Effect of 5-Diazouracil on the Catabolism of Circulating Pyrimidines in Rat Liver and Kidneys

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ABSTRACT

The inhibitory effect of 5-diazouracil on the catabolism of circulating uracil and 5-fluorouracil was examined in the rat in vivo. Measurements of the activity of the entire enzymatic pathway of uracil catabolism in the cytosolic supernatant of different rat organs as well as the determination of the total amount of 5-fluorouracil catabolites, accumulated in these tissues, served to clarify their role in the complete systemic breakdown of uracil or 5-fluorouracil. The activity of the enzymatic pathway involved in uracil catabolism was estimated from the 4CO2 produced from [2-14C]uracil in the cytosolic supernatants. Complete degradation of uracil was detected only in the liver and, at a much lower rate, in the kidneys.

Fifteen min after the i.p. injection of a tracer dose of 5-fluoro[6-14C]uracil, more than 90% of the total radioactivity in blood plasma was associated with 5-fluorouracil catabolites. The relative amount of the major catabolite α-fluoro-β-alanine and of dihydrofluorouracil in blood plasma was considerably suppressed after a pretreatment with 5-diazouracil inversely correlated with a 27-fold increase in the absolute amount of unchanged 5-fluorouracil.

Control animals accumulated by far the highest amount of total acid-soluble radioactivity from 5-fluoro[6-14C]uracil in liver and kidneys. Total radioactivity in all other organs was much lower and was comparable to the amount of label in blood plasma. In liver and kidneys, the sum of total acid-soluble catabolites including dihydrofluorouracil, α-fluoro-β-ureidopropionic acid, and α-fluoro-β-alanine made up more than 98% of the label correlating with minimal salvage utilization of the base analogue in both organs.

Injection of 5-diazouracil 2 h before a tracer dose of 5-fluoro[6-14C]uracil strongly inhibited the accumulation of labeled catabolites in liver and kidneys causing a fall in total acid-soluble radioactivity in both tissues by 75 and 66%, respectively. In blood plasma and all other organs, however, pretreatment with 5-diazouracil was followed by a 2.5-fold enhancement of the radioactivity contents, mostly due to the appearance of unchanged 5-fluorouracil. Under these conditions, there was a 2.6- to 4-fold increase in the relative proportion of cis-diol group-containing anabolics of 5-fluorouracil in liver and in kidneys.

Within 2 h, 12.7% of the administered radioactivity from 5-fluorouracil was excreted into bile. 5-Diazouracil lowered the biliary excretion of radioactivity to 2% of the injected dose.

Our studies indicate a strong inhibition of the catabolism of 5-fluorouracil and uracil by 5-diazouracil in the rat in vivo. Complete degrada-
tion of circulating pyrimidine bases was restricted to rat liver and kidneys, with the major part occurring in liver. Inhibition of the catabolic pathway by 5-diazouracil considerably increased the bioavailability of 5-fluorouracil.

INTRODUCTION

In mammalian cells the requirements for pyrimidine nucleotides are satisfied by synthesis de novo as well as by nucleotide formation from preformed pyrimidines in the salvage pathway (1). Micromolar concentrations of pyrimidines in blood plasma represent the extracellular source for salvage nucleotide synthesis in many tissues (1–4). For a certain species, salvage activities are different for circulating uridine, cytidine, thymidine, uracil, or pyrimidine deoxyribonucleosides and markedly vary from one type of tissue to the other (2, 4). The chemotherapeutic efficiency of the antimetabolite FURA1 depends on its conversion to active FURA nucleotides via the salvage pathway (5). However, the salvage utilization of uridine (2), uracil (4, 6, 7), and FURA (8, 9) often is low under in vivo conditions. Mammalian cells do not possess a specific uracil phosphoribosyltransferase activity allowing conversion of uracil into UMP in one enzymatic step (10). Moreover, at physiological pH, uracil in contrast to FURA is only a poor substrate for orotate phosphoribosyltransferase (EC 2.4.2.10) (10, 11). In vivo, uridine phosphorylase (EC 2.4.2.3) appears to be primarily involved in uridine catabolism rather than in nucleoside formation from uracil or FURA (11). Accordingly, most of the uracil originating from uridine phosphorolysis or of the exogenously supplied FURA is catabolized or excreted in urine (6–9).

