MOLECULAR PHARMACOLOGY, 13, 901-910

Anthracycline Antibiotic Augmentation of Microsomal Electron Transport and Free Radical Formation

NICHOLAS R. BACHUR, SANDRA L. GORDON, AND MALCOLM V. GEE

Laboratory of Clinical Biochemistry, Baltimore Cancer Research Center, National Cancer Institute, Baltimore, Maryland 21211

(Received March 11, 1977)
(Accepted May 18, 1977)

SUMMARY

BACHUR, NICHOLAS R., GORDON, SANDRA L. & GEE, MALCOLM V. (1977) Anthracycline antibiotic augmentation of microsomal electron transport and free radical formation. *Mol. Pharmacol.*, 13, 901-910.

Adriamycin, daunorubicin, and other anthracycline antibiotics dramatically augment electron flow from NADPH to molecular oxygen in rat liver microsomes and heart sarcosomes. This process is enzymatic, is dependent on NADPH (K_m = 420 μM), has a pH optimum of 8.0–8.5, and is inducible by phenobarbital. The K_m values for the anthracycline antibiotics range from 100 to 400 μM. Although SKF 525-A and carbon monoxide do not inhibit the anthracycline stimulation, α-tocopherol and *p*-hydroxymercuriphenylsulfonic acid inhibit both anthracycline-augmented and endogenous oxygen consumption. Electron spin resonance studies show that the anthraquinone nucleus of the anthracycline is reversibly converted to a free radical semiquinone, which serves to shuttle electrons to oxygen. This enhanced free radical formation may function as the toxic and/or active principle of anthracycline chemotherapy.

INTRODUCTION

The anthracycline antibiotics adriamycin and daunorubicin are effective anticancer chemotherapeutic agents (1, 2). Structurally, these antibiotics have potential for many molecular interactions, and they act at several biochemical levels. They bind to and inhibit the enzymatic processing of DNA (3), they inhibit mitochondrial electron transport (4), they affect membrane organization (5), and they are substrates for intracellular enzymes (6, 7).

The quinone-hydroquinone character of these antibiotics suggests an oxidation-reduction potential in biological systems which has attracted the interest of several investigators. They have postulated the involvement of anthracycline antibiotics in coenzyme Q-mediated mitochondrial functions (8), as oxidative-reductive forms (9), and in free radical formation (10).

In our research on anthracycline antibiotic pharmacology we are examining reciprocal effects of subcellular systems and anthracyclines. The present study focuses on the interactions between microsomes and these antibiotics. Microsomes from mammalian tissues metabolize adriamycin, daunorubicin, and other anthracycline analogues primarily to aglycone products (11). Whereas microsomes catalyze several anthracycline biotransformations, the most prominent microsomal action on adriamycin and daunorubicin is the NADPH-dependent, reductive glycosidic cleavage (12). Although the microsomes biotransform the anthracyclines, the anthracyclines also affect the microsomes. Microsomal pyridine nucleotide utilization, oxygen consumption, and free radical formation are greatly enhanced by the anthracyclines. We describe these phe-

ISSN 0026-895X

nomena and some characteristics of this enhancement.

MATERIALS AND METHODS

Adriamycin HCl was provided by Adria Labs, and daunorubicin HCl was supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute. Both were purified as previously described (13). Carminomycin was provided by Professor G. Gauze, Antibiotic Institute, U. S. S. R. Daunorubicin aglycone and daunorubicinol were prepared as previously described (14). Adriamycin-Fe^{3+} complex was prepared by combining 1 mole of adriamycin HCl and 4 moles of ferric chloride; this substance was used on the same day. Pyridine nucleotides and flavin coenzymes were purchased from P-L Biochemicals. Sodium deoxycholate, coenzyme Q, *p*-hydroxymercuriphenylsulfonic acid, EDTA, α-tocopherol succinate, and Triton N-101 were purchased from Sigma. SKF 525-A (β-diethylaminoethyl diphenylpropyl acetate) was the generous gift of Smith Klein & French Laboratories. Renex 31 was a gift from Dr. B. S. S. Masters, Southwestern Medical School, Dallas, Tx.

Liver microsomes (15) and mitochondria (16) and heart sarcosomes (17) were prepared from male Sprague-Dawley rats (150–200 g). Mitochondria were used on the day of preparation, but microsomes could be stored at −20° and used up to 4 weeks later. Triton N-101-treated liver microsomes were prepared daily. Thawed liver microsome suspensions in 0.15 M KCl–0.1 M potassium phosphate buffer, pH 7.4, were made up to 2% (v/v) Triton N-101 and allowed to stand for at least 30 min in ice prior to use. Protein was measured as described by Lowry *et al.* (18).

