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Daunorubicin-induced Mammary Tumors in the Rat

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Abstract—*Eleven of 24 female Sprague–Dawley rats given a single i.v. injection of daunorubicin (10 mg/kg) developed mammary tumors within 8 months after the injection. Four of 12 rats given an intramammary injection of daunorubicin (4 or 8 μg) developed five mammary tumors in the injected area within 6.5 months of injection. Tissue distribution studies using tritiated daunorubicin revealed that the liver, kidney, lung, heart, and intestine had higher daunorubicin concentrations than mammary tissue during the first 24 h after i.v. injection. However, depletion of the drug from the internal organs was more rapid than from mammary tissue. Differences in ability to metabolize daunorubicin were compared in homogenates of isolated mammary epithelial cells and hepatocytes by high-performance liquid chromatography: after 90 min, hepatocytes metabolized about 70% of daunorubicin, whereas mammary epithelial cells did not metabolize the drug. Tritiated daunorubicin injected directly into rat mammary gland showed no metabolism in 24 h, and the drug did not get into the circulation. These results suggest that retention of daunorubicin because of the inability of mammary tissue to metabolize the drug is a cause of drug-induced mammary tumors in female Sprague–Dawley rats.*

INTRODUCTION

NUMEROUS antineoplastic agents are carcinogens, and second malignancies in cancer patients treated with single or multiple drugs have been documented [1]. These drugs include doxorubicin, daunorubicin, actinomycin D, cyclophosphamide nitrosourea, procarbazine, and mitomycin C. The anthracycline antibiotics daunorubicin and doxorubicin (14-hydroxydaunorubicin) have been used extensively since 1970 for the treatment of human cancers. The major toxicities of the anthracycline antibiotics are cardiotoxicity and carcinogenicity. Sieber *et al.* [2] reported that one of 10 healthy monkeys studied died of acute myeloblastic leukemia after treatment with doxorubicin at a cumulative dose of 324 mg/m^2. The induction time for cancer development was 29 months. Bertazzoli *et al.* [3] reported a high incidence of mammary tumors in a small group of Sprague–Dawley (SD) rats treated with single doses of doxorubicin. It was later confirmed by us and others [4–8] that tumor induction by anthracyclines in rats is common in the mammary gland.

We have investigated the metabolism of daunorubicin *in vitro* and *in vivo* and offer the results of our metabolic studies as an explanation of the apparently selective tumorigenesis in mammary tissue by daunorubicin. To substantiate the carcinogenic capability of the drug, direct intramammary injection of daunorubicin was performed.

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MATERIALS AND METHODS

Experimental animals

Seven- to eight-week-old female noninbred SD rats were used throughout the study. The animals were barrier-reared and were purchased from a local vendor (Timco, Houston, Texas, U.S.A.). Daunorubicin at a dose of 10 mg/kg (60 mg/m^2) was injected through a butterfly infusion set into the tail veins of 32 rats, and the vein injection was assured by return blood flow. Residual daunorubicin was flushed from the infusion set into the vein with an additional 0.5 ml of physiological saline. The 24 rats that did not die of acute renal toxicity were killed 244 days after i.v. injection of the drug. Sixteen rats injected with 1.0 ml physiological saline were also observed for 244 days as controls.

Further, 12 rats were divided into two groups based on site of injection (Fig. 1). Daunorubicin was injected s.c. into these rats in the region of thoracic (2L, 2R) and abdominal (5L, 5R) mammary glands [9] at a concentration of 0, 2, 4, or 8 μg in 100 μl of saline. These animals were observed for 6.5 months to study mammary carcinogenesis. At necropsy, samples of palpable mammary

