American Journal of Pathology, Vol. 128, No. 2, August 1987

Aortic Endothelial Cell Proteoheparan Sulfate

II. Modulation by Extracellular Matrix

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The effects of extracellular matrix components on proteoheparan sulfate biosynthesis was studied for bovine aortic endothelial cells in tissue culture. When the cells were maintained on a variety of different purified components of the extracellular matrix, the cells expressed the same three species of proteoheparan sulfates as the cells cultured on tissue culture plastic (HS I, HS II, and HS III). However, the amounts of the three species recovered from the tissue culture medium were found to be dependent on the substrate on which the cells are grown as well as on other factors. In comparison with plastic, much less HS I was found in the medium of cells maintained on substrates containing diverse matrix molecules, whereas the amounts of HS II and HS III essentially remained the same. In contrast, when bovine aortic organ cultures were analyzed under pulsatile flow, marked differences in the profile of proteoheparan sulfate biosynthesis were observed: HS I was found exclusively associated with the plasma membrane of the endothelial cells; HS II was localized only to the subendothelial matrix; and HS III represented the only proteoheparan sulfate species in the medium. This distribution is consistent with polarized secretion and deposition into the subcellular matrix of HS III and retention of HS I in the plasma membrane in the organ culture situation, a biosynthetic phenotype which can only be approximated at best by maintaining the endothelial cells on a substrate other than plastic. When aortic media (devoid of endothelial cells) was placed in organ culture, no HS III could be detected, which suggested that the vascular endothelial cell is the major cell type responsible for its synthesis in organ culture. Thus, the extracellular matrix, depending upon its composition and organization, may play an important role in stabilizing cell polarity and thereby contribute to maintenance of the differentiated phenotype appropriate for the endothelial cell. (Am J Pathol 1987, 128:299–306)

THE VASCULAR endothelial cell (EC) is a metabolically active cell with a variety of functions, which, in part, depend on its location in a particular vascular bed and on local conditions.[1] In order to express some of these functions, the ECs have to be anchored to a substrate, which in most instances is a basement membrane. Contact with other components of connective tissue under certain conditions, eg, angiogenesis, modulates or influences the behavior of microvascular ECs in dramatic ways.[2–4] It has been demonstrated that the extent of cell attachment, spreading, migration, and proliferation, as well as the formation of tubelike structures and the synthesis of collagen by ECs, is affected by the matrix on which the cells are maintained and studied.[3,5–9] One crucial question that obviously has to be raised is whether the chosen culture conditions accurately reflect physiologic conditions and thereby provide a basis for meaningful results. As discussed in the preceding paper, proteoheparan sulfates produced by ECs in culture include a plasma membrane (cell surface) and two secreted species, one of which is deposited in the basement membrane or subcellular matrix.[10,11] Recently, several groups have demonstrated the existence of several endothelial cell-derived heparan sulfate and heparinlike molecules which appear to exert

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Supported in part by USPHS Grants HL-28373 (J.A.M.) and AM-30556, Yale University Liver Center Pilot Project 9 (H.F. and R.K.), the Yale Fluid Research Fund (J.A.M. and H.F.), Swebilius Cancer Research Fund (B.M.P.), and the Deutsche Forschungsgemeinschaft (R.K.).

Accepted for publication March 18, 1987.

significant effects on hemostasis, on the proliferation of vascular smooth muscle cells, and on ECs.[12-16] Much of this work has been done with molecules isolated from the medium and cell layer fractions of cultured ECs; and because culturing cells may have altered some of the functions they express *in vivo,* progress in understanding these phenomena has been difficult. In light of this, we have compared the biosynthetic phenotype of endothelial cells maintained in aortic organ culture with cells grown on plastic and on purified matrix components. Ideally, this approach may permit the establishment of *in vitro* conditions which would best mimic the metabolism of proteoheparan sulfate metabolism *in vivo.* In this study we found the synthesis and localization of the various EC proteoheparan sulfate products (HS I, HS II, and HS III) to depend on the culture conditions. Although culture of the cells on various matrix components did not completely mimic the organ culture proteoheparan sulfate profile, dramatic differences were apparent when the matrix-coated cultures were compared with the cells cultured on tissue culture plastic.

Materials and Methods

Carrier-free $Na_2{}^{35}SO_4$ was purchased from New England Nuclear (Boston, Mass). Heparinase, heparitinase, and chondroitinase ABC were purchased from Miles (Chicago, Ill). Collagenase was purchased from Biofractions (Lynbrook, NY) and further purified as previously described.[11] Papain was purchased from Boehringer Mannheim GmbH (Mannheim, West Germany). Sephadex G-25, G-50, Sepharose CL-4B and 6B, and DEAE-Sephacel were obtained from Pharmacia (Uppsala, Sweden).

