The Comparative Utilization of Uracil-2-C\(^{14}\) by Liver, Intestinal Mucosa, and Flexner-Jobling Carcinoma in the Rat*

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(McArdle Memorial Laboratory, The Medical School, University of Wisconsin, Madison, Wis.)

Although it has been shown that the injection of N\(^{15}\)-labeled pyrimidines into normal adult rats does not lead to appreciable labeling of the total nucleic acids (1, 19), Rutman et al. (20) have made the interesting observation that uracil-2-C\(^{14}\) is incorporated into the nucleic acids of rat hepatomas induced by 2-acetylaminofluorene to a considerably greater extent than into those of normal liver. Because of the possible chemotherapeutic implications of this finding (10) and as a continuation of the work in this laboratory on the biosynthesis of nucleic acids (11, 16, 18, 23), experiments were undertaken to determine whether uracil is also incorporated into the nucleic acids of transplantable tumors or into other normal growing tissues. The three tissues chosen were rat liver (both of normal and tumor-bearing animals), intestinal mucosa, and the Flexner-Jobling carcinoma. The results show that the transplanted tumor exhibits an elevated utilization of uracil for nucleic acid biosynthesis comparable to that found by Rutman et al. (20) for the primary hepatoma and that uracil is also well utilized by intestinal mucosa. An assessment of the comparative importance of uracil and orotic acid as building blocks in these tissues has been made, and some in vitro experiments designed to give information of the mechanisms of these conversions have been carried out.

MATERIALS AND METHODS

In Vivo Experiments

Female albino rats, obtained from the Holtzman Rat Co., Madison, Wis., weighing 170-200 gm., some bearing 10-day-old ventral subcutaneous transplants of the Flexner-Jobling carcinoma, were given injections intraperitoneally of the labeled uracil or orotic acid. The respiratory carbon dioxide, urine, and feces were collected in metabolism cages; the urine was plated directly, and the CO\(_2\) precipitated and counted as BaCO\(_3\). The rats were sacrificed at 2 and 24 hours and the desired tissues dissected. Intestinal epithelium was obtained from the uppermost 4-6 inches of small intestine. The section was flushed with 10 ml. of ice-cold saline, then slit, and the mucosa was scraped into a petri dish containing cold saline. Each tissue was homogenized, and the RNA, DNA, and acid-soluble nucleotides were isolated in the conventional manner (13, 15, 23). The ribonucleotides obtained by alkaline hydrolysis were separated by Dowex-1 chromatography (13), as were the deoxyribonucleotides obtained from DNA by DNA-ase followed by snake venom diesterase treatment. The samples were plated by evaporation and counted in gas-flow counters. They were then eluted from the plates and analyzed for nucleic acids by colorimetric reactions (22) or for nucleotides by ultraviolet spectrophotometry (15).

In the experiment in which orotic acid and uracil were compared, the combined nucleic acids were hydrolyzed with hot perchloric acid to the pyrimidine bases (17), which were separated on Dowex-1 in the ammonium chloride buffer system of Cohn (5). The uracil was freed of salt by adsorption on charcoal columns and elution with 10 per cent pyridine before it was counted.

In all in vivo experiments, the rats were each given 0.180 millimoles of uracil-2-C\(^{14}\) or orotic acid-4-C\(^{14}\) with specific activities of 51 µc/millimole.

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The dihydrouracil-2-C\(^{14}\) was prepared from uracil by low-pressure hydrogenation in acetic acid at room temperature.
with a platinum oxide catalyst and purified by recrystallization.

**In Vitro Experiments**

The high-speed supernatant system employed was essentially that of Hurlbert and Reichard (14). The tissue was homogenized in 4 volumes of a solution containing 0.15 M KCl, 0.01 M MgCl₂, 0.015 M potassium phosphate, pH 7.2, and was centrifuged for 45 minutes at 100,000 × g in a Spinco preparative ultracentrifuge. In contrast to the Hurlbert and Reichard procedure, the clear supernatant was used for incubation without being dialyzed. For incubation the "hexose diphosphate system" of Hurlbert and Reichard was used. To 2 ml. of the tissue supernatant was added an equal volume of a master mix containing 20 μmoles of hexose diphosphate, 1.5 μmole of DPN, 120 μmole of nicotinamide, and 0.5 μ mole of labeled uracil, dihydrouracil, or orotic acid. The incubations were carried out in air at 37° C. for 40 minutes in 25-ml. Erlenmeyer flasks or in Warburg flasks for the collection of CO₂. At the end of the incubation the proteins were precipitated with perchloric acid (final concentration, 0.4 M), centrifuged, and washed twice against time.

