The Molecular Size and Shape of Liver Glycogen

By ROBERT GEDDES, JOHN D. HARVEY and PETER R. WILLS
Departments of Biochemistry and Physics, University of Auckland, Auckland, New Zealand

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The molecular-weight distribution of liver glycogen has been established from the analysis of sedimentation rates of fractions separated on sucrose density gradients and from the direct measurement of the diffusion coefficients of these fractions by laser-intensity-fluctuation spectroscopy. Hydrodynamic studies indicated that all fractions of glycogen of mol.wt. exceeding 25×10^6 had about 1.1 g of water per g of polysaccharide associated with them. The hydration and hydrodynamic behaviour of all fractions of mol.wt. exceeding 25×10^6 was similar, whereas smaller fractions behaved anomalously, indicating a substantially different overall structure.

Liver glycogen has both large molecular size and great polydispersity. Because of the latter property, any observations on its size are of little significance without knowledge of the distribution of the different-sized particles. Electron microscopy (Drochmans, 1962, 1966) has shown glycogen to be composed of single spheres (β -particles) and clusters of spheres (α -particles). The α -particles appear to be held together by covalent bonds, since hydrogen-bond-breaking reagents do not affect them (Orrell & Bueding, 1964).

Previous attempts (Larner *et al.*, 1956; Bryce *et al.*, 1958*b*) to measure accurately the molecular-weight distribution of glycogen used the Schlieren patterns obtained in the analytical ultracentrifuge and involved essentially the method of Baldwin (1954). The procedure involves a tedious series of manipulations and depends on the accuracy of independent measurements of the s^0 -mol.wt. relationship, which was found to be impossible to define accurately (Bryce *et al.*, 1958*b*). Later experiments (Mordoh *et al.*, 1966) with a sucrose gradient relied on the calibration of the gradient by only one sample, and were troubled by variable amounts of glycogen appearing in a pellet, making difficult calculations of the mean molecular weight (Parodi *et al.*, 1970). This method also relied on the calculations described by Martin & Ames (1961) and yielded only approximate molecular weights, since fine hydrodynamic data were unavailable.

Attempts to measure the molecular-size distributions from electron micrographs have either been limited to muscle glycogen (Wanson & Drochmans, 1968), which contains only β -particles, or have been limited by the difficulties of measuring accurately the dimensions of large, irregular particles (Laskov & Gross, 1965; Burchard *et al.*, 1968).

Sedimentation-coefficient distributions have been described by a number of authors (Orrell & Bueding,

1964; Krisman & Parodi, 1970; Geddes, 1971), but are of limited interest, since little information is available on the hydrodynamic behaviour of large glycogen particles.

Although molecular weights can be accurately calculated by application of the Svedberg equation, its usage has been seriously limited because of difficulties in measuring the diffusion coefficient (D) and the large errors involved in this. However, the use of the relatively new technique of laser-intensity-fluctuation spectroscopy (see, for example, Chu, 1974) enables D to be measured rapidly, and with high precision. The technique has been used on particles of a size similar to that of glycogen (Farrell *et al.*, 1974).

Other factors affecting the molecular size of glycogen are the method of extraction (Orrell & Bueding, 1964), and the lability of the glycogen during the period when the tissue is being removed (Geddes & Rapson, 1973). The experiments described in the present paper used glycogen extracted by a cold-water method (Laskov & Margoliash, 1963), which yielded apparently undegraded material; the livers were removed from the rats or rabbits and placed in liquid N_2 within 40 s of death.

The present paper aims to provide a thermodynamically sound molecular-weight distribution and to indicate, within the capabilities of current hydrodynamic theories, the shape of the very large glycogen particles. Results of application of these methods to the glycogens extracted from cell sub-fractions are described in the preceding paper (Geddes & Stratton, 1977).

Experimental

General

Reagents were of analytical grade. Water was glass-distilled, having previously been de-ionized.

