A role of the liver in the physiological regulation of circulating purines and pyrimidines has been suggested by numerous studies (6, 12, 16, 17). This laboratory (6) and others (14) have demonstrated that the liver is capable of rapid and essentially complete removal of incoming uridine derived from intestinal absorption and the peritoneal organs. Despite this extensive degradation, the concentration of uridine in blood exiting the liver is at least as great as that in the portal vein and hepatic artery. This uridine has been shown to be derived from de novo synthesis as well as turnover of the liver RNA and uracil nucleotide pools (6).

The ability to control the availability of endogenous nucleosides from circulation may be the limiting factor in the action of some inhibitors of pyrimidine synthesis de novo because tumors and normal tissues differ in their ability to transport and salvage nucleosides (5). Rescue of some normal tissues from 5-fluorouracil toxicity by very large doses of uridine has been reported (13). This requirement for large doses to overcome the rapid degradation of uridine provides further impetus to develop an understanding of the sites and cellular basis for uridine homeostasis.
of 85% for the hepatocytes and 90–95% for the nonparenchymal cells before use and at least 80% at the end of uptake experiments. The purity of the hepatocyte suspensions ranged from 92 to 95%. The nonparenchymal cell preparations were >95% pure, validated by esterase staining and microscopic evaluation of the cell suspension, and typically contained 55–60% of Kupffer cells and 35–40% of endothelial cells.

Transport studies. To prepare cells for transport studies, the suspension was pelleted at 500 g for 5 min in a Sorvall GLC-4 centrifuge. The pellet was resuspended twice in Na+-free (150 mM chloride choline) Hanks' balanced salt medium plus 5.5 mM D-glucose and 4 mM HEPES buffer (pH 7.4) and centrifuged at 500 g for 5 min. The pellet was resuspended with an appropriate medium (± Na+ with choline replacement and ± nitrobenzilthioninosine (NBMPR) to give a final cell density of 5 × 10⁸ cells/ml).

The transport of [³H]Juridine was initiated by mixing 30 µl of cell suspension with 60 µl of radioactive substrate in a 1.5-ml Eppendorf Microfuge tube. At appropriate time intervals, 60 µl of the mixture were placed in an "oil-stop tube" consisting of a 400-µl Eppendorf Microfuge tube containing 30 µl of 15% TCA (100 µl for HPLC studies) and centrifuged in a Beckman model B Microfuge for 30 s at 10,000 g. Microfuge tubes were then cut through the oil layer, and radioactivity in each half was determined as previously described (4). Time-zero values, attributable to the extracellular radioactivity trapped in the cell pellet, were determined by centrifugation of 20 µl of cell suspension through a layer of radioactive substrate (40 µl) placed over the oil in the oil-stop tube. The intracellular volume was calculated in all experiments with [³H]H₂O to determine total water space and [¹⁴C]inulin for estimation of extracellular space (4).

Uridine and uracil metabolism. Liver cells were incubated with 5 µM [³H]Juridine or uracil at 22–24°C for various time periods. The reaction was terminated by the oil-stop method described previously. Supernatants were immediately removed from the oil-stop tube and mixed with 100 µl of 15% TCA. Both supernatant and cell pellet fractions were then extracted with an equal volume of trichloroacetic acid (TCA) (500 µl) for HPLC studies and centrifuged in a Beckman model B Microfuge for 30 s at 4°C. Microfuge tubes were then cut through the oil layer, and radioactivity in each half was determined as previously described (4). Time-zero values, attributable to the extracellular radioactivity trapped in the cell pellet, were determined by centrifugation of 20 µl of cell suspension through a layer of radioactive substrate (40 µl) placed over the oil in the oil-stop tube. The intracellular volume was calculated in all experiments with [³H]H₂O to determine total water space and [¹⁴C]inulin for estimation of extracellular space (4).

