[CONTRIBUTION FROM THE LABORATORY OF PHYSICAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

Some Physical Chemical Characteristics of Glycogen

BY WILBUR B. BRIDGMAN

The research described in this report was undertaken with a two-fold purpose. First, the particle size of glycogen has not been studied by sedimentation methods, and further information regarding its physical characteristics may be of value to the biological worker in understanding the relationship of glycogen to animal metabolism. Second, it was desired to gain experience and information in the application of ultracentrifuge experiments to inhomogeneous systems where the particle size varies continuously in contrast to the discrete size classes generally found in protein systems.

Much of the literature about glycogen is concerned with its determination in tissue and its relation to metabolism. While glycogen is widely distributed throughout the bodies of animals, about one-half of the total amount is concentrated in the liver. This organ is therefore the logical raw material for the preparation of glycogen. Two principal methods have been used for its extraction. The older method involves heating with concentrated alkali as the primary step. More recently the first extraction has been made with a dilute solution of trichloroacetic acid. Both methods have been shown to agree in the amount of glycogen obtained and no differences have been shown between the materials prepared by these two methods.[1] The properties of the glycogen samples that were compared were rotatory power, ash content, iodine color and reducing power. It seemed possible that the ultracentrifuge might be used to show differences in particle size that would not affect the properties previously studied.

In previous attempts to measure the particle size, osmotic pressure determinations[2] on glycogen or its derivatives have indicated high molecular weights (500,000 to 3,500,000). Early attempts to deduce the molecular size from substitution and end-group reactions were interpreted as indicating a much smaller molecule. In recent years these data have also been shown to be consistent with macro-molecules.[3] The only previous account of ultracentrifuge measurements with glycogen that the author has found in the literature is a statement by Mystkowski[4] that in preliminary experiments sedimentation of glycogen occurred at speeds of 17,000 to 25,000 r. p. m. and that it was very polydisperse.

Preparation

The glycogen used in this research was prepared from rabbit livers.[5] Two methods of preparation were used. Method A used 3% trichloroacetic acid to extract the glycogen from the liver and was essentially the same as that described by Sahyun and Alsberg.[6] In method B small pieces of liver were digested in concentrated solutions of potassium hydroxide on a steam-bath.[7] In both cases the glycogen was purified by dissolving in water and reprecipitating with ethyl alcohol three or four times.

The glycogen was kept in two forms. Some of the material was dried to constant weight in a vacuum desiccator over calcium chloride at room temperature. In other cases water solutions were dialyzed against distilled water to remove traces of salts or alcohol. The concentration of these stock solutions was determined by evaporation to dryness, assuming all non-volatile material to be glycogen. The following paragraphs describe the treatments applied to the individual samples of glycogen used in this investigation.

Glycogen II.—Five rabbit livers were treated by method A. About half of the material from the last precipitation was dried. This gave 2.4 g. of glycogen, designated as IIADd. The remainder was dissolved and dialyzed. This made a 7.14% stock solution, IIADl.

Glycogen IIIA.—Four rabbit livers were treated by method A. About half of this glycogen was dried from the last precipitation, giving 1.2 g. of IIIADd. The remainder, IIIADl, was used for the dialyzed stock solution of concentration 2.07%.

Glycogen IIIB.—Three livers obtained at the same time as those used in IIIA were worked up by method B, using 50% potassium hydroxide as solvent. The yield from this preparation was very small. All of the material obtained was dissolved to form a stock solution, IIIBDl, of 0.4% concentration.

Glycogen VA.—A single liver was frozen in dry-ice as soon as it was removed from the rabbit. The liver was crushed in the frozen state and divided into two approximately equal portions. One portion was treated by method A. All of the material was dialyzed in this case.

(1) D. J. Bell and F. G. Young, *Biochem. J.*, **28**, 882 (1934).

(2) H. B. Oakley and F. G. Young, *ibid.*, **30**, 868 (1936); S. R. Carter and B. R. Record, *J. Chem. Soc.*, 664 (1939).

(3) W. N. Haworth, *Chem. and Ind.*, **17**, 917 (1939). K. H. Meyer, "Recent Developments in Starch Chemistry," *Advances in Colloid Science*, Interscience Publishers, New York, N. Y., 1942, pp. 143–179.

(4) E. M. Mystkowski, *Biochem. J.*, **31**, 716 (1937).

