Glycosaminoglycans in Human Neutrophils and Leukemic Myeloblasts: Ultrastructural, Cytochemical, Immunologic, and Biochemical Characterization

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Chondroitin sulfate is known to be present in normal and leukemic myeloid cells; however, its definitive subcellular location and association with other glycosaminoglycans (GAGs) has not been demonstrated. We have studied the type and distribution of GAGs in neutrophil granule subpopulations of normal and leukemic myeloid cells using ultrastructural, cytochemical, immunologic, and biochemical methods. At the ultrastructural level, high-iron diamine-thiocarboxyhydrase-silver proteinate (HID-TCH-SP) stained sulfated glycoconjugates selectively in immature primary granules of normal promyelocytes and Auer rods and immature granules of leukemic myeloblasts. Staining was weak or absent in mature primary granules, whereas tertiary granules stained moderately. Primary granule staining with HID-TCH-SP was greatly diminished by prior treatment of the specimens with chondroitinase ABC and/or nitrous acid, indicating the presence of chondroitin sulfate and N-sulfated glycosaminoglycan. Immunostaining of myeloid cells with a rabbit antichondroitin 4-sulfate and f-ritin-conjugated goat anti-rabbit IgG sequence resulted in staining of most primary granules. Biochemical analysis of GAGs from leukemic myeloblasts containing primary granules and Auer rods, but lacking secondary and tertiary granules, revealed $8 \times 10^2$ mole of uronic acid/cell and electrophoretic and sulfaminohexose analysis showed 60%-70% chondroitin sulfate AC of heterogeneous molecular weight, 20%-30% of a GAG that most closely resembled heparan sulfate, and 10% dermanan sulfate. The lack of significant HID-TCH-SP staining of sulfate in sites other than Auer rods and primary granules in leukemic myeloblasts indicates that these granules contain the chondroitin, dermatan, and heparan sulfate isolated from the same specimen. Similar GAGs are present in primary granules of normal cells as evidenced by their cytochemical and immunochemical methods. Thus, these studies demonstrate a heterogeneous population of GAGs not previously identified and localize these substances to the primary granule of leukemic and normal cells.

Knowledge of the type and distribution of glycosaminoglycans (GAGs) in neutrophils is a requisite for understanding their role in normal and abnormal leukocyte development. However, neither biochemical nor cytochemical analysis alone is capable of identifying specific GAGs in discrete neutrophil organelles. Chondroitin sulfate AC and hyaluronic acid have been biochemically identified in human blood and rabbit marrow specimens. Since marrow and blood contain a heterogeneous population of sulfate-containing hematopoietic cells, and the neutrophils present contain more than one type of granule, biochemical analysis of whole cells provides no information regarding localization. On the other hand, although electron microscopic studies have localized sulfate and/or carboxyl groups in primary granules using cationic dyes and radioautographic methods, little information is provided concerning the polymers to which these functional groups are attached.

The function of GAGs in neutrophil lysosomes is not known. Immature primary granules appear more azurophilic and/or basophilic by light microscopy and demonstrate stronger ultrastructural cytochemical staining of both sulfated and vicinal glycol-containing complex carbohydrates than mature primary granules of rabbit heterophils. This loss of staining in rabbit mature primary granules presumably results from masking of the sulfate groups rather than their removal, since radiosulfate labeling persists in mature primary granules of rabbits and dogs. The cytochemical observation of a similar intragranular distribution and masking of anionic glycoconjugates and acid phosphatase, together with the biochemical observation that GAGs inhibit lysosomal enzymes, has resulted in speculation that the primary granule GAG functions to inactivate some enzymes and to facilitate their storage in lysosomes.

The purpose of the present study was to determine the nature and distribution of GAGs in normal and leukemic human myeloid cells. Cytochemical and immunochemical methods were used to characterize GAG in situ. The results of these studies showed that almost all of the GAGs were found in immature primary granules. Leukemic leukocytes containing a pure population of primary granules and lacking other organelles containing stable amounts of sulfate were selected for biochemical analysis so that a comparison could be made with cytochemically and immunologically stained primary granules of normal cell preparations.
MATERIALS AND METHODS
Ultrasound Morphology and Cytochemistry

