FACTORS AFFECTING PRODUCTION AND CLARIFICATION OF DEXTRAN

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Dextran, a glucose polymer, has been known since the nineteenth century. It was sometimes encountered as a slime in large globular masses during the processing of cane and beet sugar, where it increased the viscosity of the sugar solution and retarded filtration and crystalization. When these globular masses were examined microscopically, they were found to be made up largely of chains of encapsulated cells which were later classified by Hucker and Pederson (1930) as belonging to the family *Coccaceae*, tribe *Streptococceae*, genus *Leuconostoc*, species *L. mesenteroides* and *L. dextranicum*. Niven *et al.* (1941) reported that a few strains of *Streptococcus salivarius* and *Streptococcus bovis* are also able to synthesize, from sucrose and raffinose, an insoluble carbohydrate which seems to be a dextran.

Conditions for the preparation of dextran were determined by Tarr and Hibbert (1931), who stated that sucrose was the only suitable carbohydrate substrate for the production of dextran. Many investigators have reported that certain accessory substances are necessary for the growth of *Leuconostoc* (Snell *et al.*, 1938, Bohonos *et al.* 1941, 1942; Gaines and Stahly, 1943; Whiteside-Carlson and Carlson, 1949a and 1949b). The addition of various substances have been shown to increase the yield of dextran (Carruthers and Cooper, 1936; Stacey and Youd, 1938; Bouillene, 1938; Daker and Stacey, 1939, Hassid and Barker, 1940; Stacey, 1942; and Stahly, 1943).

The enzymatic synthesis of dextran has been described by Hehre (1941, 1946 and 1951) and Hehre and Sugg (1942). Rapid formation and high yield of the enzyme were reported by Koepsell and Tsuchiya (1952). Certain cultural factors affecting dextransucrase production were studied by Tsuchiya *et al.* (1952). The yield of dextran produced by *Leuconostoc* from sucrose and the characteristics of dextran vary considerably with different strains.

The present report describes (a) effects of some factors on dextran production, (b) factors affecting enzymatic degradation of dextran through extended incubation, and (c) dextran opalescence and methods of clarification.

Material and methods

Active cultures of *L. mesenteroides* 683 and B-512 and *L. dextranicum* "elai"[1] were used in this study. For the dextran production two comparative media were used having the following composition per liter.

Medium No. 1. Sucrose 150.0 g., Peptone 2.5 g., Yeast extract 2.5 g., K_2HPO_4 5.0 g., NaCl 2.5 g., and a water extract of sugar refining charcoal 2.0 ml.

Medium No. 2. Sucrose 150.0 g., acid hydrolyzed casein 5.0 g., Yeast extract 1.0 g., K_2HPO_4 5.0 g., NaCl 2.0 g., and $MgSO_4$ 0.022 g.

Sterilization was effected by autoclaving at 15 lbs. pressure for 15 min.

Determination of pH. The pH measurements were made using a Fisher Titrimeter.

[1] *L. mesenteroides* 683, was received from Dr. Carl S. Pederson, New York State Agri. Exp. St., Geneva, N. Y. This culture was originally designated *Betacoccus arabinosaceus* No. 6, from the Orla Jensen collection. *L. mesenteroides* B-512 was acquired from Dr. Kenneth Raper, Northern Regional Research Laboratory, Peoria, Ill. *L. dextranicum* "elai" was obtained from the University of California.

Total Acids. A 10 ml. aliquot of fermented culture media was titrated against 0.1 N NaOH with phenolphthalein indicator. The number of millimoles of total acids was calculated from the volume of alkali used.

Precipitation of dextran, drying and viscosity measurements were carried out as reported previously (Hamdy, 1953).

EXPERIMENTAL RESULTS

Some Factors Affecting Dextran Production

Cultures and nutrients. To determine the relative yields of dextran from the three cultures under investigation, in two different media, six Erlenmeyer flasks, each containing 5 l. of media were inoculated with active culture. After incubation for 5 days, the fermented media were clarified and the dextran in each flask was precipitated, dried and weighed, and the percentage yields were calculated. The results recorded in table 1 indicate that the strain as well as the composition of the medium has an effect on the yield and that medium No. 2 is more satisfactory for the production of dextran than medium No. 1.

