Proteoglycans synthesized by chondrocytes in alginate beads are found in two compartments: the cell-associated matrix and the further removed matrix (Häuselmann, H. J., Aydelotte M. B., Schumacher B. L., Kuettner K. E., Gitelis, S. H., and Thonar, E. J.-M. A. (1992) Matrix 12, 116-129). To study the metabolism of aggrecan in these two compartments, mature articular chondrocytes in alginate beads were pulsed with [35S]sulfate for 30 min or 16 h on day 7 of culture and then chased in isotope-free medium for up to 21 days. At different times, the two matrix pools were separately isolated, and the [35S]-proteoglycans quantified, purified, and characterized. Radiolabeled aggrecan molecules exhibited a very long average half-life in the beads (t1/2 = 95 days). In contrast, small non-aggregate proteoglycans, which made up approximately 4% of the [35S]-proteoglycans synthesized, were rapidly lost from the beads (t1/2 = <24 h). Approximately half the [35S]-aggrecan subunits, representing mostly molecules which showed a delay in ability to form aggregates in the presence of exogenous hyaluronan and link protein, spent only a short time (t1/2 = 4 h) in the cell-associated matrix before moving into the further removed matrix. They exhibited a much longer average half-life in the beads than [35S]-aggrecan molecules which became resident of the cell-associated matrix (t1/2 = >85 days versus 15 days). Radiolabeled aggrecan subunits in the two matrix compartments had a similar average hydrodynamic size and polydispersity; importantly, the size of these molecules did not change during the chase period. Catabolism of [35S]-aggrecan in the cell-associated matrix was the only significant contributor to the appearance in the medium of partially degraded [35S]-aggrecan which had lost the ability to bind to hyaluronan. These results strongly suggest aggrecan molecules which reside in the pericellular and territorial matrix compartments in close proximity to the chondrocytes have a much faster rate of turnover than their counter-

The extracellular matrix of articular cartilage is composed of proteoglycans (PGs), collagen, and matrix proteins which are organized in the matrix to give the tissue its ability to act as a shock absorber at the end of long bones (1, 2). The matrix of mature articular cartilage is made up of three compartments (3). The pericellular (or lacunar) matrix surrounds the chondrocyte plasmalemma; it is a very thin rim characterized by the absence of cross-banded fibrillar collagen but is very rich in PGs. The territorial (or capsular) matrix lies adjacent to this; it has a fine network of fibrillar (cross-banded) collagen extending around individual chondrocytes and groups of chondrocytes termed chondrons. The interterritorial and outermost matrix constitutes the largest domain; it is characterized by cross-banded collagen fibrils or fibers running in parallel and interspersed with PGs.

Most of the collagen molecules in human adult articular cartilage are believed to have extremely long half-lives in vivo (>100 years) (4). However, the minor collagens, i.e. types IX and XI, which play important roles in the formation and stability of the cross-linked fibrils (5), may have a faster rate of turnover than type II, the collagen which makes up 90-95% of the mature fibril (6). It is generally agreed that cartilage PGs, including aggrecan (the most abundant PG in this tissue), have a faster rate of turnover than the collagens (4, 7). Aggrecan is synthesized as a monomer which consists of a core protein to which are covalently attached numerous chains of chondroitin sulfate, keratan sulfate, and N-linked as O-linked oligosaccharides (1). The core protein has three globular domains (G1, G2, and G3) separated by two non-globular regions, the longest of which contains most of the carbohydrate side chains (8, 9). The G1 domain interacts specifically with hyaluronan (HA) and link protein molecules to form the large sized aggregates which become firmly entrapped within the fibrillar collagen network (1, 2, 9). Studies of the turnover of PGs in vivo have suggested that the articular cartilage matrices in adult guinea pigs (10) and rabbits (11) contain at least two pools of PG molecules with different rates of turnover. Support for this contends was presented by Maroudas et al. (7) who measured racemization of aspartic acid in aggrecan and its fragments to estimate the average age of aggregating PG molecules in adult

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human articular cartilage. Their results suggest that the average half-life of the aggregating PGs is approximately 2 years, but that a pool of the aggrecan molecules has a much faster rate of turnover. Autoradiographical studies of adult articular cartilage explants labeled briefly in vitro with \(^{35}\)S-sulfate and then cultured for various periods of time in isotope-free medium have suggested that the metabolism of PGs is much more dynamic in the matrix which surrounds individual chondrocytes or groups of chondrocytes (chondrons) than in the abundant interterritorial matrix which is further removed from the cells (1, 12, 13). Based on these observations, Hascall et al. (2) recently proposed that PGs exhibiting a high rate of turnover represent a distinct pool of molecules located in close proximity to the chondrocyte membrane.

