JOURNAL OF BACTERIOLOGY, Oct. 1984, p. 288–293
0021-9193/84/100288-06$02.00/0

Vol. 160, No. 1

Mutagenesis of *Neisseria gonorrhoeae*: Absence of Error-Prone Repair

L. A. CAMPBELL[1]† AND R. E. YASBIN[2]*

Department of Biochemistry, Microbiology and Molecular Biology, Pennsylvania State University, University Park, Pennsylvania 16802,[1] and Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642[2]

Received 9 May 1984/Accepted 16 July 1984

The lethal and mutagenic effects of various mutagens on *Neisseria gonorrhoeae* were investigated. Lethality studies demonstrated that *N. gonorrhoeae* was relatively sensitive to ethyl methanesulfonate, UV light, and methyl methanesulfonate. Although *N. gonorrhoeae* was readily mutated by ethyl methanesulfonate and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine for the three genetic markers assayed, no increase in the mutation frequency was observed for any of the selective markers after UV irradiation or methyl methanesulfonate treatment. These results suggest that *N. gonorrhoeae* lacks an error-prone repair mechanism.

Infection with *Neisseria gonorrhoeae* can result in a variety of clinical manifestations which include gonorrhea, pharyngitis, pelvic inflammatory disease, arthritis, and disseminated gonococcal infection (21). This organism demonstrates a wide diversity of phenotypic types and has the capacity to alter its antigenic, structural, and pathogenic states (10, 13, 26). The molecular mechanisms responsible for these various phase transitions are poorly understood.

One of the factors that has impeded genetic studies of virulence mechanisms and the extension of the map of the gonococcal genome is the low efficiency of mutagenesis of this organism in the laboratory. To understand the processing of DNA damage in *N. gonorrhoeae* and the mechanisms by which it avoids mutations, our laboratory has focused on delineating the DNA repair capacities of this organism. To date, *N. gonorrhoeae* has been shown to lack photoreactivation and to possess an excision repair system (1, 2). The purpose of this study was to assess the mutability of *N. gonorrhoeae* by both direct and indirect mutagens.

Direct mutagens cause mutations by mispairing mechanisms either involving template or nucleotide presursors (16, 19). Alternatively, indirect mutagens act by inducing a postreplication repair system that is error prone (16, 19). Hence, mutations resulting from their action are caused by misrepair. In *Escherichia coli*, error-prone repair is one of the many damage-inducible functions that have been collectively designated as the SOS response (18, 32). Expression of error-prone repair in *E. coli* is dependent on the *recA*$^+$, *lexA*$^+$, and *umuC*$^+$ gene products (9, 30).

Many organisms have been identified which lack the capacity to be mutagenized by indirect mutagens. As these organisms include *Haemophilus influenzae*, *Micrococcus radiodurans*, *Streptococcus pneumoniae*, *Streptococcus mutans*, and *Streptococcus sanguis*, the common factor that emerges from this information is that naturally competent organisms appear to lack error-prone repair (24, 33). Thus, another facet of this endeavor was to investigate further the relationship between competency and error-prone repair by examining the potential of a competent organism, *N. gonorrhoeae*, to be mutagenized by indirect mutagens.

* Corresponding author.
† Present address: Department of Microbiology, School of Medicine and Dentistry, University of Rochester, Rochester, NY 14642.

MATERIALS AND METHODS

Strains. The bacterial strains used are listed in Table 1.

Media and chemicals. Liquid cultures of *N. gonorrhoeae* were routinely grown in GCK broth, which is composed of GC medium base (Difco Laboratories) and 1% Kellogg supplements (10). Liquid cultures of *Bacillus subtilis* were grown in Penassay broth (Antibiotic Medium no. 3; Difco Laboratories) and plated onto Tryptose Blood Agar base (Difco). Cultures of *E. coli* were grown in and plated on LB medium (15). All amino acids, ethyl methanesulfonate (EMS), and methyl methanesulfonate (MMS) were obtained from Sigma Chemical Co. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was obtained from Aldrich Biochemicals.

