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Dose-Dependent Elimination of 5-Fluoro-2′-deoxyuridine in the Monkey[1] (42487)

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Abstract. The kinetics of 5-fluoro-2′-deoxyuridine (FdUrd) and 5-fluorouracil (FUra) disposition after bolus intravenous injection were determined in anesthetized rhesus and cynomolgus monkeys. FdUrd disappearance from plasma was an apparent triexponential process with average half-lives of 0.5, 2, and 8 min; FUra disappearance was biphasic with average half-lives of 2 and 13 min. After FdUrd injection, FUra reached peak plasma concentrations of 15–30% of the initial FdUrd concentrations within 3 min, and then disappeared more slowly than FdUrd. Total FdUrd clearance fell from 105 to 73 to 56 ml/kg/min as the dose increased from 10 to 20 to 40 mg/kg. Metabolic clearance was about 85% of total clearance and fell similarly with increasing dosage. Total and metabolic FUra clearances were about 30% of FdUrd values at an equimolar dose. Renal FdUrd clearance exceeded glomerular filtration rate and was decreased by probenecid, indicating tubular secretion; renal FUra clearance was close to glomerular filtration rate. There was no apparent correlation between dose and renal clearance or volume of distribution. It was concluded that FdUrd, like FUra, is eliminated primarily by a dose-dependent process. The metabolic basis of the dose-dependent kinetics remains to be determined.

5-Fluorouracil (FUra) and its nucleoside analog 5-fluoro-2′-deoxyuridine (FdUrd), introduced nearly 30 years ago by Heidelberger *et al.* (1, 2), are used in the treatment of several types of cancer (3). Relatively low response rates and short durations of response have led to continuing attempts to improve the therapeutic usefulness of the drugs (4–6). Metabolism of the fluoropyrimidines is a critical determinant of activity. Both are prodrugs and must be metabolically activated in target cells to cytotoxic nucleotides, which produce the observed biological effects. FdUrd, in a reaction catalyzed by thymidine kinase, is directly converted to 5-fluoro-2′-deoxyuridine monophosphate (FdUMP), a potent inhibitor of thymidylate synthetase and consequently of DNA synthesis (7). FUra, through a series of reactions, is also converted to (FdUMP) (8). In addition, FUra can be metabolized to 5-fluorouridine triphosphate, which is incorporated into RNA, contributing to the cytotoxic effects. Metabolism by opposing catabolic pathways leads to the formation of inactive products. FdUrd is converted by nucleoside phosphorylases (9) to FUra, which in turn undergoes reduction and hydrolysis with the ultimate formation of carbon dioxide, ammonia, and α-fluoro-β-alanine (10). In some species, urea is also a major catabolic product (11).

In vitro, FdUrd, which is metabolically closer to the cytotoxic nucleotide FdUMP, is a much more potent anticancer agent than FUra (12, 13). However, *in vivo* the drugs are essentially equipotent after iv bolus administration (2, 14). This observation has been attributed to the rapid conversion of FdUrd to FUra *in vivo* (8). Although the kinetics of this conversion have not been studied, FUra has been detected in blood (15), urine (16), and tissues (17) after FdUrd administration. For example, Ensminger *et al.* (15) demonstrated FUra in blood as early as 30 sec after FdUrd injection in cancer patients. Further, Mukherjee *et al.* (16) found that 67% of an iv dose of [2-^{14}C]FdUrd was excreted in the expired air as $^{14}CO_2$ in cancer patients over a 12-hr period; the $^{14}CO_2$ was presumably derived from [2-^{14}C]FUra formed from the [2-^{14}C]FdUrd.

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The pharmacokinetics of FUra have been extensively investigated, but relatively little information is available on the kinetics of FdUrd disposition. The present studies in the monkey were undertaken to determine whether the elimination of FdUrd, like that of FUra, is dose dependent and to further examine the renal handling of the drugs.

Materials and Methods. *Animals.* Rhesus monkeys (*Macaca mulatta*), originally imported from India, were retired breeders and were kindly provided by Dr. Robert D'Amato of Procter and Gamble (Cincinnati, OH) and by the University of Louisville Animal Care Center. Cynomolgus monkeys (*Macaca fasicularis,* also known as the crab-eating macaque) were kindly provided by Dr. Theodore Lawwill, presently at the Department of Ophthalmology, University of Kansas. The cynomolgus monkeys had received retinal exposure to laser light about 2 weeks before use in this study. No systemic effects were observed. Experiments were performed in nine rhesus and five cynomolgus monkeys; analogous results were obtained in both species. The animals weighed 5.0 ± 0.5 kg (mean ± SE).