Enzyme induction. Rats were injected intraperitoneally with phenobarbital (130 mg/kg) each day for 3 days and killed on day 5 for microsome isolation.

Oxygen measurements. The oxygen content of reaction mixtures was measured with a Clark electrode in a Yellow Springs model 53 Instrument. The reaction mixture (final volume, 4 ml) contained 0.2 M potassium phosphate buffer (pH 8.0), 5 mM NADPH, and 2–5 mg of microsomal protein. Buffer and water (approximately 3.5 ml) were aerated by bubbling with air at 25° for 3 min. Microsomes were added, and the mixture was equilibrated at 37° for 2 min while being monitored for oxygen content. NADPH was added, and endogenous oxygen consumption was assessed. Anthracycline antibiotic (0.5 mM) was then added, and the change in oxygen consumption was determined. Oxygen consumption rates were measured during the initial linear electrode response and were calculated on the basis of a dissolved oxygen content in the reaction mixture of 0.63 μmole. NADPH oxidation was measured at 340 nm in an Aminco DW-2 spectrophotometer.

Anthracycline antibiotic metabolism. Anthracycline antibiotics and products of microsomal metabolism were extracted from the reaction mixtures and quantified by fluorometric assay as previously described (12).

RESULTS

It is well established that liver microsomes consume oxygen using NADPH as a cosubstrate (19). This endogenous oxygen consumption was stimulated several fold when either adriamycin or daunorubicin was introduced into the reaction mixture (Fig. 1). The accelerated oxygen utilization depends on all three components: microsomes, NADPH, and anthracycline antibiotic (Table 1). Similarly, NADPH oxidation by the microsomes was greatly accelerated by anthracycline antibiotics (Table 1). Oxygen use responded linearly to microsomal protein addition (Fig. 2), but boiled microsomes did not catalyze oxygen consumption.

Since microsomes biotransform adriamycin and daunorubicin, we examined the relationship of anthracycline antibiotic metabolism to oxygen consumption. Microsomes were incubated with NADPH and daunorubicin, and oxygen consumption and anthracycline metabolism were measured (Fig. 3). During the oxygen consumption phase (aerobic), daunorubicin remained unchanged and no metabolites were seen. After the oxygen had been consumed (anaerobic), daunorubicin was re-

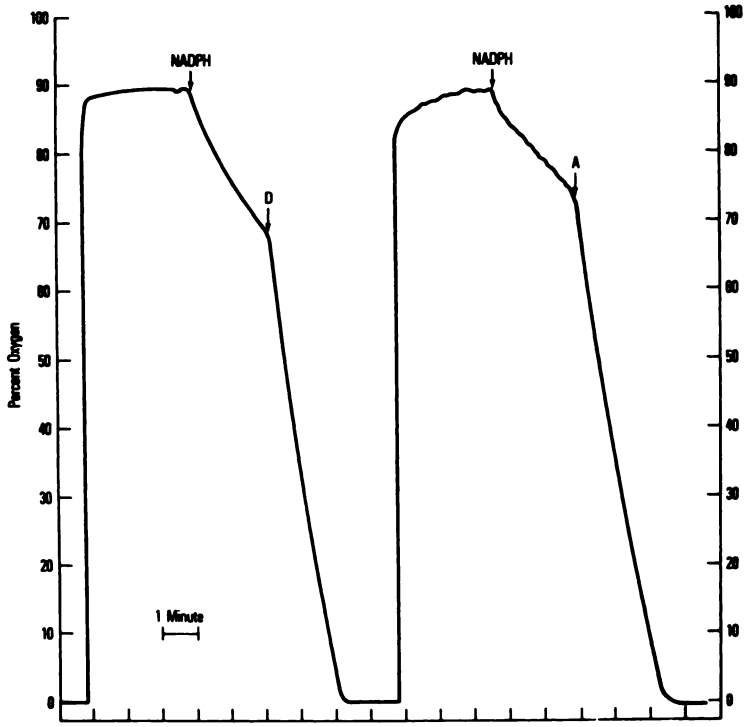


FIG. 1. *Oxygen consumption by rat liver microsomes before and after adriamycin and daunorubicin addition*

Conditions for the reaction are described in MATERIALS AND METHODS.

TABLE 1

Requirements for oxygen and NADPH consumption

For oxygen consumption, the complete 4-ml reaction mixture contained 0.2 M potassium phosphate buffer (pH 8.0), 5 mM NADPH, 0.5 mM daunorubicin, and 3.26 mg of microsomal protein. For NADPH oxidation, the complete 1-ml reaction mixture contained 0.2 M potassium phosphate buffer (pH 8.0), 0.02 mM NADPH, 1 mM adriamycin or daunorubicin, and 1.19 mg of microsomal protein. NADPH oxidation was assayed spectrally at 340 nm.

