Mucopolysaccharides in beef retina and small cerebral vessels

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On Ecteola columns in 0.05M HCl (pH 1.3) the sulfate groups of mucopolysaccharides are dissociated while the carboxyl groups are not. In 0.05M acetate buffer (pH 4.8), the carboxyl groups are dissociated and those mucopolysaccharides which do not bind to Ecteola at the lower pH can be resolved. These properties have been utilized to resolve mixtures of beef retina and small beef cerebral blood vessel mucopolysaccharides in order to further characterize and partially identify those mucopolysaccharides which are associated with the nervous elements and those which are associated with the blood vessels. These studies reveal that the retina contains a complex mixture of mucopolysaccharides while the small cerebral vessels contain a more clearly defined mixture.

The first attempts to identify the alcian-blue and periodic acid-Schiff positive materials located in the region of the retinal receptors1, 2 demonstrated that they contain hexosamine and possibly mucopolysaccharides. 4, 5 On the basis of electrophoretic separations, the material isolated from beef retina appeared to contain at least two metachromatic substances. These substances were resolved on cellulose columns and showed electrophoretic mobilities similar to authentic chondroitin and keratan sulfates. Treatment of the isolated retinal mucopolysaccharides with testicular hyaluronidase resulted in hydrolysis of the mucopolysaccharide with the lower electrophoretic mobility. Such results suggest that retina does not contain keratan sulfate. However, the definitive identification of retinal mucopolysaccharides has not yet been accomplished.*

A recent study6 has supported the earlier observations and has shown that two electrophoretically separable mucopolysaccharides can be isolated from pigment epithelium. The mucopolysaccharides isolated from pigment epithelium also appear to be a complex mixture. However, in both retina and pigment epithelium the possibility that they contain hyaluronic acid and keratan sulfate has been eliminated. 4, 5, 6

In approaching the problem of chemical identification when the whole retina is used as a source of mucopolysaccharides, the contributions from the biosynthetic sites, nervous elements, and blood vessels must be considered. The biosynthetic sources of retinal mucopolysaccharides have been investigated and the evidence

*Similar results have been obtained with human retina (unpublished observations of B. Wortman).

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suggests epithelial\(^8\) and Müller cells.\(^7\) Blood vessels are also known to have the capacity to synthesize mucopolysaccharides.\(^6\) In addition mucopolysaccharides have been identified in central nerve cells,\(^11\) in brain,\(^12\) and in large blood vessels.\(^14\) Mucopolysaccharides in nervous tissue have tentatively been identified as hyaluronic acid, chondroitin 4-sulfate, and dermatan sulfate.\(^11\) Descriptions of mucopolysaccharides in blood vessels have dealt primarily with the aorta and not the small blood vessels. Human aorta contains neutral and acid mucopolysaccharides, the latter being hyaluronic acid, heparitin sulfate, and chondroitin 6-sulfate, which is the major constituent.\(^15\) In beef aorta, chondroitin 4-sulfate rather than 6-sulfate is the major constituent.\(^6\) The mucopolysaccharide content of any tissue may vary with the age\(^10\) and the nutritional state\(^9\) of the animal.

The following communication is an extension of the previous reports which demonstrated that mucopolysaccharides can be isolated from retina.\(^4\) This study presents the further resolution of retinal mucopolysaccharides by ion-exchange chromatography as well as chemical characterization of these materials. In addition, comparisons are made with mucopolysaccharides isolated from the small cerebral vessels and the mixed blood vessel neural tissue of the retina.

**Materials and methods**

**Beef retina.** The anterior pole of fresh (2 to 3 hours post mortem) beef eye was removed by cutting 5 to 7 mm. posterior to the limbus and the remaining posterior pole inverted. The vitreous was discarded, the retina removed from its attachments, rinsed in distilled water, and homogenized in cold acetone. The dry powder was then used for the study.

**Beef cerebral vessels.** Small blood vessels (<1 mm.) were removed from fresh (2 to 3 hours post mortem) beef meninges and dissected free of adhering connective tissue. Excess blood was removed by rinsing in distilled water. The vessels were then homogenized in cold acetone and the dry powder was used for the study.

**Isolation of mucopolysaccharides.** Retinal and cerebral blood vessel acetone powders were subjected to proteolytic digestion and the mucopolysaccharides recovered and partially purified by previously described techniques.\(^4\) Anion-exchange columns. Ecteola with a binding capacity of 0.3 mEq. per gram was washed and the columns prepared and equilibrated as previously described.\(^18\) Isolated mucopolysaccharides were dissolved in a minimal amount of distilled water and applied to columns which were eluted with either 0.05M HCl (pH 1.3) or 0.05M acetate buffer (pH 4.8) according to the experimental conditions.