Collagen Types I, III, IV, and V, laminin, and fibronectin were isolated and purified as previously described.[3]

Bacteriologic plastic Petri dishes (35-mm Falcon #1008) were coated with various matrix components as described.[9]

Bovine aortic endothelial and medial smooth muscle cells were isolated and cultured as previously described.[17]

Short-term (24-hour) bovine calf aortic organ cultures were established as follows: Fresh calf aortas were obtained at the local slaughterhouse and transported in ice-cold phosphate-buffered saline (PBS) containing 1000 U/ml penicillin and 1 mg/ml streptomycin. The intercostal arteries were ligated and pairs of aortas were connected in series by sterile glass cannulas on a sterile bench. The pairs of aortas were then connected to a reservoir containing 100 ml of sterile MEM containing 20% heat-inactivated fetal calf serum and aerated with a gas mixture of 5% CO_2: 95% air. The medium was pumped through the aortas in the direction of normal blood flow with a peristaltic pump (Millipore #XX 80 200 00) at a rate of 95 ml/min. The medium reservoir and aortas were maintained at 37 C in a humidified atmosphere (Figure 1). For pulse and pulse-chase labeling experiments 100 ml of sulfate-deficient DMEM with 20% heat-inactivated fetal calf serum containing 5 mCi ^{35}S-sulfate was used. After 10-, 20-, 40-, 60-, and 120-minute pulses, 1-ml aliquots were removed for analysis. After 8 hours the medium was removed and stored at −70 C for analysis. In pulse-chase experiments the ^{35}S-sulfate-containing medium was exchanged with MEM containing cold sulfate after a 2-hour incubation and exchanged again after 30 minutes, 2, 8, and 24 hours. All medium fractions were stored frozen prior to analysis.

Short-term (24-hour) tissue explant cultures of bovine calf aorta were established as follows: 1-sq mm pieces (approximately 10 g wet weight per experiment) were prepared by carefully removing the adventitia and the EC layer. The tissue pieces were incubated with 10 ml of sulfate-deficient DMEM + 10% heat-inactivated fetal calf serum containing 100 μCi ^{35}S-sulfate/ml. In one experiment the tissue was incubated for 20 hours, and in pulse-chase experiments 4-hour incubations followed by 16-hour chase periods were used. Medium fractions were stored frozen prior to analysis.

For tissue culture experiments the cells were plated in 9.6-sq cm tissue culture dishes (Falcon #3001) and Falcon bacteriologic plastic dishes (Falcon #1008) coated with human placental pepsin soluble Type I collagen, Type III collagen, murine EHS tumor Type IV collagen, EHS tumor laminin, and human plasma fibronectin as previously described.[25] At confluency

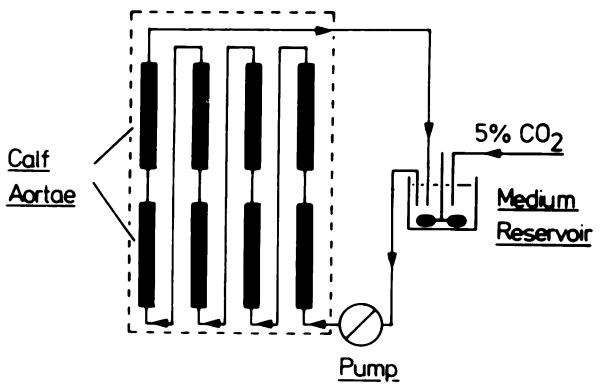

Figure 1—Schematic of bovine aorta organ culture used in pulse and pulse-chase experiments.

cultures were incubated with sulfate-deficient medium for 60 minutes and then incubated with 4 ml of medium containing 400 μCi ^{35}S-sulfate for 8 hours. After various incubation times (30 minutes, 60 minutes, 90 minutes, 180 minutes, and 8 hours), 0.5-ml aliquots were removed and stored frozen for analysis. For pulse-chase labeling experiments cells were plated, grown, and incubated for 2 hours with ^{35}S-sulfate-containing medium as described above. Following this the cultures were washed ten times with ice-cold PBS containing 1 mM $MgSO_4$ and then with 4 ml of regular DMEM. After various incubation times (30 minutes, 90 minutes, 180 minutes, 8 hours, and 24 hours) media were removed and replaced with fresh media. All medium samples were stored frozen prior to analysis.