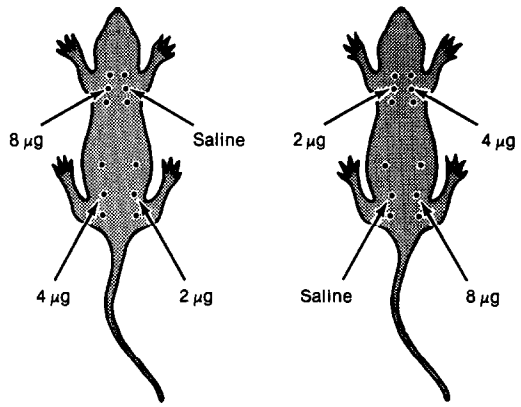

Fig. 1. Localized injection of daunorubicin into mammary tissue in female Sprague–Dawley rats. There are six pairs of mammary glands in each rat, conventionally numbered 1–6 from the cervical region to the inguinal region. Daunorubicin was injected into the second and fifth pairs of both right and left glands (2R, 2L, 5R, and 5L). The sites of injection were divided into these two groups to avoid the possibility that the cervical and thoracic regions were more sensitive to daunorubicin than the inguinal region [26].

tumors and major organs, including the lungs, liver, kidneys, spleen, and lymph nodes from rats given i.v. or s.c. injections of daunorubicin, were removed and placed in neutral phosphate-buffered 10% formalin. The tissues were processed for light microscopy by standard methods. Paraffin-embedded tissues were sectioned at 3–6 μm and stained with hematoxylin and eosin. The mammary tumors were classified according to published criteria [9]. In addition, four rats divided similarly into two groups were injected s.c. with [^{3}H]daunorubicin at the same concentrations. These four rats were killed 24 h after drug injection for studies of drug distribution.

Metabolism and distribution of daunorubicin

[^{3}H]Daunorubicin, ([^{3}H](G)daunorubicin, 1.5 Ci/mmol, New England Nuclear, Boston, MA, U.S.A.), purified by high-performance liquid chromatography, was given i.v. to investigate the metabolism of the drug *in vivo*. Fifteen animals, three at each time point, were killed 0.5, 1, 4, 12 and 24 h after the injection of daunorubicin (10 mg/kg, 10 μCi). Tissues were collected and homogenized prior to quantitation of the radioactivity. The analysis of [^{3}H]daunorubicin distribution was obtained by oxidation of approximately 0.25 g of each tissue sample with a Packard Tri-Carb tissue oxidizer (Packard Instrument, Downers Grove, IL, U.S.A.). Scintillation counting was accomplished with a Beckman LS 7500 scintillation counter (Beckman Instruments, Irvine, CA, U.S.A.) equipped with a quench-corrected counting program.

The radioactive drug was used to determine the location and the concentration–radioactivity relationship after s.c. injection of the drug in the four rats, as described above. For mammary tissues, the radioactivity was counted in tissues harvested in the immediate injected areas (averaging 0.2 g wet tissue) and the surrounding areas (averaging 0.5 g wet tissue). One gram of liver tissue and 1–2 ml of serum were obtained 24 h after drug injection. The tissues were homogenized prior to the quantitation of radioactivity.

The *in vitro* metabolism of daunorubicin was compared with the homogenate of isolated mammary epithelial cells and hepatocytes or perfused rat liver. Rat hepatocytes were obtained from virgin female rats as described elsewhere [10], and 2×10^8 viable cells were routinely isolated from 10 g of liver from each animal. Mammary epithelial cells were isolated from virgin female SD rats using a modification of the procedures of Moon *et al.* [11] and Wiepjes and Prop [12] as described previously [13]. The purity of the mammary epithelial cells was estimated to be 80%. Approximately 1×10^8 cells could be obtained from 5 g of the mammary fat. Usually, 90–94% of these cells were alive by trypan blue exclusion for over a 24-h period in Hank's balanced salt solution. The protein concentration in cell homogenates was determined by using the Bio-Rad (Richmond, CA, U.S.A.) protein assay based on increased visible light absorption at 595 nm of protein-bound dye.

