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The Molecular Compression of Dextran

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The suggestion is made that, in solution, the flexible-chain molecules of dextran can undergo an osmotic compression as concentration is increased. Approaches are developed describing the molecular shrinkage (i) as arising from intra- and inter-molecular forces, (ii) based on the molecular characteristics of the dextran, and (iii) as estimated by viscosity measurements. Comparison with the macroscopic shrinkage of cross-linked dextran (Sephadex) beads [Edmond, Farquhar, Dunstone & Ogston (1968) *Biochem. J.* **108**, 755–763] is made. In all systems studied, the experimental estimates of compression, both from gel-shrinkage and viscosity measurements were in reasonable agreement with theoretical predictions. The interpretation of the viscosity concentration-dependence was applied to compact structures (albumin and Percoll). Their behaviour was in marked contrast with that of dextran. It is noted that molecular compression may be important in considering transport processes in and thermodynamic properties of concentrated systems.

Many of the various biological properties of the intercellular matrix of connective tissues have been discussed in terms of the molecular characteristics and physico-chemical behaviour of the high-molecular-weight polysaccharides (glycosaminoglycans linked to proteins) that are associated with the aqueous phase of the matrix (Balazs, 1970). It has been suggested (Ogston, 1966; Laurent, 1966) that, at physiological concentrations, the polysaccharide coils overlap with each other and form a continuous three-dimensional network throughout the extracellular space.

Furthermore, it has been postulated that such a system would act as a permeability barrier to the transport of macromolecular agents (Laurent, 1966) and would also affect intercellular obligate transport involved in tissue turnover and deposition. Such effects have been investigated in detail in simple model systems (Laurent *et al.*, 1963; Ogston *et al.*, 1973; Preston & Snowden, 1973; Laurent *et al.*, 1975, 1976).

Although it was suggested many years ago (Weissberg *et al.*, 1951; Maron *et al.*, 1959) little attention has been paid to the consideration that flexible polymeric molecules may undergo osmotic compression as the macromolecular concentration of the solution (or environment) is increased. This type of molecular compression is thought to arise from a balance between the intra- and inter-molecular repulsions of the polymer chain segments. Such a decrease in the overall effective molecular volume of the connective-tissue polysaccharides *in vivo* should be considered in any discussion of transport phenomena within the tissues. This view of the effect of increasing concentration on molecular configuration is alternative to that of molecular overlap or interpenetration.

It was previously suggested that estimates of the decrease in coil volume could be made from the derivatives of viscosity–concentration curves (Weissberg *et al.*, 1951; Simha & Zakin, 1960, 1962), although some reservation was expressed as to its use as a quantitative measure. Evidence of molecular compression has been obtained from an experimental investigation by light-scattering on concentrated solutions of synthetic flexible polymers (Benoit & Picot, 1966). More recently, Daoud *et al.* (1975) obtained direct evidence, by using small-angle neutron-scattering data, that the radius of gyration of polystyrene decreased with increasing polymer concentration.

In the present paper we have chosen to examine the properties of the flexible polysaccharide dextran. Viscosities up to concentrations of 0.2 g/ml have been measured and an interpretation offered. In the Theory section, equations are developed describing the shrinkage of dextran (i) as estimated by viscosity and (ii) based on the molecular characteristics of the polymer. Comparison with the macroscopic shrinkage of cross-linked-dextran (Sephadex) beads (Edmond *et al.*, 1968) is made.

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Theory

Osmotic molecular shrinkage

The configuration of a flexible macromolecule in solution is the resultant of the internal free energy of dilution and of the entropy decrease involved in the extension of its chains.

In a solution, the chemical potential of solvent (water) is decreased to an extent measured by the total osmotic pressure of the solution and expressed empirically by

$$\pi/RT = c/\bar{M}_n + A_2c^2 + A_3c^3 + A_4c^4 + \cdots \quad (1)$$

where $\bar{M}_n$ is the number-average molecular weight and c the concentration (mass per unit volume) of the solute. A_2, A_3 etc. are constants that may be either predicted on theoretical grounds or determined empirically.

At finite concentration, the lowering of the chemical potential of the solvent would be expected to affect its equilibrium between the interior of the molecule and its environment, resulting in the latter's shrinkage to achieve a new balance between the internal osmotic pressure and the configurational entropy.

Such shrinkage, by affecting the volume exclusion between neighbouring solute molecules, should in turn affect the values of A_2, A_3 etc., each becoming variable with composition. That is, we should be able to express osmotic pressure by an equation alternative to eqn. (1), namely

$$\pi/RT = c\bar{M}_n + A_2^* c^2 + A_3^* c^3 + A_4^* c^4 + \cdots \quad (1a)$$

where A_2^*, A_3^*, A_4^* etc. are variable with concentration. If their values can be represented by a convergent power series in c:

$$\begin{aligned} A_2^* &= p_0 + p_1 c + p_2 c^2 + \cdots \\ A_3^* &= q_0 + qc + q_2 c^2 + \cdots \\ A_4^* &= r_0 + r_1 c + r_2 c^2 + \cdots \end{aligned} \quad (2)$$

where p, q, r etc. are constant coefficients, then putting eqn. (2) in eqn. (1*a*) and collecting powers of c

$$\pi/RT = c/\bar{M}_n + p_0 c^2 + (p_1 + q_0)c^3 + (p_2 + q_1 + r_0)c^3 + \cdots \quad (3)$$