(5) Grateful acknowledgment is made of the assistance of Drs. W. H. Jaeschke and E. A. Birge, Jr., of the College of Medicine, for assistance in obtaining these livers

(6) M. Sahyun and C. L. Alsberg, *J. Biol. Chem.*, **89**, 33 (1930).

(7) M. Sahyun, *ibid.*, **93**, 227 (1931); N. R. Blatherwick, P. J. Bradshaw, M. E. Ewing, H. W. Larson and S. D. Sawyer, *ibid.*, **111**, 537 (1935).

A 25-cc. sample of the dialyzed solution was evaporated to dryness. This gave 0.3966 g. of a residue which was designated VADd. The bulk of the solution was used as stock solution VADl.

Glycogen VB.—The other half of the liver used in preparation VA was treated by method B, using 30% potassium hydroxide as solvent. After the initial treatments with acid and alkali, respectively, the two samples VA and VB were purified simultaneously. Each sample received identical treatment in the process of purification. Drying 25 cc. of dialyzed solution gave 0.3884 g. of VBDd. The bulk of the solution was used for VBDl. Glycogen VBDd had an orange discoloration while VADd was clear white in appearance.

Experimental Results

Sedimentation Constants.—Sedimentation velocity experiments were performed in both the Svedberg oil turbine "velocity" ultracentrifuge and the Svedberg electrically driven "equilibrium" ultracentrifuge. At a speed of 18,000 r. p. m., which can be obtained with either centrifuge, sedimentation was rapid. In both centrifuges the solution was at a distance from 5 to 7 cm. from the center of rotation. Observation of the redistribution of the components of the solution was made by the Lamm scale displacement method. The sedimentation constant, s, was calculated by the procedure given by Svedberg and Pedersen.[8] Because of the heterogeneity of the material, the graphs of scale displacement, Z, against distance from the center of rotation had quite broad peaks, making it difficult to locate the maxima accurately. As a result, individual calculations of s for successive time intervals during a given experiment fluctuated widely. Average values of s from separate experiments on the same or comparable solutions show agreement with $\pm 5\%$ in most cases. Satisfactory agreement was found between sedimentation constants measured with the two centrifuges. However, the velocity centrifuge was considered to be more reliable. In some of the experiments in the equilibrium ultracentrifuge, very sharp boundaries were observed that were attributed to convection rifts because of their abnormal behavior and because of the failure to observe them in duplicate experiments in the velocity machine. The results obtained with the various samples are summarized below.

Glycogen II.—Ten measurements were carried out on solutions of this preparation. The concentration of glycogen was varied from 0.8 to 2.86%. Nine experiments were performed in the velocity ultracentrifuge, five at 42,000 r. p. m. and four at 18,000 r. p. m. One experiment was performed in the equilibrium ultracentrifuge at 17,500 r. p. m. The first two experiments made on the dialyzed material indicated the presence of two boundaries. Only the slower moving peak was sufficiently well-defined to permit the calculation of a sedimentation constant. All the remaining experiments showed one broad peak. The average values of s_{20} for these ten experiments varied from 60 to 70 S (one Svedberg unit, S, $= 1 \times 10^{-13}$ c. g. s. units). The average for the ten experiments was $s_{20} = 64.8\ S$. One of the measurements with glycogen IIDd was made more than a year after the preparation of the sample. This experiment gave $s_{20} = 60.2\ S$. An attempt to correlate s_{20} with the concentration of glycogen indicated a slight increase of s as the concentration was decreased. This trend was so small in comparison to the fluctuations of the individual values of s_{20}, that no significance was attached to it and no attempt was made to extrapolate s_{20} to infinite dilution. Likewise the solvent was varied from distilled water to 1% sodium chloride without producing any significant change. It was thus concluded that s_{20} was independent of the concentration of glycogen or of salt in the range studied within the accuracy of the observations. Subsequent experiments used 0.1% sodium chloride as solvent.

Glycogen IIIA.—Two experiments with glycogen IIIAdl and two with glycogen IIIADd were carried out at 17,000 r. p. m. in the equilibrium ultracentrifuge. These experiments showed two boundaries that gave s_{20} values in the range 65 to 88 S. A duplicate experiment in the velocity centrifuge at 18,000 r. p. m. indicated a single broad peak with $s = 61\ S$. It was concluded that convection must have occurred in the earlier observations and that this last value is the best that can be obtained under present conditions.

