THE REVERSION OF STABILIZED STRAINS OF L-FORMS OF PATHOGENIC STAPHYLOCOCCI

COMMUNICATION I. SPECIAL FEATURES OF THE PROCESS OF REVERSION

S. V. Prozorovskii

From the Department of Microbiology (Head – Active Member AMN SSSR V. D. Timakov) of the Second Moscow State Medical Institute (Director – M. G. Sirotkina)

(Received September 6, 1958. Presented by Active Member Acad. Med. Sci. USSR, V. D. Timakov)

The L-forms of bacteria are reactive formations which grow in the form of characteristic L-colonies in agar, having a characteristic morphological structure consisting of spheres, vacuoles and tiny granules, and sharply distinguishable from the original strains by their biological properties. The L-forms are extremely highly resistant to the action of the factors which were responsible for their formation. The process of formation of L-forms has been carefully studied in several bacterial species [6, 4, 1 and others]. Less attention has been paid to their biological properties [8, 5, 7, 1 and others], and there are only individual papers devoted to the study of the processes of reversion of L-forms back to the original strains [3, 4, 2].

In this connection it appeared to be of interest to obtain L-forms of the pathogenic staphylococci by means of the action of penicillin, and to study the laws governing the processes of reversion of these formations and the biological properties of strains undergoing reversion.

EXPERIMENTAL METHOD

L-forms of pathogenic staphylococci were obtained on 0.3% agar, prepared in Hottinger's broth with the addition of 20% of normal horse serum, 5% sodium chloride and various concentrations of penicillin (from 1 to 10,000 units/ml). In the same medium, but with higher concentrations of penicillin (from 1000 to 10,000 units/ml) successive passage (6-7 subcultures) of the strains of L-forms thus obtained was carried out in order to stabilize them in this state. Stabilization of the strains was proved by control experiments which demonstrated complete absence of viable cocci among the L-form colonies.

In order to obtain reversion it was necessary to provide conditions which would exclude the action of penicillin as a factor causing the formation and stabilization of L-forms, and would, at the same time, be favorable for the development of both L-forms and for the growth of staphylococci. These conditions were satisfied by 0.3% agar with 20% serum and 5% sodium chloride. On to this medium were subcultured strains 75L, 5L, 8HL, Lossmanov-L (after 6-7 passages through a medium containing penicillin) and 75L (after 13 passages through a medium with penicillin). In order to procure reversion, passages of the strains of L-forms were made every 14 days, during which time the cultures were kept continually at a temperature of 37°. Before every serial passage a note was made of the character of growth, and phase-contrast microscopy of the colonies was carried out. At the same time as each subculture into serum-salt agar was made, a control inoculation was performed into Hottinger's broth to which was added 1% glucose and 5% serum. Growth of cocci in these tubes was shown only after reversion of the L-forms in the serum-salt agar. Passage through a penicillin-free medium was continued for 5 months, and altogether 10 passages were made.