Pharmacokinetic experiments. Monkeys received food and water *ad libitum* until the evening before the experiment and only water thereafter. Animals of either sex were anesthetized with ketamine hydrochloride, 5 to 8 mg/kg im, supplemented with sodium pentobarbital iv as needed. After insertion of a tracheal tube and removal of the eyes for electron microscopic studies, the ureters and common bile duct were cannulated and the cystic duct was ligated. A branch of a femoral vein was cannulated for fluid and drug administration, and a branch of a femoral artery was cannulated for blood sampling. Acetate Ringer solution, containing up to 5% mannitol, was infused iv at a rate sufficient to produce a sustained urine flow of 1 to 3 ml/min. The acetate Ringer was composed of the following (in m*M*): NaCl, 120.9; KCl, 16.2; $MgSO_4$, 1.2; $CaCl_2$, 0.5; Na_2HPO_4, 8.2, KH_2PO_4, 1.8; Na acetate, 9.9.

The fluoropyrimidine, dissolved in saline, was injected iv over exactly 10 sec, with time zero taken as the midpoint of the injection period. Arterial blood samples were collected in heparinized tubes, which were immediately placed in ice and centrifuged at 5°C within 15 min to minimize the metabolism of FdUrd to FUra by granulocytes (18) after removal from the body. Preliminary experiments, in which the metabolism of FdUrd to FUra by whole blood *in vitro* was assessed using a high-performance liquid chromatographic method for fluoropyrimidine analysis, showed conversions of 2% of the total FdUrd (initial concentration 203 μM) in 30 min at 5°C and 17% in 30 min at 37°C. Blood samples were taken every 15 to 30 sec for the first 5 min, and then less frequently over the next 90 min. The volume collected increased from 0.3 ml for initial samples up to 4 ml for final samples. A total of 26–30 ml, equivalent to about 10% of initial blood volume (19), was collected; removed blood was replaced with iv fluids. Serial timed urine and bile samples were collected in tared tubes; volume was determined by weighing. Urine collection intervals gradually increased from 30 sec for early samples to 15 min for later samples. Bile was collected over 15-min intervals.

In four animals a second experiment of the same procedure was performed 30 min later. In two cases, probenecid, an inhibitor of the organic anion secretory system of the proximal renal tubule (20), was administered iv (50 mg/kg) before the second experiment.

Chemical analyses. FdUrd and FUra concentrations were determined by a gas–liquid chromatographic method described in detail previously (21). Briefly, plasma, urine, and bile samples, to which internal standard had been added (5-chloro-2′-deoxyuridine for FdUrd analyses and 6-methyluracil for FUra analyses) were prepared for gas–liquid chromatography by sequential cation-exchange and anion-exchange column chromatography. The extracted fluoropyrimidines were derivitized by on-column reaction with *p*-tolyltrimethylammonium hydroxide in methanol, and quantified by nitrogen-sensitive detection. Columns packed wtih 3% SP-2100 on Supelcoport were used for FdUrd analyses and columns packed with 0.75% Carbowax 20M–5% KOH on Chromsorb G (22) were used for FUra analyses. The fluoropyrimidines and internal standards were well separated from the potentially interfering endogenous compounds 2′-deoxyuridine, uridine, and uracil. Standards were prepared by addition of varying known amounts of FdUrd or FUra and a constant

amount of internal standard to blank plasma, urine, or bile. Linear standard curves (peak area ratio method) were obtained for plasma containing 0.1–81 μM FdUrd or 1.5–7.9 μM FUra, and for urine containing 0.8–4.0 μM FdUrd or 1.5–7.9 μM FUra. The correlation coefficients of the standard lines were typically 0.9999 or higher and the SE of peak area ratios were less than 10% of the mean (triplicate determinations). The amount of drug in experimental samples was calculated from the slope and vertical axis intercept of the standard curve, and the peak area ratio of the sample. A measurable and predictable amount of FUra, equivalent to 4 to 7% of the FdUrd injected, was formed from FdUrd on the gas–liquid chromatographic column, requiring correction of FUra concentrations measured in the presence of FdUrd (21). FUra derived from chromatographic decomposition of FdUrd accounted for 20, 10, and 3% of total FUra detected at 1, 2, and 5 min, respectively, after FdUrd administration. Although all plasma and urine FUra concentrations were corrected for FdUrd decomposition, there was little difference between corrected and non-corrected values beyond 5 min after FdUrd injection because the FUra concentrations were 5- to 10-fold the simultaneous FdUrd concentrations at those times.

Plasma protein binding of FdUrd was determined by the method of Toribara *et al.* (23).

