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Structural Characterization of Proteoglycans Produced by Testicular Peritubular Cells and Sertoli Cells*

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The structural characteristics of proteoglycans produced by seminiferous peritubular cells and by Sertoli cells are defined. Peritubular cells secrete two proteoglycans designated PC I and PC II. PC I is a high molecular mass protein containing chondroitin glycosaminoglycan (GAG) chains (maximum 70 kDa). PC II has a protein core of 45 kDa and also contains chondroitin GAG chains (maximum 70 kDa). Preliminary results imply that PC II may be a degraded or processed form of PC I. A cellular proteoglycan associated with the peritubular cells is described which has properties similar to those of PC I. Sertoli cells secrete two different proteoglycans, designated SC I and SC II. SC I is a large protein containing both chondroitin (maximum 62 kDa) and heparin (maximum 15 kDa) GAG chains. Results obtained suggest that this novel proteoglycan contains both chondroitin and heparin GAG chains bound to the same core protein. SC II has a 50-kDa protein core and contains chondroitin (maximum 25 kDa) GAG chains. A proteoglycan obtained from extracts of Sertoli cells is described which contains heparin (maximum 48 kDa) GAG chains. In addition, Sertoli cells secrete a sulfoprotein, SC III, which is not a proteoglycan. SC III has properties similar to those of a major Sertoli cell-secreted protein previously defined as a dimeric acidic glycoprotein. The stimulation by follicle-stimulating hormone of the incorporation of $[^{35}S]SO_4^{2-}$ into moieties secreted by Sertoli cells is shown to represent an increased production or sulfation of SC III (*i.e.* dimeric acidic glycoprotein), and not an increased production or sulfation of proteoglycans. Results are discussed in relation to the possible functions of proteoglycans in the seminiferous tubule.

In the testis, the boundary tissue of the seminiferous tubule consists of mesenchymal cells around the perimeter of the tubule wall (peritubular cells), separated by a basal lamina from adjacent epithelial cells within the tubule (Sertoli cells and germinal cells). As in other organs, it appears likely that mesenchymal cell-epithelial cell interactions occur during the formation and deposition of the extracellular matrix (ECM[1]). Peritubular cells and Sertoli cells have been shown to act cooperatively in co-culture to synthesize different ECM components (1, 2), and to deposit them as extracellular fibrils and a basal lamina-like structure. In contrast, monocultures of Sertoli cells or peritubular cells do not form a basal lamina (2).

In the intact tubule, Sertoli cells provide the cytoarchitectural arrangements which allow adjacent germinal cells to develop during spermatogenesis (3). From *in vitro* studies, it appears likely that peritubular cells are required to facilitate Sertoli cell functions. This inference is based on observations that peritubular cells contribute components required for ECM formation (Types I and IV collagen and fibronectin) (1), and peritubular cells in culture also release a protein into the medium which stimulates Sertoli cells to synthesize transferrin and several other proteins (4).

Proteoglycans constitute important components of the ECM and are thought to play an essential role in ECM deposition (5, 6). Since Sertoli cells and peritubular cells act cooperatively in co-culture to synthesize and deposit ECM components, it was of interest to determine the types of proteoglycans produced by each cell population.

The characteristics of proteoglycans synthesized by ovarian granulosa cells have been described (6–9), but those produced by testicular cells have not. The complex cellular and secreted proteoglycans synthesized by ovarian granulosa cells are thought to be implicated in the maintenance and control of folliculogenesis, particularly in antrum formation (6, 10). Since granulosa cells and Sertoli cells share many properties (11), it was of interest to compare proteoglycans synthesized by the two cell types. Sertoli cell-enriched preparations in culture have been shown to incorporate $[^{35}S]$sulfate into proteoglycans (12), but no such study has been performed with peritubular cells.

Data to be presented in this paper demonstrate that Sertoli cells in culture synthesize and secrete proteoglycans which contain both chondroitin and heparin glycosaminoglycan (GAG) chains.[2] In contrast, peritubular cells in culture produce proteoglycans of higher molecular mass which contain chondroitin GAG but not heparin GAG chains. Proteoglycans synthesized by each of the cell types are shown to be different from those produced by ovarian granulosa cells.

MATERIALS AND METHODS

Cell Culture—Sertoli cells were isolated from testes of 20-day-old Wistar rats (13). Tubule fragments were sequentially treated first with trypsin, followed by treatment with collagenase, and then hyaluronidase by procedures developed in this laboratory (14). The

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[1] The abbreviations used are: ECM, extracellular matrix; GAG, glycosaminoglycan; DAG, dimeric acidic glycoprotein; MEM, Eagle's minimal essential medium; GdnHCl, guanidine hydrochloride; SDS, sodium dodecyl sulfate; FSH, follicle-stimulating hormone; FIRT, a mixture containing FSH, insulin, retinol, and testosterone.

[2] The term chondroitin is used to indicate chondroitin sulfate polysaccharide chains. The term heparin is used to indicate heparin sulfate and/or heparan sulfate polysaccharide chains without differentiation between the two.

Sertoli cell-enriched preparations were plated in 24-well (1 ml) Linbro plates at approximately 10^6 cells/well. If larger amounts of medium were required, cells were plated in 150-mm diameter Petri dishes at a similar cell density. Sertoli cells were cultured in serum-free, chemically defined Eagle's minimal essential medium (MEM), supplemented as previously described (15). The percentage of peritubular cells in the Sertoli cell-enriched preparation was less than 1% (14).

Peritubular cells were isolated from the supernatant fraction of the collagenase-treated tubules (16) and were cultured in 150-mm diameter Petri dishes in MEM containing 10% calf serum. The cells were grown to confluence in 5 days and were then subcultured at 25% density in the same medium until they reached confluence in 4 days. The serum was then removed and the cells were incubated for 6 h in serum-free MEM, at which time the medium was discarded and replenished. This was designated time 0 of serum-free peritubular cell culture, and all experiments and radiolabeling were done in serum-free medium from that time forward.

Granulosa cells were isolated from diethylstilbestrol-primed 20-day-old Wistar rats by procedures previously described (17) and were cultured in serum-free MEM.

Some cells, as described under "Results," were treated with different hormones and retinol at the following concentrations: ovine follicle-stimulating hormone (FSH, S-16, NIH, 100 ng/ml), insulin (5 μg/ml), retinol (0.36 μM), and testosterone (1 μM).

Sample Collection and Radiolabeling—Cells were radiolabeled with [^{35}S]sulfate (10 μCi/ml) in sulfate-free MEM, unless otherwise specified, or [^{35}S]methionine (5 μCi/ml) and [^{3}H]glycine (5 μCi/ml) in methionine and glycine-free MEM. Radiolabeled medium collected was centrifuged (1,000 × *g* for 30 min at 4 °C) to remove cell debris. After appropriate treatment of samples, as described under "Results," guanidine hydrochloride (GdnHCl) was added to a final concentration of 4 M. When samples needed to be concentrated and/or desalted, an Amicon ultrafiltration system was used with a YM-10 membrane (10,000 molecular weight exclusion limit). To obtain radiolabeled cellular components, cells were washed at 4 °C with MEM, then scraped from the plate, suspended in water, and centrifuged at 15,000 × *g* for 30 min at 4 °C. The pellet was dissolved in 4 M GdnHCl containing 0.1 mM phenylmethylsulfonyl fluoride, 5 mM benzamidine, and 1 mM EDTA. Samples not used immediately were stored at −70 °C for a maximum of 7 days before analysis.

The total amount of [^{35}S]sulfate incorporated into secreted components (Fig. 1) was determined by harvesting the radiolabeled medium, adding GdnHCl to a final concentration of 4 M, desalting on a Bio-Rad P-6 gel filtration column to remove free sulfate, and then measuring the radioactivity in the void volume, expressed as disintegrations/min/μg of cell DNA. [^{35}S]Sulfate incorporation into total cellular sulfoproteins (Fig. 1) was determined by washing the cells with MEM, removing the cells from the plate by trypsin treatment (0.17% w/v, for 15 min at room temperature) followed by sonication, and measurement of radioactivity in aliquots by scintillation spectrometry. Amounts of DNA were determined in other aliquots with the ethidium bromide-fluorescence assay of Karstan and Wollenberger (18), as modified (19).