We recently have demonstrated that chondrocytes isolated from adult articular cartilage and cultured in alginate beads remain phenotypically stable for up to 8 months (14, 15). In this negatively charged gel, the cells reestablish a cell-associated matrix rich in PGs, especially aggrecan (14), and a cross-linked collagen network from joining collagen types II, IX, and XI (15). A major advantage of the alginate bead system is that solubilization of the beads under assay conditions followed by mild centrifugation permits the separation of the cells with their cell-associated matrix from matrix molecules which reside in matrix further removed from the chondrocytes (14). In this report, we describe experiments designed to study the metabolism of PG molecules in these two morphologically distinct matrix pools. We describe the movement of a proportion of newly synthesized aggrecan molecules from the cell into the cell-associated matrix where these molecules become resident and of the remainder into the further removed matrix where they accumulate into aggregates. The structure and turnover of PGs in these two matrix pools were analyzed in detail.

**EXPERIMENTAL PROCEDURES**

**Materials**

Pronase was purchased from Calbiochem, collagenase P (Clostridium histolyticum) from Boehringer Mannheim, and pepsin, sodium cacodylate, and CHAPS from Sigma. Fetal bovine serum was from HyClone, Logan, UT. Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium from National Diagnostics, Manville, NJ. All other chemicals were reagent grade and purchased from several different companies. Aggrecan monomer (D1) at 1 mg/ml was then added to each aliquot and resuspended at a density of 4 x 10^6 cells/ml in sterile alginate solution (1.2% alginate in 0.15 M NaCl). The cell suspension was then slowly expressed through a 22-gauge needle and dropped into a 102 mm CaCl₂ solution. The beads were allowed to polymerize in this solution for 10 min before two consecutive washes with 10 vol of 0.15 M NaCl followed by washes in DMEM/F-12 (1:1). The beads were placed in culture medium (500 beads/25 ml) consisting of DMEM/F-12, 10% fetal bovine serum, 25 µg/ml ascorbic acid, 10 µg/ml gentamicin. Batch cultures were kept at 37 °C in a humidified atmosphere of 5% CO₂. The medium was replaced daily. Each bead contained approximately 40,000 chondrocytes.

**Labeling of Chondrocytes with \(^{35}\)S-sulfate**

**30-min Pulse Experiment—**On day 7 of culture, 300 beads were incubated in 5 ml of fresh complete medium. After 1 h, the medium was replaced with fresh complete medium containing \(^{35}\)S-sulfate at 50 µCi/ml. After 30 min, the medium was harvested, and the beads were washed extensively (2 x 30 min at 4 °C with cold medium supplemented with 1.5 mM MgSO₄ and 1 x 30 min at 4 °C with cold complete medium), using gentle rocking at all times to promote the removal of unincorporated radiolabel. The labeled washes containing less than 0.1% of the total incorporated \(^{35}\)S radioactivity were not analyzed further.

After the washing procedure, the beads were placed into 24-well culture plates (9 beads/well) and incubated in 0.4 ml of complete medium. After various times of chase (up to 24 h), medium was removed from individual wells, and the beads were dissolved by incubation for 10-15 min at 4 °C in 10 volumes of 5 mM sodium citrate, 30 mM Na₂EDTA, 0.15 M NaCl, pH 6.8. The resulting suspension was centrifuged at 900 rpm for 10 min at 4 °C to sediment the cell-associated matrix (in the pellet) from constituents of the further removed matrix (in the supernatant) (14). The cell pellets were then extracted for 48 h at 4 °C with 4 ml guanidine HCl in the presence of proteinase inhibitors (10 mM Na₂EDTA, 0.1 mM 6-aminohexanoic acid, 5 mM benzamidine hydrochloride, 10 mM N-ethylmaleimide, and 0.5 mM phenylmethylsulfonyl fluoride) (14, 19), the suspension then was centrifuged at 5,000 rpm for 10 min at 4 °C to remove particulate matter. This procedure solubilized >98% of the \(^{35}\)S-PGs in the pellet fraction. For each time point, the supernatant and corresponding 4 ml guanidine HCl extract were separately frozen at −80 °C until analyzed. The results presented below for the two matrix pools at each time point represent the mean of data obtained from three separate cultures of nine beads each.

