Compartmentalization of Uridine and Uridine 5'-Monophosphate in Rat Liver Slices*

JOHN K. TSENG AND ERLIO GURPIDE

From the Department of Obstetrics and Gynecology and the Department of Biochemistry, Mount Sinai School of Medicine (CUNY), New York, New York 10029

SUMMARY

The metabolism of exogenous uridine, uridine 5'-monophosphate, and uracil by rat liver slices was studied using in vitro superfusion systems. Mixtures of 3H- and 14C-labeled uridine, uridine 5'-monophosphate, uracil, and orotic acid were superfused at a constant rate until an isotopic steady state was achieved. Isotopic ratios and concentrations of labeled pyrimidines were measured in tissue and perfusate. The results of these experiments indicated marked compartmentalization in the intracellular distribution of uridine 5'-monophosphate, uridine, and uracil. On the basis of a model justified by the experimental findings, rates of metabolism of exogenous pyrimidines in the plasma membrane and rates of passage of these compounds through the membranes were estimated.

As a continuation of studies aiming to determine the quantitative importance of circulating nucleosides and nucleotides as a source of intracellular pyrimidines (1, 2), the in vitro uptake of uridine and uridine 5'-monophosphate by rat liver was investigated. In the experimental design used, a mixture of two precursors, one labeled with 3H and the other with 14C, were superfused over tissue slices and concentrations of labeled precursors and products isolated from perfusate and from tissue were measured at the isotopic steady state. These experiments demonstrated intracellular compartmentalization of UMP, uridine, and uracil. They also provided evidence of complete hydrolysis of UMP to uridine, and partial conversion of uridine to uracil in the cell membrane. Furthermore, the isotope data was used to evaluate the fraction of the perfused uridine that was transported into the cells.

MATERIALS AND METHODS

Tracers and Measurement of Radioactivity—[6-3H]Uridine (specific activity 3 Ci per mmole, Amersham/Searle), [5-3H]UMP (specific activity 21.9 Ci per mmole, Schwarz/Mann), [2-14C]uracil (specific activity 55 Ci per mole, New England Nuclear), [2-14C]uridine (specific activity 58 Ci per mole, Amersham/Searle) were purified by paper chromatography using systems described in a previous publication (1). [14C]Orotic acid (specific activity 55 Ci per mole, Schwan/Mann) was used as received from the supplier. Crystalline uracil, uridine, UMP, and UTP were purchased from Sigma Chemical Co.

Radioactivity in the isolated compounds was measured in a liquid scintillation spectrometer (Tri-Carb, Packard Instrument Co.) after dissolving the dry sample in 0.5 ml of water and mixing the solution with 10 ml of Insta-gel (Packard Instrument Co.).

Superfusion Conditions—Liver, obtained from female Sprague-Dawley rats killed by cervical dislocation, was cut in small pieces with scissors and sliced with a Sorvall TC-2 tissue sectioner set at 200 μm. The slices were thoroughly washed with 0.9% NaCl solution and about 400 mg were placed in the chamber in the superfusion apparatus described elsewhere (3). Essentially, the perfusion apparatus consisted of a glass syringe connected by a glass tubing to a chamber limited by a fritted glass disc and a Teflon filter outlet (bed support for LC-1/2 column from Chromatronics, Inc., Calif.) attached to a Teflon tube. A Krebs-Ringer bicarbonate buffer containing 1 mg per ml of glucose, and the labeled pyrimidines and saturated with 95% O2-5% CO2 at 37° and pH 7.4, was forced from the syringe through the chamber at the rate of 20 ml per hour using a Sage pump, model 352. The chamber and connecting tubing were immersed in a 37° water bath. The perfusion was continued for 120 min and 4 to 5 fractions of perfusate were collected in tubes immersed in methanol-ice bath. When the perfusion was stopped, the tissue slices were rapidly washed with cold saline and homogenized in methanol-containing carriers of the compound to be isolated. An equal volume of water was then added. The precipitated proteins and nucleic acids were separated from the supernatant by centrifugation, washed with a 1:1 mixture of methanol-water, and used to measure DNA by the method of Burton (4).

Extraction of nucleosides and nucleotides from homogenates with methanol-water or with trichloroacetic acid were found to be equally efficient.

Carriers of the compounds to be isolated were added to known volumes of the collected perfusate fractions. The procedures for the isolation of uracil, uridine, and UMP from these samples and from the supernatant of the tissue homogenates have been described in a previous publication (2). UTP was isolated by ascending paper chromatography using the system 95% ethanol-
1 M ammonium acetate, 7:3 (RF values: uracil 0.70; uridine, 0.70; UMP, 0.33; UTP, 0.05).

