Recovery of Bacteria from Damages Induced by Heat

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SUMMARY

When *Escherichia coli* B was heated at 52° and subsequently incubated at 20° or at 37° in phosphate buffer, there was an increase in the number of viable organisms. No such increase was observed when the treated suspension was incubated at 0.5°. Untreated organisms did not increase in number when incubated in phosphate buffer, suggesting that the increase in viable number of heat-treated organisms was not due to division. The observed increase in number of viable organisms was time-dependent and complete in about 60 min., but could be stopped by exposing the treated organisms to X-ray doses greater than 1.5 krad., before or after the heat treatment. Growing the organisms in the presence of chloramphenicol 100 µg./ml. before heat treatment also blocked the repair mechanism, but this could be re-established by growth in the absence of chloramphenicol for 30 min. before heat treatment.

INTRODUCTION

The existence of a repair system in bacteria has been established and found to be of a general nature in that it operates on damage induced by various types of agents, such as X-rays, ultraviolet light, and radiomimetic agents (Setlow, 1966).

Recovery in *Escherichia coli* cultures damaged with heat has been shown by Heinmetz, Taylor & Lehman (1954), and the number of organisms surviving an exposure to heat found to be dependent on the use of different plating media (Busta & Jezeski, 1963; Heather & van der Zant, 1957*a*, 1958), the post-incubation temperature in liquid media and the pH value of the plating media (Heather & van der Zant, 1957*b*). In the present paper, recovery phenomenon in heated *Escherichia coli* populations in a medium not supporting growth has been examined.

METHODS

Escherichia coli B organisms were grown in the tris+glucose medium of Maaløe & Hanawalt (1961). Overnight cultures were diluted 50 times with the same medium and growth continued, with aeration, at 37°. Rapidly growing organisms were collected by centrifugation and freed from nutrients by three washings with ice-cold M 15-phosphate buffer (pH 6.9). The organisms were then suspended in warmed phosphate buffer and heated at 52° in a constant temperature waterbath for various times. After the selected periods of heating the suspension was divided into three parts, one of which was placed immediately at 0.5° and the others at 20° and 37° and held at these temperatures. The colony count of bacteria was determined at selected time intervals and the initial viable count of bacteria determined from an unheated sample. In different experiments, this concentration of bacteria varied in the range 5×10^8 to 8×10^8

colonies/ml. The number of viable bacteria was determined by plating suitable dilutions of each suspension on nutrient agar. Suspension (0.1 ml.) was spread on the agar surface and the visible colonies counted after incubation at 37° for 18 hr.

The influence of X-irradiation on the recovery of heated organisms was studied by irradiating the organisms with X-rays and then heating at 52° for the required time or, alternatively, first heating at 52° and then irradiating with X-rays. The samples were then incubated at 20° in phosphate buffer and the concentrations of viable bacteria determined at intervals.

X-irradiation of the bacteria was done in the cold in a special brass holder in a small Petri dish in contact with ice from a Muller MG 150 tube run at 80 kV, 9 mA. The X-ray doses at various positions of irradiation were measured by FeSO₄ dosimetry. The influence of protein synthesis on the recovery of bacteria was studied by growing organisms for 30 min. in the presence of chloramphenicol 100 µg./ml. This concentration of chloramphenicol was chosen because previous experiments showed that the increase in extinction at 450 mµ was completely stopped at such a concentration of chloramphenicol. After this incubation in the presence of chloramphenicol, organisms were washed free from chloramphenicol and exposed at 52° for 40 min. Subsequently, the organisms were incubated at 20° in phosphate buffer. This experiment was varied by allowing growth of bacteria to occur in the absence of chloramphenicol for 20 and 30 min. after the chloramphenicol treatment.

RESULTS

The influence of incubation at 20° on the survival of heat-exposed *Escherichia coli* organisms is shown in Fig. 1. For convenience, the initial % survivors in all cases was calculated to 100. The actual number of survivors under different conditions of treatment is shown in Table 1. Clearly there was an increase in the number of survivors during incubation; this increase was very steep for the first 30 min. and reached a maximum value in about 60 min.

When the organisms were exposed to heat for brief periods of time, the subsequent increases were small. The increase in number of survivors on incubation was greater when the number of heat survivors was small; however, an increase in number was observed in all cases. Unheated bacteria remained fully viable in control experiments under identical post-heating conditions. In all cases the maximum increase in number of survivors was reached in 60 min. and thereafter there was no change for the rest of the 3 hr period of the experiment. In Table 1, the results of holding the heated organisms at different incubation temperatures in phosphate buffer are given. No increase in survivors was observed when heated organisms were incubated at 0.5°; at 37 and 20° the results were similar. However, the recovery was prominent at 20° for the organisms exposed to shorter periods of heating. Later experiments on the recovery were therefore done at the incubation temperature 20°.

