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Mutagenesis by Methylation
N-METHYL-N'-NITRO-N-
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Hundreds of examples of the use of MNNG for induction of bacterial mutants have been reported since the method was first described (1,2). We have previously described (3) the induction of mutants of Euglena gracilis (4,5); to this example may now be added mutagenesis of Coprinus lagopus oldia (6) and of Saccharomyces cerevisiae (7). Mutagenesis of the Escherichia coli genome was found to be non-random when stationary phase cells were used (8,9). MNNG is also used in studies of the reversion of mutants (10). An interesting application has been the induction of mutants producing antibiotics different from but related to neomycin (11). Further studies of the mechanism of mutagenesis by MNNG indicated inhibition of synthesis of DNA and, to a lesser extent, of RNA in Escherichia coli, while development of resistance was indicated by decreases in uptake of MNNG and of methylation of DNA and RNA (12). Moreover cell-free protein synthesis was reduced by loss of template activity of DNA and by inactivation of ribosomes due to degradation of ribosomal RNA (12). Another mechanism of mutagenesis and carcinogenesis by MNNG may be the occurrence of single-strand breaks in DNA as observed in Escherichia coli (13). A repair mechanism may excise 7-methylguanine from DNA of Euglena gracilis methylated with N-methyl-N-nitroso-p-toluene-sulfonamide (DIAZAL®) (14). The stimulation of mutagenesis of DNA with MNNG in vitro by thiols (15) was confirmed (16) and the products of reaction of MNNG with cysteine were determined (17). On the other hand cysteine appears to protect against mutagenesis by MNNG in plants (18). Like other compounds acting on the conformation of DNA, MNNG induces synthesis of the pyrrole-epipodophyllotoxin in Pismus sativum L. (19). The action of 14C-methyl-MNNG and 14C-guanidino-MNNG on ascites hepatoma cells in vitro caused incorporation of both labels by histone; after enzymic hydrolysis the label appeared in isolated homocarboxylic (20), confirming an earlier report (21). Detailed studies of the methylation of DNA in cultured mammalian cells by MNNG and its enhancement with thiols have also been described (22). It is a special interest that MNNG methylates the same positions as other alkylating agents (dimethyl sulfate, methyl methanesulfonate) in TMV-RNA, but more slowly (23), yet MNNG is highly mutagenic when acting on TMV itself and the pattern of methylation differs from that of TMV-RNA (24, 25).

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(9) E. Cerdà-Ochoa, J. Gen. Microbiol. 73, 703 (1968).

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