Reaction mixture	Oxygen consumption	NADPH oxidation	
		Adriamycin	Daunorubicin
	nmoles/mg protein/min		
Complete system	75.5	11.3	12.0
−Anthracycline	17.8	0.01	0.01
−NADPH	0.8		
−Microsomes	1.0		

ductively cleaved to the deoxyaglycone as long as NADPH was available. From these data we conclude that daunorubicin facilitates electron flow from NADPH to oxygen but is not irreversibly altered in the reaction, since no metabolites were found and no loss of daunorubicin occurred during the aerobic phase of the reaction. Daunorubicin metabolism did not occur until the anaerobic phase, as previously reported (12).

The responses of the anthracycline-stimulated oxygen consumption to hydrogen ion concentration and buffer concentration were determined. Both Tris and phosphate buffers gave optimal daunorubicin-stimulated oxygen metabolism from pH 8.0 to 8.5 (Fig. 4), but phosphate yielded higher rates than Tris. Endogenous microsomal oxygen consumption changed little between pH 8.0 and 6.5. The anthracycline-stimulated oxygen consumption was sensitive to buffer concentration, and maximal rates were obtained at 0.2 M phosphate. This buffer concentration was used thereafter.

Endogenous microsomal oxygen con-

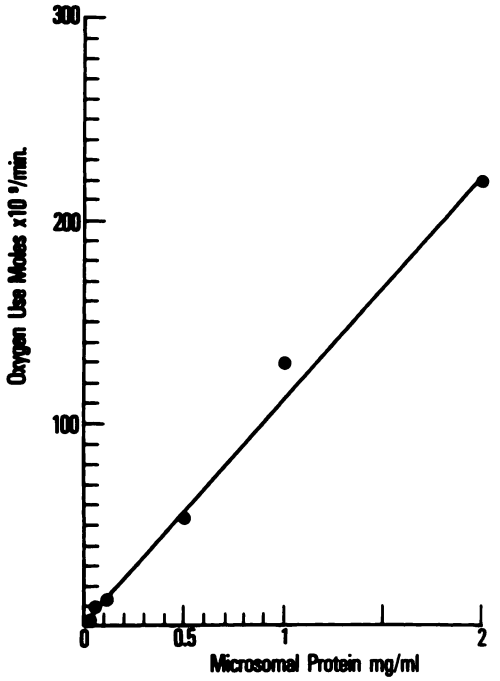


FIG. 2. *Dependence of anthracycline antibiotic-augmented oxygen consumption on microsomal protein*

The reaction mixture is described in MATERIALS AND METHODS. Daunorubicin was the anthracycline used.

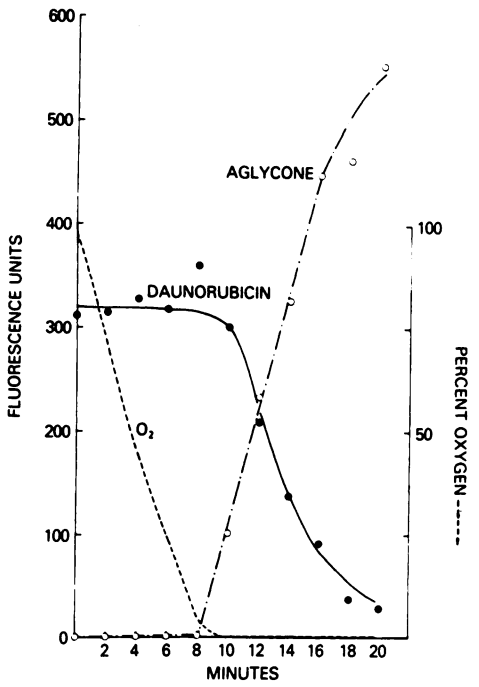


FIG. 3. *Relationship of anthracycline antibiotic-augmented oxygen consumption to anthracycline biotransformation*

The reaction conditions are described in MATERIALS AND METHODS. Following addition of daunorubicin (0.5 mM) to the reaction vessel (zero time), commencing augmented oxygen consumption, 10-μl samples were taken at the indicated times and were mixed with 40 μl of ethanol. A 10-μl portion of the ethanol extract was applied to silica gel thin-layer plates and chromatographed in chloroform-methanol-acetic acid-water (80:20:14:6). The developed chromatograms were examined under 253.7 nm light to detect fluorescent anthracycline antibiotics and metabolites. Fluorescent spots were scraped from the plate, mixed with 2 ml of 0.6 N HCl-95% ethanol, centrifuged, and measured for fluorescence (excitation, 470 nm; emission, 585 nm). The remaining 40 μl of ethanol extract were mixed with 2 ml of 0.6 N HCl-95% ethanol and were analyzed for total drug fluorescence. - - -, oxygen; ●——●, daunorubicin; O·-·-O, Tris-HCl buffer.

