Cancer Chemother Pharmacol (1982) 10: 11–15

Cancer Chemotherapy and Pharmacology

Effect of Cyclophosphamide Pretreatment on the Short-Term Disposition and Biliary Excretion of Adriamycin Metabolites in Rat

Neil Hartman, Peter J. Basseches, and Garth Powis

Division of Developmental Oncology Research, Department of Oncology, Mayo Clinic, Rochester, MN 55905, USA

Summary. *The effect of pretreatment with cyclophosphamide 180 mg/kg upon the short-term disposition of adriamycin in anesthetized rat 4 days later was studied. There was a significant decrease in plasma adriamycin clearance, from 125 to 48 ml/min/kg, and a significant decrease in the apparent volume of the peripheral compartment of adriamycin distribution, from 51.7 to 25.6 l/kg, in cyclophosphamide-pretreated as against control rats. Biliary excretion of adriamycin over 2.5 h was increased significantly by 114% in cyclophosphamide-pretreated rats and there was a small but nonsignificant increase in biliary adriamycinol excretion and a decrease in excretion of adriamycin aglycones. Cyclophosphamide pretreatment was associated with an 83% increase in bile flow. Cyclophosphamide pretreatment had no significant effect upon the utilization of adriamycin or upon the formation of adriamycin metabolites by rat isolated hepatocytes. The results suggest that NADPH-cytochrome P-450 reductase, which is decreased 40% by cyclophosphamide pretreatment, is not rate-limiting in elimination of adriamycin. Biliary excretion of adriamycin is increased when plasma adriamycin clearance is decreased, suggesting that cyclophosphamide pretreatment affects a pathway besides biliary excretion that is responsible for the short-term removal of adriamycin from plasma.*

Introduction

Adriamycin and cyclophosphamide used in combination have been shown to be beneficial in several clinical regimens [7, 18]. Administered concurrently to tumored animals these agents show therapeutic potentiation [6]. Cyclophosphamide decreases the activity of several hepatic enzymes involved in drug oxidation and conjugation [12]. Marinello et al. [15] have reported a decrease in hepatic microsomal NADPH-cytochrome P-450 reductase in rat following cyclophosphamide administration. Reductive cleavage of adriamycin to deoxyadriamycin aglycone is catalyzed by microsomal NADPH cytochrome P-450 reductase [2, 3]. Aglycones lack the antitumor acitivity of the parent anthracyclines [8]. Changes in the activity of hepatic NADPH-cytochrome P-450 reductase might alter the metabolism and lead to changes in the biological activity of adriamycin. We report the effect of cyclophosphamide pretreatment on the metabolism of adriamycin by rat isolated hepatocytes and on the short-term plasma elimination and biliary excretion of adriamycin and metabolites of adriamycin in the anesthetized rat.

