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^{35}S-Labeled Glycosaminoglycans of the Mouse Prostate and its Secretion by Organ Culture (40712)

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By established histochemical methods for staining glycosaminoglycans (GAGs), the secretions of carcinomatous human prostates are stainable; however, secretions of the normal prostates are not stainable (1). Hence, it has been accepted that prostate tissues having GAG-stainable secretions are not likely to be normal.

The seeming absence of secretion GAGs in the normal prostate was questioned by recent findings (2). When [^{35}S]sulfate was administered to normal mice, this GAG precursor was rapidly incorporated into macromolecules of the normal prostate. Subsequently, most of these ^{35}S-labeled molecules were secreted as normal secretory products by the epithelial cells. Moreover, these labeled macromolecules lost most of their ^{35}S upon reaction with methanolic HCl, a reaction known to cleave the sulfate ester groups of GAGs.

Examination of prostate secretions uncontaminated by secretions of other organs should clearly resolve whether or not GAGs are present in the secretion of the normal prostate gland.

In this report, organ culture techniques were used to obtain ^{35}S-labeled prostates and their secretions; and the ^{35}S-GAGs of the prostates and secretions were isolated, characterized, and quantitated.

Materials and methods. Organ culture of prostate glands. The lateral prostates were removed under aseptic conditions with the aid of a dissecting microscope from 6-week-old, male, ICR mice (purchased from TIMCO Breeding Labs, Houston, Tex.). The prostates were maintained in organ culture according to the method given in Ref. (3), but with a few modifications.

Immediately upon removal, the prostates were kept on ice in a "holding medium," which was made up of medium 199 supplemented with fetal bovine serum (15%, v/v; Rehatuin F. S., from Reheis Chemical Co., Phoenix, Ariz.) and chick embryo extract (3%, v/v; GIBCO, Grand Island, N.Y.), containing penicillin (250 units/ml), and streptomycin (250 μg/ml, GIBCO).

Just prior to incubation, the left and right lateral prostates of each mouse were rinsed with cold "rinse medium" (similar to the "holding medium," but having 2.5 times less penicillin–streptomycin) and placed on a 1-cm^2 lens paper (4) supported on a stainless-steel grid in the center well of a 60 × 15-mm organ culture dish (Falcon 3011). The absorbent ring in the outer well was moistened with 1.0 ml saline; and to the center well was added 0.8 ml of "labeling medium" (similar to the "rinse medium," but containing 80 μCi of $H_2{}^{35}SO_4$ (Carrier free, New England Nuclear, Boston, Mass.). (Just prior to using, the $H_2{}^{35}SO_4$ was passed through a 0.22-μm filter, Millex; Millipore Corp., Bedford, Mass.). The culture dishes, loaded on individual shelves of a 10-shelf stainless-steel rack, were placed in a jar. After flushing with a mixture of O_2 (95%) and CO_2 (5%) at 125 ml/min for 25 min, the jar was sealed and incubated at 37.5°C. The 100-hr samples were again fed the "labeling medium" at 50 hr of incubation; all other samples were fed only once.

Radioactivities of prostates and their secretions. Immediately following incubation, the prostates were immersed in 10% formalin solution containing 0.1% each of Na_2SO_4 and hexadecyltrimethylammonium bromide; they were washed at regular intervals with the solution and then with ethanol, as described (2). The fed "labeling media" of each organ culture group were combined and mixed with 3 vol of ethanol solution (1g of potassium acetate in 99 ml of

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99% ethanol) and kept overnight in a refrigerator. After spinning in a clinical centrifuge, the precipitate (hereafter referred to as secretion) was washed once each with the ethanol solution diluted with water (3:1, v/v) and with ethanol.

The washed secretions and prostates were extracted three times with toluene:ethanol (3:2, v/v) (5), twice with ethanol, and dried to constant weight. These samples were then solubilized to a clear solution in glass vials at 60°C with a mixture of 70% $HClO_4$ (0.1 ml) and 30% H_2O_2 (0.2 ml) (2, 6). When cooled to room temperature, 10 ml of Aquasol (New England Nuclear) was added to the contents of each vial and measured for radioactivity in a liquid scintillation spectrometer (Tri-Carb; Packard Inst. Co., La Grange, Ill.).

