

Association of an Extracellular Protein (Chondrocalcin) with the Calcification of Cartilage in Endochondral Bone Formation

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ABSTRACT We examined bovine fetal epiphyseal and growth plate cartilages by immunofluorescence microscopy and immunoelectron microscopy using monospecific antibodies to a newly discovered cartilage-matrix calcium-binding protein that we now call chondrocalcin. Chondrocalcin was evenly distributed at relatively low concentration in resting fetal epiphyseal cartilage. In growth plate cartilage, it was absent from the extracellular matrix in the zone of proliferating chondrocytes but was present in intracellular vacuoles in proliferating, maturing and upper hypertrophic chondrocytes. The protein then disappeared from the lower hypertrophic chondrocytes and appeared in the adjoining extracellular matrix, where it was selectively concentrated in the longitudinal septa in precisely the same location where amorphous mineral was deposited in large amounts as demonstrated by von Kossa staining and electron microscopy. Mineral then spread out from these "nucleation sites" to occupy much of the surrounding matrix. Matrix vesicles were identified in this calcifying matrix but they bore no observable morphological relationship to these major sites of calcification where chondrocalcin was concentrated. Since chondrocalcin is a calcium-binding protein and has a strong affinity for hydroxyapatite, these observations suggest that chondrocalcin may play a fundamental role in the creation of nucleation sites for the calcification of cartilage matrix in endochondral bone formation.

Recently, we described a protein that was purified from fetal epiphyseal cartilage (1), that exists as a 70,000-mol-wt dimer of 35,000-mol-wt subunits. The protein binds strongly to hydroxyapatite and increases in concentration at the time when the secondary center of ossification appears in the epiphysis. This protein is unrelated to any other known cartilage or bone proteins (1). We prepared a monospecific antiserum to this protein and have now demonstrated immunohistochemically that it is intimately associated with the major calcification of cartilage matrix that occurs in the lower hypertrophic zone of the growth plate. These observations are described in this article. They present the basis for a new understanding of cartilage calcification. We have called the protein chondrocalcin in view of its calcium-binding properties (P. Hauschka, H. V. Choi, and L. C. Rosenberg, unpublished results), its affinity for hydroxyapatite (1), and its presence in the calcified cartilage of growth plate, which is shown here.

MATERIALS AND METHODS

Isolation of Chondrocalcin and Other Molecules: Bovine fetal epiphyseal cartilage from the third trimester was extracted with 4 M guanidine hydrochloride as described previously (1). The extract was dialyzed to associative conditions and centrifuged with cesium chloride to produce density gradient fractions A1 (high density) to A6 (low density). Chondrocalcin was isolated from fractions A5 and A6 by diethylaminoethyl cellulose chromatography followed by affinity chromatography with affi-gel blue and hydroxyapatite. The protein was finally purified by dissociative chromatography in 4 M guanidine hydrochloride on Sephacryl S-200.

Cartilage proteoglycan isolated as fraction A1D1D1 from bovine fetal epiphyseal cartilage, cartilage link protein from bovine nasal cartilage, and type II collagen from bovine nasal cartilage were isolated and purified as described before (2).

Antisera and Immunoreagents: The rabbit antiserum (R102) to bovine epiphyseal chondrocalcin has already been described (1). Preimmune serum was collected before immunization (3). Nonimmune rabbit and pig sera, pig antiserum to rabbit F(ab')₂ (3), and soluble immune complexes of rabbit antiperoxidase and horseradish peroxidase (4) have been described. Concentrated immunoglobulin G (IgG) was prepared by ammonium sulphate precip-

itation of serum (5). F(ab')₂ subunits of antibody IgG were produced by pepsin digestion of concentrated IgG (3) and purified by chromatography on Ultragel ACA 44 (Pharmacia Fine Chemicals; Piscataway, NJ) in phosphate-buffered saline (PBS; see reference 5). For localization work, R102 (Fab')₂ at 14.8 mg/ml in PBS was absorbed for 1 h at 37°C with purified chondrocalcin (where indicated) and always with purified epiphyseal proteoglycan (fraction A1D1 treated with chondroitinase ABC; see reference 5) each at 1 mg/ml (final native concentration). Immunoprecipitates and other precipitates were removed immediately prior to use by centrifugation (3). Protein concentrations of IgG and F(ab')₂ were determined spectrophotometrically (3). The matrix protein was also determined spectrophotometrically, assuming E_{1%¹cm}²⁸⁰ at 280 nm equals 1.0. Immediately before use, F(ab')₂ immunoreagents were reduced with 5 mM cysteine to Fab' (3).

Enzyme-linked Immunosorbent Assays: These were performed with ammonium sulphate concentrated immunoglobulins at 22.6 mg/ml essentially as previously described (5). Purified bovine chondrocalcin and fetal epiphyseal cartilage proteoglycan (A1D1D1) were bound at concentrations of 0.044 and 5 µg, respectively, in 50-µl volumes, per well. Preimmune or nonimmune immunoglobulin was used to control these assays, with similar results. Titers were recorded as the reciprocals of the greatest dilution at which immunoreactivity was observed.

PAGE and Immunoblotting: Electrophoresis in 10% SDS PAGE and 5% (vol/vol) mercaptoethanol with subsequent electroblotting at 0.3 A for 16 h onto nitrocellulose using Biorad Trans-Blot equipment (Bio-Rad Laboratories; Richmond, CA) was carried out as otherwise reported (6, 7). For SDS PAGE, 15 µg dry weight of sample in 10 µl of sample buffer were applied to each track. Nitrocellulose-bound proteins were washed and stained as described before (7) but with either antiserum R102 or nonimmune rabbit serum diluted to 10% (vol/vol) in 3% (wt/vol) bovine serum albumin in PBS.

Growth Plates and Tissue Preparation: Primary bovine fetal growth plates from proximal tibiae were isolated as described before (8). For immunofluorescence and immunoelectron microscopy of extracellular chondrocalcin, tissues were usually fixed immediately in 2% formaldehyde with 2% glutaraldehyde in PBS for 1 h at 4°C. After washing in cold PBS for 5 min and in PBS containing 5% nonimmune pig serum for 30 min at 4°C, tissue blocks were decalcified (where indicated) in 0.5 M EDTA, pH 7.4, with proteinase inhibitors at 4°C for 16 h (8). To demonstrate intracellular chondrocalcin with immunofluorescence, unfixed tissues were decalcified, frozen, and fixed in 4% formaldehyde after sectioning. Tissues fixed in formaldehyde and glutaraldehyde were sectioned unfrozen at 20 µm with a Sorvall TC2 chopper (Beckman Instruments, Inc.; Palo Alto, CA) as before for electron microscopy (4). Fixed and unfixed tissues were frozen in 7% gelatin in liquid nitrogen for frozen sectioning at 4 µm (3) for immunofluorescence microscopy of chondrocalcin.

Immunofluorescence Microscopy: Frozen sections of fixed or unfixed tissue were attached to microscope slides pre-coated with 2% gelatin which had been air dried. They were fixed in 4% formaldehyde for 5 min at room temperature, washed for 30 min in PBS, and subsequently treated with chondroitinase ABC for 1.5 h at 37°C as previously described (3). After rinsing in PBS containing 5 mM cysteine for 10 min, they were treated at room temperature for 30 min either with Fab' from rabbit antiserum R102 to chondrocalcin (with or without preabsorption with chondrocalcin) or with Fab' from preimmune or nonimmune rabbit sera, each at a concentration of 3.7 mg/ml in freshly prepared 5 mM cysteine in PBS to produce monovalent Fab'. Subsequent washing and staining of sections with fluorescein-labeled pig Fab' anti-rabbit Fab' and fluorescence microscopy and other information were as described earlier (3).