Reprint requests should be addressed to G. Powis

Materials and Methods

Male Sprague-Dawley rats weighing 250–300 g were used for all studies except those involving collection of blood, when rats weighing 350–400 g were used. Cyclophosphamide, 180 mg/kg unless otherwise specified, was administered IP 4 days prior to study. Hepatocytes were isolated by the method of Stewart and Inaba [24]. Cell viability measured by trypan blue exclusion was routinely over 80%. Hepatocytes were suspended in 10 ml Dulbecco's phosphate-buffered saline containing 10 m*M* glucose at 10^7 cells/ml and prewarmed to 37° C with gentle shaking under air or N_2 for 5 min. The reaction was initiated by addition of adriamycin to 8.5 μg/ml. Aliquots of hepatocyte incubation medium were removed for assay at 15-min intervals over 90 min. Incubations were conducted in the dark to prevent photodecomposition of adriamycin [30]. Previous studies have shown that under anaerobic conditions hepatocytes remain viable and capable of catalyzing drug metabolism for a period of hours [18]. For studies on adriamycin distribution rats were anesthetized with IP pentobarbital, 50 mg/kg, and body temperature maintained at 37° C. Adriamycin, 20 mg/kg, was injected through a cannula in the left iliac vein. Aliquots of blood, 1 ml, were collected into heparinized tubes at various times up to 3 h from a cannula in the left iliac artery. Biliary excretion studies were conducted in a separate group of rats under similar conditions except that the bile duct was cannulated and bile collected at 30-min intervals. Pentobarbital anesthesia does not affect bile flow in the rat [14]. Bile was collected into preweighed vials protected from light with aluminum foil. Hepatocyte incubation medium, 1 ml, or plasma, 0.4 ml, were mixed an equal volume of 0.1 *M* borate buffer, pH 9.8, and extracted with 6 ml chloro form: methanol (4 : 1) as described by Baurain et al. [4]. Of the organic phase, 100 μl was injected directly into the HPLC. Bile samples were diluted with five volumes of water before direct injection into the HPLC. Adriamycin and metabolites in hepatocyte incubations were assayed by a modification of the HPLC method of Baurain et al. [4] on a Whatman PXS 10/25 PAC 10 μ column under isocratic conditions with chloroform/methanol/glacial acetic acid/3 m*M* $MgCl_2$ (72 : 21 : 2 : 3) at a flow of 0.5 ml/min. Adriamycin and metabolites in plasma and bile samples were assayed by a modification of the method of Andrews et al. [1] on a Waters μ-Bondapac Phenyl column under isocratic condition with tetrahydrofuran/0.01 *M* sodium phosphate, pH 4.0 (30 : 70) at a flow of 1 ml/min. Detection in both cases was by fluorescence with a Schoeffel Model F5970 Spectrofluoromonitor (Kratos Inc., Westwood, New Jersey) at

0344-5704/82/0010/0011/$ 01.20

an excitation wavelength of 482 nm and a 550 nm emission cutoff filter. NADPH-cytochrome P-450 reductase in liver homogenate or homogenate of isolated hepatocytes was measured by the method of Yasukochi and Masters [30]. Adriamycin was supplied by the Drug Synthesis and Chemistry Branch, Division of Drug Treatment, National Cancer Institute, Bethesda, Maryland. Adriamycin metabolites were prepared as described by Andrews et al. [1], Cyclophosphamide was obtained from Mead-Johnson, Evansville, Indiana.

Pharmacokinetic analysis of adriamycin plasma concentration data was conducted using the NONLIN [16] computer program. The biexponential decline in plasma concentration was fitted by nonlinear least-squares regression analysis to the equation $C = Ae^{-\alpha t} + Be^{-\beta t}$, where C is the concentration of adriamycin at time t after administration, A and B are the intercepts at $t = 0$, and α and β are the fast and slow disposition rate constants. A weighting factor of C^{-2} was used. Half-lives and apparent volumes of distribution were calculated from disposition rate constants and zero time intercepts as described by Gibaldi and Perrier [11]. Allowance was made for an infusion time of 1 min. Total plasma clearance of adriamycin, $\overline{Cl}$, was calculated from the dose of adriamycin administered divided by the area under the concentration-time curve extrapolated to infinity using the trapezoidal rule [11]. Groups of data were analyzed for statistical significance using Student's t-test [23].

Results

Pretreatment with cyclophosphamide, 180 mg/kg, decreased NADPH-cytochrome P-450 reductase activity in homogenates of isolated hepatocytes and whole liver by up to 46% (Table 1). This is similar to the inhibition of hepatic microsomal NADPH-cytochrome P-450 reductase by cyclophosphamide pretreatment reported by Marinello et al. [15]. There was no difference in the decrease in NADPH-cytochrome P-450 reductase produced by cyclophosphamide in 250- to 300-g rats and 350- to 400-g rats (results not shown). Higher doses of cyclophosphamide produced only small additional decreases in NADPH-cytochrome P-450 reductase activity and resulted in the death of some animals.