sumption varied from day to day and with different preparations of microsomes. Often the endogenous oxygen consumption increased several fold during a single day with a single batch of microsomes. With high endogenous oxygen metabolism in microsomes, small rate changes were difficult to quantify. Therefore we sought techniques to slow or eliminate the endogenous oxygen consumption without affecting the anthracycline stimulation phenomenon. Several detergents were tested for their effects on endogenous and anthracycline-stimulated oxygen metabolism. Whereas deoxycholate and Renex 31 did not give suitable differential rates, Triton N-101 lowered the endogenous oxygen consumption to negligible rates without affecting anthracycline stimulation (Table 2). Where indicated, microsomes were thereafter treated with 2% Triton N-101.

Using the Triton N-101-treated microsomes, we examined the cofactor requirements for the anthracycline augmentation of microsomal electron transport (Table 3). NADPH supported maximal endogenous microsomal oxygen consumption, which was stimulated 14-fold upon the addition of daunorubicin. Other pyridine nucleotides and flavin cofactors were much less effective. The apparent K_m for NADPH in the

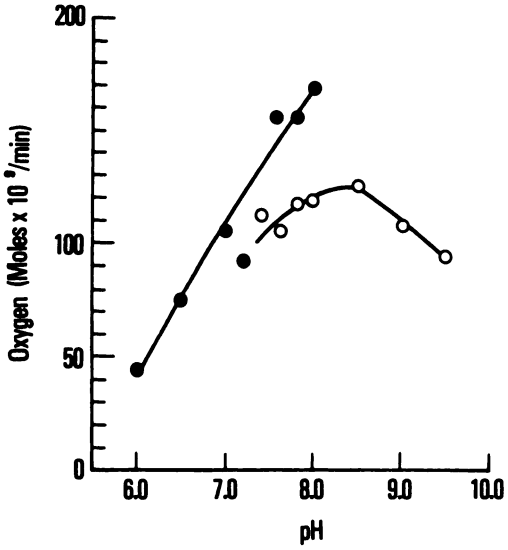


FIG. 4. *pH profile of anthracycline antibiotic-augmented microsomal oxygen consumption*
●——●, potassium phosphate buffer; ○——○, Tris-HCl buffer.

TABLE 2

Detergent effects on microsomal oxygen metabolism

The assay conditions for the reaction and the Triton N-101 treatment are described in MATERIALS AND METHODS. These reactions contained 4.2 mg of microsomal protein per sample and daunorubicin as the anthracycline.

Triton N-101	Oxygen consumption	
	Endogenous	Anthracycline-augmented
%	*nmoles/mg protein/min*	
	15	20
0.2	23	23
0.5	6	17
1.0	1	18
2.0	0	21
5.0	1	17

Triton-microsomal system is 420 μM.

Several anthracycline antibiotic analogues (see Fig. 7 for structures) were compared for their action on Triton N-101-treated microsomes. All the water-soluble anthracycline antibiotics stimulated oxygen consumption in accordance with saturation kinetics (e.g., adriamycin, Fig. 5). These reaction velocity data fit Michaelis-Menten kinetics (20) and were plotted according to Lineweaver and Burk (21) to determine the kinetic constants (Fig. 5). Of the anthracycline antibiotics tested, carminomycin was the most active, although all the analogues possessed similar K_m and V_{max} values (Table 4). The nearly water-insoluble daunorubicin aglycone could not be assayed under these conditions but was solubilized in Triton N-101 (2%) for assay. Although no kinetic constants could be determined for the aglycone, the compound did augment oxygen consumption (daunorubicin aglycone, 12.3 nmoles of O_2 per milligram of protein per minute; daunorubicin, 85.0 nmoles/mg of protein per minute; endogenous, 4.1 nmoles/mg of protein per minute).

TABLE 3

Cofactor requirement for microsomal oxygen consumption

The reaction mixture and technique are described in MATERIALS AND METHODS. Triton N-101-treated microsomes (3.96 mg/sample) were used as enzyme source. The added anthracycline was daunorubicin.