Comparing eqn. (3) with eqn. (1) we find that

$$\begin{aligned} p_0 &= A_2 \\ p_1 + q_0 &= A_3 \text{ etc.} \end{aligned} \quad (4)$$

Assuming only entropic interactions between spherical molecules, we may identify

$$A_2^* = \frac{U'_{22}}{2\bar{M}_w^2} \left(\text{cf. } A_2^{*0} \equiv A_2 \equiv \frac{U_{22}}{2\bar{M}_w^2} \right) \quad (5)$$

where U'_{22} is the self co-volume of the solute at a particular concentration c corresponding to the value of A_2^* at that concentration. $\bar{M}_w$ is the weight-average molecular weight of solute. The effective specific volume v'_c at the same concentration is defined by

$$U'_{22} = 8v'_c \bar{M}_w \quad (6)$$

Combining eqns. (2), (5) and (6)

$$\frac{4v'_c}{\bar{M}_w} = p_0 + p_1 c + p_2 c^2 = \frac{4v^0}{\bar{M}_w} + p_1 c + p_2 c^2 \quad (7)$$

where

$$p_0 = \frac{4v^0}{\bar{M}_w} = A_2 \quad (8)$$

Rearrangement of eqn. (7) gives

$$v'_c/v^0 = 1 + p'_1 c + p'_2 c^2 + \cdots \quad (9)$$

where p'_1, p'_2 etc. are constant coefficients.

By use of eqn. (8), we can estimate v^0 from the empirical A_2, but we must seek other evidence for the values of p'_1 etc.

As has been pointed out by Flory (1953), a close parallel exists between the molecular expansion of a single polymer molecule and the osmotic swelling of a macroscopic three-dimensional gel. It has been shown (Edmond *et al.*, 1968) that the shrinkage of cross-linked dextran (Sephadex) beads, when immersed in solutions of a non-penetrating solute, can be expressed empirically as

$$\frac{V'}{V^0} = 1 + kc + k'c^2 + \cdots \quad (10)$$

where V^0 is the bead volume in solvent and V' the volume in a solution of non-penetrating solute of concentration c.

If we simplify the situation in the dextran solution and consider that there is no inter-penetration of the molecular domains of a dextran particle by the neighbouring dextran molecules, then it may be expected that a separate dextran molecule may behave similarly to a Sephadex bead of the same fully swollen volume per grain; that is, a direct quantitative comparison can be made between eqn. (10) and eqn. (9) if the specific volume of the dextran sample (v^0) is similar in value to the Sephadex-bead volume (V^0).

Molecular shrinkage based on the characteristics of the polymer molecule

One of us (Ogston, 1962) previously discussed the effect on the configuration of a single polymer molecule of exposure to a second macromolecular component. The resulting shrinkage was discussed in terms of the differential osmotic effect between the intra- and extra-molecular phases. By using a

similar approach and the same notation as in Ogston (1962), we have for the binary system (where a single polymer molecule is exposed to like polymer molecules) corresponding to eqn. (50) of Ogston (1962).

$$(\mu_1' - \mu_1'') = -\frac{RTM_1}{10^3}\left[-\left(m_2'' - \frac{10^3}{M_1}\ln \gamma_1''\right) - \frac{10^3}{M_1}\ln \gamma_1'\right] \quad (11)$$

where the intramolecular phase is denoted by $'$, the extramolecular phase by $''$, subscript 1 refers to solvent (water), m_2'' represents the molal concentration of the solution with respect to the polymer (component 2), γ_1'' expresses the non-ideality of the water in the solution, γ_1' expresses the non-ideality of the water in the polymer molecule, regarded as a separate intramolecular phase. If we use m_2' for the internal molal concentration of the molecule, d' for the non-ideality coefficient within the molecule, and d'' for the non-ideality coefficient of the solution (in case they may be different), then eqn. (11) becomes

$$(\mu_1' - \mu_1'') = -\frac{RTM_1}{10^3}\left[-m_2'' - \frac{d''}{2}(m_2'')^2 + \frac{d'}{2}(m_2')^2\right] \quad (12)$$

We may proceed as in Ogston (1962), who followed the approach of Flory (1953).

Noting that $m_2' = 1000/N\phi$, where ϕ is the effective molecular volume and N is Avogadro's Number, we obtain

$$(\alpha^5 - \alpha^3) = -\frac{N\phi_0\alpha^6}{10^3}\left(m_2'' + \frac{d''}{2}(m_2'')^2\right) + \frac{10^3 d'}{2\phi_0 N} \quad (13)$$

α is the linear expansion factor from the 'unperturbed' condition of the chain, so that

$$\phi = \phi_0\alpha^3 = \frac{4\pi}{3}(\overline{S_0^2})^{3/2}\alpha^3 \quad (14)$$

where $(\overline{S_0^2})$ is the mean-square unperturbed radius.

