[CANCER RESEARCH 43, 546–550, February 1983]
0008-5472/83/0043-0000$02.00

Improved Therapeutic Benefits of Doxorubicin by Entrapment in Anionic Liposomes[1]

Eric A. Forssen[2] and Zoltán A. Tökés[3]

Comprehensive Cancer Center and Department of Biochemistry, University of Southern California, Los Angeles, California 90033

ABSTRACT

When used as drug carriers, anionic liposomes can reduce the chronic cardiac toxicity and increase the antileukemic activity of doxorubicin (DXN; Adriamycin). Continuing investigations, reported here, have now established the therapeutic benefits of this mode of drug delivery. Liposome encapsulation caused a prolonged elevation in DXN plasma levels and a 2-fold reduction in the exposure of cardiac tissue to the drug. This reduction, however, was not proportional to the substantial decrease in chronic heart toxicity observed in the earlier study. *In vivo* studies have demonstrated that the entrapped drug retains its full activity against Sarcoma 180 and significantly increases its action against Lewis lung carcinoma, as measured by reduced tumor volume. The increased antineoplastic activity was again not proportional to the increased association of drug with tumor tissue. The effect of liposome entrapment on the immune-suppressive activity of DXN was also examined to determine if factors other than the direct delivery of drug to tumor tissue might improve the therapeutic response. The suppression of the humoral immune response and peripheral leukocyte counts by free DXN was nearly abolished when the drug was administered in the liposome form. These experiments suggest that the improved therapeutic effect of encapsulation may be the outcome of three different mechanisms: (*a*) altered disposition into subcellular compartments, which reduces cardiotoxicity; (*b*) increased plasma drug exposure to tumor cells; and (*c*) significant reduction in the immune suppressive activity of DXN.

INTRODUCTION

The broad spectrum of the antitumor activity of DXN[4] has made it a widely used cancer chemotherapeutic agent (6, 9). Its therapeutic potential has been limited, however, by both its chronic cardiac toxicity (16, 17, 18, 19) and its severe immune suppression (28, 29, 32). Structural analogues (5, 8), combination therapy with potentially protective compounds (1, 27), and specialized drug carriers (2, 26, 31) for DXN have been examined in an effort to extend its therapeutic benefits. Previous investigators have reported that, when used as drug delivery vehicles, anionic liposomes can minimize the chronic cardiac toxicity of DXN while maintaining or improving its antileukemic effect (11, 21). The Sarcoma 180 and Lewis lung carcinoma tumor models have been used to compare the chemotherapeutic activities of DXN-liposomes and free drug. The relative uptake for free and entrapped DXN by leukemic cells and solid tumor tissues has also been determined. In addition, we now report that liposome entrapment reduces the immune suppressive effect of DXN as measured by hemagglutinating antibody titers.

MATERIALS AND METHODS

Preparation of Liposomes. DXN, phospholipids, and cholesterol were purchased from Sigma Chemical Co. (St. Louis, Mo.). All compounds were tested for purity by thin-layer chromatography using methods described previously (10). The lipids were found to be pure or to contain only trace amounts of cholesterol. DXN was found to be pure on silica gel thin-layer chromatography under conditions used previously for the separation of DXN from its metabolites (30). A 2-step method, described in an earlier report (10), has been used in order to avoid the precipitation of a DXN-phosphatidyl serine complex which has been found to occur with direct mixing in a one-step procedure. Briefly, a complex of DXN and PC is formed by adding a solution of the drug (8 mg/ml) in 0.077 M NaCl to the dried lipid in a DXN:PC molar ratio of 1:2. This mixture is warmed in a water bath to 45–50° and sonicated until clear (about 5 min) under nitrogen atmosphere using a needle probe sonicator (Braun Sonic, 1410) set at 100 watts. The complex is then added to a dried mixture of PC, phosphatidylserine, and cholesterol at a molar ratio of 0.6:0.2:0.3 per mol of DXN. This second liposome suspension is also sonicated at 100 watts for 5 min. Entrapped DXN is separated from unentrapped drug by gel filtration on Sephadex G-50 eluting with 0.154 M NaCl. With this procedure, between 5 and 10% of the starting DXN can be entrapped. Liposomes are cooled to 0° and used within 1 hr of preparation.

