656. *Molecular-weight Studies of Dextran.*

By G. C. Booth and V. Gold.

A sample of a British dextran (" Intradex ") has been fractionated by a precipitation method, and the molecular weights have been determined by osmotic-pressure, light-scattering, and viscosity measurements. Indirect tests of chain branching, based on the viscosity measurements, suggest that the dextran behaves as an unbranched coiled polymer in solution. Viscosity measurements on aqueous solutions of mixtures of dextran and bovine blood proteins do not reveal any interaction between these solutes.

It is generally recognised that dextran is of variable composition. Extensive studies on its characterisation and molecular weight by a variety of physical measurements have been carried out in the United States,[1-5] chiefly on material synthesised by the bacterial strain NRRL-B512 of *Leuconostoc mesenteroides*, and in Sweden on Swedish dextran.[6] Some investigations have also dealt with physical measurements of British dextran, synthesised by the organism designated *Betacoccus arabinosaceous*, Birmingham,[7,8] but no corresponding correlation of molecular weights obtained by different methods has been published. The purchasing specification of the Ministry of Health lays down measurements of intrinsic viscosity and ease of excretion following intravenous injection in rabbits as criteria of the molecular-weight range of the sample. Neither of these properties is a

[1] Weissberg and Isbell, " Molecular Properties of Plasma Substitutes," Nat. Bur. Stand. Report 1713 (1952).
[2] Wolff, Mehltretter, Mellies, Watson, Hofreiter, Patrick, and Rist, *Ind. Eng. Chem.*, 1954, **46**, 370.
[3] Senti, Hellman, Ludwig, Babcock, Tobin, Glass, and Lamberts, *J. Polymer Sci.*, 1955, **17**, 527.
[4] Arond and Frank, *J. Phys. Chem.*, 1954, **58**, 953.
[5] Yang and Foster, *J. Polymer Sci.*, 1955, **18**, 1.
[6] Ingelman and Halling, *Arkiv Kemi*, 1949, **1**, 61.
[7] Ogston and Woods, *Trans. Faraday Soc.*, 1954, **50**, 635.
[8] Martin, *Chem. and Ind.*, 1955, 184.

direct indication of the molecular weight, although American [1-3] and Swedish [6] workers have published empirical correlations between intrinsic viscosities and molecular weights, as determined by other methods, for dextrans of American and Swedish origin respectively. However, the present studies seem to indicate that the dependence of intrinsic viscosity on molecular weight is different for dextrans of different origin and to confirm the American workers' warning that their viscosity–molecular-weight relation [1] applies only to materials resulting from the acid hydrolysis of native dextrans produced by the NRRL-B512 strain of *Leuconostoc mesenteroides*.

In the present work a specimen of a British clinical dextran (" Intradex ") has been fractionated by a precipitation procedure closely similar to that used in the American studies.[1] The molecular weights of some of the fractions were determined by osmotic pressure and light-scattering methods. In addition, the intrinsic viscosities of all fractions

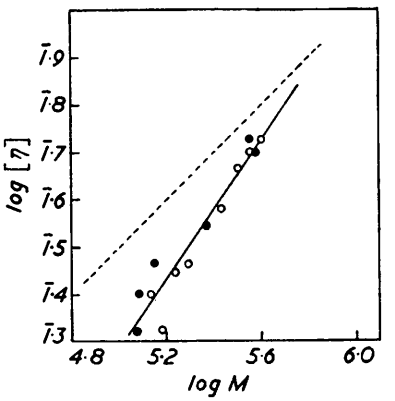


FIG. 1. *Relation between* log [η] *and* log M.
○ $\bar{M}_n$. ● $\bar{M}_w$. *The broken line shows the relation between* log [η] *and* log M *for* NRRL-B512 *dextran.*[1, 16]

were determined. These measurements enabled the formulation of an approximate empirical relation between intrinsic viscosity and molecular weight (Fig. 1) and hence, by interpolation, the determination of the molecular weights of all the fractions.

