Determination of the Molecular Weight Distribution of Chondroitin Sulfate B by Sedimentation Equilibrium*

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The discovery that purified protein preparations consist of molecules which are monodisperse with respect to molecular weight was originally entirely unexpected (1). Polymerization processes in general are expected to yield products which contain molecules with a wide range of molecular size, and only a template mechanism of synthesis can reasonably be expected to yield size homogeneity. It is now well established that the biosynthesis of most proteins, in fact, occurs through a template mechanism, and their homogeneity with respect to both size and amino acid sequence is an accepted fact.

In molecular weight studies on macromolecular carbohydrates, the method has frequently been adapted from procedures designed originally for the study of proteins. Single values for molecular weight and other physical parameters have been reported. For chondroitin sulfate A, for example, various reports have given single value molecular weights which range from 18,000 to 150,000 (2, 3).

There is, however, no a priori reason to suggest that the biosynthesis of polysaccharides involves a template mechanism. It is more likely that they are polymerized by a relatively random process which would lead to a heterogeneous product. Bridgman (4) found indications as early as 1942 that glycerogen must be highly heterogeneous with respect to molecular weight, and Williams and Saunders (5), in 1954, carried out an elegant study showing that dextran possesses a wide distribution of sedimentation coefficients and, therefore, also of molecular size.

One of the best ways to investigate this problem is to use the method of sedimentation equilibrium. This method permits precise measurement of the distribution of solute in a column of liquid in a constant centrifugal field. There are simple tests to decide whether the distribution is that expected of a homogeneous solution. If it is not, then the distribution of molecular weights in the sample can be computed from the observed solute distribution. Theoretical equations required for such an analysis are given by Svedberg and Pedersen (1), in a series of definitive papers by Wales (6) and Wales, Adler, and Van Holde (7), and, more recently, by Van Holde and Baldwin (8), by Fujita (9), by Yphantis (10), and by Hermans (11). Such equations are rigorously true for thermodynamically ideal solutes only. Efforts to cope with the problem of nonideality have been made, but they cannot be considered exact.

A recent development in the technique of ultracentrifugation permits examination of the results by Rayleigh interference optics (10, 12, 13), and this allows the use of extremely dilute solutions. Under these conditions, the effects of nonideality are likely to be small. In the present study, we have used chondroitin sulfate solutions which, before the onset of sedimentation, had concentrations of the order of 0.1 g per liter. The solvent was 0.5 M NaCl, chosen to repress electrostatic interactions. It is thus considered unlikely that thermodynamic nonideality can have an important effect on the results, and, at least in a first study, the use of equations designed for ideal solutions is justified.

**EXPERIMENTAL PROCEDURE**

Preparation of Chondroitin Sulfate—Chondroitin sulfate B was prepared from pig skin by proteolytic digestion and alcohol fractionation. Analyses were performed for hexosamine (15), for uronic acid by CO₂ release (16) and by colorimetry (17, 18), and for sulfate (19) and nitrogen (micro-Kjeldahl). Values are listed in Table I. Further analysis of this preparation revealed the presence of several amino acids, which comprised about 6% of the total weight. It is not known if these represent covalently bound material. The polysaccharide yielded a single peak when chromatographed on DEAE-Sephadex A-25 or Ecteola cellulose, contained no glucosamine (20), and was resistant to digestion by testicular hyaluronidase.

Ultracentrifugal examination of the sample revealed the presence of some heavy material, as shown in Fig. 1. This material, which represents about 15% of the sample, was assumed to be a contaminant. The conditions under which the sedimentation equilibrium experiments were conducted were such that this heavy component would be predominantly concentrated in a thin layer at the bottom of the solution column, and would not contribute to the interference fringes which are the raw experimental data on which this study is based. The results to be presented thus describe only the major component shown in Fig. 1.

Solutions for measurement were prepared by dilution of stock solutions, the concentrations of which were determined on the basis of uronic acid analyses.

Sedimentation Equilibrium—Use was made of a Spinco model E ultracentrifuge equipped with Rayleigh interference optics and a temperature control unit. The optics were aligned so that the cell was observed in collimated light parallel to the axis of rotation. An AN-J rotor was used at speeds of 17,250 r.p.m. and below; an AN-E rotor was used at speeds above 17,250 r.p.m. Cells were equipped with sapphire windows and with 30-mm standard, double sector, aluminum-filled epoxy resin centerpieces. The solution channel was filled with 0.05 ml of fluoro-
carbon FC-43 base fluid and approximately 1.00, 0.65, or 0.25 ml of solution. The solvent channel was filled with a volume of solvent 0.05 ml greater than the volume of solution. These solution volumes yielded solution column heights of 11.6, 7.4, and 3.1 mm, respectively. Solutions were filtered through micropore filters to eliminate dust and dialyzed against the solvent before use. A Kodak type 77-A filter, placed over the light source, transmitted predominantly light with a wavelength of 546 nm.

