PREPARATION AND CHARACTERIZATION OF DEXTRAN FROM LEUCONOSTOC MESENTEROIDES

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In conjunction with investigations on the α-1,6-glucosidic linkage in starch, it was desirable to conduct comparative studies on other substances containing this linkage. The dextran from Leuconostoc mesenteroides, a polysaccharide having predominantly α-1,6-glucosidic linkages, was selected to be used directly in these comparative studies and to provide a source of simpler substances containing this linkage of rare occurrence. Experiments were designed to provide a dependable source of dextran of high purity and of reproducible high viscosity. Improvements have been made over methods previously described for the preparation of this polysaccharide (1-4), and procedures have been established for the preparation of dextrans of low as well as of high viscosities.

Factors Influencing Dextran Production—Numerous factors, only a few of which have been studied (1, 4), appear to influence the properties and the amount of the dextran and of the by-products produced from sucrose by cultures of Leuconostoc mesenteroides. A factor of outstanding importance is the strain of the organism (5), which appears to determine whether the dextran is water-soluble or water-insoluble (3, 6). The structural basis for this difference in solubility is not known. Previously reported dextrans, most of which originated from different strains of Leuconostoc mesenteroides, have varied in other physical properties. Dextran has been obtained in yields of 18 (3) and 25 per cent (4), with specific rotations of +178° to +184° (2, 6, 7), of 195° (8), and of 198° (9). It has been reported to have high (2, 4, 6) and low (1, 9) contents of nitrogen, phosphorus, and ash. Some viscosity data for dextran are not on a comparable basis (7, 8) and other data present unexplained variations (5).

The optimum pH range for the enzymatic synthesis of dextran has been shown by Hehre to be 4.0 to 6.0 (7, 10). When preparing dextran from cultures of Leuconostoc mesenteroides, neutralization of the acidic by-products has been reported to increase the yield of dextran (1, 4, 11). The incubation time used previously for the production of dextran has

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varied from 18 hours to 20 days (1-4). Methods reported for determining when dextran formation was complete have involved isolating and weighing the dextran from an aliquot of the medium (1) and measuring the viscosity of the culture medium (12).

In the present investigation, the data obtained are for Leuconostoc mesenteroides NRRL B-512 and its water-soluble dextran. Observations are reported here on the effect of pH and the composition of the culture medium, aeration, and incubation time on the yield and properties of this dextran. The manner of inoculation has been held constant.

Production of High Viscosity Dextran—The viscosity of dextran from Leuconostoc mesenteroides NRRL B-512 was found to be influenced greatly by cultural conditions. However, when these conditions were controlled, dextrans of high or of low viscosity were obtained as desired.

The procedure adopted for the preparation of dextran utilized an un-aerated medium containing sucrose in 10 per cent concentration and buffered only with the mineral nutrients present. This was inoculated heavily with a rapidly growing culture of Leuconostoc mesenteroides NRRL B-512 and incubated at 25°C. The course of dextran production was followed by measurement of the viscosity of the culture medium. The formation of dextran was paralleled by an increase in viscosity until, after about 24 hours incubation, the viscosity reached a maximum and dextran formation appeared to be completed. As is shown in Table I, these changes were accompanied by a decrease in pH from the initial value of about 7.0 to about 4.6 at the time of maximum viscosity.

Dextrans such as Dextrans A and D of Table II, which were isolated from the culture media at maximum viscosity, are called high viscosity dextrans. These dextrans after purification had characteristically high viscosities, highly positive optical rotations, and high purity.

The reproduction of results from preparations of high viscosity dextrans is demonstrated by Dextrans A and D (Table II) and by another typical high viscosity dextran which had a relative viscosity of 2.230 and was obtained in 26.8 per cent yield. Dextran B is not a typical high viscosity dextran (see Table I, foot-note).

In all cases, the products called dextrans were quantitatively precipitated as gummy masses by the addition of an equal volume of absolute ethanol to the culture medium. The technique used for isolating purified dextrans from aqueous solutions and for drying the product gave finely divided fluffy dextrans, which dispersed readily in water and underwent chemical reaction with ease when in the dry state.1

Production of "Autolyzed" Dextran—The viscosity of the culture medium containing dextran decreased when incubation was extended beyond the

time of maximum viscosity. This decrease in viscosity, as is shown in Table I, was rapid at first and gradually became slower until a very low value was reached. During this incubation period, the pH of the culture medium decreased slowly from 4.6 to a steady value of 3.7.