TABLE 1

Effect of medium and culture of Leuconostoc on the yield of dextran

Cultures	Percentage Yield*	
	Medium No. 1	Medium No. 2
L. mesenteroides 683	68.0	75.0
L. mesenteroides B–512	53.0	68.0
L. dextranicum "elai"	42.0	66.0

*Based on glucose available from sucrose

It was noticed that during the fermentation of *L. mesenteroides* 683, dextran settled to the bottom of the flask in a very viscous layer, which contained some dextran in the form of hard masses or clumps. When the flasks were shaken manually, all the viscous layer, except the clumps, became uniformly dispersed. Upon standing for several hours, the viscous layer reappeared. This property might be of value, especially in large scale production. *L. mesenteroides* B-512 produced a polymer which was much easier to handle, i.e., it was clarified and precipitated more easily than that of 683 and "elai". *L. dextranicum* "elai" produced a type of dextran which was very difficult to clarify and to dissolve in distilled water. These gross differences in properties may be due to the different molecular structures of the polymers produced.

Extent of incubation. The dextran fermentation was followed during prolonged incubation using different strains and the two media. In the previous experiment it was noticed that the fermented liquor of *L. mesenteroides* 683 contained some clumps of dextran. In order to avoid error in sampling during the course of the fermentation, small Erlenmeyer flasks containing media were used so that at various time intervals analyses were carried out using the entire contents of the flasks. Several flasks of each medium were inoculated with strains 683 and B-512, respectively. Analyses were made to study the effect of extended incution on total acid production, pH, the dextran yield and the viscosity of 6 percent aqueous solution of the dextran formed.

Duplicate experiments were conducted and the average results are plotted in figures 1, 2 and 3, from which the following information was obtained.

(1) A sharp drop in the pH of all the fermented media occurred during the first 40 hr. of incubation, followed by a further slight decrease up to 110 hr. after which a steady value of 3.8 was maintained.

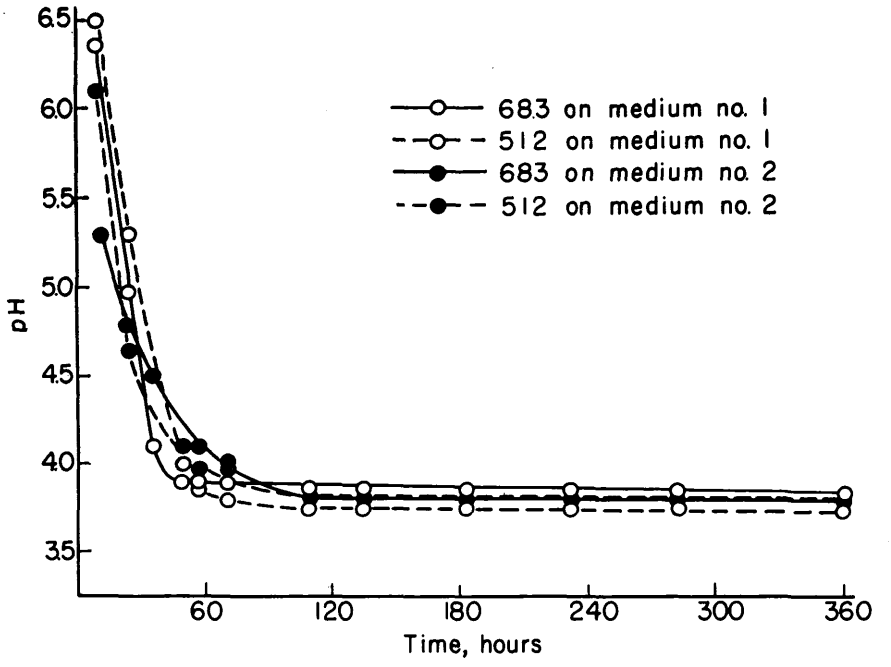

FIGURE 1. Relation between time and pH during the dextran fermentation, using different strains on comparative media.