URIDINE HEPATIC CATABOLISM

RESULTS

To define the components and mechanisms of uridine homeostasis, cells of the rat liver have been separated into parenchymal (hepatocytes) and nonparenchymal (largely Kupffer and endothelial cells) populations. This has revealed a sharp dichotomy in the metabolic potential of these cell types. For these experiments a uridine concentration of 5 µM was chosen to approximate physiological levels. Very active catabolism of uridine occurs in the nonparenchymal cell population (Fig. 1), but uridine catabolism was essentially undetectable in hepatocytes. By contrast, the uracil generated by uridine phosphorylase (Fig. 2) was almost completely eliminated by dihydrouracil dehydrogenase (20), reduced by 90% the catabolism of uracil in hepatocytes. This compartmentalization of the catalytic pathways seen in whole cells is reflected in the relative activity of the two enzymes, uridine phosphorylase and dihydrouracil dehydrogenase, in extracts of the two cell lines (Fig. 3). We also determined the uridine phosphorylase activity in extracts of two macrophage cell lines, P388D1, and J774A.1. As reported in Table 1, extracts from each cell type had phosphorylase activity...
at least 10 times higher than that in hepatocyte extracts.

Another major factor in uridine homeostasis may be membrane transport. Hepatocytes were found to exhibit a concentrative transport mechanism for uridine that is highly dependent on cotransport with Na\(^+\). This system has properties similar to Na\(^+\)-dependent nucleoside transporters in kidney and intestinal cells. Intracellular concentrations of free \([3H]\)uridine rapidly exceed media concentrations (Fig. 4), and the process is not sensitive to NBMPR. Although phosphorylation of

**Table 1.** Uridine phosphorylase activity in rat liver hepatocytes, nonparenchymal cells, and macrophages (P388D\(_1\) and J774A.1 cells)

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Uracil Phosphorylase Activity, nmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hepatocytes</td>
</tr>
<tr>
<td>5</td>
<td>0.7</td>
</tr>
<tr>
<td>15</td>
<td>0.9</td>
</tr>
<tr>
<td>30</td>
<td>1.2</td>
</tr>
<tr>
<td>60</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Values represent means of 2 separate determinations. Uridine phosphorylase activity was evaluated in cell extracts incubated with reaction mixture containing 200 µM \([3H]\)uridine in 50 mM Tris buffer (pH 7.4), NaF (4 mM), dithiorthreitol (2 mM), MgCl\(_2\) (2 mM), and potassium phosphate (1 mM) in a final volume of 100 µl at 37°C for indicated time. Protein concentrations were determined by Bradford method.
uridine to nucleotide form could concentrate uridine radioactivity, under these experimental conditions HPLC and TLC analysis revealed that <10% of uridine was converted to uracil nucleotides.

The Na⁺-dependent uridine transport in hepatocytes exhibits typical Michaelis-Menten kinetics. In studies ranging from 5 to 200 µM uridine, we observed an apparent Michaelis constant (Kₘ) of 46.8 ± 4 µM and a maximal velocity (Vₘₐₓ) of 6.1 ± 0.5 pmol·µl⁻¹·s⁻¹. The purine nucleosides adenosine, guanosine, and inosine were good inhibitors of this concentrative mechanism, whereas the pyrimidine nucleosides thymidine and cytidine were much less effective (Table 2).

Uridine uptake was evaluated in nonparenchymal cells, and in this cell population we observed a Na⁺-dependent concentrative transport mechanism for uridine very similar in its efficiency and capacity to that described for the hepatocytes with Kₘ of 34.8 ± 4.0 µM and Vₘₐₓ of 4.83 ± 0.65 pmol·µl⁻¹·s⁻¹.

