A STRAIN OF *ESCHERICHIA COLI* WITH AN UNUSUALLY HIGH RATE OF AUXOTROPHIC MUTATION[1]

AVRAM GOLDSTEIN[2] AND JANE S. SMOOT

Department of Pharmacology, Harvard Medical School, Boston, Massachusetts

Received for publication April 25, 1955

The spontaneous rates of various well-defined mutations in bacteria generally range between 10^{-10} and 10^{-5} per cell per generation (Braun, 1953). Although some kinds of heritable change apparently occur much more frequently, these may not be truly mutational in origin. Thus transduction or other types of induced transformation, the emergence of recessive traits in diploid organisms, and recombination may sometimes yield high proportions of stable variants. As far as we are aware, the spontaneous occurrence of auxotrophs, each presumably carrying a single mutant gene, at a total frequency of several per cent in a wild-type population, has not been described.

In November 1950 we obtained a smooth motile strain of *Escherichia coli* from the Department of Bacteriology, Harvard Medical School, to use in studies on streptomycin dependence (Goldstein, 1954). Subsequently we discovered that populations of this organism, on nutrient medium, normally contain a variety of auxotrophic mutants, at a mean frequency of about four per cent. The mutability has persisted through several single-colony isolations and through many transfers on minimal medium, for more than four years. Our purpose here is to report some of the important properties of this strain.

METHODS

The organism used was a motile gram negative rod, which produced smooth round opalescent white colonies on nutrient agar. Glucose, lactose, maltose, mannitol, arabinose, and salicin are fermented, with gas production. Litmus milk is acidified and coagulated without peptonization. Gelatin is not liquefied. The strain is indole-positive and urease-negative. It is catalase-positive when grown on nutrient broth or a glucose-inorganic salt medium. The auxotrophic mutants also contain catalase. The organism corresponds to the type description (Breed *et al.*, 1948) of *E. coli* and has been designated as the Harvard strain, ATCC 11887, NCTC 9561.

Two kinds of solid and liquid media were used, nutrient broth and agar (Difco), and a "minimal" broth and agar of the following composition: Na_2HPO_4, 0.04 M; NaH_2PO_4, 0.022 M; KCl, 0.01 M; Na_2SO_4, 0.001 M; $MgSO_4$, 0.4 mM; NH_4Cl, 9.2 mM (0.05 per cent); glucose, 11.1 mM (0.2 per cent); agar (when desired), 1.5 per cent; pH 7.0. Incubation was at 38 C. Colonies on nutrient agar were about 1.5 mm in diameter after 24 hours, whereas those on minimal agar appeared more slowly and were invisible in 24 hours. By 48 hours, however, the colonies on minimal agar were larger than those on nutrient agar and much more mucoid. The heavy final growth and creamy consistency on minimal agar favor spreading so that adjacent colonies readily coalesce. This property makes it easy to miss auxotrophs that show even a moderate tendency to revert, because as few as 20 or 30 colonies growing in a small test streak quickly fill the area with heavy confluent growth.

The rapid growth, after a 24-hour lag on minimal agar, does not reflect a true adaptation to minimal medium, since such rapidly growing cells fail to form colonies in 24 hours when transferred to fresh minimal agar. The lag is most striking when relatively few cells are implanted on a plate, and disappears entirely when large implants are used; thus a direct streak with a loop, or a replication by means of velvet grows out heavily as early as 16 hours. All the experiments described in this paper were done under conditions permitting wild type growth on minimal agar within 24 hours.

The high mutant frequency made special screening methods unnecessary. Instead a dilution of the population to be tested could be plated on

[1] This investigation was supported by U. S. Public Health Service Grant E-339 and by the Eugene Higgins Trust.