Tumor Cell Lines. Solid Sarcoma 180 and Lewis lung carcinoma tumors were supplied by Dr. T. Khwaja of the Animal Tumor Resource Facility, University of Southern California Comprehensive Cancer Center. Sarcoma 180 tumors were implanted s.c. by trocar needle as 2-mm fragments into the right flanks of Swiss-type mice. Treatment with either free DXN, DXN-liposomes, free DXN mixed with empty liposomes, or 0.154 M NaCl was started at 7 days after implantation. Mice received 3 i.v. doses, each in DXN (5 mg/kg), spaced at 7-day intervals. Female C57BL/6 mice received s.c. implants of 2-mm pieces of Lewis lung tumor in their right flanks by trocar needle. Two days after implantation, treatment was begun with doses of either free DXN, DXN-liposomes, free DXN plus empty liposomes, or 0.154 M NaCl. Mice received 3 injections each of DXN (5 mg/kg) at 7-day intervals. During the course of these experiments, tumor volumes were estimated using the formula: volume (cu mm) = ½ × length (mm) × width (sq mm), as determined using caliper measurements.

Mice. All mice were supplied by Simonsen Laboratories, Gilroy, Calif. Female SW mice weighing 22 to 26 g were used in drug level studies, immune suppression tests, and Sarcoma 180 tumor studies. Adult female C57BL/6 mice weighing 18 to 22 g were used in Lewis lung tumor studies.

DXN Tissue Distribution. Swiss-type mice were given DXN i.v. at a dose of 5 mg/kg in either the free or entrapped form and sacrificed by

[1] Supported by a grant from the Weingart Foundation and by National Cancer Institute Fellowship Grant CA 06481. We are grateful to the Weingart Foundation for its generous support.
[2] Recipient of Public Health Services Grant CA 06481.
[3] To whom all correspondence should be addressed, at Cancer Research Laboratory, 1303 N. Mission Road, Los Angeles, California 90033.
[4] The abbreviations used are: DXN, doxorubicin; PC, phosphatidylcholine; HRBC, human red blood cells.
Received January 4, 1982; accepted November 5, 1982.

cervical dislocation at various times from 0.5 to 24 hr. Mice bearing Sarcoma 180 tumors were sacrificed at 1, 4, and 24 hr. C57BL/6 mice bearing Lewis lung carcinomas were treated i.p. with DXN in either the free or entrapped form at the same dose and then sacrificed at 4 and 24 hr after injection. Blood samples were drawn by cardiac puncture. Upon excision, cardiac and tumor tissue samples were minced and washed with ice-cold 0.154 M NaCl solution.

Fluorescence Assays. Plasma and tissue concentrations were determined as DXN equivalents using a modification of the procedure of Benjamin *et al.* (4). This technique did not distinguish between the parent drug and its fluorescent metabolites. Samples were stored frozen until ready for assay. To 0.1-ml volumes of plasma was added 0.9 ml of 75% ethanol-0.45 N HCl. Cardiac and tumor tissue samples, about 100 and 200 mg, respectively, were homogenized in siliconized glass centrifuge tubes with 2.0 ml of the ethanol-hydrochloride. Cells were disrupted using a needle probe sonicator set at 150 watts, 10 sec for plasma and 30 sec for cardiac and tumor tissue samples. Sonicated samples were then centrifuged at 40,000 × *g* for 20 min, and the clear supernatants were removed and stored at −20° until assayed. These extracts were compared against a series of DXN standards in ethanol-hydrochloride along with control tissue extracts from untreated mice. Fluorescence was measured in an Aminco-Bowman spectrophotofluorometer using an excitation and emission wavelengths of 474 and 585 nm, respectively. The relative tissue-drug exposure is defined as the area under the concentration *versus* time curve and is determined as:

$$\text{Area} = \int_{t=0}^{t=24} C\,dt$$

where *C* is the concentration in DXN fluorescent equivalents. Volumes of distribution and half-lives were determined using techniques described by Gibaldi and Perrier (13).