Dextrans such as Dextrans C and E of Tables I and II, which were isolated from culture media of greatly reduced viscosity, are called "autolyzed" dextrans. In physical properties the "autolyzed" dextrans differ from the corresponding high viscosity dextran controls (Dextrans B and D) mainly in their lower viscosities. The viscosities of the "autolyzed" dextrans were inversely proportional to the duration of incubation; their yields were 2.1 to 2.5 per cent lower than those of their high viscosity controls.

### Table I

**Effect of Extended Incubation at 25° on pH and Viscosity of Unbuffered Culture Media**

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>pH</th>
<th>Absolute viscosity</th>
<th>Fraction isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td></td>
<td>centipoises</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.5</td>
<td>2</td>
<td>Dextran B*</td>
</tr>
<tr>
<td>23</td>
<td>4.9</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>4.5</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>4.45</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>4.4</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>3.85</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>191</td>
<td>3.7</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>335</td>
<td>3.7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>384</td>
<td>3.7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>503</td>
<td>3.7</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

* These dextrans were isolated from one-half of their respective culture media, and the incubation of the other half was continued. When the isolation of Dextran B was actually begun, the viscosity of this part of the culture medium had decreased to 59 centipoises.

Production of Dextran in Aerated and in Buffered Culture Media—Aeration of culture media was found to be unfavorable to dextran formation. Data on the pH and viscosity changes in an aerated, unbuffered culture medium are given in Table III, and the data on the product isolated therefrom, Dextran F, are given in Table II. As compared with the results from
unaerated culture media, aeration decreased the rate of formation, the yield, and the viscosity of the dextran, and did not prevent the culture medium from passing through a maximum viscosity. These results are not in conflict with the view of Hehre (10) that the action of the dextran-

**TABLE II**

Data on Dextran from Various Culture Media

<table>
<thead>
<tr>
<th>Dextran</th>
<th>Absolute viscosity of culture medium from which dextran was isolated</th>
<th>Yield</th>
<th>N</th>
<th>P</th>
<th>Relative viscosity at 25°, 0.5 per cent in water*</th>
<th>[α] D (in 1 N NaOH, C = 1)</th>
<th>Alkali No.†</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>446</td>
<td>25.3†</td>
<td>0.017</td>
<td>0.005</td>
<td>2.253</td>
<td>+203</td>
<td>0.0</td>
</tr>
<tr>
<td>B</td>
<td>73</td>
<td>24.7</td>
<td>0.033</td>
<td>0.008</td>
<td>2.003</td>
<td>201</td>
<td>0.0</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>22.2</td>
<td>0.022</td>
<td>0.011</td>
<td>1.414</td>
<td>199</td>
<td>0.6</td>
</tr>
<tr>
<td>D</td>
<td>203</td>
<td>23.7</td>
<td>0.032</td>
<td>0.007</td>
<td>2.133</td>
<td>200</td>
<td>0.1</td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>21.6</td>
<td>0.010</td>
<td>0.004</td>
<td>1.565</td>
<td>200</td>
<td>0.3</td>
</tr>
<tr>
<td>F</td>
<td>14</td>
<td>&lt;14.7</td>
<td>0.000</td>
<td>&lt;0.002</td>
<td>1.719</td>
<td>202</td>
<td>0.0</td>
</tr>
<tr>
<td>G</td>
<td>647</td>
<td>24.0†</td>
<td>0.000</td>
<td>&lt;0.002</td>
<td>1.719</td>
<td>202</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* The relative viscosities in 0.1 M calcium acetate of Dextrans A, C, and G were 2.235, 1.405, and 1.683, respectively. In 1.0 M calcium acetate, the value for Dextran A was 2.298.

† To serve as a basis for comparison, the following alkali numbers are quoted from (13): defatted corn-starch 11.0; corn amylose 20.2; corn amylpectin 5.9.

‡ The ash content of these representative dextrans was 0.02 per cent.

