STUDIES OF THE STREPTOMYCIN-RESISTANCE SYSTEM OF MUTATIONS IN E. COLI * †

M. DEMEREC

Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, New York

Received December 3, 1950

UNIQUE opportunities for the study of mutations, both spontaneous and induced, have been offered by what we may call the " phage-resistance system " of bacterial changes from sensitivity to resistance to bacteriophages. In studying this system it is easy to work with large numbers of bacteria (1×10^8 per Petri dish); and if sufficient quantities of phage are added, all sensitive bacteria will be eliminated whereas the resistant will survive and form colonies. A spontaneous mutation rate of about 1×10^{-8} can easily be measured by this method, and induction of mutations at a rate as low as 1×10^{-7} can be detected. It has been used in a considerable amount of research dealing with spontaneous mutability and mutability induced by X-rays, ultraviolet rays, and chemicals. In fact, for several years it was the only known method of obtaining quantitative measurements of low mutation rates.

For some time I have been on the lookout for another system, capable of being studied by similar quantitative methods, that would serve to check and confirm results obtained with the phage-resistance system. We now have found that mutations resulting in resistance to and dependence on streptomycin, and back-mutations to nondependence, have certain advantages over phage-resistance mutations for use in quantitative studies of mutability. I will describe here the results of several studies of spontaneous and induced mutability based on this streptomycin-resistance system. Summaries of these studies have been published in the Carnegie Institution of Washington Year Books Nos. 48 and 49 (DEMEREC, WALLACE, WITKIN and BERTANI 1949; DEMEREC *et al.* 1950).

MATERIAL AND NOMENCLATURE

The experiments were made with Strain *B/r* of *Escherichia coli,* which is a radiation-resistant mutant of strain *B* (WITKIN 1947). Its resistance to radiation was a distinct advantage in the irradiation experiments, for it permitted treatment with higher doses. In one experiment we used strain *B/r/6,* a mutant of *B/r* that is resistant to phage 6 of the T series. Several other phages of the T series were used in tests made to detect and eliminate possible contaminations.

* Part of the cost of the accompanying tables has been paid by the GALTON AND MENDEL MEMORIAL FUND.

† This study was conducted in part under a grant from the AMERICAN CANCER SOCIETY, through the COMMITTEE ON GROWTH of the NATIONAL RESEARCH COUNCIL.

Second Printing 1965 / University of Texas Printing Division, Austin

Dihydrostreptomycin sulfate was used in the preparation of media containing the desired concentrations of streptomycin.

This paper will employ the system of nomenclature described previously (DEMEREC and FANO 1945). The symbol *B* represents the original strain of *E. coli*, a bar (/) indicates " resistant to," and the symbols following the bar stand for materials to which the mutant of strain *B* is resistant. Thus *B/S* is a mutant of strain *B* resistant to streptomycin; *B/Sd* is a mutant not only resistant to but dependent on streptomycin. Numbers indicate phages of the T series (*T1, T2, . . . T7*); *B/1* is thus a mutant of strain *B* resistant to phage *T1*. Since *B/r* was the strain used in all these experiments (except number 13 of table 2), the mutants studied were actually *B/r/S* and *B/r/Sd*. For the sake of simplicity, however, the "*r*" symbol will be omitted throughout the paper. Capital *R* is the symbol for reversions (back-mutations) from dependence (*B/Sd*) to nondependence.

ACTION OF STREPTOMYCIN ON E. COLI

It was of interest first to determine the toxic effect of streptomycin on *E. coli*. Previous experiments (DEMEREC 1948) had shown that concentrations up to 1.5 micrograms per milliliter do not kill bacteria grown in an aerated broth culture. With increasing concentrations between 1.5 and 20 μg, the number of survivors decreases rapidly to about 6 per 10^8. Beyond this point, continued increases have very little further effect on the proportion of survivors. The earlier experiments had shown that bacteria surviving concentrations higher than about 4 μg per ml are mutants that have greater resistance to streptomycin than the parent strain. Survivors of concentrations of 20 μg are regarded here as completely resistant.

That streptomycin kills bacteria before a division is accomplished is suggested by the experiments summarized in table 1. In these experiments sam-

TABLE 1

Percent of survivors among resting bacteria kept for various periods in broth containing different concentrations of streptomycin at 37° C.

