FACTORS AFFECTING THE DEGRADATION PROCESSES FOR DEXTRAN

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The usefulness of dextran as a plasma volume expander is highly dependent upon its molecular weight and homogeneity. Grönwall and Ingelman (1945) reported that the molecular weight of the native dextran is in the order of many millions and that its use in this form will cause injurious reactions.

Ingelman and Halling (1949) found that the high molecular weight dextran enhances the sedimentation tendency of the red blood corpuscles and that the lower the molecular weight of the polymer, the less is this action.

In the production of such a material for use as a blood expander, it is necessary either to control the fermentation to produce the desired molecular size as reported by Hehre (1953), Koepsell *et al.* (1953), *Nadel et al.* (1953), Spendlove (1953) and Tsuchiya *et al.* (1953) or degrade the high molecular weight dextran as established by Grönwall and Ingelman (1945) (1948) (1953), Ingelman (1948), Lockwood *et al.* (1951), Renfrew and Cretcher (1949), Stacey (1951), Stacey and Pautard (1952), Stoycos (1954), Tsuchiya *et al.* (1952), Whiteside-Carlson and Carlson (1952), Wolff *et al.* (1953 and 1954), to a value where the majority of the degraded polymer will have molecular weights in the order of the plasma proteins (about 75,000 ± 25,000) as reported by Pulaski (1952).

This investigation was concerned with the study of the factors affecting the production of degraded dextran and the molecular weight distributions obtained using different procedures of depolymerization, namely, acid hydrolysis, enzyme degradation and ultrasonic vibration.

MATERIALS AND METHODS

Preparation of Dextran Samples

Culture. *Leuconostoc mesenteroides* N.R.R.L. B–512 used. Dextran produced by this organism has been previously characterized by Wilham *et al.* (1953). The culture was kept under refrigeration at 6° C and transfers were made every three weeks. For dextran production the culture was activated by several passages through the same media as used for the fermentation procedure, allowing 12 hr. incubation between each inoculation.

Media. For the stock culture, the medium used had the following composition per liter: sucrose 40.0 g., yeast extract 1.0 g., acid hydrolyzed casein 5.0 g., K_2HPO4 5.0 g., NaCl 2.0 g., $MgSO_4$ 0.022 g. and agar 15 g. For the production of dextran, the previous medium was used except that the sucrose content was 150.0 g. and agar was omitted.

Sterilization, inoculation and incubation. Sterilization of media, effected by autoclaving at 15 lbs. for 15 min., was followed by cooling and inoculation with the active culture. For the production of high molecular weight dextran, the culture medium was incubated at 25° C for 3 days, after which the dextran was precipitated.

Precipitation of dextran. The fermented liquor was diluted, necessary to decrease viscosity, with distilled water and filtered through large bacterial filters (Seitz or Berkefeld). Isopropyl alcohol was added to the filtrate to give 60 percent by volume, and the mixture was kept in the refrigerator for 20 hr. The supernatant solution was then decanted and the concentrated gel phase of the dextran was added drop-wise to 99 percent isopropyl alcohol while

the alcohol was stirred vigorously in a Waring Blendor. By use of this procedure the gummy stage was avoided and the dextran was precipitated as a very fine flocculent powder, which was separated from the alcohol by filtration. Mechanical stirring of the precipitated dextran was repeated in 99 percent isopropyl alcohol after which the dextran was filtered and dried in vacuum over anhydrous calcium chloride at 25° C.

Measurement of dextran concentration. The dextran solutions for viscosity measurements were made up to known concentrations in volumetric flasks by adding a known weight of dried dextran and filling to the mark with distilled water. The concentration of each solution was checked with the Bausch and Lomb dipping refractometer.

Depolymerization Methods

Enzymatic degradation. Production of enzyme degraded dextran B–512 is based on incubation of the fermented media, extended beyond the time for maximum viscosity, as reported by Jeanes *et al.* (1948). A six liter Erlenmeyer flask, containing 5.5 lit. of sterile medium, was inoculated with an active culture of *L. mesenteroides* B–512 using 5 percent inoculum. The flask was incubated for 3 wk., during which time it was shaken at intervals of 3 days. The fermented medium was diluted and clarified by passage through a bacterial filter. The degraded dextran was recovered by precipitation using isopropyl alcohol.