**TABLE III**

Effect of Aeration on pH and Viscosity of Unbuffered Culture Medium

<table>
<thead>
<tr>
<th>Operation</th>
<th>Incubation time</th>
<th>pH of culture medium</th>
<th>Absolute viscosity of culture medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kr.</td>
<td></td>
<td>centipoises</td>
</tr>
<tr>
<td>Aeration started</td>
<td>0</td>
<td>7.5</td>
<td>2</td>
</tr>
<tr>
<td>&quot; stopped</td>
<td>23.5</td>
<td>5.2</td>
<td>5</td>
</tr>
<tr>
<td>Dextran F isolated</td>
<td>47.5</td>
<td>4.4</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>4.3</td>
<td>14</td>
</tr>
</tbody>
</table>

* About 20 liters of air per hour were bubbled through the 6 liters of culture medium containing a small amount of lard oil to prevent foaming.

synthesizing enzyme does not appear to be "coupled with or dependent upon oxidative processes."

No advantage to dextran formation has been found by buffering the culture medium with calcium carbonate. Culture media buffered at pH
A. JEANES, C. A. WILHAM, AND J. C. MIERS

7.0 to 6.4 with calcium carbonate reached their maximum viscosity in 48 to 56 hours, and immediately the viscosity began to decrease rapidly. A dextran isolated from such a medium at its maximum viscosity is Dextran G of Table II. Comparison with the corresponding values for unbuffered preparations shows that buffering with calcium carbonate resulted in a marked increase in the viscosity of the culture medium, no increase in the yield, and a decrease in the viscosity but no decrease in the purity of the purified dextran.

Aerated media buffered with calcium carbonate did not pass through a maximum viscosity; the viscosity of such a medium increased slowly over a period of 30 days. Under these conditions the rate of dextran formation was decreased as compared with an unbuffered, buffered medium.

Other Polysaccharide Fractions—In addition to dextran, culture media of Leuconostoc mesenteroides NRRL B-512 contained other polysaccharides in small amounts which were precipitated by adding ethanol to the media to make ethanol concentrations of 65 and 75 per cent. The amounts and properties of the fractions obtained varied with the conditions of production, but all fractions appeared to contain combined fructose. Some of the fractions were levans, and some became insoluble in water after one precipitation.

It has not been reported previously that Leuconostoc mesenteroides produces both dextran and levan. A few strains of Streptococcus bovis and of Streptococcus salivarius have been observed to produce both levans and dextrans (14).

EXPERIMENTAL

The medium used for growth of Leuconostoc mesenteroides NRRL B-512 and for the preparation of dextran was the same as that used by Hassid and Barker (2), except that 0.1 per cent of sodium chloride was added, as recommended by Tarr and Hibbert (1). The dipotassium hydrogen phosphate, in 5 per cent solution, was sterilized separately and added aseptically to the cool, sterile solution of the other components. Sterilization was effected by autoclaving at 15 pounds per sq. in. for 30 minutes.

Preparation of Inoculum—A culture of the organism was prepared by inoculating one standard loopful of rapidly growing stock culture into 125 cc. of sterile medium contained in a 300 cc. Erlenmeyer flask. This was shaken mechanically for 24 hours at 25°, and then transferred to 500 cc. of medium in a 3 liter Fernbach flask. After standing for 24 hours at 25°, this culture, totaling 625 cc., was transferred to 3 liters of the medium for the preparation of dextran.

The preferred incubation time of 24 hours for the 625 cc. inoculum was adopted on the basis of experimental observations. When 24 hour inocula
were used, culture media for dextran production reached high maximum viscosities such as that of the typical Dextran A of Table II. When 36 or 48 hour inocula were used, as for Dextrans D and B, respectively, progressively lower maximum viscosities of the culture media were obtained (Table II). It appears that the incubation time for the 625 cc. inocula influenced the viscosity of these unbuffered culture media.

Preparation of High Viscosity Dextran—12 liters of medium were sterilized in a 20 liter Pyrex bottle which was equipped with a siphon for the aseptic withdrawal of test samples. Incubation was at 25°. The pH values for this preparation culture were 7.1 after inoculation, 4.95 at 24 hours incubation time, and 4.75 at 26 hours. At incubation times of 24 and 26 hours the absolute viscosity of the medium was 438 and 446 centipoises, respectively. The viscous material appeared to be homogeneously dispersed in the cloudy culture medium and did not settle out.

Supercentrifugation of the culture medium was started at the end of 26 hours incubation. The residue consisted largely of bacterial cells. Absolute ethanol to make 35 per cent by volume was stirred into the centrifugate, and the solution was again passed through the supercentrifuge to remove the remaining small amount of bacterial cells. The centrifugate was stirred mechanically while the ethanol concentration was made up to 50 per cent by volume. The dextran separated as a gummy mass from which the supernatant was decanted. The dextran was kneaded to remove mother liquors, and was washed three times by kneading with 50 per cent ethanol. It was then dissolved in 11 liters of water and precipitated by addition of an equal volume of ethanol. The silvery looking mass was again isolated, kneaded, and washed as before. This cycle of reprecipitation and washing was repeated twice more.

