Proteoglycans Synthesized by Chondrocytes of Human Nonarthritic and Osteoarthritic Cartilage (42860)

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Abstract. **Chondrocyte cultures were developed from the cell outgrowths of explanted human nonarthritic and osteoarthritic human cartilage. Two significant differences in sulfated proteoglycan synthesis were demonstrated between the chondrocytes obtained in this manner. With $^{35}SO_4$ to measure newly synthesized proteoglycan, we found that chondrocytes derived from osteoarthritic cartilage secreted significantly less ($P < 0.05$) high density proteoglycan into the culture medium than did chondrocytes from nonarthritic cartilage after 20 hr of radiolabeling. This reduced amount of high density proteoglycan was sustained when chondrocytes were maintained in unlabeled culture medium ("chase" medium). In addition, the osteoarthritic chondrocytes secreted an increased amount of low density proteoglycan when compared with their nonarthritic counterparts. The elution profile of secreted high density proteoglycan isolated from the osteoarthritic chondrocyte culture medium was assessed by gel filtration on Sepharose CL-2B and revealed the presence of two proteoglycan subpopulations (K_{av}, 0.25, 0.58), whereas only one proteoglycan series (K_{av}, 0.37) was seen in the high density fraction of nonarthritic chondrocyte culture medium. Similar gel filtration profiles were also obtained when chondrocytes were maintained in chase medium. The results of this study demonstrated that stable differences in proteoglycan synthesis, but not in intracellular processing, exist between nonarthritic and osteoarthritic chondrocytes. The findings are noteworthy in that these differences were not previously apparent when organ-cultured cartilage was used to assess putative alterations in proteoglycans between the two groups.** [P.S.E.B.M. 1989, Vol 190]

The time continuum in the development of human osteoarthritis (OA) from initial pathogenesis to clinical diagnosis can span 30 years or more. Clinically, the disease often terminates in total joint arthroplasty. The pathogenesis of OA remains obscure, and it has long been debated whether stable and authentic changes occur in chondrocytes during the development of OA (1, 2). A controversy persists whether chondrocytes in OA cartilage are "normal," but rather are adversely influenced by mechanical transduction signals *in situ* (3–6).

Several studies argue that the cartilage extracellular matrix influences the phenotypic stability of chondrocytes, OA chondrocytes included (7). A tenet of this hypothesis is that chondrocytes released from their extracellular matrix and maintained as cell cultures modulate their chondrogenic phenotype-producing macromolecules that are not resident in the parent tissue.

Human chondrocytes have been studied in primary culture after outgrowth from explants (8, 9). The cells synthesized and secreted quantitatively less hydrodynamically large proteoglycan than was found in the tissue maintained by explant techniques (10). Nevertheless, the quality of the proteoglycans secreted into the culture medium appeared to be similar to the tissue-derived newly synthesized proteoglycans. Seminal studies by Mankin *et al.* (1, 3) suggested that the hypermetabolic state of OA cartilage exemplified by increased incorporation of several radiolabeled metabolic precursors is also expressed when OA chondrocytes are compared with nonarthritic counterparts (11). Quantitative increases in $^{35}SO_4$ incorporation by OA chondrocytes have been confirmed by other studies (12). Other extracellular matrix proteins are also apparently increased

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in amount in OA (13). In addition, human chondrocytes derived from OA cartilage were shown to secrete higher levels of neutral metal-dependent enzymes which could degrade proteoglycan (14). These prior studies suggested that potentiation of gene expression under environmental stimuli increased proteoglycan and enzyme synthesis and that this was maintained in cell culture. These results led to the present investigation in which proteoglycans synthesized by chondrocytes derived from human nonarthritic and OA cartilage were compared.

