Spontaneous Mutations in Bacteria

J. BENNETT CLARK, University of Oklahoma, Norman

The fundamental aspects of the appearance of spontaneous mutants in a population of micro-organisms has been extensively discussed in the literature (12, 13). These different mutant characters which are found in any bacterial population have been shown to arise spontaneously by fluctuation tests (14) and by direct experimental tests (15). The presence of such mutant cells in a bacterial clone can obscure induced mutations, be mistaken for induced mutations by inadequate interpretation of factors of selection due to population dynamics, or can lead to calculation of false induced mutation rates. These factors have been analyzed and circumvented by Stone, *et al.* (18).

The exact cause of spontaneous mutations is unknown. From a genetic standpoint bacteria are in all probability little more unstable than other

forms of life. Auerbach, *et al.* (1) consider that chemical conditions created by the cell are sufficient to cause mutation; this would imply that in the metabolic processes of the cell, certain metabolic waste products are formed which are in themselves mutagenic. This is plausible provided the definition of a chemical mutagen is not restricted to those compounds which react directly with genic material. A more tenable hypothesis would be that in most cases the appearance of spontaneous mutants is due to inexact gene duplication during cell division. This latter theory is substantiated by the work of Haas, *et al.* (10) who showed that the spontaneous mutation rate in *Escherichia coli B* to virus resistance increased as the simplicity of the medium was increased. Their interpretation of these results is that in the more simple media, the organism must carry out more steps for the synthesis of gene material, so that the chance for inexact replication in the synthesis is increased. Wyss (21) has demonstrated that changes in environmental conditions away from optimum will affect the rate of spontaneous mutation. He found that adverse factors such as altered temperature and pH can alter the metabolism of a cell to such an extent that gene synthesis can be affected. This and other experimental evidence renders the altered synthesis hypothesis tenable.

One of the major aspects of microgenetics is to determine the chemical composition of the gene by determining the effects of chemical mutagens on the phenotypic expressions of the organism. This aspect depends to a great extent on the assumption that the mutagen reacts directly with the genic entity, and thus chemical characteristics of the gene can be determined by a study of the chemical reaction involved. On this assumption a large number of chemicals have been tested for mutagenic properties, and a surprising number have been shown to be capable of inducing mutations (2, 20). However, in many cases, it is probable that the mutagen did not react with the gene directly, but instead interfered in some metabolic pathway which caused an inexact duplication of the gene in the daughter cell. Thus the apparent induced mutation could well be merely a change in the spontaneous mutation rate in the clone. This latter possibility has been minimized in some cases by the use of "zero-point" mutations as a means of eliminating factors of selection (7, 8). In the "zero-point" mutations the mutant cells are tested for before cell division takes place. However, the inaccuracy of absolutely controlling cell division tends to invalidate some of this work.

Preliminary laboratory investigation of the altered synthesis hypothesis has been promising although unequivocal proof of this theory is still to be found. Various enzyme inhibitors have been found to be mutagenic (2, 11, 13), and the use of competitive inhibitors (16) has increased the total number of mutants in a bacterial clone (2). Such mutations would not be due to direct action with the gene. Colwell (5, 6) found that 2-methyl, 1-4, naphthoquinone would inhibit growth of fungi and bacteria by reacting with sulfhydryl groups and in a similar manner cause small colony variants of *E. coli* to be formed. The same compound also causes mutation to antibiotic resistance in *Micrococcus aureus* (2). The nucleic acid portion of the nucleoprotein-gene complex contains no sulfhydryl groups and probably few exist in the protein part of the molecule. Therefore it would appear more probable that the function of this compound would be interfering with the action of sulfhydryl containing enzymes which are involved in gene synthesis. This is substantiated by the experiments of Wyss (21) which showed the effects on spontaneous mutation rate of various sulfhydryl levels in the nutrient substrate, and by Clark *et al.* (4) who demonstrated the same mutational changes by reacting menadione with the substrate prior to inoculation. Thom and Steinberg (17, 19) found several chemicals to be mutagenic on several of the *Aspergilli*. These included compounds which are reactive with free amino groups, such as nitrous acid, ninhydrin, chloramin T, potassium iodide, and hexamethylenamine.

reversal of the mutations would take place at alkaline pH by adding d-lysine and sodium thiosulfite to the culture. These results are indicative of a direct reaction between the mutagen and the gene complex. The same chemicals have been found to induce antibiotic resistant mutants in *Micrococcus aureus* (2). However, treatment of the nutrient substrate with an acidified sodium nitrite solution, and then readjusting the pH of the medium prior to inoculation resulted in the same type of mutation. It would therefore seem plausible that these mutations were merely an increase over the spontaneous rate normally found, which increase was caused by a lowering of the availability of alpha-amino compounds for various metabolic processes, including those involved in gene synthesis.