The activity of the first step in enzymatic uracil or FURA degradation, dihydrouracil dehydrogenase (EC 1.3.1.2), has been demonstrated in several tissues and tumors of humans (12) and rats (13–16). Dihydropyrimidinase (EC 3.5.2.2) is the subsequent enzyme in pyrimidine base degradation and has been located in liver and kidneys of rats (17). It is absent from or showed only little activity in normal extrahaemoplastic human tissues but was highly active in several solid tumors (12). The last enzyme of the catabolic pathway is β-ureidopropionase (EC 3.5.1.6) which demonstrates a similar distribution pattern like dihydropyrimidinase in both species (12, 14). In normal tissues of the rat, the complete enzymatic equipment for uracil degradation thus was detected only in liver (12–14, 18–25) and in kidneys (12).

Many attempts have been made to improve the chemotherapeutic effectiveness of FURA by its combination with other antitumor agents (26) or with naturally occurring pyrimidines (27–30). Protection of FURA from catabolism has been shown to be a major effect of its combination with high doses of thymidine (31) leading in some tumors to potentiation of its antineoplastic activity. 5-Diazouracil is an irreversible inhibitor of dihydrouracil dehydrogenase (32, 33) and has been used to increase the incorporation of FURA into RNA of rat leukemia cells and of normal tissues (21). The aim of our study was to further clarify the role of rat liver and kidneys in the catabolism of FURA or uracil and to investigate the effect of a prior dose of 5-diazouracil on the metabolic profile of FURA in blood plasma, on the total FURA catabolite contents in different tissues, and on the biliary excretion of radioactive after administration of [6-14C]FURA.

MATERIALS AND METHODS

Chemicals and Isotopes

[2-14C]Uracil (52 Ci/mol) and 5-fluoro[6-14C]uracil (56 Ci/mol) were from Amersham Buchler (Braunschweig, Federal Republic of Germany). Radiochemical purity of the isotopes was controlled by reversed phase HPLC and by counting of the eluate fractions collected every 20

1The abbreviations used are: FURA, 5-fluorouracil; HPLC, high performance liquid chromatography.
s. Purity was at least 95% for each of the isotopes. FUra and 5-diazouracil were obtained from Sigma Chemical Co. (St. Louis, MO) and from ICN Pharmaceuticals (Plainview, NY), respectively. Bases and nucleosides were purchased from Boehringer Mannheim (Mannheim, Federal Republic of Germany). α-Fluoro-β-alanine and dihydrofluorouracil were kindly supplied by Hoffmann-La Roche (Basel, Switzerland). All other chemicals were of the highest purity available from E. Merck AG (Darmstadt, Federal Republic of Germany).

Preparation of Tissue Extracts

Female Wistar rats (Ivanovas, Kisslegg, Federal Republic of Germany), weighing 150 to 180 g, had free access to water and a carbohydrate-rich 20% protein diet (Altromin, Lage, Federal Republic of Germany). 5-Diazouracil (5 mg/kg body weight) (21) usually was administered i.p. 2 h before the injection of radioactive pyrimidines.

The different organs used for analysis of the complete pathway of uracil catabolism were quickly removed under light pentobarbital anesthesia (45 mg/kg body weight) and homogenized at 4°C with a motor grinder. The homogenate was first centrifuged at 30,000 x g for 30 min at 4°C and the supernatant was recentrifuged at 105,000 x g for 1 h at 4°C. The 105,000 x g supernatant fluid (cytosol) was used to estimate the complete catabolic pathway (14). The assay was run in duplicate under conditions where the activity was linear with time and enzyme concentration. The complete degradation of uracil was measured by the production of 14CO2 from [2-14C]uracil (0.5 μCi; 0.5 μCi/ml) (14) using a procedure for quantitating labeled CO2, which has been described recently (24). The reaction was stopped by the addition of 0.1 ml 2.5 M perchloric acid. Incubation at 37°C was continued for 45 min before the radioactivity trapped in 1 ml 1 M KOH was determined. Determination of protein concentration was carried out by the method of Bradford (34) using γ-globulin as a standard.

For metabolic studies, the different rat organs were freeze-clamped, extracted with cold perchloric acid, and neutralized with KHCO3 as described (35). Aliquots of the homogenate or the acid-soluble supernatant were counted in Instant Scint Gel (Packard Instrument Company, Downers Grove, IL) at an efficiency of 79%. The neutralized acid-soluble supernatants were used for metabolite analyses. Blood was withdrawn from the portal vein with heparinized syringes, immediately followed by centrifugation and ultrafiltration through Amicon MPSI filters (Amicon, Witten, Federal Republic of Germany).

