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Effect of Irradiated Medium on Strains of *Escherichia coli* Deficient in known Repair Systems of Ultraviolet Damage*

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Investigations on the lethal effects of gamma-ray irradiated medium on strains of *Escherichia coli* deficient in known systems of dark repair of UV damage revealed the possibility that this damage is not tackled by excision repair. Increased sensitivity of the *rec* A strain over *rec*$^+$, in contrast to the increased sensitivity of *uvr*$^+$ over *uvr* A, indicated the involvement of the recombinational process in the repair of this damage.
Irradiated salt solution and, to a lower extent, irradiated glucose, were some components of the irradiated minimal medium responsible for lethality. An inverse relationship between lethality and inoculum size was indicated suggesting lethality to be a function of the molecules of toxic substance generated per cell in the medium. A parallel observation was that the sensitivity of repair deficient strain increased with increase in gamma ray dose given to the medium.

Key Words: *Escherichia coli*, γ-ray **irradiated medium, UV repair deficient strains**

Introduction

Ionizing radiations are being increasingly advocated for sterilization of food and feed (Solan 1981) and bacteriological media (Altman et al. 1979). Since ionizing radiations have a component of indirect action as revealed by lethal and mutagenic action of irradiated media in bacteria (Chopra 1969, Aiyar & Rao 1977), plants (Swaminathan et al. 1962, Holsten et al. 1965), *Drosophila* (Swaminathan et al. 1963, Rinehart & Ratty 1965) and mammalian system (Kesavan & Swaminathan 1966, Hill & Berry 1967), it would be useful to understand the nature of biological lesions produced by irradiated media. The use of UV repair deficient strains of bacteria (Tatsumi & Nishioka 1977) has provided important means for classifying chemicals with DNA-modifying properties. A rapid *rec* assay has been devised in *B. subtilis* (Kada et al. 1972) and *E. coli* (Ichinotsubo et al. 1977) for screening chemical mutagens and carcinogens. We present

*This paper is dedicated to Dr M S Swaminathan on his 60th birthday which falls due on 7 August, 1985

information in this paper on the nature of action of cytotoxic and mutagenic principle in irradiated medium by employing repair deficient strains of bacteria.

Materials and Methods

(*i*) *Bacterial strains*: A list of strains used is given in table 1.

Table 1 *Strains* of Escherichia coli *used*

Strain	Genotypes related to DNA repair	Auxotrophic marker	Derived from	Reference
WP 2	*uvr*+	trp−	B/r	Witkin, 1967[a]
WP 2 *uvrA*	*uvrA*	trp−	WP 2	,,
KMBL 146WT	*rec*+	trp−	—	Mattern. et al. 1966[b]
KMBL 146 *recA*	*recA*	trp−	KMBL 146WT	,,

[a] The strains WP2 & WP2 *uvrA* were kindly suplied by Dr E M Witkin

[b] Strains KMBL 146 WT and its recombination deficient mutants were kindly supplied by Dr A Rorch

(*ii*) *Medial composition*: The minimal medium of Haas and Doudney (1957) supplemented with 0.4% (w/v) glucose and L-tryptophan (20 μg/ml) where required was used.

(*iii*) *Irradiation*: Gamma radiation at a rate of 15,000 rads per minute was obtained from a 2000 Ci ^{60}Co, Gamma Cell. Radiation exposures were given to the minimal medium or its glucose and salt component separately. In each case equalization of the volume of the solvent water was ensured. Starting inoculum for incubation in irradiated medium was about 5×10^6 cells/ml.

(*iv*) *UV irradiation*: The bacteria were grown to log phase (approx 5×10^8 cells/ml) A 3 ml cell suspension sample was placed in 5 cm (diameter) Petri dish, gently shaken and irradiated from a germicidal lamp giving 90% of its emission in wave-length 2537Å.

Dilution of cells for determining survival was made in physiological medium (0.01M, Mg_2SO_4).

Results and Discussion

Lethality

The strains that are isogenic for *uvr*^{A} show differences in sensitivity to incubation in irradiated medium (figure 1A). Incubation of *uvr*+ strain in irradiated medium for 4 hr. reduced the survival to 29.6% whereas in *uvr*^{A} there was only inhibition of growth which lasted for 4 hr but there was no killing. In strain KMBL 146 WT (*rec*+) 9hr incubation reduced the survival to 66.1% whereas in the *rec*^{A} derivative survival was 6.2% for the same period of incubation (figure 1B).

