oxidation product. This refutes the suggestions that the enamine is the normal intermediate.[8,9]

(8) H. R. Dakin, *J. Biol. Chem.*, **67**, 341 (1926).

(9) G. Taborsky, *Yale J. Biol.*, **27**, 267 (1955).

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SEDIMENTATION CHARACTERISTICS OF GLYCOGEN[1]

Sir:

Glycogens which exhibit extremely high molecular weight have been obtained by extraction of this polysaccharide with water at low temperatures (0 to 4°) from *Ascaris* muscle and from rabbit liver.

Glycogen readily sedimented on ultracentrifugation (50,000 to 60,000 × g; 0 to 4°). Under these conditions over 95% of the total liver glycogen was recovered in the residue. Protein and other impurities were removed by differential centrifugation, repeated mechanical shaking with a mixture of chloroform and octyl alcohol[2] and treatment with trypsin and chymotrypsin. The analyses of purified samples (dried *in vacuo* at 75° to constant weight) which contained no detectable protein (less than 0.005%) yielded the theoretical values for glycogen (Calcd. for $(C_6H_{10}O_5)_n$: C, 44.44; H, 6.22; O, 49.34. Found: C, 44.59; H, 6.59; O, 49.47).

Ultracentrifugal analyses indicated weight average sedimentation coefficients of 300 to 1000 svedberg units and corresponding minimal weight average molecular weights of 50 to 200 million. In the absence of reliable diffusion measurements the molecular weight is stated as the *minimum* possible for a material having this sedimentation rate.

Sedimentation coefficient distributions showed extreme polydispersity and marked skewness, a typical sample including small amounts below 25 svedbergs and significant amounts above 2000 svedbergs, with the weight average near 800 svedbergs. Glycogens extracted from both rabbit liver and *Ascaris* muscle yielded sedimentation distributions of similar shape. However, the sedimentation coefficients for liver glycogen were

(2) M. G. Sevag, *Biochem. Z.*, **273**, 419 (1934).

approximately twice those of *Ascaris* muscle glycogen.

The two conventional methods used for the extraction of glycogen consist of treatment with either high concentrations of sodium or potassium hydroxide[3,4] at 100° for several hours or with solutions of trichloroacetic acid[5,6] at temperatures ranging between 0 and 20°. The molecular weight of water extracted glycogen was found to be at least 10 times that of glycogen extracted with cold trichloroacetic acid and 50 to 100 times that of the alkali extracted material. Furthermore, heating above 90° resulted in a progressive degradation of water extracted glycogen.

Because of the observed high molecular weights the possibility of aggregation of glycogen molecules[7] was tested using procedures known to disrupt electrostatic bonds. Samples treated with urea ($8M$, 0°,[8] one week), guanidine hydrochloride ($8M$, 0°,[8] two days) or with anionic, non-ionic or cationic detergents (sodium dodecyl sulfate, Tween 80, cetyltrimethylammonium bromide) (1%, 20°, 24 hours) showed no change detectable by sedimentation analysis. Also, no changes were found on repeated freezing and thawing, on repeated precipitation, drying and redissolving and after anaerobic incubation in 0.01 N KOH (20°, 24 hours). In addition, aliquots taken at various stages of the purification procedure and containing progressively lower concentrations of contaminating protein (from 1% to less than 0.005%) showed identical sedimentation characteristics, indicating that the presence or absence of protein bears no relationship to the molecular size of glycogen. On the basis of these observations the hypothesis that the high molecular weight of water extracted glycogen is the result of aggregation receives no support.

Measurements of diffusion rates are in progress and should permit accurate estimates of molecular weight.

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(7) A. Lazarow, *Anat. Rec.*, **84**, 31 (1942).

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