TURBIDIMETRIC MEASUREMENT OF ACID MUCOPOLYSACCHARIDES AND HYALURONIDASE ACTIVITY

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The turbidimetric methods here described are based on the formation of relatively insoluble complexes between isolated acid mucopolysaccharides and cetyltrimethylammonium bromide. Some of the properties of these complexes have recently been described by Scott (1). We found that the amount of turbidity developed when the cetyltrimethylammonium bromide is added to a solution of acid mucopolysaccharide is proportional to the amount of acid mucopolysaccharide in the system, and that no turbidity develops when cetyltrimethylammonium bromide is added to chondroitinsulfate or hyaluronate which has been depolymerized by incubation with testicular hyaluronidase.

Reagents—

Acetate buffer. 0.2 M sodium acetate-acetic acid, pH 6, to which NaCl is added to give a concentration of 0.15 M.

Acid mucopolysaccharide solution. 50 mg. of sodium chondroitinsulfate or sodium hyaluronate are dissolved in 100 ml. of the acetate buffer.

Cetyltrimethylammonium bromide reagent. 2.5 gm. are dissolved in 100 ml. of 2 per cent NaOH.

Measurement of Acid Mucopolysaccharide

1 ml. of acetate buffer containing from 10 to 200 γ of acid mucopolysaccharide is delivered into each experiment test-tube. 1 ml. of acetate buffer is used as a blank in another tube. The tubes are immersed in a water bath maintained at 37.5°. After 10 to 15 minutes, 2 ml. of the cetyltrimethylammonium bromide reagent are added to each test-tube. After mixing has been insured by inversion, the content of each tube is transferred to a cuvette, and the optical density of each sample is read within 10 minutes against the blank. A Beckman DU spectrophotometer,

1 Obtained from the Amend Drug and Chemical Company, Inc., New York.
2 Sodium chondroitinsulfate was obtained through the courtesy of Dr. D. Dzie-wiatkowski. On analysis, it was found to have nitrogen 2.7, sulfate sulfur 5.3, glucuronic acid 32.4, and hexosamine 22 per cent.
3 Sodium hyaluronate was obtained through the courtesy of Dr. R. Greif. On analysis, it was found to have nitrogen 4.43, sulfate sulfur 0.4, glucuronic acid 24.8, and hexosamine 22.7 per cent.
with photomultiplier attachment, has been employed in this work, and readings were taken at $\lambda = 400 \text{ m\m}$, with a slit width of 0.15 mm.

Specificity—Different compounds have been tested under the conditions described for their capacity to produce turbidity with the cetyltrimethylammonium bromide reagent. In Table I the amount of turbidity given by 100 $\gamma$ of each compound is expressed as per cent of the turbidity produced by 100 $\gamma$ of sodium hyaluronate. It is noteworthy that the pH during the development and reading of turbidity is 12.5.

![Graph](http://www.jbc.org/)

**Fig. 1.** The relation between the turbidity as measured at $\lambda = 400 \text{ m\m}$ and the amount of sodium chondroitin sulfate or sodium hyaluronate.

**Measurement of Hyaluronidase Activity**

The above method has been used for the determination of the activity of different preparations of hyaluronidase. The activity of even partially purified preparations of this enzyme, prepared according to Tint and Bogash (2) with an activity of 2174 turbidity reducing units per mg. of nitrogen, or rather crude ones prepared according to Hahn (3) with an activity of 76 turbidity reducing units per mg. of nitrogen, can be determined. Different quantities of such enzyme preparations, dissolved in 0.6 ml. of acetate buffer, are incubated at 37.5$^\circ$ with 200 $\gamma$ of either chondroitin sulfate or hyaluronate, dissolved in 0.4 ml. of acetate buffer. By carrying out the recommendations of Meyer and Rapport (4), the incubation time has been limited to 15 minutes. At the same time, standards containing
200, 100, 50, and 25 \gamma of substrate and one blank containing buffer alone and another with enzyme alone are prepared. In our experience, the optical densities given by the enzyme preparations after addition of the cetyl-

Table I

Specificity of Turbidimetric Assay

<table>
<thead>
<tr>
<th>Substance (100 \gamma of each) tested</th>
<th>Relative turbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hyaluronate</td>
<td>100</td>
</tr>
<tr>
<td>&quot; chondroitinsulfate</td>
<td>133</td>
</tr>
<tr>
<td>&quot; heparinate</td>
<td>143</td>
</tr>
<tr>
<td>Nucleic acid (from yeast)</td>
<td>4.3</td>
</tr>
<tr>
<td>Ribose nucleic acid</td>
<td>2.8</td>
</tr>
<tr>
<td>Deoxyribonucleic acid (sperm)</td>
<td>3.5</td>
</tr>
<tr>
<td>Glycogen</td>
<td>20</td>
</tr>
<tr>
<td>Starch (soluble)</td>
<td>0.004</td>
</tr>
<tr>
<td>Inulin</td>
<td>0.012</td>
</tr>
<tr>
<td>Crystalline bovine plasma albumin</td>
<td>2.4</td>
</tr>
<tr>
<td>Acacia</td>
<td>74</td>
</tr>
<tr>
<td>Sodium polyanethol sulfonate (Liquoid &quot;Roche&quot;)</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Fig. 2. The relation between the amount of sodium hyaluronate depolymerized during 15 minutes of incubation at 37.5\degree and the quantity of testicular hyaluronidase (expressed as micrograms of nitrogen), prepared according to Hahn (3).
trimethylammonium bromide reagent have always equaled those given by the acetate buffer. At the end of the incubation period, addition of 2 ml. of cetyltrimethylammonium bromide reagent stops the enzymatic reaction (bringing the pH to 12.5) and produces the turbidity. After mixing by inversion, the optical densities are determined as previously described.

The optical densities given by the standards, plotted against the amount of acid mucopolysaccharide present in each of them, constitute the calibration line to which are referred the optical densities of the tubes containing the enzyme. It is, therefore, possible to ascertain the amount of acid mucopolysaccharide depolymerized by each quantity of enzyme by subtracting the residual amount of substrate from that originally present in each tube (Fig. 2).

The unit of enzymatic activity is expressed as the quantity of enzyme which produces a 50 per cent reduction of the turbidity given by the initial quantity (200 γ) of substrate.

DISCUSSION

The method here described recommends itself for its simplicity. Each of the several protein-containing reagents proposed for the classical turbidimetric method, derived from the original one of Kass and Seastone (5), is unstable, and it is difficult to reproduce batches with identical properties. On the other hand, the cetyltrimethylammonium bromide reagent used in the method here proposed is rapidly prepared and is stable indefinitely. Furthermore, the cetyltrimethylammonium bromide reagent stops the enzymatic reaction and produces the turbidity at the same time. Variations in the molarity of the acetate buffer at pH 6 (from 0.1 M to 0.3 M), or variations in the pH of a 0.2 M acetate buffer (from pH 5 to pH 6), do not affect appreciably the degree of turbidity produced with any given amount of acid mucopolysaccharides. If, however, the acid mucopolysaccharide is dissolved in distilled water instead of in salt solution, much less turbidity develops on addition of the cetyltrimethylammonium bromide.

SUMMARY

Simple, rapid, and reproducible methods for the determination of (a) 10 to 200 γ of isolated acid mucopolysaccharides and of (b) hyaluronidase activity have been described.

BIBLIOGRAPHY

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