The metabolism of adriamycin by isolated hepatocytes under anaerobic conditions is shown in Fig. 1. Cyclophosphamide pretreatment had no significant effect upon the utilization of adriamycin or upon the appearance of deoxyadriamcin aglycone or deoxyadriamycinol aglycone metabolites.

A typical chromatogram of adriamycin and metabolites in bile of rat is shown in Fig. 2. The effect of cyclophosphamide pretreatment on the biliary excretion of adriamycin and its metabolites in anesthetized rat following an IV dose of adriamycin, 20 mg/kg, is shown in Fig. 3. Cyclophosphamide pretreatment produced an increase in biliary excretion of adriamycin over 2.5 h from (± SEM) 8.4% ± 0.9% of the dose administered in control rats to 18.0% ± 1.7% in cyclophosphamide-pretreated rats ($P < 0.01$). There was a trend towards an increase in biliary excretion of adriamycinol from

Table 1. Inhibition of NADPH cytochrome P-450 reductase by cyclophosphamide

	Deaths	NADPH cyt. P-450 reductase nmol/min/mg	Inhibition %
Hepatocytes			
Control	0/3	57.9 ± 7.9	–
CP 180 mg/kg	0/4	34.8 ± 1.7	40
Whole Liver			
Control	0/3	60.8 ± 6.8	–
CP 180 mg/kg	0/3	33.0 ± 4.8	46
CP 250 mg/kg	1/3	32.6	46
CP 300 mg/kg	1/3	24.4	60

Values are mean ± SEM. Activity was measured in homogenates of isolated hepatocytes or whole liver 4 days after cyclophosphamide IP. Deaths refer to the number of animals dying within 4 days

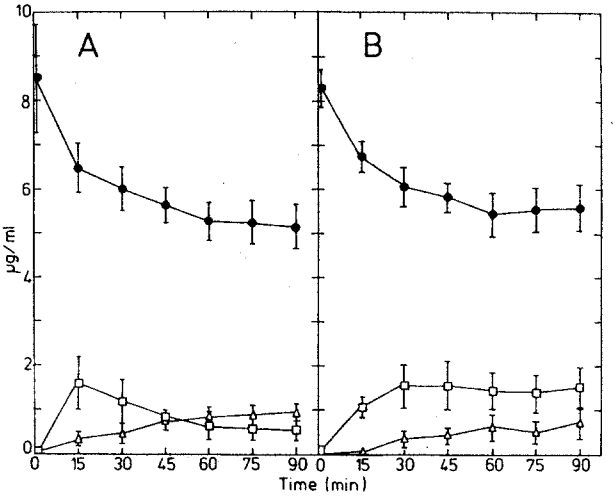

Fig. 1 A and B. Metabolism of adriamycin by isolated rat hepatocytes under anaerobic conditions. Isolated rat hepatocytes were incubated at 10^7 cells/ml in Dulbecco's phosphate-buffered saline containing 10 m*M* glucose and adriamycin at 37° C. **(A)** control hepatocytes ($n = 3$); **(B)** hepatocytes from rats pretreated with cyclophosphamide 180 mg/kg ($n = 4$). (●) adriamycin; (□) deoxyadriamycin aglycone; (△) deoxyadriamycinol aglycone. Each *point* is the mean of n observations, *bars* show SEM

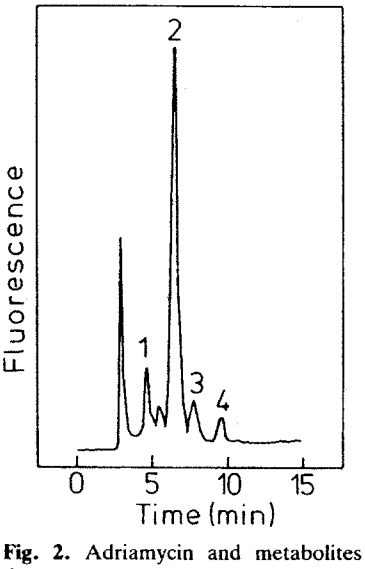

Fig. 2. Adriamycin and metabolites in bile of anesthetized rat. Chromatograph of bile collected between 30 and 60 min after an i.v. dose of adriamycin, 20 mg/kg. Peaks: 1, adriamycinol; 2, adriamycin; 3, deoxyadriamycinol aglycone; 4, deoxyadriamycin aglycone

