(4) T. R. Weibrauch, H. J. Prior, D. Höffler, and J. Kreiglstein, *Klin. Wochenshr.*, **52**, 842 (1974).
(5) D. Phaneuf and H. C. Neu, *Antimicrob. Agents Chemother.*, **16**, 625 (1979).
(6) R. D. Libke, J. T. Clarke, E. D. Ralph, R. P. Luthy, and W. M. M. Kirby, *Clin. Pharmacol. Ther.*, **17**, 441 (1975).
(7) E. Kalkani and N. Marketos, *Antimicrol. Agents Chemother.*, **9**, 89 (1976).
(8) J. S. Tan and S. J. Salstrom, *ibid.*, **11**, 698 (1977).
(9) P. Chanbusarokum and P. R. Murray, *ibid.*, **14**, 505 (1978).
(10) H. J. Harwick, P. Weiss, and F. R. Fekety, *J. Lab. Clin. Med.*, **72**, 511 (1968).
(11) E. R. Ervin and W. E. Bullock, *Antimicrob. Agents Chemother.*, **9**, 94 (1976).
(12) R. G. Tucker, *Nature (London)*, **173**, 85 (1954).
(13) C. J. Perret, *ibid.*, **174**, 1012 (1954).
(14) D. C. Grove and P. R. Randall, in "Assay Methods of Antibiotics," Medical Encyclopedia, New York, N.Y., 1955, p. 16.
(15) P. J. Weiss, *Antibiot. Chemother.*, **9**, 660 (1959).
(16) R. M. Herriot, *J. Biol. Chem.*, **194**, 725 (1946).
(17) M. W. Brandriss, M. L. Denny, M. A. Huber, and H. G. Steinman, *Antimicrob. Agents Chemother.*, **1962**, (627).
(18) T. Yasuda and S. Shimada, *J. Antibiot.*, **24**, 290 (1971).
(19) F. W. Staab, E. A. Ragan, and S. B. Binkley, *Abstracts of the 109th Meeting American Chemical Society 3B*, (1946).
(20) G. E. Boxer and P. M. Everett, *Anal. Chem.*, **21**, 670 (1949).
(21) F. R. Batchler, E. B. Chain, R. Hardy, K. R. L. Mansford, and G. N. Rolinson, *Proc. R. Soc. London, Ser. B*, **154**, 498 (1961).
(22) D. M. Lichtenwalner, B. Suh, B. Lorber, and A. Sugar, *Antimicrob. Agents Chemother.*, **16**, 210 (1979).
(23) J. M. Blaha, A. M. Knevel, and S. L. Hun, *J. Pharm. Sci.*, **64**, 1384 (1975).
(24) K. Tsuji and J. H. Robertson, *ibid.*, **64**, 1542 (1975).
(25) A. Tsuji, E. Miyamoto, O. Kubo, and T. Yamana, *ibid.*, **68**, 812 (1979).
(26) E. R. White, M. A. Carrol, J. E. Zarembo, and A. D. Bender, *J. Antibiot.*, **28**, 205 (1975).
(27) E. R. White, M. A. Carrol, and J. E. Zarembo, *ibid.*, **30**, 811 (1977).
(28) T. L. Lee, L. D'arconte, and M. A. Brooks, *J. Pharm. Sci.*, **68**, 454 (1979).
(29) W. A. Vadino, E. T. Sugita, R. L. Schnaare, H. Y. Ando, and P. J. Niebergall, *ibid.*, **68**, 1316 (1979).
(30) R. M. Bannatyne and R. Cheung, *Antimicrob. Agents Chemother.* **16**, 43 (1979).
(31) D. C. Rapson and A. E. Bird, *J. Pharm. Pharmacol.*, **15**, 222T (1963).
(32) R. H. Kwan, Thesis, University of Toronto, Toronto, Canada (1980).
(33) R. Sutherland, J. Burnett, and G. N. Rolinson, *Antimicrob. Agents Chemother.*, **1970**, 390.
(34) R. D. Libke, *Clin. Pharmacol. Ther.*, **17**, 441 (1975).
(35) M. J. Perry and H. C. Neu, *Chemother. Proc. Int. Congr. Chemother., 9th*, (1975), 5.

ACKNOWLEDGMENTS

Supported by a generous grant from Beecham Laboratories, Pointe Claire, Quebec.

The authors also wish to acknowledge the kind cooperation of Dr. R. M. Bannatyne and Ms. R. Cheung of the Department of Bacteriology, The Hospital for Sick Children, Toronto in performing the microbiological assays of ticarcillin and for their helpful comments.