Glycogen IIIB.—Five experiments were performed in the equilibrium ultracentrifuge during the interval from eleven to forty-five days after the livers were obtained. The first two experiments indicated double peaks. The slower peak corresponded to values of $s_{20} = 138$ and $152\ S$, respectively, and the faster peak gave $s = 296$ and 441. The third experiment, carried out a week later than the first, gave a single broad peak with $s_{20} = 244\ S$. The next two experiments,

(8) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, New York, N. Y., 1940.

performed at approximately two week intervals, gave single broad peaks, with $s_{20} = 120$ and 92 S. A check experiment in the velocity ultracentrifuge with this last sample gave the same type of peak with $s = 105$ S. This behavior suggests a change had taken place in the material on standing, with the final state being a single maximum distribution with the maximum corresponding to $s_{20} = 100$ S.

Glycogen VA.—Two solutions of glycogen VADl gave values of $s_{20} = 85$ and 81 S. There was a suggestion of a second peak of lighter material (s_{20} of the order of magnitude of 10 S) (see Figs. VI and VII). Two experiments were also made with solutions of glycogen VADd. One of these gave $s_{20} = 79$ S. The other was inconclusive as the first two exposures showed a single maximum peak but in later pictures this had broken up into a series of four peaks that bore no resemblance to any of the other three experiments. The average of the three experiments is $s_{20} = 82$ S.

Glycogen VB.—Two solutions of glycogen VBDl and one of glycogen VBDd gave values of $s_{20} = 67$, 75, and 77 S, respectively. The average is $s_{20} = 73$ S. All experiments with glycogen VA and VB were made in the velocity ultracentrifuge.

Diffusion Constants.—Five diffusion experiments were performed in a glass Lamm diffusion cell. Observations of the blurring of the boundary were made by the scale line displacement method. The diffusion constant was calculated by means of the expression

$$D = \sigma^2/2t$$

where t is the time and σ is the standard deviation of the curve obtained by plotting the scale displacement, Z, against position in the cell. A method of second moments was used for evaluating σ. Values of D_{20} calculated from individual exposures during these five experiments are shown graphically in Fig. 1. The abscissas are times after the formation of the boundary. The observed values of D are seen to fall into two groups. The results for glycogen IIIB are distinctly lower than the other values which are fairly consistent among themselves. The three values for glycogen IIIB have an average $D_{20} = 0.69 \times 10^{-7}$. The average of all the remaining values is $D_{20} = 1.42 \times 10^{-7}$. Figure 1 shows that there is a definite trend toward lower values as time increases. It is often observed that diffusion constants decrease from an initial value to a limiting value that is considered to be the true value.

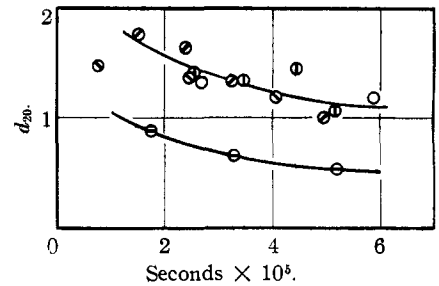

Fig. 1.—Variation of diffusion constant with time after formation of boundary: O, glycogen IIDd; ⊘, glycogen IIIADl; ⊖, glycogen IIIBDl; ⊗, glycogen IIDd; ⊕, glycogen VADd.

While no limiting value is clearly indicated by the values in Fig. 1, they can be interpreted as approaching a value of $D_{20} = 1.1 \times 10^{-7}$ for the main group and $D_{20} = 0.5 \times 10^{-7}$ for glycogen IIIB. If the individual values are plotted against the reciprocal of the time and extrapolated by a straight line to infinite time, lower values of $D_{20} =$ 0.8 and 0.35×10^{-7}, respectively, are obtained. This extrapolation may overcorrect the value since the diffusion constants should eventually become constant, instead of continuing to decrease. It is thought that the values obtained from Fig. 1 represent the best interpretation of the data.

The experimental line displacement–distance curves were compared to a normal distribution curve by a transformation of coördinates. In Figs. 2 and 3 the points represent experimental data obtained with glycogens IIIB and VA, respectively, and the solid line is the ideal distribution curve plotted on the same scale. The other three experiments gave results similar to those which form Fig. 2, *i. e.*, good agreement with the ideal curve. For a curve of ideal shape the value of D calculated from the area and maximum height

$$D = A^2/tH^2_{\text{max.}}$$

should be the same as that calculated by the first method. Good agreement was found between the values of D calculated by the two methods in all cases except for glycogen VA. In this case the height–area method gave consistently lower values.