Treatment with DNA-damaging agents. Cultures were routinely grown with aeration at 275 rpm on a Gyratory shaker (New Brunswick Scientific Co.) at 37°C to an optical density of 100 Klett units as measured by a Klett-Summerson colorimeter with filter no. 66. For survival determinations, samples were either washed once, suspended in GCK broth, and plated at the appropriate dilutions or were immediately diluted into Spizizen salts (25) and plated. For mutagenesis experiments, 1-ml portions were removed after the appropriate exposure time, centrifuged for 1 min at 8,000 × *g* at room temperature, and resuspended in 1 ml of GCK broth. The samples were then added to 9 ml of GCK broth in a 250-ml DeLong flask. Cells were incubated for 24 or 48 h at 35°C in 7% CO_2 (without aeration) before plating onto selective medium. The selective antibiotic medium was GCK broth containing either rifampin (0.125 μg/ml) or nalidixic acid (0.7 μg/ml). Prototrophs of strain F62 were selected by plating onto GGM medium lacking proline (14). After incubation, any putative mutants were isolated from individual colonies and tested on the appropriate selective medium. The mutation frequency was determined by dividing the number of mutants by the number of survivors after 24 or 48 h of incubation.

Specific treatments with alkylating agents. EMS was added to a final concentration of 1, 2, or 5% (vol/vol). Treatments with MMS used a final concentration of 0.01, 0.025, or 0.05% (vol/vol). For treatment with EMS or MMS, cultures were incubated at 37°C with aeration for various time periods.

MNNG was prepared at 1 mg/ml in 0.2 M sodium acetate buffer, pH 5.5, and frozen in 1-ml aliquots at −20°C until

TABLE 1. Bacterial strains[a]

Strain	Genotype or phenotype	Source
N. gonorrhoeae		
WLPI (ATCC 19424)	*met-1*	F. E. Young[b]
F62	*pro-1*	F. E. Young
FA19	Wild type	P. F. Sparling[c]
FA248	$Met^- Pro^-$	P. F. Sparling
HS208	$Arg^- Val^- Hyp^- Ura^- Ile^-$	F. E. Young
FA534	*rif-7* (No plasmids)	P. F. Sparling
FA514	*str-12* (Contains pF10)	P. F. Sparling
FA583	*nal-1 arg-1 met-1 pro-1*	P. F. Sparling
HVC1	Not determined	Clinical isolate[d]
B. subtilis		
BR151	*trpC2 metB10 lys-3*	R. E. Yasbin
YB886	*trpC2 metB5 xin-1* SPB^-	R. E. Yasbin
YB1005	*trpC2 metB5 xin-1 uvrA42* SPB^-	R. E. Yasbin

[a] Where allele numbers have not been assigned for the strain, the auxotype is designated.
[b] University of Rochester, N. Y.
[c] University of North Carolina, Chapel Hill.
[d] Pennsylvania State University, University Park.

needed. MNNG was added to cells at a final concentration of 10 μg/ml. Cultures were incubated with or without aeration at 37°C.

UV irradiation. Before UV irradiation of cells, cultures were pelleted by centrifugation (10,000 rpm, 4°C) for 10 min and resuspended in the original volume in Spizizen salts. The UV source was a single germicidal lamp (model G8T5; Sylvania) with a maximum output at 254 nm. The flux of the UV source was 0.8 to 1.0 W/m^2 as measured by a Blak-Ray J-225 meter (Ultraviolet Products). Ten milliliters of bacteria (10^8 CFU/ml) were UV irradiated in a 100-mm glass petri dish with constant stirring during exposure.

RESULTS

Sensitivity of *N. gonorrhoeae* to DNA-damaging agents. Mid-exponential-phase cells of various strains of *N. gonorrhoeae* were exposed to different DNA damaging agents which included UV, MMS, EMS, and MNNG. Although a heterogeneous response was observed, all strains of *N. gonorrhoeae* tested were more sensitive to UV irradiation or MMS treatment than was repair-proficient *B. subtilis* BR151 (Fig. 1). Based on the prediction of the target theory (17), one would expect that if both organisms possess equal repair capacities, *N. gonorrhoeae* would be slightly more resistant to UV than *B. subtilis* would be, since the *B. subtilis* genome is larger (2×10^9 to 3×10^9 daltons).

