PURIFICATION AND PROPERTIES OF 
D-GLUCOSE-6-PHOSPHATE 
DEHYDROGENASE*

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D-Glucose-6-phosphate dehydrogenase (Zwischenferment) was discovered by Warburg and Christian in 1931 (1, 2). An active preparation was obtained then from horse erythrocytes and in 1932 from the Lebedev juice made from brewers' yeast (3). Because of its extreme specificity, this enzyme has become an important analytical tool. Various preparations of it have been described, the first being those of Warburg and Christian (1-5) who found that the active protein could be precipitated from diluted Lebedev juice by acidification with carbon dioxide. We have been able to effect a 5- to 8-fold purification of the dehydrogenase by this method, but the conditions are not reproducible from one lot of yeast to another. Negelein and Gerischer (6) were able to obtain a preparation about 65-fold purified, in small yield, after an intricate fractionation procedure. More recently Kornberg has described a duplicable procedure which yields a product purified 20-fold and in good yield (7). In this paper we report the further purification of the enzyme and some of its properties.

EXPERIMENTAL

Materials and Methods—The pure crystalline barium salt of D-glucose-6-phosphate (G-6-P) and triphosphopyridine nucleotide (TPN) of 72 per cent purity was obtained from the Sigma Chemical Company. The d-lactone of gluconic acid was a commercial sample from General Biochemicals, Inc. Ribose nucleic acid (RNA) and protamine sulfate were purchased from the Nutritional Biochemicals Corporation, while ethylenediaminetetraacetic acid (EDTA) was a product, recrystallized three times, from the Alrose Chemical Company. Adenosine triphosphate (ATP) was purchased from the Pabst Laboratories.

Calcium phosphate gel was prepared by the method of Keilin and Hartree (8) and alumina Cγ by a slight modification of the method of Willstätter (9). The former gel had 16 mg., while the latter had 19 mg. dry weight of solids per ml.

* This work has been supported in part by a grant from the Nutrition Foundation, Inc.
Reduced TPN (TPNH) was prepared by treatment of the nucleotide with \( \text{Na}_2\text{S}_2\text{O}_4 \), followed by precipitation with acetone (10).

Dried brewers' yeast was generously supplied by Anheuser-Busch, Inc., of St. Louis. The yeast was washed with cold water by decantation, following its removal from the fermentation vats, and then was cold-pressed and cold-dried in an air stream at 1–2°C. The dry yeast was stored at 5°C. Enzyme preparations of the same activity were obtained from this yeast over a period of 6 months.

Protein was determined by the method of Robinson and Hogden (11), or, occasionally, by the method of Lowry et al. (12). When the latter method was used, care was taken to wash the protein repeatedly with 5 per cent trichloroacetic acid to free it from any adhering ammonium sulfate. Crystalline bovine plasma albumin (Armour) of known nitrogen content (Kjeldahl) was used as a standard.

The assay for the enzyme was done in the 1 cm. cell of the model DU Beckman spectrophotometer. The routine assay system contained 1.6 ml. of water, 1.0 ml. of 0.19 M tris(hydroxymethyl)aminomethane (Tris), pH 8.0, 0.10 ml. of 0.30 M MgCl₂, 0.10 ml. of 0.025 M dipotassium glucose-6-phosphate, pH 7.0, and 0.20 ml. of 0.002 M TPN, previously adjusted to pH 6.8 with KOH. 1 unit of enzyme is defined as that quantity added to the above assay system which produces an optical density change at 340 mμ of 2.07 units per minute per 3 ml. of reaction mixture (1.0 μmole of TPN reduced per minute) at 25°C. Optical density readings were made at intervals of 15 seconds and the activity was determined from the initial linear rate.

