

## Mutagenesis and repair of DNA damage caused by nitrogen mustard, *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU), streptozotocin, and mitomycin C in *E. coli*

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### Summary

Cytotoxicity and mutagenesis by streptozotocin, BCNU, nitrogen mustard, and mitomycin C were evaluated in *E. coli* mutants deficient in SOS repair, SOS-mediated mutagenesis, the adaptive response, and mutants that engage in aberrant mismatch repair. The results demonstrate that premutagenic lesions are caused by nitrogen mustard, BCNU and streptozotocin that are not repaired by *ada* or recognized by *umuDC*. Further, *recA* mutants were hypomutable after exposure to nitrogen mustard, BCNU, and streptozotocin compared to wild type. With the exception of the monofunctional nitrosourea, streptozotocin, both *recA* and *uvrA* gene products contribute to the repair of DNA damage caused by the alkylating agents tested. In the case of streptozotocin, although *recA* mutants were more sensitive than wild type, *uvrA* mutants were not. Moreover, while *ada* and *alkA* *E. coli* mutants showed increased sensitivity to streptozotocin, they were not more sensitive to the other alkylating agents evaluated.

While prior studies have elucidated the role of the adaptive response with respect to mutagenesis and repair of DNA damage by methylating and ethylating agents such as *N*-methyl-*N'*-nitro-*N'*-nitrosoguanidine (MNNG), relatively little is known about the contribution of repair mechanisms to mutagenesis and repair by other classes of agents than alkylate DNA (Jeggo et al., 1977; Samson and Cairns, 1977; Lindahl et al., 1982; Karran et al., 1982; Evensen and Seeberg, 1982; McCarthy et al., 1984). In this report we analyze

the involvement of a broad spectrum of repair mechanisms in repair and mutagenesis by various alkylators. The agents evaluated were BCNU (a bifunctional nitrosourea), streptozotocin (a monofunctional nitrosourea), nitrogen mustard (a bifunctional alkylator), and mitomycin C (an anti-tumor antibiotic that alkylates DNA).

Others have demonstrated that SOS repair ameliorates the cytotoxic effects of BCNU, nitrogen mustard and mitomycin C in *E. coli* and that *uvr* endonuclease contributes to the repair of a broad spectrum of bulky DNA adducts (Kohn et al., 1965; Murayama, 1972; Kacinski et al., 1985). The precise way by which SOS repair mediates mutagenesis by these agents, and in particular the contribution of the *umuDC* gene products to

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mutagenesis, is unclear. Further, because we previously have noted that aberrant mismatch repair markedly affects survival after exposure of *dam* *E. coli* mutants to MNNG and *cis*-platinum, we wished to evaluate whether aberrant mismatch repair similarly contributes to mutagenesis and repair by a broad spectrum of alkylating agents (Karran and Marinus, 1982; Fram et al., 1985).

Another principal aim was to assess the involvement of the adaptive response in mutagenesis and repair by these agents. While the levels of *O*<sup>6</sup>-alkyl-guanine-DNA alkyltransferase have been found to correlate both with survival and the induction of DNA interstrand cross-links by bifunctional nitrosoureas, the role of *O*<sup>6</sup>-alkyl-guanine-DNA alkyltransferase in repair and mutagenesis by these and other classes of alkylators was not further analyzed at the time (Brent et al., 1985; Erickson et al., 1980).

Our results demonstrate that mutagenesis by nitrogen mustard, BCNU, and streptozotocin involve repair mechanisms that are independent of *ada* and *umuDC*. Further, because *recA* cells are hypomutable compared to wild type, a role for a *recA* protein in mutagenesis by streptozotocin and BCNU is likely. With the exception of the monofunctional nitrosourea, streptozotocin, *recA* and *uvrA* gene products are involved in the repair of

DNA damage caused by these agents. While *recA* mutants are more sensitive than wild type to streptozotocin, *uvrA* mutants are not. The adaptive response contributes to repair of DNA damage induced by streptozotocin in contrast to the other alkylators evaluated. Lastly, aberrant mismatch repair solely affected mutagenesis by streptozotocin.

## Materials and methods

### Bacterial strains

The *E. coli* K-12 strains employed in the experiments are described in Table 1. GW2100 and the plasmid pBAR were gifts from Dr. G. Walker (MIT) and Dr. B. Demple (Harvard University), respectively.

### Chemical reagents

Nitrogen mustard and streptozotocin were obtained from Sigma Chemical Corporation (St. Louis, MO). A stock of 10<sup>-2</sup> M nitrogen mustard was prepared in 0.01 N HCl and kept at -20°C. Streptozotocin was prepared just prior to use in phosphate buffer. BCNU was a gift from Bristol-Myers Company (Syracuse, NY) and the Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD). BCNU was dissolved in

TABLE 1  
BACTERIAL STRAINS AND PLASMIDS

Strain	Synonym	Genotype
AB1157	Wild type	<i>thr-1 leuB6 thi-1 argE3 hisG4 proA2 lacY1 galK2 mtl-1 xyl-1 ara-14 rpsL31 tsx-33 glnV44 rfbD1 kdgK51</i>
AB2487	<i>recA</i>	As AB1157 but <i>recA13</i>
AB2500	<i>uvrA</i>	AS AB1157 but <i>uvrA6 thyA15 drm-2</i>
GM2927	<i>dam</i>	As AB1157 but <i>dam-13::Tn9</i>
GW2100	<i>umuDC</i>	As AB1157 but <i>umuC::Tn5</i>
GW5352	<i>ada</i>	As AB1157 but <i>ada::Tn10</i>
MS23	<i>alkA</i>	As AB1157 but <i>alkA his<sup>+</sup></i>
GM112	Wild type	<i>thr-1 leuB6 thi-1 metB1 hisG4 proA2 lacY1 galK2 ara-14 rpsL31 tsx-33 glnV44 rfbD1 kdgK51</i>
GM113	<i>dam-3</i>	As GM 112 but <i>dam-3</i>
GM3144		pMQ148/GM2927
GM1685		F- <i>lacI<sup>a</sup> lac ΔM15 pro<sup>+</sup>/GW5352</i>
GM3152		pBAR/F- <i>lac<sup>a</sup> lac ΔM15 pro<sup>+</sup>/GW5352</i>

All strains are derivatives of *E. coli* K-12. Plasmid pMQ148 is a derivative of pBR322 which carries the *dam* gene and is the same as *pdam118* (J.E. Brooks et al., 1983). GM3144 (pMQ148/GM2927) is phenotypically *dam<sup>+</sup>*. pBAR has the *ada* gene from *E. coli* B inserted into the vector pEMBL (Demple et al., 1985). While GM1685 has an *ada<sup>-</sup>* phenotype, GM3152, which is transformed with pBAR is *ada<sup>+</sup>*.

