Time-Related Distribution Profiles of Sulfated Glycosaminoglycans in Cells, Cell Surfaces, and Media of Cultured Rat Liver Fat-Storing Cells (43193)

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Abstract. **Fat-storing cells (perisinusoidal lipocytes, Ito cells), the principal proteoglycan-producing cell type in liver, were maintained for various times in primary and secondary culture to monitor the amount and pattern of [^{35}S]sulfate-labeled glycosaminoglycans (GAG) in the cells, on the cell surface, and in the medium. In parallel with the phenotypic modulation of fat-storing cells toward myofibroblast-like cells, intracellular GAG decrease progressively, whereas cell surface-bound and medium GAG increase severalfold. These changes are associated with time-dependent alterations of the pattern of GAG in the various compartments. Dermatan sulfate is the most prominent intracellular GAG type in primary cultures, but on the cell surface and in the medium chondroitin sulfate prevails and reaches almost 70% of all medium GAG in secondary cultures. The results point to a highly dynamic expression of the specific types of GAG in the cellular and extracellular compartments of fat-storing cell cultures that seems to accompany the spontaneous transformation into myofibroblast-like cells. The latter one is a mainly chondroitin sulfate-producing cell type, whereas the initial fat-storing cell generates predominantly dermatan sulfate.** [P.S.E.B.M. 1991, Vol 196]

The extracellular matrix of human and animal liver contains, besides several types of collagens (1–4), a number of glycoconjugates, among which sulfated proteoglycans constitute a major fraction (5–8). Proteoglycans are composed of an as yet not clearly characterized core protein (9) and *N*- or *O*-linked polysaccharides (glycosaminoglycans) (10–12). The glycosaminoglycans (GAG) represent unbranched chains of repeating disaccharide units of substituted hexosamines and hexuronate that become highly anionic through *N*- or *O*-sulfation and acetylation. In liver, three main types are known that are, in order of decreasing concentrations, heparan sulfate (more than 60% of total GAG), dermatan sulfate, and chondroitin-4,6-sulfate isomers (5–8, 13). As a pure glycosaminoglycan not attached to a core protein moiety, hyaluronan (14) is present in low amounts in normal liver extracellular matrix (6–8). The amount of sulfated proteoglycans in normal liver tissue is low, but in pathologic states such as liver fibrosis (6–8, 15) and hepatocellular carcinoma (16, 17), the total amount increases severalfold and the profile of GAG changes dramatically, leading to a strong absolute and relative increase of dermatan sulfate and chondroitin sulfate and a fractional decrease of heparan sulfate (lower than 50%) (18).

Fat-storing cells termed also perisinusoidal lipocytes, vitamin A-storing cells, and Ito cells (19, 20) have been identified as the main cellular source of sulfated GAG in liver as deduced from their ability *in vitro* to synthesize and secrete a broad spectrum of sulfated GAG (21, 22) and hyaluronan (23). The pattern of secreted GAG resembles that established in fibrotic liver extracellular matrix (24). However, the data reported on the fractional secretion rate and on the distribution profile of sulfated GAG are not consistent (21, 22), which suggests that the GAG profile of fat-storing cells

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in the medium and in the cell layer might change in parallel with the phenotypic transformation of fat-storing cells to myofibroblast-like cells. The latter type of cells evolves spontaneously *in vitro* but is present also in liver tissue in areas of necroinflammation (25–31). This cell type is supposed to be the pathogenetic important site of (perisinusoidal) fibrogenesis (32, 33). Myofibroblast-like cells develop from activated fat-storing cells via an intermediate phenotype, i.e., the transitional cell (27, 28). Up to now, no data have been available on the time-dependent changes of the expression of sulfated GAG in the cell layer, medium, and pericellular fraction of fat-storing cells during primary and secondary culture in which myofibroblast-like cells become prominent.

