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 $^{14}$CO$_2$ 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, a-fluoro-$\beta$-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, a-fluoro-$\beta$-ureidopropionic acid, and a-fluoro-$\beta$-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 3-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 degradation 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 on 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 circulatory 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 FURA$^3$ 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 phosphorolase (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 catalyzed 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 extrahepatic human tissues but was highly active in several solid tumors (12). The last enzyme of the catabolic pathway is $\beta$-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 was determined 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 radioactivity 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
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). a-Fluoro-ß-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 motorized homogenizer in 2 volumes of a medium containing 0.25 M sucrose, 20 mM Tris-HCl (pH 7.4, at 37°C), 10 mM ß-mercaptoethanol, and 0.1 mM EDTA (14). 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 mM; 0.5 mCi/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 Scan Gel (Packard Instrument Company, Downers Grove, IL) at an efficiency of 79%. The neutralized acid-soluble supernatants were used for metabolic analysis. Blood was withdrawn from the external jugular vein after anaerobic 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 supernatant of different tissues after i.p. administration of radioactive uracil or FUra. Anabolites like ribonucleosides and ribonucleotides contain cis-diol groups allowing their selective binding to Affi-Gel 601 (Bio-Rad Laboratories, Richmond, CA) (36). Uracil, FUra, and their catabolites as well as the recently discovered 5-fluorouracil glucuronide (37, 38) lack such a group and do not bind to the gel. Phenylboron affinity chromatography of a mixture of 500 to 900 μM of the acid-soluble supernatant of the different organs and 2.5 mM ammonium acetate (pH 8.8) (10% v/v) was performed as described (3, 36). The eluate containing bases, deoxynucleosides, catabolites, and possibly the 5-fluorouracil conjugate (37, 38) was collected from the column including a subsequent wash with 10 mM 0.25 M ammonium acetate (pH 8.8). Elution of cis-diol group-containing anabolites was performed with 6 ml of 0.1 M formic acid. The eluates were lyophilized to dryness, and the residue was dissolved in 200 μl (formic acid fraction) or 300 μl (ammonium acetate fraction) 0.01 M NH4H2PO4 adjusted to pH 5. Aliquots of both fractions were counted. Dihydrofluorouracil was completely converted into α-fluoro-ß-alanine under the conditions used for preparation of the tissue extracts as was tested by the inclusion of further amounts of blood plasma containing labeled FUra catabolites to the organ samples before the homogenization procedure. In the different organs, α-fluoro-ß-alanine thus represented the sum of total acid-soluble FUra catabolites including dihydrofluorouracil, α-fluoro-ß-ureidopropionic acid, and α-fluoro-ß-alanine.

Reversed Phase High Performance Liquid Chromatography. Subsequent to phenylboronate affinity chromatography, 50 μl of the lyophilized and reconstituted ammonium acetate fraction were analyzed by reversed phase HPLC using a procedure described for the analysis of ribonucleosides (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 Scan 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-ß-alanine, and possibly α-fluoro-ß-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-ß-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-diazauracil (5 mg/kg).

RESULTS

Localization of the Entire Pathway of Uracil Catabolism. The activity of the entire pathway of uracil catabolism including dihydrouracil dehydrogenase, dihydropropyrimidinase, and β-ureidopropionase 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-Diazauracil 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-diazauracil as compared to control rats (Table 1). Two h after FUra (100 μmol/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-diazauracil. 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 μmol/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-ß-alanine versus 7.6 ± 0.7% after 5-diazauracil pretreatment (Fig. 1). This inhibition of FUra catabolism was reflected by the increase in the relative amount of unchanged FUra in

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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>Radioactivity (µCi/liter)</td>
<td>No pretreatment</td>
<td>+ 5-Diazauracil</td>
<td>No pretreatment</td>
</tr>
<tr>
<td>[6-14C]FUra (t = 15 min)*</td>
<td>19.0 ± 2.9^d</td>
<td>32.8 ± 3.8</td>
<td>6.4 ± 0.9</td>
</tr>
<tr>
<td>[6-14C]FUra (t = 2 h)*</td>
<td>6.4 ± 0.9</td>
<td>12.7 ± 1.1</td>
<td>2.8 ± 0.45</td>
</tr>
<tr>
<td>[2-14C]Uracil (t = 2 h)*</td>
<td>2.8 ± 0.45</td>
<td>14.6 ± 1.3</td>
<td>2.8 ± 0.45</td>
</tr>
</tbody>
</table>

