Absence of Strand Breaks in Deoxyribonucleic Acid Treated with Metronidazole

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The deoxyribonucleic acid (DNA)-degrading potential of metronidazole was evaluated *in vitro* by three techniques: determination of melting curve, measurement of viscosity, and centrifugation in neutral or alkaline sucrose gradients. Studies were performed on calf thymus DNA and on ³H-labeled or unlabeled pneumococcal and T7 phage DNA after treatment with metronidazole alone or metronidazole reduced by sodium dithionite in the presence of DNA. This latter process is known to elicit covalent binding of metronidazole to DNA. Reduced or unreduced metronidazole had no effect on the melting properties, viscosity, or sedimentation velocity of the nucleic acids studied. Sodium dithionite alone, however, caused a 25% decrease in the intrinsic viscosity of pneumococcal DNA, and decreased the sedimentation velocity of pneumococcal and T7 phage DNA in both neutral and alkaline sucrose gradients. These data suggest that degradation of DNA is not important in the interaction of metronidazole with nucleic acids, an interaction assumed relevant to the cytotoxic, radiosensitizing, and mutagenic activities of this compound.

Metronidazole [Flagyl; 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] is cytotoxic to anaerobic microorganisms (5, 10), radiosensitizing for anaerobic mammalian tumor cells (8), and mutagenic in certain bacteria (16, 18, 30, 33). Although the mechanism of these effects is unclear, reduction of the nitro group is a common prerequisite (8, 18, 25, 30), and interaction with deoxyribonucleic acid (DNA) is a suggested mechanism of action (14, 27, 35). We have reported that metronidazole reduced by sodium dithionite *in vitro* covalently binds to guanine and cytosine of mammalian, bacterial, and phage DNA (15a). Edwards (4) has reported that metronidazole reduced by sodium dithionite *in vitro* alters the melting profile and sedimentation coefficient of calf thymus and T2 phage DNA, concluding that the reduced drug causes single- and double-strand breaks in these polynucleotides. Either or both of these two modes of drug-nucleic acid interaction (i.e., covalent binding to DNA or DNA degradation) could theoretically account for the major actions of metronidazole *in vivo*.

In studies presented here, we observed no alteration in the melting profile, viscosity, and sedimentation rates in neutral or alkaline sucrose gradients of mammalian, bacterial, or phage DNA exposed to metronidazole *in vitro*

under conditions known to elicit covalent binding of this drug to nucleic acids.

MATERIALS AND METHODS

Materials. Calf thymus DNA with an average molecular weight of 6×10^6 to 7×10^6 was obtained commercially from General Biochemicals (Chagrin Falls, Ohio) and purified by precipitation and repeated deproteinization by chloroform (22). DNA from a wild-type strain of *Diplococcus pneumoniae* (R36A) was prepared as previously described (13). Pneumococcal [³H]DNA was prepared by biosynthetic labeling: a thymidine-requiring and streptomycin-resistant mutant (TVS₁) of pneumococcus (31) was grown in synthetic medium supplemented with 1.2 μ g and 100 μ Ci of [³H]thymidine per ml. The specific activities of DNA preparations were 4×10^5 to 5×10^5 dpm per μ g of DNA. T7 phage [³H]DNA was also prepared by biosynthetic labeling as above. T7 phage DNA was isolated by a modification of the method of Marmur (22) in which phenol was used after phage purification by CsCl density centrifugation. T7 phage [³H]DNA thus prepared had a specific activity of approximately 10^5 dpm per μ g of DNA.

Metronidazole (molecular weight, 171) and [¹⁴C]metronidazole {1-(2-[U-¹⁴C]hydroxyethyl)-2-methyl-5-nitroimidazole} were kindly supplied by Searle Laboratories (Chicago, Ill.). The latter had a specific activity of 17 μ Ci/mg. Metronidazole has a molar extinction coefficient of $\epsilon_{320} = 9,310$ (35).

Solutions of sodium dithionite (powder purified, Fisher Scientific Co., Springfield, N. J.) were carefully prepared in double-distilled water under strict anaerobic conditions immediately before use.

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Melting temperature measurements. DNA melting and cooling curves were measured with a Gilford (model 240, Gilford Instrument Laboratories, Inc., Oberlin, Ohio) temperature-controlled recording spectrophotometer as described by Iyer and Szybalski (15). The precision of this technique in our hands is approximately 4%.

