ON THE MECHANISM OF PYRIMIDINE METABOLISM BY YEASTS

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Knowledge of the mechanisms involved in pyrimidine metabolism is fundamental to the biochemistry of the nucleic acids. Cerecedo, Emerson, and Stekol (1-7) pioneered this field in their work with dogs. Their experiments, conducted by feeding the in vitro oxidation products of uracil and the estimation of excreted urea, culminated in the proposal of a mechanism for pyrimidine metabolism (8). More recently, isotopically labeled cytosine was administered to rats and found to be catabolized partly to urea (9).

The present investigation extends the previous work on the metabolism of nucleic acid derivatives by yeasts (10) to provide more complete coverage of pyrimidines and related compounds. The oxidative mechanism proposed by Cerecedo and his collaborators for the degradation of uracil into urea was found inoperative in yeasts. Unlike soil bacteria (11-13), the yeasts were incapable of oxidizing uracil and thymine into barbituric acid and 5-methylbarbituric acid, respectively. On the basis of growth studies with yeasts the following metabolic pathway for the assimilation of pyrimidines by yeasts is suggested: cytosine → uracil → hydrouracil → hydroorotic acid → urea.

EXPERIMENTAL

The yeast cultures employed were Saccharomyces cerevisiae Hansen, requiring pantothenic acid and biotin as growth factors (14), and Torula utilis (Northern Regional Research Laboratory). The techniques for the preparation of the yeast inocula and the measurement of yeast growth were described previously (14). The composition of the basal medium was also reported earlier (15).

The yeasts were incubated at 30° with shaking in 18 mm. (outside diameter) test-tubes and the extent of growth was determined after 16, 24, and 40 hours by reading the percentage light absorption on a Lumetron 400 colorimeter equipped with a gray glass and wire screen filter. Growth data acquired for S. cerevisiae Hansen and T. utilis propagated on ammon-

1 The microanalytical work was performed by Mr. Joseph F. Alicino, Metuchen, New Jersey.
ium sulfate as the sole source of nitrogen were published earlier (10). Each compound tested was furnished to the yeasts in a quantity corresponding to 1.0 mg. of nitrogen per tube. The compounds were used as sole nitrogen sources and also with supplementation by 0.1 mg. of nitrogen in the form of ammonium sulfate. The results presented are based upon the data obtained from at least six tubes.

| Acetylurea | 2-Methyl-5-ethoxymethyl-6-hydroxypuridine |
| Acrylamide | 6-Methylthiouracil |
| Allylurea | 6-Methyluracil |
| 2-Amino-4-methyl-6-chloropyrimidine | Methylurea |
| 2-Amino-4-methyl-6-hydroxypyrimidine | 5-Nitrobarbituric acid |
| 2-Aminopyrimidine | Orotic acid |
| 5-Aminouracil | Oxamide |
| Barbituric acid | Oxamic acid |
| Biuret | Oxythiamine |
| Cyanoacetamide | Pteroic acid |
| 2,5-Dimethyl-6-hydroxypyrimidine | Semicarbazide |
| Folic acid | Succinamic acid |
| Hydantoic acid | Succinimide |
| Hydantoin | Succinyurea |
| Hydouracil-5-carboxylic acid | Thiamine |
| 2-Hydroxypyrimidine | Thiouracil |
| Isobarbituric acid | Thioura |
| Isocytosine | Thymine |
| Isodialuric acid | " glycol |
| Malonamide | Uracil-5-carboxylic acid |
| 2-Methyl-5-aminomethyl-6-aminopyrimidine | Uramil |
| 4-Methylcytosine | Urethane |
| 2-Methyl-5-cyano-6-aminopyrimidine | Ureidosuccinic acid |
| 2-Methyl-5-ethoxymethyl-6-aminopyrimidine | Xanthopterin |

Compounds which were not utilized as nitrogen sources for growth by either S. cerevisiae or T. utilis are listed in Table I. The growth data obtained with compounds serving as nitrogen sources for one or both yeasts are shown in Table II.

Hydouracil—2.24 gm. (0.02 mole) of uracil were dissolved in 200 ml. of hot glacial acetic acid. After the solution had cooled to room temperature, 300 mg. of Adams' catalyst were added. The mixture was hydrogenated at about 25° under an initial pressure of 40 pounds per sq. in. When the
reduction was complete (5.5 hours), the catalyst was removed by filtration and the filtrate was poured into 500 ml. of hexane. The white precipitate collected by filtration was washed thoroughly with hexane, dried, and recrystallized from water. The product weighed 1.67 gm. and melted at 275–276°. Brown and Johnson (16) reported a melting point of 272–274°; Lengfeld and Stieglitz (17) reported 272°. A mixed melting point with an authentic sample of hydrouracil obtained from Dougherty Chemicals showed no depression.