Partial Specific Volume.—Densities of a series of water solutions of glycogen IIDd were determined pycnometrically at 25°. The most concentrated solution contained 1% glycogen. The apparent partial specific volume calculated from the density was 0.65.

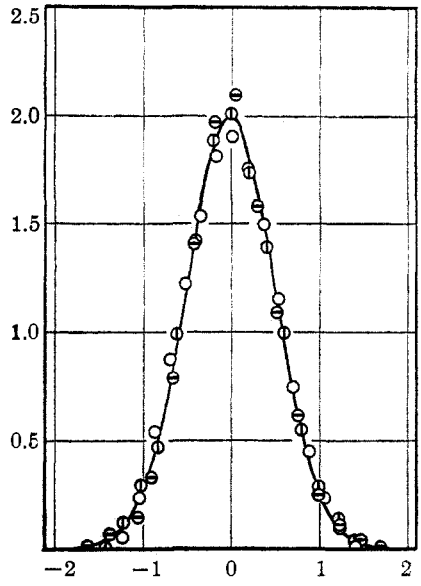

Fig. 2.—Comparison of normal distribution curve with scale line displacements obtained from diffusion experiment on glycogen IIIBDl. Solid line is theoretical curve. Points are calculated from experimental data at various times after the formation of the boundary: ⊖, 174,000 sec.; ⊕, 329,000 sec.; ○, 521,000 sec.; arbitrary units.

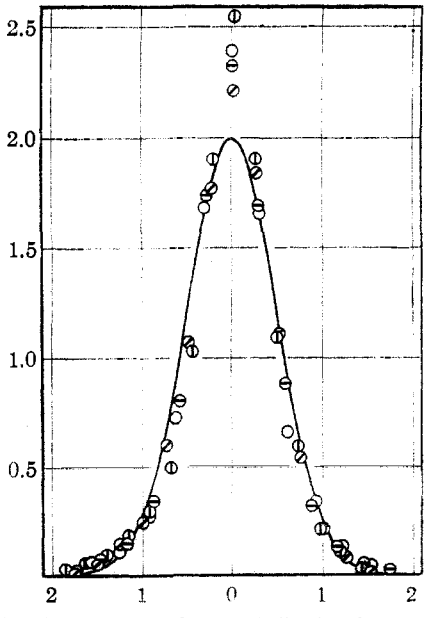

Fig. 3.—Comparison of normal distribution curve with the scale line displacements obtained from diffusion experiment on glycogen VADd. Solid line is theoretical curve. Points are calculated from experimental data at various times after the formation of the boundary: ○, 254,000 sec.; ⊖, 347,000 sec.; ⊕, 432,000 sec.; ⊘, 518,000 sec.; arbitrary units.

Refractive Index.—Refractive indices of the solutions used in the density determinations were measured with a dipping refractometer. The value of the refractive index increment,[8] α, was found to be 1.38×10^{-3} for the sodium D line at 25°.

Discussion of Results

Sedimentation.—Sedimentation experiments with glycogen II performed at different centrifuge speeds appeared to give peaks of the same width when the boundary had moved a given distance from the meniscus. This suggested that the blurring of the boundary was due to inhomogeneity of the material and that diffusion was negligible during the time of the experiment. In such a case the shape of the sedimentation curve can be used to obtain the particle size distribution in the sample.

Mathematical functions relating the Z *vs.* x curves obtained at different times during the same experiment have been derived on the assumption of no diffusion taking place during the experiment.[8,9] If the experimental curves can be superimposed when transformed to the same basis by these relationships, that is proof that diffusion has been negligible.

These transformations were applied to the data from two of the experiments. The results are shown graphically in Figs. 4 and 6. In the