Materials and Methods

Materials. Dulbecco's modified Eagle's medium (DMEM)-high glucose (4.5 g/liter), with or without $MgSO_4$, Gey's balanced salt solution (GBSS), penicillin-streptomycin, Fungizone, and mycostatin were from Grand Island Biological Co. (Grand Island, NY). Fetal bovine serum non-heat-inactivated was from KC Biological (Lenexa, KS). Testicular hyaluronidase and trypsin were from Worthington Biochemicals (Freehold, NJ). Carrier-free $Na_2{}^{35}SO_4$ (~43 Ci/mg sulfate) and chondroitin lyase ABC and AC-II were from ICN (Irvine, CA). Sepharose gel filtration media were from Pharmacia Fine Chemicals Co. (St. Louis, MO). Guanidine hydrochloride (GuHCl; ultrapure) was from Bethesda Research Laboratories (Gaithersburg, MD). Cesium chloride (density gradient grade) was from U.S. Biochemical Corp. (Cleveland, OH). All other chemicals were of ACS grade or their equivalent and were from Fisher Scientific Co. (Springfield, NJ).

Source of Tissue and Chondrocyte Culture. Articular cartilage was obtained fresh from the operating room at the time of orthopaedic surgical intervention. "Normal," or nonarthritic, cartilage was obtained from the femoral head ($n = 8$) or knee ($n = 2$) of patients (mean age, 32.6 years; range, 3–80 years) with the diagnosis of avascular necrosis or tumor of bone, but sparing the joint cartilage. The inclusion of samples from patients with the diagnosis of avascular necrosis as a source of "nonarthritic" cartilage was based on the findings of Mankin *et al.* (15) that such cartilage was histochemically similar to nonpathologic cartilage. Resident cartilage generally described as fibrillated and to a lesser extent discolored nonfibrillated tissue was obtained from the femoral heads of patients ($n = 9$) (mean age, 63 years; range, 41–80 years) at the time of total hip arthroplasty for debilitating OA. Subchondral bone and capsular tissue were excluded by dissection. A portion of each sample was processed for histologic grading. Five-micrometer sections embedded in paraffin and stained with hematoxylin and eosin, safranin O/fast green, or toluidine blue (16) were routinely evaluated.

Cartilage from each specimen was minced under sterile conditions into approximately 2- × 2-mm^2 pieces. For studies of chondrocyte outgrowths, tissues were treated with 0.05% testicular hyaluronidase for 3 min at 37°C and then rinsed thrice with GBSS to remove synovial mucins and potentially contaminating synoviocytes (9). Approximately 15–20 tissue fragments were evenly distributed into each 60-mm × 15-mm tissue culture dishes. The cultures were replenished at regular intervals with fresh culture medium. DMEM containing $MgSO_4$ supplemented with 10% fetal bovine serum, 1% Fungizone, and 0.1% each of penicillin-streptomycin and mycostatin was used. Cultures were incubated in 10% CO_2/90% air and examined microscopically every other day for evidence of cell outgrowth. When cells became confluent (usually 30–40 days), the tissue samples were removed and the remaining adherent cells were detached by treatment with 0.25% trypsin for 15 min at 37°C followed by rinsing with GBSS to loosen any remaining cells. The resultant cell suspension was centrifuged and the cell pellet resuspended in 5 ml of DMEM and gently mixed. This cell suspension was subdivided into multiple 1.5-cm microwells at an initial density of 1.5×10^5 cells/well. Each well received 1 ml of sulfate containing DMEM supplemented with 10% fetal bovine serum.

Radiolabeling and Extraction of Proteoglycans. Two days after subculture, chondrocytes confirmed as confluent by phase microscopy were incubated with serum containing DMEM with $MgCl_2$ substituted for $MgSO_4$ (17) and 20 μCi/ml $Na_2{}^{35}SO_4$ (radiolabeling medium). After 20 hr the radiolabeling medium was removed and cells were rinsed with GBSS. The medium-GBSS mixture was diluted in an equal volume of 8 *M* GuHCl/0.2 *M* sodium acetate (pH 5.8) with proteinase inhibitors (18). To assess potential differences in processing of intracellular proteoglycans between nonarthritic and OA chondrocytes, cells were incubated in sulfate containing DMEM supplemented with serum, for varying periods of time following incubation with $^{35}SO_4$ ("chase" medium). This chase medium was removed at 24-hr intervals over a 4-day period. In some cases, chase medium was removed from one fourth of the cultures on Days 1, 2, 3, and 4, whereas in other cases, medium was removed only at Day 2, Day 4, or both. On each day, cells were examined by phase microscopy to ensure that monolayers were intact. The medium was combined with an equal volume of GBSS which was used to rinse the cell layer. This mixture was diluted with an equal volume of 8 *M* GuHCl and kept for 24 hr at 4°C. The culture medium was dialyzed exhaustively and freeze dried. In a number of cases the resident chondrocytes were solubilized in buffered 4 *M* GuHCl, dialyzed, and freeze dried.

