ACRIFLAVINE INHIBITION OF DARK REPAIR AND LATE GENERATION DEATH

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Acriflavine enhances the level of γ and U.V. damage in some bacteria (Alper 1963, Witkin 1963) and of U.V. damage in some stages of cell development in diploid spores of Chlamydomonas reinhardi (Davies 1965). It is known to block host cell reactivation (Feiner and Hill 1963), and to have a greater effect in systems possessing than in those lacking certain repair activities (Alper 1963). Furthermore in the experiments with Chlamydomonas (Davies 1965) it decreased survival in those stages having sigmoid survival curves and large shoulder regions (Fig. 1), whereas in those stages showing exponential responses it had no effect. On the basis of these findings acriflavine is considered to exert its effect by inhibiting certain repair processes.

Experimental findings now indicate that in Chlamydomonas this acriflavine effect, though operating very soon after irradiation, manifests itself only after several cell generations have elapsed.

Materials and Methods. Wild type haploid strains of C. reinhardi of + and - mating type were maintained, and diploid spores produced by the standard techniques described elsewhere (Davies 1965). A Phillips 30 W TUV germicidal lamp (90% of the output at 2537 Å) and a 4000-curie ^{60}Co Hot Spot, were the sources of irradiation. The incident energy deposited by the former at the lamp to target distance used was 125 ergs/mm^2 per sec, and the dose rate in the Hot Spot was 17,500 rads per min.

The organisms were grown under 500 c. light from "Daylight" fluorescent tubes; photoreactivation (1 hour at 1000 ft. c.) was also carried out using these same tubes. The criterion of survival was the production of a 100-cell colony from a

single irradiated cell. Acriflavine was incorporated in the post-irradiation agar medium and used at concentrations of 8-15 μgms/ml - these concentrations having no effect on viability in the controls.

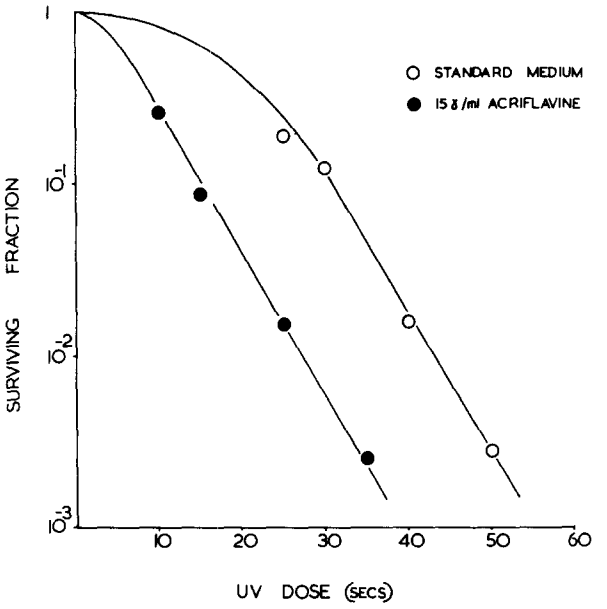

Fig. 1 Survival of UV irradiated diploid spores under different post-irradiation conditions. UV dose 125 ergs/mm^2 per sec.

Results. The response of haploid cells exposed to U.V. corroborates the interpretation that acriflavine acts by inhibiting repair. The survival curves of these cells have only a small shoulder region in comparison with the diploids, and if this is indicative of a lesser repair capacity (Haynes 1964) then the lack of an acriflavine effect is readily understood (Fig. 2).

In contrast to the response obtained following exposure of cells to U.V., acriflavine had no effect on the survival of diploid Chlamydomonas cells exposed to γ radiation (Fig. 3). These cells have elsewhere been shown to have the capacity to repair γ damage (D. R. Davies, in press). Thus acriflavine interferes with some step in U.V. repair which is not necessary for γ repair. If an analogy can be made

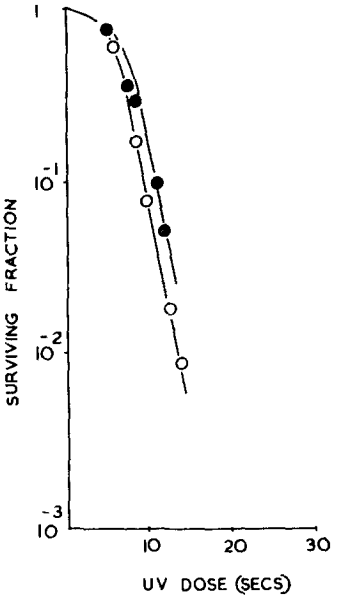

Fig. 2 Survival of UV irradiated haploid cells under different post-irradiation conditions.
0 ... standard medium; ● ... plus acriflavine. UV dose 125 ergs/mm^2 per sec.

