Contrasts in the Actions of Protein Antibiotics on Deoxyribonucleic Acid Structure and Function†

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ABSTRACT: The protein antibiotics neocarzinostatin (NCS), macromomycin (MCR), and auromomycin (AUR), which is closely related to MCR, have been compared for their in vitro and in vivo actions on deoxyribonucleic acid (DNA). NCS, markedly stimulated by 2-mercaptoethanol, is much more active in inducing strand scissions in superhelical pMB9 and linear duplex λDNA than AUR, which is slightly inhibited by 2-mercaptoethanol. Purified MCR, even at very high levels, does not give any significant amount of cutting with either DNA substrate. 2-Propanol stimulates the activity of NCS but inhibits that of AUR. On the other hand, the antioxidant α-tocopherol strongly inhibits DNA breakage by both drugs. The intercalating drugs ethidium bromide, daunorubicin, proflavin, and actinomycin D at low concentrations inhibit DNA scission by AUR. The levels of intercalators required to inhibit NCS activity to comparable levels are about 10 times higher than those for AUR. Although MCR has virtually no in vitro DNA cutting activity, it is, like AUR and NCS, cytotoxic, as measured by the inhibition of DNA synthesis and induction of DNA strand breakage in HeLa cells.

The antitumor antibiotic neocarzinostatin (NCS)[1] is a single-chain acidic protein of molecular weight 10 700 with two disulfide bonds (Ishida et al., 1965; Meienhofer et al., 1972). There is considerable evidence that the cytotoxic action of NCS is closely related to its ability to affect DNA structure and function. NCS selectively inhibits DNA synthesis in sensitive cells (Ono et al., 1966; Homma et al., 1970; Sawada et al., 1974; Beerman & Goldberg, 1977) and induces DNA strand breakage (Beerman & Goldberg, 1974, 1977; Sawada et al., 1974; Tatsumi et al., 1974; Ohtsuki & Ishida, 1975a,b; Beerman et al., 1977) and DNA repair synthesis in whole cells (Tatsumi et al., 1975; Kappen & Goldberg, 1978b; Hatayama & Goldberg, 1979) and isolated nuclei (Kappen & Goldberg, 1978b). Furthermore, this drug primarily introduces single-strand breaks in helical and linear duplex DNA in vitro in a reaction greatly stimulated by a sulfhydryl compound (Beerman & Goldberg, 1974; Beerman et al., 1977; Poon et al., 1977). The breaks occur almost entirely at deoxythymidylic acid and, to a lesser extent, at deoxyadenylic acid residues (Poon et al., 1977; Hatayama et al., 1978; D'Andrea & Haseltine, 1978). We have recently reported that while mercaptans activate the DNA cutting reaction by NCS, they also inhibit it at high concentrations and rapidly inactivate NCS on preincubation in the absence of DNA. In addition, alcohols greatly stimulate the in vitro reaction, but the antioxidant α-tocopherol strongly inhibits the in vitro reaction (Kappen & Goldberg, 1978a).

Macromomycin (MCR), produced by *Streptomyces macromomyceticus* (Chimura et al., 1968), is a weakly acidic protein (molecular weight 12 500) that is active against several experimental murine tumors (Lippman et al., 1975) and cell cultures (Kunimoto et al., 1972). MCR inhibits DNA synthesis (Kunimoto et al., 1972; Goldberg et al., 1977; Suzuki et al., 1978; Im et al., 1978; Beerman, 1978) and induces DNA strand scissions in vivo (Goldberg et al., 1977; Suzuki et al., 1978; Beerman, 1978). In preliminary studies, it was reported that MCR failed to cut DNA in vitro (Goldberg et al., 1977; Beerman, 1978). On the other hand, such a reaction has been recently described (Sawyer et al., 1978). Many of the biological studies reported earlier were carried out with MCR preparations of limited purity and stability. Recently, however, methods have been published for the purification of MCR to chemical homogeneity (Im et al., 1978; Yamashita et al., 1976) and certain differences in the properties of the preparations are observed. In addition, isolation of auromomycin (AUR) during purification of MCR from the culture filtrates has raised questions on the purity and authenticity of the MCR preparation. It is reported (Dr. M. Kanazawa, Kanegafuchi Chemical Industries Co., Osaka, Japan, personal communication) that AUR is identical with MCR in most chemical and biological activities and differs from MCR in (a) the presence of a chromophore absorbing at 355 nm, (b) being active against gram-negative as well as gram-positive bacteria, and (c) having a higher toxicity in experimental tumor systems.