We may convert eqn. (13) into weight/volume terms (without much error) by writing $m_2 = 10^3 c/M$ where c is the polymer concentration (g/ml); by replacing dm_2 terms by the corresponding second and third virial coefficients on a weight/volume basis, eqn. (13) becomes

$$(\alpha^5 - \alpha^3) = -N\phi_0\alpha^6(c/M + A_2'' c^2 + A_3'' c^3) + \frac{1}{N\phi_0} \times \left[A_2'(M)^2 + \frac{A_3'(M)^3}{N\phi_0\alpha^3}\right] \quad (14a)$$

In order to use eqn. (14a) to estimate the change in α and hence in the molecular volume with change in polymer concentration, we must first establish the molecular characteristics of the chain at infinite dilution; we shall denote this state by the superscript ^{0}. An estimate of α^0 can be obtained by putting $c = 0$, expressing α^0 in terms of $(\overline{S^2})^0$ and $(\overline{S_0^2})$, that is

$$(\alpha^0)^3 = \left[\frac{(\overline{S^2})^0}{(\overline{S_0^2})}\right]^{3/2}$$

and by using an experimentally derived value of $(\overline{S^2})^0$. Next, by putting various values of α into eqn. (14a) and assuming $A_2'' \equiv A_2'$ and $A_3'' \equiv A_3'$, the corresponding values of c are obtained by solving the resulting cubic equation.

Molecular shrinkage as estimated by viscosity measurements

If it is assumed that a solution of a given concentration of solute can be regarded as the 'solvent' for further addition of solute, and that the viscosity increment that results obeys the Stokes–Einstein relationship, then

$$\frac{1}{\delta c}\left(\frac{\delta\eta_c}{\eta_c}\right) = 2.5v_c' \quad (15)$$

when η_c is the viscosity of a solution of concentration c, and δc is the concentration increment.

Eqn. (15) integrates to

$$\ln(\eta_c/\eta_0) \equiv \ln \eta_{\text{rel.}(c)} = 2.5\int v_c' dc \quad (16)$$

and if we can express the change in specific volume as a power series in c

$$v_c'/v^0 = 1 + \kappa c + \kappa' c^2 + \cdots \quad (17)$$

where κ, κ' etc. are constants and v^0 is specific volume of solute at infinite dilution, then

$$\ln \eta_{\text{rel.}(c)}/c = 2.5v^0\left(1 + \frac{\kappa c}{2} + \frac{\kappa' c^2}{3} + \cdots\right) = [\eta]\left(1 + \frac{\kappa c}{2} + \frac{\kappa' c^2}{3} + \cdots\right) \quad (18)$$

where $[\eta] = 2.5v^0 \equiv$ intrinsic viscosity.

Thus we have

$$\ln \eta_{\text{rel.}(c)}/c[\eta] = 1 + \frac{\kappa c}{2} + \frac{\kappa' c^2}{3} \quad (18a)$$

It should be noted that eqn. (18*a*) relates the inherent viscosity $\ln \eta_{\text{rel.}}/c$ to the constants κ and κ' etc., which by eqn. (17) describes the change in volume of solute with increasing concentration. The function of the left-hand side of eqn. (18a) is dimensionless, since it is a ratio of quantities that can be identified as representing molecular volumes. If we are to use the viscosity of the solution as a measure of the molecular compression of the solute, then the values of κ (and κ') and k (and k') that satisfy eqns. (18a) and (10) respectively are to be compared.

Experimental and Results

Materials

Dextrans. As suggested in the Theory section, if a comparison is to be attempted of the osmotic shrinkage properties of a dextran gel (Sephadex) and of the molecular compression of a dextran chain, then the two materials must be 'matched'. That is, the effective specific volume of the dextran sample at zero concentration, as given by eqn. (8), must be similar in value to the volume/g of the fully swollen gel bead. To this end, three dextran samples with $\bar{M}_w/\bar{M}_n$ less than 1.5 were supplied to us by Dr. K. Granath (Pharmacia, Uppsala, Sweden). These are referred to below by 10^{-3} times their $\bar{M}_n$ values, i.e. dextran 52.8, 122.0 and 261.0. Table 1 summarizes the physical data for these samples and also lists the 'matching' Sephadex grade.

Dextran gels (Sephadex). The results on the macroscopic shrinkage of Sephadex to be used in the present comparative study are those reported by Edmond *et al.* (1968) and are given in Table 2. The shrinkage behaviours of Sephadex grades G-75, G-100 and G-200 are reported (columns 3, 5 and 7 of Table 2) in terms of the concentration of a non-penetrating solute, dextran T500 (batch T5406; $\bar{M}_w = 4.2\times10^5$; $\bar{M}_n = 1.53\times10^5$) (column 1 of Table 2). The corresponding osmotic pressure of the solutions of this dextran can be calculated from

$$\pi/RT = c/1.53\times10^5 + 3.41\times10^{-4}c^2 + 3.35\times10^{-3}c^3$$

(Edmond *et al.*, 1968)

and are given in column 2 of Table 2. It is necessary, in turn, to find the concentrations of 'matched' dextrans that correspond to these osmotic pressures. This is achieved as described below by use of the molecular weights and virial coefficients given in Table 1. These concentrations are reported in columns 4, 6 and 8 of Table 2.