In experiments to determine hormonal influences on the incorporation of [^{35}S]sulfate into proteins synthesized by Sertoli cells (Table I), cells were cultured in the presence and absence of different hormones for 7 h. Cells were then radiolabeled for 48 h in medium containing 10 μCi of [^{35}S]sulfate at a final concentration of 0.4 mM sulfate. Total amounts of radioactivity in medium and cells were determined as described above.

Enzymatic and Chemical Treatments—Enzymatic digestions and chemical treatments were performed on samples prior to the addition of GdnHCl, or on samples isolated from gel filtration columns which had been concentrated and desalted by ultrafiltration in less than 1 h at 4 °C in the presence of protease inhibitors. Alkaline borohydride treatment was done in 0.05 M NaOH at 45 °C for 18 h in the presence of 1 M sodium borohydride (20). Samples were neutralized with acetic acid, brought to a concentration of 4 M GdnHCl, and stored at −70 °C until analyzed. Enzymatic digestions were performed for 1 and 3 h at 37 °C with the following enzymes: chondroitinase lyase ABC (Sigma, 25 and 100 milliunits/sample); chondroitinase lyase AC II (Sigma, 50 and 250 milliunits/sample); heparinase (Miles, 50 and 500 milliunits/sample); or tosylphenyl chloroketone-treated trypsin (Sigma, 5 μg/sample). Digestions were performed in 250 μl of 0.15 M NaCl containing 0.5 mM benzamidine and 50 mM Tris/HCl at pH 7.5. To terminate the digestion, GdnHCl was added to the samples to give a final concentration of 4 M, and samples were stored at −70 °C until chromatographed. Digestion conditions used were slight modifications of procedures described by others (10). To determine possible contamination of the glycosidic enzymes with proteases, a ^{125}I-fibrin degradation assay (21) was employed which was capable of detecting activity equivalent to that present in 2 ng of trypsin. In the presence of 5 mM benzamidine, no protease activity was detected with this assay in chondroitinase lyase ABC, AC II, or heparinase. However, in the absence of benzamidine, AC II did contain detectable protease activity. In confirmation of information reported by the supplier, heparinase contained detectable quantities of chondroitinase ABC and heparanase. It was therefore possible to establish heparin identity only in those proteoglycans which were resistant to chondroitinase ABC, and it was not possible to differentiate between heparin and heparan sulfate with enzymatic digestion procedures described.

Column Chromatography—Sepharose CL-2B, CL-4B, and CL-6B (Pharmacia Fine Chemicals) gel filtration columns (0.7 × 100 cm) and Bio-Gel P-6 (Bio-Rad) gel filtration columns (1.7 × 20 cm) were equilibrated with a 4 M GdnHCl solution containing 50 mM Tris, pH 7.5. All chromatography was done at 4 °C, and 1.2-ml fractions were collected from each column, using the equilibration solution for elution. As a preliminary treatment of the columns, 1 ml of a 4 M GdnHCl extract of bovine nasal cartilage (10 mg/ml) was applied and eluted. This pretreatment of the columns removed nonspecific binding sites, and increased yields to greater than 95% recoveries of the applied radioactivity. Radioactivity (disintegrations/min) was determined by scintillation spectrometry, using ACS liquid scintillation fluid (Amersham Corp.).

Electrophoresis and Fluorography—Radiolabeled proteins were electrophoretically analyzed on sodium dodecyl sulfate (SDS) 5–15% polyacrylamide gradient slab gels under reducing conditions with the Laemmli buffer system (22). Gels were fluorographed with the use of 2,5-diphenyloxazole in acetic acid as previously described (23).

RESULTS

Radiolabeled Sulfate Incorporation into Sulfoproteins

Sertoli cells and peritubular cells were radiolabeled with [^{35}S]sulfate for 48-h periods at specific times during cell culture, and the amounts of sulfate incorporated into secreted and cellular labeled proteins were determined (Fig. 1). Sulfate incorporation into components secreted by Sertoli cells maintained under basal conditions increased slightly between days 4 and 8 of culture (Fig. 1*A*). [^{35}S]Sulfate incorporation into cellular moieties remained constant during this period, and the specific activity/μg of DNA was twice that of the secreted material (Fig. 1*B*). Cells were incubated from time 0 of culture in the absence or presence of a mixture containing FSH, insulin, retinol, and testosterone (FIRT). Treatment with FIRT significantly stimulated the secretion of labeled proteins by Sertoli cells, with a maximum effect observed on day 4 of culture (Fig. 1*A*). However, FIRT had a less pronounced effect on the incorporation of [^{35}S]sulfate into proteins in extracts of Sertoli cells (Fig. 1*B*).

The incorporation of [^{35}S]sulfate into labeled components by peritubular cells, both secreted and cellular, was maximal at day 2 of culture, and declined rapidly by day 4 to a basal level which remained constant (Fig. 1, *C* and *D*). The relative amounts of peritubular cell-secreted and cellular ^{35}S-labeled proteins were the same except during the first 48-h collection, during which a higher specific activity was observed in the secreted components. Treatment of peritubular cells with FIRT had no detectable influence on the incorporation of labeled sulfate into secreted or cellular moieties during the 8 days of culture (Fig. 1, *C* and *D*).

Effects of Altering the Duration of Radiolabeling—Incubation of Sertoli cells with [^{35}S]sulfate for varying time periods, starting on day 2 of culture, resulted in similar chromatographic profiles of labeled secreted materials, with increasing amounts incorporated during longer periods of incubation (Fig. 2*A*). Simliar results were obtained with peritubular cells which were labeled starting at time 0 of serum-free cell culture

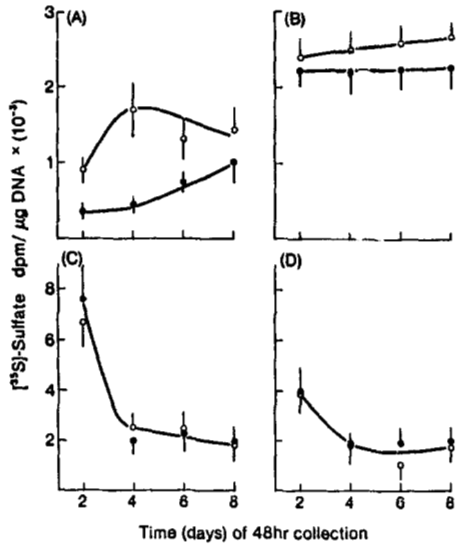

FIG. 1. **Radiolabeled sulfate incorporation into proteins.** Sertoli cells and peritubular cells were cultured in the absence (●) or presence (○) of FSH (100 ng/ml), insulin (5 μg/ml), retinol (0.36 μM), and testosterone (1 μM). Cells were incubated in [^{35}S]sulfate for 48 h at specified times of culture, after which radiolabeled medium and cells were collected. Incorporation of [^{35}S]sulfate into secreted and cellular proteins was determined, and is expressed as disintegrations/min/μg of DNA × 10^{-3}. *A*, Sertoli cell-secreted ^{35}S-labeled proteins; *B*, Sertoli cell-associated ^{35}S-labeled proteins; *C*, peritubular cell-secreted ^{35}S-labeled proteins; *D*, peritubular cell-associated ^{35}S-labeled proteins. Values are expressed as the mean ± S.D. for triplicate samples in three separate experiments (n = 9) of [^{35}S]sulfate incorporated per μg of cell DNA.

(Fig. 2*C*). In subsequent experiments, extended radiolabeling times of 48 and 72 h were chosen for both cell types.

Effects of Altering the Day of Culture of Radiolabeling—To determine if the culture period in which the cells were radiolabeled had any influence, the cells were incubated in the presence of [^{35}S]sulfate for 48 h between days 0 and 2, and between days 6 and 8 of culture. The chromatographic profiles of peritubular cell-secreted sulfoproteins were similar for the two periods, except for a reduced [^{35}S]sulfate incorporation on day 8 of cell culture (Fig. 2*D*). The Sertoli cell-secreted sulfoprotein chromatographic profiles, however, were different during the two radiolabeling periods. A decreased amount of the higher molecular mass material (K_{av} of 0) was detected in cells incubated with labeled sulfate on day 2 (Fig. 2*B*). The profiles obtained from labeling on day 8 or on day 5 were essentially the same (Fig. 2, *A* and *B*).

