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A Chemical Perspective on the Anthracycline Antitumor Antibiotics

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The anthracycline antitumor antibiotics occupy a central position in the chemotherapeutic control of cancer. They remain, however, antibiotics of the last resort and thus exhibit toxicity both to the neoplasm and to the host organism. As part of the continuing effort to dissociate the molecular processes responsible for these two separate toxicities, attention has been drawn to the intrinsic redox capacity of their tetrahydronapthacenedione aglycone moiety, and to the possible expression of this redox activity against those biomolecules for which anthracyclines have a particular affinity (polynucleotides and membranes). This review is a synopsis of the present trends and thoughts concerning this relationship, written from the point of view of the intrinsic chemical competence of the anthracyclines and their metabolites. While our ignorance is profound—the precise molecular locus of the antitumor expression of the anthracyclines remains unknown—there is now evidence that the relationship of the anthracyclines to the DNA (possibly requiring enzymatic cooperation) and to the membranes, with neither event requiring redox chemistry, may comprise the core of the antitumor effects. The adventitious expression of the redox activity under either aerobic conditions (in which circumstances molecular oxygen is reduced) or anaerobic conditions (in which circumstances potentially reactive aglycone tautomers are obtained) is therefore thought to contribute more strongly to the host toxicity. Yet little remains proven, and the understanding of the intrinsic chemical competence can do little more than lightly define the boundaries within which are found these and numerous other working hypotheses.

Introduction

Over the past decade the anthracycline antitumor antibiotics (Fig. 1), exemplified by Adriamycin (doxorubicin) and daunomycin (daunorubicin), have attained a central position in the chemotherapeutic control of cancer (*1–8*). As a consequence of their relative breadth and potency, considerable effort has been expended to discern the molecular mechanisms of their activity. Progress has, however, been painstaking, and only recently has order begun to emerge from chaos. This review is a distillation of the current thoughts and ideas emerging from these experimental endeavors, with a particular emphasis upon the role of redox transformations. The focus is placed upon the relevant chemical and biochemical events, and as such, medical progress with the anthracyclines is not covered. Literature citations are selective, and are drawn from the primary literature of the past three years, through the summer of 1984.

A systematic organization to this paper is made most difficult by the extraordinary complexity exhibited by the anthracyclines in the living organism. There is now a consensus that multiple pathways exist for the expression of anthracycline cytotoxicity, and that the full expression of any one anthracycline is a linear combination of all pathways. Unraveling these pathways into those that are desirable (toxicity to the neoplasia) and into those that are not (toxicity to the organism) is an enigma, only recently rendered less inscrutable. Accordingly, a chemical organization is chosen, where progress in the chemistry of each pathway is evaluated and an effort then made to place the chemistry into the appropriate biological context.

We begin with an evaluation of the basic redox chemistry of the anthracyclines, and explore the enzymology appropriate to mediating these reactions. The relevance of the cellular DNA as a target of the anthracyclines—with or without redox activation—and the potential consequences of metal ion chelation are then covered. At this point the enormous separation that yet exists between the chemical and biochemical knowledge will be apparent. A summation of the capacity of the cell membrane as a target is provided and is followed by a synopsis of the role of redox processes to the antineoplastic activity. From these perspectives, the working hypothesis is suggested that the redox capacity of the anthracyclines may represent an undesirable pathway, while nucleic acids and membranes may represent the focus for the desirable expression of anthracycline activity. Yet the precise chemistry remains elusive and shall remain so until the precise locus for the expression of anthracycline antitumor activity has been identified to the satisfaction of all interested parties. By this fact, all commentary within this review is qualified.

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Redox Chemistry of the Anthracyclines

Anaerobic Chemistry: The Chemistry of the Quinone Methide

Metabolic transformation of a drug as a prerequisite to the expression of its biological activity is now understood to be a common occurrence. Moore (*9,10*) was the first to note that the anthracyclines (among others) comprise a family where bioreduction could lead to reactive intermediates capable of nucleophile alkylation ("bioreductive alkylation") (*11*). A most reasonable inquiry, then, concerning the anthracyclines is the relationship of this chemistry to their biological activity. Do they alkylate biomolecules, and, if so, does this process define the antitumor activity, the general toxicity, neither, or both? Only most recently has our understanding of the anthracycline chemistry progressed to a point where the experimental design meaningfully addresses the kinetic and chemical competence of Moore's intermediates (quinone methides). While a definitive answer has not been possible, the general outlines of the quinone methide chemistry are now defined, and a description of these outlines comprises this section.

To begin with, quinone methide formation is an anaerobic event that may occur once and only once for any one individual anthracycline molecule. Anthracyclines, as quinones, are intrinsically capable of redox chemistry. At carbon 7 is found an oxygen substituent, usually a glycoside, that is transformed from a stable functional group in the quinone to a stable leaving group in the hydroquinone. As the hydroquinone itself is quite prone to oxidation by O_2, only when O_2 is absent will it persist long enough (and this need not be long at all) for this leaving group ability to be expressed. Intramolecular loss of this C-7 substituent provides the quinone methide intermediate [Eq. (1)]. The quinone methide may be envisioned to behave as a nucleophile, to react with an electrophile to yield a C-7 functionalized quinone; or to behave as an electrophile to yield a C-7 functionalized hydroquinone. This latter hydroquinone may re-eliminate to the quinone methide unless a suitable oxidant is present to convert this adduct to the stable quinone state (*12,13*). If no suitable reagent to react with the quinone methide is present, it will react with a proton (an electrophile) to provide a 7-deoxy aglycone or react with itself to provide a 7,7′-dimer. In both instances the quinone methide character is irrevocably lost.

The balance between these various reactions is determined by the overall substitution of the particular

