of 2:3-4:5-di-isopropylidene-d-fructopyranose on treatment with acid acetone under suitable conditions.

2. The reducing substances other than fructose are partly fermented by yeast, and this fraction has been accounted for by D-glucose, which was estimated by the disappearance of reducing substances on treatment with the specific enzyme, glucose oxidase.

3. A part of the reducing substances in solutions deproteinized by the use of acid cadmium sulphate and sodium hydroxide, and a greater part of those in solutions deproteinized by chloroform and ethanol, are not fermented by baker’s yeast; no evidence was obtained for the presence of any reducing sugar other than D-fructose and D-glucose.

4. The implications of these findings are discussed.

We wish to place on record our gratitude to the late Sir Joseph Barcroft, F.R.S., for the opportunity to investigate this problem, and for his encouragement, given so generously and in so many ways. We are indebted to Dr T. Mann and Mr S. W. Cole, for their advice and help in the course of the work; to Miss V. Moyle, Mr M. W. S. Hitchcock, and Dr S. M. Partridge for analyses which they performed for us; and to the members of the Agricultural Research Council Unit of Animal Physiology for their friendly co-operation.

We wish also to thank Prof. A. C. Chibnall, F.R.S., for his interest in the progress of the research.

One of us (D. J. B.) acknowledges a grant for research from the Agricultural Research Council; the other (J. S. D. B.) was in the full-time employment of the Council when the work was begun.

REFERENCES


The molecular weight of unsubstituted glycogen has, up to the present, been studied in but a few instances. Oakley & Young (1936) have reported, for samples from rabbit liver and muscle, values of 1·2-2·3 x 10⁶ for the former, and 0·7-1·8 x 10⁶ for the latter material. These results were obtained by use of a delicate method of osmotic-pressure measurement on solutions in 0·1 M-calcium chloride. These authors claim that both alkaline extraction of the tissue (the so-called Pflüger method) and water extraction followed by precipitation by acetic acid (Bell & Young, 1934) yield products of the same general molecular size. Oakley & Young (1936) further noted that glycogen solutions when salt-free manifested markedly greater osmotic pressures than in presence of Ca²⁺ and Cl⁻. Bridgeman (1942) determined the sedimentation and diffusion constants of rabbit-liver glycogen and derived from these an average molecular weight of about 4 x 10⁹. Glycogen from tubercle bacilli has been shown by Chagaff & Moore (1944), using similar methods, to have a molecular weight of 12-13 x 10⁹. These are the only reported molecular weight determinations using the ultracentrifuge.

Physicochemical Observations on some Glycogens

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AND R. CECIL AND A. G. OGSTON, Department of Biochemistry, University of Oxford

(Received 17 July 1947)
Our object in the present work was to compare certain physical properties of a number of glycogen preparations.

EXPERIMENTAL AND RESULTS

Preparation of material. Preparations were made from the appropriate tissue either by water extraction or by the Pfüger technique. In each instance final purification was effected by fourfold precipitation by acetic acid (Bell & Young, 1934). Relevant data are summarized in Table 1.

Table 1. Glycogen specimens examined

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Source</th>
<th>Method of isolation (P, by Pfüger technique; W, by water)</th>
<th>No. of residues in average unit chain</th>
<th>References to previous work on specimen used</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Horse muscle</td>
<td>P</td>
<td>11-12</td>
<td>Bell (1937)</td>
</tr>
<tr>
<td>II</td>
<td>Rabbit muscle</td>
<td>P</td>
<td>11-13</td>
<td>Bell (1944)</td>
</tr>
<tr>
<td>III</td>
<td>Human muscle</td>
<td>P</td>
<td>12</td>
<td>Bell (1948)</td>
</tr>
<tr>
<td>IV</td>
<td>Rabbit liver</td>
<td>P</td>
<td>12</td>
<td>Bell (1936)</td>
</tr>
<tr>
<td>V</td>
<td>Rabbit liver</td>
<td>W</td>
<td>12</td>
<td>Bell (1935)</td>
</tr>
<tr>
<td>VI</td>
<td><em>Ascaris lumbricoides</em></td>
<td>P</td>
<td>12-13</td>
<td>Baldwin &amp; King (1942)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13-14</td>
<td>Bell (1944)</td>
</tr>
</tbody>
</table>

Table 2. Molecular weights of glycogen specimens

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Solvent</th>
<th>(\eta/\eta_0 - 1/C)</th>
<th>(D_{20} \times 10^7)</th>
<th>(S^{20}_{20}) corr. (\times 10^{12})</th>
<th>Mol. wt.* from (S^{20}<em>{20}) and (D</em>{20})</th>
<th>Mol. wt. from osmotic pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Water</td>
<td>0.074</td>
<td>1.50</td>
<td>63</td>
<td>2.9 (\times 10^4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m-NaCl</td>
<td>0.074</td>
<td>1.55</td>
<td>58</td>
<td>2.6 (\times 10^4)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Water</td>
<td>0.060</td>
<td>1.65</td>
<td>57</td>
<td>2.4 (\times 10^4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m-NaCl</td>
<td>0.120-0.065</td>
<td></td>
<td>0.5 (\times 10^6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Water</td>
<td>0.055</td>
<td>1.27</td>
<td>81</td>
<td>4.4 (\times 10^6)</td>
<td>2.0 (\times 10^6)</td>
</tr>
<tr>
<td></td>
<td>m-NaCl</td>
<td>0.075-0.063</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Water</td>
<td>0.135</td>
<td>6.25</td>
<td>75</td>
<td>4.3 (\times 10^6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m-NaCl</td>
<td>1.80</td>
<td>18</td>
<td>0.1 (\times 10^6)</td>
<td></td>
<td>0.5 (\times 10^6)</td>
</tr>
</tbody>
</table>

* Calculated from the equation mol. wt. = \(\frac{RTS}{D(1 - V_p)}\), where R = gas constant; T = absolute temperature; S = sedimentation constant; D = diffusion constant; \(V_p\) = partial specific volume and \(p\) = density of solvent.