Phenylboronate Affinity Chromatography. Phenylboronate affinity chromatography allowed the separation of pyrimidine bases and their catabolites from anabolites present in the neutralized acid-soluble supernatants of different organs and 2.5 M ammonium acetate (3, 36). The sample was injected onto two C18-Bondapak columns (600 x 3.9 mm) connected in series with a Guard-PAK precolumn module containing a C18-Bondapak insert (Waters, Königstein, Federal Republic of Germany). The eluent was composed of 0.01 M NH4H2PO4 adjusted to pH 5 and 6% methanol (v/v). The flow rate was 1 ml/min at 35°C. The isocratic HPLC system was from Du Pont De Nemours (Bad Nauheim, Federal Republic of Germany). Fractions of the eluate were collected every 10 or 20 s and counted in Instant Scint Gel. Recovery of FUra after affinity chromatography and HPLC was 85%.

Blood plasma was deproteinized by ultrafiltration and directly injected onto the HPLC columns allowing the separation of labeled 5-fluorouracil, dihydrofluorouracil, α-fluoro-β-alanine, and possibly α-fluoro-β-ureidopropionate associated with a fourth labeled minor peak. Except for the latter peak, standards were available which demonstrated identical retention times as compared to the labeled catabolites, when UV absorbance was recorded at 200 nm. Under the conditions defined above, retention times of the unlabeled markers, α-fluoro-β-alanine, dihydrofluorouracil, and FUra were 5.51 ± 0.06, 7.48 ± 0.07, and 8.25 ± 0.08 min, respectively (mean of 5 separations ± SD).

Collection of Bile

The peritoneal cavity of female Wistar rats (240 ± 32 g) was opened after tracheotomy under pentobarbital anesthesia. A Teflon cannula (outer diameter, 0.9 mm; inner diameter, 0.5 mm) was introduced into the bile duct. Bile was collected over 10-min periods for up to 2 h after [6-14C]FUra (8 μCi/250 g rat) had been injected into the superior mesenteric artery. The samples were weighed and counted. Bile flow remained constant in either the presence (131 ± 54 mg/10 min) or the absence (148 ± 35 mg/10 min) of a prior dose of 5-diazouracil (5 mg/kg).

RESULTS

Localization of the Entire Pathway of Uracil Catabolism. The activity of the entire pathway of uracil catabolism including dihydrouracil dehydrogenase, dihydropyrimidinase, and β-ureidopropionate was measured in the cytosol of spleen, lungs, thymus, brain, intestine, kidney, and liver via the formation of radioactive CO2 from [2-14C]uracil (14). The highest activity of the pathway was found in the liver of rats [52.8 ± 3.8 nmol CO2 x (mg protein)-1 x h-1]. The kidneys possessed 10% of the catabolic activity detected in the liver (5.3 ± 0.9 nmol CO2 x (mg protein)-1 x h-1). All other tissues tested did not possess the complete degradative pathway of uracil leading to the release of CO2.

Effect of 5-Diazouracil on the Metabolite Pattern of Labeled FUra in Blood Plasma. Total radioactivity in blood plasma 15 min after a tracer dose of [6-14C]FUra was increased 1.7-fold after pretreatment of the rats with 5-diazouracil as compared to control rats (Table 1). Two h after FUra (100 μCi/kg; 0.26 Ci/mol) there was still a 2-fold rise in total radioactivity when the animals had received a prior dose of 5-diazouracil. Catabolism of [2-14C]uracil does not lead to a labeling of β-alanine. Reversed phase HPLC of deproteinized blood plasma revealed that the 5-fold increase in total radioactivity after administration of [2-14C]uracil (15 μCi/kg) and inhibition of uracil degradation (Table 1) was exclusively due to the presence of unchanged uracil (data not shown).

Fifteen min after injection of FUra 75.9 ±19% (n = 4) of the total radioactivity in blood plasma was associated with α-fluoro-β-alanine versus 7.6 ± 0.7% after 5-diazouracil pretreatment (Fig. 1). This inhibition of FUra catabolism was reflected by the increase in the relative amount of unchanged FUra in...
CATABOLISM OF CIRCULATING PYRIMIDINES

Table 1. Effect of 5-diazouracil on the elimination of pyrimidine-derived radioactivity from rat blood plasma.