0.9% ± 0.5% of the dose of adriamycin administered in control rats to 1.8% ± 0.8% in cyclophosphamide-pretreated rats but the effect was not significant ($P > 0.05$). For convenience, biliary excretion of deoxyadriamycin aglycone and deoxyadriamycinol aglycone is shown combined. Deoxyadriamycin aglycone formed 54% of the total. Biliary excretion of adriamycin-derived aglycones over 2.5 h decreased from 1.1% ± 0.4% of the dose of adriamycin administered in control rats to 0.7% ± 0.4% in cyclophosphamide-pretreated rats, but the effect was not significant ($P > 0.05$). Cyclophosphamide pretreatment was associated with a significant increase in the bile flow (Fig. 4) with a flow of 0.42 ± 0.04 ml/h/100 g body weight in cyclophosphamide-pretreated rats as against 0.23 ± 0.03 ml/h/100 g body weight in control rats ($P < 0.05$).

Plasma concentrations of adriamycin following an IV dose of 20 mg adriamycin/kg in control and cyclophosphamide-pretreated rats are shown in Fig. 5. Disappearance of adriamycin from plasma was biphasic. A slower third phase of adriamycin elimination [28] was not seen in the present study because of the short time over which blood samples were collected. Pharmacokinetic parameters are shown in Table 2. There was no significant difference between the half-lives for plasma adriamycin in the two groups although two of four cyclophosphamide-pretreated animals showed a considerably lengthened post-distributive half-life, in one case up to 754 min, accounting for an increase in the mean post-distributive half-life in this group. The apparent volume of the peripheral compartment of adriamycin distribution (V_2) was significantly decreased by 51% in cyclophosphamide-pretreated animals, and total plasma adriamycin clearance was decreased by 61%.

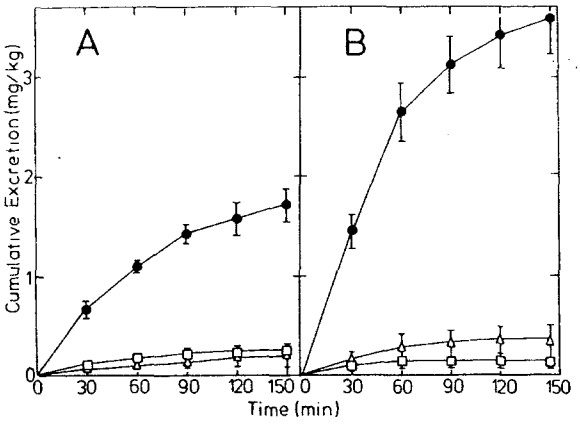

Fig. 3 A and B. Excretion of adriamycin and metabolites in bile of rat. Bile was collected from anesthetized rats receiving adriamycin 20 mg/kg. (**A**) control rats ((*n* = 4); (**B**) rats pretreated with cyclophosphamide 180 mg/kg (*n* = 4). (●) adriamycin; (△) adriamycinol; (□) combined adriamycin a glycones. Each *point* is the mean of *n* observations, *bars* show SEM

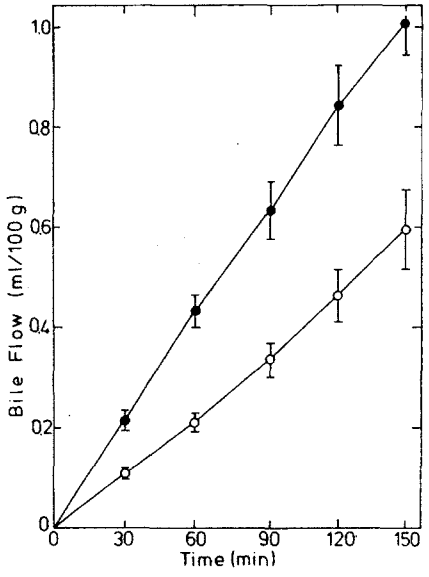

Fig. 4. Bile production in anesthetized rats with a cannulated bile duct. (○) control rats (*n* = 4); (●) rats pretreated with cyclophosphamide 180 mg/kg (*n* = 4). Each *point* is the mean of *n* observations; *bars* show SEM