Chemicals. FdUrd was kindly provided by Dr. W. E. Scott of Hoffmann–LaRoche, Inc. (Nutley, NJ). All other pyrimidine bases and nucleosides were obtained from Sigma Chemical Co. (St. Louis, MO). The ion-exchange resins were obtained from Bio-Rad Laboratories (Richmond, CA), the *p*-tolyltrimethylammonium hydroxide was from Southwestern Analytical Chemicals (Austin, TX), and the gas–liquid chromatographic packings were from Supelco, Inc. (Bellefonte, PA).

Pharmacokinetic calculations. Plasma drug concentrations were plotted against time on semilogarithmic paper. FdUrd plasma disappearance curves were readily resolved into three apparent first-order phases, and FUra curves into two phases, by the method of residuals (24). The disposition rate constant and coefficient of each phase were estimated with the ESTRIP computer program (25). Using these values as initial parameter estimates, plasma data were fitted to triexponential or biexponential elimination equations by the NONLIN 84 computer program (26). The terminal elimination rate constant, β, was also estimated from urine data from the slope ($-\beta/2.303$) of the terminal portion of a semilogarithmic plot of amount of drug remaining to be excreted vs time (24).

Total body clearance (Cl_{total}) of FdUrd was calculated as dose/AUC, where AUC is the total area under the plasma concentration vs time curve. AUC was estimated from $A'/\alpha' + A/\alpha + B/\beta$, where α', α, and β are the first-order disposition rate constants, and A', A, and B are the corresponding zero-time intercepts. AUC for FUra was calculated as $A/\alpha + B/\beta$. Estimation of AUC by the trapezoidal rule (24) gave analogous results. Cl_{total} values calculated by the two AUC estimation methods varied by an average of 3.4% for FdUrd experiments and 7.3% for FUra experiments. Renal clearance (Cl_{renal}) was calculated as the product of the fraction of the dose excreted unchanged in the urine and Cl_{total}. Metabolic clearance ($Cl_{metabolic}$) was estimated as the difference between Cl_{total} and Cl_{renal}. The apparent overall or pseudo-steady-state volume of distribution (V_d) was calculated as Cl_{total}/β (24). Half-life ($t_{1/2}$) was calculated as ln 2 divided by the first-order disposition rate constant.

The fraction of the iv FdUrd dose converted to FUra was estimated from $Cl_{FUra}(AUC_{FUra})_{FdUrd}/dose_{FdUrd}$, where Cl_{FUra} is Cl_{total} for FUra obtained in separate experiments with equimolar doses of FUra, and $(AUC_{FUra})_{FdUrd}$ is the total area under the plasma FUra concentration vs time curve after FdUrd administration (24).

Results. In the presentation of results all pharmacokinetic values are referenced to total plasma drug concentrations. The plasma protein binding of FdUrd was negligible, ranging from 0 to 2.5% over the concentration range of 12 to 1200 μM. Other investigators have reported no significant binding of FUra in human, dog, or rat plasma (27–29). Pharmacokinetic parameters for FdUrd and FUra elimination are presented in Tables I–III.

FdUrd experiments. The time course of changes in plasma FdUrd and FUra concentrations after bolus iv administration of FdUrd (20 mg/kg, 81.2 μmol/kg) is shown in Fig. 1A. Curves obtained with higher and lower doses

TABLE I. PLASMA PHARMACOKINETIC PARAMETERS FOR INTRAVENOUS FdUrd AND FUra IN THE MONKEY

	FdUrd			Fura
Parameter Dose, mg/kg:	10 (n = 2)	20 (n = 3)	40 (n = 2)	10 (n = 4)
Parent drug[a]				
A′, μM	361 (361)[b]	689 (518–912)	4430 (3980–4880)	
A	17[c]	189 (51–434)	625 (425–825)	685 (534–846)
B	6.7 (3.4–10)	18 (12–27)	43 (35–51)	76 (36–109)
$t_{1/2}\alpha'$, min	0.58 (0.53–0.62)	0.64 (0.19–0.93)	0.16 (0.11–0.21)	
$t_{1/2}\alpha$	2.5[c]	2.1 (1.1–3.6)	1.6 (1.6)	2.1 (1.4–2.6)
$t_{1/2}\beta$	6.8 (5.4–8.2)	8.4 (3.6–11)	8.0 (7.3–8.7)	14 (10–16)
Vd, ml/kg	1038 (789–1287)	869 (394–1072)	640 (631–649)	465 (349–529)
FUra derived from FdUrd				
Peak conc, μM	103 (101–104)	165 (139–198)	352 (277–426)	
$t_{1/2}\beta$, min	8.7 (7.3–10)	18 (14–25)	19 (17–21)	
AUC, nmol · min/ml	690 (579–800)	1855 (1601–1938)	5666 (5621–5711)	

