References

Ames, B. N., and Dubin, D. T. (1960), *J. Biol. Chem. 235*, 769.

Bray, G. A. (1960), *Anal. Biochem. 1*, 279.

Iyer, V. N., and Szybalski, W. (1963), *Proc. Natl. Acad. Sci. U.S. 50*, 355.

Iyer, V. N., and Szybalski, W. (1964), *Science 145*, 55.

Korn, D., and Weissbach, A. (1962), *Biochim. Biophys. Acta 61*, 775.

Lefemine, D. V., Dann, M., Barbatschi, F., Hausmann, W. K., Zbinovsky, V., Monnikendam, P., Adam, J., and Bohonos, N. (1962), *J. Am. Chem. Soc. 84*, 3184.

Lipsett, M., and Weissbach, A. (1965), *Biochemistry 4*, 206 (this issue; 2nd following paper).

Marmur, J. (1961), *J. Mol. Biol. 3*, 208.

Nirenberg, M. W., and Matthei, H. J. (1961), *Proc. Natl. Acad. Sci. U.S. 47*, 1588.

Otsuji, N., Sekiguchi, M., Iijima, T., and Takagi, Y. (1959), *Nature 184*, 1079.

Pricer, W. E., Jr., and Weissbach, A. (1965), *Biochemistry 4*, 200 (this issue; following paper).

Shiba, S., Terawaki, A., Taguchi T., and Kawamata, J. (1959), *Nature 183*, 1056.

Tissières, A., Watson, J. D., Schlessinger, D., and Hollingworth, B. R. (1959), *J. Mol. Biol. 1*, 221.

Webb, J. S., Cosulich, D. B., Mowat, J. H., Patrick, J. B., Broschard, R. W., Meyer, W. E., Williams, R. P., Wolf, C. F., Fulmor, W., Pidacks, C., and Lancaster, J. E. (1962), *J. Am. Chem. Soc. 84*, 3185.

Weissbach, A., and Korn, D. (1964), *Biochim. Biophys. Acta 87*, 621.

Enzymatic Utilization and Degradation of DNA Treated with Mitomycin C or Ultraviolet Light*

W. E. Pricer, Jr., and A. Weissbach

ABSTRACT: *Escherichia coli* K12λ DNA, alkylated with mitomycin C *in vitro*, is utilized one-third to one-fourth as well as native DNA when tested as primer for *E. coli* DNA or RNA polymerase. The alkylated DNA is also degraded at a slower rate than native DNA by three distinct exonucleases obtained from *E. coli:* the λ-exonuclease associated with λ-phage formation, the phosphodiesterase (Exo I) which is specific for single-stranded DNA, and the exonuclease associated with DNA polymerase (Exo II).

However, the RNA-inhibitable endonuclease, obtained from *E. coli*, and pancreatic endonuclease attack alkylated DNA at almost the same rate as native DNA. DNA, irradiated with ultraviolet light, acts the same as alkylated DNA with the aforementioned enzymes.

The ability of reduced mitomycin C to alkylate and attach to nucleic acids (Weissbach and Lisio, 1965) raises the question of how a DNA molecule containing such attached antibiotic residues will react with the various enzymes concerned with nucleic acid metabolism. This question is of particular relevance in view of the findings of Shiba *et al.* (1959) who reported that bacterial cells exposed to mitomycin C cease to make DNA but continue to synthesize RNA and protein.

In this paper DNA alkylated with mitomycin C has been compared to native DNA with the following enzymes: DNA polymerase, RNA polymerase, endodeoxyribonucleases, and exodeoxyribonucleases. We have also compared in these enzyme systems a DNA which had previously been irradiated with ultraviolet light and found it to resemble the mitomycin C-alkylated DNA in its behavior. In general, the alkylated or irradiated DNA is utilized or degraded at a slower rate than normal DNA by the above-mentioned enzymes. However, endonucleases cannot significantly distinguish normal DNA from alkylated or irradiated DNA.

Materials and Methods

DNA was prepared from *Escherichia coli* K12λ cells by the method of Marmur (1961). The preparation of K12λ [^{3}H]DNA has been previously described (Weissbach and Korn, 1963). Mitomycin C was purchased from the Kyowa Hakko Kogyo Co. (Tokyo). Salmon sperm DNA and nucleoside triphosphates were purchased from Calbiochem. [8-^{14}C]ATP was a product of Schwarz Biochemical Corp. and was adjusted, with nonradioactive ATP, to a specific activity of 0.69 μc/μmole. Crystalline pancreatic DNAase was a product of the Worthington Biochemical Corp. DNA polymerase