**Enzyme Preparation. Autolysis**—Dry brewers' yeast (200 gm.) was ground to a fine powder in a mortar and then allowed to autolyze in 600 ml. of distilled water for 6 hours at 37°C. Table I presents the analytical data on such an autolysate as well as on the fractions obtained from it as described below. Other preparations of brewers' yeast might require somewhat different conditions of autolysis for optimal yield of the dehydrogenase. The autolysate was centrifuged in the cold at 15,000 × \( g \) for 15 minutes. The opalescent supernatant fluid (350 to 380 ml.) was kept frozen overnight without change in activity. All operations hereafter were carried out between 0–4°C unless otherwise stated.

**Protamine Precipitation and Ammonium Sulfate Fractionation**—The nucleic acid in the autolysate was precipitated by the addition of 0.5 its volume of 2 per cent protamine sulfate solution, removed by centrifugation at 15,000 × \( g \) for 15 minutes, and discarded. The supernatant fluid was fractionated by the addition of unneutralized ammonium sulfate saturated at 25°C. The protein fraction which precipitated between 0.575 and
0.65 saturation was collected by centrifugation and dissolved in 100 ml. of 0.001 M EDTA, pH 7. This solution could be kept frozen for several days.

**Calcium Phosphate Gel Adsorption**—The solution was dialyzed against two 3 liter portions of 0.001 M EDTA, pH 7, for a total time of 3 hours. Dialysis could not be continued beyond this time without loss of enzymatic activity. After removing any precipitate present, the activity of the dialyzed solution was determined. For every 100 units of enzyme present, 3.1 ml. of calcium phosphate gel were added and allowed to stand for 5 minutes with frequent stirring before centrifugation at 1000 \( \times g \). Without washing the gel the enzyme was eluted with 6.2 ml. of 0.3 M potassium phosphate buffer, pH 8, for every 100 units of activity present originally. The eluate could be kept frozen for several months without loss of activity.

**Ethyl Alcohol Fractionation**—The eluate was dialyzed for 8 to 8.5 hours against several 2500 ml. portions of a solution 0.02 M in MgCl\(_2\) and 0.001 M in EDTA, pH 7.0. Excessive loss of enzyme occurred when dialysis was continued for a longer time. A shorter time was insufficient for the re-

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**Table I**

**Purification of D Glucose 6 phosphate Dehydrogenase**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Over-all recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml.</td>
<td>units</td>
<td>units/mg.</td>
<td>per cent</td>
</tr>
<tr>
<td>Autolysate</td>
<td>350</td>
<td>1050</td>
<td>0.13</td>
<td>100</td>
</tr>
<tr>
<td>((\text{NH}_4)_2\text{SO}_4) fraction, 0.575-0.65 saturated</td>
<td>110</td>
<td>725</td>
<td>0.34</td>
<td>69</td>
</tr>
<tr>
<td>(\text{Ca}_3\text{(PO}_4) gel eluate</td>
<td>45</td>
<td>562</td>
<td>3.45</td>
<td>53</td>
</tr>
<tr>
<td>Ethanol (9-20% volume per volume)</td>
<td>13</td>
<td>245</td>
<td>10.0</td>
<td>23</td>
</tr>
<tr>
<td>Alumina Cy eluate</td>
<td>10.5</td>
<td>290</td>
<td>16.7</td>
<td>21</td>
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<tr>
<td>Column Fraction* 1†</td>
<td>2</td>
<td>5.4</td>
<td></td>
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<tr>
<td>&quot;</td>
<td>2</td>
<td>25.4</td>
<td>86.0</td>
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<td>2</td>
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<tr>
<td>Column Fraction* 5</td>
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<td>17.4</td>
<td>65.0</td>
<td></td>
</tr>
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<tr>
<td>&quot;</td>
<td>2</td>
<td>17.4</td>
<td>65.0</td>
<td></td>
</tr>
<tr>
<td>Total (Fractions 1-7)</td>
<td>13.5†</td>
<td>113.6†</td>
<td></td>
<td>19†</td>
</tr>
</tbody>
</table>

* 120 units of enzyme activity added to column.
† First 0.40 saturated \((\text{NH}_4)_2\text{SO}_4\) eluate.
$ Total in Fractions 1 through 7.