Interference patterns were photographed on Kodak spectrographic plates, emulsion type II-G, and were measured with a Gaertner two-coordinate comparator. After alignment of the radial direction along the x coordinate of the comparator, any one fringe was selected for measurement of the y coordinate at suitable intervals of the x coordinate. A correction for minor effects of cell window distortion was made by running a blank in the same cell, with water in both sectors, as described by Yphantis (10). The value of the y coordinate at the upper meniscus was subtracted from all readings. The final result is expressed as the fringe displacement at any radial position r, and is designated \( f - f_0 \).

Photographs were taken at frequent intervals in each run, starting from zero time and extending to 8 days. Equilibrium was judged to be present when the fringe displacement \( f - f_0 \) measured at some point near the bottom of the cell asymptotically approached constant values. The typical time dependence of \( f - f_0 \) is shown in Fig. 2. The method of LaBar and Baldwin (12) was used to estimate the closeness of approach to equilibrium for most of the experiments. In each case, \( f - f_0 \) for the final photograph was estimated to be within 0.5% or less of its equilibrium value.

Under the conditions outlined here, the spacing between adjacent interference fringes was found to be 295 \( \mu \) on the comparator scale.

In a study involving a heterogeneous material, the locations of the upper and lower menisci of the solution column (designated \( r_a \) and \( r_b \)) become especially important. We measured these positions by both schlieren and interference optics, and found no appreciable difference between them. Values of \( r_a \) and \( r_b \) were measured at the beginning of each run, before appreciable sedimentation had occurred, and also after equilibrium had been established. No change was observed in \( r_a \), but measurements of \( r_b \) made at the end of a run were slightly ambiguous because of the presence at the lower meniscus of the thin layer of heavy contaminant, to which reference was made earlier. The presence of this layer was reflected in broadening of the apparent meniscus line. If the lower side of the broadened line was used to determine \( r_b \), then values were found which agreed with \( r_b \) values obtained at the beginning of each run.

It is evident that one of the consequences of the presence of the thin layer of heavy material at the bottom of the solution column is to make it impossible to read the location of interference fringes all the way to the position \( r = r_b \). A short extrapolation of the fringes was therefore required. The distance

\[ \text{TABLE I} \]

Analytical data for chondroitin sulfate B as used for this study*

<table>
<thead>
<tr>
<th></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexosamine</td>
<td>29.8</td>
</tr>
<tr>
<td>Uronic acid (COO·)</td>
<td>30.7</td>
</tr>
<tr>
<td>Uronic acid (orcinol)</td>
<td>33.4</td>
</tr>
<tr>
<td>Uronic acid (carbazole)</td>
<td>15.1</td>
</tr>
<tr>
<td>Sulfate</td>
<td>15.7</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>2.48</td>
</tr>
</tbody>
</table>

* Analyses performed on the calcium salt, dried in a vacuum over P₂O₅.

FIG. 1. Sedimentation velocity pattern of chondroitin sulfate B in 0.5 M NaCl at 25°. A double sector cell was used, with solvent on one side and chondroitin sulfate solution (5 g per liter) on the other. The speed was 59,780 r.p.m., and the photograph was taken 30 minutes after attainment of full speed. The sedimentation equilibrium results reported in this paper refer to the major component shown in this figure.

FIG. 2. Sample plot showing attainment of sedimentation equilibrium. The fringe displacement was measured at a fixed radial position near the bottom of the solution column.
Molecular Weight Distribution of Chondroitin Sulfate B

