A REDUCED PYRIDINE NUCLEOTIDE PYROPHOSPHATASE*

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Enzymes are known which can split DPN\(^1\) at several different sites. The various types of cleavage are summarized in the following reactions:

\[
\begin{align*}
&\text{DPN} + \text{H}_2\text{O} \rightarrow \text{nicotinamide} + \text{adenosine diphosphate ribose} \quad (1) \\
&\text{DPN} + \text{H}_2\text{O} \rightarrow \text{NH}_3 + \text{deamino-DPN} \quad (2) \\
&\text{DPN} + \text{PP} \rightarrow \text{ATP} + \text{nicotinamide mononucleotide} \quad (3) \\
&\text{DPN} + \text{H}_2\text{O} \rightarrow 5'-\text{AMP} + \text{nicotinamide mononucleotide} \quad (4)
\end{align*}
\]

Reaction 1 is catalyzed by DPNase and has been observed in higher animals (1, 2), in Neurospora (3), and in some bacteria (4). Reaction 2 is catalyzed by a deaminase found in Taka-Diastase (5). Reaction 3 is the DPN pyrophosphorylase from hog liver, as described by Kornberg (6); it is the only reversible reaction of those shown. The pyrophosphatase splitting shown in Reaction 4 has been observed in preparations of plants, animals, and bacteria.

The specificity of different pyrophosphatases toward DPN and DPNH varies from one enzyme to another. The highly purified potato tuber pyrophosphatase, reported by Kornberg and Pricer (7), splits DPN twice as fast as DPNH. A kidney particle preparation, on the other hand, splits DPNH about twice as fast as DPN, when tested by Kornberg and Lindberg (8). As will be reported elsewhere, many animal pyrophosphatases split DPNH more rapidly than DPN (9).

In this communication we shall report on a purified nucleotide pyro-

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1 The abbreviations used in this paper are DPN and DPNH, oxidized and reduced forms of diphosphopyridine nucleotide; TPN and TPNH, oxidized and reduced forms of triphosphopyridine nucleotide; ATP, adenosine triphosphate; ADP, adenosine diphosphate; 5'-AMP, 5'-adenylic acid; FAD, flavin adenine dinucleotide; Tris, tris(hydroxymethyl)aminomethane; PP, inorganic phosphate.
phosphatase from pigeon liver which splits DPNH but does not attack oxidized DPN.

**Materials and Methods**

Monoesterase was prepared from human hypertrophied prostate glands according to Markham and Smith (10). Preparation of the specific 5'-AMP deaminase was according to the method of Nikiforuk and Colowick (11). Yeast alcohol dehydrogenase was obtained from the Worthington Biochemical Corporation. The lactic dehydrogenase was prepared by the method of Neilands (12). The acetone powder of pigeon livers was prepared according to the procedure of Kaplan and Lipmann (13).

The analogues of DPN were prepared as described by Kaplan and Ciotti (14). Synthesis of diadenosine 5'-pyrophosphate was described by Shuster et al. (15). Deamino-DPN was prepared as described previously (16, 5), and the α isomer of DPN was obtained by the method of Kaplan et al. (17). Reduced pyridine dinucleotides were prepared enzymatically by yeast alcohol dehydrogenase (18) or chemically by hydrosulfite (19). The millimolar extinction coefficients (E) used were as follows: 7.8 for the reduced 3-acetylpyridine analogue of DPN and 6.3 for DPNH and other reduced analogues (14, 20, 21). DPN was obtained from the Pabst Laboratories and FAD from the Sigma Chemical Company.

The protein concentration was measured by a modified method of Lowry et al. (22). Phosphate was determined by the procedure of Fiske and Subbarow (23).

**Assay of DPNH Pyrophosphatase**—Assay of pigeon liver DPNH pyrophosphatase was performed in 0.1 M Tris, pH 7.5, 0.01 M MgCl₂, and 2 × 10⁻³ M DPNH in a total volume of 1 ml. During incubation at 37° aliquots were removed at zero and subsequent times, and the amount of DPNH was measured by yeast alcohol dehydrogenase and acetaldehyde. The absorption at 340 μm of reduced nicotinamide mononucleotide, the presumed product of DPNH splitting, is the same as that of DPNH. Therefore, the increase of absorption after oxidation of the DPNH by alcohol dehydrogenase and acetaldehyde represents reduced nicotinamide mononucleotide produced by the pyrophosphatase. Such an assay is represented in Table I. A unit of activity for the pyrophosphatase, with DPNH as substrate, was defined as an increase of 0.001 in optical density of the reaction mixture at 340 μm in 40 minutes after the sample had completely reacted with alcohol dehydrogenase and acetaldehyde. The specific activity is expressed in units per mg. of protein.

