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# Mutator Factor in *Neisseria meningitidis* Associated with Increased Sensitivity to Ultraviolet Light and Defective Transformation

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A variant of *Neisseria meningitidis* was found to carry a mutator factor which endowed the bacteria with generalized genetic instability. The reversion frequencies of several biochemical mutants were increased up to 1,000-fold when the factor was introduced. The factor is not unidirectional in preference, since the mutator induced mutants generally reverted with increased frequency in its presence. There could be found no indication of insufficient synthesis of nucleic acid precursors. Attempts to demonstrate an unusual, mutagenic base incorporated in deoxyribonucleic acid (DNA) were negative. Strains carrying the mutator factor had significantly increased sensitivity to ultraviolet light. A mutation to a more ultraviolet-resistant type coincided with a disappearance of the mutator property. The presence of the mutator factor in a competent strain resulted in a reduction of the transformation frequency to between 0.5 and 5% of that in the parental strain. A mutation to the more ultraviolet-resistant type resulted in simultaneous loss of the mutator property and reestablishment of a normal transformation efficiency. It has been suggested that this mutator factor may represent a defect in the DNA repair mechanism, which is also of importance for genetic recombination. The mutator factor showed cotransformation with the locus for streptomycin resistance, but a true linkage could not be proved.

Mutator factors have been found in several laboratory strains of *Escherichia coli* (4, 29, 31) as well as in *Salmonella typhimurium* (20).

After the casual finding of a mutator clone in a laboratory strain of *Neisseria meningitidis* (14), a procedure was established which showed that genetic instability arises in populations of *N. meningitidis* with a frequency sufficiently high to permit the isolation of a collection of mutator variants (K. Jyssum and S. Jyssum, Acta Pathol. Microbiol. Scand., *in press*). The present paper deals with one of four mutator variants isolated in an experiment with this selective procedure. This particular mutator strain may be of some general interest since its mechanism of action seems to be of a nature which has been postulated during discussions of possible mechanisms of increased spontaneous mutability (25, 34).

## MATERIALS AND METHODS

*Strains.* The mutator variant F9 *his mutR1* $cp^-$ was one of four isolates from *N. meningitidis* previously described (K. Jyssum and S. Jyssum, Acta Pathol. Microbiol. Scand., *in press*). The mutator factor has been indicated by the symbol *mutR1*. Genetic competence is indicated by $cp^+$ and incompetence which does not revert to competence by $cp^-$ (12, 13). Genetic experiments were performed with the following mutants obtained from the wild-type strain M1 of group B after ultraviolet (UV) light irradiation or nitrous acid treatment of cells: M1-18 *his gly* $cp^+$, M1-8 *his arg* $cp^+$, M1-5 *his hom* $cp^+$, M1-48 *his cys* $cp^+$, M1-6 *his pro* $cp^+$. The *str* marker was a single-step high-level resistant mutant (12). A mutant unable to metabolize glucose as well as maltose, indicated by the symbol *glc/mal*, probably represents a mutation in the hexokinase-synthesizing system (K. Jyssum and S. Jyssum, Acta Pathol. Microbiol. Scand., *in press*). Mutants of *E. coli* requiring adenine or guanine for growth were made by a technique employing nitrous acid (17). A thymine-requiring mutant of *E. coli* 15 was obtained from W. B. Gundersen, University of Oslo, Oslo, Norway.

*Genetic procedures.* Media, deoxyribonucleic acid (DNA) preparation, and the transformation procedures followed the previously described technique (12, 15, 18). The transformation system used to introduce the mutator factor into normal receptor strains has been described before (K. Jyssum and S. Jyssum, Acta Pathol. Microbiol. Scand., *in press*).

Additional technical procedures or modifications have been described in the experimental section.