[a] NONLIN 84 computer program estimates.
[b] Values are means (range).
[c] One plasma disappearance curve resolved into only two components.

had a qualitatively similar appearance. The FdUrd concentration decreased rapidly to about 1 to 2% of the initial concentration over the first 10 min, and then decreased more slowly with a terminal half-life ($t_{1/2}\beta$) of 3.6 to 10.9 min. The logarithm plasma concentration vs time curves could be resolved into three separate apparent first-order phases, designated β, α, and α'. There was no apparent correlation between dose and the $t_{1/2}$'s of the three phases (Table I). However, mean Cl_{total} of FdUrd decreased progressively from 105 to 73 to 56 ml/kg/min as the dose was increased from 10 to 20 to 40 mg/kg (Table III). Similarly, mean $Cl_{metabolic}$, which was about 85% of Cl_{total}, decreased progressively from 92 to 65 to 46 ml/kg/min as the dose was increased from 10 to 20 to 40 mg/kg. These observations suggested the possibility of a dose-dependent process in FdUrd elimination. Dose-dependent elimination was also indicated by the lack of superposition of logarithm FdUrd concentration/dose vs time curves obtained with different doses (Fig. 2A).

TABLE II. URINARY PHARMACOKINETIC PARAMETERS FOR INTRAVENOUS FdUrd AND FUra IN THE MONKEY

	FdUrd			FUra
Parameter Dose, mg/kg:	10 (n = 2)	20 (n = 3)	40 (n = 2)	10 (n = 4)
Parent drug:				
$t_{1/2}\beta$[a] min	12 (12–13)[b,c]	9.3 (1.4–12)	13 (7.3–18)	17 (9.8–21)
% of dose excreted unchanged	15 (15)	12 (9–14)	18 (17–18)	16 (13–19)
FUra derived from FdUrd				
$t_{1/2}\beta$, min	7.9 (7.3–8.4)	16 (9.6–26)	17 (16–17)	
% of dose excreted as FUra	5.0 (4.2–5.8)	5.9 (2.6–8.9)	7.8 (7.4–8.2)	

[a] Obtained from terminal slope of amount of drug remaining to be excreted vs time plots.
[b] Values are means (range).
[c] In one case urinary FdUrd data were obtained from second experiment in the same animal.

TABLE III. CLEARANCES OF INTRAVENOUS FdUrd AND FUra IN THE MONKEY

Clearance, ml/kg/min	FdUrd Dose, mg/kg: 10 (n = 2)	FdUrd 20 (n = 3)	FdUrd 40 (n = 2)	FUra 10 (n = 4)
Cl_{total}[a]	105 (101–109)[b]	73 (68–75)	56 (52–60)	24 (22–26)
Cl_{renal}[c]	13 (11–15)	8.4 (5.9–11)	9.8 (9.0–11)	3.7 (3.1–4.1)
$Cl_{metabolic}$[d]	92 (86–98)	65 (63–66)	46 (43–49)	20 (18–22)

[a] Calculated from dose/AUC.
[b] Values are means (range).
[c] Calculated from Cl_{total} × fraction of dose excreted unchanged.
[d] Calculated from $Cl_{total} - Cl_{renal}$.

Semilogarithmic plots of the amount of FdUrd remaining to be excreted vs time after injection yielded multiphasic curves with average terminal excretion $t_{1/2}$'s of 9 to 13 min (Fig. 1B, Table II). These values were somewhat higher than the average $t_{1/2}\beta$ values of 7 to 8 min for plasma disappearance. The Cl_{renal} of FdUrd was 6 to 15 ml/kg/min and accounted for excretion of 9 to 18% of the dose in an unchanged form (Tables II and III). Most of the urinary excretion occurred in the first 10 min after injection. There was no clear relation between dose and Cl_{renal}.

FUra appeared rapidly in the plasma after FdUrd injection (Fig. 1A). The FUra concentration was about 5 to 20% of the FdUrd concentration at 15 sec after dosing and increased to a peak value about 15 to 30% of the initial FdUrd concentration within 3 min. The FUra concentration decreased more slowly than the FdUrd concentration; the terminal plasma $t_{1/2}$ was 7 to 25 min. The pattern of urinary excretion of FUra closely paralleled that of FdUrd (Fig. 1B) and accounted for elimination of 3 to 9% of the FdUrd dose. The terminal urinary excretion $t_{1/2}$'s for FUra ranged from