The losses during the isolation of each of these compounds were estimated by measuring spectrophotometrically at 262 nm (Beckman DU II) the recoveries of the added carrier. Concentrations of labeled compounds were expressed as counts per min per ml of perfusate or as counts per min per mg of DNA in tissue. The latter concentration was also given as counts per min per g of tissue as the analysis of several samples indicated that each gram of rat liver contains 2.41 mg of DNA, in agreement with published reports (5).

Normalization of Data—The concentrations of labeled compounds in the perfusion medium (counts per min per ml) were approximately the following: \[^3H\]UMP, 50,000; \[^{14}C\]orotic acid, 20,000; \[^{14}C\]uridine, 10,000; \[^{3}H\]uridine, 50,000; and \[^{14}C\]uracil, 10,000. In order to compare results obtained from different experiments, the data was normalized by expressing the experimental data in terms of a hypothetical concentration of perfused tracers of 10,000 cpm per ml.

RESULTS

The normalized results from 11 experiments in which a mixture of \[^3H\]UMP and \[^{14}C\]uridine was perfused over slices of rat liver are shown in Table I.

Table II shows the normalized results obtained from other experiments in which either a mixture of \[^3H\]UMP and \[^{14}C\]orotic acid, of \[^3H\]uridine and \[^{14}C\]orotic acid, or of \[^3H\]uridine and \[^{14}C\]uracil was perfused.

These results are used, first, to construct a model, and, second, to estimate the fractions of perfused UMP and uridine that are metabolized in the cell membrane, transported to the inside of the cell, or released back into the medium.

Model (Fig. 1)

Conversion of UMP to Uridine in Cell Membranes

The model in Fig. 1 shows that all the UMP reaching the cell membranes is converted to uridine and that none enters the cells.

The results shown in Tables I and II provide evidence supporting this conclusion, as follows.

1. The \(^{14}C/^{3}H\) ratio in uridine isolated from tissue at the end of the perfusion of a mixture of \[^3H\]UMP and \[^{14}C\]uridine (Table I) is identical with the \(^{14}C/^{3}H\) ratio in the perfused mixture, i.e. \((^{14}C/^{3}H)_{\text{uridine, tissue}} = (^{3}H/^{14}C)_{\text{perfused}}\).

Assuming a normalized perfused ratio of 1, the isotope ratio of intracellular uridine was found to be 1.01 ± 0.15 (mean ± S.D., n = 11). This is the result to be expected if the same fractions of perfused UMP and uridine reach the cell surface, and if all of the UMP is converted to uridine in the cell membrane.

2. The normalized \(^{14}C/^{3}H\) ratio in UMP isolated from tissue during the perfusion of \[^3H\]UMP and \[^{14}C\]orotic acid (0.27 and 0.26, Table II) is the same as the normalized \(^{14}C/^{3}H\) ratio in the perfused mixture of tracers (Table I). This result would be expected if intracellular UMP were solely derived from uridine.

3. The \(^{14}C/^{3}H\) in UMP isolated from tissue following the perfusion of a mixture of \[^{14}C\]orotic acid and \[^{14}C\]uridine (0.27 and 0.26, Table II) is the same as the \(^{14}C/^{3}H\) obtained during the perfusion of \[^{14}C\]uridine and \[^{14}C\]orotic acid (0.28, Table II). This similarity of ratios indicates that superfused \[^{3}H\]UMP and \[^{14}C\]uridine are equivalent in labeling UMP inside the cell, where \[^{14}C\]UMP is assumed to be formed from \[^{14}C\]orotic acid.

In the interpretation of these experiments, it was considered that uridine and UMP isolated from the tissue correspond to the compounds inside the cell, implying that the amounts of uridine and UMP associated with the membrane are negligibly small or are removed during the washing of the perfused slices before extraction. Experimental findings support this assumption. Thus, during perfusion of a mixture of \[^{3}H\]UMP and \[^{14}C\]orotic acid, equal isotope ratios in uridine and UMP isolated from tissue (0.31 and 0.26 for uridine, 0.27 and 0.26 for UMP, Table II) are found. In these experiments, the \(^{14}E/^{3}H\) ratios in uridine in the perfusate were very high (200 and 94). Therefore, since the excess \(^{3}H\) in uridine in the perfusate is expected to derive from conversion of \[^{3}H\]UMP to uridine in the membrane, it was concluded that the membrane pool, both of uridine and UMP, did not show a significant enrichment in labeled UMP or uridine.

