

LOSS OF PATHOGENICITY BY RADIORESISTANT STAPHYLOCOCCI

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By repeated irradiation of pathogenic strains of staphylococci with Co^{60} γ rays, radioresistant variants of them were obtained. The radioresistant strains were found to have lost their features of staphylococcal pathogenicity, including production of plasmocoagulase, deoxyribonuclease, and α - and Δ -hemolysins, their dermonecrotic and lethal activity, and titratability with bacteriophages; no changes were found in the nucleotide composition of the DNA of the radioresistant variants compared with the original strains.

Under the influence of ionizing radiation a number of the features of microorganisms can be considerably altered [7, 13, 14]. Repeated γ -ray irradiation changed the radiosensitivity, biochemical and cultural properties, ultrafine structure, and enzyme activity of staphylococci [1, 2, 6, 7]. The object of the present investigation was to study the effect of γ -ray irradiation on the pathogenicity of staphylococci.

EXPERIMENTAL METHOD

Two radioresistant variants obtained from pathogenic strains of *Staphylococcus aureus* — strain no. 73 (isolated from a patient with acute laryngitis) and strain V-445 (obtained from a patient with mastitis) — were studied. The radioresistant strains were obtained by Z. G. Pershina by repeated irradiation of the strains with Co^{60} γ rays by the method described previously [7]. The spectrum of hemolysins produced by the organisms was determined by the method of Elek and Levy [8] with neutralization by specific antitoxic serum. The titers of γ - and Δ -toxins, the minimal dermonecrotic dose in rabbits, and the phage-type of the strains using phage in a dose of 100 TP, the deoxyribonuclease activity, plasma coagulation, proteolytic properties, radioresistance, and antibiotic-resistance of the staphylococci, were determined by the usual methods [3, 4, 7], and the nucleotide composition of the DNA in cells of "wild" type and of the radioresistant variants was determined by paper chromatography and from the melting temperature [5].

EXPERIMENTAL RESULTS

The pathogenic strains of staphylococci, after repeated Δ -ray irradiation in subbactericidal doses, became much more resistant to the action of radiation. For instance, whereas LD_{90} for the original staphylococcal strains no. 73 and V-445 was 27.0 and 17.5 krad respectively, for the radioresistant strains it was 80 and 68 krad respectively. Meanwhile, the base ratio of the DNA $(G + C)/(A + T)$ of staphylococci with increased radioresistance was identical with that of the original bacteria (0.51 for strain no. 73 and 0.47 for strain V-445).

Hemolysin production by staphylococci is one of the principal criteria of the pathogenicity of the strains [9, 10]. The toxins of staphylococci (α and Δ), being extracellular proteins, possess hemolytic, lethal, cytopathogenic, and dermonecrotic properties and may play an important role in the pathogenesis of staphylococcal infections.

Differences between the hemolysin production of the original strains of staphylococci and the radioresistant variants must be emphasized: strain no. 73 formed α -toxins and strain V-445 formed α - and

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TABLE 1. Signs of Pathogenicity of Original and Radioresistant Strains of Staphylococci

Strain of staphylococcus	Spectrum of hemolysins produced, after Elek and Levy [8]	Minimal hemolytic dose of toxins		Phage-type (dose of bacteriophages 100 TP)	Minimal dermonecrotic dose 10^6 of bacterial cells)	Coagulase production	Deoxyribonuclease production
		α	Δ				
Strain no. 73; original	α -Toxin	1:20	0	Not typed	200	+	+
radioresistant	ε -Toxin	0	0	The same	No necrosis in a dose of 400 million	—	—
Strain V-445; original	α -Toxin	1:160	1:40	52, 52A, 80, 81	200	+	+
radioresistant	ε -Toxin	0	0	Not typed	No necrosis in a dose of 400 million	—	—

Δ -toxins, whereas the radioresistant strains had completely lost their ability to form these hemolysins. The writers found that radioresistant variants formed a hemolysin which differed from the α and Δ types and which produced lysis (incomplete) of human group I (0) red cells. By the morphological picture of hemolysis on blood agar this hemolysin was similar to that described by Elek and Levy [8] as ε -toxin, produce by nonpathogenic staphylococci.

The results showing the hemolytic titers of the toxins, dermonecrotic activity, phage-type, and coagulase and deoxyribonuclease production of the original and radioresistant strains are given in Table 1.

As Table 1 shows, the radioresistant strains of staphylococci lost their ability to form α - and Δ -toxins both on solid and on liquid medium; they did not cause necrosis of the rabbit's skin after intradermal injection of $1, 2$, or 4×10^8 bacterial cells. Intradermal injection of 2×10^8 bacteria cells of the original strains of staphylococci led to the appearance of dermonecrotic reaction with the characteristic picture of inflammation.

The original strain V-445, which belongs to phage group 1, was typed by four phages; the radioresistant variant was no longer typed by phages in a dose of 100 TP. Radioresistant strains did not possess those essential features of staphylococcal pathogenicity such as production of plasmocoagulase and deoxyribonuclease, and in addition they had lost their lethal properties, as experiments on mice showed. Intraperitoneal injection of the original strain of staphylococcus V-445 in a dose of 2×10^9 bacterial cells caused death of albino mice weighing 18–20 g after 48 h ($LD_{50} 1.4 \times 10^9$), whereas injection of a culture of the radioresistant variant in a dose of 2×10^{10} (intraperitoneally) did not cause death of the mice. The radioresistant strains were much more sensitive than the original to the action of antibiotics. For instance, in their sensitivity to penicillin they differed by 2.5×10^4 times, and to streptomycin by 8 times. Sensitivity to aurantin was unchanged.

A study of the proteolytic activity showed that the radioresistant strain could not liquefy gelatin, whereas a culture of the original strain V-445 liquefied gelatin on the 2nd–3rd day after seeding. Strain Staphylococcus aureus no. 73 had no proteolytic activity either in its original state or as the radioresistant variant; after irradiation three or four times ability to liquefy gelatin appeared, but was again lost after the eighth irradiation of the strain in a dose of 200 krad.

The radioresistant strains of staphylococci obtained by repeated γ -ray irradiation thus differed from the original strains in a number of properties; they had lost their features of pathogenicity, although the nucleotide composition of their DNA remained unchanged.

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