*Demonstration of genetic lability.* Genetic lability was usually scored by virtue of the high frequency in the population of mutants resistant to streptomycin (11). In streptomycin-resistant (Str-r) clones, genetic lability was either scored by the unusually high number of back mutants in an amino acid-requiring mutant, or by an increased number of glucose- or maltose-positive colonies in a *glc/mal* mutant upon streaking on glucose or maltose plates (K. Jyssum and S. Jyssum, Acta Pathol. Microbiol. Scand., *in press*).

*Determination of mutation rates.* The mutation rates were first determined from the number of tubes which contained no mutants according to the method of Luria and Delbrück (21), as previously described (11). During examinations of the antimutagenic effect of growth supplements, the mutation rates were determined by a method based on the number of clones appearing during growth on solid medium (32). The method was modified by the application of streptomycin at any desired time by the layering technique generally used in transformation of Str-r (18). The mutation rate was calculated according to the equation: $a = (ln\ 2)(R_2 - R_1)/(N_2 - N_1)$, where $R_1$ and $R_2$ are the number of mutant clones present at times 1 and 2, respectively, and $N_1$ and $N_2$ are the number of bacteria present per plate at the same times (23, 32).

*Hydrolysates of DNA.* Hydrolysates from *N. meningitidis* DNA were prepared with HCl (2). The hydrolysates were carefully neutralized and used as growth supplements. In experiments with *E. coli* mutants, the hydrolysates were used in quantities which yielded the same growth response as was yielded by optimal quantities of the growth factor actually required.

*Determination of UV sensitivity.* The technique followed that previously used in this laboratory (16), with a Philips germicidal lamp (15 w) as the source of irradiation. Exposure was performed at a distance of 40 cm with a dose rate of approximately 16.2 ergs per sec per mm². The cells were suspended in the mineral salts of the basal medium which have no UV-absorbing activity (7). Volumes of 2.5 ml were irradiated in sterile petri dishes with constant agitation.

## RESULTS

*Mutator-induced reversions of nutritional mutants.* The general characteristics of the unstable variant F9 *his mutR1 cp*⁻ and the types of spontaneous mutants observed have been described (K. Jyssum and S. Jyssum, Acta Pathol. Microbiol. Scand., *in press*). By transformation, the mutator factor was introduced into several recipients so as to occur in combination with various genetic markers that had previously been obtained after treatment of the cells with UV light or nitrous acid. In this way, the influence of the factor could be roughly estimated (Table 1). It seems that the reversion frequencies are increased 100- to 1,000-fold in all the markers tested when the mutator factor is introduced into the strain. With a view to the high frequencies actually observed, and since microcolonies may develop from colony-forming units plated in the absence of supplements with resulting mutation after plating (15), it was felt that the values reported represent maximal values, and are not reliable for estimating mutation rates. An examination of mutation rates with a more adequate technique seems to substantiate this assumption (Tables 3 and 4).

*Mutator-induced reversion of mutator-induced auxotrophs.* The stability of mutants appearing in the mutator strain was examined according to the procedures of Yanofsky et al. (33) by determining the reversion frequencies of a group of mutator-

TABLE 1. *Spontaneous and mutator-induced reversion of auxotrophs of N. meningitidis*