Preparation of glycogen

Glycogen was prepared as described in the preceding paper (Geddes & Stratton, 1977) with the exception that the main source of livers was from rabbits (New Zealand White). No significant distinction was detected in distributions obtained from glycogens from rat or rabbit livers, and all conclusions are taken as applying to both.

Preparation of subfractions of glycogen

Glycogen was subfractionated on sucrose gradients as described in the preceding paper (Geddes & Stratton, 1977) by using a Beckman Spinco L2-50 preparative ultracentrifuge equipped with a SW25.2 rotor. The acceleration and deceleration periods were followed carefully to allow accurate measurement of $\omega^2 t$.

Concentrations of glycogen

Glycogen concentrations were measured by using an iodine-iodide reaction (Drochmans, 1966).

Calculation of weight-average sedimentation coefficients

The weight-average sedimentation coefficients of the various glycogen sub-fractions obtained from the sucrose gradients were calculated by using the equation of Martin & Ames (1961) in combination with simple empirical equations (Barber, 1966) to express the sucrose density and viscosity as a function

of its concentration. The equations proposed by Dingman (1972) were found to be unsatisfactory.

It has been established by separate experiments that the sedimentation coefficients of glycogens of the very large sizes described in the present paper have no significant dependence on concentration (Fig. 1). This means that the values calculated by the above procedure are s^0 values. (The superscript has been omitted for convenience in further arguments.) The sedimentation coefficient of the small glycogen particles (fractions 17–20) will show some slight dependence on concentration, as has been described (Larner *et al.*, 1956; Bryce *et al.*, 1958*b*). This dependence has been applied to calculations on the small particles.

Measurement of D

Diffusion coefficients were determined by laser-intensity-fluctuation spectroscopy. Detailed discussions of the theory of this method are available in a number of reviews (Chu, 1974). Samples of glycogen in 0.1 M-NaCl (200 μ l, 0.5–10.0 mg/ml) were placed in specially constructed glass cuvettes (internal dimensions 20 mm $\times$ 10 mm $\times$ 2 mm) and transferred into the path of a focused beam from an argon laser (model 165-03; Spectra Physics, Mountain View, CA, U.S.A.). The scattered light was collected at an angle of 90° through two collimating apertures (of 0.5 mm diameter) mounted 1 m apart, and detected by a 56 AVP photomultiplier tube. The autocorrelation function of the photomultiplier anode current was determined by a Hewlett-Packard model 3721A correlator interfaced directly to a PDP11 computer.

Fig. 1. Concentration-dependence of the diffusion coefficient (a) and sedimentation coefficient (b) of glycogen. Measurements were performed on various fractions of glycogen in 0.1 M-NaCl solution at 25°C, and the results are typical of those obtained. Diffusion coefficients were measured as described in the Experimental section. Sedimentation coefficients were measured in a Beckman-Spinco model E ultracentrifuge equipped with Schlieren optics and an An-D rotor.

Temperature was always constant within 0.1°C over the period of the experimental measurements (Harvey, 1973).

For some samples, measurements were made in the presence of sucrose within 1 h of their fractionations. After correction for viscosity and refractive index the diffusion values were within the limits of error of the same samples measured in 0.1 M-NaCl.

In agreement with previously published results (Bryce *et al.*, 1958b), no dependence of D on concentration was found (Fig. 1). Thus our values are equivalent to D^0 values.

Problem of polydispersity

For a polydisperse system there will be a distribution of diffusion coefficients and the autocorrelation function will then be a combination of exponentials. It is therefore essential to check that pronounced curvature has not been introduced into the logarithm of the autocorrelation function. Consequently this logarithmic function was fitted to a quadratic equation by using the first 10, 20, 30 or 40 data-points. For all fractions the coefficient of the linear term of the quadratic showed no significant dependence on the number of data-points used, indicating that a reliable mean value of the diffusion coefficient could be estimated from this coefficient (this mean value will be the z -average diffusion coefficient) (Pusey, 1973).

Calculation of molecular weight

Molecular weights for the fractions were calculated by using the Svedberg equation. Since these molecular weights arise from the ratio of a weight-average sedimentation coefficient to a z -average diffusion coefficient, they will be weight-average values (Pusey, 1973).

