MUTAGENIC ACTION OF THE LUMINOUS FLUX OF IONIZED PLASMA

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Successful attempts have been made in recent years to use the energy of ionized plasma in medicine. Encouraging results have been obtained with tissue cutting by a plasma jet, coagulation of blood vessels, removal of affected organs, excision of necrotic masses, and so on [2-4]. However, many problems concerned with the biological action of plasma radiation on cells, on tissue substrates, and on the body as a whole, remain unclear. One of the most urgent problems is elucidation of the effect of radiant energy on the genetic apparatus, for short-wave radiation (20-30 nm), characteristic of the spectrum of ionized helium, which is used in plasmotrons for medical application, may possess some degree of penetrating power and may give rise to a mutagenic effect. In the last case, serious limitations would be imposed on the therapeutic use of this method.

The aim of this investigation was to study the effect of the luminous flux of ionized plasma on the genetic apparatus of the cell.

EXPERIMENTAL METHOD

Experiments were carried out on two objects, an auxotrophic strain of Escherichia coli and somatic cells of the fruit fly Drosophila. The bacteria were grown in nutrient broth at 37°C. The bacterial suspension in a volume of 3 ml was poured into Petri dishes 40 mm in diameter in a concentration of $1 \cdot 10^8$-$3 \cdot 10^8$ microbial cells in 1 ml, and irradiated with a lasma jet for 3, 5, and 10 min, with continuous shaking, and at a distance of 10 cm, on an SUPR-3M helium apparatus with a current of 20 A and arc voltage of 30 V. To abolish the heating effect, a vacuum pump was used. The concentration of viable cells and the frequency of back mutations (FBM) to the phototrophic state for amino acids (proline or methionine) synthesis, were determined in irradiated and unirradiated suspensions [1]. To increase the reliability of the investigation and to test the effect of the plasma flux on the genetic apparatus of a more highly organized object, a parallel study was carried out on a eukaryotic organism, the fruit fly Drosophila. The short-term but sufficiently informative method of somatic mosaicism, which enables genetic events such as gene mutations, deletions, and loss of chromosomes to be taken into account, was used [5, 6]. The parents were males and females of two different genotypes, so that somatic mosaicism could be detected for alleles of the White gene [6]. Heterozygous offspring of the first generation, and 1.5-day-old larvae were treated with the plasma jet under conditions analogous to those of the experiments with the bacterial suspension. Experimental and control individuals at the age of 3-5 days after complete formation of the eye pigment were examined with a binocular loupe (15 × 2). Individuals with a mosaic pattern of distribution of color of their eye cells, and showing double and single spots, were counted. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The microbiological studies showed that irradiation of bacterial suspensions for 10 min reduced their survival rate by an order of magnitude. On phase-contrast microscopy, the microbial population was seen to contain a very small number (up to 5-7%) of threadlike cells,

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TABLE 1. Effect of Luminous Flux of Ionized Plasma on Genetic Apparatus in Ommatidial Cells of Eyes of Female Drosophila

Experimental conditions	Total number of eyes studied	Somatic mosaicism				p
		single spots		double spots		
		absolute	%±m	absolute	%±m	
Without irradiation	1676	5	0,30±0,14	6	0,36±0,14	<0,05
Irradiation, min						
3	892	3	0,34±0,20	5	0,56±0,24	<0,05
5	976	4	0,41±0,20	8	0,82±0,29	<0,05
10	1080	5	0,46±0,20	8	0,74±0,26	<0,05

TABLE 2. Effect of Luminous Flux of Ionized Plasma on Gene Mutations in Ommatidial Cells of Eyes of Female Drosophila

Experimental conditions	Total number of eyes studied	Number of mosaic spots	%±m	p
Without irradiation	1945	10	0,51±0,16	<0,05
Irradiation, min				
3	896	4	0,45±0,22	<0,05
5	1140	4	0,35±0,17	<0,05
10	994	5	0,50±0,22	<0,05

with the formation of microcolonies of actively dividing cells. FBM for methionine in the unirradiated bacterial suspension was not more than $2 \cdot 10^{-7}$, whereas for proline it was $1.3 \cdot 10^{-8}$ ($p < 0.05$). After irradiation FBM for methionine was virtually identical with the control values ($3.4 \cdot 10^{-7}$ for an exposure of 3 min, $3 \cdot 10^{-7}$ for 5 min, and $3.2 \cdot 10^{-7}$ for 10 min; $p < 0.05$). FBM for proline with an exposure of 3 min to the plasma was $1.6 \cdot 10^{-8}$, for 5 min $5 \cdot 10^{-8}$, and for 10 min $7 \cdot 10^{-8}$ ($p < 0.05$).

Thus irradiation of a suspension of an auxotrphic strain of Escherichia coli by ionized plasma reduces the density of the bacterial population by one order of magnitude. The frequency of back mutations for proline and methionine, induced by irradiation, does not differ significantly from the spontaneous level with respect to the same feature in the control.

Table 1 gives data reflecting the effect of luminous flux of ionized plasma on recombination and other genetic events in somatic cells of the eye of female Drosophila. Although exposure for 5 min gives rise to recombination (0.82% compared with 0.36% in the control group), there was no increase in the percentage of single spots in this case, which can be taken on the whole as evidence of the absence of genetic after-effects of irradiation. In all the remaining cases, no statistically significant changes were found in the parameters studied.

The level of gene mutations in somatic cells of Drosophila males remained virtually unchanged, whatever the duration of irradiation (Table 2). The action of plasma does not effect the survival rate of the imago, with the three exposures studied.

The investigations thus showed that under strict experimental conditions, approximating to conditions of clinical use of plasma energy for therapeutic purposes, a mutagenic effect was virtually absence, at least on the two experimental objects used. However, the sphere of application of the method, as also the working conditions of the plasmotron, its technical specification, and also the method of exposure to it, are already sufficiently varied and are continually being widened. Further research into all aspects of the biological action of ionized plasma on the living organism is required.

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