Immunoelectron Microscopy: 20-µm-thick formaldehyde-glutaraldehyde-fixed, unfrozen sections of decalcified or nondecalcified cartilage were rinsed in PBS and then digested for 3 h at 37°C with chondroitinase ABC at 2.5 times the concentration used before (3). After rinsing in PBS containing 5 mM cysteine for 30 min, floating sections were treated for 7.5 h with 600-µl volumes of R102 Fab' antichondrocalcin (3.7 mg/ml) or nonimmune rabbit Fab' (3.7 and 0.7 mg/ml). These reagents were always used in the presence of 5 mM cysteine. Sections were then washed for 1 h in 5 mM cysteine in PBS. They were subsequently treated with a pig anti-rabbit Fab' bridging serum for 2 h as previously described (4), rinsed for 1 h in PBS, and then treated for 1 h with soluble complexes of rabbit antiperoxidase and peroxidase (4). Sections were finally reacted for peroxidase and treated with osmium tetroxide, embedded in Spurr resin, sectioned, and counter-stained with uranyl and lead (4). All these procedures were conducted at room temperatures unless otherwise stated.

Matrix vesicles and the discrete focal staining for chondrocalcin were measured in micrographs of decalcified sections. The diameters of matrix vesicles were recorded in one dimension only. Due to their rather irregular shape, sites of chondrocalcin staining were measured twice in two dimensions at right angles to each other, intersecting each other equally. The products were added. Means were determined for each particle in nanometers. Standard deviations

of stained particles were determined.

Von Kossa's Stain for Calcium: Undecalcified sections fixed in 10% formalin were stained with von Kossa's stain and counterstained with 0.1% basic fuchsin in 70% ethanol.

RESULTS

Specificity of Rabbit Antiserum R102 to Chondrocalcin

Antiserum R102 reacted strongly in ELISA assays with purified chondrocalcin to give a titer of 1,024: it did not react with link protein or with type II collagen but reacted very weakly with cartilage proteoglycan (titer of 8). The latter reaction was selectively removed by absorption of the antiserum for 1 h at 37°C with proteoglycan as indicated in Materials and Methods. To further assess the specificity of antiserum R102, we subjected density gradient fractions A1 to A6 of extracted fetal epiphyseal cartilage, from which the protein was purified, to SDS PAGE under reducing conditions followed by electroblotting and staining of the nitrocellulose fingerprint with antiserum R102. This revealed that the antiserum reacted intensely only with the reduced molecule seen here as a doublet of subunits of ~35,000 mol wt (Fig. 1). The residual reaction of the 70,000-mol-wt species corresponds to that observed with the unreduced molecule (data not shown). Also, the minor reactions with species <35,000-mol-wt are due to degradation products of the 35,000-mol-wt protein (unpublished data). The minor reactions shown here cannot be detected in immunolocalization with the techniques employed (data not shown). The results also reveal that chondrocalcin is absent from fraction A1, which contains proteoglycan aggregates, but is present in all other fractions, being most concentrated in the upper half of the gradient. In view of the small size of chondrocalcin, this observation indicates that it

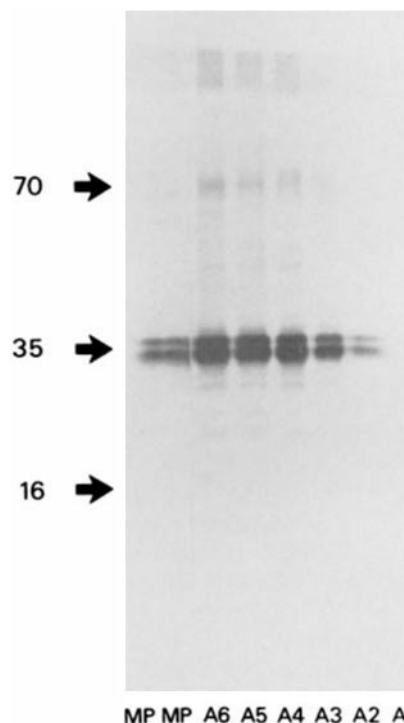


FIGURE 1 Immunoblotted SDS PAGE of purified chondrocalcin (MP) and fractions A1 through A6 from a 235-d-old fetus. Purified MP was run at 15 and 10 µg per track, reading from left to right. Molecular weight measures, × 10⁻³.

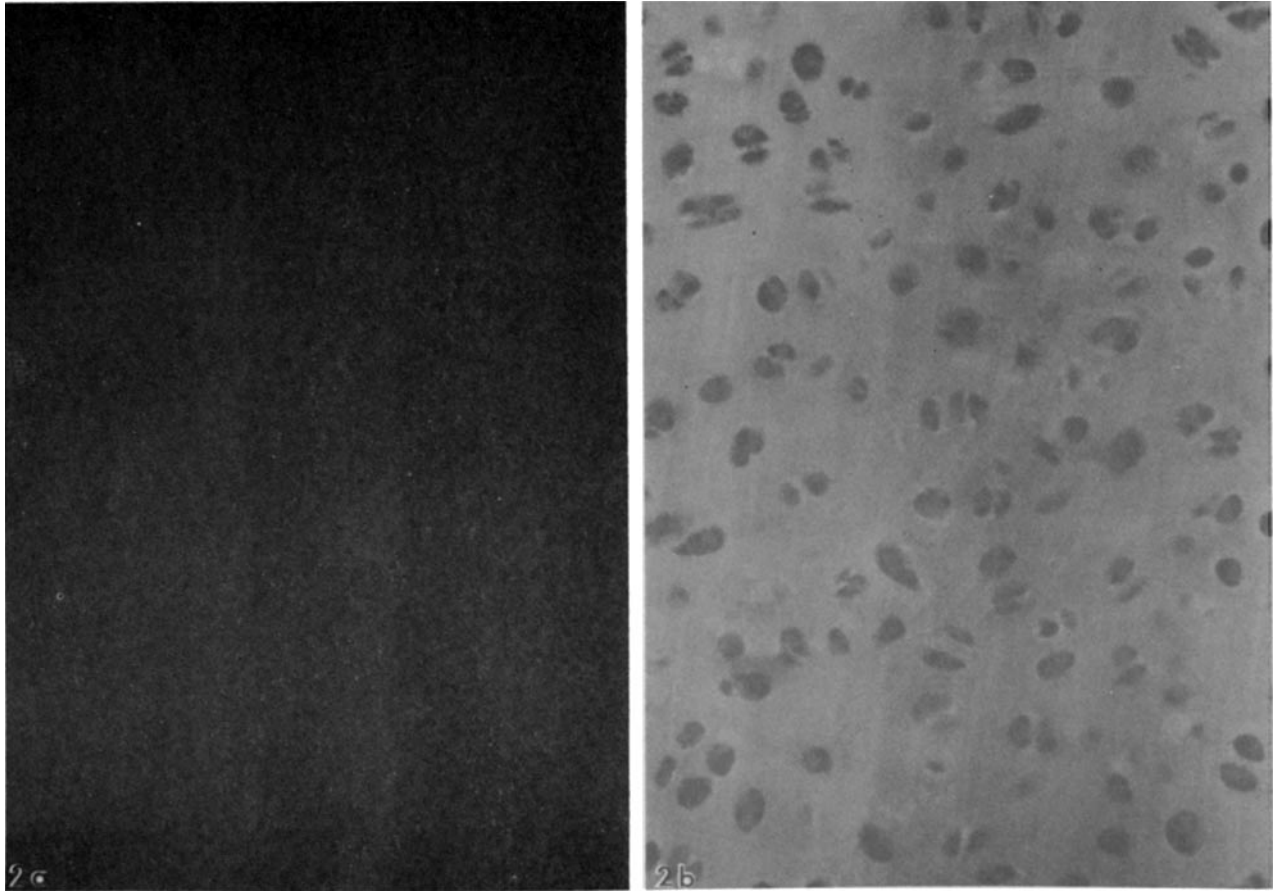


FIGURE 2 The localization of chondrocalcin in resting epiphyseal cartilage of a 181-d-old fetus. Tissue was immediately fixed in 2% formaldehyde and 2% glutaraldehyde, decalcified, and sectioned: (a) was stained initially with nonimmune rabbit Fab' and (b) with R102 Fab' to chondrocalcin. (a) $\times 375$. (b) $\times 235$.

may interact with molecules of higher buoyant density as yet unidentified.