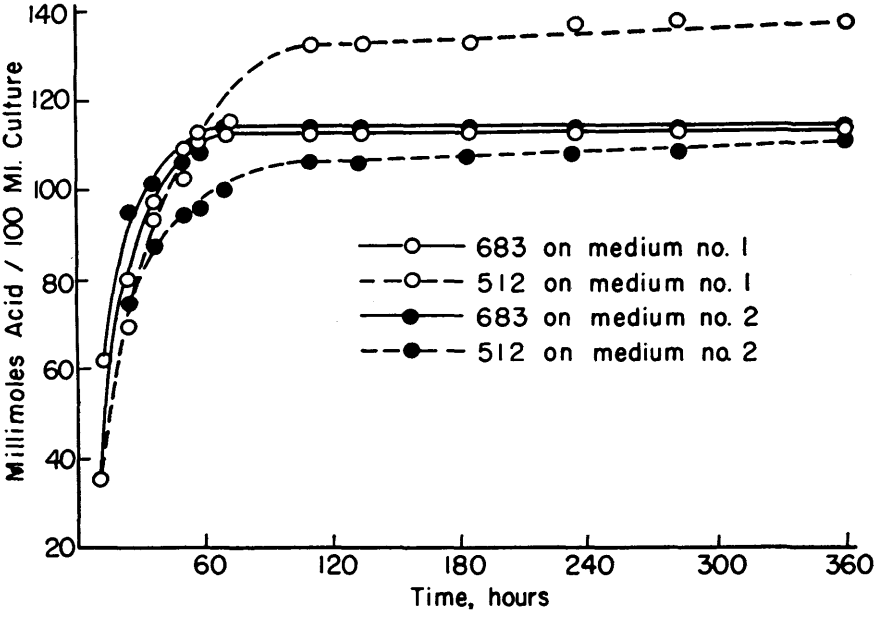

FIGURE 2. Relation between time and total acids in dextran fermentation, using different strains on comparative media.

(2) The amount of total acids increased, following the extended incubation, up to from 72 to 110 hr., after which no significant change was detected.

(3) The relative viscosities of 6 percent aqueous solutions of dextran reached a maximum at approximately 40 hr. after which the viscosities decreased rapidly

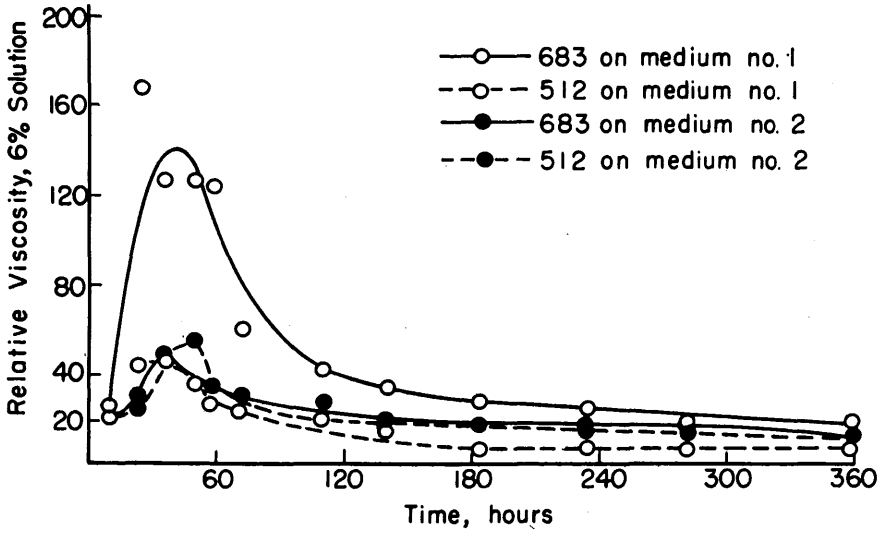

FIGURE 3. Relation between time and relative viscosity of 6 percent aqueous solution of dextran produced by different strains on comparative media.

