

## Fluorouracil: Biochemistry and Pharmacology

By Herbert M. Pinedo and Gode Fridus J. Peters

Fluorouracil (5FU) is still considered the most active antineoplastic agent in the treatment of advanced colorectal cancer. The drug needs to be converted to the nucleotide level in order to exert its effect. It can be incorporated into RNA leading to interference with the maturation of nuclear RNA. However, its conversion to 5-fluoro-2'-deoxy-5' monophosphate (FdUMP) leading to inhibition of thymidylate synthase (TS) and subsequently of DNA synthesis, is considered to be its main mechanism of action. In the presence of a folate cofactor a covalent ternary complex is formed, the stability of which is the main determinant of the action of 5FU. Resistance against 5FU can be mainly attributed to aberrations in its metabolism or to alterations of TS, eg, gene amplification, altered kinetics in respect to nucleotides or folates. Biochemical modulation of 5FU metabolism can be applied to overcome resistance against 5FU. A variety of normal purines, pyrimidines, and other antimetabolites have

been studied in this respect, but only some of them have been clinically successful. Delayed administration of uridine has recently been shown to "rescue" mice and patients from toxicity, while pretreatment with leucovorin is the most promising combination to enhance the therapeutic efficacy. 5FU is frequently administered in an intravenous (IV) injection, and shows a rapid distribution and a triphasic elimination. The nonlinearity of 5FU pharmacokinetics is related to saturation of its degradation. Continuous infusion of 5FU led to different kinetics. Regional administration, such as hepatic artery infusion, offers a way to achieve higher drug concentrations in liver metastases and is accompanied by lower systemic concentration. The current status of the biochemical and pharmacokinetic data is reviewed.

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FLUOROURACIL (5FU) has been used for several decades for the treatment of various types of cancer.<sup>1,2</sup> The mechanism underlying the action of this compound is complex and depends on the type of tissue, ie, whether normal or tumor tissue is involved. In attempts to increase the antitumor activity and limit toxicity, various analogues of 5FU such as Ftorafur and 5'-deoxy-5-fluorouridine (5'dFUR) have been studied clinically<sup>3</sup> and combinations of 5FU with other antimetabolites or natural compounds have been evaluated in efforts to improve the therapeutic index.<sup>4-12</sup> The present review deals with the biochemical activation and the inactivation of 5FU, various mechanisms underlying its action, resistance to and biochemical modulation of 5FU, metabolic aspects, and some new data on the pharmacology of the drug.

### BIOCHEMICAL ACTIVATION

The initial metabolism of 5FU to nucleotides such as fluorouridine 5'-triphosphate (FUTP) and 5-fluoro-2'-deoxyuridine-5' monophosphate (FdUMP) is essential for its action.<sup>1,3,4,13</sup> Several enzymes belonging to pyrimidine metabolism are required for the conversion of 5FU to nucleotides (Fig 1). FdUMP can be formed from FUMP

via reduction of FUDP. The extent of growth inhibition by 5FU may be correlated with the activity of one or more of the enzymes catalyzing the initial metabolism of 5FU.<sup>3</sup> For some cell lines orotate phosphoribosyl-transferase (OPRT) has been shown to play a major role in the initial metabolism, whereas for other cells uridine phosphorylase is more important.<sup>14</sup> Nucleotides formed via the direct pathway (via OPRT) and the indirect pathway (via FUR) are incorporated into different RNA fractions.<sup>15</sup> Sensitivity of cell lines and tumors to 5FU might also depend on the availability of cosubstrates required for the con-