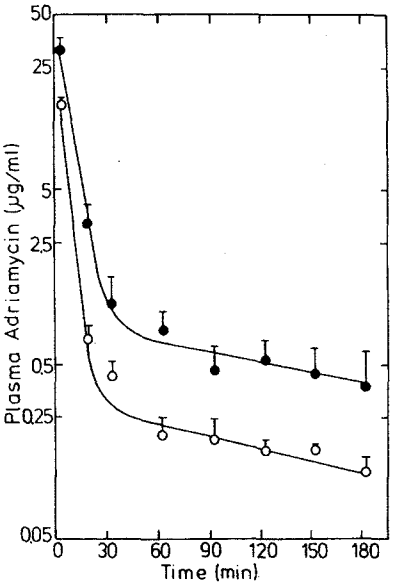

Fig. 5. Effect of cyclophosphamide pretreatment on plasma adriamycin in rat. (○) control rats (*n* = 3); (●) rats pretreated with cyclophosphamide 180 mg/kg (*n* = 4). Each *point* is the mean of *n* observations; *bars* are SEM. *Continuous lines* are computer fits to the data

Table 2. Pharmacokinetic parameters of adriamycin on control and cyclophosphamide-pretreated rats

	$t_{1/2}\alpha$ min	$t_{1/2}\beta$ min	V_1 l/kg	V_2 l/kg	$\overline{Cl}$ ml/min/kg
Control (3)	2.7 ± 0.0	100.9 ± 18.1	0.72 ± 0.12	51.70 ± 4.50	124.6 ± 11.8
CP (4)	4.2 ± 0.7	308.7 ± 163.0	0.52 ± 0.12	25.56 ± 6.46[a]	48.0 ± 17.4[a]

Values are mean ± S.E. of mean. Figures in parentheses give numbers of animals. $t_{1/2}\alpha$ and $t_{1/2}\beta$ are half-lives, V_1 and V_2 apparent volumes of distribution, $\overline{Cl}$ is total body plasma clearance

[a] $P < 0.05$

Discussion

Previous studies have shown adriamycin is rapidly cleared from the plasma of rat and that biliary excretion is a major pathway for elimination of the drug [13, 26, 27, 32]. Yesair et al. [32] found excretion of 7% of an IV dose of adriamycin in the bile of unanesthetized rats over 24 h. Israel et al. [13] and Wilkinson et al. [28] reported 20% of an administered dose of adriamycin excreted over the same period. Tavoloni and Guarino [26] reported 27% of a dose of adriamycin was excreted in the bile over a 3-h period as adriamycin and fluorescent metabolites in anesthetized rats. In the present study employing anesthetized rats, 8.4% of a dose of adriamycin was excreted as unchanged adriamycin in the bile of control animals over a period of 2.5 h, 0.9% as adriamycinol and 1.1% as aglycone metabolites of adriamycin. The rat differs from the human, where adriamycin metabolites account for almost half the adriamycin equivalents excreted in the bile [21]. Pretreating rats with cyclophosphamide resulted in a 83% increase in bile flow and a 114% increase in biliary adriamycin excretion. In cyclophosphamide-pretreated rats 18.0% of a dose of adriamycin was excreted in the bile as unchanged adriamycin in 2.5 h, 1.8% as adriamycinol and 0.7% as aglycone metabolites of adriamycin. A possible explanation for the increased biliary excretion of adriamycin is that it is a direct consequence of the increased flow of bile. Tavoloni and Guarino [26], using sodium taurocholate to inhibit bile secretion, found that biliary excretion of adriamycin equivalents in rat was directly proportional to bile flow. They suggested that entry of water into the canalicular lumen was the primary determinant of the rate of adriamycin excretion in bile. It is possible in the present study that part of the increase in the biliary excretion of adriamycin was related to the increase in plasma adriamycin concentrations in cyclophosphamide-pretreated animals. The increase in plasma adriamycin concentration in cyclophosphamide-pretreated animals appears to be due primarily to a change in the apparent volume of distribution for adriamycin. This can only partly be explained by a loss of body weight, which was 11.6% over 4 days following cyclophosphamide treatment. The area under the concentration-time curve for plasma adriamycin increased 2.8-fold following cyclophosphamide pretreatment. It should be pointed out, however, that the calculation of area under the curve did not take into account the slow terminal phase of plasma adriamycin elimination [28], which was not measured in this study.

