Medium and cell-associated proteoglycans produced by human embryo lung fibroblasts (IMR-90) in culture were analyzed after 48-h uptake of Na<sub>2</sub>SO<sub>4</sub>. The culture medium contained three proteoglycan fractions which could be separated from one another by density gradient centrifugation and gel chromatography under dissociative conditions. The most dense component eluted from Sepharose 2B with peak $K_v = 0.4$ and contained predominantly heparan sulfate chains with $M_2 \approx 40,000$. A somewhat less dense component had peak $K_v = 0.29$ and chondroitin sulfate chains with $M_2 \approx 40,000$. The least dense proteoglycan ($\rho < 1.46$) was the predominant proteoglycan component of the medium. It eluted from Sepharose 2B at $K_v = 0.68$ and was composed primarily of dermatan sulfate-containing chains with $M_2 \approx 25,000$.

Cell-associated proteoglycans were solubilized by treatment with a zwitterionic detergent followed by extraction in 4 M guanidine HCl. By Sepharose 2B chromatography, the cells contained a heparan sulfate proteoglycan fraction similar to the most dense component of the medium. This fraction was removed from the cell surface by trypsin treatment but was not significantly displaced by exogenous heparin. The cells also contained a smaller heparan-sulfate-containing component ($K_v = 0.74$) which was not sensitive to papain digestion or alkaline treatment and may not be a proteoglycan. This component was diminished by 50% during 24-h case and probably represents an intracellular storage or degradation pool.

Glycosaminoglycans produced by fibroblasts in culture include hyaluronic acid, chondroitin sulfates, and heparan sulfate. Studies of the production and turnover of these glycosaminoglycans by cultured cells indicate that fibroblasts from specific tissues elaborate a distinct glycosaminoglycan profile (1, 2), and that certain glycosaminoglycans are preferentially located in specific cell compartments. Heparan sulfate, for example, is found at the cell surface (3), and dermatan sulfate is found primarily in the medium (4, 5).

Because the sulfated glycosaminoglycans of cartilage matrix have been demonstrated to be organized into proteoglycan molecules consisting of several polysaccharide chains linked to a core protein (6, 7), it is often assumed that all of the sulfated glycosaminoglycans are in a proteoglycan organization. There are few characterizations of proteoglycans produced by noncartilage cells in culture, however, heparan sulfate from the surface of Chinese hamster cells was first demonstrated to be proteoglycan by Kraemer and Smith (8). Subsequently, proteoglycan organization for both medium and surface heparan sulfates from human skin fibroblasts (9) and a chondroitin sulfate component from human glial cells (10) was determined. Heparan sulfate proteoglycans from the surface of rat liver cells (11) and a hepatoma (12) have been characterized. Both medium and cellular fractions from cultures of human embryonic skin fibroblasts contained large proteoglycans composed of chondroitin sulfate plus smaller proteoglycan fractions containing heparan sulfate and dermatan sulfate as well (13). Chondroitin sulfate proteoglycans released to the medium by adult rat lung cells were of one size class although cell-associated proteoglycans were of two sizes, the larger containing only chondroitin sulfate and the smaller also containing dermatan sulfate and heparan sulfate (14). Molecular characterizations of proteoglycans produced by cells in culture are valuable in developing ideas about the role of glycosaminoglycans as well as in defining the cellular origins of proteoglycans from specific tissues.

Glycosaminoglycans at cell surfaces have been implicated in such basic characteristics as cellular adhesion (15) and the related phenomena of aggregation (16) and motility (17). Glycosaminoglycans in extracellular matrix are thought to play an important physiological role in the permeability and hydration of tissues (18). Multiple interactions of fibronectin and collagen with glycosaminoglycans (particularly highly sulfated heparans) could play a major role in the deposition of insoluble extracellular matrix as well as in regulation of interactions between cells and their molecular environment (18-20). The present report describes the isolation and characterization of three proteoglycan fractions from the medium of lung fibroblasts growing on a polystyrene substrate. In addition, a surface-associated proteoglycan fraction and an intracellular glycosaminoglycan component which may not be proteoglycan are described. These results form a base-line for future investigation of the relationships between specific alterations in cellular behavior and the production and processing of fibroblast proteoglycans.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Human embryo lung fibroblasts (IMR-90) have been used in this study (21). These cells were obtained from the Institute for Medical Research, Camden, NJ and maintained at 36°C in a humified environment of 4% CO<sub>2</sub> in Eagle’s minimal essential medium supplemented with 10% newborn bovine serum. Periodic examinations for mycoplasma performed on cultures from our laboratory by the National Institute on Aging Mycoplasma Testing Service were all negative. Experiments of this study utilized cells in the proliferative phase of their in vitro lifespan; only population doubling levels 15-35 were used.