Concomitant Adsorption and Stability of Some Anthracycline Antibiotics

E. TOMLINSON *x and L. MALSPEIS

Received October 20, 1981, from the *Division of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, Ohio State University, Columbus, OH 43210.* Accepted for publication December 14, 1981. *Present address: Department of Pharmacy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands.

Abstract □ Using liquid scintillation counting and liquid chromatographic techniques, it has been demonstrated that the anthracycline antibiotics, doxorubicin hydrochloride, *N*-trifluoroacetyladriamycin-14-valerate, and *N*-trifluoroacetyladriamycin-14-octanoate, can be strongly adsorbed to the walls of containers, depending on the nature of both the container material and the solute. It also has been shown that the esters are unstable in the chemical growth media commonly used in cell culture studies. Both the adsorption and stability effects are suggested as being factors which should be carefully considered in interpretation of the *in vitro* and perhaps *in vivo* activities of the anthracycline esters.

Keyphrases □ Anthracyclines—concomitant adsorption and stability, esters, cell culture studies □ Antibiotics—anthracyclines, concomitant adsorption and stability, cell culture studies □ Adsorption—concomitant adsorption and stability of some anthracyclines, cell culture studies

The anthracycline antibiotic, doxorubicin hydrochloride (I), has a wide range of antitumor activity (1). Although I given singly or in combination is now used extensively in the treatment of a variety of tumors (2, 3), its clinical value is limited due to its acute myelotoxicity and to the frequent development of irreversible cardiomyopathy [normally observed when the accumulated dose is >550 mg m^{-2} (4)]. The use of structural analogs of I has been proposed (5, 6) as a means of obviating these two effects; in this respect particular interest has been given to the proposed use of the esters of I and its *O*- and *N*-acetylated derivatives (7–14).

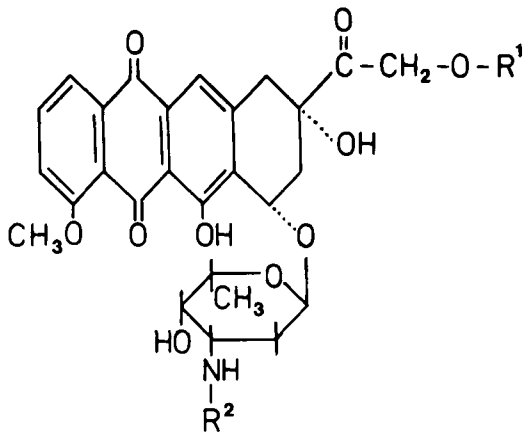
During a study examining the uptake of I and its analogs into tumor cells *in vitro* using both cell monolayers and suspensions, effects were observed on the mass balances of some radiolabeled anthracyclines which could only be reconciled in terms of solute adsorption to container walls. Accordingly, the role of adsorption and stability on the solution properties of I and two of its *N*-trifluoroacetyl esters (*i.e.*, the 14-valerate and the 14-octanoate) have been investigated more completely. The present report presents the results found in this investigation.

EXPERIMENTAL

Chemicals—Anthracycline antibiotics were obtained commercially[1]. ^{14}C-Labeled antibiotics were synthesized as described previously (15). Samples of solid I, *N*-trifluoroacetyladriamycin-14-valerate (II), and *N*-trifluoroacetyladriamycin-14-octanoate (III) had specific activities of 17.1, 22.5, and 28.3 μCi $\mu mole^{-1}$, respectively.

[1] Adria, Ohio.

0022-3549/82/01000-1121$01.00/0



	R^1	R	k′
I	H	H	
II	$CO(CH_2)_3.CH_3$	$COCF_3$	1.6
III	$CO(CH_2)_6.CH_3$	$COCF_3$	3.1
IV	H	$COCF_3$	0.6

The Hanks culture media used was an isotonic buffered (pH 7.2–7.6) solution[2] containing essential salts and glucose, together with 10% fetal calf serum, streptomycin, penicillin, gentamicin, and glutamine. For studies examining only adsorption effects, the Hanks solution contained only the essential salts and glucose.

Analysis—^{14}C-labeled anthracycline was assayed using a liquid scintillation counter[3]. Unlabeled anthracycline was assayed by high-performance liquid chromatography (HPLC) using a stationary phase packing of RP-18[4]: mobile phase, methanol–water (9:1); fluorescence detection[5] with 239 nm excitation and a 550 nm cutoff filter; potassium dichromate as nonretained compound; and quantitation using total integrated peak areas[6]. During assay care was taken to ensure that inner-filter effects (16) were avoided by measuring only in the linear portion region of the anthracycline concentration *versus* peak area relationship. The good reproducibility of the method (coefficient of variation <1.0%) permitted the use of an internal standard to be avoided. The chromatographic capacity ratios measured using this system were 1.6, 3.1, and 0.6 for II, III, and IV, respectively.