<table>
<thead>
<tr>
<th>Pyrimidine</th>
<th>[6-14C]FUra (t = 15 min)</th>
<th>[6-14C]FUra (t = 2 h)</th>
<th>[2-14C]Uracil (t = 2 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pretreatment</td>
<td>19.0 ± 2.8^a</td>
<td>32.8 ± 3.8</td>
<td>2.8 ± 0.45</td>
</tr>
<tr>
<td>+ 5-DiazoUra</td>
<td>12.7 ± 1.1</td>
<td>14.6 ± 1.3</td>
<td></td>
</tr>
</tbody>
</table>

^a Time interval between pyrimidine injection and blood withdrawal.
^b Mean ± SD from 3–6 rats.

Fig. 1. Effect of 5-diazouracil (5-DiazoUra) on the metabolite pattern of [6-14C]FUra in rat plasma, liver, kidney, and intestine. Portal vein blood was deproteinized by ultrafiltration and used for reversed phase HPLC (36). FUra and its catabolites were purified from the acid-soluble supernatant of the organs by boron affinity gel chromatography and separated by reversed phase HPLC. The eluate from the columns was collected every 10 or 20 s and counted. Similar chromatograms were obtained from 4 rats. The value given on the ordinate must be multiplied by 10^3 (left) or 10^2 (right).

Fig. 2. Excretion of Radioactivity from [6-14C]FUra into Bile. The administration of [6-14C]FUra to rats was followed by the appearance of increasing amounts of radioactivity in bile during the first 20 min after injection of the label. At later time points the rate of biliary excretion of labeled metabolites slowly declined. Within 2 h 12.7% of the injected radioactivity was collected in bile. Prior inhibition of pyrimidine catabolism by 5-diazouracil considerably suppressed the label in bile to 2% of the administered dose.

DISCUSSION

FUra has been shown to be rapidly catabolized in the whole animal in vivo (8) and in isolated hepatocytes (23). Up to 50% of a hepatic arterial infusion (39) or 67% of an i.p. dose (40) of FUra is extracted in a single pass by the human liver. From these and other studies (6–8, 12–15, 18–25) it was concluded that the liver plays a key role in the degradation of uracil pyrimidines. The results from our studies in rats in vivo after administration of a tracer dose of [6-14C]FUra support the assumption that the liver is the primary site in FUra catabolism. As early as 15 min after injection of the label, total acid-soluble radioactivity in the liver accumulated to 440 ± 38 μCi/kg organ wet weight (Table 2) with more than 99% of the label associated with intact FUra. Total radioactivity was determined in heparinized and deproteinized blood plasma.

Radioactivity (μCi/liter)

Control

<table>
<thead>
<tr>
<th>Pyrimidine</th>
<th>No pretreatment</th>
<th>5-Diazouracil</th>
</tr>
</thead>
<tbody>
<tr>
<td>[6-14C]FUra (t = 15 min)</td>
<td>19.0 ± 2.8</td>
<td>12.7 ± 1.1</td>
</tr>
<tr>
<td>[6-14C]FUra (t = 2 h)</td>
<td>32.8 ± 3.8</td>
<td>14.6 ± 1.3</td>
</tr>
<tr>
<td>[2-14C]Uracil (t = 2 h)</td>
<td>2.8 ± 0.45</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD from 3–6 rats.

control blood plasma from 5.1 ± 1.4% (n = 4) to 88.4 ± 15.6% (n = 4) after 5-diazouracil. The absolute amount of the base was increased by a factor of 27 in pretreated rats (Table 1). Dihydrofluorouracil amounted to 15.1 ± 5.0% in control plasma and 2.9 ± 0.7% in pretreated rats (Fig. 1). Another minor radioactive peak which eluted between the two other FUra catabolites from the HPLC columns possibly represented α-fluoro-β-ureidopropionic acid.

Effect of 5-Diazouracil on the Sum of Total FUra Catabolites in Different Organs. Total acid-soluble radioactivity was measured in 6 different rat organs 15 min after i.p. administration of [6-14C]FUra (Table 2). Liver and kidneys accumulated by far most of the radioactivity injected. Spleen, intestine, lungs, and thymus contained similar amounts of label as were found in the liver and kidneys after 5-diazouracil resulted from a severe depletion of 5-fluorouracil catabolites paralleled by the appearance of the unchanged base in both organs (Fig. 1; Table 2). In addition to the experiments with FUra, we investigated the changes in [2-14C]Uracil metabolism in 5 different organs 2 h after administration of the labeled pyrimidine (Table 3). 5-Diazouracil prevented the loss of label via exhalation of radioactive CO₂ and caused an increase in total radioactivity of liver and kidneys including the label in unchanged uracil, in acid-soluble nucleotides, and in nucleic acids.

