A dehydrogenase catalyzing the oxidation of glucose-6-phosphate by triphosphopyridine nucleotide (TPN) has been shown to be widely distributed in higher plants (2, 5). This finding suggested that plants might form pentose by a TPN-dependent, two-step oxidation sequence similar to that already described in other organisms (6, 8, 17) and shown in equations 1 and 2.

(1) \( \text{glucose-6-phosphate} + \text{TPN}^+ + \text{H}_2\text{O} \rightarrow \text{6-phosphogluconate} + \text{TPNH} + 2\text{H}^+ \)

(2) \( \text{6-phosphogluconate} + \text{TPN}^+ \rightarrow \text{pentose phosphate} + \text{CO}_2 + \text{TPNH} \)

This paper presents evidence that enzyme systems catalyzing reaction (2) are widespread in higher plants. After this work was started, evidence for the presence of such enzymes in peas and spinach was presented by Axelrod and Bandurski (3).

Materials and methods

The barium salt of 6-phosphogluconate was prepared according to the method of Seegmiller and Horecker (18). Solutions were prepared for use by dissolving the material in HCl, removing the barium with potassium sulphate, and adjusting the pH to 7.4. Various samples of barium phosphogluconate contained 1.7 to 2.0 \( \mu \text{M} \) of organic phosphorus per mg. and 0.01 to 0.03 \( \mu \text{M} \) of inorganic phosphorus per mg. The fructose content, according to the method of Roe (16), was not more than 0.03 \( \mu \text{M} \) per mg., and the glucose-6-phosphate content, according to assay with glucose-6-phosphate dehydrogenase (11) was 0.08 to 0.13 \( \mu \text{M} \) per mg. of the barium salt. The phosphogluconate content was calculated from the organic phosphorus content, after correction for the impurities. Ribose-5-phosphate was prepared by acid hydrolysis of adenylic acid or adenosine triphosphate according to the procedure described by LePage and Umbreit (12) and Albaum and Umbreit (1). A preparation of TPN\(^+\) of 70% purity was generously supplied by Dr. M. A. Mitz of Armour Laboratories. Oxidized glutathione (GSSG) was purchased from Schwartz or was prepared by oxidizing GSH with \( \text{H}_2\text{O}_2 \) as previously described (5). Tris (trishydroxymethyl amino-
methane) was purchased from Commercial Solvents Corporation and re-
crystallized from an acetone-water mixture. Pentose was determined ac-
cording to the method of MEJBAUM (13). The change in optical density was
measured at 660 m,m, after heating in a boiling water bath for 25 minutes.
Standards were made from D-ribose.

Except for the case of wheat germ and turnip root, the enzyme prepara-
tions were made by grinding the plant, with sand if necessary, but without
the addition of water. Leaves were ground in the frozen state; fruits and
roots were peeled and ground in a meat grinder. The coarse solids were
strained off through cheesecloth and the smaller particulate matter was
removed by centrifugation in the cold at 22,000 x g. All extracts were
dialed against 0.025 M phosphate buffer (pH 7.4) for 24 hours at 3° C.
Turnip root was peeled and ground in a meat grinder. The pulp was frozen
with an equal volume of water, thawed, and squeezed through cheesecloth.
Centrifugation and dialysis were then carried out as before. Dialyzed water
extracts of wheat germ prepared as previously described (5) gave a high
value in the orcinol test for pentose which was a disadvantage when accu-
rate determinations of pentose formation were desired. A fractionation pro-
cedure was devised which gave a protein preparation which was rich in
enzymes, but contained relatively little nucleic acid. This wheat germ pro-
tein fraction will be referred to as the WP preparation.