Cells were seeded into Corning polystyrene culture flasks (75 cm<sup>2</sup> or 150 cm<sup>2</sup>) in 8 or 16 ml of medium, respectively. Labeling was initiated after 4-5 days, as cultures were just reaching confluency, by...
addition of fresh medium containing Na$_2$SO$_4$, (5-20) $\mu$Ci/ml, 0.8-0.4 Ci/mmol, New England Nuclear) and sometimes [H]$^3$H$^1$serine (4 $\mu$Ci/ml, 4 Ci/mmol, ICN Pharmaceuticals, Inc.). Uptake of radioisotopes was continued for 48 h. Previous experiments have shown that accumulation of $^35$S-labeled glycosaminoglycan ([$^35$S]GAG) in the medium was linear for up to 5 days, whereas surface and intracellular accumulation proceeded at a steady state within 48 h. Dissociative conditions were initiated by rinsing the labeled monolayer three times in minimal essential medium without serum and then incubating in medium + 10% serum.

Isolation of Proteoglycans—Proteoglycans were obtained from various experiments on two 150-cm$^2$ cultures in the following way. After 48-h uptake of radioisotopes, medium was decanted and cold guanidine hydrochloride added to make it 4 M; the solution was then adjusted to 0.1 M Na$_2$EDTA, 0.05 M sodium acetate, and 0.005 M benzamidine/HCl and stored at 4°C. The cells of each flask were rinsed four times with 10 ml of Earle's balanced salt solution. Five ml of 4% Zwittergent (Calbiochem) in 0.05 M sodium acetate with added protease inhibitors was added to each flask and the cells scraped immediately from the growth surface with a rubber hoe. This cell suspension was then placed in the second flask. A 5-ml rinse of the first flask was also added to the second. Cells in 10 ml of 4% Zwittergent were left at room temperature for 20 min. After scraping all cells from the flask, it was rinsed twice with 5 ml of 0.5 M guanidine hydrochloride bringing the extract conditions to 4 M guanidine hydrochloride and 2% Zwittergent. Extraction was allowed to proceed for 24 h at 4°C. The extract appeared clear, and centrifugation at 10,000 $\times$ g for 30 min, 4°C removed a steady state within 48 h. Dissociative conditions were achieved by rinsing the labeled monolayer three times in minimal essential medium without serum and then incubating in medium + 10% serum.

Analytical Procedures for Glycosaminoglycans—The quantity of [$^35$S]glycosaminoglycan in each fraction was determined by counting the cetylpyridinium chloride precipitate of protease-digested samples trapped on a Millipore filter. The glycosaminoglycan composition of each fraction was determined by digestion with chondroitinase ABC or chondroitinase AC (Miles Laboratories, Inc.), separation of the disaccharides produced by thin layer chromatography, and determination of percentage of $^35$S-labeled material at each position. The details of these procedures have been previously described (5). Material which does not migrate on the thin layer chromatography plate from 0 to 5 cm may contain heparan sulfate, and the amount of this material corresponds closely to the amount of the original sample which is cleaved to smaller fragments by treatment with nitrous acid. Dermatan sulfate content was calculated by comparison of the amount of $^35$S in the chondroitin 4-sulfate position following digestion with chondroitinase ABC and chondroitinase AC, although it is recognized that this method might underestimate the iduronic acid residues in a heteropolymer (see Ref. 22).