Concentration of streptomycin (μg/ml)	% of survivors after exposures of						
	60	80	100	120	140	180	24 hours
	minutes						
3	93	100	79	67			< 0.6*
4	100	78	84	67			< 0.7
5	100	81	85	78			< 0.8
6	93	85	65	37			< 0.6
7		94	84	59	47	33	< 0.3
10		76	65	62	41	20	< 0.3
12		76	68	51	30	11	< 0.4
15		88	69	53	32	25	< 0.4
20		66	46	38	26	15	< 0.3

* Living bacteria were not found in samples originally containing between 130 and 368 bacteria.

ples of about 16,000 bacteria were placed in tubes containing 5 ml of broth to which streptomycin had been added in the quantities indicated. The tubes were incubated at 37°C, and at stated intervals 0.1-ml samples were plated to determine the number of living bacteria present in the cultures. Results showed that the number of living bacteria decreased with the length of exposure to streptomycin. Microscopic observations of bacteria plated on broth agar containing 5 μg of streptomycin per ml did not reveal any divisions in the bacteria so exposed. From this it can be inferred that killing by streptomycin does not require division of the bacteria, and that in this respect streptomycin differs from penicillin.

SPONTANEOUS MUTABILITY

Rate of mutation to streptomycin resistance. LURIA and DELBRÜCK (1943) developed two methods for measuring low mutation rates in bacteria. Both methods use a large number of small independent cultures, started from inocula small enough not to include any mutants, and register the mutants present in these cultures after bacterial growth has stopped. One method calculates mutation rate from the percentage of cultures containing no resistant mutants, the other from the average number of mutants per culture. A prerequisite of the second method is that the mutant bacteria divide at the same rate as nonmutants. Since this is not true of streptomycin-resistant mutants, only the first method can be used in our work. The formula yielding the mutation rate (a) per bacterial generation is:

$$a = -\frac{1.6}{N} \log_{10} p$$

in which N represents the average number of bacteria per culture, and p the percentage of cultures containing no resistant mutants.

The data of four experiments are given in table 2. Two of these used strain *B/r,* one strain *B/6,* and one strain *B/r/1.* To isolate resistant mutants, the whole content of each of the independent cultures was plated into agar containing 25 μg of streptomycin per ml. The results of these experiments indicate that the mutation rate is about 1×10^{-9} per bacterial generation, and that it is not appreciably affected by the strain used in tests.

Properties of resistant mutants. Resistant mutants that originated independently were found to include several distinct types. The most striking differentiation among them was with regard to dependence on streptomycin. An analysis of 208 showed that 124, or 60 percent, were dependent on streptomycin for their growth; that is, they were able to grow fully only on medium containing streptomycin, and passed through only a few divisions on medium lacking streptomycin. Other conditions being equal (*i.e.,* number of bacteria in culture, concentration of streptomycin in the parent culture), the number of divisions undergone by dependent bacteria on medium lacking streptomycin is a constant property of individual mutants, which vary greatly in this respect.

TABLE 2

Distribution of numbers of streptomycin-resistant mutants in a series of similar cultures in 1 ml synthetic medium.

Experiment No. and Strain	11 B/r	12 B/r	13 B/6	14 B/r/1
Resistant bacteria	No. of cultures			
0	25	56	19	63
1	17	22	25	18
2	12	10	21	6
3	8	6	21	0
4	2	5	9	2
5	2	0	2	1
6–10	3	4	4	0
10–50	1	0	1	3
50–100	0	0	0	1
> 100	1	0	0	9
Total	71	103	102	103
Aver. no. bacteria ($\times 10^9$)	1.35	0.48	0.44	0.28
Mutation rate ($\times 10^{-9}$)	0.73	0.91	2.6	1.3

Different dependent (*B/Sd*) and nondependent resistant (*B/S*) mutants differ considerably in their rates of growth. Many of them are "slow growers"—that is, types that when plated on broth-agar medium require 48 hours or longer to develop colonies of the size reached by the original strain in 24 hours.