Viscosity measurements. Viscosity measurements were made at 25°C with a Zimm-Crothers rotating apparatus (model A, Beckman Instruments, Inc., Fullerton, Calif.) as described (2, 36), using samples dialyzed overnight at 4°C into 200 volumes of a solution of 15 mM sodium chloride and 2 mM citric acid, pH 7.4, containing 1 mM ethylenediaminetetraacetate (EDTA). The precision of this technique in our hands is approximately 5%.

Sucrose gradient centrifugation measurements. Linear 5 to 20% neutral and alkaline sucrose gradients were prepared immediately before centrifugation. Neutral sucrose gradients contained 900 mM NaCl-10 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.5)-1 mM EDTA. Alkaline sucrose gradients contained 700 mM NaCl-0.3 N NaOH-1 mM EDTA, and the pH was adjusted to approximately 12 immediately before use. A total of 100 to 200 μ l of sample was layered on a 4.6-ml gradient in 5-ml polyallomer tubes, and the tubes were centrifuged at 20°C in a Beckman L3-50 ultracentrifuge with an SW 50.1 swinging-bucket rotor. In experiments with pneumococcal [3 H]DNA, the samples were diluted five times with 15 mM sodium chloride and 2 mM citric acid, pH 7.4, before layering on neutral sucrose gradients and centrifuged for 180 min at 35,000 rpm. Samples were layered directly on alkaline sucrose gradients and centrifuged for 195 min at 39,000 rpm. In experiments with T7 phage [3 H]DNA, 100- to 200- μ l samples were layered directly on neutral and alkaline sucrose gradients and centrifuged for 170 min at 44,000 rpm.

After centrifugation, the bottoms of the polyallomer tubes were punctured, and 8-drop fractions were collected directly into 38 to 40 liquid scintillation counting vials (Wheaton Scientific, Millville, N. J.). To each vial 0.4 ml of distilled water and 5 ml of Beckman Ready-Solv HP liquid scintillant were added, and radioactivity was measured by liquid scintillation spectrometry. The precision of this technique in our hands is approximately 2%.

Binding of [14 C]metronidazole to DNA. The binding of [14 C]metronidazole to native calf thymus and pneumococcal DNA was tested as described (15a, 32).

Experimental procedure. Since our previous studies indicated that binding of metronidazole was affected by the type of nucleic acid, the drug-to-DNA ratio in the reaction mixture, and the ionic composition of the reaction buffer, these factors were varied in the present study. On this basis, four experimental procedures were used.

Method 1. The first method involved the same experimental conditions as reported by Edwards (4): 2 μ mol of metronidazole was reduced by exactly 5 μ mol of sodium dithionite in the presence of 6 μ mol (moles of mononucleotide) of native calf thy-

mus DNA (1:3, drug-DNA molar ratio) for 1 h at 25°C in 1.5 mM HCl-150 μ M trisodium citrate, pH 7.0. Anaerobic conditions were maintained by continuous bubbling of He gas (chromatographic grade, Union Carbide, Keasby, N. J.) into the solution. The reaction mixture was then opened to room air and immediately dialyzed at 4°C for 48 h against 333 volumes of 1.5 mM NaCl-15 μ M trisodium citrate, pH 7.0, changed three times. A sample containing DNA alone was prepared as a control in an identical fashion. Melting curve and viscosity measurements were then performed on portions of the samples as described.

Method 2. The second procedure differed from Method 1 in the following manner: the drug-to-calf thymus DNA molar ratio was increased from 1:3 to 2:1; the ionic composition of the buffer was 15 mM NaCl-2 mM citric acid, pH 7.4; and separation of DNA from excess metronidazole was performed by chromatography on Sephadex G-100 (32). Control samples included DNA alone, DNA exposed to unreduced metronidazole, and DNA exposed to sodium dithionite. Melting curve and viscosity experiments were performed on all samples after dialysis, as described above.