95% ethanol and stored at  $-20^{\circ}\text{C}$ . *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was obtained from Sigma and a 1-mg/ml stock was prepared in 0.01 M sodium acetate, pH 6.0 and frozen at  $-20^{\circ}\text{C}$ .

#### *Cytotoxicity and mutagenesis studies*

Cells were grown in brain-heart medium to mid-exponential phase, harvested and resuspended in phosphate buffer for 60 min at  $37^{\circ}\text{C}$ . 1 mM thymine was added when GM113 was employed. Cells were exposed for 30 min at  $37^{\circ}\text{C}$  to drug, washed twice, and then plated on brain-heart nutrient agar. Colonies were counted after incubation overnight at  $37^{\circ}\text{C}$ . Experiments were also performed with cells in mid-exponential growth phase, not starved prior to drug exposure. The results were very similar. Ampicillin (40  $\mu\text{g}/\text{ml}$ ) was added to plates when strains containing ampicillin-resistant plasmids were used (GM3152) and was also added to media at 20  $\mu\text{g}/\text{ml}$  during incubations at  $37^{\circ}\text{C}$ .

Mutation frequency was assessed by inoculating 1 ml of brain-heart media with a 10- $\mu\text{l}$  aliquot of cells after drug exposure, incubating overnight at  $37^{\circ}\text{C}$ , and then plating onto nutrient agar containing rifampicin (100  $\mu\text{g}/\text{ml}$ ). Mutants of *E. coli* which are resistant to rifampicin have altered RNA polymerase; these mutations map at the *rif* locus (Miller, 1972). Total numbers of viable bacteria were determined by plating onto nutrient agar plates. Results of representative experiments are shown. All values for % survival and mutation frequency represent the mean of duplicates. Duplicate values did not differ by more than 10% of the mean. All experiments were performed at least twice and in most instances 3 times.

In some of the experiments analyzing the contribution of the adaptive response to mutagenesis and cytotoxicity, *ada* mutants containing a plasmid with *F-lacI<sup>q</sup>* were transformed with pBAR. The latter plasmid contains the *ada* gene under the control of a *lac* repressor (Demple et al., 1985). This strain (GM3152) was developed so as to permit induction of the adaptive response by 1 mM isopropylthiogalactopyranoside (IPTG). The system was evaluated by exposing exponentially growing cells to MNNG 0–100  $\mu\text{g}/\text{ml}$  for 5 min at  $37^{\circ}\text{C}$ . Strains GM1685 (an *ada* mutant),

GM3152 (*ada*<sup>+</sup>), and GM3152 incubated with 1 mM IPTG during growth phase were assessed for cytotoxicity and mutagenesis by MNNG as previously described. Because mutagenesis and cytotoxicity did not differ in induced cells compared to cells not exposed to IPTG (see Table 2), subsequent experiments with alkylating agents were performed without IPTG.

#### Results

The contribution of SOS-mediated repair in preventing cytotoxicity by mitomycin C, nitrogen mustard, streptozotocin and BCNU is shown in Figs. 1 and 2. When cells were starved for 1 h and exposed to drug for 30 min at  $37^{\circ}\text{C}$ , *recA* mutants were markedly more sensitive to these agents than wild type. In contrast to *recA* cells *uvrA E. coli* cells were not more sensitive to streptozotocin although more sensitive than wild type to the other agents analyzed (see Fig. 2).

*E. coli dam* mutants, which engage in aberrant mismatch repair, are more sensitive than wild-type cells to the alkylating agent MNNG (Karran and Marinus, 1982). Cytotoxicity in *dam E. coli* mutants was therefore evaluated after exposure to mitomycin C, nitrogen mustard, streptozotocin, and BCNU. As shown in Fig. 3, *dam-3 E. coli* cells were no more sensitive than wild-type cells following a 30-min incubation with any of the alkylating agents. To insure that low levels of adenine methylase in starved, wild-type cells was not the basis for a negative result, experiments were also done with *dam* mutants transformed with pMQ148, a multi-copy plasmid containing the *dam* gene (J.E. Brooks et al., 1983). These cells were not starved prior to drug exposure. Survival of *dam* mutants transformed with pMQ148 (GM3144) which are phenotypically *dam*<sup>+</sup> was the same as that of *dam* mutants (GM2927) after exposure to any of the agents (data not shown).

The role of the adaptive response in preventing cytotoxicity by these drugs is evaluated in Fig. 4. Only streptozotocin caused enhanced cytotoxicity to an *ada* mutant.

These results were confirmed by evaluating cytotoxicity in an *ada* mutant (GM1685) and an *ada* mutant transformed with the multi-copy plasmid pBAR which contains the *ada* gene