Growth rates of nine *B/Sd* and two *B/S* strains were studied by inoculating broth with bacteria, incubating the cultures at 37°C, and removing samples at intervals to assay the number of bacteria. The concentration of streptomycin in the medium in which the *B/Sd* strains were grown was 10 μg per ml. The lag period—that is, the time required for doubling the original number of bacteria—varied in these nine strains from 110 to 210 minutes; and the time taken for completion of one division varied from 45 to 100 minutes. Since the lag period for the *B/r* strain is 60 minutes, and a division is completed in about 20 minutes, it is evident that these *B/Sd* mutants, selected at random, required a considerably longer time both to start growing and to complete a division. The two *B/S* strains were grown in broth without streptomycin and in broth containing 10 μg of streptomycin per ml. The presence or absence of streptomycin made no difference in the growth of these two strains. For both, the lag period was about 65 minutes and the division time about 25 minutes.

Three other mutant strains of *B/S* were grown in mixtures with *B/r*, to test their survival values when competing with the original strain. Results of these tests, given in table 3, show that all three were at a disadvantage as compared with *B/r*.

Observations made on these five *B/S* and many *B/Sd* mutants indicated that none of them grew as well as strain *B/r* in medium without streptomycin.

TABLE 3

Results of experiments in which B/r and B/S bacteria were grown together in broth cultures.

B/S Mutant No.	Beginning of experiment		End of experiment	
	No. of bacteria	Proportion B/r : B/S	No. of bacteria	Proportion B/r : B/S
5	6.6×10^3	5.5 : 1	3×10^8	130 : 1
	1.13×10^4	48 : 1	3.9×10^8	1.6×10^3 : 1
6	7.1×10^3	3.4 : 1	2.5×10^8	2.5×10^3 : 1
	1.13×10^4	83 : 1	2.3×10^8	1.6×10^4 : 1
7	2.6×10^4	1 : 1.3	2.3×10^8	10 : 1
	2.3×10^4	77 : 1	2.3×10^8	2×10^3 : 1

In such a medium the *B/Sd* bacteria divided only a few times and the *B/S* grew at a slower rate.

Back-mutations from dependence to nondependence. When bacteria of a *B/Sd* strain are plated on agar containing no streptomycin, they undergo one or several normal divisions, and then in some strains pass through several additional abnormal divisions, giving rise to elongated filaments or "snakes," which increase in length up to about ten times the length of a bacterium and then stop growing. Observations made by BERTANI (1951) indicate that mutations to nondependence occur during the normal divisions but not in the filaments. Mutant cells are able to grow normally on the broth-agar medium, and form colonies.

The number of divisions is influenced by several environmental factors, chiefly by the amount of streptomycin present in the medium in which the bacteria were cultured, and to a lesser degree by the number of bacteria plated on the agar. If these conditions are kept constant, the average number of divisions for any one strain will also be constant. The different strains, however, show striking differences with regard to the number of normal and abnormal divisions they pass through.

The dependent strain *B/Sd-4* was used in our studies of spontaneous and induced back-mutations (reversions). Various biological properties of this strain have been investigated in detail by BERTANI (1951). Here I will give a brief summary of the procedure followed in experiments to be mentioned in this paper.

B/Sd-4 bacteria were grown under aeration, either in broth or in a synthetic medium (M9) containing 10 μg of streptomycin per ml. After the bacteria were washed—by centrifuging, decanting the supernatant, shaking up the pellet, and repeating the same processes again—they were plated on broth-agar medium containing no streptomycin. In experiments to observe back-mutations, care was taken to plate no more than 5×10^7 living bacteria per Petri dish. Back-mutants were scored after 72 hours of incubation. In doubtful cases, the observations were checked with a low-power wide-field binocular microscope. In preliminary tests, scoring had been done after 48, 72 and

96 hours of incubation. An increase in number of back-mutants was observed between the 48-hour and 72-hour scorings, but there was no further increase at 96 hours.

