Eur J Cancer Clin Oncol. Vol. 19, No. 3, pp. 419–426, 1983.
Printed in Great Britain.

0277-5379/83/030419-08$03.00/0

Subcellular Distribution of Adriamycin in the Liver and Tumor of 3LL-bearing Mice*

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Abstract—*The apparent subcellular distribution of adriamycin (ADM) was investigated in the liver and primary or metastatic tumor tissue of C57BL/6 mice bearing i.m. 3LL. ADM was measured by a fluorimetric assay in the various cell components, nuclei (N), mitochondria (MT), microsomes (M) and soluble fraction of cytoplasm (SF), either* in vitro *at various times of incubation or* in vivo *after drug injection. In both experimental conditions more than 50% of ADM accumulated in nuclei, whereas only a proportionally low amount of drug has recovered in the other fractions. However, a progressive increase in the percentage of drug stored in M and particularly in MT was noted* in vivo *in both liver and tumor, reaching in MT 3 times the starting amount on a percentage basis* 24 hr *after drug treatment. The elimination half-life of ADM was consistently longer in MT and M than in nuclei and total liver, suggesting that M and particularly MT have a higher capacity than nuclei to retain the drug. Work is in progress to evaluate whether this higher ADM accumulation at these subcellular sites is related to higher specific affinity or more persistent binding, like covalent binding to macromolecules, possibly accounting for the mitochondrial injury usually observed after treatment with ADM.*

INTRODUCTION

ADRIAMYCIN (ADM) is an anthracycline antibiotic with therapeutic activity against a variety of neoplasms [1–3]. It unfortunately also has a number of undesirable toxic side-effects [4–7].

Characterization of its pharmacokinetics and pharmacodynamics has been the object of a series of experimental and clinical studies. It was found that the drug rapidly leaves the blood compartment and accumulates to a large extent in tissues [8–11]. *In vitro*, the ADM concentration in different normal or tumoral cell lines is higher than the extracellular concentration, depending on the dose, time of contact and exposure conditions [12–15].

As regards transport across the cell membrane, inward diffusion and active efflux have been postulated by Peterson and Trouet [16] and Skovsgaard [15].

It has been also reported that the cytotoxic activity of ADM on different cell lines *in vitro* or tumor systems *in vivo* cannot always be correlated to the total intracellular amount or tissue content of drug [13, 17, 18]; whether a different subcellular localization, i.e. different concentrations of ADM at intracellular targets, explains differences in response is not yet known. Therefore studies characterizing ADM subcellular localization may prove helpful in clarifying its cellular pharmacology and consequently its mode of action.

On account of the reported high affinity of ADM for nucleic acids and its capacity for intercalating with DNA [19, 20], the studies described in the literature, either *in vitro* on cultured cell lines [21, 22] or *in vivo* on tissues of ADM-treated animals [23, 24], concentrate on identifying and quantitating drug localization to nuclei, mainly neglecting other subcellular structures. These studies have shown that more than 60% of the intracellular ADM content is actually localized in the nuclei, though a small part was also found in lysosomes [21]. A recent report by Blanchard *et al.* [25] on the subcellular localization of ADM in rat heart ventricles after i.v. perfusion, while confirming the major association to nuclear DNA, considered the possibility that a small part of drug might be

Accepted 28 September 1982.
*This work was supported by CNR (Progetto Finalizzato 'Control of Neoplastic Growth' No. 80.01593.96).

found in mitochondria as well as lysosomes. This may be relevant in relation to the mitochondrial injury observed [26–29] as a result of ADM treatment.

This report describes an *in vivo* and *in vitro* study comparing the apparent subcellular distribution of this anthracycline antibiotic in various cell components corresponding to the nuclear, mitochondrial, microsomal and soluble fractions of cytoplasm of normal or neoplastic tissue cells of mice; the liver was chosen as representative of a normal host tissue and the Lewis lung carcinoma, primary or metastatic, is the tumor tissue.

MATERIALS AND METHODS

In vivo

C57BL/6 male mice obtained from Charles River, Italy were used for these experiments. The experimental tumor employed was the Lewis lung carcinoma (3LL) implanted intramuscularly (2×10^5 cells/0.1 ml/mouse). Intravenous implantation (10^5 cells/0.1 ml/mouse) was utilized when a large amount of metastatic material was required.