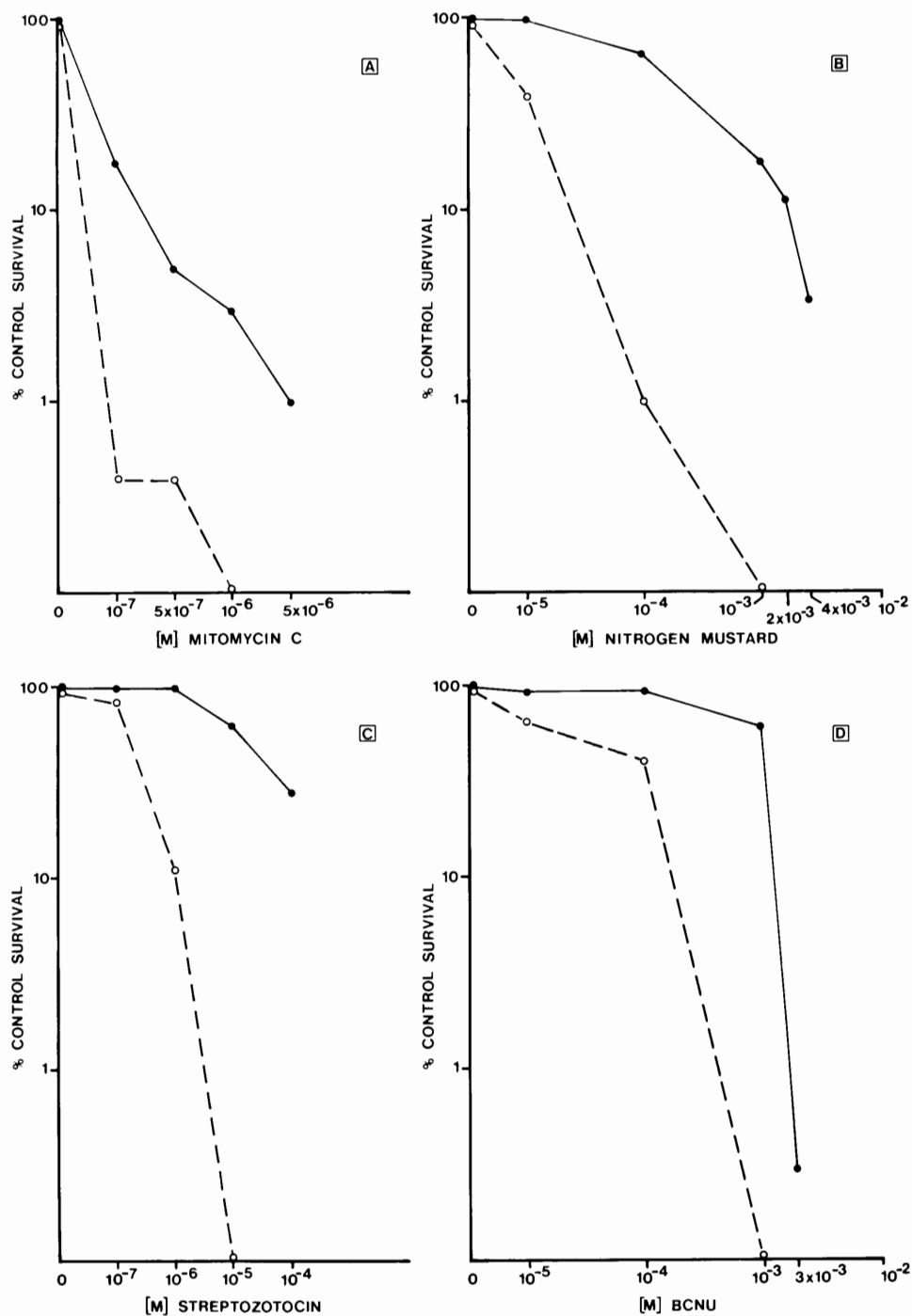


Fig. 1. Cytotoxicity by alkylating agents in *recA* and wild-type *E. coli*. Cells were grown to mid-exponential growth phase, resuspended in buffer, starved 1 h, and then exposed to drug for 30 min at 37°C. Cells were washed twice and survival analyzed by plating on nutrient agar after overnight incubation at 37°C. ○, *recA*; ●, wild-type *E. coli*.

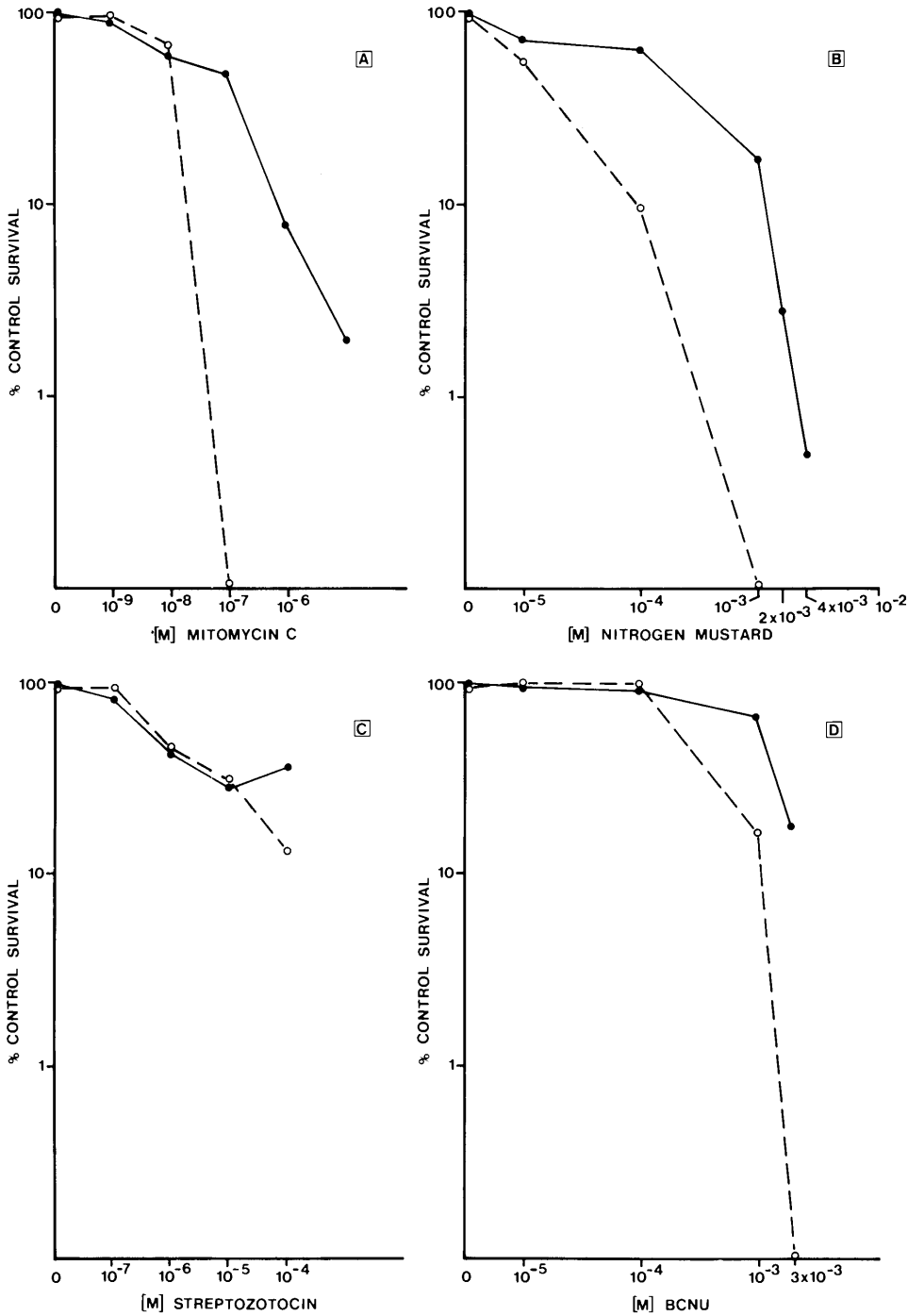


Fig. 2. Cytotoxicity by alkylating agents in *uvrA* and wild-type *E. coli*. Cells were grown to mid-exponential growth phase, resuspended in buffer, incubated for 1 h at 37°C, and then treated with drug for 30 min at 37°C. After cells were washed twice, viability was assessed as described in the text. ○, *uvrA*; ●, wild-type *E. coli*.