Method 3. A total of 1 μ mol of pneumococcal [3 H]DNA was mixed with 2 μ mol of metronidazole in 1.5 ml of 17 mM phosphate buffer, pH 7.4; 5 μ mol of sodium dithionite was added, and the solution was incubated anaerobically for 45 min at 25°C. Control samples included DNA alone, DNA exposed to unreduced metronidazole, and DNA exposed to sodium dithionite. Viscosity and neutral and alkaline sucrose gradient centrifugation measurements were made on all samples as described.

Method 4. The fourth method was identical to method 3, except T7 phage [3 H]DNA was used, and the drug-to-DNA molar ratio in the reaction mixture was increased to 500:1. Control samples also included DNA alone, DNA exposed to unreduced metronidazole, and DNA exposed to sodium dithionite. Neutral and alkaline sucrose gradient centrifugation measurements were made on all samples as described.

RESULTS

Melting curve measurements. Figure 1 shows the melting curves of native calf thymus DNA alone and after exposure to dithionite-reduced metronidazole, as described in methods 1 and 2. Dithionite-reduced metronidazole had no effect on the melting temperature, transition profile, or cooling curve of DNA at either drug-to-DNA molar ratio used. Unreduced metronidazole also had no effect on the parameters measured (data not shown). Data obtained with method 2 (Fig. 1B) have been previously published (15a) but are included here for comparison.

Viscosity measurements. Figure 2 shows results of viscosity measurements on calf thymus and pneumococcal DNA. As with melting temperature determinations on the same sam-

FIG. 1. Melting curves of calf thymus DNA. (A) Curves of DNA alone (—) and after exposure to dithionite-reduced metronidazole (---) in 1.5 mM NaCl-0.15 mM trisodium citrate, pH 7.0. Conditions of experiment as described in method 1. (B) Curves of DNA alone (—) and after exposure to dithionite-reduced metronidazole (---) in 15 mM NaCl-2 mM citric acid, pH 7.4. Conditions of experiment as described in method 2.

FIG. 2. Viscosity of pneumococcal and calf thymus DNA. For pneumococcal DNA, measurements were made on DNA alone (●) and after exposure of DNA to metronidazole (○), dithionite-reduced metronidazole (▲), or dithionite alone (△), as described in method 3. For calf thymus DNA, measurements were made on DNA alone (●) and after exposure to metronidazole (○), dithionite-reduced metronidazole (▲), or dithionite alone (△), as described in method 1 (—) and method 2 (---).

ples, the viscosity of native calf thymus DNA was unaffected by dithionite-reduced or unreduced metronidazole at either (1:3 or 2:1) drug-to-DNA molar ratio. Sodium dithionite alone

also had no effect. Similarly, dithionite-reduced or unreduced metronidazole did not affect the viscosity of pneumococcal DNA. Sodium dithionite alone, however, caused a 25% decrease in the intrinsic viscosity of pneumococcal DNA.

Sucrose gradient centrifugation. Figures 3 and 4 show results of neutral and alkaline sucrose gradient centrifugation studies for pneumococcal and T7 phage [^3H]DNA. As with viscosity determinations on the same samples, neither unreduced nor dithionite-reduced metronidazole had any effect on the sedimentation profile of pneumococcal DNA in neutral or alkaline gradients. Paralleling the viscosity studies, sodium dithionite alone caused pneumococcal DNA to sediment more slowly in both neutral and alkaline gradients. Figure 4 shows that the sedimentation profile of T7 phage DNA was also unaffected by unreduced or reduced metronidazole on either gradient. Dithionite alone, however, as with pneumococcal DNA, caused a shift in the sedimentation profile of T7 phage DNA, which was most prominent on the alkaline gradient.

FIG. 3. Neutral (A) and alkaline (B) sucrose gradient centrifugation of pneumococcal [^3H]DNA. Measurements were made on DNA alone (—), and after exposure of DNA to metronidazole (.....), dithionite-reduced metronidazole (---), or dithionite alone (-.-.-), as described in method 3.

FIG. 4. Neutral (A) and alkaline (B) sucrose gradient centrifugation of T7 phage [^3H]DNA. Measurements were made on DNA alone (—) and after exposure of DNA to metronidazole (·····), dithionite-reduced metronidazole (---), or dithionite alone (- · - · -), as described in method 4.