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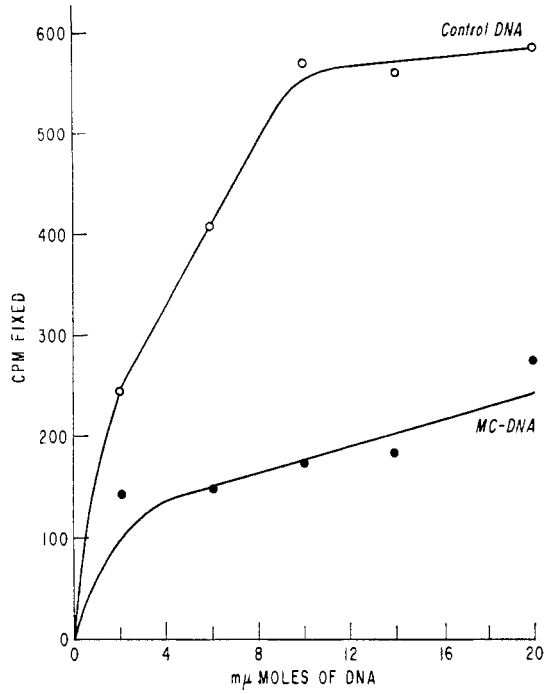

FIGURE 1: MC-alkylated DNA as a primer for DNA polymerase. Reaction mixtures, 0.3 ml, contained 30 μmoles of Tris-ethanolamine buffer, *p*H 8.75; 0.75 μmole of $MgCl_2$; 0.25 μmole of EDTA; 12 mμmoles of [^{3}H]deoxynucleoside triphosphates, containing 3.6 × 10^4 cpm; 2–20 mμmoles (as phosphate) of MC-DNA or control DNA (preparation C); and 0.5 unit of DNA polymerase. Incubation was for 20 minutes at 37°. A DNA polymerase unit is 1 mμmole of substrate converted to an acid-insoluble form in 1 hour at 37°.

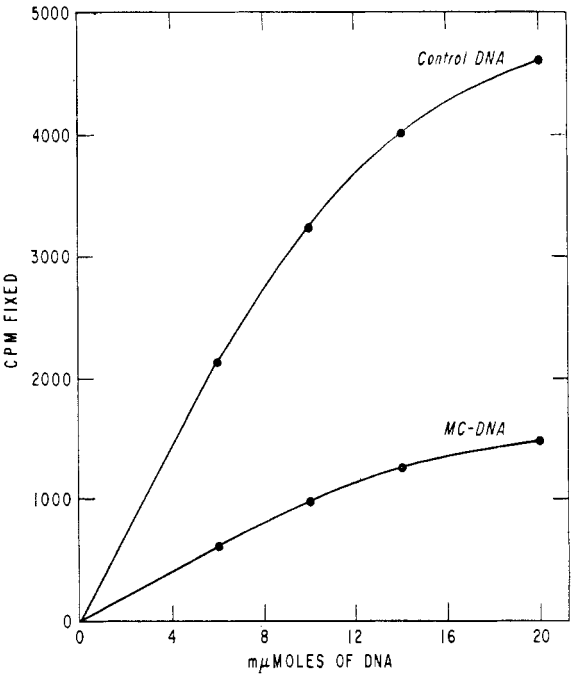

FIGURE 2: MC-alkylated DNA as primer for RNA polymerase. Reaction mixtures (0.25 ml) contained 20 μmoles of Tris-ethanolamine buffer, *p*H 8.0; 1.0 μmole of $MgCl_2$; 0.25 μmole of $MnCl_2$; 0.3 μmole of 2-mercaptoethanol; 10 units of RNA polymerase; 6–20 mμmoles of DNA (preparation C); 88 mμmoles each of GTP, UTP, CTP, and [8^{14}C]ATP. After incubation at 37° for 20 minutes the "Acid-insoluble Radioactivity" was measured as described under Materials and Methods. The enzyme unit refers to 1 mμmole of substrate converted to an acid-insoluble form in 1 hour at 37°.

was prepared by the method of Pricer and Weissbach (1964). RNA polymerase (ammonium sulfate fraction, step V) was prepared by the method of Chamberlin and Berg (1962) and was the kind gift of Dr. Maxine F. Singer. *E. coli* phosphodiesterase (Lehman, 1960) was generously donated by Dr. I. R. Lehman. *E. col* K12λ endonuclease (DEAE-cellulose, peak I) was prepared as previously described (Weissbach and Korn, 1963) as was the λ-exonuclease (Korn and Weissbach, 1963, 1964). Hyamine, 2,5-diphenyloxazole, and 1,4-bis-2(5-phenyl(oxazolyl)benzene were purchased from the Packard Instrument Co.

Reaction of DNA with Mitomycin C. Alkylation of DNA by mitomycin C was accomplished after sodium hydrosulfite reduction of the antibiotic (Weissbach and Lisio, 1965; Iyer and Szybalski, 1964). Three different DNA preparations containing varying numbers of mitomycin C residues (mitomycin C-alkylated DNA) were used in these experiments. The number of mitomycin C residues attached to the DNA was determined by carrying out parallel experiments with [^{3}H]mitomycin C and nonlabeled DNA as described in the preceding paper (Weissbach and Lisio, 1965). The concentration of DNA is expressed as μmoles of nucleotide-phosphorus as determined by the method of Ames and Dubin (1960).