Potassium β-Ureidopropionate—This compound was prepared from β-

<p>| Table II |
| Compounds Assimilated by T. utilis and S. cerevisiae |</p>
<table>
<thead>
<tr>
<th>Compound</th>
<th>S. cerevisiae</th>
<th>T. utilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetamide</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Acetamidine</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>β-Aminopropionamide</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>Asparagine</td>
<td>95</td>
<td>90</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Cytosine</td>
<td>30</td>
<td>95</td>
</tr>
<tr>
<td>Ethyl β-aminopropionate</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>Formamide</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>Hydroorotic acid</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Hydouracil</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5-Methylcytosine</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Oxaluric acid</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Propionamide</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>Uracil</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>β-Ureidopropionic acid</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

alanine and potassium cyanate by the method of Lengfeld and Stieglitz (17).

Ureidosuccinic Acid—This compound was synthesized from aspartic acid and potassium cyanate as described by Nyc and Mitchell (18).

Hydroorotic Acid—This compound was prepared from maleic acid and urea according to the procedure of Rachstéz and Cavallini (19).

CsHsOaN. Calculated. C 37.97, H 3.79, N 17.72

Found. " 37.93, " 4.01, " 17.92

β-Aminopropionamide Hydrochloride—The method of Carlson (20) was modified. 840 mg. (0.01 mole) of cyanoacetamide were dissolved in 100 ml. of glacial acetic acid and hydrogenated for 3 hours in the presence of
200 mg. of platinum oxide at an initial pressure of 38 pounds per sq. in. The catalyst was filtered off and washed with about 10 ml. of glacial acetic acid. The combined filtrate and washings were concentrated to about 25 ml. by vacuum distillation. To the concentrated solution were added 1.0 ml. of concentrated HCl and 300 ml. of hexane. The mixture was refrigerated for several hours before collecting the precipitated hydrochloride. The material was dissolved in slightly more than the minimum volume of water. Then concentrated HCl was added to pH 1. Alcohol (about 100 ml.) was added, followed by ether to produce turbidity. The white precipitate collected after refrigeration weighed 815 mg. and melted at 146° (Franchimont and Friedmann (21) reported a melting point of 149°).

\[
\text{C}_3\text{H}_6\text{O}_3\text{NCl} \quad \text{Calculated, N 22.49; found, N 22.64}
\]

**Succinylurea**—Urea and succinic anhydride were fused according to the method of Pike (22). The product melted at 230° after softening at about 200°. Pike reported 203–205° as the melting point.

\[
\text{C}_4\text{H}_6\text{O}_4\text{N} \quad \text{Calculated, N 17.50; found, N 17.52}
\]

**Hydouracil-5-carboxylic Acid**—Uracil-5-carboxylic acid (130 mg.) was dissolved in 80 ml. of warm glacial acetic acid. After the solution cooled to room temperature, 200 mg. of platinum oxide were added and the mixture was hydrogenated for 6 hours at a pressure of 50 pounds per sq. in. The catalyst was removed by filtration and the filtrate, suitably diluted, was found to contain no starting material by ultraviolet spectrophotometry. The addition of 600 ml. of hexane to the filtrate produced a precipitate which was collected after refrigeration for several hours. The dried product weighed 104 mg. It was found to darken above 235° and to melt at 255–256°.

\[
\text{C}_3\text{H}_6\text{O}_2\text{N}_2 \quad \text{Calculated, N 17.72; found, N 17.95}
\]

RESULTS AND DISCUSSION

Hahn and Haarmann (23) discovered the ability of yeast autolysates to convert cytosine into uracil and 5-methylcytosine into thymine. Chargaff and Kream (24) prepared cell-free extracts of yeast which hydrolyzed cytosine into uracil. Their findings were substantiated by our previous work (10, 25), in which it was demonstrated that *S. cerevisiae* utilized only 1 nitrogen atom of cytosine for growth and was incapable of any growth on uracil, the corresponding deaminated compound, and that both *S. cerevisiae* and *T. utilis* were able to assimilate 1 nitrogen atom of 5-methylcytosine, but no nitrogen from thymine. The specificity of cytosine deaminase was evident from the failure of either yeast to grow on amino nitrogen...
from 4-methylcytosine, 2-aminopyrimidine, iso cytosine, and other aminopyrimidines listed in Table I.