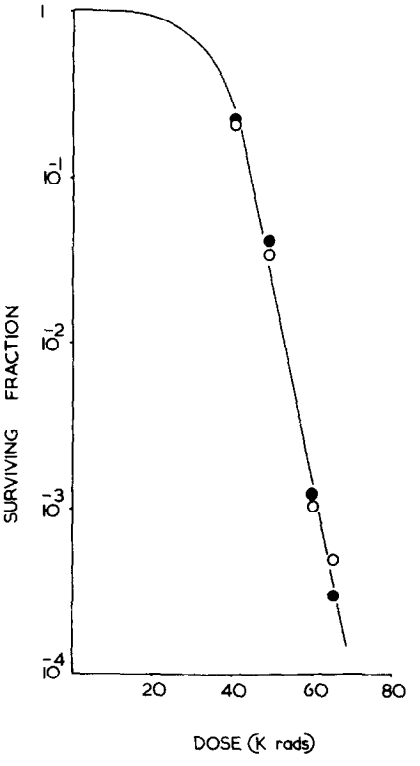

Fig. 3 Survival of γ irradiated diploid spores under different post-irradiation conditions. 0 ... standard medium, ● ... plus acriflavine.

with the responses of U.V.-sensitive bacteria (Emmerson and Howard Flanders 1965) that step may be the one involving the initial cut or excision in the D.N.A. Following U.V. exposure this excision must occur before D.N.A. breakdown and dark repair can be accomplished, but γ radiation may itself produce such breaks and so this step may not be necessary.

Photoreactivation (PR) of U.V. damage is not inhibited by acriflavine (Fig. 4, and Witkin 1963). However, the PR effect is not dose-modifying in its presence - a considerable part of that damage normally reparable by a dark repair process can

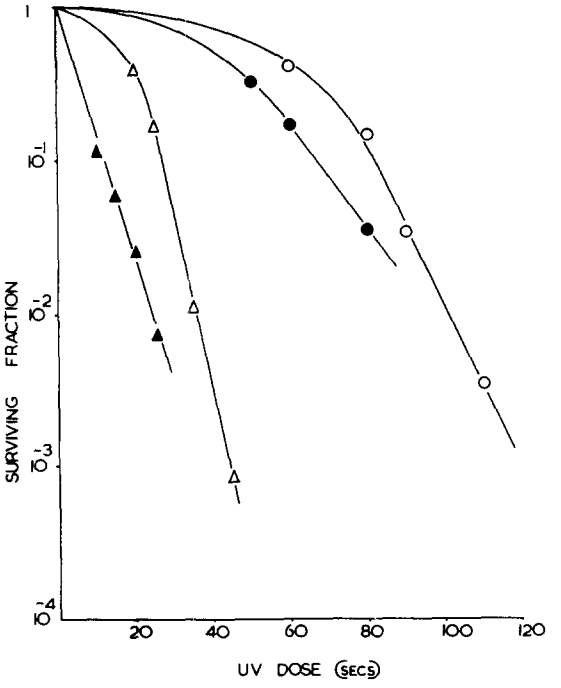

Fig. 4 Survival of UV irradiated diploid spores in the presence (O) and absence (Δ) of photoreactivation. Open symbols, standard medium; closed symbols, plus acriflavine. UV dose 125 ergs/mm^2 per sec.

be repaired by PR. A similar pattern of response may well occur in Witkin's data (Fig. 1 1963) though she considers PR to be dose-modifying in the presence and absence of acriflavine. If the primary lesions repaired by PR are thymine or other pyrimidine dimers (Setlow 1965) then it can be assumed that a considerable proportion

of the lesions repaired by the dark system, and with which repair acriflavine interferes, are also these dimers. It is unlikely that the PR effect is due to a photoprotective process (Jagger 1964); if photoprotection can occur in this organism then it would occur even under "dark conditions", as the organism is maintained under the fluorescent lights used for PR, at all times up to the onset of U.V. or γ irradiation.

