GENETICS OF RESISTANCE TO RADIATION IN *ESCHERICHIA COLI*[1,2]

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INTRODUCTION

A MUTATION in *Escherichia coli*, leading to heightened resistance to ultraviolet radiation and x-rays, was described in an earlier publication (WITKIN 1946). Evidence was presented that this mutation occurs spontaneously in cultures of the parent strain. In the present paper, a more complete account of these preliminary investigations will be given, as well as additional experiments to clarify the genetic basis of resistance to radiation.

This mutation is one of a number of bacterial variations which have been found, in recent years, to exhibit striking similarities to mutational changes in higher plants and animals. Since the standard techniques of Mendelian genetics have not been applicable to bacteria, these analogies afford the only available evidence that bacterial heredity is gene-controlled. Among the similarities between variations in bacteria and mutations in higher organisms which are most suggestive of a common underlying genetic basis are the following: (1) Many variations in morphological and physiological characters occurring in cultures of bacteria are transmissible, unchanged, through numerous generations, and are therefore considered to be stable and heritable (see, for example, MASSINI 1907, LEWIS 1933, SEVERENS and TANNER 1945). (2) The spontaneous origin of certain stable and heritable variations, independent of the specific treatments used to detect them, has been proven in a few cases by means of special techniques (LURIA and DELBRÜCK 1943, DEMEREC 1945, RYAN, SCHNEIDER and BALLENTINE, 1946). (3) Different characters within a strain may vary independently of one another (REED 1937, AUDUREAU 1942). In one instance, the rate of mutation to a particular variant type was found to be the same for strains differing by one or more other mutational steps (DEMEREC and FANO 1945). (4) Physical and chemical agents known to be effective in increasing the rates of mutations in higher organisms have similar effects on bacteria (for the effects of x-rays, see HABERMAN and ELLSWORTH 1940, GRAY and TATUM 1944; for x-rays and ultraviolet, DEMEREC 1946; for mustard gas, TATUM 1946). The mutations induced by these agents

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in bacteria, as in higher organisms, seem to be random and nonspecific. (5) Certain variations in bacteria leading to altered growth requirements and synthetic abilities (ROEPKE, LIBBY and SMALL 1944, GRAY and TATUM 1944) are close analogues of the biochemical mutations in Neurospora, a sexually reproducing microorganism in which the existence of gene-controlled heredity has been established with certainty.

Bacterial variations which meet the tests of stability and heritability, of spontaneous origin at more or less constant rates, of responsiveness to known mutation-inducing agents, etc., are likely to be the most promising materials for the investigation of bacterial heredity. Mutations involving resistance to destructive agents, such as bacteriophage (LURIA and DELBRÜCK 1943), penicillin (DEMEREC 1945) or radiation, are especially suitable for genetic analysis, since resistant mutants can be detected easily in cultures of sensitive bacteria.

Apart from its amenability to investigation, resistance to radiation may be of particular significance for genetics. The fact that radiobiological results obtained in the study of the bactericidal effect of radiation parallel those obtained for the production of mutations in *Drosophila* (see LEA 1946) suggests that the killing of bacteria by radiation may be mediated through an effect on some center of genetic importance. This possibility is also supported by the similarity between the absorption spectrum of nucleic acids and the efficiency spectrum for the killing of bacteria by ultraviolet radiation (GATES 1928). A mutation which alters the sensitivity of bacteria to radiation, therefore, is likely to effect a basic change in the genetic economy of the organism.

Since the early researches of DOWNES and BLUNT (1877), the bactericidal action of radiation has been a prominent subject for radiobiological investigation. Differences in sensitivity within a strain have often been reported, but these have usually been found to depend upon transient physiological factors, such as the age of the culture. Inherited differences in sensitivity to ultraviolet radiation among clones of *E. coli* were described by RENTSCHLER, NAGY and MOUROMSEFF (1941), and HOLLAENDER (1942) noted that one in a million bacteria in a population of *E. coli* could survive very high doses of radiation.

It was our purpose in these experiments to determine the extent to which resistance to radiation may be considered a true mutational change, and to establish its genetic basis insofar as possible.

MATERIAL AND METHODS

Strain *B* of *Escherichia coli* and mutants derived from this strain were used throughout these experiments. The original culture, obtained from DR. M. DEMEREC, was diluted and plated on agar so as to give a few well-separated colonies after incubation. A stock culture was prepared by inoculating one of these colonies on a nutrient agar slant. The stock was carried by subculturing the slant every two months. Stocks of mutant strains were carried in the same way. Cultures to be used for comparable experiments were inoculated with samples from the same slant.

The media used, unless otherwise specified, were Difco nutrient agar and

Difco nutrient broth. The synthetic medium used in certain experiments consisted of a buffered solution of inorganic salts with ammonium chloride as the source of nitrogen, and glucose as the sole carbon source.

The source of ultraviolet radiation was a General Electric germicidal lamp, of the low-pressure mercury vapor type, emitting ultraviolet radiation about 80 percent of which is of wave length 2537 Å. The output was stabilized by an automatic regulator, and the intensity at a distance of 92 cm from the source was about 4.2 ergs per square millimeter. The lamp was calibrated in absolute units by DR. R. LATARJET.

In most of the experiments, bacteria to be irradiated with ultraviolet were taken from 24-hour broth cultures, and diluted quantitatively in broth. Measured samples were spread evenly on the surface of nutrient agar Petri dishes with a sterile glass rod. The plates were then exposed to the radiation, and colony counts were made after 24 hours of incubation. Survival was measured by comparison with nonirradiated control plates. Special methods used in certain instances will be described in connection with the specific experiments.

Irradiation with x-rays was conducted at MEMORIAL HOSPITAL in New York City, through the courtesy of MR. L. D. MARINELLI, and with the assistance of MISS E. FOCHT. The tube was operated at 180 kv., and the intensity was 2050 roentgens per minute at the distance used. The radiation was administered without special filtration, but before reaching the bacteria the rays passed through the glass walls of the x-ray tube and of the tube containing the bacteria.

Bacteria to be irradiated with x-rays were taken from undiluted 24-hour aerated broth cultures, and were exposed in small, thin-walled glass tubes. Measured dilutions were made after irradiation, and were plated out. Colony counts were made after incubation, and were compared with nonirradiated controls.

Assays to determine the titre of liquid cultures were made by the usual method of plating measured dilutions and making colony counts. Similar assay methods were used in the preparation of growth curves, starting the cultures with measured inocula, and sampling at intervals over a 24-hour period.

EXPERIMENTAL

Isolation of the Resistant Mutant

From a culture of strain *B*, a sample of about 5×10^4 bacteria was taken, and irradiated with an ultraviolet dose of 1000 ergs/mm^2. Four colonies were found to have developed after incubation, indicating that only four bacteria had survived the treatment. Each of the colonies was inoculated into a tube of broth, and, at the same time, four control cultures were started by inoculating into broth single colonies from a nonirradiated plate. The sensitivity to ultraviolet of the two sets of cultures was compared, by irradiating samples from each of the cultures with two test doses. The results are shown in table 1. The cultures derived from the four survivors of the original irradiation were characterized by considerably greater resistance to ultraviolet than corresponding cultures from control colonies.

One of these four resistant cultures (no. 1) was selected to serve as a stock resistant strain for further study, and was established on an agar slant. This strain was designated as strain *B/r* (*B* resistant to radiation).

Some Properties of the Resistant Strain

(1) Stability of Strain *B/r*

The resistant strain *B/r* has been carried through over 50 successive subcultures in broth, and for a period of over two years on agar, with frequent

TABLE 1

Sensitivity to ultraviolet of bacteria surviving irradiation with a high dose of ultraviolet.

Origin of control cultures: single colonies from a non-irradiated plate seeded with bacteria from strain *B*. Origin of experimental cultures: single-colony survivors from plates seeded with bacteria from strain *B*, and irradiated with a dose of 1000 ergs/mm².

CULTURE	TREATMENT: 90 ergs/mm²		TREATMENT: 500 ergs/mm²	
	CELLS PER SAMPLE	SURVIVAL PERCENT	CELLS PER SAMPLE	SURVIVAL PERCENT
Control—1	520	3.5	1040	0.4
Control—2	481	5.4	962	0.5
Control—3	456	2.9	912	0.9
Control—4	509	5.9	1018	0.2
Experimental—1	455	92.9	890	37.7
Experimental—2	520	97.5	1040	40.0
Experimental—3	490	98.3	980	43.2
Experimental—4	512	92.3	1024	38.1

single-colony isolations. No change has been observed in sensitivity to ultraviolet, or in other characteristics studied. Resistance to ultraviolet may, therefore, be considered a stable, heritable character.