| Auxotroph | Marker tested | Mutation frequency[a] | | Increase in mutator strain |
|---|---|---|---|---|
| | | Parent | *mutR1* | |
| *pro* | *str* | $3.3 \times 10^{-9}$ | $1.0 \times 10^{-6}$ | $3.1 \times 10^{2}$ |
| | *pro* | $8.8 \times 10^{-9}$ | $>7.5 \times 10^{-5}$ | $>9.0 \times 10^{3}$ |
| *arg* | *str* | $1.5 \times 10^{-9}$ | $1.6 \times 10^{-6}$ | $1.0 \times 10^{3}$ |
| | *arg* | $1.0 \times 10^{-8}$ | $1.8 \times 10^{-5}$ | $1.0 \times 10^{3}$ |
| *his* | *str* | $<8.5 \times 10^{-9}$ | $4.7 \times 10^{-6}$ | $>5.5 \times 10^{2}$ |
| | *his* | $1.3 \times 10^{-8}$ | $>4.1 \times 10^{-5}$ | $>3.2 \times 10^{3}$ |
| *hom* | *str* | $3.1 \times 10^{-8}$ | $8.7 \times 10^{-6}$ | $2.8 \times 10^{2}$ |
| | *hom* | $<5.9 \times 10^{-10}$ | $1.8 \times 10^{-6}$ | $>3.1 \times 10^{3}$ |
| *gly* | *str* | $1.3 \times 10^{-9}$ | $5.2 \times 10^{-6}$ | $4.0 \times 10^{3}$ |
| | *gly* | $1.9 \times 10^{-9}$ | $>9.4 \times 10^{-6}$ | $>4.7 \times 10^{3}$ |
| *cys* | *str* | $2.1 \times 10^{-8}$ | $3.8 \times 10^{-6}$ | $1.8 \times 10^{2}$ |
| | *cys* | $5.2 \times 10^{-9}$ | $2.4 \times 10^{-5}$ | $4.6 \times 10^{3}$ |

[a] Ratio of the number of colonies found on minimal plates or on Str plates to the total number of colony-forming units plated, as determined by plate counts on complete agar media.

induced auxotrophs. The frequency of mutants in a mutator culture is sufficiently high to ensure the recovery of auxotrophs after subculture and single-colony isolation from the surface of complete media. Since this is not regularly possible from nonmutator cultures, it is assumed that the mutants examined were mutator-induced. Of 11 isolates studied in detail, all reverted with a frequency that can be considered to reflect a pronounced effect of the mutator factor (Table 2). The UV sensitivity of the mutator strain being known (*see below*), it became possible to prepare mutator-negative stocks of mutator-induced mutants by selecting UV-resistant variants in populations of the mutator strain. In Table 2, the mutants 1, 2, 10, and 11 have been "stabilized" according to the technique described below. The results seem to represent a decisive argument in favor of the hypothesis that the lability observed in the mutator-induced mutants is indeed due to the mutator factor.

*Attempts to demonstrate insufficiency of base synthesis or mutagenic base analogues in strains carrying the mutator factor.* An early finding from the factor *mutR1* was that its presence in a *N. meningitidis* strain coincided with a retardation of the growth rate (K. Jyssum and S. Jyssum, Acta Pathol. Microbiol. Scand., *in press*). Since it has been proposed that a quantitative insufficiency of a base may result in increased mutation (29), attempts were made to find a growth supplement which could abolish the growth retardation and at the same time reduce the frequencies of mutants. Growth rates were determined, and numbers of Str-r mutants measured in cultures supplemented with DNA, ribonucleic acid, DNA hydrolysates, nucleotides, nucleosides, purines, and pyrimidines. But it was not possible to demonstrate any significant effect on the generation time nor on the number of mutants which accumulated.

Base synthesis may also be involved in the mutator effect if there is a qualitative change in base synthesis, resulting in a gene product which is mutagenic (19). Since it has been possible to suppress the mutagenic activity of base analogues with deoxyribonucleosides (8), attempts were made to suppress the activity of the mutator factor in the same way. In these experiments, the test strains were grown on basal media with the addition of the drug to be tested for "antimutagenic activity," usually in the concentration 200 μg/ml. No significant antimutagenic effect was found (Table 3). A small difference between the system with added nucleotides and the control system has not been considered significant. In some experiments, guanine seemed to increase the mutation rate. This seemed to be a technical error because of the low solubility of guanine. When the medium was very carefully prepared in order to avoid precipitation, the effect disappeared. It was also noted that neither guanosine triphosphate nor deoxyguanosine had such an effect.

