The Loss of Phenotypic Traits by Differentiated Cells In Vitro

VII. Effects of 5-Bromodeoxyuridine and Prolonged Culturing on Fine Structure of Chondrocytes.

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CHONDROCYTES in the vertebrae of 10-day embryos are largely postmitotic,[1] and are surrounded by a metachromatic matrix into which they deposit newly synthesized chondroitin sulfate. When these cells are liberated from their matrix and cultured as single cells, they are induced to enter the mitotic cycle. The daughter cells are round or polygonal and adhere together forming multicellular colonies.[2–4] Many of the cells in these colonies deposit an extracellular metachromatic matrix, and studies with labeled sulfate and glucosamine have demonstrated that they synthesize chondroitin surfate.[5,6]

Prolonged culturing, or treatment with the thymidine analog 5-bromodeoxyuridine (BUdR) transforms polygonal, sessile and smooth-surfaced chondrocytes synthesizing chondroitin sulfate into flat, ameboid cells with irregular surfaces that do not deposit chondroitin sulfate.[1,7–9] The progeny of chondrocytes that no longer express the chondrogenic phenotype, though reared under conditions that permitted chondrogenesis in their progenitor cells, have been termed *dedifferentiated* or *altered* chondrocytes.[1]

Recently, it has been shown that the activity of enzymes involved in the synthesis of chondroitin sulfate, the sulfokinase and UDPGNAC-4-epimerase systems, is less than 10% of normal in altered cells.[6] However, the activity of enzymes, such as alkaline phosphatase, cytochrome

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oxidase and β-glucuronidase, in these cells is similar to that of normal chondrocytes.

Prolonged exposure to the *in vitro* environment, or treatment with BUdR, alters the ability of chondrocytes to synthesize chondroitin sulfate without, however, interfering with their capacity for continued proliferation.[1,9] BUdR-altered chondrocytes and chondrocytes altered by protracted culturing appear to be morphologically similar.

In the present study, the fine structure of cultured chondrocytes has been examined and compared with the fine structure of intact vertebral cartilage. In general, the fine structure of chondrocytes grown as clones or pellets resembles that of chondrocytes *in vivo*. Significant alterations in the fine structure of chondrocytes are observed after prolonged culturing or treatment with BUdR. The correlation between structural alterations and the concurrent cessation of matrix synthesis are discussed.

Materials and Methods

Cultivation

Chondrocytes from vertebrae of 10-day chick embryos were liberated from their surrounding matrix by incubation for 2.5 hr in trypsin-collagenase mixture containing 0.1% trypsin, 1.4 mg/ml collagenase, 10% chick serum in calcium-magnesium-free Simm's balanced salt solution.[2] After disaggregation of the cells by flushing through a small-bore pipet, a dilute cell suspension was made in the nutrient medium. The medium used in this experiment was Hams F-10 with 2 times the specified concentration of amino acids and sodium pyruvate (Grand Island Biological Co.). This was supplemented with 10% fetal calf serum, 1% bovine serum albumin, 1.5 g/liter sodium bicarbonate and 30 units/ml penicillin-streptomycin. Single cells were cultured from this cell suspension to eliminate the overgrowth of chondrogenic cells by contaminant nonchondrogenic cells inadvertently included in culture. Procedures for establishing chondrogenic cultures with a single cell have been described.[9] In some experiments the primary cultures derived from single cells were dissociated and spun down into a pellet. After 24 hr of incubation in the bottom of a centrifuge tube, pellets were transferred to Millipore filter rafts (Millipore Corp.) and cultured in an organ culture dish for 2 days.

Material Examined by Electron Microscopy

The types of tissues examined included (1) normal 10-day chick embryo vertebral bodies prior to cultivation; (2) clonal cultures of chondrocytes grown, with or without exposure to culture medium containing 1 μg/ml of BUdR; (3) chondrocytes cultured as pellets; (4) BUdR-altered chondrocytes cultured as pellets; (5) clonal cultures of dedifferentiated chondrocytes that had been maintained *in vitro* for 1 month.

Cultures were fixed and examined at intervals after cultivation. Non-BUdR-treated clonal cultures were fixed for examination on Days 7, 8, 10, 11, 13 and 30 after cultivation. BUdR was added to clonal cultures on Days 4, 7 and 8. The

BUdR-treated cultures were fixed for examination at 8 hr and 2, 4 and 5 days after the addition of BUdR.

Preparation of Tissues for Electron Microscopy

The cultures were washed once in 4 C aqueous NaCl (0.9%), and then fixed for 90 min in 0 C cacodylate-buffered [10] 2.5% distilled glutaraldehyde (Polysciences Inc.) After fixation, the tissues were given three 30-min rinses in cacodylate buffer at 0 C, then postfixed at 0 C in veronal acetate-buffered osmium tetroxide,[11] and dehydrated in graded alcohols and propylene oxide. Tissues were embedded in a 4:1 mixture of Epon 812.

After fixation, pellets were cut into blocks approximately 1 mm in diameter and dehydrated and embedded. In some instances, larger colonies of chondrocytes were prefixed in Petri dish cultures, scraped free with stainless-steel or Teflon spatulas, and handled as separate tissue blocks. In other instances, both fixation and dehydration were done in the Petri dishes; the embedding was carried out *in situ* following the method of Zagury and Pappas.[12]

One-micron-thick sections were cut for light microscopy using glass knives. These sections were stained with toludine blue as previously described.[13] Thin sections, approximately 500 Å thick, were cut with du Pont diamond knives. All sectioning was carried out on an LKB Ultrotome III.

Thin sections were routinely stained for 5 min with a 3% aqueous solution of uranyl acetate followed by staining with lead citrate for 5 min.[14] Some sections were stained with 10% aqueous phosphotungstic acid (PTA) for 30 min to demonstrate collagen fibrils. Thin sections were examined and photographed with an RCA EMU-3G or Phillips EM-300 electron microscope.

Hyaluronidase Digestion of Cultures

Pellet cultures were fixed for 90 min prior to hyaluronidase digestion in 4% formaldehyde dissolved in Millonig's [15] phosphate buffer (pH 7.4). After fixation, the pellets were given three 30-min rinses in 0.1 M phosphate buffer (pH 7.4) at 4 C, and then incubated overnight at 37 C in 0.015% hyaluronidase (Sigma Type IV, 750 units/mg) dissolved in 0.1 M phosphate buffer (pH 5.5). Control cultures were incubated in similar buffer that did not contain hyaluronidase. After digestion, the pellets were given two 15-min rinses in 0.1 M phosphate buffer (pH 5.5) at 4 C, postosmicated and processed as described above for light and electron microscopy.

Results

Ten-Day-Old Chick Embryo Vertebral Cartilage

Light microscopic examination showed typical chondrocytes separated by a metachromatic matrix. A similar overall appearance was demonstrated by electron microscopy (Fig 1). The cells were ovoid with occasional microvillous projections. The nuclei were also ovoid, not deeply indented and contained 1–3 nucleoli of moderate size. Mitoses were rarely observed. The moderate amount of cytoplasm (Fig 2) contained prominent rough endoplasmic reticulum and Golgi complexes, rounded or ovoid mitochondria, plus occasional lipid droplets, microtubules and cytoplasmic filaments.

