CRYSTALLINE D-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE FROM RABBIT MUSCLE*

BY GERTY T. CORI, MILTON W. SLEIN, AND CARL F. CORI

(From the Department of Biological Chemistry, Washington University School of Medicine, St. Louis)

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The reaction catalyzed by this enzyme consists in the oxidation of a \(-\text{CHO}\) to a \(-\text{COO} \cdot \text{PO}_4\text{H}_2\) group and represents the first oxidative step in the degradation of carbohydrate in the tissues. Diphosphopyridine nucleotide (DPN) and inorganic phosphate are necessary for this reversible enzymatic reaction. The equilibrium has been investigated by Warburg and Christian (1), Drabkin and Meyerhof (2), and Meyerhof and Oesper (3). When inorganic phosphate is replaced by arsenate, the reaction becomes irreversible. In both instances the reaction can be followed by measuring the appearance of reduced DPN spectrophotometrically at 340 m\(\mu\), a method originally introduced by Warburg and Christian.

The substrate for this enzyme is formed from fructose-1,6-diphosphate through the action of aldolase, the enzyme described in the preceding paper.

\[
\begin{align*}
\text{Fructose-1,6-diphosphate} & \xrightarrow{\text{Aldolase}} \text{d-Glyceraldehyde-3-phosphate} \xrightarrow{\text{Dehydrogenase}} \text{d-Glyceric acid-3-phosphate} \\
& \xleftarrow{\text{Isomerase}} \text{dihydroxyacetone phosphate}
\end{align*}
\]

This sequence of reactions, originally proposed by Embden and Meyerhof, could be demonstrated by means of the crystalline enzyme preparations because they are free of isomerase (Fig. 1). In the presence of aldolase, dehydrogenase, arsenate, and DPN, glyceraldehyde phosphate only disappears and the yield is 1 mole of triose phosphate oxidized per mole of hexose diphosphate added; when, in addition, a purified preparation of triose phosphate isomerase (5) is added, dihydroxyacetone phosphate disappears and a second mole of triose phosphate is oxidized.\(^1\)

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\(^1\) This provides a sensitive method for the quantitative determination of hexose diphosphate and triose phosphate, either separately or combined, depending on the order of addition of the three enzymes. This method will be described in a subsequent publication.
The preparation of the crystalline enzyme was described in a preliminary report (6) and shortly thereafter a note appeared by Dixon and Caputto (7) in which crystallization was achieved by another method of preparation. The enzyme had previously been crystallized from yeast by Warburg and Christian (1). In confirmation of them it was found that the enzyme, when used in much higher concentration, also oxidizes D-glyceraldehyde.

The present paper contains details of preparation, data on the properties of the enzyme protein, and kinetic measurements, while the papers which follow contain data on the prosthetic group and on amino acid composition. The enzyme constitutes about 7 to 12 per cent of the extracted proteins and the yield is about 300 mg. per 100 gm. of muscle.

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**FIG. 1.** Spectrophotometric measurement of triose phosphate oxidation with fructose diphosphate as substrate. Curve A, the reaction mixture consisted of fructose diphosphate, aldolase, dehydrogenase, DPN, and arsenate. 1 mole of triose phosphate was oxidized (or DPN reduced) per mole of fructose diphosphate added. Curve B, the reaction mixture contained in addition triose phosphate isomerase. 2 moles of triose phosphate were oxidized per mole of fructose diphosphate. The dotted line indicates the amount of reduction of DPN expected from the amount of fructose diphosphate added. Fructose diphosphate was determined by the phenylhydrazine method of Deuticke and Hollmann (4).
EXPERIMENTAL

Method of Preparation—A rabbit is injected intravenously with a lethal dose of amytal. It is rapidly skinned and the leg and back muscles are excised and weighed. All further steps are carried out in a cold room. The muscles are passed through a meat grinder, extracted immediately with 1 volume of 0.03 N KOH for 10 minutes with occasional stirring, and strained through gauze. The extraction is repeated and the residue is suspended in 0.5 volume of water for 5 minutes and strained as above. The pH, measured with the glass electrode in the combined extracts, varied in six different preparations from 6.6 to 7.2.