Associative Column Chromatography. The hydrodynamic size of newly synthesized proteoglycans radiolabeled with $^{35}SO_4$ were characterized by Sepharose CL-2B molecular sieve chromatography (0.5 cm × 115 cm) eluted with 0.5 *M* sodium acetate (pH 5.8).

Aliquots of eluted fractions were measured for incorporated $^{35}SO_4$ by liquid scintillation spectrometry in a Packard model 3255 liquid scintillation counter (Beckman, Palo Alto, CA). Based on prior studies (10), elution fractions were defined on the basis of hydrodynamic size. These were pooled Fractions I (K_{av}, 0.05), II (K_{av}, 0.2), III (K_{av}, 0.6), and IV (K_{av}, 0.9). The relative percentage of total incorporated radioactivity comprising each of these pooled fractions was calculated for proteoglycans secreted into the medium. Fractions comprising each of these subpopulations were pooled for further study.

Isopyknic Cesium Chloride Density Gradient Ultracentrifugation. The elution profiles of newly synthesized proteoglycans were similar to those previously described in studies of nonsubcultured cell outgrowths (10). High density proteoglycan (Fraction D1) derived from chromatographic pooled eluates was subjected to aggregation studies in which 2.5 μg of human umbilical cord hyaluronic acid and 25 μg of bovine cartilage link protein in Tris buffer or buffer was incubated and rechromatographed on Sepharose CL-2B (10). These previous studies demonstrated that Fraction D1 derived only from pooled Fraction I formed proteoglycan (PG) aggregates, which eluted in the void volume of Sepharose CL-2B whereas pooled Fractions II, III, and IV did not aggregate. Based on these previous studies (10), we elected to pursue further analysis of high density proteoglycan derived from pooled Fraction II, as we were interested in whether these apparently nonaggregating PG differed between nonarthritic and OA chondrocytes. Thus, pooled Fraction II PG was separated into component PG subclasses based on buoyant density by gradient ultracentrifugation under dissociative conditions (4 *M* GuHCl/0.1 *M* sodium acetate, pH 5.8). The initial CsCl concentration was 1.5 g/ml. Samples were centrifuged for 45 hr at 10°C (g_{av} = 81,900) in a Beckman L8-55 ultracentrifuge using a SW 50.1 rotor. The gradient tubes were cut into four 1.2-ml fractions. Final isopyknic densities were assessed (D1 > 1.6 g/ml) and $^{35}SO_4$ incorporation measured after exhaustive dialysis.

Sepharose CL-2B Column Chromatography. The D1 fraction from pooled Fraction II and in some cases other pooled fractions (i.e., III/IV) was eluted from Sepharose CL-2B (1.0 cm × 115 cm) with 4 *M* GuHCl/0.1 *M* sodium acetate (pH 5.8).

Glycosaminoglycan Content. The glycosaminoglycan content of proteoglycans was studied after alkaline sodium borohydride reduction for 48 hr at 45°C (19). This was followed by chromatography on Sepharose CL-6B (0.5 cm × 115 cm) eluted with 0.5 *M* sodium acetate (pH 5.8). Aliquots of eluted fractions were measured for radioactivity. Fractions within the eluted pooled peak were dialyzed and then treated with chondroitin lyase ABC or AC-II for 24 hr followed by rechromatography on Sephadex G-100 (19). Radioactivity recovered near or at the column V_t was considered enzyme-susceptible material.

Statistical Analysis. The percentage of total radioactivity in pooled Sepharose CL-2B associative chromatogram peaks, and CsCl gradient fractions were compared among nonarthritic and separately among OA samples using Student's *t* test (two-tailed). Computation of mean value and calculation of probability for comparison between nonarthritic and OA samples were obtained from the pooled estimate of variance.

