Sulfated Mucopolysaccharide Production by Synovial Sarcoma Cells in Vivo and in Tissue Culture

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SUMMARY

Tissue cultures of synovial sarcoma exposed to sulfuric acid, \(^{35}\)S incorporate the isotope into nondialyzable radioactivity. Gel filtration and electrophoretic analysis of the nondialyzable \(^{35}\)S-labeled material extracted from the cultured cells and their growth medium demonstrate the ability of synovial sarcoma cells to synthesize and secrete various sulfated mucopolysaccharides. The sulfated species that is predominant inside the cell appears to be a heparin-related mucopolysaccharide. These findings clarify the histochemical observations that indicate the presence, in vivo, of mucopolysaccharide species resistant to degradation by hyaluronidase and chondroitinase ABC. The electrophoretic pattern of the sulfated species extracted from the synovial sarcoma cell cultures is quite different from that of the \(^{35}\)S-labeled mucopolysaccharides found in a line of fibroblasts. The electron microscopic study of the tumor and of the cultured cells discloses that synovial sarcoma cells retain in vitro the ultrastructural features displayed in vivo and confirms that the cultures used for these studies were indeed composed of synovial sarcoma cells.

INTRODUCTION

It is generally assumed that the neoplastic cells of synovial sarcomas, by virtue of their kinship with the synovial membrane, produce hyaluronic acid (15). On the other hand, Enzinger (7) has pointed out that the stainable mucinous material found in synovial sarcomas is resistant to digestion by hyaluronidase. Similar histochemical observations suggesting that mucopolysaccharide species other than hyaluronic acid are present in the secretory material of synovial sarcoma have been made in this laboratory. In this report the secretory activities of synovial sarcoma cells in vivo and in vitro are compared. These studies demonstrate that, in addition to hyaluronic acid, synovial sarcoma cells synthesize and secrete, both in vivo and in tissue culture, a spectrum of sulfated mucopolysaccharides which includes a species that has some of the properties usually associated with heparin-related mucopolysaccharides.

MATERIALS AND METHODS

The tumor involved the lower portion of the popliteal region and the calf of a 12-year-old boy. Superficially, the neoplasma was well delimited from the overlying skin and s.c. tissue, whereas in the depth it invaded the soft tissues without reaching any osseous or articular structures.

Light Microscopy. A large number of blocks of neoplastic tissue were fixed in 10% phosphate-buffered formalin, pH 7 (14), and embedded in paraffin. Sections were stained with hematoxylin-eosin (2), Mason's trichrome (2), Gomori for reticulin (10), and PAS \(^3\) [McManus type (19)] with and without prior diastase digestion. To test the activity of the diastase preparations, we incubated sections of liver with and without enzyme for 2 hr at 37° and then stained with the PAS technique. The concentration of the enzyme was 0.1% in 0.004 M acetate buffer, pH 5.5. Staining with mucicarmine (14), colloidal iron (12), colloidal iron-PAS (21), and Alcian blue (17) was carried out before and after digestion of the sections with bovine testicular hyaluronidase, 250 units/ml in 0.1 M phosphate buffer, pH 6, for 20 hr at 37°, and with chondroitinase ABC, 5 units/ml in 0.05 M Tris, 0.06 M sodium acetate, 0.05 M sodium chloride, and bovine serum albumin, 100 \(\mu\)g/ml, pH 8.5, for 20 hr at 37°. Control tissues included umbilical cord, cartilage, and aortic wall.

Tissue Culture. The neoplastic tissue was finely minced under sterile conditions, suspended in growth medium, and explanted in Falcon plastic flasks (30 ml). The cultures were washed and fed twice weekly with F12 medium supplemented with 10% fetal calf serum.