All other chemicals were commercially available analytical reagents.

Methods

Solutions. Stock solutions of dextrans were made up by weight in distilled water, with heating to 95°C as required. Conversion into weight/volume concentrations was carried out on the basis of dry weight of solids [measured by heating over P_2O_5 for 24h at 105°C and 133.3Pa (1mmHg) pressure] and the partial specific volume of 0.60ml/g (Edmond *et al.*, 1968).

Table 1. *Characeristics of dextran samples*

Sample	$10^{-3}\times\bar{M}_n$	$10^{-3}\times\bar{M}_w$	Virial coefficients: $10^4\times A_2$ ($cm^3\cdot mol\cdot g^{-2}$)	Virial coefficients: $10^3\times A_3$ ($cm^6\cdot mol\cdot g^{-3}$)	Intrinsic viscosity (ml/g)	v^0 from eqn. (8)	Matching Sephadex: Grade	Matching Sephadex: Bead volume†
Dextran 52.8	52.8*	77.5*	5.1	2.0	27.0	9.9	G-75	10.3
Dextran 122.0	122.0*	152.0*	3.34	4.0	37.9	12.8	G-100	13.1
Dextran 261	261*	321*	3.95	5.23	49.6	31.7	G-200	27.4

* From K. Granath (personal communication).
† From Edmond *et al.* (1968).

Table 2. *Shrinkage charcteristics of Sephadex beads*

$10^2\times$[dextran 500] (g/ml)*	Osmotic pressure ($10^6\pi/RT$)*	Sephadex ... Shrinkage of beads: G-75 V'/V^{0}*	G-75 $10^2c_{52.8}$ (g/ml)	G-100 V'/V^{0}*	G-100 $10^2c_{122.0}$ (g/ml)	G-200 V'/V^{0}*	G-200 $10^2c_{261.0}$ (g/ml)
2.29	0.367	0.94	1.4	0.90	2.2	0.78	2.20
4.57	1.33	0.88	3.5	0.74	4.4	0.61	4.36
8.45	5.01	0.68	7.4	0.55	8.2	0.42	7.7
10.97	9.24	0.62	10.1	0.47	10.6	0.36	9.9
15.31	21.02	0.43	15.0	0.36	14.7	0.27	13.75
16.91	27.05	0.42	16.7	0.34	16.2	0.24	15.1
20.57	44.93	—		0.31	19.6	0.22	18.4

* From Edmond *et al.* (1968).

Measurement of the osmotic pressures of dextrans. This was carried out by the measurements of the non-ideality coefficients, α_1^* and α_2^* by sedimentation equilibrium as described by Nichol *et al.* (1967) with the use of their eqns. (6a) and (7a) or by a curve-fitting procedure of their eqn. (4) (present table 1). The osmotic pressure/concentration relationships were calculated by

$$\pi/RT = c/\bar{M}_n + \frac{\alpha_1^* c^2}{2} + \frac{2\alpha_2^* c^3}{3} = c/\bar{M}_n + A_2c^2 + A_3c^3$$

Viscosities. Viscometry was carried out at 25°± 0.01°C with a size-100 Cannon-Ubbelohde semi-micro viscometer. All additions to the viscometer were made by weight. Densities (ρ) of the stock dextran were measured at 25°C in 2cm³ pycnometers or in a precision density meter (DMA02C, Anton Parr, Graz, Austria) and found to agree with the relationship $\rho = \rho_0\ (1-\bar{v}c)+c$, where ρ_0, solvent density = 0.9972g/ml, $\bar{v} = 0.60$ml/g and c is dry-weight concentration of dextran (g/ml). The observed flow times t_s of the various dilutions of dextran were converted into relative viscosities by use of

$$\eta_{rel.} = \rho/\rho_0 \cdot t_s/t_0$$

where t_0 is the solvent time and ρ/ρ_0 was calculated from the relationship given above. Ranges in values of $\eta_{rel.}$ covered were 1–15.8, 1–48.7 and 1–27.2 for dextrans 52.8, 122.0 and 261.0 respectively.

The inherent viscosity $[(1/c)\ln\eta_{rel.}]$ is shown as a function of c for the three dextran samples in Fig. 1. The intrinsic viscosity, $[\eta]$ was calculated from the linear extrapolation of $[(1/c)\ln\eta_{rel.}]$ by using points corresponding to $\eta_{rel.} < 2.5$, to zero solute concentration according to the following equation (Kraemer, 1938).

$$\frac{1}{c}\cdot\ln\eta_{rel.} = [\eta] - k''[\eta]^2c \qquad (19)$$

The values of $[\eta]$ obtained agree to within 1% of the values measured independently by Dr. K. Granath (personal communication) (Table 1).

It is evident from Fig. 1 that the plots of $[(1/c)\ln\eta_{rel.}]$ versus concentration display a continuous curvature in accordance with the general behaviour of concentration-related changes in viscosity and as is suggested by eqn. (18).