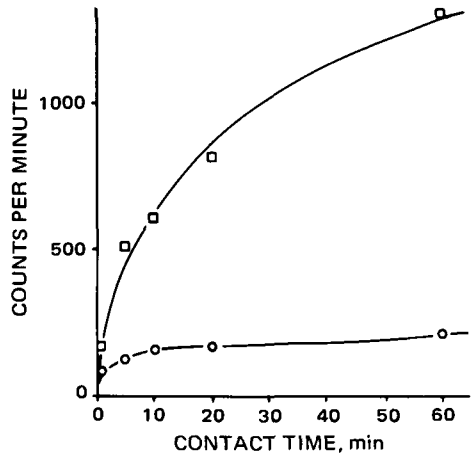


Figure 1—*Total radioactivity (cpm) of 0.5 ml of sodium lauryl sulfate lysis wash after incubation of benzpyrene-induced adenocarcinoma tumor cells (in monolayer aspect) for varying drug solution contact times using polyethylene petri dish containers. Key: (○) I; (□) II.*

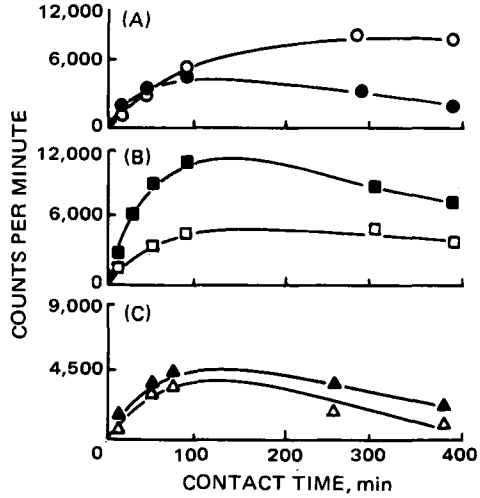


Figure 2—*Total radioactivity (cpm) of 0.5 ml of sodium lauryl sulfate lysis wash after various incubation times with antibiotic solution at 37° in the absence (closed data points) and presence (open data points) of tumor cells (in the monolayer aspect) using polyethylene petri dish containers. Key: (○●) I; (□■) II; (△▲) III.*

Adsorption-Stability Studies—*With Tumor Cells*—Hamster benzpyrene-induced adenocarcinoma cells were prepared as monolayers by seeding 10^5 cells into either 35-mm diameter sterile polyethylene petri dishes[7], or glass (silica) monolayer growth tubes (area of growth was ~2 cm^2) followed by incubation at 37° for 24 hr. The incubation media was removed by suction, and 1 ml of Hanks media was added to wash the cells. This solution was then removed and 1 ml of radiolabeled anthracycline was added (2 μM in Hanks media). Tubes or dishes were then placed in an incubator at 37°. After appropriate incubation times, the drug solution was removed from the culture vessels by suction, and these vessels were washed three times with 1 ml of Hanks solution; 1 ml of 0.5% sodium

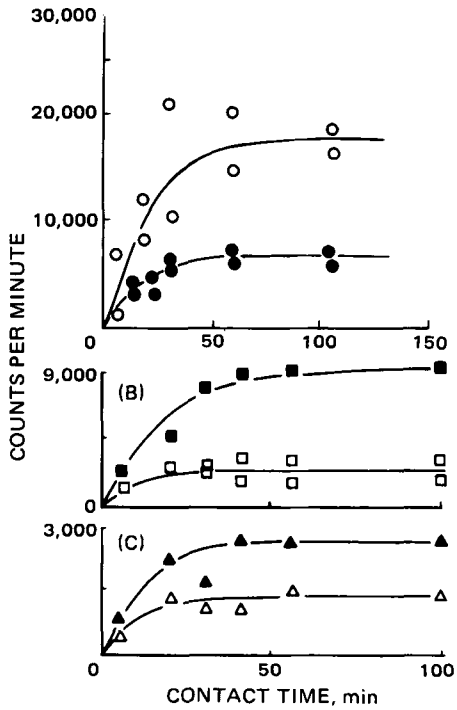


Figure 3—*Total radioactivity (cpm) of 0.5 ml of sodium lauryl sulfate lysis wash after various incubation times with antibiotic solution at 37° in the absence and presence of benzpyrene-induced adenocarcinoma tumor cells (monolayers) using silica glass culture tubes. Key: (○●) I: (□■) II; (△▲) III, and as Fig. 2.*

[2] Grand Island Minimum Essential Media.
[3] Packard Tricarb, model 3375.
[4] Spherisorb ODS 5 μm, (PhaseSep).
[5] Schoeffel flow through detector, model GM 970.
[6] Infotronics, model 308.
[7] Corning #25000, Corning Works, Corning, N.Y.