We wish to thank Dr. C. DeLuca for the preparation of the monoesterase, Mr. P. E. Stolzenbach for the preparation of the analogues of DPN and the lactic dehydrogenase, and Dr. L. Shuster for the diadenosine 5'-pyrophosphate.
DPN pyrophosphatase activity was determined similarly; DPN was substituted for DPNH in the incubation mixture, and its disappearance was followed with alcohol dehydrogenase and ethanol.

TPNH pyrophosphatase activity was also tested in the same way as the DPNH activity, but lactic dehydrogenase and sodium pyruvate were used in place of the alcohol dehydrogenase system to measure TPNH disappearance.

An additional method for following pyrophosphatase activity involved the use of a phosphomonoesterase. The release of two mononucleotides from DPNH by the pyrophosphatase results in the appearance of two monoester phosphate groups; the presence of an excess of monoesterase would then result in the release of 2 inorganic phosphate molecules for each molecule of DPNH split. The reaction sequence would be as follows:

\[
\begin{align*}
\text{DPNH} & \rightarrow 5'\text{-AMP} + \text{reduced nicotinamide mononucleotide} \\
5'\text{-AMP} & \rightarrow \text{adenosine} + P \\
\text{Reduced nicotinamide mononucleotide} & \rightarrow \text{reduced nicotinamide riboside} + P
\end{align*}
\]

Net, DPNH \rightarrow 2P + adenosine + reduced nicotinamide riboside

An excess of prostatic phosphatase was used in the pyrophosphatase incubation mixture; this monoesterase has an optimum at pH 5.5, but it was sufficiently active at pH 7.5. The extent of pyrophosphatase activity

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**Table I**

Assay of DPNH Pyrophosphatase by Yeast Alcohol Dehydrogenase

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>( E_{420} ) Before dehydrogenase addition</th>
<th>( E_{420} ) After dehydrogenase addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.580</td>
<td>0.001</td>
</tr>
<tr>
<td>25</td>
<td>0.578</td>
<td>0.048</td>
</tr>
<tr>
<td>50</td>
<td>0.618</td>
<td>0.102</td>
</tr>
<tr>
<td>75</td>
<td>0.599</td>
<td>0.138</td>
</tr>
<tr>
<td>205</td>
<td>0.585</td>
<td>0.311</td>
</tr>
</tbody>
</table>

The reaction consisted of \( 4.4 \times 10^{-2} \) m DPNH, 0.01 m MgCl\(_2\), 0.1 m Tris, pH 7.5, 2 per cent NaCl, monoesterase, and pigeon liver pyrophosphatase (acetate eluate). Aliquots of 0.06 ml. were removed at zero and subsequent times and added to a cuvette containing 2.8 ml. of 0.1 m phosphate, pH 7.5. Absorption at 340 m\(\mu\) was noted (before addition of dehydrogenase), and 0.1 ml. of 0.5 m acetaldehyde and 0.02 ml. of alcohol dehydrogenase were then added. Complete oxidation occurred in 1 to 2 minutes, and the absorption at 340 m\(\mu\) was again noted (after addition of dehydrogenase).
in this monoesterase preparation was very slight and frequently undetectable.

**Results**

**Enzyme Purification**—Extraction of the soluble constituents of pigeon liver acetone powder was accomplished by grinding 5 gm. of the powder with 70 ml. of 0.02 M sodium bicarbonate. Undissolved particles were removed in the Servall angle head centrifuge and discarded. The solution was then brought to pH 6 with 0.5 N HCl and warmed to 63-65° in a water bath at 80°. With this acidification an easily sedimentable, curd-like precipitate was formed, which was discarded after centrifugation.