PREPARATION A. The incubation mixture contained 0.75 μmole ofK12 λ [^{3}H]DNA, 0.286 μmole of mitomycin C, and 0.60 μmole of sodium hydrosulfite in a total volume of 0.83 ml of 0.15 M sodium chloride–0.015 M sodium citrate. DNA alkylated in this manner contains 1 mitomycin residue per 452 nucleotides and had 351 cpm/mμmole.

PREPARATION B. K12λ [^{3}H]DNA (8.0 μmoles), 1.43 μmoles of mitomycin C, and 3.0 μmoles of sodium hydrosulfite were incubated in a total volume of 3.6 ml. The DNA isolated from this reaction mixture contained 1 mitomycin C residue per 854 nucleotides and had 449 cpm/mμmole.

PREPARATION C. The incubation mixture (4.35 ml) contained 1.5 μmoles of K12λ DNA, 1.43 μmoles of mitomycin C, and 3.0 μmoles of sodium hydrosulfite. The isolated DNA contained 1 mitomycin residue per 1450 nucleotides.

In all cases, incubations and DNA isolations were performed at room temperature. The isolation of DNA was performed rapidly (within 30 minutes) and the recovery was about 95% in these experiments. The

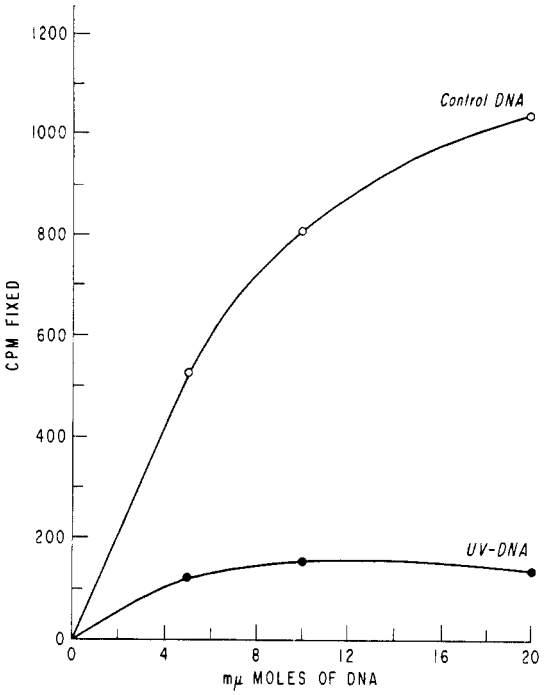

FIGURE 3: UV-DNA as primer for DNA polymerase. Reaction mixtures and incubation time were as described in Figure 1 except that 1.0 unit of DNA polymerase and 5–20 mμmoles of UV-DNA (preparation 2) were used.

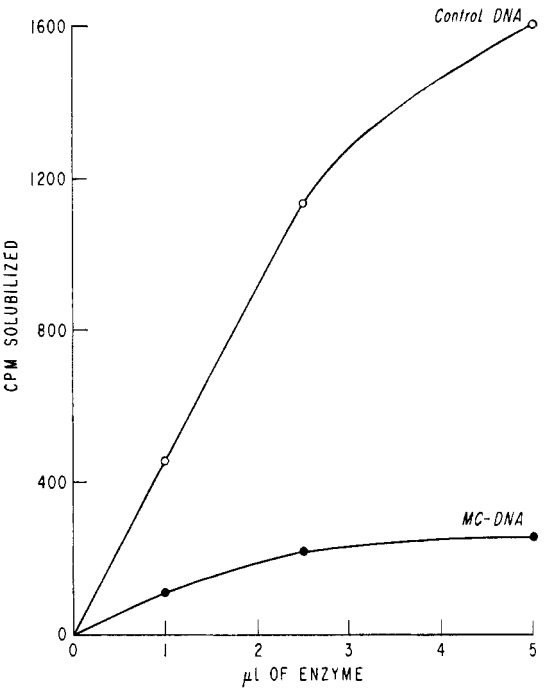

FIGURE 5: The degradation of MC-alkylated DNA by the λ-exonuclease. Incubation mixtures (0.2 ml) contained 30 μmoles of Tris-ethanolamine buffer, *p*H 10.5; 0.5 μmole of $MgCl_2$; 0.5 μmole of 2-mercaptoethanol; 15 mμmoles of [^{3}H]DNA (preparation B); and 1–5 μl of λ-exonuclease (38 units/ml). Mixtures were incubated at 37° for 20 minutes, after which acid-soluble radioactivity was measured according to Weissbach and Korn (1963).