Mice received a single i.v. dose (15 mg/kg) of adriamycin and subcellular fractionation was performed 1, 2, 24 and 48 hr after this injection.

In vitro

In vitro studies with 3LL cells were carried out by incubating 7×10^6 cells obtained by mechanical disruption of a 14-day-old i.m. 3LL, weighing about 2 g, in 2 ml of phosphate buffer (pH 7.4 and osmolality 285 mosm/kg) in the presence of ADM (10 μg/10^6 cells) at 37°C for 5 or 30 min. At the end of incubation 8 ml of ice-cold buffer were added and the tubes were centrifuged for 10 min at 200 *g* at 4°C. The resulting pellets were washed with phosphate buffer, resuspended in 5 ml of 0.25 M sucrose (pH 7.4) and processed for fractionation as described hereafter.

Subcellular fractionation

Liver and neoplastic tissue obtained immediately after decapitation of mice, and tumor cells obtained after incubation, were homogenized for 2 min in 10 vol. of 0.25 M sucrose, pH 7.4, using a glass homogenizer with a Teflon pestle (Bellco Model, average clearance 0.010–0.015 cm) driven by an electric motor at approximately 1000 rev/min.

The method of fractionation described by Hogeboom [30] was then applied, with some modifications [31]. Ten milliliters of the homogenate (1 g or 7×10^6 cells) was layered over 10 ml of 0.34 M sucrose, pH 7.4, and centrifuged for 10 min at 700 *g*. The supernatant (S_1) was removed and the resulting pellet was resuspended in 10 ml of 0.00018 M $CaCl_2$ in 0.25 M sucrose, pH 7.4, and layered over 20 ml of 0.00018 M $CaCl_2$ in 0.35 M sucrose, pH 7.4. The mixture was centrifuged for 10 min at 700 *g*.

These operations were repeated three times and the final pellet represented the nuclear fraction (N); the three supernatants were combined and called 'wash' (W).

The supernatant obtained from the first centrifugation (S_1) was centrifuged for 10 min at 5000 *g*. The supernatant (S_2) was transferred to a second tube and the pellet resuspended in 5 ml of 0.25 M sucrose, pH 7.4, and centrifuged for 10 min at 24,000 *g*. This operation was repeated twice. The remaining pellet represented the mitochondrial fraction (MT).

The combined supernatants obtained from the isolation of mitochondria and S_2 were made up to a volume of 35 ml and centrifuged for 70 min at 50,000 *g*. The pellet thus obtained represented microsomes (M) and the final high-speed supernatant was called soluble fraction (SF).

The subcellular preparations were identified and their purity checked, as suggested by other authors for liver [32] or for tumor tissues [32–34], by measuring the protein content [35] together with the content of some biochemical markers associated with the various particles, i.e. lactate dehydrogenase (LDH) for the soluble fraction (kit by Boehringer), succinic dehydrogenase (SDH) for the mitochondrial fraction [36] and aniline hydroxylase for the microsomal fraction [37], in each subcellular fraction. DNA contamination in the cytoplasmic, mitochondrial and microsomal fraction was verified by the assay described by Burton [38] and was found to be within the ranges described for DNA content in the extranuclear fractions [39].

ADM assay

ADM was measured after treating the subcellular fractions with 33% $AgNO_3$ to release DNA-bound drug, and extraction with *n*-butyl alcohol [40]. Recovery was 70 ± 3% and sensitivity was 0.02 μg/ml, corresponding to 0.7 μg/soluble fraction or nuclear washing of 1 g tissue, and to 0.02 μg/mitochondrial, microsomal or nuclear preparation of 1 g tissue.

The drug concentrations are actually ADM equivalents as *n*-butyl alcohol extracted both the primary compound and its metabolic derivatives. A scanning fluorescence technique [41, 42] was utilized to check the relative contributions of unchanged ADM and metabolites to the total fluorescence. In agreement with observations in tissues of ADM-treated mice [9], where unmetabolized ADM present in the total drug fluorescence amounted to approximately 100%,

only traces of the reduced ADM metabolite adriamycinol were found in the cell fractions of the liver or tumor tissue after *in vivo* treatment, as reported by Blanchard *et al.* for the heart ventricle [25].