Analogous results were obtained after treatment of several strains of *N. gonorrhoeae* with 1% EMS or 10 μg of MNNG per ml for different time periods since all strains tested were more sensitive to these alkylating agents than was repair-proficient *B. subtilis* BR151 (data not shown). These same lethality studies were also done by using a repair-proficient *E. coli* isolate that had survival levels comparable to those

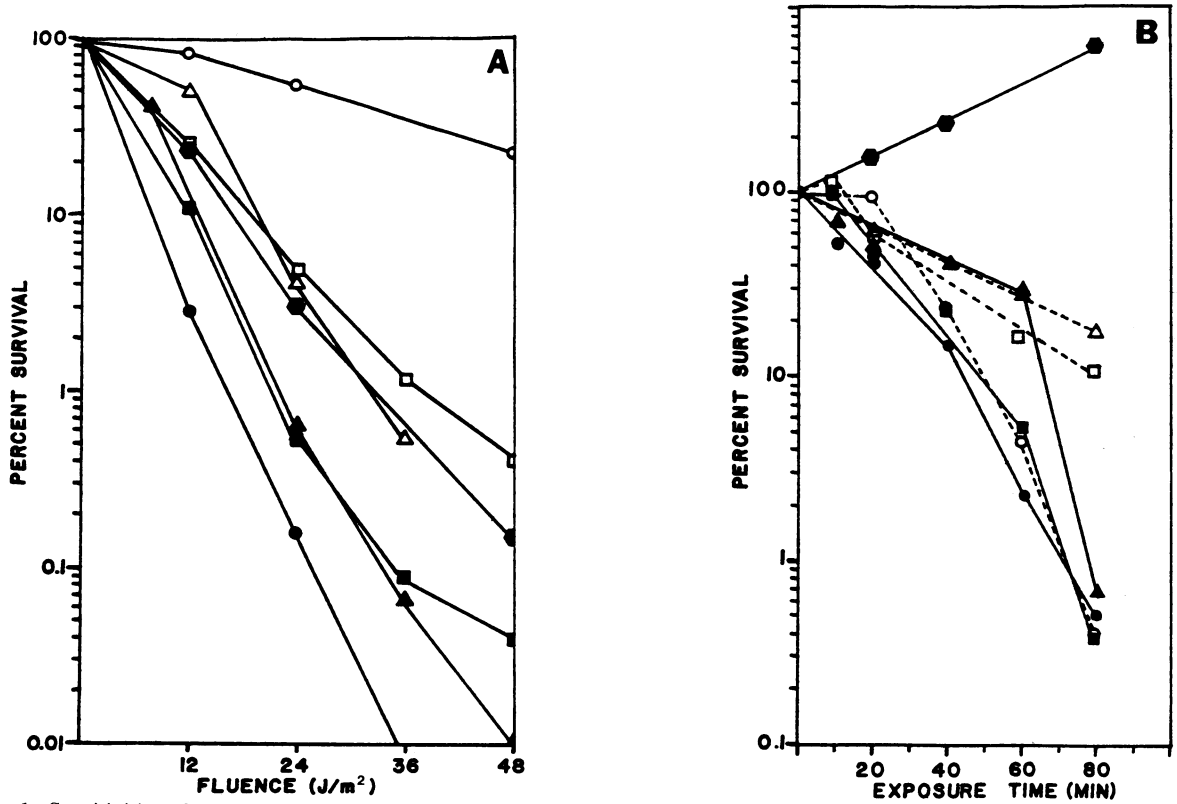

FIG. 1. Sensitivities of various strains of *N. gonorrhoeae* to UV irradiation and MMS treatment. (A) Sensitivity of *N. gonorrhoeae* to UV irradiation. Different strains of *N. gonorrhoeae* were UV irradiated as described in the text. Symbols: □, strain WLPI; ⬬, strain FA248; △, strain FA534; ■, strain F62; ▲, strain FA19; ●, strain FA514; ○, *B. subtilis* BR151. (B) Sensitivities of *N. gonorrhoeae* to MMS treatment. Mid-exponential-phase cells were incubated at 37°C with aeration in GCK broth containing a final concentration of 0.01% MMS (vol/vol) for various time periods as described in the text. Symbols: □, strain FA248; △, strain HS208; ▲, strain FA19; ●, strain HVC-1; ○, strain FA514; ■, strain WLPI; ⬬, *B. subtilis* BR151.

for *B. subtilis* BR151 (data not shown). These observations suggested that *N. gonorrhoeae* was deficient in a repair pathway(s) that renders it more susceptible to the lethal effects of these physical and chemical agents.