Immunofluorescence Microscopy of Chondrocalcin in Epiphyseal Cartilage and Growth Plate

Chondrocalcin was localized as even diffuse staining of moderate intensity throughout the matrix of epiphyseal cartilage outside of the primary and secondary growth plates (Fig. 2*b*). Control sections that had been initially treated with antibody Fab' absorbed with purified matrix protein, with preimmune or nonimmune rabbit Fab' did not stain (Fig. 2*a*).

On entering the proliferative zone of the primary growth plate, staining for matrix protein disappeared rapidly until it was almost or completely absent (Fig. 3) and stayed as such up to and including the upper hypertrophic zone (Fig. 4*b*). Intense intracellular particulate staining was observed in the proliferative (Fig. 3), maturing and upper hypertrophic zones. This was not detected elsewhere and was only seen in tissue extracted with EDTA before fixation. In the lower part of the hypertrophic zone, chondrocalcin was detectable in the extracellular matrix as intense staining present in pericellular and territorial sites limited to the longitudinal septa (Fig. 4*b* and *c*). Intracellular staining was no longer visible. This extracellular growth plate staining for chondrocalcin, which was of an intensity far greater than that seen elsewhere, was detectable in unextracted tissue and persisted in tissues which had been pre-extracted with EDTA before fixation. In other re-

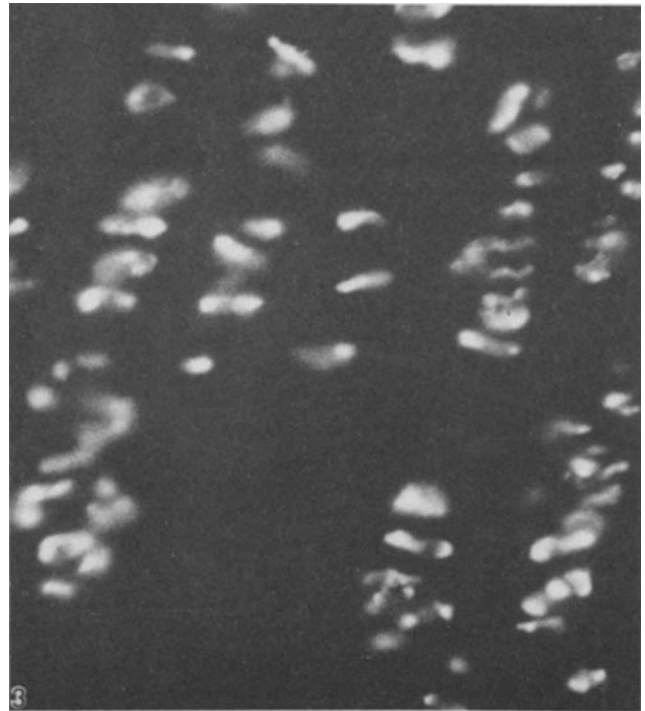


FIGURE 3 The presence of chondrocalcin in cells of the proliferative zone of growth plate from a 179-d-old fetus. The tissue was decalcified unfixed with EDTA, then frozen, sectioned, fixed in 4% formaldehyde for 5 min, and stained with rabbit 102 antibody Fab' to chondrocalcin. Intense particulate vacuolar fluorescence was observed within cells: there was no extracellular staining. $\times 490$.

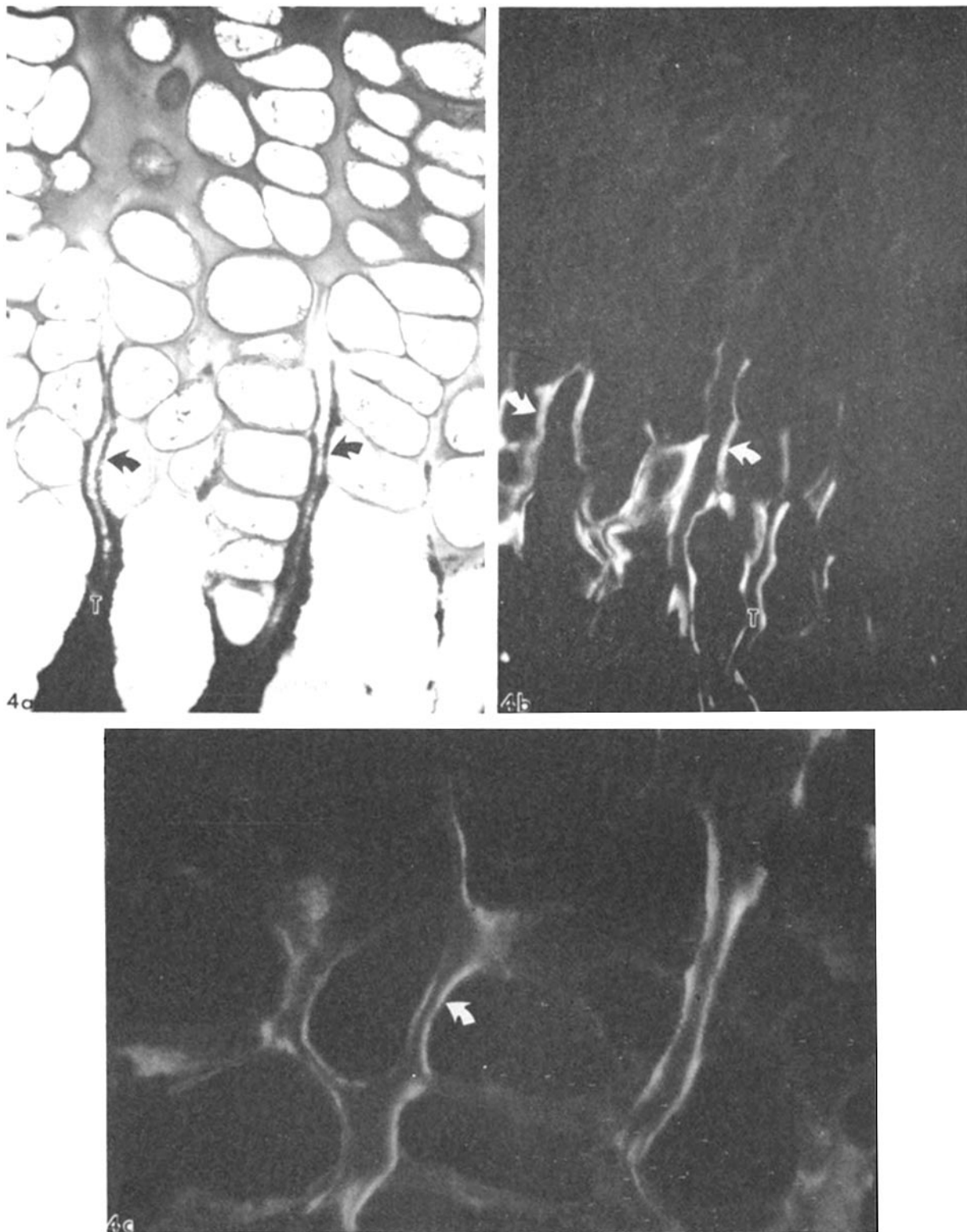


FIGURE 4 The association of calcification with chondrocalcin in the hypertrophic zone of a growth plate from a 181-d-old fetus. (a) Undecalcified section of formalin-fixed tissue treated with von Kossa's stain to show mineral (arrows) in perilacunar and territorial sites in the longitudinal septa of the lower hypertrophic zone and in the calcified trabeculae (T) of the metaphysis. (b and c) Tissue was fixed in 2% formaldehyde and 2% glutaraldehyde, decalcified, sectioned, and stained with rabbit 102 antibody Fab' to chondrocalcin. Under these conditions of tissue preparation, chondrocalcin was only detectable in extracellular sites. Intense fluorescence was seen in the lower hypertrophic zone in the same perilacunar and territorial sites in the longitudinal septa (arrows). This staining persisted at the edges of the calcified trabeculae (T) in the metaphysis. There was no staining for chondrocalcin in the matrix of the upper hypertrophic zone: the transverse septae were unstained. (a) $\times 684$. (b) $\times 268$. (c) $\times 1,368$.

