138. *Physicochemical Studies on Starches. Part XII.* The Molecular Weight of Glycogens in Aqueous Solution.*

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Molecular weights are presented for 23 samples of glycogens isolated from various biological sources. Ultracentrifugal analysis showed that most of the samples were polydisperse. The molecular weights of the main components lie in the range (3—9) $\times 10^6$. The polydisperse nature of the glycogens has been confirmed by light-scattering measurements. The effects of varying the isolation procedure, and of alkali, on the molecular weight have also been studied.

Glycogen and amylopectin are both highly branched, essentially α-1 : 4-linked glucosans. However, their hydrodynamic properties are completely different. This must be related to fundamental differences in fine structure and molecular shape.[1,2] In this paper, we describe the solution properties and hydrodynamic behaviour of glycogens isolated from a variety of biological sources. Estimations of molecular weight and its distribution have been obtained, and the effects of variations in the method of isolation, and of alkali, on the molecular weight have been studied. A preliminary account of some of this work has already appeared.[3]

Experimental

Sedimentation Measurements.—The methods described in Part XI [4] were employed. M- and 0·1M-sodium chloride and 0·2M-potassium hydroxide were used as solvents.

The sedimentation constant (S_{20}) was virtually independent of the solvent, and the majority of the measurements were carried out in either M- or 0·1M-sodium chloride. Results were corrected to water at 20°.

The apparent amount of each component in a resolvable polydisperse system was estimated by direct measurement of the areas under the refractive-index gradient curves. An enlarged image (3×) of the photographic plates was projected on smooth paper and the upper outline traced. An image of the base line (from a comparative run with solvent alone in the cell) was then superimposed by alignment of the reference lines, and traced on. The refractive-index gradient curves were carefully divided, in the usual manner, on the assumption that each component had a symmetrical distribution, and the appropriate areas between the peaks and the base-line were measured with a planimeter. Values were expressed to the nearest 5%.

Estimations of the polymolecularity of the major component of some of the glycogen samples were obtained by using Gralen's function,[5] dB/dX, where B is an estimate of the "width" of the sedimentation gradient curve and is equal to H/A (A = area of the Schlieren diagram; H = the height of the maximum ordinate), and X = the distance of the peak from the axis of rotation. In all instances, B varied linearly with X. Although this function should be extrapolated to infinite dilution, the value at $c = 1$ g. per 100 ml. was taken as a standard for comparison of the polymolecularity of different samples.

Diffusion Measurements.—The method is outlined in Part X.[6] The solvent was 0·1M-sodium chloride, and values of the diffusion constant (D_m) were calculated by the moment method.

Partial Specific Volume.—The partial specific volume ($\bar{V}$) of glycogen was taken as 0·62, the value calculated from density measurements on aqueous solutions of one sample.

Light-scattering Measurements.—The apparatus and the methods used to clarify and dilute the glycogen solutions were similar to those previously described for the *Zea mays* polysaccharides,[6] 0·1M-sodium chloride being the solvent. Although 15% aqueous magnesium

* Part XI, preceding paper.

[1] Part IV, Bryce, Cowie, and Greenwood, *J. Polymer Sci.*, 1957, **25**, 251.
[2] Greenwood, *Adv. Carbohydrate Chem.*, 1956, **11**, 335: Manners, *ibid.*, 1957, **12**, 261.
[3] Greenwood and Manners, *Proc. Chem. Soc.*, 1957, 26.
[4] Preceding paper.
[5] Gralén, Inaugural Diss., Uppsala, 1944.
[6] Part X, Greenwood and Das Gupta, *J.*, 1958, 703.

chloride has been recommended,[7,8] we found this solvent to have no advantages. The value of the molecular weight of a given sample was the same in both the above solvents. Glycogen solutions were clarified by careful filtration (cf. ref. 8) through sintered glass (G4). Repeated filtration caused some small loss in turbidity, whilst little improvement occurred in the apparent dissymmetry. (For example, a sample after one filtration had $M = 8{\cdot}4 \times 10^6$, dissymmetry = 1·41; after five filtrations, $M = 7{\cdot}7 \times 10^6$, dissymmetry = 1·34, the concentration being assumed to be unchanged by filtration.) Solutions were therefore filtered once, before dilution. This procedure gave reproducible results. Hc/τ was independent of c for all samples. The particle scattering factor ($P_{90°}$) was calculated from the dissymmetry, the molecules being assumed to be spherical.[6] The refractive-index increment (dn/dc) for glycogen was found to be 0·146 (c = g./ml.) in 0·1M-sodium chloride at 546 mμ.