Human marrow cells were obtained after informed consent by needle aspiration of the posterior iliac crest from a normal volunteer and patients (n = 4) undergoing routine staging for malignancy (without marrow involvement) and patients (n = 4) with Auer-rod-positive acute myelogenous leukemia (3 untreated at diagnosis and 1 in relapse 2 wk off chemotherapy). For light microscopy, bone marrow, without an anticoagulant, was smeared on glass slides, air dried, and fixed for 2-5 min in 10% neutral buffered formalin. For electron microscopy, all specimens were collected in heparin or citrate. After centrifugation at 1500 g for 3 min, the buffy coats were removed, fixed, and minced in 2.7%-3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.35, for 60 min at 4°C and rinsed 3 times in 0.1M cacodylate 7 g/dl sucrose buffer, pH 7.35. Cytochemical staining was carried out en block as outlined below. Some specimens were postfixed in 1% OsO<sub>4</sub>, and all specimens were routinely dehydrated and embedded in Spurr low viscosity medium. Thin sections of morphological specimens were counterstained with uranyl acetate and lead citrate, whereas cytochemical preparations were not counterstained. The thin sections were examined in a Philips 300 electron microscope at an accelerating voltage of 60 kV.

HID staining was performed as a preembedding procedure as described previously. For light microscopy, slides were immersed in the HID solution for 18 hr. For electron microscopy, tissue fragments or suspended cells were stained for 18 hr at 22°C in the HID solution (pH 1.4), which was prepared by adding 1.4 ml of 40% FeCl<sub>3</sub> to a fresh dilution solution containing 120mg of N,N-dimethyl-m-phenylenediamine (HCl); and 20 mg of N,N-dimethyl-p-phenylenediamine (HCl) in 50 ml of H<sub>2</sub>O. Control specimens were incubated for 18 hr at 22°C in a MgCl<sub>2</sub> solution prepared by adding 1.4 ml of MgCl<sub>2</sub> to 50 ml of H<sub>2</sub>O and adjusting the pH to 1.4 with 0.1 N HCl. Some specimens were postfixed in 1% or 2% OsO<sub>4</sub>, whereas others were not. HID staining was enhanced in osmicated and unomicated specimens by staining thin sections with thiocarbohydrazide and silver proteinate (TCH-SP) as described by Sannes et al. The former reagent binds aldehyde groups in the iron diamine complex and reduces silver proteinate at the site, producing an electron-dense reaction product.

HID staining was also evaluated after enzyme digestion with testicular hyaluronidase to remove chondroitin 4- and 6-sulfate, chondroitinase ABC to remove chondroitin sulfate and dermatan sulfate, or chemical degradation with nitrous acid to remove N-sulfate of heparan or heparin. Finely minced (3-4 pieces) human hyaluronidase, or chemical degradation with nitrous acid to remove N-sulfate of heparan or heparin. Finely minced (3-4 pieces) human specimens were incubated for 18 hr at 22°C in a MgCl<sub>2</sub> solution prepared by adding 1.4 ml of MgCl<sub>2</sub> to 50 ml of H<sub>2</sub>O and adjusting the pH to 1.4 with 0.1 N HCl. Some specimens were postfixed in 1% or 2% OsO<sub>4</sub>, whereas others were not. HID staining was enhanced in osmicated and unomicated specimens by staining thin sections with thiocarbohydrazide and silver proteinate (TCH-SP) as described by Sannes et al. The former reagent binds aldehyde groups in the iron diamine complex and reduces silver proteinate at the site, producing an electron-dense reaction product.

Immunostaining of Chondroitin Sulfate

Rabbit antichondroitin sulfate (RACS) was obtained courtesy of Dr. L. C. Caterson (University of Alabama at Birmingham). The preparation of this antiserum and its specificity have been previously described. The antiserum was obtained after immunization of rabbits with chondroitinase ABC-digested bovine nasal cartilage proteoglycan and contains antibodies that recognize the unsaturated uronic residue linked to N-acetylgalactosamine 4-sulfate. The antiserum does not react with intact chondroitin sulfate, and accordingly, tissues require brief digestion with chondroitinase ABC prior to immunostaining. Two aliquots of antiserum were used with titers of 1:6000 and 1:2000 by radioimmunoassay.

