ON THE NONADAPTIVE NATURE OF CHANGE TO FULL STREPTOMYCIN RESISTANCE IN ESCHERICHIA COLI

HOWARD B. NEWCOMBE AND JOHN McGREGOR

Atomic Energy Project, National Research Council, Chalk River, Ontario, Canada

Received for publication May 21, 1951

The change from streptomycin sensitivity to full streptomycin resistance (ability to grow in concentrations of 1,000 to 100,000 units per ml) can occur as a single-step alteration in the cells of certain bacteria in the complete absence of the drug. In *Escherichia coli*, strain B/r, such changes take place with a frequency of one or two in every 10^{10} cell divisions (Newcombe and Hawirko, 1949).

Resistance to streptomycin is of two kinds, simple resistance and dependence. From crossing experiments using the sexually fertile *E. coli*, strain K 12 (Lederberg, 1947), it appears that both kinds of resistance arise through mutation in the same gene locus (Demerec, 1950; Newcombe and Nyholm, 1950*a*), and the approximate position of this locus has been determined with respect to other genes on the chromosome (Newcombe and Nyholm, 1950*b*).

No chemical substance has yet been shown to act with a high degree of specificity on a particular gene locus to cause it to mutate. It would be surprising therefore if streptomycin influenced the rate of mutation to streptomycin resistance. However, as this possibility has not been ruled out by any previous work, it seemed desirable that it should be tested experimentally. For this purpose mutation rates from partial resistance to full resistance were determined in the presence and in the absence of the drug.

METHODS

Using *E. coli*, strain B/r (Witkin, 1947), partially resistant lines were obtained by serial subculture in increasing concentration of streptomycin. These lines have been designated B/r/sr32a, B/r/sr32b, B/r/sr64, and B/r/sr128, the number indicating the maximum drug concentration (in units per ml, or parts per million) in which growth would take place from small inocula. Lines B/r/sr32a and b were obtained independently from B/r; lines B/r/sr64 and 128 were derived from B/r/sr32b.

Mutation rates to streptomycin resistance (i.e., simple resistance and dependence) were determined for growth in the presence and in the absence of streptomycin, using series of one ml broth cultures started from small inocula (100 to 1,000 cells), and applying the formula,

$$a = -(\log_e 2)\ (\log_e P_0)/N$$

where a is the mutation rate per bacterium per division cycle, P_0 is the proportion of cultures in which there are no mutants, and N is the number of bacteria per culture (derived from formulas 4 and 5 of Luria and Delbrück, 1943). Mutation rates estimated by this formula are unaffected by differences in the rela-

tive growth rates of mutant and parent forms. Mutants were detected by plating whole cultures with melted agar containing 1,000 units of streptomycin per ml. Colonies which developed were tested for their ability to grow in liquid broth cultures containing the drug in the same concentration and for their ability to grow when streaked on duplicate plates of nutrient agar, the one containing no streptomycin and the other containing 1,000 units of streptomycin per ml. In this manner mutants were categorized as simple resistant or dependent.

Rates of mutation to resistance to phage T1 were determined in a similar manner except that 0.2 ml cultures were used and mutants were detected by spreading whole cultures on the surface of nutrient agar which had previously been coated with a suspension of the phage.

TABLE 1

Mutation rate to streptomycin resistance (and dependence). Effect of presence of streptomycin during growth

(Using a partially resistant line, B/r/sr32a, and 32 units of the drug per ml. For each estimate of rate one hundred test cultures of 1 ml each were used, and N was estimated by assay of four sample cultures. The rates for the combined data were based on the whole 1,000 cultures, and a value of N which is the mean of the values for the individual experiments.)

EXPERIMENT	STREPTOMYCIN ABSENT			STREPTOMYCIN PRESENT		
	N ($\times 10^8$)	P_0	Mut. rate ($\times 10^{-10}$)	N ($\times 10^8$)	P_0	Mut. rate ($\times 10^{-10}$)
A	1.8	0.82	7.8	2.8	0.72	8.1
B	5.7	0.85	2.0	3.5	0.74	5.9
C	5.0	0.89	1.6	3.5	0.81	4.1
D	5.3	0.82	2.6	4.0	0.79	4.1
E	4.2	0.55	9.9	3.0	0.48	16.9
F	3.7	0.93	1.4	3.0	0.64	10.3
G	1.8	0.90	3.7	1.8	0.84	6.1
H	3.0	0.84	4.0	1.2	0.82	11.2
I	4.5	0.79	3.6	2.9	0.78	5.9
J	4.4	0.69	5.8	3.6	0.74	5.6
Combined	3.9	0.81	3.7	2.9	0.74	7.2

EXPERIMENTAL RESULTS

Mutation rate to streptomycin resistance (and dependence) in line B/r/sr32a proved to be higher when growth took place in the presence of streptomycin (table 1). This difference is statistically significant (table 3) and could be the result of: (1) a specific action of the drug on the streptomycin locus, (2) a slight nonspecific mutagenic action, or (3) some nonmutagenic effect which nevertheless influences the estimation of rate by this method (as, for example, through causing the bacteria to remain together in small clusters or chains so that the number of viable cells in cultures grown in the presence of streptomycin would be underestimated).

