*J. Pharm. Pharmac.*, 1967, **19**, 740–743 Received April 7, 1967

# A note on some changes in the physical properties of *Escherichia coli* after heat treatment

DIANN HARRIES AND A. D. RUSSELL

Suspensions of *Escherichia coli* in water at 50, 55, 60 and 100° showed an increase in extinction and nephelometer readings, the rate of increase depending on the temperature. This is considered to represent varying degrees of alteration in bacterial cytoplasmic protein. No such increases at any temperature occurred with cells held in 0·9% w/v sodium chloride. Lysozyme induced no lysis in cells which had been heated in water or in saline.

IN 1967, Russell & Harries observed that suspensions of *Escherichia coli* which were held at temperatures of 50–60° and at 100° leaked intracellular constituents into the surrounding medium, the rate of leakage depending on the temperature used. Leakage was not prevented by 0·33 M sucrose. These authors also found that heated suspensions showed an increase in extinction. Heated and unheated suspensions of *E. coli* have been examined further for this effect; the results are now reported.

## Experimental

Details of the organism (*E. coli* Type 1), its growth and the preparation of suspensions containing about $10^9$ viable cells/ml in water and in 0·33 M sucrose, and the heating procedure have been described previously (Harries & Russell, 1966; Russell & Harries, 1967). In some experiments, the suspending medium in which the cells were heated consisted of 0·9% w/v sodium chloride.

The temperatures were 50°, 55°, 60° and 100°. Samples of suspensions held at these temperatures were removed at intervals, and the extinction at 500 mμ read in the SP 600 spectrophotometer, using 1 mm cells and the appropriate blank (suspension medium only); further samples were used for measuring turbidity with the EEL nephelometer.

*Effect of lysozyme on heated and unheated suspensions.* Concentrated solutions of egg-white lysozyme (British Drug Houses, Ltd., London) were prepared and sterilized by filtration. Unheated suspensions, and suspensions held at the desired temperature for 30 min in water or 0·9% w/v sodium chloride, were centrifuged at 2000 rev/min for 40 min, the supernatant fluids removed, and the pellets resuspended in M/15 phosphate buffer, pH 7·4. Each suspension was divided into 2 × 9 ml aliquots: to one was added 1 ml of a lysozyme solution, to give the desired final concentration; to the other was added 1 ml of sterile water. All aliquots were incubated at 37°. Samples were removed at intervals, diluted 1 in 5 with phosphate buffer, pH 7·4, and the extinction at 500 mμ determined, as previously described, using the appropriate blank. Samples were also examined microscopically (phase-contrast, ×400). Experiments were also made in which water replaced the phosphate buffer.

From the Welsh School of Pharmacy, Welsh College of Advanced Technology, Cardiff.

## Results and discussion

Both the extinction and nephelometer readings of aqueous suspensions of *E. coli* increased after being maintained at various temperatures, the rate of increase depending on the temperature (Fig. 1). An increase in extinction may represent either gross changes in bacterial cytoplasmic protein (Beckett, Patki & Robinson, 1959; Gilby & Few, 1960), or a decrease in bacterial cell volume (Mager, Kuczynski & others, 1956; Avi-Dor, Kuczynski & others, 1956; Brock, 1958; Bernheim, 1963),

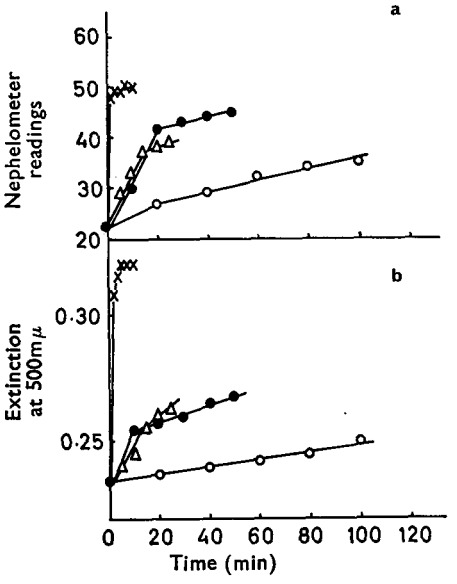


FIG. 1. Changes in (a) nephelometer readings, and (b) extinction, of aqueous suspensions of *E. coli* held at various temperatures. 50°, ○——○; 55°, △——△; 60°, ●——●; 100°, ×——×.

