of 2:3-4:5-di-*iso*propylidene-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 small 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.

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Physicochemical Observations on some Glycogens

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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 $1{\cdot}2$–$2{\cdot}3 \times 10^6$ for the former, and $0{\cdot}7$–$1{\cdot}8 \times 10^6$ for the latter material. These results were obtained by use of a delicate method of osmotic-pressure measurement on solutions in 0·1 N-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 Ca^{++} and Cl^-. Bridgeman (1942) determined the sedimentation and diffusion constants of rabbit-liver glycogen and derived from these an average molecular weight of about 4×10^6. Glycogen from tubercle bacilli has been shown by Chargaff & Moore (1944), using similar methods, to have a molecular weight of 12–13×10^6. These are the only reported molecular weight determinations using the ultracentrifuge.

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 Pflüger technique. In each instance final purification was effected by fourfold precipitation by acetic acid (Bell & Young, 1934). Relevant data are summarized in Table 1.

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 the Pflüger method (sample IV) was more polydisperse than that made by water extraction (sample V). The mean sedimentation constants, corrected to the values for water at 20° (S_{20} corr.), are given in Table 2.

Partial specific volume. V was calculated both from the densities of aqueous solutions and from the density of dry glycogen samples using diethyl β-ketoglutarate. (This liquid was chosen for the reasons that it is not appreciably volatile, has relatively low viscosity and rapidly wets glycogen without dissolving it.) There appear to be no

Table 1. *Glycogen specimens examined*

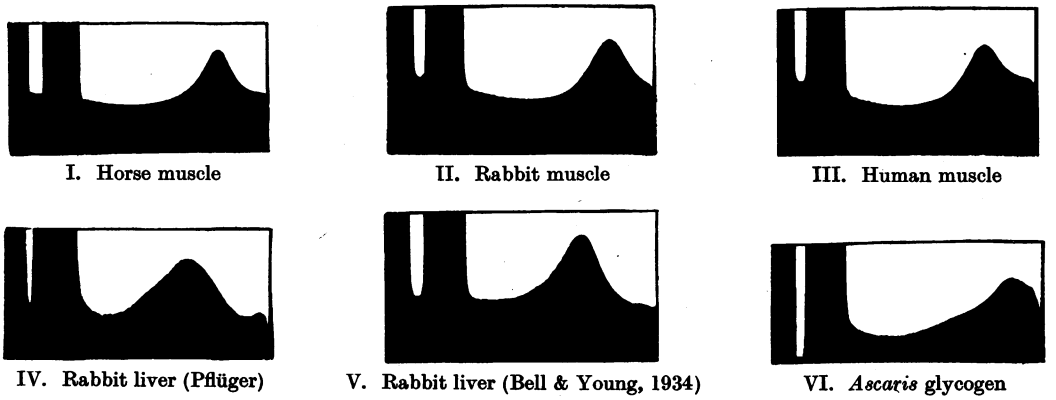
Serial number	Source	Method of isolation (P, by Pflüger technique; W, by water)	No. of residues in average unit chain	References to previous work on specimen used
I	Horse muscle	P	11–12	Bell (1937)
			11–13	Bell (1944)
II	Rabbit muscle	P	12	Bell (1948)
III	Human muscle	P	11	Halsall, Hirst & Jones (1947)
IV	Rabbit liver	P	12	Bell (1935)
V	Rabbit liver	W	12	Bell (1935)
VI	*Ascaris lumbricoides*	P	12–13	Baldwin & King (1942)
			13–14	Bell (1944)

