INHIBITION OF HOST CELL REACTIVATION IN PHAGE T1 BY CAFFEINE. +)

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Evidence has been compiled that host cell reactivation (HCR) of UV irradiated phage is due to direct repair of UV lesions (Sauerbier, 1961; 1962a, b). To explain our results we proffered the hypothesis of an enzymatic reversion of the UV photoproducts in the phage and bacterial deoxyribonucleic acid (DNA). Subsequent experiments by Metzger (1963) strongly support this view, although proof is still lacking. (See also: Howard-Flanders et al., 1962; Hill, 1958; Rörsch et al., 1963; Harm, 1963.)

The experiments described in this paper demonstrate (1) that HCR can be selectively inhibited by caffeine, and (2) that the host cell reactivable UV photoproduct is somehow eliminated by HCR from the DNA of UV irradiated T1 without requiring either synthesis of T1-messenger ribonucleic acid (m-RNA) or phage replication.

Experiments: The UV survival of T1 is determined after preadsorption to coli B, and plating on B_{s-1} (Hill, 1958), (Fig. 1, curve A). When T1 is preadsorbed in the presence of caffeine (2 gr/100 ml), diluted through buffer containing caffeine, and plated on caffeine supplemented plates (0.75 gr of caffeine/100 ml

+) Dedicated to Professor Dr. Boris Rajewsky on the occasion of his 70th birthday.

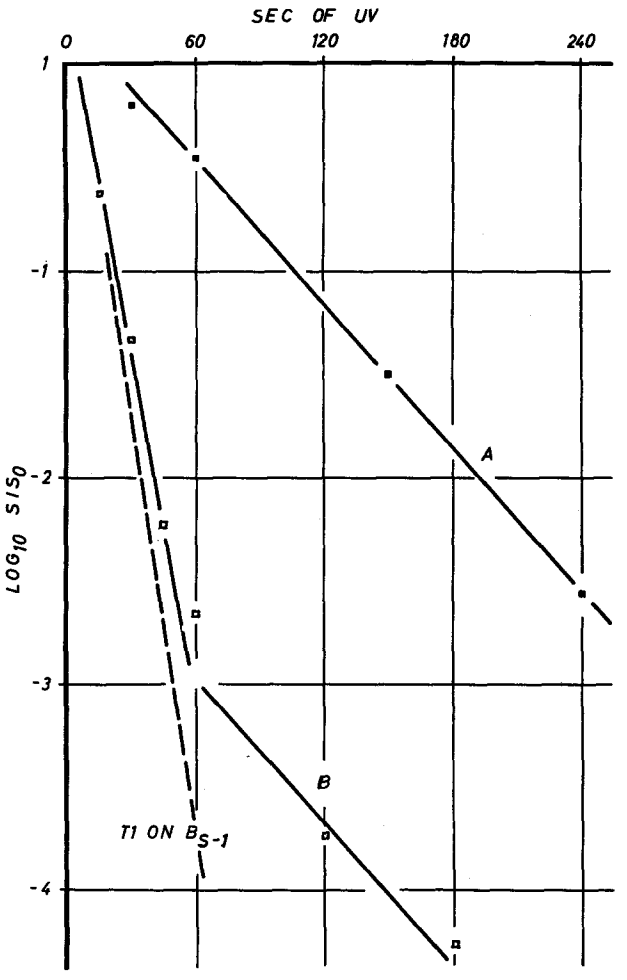

Fig. 1 Inhibition of HCR by caffeine. Survival of UV irradiated T1. Curve B: with caffeine. Curve A: without caffeine. Broken line: control plating on host without HCR.

in the top agar), curve B, Fig. 1, is obtained. HCR is abolished in more than 99% of the complexes. Direct plating of UV irradiated T1 with coli B on caffeine supplemented plates leads to the same result. There is no effect of caffeine on the UV survival of T1 when plated with B_{s-1}. (In these experiments adsorption bacteria were grown in M-9 (Anderson, 1949) to

