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Inhibition of Plasma Membrane NADH Dehydrogenase by Adriamycin and Related Anthracycline Antibiotics

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Abstract

Doxorubicin (adriamycin) is cytotoxic to cells, but the biochemical basis for this effect is unknown, although intercalation with DNA has been proposed. This study suggests that the cytotoxicity of this drug may be due to inhibition of the plasma membrane redox system, which is involved in the control of cellular growth. Concentrations between 10^{-6}–10^{-7} M adriamycin inhibit plasma membrane redox reactions >50%. AD32, a form of adriamycin which does not intercalate with DNA, but is cytotoxic, also inhibits the plasma membrane redox system. Thus, the cytotoxic effects of adriamycin, which limit its use as a drug, may be based on the inhibition of a transplasma membrane dehydrogenase involved in a plasma membrane redox system.

Key Words: Plasma membrane; NADH dehydrogenase; antitumor drugs; adriamycin; anthracycline.

Introduction

Adriamycin and related anthracycline antibiotics are widely used in cancer therapy (Carter, 1975). The inhibition of cell division is postulated to depend on intercalative binding to DNA (Waring, 1981). This hypothesis does not fully explain the mitogenic effects of these drugs, since adriamycin and daunomycin can inhibit mitosis under conditions in which the DNA synthesis is unaffected (Silvestrini *et al.*, 1970, 1973). Analogs of adriamycin, such as *N*-acetyl daunomycin or *N*-trifluoroacetyl adriamycin-14-valerate (AD32) show very little interaction with DNA, and do not concentrate in the cell nucleus, yet they inhibit mitosis (DiMarco *et al.*, 1965; DiMarco, 1975; Kanter and Schwartz, 1979; Waring, 1981). For this reason additional targets

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for the cytotoxic action of adriamycin have been sought (Goldman *et al.*, 1978; Grunicke *et al.*, 1979). Effects of the drug on the surface properties of sarcoma cells have been described (Murphree *et al.*, 1976) and Tritton and Yee (1982) have shown that adriamycin bound to agarose beads, which cannot enter the cell, retains its cytotoxicity to sarcoma cells. Likewise, the cytostatic action of adriamycin coupled to polyglutaraldehyde microspheres has been demonstrated on human leukemia cells and rat hepatocytes (Tökes *et al.*, 1982; Rogers *et al.*, 1983). Adriamycin has also been shown to stimulate oxygen radical formation through microsomal electron transport (Bachur *et al.*, 1977, 1979; Sato *et al.*, 1977; Goodman and Hochstein, 1977). The inhibitory action of this drug on mitochondrial oxidative phosphorylation has been reported (Gosalvez *et al.*, 1974; Muhammed *et al.*, 1982). The drug can also complex with tightly bound cardiolipin to inhibit mitochondrial cytochrome oxidase (Goormaghtigh *et al.*, 1982) and has also been proposed to react with the cytoskeleton (Na and Timasheff, 1977). A transplasma membrane redox system has been found in all cells which have been examined (Goldenberg, 1982; Clark *et al.*, 1981). It contains a hormone-sensitive NADH dehydrogenase which can regulate metabolic functions (Löw *et al.*, 1979; Crane *et al.*, 1982a) and can energize selected amino acid transport (Ohasawa *et al.*, 1980). The transmembrane redox system has also been shown to stimulate the growth of melanoma cells by replacing the essential components of calf serum (Ellem and Kay, 1983). We have previously reported that adriamycin can inhibit the plasma mebmrane NADH dehydrogenase activity (Crane *et al.*, 1980). In this paper we describe the effects of adriamycin and related anthracycline drugs on plasma membrane NADH dehydrogenase activity at concentrations equivalent to those used in therapy and inhibition of cell division (Waring, 1981).

Methods

Mouse liver plasma membrane was prepared by the procedure of Goldenberg *et al.* (1979) and liver endoplasmic reticulum according to Morrë *et al.* (1972). Pig erythrocyte plasma membrane preparation was adapted from Steck and Kant (1974). NADH-ferricyanide reductase activity was measured in an Aminco DW2a spectrophotometer in the dual-beam mode, substracting the absorbance at 500 nm from 420 nm to follow the reduction of ferricyanide. A 2.8-ml reaction mixture contains 0.05 M sodium phosphate, pH 7.0, 0.25 mM potassium ferricyanide, 20 μM NADH, and 12–50 μg membrane protein. The extinction coefficient for ferricyanide reduction ΔA_{420} equals 1 $mM^{-1} \cdot cm^{-1}$. NADH-cytochrome *c* reductase was measured with the dual beam at 550 nm minus 541 nm. ΔA_{550} equals 19 $mM^{-1} \cdot cm^{-1}$. The 2.8-ml reaction mixture contained 0.05 M sodium phosphate buffer, pH 7.0,

1 mM potassium cyanide, 20 μM NADH, 90 μg cytochrome *c*, and 25 μg membrane protein (Ramasarma *et al.*, 1980).

