Pharmacokinetics of Aryldihydro-*s*-triazines with Antifolate Activity I: Metabolism and Excretion by Rats

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Abstract □ The metabolism and excretion of three aryldihydro-*s*-triazines were studied using rats. The compounds investigated were 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazine (I), the prototype of the series; 4,6-diamino-1-(3,4-dichlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine (II); and *N*-(*m*-tolyl)-*p*-(4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)hydrocinnamide (III). All three compounds were excreted rapidly. Urinary excretion was favored by a lower molecular weight, and biliary excretion was favored by a higher molecular weight. The overall elimination rates of I and III were similar, I being excreted predominantly in urine and III (considerably metabolized) in bile. The overall elimination rate of II, excreted mainly in urine, was lower than for the other two triazines. A large percentage (and probably all) of the dose of I and II was unmetabolized. Metabolism of III does not appear to involve the phenyldihydrotriazine moiety. This broad view of the metabolism and excretion of the three triazines gives no particular insight into why II, although a more potent inhibitor of dihydrofolate reductase and of L-1210 tumor cell cultures, has been found to compare very poorly with III in its *in vivo* antineoplastic activity in rats.

Keyphrases □ Triazines, various substituted—metabolism and excretion in rats □ Metabolism—various substituted triazines in rats □ Excretion—various substituted triazines in rats

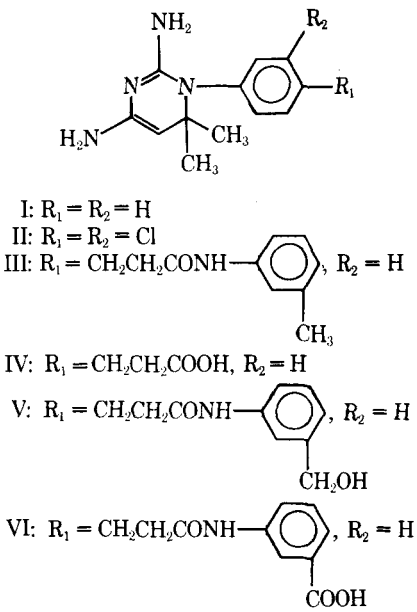
Clinical use of methotrexate has demonstrated that dihydrofolate reductase enzyme inhibitors can curb development of certain human neoplasms. The clinical effect derives from mitosis inhibition, which, apparently, is dependent on this enzyme. Since methotrexate exhibits little tissue specificity in its enzyme inhibition, most cells with rapid mitosis rates are affected, whether normal or neoplastic. As a result, clinical use of methotrexate is accompanied by serious toxic manifestations.

Isoenzyme selectivity favoring dihydrofolate reductase inhibition specifically from neoplastic tissue has been obtained *in vitro* with active-site-directed irreversible inhibitors based on 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazine (I) (1, 2). However, while many isoenzyme-selective compounds exhibited impressive ability to prolong survival of experimental animals bearing tumors, they also displayed considerable toxicity. Furthermore, the relative *in vivo* antineoplastic potencies of the aryldihydrotriazines did not always correlate with their relative potencies against the enzyme or against tumor cell cultures *in vitro*.

Despite the antineoplastic activity of many aryldihydrotriazines, little information is available on their metabolism or pharmacokinetics. To gain insight into why certain aspects of their *in vitro* behavior are not translated into the anticipated *in vivo* effects, a pharmacokinetic investigation was conducted. Metabolism and excretion data are presented.

EXPERIMENTAL

Synthetic Methods—1-(Substituted-phenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazines were prepared by the three-component synthesis of Modest (3). 4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazine (I) as the hydrochloride was recrystallized from water, mp 200–204° (uncorrected) [lit. (3) mp 200–203°]. 4,6-Diamino-1-(3,4-dichlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine (II) as the hydrochloride was recrystallized from water, mp 207–212° [lit. (3) mp 207–212°].



N - (*m*-Tolyl)-*p*-(4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)hydrocinnamide (III) was synthesized as the *p*-toluenesulfonate salt in a manner similar to that for synthesis of the ethanesulfonate salt (2), mp 210–213° (recrystallized from ethanol). The product and its intermediates had appropriate NMR and UV spectra.