If it is desired to prepare both aldolase and dehydrogenase, 1 volume of saturated (NH₄)₂SO₄ solution, pH 7.5 to 7.8 (saturated at room temperature and pH adjusted with ammonia), is added to the extract (0.5 saturation) and the mixture placed in an ice bath for about 0.5 hour. From the time of killing the rabbit to the addition of the salt solution not more than 1.5 hours should elapse. The mixture is filtered through folded paper (Whatman No. 1), and to the clear filtrate more saturated (NH₄)₂SO₄ solution is added to bring the saturation to 0.52 (4 ml. to each 100 ml.). The pH should be 7.6 to 7.8. This solution either is left at 5°C for 2 to 3 days or is warmed up very slowly to 20°C, then returned to 5°C. This procedure speeds up the rate of crystallization of aldolase (8). When a heavy mass of aldolase crystals (which do not settle) has formed, they are separated by filtration or centrifugation.

When it is not desired to prepare aldolase, saturated (NH₄)₂SO₄ solution is added to 0.52 saturation in one step, followed immediately by filtration or by centrifugation in a high speed angle centrifuge.

To each 100 ml. of filtrate at 0.52 saturation are added 13 gm. of solid (NH₄)₂SO₄, which brings the saturation to 0.72. As soon as the salt is dissolved by gentle stirring, the solution is filtered through folded paper, the filtrate being poured back on the filter until it is perfectly clear. Filtration is fairly rapid and the pH of the filtrate is about 7.5. To the filtrate, 15 per cent ammonium hydroxide solution is added dropwise with shaking until the pH is 8.2 to 8.4. The pH is measured with a glass electrode, or with metacresol purple as indicator, in an aliquot of the 5 times diluted filtrate. Crystals appear in several hours, and even without seeding a large crop of crystals (which do not settle) forms overnight. The suspension of the crystals may be left standing for several days to increase the yield. The crystals are separated by filtration through folded paper (Whatman No. 1). Filtration is rather rapid at first but eventually slows down; by the use of an automatic filtration device all the material from 300 to 500 gm. of muscle will pass through a filter of 24 cm. diameter overnight. The still moist crystalline precipitate is scraped off the paper with a spatula.
and dissolved in 40 to 80 ml. of water. Paper fibers, MgNH₄PO₄ crystals, and shreds are removed by centrifugation. To the clear, slightly reddish yellow solution are added 2 volumes of saturated (NH₄)₂SO₄ solution, pH 8.2 to 8.4, for each volume of water used in the solution of the crystals. There is no immediate precipitation of protein. In less than 1 hour, crystals begin to appear and in 24 hours a thick shimmering suspension of crystals has formed. Further recrystallizations are carried out in the same manner. The yellowish color is eventually removed with the mother liquors and the suspension appears pure white. By this procedure thirty-four preparations of the enzyme have been made in the past 2 years. There

![Crystalline \(\alpha\)-glyceraldehyde-3-phosphate dehydrogenase; \(\times 260\)](image)

was no case of failure and the yield was 1 gm. or more of enzyme per rabbit.²

The first crystals usually appear in the form of rosettes, which are apparently made up of clusters of fine needles. Closer observation reveals that the crystals consist of diamond-shaped plates which stand on edge. On recrystallization the crystals remain separate. Because of the thinness of the plates and the closeness of the refractive indices of the crystals and the mother liquor, satisfactory photomicrographs were obtained only when the crystals were stained with methylene blue. The crystals shown in Fig.

² It should be emphasized that, in order to obtain a good yield of crystalline dehydrogenase, the rapidly excised muscles must be ground and extracted without delay. When the muscles were left at 5° for 1 hour, or when 0.5 hour elapsed between the time the muscles were ground and placed in the extraction fluid, no, or only a small, yield of crystalline enzyme could be obtained.
2 were obtained by allowing the enzyme to recrystallize slowly from 0.6 saturated \((\text{NH}_4)_2\text{SO}_4\) solution and are larger than those ordinarily obtained from 0.66 saturated \((\text{NH}_4)_2\text{SO}_4\). Both the diamond shape of the crystals and the simulated rod shape due to the crystals standing on edge are seen in the photograph.