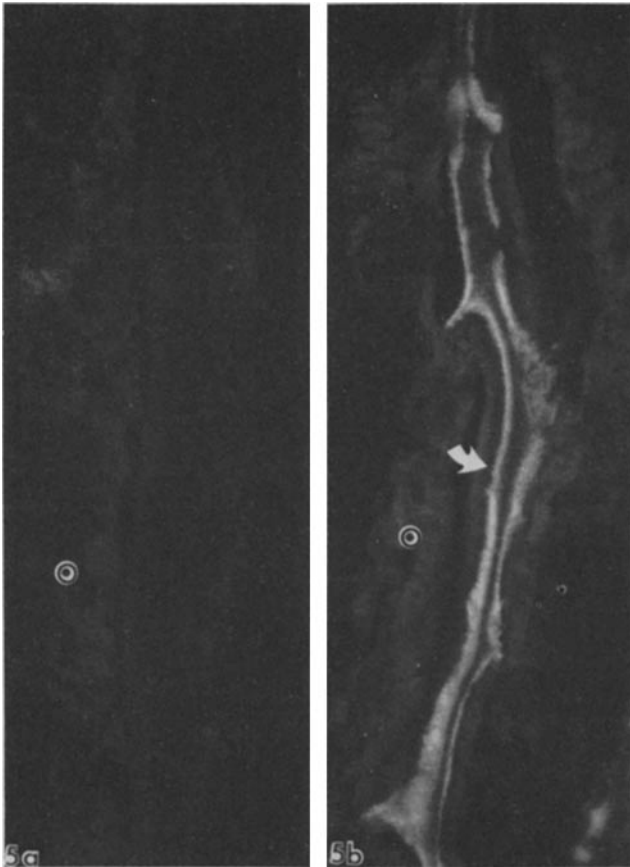


FIGURE 5 Immunofluorescence staining (arrow) of chondrocalcin in the calcified trabeculae of the metaphysis at the edges of the calcified cartilage of a 181-d-old fetus. The woven bone, osteoid and osteoblasts (O) were unstained. Tissue was fixed in 2% formaldehyde and 2% glutaraldehyde, extracted in EDTA, and sectioned: (a) stained with nonimmune rabbit Fab' and (b) with rabbit 102 antibody Fab' to chondrocalcin. (a) $\times 310$. (b) $\times 285$.

gions, such as in the epiphyseal cartilage matrix, much of the extracellular chondrocalcin was not detectable after extraction before fixation (results not shown). This intense growth plate staining was present in exactly the same sites as we observed calcification as revealed by the von Kossa stain (Fig. 4a). The staining for chondrocalcin extended into the metaphysis where it coated the edges of the trabeculae of calcified cartilage (8) that extended from the longitudinal septa (Fig. 4b). With the advent of woven bone formation by adherent osteoblasts, chondrocalcin persisted at the now covered edges of the calcified cartilage (Fig. 5b) but was absent from the demineralized woven bone and from calcified and decalcified osteoid remote from the metaphyseal junction (Fig. 5b). Controls were always negative (Fig. 5a).

Immunoelectron Microscopy

Examination of the nondecalcified lower hypertrophic zone revealed that the chondrocalcin observed in the longitudinal septa with immunofluorescence is first seen as amorphous staining in discrete focal sites adjacent to hypertrophic cells (Fig. 6). At higher magnifications, this intense immune staining (Figs. 7b and 8b) was clearly distinguishable from the less electron-dense amorphous mineral observed in the same sites in control sections treated initially with nonimmune rabbit Fab' (Figs. 7a and 8b). Moreover, the mineral was present

wherever chondrocalcin was detected, as revealed by a careful comparison of Fig. 7a with 7b and of Fig. 8a with 8b. Crystalline mineral was observed in the lowermost hypertrophic zone (Fig. 8c) and in the calcified metaphyseal cartilage.

Decalcification of this cartilage matrix (as performed for Fig. 4, b and c) demonstrated that the electron density identified as mineral in control nondecalcified cartilage was no longer present (Fig. 9a). With antibody staining, chondrocalcin was still observed in exactly the same sites (Figs. 9b and 10a). The staining was similar, although much less electron-dense, to that observed without decalcification. The staining was measured in spite of its irregular form ($644 \text{ nm} \pm 275$, $n = 34$). Closer to and at the junction with the metaphysis, chondrocalcin staining persisted and was often more defined in shape and suggestive of being present in "crystal ghosts" left after decalcification (Fig. 10, b and c). This focal staining corresponded in shape, size, and location to that seen in calcified sections such as is observed in Figs. 7b and 8b. In decalcified sections, matrix vesicles were clearly identified by their electron density, size ($78.5 \text{ nm} \pm 18.8$, $n = 13$), and the presence of a unit membrane surrounding them (Fig. 10a, b, and d). They showed no recognizable association with chondrocalcin staining and did not stain for chondrocalcin. They were, however, often observed in the decalcified matrix in the immediate vicinity of chondrocalcin.

The focal most intense calcification of cartilage matrix thus occurred wherever chondrocalcin was detected. The calcification process then spread out from these "nucleation sites" into the surrounding matrix. This is clearly shown in Fig. 11, which compares cartilage stained for chondrocalcin with (b, d, f, and h) or without (a, c, e, and g) prior decalcification. From focal sites (Fig. 11a), calcification extended outwards (Fig. 11c) into the surrounding matrix while chondrocalcin persisted mainly in relatively discrete sites (Fig. 11, b, d, and f). The calcification front at the edges of the longitudinal septa which extended into the metaphysis (Fig. 11, e and g) then formed the edge of the calcified matrix, corresponding to the lamina limitans (L in Fig. 11h) onto which osteoblasts then settled and manufactured osteoid and then bone (Fig. 11h). This occurred only after the outer noncalcified matrix had been lost. By this time, chondrocalcin was often more difficult to detect (Fig. 11h): it may have been partly lost from the matrix in vivo or during decalcification.

DISCUSSION

The calcification of the extracellular matrix of cartilage growth plate is a primary event in endochondral bone formation. The biochemical mechanisms whereby this is initiated and regulated have been the subject of much research and speculation. While much emphasis has been placed on the initiating role of matrix vesicles (9-13), their importance has been questioned recently (14).

The present studies reveal an intimate association between a newly described protein we call chondrocalcin and cartilage calcification in endochondral bone formation. Chondrocalcin is deposited precisely where we observe the initial major focal calcification of cartilage in the lower hypertrophic zone. This occurs in the immediate vicinity of matrix vesicles but not at the same sites occupied by them. This calcification is concentrated in discrete pericellular and territorial sites in the longitudinal septa of the lower hypertrophic zone where chondrocalcin is deposited. It is likely that chondrocalcin and mineral