[2] Present address: Department of Pharmacology, Stanford University School of Medicine, San Francisco, California.

nutrient media so as to yield about 100 colonies per plate. The colonies were then fished individually onto minimal and nutrient plates either directly or through a dilution step. In later experiments, the velvet replica-plate method (Lederberg and Lederberg, 1952) was used to transfer all colonies simultaneously onto the test plates. After incubation for 24 hours at 38 C, inspection revealed which colonies did not grow on minimal agar. These could be isolated from the original nutrient plate ("master plate") for further study. Whenever a colony on the master plate gave rise to an unusually small colony on the minimal replica-plate, it was tested further because many such proved to be auxotrophs. When colonies were replicated by velvet, the implant was not only large but was confined to a relatively small area. Though the colony being replicated might consist almost wholly of auxotrophs, a few reverted prototrophs might result in a small replica colony on minimal agar.

Our data on auxotrophic mutants refer only to those strains which persistently fail to grow on minimal agar even after subculture on nutrient agar. We also regularly observed strains that grew poorly or not at all in early testing, but after a cycle of growth on nutrient agar could no longer be distinguished from wild types. This finding implies frequent back-mutation to prototrophy, an interpretation consistent with the following additional observations: When colonies on a nutrient master plate were tested by streaking, fewer auxotrophs were found when the organisms were streaked directly on test plates than when they were diluted prior to plating. The apparent mutant yield also was lower when velvet replication was used, presumably because the implants were unavoidably large. Therefore, auxotrophic colonies growing on nutrient master plates are best tested while they are small and before many back-mutant clones develop.

Evidently, our method does not detect mutants with very high reversion rates, mutants that form colonies of normal size when fed syntrophically by adjacent colonies on the minimal test plate, nor incompletely blocked auxotrophs that grow slowly on minimal agar. Consequently, the mutant frequencies reported here must be minimum estimates, the exact degree of underestimation of the true frequencies being unknown.

We identified the growth requirements of the auxotrophs by streaking or by replication on minimal plates enriched with acid hydrolyzate of casein, and mixtures of purines, pyrimidines, nucleosides, and vitamins; and then onto plates enriched with amino acids and other components as described by Lederberg (1950), except as follows: Vitamin K was not included, but *p*-hydroxybenzoic acid, pimelic acid, and β-alanine were. Nicotinamide was used instead of nicotinic acid. Cytosine was omitted from the purine-pyrimidine group; adenosine and guanosine were the only nucleosides, and yeast adenylic acid the only nucleotide used. Spot-testing the supplements on a minimal plate heavily seeded with the auxotroph in question was equally effective. Each mutant was tested on all supplements, even after showing growth on one supplement, in order to detect alternative requirements. Mutants failing to grow on individual components were tested on mixtures, in order to detect multiple requirements. Mutants failing to grow on any defined medium were tested with the addition of yeast extract.

RESULTS

Frequency of auxotrophic mutants in wild type populations. When a broth tube is inoculated from a nutrient slant, the resulting population includes mutants from two sources: (a) the progeny from the inoculum, and (b) the progeny from new cells arising during growth in the broth. The high mutant frequency made it almost impossible to avoid the introduction of mutants with the inoculum. The number introduced also varied, reflecting the fluctuation in mutant frequency between different stock slants and even between different areas of the same slant. In most studies of mutant frequency, we considered isolated colonies on nutrient agar as our populations for study. Each colony grew independently, was about 4 mm in diameter at 48 hours, and contained 100 to 200 $\times$ 10^6 viable cells. After appropriate dilution, a colony was suspended in water and plated on nutrient agar. The resulting daughter colonies were then tested. If the colony under test had originated from an auxotrophic mutant, most of its cells would be auxotrophs. This situation could be recognized easily.