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1 All concentrations of \((\text{NH}_4)_2\text{SO}_4\) and of ethanol described in this procedure were calculated on the assumption of additive volumes.
moval of enough phosphate ion to permit a successful ethanol fractionation. At the end of dialysis the solution was centrifuged, and the supernatant fluid at 0° was adjusted to pH 5.9 (Beckman pH meter set at 10°) by the addition of 0.1 N HCl. 1 ml. of 95 per cent ethanol (at -6°) was added to the enzyme solution at 0°, and the solution was transferred immediately to a bath at -6°. The solution was made 9 per cent in 95 per cent ethanol, and the precipitate was quickly removed by centrifugation for 2 minutes at 1000 X g by using tubes and cups precooled to -10°. To the faintly turbid supernatant fluid at -6° enough 95 per cent ethanol at -6° was added to make its final concentration 20 per cent. The precipitate containing the enzyme was removed by centrifugation as above for 5 minutes. The precipitate was dissolved immediately in 10 to 12 ml. of 0.02 M MgCl2-0.001 M EDTA, pH 7. Occasionally, it was necessary to add enough MgCl2 to make its final concentration 0.05 M to effect solution of most of the precipitate. Any undissolved protein was removed by centrifugation. The enzyme was not stable on standing in this solution, and the preparation was carried at once through the next step.

**Alumina Cγ Adsorption**—For every 100 units of enzyme present 0.86 ml. of alumina Cγ was added. After 5 minutes the gel was removed by centrifugation, and, without washing it, the enzyme was eluted with 4.3 ml. of 0.10 M potassium phosphate-0.001 M EDTA buffer, pH 8.0, for every 100 units of enzyme originally present. After removing the gel by centrifugation at low speed, 3.5 ml. of 0.25 M glycylglycine buffer, pH 7.2, were added to the eluate. The solution was lyophilized, the white powder remaining was dissolved in 3 ml. of cold distilled water, and the mixture was centrifuged. The supernatant solution was quite stable when frozen.

**Chromatography on Starch-Celite**—The enzyme was purified further by applying the type of chromatography used by Fischer and Hilpert (13). A starch-Celite column was prepared by mixing 18 gm. of corn-starch with 18 gm. of Celite and packing the mixture into a 2 cm. X 18 cm. tube. Air pressure equivalent to 16 cm. of mercury was used to facilitate this and subsequent operations. 1 day before use, the column was washed at 4° under pressure with 50 ml. of cold distilled water followed by 50 ml. of 0.50 saturated ammonium sulfate, prepared, as were all ammonium sulfate solutions for this column, by suitable dilution of the neutralized (with NH4OH to pH 7) salt solution saturated at 4°.

The enzyme solution from the preceding step was thawed, and that volume which contained 120 units of activity was brought to 0.56 saturation with the cold, neutralized, saturated ammonium sulfate. The faint precipitate which formed was removed by centrifugation in the high speed head of the International centrifuge.

The supernatant solution was added to the column and an equal volume of fluid was displaced by air pressure. 30 ml. of 0.60 saturated ammonium
sulfate were added to the column and forced through at a flow rate of 0.5 ml. per minute. After all of this solution had passed through, it was replaced by a 0.40 saturated solution. As this latter eluent was forced through the column and collected in 2 ml. fractions, the enzyme was found to emerge with the front of the 0.40 saturated ammonium sulfate. In the eluates containing the enzyme the protein concentration was very low; nevertheless, the enzyme was stable and withstood repeated rapid freezing and thawing.

The activity of the best fractions from the starch column varies from as low as 70 units per mg. of protein to as high as 120 units per mg. of protein, corresponding to a turnover number of 12,000 moles of substrate per $10^8$ gm. of protein per minute at pH 8.0 at $25^\circ$. Thus, the over-all procedure for D-glucose-6-phosphate dehydrogenase purification results in a product 600 to 900 times purified from the Lebedev juice. The final preparation is free of phosphoglucomutase, hexose-6-phosphate isomerase, and 6-phosphogluconic acid dehydrogenase. However, hexokinase is still present, the ratio of the activities being approximately 40:1 in a column fraction with specific activity of 80 units per mg. of protein. The hexokinase assay was made via the glucose-6-phosphate dehydrogenase system by determining the initial rate of glucose utilization in the presence of an excess of all reactants. A preparation in which the hexokinase has been removed until the ratio of activities is 130:1 can be obtained as described below.