Adriamycin was generously supplied by Dr. A. Ghione Farmitalia, Milan. Carminomycin, marcellomycin, and aclacinomycin were kindly supplied by Dr. S. T. Crooke, Bristol Laboratories, Syracuse. *N*-Trifluoroacetyl adriamycin-14-valerate (AD32) and aclacinomycin were supplied by Dr. M. Suffness, Division of Cancer Treatment, National Cancer Institute.

Results

Plasma membrane redox reactions can be studied by means of NADH-ferricyanide and NADH-cytochrome *c* reductase activities. These reactions can be inhibited by various anthracycline drugs. Those tested here include adriamycin, daunomycin, carminomycin, marcellomycin, aclacinomycin, and quinizarin as a control compound.

Incubation of various types of plasma membrane with 10^{-6} M adriamycin for 3 min leads to inhibition of plasma membrane redox reactions. For example, the NADH-ferricyanide reductase activity of mouse liver plasma membrane decreases after incubation with adriamycin (Fig. 1A), while a

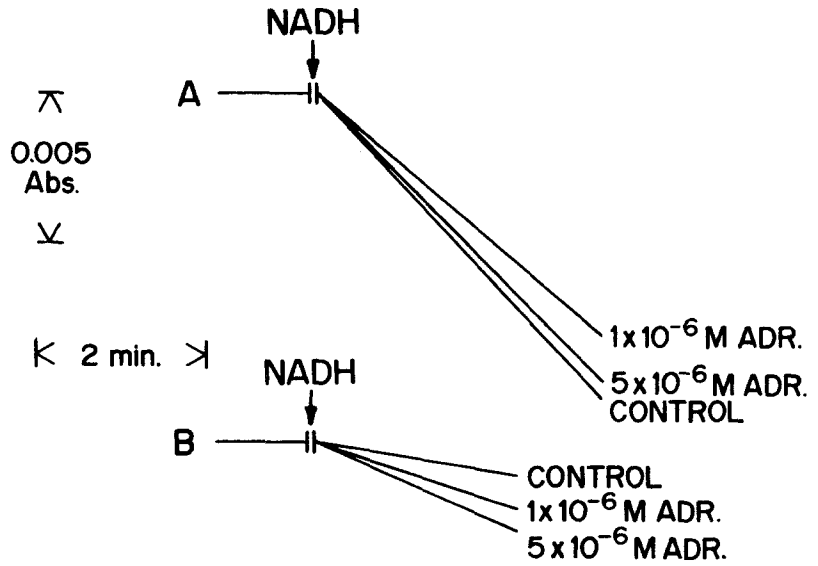

Fig. 1. NADH-ferricyanide reductase activity of mouse liver plasma membranes after a 3-min incubation with adriamycin (1 and 5 μM). Conditions: 12 μg membrane, 0.25 mM $K_3Fe(CN)_6$, 0.05 M potassium phosphate buffer, 20 μM NADH. (A) untreated membrane; (B) membrane heated at 100°C for 3 min.

Table I. The Effects of Addition Sequence on the Extent of Inhibition of NADH-Ferricyanide Reductase Activity by Adriamycin in Mouse Liver Plasma Membrane[a]

Incubation with	Start reaction with	Ferricyanide reduction rate corrected for heat inactivated control (nmol/min/mg protein)
Membrane + $Fe(CN)_6^{-3}$	NADH	1001 ± 37
Membrane + NADH	$Fe(CN)_6^{-3}$	931 ± 11
Membrane + ADR 10^{-6} M + $Fe(CN)_6^{-3}$	NADH	407 ± 11
Membrane + ADR 10^{-6} M + NADH	$Fe(CN)_6^{-3}$	349 ± 25
Membrane only	NADH and $Fe(CN)_6^{-3}$	991 ± 55
Membrane + ADR 10^{-6} M	NADH and $Fe(CN)_6^{-3}$	434 ± 27

[a]Incubation of reactants for 3 min as indicated, followed by final additions to start the reaction. The rates are given with standard errors of means for triplicate assays. ADR, adriamycin.

heat-inactivated plasma membrane sample is inactive (Fig. 1B). To see the full extent of inhibition by adriamycin, individual blanks of each concentration from the active versus heat-inactivated samples are used. No diffrence is caused by the order of addition. This shows that the inhibitor effects of adriamycin are not derived from the oxidation products of adriamycin (Table I). With shorter than 3 min incubation times, higher concentrations of adriamycin are required (Crane *et al.*, 1980).

The NADH-ferricyanide reductase activity of mouse liver plasma membrane begins to be inhibited at adriamycin concentrations of 5×10^{-8} M, with maximum inhibition at 10^{-6} M. The half maximum inhibition is at 10^{-7} M. At concentrations above 10^{-6} M the extent of inhibition decreases (Fig. 2A). A similar inhibition of NADH-ferricyanide reduction by adriamycin is shown by porcine erythrocyte plasma membranes. Maximum inhibition is at 10^{-6} M, but half maximum is at 3×10^{-7} M (Fig. 3A). At concentrations above 10^{-6} M, there is a remarkable decrease in the extent of inhibition, and stimulation is observed at concentrations above 10^{-5} M (not shown). The other anthracycline analogs of adriamycin show similar patterns of inhibition of the NADH-ferricyanide reductase activity (Figs. 2A, 3A) with both mouse liver and porcine erythrocyte plasma membranes. Aclacinomycin and marcellomycin tend to be less inhibitory than adriamycin or daunomycin. These differ primarily in the presence of deoxyfucose instead of daunoseamine substituents (Daskal *et al.*, 1978).