The mechanism proposed by Cerecedo et al., for the metabolism of uracil in dogs is illustrated in Diagram 1. The initial attack upon uracil was represented as oxidation at the 5 position to yield isobarbituric acid. This was not the manner of uracil metabolism by yeasts. *T. utilis*, a yeast culture which assimilated all uracil nitrogen, was unable to utilize any nitrogen from isobarbituric acid. The further finding that *T. utilis* did not assimilate barbituric acid showed that the 4 position of uracil was not oxidized by this yeast as it was in soil bacteria (11-13). The failure of either isodialuric acid or alloxan to promote growth of *T. utilis* eliminated the possibility of simultaneous oxidation at the 4 and 5 positions of uracil.

![Diagram 1. Mechanism of uracil metabolism in dogs proposed by Cerecedo et al.](http://www.jbc.org/)

These observations were regarded as precluding oxidation as the initial reaction of uracil catabolism by yeast.

Carboxylation was eliminated as the first step in the assimilation of uracil because of the inability of both yeasts to grow on uracil-5-carboxylic acid or on orotic acid (uracil-4-carboxylic acid), a naturally occurring compound (26) utilized for pyrimidine biosynthesis in rats (27, 28), in slices of rat liver (29), and in *Lactobacillus bulgaricus* (30).

The enzymatic hydrolysis of uracil was considered possible at three points, namely, at the 1,2, the 2,3, and the 1,6 amide linkages. Hydrolysis at either the 1,2 or the 2,3 position of uracil would yield unstable carbamic acid derivatives, both of which would be converted into β-aminoacrylamide by decarboxylation. No synthesis of this compound has been devised. Malonamide, which differs from β-aminoacrylamide only by its possession of an additional atom of oxygen, was tested. It supported no growth of either yeast. Acrylamide was also useless as a nitrogen source. Hydroly-
tic cleavage of uracil at the 1,6 linkage would yield \( \beta \)-ureidoacrylic acid, another unknown compound. Hence we were unable to eliminate the possibility of uracil utilization by a mechanism involving hydrolytic cleavage.

Attention was turned to consideration of reduction as the step initiating uracil catabolism. Hydrouracil was tested and found, as uracil, to be assimilated completely by \( T. \) utilis, but to support no growth of \( S. \) cerevisiae. This finding led to scrutiny of possible metabolic degradation products of hydrouracil. Hydrolytic cleavage at the 1,2 or the 2,3 position was re

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{COOH} \\
\text{OC} & \quad \text{CH}_2 \\
\text{HN} & \quad \text{CH}_3 \\
\beta\text{-Ureidopropionic acid}
\end{align*}
\]

\[
\begin{align*}
\text{HN} & \quad \text{CO} \\
\text{OC} & \quad \text{CH}_2 \\
\text{HN} & \quad \text{CH}_2 \\
\text{Hydroorotic acid}
\end{align*}
\]

\[
\begin{align*}
\text{HN} & \quad \text{CO} \\
\text{OC} & \quad \text{CHCOOH} \\
\text{HN} & \quad \text{CH}_3 \\
\text{Hydrouracil-5-carboxylic acid}
\end{align*}
\]

\[
\begin{align*}
\text{HN} & \quad \text{CO} \\
\text{OC} & \quad \text{CH}_2 \\
\text{HN} & \quad \text{CH}_3 \\
\text{Hydrouracil-4-carboxylic acid (hydroorotic acid)}
\end{align*}
\]

\[
\begin{align*}
\text{HN} & \quad \text{CO} \\
\text{OC} & \quad \text{CH}_2 \\
\text{HN} & \quad \text{CH}_3 \\
\text{Hydrouracil-5-carboxylic acid}
\end{align*}
\]

\[
\begin{align*}
\text{HN} & \quad \text{CO} \\
\text{OC} & \quad \text{CHCOOH} \\
\text{HN} & \quad \text{CH}_3 \\
\text{Hydrouracil-5-carboxylic acid (hydroorotic acid)}
\end{align*}
\]

Diagram 2. Investigated pathways of hydrouracil catabolism

garded as leading to the formation of \( \beta \)-aminopropionamide via the unstable carbamic acid products. Hydrolysis of hydrouracil at the 1,6 position would yield \( \beta \)-ureidopropionic acid. Enzymatic carboxylation of hydrouracil could produce either hydrouracil-4-carboxylic acid (hydroorotic acid) or hydrouracil-5-carboxylic acid. These possible conversions are shown in Diagram 2.

The four possible degradation products of hydrouracil were synthesized and tested. Hydrouracil-5-carboxylic acid did not support growth of either yeast and thus was eliminated as a catabolic product of hydrouracil which supported full growth of \( T. \) utilis. \( \beta \)-Ureidopropionic acid and \( \beta \)-aminopropionamide served as excellent nitrogen sources for \( T. \) utilis, but permitted no growth of \( S. \) cerevisiae. It is suggested that both compounds
were converted into β-alanine which has the same pattern of assimilation, namely, complete utilization by T. utilis and no utilization by S. cerevisiae. Hydroorotic acid was found to support full growth of both yeast cultures. This result was of particular significance, since it represented the only instance in which the nitrogen bound in a pyrimidine ring was assimilated by S. cerevisiae. Cytosine and 5-methylcytosine supported growth of S. cerevisiae, but only to the extent afforded by their free amino groups (10, 25).