The present data do not enable us to discriminate between interpretations

(2) and (3). However, evidence against the first of the previous interpretations (specific mutagenic action) was obtained from studies of the effect of the drug on mutation to phage T1 resistance, a change which is unrelated to the streptomycin response mutations and which involves a gene quite widely separated from the streptomycin locus on the chromosome map. The rate of this mutation showed a similar increase in the presence of the drug (table 2). The increase is statistically significant at the 5 per cent level and does not differ significantly from that for mutation to streptomycin resistance (table 3). Thus, streptomycin, in the concentrations used in these experiments, has no detectable specific effect upon mutation of the streptomycin locus.

This does not rule out the possibility of bacteriostatic or lethal concentrations of the drug having a specific mutagenic action. Such an action is unlikely, however, since equal samples from a culture of strain B/r yield equal numbers of resistant colonies when plated with melted agar containing streptomycin in any concentration from 100 to 10,000 units per ml.

TABLE 2

Mutation rate to phage T1 resistance. Effect of presence of streptomycin during growth

(Using a partially resistant line, B/r/sr32a, and 32 units of the drug per ml. For each estimate of rate one hundred test cultures of 0.2 ml each were used.)

EXPERIMENT	STREPTOMYCIN ABSENT			STREPTOMYCIN PRESENT		
	N ($\times 10^8$)	P_0	Mut. rate ($\times 10^{-8}$)	N ($\times 10^8$)	P_0	Mut. rate ($\times 10^{-8}$)
A	1.3	0.73	0.17	0.7	0.64	0 42
B	0.6	0.57	0.61	0.5	0.57	0.69
C	1.1	0.51	0.41	1.0	0.61	0.34
D	1.0	0.27	0.91	0.7	0.28	1.23
E	1.1	0.54	0.39	0.8	0.54	0.53
F	0.8	0.50	0.57	0.6	0.43	0.91
G	1.2	0.60	0.29	0.7	0.52	0.65
H	0.9	0.64	0.35	0.7	0.61	0.26
I	1.0	0.57	0.39	0.5	0.50	0.96
J	0.7	0.68	0.38	0.5	0.61	0.69
Combined	1.0	0.56	0.45	0.6	0.54	0.67

Nature of the partially resistant lines. Partial resistance might arise through: (1) mutation at the streptomycin locus, (2) mutation at a locus (or loci) in some other position on the chromosome, or (3) nongenic change (cytoplasmic adaptation), or through a combination of these.

Changes in the streptomycin locus apparently contribute to the partial resistance, since in the lines used in the present experiments mutation to full resistance is more frequent than in the strain B/r from which they were derived (see tables 1, 4, and 5). This is not the result of a general increase in mutability since mutation rate to phage resistance is unaltered in the partially resistant lines. Further, similar levels of partial resistance seem to be associated with

quite different changes in the streptomycin locus, since (a) the mutation rates to full resistance are different in strain B/r/sr32a as compared with B/r/sr32b (and the lines derived from it), and (b) the mutation patterns are different,

TABLE 3

Statistical significance of the differences shown in tables 1 and 2, using the t test

	t	P*
† Mutation to streptomycin resistance (and dependence) ($\times 10^{-10}$)		
Avg. of rates in absence of drug 4.24 ± 2.66 Avg. of rates in presence of drug 7.82 ± 3.78	2.33	0.02–0.05
† Mutation to phage resistance ($\times 10^{-8}$)		
Avg. of rates in absence of drug 0.447 ± 0.195 Avg. of rates in presence of drug 0.668 ± 0.286	2.00	0.05
Ratios of mutation rates determined in the presence to those determined in the absence of the drug		
Avg. of ratios for streptomycin resistance 2.4 ± 1.78 Avg. of ratios for phage resistance 1.6 ± 0.70	1.23	0.20

* The averages may be said to differ significantly where P is equal to, or less than, 0.05. The number of degrees of freedom is 18 in all comparisons.

† It should be noted that the averages of the rates differ slightly from the corresponding rates based on the combined data.