TABLE 2

Effect of extended incubation on the percentage yield of dextran*

Incubation time (hours)	*L. mesenteroides 683*		*L. mesenteroides B-512*	
	Medium No. 1	Medium No. 2	Medium No. 1	Medium No. 2
12	13.2	23.8	33.3	28.2
24	46.6	32.2	36.6	35.0
36	48.8	68.0	44.4	46.0
49	68.8	65.0	53.2	65.0
58	58.4	64.0	45.0	68.0
72	53.2	60.0	35.0	65.0
110	52.2	59.6	30.0	63.0
135	51.0	58.0	30.0	62.0
185	50.0	56.0	30.0	60.0
234	50.0	55.0	27.0	58.0
282	50.0	52.0	25.4	56.0
360	50.0	50.0	23.2	51.0

*Percentage yield based on the glucose available from sucrose.

but then slowed down after 110 hr. When the variations of pH and of relative viscosity during incubation are compared, it is evident that a decrease in relative viscosity ensued after the pH dropped below 4.0.

(4) The yield of dextran reached a maximum value at 49 hr. incubation time (table 2). In general this was followed by a marked decrease up to 72 hr., after which a less rapid decrease in yield was noted. This may be explained on the basis of degradation of dextran through incubation, which forms low molecular

weight polymer that is not precipitated when 60 percent isopropyl alcohol is added for the precipitation of the dextran.

Mechanical shaking. The two media and *L. mesenteroides* 683 and B-512 were used. The procedure for the preparation of the media, their inoculation and the serial analysis was the same as in the previous experiment except that the flasks were placed in a mechanical agitator (shaker) during the incubation period. The following results were obtained:

(1) A drop in the pH value occurred during the first 53 to 75 hr. after which no further decrease occurred.

(2) There was an increase of total acids up to 53 hr. after which no measurable increase was detected.

(3) There was a slight increase in the relative viscosity of the dextran produced by *L. mesenteroides* 683 followed by a slight decrease.

TABLE 3

Effect of varying concentrations of sucrose, glucose and sodium chloride on dextran production and its viscosity

Flask No.	Percent Concentration			% yield	Relative Viscosity 6%
	sucrose	glucose	sodium chloride		
1	10	—	—	69.2	52.8
2	15	—	—	70.4	48.0
3	20	—	—	65.9	36.9
4	25	—	—	62.4	25.0
5	30	—	—	58.7	18.2
6	35	—	—	64.2	8.1
7	5	5	—	70.2	51.0
8	5	10	—	65.3	48.2
9	5	15	—	48.0	32.4
10	5	20	—	40.0	35.0
11	5	25	—	37.6	34.8
12	5	30	—	32.0	31.4
13	10	—	0.5	54.3	40.2
14	10	—	1.0	37.8	39.8
15	10	—	2.0	32.3	32.2
16	10	—	3.0	21.0	20.7
17	10	—	4.0	18.9	20.3
18	10	—	5.0	15.2	18.9

(4) The yield of dextran by *L. mesenteroides* B-512 was negligible and by strain No. 683 was very poor in both media indicating that mechanical shaking is unfavorable for dextran production.

Periodic neutralization. Two large flasks each containing 10 l. of medium No. 2 were inoculated with *L. mesenteroides* B-512 using 5 percent active inoculum, which had been aseptically neutralized to pH 7.2 prior to its addition to the flasks. The flasks were incubated for 50 hr. and examined every 3 hr. by aseptically withdrawing an aliquot for pH measurements.

Sterile 5 N NaOH was added to one flask only, to readjust the pH to 6.5 while the other flask was left to proceed in normal fermentation. This neutralization was repeated every 3 hr. for the first 24 hr. of incubation.

The dextran was recovered, dried and weighed. This experiment was repeated and the average results are given below:

(1) The relative viscosity attained in the neutralized culture was significantly greater than in the unneutralized culture.

(2) Eighty-five percent of the theoretical yield of dextran was obtained in the neutralized but only 65 percent in the un-neutralized culture.

(3) A 6 percent aqueous solution of the dextran from the neutralized culture had a relative viscosity of 45.0 while that of the untreated culture was 23.0.

Concentrations of sucrose, glucose and sodium chloride. Eighteen 500 ml. Erlenmeyer flasks each containing 250ml. of medium No. 2 were inoculated with *L. mesenteroides B-512* and incubated for 48 hr. at 25°C. Six of these flasks contained the following concentrations of sucrose: 10, 15, 20, 25, 30 and 35 percent. Six flasks contained 5 percent sucrose and glucose in concentrations of 5, 10, 15, 20, 25 and 30 percent. The remaining six flasks each contained 10 percent sucrose plus the following concentrations of NaCl; 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 percent.