Measurement of $\bar{v}$

The partial specific volume $\bar{v}$ of a substance can be determined by measurement of the density ρ' of solutions of known concentration c . If ρ_0 is the solvent density then:

$$\bar{v} = \lim_{c \rightarrow 0} \frac{1}{\rho_0} \left(1 - \frac{\rho' - \rho_0}{c} \right) \quad (\text{Kratky } et al., 1973)$$

Solutions of known glycogen concentration were prepared by dissolving an accurately weighed quantity of previously dehydrated glycogen in a known volume of pure water. The density of these solutions, whose concentrations varied from approx. 2–20 mg/ml, was determined by using a 50 ml 20°C density bottle. All weights were determined with a Mettler type H16 electronic balance (E. Mettler Ltd., Zurich, Switzerland), and all measurements were performed at 20°C. Results are shown in Table 1.

Viscosity measurements

The intrinsic viscosity ($[\eta]$) of the glycogen fractions was determined with a KIMAX size-50 viscometer. All measurements were performed at $25.00 \pm 0.02^\circ\text{C}$, in a thermostatted water bath (P. M. Tamson N.V., Zoetermeer, The Netherlands). A 5.0 ml portion of each sample (or dilutions of it) was placed in the viscometer and the flow time measured to within ± 0.04 s by using electronic stopwatches. The concentration of each sample was determined by using the phenol/sulphuric acid method (Dubois *et al.*, 1956).

No significant dependence of $[\eta]$ on c was found for any of the fractions. Random errors in the determination of $[\eta]$ made it difficult to extrapolate accurately to the zero concentration, so it was assumed that no appreciable dependence on c existed, and $[\eta]$ was taken to be the mean of the $[\eta]$ values obtained at each concentration of a particular fraction. All measurements of $[\eta]$ were made in distilled water. Measurements of $[\eta]$ in 0.1 M-NaCl solution for six fractions showed no significant difference from the values measured in water.

Studies on models

Models of glycogen α -particles were constructed by carefully gluing together 5.25 mm-diameter silica spheres. With all the models it was found that the contribution of the adhesive to the overall weight was negligible. Models consisting of more than two spheres were made to have differing, though compact, symmetries. The various symmetries contributed only slightly to the frictional drag on the model (see Fig. 4 below).

The models were allowed to sediment, under gravity, through glycerol in a Perspex cylinder (radius 16 cm, height 38 cm) in a room maintained at $5^\circ\text{C} (\pm 0.5^\circ\text{C})$ during the period of any measurement. Sedimentation of the models was timed over a region starting 5 cm from the surface to 3 cm from the bottom of the cylinder. It was ensured that air was expelled from the spaces between the spheres by the

Table 1. *Partial specific volume of glycogen*
For details, see the text. The glycogen was a polydisperse sample which was dehydrated before use.

[Glycogen] (mg/ml)	Partial specific volume
	($\bar{v}$) (ml/g)
2.48	0.64 ± 0.08
3.16	0.62 ± 0.06
5.45	0.62 ± 0.04
10.22	0.65 ± 0.02
19.01	0.62 ± 0.01
Average	0.63 ± 0.02

injection of glycerol into these spaces. The possibility of wall effects interfering with the sedimentation had already been eliminated (Harvey *et al.*, 1974).