RESULTS

Extraction of Proteoglycans—The fibroblast cultures were divided into two components, the soluble or extracellular material decanted off the monolayer with the culture medium and the material remaining with cells after rinsing of the monolayer. After 48-h uptake of Na$_2$SO$_4$, approximately 90% of the $^35$S-labeled proteoglycan was in the medium. The cell-associated proteoglycans were solubilized completely by a combination of detergent treatment followed by extraction with 4 M guanidine hydrochloride. When detergent was omitted, only about 25% of the cell-associated proteoglycans were solubilized; the rest remained in a viscous pellet.

Gel Filtration of Fibroblast Proteoglycans: Dissociative Conditions—Proteoglycans of the medium were separated into two classes on the basis of differences in hydrodynamic size by Sepharose 2B filtration (Fig. 1). The larger material (M-J) formed a typically broad and indistinct peak, while the smaller material (M-II) formed a sharp peak included 62% of the radioactivity. This latter peak was characterized by a high percentage of chondroitin 4-sulfate and dermatan sulfate, while the larger material was mostly heparan sulfate and chondroitin 6-sulfate (Table I).

Cell-associated proteoglycans were also separated into two size classes by Sepharose 2B filtration (Fig. 1b). The elution positions of these peaks did not correspond to those of the medium, and each peak contained approximately the same amount of radioactivity. The larger of the cell-associated peaks (C-I) was characterized by a high percentage of heparan sulfate. If the cell layer was not treated with detergent prior extraction with guanidine hydrochloride, the Sepharose 2B profile of that material which was solubilized demonstrated a shoulder preceding the main peak (C-I, Fig. 1c) which contained heparan sulfate as the only glycosaminoglycan. This suggests that the Sepharose 2B void volume peak may represent micelles or aggregates of membrane-associated proteoglycan, although the presence of detergent-activated degradative enzymes has not been excluded.

Further Characterization of Proteoglycans in the Medium—Density gradient centrifugation of medium under dissociative conditions demonstrated the presence of proteogly-
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The Sepharose 2B profiles of proteoglycans in fractions D1–D4 are shown in Fig. 2. A predominant peak in the most dense (D1) fraction eluted at a position (Kav = 0.39) which did not show distinctly prior to

density-dependent separation. The glycosaminoglycans of this dense peak were primarily heparan sulfate (Table II). At a somewhat lower density (D2), proteoglycans of two distinct sizes were seen. The larger of the D2 proteoglycans contained both heparan sulfate and chondroitin 6-sulfate and also a significant percentage of dermatan sulfate, which was absent in the larger D2 proteoglycan.

The D3 fraction demonstrated two proteoglycan peaks similar in elution position to those of D2. At this density, the smaller dermatan-sulfate-containing material was the major component. This material (D3-II) shows a hydrodynamic size (Kav = 0.68) similar to that of the major component of medium prior to density-dependent separation (M-II, Fig. 1e). In other experiments, the densities of D2 and D3 were slightly different and it was noticed that the smaller, dermatan-sulfate-rich component was a major constituent when the D2 density was at 1.46. The lightest fraction, D4, was also enriched for this smaller component, but it was not used for further characterizations.

The distinct buoyant densities, glycosaminoglycan compositions, and elution positions suggest the presence of three different proteoglycan fractions in the medium of lung fibro-
The dense D1 component migrated in the void volume. Each of these has been further characterized.

The larger component of D2 also eluted from Sepharose 6B in the void volume. Elution from Sepharose 2B and 4B indicates that it is larger than the heparan sulfate proteoglycan of the D1. Elution of the free [35S]GAG chains following alkaline treatment was also at Kav = 0.35. The ratio of chondroitin 6-sulfate to chondroitin 4-sulfate in this material was about 7:1.

The smaller component of D3 and D4 and eluted from Sepharose 6B at Kav = 0.30 (Fig. 3a). Papain digestion or alkaline treatment shifted the elution position to a peak at Kav = 0.45, corresponding to a chain length of approximately 25,000 daltons.

**Further Characterization of Proteoglycans of the Cell Layer**—The Sepharose 2B profile of proteoglycans extracted from the cell layer by detergent/guanidine hydrochloride showed two peaks of [35S]-labeled material having Kav = 0.40 and 0.74 (Fig. 1b). Density gradient centrifugation of this material under conditions similar to that performed on the medium (starting density = 1.47) did not reveal additional components (data not shown). Both components were primarily in the most dense fraction (ρ = 1.54, 75% of [35S]-labeled material) with the remaining material of both peaks found in the D2 (ρ = 1.45). Very little material of either size was recovered from the D3 or D4.