The intercellular matrix was composed of numerous intertwining collagen fibrils measuring approximately 180 Å in width (Fig 3 and 4), plus numerous dense, ovoid or spindle-shaped matrix granules (Fig 3). Such granules have been shown to contain trypsin-digestible protein [16] and hyaluronidase-digestible polysaccharide.[16,17] In embryonic vertebrae, the matrix granules were irregularly shaped (Fig 3) and demonstrated a maximum dimension of approximately 500 Å. Often granules were attached, either to collagen fibrils, or to delicate intergranular fibrils of variable thickness (usually less than 50Å). The latter showed no evidence of banding and no selective PTA staining (Fig 3). Collagen fibrils were recognized by their strong affinity for PTA (Fig 4) but did not demonstrate a definite 640-Å banding pattern.

Fine Structure of Cultured Chondrocytes

In general, the fine structure of chondrocytes cultured as clones and pellets (Fig 5 and 6) closely resembled the fine structure of embryonic vertebral chondrocytes. Light microscopy showed that in both pellet cultures and central areas of clonal colonies, the chondrocytes were rounded or ovoid, with abundant cytoplasm and in most instances were separated from one another by a metachromatic matrix. The maximum dimension of cultured chondrocytes was somewhat larger than that of vertebral chondrocytes (compare Fig 5 and 1). At the edges of cultures, where matrix was minimal, the cells were more irregularly shaped and flattened.

Electron microscopy of both pellets and clones revealed chondrocytes with cytoplasm containing abundant rough endoplasmic reticulum and prominent Golgi complexes (Fig 5 and 6). Nuclei were round or ovoid and contained one or two large nucleoli. Nuclear pores were observed occasionally. Glycogen was present within the cytoplasm in the form of irregular particles similar to those seen in embryonic vertebral chondrocytes (Fig 2), but it was less clumped and more diffusely distributed. Membrane-bounded intracytoplasmic dense bodies (Fig 9), microtubules and cytoplasmic filaments were seen occasionally, but they were much more prominent after exposure to BUdR, as described below. Pelleted chondrocytes contained more numerous intracytoplasmic, lipid droplets than did chondrocytes in clonal cultures.

Matrix in Chondrogenic Cultures

As in intact vertebral cartilage, a prominent feature of both pellet and clonal aggregates was the presence of numerous electron-dense matrix granules in the intercellular substance (Fig 7). The maximum

dimension of granules was similar in clonal and pellet cultures—approximately 400 Å.

Overnight incubation in hyaluronidase removed all recognizable matrix granules and intergranular fibrils. Occasionally, electron-dense debris was seen clinging to collagen fibrils. In control cultures, incubated overnight in buffer, matrix granules were well preserved.

Collagen fibrils, recognizable by their affinity for PTA, were considerably reduced in number in chondrogenic cultures. In pellets, occasional clumps of fibrils were noted (Fig 8). They measured approximately 200 Å in width and did not demonstrate a pattern of cross-banding (Fig 8). These PTA-staining fibrils were rarely observed in clonal cultures.

Linear extracellular fibrils, measuring approximately 100 Å in width, which did not demonstrate a marked affinity for PTA or a recognizable periodicity, were infrequently seen in matrices of pellets and clones. This type of fibril was much more prevalent in BUdR-treated cultures (Fig 11) and in 1-month-old cultures.

BUdR-treated Cells and Matrix

First-passage clonal cultures were treated on Day 8 with 1 μg BUdR/ml. At this time there was matrix present. After 48 hr, a marked flattening of cells and nuclei was apparent. The cells assumed irregular shapes and had long pseudopodial processes as viewed by light microscopy.

The most striking structural alteration in the cytoplasm in response to BUdR treatment was the drastic reduction in rough endoplasmic reticulum and Golgi apparatus (Fig 9). Coupled with this reduction was an apparent increase in free ribosomes, seemingly unattached to membranes but frequently arranged as rosettes or in spiral configurations (Fig 9 and 10). Another prominent feature of the cytoplasm was the presence of a substantial number of cytoplasmic filaments (Fig 9 and 10). These filaments were approximately 100 Å in diameter (Fig 10) and were often arranged in bundles which coursed throughout the cytoplasm, forming an interlacing network (Fig 9). Microtubules also were observed frequently (Fig 10). Mitochondria appeared more elongate and branched than in comparable untreated cultures, but this may have been due to a more favorable plane of section caused by the extreme flattening of the cells. In BUdR-treated chondrocytes, the areal dimensions of the cytoplasm, nuclei and nucleoli may be increased three- to ten-fold compared to those in normal chondrocytes.[7] At present it is not known whether this reflects an increase in volume or whether it is only due to extreme flattening of the cell.

The extracellular matrix of BUdR-treated colonies contained many 100-Å linear fibrils and few matrix granules (Fig 11). Collagen fibrils were not identified. The linear, nonperiodic, 100-Å fibrils appeared, at times, to be continuous with cytoplasmic filaments of the same dimension across the plasma membrane. In cultures exposed to BUdR from Day 4, matrix granules never appeared. When BUdR was added on Days 7 or 8 of cultivation, some matrix granules persisted. Examination of the latter cultures at 2–5 days after the addition of BUdR revealed a marked reduction in the number of matrix granules (compared with non-BUdR-treated control cultures of equal age), and often the BUdR-treated granules appeared less plump (compare Fig 7 and Fig 11).

Normal chondrocytes grown as pellets formed adherent aggregates and synthesized cartilage matrix. BUdR-treated chondrocytes grown as pellets did not adhere to each other, did not synthesize a cartilage matrix and proceeded to undergo degeneration under these conditions. Electron microscopic examination of the pellets of BUdR-altered cells revealed extensive necrosis. All of the cells appeared swollen and vacuolated with complete or partial disruption of the plasma membrane and no extracellular matrix. Although a pellet environment is not conducive to the survival of BUdR-altered cells, such cells grow for several generations if cultured at high density as a monolayer.[4]

Prolonged Cultivation

Chondrocytes maintained in monolayer culture for 1 month showed flattening and cytoplasmic and nuclear alterations which were indistinguishable from those seen in the BUdR-treated cells. The intercellular substance contained numerous 100-Å linear fibrils, a few PTA-stained collagen fibrils and no matrix granules.

Discussion

Characteristics of Cartilage In Vitro

The fine structure of functioning chondrocytes in clones and pellets *in vitro* is similar to chondrocytes *in vivo*.[18] The cells have the same polygonal shape, sparse cell contacts and prominent rough endoplasmic reticulum and Golgi complexes.

The matrix of cultured cartilage is metachromatic [1] when stained with toluidine blue, indicating the presence of acid proteinpolysaccharides. The electron microscopic examination of cultured cartilage supports the view that the matrix proteinpolysaccharide is associated

with discrete granules, which are hyaluronidase digestible. These granules are a constant and characteristic feature of many cartilage matrices including limb bud,[16,19,20] growing epiphyseal plate,[17,21,22] bovine nasal cartilage [23] and induced cartilage.[13] Recently they were identified in cultures of reaggregating chick embryo chondrocytes.[24]

As stated above, they are digestible with trypsin [16] and hyaluronidase,[16,17] and it has been shown that they are partly digestible with papain.[25] Matrix granules are also extractable with dissociating solvents that selectively dissolve proteinpolysaccharides.[23] Thus, it is likely that matrix granules represent proteinpolysaccharide in cultured cartilage matrix, as they do *in vivo*.