Quantitation of daunorubicin and metabolites

High-performance liquid chromatographic (HPLC) quantitation of daunorubicin and metabolites was a modification of previously published procedures [14, 15]. Gradient elution and detection were achieved with two Constrametric pumps, a gradient master programmer, and Spectromonitor III ultraviolet monitor (Laboratory Data Control, Riviera Beach, FL, U.S.A.) and mini-lab integrator (Columbia Scientific Industries, Austin, TX, U.S.A.). The separations were achieved using a Whatman Partisil 10 μm octadecylsilane (ODS) 250 × 4.5 mm column (Whatman, Clifton, NJ, U.S.A.) equipped with nine pellicular ODS precolumns. The initial eluant was 26% acetonitrile (Burdick and Jackson Labs, Muskegon, MI, U.S.A.), 74% 0.01 M phosphoric acid, pH 3.5, increasing over 22 min at 2.0 ml/min to 38% acetonitrile and 62% 0.01 M phosphoric acid. Peaks were monitored at 254 nm and 0.1 AUFS.

Standards for daunorubicin and its metabolites were generously supplied by Dr. John A. Benvenuto of the Department of Medical Oncology at this institute. The capacity factors (K') of daunorubicin, daunorubicinol, daunorubicin aglycone, 7-deoxydaunorubicinol aglycone, and 7-deoxydaunorubicin aglycone were 8.1, 10, 12, 13, and 16, respectively, in this system.

*Table 1. Mammary tumors in female Sprague–Dawley rats given intravenous daunorubicin**

No. rats injected	No. rats surviving	No. with tumors	No. of tumors	Adenocarcinoma	Fibroadenoma	Mean induction time (days)	Rats with severe glomerulonephrosis
32	24	11 (46%)†	13	11 (85%)‡	2 (15%)	122	16 (67%)§

*A single daunorubicin injection of 10 mg/kg. In addition, 16 rats were injected with 1.0 ml of saline and observed for 244 days. None had any tumor. Eight out of 32 rats died of kidney failure.
†Percentage of surviving animals with tumors.
‡Percentage of number of this classification.
§Percentage of surviving animals with this condition.

RESULTS

Mammary tumor induction by i.v. or intramammary injection of daunorubicin

Eight of 32 rats given i.v. daunorubicin (10 mg/kg) died with drug-induced kidney toxicity the first 8 weeks after receiving the drug. None of these had mammary tumors. The remaining 24 animals were killed at 244 days after injection, and mammary tumors were present in 11 of 24 (Table 1). Among these 24 animals, one had multiple tumors—two adenocarcinomas and one fibroadenoma. Tumor induction time ranged from 56 to 224 days. In two animals with mammary adenocarcinomas, the tumors invaded subjacent muscle; however, no metastatic disease was found in any animal. There were four animals with marked lymphoid hyperplasia in their lymph nodes and spleens. Sixteen (67%) of the surviving animals had severe glomerulonephritis. In the 16 animals receiving intravenous physiological saline only, no tumors developed throughout the experimental period.

Table 2 illustrates the incidence of mammary tumors in the 12 rats that received intramammary daunorubicin. Five tumors developed in four of the 12 rats. The animal with two tumors had one at the site of 4 μg injection and the other where 8 μg was given. The five tumors observed were three adenocarcinomas (one with 4 μg and two with 8 μg) and two fibroadenomas (both with 8 μg). One adenocarcinoma, after the injection of 8 μg daunorubicin, was in the thoracic area (2R), and the four others were in the abdominal regions (one at 5R and three at 5L). The incidence of tumor induction correlated well with the drug dosage. There was a significant difference by 2 × 2 contingency table analysis [16] in the incidence of tumor induction between the drug dosage of 0 or 2 μg compared with 8 μg ($P < 0.005$). There was no statistical difference between 4 and 8 μg or between 0 or 2 μg when compared with 4 μg.