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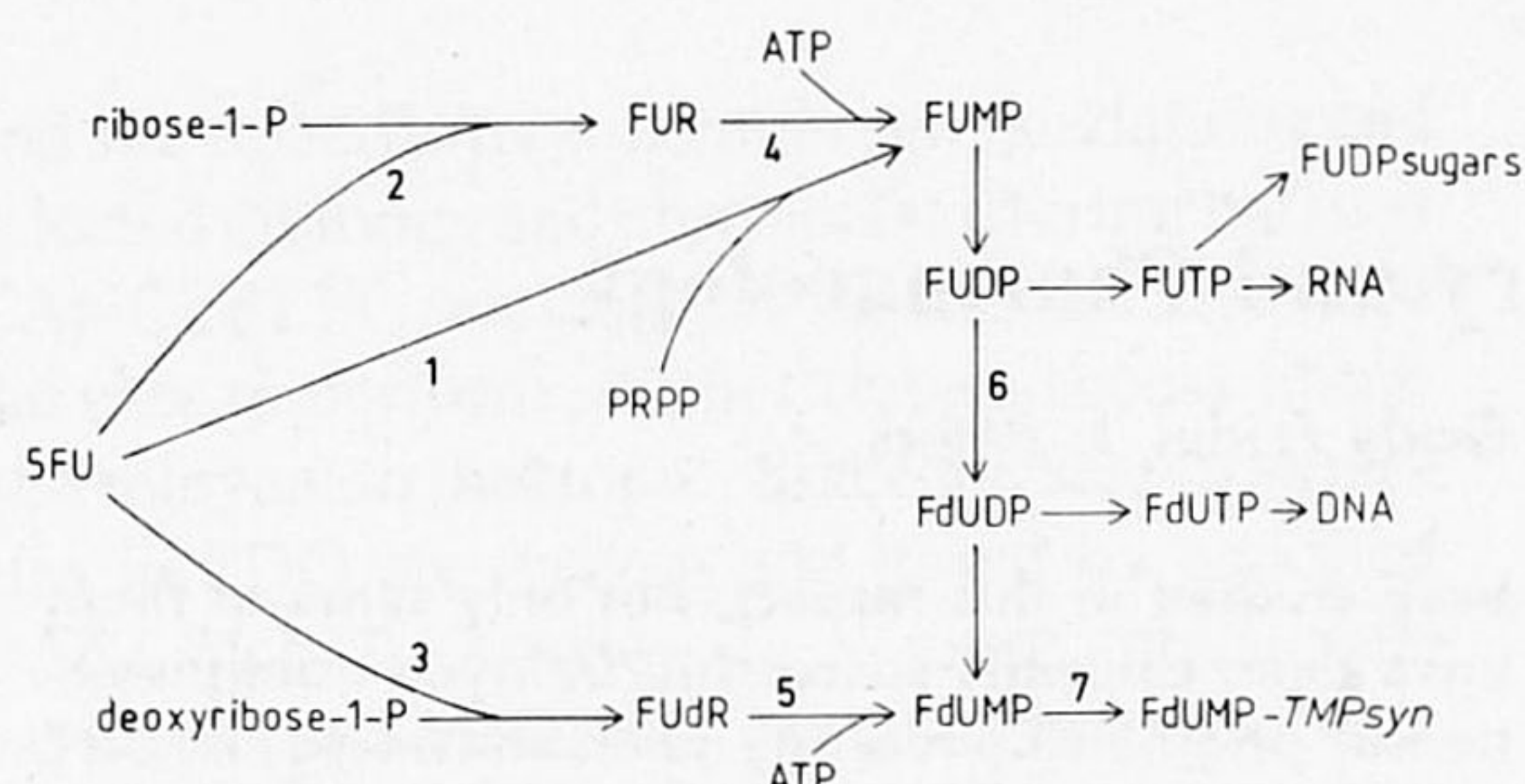
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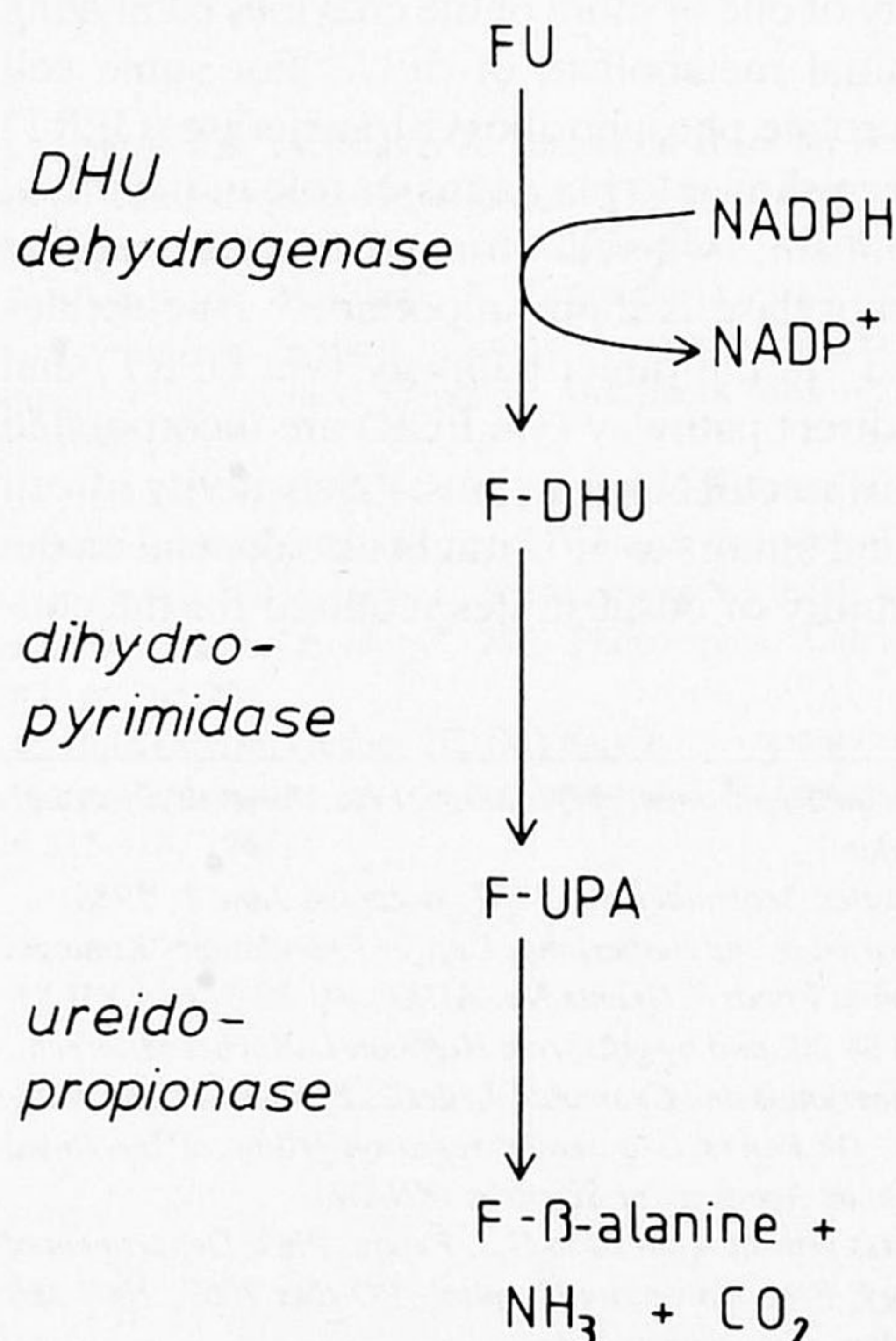
**Fig 1. Metabolism of 5FU.** The enzymes involved are: **1, Orotate phosphoribosyl-transferase (OPRT); 2, uridine phosphorylase; 3, thymidine phosphorylase; 4, uridine kinase; 5, thymidine kinase; 6, ribonucleotide reductase; 7, thymidylate synthase (TMPsyn or TS); 5-fluorouridine, FUR; 5-fluoro-2'-deoxyuridine, FUDR; phosphoribosylpyrophosphate, PRPP; 5'-fluorouridine-5' monophosphate, FUMP; 5-fluorouridine-5'-diphosphate FUDP; 5'-fluoro-2'-deoxyuridine-5'-fluoro-2'-deoxyuridine-5'-triphosphate, FdUTP.**

version of 5FU to active nucleotides,<sup>13</sup> as discussed in the section on modulation.