Under the circumstances described above, *B/Sd-4* bacteria pass through 2.7 normal divisions before beginning the formation of snake-like filaments. After 72 hours of incubation, a plate on which about 5×10^7 bacteria have been spread will show, in addition to a very thin layer of growth on the surface, a few scattered colonies representing mutants. A count of these colonies will determine how many mutations to nondependence have occurred; and if the number of plated bacteria is known the mutation rate per specified number of bacteria can be calculated. An estimate of the mutation rate per bacterial division can be obtained by dividing the mutation rate per plated bacteria by a factor of 6, which represents an estimate of the increase in number of *B/Sd-4* bacteria raised on 10 μg of streptomycin per ml when plated on medium without streptomycin. The factor is greater than 6 if the bacteria are raised in a medium containing more than 10 μg of streptomycin per ml.

The rate of spontaneous mutation of *B/Sd-4* to nondependence is about 1.4×10^{-8} per bacterial generation. Detailed data are given by BERTANI (1951).

MUTATIONS IN *B/Sd-4* INDUCED BY RADIATIONS

Ultraviolet rays. Bacteria raised in an aerated culture containing broth and 10 μg of streptomycin per ml were used in these experiments. The procedure was as follows:

(1) Bacteria were washed in saline to remove excess streptomycin.
(2) Bacterial suspension was assayed by plating, to determine concentration.
(3) Five control plates were started by plating 0.1 ml of a 10^{-3} dilution of bacterial suspension on broth-agar plates, to determine spontaneous mutation rate.
(4) A thin layer (about 7 ml) of bacterial suspension in saline was placed in the bottom part of a Petri dish and exposed to ultraviolet rays from a germicidal lamp (2537 Å), with continuous shaking during exposure.
(5) A sample of the irradiated suspension was assayed to determine the proportion of bacteria surviving treatment. Dosage was calculated from the survival ratio (using the chart published by DEMEREC and LATARJET 1946) and the exposure time.
(6) 0.1-ml samples of the irradiated suspension were plated on broth-agar plates to determine the frequency of mutations induced in the treated bacteria.
(7) Assay plates were scored after 24 hours of incubation at 37°C; and control and experimental plates were scored after 72 hours of incubation.
(8) Frequency of induced mutations per 10^8 bacteria plated was computed.

Microscopical examination of irradiated *B/Sd-4* bacteria revealed that they divide on broth-agar medium to the same extent as nonirradiated bacteria, so that the same factor can be used for computing mutation rate per bacterial division.

Data on the numbers of mutations induced by different doses are given in table 4 and presented graphically in figure 1. These results will be considered in the Discussion.

X-rays. Except for the actual treatment of bacteria, the procedure used in X-ray experiments was identical to that used with ultraviolet. Treatment was given in small glass test tubes, each containing 4.5 ml of bacterial suspension. As a rule, nine test tubes were exposed simultaneously and then removed one

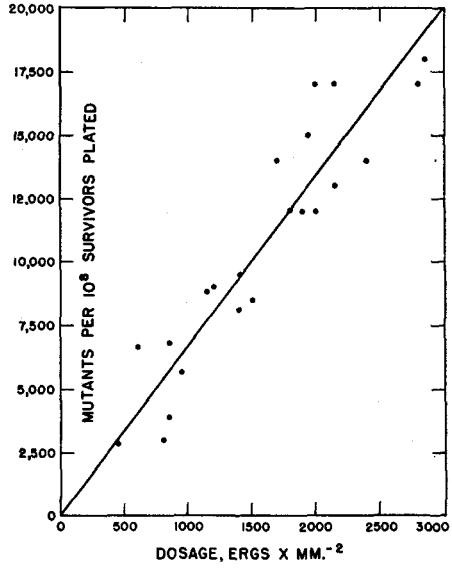

FIGURE 1.—Plot of data on mutants (both sensitive and resistant) induced by treating strain *B/Sd-4* of *E. coli* with different doses of ultraviolet rays (2537 Å).

by one at intervals representing the desired length of exposure. Intensity of the X-rays was measured with a Victoreen dosimeter, and the dosage was determined by length of exposure. The X-ray machine was run at 140 kv and 8 ma. At the exposure distance used, the dose was about 30 roentgens per second.

The results of the X-ray experiments are summarized in table 5 and presented graphically in figure 2.