An example of the yield and activity on recrystallization is given in Table I. It may be seen that the enzyme crystallizes from a 0.14 per cent protein solution in which it constitutes about one-half of the protein present. About 10 per cent of the enzyme is lost in the mother liquor, while on recrystallization from more concentrated solutions the loss is 5 to 8 per cent. The specific activity of the crystals increases slightly on recrystallization, while that of the mother liquor increases very markedly and approaches that of the crystals. The enzyme is not completely stable when in solution in ammonium sulfate and this may account for the fact that the specific activity in the mother liquor did not reach that of the crystals.

Why 0.03 N alkali rather than water is used for the extraction of muscle in the preparation of the dehydrogenase is shown in the following experiment.

1 part of ground rabbit muscle was extracted with water (pH of extract 6.1) and another part with 0.03 N KOH (pH of extract 7.1), followed in each case by the procedure outlined for the preparation of the dehydrogenase. The water extract yielded only a trace of crystals, while a large crop was obtained from the alkaline extract. Both preparations were analyzed for protein and specific activity by sampling the well mixed 0.72 saturated \((\text{NH}_4)_2\text{SO}_4\) solution. The water extract yielded 0.69 mg. of protein per ml. and a specific activity of \(5.12 \times 10^8\); the respective values for the alkaline extract were 1.43 mg. of protein and \(8.55 \times 10^8\).

### Table I

Re crystallization of Dehydrogenase

The enzyme was prepared from 500 gm. of rabbit muscle by the method described in the text.

<table>
<thead>
<tr>
<th>Crystallization</th>
<th>Protein in crystals</th>
<th>Mother liquor</th>
<th>Specific activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
<td>ml.</td>
<td>gm.</td>
</tr>
<tr>
<td>1st.</td>
<td>1.56</td>
<td>2100</td>
<td>1.42</td>
</tr>
<tr>
<td>2nd</td>
<td>1.43</td>
<td>206</td>
<td>0.13</td>
</tr>
<tr>
<td>3rd.</td>
<td>1.35</td>
<td>214</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* The specific activity (at 25°) was obtained by dividing the bimolecular rate constants by the mg. of protein per ml. of reaction mixture.
activity. From this it may be calculated that the extract prepared with alkali contained 3.5 times more enzyme than the water extract, and it is therefore not surprising that few crystals were obtained from the latter. Further investigation is required to explain the low yield of the enzyme from water extracts.

**Electrophoresis**—The electrophoretic behavior of d-glyceraldehyde phosphate dehydrogenase has been investigated with the technique described for aldolase (8). Experiments have been performed at 2° over the pH range 5.1 to 8.45.

The recrystallized enzyme is electrophoretically homogeneous over the pH range 6.2 to 7.7. Fig. 3, from an experiment at pH 7.4, shows a single peak exhibiting a slight skew. It was found that the enzyme solution (7 mg. per ml.) had lost 7 per cent of its activity during the dialysis period of 20 hours which preceded the electrophoresis. In solutions more acid than pH 6.2 or more alkaline than pH 7.7, instability of the enzyme interferes with electrophoretic measurements. When the enzyme was dialyzed against acetate buffers of pH 5.1 to 5.4 for 20 hours, it lost about 70 per cent of its activity. No appreciable turbidity developed, but a considerable part of the protein became insoluble in 0.3 saturated (NH₄)₂SO₄ solution at pH 8.2, indicating denaturation. During electrophoresis at this low pH range several minor peaks appeared besides a major one. In veronal buffer of pH 8.45, pronounced turbidity developed during electrophoresis, nearly masking the moving boundaries.