Wheat germ was extracted for 30 minutes at room temperature with four
volumes of distilled water. The extract was strained through cheesecloth
and centrifuged at 20,000 x g, for 20 minutes at 4° C. All subsequent opera-
tions were carried out in a cold room at this temperature. The supernatant
was treated with MnCl2 to remove nucleic acid, by a modification of the
method described by KAUFMAN et al. (10). For each liter of extract, 20.4
ml. of 1.0 M MnCl2 were added slowly with stirring. The solution, which
had a final pH of about 5.5, was allowed to stand overnight. The heavy
precipitate which formed was centrifuged off at 3,000 x g. The supernatant
was stored at -15° C for 48 hours, thawed rapidly and centrifuged again at
20,000 x g, for 20 minutes to remove additional precipitate which had ap-
peared. The pH was adjusted to 7 with concentrated NH4OH, and 250 gm.
of solid (NH4)2SO4 were added slowly for each liter of solution. Mechani-
cal stirring was used during these operations, and the pH was kept at 7 by
further addition of NH4OH. After 24 hours, the bulky precipitate was
centrifuged down and discarded. To each liter of supernatant, 75 gm. of
(NH4)2SO4 were then added with stirring. The precipitate was centrifuged
down and dissolved in a minimal volume of 0.025 M phosphate buffer,
pH 7.4, and dialyzed with stirring against the same buffer to remove the
(NH4)2SO4. The final volume was about 260 ml. of protein solution for
every kilogram of wheat germ originally employed. The original extract
contained about 30 mg. dry weight per milliliter after dialysis. Measure-
ment of the ratio of the optical densities at 280 and 260 m,m indicated that
about 13% of this material was nucleic acid (19). The amount of nucleic
acid removed by the MnCl2 treatment varied in different preparations, but
it was always possible by repetition of the procedure to bring the nucleic acid content down to about 1% or less. The final dry weight of this WP preparation was about 40 mg./ml.

Results

Demonstration of phosphogluconic dehydrogenase

All of the plant preparations contained the enzyme glutathione reductase (2, 5), which catalyzes the reaction shown in equation 3.

(3) \[ \text{GSSG} + \text{TPNH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{TPN}^+ \]

This system may be coupled with any reaction in which TPN is reduced, such as the reactions shown in equations 1 and 2. The presence of phosphogluconic dehydrogenase, therefore, could be easily detected by demonstrating that GSSG was reduced when phosphogluconate, GSSG, and a catalytic amount of TPN were incubated with the plant preparation to be tested. Table I contains representative results obtained with enzyme preparations

### Table I

**Reduction of glutathione by 6-phosphogluconate.**

<table>
<thead>
<tr>
<th>Source</th>
<th>Amount of extract</th>
<th>Time</th>
<th>Phosphogluconate added</th>
<th>GSSG reduced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml.</td>
<td>min.</td>
<td>µmoles</td>
<td>Complete system</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>0.5</td>
<td>45</td>
<td>3.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Cantaloupe fruit</td>
<td>1.6</td>
<td>30</td>
<td>10.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Parsley leaf</td>
<td>1.0</td>
<td>30</td>
<td>10.0</td>
<td>7.6</td>
</tr>
<tr>
<td>Spinach leaf</td>
<td>1.0</td>
<td>30</td>
<td>5.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Parsnip root</td>
<td>1.0</td>
<td>30</td>
<td>5.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Cucumber fruit</td>
<td>1.6</td>
<td>45</td>
<td>10.0</td>
<td>7.3</td>
</tr>
<tr>
<td>Turnip root</td>
<td>1.6</td>
<td>60</td>
<td>10.0</td>
<td>2.9</td>
</tr>
</tbody>
</table>

made from seven different sources. Enzyme and phosphogluconate were incubated at 30°C in 3.7 x 10^{-2} M tris buffer of pH 7.4 with 9 µM GSSG and 30 µM TPN; total volume, 2.7 ml. The reaction was stopped by the addition of 0.3 ml. 20% HPO₃, the precipitate was centrifuged down, and an aliquot of the supernatant was titrated with iodate as previously described (2, 5). The enzyme preparation of wheat germ was the dialyzed water extract. The enzyme preparation of cucumber fruit consisted of the dialyzed juice concentrated to one third of the original volume by drying in the frozen state. Controls were always run in which TPN, GSSG, and phosphogluconate were omitted separately. The first two controls were uniformly low and therefore have not been included. The third control without substrate is given because it sometimes reaches an appreciable value.