Results

Results of experimental measurements on 20 glycogen fractions are shown in Table 2. The molecular-weight distribution averaged over 30 samples isolated from different livers is shown in Fig. 2. It is obvious that glycogen contains material of sizes exceeding even that of some viruses, e.g. reovirus 1.3×10^8 (Farrell *et al.*, 1974) and tobacco mosaic virus 4×10^7 (Anderer, 1963). No close comparison can be made between this distribution and others (e.g. Parodi, 1967; Parodi *et al.*, 1970) obtained with the method described by Mordoh *et al.* (1966) because of the crudeness of calibration of the latter method. However, it is clear that these authors are assigning much higher molecular weights in comparison with the sedimentation coefficients which they calculated. For example, for values of 500S and 4000S, molecular weights based on the calculations of Mordoh *et al.* (1966) give values of 1×10^8 and 2×10^9 respectively, whereas the calculations in the present paper yield values for mol.wt. of 0.65×10^8 and 1.07×10^9 . Inspection of Table 2 also indicates that 20% of the glycogen is above a value (arbitrarily chosen) of 5×10^8 . A similar analysis attempted on Fig. 1 of the paper by Parodi (1967) yielded values varying between approx. 30 and 40%. It seems possible that workers using the extraction technique recommended by Mordoh *et al.* (1966) have lost some of the low-molecular-weight glycogen, perhaps by failing to use a sufficient volume of ethanol to precipitate glycogen from aqueous solution [Mordoh *et al.* (1966) recommend the use of 2 volumes, whereas other workers generally use 3 volumes], or by failure to add a lithium salt to check that all the glycogen had indeed been precipitated by the addition of ethanol. Both these precautions were observed in all the preparations described in the present paper, and the reproducibility (as shown in Fig. 2) that was obtained from the combined results from 30 livers was remarkably good and was not observed by the other method (Parodi, 1967).

Wanson & Drochmans (1968) showed that the mean sedimentation coefficient of β -particles of glycogen is about 130S, and a rough calculation shows that their molecular size must be of the order of 10^7 daltons. This means that they will, practically exclusively, be located in fractions 19 and 20.

Preliminary electron-microscopic studies have confirmed that 'average' particles, even in fraction 18, are dimers or trimers, in agreement with the above. Further, as the preliminary results in Table 3 show, the size of the β -particle subunits are similar to those reported previously for glycogens from liver

(Drochmans, 1962; Laskov & Gross, 1965). There is a suggestion in these preliminary results that in the very-low-molecular-weight fractions, such as fraction 18, the formation of the β -particles, even though they are joined in dimers and trimers, may be incomplete. Hydrodynamic studies (see below) have shown this to be a reasonable conclusion.

Hydrodynamic studies

The problems of defining a macromolecular size for a hydrated particle in solution have been clearly described by Ogston (1953) and by Tanford (1961). The specific volume of the macromolecule in solution, V' , will be defined by the following equation:

$$V' = \bar{v} + \delta \bar{v}_0$$

where $\bar{v}$ and $\bar{v}_0$ are the partial specific volumes of the solute and solvent respectively, and δ is the number of grams of solvent associated with 1 g of the unsolvated macromolecule. Because of the nature of the available theories it is necessary to define an equivalent hydrodynamic sphere that is of the same volume as the real solvated particle. The radius of such a sphere is defined as:

$$r = \left[\frac{3M}{4\pi N} (\bar{v} + \delta \bar{v}_0) \right]^{1/3} \quad (1)$$

Fig. 2. Molecular-weight distribution of liver glycogen. The calculations from which this is derived are described in the text. The distribution is an average obtained from glycogens isolated from 30 different rabbit livers (Geddes & Stratton, 1977). Error bars indicate the relative standard deviation in the concentration for a typical fraction. The mean concentration in each fraction was divided by the molecular-weight width of the fraction so that the abscissa could be transported from fraction number to molecular weight. The distribution was then normalized. The inset shows details of the high-molecular-weight region on an expanded scale.

Table 2. *Physical characteristics of glycogen fractions*

The sedimentation coefficients ($s_{20,w}^0$), the diffusion coefficients ($D_{20,w}^0$) and the intrinsic viscosity were measured as described in the Experimental section. The mol.wts. were calculated from the Svedberg equation:

$$M = \frac{RTs_{20,w}^0}{D_{20,w}^0(1 - \bar{v}\rho)}$$

Fraction no.	$s_{20,w}^0$ (S)	$10^8 \times D_{20,w}^0$ (cm ² /s)	$10^{-6} \times$ Mol.wt.	Intrinsic viscosity $[\eta]$ (ml/g)
20	24	2.56	6	14.3
19	76	2.35	21	8.5
18	157	4.45	23	6.48
17	260	5.90	29	6.03
16	379	5.87	42	6.10
15	511	5.06	66	6.94
14	656	4.32	100	6.94
13	810	3.63	150	6.43
12	974	3.36	190	6.39
11	1146	3.01	250	7.35
10	1325	2.74	320	7.08
9	1510	2.73	360	6.75
8	1702	2.75	410	6.49
7	1899	2.42	520	6.45
6	2104	2.57	540	6.56
5	2323	2.27	670	7.15
4	2564	2.41	700	7.49
3	2858	2.21	850	7.10
2	3290	2.17	1000	6.82
1	4106	2.49	2080	7.06
Estimated error	$\pm 4\%$	$\pm 4\%$	$\pm 10\%$	$\pm 6\%^*$

* Excluding fractions 19 and 20, where errors were estimated at $\pm 40\%$ because of the small amounts of sample.

Table 3. *Electron-microscopic measurements of glycogen particles*

Selected fractions of glycogen were negatively stained with uranyl acetate and were photographed at a magnification of approx. 50000 $\times$ in an electron microscope (by courtesy of Associate-Professor S. Bullivant, Department of Cell Biology, Auckland University). Particles in the photographs were then randomly counted and measured.

Fraction no.	No. of particles counted	Weight-average no. of β -particles per α -particle (n_w)	Arithmetic mean diameter of β -particles (d) (nm)
18	176	2.6	21
16	157	4.0	25
14	143	8.0	25

This is the f obtainable from the diffusion coefficient, and r is the radius of the equivalent hydrodynamic sphere.

$$f = \frac{kT}{D^0} \quad (3)$$

It should therefore be noted that f depends on two unknowns, namely f/f_0 and δ . Tanford (1961) defined another quantity $f_{\min.}$, the frictional ratio for the unsolvated particle:

$$f_{\min.} = 6\pi\eta r_{\min.} \quad (4)$$

where

$$r_{\min.} = \left(\frac{3M\bar{v}}{4\pi N} \right)^{1/3} \quad (5)$$

Use of the ratio f/f_0 rather than $f/f_{\min.}$ allows an interpretation of the observed hydrodynamic quantities as a function of shape only. Scheraga & Mandelkern (1953) disputed the use of eqn. (4) and define the function β from which the effective hydrodynamic shape could be estimated without recourse to estimations of the degree of hydration. This, naturally, will only give the effective solution dimensions of the macromolecule with its associated water, and cannot

where M is the molecular weight of the particle and N is Avogadro's number, and the frictional coefficient (f) of the real solvated particle is given by:

$$f = \left(\frac{f}{f_0} \right) 6\pi\eta r \quad (2)$$

Fig. 3. Effect of molecular weight on various hydrodynamic parameters of glycogen

In all functions, variations outside of the scale depicted have been omitted. These variations only occurred in the low-molecular-weight fractions. The numerical data for all fractions are summarized in Table 4. The function $\psi^{3/2}(J)\xi^{-1/2}(J)$ has been described by Ogston (1953). The Scheraga-Mandelkern function, β , was calculated from:

$$\beta = \frac{sN[\eta]^{1/3}\eta_0}{M^{2/3}(1-\bar{v}\rho)}$$

The viscosity increment (ν) (Simha, 1940) was calculated from:

$$\nu = \frac{M[\eta]}{NV_e}$$

where V_e may be put equal to $M\bar{v}/N$, yielding a $\nu_{\max}$, which refers to the anhydrous molecule, or V_e may be calculated from eqn. (2), assuming a sphere, and yielding $\nu_{\min}$. $f/f_{\min}$ may be deduced from eqns. (3), (4) and (5).

be directly related back to the anhydrous particle. Calculations for various hydrodynamic parameters with the associated errors for all 20 glycogen sub-fractions are shown in Fig. 3 and Table 4. Regardless of the precise physical significance of each individual value, it is clear that for fractions 1-16 inclusive the effective hydrodynamic conformation of the glycogen molecules does not change, even though the average molecular weight of the molecules varies between 3×10^7 and 1×10^9 . The low-molecular-weight fractions (17-20) do show significant differences in hydrodynamic behaviour (see Table 4).