Results

Histologic Assessment. Nonarthritic samples resembled the so-called "A"-type tissue previously described (20). This tissue "type" was characterized by normal or slightly reduced cellularity and a diminution of pericellular but not interterritorial metachromasia. OA samples were primarily of the "B" type but often a mixture of A- and B-type tissue was seen. B tissue was characterized primarily by extensive depletion of interterritorial extracellular matrix proteoglycan. Cloning of 3–12 cells was observed. The cells were small and spheroidal in shape and arranged in columnar or ovoid clusters and were most frequently found as a component of the B tissue type.

Kinetics of $^{35}SO_4$ Incorporation. As reported previously (10), the incorporation of $^{35}SO_4$ was essentially linear over the 20-hr radiolabeling period. In preliminary experiments, radiolabeling periods of 3 and 6 hr were studied, in addition to the more typical 20-hr period. An analysis of the newly synthesized proteoglycans at these earlier time points showed a reduced amount of high density proteoglycan (Fraction D1) compared with the 20-hr time point. Therefore, the latter time point was chosen for the radiolabeling period.

Proteoglycans Associated with the Cell Layer. The profile of newly synthesized proteoglycans associated with the chondrocytes was studied by Sepharose CL-2B chromatography after 2–4 days of maintenance in chase medium following the radiolabeling period (Table I). There was no apparent increase in the total amount of newly synthesized proteoglycans (i.e., all proteoglycan populations) retained by OA chondrocytes compared with their nonarthritic counterparts. However, OA chondrocytes apparently retained less of the hydrodynamically large proteoglycans (Fractions I/II) in the cell-associated fraction than did the nonarthritic chondrocytes (Table I). One nonarthritic sample appeared to be significantly different than the three other samples examined. In the one sample, only 2.6% of the incorporated $^{35}SO_4$ was found in Fractions I/II, whereas an average of 54.9% was found for the three other samples examined.

Proteoglycans Secreted into the Culture Medium. As previously reported (10), the majority of

incorporated $^{35}SO_4$ eluted with a large K_{av} (0.9) on Sepharose CL-2B (Table II). No differences were seen between nonarthritic and OA chondrocytes, even when the smaller number of age-matched samples (fifth–seventh decade; nonarthritic, $n = 4$; OA, $n = 5$) were compared. When the newly synthesized proteoglycans secreted into the chase medium at 2 and 4 days following the radiolabeling period was evaluated, there was a marked shift toward proteoglycans of larger hydrodynamic size (chromatographic pooled Fractions I and II) and 2- to 3-fold increase in the amount of small proteoglycan (K_{av}, 0.6, III) at the apparent expense of the small proteoglycan (K_{av}, 0.9, IV) in the medium. These results showed that during the 20-hr radiolabeling period, there was secretion of primarily small proteoglycans and the hydrodynamically larger proteoglycans were secreted to a significantly lesser extent. Although only a few samples were assessed after 24 hr of switching to chase medium after the radiolabeling period, there was already at that time an increase in the hydrodynamically larger proteoglycan (data not shown). We have previously shown (10) that 48.9% of the $^{35}SO_4$ in pooled Fraction III reached equilibrium in the most dense D1 fraction, whereas for pooled Fraction IV, 46.6% was found in the D1. Thus, almost 50% of the recovered $^{35}SO_4$ in these proteoglycan subpopulations was found in a fraction known to contain proteoglycan monomer.

Distribution of Pooled Fraction II from Sepharose CL-2B in CsCl Density Gradients. The amount of $^{35}SO_4$ in Fraction II increased almost 5-fold when proteoglycan secreted after the 20-hr radiolabeling time was compared with proteoglycans secreted after switching to chase medium for 2 days (Table II). The distribution of incorporated $^{35}SO_4$ in seven individual samples in CsCl density gradients was further evaluated. OA chondrocytes secreted significantly less high density proteoglycan (Fraction D1) into the medium than chondrocytes from nonarthritic cartilage (Table III). After 2 days in chase medium, this reduction in the amount of high density proteoglycan persisted. There was, in addition, a significantly greater amount of low density proteoglycan (Fractions D3/D4) in the radiolabeling medium of the OA chondrocytes, but no differences were found in the middle density (D2) fraction (Table III).