whereas an increase in nephelometer readings, in the absence of cell multiplication, may be associated with an increase in bacterial cell size, although such increased turbidity would also occur on coagulation of proteins. Despite the lack of adequate experimentation in this field to date (Hansen & Riemann, 1963), the coagulation of proteins has been put forward as the reason for the death of bacteria by moist heat (see for example, Sykes, 1965). However, when suspensions of *E. coli* were held in saline and exposed to temperatures of 50°–60° and 100°, nephelometer readings did not increase, and there was a small, but detectable and reproducible, decrease in extinction (Fig. 2). The reasons for these findings are not apparent, particularly as it is known that the protoplasm of *E. coli* cells becomes coarsely granular during their heating in saline (Heden & Wyckoff, 1949; Hansen & Riemann, 1963).

Because sucrose has been used to stabilize spheroplasts of this strain of *E. coli* (Barnett & Russell, 1967), and is thus relatively non-penetrating

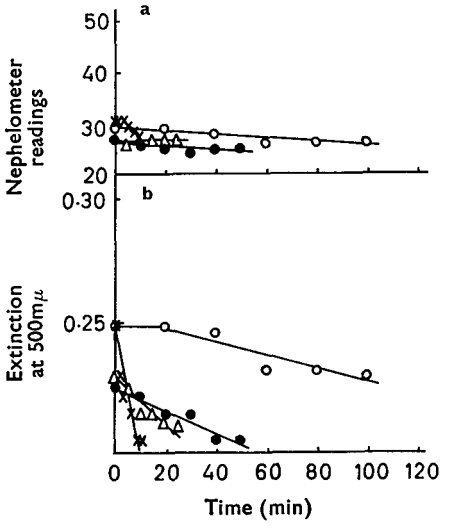


FIG. 2. Changes in (a) nephelometer readings, and (b) extinction, of suspensions of *E. coli* held in 0·9% w/v sodium chloride at various temperatures. 50°, ○——○; 55°, △——△; 60°, ●——●; 100°, ×——×.

into this organism, its effect, at a concentration of 0·33 M, on the extinction of heated cells was investigated. The results indicated that there was a similar response to that demonstrated by cells suspended in water.

The leakage of intracellular constituents from heated suspensions of this organism is not prevented by sucrose (Russell & Harries, 1967). Thus, one of the main effects of moist heat is on the cytoplasmic membrane. It is of interest, in this context, to consider the effect of heat in the Ziehl-Neelsen staining method for *Mycobacterium tuberculosis*; the effect of heat here is to render the waxy material more permeable to aqueous dyes, and this could depend on the existence of a semi-permeable membrane around the organisms which allows fuchsin to diffuse into the cell, but prevents acid fuchsin from diffusing out (Wilson & Miles, 1964).

The effect of lysozyme on heated and unheated (control) suspensions was examined because (*a*) it had previously been found (Russell & Harries, 1967) that the total counts of suspensions heated in water remained constant, which suggested that there was no significant effect on the cell wall of the organism; (*b*) it had been shown by Hoffman, Valinda & Frank (1966) that cells of *E. coli* which had been grown at 45° became swollen when suspended in distilled water, but not in 0·8% w/v sodium chloride, and were lysed when treated with egg-white lysozyme; (*c*) it was hoped that, by treating with lysozyme cells that had been heated in water and saline, information would be obtained on the nature of the differences observed earlier. Accordingly, the effects of lysozyme in phosphate buffer at 37° on cells previously held for 30 min in water or saline at 20°, 50°, 55° and 60° were investigated; the enzyme was used at concentrations of 0, 2·5, 25 and 250 μg/ml. In no instance was there any evidence

of lysis, as determined at 500 mμ or microscopically, over a period of $3\frac{1}{2}$–$4\frac{1}{2}$ hr. It must also be pointed out that at the highest lysozyme concentration used, some precipitation (agglutination of cells) occurred; this readily dispersed on shaking to give an even turbidity. A similar finding was made by Salton (1953). The replacement of phosphate buffer with water or with buffer pH 6·2 had no effect on the action of lysozyme.