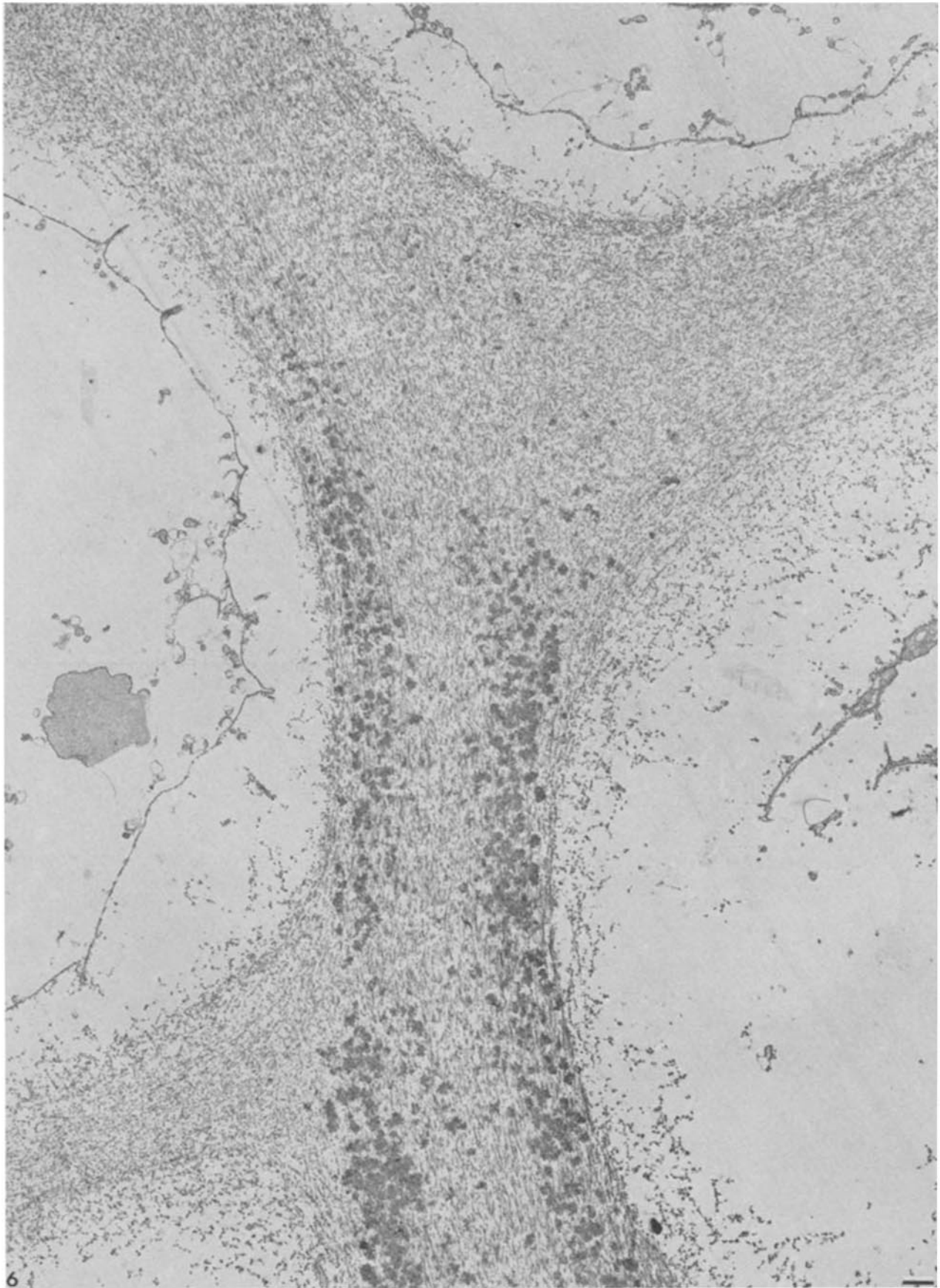


FIGURE 6 Low power electron micrograph to show the first appearance of mineral and staining for chondrocalcin in a longitudinal septum of the lower hypertrophic zone of a 151-d-old fetus. Undecalcified tissue was fixed in 2% formaldehyde and 2% glutaraldehyde, sectioned, and stained with rabbit R102 Fab' to chondrocalcin. The bottom of the figure is closest to the metaphysis. Bar, 1 μ m. \times 5,300.

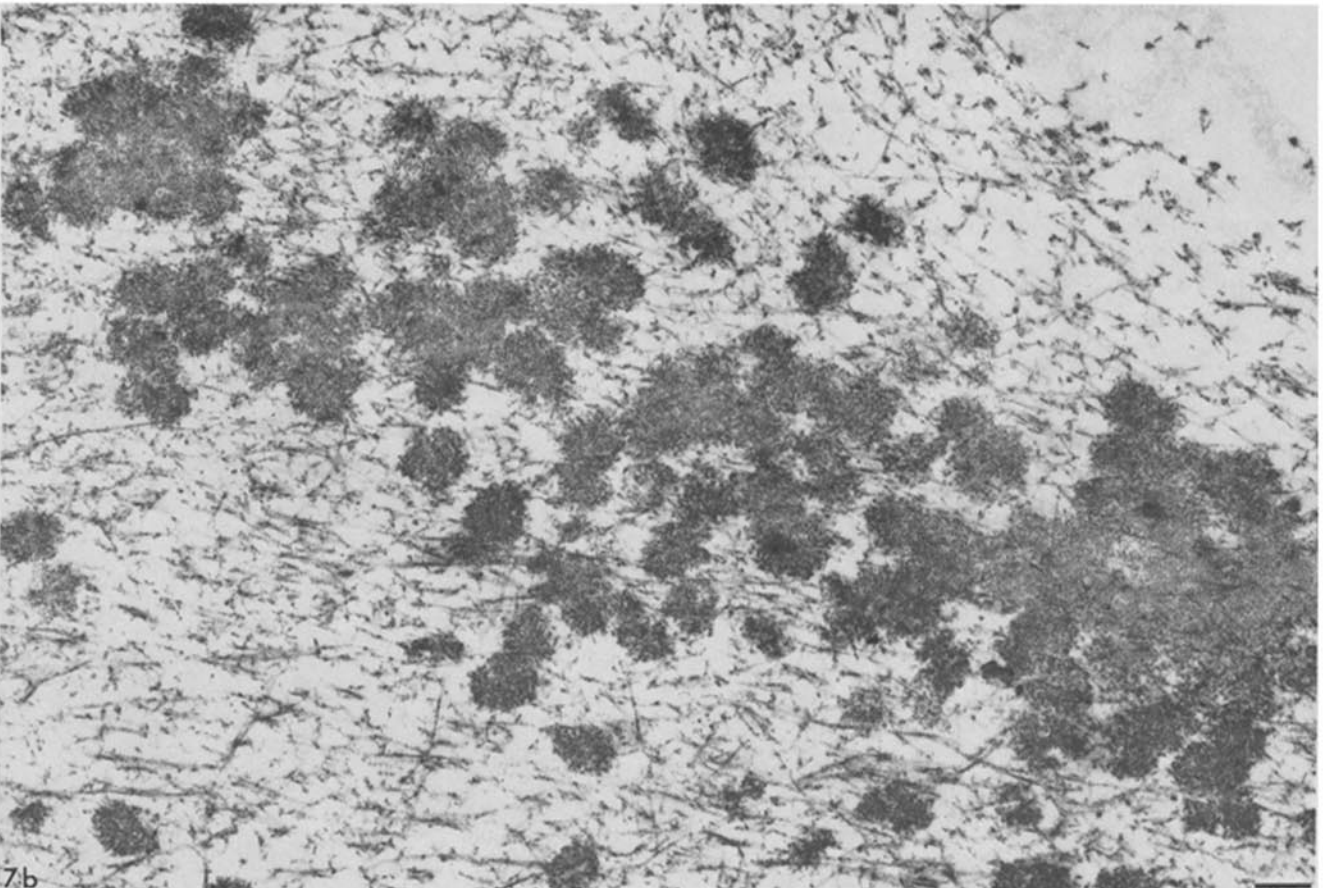
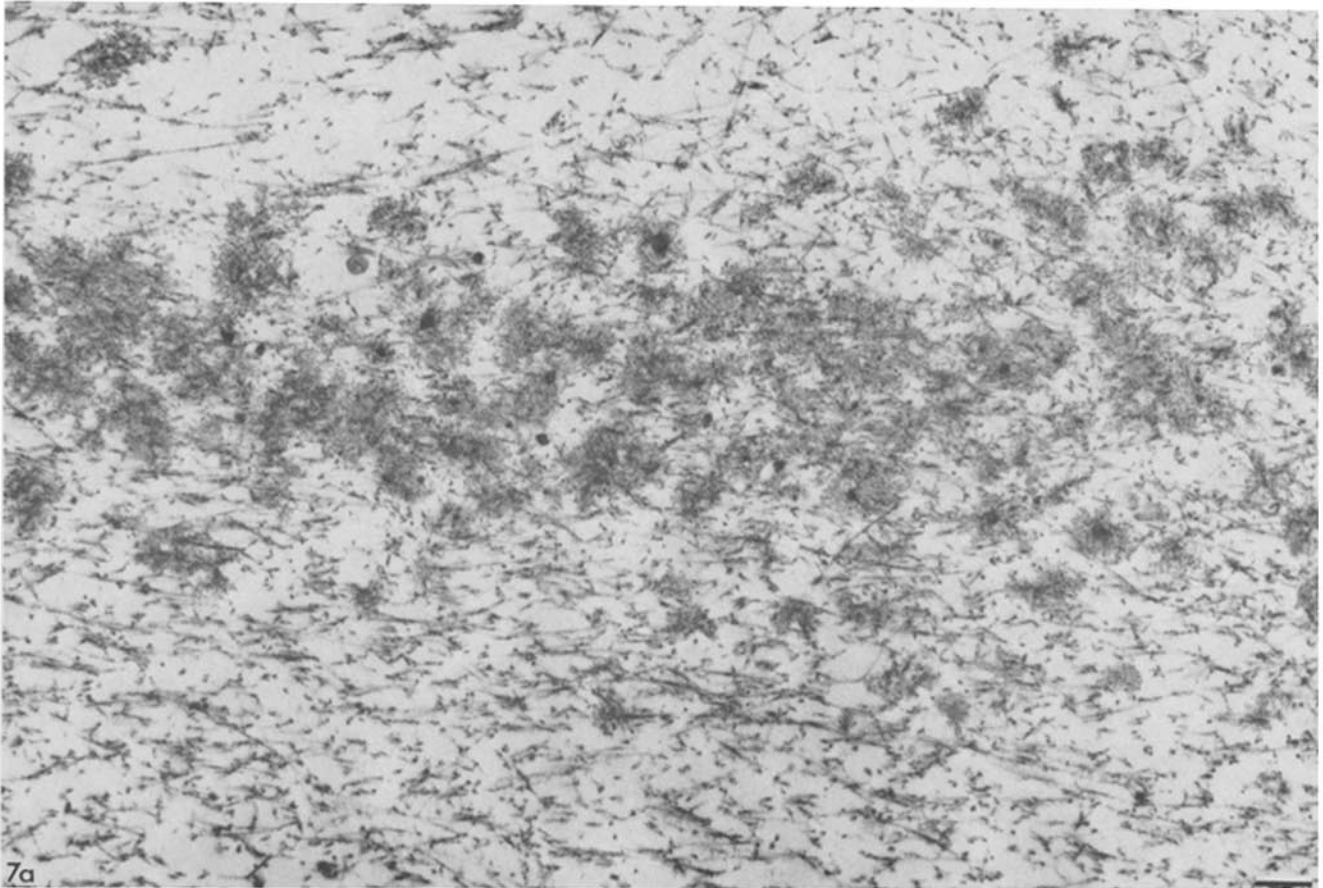