Mutability of *N. gonorrhoeae*. In choosing mutagens for this study, the main consideration was to include representatives of both direct mutagens and indirect mutagens. Two examples of direct mutagens were EMS (20) and MNNG (6). Mutagenesis by these alkylating agents is largely independent of the *recA* gene in *E. coli* (6, 8), and thus, mutations readily occur in organisms that lack an error-prone repair system. Conversely, UV irradiation and MMS were chosen as they are well-defined examples of indirect mutagens (3, 31). Mutations resulting from treatment with UV or the alkylating agent MMS are caused by the induction of a postreplication repair system that is highly error prone (16, 32).

EMS mutagenesis. Treatment of strain F62 with 1% EMS for various time periods resulted in a substantial increase in number of nalidixic acid-resistant (Nal^r) mutants, rifampin-resistant (Rif^r) mutants, or revertants from the proline auxotrophic phenotype to prototrophy (Pro^+) (Fig. 2A). Incubation of EMS-treated cells for 48 h instead of 24 h before selective challenge did not result in any increase in the number of mutants obtained (Fig. 2A). Identical results were obtained with strain WLPI (data not shown). For both strains, optimal mutation frequencies were obtained after a 60-min exposure to EMS which resulted in survival levels of 10 to 25% for strain WLPI and 0.4 to 2% for strain F62. However, depending on the particular batch of EMS used, occasionally an 80-min exposure resulted in a slightly higher mutation frequency.

Increasing the concentration of EMS to 2% (vol/vol) decreased the exposure time required for maximum mutagenesis but did not significantly alter the number of mutants obtained (data not shown). A concentration of 5% (vol/vol) EMS drastically decreased the mutation frequency because of the increased lethality.

MNNG mutagenesis. Treatment with 10 μg of MNNG per ml for various time periods resulted in an increased mutation frequency for all markers selected in strain F62 (Fig. 2B). Strain WLPI was also readily mutated to both Nal^r and Rif^r by MNNG (data not shown). Quantitatively, variability was observed in the degree of augmentation depending on the particular batch of MNNG used. However, both strains were always readily mutated by this agent.