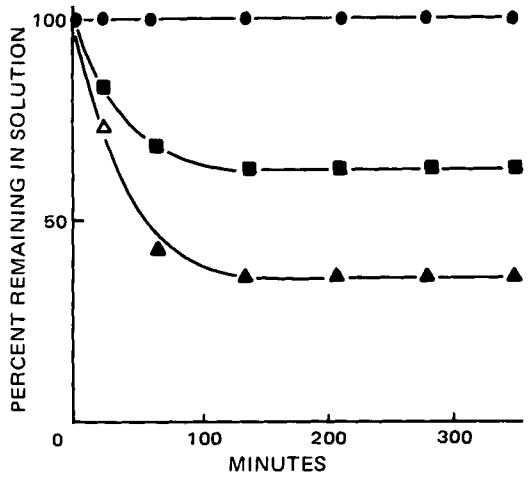


Figure 4—*Percent total radioactivity in Hanks solution with time (pH 7.4, 37°) using a polypropylene container and 1 and 2 μM initial concentrations of antibiotic. Key: (●) I; (■) II; (▲) III, and as Fig. 2.*

lauryl sulfate (as cell digestion agent) was added. After an additional 10 min, 0.5 ml of this digestion fluid was transferred to 19.5 ml of scintillation cocktail and the radioactivity measured. All data given are the mean of three separate determinations carried out in duplicate. For the blank experiments, the described procedure was followed; however, no cells were present in the culture vessels.

Absence of Tumor Cells—Solutions (1 or 2 μM) of the anthracyclines were prepared in Hanks media by dilution of a 100- or 200-μM stock methanolic solution of compound, which was stored in the dark at 0° for not longer than 7 days. (Storage of the stock solution for this length of time was shown by HPLC analysis to be possible without any significant deterioration in the solution.) The dilution was performed by adding 0.1–0.4 ml of stock solution to 19.6–19.9 ml of Hanks media at 37°. The solutions were stirred at 100 rpm using an overhead stirrer and a stirring paddle constructed of the same material as the container. Containers examined were 150-ml silica glass beakers siliconized by immersion in 5% silicone solution with oven drying, 100-ml polytetrafluoroethylene beakers, 50-ml polypropylene conical tapered tubes, and 100-ml stainless steel beakers. All containers were new when used and were discarded after use. For assay using HPLC, 5-, 10-, or 20-μl samples were taken using an HPLC syringe[8] aged using a 2-μM solution of antibiotic. For assay by scintillation counting, 0.1-ml samples were taken using an automatic syringe having polypropylene tips, transferring in the minimum amount of time to 19.9 ml of scintillation cocktail fluid. Anthracycline solutions were kept in the dark whenever possible. All data given are the means of three separate determinations.

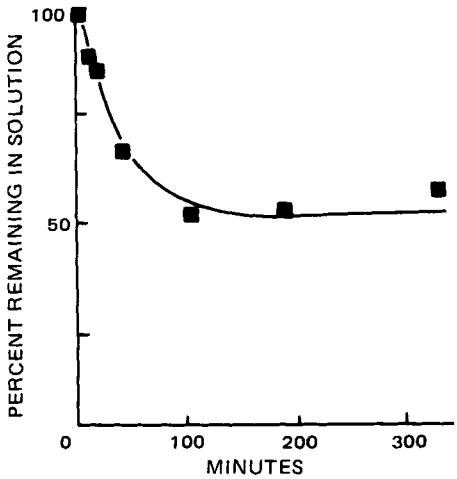


Figure 5—*Percent total radioactivity in Hanks solution with time (pH 7.4, 37°) using a siliconized glass container and 1 and 2 μM initial concentrations of II.*

[8] Hamilton HPLC syringe.