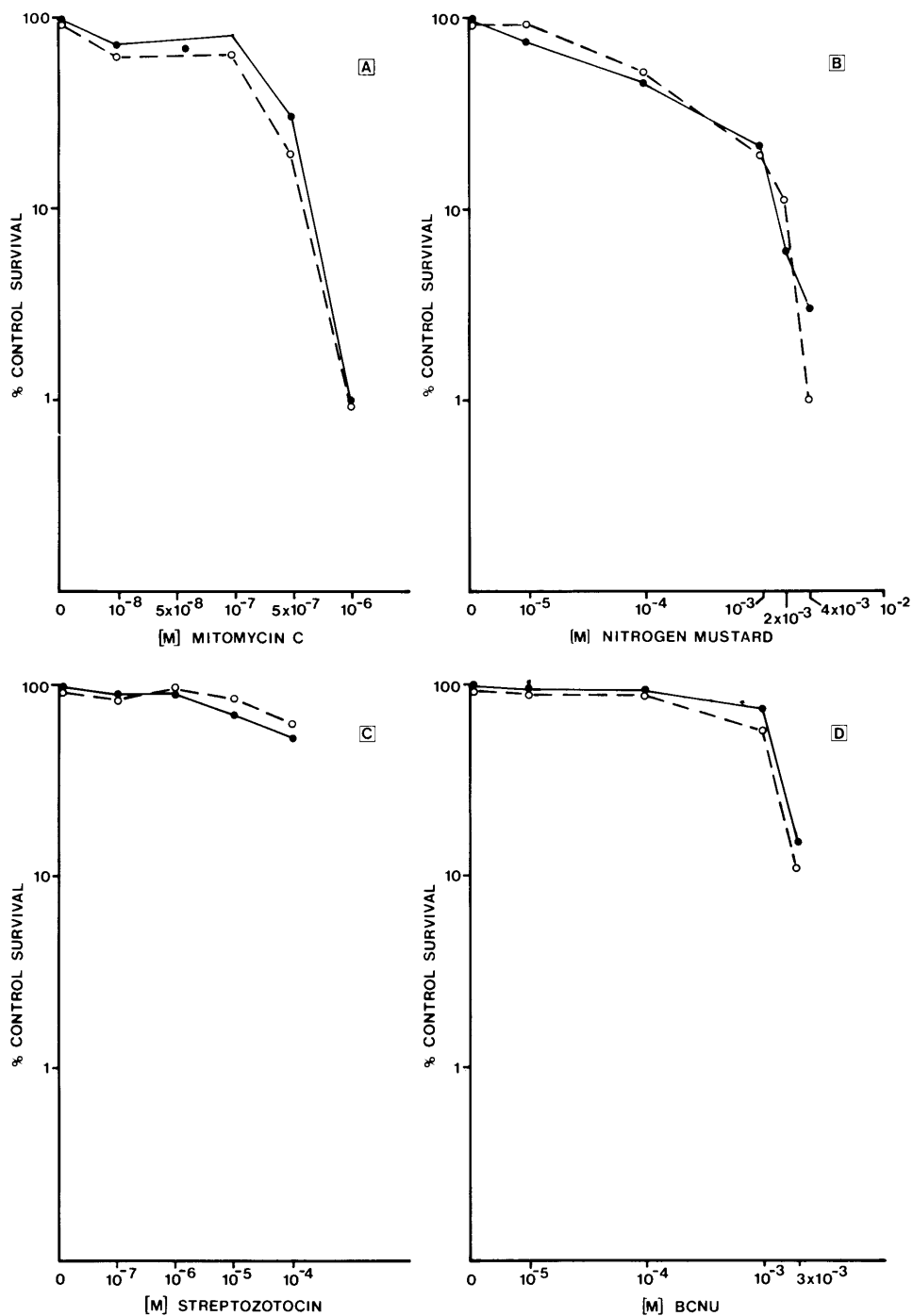


Fig. 3. Cytotoxicity by alkylating agents in *dam-3* and wild-type *E. coli*. Cells were grown to mid-exponential growth phase, resuspended in minimal salts, incubated for 1 h at 37°C, treated with drug for 30 min at 37°C. Cells were washed twice and viability analyzed as described in the text. ○, *dam-3*; ●, wild-type *E. coli*.