The results in table 1, section I, show a mutant frequency fluctuating between 1.3 per cent and 15.0 per cent of the total number of viable cells

TABLE 1

Frequency of auxotrophic mutants in wild-type populations

Source of Colonies Tested	No. of Cols. Tested	Auxotrophs Found	Mutant Frequency (%)
I. Diluted water suspension of colony growing on nutrient agar	292	4	1.4
	(275)*	(272)*	(98.9)*
	175	11	6.3
	362	6	1.7
	318	4	1.3
	183	27	14.8
	311	5	1.6
	237	3	1.3
	180	27	15.0
	223	6	2.7
Total	2,281	93	4.1
Mean of the individual frequencies	—	—	5.1
II. Diluted 24-hour nutrient broth growth of inoculum from nutrient slant	576	8	1.4
	1,057	16	1.5
	1,666	74	4.4
	1,916	32	1.7
Total for experiments on nutrient-grown populations (I, II)	7,496	223	3.0
Mean of the individual frequencies	—	—	4.2
III. Diluted 48-hour nutrient broth growth from single cell grown on minimal agar	512	5	1.0
IV. Diluted water suspension of colony growing on minimal agar	198	1	0.5
	287	0	0.0
	257	1	0.4
	141	1	0.7
	354	0	0.0
	278	1	0.4

* The colony under test here was obviously an auxotroph itself. This experiment is, therefore, excluded from all calculations and from the fluctuation test referred to in the text.

in independently grown wild-type colonies on nutrient agar. The mean frequency in this series was about 5 per cent, but the most probable frequency is closer to 2 per cent, since the distribution is asymmetric. If the data are treated as a fluctuation test, Chi-square analysis indicates marked inhomogeneity ($P < 0.001$). Thus, the auxotrophs under investigation arise de novo, spontaneously, independently, and in the fluctuating manner typical of mutational events, within wild-type colonies growing on nutrient agar. The data of section II, from experiments with nutrient slants, are wholly consistent with the above estimates of mutant frequency. The mean of the individual frequencies for all experiments on nutrient-grown populations (Sections I and II combined) is 4.2 per cent and this is our best estimate of the degree of mutability in the Harvard strain. Section III shows that mutants can also originate in a nutrient broth culture tube inoculated with a single wild-type cell and containing a final population of 515×10^6 viable cells in 5 ml after 48 hours. Section IV demonstrates that colonies growing on minimal agar may also contain auxotrophs but the frequency is much lower, presumably because the mutants cannot form clones on the minimal medium.

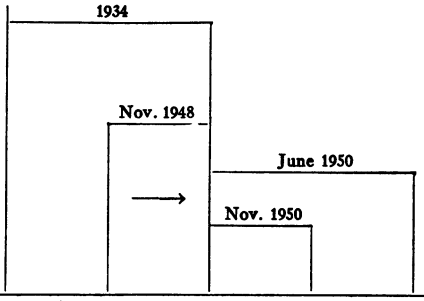
Origin of the increased mutability. Having established the unusually high frequency of auxotrophs originating de novo in wild-type populations of this organism, we investigated the history of the strain with a view to discovering, if possible, when its peculiar genetic behavior had originated. We were fortunate to find several stock cultures with well-documented histories in the Department of Bacteriology. The parent strain had been obtained by the late Dr. J. H. Mueller about 20 years ago. A stock slant was maintained on tryptic digest agar, in his own refrigerator, for his own use. Shortly after its acquisition, a subculture was made for teaching use. This slant (also on tryptic digest agar) was maintained separately, with other classroom materials, and, from time to time, was purified through single-colony isolations. Our strain was obtained in November 1950, from this class slant, and maintained by us, through several single-colony purifications on nutrient agar, and on minimal agar. A broth culture of the class strain was frozen in June 1950 and stored. Finally, a subculture of the class slant had been maintained by Dr. H. E. Umbarger since 1948. Thus, we had available for study five different stock cultures which had originated at different times, more or less directly, from the original parent strain.

Having established (table 1) that the best estimate of mean mutant frequency in the Harvard

TABLE 2

Origin of the increased mutability of the Harvard strain

The genealogy of each culture is shown in the usual way as a line diagram. In the columns, for the various strains, are shown results of testing for mutant frequency. The arrow indicates when, and in what culture, the unusual mutability of the Harvard strain originated.