In order to indicate more clearly the higher order effects of concentration on viscosity, eqn. (19) is written in the 'reduced' form:

$$\frac{1}{[\eta]}\left(\frac{\ln\eta_{rel.}}{c[\eta]} - 1\right) = -k''c \qquad (19a)$$

A plot of the left-hand side of eqn. (19a) versus concentration for the three dextran samples is given in Fig. 2. The plot reflects the effect of intermolecular

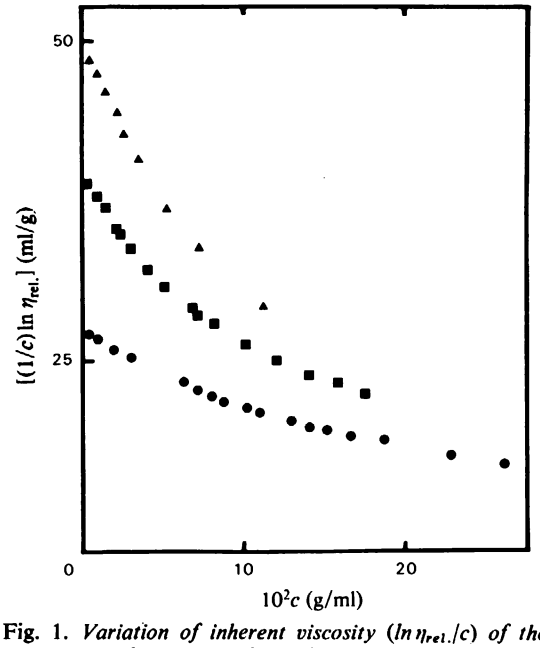

Fig. 1. *Variation of inherent viscosity* $(\ln\eta_{rel.}/c)$ *of the dextran samples with concentration*
●, Dextran 52.8; ■, dextran 122; ▲, dextran 261.

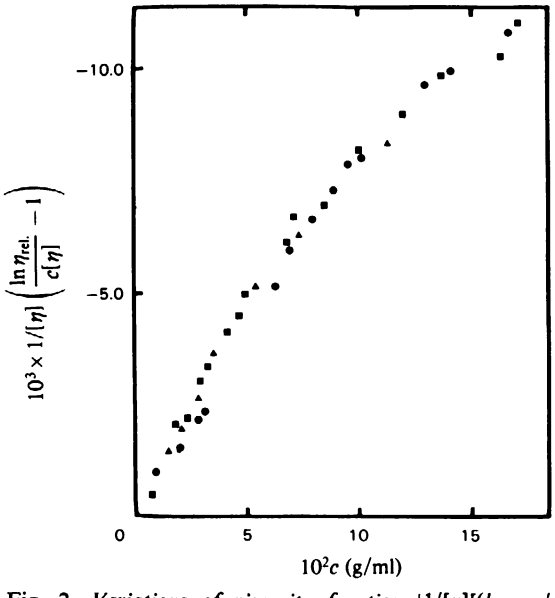

Fig. 2. *Variations of viscosity function* $\{1/[\eta][(\ln\eta_{rel.}/c[\eta]) - 1]\}$ *with concentration of solute*
●, Dextran 52.8: ■, dextran 122.0; ▲, dextran 261.

interactions, free of the primary effect of molecular weight. It is seen that the viscosity function displays no marked dependence on molecular size even up to concentrations of 0.175g/ml. The initial slope of the plot yields a value of k'' of 0.07, in good agreement with results of previous workers (Granath, 1958).

In view of the simplifying assumptions made in our treatment of the viscosity data as an estimate of molecular compression (see above), it is doubtful whether, if curve-fitting was carried out to higher powers of c to obtain estimates of κ and κ' (eqn. 18), the results would have real significance. Thus we have chosen to consider any further interpretaion of the estimated compression, by use of either eqn. (10) or eqn. (18a) in terms of an approximate linear dependence on c. That is, eqn. (10) becomes

$$V'/V^0 = 1+kc \qquad (10a)$$

and eqn. (18a) becomes

$$(\ln \eta_{rel.}/c\,[\eta]) = 1+\frac{\kappa c}{2} \qquad (18b)$$

A direct comparison of the macroscopic compression of dextran gels (Sephadex) and of the molecular compression of dextran chains, as estimated by viscometry, can be made by slight rearrangements of eqns. (10a) and (18b). If we define a quantity, the fractional compression (∇), given by

$$\frac{V^0-V'}{V^0} (\equiv 1-V'/V^0),$$

we see that from eqn. (10a)

$$\nabla = 1-V/V^0 = -kc \qquad (20)$$

and this is to be compared with the viscosity function from eqn. (18b).

$$2(\ln \eta_{rel.(c)}/c\,[\eta]-1) = \kappa c \qquad (21)$$

Figs. 3(*a*), 3(*b*) and 3(*c*) show such plots of the compression of the Sephadex gels (eqn. 20) and the molecular compression of the matched dextrans (eqn. 21).