Medium samples were desalted on Sephadex G-25 columns (1.0 × 20 cm) in 0.5 M NH_4HCO_3, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM EDTA, lyophilized, and digested with 0.5 U chondroitinase ABC in 50 mM Tris-HCl, 0.1 M NaCl, 1 mM PMSF, 10 mM N-ethylmaleimide (NEM), 1 mM EDTA, pH 7.5. Aliquots were then chromatographed on a Sepharose CL-6B column (0.5 × 60 cm) with 0.13 M Tris-HCl, 1 mM PMSF, 10 mM NEM, 1 mM EDTA, pH 7.5. Fractions of 200 μl were collected and analyzed for radioactivity. Alkali digestion of proteoglycans was accomplished by incubation in 0.5 M NaOH at 4 C for 12 hours. Digestion of proteoglycans with a mixture of heparinase and heparitinase was performed in 0.05 M Tris-HCl, 0.1 M NaCl, 5 mM $CaCl_2$, 1 mM PMSF, 10 mM NEM, pH 7.3, at 37 C for 2 hours. Materials from both digestions were analyzed by Sepharose CL-6B chromatography under the conditions described above.

For analysis of cell layer materials from the aortic organ cultures, aortas were cut open longitudinally, and the endothelial cells and subendothelial matrix were removed by a single scrape with a scalpel blade. This material was suspended in 50 mM Tris-HCl, 0.25 M sucrose, 10 mM NEM, pH 7.2, and sonicated at 0 C with a Model W 185 D Sonifier cell disruptor (Heat Systems—Ultrasonics Inc., Plainview, NY) three times for 30 seconds with 1-minute interruptions between each sonication step. The suspension was then incubated with 1 unit chondroitinase ABC at room temperature for 15 minutes. After this the material was digested with 20 units collagenase overnight at 4 C after the addition of 20 μl of 2 M NaCl, 50 mM $CaCl_2$. The material was then centrifuged at 100,000*g* at 4 C for 45 minutes (Ti 65 rotor, Beckman L5-65 ultracentrifuge) and the supernatant chromatographed on a Sepharose CL-6B column (0.5 × 60 cm) with 0.13 M Tris-HCl, 0.1% sodium dodecyl sulfate (SDS), 1 mM PMSF, 10 mM NEM, 1 mM EDTA, pH 7.2, as eluant. Fractions of 200 μl were collected and assayed for radioactivity. The pellet was solubilized in 0.1 M Tris-HCl, 7.0 M urea, 2.0% SDS, 0.2% beta-mercaptoethanol, 1 mM PMSF, 10 mM NEM, 1 mM EDTA, pH 7.2, and chromatographed on Sepharose CL-6B as described above.

Data manipulation and statistical analyses were performed on an Apple II+ microcomputer with a Visicalc/Visiplot/Visitrend spreadsheet and linear regression fit analysis.

Representative samples of aortas, after organ culture, before and after mechanical scraping, were prepared for light microscopy as previously described. Briefly, 1 sq mm tissue samples were fixed in 1.0% glutaraldehyde buffer overnight at 4 C, washed, embedded in Epon 812, and sectioned at 1.0 μ on an LKB ultramicrotome. Toluidine blue-stained sections were examined by light microscope and photographed with an Olympus photomicroscope equipped with a PM 10AD automatic camera system and Kodak Technical Pan film.[3]

Results

Biosynthesis of Medium Proteoheparan Sulfates: A Comparison of Tissue and Organ Culture

The results in Figure 2A illustrate the striking differences between the elution patterns of the ^{35}S-labeled medium proteoheparan sulfate species obtained from tissue culture and organ culture. In contrast to the tissue culture medium, which contains predominantly HS I at long incubation times, no HS I was found in the organ culture medium. Additionally, HS III appears as the predominant proteoheparan sulfate in the organ culture medium over all pulse periods tested (up to 8 hours). During shorter pulse periods (15–120 min) (Figure 2B) HS III was also found to be the only proteoheparan sulfate detected in the organ culture medium. In pulse-chase experiments of organ cultures, HS III was the only proteoheparan sulfate detected in the medium (data not shown), in contrast to the findings in tissue culture, in which HS I represents the major species.[13] As observed with tissue culture-derived HS III, the organ culture-derived material was only partially sensitive to heparitinase and heparinase (50%). Furthermore, the chromatographic behavior of HS III from both sources was identical with respect to elution from DEAE-Sepharose and Sepharose CL-6B, as well as number and size of the glycosaminoglycan side chains (data not shown).