Precipitation and recovery of the dextran followed by determinations of yield and relative viscosity of 6 percent aqueous solutions were then made. The results in table 3 indicate:

TABLE 4

The effect of neutralizing and autoclaving the culture medium upon the type of dextran formed, as shown by viscosity measurements of a 6 percent solution

Incubation Time	Relative Viscosity		
Hours	Culture I	Culture II	Culture III
48	46.0*	52.5*	35.0
48	40.2**	38.0**	35.0
72	33.5	35.2	31.5
96	30.0	28.6	27.4
120	29.0	31.8	24.0
144	27.4	31.6	20.5
168	30.0	30.0	17.3
192	29.0	33.0	15.0
216	28.0	31.9	11.0
240	27.0	30.5	10.2
264	26.2	29.0	9.5
288	27.0	29.2	9.0
312	27.2	31.0	8.8

Culture I Neutralized and Autoclaved
Culture II Autoclaved
Culture III Normal Fermentation
*Before Autoclaving
**After Autoclaving

(1) The concentration of sucrose has relatively little effect upon the percentage dextran yield but has a pronounced effect upon the molecular weight of the dextran formed as measured by the viscosity of 6 percent aqueous solutions.

(2) Varying the concentration of glucose and sodium chloride had a marked effect upon the yield of dextran obtained from 10 percent sucrose and a lesser effect on the viscosity of 6 percent solutions; the higher the concentration of these materials, the lower were the yield and the viscosity of the dextran.

Enzymatic Hydrolysis of Dextran

Confirmation of enzymatic mechanism. It has been shown that degradation of dextran can be accomplished through extended incubation of the culture medium (Hamdy, 1953). Jeanes *et al.* (1948) reported that this hydrolysis is due to "autolysis of the *Leuconostoc* cells". The following experiments were conducted to investigate this mechanism and to study some factors affecting the degradation of dextran upon extended incubation of the culture.

Three flasks, each containing 1500 ml. of medium No. 2, were inoculated with *L. mesenteroides* B-512 and incubated for 48 hr. The pH of the fermented cultures was measured and found to be 4.0. One culture was neutralized with 5 N

NaOH and autoclaved for 20 min. at 15 lbs. The second culture was sterilized by autoclaving without prior neutralization. The third culture was allowed to proceed normally. Aliquot portions were taken aseptically from each flask every 24 hr. The dextran was precipitated and its relative viscosity was measured in a 6 percent aqueous solution. The results recorded in table 4 indicate that heat sterilization destroyed the hydrolytic component in the fermented media and that a slight decrease in viscosity, upon extended incubation, was noted in both cultures I and II.

TABLE 5

The effect of removal of bacterial cells by filtration of the culture medium upon the degradation of dextran

Time (hours)	Relative Viscosity	
	Filtered	Unfiltered
48	21.4	20.4
72	21.4	16.5
96	21.0	14.2
120	21.0	8.0
144	20.8	6.5
168	21.3	4.2
192	21.0	3.6

TABLE 6

The effect of temperature on the enzymatic degradation of dextran as determined by viscosity measurements of the culture median

Incubation Time (Hours)	Temperature of Incubation C					
	7	20	30	37	45	50
	Relative Viscosity					
0	23.2	23.6	25.4	24.8	28.8	22.2
24	22.6	15.3	15.8	19.6	25.6	20.0
48	22.0	11.0	11.4	16.1	23.4	19.0
72	20.9	10.0	10.2	16.0	22.5	18.0
96	19.0	8.3	8.5	15.2	20.6	17.5
120	18.0	6.8	8.3	15.0	20.4	15.3
144	17.0	6.6	8.0	14.0	20.0	14.7
168	16.0	6.2	7.7	13.6	19.6	14.4
192	15.4	6.0	7.2	13.0	19.4	14.2
216	15.2	6.0	7.0	11.4	19.4	14.0

A further evidence for enzymatic degradation was demonstrated by an experiment in which two liters of medium No. 2 were inoculated with *L. mesenteroides* B-512 and incubated for 48 hr. after which time the fermented medium was divided into two parts; one part was passed through a Seitz filter while the other was left for continued incubation. The degradation was followed every 24 hr. by viscosity measurements of the culture media. The results in table 5 show that sufficient quantity of the hydrolytic enzyme is not present in the culture filtrate after 48 hr. incubation to produce a detectable degradation of the dextran, and further indicate that the hydrolytic enzymes are probably liberated upon autolysis of the bacterial cells.