	Mueller	Umbarger	Class	Harvard	Class (Frozen)	K-12
No. mutants/total cells in different experiments	0/249 0/505 0/607 1/638	0/200	17/350	38/500 3/183 6/515	0/200 0/569	1/523 0/755
Max. per cent mutants in stable strains (upper 99% confidence limit)	0.34	2.30	—	—	0.60	0.52
Per cent mutants in unstable strains			4.9	3.9*		

* An estimate based on frequencies in a larger number of experiments (table 1) is 4.2 per cent.

strain was 4.2 per cent, we sought to determine which of the other strains displayed a comparable degree of mutability. A fresh nutrient-grown population was tested in each experiment. The results are summarized in table 2. Only the class strain currently in use yielded a mutant frequency of the same order as the Harvard strain. Populations of the other strains contained only an occasional auxotroph. Upper 99 per cent confidence limits, calculated readily from the Poisson distribution, show that the mutant frequency is very significantly lower in these strains than in the class and Harvard strains.

The relationship of the class and Harvard strains to the others (table 2) indicates clearly that the mutability of the Harvard strain originated between June and November of 1950 in the Department of Bacteriology. The culture frozen in June 1950 (designated MSFB) is the nearest genetically stable ancestor of the Harvard strain. It is available on request from the authors. As there is no record or recollection of any extraordinary environmental circumstance at this time, it must be assumed that the mutability itself, having arisen as a mutational event, was selected accidentally during a single-colony purification and thus perpetuated. The negative results with other cultures (including the K-12 strain) exclude artifacts, misinterpretations, and unusual conditions in our laboratory as explanations of the apparent mutability of the Harvard strain.

Types of auxotroph obtained. Table 3 presents the results of two typical experiments, performed at different times, with different stock cultures. The auxotroph frequencies were 4.4 and 1.7 per cent, the significant difference ($P < 0.001$) reflecting the expected fluctuation between independent cultures. Characterization of the auxotrophs obtained in these experiments establishes two important facts: (1) The nutritional requirements are diverse. The pathways for synthesis of amino acids, vitamins, purines, and pyrimidines are involved. The finding that requirements for amino acids (especially histidine) are most frequent accords with general experience. (2) The nutritional requirements are single in most cases. Only 5 to 6 per cent have a multiple requirement. Such a pattern is characteristic of auxotrophic

mutation involving single genes, in contrast to linkage-group effects such as result from recombination. The diversity of the single requirements suggests that the genetic instability is not confined to the vicinity of any single gene locus.

TABLE 3

Analysis of growth requirements of auxotrophs obtained from two wild-type populations

Experiments A and B were done at different times, using different nutrient slants of wild-type Harvard strain. Inocula (about 10^6 viable cells) were grown in nutrient broth to 84.5 and 97.2 × 10^6 viable cells per ml, respectively. Dilute implants were made on nutrient plates and the resulting colonies were replicated by velvet onto minimal and nutrient agar. Subsequent testing was done by enriching minimal agar as described in the text. Data in the table are the numbers of colonies of each mutant type obtained, each such colony representing a single cell of that type in the wild-type population. One cannot distinguish here between a single large mutant clone and several smaller clones of the same mutant type.

Requirement	Experiment A	Experiment B
None*	14	9
Histidine†	53	14
Proline	3	0
Methionine	2	0
Cysteine‡	3	1
Isoleucine	1	0
Arginine	3	0
Lysine	0	5
Tryptophan	0	3
Tyrosine	1	0
Tyrosine or phenylalanine	0	2
B_{12} or methionine	1	0
Nicotinamide	1	0
Purine§	1	1
Uracil	1	2
Arginine plus Pyridoxamine	1	0
Casein hydrolysate (acid)¶	3	0
Casein hydrolysate plus vitamins¶	0	2
Yeast extract‖	0	2
(a) Total colonies originally tested	1,666	1,916
(b) Total apparent auxotrophs on first test	88	41
(c) Total stable auxotrophs	74	32
as per cent of (a)	4.4%	1.7%
Type of growth requirement		
Single requirement or alternative single requirements	70	28
as per cent of (c)	94.6%	87.4%
Not completely determined	0	2
as per cent of (c)	0%	6.3%
Definite or probable multiple requirement	4	2
as per cent of (c)	5.4%	6.3%