Heating of suspensions at 100° for more than 10 min resulted in clumping and precipitation of the cells, and such suspensions were therefore not used for studying subsequent lysozyme action.

Thus, the results suggest that moist heat at temperatures of 50–60° does not damage the outer layers of the cell wall of *E. coli* sufficiently to permit the lysozyme molecule to reach its mucopeptide substrate (Salton, 1957, 1964), although lysozyme might not induce lysis in cells in which the protein had been coagulated (Salton, 1953). However, Carson & Eagon (1966) showed that with *Pseudomonas aeruginosa*, the mucopeptide component is not solely responsible for the structural integrity of the cell wall, and this may be so also for the wall of other Gram-negative organisms.

The overall result of our experiments is to indicate that moist heat causes an alteration in the cytoplasmic protein of *E. coli* cells suspended in water, but thermal injury cannot be reconciled with damage to the cell wall. Although protein coagulation could be put forward as being the primary reason for thermal damage, this is not necessarily so, as there is also a release of intracellular constituents (Russell & Harries, 1967). Moreover, in at least some bacterial species (Strange & Shon, 1964; Iandolo & Ordal, 1966) there is a breakdown of ribonucleic acid in heated cells. It is interesting to note that until recently, no really critical experiments had been made to investigate the nature of thermal death in non-sporing bacteria (Wood, 1956; Hansen & Riemann, 1963).

## References


Avi-Dor, Y., Kuczynski, M., Schatzberg, G. & Mager, J. (1956). *J. gen. Microbiol.*, **14**, 76–83.

Barnett, M. I. & Russell, A. D. (1967). *J. Med. Lab. Tech.*, **24**, 113–118.

Beckett, A. H., Patki, S. J. & Robinson, A. E. (1959). *J. Pharm. Pharmac.*, **11**, 367–373.

Bernheim, F. (1963). *J. gen. Microbiol.*, **30**, 53–58.

Brock, T. D. (1958). *Can. J. Microbiol.*, **4**, 65–71.

Carson, K. J. & Eagon, R. G. (1966). *Ibid.*, **12**, 105–109.

Gilby, A. R. & Few, A. V. (1960). *J. gen. Microbiol.*, **23**, 19–26.

Hansen, N. H. & Riemann, H. (1963). *J. appl. Bact.*, **26**, 314–333.

Harries, D. & Russell, A. D. (1966). *Experientia*, **22**, 803–804.

Heden, C. G. & Wyckoff, R. W. G. (1949). *J. Bact.*, **58**, 153–160.

Hoffman, H., Valinda, J. & Frank, M. E. (1966). *Ibid.*, **91**, 1635–1636.

Iandolo, J. J. & Ordal, Z. J. (1966). *Ibid.*, **91**, 134–142.

Mager, J., Kuczynski, M., Schatzberg, G. & Avi-Dor, Y. (1956). *J. gen. Microbiol.*, **14**, 69–75.

Russell, A. D. & Harries, D. (1967). *Appl. Microbiol.*, **15**, 407–410.

Salton, M. R. J. (1953). *J. gen. Microbiol.*, **9**, 512–523.

Salton, M. R. J. (1957). *Bact. Rev.*, **21**, 82–100.

Salton, M. R. J. (1964). *The Bacterial Cell Wall*, Amsterdam: Elsevier.

Strange, R. E. & Shon, M. (1964). *J. gen. Microbiol.*, **34**, 99–114.

Sykes, G. (1965). *Disinfection & Sterilization*, 2nd edn, London: Spon.

Wilson, G. S. & Miles, A. A. (1964). Topley & Wilson's *Principles of Bacteriology & Immunity*, vol. 1, 5th edn, London: Arnold.

Wood, T. H. (1956). *Adv. biol. med. Phys.*, **4**, 119–165.