The dextran, redissolved in 2.5 liters of water, was precipitated by slowly adding 100 cc. portions of the solution to 500 cc. of absolute ethanol, which was agitated in a Waring blender. The precipitates were combined, collected on a filter, washed twice by resuspension in 4 liters of absolute ethanol, and filtered. The product was dried in vacuo over anhydrous calcium chloride at room temperature. The weight of the product (dry basis) was 361 gm., 25.3 per cent of the initial weight of sucrose, or 50.6 per cent of the glucose available from the sucrose. Dextran A was shown not to contain carbohydrates small enough to dialyze through Visking cellulose membranes. Other data on this product (Dextran A) are given in Table II.

Preparation of "Autolyzed" Dextran—The changes in pH and viscosity during the preparation of two "autolyzed" dextrans are given in Table I and data on the purified products are given in Table II. The time of incubation of the 625 cc. inocula was 48 hours for Culture Medium BC and
36 hours for Culture Medium DE. 6 liter quantities of culture media were used. At 33 hours incubation, when Culture Medium BC was slightly past its maximum viscosity, one-half of it was removed aseptically and the high viscosity control, Dextran B, was isolated. The remaining half of the culture medium was allowed to stand at 25° for a total of 503 hours. The product insoluble in 50 per cent ethanol, "autolyzed" Dextran C, was then isolated in the usual way. In Culture Medium DE, the high viscosity control, Dextran D, was isolated from half of the culture medium at the time of maximum viscosity, and the "autolyzed" Dextran E was isolated from the remaining half of the medium after a total incubation of 273 hours.

The viscosities of Culture Media BC and DE, although of markedly different maximum values, decreased to 20 centipoises in 90 to 100 hours. After this time, the rate of change in viscosity was about the same in both media. At about 200 hours incubation, the pH of the culture media had reached a steady value of 3.7, and from then on changes in viscosity were very slow. This seems to be a practical time to isolate "autolyzed" dextrans.

Preparation of Dextran in Presence of Calcium Carbonate—Except as otherwise stated, all conditions and manipulations were the same as have been described for unbuffered culture media.

The 625 cc. inoculum contained 2 per cent of calcium carbonate and was incubated for 48 hours at 25° with occasional shaking. When this was transferred to the medium for preparation of dextran, 2 per cent sterile calcium carbonate was also added and kept suspended by occasional swirling during incubation of the culture medium. After inoculation, this culture medium had a pH of 7.0 to 7.1, and a viscosity of 2 centipoises.

When the culture medium was not aerated, the pH values were 6.5 at 22 hours and 6.4 at 47 hours; the corresponding viscosities were 4 and 879 centipoises, respectively. At 50 hours, isolation of the dextran was started. The culture medium was diluted with about one-third its volume of water, and supercentrifuged. The pH of the centrifugate was adjusted to 4.4 with acetic acid, and absolute ethanol was added to give an ethanol concentration of 35 per cent by volume. The mixture was then passed twice through the supercentrifuge and the dextran, isolated in the usual way, gave a 24 per cent yield. Other data for this Dextran G are given in Table II. The low nitrogen and phosphorus contents indicate that the procedure for purification of this dextran was more efficient in removing bacterial cells than that described for high viscosity dextran.

When the culture medium was aerated, the viscosities at 42, 50, 66, and 72 hours incubation time were 29, 122, 580, and 768 centipoises, respectively. During this time the pH was near 5.8. After 90 hours, when the
viscosity was 786 centipoises and the pH was 6.0, the dextran was isolated. The yield was 22 per cent, and the relative viscosity of the dextran was 1.006.

**Effect of Sterilization on Decrease in Viscosity**—An experiment was conducted to determine whether a decrease in viscosity would occur in a dextran preparation medium in which bacterial and enzyme action had been stopped by autoclaving. The usual unbuffered medium was inoculated with a 625 cc. inoculum which had been incubated 44 hours. After 24 hours incubation, the pH was 4.65 and the viscosity was 171 centipoises. The medium was autoclaved at 15 pounds per sq. in. for 30 minutes, and cooled quickly. The pH was still 4.65 and the viscosity was 138 centipoises. The pH was adjusted to 4.2 with sterile butyric acid solution, and the mixture was kept at 25° for a time which is expressed as a continuation of the incubation period. The pH remained constant, and the viscosity at 50, 121, and 174 hours was 135, 129, and 125 centipoises, respectively. Although a slow change in viscosity occurred under these conditions, the viscosity of this solution at 174 hours incubation was roughly 10 times the viscosities observed at comparable times for media in which normal autolysis had occurred (see Table I).