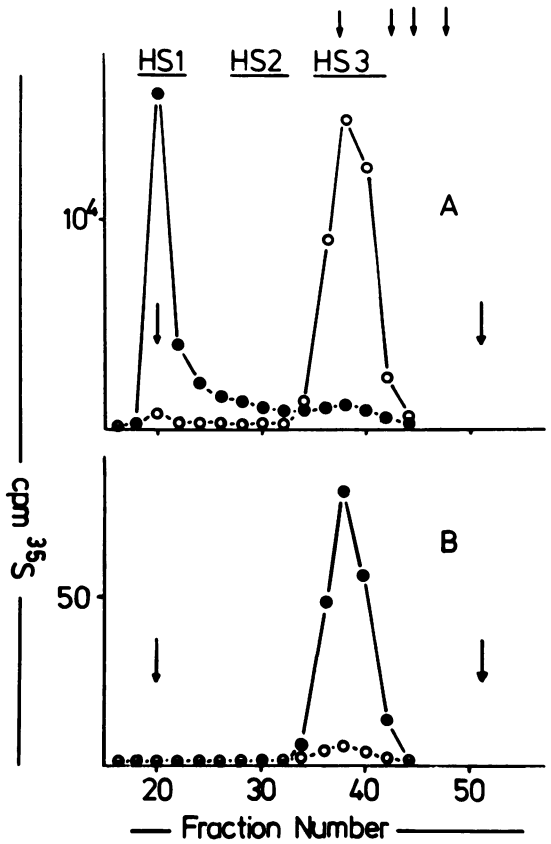

Figure 2—Sepharose CL-6B chromatogaphy of a chondroitinase ABC digest of medium ^{35}S-proteoglycan from tissue and organ culture. **A**—Confluent bovine aortic endothelial cell (BAEC) cultures (~ 8×10^6 cells) were incubated with 50 μCi ^{35}S-sulfate-containing medium for 8 hours (*closed circles*). One calf aorta (~ 20 cm) was perfused with 100 μCi/ml ^{35}S-sulfate-containing medium at 100 ml/min flow for 8 hours at 37 C (*open circles*). The medium was desalted on Sephadex G-50, digested with 1 unit of chondroitinase ABC, and chromatographed on a Sepharose CL-6B column (0.5 × 60 cm) with a 0.13 M Tris-HCl, 1 mM PMSF, 10 mM NEM, 1 mM EDTA, pH 7.2, buffer. Fractions of 200 μl were collected at 1 ml/hr and analyzed for radioactivity. *Arrows* indicate V_o and V_t. **B**—Perfusate aliquots from 40-minute (*open circles*) and 120-minute (*closed circles*) pulse experiments of an aortic organ culture. Analysis was performed as described above.

Biosynthesis of Medium Proteoheparan Sulfates: Matrix Effects

Figure 3A illustrates the effects of matrix-derived molecules on the proteoheparan sulfate biosynthetic profile of the cultured cells. When collagen Types I/III, IV, V, laminin, or fibronectin was used to coat bacteriologic plastic Petri dishes, similar changes in the heparan sulfate proteoglycan profile were observed. Namely, the amount of medium HS I was considerably less than that observed for the cells cultured on tissue culture plastic, whereas the amounts of HS III produced remained essentially unchanged for all substrates tested. The differences in HS I accumulation in the medium fraction can be clearly seen in