Isolation of ^{35}S-GAGs of prostates and their secretions. In another set of experiments, immediately after incubation, the prostates were immersed in and washed several times with acetone over a period of 1 to 4 days. The fed "labeling media" of each group were combined, mixed with the ethanol solution, and the precipitated secretions washed as above. Both the washed secretions and prostates, after extracting with the organic solvents and drying to constant weight, were digested with papain (papaya latex, twice crystallized; Sigma Chemical Co., St. Louis, Mo.) as described (7). These digests were centrifuged at an average of 40,000*g* for 30 min, and the pellets were washed once with water; the supernates of digests and wash were combined with their respective NaOH extracts, below. The washed pellets were mixed with 0.5 *N* NaOH and kept at 4°C overnight. When neutralized with equimolar amounts of acetic acid, the prostate samples remained clear; however, the secretion samples became milky, so they were centrifuged and their pellets washed once with water. All of the supernates were combined with their respective papain extracts, above, and then dialyzed against deionized water in either 6-mm Visking tubing or Spectrapor 1 (Spectrum Medical Industries, Inc., Los Angeles, Calif.). (Before using, the tubings were washed for 15 min with running hot water and 15 min with running deionized water.) The retentates were reduced in volume and those of the prostates were designated prostate ^{35}S-GAGs; those of the secretion, secretion ^{35}S-GAGs.

Radioactivities of the NaOH-insoluble residues were measured after they were solubilized in the $HClO_4-H_2O_2$ mixture and mixed with Aquasol.

Analyses of prostate and secretion ^{35}S-GAGs. Aliquots of prostate and secretion ^{35}S-GAGs were digested for 1 hr at 37.5°C with twice the recommended amounts of either chondroitinase ABC (ABCase) or chondroitinase AC (ACase) (Miles Research Products, Elkhart, Ind.) (7, 8). Each digest was extracted by mixing with twice its volume of cold *n*-butanol:acetic acid (2:1, v/v), chilling in an ice bath for 30 min, and centrifuging. The pellet was extracted three times by suspending it in cold water equal to the volume of the digest and proceding as in above.

The respective butanol–acetic acid extracts were combined, chilled, and dried under reduced pressure over NaOH and $CaCl_2$ pellets. For chromatographic separation, the dried extracts were taken in water and streaked across Whatman No. 1 paper. After desalting with *n*-butanol:ethanol:water (13:8:4) for 24 hr, they were developed with *n*-butanol:acetic acid:water (2:1:1) for 18 hr and then with *n*-butanol:1 *N* NH_4OH:acetic acid (2:1:1) for 18 hr (9). The radioactive areas, visualized by radioautography on RP Royal X-Omat (Eastman Kodak Co., Rochester, N.Y.), were cut out and eluted with water. Because some of the cutouts retained residual amounts of radioactivity, appropriate corrections were applied to the values presented in Tables I and II. Radioactivity was measured by liquid scintillation counting; and hexuronic acid content, by the *m*-hydroxydiphenyl method (10).

Digestions with chondro-4-sulfatase (4Sase) and chondro-6-sulfatase (6Sase) (Miles Research Products) were at six times the recommended enzyme concentrations (8). Control samples were identical to the digestion mixtures but with boiled enzymes. All the samples were incubated at 37.5°C for 30 min, lyophilized, and chromatographed as given above.

Reference samples of 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-6-*O*-sulfo-D-galactose (ΔDi-6S), 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-4-*O*-sulfo-D-galactose (ΔDi-4S), and 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose (ΔDi-0S) were purchased from Miles Research Products.

Results. [^{35}S]Sulfate was incorporated into the lateral prostates of mice with increasing time of organ culture, up to 100 hr (curve I, Fig. 1). However, the ^{35}S incorporation into the prostate secretions was delayed by about 20 hr (curve II, Fig. 1). The amount of ^{35}S incorporated into the 100-hr samples was about 0.03% of the total media ^{35}S, fed at the beginning and at 50 hr. The total uptake of ^{35}S by the 100-hr incubated prostates was less than 1% of the media ^{35}S, thus, the media ^{35}S available to the prostates remained practically constant during the culture.

^{35}S-GAGs were extracted with papain and 0.5 *N* NaOH. The radioactivities of the extracted residues averaged 0.49 and 2.88%, respectively, of prostate and secretion ^{35}S-GAGs.