Acid hydrolysis. Partial acid hydrolysis of a 6 percent solution of high molecular weight dextran was accomplished using 0.1 N HCL at 60° C in a water bath. The hydrolysis was followed by measuring the relative viscosity of the product. The degradation was stopped by cooling and neutralization with 5 N NaOH at relative viscosities between 2.0 and 3.0. The partially hydrolyzed dextran was precipitated as previously described.

Ultrasonic depolymerization. In this investigation two types of apparatus were used, a model U–300 Ultrason and a Radio Sonorator model RS–2 operating at frequencies of 450,000 and 250,000 cycles per second, respectively.

For the degradation process, the aqueous solution of dextran was subjected to the ultrasonic waves. Irradiation was made in 15 min. periods since it was necessary to stop the ultrasonic treatment to cool the quartz crystal.

Fractional Precipitation

Degraded dextran consists of various molecular weights. Fractionation experiments using isopropyl alcohol were conducted to investigate the molecular weight distribution and to obtain homogeneous dextran fractions for physicochemical studies. The optimum conditions for fractional precipitation of dextran with isopropyl alcohol were studied and a procedure was developed for the separation of the two liquid phases which are formed when alcohol is added to dextran solution, up to alcohol concentration of 50 percent by volume.

(1) An aqueous solution of low concentration of dextran (1–3 percent) was used, since, in a series of fractionation experiments, it was found that the lower the dextran concentration, the more efficient was the fractionation procedure.

(2) Under mechanical stirring, the desired amount of isopropyl alcohol was added slowly, while the temperature was kept at 10 to 12° C. This temperature was chosen to permit the use of lower alcohol concentrations in the precipitation of dextran.

(3) The temperature of the solution was raised to 25 to 30° C to redissolve the precipitated fraction and any low molecular weight dextran which might be precipitated by momentarily high local concentration of alcohol occurring during step 2. Then the clear solution was cooled to 10 to 12° C and allowed to stand overnight at this temperature.

(4) To separate the gel phase from the supernatant solution, the system was cooled quickly to 0 to 2° C to cause the gel phase to freeze and adhere to the

bottom of the bottle. "A cloudiness or a pseudo-fraction appeared in the upper liquid phase on cooling at 4° C but did not settle out. On subsequent warming of the separated supernatant solution to 10 to 12° C, the cloudiness disappeared." The upper liquid phase, which does not freeze because of its higher alcohol content, was decanted easily, insuring a clean-cut separation.

(5) Lower molecular weight fractions were obtained by repeating steps 2 through 4 on the supernatant solutions from the preceding fractionations. By measuring the volume of the supernatant solution, the amount of alcohol necessary to precipitate the next fraction was calculated.

(6) Each fraction was redissolved in warm water (50° C) and the dextran samples were then precipitated with isopropyl alcohol, dried and weighed. Each fraction was sub-fractionated in the same manner as outlined in steps 1 through 5.

Viscosity Measurements

The viscosity measurements were made at 25° ± 0.05° C using a No. 50 Ostwald-Fenske viscosimeter and dextran concentrations between 0.2 and 0.6 percent. Specific viscosities were obtained from the relationship,

$$\eta sp = \eta/\eta_o - 1$$

where

η = viscosity of dextran solution
η_o = viscosity of distilled water

Intrinsic viscosities, $[\eta]$ in deciliters/gram, were obtained by plotting the reduced viscosity, $\eta sp/c$, versus concentration, c, in percent by weight, and extrapolating the reduced viscosity values to zero concentrations.

Light-Scattering

The instrument used in this investigation was the B–S light-scattering photometer designed by Brice *et al.* (1950). The refractive index increments were determined with the B–S differential refractometer. Slight amounts of dust particles scatter light considerably and give rise to erroneous results. Consequently, the dextran solutions were filtered through an ultrafine sintered glass filter into a semi-octagonal cell and light-scattering measurements were made at 0, 45, 90 and 135 degrees to the emergent beam for both blue and green light to obtain values for both turbidity, τ, and dissymetry, Z. The weight-average molecular weight was then calculated using the following relationship which was established by Debye (1941) and Debye *et al.* (1946):

$$H\,(c/\tau) = 1/M + 2Bc$$
$$H\,(c/\tau)_{c=o} = 1/M$$

Where H is the refraction constant, M is the solute weight-average molecular weight, B is a constant depending on the solvent and "c" is the concentration in grams per milliliter. The turbidity, τ, was made using dextran concentrations between 0.03 and 0.6 percent.