Fig. 4 shows the variation in electrophoretic mobility from pH 6.2 to 7.7 in phosphate buffer of ionic strength 0.1. The ascending and descending mobilities were generally quite close, and have been averaged. A free-hand curve drawn through the points establishes the isoelectric point under these conditions at pH 6.55.

![Fig. 3. Electrophoretic schlieren diagram of recrystallized d-glyceraldehyde-3-phosphate dehydrogenase after 4 hours at 2° in phosphate buffer, pH 7.4, ionic strength 0.1.](https://example.com/fig3.png)
Activity Measurements—A portion of the crystal suspension is centrifuged sharply, drained, and dissolved in 0.03 M sodium pyrophosphate, pH 8.5, in the cold to give a concentration not less than 1 mg. of protein per ml. This stock solution is stable for 2 to 3 hours at 0°, but is unstable at room temperature. For activity tests this solution is diluted with pyrophosphate buffer containing cysteine. Dilution in the absence of cysteine results in loss of enzyme activity. Cysteine cannot be replaced by a protective protein such as crystalline serum albumin. To give an example (which incidentally describes the procedure usually followed), 0.1 ml. of a stock solution (410 γ of protein) was diluted to 4 ml. (a) with 0.03 M pyrophosphate, pH 8.5, (b) with 0.004 M cysteine in 0.03 M pyrophosphate, (c) with 0.03 M pyrophosphate containing 225 γ of serum albumin, and (d) with a solution containing cysteine, pyrophosphate, and albumin. As soon as each dilution was made, 0.1 ml. (10 γ) was transferred to a silica absorption cell containing 2.7 ml. of a 0.004 M cysteine-0.03 M pyrophosphate-DPN mixture. After the mixture stood for 7 minutes at room temperature, the reaction was started by the addition of 0.2 ml. of a mixture of triose phosphate and arsenate. Density readings at 340 μμ were taken at 1 minute intervals in the Beckman spectrophotometer. Bimolecular rate constants were calculated from the 1 and 2 minute readings. These showed that Sample a had 45 and Sample c 23 per cent less activity than Samples b and d, which gave the same rate constant.

The usual composition of the reaction mixture in moles per ml. was 2.5 \times 10^{-7} for the D component of D,L-glyceraldehyde phosphate, 1 \times 10^{-7}

\footnote{We are indebted to Dr. H. O. L. Fischer, Dr. E. Baer, and Dr. H. A. Lardy for several samples of synthetic D,L-glyceraldehyde phosphate (9). Determinations of}

![Fig. 4. Electrophoretic mobility of D-glyceraldehyde-3-phosphate dehydrogenase in sq. cm. volt⁻¹ sec⁻¹ \times 10^6 at 2° in phosphate buffers of ionic strength 0.1.](http://www.jbc.org/issue3/issue30141017/fig4.jpg)
GLYCERALDEHYDE PHOSPHATE DEHYDROGENASE

DPN, $6 \times 10^{-6}$ arsenate, $3 \times 10^{-5}$ pyrophosphate, $4 \times 10^{-6}$ cysteine, pH 8.5. The reference cell contained the same reactants with the exception of the enzyme. The concentrations of triose phosphate and DPN are about 6 and 2.5 times higher, respectively, than those required to give one-half saturation of the enzyme. Bimolecular rate constants were calculated from the equation

$$K = \frac{2.3}{t(a - b)} \log \frac{b(a - x)}{a(b - x)}$$

where $a$ is the initial concentration of triose phosphate, $b$ that of DPN, and $x$ the amount of reduced DPN formed in time $t$ (minutes), all expressed in moles per ml. Reduced DPN was calculated from the spectrophotometric readings by means of the relation, $(2.3 \log I_0/I)/1.45$. This corresponds to the $\beta$ coefficient for pure reduced DPN determined by Ohlmeyer (10). In some cases equal concentrations of triose phosphate and DPN were used in which case the equation reduces to

$$K = \frac{x}{t(a - x)}$$

For protein determinations an aliquot of the stock solution was diluted with water and read immediately at the wave-length of maximum absorption (276 m$\mu$) in the spectrophotometer. The conversion factor, based on micro-Kjeldahl determinations, was 1.9; hence $(2.3 \log I_0/I)/1.9 = \text{mg. of protein per ml.}$ In some cases protein was determined by the biuret method of Robinson and Hogden (11) or, in the case of dialyzed enzyme solutions, according to the modification of this method by Weichselbaum (12). For comparison of specific activities, the rate constants were divided by mg. of protein present per ml. of reaction mixture.