Adriamycin is not extensively metabolized in rat. Less than 1.0% of the total dose administered was excreted as adriamycinol in the bile, and no adriamycinol was detected in plasma. Other workers have also found little or no adriamycinol excreted in bile or in plasma of rat [5, 9, 13, 22]. This is in contrast to human, where plasma concentration of adriamycinol can approach those of adriamycin [1, 9] and adriamycinol is a major metabolite in bile [20, 29]. Low concentrations of adriamycin-derived aglycones were found in rat bile, confirming a previous report by Shinozawa et al. [22], although it has been claimed that no free aglycones are seen in rat bile [29]. In vitro studies with rat isolated hepatocytes failed to show formation of appreciable amounts of adriamycinol under aerobic or anaerobic conditions, although it is possible that adriamycinol is further metabolized to form conjugates [25] which are not detected by the assay procedure. Under anaerobic conditions formation of deoxyadriamycin aglycone and deoxyadriamycinol aglycone is seen by isolated hepatocytes. These aglycones are formed by reductive cleavage of adriamycin and adriamycinol by NADPH-cytochrome P-450 reductase [3]. Aglycone formation is inhibited under aerobic conditions due to redox-cycling of the semiquinone intermediate formed by reduction of adriamycin by NADPH-cytochrome P-450 reductase [2]. Pretreating rats with cyclophosphamide, which decreases hepatic NADPH-cytochrome P-450 reductase by 40%, had no significant effect upon the formation of aglycone metabolites by rat isolated hepatocytes. Cyclophosphamide pretreatment similarly had no significant effect upon the secretion of aglycone metabolites in rat bile. It appears from these results that NADPH-cytochrome P-450 reductase activity is not rate-limiting in the formation of aglycone metabolites from adriamycin.

Adriamycin is rapidly removed from plasma following its IV administration. The initial phase of removal, which follows the rapid blood distribution phase, is probably due to uptake of adriamycin from vascular and extracellular compartments into an intracellular compartment exhibiting a large apparent volume of distribution [2]. Biliary excretion may also contribute to this phase of elimination. Tavoloni and Guarino [26] reported an increase in plasma adriamycin equivalents in proportion to the inhibition of excretion of adriamycin in bile in rat produced by sodium taurolithocholate in the first 3 h following adriamycin administration. In the present study, however, biliary excretion of adriamycin increased in cyclophosphamide-pretreated rats at a time when plasma adriamycin clearance was decreased. This suggests that cyclophosphamide pretreatment inhibits a process which removes adriamycin from plasma, overcoming any contribution of an increased biliary adriamycin excretion to the initial phase of adriamycin clearance. The process responsible for removing adriamycin from plasma which is inhibited by cyclophosphamide pretreatment may be uptake of adriamycin into tissues. If uptake of adriamycin into tissues is decreased by cyclophosphamide pretreatment it may be that at later time release of adriamycin from tissues and binding sites, which is thought to be responsible for the slow terminal phase of plasma adriamycin elimination [2, 20], is also decreased. It is possible that the decrease in short-term adriamycin plasma clearance that we see in cyclophosphamide-pretreated animals might be compensated by an increase in adriamicin clearance during the terminal phase of plasma elimination, although this was not studied.