Aliquots of ^{35}S-GAGs of 20-, 30-, and 50-hr cultured samples of prostates and their secretions were depolymerized with either ABCase or ACase. The depolymerized components were separated by paper chromatography and then radioautographed. Radioautographs of the 50-hr samples are presented in Figs. 2 (prostate) and 3 (secretion). Figure 2 shows that the radioactive components of prostate ^{35}S-GAGs are separated into bands of various densities, which reflect their relative ^{35}S content. Moreover, some of the bands in row A (ABCase) did not have similar configurations and densities to their counterparts in row B (ACase). In contrast to Fig. 2, Fig. 3 shows that the ABCase (row A) bands were almost identical to those of the ACase (row B) bands. Further comparison shows that bands 3 and 6 were not present in Fig. 3. The respective radioautographs of the depolymerized components of the prostate and secretion ^{35}S-GAGs for the 20- and 30-hr time points were identical to those of Fig. 2 and 3, except for their densities; that is, lower density with shorter time of culture. The radioactive bands were numbered from "O" (for origin) to 6. Their radioactivities are presented in Tables I and II and summarized in Table III.

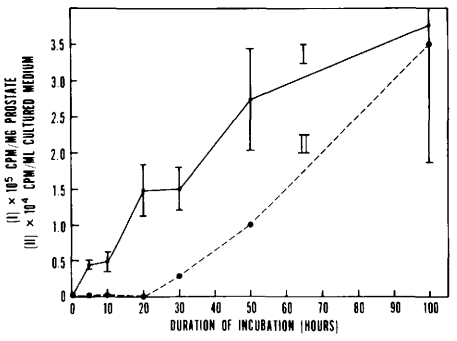

FIG. 1. [^{35}S]Sulfate incorporation into organ-cultured prostates and their secretions. Curve I, prostates; curve II, secretions, corrected for controls. Controls ("labeling medium" incubated from 0 to 100 hr without prostates) had an average of 330 cpm/ml medium.

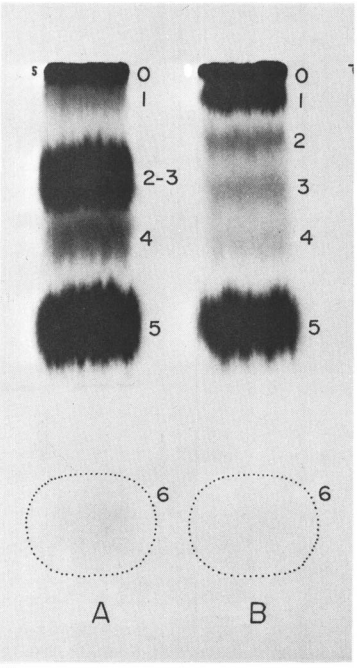

FIG. 2. Radioautograph of paper chromatogram. ^{35}S-GAGs of prostates, maintained in culture for 50 hr, were digested with either ABCase (A) or ACase (B) and the products separated by paper chromatography.

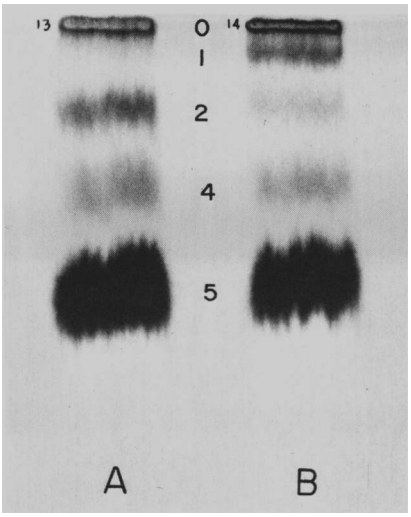

FIG. 3. Radioautograph of paper chromatogram. ^{35}S-GAGs of secretions, from 50-hr prostate-cultured media, were digested with either ABCase (A) or ACase (B) and the products separated by paper chromatography.

Several differences between the ABCase and ACase products of prostate ^{35}S-GAGs can be seen in Table I. Band O's of the ABCase groups (20-, 30-, and 50-hr cultures) had 25–34% of the total radioactivity; those of the ACase groups had 67–75%. The radioactivities (cpm) of band 1s of ABCase groups were one-half those of ACase; however, radioactivities of bands 4s, 5s, and 6s of ABCase groups were twice those of the ACase. The specific radioactivities (S.A.) of band 2s, 4s, and 5s of ABCase groups were greater than those of the ACase by 3.2, 1.2, and 2.4, respectively.