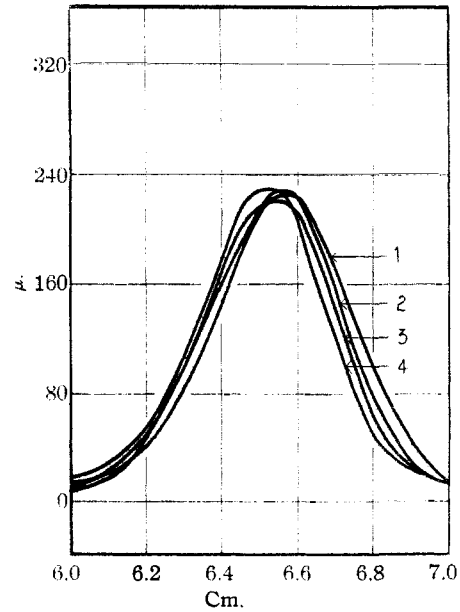

Fig. 4.—Scale line displacement *vs.* distance from center of rotation for a sedimentation experiment on glycogen IIDd. Curves corresponding to different times after the start of sedimentation have all been transformed to the same time (60 min.) assuming diffusion to be negligible: 1, 30 min.; 2, 40 min.; 3, 50 min.; 4, 60 min.

(9) R. Signer and H. Gross, *Helv. Chim. Acta*, **17**, 726-735 (1934).

case of Fig. 4 (a 1% solution of glycogen IIDd at 18,000 r. p. m.) the agreement is very satisfactory. The maximum heights of the four curves agree very well. The horizontal shifting of the curves may be due to error in the determination of the position of the meniscus or the starting time of the experiment. Both of these quantities are important in the calculations. The beginning of sedimentation was taken as the time when the ultracentrifuge had reached operating speed. Actually some sedimentation has probably occurred during the period when the machine is coming up to speed (about seven minutes in this case). The agreement of these curves is taken as proof of the absence of diffusion during this experiment. In Fig. 5 is given a distribution curve indicating the relative concentrations of components as a function of the sedimentation constant. This distribution curve has been constructed from the last curve of Fig. 4 by using the expressions

$$s = \ln \frac{x}{x_0} \Big/ t\omega^2$$

and

$$\frac{dc}{ds} = \left(\frac{x}{x_0}\right)^2 xt\omega^2 \frac{dc}{dx}$$

The scale displacement, Z, is used to evaluate dc/dx.[8]

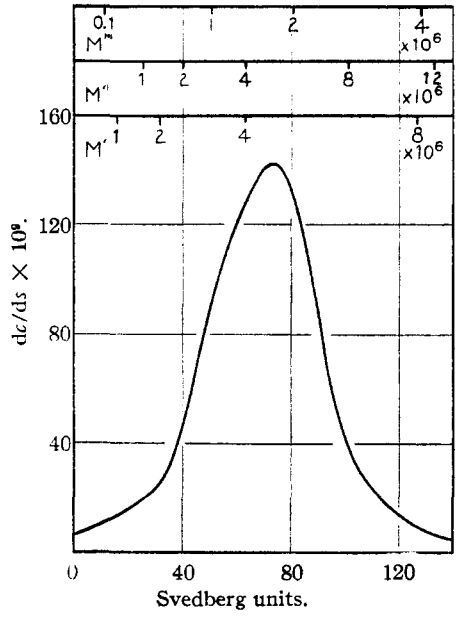

Fig. 5.—Distribution curve calculated from curve 4 of Fig. 4 showing variation of concentration as a function of the sedimentation constant.

Figure 6 shows the results of attempting to superimpose the data from an experiment with

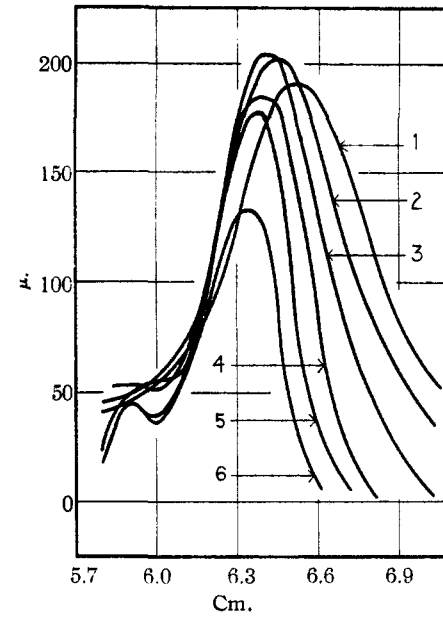

Fig. 6.—Scale line displacement *vs.* distance from center of rotation for a sedimentation experiment on glycogen VADd. Curves corresponding to different times after the start of sedimentation have all been transformed to the same time (53 min.) assuming diffusion to be negligible: 1, 13 min.; 2, 23 min.; 3, 38 min.; 4, 53 min.; 5, 68 min.; 6, 83 min.