The effect of inhibition of protein synthesis on the observed increase in number is shown in Fig. 2. In this experiment, the initial heat survival was 0.008% in all cases. The organisms that were exposed to chloramphenicol for 30 min. before exposure to heat showed no subsequent increase or decrease in the number of survivors on incubation at 20°; but by allowing growth in the absence of chloramphenicol for 20 and 30 min. before heat-exposure, organisms again showed the increase in survival

number on subsequent incubation in phosphate buffer, at 20°. However, the increase in number was small for the organisms that were allowed to grow in the absence of chloramphenicol for only 20 min.

Thus there was a gradual increase in number of survivors on incubation of bacteria grown in the absence of chloramphenicol for 0 to 30 min. The organisms grown for

Fig. 1

Fig. 2

Fig. 1. Effect of incubation in buffer at 20° on the survival of heat-damaged bacteria. Initial heat-survivors: ■, 0.008%; □, 0.01%; ▲, 0.07%; △, 1.4%; ●, 2.2%; ○, 100% (unheated).

Fig. 2. Influence of protein synthesis on the recovery of heat-damaged bacteria. ○, Protein synthesis not inhibited, prior to heating. ▲, Protein synthesis inhibited by growing cells in presence of 100 µg./ml. of chloramphenicol for 30 min. prior to heating. △, Protein synthesis inhibited, but prior to heating, 20 min. of growth in absence of the inhibitor (chloramphenicol) was allowed. ●, Protein synthesis inhibited but prior to heating 30 min. of growth in absence of the inhibitor (chloramphenicol) was allowed.

Table 1. *Post-incubation of heated bacteria at 0.5, 20 and 37°*

Temperature of post-incubation (°C)	Initial heat survivors (%)	Percentage surviving at incubation times (hr)					
		½	1	1½	2	3	4
0.5	2.76	2.4	2.9	2.5	2.86	2.7	2.5
0.5	0.17	0.19	0.15	0.17	0.16	0.15	0.17
0.5	0.027	0.026	0.028	0.027	0.020	0.027	0.027
0.5	0.0015	0.002	0.0016	0.0016	0.002	0.0011	0.001
20	2.20	7.6	5.2	4.4	6.4	4.8	6.4
20	1.4	6.0	6.7	7.0	5.8	7.0	6.7
20	0.07	1.1	1.27	1.14	1.28	1.4	1.14
20	0.01	0.07	0.33	0.316	0.35	0.31	0.32
20	0.008	0.25	0.32	0.37	0.32	0.37	0.33
37	1.5	6.0	6.1	7.0	5.8	6.0	6.8
37	0.07	0.29	0.43	0.43	0.33	0.5	0.47
37	0.015	0.077	0.12	0.12	0.11	0.16	0.12
37	0.008	0.25	0.17	0.2	0.3	0.2	0.18
0.5	100	99	98.9	100	100	101	99.5
20	100	100	98	98.8	99.2	100	100
37	100	98.4	99	100	100	102	98.8

0 min. before heat treatment showed no subsequent increase in number; organisms grown for 20 min. showed some increase; but only the organisms grown for 30 min. after chloramphenicol treatment showed the same increase as that given by organisms not exposed to chloramphenicol.

Fig. 3. Effect of X-irradiation on the recovery of heat-damaged bacteria. Curves: (a) irradiated before heating and (b) irradiated after heating. Doses used; $\circ$, 0.268 krad.; $\bullet$, 0.804 krad.; $\triangle$, 0.877 krad.; $\blacktriangle$, 1.072 krad.; $\square$, 1.608 krad.; $\blacksquare$, 4.021 krad.; $\odot$, 6.432 krad.

In Fig. 3, the influence of X-irradiation on the observed increase in number of survivors of heat treatment is shown. In all cases, survivors were at the level of 0.008%. The organisms were exposed to various doses of X-rays either before or after the heat treatment and then they were incubated in phosphate buffer at 20°. Then X-irradiation affected the increase in number of heat survivors. When the X-ray doses were greater than 1.5 krad., no increase was observed. This was true both for pre- and post-X-ray treatment. At X-ray doses up to 2 krad., there was a gradual suppression in the observed increase in number of post-heat survivors.