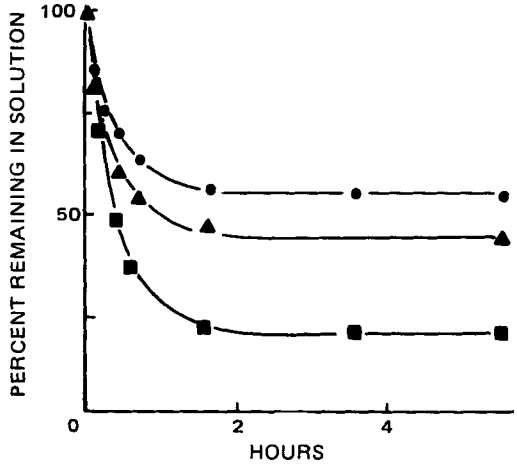


Figure 6—*Percent total radioactivity remaining in Hanks solution with time (pH 7.4, 37°) using a polytetrafluoroethylene container and 1 and 2 μM initial concentrations of antibiotic. Key: (●) I; (■) II; (▲) III.*

RESULTS AND DISCUSSION

Adsorption effects by large hydrophobic drugs have been reported (17–23) with respect to their apparent loss from solution onto and into the walls of their containers. The adsorption of I onto container walls, although a recognized phenomenon, has been noted only briefly in the literature as the probable cause of the observed nonideal behavior of I in its analysis (24) and during its sampling from solution (25). In addition, the use of various cosolvent extraction schemes have been proposed (24, 26, 27) to overcome these effects. Although it has been shown that I undergoes self-association in water (28), others have suggested (29) that no evidence for air–water interfacial adsorption of I can be found.

Although the *in vivo* metabolism of I and its esters have been reported (11), little attention has been given to their solution stability. Kaniewska (30) showed that the aqueous stability of I over a temperature range of 50–60° and a pH range of 2.6–5.5 is greater at lower temperatures and the higher pHs, with a half-life of 136 hr in water at 25° and pH 5.5 (0.2% phosphate buffer) being suggested by extrapolation. Other workers have studied recently the photostability of doxorubicin (31) and the stability of some anthracyclines in infusion fluids (32).

Using the described procedures, Fig. 1 shows the amount of radioactive anthracycline antibiotic determined in the cell digestion fluid after various contact times. Similar cell uptake kinetics are reported often in the literature for these molecules and show that the more hydrophobic the molecule, the greater its rate and extent of uptake by cells (7, 8, 14, 33, 34). However, using the same digestion technique, but in the absence of cells, it can be demonstrated (Figs. 2 and 3) that for I, II, and III, more or less anthracycline antibiotic can be extracted from the system, de-

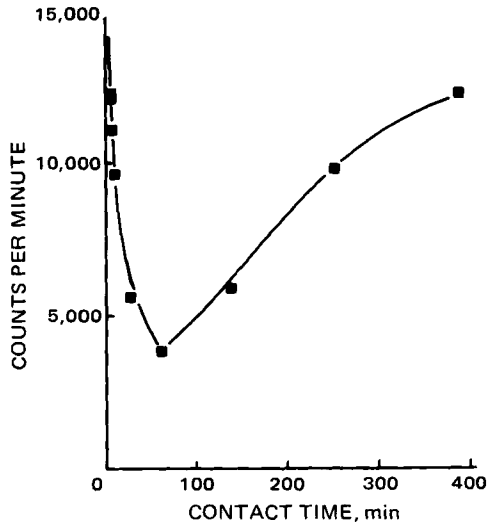


Figure 7—*Percent total radioactivity in Hanks solution (pH 7.4, 37°), for II (2 μM) using a stainless steel container.*

Table I—Percent Maximum Loss of Anthracycline from Solution to Container Wall

Material	Percent Maximum Loss[a] Compound I	II	III	IV[b]
Polypropylene	0	32 (110 min)	67 (190 min)	6 (20 min)
Polytetrafluoroethylene	45 (60 min)	83 (100 min)	57 (100 min)	—
Siliconized glass	0	50 (100 min)	74 (90 min)	—
Stainless steel	—	68 (60 min)	—	—
Glass tubes[c]	7.3 (5 min)	30 (60 min)	8.5 (25 min)	—
Polyethylene petri dishes[c]	4.6 (50 min)	8.7 (70 min)	11 (50 min)	—

[a] Initially 1- or 2-μM solution of radioactive-labeled molecule in Hanks media at 37° in the absence of cells and assayed using scintillation counting; number in parentheses, time for maximum adsorption. [b] By HPLC. [c] Solution 2 μM left in contact, no stirring, all external solution removed by suction, surface washed four times, 1 ml of sodium lauryl sulfate to surface for 30 min, aliquot of this control.

Table II—Rate Constants According to Scheme I

Rate constant	k, hr^{-1} II	III
k_{12}	1.900	2.150
k_{21}	0.005	0.001
k_{13}	1.150	1.150
k_{34}	0.012	0.012
k_{43}	0.001	0.001
k_{35}	0.085	0.085

pending on the analog and the type of container material used. This suggests an adsorption of anthracycline onto the container walls.