The viscosity measurements also allow some tentative conclusions about the structure of the dextran molecule in solution and its interaction with bovine blood proteins.

EXPERIMENTAL

Dextran was obtained in 1952 from Dextran Limited (Aycliffe, Co. Durham) (now part of Glaxo Laboratories Ltd.). Two specimens were employed in this investigation, both produced by the bacterial strain *Betacoccus arabinosaceous*, Birmingham: (i) Clinical dextran (" Intradex "), produced from a crude native polysaccharide by thermal degradation [9] and fractionally precipitated by acetone, fractions of high and low molecular weight being discarded. The intrinsic viscosity of the batch used was recorded by the firm at the time of its manufacture and was 0·35 decilitre g.$^{-1}$ at 37°, and the weight-average molecular weight was stated to be 175,000. It was supplied as a 6% solution, containing also 0·90% of sodium chloride. (ii) Natural polysaccharide from the same source.

Fractionation of Clinical Dextran.[1]—The complete contents of a 540 ml. bottle of " Intradex " were diluted to 2 l., with the addition of phenylmercuric acetate (0·1 g.) as fungicide. The solution was kept at 25° and methanol added in portions until the bulk of the liquid was permanently cloudy. The flask was then fitted with a mercury-sealed stirrer and heated at 45°. The heating bath was then allowed to cool slowly (36 hr.) to 25°. Stirring was stopped for 6 hr., during which the coacervate formed settled. The supernatant liquid was decanted, filtered through sintered glass, returned to the flask, and treated with more methanol to obtain the second fraction, and similarly for further fractions. The gel was dissolved in water and separated from the solution, in a spongy white and readily soluble form, by freeze-drying. The details of the main fractionation (B) [following a trial fractionation (A)] are given below.

[9] B.P. 719,382.

To obtain some fractions of high molecular weight, a sample of undegraded dextran was heated with aqueous hydrochloric acid, and the hydrolysate was subjected to a similar fractionation (specimen C).

No. of fraction	B1	B2	B3	B4	B5	B6	B7	B8	B9
Vol. % of methanol in soln.	45·6	46·7	47·7	49·0	50·3	51·7	53·8	56·9	76·4
Wt. of dry dextran in fraction	4·5	3·75	2·7	5·5	1·4	3·4	2·1	2·7	0·5

Unaccounted for : 5·8 g.

Infrared Spectra.—For identification the infrared spectra of Nujol mulls of undegraded dextran and of fractions A2b, A5, and B1 were determined on a Grubb–Parsons double-beam spectrometer. The bands observed in the diagnostically interesting region (768, 798, 846, and 917 cm.$^{-1}$) agree tolerably well with those reported [10] for dextran of the same origin (768, 793, 841, and 917 cm.$^{-1}$). The presence of the band at *ca.* 798 cm.$^{-1}$ (attributed to α-1 : 3-links [11]) is particularly significant in showing that the specimen of our investigation did not belong to the variant of British dextran isolated more recently.[12]

Analysis of Dextran Solutions.—This was carried out by freeze-drying, of measured amounts of solution, to constant weight.

Osmotic-pressure (π) *Measurements.*—These were carried out at 25° with a slightly modified form of the osmometer described by Weissberg.[13] *cyclo*Hexane was used in the capillary, as it is more freely moving than aqueous solutions. With suitably prepared nitrocellulose membranes equilibrium was attained after about 36 hr. and no diffusion of dextran through the membrane could be detected.

The number-average molecular weight ($\bar{M}_n$) of each fraction was calculated from measurements on a series of solutions of different concentrations (c). A graph of (π/c) against c was drawn. The intercept $(\pi/c)_{c\to 0}$ was obtained by linear extrapolation and used to calculate $\bar{M}_n$ according to the equation $\bar{M}_n = \boldsymbol{R}T[(\pi/c)_{c\to 0}]^{-1}$. The results are tabulated.