**16-h Pulse Experiment—**Three hundred beads prepared on day 7 as described above were labeled with 20 µCi/ml of \(^{35}\)S-sulfate for 16 h. The beads were then washed at 4 °C, twice with 30 ml of fresh culture medium supplemented with MgSO₄ at 1.5 mM (45 min each), and then twice with 30 ml of normal complete medium (45 min each), using gentle rocking at all times to promote the removal of unincorporated radiolabel. After incubation, the beads were washed for times up to 21 days in complete medium. The medium was changed daily from 10% fresh medium to fresh medium. For each time point studied, the beads were collected, solubilized and \(^{35}\)S-PGs in each of the two matrix pools isolated and stored as frozen as described in the protocol for the 30-min pulse. The results for the medium and the two matrix pools at each time point represent the mean of data obtained from three separate cultures.

**Quantification of Total and Radiolabeled PGs**

Aliquots from each chase medium and supernatant fraction were first made 4 ml by diluting 1:1 with 8 ml guanidine HCl. At each time point, total PG content in the supernatant fraction and 4 ml guanidine HCl extract was measured by a previously described immunoelectrophoretic assay (20). An equal volume of a 4 ml guanidine HCl containing aggrecan monomer (D1) at 1 mg/ml was then added to each aliquot and to aliquots from each 4 ml guanidine HCl extract of the cell pellet. The samples were then subjected to chromatography on Sephadex G-25 (PD-10) columns equilibrated and eluted with 4 ml guanidine HCl. Radiolabeled PGs eluting in the void volume (V0) of the column were quantified by liquid scintillation counting (21).

**Determination of PG Size on Sepharose CL-2B**

Aliquots from selected media and supernatant fractions made 4 ml by the addition of an equal volume of 8 ml guanidine HCl, and 4 ml guanidine HCl extract were electrophoresed on a Sephacryl S-200 column in 2x SSC (1 x SSC = 150 mM NaCl, 15 mM Na₂CO₃, 0.05 x Na acetate, 0.05% CHAPS, pH 6.0. Fractions (approximately 1.0 ml) were collected and aliquots assayed for radioactivity by
liquid scintillation counting. The void volume (Vv) and total volume (Vt) of the column were determined using high molecular weight soluble proteins in the supernatant of a micrococcus suspension and free \[^{35}S\]sulfate, respectively (14). The partition coefficient of PGs in each column fraction was calculated as follows: \( K = (V_t - V_V)/V_V \) where \( V_t \) represents the elution volume for each column fraction.

**Determination of Aggregating Ability of PGs**

High molecular weight HA (Healon) and bovine link protein were added to selected samples (media, supernatant fractions, and extracts of the cell pellet) in 4 M guanidine HCl, 0.1 M NaSO\(_3\), 0.05 M sodium acetate, pH 6.0, containing the protease inhibitors at the concentrations listed above, to make the ratios of PG/HA ~20 (w/w) and of PG/link protein ~1 (mol/mol). After incubation for 3 h at 4 °C, the samples were brought to associative conditions by dialysis at 4 °C for 24 h against 0.05 M sodium acetate, pH 6.8. To determine what proportion of the aggregating molecules had formed aggregates, the samples were subjected to chromatography on a Sepharose CL-2B column (0.6 x 100 cm) equilibrated and eluted with 0.05 M sodium acetate, pH 6.8. Fractions (~1 ml) were collected and aliquots assayed for radioactivity. Because alginate in the supernatant fractions of the solubilized beads caused rapid clogging of the Sepharose CL-2B column (associative conditions), the aggregating ability of \[^{35}S\]-aggrecan molecules in these fractions was assessed using a Sephacryl S-1000 column (0.6 x 100 cm) instead. The \( V_t \) and \( V_V \) of the columns were determined in each case using purified aggregated \[^{35}S\]-PGs and free \[^{35}S\]sulfate, respectively. Partition coefficients were calculated as described above. Radiolabeled PGs eluting in the \( V_t \) of the Sepharose CL-2B column were considered to represent aggregates. In the case of the Sephacryl S-1000 column, \[^{35}S\]-PGs eluting in the \( V_V \) as well as in the “shoulder” of the \( V_t \) peak (partition coefficients <0.28) were considered to represent aggregates; PGs in this shoulder were shown to migrate in the \( V_V \) of the Sepharose CL-2B column and thus probably represent aggregates of small size.