Cofactor (1 mM)	Oxygen consumption	
	Endogenous	Anthracycline-augmented
	nmoles/mg protein/min	
None	0	0
NADPH	1.6	22.1
NADP	0.8	1.3
NADH	0.3	2.4
NAD	0	1.2
FAD	0	0.8
FMN	1.6	1.6

Endogenous oxygen consumption was inhibited by Triton N-101, as noted previously (Table 2), and also by EDTA and α-tocopherol (Table 5). *p*-Hydroxymercuriphenylsulfonate completely inhibited both endogenous and stimulated oxygen consumption. Although SKF 525-A, hexobarbital, and aspirin increased oxygen consumption modestly, these changes are insignificant compared with the nearly 10-fold augmentation produced by daunorubicin. These agents also did not significantly affect the anthracycline stimulation. Endogenous oxygen consumption was not altered by cyanide, but this agent increased the anthracycline stimulation. Coenzyme

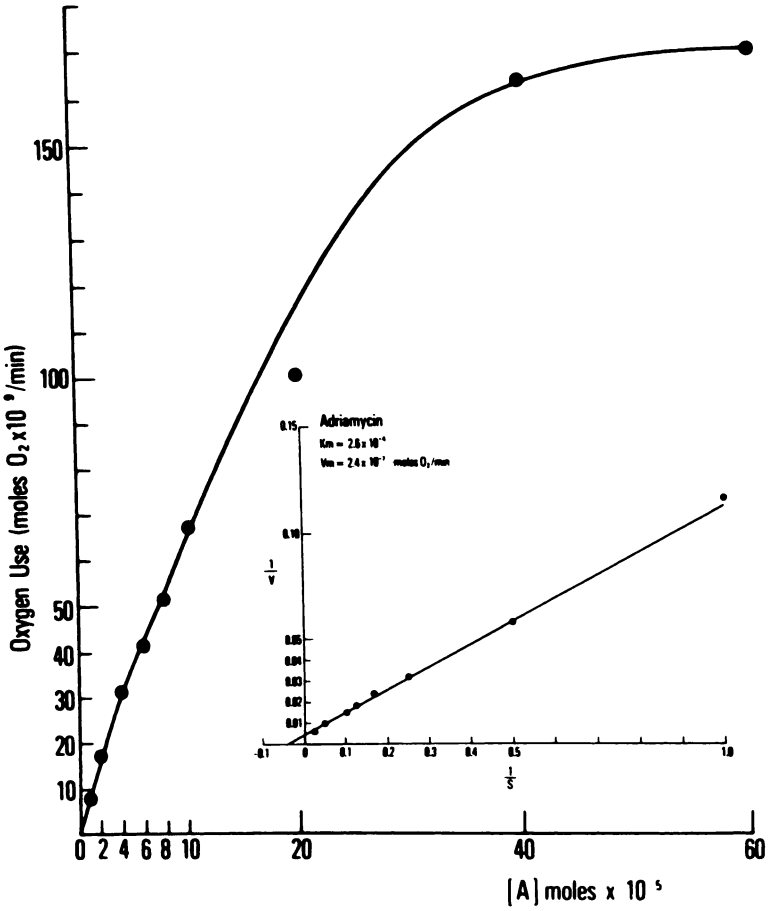


FIG. 5. *Relationship of anthracycline antibiotic (adriamycin) concentration to oxygen uptake and Lineweaver-Burk plot for adriamycin*
[A] = adriamycin concentration.

TABLE 4

Kinetics of anthracycline-augmented microsomal oxygen consumption

The kinetic constants were determined by assessing the rate of oxygen consumption according to Lineweaver and Burk (21). The reactions were carried out as described in MATERIALS AND METHODS, using at least five substrate concentrations over a 10-fold range and 3.5 mg of Triton N-101-treated microsomal protein.

Anthracycline	K_m	V_{max}
	μM	*nmoles/min*
Daunorubicin	390	350
Adriamycin	260	240
Daunorubicinol	250	230
Carminomycin	110	770
Adriamycin-Fe_4^{3+}	250	620

Q moderately increased both endogenous and daunorubicin-stimulated oxygen consumption. α-Tocopherol, a free radical scavenger and antioxidant, inhibited the endogenous oxygen consumption about 60% and reduced the antibiotic stimulation nearly 80%. The sulfhydryl compounds reduced glutathione and cysteine had little effect on either reaction.

Since it is technically difficult to assess carbon monoxide for inhibition of microsomal oxygen use, we analyzed the effect of this cytochrome P-450 inhibitor on NADPH oxidation rates. Enzymatic reaction mixtures as described in Table 1 (2) containing 0.113 mg of Triton-treated liver microsomal protein were assayed for dau-

TABLE 5

Effectors of microsomal oxygen consumption

The assays were conducted as described in MATERIALS AND METHODS, with daunorubicin as the anthracycline effector.

