MECHANISM OF CHEMICAL MUTAGENESIS

III. INDUCED MUTATIONS IN SPHEROPLASTS OF Escherichia coli

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Earlier in the course of these studies on chemical mutagenesis (Iyer and Szybalski, 1958; Steinman et al., 1958), V. N. Iyer evaluated the feasibility of substituting penicillin protoplasts prepared by the method of Lederberg (1956) for intact Escherichia coli cells. It was reasoned that the greater permeability of these structures should facilitate the penetration of mutagens, particularly those which, like MnCl₂, require osmotic decompression for effective entry into the cell (Demerec and Hanson, 1951; Roberts and Aldous, 1951). Preliminary results, however, indicated that technical difficulties associated with the high osmotic fragility of penicillin protoplasts presented too great a hindrance to their efficient utilization in studies of this type. The properties of LiCl-induced spheroplasts of E. coli (Pitzurra and Szybalski, 1959), in particular their stability in saline and in distilled water, promised to be more suitable.

MATERIALS AND METHODS

A streptomycin-dependent strain, Sd-4, of E. coli, obtained through the courtesy of Dr. M. Demerec, was used throughout these studies. A suspension of spheroplasts was prepared by washing off into either saline or 20 per cent sucrose solution a 24-hr growth of E. coli cells on a cellophane membrane over protoplasting agar (nutrient agar containing 100 μg streptomycin per ml, 10 μg leucine per ml, and 2.5 per cent LiCl), according to the procedure described by Pitzurra and Szybalski (1959). The cells were centrifuged, washed, and resuspended in the same media to which was added one of the following mutagens: MnCl₂ at a concentration of 400 μg per ml, or triethylene melamine (TEM) at 10 μg per ml. Exposure to the mutagen was continued at 34 C for a period of 2 hr. The protoplast-like cell form was retained both in saline and in 20 per cent sucrose during the period of mutagen treatment, as confirmed by microscopic observations and counts. Thereafter, the cells were plated at appropriate dilutions on streptomycin-containing nutrient agar to assess survival, and on nutrient agar to determine the number of streptomycin-independent mutants. In each experiment, the following four suspensions were prepared for mutagen treatment: two suspensions of spheroplasts, one in 20 per cent sucrose solution and another in saline, and normal bacillary forms in the same two suspending media. The method for determining the numbers of spontaneous and induced mutants has been described previously by Iyer and Szybalski (1958).

RESULTS AND CONCLUSIONS

The results of these studies are summarized in table 1. The frequencies of both spontaneous and MnCl₂- or TEM-induced mutations in saline-suspended normal bacillary forms were not significantly different from the values obtained for LiCl-induced spheroplasts of E. coli suspended in the same medium. On the other hand, in 20 per cent sucrose solution, the mutagenic action of both mutagens on bacilli and spheroplasts was abolished altogether. This effect of sucrose (see also Demerec and Hanson, 1951), if extracellular, would be different from the antimutagenic effects described in an earlier paper of this series (Steinman et al., 1958). The similarity in induced mutational behavior between LiCl spheroplasts and normal bacillary forms indicates that there is little difference between the permeability barriers of these cell forms for the mutagens studied. The magnitude of the mutagenic effect of Mn⁺⁺ ions on the spheroplasts did not begin to approach their effect on cells made more permeable by pre-
TABLE 1

Comparison of spontaneous and induced mutation frequencies in spheroplasts and in bacillary forms of Escherichia coli

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>Bacillary Forms</th>
<th>Spheroplasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium</td>
<td>Saline</td>
</tr>
<tr>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous mutants/10⁸</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MnCl₂</td>
<td>Survival %</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Induced mutants/10⁸</td>
<td>28</td>
</tr>
<tr>
<td>TEM†</td>
<td>Survival %</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>Induced mutants/10⁸</td>
<td>1600</td>
</tr>
</tbody>
</table>

* Pretreatment in 0.3 M NaCl, as described by Demerec and Hanson (1951), resulted in 9.8-fold higher frequency of induced mutants.
† Triethylene melamine.

Summary

Frequencies of spontaneous and induced mutations were found nearly identical for normal bacillary forms and for LiCl-induced spheroplasts of Escherichia coli suspended in saline solution. Triethylene melamine and MnCl₂ served as mutagens. In 20 per cent sucrose solution the mutagenicity of these compounds was nearly abolished.

References