(2) Sensitivity of Strain *B/r* to Ultraviolet

The curves of survival of strains *B* and *B/r* as a function of ultraviolet dose are shown in Figure 1. Two sets of curves are represented, one set (A) obtained when the irradiation is administered to bacteria spread on the surface of agar medium, and the other set (B) when the bacteria are irradiated in liquid suspension. For purposes of comparing the sensitivity of the parent and mutant strains, the curves made under one or the other of these conditions should be examined. In addition to the difference in level of sensitivity, there is a marked difference in the shape of the survival curves of the parent and mutant strains. For this reason, a numerical coefficient to express the difference in sensitivity between the two strains would be meaningless.

The significance of the difference in sensitivity observed when irradiation is carried out on solid or in liquid medium will be considered in the Discussion.

(3) Sensitivity of Strain *B/r* to X-rays

The survival curves of strains *B* and *B/r* with x-rays are shown in figure 2. The mutant strain exhibits relative resistance to x-rays, as well as to ultraviolet. The shape of the x-ray survival curve of strain *B/r* is linear, in contrast to the sigmoid curve obtained for this strain with ultraviolet.

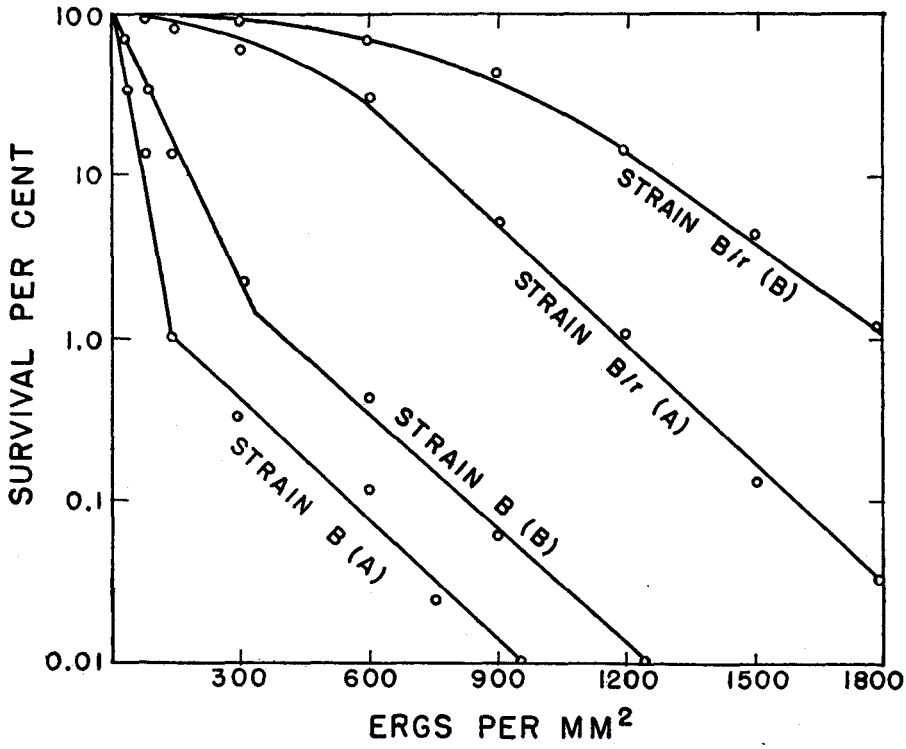

FIGURE 1.—Sensitivity of strains *B* and *B/r* to ultraviolet radiation.
(A) bacteria irradiated on surface of solid medium.
(B) bacteria irradiated in liquid suspension.

The survival curves for the parent strain *B* with both ultraviolet and x-rays show a change of slope at about one percent survival. The change in rate of death at this point cannot be accounted for by the presence of resistant mutants in the normal samples.

(4) Growth Characteristics of Strain *B/r*

The growth rates of strains *B* and *B/r* in broth at 37°C were compared. Differences in growth rates between the two strains are confined to the lag

phase before the onset of logarithmic growth, and to the final titre. The lag phase of the resistant strain, defined as the time required for the doubling of the initial inoculum, was found to be one hour, as compared with ninety minutes for the parent strain. The generation time (19 minutes) was the

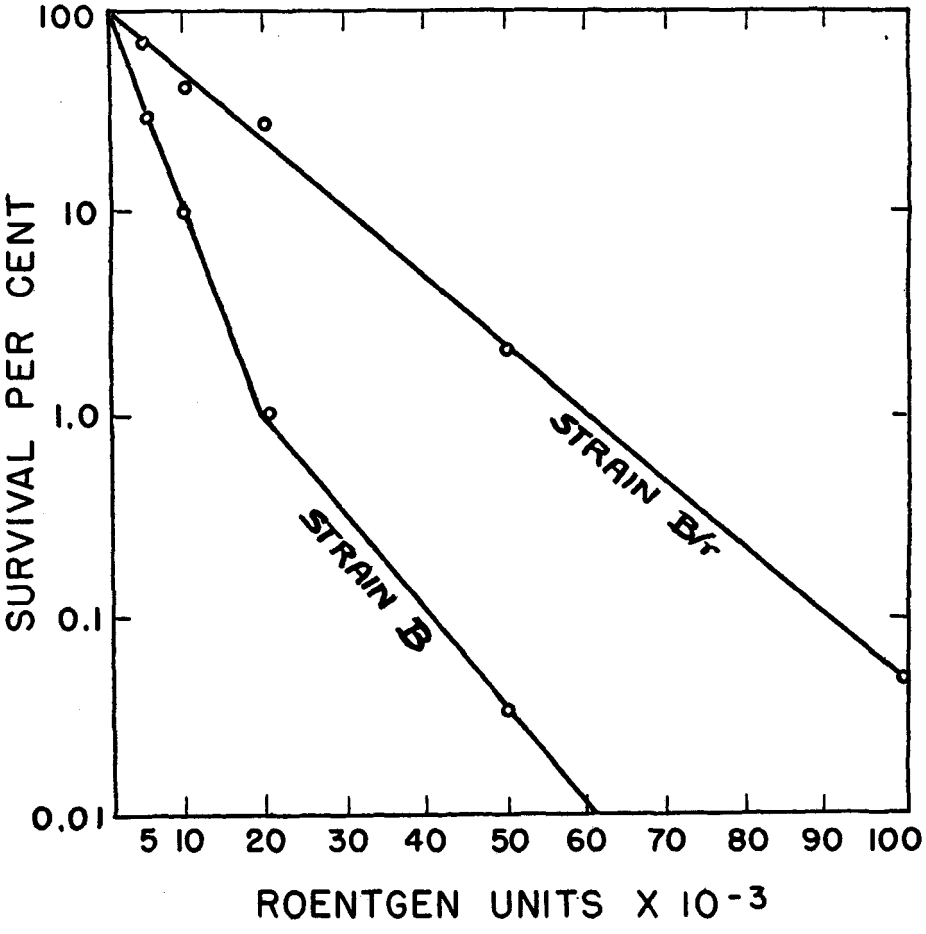

FIGURE 2.—Sensitivity of strains *B* and *B/r* to x-rays.

same for both strains, but the final titre of resistant cultures is usually slightly lower than that of sensitive cultures.

Both strains are able to grow luxuriantly in synthetic medium without added growth factors.

(5) Behavior of Individual Cells after Irradiation with Ultraviolet

Another difference between sensitive and resistant bacteria was observed by examining under the microscope bacteria irradiated with low doses of ultraviolet. It is known (GATES 1933) that ultraviolet radiation, in low doses, is

one of a number of agents which exert a specific inhibiting effect on the division of bacterial cells, without affecting growth in length, resulting in the production of long filaments.

Samples from the parent strain (*B*) were irradiated with a dose of 50 ergs per mm^2, incubated, and examined at intervals on the surface of the agar plate under high-power magnification. Almost every cell was seen to elongate into a filament, reaching a length of 50–100μ within three to four hours after treatment. No evidence of division was observed until after four hours of incubation.

Samples from the resistant strain (*B/r*) were irradiated with the same dose, incubated, and examined in the same way. Almost every cell began to divide

TABLE 2

Sensitivity of strains B and B/r to penicillin and sodium sulfathiazole.