When it was found that degradation of dextran through extended incubation was apparently due to enzymes liberated upon autolysis of cells, it was decided to investigate factors affecting this degradation.

Effect of temperature. Six flasks each containing 1500 ml. of medium No. 2, were inoculated with *L. mesenteroides* B-512 and incubated until maximum vis-

cosity had been reached. Then the effect of temperature on degradation was studied by incubating the flasks at 7°, 20°, 30°, 37°, 45°, and 50°C, respectively. Viscosity measurements of the culture medium were conducted at 25°C to follow the depolymerization. The results in table 6 indicate that the most favorable temperature for the enzymatic hydrolysis seems to be in the range of 20° to 30°C.

Effect of sucrose concentration. Five flasks, containing ingredients of medium No. 2, except for sucrose which was added in concentrations of 5, 10, 15, 20 and 30 percent, respectively, were inoculated with *L. mesenteroides* B-512 and incubated for a period of 240 hr., approximately 192 hr. after maximum viscosity

TABLE 7

Effect of sucrose concentration on the molecular size and enzymatic degradation of dextran, as shown by viscosity measurements of 6 percent aqueous solutions of dextran formed by L. mesenteroides B-512

Incubation Time (Hours)	Relative viscosity of 6 percent of dextran produced in the following sucrose concentrations				
	5%	10%	15%	20%	30%
48	68.1	55.7	47.1	32.1	24.0
72	56.2	52.6	41.2	26.0	18.0
96	54.3	48.2	34.5	21.8	12.0
120	44.1	46.5	27.0	12.0	8.4
144	43.9	35.2	22.0	8.4	6.2
168	43.9	34.8	18.0	6.0	5.9
192	42.7	34.3	16.2	6.2	5.7
216	41.8	34.1	15.8	6.0	5.2
240	41.0	32.0	12.0	5.7	4.6

TABLE 8

Effect of strains and species of Leuconostoc on the enzymatic degradation of dextran, as shown by relative viscosity measuremenls of the culture media

Incubation Time (hours)	*Leuconostoc* cultures used		
	B-512	683	elai
48	65.2	306.0	69.6
72	41.9	187.5	69.4
96	21.5	135.0	69.0
120	18.1	113.4	66.0
144	16.8	101.1	66.3
168	14.2	96.7	65.2
192	12.7	94.3	67.8
216	11.3	93.3	66.5
240	10.8	92.0	67.0

had been reached. The enzymatic degradation was followed by viscosity measurements and the results (table 7) show that the sucrose concentration has little effect upon the rate of enzymatic degradation while it has a striking effect on the dextran produced. The greater the sucrose concentration, the smaller is the molecular weight of dextran synthesized and degraded accordingly.

Effect of culture. Two strains of *L. mesenteroides*, B-512 and 683, and *L. dextranicum* "elai" were used. Each culture was inoculated into a one liter flask containing 500 ml. of medium No. 2. After 48 hr. incubation the relative

viscosities of the culture media were determined. Incubation was extended an additional 8 days during which time the degradation was followed by relative viscosity measurements of the culture media. Table 8 indicates that strains B-512 and 683 showed considerable enzyme degrading activity while *L. destranicum* "elai" apparently did not. However, the lack of decrease in relative viscosity observed for *L. dextranicum* "elai" cannot be interpreted as indicating the lack of enzymic degrading activity since fractionation of the recovered dextran revealed that hydrolysis does occur as demonstrated by the recovery of a large fraction of low molecular weight polymer. Further investigation should be made before an explanation may be given for the anomalous viscosity behavior.