Viscosity measurements. These were made in an Ostwald-type viscometer having a flow time for water of about 3 min. Series of solutions of each sample of glycogen, in concentrations ranging from 0.2 to 2.0%, were examined. Except where indicated in Table 2, we observed no marked dependence of the specific viscosity upon concentration.

Diffusion rates. These were observed on 1% solutions (in m-NaCl) of the glycogens in a cell of the type described by Lamm & Polson (1936). Diffusion constants were calculated by the inflexion-point method and distribution curves over the boundary were obtained by the Philpot (1938) optical system. The diffusion constants \(D_{20}\) recorded in Table 2 are corrected to diffusion in water at 20°.

Sedimentation rates. The sedimentation velocities were observed in the Svedberg oil-turbine ultracentrifuge by the method of Philpot (1938). Fig. 1 shows specimen diagrams. The thicknesses of the sedimenting boundaries were in all instances much greater than could be accounted for by diffusion, showing that the samples were markedly polydisperse. The glycogen of rabbit liver prepared by
significant differences between the densities of glycogen samples from different sources, and the calculated partial specific volume was equal to 0.64–0.66.

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**Osmotic pressure.** This was measured by the method of Adair (1925). The concentration of glycogen after equilibrium was determined polarimetrically. A series of experiments over a range of concentrations of from 1 to 7% of solute showed that the apparent molecular weight calculated from osmotic pressure is markedly dependent on concentration. It was difficult to apply the extrapolation to zero concentration recommended by Adair & Robinson (1930) as the pressure exerted by dilute solutions was very small and the results obtained yielded, therefore, only rough approximations.

**DISCUSSION**

From the observations recorded in Table 2, it is clear that glycogens rank among the largest known metabolites, and that significant differences of molecular size occur according to the source from which the glycogen is obtained. Although all the samples were polydisperse and although the degree of polydispersity evidently depends on the method of isolation, their general uniformity suggests that these values may be valid for glycogen as it occurs in the cell.

The lower values of molecular weight obtained by osmotic measurement are to be expected in view of the polydispersity of the material, since osmotic pressure depends on the 'number average' while sedimentation and diffusion depend on the 'mass average' of molecular species.

The lowering of the osmotic pressure by salts, already noticed by Oakley & Young (1936), is difficult to account for. Disaggregation is unlikely in view of the lowering of viscosity produced by salt. A Donnan lowering is unlikely in view of the absence of ionizable groups in glycogen, though compensa-

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**Fig. 1.** Sedimentation diagrams. The following are the values of the speed of rotation (rev./sec.) and the time after reaching full speed at which the diagrams were obtained (roman numbers refer to the glycogen specimens: see Table 1). I, II, III: 450; 25 min. IV: 500; 30 min. V: 315; 60 min. VI: 625; 20 min. Concentration of glycogen: in each case 1 g./100 ml. of solvent.

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**SUMMARY**

1. The average molecular weights of samples of muscle glycogen have been shown, from sedimentation and diffusion data, to be of the order of 2.4–2.9 x 10^6.
2. Samples of liver glycogen have molecular weights of 4.4–4.5 x 10^6. Alkaline extraction of the glycogen from its parent tissue does not effect very serious degradation.
3. Glycogen from the whole tissues of Ascaris lumbricoides has a much smaller molecular weight, namely 0.70 x 10^6.
4. Osmotic pressure measurements have been shown to lead to estimations of molecular weight considerably smaller than those afforded by sedimentation-diffusion methods.
5. In all instances the glycogens were found to be polydisperse with respect to molecular size.
6. The observations of Oakley & Young on the effect of salts on the apparent molecular weight by osmotic pressure have been confirmed.

The authors are indebted to Prof. A. C. Chibnall, F.R.S., for his interest. One of us (D.J.B.) acknowledges with thanks a grant from the Agricultural Research Council.
Partial Amino-acid Compositions of some Plant-leaf
Protein Preparations: the Arginine,
Histidine and Lysine Contents

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In an earlier publication (Lugg & Weller, 1944) the
amide, tyrosine, tryptophan, cyst(e)ine (i.e. cystine
plus cysteine calculated as cystine) and methionine
contents of some extracted plant-leaf protein pre-
parations were reported. These preparations, one
each from leaves of Phalaris tuberosa L., Hordeum
muriunum L., Medicago sativa L., and M. denticulata
Willd., were judged, both from the mode of pre-
paration and from analytical evidence, to be reason-
able representative of the whole proteins in the
leaf materials. The arginine, histidine and lysine
contents of these same preparations have now been estimated
and it is one purpose of this article to record the
values obtained. The question of the existence of
hydroxyllysine in these preparations has also been
considered.

EXPERIMENTAL

Methods. The arginine and lysine contents of the
preparations were estimated by methods based essentially
upon those of Tristram (1939). Histidine contents were at
first estimated by Tristram's (1939) method, but for reasons
to be discussed, the resulting values were considered
unsatisfactory and recourse was had to an adaptation of the
method of Vickery & Winternitz (1944). A modification of
the Kapeller-Adler's (1934) colorimetric method of estimating
histidine, recommended by Block (1937), was unsatis-
factory in our hands.

Hydrolysis. In Tristram's experience the hydrolysis of
plant-leaf protein preparations for the purpose of estimating
the contents of these three amino-acids could be performed
satisfactorily by heating the protein with about ten times
its weight of $4\times H_2SO_4$ for 18-22 hr. at the boiling point of

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Melbourne, Melbourne, N. 3, Victoria.

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