MUTATIONS IN *B/r* INDUCED BY ULTRAVIOLET RADIATION

Extensive testing showed that induced mutants cannot be observed if radiation-treated bacteria are plated on or in medium containing streptomycin immediately after treatment. This fact indicated that irradiated bacteria have to undergo division before the mutations induced by the radiation can be ex-

TABLE 4

Summary of experiments with B/Sd-4 on relation between dose of ultraviolet radiation (2537 Å) and number of induced nondependent mutants (both streptomycin-sensitive and streptomycin-resistant).

Exper. No.	Survivors (%)	Dosage (ergs)	No. of bacteria		No. of mutants	
			Per plate	Total	Total	Per 10^8 plated bacteria
6-3	53.4	450	4.8×10^6	1.9×10^7	565	2,850
8-2	41.5	600	5.8×10^5	1.7×10^6	114	6,700
5-3	29.4	800	2.1×10^6	6.3×10^6	192	3,000
6-4	22.2	850	2.0×10^6	6.0×10^6	408	6,800
3-3	22	850	2.5×10^7	1.25×10^9	4886	3,900
4-3	18.2	950	2.8×10^6	8.4×10^6	476	5,700
6-5	9.7	1150	8.7×10^5	2.6×10^6	229	8,800
8-3	7.9	1200	5.5×10^5	1.1×10^6	100	9,100
4-4	4.2	1400	3.3×10^6	6.6×10^6	629	9,500
5-4	4.1	1400	1.5×10^6	3.0×10^6	246	8,200
7-3	2.8	1500	2.6×10^6	1.3×10^7	1099	8,500
6-6	1.3	1700	1.2×10^5	6.0×10^5	85	14,000
7-4	0.76	1800	7.1×10^5	3.6×10^6	441	12,000
8-4	0.56	1900	7.8×10^5	2.3×10^6	276	12,000
3-4	0.45	1950	1.0×10^6	2.0×10^7	297	15,000
4-5	0.32	2000	5.0×10^5	1.0×10^6	172	17,000
5-5	0.35	2000	2.5×10^5	5.0×10^5	62	12,000
6-7	0.19	2150	1.7×10^4	8.5×10^4	11	13,000
7-5	0.15	2150	1.4×10^5	5.6×10^5	97	17,000
8-5	0.05	2400	7.0×10^4	2.1×10^5	30	14,000
7-6	0.012	2750	1.1×10^4	5.5×10^4	15	27,000
5-6	0.011	2800	8.0×10^3	4.8×10^4	8	17,000
4-6	0.009	2850	1.4×10^4	2.8×10^4	5	18,000

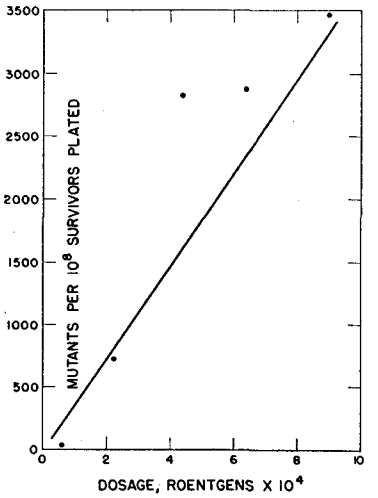

FIGURE 2.—Plot of data on mutants (both sensitive and resistant) induced by treating strain *B/Sd-4* of *E. coli* with different doses of X-rays.

TABLE 5

Summary of experiments with B/Sd-4 on relation between X-ray dosage and number of induced nondependent mutants (both streptomycin-sensitive and streptomycin-resistant).

Exper. No.	Dosage (roentgens)	Survivors (%)	No. of bacteria		No. of mutants	
			Per plate	Total	Total	Per 10^8 plated bacteria
3-3	6,000	52.5	1.6×10^7	3.2×10^7	1464	46
1-3	22,000	8.85	2.0×10^6	2.0×10^7	147	735
3-5	44,000	0.66	2.0×10^7	4.0×10^7	1128	2820
1-4	46,000	0.491	1.1×10^6	1.1×10^7	129	1170
3-6	64,000	0.063	1.9×10^6	1.6×10^7	446	2890
2-6	68,000	0.04	1.5×10^6	1.5×10^7	311	2080
2-7	90,000	0.00306	1.14×10^5	1.1×10^6	38	3460

pressed. The following technique was developed for studies of such induced mutations:

(1) The culture was assayed by plating, to determine the concentration of bacteria. When ultraviolet treatment was to be given, bacteria were cultured in transparent synthetic medium (M9).

