STUDIES ON HUMAN GLYCOGEN

II. SEDIMENTATION IN THE ULTRACENTRIFUGE*

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Early investigations of the size of glycogen by osmotic pressure measurements indicated molecular weights of 500,000 to 3,500,000 for this substance (1, 2). Since that time several studies of glycogen have been made in the ultracentrifuge. Bridgman (3) reported that rabbit liver glycogen is inhomogeneous with the maximum component, having a sedimentation constant of about 70 S and exhibiting a spread of values from 20 to 120 S. Similar findings were reported by Bell et al. (4) for glycogen samples of different origin. Chargaff and Moore (5) reported the isolation from tubercle bacilli of a glycogen which had an average particle size somewhat greater than those obtained from animal tissues.

In the present study, the objective was a comparison of glycogen samples from normal individuals and from those having glycogen disorders. All the samples were purified in the same manner in order to obtain comparative results (6) since it is recognized that different methods of handling may be reflected in the physical properties of the glycogen. A single sample of glycogen from normal human muscle studied by Bell et al. (4) did not differ significantly from the animal preparations. In the present investigation it was found that glycogen from a patient (N. D.) with a glycogen storage disorder of muscle1 differed from normal samples in the distribution of sedimenting components.

Preparations of human liver glycogen show two distinct sedimenting peaks, indicating that the distribution of particle sizes is not continuous. These two kinds of liver glycogen have been separated by chemical fractionation and by differential centrifugation in the preparative ultracentrifuge. The glycogen from a single case of liver glycogen storage disease (von Gierke's disease) did not show the heavy material found in preparations from normal individuals.

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Studies were made in the Spinco (Specialized Instruments Corporation) electrically driven model E ultracentrifuge, with the controls and procedures previously described (7). All the runs were conducted at speeds between 15,000 and 20,000 r.p.m. at room temperature. The samples were freshly prepared by dissolving in molar sodium chloride at a concentration of 1 per cent. Sedimentation constants were calculated from the peak positions and incorporate the usual corrections for the density and viscosity of the medium (8). The constants (s20,w) are given in Svedberg units where $S = 1 \times 10^{-13}$.

**Liver Glycogen**

Bridgman (3) has shown for rabbit liver glycogen that little or no diffusion occurs in the short time that glycogen samples are sedimented in the ultracentrifuge. In view of the large sedimentation and low diffusion constants exhibited by glycogen (3, 4), this is to be expected. The spreading of the boundaries is then due mainly to the polydispersity of the glycogen samples with the peak position representing the most probable sedimentation value for a family of molecules of different sizes grouped about a mode. In the present study, when two “components” were found, that is two peak positions, estimates were made of the relative amounts distributed about each peak, as though each of these were present in a relatively symmetrical manner. The values are given to the nearest 5 per cent. This arbitrary procedure was used to give a comparison of the different samples. Good agreement was found in different runs with the same material, and samples of glycogen from the same individual (N. D.), obtained first from a liver biopsy and some weeks later on autopsy, were in excellent accord (Table I). References to heavier and lighter “components” are used for convenience in referring to these families of molecules.

The results for human liver glycogen are presented in Table I, together with samples from rat and rabbit liver for comparison. These data show that all preparations sedimented with a peak position which gave $s_{20,w}$ values in the range of 60 to 100 S in good agreement with the range for peak positions previously reported by Bridgman (3) and by Bell et al. (4) with samples of rabbit liver glycogen. In contrast to the animal preparations, all human liver glycogens except one exhibited 20 to 55 per cent of a secondary peak representing heavier material which was clearly distinguishable from the lighter fraction. The sedimentation constants for the peak of this fraction have varied from 150 to 300 S, with a spread of values overlapping the light component on one end of the scale and containing material with values as high as 450 S. The absence of the secondary heavy
component in the sample from subject P. N. is of particular interest since it was obtained from a patient showing the symptoms of von Gierke's disease (6). Representative sedimentation patterns of human liver glycogen samples are illustrated in Fig. 1, B, C, D, and E.²

The sample of rabbit liver glycogen (Fig. 1, A) shows a small secondary peak of lighter material with \( s_{20w} = 24 \) S. Such a peak was observed by Bridgman (3) in some samples.

Fractionation of Liver Glycogen—The heavy fraction of a glycogen preparation from one normal individual (autopsy (P. F.); see Fig. 1, B) was precipitated by the addition to the aqueous solution of glacial acetic acid to a concentration of 67 per cent volume per volume as described below. The lighter fraction was obtained by ultracentrifugation from the glycogen remaining in the supernatant.