Since the calculation of the frictional ratio is based on the anhydrous volume, it is, in fact, $f/f_{\min}$. [A note on the effect of polydispersity of $f/f_{\min}$. It can be shown that, in a polydisperse system, the frictional ratio (as estimated from $\bar{D}_z$ and $\bar{s}_w$) is given by $\lambda \times f/f_{\min}$, if all molecules have the same frictional ratio, where:

$$\lambda = \left(\frac{\sum_{ijk} n_i M_i^2 n_j M_j^2 n_k M_k}{\sum_{ijk} n_i M_i^{5/3} n_j M_j^{5/3} n_k M_k^{5/3}} \right)^{1/3}$$

if there are n_i molecules of M_i mol.wt. etc. Calculation of λ over the distribution described in the present paper has shown it to be equal to 1.00 ± 0.01 and consequently it has been ignored.] f/f_0 , however, can be estimated independently (see below) by the use of models.

The β function of Scheraga & Mandelkern (1953), like the other properties mentioned, remains effectively constant over the range of fractions from 1 to 16. Its average value ($2.06 \times 10^6 \pm 0.17 \times 10^6$) is in agreement with results obtained with the similar macromolecule, acid-hydrolysed dextran NRRL B-512 (Senti *et al.*, 1955).

Anomalous fractions

From Table 4 it is clear that the low-molecular-weight fractions show anomalous hydrodynamic behaviour. The reason for this is probably that the β -subunits of the glycogen particles have not been

Table 4. Hydrodynamic parameters of glycogen molecules

The derivations of all the functions, except ν , are described in the legend to Fig. 3. The derivation of ν , the real viscosity increment, is described in the text. n.c., Not calculated.

Fraction no.	$f/f_{\min}$	$\nu_{\min}$	$\nu_{\max}$	β	$\psi^{3/2}(J)\xi^{-1/2}(J)$	ν
1-16	1.67 ± 0.11	2.38 ± 0.69	10.82 ± 0.53	2.06 ± 0.17	0.66 ± 0.07	$3.85 \pm 0.59^*$
17	1.88	1.45	9.57	1.76	0.82	n.c.
18	2.68	0.53	10.29	1.26	1.36	n.c.
19	5.24	0.09	13.49	0.72	3.24	n.c.
20	7.30	0.06	22.70	0.60	4.11	n.c.

* Excluding fraction 1, which was anomalously high.

Fig. 4. Frictional ratios of glycogen particles $f/f_{\min.}$ is calculated from the equation:

$$\frac{f}{f_{\min.}} = \frac{kT}{D\eta} \left(\frac{N}{162\pi^2\bar{v}M} \right)^{1/3}$$

which is easily derived from eqns. (3), (4) and (5), and refers to the experimentally observed frictional characteristics of the real glycogen particles. f/f_0 is the ratio of the observed sedimentation time for models of glycogen α -particles as compared with the time computed for a spherical particle of the same weight and density. The models have been placed on the abscissa by assuming a mol.wt. for β -particles of 12×10^6 . Where different (compact) models incorporating an equal number of spheres were constructed, the results are displaced slightly for clarity. The inset shows the full range of frictional ratios observed.

consume with its $\alpha 1 \rightarrow 4$ -linked glucose chains (Stetten & Katzen, 1961) and consequently the less permeable to solvent the particle will be. The results from the smaller molecules indicate that in these particles the solvent can flow through them, thereby disturbing the smooth flow lines of solvent flowing around them. The larger particles, having a denser surface, appear to be relatively impermeable to solvent flow and hence behave as if comprised of fairly ideal spherical particles.