### Table I

<table>
<thead>
<tr>
<th>urc</th>
<th>urd</th>
<th>urc/urd</th>
<th>urc</th>
<th>urd</th>
<th>urc/urd</th>
</tr>
</thead>
<tbody>
<tr>
<td>[^{3}H]</td>
<td>[^{14}C]</td>
<td>[^{3}H]</td>
<td>[^{14}C]</td>
<td>[^{3}H]</td>
<td>[^{14}C]</td>
</tr>
<tr>
<td>470</td>
<td>10</td>
<td>340</td>
<td>390</td>
<td>0.36</td>
<td>1.20</td>
</tr>
<tr>
<td>630</td>
<td>10</td>
<td>630</td>
<td>910</td>
<td>0.69</td>
<td>1.14</td>
</tr>
<tr>
<td>390</td>
<td>10</td>
<td>390</td>
<td>570</td>
<td>0.47</td>
<td>1.12</td>
</tr>
<tr>
<td>900</td>
<td>10</td>
<td>900</td>
<td>660</td>
<td>0.45</td>
<td>1.22</td>
</tr>
<tr>
<td>400</td>
<td>10</td>
<td>400</td>
<td>810</td>
<td>0.52</td>
<td>1.04</td>
</tr>
<tr>
<td>490</td>
<td>10</td>
<td>490</td>
<td>930</td>
<td>0.50</td>
<td>1.20</td>
</tr>
<tr>
<td>600</td>
<td>10</td>
<td>600</td>
<td>970</td>
<td>0.33</td>
<td>1.20</td>
</tr>
</tbody>
</table>

All isotopic data were normalized by assuming that 10,000 cpm per ml of each tracer was superfused.
TABLE II

Normalized isotopic data from superfusions of rat liver slices

<table>
<thead>
<tr>
<th>Compounds perfused</th>
<th>Concentrations in perfusate</th>
<th>Concentrations in tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>eH</td>
<td>eC</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[3H]Uridine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[14C]Orotic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uridine</td>
<td>8,320</td>
<td>25</td>
</tr>
<tr>
<td>Uracil</td>
<td>740</td>
<td>36</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[3H]Uridine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[14C]Orotic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uridine</td>
<td>8,080</td>
<td>26</td>
</tr>
<tr>
<td>Uracil</td>
<td>730</td>
<td>35</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[3H]UMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[14C]Orotic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UMP</td>
<td>3,680</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Uracil</td>
<td>4,600</td>
<td>24</td>
</tr>
<tr>
<td>Experiment 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[3H]UMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[14C]Orotic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UMP</td>
<td>3,900</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Uracil</td>
<td>4,300</td>
<td>48</td>
</tr>
<tr>
<td>Uracil</td>
<td>720</td>
<td>50</td>
</tr>
<tr>
<td>Experiment 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[3H]Uridine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[14C]Uracil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uridine</td>
<td>7,600</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Uracil</td>
<td>1,500</td>
<td>7900</td>
</tr>
</tbody>
</table>

Fig. 1. Intracellular compartmentalization of UMP, uridine, and uracil in rat liver. The model illustrates the bicompartmental distribution of the pyrimidines detected during experiments in which 3H- and 14C-labeled uridine, UMP, uracil, and orotic acid were superfused over rat liver slices. One of these compartments was identified with the plasma membrane. The numbers associated with the arrows indicate the fraction of perfused UMP (or uridine) involved in each of the processes studied under the experimental conditions used (Krebs-Ringer bicarbonate buffer saturated with 95% O2-5% CO2, containing 1 mg per ml of glucose, 2 ng per ml of UMP, and 50 ng per ml of uridine). The numbers in parentheses are calculated assigning a value of 100% to the rates at which the exogenous compounds reach the cell surfaces from the medium. OA, orotic acid; Urd, uridine; Ura, uracil.

not influence the isotope ratios of the compound isolated from the washed tissue slices.

Complete conversion of UMP to uridine in the cell membrane is not surprising in view of the high specific activity of 5'-nucleotide found in the plasma membrane during centrifugal fractionation of rat liver homogenates (6-9).

The model indicates that no UMP is released from inside the cell to the medium as UMP. The evidence supporting this conclusion derives from results obtained from experiments in which a mixture of [3H]UMP and [14C]orotic acid, or a mixture of [3H]-UMP and [14C]uridine were perfused. In all of these experiments, the UMP isolated from the tissue was labeled both with 3H and 14C, but the UMP isolated from the perfusate contained only 3H (Tables I and II).