Effect of aeration. Two flasks, each containing one liter of medium No. 2. were inoculated with *L. mesenteroides* B-512 and incubated for 24 hr. One flask was then aerated during enzyme degradation using a moderate flow of sterile air while the other was not. Degradation of dextran was followed by viscosity measurements of the culture media. The results (table 9) indicate that aeration has no significant effect on the enzymatic hydrolysis of dextran.

TABLE 9

Effect of aeration on the enzymatic degradation of dextran as shown by the relative viscosity of the culture medium using L. mesenteroides B–512

Incubation Time (hours)	Relative viscosity of culture medium	
	Aerated	Stationary
24	25.6	26.4
48	15.3	16.7
72	11.0	11.5
96	10.0	10.8
120	8.3	8.7
144	6.4	6.8
168	6.0	5.8
192	5.6	5.4

Clarification of Dextran

Dextran may be water-soluble, partially soluble or insoluble depending upon the strain of the organism which produces it. *L. mesenteroides* B-512 and 683 produce water-soluble polymers while *L. dextranicum* "elai" produces a partially soluble dextran.

An aqueous solution of a water-soluble dextran has a faint opalescence which varies greatly from a white cream color to a blue tinge. This opalescence is undesirable for a plasma volume expander. The following experiments were conducted to investigate the cause for this opalescence and to establish a rapid, reliable procedure for the dextran clarification.

Dextran opalescence. Stained smears and hanging drop slides were made from an aqueous solution of unhydrolyzed dextran produced by *L. mesenteroides* 683. Bacterial cells, which were found to be present, were counted and an average value per field was determined. The aqueous solution was centrifuged for several hours, after which a noticeable decrease in the opalescence was detected. The supernatant was decanted and the white residue recovered, from which stained smears and hanging drop slides were made. Bacterial counts were performed from both preparations and an average value per field was determined and the following information was obtained:

(1) The supernatant had a bacterial content considerably lower than the original aqueous solution of dextran.

(2) The residue was found to be mostly bacterial debris with particle diameters in the size range, 0.2 to 0.5 μ. In comparison, the normal cells of *Leuconostoc* cultures have diameters in the range of 0.8 to 1.0 μ.

(3) When these bacterial cells were resuspended in water, a white opacity was noticed comparable to that of the original aqueous solution of dextran. It can be stated that the opalescence was mostly due to the bacterial debris.

Methods of clarification

(A) *Use of super-cell and norit.* Activated charcoal (norit) was added to a 5 percent aqueous solution of crude dextran; the mixture was heated to 80°C and filtered while hot through a pad of super-cell in a Buchner funnel under vacuum. Various thicknesses of the super-cell pad were used to establish the most efficient thickness. Clarification was accomplished, but it was noticed that the super-cell adsorbed a considerable amount of dextran from the solution.

(B) *Clarification during acid hydrolysis.* One hundred twenty-five grams of crude dextran were partially hydrolyzed in 2 l. of 0.1 N HCl at 60°C in a water bath. The hydrolysis was followed by viscosity measurements and was stopped by neutralization when the relative viscosity reached a value of 2.8. The solution was filtered through a Berkefeld filter and the dextran was precipitated. It was noticed that the opacity of the dextran solution was markedly reduced during hydrolysis and completely absent after filtration.

(C) *Filtration of dextran.* Seitz and Berkefeld filters were used to eliminate the bacterial cells from the aqueous solution of 6 percent crude dextran. It was found that a repeated filtration was necessary to clear the dextran solution as some of the bacterial debris passed through the filter.

The fermented culture medium was diluted with distilled water, using 20 percent by volume, and filtered through large Seitz filters (capacity—2 l.). The dextran was precipitated and it was found that an aqueous solution of this dextran was clear. This method proved to be quick and efficient.

DISCUSSION

The production of the high molecular weight polysaccharide dextran, for use as a plasma expander, has been given considerable attention by many investigators. It has been shown in this investigation that there are many factors involved in this fermentation. The selection of the appropriate culture and maintenance of its nutritional requirements; i.e., amino acids, vitamins, and minerals, were found to be highly significant factors. *Leuconostoc mesenteroides* B-512 and medium No. 2, which contained other than sucrose, hydrolyzed casein, yeast extract, potassium monohydrogen phosphate, sodium chloride and magnesium sulfate, gave a high yield of dextran, which was easily clarified and precipitated as a very fine powder. The periodic neutralization early in the fermentation was found to increase the yield of this high molecular weight polymer. This may be explained on the basis of the optimum pH for production and activity of the dextransucrase enzyme.

