have much in common.

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REFERENCES

ANDERSON, H. C. (1969). Vesicles associated with calcification in the matrix of epiphyseal cartilage. *Journal of Cell Biology* **41**, 59–72.

ANDERSON, H. C., MATSUZAURA, T., SAJDERA, S. W. & Ali, S. Y. (1970). Membranous particles in calcyfying cartilage matrix. *Transactions of the New York Academy of Sciences, Series 2*, **32**, 619–630.

ANDERSON, C. E. & PARKER, J. (1968). Electron microscopy of the epiphyseal cartilage plate. *Clinical Orthopaedics and Related Research* **58**, 225–241.

ANDERSON, H. C. & SAJDERA, S. W. (1971). The fine structure of bovine nasal cartilage. Extraction as a technique to study proteoglycans and collagen in cartilage matrix. *Journal of Cell Biology* **49**, 650–663.

BLOOM, W. & FAWCETT, D. W. (1968). *A Textbook of Histology*, 9th ed. Philadelphia: W. B. Saunders Co.

BONUCCI, E. (1970). Fine structure and histochemistry of 'calcifying globules' in epiphyseal cartilage. *Zeitschrift für Zellforschung und mikroscopische Anatomie* **103**, 192–217.

BONUCCI, E. (1971). The locus of initial calcification in cartilage and bone. *Clinical Orthopaedics and Related Research* **78**, 108–139.

CAMERON, D. A. (1963). The fine structure of bone and calcified cartilage. *Clinical Orthopaedics and Related Research* **26**, 199–328.

CRISSMAN, R. S. & LOW, F. N. (1974). A study of fine structural changes in the cartilage-to-bone transition within the developing chick vertebra. *American Journal of Anatomy* **140**, 451–470.

FELL, H. B. (1925). The histogenesis of cartilage and bone in the long bones of the embryonic fowl. *Journal of Morphology and Physiology* **40**, 417–459.

FITTON-JACKSON, S. (1960). Fibrogenesis and the formation of matrix. *In Bone as a Tissue* (ed. K. Rodehl *et al.*), pp. 165–185. New York: McGraw-Hill. Cited by Anderson & Parker (1968).

JACKSON, D. S. & BENTLEY, J. R. (1968). Collagen-glycosaminoglycan interactions. In *Treatise on Collagen*, Vol. 2, pp. 189–214 (ed. G. N. Ramachandran & B. S. Gould). New York: Academic Press.

KARNOVSKY, M. J. (1965). A formaldehyde-glutaraldehyde of high osmolarity for use in electron microscopy. *Journal of Cell Biology* **27**, 137 A.

KEECH, M. K. (1961). The formation of fibrils from collagen solutions. IV. Effect of mucopolysaccharides and nucleic acids: An electron microscopic study. *Journal of Biophysical and Biochemical Cytology* **9**, 139–209.

LOWTHER, D. A. (1963). Chemical aspects of collagen fibrillogenesis. In *International Review of Connective Tissue Research* (ed. D. A. Hell), pp. 64–126. New York: Academic Press.

LUFT, J. H. (1971*a*). Ruthenium red and violet. I. Chemistry, purification, methods of use for electron microscopy and mechanism of action. *Anatomical Record* **171**, 347–368.

LUFT, J. H. (1971*b*). Ruthenium red and violet. II. Fine structural localization in animal tissues. *Anatomical Record* **171**, 369–415.

LUTFI, A. M. (1974). The ultrastructure of cartilage cells in the epiphyses of long bones in the domestic fowl. *Acta anatomica* **87**, 12–21.

MARTIN, A. V. (1954). An electron microscopic study of the cartilaginous matrix in the developing tibia of fowl. *Journal of Embryology and Experimental Morphology* **2**, 38–48.

MATUKAS, V. J. & KRIKOS, G. A. (1968). Evidence for changes in protein polysaccharide associated with the onset of calcification in cartilage. *Journal of Cell Biology* **39**, 43–48.

MATUKAS, V. J., PANNER, B. J. & ORBISON, J. L. (1967). Studies on ultrastructural identification and distribution of protein-polysaccharide in cartilage matrix. *Journal of Cell Biology* **32**, 365–377.

MILLER, E. J. & MATUKAS, V. J. (1969). Chick cartilage collagen. *Proceedings of National Academy of Science* **64**, 1264–1268.

NOVIKOFF, A. B. (1963). *Lysosomes. Ciba Foundation Symposium*, 36. Boston, Massachusetts: Little, Brown & Co.

OLSON, M. D. & LOW, F. N. (1971). The fine structure of developing cartilage in the chick embryo. *American Journal of Anatomy* **131**, 197–216.

REVEL, J. P. & HAY, E. (1963). An autoradiographic and electron microscopic study of collagen synthesis in differentiating cartilage. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **61**, 110–144.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–212.

SALEMA, R. & BRANDÃO, I. (1973). The use of Pipes buffer in the fixation of plant cells for electron microscopy. *Journal of Submicroscopic Cytology* **5**, 79–96.

SERAFINI-FRACASSINI, A. & SMITH, J. W. (1974). *The Structure and Biochemistry of Cartilage*. Edinburgh & London: Churchill Livingstone.

SPURR, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* **26**, 31–43.

TAKUMA, S. (1960). Electron microscopy of the developing cartilaginous epiphysis. *Archives of Oral Biology* **2**, 111–119.

TAPPEL, A. L. (1969). *In Lysomes in Biology & Pathology* (ed. J. T. Dingle & H. B. Fell), vol. 2, p. 207. Amsterdam: North Holland Publishing Co.

THYBERG, J., LOHMANDER, K. & FRIBERG, U. (1973). Electron microscopic demonstration of proteoglycans in guinea pig epiphyseal cartilage. *Journal of Ultrastructure Research* **45**, 407–427.

THYBERG, J. & FRIBERG, U. (1970). Ultrastructure and acid phosphatase activity of matrix vesicles and cytoplasmic dense bodies in the epiphyseal plate. *Journal of Ultrastructure Research* **33**, 554–573.

THYBERG, J. & FRIBERG, U. (1971). Ultrastructure of the epiphyseal plate of the normal guinea pig. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **122**, 254–272.

WISE, D. R. & JENNINGS, A. R. (1973). The development and morphology of the growth plates of two long bones of the turkey. *Research in Veterinary Science* **14**, 161–166.

WOLBACH, S. B. & HEGSTED, D. M. (1952). Endochondral bone growth in the chick. *Archives of Pathology* **54**, 1–12.