RESULTS

Enzymatic Degradation

The following studies were carried out to investigate the molecular weight distribution of enzyme degraded dextran (Hamdy, 1953) previously described by Jeanes *et al.* (1948) as "autolyzed" dextran.

Two liters of 2 percent aqueous solution of that dextran with an $[\eta]$ value of 0.33 were subjected to fractional precipitation. Five main fractions were obtained (E_1–E_5) at the following alcohol concentrations: 32.5, 37.5, 42.5, 47.5 and 55.0 percent. The percentage yields of the fractions were calculated and their intrinsic viscosities were determined. This experiment was duplicated with almost the same results and the averages are recorded in table 1. The first two fractions E_1 and E_2 were further separated into six and four subfractions, respectively.

Intrinsic viscosity measurements were performed on all the fractions and subfractions while light-scattering experiments were carried out on some of each, and the results are presented in table 2 indicating a wide range of molecular weights.

Acid Hydrolysis

Studies using partial acid hydrolysis were made to investigate the effects of certain factors on the degradation and the molecular weight distribution.

Effect of acid concentration and time. A 6 percent solution of high molecular weight dextran (B–512) was hydrolyzed in different concentrations of hydrochloric acid (0.05, 0.10, 0.15 and 0.20 N) in a water bath at 60° C. The hydrolysis was followed by relative viscosity measurements of the dextran solution at different

TABLE 1

Results of fractional precipitation of two liters of 2 percent aqueous solution of enzyme degraded dextran B-512 with an [η] value of 0.33

Fractions	% IPA	wt. of fractions in g.	% yield*	[η]	Mol. Wt.**
E_1	32.5	19.8	49.5	0.48	———
E_2	37.5	5.8	14.5	0.36	129,600
E_3	42.5	2.7	6.8	0.27	100,000
E_4	47.5	2.4	5.9	0.18	48,000
E_5	55.0	1.6	4.0	0.14	27,000

*Percent yield, based on the initial weight of the fractionated dextran.
**Molecular weight as determined by light-scattering.

TABLE 2

Molecular weight distribution of enzyme degraded dextran B-512

Fraction	Subfraction	% IPA	% yield	[η]	Mol. Wt.
E_1	a	30.0	37.4	0.68	———
	b	32.5	1.4	0.58	500,000*
	c	35.0	4.0	0.50	355,000‡
	d	37.5	3.4	0.35	175,000*
	e	45.0	1.3	0.25	89,000‡
	f	55.0	1.0	0.17	43,500*
E_2	a	35.0	10.8	0.39	214,000‡
	b	37.5	1.8	0.26	96,000*
	c	45.0	0.8	0.20	57,000*
	d	55.0	0.8	0.10	14,000‡

*Obtained by light-scattering method.
‡Calculated from intrinsic viscosity and the equation $M = 1.42 \times 10^6 \times [\eta]^2$ (Hamdy, 1953).

time intervals. Figure 1 presents the results of the measurements, which indicate that the rate of decrease of viscosity is greatest at the higher acid concentrations and is most rapid in the early stages of hydrolysis.

Effect of temperature. Hydrolysis of 6 percent solutions of dextran in 0.1 N hydrochloric acid was effected in a water bath at temperatures of 45°, 70°, 80° and 98° C., respectively. The hydrolysis was followed by viscosity measurements at 20 min. intervals. The results, recorded in table 3 show that the resulting rate of drop in viscosity increases with increasing temperature.

Molecular weight distribution. Two liters of 2 percent aqueous solution of partially acid-hydrolyzed dextran, which had an intrinsic viscosity of 0.19, were fractionated to determine molecular weight distribution. Intrinsic viscosity measurements were made on the fractions and the results are given in table 4. All the fractions, with the exception of G_5, were refractionated. Intrinsic viscosity

measurements were made on all the sub-fractions while light-scattering determinations were performed on some of them; the results recorded in table 5 indicate a wide range of molecular weights.

