# Induction and Prevention of Chondrocyte Hypertrophy in Culture

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*Abstract.* Primary chondrocytes from whole chick embryo sterna can be maintained in suspension culture stabilized with agarose for extended periods of time. In the absence of FBS, the cells remain viable only when seeded at high densities. They do not proliferate at a high rate but they deposit extracellular matrix with fibrils resembling those of authentic embryonic cartilage in their appearance and collagen composition. The cells exhibit many morphological and biochemical characteristics of resting chondrocytes and they do not produce collagen X, a marker for hypertrophic cartilage undergoing endochondral ossification. At low density, cells survive in culture without FBS when the media are conditioned by chondrocytes grown at high density. Thus, resting cartilage cells in agarose cultures can produce factors required for their own viability.

Addition of FBS to the culture media leads to profound changes in the phenotype of chondrocytes seeded at low density. Cells form colonies at a high rate and assume properties of hypertrophic cells, including the synthesis of collagen X. They extensively deposit extracellular matrix resembling more closely that of adult rather than embryonic cartilage.

THE phenotype of cartilage cells is subject to extensive modulation in situ as well as in tissue culture (Kosher, 1983). In cartilage, cell differentiation is a multistep process starting from chondrogenic mesenchymal cells and eventually resulting in mature, hypertrophic chondrocytes with high metabolic activity. Usually, that stage is reached only in cartilage undergoing endochondral ossification during development, growth, or repair of bones. In permanent cartilage, e.g., normal articular cartilage close to the joint surface, terminal chondrocyte differentiation does not take place but is interrupted at the stage of resting chondrocytes. These cells display only moderate metabolic activity sufficient for normal tissue turnover.

Chondrocyte differentiation is accompanied by a switch of gene activation. Mesenchymal cells produce collagens I and III, whereas chondrocytes synthesize cartilage specific proteoglycans and collagens II, IX, and XI. Hypertrophic chondrocytes also produce collagen X, a biochemical marker for this stage of cell differentiation (Schmid and Conrad, 1982; Gibson et al., 1982; Capasso et al., 1984; Schmid and Linsenmayer, 1985; Solursh et al., 1986).

In tissue culture, chondrocytes can differentiate from mesenchymal cells (Solursh, 1982), a process inhibited by viral transformation (Adams et al., 1982; Allebach et al., 1985; Finer et al., 1985), or by retinoic acid (Benya et al., 1988) or 5-bromodeoxyuridine (Abbot and Holtzer, 1968; Mayne et al., 1975). On the other hand, chondrocyte differentiation is induced by transforming growth factors $\beta$ (Seyedin et al., 1985, 1987), or pharmacological agents disrupting cytoskeletal structures (Zanetti and Solursh, 1984; Benya et al., 1988). The significance of appropriate cell–matrix interactions or other exogenous factors has also been emphasized (Solursh et al., 1984, 1986). The differentiation of chondrocytes from mesenchymal cells is reversible with respect to morphological criteria and to the production of specific matrix macromolecules (von der Mark et al., 1977; von der Mark, 1980). The mesenchymal phenotype is expressed by chondrocytes in monolayer culture, particularly upon prolonged culture (Mayne et al., 1976) or upon repeated passage (Benya and Brown, 1986) whereas the chondrocytic phenotype is stabilized in cell suspensions in agarose (Horwitz and Dorfman, 1970; Benya and Shaffer, 1982) or collagen gels (Gibson et al., 1984). Chondrocytes in suspension can be maintained in the resting state for a limited period of time if they are derived from resting cartilage (McClure et al., 1988) but they eventually become hypertrophic cells, irrespective of their stage of differentiation at the time of removal from the tissue (Tacchetti et al., 1987; Castagnola et al., 1988).

In superficial zones of articular cartilage, resting cells maintain their phenotype permanently. However, these cells, too, can proliferate and even become hypertrophied but this process usually is associated with pathological cartilage degeneration, such as osteoarthritis (Sokoloff, 1979). The exact circumstances which promote chondrocyte hypertrophy still are ill defined and culture conditions for permanent maintenance of resting chondrocytes have not yet been reported. Here, we describe such conditions. We provide evidence for the importance of chondrocyte product(s) for the viability of resting chondrocytes. Serum factor(s) can promote hypertrophy in chondrocytes.

## Materials and Methods

### Isolation of Chondrocytes

Sternal cartilages were explanted under sterile conditions from 17-d-old chick embryos. 15 sterna at a time were incubated in 60-mm Petri dishes in 5 ml of DME containing 1 mg/ml of chymotrypsin (Serva, Heidelberg, FRG) and 2 mg/ml of collagenase (grade CLS; Worthington Biochemical Corp., Freehold, NJ). After 30 min, the sterna were washed with fresh DME and were incubated for 12 h in 5 ml of DME containing 2 mg/ml of collagenase. The tissue fragments were disintegrated mechanically by repeated aspiration with a pipet and matrix free cells were passed through three layers of 100 $\mu$m pore-size Nylon tissue. Cells were centrifuged at 250 $g$ for 5 min, washed with PBS containing 2.5 mM KCl, passed through three layers of 20 $\mu$m pore-size Nylon tissue, recentrifuged, and suspended in an appropriate volume of DME.

### Agarose Cultures

Agarose cultures were carried out either at cell densities of 1.5–2.5 $\times$ $10^6$ cells/ml (low) of agarose gel in the presence of 10% FBS as described by Benya and Shaffer (1982) or in the absence of FBS at cell densities of 1.1–2.5 $\times$ $10^7$ cells/ml (high). In some experiments, they were grown at 1.5 $\times$ $10^6$ cells/ml without FBS but in the presence of a medium containing 1/4 vol of DME conditioned by agarose cultures at high cell density. The media contained 50 $\mu$g/ml, each, of $\beta$-aminopropionitrile fumarate and ascorbate (Fluka AG, Buchs, Switzerland) and were replaced after 2–3 d over periods of up to 6 mo.