Quinizarin, a dihydroxy anthroquinone analog of the anthracyclines, gives no inhibition of the NADH-ferricyanide reductase activity with either mouse liver or pig erythrocyte plasma membrane (Figs. 2A, 3A).

The NADH-cytochrome *c* reductase activity of both mouse liver and pig erythrocyte plasma membrane is also inhibited after a 3-min incubation period with 10^{-6} M adriamycin, with maximum inhibition approaching 50%

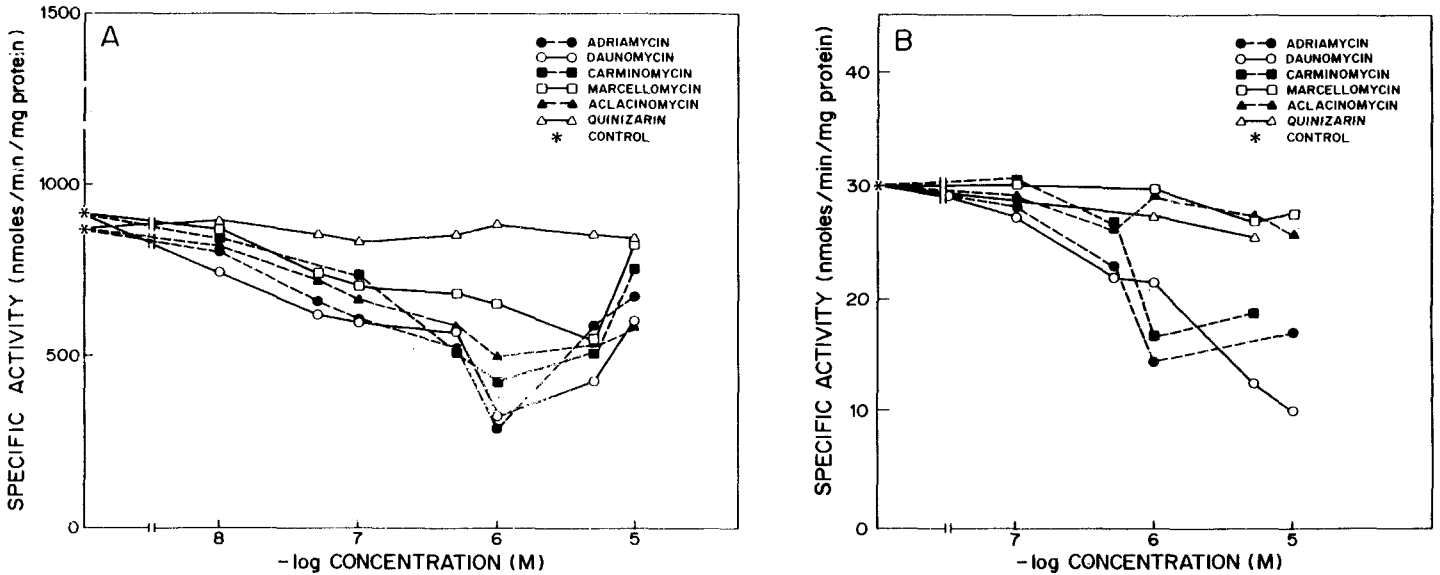

Fig. 2. The effects of anthracycline antibiotics on NADH dehydrogenases of mouse liver plasma membrane. (A) NADH-ferricyanide reductase; (B) NADH-cytochrome *c* reductase. Assays as described under Methods. Three-minute incubation of drugs with membrane before adding NADH and ferricyanide to start the reaction.