In general, it is evident that there is reasonable agreement between these experimental estimates of compression for all three cases investigated, that is, $-k$ of eqn. (20) is similar in value to κ of eqn. (21).

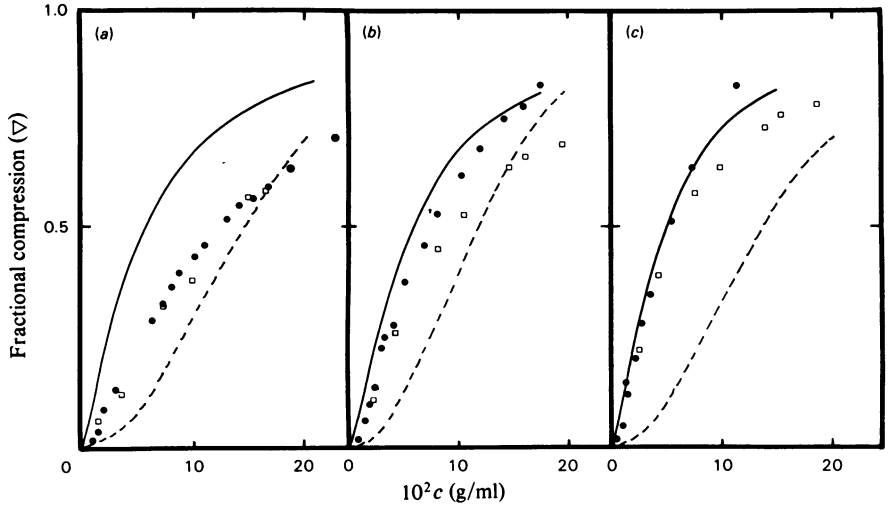

Fig. 3. *Variation of fractional compression* (▽) *of the cross-linked dextran gels* (*Sephadex*) (*eqn.* 20, □) *and of the matched molecular dextran sample* (*eqn.* 21, ●) *with solute concentration*

(*a*) Sephadex G-75–dextran 52.8; (*b*) Sephadex G-100–dextran 122; (*c*) Sephadex G-200–dextran 261. ——, Comparable plot of $1-(\alpha/\alpha^0)^3$ as calculated by use of eqn. (14a) (see the text); – – – –, plot of $(1-V'/V^0)$ as estimated by use of eqn. (4) of Simha & Zakin (1962). This takes the form of

$$(\alpha^0)^2(V'/V^0)^{5/3}-(V'/V^0)+\{8^{-2/3}\beta\,[(\overline{S^2})^0]^{3/2}/\alpha^0\}\,(V'/V^0)^{5/3}\times[1+2(\alpha^0)^2/3(V'/V^0)^{2/3}]^{1/2} =$$
$$(V'/V^0)^{5/3}[1+2(\alpha^0)^2/3]^{5/2}\times[(\alpha^0)^2-1]/[1+2(\alpha^0)^2/3\,(V'/V^0)^{2/3}]^{5/2}$$

Where β, the internal osmotic pressure is given by

$$\beta = 4/3\pi N(A_2c^2+A_3c^3)$$

By putting values of V'/V^0 into above equation the corresponding value of β is obtained, which in turn can be solved for the corresponding value of c.

With the Sephadex G-75–dextran 52.8 system, the agreement between the two sets of results is excellent over the entire concentration range studied, whereas with the higher grades of Sephadex, the higher-order terms of eqn. (10) and eqn. (18) may be significant.

Included in Fig. 3 are the theoretical estimates of the decrease in molecular volume with the change in dextran concentration as calculated by use of eqn. (14a). The plots of $[1-(\alpha/\alpha^0)^3]$ against concentration are shown. The procedure for calculating the estimated change in volume was given above. We have used the ralationship, derived by Granath (1958) for dextran polymers, of $[(\overline{S^2})^0]^{1/2}=0.066\ (\bar{M}_w)^{0.43}$, to obtain values of the root-mean-square radius of the molecule (nm) at infinite dilution.

It is seen that eqn. (14a) does predict decreases in the molecular volume of the dextrans comparable with those estimated by the experimental data. The agreement for the Sephadex G-100–dextran 122 and Sephadex G-200–dextran 261 systems is reasonably good considering the assumptions made.

Discussion

Flory (1953) pointed out in some detail that a close similarity exists between the swelling of a macroscopic three-dimensional gel network and the osmotic action of a solvent on the molecular expansion of a flexible polymer molecule in solution. In the present investigation we have attempted to make such a comparison for the dextran molecule in an aqueous environment. The macroscopic swelling of cross-linked dextrans (Sephadex) is well established, and a quantitative examination of its behaviour was made by one of us (A. G. O.) several years ago (Edmond *et al.*, 1968).

It is noteworthy that, in that study, the estimates of the second and third virial coefficients of the internal chains of dextran of the Sephadex grades were not very different from those of the parent (non-cross-linked) material.