The pathways investigated in the catabolism of hydroorotic acid are shown in Diagram 3. They consisted in hydrolysis at the 1,6 position to ureidosuccinic acid, reduction at the 3,4 position to succinylurea, and hydrolysis at either the 1,2 or 2,3 position to form asparagine after de-
urea, hydroorotic acid, and asparagine upon the biotin concentration of the growth medium is illustrated in Fig. 1. The high biotin level needed for good growth on hydroorotic acid indicated that this compound was catabolized to urea. Asparagine was an effective nitrogen source at low biotin concentrations; obviously its assimilation was not routed through urea. The mechanism of asparagine assimilation was regarded as involving conversion into aspartic acid by the action of asparaginase, an enzyme found in yeast by several groups of investigators (34), and the release of ammonia from aspartic acid to form fumaric acid. Aspartase, the enzyme which effects the latter conversion, was reported present in yeast autolysates (35). This interpretation was supported by the present findings that, whereas aspartic acid was an excellent nitrogen source for both yeast cultures, succinamic acid, obtainable from asparagine by deamination, supported no growth of either culture, and \( \beta \)-aminopropionamide, obtainable by decarboxylation of asparagine, supported growth of only \( T. \) utilis.

The preceding considerations led to the proposal of a metabolic pathway for the assimilation of pyrimidines by yeasts. The scheme is presented in Diagram 4.

### TABLE III

**Growth of \( S. \) cerevisiae on Asparagine, Hydroorotic Acid, and Urea in Presence of Pantothenic Acid and \( \beta \)-Alanine**

<table>
<thead>
<tr>
<th>As (NH(_4))SO(_4) mg.</th>
<th>Test compound</th>
<th>Pantothenic acid, ( \gamma ) per tube</th>
<th>( \beta )-Alanine, ( \gamma ) per tube</th>
<th>Growth( \dagger ) after 16 hrs.</th>
<th>40 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Asparagine</td>
<td>5</td>
<td>8.7</td>
<td>12.8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Asparagine</td>
<td>5</td>
<td>9.4</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Hydroorotic acid</td>
<td>5</td>
<td>6.5</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Urea</td>
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<td>9.0</td>
<td>13.5</td>
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<td>2</td>
<td>Asparagine</td>
<td>1.7</td>
<td>8.6</td>
<td>12.6</td>
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<td>Hydroorotic acid</td>
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<td>1.7</td>
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<td>7.0</td>
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<td>10.8</td>
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</tr>
<tr>
<td>2</td>
<td>Urea</td>
<td>1.7</td>
<td>8.5</td>
<td>13.0</td>
<td></td>
</tr>
</tbody>
</table>

\* Furnished to the extent of 1.0 mg. of nitrogen per tube.

\( \dagger \) The growth data are expressed in terms of optical density \( \times 10 \).
Fig. 1. Effect of biotin concentration upon the extent of growth of *S. cerevisiae* on asparagine (Curve 1), ammonium sulfate (Curve 2), urea (Curve 3), and hydro-orotic acid (Curve 4) after 24 hours.

Diagram 4. Mechanism proposed for the catabolism of pyrimidines by yeasts.
PYRIMIDINE METABOLISM BY YEASTS

The authors are grateful to Professor L. R. Cerecedo of Fordham University for samples of isobarbituric acid, isodialuric acid, thymine glycol, and orotic acid, to Dr. Morris Soodak of the Massachusetts General Hospital for a sample of oxythiamine, and to Dr. George H. Hitchings of the Wellcome Research Laboratories for a sample of uracil-5-carboxylic acid.

SUMMARY

1. The assimilation of nitrogen from 64 compounds by Torula utilis and Saccharomyces cerevisiae Hansen was investigated. T. utilis utilized practically all of the nitrogen from fifteen of these compounds, whereas only six supported the growth of S. cerevisiae.

2. T. utilis assimilated all of the nitrogen of uracil for growth, but failed to grow on isobarbituric or isodialuric acid. This showed that the yeast did not catabolize uracil by the pathway followed in dogs.

3. A mechanism for the catabolism of pyrimidines by yeasts was suggested from structural considerations and growth data.

4. The conventional scheme of asparagine catabolism received support in this work.

BIBLIOGRAPHY


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