Proportionality could be tested for only a limited range of enzyme concentrations (0.8 to 5 $\gamma$ per ml.), because at higher enzyme concentrations the rate of reaction was too fast to permit accurate galvanometer readings. Within the range tested, proportionality was satisfactory. The bimolecular rate constant decreased somewhat with time. This was the case in the presence or absence of cysteine, as well as when glutathione was substituted for cysteine. For example, with 4 $\gamma$ of protein per ml. the rate constants for 1, 2, and 3 minutes were 2.59, 2.49, and $2.44 \times 10^6$, respectively, giving a specific activity for the 1st minute of $(2.59 \times 10^6)/0.004 = 6.5 \times 10^3$ at $24^\circ$.

alkali-labile $P$ or of the amount of DPN reduced enzymatically were used to calculate concentrations.

In most of the experiments a sample of 50 per cent purity prepared in this laboratory was used.
An example of the activating effect of cysteine is given in Fig. 5. Separate tests have shown that the effect of cysteine on the enzyme at room temperature is not instantaneous; maximum activity is reached in 5 to 7 minutes and is thereafter maintained for about 30 minutes, followed by a decline in activity after longer periods of incubation at room temperature. In order to test the enzyme under optimal conditions, it is diluted with and then kept for 7 minutes in the cysteine-pyrophosphate buffer in the presence of DPN; the reaction is then started by the addition of triose phosphate plus arsenate.

Triose phosphate reacts with cysteine, but this source of error is small because of the low concentration of cysteine (0.004 M), because triose phosphate is added last, and because the reaction is measured for only 2 minutes. When \(2.5 \times 10^{-4} \text{ M}\) triose phosphate is incubated with \(4 \times 10^{-3} \text{ M}\) cysteine for 6 minutes at room temperature before the enzyme is added, the loss of triose phosphate reduces the rate of the reaction by about 20 per cent. Cysteine is a disturbing factor in the measurement of the equilibrium of the reaction in the presence of phosphate. After apparent
equilibrium has been reached, reduced DPN is slowly reoxidized, due to the removal of triose phosphate by cysteine. Glutathione reacts much more slowly with triose phosphate, as is shown by the fact that the equilibrium position is maintained unchanged for 10 minutes; furthermore the same equilibrium is reached in the presence and absence of glutathione. It has been found that glutathione is not as effective as cysteine in maintaining dilute enzyme solutions at the level of maximum activity; hence cysteine is preferred for rate, while glutathione is preferred for equilibrium measurements.

 Stability—Enzyme crystals left suspended in 0.66 saturated (NH₄)₂SO₄ and stored in the refrigerator lost 50 per cent of their original activity after 2.5 months. The small amount of enzyme which remains in solution in 0.66 saturated (NH₄)₂SO₄, pH 8.3, is stable for at least 24 hours at 3°.

 In weak salt solution, stability of the enzyme is dependent, among other factors, on pH. An enzyme solution (1 mg. per ml.) was incubated for 30 minutes at 30° in acetate buffer at pH 5.5 and in pyrophosphate buffer at pH 7.3; the loss of enzyme activity was 27 per cent in the former and 17 per cent in the latter case. More dilute enzyme solutions are inactivated more rapidly under these conditions.

 It has been found, in confirmation of Rapkine (13), that DPN exerts a protective effect on dilute enzyme solutions in the absence of cysteine. For example, an enzyme solution (2.5 γ per ml.) incubated at room temperature for 10 minutes in the presence of DPN, but in the absence of cysteine, retained 85 per cent of its activity when compared with a sample incubated with cysteine in the usual way. When incubated without DPN the enzyme was almost completely inactive.