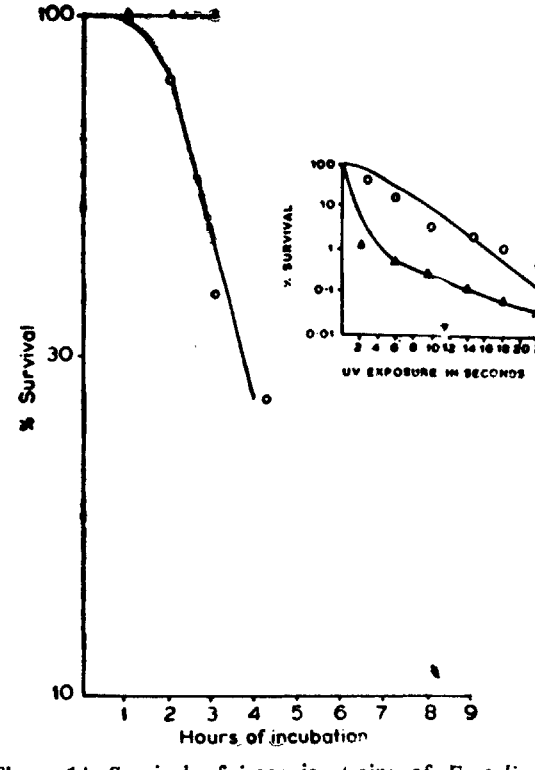

Figure 1A Survival of isogenic strains of *E. coli* differing for excision repair when incubated in minimal medium irradiated with 100 kr. gamma rays, (▲) *uvrA*, (O) *uvr*+, *inset shows survival of uvr*+ *and uvrA strains* when exposed to *uv* light (O) *uvr*+ (▲) *uvrA*

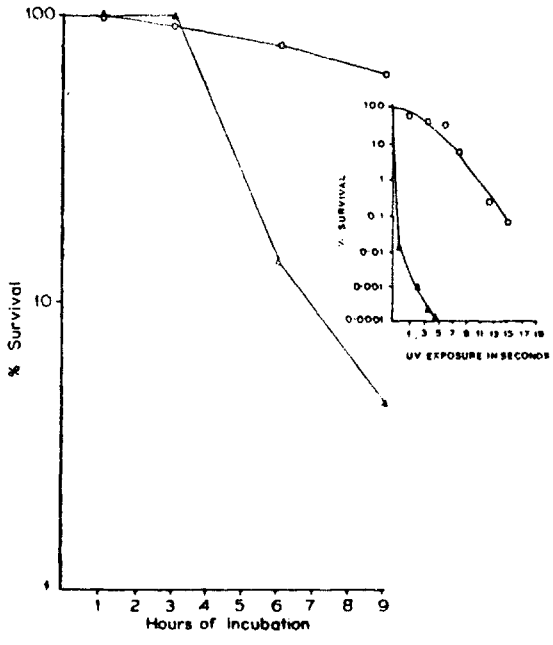

Figure 1B Survival of *E. coli* isogenic strains for recombinational repair on incubation in minimal medium irradiated with 100 kr of gamma rays (▲) *recA* (O) *rec*+; inset represents survival of *rec*+ and *recA* stain when exposed to uv light (▲) *recA*, (O) *rec*+

The effect of irradiated medium was studied at three inoculum densities (3.4×10^6, 10^5 and 10^4 cells/ml) for *uvr*+ and *uvr* A strains (figure 2) by fitting an (regression) equation of lethality (number of cells surviving per ml) on incubation period (hours) in irradiated medium (table 2). The relationship was observed to be curvilinear at the lowest density while at the other two densities studied, it was linear during the incubation period 1 to 4 hr. Such effects of irradiated medium and inoculum size on lethality have earlier been reported and explained (Chopra 1969, Pollard 1970, Keller & Pollard 1977).

Dose Response Relationship

The effect of irradiated medium was found to increase with increase in the dose of irradiation (table 3). Inhibition of growth was observed at lower doses and killing was induced at higher doses.

Component of Irradiated Medium Responsible for Lethality

With irradiation of only the glucose component of the medium, the *uvr*+ strain registered a survival of 87.6% for a 2 hr

