A MICROCOLORIMETRIC METHOD FOR THE DETERMINATION OF 1,2-PROPANEDIOL PHOSPHATE*

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Recent interest in the metabolism of 1,2-propanediol phosphate (1–9) has made desirable the development of a specific assay procedure for the determination of this compound in tissues. The extreme stability of the phosphorylated compound to strong acid and to alkali (1, 10) and its ready hydrolysis to 1,2-propanediol and phosphate by both acid and alkaline phosphatase (1) have led to the development of an assay procedure based on the oxidation of 1,2-propanediol to acetaldehyde and formaldehyde by periodate (11). An aeration train has been utilized by this method similar to that described by Shinn and Nicolet (12), in which the aeration is carried out at pH 7.0 to 7.2 and thereby acetaldehyde is selectively separated from formaldehyde and trapped in a solution of sodium bisulfite. Acetaldehyde is then condensed with p-hydroxydiphenyl in concentrated sulfuric acid as described by Eegriwe (13) and its concentration determined colorimetrically.

EXPERIMENTAL

Reagents—Sodium periodate, 80 μmoles per ml., sodium salt being preferred to the potassium salt because of its greater solubility in water. Phosphate buffer, 1.0 M, was prepared in the usual manner and the pH adjusted to 7.1; an aqueous sodium bisulfite solution, 5 per cent, was prepared daily. An alkaline solution of p-hydroxydiphenyl was prepared according to previously described procedures (14), and sulfuric acid, c.p., and water-pumped nitrogen were used in the analytical method to be described. Partially purified intestinal alkaline phosphatase was prepared by the method of Schmidt and Thannhauser (15). The purification was carried up to the last step (15) and allowed to remain in 0.02 N ammonium

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acetate buffer. The phosphatase preparation was divided into 5 ml. aliquots and kept frozen until needed. At this time, 5 ml. aliquots of enzyme were diluted to 40 ml. with Veronal buffer, pH 9.6.

**Methods**

**Acetaldehyde** Acetaldehyde was determined by the method of Eegriwe (13) as modified by Barker and Summerson (14), except that the copper-lime purification was unnecessary in this case and therefore omitted.

![Fig. 1. Apparatus used for selective separation of acetaldehyde from formaldehyde.](http://www.jbc.org/)

1,2-Propanediol—An oxidation-aeration train was constructed as depicted in Fig. 1. Eight of these units were set up in parallel manner for multiple analyses, all eight being connected to a manifold which in turn was connected to a cylinder of nitrogen. Since the filter sticks used for dispersing the gas were of varying porosity, it was necessary to fit each unit with a screw clamp for regulating the flow of nitrogen. An aliquot of 1,2-propanediol solution, pH 7.0 to 7.2, in a volume of not more than 4 ml.

1 The following abbreviations are used throughout this paper: PD, 1,2-propanediol; PDP, 1,2-propanediol phosphate.
was added to Tube I. In order to get reproducible results, it is desirable
to have at least 0.1 pmole of 1,2-propanediol. 3 ml. of phosphate buffer
(1.0 M, pH 7.1) were added, followed by 1 drop of Dow Corning antifoam
A.

1 ml. of 5 per cent sodium bisulfite solution was added to Tube II, and
then 9 ml. of water. Lastly, 0.5 ml. of sodium periodate solution was
added to Tube I, and immediately thereafter each unit (Tubes I and II)
was completely assembled and aeration with nitrogen begun. The aera-
tion proceeds at a rapid rate under a nitrogen pressure of approximately
5 pounds per sq.in. for 2 hours. Acetaldehyde was trapped in the sodium
bisulfite solution and determined by the method outlined above. The
data presented in Table I were obtained by the above procedure.

### Table I

**Quantitative Determination of 1,2-Propanediol**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>PD added</th>
<th>PD found</th>
<th>Per cent recovery*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmoles</td>
<td>µmoles</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.54</td>
<td>6.75</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>7.54</td>
<td>6.61</td>
<td>89.5</td>
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<tr>
<td>4</td>
<td>3.86</td>
<td>3.42</td>
<td>89</td>
</tr>
<tr>
<td>5</td>
<td>3.86</td>
<td>3.71</td>
<td>90.5</td>
</tr>
</tbody>
</table>

Average recovery: 92

* Micromoles found per micromoles added × 100.

**1,2-Propanediol Phosphate**—5 ml. aliquots of a solution, pH 8 to 9, con-
taining 1,2-propanediol phosphate (not more than 2 µmoles²), were added
to a series of test-tubes. 3 ml. of Veronal buffer (0.1 M, pH 9.6) were
added to the tubes containing the sample, followed by 0.5 ml. of magne-
sium chloride (15 per cent). 0.5 ml. of a solution of partially purified in-
testinal alkaline phosphatase was added and the tubes were incubated for
3 hours at 43° in a constant temperature water bath. An additional 0.5
ml. of the phosphatase solution was added and the incubation continued
for another 3 hours, making the total incubation period 6 hours. Phos-
phatase activity was then stopped by addition of 0.5 ml. of 100 per cent
trichloroacetic acid. Each sample was set up in duplicate incubation tubes
and enzyme activity was stopped at zero time in 1 tube of each pair. Both
tubes were then incubated for 6 hours as described above. Proteins were
removed from the incubation tubes by centrifugation and the pH of the

² The activity of the phosphatase determines to a large extent the upper limits of
1,2-propanediol phosphate that one may use.
1,2-propanediol phosphate supernatant fluid adjusted to 7.0 to 7.2. Appropriate aliquots of these supernatant fluids were analyzed for 1,2-propanediol by the method described above. The molar concentration of 1,2-propanediol phosphate may then be determined by the following formula.