For subsequent experiments, media from the 72-h collection and radiolabeling periods from days 2 to 5 of Sertoli cell culture and from days 0 to 3 of serum-free peritubular cell culture were employed as sources for proteoglycans to be isolated and characterized and for the analysis of the hormonal regulation of sulfoprotein production.

Comparison of Sulfoproteins Secreted by Granulosa Cells and by Sertoli Cells

Granulosa cells were cultured and radiolabeled with [^{35}S] sulfate during the initial 48 h of culture. The secreted [^{35}S] sulfate-labeled proteins were applied to a CL-2B gel filtration column, and the chromatographic profile was compared with that obtained with Sertoli cell-secreted ^{35}S-labeled proteins. The granulosa cells secreted sulfate-labeled material which had K_{av} values of 0.15 and 0.25. In contrast, Sertoli cells secreted labeled sulfoproteins having K_{av} values of 0.5 and 0.75 (Fig. 3).

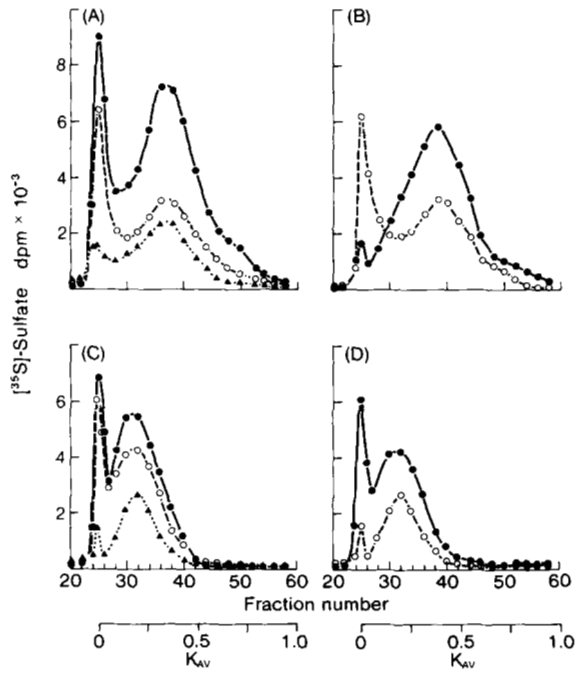

FIG. 2. **Time and duration of radiolabeling periods.** Cell cultures were radiolabeled with [^{35}S]sulfate, and secreted proteins were collected and chromatographed on Sepharose 6B gel filtration columns. *A*, Sertoli cells were radiolabeled on day 2 of culture for 12 (▲), 48 (○), and 72 (●) h. *B*, Sertoli cells were radiolabeled for 48 h between days 0 and 2 (●) and 6 and 8 (○) of culture. *C*, peritubular cells were radiolabeled on day 0 of culture for 12 (▲), 48 (○), and 72 (●) h. *D*, peritubular cells were radiolabeled for 48 h between days 0 and 2 (●) and 6 and 8 (○) of culture. Data are expressed as disintegrations/min of [^{35}S]sulfate/fraction, and are normalized per culture well from which the sample was collected. Each well contained approximately 1.5 μg of cell DNA in various experiments, with a coefficient of variation of less than 5% in DNA content between wells in a given experiment.

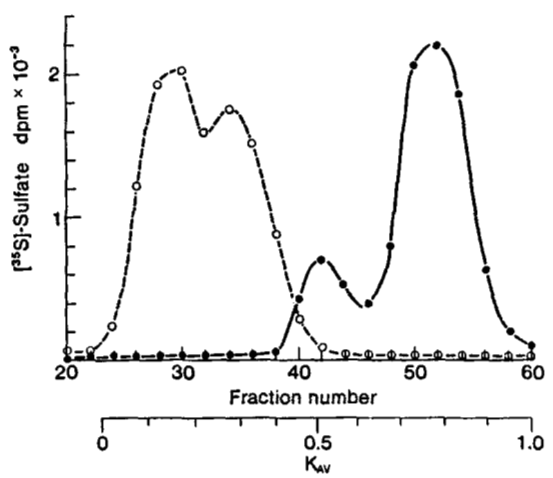

FIG. 3. **Comparison of Sertoli cell- (●) and granulosa cell- (○) secreted sulfate-labeled proteins.** Sertoli cells were cultured in the presence of [^{35}S]SO_4^{2-} on day 2 of culture for 72 h and granulosa cells were cultured in the presence of [^{35}S]SO_4^{2-} on day 0 of culture for 48 h. Radiolabeled medium was collected and applied to a Sepharose CL-2B gel filtration column.

Isolation of Secreted Proteoglycans

The radiolabeled proteins secreted by Sertoli cells were applied to CL-4B and 6B columns. The void peak obtained by chromatography on the 6B column had a K_{av} of 0.1 when chromatographed on a 4B column (Fig. 4, *A* and *B*). Similar

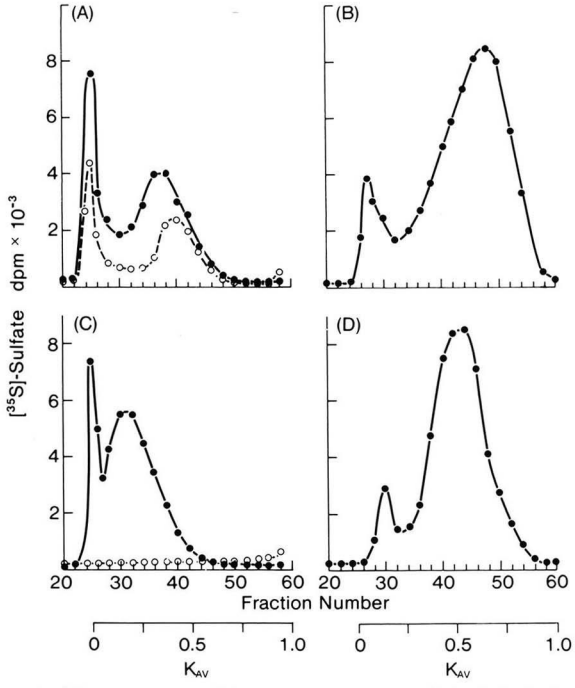

FIG. 4. **Chromatographic profiles of sulfate-labeled proteins secreted by Sertoli cells and peritubular cells in culture.** *A*, Sertoli cell-secreted sulfate-labeled proteins applied to a Sepharose CL-6B gel filtration column. Prior to application, samples were incubated in the absence (●) or presence (○) of chondroitinase ABC. *B*, Sertoli cell-secreted sulfate-labeled proteins applied to a Sepharose CL-4B column. *C*, peritubular cell-secreted sulfate-labeled proteins applied to a Sepharose CL-6B gel filtration column. Prior to application, samples were incubated in the absence (●) or presence (○) of chondroitinase ABC. *D*, peritubular cell-secreted sulfate-labeled proteins applied to a Sepharose CL-4B gel filtration column.