Collagen in Cultured Cartilage

The matrix of chondrocyte clones and pellets differs from that of embryonic vertebrae in that the number of PTA-stainable collagen fibrils is reduced. This finding is in good agreement with the fact that the medium (F-10) used in these experiments does not favor the synthesis of collagen.[26] However, the ability of the chondrocytes to proliferate and to synthesize chondroitin sulfate does not seem to be affected when they are cloned in this medium. In contrast to F-10, a medium containing embryo extract (2 parts balanced salt solution, 2 parts horse serum, and 1 part embryo extract—2:2:1) favors the synthesis of collagen by the chondrocytes. The collagen synthesized by cells grown in 2:2:1, as estimated by the conversion of ^{14}C-proline to ^{14}C-hydroxyproline, is 3–5 times more than that synthesized by the cells reared in F-10.[26] One possible explanation for the reduced collagen in the matrix is that the medium used in these experiments was lacking in one or more factors necessary for collagen synthesis (—*eg*, ascorbic acid). It is interesting to emphasize, in this regard, that chondroitin sulfate and collagen, two intimately associated components of cartilage matrix, apparently can be synthesized to some degree independently of each other. The absence of banding from very small collagen fibrils, as seen in the present material, has been reported in other cartilages[13] and possibly could result from masking of fibrils by amorphous mucosubstances, as suggested by van den Hoof.[27]

Matrix granules appear to be linked together despite the reduced number of collagen fibrils in the cultured cartilage. Linkage was often effected by extremely delicate intergranular fibrils of variable width, usually less than 50 Å. Similar fibrils have been described in association with matrix granules in embryonic cartilage,[16,20] in induced cartilage,[13] and in lacunae of epiphyseal cartilage.[17] The fact that they are removed

by hyaluronidase in cultured cartilage and in epiphyseal plate,[17] and by dissociating solvents in bovine nasal cartilage[23] would support the idea that they are noncollagenous.

Effect of BUdR and Prolonged Cultivation

BUdR and prolonged cultivation cause chondrocytes to become flattened and to increase in surface area.[3,9,28] Electron microscopic inspection suggests that there is an increase in cytoplasm and nucleoplasm. Careful measurements of cell volume will be required to document this point.

Linear fibrils of uniform approximately 100-Å width, without apparent cross-banding or special affinity for PTA, are prominent in the matrix of cultured cartilage after BUdR treatment or prolonged cultivation. It is possible that these fibrils represent cytoplasmic filaments of chondrocytes extruded or shed into the matrix. Cytoplasmic filaments occasionally appear to be in continuity with linear matrix fibrils across the plasma membrane. However, such an appearance of continuity may be produced artifactually by tangential sectioning.[29] Further studies involving sections cut in various planes are necessary to clarify this point.

Filaments similar to the 100-Å cytoplasmic filaments observed in the altered chondrocytes have been observed in the cytoplasm of many other cultured cells, as well as in normal cells. These filaments have been implicated in maintaining cell rigidity and in playing a role in cell movement.[30] It is interesting to note that an increase in cytoplasmic filaments correlates with the initiation of ameboid activity and cell extension in BUdR-altered or dedifferentiated chondrocytes.

The presence of a well-developed ergastoplasm and Golgi complex has been correlated with synthesis and secretion of proteins for export.[31,32] Also, the Golgi complex has been shown to be an important site of synthesis [33] and sulfation [34,35] of proteinpolysaccharides in chondrocytes. Thus, it is not surprising that cells that are inactive in the synthesis and secretion of proteinpolysaccharide should be lacking in the development of ergastoplasm and Golgi complexes. The disappearance of matrix granules after BUdR treatment is further structural evidence of diminished porteinpolysaccharide synthesis.

BUdR reversibly inhibits the synthesis of myosin in myoblasts,[36–38] hyaluronic acid in amnion cells,[39] pigment in retinal cells,[40] and chondroitin sulfate in chondrocytes.[3,41] BUdR-suppressed cells exhibit an increase in cell surface and in ameboid activity. That this curious effect acts selectively upon cell-specific molecules is shown by the fact

that cell division, mitosis, alkaline phosphatase and β-glucuronidase activity, and absorption spectra of several cytochromes are unaffected in BUdR-suppressed cells.[3,6]

The effect of BUdR on a variety of cell types suggests that a more general on-off switch, common to several different kinds of cells, may control the synthesis of cell-specific molecules. For example, little is known about how cells assign priorities to synthetic activities, but one mechanism might involve the cycling of endoplasmic reticulum (ER), Golgi and plasma membranes. Under conditions in which the cell surface is expanded, the membrane *stock* of the cell might be tied up primarily in plasma membrane. Endoplasmic reticulum and Golgi membranes would be reduced. In the absence of sufficient ER and Golgi, cell-specific molecule syntheses in a variety of cell types would be prevented. Thus, rather than directly interfering with the transcription for chondroitin sulfate, myosin or hyaluronic acid, it is possible that BUdR may act indirectly by initially interfering with the production of some common surface molecule [3,9] whose loss or abnormal composition would result in plasma membrane expansion, loss of ER and Golgi, and subsequent cessation of cell-specific molecule synthesis.

Summary

The fine structure of cultured chicken embryo cartilage has been studied and compared to that of 10-day-old embryo vertebral cartilage *in vivo*. Pellet and clonal cultures closely resemble normal vertebral cartilage. The cultured chondrocytes have a rounded or polygonal shape and are separated by an extracellular matrix; their cytoplasm contains numerous profiles of rough endoplasmic reticulum and prominent Golgi complexes. The intercellular matrix of cultured cartilage contains numerous proteinpolysaccharide matrix granules as does the normal vertebral cartilage. Collagen fibrils, which were recognized by their affinity for phosphotungstic acid, are less abundant in chondrocyte cultures than in intact vertebral cartilage.

The effects on chondrocyte fine structure of prolonged cultivation or treatment with the thymidine analog, 5-bromodeoxyuridine (BUdR), have been examined. Both procedures change the polygonal chondrocytes into flattened *fibroblast-like* amoeboid cells. These altered chondrocytes do not deposit a metachromatic extracellular matrix. Rough endoplasmic reticulum and Golgi complexes are less prominent, while free ribosomes, microtubules and cytoplasmic filaments are more numerous in the altered chondrocytes. Matrix granules are greatly reduced in size and number, or are completely absent after BUdR in-

corporation or prolonged cultivation. The correlation between fine structural alterations and known biochemical alterations caused by BUdR or prolonged cultivation is discussed.