In contrast to those of the prostate (Table I), the ^{35}S distribution of the ABCase and ACase products of secretion ^{35}S-GAGs (Table II) were rather similar.

The radioautographs presented in Fig. 2 and 3 and the radioactivities given in Tables I and II represent depolymerized components of prostate and secretion ^{35}S-GAGs that have been digested to completion by

TABLE I. PROSTATE ^{35}S-GAGS. RADIOACTIVITIES OF CHROMATOGRAPHICALLY SEPARATED COMPONENTS OF ^{35}S-GAGS DIGESTED WITH ABCASE OR ACASE[a]

Duration of culture (hr)	Band (No.)	ABCase			ACase		
		%	cpm	S.A.[b]	%	cpm	S.A.[b]
20	O	34.3	48,400	3,000	67.8	83,600	6,100
	1	2.6	3,600	663	5.3	6,510	1,090
	2	17.9[c]	25,300[c]	3,420[c]	2.5	3,030	1,290
	3				2.1	2,580	713
	4	5.0	7,080	887	3.1	3,780	698
	5	39.4	55,600	8,270	18.8	23,200	3,020
	6	0.8	1,180	—[d]	0.5	579	—[d]
30	O	34.2	59,100	3,760	75.2	121,000	7,480
	1	2.6	4,410	1,190	4.9	7,930	2,230
	2	18.9[c]	32,700[c]	4,340[c]	1.7	2,740	1,840
	3				1.6	2,650	1,130
	4	4.2	7,200	990	1.8	2,810	824
	5	39.2	67,700	10,200	14.4	23,100	4,010
	6	0.9	1,580	—[d]	0.4	660	—[d]
50	O	25.2	72,500	3,760	67.4	162,000	7,800
	1	1.9	5,570	1,130	5.4	13,000	2,210
	2	21.1[c]	60,600[c]	10,200[c]	1.8	4,380	2,200
	3				1.8	4,250	1,380
	4	3.4	9,740	1,240	1.2	2,910	1,000
	5	47.3	136,000	21,900	21.8	52,500	9,270
	6	1.0	2,960	—[d]	0.6	1,370	—[d]

[a] Each culture group (20, 30, and 50 hr) consisted of prostates from 20 mice.
[b] Specific activity: cpm/μg hexuronic acid equivalent.
[c] Values apply to band 2–3.
[d] Presence of hexuronic acid was not detectable by the *meta*-hydroxydiphenyl method (10).

TABLE II. SECRETION ^{35}S-GAGS. RADIOACTIVITIES OF CHROMATOGRAPHICALLY SEPARATED COMPONENTS OF ^{35}S-GAGS DIGESTED WITH ABCASE OR ACASE[a]

Duration of culture (hr)	Band (No.)	ABCase			ACase		
		%	cpm	S.A.[b]	%	cpm	S.A.[b]
20	O	5.0	464	20	8.5	614	50
	1	5.5	515	21	10.2	738	54
	2	11.4	1,070	50	7.6	552	37
	3[c]						
	4	6.9	642	46	11.3	819	52
	5	71.2	6,660	323	62.5	4,530	246
30	O	4.4	789	43	8.5	1,130	80
	1	4.1	730	43	9.0	1,190	86
	2	14.2	2,550	102	7.5	1,000	136
	3[c]						
	4	12.1	2,180	122	12.8	1,700	137
	5	65.2	11,700	660	62.2	8,270	387
50	O	5.0	3,220	104	6.9	3,130	305
	1	3.1	2,010	137	9.2	4,150	356
	2	10.6	6,790	504	5.9	2,650	416
	3[c]						
	4	11.0	7,040	395	10.8	4,900	338
	5	70.2	45,000	2,060	67.2	30,400	1,370

[a] Each culture group consisted of secretions pooled from 20 dishes; each dish contained the left and right lateral prostates of one mouse and 0.80 ml of medium.
[b] Specific activity: cpm/μg hexuronic acid equivalent.
[c] Band 3 was not present in the digests of secretion ^{35}S-GAGs.