**RESULTS**

In Vivo Experiments.—The excretion of the 2-carbon of uracil by rats bearing the Flexner-Jobling carcinoma is shown in Chart 1. It is apparent that there was extensive oxidation to respiratory carbon dioxide, which was virtually complete by 4 hours. At the end of 24 hours, there was a urinary excretion of 35 per cent of the administered radioactivity and a negligible amount in the feces, so that at the end of 1 day 95 per cent of the rather large dose given (20 mg.) had been metabolized or excreted. The patterns of excretion were essentially the same for rats with and without tumors and were very similar to those reported by Rutman et al. (20) for rats with induced hepatomas.

The specific radioactivities of the total nucleic acids, the DNA and RNA pyrimidine nucleotides, taken 24 hours after uracil-2-C¹⁴ administration, and the 2-hour acid-soluble pyrimidine nucleotides are given in Table 1. It was not possible to obtain a recognizable acid-soluble nucleotide chromatogram from intestinal mucosa with the quantities isolated in these experiments.

It will be noted that in the liver of normal rats some incorporation of uracil into the acid-soluble and nucleic acid pyrimidine nucleotides occurred, although to only a small extent. In the livers of the tumor-bearing animals, however, there was a two- to fivefold increase in its utilization. It is of interest to note that we have previously shown (23) that, whereas the incorporation of glycine and inorganic phosphate into the DNA of the livers of tumor-bearing rats is increased relative to the normal, the incorporation of those precursors into RNA nucleotides was not affected by the presence of the tumor, contrary to the present case with uracil as the precursor, where there is an elevated incorporation into RNA nucleotides.

A relatively higher incorporation occurred in the case of the tumor transplants. The specific activities of the acid-soluble pyrimidine ribonucleotides were 8–9 times higher than the corresponding

**chart 1**

Excretion of uracil-2-C¹⁴. Per cent of dose against time.

With 0.4 M perchloric acid. The combined supernatant fluids were neutralized with KOH and applied to columns after removal of the potassium perchlorate.

Chromatography.—The various compounds of the acid-soluble fraction were separated on columns (1 X 20 cm.) of Dowex-1-formate. To facilitate the identification of the various compounds of interest, since only total rather than specific activities were desired, carrier UMP, UDP, UTP, and orotic acid were added. The samples were made up to pH 11–12, under which conditions uracil is retained on the column but dihydrouracil was made to pH 12, kept overnight, and the β-ureidopropionic acid was eluted from charcoal as above. Therefore, the total β-ureidopropionic acid represents the sum of the dihydrouracil and β-ureidopropionic acid present at the end of the incubation; the procedure does not permit their separate analyses.

The individual compounds were identified chromatographically and by the ratio of their ultraviolet absorption at 260 and 275 mµ. All samples were plated directly and counted in internal flow counters to at least 10 per cent statistical significance and were corrected for self-absorption when necessary.

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A relatively higher incorporation occurred in the case of the tumor transplants. The specific activities of the acid-soluble pyrimidine ribonucleotides were 8–9 times higher than the corresponding
nucleotides of normal liver; the corresponding increase in the RNA nucleotides was three- to six-fold. A still greater (six- to 24-fold) utilization of uracil for DNA biosynthesis was observed. The increased utilization of uracil for RNA biosynthesis in the Flexner-Jobling transplants relative to normal liver was somewhat less than that observed by Rutman et al. (20) in the case of primary hepatoma.

That this phenomenon is not specific for tumors is shown by the even greater incorporation of uracil into the RNA nucleotides of intestinal mucosa, although in DNA the utilization was not so high as in the tumor. The specific activities of the nucleic acids of intestinal mucosa did not differ significantly in normal and tumor-bearing rats.