FIGURE 7 Electron micrographs to show the close association between mineral and chondrocalcin in the lower hypertrophic zone in a 151-d-old fetus. Undecalcified tissue was fixed in 2% formaldehyde and 2% glutaraldehyde, sectioned, and stained with either rabbit antibody R102 Fab' to chondrocalcin (b) or nonimmune rabbit Fab' (a) when only mineral was detectable. Bars, 500 nm. $\times 15,000$.

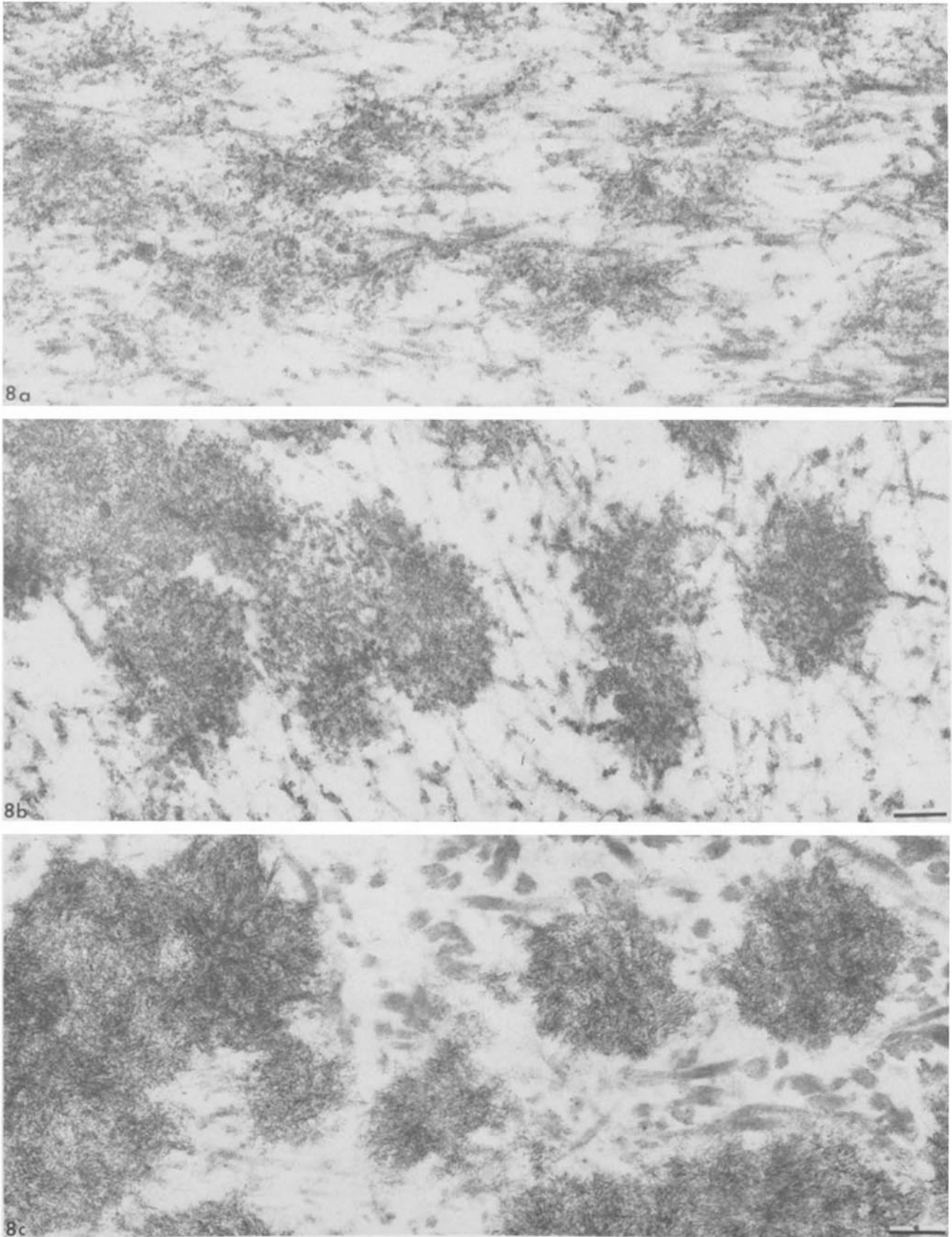


FIGURE 8 Electron micrographs to show at higher magnification the co-existence of mineral and chondrocalcin in the lower hypertrophic zone (*b*) of a 151-d-old fetus and in the junctional zone with the metaphysis (*c*). *a* shows mineral only and is the nonimmune rabbit Fab' control for *b*. *c* and *b* have been stained with rabbit antibody Fab' to chondrocalcin. In *a* and *b* amorphous calcium phosphate is present, whereas in *c* it is crystalline. Undecalcified tissue was fixed as in Fig. 7. Bars, 200 nm. (*a* and *b*) $\times 47,000$. (*c*) $\times 51,700$.