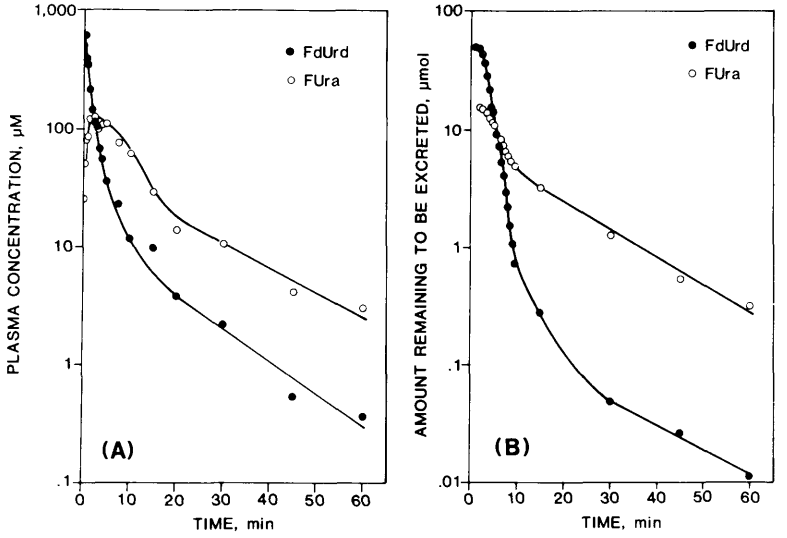

FIG. 1. Kinetics of FdUrd elimination in the monkey. Representative experiment in which FdUrd (20 mg/kg, 81.2 μmol/kg) administered by iv bolus to a 7-kg rhesus monkey. (A) Time course of changes in plasma FdUrd and FUra concentrations; (B) time course of urinary excretion of FdUrd and FUra expressed as amount of drug remaining to be excreted vs time.

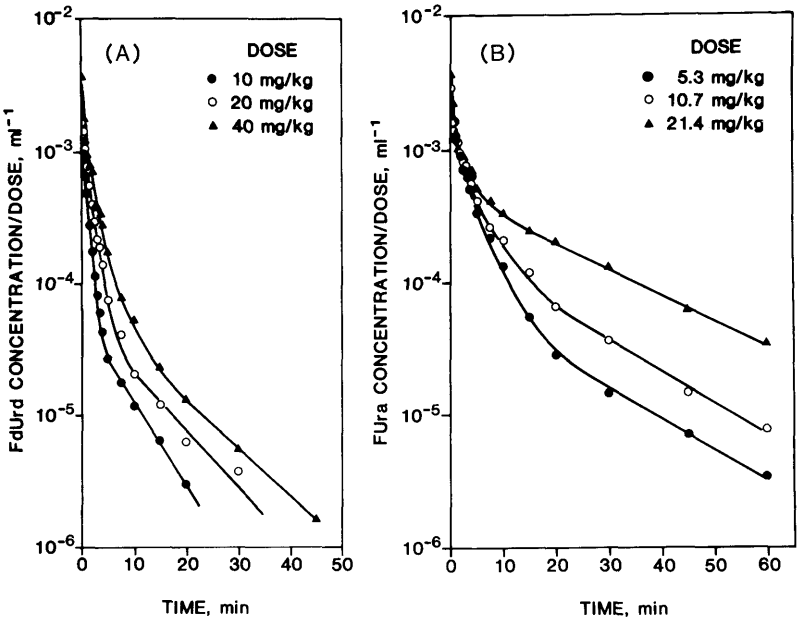

FIG. 2. Lack of superposition of logarithm plasma concentration/dose vs time curves obtained with different doses of FdUrd and FUra. (A) FdUrd: data symbols represent average values for all experiments with FdUrd; (B) FUra: data symbols for 10.4 mg/kg dose represent average values for four animals; other curves represent single experiments at each dose.

7 to 26 min, and were close to the terminal plasma $t_{1/2}$'s. Biliary excretion of FdUrd and FUra was negligible, accounting for elimination of less than 0.01% of the dose. The V_d of FdUrd ranged from 394 to 1287 ml/kg (Table I). There was no apparent correlation between FdUrd dose and V_d.

Second FdUrd experiments were performed in four animals, two of which received probenecid, 50 mg/kg iv at the end of the first FdUrd experiment. Because different doses of FdUrd were used in nontreated (10 and 40 mg/kg doses) and probenecid-treated (20 mg/kg doses) groups, the results were expressed as percentages of control (first experiment) values. In nontreated animals Cl_{total} was 27% lower and Cl_{renal} was 13% lower in the second experiment than in the first, but there was no change in the fraction of the drug excreted unchanged in the urine. In contrast, in probenecid-treated animals there was a greater (43%) decrease in Cl_{total} in the second experiment due mainly to a dramatic (84%) decrease in Cl_{renal}, which was associated with a 75% decrease in the fraction of the dose excreted unchanged in the urine.