Further Removal of Hexokinase—The preparation was carried as far as the ethanol fractionation described above. Instead of adsorbing the enzyme on alumina Cγ, 2.0 ml. of 0.25 M glycylglycine buffer, pH 7.2, were added for every 5 ml. of enzyme solution. The solution was dried by lyophilization and the powder was dissolved in enough cold water to give a final concentration of 60 units per ml. To 0.4 ml. of this solution was added 1.0 ml. of 1 M potassium acetate buffer, pH 4.7, followed by dilution with water to 8.0 ml. After standing at $0^\circ$ for 10 minutes, the enzyme was precipitated by the addition of 3.0 ml. of 0.3 per cent RNA, pH 4.8. After standing for 10 minutes in ice the precipitate was removed by centrifugation. Solution of this precipitate in 0.25 M glycylglycine buffer, pH 7.2, gave a stable preparation in which the D-glucose-6-phosphate dehydrogenase had a specific activity of 20 units per mg. of protein. The yield was 50 per cent, starting with the ethanol-fractionated product. It has been observed that precipitation of the enzyme-RNA complex does not occur in solutions with high phosphate ion concentration. We have been unable to remove the last traces of hexokinase by reprecipitation with RNA.

Properties of Enzyme—Fig. 1 illustrates the effect of pH on the activity of the enzyme under the conditions of the standard assay procedure. The maximal activity occurs at about pH 8.5. The data for Fig. 1 were ob-
tained with organic acids and bases and their potassium and hydrochloride salts. As shown in Fig. 1 the activity was independent of the buffer used at pH 6.8, 7.55, and 9.0.

The effect of temperature on enzyme activity was studied by allowing the reaction to proceed at pH 7.4 in the presence of hydroxylamine which reacts with the lactone of 6-phosphogluconic acid to form the corresponding hydroxamic acid (14, 15) which can be determined quantitatively (16).

Thus, when the enzymatic reaction was allowed to go to completion in the presence of hydroxylamine under conditions in which the amount of TPN was limiting, the color obtained in the hydroxamic acid test over the range from 0 to 1 amole of nucleotide was found to be proportional to the amount of TPN added and, by inference, to the amount of lactone formed. By equating hydroxamic acid color with TPNH formation the molar extinction coefficient of the iron-hydroxamic acid complex of 6-phosphogluconic acid was found to be identical to that of gluconic acid itself, as determined by using a standard solution of the δ-lactone of the latter substance. It has been reported previously (15) that these two substances do not give an equal amount of color on a molar basis in the hydroxamic acid test.
Fig. 2 demonstrates the dependence of enzymatic activity on temperature. The rate of the enzymatic reaction at 28° was linear for the first 5 minutes; it was stopped after exactly 3 minutes by the FeCl₃-HCl reagent of Lipmann and Tuttle (16), and the amount of hydroxamic acid formed in this interval was taken to be proportional to the specific reaction rate. Fig. 2 shows the log₁₀ of the rate over the first 3 minutes determined in this manner plotted as a function of 1/T where T is the absolute temperature. From the slope of this plot the apparent energy of activation of the enzymatically catalyzed oxidation of glucose-6-phosphate was calculated to be 7070 calories per mole over the temperature range 20–37°. The Q₁₀ between 25–35° is 1.48.