Hydrodynamic Size of the D1-Proteoglycan Iso-

Table I. Retention of Newly Synthesized Proteoglycans in the Cell-Associated Fraction[a]

Group	Retained in cell layer (%)	Distribution of $^{35}SO_4$ on Sepharose 2B (%)[b]			
		I	II	III	IV
Nonarthritic	18.0 ± 8.5	18.0 ± 12.8	23.1 ± 14.1	46.4 ± 17.1	13.1 ± 11.0
OA	18.6 ± 10.6	5.7 ± 2.4	15.2 ± 5.9	53.7 ± 18.9	25.4 ± 15.7

[a] Newly synthesized proteoglycan in the cell layer collected after maintenance in "chase" medium for 2 to 4 days was solubilized with 4 *M* GuHCl/0.1 *M* sodium acetate buffer (pH 5.8) containing protease inhibitors. After dialysis, the radioactivity retained was used as a measure of the retention in the cell-associated fraction. An aliquot of this nondialyzable fraction was chromatographed on Sepharose CL-2B eluted with 0.5 *M* sodium acetate (pH 5.8).
[b] Nonarthritic, $n = 4$; OA, $n = 5$, Mean ± SD. The Δ% comparing the distribution of $^{35}SO_4$ on Sepharose CL-2B of nonarthritic and OA chondrocytes was Fraction I, −216%; Fraction II, −51.9%; Fraction III, +15.7%; and Fraction IV, +93.9%.

Table II. Sepharose CL-2B Elution Profile of $^{35}SO_4$-labeled PG[a]

Sepharose CL-2B subpopulation	Pooled fraction (%)[b]					
	Nonarthritic			OA		
	20 hr[c]	2 days[d]	4 days[d]	20 hr	2 days	4 days
I	4.3 ± 3.2	19.1 ± 9.0	17.2 ± 13	3.0 ± 3.1	14.0 ± 7.9	8.3 ± 3.6
II	4.8 ± 3.4	23.2 ± 9.1	21.4 ± 14	4.2 ± 3.7	25.2 ± 8.0	22.0 ± 3.1
III	10.3 ± 5.2	28.3 ± 7.1	23.5 ± 8.6	11.1 ± 3.8	40.4 ± 13.0	35.2 ± 5.7
IV	80.6 ± 10.8	29.4 ± 17	38.1 ± 30.3	81.7 ± 8.1	20.4 ± 14.0	34.5 ± 9.0

[a] Chondrocyte cultures were developed from explant outgrowths by treatment with trypsin and replating (see Materials and Methods for details). Cells were incubated with $^{35}SO_4$ for 20 hr. The medium was exhaustively dialyzed and chromatographed on Sepharose CL-2B. The cells were incubated further for 1 to 4 days in chase medium containing sodium sulfate. The medium was harvested, dialyzed, and chromatographed. No significant difference existed between the chromatographic profile of radiolabeling medium between normal and OA chondrocytes. For both normal and OA chondrocytes, a significant ($P < 0.01$–0.001) difference was found when the profile of radiolabeling medium was compared with chase medium at 2 or 4 days. Nonarthritic, $n = 10$; OA, $n = 9$.
[b] Mean ± SD.
[c] Twenty-hour radiolabeling.
[d] Two or 4 days in chase medium.

Table III. Distribution of Pooled Fraction II from Sepharose CL-2B in CsCl Density Gradients[a]

Group	Density gradient fraction (%)[b]							
	Radiolabeling medium				Chase medium			
	D4	D3	D2	D1	D4	D3	D2	D1
Nonarthritic	11.8 ± 5.3 (11.6 ± 5.9)	12.9 ± 3.8 (12.7 ± 4.2)	16.0 ± 5.2 (14.6 ± 5.1)	59.3 ± 11.6 (61.1 ± 12.7)	16.4 ± 7.5	14.9 ± 3.8	16.0 ± 3.9	52.7 ± 7.2
OA	20.2 ± 9.3**	18.8 ± 5.9**	15.5 ± 5.6	45.5 ± 13.7**	19.6 ± 4.7	19.0 ± 4.8	17.6 ± 2.7	43.8 ± 10.8