glycogen VBDl. With the exception of the first and last curves, the agreement is quite satisfactory on the left side of the peak. Toward the bottom of the cell there is a marked decrease with increasing time. This may be accounted for in several ways. These curves were obtained during the period from thirteen to eighty-three minutes after the centrifuge had reached operating speed. The curves of Fig. 4 cover the interval from thirty to sixty minutes. Thus any error in the position of the meniscus or the initial time will be much more apparent in Fig. 6 than in Fig. 4. Furthermore, the calculations show that positions corresponding to size classes in the leading edge of the peak in the early pictures will fall beyond the bottom of the cell in the last exposures (*i. e.*, the time is sufficient for some of the material to have sedimented to the bottom of the cell). This fact makes questionable the usual practice of using the s values at the bottom of the cell to determine the base line. In the later exposures of this experiment no portion of the cell could be expected to have the original unchanged concentration present. The curves given in Fig. 6 were obtained by drawing in a base line in the conventional manner. Another cal-

culation in which the displacements measured in the comparator were used directly without any base line correction gave somewhat more erratic results but did not show any essential differences from Fig. 6.

In spite of the failure of the curves to superimpose exactly there is considerable indication that diffusion is not the cause of the discrepancy. The maximum heights of the curves (omitting the last two) agree within 10%. The heights of the original experimental curves, *i. e.*, before the transformation was made, varied tenfold. The original curves of Z against x without any base line correction are shown in Fig. 7. The first two curves have been corrected for differences in the optical arrangement so as to be comparable with the rest. The broken lines in Fig. 7 are the theoretical functions for the variation of the height of the peak with position in the cell. The upper one is for the case of a homogeneous material, the blurring being entirely due to diffusion. The lower one represents the case of no diffusion. The observed heights of the curves agree very well with the prediction for no diffusion. If blurring is due to diffusion, the application of the transformation

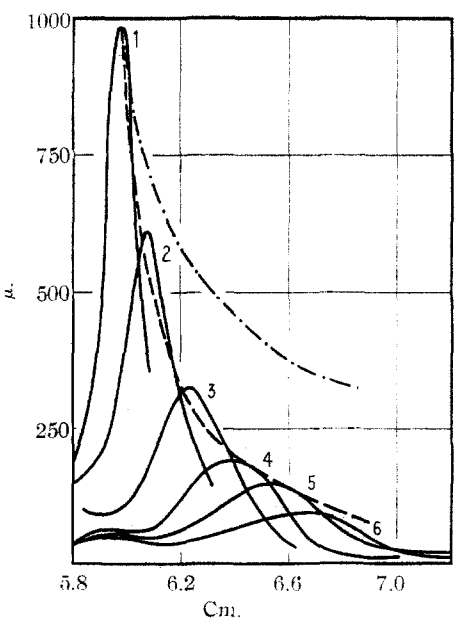

Fig. 7.—Scale line displacement *vs.* distance from center of rotation for a sedimentation experiment on glycogen VADd. Solid lines are observations of the boundary at various times: 1, 13 min.; 2, 23 min.; 3, 38 min.; 4, 53 min.; 5, 68 min.; 6, 83 min. Broken lines are the theoretical functions for the decrease in maximum height of the curves based on different assumptions: · — · — · — homogeneous material with diffusion; – – – – inhomogeneous material with negligible diffusion.

equations will result in the later curves being higher than the curves obtained from exposures at an earlier time.[9] Actually the trend of Fig. 6 is in the opposite direction. In spite of the evidence pointing to the absence of diffusion in this experiment, the failure of the individual curves to superimpose completely makes it undesirable to calculate a size distribution curve from any one of them.

The later curves in both Figs. 6 and 7 show a small but definite peak of lighter material that was not resolved in the earlier exposures.

Analysis of the transformation equations shows that the scale line displacements corresponding to the more rapidly moving components decrease more rapidly than those for the components which move more slowly. This means that as sedimentation proceeds the maximum of the Z curve shifts toward the lighter components. Thus a sedimentation constant calculated from the maxima of successive Z curves might be expected to decrease as the length of the experiment increases and it would not represent the specific sedimentation constant for a particular component. The extent of this effect would depend upon the shape of the distribution curve, being greatest for a curve with a very broad maximum. A calculation applied to the data for the thirty-minute and sixty-minute curves in Fig. 4 showed that in this case the apparent sedimentation constant calculated from the maximum points would differ by less than 2% from the actual sedimentation constant of the component in the maximum at one time. In view of the experimental error in determining the position of the maximum this effect is not considered for correction. This effect does not explain the shifting of the maxima in Figs. 4 and 6 since the transformations should correct the apparent shift of the maximum caused by the more rapid separation out of the heavier components.