Table 2. Mammary tumors in rats given intramammary daunorubicin

Daunorubicin (μg) Dose (μg)	No tumors/No. animals
0	0/12
2	0/12
4	1/12
8	4/12

Daunorubicin distribution and metabolism after i.v. and intramammary injection

Tissue levels of [^{3}H]daunorubicin were determined in rats after i.v. injection of 10 mg/kg of the drug. Figure 2 presents tissue levels in various organs as daunorubicin equivalents of tritium radioactivity and expressed as micrograms per gram of tissue wet weight. A wide variation in tissue levels of daunorubicin was noted. Mammary tissue exhi-

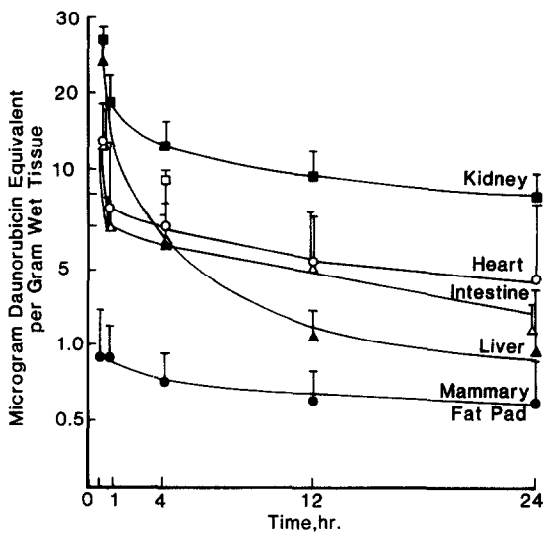

Fig. 2. Clearance of tritium radioactivity in kidney (■), heart (○), intestine (△), liver (▲) and mammary fat pad (●) after the injection of [^{3}H]daunorubicin (10 mg/kg) into a tail vein of female rats. The purity of ^{3}H daunorubicin ranged from 82 to 85% according to HPLC. The peak corresponding to daunorubicin was collected and lyophilized prior to drug injection, and the material thus obtained had a purity greater than 99%. The points represent the means of the determinations from three animals ± 1 S.D.

bited the lowest levels of daunorubicin at all times sampled after i.v. injection of daunorubicin. Liver, along with kidney and lung, exhibited one of the highest tissue radioactivity levels, with 14 μg daunorubicin equivalent/g at 30 min after drug injection. Tissue depletion of daunorubicin was rapid in the liver, while mammary tissue levels varied little after 24 h. Less than 40% of radioactivity of [^{3}H]daunorubicin was eliminated from the mammary tissue area, as compared with more than 90% from liver 24 h after drug administration. The estimated drug elimination rate constant (K_{el}) in liver (0.746 h^{-1}) was 60 times greater than that observed in the mammary fat pad (0.012 h^{-1}). The estimated areas under the curves based upon an open two-compartment model in mammary fat pad was 1.5 times greater than that in liver [17].

The nonlinear drug clearance *in vivo* suggested a rapid metabolism of daunorubicin in the liver. The linear drug clearance in mammary tissue areas indicated insignificant metabolism of daunorubicin. The extent of metabolism of daunorubicin *in vivo* was analyzed using HPLC. The two major metabolites of the liver were daunorubicinol and 7-deoxydaunorubicinol aglycone. A high quantity of daunorubicinol was detected in the liver 24 h after drug administration (Table 3), but no daunorubicin was seen at this time. The presence of metabolites was confirmed by tritium radioactivity. No metabolic products of daunorubicin in mammary tissue could be found 1 h after the injection. Because of the low levels of drug present, results of drug metabolism at 4–24 h could not be obtained.

In an effort to confirm that the drug was not metabolized, [^{3}H]daunorubicin was injected into the mammary tissues of an additional four rats. These four rats were killed 24 h after the injection. The mammary tissue areas where [^{3}H]daunorubicin was injected were scraped off, and the radioactivity was counted. The radioactivity increased in proportion to the increase in the amount of drug injected ($n = 16$ areas, $r = 0.6164$, $P < 0.01$) in the mammary tissue area. The radioactivity was exclusively associated with daunorubicin by HPLC (data not shown). Daunorubicin was localized in a tissue mass less than 1 g surrounding the mammary gland area 24 h after the drug injecion. There was no detectable radioactivity in the liver or serum 24 h after drug injection.