Materials and Methods

Isolation, Characterization, and Culture of Fat-Storing Cells from Rat Liver. Fat-storing cells (perisinusoidal lipocytes) were prepared from 1-year-old male Sprague-Dawley rats (body weight 500–700 g) receiving a standard rat diet containing 1500 units of vitamin A/kg. Nonparenchymal liver cells were isolated by the Pronase-collagenase method of De Leeuw *et al.* (34) in a sequence of non- and recirculating perfusions incorporating some minor modifications described in detail elsewhere (22). Fat-storing cells were purified from the nonparenchymal liver cell suspension by single-step density gradient centrifugation with Nycodenz (analytical grade; Nyegaard and Co. AS, Oslo, Norway), as described previously (22). The purity of freshly isolated cells was at least 90%, cell viability checked by trypan blue exclusion was more than 80%, and the yield ranged from $10 \cdot 10^6$ to $50 \cdot 10^6$ cells/liver. After the first change of medium, most of the contaminating cells were removed, and the monolayers were essentially free of impurities. Fat-storing cells were identified by their typical light microscopic appearance, transmission electron microscopy (22), positive indirect immunofluorescence staining for desmin (35), and vitamin A-specific autofluorescence at an excitation wavelength of 328 nm (22). Impurities were recognized by peroxidase staining and latex phagocytosis as reported previously (36). The cells were seeded at an initial density of $0.40 \cdot 10^6/10$ cm^2 and maintained as monolayers in 6-well culture plates (Falcon; Becton & Dickinson, Oxnard, CA) in 2 ml of Dulbecco's modification of Eagle's medium (Flow Laboratories GmbH, Bonn, Federal Republic of Germany) containing 4 mmol of L-glutamine/liter, penicillin (100 kU/liter), streptomycin (100 mg/liter), and 10% fetal calf serum (Boehringer GmbH) in a humidified atmosphere at 5% CO_2 and 95% air. The first change of medium was made about 20 hr after seeding, and the following changes were made at 2-day intervals. For subcultivation, 10-day-old primary cultures were trypsinized (10 min, 0.5 g/liter trypsin, 0.2 g/liter EDTA in phosphate-buffered saline, pH 7.4) and seeded at a 1:5 split ratio. The effect of trypsinization and passage on GAG synthesis was studied also with cells at the third day of culture. One portion of cultured cells was trypsinized as described above and reseeded with the same cell number at a plating efficiency of 64%, i.e., at 40% lower than the initial density, whereas the other portion was maintained in primary culture including only a medium change. Two days after this procedure, the trypsinized and parent cultures were labeled and processed as described below.

Isolation and Determination of Total Glycosaminoglycans. At various times of culture, fat-storing cells were incubated for 48 hr with 50 μCi/ml (1.85 MBq/ml) medium of [^{35}S]sulfate (18.5–22.2 GBq/mmol; New England Nuclear, Boston, MA) for labeling of sulfated GAG, which were separately isolated from the cell layer, cell surface, and medium, respectively. The addition of the isotope was preceded by a change of medium. After aspiration of the medium, the cell layer was rinsed five times with phosphate-buffered saline containing unlabeled GAG and, thereafter, treated for 20 min at 37°C with trypsin (0.5 g/liter):EDTA (0.2 g/liter; Boehringer Mannheim, GmbH) in phosphate-buffered saline, during which time the cells detached from the well and membrane-associated GAG were released. The cell suspension was cooled and centrifuged (5 min, 1000*g*, 4°C). The supernatant containing the trypsin-removable, previously cell surface-bound GAG, the resuspended cell pellet (containing intracellular GAG), and the medium (after centrifugation for 6 min, 1000*g* at 4°C), respectively, were proteolyzed with papain (EC 3.4.22.2; Boehringer GmbH) for 48 hr at 60°C in 0.1 *M* sodium acetate, pH 6.2 (22). Proteolysis was terminated by cooling the samples in ice. The supernatant obtained after centrifugation (4500*g*, 10 min, 4°C) was mixed with unlabeled GAG (hyaluronic acid, heparin, chrondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate; all from Sigma) used as carrier (each at a final concentration of 0.12 mg), and total GAG were bound during an incubation of 45 min to a batch of DEAE-Sephacel (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with 0.1 *M* sodium acetate, pH 6.2 (37). After washing the resin seven times with 0.3 *M* sodium acetate, total GAG were eluted with 2 ml of 2.2 *M* NaCl and precipitated for 12 hr at room temperature with 4 ml of absolute ethanol. After centrifugation (4500*g*, 20 min) the sediment was washed twice with 3 ml of absolute ethanol to remove NaCl. The final sediment was dissolved in 1 ml of water, of which a 100-μl aliquot was counted for total GAG radioactivity, which was referred to the DNA content of the culture. The efficiency of proteolytic GAG extraction from cells and medium was more than 90%.