Precipitation of the pyrophosphatase could be accomplished by the addition of protamine sulfate (0.2 per cent in 0.04 M Tris, pH 7.5) by using a ratio of protamine solution to heated supernatant fluid of 1.5:1.0. Essentially all the activity was removed from solution by this protamine treatment; if much activity was detected in the supernatant fluid, more protamine solution was added. An active protein was eluted from the protamine precipitate with 0.2 M acetate, pH 4.8. Two elutions, totaling one-fifth the volume of the original extract, were usually sufficient to give maximal recovery of the enzyme.

Addition of alumina Cγ to the combined acetate eluates removed all activity from solution when the proportion of alumina Cγ (15 mg. per ml.) to acetate eluate was one-half. The enzyme was eluted with a 5 per cent solution of ammonium sulfate at pH 8.5. Most of the activity was usually recovered by three elutions of a combined volume equal to one-fourth the volume of the original extract. These eluates were combined and saturated with solid ammonium sulfate. The precipitate was dissolved in 0.1 M Tris-0.01 M MgCl₂, pH 7.5, or in Tris alone. This fraction will be referred to as the purified enzyme. A summary of a purification procedure is presented in Table II.³

**Stability of Enzyme**—Storage of the purified enzyme for 5 days at -20° resulted in about 30 to 50 per cent loss of activity, although the enzyme was stable in storage prior to the protamine step. Storing at 4° was no less detrimental to the purified enzyme; storage in water rather than in Tris-Mg buffer also led to relatively rapid destruction of the enzyme. The presence of glutathione or cysteine did not prevent loss of activity.

**Demonstration of Pyrophosphatase Activity**—The action of the pigeon

³ Since this work was completed, it was found that the DPNH pyrophosphatase occurred, exclusive of activity on DPN, in the soluble fraction of pigeon liver (supernatant fluid from centrifugation at 105,000 × g). The specific activity was about 280; thus this fraction would probably be preferable to the acetone powder as starting material for purification of the enzyme (9).
liver enzyme was shown to be that of a nucleotide pyrophosphatase in the following manner: If DPNH is hydrolyzed at the pyrophosphate linkage, then a mole each of 5'-AMP and reduced nicotinamide mononucleotide should be produced. The presence of a monoesterase would result in the release of 2 moles of phosphate for each mole of DPNH split. In Fig. 1 it may be seen that, in the presence of the prostatic phosphatase, 2 moles of phosphate were produced for each mole of DPNH split. The presence

### Table II

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Specific activity</th>
<th>Total activity</th>
<th>Recovery per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicarbonate extract</td>
<td>53</td>
<td>57</td>
<td>92,400</td>
<td>100</td>
</tr>
<tr>
<td>Supernatant liquid treated at 65°</td>
<td>43</td>
<td>83</td>
<td>44,900</td>
<td>49</td>
</tr>
<tr>
<td>Acetate eluates, combined</td>
<td>11</td>
<td>720</td>
<td>21,340</td>
<td>23</td>
</tr>
<tr>
<td>Ammonium sulfate eluates, combined</td>
<td>17</td>
<td>1450</td>
<td>11,520</td>
<td>12</td>
</tr>
<tr>
<td>&quot; ppt.</td>
<td>2.5</td>
<td>1040</td>
<td>6,900</td>
<td>7.5</td>
</tr>
</tbody>
</table>

For definition of units see the text.
of some monoesterase activity in the pyrophosphatase preparation was indicated by the release of inorganic phosphate in the absence of added monoesterase.

The appearance of 5'-AMP during DPNH splitting was also followed with the specific 5'-AMP deaminase. It was found that 1.8 μmoles of 5'-AMP were produced when 1.95 μmoles of DPNH had been split by the heated supernatant fraction.

**TABLE III**

*Relative Activity of Pigeon Liver Pyrophosphatase on Various Substrates*

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Activity (per cent)</th>
<th>Substrate</th>
<th>Activity (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPNH (enzymatic)</td>
<td>100</td>
<td>α isomer of DPNH</td>
<td>28</td>
</tr>
<tr>
<td>&quot;</td>
<td>108</td>
<td>&quot;   &quot;   &quot; DPN</td>
<td>23</td>
</tr>
<tr>
<td>3-Acetylpyridine analogue of DPNH</td>
<td>216</td>
<td>DPN</td>
<td>0</td>
</tr>
<tr>
<td>Adenosine diphosphate ribose</td>
<td>195</td>
<td>TPN</td>
<td>0</td>
</tr>
<tr>
<td>TPNH</td>
<td>99</td>
<td>Deamino-DPN</td>
<td>0</td>
</tr>
<tr>
<td>Diadenosine 5'-pyrophosphate</td>
<td>62</td>
<td>3-Acetylpyridine analogue of DPN</td>
<td>0</td>
</tr>
<tr>
<td>FAD</td>
<td>50</td>
<td>ADP</td>
<td>0</td>
</tr>
<tr>
<td>Deamino-DPNH</td>
<td>45</td>
<td>ATP</td>
<td>0</td>
</tr>
</tbody>
</table>

