at $-10°$. This is true of diluted solutions of the 30–60% ammonium sulfate fraction and of purified eluates from gels. The pyrophosphorylases for UDP-D-galactose and for deTDP-D-glucose are considerably more stable.

Optimal conditions for deTDP-D-galactose pyrophosphorylase activity were found to be 25–30°, pH 8.2 (in 0.04 M Tris buffer), and the presence of 3.3 mM $MgCl_2$ in the incubation mixture. Mn^{2+} and Co^{2+} were less effective than Mg^{2+}. *p*-Chloromercuribenzoate (10^{-4} M) inhibited the activity completely. Some nucleotides (UMP, deTMP, deUMP, and AMP) inhibited the reaction by 40–50% at a concentration of 1.5 mM; thymine or thymidine at the same concentration had no effect. K_m for α-D-galactose 1-phosphate was found to be 1.6 mM, and that for deTTP, 1 mM.

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Molecular weight of the phospho and dephospho forms of phosphoglucomutase

The molecular weight of phosphoglucomutase (D-glucose-1,6-diphosphate: D-glucose-1-phosphate phosphotransferase, EC 2.7.5.1) has a special importance because (a) it is a relatively high-molecular-weight protein which may have only one active site per molecule, (b) its molecular weight is of importance in determining the degree of phosphorylation, and (c) the phosphorylation and dephosphorylation of the enzyme might be accompanied by molecular-weight changes. KELLER, LOWRY AND TAYLOR[1] found a molecular weight of 74 000 using sedimentation and diffusion coefficient measurements on enzyme crystallized from ammonium sulfate[2]. The advent of more highly purified preparations of phosphoglucomutase[3,4] and the development by YPHANTIS[5] of a simple accurate equilibrium centrifugation method has led us to redetermine this value.

Crystalline phosphoglucomutase obtained by the method of NAJJAR[2] was chromatographed on carboxymethyl cellulose columns to obtain pure phospho and dephospho forms of the protein[3,4]. These forms were then tested in the Tiselius moving-boundary electrophoresis apparatus. Isoelectric points of both proteins were found to be near pH 7.0. There were, however, minor differences between the iso-

electric points of the two forms since the dephospho form was found to move slightly at a pH at which the phospho form did not migrate. The molecular weights of these two enzymatic forms were then determined by the equilibrium ultracentrifuge method[5] using a column of solution 2.5 mm in height at speeds high enough so that the concentration at the meniscus went essentially to zero. Using these conditions equilibrium was reached in about 8 h. Samples from which final calculations were made were, however, allowed at least 16 h at constant speed prior to measurement. Using the multichannel cell three concentrations in the range 0.01–0.1% were studied simultaneously. Concentrations in the cell were determined by measuring interference fringes.

In the molecular weight formula of Eqn. 1,

$$M = \frac{RT(d\ln c/dr)}{r(1 - \bar{v}\varrho)\omega^2} \quad (1)$$

the partial specific volume, $\bar{v}$, was calculated from the amino acid composition[4] by conventional methods[6,7] and the density of the solvent, ϱ, was determined with the Westphal balance. The equilibrium runs were performed at pH 7 in sodium cacodylate buffer of ionic strength 0.15 in the Spinco model E ultracentrifuge at speeds of 25 980 and 29 500 rev./min, and at temperatures of 25° and 22°, respectively. Photographs were taken on Kodak spectroscopic plates type II-G. Measurements were made with a Gaertner tool-makers microscope.

Figs. 1a and 1b show the actual experimental data obtained in two typical runs. Such data on a variety of preparations were then used to calculate the values shown in Table I. From the data presented in this Table, one can conclude that the molecular weight of phosphoglucomutase is 62 000 and that both the phospho and dephospho forms have the same molecular weight. The error calculated for a method of this sort is approx. 4% at a maximum and the values in Table I are well within this tolerance. The linearity of the plots in Fig. 1 is added support for the purity of the preparations.

There are several possible explanations for the difference between this value for the molecular weight and the earlier value of 74 000, which was obviously determined

TABLE I

MOLECULAR WEIGHTS OF DIFFERENT PREPARATIONS OF THE PHOSPHO AND DEPHOSPHO FORMS OF PHOSPHOGLUCOMUTASE

Form of phosphoglucomutase	*Concentration (mg/ml)*	*Speed*	*Temperature*	*Mol. wt.*
Phospho	1.0	25 980	25°	61 000
Phospho	0.1	25 980	25°	62 600
Dephospho	1.0	25 980	25°	61 900
Phospho	1.0	29 500	22°	64 000
Phospho	0.1	29 500	22°	61 000
Dephospho	1.0	29 500	22°	61 700
		Average phospho form		62 000
		Average dephospho form		61 800

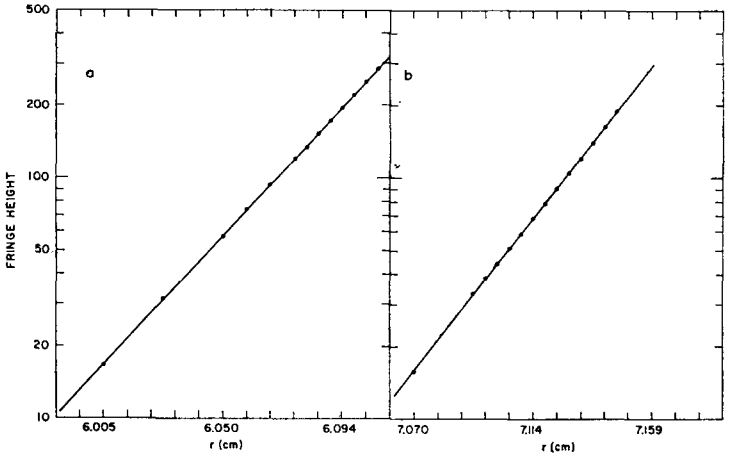

Fig. 1. In this figure are shown plots of the fringe height on a logarithmic scale *versus* r, the distance from the center of rotation for centrifuge speeds of 25 980. (a) the phospho form of phosphoglucomutase and (b) the dephospho form of phosphoglucomutase.

with care. One possibility is that the earlier samples of phosphoglucomutase were contaminated with a high-molecular-weight protein. When crystalline protein was isolated by the method of NAJJAR[2] and then chromatographed, we found that it contained 40–50% of a protein having little or no enzymatic activity. If this protein were of high molecular weight it could cause such an error in the velocity ultracentrifugation runs. Other possibilities are an error in the diffusion coefficient due to the schlieren scanning method or in the correction factor used to calculate the effect of temperature on the water viscosity. Although the equilibrium method is also subject to errors, it is less complex and less dependent on assumptions and this together with the improved purification of the protein leads us to conclude that the true molecular weight of purified phosphoglucomutase from rabbit muscle is 62 000.

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