	CONCENTRATION OF PENICILLIN—UNITS/ML			CONCENTRATION OF SODIUM SULFATHIAZOLE—MOLES/ML		
	20	40	60	1×10^{-6}	2.5×10^{-6}	5×10^{-6}
Growth of strain *B*	+	−	−	+	−	−
Growth of strain *B/r*	++	++	−	++	++	+

++ = Full turbidity after 72 hours.
+ = Less than full turbidity after 72 hours.
− = No visible turbidity after 72 hours.

within one hour, the normal lag, and formed a microcolony of 50–100 normal-sized cells within three to four hours.

Strain *B/r* thus exhibits resistance not only to the lethal action of ultraviolet radiation, but also to its inhibitory effect on cell division. The technical usefulness of this difference for the genetic analysis of the mutation will be apparent in a later section.

(6) Sensivitity of Strain *B/r* to Penicillin and Sulfathiazole

Penicillin and sulfathiazole are antibacterial agents which are also known to cause inhibition of cell division, with production of elongated filaments, when administered to sensitive cells in low concentrations. The possibility was considered that the resistance of strain *B/r* to radiation might extend to other agents exerting a specific division-inhibiting effect. Experiments were conducted to compare the sensitivity of strains *B* and *B/r* to penicillin and sodium sulfathiazole. Table 2 gives the results, which indicate that the mutant *B/r* is relatively resistant to these agents, as well as to ultraviolet radiation and x-rays.

A Technique for the Quantitative Detection of Radiation-Resistant Bacteria in Samples from Sensitive Cultures

The genetic analysis of mutations in bacteria is dependent upon selective procedures which permit the determination of the number of mutants present in samples from parent cultures. Since the resistance to radiation characterizing strain *B/r* is relative, rather than absolute, no such procedure was readily available.

If spontaneous mutation is wholly or partially responsible for the change in sensitivity, a sensitive culture must contain, prior to irradiation, a certain proportion of resistant cells. The survival curves for sensitive and resistant bacteria show that a dose sufficiently high to eliminate all or most of the sensitive bacteria will, at the same time, eliminate most of the resistant cells. Treatment with a high dose of ultraviolet will thus permit the recovery of only a small fraction of the resistant bacteria initially present in the sample.

A special method, designated as the double-irradiation technique, was developed, whereby all the resistant bacteria in a normal sample could be detected selectively. This method was utilized in many of the experiments to be reported below, and will therefore be described in some detail.

The technique makes use of the difference, described above, in the behavior of individual bacteria of the parent and mutant strains after irradiation with low doses of ultraviolet. It will be remembered that sensitive bacteria, irradiated with a low dose, form undivided long filaments, 50–100μ in length, after three to four hours of incubation. Resistant bacteria, irradiated with the same dose, form microcolonies of 50–100 cells of normal size after the same period of incubation. The dose employed (50 ergs per mm^2) kills all but 10 percent of the sensitive bacteria, but permits the survival of 100 percent of the resistant mutants.

Measured samples from cultures of strain *B*, strain *B/r* and artificially prepared mixtures of the two strains were spread on agar plates, irradiated with a dose of 50 ergs per mm^2 and incubated for three hours at 37°C. At the end of the incubation period, each of the plates was irradiated a second time, with a dose of 700 ergs per mm^2. It will be seen from the survival curve (figure 1) for strain *B/r* that this dose allows the survival of 20 percent of the resistant bacteria. Therefore, about 10–20 cells in each resistant microcolony of 50–100 cells (formed during the incubation period between the first and second irradiations) should survive the second treatment. If at least *one* cell in each resistant microcolony survives the second irradiation, a visible colony should form, after suitable incubation, for every resistant bacterium originally present in the sample.

On the other hand, the second dose of 700 ergs per mm^2 permits the survival of only 0.05 percent of the sensitive bacteria. Since 90 percent of the sensitive cells are eliminated by the first irradiation, the overall survival of sensitive bacteria after the two treatments should be about .005 percent. If the original sample is no larger than 2×10^4 cells, therefore, it should be possible to eliminate *all* the sensitive bacteria by the same treatment which insures 100 per-

cent survival of resistant bacteria. This will be possible, of course, only if the filamentous cells produced by the first irradiation behave like single bacteria rather than like chains of bacteria in their sensitivity to radiation. LEA, HAINES and COULSON (1937) found that this was true of long forms produced by gamma rays.

Table 3 gives the results of an experiment to determine the validity of this technique. When samples from cultures of strain *B* were given the double-

TABLE 3

Reliability of double-irradiation technique for selective recovery of radiation-resistant bacteria in mixture with sensitive bacteria.

Double-irradiation technique: first dose of 50 ergs/mm² followed by three hours of incubation and second dose of 700 ergs/mm².

CULTURE	SAMPLE	INITIAL NUMBER OF CELLS PER SAMPLE		COLONY COUNT AFTER TREATMENT AND INCUBATION	
		B	*B/r*	SENSITIVE	RESISTANT
Normal (*B*)	1			0	1
	2			0	0
	3			0	0
	4			0	1
		Av. 11,200	—	—	—
Resistant (*B/r*)	1			0	114
	2			0	91
	3			0	120
	4			0	130
		—	Av. 111±9.0	—	Av. 113.7±16.5
Mixture (*B*+*B/r*)	1			0	94
	2			0	112
	3			0	106
	4			0	96
		Av. 9,400	Av. 106±8.5	—	Av. 102.0±8.5

irradiation treatment, the only survivors obtained proved to be resistant mutants. When samples from cultures of the resistant strain *B/r* were treated, the number of colonies obtained corresponded to the number of bacteria originally plated. When an artificial mixture of the two strains was treated, only resistant colonies were obtained, and these corresponded in number to the proportion of resistant bacteria in the mixed sample originally plated. Every colony was characterized with respect to sensitivity or resistance by a simple and rapid diagnostic test. An inoculum from each colony to be tested was suspended in a small volume of broth, and a loopful of the suspension was drawn across a small segment of an agar plate. About twenty colonies could be

tested on a single plate. The plates were irradiated with a dosé of 50 ergs per mm^2, and incubated at 37°C for three hours. Each segment was then examined under the microscope. If the colony tested was composed of sensitive bacteria, long thread-like filaments were observed; if the colony consisted of resistant cells, microcolonies of 50–100 cells of normal size were seen. The difference between the long sensitive filaments and the compact resistant microcolonies is so striking that the diagnosis can be made in a few seconds, under low power magnification.

The results of the experiment reported in table 3 indicate that the double-irradiation technique is effective in providing an absolute selective procedure for the detection of radiation-resistant mutants.

The technique can be modified to determine the number of resistant mutants in samples as large as 10^7, by extending the time of incubation between irradiations up to five hours, and increasing the second dose up to 1800 ergs per mm^2. When large samples are used, however, sensitive bacteria occasionally survive, probably because of screening by the dense network of elongated cells which develops after the first irradiation. When samples of about 10^6 bacteria or more are used, the diagnosis of each surviving colony for sensitivity or resistance becomes a necessary part of the procedure.

Origin of the Change from Sensitivity to Resistance

The proof of the spontaneous origin of hereditary variations in bacteria at more or less constant rates under given conditions, is an important means of establishing the underlying similarity of these changes to mutations in higher organisms. In the case of radiation-resistance, this problem is of particular interest, since ultraviolet radiation and x-rays are known to be effective mutation-inducing agents.

The following possible modes of origin of the change from sensitivity to resistance may be postulated: (1) The change may be induced by the radiation in a certain number of cells in an initially homogeneous population, or (2) the change may be a spontaneously occurring mutation, and prior to the treatment the culture contains a certain number of resistant mutants. In this case (a) the radiation may act merely as a selective agent, or (b) it may act as a selective agent, but also as an inducing agent, increasing the rate of mutation to resistance.

The method used to test these hypotheses was developed by LURIA and DELBRÜCK (1943), and was applied by them and by DEMEREC (1945) to the study of bacterial mutations. The method involves the following considerations:

If the change is entirely induced by the radiation, the number of resistant bacteria obtained from a sample will depend upon the probability that an induced change will occur in any bacterium. This probability should be the same for all bacteria under similar physiological conditions. Therefore, the number of resistant bacteria in samples from a series of similar, independent cultures should show fluctuations no greater than those shown by the number of resistants in a series of samples from a single culture. These fluctuations

TABLE 4

Number of resistant bacteria in samples from independent cultures, and in samples from single cultures.

Number of resistant bacteria determined by double-irradiation technique: first dose of 50 ergs/mm² followed by 5 hours of incubation, and second dose of 1500 ergs/mm².