* These failed to grow on minimal agar in 24 hours in the initial test but subsequently grew in a manner indistinguishable from wild type. They may have been mutants that reverted during the single passage on nutrient agar.

† There were at least two types of histidine requirement. Both could be met by acid-hydrolyzed casein but only one by enzyme-hydrolyzed casein.

‡ For one mutant in each experiment methionine plus a vitamin mixture replaced cysteine.

§ Adenine or guanine or hypoxanthine.

¶ As these would not grow on any single amino-acid or any one screening group of amino-acids they presumably required two or more from different groups. Some also had a vitamin requirement. They were not further studied.

‖ No other supplement or mixture of supplements sufficed.

In studies on streptomycin resistance and dependence, we found that the unusual mutability does not involve all genetic systems to the same degree. The frequency of mutants resistant to 10 μg streptomycin per ml, estimated from data of a great many experiments, was about one in 60,000 cells; and of mutants dependent upon streptomycin, about one in 10,000 cells (Goldstein, 1954). Since the resistant mutants are known to grow at about the same rate as the wild type in ordinary nutrient medium, one can calculate the mutation rate to be approximately 7×10^{-7} per cell per division cycle. This is within the range found by other invesitgators (Braun, 1953). On the other hand, the mutation rate from wild type to dependence must be phenomenally high. Dependent mutants form clones of very limited size in the absence of streptomycin (Gold-

stein, 1954), and it follows that the mutation rate is not very much lower than the observed mutant frequency, or about 1×10^{-4} per cell per division cycle. In contrast to the situation found (Newcombe and Hawirko, 1949) in other strains of *E. coli* mutation to dependence, in the Harvard strain, is much more frequent than mutation to a moderate degree of resistance.

Of 25 streptomycin-dependent strains, each isolated in a different experiment, four were found to be auxotrophs, each with a different single requirement. Likewise, a streptomycin-dependent but otherwise prototrophic population was found to contain 4.9 per cent of cells that were both streptomycin-dependent and auxotrophs. These results seem to show that the mutations to streptomycin dependence and to auxotrophy, in this strain, are quite independent processes. We do not consider four auxotrophs among 25 streptomycin-dependent strains to be significantly higher than expected, especially since the data in table 1 show the particular wild-type slant used as starting material for the isolation of dependent mutants may have contained as many as 16 per cent auxotrophs.

The independence of successive mutations is also shown in the experiment summarized in table 4. A uracil-less auxotroph was used as starting material. Progeny of this mutant were screened for the presence of cells with additional growth requirements, and several types of double auxotroph were found. The unusual mutability of the strain persists after a first mutation has occurred, so that double auxotrophs arise as frequently in a population of single auxotrophs, as did the latter in a wild-type population.

Effects of ultraviolet irradiation. In two experiments summarized in table 5 we compared the frequency of auxotrophic mutants in a wild-type population before and after ultraviolet irradiation. A turbid suspension was exposed to a General Electric germicidal lamp for one minute, killing 97 to 99 per cent of the cells, as measured by viable count on nutrient agar. In experiment A the irradiated organisms grew on minimal agar and only a few auxotrophs were initially present. In experiment B a nutrient slant containing a high proportion of auxotrophs was used. In both cases there was an apparent increase in auxotroph frequency. At least in experiment B some new auxotrophs must have been produced, unless those initially present were killed more slowly than the wild type. Irradiation also produced, in both experiments, a great many colonies unable to grow on minimal agar when first tested. Later, after further growth on nutrient agar, the colonies regained this capacity. Such apparently unstable auxotrophs are characteristic of the Harvard strain under normal conditions. Their usual frequency is much increased by irradiation, here from 16.4 to 42.0 per cent and from 13.4 to 39.6 per cent in experiments A and B, respectively.