### Determination of Cell Numbers and Thymidine Incorporation

After various culture periods, agarose cultures in 35-mm dishes were exposed for 24 h to media containing 5 $\mu$Ci/ml of [methyl-$^3$H]thymidine (Amersham International, Amersham, UK; 74 Ci/mmol). Whole cultures were then supplemented with 1/10 vol of 0.25 M Hepes containing 1% Triton X-100 and 10 mg/ml of pronase (Boehringer Mannheim GmbH, Mannheim, FRG) and were incubated for 24 h at 55°C. After sonication for 1 min, agarose was removed by centrifugation and the reaction mixtures were dialyzed against distilled water. Aliquots were analyzed for nondialyzable radioactivity by liquid scintillation counting and the DNA content was determined by the fluorimetric assay of Labarca and Paigen (1980). The assay was calibrated with suspensions of chondrocytes in DME at various dilutions and with freshly prepared agarose cultures containing known amounts of cells.

### Synthesis of Collagens and Proteoglycans

Parallel 35-mm culture dishes were labeled either with 1 $\mu$Ci/ml of [U-$^{14}$C]proline (Amersham International; 285 mCi/mmol) or with 2.5 $\mu$Ci/ml of $^{35}SO_4$ (Amersham International; 1,209 Ci/mmol) for 24 h.

Proline-labeled cultures were frozen, thawed, and treated with 10 ml of 0.5 M acetic acid, containing 0.2 M NaCl and 1 mg/ml of pepsin (Serva) for 48 h at 4°C. Samples were neutralized by addition of NaOH, adjusted to 1 M in $Na^+$-ion concentration by addition of solid NaCl, and extracted for 24 h at 4°C. Agarose was removed by centrifugation, the collagens were precipitated with 176 mg/ml of $(NH_4)_2SO_4$, washed twice with 2 ml of a mixture of 3 vol of ethanol and 1 vol of 0.4 M NaCl, 0.1 M Tris-HCl, pH 7.4, and dissolved in 0.2 ml 0.5 M Tris HCl, pH 6.8, containing 2% SDS. Aliquots were counted by liquid scintillation or analyzed by SDS-PAGE on 4.5–15% gradient gels, followed by staining with Coomassie blue or by fluorography.

Sulfate-labeled cultures were extracted with 10 ml of 4 M guanidine hydrochloride, 10 mM EDTA, 50 mM sodium acetate, pH 6.5, overnight at 4°C. After removal of the agarose by centrifugation, the samples were exhaustively dialyzed against distilled water and nondialyzable radioactivity was determined by liquid scintillation counting.

### Light and Electron Microscopy

Cultures were directly photographed in a phase contrast light microscope. Pellets of agarose cultures were excised with a 6-mm skin biopsy punch, fixed in 2% phosphate-buffered glutaraldehyde at pH 7.4, dehydrated in graded ethanol and embedded in Epon. Semithin sections stained with methylene blue, Azur II (1%), were examined in the light microscope for orientation. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips 400 electron microscope.

### Immunogold Staining of Fibrils

Whole cultures were homogenized by a brief treatment with a Polytron homogenizer and fibril fragments were isolated by differential centrifugation (Vaughan et al., 1988), adsorbed onto EM-grids and reacted with antibodies to collagens II, IX, and XI, and colloidal gold particles coated with goat antibodies to rabbit immunoglobulins (Bruckner et al., 1988; Mendler et al., 1989).

## Results

Primary chick embryo sternal cells were cultured in agarose gels (1.5–2.5 $\times$ $10^6$ cells/ml agarose suspension) in DME containing 10% FBS essentially as described by Benya and Shaffer (1982) and by Castagnola et al. (1986). Under these conditions, they strongly proliferated during the first week in culture (Table I and Fig. 1). Thereafter, the number of cells remained approximately stable for another week and, finally, even decreased somewhat. After 14 d in culture, the cells became very large (Fig. 2 *A*) and often formed colonies.

Chondrocytes grown under these or similar conditions are well-known to undergo terminal differentiation to the hypertrophic stage (Castagnola et al., 1986, 1988; Tacchetti et al., 1987; Gibson et al., 1982). Our results were in agreement with these earlier observations in that, after 2 wk, the cells showed many characteristics of hypertrophic chondrocytes. Their volume steadily increased over this period of time. The cells had euchromatic nuclei characteristic for transcriptionally active cells. Abundant and dilated rough endoplasmic reticulum and large amounts of Golgi vesicles were apparent indicating that the cells were intensively engaged in protein synthesis and secretion. Glycogen particles were thinly dispersed throughout the cytoplasm again suggesting that the cells were metabolically highly active (Fig. 3, *A* and *B*).

Hypertrophied chondrocytes produce collagen X, a biochemical marker for this stage of differentiation. After limited digestion with pepsin, chicken collagen X consists of three identical polypeptides of a molecular mass of 45 kD forming a triple helix lacking interchain disulfide bonds (Schmid and Conrad, 1982). The protein, therefore, can easily be identified in mixtures of pepsin digested cartilage collagens by SDS-PAGE based on the size of its polypeptides and its indifference towards reducing agents. In a series of papers, Capasso et al. (1984) and Castagnola et al. (1986, 1988) have shown with a large variety of techniques that chicken chondrocytes extensively produce collagen X when grown under the above conditions. To confirm these observations, we extracted the collagens from the whole cultures by pepsin digestion and subjected them to analysis by SDS-PAGE. Large amounts of fibril collagens II, IX, and XI were found. In addition, a band corresponding to a molecular mass of 45 kD was present which was susceptible to digestion with bacterial collagenase (not shown) and which was insensitive to treatment with 2-mercaptoethanol (Fig. 4, lanes *1* and *3*). This protein, therefore, was identified as collagen X and was a prominent biosynthetic product already after 1 wk (Fig. 4, lane *5*) and became the major collagen species after 2 wk (Fig. 4, lane *7*). Omission of $\beta$-aminopropionitrile fumarate from the media resulted in a lowered extractability of collagens II, IX, and XI, as expected, but collagen X remained unaffected.

