THE CATABOLISM OF URACIL IN VIVO AND IN VITRO*

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Although there have been noteworthy advances in our knowledge of the
metabolism of the purine components of nucleic acids, comparable progress
is yet to be made in regard to the pyrimidine components (1, 2). It is the
purpose of this communication to report on the catabolism of uracil, and,
in particular, on a new reaction involving its ureido carbon (3). In the
light of these findings and our prior report (4) that uracil is an effective
precursor of nucleic acids in the acetaminofluorene-treated rat, it would
appear that current concepts of pyrimidine metabolism require revision.

Methods

For studies of metabolism in vivo, male albino rats, averaging 255 gm.
(range, 220 to 300 gm.), were injected with the indicated doses, per rat,
of uracil-2-C14 in isotonic saline. Absorption of the largest dose employed
was shown to be complete within 1 hour. Food was withheld during the
test period. After intraperitoneal injection, the animals were placed in
glass metabolism cages equipped for urine collection, and the expired air
was collected in 4 N sodium hydroxide. Aliquots were precipitated with
M barium chloride at chosen time intervals. The radioactive barium carbo-
nate was extensively washed with CO2-free distilled water and then dried
with anhydrous solvents. The dried carbonate was weighed and assayed
for radioactivity.

At the termination of the experiments, the rats were sacrificed and the
contents of the bladder added to the terminal urine samples, which in-
cluded cage washings. In addition to assays of the total radioactivity of
the diluted urines, specimens in center well collection flasks were treated

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tute, National Institutes of Health, United States Public Health Service.
1 The uracil-2-C14 used in these experiments was generously synthesized for us by
Dr. C. S. Miller of Sharp and Dohme, Inc., North Wales, Pennsylvania, by the
procedure of Johnson and Flint (35) with urea-C14 and malic acid as starting materi-
als. Tests of the purity of the product showed that 95 to 100 per cent of the radio-
activity moved with the Rf of uracil on both butanol-water (5) and tert-butanol (0.8
N HCl) (6) chromatograms. The synthetic material had an E M value of 7.88 × 106
at its maximum of 258 m£ in acid medium and a 260:280 m£ absorption ratio of 6.48.
with urease, and the evolved $^{14}\text{CO}_2$ was assayed as in the case of the respiratory samples. Uracil was isolated from the urine by means of paper chromatography in $n$-butanol (5) or in tert-butanol (0.8 N HCl) (6). Urea was also isolated from the $n$-butanol papers by Dent's procedure (7) for locating the spot. The chromatograms were examined for ultraviolet-absorbing areas, and, after removal of the uracil and urea spots, the remaining strips were examined for residual radioactivity, which was found to be negligible.

In a number of experiments, comparison was made between the partition of the uracil-2-$^{14}$ and an equimolar intraperitoneal dose of urea-$^{14}$ in saline.2

Studies of $^{14}$O$_2$ formation in vitro were performed on surviving liver slices prepared from the pooled livers of two or more rats according to procedures of Melchior and Tarver (8), as well as on liver homogenates and subcellular fractions prepared according to the isotonic sucrose procedure of Hogeboom et al. (9). Incubations were conducted in center well flasks and the radioactivity assayed as for the respiratory CO$_2$ samples. After incubation, the contents of the flasks were denatured with 5 per cent trichloroacetic acid. The acid-soluble components were freed of precipitant by ether extraction, followed by boiling for 1 hour, and the ultraviolet spectra were obtained in a Beckman DU spectrophotometer. In a number of the homogenate experiments, the trichloroacetic acid-insoluble residues were repeatedly washed with 1 per cent trichloroacetic acid saturated with inert uracil and then freed from lipides by hot solvent extraction. The nucleic acids were extracted by means of the Schneider procedure (10) and subjected to radioactivity assay and ultraviolet estimation of nucleic acid content. Control incubations with trichloroacetic acid-denatured homogenate samples were run in parallel with the test samples.

For assay of radioactivity, samples were deposited on 1 cm. glass planchets and measured with a gas flow counter in the Geiger region, self-absorption factors being applied when necessary.