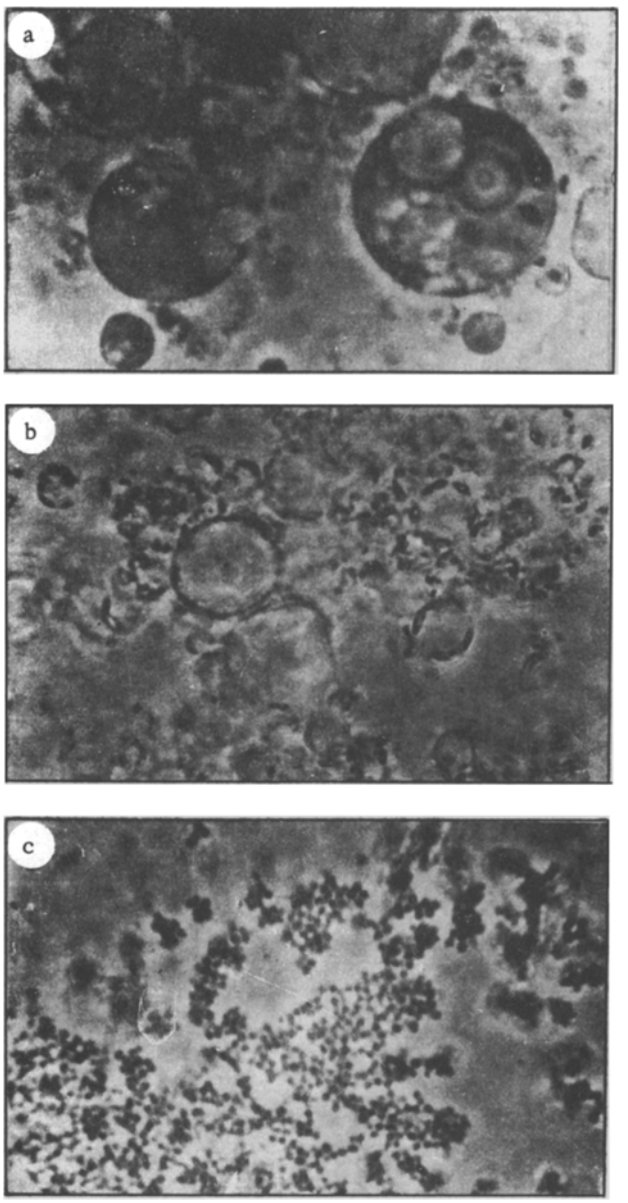

Fig. 1. Stages in the reversion of stabilized cultures of L-forms of pathogenic staphylococci. Phase-contrast microscopy. Objective 90×, ocular 15 ×.
a)Giant spheres with marked vacuolization. Separate granular forms; b) vacuoles with thin walls, inside which are inclusions of various sizes and shapes; c) pure culture of staphylococci, reverting from a stabilized culture of L-forms.

EXPERIMENTAL RESULTS

Of the five strains of L-forms of pathogenic staphylococci selected for the experiment, reversion was obtained in three – 75L (previously subcultured on 7 occasions into medium containing penicillin), 5L, and Lossmanov-L. The two remaining strains – 8HL and 75L (after 13 passages through medium with penicillin) retained their ability to develop as L-forms even after passage for 5 months in the absence of antibiotic.

Analysis of the results given in the table shows that the intensity of the reversion process depended, on the one hand, on how deeply stabilized was the strain of the L-form, and on the other hand, on the individual properties of the strain of staphylococcus used for production of the L-forms.

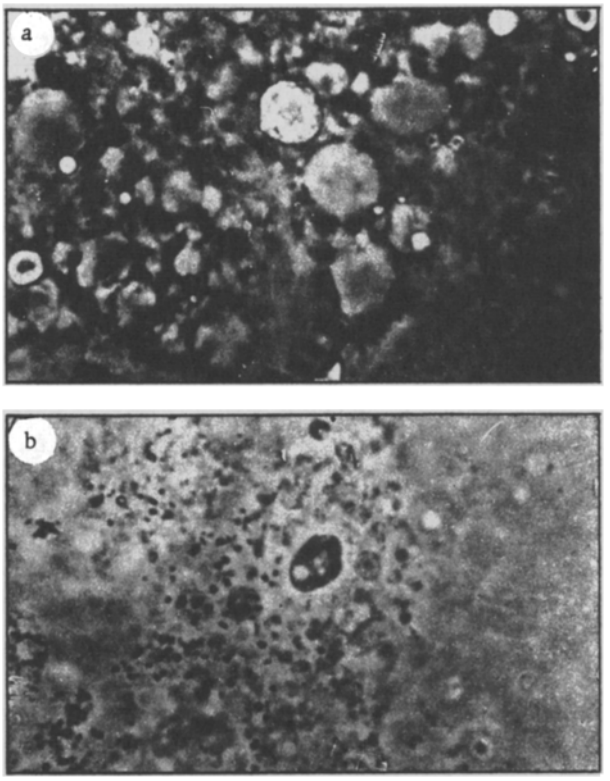

Fig. 2. Stages in the reversion of stabilized cultures of L-forms of pathogenic staphylococci.
a)Clear, light-refracting bodies among spheres, vacuoles and granular elements of L-forms. Magnification × 1350; b) area of large granules, approximately resembling staphylococci in their external appearance. Magnification × 1350.

In confirmation of the first statement was reversion of the cultures of strain 75L. Although obtained from the same strain, these cultures behaved differently in the conditions bringing about reversion. A culture of 75L, used in the experiment after 7 passages on agar containing penicillin, reverted at the 3rd subculture, whereas a culture of 75L, after 13 preliminary passages on a medium containing penicillin, showed no reversion to staphylococci after 10 subcultures in corresponding conditions.