*Table I. Cell Proliferation as Derived from DNA Quantification*

| Experiment | Presence of 10% FBS | Cells/ml agarose ($\times 10^{-6}$) at day 1 | 3 | 8 | 15 | 29 |
|---|---|---|---|---|---|---|
| 1 | − | 14.7 | 16.8 | 19.1 | 19.1 | 23.2 |
| | − | 15.2 | 18.2 | 19.6 | 20.6 | 18.3 |
| | + | 2.08 | 2.88 | 3.64 | 3.12 | 1.17 |
| | + | 2.30 | 2.73 | 3.53 | 4.03 | 1.39 |
| 2 | − | 16.8 | 18.4 | 23.7 | 29.6 | 27.7 |
| | − | 17.6 | 19.4 | 24.6 | 26.0 | 23.6 |
| | + | 2.43 | 4.06 | 7.38 | 8.57 | 4.43 |
| | + | 2.28 | 4.43 | 6.29 | 9.39 | 4.38 |

The number of cells was calculated based on an estimate of 7.7 pg DNA per cell (Kim et al., 1988). The inoculation density was $1.5 \times 10^6$ cells/ml (cultures with FBS) or $15 \times 10^6$ cells/ml (cultures without FBS).

The terminal chondrocyte differentiation proceeds rapidly under the above culture conditions. Since we suspected that serum factors were responsible for this phenomenon we developed culture conditions avoiding FBS in the media.

Differentiation in monolayer culture of limb bud mesenchyme cells into chondrocytes in part depends on their density (Solursh and Meier, 1974). Further, there are indications that this process is stimulated by products secreted by differentiated chondrocytes even in chemically undefined media rich in hormones and growth factors (Solursh and Reiter, 1975). In monolayer culture, however, chondrocytes undergo dedifferentiation and, for this reason, it is difficult to study their differentiation at late stages. To avoid this complication, we adopted the agarose culture system. However, in the experiments described above, cells were seeded at low densities if compared with those in cartilages. For example, there are $4 \times 10^7$ cells/ml in hypertrophic zones, and $2.2 \times 10^8$ cells/ml in proliferating zones of rat growth plate (Hunziker et al., 1987).

Therefore, we addressed the question whether cells at higher densities depended on serum components in the culture media. We found that chondrocytes survived for at least 6 mo in DME without FBS when the cultures were started with $1.1\text{–}2.5 \times 10^7$ cells/ml agarose suspension. Their number increased only by ~40% during the first week in culture and, thereafter, remained stable for at least 4 wk (Fig. 1). When freshly seeded, the cells were well-dispersed and spherical. Most of them remained in that state but some cells became discoidal after more than 24 h in culture and usually appeared as pairs, presumably arising from a single cell division (Fig. 2 *B*).

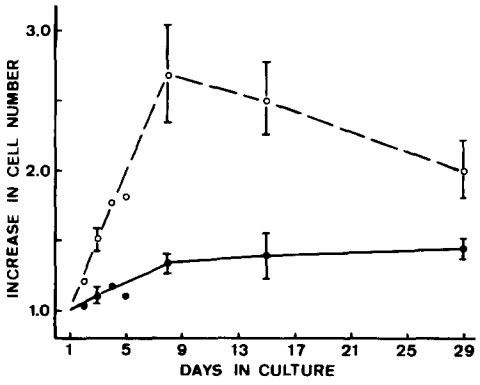



*Figure 1.* Cell proliferation calculated from DNA content of culture dishes. (○) Cultures with $1.5\text{–}2.5 \times 10^6$ cells/ml and with 10% FBS and (●) cultures with $1.5\text{–}2.5 \times 10^7$ cells/ml initial cell densities and without FBS. Standard deviations from mean values are given when samples were measured at least in quadruplicate. Duplicates appear as mean values only.

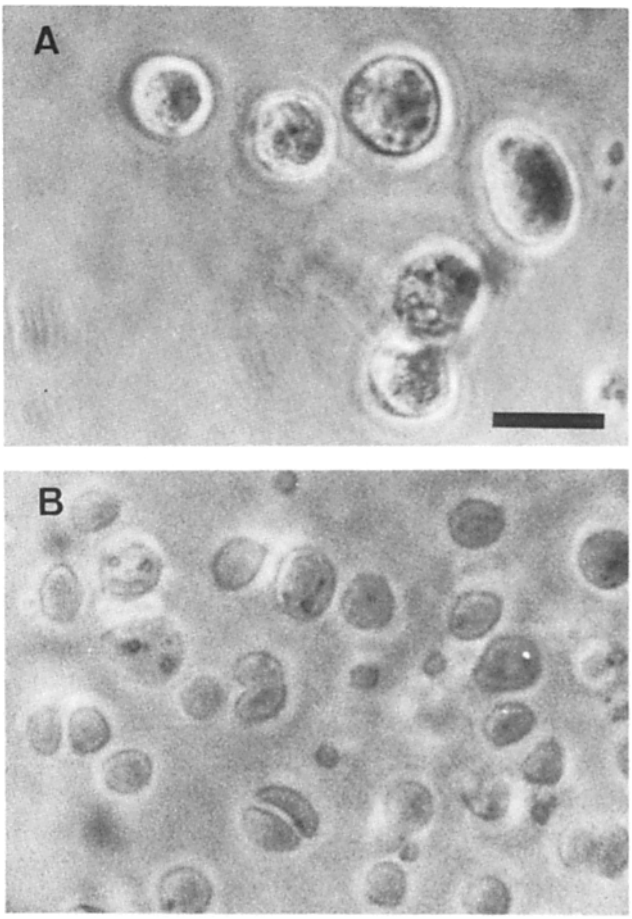



*Figure 2.* Phase contrast micrograph of chondrocytes after 2 wk in agarose culture in the presence (*A*) or in the absence (*B*) of 10% FBS in the culture medium. Bar, 20 μm.