TABLE III. SUMMARY. ^{35}S DISTRIBUTION OF PROSTATE AND SECRETION ^{35}S-GAGS

Prostate ^{35}S-GAGS	%[a]
Chondroitin-4-sulfate	18.3
Chondroitin-6-sulfate	3.1
Highly sulfated component of chondroitin-4/6-sulfate	2.0
Dermatan sulfate	23.7
Highly sulfated component of dermatan sulfate	15.5
Heparan sulfate	31.2
"Heparan sulfate oligosaccharide"	2.4
Hybrid component (Chondroitin sulfate-dermatan sulfate)	2.8
Unknown I (Band 3)	1.8
Unknown II (Band 6)	0.4
Total	101.2
Secretion ^{35}S-GAGS	
Chondroitin-4-sulfate	64.0
Chondroitin-6-sulfate	11.6
Highly sulfated component of chondroitin-4/6-sulfate	6.0
Dermatan sulfate	4.9
Highly sulfated component of dermatan sulfate	5.1
Heparan sulfate	4.8
"Heparan sulfate oligosaccharide"	4.2
Hybrid component (Chondroitin sulfate-dermatan sulfate)	5.2
Total	105.8

[a] Average percentages of radioactivity derived from Tables I and II.

either ABCase or ACase. Completion of enzyme action was evidenced by the absence of substrate ^{35}S-GAGs from both the extracts and residues of the enzyme digests. In row I of Figs. 4B (secretion) and C (prostate), the substrates of ACase, chondroitin-4-sulfate and chondroitin-6-sulfate, were absent (zone C) while the nonsubstrates, heparan sulfate (zone H) and dermatan sulfate (zone D), were present. Similarly, in row II of Figs. 4B and C, the substrates of ABCase, dermatan sulfate and chondroitin sulfates, were absent; however, the nonsubstrate, heparan sulfate, was present. The extracts of ABCase- and ACase-digested prostate ^{35}S-GAGs likewise showed the absence of the respective substrates (Fig. 4A).

Gel-filtration studies affirmed the presence of heparan sulfate and the absence of substrate ^{35}S-GAGs in the extracts of ABCase and ACase digests. Treatment of the ^{35}S-GAG under the major peak of curve II (ABCase), Fig. 5, with nitrous acid abolished that peak (curve II, Fig. 6), as would be expected with heparan sulfate

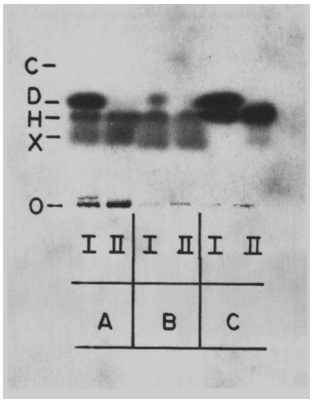

FIG. 4. Radioautograph of cellulose membrane electropherogram. Samples were applied to membrane at the origin (O) in the Beckman Microzone Electrophoresis chamber and a constant current of 10 mA was applied for 25 min using 0.05 *M* barium acetate–0.10 *M* acetic acid buffer (14). The direction of migration was from bottom to top, toward the anode. Band H corresponds to that of authentic heparan sulfate; band D, to dermatan sulfate; position C, to chondroitin sulfate; band X, unidentified component, corresponds to glycoprotein. (A) Materials eluted from band Os of prostate ACase (row I) and ABCase (row II). (B) Butanol–acetic acid extracted residues of secretion ^{35}S-GAGs digested with ACase (row I) and ABCase (row II). (C) Butanol–acetic acid extracted residues of prostate ^{35}S-GAGs digested with ACase (row I) and ABCase (row II).

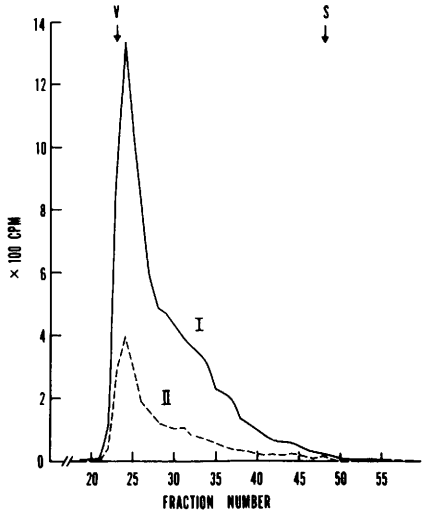

FIG. 5. Gel-filtration profiles of materials eluted from band Os of prostate ABCase and ACase samples (re: Fig. 4A). Aliquots of 80 μl were added to the tops of Bio-Gel A-0.5m columns (0.6 × 102 cm), previously equilibrated with 0.025 *M* NaCl. Hydrostatic pressure was adjusted to 45 cm and 11-drop (0.5 ml) fractions were collected with 0.025 *M* NaCl as the eluent, flowing at 3 drops/min. Curve I, ACase-digested sample; curve II, ABCase-digested sample. V, Blue Dextran 2000 (Pharmacia) void volume; S, [^{35}S]sulfate void volume.