Metabolism of daunorubicin in isolated hepatocytes and mammary cells

Metabolism of daunorubicin was investigated in the homogenates of enzymatically isolated hepatocytes or perfused liver and mammary epithelial cells. Figure 3 shows the drug and metabolite clearance from hepatocyte and mammary epithelial cell homogenates incubated with daunorubicin. Metabolism of daunorubicin in the hepatocyte homogenate was extensive, with only 31% of the original daunorubicin present after 90 min. The major metabolites were daunorubicinol, 7-deoxydaunorubicinol aglycone, and 7-deoxydaunorubicin aglycone. The presence of metabolites was verified utilizing [^{3}H]daunorubicin in some incubations. The observed drug metabolism level was much lower in mammary cell homogenates than in liver at equal protein concentrations. Ninety-nine per cent of the original daunorubicin was still present after 90 min incubation with mammary cell homogenates.

DISCUSSION

The anthracycline antibiotics daunorubicin and doxorubicin are mutagens [18], and the metabolism of anthracycline antibiotics alters the mutagenicity of doxorubicin. The latter is evident in that the

Table 3. Daunorubicin and metabolites in liver and mammary tissue following an intravenous injection of 10 mg/kg

Tissue	Time after injection (h)	Total all compounds* (mg)	Percentage of total daunorubicin and metabolites			
			Daunorubicin	Daunorubicinol	7-Deoxydaunorubicinol aglycone	Daunorubicin aglycone
Liver	0.5	18	72	14	11	2
	1	9.1	78	14	6	N.Q.
	4	4.0	42	36	8	8
	24	0.4	N.Q.	78	18	N.Q.
Mammary fat pad	0.5	0.5	98	0	0	N.Q.
	1	1.0	98	0	0	N.Q.

*Expressed as micrograms of daunorubicin equivalents per gram of wet tissue. This quantity was obtained by the summation of the quantities of all daunorubicin-related compounds detected by HPLC. The identification of the peak was by co-elution of authentic compounds and by tritium radioactivities.

N.Q., peak area not quantifiable. The peak area was below the reliable limit for quantitation, the <15,000 integrated area units at 0.01 AUFS.

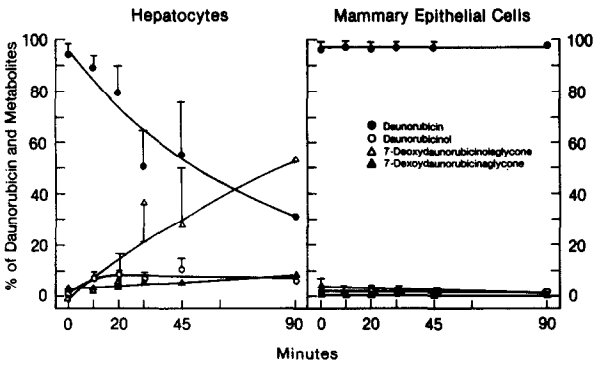

Fig. 3. Daunorubicin and metabolites in mammary epithelial cells (n = 2–4) *and hepatocytes* (n = 2–5) *homogenates. Incubation conditions were: protein,* 4.0 mg/ml; *daunorubicin,* 8.0×10^{-5} M; *NADPH,* 8.3×10^{-4} M; *and Tris,* 0.05 M *in a total of* 3.0 ml *at* 37°C. *At each time point,* 0.5 ml *was taken out for analysis. In two experiments,* 2 μCi [^{3}H]*daunorubicin (HPLC-purified) was added into the incubates of hepatocytes or mammary epithelial cell homogenates. There were five peaks containing tritium radioactivity; four of them are listed in the figures. The fifth was found to be daunorubicin aglycone an autodegradation product of daunorubicin. Less than* 1% *radioactivity accounted for this product.*