Table 2. *Molecular weights of glycogen specimens*

Specimen	Solvent	$\frac{\eta/\eta_0 - 1}{C}$	$D_{20} \times 10^7$	S_{20} (corr.) $\times 10^{13}$	Mol. wt.* from S_{20} and D_{20}	Mol. wt. from osmotic pressure
I	Water	0·074	—	—	—	—
	M-NaCl	—	1·50	63	$2{\cdot}9 \times 10^6$	—
II	Water	0·074	—	—	—	—
	M-NaCl	0·055	1·55	58	$2{\cdot}6 \times 10^6$	—
III	Water	0·060	—	—	—	—
	M-NaCl	—	1·65	57	$2{\cdot}4 \times 10^6$	—
IV	Water	0·120–0·065	—	—	—	$0{\cdot}5 \times 10^6$
	M-NaCl	—	1·27	81	$4{\cdot}4 \times 10^6$	$2{\cdot}0 \times 10^6$
V	Water	0·075–0·063	—	—	—	—
	M-NaCl	—	1·21	75	$4{\cdot}3 \times 10^6$	—
VI	Water	0·135	6·25	—	—	$0{\cdot}1 \times 10^6$
	M-NaCl	—	1·80	18	$0{\cdot}70 \times 10^6$	$0{\cdot}5 \times 10^6$

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

significant differences between the densities of glycogen samples from different sources, and the calculated partial specific volume was equal to 0·64–0·66.



I. Horse muscle II. Rabbit muscle III. Human muscle

IV. Rabbit liver (Pflüger) V. Rabbit liver (Bell & Young, 1934) VI. *Ascaris* glycogen

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: 400; 30 min. V: 315; 60 min. VI: 625; 20 min. Concentration of glycogen: in each case 1 g./100 ml. of solvent.

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 compensation of a charge-effect would account also for the lowering of viscosity. The effect of salt on viscosity might be accounted for by a decreased asymmetry of the molecule; this might be accompanied by a reduction of the number of its possible configurations which would account, on statistical grounds, for the reduction of osmotic pressure.

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 $2{\cdot}4–2{\cdot}9 \times 10^6$.

2. Samples of liver glycogen have molecular weights of $4{\cdot}4–4{\cdot}3 \times 10^6$. 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 $0{\cdot}70 \times 10^6$.

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.

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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 preparations were reported. These preparations, one each from leaves of *Phalaris tuberosa* L., *Hordeum murinum* L., *Medicago sativa* L., and *M. denticulata* Willd., were judged, both from the mode of preparation and from analytical evidence, to be reasonably 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 hydroxylysine 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 (1934) colorimetric method of estimating histidine, recommended by Block (1937), was unsatisfactory 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 4M-H_2SO_4 for 18–22 hr. at the boiling point of the mixture (approx. 110°). Whilst it is unlikely that these conditions would secure the maximum 'net production of amide NH_3 plus amino-acid "amino" groups' (see Lugg, 1946), they may achieve almost complete liberation of these particular amino-acids from such proteins. In our work, about 2·7 g. of the air-dried preparation (about 2·5 g. dry wt.) were heated for *c.* 1 hr. with 25 ml. of 4M-H_2SO_4 at 100° and then at the boiling point of the mixture for 21–22 hr., which is the upper region of Tristram's (1939) range.

Estimation of arginine, histidine and lysine. The excessive variability of replicate histidine values estimated by Tristram's (1939) method and the extreme uncertainty of values obtained by the Conrad & Berg (1937) modification of Kapeller-Adler's (1934) method (tints of standard and test colour solutions frequently differed markedly) made it desirable to find a more satisfactory procedure. The Vickery & Winternitz (1944) method, in which the histidine is estimated gravimetrically as the di-3:4-dichlorobenzene sulphonate (histidine-DBS), was adapted for use in a general procedure for the estimation of arginine and histidine.

In this procedure insufficient Ag_2O and H_2SO_4 for precipitation of Ag_2SO_4 to occur were used, and larger quantities of $AgNO_3$ than are needed in Tristram's (1939) procedure, had to be employed. This and subsequent steps were taken to prevent the introduction of variable amounts of H_2SO_4 and/or HCl, in the presence of which we found the solubility of histidine-DBS to be appreciably increased. Urea was added in small quantities to decompose any nitrous acid which may have been present (from decomposition of nitrate by reducing substances in acid solutions). Tristram's procedure was then followed for the separation of the arginine- and histidine-silver compounds and for the isolation of the arginine and lysine as monoflavianate and monopicrate, respectively.

The histidine was liberated from the silver compound by treatment with H_2S (Vickery & Winternitz, 1944, used HCl) and was then precipitated as histidine-DBS in the

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