Normal human marrow cells were resuspended in 2.7% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.35, for 15 min at 4°C. The cells were then centrifuged and rinsed in 0.1 M cacodylate 7 g/dl sucrose buffer. To facilitate penetration of immunologic reagents, the cells were then resuspended in 1% saponin in H<sub>2</sub>O for 1 hr at 50-55°C, and rinsed in phosphate-buffered saline (PBS). The cells (0.02-0.05 ml packed cell volume) were then exposed for 1 hr to a 0.2 U/ml chondroitinase ABC solution in 0.1 M Na(CH<sub>2</sub>COO)/0.1 M Triss-HCl buffer (pH 7.3), with 0.2% bovine serum albumin. The cells were then resuspended in RACS diluted 1:10 with PBS-1% saponin and incubated at 22°C for 1 hr. After thorough rinsing with PBS-1% saponin, some specimens were exposed for 1 hr at 22°C to a normal goat serum (NGS) diluted 1:10 in PBS-1% saponin to block nonspecific binding of ferritin-conjugated goat anti-rabbit IgG (F-GAR). The cells were then rinsed in PBS-1% saponin and exposed for 1 hr at 22°C to F-GAR (Miles Laboratories, Inc.) diluted 1:10 in PBS-1% saponin. Control specimens were comparably processed with either omission of the chondroitinase ABC step or substitution of RACS with normal rabbit serum diluted 1:10 in PBS. After rinsing in PBS-saponin the cells were postfixed in 1% OsO<sub>4</sub> and embedded as described above. Thin sections were examined without counterstaining.

Biochemical Analysis

GAGs were isolated using methods described previously in detail from 200 ml of packed leukemic leukocytes (less than 1% of the cell mass consisted of platelets) taken during therapeutic leukopheresis of an untreated patient with acute myelogenous leukemia. Because the cytochemical methods are sensitive only to carbohydrate functional groups, the GAGs were isolated and identified as free chains (rather than as proteoglycans) for comparison to the biochemical results. Briefly, the procedure involves digestion with 0.5 M NaOH, "Pronase," precipitation of nucleic acid with trichloroacetic acid, and gel filtration on Bio-gel P-4 to isolate GAGs larger than 2000 daltons. The GAGs were extracted from the void volume fraction as their hexadecylpyridinium complexes. Colorimetric analyses were carried out for uronic acid, hexosamine, and sulfaminohexose.

The isolated GAG mixture was analyzed by electrophoresis combined with chemical and enzymatic degradation to confirm the presence of heparan sulfate, chondroitin 4/6-sulfate (AC), dermatan sulfate, hyaluronate, and heparin as previously described. The buffers used were 0.2 M calcium acetate and 0.1 M HCl. Reference GAG was obtained from Drs. J. A. Cifoneili and M. B. Mathews (University of Chicago). The alcian-blue-stained electrophorograms were scanned with a Zeineh solt-laser scanner (LKB Instruments). The molecular weight and molecular weight distribution of the purified GAG preparation were estimated by chromatographing 1 μmol of glycosaminoglycan uronate on a column of agarose 0.5M.
Three markers, a 30,000-dalton chondroitin sulfate, a 15,000-dalton commercial heparin preparation, and an 8000-dalton chondroitin sulfate fraction, were also chromatographed on the column for calibration. The markers were calibrated against primary standard chondroitin sulfate fractions provided by Wasteson.27

RESULTS

Morphology

The ultrastructural morphology of normal marrow myeloid cells and leukemic myeloblasts was similar to that described previously.26,29 Consequently, only those observations related to GAG localization are described. Promyelocytes contained a well developed Golgi area and numerous primary granules (0.3–0.5 μm in diameter) in various stages of maturation. Immature primary granules contained a lucent rim with an electron-dense center, whereas fully mature granules contained osmiophilic material that extended to the granule periphery. Several of these latter granules contained a central electron-lucent crystalloid. Myelocytes contained several less dense secondary granules (0.3–0.4 μm in diameter), which lacked affinity for osmium and for lead and uranyl counterstains. Smaller (0.2 μm in diameter) moderately dense granules presumed to be tertiary granules were observed in late (segmented nuclei) neutrophils. Secondary granules generally outnumbered primary granules in segmented neutrophils.

Leukemic myeloblasts often contained numerous dense pleomorphic granules 0.2–0.5 μm in diameter. Auer rods were observed in all patient samples (n = 4). These elongated granules, averaging 0.5 μm in width and 3 μm in length, contained moderately dense material, occasionally with a periodic structure.

Cytochemistry

HID-TCH-SP stained primary granules in osmi- cated and unosmicated specimens similarly. The most immature granules, or relatively lucent condensing vacuoles, stained weakly; more condensed immature primary granules stained intensely; whereas staining was decreased or absent in fully condensed mature primary granules (Figs. 1 and 2). Weak to moderate reactivity was present in tertiary granules (Fig. 3A), but none was observed in secondary granules. Weak staining was observed in the mature face of Golgi lamellae of promyelocytes and segmented neutrophils (Fig. 3B), but was absent in myelocytes. No staining was observed in tubulo-vesicular structures, mitochondria, or on the cell surface of normal neutrophilic myeloid cells.

