EFFECT OF X-RAY IRRADIATION ON THE FREQUENCY OF GENETIC RECOMBINATION IN *Escherichia coli* AND ON THE PROPERTIES OF ITS RECOMBINANTS

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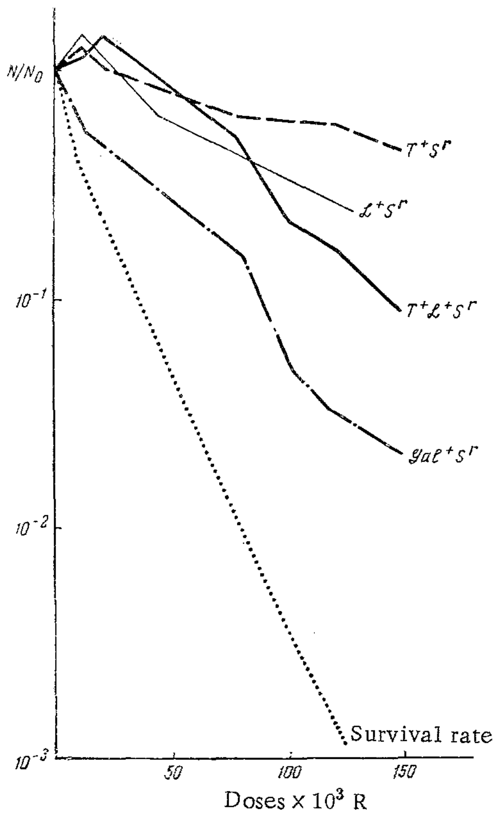
It is still uncertain whether ionizing radiation can cause profound changes in the chromosomes of bacteria, including changes in their configuration, or whether its action is limited to the induction of mutations in specific loci.

The most suitable method of solving this problem is by crossing sex-differentiated strains of E. coli, followed by genetic analysis of their recombinants. By using this method, Wilson [3] studied E. coli crosses in which the donor strain was exposed to γ-ray irradiation in large doses, and found that the frequency of recombinants exhibiting linkage between the threonine and lactose loci decreases with an increase in the dose of radiation. On the basis of these observations it was suggested that ionizing radiation causes breaks in the chromosomes of the donor bacterial cells. According to other findings [2], indicating that the frequency of selective signs in bacterial recombinants also falls during irradiation with large doses of x-rays, it must be assumed that ionizing radiation produces "points of fragility," at which breaks occur during conjugation, and also induces changes interfering with the transmission of the donor's chromosome segment to the recipient.



Effect of x-ray irradiation on transmission of selective signs.

In a previous investigation [1] we studied the effect of high-energy protons on genetic recombination in E. coli. However, in contrast to the investigations cited above, irradiation of donor cells caused an increase in the number of recombinants and in the frequency of transmission of nonselective signs. It is important to note that the bacteria in this case were irradiated with comparatively small doses of protons.

In the present study the effect of various doses of x-ray irradiation on genetic recombination in E. coli was investigated. The effect of x-ray irradiation on transmission of the ability to synthesize threonine and leucine (both together), to ferment lactose and galactose, and to synthesize threonine and leucine (separately), and also on the transmission of a number of nonselective signs was analyzed.

METHOD

Donor and recipient strains of E. coli were used in the experiments. The donor strain E. coli HfrH is thiamine-dependent (B_1^-) and sensitive to streptomycin (S^s), phages of the T group, and phage λ. The order of transmission of signs to the cells of

TABLE 1. Genetic Structure of $T^+L^+S^r$ and Gal^+S^r Recombinants (Transmission of nonselective signs as a percentage of number of recombinants)

Doses of irradiation (in R)	$T^+L^+S^r$				Gal^+S^r					
	Nonselective signs									
	Lac^+	Gal^+	T_1^S	λ^S	Lac^+	T^+L^+	T^+	L^+	T_1^S	λ^S
0	14,6	15,5	82,2	75,7	72,7	100	89,0	100	70,3	75,7
10×10^3	13,3	12,1	83,5	82,5	72,0	100	86,6	100	73,4	78,7
20×10^3	6,1	6,1	92,5	75,6	75,9	100	89,9	100	78,5	78,5
80×10^3	2,6	3,3	74,0	76,0	84,2	100	89,5	100	71,1	73,7
100×10^3	5,3	7,0	82,5	65,0	64,4	100	85,8	100	71,5	64,3
120×10^3	2,3	5,1	83,5	66,3	71,4	100	85,7	100	71,5	57,2
150×10^3	1,4	2,1	79,0	70,9	77,7	100	100	100	55,6	55,6

the recipient strain is as follows: threonine (T)–leucine (L)–lactose (Lac)–galactose (Gal). The recipient strain E. coli PA678F is streptomycin resistant (S^r) and is characterized by loss of the independent ability to synthesize threonine, leucine, and thiamine, and to ferment lactose, galactose, xylose, mannitol, and maltose, and it is resistant to phases T_1, T_5, T_6, and λ.