Anal.—Calc. for $C_{28}H_{34}N_6O_4S$: C, 61.1; H, 6.2; N, 15.3. Found: C, 61.6; H, 6.4; N, 14.9.

p - (4,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)hydrocinnamic acid (IV) was prepared as the ethanesulfonate salt from methanolic acetone, mp 191–193° [lit. (4) mp 191–193°].

N - (*m*-Carboxyphenyl)-*p*-(4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)hydrocinnamide (VI) was synthesized as the hydrochloride. *m*-Aminobenzoic acid was esterified by refluxing in an ethanolic solution of hydrogen chloride. Ethyl *m*-aminobenzoate was refluxed with *p*-nitrocinnamoyl chloride in toluene. The resultant ethyl *N*-(4-nitrocinnamoyl)-3-aminobenzoate was hydrogenated under 1900 psi of hydrogen using platinum oxide catalyst. Ethyl *N*-[3-(4-aminophenyl)propionyl]-3-aminobenzoate was hydrolyzed (85 hr in a solution of 1% NaOH in 80% methanol under nitrogen), giving *N*-[3-(4-aminophenyl)propionyl]-3-aminobenzoic acid. This was converted to VI, which was recrystallized from water, mp 184°. All IR, NMR, and UV spectra and elemental analyses of the product and intermediates were consistent with the assigned structures. Overall yield was 43%.

Anal.—Calc. for $C_{21}H_{25}ClN_6O_3 \cdot H_2O$: C, 54.5; H, 5.9; N, 18.2. Found: C, 54.3; H, 6.1; N, 17.8.

The radiolabel for I was derived from ^{3}H-aniline[1]; for the other triazines, it was derived from ^{14}C-dicyanodiamide[2]. Radioactive triazines were recrystallized to constant specific activity and gave one radioactive peak[3] by TLC on silica gel GF, corresponding to the sole UV-detectable spot (specific activities in disintegrations per minute per milligram: I,

[1] Radiochemical Center, Amersham, United Kingdom.
[2] International Chemical and Nuclear Corp.
[3] Nuclear Chicago Actigraph III radioscanner, G. D. Searle and Co.

8.02×10^6; II, 1.42×10^6; III, 2.04×10^6; IV, 1.82×10^5; and VI, 1.60×10^5).

Radiotracer Techniques—Radioisotope levels were measured by liquid scintillation spectrometry[4] using Bray's scintillation solution (5). Aliquots (300 mg) of dried feces, ground with sand, were digested for 1 hr at 40° in 1 ml of 1 *M* KOH (aqueous) before 10 ml of Bray's solution was added; counting was done 24 hr later.

Reverse isotope dilutions were performed by adding known amounts of nonradioactive aryldihydrotriazine to aliquots of radioactive urine or bile. The solutions were evaporated to dryness *in vacuo* below 40°, and the residues were crystallized to constant specific activity from the appropriate solvents. Prior elution with methanol from an ion-exchange column[5] aided crystallization from samples containing II.

GLC Techniques—A GLC assay was devised to differentiate and quantitate potential metabolites of III after hydrolysis of the amide bond common to these compounds.

Hydrolysis of Triazines—Bile was collected in a 5-ml ampul and diluted to 1.5 ml with water. Internal standard solution[6] (200 μl) and then 1.0 ml of methanolic potassium hydroxide solution (10 *N*) were added. The walls of the ampul were washed down with 0.5 ml of methanol before sealing under nitrogen. After shaking, the ampul was stored at 40° for 7 days.

Extraction of m-*Toluidine and* m-*Aminobenzyl Alcohol*—After cooling, the contents of the ampul were transferred to a 15-ml graduated glass centrifuge tube, followed by ethereal (2 × 5 ml) and aqueous (0.5 ml) washings from the ampul. The centrifuge tube was agitated and then centrifuged. The ether phase (Extract 1) was transferred to a 15-ml tapered evaporation tube.