2×10^8 cells/ml, chloramphenicol (CMP, 33 γ/ml) was added, and incubation was continued for 30 minutes at 37°. The bacteria were then shifted to T1 absorption medium (T1-ADS; Watson, ref. Benzer, 1952) containing M/500 KCN. Following the CMP treatment all bacteria perform HCR of T1 with the same efficiency as the 30% fraction at direct plating of UV irradiated T1 (e.g. see Sauerbier, 1962c). Irradiations were performed with a low pressure mercury vapor lamp, OSRAM HNS 12, emitting predominantly the 254 mμ line with an intensity of about 3 ergs/mm^2 sec at sample distance.

The second type of experiments was designed to determine (1) whether HCR occurs in the absence of m-RNA and of DNA synthesis, and (2) to measure the time course of HCR. As bacterial host we used the adenine and arginine requiring coli strain B94 (Gollub and Gots, 1959), in which RNA and DNA synthesis can be blocked by adenine withdrawal (Volkin, 1960). As a control we infected washed B94 with T1 and incubated in aerated M-9 at 37° for 120 minutes, in which time no progeny phage was released. (Strain B94 was kindly supplied to our lab by Dr. E. Volkin.)

Experiment: B94, thoroughly starved for adenine and for arginine, was infected with UV irradiated T1 and incubated in in M-9 (+ 6-azauracil, 250 γ/ml; see Pardee and Prestidge, 1960). Then, HCR was blocked at different times after infection by plating the complexes with B_{s-1} on caffeine supplemented tryptone plates. The fraction of plaque forming complexes is given in Fig. 2, curve B. In a rapidly increasing fraction of the complexes HCR becomes insensitive to caffeine. After 10-15 minutes most complexes apparently had performed HCR in the absence of m-RNA and of DNA synthesis. (Attention is drawn to the fact that the block

of HCR must be delayed by the time needed to diffuse the caffeine into the cells.) The result is in good agreement with the lengthening of the latent period of UV irradiated T1 from 13 to about 20 minutes. When the complexes are plated on plates free of caffeine - i.e., if HCR is not blocked - Fig. 2, curve A is obtained.

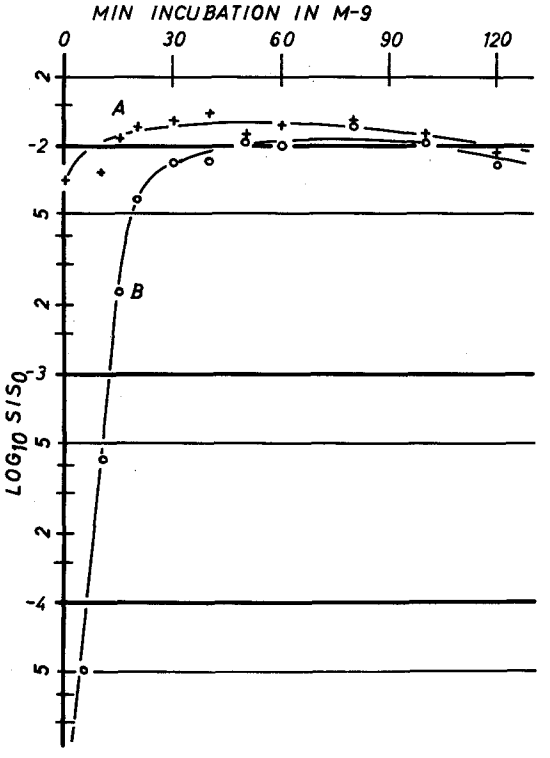

Fig. 2 Time course of HCR in the absence of m-RNA and of DNA synthesis. Plating on caffeine plates at various times after infection. Curve B: caffeine plate. Curve A: control plate. Survival of UV irradiated T1 (150 secs), preadsorbed to B94 (starved for adenine and arginine), and shifted at time zero to M-9 (+ 250 γ/ml 6-azauracil) at 37°.