Testing for Immune Suppression. HRBC used for immunization were collected in heparinized test tubes, centrifuged, and washed 3 times with phosphate-buffered saline to remove serum proteins. Swiss mice were immunized s.c. with 0.1 ml of a 10% suspension of HRBC in phosphate-buffered saline. This immunization was followed at 24 and 48 hr by treatment with DXN (5 mg/kg) in either the free or liposome-entrapped form or with i.v. 0.154 M NaCl for controls. Mice were reimmunized 7 days later, and drug treatments were repeated. Five mice/treatment group were used in each experiment. Whole-blood samples used for leukocyte counts were obtained by tail vein bleeding at 2 weeks. Leukocyte counts were performed using a hemocytometer or Coulter Counter following established procedures (12). Determination of antibody titers to HRBC was made for plasma samples obtained by retroorbital bleeding 4 weeks after the second immunization. Hemagglutination titers were carried out in duplicate using microtiter plates (Dynatech Laboratories, Alexandria, Va.). Titer levels were expressed as the highest serum dilution capable of the complete agglutination of a 2% suspension of HRBC.

RESULTS

Following an i.v. injection of 5 mg/kg in Swiss mice, a biphasic rate of clearance was observed for both forms of the drug during the 24-hr study. At 0.5 hr following administration, the plasma level of 8.8 μM for entrapped DXN was more than 4 times greater than that for free drug at 1.8 μM. During the distribution phase (up until 4 hr after administration), plasma levels fell rapidly, with half-lives of 2.3 hr for the entrapped and 1.1 hr for the free drug. At 4 hr, the plasma levels for entrapped drug had decreased to 1.0 μM, while free drug levels had declined to 0.6 μM. During the later elimination phase, from about 4 hr on, the removal of total DXN fluorescent equivalents occurred at about the same rate for both forms of drug delivery; thus, plasma half-lives for entrapped and free DXN were 8.3 and 8.1 hr, respectively. Encapsulation of DXN in anionic liposomes caused a reduction in its volume of distribution. Thus, mice receiving the entrapped drug displayed a distribution volume of only 122 ml as compared to a volume of 208 ml for free DXN. The alteration in volume of distribution was most probably due to an inhibition of tissue binding as caused by liposome entrapment. This is also supported by a comparison of areas under the plasma concentration *versus* time curves, as indicated by an area for entrapped drug at 22.9 μM-hr, which is more than twice that for free DXN at only 9.2 μM-hr.

Levels of DXN and its fluorescent metabolites in the heart were also determined. Despite the higher levels of DXN equivalents produced in the plasma with liposome-mediated delivery, the cardiac tissue accumulated less fluorescent material than was observed for free drug. With administration of free DXN, cardiac tissue showed an increase in fluorescence from 0.5 to 1 hr. In contrast, a continuing decrease in fluoresence was noted over the same time period after administration of entrapped drug. Beyond 1 hr, DXN tissue equivalents decreased at similar rates for both forms of the drug. Comparison of areas under the tissue concentration *versus* time curve indicates that the relative cardiac tissue exposure for free DXN (57.1 ng-hr/mg tissue) was more than twice as great as that for the entrapped drug (27.4 ng-hr/mg tissue).