**Effect of Variation of Medium**—When the usual unbuffered medium was supplemented with 0.5 mg. of manganous sulfate monohydrate per liter (15), the incubation times for the two inocula and for the preparation culture medium were 16, 9, and 6 hours, respectively. The purified dextran, obtained in 24 per cent yield, had a specific rotation of +200.1° and a relative viscosity of 2.255. By using an unaerated, calcium carbonate-buffered medium to which 1 mg. of manganous sulfate monohydrate per liter had been added, the incubation time was 13 hours for both of the inocula as well as for the preparation culture medium. The purified dextran, obtained in 27 per cent yield, had a specific rotation of +200.8° and a relative viscosity of 1.673. Extension of the incubation time resulted in inactivation of the bacteria in the unbuffered inocula and in a decrease in viscosity of both the buffered and the unbuffered culture media.

Substitution of the mineral constituents of Dunn et al. (16) (with only 0.1 the concentration of ferrous and manganous sulfates recommended) for the mineral constituents of our medium resulted in no significant increase in dextran production. Inclusion in the medium of corn steep liquor, autolyzed yeast, or Bacto-tryptone appeared to be of no advantage.

**Other Polysaccharide Fractions**—From the 50 per cent ethanolic dextran mother liquors, after supercentrifugation to remove a small amount of dextran, fractions insoluble in 65 and 75 per cent ethanol were successively precipitated. The fractions were purified and isolated as white powders in a manner analogous to that described for dextran.
The fractions from unbuffered "autolyzed" media (see Table I) were homogeneously water-soluble, and their yields totaled 5 per cent of the initial weight of sucrose in the medium; dialysis reduced their nitrogen and phosphorus contents from about 0.04 and 0.20 per cent, respectively, to 0.02 per cent, but did not effect any significant change in other properties. These fractions had $[\alpha]_D^{25} = +55^\circ$ to $+133^\circ$ (in 1 N sodium hydroxide, $C = 1$), relative viscosities of 1.060 to 1.134 (0.5 per cent concentration in water, 25$^\circ$), alkali numbers of 4 to 13, and contained combined fructose. Exposure in air having 100 per cent relative humidity at 25$^\circ$ converted them from a state which produced amorphous x-ray patterns to states from which x-ray diffraction line patterns characteristic of dextran were obtained (17).

The fractions from unbuffered, high viscosity culture media totaled about 4 per cent in yield. One fraction, isolated in 2.6 per cent yield, had $[\alpha]_D^{25} = -23^\circ$, an alkali number of 1, and produced only amorphous x-ray patterns (17). Another fraction, which became water-insoluble during isolation, gave a dextran x-ray line pattern without further treatment.

Fractions totaling 3.4 per cent in yield were obtained from a calcium carbonate-buffered culture medium. A fraction, obtained in 2.8 per cent yield, gave $[\alpha]_D^{23} = -54^\circ$ and an alkali number of 0.

Tests for ketose, which is assumed to be fructose, in these fractions were made by allowing some of the dry carbohydrate to stand in 85 per cent phosphoric acid at room temperature (18). Under these conditions, fructose, sucrose, inulin, and calcium 5-ketogluconate developed dark brown to black colors within 24 hours, but neither glucose nor dextran produced any color. Some fractions produced dark brown to black colors, and others produced light yellow to tan colors when tested in this way. The intensity of color appears to indicate the relative amount of combined fructose present. Further evidence of the presence of much fructose in fractions which produced dark colors was provided by their negative or low positive optical rotations.

Preparation of Water-Insoluble Dextran—For comparison with the watersoluble dextran from Leuconostoc mesenteroides NRRL B-512, the water-insoluble dextran from Leuconostoc mesenteroides NRRL B-523 was prepared. Our usual unbuffered sucrose medium was used. Because of the slowness of growth of the organism, 48 hours incubation was required. The culture medium became viscous with insoluble gelatinous particles. Microscopic examination revealed a heavy growth of non-capsulated bacteria, and discrete particles of gelatinous material. Microscopic examination revealed a heavy growth of non-capsulated bacteria, and discrete particles of gelatinous material.