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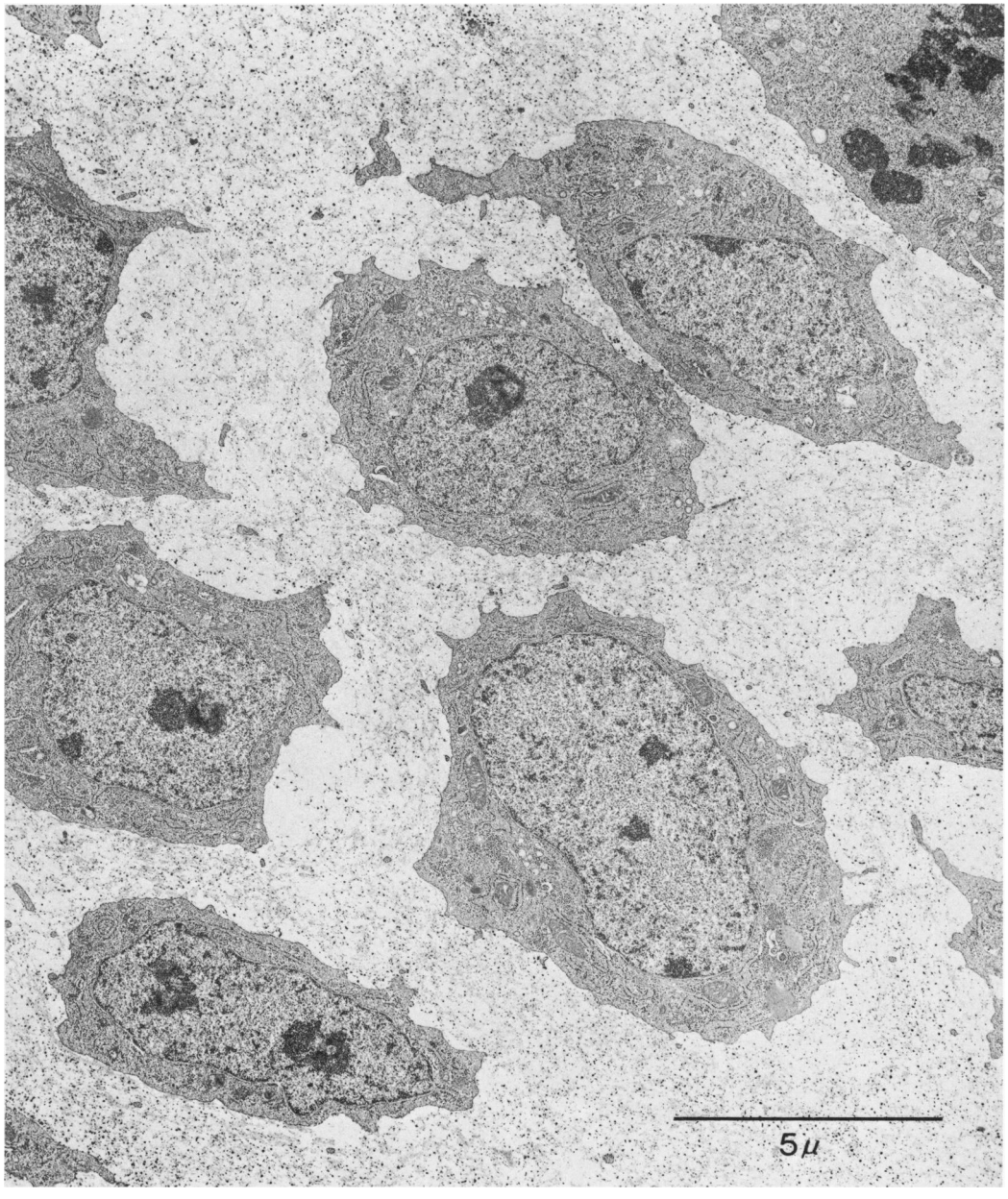
Legends for Figures

Fig 1. Chondrocytes and matrix of 10-day-old chicken embryo vertebra. Cytoplasm is not abundant, but contains notable amounts of rough endoplasmic reticulum and Golgi apparatus. Chondrocyte is seen in mitosis at upper right. Matrix contains numerous, tiny, electron-dense matrix granules. Lead citrate and uranyl acetate. × 7300.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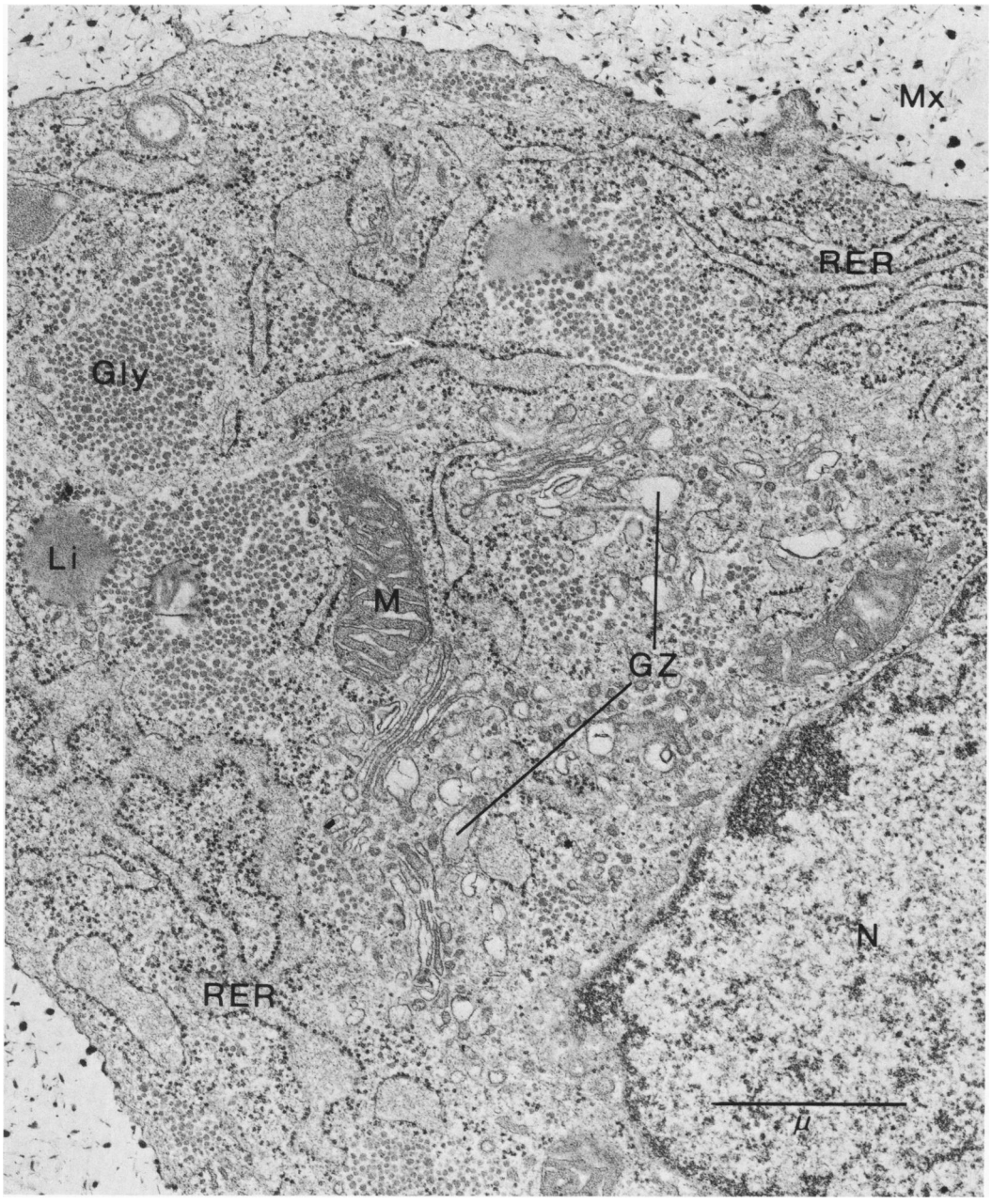


Fig 2. Cytoplasm and nucleus (*N*) of 10-day-old chicken embryo vertebral chondrocyte. Cytoplasm contains prominent rough endoplasmic reticulum (*RER*), large Golgi zone (*GZ*), ovoid mitochondria (*M*), clusters of glycogen particles (*Gly*) and scattered lipid droplets (*Li*). Matrix (*Mx*) contains faintly staining collagen fibrils and numerous, dense matrix granules. Lead citrate and uranyl acetate. × 27,200.