Na⁺-dependent transport is known to coexist with facilitated diffusion and passive equilibration in different proportions, depending on cell type. We have determined the number of NBMPR binding sites (1.5 ± 0.14 × 10⁵/cell) and their affinity (0.6 ± 0.2 nM) in the hepatocyte populations. These values are in the range seen for many other cell types. However, measurement of uridine entry into hepatocytes in Na⁺-free (choline replacement) media in the presence and absence of 10 µM NBMPR indicates that at nonphysiological concentrations of uridine above 50 µM, the predominant mechanism is nonsaturatable and presumed to be passive diffusion or at least NBMPR insensitive (Fig. 4). This may be an intrinsic property of hepatocytes or be consequent to the collagenase treatment that may alter the plasma membrane in a subtle manner to permit diffusion of uridine at high concentrations without permitting entry of trypan blue (cells exhibit >80% trypan blue exclusion). The very slight difference between intra- and extracellular concentrations of uridine at these unphysiological concentrations of uridine up to 2,000 µM may reflect small errors in the estimation of the intracellular volume by [¹⁴C]inulin and [³H]H₂O. These factors may explain why hepatocytes in suspension are unable to generate more than threefold greater concentrations of uridine in the presence of Na⁺, whereas in the intact animal liver concentrations of uridine are about 10-fold greater than seen in the plasma (4).

The metabolism of [³H]uracil in hepatocytes is associated with an extensive accumulation of radioactivity within the cell. Intracellular uracil rapidly equilibrates with the medium concentration of uracil, but as time progresses most of the radioactivity is present as β-alanine. This end product of the uracil catabolic pathway is avidly retained within cells by a Na⁺-dependent concentrative mechanism (Fig. 5) with an apparent Kₘ of 38.8 ± 5.9 µM that has not previously been reported in liver cells (data not shown). GABA as well as hypotaurine, the sulfonic acid analog of β-alanine, are potent inhibitors of this process with an apparent inhibitory constant of 20.0 ± 1.8 and 20.1 ± 1.3 µM, respectively. α-Fluoro-β-alanine, the primary catabolic product of 5-fluorouracil, also exhibits inhibi-

### Table 2. Inhibitory effects of purine and pyrimidine nucleosides on sodium-dependent uridine transport in rat hepatocytes

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>IC₅₀, µM</th>
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<tbody>
<tr>
<td>Adenosine</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Guanosine</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Inosine</td>
<td>50 ± 3</td>
</tr>
<tr>
<td>Cytidine</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Thymidine</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

Values represent means of 4 determinations in each of 2 experiments. Transport of uridine was determined as described in MATERIALS AND METHODS. Competing nucleosides were mixed with [³H]uridine (5 µM) before addition to suspension of hepatocytes (4–6 × 10⁶ cells/ml).
tory properties in this transport system, albeit less efficiently. All three compounds displayed competitive inhibition kinetics (data not shown). The intermediate degradation products of uracil, N-dihydrouracil and N-carbamyl-β-alanine were not detected in agreement with a previous report (30) and in contrast to 5-fluorouracil catabolism where these intermediates can accumulate (29).

In nonparenchymal cells nonconcentrative entry of uracil occurred, but analysis of intracellular radioactivity indicated that >85% was present as uracil after 20 min of incubation. Extracellular radioactivity from each time point also indicated the absence of uracil degradation. Thus uracil is the end product of uridine degradation in the nonparenchymal cell population, and only hepatocytes are responsible for uracil degradation in rat liver. The enzymatic basis for this difference in uracil catabolism in two cell populations can be seen in the dihydropyrimidine dehydrogenase activity in cell-free extracts of both cell types (Fig. 3, bottom).

**DISCUSSION**

The discrete compartmentalization of uridine metabolism between two cellular compartments of the liver affords a partial insight into the apparent paradox of essentially complete catabolism and replacement of plasma uridine in a single pass. Previous studies indicated relatively high phosphorylase activity in liver extracts (26). Although it had been shown that macrophages and Kupffer cells possess considerable uridine phosphorylase (7), the virtual absence of this enzyme activity in hepatocytes was unexpected, particularly since they comprise at least 80% of the liver mass.