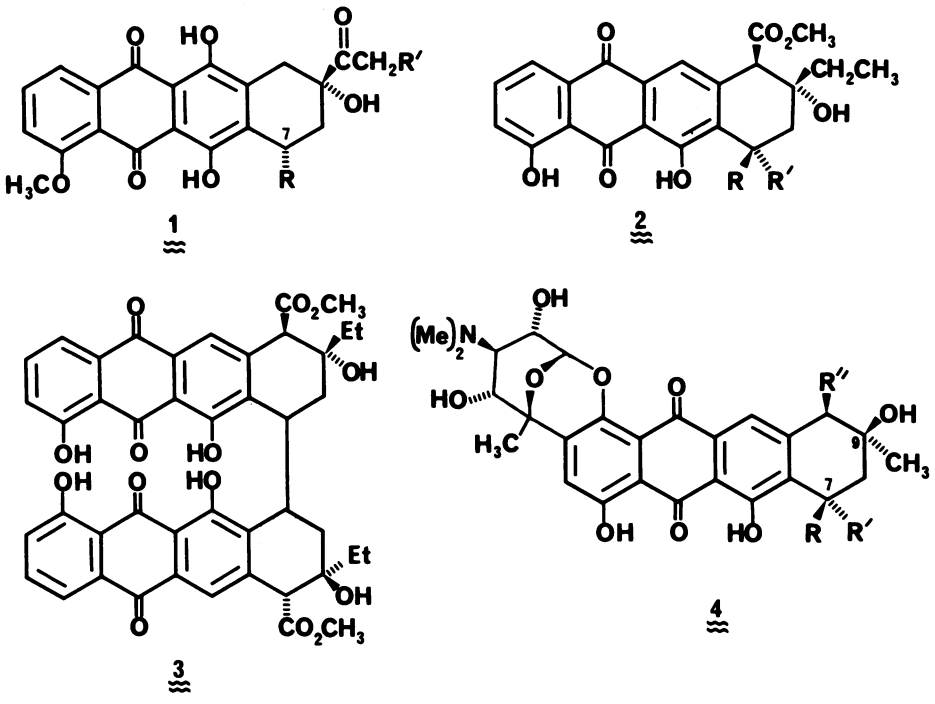
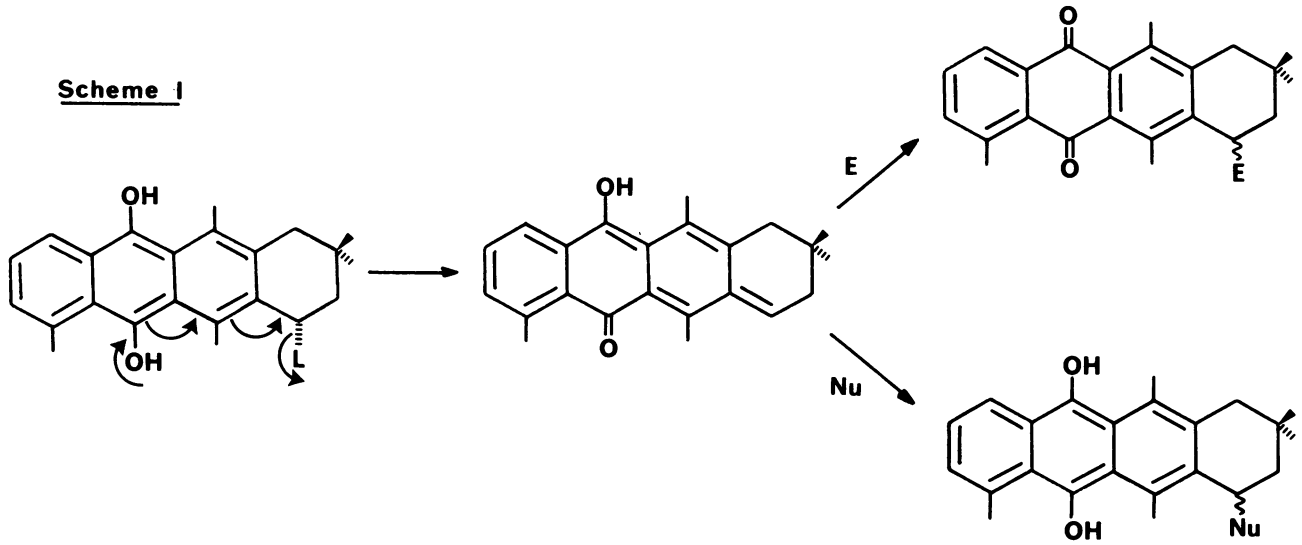


FIGURE 1. Anthracyclines and anthracyclinones. (*1*) Daunomycin family: (*1a*) daunomycin (R = L- daunosamine, R′ = H); (*1b*) 7-deoxydaunomycinone (R = R′ = H); (*1c*) daunomycinone (R = OH, R′ = H); (*1d*) Adriamycin (R = L-daunosamine, R′ = OH); (*1e*) epirubicin (R = 4′-epi-L-daunosamine, R′ = OH). (*2*) Aclacinomycin family: (*2a*) aclacinomycin A (R′ = L- rhodosamine-2-deoxy-L-fucose-L-cinerulose A, R = H); (*2b*) 7-deoxyaklavinone (R = R′ = H). (*3*) Bi(7- deoxyaklavinon-7-yl). (*4*) Nogalamycin family: (*41*) nogalamycin (R = H, R′ = nogalose, R″ = CO_2CH_3); (*4b*) 7-deoxynogalarol (R = R′ = H, R″ = CO_2CH_3); (*4c*) 7R-nogamycin (R = nogalose, R′ = R″ = H); (*4d*) menogarol (R = OCH_3, R′ = R″ = H); (*4e*) epimenogarol (R′ = OCH_3, R = R″ = H); (*4f*) 7-deoxynogarol (R = R′ = R″ = H).

Scheme I



anthracycline. At present, three different subgroups are recognized; it is likely, however, that others exist as not all anthracyclines have been carefully examined. These three groupings do contain those anthracyclines of primary clinical interest. The first consists of 11-hydroxy anthracyclines (including daunorubicin, Adriamycin, and epirubicin; and presumably including Ad-32 and esorubicin); the second the 11-unsubstituted anthracyclines (including aclacinomycin, 11-deoxydaunomycin, and marcellomycin); and the third the nogalamycin family (including nogalamycin itself and menogarol). Comparison of the three families indicates that the two features of the quinone methide most strongly affected are the rate constant for quinone methide formation and the balance between the electrophilic and nucleophilic character of the quinone methide.

Access to the quinone methide from daunomycin may be had from anaerobic reduction of the quinone in either organic or aqueous solvent. The reducing agent used by Koch and colleagues (*14–17*) in their extensive study of the quinone methide in organic solvent is the *mero*-stabilized 3,5,5-trimethyl-2-oxomorpholin-3-yl radical. This is a uniquely suitable reducing agent for quinone methide production in that it is stable in the absence of an oxidant, a strong and rapid reducing agent in the presence of an oxidant, and possesses complete regiochemistry for quinone reduction over C-13 carbonyl reduction. Daunomycin reduction in aqueous solution is most conveniently done with NAD(P)H as the penultimate reducing agent and an enzyme catalyst, NADP:ferredoxin oxidoreductase from spinach (*18*), as the ultimate reducing agent. In both cases, spectroscopic examination reveals the essentially immediate formation of the quinone methide from the daunomycin hydroquinone, observing at the λ_{max} of the quinone methide near 600 nm. Without an appropriate electrophile or nucleophile present, it decays by solvent protonation at C-7. This reaction has apparent first order kinetics, and indicates a lifetime for the quinone methide of approximately one minute ($t_{1/2}$ for the decay for the quninone methide is 15 sec at pH 8.0, 30°C).

The daunomycin-derived quinone methide displays nucleophilic character toward electrophiles of comparable reactivity to H^+. The one functional group found to qualify thus far is the aldehyde (*15,16,18*). Quinone methide trapping at the *re* face is followed by intramolecular hemiacetal formation at the C-13 carbonyl. Weaker electrophiles, such as oxidized glutathione, do not react to yield stable adducts. Whether the bimolecular rate constant is too low, or whether it is that hemiacetal formation is a required event to stabilize the adduct (vide infra), is undecided. Since, however, suitable electrophiles *in vivo* are uncommon, and the bimolecular rate constant even for aldehydes low (~1 M^{-1}-sec^{-1}), it may safely be speculated that only rather common and innocuous electrophiles—such as glyceraldehyde-3-phosphate, as a representative glycolytic intermediate—may react. The one qualification to this speculation is the possibility that quinone methide formation occurs after complexation of the anthracycline to a critical cellular target, where an otherwise weakly reactive functional group is compelled to participate by its proximity.

The daunomycin-derived quinone methide may also undergo trapping by nucleophiles. The formation of a stable adduct from nucleophilic trapping of the quinone methide requires a subsequent oxidation step; the subtleties of these reactions are discussed elsewhere (*12*). Only one nucleophile, *N*-acetylcysteine, has been found thus far to have significant bimolecular reactivity to this quinone methide (K. Ramakrishnan and J. Fisher, unpublished data). A comprehensive examination of the nucleophile reactivity has yet to be made, and in any case the identical caution with respect to specific trapping as is made in the preceding paragraph applies here as well.

Aclacinomycin A is the representative anthracycline

from the second anthracycline family with respect to quinone methide reactivity, the 11-deoxy anthracyclines. This anthracycline contains several other features that differentiate it from the daunomycin family, including a C-7 trisaccharide, an unoxidized carbon at C-14, and the 10*R*-carbomethoxy moiety. Initial antitumor evaluation suggests it to be a somewhat less potent, but also less toxic, anthracycline. Its clinical efficacy is now being established.

Like daunomycin, the aclacinomycin hydroquinone rapidly eliminates to the quinone methide. This quinone methide is not as stable as that from daunomycin, primarily as a consequence of a second reaction pathway—in addition to solvent protonation—available to it. Examination of the reaction products obtained in the absence of other nucleophiles or electrophiles shows that two compounds are formed: 7-deoxyaklavinone, from solvent protonation; and bi-(7-deoxyaklavinon-7-yl), a 7,7′-dimer from quinone methide coupling (*17*).