	SAMPLES FROM INDEPENDENT CULTURES				SAMPLES FROM SINGLE CULTURES		
EXPT. NO.	1	2	3		1	2	3
AV. NO. CELLS PER SAMPLE	1×10^6	1.1×10^6	9.5×10^5		9.7×10^5	1×10^6	1.2×10^6
SAMPLE NO.				SAMPLE NO.			
1	0	4	5	1	8	0	13
2	12	8	13	2	10	1	9
3	8	15	61	3	5	3	11
4	8	12	10	4	9	1	14
5	19	0	1	5	7	0	9
6	98	14	12	6	9	0	8
7	7	1	2	7	15	2	12
8	5	76	8	8	8	1	13
9	14	11	0	9	6	4	7
10	24	42	13	10	16	1	15
11	—	—	9	11	—	—	8
12	—	—	7	12	—	—	8
13	—	—	18	13	—	—	14
14	—	—	0	14	—	—	9
15	—	—	11	15	—	—	13
16	—	—	8	16	—	—	6
17	—	—	116	17	—	—	11
18	—	—	12	18	—	—	7
19	—	—	10	19	—	—	10
20	—	—	6	20	—	—	18
Average	19.5	18.3	16.1		9.3	1.3	10.8
Variance	764.7	574.7	1509.8		12.9	1.8	6.9
χ^2	373.3	279.6	844.0		12.5	11.6	12.2
P	—	—	—		0.1–1.2	0.2–0.3	0.8–0.9

should be due only to sampling error, and in both cases the distribution of the number of resistant bacteria should constitute a Poisson series, with the variance approximately equal to the mean.

If the change is a spontaneous mutation, the number of resistant bacteria obtained from a given sample depends upon (1) the probability that any bacterium will mutate during its lifetime, and (2) the time of occurrence of mutations during the growth of the culture, since all bacteria descended from mutated cells will be resistant. In this case, the number of resistants in samples

from a series of similar, independent cultures should show large fluctuations (see LURIA and DELBRÜCK 1943) and the variance should be significantly higher than the mean.

Table 4 gives results of experiments to determine the number of resistant bacteria in samples from a series of similar, independent cultures, and in samples from a single culture. Every culture was started with an inoculum of about 20 cells from the sensitive strain, in a volume of 1 ml. of broth. The maximum difference in final titre of the cultures in any series was 12 percent.

In all three experiments, the number of resistant bacteria in samples from a single culture shows fluctuations satisfactorily accounted for by sampling errors. This indicates that the method of plating and irradiating does not introduce fluctuations beyond those expected on the basis of sampling.

The number of resistant bacteria in samples from a series of independent cultures, in all three experiments, shows fluctuations much greater than can be accounted for by sampling errors. The variance is significantly higher than the mean in every case, and the fluctuations are of the type to be expected according to the hypothesis of spontaneous mutation.

Although these experiments confirm the hypothesis of spontaneous origin of resistance to radiation, the possibility remains that the treatment used to detect these mutants induces the change in an additional number of sensitive bacteria. Under the conditions of the double-irradiation technique, however, there is only one class of induced mutation that could possibly appear, namely, those which become phenotypically resistant with no delay. Since no phenotypically sensitive cell can survive the treatment, those sensitive bacteria in which a mutation is induced, but does not take effect immediately, will have no chance to survive. DEMEREC (1946) has shown that, in the same strain of *E. coli*. the majority of ultraviolet-induced mutations to bacteriophage-resistance take effect only after cell division has occurred.

Direct experimental evidence was obtained which further weakens the likelihood that any significant number of the resistant mutants detected by the double-irradiation technique is due to induction. It has been mentioned that the doses used in this technique, depending upon the size of the sample, can vary from 700 to 1800 ergs per mm^2. An experiment was conducted to determine the number of resistant bacteria in several samples from the same culture, using doses throughout this range. The proportion of mutants in all of these samples was the same within the limits of sampling error. If a significant fraction of these mutants arises by induction, their number should vary with the dose.

An Estimate of the Mutation Rate

It is possible to estimate the rate of bacterial mutations from experiments of the type described above by solving the following equation: $r = aN_t ln(CaN_t)$ (for derivation see LURIA and DELBRÜCK 1943), where r is the experimental average of the number of mutants in a series of similar cultures, N_t is the number of bacteria at the time of observation, C is the number of cultures and a is the mutation rate.

LURIA and DELBRÜCK have plotted a series of curves relating the observed values of r to aN_t for various values of C. The estimated mutation rate for radiation-resistance, obtained from these curves, is about 10^{-5} mutations per bacterium per generation.

Induction of the Mutation to Radiation-resistance by Ultraviolet

It has been demonstrated that resistance to radiation is due to a spontaneous mutation, and that, under the conditions of the double-irradiation technique, the mutants are passively selected by the radiation. Experiments were conducted to determine whether, under suitable conditions, the mutation could be induced by radiation.

TABLE 5

Induction of mutations to radiation-resistance by treatment with a high dose of ultraviolet.

Culture used: 24-hour aerated culture of strain *B* in nutrient broth.
Spontaneous frequency of radiation-resistant mutants, determined by double-irradiation
Treatment: Experiment no. 1—.001%
Experiment no. 2—.005%

	INITIAL NO. OF BACTERIA	DOSE ADMINISTERED	AV. NO. OF COLONIES PER PLATE	TOTAL NO. OF COLONIES TESTED FOR RES. TO U.V.	NO. OF COLONIES RES. TO U.V.	% OF RES. MUTANTS AMONG SURVIVORS
Experiment no. 1						
Control	2×10^2	None	2×10^2	500	None	—
Experimental	2.4×10^8	3800 ergs/mm^2	26	434	21	4.9
Experiment no. 2						
Control	1.7×10^2	None	1.7×10^2	365	None	—
Experimental	2.0×10^8	3800 ergs/mm^2	30	450	15	3.3

The results of these investigations are shown in table 5. Samples taken from a 24-hour aerated culture of strain *B* were irradiated with a dose of about 3800 ergs per mm^2. Nonirradiated control plates were spread with suitably diluted samples from the same culture. After incubation, several hundred colonies from the treated plates, and a similar number from the control plates, were isolated and diagnosed for resistance to ultraviolet by the usual method of microscopic examination. A determination of the spontaneous frequency of mutants in the original culture was made by the double-irradiation method. The frequency of radiation-resistant mutants among the survivors of the high-dosage irradiation was found to be 4.9 percent in one experiment, and 3.3 percent in the other. No mutants were found among the control colonies in either experiment. The spontaneous frequency of mutants in the original

culture was about .001 percent in one experiment, and .005 percent in the other.

Although the values of 4.9 percent and 3.3 percent may be slightly exaggerated by the differential survival of resistant mutants, these experiments indicate that the rate of mutation to radiation-resistance can be increased considerably by high doses of ultraviolet.

The Number of Independent Mutations Resulting in Resistance to Radiation

In the foregoing section, resistance to radiation has been discussed as if a single mutation were responsible for this character. Techniques comparable to the analysis of breeding ratios and gene localization are not available to investigators of bacterial heredity, and it is necessary to approach this question with indirect and less satisfactory methods. A certain amount of information can be gained if the mutant differs from the parent strain in a number of independently demonstrable phenotypic characters. If a series of unrelated strains of the mutant is isolated, and tested with respect to all of these properties, it may be possible to divide them into a number of distinct classes, differentiated on the basis of one or more secondary characteristics. In favorable instances, the number of classes differentiated in this way may be shown to correspond to a minimum number of separate mutations, each capable of producing the primary phenotypic character, which may or may not be associated with the various secondary changes.

An analysis of this type was made of a group of 50 unrelated strains of the radiation-resistant mutant. Each of the strains was isolated from a different culture of strain *B*, started with an inoculum of about 20 bacteria. A sample from each of the cultures was subjected to the double-irradiation treatment, and one resistant colony from each plate was selected and established on a agar slant.

The properties chosen for analysis were those whereby the original mutant strain *B/r* was known to differ sharply from the parent strain: sensitivity to ultraviolet radiation, x-rays, penicillin and sulfathiazole; lag phase in broth at 37°C, and filament formation after exposure to an ultraviolet dose of 50 ergs/mm^2. All 50 strains were characterized with respect to each of these properties, except that only ten strains were tested for resistance to x-rays. The results are summarized in table 6. The 50 unrelated strains were found to be identical with respect to all of the properties examined, except resistance to penicillin and sulfathiazole. Four classes of mutants were differentiated on the basis of these characters: 21 strains were resistant to both of these agents; eight were resistant to penicillin, but not to sulfathiazole; five were resistant to sulfathiazole, but not to penicillin, and 16 were resistant to neither of these agents.