DISCUSSION

The observed increase in number of survivors after heating at 52° and incubation at 20 and 37° was not likely to be due to limited division of the organisms. This follows from the fact that under identical conditions of incubation organisms not heated did not increase in number. Thus the observed increase must have been due to repair of the lesions induced by heating the organisms. In other experiments, bacteria after heating were freed from the media in which they had been heated and were incubated at 20 and 37° in fresh phosphate buffer. The results obtained were identical with those in which the medium was not changed. This clearly indicated that recovery was not due to assimilation of products which leaked from the bacteria after heat treatment. However, in absolute terms, the number of organisms recovering from the damage was not high. In all cases, the observed recovery was in a medium which could not sustain growth, and in this respect the experiments presented here differ from those reported by Iandolo & Ordal (1966). These authors made experiments in complete growth media and the observed recovery was very high; but the present experiments show that even in the absence of growth nutrients a limited amount of recovery was possible. This recovery was suppressed at 0.5°, indicating that some metabolism was necessary; this was further supported by the observation that growth in presence of chloramphenicol (100 µg./ml.) for 30 min. stopped the process of recovery. The recovery phenomenon was fully re-established when the organisms were subsequently allowed to grow in the absence of chloramphenicol for a period of at least 30 min. before exposure to heating. Thus a direct involvement of protein in the mechanism is indicated. There is no evidence that chloramphenicol inhibits the synthesis of RNA (Maaløe & Kjeldgaard, 1966). Thus even if the involvement of RNA is postulated (Iandolo & Ordal, 1966), it cannot be direct.

A feature of the repair phenomenon is that it was completely inhibited by a small dose of X-rays. This X-ray dose was of the order at which colony formation was affected. Also, whether the exposure was made before or after the heat treatment was immaterial. So it would seem that the radiation acted on a system which had the same sensitivity to radiation before and after heat treatment. Since inhibition of protein synthesis stopped the recovery process completely, it is tempting to suggest that irradiation also acts through the same pathway. However, the ultimate results of protein inhibition and of X-irradiation were capable of suppressing the recovery. No effect on any of the synthetic abilities of cell were observed to follow these low doses of X-rays. The division ability of heat survivors was more sensitive to radiation than that of the control bacteria. Thus the process of recovery may be unrelated to the division ability of the bacteria because, in these experiments, pre- and post-X-ray exposures had the same effect on the recovery of heat-damaged organisms. The bacteria which recovered from heat-induced lesions had the same sensitivity to X- and u.v.-irradiation as of untreated organisms. They also showed photo-reactivation and liquid-holding recovery, indicating that the recovered organisms were not different from the untreated bacteria in these respects.

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REFERENCES

- BUSTA, F. F. & JEZESKI, J. J. (1963). Effects of sodium chloride concentration in an agar medium on growth of heat-shocked *Staphylococcus aureus*. *Applied Microbiology* **11**, 404.
- HEATHER, C. D. & VAN DER ZANT, W. C. (1957*a*). Effects of plating medium on the survival of heat-treated *Pseudomonas fluorescens*. *Food Research* **22**, 164.
- HEATHER, C. D. & VAN DER ZANT, W. C. (1957*b*). Effects of temperature and the time of incubation and pH of plating medium on enumerating heat-treated *psychrophilic bacteria*. *Journal of Dairy Science* **40**, 1079.
- HEATHER, C. D. & VAN DER ZANT, W. C. (1958). Effect of metabolites on the viability of heat-treated *Pseudomonas fluorescens*. *Food Research* **23**, 126.
- HEINMETZ, F., TAYLOR, W. W. & LEHMAN, J. J. (1954). The use of metabolites in the restoration of the viability of heat and chemically inactivated *Escherichia coli*. *Journal of Bacteriology* **67**, 5.
- IANDOLO, J. J. & ORDAL, Z. J. (1966). Repair of thermal injury of *Staphylococcus aureus*. *Journal of Bacteriology* **91**, 134.
- MAALØE, O. & HANAWALT, P. C. (1961). Thymine deficiency and the normal DNA replication cycle. I. *Journal of Molecular Biology* **3**, 144.
- MAALØE, O. & KJELDGAARD, N. O. (1966). *Control of macromolecular synthesis*, p. 16. New York & Amsterdam: W. A. Benjamin, Inc.
- SETLOW, R. B. (1966). The repair of molecular damage to DNA. In *Radiation Research* (G. Silini), p. 525. Amsterdam: North-Holland Publishing Company.