The larger cellular component (C-1) was composed primarily of heparan sulfate and eluted in the V0 from Sepharose 6B. Following either extensive papain digestion or alkaline/borohydride treatment, this component migrated at Kav = 0.35 (Fig. 3b).

The smaller cellular component (C-II) eluted from Sepharose 6B at a Kav of 0.42 initially, and this elution position was not shifted by papain digestion or alkaline treatment (Fig. 3c). By these criteria, therefore, the cellular [35S]glycosaminoglycans of peak II may not be proteoglycans, but are polysaccharide chains with Mtr = 28,000.

Variations in the relative heights of the two cell-associated peaks have been occasionally noticed. Although they usually contain approximately equal amounts of [35S]SO4, there were several experiments in which the first peak was substantially larger than the second (compare Fig. 1b and Fig. 4a). This difference could not be related to brand of culture flask, passage number of the cultures, or washing of the cell layer prior to extraction. Although of great interest, factors which control the relative amounts of these two components have not been determined.

**Location of Cell-associated Proteoglycans**—The Sepharose 4B profile of cell-associated components shows two peaks with Kav = 0.20 and 0.60 (Fig. 4a). Approximately 50% of the cell-associated proteoglycans were released by gentle trypsin treatment (0.1 mg/ml, 15 min) which did not detach the cells or increase trypan blue permeability. Most of this trypsin-removable material eluted as a single peak on Sepharose 4B, with Kav = 0.20 (Fig. 4b). A small percentage was retarded even more. Following trypsin treatment, the proteoglycans of the remaining cell layer were extracted by detergent/guanidine hydrochloride. They eluted from Sepharose 4B in the usual two positions (Fig. 4c); however, the first peak was greatly diminished. The trypsin-removable material eluted from Sepharose 6B in the V0 and at Kav = 0.35 following alkaline/borohydride treatment (Fig. 3d). Glycosaminoglycan analysis of the trypsin-removable material indicated that it was ~85% heparan sulfate.

Following a 24-h chase period, the elution characteristics of proteoglycans released to the medium, removable from the surface with trypsin and remaining with the cells, were determined. The apparent size of each of the components was not altered during the chase interval. Although both size classes were lost from the cell, material of the larger size class appeared in the medium (Fig. 5a) and net loss from the culture was less than 10%. This material of the chase medium eluted

| Table II: Glycosaminoglycan composition of proteoglycans from medium after CoCl2 density gradient centrifugation |
| Fraction | Heparan sulfate | Chondroitin 6-sulfate | Chondroitin 4-sulfate | Dermatan sulfate |
|---------|----------------|-----------------------|-----------------------|----------------|
| D1      |                |                       |                       |                |
| I       | 74             | 20                    | 3                     | 3              |
| D2      |                |                       |                       |                |
| I       | 11             | 74                    | 10                    | 5              |
| II      | 34             | 49                    | 5                     | 21             |
| D3      |                |                       |                       |                |
| I       | 25             | 61                    | 8                     | 6              |
| II      | 12             | 30                    | 21                    | 37             |
| D4      |                |                       |                       |                |
| I       | 27             | 49                    | 11                    | 13             |
| II      | 20             | 41                    | 16                    | 23             |

Fig. 3: Sepharose 6B chromatographic profiles of proteoglycan fractions before and after papain digestion or NaOH treatment. Peaks were collected after 2B chromatography and run on Sepharose 6B in 4 M guanidine HCl before (——) and after (----) papain digestion or alkaline treatment. Samples c, b, and c are fractions collected as in Fig. 1; d is from the large peak of Fig. 4b. Details of treatments are given under "Experimental Procedures."
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...released continuously by the cells...

...of several proteoglycans in the medium during labeling and can be released by gentle trypsin treatment) and is also...glycan which accumulates at the cell surface (from which it...

...sulfate content. These results suggest that human lung fibroblasts in culture are producing a large heparan-sulfate proteoglycan...