By combining values of the sedimentation constant, diffusion constant and partial specific volume, molecular weight and shape factor data for glycogens can be obtained.[8] In Table I are summarized average values of s_{20} and D_{20} for each of the samples of glycogen which were studied, together with the values of molecular weight and shape factor calculated from them. Aside from glycogen IIIB the variations between the samples are not considered significant. In preparation IIIB a glycogen of definitely greater particle size was ob-

tained. This may be connected with the small yield of glycogen obtained in this preparation. It seems likely that an unintentional fractionation may have occurred in the extraction or purification of this sample. It is of interest in showing that glycogen exists or can be prepared in samples of varying size.

TABLE I

MOLECULAR KINETIC DATA FOR GLYCOGENS

Glycogen	S_{20} (S units)	$D_{20} \times 10^7$	f/f_0	$M \times 10^{-6}$
II	65	1.1	1.90	4.1
IIIA	61	1.1	1.94	3.9
IIIB	100	0.5	2.78	13.9
VA	82	1.1	1.76	5.2
VB	73	1.1	1.83	4.6

Some experiments, particularly those with glycogen IIIB, suggested that when first prepared larger sized particles were present. On standing in water solution, the distribution changed to a function with a single maximum at a lower molecular weight. The evidence for this is, however, not conclusive. This possibility is of interest in the light of K. Meyer's conclusion that starch undergoes a continual aggregation in water solution.[10]

Samples of glycogen VA and VB, which should provide the most critical test for any differences in the products obtained by the acid method or basic method, do not show significant differences.

A shape factor of 1.9 corresponds to an ellipsoid of revolution having an axis ratio of 1 to 18 for a prolate ellipsoid or a ratio of 1 to 25 for an oblate ellipsoid. This dissymmetry is much greater than other investigations of glycogen have indicated. A larger value of the diffusion constant would lower the shape factor.

In order to translate the sedimentation constants used as abscissa in Fig. 5 into molecular weight, it is necessary to make assumptions regarding the particle shape. At the top of Fig. 5 are three molecular weight scales based on different assumptions. M' is calculated on the assumption that the diffusion constant, 1.1×10^{-7}, is the same for all components. M'' is based on the assumption that the shape factor 1.9 is constant for all species. M''' is the value for spherical particles. Probably no one of these scales represents the actual relationship exactly, but together they give an indication of the range of sizes possible.

(10) K. H. Meyer (see ref. 3), p. 146–165.

An interpretation of diffusion measurements on polydisperse material has been made by Gralén.[11] His investigation shows that for a polydisperse system the value of the diffusion constant calculated will be greater, the higher the moments used in its calculation. This difference in diffusion constant, calculated by the different moments, can be used as a measure of polydispersity but requires very accurate data. The results with glycogen lead to the conclusion that comparison of the observed scale line displacement curve with the Gaussian error curve is not a sensitive test of homogeneity. The one diffusion experiment which did not show good agreement with the normal distribution curve was with sample VA where the ultracentrifuge also had indicated some lighter material grouped about a second maximum (see Figs. 6 and 7).

Apparently, polydispersity of the degree observed here is not sufficient to cause marked deviation from the ideal case as long as the particle size distribution can be described by a simple curve with a single maximum.

There is no proof that the principal component in sedimentation would be the principal factor in determining the diffusion constant. The molecular weight calculated from sedimentation and diffusion may be questioned on this basis that the two average values do not necessarily apply to the same component. A process of fractionation followed by sedimentation and diffusion studies with the more homogeneous fractions should be very helpful in interpreting data of this type.

Acknowledgment.—The author wishes to express his appreciation to Professor J. W. Williams for his coöperation and advice in the preparation of this report.

Summary

Glycogen solutions prepared by either the acidic or basic method give an inhomogeneous product. The bulk of the material prepared by the methods used lies in the range of sedimentation constant from 20 to 120 S with the maximum component having a value of $s_{20} = 70\ S$. This maximum corresponds to a molecular weight of 2,000,000 if the particle is spherical or a molecular weight of 4,000,000 if the measured diffusion constant can be used to evaluate the frictional resistance to sedimentation. It has not been proven that the glycogen in the tissue is of this same par-

(11) N. Gralén, *Kolloid. Z.*, **95**, 188 (1941).

ticle size or that this particle represents the chemical molecule rather than an aggregate. This investigation indicates the possibility of further work on the natural state of glycogen, methods of its preparation and the interpretation of experimental results on inhomogeneous systems in general.