Chondroitinase ABC digestion resulted in a 50%–75% decrease in HID-TCH-SP staining of normal primary granules. A 50%–60% reduction in staining was observed after digestion with testicular hyaluronidase. A 20%–30% reduction in staining was noted after nitrous acid digestion. Sequential chondroitinase and nitrous acid treatment resulted in a greater than 75% reduction in staining. The reduction in staining was observed at both the light and electron microscopic level, but was easier to evaluate at the light microscopic level where the color intensity of several cells could be evaluated simultaneously.

At the light microscopic level, HID staining of marrow smears resulted in brown-purple staining of Auer rods in leukemic myeloblasts. HID-TCH-SP intensely stained most Auer rods as well as the immature primary granules at the ultrastructural level (Figs. 4 and 5). A few Auer rods lacked staining similar to that observed for mature primary granules (Fig. 6). HID-TCH-SP staining was confined to cytoplasmic granules and Golgi vesicles in the patient samples, which were also used for biochemical analysis. A few leukemic cells from one patient demonstrated cell surface staining with HID-TCH-SP, which conceivably could have resulted from degranulated GAG adherent to the cell surface. Similarly, some leukemic cells (<10% of cells) from 2 patients contained mitochondria with weak HID-TCH-SP staining. This latter staining pattern was encountered in the few cells of presumed monocytic origin as described previously.30 The possibility that some GAG was extracted during tissue processing could not be ruled out. Nevertheless, the specimens from which biochemical studies were done lacked both cell surface and mitochondrial staining.

Control specimens exposed to an acid MgCl2 solution instead of HID lacked significant TCH-SP staining (Fig. 7). Osicated specimens not exposed to acid MgCl2 or HID did demonstrate some fine stain precipitates in phospholipid containing mature primary granules. Also, very weak staining of some membranes and nuclear chromatin was observed. Unosmicated control specimens always lacked TCH-SP staining.

The distribution of acid phosphatase activity was similar to that observed for HID-TCH-SP (Figs. 8 and 9). Immature primary granules stained strongly, whereas staining was not observed in mature primary granules. Secondary granules lacked staining, and tertiary granules stained moderately. The Golgi vesicles in promyelocytes, band, and polymorphonuclear cells stained strongly.

Immunostaining

Immunoferritin particles were observed in immature and mature primary granules of normal human neutrophilic myeloid cells (Fig. 10). Staining was
Fig. 1. This promyelocyte from a normal marrow specimen contains several HID-TCH-SP-reactive (P-1) and unreactive primary granules (P-2) as well as condensing vacuoles (CV) with sparse stain deposits (enlarged in inset). Specimen postfixed in OsO₄ (×12,500; inset, ×37,000).

Fig. 2. Variable HID-TCH-SP staining is present in primary granules (P-1) of this promyelocyte. Some primary granules are unreactive (P-2). Unlike Fig. 1, this specimen is unosmicated and consequently lacks fine background TCH-SP staining. (N, nucleus; Nu, nucleolus; ×34,000.)

Fig. 3. (A) This segmented neutrophil contains a few HID-TCH-SP reactive primary granules (P-1), but the majority are unreactive (P-2). Secondary granules lack staining. (B) An enlargement of the central portion of the neutrophil demonstrating fine staining of Golgi (G) vacuoles (arrows) at the maturing face. Tertiary granules (T) stain moderately. N, nucleus; C, centriole. Specimen postfixed in OsO₄. (A—×12,500; B—×38,400.)
The total yield of glycosaminoglycan, measured as uronate, was \(8 \times 10^{-17}\) mole/cell. Analysis of the

Biochemical Analysis

Some cells contained cytoplasmic and plasmalemmal ferritin particles, which appeared to represent non-specific staining.

Fig. 4. An Auer rod (arrows) in this leukemic myeloblast stains intensely with the HID-TCH-SP method. The plasmalemma and mitochondria (M) lack staining. This cell is from the same specimen processed for biochemical analysis. N. nucleus. Specimen postfixed in OsO\(_4\) (x 17,000).

Fig. 5. The Auer rod (A) in this leukemic myeloblast stains strongly with HID-TCH-SP. Some areas (small arrows) contain an increased concentration of reactive material possible resulting from recent fusion of primary granules or their precursors. Primary granules (large arrows) demonstrate similar staining. Unosmicated specimen (x 31,250).