Vol. 239, No. 12

TABLE II
Summary of molecular weight data

<table>
<thead>
<tr>
<th>Nominal initial concentration</th>
<th>Rotor speed</th>
<th>Column height</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/litre*</td>
<td>r.p.m.</td>
<td>mm</td>
<td>$\bar{M}_n^o$</td>
</tr>
<tr>
<td>0.175</td>
<td>15,250</td>
<td>3.4</td>
<td>27.3</td>
</tr>
<tr>
<td>0.146</td>
<td>17,350</td>
<td>3.3</td>
<td>27.3</td>
</tr>
<tr>
<td>0.146</td>
<td>10,589</td>
<td>11.8</td>
<td>27.3</td>
</tr>
<tr>
<td>0.146</td>
<td>12,410</td>
<td>11.9</td>
<td>27.3</td>
</tr>
<tr>
<td>0.146</td>
<td>17,250</td>
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<td>23.5</td>
</tr>
<tr>
<td>0.146</td>
<td>20,410</td>
<td>4.4</td>
<td>23.5</td>
</tr>
<tr>
<td>0.0875</td>
<td>13,410</td>
<td>7.4</td>
<td>26.3</td>
</tr>
<tr>
<td>0.0875</td>
<td>17,250</td>
<td>4.4</td>
<td>26.3</td>
</tr>
<tr>
<td>0.0875</td>
<td>20,410</td>
<td>7.4</td>
<td>23.0</td>
</tr>
</tbody>
</table>

Average values

$M_n^o / M_w^o$ = 1.2

$M_z^o / M_w^o$ = 1.5

* Nominal concentrations include heavy impurity which does not contribute to the results.

† $M_n^o$ values are necessarily equal when initial concentrations are equal, by virtue of the method used to determine $f_0$.

where $r_b$ represents the position of the lower meniscus, $C_0$ is the initial uniform concentration of solute in the solution submitted to sedimentation, and $f_e = K C_0$.

Equations 2 and 3 show that both $f_0$ and $a$ curve of $f$ versus $r$, which were plotted by the method used to determine $f_0$. The observed values of $f - f_a$ may be integrated over the entire solution column to yield

$$\int_a^b (f - f_a) r dr = K \int_a^b C r dr - K \int_a^b C r dr$$

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Equations 2 and 3 show that both $f_0$ and $a$ could be determined from a single run if $f_a$ were negligibly small. This information would be sufficient to determine the entire molecular weight distribution of the solute. In general, the desired condition ($f_0 = 0$) could be attained by use of a sufficiently high rotor speed or a sufficiently long solution column, but if the solute contained molecules which vary over a wide range of molecular weights, as is true in the present study, the condition required to make $f_a \approx 0$ for the lighter molecules would lead to unmanageable crowding of the heavier molecules at the bottom of the solution column.

A procedure frequently used under these circumstances is to determine $f_a = K C_0$, by an independent experiment, e.g. by use of a synthetic boundary cell, or from previous knowledge of both $K$ and $C_0$. It is not certain, however, that this is a safe procedure.

The primary data obtained in this study consist of interference patterns such as that shown in Fig. 3. The vertical position ($f$) of any fringe at a distance $r$ from the center of rotation is proportional to the concentration of solute at that point.

$$f = \delta h (dn/dC) C/\lambda = K C$$

where $\delta$ is the distance between adjacent fringes in microns, as measured on the comparator, $h$ the thickness of the solution column in the ultracentrifuge cell, $dn/dC$ the refractive increment of the solute (assumed independent of molecular weight and of concentration), $\lambda$ the wave length of light used, and $C$ the concentration. The values for the various parameters in Equation 1 are listed in "Experimental Procedure." With these values the constant, $K$, of the equation, with $f$ in microns and $C$ in g per liter, becomes equal to 2593.

Under the conditions used by us, a fringe position corresponding to zero concentration cannot be established. Thus, observed values of $f$ are only relative, containing an unknown additive constant. However, the difference between the $f$ values at any two positions is given directly by the data, and we have therefore based all calculations on values of $f - f_a$, where $f_a$ is the fringe height at the upper meniscus ($r = r_0$). Where $C_a$ is the concentration at that position,

$$f - f_a = K (C - C_a)$$

The observed values of $f - f_a$ may be integrated over the entire solution column to yield

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SUMMARY

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A procedure frequently used under these circumstances is to determine $f_a = K C_0$, by an independent experiment, e.g. by use of a synthetic boundary cell, or from previous knowledge of both $K$ and $C_0$. It is not certain, however, that this is a safe procedure.
when very dilute solutions are used, as in the present experiments. Adsorption of solute may occur in the syringe used to fill the ultracentrifuge cell, and in the cell itself. If the initial solution is very dilute, this adsorption may remove a substantial fraction of solute from the solution, and diminish $f_0$ below the value calculated on the basis of an independent experiment. For the chondroitin sulfate sample, which is the subject of this investigation, there is an even more important factor which makes an independent determination of $f_0$ unattractive. This is the fact, already mentioned above, that the sample contains a heavy component which does not contribute to the fringe pattern at sedimentation equilibrium. This component would, however, contribute to the value of $f_0$ obtained by independent means.