MADISON, WISCONSIN RECEIVED JUNE 29, 1942

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Structure of Copolymers of Vinyl Chloride and Vinyl Acetate[1]

BY C. S. MARVEL, GIFFIN D. JONES, T. W. MASTIN AND G. L. SCHERTZ

It is widely recognized that the simultaneous polymerization of two monomers in a mixture leads to products that are quite different from the mixtures obtained by polymerizing the two monomers separately and then combining the polymers. The copolymerization of the monomers thus must lead to mixed units of two monomers in a single polymer chain.

Considerable experimental evidence supporting this generally accepted fact can be cited. Hill, Lewis and Simonsen[2] have made copolymers of butadiene and methyl methacrylate and then ozonized them. The ozonolysis products were such as to show that in general the methyl methacrylate units were sandwiched between butadiene units and that the latter were usually attached by the 1 and 4 carbon atoms in the chain. There was also evidence of direct union between butadiene units and between methyl methacrylate units in the polymer chain. Thus in this particular case almost every possible type of union between the monomers seems to have occurred when the mixture of monomers was polymerized.

Staudinger,[3] Norrish,[4] and others[5] have shown by their studies of copolymers of styrene and *p*-divinylbenzene that cross-linking of chains occurs due to the participation of the divinylbenzene in the reaction. Hence real copolymers must form.

Staudinger and Schneiders[6] have shown that a copolymer of vinyl chloride and vinyl acetate can be separated into products containing varying amounts of chlorine by means of fractional precipitation. This suggests considerable non-homogeneity of product.

Previous work in this Laboratory has shown that vinyl chloride when polymerized alone gives a 1,3 product[7] and likewise when vinyl acetate alone polymerizes,[8] a 1,3 product results. The present work on the copolymers of these two compounds was undertaken to see whether the two monomers entered the copolymer chains in the same arrangement. The first samples examined were some high-chlorine experimental "Vinylites" prepared in the research laboratories of Carbide and Carbon Chemicals Corporation and furnished to us with that Company's permission by Dr. G. H. Young at Mellon Institute. An attempt was made to study the distribution of chlorine in the polymer chain by statistical methods using the dehalogenation of the polymer by zinc.[7] The results of these experiments seemed to be what was expected for a chance distribution in the polymer chains of vinyl chloride and vinyl acetate units.[9]

Next an attempt was made to apply the same procedure to some low-chlorine vinyl chloride–vinyl acetate copolymers made in our own Laboratory. In early results we found the chlorine was removed in far greater quantities than should have been the case if the vinyl chloride units were distributed in the polymer chains according to chance alone. The dehalogenation experiments did not prove to be too satisfactory and were, in fact, not readily reproducible but they did prove that the chlorine atoms were closer together than chance alone would explain.

At this stage of our work, Wall[10] pointed out that two monomers may have quite different tendencies to enter the growing chain of the copolymer. If the two monomers do enter the chain at different rates the polymer found will not have a uniform composition. Wall's equation[10] can be used to express this relation. When α is 1, the

(1) This paper was first presented at the Gibson Island Conference on Polymers in July, 1941, and it is the fourteenth communication on the structure of vinyl polymers. For the thirteenth see THIS JOURNAL, **64**, 1675 (1942).

(2) Hill, Lewis and Simonsen, *Trans. Faraday Soc.*, **35**, 1073 (1939).

(3) Staudinger, *ibid.*, **32**, 323 (1936).

(4) Norrish and Brookman, *Proc. Roy. Soc.* (London), **163A**, 205 (1937).

(5) Blaikie and Crozier, *Ind. Eng. Chem.*, **28**, 1155 (1936).

(6) Staudinger and Schneiders, *Ann.*, **541**, 151 (1939).

(7) Marvel, Sample and Roy, THIS JOURNAL, **61**, 3241 (1939).

(8) Marvel and Denoon, *ibid.*, **60**, 1045 (1938).

(9) Wall, *ibid.*, **62**, 803 (1940); *ibid.*, **63**, 821 (1941).

(10) Wall, *ibid.*, **63**, 1862 (1941).