Evidence for the conversion of uridine to uracil in the cell membrane derives from experiments in which a mixture of [14C]uridine and [3H]orotic acid or a mixture of [3H]-UMP and [14C]orotic acid were perfused. In these experiments, the isotope ratios in uracil isolated from the perfusate and the tissue were different (Table II). This result indicates that uracil originates in two different compartments in the cell, most likely representing uridine distributed either in the plasma membrane or inside of the cell.

It appears from the results of Canelakis et al. (10) that all the catabolism of uracil is effected by soluble enzymes which are not present in the membrane. He demonstrated that whole slices of rat liver and unfortified supernatant fractions metabolize uracil equally well.

The conversion of uracil to UMP occurring inside the cell appears to involve uridine as an obligatory intermediate in mammalian liver (11, 12).

Estimation of Rates of Uptake of Exogenous Uridine and UMP

The fractions of the perfused tracers involved in some of the processes represented in the model were calculated from experimental data, as follows.

UMP from Medium to Cell Membranes (a)

Since, as shown in the model, exogenous UMP reaching the cells is converted completely to uridine and does not return to the medium as UMP, the fraction of perfused UMP bypassing the tissue equals the ratio of the concentration of tritiated UMP
in the perfusate and in the perfusion medium. Therefore, the fraction of perfused UMP reaching the cell membrane is
\[ a = \left( \frac{c_{\text{UMP}}^{3H}}{c_{\text{UMP}}^{14C}} \right) \left( \frac{c_{\text{UMP}}^{3H}}{c_{\text{UMP}}^{14C}} \right) = 1 - \left( \frac{c_{\text{UMP}}^{3H}}{c_{\text{UMP}}^{14C}} \right), \]
where \( c_{\text{UMP}}^{3H} \) is the concentration of \(^{3H}\)UMP in the perfusion medium, and \( c_{\text{UMP}}^{14C} \) denotes the concentration of the tracers in the perfusate. The value of this fraction depends upon the amount of tissue perfused, the thickness of the slices, the flow rate, and the geometry of the perfusion chamber. Assuming that the number of molecules reaching the cells is proportional to the concentration of the compound in the perfusion medium, and to the weight of tissue, the following equations are valid:
\[ a = \frac{w_{\text{tissue}}}{w_{\text{tissue}}} \left( \frac{c_{\text{UMP}}^{3H}}{c_{\text{UMP}}^{14C}} \right), \]
where \( w_{\text{tissue}} \) is the weight of tissue, and \( \phi \) is the flow rate. Therefore, at a constant flow rate and within certain limits of variation of \( w \), the fraction, \( a \), should be proportional to the weight of tissue.

In the series of experiments shown in Table 1, 300 mg of tissue was used and the average value found for the fraction, \( a \), was 0.50. Therefore, a value of \( a = 0.66 \) would be expected if 400 mg of tissue were perfused, in agreement with the average of 0.62 obtained experimentally (Experiments 3 and 4, Table I).

**Uridine from Medium to Cell Membrane**

If the fraction, \( a \), is determined by the number of perfused molecules of UMP reaching cell surfaces, the same value could be assigned to the fraction of perfused uridine reaching the cells, since the diffusion coefficients of uridine and UMP in the buffer solution can be expected to be similar.

**Uridine (or UMP) Released from inside Cells to Medium as Uridine (c)**

According to the model, the amount of labeled uridine to be found in the perfusate when labeled uridine is perfused originates from three sources: uridine bypassing the tissue, uridine released from the membrane back to the medium without entering the cell, and uridine released to the medium from inside the cells. The amount of \(^{3H}\)uridine in the perfusate derived from the last source can be estimated when \(^{14C}\)orotic acid is added to the perfusion medium. In this case, uridine inside the cells released to the medium can be expected to have the isotope ratio found in uridine isolated from the tissue at the end of the perfusion. Therefore, as described in a previous publication (3),
\[ c' = \left( \frac{^{3H}}{^{14C}} \right)_{\text{uridine, tissue}} \times \left( \frac{c_{\text{UMP}}^{3H}}{c_{\text{UMP}}^{14C}} \right). \]
The average value for \( c' \) obtained from Experiments 1 and 2 (Table I) is 0.0011.

Since the fraction of perfused uridine or UMP reaching the cell is expected to be the same, and the conversion of UMP to uridine in the cell membrane was found to be complete, a similar value for \( c' \) can be expected from experiments in which \(^{3H}\)UMP and \(^{14C}\)orotic acid are perfused, if the following expression is used:
\[ c' = \left( \frac{^{3H}}{^{14C}} \right)_{\text{uridine, tissue}} \times \left( \frac{c_{\text{UMP}}^{3H}}{c_{\text{UMP}}^{14C}} \right). \]
The average value for \( c' \) obtained from Experiments 3 and 4 is 0.0009.