\[
[\text{PD after phosphatase action}] - [\text{PD before phosphatase action}] = [\text{1,2-propanediol phosphate}]
\]

The data presented in Table II are typical of the data obtained after analysis of 1,2-propanediol phosphate by the above procedure.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Incubation time</th>
<th>PDP added</th>
<th>PDP found*</th>
<th>Per cent recovery†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hrs.</td>
<td>µmoles</td>
<td>µmoles</td>
<td></td>
</tr>
<tr>
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<td>0</td>
<td>12.2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>12.2</td>
<td>10.8</td>
<td></td>
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<tr>
<td>2</td>
<td>0</td>
<td>18.15</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>18.15</td>
<td>15.2</td>
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<td>0</td>
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<td>4</td>
<td>6</td>
<td>2.0</td>
<td>1.9</td>
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</table>

* Average of four experiments.
† Micromoles found per micromoles added × 100.

RESULTS AND DISCUSSION

Effect of Time on Liberation of Phosphate and 1,2-Propanediol from 1,2-Propanediol Phosphate—1,2-Propanediol was liberated by phosphatase action on PDP at a rate roughly parallel to that at which phosphate was liberated only after 4 to 6 hours of incubation with the enzyme. Unexpectedly, PD appeared to be liberated less rapidly than phosphate during short periods of incubation with phosphatase. It had been previously noted (1) that crude preparations of enzymes from the gut of the rat would liberate phosphate from the natural isomer of PDP at a rapid rate. It is assumed that the slower rate of enzyme action on dl-PDP exhibited by purified intestinal phosphatase may in part be due to the influence of the unnaturally occurring isomer of PDP on the enzyme. It is possible that the lack of agreement between phosphate and PD liberated in early phases of incubation may also be attributed to the effect of the unnaturally occurring isomer of PDP. Consequently, it was necessary to select a period of 6 hours incubation of the enzyme with PDP in order that complete agreement might be assured between the amount of phosphate and the
amount of PD liberated. Three aliquots of a standard 1,2-propanediol phosphate solution were incubated with intestinal alkaline phosphatase. Phosphatase action was halted by addition of trichloroacetic acid at 0, 10, 20, 30, 40, 50, 60, 120, 180, and 360 minutes. The protein-free filtrates were then analyzed for 1,2-propanediol and phosphate. Phosphate was determined by the method of Taussky and Shorr (16), and 1,2-propanediol by the method described above. The results are presented in Fig. 2.

_Fig. 2._ The effect of time on the liberation of phosphate (□) and of 1,2-propanediol (X) from 1,2-propanediol phosphate by intestinal alkaline phosphatase.

Interfering Substances—Few compounds in tissues have been described which are known to yield acetaldehyde upon periodate oxidation. A series
acetylmethylcarbinol. The phosphorylated compounds were subjected to intestinal alkaline phosphatase action for 6 hours at pH 9.6 and at 43° before they were analyzed for acetaldehyde-producing ability. Of this series of compounds, only rhamnose, 2,3-butylene glycol, acetylmethylcarbinol, and threonine gave positive results and appeared to be converted quantitatively to acetaldehyde. This interference was to be expected from their chemical structure. Rhamnose, 2,3-butylene glycol, and acetylmethylcarbinol have not been reported to be widely distributed in tissues. Threonine, an essential amino acid, has not been found in the free form in biological tissues in sufficient concentration to interfere in this determination (17-20).

Absorption curves of the color produced when acetaldehyde, formaldehyde, and a mixture of the two are condensed with p-hydroxydiphenyl are similar with absorption maxima at 560, 610, and 585 mp respectively. It was found that 60 per cent of the formaldehyde actually present in a mixture of the two aldehydes may be determined as acetaldehyde if read at 560 mp. For this reason it was found necessary to separate the two aldehydes quantitatively. In the determination as described, care has been taken to prevent aeration of formaldehyde by controlling the pH of the solution with a buffer. If, however, the concentration of formaldehyde precursors, such as glucose or glycerol, present in the periodate oxidation tube exceeds the molar ratio of 50:1 with respect to acetaldehyde, it may be necessary to suppress the aeration of formaldehyde without suppressing that of acetaldehyde by addition of an amino acid such as glycine or alanine. This technique was previously used by Shinn and Nicolet (12). After the development of the color, it is possible to determine qualitatively whether there has been contamination by formaldehyde. Trace contamination with formaldehyde will produce a blue-violet solution, and larger amounts will completely mask the true violet color formed by acetaldehyde by producing a deep blue-colored solution. It was not found necessary to use an amino acid to suppress formaldehyde aeration except in extreme conditions, which one rarely encounters. It is not recommended for routine work; indications for its use are self-evident upon development of the color reaction.

The method described has not been used extensively in analysis for actual concentration of PD and PDP in animal tissues. However, it has been found to be quite good in determining the disappearance or appearance of these compounds in metabolic experiments with yeast cells.

SUMMARY

A microcolorimetric method has been described for the determination of 1,2-propanediol phosphate as 1,2-propanediol. The phosphorylated com-
pound is hydrolyzed to 1,2-propanediol and phosphate, after which the glycol is oxidized by periodate to acetaldehyde and formaldehyde. Acetaldehyde is selectively aerated from the mixture and collected in a bisulfite trap, and its concentration is determined colorimetrically.

BIBLIOGRAPHY

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