DPNH, deamino-DPNH, and 3-acetylpyridine analogue of DPNH were assayed by alcohol dehydrogenase; TPNH was assayed by lactic dehydrogenase. The remaining compounds were assayed by measuring the phosphate produced by the action of monoesterase on the mononucleotides produced by the pyrophosphatase. All values were calculated from the amount split in 60 minutes. In the alcohol and lactic dehydrogenase assays the reaction ingredients were 0.1 M Tris, pH 8.5, 0.01 M MgCl₂, heated supernatant fluid, and 1.5 × 10⁻³ M substrate. In the phosphate assays the reaction contained 0.1 M Tris, pH 7.5, 0.01 M MgCl₂, monoesterase, purified pyrophosphatase, and 1.0 × 10⁻³ M substrate, except for FAD which was 0.54 × 10⁻³ M. DPNH at 1 and 0.54 × 10⁻³ M was split at the same rate. All reduced dinucleotides were prepared with hydrosulphite, except the DPNH (enzymatic) which was prepared with yeast alcohol dehydrogenase and ethanol.

**Specificity**—A variety of compounds was readily split by the pigeon liver enzyme, as shown in Table III. TPNH was fully active in the system, but deamino-DPNH was split at a considerably reduced rate. Alteration of the nicotinamide moiety of the DPNH also affected activity; a substrate more reactive than DPNH was obtained by replacing the amide NH₂ with CH₃ (the 3-acetylpyridine analogue of reduced DPN) or by completely removing the nicotinamide ring (adenosine diphosphate ribose). Further variation of the DPNH structure, *e.g.*, diadenosine 5'-pyrophosphate and FAD, resulted in diminished but still significant activity. In contrast to
the potato nucleotide pyrophosphatase (7) the pigeon liver enzyme did not split ADP or ATP. The oxidized forms of the pyridine nucleotides, except for the \( \alpha \) isomer of DPN, were not split.

**Metal Requirement**—The purified pyrophosphatase had a nearly absolute requirement for metal ions. With \( 1 \times 10^{-3} \text{ M DPNH} \) as substrate, stimulation was maximal with \( 6 \times 10^{-3} \text{ M MgCl}_2 \); the rate was 1 per cent of maximal in the absence of magnesium ion.

**Substrate Saturation**—Substrate saturation was determined by using DPNH and the purified enzyme. The \( K_m \) was found to be approximately \( 1.0 \times 10^{-4} \text{ M} \).

**pH Optimum**—The pH optimum was rather broad and found to be between pH 8.0 and 8.5. At pH 7.5 about 65 to 75 per cent of the optimal activity was obtained; many of the experiments involved the use of the prostatic phosphatase which was active at pH 7.5 but inactive at pH 8.5.

**Inhibitors**—Table IV shows that 5'-AMP is a potent inhibitor of DPNH splitting and that ADP and ATP also inhibit strongly despite the fact that they are not split by the enzyme. The splitting of DPNH is not inhibited by the corresponding inosinic nucleotides although deamino-DPNH is attacked by the enzyme. DPN, at concentrations equivalent to DPNH, inhibits only slightly. Sodium fluoride and pyrophosphate are strong inhibitors, but only at concentrations greater than those at which the adenine nucleotides were effective; sodium phosphate did not inhibit. The inhibition by adenosine diphosphate ribose is quite likely due to competition with DPNH as a substrate for the hydrolysis reaction. Adenosine failed to inhibit, indicating that the stimulatory effect of the prostatic

---

**Table IV**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Inhibitor concentration</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-AMP</td>
<td>2</td>
<td>73</td>
</tr>
<tr>
<td>ADP</td>
<td>2</td>
<td>54</td>
</tr>
<tr>
<td>ATP</td>
<td>2</td>
<td>46</td>
</tr>
<tr>
<td>Adenosine</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>DPN</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Adenosine diphosphate ribose</td>
<td>0.5</td>
<td>47</td>
</tr>
<tr>
<td>NaF</td>
<td>10</td>
<td>82</td>
</tr>
<tr>
<td>Sodium pyrophosphate</td>
<td>25</td>
<td>66</td>
</tr>
</tbody>
</table>