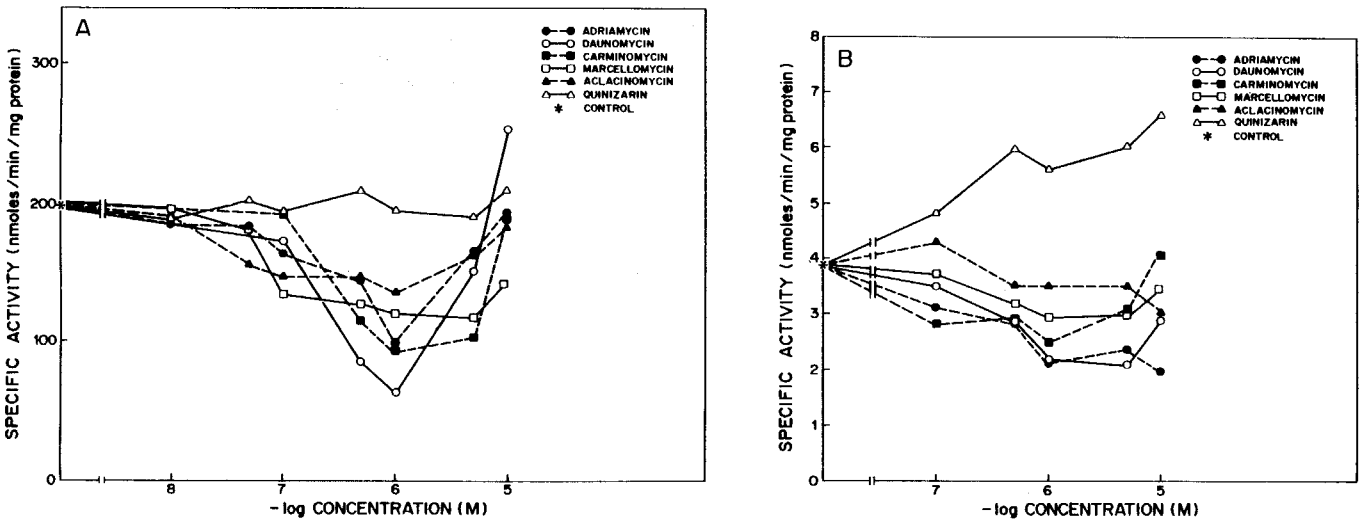

Fig. 3. The effects of anthracycline antibiotics on NADH dehydrogenases of pig erythrocyte membrane. (A) NADH-ferricyanide reductase; (B) NADH-cytochrome *c* reductase.

of the control rate (Figs. 2B, 3B). This inhibition is less than the inhibition of ferricyanide reduction in mouse liver plasma membrane and is similar to the inhibition of ferricyanide reduction observed for pig erythrocyte membranes. Note that half maximum inhibition of the liver membrane cytochrome *c* reductase activity is found at a higher adriamycin concentration than observed for erythrocyte membrane cytochrome *c* reductase. The pig erythrocyte membrane characteristically has a very low NADH-cytochrome *c* reductase activity compared to other plasma membranes (Crane *et al.*, 1982a; MacKellar, 1980).

Daunomycin and carminomycin show inhibition similar to adriamycin with the NADH-cytochrome *c* reductase of liver and pig erythrocyte plasma membranes. Aclacinomycin and marcellomycin, however, give no inhibition of cytochrome *c* reductase in mouse liver plasma membrane (Fig. 2B) and less inhibition in pig erythrocyte plasma membrane. Quinizarin does not affect the NADH-cytochrome *c* reductase activity in mouse liver plasma membrane and causes a stimulation in pig erythrocyte membranes (Figs. 2B, 3B).

Adriamycin does not inhibit the NADH-ferricyanide reductase or the NADH-cytochrome *c* reductase activity of mouse liver endoplasmic reticulum membranes. At higher concentrations (above 10^{-6} M) it clearly shows a stimulation of these activities (Fig. 4A, B). This stimulation is consistant with the adriamycin effects observed on NADH-cytochrome *c* reductase activities of rat liver endoplasmic reticulum (Goodman and Hochstein, 1977). Among the other anthracycline compounds, carminomycin tends to cause stimulations similar to adriamycin, whereas the others induce less stimulation.

N-Trifluoroacetyl adriamycin-14-valerate (AD32), which has been proposed to exert cytotoxic effects at the plasma membrane (Waring, 1981), shows inhibition of both NADH-ferricyanide reductase and NADH-cytochrome *c* reductase activity in both plasma membranes. Inhibition starts at 3×10^{-6} M and reaches a maximum at 1.3×10^{-5} M for both activities (Fig. 5).

Discussion

This communication describes the effects of various anthracycline drugs on plasma membrane redox reactions. Adriamcyin, daunomycin, and carminomycin strongly inhibit plasma membrane NADH-ferricyanide reductase and NADH-cytochrome *c* reductase activities, while aclacinomycin and marcellomycin show lesser effects on the membranes tested. Such differential degrees of inhibition could be the basis for the degrees of antitumor action by these compounds (DuVermay *et al.*, 1977). We would like to propose that the mechanism by which these drugs cause cytotoxic effects is related to the degree of inhibition of plasma membrane dehydrogenase activities.