The antitumor activities of free and encapsulated DXN were compared for their ability to suppress the growth of solid Sarcoma 180, as shown in Chart 1. Three i.v. treatments were administered at weekly intervals to Swiss mice bearing the tumors as s.c. implants. At 23 days postimplantation, liposome-entrapped DXN appeared to be as active as free drug for inhibiting tumor growth. Free drug mixed with empty liposomes was less effective than either entrapped or free drug alone. The levels of DXN-associated fluorescence in Sarcoma 180 tumor tissue were determined at 1, 4, and 24 hr after i.v. administration. One hr after injection, free DXN produced 1.35 ng of DXN equivalents/mg of tissue, which was significantly greater ($p < 0.05$) than the 0.69 ng of DXN equivalents/mg of

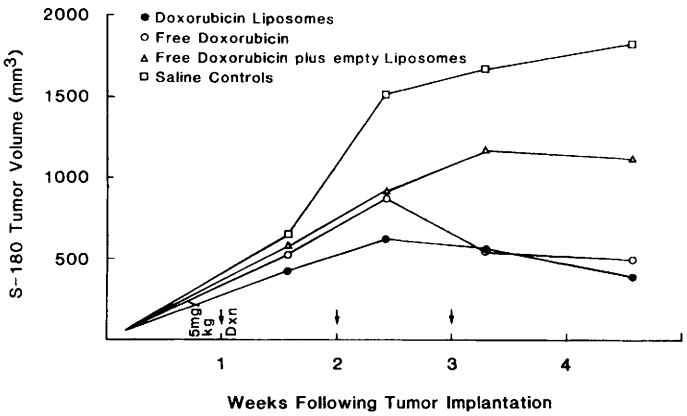

Chart 1. Antitumor activities of free and liposomal DXN were compared using the Sarcoma 180 solid tumor model. Swiss mice received 3 courses of i.v. DXN therapy (5 mg/kg) at 7-day intervals beginning 1 week after s.c. tumor implantation. Five mice/treatment group were used. Both free and entrapped DXN demonstrated significant activities in comparison to the 0.154 M NaCl solution controls ($p < 0.05$).

tissue produced by liposome-entrapped drug. This difference was negligible after 4 hr, because the 2-dosage forms produced nearly equivalent tissue levels. At 24 hr, mice receiving entrapped drug had levels of DXN-associated tumor tissue fluorescence which were not significantly different from those of mice receiving the free drug.

The *in vivo* activities of free and entrapped DXN against Lewis lung tumor are shown in Chart 2. In terms of tumor volume, both forms produced significant inhibition of tumor growth ($p < 0.001$). However, liposomal DXN demonstrated greater antitumor activity than did free drug. In terms of volume, the tumors in the DXN-liposome treatment group were inhibited to 31.9 and 43.0% of controls at 20 and 23 days, respectively. Free DXN, on the other hand, was able to limit tumor growth to only 51.9 and 63.4%. Equivalent treatment with free drug plus empty liposomes demonstrated very little inhibitory activity, because tumor growth in this group was not significantly different from that of controls ($p > 0.2$). Levels of DXN-fluorescent equivalents in Lewis lung tumor tissue were determined following i.p. drug administration. For the times monitored in these experiments, entrapped DXN resulted in less tumor-associated fluorescence than did free drug. Mice which received liposome DXN demonstrated tumor levels of only 0.25 and 0.58 ng/mg (in DXN equivalents) at 4 and 24 hr, respectively. In contrast, those animals which received free drug had tumor levels of 0.43 and 0.78 ng/mg, respectively, for the same time points. The i.p. injection of entrapped drug produced higher plasma levels of DXN-associated fluorescence than did free drug. This indicates that liposome entrapment does not cause a decrease in the transfer of DXN from the peritoneal cavity into the systemic circulation.

Mice immunized with HRBC and treated with only 0.154 M NaCl were able to produce a marked titer of hemagglutinating antibody (Chart 3). Administration of free DXN, 5 mg/kg/dose