Isolation of Glycogens.—Unless otherwise stated, samples of glycogen had been isolated from the tissue by the classical Pflüger method involving digestion with 30% aqueous potassium hydroxide at 100°, followed by precipitation of the glycogen with ethanol and with acetic acid.[9] Commercial samples of glycogen from British Drug Houses Ltd. (I), and Nutritional Biochemicals Corporation, Ohio, U.S.A. (II), were also examined. Methylated horse-muscle glycogen was kindly provided by Dr. D. J. Bell.

Results and Discussion

Sedimentation Coefficients.—Typical sedimentation data are shown in Table 1. It was apparent that for all the glycogens studied in detail, the sedimentation constant (S_{20}) was dependent on the concentration (c), and varied by about 10% for a 1% change in concentration. This is in general agreement with Larner, Ray, and Crandall's results,[10] but, whilst these authors suggested that S_{20} was a function of c^2, our values were best

Table 1. *Typical sedimentation results.*

		$10^{13}S_{20}$ at c (g./100 ml.)							
Glycogen sample	Solvent	1·0	0·75	0·50	0·25	0·16	0·125	0·08	0 (extrapol.)
Ascaris lumbricoides ...	0·1M-NaCl	47	47	47	48	—	—	—	48
Brewer's yeast	0·1M-NaCl	56	—	60	61	—	62	—	64
,, ...	1M-NaCl	54	—	—	—	—	—	—	—
,, ...	0·2M-NaOH	56	58	60	61	62	—	63	64
Commercial, II	0·1M-NaCl	65	67	69	71	—	—	—	73

represented by a linear function. The relations was expressed by $S_{20} = (S_{20})_0(1 - kc)$, where $(S_{20})_0$ is the value of S_{20} at infinite dilution, and c was expressed in g./100 ml. With the exception of the *Ascaris lumbricoides* glycogen (which was relatively concentration-independent; see Table 1), the average value of k was 0·10 ± 0·02. Values of $(S_{20})_0$ for glycogens examined at only one concentration were therefore calculated from this value, and are shown in parentheses in the second and third columns of Table 2.

Molecular Weight and its Distribution.—Table 2 shows the results of the sedimentation measurements for the 23 samples examined. Typical sedimentation diagrams are shown in the Figure. Most samples proved to be polydisperse on ultracentrifugation. Diagrams *a* and *b* (for oyster and *Helix pomatia* glycogen) illustrate the type of Schlieren diagram observed for the most obviously polydisperse samples. This feature is unusual, although Polglase, Brown, and Smith [11] reported similar results for samples of human-liver glycogen. The amounts of main components quoted in the Table are only approximate as no attempt was made to correct for boundary anomaly effects.[12] For many samples, an extremely wide molecular-weight distribution was indicated; the leading sedimentation boundary

[7] Putzeys and Verhoeven, *Rec. Trav. chim.*, 1949, **68**, 817.
[8] Stetten, Katzen, and Stetten, *J. Biol. Chem.*, 1956, **222**, 587.
[9] Bell and Manners, *J.*, 1952, 3641; Manners and Archibald, *J.*, 1957, 2205.
[10] Larner, Ray, and Crandall, *J. Amer. Chem. Soc.*, 1956, **78**, 5890.
[11] Polglase, Brown, and Smith, *J. Biol. Chem.*, 1953, **199**, 105.
[12] See, *e.g.*, Trautman, Schumaker, Harrington, and Schachman, *J. Chem. Phys.*, 1954, **22**, 555.

was extremely asymmetric and reached nearly to the bottom of the cell after a short time of centrifugation. It was difficult to prove whether or not this leading boundary was a second component, and hence no attempt was made to estimate either its amount or its approximate sedimentation constant. Samples which showed this probable fast component are indicated by the symbol S_{20}(F) in the Table. In some other samples, a corresponding asymmetric lower molecular weight distribution was apparent. Again, no analysis of this was attempted and this is indicated by the symbol S_{20}(S) in Table 2.

TABLE 2. *Sedimentation results.*

Glycogen sample	$10^{13}S_{20}$ of components [a] major	minor	Major component (%)	dB/dx [b]	$10^{-6}M$	f/f_0 [d]
	(a) *Mammalian livers*					
Cat I	75	F, S	—	0·8	4·4	—
,, IV	84	F, S	—	—	4·9	—
,, VI	(102)	F, S	—	1·0	5·9	—
Human (glycogen-storage disease)	(53)	(220)	70	1·8	3·1	—
Fœtal sheep	110	F	—	1·1	6·4	—
Fœtal pig	(49)	(11)	70	0·8	2·9	—
Rabbit II	94	—	95+	—	5·5	1·7
,, (fructose-infused *)	(80)	F	—	1·1	4·7	—
,, (galactose-infused *)	(153)	S	—	—	9·0	—
,, (normal *)	(145)	F	—	1·8	8·4	—
	(b) *Mammalian muscles*					
Horse (methylated)	23	—	95+	—	2·8	1·4
Human	(85)	(20)	85	0·8	4·9	—
Rabbit I	79	—	95+	0·7	4·6	1·9
	(c) *Other glycogens*					
Ascaris lumbricoides	48	F	—	—	2·8	—
Brewer's yeast	64	—	95+	—	3·7	2·0
Commercial, I	24	—	95+	—	0·7	1·9
,, II	73	—	95+	—	4·0	1·7
Helix pomatia II	(63)	(300, 7)	80	0·9	3·6	—
Mytilus edulis I	(93)	F	—	0·9	5·4	—
Oyster *	(45)	(90, F)	—	—	2·6	—
Tetrahymena pyriformis I	(69)	S	—	—	4·0	—
Trichomonas foetus I	(70)	S	—	—	4·0	—
Trichomonas gallinae I	(84)	S	—	—	4·9	—