Studies on models

Models of glycogen α -particles, constructed by gluing together silica beads, were used to determine the effect of shape alone on the frictional properties of the particles. The results are shown in Fig. 4. Only in the cases of the small models (three to five particles) did the possible isomers make any significant difference to f/f_0 . Once the number of particles was sufficient, if it was assumed that the structure of the model α -particle was compact rather than a linear collection of spheres, then differences in f/f_0 (arising from different closely packed groupings of spheres of the same overall weight) were within experimental error. The results show that the effect of shape alone would give rise to an f/f_0 of value approx. 1.2, whereas the experimentally measured $f/f_{\min.}$ is 1.7 ± 0.1 for fractions 1-16 (Table 4). It should be noted that other authors (for example, Bryce *et al.*, 1958a) have used a variant of eqn. (2) in their derivations:

$$f = 6\pi\eta r'$$

It is clear that r' refers to the effective radius rather than the radius of the equivalent hydrodynamic sphere. Values of r' calculated on this basis will clearly be too high by a factor of f/f_0 .

When this value f/f_0 is applied to eqn. (2), the radius of the real solvated particle, r , may be calculated, and from this a value of δ , the amount of solvent bound (g/g), may be calculated from eqn. (1). The average value of δ for fractions 1-16 is 1.1 ± 0.3 g/g, which is of the same order as that calculated (Harvey *et al.*, 1974) for a virus of mol.wt. 1.3×10^8 .

Direct calculation of the real viscosity increment (ν) (Simha, 1940) rather than the maximum and minimum values shown in Fig. 3:

$$\nu = \frac{[\eta]}{V'}$$

and

$$V' = \frac{V_e N}{M}$$

fully formed (or have been partially degraded), as was suggested by the preliminary electron-microscopic study reported above. Because of the nature of its regularly branched sub-structure, the larger the β -particle the more of its own surface area it will

where V_e is the volume of the equivalent hydrodynamic sphere. Clearly:

$$V_e = \frac{4}{3} \pi r^3$$

and r is directly accessible from eqn. (2) when f/f_0 is known. The average value of v for the high-molecular-weight fractions is 3.9 (Table 4), whereas the value for an impermeable sphere is 2.5. It could not be evaluated for the anomalous fractions 17–20 because of the lack of reliable f/f_0 values.

Conclusions

Our experiments have provided a thermodynamically sound confirmation for the uniquely large molecular weight of glycogen and its great polydispersity. The molecular weight of glycogen is rivalled only by the sizes reported for isolated samples of nucleic acid. Clearly the glycogen molecule is constructed to perform its metabolic role of glucose supplier most efficiently. The single spheres (β -particles) of glycogen could not continue to increase in size as spheres because they would consume their own surface area (Stetten & Katzen, 1961) and eliminate the accessibility of enzymes to the non-reducing chain ends. Indeed, even the action of some α -amylases on 'normal' glycogen is restricted by the size of the protein molecule (Geddes, 1968). Having an agglomeration of the β -particles provides a very great density of non-reducing chain ends without any appreciable contribution to the osmotic pressure. The polydispersity arises partially from the non-specificity of synthesis with respect to molecular size and partially from the compartmentation of metabolism (Geddes & Stratton, 1977).

From the hydrodynamic analyses and the experiments with the models of α -particles even the most massive particles are seen to assume a smooth hydrodynamic shape when in solution. Hydration increases the effective radius of the particles by about 40%. As pointed out by Ogston (1953), the amount of water which we have shown (1.1 g/g of polysaccharide) to be associated with the particles is some sort of average and could well be different under different hydrodynamic conditions. For example, we (and most other workers) have measured viscosity under conditions that were not purely Brownian (capillary flow), and diffusion under Brownian conditions, hence it is possible that the loosely associated water in the hydration region surrounding the molecules may differ in amount under these different experimental conditions, and our measurements may represent the average between these two states. [This point was originally made by Mehl *et al.* (1940).] It is clear both from the direct experimental results and from the model studies that above 25×10^6 daltons

the shape alone causes no measurable effect upon the hydrodynamic behaviour of the particle. Below this size 'incomplete' β -particles behave anomalously and cannot be approximated easily by model studies. These particles appear to be porous to solvent, thus increasing their frictional ratio drastically.

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