Therefore, it can be concluded that only a very small fraction of perfused uridine or UMP returns as uridine to the medium from inside the cell.

**Uridine from Membrane to Medium (d)**

Since the contribution of labeled uridine from inside the tissue to uridine in the perfusate was found to be negligible, the fraction of perfused uridine which is released back into the medium from the membrane can be easily calculated by subtracting from the total fraction of perfused uridine which appears in the perfusate \( \left( \frac{c_{\text{uridine}}^{3H}}{c_{\text{uridine}}^{14C}} \right) \) the fraction that bypasses the tissue completely \( (1 - a) \). Thus, in experiments in which \(^{3H}\)uridine was perfused, \( d = \left( \frac{c_{\text{uridine}}^{3H}}{c_{\text{uridine}}^{14C}} \right) - (1 - a) \). The values of \( d \) calculated from Experiments 1 and 2 (Table II) were 0.46 and 0.44, respectively.

The fraction, \( d \), was also estimated from experiments in which \(^{3H}\)UMP was perfused. According to the model, the fraction, \( d \), will equal the ratio of tritiated uridine in the perfusate and tritiated UMP perfused, i.e., \( d = \left( \frac{c_{\text{uridine}}^{3H}}{c_{\text{UMP}}^{3H}} \right) \). The values for \( d \) estimated from Experiments 3 and 4 were 0.45 and 0.43, respectively. The average of these values and of those obtained from perfusion of \(^{3H}\)uridine is 0.45.

**Uridine (or UMP) Released to Medium as Uracil**

**Total (c)**—The calculation of this fraction is straightforward:
\[ e = \frac{c_{\text{uracil}}^{14C}}{c_{\text{uridine}}^{14C}} \]
when \(^{3H}\)uridine is perfused and \( e = \frac{c_{\text{uracil}}^{14C}}{c_{\text{UMP}}^{3H}} \) when \(^{3H}\)UMP is perfused. The values of \( e \) from Experiments 1, 2, and 4 were 0.074, 0.073, and 0.072, with an average of 0.073.

**From Uridine inside Cells (g)**—Since any uracil in the perfusate originating from uridine inside the cells would have the same isotope ratio as uridine isolated from the tissue, it follows that \( \left( \frac{^{3H}}{^{14C}} \right)_{\text{uridine, tissue}} \) multiplied by the concentration of \(^{14C}\)uracil in the perfusate equals the concentration of \(^{3H}\)uracil in the perfusate that derives from uridine inside the cells. Therefore,
\[ g = \left( \frac{^{3H}}{^{14C}} \right)_{\text{uridine, tissue}} \times \left( \frac{c_{\text{uracil}}^{14C}}{c_{\text{uridine}}^{14C}} \right). \]
When \(^{3H}\)UMP and \(^{14C}\)orotic acid are perfused, the corresponding formula is:
\[ g = \left( \frac{^{3H}}{^{14C}} \right)_{\text{uridine, tissue}} \times \left( \frac{c_{\text{uracil}}^{14C}}{c_{\text{UMP}}^{3H}} \right). \]
The values from Experiments 1, 2, and 4 are 0.0013, 0.0017, and 0.0013 with an average of 0.0015. Therefore, since \( g \) is much smaller than \( e \), it can be concluded that the bulk of the uracil appearing in the medium during perfusion of uridine or UMP results from the conversion of uridine to uracil in the cell membrane.

**Uridine (or UMP) to Uracil in Cell Membranes (b)**

Since uracil appearing in the perfusate during perfusions of uridine or UMP is almost exclusively derived from the conversion of uridine to uracil in the cell membrane, it follows that \( b \) should be equal to the total fraction of perfused uridine or UMP converted to uracil in the perfusate \( (c) \) plus the fraction of uracil formed in the cell membrane that is not released to the medium \( (f) \). The fraction \( f \) was estimated by perfusing \(^{14C}\)uracil over tissue slices. Thus, \( f = \left( \frac{c_{\text{uracil}}^{14C}}{c_{\text{uridine}}^{14C}} \right) / \left( c_{\text{uracil}}^{14C} \times c_{\text{uridine}}^{14C} \right) \). The results of Experiment 5 (Table II) indicate that \( f = 0.34 \). Therefore, \( b = 0.11 \).