Loss of Radioactive Label from Solution—Figures 4–7 show how the loss of anthracycline is affected by the type of container material in use. Although polypropylene has no adsorbent properties, glass, polyethylene, polytetrafluoroethylene, and stainless steel do have adsorbent properties for I. Both esters are taken up well by all the materials studied, with the more hydrophobic molecule being taken up at a faster rate and to a greater extent. Table I is a summary of these effects. The unusual phenomenon found with stainless steel will be discussed.

All three anthracycline antibiotics studied can be lost from aqueous solution solely by adsorption or sorption. The extent of this adsorption depends on the nature of the container material used. Similar effects have been observed (19) for hydrophobic amines on wall adsorption material dependency. The present results indicate strongly that it is necessary to take care in practice to avoid these effects by: (*a*) reducing the number of wall surface contacts made; (*b*) choosing a container material with care;

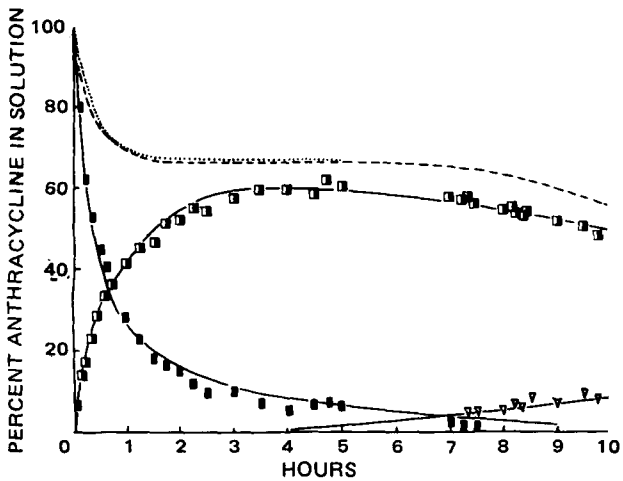


Figure 8—*Concomitant adsorption and stability of II at pH 7.4 and 37° using polypropylene containers. Solid lines are generated using the model given by Scheme I and the rate constants of Table II. Key: (■) II; (◨) IV; (▽) breakdown product of IV; (·····) total radioactivity found in solution; (- - -) the total normalized HPLC peak area.*

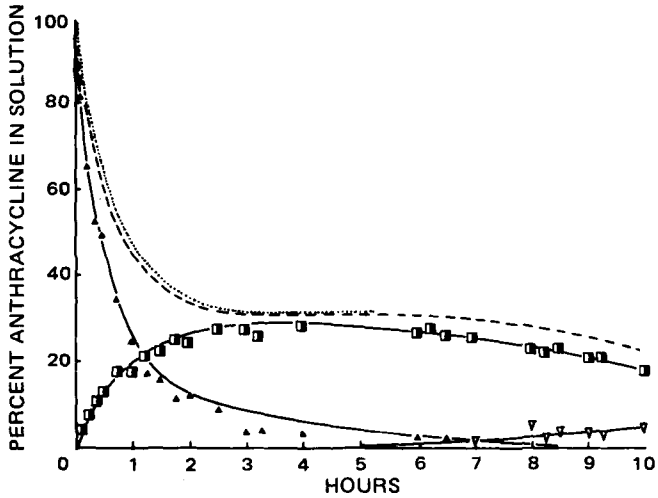


Figure 9—*Concomitant adsorption and stability of III at pH 7.4 and 37° using polypropylene containers. Solid lines are generated as for Fig. 8. Key: (▲) III; (◨) IV; (▽) breakdown product of IV; (·····) total radioactivity found in solution; (- - -) the total normalized HPLC peak area.*

(*c*) making use of possible preaging of containers with compound before use; and (*d*) using cosolvents (19, 24, 26, 27).

Concomitant Stability and Adsorption of II and III—Israel *et al.* (11, 12) demonstrated that *in vivo* II is not metabolized extensively to I, but that *N*-trifluoroacetyladriamycin (IV) and *N*-trifluoroacetyladriamycinol are important metabolites of this drug. In addition, the lack of DNA binding of II suggests that its *in vivo* antitumor activity is due to either its conversion to a DNA-binding metabolite or to a mechanism of action different from that accepted (10) for I. Thus, since II and other esters of I are proposed as antitumor agents with a high therapeutic index, and with the knowledge that both the esters examined in this study are adsorbed onto container walls, it was appropriate to examine ester stability in conjunction with these adsorption effects. Figures 8 and 9 give the combined HPLC and radioactivity information for II and III. The initial breakdown product of both II and III is considered to be IV, since the chromatographic capacity ratio of this product corresponded exactly to the value for authentic samples of IV. The second breakdown product had a chromatographic capacity factor which indicated that it was more hydrophobic than the parent molecule.