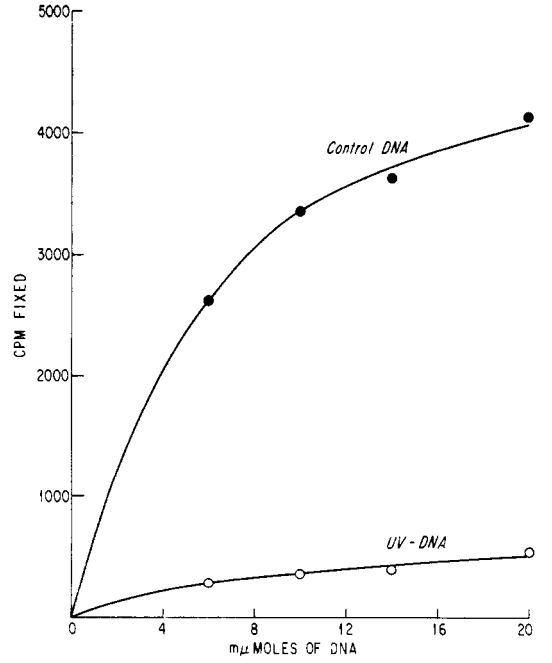

FIGURE 4: UV-DNA as primer for RNA polymerase. Incubations were identical to those described in Figure 2 except that UV-DNA (preparation 1) was used.

final ethanol precipitates of DNA were dissolved in 0.015 M sodium chloride–0.0015 M sodium citrate. Control samples of DNA were treated and isolated as described for preparations A, B, and C except that mitomycin C was omitted from the incubations.

Irradiation of DNA with Ultraviolet Light. DNA samples, dissolved in 0.015 M sodium chloride–0.0015 M sodium citrate, were irradiated at a distance of 4.5–5.3 cm by a Mineralight lamp, Model R-51 (Fisher Scientific Co.). Continuous stirring was maintained throughout the irradiation.

Preparation 1: K12λ [^{3}H]DNA, 2.25 μmoles in 3 ml, was irradiated for 120 minutes and contained 400 cpm/mμmole.

Preparation 2: K12λ DNA, 0.40 μmole in 2 ml, was irradiated for 90 minutes.

Enzyme Assays. DNA polymerase activity was assayed as previously described (Pricer and Weissbach, 1964), except that at the end of the incubation period the acid-insoluble material was collected and washed in the manner of Lehman *et al.* (1958). To the incubation mixture were added 0.2 ml of salmon sperm DNA (2.5 mg/ml) and 2 ml of cold water. Cold 6% perchloric acid (0.5 ml) was slowly added with stirring. After several minutes at 0°, the precipitate was collected by centrifu-

gation and dissolved in 0.30 ml of 0.2 N NaOH. Two ml of cold water was added and the DNA was reprecipitated with 0.5 ml of 6% perchloric acid. The precipitate was collected by centrifugation, dissolved in 0.5 ml of hyamine, and incubated in covered tubes at 57° for 10 minutes. The samples were transferred to 10 ml of toluene scintillator fluid (Weissbach and Korn, 1964) and examined for radioactivity.

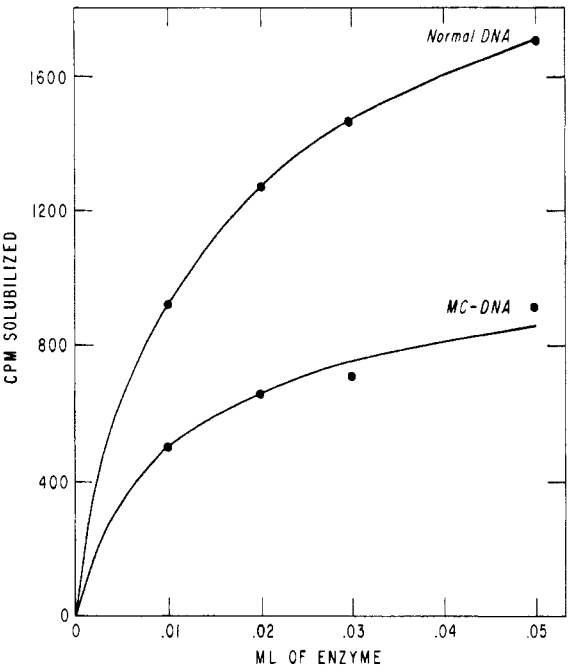

FIGURE 6: The degradation of MC-alkylated DNA by the DNA polymerase–associated exonuclease. Assay mixtures (0.2 ml) contained 30 μmoles of Tris-glycine buffer, *p*H 9.0; 2.0 μmoles of $MgCl_2$; 0.3 μmole of 2-mercaptoethanol; 0.05 μmole of *E. coli* s-RNA; 15 mμmoles of [^{3}H]DNA (preparation A); and 0.01–0.05 ml of DNA polymerase (10,000 polymerase units/ml) which had been dialyzed against 0.01 M Tris buffer, *p*H 7.4, to remove phosphate. Incubations were carried out for 60 minutes at 37° and acid-soluble radioactivity was then determined (Weissbach and Korn, 1963).

RNA polymerase activity was assayed as described by Chamberlin and Berg (1962). The acid-insoluble material from these incubations was collected and washed as described in the DNA polymerase assay except that the perchloric acid precipitate was dissolved in 0.5 ml of 0.2 M Tris-ethanolamine buffer, *p*H 8.0.