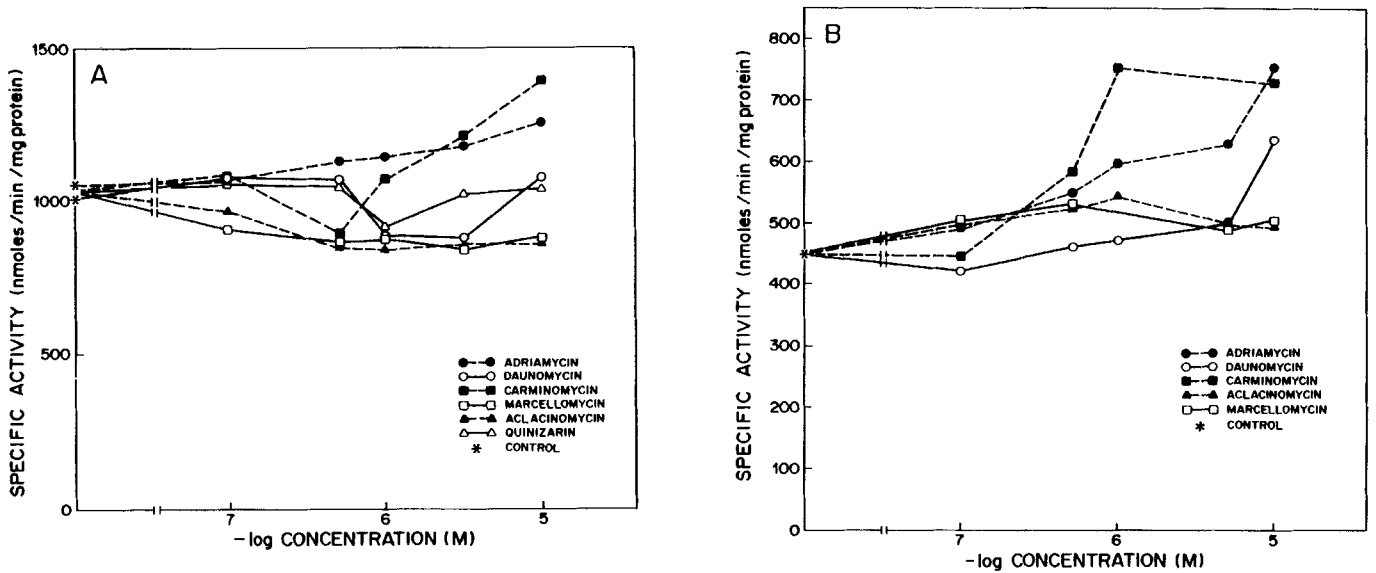

Fig. 4. The effects of anthracycline antibiotics on NADH dehydrogenases of mouse liver endoplasmic reticulum membrane. (A) NADH-ferricyanide reductase; (B) NADH-cytochrome *c* reductase.

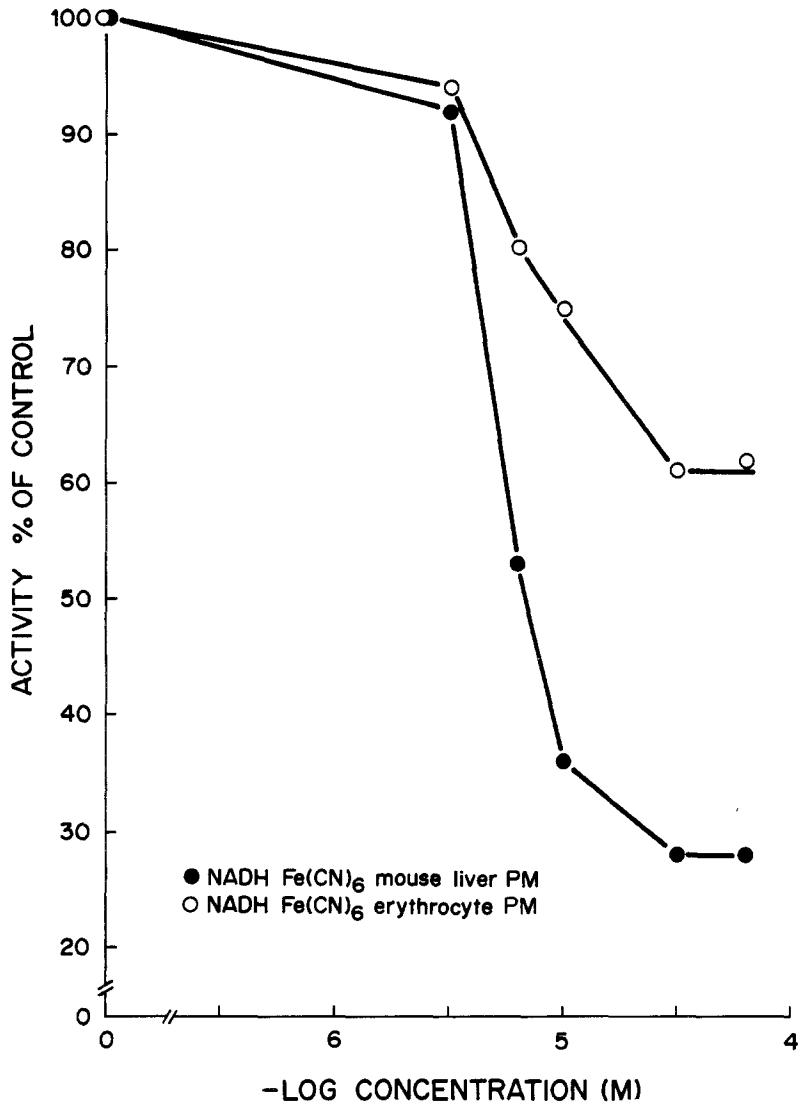

Fig. 5. The effects of AD 32 on NADH-ferricyanide reductase of mouse liver plasma membrane and pig erythrocyte plasma membrane.

Transmembrane electron transport is known to control the hormone response by cells (Löw *et al.*, 1979; Zederman *et al.*, 1977; Löw and Werner, 1976) and the growth of melanoma cells (Ellem and Kay, 1983). The redox system is also necessary for iron uptake by plant cells [Bienfait *et al.*, 1982; Römheld and Marschner, 1983), and a similar function in relation to transferrin iron has been suggested for animal cells (Crane *et al.*, 1982b).