Most strikingly, however, the cells grown at high density in the absence of FBS showed many characteristics of resting cells. They were conspicuously smaller than the hypertrophied cells described above (compare Fig. 2 *A* with *B*). Many of their suprastructural features also were clearly those of resting cartilage cells (Fig. 3 *C*). The nuclei had extensive, darkly contrasting heterochromatic areas indicating that transcription of DNA proceeded at moderate level. Rough endoplasmic reticulum was not very prominent and usually was not dilated and Golgi vacuoles were less conspicuous. These observations are consistent with a lower level of synthesis of extracellular proteins in these cells. The presence of large quantities of condensed cytoplasmic glycogen particles and well-developed intermediate filaments suggested that the cells generally had a low metabolic activity.

The cells initially produced large amounts of extracellular matrix macromolecules. This was reflected by high rates of incorporation of $^{14}$C-labeled proline into pepsin-resistant collagens (Fig. 5 and Fig. 4, lanes *6* and *8*). $^{35}SO_4$ was also

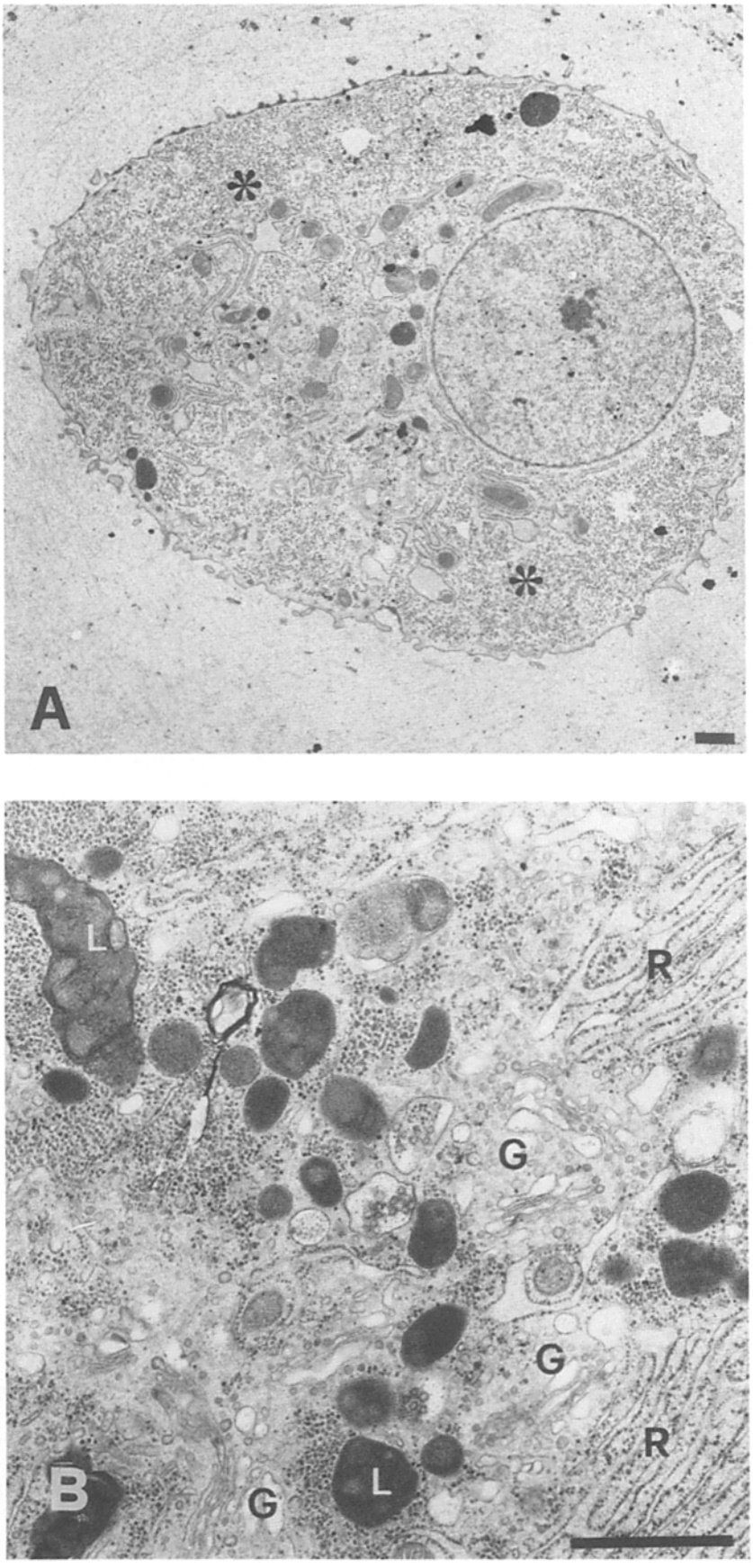
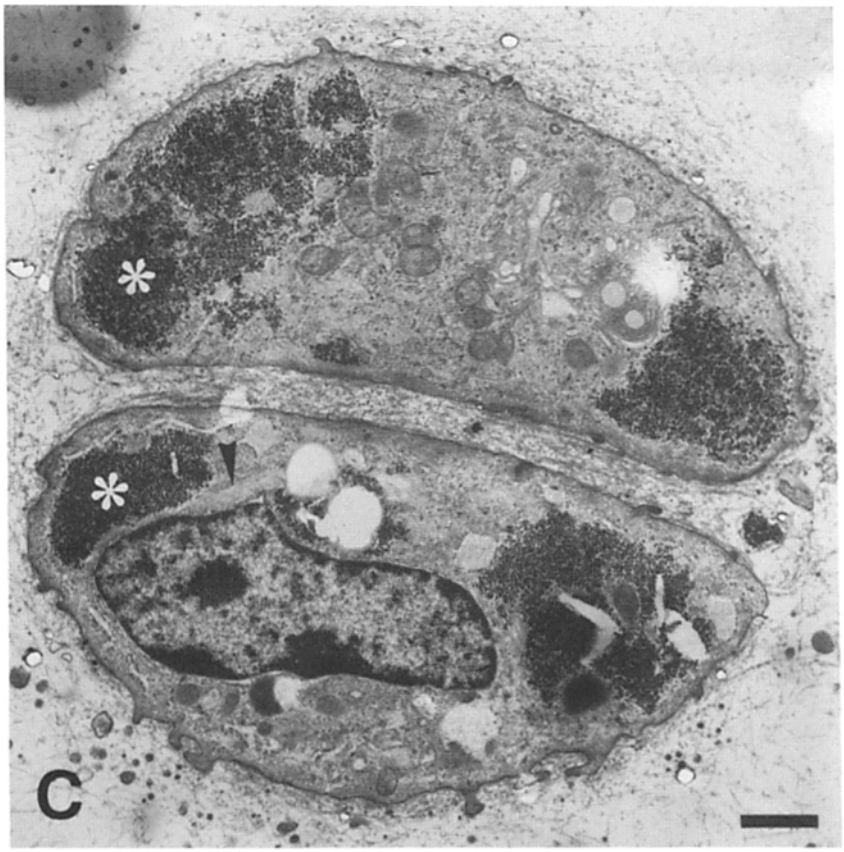