Morgan et al. [17] reported that administration of cyclophosphamide to patients with breast cancer 2 days prior to a low dose of adriamycin resulted in slower plasma elimination of total drug fluorescence, with $t_{1/2}\alpha$ 20 min and $t_{1/2}\beta$ 3 h, as against $t_{1/2}\alpha$ 10 min and $t_{1/2}\beta$ 1.5 h in patients not receiving cyclophosphamide. Evans et al. [10] reported that in children receiving adriamycin and cyclophosphamide concurrently the area under the concentration-time curve for serum adriamycin showed a small reduction and the area under the concentration-time curve for adriamycinol increased almost two-fold over that of the corresponding curve for subjects receiving adriamycin alone. The increase in area under concentration-time curves for plasma adriamycin in cyclophosphamide-treated rats is consistent with the changes seen in human, but no effect was seen on plasma adriamycinol concentrations. As noted previously, rat differs from human in forming little adriamycinol under normal conditions.

In summary, pretreatment of rats with cyclophosphamide decreases adriamycin plasma clearance and decreases the apparent volume of distribution of adriamycin administered

4 days later. There is an increase in bile flow and an increase in excretion of adriamycin in the bile of cyclophosphamide-pretreated rats. Cyclophosphamide pretreatment has no significant effect upon the excretion of adriamycin metabolites in rat bile or upon the utilization of adriamycin and the formation of adriamycin metabolites by rat isolated hepatocytes. A decrease in hepatic NADPH-cytochrome P-450 reductase activity produced by cyclophosphamide pretreatment is not associated with a significant decrease in formation of aglycone metabolites from adriamycin.

Acknowledgement. This work was supported by American Cancer Society Grant CH143.