In the present study, we show that there are differences and similarities in the stimulation or inhibition of the in vitro DNA cutting activities of NCS and AUR in the presence of many agents, including several drugs that intercalate DNA. On the other hand, highly purified MCR appears not to cut DNA in vitro. Of particular significance is the finding that comparison of the in vitro and in vivo activities of one antibiotic with another fails to reveal a consistent correlation pattern between the two types of activities.

Materials and Methods

NCS (clinical form) was generously provided by Dr. W. T. Bradner of Bristol Laboratories, and MCR and AUR were obtained from Kanegafuchi Chemical Industries Co. through the courtesy of Dr. Umezawa. MCR and AUR were further purified to homogeneity, as determined by acrylamide gel electrophoresis and isoelectric focusing (p*I* 5.4), by chromatography on Sephadex G-100 and DEAE-cellulose (T. S. A. Samy, unpublished experiments). The MCR was essentially free of contaminating AUR, as also determined by UV absorption at 355 nm and fluorescence emission at 430 nm (excitation at 360 nm) (through the courtesy of Dr. M. A. Napier). Ampholines (LKB-1809) were purchased from LKB Productor AB (Bromma, Sweden). D-α-Tocopherol succinate

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[1] Abbreviations used: NCS, neocarzinostatin; MCR, macromomycin; AUR, auromomycin; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; EDTA, (ethylenedinitrilo)tetraacetic acid.

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and chloroquine were products of Sigma Chemical Co. Ethidium bromide, proflavin, and actinomycin D were purchased from Calbiochem. Daunorubicin hydrochloride was obtained from the National Cancer Institute.

[*methyl*-^{3}H]Thymidine-labeled λDNA was prepared as described by Kappen & Goldberg (1977). The plasmid DNA (pMB9) was isolated by gentle lysis of the cells (*Escherichia coli* HMS 174pMB9) with lysozyme and detergent, followed by centrifugation to remove chromosomal DNA (Clewell & Helinski, 1969). Further treatment included banding in a CsCl gradient containing ethidium bromide at 200 μg/mL; the ethidium bromide was subsequently removed with Dowex 50 resin. The DNA preparation contained 85–90% of the supercoiled form (form I), and the rest was the open circular form (form II). Electrophoresis on acrylamide gels was carried out according to Reisfeld et al. (1962) and Weber & Osborn (1969). Isoelectric focusing was performed on a linear sucrose gradient containing 2% carrier ampholyte, pH 3.5–10.

The standard in vitro DNA cutting reaction contained 50 mM Tris (pH 8.0) (100 mM at high drug levels), 10 mM 2-mercaptoethanol when present, and the drugs at levels indicated in the figure and table legends. After incubation at 37 °C, the reaction mixture was analyzed on 5–20% alkaline sucrose gradients containing 0.7 M NaCl, 0.3 M NaOH, and 1 mM EDTA. The samples were centrifuged at 20 °C in a Beckman SW 50.1 rotor for 2 h at 49 000 rpm (λDNA) or at 40 000 rpm (pMB9 DNA). The gradient fractions, after neutralization with HCl, were mixed with 10 mL of Scintiverse, and the radioactivity was determined in a liquid scintillation spectrometer. The reaction products with pMB9 DNA were also separated electrophoretically on 1% agarose gels in a buffer containing 40 mM Tris, 5 mM sodium acetate, and 1 mM EDTA, pH 7.9. The radioactivity in gel slices was determined after digestion with 10 mL of a mixture of protosol–liquiflor–toluene (5:4:91 v/v). Strand breaks in λDNA were calculated by using the equation of Abelson & Thomas (1966). Since in both sucrose gradient and gel analyses pMB9 (form I) DNA is well separated from the nicked forms, the extent of the reaction was measured from the amount of form I remaining after treatment. Strand breaks (μ) can be calculated from the equation $\mu = -\log$ form I.