Ultrasonic Depolymerization

Procedure. A 6 percent solution of dextran in distilled water was subjected to ultrasonic vibrations for a total period of 4.0 hr., using model U–300 ultrason, a voltage input of 2.2 k.v. and a current input of 250 milliamps, *i.e.*, a power input of 550 watts. The degradation was followed by viscosity measurements as shown in table 6. The degraded polymer was recovered and its intrinsic viscosity was found to be 0.65. Preliminary fractionations were performed on 500 ml. of a 2 percent aqueous solution of the degraded polymer. The yields of the fractions

TABLE 3

The effect of temperature on the hydrolysis of dextran by 0.1 N hydrochloric acid

TIME OF HYDROLYSIS, MIN.	RELATIVE VISCOSITY OF 6% SOLUTION AT VARIOUS TEMPERATURES			
	45. C	70. C	80. C	98. C
0	23.0	23.0	23.0	23.0
20	21.4	12.2	11.0	1.7
40	21.2	5.8	5.7	1.3
60	21.0	4.3	3.7	1.3
80	20.5	3.5	3.4	1.3
100	19.8	3.1	2.6	1.3
120	19.2	2.8	2.4	1.3

TABLE 4

*The intrinsic viscosity and molecular weight distribution of various fractions of acid hydrolyzed dextran B-512**

Fraction	% IPA	wt. of fraction in g.	% yield	$[\eta]$	Mol. Wt.**
G_1	35.0	13.2	33.0	0.30	127,000
G_2	40.0	8.7	21.8	0.19	55,000
G_3	45.0	9.4	23.6	0.16	33,500
G_4	50.0	4.1	10.3	0.11	17,500
G_5	55.0	2.2	5.5	0.09	12,000

*This acid hydrolyzed dextran had an intrinsic viscosity of 0.19.
**Obtained by light-scattering method.

were calculated and their intrinsic viscosities determined. The results are given in table 7, which, with table 6 show that: (1) high molecular weight dextran is depolymerized by ultrasonic vibrations using a power input of 550 watts; (2) a total of 83.6 percent of the degraded polymer had an intrinsic viscosity value of almost the same as that before fractionation; (3) the intrinsic viscosity of the degraded dextran was greater than the desired value (0.255 ± 0.035) for a plasma volume expander. Therefore, the following experiments were conducted to investigate certain factors affecting the ultrasonic depolymerization.

Effect of dextran concentration. Various concentrations of aqueous solutions of dextran were subjected to ultrasonic vibrations using a power input of 430 watts. Depolymerization was followed by viscosity measurements; the results are given in figure 2.

Effect of the molecular weight of dextran. The previous experiment showed that the ultrasonic treatment of dextran led to a large drop in viscosity in the first 60 to 90 min., followed by a small decrease beyond 90 min. The following experiment was conducted to attempt to explain this observation.

A native dextran with a specific viscosity of 1.87 for a one percent aqueous solution and an enzyme degraded dextran with a specific viscosity of 0.45, also in one percent solution, were subjected to the same treatment at a frequency of 450 k.c., using a power input of 430 watts. Depolymerization was followed by viscosity measurements. The results (figure 3) show that the high molecular weight dextran was degraded rapidly, while the low molecular weight polymer was only slightly affected by the depolymerization process. Therefore, it can be stated that the ultrasonic treatment acts on the large molecules of dextran much more rapidly than on the small molecules.

Effect of power input. In the previous experiments, it was found that the dextran concentration and its molecular weight are important factors in the degradation process and with high concentrations and high molecular weights, a longer period of exposure to ultrasonic waves was required to reach the desired molecular size. It was decided, therefore, to study the effect of power input (intensity) of the ultrasonic vibration on the degradation of dextran.

TABLE 5

The intrinsic viscosity and molecular weight distribution of various sub-fractions of acid hydrolyzed dextran B-512

Fraction	% IPA	Subfraction	%** yield	$[\eta]$	Mol. Wt.
G_1	32.5	a	7.5	0.38	205,000*
	35.0	b	9.2	0.30	127,800
	40.0	c	6.1	0.22	69,000
	45.0	d	1.1	0.19	55,500
	55.0	e	7.5	0.16	36,000*
G_2	37.5	a	13.5	0.28	111,000
	40.0	b	1.1	0.21	63,000*
	42.5	c	2.3	0.18	46,000
	45.0	d	1.4	0.15	32,000*
	50.0	e	0.5	0.12	20,000
	55.0	f	1.4	0.08	9,000*
G_3	40.0	a	9.5	0.17	41,000
	42.5	b	6.0	0.15	32,000
	50.0	c	2.3	0.12	20,000
	55.0	d	2.6	0.06	51,000*
G_4	42.5	a	4.0	0.14	28,000*
	45.0	b	1.4	0.11	18,000*
	50.0	c	1.6	0.09	12,000
	55.0	d	1.4	0.04	2,500*

*Calculated from intrinsic viscosity and the equation $M = 1.42 \times 10^6 \times [\eta]^2$ (Hamdy, 1953).
**Yield based on the original material for fractionation .

