STUDIES ON THE METABOLISM OF THYMINE-2-C\textsuperscript{14} BY THE RAT* 

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It has been clearly established (1, 2), through the use of N\textsuperscript{15}-labeled material, that the utilization of free thymine for the synthesis of deoxyribonucleic acid (DNA) by the rat is extremely limited. However, these studies afforded but little information concerning the rate and extent of the metabolism of thymine. In the experiments reported here, thymine-2-C\textsuperscript{14} has been found to be more rapidly and extensively catabolized by this species than was heretofore appreciated. In marked contrast to the results obtained after administration of uracil-N\textsuperscript{15} and thymine-N\textsuperscript{15} to rats (2), and of uracil-N\textsuperscript{15} to human subjects (3), only a small amount of the tracer was found to be excreted via the urine. A large portion (55 per cent) of the isotope was excreted, however, in the respiratory CO\textsubscript{2} during a period of 4 hours following intraperitoneal administration of thymine-2-C\textsuperscript{14}. Experiments with rat liver slices also have shown that thymine-2-C\textsuperscript{14} is rapidly catabolized by this tissue to give rise to C\textsuperscript{14}O\textsubscript{2}. 

In agreement with the findings of Brown, Roll, and Weinfeld (1), a small but significant amount of the administered isotope was found in the thymine moiety of DNA of regenerating rat liver, and a lesser amount in the DNA of other tissues. 

EXPERIMENTAL 

Synthesis of Labeled Compounds—Barium cyanamide-C\textsuperscript{14} was prepared from BaC\textsubscript{14}O\textsubscript{3} by the method of Zbarsky and Fisher (4), and thiourea-C\textsuperscript{14} from the crude barium cyanamide by the method of Bills and Ronzio (5). Thymine-2-C\textsuperscript{14} was synthesized\textsuperscript{1} from thiourea-C\textsuperscript{14} according to the procedure of Bennett (6) and had a specific activity of 6.0 × 10\textsuperscript{7} c.p.m. 

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per mg., as measured in a windowless flow counter by techniques pre-
viously described (7).

Treatment of Experimental Animals—Partially hepatectomized, Sprague-
Dawley strain, female rats averaging 150 gm. in weight were used in these
studies. The animals were housed in individual metabolism cages and were
maintained on a diet of Purina chow pellets and water.

1 hour after partial hepatectomy 1 mg. of dilute thymine-2-C\(^{14}\) (final
specific activity, 1.0 \(\times 10^7\) c.p.m. per mg.) dissolved in water (2 ml.) was
administered to each animal by intraperitoneal injection. This dosage
was repeated at intervals of either 12 or 24 hours for 3 or 4 days, as indi-
cated in Tables III and IV. The animals were sacrificed by decapitation
24 hours after the last injection; the organs to be investigated were re-
moved quickly, frozen in a dry ice-ethanol bath, and stored at \(-30^\circ\).

The methods used for the isolation of the nucleic acids from tissues, for
the preparation of nucleotides and free bases for counting, and for the
measurement of radioactivity have been described previously (7).

For the collection of respiratory CO\(_2\) the animals were housed in closed
metabolism cages, which also permitted the collection of urine and feces
separately. The CO\(_2\) was swept into NaOH traps with a continuous
stream of CO\(_2\)-free air. The carbonate was precipitated from a suitable
aliquot of the NaOH solution as BaCO\(_3\), the radioactivity of which was
determined as previously described (7).

Experiments in Vitro—Freshly prepared rat liver slices (1.0 gm.), sus-
pended in Krebs' phosphate-saline buffer (8) (3 ml.), were incubated with
thymine-2-C\(^{14}\) in Warburg vessels (15 ml.) at 37-38\(^\circ\), with air as the gas
phase. The inset contained 5 per cent CO\(_2\)-free NaOH (0.5 ml.). At
appropriate times, 20 per cent trichloroacetic acid (0.3 ml.) was added from
the side arm and the flask was shaken for an additional 5 minutes. The
NaOH was then quickly removed from the inset, the inset was washed with
water, and the washings were added to the NaOH solution. The carbonate
was precipitated and counted as BaCO\(_3\).

RESULTS AND DISCUSSION

The data presented in Table I show that thymine is very rapidly catab-
olized by the rat and that the carbon of position 2 is excreted largely as
CO\(_2\) (about 60 per cent in 24 hours). A very small amount of the adminis-
tered radioactivity was excreted in the urine and feces during a 24 hour
period. Urine was collected under toluene at 12 hour intervals during a
total period of 76 hours from the four animals used to obtain the data re-
corded in Table IV. During the first 60 hours of this experiment the four
animals received a total of 240,000,000 c.p.m., of which only 8,700,000
c.p.m. (3.6 per cent) were excreted in the urine during 76 hours. It would
appear, therefore, that most of the intraperitoneally administered thymine was catabolized by the rat during this period.