Efforts to trap this quinone methide with the aldehydes capable of adduct formation with the 7-deoxydaunomycinone quinone methide tautomer, have not been successful. This does not, however, necessarily abrogate the quinone methide of nucleophilic character; it does, after all, react with the proton. A possible explanation is that aldehyde adducts do form, but the absence of the C-14 ketone precludes the stabilizing formation of the hemiacetal, and the adducts revert. Indeed, preliminary experiments indicate that an aldehyde adduct can be obtained from 11-deoxydaunomycin (which has the C-14 ketone). This anthracycline may best represent the structural and mechanistic bridge between daunomycin and aclacinomycin.

The aclacinomycin-derived quinone methide is more easily trapped by nucleophiles in the presence of oxidants than is that from daunomycin. These nucleophiles are, however, limited to thiols, and do not include more relevant nucleophiles such as enols or phosphate esters (such as GMP). Although negative results with GMP may reflect on the possibility of covalent DNA modification, it must be remembered that mononucleotides significantly underestimate the reactivity of oligonucleotides as nucleophiles (*19,20*).

Nogalamycin is the parent structure for the third family of anthracycline-derived quinone methides. It differs fundamentally from the preceding two by having its aminoglycoside fused to the D, rather than the A, ring; by a neutral sugar at C-7; and by the uncommon 7*S*, 9*S*, 10*R* (epimeric at C-9) configuration (*21,22*). Interestingly, the nogalamycin derivative with the most promising clinical antitumor efficacy, menogarol, is a semisynthetic derivative where the C-7 glycoside is lost and replaced with an epimeric methoxy. This change is observed to significantly weaken the *in vitro* DNA affinity (*23,24*). The chemistries of nogalamycin and menogarol upon anaerobic reduction display very different characteristics compared to the other two classes. Rather than immediately yielding the quinone methide, as is the case for daunomycin and aclacinomycin, their respective hydroquinones slowly decay to the quinone methide, ultimately giving the 7-deoxy aglycone. Thus, quinone methide formation, and not its protonation, is rate-limiting for these products. Trapping experiments have not been attempted, as it is likely that the quinone methide has an insufficient steady state concentration to afford appreciable bimolecular reactivity.

A reasonable hypothesis for this dramatic change in the rate of quinone methide formation is that this intramolecular elimination is under stereoelectronic control, and that a periplanar orientation between the intramolecular nucleophile, the π bond, and the leaving group, the C-7 substituent, is required to minimize the activation energy. Thus, while for both daunomycin and aclacinomycin the leaving group has available the more labile pseudoaxial position, this may not be the case for nogalamycin. However menogarol, being epimeric at C-7, has the pseudoaxial position available (*25*). Yet it reacts only somewhat faster than nogalamycin (*18*). Since within this family leaving group stability does not influence the reaction rate, it must be concluded that stereoelectronics alone is insufficient to account for the slow rate of quinone methide formation within the nogalamycin family. Interestingly—and in something of a contrast to this conclusion—Arcamone and colleagues (*26*) have been able to apply with excellent success these same stereoelectronic principles to account for the relative ability to yield the quinone methide within the daunomycin and 6-deoxydaunomycin families. Clearly the full complexity of this problem has yet to be appreciated.

This same conclusion pertains to the chemistry of the quinone methide itself. Only first indications of its behavior are available for three anthracycline classes; at least two anthracycline families of clinical interest are as yet unevaluated (4-demethoxydaunorubicin and 6-deoxydaunorubicin). Nonetheless all presently available evidence indicates that the bimolecular reactivity of these quinone methides is surprisingly low. Krohn and colleagues have, however, provided excellent circumstantial evidence that under chemically more vigorous conditions a greater expression of reactivity may be manifested (*27*). If, then, quinone methide formation is a particular event for the expression of the anthracycline antitumor activity, a common behavior must be enforced by the target to which the anthracycline is complexed. Since quinone methide formation occurs only once, the anthracycline must form this complex prior to reduction. It is concluded that without a molecular knowledge of that target, the importance of the anaerobic reductive activation of the anthracyclines is not ascertainable.

Chemistry of the Anthracycline Semiquinone

As quinones, anthracyclines are capable of accepting electrons in one electron increments, thus forming semiquinone radicals. The relationship of these radicals

to other biological phenomena remains a major research focus. The basic aspects of the daunomycin semiquinone's chemistry are now established. One-electron reduction of the quinone by electrochemical (*28,29*), chemical (*16*) or enzymatic (*30*) means leads to an EPR signal characteristic of the daunomycin semiquinone (*31,32*). The disproportionation equilibrium lies well toward the hydroquinone and quinone, as spectrally detectable quantities of this radical are not observed during titrations. This is equally true for the aclacinomycin semiquinone (*17*). Lown and co-workers (*28*), by electrochemical measurement, provided the first value for the two electron reduction midpoint potential of -0.64 V (vs. SCE) for daunomycin; further study with additional anthracyclines indicates that there are substantial substituent effects on this potential (ranging from -0.70 V for carminomycin to -0.50 V for aclacinomycin) (*33*). As would be expected, the daunomycin semiquinone is a labile and powerful reductant toward one-electron acceptors, including metals (*34*) and organic oxidants (*35*), and last but not least oxygen (*36*). Electron transfer to oxygen is sufficiently fast to prevent the reductive elimination of the C-7 substitutent under aerobic conditions. A detailed pulse radiolysis study has established the one electron reduction potential for Adriamycin as -0.328 V (vs. NHE, pH 7), which may be combined with the one electron potential for O_2 of -0.155 V to provide an equilibrium constant of 0.0012 for electron transfer from O_2^- to Adriamycin, to give O_2 and the semiquinone (*36*). In all the above respects the semiquinone chemistry is rather ordinary.

There are, however, two aspects to the semiquinone chemistry that remain uncertain. In order to place its involvement in lipid peroxidation in a reasonable chemical setting, it has been presumed that the semiquinone is competent for the reduction of alkyl peroxides to the hydroxyl radical (*37,38*). Indeed, spin-trapping experiments are consistent with this notion (*38,39*). Direct evidence for this reaction has been provided by Kalyanaraman et al. (*40*), where the effect of H_2O_2 on the steady state semiquinone concentration during enzymatic reduction has been used to estimate a bimolecular rate constant of about 10^3-10^4 $M^{-1}sec^{-1}$ for this reaction. On the other hand Houee-Levin et al. (*41*), using excess CO_2^- as reductant, find no evidence for this reaction but rather for $2e^-$ reduction, from the hydroquinone, to the peroxide to yield water. A most reasonable explanation is that both reactions are possible, with the balance between the two determined by the availability of electrons to the anthracycline. However, with respect to the semiquinone reduction of peroxides the contribution from metal catalysis remains problematical (*40,42*). The second uncertainty is the contribution of the semiquinone to the reductive deglycosylation reaction. A prevalent assumption in the literature is that this reaction is reserved to the semiquinone, and not to the hydroquinone. In support of this notion are the observations that the one electron-transferring flavoenzymes are quite generally more active as catalysts of this reaction than the two-electron transferring dehydrogenases (*43*), and the good correlation between the semiquinone steady state concentration and extent of 7-deoxy aglycone formed (*30*). However, the first observation may reflect more that these enzymes are generally stronger reducing agents, and have a lower substrate specificity. Since they *are* capable of overall two electron transfer, they may produce the hydroquinone as well as the semiquinone. The second observation applies equally well to the hydroquinone as to the semiquinone, since in the course of anthracycline reduction the semiquinone may be seen in direct proportion to the overall forward velocity to the hydroquinone. Thus, these data alone will not distinguish the semiquinone from the hydroquinone as a competent intermediate for this reaction. Further, the failure of previous efforts to detect hydroquinone intermediates is explained both by a lack of knowledge as to their properties—it was not certain what to look for—and as a result of the aqueous insolubility of many of the 7-deoxy aglycones. Aglycone precipitation may be quite competitive with reduction. Nonetheless under the appropriate experimental circumstances the competence of the anthracyclines for overall two electron reduction is easily established (*44,45*); moreover their competence in the elimination reaction to the 7-deoxy aglycone quinone methide tautomer is firmly established (*12,16,17*). Thus while the semiquinone has not been excluded from this reaction, neither is there any evidence that demands it be included. A final point to be made concerns the product of the (as yet unproven) elimination reaction from the semiquinone glycoside, the C-7 benzyl radical. This neutral radical has been frequently spoken of as a potentially reactive intermediate, in accounting for the presumed covalent labeling of macromolecules upon anaerobic anthracycline reductive activation. The reactivity of this radical is questionable. Radicals behave as reducing agents, as electrophiles toward each other, and less frequently as radical abstractors. Given the resonance stabilization available to the C-7 benzylic radical and the behavior of the hydroquinone-derived quinone methide, it appears most unlikely that it would be chemically competent for bimolecular attack as an electrophile on cellular macromolecules.