#### 5FU INACTIVATION

5FU can be inactivated by degradation to 5-fluorodihydrouracil (F-DHU) (Fig 2). Further degradation of 5FU has been studied as far as

#### Catabolism of 5-Fluorouracil (FU)



**Fig 2. Degradation of 5FU dihydrouracil, DHU; 5-fluoro-ureido propionate, FUPA.**

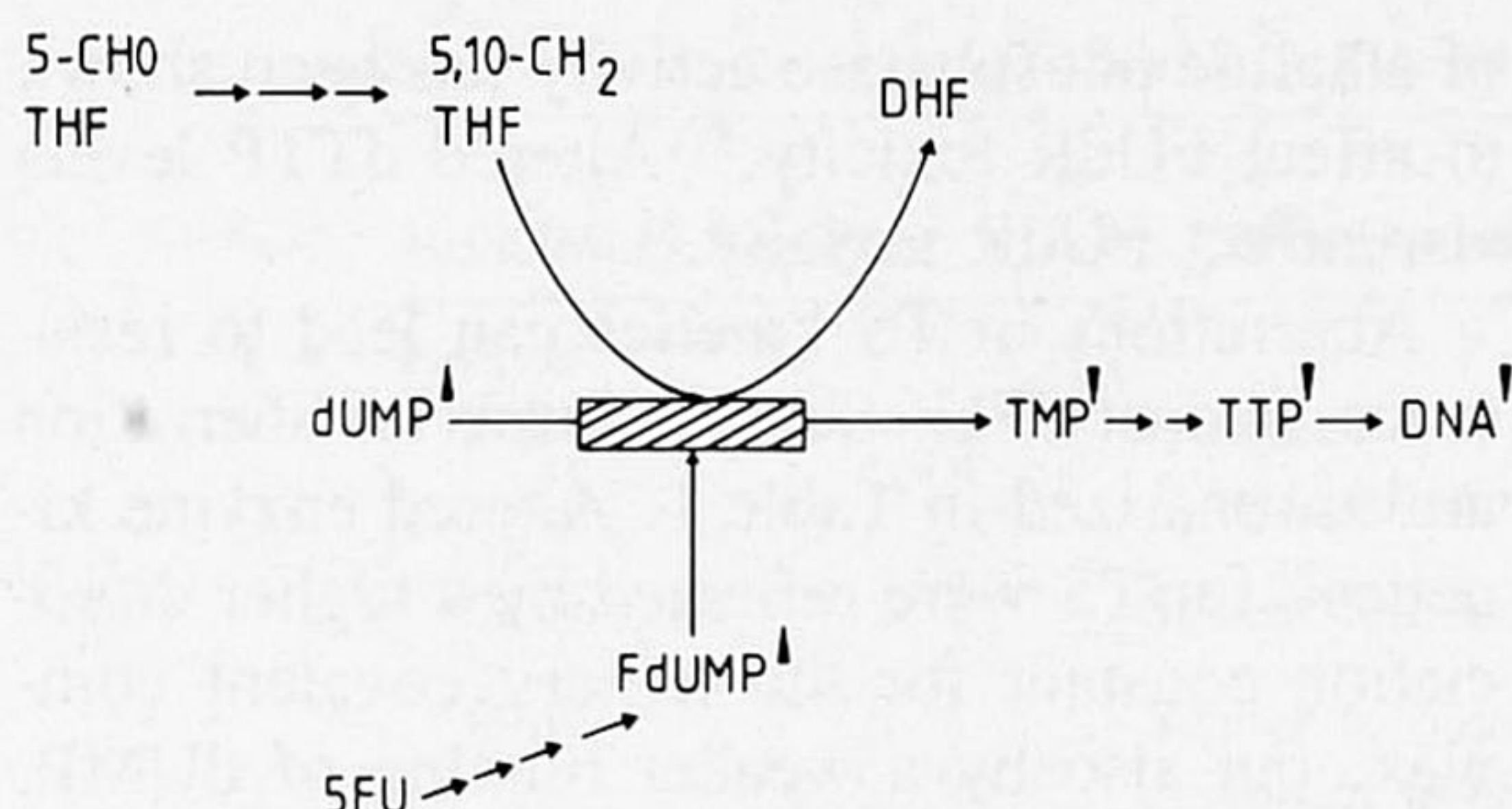
5-fluoro-alanine (F-BAL), and further metabolism of F-BAL is not unlikely.  $\beta$ -alanine itself is a substrate for carnosine, but it can also be converted to acetate. Similarly, F-BAL can be converted to fluoroacetate, which has been related to neurotoxicity.<sup>16</sup> 5FU degradation occurs in all tissues, but tumor tissue contains very small amounts of dihydrouracil dehydrogenase.<sup>17</sup> The activity of this enzyme, while occurring in the kidney, is most intense in the liver,<sup>18</sup> which means that the liver plays an important role in 5FU degradation and elimination.

It has been shown in patients that large amounts of 5FU are degraded to F-DHU,<sup>19</sup> and fluorine-19 (19F) nuclear magnetic resonance (NMR) has been used to show that, in vivo, F-DHU is rapidly degraded further to F-BAL.<sup>20</sup> Recently, a new catabolite of 5FU was detected in bile, and was identified as an N-cholyl-2-fluoro- $\beta$ -alanine conjugate by NMR<sup>21</sup> and enzymatic methods.<sup>22</sup> In vivo inhibition of 5FU degradation has been thought to increase the availability of 5FU to tumors. However, it has been reported that an improved therapeutic index was not observed clinically after the administration of thymidine and 5FU,<sup>23</sup> but in rats toxicity appeared to be increased.<sup>24</sup> Impaired 5FU degradation due to a deficiency of dihydrouracil dehydrogenase led to a dramatic and fatal increase of 5FU toxicity.<sup>25</sup>

It may be concluded that inhibition of 5FU degradation probably will not improve therapeutic efficacy, since toxicity increases as much as or even more than the antitumor activity.

#### INHIBITION OF THYMIDYLATE SYNTHASE BY FdUMP

It has long been recognized<sup>26-28</sup> that inhibition of TS by FdUMP is one of the main mechanisms underlying 5-FU action (Fig 3). Initial studies have been performed with the methotrexate (MTX)-resistant mutant from *Lactobacillus casei*.<sup>26</sup> Since the older studies have been reviewed extensively by Danenberg<sup>27</sup> and Danenberg and Lockshin, this review will be limited to clinically relevant aspects. Enzyme kinetic studies in various systems revealed a rather low Michaelis-Menten constant ( $K_m$ ) of about 2  $\mu$ mol/L for dUMP.<sup>27-29</sup> Without preincubation the inhibition constant ( $K_i$ ) for FdUMP appeared to be competitive,<sup>27</sup> with a  $K_m/K_i$  ratio of about 1,000.