No negative results were obtained in these experiments. Considerable quantitative variations were found, however, even with different preparations made from the same source. The figures must not be regarded as a quantitative measure of the amount of phosphogluconic dehydrogenase present in the plant, except in the sense that they set a minimal value (2).
Phosphogluconic dehydrogenase was also demonstrated spectrophotometrically by measurement of the increase in light absorption at 340 m\(\mu\) when enzyme and substrate were incubated with TPN. The results confirmed the conclusions reached on the basis of the titration procedure. The specificity of the enzyme for pyridine nucleotide was likewise tested spectrophotometrically. No evidence for any reduction of diphosphopyridine nucleotide by phosphogluconate was ever found, even with enzyme mixtures that caused an almost immediate reduction of 0.2 \(\mu\)M of TPN.

**Pentose and CO\(_2\) Production from Phosphogluconate**

In another series of experiments, analyses were made for pentose and CO\(_2\) formed during the reduction of GSSG by phosphogluconate. Representative results of these experiments are shown in table II.

### TABLE II

**COMPARISON OF GLUTATHIONE REDUCTION WITH FORMATION OF PENTOSE AND CO\(_2\).**

<table>
<thead>
<tr>
<th>Source</th>
<th>Amount of extract</th>
<th>Time</th>
<th>GSSG reduced</th>
<th>CO(_2) formed</th>
<th>Pentose formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat germ (WP)</td>
<td>0.3 ml.</td>
<td>45</td>
<td>8.6 (\mu)moles</td>
<td>6.0 (\mu)moles</td>
<td>5.6 (\mu)moles</td>
</tr>
<tr>
<td>Cantaloupe fruit</td>
<td>1.0 ml.</td>
<td>60</td>
<td>6.0 (\mu)moles</td>
<td>3.9 (\mu)moles</td>
<td>1.3 (\mu)moles</td>
</tr>
<tr>
<td>Parsley leaf</td>
<td>0.7 ml.</td>
<td>10</td>
<td>6.0 (\mu)moles</td>
<td>3.9 (\mu)moles</td>
<td>2.1 (\mu)moles</td>
</tr>
<tr>
<td>0.7 ml.</td>
<td>20</td>
<td>6.9</td>
<td>5.5 (\mu)moles</td>
<td>2.3 (\mu)moles</td>
<td>1.6 (\mu)moles</td>
</tr>
<tr>
<td>0.7 ml.</td>
<td>40</td>
<td>8.7</td>
<td>6.0 (\mu)moles</td>
<td>6.0 (\mu)moles</td>
<td>1.6 (\mu)moles</td>
</tr>
<tr>
<td>Spinach leaf</td>
<td>0.7 ml.</td>
<td>15</td>
<td>2.7 (\mu)moles</td>
<td>1.9 (\mu)moles</td>
<td>2.2 (\mu)moles</td>
</tr>
<tr>
<td>0.7 ml.</td>
<td>45</td>
<td>2.7</td>
<td>1.9 (\mu)moles</td>
<td>2.1 (\mu)moles</td>
<td>1.7 (\mu)moles</td>
</tr>
<tr>
<td>0.7 ml.</td>
<td>90</td>
<td>3.8</td>
<td>2.1 (\mu)moles</td>
<td>3.8 (\mu)moles</td>
<td>1.4 (\mu)moles</td>
</tr>
<tr>
<td>Cucumber fruit</td>
<td>1.6 ml.</td>
<td>15</td>
<td>4.0 (\mu)moles</td>
<td>2.3 (\mu)moles</td>
<td>3.8 (\mu)moles</td>
</tr>
<tr>
<td>1.6 ml.</td>
<td>30</td>
<td>7.0</td>
<td>6.4 (\mu)moles</td>
<td>6.7 (\mu)moles</td>
<td>6.7 (\mu)moles</td>
</tr>
<tr>
<td>1.6 ml.</td>
<td>60</td>
<td>9.1</td>
<td>7.4 (\mu)moles</td>
<td>4.8 (\mu)moles</td>
<td>4.8 (\mu)moles</td>
</tr>
<tr>
<td>Turnip root</td>
<td>1.6 ml.</td>
<td>20</td>
<td>1.8 (\mu)moles</td>
<td>0.6 (\mu)moles</td>
<td>0.4 (\mu)moles</td>
</tr>
<tr>
<td>1.6 ml.</td>
<td>40</td>
<td>2.8</td>
<td>1.5 (\mu)moles</td>
<td>1.4 (\mu)moles</td>
<td>1.4 (\mu)moles</td>
</tr>
<tr>
<td>1.6 ml.</td>
<td>60</td>
<td>3.2</td>
<td>1.6 (\mu)moles</td>
<td>1.6 (\mu)moles</td>
<td>1.6 (\mu)moles</td>
</tr>
</tbody>
</table>