The second statement follows from the course of reversion of cultures of strains Lossmanov-L and 8HL. Despite the fact that these strains, in contrast to 5L and 75L (7th passage) had been subcultured only six times on medium containing penicillin, they showed considerable resistance to reversion on medium without penicillin. Strain Lossmanov-L reverted at the 8th passage, but strain 8HL preserved all the properties of the L-forms up to and including the 10th passage.

The course of the morphological changes observed during passage of stabilized cultures of L-forms on a medium not containing penicillin, and during reversion of L-forms into the original strain, showed highly distinctive features in the individual strains. Depending on the character of these changes, all the strains used in the reversion experiment could be divided into three groups.

Reversion of Stabilized Cultures of L-Forms of Pathogenic Staphylococci during Passage on Serum-Salt Agar without Penicillin

Strains selected for the experiment: L-forms		Time of passage									
		1-month		2-months		3-months		4-months		5-months	
Name of strain	Number of preliminary passages on medium containing penicillin	1st passage	2nd passage	3rd passage	4th passage	5th passage	6th passage	7th passage	8th passage	9th passage	10th passage
75 L	7	L-form	L-form	reversion							
5 L	7	L-form	L-form	reversion							
Lossmanov L	6	L-form	L-form	L-form	L-form	L-form	L-form	L-form	reversion		
8HL	6	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form
75 L	13	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form	L-form

The first group included strains 75L (7th passage) and 5L. In the first days of development on a medium without penicillin, these cultures grew with the appearance of typical L-forms, but marked vacuolization began to show itself as giant forms, up to 25-30 μ in diameter and with the development of granularity (Fig. 1, a). At the end of the second passage, the colonies consisted entirely of large (from 5 to 15 μ) vacuoles with thin walls, inside which were round and elongated inclusions, varying in thickness (Fig. 1, b). At the beginning of the 3rd passage the number of inclusions as described above, and of small granular formations increased. Such a picture immediately preceded the discovery under the microscope of proliferating cocci, a pure culture of which is shown in Fig. 1, c, after isolation on an agar plate.

The second group consisted of strain Lossmanov-L. During the first passages on medium without penicillin, this strain did not differ in its morphology from typical cultures of L-forms, but at the 3rd passage there appeared numerous clear, light-refracting bodies, the number of which increased sharply in the 5th, 6th and 7th passage (Fig. 2, a). At this time there appeared large areas of coarser granules, resembling cocci in their outward appearance (Fig. 2, b). A similar picture immediately preceded the appearance of coccal forms.

The third group included strains 8HL and 75L (13th passage). Throughout all 10 passages these strains grew readily on serum-salt agar with the appearance of typical L-forms. Only at the 9th-10th passage of strain 75L (13th passage) was the appearance of numerous vacuoles observed, with thin walls and inclusions of various sizes and shapes.

Stabilized cultures of L-forms of pathogenic staphylococci thus possessed the ability to undergo subculture for $1\frac{1}{2}$ to 5 months on serum-salt agar without penicillin as L-forms. The rate of reversion depended on the duration of preliminary passage of the culture of L-forms in the presence of penicillin and on the individual properties of the original strain. Significant differences were observed in the morphology of the various stages of reversion of the individual L-form cultures.

SUMMARY

The author studied the process of reversion of the stabilized cultures of the L-forms of pathogenic staphylococci under the effect of penicillin. The passage of the L-form stabilized cultures on the serum-salt agar without penicillin was possible for from $1\frac{1}{2}$ to 5 months. The rate of the reversion process depended on the duration of the passage of the L-form in the presence of penicillin, and on the individual properties of the initial strain. Significant variations were observed in the morphology of individual stages of reversion of different L-form cultures.

LITERATURE CITED

[1] V. S. Levashov, Antibiotiki, 2, 12-18 (1957).

[2] V. D. Timakov, G. Ya. Kagan, V. S. Levashov et al., Variation among Microorganisms, 2, 353-362, (1957). [In Russian].

[3] L. J. Dienes, Bact. 57, 529-546 (1949).

[4] L. Dienes, Bact., 66, 274-278 (1953).

[5] R. Minck and A. Minck, Compt. r. Soc. Biol.,145, 927-929 (1951).

[6] R. Tulasne, Rev. immunol, 15, 223-224 (1951).

[7] R. Tulasne and J. Lavillaureix, Compt. rend. Soc. Biol., 48, 2080-2082 (1954).

[8] H. Weinberger, S. Madoff and L. Dienes, J. Bact., 59, 765-775 (1950).