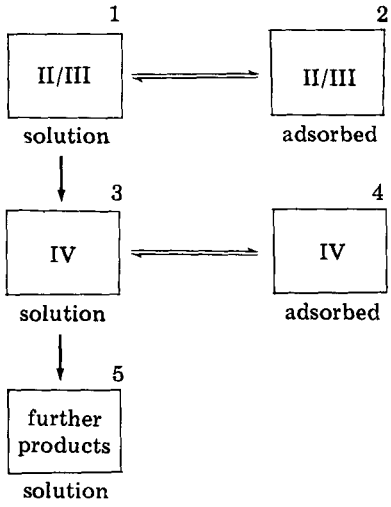
Although the initial HPLC data up to 4 hr indicate that the total anthracycline mass detected is equal to that found using radioactivity measurements, the subsequent falls in the total fluorescent peak values (Figs. 8 and 9) suggest that the first breakdown product is itself weakly adsorbed onto the polypropylene container wall. These data suggest that the solution equilibria for II and III in aqueous solution at pH 7.4 can be described by Scheme I when adsorption effects are in evidence. These schemes have been modeled using an analog computer[9], and the lines drawn in Figs. 8 and 9 are computer generated using the rate constants determined (Table II).

Good agreement is obtained between the experimental results and the theoretical results using Scheme I as a model of the equilibria occurring in these systems. Thus, the rapid loss of either ester from solution can be attributed to both adsorption and chemical transformation to IV.

Notable features of the rate constants are that the desorption steps are small, and, although for III adsorption is faster, for neither ester can hydrolysis or adsorption be described as the rate-determining step in the loss process. Combination of the peak areas for either ester and IV (normalized for differences in extinction) gives mass balances equivalent to those found using radioactivity measurements. Compound IV is unstable to a small extent and also adsorbs, which is confirmed by inspection of Fig. 10 where the results of an HPLC examination of the behavior of this compound in Hanks media are given.

If it is assumed that the ester is stable in the adsorbed state, then the container acts as a reservoir. For the polypropylene material, since the degradation products of the esters are less adsorbed due to their lower hydrophobicity, it follows that the relationship between total anthracycline concentration in solution and time should exhibit a minimum. Due to the slow rates of desorption (Table II), these minima are not seen

[9] Type RA 742, Telefunken.



Scheme I

within the experimental time, although there is some indication of this effect in the case of II and siliconized glass (Fig. 5). A similar phenomenon would be observed if the ester were unstable at the container surface resulting in the production of a species with a high desorption rate. This effect is observed for II adsorbed at the stainless steel surface (Fig. 7). If this were the case, the altered stability of II at the stainless steel surface could be indicative of metal ion catalysis, which is possible since the structures of these anthracycline antibiotics suggest that they can act as chelating agents.

The results indicate that anthracycline antibiotics can adsorb strongly onto the walls of containers, and that the rate and extent of adsorption depends on both the nature of the anthracycline and the wall material

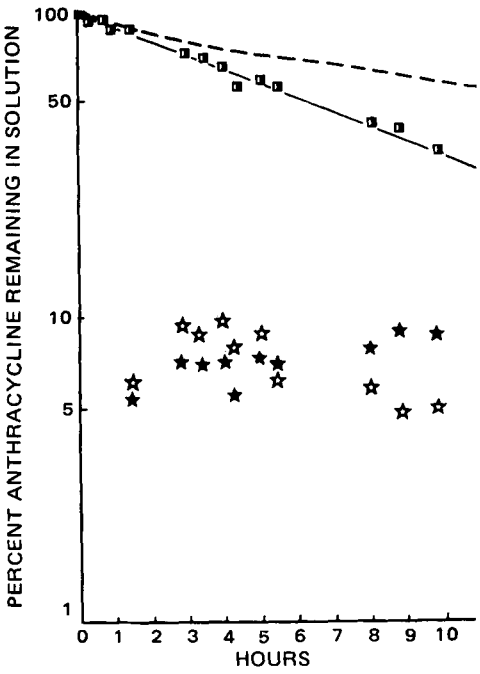


Figure 10—*Stability of IV in Hanks media (pH 7.4, 37°) using polypropylene containers. Key: (- - -) total peak area obtained by summation of the IV HPLC peak area (■) and the peak areas of its two fluorescent breakdown products (☆ ★).*

used. It is believed that these effects have an importance in the analysis of these compounds, as well as in those studies attempting to measure the effect of these antibiotics in living cell cultures. Finally, it can be argued that the combined effects of adsorption and ester instability have implications for the bioavailability and delivery of these drugs.