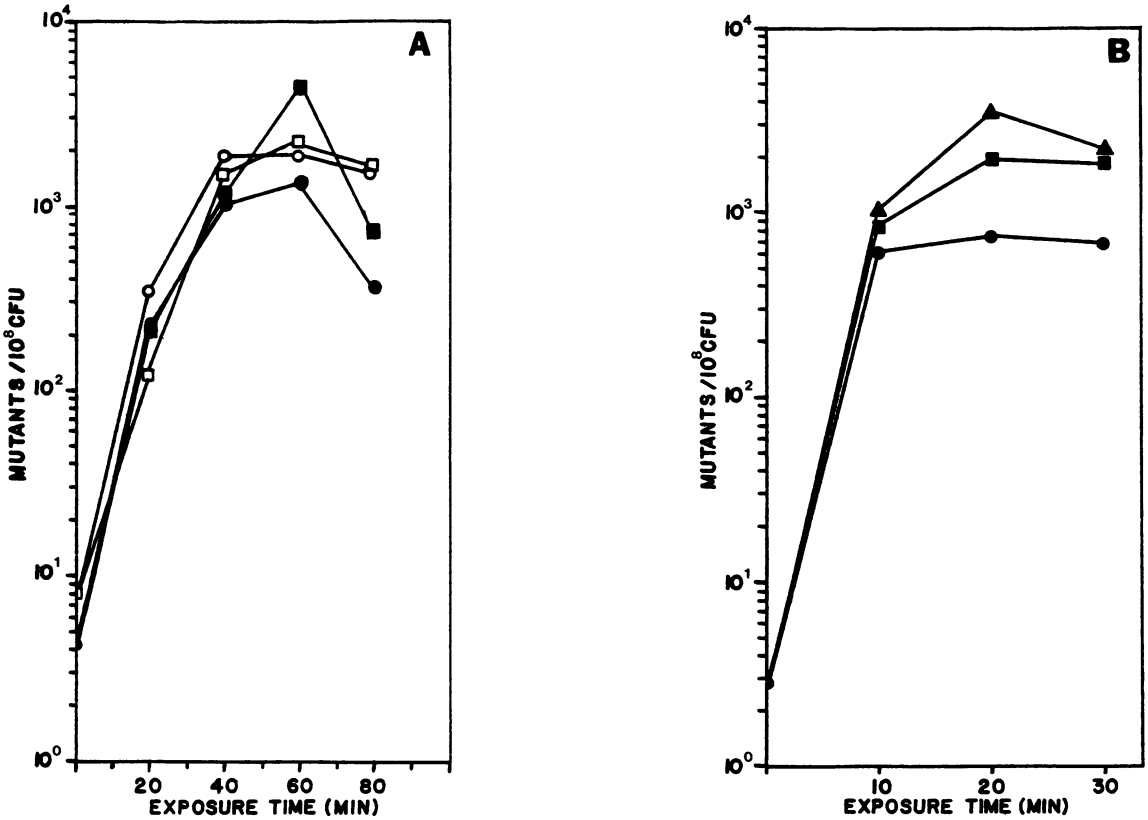

FIG. 2. Mutagenesis of strain F62 by EMS and MNNG. (A) EMS mutagenesis of strain F62. Mid-exponential-phase cells of strain F62 were incubated in medium containing 1% EMS (vol/vol) for various time periods as described in the text. After 24 or 48 h of incubation, cells were plated onto the appropriate selection medium and incubated for 72 h. The percent survival at each time point was as follows: 20 min, 43; 40 min, 8.4; 60 min, 0.9; 80 min, 0.04. Data are representative of three separate experiments. Symbols: ●, Rif^r mutants after a 24-h posttreatment incubation; ■, Nal^r mutants after a 24-h posttreatment incubation; ○, Rif^r mutants after a 48-h posttreatment incubation; □, Nal^r mutants after a 48-h posttreatment incubation. (B) MNNG mutagenesis of strain F62. Mid-exponential-phase cells of strain F62 were incubated in 10 μg of MNNG per ml at 37°C without aeration as described. This treatment decreased the survival to 1.5% after a 30-min incubation and was used for the selection of Rif^r and Nal^r mutants. Alternatively, Pro^+ revertants were isolated after treatment of strain F62 with 10 μg of MNNG at 37°C with aeration. This treatment decreased the survival to 0.03% after a 30-min incubation. Data are representative of three separate experiments. Symbols: ▲, Rif^r mutants; ■, Nal^r mutants; ●, Pro^+ revertants.

TABLE 2. MMS mutagenesis of strain F62[a]

Exposure time (min)	% Survival	No. of Rif^r mutants/10^7 CFU after 48 h[b]	No. of Nal^r mutants/10^7 CFU after 48 h[b]	No. of Pro$^+$ revertants/10^7 CFU after:		
				72 h	96 h	120 h
0	100	<0.6	<0.6	0.8	0.8	0.8
10	31.3	<0.3	<0.3	1.0	1.2	1.2
20	1.1	<0.3	0.3	0.3	0.3	0.3
30	0.06	<0.5	<0.5	0.2	0.2	0.4

[a] MMS concentration, 0.025%. Data are representative of three experiments. The mutation frequencies marked < were cases in which no resistant colonies were observed per set of duplicate plates. The number shown is the calculated mutation frequency had there been one such colony.

[b] Plates were incubated for up to 120 h with no change in the mutation frequencies.

MMS mutagenesis. No increase in mutation frequency was observed for any of the markers selected in strain F62 after exposure to 0.025% MMS for various time periods (Table 2). Increasing the concentration to 0.05% MMS resulted in an increased lethality but not any increase in the mutation frequency (data not shown). Analogous results were obtained for strain WLPI (data not shown).

UV mutagenesis. Strain F62 was exposed to varying fluxes of UV irradiation to inflict different degrees of damage to the DNA. This treatment did not result in any augmentation of the mutation frequency for any marker assayed (Table 3). Identical results were obtained with strain WLPI. In addition, the incubation time (24 to 48 h) before plating on the selective medium had no effect on the number of mutants obtained (data not shown).