Determination of Specific Types of Glycosami-

noglycans. For analysis of specific types of glycosaminoglycans, total GAG were subjected to consecutive degradations with nitrous acid to yield the incorporation of label into heparan sulfate and to enzymatic digestion with chondroitin AC- (EC 4.2.2.5) and -ABC-lyases (EC 4.2.2.4) (Seikagaku Fine Chemicals, Tokyo, Japan) to obtain the fractions of chondroitin 4,6-sulfate and dermatan sulfate, respectively. Analytical details of the procedure are reported elsewhere (22, 37). Total degradation efficiency was determined by the degradation of biosynthetically labeled rat liver heparan sulfate and of *in vitro* *N*-[^{3}H]acetyl-labeled chondroitin sulfate and dermatan sulfate (Sigma) (38); the efficiency was found to be between 85 and 96% (22). All experiments were performed in triplicate cultures; SD was found to be between 8 and 17%.

General Techniques. The amount of cells was estimated by fluorometric determination of DNA (39) using calf thymus DNA (type I; Sigma Chemical Co., Munich, Federal Republic of Germany) as a standard. Cell viability was determined with the trypan blue exclusion test.

Results

General Characterization of Cultured Fat-Storing Cells. Fat-storing cells were characterized in early cultures by abundant fat droplets expressing intense (vitamin A) fluorescence. On the fourth day, the cells showed long cellular extensions and a typical stellate shape (Fig. 1A). The cultures were free of hepatocytes, and phagocytosing and peroxidase-positive cell contaminations were estimated to be less than 5%. More than 85% of fat-storing cells on the fourth day excluded trypan blue. With advancing culture time, the number and volume of fat droplets decreased and the cells flattened (Fig. 1, B and C). During this time, the cells became strongly positive for desmin and the α-actin isoform of smooth muscle cells as shown previously by indirect immunoperoxidase staining (24). These changes were even more pronounced in secondary cultures of fat-storing cells (Fig. 1D), which were essentially free of fat droplets gaining myofibroblast-like appearance.

Distribution and Composition of Sulfated Glycosaminoglycans during Culture of Fat-Storing Cells. During primary culture of fat-storing cells, the distribution profile of total GAG changes significantly (Fig. 2). The amount of labeled intracellular GAG decreased with advancing culture time from initially 293 kBq/mg DNA (fourth day) to 98 kBq/mg DNA (14th day). At the sixth day of secondary culture, only 13 kBq/mg DNA of sulfate-labeled GAG were found in the cell layer. In contrast to the decrease of intracellular GAG, there was a doubling of cell surface-bound GAG from the fourth to the 14th day of primary culture with a further 5.8-fold increase in secondary cultures (sixth day). The most prominent quantitative changes were noticed in the medium fraction resulting in a 5.6-fold increase during primary culture (fourth to 14th day) and 22-fold elevation in secondary culture when compared with the fourth day of primary culture (Fig. 2). The strong changes of the relative distribution of total sulfated GAG during primary and secondary culture are further substantiated by the ratios given in