*Figure 3.* (*A*) Chondrocyte grown in culture medium containing 10% FBS showing signs of hypertrophy. Note that the volume of the cell is drastically increased. Nuclear chromatin is finely dispersed and a considerable amount of glycogen particles are seen in the cytoplasm (*). (*B*) Detail of chondrocyte grown in medium containing 10% FBS. The cytoplasm of the hypertrophic cell shows abundant rough endoplasmic reticulum (*R*), a prominant Golgi apparatus (*G*), and many lysosomal particles (*L*). (*C*) A pair of chondrocytes grown in medium without FBS. Note that the small discoidal cells exhibit structural features characteristic of resting cartilage cells, condensed nuclear chromatin, moderate amounts of cellular organelles, bundles of intermediate filaments (*arrowhead*), and caps of densely packed glycogen particles (*). Bars, 1,000 nm.




incorporated into nondialyzable material indicating that proteoglycans were synthesized at high rates. The production of radioactively labeled collagens and proteoglycans rapidly decreased to a few percent of the initial values (Fig. 5) while the number of cells still increased as estimated by DNA content (Table I and Fig. 1) and by the incorporation of radioactively labeled thymidine (Fig. 5). We could not perform such experiments with cells grown at low density because FBS interfered with metabolic labeling of these macromolecules. Most importantly, however, collagen X was not detectable in cultures without FBS even after at least 8 wk whereas the fibril collagens were extensively produced (Fig. 4, lanes *2*,