The reaction mixture contained 0.1 M Tris, pH 7.5, 0.01 M MgCl₂, \( 1.6 \times 10^{-2} \text{ M DPNH} \), and purified pigeon liver enzyme. DPNH splitting was followed by the yeast alcohol dehydrogenase assay.
monoesterase, previously noted, was due to the conversion of 5'-AMP to adenosine. In this connection, it was found that the addition of prostatic enzyme would overcome the inhibition of added 5'-AMP.

**Number of DPNH Pyrophosphatases**—In later experiments (9), it was shown that a microsomal pyrophosphatase was present in pigeon liver that split both DPNH and DPN; in the same studies, the DPNH-specific pyrophosphatase was found in the soluble fraction. An attempt to detect a second pyrophosphatase in the enzyme extracted from the acetone powder is given in Table V. The pyrophosphatase activity present in three fractions, obtained during the purification procedure, was measured against three different substrates. The rate of splitting of adenosine diphosphate ribose and the 3-acetylpyridine analogue of reduced DPN remained essentially the same relative to each other and to DPNH. Although these preparations had similar specificities for the three substrates listed, the crudest fraction was able to split oxidized DPN while the purified fraction was not. This suggested the possibility that two pyrophosphatases were present in the crude fraction. This possibility has been substantiated in experiments on cellular fractionation of pigeon liver (9).

**Effect of Purified Pyrophosphatase on Alcohol Dehydrogenase Equilibrium**—The physiological role of the DPNH pyrophosphatase is not clear, but one possible function could be the displacement of an equilibrium in a reaction in which DPN was being reduced. The feasibility of this idea was tested with yeast alcohol dehydrogenase to catalyze the reduction of DPN when a limiting amount of ethanol was present; approximately one-third of the DPN was reduced at equilibrium before the splitting enzyme was added. As seen in Fig. 2, the absorption continued to increase in the cuvette containing the pigeon liver enzyme. The amount of DPNH converted to reduced nicotinamide mononucleotide was demonstrated by add-

### Table V

*Comparison of Activity of Three Enzyme Fractions*

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Bicarbonate extract</th>
<th>Heated supernatant fluid</th>
<th>Purified enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPNH</td>
<td>0.72</td>
<td>0.57</td>
<td>0.37</td>
</tr>
<tr>
<td>Adenosine diphosphate ribose</td>
<td>1.35</td>
<td>1.08</td>
<td>0.94</td>
</tr>
<tr>
<td>3-Acetylpyridine analogue of DPNH</td>
<td>1.46</td>
<td>1.20</td>
<td>0.92</td>
</tr>
<tr>
<td>DPN</td>
<td>0.12</td>
<td>0.05</td>
<td>0</td>
</tr>
</tbody>
</table>

Activity expressed as micromoles of phosphate released per ml. per 30 minutes. The reaction mixture contained 0.1 M Tris, pH 7.5, 0.01 M MgCl₂, and monoesterase. DPNH was 8 × 10⁻⁴ M, adenosine diphosphate ribose was 1.0 × 10⁻³ M, 3-acetylpyridine analogue of DPNH was 1.0 × 10⁻³ M, and DPN was 1.0 × 10⁻² M.
ing acetaldehyde at the end of the reaction. It is evident from the curves in Fig. 2 that the DPNH produced in the oxidation reaction was split, and, as a result, oxidation of ethanol was continued beyond the equilibrium point of the reaction catalyzed by the alcohol dehydrogenase.

![Figure 2](http://www.jbc.org/)

**Fig. 2.** Effect of purified pyrophosphatase on an oxidation-reduction reaction. The complete reaction mixture consisted of $0.12 \times 10^{-3} \text{ M DPN}$, $0.01 \text{ ml. of 10 per cent ethanol}$, alcohol dehydrogenase, $0.1 \text{ m Tris, pH 7.5}$, $0.01 \text{ m MgCl}_2$, purified pigeon liver enzyme. Total volume $3.0 \text{ ml}$. Incubation at $37^\circ$ was in the test tube. Solution transferred to cuvette for optical density determination. 
- $\bullet$, complete system; 
- $\bigcirc$, no purified pigeon liver pyrophosphatase; 
- $\triangle$, no alcohol dehydrogenase, no purified pyrophosphatase.