 Dissociation Constants of Substrates—The concentrations of triose phosphate and DPN were varied over a 10-fold range in enzymatic tests at pH 8.5. Satisfactory straight lines were obtained when the reciprocal of concentration was plotted against the reciprocal of the log I₀/I reading at 1 minute. The values obtained from these graphs were 5.1 × 10⁻⁵ moles per liter for d-glyceraldehyde phosphate and 3.9 × 10⁻⁵ moles per liter for DPN. For the yeast enzyme, Warburg and Christian found 3.2 × 10⁻⁵ moles per liter for DPN at pH 7.5 with D,L-glyceraldehyde as substrate. When recalculated by the method indicated above, the value would be about 5 × 10⁻⁵ moles per liter.

 Activity at Different pH Values and Turnover Number—In these experiments, the enzyme was saturated with both glyceraldehyde phosphate and DPN by using initial concentrations of each of 4.8 × 10⁻⁴ moles per liter. The initial rate of the reaction (obtained by extrapolation to zero time)

⁵ A preparation of 80 per cent purity as determined by enzymatic reduction was kindly supplied by Dr. A. Kornberg.
was the same at pH 8.6 and 9.0; at pH 8.1, 7.7, and 7.1 it was 81, 57, and 15 per cent, respectively, of the rate obtained at the higher pH values.

Because of the instability of the enzyme protein, it has not been possible, so far, to obtain satisfactory values for the diffusion and sedimentation constants. The turnover number for 100,000 gm. of protein corresponds to a reduction of 6700 moles of DPN per minute at pH 8.6 and 27°. From the data given above, it may be seen that the turnover number at the pH of muscle would be considerably lower. The high concentration of this enzyme in muscle may be related to this relatively low catalytic activity.

Fig. 6. Rate of inactivation of glyceraldehyde phosphate dehydrogenase by 0.0001 M iodoacetate at 0°, pH 7.1. Activity was measured at 27° at pH 8.5. Curves 1 and 2, control samples tested after being kept for 1 and 30 minutes at 0°. Curves 3, 4, and 5, samples tested after being kept for 1, 15, and 30 minutes in the presence of iodoacetate at 0°.

A twice crystallized sample of yeast glyceraldehyde phosphate dehydrogenase, prepared in this laboratory by Dr. E. G. Krebs by a modification of the method of Warburg and Christian, was tested under the same conditions as the muscle enzyme. Dr. Krebs found that the crystalline yeast enzyme requires a reducing agent such as cysteine for full activity, a fact which is not mentioned by Warburg and Christian. The turnover number of the yeast enzyme was of the same order of magnitude as that of the muscle enzyme.

Iodoacetate—The well known inhibition of lactic acid fermentation in muscle and of alcoholic fermentation in yeast has been shown to be at the triose phosphate level (14) when low concentrations of iodoacetate are used. That the triose phosphate dehydrogenase may be classified as an "-SH" enzyme has been established by the work of Rapkine and Trpinac (15–17). It has also been shown that the inhibition by iodoacetate is not instantane-
ous and that it cannot be reversed by cysteine. It seemed of interest to repeat some of these observations with the crystalline enzyme preparation.

The curves in Fig. 6 show the rate of inactivation of the enzyme by $10^{-4}$ M iodoacetate at $0^\circ$ and pH 7.1. No cysteine was used in this experiment and consequently relatively large amounts of enzyme (about 100 γ) had to be used in the activity measurements, which were carried out at $27^\circ$. A control sample of the enzyme was kept at $0^\circ$ in the absence of iodoacetate in order to determine the amount of spontaneous inactivation.

![Fig. 7. Irreversibility of the iodoacetate inhibition of glyceraldehyde phosphate dehydrogenase by cysteine.](http://www.jbc.org/)

The enzyme lost 15 per cent of its activity in 30 minutes without iodoacetate and 15, 70, and 85 per cent in the presence of iodoacetate after incubation periods of 1, 15, and 30 minutes.