Assay of DNA synthesis in HeLa S_3 cells grown in spinner culture was essentially as described by Beerman & Goldberg (1977)). Cells (2.5 mL, 3×10^5/mL) were preincubated with the drug at 37 °C for 30 min in a shaker bath before the addition of [^{3}H]thymidine (50 Ci/mmol, 1 μCi). The radioactivity incorporated into DNA at 45 min was determined. For measurement of the DNA strand breakage, the cells (3×10^5/mL) were prelabeled with [^{14}C]thymidine (50 mCi/mmol, 0.1 μCi/mL) for 18–20 h. The labeled cells (4×10^5/mL) taken up in fresh medium were incubated with the drug at 37 °C for 30 min. The cells were then collected by centrifugation and were washed once with phosphate-buffered saline (0.01 M sodium phosphate and 0.15 M NaCl, pH 7.2). One hundred microliters of cell suspension in phosphate-buffered saline (2×10^5 cells) was placed onto 4.5 mL of a 5–20% alkaline sucrose gradient overlaid with 0.5 mL of 0.3% Sarkosyl in 2.5% alkaline sucrose. The samples were centrifuged at 20 °C in a Beckman SW 50.1 rotor at 40 000 rpm for 40 min. The trichloroacetic acid insoluble radioactivity in the gradient fractions was determined as described by Beerman et al. (1977).

Results and Discussion

The in vitro DNA cutting activity of NCS and AUR, as measured by alkaline sucrose gradient analysis, is shown in

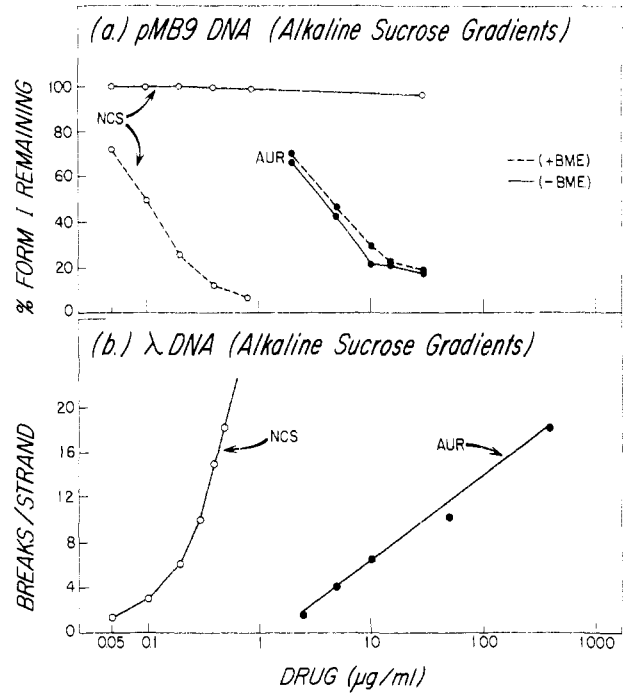

FIGURE 1: Strand breakage in pMB9 and λDNA by NCS and AUR. (a) Standard reactions (0.1 mL) contained 0.94 μg (1.8×10^4 cpm) of superhelical pMB9 DNA and varying amounts of the drugs in the presence and absence of 2-mercaptoethanol. The data have been adjusted as though there was 100% form I at the start of the reaction. (b) λDNA (0.4 μg, 2.4×10^4 cpm) was incubated with the drugs in a final volume of 0.1 mL. 2-Mercaptoethanol was added only to the samples containing NCS. After incubation for 30 min, the reaction mixtures were analyzed on alkaline sucrose gradients.