Curve B, Fig. 2, yields no information on the kinetics of HCR, since two models are compatible with the results.

(1)HCR is a randomly occurring, instantaneous event in each bacterial cell. At any given time, there are two populations of complexes: those in which HCR has occurred and those in which HCR has not yet occurred. Longer times of incubation increase the probability that HCR has occurred in any given complex.

(2)HCR is a gradually occurring process in every complex - i.e., there is only one population of complexes. Each reactivable UV lesion is reactivated with equal probability. The probability of being reactivated increases with longer times of incubation.

If complete T1 UV survival curves are measured with B94 (pre-treated as described above), assumption (1) would predict a series of biphasic curves when caffeine is added at different times after infection. Assumption (2) predicts monophasic curves with different slopes. Fig. 3 shows that (2) is essentially correct. Thus, we may conclude from Fig. 2, curve B, that HCR is proceeding in every complex at approximately the same rate.

Discussion:

(1) These experiments show that caffeine in concentrations of about 10^{-2}M is an efficient and selective inhibitor of HCR in phage T1. (2) HCR is not inhibited by caffeine, added after prolonged periods of incubation (Fig. 2). This implies that within a relatively short time HCR has achieved a state in which it is no longer sensitive to caffeine. (3) The occurance of HCR in the absence of m-RNA and of DNA synthesis suggests that HCR directly alters the reactivable UV photo-product in the phage DNA.

We have no detailed knowledge of the mode of caffeine action on HCR. Since photoreactivation proceeds in the presence of

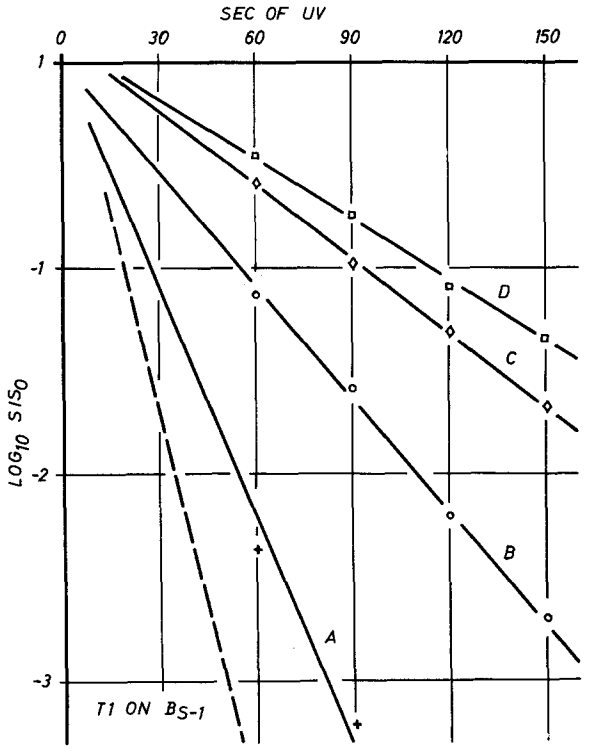

Fig. 3 Survival curves after intermediate degrees of HCR. UV survival of T1, preadsorbed to CMP treated, and adenine-arginine starved B94, plated on caffeine plates after various times of incubation in M-9 (+ 250 μ/ml 6-azauracil) at 37°. Curve A, after 0-3 min; B, after 10 min; C, after 30 min. Curve D is plated after 40 min on plates without caffeine.

caffeine (Metzger, 1963, personal communication), it probably does not form a complex with the reactivable UV photoproduct thus sterically inhibiting the formation of an enzyme-substrate complex. More likely, the caffeine is bound reversibly to the HCR enzyme itself, since it can be readily diluted out without affecting HCR under conditions where de novo synthesis of HCR enzyme is impossible.

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