Electron Microscopy. Representative portions of the tumor were cut into 1-cm blocks, fixed immediately in cold glutaraldehyde (2% in Sorensen's phosphate buffer, pH 7.4) for 3 hr, repeatedly washed in the buffer, postfixed in 2% buffered osmium tetroxide for 2 hr at 4°, and embedded in Epon 812. Thin sections (<1 \(\mu\)m) were stained with toluidine blue for light microscopic control. Ultrathin sections were mounted on Parlodion-coated grids and stained with uranyl acetate and lead citrate. Cultures of tumor cells were prepared for electron microscopy as follows. Eight-day-old cultures were fixed in the original culture vessel, described above, prestained with uranyl acetate (2% in ethanol), and embedded in Epon 812. After polymerization, colonies were washed with sodium acetate, 0.05 M, and embedded in Epon 812. After polymerization, colonies were selected with the phase microscope for ultrathin serial sectioning parallel with and vertical to the growth surface.

Preparation of \(^{35}\)S-labeled Mucopolysaccharide Extracts for Gel Filtration and Electrophoretic Analysis. The procedure used for the preparation of \(^{35}\)S-labeled mucopolysaccharide extracts takes advantage of the fact that mammalian cells do...
not introduce sulfate into cystine or methionine (11); sulfated mucopolysaccharides are therefore the only macromolecules that are labeled when cultured mammalian cells are exposed to sulfuric acid.\textsuperscript{35}S (8). To obtain \textsuperscript{35}S-labeled mucopolysaccharides, we exposed 14-day-old cultures of synovial sarcoma cells to sulfur-free growth medium supplemented with carrier-free sulfuric acid.\textsuperscript{35}S (25 \mu Ci/ml). After 24 hr the medium was pooled and frozen. The cells were washed 3 times with 5 ml of Hanks' balanced salt solution and frozen.

\textsuperscript{35}S-labeled mucopolysaccharide extracts were prepared as follows. Medium and cells were digested with papain (20) for 24 hr. The digests were made 5\% with respect to trichloroacetic acid and centrifuged, and the supernatants were decanted and dialyzed as described previously (1). The dialyzates were freeze-dried and used for gel filtration and electrophoretic analysis.

The fraction of \textsuperscript{35}S-labeled mucopolysaccharides resistant to hyaluronidase degradation was obtained by incubating nondialyzable \textsuperscript{35}S radioactivity for 150 min at 37\° with 0.8 mg of testicular hyaluronidase per ml of 0.14 M NaCl-10\textsuperscript{-2} M sodium acetate buffer, pH 5.4 (22). Two \mu g of each of the authentic compounds (hyaluronic acid, chondroitin sulfate A, chondroitin sulfate B, and chondroitin sulfate C) were added to the incubation mixture. Control tubes were incubated without the enzyme. The undegraded radioactivity was then isolated by gel filtration as described below, freeze-dried, and used for electrophoretic analysis on cellulose acetate strips. After the electrophoretic run, the strips were stained with Alcian blue (23). No trace of material with the electrophoretic mobility of hyaluronic acid, chondroitin sulfate A, or chondroitin sulfate C was detected in the strips streaked with aliquots of the mixtures incubated with the enzyme. Chondroitin sulfate B did not appear to be appreciably degraded under the conditions used in these studies: this fact was determined by comparing the densitometer tracing (23) of strips streaked with incubation mixtures digested with the enzyme to the tracing of strips streaked with mixtures incubated without enzyme.

The fraction of the nondialyzable \textsuperscript{35}S radioactivity resistant to degradation by chondroitnase ABC was prepared by incubating \textsuperscript{35}S-labeled mucopolysaccharides with the enzyme as described for the tissue sections. In addition to the nondialyzable \textsuperscript{35}S radioactivity the incubation mixture contained 2 \mu g of each of the authentic compounds. After 150 min the undegraded radioactivity was isolated by gel filtration as indicated below and used for electrophoretic analysis. No trace of any of the authentic compounds added to the incubation mixture was found when the strips were stained with Alcian blue.