Extraction of m-*Aminobenzoic Acid*—The aqueous phase was cooled in an ice bath. After addition of 1.5 ml of 10 *N* HCl and sufficient water (to 5 ml) to redissolve the resultant potassium chloride precipitate, it was extracted with ether (5 ml) and the ether was discarded. Then the aqueous phase was diluted to 7 ml with water, and 1.0 ml of a sodium oxalate solution (20 mg/ml) and 0.5 ml of 10 *N* NaOH were added. The pH was then adjusted to 3.8–4.1. The resulting solution was extracted twice with 5 ml of ether, and the ether extracts were combined (Extract 2) in an evaporation tube.

Preparation of Ether Extracts—The volume of each ether extract was reduced to about 100 μl on a water bath at 40°. (Superheating was prevented by use of a fine-glass boiling stick.) Fresh ether (10 ml) was immediately added. After agitation of the tube, the ether volume was reduced to about 20 μl and the tube was stoppered and placed directly in ice.

Prior to chromatography, Extract 1 was centrifuged (~2000×*g* for 5 min). To Extract 2, 200 μl of an ethereal solution of diazomethane (6) was added and the extract was restoppered. After a minimum of 30 min, the volume of ether was again reduced to about 20 μl. The tube was stoppered and placed in ice until sample analysis.

GLC—All analyses were performed under isothermal conditions using the chromatograph[7] in the single-column mode. The Pyrex glass column (1.5-m length, 4-mm bore) was packed with 3% OV-17 on 100–120-mesh Gas Chrom Q[8]. Gas flow rates were: carrier gas (nitrogen), 40 ml/min; hydrogen, 40 ml/min; and air, 550 ml/min.

Column temperatures and retention times were: *m*-toluidine, 102°, 4.25 min (internal standard, 2,4-dimethylaniline, 6.75 min); *m*-aminobenzyl alcohol, 143°, 4.75 min (2,4,6-trichloroaniline, 5.75 min); and *m*-aminobenzoic acid, 143°, 8.0 min (*o*-aminobenzoic acid, 4.25 min; both acids chromatographed as their methyl esters). The flame-ionization detector was coupled to a recorder[9] equipped with an integrator[10].

Standard curves were constructed after known amounts of triazines added to blank bile samples were processed. Since an authentic sample of *N*-[*m*-(hydroxymethyl)phenyl]-*p*-(4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)hydrocinnamide (V) was not available, the assay for this metabolite was based on a standard curve for *m*-aminobenzyl alcohol carried through the entire procedure. Since III underwent complete hydrolysis, allowing virtually total recovery of *m*-toluidine (95, 97, or 101% recovery from 109, 210, or 1920 μg of III added to bile, respectively), it was assumed that V would similarly yield stoichiometric proportions of *m*-aminobenzyl alcohol.

Table I—Excretion of Aryldihydrotriazines after Intraperitoneal Dosage in Rats [a] (Expressed as Percent of Dose ± *SE*)

Sample	I	II	III
Urine			
0–24 hr	85 ± 2.0	57 ± 1.5	27 ± 1.0
24–48 hr	0.8 ± 0.2	3.3 ± 0.4	0.6 ± 0.1
48–72 hr	0.3 ± 0.1	0.8 ± 0.1	0.31 ± 0.02
72–100 hr	—	—	0.24 ± 0.04
Feces			
0–24 hr	9.6 ± 2.2	19 ± 1.9	37 ± 5.5
24–48 hr	2.0 ± 1.0	5.1 ± 1.3	20 ± 4.6
48–72 hr	0.1 ± 0.08	1.9 ± 0.4	1.8 ± 0.4
72–100 hr	—	—	0.6 ± 0.2
Total recovered	98	87	88

[a] Five milligrams of radiolabeled compound was given to each rat. Three rats received I, three rats received II, and six rats received III.

GLC–Mass Spectrometry—Bile samples were prepared as for GLC, except that internal standards were not used. A quadrupole instrument[11] was employed to scan chemical-ionization spectra of components of interest. The GLC column (glass, 1.5-m length, 2-mm bore) was packed with 3% OV-225 on 100–120-mesh Gas Chrom Q[7]. Methane was the carrier gas (flow rate of 20 ml/min). Column temperatures of 140° for *m*-toluidine and 200° for both *m*-aminobenzyl alcohol and methyl *m*-aminobenzoate resulted in retention times of 1–2 min. The source temperature was 50°, and the ionization energy was 165 ev.