Table I: Strand Breakage in Superhelical pMB9 DNA by NCS and AUR[a]

drug (μg/mL)	percent form I	percent form II	percent form III
none	72.3	25.5	2.2
NCS (0.2)	20.9	75.7	3.4
NCS (0.3)	13.5	83.0	3.5
NCS (0.4)	5.7	90.4	3.9
AUR (10)	24.7	69.4	5.9
AUR (20)	18.1	74.0	7.9
AUR (100)	4.3	87.7	8.0
AUR (800)	1.0	86.5	12.5

[a] Incubations similar to those in Figure 1a contained AUR without or NCS with 2-mercaptoethanol. The reaction products were analyzed by agarose gel electrophoresis. In the absence of drug, the percent of the different forms of the DNA was the same whether or not 2-mercaptoethanol was present.

Figure 1. With superhelical pMB9 DNA as the substrate, NCS is more potent than AUR (Figure 1a and Table I). Fifty-percent reaction is obtained at 0.1 and 3.5 μg/mL NCS and AUR, respectively. When the strand breakage of linear duplex λDNA was measured, NCS is again more active than AUR. In contrast to NCS and AUR, even at very high levels of purified MCR (500 μg/mL) no significant amount (<10% conversion of form I to form II) of strand breakage was obtained with supercoiled (or linear duplex) DNA. In fact, we have found that MCR specifically blocks AUR-induced DNA strand scission (Kappen & Goldberg, 1979). On the other hand, with different batches of crude MCR, a low level of DNA strand breakage was observed, presumably due to contaminating AUR, as judged by UV absorption and fluorescence measurements. It is of interest that both NCS (Samy et al., 1974; Napier et al., 1979) and AUR possess chromo-

Table II: Effect of 2-Propanol and α-Tocopherol on Strand Breakage in pMB9 DNA[a]

	% form I pMB9 DNA remaining		
	no addn	+2-propanol (1 M)	+α-tocopherol (0.75 mM)
expt 1			
NCS (0.25 μg/mL)*	45	11	
NCS (400 μg/mL)	93	92	
AUR (16 μg/mL)	37	75	
expt 2			
NCS (0.5 μg/mL)*	19		84
AUR (10 μg/mL)	51		93

[a] Incubations were similar to those in Figure 1a except that the standard reaction contained 1.45 μg of pMB9 DNA. 2-Mercaptoethanol was present only where marked by an asterisk. Following incubation, the reaction mixtures in expt 1 were analyzed electrophoretically on agarose gels and those in expt 2 were centrifuged on alkaline sucrose gradients. The amount of ethanol (1.5%) included with the α-tocopherol had no significant effect on the control or the drug-treated DNA. pMB9 DNA used in these experiments contained 79% form I and 21% form II. The data have been adjusted as though there was 100% form I at the start of the reaction.

phores absorbing at ~360 nm and cut DNA in vitro, whereas MCR from which the 360-nm chromophore has been removed lacks this activity.

We have earlier shown (Beerman & Goldberg, 1974; Beerman et al., 1977; Kappen & Goldberg, 1978a) that sulfhydryl compounds markedly enhance the in vitro DNA scission by NCS, although at very high levels the drug alone will cut DNA. As expected, 2-mercaptoethanol stimulates at least 10000-fold the reaction of NCS with pMB9 DNA. By contrast, 2-mercaptoethanol, if it has any effect, slightly inhibits the activity of AUR and has no effect on MCR activity. The stimulation of NCS activity appears to be due to the generation of an active species of the drug after reduction of its disulfide bond(s) (Kappen & Goldberg, 1978a). Such a mechanism appears not to be involved in the case of AUR.

NCS primarily introduces single-strand breaks in helical DNA. For examination of whether this is also true of AUR, pMB9 form I DNA was incubated under standard conditions with varying levels of the drugs and the products were analyzed by agarose gel electrophoresis. Introduction of a single nick converts the superhelical molecule (form I) to the open circular (form II) molecule, and a double-strand break will produce a linear (form III) molecule. The results of these experiments are summarized in Table I. At levels of drugs where ~20% form I remains, AUR gives rise to 2 to 3 times as much form III as produced by NCS. This suggests that in the case of AUR, double-strand breaks occur as an independent event and not solely as a result of the random coincidence of single-strand breaks within a small number of base pairs. In this regard, AUR can be compared with bleomycin, which has been shown to produce specific double-strand breaks in DNA (Povirk et al., 1977; Lloyd et al., 1978).