Therefore in our conditions total fluorescence, mostly associated with the unchanged compound, can be taken as an estimate of the ADM content. The half-life of ADM disappearance from the various fractions was calculated by linear regression analysis according to the least-squares method.

RESULTS

The apparent *in vivo* subcellular distribution profile of a drug obtained by ultracentrifugation techniques may be significantly affected by redistribution artifacts arising in the course of fractionation [43]. To check whether any such artifacts were involved in the case of adriamycin, we added the compound to liver or 3LL metastatic cells in the homogenizer (Fig. 1). The concentrations of ADM were chosen in order to approximate the drug concentrations found after therapeutic doses *in vivo* in the liver and tumor, with 5–10 times higher levels observable in the liver ([9]; see also Tables 2 and 3).

The drug distribution pattern in the subcellular fractions was very similar for liver and tumor; in the range of the three ADM concentrations tested, ADM fluorescence distributed from the soluble to the particulate fractions to a varying extent, being found predominantly in the nuclear fraction (59–77% of the drug recovered). In the liver the percentage was lower at the higher drug dose as if the nuclei had become saturated. Fifteen to seventeen percent of ADM was found in the nuclei wash; the lower the percentage of drug found in the nuclei the higher was the proportion in the wash.

In both tissues the mitochondrial and microsomal fraction together concentrated less than 10% of the total ADM fluorescence recovered, the percentage in microsomes appearing to increase as a function of the dose.

With the same percentages of ADM in the nuclear material of liver and tumor cells, the relative drug amount bound to the mitochondrial and microsomal preparation of tumor cells seemed slightly less, probably because of the frequently altered content of these organelles in neoplastic tissues.

If intact 3LL cells are incubated *in vitro* in the presence of ADM (Table 1), as much as 26% of the initial starting fluorescence concentration of the medium is recovered in the cells after 30 min incubation. Of this amount more than 90% is present in the nuclear fraction and its wash and less than 5% altogether is found in mitochondria and microsomes.

After 30 min incubation no marked shifts in the percentages of the drug in the various fractions are observable compared to the 5-min picture. The *in vivo* disposition in the different subcellular fractions of the residual drug in the liver and tumor tissue of 3LL-bearing mice treated with

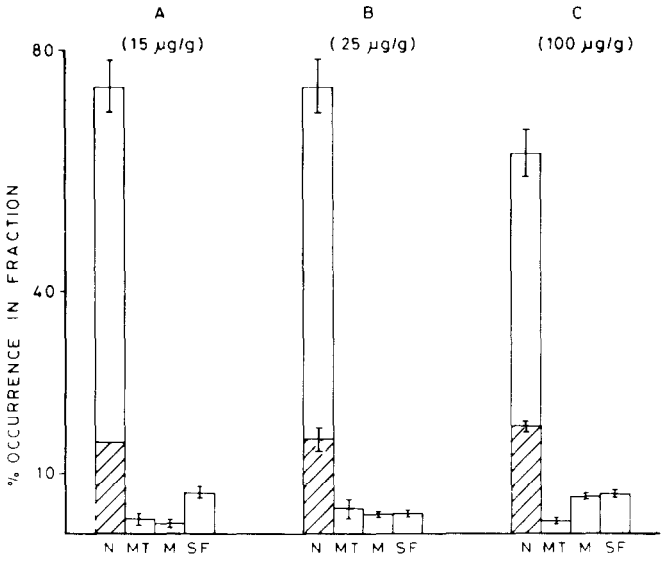

Fig. 1. Distribution pattern in subcellular fractions from the liver and 3LL lung tumor of adriamycin added in the homogenizer. The columns indicate mean values of 2 experiments, each based on 3 samples for the liver and values of pooled metastatic tissue from 6 mice 23 days after i.m. transplant for pulmonary 3LL. Values refer to the percentage ± S.E. of ADM found in each fraction (the sum of the ADM content in the 5 fractions prepared from the same liver was made equal to 100%). Adriamycin recovery from the various fractions was 74% in the tumoral tissue, and 75 and 82% respectively for the 25 and 100 μg/g doses in the liver. N = □ nuclei, ■ = wash, MT = mitochondria, M = microsomes, SF = soluble fraction. A = metastatic 3LL; B and C = liver.