(11). Similar treatment of the ^{35}S-GAGs under curve I (ACase), Fig. 5, only diminished its peak (curve I, Fig. 6), thus, indicating the loss of heparan sulfate and the presence of dermatan sulfate.

That the butanol–acetic acid extractions were quantitative was ascertained by the absence of the enzyme-depolymerized components of ^{35}S-GAGs in the extracted residues. Radioautographs showed that all of the radioactivity of the residues had remained at the origin of their paper chromatograms, even though the cochromatographed reference disaccharides (visible under ultraviolet light) had moved to their expected positions.

Of the total ^{35}S in the prostate ^{35}S-GAG samples, butanol–acetic acid solution extracted about 96 and 94% (averages of the 20-, 30-, and 50-hr samples), respectively, from their ABCase and ACase digests; however, from the respective secretion

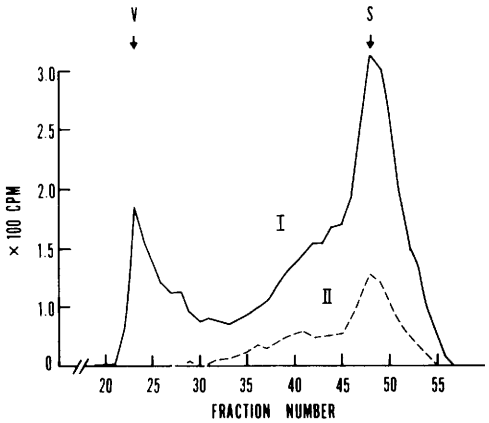

FIG. 6. Gel-filtration profiles of ^{35}S-GAGs after nitrous acid treatment. Fractions No. 22–26 of Fig. 5 of ABCase and ACase samples were pooled separately, dialyzed, concentrated, and then mixed with 0.20 vol each of glacial acetic acid and 18% ammonium nitrate (11). After 80 min at room temperature, the mixtures were frozen in an acetone–dry ice bath and lyophilized. The residues were dissolved in water and subjected to gel filtration as described in Fig. 5.

^{35}S-GAG digests, only about 69 and 58% of the total ^{35}S were extracted. Since the ^{35}S-GAGs were digested to completion and their ^{35}S-components completely extracted and quantitated, it appears that the ^{35}S of the prostate samples was almost exclusively incorporated into GAGs, while only about half of the ^{35}S of the secretion samples was incorporated into GAGs. Electropherogram, Fig. 4B, shows much of the butanol–acetic acid-extracted ^{35}S-residues of the secretion samples occupied the position of glycoprotein.

Band 1 eluates of ABCase samples of both prostate (Fig. 2A) and secretion (Fig. 3A) contained heparan sulfate-like oligomer (polymeric heparan sulfate remained at the origin). The presence of the 0.04 *N* HCl-labile *N*-$^{35}SO_3$ group of the oligomer was demonstrated by the appearance of [^{35}S]sulfate in its acid hydrolysate (Fig. 7A) (12). Hence, the ^{35}S-substance of band 1 of both secretion and prostate ABCase samples is designated "heparan sulfate oligosaccharide" (Table III).

A hybrid component of chondroitin sulfate-dermatan sulfate was found in band 1 of the ACase samples of both prostate (Fig. 2B) and secretion (Fig. 3B). This component was further degraded by ABCase (Fig. 7B). Thus, the differences between the respective band 1s of ABCase and ACase samples are presented as the hybrid component in Table III. Hybrid GAGs of dermatan sulfate have been reported for other tissues (13).