Acriflavine is effective in inhibiting dark repair only in the first few hours after U.V. exposure (Fig. 5), and an acriflavine challenge 24 hours after

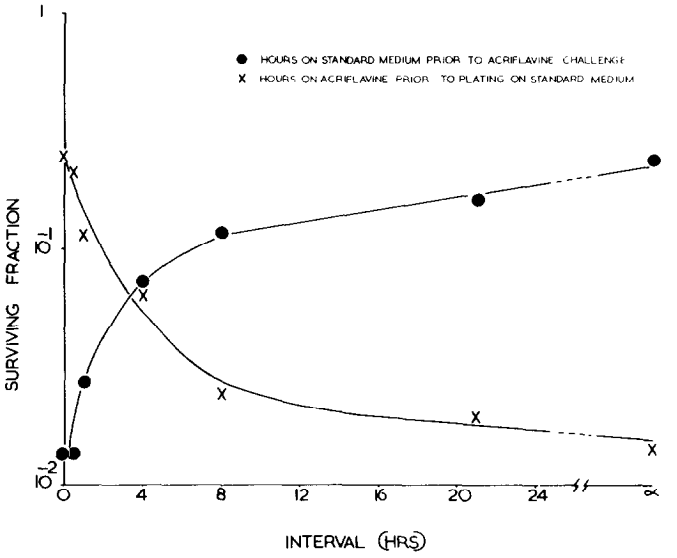

Fig. 5 Effect of an acriflavine challenge after various post-irradiation intervals, and of different periods of acriflavine treatment, on the survival of UV irradiated (3750 ergs/mm^2) diploid spores.

irradiation has little effect. (The time to the first division in unirradiated cells is 9 hours, but after exposure to a dose giving 20% survival it is approximately 40 hours.) However, the inhibition of dark repair mainfests itself by enhancing lethality several cell generations away from that which was irradiated. In the absence of acriflavine the cells almost invariably die before cell division (Figs. 6 and 7). The significant fact is that those cells which are normally

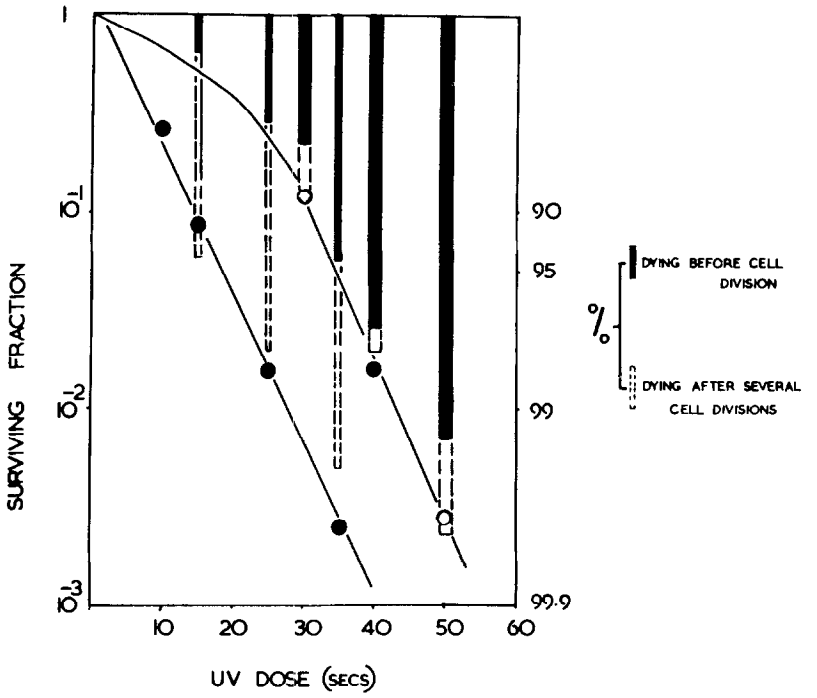

Fig. 6 Stage at which UV irradiated diploid spores die, in the presence and absence of acriflavine post irradiation. UV dose 125 ergs/mm^2 per sec.

rescued by dark repair but which in the presence of acriflavine are killed, die after dividing several times to form a colony.

