A STUDY OF THE MUTATIONAL VARIABILITY OF E. coli K-12 λ IRRADIATED WITH NUCLEAR PARTICLES IN AN IRT-1000 REACTOR

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At the present time there are almost no papers in the literature devoted to a study of the effect of neutrons on bacteria. In 1961 there was a paper published [7] in which the effect of neutron and γ -radiation on the microflora of soil planted with alfalfa was described.

The object of the present investigation was a study of the effect of neutrons on the biological properties of E. coli. The survival rate, morphological changes in the cells and the frequency of biochemical mutations upon irradiation of the bacterial cultures with different neutron doses were determined.

EXPERIMENTAL METHOD

A strain of E. coli K-12 λ was used in the work. A suspension of the bacteria in physiological solution washed from a 24 h agar culture was submitted to irradiation. The density of the suspension was 1 billion/ml. 4.5 ml of the prepared suspension was placed in a quartz weighing bottle with a capacity of 5 ml. A nonirradiated portion of the suspension served as the control.

Treatment of the bacteria with neutrons was carried out in the vertical canal of the IRT-1000 reactor at a temperature of about 30°. The output of the source was 15 rad/sec. The irradiation time was determined by the required dose. Doses from 3000 to 60,000 rad were tested.

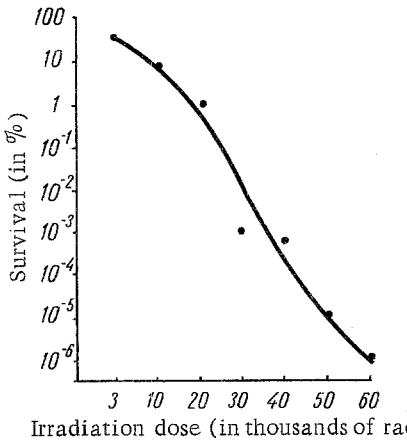

Fig. 1. Survival rate of E. coli K-12 λ upon irradiation with different doses of neutrons.

The composition of the beam through which the radiation passed was nonuniform. Besides neutrons possessing various energy reserves (mostly high speed), γ -rays composing about 10% of the absorbable dose were recorded.

It is important to note the presence of induced radioactivity which was recorded at the conclusion of irradiation. Measurements made with dosimeters showed that this is β -radiation with energies of 1 Mev (at a dose of 3000 rad) and 1.4 Mev (10,000 rad). It was established that the induced radioactivity quickly decreases in the course of time which indicates the formation of short-lived isotopes. At the end of 24 h the radioactivity had almost completely disappeared.

In an evaluation of the data obtained it is necessary to consider both the nonuniformity of beam composition and the induced radioactivity; however, the majority of the changes occurring in the bacterial culture can be explained by the primary action of neutrons.

The bacteria from the irradiation suspension were examined $1^1/_2$ h after irradiation.

The survival rate was determined by inoculating various dilutions of the experimental and control suspension on a meat peptone agar plate and subsequent calculation of the number of colonies which developed.

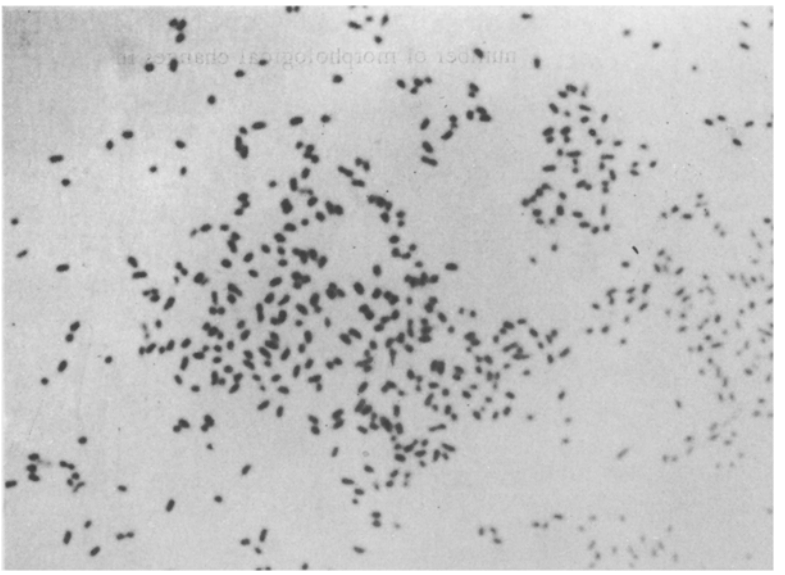

Fig. 2. Cellular morphology of a 24 h E. coli agar culture in physiological solution. (Ob. 90, oc. 7).