Animal Techniques—Male Wistar rats[12], 250–300 g and 3–6 months old, were used after maintenance for 1–4 months in an environment untreated with insecticides. They received a commercial diet formulated for laboratory mice and rats. No animal was used for experimentation more than once.

Samples of urine and feces were collected from animals housed in cages equipped with a urine–feces separator. They were fed daily, and water was available *ad libitum*. When serial urine samples were required as frequently as every 2 hr, rats were water loaded intraperitoneally with 2 ml of saline 1 hr prior to dosage, immediately after dosage, and after collection of each urine sample. Rats were induced to urinate by compelling them to inhale ether fumes at specified times (7).

Bile was collected from cannulated rats under urethan anesthesia.

RESULTS

Gross Excretion Pattern—A dose[13] of 5 mg ip of I, II, or III was given to each of 12 rats (Table I). All three compounds were excreted rapidly, with 95, 76, and 64% of the radioactivity from a dose of I, II, and III, respectively, being collected in the first 24 hr. Moreover, an additional amount of III, as much as 20% of the dose, may have already been excreted *via* the bile into the GI tract at the time of collection of the 24-hr feces.

The well-documented trend toward excretion of larger molecules in the bile and feces (8) was in evidence with this series of compounds; 12, 26, and 60% of the radioactive dose were excreted in the 72-hr feces of animals dosed with I, II, and III, respectively (having molecular weights of 217, 286, and 378, respectively).

Urinary Excretion—Representative urinary excretion rate plots in Fig. 1 show that, initially, the urinary excretion of all three compounds was rapid, in accordance with the considerable polarity of the common triazine moiety (pKa of about 11) (3). Within 1 hr, 50% of the injected I appeared in the urine; within 2 hr, 70–75% appeared in the urine. The less polar II and III were excreted in the urine at a rate initially less than that of I. Thus, in the 2 hr following injection, only 30% of the dose of II and 20% of III were excreted in the urine.

Biliary Excretion–The greater susceptibility of the high molecular weight triazine to biliary excretion, inferred from the fecal data (Table I), is confirmed by biliary excretion rate plots (Fig. 2) derived from anesthetized animals dosed intravenously with 1 mg of triazine[14]. A high

[4] Tri-Carb model 3314 with automatic external standardization, model 3950, Packard Instrument Co.

[5] XAD-2, BDH Chemicals Ltd.

[6] 2,4-Dimethylaniline (270 μg/ml), 2,4,6-trichloroaniline (25 μg/ml), and *o*-aminobenzoic acid (115 μg/ml) in 0.1 *N* HCl.

[7] Pye Series 104, model 64, W. G. Pye and Co. Ltd.

[8] Applied Science Laboratories.

[9] Speedomax type G, model S, 60000 series, Leeds and Northrup Co.

[10] Model 203, Disc Instruments Inc.

[11] A 3200E gas chromatograph–mass spectrometer (9500 gas chromatograph) with MS data system 6000 and digital (zeta) plotter, Finnigan Corp.

[12] University of Sydney Animal House, Castle Hill, New South Wales.

[13] All triazines were injected in a 10-mg/ml solution in 60% dimethyl sulfoxide in water.

[14] Reliable intraperitoneal dosage was not possible after the abdominal operation for biliary cannulation, and the lethal dose of any of the triazines by the intravenous route was little more than 1 mg.