Results

In Vivo—The excretion of radioactivity observed after injection of graded doses of uracil-2-$^{14}$ is presented in Fig. 1. The most prominent feature of this excretion is the rapid and extensive conversion of the ureido carbon of the pyrimidine to respiratory $^{14}$O$_2$. This conversion is nearly quantitative at the lower dose levels, but accounts for a decreasing percentage of the uracil as the dose is increased. As the dose increases, the

2 The urea-$^{14}$ used for synthesis and for animal experiments was obtained from Technical Associates, Glendale, California. The material released 97 to 100 per cent of its radioactivity upon treatment with urease.
percentage of the radioactivity present in the urine increases. Although urea-C\textsuperscript{14} accounts in part for this increase, this component is negligible at the lowest dose level, while at the highest it accounts for no more than 10 per cent of the administered isotope. By means of paper chromatography (5, 6), uracil is shown to account for the non-urea radioactivity of the urine. The isolated uracil shows no evidence of dilution or alteration, since its specific activity is the same as that of the original, and other ultraviolet-absorbing materials are absent from the chromatograms.

Time studies of the partition of the radioactivity following a 20 mg. dose of the uracil are presented in Fig. 2. These data indicate that urinary excretion of the isotope is virtually complete in 3 hours, while the formation of respiratory C\textsuperscript{14}O\textsubscript{2} continues well beyond this period, doubling its 3 hour value by the end of the experiment. Fig. 2 also includes a comparison of the fate of injected urea-C\textsuperscript{14} with that of the uracil. It can be seen that the primary pathway of loss of the urea isotope is urinary, accounting for 82 per cent of the dose in 6 hours. The 18 per cent conversion of the urea to

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**Fig. 1.** Partition of radioactivity from uracil-2-C\textsuperscript{14}. Each point represents a 3 hour collection from three or more adult male albino rats receiving the indicated doses via the intraperitoneal route. Urea-C\textsuperscript{14} was determined by urease treatment and confirmed by chromatography. The figures in parentheses represent the per cent of the dose present in the urine as uracil, determined by chromatography.
respiratory $\text{C}^{14}\text{O}_2$ is to be compared with the 6 hour value of 55 per cent for the uracil.

*In Vitro*—Liver slice or homogenate systems actively form $\text{C}^{14}\text{O}_2$ from uracil-2-$\text{C}^{14}$. From Table I, it can be seen that surviving slices retain a major share of the *in vivo* activity, while cell disruption results in a sharp decrease in this activity. Fractionation of the whole homogenate into subcellular components (9) localized the CO$_2$-releasing reaction in the particle-free supernatant fraction. The activity recoverable in this fraction greatly exceeds that shown by a quantity of whole homogenate containing an equal

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

**FIG. 2.** Comparison of the partition of radioactivity from uracil-2-$\text{C}^{14}$ and urea-$\text{C}^{14}$. Each bar represents results from three or more adult male albino rats receiving either 20 mg. of uracil or 11 mg. of urea. The height of the bars represents the total elimination of the isotope at the indicated time interval after injection via the intraperitoneal route.

amount of supernatant fraction, suggesting an inhibitory effect on the part of the unresolved homogenate. This effect is noticeable when the activities are compared on the basis of protein content ("activity coefficient" in Table I). Table II shows a typical substrate saturation effect in $\text{C}^{14}\text{O}_2$ formation from increasing concentrations of the uracil.

Efforts have not yet been made to explore the cofactor requirements of this system. However, both adenosine and ATP have a marked stimulating influence on the rate of carbon dioxide release from uracil. As shown in Table III, these supplements accelerate the reaction in both the whole homogenate and the supernatant fraction by 100 per cent or more. Following a number of the experiments with the supernatant fraction, the ultraviolet spectra of the acid-soluble components were compared. For
this purpose, acid-soluble spectra were also obtained for control flasks incubated with each supplement but without uracil (supplement blanks) or

**Table I**

*Release of $^{14}O_2$ from Uracil by Liver Slices, Whole Homogenates, and Cell Fractions*

50 ml. center well incubation flasks contain $10^{-4}$ M adenosinetriphosphate (ATP), $10^{-4}$ M MgCl$_2$, and 18 mg. of glucose in a final volume of 10 ml. of $10^{-2}$ M glycine buffer at pH 7.4. Immediately prior to incubation, 2 mg. of uracil-$2-C^{14}$ ($4 \times 10^6$ c.p.m.) and tissue preparation were added, the contents gassed with 95 per cent O$_2$-5 per cent CO$_2$ and stoppered, 1 ml. of 4 N KOH was injected into the center well, and the flasks were incubated for 1 hour at 37.5° with shaking.