Glycogen (508 mg.) prepared as previously described (6) was dissolved in water to give a final volume of 10 ml. Glacial acetic acid (21 ml.) was added slowly with stirring, and the mixture was centrifuged for 1 hour at 2000 r.p.m. The precipitate was dissolved in water and freed of acetic

² For preparations of human liver glycogen, it was generally necessary to make two series of photographs, the first set for estimating the sedimentation constant of the heavier fraction and the second set for the lighter material. The patterns chosen for illustration were selected to demonstrate the presence of both peaks at the same time.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Speed r.p.m.</th>
<th>Component 1 ( s_{20w} ) Amount</th>
<th>Component 2 ( s_{20w} ) Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>20,410</td>
<td>96 99+</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>19,160</td>
<td>95 90</td>
<td>24 10</td>
</tr>
<tr>
<td>Human (normal) autopsy (P. F.)</td>
<td>20,410</td>
<td>68 45</td>
<td>300 55</td>
</tr>
<tr>
<td>&quot; (J. H. W.)&quot;</td>
<td>15,220</td>
<td>63 80</td>
<td>150 20</td>
</tr>
<tr>
<td>&quot; biopsy (J. K.)&quot;</td>
<td>15,220</td>
<td>97 65</td>
<td>300 35</td>
</tr>
<tr>
<td>&quot; (glycogen storage) biopsy (N. D.)&quot;</td>
<td>20,410</td>
<td>74 65</td>
<td>220 35</td>
</tr>
<tr>
<td>&quot; &quot; &quot; autopsy &quot;</td>
<td>15,220</td>
<td>72 70</td>
<td>220 30</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>15,220</td>
<td>75 65</td>
<td>160 35</td>
</tr>
<tr>
<td>&quot; (von Gierke's) biopsy (P. N.)</td>
<td>15,220</td>
<td>75 99+</td>
<td></td>
</tr>
<tr>
<td>&quot; (normal) P. F., Fraction 1</td>
<td>15,220</td>
<td>5 99+</td>
<td>300 95</td>
</tr>
<tr>
<td>&quot; &quot; &quot; Fraction 2</td>
<td>19,160</td>
<td>58 99+</td>
<td></td>
</tr>
<tr>
<td>&quot; (glycogen storage) N. D., Fraction 1&quot;</td>
<td>15,220</td>
<td>220 99+</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>19,160</td>
<td>59 99+</td>
<td></td>
</tr>
</tbody>
</table>

* This sample was purified only by alcohol precipitation; it was not given the acetic acid treatment used for the other samples (6).
acid by several successive precipitations from water with ethanol. After washing with alcohol and acetone and drying at 94° in vacuo, the product weighed 72 mg. (P. F.; Fraction 1). \( [\alpha]_s^20 = +198° \pm 5° \) (1 per cent, m NaCl).

The glycogen remaining in solution in 67 per cent acetic acid was precipitated by the further addition of 15 ml. of acetic acid. The precipitate was collected, washed, and dried in the usual manner; yield, 390 mg. This material was dissolved in 26 ml. of aqueous m sodium chloride. The solution was centrifuged for 90 minutes at 19,160 r.p.m. in the preparative head of the ultracentrifuge. A pellet of gelatinous precipitate formed at the bottom of the centrifuge tubes, and the supernatant liquid (22 ml.) was removed without disturbing this precipitate. The glycogen was precipitated from this supernatant by the addition of ethanol (28 ml.) and was purified and dried in the usual way; yield, 108 mg. (P. F.; Fraction 2). \( [\alpha]_s^20 = +190° \pm 2° \) (1 per cent, m NaCl).

By the same procedure, fractionation of a glycogen preparation (1.80 gm.) from liver obtained at autopsy of the patient having glycogen storage disorder of muscle (N. D.) gave a fraction (295 mg.) insoluble in 67 per cent acetic acid (N. D.; Fraction 1) and a lighter fraction (430 mg.) from the ultracentrifuge (N. D.; Fraction 2). N. D.; Fraction 1, \( [\alpha]_s^{20} = +198° \)
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$\pm 6^\circ$ (0.5 per cent m NaCl); Fraction 2, $[\alpha]^{20}_D = +194^\circ \pm 2^\circ$ (1 per cent m NaCl).

