HOST CELL REACTIVATION OF GAMMA-RAYED T1

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In earlier work (Sauerbier, 1961; 1962a, b) we had postulated the existence of a host cell reactivating (HCR) enzyme. This enzyme should be capable of dark repair of ultraviolet light (2537 Å) damage to the DNA of several phages as T1, T3, T7, λ , P22. By this repair, UV damage is eliminated from the DNA of T1 (Sauerbier, 1964a). Thymine dimer, which to the present state of knowledge is the major photoproduct responsible for UV inactivation (Setlow and Setlow, 1962), is not monomerized by HCR in T1 (Sauerbier, 1964b). Recent findings (Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964) show that HCR excises thymine dimers from UV irradiated bacterial DNA in form of di- or oligonucleotides. Thus, HCR might be a versatile repair agent capable of eliminating various kinds of damages from DNA. It is, therefore, conceivable that also DNA damaged by ionizing radiations is subjected to repair by HCR.

To test this notion, the survival of gamma-rayed T1 was determined under conditions of HCR, and when HCR was eliminated. We employed three different methods to prevent HCR: (1) Parallel plating on indicator bacteria capable of performing HCR, and incapable of HCR (Hill 1958; Harm, 1963; Rörsch et al., 1963). (2) Inhibition of HCR by caffeine (Sauerbier, 1964a; Metzger, 1964). (3) Elimination of HCR by UV irradiation of the host bacteria (Garen and Zinder, 1955).

Irradiations of T1 were performed with Cobalt-60 gamma sources $^{+}$) (dose rate about 100 krad/hour), $^{++}$) (dose rate about 250 krad/hour). At irradiation phages were suspended in a 5-fold nutrient broth solution

(40 g Difco nutrient broth, 5 g NaCl, 1 l H_2O) to minimize indirect radiation effects.

For UV irradiation of bacteria a low pressure mercury vapour lamp (OSRAM HNS12) was used.

Fig. 1 shows the survival of gamma-rayed T1 when plated with indicator bacteria E. coli B and B_{s-1}, respectively. Up to 22% of the lethal damage is repaired by HCR. Parallel plating with E. coli K12 hcr^+, and with K12 hcr^- (Harm, 1963); and with E. coli C syn^+, and with C syn^- (Rörsch et al., 1963) yielded exactly the same differences in survival.

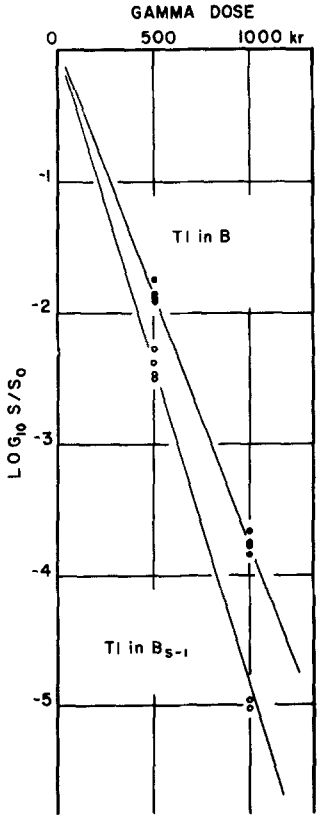

Fig.1

Survival of Co-60 gamma-rayed T1 on E. coli B (capable of HCR, upper curve), and on strain B_{s-1} (incapable of HCR, lower curve).

+) Irradiated at Corvallis; dose rate about 100 krad/hour.

Elimination of HCR by caffeine: To test the effect of caffeine on the survival of gamma-rayed T1, we measured the survivors in C syn^+, and

in C syn in the presence and in the absence of caffeine; (Experimental conditions were those described by Sauerbier, 1964a). If HCR really causes the higher survival of gamma-rayed T1 in C syn^+ (or in B, respectively), then we should expect (1) that caffeine lowers the survival in syn^+ to the values obtained with syn^-, and (2) that in syn^- the survival is not affected by caffeine. The values, shown in table 1, affirm these expectations.

TABLE I % survival^{+++}) of gamma-rayed T1 in C syn^+, and in C syn^- in the presence and in the absence of caffeine (mean values of 2 experiments). Irradiated at Köln, dose rate about 250 krad/h.

Dose in krad		0	250	500
C syn^+	- caffeine	100	1.05	0.038
	+ caffeine	100	0.36	0.0069
C syn^-	- caffeine	100	0.48	0.0065
	+ caffeine	100	0.475	0.0070

$^{+++}$)The sensitivity of T1 to gamma-irradiation, as determined following irradiation at Köln, is twice as high as the value obtained at Corvallis, Oregon. This difference might be real, depending on the dose rate, or might be due to inaccurate dose estimations. It does not influence our considerations.

Elimination of HCR by UV irradiation of host bacteria: In these experiments the survival of extracellularly gamma-rayed T1 was determined when adsorbed to C syn^+, and to UV irradiated C syn^+ (having received an incident 254 mμ UV dose of 1800 erg/mm^2; irradiated at 2 x 10^8 cells/ml in T1-adsorption medium, Watson, ref. Benzer, 1952). The results are shown in table 2.

TABLE II % survival of extracellularly gamma-rayed T1 in C syn^{+}, and in C syn $^{+}$, which has been UV irradiated prior to T1 infection (1800 erg/mm^{2} incident dose; mean values of 2 experiments). Irradiated at Köln, dose rate about 250 krad/h.

Dose in krad	0	250	500
unirradiated host bacteria	100	0.885	0.0355
UV irradiated host bacteria	100	0.172	0.0020

The lowered survival in UV irradiated hosts yields another evidence for the repair of gamma-ray damaged DNA by HCR.

In analogy to what is known with UV inactivated T1, we present three lines of evidence, which demonstrate HCR in gamma-rayed T1. This might mean that, either a considerable fraction of the gamma ray damage is identical to UV damage, or, that damages are different, but HCR is capable of repairing more than one type of damage.

Thymine dimer seems to be the major host cell reactivable (Sauerbier, 1961; Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964) and photoreactivable (Wulff and Rupert, 1962) UV photoproduct in DNA. Since the extent of photoreactivation with gamma-rayed T1 is very small compared with the extent of HCR (own observations), it is likely that little or no thymine dimer had been formed by the gamma irradiation (see also Wacker, 1963). We, therefore, interpret our results as follows: Repairable gamma and UV damages are different, but HCR is capable of repairing several types of damages to the DNA.

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