**Histology**

Day 7 beads were solubilized by the addition of 10 volumes of 55 mM sodium citrate, 90 mM NaCl, pH 6.8. The chondrocytes with their cell-associated matrix were recovered by mild centrifugation as described above and then resuspended in this buffer. After simultaneous fixation and Alcian blue staining, as described previously (14, 18), the cells and their cell-associated matrix were visualized by light microscopy. Day 7 beads also were incubated for 20-15 min in 100 mM BaCl\(_2\) to replace the calcium multivalent ions by barium ions which cause irreversible polymerization of the alginate molecules and thus help promote retention of the alginate bead shape while fixing overnight in 2.5% glutaraldehyde, 10% cetyl pyridinium chloride, 2% sucrose. The fixed beads were then embedded in paraffin followed by sectioning (5-μm thick sections) and processing for immunostaining with the 1/20/5-/~4 anti-keratan sulfate monoclonal antibody, as described previously (14).

**Autoradiography**

Algin beads labeled for 30 min with \[^{35}S\]sulfate on day 7 of culture were rinsed extensively and then subjected to autoradiography, either immediately or after 24 h of chase in fresh medium. The results shown in Fig. 2, A and B, suggest that most of the \[^{35}S\]-labeled macromolecules were still present in the cell or the cell-associated matrix at the end of the 30 min pulse. After 24 h of chase, the radiolabel showed a more diffuse distribution (Fig. 2, C and D). While radiolabeled molecules were still found at a higher concentration in the vicinity of the cells, a much greater proportion of the \[^{35}S\]-labeled macromolecules appeared to have reached the further removed matrix.

**Kinetics of Movement of Newly Synthesized \[^{35}S\]-PGs**

At the end of the 3 x 30 min rinses (at 4 °C) which followed the 30-min pulse with \[^{35}S\]sulfate, 80% of the newly synthesized \[^{35}S\]-PGs were recovered with the chondrocytes in the pellet (Fig. 3A). Most of the \[^{35}S\]-PGs in this fraction probably were present in the cell-associated matrix since the time between glycosaminoglycan chain synthesis in the Golgi apparatus and the appearance of PG molecules extracellularly is short (5-10 min) (25). During the 24-h chase period, approximately 45% of these \[^{35}S\]-PGs moved rapidly (\( t_{1/2} = 3.4 \) h) out of this matrix pool into the further removed matrix compartment. Fig. 3B shows that the disappearance of this population of \[^{35}S\]-PGs from the cell-associated matrix followed first order kinetics. The content...
Fig. 2. Autoradiographs of chondrocytes labeled with [35S]sulfate for 30 min, before and after a 24-h period of chase. After 7 days in culture, the cells were pulsed with [35S]sulfate for 30 min, without (A, B) or with (C, D) subsequent culture for 24 h in fresh complete medium as described in the text. The beads were fixed, embedded, and sectioned, and the sections were processed for autoradiography. After development, the sections were stained with methylene blue and azure II and then photographed using bright-field (A, C) and dark-field (B, D) microscopy. Bar = 20 μm.

Fig. 3. Kinetics of movement of newly synthesized 35S-proteoglycans from the cell-associated matrix to the further removed matrix. On day 7 of culture in alginate, chondrocytes were labeled for 30 min with [35S]sulfate and, after washing of the beads, cultured in isotope-free medium for up to 24 h. At each time point, medium was collected, and the beads were processed to isolate and quantify 35S-PGs present in the cell-associated matrix and further removed matrix, respectively. The value at each time point reflects the mean of the analysis of three separate cultures; standard deviations were in all cases <5%. A, the counts/min values for 35S-PGs in the cell-associated matrix were fitted to the “single exponential decay” equation: $y = ae^{-bt} + c$, starting at $a + c$ when $x = 0$ and decreasing to $c$ with increasing $x$ when $b > 0$. The counts/min values for 35S-PGs in the further removed matrix were fitted to the “single exponential rise to maximum” equation: $y = a(1 - e^{-bt}) + c$, which starts at $c$ and rises to $a + c$ with a time constant of 1/b. B, for the cell-associated matrix, the counts/min value for 35S-PGs remaining in this pool at the 24 h time point (cpm$_{\text{min}}$) was deducted from radioactivity present at each time point (cpm$_{\text{tot}}$). The linearity of the curve drawn from these points shows that the disappearance of the cell-associated matrix followed first order kinetics.

of 35S-PGs in the beads decreased by only 5% during the chase period; approximately half of these radiolabeled PGs were recovered from the medium (Fig. 3A).