TABLE 2. *Reversion frequencies of some mutator-induced mutants*

| Mutant No. | Markers | Marker tested | Mutation frequency[a] |
|---|---|---|---|
| 1 | *gly thr mutR1* | *str* | $7.3 \times 10^{-6}$ |
| | | *thr* | $6.3 \times 10^{-5}$ |
| 1b[b] | *gly thr* | *str* | $1.4 \times 10^{-9}$ |
| | | *thr* | $3.2 \times 10^{-10}$ |
| 2 | *gly arg mutR1* | *str* | $5.7 \times 10^{-6}$ |
| | | *arg* | $>6.9 \times 10^{-5}$ |
| 2b[b] | *gly arg* | *str* | $2.6 \times 10^{-9}$ |
| | | *arg* | $4.1 \times 10^{-9}$ |
| 3 | *gly nic mutR1* | *str* | $1.5 \times 10^{-6}$ |
| | | *nic* | $7.6 \times 10^{-4}$ |
| 4 | *gly trp his mutR1* | *str* | $1.9 \times 10^{-5}$ |
| | | *trp* | $3.6 \times 10^{-6}$ |
| 5 | *gly trp his mutR1* | *str* | $1.4 \times 10^{-5}$ |
| | | *trp* | $2.6 \times 10^{-6}$ |
| 6 | *gly trp his mutR1* | *str* | $1.2 \times 10^{-5}$ |
| | | *trp* | $2.4 \times 10^{-6}$ |
| 7 | *gly his mutR1* | *str* | $3.4 \times 10^{-5}$ |
| | | *his* | $>6.4 \times 10^{-5}$ |
| 8 | *gly his mutR1* | *str* | $9.6 \times 10^{-6}$ |
| | | *his* | $1.7 \times 10^{-5}$ |
| 9 | *gly his mutR1* | *str* | $2.8 \times 10^{-6}$ |
| | | *his* | $>4.5 \times 10^{-5}$ |
| 10 | *gly his mutR1* | *str* | $1.4 \times 10^{-6}$ |
| | | *his* | $1.6 \times 10^{-5}$ |
| 10b[b] | *gly his* | *str* | $3.6 \times 10^{-9}$ |
| | | *his* | $5.2 \times 10^{-9}$ |
| 11 | *his glc/mal mutR1* | *str* | $3.6 \times 10^{-6}$ |
| | | *glc/mal* | $4.2 \times 10^{-6}$ |
| 11b[b] | *his glc/mal* | *str* | $1.1 \times 10^{-9}$ |
| | | *glc/mal* | $5.2 \times 10^{-10}$ |

[a] Determined as in Table 1.

[b] Stable variants isolated as UV-resistant clones by the technique described in the text.

Attempts were also made to demonstrate the presence of mutagenic bases in DNA from *N. meningitidis* strains carrying the mutator factor. No significant mutagenic effect could be observed when *E. coli* auxotrophs were grown on DNA hydrolysates from mutator strains (Table 4). A slight increase in the mutation rate of the mutant K-12 *ade*, when grown on hydrolysates from *N. meningitidis*, was constantly observed. If this is significant, it could be the effect of a slight adenine starvation (3), although no retardation of the growth rate could be observed. If the effect is caused by the DNA hydrolysate, the causative agent cannot be specific for the mutator strain, since a comparison between hydrolysates from DNA with a mutator factor (F10 *gly mutR1*) and without (18 *his gly* $cp^+$) gives no evidence of a specific mutagenic effect of the former.

*Inactivating effect of UV light on strains carrying the mutator factor.* It is known that defective repair mechanisms in *E. coli* (9, 28), as well as in *Bacillus subtilis* (6), may result in a phenotype with increased sensitivity to UV light. To find out whether the mutator strain of *N. meningitidis* could be defective in repair of lesions of DNA, UV light survival determinations were performed. The relative UV susceptibilities of the strain M1–6 *his pro* and the mutator variant 2B.2 *his mutR1* (Fig. 1) demonstrate that strains with the mutator factor have an increased sensitivity to UV irradiation. This was a constant finding which was not influenced by a change of the growth medium. In agreement with earlier findings (16), the competence factor *cp* seems to have no significant influence on sensitivity to UV light.