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

Disappearance of 1-µCi [6-14C]FUra into nucleosides or nucleotides via the salvage pathway was almost negligible under the conditions of our experiments. Measurable amounts of cis-diol group-containing acid-soluble anabolites were detected only in liver and kidneys. Pretreatment of the animals with 5-diazouracil increased the relative proportion of this anabolic fraction in the acid-soluble pool from 0.56 ± 0.22 to 2.23 ± 0.37% in liver and from 1.51 ± 0.09 to 3.9 ± 0.54% in the kidneys. In the other organs tested, the radioactivity in FUra anabolites binding to the phenylboronate gel was below or close to the detection limit of our methods. In the different tissues, α-fluoro-β-ureidopropionic acid, α-fluoro-β-ureidopropionic acid, and α-fluoro-β-alanine, due to the instability of the former two compounds under our experimental conditions. Accordingly, it was the only catabolite which could be measured in the organs (Fig. 1). The 5-diazauracil-induced increase in total acid-soluble radioactivity of spleen, intestine, lungs, and thymus was caused by a strong rise in the relative and absolute amount of unchanged FUra as indicated in Table 2. In the absence of the inhibitor, intact FUra could not be detected in any organ except for spleen and intestine. The latter two organs could have been contaminated by i.p. FUra. The fall in total acid-soluble radioactivity of liver and kidneys after 5-diazauracil 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 in 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-diazauracil 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 further demonstrate that the kidneys also accumulate FUra catabolites amounting to 275 µCi/kg, wet weight. This is in line with the presence of the complete enzymatic pathway for uracil catabolism in rat kidney.
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>anabolics</th>
<th>catabolites</th>
<th>FUra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>44 ± 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>0</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>

* Mean ± SD from 5 rats.
* ND, not detectable under our experimental conditions.

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

<table>
<thead>
<tr>
<th>Organ</th>
<th>Radioactivity (μCi/kg, wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.66 ± 0.86*</td>
</tr>
<tr>
<td>Kidneys</td>
<td>5.35 ± 1.28</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.62 ± 0.19</td>
</tr>
<tr>
<td>Intestine</td>
<td>2.88 ± 0.90</td>
</tr>
<tr>
<td>Lungs</td>
<td>1.60 ± 0.47</td>
</tr>
</tbody>
</table>

* Mean ± SD from 4 rats.

Fig. 2. Effect of a prior treatment with 5-diazouracil (5-DiazOura) on the amount of radioactivity from [6-14C]FUra (5 μCi/150 g rat; 56 Ci/mol) excreted into bile. Bile was collected every 10 min and counted. Points, means of four animals; bars, SE.

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

neous. The proportion of FUra breakdown occurring in the kidneys themselves, however, is difficult to assess from the FUra catabolites present in the tissue. A considerable portion of these catabolites presumably originated from FUra degradation in the liver, since more than 90% of an administered FUra dose is excreted via the kidneys into urine (9, 41). α-Fluoro-β-alanine is the major excreted metabolite which makes up 79% of the total label in urine (9). In the cytosol of rat kidneys, formation of labeled CO2 from [2-14C]Ura occurred at 10% of the rate detected in the supernatant of rat liver homogenates. All the other tissues tested did not possess the complete enzymatic equipment for uracil degradation. In contrast to the subsequent catabolic enzymes, dihydouracil dehydrogenase has been identified in many rat tissues (42, 43). The amount of α-fluoro-β-alanine in supernatants of organs lacking dihydrouracilidinase or β-ureidopropionase originated from dihydrofluorouracil converted into α-fluoro-β-alanine during workup of the samples or from the degradative products present 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 dihydrofluorouracil (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 dihydrofluorouracil (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 dihydrofluorouracil 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 dihydrofluorouracil 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 and 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).
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 nm) 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 depressed the proportion of label in bile from 12.7% to 2%. It remains to be established whether the recently discovered glucuronide 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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References

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