Binding of [^{14}C]metronidazole to DNA. [^{14}C]metronidazole bound to native calf thymus DNA when the drug was reduced by sodium dithionite in the presence of DNA as previously reported (15a). The binding ratio (molecules of drug per 10^3 nucleotide units) was 0.73. Under similar conditions, [^{14}C]metronidazole bound to pneumococcal DNA with a binding ratio of 0.60.

DISCUSSION

The major findings of these studies are that metronidazole does not affect the melting temperature, intrinsic viscosity, or sedimentation velocity of mammalian, bacterial, and phage DNA. This lack of effect persists even after reduction of metronidazole by sodium dithionite, a process shown *in vitro* to elicit covalent binding of the drug to native and denatured DNA, ribonucleic acid, and synthetic polynucleotides. Though large numbers of chemical agents are presumed to exert their major biological effects by inducing single- and/or double-strand breaks in DNA (i.e., streptonigrin and its analogs, nitrofurans, bleomycin) (1, 11,

19, 20, 23, 26, 34), other substances (cross-linking agents such as mitomycin C, intercalating agents such as daunomycin or acridine dyes) bind to DNA without affecting its integrity (17, 32, 37). Results presented here, as well as data previously published, indicate that reductively activated metronidazole covalently binds to DNA but does not degrade it. Additional studies are warranted to determine the exact mode of binding of reductively activated metronidazole to DNA, to identify the activated species of metronidazole actually bound, and to explore other possible types of metronidazole-nucleic acid interaction, such as free radical modification of individual bases (7).

These data fail to confirm those of Edwards, who reported that dithionite-reduced metronidazole caused single- and double-strand breaks in T2 phage and calf thymus DNA (4). We chose to use viscometry rather than sucrose gradient centrifugation to evaluate the effect of metronidazole on calf thymus DNA, since this molecule is rather non-homogeneous. The validity of the viscometry measurements is supported by the agreement between our data and published values (Table 1). In addition, the decreased intrinsic viscosity of pneumococcal DNA observed with dithionite alone demonstrates that our system detected changes in viscosity of the solutions studied when they occurred. Nevertheless, even employing identical methods (i.e., melting curve measurements) and reaction conditions (type of DNA, drug-to-DNA molar ratio, incubation time, reduction agent and procedure, ionic strength, etc.), our results were still negative. Thus, differences in procedures are not likely to account for the different results from those of Edwards (4), and we are unable to explain the differences observed.

The validity of the negative results with mammalian DNA was supported by consistently negative results both employing DNA from other sources (i.e., bacterium and phage) under various reaction conditions, as well as using the additional technique of sucrose gradient centrifugation. At a drug-to-pneumococcal DNA molar ratio of 2:1, we were unable to demonstrate any drug effect by either viscometric measurements or neutral and alkaline sucrose gradient centrifugation of the same samples. Even at a drug-to-T7 phage DNA molar ratio of approximately 500:1, no differences were seen in the sedimentation profiles on neutral or alkaline sucrose gradients between DNA alone or DNA exposed to reduced or unreduced metronidazole. Thus, negative results were found with DNA from three different sources with three different techniques studied

TABLE 1. Experimentally determined and published viscometric data

DNA	Intrinsic viscosity ^a		Mol wt ($\times 10^{-6}$) ^b	
	Measured	Published	Calculated	Published
Calf thymus	43.4	30.7–53.4 (3, 28)	6.7	3.0–6.0
Pneumococcal	71.0	52.0–99.0 (6)	13.5	8.8–21.8 ^c

^a Determined by extrapolation of reduced viscosity values to zero DNA concentration (2).

^b Calculated from intrinsic viscosity data, using the Zimm-Crothers modification of the Mandelkorn-Flory equation (2, 21).

^c Calculated from intrinsic viscosity data of Eigner and Doty (6), using the Zimm-Crothers modification of the Mandelkorn-Flory equation (2, 21).

under various conditions previously shown to promote binding of metronidazole to nucleic acids.