(2) A thin layer of bacterial suspension was irradiated, with continuous shaking.

(3) A sample of the irradiated suspension was assayed by plating to determine the proportion of survivors.

(4) Samples were plated on broth agar containing 25 μg of streptomycin per ml, to determine the " background " number of resistant mutants present.

(5) Samples were plated on plain broth agar and incubated for a certain period (2 hours or longer) so that the bacteria would divide. Since the lag period varies with dosage, the incubation period was adjusted accordingly.

(6) After incubation, the plates were refrigerated to stop growth.

(7a) Growth of the bacteria during incubation was determined on 3 plates by washing with 10 ml of broth and assaying the suspension.

(7b) Onto each of the remaining plates was poured 4 ml of soft agar (0.7 percent) containing 200 μg of streptomycin per ml. After the agar had set these plates were kept at 16°C for 2 hours, allowing time for the streptomycin to act on sensitive bacteria. The plates were then incubated at 37°C, and after 48 hours the colonies were counted. Under these circumstances, only resistant bacteria survive and form colonies; therefore, after the number of background mutants had been deducted, the counts showed the number of induced mutants that were present after the treated bacteria had multiplied a definite number of times.

A summary of the data of these experiments with ultraviolet treatment is given in table 6. Here, again, dosage was calculated from the percentage of

survivors. A fresh culture of bacteria was used for each experiment. Examination of the table shows that the total number of mutants in any one experiment depended not only on the dose used in treatment but also on the subsequent stage of division at which mutants were scored. Since it would be a very difficult and laborious process to obtain material that had received different treatments but undergone the same number of divisions after treatment, the material and method described here are not suitable for quantitative studies of induced mutations. In this case it is not feasible to work with " endpoint " mutants, as has been done successfully in studies with phage resistance

TABLE 6

Summary of experiments with B/r on induction of mutations to streptomycin resistance by ultraviolet rays.

Exper. No.	Survivors (%)	Dosage (ergs)	Incubation (hours)	No. of bacteria			No. mutants per 10^8		
				Plated	Washed	Increase (×)	Total	Plated	Washed
14-3	22.4	800	2	1.2×10^8	1.46×10^8	1.07	50	39.7	34.3
-4			4	1.02×10^8	1.24×10^8	1.2	346	339	279
-5			5	1.02×10^8	6.04×10^8	5.9	702	687	116
15-3	12.9	1000	2½	6.0×10^7		0	104	174	174
-4			3½	6.0×10^7	1.44×10^8	2.4	336	560	233
12-2	13.5	1000	3	2.24×10^7	3.14×10^7	1.4	116	535	383
7	14.5	1000	3½	5.8×10^7	1.77×10^8	3	341	588	192
16-2	11.5	1050	2½	9.36×10^7		0	167	179	179
-3			3½	6.02×10^7	9.12×10^7	1.3	347	576	380
-4			4½	1.87×10^7	2.0×10^8	10.7	182	1000	91
-5			5½	6.28×10^6	9.0×10^8	128	130	2167	14.4
3	10.6	1100	3	1.7×10^7	3.6×10^7	2.1	632	3750	1770
13-4	11	1100	2	6.18×10^7		0	83	134	134
-5			3	5.15×10^7	6.4×10^7	1.28	304	590	475
-6			4	5.15×10^7	6.5×10^8	13	1221	2368	1882
4	8	1200	3	2.05×10^7	2.4×10^7	1.2	165	825	688
5	5.6	1350	3	2.3×10^7		0	149	650	1120
20-3	2	1600	4	7.08×10^7	1.0×10^8	1.42	410	580	410
-4			4½	5.9×10^7	3.6×10^8	6.08	623	1056	174
12-3	1.34	1700	3½	6.66×10^6	7.2×10^6	1.1	40	610	555
19	1.35	1700	4	4.5×10^7	4.8×10^7	1.07	180	400	375
			4½	4.5×10^7	1.48×10^8	3.3	361	804	244
17	0.55	1900	4	1.98×10^6	3.0×10^6	1.5	12	606	400
11	0.2	2100	3½	8.96×10^5	3.5×10^6	3.9	10	1115	286

(DEMEREC and LATARJET 1946), because of the technical difficulties of applying streptomycin to colonies of cells after they have reached a certain size. One of the main requirements of the method is that cells belonging to different colonies not be mixed together when the streptomycin is applied; and a suitable way of preventing this, after the number of cells per colony has increased beyond a certain stage, has not yet been found.