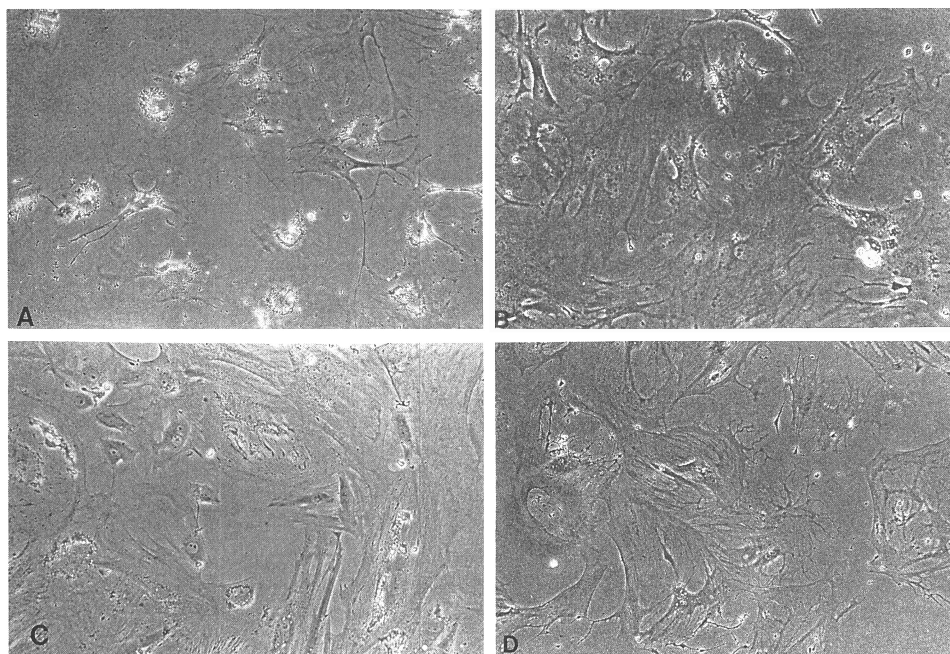

Figure 1. Phase-contrast microscopy of fat-storing cells at the fourth (A), ninth (B), and 12th (C) day of primary culture and at the eighth day of secondary culture (D) (original magnifications: A–C, ×140; D, ×70).

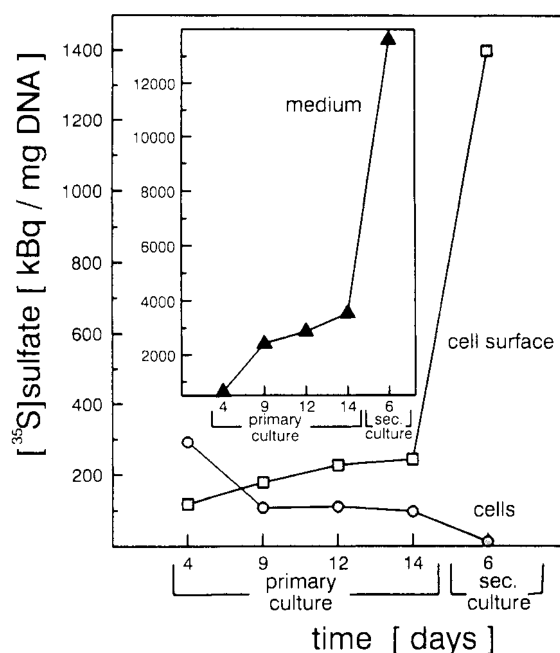

Figure 2. Distribution of total sulfated glycosaminoglycans in the medium, cell layer, and on the cell surface of fat-storing cells at various times of primary culture and at the sixth day of secondary culture. Two days before the times indicated, medium was renewed and cells were exposed for 48 hr with 50 μCi of [^{35}S]sulfate/ml. At these time points, GAG were isolated separately from cells, medium, and cell surface by proteolytic extraction and referred to the DNA content of the culture. Mean values of triplicate experiments are shown; SD was lower than 17%.

Table I. There are continuous declines of the cell to cell surface and cell to medium ratios with progressive culture time leading to extreme reductions in secondary cultures. Slight decreases were noticed also for the cell surface to medium ratios.

In conjunction with the changes of total GAG distribution, the pattern of sulfated polysaccharides in the various compartments varies with culture time (Fig. 3). In the cell layer dermatan sulfate prevails, comprising between 50 and 64% of total GAG with no significant modulation during culture time, but in secondary cultures this fraction declines below 40%. Chondroitin sulfate, representing initially only 9% of total intracellular GAG, increases gradually up to 30% (14th day) with a further strong increase up to 52% in secondary cultures (Fig. 3). The fraction of heparan sulfate declines by more than 50% from initially 30% of total intracellular GAG. The pattern of cell surface-bound GAG was found to be distinct from that of intracellular and medium GAG (Fig. 3). Chondroitin sulfate is the most prominent GAG type expressed on the cell surface of fat-storing cells, and its fraction (around 40%) does not change significantly with culture time, even in secondary cultures. Cell surface dermatan sulfate (initially 23%) and heparan sulfate (initially 40%) decline in advanced cultures, but in secondary cultures the fraction of dermatan sulfate is almost 40%. In the medium of lipocytes, chondroitin sulfate accumulates progressively with culture time from 19% at the fourth day up to 66% in secondary cultures (Fig. 3). This increase is paralleled by a strong decrease of dermatan sulfate from 70% down to 29% in secondary cultures, but the fraction of heparan sulfate in the medium is low and remains essentially constant in primary and secondary cultures.