**Fig 3. Inhibition of TS by FdUMP (hatched bar) leading to accumulation of dUMP and FdUMP, depletion of TMP and TTP, and inhibition of DNA synthesis. TS catalyzes the conversion of dUMP to TMP. 5-CHO-THF, 5-formyl-tetrahydrofolate (leucovorin); DHF, dihydrofolate; 5,10-CH<sub>2</sub>THF, 5,10-methylene-tetra hydrofolate.**

FdUMP forms a reversible but tight-binding covalent bond with TS in the presence of 5,10-CH<sub>2</sub> tetrahydrofolate (THF),<sup>27-30</sup> which is precipitable with trichloroacetic acid.<sup>30</sup> The *in vivo* recovery of uninhibited TS differed among various tumors and cell lines.<sup>31-34</sup> Retention of TS inhibition was also dependent on the ratio between free dUMP and FdUMP levels.<sup>33</sup> The presence of folate cofactor appeared to be correlated with the extent of enzyme inhibition and the retention of the complex.<sup>33-36</sup> Not only enzyme inhibition, but also the enzyme level before treatment was related to growth inhibition by 5FU.<sup>33</sup> The degree of inhibition of TS and the persistence of inhibition are essential factors for maximal *in vivo* growth inhibition by either 5FU or FUdR. A relationship has been reported to exist between low sensitivity to 5FU and rapid disappearance of FdUMP.<sup>37,38</sup> Retention of the inhibition of TS is dependent on the binding of FdUMP and the stabilization of the ternary complex by 5,10 CH<sub>2</sub> THF<sup>33,38-40</sup> or one of its polyglutamates.<sup>33,41</sup> The naturally occurring cofactor for TS is a polyglutamate.<sup>42,43</sup> The *K<sub>i</sub>* for FdUMP in the presence of folylpolyglutamate is lower than with the monoglutamate.<sup>44</sup> A noncovalent complex of FdUMP with TS that is less stable can also be formed.<sup>27,28</sup> Folates appear to be essential for the formation of a covalent complex. Evidence has been presented that tumors of patients responding to 5FU show greater inhibition of TS than tumors of patients with progressive disease.<sup>45</sup>

#### 5FU INCORPORATION INTO RNA

In most cells and tissues 5FU will be converted not only to FdUMP, but also to FUTP which can

be incorporated into all classes of RNA in tumor cells, mainly into nuclear RNA.<sup>46</sup> Processing of nuclear RNA into cytoplasmic rRNA is in all probability the essential factor leading to cytotoxicity. Initially, it was demonstrated *in vitro* that the amount of 5FU incorporated into RNA correlated with the sensitivity to 5FU of various cell lines<sup>47-50</sup> and *in vivo* with the antitumor effect of 5FU<sup>51</sup> and with gastrointestinal cytotoxicity.<sup>52</sup> Although 5FU is incorporated into most species of RNA, toxicity was not correlated with incorporation in all of these species.<sup>53,54</sup> The cytotoxicity due to incorporation of 5FU into RNA is mainly determined by the incorporation of 5FU into nuclear RNA.<sup>51,55-57</sup> Recently, more evidence has been presented that misincorporation into RNA might be associated with a block in processing and/or nuclear cytoplasmic transport.<sup>58-61</sup> Thus, in all likelihood, 5FU incorporation into RNA produces cytotoxicity by interference with the maturation of nuclear RNA.

#### 5FU INCORPORATION INTO DNA

5FU incorporation into DNA has long been considered a very unlikely event, and not contributory to 5FU cytotoxicity. Intracellular FdUTP is hydrolyzed by dUTPase<sup>62</sup>; FdUTP incorporated into DNA is thought to be removed by uracil-DNA glycosylase.<sup>63</sup> Despite these protective mechanisms, some 5FU residues can be incorporated into DNA.<sup>63-71</sup> 5FU incorporation has been shown to be enhanced by MTX,<sup>69</sup> but increased excision of 5FU residues<sup>63</sup> has also been reported. A relation between 5FU incorporation and cytotoxicity has been postulated.<sup>63,72</sup> 5FU is capable of inducing DNA strand breaks,<sup>67,73</sup> which might also be related to inefficient DNA repair of normally occurring defects.<sup>73</sup> Thus, the extent to which 5FU incorporation into DNA, the subsequent excision, its effect on DNA repair, and the induction of strand breaks are related to 5FU cytotoxicity is not yet clear.

#### 5FU NUCLEOTIDE SUGARS

Uridine metabolites occur predominantly as nucleotide sugars, such as uridine 5'-diphosphate (UDP)-glucose and UDP-N-acetyl-hexoseamines. These sugars are substrates for glycosyltransferases, which catalyze the glycosylation of proteins and lipids, and are important for cellular functions involving, for example,

cell-surface glycoprotein and glycolipid receptors, differentiation markers, and recognition determinants. It has been shown that 5FU can be a substrate for the synthesis of FUDP sugars, such as FUDP-hexoses,<sup>74-76</sup> FUDP-hexoseamines, and FdUDP-N-acetylhexoseamines.<sup>77-79</sup> From these findings it is clear that FUDP sugars are formed, but their effect on the nucleotide sugar metabolism and glycosylation warrants further investigation.

### RESISTANCE TO 5FU

5FU resistance, whether intrinsic or acquired, is usually caused by aberrations in the metabolism of 5FU or altered effects of 5FU-metabolites. The normal metabolism has been discussed in the preceding sections, and aberrations are summarized in Table 1. Generally, studies on 5FU resistance have been performed by comparison of several tumor cell lines with different sensitivity to 5FU or selection of a 5FU-resistant subpopulation from a sensitive tumor or cell line. It has already been shown by Reyes and Hall<sup>80</sup> and Kessel et al<sup>81</sup> that tumors with a low level of anabolism have a low sensitivity to 5FU. OPRT may be the limiting enzyme for 5FU anabolism.<sup>80</sup> 5FU transport across the cell membrane might not limit its activity, but FUdR resistance was found to be related to deficiency in its transport.<sup>82</sup> Depletion of cosubstrates, ie, (deoxy)Ribose-1-P or PRPP,<sup>4</sup> seems to limit the anabolism of 5FU, as suggested by indirect evidence. Increased availability of Ribose-1-P,<sup>15</sup> deoxy-Rib-1-P,<sup>39,83</sup> or PRPP<sup>4</sup> enhanced the sensitivity to 5FU. Enhanced nucleotide catabolism due to a high level

of alkaline phosphatase activity has been shown to affect FUdR toxicity.<sup>84</sup> Altered dTTP levels also affect FUdR toxicity.<sup>85</sup>