Table 1. *Distribution pattern of ADM in the subcellular fractions of 3LL cells incubated* in vitro *in the presence of the drug*

Incubation (min)	N*	Percentage distribution in: MT	M	SF
—	94.0	2.3	1.7	2.0
5	96.0	1.0	1.1	1.9
30	96.3	0.9	1.2	1.6

3LL cells (3.5×10^6/ml) from an i.m. tumor were incubated at 37°C and pH 7.4 in the presence of ADM (10 μg/10^6 cells). After 30 min incubation ADM fluorescence recovered in intact cells amounted to 26% of the starting fluorescence. The figures are the average of 2 samples. The sum of the ADM content in the four fractions was made equal to 100%. N = nuclei, MT = mitochondria, M = microsomes, SF = soluble fraction.
*Washing of nuclei is included in this value, as separation of nuclei from their wash was not technically possible.

ADM was investigated at different times after administration (Tables 2 and 3, Fig. 2). The concentration in the nuclear fraction amounted to more than 70% of the total ADM fluorescence, and the percentage seemed to decrease in the liver around 24 hr after treatment. In the nuclei of the neoplastic tissue at the same time there was no substantial reduction in the relative ADM content.

The fractions representing mitochondria and microsomes in the tumoral tissue, as already observed in the *in vitro* profile, accumulated the drug much less than the liver and no drug was detectable in the soluble fraction of 3LL metastases when the animal was treated. However, the ADM fluorescence recovered in M and particularly in MT of either hepatic or neoplastic tissue seemed to increase as a percentage with time, reaching 13.7 and 7.5% in the MT of liver and tumor respectively 24 hr after treatment, compared to 5.5 and 2.7% 1 hr after treatment. The same trend, although less marked, was observable in M of both tissues.

The distribution pattern in terms of absolute concentrations of ADM in the various liver fractions (Fig. 2) as compared to the drug content in the liver prior to fractionation indicated that the drug disappearance rate, while similar in whole liver and nuclei, was slower in the mitochondrial and microsomal fractions. Calculation of the elimination half-life for the different subcellular sites indicated a $t_{\frac{1}{2}}$ of 19.8 and 16.2 hr respectively for MT and M compared to 12.5 and 12.3 hr for the whole liver and nuclei, thus suggesting a longer persistence of ADM in MT and M.

DISCUSSION

Previous studies on the subcellular distribution of ADM have shown that the drug does not

Table 2. In vivo *subcellular distribution profile of adriamycin in the liver of C57BL/6 mice after i.v. injection of* 15 mg/kg

Time after treatment	Liver concentration (μg/g)	Percentage distribution in: N	W	MT	M	SF
Added in the homogenizer	25	73.7 ± 4.7	15.7	4.2 ± 1.4	3.0 ± 0.05	3.3 ± 0.01
60 min	90.6 ± 0.01	72.2 ± 0.01	14.7	5.5 ± 0.9	3.3 ± 0.3	4.4 ± 0.4
2 hr	67.9 ± 5.4	71.5 ± 6.2	15.9	6.1 ± 0.3	3.5 ± 1.5	3.0 ± 0.3
24 hr	14.2 ± 1.1	56.3 ± 1.7	26.7	13.7 ± 0.9	4.2 ± 0.04	n.d.
48 hr	6.6 ± 0.2	49.3 ± 8.4	36.9	9.5 ± 1.1	4.3 ± 0.16	n.d.

n.d. = not detectable. Figures are mean values ± S.E. of 2 experiments, each with 2 mice per time. The sum of the ADM content in the 5 fractions of the same liver, which was made equal to 100%, represents 75.4% of the concentration in the liver at 0 min, 64% at 60 min, 71% at 2 hr, 71% at 24 hr and 96% at 48 hr. N = nuclei, W = wash, MT = mitochondria, M = microsomes, SF = soluble fraction.