[a] Fraction II from Sepharose CL-2B chromatography was analyzed in CsCl density gradients (see Materials and Methods for details). A significant reduction ($P < 0.05$)** was found in the most dense D1 fraction of the radiolabeling medium when nonarthritic ($n = 9$) and OA ($n = 7$) chondrocytes were compared. There was no difference between the chondrocytes in the middle density D2 fraction. However, there was a significant increase ($P < 0.05$)** in low density proteoglycans (D3/D4) of the radiolabeling medium in the OA chondrocytes. There was no significant difference in the 2-day chase medium profile (normal, $n = 7$; OA, $n = 7$). The values in parentheses in the nonarthritic radiolabeling medium represent values matched to similar samples obtained from chase medium. The total $^{35}SO_4$ recovered from pooled Fraction II used in this analysis was: nonarthritic, radiolabeling medium, 2.7 ± 0.47 × 10^3 cpm; nonarthritic, chase, 1.27 ± 0.64; OA, radiolabeling medium, 2.92 ± 0.67; OA, chase, 1.2 ± 0.58, $n = 6$. In one sample, the amount of incorporated $^{35}SO_4$ in pooled Fraction II was not recorded.
[b] Mean ± SD.

lated from Pooled Fraction II. The elution profile of proteoglycan in the D1 fraction was chromatographed on Sepharose CL-2B (Fig. 1). When nonarthritic chondrocytes were examined, proteoglycan eluted from the column as a symmetrical peak (K_{av}, 0.37). A small shoulder of incorporated $^{35}SO_4$ was frequently present, K_{av}, 0.75 (Fig. 1A). The size and elution profile of these secreted proteoglycans was unaltered 2 days after switching to the chase medium (Fig. 1B). A representative profile from OA chondrocytes, however, showed two distinct proteoglycan populations instead of one, plus the additional shoulder (Fig. 1C). One proteoglycan subpopulation eluted with a K_{av} considerably smaller (K_{av}, 0.25) than that of the single peak determined for nonarthritic chondrocytes. The K_{av} of the other proteoglycan subpopulation (0.58) was considerably larger. In a typical experiment, 39% of the incorporated $^{35}SO_4$ was associated with the hydrodynamically large proteoglycan and 61% was associated with the smaller proteoglycan. Neither the distribution of incorporated $^{35}SO_4$ nor the hydrodynamic size of the proteoglycans changed 2 or 4 days after switching to chase medium (Fig. 1D).

The hydrodynamic size of pooled Fraction II-D1 proteoglycan from nonarthritic and OA chondrocytes is shown for individual samples in Figure 2. These results demonstrated a considerable uniformity in this type of analysis among the different chondrocyte samples. In one case of OA chondrocytes, only one proteoglycan subpopulation was detected, and in this case it was just the hydrodynamically smaller proteoglycan.

Newly Synthesized Glycosaminoglycans. No differences were detected in the glycosaminoglycan chain size (K_{av}, 0.5–0.6 on Sepharose CL-6B) in pooled Fraction II-D1 when nonarthritic and OA chondrocytes were compared (data not shown). Digestion of newly synthesized glycosaminoglycans with either chondroi-