A comparable study of the behaviour of the dextran polymer requires physical measurements into the semi-dilute region, that is up to concentrations of 0.3g/ml, where the chains overlap strongly. Interpretation of the measurements in terms of the molecular compression of the polymer molecule is difficult. The early work of Simha and co-workers (Weissberg *et al.*, 1951; Simha & Zakin, 1960, 1962) suggested that viscosity measurements could be used, but reservations were expressed as to its use as an adequate measure of the decrease in coil volume. Benoit & Picot's (1966) use of light-scattering to show that the radius of gyration of polystyrene decreased with increase of polymer concentration was limited to concentrations below 0.015g/ml. A direct measure of the decrease in molecular volume of polystyrene in the semi-dilute region has been obtained from neutron-scattering experiments on the ^{2}H-labelled polymer (Daoud *et al.*, 1975).

We chose to re-examine the variation of viscosity with concentration because of its experimental simplicity. The relation between viscosity and concentration in the semi-dilute range is determined by (*a*) long-range hydrodynamic interactions of single molecules, (*b*) formation of aggregate molecules and (*c*) osmotic compression of the polymer coil arising from short-range interchain-segment repulsions. For simplicity, we neglect (*b*), so that the apparent-viscosity increment defined in eqn. (15) is regarded as depending only on (*a*) and (*c*). By assuming that the solution to which a further molecule is added can be regarded as a uniform solvent medium of the same viscosity, we should have taken care, at least approximately, of the effects of hydrodynamic interaction (*a*). It may be that, at the higher concentrations studied, this approach is oversimplified and may lead to underestimation of the decrease in coil volume. Alternatively, if interpenetration and entanglement of the coil segments takes place to any marked extent (which would be contrary to our simplifying assumptions made above), then the apparent-viscosity increment may overestimate the extent of molecular compression.

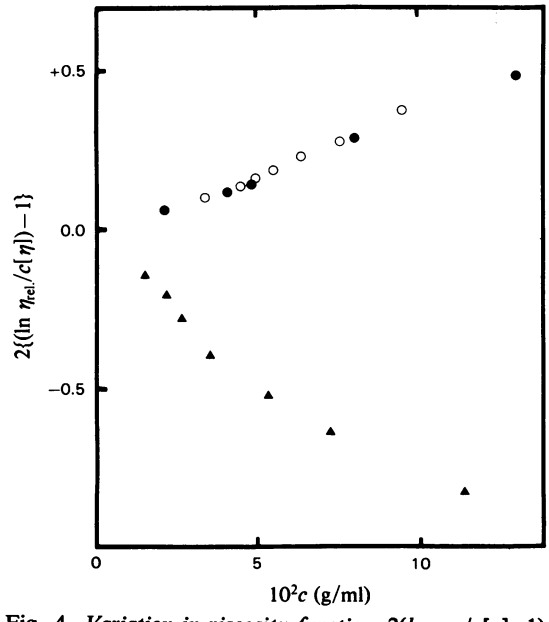

Fig. 4. *Variation in viscosity function,* $2(\ln \eta_{rel.}/c[\eta]-1)$, *with concentration*

○, Bovine serum albumin; ●, Percoll; ▲, dextran 261. Albumin data are from McMillan (1974) and Percoll data are from T. C. Laurent (unpublished work).

The reduced form of the viscosity plot given in Fig. 2 suggests that the higher-order viscosity effects are relatively independent of the molecular weight of the polymer. In this concentration range it appears that the molecules may lose their individual integrity, and the changes in viscosity reflect interchain-segment interaction.

We decided to compare the form of the plot of eqn. (21) when this relationship is applied to viscosity data of structures or molecules that are considered to be compact and incapable of undergoing any marked decrease in molecular volume. Data on serum albumin are available from McMillan (1974) and on Percoll, a commercial preparation of silica particles (Pharmacia A.B., Uppsala, Sweden) were given to us by Professor T. C. Laurent (unpublished work), University of Uppsala, Uppsala, Sweden. The results of these calculations are shown in Fig. 4, where the data on dextran 261 are included for comparison. It is evident that the two compact particles display similar behaviour, the viscosity function $2(\ln \eta_{rel.}/[\eta]c-1)$ having positive values. This is in marked contrast with that of the dextran, where we have interpreted the negative values of the function as indicating a decrease in molecular volume. Following the reasoning given above, the positive nature of this function for albumin and Percoll may be understood in terms of an apparent increase in the molecular volume arising from hydrodynamic interactions not taken care of in our simplifying assumptions.

As suggested by Weissberg *et al.* (1951), it may be convenient for a discussion of the solution viscosities in terms of the present compression effects to introduce a reduced concentration scale. An obvious choice of a normalizing concentration is c^*, the concentration at which significant overlap of molecular domains occurs, given by Simha & Zakin (1960) as $c^* = 1.08/[\eta]$. However, this quantity should be corrected for the compression of molecules at the concentration of incipient overlap, that is the scale of concentration is $c/(c^*)'$, where $(c^*)' = c^*(V^0/V')$.