Two portions of a 2 percent aqueous solution of high molecular weight dextran were subjected to ultrasonic vibrations. One part was irradiated using a power input of 430 watts for a period of 3.5 hr. The other part was treated for the same length of time with a power input of 630 watts. Degradation was followed by viscosity measurements. The results are shown graphically in figure 4 which indicates that: (1) the initial rates of degradation as measured by reduced viscosity are approximately the same for power input of 430 and 630 watts; (2) for long irradiation times the molecular sizes produced by 630 watts are smaller than those produced by 430 watts.

Molecular weight distribution. Approximately 50 g. of high molecular weight dextran were subjected to ultrasonic vibration using a Radio Sonorator model RS–2 under the following controlled conditions: (1) the use of 4 percent aqueous solution; (2) the use of 430 watts power input for the first period of degradation (8 hr.); and (3) the use of 600 watts power input for the second period of degradation (8.5 hr.); to reach an intrinsic viscosity value of 0.24–0.26.

The degraded polymer was subjected to fractional precipitation using two liters of 2 percent aqueous solution.

Five main fractions were obtained using varying concentrations of isopropanol. Table 8 presents the data for the percentage yield and the intrinsic viscosities of the fractions, indicating that 94.0 per cent of the degraded dextran was in the range of that suitable for a plasma expander, and that 92.0 percent of the frac-

