A NEW CHEMICAL MUTAGEN FOR BACTERIA, 1-METHYL-3-NITRO-1-NITROSOGUANIDINE

Joseph D. Mandell and Joseph Greenberg

Department of Biological Sciences
Stanford Research Institute
Menlo Park, California

Received November 9, 1960

There have been numerous reports of the chemical induction of mutations in bacteria. The classes of chemicals possessing this characteristic are greatly varied and include many kinds of chemical functional groups. The compound 1-methyl-3-nitro-1-nitrosoguanidine (NG) may now be added to this list.

In the course of an investigation of genetically stable mutants resistant to NG (Mandell, Woody and Greenberg, 1960) it was noted that the number of mutants arising on plates appeared to be abnormally high. It was suspected that NG might be inducing mutations to resistance to itself rather than simply selecting spontaneously arising mutants. An experiment based on the fluctuation test of Luria and Delbrück (1943) was performed to test this idea. Forty-four 2-ml cultures of Escherichia coli strain S, each started from an average 4 cells/tube, were grown overnight to an average cell density of 1.1×10^9/ml and 0.1 ml of each was spread on tryptone agar plates containing 2 μg/ml of NG. Tryptone agar contains (per liter) tryptone 10 g, glucose 1 g, sodium chloride 5 g, agar 12 g, and is adjusted to pH 5.5 with hydrochloric acid. On this solid medium 2 μg/ml NG is sufficient to inhibit the parent sensitive strain S but not the first-step resistant mutants, S/Ng 1. The results presented in Table I reveal the variance to be far less than the mean -- even a smaller variation than would be characteristic of a Poisson distribution. The mutants must all have arisen at the same time, and not random in time during the growth of the culture. It was, therefore, concluded that

TABLE I

NUMBER OF *E. COLI* STRAIN S CELLS RESISTANT TO 2 μg/ml OF NG IN AGAR IN SAMPLES TAKEN FROM A SERIES OF INDEPENDENT CULTURES

Culture No.	Number of Resistant Colonies	Culture No.	Number of Resistant Colonies
1	1030	23	868
2	478	24	548
3	680	25	364
4	568	26	331
5	444	27	441
6	556	28	372
7	672	29	1274
8	506	30	460
9	1444	31	456
10	546	32	614
11	470	33	430
12	311	34	891
13	423	35	510
14	400	36	452
15	432	37	468
16	672	38	496
17	1452	39	322
18	462	40	482
19	506	41	622
20	468	42	736
21	412	43	638
22	384	44	454

Mean 580.7
Variance 267.7

NG is a mutagen at least in so far as inducing mutations to resistance to itself is concerned. The hypothesis that NG is indeed a general mutagen was confirmed by demonstrating the presence of auxotrophs among the survivors of NG treatment. *E. coli* strain H auxotrophs were identified as colonies which failed to grow on glucose-salts medium when replica plated from complete medium (Lederberg and Lederberg, 1952). At a survival level of about 10^{-4}, approximately 10% of the colonies were found to be auxotrophs with growth requirements for amino acids, vitamins, purines, pyrimidines, etc. A more detailed account of NG induced auxotrophs will be reported elsewhere. Incidentally, it was noted that in contrast to the induction of mutants in *E. coli* strain B by nitrous acid (Kaudewitz, 1959) in which mixed mutant clones were not observed, most of the mutant clones arising after nitrosoguanidine treatment

were found to be mixtures, composed most often of strains differing in colony size or morphology and occasionally of strains differing in their growth requirements.

NG is known to decompose under acid conditions to yield nitrous acid and under alkaline conditions to yield diazomethane. These two alternative possibilities to account for the mutagenicity of NG have been eliminated by the conditions of the experiments described above. NG yields neither diazomethane nor nitrous acid at pH 5.5. Slow liberation of nitrous acid was found to occur only in 0.1 M hydrochloric acid. Furthermore, strains S/Ng 1 and S/Ng 2 (first- and second-step NG resistant mutants) were as sensitive as was the parent strain S to inhibition by nitrite in the medium. It is therefore concluded that the nitronitrosoguanidine structure, rather than a breakdown product, is the mutagenic agent.

The technical assistance of Miss Pearl Woody with some of the experiments is gratefully acknowledged. 1-Methyl-3-nitro-1-nitrosoguanidine is available commercially. The parental strains of E. coli S and H were obtained through the courtesy of Dr. A. D. Hershey.

This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, U.S. Public Health Service, Contract No. SA-43-ph-3070 and Grant No. CY-4548.

REFERENCES

Kaudewitz, F. Production of Bacterial Mutants with Nitrous Acid. Nature, 183, 1829-30 (1959).

Lederberg, J. and E. M. Lederberg. Replica Plating and Indirect Selection of Bacterial Mutants. J. Bact., 63, 399-406 (1952).

Luria, S. E. and M. Delbrück. Mutations of Bacteria from Virus Sensitivity to Virus Resistance. Genetics, 28, 491 (1943).

Mandell, J. D., P. L. Woody, and J. Greenberg. Resistance and Cross-Resistance of Escherichia coli mutants to anticancer agents. 1-Methyl-3-nitro-1-nitrosoguanidine. J. Bact., in press.