Band 2–3 was present only in ABCase samples of prostate (Fig. 2A). In this band, highly sulfated components susceptible to sulfatases were present (Fig. 7C). They were partially desulfated, separately by 4Sase (row II) and 6Sase (row III) and by both sulfatases together (row IV). Thus, band 2–3 consisted of at least three components and one or two of these had sulfate ester groups at the 4 and 6 positions of the terminal hexosamine. These results, however, are not identical to those reported for human urinary GAGs (9); nevertheless, they do show the presence of highly sul-

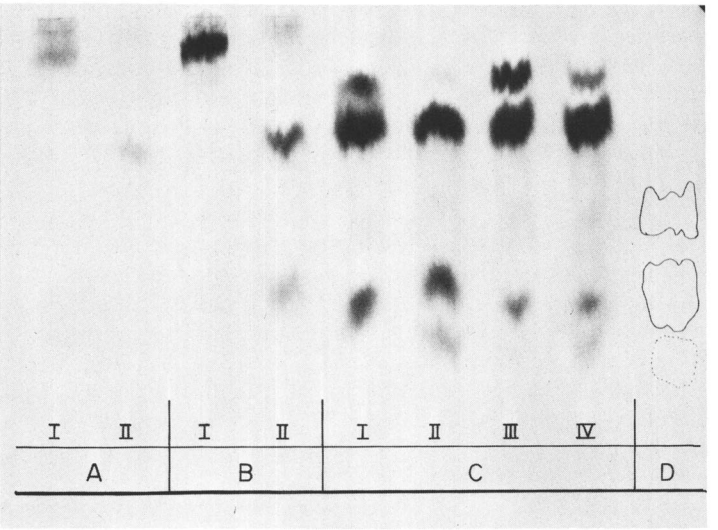

FIG. 7. Radioautograph of paper chromatogram showing the effects of various tests applied to the components isolated from ABCase and ACase digests of prostate ^{35}S-GAGs. (A) An aliquot of pooled band 1s of ABCase digests (Fig. 2A) made 0.04 *N* HCl was sealed in a glass tube and heated at 100°C for 90 min (row II); the control (row I) was heated after the 0.04 *N* HCl was immediately neutralized with NaOH. (B) An aliquot of pooled band 1s of ACase digests (Fig. 2B) was incubated with ABCase (row II); the control (row I) was incubated with boiled ABCase. (C) Aliquots of pooled band 2–3s of ABCase digests (Fig. 2A) were incubated with either 4Sase (row II), 6Sase (row III), 4Sase plus 6Sase (row IV), or with boiled 6Sase plus 4Sase (row I). (D) Tracings of authentic ΔDi-6S, ΔDi-4S, and an impurity viewed in ultraviolet light, from top to bottom—the direction of solvent flow.

fated sections in mouse prostate dermatan sulfate. The control (row I) somewhat failed to reproduce its original pattern (band 2–3, Fig. 2A), probably due to residual action of 6Sase.

Highly sulfated component of ^{35}S-GAGs was also present in band 2 of prostate ACase and both ABCase and ACase samples of secretion. The component was not affected by ABCase, but it was desulfated by 4Sase and 6Sase. Thus, the band 2 component of the ACase samples of prostate and secretion is listed as the highly sulfated component of chondroitin-4/6-sulfate in Table III. The highly sulfated component of secretion dermatan sulfate is given as the difference between the band 2s of ABCase and ACase samples; however, that of the prostate is given as the difference between band 2–3 (ABCase) and bands 2 plus 3 (ACase).

ABCase, 4Sase, and 6Sase had no effect on the radioactive component of band 3 of prostate ACase samples. This component is listed as Unknown I in Table III.

Band 4 corresponds to authentic ΔDi-6S, the disaccharide unit of chondroitin-6-sulfate produced by either ABCase or ACase. The average of both ABCase and ACase samples is presented as chondroitin-6-sulfate in Table III.

Band 5 corresponds to ΔDi-4S, the disaccharide unit of chondroitin-4-sulfate produced by either ABCase or ACase; it is also the ABCase product of dermatan sulfate. Because ΔDi-4S is derivable from both chondroitin-4-sulfate and dermatan sulfate, the usual practice in calculating the amounts of dermatan sulfate in a mixture of GAGs is to subtract the amount of ΔDi-4S produced by ACase from the amount produced by ABCase (8) (on the assumption that the ACase values represent total chondroitin-4-sulfate and those of the ABCase the total of both chondroitin-4-sulfate and dermatan sulfate). It is noteworthy that in so doing, the amounts of the highly sulfated component would be excluded and the amounts of dermatan sulfate underestimated: 23.7%, instead of 39.2% for the prostate ^{35}S-GAGs; 4.9%, instead of 10.0% for the secretion ^{35}S-GAGs (Table III).