The time-dependent changes of the amounts of specific types of GAG calculated from total sulfated GAG and the distribution pattern are summarized in Figure 4. The intracellular amounts of heparan sulfate and dermatan sulfate decrease 26- and 20-fold, respectively, when initial primary and secondary cultures are compared, whereas chondroitin sulfate remains almost constant due to its fractional increase (Fig. 3). The expression of chondroitin sulfate and dermatan sulfate on the cell surface of secondary cultures is about 13- and 24-fold stronger than in primary cultures at the fourth day. In the medium of secondary cultures, we found 75-fold higher amounts of accumulating chondroitin sulfate than in 4-day-old primary cultures. The corresponding increases of heparan sulfate and dermatan sulfate were 16- and 9-fold, respectively. The strong changes of the distribution of specific GAG during primary and secondary cultures are summarized in Table II, which shows not only significant GAG-type specific differences of the distribution ratios but also the most prominent aberrations occurring in secondary cultures.

Effect of Cell Trypsinization and Early Passage on Distribution and Composition of Glycosaminoglycans. As shown above, trypsinization and passage of 2-week-old cultures are associated with a strong increase of total GAG labeling. To see whether this observation is due to the days in culture or to trypsinization/reseeding itself with a possible removal of a small number of potentially contaminating nonparenchymal

Table I. Relative Distribution of Total Sulfated Glycosaminoglycans in Fat-Storing Cell Cultures

GAG distribution	Primary culture				Secondary culture, Day 6
	Day 4	Day 9	Day 12	Day 14	
Cell/cell surface	2.5	0.61	0.49	0.40	0.009
Cell/medium	0.46	0.04	0.04	0.03	0.0009
Cell surface/medium	0.19	0.07	0.08	0.07	0.10

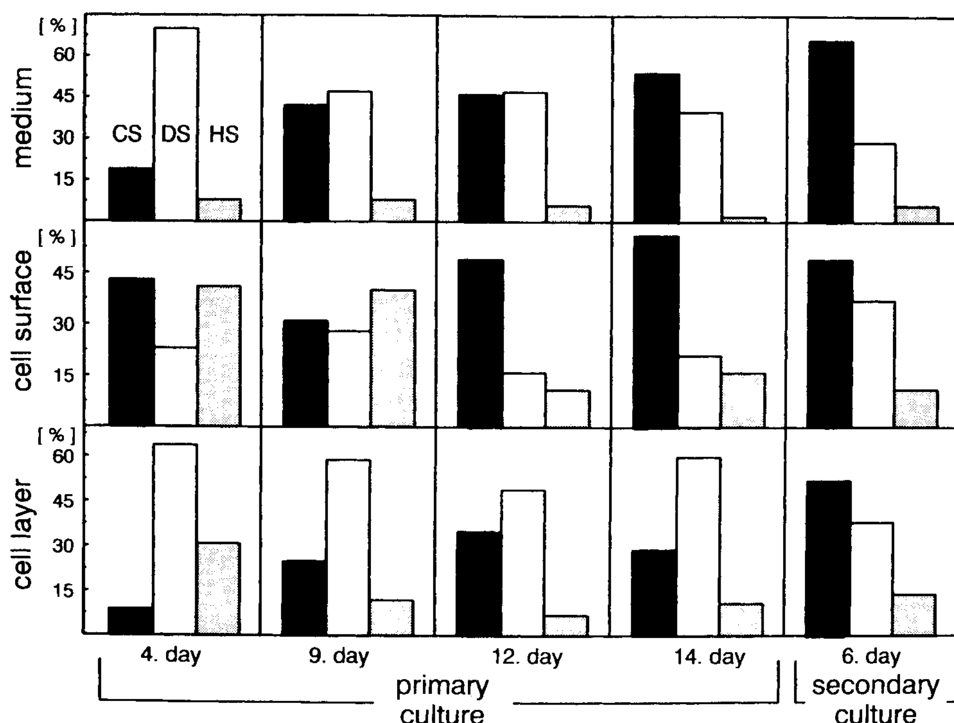

Figure 3. Pattern of specific types of sulfated glycosaminoglycans in the medium, cell layer, and on the cell surface of fat-storing cells at various times of primary culture and at the sixth day of secondary culture. Each type of GAG is expressed as percentage of total sulfated GAG. CS, chondroitin sulfate; DS, dermatan sulfate; HS, heparan sulfate.

cells producing inhibitory effects on GAG synthesis, fat-storing cells were trypsinized at the third day, reseeded at a 40% less than original density (plating efficiency, 64%), labeled, and compared with the parent, nontrypsinized culture. Morphologically there was no difference between both cultures noticed. The data presented in Figure 5 show that the synthesis of total labeled medium GAG was enhanced 2.2-fold in trypsinized cultures. Smaller degrees of stimulation were noticed for the cell surface (1.3-fold) and cellular (1.4-fold) fraction of total GAG. Compared with the data in Fig. 2, the magnitude of increases of GAG labeling by trypsinization is much smaller than that observed with the passaged older cultures. The profile of specific GAG in medium, cell surface, and cells was not changed significantly by trypsinization/passage (Fig. 6), which implies that mainly the labelings of chondroitin sulfate in the medium and cell fraction and, to a small degree, also on the cell surface were enhanced. The increases in trypsinized cultures of labeled dermatan sulfate and heparan sulfate were much smaller than that of chondroitin sulfate (Fig. 5).