Table 3. In vivo *subcellular distribution profile of ADM in the 3LL pulmonary tumor after i.v. injection of* 15 mg/kg

Time after treatment	Pulmonary 3LL concentration (μg/g)	Percentage distribution in: N	W	MT	M	SF
Added in the homogenizer	15	74.1	15.2	2.4	1.6	6.7
60 min	11.4 ± 0.7	70.3	25.0	2.7	2.0	n.d.
24 hr	6.5 ± 1.5	67.0	23.2	7.5	2.3	n.d.

n.d. = not detectable. Figures are mean values of 2 samples of pooled metastatic tumor from 6 mice per time. The sum of the ADM content in the 5 fractions of the same tumor tissue, which was made equal to 100%, amounted to 56% of the concentration in 3LL metastases at 0 min, 62% at 60 min and 70% at 24 hr. N = nuclei, W = wash, MT = mitochondria, M = microsomes, SF = soluble fraction.

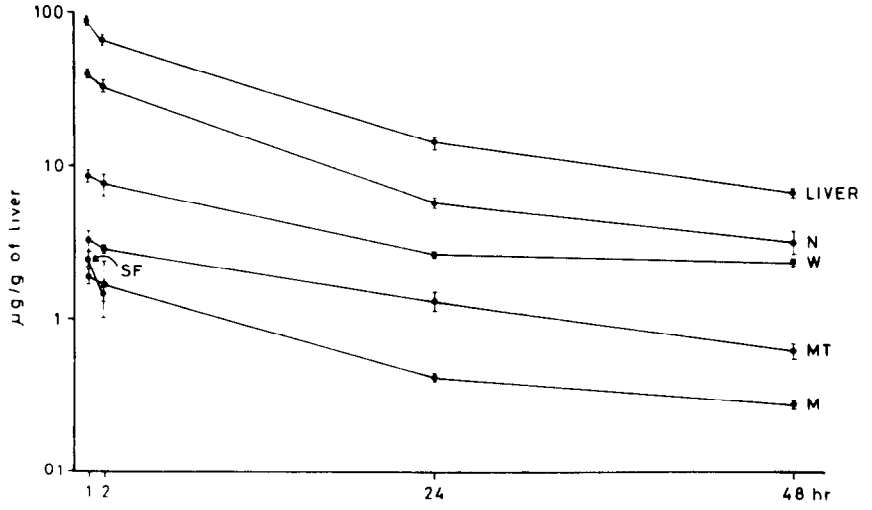

Fig. 2. In vivo *subcellular distribution of adriamycin in the liver of C57BL/6 mice after i.v. injection of* 15 mg/kg. *Figures are mean values ± S.E. of 2 experiments, each with 2 mice per time. N = nuclei, W = nuclei wash, MT = mitochondria, M = microsomes, SF = soluble fraction.*

distribute uniformly throughout the cell, but is stored preferentially in the nuclear fraction [21–23]. Our findings, obtained *in vitro* and also *in vivo* in tumor and liver excised from drug-treated mice at different times after administration, lend consistency to this observation.

The high affinity of ADM for DNA [12] and its specific inhibitory effect on nucleic acid synthesis [14] might explain the very rapid, large-scale localization of ADM in the nuclear fraction (assuming that all the drug found is bound to nucleic acids), and this seems relevant in relation to the described intercalation with DNA [19, 20]. However, our results suggest that neoplastic and normal cells behave somewhat differently in accumulating and retaining the drug at the nuclear site. In spite of quantitatively similar nuclear storage of ADM, its cellular pharmacokinetics differ in liver and tumor cells with some apparent selectivity for neoplastic cells. In fact, 24 hr after treatment the nuclear ADM, as a percentage, shows a tendency to decrease in the liver, but no changes are evident at the same time in the neoplastic tissue, in good agreement with the faster disappearance rate of ADM observed in the liver as a whole compared with tumor tissue (see Tables 2 and 3; Broggini *et al.* [9]). This different capacity to retain the compound in the nuclei of normal and tumoral cells may be related to a different efflux of ADM from the nuclear membrane in the two types of cells and certainly merits further investigation. In this regard the active outward transport of ADM through the nuclear membrane, which may have been postulated similarly to the plasma membrane [15, 16], could be impaired because of energy deficiency in tumor cells [33].