In *E. coli*, a substantial increase in the mutation frequency is observed in excision repair-deficient strains after UV irradiation in comparison with excision repair-proficient strains (32). Since *N. gonorrhoeae* possesses an excision repair system (2) that functions on pyrimidine dimers, this capacity was chemically inhibited by the addition of caffeine (0.5 to 1.0 mg/ml), a known inhibitor of excision repair in procaryotes (28), including *N. gonorrhoeae* (2), to the postirradiation incubation media. The use of caffeine to inhibit excision repair was necessitated since no excision repair-deficient mutant of *N. gonorrhoeae* has yet been obtained. No increase in the number of Rif^r or Nal^r mutants was observed after a postirradiation incubation of the cells in a caffeine-containing medium (data not shown). This result further confirmed the immutability of *N. gonorrhoeae* by UV irradiation.

DISCUSSION

The classical genetic approach for studying pathogenic mechanisms and phase transitions in *N. gonorrhoeae* has been hampered by the limitations of genetic transfer systems in this organism and by the difficulty encountered in generating mutations. The purpose of this investigation was to gain better understanding of the processing of DNA damage in this organism and its relation to mutation fixation. Lethality studies demonstrated that although a heterogeneous response was observed, all strains of *N. gonorrhoeae* tested were relatively sensitive to UV irradiation, EMS, and MMS in comparison with repair-proficient strains of *B. subtilis* and *E. coli*. The difference in the UV sensitivities of *N. gonorrhoeae* and *B. subtilis* cannot be attributed to the absence of the dimer-specific photoreactivation pathway since both organisms lack this repair system (1, 11). The sensitivity of *N. gonorrhoeae* to these DNA-damaging agents suggested that this organism might lack a form of postreplication repair. Significantly, organisms that lack this system are more sensitive to the lethal effects of UV, EMS, MMS, and bifunctional alkylation agents (3, 29). It is important to note that strain WLPI is also extremely sensitive to mitomycin C (MIC, 0.015 μg/ml; unpublished data). Other experiments have suggested that the sensitivity of *N. gonorrhoeae* to the alkylating agents EMS and MNNG is at least partly attributed to the absence of an adaptive response to the lethal effects of these agents (Campbell and Yasbin, manuscript in preparation).

The mutagenesis studies revealed that *N. gonorrhoeae* was readily mutated for the three phenotypes assayed by the direct mutagens EMS and MNNG. Conversely, no significant increase above the spontaneous mutation frequency was observed for any markers after treatment with the indirect mutagens MMS and UV irradiation. As organisms that lack the inducible repair system are immutable by UV irradiation and MMS but are readily mutated by EMS and MNNG, these results strongly suggest that *N. gonorrhoeae* lacks this inducible type of error-prone repair.

As defined by UV immutability, *N. gonorrhoeae*, *M. radiodurans* (27), *S. pneumoniae* (5), *S. mutans* (24), *S. sanguis* (24), and *H. influenzae* (12, 23) all lack error-prone repair. Since all of the aforementioned bacteria are competent for DNA-mediated transformation, the intriguing possibility arises that the competency is incompatible with error-prone repair (24, 33). One exception to this observation is the demonstration of chromosomal mutagenesis by MMS treatment and by UV irradiation in *B. subtilis* (22; R. Miehl-

TABLE 3. UV mutagenesis of strain F62[a]

Trial	Fluence (J/m^2)	% Survival	No. of Rif^r mutants/10^7 CFU after 48 h[b]	No. of Nal^r mutants/10^7 CFU after 48 h[b]	No. of Pro$^+$ revertants/10^7 CFU after:		
					72 h	96 h	120 h
1	0	100	<0.3	0.5	0.6	1.1	1.1
	12	11.1	0.3	0.8	0.7	1.1	1.1
	24	0.55	0.3	<0.2	1.5	1.8	1.8
	36	0.09	0.5	<0.3	<0.2	<0.2	<0.2
	48	0.04	0.5	<0.3			
2	0	100	<0.4	<0.4			
	4	32.2	<0.3	<0.3			
	8	14.2	<1.2	<1.2			
	16	0.95	<0.4	0.4			
	24	0.21	<0.8	<0.8			

[a] Data are representative of three experiments. For trial 1, the maximum number of colonies observed on any set of plates was two or three.

[b] Plates were incubated for up to 120 h with no change in the mutation frequencies.