A Redox-Incapacitated Anthracycline

A singular anthracycline analog is 5-iminodaunorubicin, developed by Acton and colleagues (*45*). This analog provides an excellent contrast to daunorubicin, as replacement of the oxygen with nitrogen virtually abolishes its redox capability, but not antitumor activity. By virtue of its quinone imine C-ring, it at once becomes an analog both more difficult to reduce and more difficult to reoxidize (*47*). Hence 7-deoxy metabolites are not observed *in vivo*, and its toxicity toward cardiac tissue is substantially diminished (*48,49*). Nor is it a pro-drug for daunorubicin; the imine moiety is quite hydrolytically stable. Its poor redox ability is somewhat surprising and must clearly be related to the overall electron-donating substitution elsewhere in the

anthracycline (at C-4, 6 and 11); quinone imines having even modest electron-withdrawing substitution are very potent electrophiles (*50,51*). The absence of redox capability has the following consequences. Futile oxygen cycling is virtually absent compared to daunorubicin (*48*), and lipid peroxidation is therefore abolished (*52–54*). The quinone imine also influences other properties. 5-Iminodaunorubicin is a poor inhibitor of nuclear rRNA synthesis, and although it possesses typical behavior toward DNA as an intercalator, it has a somewhat smaller affinity (*55*). As discussed more fully below, this intercalation also affects the topoisomerase II enzyme but in a fashion still dissimilar to daunorubicin (*56*). Last, it provides excellent evidence for redox activity contributing more toward undesirable toxicity than tumor toxicity; it retains antineoplastic activity at potencies quite comparable to daunorubicin *in vitro* and somewhat less *in vivo* (*56*). It is not, however, a nontoxic anthracycline, as elevated dosing does result in toxicity believed to be associated with a membrane effect, and not originating from redox activity (*49*). Thus while this anthracycline in not necessarily an improved antitumor antibiotic—although it is certainly being evaluated for this possibility—it (as well as other new derivatives) offers optimism that substantial improvements in the therapeutic ratio may be attained by subtle structural alterations.

Metal Ion Modulation of Redox Activity

An aspect that has only recently received its proper scrutiny is the effect of metals, particularly Fe(II), Fe(III), and Cu(II), on the anthracycline redox chemistry. The ability of metals to strongly potentiate aerobic peroxidation has been recognized for some time (*57,58*) as has the chelating ability of 11-hydroxy anthracyclines, such as daunomycin and Adriamycin (*6*). The consequences of metal chelation to daunomycin appear to be dramatic, resulting in an overall increase in electrophilicity, as would be expected. Metal chelation occurs to the 11,12 oxygens (*6*), providing a complex that is reduced by simple sulfhydryls (glutathione, cysteine) whereas the uncomplexed anthracycline is not. Under aerobic conditions, the electrons derived from the sulfhydryl are passed to O_2, providing H_2O_2 that is used in the complex-dependent degradation of lipids, deoxyribose, and DNA (*59–63*). With respect to this latter reaction, Myers et al. (*64*) have provided evidence that not only is the complex significantly more active than either component alone, but that complex association to DNA is specific, remarkably stable, and fundamentally different than the intercalation that occurs with the free anthracycline. The chemistry associated with the DNA cleavage suggests that the anthracycline serves primarily to stabilize the DNA complex, rather than to assume a direct redox role (this is then reserved to the iron). Although there exists no direct evidence that the iron-anthracycline complex forms *in vivo*, there are substantial circumstantial data consistent with this notion, summarized by Eliot et al. (*63*), which further indicate that the complex may represent a component of the cardiac toxicity.

Even fewer data are available concerning the chemistry of this complex under hypoxic conditions. Zweier (*65*) has described direct evidence for the complex's role in O_2 reduction, and observes that under anaerobic conditions the Fe(III)–Adriamycin complex slowly converts to the Fe(II) complex, presumably using the anthracycline as the reducing agent. Admission of O_2 immediately restores the Fe(III) oxidation state. Complementary observations are also reported by Gutteridge (*66*). Myers et al. (*62*) have treated the iron complex anaerobically with glutathione; 7-deoxyadriamycinone is not formed. This suggests that quinone methide formation does not occur from the complex. However, this point has not been pressed, and further experimentation (particularly with stronger reducing agents) is necessary to settle this possibility. Should the quinone methide-iron complex form, the metal will accentuate the electrophilic character of the quinone methide and conceivably allow a broader range of reactivity than is observed for the uncomplexed quinone methide. This may also conceivably confer redox activity to an anthracycline otherwise thought inert, in particular 5-iminodaunorubicin (vide supra). Finally, metal chelation does not occur for 11-deoxyanthracyclines (*64,67*) possibly explaining their generally lower cardiac toxicity.

Chemistry of the 7-Deoxyaglycones

A potentially interesting and important aspect of anthracycline redox chemistry that has received little attention is the chemistry of the 7- deoxyaglycones. The extensive studies on anthracycline metabolism by Schwartz and colleagues (*68,69*) have unearthed an abundance of structurally unidentified metabolites. Within the chemistry of the aglycones may be found suggestions for structures for these metabolites. Apart from this possibility, the rationale for the study of these aglycones is straightforward and easily defended. Quinone methide formation occurs subsequent to the first anaerobic reduction of the anthracycline. One may most reasonably presume then that the major product from the quinone methide—the C-7 deoxyaglycone, by solvent protonation—will also serve as a substrate for the enzyme catalyst that first gave rise to the quinone methide.

Indeed, initial examination reveals that 7-deoxydaunomycinone is reduced chemically and enzymatically with the same alacrity as the parent glycoside. This would be rather prosaic chemistry, if all that it involved was overall two electron reduction to the 7- deoxydaunomycinone hydroquinone. Recent studies indicate this hydroquinone to be a rather interesting chemical entity. While retaining the anticipated lability toward oxidants (*44,45*), if anaerobic conditions persist (to allow it a reasonable lifetime) it may behave as a strong hydride donor, as noted by Koch and colleagues (*70*); or to otherwise undergo spontaneous tautomerization to the more stable leuco tautomers [Eq. (2)]. These tautomers