*Influence of the mutator factor on transformation.* The factor *mutR1* was originally detected among progeny from an incompetent strain, and all direct descendants from the mutant F9 *his mutR1* have been constantly incompetent in transformation. But the mutator factor may also be present in competent variants of *N. meningitidis*. When mutator strains obtained by transformation of nonmutator receptors with DNA carrying the mutator factor are examined, more than 99% are found to be competent (K. Jyssum and S. Jyssum, Acta Pathol. Microbiol. Scand., *in press*). However, there is no doubt that the presence of the mutator factor has a significant influence on the transformation efficiency. Table 5 shows that the presence of competence in a strain carrying the mutator factor results in a significantly increased number of Str-r clones, as well as prototrophic ones, when DNA with the appropriate genetic markers is present in the system. Since this effect of DNA is abolished by deoxyribonuclease and is specific for the marker present in DNA, it is considered as a transformation. But the number of transformants is far less than that found in the "normal" competent strain. A further comparison of the frequency of transformation of various mutator derivatives relative to their parental strains showed that strains bearing the mutator factor were transformed at 0.5 to 5% of the rate for the parental strain, with some variation between individual isolates.

TABLE 3. *Mutation rates to str-r in mutator strains grown on basal media with various supplements*

| Strain | Supplement[a] | No. of experiments | Mutation rate[b] (avg) |
|---|---|---|---|
| 2B.2 *his mutR1* | His | 6 | $6.2 \times 10^{-8}$ |
| | His + ADP | 2 | $4.3 \times 10^{-8}$ |
| | His + ITP | 2 | $5.1 \times 10^{-8}$ |
| | His + GTP | 2 | $5.3 \times 10^{-8}$ |
| | His + TMP | 2 | $4.8 \times 10^{-8}$ |
| F10 *gly mutR1* | Gly | 4 | $1.5 \times 10^{-8}$ |
| | Gly + dAdo | 2 | $2.1 \times 10^{-8}$ |
| | Gly + dGuo | 2 | $1.5 \times 10^{-8}$ |
| | Gly | 8 | $1.7 \times 10^{-8}$ |
| | Gly + A | 5 | $2.8 \times 10^{-8}$ |
| | Gly + G | 4 | $1.7 \times 10^{-8}$ |
| | Gly + T | 5 | $3.8 \times 10^{-8}$ |
| | Gly + C | 4 | $1.2 \times 10^{-8}$ |

[a] His = histidine; ADP = adenosine diphosphate; ITP = inosine triphosphate; GTP = guanosine triphosphate; TMP = thymidine monophosphate; Gly = glycine; dAdo = deoxyadenosine; dGuo = deoxyguanosine; A = adenine; G = guanine; T = thymine; C = cytosine.

[b] Determined as described in Materials and Methods.

TABLE 4. *Mutation rates to str-r in E. coli auxotrophs grown on basal media supplemented with DNA hydrolysates from N. meningitidis*

| Strain | Supplement | No. of experiments | Mutation rate[a] (avg) |
|---|---|---|---|
| K-12 *ade* | A | 3 | $8.6 \times 10^{-10}$ |
| | Hydrolysate F10 *gly mutR1* | 3 | $1.2 \times 10^{-9}$ |
| | Hydrolysate 18 *his gly cp*$^+$ | 3 | $1.9 \times 10^{-9}$ |
| K-12 *gua* | G | 2 | $2.3 \times 10^{-10}$ |
| | Hydrolysate F10 *gly mutR1* | 2 | $4.0 \times 10^{-10}$ |
| | Hydrolysate 18 *his gly cp*$^+$ | 2 | $5.1 \times 10^{-10}$ |
| *E. coli* 15 *thy* | T | 2 | $6.8 \times 10^{-10}$ |
| | Hydrolysate F10 *gly mutR1* | 2 | $7.1 \times 10^{-10}$ |
| | Hydrolysate 18 *his gly cp*$^+$ | 2 | $4.3 \times 10^{-10}$ |

[a] Determined as described in Materials and Methods.