No. of fraction	A1	A2	A4	A5	B1	B5	B8
$10^{-3}\bar{M}_n$	384	269	172	136	394	195	153

Light Scattering.—The reduced intensity of scattered light (5461 Å) at an angle of 90° (R_{90}) was determined for dextran solutions with an apparatus of simple design constructed by Dr. P. M. Doty at the Royal Institution in 1947. A reading for the scattering of water was subtracted from all measurements. Care was taken to remove and exclude dust from all vessels and solutions. The apparatus was calibrated with purified benzene. The depolarisation was not measured and a depolarisation correction was not applied.

For each fraction of dextran studied a series of solutions was examined and a graph of (c/R_{90}) against c constructed. The intercept $(c/R_{90})_{c\to 0}$ was obtained by linear extrapolation and used to calculate the weight-average molecular weight ($\bar{M}_w$) from the equation $\bar{M}_w = [K(c/R_{90})_{c\to 0}]^{-1}$, where $K = (2\pi^2 n_0^2)(dn/dc)^2/\lambda^4 \boldsymbol{N}$ n_0 being the refractive index of the solvent and λ the wavelength of light used. The refractive-index increment (dn/dc) of solutions in the concentration range 0·19—0·84 g. per 100 g. of solution was found to be 0·148 g.$^{-1}$ ml. (cf. ref. 6). The results were :

No. of fraction	A1	A3	A5	B1	B5	B8
$10^{-3}\bar{M}_w$	362	236	121	356	142	118

Viscosity Measurements.—These were carried out at 25° in a Fitzsimmons suspended-level viscometer.[14] Intrinsic viscosities ([η]) were obtained as intercepts from graphs of $(\eta_{rel.} - 1)/c$ against c, from the definition $[\eta] = [(\eta_{rel.} - 1)/c]_{c\to 0} = [\eta_{sp.}/c]_{c\to 0}$, where $\eta_{rel.} = \eta_{soln.}/\eta_{solv.}$, and $\eta_{sp.} = (\eta_{rel.} - 1)$. The units of η are decilitres g.$^{-1}$, c being expressed as g. per 100 ml. of solution. From the slopes of these graphs, the Huggins constants k', defined according to the equation $(\eta_{rel.} - 1)/c = [\eta] + k'[\eta]^2 c$ were obtained. The results are tabulated.

[10] Barker, Bourne, Bruce, Neely, and Stacey, *J.*, 1954, 2395.
[11] Burket and Melvin, *Science*, 1952, **115**, 516.
[12] Barker, Bourne, James, Neely, and Stacey, *J.*, 19.5, 2096.
[13] Weissberg, *J. Res. Nat. Bur. Stand.*, 1952, **49**, 393.
[14] Institute of Petroleum, Standard Methods of Testing Petroleum (1952).

Fraction	In H_2O at 25° $[\eta]$ (dl. g.$^{-1}$)	k'	In 40·5% aq. MeOH at 25° $[\eta]$	Fraction	In H_2O at 25° $[\eta]$ (dl. g.$^{-1}$)	k'	In 40·5% aq. MeOH at 25° $[\eta]$
A1	0·50	0·46	—	B6	0·28	0·18	—
A2	0·38	0·37	—	B7	0·26	0·58	—
A3	0·35	0·12	0·212	B8	0·21	0·67	0·145
A4	0·28	0·70	—	C1	1·07	0·79	—
A5	0·25	0·53	—	C2	0·54	0·43	—
B1	0·53	0·50	0·252	C3	0·31	0·43	—
B2	0·45	0·30	—	C4	0·20	0·73	—
B3	0·40	0·19	—	C1a	0·55	0·79	—
B4	0·38	0·20	—	C1b	0·83	0·70	—
B5	0·29	0·43	—	H	0·46	0·33	—

Viscosity measurements were also carried out on buffered aqueous solutions of some bovine blood proteins and their mixtures with dextran. A Table of results is given below. The calculated values of $\eta_{rel.}$ in the last column were obtained from the equation :

$$\eta_{rel.} = 1 + (\eta_{sp.}/c)_{protein} \cdot c_{protein} + (\eta_{sp.}/c)_{dextran} \cdot c_{dextran}$$

which is expected to apply if there is no interaction and each solute contributes independently to the relative viscosity of the solution. The relevant values of $(\eta_{sp.}/c)$ for the dextran fractions and proteins in the buffer solutions were found by independent experiments.