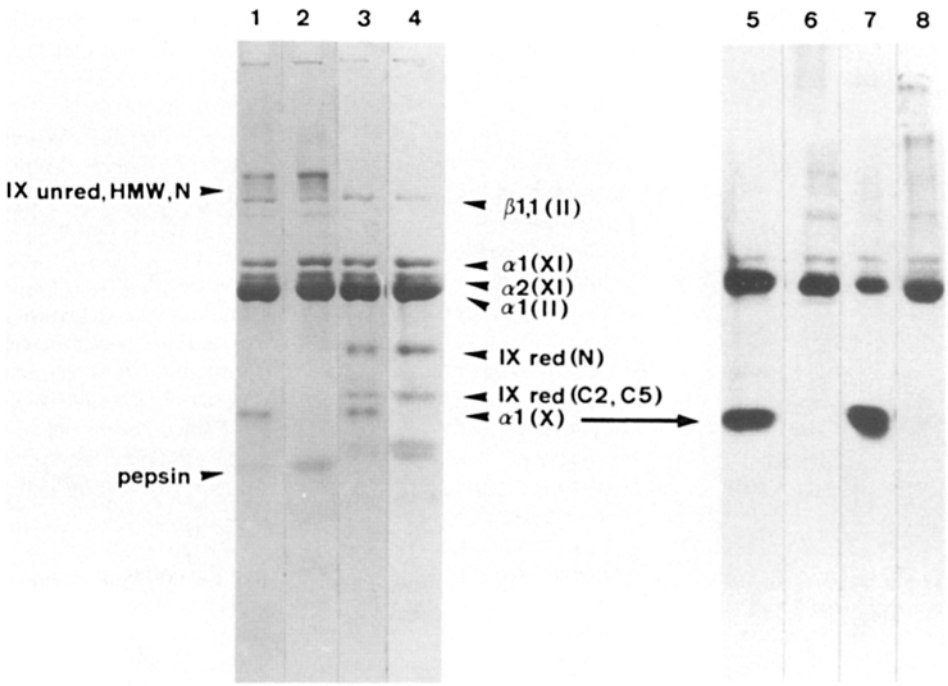



*Figure 4.* SDS-PAGE (4.5–15% gradient gel) of collagens extracted by pepsin digestion from whole cultures of chondrocytes grown with (lanes *1*, *3*, *5*, and *7*) or without (lanes *2*, *4*, *6*, and *8*) 10% FBS. Samples in lanes *3* and *4* were reduced with 2% 2-mercaptoethanol before electrophoresis. (Lanes *1–4*) Collagens synthesized by 35-mm cultures during 6 wk stained with Coomassie blue. (Lanes *5–8*) Fluorographs of gels with newly synthesized collagens radiolabeled with [$^{14}$C]proline for 24 h after 7 d (lanes *5* and *6*) and 14 d (lanes *7* and *8*) in culture.

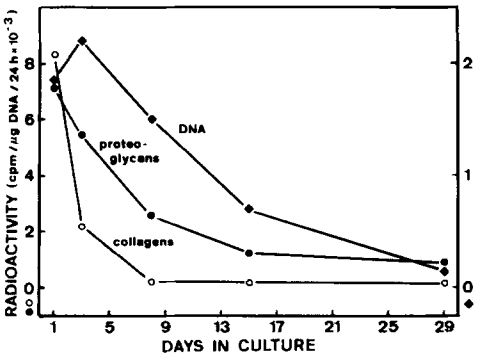



*Figure 5.* Metabolic activities of dense chondrocyte cultures grown without FBS. Collagen production was determined by incorporation of radioactively labeled proline into pepsin resistant macromolecules (○), proteoglycan synthesis by incorporation of radioactive sulfate (●), and cell proliferation by thymidine incorporation (◆), respectively. Cells were incubated with the labeled compounds for 24 h at the end of the culture periods indicated.

*4*, *6*, and *8*). Again, this did not depend on the presence of β-aminopropionitrile.

The dependence of the viability of resting chondrocytes on their density in culture suggested that these cells secreted products required for their own survival. To test this hypothesis, we initiated cultures with 1 × $10^6$ cells/ml in medium

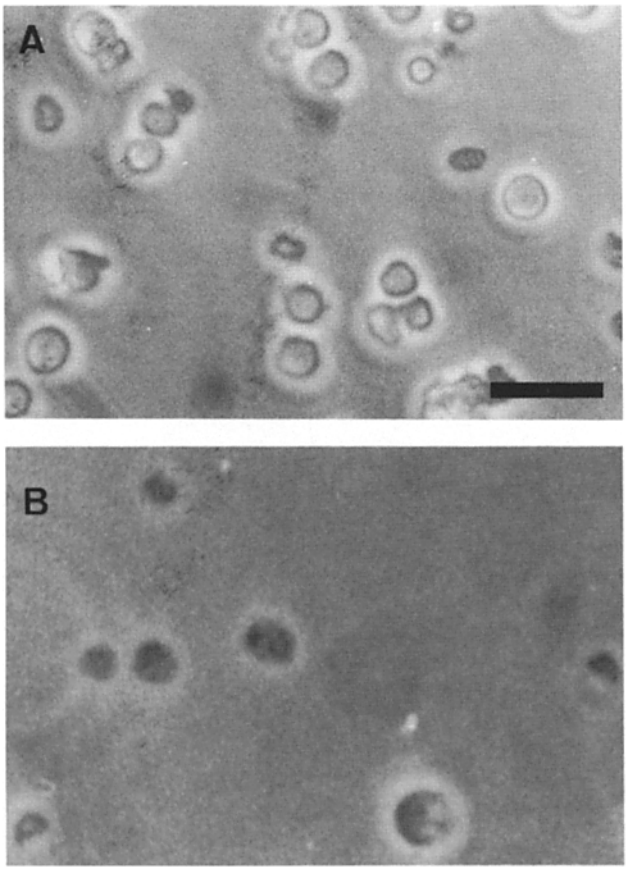



*Figure 6.* Phase contrast micrograph of chondrocytes seeded at low density (1.5 × $10^6$ cells/ml) in medium conditioned by chondrocytes grown at high density (*A*) and in DME (*B*). Note that cells were effectively protected in conditioned medium. Bar, 20 μm.

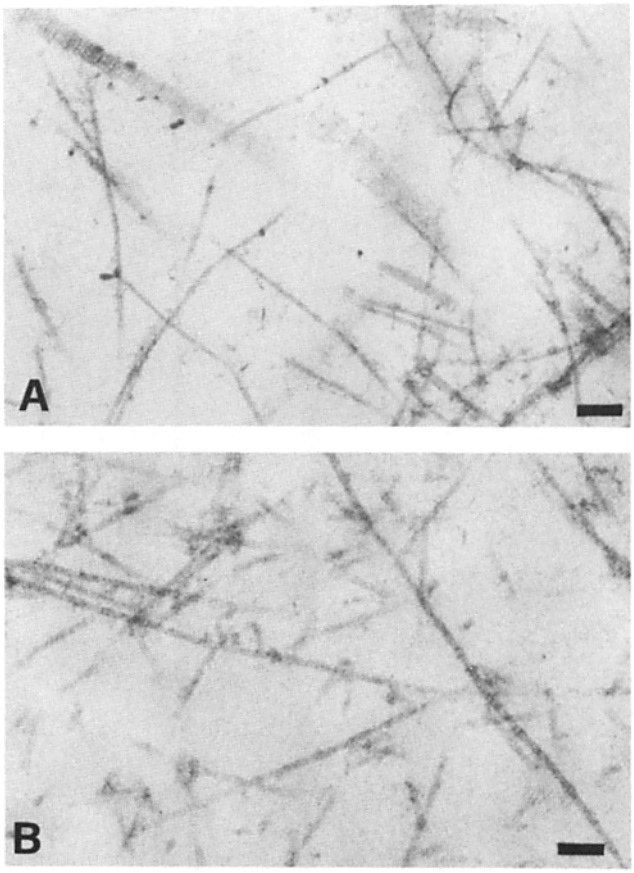



*Figure 7.* Extracellular matrix of chondrocytes grown at low density with FBS (*A*) and at high density without FBS (*B*). Note that the fine collagen fibrils have uniform diameters and wide, banded fibrils are present only in *A*. Bars, 100 nm.