The competence factor may be lost from the parent strain of the mutator stock (13). When single colonies from the competent mutator strain 2B.2 *his mutR1 cp*$^+$ were examined for competence, this property was lost in 0.5 to 6% of the colony-forming units from any competent clone. Thus, the mutator factor and competence may be dissociated during loss of the latter property.

It has been stated that the mutator property, on a limited scale, and at least compared to the competence factor, is a stable character (K. Jyssum and S. Jyssum, Acta Pathol. Microbiol.

Scand., *in press*). But the mutator property may also be lost. Variants, probably back mutants or suppressor mutants with no more than normal mutation frequencies, could be isolated on the basis of the increased UV sensitivity of the mutator variant. Colonies from bacteria surviving irradiation with approximately 300 ergs/mm² were replica-plated on two daughter plates. One of these was incubated after irradiation with 500 to 600 ergs/mm²; the other was incubated without irradiation. From the unirradiated daughter plates, all colonies that were suspected to consist of cells more radioresistant than those of the mutator phenotype were restreaked and investigated in detail. The more UV-resistant clones isolated in this way were all nonmutators. In experiments with competent mutator strains, approximately 98% of the nonmutator variants which were picked up were competent in transformation. The transformation efficiency in these "revertants" was the same as that of the normal, competent parent; i.e., they had a transformation frequency of the dimension $10^{-3}$ (15). The acquisition of the more UV-resistant phenotype coincides, therefore, with the reestablishment of the transformation efficiency which is considered normal for the parent strain.

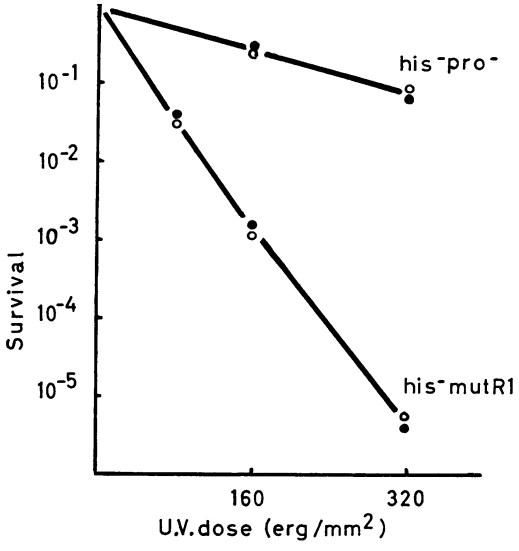


FIG. 1. *Ultraviolet light survival curves for a strain of N. meningitidis carrying the mutator factor mutR1 compared with a strain with usual mutation frequencies. Symbols:* ○, *competent strain;* ●, *incompetent strain.*

It was observed that lyophilization or maintenance of a mutator strain in the frozen state, even in the presence of glycerol (18), in many instances resulted in a very high rate of killing and what seemed to be a selective survival of "back mutants" with normal mutation frequencies and normal transformation efficiency. The more UV-resistant phenotype could be searched for in the replica-plating technique described above. Thus, it appears that freezing as well as lyophilization may result in defects in DNA that require repair in order that the microbe may be functional.

*Attempts to locate the mutator factor by cotransformation.* The following markers were examined with regard to cotransformation with the factor *mutR1*: *his*, *arg*, *hom*, *gly*, *cys*, *pro*, *str*, and the sugar marker *glc/mal*. Only transformation to Str-r showed any definite cotransformation with the mutator factor (Table 6). Because we have no method which selects for the mutator factor, the reverse experiment cannot be performed. This cotransformation being known, however, it also became possible to prepare mutator-negative stocks of mutator-induced mutants by introducing the mutator-negative allele as an unselected marker.

