Effects of High Incubation Temperature Upon the Cell Wall of *Escherichia coli*

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ABSTRACT

HOFFMAN, HEINER (New York University, New York, N.Y.), JON VALDINA, AND MICHAEL E. FRANK. Effects of high incubation temperature upon the cell wall of *Escherichia coli*. J. Bacteriol. 91:1635-1637. 1966.—*Escherichia coli* cells grown at 45 C were found to swell when suspended in distilled water, and were broken down when subjected to egg white lysozyme. Cells grown at 37 C were unaffected by these reagents. It appears, therefore, that cultivation at the upper limits of the temperature growth range leads to cell wall damage. It is suggested that this reaction may account for the favorable therapeutic effects of artificially elevated body temperature in certain infectious diseases.

It was recently reported (2) that high incubation temperature results in the appearance of a certain proportion of filamentous cells in microcultures of *Escherichia coli* on agar, although the greater part of the microculture continues to give a normal appearance. However, since the filaments appear to be of the same width as the normal length cells and apparently differ from them only in the failure to form cross walls, our results cannot be considered entirely comparable to those of Murabito and Seiser (3). These investigators had found that, at temperatures of 43 C and above, very large and thick forms may occur in microcultures of *E. coli* which probably correspond to the "megalomorphic" cells of Wahlin and Almaden (4). The swollen appearance of megalomorphic cells suggests disturbances in the form-maintaining structural components of the cell wall. In the present investigation, *E. coli* cells from broth cultivations were examined to determine whether cell wall alterations do in fact occur at high incubation temperature.

MATERIALS AND METHODS

Overnight cultures at 37 C of *E. coli* ATCC 8677 were inoculated into Brain Heart Infusion (Difco) and incubated at 37 C (air incubator) or at 45 C (water bath) for 5 hr. The cultures were then centrifuged and resuspended in sterile distilled water, in physiological saline (0.8%) solution, or in a 0.2% solution of egg white lysozyme (Worthington Biochemical Corp., Freehold, N.J.) in Brain Heart Infusion. Control suspensions in Brain Heart Infusion alone were also established. The suspensions were held at 37 C for 20 min while being agitated. The

cells were then concentrated again by centrifugation and the supernatant fluid was discarded. Smears were made for Gram staining, or wet mounts were prepared for dark phase-contrast microscopy.

RESULTS AND DISCUSSION

Hypotonicity and lysozyme were found to produce marked effects upon the cells cultivated at 45 C (Fig. 1 to 4), whereas they produced no detectable cytological effects upon the cells grown at 37 C (Fig. 5 to 8). The swelling of cells grown at 45 C when they were suspended in distilled water suggests that the cell wall components which are responsible for cell form have been structurally damaged, and the susceptibility of the cells to lysozyme suggests that the normally inaccessible lysozyme substrate in the cell wall of *E. coli* has become exposed through the changes which have occurred in the outer cell wall layers normally maintaining cell form (1).

These present results with broth cultures are in harmony with the observations of Murabito and Seiser (3) that *E. coli* in microculture may form large and thick forms at incubation temperatures of 43 C and above. Our previous results (2), which did not agree in this detail with those of Murabito and Seiser, may perhaps be due to differences in microculture techniques. The effects of high temperature of incubation upon the cell wall may be the mechanism which underlies the beneficial effects of hyperpyrexia in the therapy of certain infectious diseases. It would be of great interest in this regard to determine the influence of lyso-

FIGS. 1-8

zyme upon the therapeutic effectiveness of induced high temperature.

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FIG. 1 to 4. *Escherichia coli* series grown at 45 C in beef Brain Heart Infusion broth. Gram stain. $\times 2,500$. (1) Cells after 20 min in beef Brain Heart Infusion broth at 45 C. (2) Cells after 20 min in 0.8% sodium chloride aqueous solution at 45 C. (3) Cells after 20 min in distilled water at 45 C. (4) Cells after 20 min in 0.2% lysozyme solution at 45 C.

FIG. 5 to 8. *Escherichia coli* series grown at 37 C in beef Brain Heart Infusion broth. Gram stain. $\times 2,500$. (5) Cells after 20 min in beef Brain Heart Infusion broth at 37 C. (6) Cells after 20 min in 0.8% sodium chloride aqueous solution at 37 C. (7) Cells after 20 min in distilled water at 37 C. (8) Cells after 20 min in 0.2% lysozyme solution at 37 C.