STABILITY OF L-FORMS OF BACTERIA TO ULTRAVIOLET LIGHT

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The few studies made by a number of authors [4, 5, 7, 8] of the stability of individual species of L-form bacteria to the action of individual physical factors do not permit making theoretical generalizations in regard to the stability of all L-form bacteria. In our previous report [3] an attempt was made to study the comparative stability of the stabilized L-forms of some bacterial species—Str. haemolyticus, Pr. vulgaris, S. typhosa, S. typhimurium, the parent bacteria and forms reverting from the effect of temperature factors.

The goal of the present work was a study and comparison of the stability of forms of microorganisms enumerated above to the effect of ultraviolet light of the short-wave (bacteriocidal) spectrum. The expediency of such a study is determined by the almost complete absence of analogous experiments [4] available to us in the literature.

EXPERIMENTAL METHOD

Five strains of stabilized L-forms, 4 strains of cultures which reverted from the L-form and 5 strains of parent bacterial cultures were the objects of our investigation. L-cultures included 2 strains of the L-forms of α- and β-hemolytic streptococci (L 409 and L 196), one strain of Pr. vulgaris (LP), 1 strain of S. typhosa (L 152) and 1 strain of S. typhimurium (L 5710). Among the reverted cultures were strains of streptococci (R 190, R 196, R 409) and Pr. vulgaris (RP). The parent cultures included strains of hemolytic streptococci (No. 4 and 10-S), Pr. vulgaris (No. 3156), S. typhimurium (No. 5710) and S. typhosa (No. 5606). Methods of obtaining the strains used in the experiments and a brief description of them were given by us earlier [3]. Ten day broth cultures of the L-forms and 24 h bacterial cultures (parent bacteria and revertants) were centrifuged for 15 min at 3000 rpm. The supernatant fluid was drawn off. The packed sediment was homogenized with the addition of physiological solution. The bacterial suspensions obtained from the sediment were brought to a concentration of 0.5 billion by the optical bacterial standard of turbidity.

A BUV-15 lamp (with spectrum maximum $\lambda = 2537$ Å) was used as the source of UV radiation. The prepared bacterial suspensions were irradiated in an open Petri dish from a distance of 10 cm. The thickness of the irradiated layer of the suspension was 1-3 mm, and the radiation intensity 20 erg/mm^2/sec. Irradiation was carried out in a dark box. The duration of irradiation (mainly 1-30 min) guaranteed a lethal and sublethal effect for most of the microorganisms in the irradiated suspensions. After irradiation 0.5 ml of the bacterial suspension was placed with a spatula on agar media in Petri dishes and 0.2 ml of the irradiated L-form suspensions inoculated on semisolid (1.3% agar) or semiliquid (0.3% agar) 10% serum media with and without penicillin. At the same time, the same volumes and concentrations of nonirradiated suspensions were inoculated on appropriate media. The results of the inoculation of the bacterial cultures were calculated after 24 h and of the L-form cultures after 7 days of incubation at 37°. After this the cultures were kept for an additional 3 days in diffuse daylight at room temperature for the development, possible under these conditions, of reactivated microorganisms [1]. Each series of experiments was done twice and in counting the colonies which developed the results of both series were considered.

EXPERIMENTAL RESULTS

The stability of the L-forms, as seen from the table, depended on the species to which the culture belonged. In comparison with the parent bacteria the L-forms of proteus (LP) exposed for 30 sec were less stable to UV irradiation,

Comparative Stability of L-forms, Parent Bacteria and Revertant Forms to Short-Wave UV Irradiation