It is of interest to compare the present findings with those obtained in related studies by other investigators. Hurlbert and Potter (9) reported that, after a single intraperitoneal dose of orotic acid-2-C\textsuperscript{14} (1 mg.), the rat excreted 20 per cent of the isotope in the urine and 5 per cent in the respiratory CO\textsubscript{2} during a 4 hour period. Rutman et al. (10) found that normal rats excreted 25 per cent of an intraperitoneal dose of uracil-2-C\textsuperscript{14} (20 mg.) in the urine and 23 per cent as respiratory CO\textsubscript{2} in a period of 3 hours. Other studies in this laboratory\textsuperscript{2} have shown that, even after an oral dose of 100 mg. of unlabeled thymine, the rat excretes only 8 per cent of this compound in the urine and feces. Thus, the administration of a larger amount of labeled uracil than of labeled thymine does not account for the greater excretion in the urine of the isotopic carbon. The present results not only show that the rat has the capacity to catabolize thymine rapidly, but also suggest that this compound is metabolized more rapidly and extensively than is orotic acid or uracil by this species.

Incubation of rat liver slices with thymine-2-C\textsuperscript{14} (Table II) resulted in the formation of a considerable amount of C\textsuperscript{14}O\textsubscript{2} in a period of 2 hours; the amount formed was not altered by the presence of 100 units of potassium penicillin G per ml. of reaction mixture. No radioactive CO\textsubscript{2} was

\textsuperscript{2} Unpublished observations.

### Table I

**Excretion of C\textsuperscript{14} Following Intraperitoneal Administration to Rats of 1 Mg.**

<table>
<thead>
<tr>
<th>Time</th>
<th>Rat 1</th>
<th>Rat 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C\textsuperscript{14} excreted, c.p.m.</td>
<td>Per cent administered C\textsuperscript{14}</td>
</tr>
<tr>
<td>Respiratory CO\textsubscript{2}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>2,316,000</td>
<td>23.1</td>
</tr>
<tr>
<td>1-2</td>
<td>1,658,000</td>
<td>16.6</td>
</tr>
<tr>
<td>2-4</td>
<td>1,517,000</td>
<td>15.2</td>
</tr>
<tr>
<td>4-6</td>
<td>257,000</td>
<td>2.5</td>
</tr>
<tr>
<td>6-24</td>
<td>280,000</td>
<td>2.8</td>
</tr>
<tr>
<td>24 hr. excretion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine*</td>
<td>6,028,000</td>
<td>60.2</td>
</tr>
</tbody>
</table>

* A very small amount of radioactivity appeared in the feces, less than 0.5 per cent of the administered dose.
formed by the incubation of thymine with slices which had been heated at 80° for 5 minutes.

These studies perhaps shed some light on the pathways of pyrimidine degradation by the mammal. Cerecedo (11) proposed that thymine was degraded via thymine glycol to CO₂, urea, and acetol. However, experiments with N¹⁸-labeled thymine (2) and N¹⁸-labeled urea (12) indicated that the catabolism of thymine probably does not involve the direct formation of urea. Although urea-C¹⁴ has been reported (13) to be partially metabolized by the rat to C¹⁴O₂, evidence has been presented (14, 15) which suggests that the catabolism of urea in mice and in cats, presumably in other mammals, is due to the presence in the gastrointestinal tract of bacteria possessing urease activity. Moreover, the rapid renal clearance of urea suggests that a much larger proportion of carbon 2 of thymine would appear in the urine if urea were formed during the catabolism of this compound. Although some of the thymine degradation may be the result of bacterial activity in the gastrointestinal tract, the results of the experiments in vitro reported herein indicate that a large part of the degradation probably takes place in the liver. The present findings, together with the observations discussed above, strongly suggest that thymine is metabolized by the rat, in part at least, by a pathway other than that proposed by Cerecedo.

Fink, Henderson, and Fink (16) have reported the presence of a small amount of β-aminoisobutyric acid in rat urine after the administration of thymine or dihydrothymine, and have suggested that this compound arises by a direct conversion of the pyrimidine to this amino acid.