Aberrations in TS kinetics can lead to resistance against 5FU. Several forms of aberration are summarized in Table 1. Altered enzyme kinetics<sup>29</sup> for TS were reflected by a higher dissociation constant for the ternary covalent complex, but also by a weaker binding of dUMP. Intrinsic resistance to 5FU has been associated with high accumulation of dUMP.<sup>38</sup> The turnover of TS was higher in a resistant sub-cell line than in the sensitive cells.<sup>29</sup> A higher activity of TS was found in an FUdR-resistant sub-cell line,<sup>36</sup> possibly due to gene amplification. Amplification of the gene coding for TS has been shown,<sup>86</sup> recently also in a case of human colon cancer with acquired resistance.<sup>87</sup> The stability of the ternary complex depends on the concentrations of dUMP and FdUMP and the kinetic parameters, but also on the availability of folates. Low total folate pools were associated with 5FU resistance,<sup>35,41</sup> as well as a low proportion of polyglutamate derivatives.<sup>41</sup> It may be concluded that resistance to 5FU can be due to a variety of aberrations in 5FU-metabolism, but factors affecting TS appear to be of major clinical relevance.

### BIOCHEMICAL MODULATION OF 5FU

Biochemical modulation of anticancer agents involves the pharmacologic manipulation of the intracellular pathways of a drug. The aim is to improve the therapeutic index.<sup>10,88</sup> Modulation can be used to overcome 5FU resistance. For cell culture systems and animal models, various combinations of 5FU with other drugs have been selected on a rational basis.<sup>3,88</sup> A list of such combinations is given in Table 2, where a subdivision according to antimetabolites and naturally occurring purines and pyrimidines is made. The list is not exhaustive. Although certain combinations have only theoretical value, their use in *in vitro* studies has led to a better insight into the mechanism of action and resistance. Other combinations have more practical applications. The preclinical studies on combinations of 5FU with purines have thrown more light on 5FU activation and metabolism, but the findings have not yet been applied clinically. Purines can even provide protection against 5FU cytotoxicity by

**Table 1. Resistance to 5FU**

Deficiency of 5FU anabolism
Deficiency of 5FU transport
Depletion of essential cosubstrates
Enhanced catabolism of 5FU, FUMP, or FdUMP
Enhanced intracellular uridine concentrations
Altered dTTP levels
Alterations in thymidylate synthase
Altered enzyme kinetics
Enhanced dUMP accumulation
Decreased FdUMP retention
Rapid recovery of new enzyme synthesis
Gene amplification
Decreased stability of ternary complex
Depletion of folates
Decreased polyglutamylation of folates

Abbreviation: dTTP, deoxythymidine triphosphate.

Table 2. Biochemical Modulation of 5FU

Modulating Agent	Postulated Mechanism	Sequence of Addition	Tested Systems
<b>Purines</b> <sup>13,83,89-93</sup>			
Inosine	Increased rib-1-P might lead to increased anabolism	Pre, sim	In vitro, mice
Guanosine	Similar to inosine	Pre, sim	In vitro, mice
GMP	Similar to guanosine after conversion of GMP to guanosine	Pre, sim	In vitro, mice
Deoxyinosine	Increased dRib-1-P leads to enhanced FdUMP	Pre, sim	In vitro, mice
<b>Pyrimidines</b>			
Thymidine <sup>7,23</sup>	Inhibition of 5FU breakdown	Pre, sim	Mice, patients
Uridine <sup>6,9,12,94-97,99,101</sup>	Rescue of normal tissue by competition of UTP with FUTP	Delayed	Mice, patients, in vitro
Cytidine <sup>12,96,99,101</sup>	Similar to uridine	Delayed	Mice, in vitro
<b>Antimetabolites</b>			
Methotrexate <sup>4,88</sup>	Increased 5FU anabolism due to enhanced PRPP	Pre	In vitro, animals, patients
PALA <sup>6,8,78,102</sup>	Decrease of uracil nucleotides leads to enhanced anabolism	Pre	In vitro, animals, patients
Allopurinol <sup>103</sup>	Postulated differential metabolism of 5FU in tumors and normal tissues is used, decreased toxicity	Sim	In vitro, animals, patients
Hydroxyurea <sup>105</sup>	Inhibition of ribonucleotide reduction prevents rescue by normal nucleotides	Post	In vitro
Dipyridamol <sup>106</sup>	Prevention efflux of FUR and FUdR	Sim	In vitro
Leucovorin <sup>5,33,35,38-41,107-113</sup>	Enhanced retention of FdUMP binding to thymidylate synthase in tumors	Pre, sim	In vitro, animals, patients