DNAase assays were performed as previously described (Weissbach and Korn, 1963; Korn and Weissbach, 1963). The DNAase unit is 10^4 cpm (0.018 μmole of base) solubilized in 15 minutes at 37°.

Radioactive samples were measured in a liquid scintillation spectrometer.

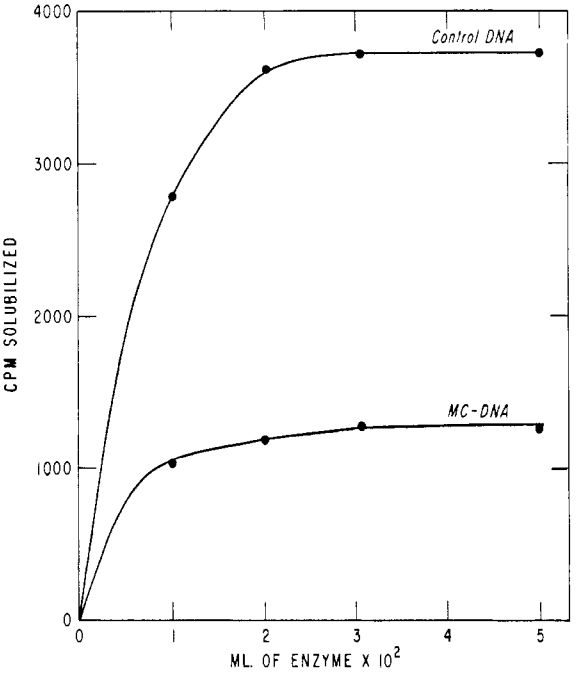

FIGURE 7: The degradation of MC-alkylated DNA by phosphodiesterase (Exo I). MC–K12λ [^{3}H]DNA (preparation A) was diluted to 0.5 μmole/ml in 0.02 M Tris buffer, *p*H 7.4–0.02 M NaCl. Both MC-[^{3}H]DNA and control [^{3}H]DNA were heated for 10 minutes at 100° and rapidly cooled in ice. Assay mixtures contained 30 μmoles of Tris-glycine buffer, *p*H 9.0; 3 μmoles of $MgCl_2$; 10 mμmoles of either heated DNA; and 0.01–0.05 ml of phosphodiesterase (180 units/ml) in a final volume of 0.3 ml. After incubation at 37° for 20 minutes, acid-soluble radioactivity was measured as previously described (Weissbach and Korn, 1963).

Results

Figures 1 and 2 show the ability of DNA polymerase and RNA polymerase to utilize as primer a DNA containing 1 mitomycin residue per 1450 nucleotides. Both the RNA and DNA polymerase utilize the control DNA three to four times better than they utilize the alkylated DNA. This difference extends over a wide concentration range of DNA. DNA damaged by ultraviolet irradiation is also a relatively poor primer for both the DNA and RNA polymerases obtained from *E. coli* (Figures 3 and 4). The DNA used as primer in these latter experiments had been irradiated for 120 minutes. However, the same effect is observed when DNA is irradiated for only 10–20 minutes under our conditions.

The ability of three different exonucleases to degrade the normal (control) and mitomycin-alkylated DNA's was also tested as shown in Figures 5, 6, and 7. Figure 5 shows that the λ-exonuclease (Korn and Weissbach, 1963) obtained from induced *E. coli* K12λ attacks the control DNA some seven times faster than it degrades the alkylated DNA. Ultraviolet-irradiated DNA

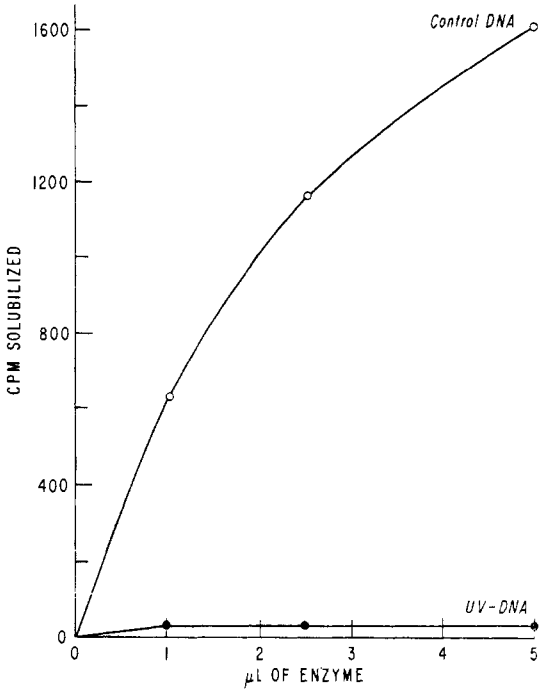

FIGURE 8: UV-DNA as substrate for the λ-exonuclease. Reaction mixtures and incubation times were as described in Figure 5 except that UV-[^{3}H]DNA (preparation 1) was used.