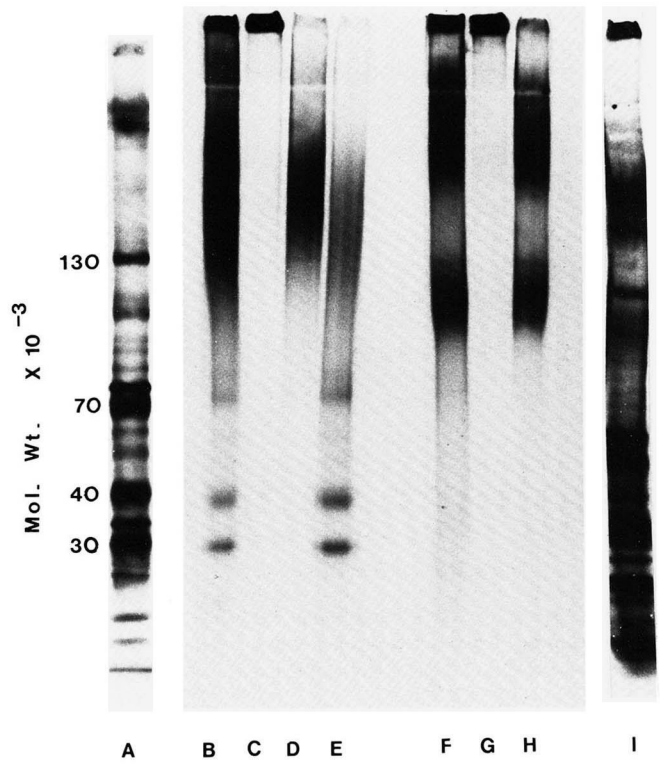

FIG. 5. **Electrophoretic analysis of Sertoli cell- and peritubular cell-secreted radiolabeled proteins.** SDS-5–15% polyacrylamide slab gel after electrophoresis was fluorographed. *Lane A*, Sertoli cell-secreted [^{35}S]methionine-radiolabeled proteins; *lane B*, Sertoli cell-secreted [^{35}S]sulfate-radiolabeled proteins; *lane C*, Sertoli cell-secreted [^{35}S]sulfate-radiolabeled protein (SC I) from fraction 25 of 6B column, Fig. 4*A*; *lane D*, Sertoli cell-secreted [^{35}S]sulfate-radiolabeled sulfoprotein (SC II) from fraction 32 of 6B column, Fig. 4*A*; *lane E*, Sertoli cell-secreted [^{35}S]sulfate-radiolabeled protein (SC III) from fraction 40 of 6B column, Fig. 4*A*; *lane F*, peritubular cell-secreted [^{35}S]sulfate-radiolabeled proteins; *lane G*, peritubular cell-secreted [^{35}S]sulfate-radiolabeled sulfoprotein (PC I) from fraction 25 of 6B column, Fig. 4*C*; *lane H*, peritubular cell-secreted [^{35}S]sulfate-radiolabeled protein (PC II) from fraction 32 of 6B column, Fig. 4*B*; *lane I*, peritubular cell-secreted [^{35}S]methionine-radiolabeled proteins.

observations on the peritubular cell-secreted ^{35}S-labeled proteins demonstrated that the void peak obtained by chromatography on the 6B column had a K_{av} of 0.15 when chromatographed on a 4B column (Fig. 4, *C* and *D*). These experiments indicate that the leading peak detected on 6B column chromatography consists of a discrete moiety, evident even after chromatography of the material on a larger molecular weight exclusion limit gel, CL-4B (Fig. 4, *C* and *D*).

The sensitivity of total secreted ^{35}S-labeled proteins to chondroitinase ABC was determined by treatment of medium samples with the enzyme preparation prior to the addition of GdnHCl. Sertoli cell-secreted sulfate-labeled proteins were sensitive to chondroitinase ABC, with a reduction in radioactivity associated with macromolecules and a shift in the second peak from K_{av} of 0.35 to 0.42 (Fig. 4*A*). This reduction in radioactivity corresponds to an increase in radioactivity in material having a K_{av} of 1.0 (data not shown). As previously observed (12), some Sertoli cell-secreted sulfate-labeled proteins were resistant to digestion by chondroitinase ABC. With peritubular cell-secreted labeled proteins, however, treatment with chondroitinase ABC removed all radioactivity (Fig. 4*C*). The sulfate label incorporated into proteins secreted by peritubular cells therefore appears to be chondroitin and/or dermatan sulfate proteoglycans.

To analyze further the sulfate-labeled proteins secreted by Sertoli cells and peritubular cells, specific fractions from the CL-6B column (Fig. 4, *A* and *C*), were desalted and analyzed on SDS-polyacrylamide gel electrophoresis and fluorography (Fig. 5). Comparison of the total secreted sulfate-labeled proteins with the isolated fractions demonstrates the ability to separate the different sulfated components by gel filtration on the 6B gel column. Proteoglycans within the gel appear as diffuse bands, presumably because of charge heterogeneity and the high carbohydrate content. The approximate molecular masses of proteoglycans referred to in Fig. 5 do not provide accurate estimations, but are used only for identification of moieties in this discussion. The void peak from the Sertoli cell 6B column (fraction 25) was too large to enter the SDS gel and remained at the top (Fig. 5, *lane C*). This Sertoli cell-secreted sulfate-labeled protein is designated SC I. A second sulfate-labeled protein was evident as a band appearing between 100 and 250 kDa (Fig. 5, *lane D*). It was obtained from the leading portion of the peak K_{av} of 0.35 on the 6B column (fraction 32 of Fig. 4*A*), and is designated SC II. A third fraction obtained from the 6B column (fraction 40) had three distinct sulfate-labeled proteins of 30, 40, and 70 kDa, with some diffuse material similar to that of SC II (Fig. 5, *lane E*). These three sulfate-labeled proteins, which are termed SC III, will be discussed subsequently. Electrophoretic profiles of [^{35}S]sulfate-labeled proteins secreted by Sertoli cells and of [^{35}S]methionine-labeled proteins secreted by Sertoli cells each had bands at 30, 40, and 70 kDa (Fig. 5, *lanes A* and *B*). It appears that the second peak of Sertoli cell-secreted proteins on the 6B column, having a K_{av} value of 0.35

(Fig. 4*A*), is made up of the two sulfate-labeled protein fractions, SC II and SC III (Fig. 5, *lanes D* and *E*).

The total peritubular cell-secreted sulfate-labeled proteins separated into three bands on the SDS gel (Fig. 5, *lane F*). The void peak of the 6B column (fraction 25 of Fig. 4*C*) was a large protein which did not enter the gel (Fig. 5, *lane G*), and is designated PC I. The second peak of the 6B column having a K_{av} of 0.20 (fraction 32 of Fig. 4*C*) had two diffuse bands: one between 90 and 110 kDa and the other between 150 and 400 kDa (Fig. 5, *lane H*). When all fractions of the second peak of the peritubular cell secreted proteins (fractions 29–34 of the 6B column, Fig. 4*C*) were analyzed separately, it was found that each contained both bands at equal ratios and intensities (data not shown). These two bands could not be separated by gel filtration. They are designated PC II, and will be discussed later. The SDS gel electrophoretic pattern of proteins labeled with [^{35}S]methionine and secreted by peritubular cells is different from that of [^{35}S]sulfate-labeled secreted proteins (Fig. 5, *lanes F* and *I*).

Characterization of Secreted Proteoglycans

The protocol for the characterization of the specific proteoglycans was to isolate the appropriate fractions from the 6B column, subject the desalted samples to base hydrolysis or trypsin digestion, and then rechromatograph the samples. The *O*-glycosidic linked oligosaccharides (*e.g.* glycosaminoglycans) released after the base hydrolysis were collected from the 6B column, desalted, and subjected to digestion with different enzymes. From this information the approximate size and the specific type of glycosaminoglycan bound to the protein were deduced.

Dextrans were used as the oligosaccharide molecular weight standards for calibrating columns (Fig. 6*A*). These markers provide a maximum estimate of the molecular weight of the sulfated glycosaminoglycans, since uncharged dextrans have a smaller Stokes radius than that of charged polysaccharides.

Sertoli cell-secreted proteoglycans SC I were obtained from fractions 23–26 of the 6B column (Fig. 4*A*), and subjected to base hydrolysis. This resulted in the formation of two peaks with K_{av} values of 0.3 and 0.75, corresponding to glycosaminoglycans with approximate molecular masses of 62 and 15 kDa, respectively (Fig. 6*A*). The 62-kDa glycosaminoglycan collected from fractions 32–40 of the 6B column (Fig. 6*A*) was concentrated, desalted, and then treated with different enzymes. This 62-kDa glycosaminoglycan was observed to be sensitive to chondroitinase ABC and AC II (Fig. 6*C*), implying that it is chondroitin sulfate. The 15-kDa glycosaminoglycan, obtained from fractions 42–52 of the 6B column (Fig. 6*A*), was found to be resistant to chondroitinase ABC digestion but sensitive to heparinase (Fig. 6*D*), implying that it is heparan sulfate. Trypsin digestion of SC I produced two fragments, each of which contained glycosaminoglycans, with peaks having K_{av} values of 0.1 and 0.65 on the 6B column (Fig. 6*A*). We interpret these data to indicate the presence of different glycosaminoglycans in SC I on separate tryptic fragments.