Gel filtration of the fraction of nondialyzable \textsuperscript{35}S radioactivity resistant to degradation by hyaluronidase was performed with a Sephadex G-25 column, 1.4 x 150 cm. All other gel filtration analyses were performed with a 0.9- x 55-cm column. Electrophoretic analysis on cellulose acetate of the various fractions was performed with 0.2 M calcium acetate, pH 7.2, as buffer (23). The \textsuperscript{35}S radioactivity present in the strip was determined as previously described (1). The only variation was that the strip was not allowed to dry and, supported by a pad of filter paper, was cut into 0.5- or 1-mm fractions with a Yeda macrotome (Yeda Research Corpora-

tion, Rehovot, Israel).

The fibroblast line from rabbit aorta used in this study was developed in our laboratory with a technique similar to that used to obtain endothelial cultures (1). The aorta, tied at both ends, was filled with an enzyme solution (0.1% Viokase-0.1% collagenase) that was withdrawn after 90 min of incubation at 37\°. This preparation was composed essentially of endothelial cells. The aorta was filled again with the enzyme solution and the incubation was continued for 30 to 40 min more. During this period of time a large number of cells resembling fibroblasts appeared in the preparation. These cells were plated directly into Falcon Petri dishes, 60 x 15 mm, and grown in F12 medium supplemented with 10\% fetal calf serum. At the time when these experiments were performed, the cells had been kept in culture for almost 1 year with weekly subcultures, and were still growing in a typical fibroblast pattern (elongated, spindle-shaped cells lined up in a parallel fashion). At this time, the mucopolysaccharide composition (as determined by electrophoresis on cellulose acetate) was identical with that found after a period of culture of 2 weeks.

Papain (code: PAP, 10 units/mg) was purchased from Worthington Biochemical Corporation, Freehold, N. J.; testicular hyaluronidase (740 units/mg) and malt diastase (type V-A) from Sigma Chemical Company, St. Louis, Mo.; chondroitnase ABC (a highly purified preparation from \textit{Proteus vulgaris}) and the authentic compounds used for electrophoretic analysis from Miles Laboratories, Inc., Elkhart, Ind.; carrier-free sulfuric acid-\textsuperscript{35}S from New England Nuclear, Boston, Mass.; butyl PBD (a primary fluor for liquid scintillation counting) and BBS3 (a solubilizer for aqueous solutions) from Beckman Instruments, Inc., Lincolnwood, Ill.

### RESULTS

#### Light Microscopy

The tumor exhibited the typical biphasic pattern of synovial sarcomas with predominance of the sarcomatous phase. The lumen of the gland-like spaces contained secretory material that stained intensely with PAS, mucicarmine, colloidal iron, and Alcian blue (Fig. 1). A fainter reaction with the above stains was observed in the ground substance of the sarcomatous areas. In addition, occasional sarcomatous cells contained granules that were deeply stained by colloidal iron and Alcian blue. Digestion of the sections by testicular hyaluronidase prevented the staining of the ground substance, whereas it did not affect the staining of the intraluminal secretory material and of the intracellular granules. Chondroitnase ABC digestion had no appreciable effect on any of the above staining reactions. No diastase-sensitive material was found with any of the light microscopic techniques.

#### Electron Microscopy

The tissue processed for electron microscopy displayed only the sarcomatous phase and no gland-like spaces. The tumor cells (Fig. 2A) contained a varying number of mitochondria, lysosomal-like bodies, and free ribosomes. The Golgi complexes and the granular endoplasmic reticulum were moderately developed. The latter was frequently dilated and contained amorphous, lightly osmophilic material. Paired cisternae were seldom noted. Fine filaments could be seen in any portion of the cytoplasm.
perhaps more frequently close to the nucleus. They were mostly haphazardly oriented or in small bundles and were occasionally grouped in tightly woven masses. The nuclei were irregularly shaped with peripheral chromatin condensation and occasional indentations of the nuclear membrane. Prominent nucleoli were frequent. The cells had irregular convoluted contours and were separated by narrow and uniform intercellular spaces faced by somewhat ill-defined plasma membranes with occasional focal thickenings reminiscent of specialized junctions. The stroma was composed of “fuzzy” material in which mature collagen fibers could be seen only rarely. In focal areas, amorphous material accumulated in a linear fashion along the tumor cells, separated from them by a narrow translucent space, suggesting the formation of a basal lamina. In some of these areas the plasma membrane presented focal thickenings reminiscent of hemidesmosomes (Fig. 2B).