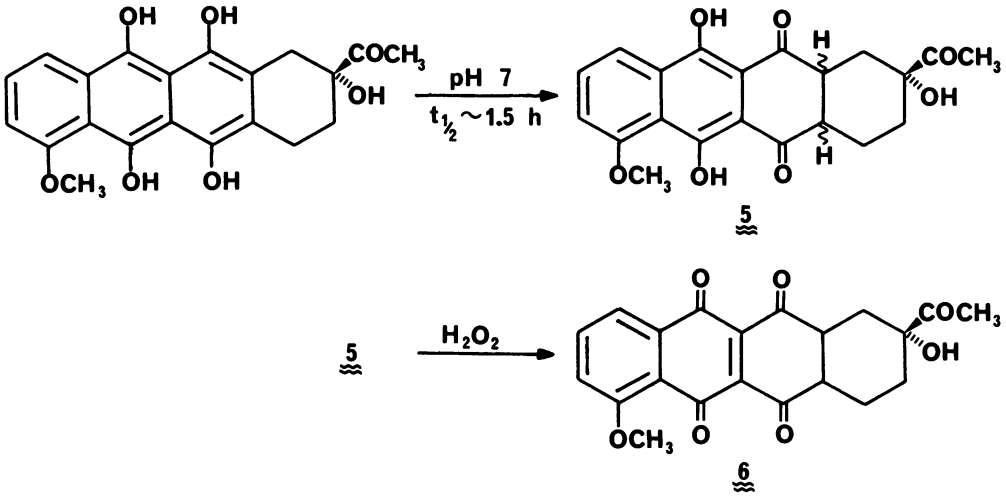
(*5*) are bright yellow and strongly blue fluorescent compounds, that in the absence of base remain quite stable to oxygen oxidation (D. Brand, unpublished data). Although the spontaneous tautomerization is slow, this reaction may be catalyzed by metal ions and it would not be at all unreasonable to anticipate the formation of these tautomers *in vivo*. Further, these *leuco* tautomers retain a capability as redox agents in that they are observed to undergo slow oxidation by H_2O_2 to yield a product tentatively identified as the quinone (*6*). This latter reaction is accelerated by horse radish peroxidase. Should this quinone indeed be the product, it may behave as a potent oxidant and possibly as an electrophile as well. While much of this chemistry is new and certainly speculative, it may offer further insight into anthracycline behavior *in vivo*, and may allow a better understanding of the metabolism of these quinones. The nature and characteristics of these reactions *in vivo* are likely to be quite different from that of the glycosides, as a consequence of the much greater lipophilicity of the aglycones.

Enzymology of Anthracycline Redox Activation

There are a number of different redox processes available to the anthracyclines. In order to determine the pathways followed *in vivo*, the identification of the biological molecules which interact with these antibiotics must be made. Since the anthracyclines are absolutely dependent on enzymatic catalysis for their reduction, a particular focus is placed upon those enzymes involved in these events. Unfortunately, it appears characteristic of reductive metabolism that the enzymes that participate are used adventitiously (*71*); that is to say there are no specific "anthracycline reductases" but rather enzymes whose catalytic capacity is usurped, and diverted to electron transfer to the oxidant. In these circumstances it is not possible to identify the enzyme(s), but rather only to make an educated determination on the basis of relative organelle ability, and relative enzyme availability within the organelle. Progress with respect to the anthracyclines continues, and a summary of the current knowledge of the enzymology associated with the biological activities of the anthracyclines may be provided.

Many of these studies have centered on the heart. This is the obvious consequence of the fact that the cardiotoxicity associated with anthracycline administration is therapy limiting, and the probable association of redox activity to this cardiotoxicity. Doroshow (*72*), beginning with the observation that O_2 consumption (and O_2^- production) by heart cell homogenates is significantly enhanced by the presence of Adriamycin, has examined the different characteristics and contributions of the cytosol, sarcosomes, and mitochondria. All catalyze aerobic anthracycline-mediated oxygen reduction. Superoxide production by the sarcosomes requires NADPH, is inhibited by $NADP^+$, and accumulates H_2O_2. This suggests that the reducing equivalents are provided by NADPH:cytochrome P-450 reductase. In the cytosol, oxygen depletion involves NADH and is significantly inhibited by allopurinol, thus implicating xanthine oxidase as the catalyst. NADH is also the reducing agent in the mitochondrion, and anthracycline-mediated O_2 reduction is inhibited by excess NAD^+ or rotenone. These facts implicate the NADH dehydrogenase (although other enzymes cannot be entirely excluded), and indeed Doroshow has provided direct evidence with a purified NADH dehydrogenase preparation (*73*).

Since many of the active antitumor anthracyclines accumulate in the nucleus, and since the nucleus contains the obvious target (but yet unproven target) of the anthracyclines, the DNA; a considerable effort has been put to determining the redox capability of the nucleus. This effort has been rewarded with the isolation of nuclear enzymes by Bachur and colleagues (*74*) capable of anthracycline reduction. Indeed, it is probable that the nucleus is generally capable of drug metabolism (*75*). The particular identity of the nuclear reductase enzyme is as yet unestablished; it may correspond to either or both cytochrome P-450 reductase or xanthine



oxidase as the nucleus has both of these enzymes. Reductive activation of the anthracyclines in the nucleus is therefore a probable event.

A requirement for nuclear anthracycline-metabolizing enzymes is presumed by the anticipated brief lifetime of the activated intermediates (*76*). For good reason, one envisions anthracyline activation at the target site, to avoid the necessity of the intermediate traversing large distances. However, one does not need to have the anthracyclines within the nucleus in order for the nuclear DNA to be damaged. In particular, Nudd and Wilkie (*77*) argue that since the mitochondrion is known to be quite susceptible to drug-mediated damage (perhaps more so than the nucleus), and since it contains DNA also quite susceptible to covalent modification (*78*), that the DNA of the mitochondrion might represent a specific target of anthracyclines. Completing the circumstantial evidence in favor of this hypothesis is the observation that mitochondrial DNA migrates to the nucleus (*79*). Nor is NADH dehydrogenase the only candidate in the mitochondrion for this reaction. Attention is drawn to this enzyme both by its classification as a low potential flavoenzyme (*43*) and by Doroshow's studies (*72,73*); however, the observation that low potential iron-sulfur centers are chemically competent anthracycline reductants (*18*) opens the range of possibilities to other enzymes. While it is possible that these may be minor contributors to the overall reduced oxygen flux, the inhibition of these enzymes if specific and potent, may contribute to cytotoxicity. It is noted in passing that nitroimidazole activation as an antiparasitic antibiotic occurs at low potential iron-sulfur centers (*80,81*). In many respects, the overall features of this hypothesis are reminiscent of Folker's hypotheses concerning the relationship of mitochondrial injury to the acute toxicity of anthracyclines (*82*). Thus, the stage for anthracycline reductive activation in these organelles is set, with the nucleic acids and membranes plausible recipients of the consequences.

Oligonucleotides as Anthracycline Targets

The relationship between the anthracyclines and nucleic acids is one that has become less distinct with time, as the measure of its complexity has evolved (*5*). As it now stands, this relationship consists of two known parts; the first involving the consequences of nonredox associated processes (primarily derived subsequent to intercalation) and the second involving the consequences of redox processes, also following DNA association (but not necessarily requiring intercalation).

Intercalation and Related Phenomena

An ability of anthracyclines to intercalate DNA was one of the first biological properties established; and for some period of time thereafter the correlation between antitumor potency and intercalation was presumed to be a requisite for antitumor ability (*1*). With the biological evaluation of new anthracyclines, found to retain antitumor activity yet with diminished affinity for DNA, the situation is now much less certain (*83*), although it cannot be disputed that within the Adriamycin family an excellent correlation exists between antibiotic efficacy and DNA affinity. There are however anthracyclines that are active; and inhibit DNA and RNA synthesis near equally (Adriamycin), or RNA preferentially (aclacinomycin), or poorly inhibit either (menogarol).

As befits their present importance in chemotherapy, Adriamycin and daunomycin have been most extensively examined with respect to DNA binding. There is general agreement that they behave as "classic" intercalators, binding through the minor groove with the BC rings lying skew to the base pairs, and the C-7 amino sugar resting well within the minor groove (*84-87*). Depending on conditions, the binding may occur cooperatively or not (*88*); and there is a distinct preference for B rather than Z DNA (*89*). Although early studies suggested a GC base pair preference, there are recent studies indicating little apparent sequence specificity (*90,91*). In this regard however, a caution must be placed upon the inference of the true biological locus from mere binding data (*92*). There is little doubt that daunomycin binding to nucleosomes is rather different than to free DNA; Chaires et al. (*93*) suggest a preference of daunomycin for genetically active DNA regions having less nucleosomal structure. In any case, DNA affinity may be virtually abolished by *N*-acylation of daunosamine with retention of antitumor activity (*94,95*). (Whether or not these are mere pro-drugs, liberating the free aminoglycoside by acylase-catalyzed hydrolysis, remains uncertain). These observations again underscore the difficulty in separating the important from the extraneous observations while the source for the molecular expression of anthracycline activity remains unknown. Given only our present knowledge of the plasticity of the DNA helix (*96–99*), we may reasonably inquire if any *in vitro* system is truly appropriate to these questions.