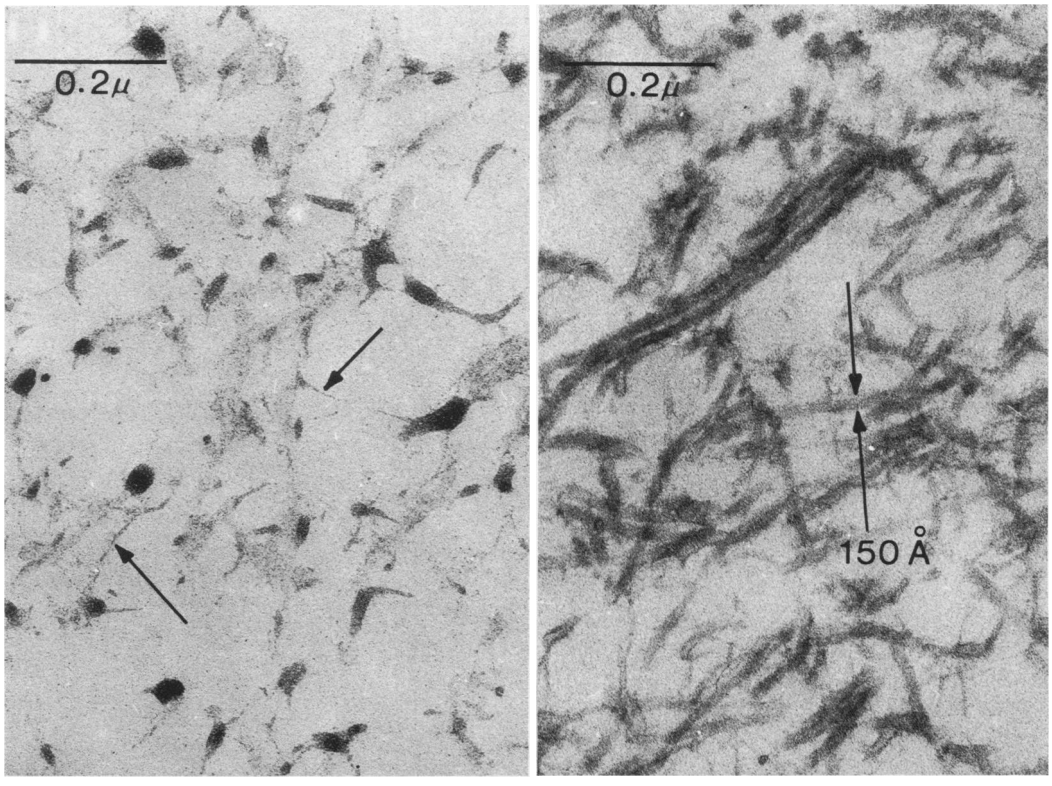
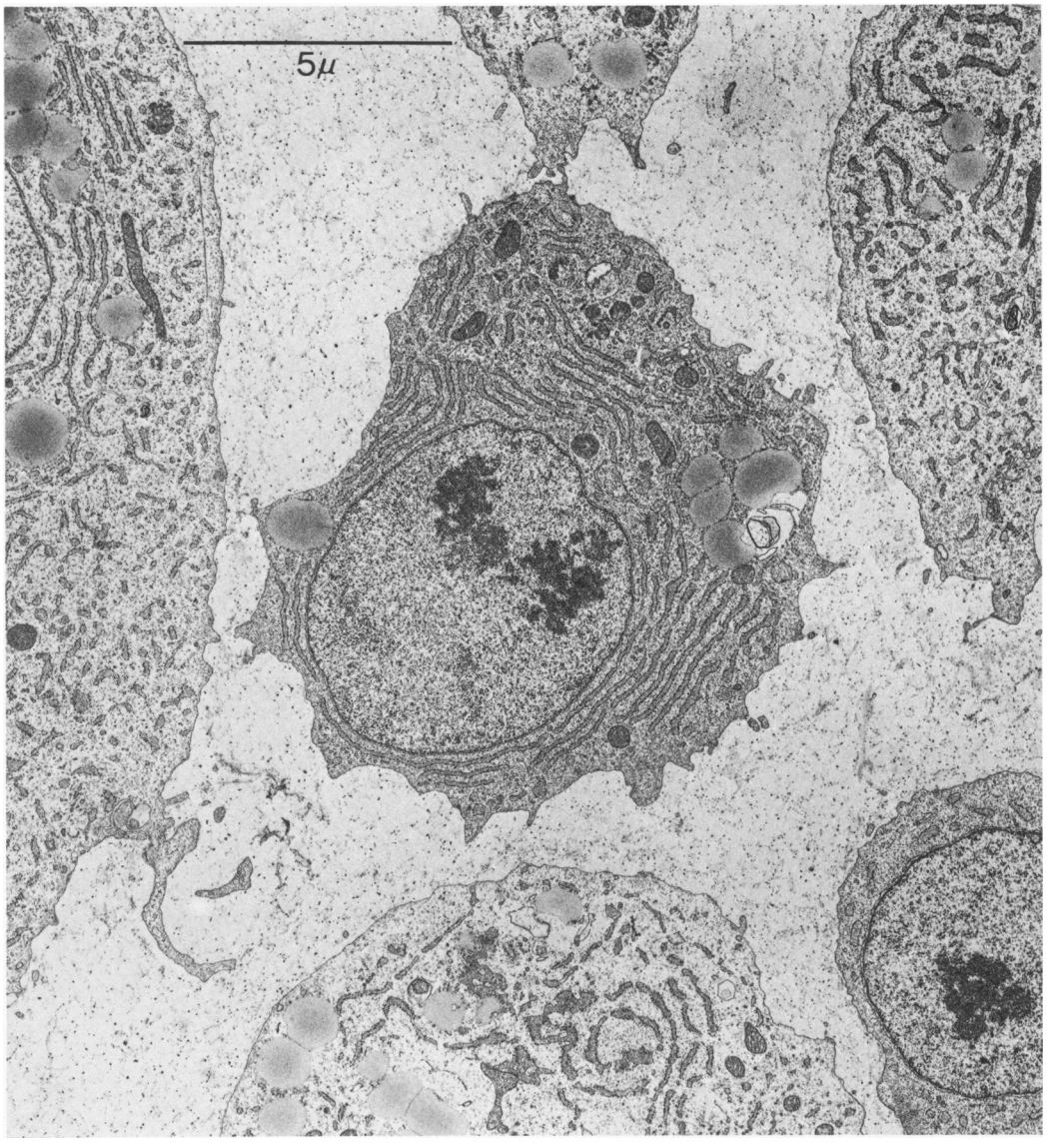


Fig 3. (*Left*) Matrix of chicken embryo vertebra, stained with lead citrate and uranyl acetate. Matrix granules, which appear irregular and very dense after lead staining, are linked to collagen fibrils, which are poorly discerned as hazy irregular shapes, or to delicate, nonbanded, intergranular fibrils of irregular thickness, usually less than 50 A, two of which are indicated by *arrows*. × 100,300.