The reactions were carried out in double arm Warburg vessels in an atmosphere of N\(_2\), with yellow phosphorus in the center well. Reaction mixtures were identical with those described for table I, except that 10 \(\mu\)M of phosphogluconate were used in all cases. After incubation, 0.3 ml. of 20\% HPO\(_3\) was tipped in, and the CO\(_2\) formed during the reaction was calculated by the standard procedure, with corrections for CO\(_2\) initially present. The contents of the vessels were centrifuged, and aliquots of the supernatants were used for pentose and GSH determinations. The results recorded in table II have been corrected for the small amounts of GSSG reduction, and pentose and CO\(_2\) formation which occurred in the absence of added phosphogluconate. With most of the sources studied, the course of the reaction was followed with time, and a number of these experiments have been included.

In all instances where a given enzyme preparation catalyzed a reduction...
of GSSG by phosphogluconate, CO₂ and pentose were found to be reaction products, but the amounts of CO₂ and pentose formed were always smaller than the amount of GSSG reduced. The above discrepancies might be accounted for both by the formation of pentoses with orcinol spectra different from that of ribose, and by the further metabolism of the pentose. The orcinol spectra of the reaction products was, therefore, examined. The parsley leaf preparation and the WP fraction from wheat germ were selected for these studies, since preliminary experiments indicated that they give quite different results. With parsley, an absorption maximum at 660 mµ was predominant at 10 minutes, but in succeeding time intervals, the spectrum shifted to give a peak at about 580 mµ. The absorption maximum at 660 mµ is characteristic of pentoses, and the seven-carbon sugar, sedoheptulose, has been reported to give a maximum at 600 mµ (9), whereas hexoses give absorption at shorter wave lengths. The orcinol spectra obtained with parsley indicated, therefore, that a pentose product formed initially was further metabolized, possibly in part to sedoheptulose. With wheat germ, on the other hand, there was a steady rise of the band at 660 mµ with no decline at later time intervals. The pentose which was formed was apparently not further metabolized. Figure 1 shows the characteristic difference in the orcinol spectra finally observed with the enzymes from the two sources.

Positive results were obtained when the qualitative color test specific for the seven-carbon ketose (4, 14) was applied to the incubation mixture with parsley leaf enzyme, but not with wheat germ. The spectral changes

![Graph showing absorption spectra](https://www.plantphysiol.org/)

**Fig. 1.** The absorption curve shown for parsley was determined on a filtrate from a mixture set up as in table II for a 40-minute incubation period. The one for wheat germ was obtained from a mixture comparable to that in table II except that 0.5 ml. enzyme was used and the incubation time was 30 minutes. Absorption spectra were obtained from mixtures incubated with and without phosphogluconate. The curves represent the differences between these spectra.
of the orcinol test and the results of the heptulose color test suggested that
the parsley leaf preparation contained an enzyme system metabolizing pen-
tose in a manner similar to that described by Horecker and Smyrniotis (9).
Such a system appeared to be absent in the wheat germ preparation. The
action of the enzymes on ribose-5-phosphate was further investigated in
order to obtain confirmation of these conclusions.