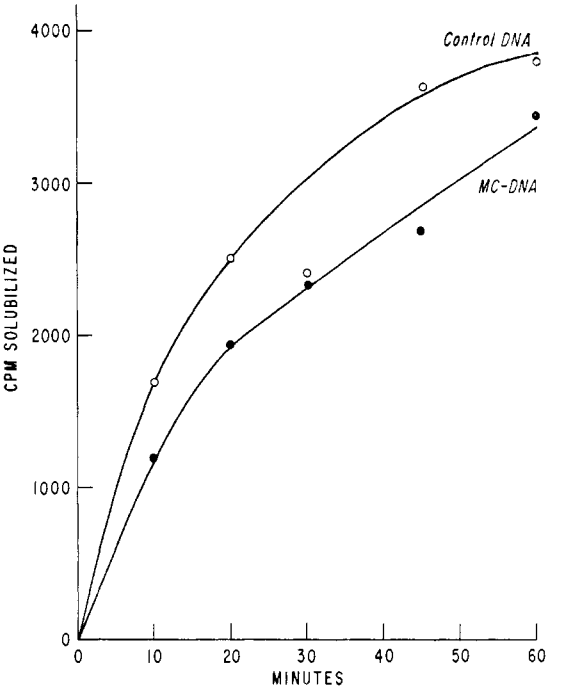

FIGURE 9: MC-alkylated DNA as substrate for pancreatic DNAase. Incubation mixtures (1.6 ml) contained 240 μmoles of Tris-ethanolamine buffer, *p*H 7.5; 4.0 μmoles of $MgCl_2$; 1.6 μmoles of 2-mercaptoethanol; 3.2 μg of pancreatic DNAase; and 120 mμmoles of [^{3}H]DNA (preparation B). Mixtures were incubated at 37°. Aliquots (0.2 ml) were removed at the indicated times for measurement of acid-soluble radioactivity (Weissbach and Korn, 1963).

is an even poorer substrate for the λ-exonuclease since it is attacked at one-tenth to one-thirtieth the rate of the control DNA (Figure 8).

The exonuclease associated with highly purified preparations of *E. coli* K12λ DNA polymerase (Pricer and Weissbach, 1964) also attacks mitomycin-alkylated DNA at a slower rate than normal DNA (Figure 6). These experiments were run in the presence or absence of added RNA (to suppress any possible *E. coli* endonuclease activity) with identical results. The experiments with the λ-exonuclease and DNA polymerase-associated exonuclease were performed with native double-stranded DNA. DNA, alkylated with mitomycin C and then heat denatured, also becomes a poorer substrate for the single-stranded DNA-specific exonuclease (Exo I)[1] described by Lehman (1960) (Figure 7). The heated control DNA is degraded three times faster than the heated mitomycin C-alkylated DNA.

In contrast to the results obtained with the exonucleases, it was found that endonucleases cannot distinguish a control DNA from one containing attached mitomycin C residues. Both the RNA-inhibitable endonuclease from *E. coli* (Lehman *et al.*, 1962) and the pancreatic endonuclease (Kunitz, 1950) attack the control and alkylated DNA's at about equal rates (Figures 9 and 10). The inability of the *E. coli* endonuclease to distinguish normal and alkylated DNA's is reflected in the ability of this enzyme to degrade the control and ultraviolet irradiated DNA's. Figure 11 shows that DNA, irradiated for 120 minutes, is still degraded at about 55% the rate that normal DNA is. This is to be contrasted to the results obtained with the λ-exonuclease (Figure 8) where the same UV-DNA is degraded at a rate of 3–10% that of the control DNA.

Discussion

DNA, alkylated with mitomycin C or irradiated with ultraviolet light, is degraded at a slower rate than untreated DNA by three distinct exonucleases obtained from *E. coli*. These three exonucleases act by removing, in a sequential manner, 5′-nucleotide monophosphates from the end of the DNA chain (Lehman, 1960; Lehman and Richardson, 1964; Korn and Weissbach, 1963). It is possible, therefore, that the presence of a mitomycin residue, or a mitomycin-induced "cross-link" (Iyer and Szybalski, 1963), in the DNA would act as a block to the action of such exonucleases. Similarly, base dimerization in DNA caused by ultraviolet irradiation (Setlow *et al.*, 1963) should also affect the rate of degradation of DNA by exonucleases as was,

[1] Abbreviations used in this work: Exo I, single-stranded DNA-specific exonuclease; MC, mitomycin C; UV-DNA, ultraviolet-irradiated DNA.