[a] For values in parentheses and meaning of F and S, see text. [b] Value for main component at c (total) = 1·00 g./100 ml. [c] Molecular weight calculated from $M = \boldsymbol{R}T(S_{20})_0/(1 - \bar{V}\rho)D_m$. [d] Frictional ratio calculated from $f/f_0 = 10^{-8}[(1 - \bar{V}\rho)/D_m{}^2(S_{20})_0\bar{V}]^{1/3}$.

* Samples kindly supplied by Dr. M. Schlamowitz.

Diffusion measurements showed that for methylated horse muscle the diffusion coefficient (D_m) = $1{\cdot}0 \times 10^{-7}$; for brewer's yeast glycogen, $D_m = 1{\cdot}1 \times 10^{-7}$; for commercial glycogen I, $D_m = 2{\cdot}0 \times 10^{-7}$; and for commercial glycogen II, $D_m = 1{\cdot}1 \times 10^{-7}$ The molecular weights shown in Table 2 for the main components in the other samples are calculated by assuming a value of $1{\cdot}1 \times 10^{-7}$ for D_m in agreement with other workers.[2] All the molecular weights are in the range (3—9) $\times 10^6$, and, together with the values of the frictional ratio (f/f_0), are of the same order as those previously reported from sedimentation and diffusion measurements.[2,13] It should be noted, however, that the values for rabbit liver and muscle are considerably lower than those recently reported by Stetten, Katzen, and Stetten [8] (see below).

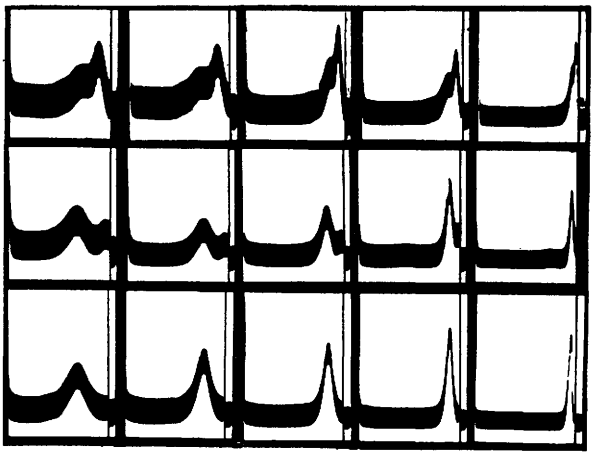
The values of (dB/dx) confirm qualitatively the extremely polymolecular nature of glycogen (cf. ref. 5) in agreement with the distributions evaluated by Larner and his

[13] Cori, *Makromol. Chem.*, 1956, **20**, 169.

co-workers.[10] Further, in agreement with these authors, mammalian-muscle glycogens appear to be less polymolecular than liver glycogens.

The polydisperse nature of most of the samples studied was confirmed by turbidimetric measurements. Although the molecular weight from these measurements is a true weight-average whilst that from sedimentation and diffusion is less well-defined,[14] the results from both these methods on a given polymolecular sample should be of the same order of magnitude. Polydispersity, particularly if it involves components of high molecular

Typical sedimentation diagrams. For all samples, c = 1 g./100 ml.; solvent, 1·0M-sodium chloride; speed = 20,000 r.p.m. Movement of the peaks is from right to left. The figures in parentheses after the times indicate the angle of the Schlieren bar.