In the above experiment only a small part of the enzyme was in the active or "reduced" form. This is shown by the fact that the enzyme had only about 10 per cent of the activity it had when tested in the presence of cysteine. The inactive or "oxidized" form of the enzyme does not react with low concentrations of iodoacetate and is converted to the reduced form by cysteine; this simulates a reversal of the iodoacetate inhibition by cysteine.
In Fig. 7 are shown the effect of iodoacetate on a fully reduced enzyme and the non-reversibility of the inhibition by cysteine. A solution of 2 mg. of enzyme per ml. in 0.03 M cysteine-pyrophosphate buffer, pH 8.5, was kept for 15 minutes at 24° in order to reduce the enzyme. This solution was then stored at 0° and aliquots were diluted 30-fold with 0.004 M cysteine-pyrophosphate as needed. As soon as each dilution was made 0.1 ml. of the dilution (7 γ of enzyme) was treated as follows. In Curve A, 0.1 ml. was incubated for 7 minutes in 0.004 M cysteine-pyrophosphate pH 8.5, and triose phosphate was added to start the reaction; in Curve B, 0.1 ml. was added to the otherwise complete, but cysteine-free reaction mixture, the rate of reaction in Curves A and B being nearly the same; in Curve C, 0.1 ml. was added to start the reaction in the same way as for Curve B, except that the reaction mixture contained iodoacetate. Curve C shows that even at 23° the inhibition by iodoacetate (final concentration 4 × 10^-4 M) requires several minutes for completion and that addition of cysteine, at a time at which the reaction has practically stopped, did not remove the inhibition. In contrast to this is the reactivation by cysteine of the oxidized form of the enzyme, Curve D. In Curve D the enzyme (70 γ per ml.) had been allowed to remain at 22° in the absence of cysteine for 30 minutes. The initial rate in the absence of cysteine was insignificant, but the addition of cysteine rapidly reestablished activity.

The authors wish to thank Mr. Robert Loeffel and Dr. A. A. Green for carrying out the measurements of electrophoretic mobility reported in this paper.

**SUMMARY**

1. The enzyme d-glyceraldehyde-3-phosphate dehydrogenase has been isolated and crystallized by a method involving fractionation with ammoniacal (NH₄)₂SO₄ solution of an extract of rabbit muscle prepared with dilute alkali. The enzyme crystallizes from a dilute solution (about 0.1 per cent) of purity level of about 0.5, when the saturation with (NH₄)₂SO₄ is 0.72 and the pH 8.2 to 8.4. 1 gm. or more of crystalline enzyme is obtained from 500 gm. of muscle. On recrystallization from more concentrated enzyme solutions at 0.66 saturation with (NH₄)₂SO₄ only a slight gain in specific enzyme activity results.

2. Aldolase and the dehydrogenase can be prepared from the same muscle extract.

3. The recrystallized enzyme is electrophoretically homogeneous over the pH range 6.2 to 7.7. In phosphate buffer, ionic strength 0.1, the isoelectric point is at pH 6.55.

4. Activity of the enzyme was measured spectrophotometrically accord-
ing to the method of Warburg and Christian. In order to obtain maximum activity the enzyme has to be diluted and preincubated in a cysteine (or glutathione) solution.

5. In weak salt solution the enzyme has highest stability around neutrality. At pH 5.2 or 9 it is rapidly denatured even at 0°.

6. The enzyme has one-half maximum activity when the concentration of glyceraldehyde-phosphate is $5.1 \times 10^{-5}$ moles per liter. The corresponding value of DPN is $3.9 \times 10^{-5}$ moles per liter.

7. The enzyme activity is highest between pH 8.6 and 9 and drops off sharply on the acid side, so that at pH 7.1 it is only 15 per cent of the maximum rate.

8. Iodoacetate in low concentration ($10^{-4}$ M) inhibits the enzyme. This inhibition, even at 27°, is not instantaneous. Cysteine does not reverse the inhibition.

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CRystalline d-Glyceraldehyde-3-Phosphate Dehydrogenase from Rabbit Muscle

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