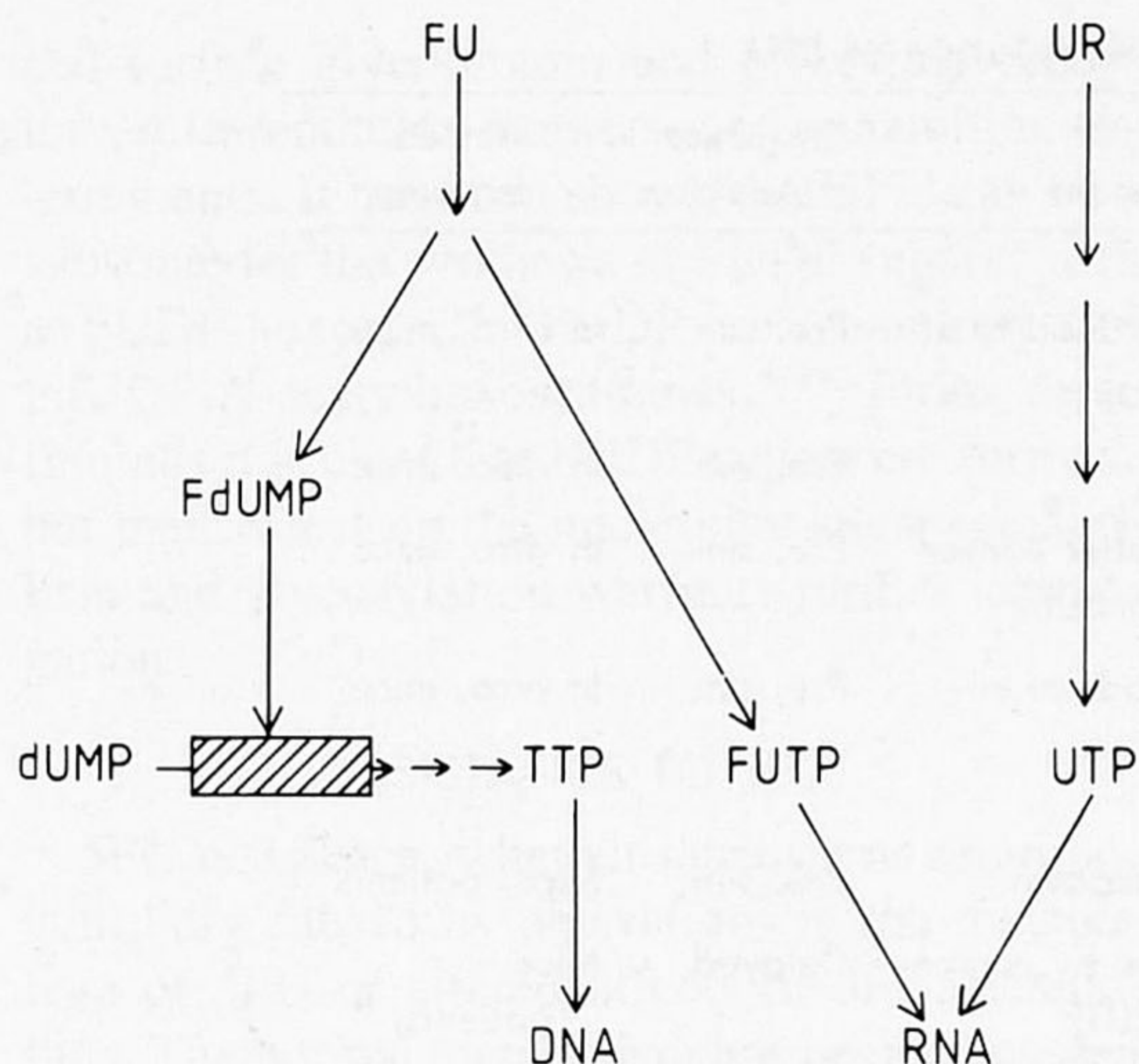
Abbreviations: pre, pretreatment; sim, simultaneous; post, posttreatment; rib-1-P, ribose-1-P; dRib-1-P, deoxyribose-1-P; GMP, guanosine-5'-phosphate.

depletion of PRPP leading to inhibition of activation.<sup>89,90</sup>

Combinations of 5FU with pyrimidines have been studied in both animal models and patients. 5FU combined with thymidine led to increased toxicity<sup>7,23</sup> caused by enhancement of the anabolism of 5FU in normal tissues. An interesting scientifically based combination is that of 5FU and uridine,<sup>8,9</sup> chosen on the hypothesis that 5FU antitumor activity consists mainly of inhibition of TS whereas 5FU toxicity is caused by incorporation of 5FU into RNA<sup>52,94</sup> (Fig 4); if high-dose uridine is administered several hours after 5FU, the binding of FdUMP to TS will not

be affected but UTP will replace FUTP in RNA.<sup>8,9</sup> Preliminary results of a phase I study<sup>11,12,95,96</sup> indicated that delayed uridine administration prevented myelosuppression induced by 5FU.<sup>95</sup> Since cytidine, too, can prevent 5FU toxicity in mice,<sup>10</sup> its clinical application should be considered.

The selectivity of uridine "rescue" might be related not only to a different mechanism of action in tumors and normal tissues, but also to the metabolism of uridine. Darnowsky and Handschumacher<sup>97,98</sup> showed that uridine concentrations in murine tissues are much higher than in plasma, and suggested a concentrative mecha-



**Fig 4. Proposed mechanism of uridine (UR) "rescue." FdUMP bound to TS will not be affected by delayed UR, while FUTP formed from UR will interact with FUTP for incorporation into RNA.**

nism for uridine uptake by tissues and especially by tumors.<sup>98</sup> This might be related to a selective effect of delayed uridine administration on 5FU-induced myeloid toxicity. The relative increase in plasma uridine is much higher than that occurring in tumor tissue.<sup>11,12,99</sup> If the concentrations are similar in plasma and bone marrow, uridine might have a stronger effect on bone marrow than on other tissues since it is an important precursor for pyrimidine nucleotide synthesis in lymphoid cells.<sup>100</sup> Uridine treatment was complicated by the development of fever in both humans and the rabbit,<sup>11,96</sup> whereas mice developed hypothermia.<sup>99</sup> Fever in humans could be prevented by a complicated schedule of intermittent administration.<sup>11,96</sup>

Although experimental data on MTX plus 5FU<sup>4</sup> have encouraged clinical studies, the results of treatment have been disappointing. There are some data indicating that a longer interval between MTX and 5FU may increase the response rate in humans.<sup>10,88</sup> The combination of N-phosphonacetyl-L-aspartate (PALA) and 5FU has given no clinical benefit.<sup>6,102</sup>

5FU and allopurinol were combined because metabolites of allopurinol were expected to inhibit 5FU anabolism catalyzed by OPRT in normal tissues but not in tumor tissues.<sup>103</sup> It was postulated that uridine phosphorylase would be important for 5FU anabolism in tumor tissue.<sup>104</sup>

This combination, which has not yet shown any clinical advantage, may require further evaluation when more is known about 5FU metabolism in human tumors and healthy tissues.

An interesting combination is that of 5FU and hydroxyurea,<sup>105</sup> which exploits the cell-phase specificity of 5FU at low concentrations.<sup>48</sup> The combination of 5FU and dipyridamole<sup>106</sup> is based on the nucleoside transport-inhibiting properties of dipyridamole, which does not affect 5FU uptake but inhibits efflux of FUR and FUDR. Neither of the last two combinations has received sufficient clinical investigation.