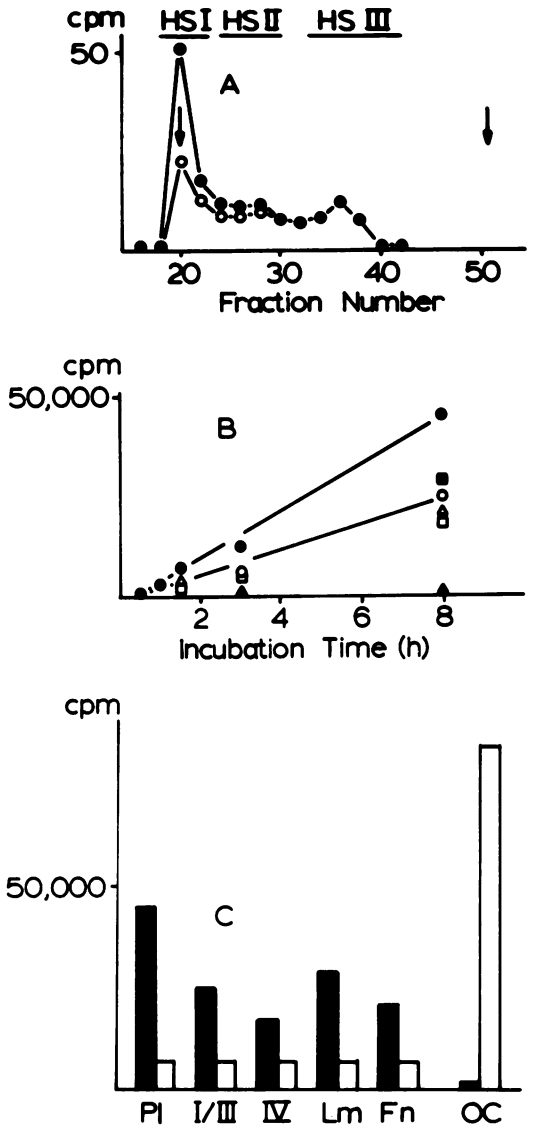

Figure 3—Matrix effects on medium proteoheparan sulfate biosynthesis by confluent BAECs. **A**—Quantitative analysis of Sepharose CL-6B medium fractions from confluent BAECs (~2.5×10^5 cells) grown on plastic (*closed circles*) and collagen I/III- (*open circles*) coated dishes and incubated with 4 ml of medium containing 400 μCi ^{35}S-sulfate for 8 hours. After 30, 60, 90, and 120 minutes 0.5-ml aliquots were removed, desalted, chondroitinase-digested, and chromatographed as described. Total fraction volumes were counted. Shown are representative profiles of 60-minute pulses of BAECs grown on plastic and collagen Types I and III. *Arrows* indicate V_o and V_t. **B**—Medium proteoheparan sulfate (HS I) accumulation in cultured BAECs grown on plastic (*closed circles*), collagen Types I and III (*open circles*), IV (*open squares*), laminin (*closed squares*), fibronectin (*open triangles*), and organ culture (*closed triangles*). BAECs cultured on plastic showed a linear accumulation of HS I ($r = 0.991$) over the time period studied (8 hours). BAECs cultured on all the matrix components tested also revealed a linear accumulation of HS I ($r = 0.922$); however, net accumulations were lower than those observed on plastic. Furthermore, amounts of HS I were not significantly different from each other. In contrast, aortic organ cultures exhibited no appreciable HS I accumulation. **C**—Medium proteoheparan sulfate (HS I and HS III) 8-hour accumulation by BAECs (~ 2.5×10^5/dish) grown on plastic (*PL*), on collagen Types I/III (*I/III*), IV (*IV*), laminin (*Lm*), fibronectin (*Fn*), and in organ culture (*OC*). Although HS I was found to be approximately twice as high on the plastic substrate as compared with the various matrices tested, HS III was found to be produced in roughly equal amounts in all the cultures. In contrast, organ cultures were observed to produce negligible amounts of HS I and high amounts of HS III. (HS I, *closed bar*; HS III, *open bar*).

Figure 3B. Accumulation of both species was observed to be linear ($r = 0.991$ for HS I and $r = 0.922$ for HS III). As illustrated, the rate of HS I accumulation for the cells maintained on tissue culture plastic is approximately twice that for the cells cultured on the various matrix components. These findings contrast the aortic organ culture studies, in which no appreciable HS I was observed to accumulate in the medium fraction. In addition, the dramatic differences in HS III accumulation in medium fractions are illustrated in Figure 3C. As observed, the amounts of HS III found when the cells were cultured on tissue culture plastic or the various matrix components were virtually identical. In contrast, in aortic organ cultures, HS III is the major medium proteoheparan sulfate, and only minute amounts of HS I are detected.

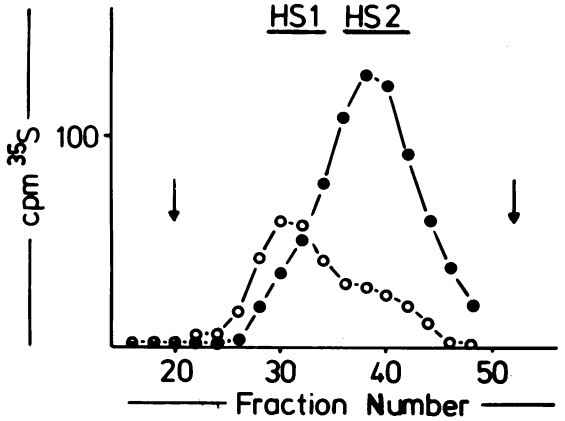

Figure 4—Sepharose CL-4B chromatography of collagenase-treated supernatant and pellet fractions of an 8-hour ^{35}S-sulfate pulse of an organ culture scraped endothelial cell/subendothelial matrix layer. The soluble material (*closed circles*) was observed to elute at the position of HS II—previously described as a matrix proteoheparan sulfate species. In contrast, the insoluble material (*open circles*), after detergent solubilization, eluted at the position of HS I—previously described as a plasmalemmal membrane proteoheparan sulfate species. See Materials and Methods for details.