**DISCUSSION**

Pyrophosphatase action on DPN and DPNH has been studied previously with a purified potato enzyme and a kidney particle preparation. Kornberg and Pricer (7) reported that the former enzyme will split DPN about twice as fast as DPNH; Kornberg and Lindberg (8) found that the kidney preparation split DPNH more than twice as fast as DPN. The purified pyrophosphatase from pigeon liver does not cleave DPN but will split DPNH. In contrast to the potato enzyme, the pigeon enzyme did not split ADP or ATP. From studies reported elsewhere (9) and work reported by Epperson (24), it seems that most pyrophosphatases from higher animals split DPNH faster than DPN.

The mechanism of pyrophosphatase action is of interest, in the case of
the pigeon liver enzyme, since this enzyme is able to split DPNH, adenosine diphosphate ribose, FAD, diadenosine 5'-pyrophosphate, and a number of analogues of DPNH, but is not able to split DPN or the oxidized analogues of DPN. Several explanations may be proposed to explain the specificity of the pigeon liver pyrophosphatase. The positive charge on the oxidized pyridine ring of DPN may interact with negative charges on the molecule and thereby cause the DPN molecule to be in a configuration unfavorable for enzymatic attack. On the other hand, other forces may play a major role in determining the susceptibility of the dinucleotide to attack. For example, molecular models of oxidized DPN and of nicotinamide mononucleotide suggest that a hydrogen bond may be present between the amide group and a phosphate moiety of the molecule. The hydrogen bond is lost upon reduction of the DPN because the nicotinamide ring’s spatial position changes when the resonating, planar ring is converted to a non-resonating, chair- or boat-shaped ring. The α isomer of DPN contains a positively charged ring and an α-glycosidic link between nicotinamide and ribose rather than the normal β bond; this compound is split by the pigeon liver enzyme. In contrast to β-DPN, models of α-DPN indicate no hydrogen binding between the amide group and the pyrophosphate grouping. This finding suggests that hydrogen bonding is a factor in the inability of the enzyme to cleave β-DPN. The two explanations are not mutually exclusive, and both may apply. Since ADP is not split and adenosine diphosphate ribose is readily attacked, the ribose group must play an indispensable role which is not readily understood at the present time.

The ability of the pigeon liver DPNH pyrophosphatase to split DPNH as it is produced in the ethanol oxidation is suggestive of an intracellular regulatory role of the enzyme in that it could displace the equilibrium of reactions that would normally proceed only slightly.

Another role this enzyme could play is in conjunction with the enzyme, DPNase. The latter enzyme hydrolyzes DPN at the nicotinamide ribose bond so as to produce nicotinamide and adenosine diphosphate ribose. No metabolic role is known for adenosine diphosphate ribose. The specific pyrophosphatase, being able to distinguish adenosine diphosphate ribose from DPN, could hydrolyze the adenosine diphosphate ribose produced by DPNase. The adenylic acid and ribose 5-phosphate produced would then be able to participate in metabolic reactions.

**SUMMARY**

1. A pyrophosphatase has been purified from pigeon liver acetone powder that is able to cleave reduced diphosphopyridine nucleotide (DPN) into

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4 The Pauling-Corey molecular models were used to portray these structures (N. O. Kaplan and L. Grossman, to be published).
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reduced nicotinamide mononucleotide and $5'$-adenylic acid. Oxidized DPN is not attacked. The properties of this purified enzyme are reported.

2. The specificity of the enzyme is such that it hydrolyzes flavin adenine dinucleotide, adenosine diphosphate ribose, and several analogues of DPNH, as well as DPNH. Adenosine di- and triphosphates, oxidized analogues of DPN (with the exception of the $\alpha$ isomer), and DPN are not split.

3. A potent inhibitor of the enzyme is $5'$-adenylic acid. Reduced nicotinamide mononucleotide is not inhibitory.

4. The possible metabolic significance of the pigeon liver nucleotide pyrophosphatase is discussed.

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A REDUCED PYRIDINE NUCLEOTIDE PYROPHOSPHATASE
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