Discussion

This study presents data that clearly show a time-dependent modulation of total GAG synthesis and, even more important, a compartment-related changing pattern of specific GAG composition in primary cultures of fat-storing cells and in first passage cultures. One of the most prominent findings is the shift of the mainly dermatan sulfate secreting early cultured fat-storing cell to a predominantly chondroitin sulfate-producing passaged cell type as shown by an (8.5-fold) increase of the medium chondroitin sulfate to dermatan sulfate ratio from 0.27 to 2.3. The described changes of total and specific GAG distribution accompanied the phenotypic modulation of cultured fat-storing cells (31, 34, 40–44), characterized by a loss of vitamin A stores, hypertrophy of rough endoplasmic reticulum, increase of mitotic activity, change of collagen gene expression, increased expression of desmin and smooth muscle α-actin, and acquisition of a more myofibroblast-like morphology (Fig. 1). The results are complementary to a previous report showing compartment (cellular versus extracellular)-related modulation of collagen synthesis in fat-storing cell cultures undergoing phenotypic changes to cells with the ultrastructural characteristics of myofibroblast-like cells (44). The transition of fat-storing cells to myofibroblast-like cells evolves spontaneously *in vitro* but occurs *in situ* in areas of hepatocellular damage and inflammatory infiltration (25–31). Presently, there are no generally accepted parameters available that differentiate clearly original fat-storing cells from developing myofibroblast-like cells. Therefore, we have not yet related the variations of specific GAG expression to a defined stage of *in vitro* transformed fat-storing cells. The immunocytochemical evaluation of the *de novo* expression of the smooth

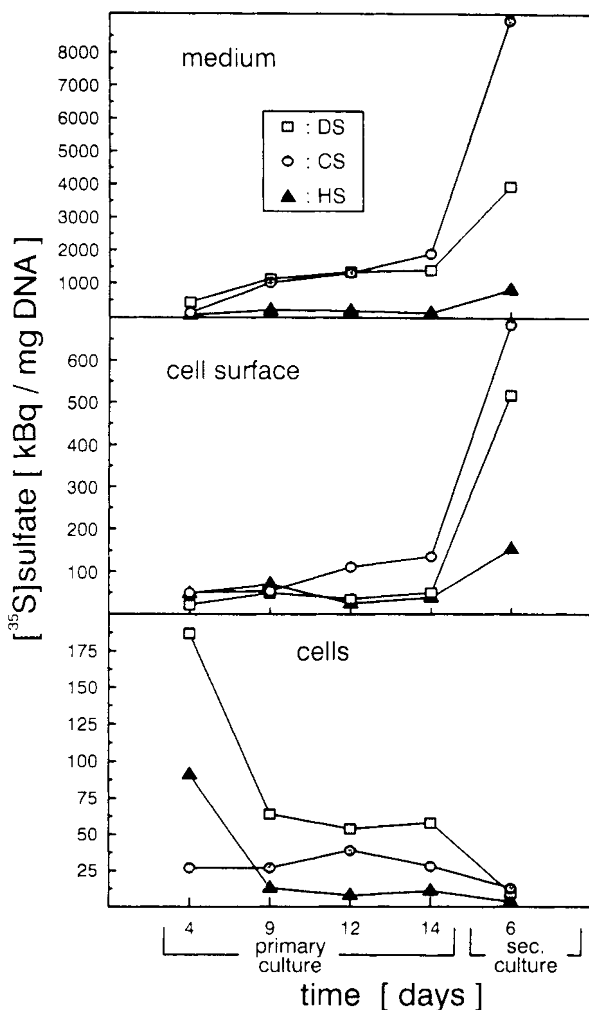

Figure 4. Distribution of specific types of sulfated glycosaminoglycans in the medium, cell layer, and on the cell surface of fat-storing cells at various times of primary cultures and at the sixth day of secondary culture. The conditions were similar to those described in the legend of Figure 1. CS, chondroitin sulfate; DS, dermatan sulfate; HS, heparan sulfate.