Among the many experiments done by us, there were a few that showed a similar increase in number of bacteria between the treatment and the application of streptomycin; in these, the factor of increase varied between 1.2 and

1.5. The data of these experiments are plotted in figure 3, which indicates a straight-line relation between ultraviolet dose and induced mutations.

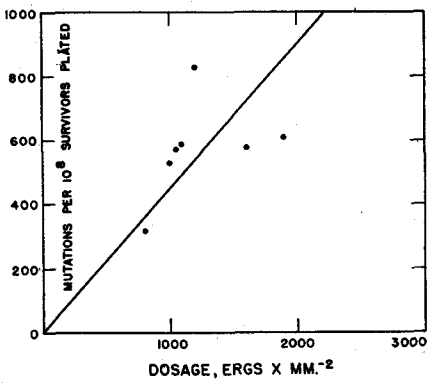

FIGURE 3.—Plot of data on mutants (both streptomycin-resistant and streptomycin-dependent) showing up after strain *B/r* of *E. coli* was irradiated with different doses of ultraviolet rays (2537 Å) and the bacteria allowed to multiply 1.2 to 1.5 times.

DISCUSSION

Studies of streptomycin-resistant mutants have revealed an interesting situation; namely, that none of the mutants investigated can compete with the original strain when grown without streptomycin. Since about 60 percent of these mutants are dependent on streptomycin, they can pass through only a few divisions without it; but even those resistant mutants that are not streptomycin-dependent seem to be at a disadvantage as compared with nonmutants. This was indicated by a study of five resistant mutants, three of which were tested in mixtures with nonmutant bacteria and two of which were studied for division rate.

Experiments with ultraviolet radiation and with X-rays showed two significant results. The first was that there are no "zero-point" mutations among the induced mutations from *B* to *B/S* and *B/Sd*, and also that there are probably none among reversions from *B/Sd*. This is in contrast to the phage-resistance system, where some of the induced mutations do not need cell division for their expression (zero-point mutants) although others require at least one cell division to take place before they show up (end-point mutants). In every irradiation experiment made in the study of induction of mutations from *B* to *B/S*, bacteria were plated, immediately after treatment, on medium containing streptomycin; therefore records of a large number of such platings are available. In no case was the proportion of mutants significantly higher than in treated controls, which offers good evidence that there were no zero-point mutants.

In experiments 15–3, 16–2, 13–4, and 13–5, listed in table 6, the numbers of mutants per 10^8 were high, although no increase in numbers of plated bacteria was detected. In these cases, however, the bacteria were incubated for 2–3 hours after treatment, and undoubtedly some of them divided, although

the number may not have been sufficiently high to be detected by the method used in the experiments. Therefore these data are not in disagreement with the conclusion that zero-point mutations do not occur among induced *B*-to-*B/S* mutations.

If zero-point mutations occurred among induced back-mutations from *B/Sd,* they would be expected to produce colonies that could be detected after incubation of about 12–15 hours. Since no unexpectedly large proportions of early-appearing colonies were noticed in any of the experiments, it seems reasonable to conclude that zero-point mutants are not present among reversions.