**Uridine (or UMP) Entry as Uridine into Cells (c)**

The fraction of perfused uridine reaching the cell surfaces \( (a) \) is equal, according to the model in Fig. 1, to the sum of the fractions converted to uracil in the membrane \( (b) \), the fraction returned to the medium as uridine \( (d) \), and the fraction entering the cell as uridine \( (c) \), i.e., \( c = a - b - d \). The release of uridine from inside the cells back into the medium is considered to be negligible. From the average values of \( a, b, \) and \( d \) given above, it can be estimated that \( c = 0.06 \).

The estimated values for the fractions of perfused uridine and UMP corresponding to processes of uptake and metabolism are indicated in the model in Fig. 1. These values depend upon the experimental conditions, e.g., flow rate, and amount of tissue perfused. Upon dividing these numbers by the fraction of the perfused tracer reaching the cells \( (n) \), the fate of exogenous uridine or UMP is more clearly seen. These values are shown in parentheses in Fig 1.
erotic acid inside the cells. Uridine, formed in the cell, is recognized in superfusions of tissue slices with the same mixture of tracers, the 3H:14C ratio was higher in uridine isolated from the perfusate than in uridine recovered from the tissue. This confirms the previous conclusion that uridine is compartmentalized in the liver for 2 hours at room temperature, are shown in Table III. Before excision, the liver was flushed in situ with heparinized saline through the portal vein while the pentobarbital anesthetized rat was exsanguinated through the vena cava. As it was observed in superfusions of tissue slices with the same mixture of tracers, the 3H:14C ratio was higher in uridine isolated from the perfusate than in uridine recovered from the tissue. This result confirms the previous conclusion that uridine is compartmentalized in the hepatocyte. [3H]Uridine formed in the cell membrane exchanges poorly with [14C]uridine formed from [14C]orotic acid inside the cells.

### TABLE III

<table>
<thead>
<tr>
<th>Perfusate (cpm/ml)</th>
<th>UMP</th>
<th>H</th>
<th>C</th>
<th>H:14C</th>
<th>Uridine</th>
<th>H</th>
<th>C</th>
<th>H:14C</th>
<th>Uracil</th>
<th>H</th>
<th>C</th>
<th>H:14C</th>
</tr>
</thead>
<tbody>
<tr>
<td>60-80 min</td>
<td>210</td>
<td>&lt;0.5</td>
<td>&gt;500</td>
<td></td>
<td>2100</td>
<td>210</td>
<td>10</td>
<td></td>
<td>650</td>
<td>47</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>80-100 min</td>
<td>250</td>
<td>&lt;0.5</td>
<td>&gt;500</td>
<td></td>
<td>1900</td>
<td>300</td>
<td>5.3</td>
<td></td>
<td>630</td>
<td>78</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>100-120 min</td>
<td>270</td>
<td>&lt;0.5</td>
<td>&gt;500</td>
<td></td>
<td>1800</td>
<td>440</td>
<td>4.1</td>
<td></td>
<td>500</td>
<td>82</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>Tissue (cpm/g)</td>
<td>570</td>
<td>1330</td>
<td>0.43</td>
<td></td>
<td>3300</td>
<td>350</td>
<td>0.94</td>
<td></td>
<td>150</td>
<td>42</td>
<td>0.36</td>
<td></td>
</tr>
</tbody>
</table>

Table IV. Normalized isotopic data from perfusion of whole liver with [3H]UMP and [14C]orotic acid.

<table>
<thead>
<tr>
<th>Perfusion time</th>
<th>Concentration of [3H]uridine in tissue</th>
<th>Experiments A</th>
<th>Experiments B</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td></td>
<td>(3H:14C=250,000 cpm/ml, 1 orotic acid per ml)</td>
<td>(14C=2,000,000 cpm/ml, 100 orotic acid per ml)</td>
</tr>
<tr>
<td>20</td>
<td>5,900</td>
<td>12,100</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>4,100</td>
<td>10,900</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>5,170</td>
<td>12,000</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>7,650</td>
<td>11,200</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>6,400</td>
<td>11,100</td>
<td></td>
</tr>
</tbody>
</table>

(about 2 to 50 ng per ml, respectively, in this series of experiments).

### Whole Liver Perfusion

The isotopic data from an experiment in which a mixture of [3H]UMP and [14C]orotic acid in Krebs-Ringer bicarbonate-glucose buffer was perfused through the portal vein of an excised rat liver for 2 hours at room temperature, are shown in Table III. Before excision, the liver was flushed in situ with heparinized saline through the portal vein while the pentobarbital anesthetized rat was exsanguinated through the vena cava. As it was observed in superfusions of tissue slices with the same mixture of tracers, the 3H:14C ratio was higher in uridine isolated from the perfusate than in uridine recovered from the tissue. This result confirms the previous conclusion that uridine is compartmentalized in the hepatocytes. [3H]Uridine formed in the cell membrane exchanges poorly with [14C]uridine formed from [14C]orotic acid inside the cells.