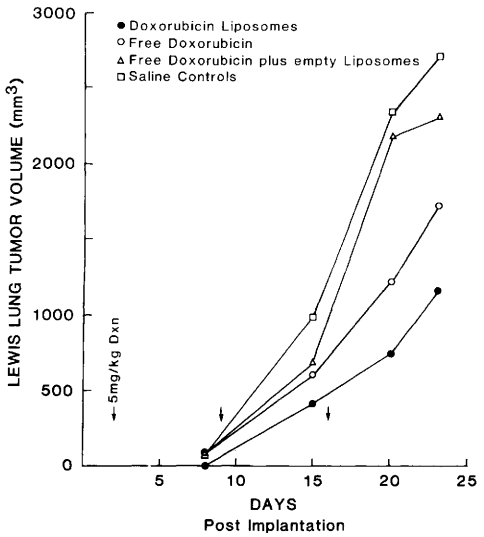

Chart 2. Antitumor activity of free and liposome-entrapped DXN against Lewis lung carcinoma. C57BL/6 mice received 3 doses of i.p. DXN (5 mg/kg) at 7- or 8-day intervals beginning 2 days postimplantation. Five animals/treatment group were used. In terms of tumor volume, both the free and the entrapped form of DXN produced significant inhibition of tumor growth ($p < 0.01$). Measurement of final tumor volume for the liposome treatment group demonstrated a 57% size reduction as compared to control, while the reduction for free drug treatment was only 37%. The significance of this observation was $0.1 > p > 0.05$.

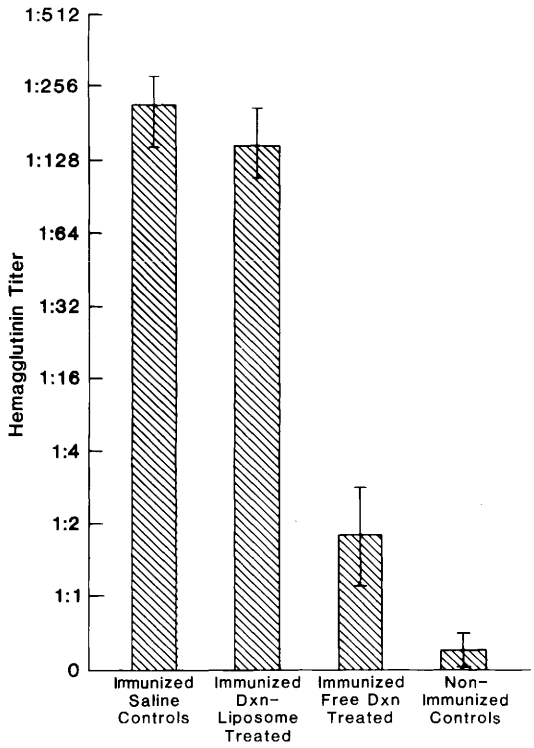

Chart 3. Antibody titers for hemagglutination. Swiss-type mice were tested for their ability to form agglutinating antibodies to xenogeneic HRBC following DXN therapy. Mice received 0.1 ml of a 10% RBC suspension in phosphate-buffered saline as s.c. injection. Mice were then treated at 24 and 48 hr following this immunization with free or liposome-entrapped DXN (5 mg/kg) or 0.154 M NaCl. One week later, the immunization and treatments were repeated. Titers are the greatest serum dilutions capable of the complete agglutination of a 2% suspension of HRBCs. *Bars*, S.D.

at 24 and 48 hr after each immunization, significantly reduced the levels of circulating antibody ($p < 0.002$). In contrast, an identical dosage regimen of liposome-entrapped drug caused a less than significant loss of humoral response ($p > 0.1$). Whole-blood samples drawn 2 weeks after the second immunization demonstrated the bone marrow-suppressive activity of DXN. Non-drug-treated controls had an average white blood cell count of $6.0 \pm 1.7 \times 10^3$/cu mm. Mice treated with free DXN demonstrated a significant leukocyte depression with an average count of only $3.6 \pm 0.8 \times 10^3$/cu mm. Administration of DXN in the entrapped form, however, prevented this suppression and yielded an average leukocyte count of $7.3 \pm 0.8 \times 10^3$/cu mm. The difference between the free and liposome-entrapped DXN groups was significant ($p < 0.01$).

DISCUSSION

We reported previously (11) on the use of liposome entrapment as a means for overcoming the cardiac toxicity of DXN while still maintaining its antileukemic action. Further evaluation to establish that liposomes could function as clinically useful carriers of DXN required the consideration of additional therapeutic parameters. In this report, we describe the uptake of free and entrapped DXN by cardiac and tumor tissues, antineoplastic activity against 2 solid tumors, and its effect on the immune system.