The possibility of a divalent metal requirement by this enzyme was suggested by Kornberg (7) who found activation by Mg²⁺ in the presence of glycylglycine buffer but not in the presence of phosphate buffer. In earlier work on this enzyme (1–6) no such requirement was observed. The purest preparations of the enzyme which we have had showed approximately 70 per cent of the maximal activity in the absence of added Mg²⁺, and this activity was not reduced by having 0.025 mM EDTA present in the reaction mixture. These facts make it unlikely that glucose-6-phosphate dehydrogenase has an absolute requirement for Mg²⁺. A study of the effect of various ions on the purified enzyme has now shown that it can be both activated and inhibited by a variety of univalent and divalent salts (Fig. 3). Potassium is more effective than sodium, and calcium is more effective
than magnesium. The effects observed are not on the stability of the enzyme, as they will increase the rate if added to the solution after the reaction has proceeded for some time. The effect of these ions appears to be on the affinity of the enzyme for its substrates. Thus, Fig. 4 shows that the $K_m$ of the enzyme for G-6-P is $5.8 \times 10^{-5} \text{ M}$ in the presence of $0.01 \text{ M MgCl}_2$, but is $6.9 \times 10^{-5} \text{ M}$ in the absence of any added MgCl$_2$. Similarly, for TPN the $K_m$ in the presence of $0.01 \text{ M MgCl}_2$ is $2.0 \times 10^{-5} \text{ M}$, while in the absence of any added MgCl$_2$ it is $3.3 \times 10^{-5} \text{ M}$ (Fig. 5).

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**Fig. 4.** Effect of MgCl$_2$ on enzymatic activity at various G-6-P concentrations. The reaction mixtures contained $6.3 \times 10^{-2} \text{ M Tris}, 2.7 \times 10^{-4} \text{ M TPN}; \text{ pH } 8.0, 25^\circ$. Enzyme (80 units per mg.) present at $0.5 \gamma$ per ml. ●, $1.0 \times 10^{-2} \text{ M MgCl}_2$; ■, no added MgCl$_2$.

**Fig. 5.** Effect of MgCl$_2$ on enzymatic activity at various TPN concentrations. The reaction mixtures contained $6.3 \times 10^{-2} \text{ M Tris}, 8.3 \times 10^{-4} \text{ M G-6-P}; \text{ pH } 8.0, 25^\circ$. Enzyme (80 units per mg.) present at $0.5 \gamma$ per ml. ●, $1.0 \times 10^{-2} \text{ M MgCl}_2$; ■, no added MgCl$_2$.

Theorell has found (17) that the enzyme was inhibited by phosphate and that this inhibition could be reversed by TPN. We have confirmed these findings with the highly purified enzyme (see Fig. 6).

Negelein and Haas found that the enzyme is inhibited by TPNH (18). Fig. 7 demonstrates that the inhibition is competitive with TPN, and, from an analysis of the curves, the inhibition constant, $K_i$, for TPNH is $2.7 \times 10^{-6} \text{ M}$. The enzyme is not inhibited by reduced diphosphopyridine nucleotide.

The enzyme is inhibited by D-glucosamine-6-phosphate (prepared by the action of yeast hexokinase on glucosamine (19)). Fig. 8 shows that this inhibition is competitive with G-6-P and that the $K_i$ is $7.2 \times 10^{-4} \text{ M}$. Neither mannose-6-phosphate nor N-acetyl-D-glucosamine-6-phosphate
(20) inhibits this enzyme. These facts emphasize the specificity of \(d\)-glucose-6-phosphate dehydrogenase.

**Equilibrium of Enzymatic Reaction**—Since it has been shown that the primary product of the oxidation of G-6-P is the \(\delta\)-lactone of 6-phosphogluconic acid (14) and that the reaction is reversible (15), it seemed of interest to determine the approximate value of the equilibrium constant of this reaction. At pH 8.0, at which the enzyme has nearly maximal activity, the lactone decomposes quickly, but at pH 6.4 and 28° the half life of the lactone has been found to be 24 minutes. It seemed possible, therefore, that at pH 6.4 in the presence of a large excess of enzyme the reaction would reach equilibrium before any appreciable amount of the lactone had decomposed. Fig. 9 illustrates several such experiments. The continued slow reduction of TPN after the initial rapid reduction reflects the slow hydrolysis of the lactone. The data in Table II give the initial concentrations of the reactants in a number of similar experiments as well as the observed value of the ratio of the equilibrium constant of the reaction, \(K\), to the hydrogen ion activity, where

\[
K = \frac{(\text{TPNH})(\text{6-phosphogluconic acid } \delta\text{-lactone})[\text{H}^+]}{(\text{TPN})(\text{G-6-P})}
\]
The concentration of 6-phosphogluconic acid δ-lactone at any time has been taken to be equal to the observed concentration of TPNH diminished by the amount of lactone which hydrolyzes in that interval of time. The