TABLE 5. *Comparison of transformation and mutation in a mutator strain*

| Strain | DNAase[a] | Selected marker per unit plated | | | |
|---|---|---|---|---|---|
| | | With DNA[b] | | No DNA | |
| | | Str-r | His⁺ | Str-r | His⁺ |
| 2B.2 *his mutR1 cp*⁻ | − | $3.2 \times 10^{-7}$ | $4.6 \times 10^{-7}$ | $2.1 \times 10^{-7}$ | $4.8 \times 10^{-7}$ |
| | + | $2.6 \times 10^{-7}$ | $6.4 \times 10^{-7}$ | $1.4 \times 10^{-7}$ | $2.6 \times 10^{-7}$ |
| 2B.2 *his mutR1 cp*⁺ | − | $1.8 \times 10^{-5}$ | $1.2 \times 10^{-5}$ | $3.8 \times 10^{-7}$ | $2.4 \times 10^{-7}$ |
| | + | $6.1 \times 10^{-7}$ | $3.2 \times 10^{-7}$ | $6.4 \times 10^{-7}$ | $4.3 \times 10^{-7}$ |
| 12 *his cp*⁺ [c] | − | $2.6 \times 10^{-3}$ | $3.1 \times 10^{-3}$ | $<1 \times 10^{-9}$ | $<1 \times 10^{-9}$ |
| | + | $<1 \times 10^{-9}$ | $<1 \times 10^{-9}$ | $<1 \times 10^{-9}$ | $<1 \times 10^{-9}$ |

[a] Refers to deoxyribonuclease added at zero-time.
[b] DNA from M1 Str-r.
[c] Parent strain.

TABLE 6. *Analysis of cotransformation between the mutator factor and some genetic markers*

| Recipient | DNA | DNA added to the system (ml)[a] | Selected marker | Scored marker | Per cent co-transformation |
|---|---|---|---|---|---|
| *glc/mal cp*$^+$ | *str* | 0.1 | Str-r | Mal$^+$ | 2.3 |
| | | 0.01 | | | 1.8 |
| | | 0.001 | | | 0.1 |
| | *str his mutR1* | 0.1 | Str-r | MutR1$^+$ | 2.8 |
| | | 0.01 | | | 2.4 |
| | | 0.001 | | | 1.5 |
| | *his mutR1* | 0.1 | Mal$^+$ | MutR1$^+$ | <0.5 |
| | | 0.01 | | | <0.5 |
| | | 0.001 | | | <0.5 |
| *glc/mal his mutR1 cp*$^+$ | *str* | 0.1 | Str-r | Mal$^+$ | 6.2 |
| | | 0.01 | | | 3.1 |
| | | 0.001 | | | 0.4 |
| | *str* | 0.1 | Str-r | MutR1$^-$ | 2.8 |
| | | 0.01 | | | 2.3 |
| | | 0.001 | | | 0.8 |
| *his cys cp*$^+$ | *his mutR1* | 0.1 | Cys$^+$ | MutR1$^+$ | <0.5 |
| | | 0.01 | | | <0.5 |
| | | 0.001 | | | <0.5 |
| *his arg cp*$^+$ | *his mutR1* | 0.1 | Arg$^+$ | MutR1$^+$ | <0.5 |
| | | 0.01 | | | <0.5 |
| | | 0.001 | | | <0.5 |

[a] DNA at a concentration of 50 μg/ml.

## DISCUSSION

The lability caused by the factor *mutR1* seems to be general, with no preference for any particular genetic marker. Auxotrophs of various types are more abundant than in the normal parent, and the auxotrophs back-mutate at a higher frequency. The factor, therefore, does not appear to be unidirectional in preference. This behavior is also typical of the "Harvard strain" of *E. coli* (34) as well as of the mutator gene in *S. typhimurium* (22). On the other hand, the mutator gene of the "Treffers strain" of *E. coli* has a marked mutational specificity (1, 33), so that mutator-induced auxotrophs are generally not reverted by the gene. It should be emphasized, however, that the results obtained with the factor *mutR1* may largely be due to mutator-induced suppressor mutations or second-site reversions.