To determine whether SC I was made up of one or two different proteoglycans, this moiety was first incubated at 37 °C in the absence or presence of chondroitinase ABC, after which it was chromatographed on a CL-4B column (Fig. 6*B*). SC I not subjected to chondroitinase treatment eluted with a K_{av} of 0.05. In contrast, SC I subjected to chondroitinase ABC treatment eluted with a K_{av} of 0.65 (Fig. 6, *B* and *D*). If two different proteoglycans had been present, one containing chondroitin GAG chains and the other containing heparin GAG chains, then chondroitinase ABC digestion should not

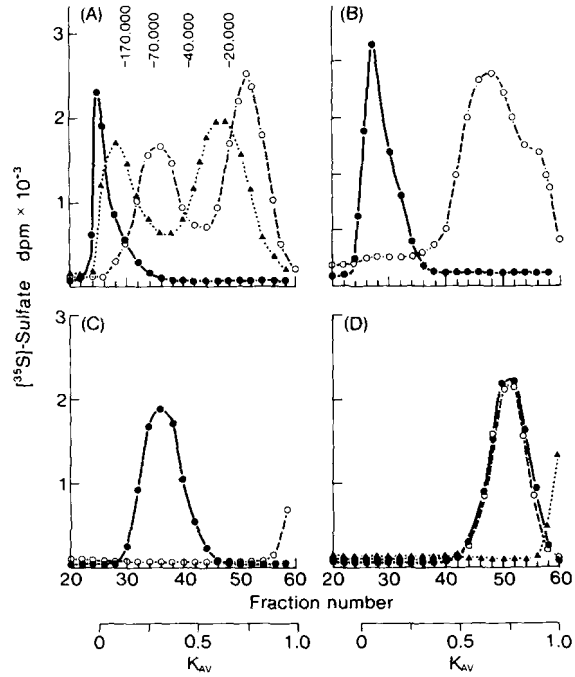

FIG. 6. **Sertoli cell-secreted [^{35}S]sulfate-labeled protein SC I.** *A*, CL-6B gel filtration chromatograph of SC I (●), base-hydrolyzed SC I (○), and trypsin-treated SC I (▲); *inset* molecular weights are dextran molecular weight standards. *B*, CL-4B gel filtration chromatograph of SC I, incubated prior to application in the absence (●) or presence (○) of chondroitinase ABC. *C*, CL-6B gel filtration chromatograph of the oligosaccharide of base-treated SC I isolated from fractions 32–40 of the 6B column in *A* that was incubated prior to application in the absence (●) or presence (○) of chondroitinase ABC or AC II. *D*, CL-6B gel filtration chromatograph of the oligosaccharide of base-treated SC I isolated from fractions 42–52 of the 6B column in *A* that was incubated prior to application in the absence (●) or presence (○) of chondroitinase ABC or heparinase (▲).

have affected the heparin proteoglycan. Consequently, a peak having a K_{av} of 0.05 with a reduced amount of radioactivity would have been observed. However, the peak with a K_{av} at 0.05 disappeared after enzymatic treatment, and a new peak appeared, having a K_{av} of 0.65, corresponding approximately to a 1500-kDa decrease in molecular weight of oligosaccharides. Chondroitinase ABC treatment had no detectable effect on the heparin GAG (Fig. 6*D*), indicating the specificity of the enzyme preparations. We interpret results presented to indicate that both the heparin and chondroitin GAGs are on the same peptide, and that SC I is a single complex proteoglycan. Additional evidence that SC I is a single proteoglycan is provided by the appearance of a single peak, having a K_{av} of 0.5, after gel filtration of SC I on Sepharose 2B (see Fig. 3).

Sertoli cell-secreted proteoglycans SC II, obtained from fractions 31–35 of the 6B column (Fig. 4*A*), were concentrated, desalted, and then subjected to base hydrolysis or to trypsin digestion (Fig. 7*A*). Base hydrolysis of SC II resulted in the formation of one peak having a K_{av} of 0.65, corresponding to a molecular mass of 25 kDa. This GAG was then collected (fractions 42–52 of the 6B column, Fig. 7*A*), concentrated, desalted, and subsequently incubated in the presence or absence of glycosidic enzymes (Fig. 7*B*). It was found that the 25-kDa GAG obtained from SC II was sensitive to chondroitinase ABC and AC II, implying that it is a chondroitin GAG. Trypsin digestion reduced the molecular weight of SC II slightly (Fig. 7*A*). From combined results, it appears that SC II is a proteoglycan with a polypeptide chain containing 25-kDa chondroitin GAG chains.

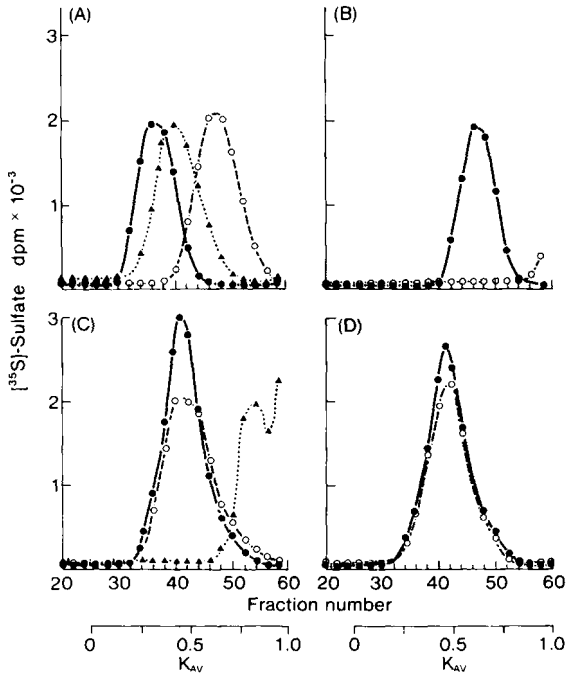

FIG. 7. **Sertoli cell-secreted [^{35}S]sulfate-labeled proteins SC II and SC III.** *A*, CL-6B gel filtration chromatograph of SC II (●), base-hydrolyzed SC II (○), and trypsin-treated SC II (▲). *B*, CL-6B gel filtration chromatograph of the oligosaccharide of base-treated SC II isolated from fractions 32–54 of the 6B column in *A* that was incubated prior to application in the absence (●) or presence (○) of chondroitinase ABC or AC II. *C*, CL-6B gel filtration chromatograph of SC III (●), base-hydrolyzed SC III (○), and trypsin-treated SC III (▲). *D*, CL-6B gel filtration chromatograph of SC III incubated prior to application in the absence (●) or presence (○) of chondroitinase ABC, AC II, or heparinase.

Sertoli cell-secreted sulfate-labeled proteins SC III, obtained from fractions 40–45 of the 6B column (Fig. 4*A*), were concentrated and desalted. Base hydrolysis of SC III had no effect on the chromatographic profile, except for a slight reduction in total radioactivity. The peak having a K_{av} of 0.45 remained unchanged (Fig. 7*C*). Trypsin digestion of SC III yielded peaks having K_{av} values of 0.8 and smaller (Fig. 7*C*). SC III was resistant to digestion with chondroitinase ABC, AC II, or heparinase (Fig. 7*D*). These results imply that SC III is not a proteoglycan, but instead is a sulfated, trypsin-sensitive, base-stable protein. A major Sertoli cell-secreted protein has been reported which contains both 30 and 40 kDa monomers (24). Antiserum produced against this protein was found to immunoprecipitate proteins having molecular masses of 30, 40, and 70 kDa (25). This same antiserum, provided by Dr. Michael Griswold (Washington State University), was used to immunoprecipitate [^{35}S]sulfate-labeled Sertoli cell-secreted proteins (see Fig. 9). It was observed that sulfoproteins having molecular masses of 30, 40, and 70 kDa in SC III were specifically immunoprecipitated, indicating that SC III has subunits and immunological properties indistinguishable from those of the protein previously described by Sylvester *et al.* (25) and called "dimeric acidic glycoprotein" or "DAG."