Excretion of Radioactivity from [6-14C]FUra into Bile. The administration of [6-14C]FUra to rats was followed by the appearance of increasing amounts of radioactivity in bile during the first 20 min after injection of the label (Fig. 2). At later time points the rate of biliary excretion of labeled metabolites slowly declined. Within 2 h 12.7% of the injected radioactivity was collected in bile. Prior inhibition of pyrimidine catabolism by 5-diazouracil considerably suppressed the label in bile to 2% of the administered dose.

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Table 2  Effect of 5-diazouracil on the contents of total acid-soluble radioactivity in different rat organs after [6-14C]FUra

<table>
<thead>
<tr>
<th>Organ</th>
<th>Acid-soluble supernatant</th>
<th>anabolites</th>
<th>catabolites</th>
<th>FUrA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µCi/kg</td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>440 ± 51</td>
<td>0.6</td>
<td>99.4</td>
<td>0</td>
</tr>
<tr>
<td>Kidney</td>
<td>275 ± 46</td>
<td>1.5</td>
<td>98.5</td>
<td>26</td>
</tr>
<tr>
<td>Spleen</td>
<td>12 ± 2</td>
<td>ND</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>Intestine</td>
<td>18 ± 2</td>
<td>ND</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td>Lungs</td>
<td>12 ± 2</td>
<td>ND</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Thymus</td>
<td>10 ± 2</td>
<td>ND</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

No pretreatment

<table>
<thead>
<tr>
<th>Organ</th>
<th>Acid-soluble supernatant</th>
<th>anabolites</th>
<th>catabolites</th>
<th>FUrA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µCi/kg</td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>110 ± 9</td>
<td>2.2</td>
<td>76.8</td>
<td>21</td>
</tr>
<tr>
<td>Kidney</td>
<td>93 ± 5</td>
<td>3.9</td>
<td>37.1</td>
<td>59</td>
</tr>
<tr>
<td>Spleen</td>
<td>30 ± 4</td>
<td>ND</td>
<td>13</td>
<td>87</td>
</tr>
<tr>
<td>Intestine</td>
<td>34 ± 11</td>
<td>ND</td>
<td>18</td>
<td>82</td>
</tr>
<tr>
<td>Lungs</td>
<td>24 ± 1</td>
<td>ND</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Thymus</td>
<td>26 ± 2</td>
<td>ND</td>
<td>18</td>
<td>82</td>
</tr>
</tbody>
</table>

+ 5-Diazouracil

* Mean ± SD of 4-5 rats are given.

Table 3  Increased radioactivity from [2-14C]uracil in different rat organs after 5-diazouracil pretreatment

Labeled uracil (15 µmol/kg; 3 Ci/mol) was injected into rats in the absence or presence of a pretreatment with 5-diazouracil. Radioactivity was analyzed in the homogenate of the different organs which had been freeze-clamped 2 h after [2-14C]uracil.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Radioactivity (µCi/kg, wet wt)</th>
<th>No pretreatment</th>
<th>+ 5-Diazouracil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.66 ± 0.86</td>
<td>11.48 ± 1.39</td>
<td>5-Diazouracil</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.35 ± 1.28</td>
<td>28.11 ± 2.77</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>1.62 ± 0.19</td>
<td>10.70 ± 0.94</td>
<td></td>
</tr>
<tr>
<td>Intestine</td>
<td>2.88 ± 0.90</td>
<td>14.64 ± 2.63</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>1.60 ± 0.47</td>
<td>9.36 ± 0.77</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SD of 5 rats.

Catabolism of circulating pyrimidines

[6-14C]FUra was injected i.p. at a dose of 5 µCi/150 g rat in the absence or presence of a prior dose of 5-diazouracil (5 mg/kg rat). Fifteen min after injection of the label, the different organs were freeze-clamped and homogenized. Alliquots of the neutralized acid-soluble supernatants were counted and analyzed for anabolites, retained by boronate affinity chromatography, for the unchanged FUra, and for the sum of FUra catabolites, including dihydrououracil, α-fluoro-β-ureidopropionic acid, and α-fluoro-β-alanine.

Table 3 shows that in each of the different organs, radioactivity was increased by a factor of 27. Under these conditions, the absolute amount of FUra in blood plasma strongly rose by a factor of 27. Inhibition by 5-diazouracil of dihydrofuroacil dehydrogenase which degrades FUra more efficiently than uracil in normal tissues and in tumors (12, 15, 16) thus represents a valuable approach in cancer chemotherapy (21) to improve the bioavailability of FUra.