...the D1 component separated from medium by density centrifugation. These results suggest that human lung fibroblasts in culture are producing a large heparan-sulfate proteoglycan which accumulates at the cell surface (from which it can be released by gentle trypsin treatment) and is also released continuously by the cells. As such, it is found as one of several proteoglycans in the medium during labeling and as the major proteoglycan of the medium during chase. The elution characteristics of this proteoglycan indicate that it could have a molecular weight as large as $10^6$ daltons and is certainly much larger than the 75,000-dalton membrane-associated heparan-sulfate proteoglycan of rat liver cells (11) or an ascites hepatoma (12). Heparan sulfate chains of the latter proteoglycans had molecular weights of ~14,000 and 21,000, respectively (three to four chains per proteoglycan), whereas the fibroblast species has polysaccharide chains of ~40,000 daltons (up to 25 chains/proteoglycan). A more accurate determination of molecular weight cannot be made until the component is available in greater quantity. We estimate that one 150-cm² flask of IMR-90 fibroblasts has no more than 1 µg of this component associated with the cells.

The chase experiment (Fig. 5) also suggests that the smaller component of the cell layer (C-II, Fig. 1b) does not represent a direct precursor of the smaller component of the medium (M-II, Fig. 1a) for it does not appear significantly in chase medium. This conclusion is further confirmed by the high heparan sulfate content of the C-II cellular component (compared with a high chondroitin and dermatan content for the M-II proteoglycan of medium) by the fact that its buoyant density is greater than that of the M-II component and its insensitivity to alkaline treatment.

Removal of Surface Proteoglycan by Addition of Exogenous Heparin—The release of surface-associated proteoglycans by addition of exogenous heparin was investigated in the lung fibroblasts. It has been reported that a significant portion of the heparan sulfate proteoglycans at the surface of rat liver...
cells are released by this treatment (24). Cells were labeled for 48 h with Na$_2^{35}$SO$_4$ (20 $\mu$Ci/ml) and [$^3$H]serine (4 $\mu$Ci/ml), rinsed with Earle’s balanced salt solution, and then sequentially incubated with BSS + heparin (a) (0.1 mg/ml, 30 min, 37 °C) and BSS + trypsin (b) (0.1 mg/ml, 15 min, 37 °C). The cell layer (c) was then extracted with 4 M guanidine HCl + 4% Zwittergent as described under “Experimental Procedures.” Two ml of each sample (equivalent to 20% of the total) was chromatographed on Sepharose CL-4B in 4 M guanidine HCl and 0.1 M sodium acetate, pH 5.8. 0.4 ml of 2-ml fractions was counted. Radioactivity eluting after 85 ml represents unincorporated labeling isotope.

Although we have generally confirmed the heparin-release results seen with rat liver cells, there is a significant difference. The amount of additional $^{35}$S-labeled cetylpyridinium chlo-

ride-precipitable material released in the presence of exogenous heparin was only 5–10% of the total in the cell layer (also Ref. 25) instead of 30–50%, as in rat liver cells (24). If exogenous heparin is releasing surface molecules from a receptor, then it must be concluded either that fibroblasts have fewer such receptors, that the larger proteoglycan of fibroblasts does not interact as well with the receptor, or possibly that the additional heparan sulfate chains per fibroblast proteoglycan provide multiple binding sites which have greatly enhanced binding avidity.

**DISCUSSION**

Previous studies of sulfated glycosaminoglycans (S-GAGs) produced by cultured human embryo lung fibroblasts (5), skin fibroblasts (2), and glial cells (4, 27) have demonstrated certain definite characteristics for their composition, distribution, and turnover. In all of these studies, the glycosaminoglycans produced included heparan sulfate, chondroitin 4- and 6-sulfate, and dermatan sulfate. Hyaluronic acid was also produced by each of these cell types. The sulfated glycosaminoglycans were distributed as soluble molecules in the culture medium, as cell-associated molecules which were released by gentle proteolysis or by treatment with EDTA, and as intracellular molecules. In each of these studies, there have been certain S-GAGs associated with particular culture compartments. The medium contained primarily chondroitin sulfates of which a great deal was dermatan sulfate; the cell-associated surface compartment was greatly enriched for heparan sulfate; and the intracellular pool contained all types of glycosaminoglycans. Under chase conditions, most of the cellular [$^{35}$S]GAGs moved out of the cell rapidly although a residual stable pool enriched for heparan sulfate was present. The trypsin-removable [$^{35}$S]GAGs left the surface with an initial half-life varying from 7–22 h, followed by much slower turnover rates. Sulfated glycosaminoglycans appeared in the medium, and heparan sulfate was an increasing percentage of the [$^{35}$S]GAGs of chase medium. The present report describes three distinct proteoglycan fractions produced by human lung fibroblasts in culture and suggests that their location and composition now make it possible to describe [$^{35}$S]GAG turnover in terms of proteoglycans.