Oyster glycogen: 8 (55°), 15 (45°), 18 (35°), 30 (30°), *and* 47 (25°) *min. after reaching full speed.*

Helix pomatia *glycogen*: 6 (65°) 14 (45°), 25 (45°) 37 (45°), *and* 50 (35°) *min. after reaching full speed.*

Brewer's yeast glycogen: 9 (65°), 17 (50°), 26 (45°), 37 (40°), *and* 52 (40°) *min. after reaching full speed.*

weight will cause disparities. Table 3 shows the results of light-scattering measurements on the ten samples which appeared to be the least obviously polydisperse on ultracentrifugation. For four of these, the agreement is reasonably good, indicating that they were only polymolecular, whilst the presence of S_{20}(F) in the other samples is convincingly illustrated

TABLE 3. *Light-scattering results.*

Sample	Uncorr. 10^{-6}M	Dissymmetry	$1/P_{90°}$	Corr. 10^{-6}M
Liver glycogens				
Cat I	10·5	1·48	1·30	13·6
,, IV	8·8	1·85	1·52	13·4
,, VI	12·8	1·67	1·40	17·9
Rabbit II *	6·9	1·20	1·13	7·8
Other glycogens				
Ascaris lumbricoides	7·1	1·40	1·26	8·9
Brewer's yeast *	4·0	1·15	1·10	4·4
Commercial, I	1·7	1·19	1·12	1·9
,, II *	4·9	1·15	1·10	5·4
Rabbit muscle I *	3·7	1·17	1·11	4·1
Tetrahymena pyriformis I	6·1	2·50	1·85	11·3

* Samples exhibiting no polydispersity.

by the higher turbidimetric molecular weight. It is therefore suggested that a given glycogen sample should be examined by both the sedimentation and the light-scattering method in order to prove unambiguously whether or not it is polydisperse. Without

[14] See, *e.g.*, Kinell and Rånby in " Advances in Colloid Science," Vol. III, Interscience, Publ. Inc., New York, 1950.

further investigations, it is not possible to decide whether polydispersity occurs in native glycogen in the tissue or is an artefact resulting from degradation during isolation. Polglase and his co-workers [11] consider that such variations occur naturally.

Effect of Isolation Procedure on Molecular Weight.—The classical Pflüger method involving digestion of tissue with 30% potassium hydroxide has often been criticised [15] on the assumption that alkaline degradation occurs. Table 4 shows the results for the determination of S_{20} for glycogen samples isolated from the halves of two rabbit livers severally with boiling water and 30% aqueous potassium hydroxide. Within experimental error, S_{20} is the same for all samples. It is concluded that, in the presence of air, the extent of degradation of glycogen by 30% potassium hydroxide solution at 100° is no greater than that which might be caused by boiling water. Similar results have been obtained by Staudinger,[16] and Bridgman [17] reported that glycogen extracted with cold trichloroacetic acid and hot alkali from two halves of a rabbit liver had a similar molecular weight. However, recent light-scattering work by Stetten, Katzen, and Stetten [8] has shown that if extraction with trichloroacetic acid is for a limited time at 0° the glycogen from rabbit liver has an average molecular weight of (11—80) $\times$ 10^6 rather than the (2—6) $\times$ 10^6 as in hot potassium hydroxide extractions. This suggests that it is difficult to avoid degradation during extraction, and that the molecular weights reported here and previously [2] may not be representative of " native " glycogen.

Effect of Dilute Alkali and Acetic Acid.—In contrast to the behaviour of hot 30% alkali, hot *dilute* alkali appears to degrade glycogen rapidly. Digestion of rabbit-liver glycogen in 8% aqueous sodium hydroxide at 100° for 1·5 hr. reduced S_{20} from 86 to 57 $\times$ 10^{-13} c.g.s. units (see Table 4), and increased the polymolecularity (as shown by a broadening of the peak of the Schlieren pattern).

It has been suggested [18] that purification of glycogen by precipitation with glacial acetic acid may render it unsuitable for ultracentrifugal analysis. However, when rabbit-liver and brewer's yeast glycogens were reprecipitated with 80% acetic acid there was no change in the value of S_{20} (see Table 4). Precipitation of glycogen by acetic acid does not, therefore, alter the hydrodynamic properties or cause degradation of glucosidic linkages to any appreciable extent.

TABLE 4. *Effect of isolation procedure on the sedimentation constant.*

Sample	Method of isolation	$10^{13}S_{20}$ at c = 1 g./100 ml.
Rabbit liver XII	Hot water	85
	30% KOH at 100°	86
Rabbit liver XIII	Hot water	76
	30% KOH	83
	30% KOH + repptn. with AcOH	83
Rabbit liver IV	30% KOH	86
	30% KOH + 8% NaOH at 100° for 1½ hr.	57
Brewer's yeast	30% KOH	64
	30% KOH + repptn. with AcOH	63

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[15] *E.g.*, Meyer and Jeanloz, *Helv. Chim. Acta*, 1943, **26**, 1784.
[16] Staudinger, *Makromol. Chem.*, 1948, **2**, 88.
[17] Bridgman, *J. Amer. Chem. Soc.*, 1942, **64**, 2349.
[18] Illingworth, Larner, and Cori, *J. Biol. Chem.*, 1952, **199**, 105.