Fig. 6. The Auer rod (A) of this leukemic myeloblast lacks staining, whereas staining is evident in some primary granules (arrows). The finding suggests masking or loss of sulfate similar to that seen in normal primary granules (cf., Figs. 1-3). N. nucleus. Specimen postfixed in OsO\(_4\) (x 26,250).

Fig. 7. This control normal marrow specimen was incubated in an acid MgCl\(_2\) solution rather than in the HID stain solution. TCH-SP does not stain primary granules (P). Specimen postfixed in OsO\(_4\) (x 20,500).

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isolated GAGs for sulfaminohexose, which is found in heparin and heparan sulfates, demonstrated $1.2 \times 10^{-17}$ mole/cell for a molar ratio of 0.15 mole sulfaminohexose/mole of uronate.

Figure 11 presents the molecular weight distribution of the isolated GAG product by showing the distribution of uronate and hexosamine, which are found in all GAG, and sulfaminohexose. Also shown are the peak elution volumes of glycosaminoglycan molecular weight markers. Uronate-containing material ranged from about 2000 daltons to around 20,000 daltons, but with a distinct peak at 10,000–12,000 daltons. The higher molecular weight material, which contained only uronate and hexosamine, corresponded to the large chondroitin sulfate band seen in electrophoresis. As shown by the sulfaminohexose distribution, the heparan sulfate was of much lower molecular weight. Indeed, no distinct maximum was seen in the distribution in the range of 2000 (approximately an octasaccharide) to about 12,000.

When the isolated GAGs were analyzed by electrophoresis in calcium acetate, a complex pattern of bands was evident. These bands could not be entirely resolved, and any estimates of relative proportions must be treated as only rough estimates. At least four broad bands were evident in calcium acetate, as shown in Fig. 12A. The fastest moving band spanned the mobilities of reference dermatan sulfate; the third band migrated slightly behind hyaluronate, and the fourth band migrated only slightly from the origin. After digestion with chondroitinase AC, a different pattern of four bands was seen (Fig. 12B). The fastest moving band in the original sample was presumably chondroitin sulfate, since it was abolished, leaving the band that migrated with dermatan sulfate. This was the fastest moving band in the pattern observed after chondroitinase AC digestion (Fig. 12B). Some material in the third band seen in the original sample appeared to be chondroitinase-AC-susceptible because the intensity was reduced, leaving two bands. Neither matched the mobility of any of the reference glycosaminoglycans. The fourth band, which was found near the origin, was not affected by chondroitinase AC. Treatment with chondroitinase ABC abolished both the large faster moving band and the smaller band that migrated with dermatan sulfate (Fig. 12C), leaving two poorly resolved bands that corresponded in mobility to those described above, and the band near the origin. Nitrous acid reduced the intensity of the poorly resolved bands migrating in the regions near reference in the heparan sulfate and dermatan sulfate and the band migrating behind hyaluronate (Fig. 12D).
order to obtain a clearer picture of the nitrous-acid-susceptible material, the chondroitinase-AC-resistant material was treated with nitrous acid (Fig. 12E). Both of the slower moving bands seen in Fig. 12B were abolished by nitrous acid treatment. Hyaluronic acid was not identified. No change in electropherograms was seen after digestion with streptomyces hyaluronidase. In 0.1 M HCl, a system used to identify heparin, no bands were seen that migrated with reference heparin. The major band migrated with chondroitin sulfate.

**DISCUSSION**

These studies have utilized cytochemical, immunologic, and biochemical methods to characterize and localize a heterogeneous population of glycosaminoglycans (GAGs) not previously recognized in human neutrophil granules and Auer rods of leukemic myeloblasts. Cytochemical results localize the majority of demonstrable GAG in immature primary granules and to a lesser extent in tertiary granules and together with biochemical results identifies this GAG in primary granules as chondroitin, heparan, and dermatan sulfate. Previous biochemical studies have identified chondroitin sulfate in leukocytes,

However, subcellular localization of the GAG could not be accomplished with the methodology employed in previous studies.