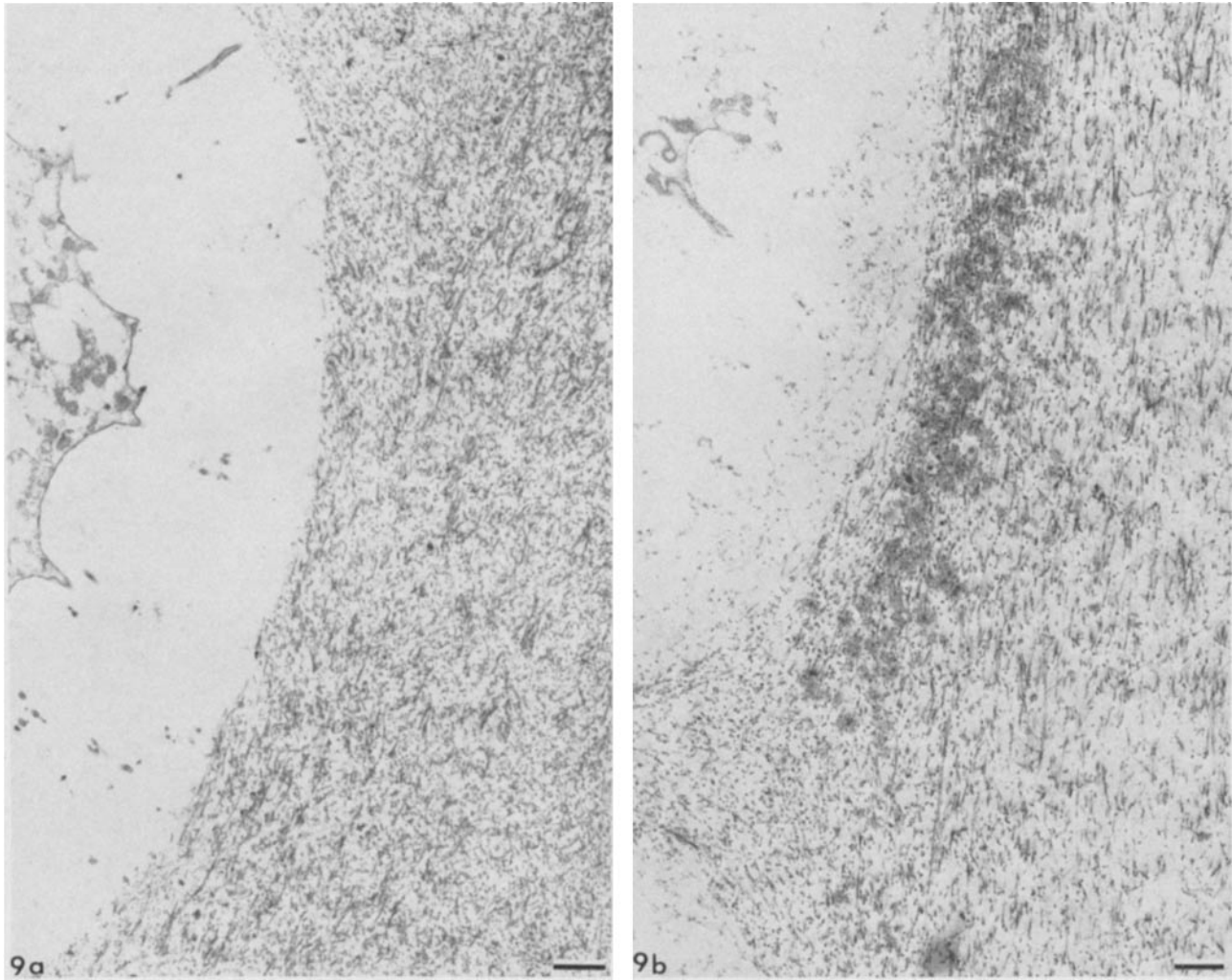


FIGURE 9 Electron micrographs to show the presence of staining for chondrocalcin in the decalcified pericellular and territorial matrix of longitudinal septa of the lower hypertrophic zone of a 181-d-old fetus. Tissue was fixed in 2% formaldehyde and 2% glutaraldehyde before extraction with EDTA and sectioning. *a* was initially treated with nonimmune rabbit Fab' and (*b*) with rabbit 102 antibody Fab' to chondrocalcin. Bars, 1 μ m. \times 7,000.

are simultaneously deposited since they were invariably observed together at the ultrastructural level: also, chondrocalcin appears to be partly lost from the matrix when it is decalcified, as indicated by immunoelectron microscopy. From these initial focal nucleation sites, calcification extended into the surrounding matrix. Chondrocalcin is a calcium-binding protein (P. Hauschka, H. V. Choi, and L. C. Rosenberg, unpublished results) that has a considerable affinity for hydroxyapatite (1). These properties would favour its role as a nucleation agent.

While chondrocalcin is selectively concentrated in the extracellular matrix of the longitudinal septa of the lowermost hypertrophic zone, it is also present in lower concentrations in epiphyseal cartilage that is not calcifying. The reason for this selective calcification of cartilage remains to be established. Since chondrocalcin is essentially absent from the matrix of the noncalcifying part of the growth plate, it is possible that a special molecular form or assembly of this protein in high concentration is required for calcification to occur. Our observations that chondrocalcin is present in intracellular sites before its extracellular appearance in the growth plate suggest that the cells of the growth plate synthesize and suddenly release chondrocalcin in the lower hyper-

trophic zone where it is required for calcification. Trace amounts of chondrocalcin have been detected in serum (1). It could be argued that this molecule accumulates in calcifying cartilage by passively binding to hydroxyapatite. Since, however, chondrocalcin has never been detected in calcifying bone (A. R. Poole, I. Pidoux, L. Rosenberg, manuscript in preparation), this passive hypothesis is unlikely.

Other studies that are still in progress indicate that chondrocalcin appears in extracellular sites of calcifying cartilage where unusually high local concentrations of proteoglycans and link protein are demonstrable with immunoelectron microscopy (A. R. Poole, I. Pidoux, and L. C. Rosenberg, unpublished results): these have not been found in noncalcifying cartilage (4). Recent evidence was presented indicating that early endochondral calcification occurred at sites rich in sulphur (15), a major component of cartilage proteoglycans: the earliest detectable event was an increase in calcium in association with focal sulphur deposits. Other work has also revealed that calcification occurs at sites where proteoglycans are concentrated (16, N. Shepard and N. Mitchell, manuscript in preparation). Thus chondrocalcin may interact with special macromolecular aggregates of proteoglycans in the cartilage matrix. The initial binding of chondrocalcin to proteoglycan