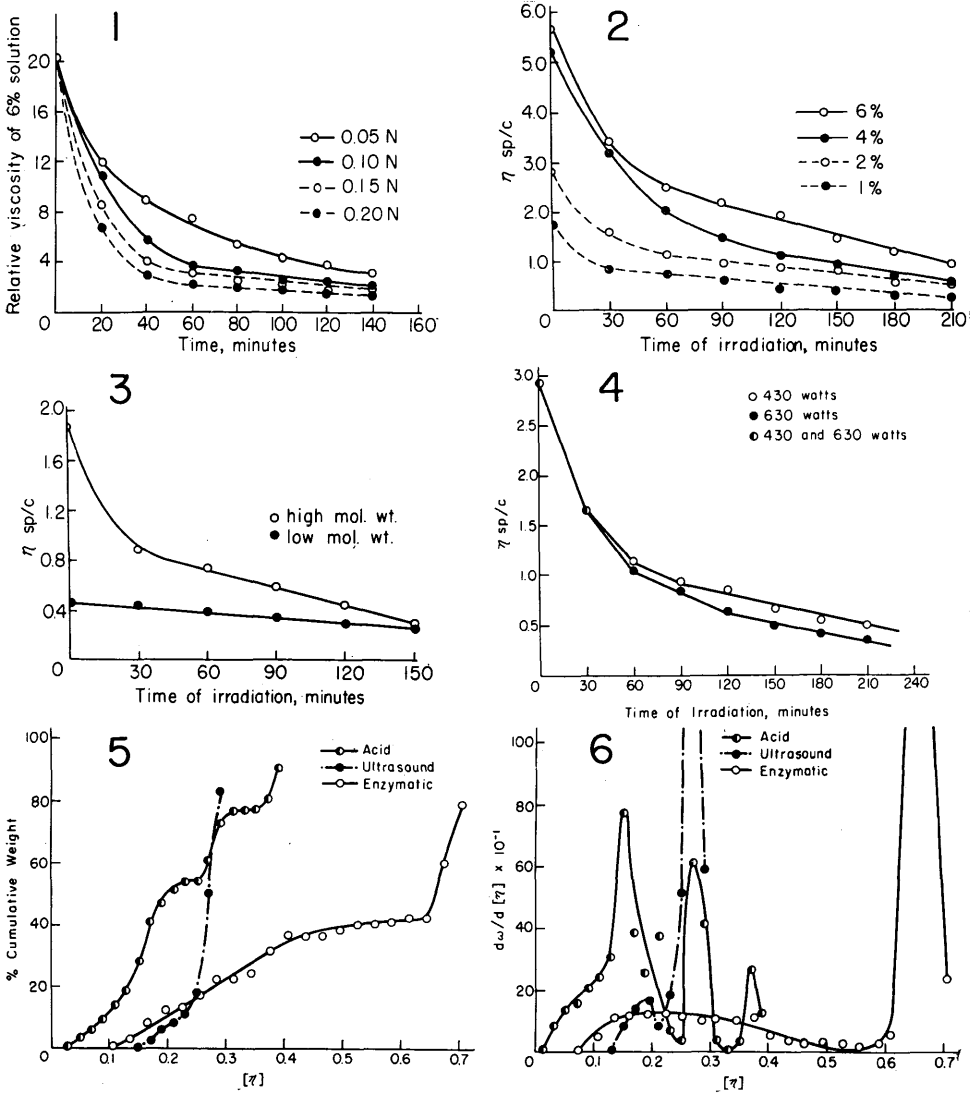

FIGURE 1. Effect of acid concentration on the hydrolysis of dextran.
FIGURE 2. Effect of dextran concentration on the ultrasonic depolymerization.
FIGURE 3. Effect of molecular weight of dextran on the ultrasonic degradation using 1% aqueous solution of dextran.
FIGURE 4. Effect of energy input on the degradation of dextran B-512 by ultrasound.
FIGURE 5. Comparison of integral distribution curves for degraded dextran B-512 resulting from different methods of depolymerization.
FIGURE 6. Comparison of differential distribution curves of degraded dextran B-512 resulting from different methods of depolymerization.

tionated polymer had almost the same [η] as that of the unfractionated polymer. Further fractionation was carried out on fractions U_1 and U_2 and the data for percentage yields of the sub-fractions (based on the original weight of the degraded dextran) and their intrinsic viscosities are recorded in table 9. It may be seen that most of the dextran is precipitated in the first and second sub-fractions, and that the intrinsic viscosities for the majority of the sub-fractions are in the range desired for a plasma volume expander.

Reducing power of degraded dextran. The reducing abilities of the polymers, resulting from the three different procedures of degradation, were investigated using Benedict solution. It was found that the acid and enzyme degraded polymers had markedly high reducing powders in contrast to the ultrasonically depolymerized dextran which was non-reducing.

TABLE 6

The effect of ultrasonic vibrations in reducing the viscosity of an aqueous solution of dextran B-512 using a power input of 550 watts

Time of irradiation, min.	ηsp/c*
0	5.88
30	3.30
60	2.50
90	2.30
120	2.00
150	1.20
180	0.89
210	0.69
240	0.67

*η sp/c values are for dextran concentration of 6%, where η sp = η rel.-1.

TABLE 7

Intrinsic viscosity distribution of an ultrasonic degraded dextran

% IPA	wt. of fractions in g.	% yield	[η]
35	7.4	73.5	0.64
40	1.0	10.1	0.63
45	0.3	3.4	0.56
50	0.1	1.0	0.49
55	not recovered	——	——

Integral and differential distribution curves. The integral distribution curve provides a simple and direct means for comparing the molecular weight or intrinsic viscosity distributions produced by the different procedures of degradation under investigation. Using the dry weights and the intrinsic viscosities of the sub-fractions obtained from fractional precipitation of the degraded polymers resulting from the three different methods, a graph was prepared plotting "Percent Cumulative Weight" precipitated against "Intrinsic Viscosity". Figure 5 represents the resultant integral distribution curves.

In the determination of molecular weight distribution, the differential curve is usually the most revealing. This curve results from plotting the slopes from the integral distribution curves against intrinsic viscosities. These slopes represent the rate of change of cumulative weight with change in the intrinsic viscosity $\frac{dw}{d[\eta]}$. In plotting, slopes were taken from the integral distribution curves at representative intrinsic viscosity intervals and plotted against the intrinsic

viscosities at which they were taken. Figure 6 shows the resultant curves for the different procedures of degradation. In this figure it may be seen that ultrasonic depolyerization results in degraded polymers, most of which lie within a narrow range of molecular weights. In contrast, acid and enzymatic degradation lead to wide ranges of molecular weights, with some evidence for tri- and bi-modal distribution, respectively. Errors in the fractionation procedure and viscosity measurements are not sufficiently large to account for the bi-modal distribution curve for enzyme degraded dextran as an artifact. But there is some possibility that the differential distribution curve for acid hydrolyzed dextran is uni-modal rather than tri-modal, since a smooth curve with only one inflection point may be drawn through the points of the integral distribution of figure 5. The important feature of the distribution obtained for acid-hydrolyzed dextran is the wide range of molecular weights which is represented.