TABLE 4

Mutation rate to streptomycin resistance and dependence. Effect of presence of streptomycin during growth

(Using three partially resistant lines and three different concentrations of the drug. For each estimate of rate one hundred test cultures of 1 ml each were used.)

EXPERIMENT	LINE (B/r)	DRUG CONC. (UNITS/ML)	STREPTOMYCIN ABSENT			STREPTOMYCIN PRESENT		
			N ($\times 10^8$)	P_0	Mut. rate ($\times 10^{-10}$)	N ($\times 10^8$)	P_0	Mut. rate ($\times 10^{-10}$)
A	/sr32b	32	3.0	0.58	12.6	1.6	0.57	24.4
B	/sr32b	32	3.1	0.55	13.4	1.8	0.56	22.4
C	/sr64	64	5.4	0.58	7.1	4.0	0.40	15.9
D	/sr64	64	3.0	0.70	8.2	2.0	0.54	21.4
E	/sr128	128	2.1	0.33	36.6	1.0	0.60	35.5
F	/sr128	128	2.2	0.63	14.7	0.4	0.67	69.6

strain B/r/sr32a mutating almost exclusively to simple resistance, and the other lines mutating at roughly equal rates to simple resistance and to dependence (data not shown).

What has been termed the streptomycin locus (in the absence of evidence for

genetic crossing over between the genes for simple resistance and for dependence) may of course be two (or more) gene loci located close together on the chromosome. If this is the case, the foregoing information would indicate that changes in both of these may be associated with the development of partial resistance.

Changes in other gene loci probably also contribute to the partial resistance. Thus, when streptomycin sensitive and resistant lines of *E. coli*, strain K 12, are crossed, the segregants which are not fully resistant show quite variable degrees of partial resistance (unpublished data). It would appear that the resistant parent contained not only the gene for full resistance, but in addition mutant alleles of other loci capable of producing partial resistance. Similar evidence for the multifactorial origin of chloromycetin resistance in *E. coli*, strain K 12, has been demonstrated by Cavalli and Maccacaro (1950).

TABLE 5

Mutation rate to streptomycin resistance (and dependence) and to phage resistance

Effect of change from streptomycin sensitivity to partial resistance.

STRAINS	NO. OF EXPERIMENTS	AVG. MUTATION RATE	SOURCE
Mutation to streptomycin resistance (and dependence) ($\times 10^{-10}$)			
B/r	4	1.3	Control experiments
B/r	8	2.1	Newcombe and Hawirko, 1949, table 2
B/r/sr32a	10	4.2	table 3
B/r/sr32b	2	13.0	table 4
B/r/sr64	2	7.0	table 4
B/r/sr128	2	20.7	table 4
Mutation to phage resistance ($\times 10^{-8}$)			
B/r	2	1.5	Control experiments
B/r	8	0.40	Newcombe, 1948, table 9
B/r/sr32a	10	0.45	table 3

It is not known whether nongenic adaptation plays any part in the development of partial resistance.

SUMMARY

The presence of streptomycin has no detectable specific effect on mutation rate from partial streptomycin resistance to full streptomycin resistance in *Escherichia coli*.

The change from sensitivity to partial resistance appears to be associated with mutations of the streptomycin locus as well as of other loci.

REFERENCES

CAVALLI, L. L., AND MACCACARO, G. A. 1950 Chloromycetin resistance in *E. coli*, a case of quantitative inheritance in bacteria. Nature, **166**, 991–992.

DEMEREC, M. 1950 Reaction of populations of unicellular organisms to extreme changes in environment. Am. Naturalist, **84**, 5–16.

LEDERBERG, J. 1947 Gene recombination and linked segregations in *Escherichia coli*. Genetics, **32**, 505–525.

LURIA, S. E., AND DELBRÜCK, M. 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics, **28**, 491–511.

NEWCOMBE, H. B. 1948 Delayed phenotypic expression of spontaneous mutations in *Escherichia coli*. Genetics, **33**, 447–476.

NEWCOMBE, H. B., AND HAWIRKO, R. 1949 Spontaneous mutation to streptomycin resistance and dependence in *Escherichia coli*. J. Bact., **57**, 565–572.

NEWCOMBE, H. B., AND NYHOLM, M. H. 1950*a* The inheritance of streptomycin resistance and dependence in crosses of *Escherichia coli*. Genetics, **35**, 603–611.

NEWCOMBE, H. B., AND NYHOLM, M. H. 1950*b* Anomalous segregation in crosses of *Escherichia coli*. Am. Naturalist, **84**, 457–465.

WITKIN, E. M. 1947 Genetics of resistance to radiation in *Escherichia coli*. Genetics, **32**, 221–248.