Effector (1 mM)	Oxygen consumption	
	Endogenous	Anthracycline-augmented
	nmoles O_2/min	
None	35	296
SKF 525-A	61	258
Hexobarbital	88	309
Aspirin	54	302
EDTA	18	252
Zn^{2+}	35	277
Fe^{3+}	49	315
CN^-	39	439
p-Hydroxymercuriphenylsulfonate	1	0
Coenzyme Q	53	447
α-Tocopherol[a]	13	68
Cysteine	49	388
GSH	53	389

[a] α-Tocopherol succinate was hydrolyzed to the free acid with 2 molar equivalents of KOH.

TABLE 6

Subcellular localization and phenobarbital induction of anthracycline antibiotic-augmented oxygen consumption

Liver cytoplasmic extracts (105,000 × *g* supernatant) were prepared from 0.2 M potassium phosphate buffer (pH 7.4) homogenates of rat liver (1 g of tissue per 5 ml). Subcellular fractions were prepared as described in MATERIALS AND METHODS. The induced organelles were prepared from phenobarbital-treated animals, and the fractions were assayed, as described in MATERIALS AND METHODS. The anthracycline was daunorubicin.

Subcellular fraction	Oxygen consumption	
	Endogenous	Anthracycline-augmented
	nmoles O_2/mg protein/min	
Liver cytoplasmic extract	0.3 ± 0.4	1.6 ± 0.0
Liver mitochondria	2.1 ± 0.3	4.3 ± 0.9
Liver microsomes	25.2 ± 2.6	93.0 ± 11.2
Induced liver mitochondria	3.4 ± 0.3	5.8 ± 0.7
Induced liver microsomes	30.9 ± 2.8	151.0 ± 3.6
Heart sarcosomes	3.3 ± 1.0	6.3 ± 1.3

norubicin-augmented NADPH oxidation in the presence and absence of carbon monoxide. No NADPH oxidation occurred in the control system. When daunorubicin was added (1 mM), the NADPH oxidation rate was 1.68 nmoles/mg of protein per minute. After an identical sample had been saturated with carbon monoxide, the daunorubicin-stimulated NADPH oxidation remained unaffected at 1.68 nmoles/mg of protein per minute.

Analyses of subcellular components from liver cells showed localization of the anthracycline-augmented oxygen consumption (Table 6). Although the liver cytosol and mitochondria contained measurable activities, microsomes were by far the most active subcellular component. Whereas liver mitochondria consumed little oxygen with NADPH as an extramitochondrial electron source, anthracyclines stimulated mitochondrial oxygen use 2-fold. Liver microsomes utilized NADPH effectively to reduce oxygen and showed a 4-fold stimulation of this process by added anthracycline antibiotic. Heart sarcosomes were not as active as liver microsomes but were stimulated by the anthracycline.

Prior treatment of the rats with phenobarbital yielded microsomes with increased endogenous oxygen consumption (1.2 times the control) and increased anthracycline stimulation (1.6 times the control) (Table 6).

In facilitating the transfer of electrons from NADPH to oxygen, the anthracycline antibiotics may participate as electron carriers, and a reduced form of the drug may exist. The quinone portion of the anthracycline molecule is thought to function in this fashion and may show a physical change. Chemically reduced anthracycline is bleached and shows a corresponding change in the absorption spectrum (data not shown). Since the anthraquinone moiety has spectral properties which are easily assayed, we analyzed the visible absorption spectrum (300–550 nm) during the microsomal reaction. No change was apparent. However, it is possible that an intermediate form exists as a free radical

that shows no detectable spectral change. We therefore investigated the possibility that the anthracycline antibiotic may function in the shuttle of single electrons as a semiquinone intermediate. An ESR scan of reaction mixtures showed the strong signal of an unpaired electron, which was dependent on the presence of anthracycline, NADPH, and the active microsomal system (Fig. 6). No free radical signal was detected in control reaction mixtures lacking any one of those components. This free radical indicates a semiquinone formation of the anthraquinone nucleus.

DISCUSSION

The anthracycline augmentation of microsomal oxygen consumption could result from the antibiotics serving (a) as substrates for the mixed-function oxidase system, (b) as allosteric effectors of microsomal electron transport, or (c) as electron transfer agents themselves. By examining the metabolic state of the anthracycline during the accentuated oxygen uptake phase, we found that the anthracycline remains structurally unchanged and that any alteration to the molecule is rapidly reversible, leaving no evidence of its effect while oxygen is available. Therefore in this system the anthracycline antibiotics do not act as substrates for a classical mixed-function oxidase.