FUra experiments. The time course of plasma disappearance of FUra after bolus iv injection (10.7 mg/kg, 82.3 μmol/kg) is shown in Fig. 3A. FUra, like FdUrd, disappeared rapidly after injection; by 10 min the concentration was about 8% of the initial value. The plasma disappearance curves could be resolved into two logarithm-linear components with initial and terminal $t_{1/2}$'s of 0.9 to 2.6 min and 8.8 to 16.1 min, respectively. Cl_{total} and $Cl_{metabolic}$ of FUra were only about 30% of the values obtained with an equimolar dose of FdUrd. To confirm the dose dependence of FUra elimination, as shown previously in many species (27–29), single additional experiments were performed with FUra doses of 2.6, 5.3, and 21 mg/kg (20, 40, and 165 μmole/kg). Dose dependence was suggested by the lack of superposition of logarithm FUra/dose vs time curves obtained with the different doses (Fig. 2B). Also, Cl_{total} fell progressively from 31 to 20 ml/kg/min, and $Cl_{metabolic}$ fell

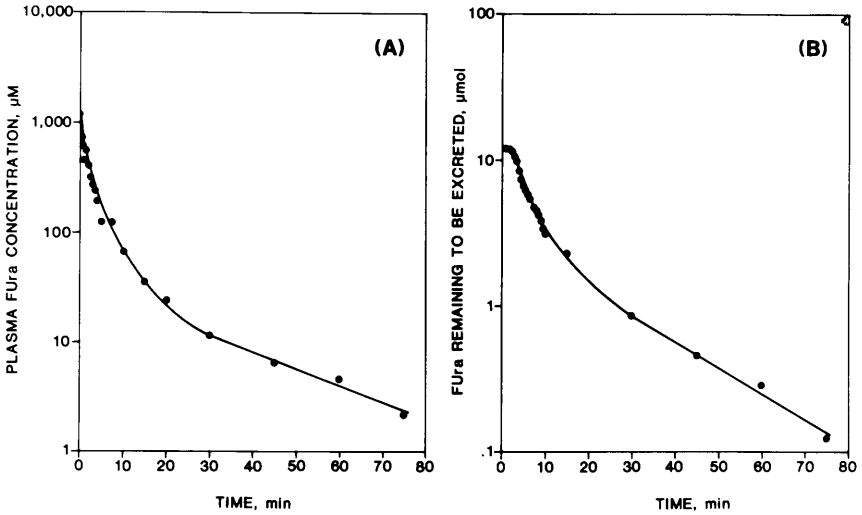

FIG. 3. Kinetics of FUra elimination in the monkey. Representative experiment in which FUra (10.7 mg/kg, 82.3 μmol/kg) administered by iv bolus to 5-kg rhesus monkey. (A) Time course of plasma FUra disappearance; (B) time course of urinary excretion of FUra expressed as amount of drug remaining to be excreted vs time.

from 28 to 18 ml/kg/min, as the dosage increased from 2.6 to 21 mg/kg.

The pattern of urinary excretion of FUra (Fig. 3B) was similar to that obtained with FdUrd. The Cl_{renal} of FUra was 1.5 to 4.1 ml/kg/min and resulted in the excretion of 8 to 19% of the dose in an unchanged form. Most excretion occurred over the first 10 min. The terminal urinary excretion $t_{1/2}$'s of 9 to 21 min were generally in close agreement with terminal plasma elimination $t_{1/2}$'s. No apparent dose dependency of urinary excretion was demonstrated. The V_d of FUra was 392 to 561 ml/kg and had no apparent correlation with dose.

Discussion. The overall patterns of disposition of FdUrd and FUra are similar in many respects, but there are large differences in the actual rates of elimination and possibly in the mechanisms of renal handling. The results of the present study demonstrate rapid and extensive breakdown of FdUrd to FUra, adding support to the hypothesis that this conversion is the basis for the observed equipotence of the two drugs after bolus administration *in vivo* (2, 14). Although the results indicate that metabolism by a dose-dependent process is the predominant route of elimination of FdUrd, significant amounts of active drug are also excreted in the urine.