Fig 4. (*Right*) Matrix of chicken embryo vertebra (comparable to Fig 3) stained with PTA. Collagen fibrils, measuring approximately 150 A in thickness, are selectively stained, while matrix granules are not apparent. Unequivocal cross-banding of collagen fibrils is not observed. × 100,300.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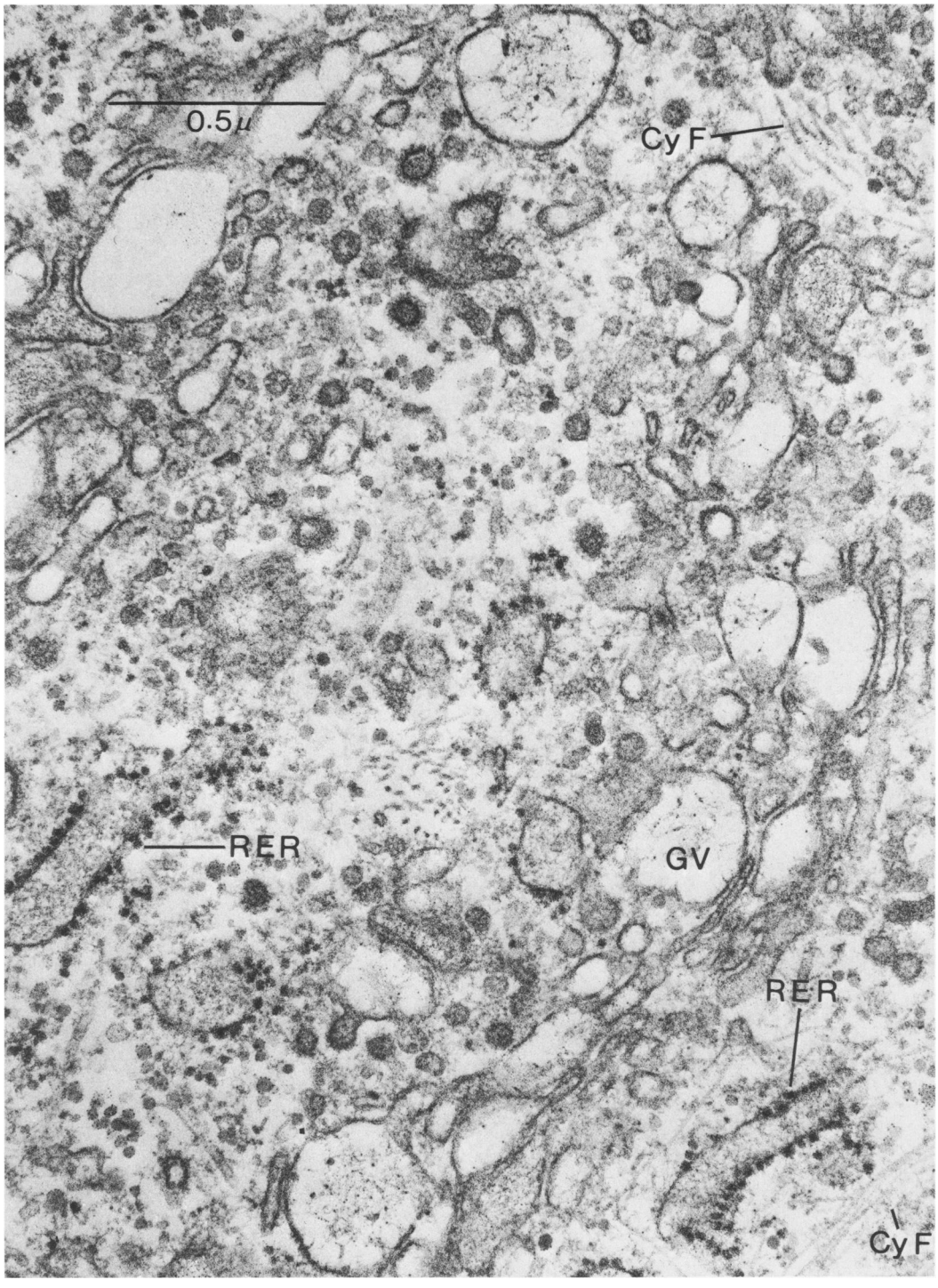
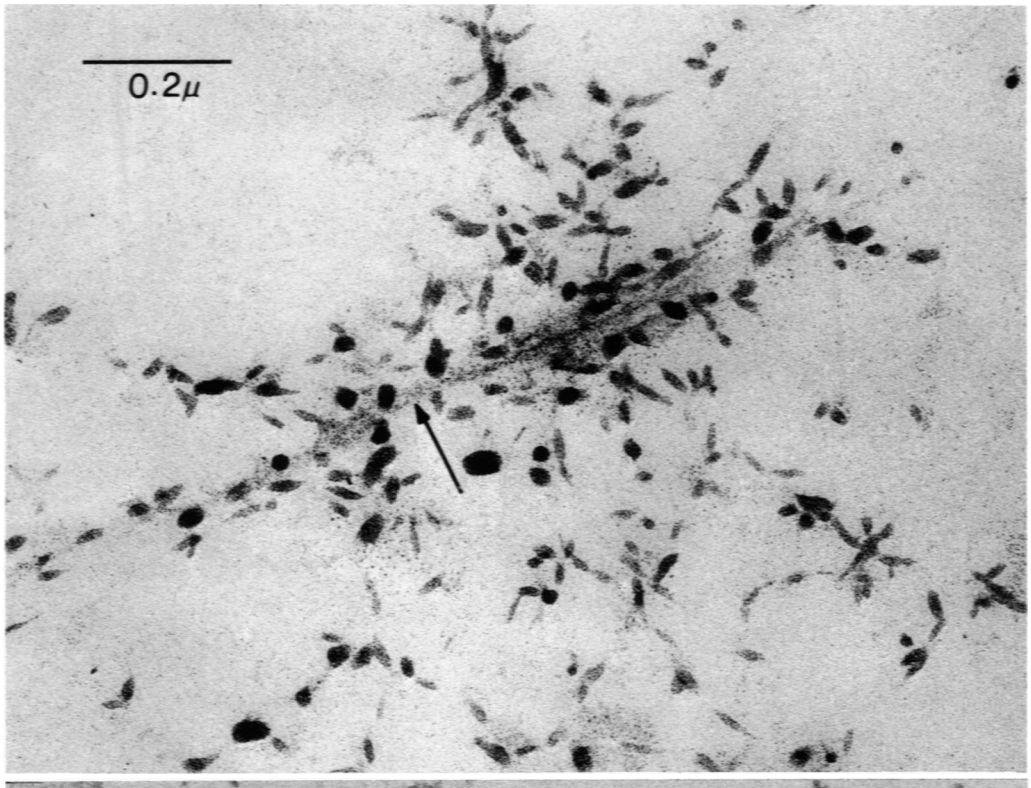


Fig 6. Cytoplasm of cultured chondrocyte containing well-developed Golgi complex composed of vesicles and lamellae arranged as inverted U. Several dilated Golgi vesicles (*GV*) enclose tiny fibrils and granules, as is often case *in vivo* for chondrocytes. Cysternae of rough endoplasmic reticulum (*RER*) are seen as well as a few cystoplasmic filaments (*CyF*) at upper and lower right. Lead citrate and uranyl acetate. × 63,000.