The $s_{20,\omega}$ values for these liver glycogen fractions are recorded in Table I and representative sedimentation patterns are shown in Fig. 1, F, G, H, and I. Although the $s_{20,\omega}$ values of P. F., Fraction 1, and N. D., Fraction 1, are different (300 S and 220 S, respectively), this is not significant in

![Fig. 2. Representative sedimentation patterns obtained on human muscle glycogen.](http://www.jbc.org/)

view of the low value ($s_{20,\omega} = 150$ S) obtained in one other normal (J. H. W.). It should be stated, however, that autopsy sample J. H. W. was obtained in very small yield from a tissue in which considerable glycogenolysis may have occurred, and may not be truly representative material.

The values obtained for the separated lighter fractions, $s_{20,\omega} = 58$ S (P. F., Fraction 1) and $s_{20,\omega} = 59$ S (N. D., Fraction 1), are somewhat lower than those obtained for these components before fractionation, $s_{20,\omega} = 68$ S (P. F.) and $s_{20,\omega} = 74$ S (N. D.). This indicates that some of the
heavier material was removed from solution in the ultracentrifuge. It would appear that further fractionation would yield a gradation of molecular sizes unlike the fractionation into two relatively distinct polymer-homologous series which has been described above. From the presently available data, there appears to be no fixed distribution of the lighter and heavier fractions in normal human liver glycogen. Since it is difficult to standardize the method of obtaining samples, undeterminable amounts of autolysis may occur between the time of obtaining the tissue and the time at which the tissue is chemically digested. The relative amounts of light and heavy materials and the average molecular distribution within each group may change during autolysis or during normal metabolism.

Preparations of "heavy glycogen" (Fraction 1) give extremely opalescent solutions in contrast to the purified lighter Fraction 2 which yields water-clear preparations. The highly turbid character of solutions of normal human liver glycogen has already been mentioned (6). It is noteworthy that solutions of the liver glycogen sample of P. N., which does not show a distinct peak for a heavy component, show only slight opalescence.

**Muscle Glycogen**

The glycogen of normal human muscle is not appreciably different in sedimentation behavior from animal glycogen samples previously studied.
(4), and the spread of curves is similar to those exhibited by animal liver glycogen preparations. Two preparations (P. F. and G. G.), considered to be normal, contained small amounts of a light component (Fig. 2, A), whereas one preparation (B. K.) showed only a single peak (Fig. 2, B). Glycogen from the patient (N. D.) having glycogen storage disease of muscle showed, in all samples examined, 30 to 40 per cent of lighter material with the peaks having $s_{20,w} = 22$ to 38 S. The two distinct peaks are clearly shown in Fig. 2, C. It should be noted that repeated determinations on separate samples of glycogen from subject N. D. gave results in good agreement with one another; the data are given in Table II. A sample of glycogen from cardiac muscle of N. D. closely resembled those from skeletal muscle.

DISCUSSION

The presence of distinct peaks for heavy and light components in human liver glycogen is indicated both by sedimentation behavior and by their separation by simple precipitation techniques. The absence of the heavy fraction in liver glycogen from P. N. may be of significance in von Gierke's disorder. In their review of glycogen storage disorders, Mason and Andersen (9) concluded, as a result of their biochemical studies, that no abnormality was present in the glycogen but that the enzyme system was unable to metabolize glycogen in a normal fashion. Our results do not contradict these findings but suggest that the fundamental enzymatic disorder may be accompanied by a change in the physical nature of the substrate and that an examination of glycogen in the ultracentrifuge may be of some importance in a study of glycogen disorders.

The samples of human muscle glycogen are similar to those obtained from animal liver in these studies and also to those in the studies by Bell and coworkers (4). However, the glycogen from the patient with glycogen storage disease of muscle (N. D.) contains substantial amounts of a lighter fraction which is present in smaller amounts in the other three samples of muscle glycogen examined. This again suggests that a physical difference in glycogen may occur as a result of what is, no doubt, fundamentally a disturbance in the enzyme system.

SUMMARY

1. Normal human liver glycogen contains two polydisperse components with sedimentation constants for the peak positions for one, in the range of 60 to 100 S, and for the other from 150 to 300 S. These have been separated from one another by fractional precipitation and by ultracentrifugation.
2. A liver biopsy from a case of glycogen storage disease of liver (von Gierke's disease) yielded a glycogen containing only the lighter material.
3. A preparation of glycogen from a case of glycogen storage disease of muscle contained a lighter fraction which was present in smaller amounts in the normal human muscle samples examined.

BIBLIOGRAPHY

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