**Results and discussion**

**Beef retina.** Two major hexuronic acid-containing substances were detected after passage of retinal mucopolysaccharides through the Ecteola column which had been equilibrated with 0.05M HCl (pH 1.3) (Forms I and II, Fig. 1, A). Form I eluted from the Ecteola before the linear salt gradient was started. When Form I was rechromatographed under similar conditions, none of Form II was detected (Fig. 1, B). Form II was eluted from Ecteola as a broad, trailing peak (Fig. 1, A), and when rechromatographed under similar conditions, most of the trailing substances were reduced and none of Form I was detected (Fig. 1, C). However, when Form I was rechromatographed at a high pH (0.05M acetate buffer, pH 4.8), a second hexuronic acid-containing substance was observed (Form IA, Fig. 1, D).

Forms I and II (Fig. 1, A) were recovered from column effluents, concentrated at reduced pressure, dialyzed against distilled water, and analyzed for chemical components (Table I). When the data in Table I are considered with those in Fig. 1, the complexity of these substances is evident. The starting material was relatively low in hexosamine and high in nitrogen; these values do not suggest a pure mucopolysaccharide. The high hexuronic acid value found in the starting material and in Form II is unusual. The carbazole test used for hexuronic acids can be influenced by glycosidic linkages and by the presence of contaminating nucleic acids or nucleoproteins.\(^19\) The analyses also indicate the
Fig. 1. Resolution of retinal mucopolysaccharides on Ecteola columns (1 by 30 cm.). Hexuronic acid, • — •, hexose O — O. A, Forty milligrams of retinal mucopolysaccharides were applied to a column which had been equilibrated with 0.05M HCl (pH 1.3) and eluted with a gradient to 1M NaCl-0.05M HCl (pH 1.3). B, Fractions 5 through 20 (in A) were applied to a column and eluted as above. C, Fractions 48 through 70 (in A) were rechromatographed as above. D, Fractions 5 through 9 (in B), were rechromatographed on a column which had been equilibrated with 0.05M acetate buffer (pH 4.8) and eluted with a gradient to 1M NaCl-0.05M acetate buffer (pH 4.8).

Fig. 2. Resolution of cerebral blood vessel mucopolysaccharides on Ecteola columns (1 by 20 cm.). Hexuronic acid • — •, hexose O — O. A, Fifteen milligrams of cerebral blood vessel mucopolysaccharides were applied to a column which had been equilibrated with 0.05M HCl (pH 1.3) and eluted with a gradient to 1M NaCl-0.05M HCl (pH 1.3). B, Fractions 3 through 9 were dialyzed against 0.05M acetate buffer (pH 4.8), and rechromatographed on a column which had been equilibrated with the same buffer and eluted with a gradient to 1M NaCl-0.05M acetate buffer (pH 4.8).
Table I. Per cent composition of beef retinal mucopolysaccharides before and after anion-exchange chromatography

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<th>Chromatographic form*</th>
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<tr>
<td></td>
<td>I</td>
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<tr>
<td>Hexuronic acid 19</td>
<td>66.2</td>
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<tr>
<td>Hexosamine 20, 21</td>
<td>2.6</td>
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<tr>
<td>Galactosamine</td>
<td>++</td>
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<tr>
<td>Glucosamine</td>
<td>+</td>
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<tr>
<td>&quot;Chondroitin&quot; and/or &quot;Chondroitin 6-&quot;SO 22</td>
<td>5.1</td>
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<tr>
<td>Nitrogen (micro-Kjeldahl)</td>
<td>10.3</td>
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<tr>
<td>Digested by hyaluronidase 23</td>
<td>19</td>
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*Same designation as that shown in Fig. 1, A.
†Form I does not yield a turbid complex with albumin.

The presence of both glucosamine and galactosamine which further suggests that retina contains a mixture of mucopolysaccharides, part of which can be hydrolyzed by testicular hyaluronidase and may be chondroitin and/or chondroitin 6-sulfate.

On the basis of behavior on Ecteola columns and chemical analyses, it is suggested that retina contains neutral-, nonsulfated acid-, and sulfated acid-mucopolysaccharides.

**Cerebral blood vessels.** For comparison and to gain insight into possible differences between the mucopolysaccharides isolated from nervous tissue and that isolated from small blood vessels, the small cerebral blood vessels from beef brain meninges were studied. When the isolated mucopolysaccharides were eluted from the anion-exchanger, Ecteola, a more clearly defined pattern was obtained than that obtained with the retinal material. Form I (Fig. 2, A) is suggestive of a nonsulfated neutral and acid-mucopolysaccharide mixture and is further supported by the separation obtained at the higher pH (Fig. 2, B). Forms II and III are suggestive of chondroitin sulfate and heparitin sulfate. These data indicate that the small blood vessels are qualitatively similar to the aorta and contain a neutral polysaccharide, hyaluronic acid, chondroitin sulfate, and heparitin sulfate.

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REFERENCES