A STUDY OF THE METABOLISM OF 2,4-DIAMINOPYRIMIDINE*

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Recent studies have revealed that various derivatives of 2,4-diaminopyrimidine exhibit profound biochemical reactivity. For example, nearly all 2,4-diaminopyrimidines and their condensed ring systems inhibit the growth of Lactobacillus casei with folic acid (1). 2,4-Diamino-p-chlorophenoxy pyrimidine was found to be a potent antimalarial in that it was active against Plasmodium gallinaceum infection in chicks (2); the activity exhibited was of the same order as that of quinine. A definite chemotherapeutic effect of 2,6-diaminopurine\(^1\) and 4-amino-N\(^{10}\)-methylfollic acid against transplanted mouse leucemia has been described (3, 4). Inhibition by 2,6-diaminopurine of the multiplication of vaccinia virus \textit{in vitro} (5) and of estrogen-induced growth in the genital tract of the female chick has been observed (6).

In addition, this purine, 2,6-diaminopurine, was found to be utilized by the rat for nucleic acid guanine synthesis (7, 8).

It was therefore felt desirable to investigate the metabolic fate of the simplest member of this series and this communication will deal with a metabolic study of 2,4-diaminopyrimidine\(^1\) and a report on its rôle in the biosynthesis of nucleic acids. The pyrimidines occurring naturally in nucleic acids, uracil (9), thymine (9), and cytosine (10), have been found to be inactive in this regard. On the other hand, orotic acid (4-carboxyuracil) is an active precursor of nucleic acid pyrimidines (11).

When rats on an otherwise normal diet were permitted to ingest 2,4-diaminopyrimidine (12), labeled with an excess of N\(^{16}\) in the 1- and 3-nitrogen atoms as well as in the 2-amino group, the compound was extensively absorbed, as evidenced by the rather high N\(^{16}\) content of the urine (Table I). No evidence of incorporation into the tissue nucleic

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\(^1\) By the older numbering system, 2,4-diaminopyrimidine can be named 2,6-diaminopyrimidine. In any case, this compound and 2,6-diaminopurine have the same pyrimidine system in common.
acids was found. The compound was excreted undegraded, since the isotope levels of its possible urinary end-products, urea, ammonia, and allantoin, were identical with the isotope values of urea and allantoin from normal rat urine. This slight enrichment in isotopic nitrogen content of urinary constituents has been previously noticed in humans on normal diets (13).

TABLE I

<table>
<thead>
<tr>
<th>Materials isolated</th>
<th>Atom per cent excess N\textsuperscript{15}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium nucleic acids</td>
<td>0.006</td>
</tr>
<tr>
<td>Nucleic acids</td>
<td>0.002</td>
</tr>
<tr>
<td>Purine hydrochlorides</td>
<td>0.003</td>
</tr>
<tr>
<td>Silver pyrimidines</td>
<td>0.004</td>
</tr>
<tr>
<td>Urea</td>
<td>0.004</td>
</tr>
<tr>
<td>&quot; control\textsuperscript{t}</td>
<td>0.005</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.005</td>
</tr>
<tr>
<td>Allantoin</td>
<td>0.004</td>
</tr>
<tr>
<td>&quot; control\textsuperscript{t}</td>
<td>0.004</td>
</tr>
<tr>
<td>Total urinary nitrogen</td>
<td>0.186</td>
</tr>
<tr>
<td>&quot; fecal nitrogen</td>
<td>0.034</td>
</tr>
</tbody>
</table>

* Consolidated-Nier ratio mass spectrometer, model 21-201, probable error ±0.001; tank nitrogen used as standard.
\textsuperscript{t} From the urine of rats on normal diets receiving no isotopic supplement.

EXPERIMENTAL

Preparation of Isotopic 2,4-Diaminopyrimidine (12)—Guanidine nitrate was prepared (14) from dicyandiamide and ammonium nitrate (containing about 32 atom per cent excess N\textsuperscript{15} in the ammonium radical).

Isotopic guanidine nitrate (2.44 gm.) and 3.0 ml. of cyanoacetaldehyde diethylacetal (10) were added to 18 ml. of absolute n-butanol in which 0.5 gm. of sodium had been dissolved, and the mixture was refluxed for 2.5 hours with constant stirring. After the mixture was cooled, the precipitated NaNO\textsubscript{3} was removed and the filtrate was acidified with sufficient 6 N H\textsubscript{2}SO\textsubscript{4} to give maximal precipitation of the crude pyrimidine, 2.94 gm.

The product, crystallized from 2 N H\textsubscript{2}SO\textsubscript{4} and dried at 110° over P\textsubscript{2}O\textsubscript{5} in vacuo, contained 8.03 atom per cent excess N\textsuperscript{15} (theory, 8 per cent).

\( \text{C}_{6}\text{H}_{6}\text{N}_{4}\cdot\text{H}_{2}\text{SO}_{4}\cdot\frac{1}{2}\text{H}_{2}\text{O} \). Calculated, N 33.3; found, N 32.9

Three adult male rats of the Sherman strain with a combined weight of
900 gm. were fed, over a period of 3 days, a total of 306 mg. of non-isotopic 2,4-diaminopyrimidine sulfate admixed with 180 gm. of moistened stock diet (Rockland rat diet complete) to which the animals had been previously accustomed. Commencing on the 4th day, and for a week thereafter, the animals were maintained on a normal diet. During this period the daily excretions, both urinary and fecal, were collected and pooled. On the 11th day the animals were fed, over a period of 3 days, a total of 306 mg. of 2,4-diaminopyrimidine sulfate (8.03 atom per cent excess N\textsuperscript{15}) admixed with 180 gm. of stock diet. The daily urine and fecal excretions were collected. The animals were sacrificed on the day following the last isotope feeding. The urinary constituents and nucleic acids were isolated by the procedures outlined in previous studies (15). The feces, urinary constituents, and the total nucleic acids of the viscera were examined for isotope content. The normal urinary constituents were also examined as controls. The results are given in Table I.

The authors wish to acknowledge the analytical assistance of Mr. Roscoe C. Funk, Jr., and Mr. John Deonarine.

**SUMMARY**

A synthesis of 2,4-diaminopyrimidine, containing an excess of N\textsuperscript{15} in the 1 and 3 positions as well as in the 2-amino group, is described. Although the compound was extensively absorbed by the rat, the data presented indicate that 2,4-diaminopyrimidine is not metabolized to ammonia, urea, or allantoin, nor is it utilized for the biosynthesis of nucleic acids.

**BIBLIOGRAPHY**

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