Earlier work (Kappen & Goldberg, 1978a) showed that alcohols and other organic solvents greatly stimulate the 2-mercaptoethanol-dependent activity of NCS, as measured by its ability to produce trichloroacetic acid soluble radioactivity from λDNA. In experiments presented in Table II, we studied the effect of 2-propanol on the conversion of pMB9 form I DNA to form II (III or smaller forms) by NCS and AUR. In agreement with previous results, the reaction with NCS in the presence of 2-mercaptoethanol (but not in its absence) is

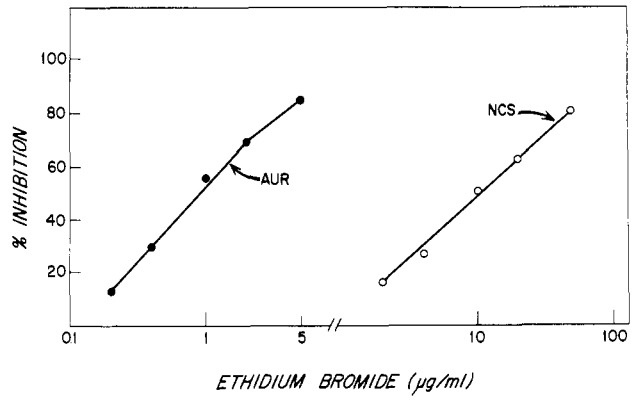

FIGURE 2: Effect of intercalating agents on strand breakage in pMB9 DNA by NCS and AUR. Reaction mixtures (100 μL) contained 0.65 μg (7×10^3 cpm) of DNA and varying levels of ethidium bromide. 2-Mercaptoethanol was added only to the reactions containing NCS. All the components except the drugs were preincubated for 5 min at 37 °C. After the addition of the drugs (AUR, 7.5 μg/mL; NCS, 0.18 μg/mL), the reaction mixtures were incubated for 30 min and then analyzed on alkaline sucrose gradients. The amount of form II (12%) present in DNA in the absence of any drug treatment has been subtracted in calculating that produced by drug treatment.

markedly increased by 2-propanol. In contrast, 2-propanol inhibits >50% the DNA strand breakage by AUR. The inhibition of AUR activity by 2-propanol is similar to that found by us and others (Sim & Lown, 1978) for bleomycin-induced DNA cleavage where OH radicals have been implicated. DNA scission by NCS requires oxygen (Kappen & Goldberg, 1978a,b; Sim & Lown, 1978; Burger et al., 1978) and is strongly inhibited by α-tocopherol (Kappen & Goldberg, 1978a). As in the case of NCS, α-tocopherol inhibits over 85% DNA cutting by AUR (Table II). Preliminary experiments also suggest a dependence on oxygen in the action of these drugs as well (data not shown).

We have also studied the effect of several compounds that bind to DNA by intercalation on the DNA cutting activity of NCS and AUR. Figure 2 shows the dose-dependent effect of ethidium bromide on the conversion of pMB9 form I to form II DNA by NCS and AUR. In these experiments the antibiotics alone converted ~75–80% form I DNA to form II. Ethidium bromide strongly inhibits DNA cutting by AUR (50% inhibition at 2.25 μM). The concentrations of ethidium bromide required to give comparable levels of inhibition of NCS activity are ~10 times higher than those for AUR. Inhibition of DNA scission by ethidium bromide is not found if it is added after incubation of the DNA with the antibiotics. Inhibition of the DNA cutting activity of NCS by ethidium bromide was also observed with linear λDNA as the substrate when the cutting was measured by alkaline sucrose gradient analysis or by the production of trichloroacetic acid soluble radioactivity. In the latter case, 50% inhibition was obtained at 7 μM ethidium bromide. Further, ethidium bromide inhibition of the DNA scission activity of both protein antibiotics could be overcome by adding an excess of DNA but not by an excess of the antibiotics. This suggests that ethidium bromide inhibits the reaction by altering the structure of the DNA substrate and not by competing with the antibiotics for the substrate. Of course, this does not rule out the possibility that an active moiety on the antibiotics (e.g., a chromophore prosthetic group) interacts with the DNA by a process such as intercalation.

