CHLORAMPHENICOL RESISTANCE IN MICROCOCCUS PYOGENES

IV. THE EFFECT OF PREINCUBATION ON RESISTANCE

T. E. WILSON AND H. H. RAMSEY

Department of Bacteriology, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma

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In an earlier report from this laboratory (Wilson and Ramsey, 1955) an antagonism between chloramphenicol and complex materials (yeast extract, proteose peptone #3) was noted. It was suggested that the antagonism observed was possibly due to stimulation of growth, rather than to direct antagonism of the antibiotic. In order to examine more critically the respective roles of stimulation and direct antagonism, experiments have been performed in which the chloramphenicol resistance of cells preincubated in both the presence and the absence of yeast extract was compared with the resistance of cells placed directly in contact with the antibiotic.

EXPERIMENTAL METHODS

The test organism, Micrococcus pyogenes var. aureus strain S, has been described (Ramsey and Padron, 1954), as have the methods of preparing the inoculum and the composition of the media employed (Wilson and Ramsey, 1955). Inoculated molecular membranes were placed on drug-free media and on media containing graded concentrations of chloramphenicol. After the desired preincubation interval, the membranes on drug-free plates were transferred to plates of the same type media containing chloramphenicol. The plates were incubated at 37°C and counts made at daily intervals for 7 days. Average total counts of triplicate membranes were used in tabulating results. The generation time on agar plates was determined by the method of Spiegelman, Sussman and Pineska (1950). The average time required for the first cell division to occur was 4 hr.

RESULTS AND DISCUSSION

The results shown in figure 1 indicate that two separate and distinct processes may operate to endow cells with a higher degree of resistance.

First, the incorporation of yeast extract protects the cells from the deleterious effect of the antibiotic. The "control" curve of figure 1A refers to membranes inoculated and placed directly on yeast extract agar supplemented with graded amounts of chloramphenicol. Comparison of this curve with the "control" curve of figure 1B, in which synthetic agar was used, indicates that yeast extract in some way directly antagonizes the inhibitory effect of the antibiotic. These results are in accord with those of Foster and Pittillo (1953a).

The second effect to be noted from the figure is that of preincubation. Allowing the cells to metabolize for 4 hr prior to exposure to chloramphenicol also endows the organisms with a higher degree of resistance. Yeast extract serves to magnify the effect of preincubation and the two effects appear to be additive. Lichstein (1955) has recently described an interesting phenomenon wherein preincubation of Escherichia coli in the presence of pyridoxal or its coenzyme protects the tryptophanase system of the organism against the inhibitory effect of isonicotinic acid hydrazide. It may well be that a similar mechanism operates in the present case. For example, preincubation may allow the cells to synthesize a necessary metabolite with which chloramphenicol interferes. At the same time, yeast extract may serve as an exogenous source of the same metabolite. Either effect would endow the organism with a higher degree of resistance, and the two effects may be additive.

It should be noted that the preincubation time employed correlates closely with the time required for growth to be initiated. Therefore, at most, the cells had undergone only one cell division prior to exposure to chloramphenicol. That the effect is not due to increased cell numbers is shown in table 1. Preincubation for as short a period as 30 min results in a striking increase in survivors and, indeed, allows growth in the presence of 10 μg chloramphenicol per ml—a
The effect of preincubation in yeast extract agar (A) and synthetic agar (B) on survival. See text for details.

**TABLE 1**

<table>
<thead>
<tr>
<th>Time of Preincubation</th>
<th>Colonies Appearing in 169 Hr on Yeast Extract Agar Containing Chloramphenicol</th>
</tr>
</thead>
<tbody>
<tr>
<td>min*</td>
<td>µg/ml</td>
</tr>
<tr>
<td>0</td>
<td>52†  68  37  37  0</td>
</tr>
<tr>
<td>30</td>
<td>76  58  58  50  6</td>
</tr>
<tr>
<td>60</td>
<td>88  96 130  94  65</td>
</tr>
</tbody>
</table>

* Preincubated on yeast extract agar without chloramphenicol.
† Expressed as per cent of colonies developing on medium without chloramphenicol.

tagonize antibiotic action, the earlier discrepancies between our results and those of Foster and Pittillo (1953a, 1953b) may be more apparent than real.

**ACKNOWLEDGMENTS**

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**SUMMARY**

Preincubation of *Micrococcus pyogenes* in the absence of chloramphenicol prior to exposure to the antibiotic results in an increased resistance to the drug. This resistance is apparently due to a high rate of metabolic activity. Enhanced resistance is also obtained by adding a complex material such as yeast extract to the medium containing chloramphenicol. The two effects appear to be additive.

**REFERENCES**