The culture medium was diluted with an equal volume of water and centrifuged at 3300 R.P.M. The residue, which was insoluble in boiling water, was dissolved in 0.75 N potassium hydroxide. This solution was neutralized with acetic acid and supercentrifuged twice to remove bacteria.
Addition of an equal volume of ethanol precipitated the polysaccharide and rendered it insoluble in water. The precipitate was washed with 50 per cent ethanol and the polysaccharide, called Fraction J, was isolated in the usual manner as a coarsely fluffy product.

The supernatant from the diluted culture medium was supercentrifuged and an equal volume of ethanol was added. The precipitate, which swelled greatly in water but did not dissolve, was washed with 50 per cent ethanol and the polysaccharide, called Fraction K, was isolated from absolute ethanol.

The yields of Fractions J and K were 9 and 7 per cent, respectively, of the initial weight of sucrose. Fraction J gave \([\alpha]_25^0 = +208^\circ\) (in 1 N sodium hydroxide, \(C = 1\)). Both fractions gave negative tests for fructose in 85 per cent phosphoric acid. The percentage of nitrogen in Fraction J was 0.008, and in Fraction K 0.020.

**Analytical Methods**—Because anhydrous dextran is very hygroscopic, samples were equilibrated with moisture in a constant humidity room (50 per cent relative humidity at 25º) where all weighings were made. Under these conditions the moisture content of the dextrans was 12 to 13 per cent. The moisture content was determined on separate samples, and all results were calculated on a dry basis.

Dextran, which was always dried in vacuo over anhydrous calcium chloride at 25º before being equilibrated in 50 per cent relative humidity, was shown by ethoxyl determination to contain no ethanol.

Viscosity measurements were made with standardized Ostwald-Cannon-Fenske viscosimeter tubes, at 25º ± 0.03º. For measurements on purified dextrans, 0.5 per cent aqueous solutions were used after filtration through fritted glass funnels to remove traces of lint. For measurements on culture media, care was taken to obtain representative samples, and samples from calcium carbonate-buffered media were filtered through fritted glass funnels before use.

Optical rotations were read with the light from a sodium vapor lamp. Solutions in 1 N sodium hydroxide were used to avoid the opalescence which high viscosity dextrans gave in water solutions. However, specific rotations in water were only a few degrees lower than those in sodium hydroxide.

Measurements of pH were made with a glass electrode. Alkali number measurements were made by the method of Schoch and Jensen (19). The values are reproducible to ±0.3. Nitrogen analyses were made by the micro-Kjeldahl procedure, and phosphorus analyses by a modification of the method of Truog and Meyer (20).

**DISCUSSION**

**Viscosity and Solubility of Dextran**—Our results show that the maximum viscosity of culture media of *Leuconostoc mesenteroides* NRRL B-512 does
not give an accurate indication of the yield or viscosity of the pure dextran after its isolation. Dextrans with relative viscosities of 2.003 to 2.253 have been isolated in yields of 24 to 25 per cent from unbuffered culture media having viscosities of 73, 202, and 446 centipoises. These differences in viscosity of the culture media appear to be related to the age of the inoculum used. The purified dextrans showed no significant difference in any other of the observed properties. From a calcium carbonate-buffered culture medium with a viscosity of 847 centipoises, dextran having a relative viscosity of 1.719 was isolated in 24 per cent yield. The very high viscosities of culture media buffered with calcium carbonate do not appear to be due to the effect of calcium ions on dextran alone, as is indicated by viscosity measurements on purified dextrans in solutions of calcium acetate (see Table II, foot-notes).

The viscosities of the purified water-soluble dextrans do not correlate with their nitrogen contents. The slight differences in nitrogen content are believed to reflect variation in the efficiency of separation of bacteria from the dextran. Likewise, the solubilities of our dextrans are not related to their nitrogen contents, for the purified water-insoluble dextran from Leuconostoc mesenteroides NRRL B-523 has a nitrogen content as low as the water-soluble dextran from Leuconostoc mesenteroides NRRL B-512. It is inconceivable that the methods employed for the isolation and purification could have contributed to the solubility of the water-soluble dextran. The solubility and viscosity of these dextrans apparently are inherent properties of the polysaccharides rather than related to combinations of the dextran with protein, as postulated by Stacey (21-23).