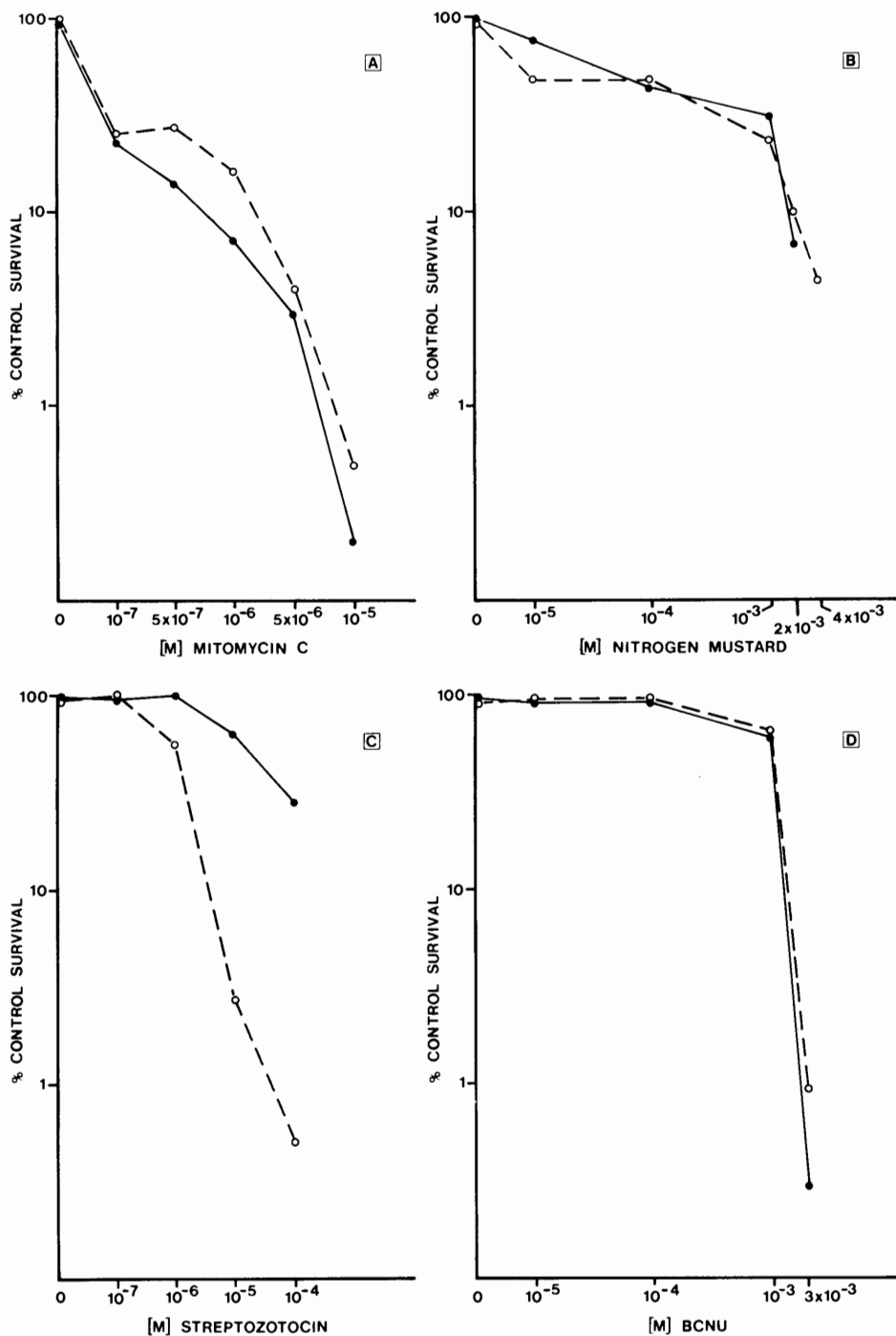


Fig. 4. Cytotoxicity by alkylating agents in *ada* and wild-type *E. coli*. Cells were grown to mid-exponential growth phase, resuspended in buffer, incubated for 1 h at 37°C and then treated with drug for 30 min at 37°C. Cells were washed twice and viability evaluated as described in the text. ○, *ada*; ●, wild-type *E. coli*.

(Demple et al., 1985). This strain (GM3152) was 5-fold less sensitive to MNNG than an *ada* mutant (GM1685) and was as resistant to MNNG as the same strain after induction of *ada* prior to drug exposure (see Table 2). Further, mutation frequency by MNNG in GM3152 was several 1000-fold reduced compared to GM1685 and was not reduced further when cells were grown in the presence of IPTG. *ada E. coli* were 10-fold more sensitive than GM3152 cells to streptozotocin (see Table 4). And as shown in prior experiments, *ada* mutants were not more sensitive than GM3152 cells to nitrogen mustard or BCNU than wild-type cells (Tables 3C and 5C).

Since *ada* mutants may be more sensitive to an agent by failing to induce synthesis of 3-methyladenine DNA glycosylase II, survival of *alkA* cells was evaluated after exposure to streptozotocin. Fig. 5 demonstrates that *alkA* cells exposed to streptozotocin are about 10-fold more sensitive than wild type. And, as is the case with the *ada* mutant, the *alkA* mutant is not more sensitive than wild type to the other agents.

TABLE 2  
MUTAGENESIS BY MNNG IN GM1685 (*ada*) AND GM3152 (pBAR/*ada*)

Strains	Concentration of MNNG ( $\mu\text{g}/\text{ml}$ )	Mutation frequency/ $10^8$ viable cells	Survival (%)
GM1685	0	0	100
	10	6464	12.7
	25	3921	<1
	50	18744	<1
	100	—	<1
GM3152	0	1	100
	10	91	39
	25	5	23
	50	6	12
	100	26	8
GM3152 *	0	0	100
	10	—	96
	25	192	58
	50	5	19
	100	91	12

Cells were grown to mid-exponential phase. GM3152 \* cells were grown in the presence of 1 mM IPTG. After resuspending cells in phosphate buffer, MNNG was added for 5 min at 37°C. Cells were washed twice and plated on nutrient agar. Mutagenesis was analyzed as described in the text.

The role of DNA repair mechanisms in mutagenesis by these agents was also analyzed (Tables 3–5). Data for 3 agents are shown because mitomycin C was not mutagenic in any of the strains tested. Mutagenesis by nitrogen mustard is remarkable in that mutation frequencies in both *umuDC* and *ada* mutants were comparable to those observed in wild-type *E. coli* (see Table 3A). *recA* cells were hypomutable compared to wild type. That this effect was primarily a result of enhanced toxicity in *recA* cells could not be excluded under these experimental conditions since *recA E. coli* cells were markedly sensitive to drug at concentrations which were mutagenic in wild-type cells (see Table 3A). The results also demonstrate that *dam-3 E. coli* cells were no more mutable by nitrogen mustard than were wild-type cells.

The role of the adaptive response in mutagenesis by nitrogen mustard was further analyzed by comparing mutation frequencies in cells that were (in effect) constitutively induced for the adaptive response with those obtained in cells of an isogenic *ada* mutant. Mutation frequency was not significantly different in GM3152 (a strain transformed with an *ada*-containing plasmid) compared to GM1685 after exposure to nitrogen mustard in each of two experiments (see Table 3C).

The induction of mutants by streptozotocin in the various strains is summarized in Table 4. As with nitrogen mustard, *umuDC* and *ada E. coli* cells yielded as many mutants as did wild-type cells. In contrast to the results obtained with nitrogen mustard and BCNU, however, *dam* mutants (GM113) were hypomutable after exposure to streptozotocin when compared to wild-type cells (GM112). This observation was confirmed by evaluating mutation frequency in another *dam* mutant (GM2927) as well as in cells of a derivative of this strain which had been transformed with pMQ148, a multi-copy plasmid that contains the *dam* gene (PMQ148/2927). As is evident from Table 4B, the mutation frequency was consistently lower in GM2927 than it was in to the same strain transformed with the *dam*-containing plasmid. Differences in toxicity do not underlie these results, since these strains did not differ significantly with respect to survival after exposure to streptozotocin (Table 4B).