### System Testing

In order to obtain evidence for the achievement of isotopic steady state during the superfusions, concentrations of labeled compounds were measured in at least three fractions of perfusate during the last hour, in all experiments. No significant differences between these values and their average were noted. Furthermore, tissue slices were superfused in parallel for different lengths of time with [3H]uridine and the intracellular concentrations of [3H]uridine were measured at the end of each perfusion. Experiments were performed using two different concentrations of uridine in the perfusion medium. The results are shown in Table IV. It can be seen that an isotopic steady state is reached after 20 min of perfusion, both at a low and a high concentration of perfused uridine. The presence of enzymatic activities in the perfusate was investigated. For this purpose, slices of rat liver were superfused with buffer under conditions identical with those used in other experiments and 1 ml samples of perfusate collected at various times were incubated for 30 min with [14C]uridine and [3H]UMP. The concentrations of labeled uridine and UMP in the medium at the end of the incubation were measured after addition of carriers. No isotope exchange was noted in samples of perfusate taken after 40 min of superfusion.

To test for the adequacy of the washing procedure to remove contaminating medium from the tissue, slices were superfused with [3H]UMP and [14C]uridine for 2 hours, transferred rapidly to a MiraCloth filter (Chicopee Manufacturing Co.), and washed with cold buffer at a rate of 18 ml per min for 8 min. Eight fractions were collected. With the exception of the 3H:14C ratio in uridine isolated from the first 3-ml fraction, the isotope ratios in all other fractions were not significantly different from that found in uridine in the thoroughly washed tissue.

To estimate the extent of metabolism of uridine during the washing procedure, a kinetic study of the conversion of uridine to uracil by an homogenate of 4 g of rat liver in 18 ml of oxygenated Krebs-Ringer bicarbonate buffer, containing 1 mg of glucose per ml, was conducted. A cooled all glass homogenizer was used, [3H]uridine (300,000 cpm per ml, 0 ng per ml) was incubated with this homogenate at 37° with shaking, samples were taken at various intervals, and the reaction was stopped by addition of 4 volumes of methanol containing uridine and uracil carrier. The results of this kinetic study are shown in Fig. 2. It is of interest to note that the intersection of the concentration curves of labeled uridine and uracil occurs at a time after the beginning of the incubation equal to the half-life of uridine (\(t_{1/2} = 7.9\ min\)). This is the result to be expected if the only reaction involving uridine were its conversion to uracil and if uracil were not further metabolized. In this case, the concentrations of product and precursor are identical when half of the initial concentration of the precursor is converted to the product. The usefulness of this relation in evaluating the existence of consecutive and simultaneous reactions is apparent.

Constancy of oxygen consumption by the tissue slices during the superfusion period was demonstrated by measuring oxygen levels in samples of perfusion medium and perfusate taken at various intervals. In collaboration with Mr. Luis Escarcena, University of Minnesota, a special all stainless steel glass superfusion apparatus was designed to eliminate losses of O2 in Teflon. Special care was placed to remove air bubbles trapped in the system. Measurements of oxygen were performed with an Astrup A-1 meter (Radiometer, Leeds Co., Cleveland, Ohio).

### DISCUSSION

On the basis of an estimate of the amount of perfused [3H]uridine that enters the cell, the endogenous production of uridine...
was determined. For this purpose, two equal portions of tissue slices were perfused in parallel under identical conditions. One of these portions was superfused with [3H]uridine and the intracellular concentration of the tracer (c_{uridine}) was determined at the steady state. The other portion was superfused with unlabeled uridine at the same concentration used in the parallel run (2 ng per ml) and the intracellular concentration of uridine (c_{uridine}) was measured by a double isotope method described elsewhere (1). Combining the results from these two experiments, it was possible to estimate the steady state specific activity of intracellular uridine that would result during perfusion of [3H]uridine. Therefore, the production rate (PR) of endogenous uridine per gram of tissue was calculated, as follows

\[
P_{\text{uridine}} = \frac{\text{rate of entry of uridine into cells}}{\text{specific activity of intracellular uridine} \times \text{weight of tissue}}
\]

\[
= \frac{c \times \phi \times \frac{\text{cpm/mL}}{\text{uridine}}}{\frac{\text{cpm/mL}}{\text{uridine}} \times \frac{\text{mg}}{\text{g tissue}}} \times \frac{\text{g}}{\text{mL}} \times \frac{\text{mg}}{\text{g}}
\]

\[
= \frac{0.06 \times 20 \text{ (ml/hour)} \times 10,000 \text{ (cpm/ml)}}{3250 \text{ (cpm/g tissue)} / 0.5 \text{ (g tissue)} \times 0.5 \text{ (g)}}
\]

\[
= 75 \text{ (cpm/g tissue) / hour)}
\]

This estimate, however, is only approximate since it depends heavily on the fraction of labeled uridine entering the cell (c). This fraction is calculated as a difference between other fractions and may be affected by considerable errors.