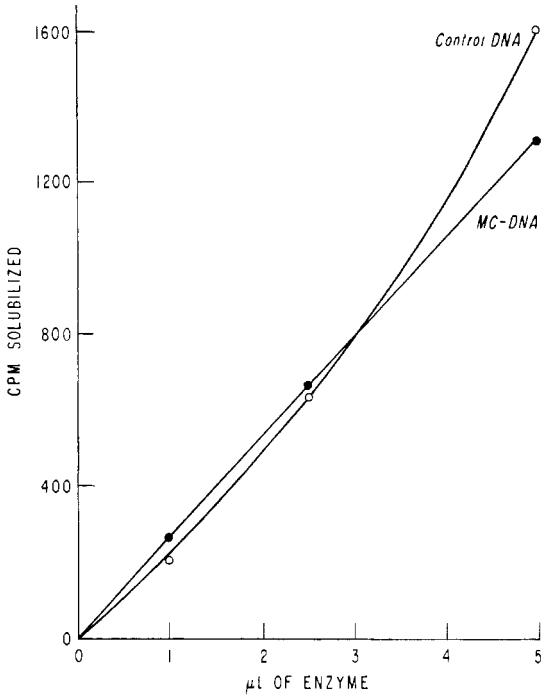

FIGURE 10: Degradation of MC-alkylated DNA by *E. coli* endonuclease. Reaction mixtures (0.2 ml) and incubations are described in Figure 5 except that *E. coli* K12λ endonuclease (1.43 units/ml) was the enzyme and the *p*H of the incubation mixture was 7.5.

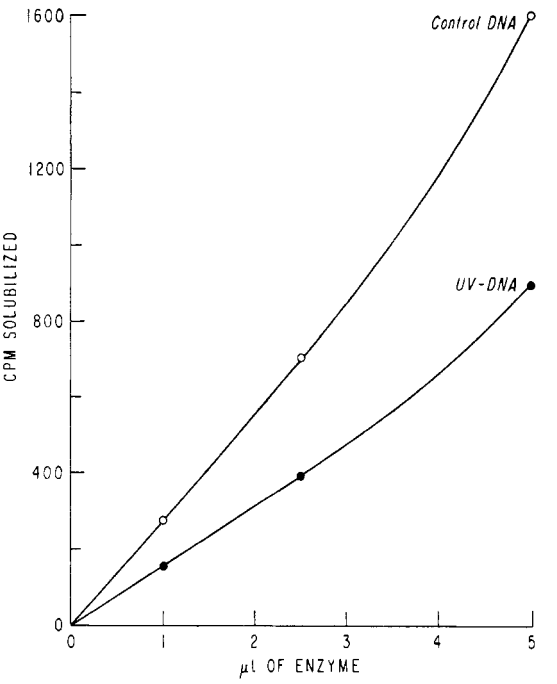

FIGURE 11: UV-DNA as substrate for *E. coli* endonuclease. Reaction mixtures are the same as in Figure 10 except that the substrate was UV-[^{3}H]DNA (preparation 1). Incubations were carried out at 37° for 15 minutes.

indeed, found in the experiments reported here. The relative inability of Exo I to degrade heat-denatured mitomycin-treated DNA (Figure 7) may also be because of the possibility that complete strand separation had not occurred during heat denaturation because of "cross-links" in the DNA (Iyer and Szybalski, 1963).

Both DNA and RNA polymerases apparently utilize DNA as a template by sequential base pairing along the DNA chain (Josse *et al.*, 1961; Weiss and Nakamoto, 1961). The presence of a mitomycin C residue in the DNA or of base dimers caused by ultraviolet irradiation might also affect the template activity of the DNA. In our studies the mitomycin C–alkylated DNA or ultraviolet radiation–damaged DNA is, in fact, utilized at an appreciably slower rate than the control DNA by both polymerases. Mitomycin C is known to inhibit potently DNA synthesis (Shiba *et al.*, 1959) and at high levels to suppress cellular RNA synthesis (Reich and Franklin, 1961). Bollum and Setlow (1963) also have previously reported that ultraviolet light lowers the ability of DNA, *in vitro*, to serve as a primer for calf thymus DNA polymerase.

On the other hand, endonucleases which attack in the interior of the DNA chain in a random fashion should not be appreciably affected by the presence of a relatively few molecules of antibiotics at scattered points along the DNA chain. This is borne out in experiments since both the *E. coli* and pancreatic endonucleases attack alkylated DNA as fast as control DNA. These enzymes can therefore avoid the alkylated or damaged regions of the DNA or are not inhibited by them.

It is of interest that mitomycin C and ultraviolet light are among the most efficient lysogenic inducers known for *E. coli* K12λ. As shown in this paper, both ultraviolet light and mitomycin C can affect DNA in a similar fashion with regard to susceptibility to various enzymes. The connection between such DNA damage, the cessation of DNA (or RNA) synthesis, and lysogenic induction remains to be clarified.

References

Ames, B. N., and Dubin, D. T. (1960), *J. Biol. Chem. 235*, 769.

Bollum, F. J., and Setlow, R. B. (1963), *Biochim. Biophys. Acta 68*, 599.

Chamberlin, M., and Berg, P. (1962), *Proc. Natl. Acad. Sci. U.S. 48*, 81.

Iyer, V. N., and Szybalski, W. (1963), *Proc. Natl. Acad. Sci. U.S. 50*, 355.