It would appear from these data that the formation from uracil of ribonucleotides and their subsequent conversion into nucleic acids may be general reactions in the rat and that the rates of these conversions vary with the tissues. Since the differences noted were most striking in the acid-soluble and nucleic acid fractions than was uracil. The same was true in intestinal mucosa, but to a considerably lesser extent. In the tumor acid-soluble fraction, radioactivity derived from uracil was present to an extent. In the tumor acid-soluble fraction, radioactivity derived from uracil was present to an extent. In the tumor acid-soluble fraction, radioactivity derived from uracil was present to an extent. In the tumor acid-soluble fraction, radioactivity derived from uracil was present to an extent. In the tumor acid-soluble fraction, radioactivity derived from uracil was present to an extent. In the tumor acid-soluble fraction, radioactivity derived from uracil was present to an extent. 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TABLE 1

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>NORMAL LIVER</th>
<th>LIVER OF TUMOR-BEARING ANIMALS</th>
<th>INTESTINAL MUCOSA</th>
<th>TUMOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA</td>
<td>15</td>
<td>23 (1.65)*</td>
<td>140 (9.8)</td>
<td>64 (4.8)</td>
</tr>
<tr>
<td>DNA</td>
<td>6</td>
<td>17 (2.5)</td>
<td>11 (1.8)</td>
<td>22 (5.7)</td>
</tr>
<tr>
<td>RNA uridylic acid</td>
<td>51</td>
<td>68 (2.2)</td>
<td>311 (10)</td>
<td>97 (5.1)</td>
</tr>
<tr>
<td>RNA cytidylic acid</td>
<td>14</td>
<td>23 (1.5)</td>
<td>76 (5.4)</td>
<td>91 (6.5)</td>
</tr>
<tr>
<td>DNA cytidylic acid</td>
<td>4.5</td>
<td>60 (1.5)</td>
<td>110 (4.4)</td>
<td>73 (6.1)</td>
</tr>
<tr>
<td>DNA thymidylic acid</td>
<td>12</td>
<td>59 (4.9)</td>
<td>73 (6.7)</td>
<td>2760 (8.5)</td>
</tr>
<tr>
<td>Acid-soluble uridylic acid</td>
<td>312</td>
<td>904 (2.9)</td>
<td>2760 (8.5)</td>
<td>2190 (7.9)</td>
</tr>
<tr>
<td>Acid-soluble cytidylic acid</td>
<td>277</td>
<td>1370 (4.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The figure given in parentheses is the ratio of the specific activity of the sample in the tissue to that of a similar sample from normal liver. The tissues from three rats were pooled for each determination.

TABLE 2

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>Liver:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-Hour acid-soluble</td>
</tr>
<tr>
<td>Liver:</td>
<td>30.5</td>
</tr>
<tr>
<td>Intestinal mucosa:</td>
<td>13.4</td>
</tr>
<tr>
<td>Flexner-Jobling Carcinoma:</td>
<td>0.23</td>
</tr>
</tbody>
</table>

* The ratio

Specific activity of sample from orotic acid-C14

Specific activity of sample from uracil-C14

The tissues from three rats were pooled for each determination.
tent about fourfold that of orotic acid; however, in the nucleic acids the two precursors were utilized to about the same extent. It is recognized that these data represent measurements made at only 2 times, and, in order for a quantitatively completely valid comparison of this sort to be made, a time study of these processes would be required. Nevertheless, the effects reported here are so striking that they leave no doubt as to the validity of the orders of magnitude expressed in the table.

In vitro Experiments.—Since it appeared from the in vivo experiments that the differences among the tissues in their utilization of uracil lay in the steps involving the conversion of the base into the acid-soluble nucleotide precursors of the nucleic acids, rather than in the process of nucleic acid biosynthesis, an in vivo study was undertaken of the conversion of uracil, dihydrouracil, and orotic acid into nucleotides in liver and tumor. The system chosen for this work was that of Hurlbert and Reichard (14) and is the microsome-free high-speed supernatant of the tissues with hexose diphosphate as the source of energy. In view of the observation of Cannelakis (2, 4) that uracil-2-C14 is extensively oxidized to carbon dioxide in liver slices and whole homogenates there was no more conversion of uracil into nucleotides than that observed in this system (unpublished).

In contrast, in both liver and tumor there was a very extensive conversion of orotic acid into the uridine nucleotides at all three levels of phosphorylation.

### TABLE 3

<table>
<thead>
<tr>
<th>PER CENT RECOVERIES OF ACID-SOLUBLE RADIOACTIVITY IN HURLBERT-REICHARD SYSTEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SAMPLE</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>C14O2</td>
</tr>
<tr>
<td>Nucleosides</td>
</tr>
<tr>
<td>Total ?-ureidopropionic acid*</td>
</tr>
<tr>
<td>Uracil</td>
</tr>
<tr>
<td>CMP</td>
</tr>
<tr>
<td>Orotic acid</td>
</tr>
<tr>
<td>UMP</td>
</tr>
<tr>
<td>UDP</td>
</tr>
<tr>
<td>UTP</td>
</tr>
<tr>
<td>UX</td>
</tr>
<tr>
<td>Total recovery of acid-soluble radioactivity</td>
</tr>
</tbody>
</table>

* This fraction also includes unchanged dihydrouracil.
For the experimental conditions, see "Materials and Methods."