**Morphology of Cultured Cells.** The outgrowth from the neoplastic explants was characterized by elongated, mostly stubby cells that migrated at mutual contact (Fig. 3A) in a pavement-like pattern. At the periphery of the colonies, the cells became irregularly shaped and spindly because of haphazardly oriented, fine cytoplasmic processes. Away from the colonies, some of the isolated cells attained fibroblast-like features, although a typical reticular fibroblastic pattern of growth was not observed. The nuclei were mostly uniform with 1 or 2 nucleoli. More nucleoli were seen in occasional cells.

The cultured cells reproduced in vitro the ultrastructural features displayed by the parent synovial sarcoma cells in vivo described above. The granular endoplasmic reticulum was focally prominent with cisternal dilations (Fig. 3A), but not as diffusely prominent as that of comparable cultures of fibroblasts. The cytoplasmic filaments, including those in tightly woven masses (Fig. 3B), were striking. Microtubules, as expected, were more numerous in vitro than in vivo. No desmosomes were seen along narrow intercellular spaces. Production of mature collagen in vitro could not be demonstrated ultrastructurally.

**Synthesis and Secretion of Various Sulfated Mucopolysaccharide Species by Cultures of Synovial Sarcoma.** Cultures of synovial sarcoma exposed to growth medium supplemented with sulfuric acid-35S incorporate the isotope into nondialyzable radioactivity that can be extracted from the cells according to the procedure outlined in “Materials and Methods.” Furthermore, about twice as much nondialyzable radioactivity is found in the supernatant medium.

For further characterization of the nondialyzable radioactivity extracted from synovial sarcoma cell cultures, 35S-labeled material extracted from the cells was applied to a column of Sephadex G-25 (Chart 1a). The radioactivity eluted with the void volume (essentially all the radioactivity recovered from the column) was incubated with hyaluronidase and reexamined by gel filtration (Chart 1b). About 55% of the radioactivity recovered from the column is eluted apparently undegraded with the void volume. When the radioactivity eluted with the void volume of the 2nd Sephadex column is analyzed by electrophoresis on cellulose acetate (Chart 1c), essentially all the counts streaked on the strip are recovered in 2 peaks. The slower moving fraction contains 70% of the radioactivity. The faster moving fraction has the same electrophoretic mobility as chondroitin sulfate B. Thus, electrophoretic analysis confirms and elucidates the histochemical findings that show that digestion of histological sections of the tumor with hyaluronidase does not affect the staining of the intracellular granules by the various techniques used to demonstrate the presence of mucopolysaccharides. Hyaluronidase causes a variable degradation of chondroitin sulfate B depending on the content in glucuronic acid (6); thus, the percentage of chondroitin sulfate B found in this study should be considered a minimum value since some degradation of this mucopolysaccharide may have occurred during the short incubation period with the enzyme which was sufficient to cause complete degradation of the authentic compounds (chondroitin sulfate A and C and hyaluronic acid) added to the incubation mixture containing the 35S radioactivity (see “Materials and Methods” and legend to Chart 1).