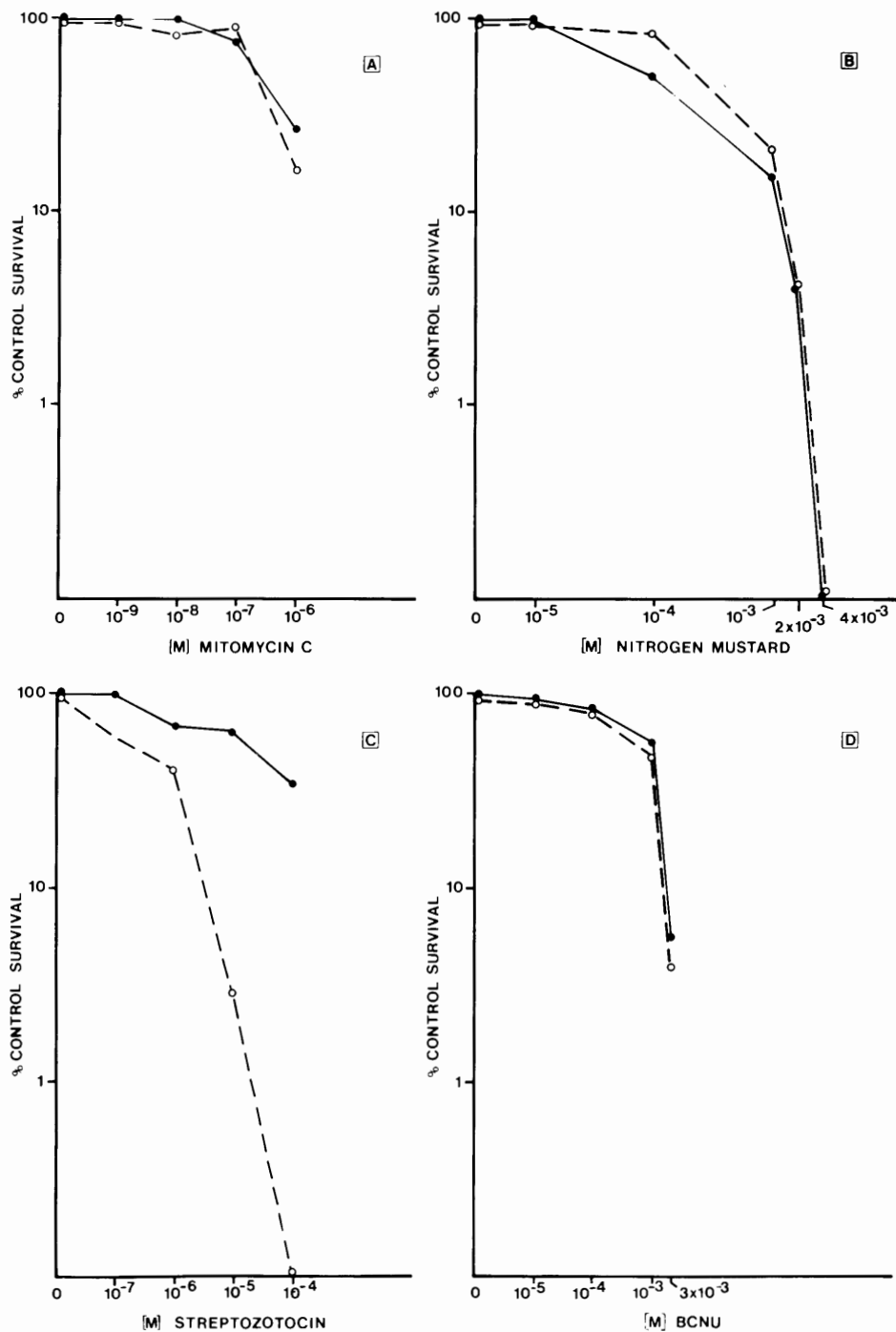


Fig. 5. Cytotoxicity by alkylating agents in *alkA* and wild-type *E. coli*. Cells were grown to mid-exponential growth phase, resuspended in buffer, incubated for 1 h at 37°C and then treated with drug for 30 min at 37°C. Cells were washed twice and viability assessed. ○, *alkA*; ●, wild-type *E. coli*.

TABLE 3  
MUTAGENESIS BY NITROGEN MUSTARD

Concentration of drug (M)	Mutation to rifampicin resistance/ $10^8$ viable cells					
	Wild type	<i>umuDC</i>	Wild type	<i>recA</i>	Wild type	<i>uvrA</i>
<b>3A. Effect of nitrogen mustard on mutation induction</b>						
0	0 (100)	0 (100)	0 (100)	0 (100)	1 (100)	0 (100)
$10^{-5}$	1 (100)	8 (78)	1 (100)	0 (40)	0 (71)	3 (56)
$10^{-4}$	41 (57)	18 (50)	20 (63)	1 (1)	2 (64)	1 (10)
$10^{-3}$	197 (13)	283 (12)	828 (17)	0 (0)	136 (17)	0 (0)
$2 \times 10^{-3}$	139 (4)	458 (4)	128 (11)	1 (0)	87 (3)	0 (0)
	Wild type	<i>ada</i>	Wild type	<i>alkA</i>		
0	0 (100)	0 (100)	0 (100)	4 (100)		
$10^{-5}$	0 (75)	1 (48)	6 (100)	0 (100)		
$10^{-4}$	1 (43)	0 (47)	0 (51)	0 (67)		
$10^{-3}$	0 (30)	12 (23)	18 (15)	18 (31)		
$2 \times 10^{-3}$	212 (6)	144 (9)	250 (4)	97 (4)		
	Wild type	<i>dam-3</i>				
<b>3B. Effect of nitrogen mustard on mutation induction in <i>dam</i><sup>-</sup> <i>E. coli</i></b>						
0	0 (100)	28 (100)				
$10^{-5}$	0 (100)	3 (100)				
$10^{-4}$	0	9 (94)				
$10^{-3}$	96 (51)	5 (32)				
$2 \times 10^{-3}$	54 (29)	206 (10)				
$4 \times 10^{-3}$	0 (1)	18 (2)				
	GM1685 ( <i>ada</i> )	GM3152 (pBAR/ <i>ada</i> )				
<b>3C. Effect of nitrogen mustard on mutation induction in GM1685 and GM3152</b>						
0	0 (100)	0 (100)				
$10^{-5}$	0 (88)	2 (60)				
$10^{-4}$	0 (77)	0 (44)				
$10^{-3}$	47 (37)	25 (11)				
$2 \times 10^{-3}$	9 (18)	8 (10)				
$4 \times 10^{-3}$	0 (1)	5 (3)				

Numbers in parentheses represent % control survival.

As with nitrogen mustard, *recA E. coli* cells were hypomutable compared to wild type after exposure to streptozotocin. This observation is not likely a result of enhanced toxicity in *recA* cells since markedly fewer mutants were induced in *recA* cells by  $10^{-6}$  M streptozotocin; conditions of exposure that were mutagenic for wild-type cells and not excessively toxic to *recA* cells.