The proteoglycan (PC I) secreted by peritubular cells, isolated from fractions 24–26 of the 6B column (Fig. 4*C*), was concentrated and desalted. Base hydrolysis of PC I yielded a peak having a K_{av} of 0.3, corresponding to a 70-kDa GAG. This 70-kDa GAG from PC I was isolated (fractions 30–40 of the 6B column, Fig. 8*A*), and found to be sensitive to chondroitinase ABC and AC II digestion (Fig. 8*B*). Trypsin digestion of PC I produced a peak having a K_{av} of 0.1, which is the tryptic peptide containing the 70-kDa chondroitin GAG chain (Fig. 8*A*).

PC II isolated from fractions 28–38 of the 6B column (Fig. 4*C*) was concentrated and desalted. Base hydrolysis of PC II yielded a peak having a K_{av} of 0.3, corresponding to a 70-kDa GAG (Fig. 8*C*). The PC II 70-kDa GAG (fractions 30–40 of the 6B column, Fig. 8*C*) was found to be sensitive to chondroitinase ABC and AC II digestion (Fig. 8*D*). Trypsin digestion of PC II produced a peak with a K_{av} of 0.22 on the 6B column (Fig. 8*C*). Since PC I and PC II each had a 70-kDa chondroitin GAG, the possibility exists that PC II was a degradation product or processed form of PC I. After incubation of radiolabeled peritubular cell-conditioned medium overnight at room temperature, in the absence of GdnHCl, it was found that only a peak corresponding to PC II, having a K_{av} of 0.2, was present (data not shown). The ratios of PC I to PC II were not constant among different preparations of peritubular cells and radiolabeled medium, again suggesting that PC II may be a degradation product or processed form of PC I.

To determine the size of the peptide core of the different proteoglycans, the following protocol was used. 1) Cells were incubated in medium containing [^{35}S]methionine and [^{3}H] glycine, after which the medium was concentrated 50-fold. 2) The concentrated medium was chromatographed on a 6B column, and specific fractions were collected, concentrated, and desalted. 3) The fractions were incubated in the absence or presence of chondroitinase ABC and heparinase. 4) Samples were analyzed with SDS-polyacrylamide gel electrophoresis and fluorography. It was found for SC II that the peptide

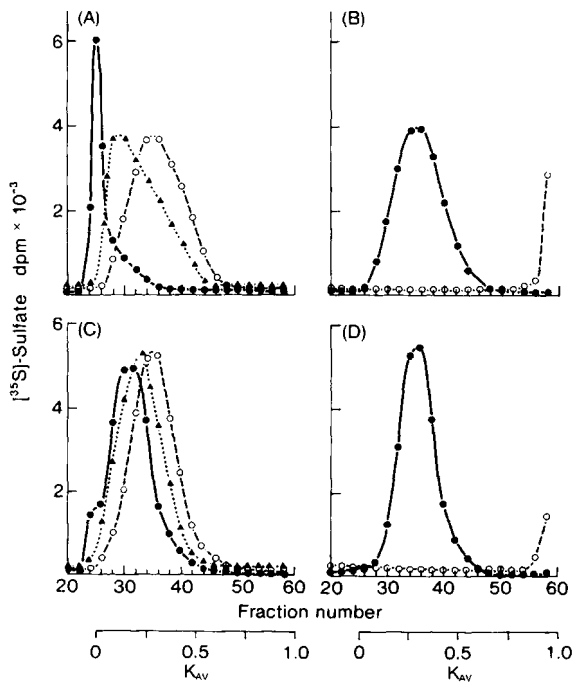

FIG. 8. **[^{35}S]Sulfate-labeled proteins PC I and PC II secreted by peritubular cells.** *A*, CL-6B gel filtration chromatograph of PC I (●), base-hydrolyzed PC I (○), and trypsin-treated PC I (▲). *B*, CL-6B gel filtration chromatograph of the oligosaccharide of base-treated PC I isolated from fractions 30–42 of the 6B column in *A* that was incubated prior to application in the absence (●) or presence (○) of chondroitinase ABC or AC II. *C*, CL-6B gel filtration chromatograph of PC II (●), base-hydrolyzed PC II (○), and trypsin-treated PC II (▲). *D*, CL-6B gel filtration chromatograph of the oligosaccharide of base-treated PC II isolated from fractions 30–42 of the 6B column in *C* that was incubated prior to application in the absence (●) or presence (○) of chondroitinase ABC or AC II.

core was 50 kDa (Fig. 9, *lanes C* and *D*), and for PC II the peptide core was 45 kDa (Fig. 9, *lanes A* and *B*).This set of procedures did not give reproducible results for PC I, SC I, or the cellular proteoglycans (data not shown).

Characterization of the Cellular Proteoglycans

Labeled proteoglycans obtained from extracts of cultured Sertoli cells or peritubular cells were chromatographed on CL-4B and CL-6B columns (Fig. 10). The cellular proteoglycans synthesized by Sertoli cells had a K_{av} of 0.1 on a 4B column, and eluted in the void volume of a 6B column (Fig. 10, *A* and *B*). Base hydrolysis of the Sertoli cellular proteoglycan generated one peak having a K_{av} of 0.4, corresponding to a 48-kDa GAG. This 48-kDa GAG was not sensitive to chondroitinase ABC or AC II, but was sensitive to heparinase (Fig. 10*A*). These results imply that the Sertoli cells produce a large cell-associated proteoglycan which contains heparin GAG chains.

The peritubular cellular proteoglycan had a K_{av} of 0.15 on the 4B column, and eluted in the void volume of the 6B column (Fig. 10, *C* and *D*). Base hydrolysis generated a peak having a K_{av} of 0.3, corresponding to a 70-kDa GAG. This GAG was sensitive to digestion with chondroitinase ABC or AC II (Fig. 10*C*). These data imply that peritubular cells produce a cell-associated proteoglycan which contains a 70-kDa chondroitin GAG similar to PC I.

Hormonal Regulation of the Incorporation of [^{35}S]Sulfate into SC III (DAG)

The addition of FIRT to the medium had no detectable effects on the formation of either secreted or cellular radiolabeled sulfoproteins produced by peritubular cells, and only slight stimulation of cellular sulfoprotein production by Sertoli cells (Fig. 1). However, FIRT addition did stimulate the incorporation of [^{35}S]sulfate into total Sertoli cell-secreted sulfoproteins, with a maximum effect observed between days 2 and 8 of culture (Fig. 1). To examine in more detail the hormonal influence on Sertoli cell-secreted sulfoprotein production, Sertoli cells were cultured in the absence or presence of hormones, and then radiolabeled with [^{35}S]sulfate between days 2 and 5 of culture. Radiolabeled medium was collected and applied to gel filtration columns to remove free sulfate. To determine the specific type of sulfoprotein whose synthesis was modulated by the hormone mixture, radiolabeled medium was digested with different glycosidic enzymes prior to gel filtration. It was found that addition of FSH alone increased total radiolabeled sulfoprotein production by over 2-fold (Table I), in confirmation of previous observations (12). In contrast, the addition of insulin, testosterone, or retinol alone did not increase the formation of labeled sulfoproteins even though addition of insulin slightly augmented the degree of stimulation by FSH (data not shown). To determine the amounts of stimulation of incorporation of labeled sulfate into chondroitin GAG, radiolabeled medium was digested with chondroitinase ABC. To determine the degree of stimulation of incorporation of labeled sulfate into heparin GAG and into non-proteoglycan sulfoprotein (*i.e.* DAG), radiolabeled medium was digested with both chondroitinase ABC and heparinase (Table I). FSH treatment had no significant influence on the incorporation of labeled sulfate into heparin GAG or chondroitin GAG (Table I). However, FSH did stimulate sulfate incorporation into the non-proteoglycan fraction (*i.e.* SC III, or DAG) by over 2-fold (Table I).