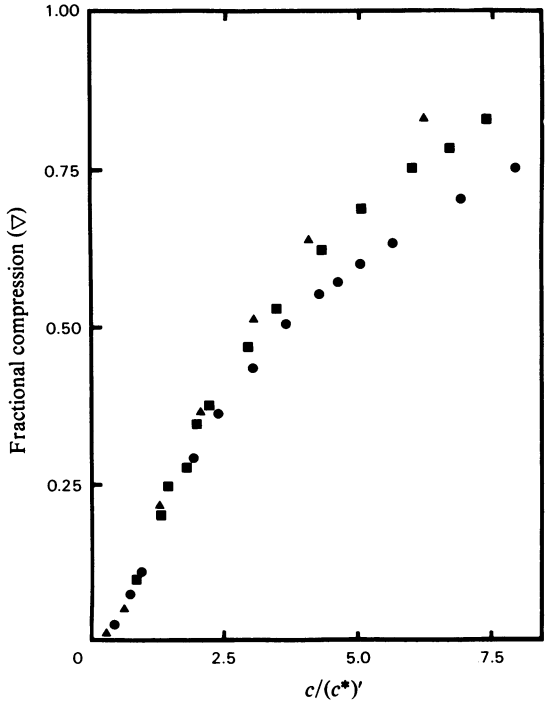

Fig. 5. *Variation of fractional compression of the dextran samples against reduced concentration scale,* $c/(c^*)'$ $(c^*)'$ is given by $c^*(V^0/V')$, where (V^0/V') is the factor allowing for the compression of the dextran molecule at the concentration of incipient overlap of molecule domains. c^* in turn is given by $1.08/c[\eta]$ (Simha & Zakin, 1960). The compression is estimated from the viscosity data by use of eqn. (21), that is, $\nabla = -2(\ln \eta_{rel.(c)}/c[\eta]-1)$. ●, Dextran 52.8; ■, dextran 122.0; ▲, dextran 261.

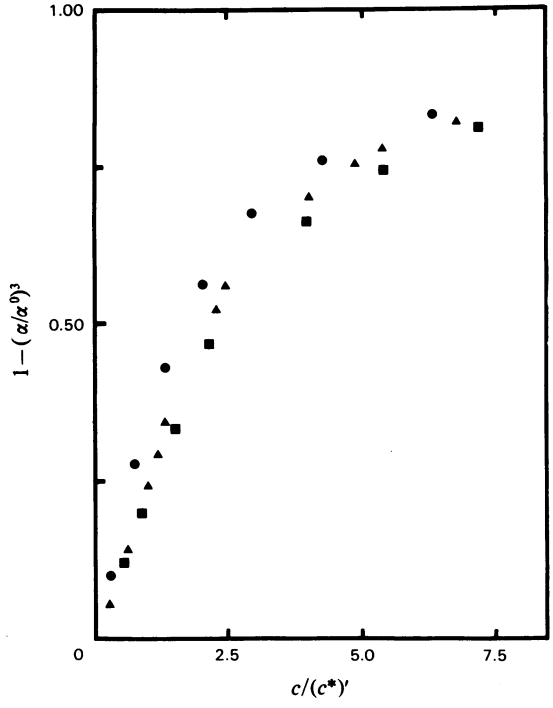

Fig. 6. *Plot of theoretical decrease in coil volume,* $1-(\alpha/\alpha^0)^3$, *against reduced concentration scale* $c/(c^*)'$ *(see legend to Fig. 5)*

The values of $(\alpha/\alpha^0)^3$ are calculated by use of eqn. (14a) (see the text). ●, Dextran 52.8; ■, dextran 122.0; ▲, dextran 261.

On this scale, it is observed that the curves of the fractional compression ∇ against concentration of the different molecular-weight dextrans are approximately superimposable (Fig. 5). It is seen that at $c/(c^*)' = 1$, the different dextrans have all undergone a compression of about 20%. Only at concentrations of $c/(c^*)' > 3$ do the curves diverge. Thus it may be considered that the variable $c/(c^*)'$ defines approximately corresponding physical states for the different molecular-weight species. This is again emphasized if the theoretical curves of $1-(\alpha/\alpha^0)^3$ (from use of eqn. 14a) are plotted against $c/(c^*)'$ (Fig. 6), where approximate coincidence is seen over the entire concentration range.

In the studies by Simha and his co-workers (Weissberg *et al.*, 1951; Simha & Zakin, 1960) an alternative theoretical approach was offered to estimate the molecular-coil shrinkage. Although it is of similar form to our expression given above, it differs in as much that it considers the pressure causing the decrease in coil volume is the internal osmotic pressure of the solution, that is, the excess of osmotic pressure over the van't Hoff molecular-weight term. We have calculated the estimated compressions by use of eqn. (4) of Simha & Zakin (1960) and these are included in Figs. 3(*a*), 3(*b*) and 3(*c*) as the broken lines.

It is seen in all the systems studied that the experimental estimates of compression from both gel shrinkage and viscosity measurements lie in between the two predicted curves. Because of the simplifying assumptions made, quantitive agreement of the several estimates of molecular shrinkage were not to be expected. However, the evidence does indicate that marked osmotic compression of flexible molecular structures occurs within the semi-dilute concentration range. Such decreases in molecular volumes may have important effects on transport processes in systems of this type, including multicomponent systems found in living organisms.

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