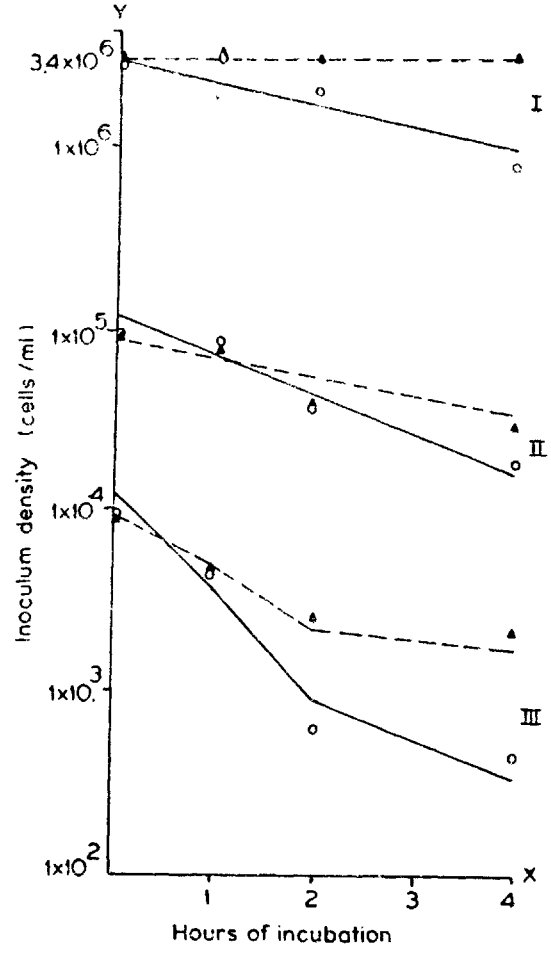

Figure 2 Effect of irradiated medium on *uvr*+ (O) and *uvrA* (▲) strains at different inoculation densities. Theoretical lines of best fit for *uvr*+ (—) & *uvrA* (—)

I *uvr*+ $Y=3.74-0.67X$

II *uvr*+ $Y=1.042-0.213X$

uvrA $Y=0.985-0.149X$

III *uvr*+ $Y=1.025-0.675X+0.107X^2$

uvrA $Y=0.987-0.527X+0.083X^2$

Table 2 *Regression equations between inoculum density and period of incubation at various inoculum sizes*

Inoculum size (cells/ml)	Strain	Regression equation	Regression co-efficient	Per cent survival in the first hour of incubation
3.4 units	uvr+	$Y = 3.74—0.67X$	*	82.1
(1 unit = 10^6)	uvr^A	—	—	100.0
1 unit	uvr+	$Y = 1.042—0.213X$	*	79.6
(1 unit = 10^5)	uvr^A	$Y = 0.985—0.149X$	*	84.9
1 unit	uvr+	$Y = 1.025—0.675X + 0.107X^2$	**	44.6
(1 unit = 10^4)	uvr^A	$Y = 0.987—0.527X + 0.083\ X^2$	**	55.0

Y, number of cells/ml, X, period of incubation in the irradiated medium (hours), *significant at 5% level **significant at 1% level

incubation, whereas irradiation of salt solution component resulted in 28.3% survival for the same strain (figure 3A). A similar observation was made with *rec* A strain (figure 3B). Survival was 27.3% for 6 hr incubation followed by resumption of normal growth when glucose solution was irradiated, and was 12.6% when the salt solution was irradiated. Further, the sensitivity of *rec*^{A} strain to the irradiated minimal medium or the irradiated salt solution component was approximately equal.

Table 3 (a) *Effect on survival of uvr+, uvr^A strains of* E. coli *incubated in medium exposed to four doses of Gamma rays*

Strain	Dose to medium (kR)	Per cent survival	Inhibition before growth resumption	
			Hr.	Min.
nvr+	25	100	0	30
	50	100	4	42
	75	73.3	—	—
	100	17.2	—	—
uvrA	25	100	0	10
	50	100	0	10
	75	100	6	42
	100	94.6	—	—

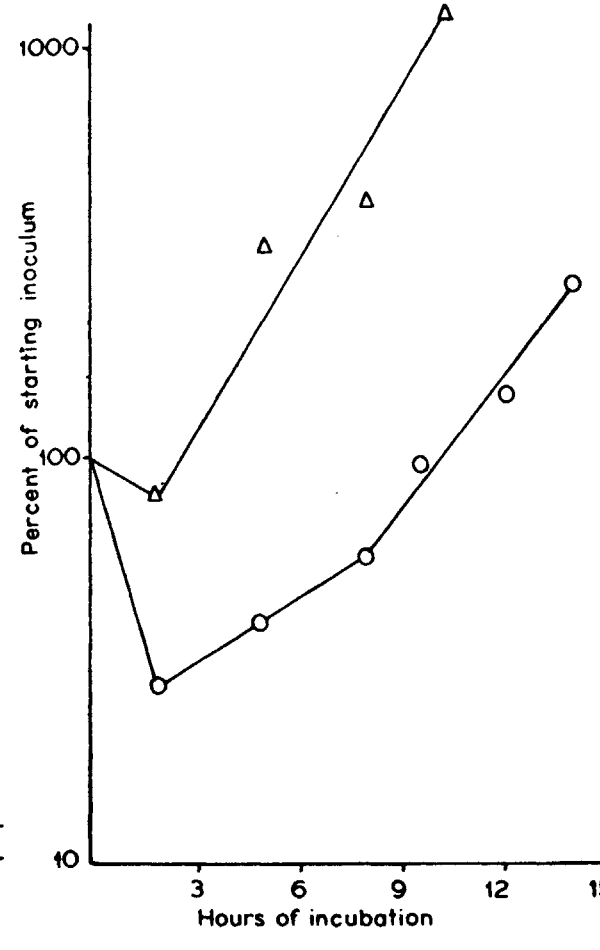

Figure 3A Survival of WP2 *urvA trp* strain treated separately with irradiated glucose solution (△) and irradiated salt solution (O)