To establish a further correlation with the histochemical findings and to characterize more completely the 35S-labeled species that migrates slower than chondroitin sulfate B, we incubated the 35S-labeled material shown in Chart 1a with chondroitinase ABC (an enzyme that does not degrade heparin and heparin-related mucopolysaccharides) and then it was applied to a Sephadex G-25 column. Forty % of the radioactivity recovered from the column is eluted with the void volume (Chart 2a). This radioactivity was examined by electrophoresis on cellulose acetate (Chart 2b). More than 94% of the radioactivity recovered from the strip is found in a single fraction that has an electrophoretic mobility slower than that of chondroitin sulfate B. It appears, then, that 40% of the total mucopolysaccharides extracted from the cell are represented by a species that may be related to heparin. Under the conditions used for these electrophoretic analyses (calcium acetate buffer, pH 7.2), the authentic compound heparan sulfate was found to have an electrophoretic mobility similar to that of chondroitin sulfate B; therefore, the fraction resistant to degradation by chondroitinase ABC has an electrophoretic mobility close to, but not identical with, that of the authentic compound heparan sulfate. A faster rate of migration of this fraction was obtained when the electrophoretic run was performed at a lower pH (pyridine-formic acid buffer, pH 3) (16).

As indicated in an earlier section, treatment with hyaluronidase does not prevent the staining with colloidal iron and Alcian blue of the material present in the lumen of the pseudoglandular structures. Since nondialyzable 35S radioactivity is found in the growth medium of synovial sarcoma cell cultures, it seemed possible that synovial sarcoma cells may secrete in tissue culture the same substances that they secrete in vivo. To elucidate this question, we performed experiments similar to those described in Chart 1 with the nondialyzable 35S radioactivity extracted from the medium. On the basis of these experiments, the amount of hyaluronidase-resistant material secreted by the cells was estimated at 42% of the 35S radioactivity eluted from the Sephadex column. The remainder of the nondialyzable 35S-labeled material extracted from the medium is likely to represent a mucopolysaccharide related to chondroitin sulfate A, since it is sensitive to degradation by hyaluronidase (as shown by its elution as retarded material from the Sephadex column). Furthermore, electrophoretic analysis of whole extracts of 35S-labeled...
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Chart 1. Electrophoretic analysis of the fraction of intracellular $^{35}$S radioactivity resistant to hyaluronidase degradation. Synovial sarcoma cell cultures were labeled with carrier-free sulfuric acid-$^{35}$S as described in "Materials and Methods." Nondialyzable $^{35}$S radioactivity extracted from the cells (see "Materials and Methods") was dissolved in 1 ml of 0.1 M NaCl and applied to a Sephadex G-25 column (a); 1 ml fractions were collected with 0.1 M NaCl as eluent with a flow rate of 20 ml/hr. An aliquot of each fraction was counted in toluene (toluene, butyl PBD, 8.5 g/liter; BBS3 10%), in a Packard scintillation spectrometer. The $^{35}$S-labeled material eluted with the void volume was incubated with hyaluronidase as indicated in "Materials and Methods" and 2 μg each of the authentic compounds hyaluronic acid, chondroitin sulfate A, chondroitin sulfate B, and chondroitin sulfate C. The digest was then examined by gel filtration (b). The $^{35}$S-labeled material eluted with the void volume of the 2nd Sephadex column was pooled, freeze-dried, and examined by electrophoresis on cellulose acetate (c) as indicated in "Materials and Methods." At the end of the electrophoretic run, the strips were stained with 0.5% Alcian blue in 3% acetic acid (23). △, position of the authentic compound chondroitin sulfate B, the only material stainable with Alcian blue present in the strip. Arrows, the elution volume of a molecule (bovine serum albumin) that is excluded from the gel.

mucopolysaccharides revealed the presence of $^{35}$S-labeled material with an electrophoretic mobility close to that of the authentic compound, chondroitin sulfate A. Using the same experimental approach shown in Chart 2, we estimated the amount of $^{35}$S-labeled material resistant to degradation by chondroitinase ABC at 21% of the total $^{35}$S-labeled mucopolysaccharides found in the growth medium.