References

1. Andrews PA, Brenner DE, Chan FTE, Kubo Hk, Bachur NR (1980) Facile and definitive determination of human adriamycin and daunorubicin metabolites by high-pressure liquid chromatography. Drug Metab Dispos 8: 152
2. Bachur NR (1979) Anthracycline antibiotic pharmacology and metabolism. Cancer Treat Rep 63: 817
3. Bachur NR, Gordon SL, Gee MV, Kon H (1979) NADPH-cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. Proc Natl Acad Sci USA 76: 954
4. Baurain R, Deprez-DeCampaneere D, Trouet A (1979) Rapid determination of doxorubicin and its fluorescent metabolites by high-pressure liquid chromatography. Anal Biochem 94: 112
5. Broggini M, Colombo T, Martini A, Danelli MG (1980) Studies on the comparative distributions and biliary excretion of doxorubicin and 4′-epi-doxorubicin in mice and rats. Cancer Treat Rep 64: 897
6. Corbett TH, Griswold DP, Mayo JG, Laster WR, Schabel FM (1975) Cyclophosphamide-adriamycin combination chemotherapy of transplantable murine tumors. Cancer Res 35: 1568
7. Davis HL, Davis TL (1979) Daunorubicin and adriamycin in cancer treatment: An analysis of their roles and limitations. Cancer Treat Rep 63: 809
8. Driscoll JS, Hazard GF, Wood HB, Goldin A (1974) Structure-antitumor activity relationships among quinone derivatives. Cancer Chemother Rep [2] 4: 1
9. Eksborg S, Ehrsson H, Andersson I (1979) Reversed-phase liquid chromatographic determination of plasma levels of adriamycin and adriamycinol. J Chromatogr 164: 479
10. Evans WE, Crom WR, Yee GC, Green AA, Hayes A, Pratt CB, Avery TL (1980) Adriamycin pharmacokinetics in children. Proc Am Assoc Cancer Res 21: 176
11. Gibaldi M, Perrier D (1975) In: Pharmacokinetics. Marcel Dekker, New York, pp 45–128 and pp 293–300
12. Gurtoo HL, Gessner T, Culliton P (1976) Studies of the effects of cyclophosphamide, vincristine, and prednisone on some hepatic oxidations and conjugations. Cancer Treat Rep 60: 1285
13. Israel M, Wilkinson PM, Pegg WJ, Frei E (1978) Hepatobiliary metabolism and excretion of adriamycin and *N*-trifluoroacetyl adriamycin-14-valerate in the rat. Cancer Res 38: 365
14. Klassen CD (1971) Studies on the increased biliary flow produced by phenobarbital in rats. J Pharmacol Exp Ther 176: 743–751
15. Marinello AJ, Berrigan MJ, Struck RF, Guengerich FP, Gurtoo HL (1981) Inhibition of NADPH-cytochrome P-450 reductase by cyclophosphamide and its metabolites. Biochem Biophys Res Commun 99: 399
16. Metzler CM, Elfring GL, McEwen AJ (1974) A package of computer programs for pharmacokinetic modeling. Biometrics 30: 562
17. Morgan LR, Hagardorn AN, Maddux B, Sutherland C, Carter RB, Krementz ET (1976) Pharmacology of weekly low-dose adriamycin in human breast cancer. Pharmacologist 18: 126
18. Muggia FM, Perloff M, Chia GA, Reed LJ, Escher GC (1974) Adriamycin (NSC-123127) in combination with cyclophosphamide (NSC-26271): A phase I and II evaluation. Cancer Chemother Rep 58: 919
19. Powis G, Svingen BA, Degraw C (1982) Iron-EDTA stimulated reduction of indicine N-oxide by the hepatic microsomal fraction, isolated hepatocytes, and the intact rat. Biochem Pharmacol 31: 293
20. Reich SD (1978) Clinical correlations of adriamycin pharmacology. Pharmacol Ther [C] 2: 239
21. Riggs CE, Benjamin RS, Serpick AA, Bachur NR (1977) Biliary disposition of adriamycin. Clin Pharmacol Ther 22: 234
22. Shinozawa S, Oda T (1981) Determination of adriamycin (doxorubicin) and related fluorescent compounds in rat lymph and gall by high-performance liquid chromatography. J Chromatogr 212: 323
23. Snedecor GW, Cochran WG (1976) Statistical methods, 6th edn. Iowa State University Press, Ames, p 59
24. Stewart AJ, Inaba T (1979) *N*-Demethylation of aminopyrine in vivo and in the isolated hepatocyte of the rat. Biochem Pharmacol 28: 461
25. Takanashi S, Bachur NR (1976) Adriamycin metabolism in man. Evidence from urinary metabolites. Drug Metab Dispos 4: 79
26. Tavoloni N, Guarino AM (1980a) Bile secretory function: A determinant of adriamycin disposition. Arch Int Pharmacodyn 245: 180
27. Tavoloni N, Guarino AM (1980b) Biliary and urinary excretion of adriamycin in anesthetized rats. Pharmacology 20: 256
28. Wilkinson PM, Mawer GE (1974) The persistence of adriamycin in man and rat. Br J Clin Pharmacol 1: 241
29. Wilkinson PM, Israel M, Pegg WJ, Frei E (1979) Comparative metabolism and excretion of adriamycin in man, monkey, and rat. Cancer Chemother Pharmacol 2: 121
30. Williams BA, Tritton TR (1981) Photoinactivation of anthracyclines. Photochem Photobiol 34: 131
31. Yasukochi Y, Masters BSS (1976) Some properties of a detergent-solubilized NADPH-cytochrome c (cytochrome P-450) reductase purified by biospecific affinity chromatography. J Biol Chem 251: 5337
32. Yesair DW, Schwarztbach E, Schuck D, Denine EP, Asbell MA (1972) Comparative pharmacokinetics of daunomycin and adriamycin in several animal species. Cancer Res 32: 1177

Received April 8, 1982/Accepted July 22, 1982