presence of rat liver microsomal or rat liver postmitochondrial fractions have reduced the drug's capacity to induce mutagenesis and malignant transformation [19]. Furthermore, inhibitors of microsomal enzyme activity in mouse cells increased the drug-induced microbial transformants. Microsomal NADPH cytochrome P-450 reductase [20], xanthine oxidase [21], and NADH-oxidoreductase [22]—all flavin-containing enzymes—catalyze the reductive glycoside cleavage of the anthracycline antibiotics. The reductive cleavage of the anthracyclines proceeds by a single electron transfer to form an anion-free radical intermediate. The free radicals may react directly with susceptible molecules such as DNA or indirectly generate other toxic free radicals, such as O_2 or OH. These free radicals may play important roles in toxicity. The biochemistry of mammary epithelial metabolism is not yet clear. However, the quantitation of enzyme activities in mammary glands [23] and cells [24] may eventually lead to a satisfactory explanation of the inability of mammary cells to catabolize daunorubicin.

Our results [13] also suggested that daunorubicin in the dose range of 1.5–10 μg per 1×10^6 cells induces single-strand DNA breaks in isolated mammary epithelial cells of female SD rats. The same drug dosages were unable to induce similar strand breaks in 3.5×10^5 to 10^6 single rat hepatocytes. The mechanism of this difference is yet unknown. However, it is not due to the difference in cellular transport or DNA repair [13].

In the current study, a dose of up to 8 μg daunorubicin was injected directly into the vicinities of nipples in the area of 2R, 2L and 5R, 5L [9]. Three considerations were made regarding the selections of the dosages: the first was that daunorubicin could induce skin lesions in rats [25], the second was that 0.9 μg daunorubicin/g of wet tissue was found 30 min after i.v. drug administration (Table 2), and the third was that drug concentration of 1.5 to 10 μg can induce DNA lesions in isolated mammary epithelial cells [13]. The direct injection procedure offers two distinct advantages to help avoid early death of the animals from systemic toxicity (Table 1) and immunosuppression induced by the drug. The results obtained by this approach further strengthen the data derived from the i.v. injection of the drug. From these results and those from the investigation of drug metabolism in isolated hepatocytes and mammary epithelial cells, it is clear that the mammary cells were unable to metabolize daunorubicin *in vitro* and *in vivo* and that this inability to metabolize and eliminate the carcinogen daunorubicin is the basis for the drug-induced mammary tumors in the animal.

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REFERENCES

1. Rieche K. Carcinogenicity of antineoplastic agents in man. *Cancer Treat Rev* 1984, **11**, 39–67.
2. Sieber SM, Correa P, Young DW, Dalgard DW, Adamson RH. Cardiotoxic and possible leukemogenic effects of Adriamycin® in non-human primates. *Pharmacology* 1980, **20**, 9–14.
3. Bertazzoli C, Chieli T, Solcia E. Different incidence of breast carcinomas or fibroadenomas in daunomycin or Adriamycin® treated rats. *Experimentia* 1971, **27**, 1029–1036.
4. Wang YM, Howell SK, Kimball JC, Tsai CC, Sato J, Gleiser CA. Alpha-tocopherol as a potential modifier of daunomycin carcinogenesis in Sprague–Dawley rats. In: Arnott MS, van Eys J, Wang YM, eds. *Molecular Interrelations of Nutrition and Cancer.* Raven Press, New York, 1982, 369–379.
5. Bucciarelli E. Mammary tumor induction in male and female Sprague–Dawley rats by Adriamycin® and daunomycin. *J Natl Cancer Inst* 1981, **66**, 81–84.
6. Marquardt H, Phillips FS, Sternberg SS. Tumorigenicity *in vivo* and induction of malignant transformation and mutagenesis in cell cultures by Adriamycin® and daunomycin. *Cancer*