Mitochondria, microsomes and the soluble fraction of cytoplasm are storage sites for only a small part of ADM and this cannot be accounted for by the DNA contamination in the various fractions. Around 15% of the total ADM recovered in the cells is present in the extranuclear fractions, and this proportion is even less when neoplastic cells are taken into consideration, possibly because of their altered microsomal, mitochondrial and cytoplasmic content.

A progressive increase in the percentage of drug bound to microsomes, particularly to mitochondria, is noted *in vivo* in both liver and tumor tissue, reaching a peak (on a percentage basis) 24 hr after drug treatment, when the percentage of nuclear concentration of the ADM residual begins to decrease, at least in the liver. At this time the percentage of drug recovered in mitochondria of the two tissues is about 3 times the starting amount.

That the ADM retention capacity of mitochondria and microsomes is proportionally higher than for nuclei is further indicated by the consistently longer elimination $t_{\frac{1}{2}}$ from MT and M compared to nuclei and total liver. This in turn can be ascribed to different efflux rates through the various cell membranes.

In view of the fact that MT and M constitute only a small part of the cell volume, the amount of ADM in these fractions would appear higher if the concentrations were expressed per unit volume.

The finding of a higher percentage accumulation of ADM in time in these cytoplasmic organelles is compatible with an actual ADM localization at these sites and reduces the possibility that the ADM measured is accounted for by the presence of nuclear DNA or lysosomes, which, as described by other authors [22, 25], should play a role in retaining the compound. A

drug's higher specific affinity or more persistent binding may be postulated in view of recent reports by our group giving evidence for the existence of more stable binding to different cell structures, in particular covalent binding to macromolecules, proteins and nucleic acids [44, 45].

Whether the differential accumulation of ADM in the various cell fractions is in any way related to its reported interaction with phospholipids [46, 47] and to a different membrane composition of the various organelles remain to be established. The high concentration of cardiolipin in the inner membrane of mitochondria and ADM's high affinity for this phospholipid could account, at least in part, for the high concentration and for the longer retention of the drug in this subcellular fraction. Since MT degeneration and functional alterations [7, 26, 27] and inhibition of DNA synthesis in MT [28] have been shown to occur after drug treatment, MT are considered targets of the toxic or therapeutic effects of ADM [48]. Severe drug-induced injury to this cell organelle may therefore be related to ADM's higher affinity for MT binding sites.

What relationship, if any, the intracellular location of this drug has to its accumulation by intact normal or neoplastic cells and to its ability to induce cytotoxicity needs further investigation.

Acknowledgements—The authors are indebted to Dr. Tiziana Mennini and Dr. Chiara Cerletti for expert advice and instructive discussion. Thanks are due to Farmitalia-Carlo Erba (Milan) for providing us with adriamycin and adriamycinol.