To examine the issue of whether the adaptive response contributes to mutagenesis more carefully, experiments were also performed assessing mutation frequency in *ada* mutants and in *ada* mutants which had been transformed with the multi-copy plasmid pBAR containing the *ada* gene (see Table 4C). The results demonstrate that exposure of proliferating cells to  $10^{-7}$ – $10^{-6}$  M

streptozotocin for 30 min at 37°C causes mutagenesis to a similar extent in both *ada E. coli* and in *ada* mutants transformed with pBAR. Exposure to concentrations of drug in the  $10^{-5}$ – $10^{-4}$  M range, however, results in a markedly higher frequency of mutation induction in *E. coli ada* mutants than in cells possessing multiple copies of the *ada* gene. This effect is not caused by enhanced toxicity since cells transformed with the *ada*-containing plasmid are, in fact, more resistant to drug than *ada E. coli* mutants (see Table 4C).

Studies on mutagenesis by BCNU are summarized in Table 5. The results demonstrate that *umuDC* and *ada E. coli* are mutated to the same extent as wild-type cells. As with nitrogen mustard and streptozotocin, *dam* mutants were not hyper-

TABLE 4  
MUTAGENESIS BY STREPTOZOTOCIN

Concentration of drug (M)	Mutation to rifampicin resistance/10 <sup>8</sup> viable cells			
	Wild type	<i>umuDC</i>	<i>uvrA</i>	
<b>4A. Effect of streptozotocin on mutation induction in <i>E. coli</i></b>				
0	7 (100)	6 (100)	0 (100)	
10 <sup>-6</sup>	586 (80)	450 (100)	526 (92)	
10 <sup>-5</sup>	3 340 (41)	4 065 (51)	4 429 (45)	
10 <sup>-4</sup>	5 351 (37)	6 235 (25)	4 360 (30)	
10 <sup>-3</sup>	6 457 (27)	5 492 (13)	2 036 (13)	
	Wild type	<i>recA</i>	<i>ada</i>	
0	0 (100)	0 (100)	0 (100)	
10 <sup>-7</sup>	0 (96)	0 (34)	1 727 (98)	
10 <sup>-6</sup>	327 (100)	0 (11)	6 710 (56)	
10 <sup>-5</sup>	3 361 (62)	0 (0)	2 514 (3)	
10 <sup>-4</sup>	4 208 (7)	0 (0)	1 204 (0)	
	Wild type	<i>alkA</i>		
0	0 (100)	0 (100)		
10 <sup>-7</sup>	51 (100)	0 (62)		
10 <sup>-6</sup>	73 (79)	357 (42)		
10 <sup>-5</sup>	8 012 (68)	2 957 (3)		
10 <sup>-4</sup>	1 564 (35)	11 745 (0)		
	GM112 (wild type)	GM113 ( <i>dam-3</i> )	pMQ148/GM2927	GM2927 ( <i>dam-13::Tn9</i> )
<b>4B. Effect of streptozotocin on mutation induction in <i>dam</i><sup>-</sup> <i>E. coli</i></b>				
0	0 (100)	0 (100)	0 (100)	4 (100)
10 <sup>-7</sup>	0 (90)	9 (86)	0 (100)	14 (89)
10 <sup>-6</sup>	460 (91)	0 (99)	3 471 (93)	655 (10)
10 <sup>-5</sup>	11 722 (70)	0 (86)	3 783 (8)	54 (12)
10 <sup>-4</sup>	17 000 (53)	0 (76)	2 021 (2)	115 (<1)
	Mutation frequency/10 <sup>8</sup> viable cells			
	GM1685 ( <i>ada</i> )	GM3152 (pBAR/ <i>ada</i> )		
<b>4C. Effect of streptozotocin on mutation in GM1685 and GM3152</b>				
0	0 (100)	0 (100)		
10 <sup>-7</sup>	1 970 (72)	364 (90)		
10 <sup>-6</sup>	10 142 (25)	6 538 (65)		
10 <sup>-5</sup>	3 043 (<1)	112 (18)		
10 <sup>-4</sup>	3 708 (<1)	0 (2)		

Numbers in parentheses represent % control survival.

mutable compared to wild type after exposure to BCNU. Further, as noted with streptozotocin and nitrogen mustard, mutation frequency was low in *recA* mutants. Although *recA* mutants were markedly sensitive to this agent (see Fig. 1), absence of *recA* gene product may underlie these findings since hypomutability occurs at survival levels associated with markedly higher mutation frequencies in wild type.

The effect of inducing the adaptive response on mutagenesis by BCNU also was analyzed. No

marked difference in mutation frequency occurred in GM3152 compared to GM1685, an *ada E. coli* mutant.

## Discussion

We have assessed the involvement of repair mechanisms in cytotoxicity and mutagenesis by 4 drugs that represent different classes of alkylators. These agents are commonly used in the therapy of patients with solid tumors. Patients who are treated

TABLE 5  
MUTAGENESIS BY BCNU

Concentration of drug (M)	Mutation to rifampicin resistance/10 <sup>8</sup> viable cells		
	Wild type	<i>umuDC</i>	<i>wvrA</i>
<b>5A. Effect of BCNU on mutation induction in <i>E. coli</i></b>			
0	0 (100)	0 (100)	0 (100)
10 <sup>-5</sup>	1 (92)	1 (100)	16 (100)
10 <sup>-4</sup>	1 (92)	1 (82)	32 (100)
10 <sup>-3</sup>	11 (65)	1005 (80)	323 (16)
3 × 10 <sup>-3</sup>	1224 (17)	2286 (24)	0 (11)
	Wild type	<i>recA</i>	<i>ada</i>
0	0 (100)	0 (100)	0 (100)
10 <sup>-5</sup>	0 (91)	0 (64)	0 (100)
10 <sup>-4</sup>	3 (92)	1 (40)	2 (93)
10 <sup>-3</sup>	64 (60)	0 (<1)	230 (67)
3 × 10 <sup>-3</sup>	0 (<1)	0 (<1)	65 (10)
	Wild type	<i>alkA</i>	
0	2 (100)	0 (100)	
10 <sup>-5</sup>	8 (98)	6 (95)	
10 <sup>-4</sup>	4 (83)	24 (82)	
10 <sup>-3</sup>	257 (57)	443 (48)	
3 × 10 <sup>-3</sup>	4053 (6)	9450 (4)	
	Wild type	<i>dam-3</i>	
0	0 (100)	3 (100)	
10 <sup>-5</sup>	4 (97)	4 (98)	
5 × 10 <sup>-4</sup>	8 (91)	10 (79)	
10 <sup>-3</sup>	26 (74)	35 (57)	
5 × 10 <sup>-3</sup>	281 (15)	543 (11)	
	GM1685 ( <i>ada</i> )	GM3152 (pBAR/ <i>ada</i> )	
<b>5C. Effect of BCNU on mutation induction in GM1685 and GM3152</b>			
0	0 (100)	2 (100)	
10 <sup>-5</sup>	1 (100)	5 (95)	
10 <sup>-4</sup>	0 (100)	8 (90)	
5 × 10 <sup>-4</sup>	48 (12)	68 (1)	
10 <sup>-3</sup>	2 (5)	0 (<1)	
5 × 10 <sup>-3</sup>	0 (2)	0 (<1)	

Numbers in parentheses represent % control survival.

with alkylating agents, particularly monofunctional nitrosoureas, have a increased risk of second malignancies (Canellos, 1984). Thus, understanding mechanisms underlying repair and mutagenesis by these agents is relevant from a basic as well as a clinical perspective.