Koepsell and Tsuchiya (1952) established that the optimum pH for the production of the enzyme dextransucrase by *L. mesenteroides* B-512 ranges from 6.5 to 7.0 in the growing culture and that dextran synthesis, in the presence of the enzyme is optimum at pH 5.0 to 5.2. It seems that periodic neutralization to keep the pH around 6.5 during the initial hours of the dextran fermentation, favors the enzyme production, which, in turn, results in a higher yield of the synthesized dextran, especially when the pH drops to 5.0 to 5.2. In contrast, the regular fermentation, where the pH drops to 6.2 within 6 hr. and then to 3.9 after 24 hr., produces less enzyme, resulting in a decreased activity for dextran production. The higher viscosity of six percent aqueous solutions of dextran

from neutralized as compared with unneutralized fermentations may be due to the more favorable conditions for dextran synthesis in the neutralized media which led logically to higher yields with higher average molecular weight.

The decrease of viscosity of dextran during extended incubation of the culture media, appears to be caused mainly by one or more thermolabile endoenzymes, probably liberated upon the death and autolysis of the *Leuconostoc* cells. The most favorable incubation temperature for this degrading enzyme was found to be in a range of 20° to 30°C. Sucrose concentration, and aeration, seem to have very little effect upon the activity of the degrading enzyme. The molecular weight distribution studies on this enzyme degraded polymer (Hamdy, 1953) revealed a wide range of molecular sizes and a very low yield of the desired molecular weight dextran, indicating the impracticality of using this method commercially.

An interpretation of the dextran opalescence based on the results of this investigation is that the proteins of bacterial cells of *Leuconostoc* which are intimately associated with the polysaccharide and imbedded within the mass of dextran become denatured on the addition of alcohol during the precipitation process and the resulting cell fragments are virtually impossible to remove by supercentrifugation or filtration. A definite relationship existed between the amount of opalescence and the amount of cellular debris. When the fermented medium was first diluted with water to decrease its viscosity and then passed through bacterial filters followed by alcohol precipitation of the dextran, the resulting product in aqueous solution was entirely free of opalescence. No appreciable loss of dextran occurred due to adsorption, as was the case when supercell filtration was used.

SUMMARY

1. Dextran production was found to be markedly affected by various factors, among which were culture used, nutrients available, incubation period, periodic neutralization early in the fermentation, mechanical shaking and sucrose concentration.
2. Medium No. 2, which contained acid hydrolyzed casein, yeast extract, potassium monohydrogen phosphate, magnesium sulfate and sodium chloride, was found to meet the desired nutritional requirements for growth and production of dextran.
3. Mechanical shaking is unfavorable for dextran production.
4. Periodic neutralization of acidity early in the dextran fermentation resulted in higher yields of high molecular weight dextran.
5. Increasing the concentration of sucrose has little effect upon the yield but results in decreasing greatly the molecular weight of the dextran formed, while increasing the concentration of glucose and sodium chloride resulted in decreasing the yield and very little effect on the molecular weight of the dextran.
6. Degradation of dextran through extended incubation was found to be mainly due to an endoenzyme or enzymes liberated upon the death and autolysis of the bacterial cells of *Leuconostoc*.
7. A range of temperatures from 20° to 30°C was found to be most favorable for the activity of the degrading enzyme.
8. Sucrose concentration as well as aeration had little effect on the enzymatic depolymerization.
9. The enzymatic degradation varied with the strain and species of *Leuconostoc*, probably due to the type of dextran formed by the different cultures and its susceptibility to the influence of the degrading enzyme.
10. Dextran opalescence in aqueous solution was found to be due to the bacterial cells and their cellular debris. Elimination of this opalescence was accom-

plished by Seitz or Berkefeld filtration of the fermented culture medium after it had been diluted, if necessary, with distilled water to decrease its viscosity.

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