These results constitute indirect evidence that at least four separate mutations to radiation-resistance are possible, distinguishable by the presence or absence of associated resistance to penicillin and sulfathiazole. This conclusion, of course, would not be justified if the resistance to penicillin and sulfathiazole associated with radiation-resistance is due to coincidence of independent muta-

tions. The relative frequencies of the four classes of mutants militate strongly against this possibility. If coincidence of three independent mutations were necessary to produce simultaneous resistance to radiation, sulfathiazole and penicillin, the rarest class should be the triple mutants. Actually, the most frequent class of mutants is that exhibiting resistance of all three agents.

Radiation-resistance as a "Modifier" of Penicillin-resistance

The experiments described in this section were designed to clarify the resistance to penicillin characterizing many radiation-resistant strains.

Curves of survival of strains *B* and *B/r* as a function of penicillin concentra-

TABLE 6

Analysis of properties of 50 unrelated radiation-resistant strains.

PROPERTIES EXAMINED	LAG PHASE IN BROTH AT 30°C	FORMATION OF FILAMENTS 3 HRS. AFTER IRRADIATION WITH 50 ERGS/MM²	RESISTANCE TO ULTRAVIOLET: DOSE AT WHICH 50% SURVIVE	RESISTANCE TO X-RAYS: DOSE AT WHICH 50% SURVIVE	RESISTANCE TO PENICILLIN: GROWTH IN 40 U/ML	RESISTANCE TO SODIUM SULFATHIAZOLE: GROWTH IN 2.5×10^{-6} MOLES/ML
Strain *B*	1 hr., 20 min.	Filaments 50–100μ in length	About 30 ergs/mm²	About 5 r	No growth	No growth
Strain *B/r*	1 hr.	No filaments	About 450 ergs/mm²	About 15 r	Full growth	Full growth
Unrelated radiation—resistant strains:						
1–10	1 hr.	No filaments	About 450 ergs/mm²	About 15 r	Full growth	Full growth
11–21	1 hr.	No filaments	About 450 ergs/mm²	—	Full growth	Full growth
22–29	1 hr.	No filaments	About 450 ergs/mm²	—	Full growth	No growth
30–34	1 hr.	No filaments	About 450 ergs/mm²	—	No growth	Full growth
35–50	1 hr.	No filaments	About 450 ergs/mm²	—	No growth	No growth

tion were made, by plating measured samples of bacteria on penicillin agar. These curves are shown in Figure 3.

Samples from cultures of strains *B* and *B/r* were then plated on agar containing concentrations of penicillin ranging from 30 to 60 units per ml, and the plates were incubated for 48 hours. Forty-five colonies from the *B* series, and 50 colonies from the *B/r* series were isolated and inoculated into broth containing no penicillin. Each of the cultures was then tested roughly for resistance to penicillin, by inoculation into broth containing three critical concentrations of penicillin. Twenty-two of the cultures in the *B* series were able to grow in higher concentrations of penicillin than strain *B*, and were therefore considered to be penicillin-resistant mutants of this strain. They were designated as *B/p*. Seventeen of the cultures from the *B/r* series were able to grow in higher concentrations of penicillin than strain *B/r*. They were

considered to be penicillin-resistant mutants of strain *B/r*, and were designated as *B/r/p*.

Curves of survival of 15 *B/p* mutants and 15 *B/r/p* mutants as a function of penicillin concentration were made. These curves are shown in figure 3,

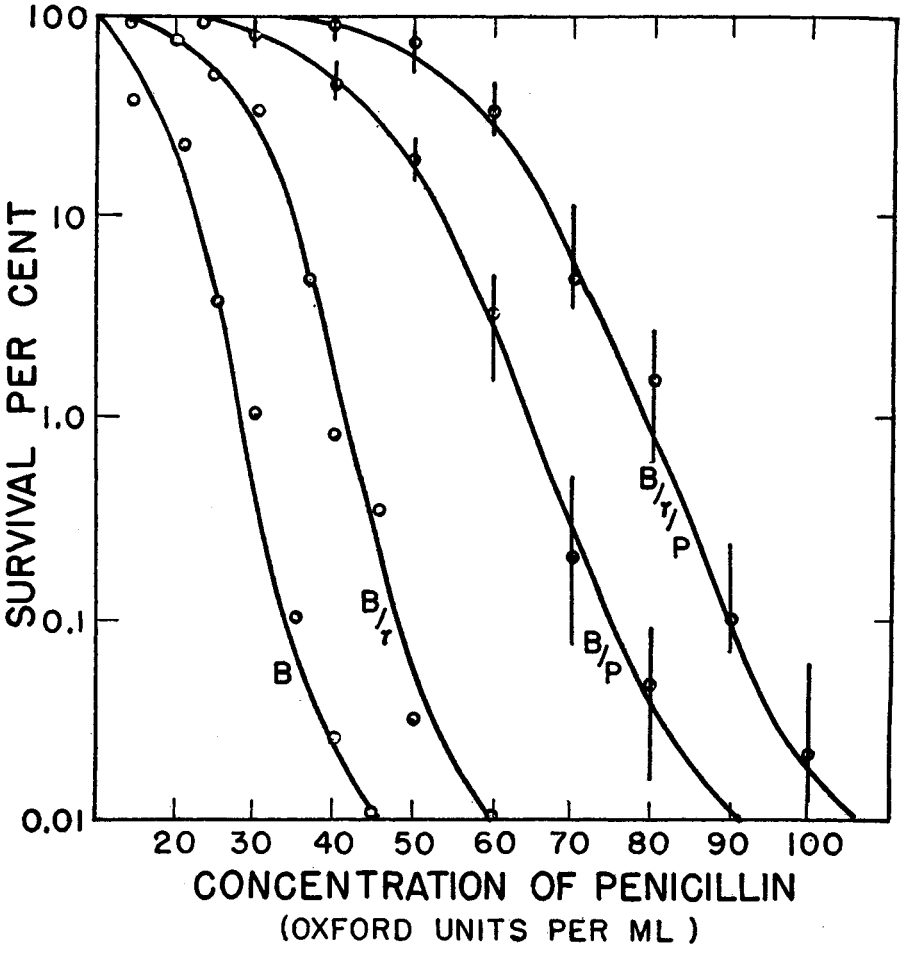

FIGURE 3.—Sensitivity of strains *B*, *B/r*, *B/p* and *B/r/p* to penicillin. Vertical lines drawn through points indicate limits of variability among 15 strains of *B/p* and 15 strains of *B/r/p*.

with the curves for each of the parent strains. The points represent average values for 15 strains, and the limits of variability are indicated by vertical lines drawn through each of the points.

When the four curves in figure 3 are compared, the following observations emerge: (1) Penicillin-resistant mutants obtained from strain *B*(*B/p*) are characterized by a higher level of resistance to penicillin than that shown by strain *B/r*. This observation supports the view that coincidence of independent

mutations is not responsible for the association of resistance to penicillin and to radiation found in strain *B/r*. (2) Penicillin-resistant mutants obtained from strain *B/r* (*B/r/p*) are more resistant to penicillin than mutants obtained from strain *B* by the same treatment. The survival curves for *B/p* and *B/r/p* are similar, but the resistance of the latter to penicillin is shifted to a higher level by an amount which corresponds approximately to the initial difference between strains *B* and *B/r*. Thus, the mutation to radiation-resistance, when it results in resistance to penicillin as well, acts as a "modifier" of an independent mutation to penicillin-resistance, adding quantitatively to its effect.

An additional observation was made which lends support to these conclusions. Ultraviolet-resistant mutants were isolated from a *B/p* mutant strain by the double-irradiation treatment, and survival curves of ten of these mutants with penicillin were made. Six of the curves were no different from those of the parent strain *B/p* but four gave curves identical with the penicillin survival curves of strain *B/r/p*. In these four strains, the resistance to penicillin was shifted to a higher level by a mutation to radiation-resistance, acting as a modifier of an independent mutation to penicillin-resistance.

Some Preliminary Experiments on Population Dynamics

The study of the factors governing selection in mixed populations of bacteria is limited, at the present time, by our inadequate knowledge of the genetic makeup of bacteria, and by the complexity of their physiological responses under conditions which are not strictly defined. To the extent that well-defined and easily detected mutants are available within a strain, however, it may become profitable to explore the behavior of artificially mixed populations of known constitution.