Causes and Effects of Decrease in Viscosity of Culture Media—The decrease which occurred in the viscosity of culture media after the formation of dextran was complete appears to be caused mainly by autolysis. The change was almost, but not completely, stopped by heat sterilization of the culture media. This viscosity change was not dependent on the pH or on aeration in unbuffered media. Aeration of buffered media prevents it.

It is not yet known whether “autolysis” results in some selective structural change in dextran, or merely produces a random decrease in molecular size.

Stability of Dextran to Alkali—The alkali numbers in Table II show that Dextrans A, B, and D are not attacked when heated with 0.1 N sodium hydroxide solution, and that Dextrans C and E are only slightly attacked. From other reactions previously reported (2, 3, 9), evidence is available which also indicates the lack of reducing power in dextran. According to the interpretation of alkali numbers (19, 24), our preparations of dextran have very few, if any, reducing groups.

From this viewpoint it is interesting to consider possible modes of termination of dextran molecules which would result in the stability of dext-
Dextran from L. mesenteroides

Tran to alkali. An unterminated cyclic structure, such as is found in the Schardinger dextrins, would be stable to alkali (13), but a cyclic structure for the molecule as a whole would not be in accordance with the pronounced filiform characteristics of water-soluble dextran (8, 17). A chain structure terminated by a unit of glucose-1-phosphate (25), of fructose combined as in sucrose (19), of a hexahydric alcohol such as mannitol, or of gluconic acid (19, 24) would be stable to alkali. Glucose-1-phosphate has been reported to be formed from sucrose by Leuconostoc mesenteroides (26), but there is no evidence that it takes part in the synthesis of dextran (27, 28). The possibility that sucrose may be converted directly into dextran without formation of a simpler intermediate (28) could result in the dextran molecule having a terminal fructose unit. No additional evidence can be cited for terminal glucose units in an oxidized or reduced state.

SUMMARY

1. A method has been established for the preparation of water-soluble dextran products of uniformly high viscosities and of high purity from cultures of Leuconostoc mesenteroides NRRL B-512 on an unbuffered, un-aerated, sucrose medium. This method was dependent on the correlations that the development of maximum viscosity in the culture medium coincided with the end of dextran formation and that thereafter the viscosity of the culture medium and of the dextran decreased.

2. Purified dextrans which were isolated from culture media at their maximum viscosity were characterized by high viscosities; purified dextrans isolated from culture media after their maximum viscosity had been passed had lower viscosities.

3. Accompanying the changes in viscosity of the culture medium, the pH decreased from an initial value of about 7.0, through about 4.6 at the time of maximum viscosity, to a steady value of 3.7 when the viscosity had become very low.

4. Modification of the selected cultural conditions by aeration of the medium or by buffering with calcium carbonate, either singly or in combination, gave no increase in the yield or viscosity of dextran.

5. The solubilities of the water-soluble dextran from Leuconostoc mesenteroides NRRL B-512 and of the water-insoluble dextran from Leuconostoc mesenteroides NRRL B-523 appear to be inherent properties of the polysaccharides and are not related to their nitrogen contents.

6. In addition to dextran, levan was produced in relatively small amounts in cultures of the strain NRRL B-512.

The use of trade names in this paper does not necessarily constitute endorsement of these products or of the manufacturers thereof.
Grateful acknowledgment is made of the generous assistance and advice of Dr. Robert G. Benedict of the Fermentation Division, who isolated *Leuconostoc mesenteroides* NRRL B-512 from syrupy root beer, and who prepared many of the inocula for our experimental work. The authors wish to express their appreciation to Dr. William C. Haynes for the preparation of some inocula, to Mr. Cecil H. Van Etten and Mrs. Mary B. Wiele of the Analytical and Physical Chemical Division for the nitrogen and phosphorus analyses, and to Mr. C. S. Wise for the alkali number measurements.

**BIBLIOGRAPHY**

CORRECTIONS


On page 1461, Vol. 176, No. 3, December, 1948, the structure for tryptophan should read

\[
\begin{array}{c}
\text{\textbf{\textsf{\textbullet}}} \\
\text{H} \\
\text{\textsf{\textbullet}} \\
\text{CH}_2\text{CHCOOH} \\
\text{NH}_2
\end{array}
\]
PREPARATION AND CHARACTERIZATION OF DEXTRAN FROM LEUCONOSTOC MESENTEROIDES

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