It is unlikely that the anthracyclines facilitate microsomal electron shuttling by functioning as allosteric effectors. The evidence against an allosteric effect lies in the hyperbolic rather than sigmoid kinetics obtained, which indicates that the anthracyclines serve as substrates according to Michaelis-Menten kinetics.

Several of the effectors provide clues to the nature of this reaction. First, SKF 525-A and carbon monoxide do not inhibit, suggesting that the electron transfer does not involve microsomal cytochrome P-450. Second, hexobarbital does not decrease the anthracycline effect, although it is a substrate for microsomal hydroxylation at the cytochrome P-450 level, as indicated by the modest stimulation of oxygen uptake. This could indicate that the anthracyclines act at an earlier step in electron transport than hexobarbital hydroxylation.

From our previous experience with chemically reduced anthracycline antibiotics, we know that reduction of the quinone oxygens to hydroquinones results in visible bleaching of the red color of the antibiotic with a corresponding change in absorption spectrum. We could see no evidence of this in the microsomal reactions. We observed only a very pronounced ESR

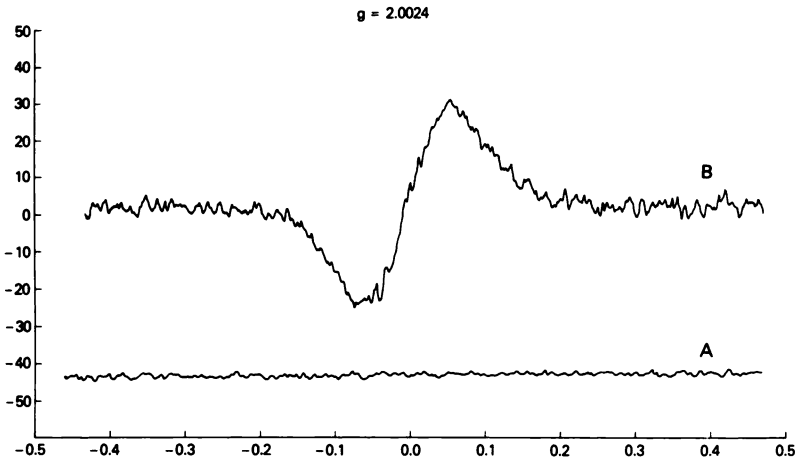


FIG. 6. *Electron spin resonance derivative spectrum of daunorubicin-microsome interaction*
The spectra were obtained with a Varian V-4502 X-band spectrometer at room temperature, 100 kHz field modulation, and 9.453 GHz microwave frequency. The reaction mixtures were the same as described in MATERIALS AND METHODS, except that A contained no daunorubicin and B contained 1 mM daunorubicin.

signal, indicating free radical formation. Since daunorubicin aglycone augments electron transfer, the anthraquinone moiety must be responsible for the single-electron shuttling. The inhibition of the reaction by α-tocopherol, an antioxidant and free radical-trapping agent, supports a free radical mechanism for the reaction.

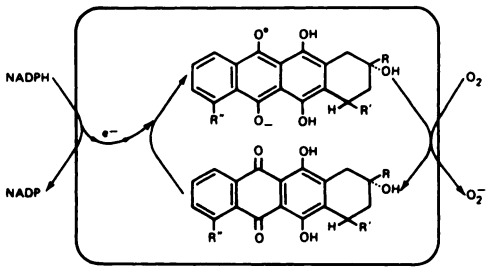
These data support the thesis that the anthracycline antibiotics participate in single-electron transfer at a point between NADPH and cytochrome P-450, shuttling electrons from some microsomal protein to molecular oxygen prior to the cytochrome P-450 stage (Fig. 7). The process is enzymatic and is inducible.

Our previous studies showed that treatment of animals with phenobarbital accentuates the toxicity of adriamycin (22). This corroborates involvement of microsomal machinery as at least partly responsible for the toxicity. Microsomes convert anthracyclines to a free radical state, which may serve to react directly with or to generate other secondary free radicals to react with susceptible molecules, such as DNA. This mechanism may be responsible for the DNA fragmentation and cellular damage associated with anthracycline action. The inhibitory activity of α-tocopherol toward free radical generation by microsomes may be responsible for its reduction of adriamycin toxicity in mice (23). This enhanced free radical generation may also be the mechanism by which the anthracyclines are lethal to resting cells, i.e., cells not in mitotic activity.

Rat heart sarcosomes show augmented oxygen consumption when stimulated by anthracyclines. Although their activity is not as high as that of liver microsomes, the free radical generation still may account for cardiotoxic damage produced by anthracycline therapy (24, 25).