Lester, personal communication). However, unlike *M. radiodurans*, *H. influenzae*, *S. pneumoniae*, and *N. gonorrhoeae*, in which the competent population can approach 100%, only 10 to 20% of the *B. subtilis* population becomes competent (4). In *B. subtilis*, the development of competency is accompanied by activation of SOS functions (33). Since error-prone repair is one of the SOS-inducible functions, it is not surprising that an increased mutation frequency was obtained in the transformed population of *B. subtilis* cells (22, 33). *N. gonorrhoeae* is competent throughout its life cycle; it could be hypothesized that the coordinate expression of competency and SOS functions, including error-prone repair, might result in an unfavorable genetic load. Thus, it could be suggested that the majority of competent organisms evolved in such a way as to remove error-prone repair from the phenomena associated with the SOS response. Although this hypothesis is attractive, it should be approached with caution. Both *Salmonella typhimurium* and *Proteus mirabilis*, two noncompetent organisms, appear to be somewhat deficient in error-prone repair (7, 30).

The absence of error-prone repair in *N. gonorrhoeae* dictates the considerations that must be applied in mutagenizing this organism and also suggests methods for augmenting the mutation frequency. Any agent that is known to act by inducing error-prone repair will be ineffective for *N. gonorrhoeae*. Conversely, emphasis should be placed on those agents that act by mispairing mechanisms. We also assayed the mutability of *N. gonorrhoeae* to hydroxylamine, another direct mutagen. Although this mutagen might prove advantageous for generating specific GC→AT transitions, the overall efficiency in *N. gonorrhoeae* is very low and, thus, does not prove warranted for routine mutagenesis (unpublished data). The overall inefficiency of most mutagens to generate high mutation frequencies in *N. gonorrhoeae* suggests that alternative methods be employed. We are presently investigating transpositional mutagenesis in *N. gonorrhoeae*. Attempts are also being made to introduce an error-prone repair system into this organism.

ACKNOWLEDGMENTS

This study was supported by contract DE-ACD2-81ER 60017 from the Department of Energy and Public Health Service contract AI-11709 from the National Institute of Allergy and Infectious Diseases. This work was done as partial fulfillment of requirements for a Ph.D. degree.

We thank Virginia Clark and Eugene LeClerc for their critical evaluations of the manuscript.