Since insulin, somatomedin A, and transferrin can stimulate the growth of certain cells and can control, or are substrates for, the transmembrane enzymes (Petrides and Bolin, 1980; Löw *et al.*, 1978), it is possible that the enzyme is involved in the control of growth.

Increasing evidence that adriamycin can be cytotoxic to cells by interacting with a component of the plasma membrane (Tritton and Yee, 1982; Tritton *et al.*, 1983; Tökes *et al.*, 1982; Rogers *et al.*, 1983), together with the evidence of a vital function for the transmembrane redox system (Ellem and Kay, 1983), suggests that the transmembrane enzyme described her could be the site for the cytotoxic effects of adriamycin.

The proton transport function associated with the plasma membrane redox system may also be related to the control of cell division (Crane *et al.*, 1982a). Alkalization of the cytoplasm has been shown to stimulate cell division (Busa and Crowe, 1983; Gerson *et al.*, 1982; Moolenaar *et al.*, 1983). The export of protons by the transmembrane enzyme would tend to increase the pH of the cytoplasm and thus stimulate mitosis. Adriamycin can inhibit the pH increase of the cytoplasm by inhibiting the transmembrane electron transport and concomitant proton flow.

The inhibition of NADH-ferricyanide reductase observed with isolated plasma membranes is primarily directed against the transmembrane dehydrogenase. We have previously described an NADH dehydrogenase located on the outside of plasma membranes (Cherry *et al.*, 1981; Crane *et al.*, 1982a), but this enzyme in lung tumor cells (Cherry *et al.*, 1981) and liver (Sun *et al.*, 1983) is only inhibited by adriamycin at above 10^{-5} M. NADH-ferricyanide reductase activity on the inner surface of plasma membranes (Steck and Kant, 1974) is primarily dependent on the NADH-cytochrome b_5 reductase (Choury *et al.*, 1981; Kitajima *et al.*, 1981). Since the activity in endoplasmic reticulum is also dependent on the NADH-cytochrome b_5 reductase and this activity is not inhibited by adriamycin, the inhibition seen in isolated plasma membranes is not an effect on the NADH-cytochrome b_5 reductase flavoprotein. It is possible that the plasma membrane may contain a component, which would link adriamycin to the cytochrome b_5 reductase, and this component is not present in endoplasmic reticulum. We do not see any adriamycin inhibition of NADH-ferricyanide reductase activity of inverted closed vesicles of human erythrocyte membranes (Grebing, unpublished), and this activity has been shown to be partly based on the cytochrome b_5 reductase (Kitajima *et al.*, 1981). In both inverted human erythrocyte membrane vesicles and in liver endoplasmic reticulum adriamycin stimulation of NADH-ferricyanide reductase at concentrations of adriamycin 10^{-5} M or higher is seen. This would be consistent with the stimulation of superoxide radical production by adriamycin, previously reported with endoplasmic reticulum NADH cytochrome b_5 reductase (Goodman and Hochstein, 1977) and mitochondrial NADH dehy-

drogenase (Davis *et al.*, 1983). More dramatic stimulations are seen with the endoplasmic reticulum NADPH dehydrogenase (Sato *et al.*, 1977; Bachur *et al.*, 1977, 1979), but this activity is very low in liver plasma membranes and is not present in erythrocyte plasma membranes (Crane *et al.*, 1979).

The fact that the NADH-cytochrome *c* reductase activity of the plasma membranes is inhibited by adriamycin, whereas the NADH-cytochrome *c* reductase of the liver endoplasmic reticulum is not, is unexpected. This activity in endomembranes is generally assumed to represent the action of the NADH-cytochrome b_5 reductase together with cytochrome b_5. The different responses observed in the two types of membrane could derive from a different enzyme or different binding sites for adriamycin on the two membranes.

Transplasma membrane redox activity can also be assayed by measuring ferricyanide reduction by intact cells (Mishra and Passow, 1969; Clark *et al.*, 1981). If the transmembrane NADH dehydrogenase is responsible for ferricyanide reduction by intact cells, we would expect reduction of ferricyanide by erythrocytes or liver cells to be inhibited by adriamycin. On the contrary, we have found that adriamycin at 10^{-6} M does not inhibit ferricyanide reduction by pig erythrocytes (Sun and Crane, 1982) or primary liver cells (Sun *et al.*, 1983). This would appear to indicate that NADH is not the substrate for ferricyanide reduction by these cells. Glutathione (Arese *et al.*, 1972) or ascorbate (Orringer and Roer, 1979) would be other suggested substrates. On the other hand, the reduction of external ferricyanide by virus-transformed liver cells and hepatoma cells (Crane *et al.*, 1983) or HeLa cells (Sun and Crane, 1982) is clearly inhibited by 10^{-6} M adriamycin. This would indicate that the transmembrane NADH dehydrogenase in tumor cells is modified and its sensitivity to adriamycin over normal cells is increased. Isolation of the plasma membrane from the erythrocyte or liver cell appears to alter the sensitivity of the enzyme, so that it corresponds to the enzyme in tumor cells with regard to adriamycin inhibition.