	$c_{dextran}$ (g./100 ml.)	$c_{protein}$ (g./100 ml.)	$\eta_{rel.}$	$\eta_{rel.}$ (calc.)
In buffer at pH 6·21				
Dextran A5 + Bovine serum albumin	0·250	0·292	1·10	1·10
	0·125	0·146	1·05	1·05
Dextran C1a + Bovine fibrinogen	0·370	0·353	1·36	1·39
	0·246	0·236	1·23	1·23
	0·185	0·176	1·15	1·16
	0·095	0·088	1·07	1·07
In buffer at pH 7·47				
Dextran C1a + Bovine globulin	0·360	0·336	1·69	1·66
	0·240	0·223	1·41	1·38
	0·120	0·167	1·29	1·28
Dextran A5 + Bovine globulin	0·528	0·501	1·25	1·24
	0·349	0·334	1·17	1·15
	0·264	0·250	1·12	1·11
	0·209	0·200	1·09	1·09
	0·174	0·167	1·08	1·07

The critical miscibility temperature for infinite molecular weight (Θ-point) was determined for 40·5% aqueous methanol from the equation $T_c = \Theta - b\Theta M^{-\frac{1}{2}}$, where T_c is the temperature at which cloudiness begins to appear (or disappear) on cooling (or warming) of a 6% or 15% solution of dextran. The Θ-point was found to be *ca.* 23° and is sufficiently close to 25° for the relative viscosity measurements in 40·5% methanol at 25° to be regarded as relating to the Θ-point.

Discussion

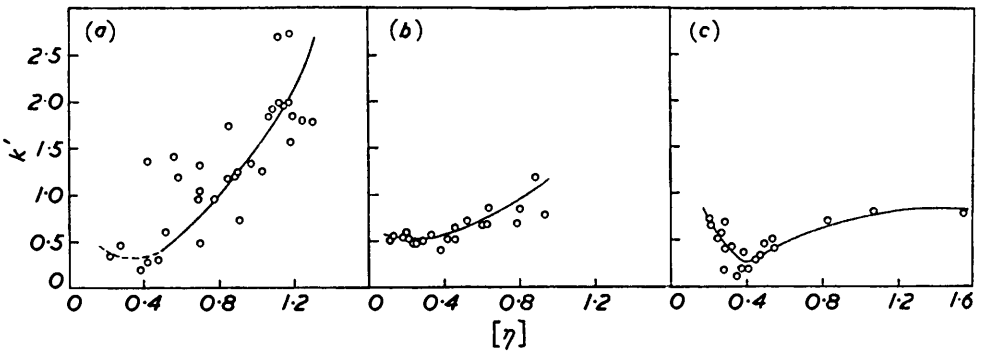
The intrinsic-viscosity determinations in 40·5% aqueous methanol allow a test for the occurrence of chain branching in the dextran molecule. According to the extension of the Flory–Fox [15] theory due to Wales, Marshall, and Weissberg [1,16] a value of less than 0·5 of the exponent a in the Mark equation $[\eta] = KM^a$, at the Θ-point, indicates chain branching, whereas the exponent has the value 0·5 for unbranched molecules. Our data lead to a value of a which is indistinguishable from 0·5 within the somewhat wide limits of experimental accuracy (*ca.* ± 0·1). The more precise data on NRRL-B512 dextran [1,16] lead to a value of 0·32. The index 0·32 indicates chain branching but it is not permissible,[1,16] on the theory mentioned, to invert the argument and regard the absence of chain branching as established for British dextran.

[15] Flory and Fox, *J. Amer. Chem. Soc.*, 1951, **73**, 1904.
[16] Wales, Marshall, and Weissberg, *J. Polymer Sci.*, 1953, **10**, 229.