For these reasons a determination of $f_0$ from the equilibrium fringe patterns themselves becomes desirable. We have made use of the fact that $f_0 - f_a$ as determined from the experimental data by Equation 3 must approach $f_0$ at high rotor speeds and large values of $r_a - r_b$. For a homogenous solute the equation for $f_a$ in a thermodynamically ideal solution would be

$$f_a = C_a = \frac{H e^{-H}}{\sinh H}$$

where $H$ is related to the molecular weight, $M$, by the equation

$$H = \frac{\omega^2}{4M(1 - \bar{v}p)\omega^2(r_a^2 - r_b^2)/RT}$$

In Equation 5, $\bar{v}$ is the partial specific volume of the solute, $\rho$ the solution density, and $\omega$ the rotor speed in radians per second. Equation 4 shows that $f_a$ should approach zero as $\omega^2(r_a^2 - r_b^2)$ becomes large, regardless of the solute molecular weight.

Ten separate determinations were carried out, as shown in Table II, at different concentrations, rotor speeds, and column heights. The observed values of $f_0 - f_a$ obtained by Equation 3 are shown as a function of $\omega^2(r_a^2 - r_b^2)$ in Fig. 4. The four runs at a nominal initial concentration of 0.0875 g per liter are grouped together, and are seen to reach an asymptotic limit of 155 μ at high values of $\omega^2(r_a^2 - r_b^2)$. The upper curve of Fig. 4 shows the five runs at a nominal initial concentration of 0.146 g per liter, plus the data for the single run at 0.175 g per liter for which the observed value of $f_0 - f_a$ has been multiplied by the ratio 0.146/0.175. The asymptotic limit for these points is seen to be 255 μ. It is concluded that $f_0 = 155, 255, and 305 μ$, respectively, for the three nominal concentrations which were used.

The nominal initial concentrations, when multiplied by the known value $K$ (Equation 1), lead to nominal $f_0$ values of 227, 378, and 454 μ, respectively. The observed $f_0$ values thus indicate that only about 70% of the total solute contributes to the observable interference fringe pattern. From the content of heavy material, discussed earlier, a figure of 85% would have been predicted. The discrepancy may represent loss of solute by adsorption, or it may simply represent a measure of the uncertainty of our experimental procedures.

With $f_a$ known, $f_a$ for each run may be determined by Equation 3, and the observed values of $f_0 - f_a$ at all points in the cell may be converted to values of $f$. Plots of $\ln f$ versus $r^2$ may then be obtained, and typical plots are shown in Fig. 5. It is clear that these plots are curved, showing that chondroitin sulfate is a substance which is heterogeneous with respect to molecular size. Such a substance must be characterized by molecular weight averages rather than by a unique molecular weight. The following averages are readily obtained from our data.\(^1\)

The weight average molecular weight, $\overline{M}_w$, is obtained directly from the ratio $(f_0 - f_a)/f_a$

$$\overline{M}_w = \frac{2RT}{(1 - \bar{v}p)\omega^2(r_a^2 - r_b^2)/RT}$$

Since the value of $f_a$ was obtained by extrapolation, on the basis that $(f_0 - f_a)/\omega^2(r_a^2 - r_b^2)$ is the same for all runs at a single

\(^1\)The designations $\overline{M}_w, \overline{M}_z, and \overline{M}_\phi$ refer to molecular weight averages of the major component of the original sample subjected to sedimentation analysis. The designations $\overline{M}_w, \overline{M}_z$, \(\overline{M}_\phi\) refer to the molecular weight average of the solution at a particular position, $r$, in the cell, after equilibrium has been reached.
concentration, the values of $M_{av}$ determined for all runs at a single concentration are necessarily the same.

The weight average of that part of the solute which lies at any position $r$ in the centrifuge cell (designated $M_{cr}$) is obtained from the slope of plots such as are shown in Fig. 5.

\[ M_{cr} = \frac{2RT \ln f}{(1 - \beta \alpha)^2} \frac{d\ln f}{dr} \]  

From the limiting value of this parameter at $r = r_b$, one can determine the $z$-average molecular weight ($M_{az}$) of the sample.

\[ M_{az} = M_{av} + \frac{(f_a/f_b)(M_{cr}/M_{av})}{1 - (f_a/f_b)} \]  

Except for the runs with the smallest values of $\omega(r_b^2 - r_a^2)$, $f_a/f_b$ is sufficiently small to allow $M_{az}$ to be equated with $M_{av}$. (Values of $M_{az}$ were not calculated from the runs with very low values of $\omega(r_b^2 - r_a^2)$.)