TABLE 8

Results of fractional precipitation of ultrasonic depolymerized dextran B-512 ($[\eta]$ 0.24–0.26)

Fraction	% IPA	wt. of fraction g	% yield	$[\eta]$	Mol. Wt.*
U_1	32.5	27.7	69.3	0.260	108,000
U_2	37.5	4.6	11.5	0.255	———
U_3	42.5	3.1	7.9	0.250	105,000
U_4	47.5	1.5	3.7	0.240	———
U_5	50.0	0.8	2.0	0.190	———

*Obtained by light-scattering method.

TABLE 9

The intrinsic viscosity and molecular weight distribution of various sub-fractions of ultrasonic degraded dextran B-512

Fraction	Sub-fraction	% IPA	% Yield	$[\eta]$	Mol. Wt.*
U_1	a	32.5	38.4	0.280	———
	b	35.0	14.6	0.275	118,000
	c	37.5	6.0	0.270	116,000
	d	42.5	1.8	0.230	———
	e	55.0	1.7	0.200	———
U_2	a	37.5	6.3	0.270	114,000
	b	40.0	3.2	0.180	———
	c	55.0	1.0	0.150	———

*Obtained by light-scattering method.

DISCUSSION

The production of degraded dextran for use as a plasma volume expander has been reported by many investigators. Studies on acid hydrolysis on dextran B–512 have been discussed by Wolff *et al.* (1954). Independently, we have obtained similar results. Increase in acid concentration and temperature resulted in a rapid decrease of viscosity.

Factors affecting the ultrasonic depolymerization of dextran B–512 have been explored. It was found that dextran concentration, its molecular weight and the power input of the ultrasonic apparatus are among the main factors that govern the degradation process.

Comparative studies on the molecular weight distribution of degraded dextran B-512 resulting from three methods of depolymerization under investigation showed that partial acid hydrolysis and enzyme degradation through extended incubation (Hamdy, 1953 and Jeanes *et al.*, 1948) led to polymers with a wide range of molecular sizes, while ultrasound produces a degraded dextran with a narrow range of molecular weights.

It would appear from the results that there is an essential difference in the mechanism of these methods of depolymerization. The acid and enzyme degradations involve cleavage of the glycosidic linkage in the molecules to form reducing low molecular weight dextran. In contrast, the cavitation action of ultrasound fractures the molecules to produce non-reducing low molecular weight dextran. This fracturing may involve carbon to carbon bonds with a preferential degradation of higher molecular weight material. The work of Schmid *et al.* (1951) on synthetic polymers supports such a mechanism of degradation. These authors stated that ultrasonic vibrations can break down macromolecules by rupturing covalent bonds. Alexander and Fox (1954) also stated that the breaking of bonds by ultrasound decreased as the molecules became smaller. This mechanism seems to be comparable to what has been reported by Prudhomme and Graber (1949) and Weissler (1950). The cavitation action, which is the important factor in the ultrasonic vibration, has been investigated by Colin (1940), Weissler et al. (1950) and Wise and Ensminger (1952).

The method developed in this work for the fractional precipitation of dextran and for cleanly separating the precipitated dextran gel from the supernatant solution phase by quick freezing of the gel phase was found to be a convenient and rapid procedure. In this method, the higher alcohol content of the solution phase made it possible to freeze the gel phase without freezing the supernatant liquid, and to pour off the supernatant solution without disturbing the gel phase.

SUMMARY

(1) A modified fractionation procedure has been developed for the separation of molecular weight size classes of depolymerized dextran in which dextran is fractionally precipitated from aqueous solution by the addition of isopropyl alcohol and then frozen to convert the gel-like phase of precipitated dextran to a solid, leaving the supernatant solution in liquid form for subsequent separation by decantation.

(2) Factors affecting the degradation of dextran by acid and ultrasound have been investigated.

(3) The molecular weight distributions for the degraded polymers resulting from acid, ultrasound and enzymatic depolymerization were determined. Acid and enzyme degradation resulted in a wide range of molecular sizes, in contrast to ultrasonic degradation which gave a narrow spectrum of molecular weights.

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