Both SOS repair and the adaptive response affect cytotoxicity caused by monofunctional and bifunctional nitrosoureas. Our studies demonstrate that *recA* and *wvrA* mutants are more sensitive than wild type to BCNU. In contrast to the

situation with BCNU, however, *recA* mutants are more sensitive to streptozotocin than wild type but *wvrA* mutants are not. The ability of BCNU to cross-link DNA (and also perhaps the bulky size of the adduct it forms with DNA) may underlie the sensitivity of the *wvrA* mutant to this drug (Ludlum and Tong, 1981). Mitomycin C, nitrogen mustard, and BCNU all cross-link DNA and probably form large DNA adducts, resulting in enhanced cytotoxicity in *wvrA* mutants compared to wild type (Ewig and Kohn, 1977; Iyer and Szybalski, 1963). Unlike the other alkylating agents tested, streptozotocin does not form DNA inter-strand cross-links (Mossman et al., 1986). Further, others have observed that *wvrA* mutants are no more sensitive than wild type to *N*-ethyl-*N*-nitrosourea (a nitrosourea that does not cross-link DNA), while *wvrA* mutants are markedly sensitive to BCNU and *N*-(2-chloroethyl)-*N*-nitrosourea, agents which both form larger adducts and cross-link DNA (Kacinski et al., 1985).

That RecA gene product mediates repair of lesions independently of its role in derepressing *wvr* endonuclease is suggested by the enhanced cytotoxicity observed in *recA* mutants as compared to *wvrA* mutants exposed to these agents. While such differences are most marked after exposure to streptozotocin, the other agents analyzed also are more cytotoxic in *recA* mutants than in *wvrA* mutants.

The involvement of the adaptive response in the repair of lesions induced by streptozotocin is suggested by the enhanced sensitivity of the *ada* mutant to this agent. Further, this effect may be explained by participation of 3-methyl-adenine DNA glycosylase in the repair of lesions induced by this agent, since *alkA* *E. coli* cells are similarly sensitive to streptozotocin.

In contrast to streptozotocin, however, *ada* mutants are no more sensitive to BCNU than wild type. In both eukaryotic cell lines and tumor xenografts, cytotoxicity by bifunctional nitrosoureas is reduced in those tumors with high levels of *O*<sup>6</sup>-alkylguanine-DNA alkyltransferase (Erickson et al., 1980; Brent et al., 1984, 1985; Kohn et al., 1981; Robins et al., 1983). Failure of DNA adducts formed by BCNU to induce the adaptive response in wild-type cells is not a likely explanation for our findings, since survival of GM3152

cells (which in effect are constitutively induced for the adaptive response) is the same as that of *ada* cells exposed to BCNU.

Our findings suggest that in prokaryotes SOS repair rather than the adaptive response is the principal mechanism underlying repair of BCNU-induced DNA damage. In support of such a view, others have noted that induction of the adaptive response in *uvrA* and *recA* *E. coli* did not enhance survival (as compared to uninduced mutants) after exposure to BCNU (Kacinski et al., 1985). Such findings may not be applicable to eukaryotic cells since prokaryotic *O*<sup>6</sup>-alkylguanidine-DNA alkyltransferase is less efficient in removing ethyl adducts than is the eukaryotic transferase (Sedgwick and Lindahl, 1982).

Our results also demonstrate that, in contrast to the results found for MNNG and *cis*-platinum (Karran and Marinus, 1982; Fram et al., 1985), aberrant mismatch repair does not alter cytotoxicity by the alkylating agents analyzed in this study. These findings probably reflect differences both in the structure of DNA adducts formed and in the sites in DNA in which these adducts occur.

Mutagenesis by the alkylators analyzed in our experiments reveals that in contrast to agents such as *cis*-platinum and MNNG, these alkylators mediate mutagenesis via pathways not affected by gene products specified by the *ada* and *umuDC* genes. Thus *ada* and *umuDC* *E. coli* are as mutable as wild-type cells after exposure to nitrogen mustard, BCNU, and streptozotocin. The involvement of *ada* in mutagenesis by streptozotocin is complex. For example, an *ada* strain transformed with a multi-copy plasmid carrying *ada* (GM3152) was hypomutable after exposure to high concentrations ( $10^{-5}$ – $10^{-4}$ M) but was normally mutable at low drug concentrations ( $10^{-7}$ – $10^{-6}$  M) (see Table 4C). These findings are not the result of differences in toxicity since an *ada* strain transformed with pBAR was more resistant to streptozotocin than an *ada* strain without pBAR. While the basis for such findings is unclear, the induction of premutagenic lesions not corrected by *ada* appears likely.

The basis for the low mutation frequency observed in *dam* *E. coli* cells as compared to wild type after exposure to streptozotocin is unclear. The findings are consistent with the involvement

of the mismatch repair mechanism in the recognition and removal of premutagenic, noncytotoxic lesions. Because *dam* *E. coli* cells fail to methylate adenine in GATC sequences at or near the replication fork, mismatch repair occurs with equal efficiency on both the parental and daughter DNA strand (Pukkila et al., 1981). More extensive albeit aberrant mismatch repair may cause the more efficient repair of premutagenic lesions induced by the drug.

Mutagenesis by BCNU, as with that by nitrogen mustard, is not affected by gene products specified by *ada*, *umuDC*, or *dam*, but is affected by *recA*. In contrast to streptozotocin, *dam* *E. coli* mutants were not hypomutable after exposure to BCNU. The basis for this finding is unclear, but differences in adduct size as well as sites of adduct formation may be relevant.

A role for the RecA gene product in mediating mutagenesis by these agents is suggested by markedly reduced mutation frequency in *recA* mutants. The RecA protein participates in mutagenesis by UV irradiation independently of its effect in depressing *umuDC* (Ennis et al., 1985) and similar events may be pertinent to mutagenesis by streptozotocin, nitrogen mustard, and BCNU. Studies analyzing the role of RecA gene product in alkylator-induced mutagenesis in greater detail are currently underway. Future directions of our work also include characterizing repair mechanisms other than those specified by *ada* and *umuDC* that mediate mutagenesis by alkylating agents.

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