Our viscosity measurements in aqueous solutions lead to the exponent 0·70 in the Mark equation, the maximum value of a being 0·8 for an unbranched coiled polymer, according to Flory and Fox's theory. This value compares with an exponent around 0·5 at low molecular weights [1,2] (and decreasing at higher molecular weight [5]) obtained for NRRL-B512 dextran and 0·34 for Swedish dextran.[6] According to Wales, Marshall, and Weissberg,[1,16] the intrinsic viscosity of hypothetical unbranched dextran is given by $[\eta] = 1{\cdot}99 \times 10^{-4}M^{0{\cdot}675}$ in the interval $11{,}000 < M < 370{,}000$. The exponent in this expression lies very close to our value but the numerical factor is too high to fit the results.

It is recognised as an empirical fact,[17] and with some theoretical justification,[18] that the relation between $[\eta]$ and the Huggins constant k' allows the detection and estimation of chain branching in polymer molecules. According to this test, a plot of k' against $[\eta]$ is horizontal for an unbranched polymer, whereas for a branched molecule k' increases with $[\eta]$ at high intrinsic viscosities. When k' is plotted against $[\eta]$ for dextran solutions (Fig. 2) it is found that k' for our specimen of British dextran increases less rapidly with $[\eta]$ than for Swedish dextran. American NRRL-B512 dextran again occupies an intermediate position. By analogy with other examples one may tentatively conclude that

FIG. 2. *Relation between intrinsic viscosity and Huggins constant k'.*



a, Swedish dextran.[6] *b,* NRRL–B512 *dextran.*[3] *c, " Intradex " (this work).*

Swedish dextran is the most highly branched, and British dextran the most nearly unbranched of the three types studied. None of the specimens gives rise to $[\eta]$–k' graphs of the precise shape found by Cragg and Fern [17] and, owing to the difficulties of precise viscometry on aqueous solutions, the experimental points from American,[1,3] Swedish,[6] and our work show a considerable scatter. Senti *et al.*[3] have suggested that k' passes through a minimum in the case of NRRL-B512, and we find that the same trend is perceptible in our own data and perhaps also in those of Ingelman and Halling.[6]

Our tentative conclusion that British dextran behaves as the most nearly unbranched of the three dextrans studied in this manner appears to conflict with the results of chemical methods which have shown British dextran from the same source to possess α-1 : 3-branches.[10] The two lines of evidence are not necessarily contradictory for, as Wales, Marshall, and Weissberg [1,16] point out, the frictional effect of branching will be lost and the molecule will behave like an unbranched chain if the branches are closely spaced so as to affect rotation about many bonds in the main chain. This could arise if the branch points occurred in small, closely packed groups. Possible structures of dextran, some of which follow this pattern, have been mentioned in the literature.[19]

The significance of molecular-weight determinations for clinical applications of dextran

[17] Speiser and Whittenberger, *J. Chem. Phys.*, 1945, **13**, 349; Walker and Winkler, *Canad. J. Res.*, 1950, **28**, *B*, 298; Manson and Cragg, *Canad. J. Chem.*, 1952, **30**, 482; Cragg and Manson, *J. Polymer Sci.*, 1952, **9**, 265; Cragg and Fern, *ibid.*, 1953, **10**, 185.

[18] Simha, *J. Res. Nat. Bur. Stand.*, 1944, **42**, 409.

[19] Dimler, Wolff, Sloan, and Rist, *J. Amer. Chem. Soc.*, 1955, **77**, 6568; Krysiak, Murawski, May, and Malec, *Acta Biochim. Polon.*, 1954, **1**, 27.

as a plasma-extender clearly depends on the extent to which measurements in aqueous solution reflect the true molecular weight of dextran particles in human blood. The problem awaits a more thorough study, but the exploratory experiments which we have carried out show that the interaction of dextran and bovine blood proteins is not detectable by viscosity measurements. In every case, the relative viscosity of a solution of a mixture of dextran and protein agreed with the value calculated by assuming independent contributions to the relative viscosity from the two solutes. The measurements therefore rule out the occurrence of appreciable association between dextran and bovine serum albumin, fibrinogen, and globulin.

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