A pretreatment with 5-diazouracil which has been shown to maximally inhibit dihydrououracil dehydrogenase in rat liver in vivo (21) considerably suppressed the amount of total radioactivity in liver and kidneys by 75 and 66%, respectively. This was in opposite to the effect on the total amount of label in blood plasma. In addition, total acid-soluble radioactivity in these organs after injection of [2-14C]uracil or [6-14C]FUra did not differ significantly from the amount of label in blood plasma.

The rapid catabolism of FUra in liver and kidneys was reflected by a high concentration of α-fluoro-β-alanine in blood plasma as early as 15 min after injection of the base analogue. In human blood plasma also dihydrofuroacil (44), α-fluoro-β-alanine, and presumably α-fluoro-β-ureidopropionic acid were detected using 19F nuclear magnetic resonance (45). The increase in blood plasma catabolites including the cytotoxic dihydrofuroacil (46) was almost completely prevented by pretreatment with 5-diazouracil (Fig. 1). Under these conditions, the absolute amount of FUra in blood plasma strongly rose by a factor of 27. Inhibition by 5-diazouracil of dihydrououracil dehydrogenase which degrades FUra more efficiently than uracil in normal tissues and in tumors (12, 15, 16) thus represents a valuable approach in cancer chemotherapy (21) to improve the bioavailability of FUra.

A pretreatment with 5-diazouracil which has been shown to maximally inhibit dihydrououracil dehydrogenase in rat liver in vivo (21) considerably suppressed the amount of total radioactivity in liver and kidneys by 75 and 66%, respectively. This was in opposite to the effect on the total amount of label in blood plasma. In all other tissues examined which demonstrated a 2-fold rise in radioactivity mostly due to the accumulation of the intact base (Table 2; Fig. 2). The 5-diazouracil-induced increase in the label from [2-14C]uracil in liver and kidney homogenates reflected the absence of radioactive uracil breakdown products since catabolism of the C-2-labeled uracil involves the loss of radioactivity via volatile 14CO2. Under these conditions the relative rise in radioactivity after 5-diazouracil was similar in each of the different organs (Table 3).

In the liver of pretreated animals the proportion of unmetabolized FUra amounted to 21% of total acid-soluble radioactivity versus 2.2% of FUra anabolites. Although the hepatic fraction of anabolites was very low as compared to the label in catabolites and unchanged FUra, there was a 4-fold and 2.6-fold increase in acid-soluble anabolite formation in the liver and kidneys after 5-diazouracil. Twenty-four h after FUra, a 3- to
4-fold enhanced utilization of the drug has also been observed in leukemia cells, liver, spleen, and small intestine of rats after the additional treatment with 5-diazauracil (21).

The methodology used did not allow to discriminate between the individual catabolites of FUra in the organ extracts. In isolated rat hepatocytes the main intracellular catabolite is dihydrofluorouracil after exposure of the cells to 30 μM FUra (23). As in the liver in vivo (Fig. 1) unmetabolized FUra has not been detected within the cells (23). Thymine (2 mm) causes a 99% inhibition of the degradative pathway in hepatocytes and is associated both with insignificant synthesis of FUra anabolites and the formation of a glucuronide of the FUra base (38). The main reason for the failure to identify the glucuronide derivative of FUra in the liver in vivo in the presence of 5-diazauracil presumably was the use of a low tracer dose of FUra. The formation of the FUra conjugate has been shown to depend largely on the intracellular concentration of the base analogue (37).

In addition to the elimination of FUra and its catabolites in urine (8, 9, 41), we found that a considerable fraction of FUra-derived radioactivity was excreted in bile (Fig. 2). 5-Diazauracil had no influence on bile flow but decreased the proportion of label in bile from 12.7% to 2%. It remains to be established whether the recently discovered glucuronidation of FUra (37, 38) is one of the radioactive metabolites in bile and whether 5-diazauracil is able to interfere with its formation.

The results of our experiments indicate that the catabolism of uracil and FUra is strongly inhibited in the whole animal in vivo by 5-diazauracil through interference with pyrimidine catabolism occurring in liver and kidneys of rats. In addition, the excretion of label from FUra into bile is considerably suppressed by 5-diazauracil. The bioavailability of FUra and its consumption for ribonucleoside or ribonucleotide synthesis is markedly increased in the acid-soluble supernatant of liver and kidneys.

ACKNOWLEDGMENTS

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