The 11-unsubstituted anthracyclines (such as aclacinomycin and marcellomycin) by all indications bind to DNA in a fashion quite similar to daunomycin (*83,85,100*). The spectral and fluorometric changes are quite analogous, and are consistent with intercalation. The geometry of the complex is also likely to be similar to daunomycin (*85,101*). Similar to the apparent lack of a base composition preference for daunomycin, there is little evidence for a base composition preference for these anthracyclines. Nogalamycin poses a particular problem as it also intercalates, in spite of the two glycosidic rings (one at each end) that are too sterically demanding to permit intercalation during normal DNA breaking. It exhibits a strict AT preference, and Collier et al. (*102*) reasonably surmise that local melting of the DNA is required for the intercalation process. Simulation of the structure of the intercalated complex places the nogalose ring snugly within the minor groove

(*22,102*), and provides a structural explanation for the pronounced decrease in DNA affinity observed for those nogalamycin analogs lacking it as a substituent. The curious feature within the nogalamycin family is that optimization of antitumor activity coincides with a substantial decrease in DNA affinity; menogarol lacks the C-10 carbomethoxy and the C-7 nogalose (but does contain a 7*R*-methoxyl) (*23,24*). Thus neither DNA affinity nor RNA synthesis inhibition correlate well with tumor cytotoxicity within the nogalamycin family; and data from the other families as well suggest this to be an even more general statement. The present understanding of the anthracycline structure-activity relationship does not demand intercalation as a necessary part of the tumor cytotoxicity.

A development that has justifiably attracted considerable attention—as its implications extend beyond the anthracyclines—is the effect of DNA intercalation on the mammalian topoisomerase II enzyme. In addition, this development may address the difficulty discussed in the preceding paragraph. Following the seminal observations of Kohn, Ross and colleagues (*103,104*) using the alkaline elution technique to monitor DNA integrity (*105*), the presence of single and double strand DNA breaks was found to be a consequence of both an intercalating agent (those that are effective include Adriamycin, 5-iminodaunorubicin, ellipticine and *m*-AMSA) and covalently bound protein at the cleavage site. The identity of this protein as the topoisomerase II is confirmed by several research groups (*104,106–111*). The role of this enzyme, summarized from these publications, in producing the DNA strand breaks is as follows. Topoisomerase II catalyzes the crossing of two double-stranded DNA segments via the generation of transient double-stranded DNA breaks involving covalent bond formation between a tyrosine of the enzyme and the DNA strand. It is an enzyme activity that is presumed to parallel transcriptional activity, and hence tumor cells might remain more sensitive to the inhibition of this enzyme's activity. Exposure to DNA, containing certain intercalated molecules, results in the formation of a stable enzyme-DNA complex, trapped at the covalent intermediate state. Denaturation then unmasks this complex—retaining the now-unfolded enzyme at the strand end—providing a lesion that appears upon *in vitro* analysis as either a single or double strand break. Likewise, removal of the intercalator without denaturation restores enzyme activity as evidenced by the resealing of the strand. On the surface, the above biochemistry is appealing in that it presents at the molecular level and in a unique fashion the antitumor antibiotic as a molecule specifically targeted to the DNA. On even slightly greater scrutiny however the complexity that must rest within this apparently straightforward process emerges, as the rationale for this event is as yet absent. Certainly not all intercalators are antitumor antibiotics, and it is necessary to correlate those that are with their relative efficacy in order to properly relate this phenomenon as a molecular event to the overall biological properties observed. There can now be no doubt that the actual details of the stabilization of the topoisomerase-intercalator DNA complex are very much influenced by the intercalator structure (*106–111*). This is most clearly evidenced by comparison of the *ortho* and *meta* AMSA isomers, which possess equivalent intercalative ability yet only the *meta* isomer possesses potent cytotoxic activity and topoisomerase inhibition (*110*). They differ also in sequence specificity (*110*), and in their single- to double-strand break ratios (*107*). Two possibilities exist for the topoisomerase-DNA complex stabilization by the intercalator. In the first, the conformational deformation in the DNA in response to intercalation is expressed in the complex stabilization; or alternatively a more subtle relationship exists between the antibiotic and the topoisomerase, perhaps with the antibiotic as an allosteric effector of sorts. Although a decision is premature, the continuing observations favor the latter choice, as argued by Liu and colleagues (*111*). Nonetheless the excellent correlation between cytotoxicity and topoisomerase stabilization within the AMSA and ellipticine families secures this phenomenon as a central focus for antitumor research. Lastly the notion of an allosteric role for the anthracyclines (as distinct from a pure intercalative role) is attractive from the point of view that there are several antitumor anthracyclines with weak *in vitro* DNA affinity that yet retain antitumor ability, such as the *N*-alkylanthracyclines (*94*) and menogarol (*24*). An unrelated yet complementary observation by Israel, Chuang, and colleagues (*94,95*) is that the *N*-alkyladriamycins retain a potent ability to inhibit eukaryotic RNA polymerases; again, this phenomenon cannot involve intercalation. It may follow that elements of the anthracycline structure as seen by the topoisomerase and other enzymes—rather than by the DNA as expressed by intercalation—define the cytotoxic relationship with DNA.

Implicit in the above discussion is that the topoisomerase inhibition is a nonredox-controlled event. This is directly confirmed experimentally, by the lack of a requirement for a reducing agent in the duplication of this event *in vitro*, and by the relative efficacy of 5-iminodaunorubicin. This analog is poorly reduced, yet retains an excellent ability to stabilize the topoisomerase-DNA cleavable complex. Whether intercalation and topoisomerase inhibition are themselves sufficient to account for all anthracycline-mediated DNA damage is by no means certain. In particular, there remains the possiblity that the redox properties enumerated in the above sections permit DNA damage. This may occur by either of two circumstances; first where aerobic redox cycling leads to oxygen-radical mediated DNA damage, and the second where anaerobic activation to a quinone methide, proximal to a susceptible functional group of the DNA, enables covalent modification to occur. Unfortunately, evidence of only the most circumstantial sort may be brought forth in support of either hypothesis. The proper relationship of the two possible redox phenomena is therefore uncertain. A summary of their present status is provided.

Anthracycline-Mediated Damage to Oligonucleotides

Based upon Lown and colleagues' observation (*112*) that aerobic anthracycline reduction resulted in DNA cleavage and their own extensive studies of anthracycline metabolism, Bachur and colleagues proposed (*113*) that the cytotoxic expression of the anthracyclines derived from the site specific generation of free radicals targeted to the DNA. Under aerobic conditions, these radicals could generate $HO^{\cdot}$ from the H_2O_2 produced concurrently, while anaerobically covalent labeling by the semiquinone-derived quinone methide was possible. The essentials of part of this proposal are supported by several experimental studies confirming the aerobic sensitivity of DNA to reductively activated anthracyclines. Berlin and Haseltine (*90*) observed that an excess of Adriamycin relative to DNA, upon enzymatic reductive activation, gave DNA strand scission during turnover (but not afterward) under aerobic conditions (much less scission is observed anaerobically). No sequence specificity is apparent. Examining a series of anthracycline analogs, Lown et al. (*114*) found an excellent correlation between the rate of microsomal reduction and the extent of aerobic degradation. Gutteridge and Toeg (*60*) observe that the efficiency of this reaction increased under "partially" anaerobic conditions, and provide evidence for an absolute metal ion requirement (as was also surmised by Lown et al.). The mechanistic events of this process are not known, but quite likely involve the hydroxyl radical (or its metal-bound equivalent) as a hydrogen atom abstractor. A possible initiation step is hydrogen atom loss from the 2′ or 3′ carbon of the deoxyribose, followed by fragmentation. Should this prove to be the case, the DNA cleavage would be analogous to what is believed to occur for bleomycin (*115*). Other perspectives in metal ion modulation of anthracycline redox processes are discussed previously.