Fig. 7. (*Top*) Matrix of cultured cartilage containing ovoid, dense matrix granules which measure approximately 400 A in maximum dimension, and are attached to one or two nonbanded fibrils, measuring approximately 150 A. The latter fibrils (*arrow*) traverse field diagonally. Most of matrix granules appear to be linked to form a reticulum (see Fig 3). However, fine, linking intergranular fibrils are not well demonstrated here. Lead citrate and uranyl acetate. × 100,300.

Fig 8. (*Bottom*) PTA-stained collagen fibrils in matrix of pelleted chondrocyte culture. Fibrils are arranged in bundle, as is often the case in cultures. Portion of plasma membrane and cytoplasm of chondrocyte is seen at left. These collagen fibrils measure approximately 220 A in thickness. As with embryonic vertebra (Fig 4), matrix granules in cultured cartilage are inapparent when stained with PTA. × 63,000.


0.2μ

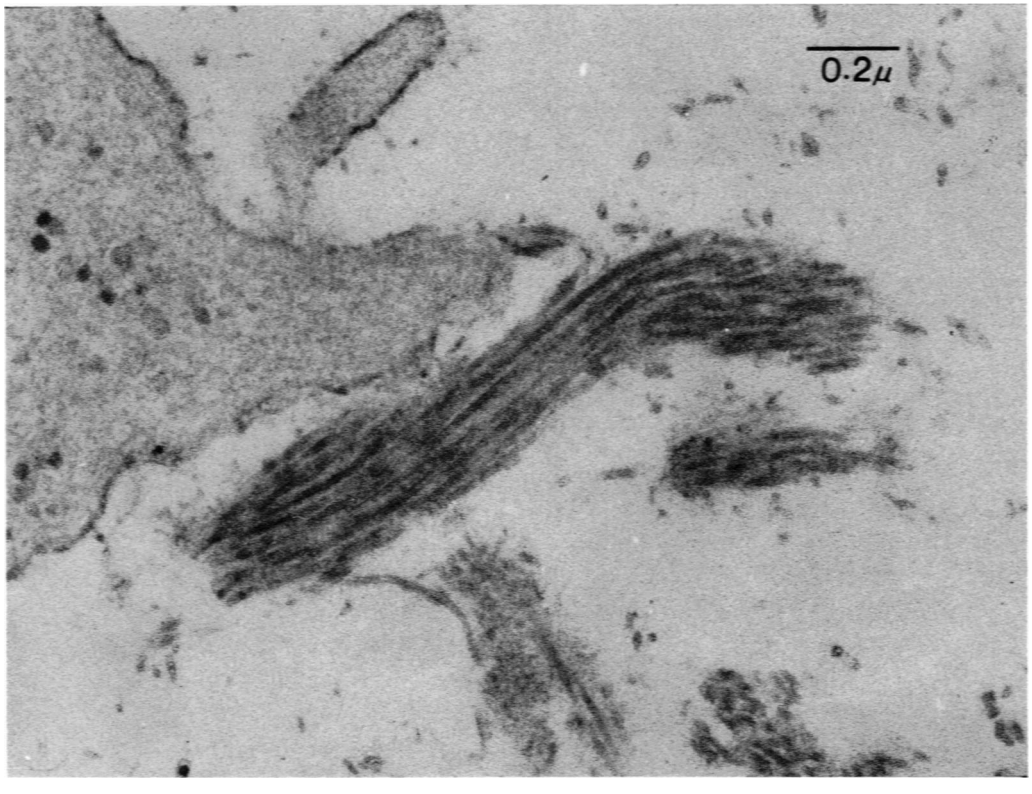

0.2μ

Fig 9. Chondrocytes, cultured for 7 days and then exposed to BUdR for 4 days. Cells and nuclei (*N*) are markedly enlarged. (Compare with Fig 1 and 5, which are equally magnified.) Cytoplasm contains numerous, nonmembrane-bounded ribosomes as well as cytoplasmic filaments (*CyF*) and elongated, sometimes branching, mitochondria (*M*). Intracytoplasmic dense bodies (*DB*) are present, as is often the case. Nuclei (*N*) vary considerably in shape, and present many nuclear pores (*NP*) at tangentially sectioned surfaces. Intercellular matrix (*Mx*) is sparse, filamentous, and contains very few electron-dense granules. Lead citrate and uranyl acetate. × 7300.