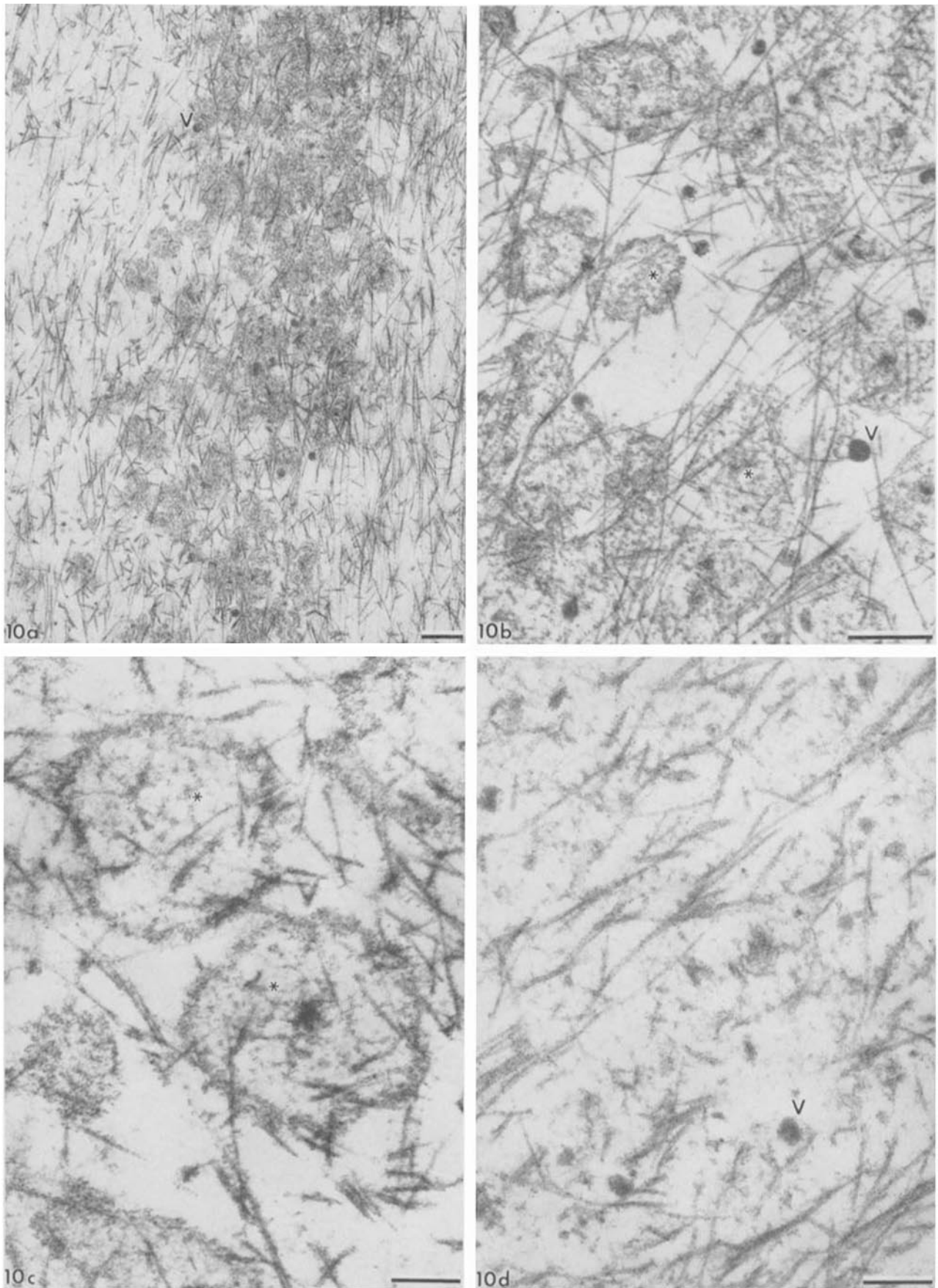


FIGURE 10 Electron micrographs to show at higher magnification the presence of chondrocalcin in decalcified matrix as shown in Fig. 9. *a*, *b*, and *c* were stained with rabbit 102 antibody Fab' to chondrocalcin and *d*, which is the control for *a* and *b*, was stained with nonimmune rabbit Fab'. *a* shows at higher magnification the staining for chondrocalcin as seen in Fig. 9 *b* (*b*) and (*c*) show staining for chondrocalcin (asterisks) in the calcified cartilage closer to and at the metaphyseal junction respectively. Stained structures with unstained electron-dense edges and electron-dense centers can be seen in *b* (*c*). Matrix vesicles (V) are visible in *a*, *b*, and *d*. (*a*) $\times 15,000$ (*b*) $\times 30,500$ (*c* and *d*) $\times 62,000$. Bars: (*a* and *b*) 500 nm; (*c* and *d*) 200 nm.

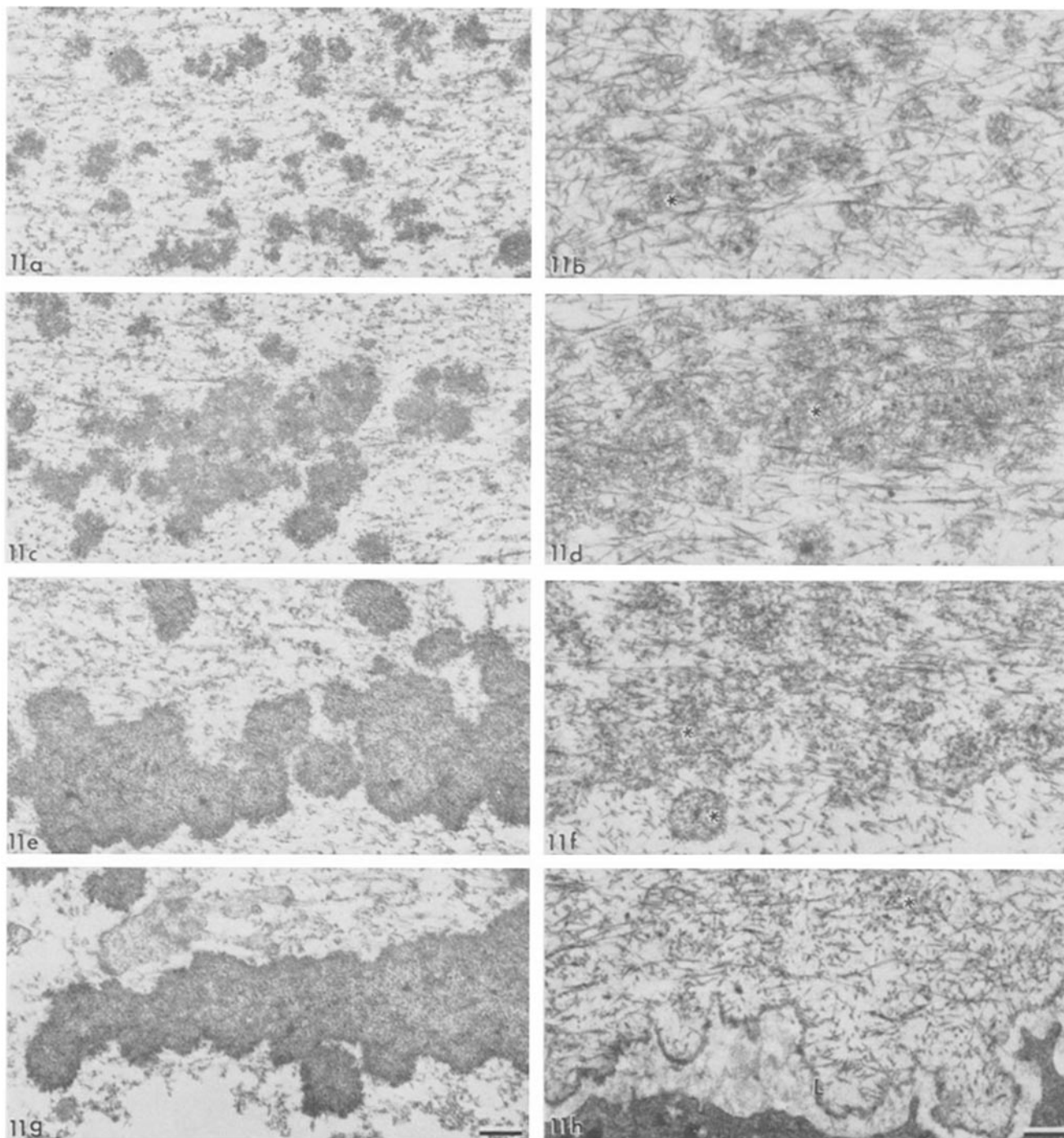


FIGURE 11 Electron micrographs to show chondrocalcin staining with (a, c, e, and g) and without (b, d, f, and h) mineral in the lower hypertrophic zone (a-d) and the metaphysis (e-h). The two sequences reading from top to bottom demonstrate chondrocalcin and initial calcification (a), the progressive spread of calcification (c, e, and g) and the appearance of chondrocalcin alone (asterisks) during these changes (b, d, f, and h). The formation of calcified edges to the trabeculae is also shown (e and g). The edge of an osteoblast can be seen in h. Tissue was otherwise prepared as in Fig. 9b. L, the lamina limitans. Bars, 500 nm. $\times 13,400$.

may be mediated by calcium. Once it has bound, chondrocalcin could act as a nucleator for mineral accretion.

Chondrocalcin has also been found to be selectively concentrated in other calcifying cartilages although it is present in relatively trace amounts in noncalcifying cartilage including articular cartilage (A. R. Poole, I. Pidoux, H. Reddi, and L. C. Rosenberg, manuscripts in preparation). As yet, it has not been found in immature (as shown here) nor mature bone and appears to be absent from tissues other than cartilage (A.

R. Poole, I. Pidoux, and L. Rosenberg, manuscript in preparation) although trace amounts have been detected in serum (1). These observations together indicate that high local concentrations of chondrocalcin are closely associated with cartilage calcification and provide us with a new perspective of cartilage calcification.

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