A related issue is the competence of the intercalated anthracycline as an oxidant. There is general agreement that intercalation severely diminishes the yield of the semiquinone from anaerobic enzymatic reduction (*32,90*). However, the inference that anthracyclines cease to behave as oxidants upon intercalation is premature. In particular, the rationalization that since the DNA now limits access or approach to the anthracyclines, they become incapable of electron transfer, is unwarranted. The prosthetic groups of cytochrome c or flavodoxin—just two examples—are well protected by the protein polypeptide, with little loss in their redox capacity. There is no reason to suppose that the anthracyclines will retain or lose redox activity before the experimental data are available. The less intense EPR signal for the semiquinone observed thus far might be a consequence of the anthracycline being unavailable due to steric reasons; or as well to an effect of the DNA on the two one electron potentials to favor even more overall two electron reduction. Further, the number of enzymatic and chemical reducing agents thus far examined is small. It is conceivable that certain enzymes are more capable than others, given a situation where perhaps the anthracycline intercalated to the mitochondrial DNA is more available for redox processes than the DNA in the nucleus. Youngman and Elstner (*116*) have presented evidence for O_2^- as a competent intercalated anthracycline reducing agent. In brief, there is simply too little experimental data concerned with the kinetic and chemical consequences of intercalation with respect to the anthracycline redox processes, to make any substantial conclusion for either aerobic or anaerobic events.

The only present evidence for covalent labeling of the DNA by anthracyclines is circumstantial, deriving from Sinha's studies (*117,118*) conducted under anaerobic conditions using both chemical and enzymatic reduction. Following anthracycline activation, the DNA is extensively purified by high salt extraction and ethanol precipitation, conditions which should remove all drug that is merely intercalated. Under these conditions, some of the anthracycline is retained, presumably covalently bound somewhere within the DNA. Significantly lower yields are obtained using the (7-hydroxy)aglycones, that are unable to intercalate. Similar experiments are reported for protein (*119*), that also purport to establish covalent labeling. Possible candidates for the reactive intermediate are the semiquinone; the seimquinone-derived quinone methide; or the hydroquinone-derived methide. As is discussed previously, only the latter is an attractive candidate in terms of established chemical competence, and its bimolecular reactivity as either an electrophile or nucleophile is low. Whether the association to DNA improves the situation (covalent reaction at C-7 thus becomes in effect an intramolecular reaction) is an open question. In brief, in spite of the extensive literature presuming a kinetic and chemical competence for anthracyclines as reductively activated pro-drugs targeted to the DNA, the available experimental evidence is meager and incomplete. There is nowhere near the level of structural detail as is presently available for mitomycin c (*120*), a quinone antitumor antibiotic that all evidence indicates operates as a bioreductive alkylator toward DNA.

This discussion on the chemical alterations to DNA effected by the anthracyclines concludes with a most interesting observation (*121*). Treatment of DNA containing intercalated Adriamycin with the *uvr*ABC endonuclease, a DNA repair enzyme specific for bulky covalent lesions, results in *no* excision of DNA. However recovery of DNA from *E. coli* bacteria, deficient in this enzyme's activity, that had been exposed to Adriamycin did result in DNA excision. Furthermore the mutant was found exquisitely sensitive to Adriamycin as a toxic agent. These circumstances indicate that the anthracyclines operate upon the DNA *in vivo* in a fashion dissimilar to mere intercalation. Kacinski and Rupp offer as a working hypothesis that a correlation may exist between the antibacterial activity and the extent of DNA damage, and that this correlation may also extend to some aspect of the anthracycline clinical activity. Clearly, this hypothesis is attractive, and will perhaps

become even more fetching when the molecular lesion involved may be described. As it now stands DNA damage via aerobic degradation, covalent labeling by the quinone methide, or even related to the topoisomerase cleavable complex provide reasonable explanations.

The Cellular Membrane as an Anthracycline Target

Membrane Association as a Cytotoxic Event

As is the case for the nucleic acids, the cellular membrane is also a potential target of both nonredox and redox anthracycline processes. This section considers the present status of the former. The rationale for a search beyond nucleic acids—and to the membrane—as the cytotoxic target for the anthracyclines is succinctly defended by Tritton and colleagues (*122*). Arguing that the absence of a correlation between the cytotoxic activity of the anthracyclines and either DNA affinity or damage demands that other possibilities be considered, they identify three criteria by which the cell membrane may be established as a drug target. The first criterion is that the drug interact with the membrane; the second that it alter the membrane's properties; and the third that this alteration be necessary and sufficient for cell cytotoxicity. There is a multitude of evidence attesting to the ability of the anthracyclines with respect to the first two criteria (*123*). A strong association of anthracyclines to membranes having lipids with acidic head groups is well proven (*124*). Further, an array of membrane properties are found altered by the presence of anthracyclines. These include phospholipid structure, glycoprotein synthesis, the transport of small molecules and ions, and membrane fluidity. With respect to membrane fluidity, an attempt has been made to correlate it with the relative amount of oxygen present, and with the development of drug resistance (*125*). Under hypoxic conditions, there was an increase in fluidity that did not correlate with differences in membrane permeability to the anthracyclines. It did, however, seem to relate to an as yet unidentified mechanism of anthracycline resistance. In general, resistant cells display a lowered capacity for anthracycline accumulation which is attributed to an enhanced drug efflux system. (Resistance also does not involve an increased capacity for anthracycline metabolism nor an increased sensitivity toward oxygen radicals) (*126*). Yee et al. (*127*) have attempted to locate an anthracycline membrane receptor by photolytic labeling. Labeling was equally successful under conditions where the drug was able and was not able to enter the cell, indicating a strong membrane interaction. The photolytic coupling is to a protein, as the bound drug is freed upon treatment with a protease. However the yield of photolytic labeling was neither specific nor saturable, did not occur at the drug transport locus, and did not affect the cell viability. Since the yield did increase in direct proportion to the cell's level of drug resistance, the protein that is labeled may be associated with the resistance process, with the membrane property most likely affected being fluidity. Thus association of anthracyclines and the alteration of membrane properties is proven. The fulfillment of the third criterion is quite a bit more problematical.

The experimental approach that has been used to separate the cell membrane activities from the intracellular processes is the use of anthracyclines covalently immobilized to polymeric microspheres (*128,129*). Provided that the drug is truly immobilized, this prevents the anthracycline from being internalized by the cell. It appears that loss of the drug from the microsphere does not occur, and that the ensuing cytotoxic events that are observed correspond to a membrane-associated cytotoxic event. At equivalent concentrations, the polymer-bound anthracyclines are equally cytotoxic as the free drug. Following exposure of the cell to polymer-bound anthracycline, cell-surface microvilli are lost and replaced with blebs; holes appear in the membrane concomitant with cell death (*130*). A reasonable question, however, is the relevance of this cytotoxic process to that produced by the free drug, which will not necessarily remain immobile at the cell membrane. The answer here is unavailable. As an illustration of the complexity and peculiarity of this overall process, a most interesting experiment by Rogers and Tokes (*131*) is offered in point. It is found that an anthracycline diastereomer (7*R*,9*R*-4-demethoxydaunorubicin) that is totally inactive as the free drug, displays potent cytotoxicity when immobilized to the polymer support. While this underscores the role of the membrane in any hypothesis for anthracycline cytotoxicity, the experiment is difficult to rationalize because of the very appreciable sensitivity (with respect to anthracycline structure) of cell cytotoxicity *in vivo*. In closing, attention is called to some recent developments concerning a possible effect of drugs in general (*132*), and anthracyclines in particular (*133–135*), on the Ca^{+2} balance. This process may influence both cell toxicity and cell resistance to the anthracyclines.