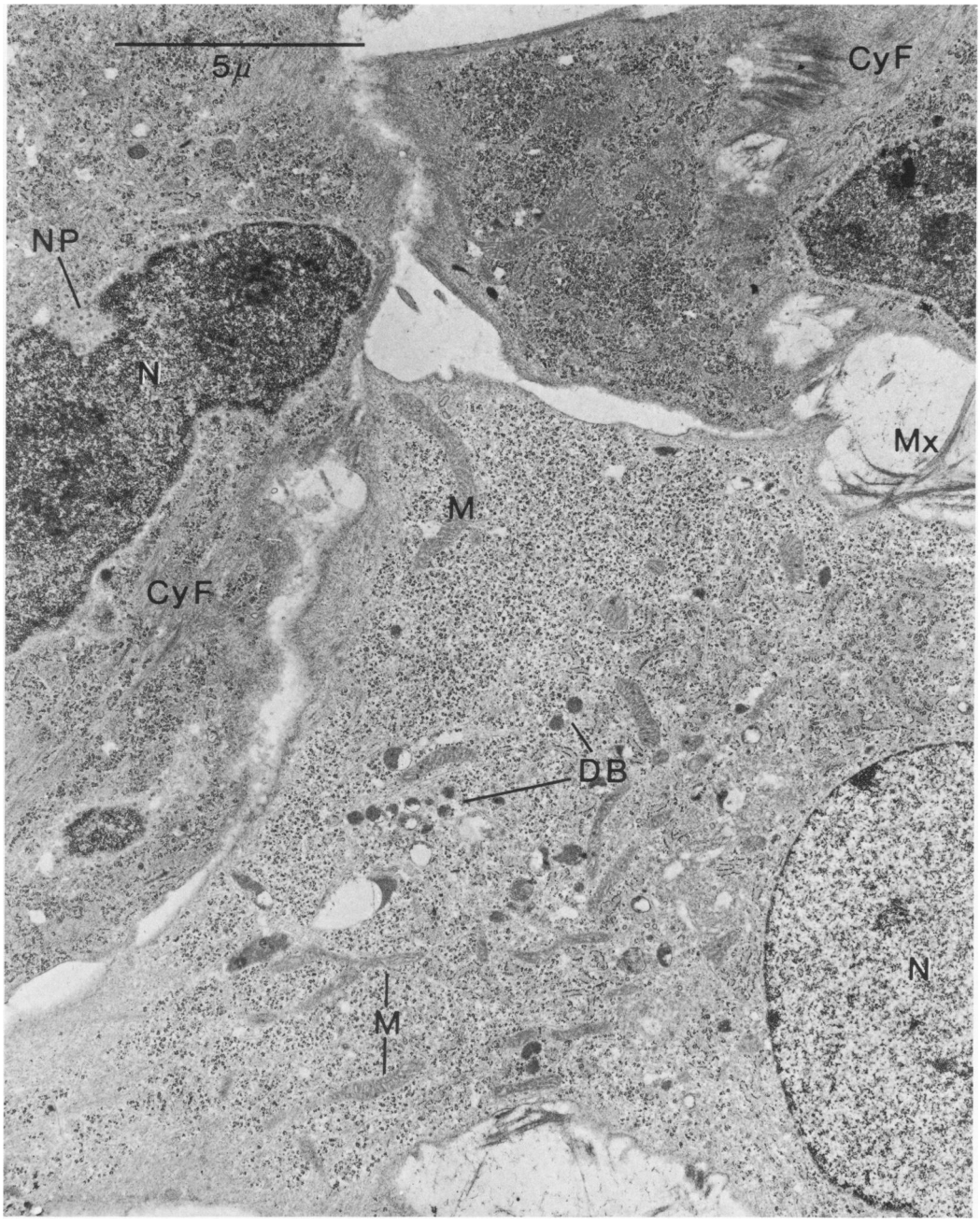
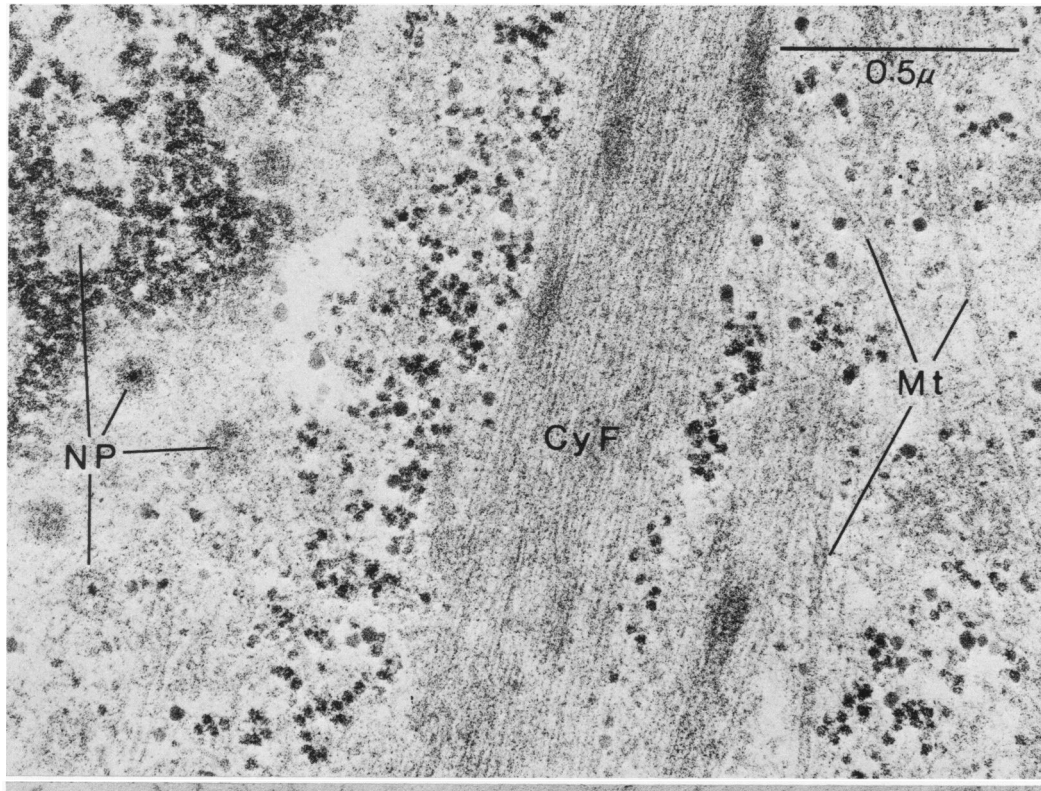
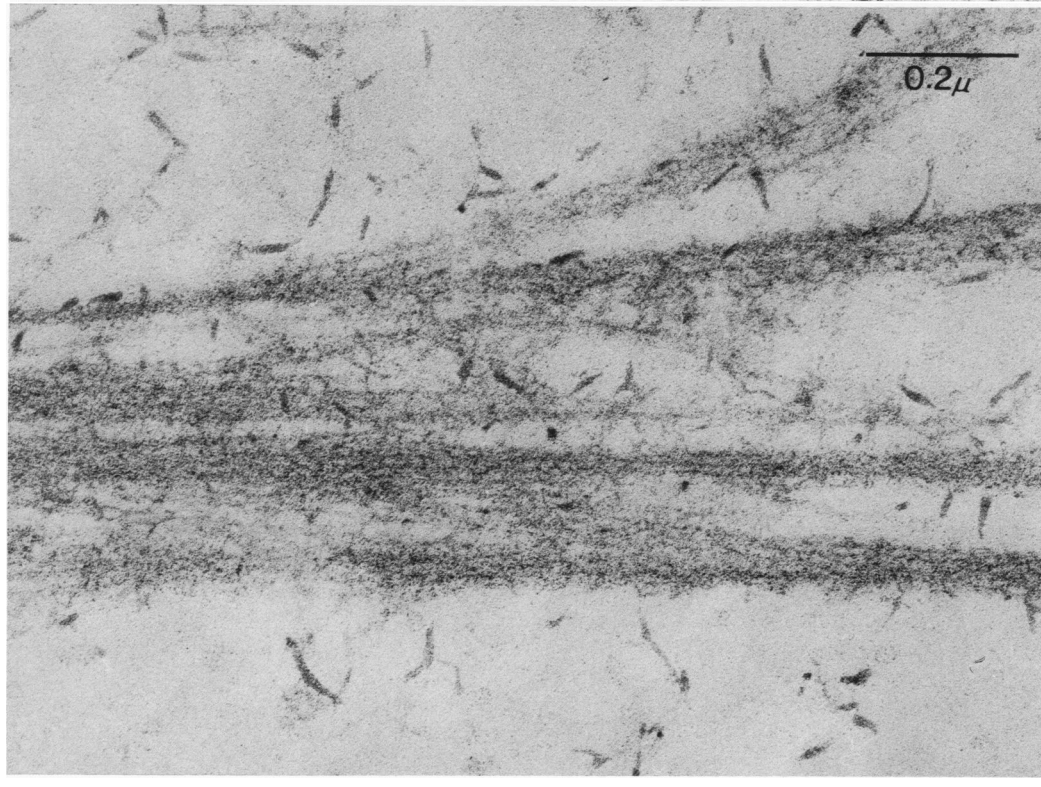

5μ
CyF
NP
N
Mx
M
CyF
DB
N
M

Fig 10. (*Top*) Detail of cytoplasm of BUdR-treated chondrocyte. Numerous cytoplasmic filaments (*CyF*), and free ribosomes are seen, as well as a few microtubules (*Mt*) and nuclear pores (*NP*) associated with tangentially sectioned nucleus at upper left. Lead citrate and uranyl acetate. × 67,200.

Fig 11. (*Bottom*) Matrix of chondrocyte culture after 4 days' exposure to BUdR. Linear, nonbanded fibrils, measuring approximately 100 A, predominate and are associated with a few probable, matrix granules, which appear less dense and less plump than in non-BUdR-treated cultures (Fig 7), or intact vertebra (Fig 3). Lead citrate and uranyl acetate. × 100,300.


0.5μ
NP
CyF
Mt


0.2μ

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