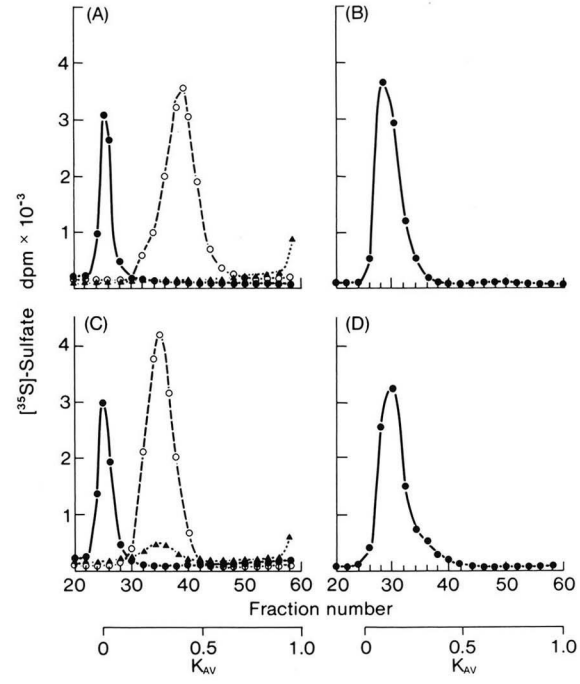

FIG. 10. **Sertoli cell and peritubular cell [^{35}S]sulfate-labeled proteins.** *A*, CL-6B gel filtration chromatograph of Sertoli cellular sulfate-labeled protein (●), base-hydrolyzed or chondroitinase ABC- or AC II-treated (○) and heparinase-treated (▲). *B*, CL-4B gel filtration chromatograph of Sertoli cellular sulfate-labeled protein. *C*, CL-6B gel filtration chromatograph of peritubular cellular sulfate-labeled protein (●), base-hydrolyzed (○) and treated with chondroitinase ABC or AC II (▲). *D*, CL-4B gel filtration chromatograph of peritubular cellular sulfate-labeled protein.

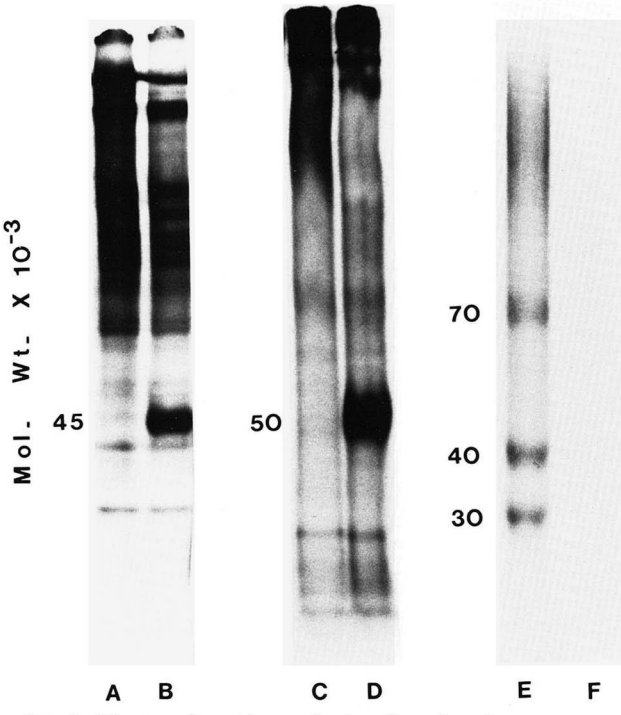

FIG. 9. **Electrophoretic analysis of proteoglycan core protein size and DAG immunoprecipitates.** SDS-5–15% polyacrylamide slab gels after electrophoresis were fluorographed. [^{35}S]Methionine- and [^{3}H]glycine-radiolabeled PC II was preincubated in the absence (*lane A*) or presence (*lane B*) of chondroitinase ABC and heparinase. [^{35}S]Methionine- and [^{3}H]glycine-radiolabeled SC II was preincubated in the absence (*lane C*) or presence (*lane D*) of chondroitinase ABC and heparinase. Immunoprecipitates of [^{35}S]sulfate-radiolabeled Sertoli cell-secreted proteins with a rabbit antiserum to DAG protein (*lane E*) and nonimmune rabbit serum (*lane F*) are shown.

TABLE I

FSH stimulation of Sertoli cell-secreted radiolabeled sulfoproteins

Sertoli cells were cultured in the absence or presence of FSH and then radiolabeled with [^{35}S]sulfate between days 2 and 5 of culture. Radiolabeled medium was collected and treated with different glycosidases before desalting or fractionation on gel filtration columns as described under "Materials and Methods." The amount of [^{35}S]sulfate associated with macromolecules was determined and is expressed as the mean ± S.D. for triplicate wells in three separate experiments (n = 9). [^{35}S]Sulfate associated with chondroitin, heparin, and nonproteoglycan (*i.e.* DAG) material was determined as shown.

Treatment of cells	[^{35}S]Sulfate enzymatic digestions		
	Control (A)	Chondroitin ABC (B)	Chondroitin ABC + heparinase (C)
	dpm/μg DNA		
None	1155 ± 104[a]	614 ± 71[a]	499 ± 40[a]
FSH	1906 ± 114[a]	1331 ± 96[a]	1146 ± 110[a]

Treatment of cells	Type sulfoprotein		
	DAG (C =)	Chondroitin (A−B =)	Heparin (B−C =)
None	499 (43%)[a]	541 (47%)	115 (10%)
FSH	1146 (60%)[a]	575 (30%)	185 (10%)

[a] Values are statistically different ($p < 0.01$) between control and FSH-treated preparations.

DISCUSSION

The present study provides a structural characterization of the proteoglycans produced by seminiferous peritubular cells and Sertoli cells. Limitations of methods used to characterize the proteoglycans will be presented prior to discussing specific structures in detail. Estimates of the molecular weights of the GAGs with the use of dextrans provides only a maximum possible molecular weight. However, we feel this is a reasonable estimate which can be used in comparing different GAGs. The procedures used to determine molecular weights of the core peptides did not give reproducible results for the large secreted or cellular proteoglycans. We therefore feel confident in reporting molecular weights only for the smaller proteoglycans. The sensitivity of the proteoglycans to glycosidic enzymes was initially determined on intact proteoglycans and subsequently on isolated GAGs. Since results obtained with intact proteoglycans confirm those presented on the isolated GAGs, it appeared unnecessary to provide both sets of data in detail. Methods used did not permit differentiation between heparin and heparan sulfate. To achieve this, other procedures will be required, such as nitrous acid treatment or antithrombin binding.

The probable structures of proteoglycans secreted by Sertoli cells and by peritubular cells can be constructed from data presented (Fig. 11). Peritubular cells secrete two proteoglycans, PC I and PC II. PC I is a large proteoglycan, having a K_{av} of 0.15 on the 4B column, and containing 70-kDa chondroitin GAG chains. The number of GAG chains on the protein core is difficult to estimate, but from the K_{av} of 0.15 on the 4B column for PC I, we speculate that 15–25 chains may be present on the protein core. PC II was a smaller proteoglycan, having a K_{av} of 0.2 on the 6B column, and also containing 70-kDa chondroitin GAG chains. It was found that PC II had a 45-kDa core protein. Due to the small change in molecular weight after either base or trypsin treatment, we speculate that one or two GAG chains are bound to PC II. PC II was found to separate into two diffuse bands on SDS gels under reducing or nonreducing conditions, and could not be separated by gel filtration. It is possible that this heterogeneity is due to a difference in the number of GAG chains on the peptide, and that the two forms have a similar Stokes radius. The observations that PC I, upon prolonged incubation at room temperature under nondenaturing conditions, generated a peak on the 6B column corresponding to PC II, and that both had a 70-kDa chondroitin GAG chain, imply that PC II may be a degradation product or processed form of PC I. However, the tryptic peptide on the GAG from PC I was larger than that from PC II. If a protease is responsible for the conversion of PC I to PC II, then an additional protease having a specificity different from that of trypsin would be required. The cellular proteoglycan produced by peritubular cells had a K_{av} of 0.15 on a 4B column, and contained a 70-kDa chondroitin GAG. These properties are similar to those found for PC I. We propose that the cellular proteoglycan and PC I are the same, and that PC I is either shed or it is the soluble secreted species. Once solubilized, PC I may be more susceptible to degradation. Alternatively, PC I could be processed into PC II.