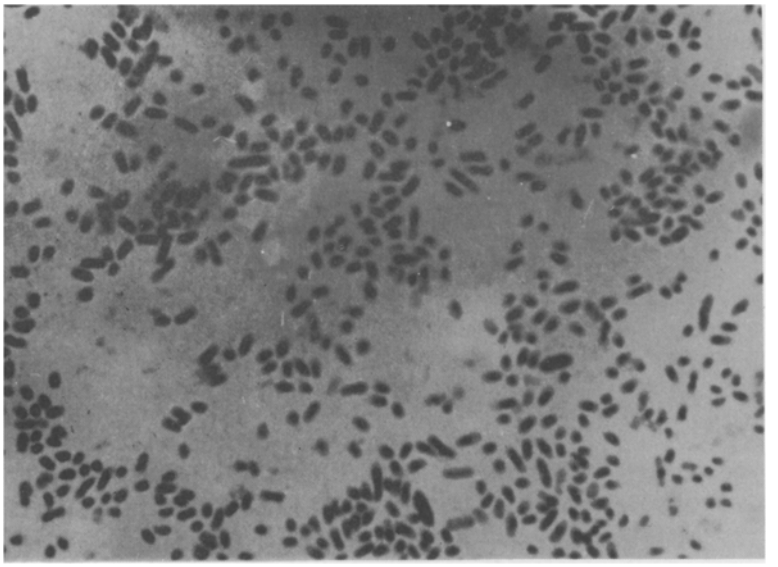

Fig. 3. The same culture $1^1/_2$ h after neutron irradiation (dose 40,000 rad). (Ob. 90, oc. 7).

EXPERIMENTAL RESULTS

The survival curve is presented in Fig. 1. As seen from Fig. 1, the LD_{50} is 2500-3000 rad, the survival rate decreases with increasing dose. However, even with a dose of 60,000 rad complete destruction of the bacteria does not occur ($1 \cdot 10^{-8}$ of the cells survive). Examination of the strains obtained from the cells surviving after irradiation with high doses (55,000 rad) showed that these bacteria possess radio-resistance of the same order as the parent strain.

Biological Effect of Nuclear Particles on E. coli K-12 λ

Dose (in rad)	No. of expts.	Survival (in %)	Mutational frequency																					
			bacterial suspensions after irradiation			In reinoculations (transfers to agar slant)																		
						1-й			2-й			3-й			4-й			5-й					
			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3			
3 000	3	45	28 793	0	0																			
6 000	3	17	48 710	0	0																			
10 000	2	10	39 646	1	$2 \cdot 10^{-5}$	14 368	1	$6 \cdot 10^{-5}$	16 294	2	$1 \cdot 10^{-4}$	23 781	0	0										
20 000	3	1	10 843	1	$9 \cdot 10^{-5}$	19 553	5	$2 \cdot 10^{-4}$	22 079	60	$2 \cdot 10^{-3}$	18 221	9	$4 \cdot 10^{-4}$										
30 000	1	0,0076	5 568	0	0	3 292	0	0	3 893	0	0	1 509	0	0										
															12 267	3	$2 \cdot 10^{-4}$	8 491	2	2 1				
															20 037	4	$2 \cdot 10^{-4}$							

Symbols: 1) number of examined colonies; 2) number of mutants; 3) mutational frequency.

From a study of Gram and Romanovsk stained preparations of E. coli prepared from the control suspension and from the suspension irradiated with various doses (the preparations were made 1-1½ h after irradiation) it was established that neutron irradiation causes a number of morphological changes in the cells.

The strain K-12 λ, was characterized by considerable polymorphism. Most of the bacteria were small straight Gram-negative rods, uniformly stained over the whole length (Fig. 2).

Immediately after irradiation (10,000 and 40,000 rad) a sharp increase in polymorphism was observed. Coccus-shaped and elongated forms appeared in large number, curved and swollen cells were found. A large part of the bacteria stained nonuniformly, a concentration of chromatin material was observed in the center or at the end of the cell (Fig. 3).