Res 1976, **36**, 2065–2069.
7. Solcia E, Ballerini L, Ballini O, Sala L, Bertazzoli C. Mammary tumors induced in rats by Adriamcyin® and daunomycin. *Cancer Res* 1978, **38**, 1444–1446.
8. Sternberg SS, Philip FS, Cronin AP. Renal tumors and other lesions in rats following a single intravenous injection of daunomycin. *Cancer Res* 1972, **32**, 1029–1036.
9. Young S, Hallowes RC. Tumours of the mammary gland. In: Tursov VS, (ed). *Pathology of Tumours in Laboratory Animals,* Vol. I, *Tumours of the Rat.* International Agency for Research on Cancer, Lyon, 1973, 31–74.
10. Billings RE, Tephly TR. Studies on methanol toxicity and formate metabolism in isolated hepatocytes. *Biochem Pharmacol* 1979, **28**, 2985–2991.
11. Moon RC, Janes DH, Young S. Preparation of fat cell 'free' rat mammary gland. *J Histochem Cytochem* 1969, **17**, 182–186.
12. Wiepjes GJ, Prop FJA. Improved method for preparation of single-cell suspensions from mammary glands of adult virgin mouse. *Exp Cell Res* 1970, **61**, 451–454.
13. Howell SK, Haidle CW, Wang YM. Daunorubicin-induced DNA lesions in isolated rat hepatocytes and mammary epithelial cells. *Biochim Biophys Acta* 1986, **868**, 254–261.
14. Eksborg S, Ehrsson H, Andersson I. Reversed-phase liquid chromatographic determination of plasma levels of Adriamcyin® and adriamycinol. *J Chromatogr* 1979, **164**, 479–486.
15. Pierce RN, Jatlow PI. Measurement of Adriamycin® and its metabolites in human plasma using reversed-phase high performance liquid chromatography and fluorescence detection. *J Chromatogr* 1979, **164**, 471–478.
16. Ostle B. *Statistics in Research: Basic Concepts and Techniques for Research Workers.* 2nd edn. Iowa State University Press, 1963, 585.
17. Ritschel WA. *Handbook of Basic Pharmacokinetics.* 2nd edn. Hamilton, IL, Drug Intelligence Publications, 1980.
18. Vig BK. Mutagenic effects of some anticancer antibiotics. *Cancer Chemother Pharmacol* 1979, **3**, 143–160.
19. Marquardt H. *DNA: The Critical Cellular Target in Chemical Carcinogenesis and DNA.* Boca Raton, FL, CRC Press, 1979, vol. 2, 159–179.
20. Oki T, Komiyama T, Tone H, Inui T. Reductive cleavage of anthracycline glycosides by microsomal NADPH–cytochrome *c* reductase. *J Antibiotics* 1977, **30**, 613–615.
21. Pan SS, Bachur NR. Xanthine oxidase catalyst reductive cleavage of anthracycline antibiotics and free radical formation. *Mol Pharmacol* 1980, **17**, 95–99.
22. Doroshow JH. Anthracycline antibiotics-stimulated superoxide, hydrogen peroxide, and hydroxyl radical production by NADH dehydrogenase. *Cancer Res* 1983, **43**, 4543–4551.
23. Ritter CL, Malejka-Giganti D. Mixed function oxidase in the mammary gland and liver microsomes of lactating rats. *Biochem Pharmacol* 1982, **31**, 239–247.
24. Medina D, Lane HW, Tracey CM. Selenium and mouse mammary tumorigenesis: an investigation of possible mechanisms. *Cancer Res* 1983, **43** (Suppl), 2460S–2464S.
25. Wang YM. Mechanism and prevention of anticancer agent-induced cancer interactions of vitamin E and daunorubicin. In: Meyskens FL, Prasad KN, eds. *Vitamins and Cancer—Human Cancer Prevention by Vitamins and Micronutrients.* Clifton, NJ, Humana Press, 1985, 213–228.
26. Rudolph R, Suzuki M, Luce JK. Experimental skin necrosis produced by Adriamcyin®. *Cancer Treat Rep* 1979, **63**, 529–537.
27. Russo J, Saby J, Isenberg WM, Russo IA. Pathogenesis of mammary carcinomas induced in rats by 7,12-dimethylbenz(a)anthracene. *J Natl Cancer Inst* 1977, **59**, 435–445.