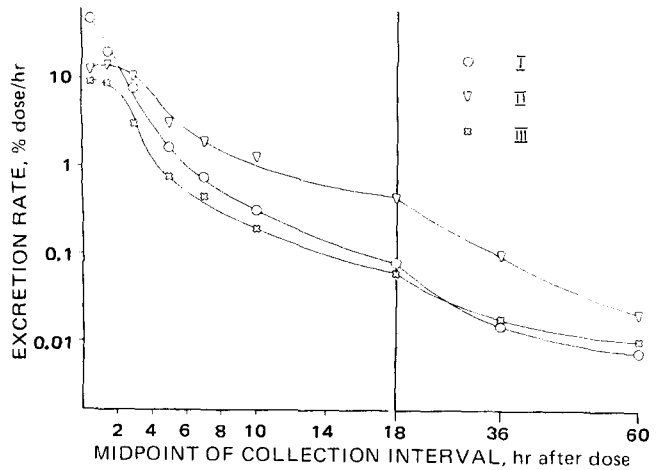


Figure 1—*Representative urinary excretion curves for aryldihydrotriazines I–III after a 5-mg ip dose.*

rate of excretion of III was achieved early after injection but rapidly subsided shortly afterward. Whereas 50% of the III dose appeared in the 3-hr bile, only 11% was excreted over the next 7 hr. The lower molecular weight I and II were excreted in the bile at lower, but more consistent, rates; 12% of the dose of I and 10% of II appeared in the 8-hr biles.

Extrapolation of the biliary excretion profiles indicated that I and II, particularly, may ultimately be excreted in the bile to a slightly greater extent than is reflected by their appearance in the feces. Furthermore, 28% of an oral dose of I and 9% of an oral dose of II were found in the 72-hr urine. The possibility of reabsorption of biliary excreted triazines from the GI tract was investigated by arranging that the bile of a dosed rat flowed into the duodenum of an undosed acceptor rat. From a comparison of the biliary excretion from acceptor rats with that from rats receiving an intravenous bolus dose directly, reabsorption of approximately 13, 24, and 3% of the biliary radioactivity from I, II, and III, respectively, was calculated. No allowance was made, of course, for involvement of metabolites.

Metabolism—Metabolism of the dosed triazines was investigated by reverse isotope dilution analysis of the excretion fluid containing the major part of the injected radioactivity. After a 5-mg ip dose of I, only the unchanged triazine (99% unchanged in one rat, 98% in another) was found in 24-hr urine containing 80% of the dose. It thus seems unlikely that any of the 90% of the dose eventually excreted in the urine was metabolized. Even if all of the compound excreted in the feces had been metabolized, which seems improbable by comparison with III (discussed later), this amount would represent only a minor fraction of the original dose.

In the 12 hr following a 5-mg ip dose of II, 52% was excreted in the urine, all as unmetabolized II (95 and 100% unchanged in two rats). The urine

Table II—Mass Spectral Data for Aromatic Amines Derived from Hydrolysis of Bile Containing Metabolites of III[a]

GLC Peak	m/e	Percent of Base Peak	Authentic Sample: Percent of Base Peak	Assignment[b]
1			*m*-Toluidine	
	108	100	100	$P + H^+$
	136	10	9	$P + C_2H_5^+$
	122	2	2	
	148	2	2	$P + C_3H_5^+$
2			*m*-Aminobenzyl Alcohol	
	106	100	100	$P + H^+ - H_2O$
	124	36	42	$P + H^+$
	94	5	7	
	134	3	3	$P + C_2H_5^+ - H_2O$
	152	1	1	$P + C_2H_5^+$
	164	0.5	Minute	$P + C_3H_5^+$
3			Methyl *m*-Aminobenzoate	
	152	100	100	$P + H^+$
	120	8	14	$P + H^+ - CH_3OH$
	180	4	2	$P + C_2H_5^+$
	166	2	10	
	192	1.5	0.5	$P + C_3H_5^+$

[a] Bile was obtained from anesthetized rats for 3 hr after a 1-mg iv dose of III. [b] P = parent compound.

