SUSCEPTIBILITY OF MILD THERMAL AND OF IONIZING RADIATION DAMAGE TO THE SAME RECOVERY MECHANISMS IN ESCHERICHIA COLI.

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Work in recent years has produced convincing evidence for the existence in bacteria and other organisms of enzyme systems capable of repairing or of mitigating the effect of damage to DNA inflicted by ionizing radiation(see, for example, Haynes, 1964; Moseley, 1968). The reasons for the existence of these often extremely efficient recovery systems are still the subject of speculation. One possibility is that damage similar to that caused by ionizing radiation is produced by other environmental factors or occurs as a consequence of normal metabolism.

Among most common lesions produced by ionizing radiation are scissions of a single strand of the DNA duplex (Freifelder, 1965; Hagen and Wellstein, 1965). Such breaks might also occur during exposure to mild heat as a result of direct hydrolysis of the phosphate diester link (c.f. Eigner, Boedtker and Michaels, 1961), or of hydrolysis following depurination (c.f. Greer and Zamenhof, 1962), or indirectly as a result of attack by released endonucleases.

In the course of studies using the alkaline sucrose gradient technique of McGrath and Williams (1966) we have obtained evidence for the production of single strand breaks in the DNA of bacteria which had been incubated at 52°C for several minutes. It seemed possible that the damage to DNA in vivo caused directly or indirectly by heating to 52° might be similar to that caused by exposure to ionizing radiation. Thus mild thermal stress might be the natural environmental factor whose effects on DNA are imitated by ionizing radiation.

Table 1. Sensitive and resistant strains of E.coli.

Strain	Source	Radiation sensitivity mutations		
B/r	Witkin			
B_{s-1}	Hill	fil	hcr	exr
K12 AB1157	Howard-Flanders		rec	
K12 AB2463				
H/r 30-R				
R 15	Kondo		exr?	
NG 30			rec	

If this hypothesis is correct then certain predictions may be made. One of these is that there should be a correlation between radiation sensitivity and heat (52°C) sensitivity in related organisms. At 52° one would expect protein denaturation to be fairly slow and not enough to affect viability for some time. Damage to DNA might thus be the dominant factor in lethality. We have examined three radiation resistant strains of Escherichia coli and four related sensitive strains, the relative radiation sensitivities of which are determined by differences in repair or recovery capacity (see Table 1). Their sensitivities to incubation at 52°C and to gamma radiation are shown in Figure 1.

It is clear that radiation sensitive bacteria are also sensitive to heat. This suggests (a) that much of the damage contributing to inactivation at 52° resembles at least in some respects lethal damage from ionizing radiation (there is considerable evidence that the latter is largely inflicted directly or indirectly on DNA, Haynes, 1964: Moseley, 1968), and (b) that cellular systems responsible for recovery from damage inflicted by ionizing radiation

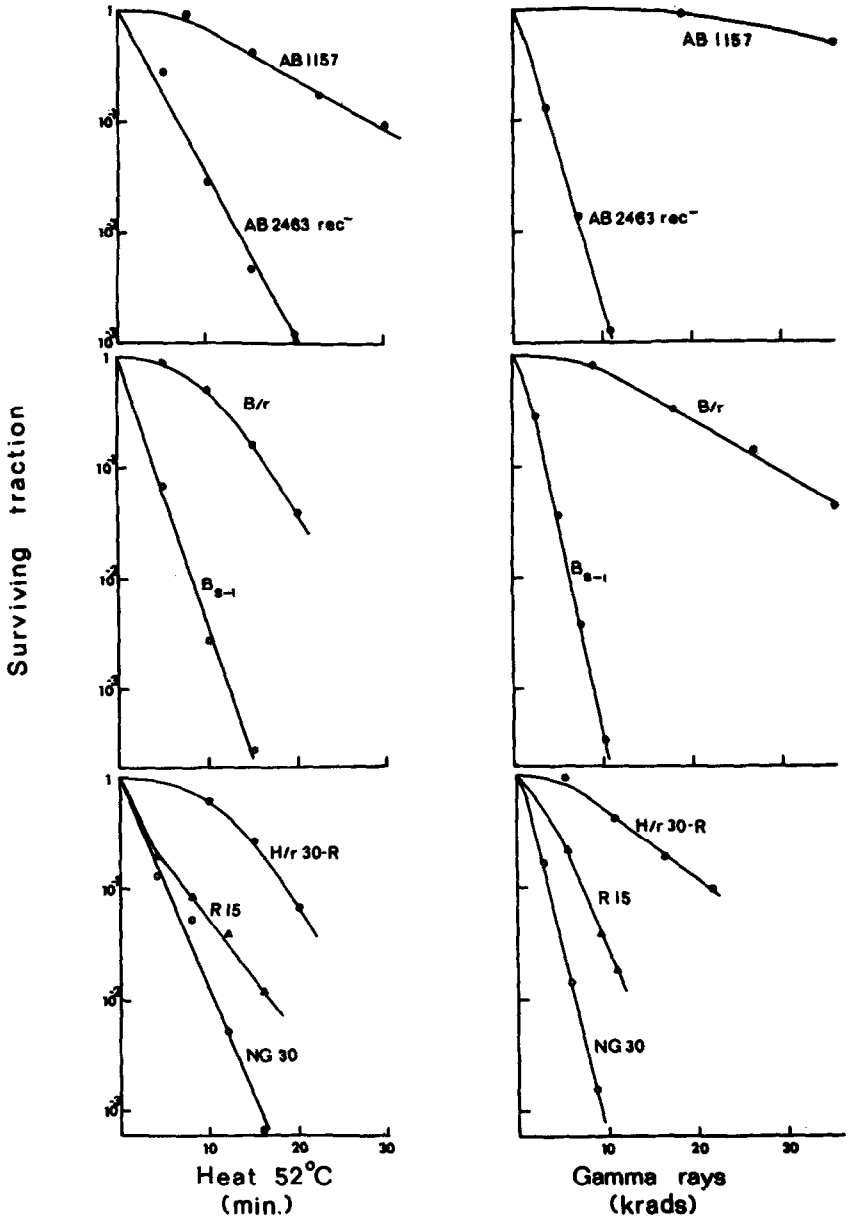

Figure 1. Survival of various strains of E.coli from exposure to gamma radiation under aerobic conditions or to a temperature of 52°C. Bacteria were grown to about $1.5 \times 10^8/cm^3$ in Oxoid Nutrient Broth No. 2, diluted after treatment in 0.067 M phosphate buffer pH 7 and plated at 37° on Oxoid Nutrient Broth No. 2 solidified with 1.5% Difco Purfied Agar. Irradiation was carried out while the bacteria were held in their growth medium below 5°C.

are also involved in recovery from damage caused during incubation at 52°C. We suggest that the normal function of these systems might be the repair or bypassing of single-stranded lesions in DNA which arise from hydrolysis of the backbone, from depurination, or from endonuclease attack during modest temperature rises or even during growth at normal temperatures (e.g. 37°C). The possible consequences of damage to DNA should obviously be considered in experiments where a heat treatment is used to inactivate cellular enzymes.

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Abstract: A correlation exists in E.coli between sensitivity to ionizing radiation and to thermal stress at 52°C. It is suggested that systems involved in recovery from damage inflicted by ionizing radiation are those which in a natural environment repair or bypass mild thermal damage, for example the breakage of single strands of the DNA duplex.

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