Mutations from *B* to *B/S* induced by ultraviolet radiation did not follow the pattern of expression observed in delayed mutations from *B* to *B/1* (DEMEREC 1946); that is, their time of appearance did not stretch over a long period during which the total number of bacteria increased manyfold. In the case of induced *B/1* mutants, only a small proportion were expressed after the first few divisions of the irradiated bacteria, whereas the great majority showed up after later divisions; and the mutation rate per bacterium per division returned to the spontaneous level only after 10 to 12 divisions. Examination of the data presented in table 6 reveals a pattern of appearance of induced mutants that is not comparable to that observed in the *B/1* material. For example, in experiment 14, after 4 hours of incubation (14–4), when the number of bacteria had increased about 1.2-fold (*i.e.,* before all the irradiated bacteria had undergone one division), the frequency of induced mutants was 339 per 10^8 bacteria surviving the treatment. In the same experiment, after 5 hours of incubation (14–5), when the number of bacteria had increased 5.9-fold, the frequency of mutants was 687 per 10^8. Similarly, in experiment 16, after 3½ hours of incubation the bacteria had multiplied 1.3-fold and the frequency of mutants was 576 per 10^8; after 4½ hours, the increase was 10.7-fold and the frequency of mutants 1000 per 10^8; and, fiinally, after 5½ hours the increase was 128-fold and the frequency of mutants about 2167 per 10^8. In this last-mentioned experiment there was an indication that some induced mutants were expressed during the period when the bacteria increased from 10-fold to 128-fold, but their number was very small compared with the increment of *B/1* mutants during the comparable period. More extensive experiments carried on in connection with another study did not show any consistent increase in the frequency of induced mutants after the treated bacteria had undergone between 2 and 4 divisions.

Recently NEWCOMBE and HAWIRKO (1949) made a study of the rate of mutation from streptomycin sensitivity to streptomycin resistance in this same strain of *coli.* They calculated the mutation rate as 10^{-10} per bacterium per division, which is about ten times lower than the rate observed in these experiments. The only apparent difference between the methods employed is that NEWCOMBE and HAWIRKO used a medium containing 64 μg of streptomycin per ml to select mutants, whereas we used a concentration of 25 μg per ml.

The second important finding brought out in this study is the straight-line relation between ultraviolet dose and number of induced mutants. This applies

to induced mutations from *B/Sd* to *R* (back-mutations to nondependence) as well as to those from *B* to *B/S*. It is quite different from the situation found in the phage-resistance system, where a multiple-hit curve was indicated for the relation between ultraviolet dose and induced mutation rate (DEMEREC and LATARJET 1946).

SUMMARY

Experimental results indicate that the rate of mutation in strain *B* (*B/r*) of *E. coli* from sensitivity to complete resistance to streptomycin is about 1×10^{-9} per bacterium per division. About 60 percent of the resistant mutants are dependent on streptomycin for their growth.

The rate of mutation from *B* to *B/S* (resistance to streptomycin) can be studied with considerable precision, since the work can be carried out with very large numbers of bacteria. The same is true for the study of back-mutation (*R*, equals reversion) from streptomycin dependence (*B/Sd*) to nondependence. The mutation rate in the mutant strain *B/Sd-4* is about 1.4×10^{-8} per bacterium per division.

Mutations from *B* to *B/S*, as well as from *B/Sd* to *R*, may be induced by ultraviolet rays and by X-rays. The dose–mutation rate relation is represented by a straight line for both radiations.

It is necessary for at least two to four cell divisions to take place before the induced mutations are expressed; " zero-point " mutants do not appear in the streptomycin-resistance system.

LITERATURE CITED

BERTANI, G., 1951 A method for detection of mutations, using streptomycin dependence in *Escherichia coli*. Genetics **36:** 598–611.

DEMEREC, M., 1946 Induced mutations and possible mechanisms of the transmission of heredity in *Escherichia coli*. Proc. Nat. Acad. Sci. **32:** 36–46.

1948 Origin of bacterial resistance to antibiotics. J. Bact. **56:** 63–74.

DEMEREC, M., and U. FANO, 1945 Bacteriophage-resistant mutants in *Escherichia coli*. Genetics **30:** 119–136.

DEMEREC, M., E. M. WITKIN, B. W. CATLIN, J. FLINT, W. L. BELSER, C. DISSOSWAY, F. L KENNEDY, N. W. MEYER and A. SCHWARTZ, 1950 The gene. Carnegie Inst. Wash. Yr. Bk. **49:** 144–157.

DEMEREC, M., and R. LATARJET, 1946 Mutations in bacteria induced by radiations. Cold Spring Harbor Symp. Quant. Biol. **11:** 38–50.

DEMEREC, M., B. WALLACE, E. M. WITKIN and G. BERTANI, 1949 The gene. Carnegie Inst. Wash. Yr. Bk. **48:** 154–166.

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

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

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