An additional finding of interest was the ability of sodium dithionite alone to decrease the viscosity of pneumococcal DNA and alter the sedimentation velocity on neutral and alkaline sucrose gradients of both pneumococcal and T7 phage DNA. When metronidazole was present, presumably oxidizing dithionite (18), these effects on DNA were abolished. Our results are consistent with the interpretation that, under the conditions of the experiment, sodium dithionite causes single- and double-strand nicks in DNA, and this degradative effect can be prevented if dithionite is oxidized by an electron acceptor such as metronidazole. The 25% decrease in intrinsic viscosity of pneumococcal DNA caused by dithionite agrees well with the results of Cone et al. (1). Using the ethidium bromide fluorescence assay (24), they found that 2 mM dithionite cleaved PM2 covalently closed circular DNA to an extent of 20%. In contrast, Edwards reported that dithionite alone had no effect on the sedimentation velocity of T2 phage DNA on alkaline sucrose gradients (4). The degradation of DNA in vitro by other reducing agents, both chemical compounds such as sodium bisulfite (12) or naturally occurring substances such as cysteine (29), is known. Free radicals derived from oxidation of sodium dithionite could be responsible for its observed degradative effect, since bisulfite probably cleaves DNA via free radical formation (12). Additional studies are warranted to clarify the mechanism of dithionite-induced degradation of DNA.

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LITERATURE CITED

1. Cone, R., S. K. Hasan, J. W. Lown, and A. R. Morgan. 1976. The mechanism of degradation of DNA by streptonigrin. *Can. J. Biochem.* 54:219–223.
2. Crothers, D. M., and B. H. Zimm. 1965. Viscosity and sedimentation of DNA from bacteriophages T₂ and T₇ and the relation to molecular weight. *J. Mol. Biol.* 12:525–536.
3. Doty, P., and B. H. Bunce. 1952. The molecular weight and shape of desoxyribose nucleic acid. *J. Am. Chem. Soc.* 74:5029–5034.
4. Edwards, D. I. 1977. The action of metronidazole on DNA. *J. Antimicrob. Chemother.* 3:43–48.
5. Edwards, D. I., M. Dye, and H. Carne. 1973. The selective toxicity of antimicrobial nitroheterocyclic drugs. *J. Gen. Microbiol.* 76:135–145.
6. Eigner, J., and P. Doty. 1965. The native, denatured and renatured states of deoxyribonucleic acid. *J. Mol. Biol.* 12:549–580.
7. Emerson, P. T. 1972. X-ray damage to DNA and loss of biological function: effect of sensitizing agents, p. 209–270. In M. Burton and J. L. Magee (ed.), *Advances in radiation chemistry*, vol 3. Wiley-Interscience, New York.
8. Foster, J. L., P. J. Conroy, A. J. Searle, and R. L. Wilson. 1976. Metronidazole (Flagyl): characterization as a cytotoxic drug specific for hypoxic tumor cells. *Br. J. Cancer* 33:485–490.
9. Grossman, L., S. L. Levine, and W. S. Allison. 1961. The reaction of formaldehyde with nucleotides and T₂ bacteriophage DNA. *J. Mol. Biol.* 3:47–60.
10. Grunberg, E., and E. Tittsworth. 1974. Chemotherapeutic properties of heterocyclic compounds: monocyclic compounds with five membered rings. *Annu. Rev. Microbiol.* 27:317–347.
11. Haidle, C. 1971. Fragmentation of deoxyribonucleic acid by bleomycin. *Mol. Pharmacol.* 7:645–652.
12. Hayatsu, H., and R. C. Miller. 1972. The cleavage of DNA by the oxygen-dependent reaction of bisulfite. *Biochem. Biophys. Res. Commun.* 46:120–124.
13. Hotchkiss, R. D. 1957. Isolation of sodium deoxyribonucleate in biologically active form from bacteria. *Methods Enzymol.* 3:692–696.
14. Ings, R. M. J., J. A. McFadzean, and W. E. Ormerod. 1974. The mode of action of metronidazole in *Trichomonas vaginalis* and other microorganisms. *Biochem. Pharmacol.* 23:1421–1429.
15. Iyer, V. N., and W. Szybalski. 1964. Mitomycin and porfiromycin: chemical mechanism of activation and cross-linking of DNA. *Science* 145:55–58.
- 15a. LaRusso, N. F., M. Tomasz, M. Müller, and R. Lipman. 1977. Interaction of metronidazole with nucleic acids *in vitro*. *Mol. Pharmacol.* 13:872–882.