Several mechanisms have been suggested for the operation of mutator factors. Since we know that thymine starvation is mutagenic as well as lethal (3), one possibility is that there is a quantitative insufficiency of a base. On the other hand, a qualitative change in base synthesis may result in the synthesis of a mutagenic base (19). While a quantitative insufficiency does not seem to have been demonstrated so far, the synthesis of an unusual base may be the mechanism of action in the mutator strain of *S. typhimurium* (19), as well as in the "Treffers strain" of *E. coli* (26). Because mutational changes are suppressible with deoxyribonucleosides when induced by base analogues, Siegel and Bryson (29) attempted to suppress the mutator activity of the mutator factor of *E. coli* B by the addition of deoxyribonucleosides to the medium. They found no effect. Similar attempts with the factor *mutR1* of *N. meningitidis* were also negative. But these experiments are far from conclusive since we know little about the actual uptake by these bacteria of the compounds tested.

The primary gene product of a mutator gene may be a mutated DNA polymerase. Speyer (30) has shown that an alteration of the gene controlling T4 DNA polymerase results in a mutator effect on other genes. Another possible mechanism is that the mutator factor may alter the internal cellular environment so that errors in replication occur (29). An increased intracellular concentration of $Mn^{++}$ might, for instance, cause mutations (24). Whether this mechanism is operative in vivo is not known.

The suggestion has been made that loss of the capacity to repair lesions normally occurring in DNA could result in a mutator phenotype (25, 34). The mutator property of the factor

*mutR1* of *N. meningitidis* coincides with a significantly increased susceptibility to UV irradiation, and a reversion of the mutator strain to a more UV-resistant type results in a simultaneous disappearance of the mutator property. It may be assumed that an increased sensitivity to UV light is due to the loss of capacity to repair lesions occurring in DNA (9, 28). Although no mechanism suggested for the operation of mutator factors has been ruled out, it may be suggested that the lability connected with the factor *mutR1* is due to loss of a repair function.

Mutants with increased susceptibility to UV irradiation resulting from defective repair mechanisms have been mapped in *E. coli* (9, 28). Among such mutants are also recombination-deficient ones (6, 10, 27). There is no doubt that the presence of the factor *mutR1* in a competent strain of *N. meningitidis* has a significant influence on the efficiency of the transformation system. Transformation frequencies in *mutR1* strains were reduced to 0.5 to 5% of those of the parental strains. It may be assumed that a repair function is lost or reduced in the mutator strain which is also of importance for genetic recombination in the process of transformation. An argument for this hypothesis is the finding that a reversion to the more UV-resistant type concomitantly confers loss of the mutator property and acquirement of normal transformation efficiency. These results are similar to those found in two recombination-deficient mutants of *B. subtilis* (6), in which the transformation frequencies were reduced to approximately 5 and 25% of that of the parental strain. But in these strains there was no evidence of genetic lability.

Elevated mutability in bacteria may be caused by mutator genes located on the bacterial chromosome (22, 29, 33, 34) or by some form of interaction between the chromosome and an element of episomic or plastid nature (5). The genetic location of the factor *mutR1* is not yet defined. Since the cotransformation between the factor and *str* does not seem to take place independently of the DNA concentration, it remains an open question whether it represents a true but weak linkage. During transformation of recipients carrying the factor *mutR1*, the dilution of DNA results in a reduction of the total number of transformants so that the number of spontaneous mutants is rapidly approached. The results obtained with more diluted DNA, therefore, may not be representative. Even if the cotransformation is not due to true linkage, it permitted the removal of the mutator allele from mutator stocks. The clones thus "stabilized" have the disadvantage of being Str-r. But it may be assumed that the normal allele corresponding to the mutator locus has been introduced. This may be a great advantage, since the back mutants isolated by the UV technique probably represent new genetic lesions in most instances, resulting from some form of suppression of the mutator genotype.

*Note.* Since completion of this manuscript, genetic instability has also been reported from a UV-sensitive mutant of *Proteus mirabilis* and from a recombination-defective mutant of *B. subtilis* (H. Böhme, Biochem. Biophys. Res. Commun. **28**:191–196, 1967).

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