Selective rescue of healthy tissue and increased FdUMP binding to TS in tumors offer a favorable basis for combination with 5-FU. Foliates are known to prolong retention of the FdUMP-TS complex.<sup>33,38-41,107,108</sup> Leucovorin has been used for this purpose. In two murine colon cancer lines pretreatment with leucovorin increased the therapeutic effect of 5FU.<sup>109</sup> Phase I and II clinical trials of 5FU-leucovorin in patients with advanced colorectal cancer showed response rates of up to 40%, which is considerably higher than those obtained with 5FU alone.<sup>5,110,111</sup> The response rate in randomized trials comparing 5FU with 5FU plus leucovorin in colorectal cancer lay between 40% and 48% for the combination and between 10% and 15% for single-agent 5FU.<sup>112,113</sup> Combination of 5FU and leucovorin with delayed uridine may also enhance antitumor activity and prevent toxicity.<sup>109</sup>

Of the combinations with 5FU tested so far, that of leucovorin with 5FU is the most promising. Since a number of different schedules are in use results might be improved by using more appropriate schedules. Both preclinical<sup>109</sup> and clinical<sup>110-113</sup> data justify the conclusion that pretreatment with leucovorin looks promising. The clinical schedule of a two-hour leucovorin infusion with a mid-infusion bolus injection of 5FU appears to give the best results, and this is in agreement with the preclinical data.

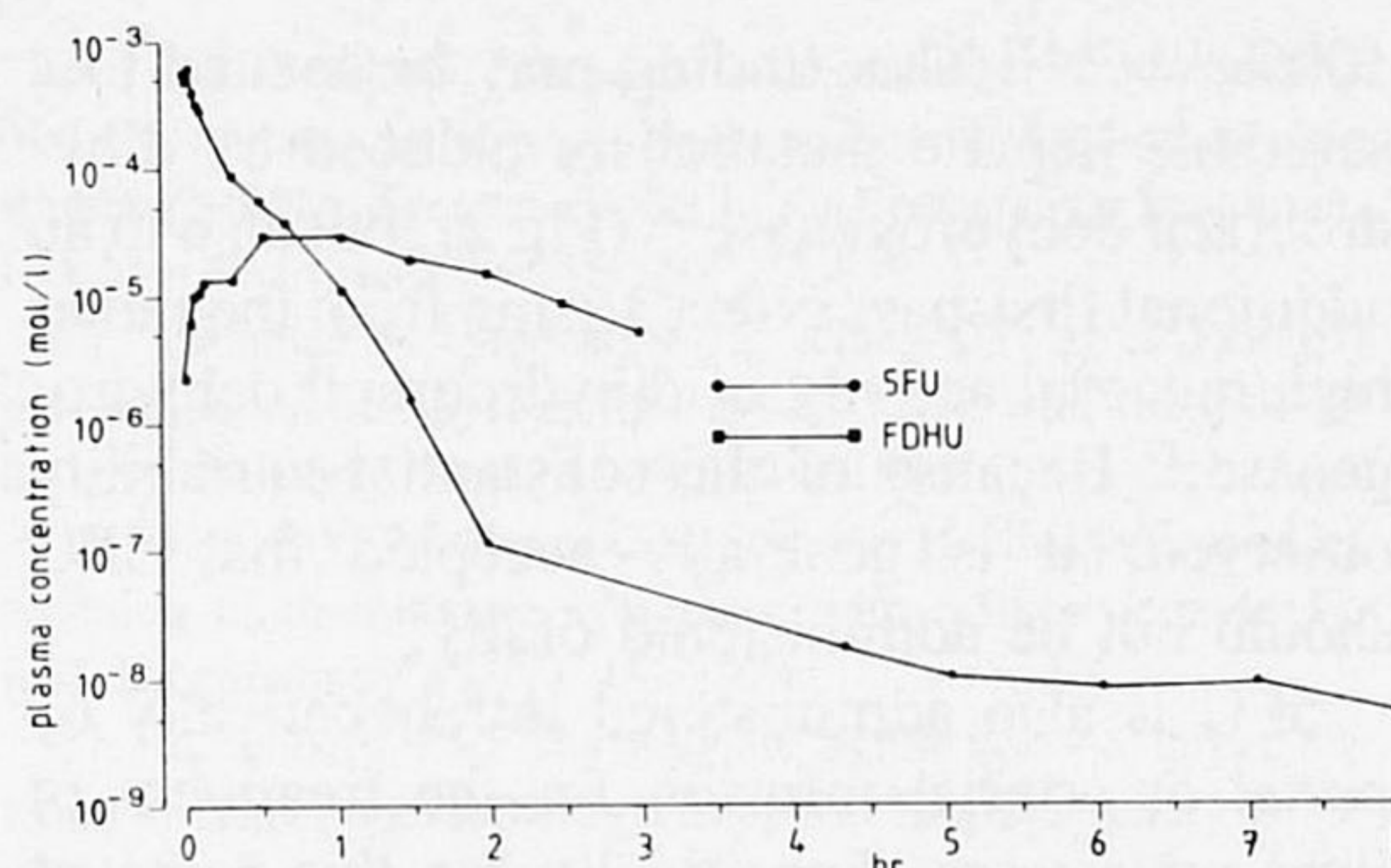
#### PHARMACOKINETICS

The pharmacokinetics of 5FU have been reviewed extensively by others.<sup>114,115</sup> Initial assays of 5FU lacked either specificity or sensitivity.<sup>115</sup> Currently, the most widely used method is high performance liquid chromatography (HPLC) combined with UV absorption with a detection

limit of 0.5 to 1.0  $\mu\text{mol/L}$  5FU.<sup>12,115,116</sup> A lower detection limit for 5FU (down to  $3 \times 10^{-9}$  mol/L; 0.3 ng/mL) can be achieved with gas-chromatography-mass-spectrometry (GC-MS).<sup>117,118</sup> Most of the pharmacokinetic studies were restricted to three hours or less, but with the sensitive GC-MS method 5FU plasma concentrations can be followed for at least eight hours after the injection,<sup>118</sup> which makes it possible to perform long-term pharmacokinetic studies.<sup>119</sup>

The pharmacokinetics of single-dose 5FU administered as an intravenous (IV) bolus injection in doses ranging between 300 and 600 mg/m<sup>2</sup> have been studied in detail,<sup>114,120</sup> and the findings are summarized in Table 3. Rapid distribution over a large volume and rapid elimination have been reported to follow peak levels lying in the millimolar range (Fig 5). The total clearance was rather high (Table 3), and comparable to the liver flow, but hepatic extraction has been estimated to be 50%.<sup>123</sup> Yet the liver is the organ with the highest level of dihydrouracil dehydrogenase activity.<sup>18</sup> The kidneys, in which the activity of this enzyme is also high, contribute to elimination by both degradation and active renal excretion, about 20% of 5FU being excreted as the parent drug.<sup>115</sup> The lungs have also been reported to be a major site of 5FU clearance.<sup>114,115,124,125</sup> Collins et al<sup>120</sup> have shown that a saturable two-compartment model can be used to describe the elimination kinetics of 5FU. Calculation gave an apparent Km of 15  $\mu\text{mol/L}$  in plasma.

