ABSTRACT

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The effect of 52°C heat treatment on *Escherichia coli* K-12 and bacteriophage λ were studied. The essential data can be summarized as under:

A. Effect of heat treatment on *Escherichia coli* K-12.

1. The redA, lux, and galA mutants of *E. coli* K-12 exposed to 52°C in Tris-NaCl buffer (0.01M, pH 8.0) were more sensitive to heat than the wild-type and λ redA and λ redB mutants.

2. Survival of bacteria was influenced by composition of the pre- and post-treatment growth medium. When heated cells were incubated in tryptone broth (TM) or minimal medium (MM) for 120 min before plating on tryptone agar, survival depended on the composition of pre-treatment and post-treatment incubation media. Recovery was maximum when heated bacteria were held in MM for 120 min. This is "liquid-holding recovery" of heated cells observed in broth but not if plated directly on tryptone agar.

3. During liquid holding in tryptone broth, redA and galA mutants also recovered but a lux mutant did not.

4. As a result of heating, the sensitivity of bacteria to ultraviolet radiation, mitomycin C and to plating on high
salt medium was enhanced. After incubation for 2 hr in tryptone broth at 37°C, the bacteria regained their normal resistance to UV, mitomycin C and tolerance to high salt medium.

5. Recovery of viability required RNA and protein syntheses. Addition of rifampicin and chloramphenical in the recovery medium interfered with recovery. Rifampicin inhibited recovery of mitomycin C resistance significantly and to large extent, recovery of plating on high salt medium. Chloramphenical, on the other hand, affected to some extent recovery of high salt tolerance and resistance to mitomycin C. Recovery of UV resistance did not require protein synthesis.

6. Heat treatment to bacteria resulted in the appearance of single strand breaks in the DNA. These breaks were repairable in the recovery medium at 37°C.

7. Less DNA was degraded in all the heated bacterial strains compared to unheated cells. This observation was made with all the bacterial strains irrespective of their genetic marker.

8. Heat treatment caused inhibition of DNA synthesis. The degree of inhibition was more in lex strains than in the wild-type bacteria. During liquid holding, recovery of DNA synthesis was observed with w.t. bacteria whereas recovery was poor with lex strains.
9. RNA synthesis was also affected by heat treatment. In wild-type bacteria, RNA synthesis resumed after 30 min if incubated in recovery medium whereas it remained inhibited up to 90 min in *lex* strain.

10. RNA degradation was observed with heated bacteria during incubation in recovery medium. The extent of degradation was more with the *lex* mutant than the wild-type strain.

11. During the 30 min of heat treatment to bacteria in Tris-Mg buffer, release of RNA precursors up to the extent of 4% of the total RNA was observed. Most of the leakage occurred during 10 min of exposure.

12. Leakage of proteins during heat treatment and degradation of proteins during liquid holding were not observed.

13. Permeability of *E. coli* to *H*-actinomycin D increased with duration of heat treatment suggesting alteration of cell membrane. Liquid holding restored normal permeability of cell membrane.

14. Heat treatment to bacteria was found to be mutagenic. The frequency of *ampicillin* and rifampicin-resistant colonies were measured. Mutations were lost when bacteria were held in tryptone broth.

**8. Effect of heat treatment on θ.**

1. Extracellular heating of phage as free particles at 52°C up to 60 min had no effect on plaque forming units (PFU).
2. Intracellular heating resulted in loss of PFU. \(\lambda\)-pola complex was most sensitive to heat specially at short periods of heat treatment. Other mutations in bacteria, such as reaA, lep and nvrA also affected survival of PFU of \(\lambda\).

3. \(\lambda\)red phage was more sensitive as compared to red\(^{-}\). The \(\text{red}^+\) and \(\text{reaA}^+\) functions seem have complementary effect specially at short exposures of heat treatment.

4. Contrary to heated \(\lambda\)galli liquid holding recovery of PFU was not observed when \(\lambda\)galli complex was held in recovery medium. An assay of \(\beta\)-galactosidase and synthesis of mRNA revealed that no transcription occurred in infected bacteria. On the other hand, in uninfected bacteria, these activities normally occurred. This suggested that recovery of PFU in recovery medium was not possible because infected bacteria could not recover.

5. The inactivation of PFU of \(\lambda\) depended very much on the state of the host. When u.e., reaA, polA hosts were heated and infected with unheated \(\lambda\), survival of PFU of \(\lambda\) declined with increase in heating of bacteria.

6. Heat treatment was not mutagenic to phage \(\lambda\), no matter how \(\lambda\) was exposed to 32\(^\circ\)C, i.e., extracellularly or intracellularly. Heating of bacteria alone did not enhance mutation frequency.
C. Purification of phage λ by gel filtration.

A method for purification of phage λ by gel filtration has been developed. The crude lysate of phage is subjected to nucleases and polyethylene glycol treatments prior to loading on Sephadex G-100 column. The purified phage is eluted in the void volume. The method is rapid, efficient and relatively inexpensive. The technique can also be used for large scale purification of phage λ.