<table>
<thead>
<tr>
<th>Tissue preparation</th>
<th>Protein present</th>
<th>C$^{14}O_2$ production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg.</td>
<td>Experiment 1</td>
</tr>
<tr>
<td>Liver slices</td>
<td>500</td>
<td>6.4 $\times$ 10$^4$</td>
</tr>
<tr>
<td>&quot; homogenate</td>
<td>550</td>
<td>2710</td>
</tr>
<tr>
<td>Liver cell fractions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>165</td>
<td>345</td>
</tr>
<tr>
<td>Microsomal</td>
<td>90</td>
<td>50</td>
</tr>
<tr>
<td>Supernatant</td>
<td>180</td>
<td>6800</td>
</tr>
</tbody>
</table>

* Determined by the biuret procedure.
† Counts per minute in CO$_2$ divided by the mg. of protein in the flask.

**Table II**

*Effect of Substrate Concentration on Release of $^{14}O_2$ from Uracil by Whole Homogenate*

Conditions as in Table I.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>C.p.m. in C$^{14}O_2$</th>
<th>Per cent of substrate decomposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
<td>Experiment 2</td>
</tr>
<tr>
<td>mg.</td>
<td>c.p.m.</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>$4 \times 10^4$</td>
<td>2090</td>
</tr>
<tr>
<td>2.0</td>
<td>$4 \times 10^4$</td>
<td>2610</td>
</tr>
<tr>
<td>10.0</td>
<td>$2 \times 10^4$</td>
<td>3825</td>
</tr>
<tr>
<td>20.0</td>
<td>$4 \times 10^4$</td>
<td>4080</td>
</tr>
</tbody>
</table>

with the supernatant fraction alone (tissue blank). After correcting for tissue or supplement blanks, the uracil incubations showed the expected shift (11) from an acid maximum of 260 m$\mu$ to an alkaline maximum of 280 m$\mu$, but this shift was absent from the supplemented incubations, and the 260:280 m$\mu$ absorption ratios were considerably reduced in the latter.
Although these changes are suggestive of the formation of uridine (5) in the presence of supplements containing nucleoside linkages, stronger evidence than qualitative shifts in spectra would be required for decision on this point. In this regard, however, it is interesting to note that the carbon dioxide-releasing activity of the supernatant fraction was considerably reduced by Tris buffers, which might reflect the type of pyrimidine nucleosidase inhibition observed by Lampen (12) for *Lactobacillus pentosus*.

The nucleic acids extracted from the whole homogenates by the trichloroacetic acid procedure (10) all showed considerable retention of radioactivity. In six such experiments, involving three or more individual samples, an average incorporation of 65 c.p.m. per mg. of nucleic acids, with a range of 52 to 103 c.p.m., was observed. In contrast, the protein residues retained 2 to 4 c.p.m. per mg., and control samples of denatured homogenate showed negligible retention. The absence of significant radioactivity in the proteins or in the denatured controls suggests that neither absorption nor metabolic fixation of Cl402 is responsible for the observed incorporation.

**DISCUSSION**

Present concepts of pyrimidine metabolism (2, 13–15) are derived principally from the balance experiments of Cerecedo and associates (16, 17). These workers, studying the utilization of uracil and postulated intermediates, concluded that the pyrimidine is split into urea and a carbon derivative. Di Carlo et al. (18), Hayaishi and Kornberg (19), and Lara (20) have given support to this formulation, Lara demonstrating an an-

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**Table III**

_Effect of ATP and Adenosine on CO2 Release from Uracil_

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Tissue preparation</th>
<th>Supplement</th>
<th>C.p.m., per flask</th>
<th>Activity coefficient</th>
<th>Per cent of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>17, 18</td>
<td>Whole homogenate</td>
<td>None</td>
<td>506</td>
<td>3.4</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10⁻³ M ATP†</td>
<td>1600</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td>32, 33</td>
<td>Supernatant fraction</td>
<td>None</td>
<td>2240</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10⁻³ M ATP†</td>
<td>3975</td>
<td>26.5</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10⁻³ M adenosine†</td>
<td>4250</td>
<td>28.3</td>
<td>190</td>
</tr>
</tbody>
</table>

* Counts per minute in CO₂ per mg. of protein in flask.
† Final concentration in reaction mixture, 10⁻⁴ M.
aerobic split of uracil to urea in a bacterial system. Support is also lent to this pathway by work with N15-pyrimidines (15, 21, 22).