Iyer, V. N., and Szybalski, W. (1964), *Science 145*, 55.

Josse, J., Kaiser, A. D., and Kornberg, A. (1961), *J. Biol. Chem. 236*, 864.

Korn, D., and Weissbach, A. (1963), *J. Biol Chem. 238*, 3390.

Korn, D., and Weissbach, A. (1964), *J. Biol. Chem. 239*, 3849.

Kunitz, M. (1950), *J. Gen. Physiol. 33*, 349.

Lehman, I. R. (1960), *J. Biol. Chem. 235*, 1479.
Lehman, I. R., Bessman, M. J., Sims, E. S., and Kornberg, A. (1958), *J. Biol. Chem. 233*, 193.
Lehman, I. R., and Richardson, C. C. (1964), *J. Biol. Chem. 239*, 233.
Lehman, I. R., Roussos, G. G., and Pratt, E. A. (1962), *J. Biol. Chem. 237*, 819.
Marmur, J. (1961), *J. Mol. Biol. 3*, 208.
Pricer, W. E., Jr., and Weissbach, A. (1964), *J. Biol. Chem. 239*, 2607.
Reich, E., and Franklin, R. M. (1961), *Proc. Natl. Acad. Sci. U.S. 47*, 1212.
Setlow, R. B., Swenson, P. A., and Carrier, W. C. (1963), *Science 142*, 1464.
Shiba, S., Terawaki, A., Taguchi, T., and Kawamata, J. (1959), *Nature 183*, 1056.
Weiss, S., and Nakamoto, T. (1961), *Proc. Natl. Acad. Sci. U.S. 47*, 100.
Weissbach, A., and Korn, D. (1963), *J. Biol. Chem. 238*, 3383.
Weissbach, A., and Korn, D. (1964), *Biochim. Biophys. Acta 87*, 621.
Weissbach, A., and Lisio, A. (1965), *Biochemistry 4*, 196 (this issue; preceding paper).

The Site of Alkylation of Nucleic Acids by Mitomycin*

Marie N. Lipsett and A. Weissbach

ABSTRACT: Alkylation of synthetic polyribonucleotides with [^{3}H]mitomycin or [^{14}C]porfiromycin is shown to proceed at least four times as easily on guanine as on the other common bases. Alkylation of s-RNA with porfiromycin is demonstrated to yield both monoguanyl- and diguanyl-porfiromycin, the latter probably arising from an interstrand linkage. The alkylated guanine moieties are labilized from the s-RNA on storage, leaving depurinated strands in these areas.

Evidence has been increasing to show that mitomycin and chemically related antibiotics may act in large measure by combining with DNA. Mitomycin C is known to prevent DNA synthesis in bacteria (Shiba *et al.*, 1959) and to cause lysogenic induction (Korn and Weissbach, 1962).

The work of Iyer and Szybalski (1964) gave the first insight into the possible mode of action. When DNA was treated with reduced mitomycin C, its physical properties were changed in a manner suggesting the formation of cross-linkages. This process, which occurs *in vivo* or *in vitro*, may represent direct alkylation of the DNA. The extent of such "cross-linking" increased roughly with the G-C content of the DNA.

Weissbach and Lisio (1965) have provided a more direct demonstration that chemically reduced mitomycin C and porfiromycin can attach to isolated DNA, s-RNA, and ribosomal RNA. Incubation of the nucleic acids with hydrosulfite-reduced [^{3}H]mitomycin C or [^{14}C]porfiromycin led to the binding of one antibiotic molecule for approximately 500 nucleotides in each case.

In view of this relatively high degree of alkylation of the nucleic acids, the site of attachment of the antibiotic to the polynucleotide became of interest. This paper describes the isolation of fragments containing both guanine and antibiotic, either mitomycin or porfiromycin, from alkylated s-RNA.

Materials and Methods

Mitomycin C was obtained from Kyowa Hakko Kogyo Co., Tokyo. Porfiromycin was obtained from the Lederle Laboratories Div., American Cyanamid Co. Tritiated mitomycin C and [1a-^{14}C]porfiromycin were prepared by the method of Weissbach and Lisio (1965). Yeast s-RNA was obtained from General Biochemicals, and was freed from any residual traces of nucleases by passing the solutions three times through a column of Amberlite IRC-50 resin equilibrated with 0.2 M phosphate, *p*H 5.55. Sephadex G-25 was obtained from Pharmacia, Uppsala. Synthetic homopolymers and poly-GU were a gift from Dr. Leon Heppel. Venom phosphodiesterase was prepared according to the method of Koerner and Sinsheimer (1957). Pentose was determined by a micromodification of the method of Dische (1951).

Experimental

Reduction of the Antibiotics. It has been shown (Iyer and Szybalski, 1963) that mitomycin or porfiromycin requires a preliminary reduction in order to serve as alkylating agents. In the present work, this was accom-

* From the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. *Received October 30, 1964.*