TABLE 4

Frequency and types of double auxotrophs

The populations studied were three colonies of a uracil-less auxotroph of the Harvard strain, growing on nutrient agar. The minimal test media contained uracil and suspected mutants were tested by adding further supplements. All the mutants isolated still required uracil and had a single additional requirement, as shown below.

Population	Total Colonies Tested	Numbers and Types of Auxotroph Found	Mutant Frequency
			%
I	388	2 histidine 1 nicotinamide 2 casein hydrolysate (acid)* 1 yeast extract†	
Total	.....	6	1.5
II	218	2 histidine 1 lysine 1 thiamin	
Total	.....	4	1.8
III	199	9 purine‡	4.5

* Would not grow on uracil plus any single amino acid.

† Would not grow on any defined medium.

‡ The purine requirement was met alternatively by adenine, guanine, or hypoxanthine.

DISCUSSION

The findings indicate that in a certain strain of *E. coli* an abrupt change occurred, leading to persistent genetic instability. The unstable line, designated the Harvard strain, does not differ obviously from its parent, except in the frequency with which various mutants arise. Auxotrophic mutants have been studied in some detail and have been shown to arise spontaneously in an

TABLE 5

Effects of ultraviolet irradiation upon auxotroph frequency

	Experiment A		Experiment B	
Source of organisms	minimal slant		nutrient slant	
Per cent killed by U.V.	99		97	
Conditions of first test	Direct streak from colonies onto minimal and nutrient agar at 21 hours.		Replication of colonies by velvet onto minimal agar at 24 hours. Master plates incubated a further 24 hours.	
Conditions of retest	Dilute streak from growth on nutrient plate of first test, onto minimal and nutrient agar.		Direct streak from colonies on master plate, onto minimal and nutrient agar.	
	Before U. V.	After U. V.	Before U. V.	After U. V.
Total colonies tested	195	200	463	187
Number failing to grow, or growing very poorly on minimal agar in 24 hours in first test	32 (16.4%)	84 (42.0%)	62 (13.4%)	74 (39.6%)
Number of stable auxotrophs after retest	1 (0.5%)	2 (1.0%)	46 (9.9%)	29 (15.5%)

ordinary wild-type population growing on nutrient media. Their frequency fluctuates significantly from population to population about a mean of approximately 4 per cent of the total number of viable cells present. Assuming equal growth rate of auxotrophs and wild type in nutrient medium, this would represent an over-all mutation rate of 1.5×10^{-3} per cell per division cycle. A wide variety of nutritional deficiencies is represented, but the requirements for growth on minimal medium can usually be met by single (or alternative single) substances. The frequency of auxotrophic double mutants in a single-mutant population is just as phenomenally high as that of initial mutants in a wild-type population, and diverse types of second mutation occur. There is some evidence of a very high frequency of streptomycin-dependent mutants, but other mutations have not yet been studied.

The evidence seems to indicate that the changes which occur in the Harvard strain are in fact single-gene mutations. The resulting auxotrophs are apparently like those occurring in normal strains at much lower spontaneous frequencies. Because the genetic instability of the Harvard strain is itself a stable hereditary characteristic it may be assumed to have a genetic basis. Comparable instances of the actions of mutator genes have been described in Drosophila (Demerec, 1937; Ives, 1950), maize (McClintock, 1951), yeast (Ephrussi and Hottinguer, 1951) and Streptomyces (Newcombe, 1953). Treffers *et al.* (1954) reported a similar case in the K-12 strain of *E. coli*, characterized by high mutation rates to streptomycin resistance as well as resistance to certain other agents.