It is clear, however, that the data reported here eliminate direct formation of urea from uracil as the primary degradative pathway, which appears, instead, to be the rupture of the pyrimidine ring at the 2 position, with release of the ureido carbon. This mechanism accounts quantitatively for the administered uracil at levels comparable to the endogenous replacement rate estimated from incorporation of orotic acid (23); at higher levels it accounts for 90 per cent of the catabolism, whereas urea formation accounts for less than 10 per cent. The possible secondary formation of the respiratory C14O2 from urea-C14 split directly from uracil cannot explain our observations, as shown by the results obtained with urea-C14 which confirm those of Leifer et al. (24).

None the less, urea-C14 is seen to arise from all but the smallest doses of the uracil. Since this small urea-C14 formation accompanies only the initial phase of respiratory C14O2 release, the direct split of a portion of the uracil to urea, as proposed for cytosine-N15 by Bendich et al. (22), cannot be excluded. Such a split appears to account for a maximum of 5 per cent of the doses employed. Furthermore, the protracted excretion of the pyrimidine nitrogens, as observed by Caren and Morton (15) for uracil-N15 and by Bendich et al. (22) for cytosine-N15, contrasts with the rapid clearance of the ureido carbon of the uracil. This contrast may reflect, in part, the more rapid turnover of bicarbonate than of urea nitrogen precursors and further supports our view that the primary release of the ureido carbon, and the consequent entrance of the nitrogens into the urea nitrogen pool, is the major degradative mechanism.

Independent evidence of this mechanism is to be seen in the demonstration by Fink et al. (25) of the formation of β-amino acids from pyrimidines, since the removal of the ureido carbon would yield the amides of the observed acids. The observation by Wang and Lampen (26) of a system which metabolizes uracil without urea formation indicates a similar mechanism in bacteria.

The applicability of in vitro systems to the study of uracil metabolism is established by this report. The activity of liver slices approximates that of the whole animal and suggests that liver may be the main site of uracil catabolism. Although homogenization reduces activity sharply, some purification of the system can be obtained by the simple expedient of preparing the particle-free supernatant fraction, the activity of which is inhibited in the whole homogenate. The observation that adenosine or ATP stimulation of C14O2 release is accompanied by changes suggestive of nucleoside formation indicates that uracil may not be the reaction substrate. Both of the supplements contain nucleoside linkages which can be utilized
by the nucleoside phosphorylases present in liver (27-30). Non-availability of substrate or of energy for formation of a nucleoside might then explain the inhibition of C\textsuperscript{14}O\textsubscript{2} release observed in the whole homogenate. The possibility that free uracil is not the substrate for CO\textsubscript{2} release might also explain the observation that urinary excretion of uracil is complete at 3 hours, at which time only 50 to 60 per cent of the total respiratory C\textsuperscript{14}O\textsubscript{2} formation has occurred.

It is interesting that the formate exchange reaction involving the ureido (C-2) carbon of hypoxanthine, which seems to require a nucleoside as substrate, is also localized in the supernatant fraction (31, 32). Since CO\textsubscript{2} has been shown to enter the ureido carbon of uracil (33, 34) and we have shown its release, the analogy to the purine reaction suggested the possibility of a CO\textsubscript{2} exchange reaction. Efforts to demonstrate this reaction with inert uracil and bicarbonate-C\textsuperscript{14} were unsuccessful in vivo and in vitro. The chromatographically isolated carrier uracil contained small amounts of C\textsuperscript{14}, insufficient to serve as evidence for the exchange reaction.

Finally, mention must be made of the possible significance of the incorporation of isotope in vitro into the nucleic acids of liver homogenates. This incorporation may indicate utilization of the uracil, as has been shown for the intact pretumor and tumor-bearing rats (4), in contrast to its non-utilization in the intact normal animal (7, 21, 22). Since the catabolism of uracil may involve nucleoside formation, the possibility arises that incorporation in the normal system in vitro, as well as in the tumor animal, reflects the activity of synthetic stages beyond the nucleoside which are present but dormant in the intact normal rat. This formulation implies that the uracil utilization results from a loss or alteration of physiological control of nucleic acid synthesis in the in vitro systems and during carcinogenesis.

**SUMMARY**

1. Release of the ureido carbon as respiratory CO\textsubscript{2} represents the major pathway of catabolism of the pyrimidine, uracil.

2. Urea formation from uracil appears to be negligible at endogenous metabolism levels and of secondary importance at higher dose levels.

3. The activity of surviving slices suggests that the liver may be the main site of uracil catabolism.

4. The enzymes responsible for the cleavage of the pyrimidine ring are localized in the supernatant fraction and may require a nucleoside as substrate.

We should like to express our deep appreciation to our assistant during this investigation, Mrs. Edith Gollub, as well as to our technician, Mrs. B. Burress.
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