REFERENCES

1. BENJAMIN RS, WIERNIK PH, BACHUR NR. Adriamycin chemotherapy: efficacy, safety and pharmacologic basis of an intermittent single high-dosage schedule. *Cancer* 1974, **33**, 19–27.
2. BLUM RH, CARTER SK. Adriamycin. A new anticancer drug with significant clinical activity. *Ann Intern Med* 1974, **80**, 249–259.
3. WANG JJ, CORTES E, SINKS LF, HOLLAND JF. Therapeutic effect and toxicity of adriamycin in patients with neoplastic disease. *Cancer* 1971, **28**, 837–843.
4. CORTES EP, LUTMAN G, WANKA J *et al.* Adriamycin (NSC-123127) cardiotoxocity: a clinicopathologic correlation. *Cancer Chemother Rep (Part 3)* 1975, **6**, 215–225.
5. LEFRAK FA, PITHA J, ROSENHEIM S, GOTTLIEB JA. A clinicopathologic analysis of adriamycin cardiotoxicity. *Cancer* 1973, **32**, 302–314.
6. MINOW RA, BENJAMIN RS, GOTTLIEB JA. Adriamycin (NSC 123127) cardiomyopathy. An overview with determination of risk factors. *Cancer Chemother Rep (Part 3)* 1975, **6**, 195–201.
7. RINEHART JJ, LEWIS RP, BALCERZAK SP. Adriamycin cardiotoxicity in man. *Ann Intern Med* 1974, **81**, 475–478.
8. BACHUR NR, HILDEBRAND RC, JAENKE RS. Adriamycin and daunorubicin disposition in the rabbit. *J Pharmacol Exp Ther* 1974, **191**, 331–340.
9. BROGGINI M, COLOMBO T, MARTINI A, DONELLI MG. Studies on the comparative distribution and biliary excretion of doxorubicin and 4'-epidoxorubicin in mice and rats. *Cancer Treat Rep* 1980, **64**, 897–904.
10. YESAIR DW, SCHWARTZBACH E, SHUCK D, DENINE EP, ASBELL MA. Comparative pharmacokinetics of daunomycin and adriamycin in several animal species. *Cancer Res* 1972, **32**, 1177–1183.
11. WILKINSON PM, MAWER GE. The persistence of adriamycin in man and rat. *Br J Clin Pharmacol* 1974, **1**, 241–247.
12. BACHUR NR, STEELE M, MERIWETHER WD, HILDEBRAND RC. Cellular pharmacodynamics of several anthracycline antibiotics. *J Med Chem* 1976, **19**, 651–654.
13. CHERVINSKY DS, WANG JJ. Uptake of adriamycin and daunomycin in L1210 and human leukemia cells: a comparative study. *J Med* 1976, **7**, 63–79.
14. MERIWETHER WD, BACHUR NR. Inhibition of DNA and RNA metabolism by daunorubicin and adriamycin in L1210 mouse leukemia. *Cancer Res* 1972, **32**, 1137–1142.
15. SKOVSGAARD T. Transport and binding of daunorubicin, adriamycin, and rubidazone in Ehrlich ascites tumour cells. *Biochem Pharmacol* 1977, **26**, 215–222.
16. PETERSON C, TROUET A. Transport and storage of daunorubicin and doxorubicin in cultured fibroblasts. *Cancer Res* 1978, **38**, 4645–4649.
17. DONELLI MG, BARBIERI B, ERBA E *et al.* *'In vitro'* uptake and cytotoxicity of adriamycin in primary and metastatic Lewis lung carcinoma. *Eur J Cancer* 1979, **15**, 1121–1129.