REFERENCES

(1) R. H. Blum and S. K. Carter, *Ann. Intern. Med.*, **80,** 249 (1974).

(2) A. Dimarco and L. Lenaz, in "Cancer Medicine," J. F. Holland and E. Frei, Eds., Lea and Febiger, Philadelphia, Pa., 1973, p. 826.

(3) T. Skovsgaard and N. I. Nissen, *Dan. Med. Bull.*, **22,** 64 (1975).

(4) E. A. Lefrak, J. Pitha, S. Rosenheim, and J. A. Gottlieb, *Cancer*, **32,** 302 (1973).

(5) P. Chandra, *Cancer Chemother. Rep. Part 3*, **6,** 115 (1975).

(6) M. Israel, E. J. Modest, and E. Frei, *Cancer Res.*, **35,** 1365 (1975).

(7) F. Arcamone *et al.*, *J. Med. Chem.*, **17,** 335 (1974).

(8) N. R. Bachur, M. Steele, W. D. Meriwether, and R. C. Hildebrand, *ibid.*, **19,** 651 (1976).

(9) S. Neidle, *Cancer Treat. Rep.*, **60,** 217 (1976).

(10) S. K. Sengupta, S. Seshadri, E. J. Modest, and M. Israel, *AACR Abstracts*, 109 (1976).

(11) M. Israel, N. J. Pegg, and P. M. Wilkinson, *J. Pharmacol. Exp. Ther.*, **204,** 696 (1978).

(12) M. Israel, P. M. Wilkinson, W. J. Pegg, and E. Frei, *Cancer Res.*, **38,** 365 (1978).

(13) G. Cassinelli, D. Ruggieri, and F. Arcamone, *J. Med. Chem.*, **22,** 121 (1979).

(14) P. M. Kanter and H. S. Schwartz, *Cancer Res.*, **39,** 448 (1979).

(15) B. R. Vishnuvajjala, T. Kataoka, F. D. Cazer, D. T. Witiak, and L. Malspeis, *J. Labeled Comp. Radiopharm.*, **14,** 77 (1978).

(16) R. B. Mikkelsen, P. S. Lin, and D. F. H. Wallach, *J. Mol. Med.*, **2,** 33 (1977).

(17) L. H. Bird, *N. Z. Sci. Technol.* **30,** 344 (1949).

(18) K. T. H. Farrer and E. C. J. Hollenberg, *Analyst*, **78,** 730 (1953).

(19) K. D. Thakker, T. Higuchi, and L. A. Sternson, *J. Pharm. Sci.*, **68,** 93 (1979).

(20) T. Mitzutani and A. Mitzutani, *ibid.*, **67,** 1102 (1978).

(21) T. Mitzutani and A. Mitzutani, *ibid.*, **69,** 279 (1980).

(22) T. Mitzutani, *ibid.*, **70,** 493 (1981).

(23) W. G. Crouthamel and K. van Dyke, *Anal. Biochem.*, **66,** 234 (1975).

(24) S. Eksborg, H. Ehrsson, B. Andersson, and M. Beran, *J. Chromatogr.*, **153,** 211 (1978).

(25) K. Danø, *Biochem. Biophys. Acta*, **323,** 466 (1973).

(26) R. Baurain, A. Zenebergh, and A. Trouet, *J. Chromatogr.*, **157,** 331 (1978).

(27) J. F. Strauss, R. L. Kitchens, V. W. Patrizi, and E. P. Frenkel, *ibid.*, **221,** 139 (1980).

(28) S. Eksborg, *J. Pharm. Sci.*, **67,** 783 (1978).

(29) F. A. Vilallonga and E. W. Phillips, *ibid.*, **67,** 773 (1978).

(30) T. Kaniewska, *Farm. Pol.*, **33,** 539 (1977).

(31) N. Tavoloni, A. M. Guarino, and P. D. Berk, *J. Pharm. Pharmacol.*, **32,** 860 (1980).

(32) G. K. Poochikian, J. C. Cradock, and K. P. Flora, *Am. J. Hosp. Pharm.*, **38,** 483 (1981).

(33) T. Skovsgaard, *Biochem. Pharmacol.*, **26,** 215 (1977).

(34) *Ibid.*, **27,** 1221 (1978).

ACKNOWLEDGMENTS

The authors thank Dr. B. S. Zwilling, Department of Microbiology, The Ohio State University, for assistance in the cell culture studies.