**DISCUSSION**

It is evident from these results and those of Rutman et al. (20) that uracil is more extensively utilized for nucleic acid biosynthesis in a primary induced rat hepatoma and in a transplantable tumor than it is in normal liver. The finding that there is appreciable conversion of uracil into nucleic acid pyrimidines in intestinal mucosa in vivo shows that this enhanced utilization of uracil is a property which, if it is general for tumors, is not specific to them. It appears that the important site of the differences among the tissues lies at the level of the conversion of uracil into acid-soluble nucleotides, rather than in the subsequent conversion of these intermediates into the nucleic acids (cf. 16). In attempting to assess in vivo the relative importance of preformed uracil and orotic acid to the economy of the various tissues, it was observed in liver that orotic acid is utilized for both acid-soluble and nucleic acid pyrimidines to a much greater extent than uracil. In intestinal mucosa, this effect was less pronounced, and in the tumor
tissue the two precursors were utilized to about the same extent.

In the in vitro studies with liver and tumor supernatant fractions (it was not possible to obtain an active system from intestinal mucosa), rapid catabolism of uracil to carbon dioxide was observed in the liver (21) but not in the tumor systems. This pathway probably predominates to such an extent that little uracil is available in liver for conversion into uridine nucleotides. It has subsequently been shown by Fink et al. (7), Grisolia and Wallach (8), and Canellakis (4) that the catabolism of uracil involves the initial reduction to dihydrouracil, cleavage to \(\beta\)-ureidopropionic acid, and hydrolysis to \(\beta\)-alanine, carbon dioxide, and ammonia; these reactions all take place in the high-speed supernatant fraction from liver.

Since dihydropyrimidine nucleotides are hydrolyzed under mild acid conditions to the free bases (6), whereas pyrimidine nucleotides are not, it appeared possible that the biological formation of uridine nucleotides might go through the thermodynamically more favorable dihydrouracil, even when uracil was supplied as the precursor. When dihydrouracil-2-C\(^{14}\) was used as the precursor in vitro, there was a somewhat higher conversion into nucleotides than that obtained with uracil-2-C\(^{14}\). However, since both tissues reduce uracil and convert it into \(\beta\)-ureidopropionic acid, further work will be required to determine which is the true intermediate.

The suggestion from this work that tumors may utilize uracil to a greater extent than most other tissues is far from proved. Nevertheless, this possibility has been followed from the aspect of tumor chemotherapy, since it suggests that pyrimidine analogs, if properly chosen, would be worth further investigation as potential chemotherapeutic agents. In the past a large number of such compounds have been tested, but none with really significant tumor-inhibitory properties had been uncovered until the demonstration by Welch and his colleagues of such activity in the case of 6-azauracil (9). This observation encouraged us to pursue the problem further and led to the successful demonstration of potent tumor-inhibitory activity in the hitherto unknown series of fluorinated pyrimidines (10).

**SUMMARY**

1. When uracil-2-C\(^{14}\) was administered to tumor-bearing rats, it was utilized for acid-soluble nucleotide formation and RNA and DNA nucleic acid pyrimidine biosynthesis to a much greater extent by the Flexner-Jobling carcinoma and intestinal mucosa than by liver.

2. In a direct comparison of the utilization of uracil-2-C\(^{14}\) and orotic acid-4-C\(^{14}\) in vitro, the latter was incorporated into acid-soluble nucleic acid pyrimidines to a much greater extent in the liver. In intestinal mucosa orotic acid was more efficiently utilized than uracil, while in the tumor the two precursors were incorporated to the same extent.

3. In the soluble, high-speed supernatant fraction obtained from homogenates of liver and Flexner-Jobling carcinoma, there was an extensive conversion of orotic acid into the acid-soluble uridine nucleotides and a much lesser utilization of uracil. There was a somewhat higher conversion of dihydrouracil into nucleotides than of uracil. Extensive catabolism of both uracil and dihydrouracil was observed in this system derived from liver; very little was obtained in the tumor system.

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The Comparative Utilization of Uracil-2-C\textsuperscript{14} by Liver, Intestinal Mucosa, and Flexner-Jobling Carcinoma in the Rat


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