“L” FORMS OF *STAPHYLOCOCCUS*

II. STUDIES ON THE MORPHOLOGY OF THE TRANSFORMATION AND ON THE REVERSIBILITY

by

J. K. SCHÖNFELD

with the technical assistance of Mrs. H. A. de Haas-Poppinga, Miss J. van der Veere, Miss J. C. Verhulst and Miss C. H. Blotkamp

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In the course of our previous studies on the action of penicillin on staphylococci (1956, 1957) the suggestion was made that a structural change may enable the staphylococci to survive. This supposed change was speculatively associated with the “L” phase. In a later publication (1959) the transformation of the staphylococci into the “L” phase and the reversibility of this phenomenon were described. As “L” colonies were never found on plain agar media containing penicillin, the question was raised whether staphylococci can survive on these media in an intermediary stage between the bacterial and the “L” phase. In order to examine such stages the present investigation was undertaken.

**Methods and materials.**

Pieces of medium inoculated with staphylococci were cut out and mounted between two coverglasses. The transformation into the “L” phase was studied by phase contrast microscopy. Time-lapse motion picture photographs were made four or eight times every minute. Every single picture of this film was examined in a reading ap-

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paratus and magnified prints were made of many pictures where important stages were considered to be present).

**Media.**

Except for a minor modification the media used were those described in a previous publication (1959). Dienes' solid medium now used contained:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>brain-heart infusion Difco</td>
<td>3.7%</td>
</tr>
<tr>
<td>Difco-agar</td>
<td>1.3%</td>
</tr>
<tr>
<td>NaCl</td>
<td>3%</td>
</tr>
<tr>
<td>(or KH$_2$PO$_4$ 5.6%</td>
<td></td>
</tr>
<tr>
<td>or Na$_2$HPO$_4$ 5.6%</td>
<td></td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

After sterilisation ascitic fluid to a final concentration of 16.5% was added. Only ascitic fluid of a 30% protein content or more was used. In all penicillin containing media the final concentration was at least 100 U/ml. For the transformation of penicillinase producers into the "L" phase a few additional drops of penicillin 10,000 U/ml and cycloserin (2.5 mg/ml) were added to the medium.

**Strains.**

All strains in this investigation had been recently isolated from patients. No. 6460, 6988 and 524 were resistant due to penicillinase production, No. 1365 was sensitive to penicillin.

**Serology, phage-typing.**

We gratefully acknowledge our indebtedness to Dr. Oeding (University of Bergen, Norway) who determined the serological pattern according to a technique published earlier (1957). We are indebted to Dr. R. Th. Scholtens (Rijks Instituut voor de Volksgezondheid, Utrecht), to Professor A. Ch. Ruys and Dr. J. Borst (University of Amsterdam) for phage-typing of the sub-strains reverted from the "L" phase. In all four strains the phage-type of the strains regained was lost after 20 passages in the "L" phase. For this reason the results are not mentioned in the tables. New types were not found.

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1) We want to express our gratitude to Prof. P. J. Gaillard (Leyden University) who gave us the opportunity to use the time-lapse motion-picture apparatus in his laboratory, and to Dr. J. P. Scherft for his valuable assistance in making the motion pictures. We are indebted to Mr. J. W. Wesseling and Mr. S. Laszlo for the magnification of these pictures.