18. DURAND RE. A correlation between adriamycin toxicity and the intracellular drug level. *Proc Am Assoc Cancer Res* 1980, **21**, 276.
19. DI MARCO A. Adriamycin (NSC-123127): mode and mechanism of action. *Cancer Chemother Rep (Part 3)* 1975, **6**, 91–106.
20. SCHWARTZ HS. Mechanisms and selectivity of anthracycline aminoglycosides and other intercalating agents. *Biomedicine* 1976, **24**, 317–323.
21. EGORIN MJ, CLAWSON RE, COHEN JL, ROSS LA, BACHUR NR. Cytofluorescence localization of anthracycline antibiotics. *Cancer Res* 1980, **40**, 4669–4676.
22. NOËL G, PETERSON C, TROUET A, TULKENS P. Uptake and subcellular localization of daunorubicin and adriamycin in cultured fibroblasts. *Eur J Cancer* 1978, **14**, 363–368.
23. KRISHAN A, GABAPATHI R. Laser flow cytometric studies on the intracellular fluorescence of anthracyclines. *Cancer Res* 1980, **40**, 3895–3900.
24. SILVESTRINI R, GAMBARUCCI C, DASDIA T. Attività biologica dell'adriamicina *in vitro*. *Tumori* 1970, **56**, 137–148.
25. BLANCHARD JC, SCHNEIDER Y-J, BAURAIN R, TROUET A. Accumulation, metabolism and subcellular localization of daunorubicin, doxorubicin and their DNA-complexes in rat heart ventricles. *Eur J Cancer* 1981, **17**, 297–305.
26. FERRERO ME, FERRERO E, GAJA G, BERNELLI-ZAZZERA A. Adriamycin: energy metabolism and mitochondrial oxidations in the heart of treated rabbits. *Biochem Pharmacol* 1976, **25**, 125–130.
27. GOSALVEZ M, BLANCO M, HUNTER J, MIKO M, CHANCE B. Effects of anticancer agents on the respiration of isolated mitochondria and tumor cells. *Eur J Cancer* 1974, **10**, 567–574.
28. HIXON SC, ELLIS CN, HAYDEN R *et al.* Adriamycin inhibition of mitochondrial DNA synthesis in rat heart and liver. *Proc Am Assoc Cancer Res* 1980, **21**, 11.
29. REVIS NW, MARUSIC N. Effects of doxorubicin and its aglycone metabolite on calcium sequestration by rabbit heart, liver and kidney mitochondria. *Life Sci* 1979, **25**, 1055–1064.
30. HOGEBOOM GH. Fractionation of cell components of animal tissues. *Methods Enzymol* 1955, **1**, 16–19.
31. SCHNEIDER RM, PETERMAN ML. Nuclei from normal and leukemic mouse spleen I. The isolation of nuclei in neutral medium. *Cancer Res* 1950, **10**, 751–754.
32. DE DUVE C, WATTIAUX R, BAUDHUIN P. Distribution of enzymes between subcellular fractions in animal tissues. *Adv Enzymol* 1962, **24**, 291–358.
33. PAWLICKA E, RZECZYCKI W. Substrate oxidation and energy production by Guerin epithelioma mitochondria. *Arch Geschwulstforsch* 1979, **49**, 124–131.
34. LONDOS-GAGLIARDI D, ZENEBERGH A, AUBEL-SADRON G. Analytical fractionation of homogenates from Ehrlich ascites tumor cells. *Bull Soc Chim Belg* 1979, **88**, 99–105.
35. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
36. SHELTON E, RICE ME. Comparison of the reduction of two tetrazolium salts with succinoxidase activity of tissue homogenates. *J Natl Cancer Inst* 1957, **18**, 117–125.
37. GILBERT D, GOLDBERG L. Liver response tests. 3. Liver enlargement and stimulation of microsomal processing enzymes activity. *Food Cosmet Toxicol* 1965, **3**, 417–432.
38. BURTON K. The conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* 1956, **62**, 315–323.
39. BLOBEL G, POTTER VR. Nuclei from rat liver: isolation method that combines purity with high yield. *Science* 1966, **54**, 1662–1665.
40. SCHWARTZ HS. A fluorometric assay for daunomycin and adriamycin in animal tissues. *Biochem Med* 1973, **7**, 396–404.
41. BENJAMIN RS, RIGGS CE JR, BACHUR NR. Plasma pharmacokinetics of adriamycin and its metabolites in humans with normal hepatic and renal function. *Cancer Res* 1977, **37**, 1416–1420.
42. WATSON E, CHAN KK. Rapid analytic method for adriamycin and metabolites in human plasma by a thin-film fluorescence scanner. *Cancer Treat Rep* 1976, **60**, 1611–1618.
43. MANARA L, MENNINI T, CERLETTI C. ^{3}H-Reserpine persistently bound *'in vivo'* to rat brain subcellular components: limited removal by peanut oil extraction. *Life Sci* 1974, **14**, 2267–2276.
44. GHEZZI P, DONELLI MG, PANTAROTTO C, FACCHINETTI T, GARATTINI S. Evidence for covalent binding of adriamycin to rat liver microsomal proteins. *Biochem Pharmacol* 1981, **30**, 175–177.
45. GARATTINI E, DONELLI MG, PANTAROTTO C. Intact rat liver nuclei catalyze

adriamycin irreversible interactions with DNA and nuclear proteins. *Toxicol Lett* Submitted for publication.

46. DUARTE-KARIM M, RUYSSCHAERT JM, HILDEBRAND J. Affinity of adriamycin to phospholipids as possible explanation for cardiac mitochondrial lesions. *Biochem Biophys Res Commun* 1976, **71**, 658–663.
47. GOLDMAN R, FACCHINETTI T, BACH D, RAZ A, SHINITZKY M. A differential interaction of daunomycin, adriamycin and their derivatives with human erythrocytes and phospholipid bilayers. *Biochim Biophys Acta* 1978, **512**, 254–269.
48. YOUNG DM. Pathologic effects of adriamycin (NSC-123127) in experimental systems. *Cancer Chemother Rep (Part 3)* 1975, **6**, 159–175.