Bacterial species	Nature of culture	Strain	No. of colonies which grew from nonirradiated suspension in control	No. of colonies which grew from suspensions irradiated for						
				30 sec	1 min	3 min	5 min	10 min	15 min	20 min	30 min
Str. haemolyt. β	I	10-S	∞		2	—	—	—	—	—	—
	L	L 196	98		2	—	—	—	—	—	—
	R	R 196	∞		754	80	56	15	0—1	2	—
Str. haemolyt. β	I	4	∞		109	120	100	1	—	—	—
	L		Stability of strain not determined								
	R	R 190	∞		1836	20	3	1	1	—	—
Str. haemolyt. α	I		Stability of strain not determined								
	L	L 409	74		2	—	—	—	—	—	—
	R	R 409	∞		10	4	4	0,1	2	1	2
Pr. vulgaris	I	3156	∞		∞	7	2	—	—	—	—
	L	LP	∞	3	—	—	—	—	—	—	—
	R	RP	∞		156	22	1	5	5	2	—
S. typhosa	I	5606	∞		55	9	1	—	—	—	—
	L	L 152	10		15	17	25	8	1	1	1
	R		Stability of strain not determined								
S. typhi murium	I	5710	∞		175	1	4	2	—	—	—
	L	L 5710	50		3	3	2	5	1	—	—
	R		Stability of strain not determined								

Note. I—parent culture; L—L-culture; R—revertant (from L-form) culture.

the L-forms of streptococcus L 196 (1 min) had as much stability and the L-forms of the salmonella L 152 and L5710 (15-30 min) were more stable.

In the L-forms of intestinal salmonella (strain L 152) under the effect of UV irradiation and with moderate exposure (1-5 min) there was observed a significant increase in L-colonies in comparison with L-growth obtained from nonirradiated suspensions in the control inoculation.

The unusual L-form growth "stimulating" effect of moderate doses of UV light, possibly, is linked with its ability to cause denaturation which stops the incipient photolytic decomposition and the escape of nucleic acids and protein from the bacterial cells and "fixes," in this way, the irradiated cellular structures [2]. A similar effect may easily dilute the approaching breakdown of L-elements suspended in an isotonic solution of NaCl and create in irradiated suspensions a relatively greater, than in the controls, number of viable, at the moment of inoculation, elements which then form L-colonies.

Reverted cultures always showed more stability to UV light than the parent bacteria. The growth of the revertants and the L-forms obtained from prolonged irradiation of bacterial suspensions (exposure of 15-30 min) was characterized by the formation of chiefly single colonies.

L-colonies grown from irradiated suspensions did not differ from L-colonies of the control either macro- or microscopically. Colonies grown from irradiated bacterial suspensions (parent and revertant) differed considerably from those of the control. The growth of irradiated cultures was more delayed. Colonies of streptococci (strains No. 4 and 10-S) grown from irradiated suspensions usually were of larger size (up to 1.5 mm) and in phase contrast microscopy consisted mainly of twin, elongated in the form of a candle flame cocci, similar to diplococci. Short and especially long chains of cocci were found in these colonies in considerably fewer number than in the control.

Upon inoculation of irradiated suspensions of proteus strain No. 3156, which under normal cultural conditions gave N-growth, after 18 h of incubation we observed the development of isolated colonies edged only with a thin (1-2 mm) zone of noticeable "creeping" growth. With phase contrast microscopy these colonies consisted of nonmotile or slightly motile rods. Two days after the cultures were kept in diffuse daylight at room temperature around their colonies arose a wide zone of typical "creeping" growth, covering all of the agar.

The proteus revertant (RP) being more stable to UV light, after 16 h of incubation of the inoculated suspension irradiated for 1-20 min at 37° produced uniform N-growth on the plates. At the original inoculation sites, however, isolated colonies appeared, clearly outlined against the background of the more retarded uniform layer of the culture and quite easily counted.

Streptococci revertant suspensions (R 190, R 196, R 409) after irradiation for 1-10 min produced slowly forming colonies, whose number increased with more prolonged (over 24 h) incubation. After prolonged irradiation (15-30 min) the number of colonies which formed initially after 24 h incubation upon subsequent longer incubation did not increase.

An analysis of the results obtained shows that in spite of the considerable variation depending on the species and strain of the individual L-cultures, their stability to UV radiation hardly differed from the stability of the parent bacteria which may be explained by the fact that in both cases the primary structural object of the action is one and the same substrate—DNA, significant damage to which leads to an impairment of the microorganisms multiplication and their death. The greater (than in the parent bacteria) stability to irradiation of some species of L-forms (S. typhosa and S. typhimurium) and revertants, possibly, is caused by the known similarity of action of ultraviolet light and penicillin [6], since the L-forms and the forms reverted from them show a particular stability to factors possessing L-transforming properties.

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