lacking FBS but conditioned by similar cells at high density. As judged by their appearance, these cells were viable for at least a week whereas they did not survive for >48 h in unconditioned medium (Fig. 6). These results confirmed that cartilage cells could support their own viability in culture by secretion of soluble products to their medium and that they did not necessarily depend on serum components.

Both types of chondrocytes in culture extensively produced extracellular matrix staining with cationic dyes. When examined in the electron microscope, the matrix closely resembled that of authentic cartilage (Fig. 7). The cells deposited a matrix consisting of a network of fibrils entrapping electron translucent, amorphous material similar to the ground substance of cartilage. In the matrix of hypertrophic cells, the fibrils largely had a uniform diameter of 20 nm but, occasionally, much wider and well-banded fibrils were seen (Fig. 7 *A*). The occurrence of thick, banded fibrils is characteristic for adult chicken cartilage. Therefore, the matrix of chondrocytes grown in the presence of FBS seems to resemble that of adult animals although the cells initially were isolated from embryonal sterna. By contrast, the resting cells produced exclusively the fine fibrils and, hence, their matrix is more closely similar to that of embryonic cartilage.

Further, we studied the structure and the collagen composition of the fibrils produced by the two types of chondrocytes in culture. Fibril fragments mechanically generated by homogenization and extraction from the cultures were indis-

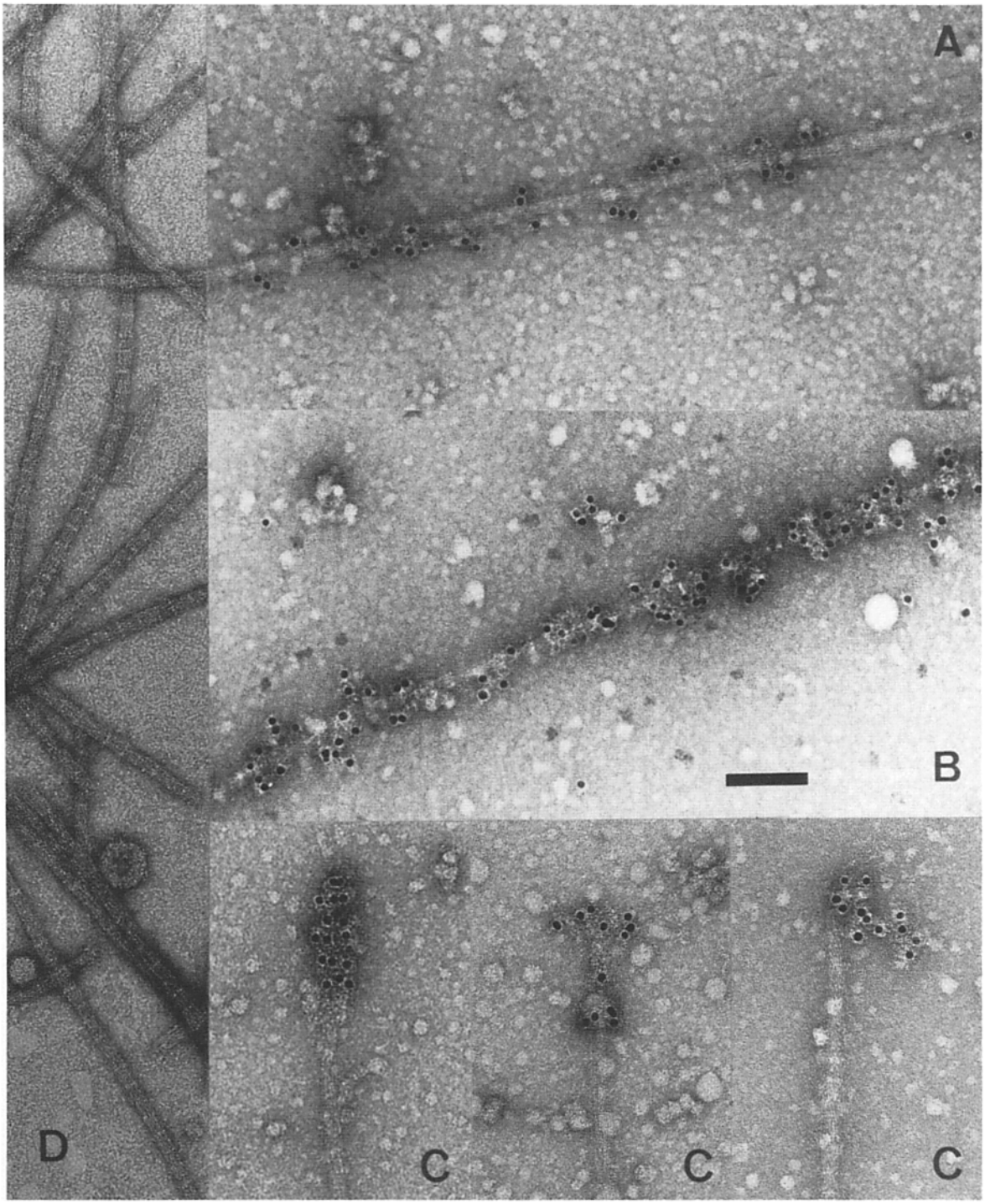



*Figure 8.* Electron micrograph of fibril fragments isolated from chondrocyte cultures in agarose after indirect immunogold labeling with antibodies to collagen II (*A*), collagen IX (*B*), and collagen XI (*C*) and after negative staining. For comparison, unlabeled fibril fragments from chick embryo sternal cartilage are shown in *D*. Bar, 100 nm.