To calculate a number average molecular weight, it is first necessary to compute the integral $\int_a^r f(r)dr$ at each point of the cell. The local number average molecular weight ($M_{nr}$) at each point in the cell is related to this integral as

\[ \frac{f}{M_{nr}} = \frac{f_a}{M_{av}} + \frac{(1 - \beta \alpha)^2}{RT} \int_a^r f(r)dr \]  

where $M_{av}$ is the value of $M_{av}$ at the upper meniscus. It is seen that $M_{av}$ could be unequivocally determined from the data if $f_a$ were zero. When $f_a$ is not zero, $M_{av}$ is indeterminate because $M_{av}$ is not known, but the error introduced thereby can be made small by calculating number average molecular weights only from runs for which $f_a$ is quite small.

It is now possible to obtain a value for $M_{av}$, the number average molecular weight of the original sample, from the relation

\[ M_{av} = \frac{1}{b} \int_a^b \frac{f_b(r_b^2 - r_a^2)}{(f_b/M_{av})dr} \]  

However, another error arises at this point from the fact that data from the upper part of the cell make an appreciable contribution to the integral in the denominator of Equation 10. Since the percentage error in determining $f$ in this part of the cell is very large, we have used a modification of a procedure used by Yphantis (10). An arbitrary value of $r$ (designated $r'$) is selected, above which the percentage error in $f$ is no longer appreciable. The value of $f/M_{av}$ at this point can be considered an unknown parameter, and $M_{av}$ elsewhere in the cell can be expressed in terms of this parameter. This can be done by rewriting Equation 9 to give

\[ \frac{f}{M_{av}} = \left( \frac{f}{M_{av}} \right)_{r'} + \frac{(1 - \beta \alpha)^2}{RT} \int_r^{r'} f(r)dr \]  

An arbitrary number ($M^*$) is now tentatively chosen for the value of $M_{av}$ at $r = r'$. Since $f$ is accurately known at this point, this is equivalent to picking a value for $(f/M_{av})_{r'}$. The value of $M_{av}$ at all values of $r$ is now automatically fixed by Equation 11, and a plot of $M_{av}$ against some suitable parameter representing position in the cell can be made. We have chosen the parameter $\varphi = \int_a^r f(r)dr/B_{av}(r_b^2 - r_a^2)$, which represents the fraction of all of the solute which lies between the upper meniscus and the point $r$.

It is a requirement of the laws of sedimentation equilibrium that $M_{av}$ is either independent of $r$, or increases as $r$ increases, and $M_{av}$ should thus be a monotonically increasing function of $\varphi$. As the sample plots of Fig. 6 show, this condition is satisfied, regardless of the choice of $M^*$, over much of the cell. Near the top of the cell, however, over a region involving perhaps 25% of the solute, the results depend strongly on the choice of $M^*$.

In fact, a single value of $M^*$ can be chosen (within narrow limits) on the basis that the final plot of Fig. 6 must resemble that shown in the middle curve of that figure. In effect, what this procedure does is to eliminate systematic errors in the determination of $f$ (such as might occur through use of a value of $f_a$ which is in error by as little as 5%), by observing their cumulative effect over a wide region of the cell.

Once $M^*$ is known, the value of $f/M_{av}$ is also established, and the number average molecular weight of the whole sample is obtained by Equation 10. All of the results obtained in this way have been summarized in Table II. It is seen that the results are subject to an uncertainty of about 10%. (The agreement between $M_{av}$ values at a single concentration is an artifact of the method of extrapolation.) It is felt that this is not an unreasonable experimental error, in view of the uncertainties introduced by the impurity in the sample and by the necessary use of extrapolation procedures.