The results of the administration, by intraperitoneal injection, of thymine-2-C¹⁴ (Tables III and IV) indicate that this compound is utilized to

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**Table II**

*Metabolism of Thymine-2-C¹⁴ by Rat Liver Slices*

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Per cent C¹⁴O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment I</td>
</tr>
<tr>
<td>30</td>
<td>2.8</td>
</tr>
<tr>
<td>60</td>
<td>7.4</td>
</tr>
<tr>
<td>90</td>
<td>11.2</td>
</tr>
<tr>
<td>120</td>
<td>15.3</td>
</tr>
<tr>
<td>120 (Heat-treated slices)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Each vessel contained 450 γ (12,000 c.p.m.) of thymine-2-C¹⁴.
† Potassium penicillin G added to a final concentration of 100 units per ml.
a small extent for the synthesis of DNA by regenerating rat liver and to a lesser extent by the other tissues investigated. As was the case with $^{15}$N-labeled thymidine (17), the incorporation of the label into DNA appears to be quite specific, as evidenced by the small amount of tracer found in

**Table III**

Specific activities of pyrimidine nucleotides, pyrimidines, and purines of nucleic acids from regenerating rat liver and combined internal organs after intraperitoneal administration of 1 mg. (1.0 x $10^7$ c.p.m.) of thymine-2-C$^{14}$ daily for 4 days.

<table>
<thead>
<tr>
<th>Isolated compound</th>
<th>Specific activity, c.p.m. per $\mu$m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regenerating liver</td>
</tr>
<tr>
<td>Adenine</td>
<td>13.7</td>
</tr>
<tr>
<td>Guanine</td>
<td>12.2</td>
</tr>
<tr>
<td>Uridylic acid</td>
<td>47.3</td>
</tr>
<tr>
<td>Uracil</td>
<td>19.1</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>42.8</td>
</tr>
<tr>
<td>Deoxyctidylic acid</td>
<td>24.4</td>
</tr>
<tr>
<td>Cytosine</td>
<td>678.0</td>
</tr>
<tr>
<td>Thymidylic acid</td>
<td>518.0</td>
</tr>
<tr>
<td>Thymine</td>
<td></td>
</tr>
</tbody>
</table>

**Table IV**

Specific activities of pyrimidine nucleotides, pyrimidines, and purines of nucleic acids of rat tissues after intraperitoneal administration of 1 mg. (1.0 x $10^7$ c.p.m.) of thymine-2-C$^{14}$ twice daily at 12 hour intervals for 3 days.

<table>
<thead>
<tr>
<th>Isolated compound</th>
<th>Specific activity, c.p.m. per $\mu$m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regenerating liver</td>
</tr>
<tr>
<td>Adenine</td>
<td>43.5</td>
</tr>
<tr>
<td>Guanine</td>
<td>27.0</td>
</tr>
<tr>
<td>Uridylic acid</td>
<td>83.2</td>
</tr>
<tr>
<td>Uracil</td>
<td>67.2</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>79.2</td>
</tr>
<tr>
<td>Deoxyctidylic acid</td>
<td>61.5</td>
</tr>
<tr>
<td>Cytosine</td>
<td></td>
</tr>
<tr>
<td>Thymidylic acid</td>
<td></td>
</tr>
<tr>
<td>Thymine</td>
<td></td>
</tr>
</tbody>
</table>
DNA cytosine and the PNA pyrimidines. The trace incorporation of $C^{14}$ into the latter compounds might be due to a minute amount of contaminant in the starting material or, more probably, to the utilization of degradation products of the thymine. The results also confirm the observations of Reichard and Estborn (17) and of Brown et al. (1) that very little if any thymine is converted to DNA cytosine.

From the data given in Tables III and IV it is evident that the administered thymine is diluted from 1000- to 5000-fold during its incorporation into DNA, depending on the amount and frequency of administration. The great rapidity of degradation of the thymine and excretion of carbon-2 as $CO_2$ are compatible with the utilization in the synthesis of DNA of a metabolic degradation product to which the 5-methyl group is still attached, rather than of thymine per se. When compared to the incorporation of pyrimidine deoxyribonucleosides or other precursors of nucleic acids, such as adenine or orotic acid, the present observations also indicate clearly that free thymine is not a prominent natural precursor of DNA thymine.

**SUMMARY**

1. During the 24 hour period following the intraperitoneal injection of 1 mg. of thymine-2-$C^{14}$, 60 per cent of carbon 2 was expired as carbon dioxide, and about 6 per cent was excreted in the urine. During the first 4 hours after the administration of the labeled thymine 55 per cent of the label appeared in the respiratory carbon dioxide, indicating an extremely rapid catabolism of this compound.

2. Incubation with rat liver slices also resulted in a significant catabolism of thymine-2-$C^{14}$ with the formation of $C^{14}O_2$.

3. Thymine-2-$C^{14}$ is utilized to a small but significant extent for the synthesis of DNA by regenerating rat liver and to a lesser extent by small intestine, spleen, and thymus.

**BIBLIOGRAPHY**

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