Among the other intercalating agents tested, daunorubicin and proflavin are nearly as potent as ethidium bromide in inhibiting the activity of NCS and AUR, whereas about 5- and 25-fold levels of actinomycin D and chloroquine, respec-

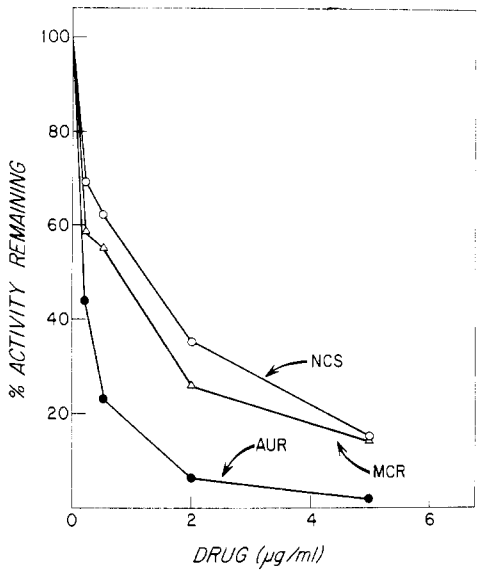

FIGURE 3: Effect of drugs on DNA synthesis in HeLa cells. Thymidine incorporation into the DNA was measured as described under Materials and Methods. In the absence of drug (100% activity), 2.4×10^4 cpm of [^{3}H]thymidine was incorporated into DNA.

tively, are required to inhibit AUR (Table III). At the highest levels of actinomycin D and chloroquine tested, NCS activity was only slightly inhibited (~10%). On the other hand, mithramycin, which like actinomycin D requires the 2-amino group of guanine to interact with DNA (Ward et al., 1965) but does not intercalate DNA (Waring, 1970), was found not to inhibit the DNA cutting activities of the protein antibiotics. All the intercalating agents, at the concentrations used in these studies, did not nick DNA in either the presence or the absence of 2-mercaptoethanol.

Various metal chelators such as EDTA (20 mM), 8-hydroxyquinoline (1.4 mM), α,α'-dipyridyl (4 mM), and deferoxamine (5 mM) fail to affect the activity of any of the antibiotics. Superoxide dismutase and catalase also had no effect.

Exposure to light destroys the activity of NCS (Ishida et al., 1965; Kohno et al., 1974; Burger et al., 1978; M. Napier and I. H. Goldberg, unpublished experiments) and MCR (Im et al., 1978). We further found that on treatment of NCS and AUR in 50 mM Tris, pH 8.0, with long-wave UV light (Mineralite lamp, Ultraviolet Products) at 15-cm distance for 30 min, AUR retained ~50% of its in vitro activity under conditions which destroyed the activity of NCS completely.

Table III: Effect of Intercalating Agents on DNA Strand Breakage by NCS and AUR[a]

	concn (μM) for 50% inhibn	
	AUR	NCS
ethidium bromide	2.25	25
daunorubicin	3.0	20
proflavin	5.2	38
actinomycin D	10	>200
chloroquine	50	>250

[a] In a series of experiments with those in Figure 2, the amount of form II produced by the drugs in the presence and absence of the compounds listed was determined.

Since the in vitro activities of the three drugs differ widely, it is of interest to compare their activities in vivo. As shown in Figure 3, MCR is about as potent as NCS in inhibiting thymdine incorporation into the DNA of HeLa cells. After 30 min of preincubation of the cells with the drug, MCR at 0.2 μg/mL inhibited DNA synthesis 41% and at 5 μg/mL the inhibition reached 85%. The levels of inhibition obtained with MCR under our experimental conditions are comparable to those reported by others in HeLa (Kunimoto et al., 1972; Suzuki et al., 1978; Beerman, 1978) and other cell lines (Im et al., 1978). AUR at 0.2 and 2 μg/mL caused 56 and 94% inhibition, respectively, and is thus somewhat more effective than NCS and MCR. We further compared the ability of these drugs to induce strand breakage in cellular DNA (Figure 4). It can be seen that at 0.25 and 1 μg/mL MCR is as active as NCS in causing DNA scission in vivo, although it is virtually inactive in the in vitro DNA cutting reaction. Similarly, AUR, which is much less active than NCS in the in vitro reaction, appears to be somewhat more potent in causing DNA breakage in cells. Whether the different selective activities in vivo and in vitro reflect different mechanisms or different active molecular forms of the antibiotics in the two types of reactions remains to be determined. The greater cytotoxic activity and the much greater in vitro activity on DNA of AUR compared with MCR may be due to the presence of the chromophore absorbing at ~360 nm in AUR. This chromohore appears to be removed during chromatography on Amberlite XAD, resulting in conversion of AUR to MCR (M. Kanazawa, personal communication).