Proteoheparan Sulfate Biosynthesis and Localization in Aortic Organ Cultures

The major differences found in the accumulation of proteoheparan sulfate in the medium of aortic organ culture prompted a more detailed, careful investigation of the proteoheparan sulfate species in the EC and intimal layers of the aorta. After incubation with ^{35}S-sulfate for 8 hours, the aortas were washed with ten changes of cold sulfate-containing medium, perfused extensively with ice-cold sulfate-containing PBS, and then opened longitudinally. The endothelium and subendothelial matrix were carefully removed as described, homogenized, and incubated with collagenase to discriminate between extracellular and plasma membrane-associated material. Chromatography of chondroitinase ABC digests on Sepharose CL-4B (Figure 4) showed that the collagenase-soluble material eluted in the same position as the matrix-associated proteoheparan ^{35}S-sulfate HS II[13] deposited by the cells in tissue culture. In contrast, the bulk of the collagenase resistant proteoheparan ^{35}S-sulfate found in the 100,000*g* pellet eluted in the same position as proteoheparan sulfate HS I, which is found in the tissue culture medium of the cells in large amounts but which in fact is a plasmamembrane component.[13]

Examination of the aortic organ cultures morphologically by light microscopy following 24 hours of culture revealed an intact EC layer resting on a subendothelial matrix and a highly organized tunica media (Figure 5a). Light-microscopic examination of the aortic organ cultures following removal of the EC layer by mechanical scraping revealed the internal elastic lamina and an underlying organized tunica media with no appreciable ECs or subendothelial matrix evident (Figure 5b).

The aortic wall is obviously composed of other cell types in addition to the luminal endothelial cells. These other cell types could contribute to the synthesis of HS III, the only proteoheparan sulfate detected in the organ culture medium, as well as to the synthesis of the other two proteoheparan sulfates isolated from the endothelium and the subendothelial matrix. For this reason we determined the possible contributions of the medial smooth muscle cells to the synthesis of proteoheparan sulfate in organ culture (Figure 6). The culture medium of labeled aortic tunica media fragments free of endothelial cells and adventitial tissue did not exhibit appreciable amounts of HS III during variable pulse periods (Figure 6a) and during an extended chase period (Figure 6b). The three major species found, although sensitive to heparinase and heparitinase digestion supporting their proteoheparan sulfate nature (data not shown), eluted at different positions on Sepharose CL-6B, as compared with HS III isolated from EC culture media. However, these studies do not rule out the possibility that overlying endothelial cells stimulate the production of HS III by the medial smooth muscle cells.

Discussion

In this report we describe proteoheparan sulfate production in cultured aortic endothelial cells grown on plastic on various extracellular matrix components, and in aortic organ culture. Although in all cases the same species of EC proteoheparan sulfates (HS I, HS II, and HS III) are found, there is a dramatic