Plasma disappearance. The very brief initial phase (α') of FdUrd disappearance was possibly a consequence of the early sampling times, reflecting the first pass of the drug bolus through the arterial sampling site before complete mixing in the plasma had occurred. The second phase (α) of disappearance was attributed to concomitant distribution and metabolism, on the basis of the rapid appearance of FUra in high concentrations. This phase contributed prominently to the decrease in plasma FdUrd concentration to about 1% of the initial value over the first 10 min after injection. The terminal phase (β) of disappearance, which became predominant at about 5 to 15 min, may be a reflection of the slow return of FdUrd to plasma from intracellular sites. Release from cells could be due to dissociation from binding sites or degradation of nucleotide metabolites, which because of large size and ionic charge are restricted to an intracellular location (30). Alternatively, inhibition of nucleoside phosphorylases by the reaction product FUra could result in a slowing of disappearance of the substrate FdUrd. This possibility was felt to be less likely, however, since the

β-phase did not become the major determinant of plasma disappearance until 7 to 20 min after the FUra peak, and by this time the FUra concentration had decreased to about 10 to 20% of the peak value.

In an early study in humans, Clarkson *et al.* (31) found a monophasic decrease in combined FdUrd and FUra plasma concentration with a $t_{1/2}$ of about 15 min. The failure to detect earlier elimination phases was probably due to the lack of early time points. Collins *et al.* (32) have pointed out that early samples are important in pharmacokinetic studies with rapidly eliminated drugs, such as FdUrd and FUra, since the early concentrations contribute greatly to calculations of area under the curve. In the present studies 78 to 96% of the total area under the curve was contributed by the plasma concentrations over the first 5 min. Omission of the early time concentrations from the data analysis would thus result in lower AUC and higher clearance estimates.

The biphasic plasma disappearance of FUra in the monkey is analogous to results obtained in humans (27). The terminal $t_{1/2}$ of 9 to 16 min in the monkey was close to the values of 8 to 20 min reported in the human (32) and 15 min in the rat (29).

The average V_d of FdUrd during the β-phase of elimination (790 ml/kg) was close to total body water volume of 693 ml/kg in the monkey (19), while $V\beta$ for FUra was about 70% of total body water volume. Widely varying $V\beta$ values have been obtained for FUra in different studies (32); the value obtained in the monkey is close to average values reported for humans.

The plasma clearance values provide some indirect information on the possible sites of removal of the fluoropyrimidines. At the lower doses of FdUrd, Cl_{total} was very high, exceeding blood flow to any individual organ except the lung, and indicating that elimination must occur at multiple sites. This is consistent with the observed simultaneous metabolism and renal excretion. $Cl_{metabolic}$ (60 to 82 ml/kg/min) exceeded hepatic plasma flow (19) by five- to sixfold, indicating that FdUrd metabolism is largely extrahepatic. This is consistent with the widespread distribution of the nucleoside phosphorylases in the body (9). One site of extrahepatic metabolism, as found in our preliminary studies, is the bloodstream. Woodman *et al.* (18, 33) have shown that circulating granulocytes contain thymidine phosphorylase, a nucleoside phosphorylase that can catalyze the conversion of FdUrd to FUra. Hepatic metabolism is nevertheless an important site of FdUrd removal as shown by several findings, including the poor oral bioavailability and effectiveness (31) of the drug, and the hepatic extraction ratio of 0.7 to 0.9 demonstrated during continuous iv infusion (15). Cl_{total} and $Cl_{metabolic}$ of FUra were much lower than those of FdUrd, possibly reflecting a more restricted distribution or activity of dihydrouracil dehydrogenase than of the nucleoside phosphorylases. $Cl_{metabolic}$ was less than twofold greater than hepatic plasma flow at the lowest dose employed in these studies, but significant extrahepatic metabolism of this drug probably also occurs since Garrett *et al.* (27) demonstrated that Cl_{total} during iv infusion was three- to sixfold greater than hepatic plasma flow in the human. Cl_{total} of FUra in the monkey was in close agreement with values obtained with comparable iv bolus doses in the human (32) but lower than values reported for the rat (29).

Since FdUrd and FUra are high extraction drugs, anesthetic-related changes in blood flow to metabolizing tissues such as the liver could alter the kinetics of elimination. Little data are available on the effects of anesthetics on the regional distribution of blood flow, but ketamine and pentobarbital, the agents employed in the present study, have opposite effects on cardiac output (34). The anesthetic combination may thus produce less of an alteration in flow to metabolizing tissues than occurs with either drug alone.

Because of the dose dependence of FdUrd elimination and the rapid elimination of FUra, it was not possible to accurately determine the fraction of the FdUrd dose converted to FUra. Calculating the extent of conversion from $Cl_{FUra}(AUC_{FUra})_{FdUrd}/dose_{FdUrd}$ gave a value of 42 to 65%. This estimate is inexact, however, since the equation is based on the assumption of first-order kinetics (24). Further, the calculated value would underestimate the true fractional conversion because any FUra formed at sites of dihydrouracil dehydrogenase activity, such as the liver (35), or at sites of

anabolic enzyme activity would be subject to further metabolic alteration before release into the circulation. Based on the known pathways of FdUrd metabolism (8), it is likely that most of the administered FdUrd that is not excreted unchanged in the urine is ultimately converted to FUra; this would amount to 82 to 91% of the dose. Similarly, it is likely that most of the FUra not excreted unchanged in the urine is eliminated as catabolic products.