This study indicates that liposome entrapment of DXN does

not block the association of drug with cardiac tissue, although most of the heart toxicity is prevented at even the high dose of 40 mg/kg (11). These experiments thus establish that the complete exclusion of DXN from the cardiac tissue is not essential for the elimination of chronic toxicity. This may be due to uptake by cells other than cardiac myocytes, such as the capillary endothelial cells in the coronary vasculature. Alternatively, the reduced toxicity could be caused by a different subcellular distribution of entrapped drug to sites not associated with toxicity. Future investigations which examine the *in vivo* disposition of entrapped DXN with respect to metabolism and subcellular distribution may help to clarify the mechanism of DXN cardiac toxicity.

Previous studies had shown that entrapment of DXN in anionic liposomes had increased its activity against ascitic leukemias (11). However, i.p. administration of liposomal drug for treatment of an ascitic tumor represented a special situation in which liposomes were applied directly to the cancer cells. A more clinically relevant measure of antineoplastic activity for DXN liposomes requires testing against solid tumors. Both Sarcoma 180 and Lewis lung solid tumors have been well characterized as models for evaluating the antitumor activity of DXN and its analogues (7) and were therefore used in this investigation. Solid Sarcoma 180 tumor normally responds well to i.v. DXN treatment. When free DXN was compared to entrapped drug, both forms displayed significant and nearly equal antineoplastic activity. When tested against Lewis lung tumor, DXN liposomes proved to have an activity exceeding that of the free drug. This is important, since new anthracycline preparations can be considered superior to DXN when they exhibit increased activity against unresponsive tumors such as Lewis lung carcinoma (3).

Compared to free drug, anionic liposomes do not deliver increased amounts of DXN to the solid tumor tissue. With the Lewis lung carcinoma model, i.p. administration of free DXN actually produced greater levels of tumor-associated fluorescence than did the entrapped drug. This was surprising in light of the higher antitumor action which had been observed for liposomes. Thus, when solid tumors are treated with either free or entrapped DXN, the absolute amount of drug delivered does not appear to directly correlate with the level of antineoplastic activity. A possible explanation for this finding is that liposomal DXN may be less immune suppressive and thus offer the host a better capacity to reject the tumor burden.

DXN exerts its greatest effect for suppression of antibody formation when it is administered shortly after immunization (15, 16), indicating that it inhibits proliferating lymphoid cells (25). Encapsulation of DXN in anionic liposomes prevented both suppression of antibody production and decreased leukocyte counts. The mechanism for this protective effect is not clear; however, it may involve an *in vivo* redistribution of injected drug. An earlier study on the organ distribution of free and entrapped DXN (11) indicated that animals treated with liposome DXN demonstrated significantly lower splenic drug levels at 4 hr post injection. Only total drug was estimated in that study, and thus, any metabolic differences caused by liposome entrapment were not detected. Studies by other investigators have shown that liposomes have an affinity for lymphoid tissues, especially when administered by the i.v. or peritoneal routes (14, 24, 27). Liposome encapsulation could spare the immune system by methods other than altering tissue pharmacokinetics. Such modifications may include changes in subcellular distribution and metabolism of liposome-entrapped drug, contributing to the observed differences in toxicity. Additional studies which focus on the uptake and metabolism of liposome-entrapped drug by specific cell types within bone marrow and lymphoid tissues will be needed in order to elucidate a mechanism for reduced toxicity.