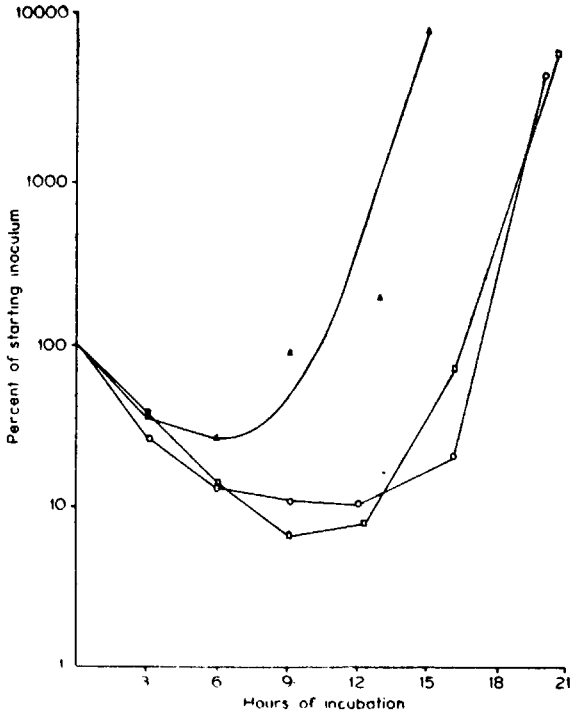

Figure 3B Survival of KMBL 146 *recA trp*⁻ strain grown separately in irradiated glucose solution (▲) irradiated salt solution (O) and in irradiated minimal medium (□)

Chopra (1966a, b, 1969) attributed the lethal effect of the irradiated medium to the radiation products of glucose in the minimal medium. In the present study, however, irradiated salt solution was found to make a major contribution to induce lethality. The difference possibly arises from differences in the chemical composition of the media irradiated. The minimal medium used in the present experiment differs from the M9 medium used earlier in its content of organic salt, sodium citrate. Schubert and Watson (1968), in *Salmonella typhimurium*, and others (Danphin & Saint Lebe 1977) have shown that the lethal effects of irradiated organic solutions is due to hydroxyalkyl peroxide derived from the interaction of carbonyl compounds with H_2O_2. One possibility

Table 3(b) *Effect on survival of rec+ and recA strains of* E. coli *incubated in medium exposed to four doses of Gamma rays*

Strain	Dose to medium (kR)	Per cent survival	Inhibition growth before resumption	
			Hr.	Min.
rec+	25	100	0	54
	50	100	2	32
	75	100	6	42
	100	78.9	—	—
recA	25	100	0	66
	50	100	3	12
	75	44.7	—	—
	100	18.8	—	—

for the major contribution of irradiated salt solution to lethality could be the production of such compounds from sodium citrate. Further, related organic salts such as sodium formate (Garrison et al. 1958, Hardwick 1960) are known to generate a range of products including glyoxal like compounds on exposure to ionizing radiation and glyoxal (HCO_2) has been found to be lethal and mutagenic for bacteria (Chopra 1966a).

Effect of Different Repair Systems on the Sensitivity of Bacteria to Irradiated Medium

In our experiments we find *rec*ᴬ strain to be more sensitive than the *rec*+ strains, implying that the recombination repair is involved in the correction of damage produced by irradiated medium. On the other hand, *uvr*+ has been found to be more sensitive to the effect of irradiated medium than the *uvr*ᴬ counterpart. *Uvr*ᴬ strain of *E. coli* B has been reported to show greater resistance to damage by X-rays under aerobic conditions compared to *uvr*+. This has been attributed to the involvement of a lesion in the sensitive strain different from the one that is normally repaired by the resistant parent (Alper 1967, 1968).

Also, Pollard (1970) has shown that cells characterized as radiation sensitive can be classified as very resistant on the criterion of oxygen respiration; Bs-1 being a notable

example. Incubation of cells in irradiated medium results in the inhibition of respiratory activity (Pollard 1970) and therefore strains with better oxygen respiration will be relatively better equipped to counter the effect of irradiated medium. The greater resistance of *urv*^{A} compared to *uvr*+ to irradiated medium may be due to such causes.

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