Both fractions were examined by electrophoresis (Chart 3). The $^{35}$S radioactivity resistant to degradation by hyaluronidase separates into 2 components: the faster moving fraction has the same electrophoretic mobility as chondroitin sulfate B and, as shown in Chart 3b, is completely degraded by chondroitinase ABC as one would expect if this species were in fact chondroitin sulfate B. Thus, according to the data obtained from the electrophoretic analysis, 65% of the material present in the fraction resistant to degradation by hyaluronidase is chondroitin sulfate B. Chart 3b also shows that the fraction isolated from the medium that is resistant to degradation by chondroitinase ABC migrates as a single component when examined by electrophoresis; this fraction has an electrophoretic mobility identical to that of the slower moving component shown in Chart 3a. The resistance to degradation by chondroitinase ABC suggests that this species, which is present both in the cells and in the material that is secreted into the growth medium, may be a heparin-related mucopolysaccharide.

Additional characterization of this species was carried out with a procedure based on the fact that heparin and heparin-related species are the only sulfated mucopolysaccharides that possess N-sulfates that are labile under conditions of mild acid hydrolysis. The presence of labile sulfate groups in the fraction resistant to chondroitinase ABC degradation was determined by incubating this fraction with 0.04 N HCl at 100°, for 90 min. The $^{35}$S radioactivity was then passed through a G-25 Sephacryl column. Sixty % of the counts were retarded in the column (Chart 4). The $^{35}$S radioactivity that was retarded in the gel bed was examined by paper electrophoresis (2); essentially all the radioactivity present in the strip was found to migrate as inorganic sulfate.

The electrophoretic pattern of the $^{35}$S-labeled mucopoly-
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Chart 2. Electrophoretic analysis of the fraction of intracellular $^{35}$S radioactivity resistant to degradation by chondroitinase ABC. Nondialyzable radioactivity extracted from synovial sarcoma cell cultures labeled with sulfuric acid-$^{35}$S was incubated with chondroitinase ABC (see text). The digest was then applied to a Sephadex G-25 column and eluted with 0.1 M NaCl at a flow rate of 20 ml/hr. The fractions between the arrows (a) were pooled and prepared for electrophoresis on cellulose acetate (b) as outlined in “Materials and Methods” and in the legend of Chart 1. At the end of the electrophoretic run, the strips were stained with Alcian blue. ■, position of the authentic compounds added to the $^{35}$S-labeled material after elution from the Sephadex column. Before the addition of the authentic compounds, the $^{35}$S radioactivity was extracted with an equal volume of water-saturated phenol. A, chondroitin sulfate A; B, chondroitin sulfate B; C, chondroitin sulfate C.

Chart 3. Electrophoretic analysis of the fractions extracted from the medium of synovial sarcoma cultures resistant to degradation by hyaluronidase and chondroitinase ABC. The fractions resistant to degradation by hyaluronidase and chondroitinase ABC present in the medium of synovial sarcoma cell cultures labeled with sulfuric acid-$^{35}$S were prepared for electrophoretic analysis according to the procedure outlined in Charts 1 and 2 (see also “Materials and Methods”). More than 94% of the radioactivity loaded on the cellulose strips was recovered in the electrophoretic fractions shown. a, electrophoretic analysis of the $^{35}$S-labeled material resistant to degradation by hyaluronidase; b, electrophoretic analysis of the fraction of nondialyzable $^{35}$S radioactivity resistant to degradation by chondroitinase ABC.

Saccharides extracted from synovial sarcoma cell cultures is quite different from that of a line of fibroblasts derived from the wall of rabbit aorta (Chart 5) and suggests a greater molecular heterogeneity. For instance, chondroitin sulfate B is present in a significant amount in the synovial tumor cells (Chart 5a). In contrast, chondroitin sulfate B is not present in an appreciable amount in the cells of fibroblastic type (Chart 5b). The difference in the electrophoretic pattern militates against the possibility that the fibroblasts present in the original inoculum could have overgrown the neoplastic cells. At the steady state, one $^{35}$S species is by far predominant among the sulfated mucopolysaccharides found inside the cells of fibroblastic type; the properties of this fraction that have been investigated so far (e.g., resistance to chondroitinase ABC, lability of sulfate groups) suggest that it may be a heparin-related mucopolysaccharide. The nature of the small radioactive peak shown in Chart 5a that migrates slower (Fractions 40 to 45) than heparan sulfate is not known at the present time.