The major proteoglycan fraction secreted by lung fibroblasts is a rather small monomer with high derman sulfate content (M-II, Fig. 1a). Its low buoyant density suggests that it is made up of few carbohydrate chains attached to a core protein. Although this is the major proteoglycan fraction secreted during $^{35}$SO$_4$ labeling, it is not found in the medium after chase. Lung fibroblasts do not demonstrate a high amount of intracellular dermatan sulfate, and the smaller intracellular pool (C-II, Fig. 1b) does not act like a precursor to this medium component. Thus, for lung fibroblasts, it seems most reasonable to suggest that the dermatan sulfate-containing proteoglycan is synthesized and rapidly secreted without intervening intracellular or surface accumulation.

A large chondroitin sulfate proteoglycan is also found in the medium. Report of this proteoglycan from lung fibroblasts added to previous reports of such a proteoglycan from glial cells (10) and skin fibroblasts (13) reinforces the conclusion that large chondroitin sulfate proteoglycans are a general product of noncartilage cells. Aggregation behavior influenced by hyaluronic acid was reported for the glial and skin fibroblast molecules (10, 13). The chondroitin sulfate proteoglycan seems to be a greater percentage of the secreted S-GAGs of skin fibroblasts (13) than of lung fibroblasts, as reported in this study.

The third proteoglycan fraction identified in the medium of lung fibroblast cultures contains a large, high density heparan...
Heparan sulfate proteoglycan solubilized by detergent from the cell surface by gentle proteolysis even after a 24-h chase incubation. This was the only heparan sulfate-containing proteoglycan demonstrated, although it is recognized that distinct heparan sulfate species of similar size having variable sulfation characteristics could be combined in the one fraction. Free heparan sulfate polysaccharides were not identified at the cell surface or in the extracellular medium. This suggests that the surface and extracellular heparan sulfate movements previously defined by following only \(^{35}S\)GAG (5) resulted from movement of proteoglycan.

The trypsin-removable fraction was never 100% heparan sulfate, and the other \(^{35}S\)GAG generally present is chondroitin 6-sulfate (6). The presence of a minor amount of the large chondroitin sulfate proteoglycan in surface material would not have been detected by filtration chromatography alone because the proteoglycan sizes and polysaccharide chain lengths are quite similar. The simplest suggestion, therefore, is that proteoglycans at the surface of fibroblasts are of both the chondroitin and heparan sulfate types. The existence of both chondroitin and heparan sulfate polysaccharides chains on the same core protein has not been rigorously excluded, however. It is not clear whether the small amount of proteoglycan which remains attached to cells after trypsin treatment (Fig. 4c, peak I) represents an intracellular precursor pool or a trypsin-inaccessible surface fraction.

It is important to understand how the trypsin-removable heparan sulfate proteoglycan is attached to the cell surface. The fact that it is not removed by washing, high salt, pH changes, or EDTA suggests that it is firmly attached (25). Furthermore, the need for detergent treatment in order to solubilize the component from whole cells suggests tight association with membrane. The boundary between cell membrane and extracellular matrix cannot be clearly defined, however, and certain components, including glycosaminoglycans and the glycoprotein fibronectin, exist within this boundary. Heparan sulfate has been shown to bind preferentially to fibronectin (19, 20), but the organization of these interactions is not yet defined. Fibronectin may be linked to a cell membrane component with secreted heparan sulfate bound to the fibronectin. On the other hand, heparan sulfate attached to a core protein which is an integral membrane constituent may be the cell surface component to which fibronectin binds.