Characterization of the Rate of Catabolism and Structure of 35S-PGs in the Two Matrix Pools

Rate of Catabolism—Approximately half the 35S-PGs synthesized during the 16-h pulse had reached the further removed matrix by the end of the pulse and the rinsing stages (Fig. 4), confirming that about half the PGs synthesized by the cells spend less than 24 h in the cell-associated matrix before moving into the further removed matrix. Radiolabeled PGs in the cell-associated matrix at that time appeared to consist of two populations with markedly different $t_{1/2}$ in this pool (4 h and 15 days, respectively); the half-lives of these radiolabeled PG populations were considerably shorter than the average half-life of 35S-PGs in the beads ($t_{1/2} = 95$ days). Importantly, 35S-PGs which reached and became localized in the further removed matrix showed no evidence of turning over in this pool during the 21 days of culture. The rate of appearance of 35S-macromolecules in the medium was faster during the first 48 h of the chase period than at later times: 3.1% of the incorporated radioactivity appeared in the medium during the first 48 h of chase versus 7.2% between days 7 and 21. Summation of the total cpm in 35S-macromolecules present in the two matrix pools, and daily medium changes revealed that approximately 5% of the radiolabeled macromolecules disappear from the system during the first 48 h of chase (Fig. 4). It is possible this represents a subpopulation of cell surface PGs which are internalized and degraded intracellularly.

Characterization of 35S-PGs Appearing in the Medium—Daily medium samples collected at the end of the first 24 h of chase (day 1) and on day 12 of the chase period were chromatographed on a Sepharose CL-2B column eluted with a dissociative solvent. Three peaks of 35S-molecules were detected in day 1 medium (Fig. 5). The average hydrodynamic size of the first population (average $K_d = 0.37-0.40$), which made up 20% of the radioactivity present, was similar to that of aggrecan molecules remaining in the bead. The average hydrodynamic size of the second population (average $K_d = 0.72$) suggests that it represents small non-aggregating PGs synthesized in small amounts by mature bovine articular chondrocytes (14, 15). Nearly half the radioactivity in day 1 medium was present in a third peak eluting in the $V_r$. This probably represented, at least in part, unincorporated [35S]sulfate which was not removed during the rinsing stages performed prior to the chase period proper. However, this radiolabeled material may be derived in part from the catabolism of PGs internalized and degraded intracellularly (26). On day 12 of the chase period, this $V_r$ fraction represented
Fig. 4. Distribution of 35S-proteoglycans in the two matrix pools at various times after synthesis in vitro. On day 7 of culture in alginate, chondrocytes were labeled for 16 h with [35S]sulfate and, after washing of the beads, cultured in isotope-free medium for up to 24 h. At each time point, 35S-PGs present in the medium, cell-associated matrix and further removed matrix were quantified as described in the text and in Fig. 3. Total refers to the sum of the counts/min values in these three pools. For the medium, the counts/min value at each time point reflects the total amount of 35S-PGs which had appeared into this compartment since the beginning of the chase period. The data for free 35S-PGs and radiolabeled molecules in the cell-associated matrix were fitted to the double exponential decay equation: \[ y = ae^{-bx} + ce^{-dx}, \]
starting at \( y = 0 \) and decreasing to \( y \) with increasing \( x \) when \( b, d > 0 \). The values for serum were fitted to the "single exponential rise to maximum" equation described in the legend to Fig. 3. The curve for the further removed matrix then was drawn automatically by the computer program.

Fig. 5. Sepharose CL-2B molecular sieve chromatography (dissociative conditions) of 35S-proteoglycans isolated from the medium and two matrix pools at various times after synthesis. Chondrocytes were pulsed with [35S]sulfate for 16 h and the radiolabel was chased for varying periods of time, as described in Fig. 4. Proteoglycans in the medium and each of the two matrix pools at selected time points were chromatographed on a Sepharose CL-2B column, equilibrated and eluted with a dissociative solvent, as described in the text. The position of elution is given in each case in terms of partition coefficients.

Fig. 6. Molecular sieve chromatography (associative conditions) of 35S-proteoglycans recombined with HA and link protein after isolation from the medium and two matrix pools. Radiolabeled proteoglycans isolated from the medium and each of the two matrix pools at various times of chase after a 16-h pulse with [35S]sulfate were allowed to reform aggregates in the presence of exogenously added high molecular weight HA (Healon) and bovine link protein, as described in the text. The proportion of 35S-proteoglycans which formed aggregates is indicated in each case and was calculated as described in the text.

only a minor proportion (approximately 5%) of the radioactivity in the medium (Fig. 5).