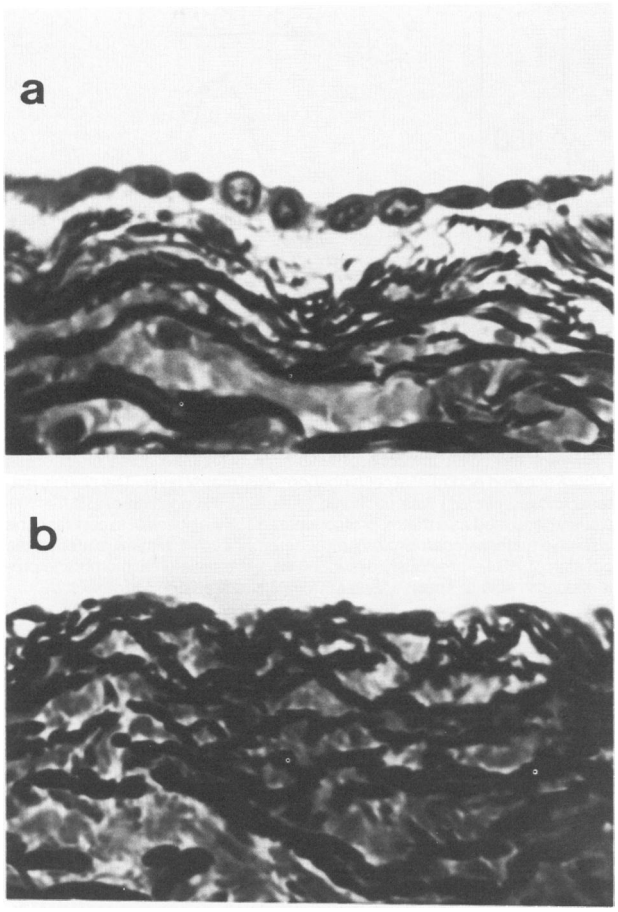

Figure 5—Light microscopy of intact (**a**) and scraped (**b**) aortas following an 8-hour pulse of ^{35}S-sulfate-containing medium and an 18-hour chase period. (Original magnification, ×500) **a**—Representative 1-μ-thick section stained with toluidine blue of a biopsy specimen of a calf aorta after organ culture. The section reveals an intact EC layer overlying the subendothelial matrix and tunica media of the vessel wall. **b**—Representative 1-μ-thick section stained with toluidine blue of a biopsy specimen of a calf aorta after organ culture and scraping off the EC/subendothelial matrix with a razor blade. The section illustrates the internal elastic lamina and the tunica media and the absence of endothelial cells and subendothelial matrix.

difference in their amounts and distribution between culture medium, cell surface, and substratum. At all time points and time periods studied ECs cultured on all the matrix components tested exhibited significantly less HS I in medium fractions, as compared with cells cultured on plastic. Furthermore, the medium fractions of aortic organ cultures contained virtually no HS I, but this molecule was found to be exclusively associated with EC plasmalemmal membranes. In the accompanying article[11] we have observed that HS I indeed can be isolated from the plasma membrane fraction of cultured endothelial cells. Because a molecule with identical properties was isolated and defined here as a component of the aortic endothelial cell layer, and because its extraction requires the use of detergents, it is likely that HS I *in vivo* is also localized in the plasma membrane. These findings are consistent with the hypothesis that the presence of HS I in the medium of cultured endothelial cells may be due to a shedding process, rather than secretion. The extent of shedding is dependent on the substrate and thus may represent an anomaly of the tissue culture system. Culture on various matrix components allows the expression of a phenotype intermediate between that observed on plastic and that in organ culture, which suggests that culture of the cells

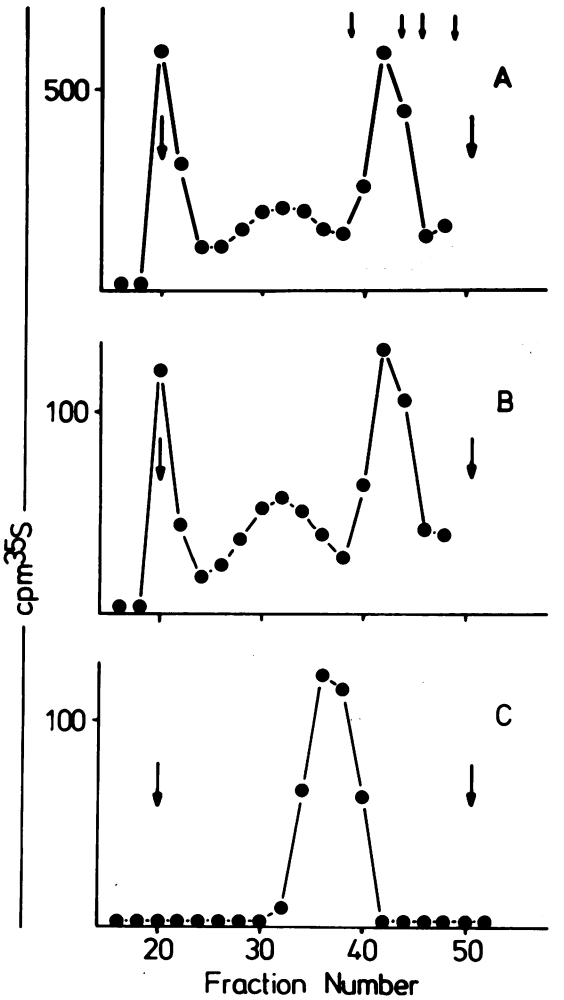

Figure 6—Sepharose CL-6B chromatography of a chondroitinase ABC digest of ^{35}S-medium proteoglycans from bovine aortic smooth muscle explant organ cultures. **A**—Small pieces of aortic media (1 sq mm) were incubated in 10 ml of sulfate-deficient DMEM + 10% fetal calf serum containing 100 μCi ^{35}S-sulfate/ml for 20 hours. After desalting on G-50 Sephadex and chondroitinase ABC digestion, aliquots were chromatographed on Sepharose CL-6B columns (0.5 × 60 cm) with a 0.13 M Tris-HCl, 1 mM PMSF, 1 mM EDTA, pH 7.3, buffer. Fractions of 200 μl were collected and analyzed for radioactivity. *Large arrows* denote V_o and V_t, respectively. *Small arrows* denote elution positions of GAG standards of 35,000, 19,000, 17,000, and 13,000 as previously described.[11] **B**—Sepharose CL-6B chromatography of representative media aliquots from a 4-hour pulse followed by a 16-hour chase revealed identical profiles as observed following 20-hour pulses. **C**—Sepharose CL-6B chromatography of endothelial cell proteoheparan sulfate HS III is given for comparison.