Recent reports from 2 other laboratories using liposome-entrapped DXN have confirmed our observations. Both acute and chronic cardiotoxicity of DXN were reduced by cationic liposomes (22, 23). The inclusion of the anionic phospholipid cardiolipin further reduced the cardiac association of entrapped DXN (23). The investigators, however, did not observe an increased antitumor activity. *In vivo* studies have shown that entrapment of DXN in liposomes does not modify the antitumor activity of the drug in mice bearing B-16 melanoma, Lewis lung carcinoma, or L1210 leukemia. The anionic and neutral liposomes used in these studies produced less cardiac toxicity as measured with chick embryonic heart cells *in vitro* (18). Other approaches which have been proposed for reducing anthracyclin-induced cardiac toxicity have included the binding of the drug to DNA and pretreatment with α-tocopherol (2, 20, 26). Both of these methods, however, have been shown to cause increased toxicity towards hematopoietic precursor cells in experimental animals (1, 15). If applicable to the clinical situation, this toxicity could severely limit the therapeutic use of these methods. Liposome-entrapped DXN, on the other hand, appears to lack immunosuppressive potential while still maintaining both its full antineoplastic action and reduced cardiotoxicity. Future studies with other animal tumor systems and preclinical toxicology will be needed to fully evaluate the therapeutic potential of this drug delivery system for cancer patients.

ACKNOWLEDGMENTS

We thank Dr. John Todd for his valuable discussions and Margaret Soh for her assistance in the preparation of this manuscript.

REFERENCES

1. Alberts, D. S., Peng, Y-M, and Moon, T. E. α-Tocopherol pretreatment increases Adriamycin bone marrow toxicity. Biomedicine (Paris), *29:* 189–191, 1978.
2. Atassi, G., Duarte-Karim, M., and Tagnon, H. J. Comparison of Adriamycin with DNA-Adriamycin complex in chemotherapy of experimental tumors and metastases. Eur. J. Cancer, *11:* 309–316, 1975.
3. Benjamin, R. S. Clinical strategy for evaluation of anthracycline analogs. Recent Results Cancer Res., *70:* 67–73, 1980.
4. Benjamin, R. S., Riggs, C. E., and Bachur, N. R. Pharmacokinetic and metabolism of Adriamycin in man. Clin. Pharmacol. Ther., *14:* 592–600, 1973.
5. Bhuyan, B. K., Blowers, C. L., and Shugars, K. D. Lethality of nogalamycin, nogalamycin analogs, and Adriamycin to cells in different cell cycle phases. Cancer Res., *40:* 3437–3442, 1980.
6. Blum, R. H., and Carter, S. K. Adriamycin: a new anticancer agent with significant clinical activity. Ann. Intern. Med., *80:* 249–259, 1974.
7. Casazza, A. M. Experimental evaluation of anthracycline analogs. Cancer Treat. Rep., *63:* 835–844, 1979.
8. Chandra, P. Role of chemical structure in biochemical activity of daunorubicin, Adriamycin, and some structural analogs: macromolecular interactions and their biologic consequences. Cancer Chemother. Rep. Part III, *6:* 115–122, 1975.
9. DiMarco, A. Daunomycin and Adriamycin. *In:* A. C. Sartorelli and D. C. Johns (eds.), Antineoplastic and Immunosuppressive Agents (Part II), pp. 593–614. New York: Springer-Verlag, 1975.
10. Forssen, E. A., and Tökés, Z. A. *In vitro* and *in vivo* studies with Adriamycin liposomes. Biochem. Biophys. Res. Commun., *91:* 1295–1301, 1979.
11. Forssen, E. A., and Tökés, Z. A. Use of anionic liposomes for the reduction