Close association between glycosaminoglycans and fibronectin at the surface of hamster fibroblasts (NIL-8) was demonstrated by their ability to be cross-linked by a photocrosslinkable reagent bound to added fibronectin (28). Large, nonproliferating late passage IMR-90 lung fibroblasts do not organize a fibrillar array of fibronectin, whereas proliferating, early passage cultures do have such a fibronectin network when visualized by indirect immunofluorescence (29). However, the late passage cells accumulate trypsin-removable heparan sulfate in quantities even greater than the early passage cells (30), indicating that fibronectin fibrils are not necessary for accumulation of the surface-associated proteoglycan. Similar elution characteristics for extracellular and trypsin-released proteoglycan do not necessarily indicate that trypsin treatment is digesting some attachment protein other than the core protein. If trypsin is clipping only a limited piece of the core protein from the proteoglycan, e.g. a cleavage just outside the membrane lipids into which the core protein inserts, the hydrodynamic size of the proteoglycan would not be noticeably different although the core protein would be smaller. It has been recently reported that the core protein of a heparan sulfate proteoglycan solubilized by detergent from Swiss mouse 3T3 cells has \(M = 20,000-30,000\), whereas the core protein of the same proteoglycan removed from the cell by proteolytic methods contains fewer hydrophobic amino acid residues and has \(M = 10,000-15,000\) (31). All of the results are consistent with cell surface proteoglycan being synthesized as an integral membrane component which is subsequently either shed to the medium or endocytosed and degraded.

The intracellular pool of nonproteoglycan \(^{35}S\)GAGs (C-II, Fig. 1b) is interesting. Because proteoglycans are synthesized by addition of monosaccharide moieties to the core protein (32), free glycosaminoglycans of this size would not be expected to add directly to a proteoglycan. Furthermore, this fraction diminished by only 50% over the 24-h chase interval and did not appear at the surface or in the medium, indicating that it is not primarily a precursor of secreted material. Sjoberg et al. (2) have suggested that the soluble cellular fraction of skin fibroblast glycosaminoglycans was a storage and degradation pool because the \(^{35}S\) to \([H]\)glucosamine ratio remained high and constant during a 24-h chase interval. The presence of an intracellular pool of large glycosaminoglycans (\(M = 28,000\)) is somewhat surprising because this chain length does not represent breakdown to single sugars or oligosaccharides produced by endoglucuronidase activity (33). The absence of smaller intracellular fragments of \(^{35}S\)GAG may indicate desulfation prior to digestion of glycosidic linkages. An intracellular pool of heparan sulfate has been described in 3T3 mouse fibroblasts (34), but these glycosaminoglycans were smaller (\(M = 6,000\)). Although it is assumed that the C-II glycosaminoglycans are from an intracellular pool, only their solubility in 4 M guanidine hydrochloride (Fig. 1c) and insensitivity to trypsin (Fig. 4c) have actually been demonstrated.

Proteoglycans from various tissues of adult bovine lung were recently isolated and characterized (35). Gas exchange tissue contained both a chondroitin and a heparan sulfate proteoglycan fraction, while pleura contained only a heparan sulfate proteoglycan; bronchiolar proteoglycan was composed primarily of chondroitin sulfate and was similar in size to the proteoglycan of nasal cartilage. The major proteoglycan isolated from porcine lung was also similar in size and composition to the cartilage proteoglycan (36). None of these tissues demonstrated a chondroitin/dermatan fraction similar to the smaller secreted proteoglycan (M-II) of human embryo lung fibroblasts in culture. The major secreted proteoglycan of cultured lung fibroblasts thus could be unique either for embryonic cells or to human cells. Alternatively, it could represent a cleaved or nonaggregated form of the molecular structure found in intact tissue.