What heritable biochemical anomaly might cause the instability? It is hard to imagine an intrinsic structural instability of chromosomal desoxyribonucleic acid or protein that would involve a great many different loci and at the same time spare others, e. g., those controlling streptomycin resistance. More plausible mechanisms currently under investigation include production of endogenous mutagen, abnormal amount or localization of a protective enzyme, and excessive desoxyribonuclease activity. The surprising inability of ultraviolet irradiation to cause a substantial increase in the frequency of stable auxotrophs perhaps suggests that whatever mechanism is activated by irradiation of normal bacteria is already maximally active in the Har-

vard strain. The significance of the normally occurring unstable auxotrophs whose frequency is increased by irradiation remains obscure although very similar observations have been reported by Peacocke and Hinshelwood (1948), Miller *et al.* (1949), and Lederberg (1950).

ACKNOWLEDGMENTS

The authors are indebted to Alexandra Shedlovsky and Barbara Wolff for assistance with some of the experiments, to Marjorie Jewell for assistance in establishing the genealogy of the strain, and to Dr. H. E. Umbarger for helpful discussions at various stages of the investigation.

SUMMARY

A strain (ATCC 11887) of *Escherichia coli* has been described in which various auxotrophic mutants arise at phenomenally high rates, leading to mutant frequencies of several per cent in ordinary wild-type populations. This strain may serve as a useful tool in studying the causes of "spontaneous" mutation.

REFERENCES

BRAUN, W. 1953 *Bacterial genetics*. W. B. Saunders Co., Philadelphia, Pa.

BREED, R. S., MURRAY, E. G. D., AND HITCHENS, A. P. 1948 *Bergey's Manual of Determinative Bacteriology*. 6th ed. The Williams and Wilkins Co., Baltimore, Md.

DEMEREC, M. 1937 Frequency of spontaneous mutations in certain stocks of *Drosophila melanogaster*. Genetics, **22**, 469–478.

EPHRUSSI, B., AND HOTTINGUER, H. 1951 Cytoplasmic constituents of heredity. On an unstable cell state in yeast. Cold Spring Harbor Symposia Quant. Biol., **16**, 75–85.

GOLDSTEIN, A. 1954 The origin of streptomycin-dependent variants of *Escherichia coli*. J. Pharmacol. Exptl. Therap., **112**, 326–340.

IVES, P. T. 1950 The importance of mutation rate genes in evolution. Evolution, **4**, 236–252.

LEDERBERG, J. 1950 Isolation and characterization of biochemical mutants of bacteria. Methods in Medical Research, **3**, 5–22.

LEDERBERG, J., AND LEDERBERG, E. M. 1952 Replica plating and indirect selection of bacterial mutants. J. Bacteriol., **63**, 399–406.

MCCLINTOCK, B. 1951 Chromosome organization and genic expression. Cold Spring Harbor Symposia Quant. Biol., **16**, 13–48.

MILLER, H., FARGHALY, A. H., AND MCELROY, W. D. 1949 Factors influencing the recovery of biochemical mutants in luminous bacteria. J. Bacteriol., **57**, 595–602.

NEWCOMBE, H. B., AND HAWIRKO, R. 1949 Spontaneous mutations to streptomycin-resistance and dependence in *Escherichia coli*. J. Bacteriol., **57**, 565–571.

NEWCOMBE, H. B. 1953 Radiation-induced instabilities in Streptomyces. J. Gen. Microbiol., **9**, 30–36.

PEACOCKE, A. R., AND HINSHELWOOD, C. N. 1948 The changes induced in Bacterium lactis aerogenes by irradiation with ultraviolet light. Proc. Roy. Soc. (London), **B135**, 454–461.

TREFFERS, H. P., SPINELLI, V., AND BELSER, N. O. 1954 A factor (or mutator gene) influencing mutation rates in *Escherichia coli*. Proc. Nat. Acad. Sci. U. S., **40**, 1064–1071.