Analysis of the nondialyzable $^{35}$S radioactivity from different culture flasks performed according to the procedure outlined above showed that the mucopolysaccharide composition of both the cells and the secretory material was reproducible. Since the culture was maintained only for a limited period of time, it is not known whether the synthetic pattern remains stable through repeated subcultures.

For determination of whether, in addition to the sulfated mucopolysaccharides, synovial sarcoma cells also synthesize hyaluronic acid, electrophoretic analysis on cellulose acetate of the $^{35}$S-labeled mucopolysaccharide extracts was per-
formed by applying to the strip an amount of radioactivity higher than that used in the previous electrophoretic runs. This analysis revealed the presence of material that could be stained with Alcian blue and had the same electrophoretic mobility as the authentic compound hyaluronic acid. No stainable material could be found in the cellulose acetate strip when, prior to the electrophoretic run, the extracts were incubated with hyaluronidase.

**DISCUSSION**

The identity of this tumor is well documented by its features which correspond to previous reports on light microscopy (24), tissue culture (18), and electron microscopy (25). The synthetic and secretory functions of the tumor cells deserve further comment. Gel filtration and electrophoretic analysis of the nondialyzable, $^{35}$S-labeled material extracted from the cells and the growth medium of synovial sarcoma cultures indicate that, as surmised from the histochemical findings, these cells are capable of synthesizing and secreting various sulfated mucopolysaccharide species. As shown by Chart 5, the electrophoretic pattern of the sulfated species extracted from synovial sarcoma cell cultures is different from that of the $^{35}$S-labeled mucopolysaccharides extracted from a line of fibroblasts; this finding tends to exclude the possibility that fibroblasts had overgrown the original neoplastic cell population at the time when the synthetic activity of these cultures was determined, although the possibility exists that fibroblasts from different organs synthesize and secrete a different spectrum of sulfated mucopolysaccharides. However, all the data available (including the ultrastructural similarities between the synovial sarcoma cells in vivo and the cells growing in our cultures as well as the electron microscopic differences between the latter and fibroblasts growing in vitro) support the conclusion that the mucopolysaccharides extracted from the cells and from the medium of our cultures of synovial sarcoma are an expression of the synthetic and secretory activity of this tumor. Some of these species are resistant to degradation by hyaluronidase and have been identified as chondroitin sulfate B and a heparin-related mucopolysaccharide. These molecules are present both inside the cell and as secretory material in the growth medium. Heparin-related species are about 40 to 50% of the total $^{35}$S-labeled mucopolysaccharides extracted from the cell but only 21% of the sulfated mucopolysaccharides extracted from the medium.

It is possible that similar studies with other tissues will reveal that heparin-related mucopolysaccharides are the sulfated species that are predominant inside the cell. This was found to be true also in the case of another cell type, the endothelial cell; in contrast to the intracellular situation, a wider spectrum of sulfated molecules was extracted from the culture medium in which the heparin-like mucopolysaccharide...
was only a small fraction of the total (1). The biological functions of these molecules are not well understood at the present time, although a few studies concerned with the presence of heparin-related species in cultured mammalian cells have appeared (4, 5, 13).

Treatment with hyaluronidase prevents the staining of the ground substance in histological sections of synovial sarcoma. Thus, it appears that none, or very little, of the 35S-species resistant to enzymatic degradation that either are found intracellularly or are secreted by the cells enter into the formation of the ground substance. This finding suggests either that the ground substance is synthesized at least in part by cells other than the tumor cells or that the material resistant to enzymatic degradation is a special fraction of the total mucopolysaccharides synthesized by the tumor cells and is not destined to become part of the ground substance.

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