muscle specific α-actin isoform has been suggested recently as a useful indicator of the transformation of fat-storing cells *in vitro* (41, 43). It would be of great interest to develop experimental devices that allow in individual cells to associate the switch of α-actin negative to positive fat-storing cells with the modulation of the GAG expression in the various cellular and extracellular compartments. Our data point out that the modulation of the GAG profile occurs early in culture, which has to be recognized when data of different studies are compared. These findings are similar to observations made on GAG synthesis of smooth muscle cells of differing phenotypes in culture (45). There, it was shown that during phenotypic transition of smooth muscle cells from an early contractile to a late, serially passaged irreversible synthetic type, total GAG synthesis increases strongly and the proportions of individual GAG change significantly (45). The trypsinization experiment of young cultures shows that the rate of total GAG synthesis seems to be sensitive to manipulations of the culture conditions like trypsinization/passage and cell density. Trypsinization and reseeding at slightly lower than the initial density (due to a plating efficiency of 64%) increased total GAG synthesis more than 2-fold without changing significantly the pattern of specific GAG in the various compartments. A much stronger increase of GAG formation was observed in late passaged cultures, which might indicate that GAG synthesis in older (more transformed) and/or post-confluent cultures of fat-storing cells reacts more sensitively to a passage than in younger (not transformed) and/or not confluent cultures. We have no indications that the increase of GAG synthesis after cell passage is due to a loss of a contaminating, potentially inhibitory cell type.

We are not aware of the functional meaning of increased expression of mainly chondroitin sulfate but also of dermatan sulfate in the peri- and extracellular compartment of transformed fat-storing cells. Recently, we have identified in fat-storing cell medium small chondroitin sulfate/dermatan sulfate proteoglycan I (biglycan) and II (decorin) in addition to a novel 101-kDa core protein containing proteoglycan (46). The latter one and decorin (47) bind to type I collagen, which suggests for them a role in collagen fibrillogenesis (48, 49). A coordinate increase in the expression of extracellular collagens and certain proteochondroitin/dermatan sulfates is likely to be a requirement for the assembly of a well-structured extracellular matrix. Furthermore, the changing composition of GAG in the extra- and pericellular matrix might have direct influences on the regulation of the proliferation activity, on specific gene expression, and on the responsiveness to certain growth factors and cytokines, since it was shown before that collagenous matrices (50, 51) and a basement membrane-like matrix, i.e., the Engelbreth-Holm-Swarm murine tumor matrix (52), exert profound effects on fat-storing cell behavior *in vitro*. If the spontaneous evolution of fat-storing cells to myofibroblast-like cells *in vitro* compares with their transition in necroinflammatory liver tissue *in situ*, one might suspect that the transformed counterparts of fat-storing cells *in situ* produce and secrete chondroitin sulfate as the major fraction. However, in liver fibrogenesis, dermatan sulfate is the predominantly accumulating GAG type (6–8), which suggests that additional mechanisms might operate to reach a preferential deposition of this special type of GAG. These mechanisms might include an additional synthesis of dermatan sulfate by some cell type other than transformed fat-storing cells (e.g., fibroblasts) and/or a fractional catabolic rate of dermatan sulfate lower than that of chondroitin sulfate. Presently, no reliable data are available on the turnover rates of specific GAG in normal and fibrotic liver, but

Table II. Relative Distribution of Specific Sulfated Glycosaminoglycans in Fat-Storing Cell Cultures

GAG distribution	GAG type	Primary culture				Secondary culture, Day 6
		Day 4	Day 9	Day 12	Day 14	
Cell/cell surface	CS[a]	0.5	0.5	0.4	0.2	0.02
	DS	8.5	1.3	1.5	1.1	0.06
	HS	1.9	0.2	0.3	0.3	0.02
Cell/medium	CS	0.2	0.03	0.03	0.01	0.001
	DS	0.4	0.06	0.04	0.04	0.002
	HS	1.8	0.07	0.05	0.09	0.004
Cell surface/medium	CS	0.4	0.05	0.08	0.07	0.07
	DS	0.05	0.04	0.03	0.04	0.1
	HS	1.0	0.4	0.2	0.3	0.2

[a] CS, chondroitin sulfate; DS, dermatan sulfate; HS, heparan sulfate.