The method used in the preliminary experiments to be described below consisted of (1) the preparation of mixtures of various proportions of strains *B* and *B/r*, (2) the maintenance of the mixed cultures under specified conditions, and (3) the periodic determination of the proportions of each strain present in the mixtures.

In the first series of experiments, four duplicate sets of mixed cultures were set up by inoculating measured volumes from fully grown broth cultures of strains *B* and *B/r* simultaneously into tubes of fresh broth. Immediately after the inoculation of the mixed cultures, assays were made to determine precisely their composition. Each culture was assayed in two ways: first, to determine the total number of bacteria, both *B* and *B/r*, per ml of the culture; second, to determine the number of resistant bacteria per ml of the mixture. The assays for the total number of bacteria were conducted by the usual method of dilution, plating and colony counts. The assays for the number of resistant bacteria were made by subjecting samples from the mixed cultures to the double-irradiation treatment. Knowing the titre of the culture as a whole, and the titre of the *B/r* component, the proportion of mutant bacteria in each of the cultures was computed.

In the first experiment, the mixtures were incubated at 37°C, and were serially subcultured in broth every 24 hours over a period of 24 days. Every

48 hours, the proportion of resistant bacteria in the current set of subcultures was determined, using the two assay methods described above. Figure 4 shows the proportion of resistant bacteria in each of the mixtures plotted as a function of time in days after the start. The curves show that no significant change in the proportions of the two strains occurred over the 24-day period. This experiment was repeated, using synthetic medium throughout instead of nutrient broth, and the results were essentially the same.

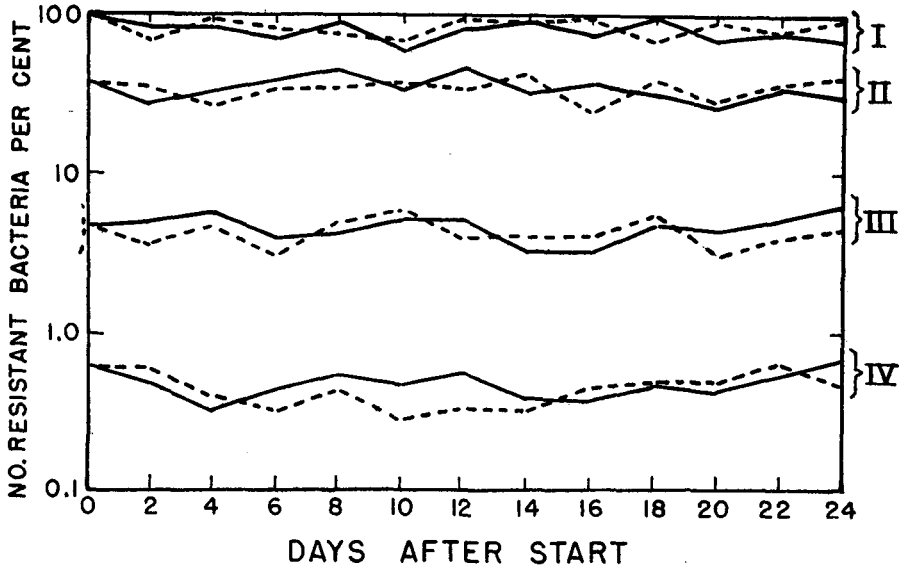

FIGURE 4.—Proportion of resistant bacteria in artificially mixed cultures of strains *B* and *B/r*, maintained by daily serial subculture.
(I) duplicate mixtures—initial proportions 1.0% *B*:99.0% *B/r*
(II) duplicate mixtures—initial proportions 37.0% *B*:63.0% *B/r*
(III) duplicate mixtures—initial proportions 95.1% *B*: 4.9% *B/r*
(IV) duplicate mixtures—initial proportions 99.4% *B*: 0.6% *B/r*

A second experiment was conducted, in which the method of maintaining the mixed cultures was modified. In this experiment, only one set of mixtures was followed, again in duplicate. The initial proportions of this set of mixtures, which were carried in nutrient broth, were approximately 80% *B/r*: 20% *B*. As in the first experiment, the mixtures were subcultural serially every 24 hours, and periodic determinations were made of the proportion of mutant bacteria. However, the initial mixed cultures, from which the series of subcultures was started, were not discarded. Instead, they were placed in the incubator, and allowed to age at 37°C for the 20-day period of the experiment. Determinations of the proportion of resistant bacteria in these aging cultures were made every 48 hours. The results of this experiment are shown in figure 5. When the mixtures were carried by daily serial subculture, as in the first experiment, no significant change in the proportion of resistant bacteria was

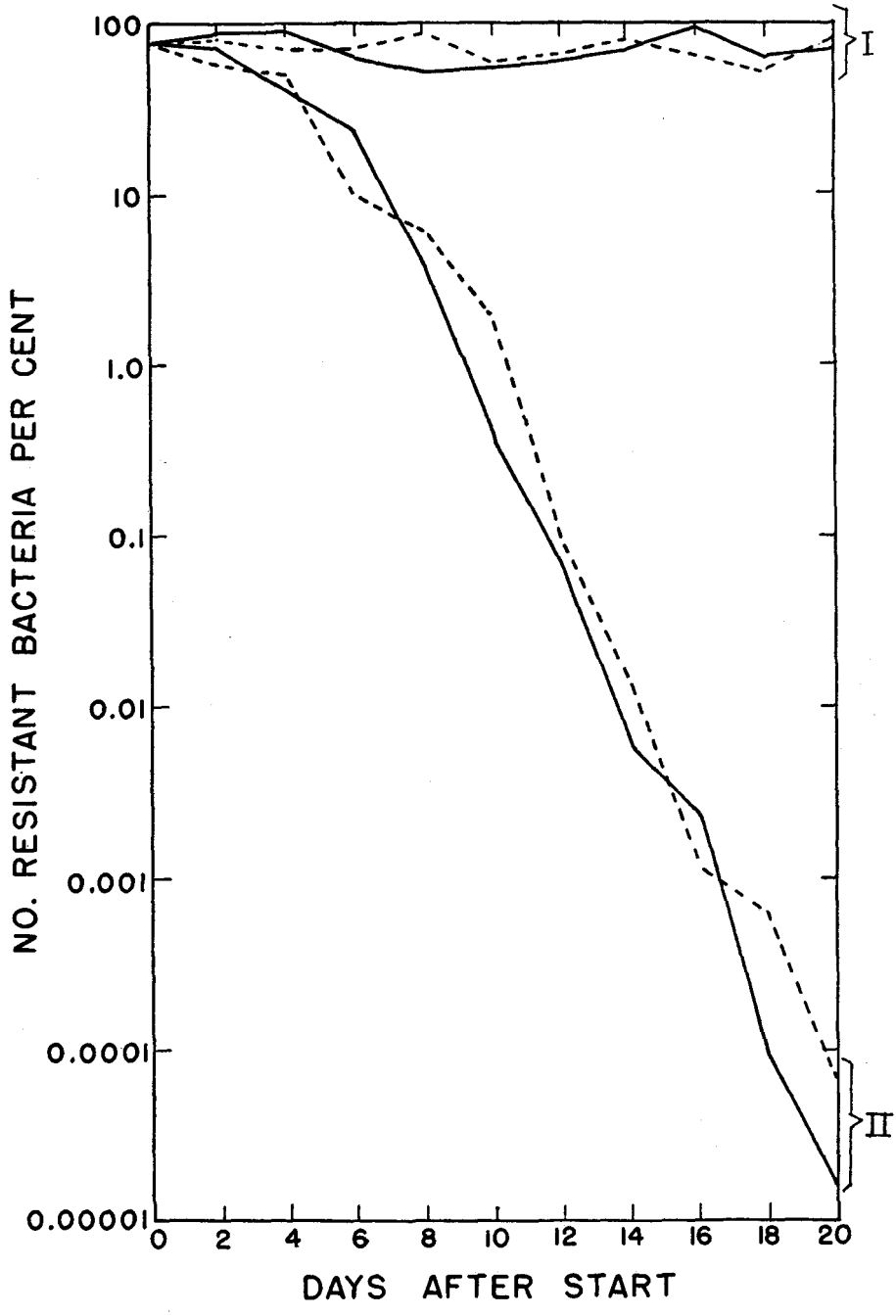

FIGURE 5.—Proportion of resistant bacteria in artificially mixed cultures of strains *B* and *B/r*.
(I) duplicate mixtures: initial proportions 20% *B*; 80% *B/r* maintained by daily serial subculture.
(II) same duplicate mixtures as (I), maintained by aging without subculture.

observed. When the cultures were allowed to age without subculture, the proportion of the *B/r* component dropped from 80 percent to about 0.00005 percent, or 1 in 2×10^6 bacteria, in 20 days. There are indications that the proportion of mutants continues to fall, until there are no mutants in a sample of 10^8 bacteria.

