Determination of Molecular Weight—The molecular weights of the holoenzyme and apoenzyme, the latter prepared by the acid-ammonium sulfate method described in a previous paper (*12*), were determined by Sephadex G-200 gel chromatography using marker proteins of known molecular weight. The results are shown in Fig. 4. The holoenzyme has a molecular weight of 160,000 and that of the apoenzyme is 80,000 (the elution pattern is shown in Fig. 8). From the results of SDS-polyacrylamide gel electrophoresis essentially only one component with a molecular weight of 79,000±4,000 was detected, as shown in Fig. 5, although, on the top of the gel, one more band was seen (possibly nonreduced holoenzyme). This suggests that the enzyme consists of two identical subunits.

As glucose oxidase is a typical glycoprotein, these semiempirical methods may not be ideal for the determination of molecular weight. Glucose oxidase also has activity even in 0.75% SDS, as indicated by Swoboda and Massey (*4*). In order to ascertain the effect of acrylamide concentration on the mobility of this enzyme

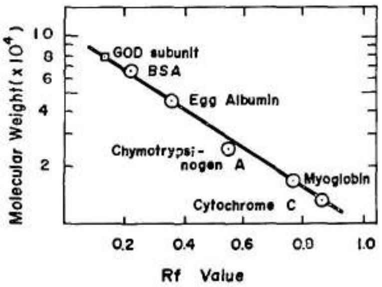

Fig. 5. Determination of the molecular weight of the enzyme by SDS-polyacrylamide gel electrophoresis (7.5% gel). Each sample was treated with 1% SDS, 1% 2-mercaptoethanol, and 5% glycerol in 250 mM phosphate buffer, pH 7.2 at 55° for 2 hr. Electrophoresis was carried out at a current of 5 milliamperes per tube for 4 hr.

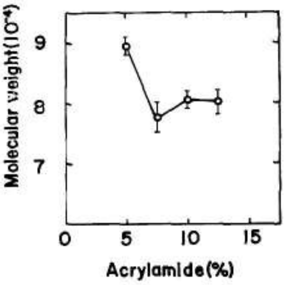

Fig. 6. Observed molecular weight of subunit of glucose oxidase calculated from the electrophoretic mobility *vs.* acrylamide gel concentration (see the legend to Fig. 5).

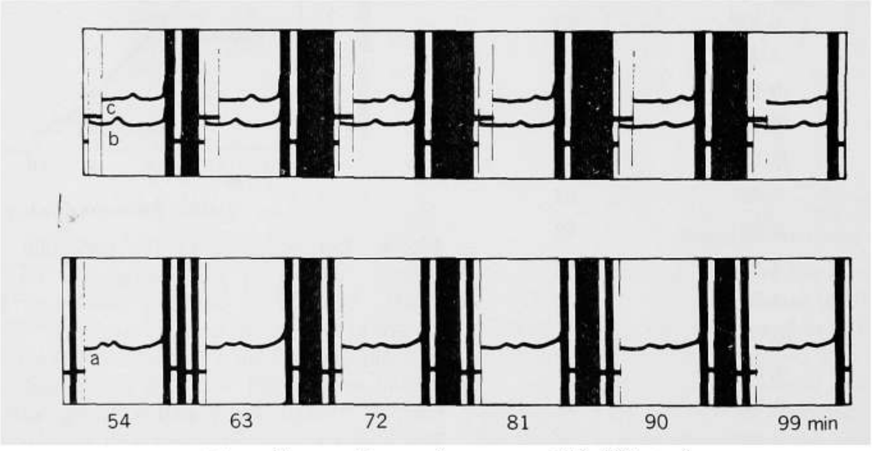

Fig. 7. Sedimentation velocity analysis of the apo and holo forms of glucose oxidase. Photographs show the sedimentation pattern of (a) apoenzyme only, (b) apoenzyme *plus* FAD (7.1 μM) after 4 hr, and (c) apoenzyme *plus* FHD (12.5 μM) after 24 hr. The schlieren diaphragm angle was 60°. Experiments were carried out in 100 mM phosphate buffer, pH 7.0.