The reciprocal relationship between uracil catabolism and uridine cleavage adds to the dichotomy of this physiological process. A possible model would be that entering uridine from both the hepatic artery and the portal circulation initially or at least predominantly encounters the endothelial and Kupffer cells that cleave it to uracil. Subsequently, uracil diffuses to hepatocytes where it is rapidly degraded. What remains to be established is the mechanism by which uridine concentrations in the hepatic vein are essentially equal to that found in the portal vein and the hepatic artery; this uridine is presumed to come from de novo synthesis in hepatocytes and possibly from the turnover of mRNA and other RNA species by 5-nucleotidase activity. Previously, it had been shown that the specific activity of exiting uridine is less than would be expected if it came solely from the acid-soluble pyrimidine nucleotide pool (6). In hepatocytes, however, most of the uridine formed by dephosphorylation of UMP is undoubtedly conserved for reutilization by the Na+-dependent active transport mechanism documented in this report. What is also not clear at this time is the nature of the chemostat that maintains the concentration of uridine in blood at about 1–3 μM despite wide variations in the concentration of uridine entering the liver (6).

The remarkable stability of uridine in suspensions of hepatocytes indicates that a previous report (7) suggesting that uridine phosphorylase activity is associated with hepatocyte membranes has limited physiological significance. It is also apparent that the ~10-fold concentration of free uridine in the whole liver (4) is predominantly in the hepatocyte population because it comprises 80% or more of the total mass.

Although the coexistence of a unidirectional Na+-dependent pathway and equilibrative facilitated diffusion establishes a concentration of uridine inside hepatocytes, the equilibrium can be shifted by appropriate agents. The minimal effect of NBMPR indicates that at concentrations up to 50 μM uridine, facilitated diffusion plays a minor role. Other experiments not presented indicate that at higher concentrations of uridine the Na+-dependent process approaches saturation, and facilitated and passive diffusion mechanisms become predominant. The pattern of inhibition by purine nucleosides at lower concentrations of uridine is consistent with previous reports with renal tubular vesicles (10, 11) and enterocytes (8, 22) and confirms the presence of a purine-specific Na+-dependent transporter on the biliary canalicular membrane (3). Other data support the existence of a Na+-dependent concentrative system found on the surface of the hepatocyte that forms the biliary canaliculi. In fact, uridine concentrations in bile are less than in blood, presumably because of the active concentration process that retains uridine (unpublished results). The transporter must also, however, be found in the basolateral membrane because hepatic concentrations of uridine rapidly respond to changes in plasma uridine to sustain a gradient of ~10 to 1 in the intact liver (4). This last observation is in agreement with a report from Ruiz-Montasell et al. (24) indicating the presence of a Na+-dependent uridine transporter in the basolateral membrane vesicles from rat liver. However, contradictory results were reported in an earlier study that demonstrated the existence of a Na+-dependent uptake only on the bile canalicular membrane (15).

Concentration of β-alanine, the product of uracil catabolism by hepatocytes, occurs by a specific transporter different from that for neutral α-amino acids (9). It is also apparently different from β-alanine transport in the brain (25). The current studies that demonstrate inhibition of transport by the neurotransmitter GABA raise the question of what may be the relevant natural substrate for this concentrative mechanism in the liver. β-Alanine is a component of CoA, a low concentration cofactor, but is found in relatively high concentrations in various tissues as carnosine and anserine, dipeptides in amide linkage with histidine or methyl histidine. Because the liver has relatively low concentrations of these dipeptides and an active dipeptidase, carnosinase, it is unlikely to be a primary site of synthesis of these peptides for distribution to other tissues. Although several roles have been postulated for these dipeptides, definitive statements cannot be made about their normal metabolic roles or the effects of altered concentrations associated with certain disease states (2).

In a variety of animal species, including humans, the pool of incoming and exiting nucleosides is distinct and...
regulated to sustain uridine concentrations of 1-3 µm. The geometry of elements permitting this homeostasis of uridine in the liver is not yet known. The biological relevance of this system is apparent in 5-fluorouracil therapy as attempts are being made to expand or contract circulating and tissue pools of uridine and uracil nucleotides by administering exogenous uridine or such agents as PALA (20), brequinar (21), and benzylacyclouridine (5).

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