Added in Proof

Since submission of this paper, the work noted in the personal communication of Dr. M. Kanazawa has been published [see Yamashita et al. (1979)].

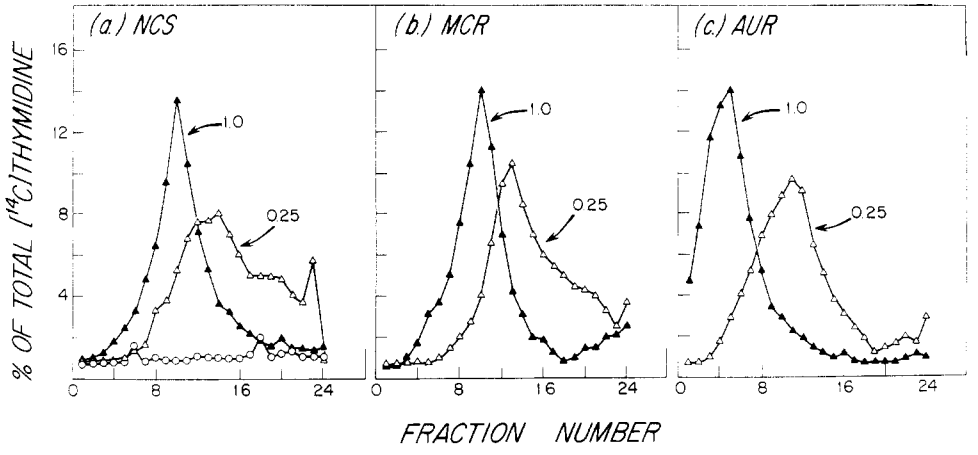

FIGURE 4: DNA strand breakage in HeLa cells by drugs. Experimental details are given under Materials and Methods. In the control containing no drug (O), ~95% of the total radioactivity was in the pellet (not shown). The recovery was 80%. The drug concentrations (μg/mL) are shown on the figure.

Acknowledgments

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References

Abelson, J. N., & Thomas, C. A., Jr. (1966) *J. Mol. Biol. 18*, 262.

Beerman, T. A. (1978) *Biochem. Biophys. Res. Commun. 83*, 908.

Beerman, T. A., & Goldberg, I. H. (1974) *Biochem. Biophys. Res. Commun. 59*, 1254.

Beerman, T. A., & Goldberg, I. H. (1977) *Biochim. Biophys. Acta 475*, 281.

Beerman, T. A., Poon, R., & Goldberg, I. H. (1977) *Biochim. Biophys. Acta 475*, 294.

Burger, R. M., Peisach, J., & Horwitz, S. B. (1978) *J. Biol. Chem. 253*, 4830.

Chimura, H., Ishizuka, M., Hamada, M. Hori, S., Kimura, K., Iwanaga, J., Takeuchi, T., & Umezawa, H. (1968) *J. Antibiot. 21*, 44.

Clewell, D. B., & Helinski, D. R. (1969) *Proc. Natl. Acad. Sci. U.S.A. 62*, 1159.

D'Andrea, A. D., & Haseltine, W. A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3608.

Goldberg, I. H., Beerman, T. A., & Poon, R. (1977) *Cancer, a Comprehensive Treatise* (Becker, F. F., Ed.) Vol. 5, pp 427–456, Plenum Press, New York and London.

Hatayama, T., & Goldberg, I. H. (1979) *Biochim. Biophys. Acta 563*, 59.