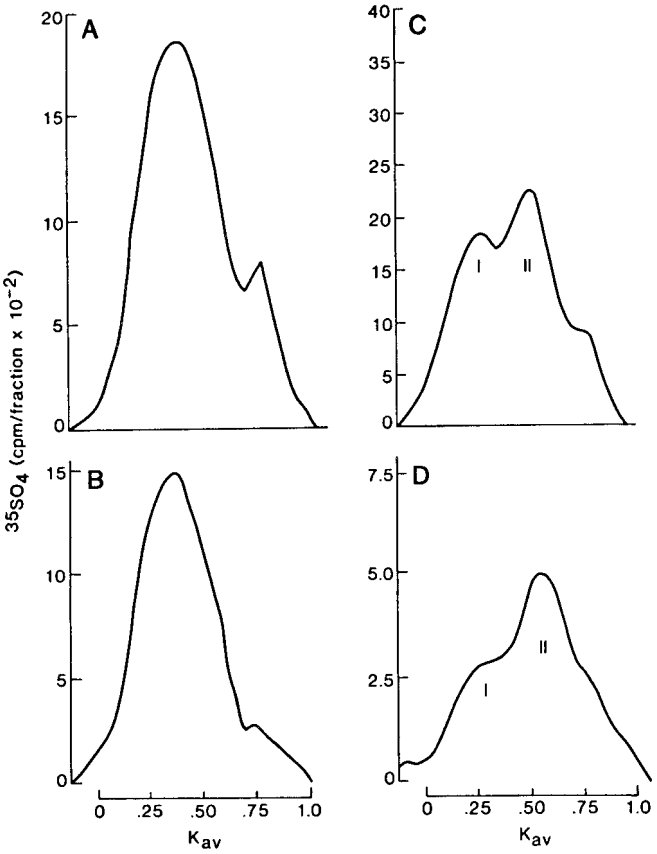

Figure 1. Sepharose CL-2B chromatography of the $^{35}SO_4$-labeled D1 fraction isolated from pooled Fraction II eluted under dissociative conditions. D1 gradient fractions prepared from the culture medium were eluted with 4 *M* GuHCl/0.1 *M* sodium acetate buffer (pH 5.8) from Sepharose CL-2B (1.0 cm × 115 cm). Nine-tenths-milliliter fractions were collected. The void volume ($K_{av} = 0$) was determined with blue dextran 2000 and the total column volume ($K_{av} = 1$) with $Na_2{}^{35}SO_4$. (A) Nonarthritic chondrocytes, 20-hr radiolabel with $^{35}SO_4$. (B) Nonarthritic chondrocytes, 2 days in "chase" medium. (C) OA chondrocytes, 20-hr radiolabel with $^{35}SO_4$. (D) OA chondrocytes, combined 2 days and 4 days in chase medium. Roman numerals I and II denote the two newly synthesized proteoglycan subpopulations found in the D1 fraction of OA chondrocytes.

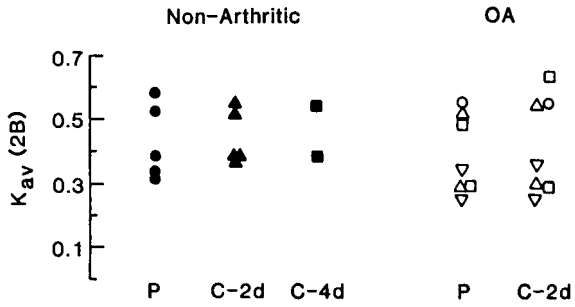

Figure 2. K_{av} determinations of D1 fractions prepared from pooled Fraction II of culture medium from nonarthritic and OA chondrocytes. Individual chondrocyte cultures were radiolabeled with $^{35}SO_4$ for 20 hr, washed, and maintained for 2 or 4 days in chase medium. D1 fractions from CsCl density gradient centrifugation were chromatographed on Sepharose CL-2B. In the nonarthritic chondrocytes, only one peak of incorporated radioactivity was found whose K_{av} did not change after 2 or 4 days in chase medium. In the OA chondrocytes, three of four D1 fractions studied were determined to have proteoglycan subpopulations I and II (see Fig. 1). These proteoglycan subpopulations were also found after 2 days of maintenance in chase media. The one D1 fraction which exhibited only the 0.58 K_{av} proteoglycan subpopulation in the 20-hr radiolabeling medium also showed only that single population in the chase medium as well. The individual OA D1 fractions showing proteoglycan subpopulations I and II are indicated by identical symbols.

tin lyase showed that they consisted of predominantly chondroitin sulfate since over 90% of the intact glycosaminoglycan chains were digested with either enzyme. These results did not change after the cells were switched to chase medium.

Discussion

Chondrocytes have been established from explant outgrowth of nonarthritic and OA cartilage. The present studies established authentic differences between the proteoglycans synthesized by these chondrocytes. In addition, these results indicated that under the high density monolayer conditions employed, the proteoglycans secreted into the culture medium during the 20 hr of radiolabeling with $^{35}SO_4$ was primarily hydrodynamically small. Only after switching to chase medium did hydrodynamically larger proteoglycan emerge from the cells. We also previously found that the small proteoglycans dominated the cell-associated fraction as well (10). Interestingly, there was no difference in this pattern between nonarthritic and OA chondrocytes.