It is also of interest to calculate the fraction of perfused orotic acid that is converted to uridine, m. The [3H] uridine obtained when [3H]orotic acid (or [3H]UMP) and [14C] orotic acid are perfused, is determined by the ratio of the fractions of each of these tracers appearing in uridine inside the cells, i.e. (\text{[3H]}: \text{[14C]})_{\text{uridine}} = c/m. In Experiments 1, 2, 3, and 4 the average (\text{[3H]}: \text{[14C]}) ratio in uridine isolated from the tissue was 0.37. Therefore, the fraction of perfused orotic acid converted to uridine (m) is

\[
m = \frac{c}{(c^{[3H]} + c^{[14C]})_{\text{uridine}}}
\]

The value of a in model in Fig. 1 can be interpreted to represent the fraction of tracer reaching the cell surfaces by diffusion. This interpretation implies that all exogenous UMP coming in contact with the cells is taken up and metabolized. It also allows to use the same value of a for different tracers present in the medium. The following argument supports this interpretation. Consider that a fraction x of perfused UMP reaches the cell surfaces but is returned to the medium. Then, (c_{uridine}^{[3H]} + c_{uridine}^{[14C]})^{[3H]} = 1 - a + x + d. In the same system, when [3H]uridine is perfused, it can be assumed that an identical fraction a, reaches the cell surfaces and has the same metabolic fate as uridine formed in the membrane from UMP. Then, c_{uridine}^{[3H]} / c_{uridine}^{[14C]} = 1 - a + d. Since the two experimental expressions described above were found not to be significantly different (Tables I and II), it follows that x = 0.

The results shown in Fig. 1 indicate that a large fraction of uridine is returned from the membrane to the medium without reaching the cell, and that uridine is converted to a large extent to uracil in the membrane. The presence of this enzymatic activity in plasma membranes of rat liver cells was demonstrated with a preparation, homogenous by electron microscopy, obtained according to the procedure described by Ray (13). It was found that thoroughly washed membranes incubated with [3H]uridine in Krebs-Ringer bicarbonate buffer yielded [3H]uracil.

The enzymatic activity was much lower in the membrane than in the original homogenate, both in absolute amount and specific activity. However, because of spatial considerations, this finding is compatible with the prominent role that the membrane phosphorlylase plays in the metabolism of exogenous uridine or uridine derived from exogenous UMP, as postulated in this article. It should be recalled that only a small fraction of uridine originating from these sources enters the cell. The results also show that conversion of UMP to uridine occurs not only in the membrane, but inside the cell as well. This finding does not necessarily contradict the concept of 5'-nucleotidase as a marker for plasma membrane since the specific activity of the enzyme in this fraction is much larger than in all other fractions (8).

The usefulness of experimental designs involving superfusion of tracers over tissue slices or cell suspensions and intracellular labeling of compounds deserve emphasis. Among other advantages, superfusion tracer techniques provide isotopic steady state data from which quantitative estimates of rates of metabolism and movement of compounds across membranes can be obtained (3, 14).

The experimental design proposed here can be used to study the dependence of the values shown in Fig. 1 upon several variables such as concentrations of perfused uridine and UMP, or addition of other nucleosides and nucleotides, plasma proteins, and hormones to the medium. Studies of this type can be expected to yield information about the processes taking place in the cell membrane. The results can also be useful to interpret properly data on incorporation of radioactive uridine into nucleic acids, a technique often used to estimate rates of RNA synthesis without adequate consideration of changes in the labeling of intracellular nucleotide pools.

Acknowledgments—The excellent technical assistance of Mrs. Constance Tolef is gratefully recognized. Mr. Luis Escarecia conducted the measurements of oxygen consumption.

---

1 J. K. Tseng and E. Gurpide, unpublished results.
REFERENCES

10. Canellakis, E. S. (1956) J. Biol Chem 221, 315
Compartmentalization of Uridine and Uridine 5'-Monophosphate in Rat Liver Slices
John K. Tseng and Erlio Gurpide