tinguishable from those obtained from authentic cartilage as judged by their appearance in the electron microscope after negative staining. They were composed of the cartilage specific collagens II, IX, and XI as revealed by indirect immunostaining with colloidal gold (Fig. 8). Labeling by antibodies to collagen XI could only be obtained at the ends of the fibril fragments where the dense packing of the collagen molecules is disrupted by strong shear forces during the preparation of the fragments. By contrast, labeling of the other collagen types uniformly occurred along the fibril fragments. Recently, we have made very similar observations with fibrils from chick embryo sterna (Mendler et al., 1989). Therefore, our results support the notion that the matrices produced in vitro by both types of cells are closely similar to cartilage matrix. Moreover, they agree with our earlier findings (Müller-Glauser et al., 1986; Mendler et al., 1989) demonstrat-

ing that collagens II, IX, and XI were uniformly distributed throughout morphologically distinct zones of chick embryo cartilage.

## Discussion

While chondrocytes at different stages of their development can easily be distinguished from each other on the basis of morphological and biochemical criteria very little is known about the signals inducing the resting, the proliferative, and the hypertrophied states, respectively. Frequently, serum or other ill-defined components of culture media are used in long term cultures of chondrocytes. Under these circumstances, precise conditions for any particular cell type cannot easily be established. There are several reports on monolayer culture of chondrocytes in chemically defined media (Choi et al., 1980; Rifas et al., 1982; Schwartz and Sugumaran, 1983; Jennings and Ham, 1983; Glaser and Conrad, 1984; Malemud and Papay, 1986). Such culture systems may be beneficial for detailed studies on chondrocyte dedifferentiation (for review, see Benya and Brown, 1986) but they will not easily allow direct insight into the mechanisms of terminal differentiation of cartilage cells.

Here, we describe long term culture conditions supporting the phenotype of resting chondrocytes. Our observations by light and electron microscopy demonstrate that these cells closely resemble resting cartilage cells. As expected for this cell type, no extensive proliferation takes place and, characteristically, the typical marker of hypertrophic cartilage, collagen X, is not detectable. These results imply that chick embryo sternal cartilage cells in agarose gels do not necessarily require the presence of serum in the culture media but, instead, depend on products they secrete themselves. If these are present at high enough concentrations because the cells are grown at high density or in conditioned media they remain viable in the absence of serum for at least several months. With serum, cells survive also at low density but they develop clear signs of hypertrophy including the production of collagen X.

Our results agree with earlier reports (Tacchetti et al., 1987; Castagnola et al., 1988) that primary chick embryo sternal chondrocytes in agarose culture can undergo terminal differentiation to the hypertrophic stage. In addition, they extend these observations by demonstrating that chondrocyte differentiation beyond the resting stage requires the action of serum factors.

Extensive cell death was not observed. Therefore, since sternal chondrocytes are initially heterogeneous in their state of maturation, it is unlikely that our culture conditions lead to a large scale selection of specialized cell populations. In addition, the conjecture of cell selection is incompatible with the results of Tacchetti et al. (1987) which demonstrate that resting cells from the caudal portion of the sterna survived and progressed towards hypertrophy in agarose culture in the presence of serum. Preliminary results indicate that primary cartilage cells from entire human fetal femoral heads respond to our culture conditions similarly to chick embryo sternal cells. Since these cells are even more heterogeneous in their state of differentiation, these observations suggest that our conclusions apply to all types of chondrocytes and, moreover, that the earlier steps of maturation of hypertrophic chondrocytes may be reversible.

Therefore, we conclude that soluble blood components play an important role in cartilage degeneration and in bone formation. Vascularization of calcifying cartilage, the site of the largest hypertrophic chondrocytes, takes place before and concomitantly with remodeling into bone. Therefore, diffusible blood components promoting hypertrophy of chondrocytes in vitro may well enter into vascularized cartilage inducing hypertrophy in chondrocytes in situ and subsequent bone formation. This notion is consistent with earlier findings that induction of chondrocyte hypertrophy was abolished upon experimental prevention of growth plate vascularization (Trueta and Little, 1960; Trueta and Morgan, 1960; Trueta and Amato, 1960).

Similar considerations may apply to the pathogenesis of certain cases of degenerative joint diseases in which traumatic or inflammatory lesions of articular cartilage are thought to play an important role (Schleyerbach, 1986). Such lesions could enhance exposure of joint cartilage chondrocytes to serum factors. As a consequence, focal hypertrophy could be induced in cells which normally remain in the resting state. Induction of hypertrophy may become self sustaining through further vascularization in repair tissue and cartilage degeneration would naturally ensue.

The identity of the serum factor(s) causing hypertrophy is unknown. However, several factors such as growth hormone (Lindahl et al., 1987), transforming growth factor $\beta 1$ and $\beta 2$ (Seyedin et al., 1985, 1986, 1987), insulin-like growth factors (Hiraki et al., 1986), multiplication-stimulating activity (Hiraki et al., 1986), or interleukins (Dingle and Tyler, 1986) are known to affect cartilage or chondrocyte metabolism (for review, see Canalis et al., 1988). Our culture system provides a unique opportunity to study directly the action of such substances on chondrocytes without interference by undefined components of the media.

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