Small proteoglycans have been found in cartilage (21) and in other tissues such as rabbit skeletal muscle (22). The report of newly synthesized small proteoglycan in the culture medium of chondrocytes isolated from rabbit rib growth cartilage (23) emphasizes that this proteoglycan subpopulation is a component of the proteoglycan phenotypic repertoire of animal and human chondrocytes. Although the function of the small proteoglycan is unknown (21), it may be topographically important for extracellular matrix structure. Its decrease rather than increase as a proportion of total Sepharose CL-2B eluted newly synthesized proteoglycan suggests that at least in this system the small proteoglycan is not a catabolic product of the larger (Fraction I/II) proteoglycans.

Previous studies of OA cartilage (24, 25) suggested a delay rather than an absolute loss of capability of newly synthesized proteoglycan to aggregate with hyaluronic acid. In the present study, attention was paid to putative differences in the nonaggregating proteoglycan subpopulation. Two fundamental differences were found between the proteoglycan isolated from the high density D1 fraction of pooled peak II nonaggregatable PG of the nonarthritic and OA chondrocytes. The OA chondrocytes secreted less proteoglycan monomer and significantly more low density proteoglycan into the culture medium. In addition, OA chondrocytes secreted at least one additional proteoglycan of small size in addition to a hydrodynamically large proteoglycan. Three of four OA chondrocyte samples demonstrated this heterogeneity in the D1 proteoglycan subpopulation. Harmand *et al.* (12) previously reported the synthesis and secretion of several proteoglycan subpopulations by OA chondrocytes. The hydrodynamic size of the proteoglycan in that study, as in this analysis differed between "normal" and OA chondrocytes. Interestingly, Harmand *et al.* (12) failed to describe the predominating small proteoglycan (pooled Fraction IV). Those results may be reconciled with the present findings in that Harmand *et al.* (12) radiolabeled cells continuously for 4 days. By that time, the amount of pooled Fraction IV was markedly reduced (Table I). Bassleer *et al.* (26) maintained chondrocytes in clusters and did not report the presence of substantial small proteoglycan. Those measurements made by immunoreactivity with specific antibody (26) did identify proteoglycans in the size range of the pooled Peaks I and II, however. Previous reports of increased retention of newly synthesized proteoglycans in the cell-associated fraction by OA chondrocytes (4, 12) received no support from the present studies. However, the OA chondrocytes retained hydrodynamically smaller proteoglycans in greater amounts than did their nonarthritic counterparts.

Significant postsynthetic transformations dominate the metabolic pathway resulting in the integration of high density proteoglycan into the extracellular matrix. In the present study, the hydrodynamic size of the secreted proteoglycan did not change with time in chase media. These results showed that under the culture conditions employed, chondrocytes obtained from nonarthritic and OA cartilage do not significantly modify in the intracellular compartment the glycosylated form of proteoglycan.

These studies support the hypothesis that phenotypic expression of proteoglycan is altered during OA. They also address the potential differences occuring when chondrocytes are compared with explant culture. For example, proteoglycan synthesis by fibrillated car-

tilage was almost indistinguishable from proteoglycan synthesis by discolored nonfibrillated cartilage of the same joint (27). In recent studies, similar proteoglycan synthesis profiles on Sepharose CL-2B were seen when normal and OA femoral head cartilage was maintained in explant culture (28, 29). Thus, the establishment of chondrocyte cultures from nonarthritic and OA cartilage appear to accentuate the potential differences in proteoglycan structure not previously appreciated in organ culture. The significance of the differences in the secreted forms of proteoglycan remains to be established. In part, differences between nonarthritic and OA chondrocytes may reflect the initial cell population derived from tissues of differing thickness (30). However, the reduced secretion of high density proteoglycan, the emergence of a small proteoglycan and the increase in low density proteoglycan by OA chondrocytes may be expected to markedly affect the long-term survival of the affected synovial joint.

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