Nonlinearity of 5FU kinetics has been described by several authors,<sup>114,115,122,124,125</sup> and is related to the saturation of 5FU catabolism. Studies on the pharmacokinetics of 5FU catabolites have been hampered by the lack of appropriate detection methods. A relatively insensitive method applied HPLC (Fig 5), and a more sensitive



**Fig 5. Representative curves for 5FU and F-DHU post-IV bolus injection of 5FU (500 mg/m<sup>2</sup>) to a colorectal cancer patient. 5FU was determined with GC-MS.<sup>118</sup> F-DHU was determined with HPLC with UV detection at 210 nm (Van Groeningen et al<sup>121</sup>); due to F-DHU instability in plasma<sup>19,122</sup> extractions and measurements were performed as soon as possible.**

method used GC with electron capture detection.<sup>19</sup> With <sup>19</sup>F-NMR, the other catabolites could also be demonstrated in human plasma.<sup>20</sup> Analysis of the cumulative urinary excretion of these catabolites showed that F-BAL was the major one followed by FUPA. F-DHU was a minor constituent of the urinary excretion products.<sup>126</sup> The high detection limit of 10  $\mu\text{mol/L}$ , which is also in the range of the peak plasma concentration,<sup>126</sup> is the major limitation of this technique. Improvement might permit investigation of the dose-dependency of 5FU pharmacokinetics in humans in relation to the in vivo behavior of F-DHU.

There is no evidence that continuous IV administration of 5FU is associated with a higher antitumor efficacy than bolus administration. These two schedules give quite different types of toxicity, mucositis being dose-limiting for infusion and myelosuppression for the bolus injection.<sup>114</sup> The pharmacokinetics of continuous 5FU infusion differ significantly from those of the IV bolus, the former having a much higher clearance value of 2 to 6 L/min,<sup>120</sup> which considerably exceeds the hepatic flow of 1.5 L/min and approaches the cardiac output. This high clearance level can be explained mainly by the high pulmonary extraction.<sup>114,115,120,124</sup> Pulmonary extraction accounted for a clearance higher than the cardiac output.<sup>115</sup> However, it has been shown that the liver and kidneys also contribute to clearance.

In the past, 5FU was administered orally; however, there is a marked variability in its bioavailability, ranging between 28% and

**Table 3. Pharmacokinetic Parameters of 5FU Administered as an IV Bolus Injection**

Parameters	Value
Peak levels	$10^{-4} - 10^{-3}$ mol/L
T <sub>1/2</sub> , $\beta$	10-20 min
MRT	12-23 min
V <sub>D</sub>	8-54 L
Total clearance	0.5-2.0 L/min

Note. Values are from doses varying between 300 and 600 mg/m<sup>2</sup>.<sup>114,115,122,124,125</sup>

Abbreviations: V<sub>D</sub>, volume of distribution; MRT, mean residence time.

100%.<sup>113,114,127</sup> This finding may be related to a saturable hepatic metabolism induced by dihydrouracil dehydrogenase<sup>120</sup> (Fig 2), but also to an additional first-pass effect arising from the rather high mucosal activity of dihydrouracil dehydrogenase.<sup>17</sup> Because of the substantial variability observed, it is generally accepted that 5FU should not be administered orally.

5FU is also administered intrahepatically by portal or arterial infusion for the treatment of liver metastases. Specifically for this route of administration, hepatic extraction and the rate of infusion determine the systemic availability. The use of rapid intrahepatic arterial infusions at a high dose (1,000 mg/m<sup>2</sup>/d) gave relatively low hepatic extraction amounting from 20% to 60%,<sup>123,128</sup> which led to a high systemic availability. With a slower infusion rate and/or lower doses (780 mg/m<sup>2</sup>/d), hepatic extraction exceeded 90%<sup>123,128</sup> and this was accompanied by low systemic toxicity. More evidence pointing to hepatic saturation was provided by the observation that 5FU levels rose significantly during the infusion.<sup>128</sup> This new pharmacokinetics information makes it possible to design better 5FU schedules for the treatment of liver metastases.

Intraperitoneal infusions offer the possibility of achieving higher drug concentrations, give optimal exposure of tumor tissue within the abdominal cavity, and provide more effective treatment of not only the liver (via the portal vein) but also of peritoneal metastases. 5FU can be administered by intraperitoneal peritoneal dialysis<sup>129</sup> or via implantable devices.<sup>130</sup> Intraperitoneal 5FU was cleared at a rate of 14 mL/min, and 82% of the 5FU administered was absorbed within four hours. Hepatic extraction was calculated to be 67%.<sup>129</sup> A 2- to 3-log difference was observed between peritoneal and plasma 5FU concentrations.<sup>129,130</sup> With continuous infusion the mean

steady-state level of 5FU in the intraperitoneal cavity was 622  $\mu$ mol/L.<sup>130</sup> Total body clearance ranged from 0.9 to 16.5 L/min,<sup>129,130</sup> which is similar to the rate seen with continuous IV infusion of 5FU. Clearance decreased with increasing 5FU concentration, which is consistent with saturable or nonlinear 5FU pharmacokinetics. It might be worthwhile to study this method of administration in an adjuvant setting after surgical removal of Dukes B2 and C colorectal cancers.

### CONCLUSIONS

Studies on the biochemistry of 5FU have yielded new detailed information on the mechanism of action of 5FU and about resistance to 5FU. Although valuable, most of this information was obtained from studies performed in vitro or in animals. Detailed biochemical studies in humans have been undertaken but are still scarce. Pharmacokinetic studies have supplied a basis for the application of new administration schedules, but improvement of the antitumor effect has not been achieved yet. Promising clinical results have been reported for treatment with 5FU plus leucovorin. Biochemical modulation seems to be the approach most likely to improve the therapeutic efficacy of 5FU; further research in this area is urgently needed.

More information about the intratumoral metabolism of 5FU is needed as well as in normal tissues. Analysis of the pharmacodynamic behavior of 5FU metabolites, such as FdUMP, and their binding to TS is essential, as is a more detailed analysis of the mechanisms leading to resistance and toxicity in humans. This information must be obtained before the selectivity of 5FU can be improved.

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