These results indicate that, under the conditions of aging broth cultures, strain *B/r* is at a distinct selective disadvantage in mixture with strain *B*. No such disadvantage is manifest under the conditions of daily serial transfer to fresh medium.

DISCUSSION

Evidence has been presented that the increased resistance to radiation exhibited by strain *B/r* is the result of a spontaneous mutation occurring in cultures of the parent strain. At least four separate mutations have been differentiated, each of which results in resistance to both ultraviolet and x-rays. but distinguishable on the basis of secondary resistance to penicillin and sulfathiazole. The estimated mutation rate, 10^{-5} mutations per bacterium per generation, should therefore be interpreted as the sum of at least four separate mutation rates.

The mutation rate was calculated on the simplifying assumption that the growth rates of mutant and parent bacteria are the same, under the experimental conditions. The dangers inherent in this assumption are evident, particularly in the light of the population dynamics experiments. These experiments show that, under different conditions of mixed culture, the relative growth rates of mutant and parent bacteria may vary widely. Estimates of mutation rates which are based on assumptions as to growth rates under given conditions may, therefore, involve considerable error.

Although mutations to radiation-resistance occur spontaneously, their rate can be increased considerably by treatment with high doses of ultraviolet. The fact that the inducing agent and the specific selective agent for the mutant are the same constitutes an obstacle to the quantitative study of the induction effect. This difficulty, however, has an interesting philosophic implication. The production of radiation-resistant mutants by radiation, along with other seemingly purposive biological events, might be interpreted by some as a manifestation of teleology. The spurious nature of such an explanation is easily exposed in this case, since it is known (1) that radiation-resistance occurs spontaneously, as well as in direct response to radiation, and (2) that radiation-resistance is only one of a number of random changes induced by radiation, although it is automatically selected by the treatment responsible for its induction.

Speculations as to the possible mechanism of resistance to radiation must be based upon a more or less definite conception of the manner in which radiation kills bacteria. The target theory (for general discussions see TIMOFÉEFF-RESSOVSKY 1937, ZIMMER 1943, LEA 1946) has provided a basis for one interpretation of the bactericidal action of radiation. This theory, which has been applied extensively to radiobiological phenomena and is supported by con-

siderable experimental evidence, will be adopted tentatively as a starting point in this discussion.

According to the simplest assumptions of the target theory, certain biological effects of radiation (in this case, the killing of bacteria) are produced whenever a "hit" or a specific number of "hits" occurs in a sensitive region, or target. A hit is defined as an elementary act of absorption of radiation energy, that is, as the production of an ionization (in the case of x-rays) or as the absorption of a photon (in the case of ultraviolet).

In the application of the target theory to the killing of bacteria by radiation, the interpretation of survival curves has played an important part. When the fraction of surviving bacteria is plotted against the dose, it is often found that each successive increment of dose kills the same *proportion* of the remaining bacteria, rather than the same *number* of bacteria. In other words, when the logarithm of the surviving fraction is plotted as a function of the dose, a straight line is obtained. Exponential survival curves of this type have been described for ultraviolet by WYCKOFF (1932), HERČÍK (1936), HOLLAENDER and CLAUS (1936), and LEA and HAINES (1940).

The central features of the target theory rest on the interpretation of the statistical law underlying the exponential curves:

$$\log N/N_0 = kD,$$

where N_0 is the initial number of bacteria, N is the number of bacteria surviving and D is the dose. The dose D, according to the target theory, represents ultimately the number of hits occurring per unit of volume, and therefore defines the probability that a hit will occur in a sensitive volume, or target. The constant k thus directly represents the size of the target, and a single hit, occurring within the target, will kill a bacterium. Certain investigators (COBLENTZ and FULTON 1924, GATES 1929, DREYER and CAMPBELL-RENTON 1936) have reported survival curves which are sigmoid in shape. Sigmoid survival curves are regarded with suspicion by some proponents of the target theory (LEA 1946), on the ground that they are obtained only when certain sources of experimental error are not rigorously controlled. *Bona fide* sigmoid curves, however, would be interpreted as signifying that two or more hits in a sensitive volume are required to kill a bacterium.

Within the framework of the target theory, how can a mutation leading to increased resistance to radiation be explained?

The survival curves for strain B, with both ultraviolet and x-rays, are exponential. Let us assume, then, that sensitive bacteria are killed whenever a single hit occurs in a sensitive volume. The ultraviolet survival curve for the resistant strain is distinctly sigmoid in shape. Technical sources of error cannot be responsible for this deviation from exponential killing, since the techniques used were identical with those involved in the preparation of the corresponding curve for strain B. This curve must be interpreted, according to the target theory, as a multiple-hit curve, indicating that several hits in a sensitive volume are required to kill a resistant bacterium. The ultraviolet curves suggest that the mutation from sensitivity to resistance changes the

mode of killing from a one-hit to a multiple-hit mechanism.

The x-ray curve for strain *B/r*, however, is a one-hit curve, differing only in slope from the x-ray curve for the sensitive strain. Since a single x-ray hit supplies considerably more energy than a single ultraviolet hit (the ratio being approximately 6:1), an effect requiring multiple ultraviolet hits can be satisfied by a single x-ray hit (LATARJET 1946). However, if the mutation involves merely a change in the energy threshold for killing which is still satisfied by a single x-ray hit, the mutant should not exhibit increased resistance to x-rays. On the basis of the x-ray data, the mutation seems to produce a decrease in the size of the target (which determines the value of *k*, and hence the slope of the curve). Or, if it is assumed that a number of possible targets exist in sensitive bacteria, a single hit in any one of which will result in death, the change may involve the inactivation of a fraction of the available targets.

In any case, there is a contradiction between the x-ray and ultraviolet data, which are not easily reconcilable according to the simplest assumptions of the target theory.

The validity of the assumption that the value of *k* is determined by the size of the sensitive volume alone, and that a hit within the target is always effective, has recently been questioned on theoretical grounds by a number of investigators (SOMMERMEYER 1938, JORDAN 1939, MULLER 1940, FANO 1942). These authors have discussed, as an additional factor to be considered as influencing the value of *k*, the probability that a hit occurring in the sensitive volume will produce a given effect. This "quantum efficiency" factor which has been applied in experimental work by WOLLMAN, HOLWECK and LURIA (1940), and by LATARJET (1946), can approach one, or can be very small, depending upon the reaction system involved. Taking into account this probability factor in determining the value of *k*, a consistent explanation of resistance to ultraviolet and x-rays can be developed.

Let us assume that, in the sensitive strain, a single ultraviolet hit will, with a certain probability, produce a primary change which will lead to the death of the bacterium. A single x-ray hit is also effective, with its own probability. Suppose, then, that the mutation to resistance raises the threshold for the production of the primary effect, so that the probability that a single ultraviolet hit will be effective approaches zero. A multiple-hit mechanism for the killing of resistant bacteria with ultraviolet may result. A single x-ray hit may still supply the energy required to meet the new threshold, so that a single-hit curve will be obtained with x-rays, *but the change in threshold decreases the probability that an x-ray hit will be effective, so that the slope of the curve, determined by k, is shifted in the direction of greater resistance.*

The parallel radiobiological results obtained for the production of mutations in Drosophila and the killing of bacteria, among other considerations, have led to the suggestion that the bactericidal effect of radiation may be due to the induction of lethal mutations (LEA and HAINES 1940, LEA 1946). On the basis of this hypothesis, the change in threshold for the production of the primary effect of radiation, discussed above as a possible result of the mutation from sensitivity to resistance, can be given a more concrete formulation.

The mutation can be envisaged as a rearrangement of the gene, or of a critical constituent of it, leading to greater stability, and consequently to decreased susceptibility to induced lethal mutation. In Drosophila, many mutated genes are known to be less susceptible to the inducing action of radiation than the original alleles (TIMOFÉEFF-RESSOVSKY 1933).