![Graph](image1)

**Fig. 8.** Inhibition by d-glucosamine-6-phosphate (Gm-6-P) of enzymatic activity at various G-6-P concentrations. The reaction mixtures contained $6.3 \times 10^{-2}$ M Tris, $2.0 \times 10^{-4}$ M TPN, $1.0 \times 10^{-2}$ M MgCl₂; pH 8.0, 25°. Enzyme (80 units per mg.) present at 0.6 y per ml. ○, no added Gm-6-P; △, $4.29 \times 10^{-4}$ M Gm-6-P; ■, $7.15 \times 10^{-4}$ M Gm-6-P.

**Fig. 9.** Enzymatic activity at different substrate concentrations. The reaction mixtures contained $6.3 \times 10^{-2}$ M maleate buffer, pH 6.40, $1.0 \times 10^{-2}$ M MgCl₂, TPN, and G-6-P as indicated; 28°. Enzyme (14 units per mg.) present at 43 y per ml. Volume, 3 ml. Curve A, $3.29 \times 10^{-4}$ M TPN, $2.94 \times 10^{-4}$ M G-6-P. At the arrow, TPN increased to $3.95 \times 10^{-4}$ M. Curve B, $3.78 \times 10^{-4}$ M TPN, $2.96 \times 10^{-4}$ M G-6-P; Curve C, $3.14 \times 10^{-4}$ M TPN, $4.42 \times 10^{-4}$ M G-6-P; Curve D, $4.42 \times 10^{-4}$ M TPN, $4.42 \times 10^{-4}$ M G-6-P.

**Table II**

*Equilibrium Constant of Glucose-6-phosphate Dehydrogenase Reaction*

Composition of reaction mixtures as in Fig. 9.

<table>
<thead>
<tr>
<th>Initial TPN, μmole per 3 ml</th>
<th>Initial G-6-P, μmole per 3 ml</th>
<th>$K_{[H^+]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0757</td>
<td>0.0884</td>
<td>1.27</td>
</tr>
<tr>
<td>0.0986</td>
<td>0.0888</td>
<td>1.22</td>
</tr>
<tr>
<td>0.0944</td>
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</tr>
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<td>0.1183</td>
<td>0.0888</td>
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</tr>
<tr>
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<td>0.0884</td>
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</tr>
<tr>
<td>0.1135</td>
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<td>1.74</td>
</tr>
<tr>
<td>0.1325</td>
<td>0.1325</td>
<td>1.55</td>
</tr>
</tbody>
</table>
latter quantity was calculated by using the observed value of 24 minutes for the half life. The values for the equilibrium constant in Table II are the averages of those obtained at 7 and 14 minutes. Curve A of Fig. 9 represents an experiment in which, after the $K/[H^+]$ was found to be 1.22 at 8 minutes, more TPN was added, and the new $K/[H^+]$ was 1.44 at 14 minutes. The average of many determinations gives a $K = (6.0 \pm 0.7) \times 10^{-7}$ M at 28°. The corresponding standard free energy change, $\Delta F^o$, is +8600 calories per mole. The data of Strecker and Korkes for the equilibrium of glucose to gluconic acid $\delta$-lactone obtained with the glucose dehydrogenase of liver give a $\Delta F^o$ of about +8000 calories per mole (21). The $\Delta F^o$ for the reduction of TPN by H₂ to (TPNH + H⁺) has been calculated by Burton and Wilson (22) to be +5400 calories per mole at 25°. Therefore, the $\Delta F^o$ for the reaction

$$G-6-P = (6\text{-phosphogluconic acid } \delta\text{-lactone}) + H_2$$

is +3200 calories per mole, corresponding to $E_0 = -0.069$ volt. At pH 6.4, $E'_0 = -0.26$ volt, while at pH 7.0, $E'_0 = -0.28$ volt. Burton and Wilson have calculated $E'_0 = -0.32$ volt for the reduction of TPN at pH 7.0.