The use of nitrogen mustards as mutagens has been fairly extensive (1, 9, 11, 13). In these cases it is assumed that the mustard mutagen reacts directly with the gene complex. However Wyss, *et al.* (22) found that treatment of the nutrient substrate with tris-(β-chloroethyl)-amine prior to inoculation with *Micrococcus aureus* resulted in a significant increase in the number of antibiotic resistant mutants. It was believed that the mustard was completely hydrolyzed at the time of inoculation because no odor was detectable and no inhibitory effect was noted. This again appears to be a case of inexact gene synthesis due to altered metabolic processes in an abnormal substrate.

In view of these preliminary experiments it seems very plausible to assume that the altered metabolism theory might be operative to a great extent in many of the supposedly induced mutations. Therefore in working with any mutagenic system one faces at least four possibilities for the mode of action of the mutagen. These are:

1. The mutagen acts directly on the gene itself.
2. The mutagen alters the permeability of the cytoplasmic membrane thus allowing the liberation of some transforming substance (20).
3. The apparent increase in mutant forms is due to uncovering of a recessive trait by stimulation of gene recombination (3).
4. The mutagen interferes with some metabolic pathway leading to gene synthesis and thus causes inexact duplication of the gene in the daughter cell.

LITERATURE CITED

1. Auerbach, C., J. M. Robson, and J. G. Carr. 1947. Chemical production of mutations. Science *105*: 243-257.
2. Clark, J. B. 1948. The chemical induction of mutations in bacteria. Master's thesis, the University of Texas, Austin.
3. ———, F. Haas, W. S. Stone, and O. Wyss. 1950. The stimulation of gene recombination in *Escherichia coli*. J. Bact. *59*: 375-379.
4. ———, O. Wyss, and W. S. Stone. 1950. Induction of mutation in *Micrococcus pyogenes* by chemical inactivation of sulfhydryl groups. Nature *166*: 340.
5. Cowell, C. A. 1946. Small colony variants of *Escherichia coli*. J. Bact. *52*: 417-422.
6. ———, and M. McCall. 1946. The mechanism bacterial and fungus growth inhibition by 2-methyl, 1-4, naphthoquinone. J. Bact. *51*: 659-670.
7. Demerec, M. 1946. Induced mutations and possible mechanisms of the transmission of heredity in *Escherichia coli*. Proc. Natl. Acad. Sci. U. S. *32*: 36-46.

8. ————, and R. Latarjet. 1946. Mutations in bacteria induced by radiation. Cold Spring Harbor Symp. Quant. Biol. *11*: 38-50.

9. Gilman, A., and F. S. Philips. 1946. The biological actions and therapeutic applications of β-chloroethylamines and sulfides. Science *103*: 409-415.

10. Haas, F., J. B. Clark, O. Wyss, and W. S. Stone. 1950. Mutations and mutagenic agents in bacteria. Am. Naturalist *84*: 261-274.

11. Lederberg, J. 1948. Problems in microbial genetics. Heredity *2*: 145-198.

12. Lederberg, J. 1951. Papers in microbial genetics, bacteria and bacterial viruses. Madison: Univ. of Wisconsin Press.

13. Luria, S. E. 1947. Recent advances in bacterial genetics. Bact. Rev. *11*: 1-40.

14. ————, and M. Delbrück. 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics *28*: 491-511.

15. Newcombe, H. B. 1949. Origin of bacterial variants. Nature *164*: 150.

16. Shive, W., and J. M. Macow. 1946. Biochemical transformations as determined by competitive analogue-metabolite growth inhibitors. I. Some transformations involving aspartic acid. J. Biol. Chem. *162*: 451-462.

17. Steinberg, R. A., and C. Thom. 1940. Mutations and reversions in reproductibility of *Aspergilli* with nitrite, colchocine, and *d*-lysine. Proc. Natl. Acad. Sci. U. S. *26*: 363-368.

18. Stone, W. S., F. Haas, J. B. Clark, and O. Wyss. 1948. The role of mutation and selection in determining the occurrence of mutations in irradiated broth. Proc. Natl. Acad. Sci. U. S. *34*: 142-149.

19. Thom, C., and R. A. Steinberg. 1939. The chemical induction of genetic changes in fungi. Proc. Natl. Acad. Sci. U. S. *25*: 329-333.

20. Witkin, E. M. 1947. Mutations in *Escherichia coli* induced by chemical agents. Cold Spring Harbor Symp. Quant. Biol. *12*: 256-269. (see discussion)

21. Wyss, O. Personal communication.

22. ————, W. S. Stone, and J. B. Clark. 1947. The production of mutations in *Staphylococcus aureus* by chemical treatment of the substrate. J. Bact. *54*: 767-772.