The difference in the spectrophotometrically determined amounts of ΔDi-4S between the ABCase and ACase samples was too small to be reliable; notwithstanding, the specific activity of dermatan sulfate appears to be much higher than those of the other GAGs (Tables I and II).

The radioactive material of band 6 did not produce any color with the *m*-hydroxydiphenyl reagent; it is listed in Table III as Unknown II.

Keratan sulfate and heparin have not been found in the prostate or its secretion. Keratan sulfate was not detectable by gel filtration (curve II, Fig. 6) and electrophoresis. By the latter method, keratan sulfate moves between dermatan sulfate and chondroitin sulfate (14); however, this position was vacant in Figs. 4A, B, and C. Keratan sulfate and heparin were not detectable in alcian blue-stained bidimensional electropherograms of both prostate and secretion ^{35}S-GAGs, nor in their radioautographs.

Discussion. The presence of secretion GAGs in the lumen of the normal prostate acinus is not demonstrable by the usual histochemical methods (1, 2, 15). However, papain digestion of the ^{35}S-labeled prostate secretion did solubilize ^{35}S-GAGs. Thus, it appears that the functional groups of secretion GAGs are normally masked by protein so that they are not available to GAG stains.

The findings that GAGs are present in the secretion of the normal prostate and that the secretion GAGs are different from those of the prostate raise the question: What is the pathophysiological significance of GAGs in the secretion of the prostate?

That highly purified GAGs (16) and tissue GAGs of normal, hyperplastic, and carcinomatous human prostates bind zinc have been reported (15, 17). While zinc is present in the normal human prostate secretion (18), the relationship of zinc to GAGs in the secretion might not be the same as in the tissue—especially since the functional groups of the normal secretion GAGs appear to be masked by protein. The secretion GAGs of the hyperplastic prostate might also be masked, since they too are not stainable by the usual methods. Nevertheless, secretion GAGs of the carcinomatous

human prostates are stainable (1). Perhaps in carcinoma of the prostate the secretion GAGs remain unmasked, since their functional groups are available to GAG stains. Or, possibly, the secretion GAGs in carcinoma are different enough from those of the normal and hyperplastic prostates so that they cannot be masked. On the other hand, the normal GAG-masking protein might be absent in carcinoma of the prostate.

Summary. Mouse prostates maintained in organ culture continued to incorporate [^{35}S]sulfate into GAGs up to 100 hr, the duration of culture. However, there was a lag phase of about 20 hr before the appearance of ^{35}S-labeled secretions in the media.

Aliquots of papain-extracted ^{35}S-GAGs of prostates and secretions from 20-, 30-, and 50-hr cultures were digested to completion with either chondroitinase ABC or AC. The depolymerized components were separated by paper chromatography and visualized by radioautography. Both the radioautographs and specific radioactivities of the components of the prostate ^{35}S-GAGs were consistently different from those of the secretion ^{35}S-GAGs. Moreover, while about 95% of the nondialyzable ^{35}S in the prostate GAG preparations was shown to be incorporated into GAGs, only about 63% of the ^{35}S of the secretion GAG preparations was incorporated into GAGs—the remainder appears to be in glycoprotein.

The ^{35}S distribution among the prostate and secretion ^{35}S-GAGs was as follows: chondroitin-4-sulfate: prostate, 18.3%; secretion, 64.0%; highly sulfated component of chondroitin sulfate: prostate, 2.0%; secretion, 6.0%; dermatan sulfate: prostate, 23.7%; secretion, 4.9%; highly sulfated component of dermatan sulfate: prostate, 15.5%; secretion, 5.1%; heparan sulfate: prostate, 31.2%; secretion, 4.8%; "heparan sulfate oligosaccharide": prostate, 2.4%; secretion, 4.2%; hybrid component of chondroitin sulfate–dermatan sulfate: prostate, 2.8%; secretion, 5.2%.

Keratan sulfate and heparin were not detectable in the mouse prostate and its secretion.

Secretion GAGs of normal prostate are not stainable by the usual histochemical methods, but they are extractable with papain. Thus, the functional groups of secretion GAGs of the normal prostate appear to be masked by protein.

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