LITERATURE CITED

1. **Campbell, L. A., and R. E. Yasbin.** 1979. Deoxyribonucleic acid repair capacities of *Neisseria gonorrhoeae*: absence of photoreactivation. J. Bacteriol. **140:**1109–1111.
2. **Campbell, L. A., and R. E. Yasbin.** 1984. A DNA excision repair system for *Neisseria gonorrhoeae*. Mol. Gen. Genet. **193:**561–563.
3. **Drake, J., and R. H. Baltz.** 1976. The biochemistry of mutagenesis. Annu. Rev. Biochem. **45:**1–27.
4. **Dubnau, D. A.** 1981. Genetic transformation in *Bacillus subtilis*, p. 148–178. *In* D. A. Dubnau (ed.), The molecular biology of the bacilli, vol. I. Academic Press, Inc., New York.
5. **Gasc, A. M., N. Sicard, J. B. Clavery, and A. M. Sicard.** 1980. Lack of SOS repair in *Streptococcus pneumoniae*. Mutat. Res. **70:**157–165.
6. **Gichner, T., and J. Veleminsky.** 1982. Genetic effects of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and its homologs. Mutat. Res. **99:**129–242.
7. **Hofemeister, J., H. Kohler, and V. D. Fillipov.** 1979. DNA repair in *Proteus mirabilis*. VI. Plasmid (R46)-mediated recovery and ultraviolet mutagenesis. Mol. Gen. Genet. **176:**265–273.
8. **Iishi, Y., and S. Kondo.** 1975. Comparative analysis of deletion and base change mutabilities of *Escherichia coli* B strains differing in repair capacities (wild type, *uvr*A$^-$, *uvr*B$^-$, *pol*A$^-$, *rec*A$^-$) by various mutagens. Mutat. Res. **27:**27–44.
9. **Kato, T., and Y. Shinoura.** 1977. Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. Mol. Gen. Genet. **156:**121–131.
10. **Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and L. I. Pirkle.** 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J. Bacteriol. **85:**1274–1279.
11. **Kelner, A.** 1964. Correlation between genetic transformability and non-photoreactivity in *Bacillus subtilis*. J. Bacteriol. **87:**1295–1303.
12. **Kimball, R. F., M. E. Boling, and S. W. Perdue.** 1977. Evidence that error prone repair is absent in *Haemophilus influenzae* Rd with a discussion of the relationship to error prone repair of alkylating damage. Mutat. Res. **44:**183–196.
13. **Lambden, P. R., J. E. Heckels, and P. J. Watt.** 1982. Effect of antipilus antibodies on survival of gonococci within guinea pig subcutaneous chambers. Infect. Immun. **38:**27–30.
14. **Lascolea, L. J., Jr., and F. E. Young.** 1974. Development of a defined minimal medium for growth of *Neisseria gonorrhoeae*. Appl. Microbiol. **28:**70–76.
15. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Miller, J.** 1983. Mutational specificity in bacteria. Annu. Rev. Genet. **17:**215–283.
17. **Okada, S.** 1970. Radiation biochemistry, p. 102–109. Academic Press, Inc., London.
18. **Radman, M.** 1975. SOS repair hypothesis. Phenomenology of an inducible DNA repair which is accompanied by mutagenesis, p. 355–367. *In* P. C. Hanawalt and R. S. Setlow (ed.), Molecular mechanisms for repair of DNA. Plenum Publishing Corp., New York.
19. **Radman, M., G. Villani, S. Boiteux, M. Defais, P. Caillet-Fauquet, and S. Spadari.** 1977. On the mechanism and genetic control of mutagenesis induced by carcinogenic mutagens, p. 903–922. *In* H. Haitt (ed.), The origins of human cancer. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
20. **Radman, M., G. Villani, S. Boiteux, A. R. Kinnells, B. W. Glickman, and J. Spadari.** 1979. Replication fidelity: mechanism of mutation avoidance and mutation fixation. Cold Spring Harbor Symp. Quant. Biol. **43:**937–946.
21. **Rein, M. J.** 1977. Epidemiology of gonococcal infections, p. 1–31. *In* R. B. Roberts (ed.), The gonococcus. John Wiley and Sons, New York.
22. **Rudner, R.** 1981. Mutagenesis during transformation of *Bacillus subtilis*. II. An increase in chemically induced mutagenesis during competency. Mutat. Res. **83:**339–343.
23. **Setlow, J. K., and N. K. Notani.** 1981. *Haemophilus influenzae* does not fit theories of post-replication repair, p. 181–186. *In* E. Seeberg and K. Kleppe (ed.), Chromosomal damage and repair. Plenum Publishing Corp., New York.
24. **Sicard, N.** 1983. Possible correlation between transformability and deficiency in error-prone repair. J. Bacteriol. **154:**995–997.
25. **Spizizen, J.** 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. Proc. Natl. Acad. Sci. U.S.A. **44:**1072–1078.
26. **Swanson, J.** 1978. Studies on gonococcus infection. XII. Colony and opacity variants of gonococci. Infect. Immun. **22:**359–364.
27. **Sweet, D. M., and B. E. B. Mosely.** 1974. Accurate repair of ultraviolet induced damage in *Micrococcus radiodurans*. Mutat. Res. **23:**311–318.
28. **Timson, J.** 1977. Caffeine. Mutat. Res. **47:**1–52.
29. **Todd, P. A., J. Bouwer, and B. W. Glickman.** 1981. Influence of DNA repair deficiencies on MMS- and EMS-induced mutagene-

sis in *Escherichia coli* K-12. Mutat. Res. **82**:239–250.
30. **Walker, G.** 1978. Inducible reactivation and mutagenesis of UV-irradiated bacteriophage P22 in *Salmonella typhimurium* LT2 containing plasmid pKM101. J. Bacteriol. **135**:415–421.
31. **Walker, G. C., and P. P. Dobson.** 1979. Mutagenesis and repair deficiencies of *Escherichia coli umu*C mutants are suppressed by plasmid pKM101. Mol. Gen. Genet. **172**:17–24.
32. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. **40**:869–907.
33. **Yasbin, R. E.** 1977. DNA repair in *Bacillus subtilis*. II. Activation of the inducible system in competent bacteria. Mol. Gen. Genet. **153**:214–225.