**DISCUSSION**

It is evident from our results that chondroitin sulfate is a heterogeneous polymer with a broad distribution of molecular weight. This suggests that the biosynthesis of this substance occurs by means of a more or less random polymerization process. The simplest possibility would be perfectly random addition polymerization, in which the rate constant for adding a new monomer unit to the polymer is independent of the length of the polymer chain. For this type of polymerization, the weight fraction of $z$-mer is

\[ W_z = zp^{x-1}(1 - p)^y \]  

where $p$ is a measure of the total extent of the polymerization,
equal to the fraction of all functional end groups in the sample which are contained in polymer bonds; i.e. $1 - p$ represents the fraction of such end groups still free to cause further polymerization. The following relations give the molecular weight averages of a polymer of this type, in terms of the molecular weight, $M_o$, of the repeating unit.

$$
\bar{M}_w = \frac{\sum x^p}{\sum x} = \frac{M_o}{1 - p} \tag{13}
$$

$$
\bar{M}_d = \frac{\sum x^p}{\sum x} = M_o(1 + p) \tag{14}
$$

$$
\bar{M}_f = \frac{1}{\sum x} = M_o(1 + 4p + p^2) \tag{15}
$$

The characteristic identifying marks of this type of polymer are the molecular weight ratios. For reasonably large values of $p$, $\bar{M}_w/\bar{M}_d = 2$ and $\bar{M}_w/\bar{M}_f = 1.5$. Chondroitin sulfate would appear not to be an ideal random polymer of this type, since the observed value of $\bar{M}_w/\bar{M}_d$ given in Table II is 1.2, compared with the theoretical value of 2.

It is important to note, however, that chondroitin sulfate is a polymer with a relatively low degree of polymerization. The experimental number average molecular weight given in Table II corresponds to an average degree of polymerization of about 45. An ideal random polymer with such a low degree of polymerization would contain appreciable quantities of low molecular weight species; i.e. the terms with the smallest values of $x$ made an appreciable contribution to the molecular weight averages given by Equations 13 to 15. However, such low molecular weight species could not be present in the polymer sample subjected to sedimentation equilibrium because the sample had been dialyzed for long periods during its preparation. Such dialysis would remove most of the smaller molecules in the preparation. Removal of these smaller polymer molecules would have a relatively large effect on $\bar{M}_w$, and a much smaller effect on $\bar{M}_d$ and $\bar{M}_f$. The ratio $\bar{M}_w/\bar{M}_d$ could thus be substantially reduced.

Actual calculations of the effect of dialysis are easily made, simply by deleting from the sums which occur in Equations 13 to 15 the terms with $x$ equal to or less than some limiting value, $t$. In other words, molecular weight averages are evaluated from Equations 13 to 15, but with the sums extending from $x = t + 1$ to $x = \infty$, instead of from $x = 1$ to $x = \infty$. Such calculations show that the ratio $\bar{M}_w/\bar{M}_d$ drops rapidly with increasing $t$, and calculated results close to the observed data are easily obtained. For example, with $p = 0.96$ and $t = 15$, one obtains $\bar{M}_w = 20,000$, $\bar{M}_d = 27,500$, and $\bar{M}_f = 38,000$. (The actual distribution which leads to these values is shown in Fig. 7.)

An alternative possibility is that the polymerization of chondroitin sulfate does not occur by a completely random process.

In the calculations of this section the monomer molecular weight is taken to be 500. This figure is based on the assumption that roughly 1 sodium ion per monomer unit is thermodynamically a part of the chondroitin sulfate molecule.

**Fig. 7.** Molecular weight distribution of chondroitin sulfate. Curve 1 is calculated according to Equation 12, with $p = 0.96$, and leads to a much greater $\bar{M}_w/\bar{M}_d$ ratio than is observed. Curve 2 is a similar calculation, except that molecules with $x \leq 15$ have been assumed to be removed by dialysis prior to ultracentrifugation, and it gives molecular weight averages in much better agreement with experiment (see text). It is to be expected that any distribution with relatively little low molecular weight material (such as the dashed line of the figure) will lead to fairly good agreement with the experimental results.

If the rate of addition of monomer units is more rapid for short chains than for longer chains, the fraction of small polymer molecules would be diminished. This would lead to deviations from the result expected on the basis of Equations 13 to 15, similar to that which results from the removal of the smaller chains by dialysis.

**SUMMARY**

The molecular weight distribution of a sample of chondroitin sulfate B has been studied by the method of equilibrium sedimentation. The sample was found to have number, weight, and $z$-average molecular weights of 23,000, 27,000, and 41,000, respectively. The precision of these results is of the order of 10%.

These results indicate that this polysaccharide is heterogeneous with respect to molecular weight, but the distribution is not as broad as would be expected for a polymer which is polymerized by a purely random process. Qualitatively, there are two ways to account for the data. (a) The polymer was originally random, but much material of low molecular weight was removed by dialysis during preparation. (b) The polymerization process occurs by continuous addition of monomer to growing chains, but the rate of addition is decreased as the chain length increases.

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