Before crossing took place, broth cultures of both strains were centrifuged for 5 min at 3000 rpm. The bacterial residue was transferred to fresh broth to give a concentration of 10^9 cells/ml.

The donor cells were irradiated with a type RUM-7 x-ray apparatus. After irradiation of donor Hfr cells with different doses of x-rays, they were crossed with recipient cells by mixing in a proportion of 1 : 10. Tubes containing the mixture were incubated at 37° for 60 min, after which samples were seeded on selective media and then incubated at 37° for 72 h. Selection of the $T^+L^+S^r$ recombinants was carried out on minimal medium with the addition of 1% glucose, thiamine (1 μg/ml) and streptomycin (150 μg/ml), and of the Gal^+S^r recombinants on minimal medium with 1% galactose, threonine and leucine (80 μg/ml of each), thiamine (1 μg/ml), and streptomycin (150 μg/ml) [1]. The T^+S^r and L^+S^r recombinants were selected on the same medium as the $T^+L^+S^r$ recombinants, with the addition of leucine to obtain T^+S^r recombinants and of threonine to obtain the L^+S^r.

The genetic structure of the recombinants (transmission of nonselective signs) was studied with reference to the results of seeding on media capable of detecting a particular sign (ability to ferment lactose was detected on meat-peptone agar with lactose and an indicator dye, sensitivity to phage T_1 on MPA with this phage, and so on).

RESULTS

The results of determination of the frequency of transmission of the studied signs from donor to recipient in relation to the dose of irradiation of the donor are illustrated by the curves given in the figure. These curves were drawn on the basis of the ratio between the number of recombinants in the crosses in which the donor was irradiated and the number of recombinants in the crosses in which the donor was not irradiated. The initial doses of irradiation that were used caused an increase in the number of $T^+L^+S^r$, T^+S^r, and L^+S^r recombinants. However, with an increase in the dose of irradiation an appreciable decrease in the number of all the recombinants took place. If the order of transmission of signs from donor to recipient is remembered, it will be easy to see that, in accordance with the curves obtained, the radiosensitivity of the selective signs was the greater, the farther along the transmitted segment of the chromosome they were situated from its initial portion, i.e., the portion first to enter the recipient cell.

The data given in Table 1 show that preliminary irradiation of the donor cells with an increase dose of x-rays caused a decrease in the number of $T^+L^+S^r$ recombinants inheriting the property of fermenting lactose and galactose. The number of Gal^+S^r recombinants inheriting the property of fermenting lactose with an increase in the dose of x-rays rose at first and then fell. A marked increase was observed in the number of recombinants inheriting sensitivity to phages T_1 and λ.

Analysis of the genetic structure of the T^+S^r and L^+S^r recombinants (Tables 2 and 3) shows that irradiation in increased doses also caused a decrease in the number of recombinants inheriting the property of fermenting lactose and galactose. It is clear from the results given in these tables that irradiation causes a disturbance in the coupling

TABLE 2. Genetic Structure of T^+S^r Recombinants (Transmission of nonselective signs as a percentage of number of recombinants)

Doses of irradiation (in R)	Nonselective signs				
	Lac+	Gal+	L+	T_I^S	λ^S
0	9	5	78	77	59
10×10^3	8	—	79	77	75
20×10^3	5	4	68	84	83,0
80×10^3	0	1,1	56,6	93,6	85,5
100×10^3	4,9	4,9	45,9	67,2	77,0
120×10^3	0	0	40,0	50,0	55,0
150×10^3	0	0	40,0	55,0	55,0

TABLE 3. Genetic Structure of L^+S^r Recombinants (Transmission of nonselective signs as a percentage of number of recombinants)

Doses of irradiation (in R)	Nonselective signs				
	Lac+	Gal+	T+	T_I^S	λ^S
0	16,2	5,0	100	86,2	82,5
10×10^3	9,0	4,0	100	60,0	63,0
20×10^3	7,3	8,8	100	82,3	88,0
80×10^3	1,3	1,3	100	58,1	—
100×10^3	0	0	100	68,0	68,0
120×10^3	0	0	100	62,8	60,0
150×10^3	0	0	100	62,8	60,0

of the T^+ and L^+ signs (the ability to synthesize threonine and leucine), for a decrease was observed in the number of T^+S^r recombinants inheriting the ability to synthesize leucine, while there was no reduction in the number of L^+S^r recombinants inheriting the ability to synthesize threonine. This latter feature was associated with the fact that the L^+ sign is transmitted to the recipient cell after the T^+ sign. The results of analysis of the transmission of nonselective signs also showed that the farther the sign is located from the initial portion of the transmitted segment of the donor's chromosome, the more sensitive it is to ionizing radiation.

Ionizing radiation evidently causes genetic changes in the transmissible material of the donor which either stimulate the process of transmission of the segment of the donor's chromosome or depress it. This choice between the two outcomes of the action of radiation on genetic recombination in bacteria depends on the dose of irradiation. So far as the possibility of breaking of chromosomes at individual points is concerned, it is not yet possible to say whether this takes place immediately after irradiation or during the process of conjugation at the "points of fragility."

LITERATURE CITED

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