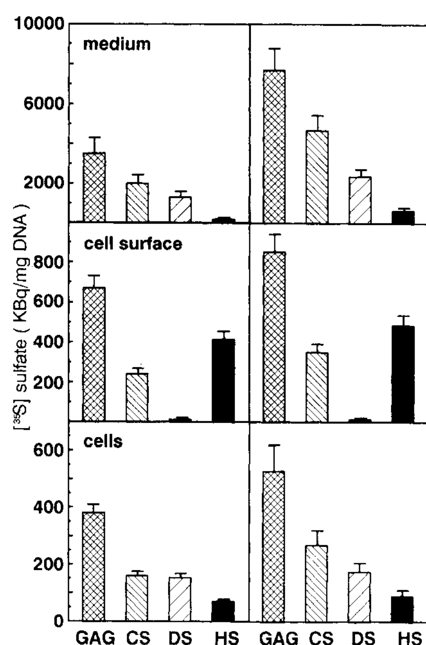

Figure 5. Distribution of total and specific types of sulfated glycosaminoglycans in the medium, cell layer, and on the cell surface of fat-storing cells at the seventh day of culture. Right panels, results of cells trypsinized and reseeded at the third culture day, kept in secondary culture for an additional 2 days before labeling for 48 hr with [^{35}S]sulfate are shown; left panels, the results obtained with the parent culture (not passaged) are given. Cell numbers similar to that of initial cultures were seeded, but the plating efficiency was 64%. Therefore, a 40% lower density of the trypsinized cells is encountered. Details of the other procedures are similar to those described in Figure 2. CS, chondroitin sulfate; DS, dermatan sulfate; HS, heparan sulfate.

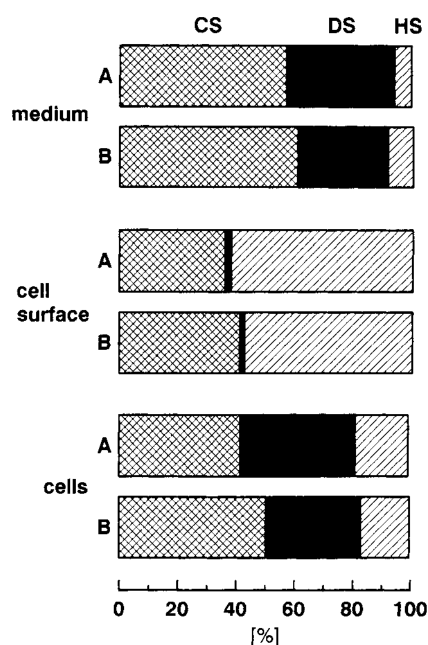

Figure 6. Pattern of specific types of sulfated glycosaminoglycans in the medium, cell layer, and on the cell surface of fat-storing cells passaged at the third day (B) and of the parent cells (A). Details and abbreviations are identical to those in Figure 5.

early work of Hutterer (53) and Aronson and Davidson (54) indicates that dermatan sulfate in contrast to other GAG types is not degraded by liver lysosomal enzymes. This finding suggests that dermatan sulfate secreted by transformed fat-storing cells accumulates in the extracellular space because of a lack of degradative enzyme activities.

The mechanisms underlying the alteration of GAG distribution and composition are not known. Although transcriptional regulation of core protein expression is likely to be involved, posttranscriptional events will be leading in the observed shift of dermatan sulfate to chondroitin sulfate since the formation of variable proportions of L-iduronic acid residues occurs by C5-epimerization of the D-glucuronic acid already incorporated into the growing polymer attached to the core protein (55). The molecular trigger of these mechanisms is not identified, but transforming growth factor β_1 (TGF-β_1) might be a candidate cytokine, since its mRNA is expressed in fat-storing cells (42), with culture time increasing activities of TGF-β are secreted (56), and TGF-β stimulates strongly the production and secretion of chondroitin sulfate (and total GAG) in primary cultures of fat-storing cells (57). Autocrine activation of chondroitin sulfate expression via TGF-β might be a possible mechanism of self-amplification of

GAG production reported here and may also occur *in situ* during fat-storing cell activation. In conclusion, the changing pattern of proteoglycans in the compartments of fat-storing cell cultures offers the possibility of multiple functional roles of proteoglycans in cellular metabolism and in regulation of liver matrix assembly during fibrogenesis.

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