Dose dependence. The lack of superposition of the logarithm plasma concentration/dose vs time curves for different doses (Fig. 2), and the progressive decreases in Cl_{total} and $Cl_{metabolic}$ with increasing dosage indicate that FdUrd is eliminated predominantly by a dose-dependent metabolic process. No clear evidence was obtained for dose-dependent plasma protein binding, distribution, or urinary excretion. Despite the demonstration of dose dependence, the individual semilogarithmic plasma disappearance curves appeared linear. Collins *et al.* (32) demonstrated that a physiologic pharmacokinetic model incorporating both linear and nonlinear processes yielded linear-appearing plasma disappearance curves similar to those observed in actual human studies with FUra.

Dose-dependent elimination of FUra has been demonstrated in the human (32), dog (28), and rat (29). For example, Garrett *et al.* (27) found that increases in FUra dose in humans resulted in decreases in Cl_{total} and $Cl_{metabolic}$ but had no effect on Cl_{renal} or V_d. No similar studies on FdUrd clearance have previously been reported.

The metabolic basis of the dose-dependent kinetics of elimination of the fluoropyrimidines is uncertain. In the case of FUra, saturation of dihydrouracil dehydrogenase, which catalyzes the rate-limiting step in catabolism of the drug (8), could account for the observed dose dependence. In the case of FdUrd, dose-dependent elimination could be a reflection of saturable breakdown of FdUrd or a reversal of the conversion of FdUrd to FUra. The conversion of FdUrd plus inorganic phosphate to FUra and deoxyribose-1-phosphate is catalyzed by pyrimidine nucleoside phosphorylases (9). Au *et al.* (36) have shown that the reaction is reversible *in vivo.* They demonstrated in humans that FdUrd can be formed from FUra when FUra is coadministered with thymidine, a precursor of deoxyribose-1-phosphate. If such reversal of the reaction became more marked with increasing FdUrd dose, due to increased deoxyribose-1-phosphate levels, this would result in an increase in the AUC of FdUrd, and decreases in the calculated Cl_{total} and $Cl_{metabolic}$.

Renal excretion. Urinary excretion was a minor but significant route of elimination of the fluoropyrimidines. Approximately 20% of the administered FdUrd was excreted as active drug (15% unchanged and 5% as FUra) and 9 to 19% of the FUra dose was excreted unchanged. Analogous results have been obtained with similar doses of FUra in the human (27, 31) and the rat (29).

The Cl_{renal} of FdUrd exceeded reported inulin clearance in the monkey (19) by fourfold and was markedly decreased by probenecid, indicating that the drug is secreted by the organic anion secretory system of the proximal tubule (20). Clarkson *et al.* (31) found that the Cl_{renal} of FdUrd during constant iv infusion in humans exceeded inulin clearance and also concluded that the drug undergoes tubular secretion. In contrast, no evidence for secretion was obtained in the dog (unpublished observations), where renal FdUrd and inulin clearances were approximately equal ($\bar{Cl}_{FdUrd}/Cl_{inulin}$ ratio 0.94 ± 0.08; mean $\pm$ SE, $n = 3$), suggesting a species difference in renal handling. The monkey thus appears to most closely resemble the human in its renal handling of FdUrd. In the monkey and rat (29), Cl_{renal} of FUra was close to reported glomerular filtration rate, indicating no net tubular transport, but studies in other species have demonstrated FUra clearances slightly above or below glomerular filtration rate (28), suggesting possible species variation in the renal handling of this drug also. It is possible that in the monkey FUra underwent tubular secretion followed by passive reabsorption (backdiffusion) resulting in no net reabsorption or secretion and in the observed Cl_{renal} values approximating glomerular filtration rate. Extensive backdiffusion of FdUrd, which is highly water soluble due to the presence of the deoxyribose group, would not be expected. Thus, most FdUrd secreted into the tubular fluid would probably be excreted in the urine. Any

differences in the extent of reabsorption of FdUrd and FUra could not be attributed to differences in the degree of ionization since the pK_a values of the drugs are nearly equal (37).

In conclusion, the present studies demonstrate dose-dependent elimination of FdUrd and suggest that the monkey may be a good model for human studies. The metabolic basis of the dose-dependent kinetics of FdUrd elimination remains to be determined.

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