on various matrix components may provide a system for maintaining the cells in an environment more closely related to the physiologic state.[2,4,7]

A similar trend is observed for the other proteoheparan sulfate species. HS II, the major matrix-associated species, is also observed in small amounts in the medium fractions of endothelial cells cultured on plastic and matrix components, but it is found exclusively in the subendothelial matrix of organ cultures. This finding is consistent with its polarized (basal) secretion *in vivo*. HS III, a minor medium form found in small amounts in endothelial cell cultures on plastic and matrix components, is the major, if not exclusive, proteoheparan sulfate species secreted into the medium of aortic organ cultures. Whether HS III is secreted into blood vessel lumens *in vivo* has yet to be determined.

The observation of matrix components and specific connective tissue elements dramatically influencing cell behavior in culture and *in vivo* is not novel and has been described in several culture systems. These examples include cultured capillary ECs, in which tube formation and basement membrane synthesis are accelerated by culture on selected matrices,[3] and Type II pneumocyte culture, in which maintenance of the Type II phenotype is accomplished on a heterologous matrix (amnion basement membrane), while progression to the Type I phenotype is observed on a pulmonary basement membrane matrix.[18,19] Other examples of matrix influencing cell behavior include matrix-driven cyto-differentiation and polarization of rat pancreatic adenocarcinoma cells *in vivo* and *in vitro*.[20–22]

Thus, the role of the extracellular matrix in the maintenance of a "differentiated" phenotype of the EC appears to be a permissive one, allowing for the maintenance of polarity of secretion of the proteoheparan sulfates HS II and HS III and for the expression of HS I exclusively as a cell surface-associated species (Figure 7).

The role of these three proteoheparan sulfate species and the potential modulation of their expression *in vivo* can only be speculated upon at this time.[3,5,23,24] The potential function of these proteoheparan sulfate species (endothelial cell HS I, HS II, and HS III) is completely unknown, but may include modulation of hemostasis as well as modulation of other endothelial and vascular smooth muscle cell behavior such as proliferation.[12,13,15,16,25,26] What specific roles other matrix components may have in affecting these biologic processes also await discovery.

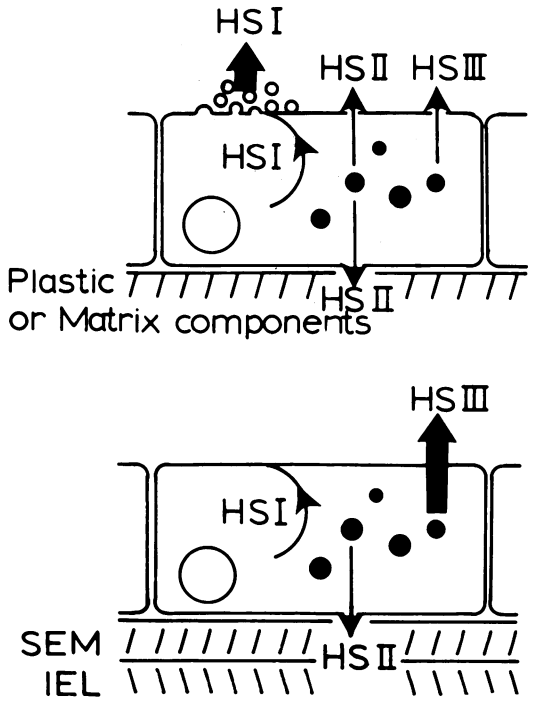

Figure 7—Schematic depicting proteoheparan sulfate biosynthesis in cultured BAECs and BAECs in organ culture. **A**—In culture on plastic or matrix components BAECs are observed to produce three proteoheparan sulfate (HSPG) species: HS I—a plasmalemmal membrane-associated species that is also shed in large amounts into the medium, being the major medium HSPG species; HS II—a secreted subendothelial matrix species also found in small amounts in the medium; and HS III—a secreted medium species found in very small amounts. **B**—In organ culture BAECs exhibit a markedly different HSPG biosynthetic profile. HS I retains its plasmalemmal membrane association, but there is no evidence of shedding into the medium. HS II is found exclusively as a matrix-associated material with no secretion into the medium. HS III is found as the major secreted HSPG in the medium. *SEM*, subendothelial matrix; *IEL*, internal elastic lamina.

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