Inhibition of ferricyanide reductase by AD32, which does not intercalate with DNA (Waring, 1981), is another point in favor of anthracycline drug action on plasma membrane redox systems to produce cytotoxicity. The higher concentration of AD32 required compared to adriamycin for inhibition of redox activity is consistant with the 12 times greater concentration of AD32 required for inhibition of mouse leukemia (Israel *et al.*, 1975) or nucleolar RNA synthesis (DiMarco *et al.*, 1965).

One of the major drawbacks of the use of adriamycin as an antitumor drug is its cardiotoxic effects. These effects have been proposed to depend on peroxidative membrane damage induced by free radical formation in association with redox systems (Bachur *et al.*, 1979; Svingen and Porvis, 1981) or on changes in calcium transport in heart muscle cells (Combs *et al.*, 1981). Since the effect we see (at lower concentrations) on the plasma membrane dehydro-

genase activity is inhibition, this is unlikely to increase oxygen radical formation. On the other hand, evidence that the plasma membrane redox system can control calcium channels in heart muscle (Löw *et al.*, 1984) suggests that the adriamycin inhibition of redox enzymes which we observe could be a basis for affecting calcium transport in heart muscle exposed to adriamycin.

Acknowledgments

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References

Arese, P., Bosia, A., and Pescamora, G. P. (1972). In *VI International Symposium Uber Structur und Function der Erythrozyten* (Rapport, S. and Jung, F., eds.), Akademie Verklag, Berlin, pp. 91–101.

Bachur, N. R., Gordon, S. L., and Malcolm, V. G. (1977). *Mol. Pharmacol.* **13,** 901–910.

Bachur, N. R., Gordon, S. L., Gee, M. V., and Kon, H. (1979). *Proc. Natl. Acad. Sci. U.S.A.* **76,** 954–957.

Bienfait, H. F., Duivenvoorden, J., and Verkerke, W. (1982). *J. Plant Nutr.* **5,** 447–450.

Busa, S. A., and Crowe, J. H. (1983). *Science* **221,** 366–368.

Carter, S. K. (1975). *J. Natl. Cancer Inst.* **55,** 1265–1274.

Cherry, J. M., MacKellar, W. C., Morré, D. J., Crane, F. L., Jacobsen, L. B., and Schirrmacher, V. (1981). *Biochim. Biophys. Acta* **634,** 11–18.

Choury, D., Leroux, A., and Kaplan, J. C. (1981). *J. Clin. Invest.* **67,** 149–155.

Clark, M. G., Patten, G. S., Crane, F. L., Löw, H., and Grebing, C. (1981). *Biochem. J.* **200,** 565–572.

Combs, A. B., Faria, D. T., Leslie, S. W., and Bonner, H. W. (1981). In *Biomedical and Clinical Aspects of Coenzyme Q,* Vol. 3 (Folkers, K., and Yamamura, Y., eds.), Elsevier, Amsterdam, pp. 137–144.

Crane, F. L., Goldenberg, H., Morré, D. J., and Löw, H. (1979). In *Subcellular Biochemistry,* Vol. 6 (Roodyn, D. B., ed.), Plenum Press, New York, pp. 345–399.

Crane, F. L., MacKellar, W. C., Morré, D. J., Ramasarma, T., Goldenberg, H., Grebing, C., and Löw, H. (1980). *Biochem. Biophys. Res. Commun.* **93,** 746–754.

Crane, F. L., Crane, H. E., Sun, I. L., MacKellar, W. C., Grebing, C., and Löw, H. (1982a). *J. Bioenerg. Biomembr.* **14,** 425–433.

Crane, F. L., Löw, H., and Clark, M. G. (1982b). In *Membranes and Transport,* Vol. 2 Martonosi, A., ed.) Plenum Press, New York, pp. 251–254.

Crane, F. L., Sun, I. L., Chou, J. Y., and Löw, H. (1983). *J. Cell Biol.* **97,** 71a.

Daskal, Y., Woodward, C., Crooke, S. T., and Busch, H. (1978). *Cancer Res.* **38,** 467–473.

Davies, K. J. A., Doroshow, J. H., and Hochstein, P. (1983). *FEBS Lett.* **153,** 227–230.

DiMarco, A. (1975). *Cancer Chem. Rep.* **59,** 90–105.

DiMarco, A., Silvestrini, R., DiMarco, S., and Dasdia, T. (1965). *J. Cell. Biol.* **27,** 545–550.

DuVernay, V. H., Prestayko, A. W., Galvan, L., Busch, H., and Crooke, S. (1977). *Proc. Am. Cancer Soc. Res.* **18,** 182.