16. Legator, M. S., T. H. Connor, and M. Stoeckel. 1975. Detection of mutagenic activity of metronidazole and niridazole in body fluids of humans and mice. *Science* 188:1118-1119.
17. Lerman, L. S. 1961. Structural considerations in the interaction of DNA and acridines. *J. Mol. Biol.* 3:18-30.
18. Lindmark, D. G., and M. Müller. 1976. Antitrichomonad action, mutagenicity, and reduction of metronidazole and other nitroimidazoles. *Antimicrob. Agents Chemother.* 10:476-482.
19. Lown, J. W., and S. K. Sim. 1976. Studies related to antitumor antibiotics. VIII. Cleavage of DNA by streptonigrin analogues and the relationship to anti-neoplastic activity. *Can. J. Biochem.* 54:446-452.
20. McCalla, D. R., A. Reuvers, and C. Kaiser. 1971. Breakage of bacterial DNA by nitrofur derivatives. *Cancer Res.* 31:2184-2188.
21. Mandelkern, L., and P. J. Flory. 1952. The frictional coefficient for flexible chain molecules in dilute solution. *J. Chem. Phys.* 20:212-214.
22. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.* 3:208-218.
23. Mohindra, J. K., and A. M. Rauth. 1976. Increased cell killing by metronidazole and nitrofurazone of hypoxic cells compared to aerobic mammalian cells. *Cancer Res.* 36:930-936.
24. Morgan, A. R., and D. E. Pulleyblank. 1974. Native and denatured DNA, cross-linked and palindromic DNA and circular covalently-closed DNA analyzed by a sensitive fluorometric procedure. *Biochem. Biophys. Res. Commun.* 61:396-403.
25. O'Brien, R. W., and J. G. Morris. 1972. Effect of metronidazole on hydrogen production by *Clostridium acetobutylicum*. *Arch. Mikrobiol.* 81:225-233.
26. Olive, P. L., and D. R. McCalla. 1975. Damage to mammalian cell DNA by nitrofurans. *Cancer Res.* 35:781-784.
27. Plant, C. W., and D. J. Edwards. 1976. The effect of tinidazole, metronidazole, and nitrofurazone on nucleic acid synthesis in *Clostridium bifermentans*. *J. Antimicrob. Chemother.* 2:203-209.
28. Reichman, M. E., S. A. Rice, C. A. Thomas, and P. Doty. 1954. A further examination of the molecular weight and size of desoxypentose nucleic acid. *J. Am. Chem. Soc.* 76:3047-3053.
29. Rosenkranz, H. S., and S. Rosenkranz. 1971. Degradation of DNA by cysteine. *Arch. Biochem. Biophys.* 146:483-487.
30. Rosenkranz, H. S., and W. T. Speck. 1975. Mutagenicity of metronidazole: activation by mammalian liver microsomes. *Biochem. Biophys. Res. Commun.* 66:520-525.
31. Seto, H., and A. Tomasz. 1974. Early stages in DNA binding and uptake during genetic transformation of pneumococci. *Proc. Natl. Acad. Sci. U.S.A.* 71:1493-1498.
32. Tomasz, M., C. M. Mercado, J. Olson, and N. Chatterjee. 1974. Mode of interaction of mitomycin C with deoxyribonucleic acid and other polynucleotides *in vitro*. *Biochemistry* 13:4878-4887.
33. Voogd, C. E., J. J. Van Der Stel, and J. J. A. A. Jacobs. 1974. The mutagenic action of nitroimidazoles. I. Metronidazole, nimorazole, dimetridazole and ronidazole. *Mutat. Res.* 26:483-490.
34. White, H. L., and J. R. White. 1966. Interaction of streptonigrin with DNA *in vitro*. *Biochim. Biophys. Acta* 123:648-641.
35. Willson, R. L., W. A. Cramp, and R. M. J. Ings. 1974. Metronidazole ('Flagyl'): mechanism of radiosensitization. *Int. J. Radiat. Biol.* 26:557-569.
36. Zimm, B. H., and D. M. Crothers. 1962. Simplified rotating cylinder viscometer for DNA. *Proc. Natl. Acad. Sci. U.S.A.* 48:905-911.
37. Zunio, F., R. Gambetta, A. DiMarco, and A. Zaccara. 1972. Interaction of daunomycin and its derivatives with DNA. *Biochim. Biophys. Acta* 277:489-498.