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REFERENCES
1. Conrad, G. W., Hamilton, C., and Haynes, E. (1977) J. Biol. Chem. 252, 6861-6875.
2. Sjoberg, L., Carlstedt, J., Coster, L., Malmström, A., and Fransson, L.-Å. (1979) Biochem. J. 178, 257-270.
3. Kraemer, P. M. (1971) Biochemistry 10, 1437-1445.
4. Glimelius, B., Norling, B., Westermark, B., and Wastesson, Å. (1978) Biochem. J. 172, 443-456.
5. Vogel, K. G., and Kendall, V. F. (1980) J. Cell. Physiol. 103, 457-467.
6. Hascall, V. C., and Sajdera, S. W. (1970) J. Biol. Chem. 245, 4920-4930.
7. Norr, H., and Hardingham, T. E. (1975) in Biochemistry of Carbohydrates, MTP International Review of Science (Whe- lan, W. J., ed) Vol. 9, pp. 153-222, Butterworths, London.
8. Kraemer, P. M., and Smith, D. A. (1974) Biochem. Biophys. Res.
Proteoglycans of Human Lung Fibroblasts in Culture

9. Kleinman, H. K., Silbert, J. E., and Silbert, C. K. (1975) Connect. Tissue Res. 4, 17-23
10. Norling, B., Glimelius, B., Westermark, B., and Wasteson, A. (1978) Biochem. Biophys. Res. Commun. 84, 914-921
11. Oldberg, Å., Kjellen, L., and Höök, M. (1979) J. Biol. Chem. 254, 8905-8910
12. Muth, S., Funakoshi, I., Ui, N., and Yamashina, I. (1980) Arch. Biochem. Biophys. 202, 137-143
13. Coster, L., Carlstedt, I., and Malmstrom, A. (1979) Biochem. J. 183, 669-681
14. Ehrlich, K. C. (1981) J. Biol. Chem. 256, 73-80
15. Colp, L. A., Murray, B. A., and Hollins, B. J. (1979) J. Supramol. Struct. 11, 401-427
16. Underhill, C., and Dorfman, A. (1975) Exp. Cell. Res. 117, 155-164
17. Bernanke, D. H., and Markwald, P. R. (1979) Tex. Rep. Bio. Med. 39, 271-285
18. Comper, W. D., and Laurent, T. C. (1978) Physiol. Rev. 58, 255-315
19. Laterra, J., Ansbacher, R., and Culp, L. A. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 6662-6666
20. Ruoslahti, E., and Engvall, E. (1980) Biochim. Biophys. Acta 631, 350-358
21. Nichols, W. W., Murphy, D. G., Cristofalo, V. J., Toji, L. H., Green, A. E., and Dwight, S. A. (1976) Science 196, 60-63
22. Yanagishita, M., Rodbard, D., and Hascall, V. C. (1979) J. Biol. Chem. 254, 911-920
23. Wasteson, A. (1971) J. Chromatogr. 59, 87-97
24. Kjellen, L., Oldberg, Å., and Höök, M. (1980) J. Biol. Chem. 255, 10407-10413
25. Vogel, K. G., and Dolde, J. (1979) Biochim. Biophys. Acta 552, 194-200
26. Deleted in proof
27. Glimelius, B., Norling, B., Westermark, B., and Wasteson, A. (1978) Exp Cell. Res. 117, 179-189
28. Perkins, M. S., Ji, T. H., and Hynes, R. O. (1979) Cell 16, 941-952
29. Vogel, K. G., Kelley, R. O., and Stewart, C. (1981) Mech. Aging Develop. 16, 285-302
30. Vogel, K. G., Kendall, V. F., and Sapien, R. E. (1981) J. Cell. Physiol. 107, 271-281
31. Lowe-Krentz, L. J., Keller, J. M., and Saidel, L. (1981) Fed. Proc. 40, 1840 (Abstract)
32. Reden, L., and Schwartz, N. B. (1975) in Biochemistry of Carbohydrates, MTP International Review of Science (Whelan, W. J., ed) Vol. 5, pp. 95-152, Butterworths, London
33. Klein, U., Kresse, H., and von Figura, K. (1976) Biochem. Biophys. Res. Commun. 69, 158-166
34. Johnston, L. S., Keller, K. L., and Keller, J. M. (1979) Biochim. Biophys. Acta 583, 81-94
35. Radhakrishnamurthy, B., Smart, F., Dalferes, E. R., Jr., and Berenson, G. S. (1980) J. Biol Chem. 255, 7575-7582
36. Sahu, S., and Lynn, W. S. (1979) J. Biol. Chem. 254, 4262-4266