of chronic doxorubicin-induced cardiotoxicity. Proc. Natl. Acad. Sci. U. S. A., *78:* 1873–1877, 1981.
12. Garvey, J. S., Cremer, N. E., and Sussdorf, D. H. Methods in Immunology, pp. 444–494. Reading, Mass.: W. A. Benjamin, Inc., 1977.
13. Gibaldi, M., and Perrier, D. Pharmacokinetics. New York: Marcel Dekker, 1975.
14. Gregoriadis, G. The carrier potential of liposomes in biology and medicine. N. Engl. J. Med. *295:* 704–770, 1976.
15. Huybrechts, M., Symann, M., and Trouet, A. Effects of daunorubicin and doxorubicin, free and associated with DNA, on hemopoietic stem cells. Cancer Res., *39:* 3738–3743, 1979.
16. Isetta, A. M., Intini, C., and Soldati, M. On the immunodepressive action of Adriamycin. Experienta (Basel), *27:* 202–204, 1971.
17. Lefrak, E. A., Pitha, J., Rosenheim, S., and Gottlieb, J. A. A clinicopathological analysis of Adriamycin cardiotoxicity. Cancer (Phila.), *32:* 302–314, 1973.
18. Maslow, D. E., Mayhew, E., Olson, F., and Rustum, Y. Reduction of inhibitory effect of Adriamycin on myocardial contraction *in vitro* by entrapment in liposomes. Proc. Am. Assoc. Cancer Res., *21:* 281, 1980.
19. Minow, R. A., Benjamin, R. S., and Gottlieb, J. A. Adriamycin cardiomyopathy—an overview with determination of risk factors. Cancer Chemother. Rep. Part III, *6:* 195–201, 1975.
20. Myers, C. E., McGuire, W., and Young, R. Adriamycin: amelioration of toxicity by α-tocopherol. Cancer Treat. Rep., *60:* 961–962, 1976.
21. Olson, F., Mayhew, E., Maslow, D., Rustum, Y., and Szoka, F. Characterization, toxicity and therapeutic efficacy of Adriamycin encapsulated in liposomes. Eur. J. Cancer Clin. Oncol., *18:* 167–176, 1982.
22. Rahman, A., Kessler, A., More, N., Sikic, B., Rowden, G., Woolley, P., and Schein, P. S. Liposomal protection of Adriamycin-induced cardiotoxicity in mice. Cancer Res., *40:* 1532–1537, 1980.
23. Rahman, A., White, G., More, N., Pradhan, S., and Schein, P. Protection of chronic cardiotoxicity of Adriamycin by liposome delivery. Proc. Am. Assoc. Cancer Res., *22:* 269, 1981.
24. Ryman, B. E., Jewkes, R. F., Jeyasingh, K., Osborne, M. P., Patel, H. M., Richardson, V. J., Tattersall, M. H. N., and Tyrrell, D. A. Potential applications of liposomes to therapy. Ann. N. Y. Acad. Sci., *308:* 281–307, 1978.
25. Schwartz, H. S., and Grindey, G. B. Adriamycin and daunomycin: a comparison of antitumor activities and tissue uptake in mice following immunosuppression. Cancer Res., *33:* 1837–1844, 1973.
26. Trouet, A. Deprez-de-Champeneere, D., and de Duve, C. Chemotherapy through lysosomes with a DNA-daunorubicin complex. Nature New Biol., *239:* 110–112, 1972.
27. Tyrrell, D. A., Heath, T. D., Colley, C. M., and Ryman, B. E. New aspects of liposomes. Biochim. Biophys. Acta, *457:* 259–302, 1976.
28. Van Vleet, J. F., Ferrans, V. J., and Weirich, W. E. Cardiac disease induced by chronic Adriamycin administration in dogs and an evaluation of vitamin E and selenium as cardioprotectants. Am. J. Pathol., *99:* 13–41, 1980.
29. Vecchi, A., Mantovani, A., Tagliabue, A., and Spreafico, F. A characterization of the immunosuppressive activity of Adriamycin and daunomycin on humoral antibody production and tumor allograft rejection. Cancer Res., *36:* 1222–1227, 1976.
30. Watson, E., and Chan, K. K. Rapid analytic method for Adriamycin and metabolites in human plasma by a thin-film fluorescence scanner. Cancer Treat. Rep., *60:* 1611–1618, 1976.
31. Widder, K. J., Morris, R. M., Poore, G., Howard, D. P., Jr., and Senyei, A. E. Tumor remission in Yoshida sarcoma-bearing rats by selective targeting of magnetic albumin microspheres containing doxorubicin. Proc. Natl. Acad. Sci. U. S. A., *78:* 579–581, 1981.
32. Young, R. C., Ozols, R. F., and Myers, C. E. The anthracycline antineoplastic drugs. N. Engl. J. Med., *305:* 139–153, 1981.