**DISCUSSION**

Although a highly purified enzyme was obtained in good yield by the procedure described above, the amount of active protein present in yeast is very small. Only 9 mg. of enzyme with a turnover number of 12,000 moles of substrate per $10^6$ gm. of protein per minute are present in the Lebedev juice from 200 gm. of dry yeast. Thus, the enzyme is only 0.09 per cent of the total extracted protein. The most highly purified enzyme obtained in 0.40 saturated (NH₄)₂SO₄ at a protein concentration of about 0.13 mg. per ml. can be precipitated only to the extent of 50 per cent by dialysis against a large volume of saturated (NH₄)₂SO₄. The precipitate so obtained cannot be freed of (NH₄)₂SO₄ without denaturing the enzyme. This fact made it impractical to obtain enough enzyme for any physical study of the protein.

The calcium phosphate gel eluate contains no 6-phosphogluconic acid dehydrogenase but is contaminated with hexose-6-phosphate isomerase and with phosphoglucomutase, as well as with hexokinase. The enzyme obtained by elution from alumina Cₓ contains no phosphoglucomutase, hexose-6-phosphate isomerase, or 6-phosphogluconic acid dehydrogenase, but contains 1 part in 15 of hexokinase estimated as described above. This enzyme is purified 130 times from the Lebedev juice and may be sufficiently pure for many kinds of analytical uses.
D-GLUCOSE-6-PHOSPHATE DEHYDROGENASE

SUMMARY

1. A preparation of d-glucose-6-phosphate dehydrogenase has been obtained from dried brewers' yeast in which the enzyme has been purified 600 to 900 times from an autolysate. The final product has a turnover number of 12,000 moles of substrate per $10^5$ gm. of protein per minute at pH 8.0 and 25°.

2. The purified enzyme is free of contamination by phosphoglucomutase, hexose-6-phosphate isomerase, and 6-phosphogluconic acid dehydrogenase. Hexokinase is still present. The activity of the enzyme in the presence of glucose and ATP was 2.5 per cent of its activity in the presence of glucose-6-phosphate. A procedure is described whereby a product having one-third as much hexokinase can be obtained.

3. The enzyme has its maximal activity at pH 8.5. The apparent energy of activation of the enzymatically catalyzed oxidation of glucose-6-phosphate at pH 7.40 was found to be 7070 calories per mole between 20-37°. The $Q_{10}$ between 25-35° is 1.48.

4. The enzyme does not appear to have an absolute requirement for any salt but can be both activated and inhibited by a variety of salts. The effect of the salt appears to be on the affinity of the enzyme for its substrates.

5. The Michaelis constants of the enzyme for TPN and for G-6-P have been determined.

6. The enzyme is inhibited by d-glucosamine-6-phosphate and the inhibition is competitive with G-6-P. The enzyme is also inhibited by phosphate ion and by TPNH. Both inhibitions are competitive with TPN.

7. The equilibrium constant of the reaction, G-6-P + TPN = TPNH + H+ + 6-phosphogluconic acid δ-lactone, has been measured at pH 6.4 and 28°. Its value is $6.0 \times 10^{-7} \text{m}$, corresponding to $\Delta F^\circ = +8600$ calories per mole. From this value and other data the $\Delta F^\circ$ for the reaction, G-6-P = 6-phosphogluconic acid δ-lactone + H₂, is $+3200$ calories per mole, corresponding to $E_0' = -0.069$ volt. At pH 7.0, $E'_0 = -0.28$ volt.

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