Thus, in the first hours after irradiation with the tested doses of neutrons the cellular morphology changes strongly and the degree of the change increases with an increasing dose.

E. coli K-12 λ is a quite stable prototrophe. According to our observations, in conformity with data in the literature [1] the frequency of spontaneous biochemical mutations is 1 in 10^5 cells. We recorded the mutations affecting the synthesis of amino acids.

Isolation of the mutants was done by the delayed enrichment method [2, 6]. It is known that for the isolation of mutants conditions in which the irradiated cells are able to multiply are necessary [3, 4, 5, 8]; therefore, we carried out 2 variations of the experiments.

In the first, irradiated cells were inoculated in meat peptone broth. After different intervals of time from the moment of inoculation—4, 5, 6, 8, and 24 h—inoculations were made from the broth into a minimal medium by the delayed enrichment method. Suspensions of bacteria irradiated with doses of 3000, 10,000 and 20,000 rad were studied. With intensive multiplication of the irradiated cells in the broth the regular increase in the mutation frequency was not found. Mutants were isolated irregularly, with a frequency varying within the limits of $1 \cdot 10^{-5}$ to $1 \cdot 10^{-3}$ in various periods after inoculation from broth.

In the 2nd variation of the experiments the irradiated culture was transferred to a meat peptone agar slant and from each transfer an inoculation was made into a minimal medium. To calculate the zero mutants inoculation of the initial irradiated suspension was carried out.

The results of this variant of the experiments are presented in the table. With irradiation doses of 3000, 6000, and 30,000 rad zero mutants were not obtained, with doses of 10,000 and 20,000 rad they appeared with a frequency somewhat greater than the frequency of spontaneous mutations. From the table it is seen also that upon transfer to a complete medium of cells irradiated with doses of 10,000 20,000 rad the mutational frequency increased, attaining a considerable increase in the 2nd transfer of the cells.

It is necessary to mention that the overall level of mutational frequency at an irradiation dose of 20,000 rad (survival 1%) is higher

than with a dose of 10,000 rad (survival 10%); however, a further increase in the dose to 30,000 rad did not lead to an increase in the frequency of the formation of mutants. We made an analysis of the biological properties of the auxotrophic variants obtained in a number of experiments. We considered as mutants those colonies which in the course of 4-5 transfers from the moment of isolation were not able to grow on a minimal medium and did not revert to the parent type or made a reversion with a lower frequency (no more than $1 \cdot 10^{-5}$).

The mutants were similar to the parent strain in many properties: they fermented glucose, lactose and mannite with the formation of acid and gas, produced indole and hydrogen sulfide, agglutinated serum against strain K-12 λ at the same or somewhat lower dilutions (maximal 1 : 10,240) and had the same sensitivity to streptomycin (5 units/ml) as the parent strain.

The majority of the mutants stably maintained the property of auxotrophism. Of 61 of the variants studied only 8 regressed completely to the metabolism of the parent strain, while 1 of them regressed after 1 month, 5 after 3, and 2 after 4 months after isolation.

An identification of 44 mutants was made according to the amino acid requirement. It was established that the majority of them (27) lost the ability to synthetize histidine. The remaining mutants needed for growth in the minimal medium the following amino acids: 6—methionine, 2—proline, 2—threonine, 2—cystine, 2—isoleucine and valine. With the addition of cerine and glycine one mutant grew in a medium containing serine, glycine or threonine, —1.

SUMMARY

A study was made of the influence of neutrons on the biological properties of E. coli K-12. The survival of bacteria irradiated with different doses of neutrons was investigated. A LD_{50} equal to 2,50—3,000 rad was established.

The morphological changes in the cells were studied immediately after irradiation. There was discovered a sharp growth in the number of coccus-shaped elongated cells, and the appearance of curved, swollen, irregularly staining cells (doses—10 and 40 thousand rad). Irradiation of E. coli with neutrons (10,000 and 20,000 rad) leads to the formation of biochemical mutants which can be isolated in passing irradiated cells in a fully adequate medium.

The mutants obtained differed from the initial strain only by disordered synthesis of some amino acid (commonly histidine) and stably retained the newly-acquired property.

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