Certain effects of radiation which lie outside the scope of the target theory can be considered as a basis for an entirely different approach to the mechanism of resistance. DALE (1940, 1942) has developed the argument that the inactivation of enzymes may be important in the lethal action of radiation. Another possibility, mentioned by LEA (1946), is that the decomposition products of proteins, or other cell constituents, produced as a result of irradiation, may act as cell poisons. With these suggestions in mind, the following observations take on significance for the problem under discussion: (1) All radiation-resistant strains are resistant to the division-inhibiting action of radiation as well as to its lethal effect, (2) many radiation-resistant strains are also resistant to penicillin, or sulfathiazole or both; (3) strains resistant to sulfathiazole and penicillin are resistant to the division-inhibiting action of these agents as well as to their lethal effects.

These facts suggest a direct relationship between the division-inhibiting action of these antibacterial agents and their lethal effect. LEA, HAINES and COULSON (1937) have presented evidence that these two effects of radiation are independent of one another. The observation that resistance to the division-inhibiting effect of ultraviolet invariably accompanies resistance to its bactericidal action is evidence that the two effects are closely related, unless the unlikely assumption is made that the mutation from *B* to *B/r* involves two independent changes.

The microscopic study of sensitive bacteria irradiated with low doses of ultraviolet yields two further significant observations: (1) bacteria which recover from the division-inhibiting effect of the treatment within a certain time give rise to colonies, that is, survive; (2) bacteria which do not recover from the division-inhibiting action of the treatment within a certain time do not give rise to colonies, that is, are killed. Thus, recovery from the inhibition of division produced by radiation seems to be associated with survival. The resistance of strain *B/r* to radiation may be due to a change which renders this strain less susceptible to inhibition of division, or more efficient in recovery. The shorter lag phase characteristic of radiation-resistant strains may be another manifestation of this property.

The inhibition of division which occurs as a result of treatment with radiation may be due to either (a) the accumulation of an inhibitor (cell poison hypothesis), or (b) the destruction of a compound necessary to division (enzyme inactivation hypothesis). The resistance of strain *B/r* may be due to its greater efficiency in either the reformation of the inactivated compound or in the destruction or neutralization of the inhibitor.

The resistance of certain radiation-resistant strains to penicillin and sulfathiazole can be considered in this connection. Although the primary actions of these substances are likely to differ from each other, and from the primary

action of radiation, all of these agents share the ability to inhibit division in sensitive cells, and to a lesser extent in resistant bacteria. The chains of reactions blocked by the primary action of each of these agents may share one or more common steps. Different mutations to resistance may alter, or provide alternative pathways for, different steps in the reaction chains. When a step common to all of the reaction chains is affected, resistance to all of these agents may result.

One experimental observation, which was briefly mentioned in an earlier section, may have some bearing on the present discussion. Figure 2 shows two sets of ultraviolet survival curves for strains *B* and *B/r*, one set obtained when the bacteria were irradiated on the surface of solid medium, and the other when the irradiation was made in a nonabsorbing synthetic liquid medium. Both strains show greater sensitivity when irradiated on solid medium. Microscopic examination showed that bacteria irradiated on solid media exhibit more prolonged inhibition of division than bacteria treated in liquid suspension. This correlation between sensitivity and susceptibility to inhibition of division is further evidence of the intimate relation between these two factors.

Differences in treatment of the bacteria before and after irradiation, as well as differences in specific components of the media, have been eliminated as the factors responsible for this effect. Reflected radiation from the agar surface has also been eliminated (HOLLAENDER 1946). The difference in sensitivity seems to depend solely upon the liquid or solid character of the medium. This suggests the possibility that cell poisons, produced in the bacteria as a result of irradiation, diffuse away more rapidly in the liquid than in the solid medium, resulting in higher survival under the former conditions, and earlier recovery from the division-inhibiting effect.

As advances are made in our understanding of the pathways through which which radiation exerts its lethal effect, more fruitful approaches to the mechanism of resistance to radiation may become available.

Finally, some remarks will be made concerning the preliminary experiments on population dynamics described above. It will be remembered that, under the conditions of daily subculture, the proportion of mutants in mixture with sensitive bacteria was maintained at the initial level over a period of 24 days. When the mixtures were allowed to age in the incubator, however, the proportion of mutants dropped sharply, approaching complete elimination during the period of the experiment.

In mixed cultures maintained in broth at 37°C, selection apparently does not operate against either strain during the early phases of growth. The stage of growth during which selection is effective against the mutant strain is the so-called "stationary" phase. During this phase, according to JORDAN and JACOBS (1944), the viable count remains constant, while the total count increases steadily. The stable viable count is thus determined by a balance between the rates of division and death. BRAUN (1946) found that selection during this phase of growth was responsible for the characteristic dissociation patterns in clones of *Brucella abortus*.

The theoretical influence of selection pressure, and of forward and reverse

mutation rates, in determining the equilibria reached in genetically mixed populations has been analyzed mathematically by WRIGHT (1931), and for bacteria specifically by DELBRÜCK (1945). In general, equilibria in cultures of strains having stable properties are determined by forward mutation rates, which may be opposed by reverse mutation, or by selection against the mutant, or both. DELBRÜCK has pointed out that mutations occurring at very low rates need not be opposed by either of these factors, since the probability is low that rare mutants will be carried over at each subculture of the stock. Mutations occurring at high rates, however, must be opposed by selection or reverse mutation or both, if the original stock is to maintain its constancy.

Under the conditions of aging broth cultures, there is no evidence that forward mutation opposes the selection against radiation-resistant mutants, with the establishment of an equilibrium. If mutation is dependent upon the occurrence of cell division, the apparent absence of mutation pressure may be related to the rarity of dividing bacteria in aging cultures.

These experiments suggest that, in the case of mutations occurring at high rates, the method of carrying the stock can be important in maintaining the constancy of its properties. If selection is operative against such a mutant only in the stationary phase of growth, the mutant would soon replace the parental type (in the absence of reverse mutation) if the stock should be carried by daily serial subculture. The mutant could be held in check, however, if the stock culture were permitted to age between subcultures.

The rate of mutation to radiation-resistance, which is of the order of 10^{-5} mutations per bacterium per generation, is apparently not sufficiently high to permit the establishment of the mutant, even under conditions of daily serial subculture of the parent stock.

Further experiments are being conducted to determine the relation between the observed selection rate and the rates of division and death of the parent and mutant strains, in pure culture and in mixture. The dependence of the selection rate upon various initial proportions of parent and mutant bacteria, and upon specific conditions of growth and constituents of the medium, seems also worthy of investigation, since the experimental control of selection pressure in mixed populations of bacteria is likely to be of primary practical importance. The value of bacteria as materials for population studies, for which they are uniquely suited in many ways, will increase with our knowledge of their genetic and physiological constitution.

SUMMARY

Strain *B* of *Escherichia coli* gives rise to a variant which is characterized by relative resistance to the lethal effects of both ultraviolet radiation and x-rays. This character is stable and heritable.

Some additional properties of radiation-resistant strain, *B/r*, derived from the parent strain by culturing a last survivor of an irradiation with ultraviolet, are described. This strain exhibits relative resistance to the division-inhibiting effect of radiation, as well as to its lethal effect, and is also relatively resistant to the lethal and division-inhibiting action of penicillin and sodium sulfathia-

zole. Strain *B/r* exhibits a shorter lag phase in broth at 37°C than the parent strain, and a somewhat lower final titre. Survival curves of strains *B* and *B/r* with ultraviolet radiation and x-rays are compared.

A technique is described whereby radiation resistant mutants can be detected quantitatively in samples from the parent strain. This technique, designated as the double-irradiation treatment, provides an absolute selective procedure for the detection of the variant.

Resistance to radiation is shown to be due to a spontaneous mutation, occurring in cultures of strain *B* at a rate of about 10^{-5} mutations per bacterium per generation.

The rate of occurrence of the mutation to radiation-resistance can be increased considerably by treatment of the parent strain with high doses of ultraviolet radiation.

At least four independent mutations are demonstrated, each of which results in resistance to radiation, but distinguishable by the presence or absence of associated resistance to penicillin, or sodium sulfathiazole or both.

The mutation to radiation-resistance, when it is accompanied by resistance to penicillin, acts as a modifier of an independent mutation to penicillin-resistance, adding quantitatively to its effect.

Some preliminary experiments in population dynamics are described. When strains *B* and *B/r* are artificially mixed and carried by daily subculture, neither strain is at a selective disadvantage. When the mixtures are aged without subculture, however, the mutant strain is at a distinct selective disadvantage approaching complete lethality.

Interpretations are offered for certain features of the results.

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