CHAPTER VII

GENERAL DISCUSSION AND CONCLUSION
Some effects of heat treatment (52°C) to E. coli and bacteriophage λ were studied. In the case of bacteria, heat treatment caused loss of colony forming ability, and *recA*, *lex* and *polA* strains were more sensitive than the wild-type, *reAc* and *uvrA* strains. Liquid holding recovery was influenced by *lex* function of E. coli. Heat treatment affected metabolism and stability of DNA and RNA and caused damage to cell membrane and enzymes. Phage λ, on the other hand, was not inactivated at 52°C as free particles. However, intracellular heating inactivated λ and the sensitivity depended largely on bacterial strain.

On the basis of these data and those reported earlier (as reviewed in Chapters IV and V), a scheme for heat lesions and their mode of recovery is being proposed (Fig. 1).

Short periods of heat treatment (low doses) cause transient local openings of DNA at various points (Iman, 1968). In vivo, these specific regions are recognised and attacked by DNase(s) resulting in nicked DNA (Goldmark and Linn, 1970; Sedgwick and Bridges, 1972). As the heat treatment prolongs, nicked ends get denatured and DNase(s) also become inactive.

The local openings induced in λDNA by extracellular heat treatment are reanimated due to slow cooling during infection. However, if λDNA was heated intracellularly in the host, nuclease(s) induce single strand breaks followed by formation of gaps which would require the activity of repair enzymes. It has been proposed earlier that single-strand
Fig. 1. Scheme of in vivo heat (52°C) induced lesions and their mode of repair in *Escherichia coli* K-12.
Breaks are not formed as a direct consequence of heat but breaks appear as a result of nucleases action (Sedgwick and Bridges, 1972). Nicked DNA will be subjected to fast rejoining by DNA polymerase I and ligase (Town et al., 1971, 1972; Srivastava, 1974), while the remaining breaks/gaps require functional *lex* and *redA* genes.

For the repair of λ DNA, the gene-products of *redA*, *uvrA*, *lex* and *red* of λ-gold and *red* of λ are required. The *uvrA* gene plays an important role in the repair of λ DNA rather than in the repair of bacterial DNA. Thus, it seems that at low doses only the lesions in the DNA are important and these lesions are repairable (Woodcock and Grigg, 1972; Chapter VI).

Contrary to radiation, heat induces multi-target damage including DNA, RNA, proteins and cell membranes. The damages other than DNA are more important at longer periods of heating (high dose). Damage to DNA would involve massive denaturation of DNA up to a certain point when nuclease(s) and other enzymes involved are inactivated by heat. DNA synthesis is inhibited and normal synthesis resumes during incubation in recovery medium.

RNA synthesis is susceptible to heat and is probably due to the attack by RNase(s) which becomes inactivated at higher doses of heat. Liquid holding requires de novo RNA biosynthesis. It is a hypothesis that *lex* gene plays a role in the control of RNA degradation after heat treatment.
Holding in recovery medium, most probably because of the inhibition of de novo host biosynthesis following phage \(\lambda\) infection which are vital for repair and multiplication of \(\lambda\).

Inactivation of repair enzymes at longer periods of heat treatment constitutes an important effect of heat. We have demonstrated inhibition of DNA degradation and inactivation of repair enzymes. All other proteins including nuclease, proteinases and enzymes of transport system may be inactivated by heat. Most of these proteins and enzymes are renatured/activated without involving de novo protein synthesis during incubation in recovery medium. For example, chloramphenicol had no effect on the recovery of normal UV resistance of heated bacteria.

Heat treatment also affects the bacterial cell wall and damages the permeability barrier, as a result permeability increases and cells become less tolerant to osmotic shock. Recovery of the cell membrane occurs in recovery medium as shown by high salt tolerance and decreased uptake of \(^{3}H\)-actinomycin D.