Hatayama, T., Goldberg, I. H., Takeshita, M., & Grollman, A. P. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3603.

Homma, M., Koida, T., Saito-Koide, T., Kamo, I., Seto, M., Kumagai, K., & Ishida, N. (1970) *Progr. Antimicrob. Anticancer Chemother., Proc. Int. Congr. Chemother., 6th, 1969 2*, 410.

Im, W. B., Chiang, C., & Montgomery, R. (1978) *J. Biol. Chem. 253*, 3259.

Ishida, N., Miyazaki, K., Kumagai, K., & Rikimaru, M. (1965) *J. Antibiot. 8*, 68.

Kappen, L. S., & Goldberg, I. H. (1977) *Biochemistry 16*, 479.

Kappen, L. S., & Goldberg, I. H. (1978a) *Nucleic Acids Res. 5*, 2959.

Kappen, L. S., & Goldberg, I. H. (1978b) *Biochim. Biophys. Acta 520*, 481.

Kappen, L. S., & Goldberg, I. H. (1979) *Biochemistry* (in press).

Kohno, M., Haneda, I., Koyama, Y., & Kikuchi, M. (1974) *Jpn. J. Antibiot. 27*, 707.

Kunimoto, T., Hori, M., & Umezawa, H. (1972) *Cancer Res. 32*, 1251.

Lippman, M. M., Laster, W. R., Abbott, B. J., Venditti, J., & Baratta, M. (1975) *Cancer Res. 35*, 939.

Lloyd, R. S., Haidle, C. W., & Robberson, D. L. (1978) *Biochemistry 17*, 1890.

Meienhofer, J., Maeda, H., Glaser, C. B., Czombos, J., & Kuromizu, K. (1972) *Science 178*, 875.

Napier, M. A., Holmquist, B., Strydom, D. J., & Goldberg, I. H. (1979) *Biochem. Biophys. Res. Commun. 89*, 635.

Ohtsuki, K., & Ishida, N. (1975a) *J. Antibiot. 28*, 143.

Ohtsuki, K., & Ishida, N. (1975b) *J. Antibiot. 28*, 229.

Ono, Y., Watanabe, Y., & Ishida, N. (1966) *Biochim. Biophys. Acta 119*, 46.

Poon, R., Beerman, T. A., & Goldberg, I. H. (1977) *Biochemistry 16*, 486.

Povirk, L. F., Wubker, W., Kohnlein, W., & Hutchinson, F. (1977) *Nucleic Acids Res. 4*, 3573.

Reisfeld, R. A., Lewis, U. J., & Williams, D. E. (1962) *Nature (London) 195*, 281.

Samy, T. S. A., Atreyi, M., Maeda, H., & Meienhofer, J. (1974) *Biochemistry 13*, 1007.

Sawada, H., Tatsumi, K., Sasada, M., Shirakawa, S., Nakamura, T., & Wakisaka, G. (1974) *Cancer Res. 34*, 3341.

Sawyer, T. H., Prestayko, A. W., & Crooke, S. T. (1979) *Cancer Res. 39*, 1180.

Sim, S-K., & Lown, J. W. (1978) *Biochem. Biophys. Res. Commun. 81*, 99.

Suzuki, H., Nishimura, T., Muto, K., & Tanaka, N. (1978) *J. Antibiot. 31*, 875.

Tatsumi, K., Nakamura, T., & Wakisaka, G. (1974) *Gann 65*, 459.

Tatsumi, K., Sakane, T., Sawada, H., Shirakawa, S., Nakamura, T., & Wakisaka, G. (1975) *Gann 66*, 441.

Ward, D. C., Reich, E., & Goldberg, I. H. (1965) *Science 149*, 1259.

Waring, M. (1970) *J. Mol. Biol. 54*, 247.

Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406.

Yamashita, T., Naoi, N., Watanabe, K., Takeuchi, T., & Umezawa, H. (1976) *J. Antibiot. 29*, 415.

Yamashita, T., Naoi, N., Hidaka, T., Watanabe, K., Kunada, Y., Takeuchi, T., & Umezawa, H. (1979) *J. Antibiot. 32*, 330.