From another perspective the cell membrane is not a target but an obstacle. If the cytotoxic target of the anthracycline rests within the cell, then the membrane must be circumvented for the drug to attain access. As different cells have different receptors, a drug delivery system that takes advantage of a unique difference between the neoplastic and normal cell would achieve both specificity and increased potency. Unfortunately as yet this difference must be sought empirically. Among the carriers so far investigated are low-density lipoproteins (*136,137*), lysosomal proteins (*138*), antibodies and immunoglobin (*139,140*), and anionic liposomes (*141*). While some of these complexes do improve activity, this may be a consequence of any number of possibilities and not necessarily to more selective targeting.

Cardiotoxicity as a Consequence of Anthracycline-Induced Lipid Peroxidation

The ability of the anthracyclines to divert electrons from low potential flavin- and metal-containing enzymes to oxygen, yielding O_2^- and H_2O_2, and ultimately the hydroxyl radical, suggests lipid peroxidation as a potentially deleterious physiological result (*6,142*). Recent evidence points to a particular contribution of this process to the cumulative cardiomyopathy, rather than to the antitumor activity (*6,47,72,142,143*). The enhancement of lipid peroxide concentration following anthracycline administration is readily demonstrated, as discussed by Thayer (*144*), who observes a dose and time dependent rise in serum neutral lipid peroxides with Adriamycin in rats. There are further data indicating an identical effect to the heart (*145,146*), under dosages which are sufficient to develop the cardiomyopathy. That this phenomenon appears in the heart, rather than in the liver—which is more active in anthracycline reductive metabolism—is believed to reflect the heart as a tissue particularly susceptible to this reaction and its consequences. The heart has a high rate of respiration and is well oxygenated. This provides opportunity. Furthermore, the relative ability of the heart to defend itself against oxygen reduction may be poorer than other tissues. It contains much lower levels of catalase and superoxide dismutase than found in the liver (*6,71*), and while the levels of the third enzyme, glutathione peroxidase, is identical to that in the liver, anthracycline administration depresses its activity. This may occur by an inadequate mobilization of selenium for this enzyme (*147*). The overall situation may be exacerbated by additional circumstances. The steady state levels of both NADPH and glutathione may fall as a consequence of the futile oxygen reduction, leading to a further deterioration in reduced oxygen detoxification. The abundance of metal and heme proteins and enzymes may also aggravate this situation by either direct metal chelation to the anthracycline or iron-heme catalyzed oxygen activation. A situation is envisioned where the heart's intrinsic ability to detoxify the reduced oxygen species is overwhelmed, at which time lipid peroxidation occurs on a scale sufficient to adversely effect the heart's performance. Together, these observations and conjectures are reasonably consistent with the delayed yet cumulative dose-dependent nature of the heart damage by the anthracyclines.

The direct involvement of lipid peroxidation in the cardiac damage is substantiated by other experimental approaches. Anthracyclines with a diminished redox capacity (such as 5-iminodaunorubicin) or an inability to chelate metals (such as 11-deoxyanthracyclines) appear *in vitro* as less cardiotoxic agents. Interestingly, this frequently coincides with a loss in antitumor potency. In contrast anthracyclines with an enhanced redox capacity (such as carminomycin or 4-demethoxydaunorubicin) are capable of enhanced lipid peroxidation and cardiotoxicity (*148,149*). A potential benefit of antioxidants may be to augment the heart's capacity in controlling free radical damage. This is well established in *in vitro* and animal studies (*6,150*), although no successful clinical strategy for minimizing the cardiotoxicity has yet to evolve. Additional studies concerned with the effect of a deliberate abolition of anthracycline redox activity (discussed below in the concluding section) are also consistent with the cardiotoxicity as an oxygen-dependent redox process. Yet, at present, the final proof remains absent. Even rather straightforward *in vitro* experiments offer puzzles not reconcilable with simple chemistry (*148*). As an example of the degree of complexity posed by this phenomenon, the developments with mitoxantrone are offered. This nonanthracycline anthraquinone antitumor antibiotic is clearly redox-impaired relative to Adriamycin (*151*), and, indeed, early studies indicated a decreased cardiotoxicity. More recently however the situation has blurred, and the possibility is presented that this antitumor antibiotic is as cardiotoxic as the anthracyclines (*152,153*). Once again, the difficulty in connecting chemical observation to physiological consequence is emphasized. We may conclude however that the sum of the available evidence supports lipid peroxidation as the cardiotoxic event, and that it is the proper relationship of lipid peroxidation as a cause and the cardiotoxicity as an effect that now merits closer attention.

Envoi

Relation of Redox Activity to the Antitumor Activity

The urgency behind the study of anthracycline chemistry derives, of course, from their antitumor efficacy. Their capacity as redox agents may, in the proper circumstances, be exerted against both cell membrane and nucleic acids, and, as likely, selected enzymes as well. Given their extraordinary potency, it is probable that only one of these targets *in vivo* suffices to destroy the neoplastic cell in a precise and methodical fashion. It is therefore with a discordant note of resignation that this review must contain the statement that neither the cytotoxic event, nor the relationship of the redox activity, has been properly placed into the context of the anthracyclines as antitumor antibiotics.

Yet new experimental approaches are emerging to attack this conundrum, drawn from the reservoir of basic chemical and biochemical knowledge now accumulated from the studies summarized in the review. In particular, there is preliminary progress indicating that catalytic redox turnover contributes a significantly greater extent to the host cytotoxicity, rather than to the antitumor activity. Several different yet conceptually related studies bear on this point. An examination of anthracycline-sensitive tumor cells suggests that neither the presence nor absence of oxygen is a component of the tumor sensitivity to the anthracycline (*154,155*). The presumption to be drawn is that the tumor cytotoxicity does not involve any redox event. On the pre-

sumption that xanthine oxidase contributes significantly to anthracycline metabolism and so serves to diminish the anthracycline titer with respect to the antitumor locus, Schwartz (*68,156*) has examined the consequences of the co-administration of a xanthine oxidase inhibitor upon survival times of animals having L1210 leukemia. Significant increases in the survival time are observed. The simplest interpretations of this effect are that anthracycline reductive activation is at best a chemically innocuous event that causes a decrease in the anthracycline potency at the antitumor locus; and at worst is a chemically damaging event that may be forestalled as the anthracycline is diverted to its proper place. Likewise, Banks et al. (*157*), recognizing the specific and efficient reduction of anthracyclines by the trimethylmorpholinyl radical, observe that the prompt administration of the precursor to this radical, subsequent to an otherwise lethal dose of Adriamycin, rescues the animal from death. This may be done without interference with the antitumor activity (T. Koch, unpublished data). This simple experiment holds the promise that strategies based upon minimizing catalytic redox activity due to the anthracycline's presence may favorably alter the therapeutic ratio. This same conclusion may be drawn from a study by Hrushesky, Eaton, and colleagues (*158*). On the premise that a transient reduction in NADPH levels might forestall toxic catalytic redox cycling while preserving the anthracycline for the antitumor locus, co-administration of methylene blue with Adriamycin in L1210 inoculated mice was examined. A significant reduction in the Adriamycin lethality is found, without any effect on the antineoplastic activity. Together, these three studies suggest that the host toxicity and the antitumor activity are dissociable processes, and offer an experimental basis for further effort to vindicate this claim.

Whether the antitumor locus is found at the nucleic acids, the membranes, or in a specific redox-controlled event remains unknown. What is clear is that the study of the basic chemical and biochemical principles by which the anthracyclines are limited, has been rewarded with the development of experimental hypotheses which allow a rational manipulation of the drugs' biological activity. It is in this simple fact that all who have labored in the anthracycline field may take comfort.

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