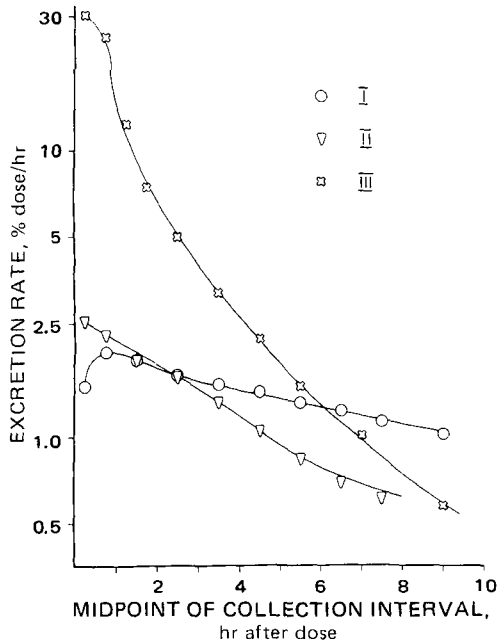


Figure 2—*Representative biliary excretion curves for aryldihydrotriazines I–III after a 1-mg iv dose.*

accumulated ultimately 65% of the dose. The chemical relationship of I and II would suggest that, if I was not metabolized, metabolism of II was not likely.

Only 59% of the radioactivity excreted in the bile in the 3 hr following a 1-mg iv dose of III was in the form of the unchanged compound.

Compound IV might be expected as a metabolite if the amide bond of III was to split. A 5-mg ip dose of IV given to each of four rats was excreted rapidly in urine (average of 83% of the dose in 2 hr, all as unmetabolized IV), with very little appearing in the feces (6.3% in 72 hr). When four rats (anesthetized) were each dosed with 1 mg iv of IV, only an average 5.3% of the radioactivity was excreted in the 3-hr bile, in contrast to 55% following injections of III. Therefore, it was not likely that IV was a major metabolite in the bile of rats receiving III.

GLC techniques were devised to identify and assay amines liberated by hydrolyzing the amide bond of III or potential metabolites. *m*-Toluidine, *m*-aminobenzyl alcohol, and *m*-aminobenzoic acid (chromatographed as the methyl ester) were verified in the hydrolysate of a 3-hr bile sample by GLC–mass spectrometry (Table II). Since I, II, and IV apparently are not metabolized, metabolism of III is probably confined to the terminal phenyl ring. In that case, *m*-toluidine, *m*-aminobenzyl alcohol, and *m*-aminobenzoic acid would be in the hydrolysate derived from III, V, and VI, respectively, in the bile.

Of the radioactive content of 3-hr bile, 99% could be accounted for in terms of these three compounds: 57% as unchanged III, 25% as V, and 17% as VI (Table III). In light of the low level of biliary excretion of I, II, and IV, the close correspondence between the amount of the chromatographed species in bile and its radioactivity[15] indicates that the chromatographed species derived exclusively from triazines excreted with the amide bond intact.

Since the extreme hydrolysis conditions used should cleave conjugates also, the GLC data indicate the biliary content of the respective triazines plus their conjugates, if present. Reverse isotope dilution analyses, however, were performed under conditions such that a measure of the diluting species only was expected. The parity in the proportion of triazine as VI in the bile, whether determined by GLC (17%) or by dilution analysis (20%) after a dose of III, suggests that VI was probably not conjugated.

Four animals dosed with 1 mg iv of VI excreted in 3-hr bile an average 46 ± 2 (*SE*) % of the dose. This excretion rate could certainly account for 9% of the dose of III as VI in 3-hr bile (Table III).

Thus, V and VI appear to be the only significant metabolites (unless V is conjugated) in the bile of rats dosed with III. There was more V than VI, but neither of these metabolites was excreted in as great a quantity as the unchanged III. It must be considered probable that V is an intermediate in the metabolism of III to VI.

[15] The triazine ring contains the ^{14}C-label of III.

Table III—Quantitative Assay of Metabolites of III in Bile [a]

Animal	III	V	VI	Total Recovery by GLC	Total Recovery by Liquid Scintillation Counting (LSC)	Percent Recovery, 100 × GLC/LSC
1	272	151	163	586	550	107
2	287	153	61	501	530	95
3	308	106	97	512	521	98
4	346	124	77	547	569	96
5	343	146	61	550	565	97
Mean	311	136	92	539	547	99
As percent biliary excretion	57	25	17	99	—	—
As percent dose	30	13	9	—	53	—

[a] Content of III, V, and VI in the 3-hr bile of anesthetized rats dosed with 1.03 mg iv of III. Data are expressed as micrograms of III equivalent to each metabolite. Assay was by GLC after alkaline hydrolysis.

