REVERSION OF STABILIZED STRAINS OF L-FORMS OF PATHOGENIC STAPHYLOCOCCI

COMMUNICATION II. THE BIOLOGICAL PROPERTIES OF REVERTED STRAINS

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As a result of reversion experiments described in the previous communication, we obtained 3 strains of staphylococci which reverted after a prolonged existence in the L-form. In the literature there are only isolated papers devoted to the study of the biological properties of strains undergoing reversion from the L-forms of bacteria [2-4].

Accordingly it was desirable to make a comparative study of the properties of the original strains of pathogenic staphylococci and of strains undergoing reversion after prolonged subculture in the L-form on media with high concentrations of penicillin (from 1000 to 10,000 units/ml). We studied the morphology, fermentation properties, resistance to the action of antibiotics, pathogenic properties and antigenic structure.

EXPERIMENTAL METHOD

The investigations were based on the generally accepted methods of study of the biological properties of the pathogenic staphylococci [1].

Immediately after reversion all the strains obtained from the L-forms of staphylococci were distinguished by their well marked polymorphism. On microscopic examination, very small, middle sized and grossly enlarged (often to the size of small spheres, 2-3 μ in diameter) staphylococci could be seen. When stained by Gram's method the majority of the cocci gave a positive stain, but from time to time Gram-negative specimens were also found. After a few subcultures on simple nutrient media, the reverted strains lost their polymorphism, stained only Gram-positive, and were in every way indistinguishable from the original strains.

The original strains of staphylococci decomposed the 5 sugars which we tested (lactose, glucose, mannitol, maltose and saccharose) with formation of acid after 24 hours. The strains reverted from L-forms also decomposed all the sugars after 24 hours, with the exception of mannitol. Strain No. 75R decomposed mannitol after 48 hours, and strain No. 5R — after 72 hours. Strain "Lossmanov R" behaved in precisely the same way in relation to mannitol, but it was distinguished from the others by the fact that it decomposed lactose not after 24 but after 48 hours.

A trial of the ability of the reverted strains to coagulate plasma showed that strain No. 5R had almost completely recovered the plasma-coagulating activity of the original strain, but strains No. 75R and "Lossmanov R" showed the intensity of this reaction to be greatly weakened in time. The hyaluronidase activity of strains No. 5R and "Lossmanov R" was reduced to one hundredth that of the original strains, and strain No. 75R to one fiftieth.

Comparative Analysis of the Serological Properties of the Original Strain of Staphylococcus and of Strains Reverted from the L-Form

Antigen	Dilution of Serum								Control	
	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1280	1 : 2560	1 : 5120	of strain	of serum
Antiserum to original strain No. 75												
№ 75 original	++++	++++	++++	++++	++++	++++	+++	++	++	±	—	—
№ 75 reverted	++++	++++	++++	+++	+++	++	++	—	—	—	—	—
Antiserum to L-form of strain No. 75												
№ 75 original	++	++++	++++	++++	++++	++++	++	++	—	—	—	—
№ 75 reverted	++	++	++	++	++	++	—	—	—	—	—	—
Antiserum to reverted strain No. 75R												
№ 75 original	++++	++++	++++	++++	++++	+++	+++	±	±	—	—	—
№ 75 reverted	++++	++++	++++	++++	+++	+++	++	++	—	—	—	—

Note: The degree of intensity of the agglutination reaction was recorded in + signs, from intensive (++++) to doubtful (±). Absence of agglutination was designated as —.

Of the tests of the pathogenicity of staphylococci, besides the ability to coagulate plasma, those of particularly great importance are the presence of hemolytic and dermonecrotic properties. In the strains reverted from L-forms, the hemolytic activity was either slightly weaker than that of the original strains or it was identical with it. The necrotic properties had recovered to the greatest extent in strain No. 5R. Zones of necrosis were observed in the same dilutions as with the original strains, although they were smaller in size. Strain No. 75R in high concentrations caused only insignificant pin-point necroses, and strain "Lossmanov R" in general produced no necrotic lesions at all, whereas in both original strains the dermonecrotic power was very pronounced.

The results of the determination of the virulence of the reverted strains by intraperitoneal inoculation of white mice also indicated the comparatively slight reduction in virulence of strain No. 5R, the considerable loss of virulence in strain No. 75R and the almost complete disappearance of virulence in strain "Lossmanov R".

The results, which have just been described, of the study of the biological properties of strains reverting from stabilized L-forms of the pathogenic staphylococci, like those given in our first communication, again testify to the great importance of the individual properties of the original strain, which determine the depth of the process of stabilization of the strain in the L-form. Staphylococci reverted from strains Nos. 5L, 75L and "Lossmanov L". As a first stage, all three strains were subcultured under identical conditions 6-7 times on media containing penicillin. At the same time, strain No. 5R was much closer in its biological properties to the original strain than No. 75R. The still greater loss of its properties by strain "Lossmanov R" may be explained by the fact that the process of stabilization of this strain in the L-form had gone much deeper than in the two preceding strains.

The comparative study of the penicillin resistance of the original and reverted strains showed that, in spite of the prolonged subculture of the L-forms in extremely high concentrations of penicillin (1000-10,000 units/ml), the strains reverting from them fully retained the high sensitivity of the original strains to the action of this antibiotic. The sensitivity of the reverted strains to the action of other antibiotics (chlortetracycline and streptomycin) was also unchanged.

A comparative analysis of the antigenic properties of the original strains and of the strains reverted from the L-forms was undertaken with strain No. 75. Three tests were performed: with antiserum to the original strain No. 75 (orig.), antiserum to the L-form and antiserum to the reverted strain No. 75R.

Concurrently with the agglutination reactions described in the table, further control experiments were carried out, in which the same antigens were used for the agglutination test, but with normal rabbit serum (the reaction in all cases was negative), and the same sera, but with heterogeneous antigens of the original and reverted strains No. 5 and "Lossmanov" (the reaction was either negative or feebly positive in dilutions of 1:10-1:20).

The results given in the table may be generalized in the form of the following conclusion: in the process of reversion the antigenic and serological properties of the original strain are restored. The antigenic links with the L-form are preserved, presumably on account of common antigens with the original strain. Taking into consideration the character of the agglutination with the heterogeneous antigens it may also be considered that the existence of bacteria in the L-form does not affect their antigenic specificity.

SUMMARY

The biological properties of reversed cultures of stabilized pathogenic staphylococci of the initial and reversed cultures demonstrated that the more profound the process of stabilization in the L-form – the greater the difference in their biological peculiarities. Individual properties of the initial strains play an important role in the stabilization degree.

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