At least two populations of primary granules are evident in specimens stained for sulfate and acid phosphatase: one population is reactive and the other nonreactive. Somewhat similar observations in the rabbit led us to propose that this loss of staining in primary granules results from masking of at least some sulfated GAG rather than removal of sulfated material during granule maturation. Immunostaining of GAG in many mature primary granules in this study further
supports the hypothesis that masking of sulfate groups is occurring. In addition, radioautographic studies of rabbit\textsuperscript{11} and dog\textsuperscript{19} have demonstrated that at least some $^{35}$S\textsubscript{4} labeling persists in mature primary granules and late neutrophils. Acid phosphatase is concomitantly masked in mature primary granules, as shown in this study, as well as in eosinophil crystallloid granules.\textsuperscript{31,32} These observations, together with biochemical observations that GAGs inhibit hydrolytic enzymes,\textsuperscript{13,14} support the hypothesis that leukocyte GAGs function by complexing with certain granule components and holding them in an inactive state to facilitate granule storage.

Localization of GAG in Auer rods is a new finding and is consistent with the observation of other primary granule components in these abnormal granules.\textsuperscript{29} The observation that some Auer rods lack HID-TCH-SP staining suggests that these abnormal granules may undergo a maturational sequence similar to that seen in normal primary granules.

Chondroitin sulfate was the predominant GAG, comprising at least 60% of the GAG as evidenced by biochemical, cytochemical, and immunologic results. We estimate that 30% of the GAG is heparan sulfate. This estimate is based on the molar ratio of sulfaminohexose to uronate (0.15) and the usual composition of heparan sulfates—roughly 50% of disaccharide residues are N-sulfated\textsuperscript{33}; the other 50%, being N-...
acetylated, do not react in the sulfaminohexose assay. The identification of dermatan sulfate as 10% of the GAG was also clear in the biochemical studies. The testicular hyaluronidase susceptibility of stained GAG in histologic samples is consistent with the presence of chondroitin 4- or 6-sulfate, but differs from the relative resistance of rabbit heterophil GAG staining to testicular hyaluronidase digestion. Presumably, the GAG content of leukocyte granules is species variable.

The identification of sulfaminohexose and nitrous-acid-susceptible electrophoretic bands establishes the presence of a GAG that most closely resembles heparan sulfate, even though the mobilities of the susceptible bands did not completely match that of the reference heparan sulfate. Nitrous acid treatment alone decreased the staining intensity markedly in the region of the electropherogram wherein heparan sulfate migrates (Fig. 12B). However, after chondroitinase AC degradation, two bands were seen that did not migrate with reference heparan sulfate (Fig. 12B) but, as shown in Fig. 12E, both are nitrous acid susceptible. Some of the unusual electrophoretic behavior of the heparan sulfate may well be a function of the low molecular weight or the presence of an attached peptide. The protease-resistant peptide could link the heparan sulfate to dermatan or chondroitin sulfate, which when removed by enzyme digestion could lead to the appearance of new bands composed of heparan sulfate and a peptide. The reference sample is quite different from the granule heparan sulfate, being at least of higher molecular weight and possible different composition. Both of these factors may well affect electrophoretic mobility. The heparan sulfate identified in electropherograms and sulfaminohexose analysis of leukemic cells is present in primary granules of normal cells on the basis of a similar decrease in sulfate staining after nitrous acid digestion of histologic samples.

The GAG in the granules must be attached to a protein core, otherwise it would have been extracted during the ultrastructural staining protocol, and no staining would have been seen in the granules. The heparan sulfate, in particular, is very heterogeneous, ranging from about 12,000 daltons, which is commonly associated with intact chains, down to sizes equivalent to about octasaccharides in length. These smaller fragments are much more abundant than are the larger chains. This distribution of molecular weights is consistent with the action of an endoglycosidase that has left "stubs" of varying lengths on the protein core during physiologic degradation. Alternately, it could represent incomplete assembly or interrupted biosynthesis. Whether this finding represents a function of the leukemic process in these cells or occurs in normal primary granules remains to be determined. However, the similar cytochemical properties of granules in both normal and leukemic specimens suggest that their biochemical properties are also similar.

In this study tertiary granules in normal leukocytes are identified cytochemically for the first time on the basis of HID-TCH-SP and acid phosphatase staining. This observation is consistent with the previously observed $^{35}$SO$_4$ incorporation into these granules. The staining of Golgi lamellae in band and segmented neutrophils presumably represents the synthesis of GAG destined for tertiary granules. The removal of much, if not all, of the HID-TCH-SP staining in these granules by testicular hyaluronidase indicates that the tertiary granule contains chondroitin sulfate AC. As in primary granules, the staining pattern of tertiary granules with HID-TCH-SP is similar to that observed for acid phosphatase.

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