**RIBOSE-5-PHOSPHATE BREAKDOWN**

No action of the wheat germ WP preparation on ribose-5-phosphate
could be detected. When ribose-5-phosphate was incubated with the parsley
leaf enzyme, however, pentose disappeared, as shown by the decrease in the
orcinol absorption band at 660 m\(\mu\). Furthermore, a reduction of GSSG
occurred when TPN and GSSG were incubated with ribose-5-phosphate in
the presence of the enzyme. For example, when 5 \(\mu\)M of ribose-5-phosphate
were incubated with one ml. of enzyme for 30 minutes, 3.5 \(\mu\)M of pentose
disappeared and 1.6 \(\mu\)M of GSSG were reduced. The oxidoreduction was
not required for pentose breakdown, since pentose disappearance was almost
as great in the absence of GSSG and TPN as in their presence. The de-
crease in the 660 m\(\mu\) absorption band was accompanied by an increase in
absorption at about 580 m\(\mu\). At the same time the qualitative heptulose
color test became positive. These results confirmed the experiments done
with phosphogluconate, and showed further that TPN could be reduced by
pentose phosphate or by some product derived from it. A parsley leaf
preparation in which glucose-6-phosphate dehydrogenase had been com-
pletely inactivated was still able to cause a reduction of GSSG in the pres-
ence of ribose-5-phosphate and TPN. This observation ruled out the possi-
bility that the reduction of GSSG observed with ribose-5-phosphate might
be due to an indirect formation of glucose-6-phosphate from pentose phos-
phate (15). Sable (17) found that yeast preparations formed triose phos-
phate from ribose phosphate; and Horecker and Smyrniotis (9) found that
when sedoheptulose phosphate was formed from pentose phosphate with a
liver enzyme preparation, triose phosphate also appeared. The possibility
that the reduction of GSSG in the TPN-ribose-5-phosphate system might be
due to a TPN-triose-phosphate dehydrogenase was therefore entertained.
Such an enzyme has been found by Gibbs (7) in pea leaves. However, the
coupled reduction of GSSG by ribose-5-phosphate in the presence of the
parsley leaf enzyme was completely uninfluenced by arsenate. A similar
coupled reduction of GSSG independent of arsenate occurs with fructose
disphosphate in the presence of the enzyme of parsley leaf and will be
further described in a subsequent paper.

**Discussion**

The wide distribution of phosphogluconic dehydrogenase and glucose-
6-phosphate dehydrogenase in higher plants suggests that this oxidative
degradation of hexose occupies a significant position in plant carbohy-
drate metabolism. The problem of the further metabolism of pentose in these
sources has been sketchily investigated in the experiments described in this
paper, and the presence of enzyme systems metabolizing pentose was proved only for the case of the parsley leaf. The data in table II show, however, that with both spinach leaf and cucumber fruit, an initial rise in pentose formation from phosphogluconate was followed by a decrease on further incubation. This may be regarded as indirect evidence that these tissues likewise metabolize pentose. AXELROD and BANDURSKI (private communication) have demonstrated pentose disappearance and sedoheptulose formation with spinach leaf enzyme.

The discrepancies in the stoichiometry shown in the results of table II could be partially explained by further metabolism of pentose. Such an explanation, however, could not account for the data obtained with wheat germ. The failure to demonstrate pentose breakdown in the case of the wheat germ preparation may have been due to inactivation of the enzymes during the procedure employed to lower the pentose content of extracts from this source.

Summary

A phosphogluconic dehydrogenase dependent on TPN has been demonstrated in wheat germ, cantaloupe and cucumber fruits, parsley and spinach leaves, and parsnip and turnip roots. Since all plant sources tested contained glutathione reductase, the oxidation of phosphogluconate was coupled with the reduction of GSSG, and pentose orcinol-reacting material and CO₂ were shown to be formed as products of such reactions. The stoichiometry indicated the occurrence of other reactions in addition to the oxidation of phosphogluconate to pentose and CO₂. A preparation from parsley leaves caused a disappearance of ribose-5-phosphate, with an appearance of the qualitative test for seven-carbon ketoses, and an unidentified TPN dependent oxidative step.

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LITERATURE CITED