Ellem, K. A. O., and Kay, G. F. (1983). *Biochem. Biophys. Res. Commun.* **112,** 183–190.
Gerson, D. F., Keifer, H., and Eufe, W. (1982). *Science* **216,** 1009–1010.
Goldenberg, H. (1982). *Biochim. Biophys. Acta* **694,** 203–223.
Goldenberg, H., Crane, F. L., and Morré, D. J. (1979). *J. Biol. Chem.* **254,** 2491–2498.
Goldman, R., Facchinetti, T., Bach, D., Raz, A., and Schmitzky, M. (1978). *Biochim. Biophys. Acta* **512,** 254–269.
Goodman, J., and Hochstein, P. (1977). *Biochem. Biophys. Res. Commun.* **77,** 797–803.
Goormaghtigh, E., Brasseur, R., and Ruyschaert, J.-M. (1982). *Biochem. Biophys. Res. Commun.* **104,** 314–320.
Gosalvez, M., Blanco, M., Hunter, J., Miko, M., and Chance, B. (1974). *Eur. J. Cancer* **10,** 567–574.
Grunicke, H., Gaulner, G., Holzweber, F., Ihlenfeldt, M., and Puschendorf, B. (1979). *Adv. Enzyme Regul.* **17,** 291–305.
Israel, M., Modest, E. J., and Frei, E. (1975). *Cancer Res.* **38,** 365–370.
Kanter, P. M., and Schwartz, H. S. (1979). *Cancer Res.* **39,** 3661–3672.
Kitajima, S., Yasukochi, Y., and Minakami, S. (1981). *Arch. Biochem. Biophys.* **210,** 330–339.
Löw, H., and Werner, S. (1976). *FEBS Lett.* **65,** 96–98.
Löw, H., Crane, F. L., Grebing, C., Tally, M., and Hall, K. (1978). *FEBS Lett.* **91,** 166–168.
Löw, H., Crane, F. L., Grebing, C., Hall, K., and Tally, M. (1979). In *Diabetes 1979* (Waldhäusl, W. K., ed.), Excerpta Medica, Amsterdam, pp. 209–213.
Löw H., Crane, F. L., Partick, E. J., and Clark, M. G. (1984). *Biochim. Biophys. Acta* (in press).
MacKellar, W. C. (1980). Ph.D. Thesis, Purdue University.
Mishra, R. K., and Passow, H. (1969). *J. Membr. Biol.* **1,** 214–224.
MacKellar, W. C. (1980). Ph.D. Thesis, Purdue University.
Mishra, R. K., and Passow, H. (1969). *J. Membr. Biol.* **1,** 214–224.
Moolenaar, W. H., Tsien, R. Y., vander Saag, P. T., and deLaat, S. W. (1983). *Nature (London)* **304,** 645–648.
Morrë, D. J., Cheetham, R. D., Nyquist, S. E., and Outracht, L. (1972). *Prep. Biochem.* **2,** 61–69.
Muhammed, H., Ramsarma, T., and Ramakrishna Kurup, C. K. (1982). *Biochim. Biophys. Acta* **722,** 43–50.
Murphee, S. A., Cunningham, L. S., Hwang, K. M., and Sartorelli, A. C. (1976). *Biochem. Pharmacol.* **25,** 1227–1231.
Na, C., and Timasheff, S. N. (1977). *Arch. Biochem. Biophys.* **182,** 147–154.
Ohsawa, M., Kilberg, M. S., Kimmel, G., and Christensen, H. N. (1980). *Biochim. Biophys. Acta* **599,** 175–190.
Orringer, E. P., and Roer, M. E. S. (1979). *J. Clin. Invest.* **63,** 53–58.
Petrides, P. E., and Bolin, P. (1980). *Biochem. Biophys. Res. Commun.* **95,** 1138–1144.
Ramasarma, T., MacKellar, W. C., and Crane, F. L. (1980). *Indian J. Biochem. Biophys.* **17,** 163–167.
Römheld, V., and Marschner, H. (1983). *Plant Physiol.* **71,** 949–954.
Rogers, K. E., Carr, B. I., and Tökes, Z. A. (1983). *Cancer Res.* **43,** 2741–2748.
Sato, S., Iwaizumi, M., Handa, K., and Tamura, Y. (1977). *Gann* **68,** 603–508.
Steck, T. L., and Kant, J. A. (1974). *Methods Enzymol.* **31,** 172–180.
Silvestrini, R., DiMarco, A., and Dasdia, T. (1970). *Cancer Res.* **30,** 966–973.
Silvestrini, R., Lenza, L., and DiFronzo, G. (1973). *Cancer Res.* **33,** 2954–2958.
Sun, I. L., and Crane, F. L. (1982). *Fed. Proc.* **41,** 737.
Sun, I. L., Crane, F. L., Chou, J. Y., Löw, H., and Grebing, C. (1983). *Biochem. Biophys. Res. Commun.* **116,** 210–216.
Svingen, B. A., and Porvis, G. (1981). *Arch. Biochem. Biophys.* **209,** 119–126.
Tökes, Z. A., Rogers, K. E., and Rembaum, A. (1982). *Proc. Natl. Acad. Sci. U.S.A* **79,** 2026–2030.
Tritton, T. R., and Yee, G. (1982). *Science* **217,** 248–150.
Tritton, T. R., Yee, G., and Wingard, L. B., Jr. (1983). *Fed. Proc.* **42,** 284–287.
Waring, M. J. (1981). *Annu. Rev. Biochem.* **50,** 159–192.
Zederman, R., Löw, H., and Hall, K. (1977). *FEBS Lett.* **75,** 291–294.