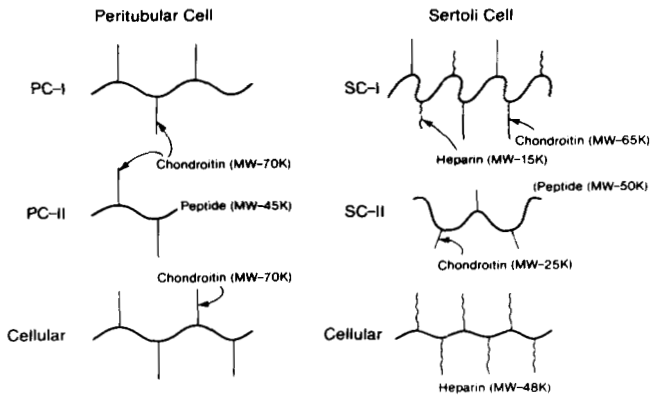

FIG. 11. **Summary of proposed structures of proteoglycans produced by Sertoli cells and peritubular cells.** Molecular masses presented for glycosaminoglycans are maximum estimates. The number of GAG chains illustrated per peptide core is arbitrary.

The Sertoli cells were found to secrete two proteoglycans, SC I and SC II. SC I is a large proteoglycan apparently containing both a 62-kDa chondroitin GAG and a 15-kDa heparin GAG. It is suggested from the shift in elution profiles after digestion with chondroitinase ABC (from a K_{av} of 0.05 to 0.65, on the 4B column) that both the chondroitin and heparin GAG were on the same SC I peptide. If the heparin GAG were on a separate peptide, no change in the elution profile would have been expected. One possible limitation to this interpretation is that a unique protease may exist as a contaminant in the chondroitinase ABC preparations which is not detectable by the ^{125}I-labeled fibrinolytic protease assay. If present, it may generate a small peak on the 4B column associated with the heparin. Due to the sensitivity and wide range of different proteases detected by the ^{125}I-labeled fibrinolytic assay previously described (26), we believe this is not a strong possibility, and that SC I is a single protein containing both chondroitin and heparin GAGs. Additional evidence in favor of this supposition is the single peak obtained when SC I was chromatographed on Sepharose 2B (Fig. 3). Unequivocal demonstration that SC I is a single proteoglycan containing both chondroitin and heparin GAGs will require further analyses, such as composite agarose/acrylamide electrophoresis, and density gradient centrifugation. The other secreted proteoglycan, SC II, was found to have a 50-kDa peptide, and contains 25-kDa chondroitin GAG chains. Due to the change in elution after base treatment, we speculate that SC II contains two to four chondroitin chains. The cellular proteo-

glycan produced by Sertoli cells was found to have a K_{av} of 0.1 on a 4B column, and it contains 48-kDa heparin GAG chains. Therefore, the Sertoli cellular proteoglycan is a large heparin proteoglycan.

The other sulfate-labeled protein secreted by Sertoli cells, designated SC III, was found not to be a proteoglycan but a sulfated protein. SC III was resistant to base hydrolysis and glycosidic enzymes, while sensitive to trypsin. Analysis on SDS-polyacrylamide gel electrophoresis demonstrated that SC III contained 30-, 40-, and 70-kDa bands. Comparison of the radiolabeled sulfated proteins with [^{35}S]methionine- and [^{3}H]glycine-radiolabeled proteins revealed the presence of the same 30-, 40-, and 70-kDa bands. It has been previously reported that Sertoli cells secrete a DAG, which contain subunits of 30 and 40 kDa (24). An antiserum against DAG was found to immunoprecipitate the 30- and 40-kDa proteins, as well as a 70-kDa protein, which was a putative precursor (25). In the present study we have utilized DAG antiserum to immunoprecipitate [^{35}S]sulfate-labeled Sertoli cell-secreted proteins. It was found that SC III was immunologically similar to DAG, and both had the same molecular weights.

Proteoglycans produced by ovarian granulosa cells have been well characterized (6–9). Both granulosa (27) and Sertoli cells (12) respond to FSH with increased sulfoprotein production. These observations provided the initial incentive to compare the proteoglycans produced by Sertoli cells with those synthesized by granulosa cells. In an analysis of the chromatographic profiles of sulfoproteins secreted by Sertoli cells and by granulosa cells, significant differences were found. The granulosa cell-secreted radiolabeled sulfoproteins had much higher molecular weights, and the structures of the proteoglycans produced by the two cell types were also different. Granulosa cells are reported to secrete a large proteoglycan containing both *N*-glycosidic bound oligosaccharides and dermatan GAG chains (7, 10). Other proteoglycans produced by granulosa cells include a small dermatan-containing proteoglycan (8), and a heparan-containing proteoglycan (9). It is therefore apparent that proteoglycans produced by granulosa cells differ greatly from the proteoglycans described in this paper which are synthesized by Sertoli cells or by peritubular cells.

Addition of FSH or dibutyryl cAMP has been reported to increase the incorporation of [^{35}S]sulfate into proteins secreted by Sertoli cell preparations. The stimulation reported did not occur in proteins containing chondroitin GAGs (12). Results of the present study demonstrate that FSH does not stimulate the incorporation of [^{35}S]sulfate into chondroitin or heparin proteoglycans, but does stimulate the incorporation of labeled sulfate into a sulfoprotein identified as SC III, equivalent to DAG (24, 25). Although FSH does stimulate proteoglycan production by granulosa cells (27), it does not do so in Sertoli cells. The stimulation by FSH of [^{35}S]sulfate incorporation into DAG may reflect an increased rate of protein synthesis, or simply an increase in labeled sulfate incorporation into pre-existing DAG. It remains to be determined whether the sulfate is associated with the peptide or carbohydrate portion of DAG.

In many organs, epithelial cells are in contact with stromal (mesenchymal) cells, separated by a basal lamina (28). The complex ECM between these two cell types is generally recognized as being functionally important (28). Peritubular cell-Sertoli cell interactions provide an example of a mesenchymal cell-epithelial cell interaction in which the cells act cooperatively to generate a basal lamina (2) and various ECM components required for extracellular fibril deposition (1). We have reported that Sertoli cells and peritubular cells in culture produce different components of the ECM, and that deposition is increased when both cell types are present (1). Since proteoglycans are an important component of the ECM (5, 6), the identification and characterization of proteoglycans produced by the two cell types is a necessary first step in determining the role that proteoglycans may play in the cooperative deposition of ECM components observed in cocultures of peritubular and Sertoli cells.

The unique properties proposed for the Sertoli cell-secreted SC I may imply a bifunctional role for this proteoglycan, since both heparin and chondroitin (GAGs) appear to be present on the same polypeptide. Heparin proteoglycans are commonly associated with the plasma membrane, such as the liver plasma membrane proteoglycan (29). It is possible that the heparin portion of SC I may bind to the cell surface, while the chondroitin portion may associate with other ECM components, or with another cell, thereby providing a bifunctional linker. Another example of a proteoglycan containing two different types of GAG chains is the major proteoglycan in cartilage which contains both keratan sulfate and chondroitin GAG chains (30). The presence of two different types of GAG chains on one peptide (SC I) secreted by Sertoli cells appears to be novel. It also introduces a complexity concerning the control of synthesis and processing of such proteoglycans (31).

Information presented has provided an initial characterization of proteoglycans produced by Sertoli cells and by testicular peritubular cells in culture. A more detailed characterization is required for each proteoglycan identified, and future investigations are needed to determine the validity of the novel structure proposed for SC I. In addition, the production of antisera against specific proteoglycans will be useful for more rigorous quantitation, and for investigations of changes during development and turnover. Further analysis of the proteoglycans produced by peritubular cells and by Sertoli cells may provide a favorable system for the investigation of the role of proteoglycans in mesenchymal cell-epithelial cell interactions.

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