DISCUSSION

Of the three triazines chosen for study, two were among folate antagonists previously tested (2) for *in vivo* antineoplastic activity; III was reported to prolong considerably the survival of rats bearing Walker 256 ascites tumor; II was a more potent inhibitor of dihydrofolate reductase and of L-1210 tumor cell cultures but, nevertheless, compared very poorly with III in its *in vivo* antineoplastic efficacy. It was anticipated that adverse pharmacokinetic properties may have been responsible for the comparative lack of *in vivo* activity by II. Thus, to investigate triazines with molecular weights both lower and higher than the molecular weight of II, the prototype of the series, I, was included.

The radioactive contents of bile and urine samples, although not giving direct information on III as the unchanged species, do indicate minimum amounts of the dose of this compound eliminated or maximum amounts excreted as this compound *per se*. The relationship between the biliary and urinary excretion data presented is then consistent with the standard pharmacokinetic assumption that urinary and biliary excretion are parallel first-order processes dependent on the blood concentration of the compound. If this model is valid, the urinary excretion rate profiles can be used to project the shape of blood concentration–time curves.

Following injection, the urinary excretion of all three triazines (or their metabolites) was initially rapid, I being the most rapidly excreted and III the least. Urinary excretion subsequently slowed as the bloodstream was depleted of these compounds. Despite an initial urinary output rate of I as much as five times that of III, the urinary excretion of these compounds (in terms of percentage of the dose) decelerated similarly. Excretion of II in the urine, however, was far more persistent than for the other two compounds (at 18 hr the urinary excretion rate of II was about five times that of the other triazines).

If the blood was being depleted of radioactivity at similar rates following injections of either I or III, it seems that biliary excretion favoring III (and its metabolites) was countering the imbalance in their urinary excretion rates. By superimposition of the biliary and urinary data, a coarse estimate of the total excretion is obtainable. In the first 2 hr after injection, 74% of the dose of I (70% in urine and 4% in bile) and 60% of the dose of III (20 and 40%) were excreted. In contrast, in the first 2 hr after injection of II, only 34% (30% in urine and 4% in bile) of the dose was excreted. By allowing for an amount of the metabolites of III being formed but not excreted in the first 2 hr, closer elimination rates of I and III and a greater discrepancy between those of II and III may be assumed.

Biliary excretion, however, appears to have been operating by a more complex mechanism than the direct first-order transfer of triazine from the bloodstream to bile. Although dosed by the intravenous (femoral) route, many animals displayed an early period of increasing biliary excretion rate. Furthermore, the biliary excretion rate of a 1-mg iv dose of I varied only two- to threefold over the first 9 hr after injection, while urinary excretion (at least by unanesthetized rats dosed with 5 mg ip) in this period accounted for 90% of the dose. It seems likely that an additional physiological compartment was intervening between blood and bile, as previously found (9).

Under these circumstances, the initial removal rate from the blood of any triazine by the biliary route would have been greater than its initial appearance rate in the bile. Thus, the approximate extent of the 2-hr elimination of III calculated above may be even more an underestimate than previously considered.

Thus, the overall elimination rates of I and III appear to be similar, I being excreted predominantly in urine and III (extensively metabolized) in bile. While all three compounds are eliminated rapidly, the overall elimination rate for II, excreted mainly in urine, is lower than for the other two triazines. Compound II, like I, does not seem to be metabolized. Metabolism of III apparently does not involve the phenyldihydrotriazine moiety.

This broad view of the metabolism and excretion of the three triazines gives no particular insight into the antineoplastic superiority of III over II *in vivo* (2). Conceivably, one metabolite of III is considerably more active as an inhibitor of dihydrofolate reductase than III itself. On the other hand, the relative pharmacokinetic properties of II and III, on the basis of their elimination behavior, appear to favor the superior activity of the more persistent II rather than explain its relative inactivity *in vivo*. Studies on blood levels of these triazines have been commenced.

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