After interaction with exogenous HA and link protein followed by dialysis, 38% of the 35S-PGs in day 1 medium eluted in the \( V_c \) of a Sepharose CL-2B column equilibrated with an associative solvent (Fig. 6A). This suggests that the 35S-PGs eluting with an average \( K_d = 0.40 \) on the Sepharose CL-2B dissociative column represent intact aggrecan molecules which have retained their ability to aggregate. These aggregating PGs made up only a very minor proportion of the 35S-macromolecules which diffused into the medium on day 12 of the chase period (Fig. 6A): most of these 35S-PGs eluted from the Sepharose CL-2B dissociative column as a major peak with average \( K_d = 0.58 \) (Fig. 5) and probably represent aggrecan molecules which have undergone proteolysis and are no longer able to bind to HA (27). Small non-aggregating 35S-PGs were not present in significant amounts in day 12 medium.

Characterization of 35S-PGs in the Two Matrix Pools—35S-PGs extracted or released from the two matrix pools consisted predominantly of a single population of molecules (average \( K_d = 0.37-0.40 \) on Sepharose CL-2B, dissociative conditions) which did not undergo a measurable decrease in size throughout the chase period (Fig. 5). The size of these molecules is consistent with the view that they represent intact aggrecan molecules. The majority (73%) of 35S-aggrecan molecules isolated from the cell-associated matrix of beads that were not subjected to the period of chase formed aggregates in the presence of exogenously added high molecular weight HA and bovine link protein, as demonstrated by their elution in the \( V_c \) of a Sepharose CL-2B column under associative conditions. A slight but reproducible increase in the functionality of the G1 domain of the 35S-aggrecan molecules in this pool was noted after 12 days of chase. In contrast, the majority of 35S-aggrecan molecules appeared to have a non-functional GI domain upon arriving in
proportion of these non-aggregating molecules gain a functional G1 domain, resulting in an increase from 34% to 69% in the aggregated form under these conditions.

DISCUSSION

The studies presented here provide new evidence of the usefulness of the alginate bead system for studying the catabolism and turnover of cartilage aggrecan. In this system, mature bovine articular chondrocytes synthesize aggrecan molecules which become incorporated into aggregates as well as small amounts of non-aggregating PGs of small size. The single most important finding of our studies is the observation that catabolism of the newly synthesized aggrecan molecules occurs almost exclusively in the cell-associated matrix (Fig. 4). The average half-life of the population of aggrecan molecules which were still present in the cell-associated matrix after 48 h of chase, when movement of intact aggrecan molecules from the cell-associated matrix to the further removed matrix had essentially stopped, was significantly shorter than that of molecules which had become resident of the further removed matrix (half-lives = 15 days versus longer than 95 days). Importantly, the disappearance of these molecules from the cell-associated matrix during the chase period correlated closely with the appearance of an equivalent amount of 35S-PGs in the medium. Further, the disappearance of the population of 35S-PGs with a t1/2 of 15 days appeared to correlate with the initial appearance of non-aggregating 35S-aggrecan fragments (average Kt = 0.57) in the medium on day 7 and their continued release into the medium through to day 12 (Fig. 5). These findings thus add considerable support for the contention (2) that turnover of aggrecan in adult articular cartilage is restricted almost exclusively to a metabolically active matrix pool which possibly corresponds to the pericellular and territorial matrix compartments which surround individual chondrocytes in cartilage (3). The aggrecan molecules in this cell-associated matrix are present as individual tethered aggregates which appear to be bound to hyaluronan receptors on the cell membrane and extend outward in a brushlike configuration (28).

In our alginate bead system, aggrecan molecules in the further removed matrix exhibited an average half-life of 24 h (Kt = 0.27). These molecules which become incorporated into aggregates as well as small amounts of non-aggregating PGs of small size. The single most important finding which have been shown to accumulate in adult articular cartilage with age (37, 38), we do not know if the G1 domains of aggrecan molecules cleaved by aggrecanase (27, 35) were retained in the alginate beads. The alginate bead culture system could prove most useful to determine how soon after synthesis this HA-binding fragment is produced and if it accumulates in one or both matrix pools.

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Metabolism of Cartilage Aggrecan in Two Distinct Pools