All the anthracycline analogues functioned as promoters of electron flow, and they possessed similar kinetic characteristics. However, carminomycin was most active; this correlates with its higher toxicity compared with other anthracycline antibiotics.[1]

[1] R. R. Johnson, personal communication.



ANTHRACYCINE ANALOGS

NAME	R	R'	R"
ADRIAMYCIN	$COCH_2OH$	DAUNOSAMINE	OCH_3
CARMINOMYCIN	$COCH_3$	"	OH
DAUNORUBICIN	$COCH_3$	"	OCH_3
DAUNORUBICINOL	$CHOHCH_3$	"	OCH_3
DAUNORUBICIN AGLYCONE	$COCH_3$	OH	OCH_3

FIG. 7. *Proposed mechanism of anthracycline interaction in microsomal electron transport, and structures of anthracycline analogues used in this study*

It is important to examine the biochemical consequences of this process to determine the reactivities of the intermediate free radical species (Fig. 7) and their potential for the formation of other reactive species. Through this mechanism we may be able to understand, predict, and possibly control the actions and/or toxicological phenomena of the anthracycline antibiotics.

ACKNOWLEDGMENTS

We appreciate the help of Dr. Hideo Kon, Laboratory of Chemical Physics, National Institute of Arthritis, Metabolic, and Digestive Diseases, in obtaining the ESR spectra, and the efforts of Mrs. Barbara Dressel in the preparation of the manuscript.

REFERENCES

1. Bonadonna, G., Beretta, G., Tancini, G., Brambilla, C., Bajetta, E., De Palo, G. M., DeLena, M., Fossati-Bellani, F., Gasparini, M., Valagussa, P. & Veronesi, U. (1975) *Cancer Chemother. Rep.*, 6, 231-246.
2. Von Hoff, D. D., Rosenzweig, M., Layard, M., Slavik, M., & Muggia, F. (1977) *Amer. J. Med.* 62, 200-208.
3. DiMarco, A., Arcamone, F. & Zunino, F. (1975) in *Antibiotics* (Corcoran, J. W. & Hahn, F. E., eds.), Vol. 3, pp. 102-128, Springer, New York.
4. Cargill, C., Bachman, E. & Zbinden, G. (1974)

J. Natl. Cancer Inst., 53, 481–486.
5. Murphree, S. A., Cunningham, L. S., Hwang, K. M. & Sartorelli, A. C. (1976) *Proc. Am. Assoc. Cancer Res.*, 17, 41.
6. Ahmed, N. K., Felsted, R. L. & Bachur, N. R. (1977) *Fed. Proc.*, 36, 723.
7. Bachur, N. R., Takanashi, S., and Arena, E. (1975) *Proc. 6th Int. Congr. Pharmacol.*, 557.
8. Iwamoto, Y., Hansen, I. L., Porter, T. H. & Folkers, K. (1974) *Biochem. Biophys. Res. Commun.*, 58, 633–638.
9. Driscoll, J. S. (1974) *Cancer Chemother. Rep.*, 4, 3–9.
10. Handa, K. & Sato, S. (1975) *Gann*, 66, 43–47.
11. Bachur, N. R. & Gee, M. (1971) *J. Pharmacol. Exp. Ther.*, 177, 567–572.
12. Bachur, N. R. & Gee, M. (1976) *J. Pharmacol. Exp. Ther.*, 197, 681–686.
13. Bachur, N. R. & Cradock, J. C. (1970) *J. Pharmacol. Exp. Ther.*, 175, 331–337.
14. Bachur, N. R. (1971) *J. Pharmacol. Exp. Ther.*, 177, 573–578.
15. Omura, T. & Takesue, S. (1970) *J. Biochem. (Tokyo)*, 67, 249–257.
16. Lynn, T. C. (1967) *J. Biol. Chem.*, 242, 984–989.
17. Martonosi, A. (1968) *J. Biol. Chem.*, 243, 71–81.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.*, 193, 265–275.
19. Orrenius, S., Ericsson, J. L. E. & Ernster, L. (1965) *J. Cell Biol.*, 25, 627–639.
20. Michaelis, L. & Menten, M. L. (1913) *Biochem. Z.*, 49, 333.
21. Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.*, 58, 658.
22. Reich, S. D. & Bachur, N. R. (1976) *Cancer Res.*, 36, 3803–3806.
23. Myers, C. E., McGuire, W. & Young, R. (1976) *Cancer Treat. Rep.*, 60, 961–962.
24. Lefrak, E. A., Pitha, J., Rosenheim, S. & Gottlieb, J. A. (1973) *Cancer*, 32, 302–314.
25. Jaenke, R. S. (1974) *Lab. Invest.*, 30, 292–304.