Discussion. The Chlamydomonas PR data indicate that the primary U.V. lesions normally repaired by the dark system are pyrimidine dimers; Setlow (1964) has shown that acriflavine retards the rate at which one of these - thymine dimers - are excised from the D.N.A. of E. coli B/r. If a similar delayed or inhibited excision occurs in Chlamydomonas then it is difficult to equate this with the delayed death. In the first place, if any D.N.A. replication can occur around the dimer, analogous to that which can occur in vitro (Bollum and Setlow 1963), then the newly synthesised strand would have either a deletion, incorrect bases incorporated, or the normal sequence restored. It is difficult to visualise why the two first lesions should almost invariably result in a delayed death.

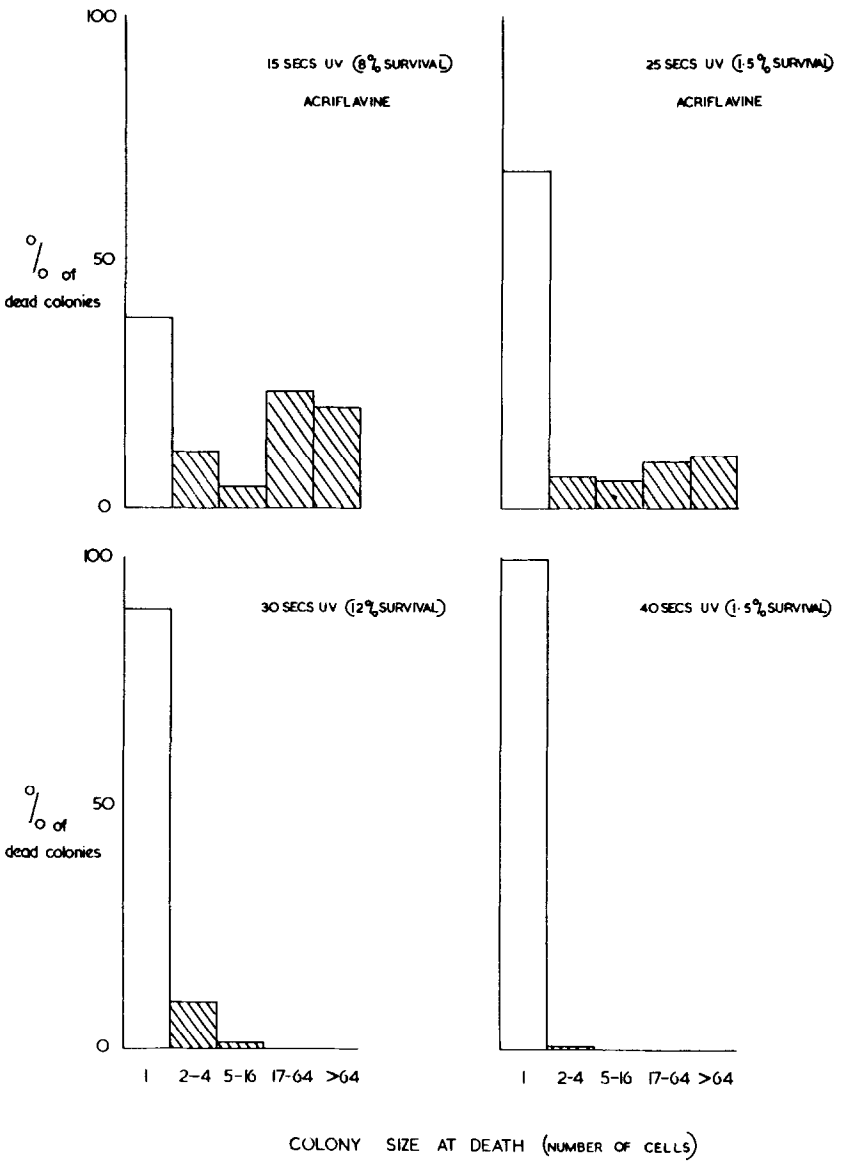

Fig. 7 The effect of acriflavine on the number of cell divisions which lethally damaged diploid spores undergo, following exposure to UV. UV dose 125 ergs/mm^2 per sec.

Secondly, even if acriflavine impedes the excision of lesions, such non-excision probably occurs also in those cells killed by U.V. irrespective of acriflavine, yet these die before dividing. There is no reason for assuming that such cells have a different class of lesions, since it has been shown (Davies 1965) that growth at a

lower temperature post irradiation results in a greater amount of dark repair, and a rescue of some of these cells. Furthermore, those cells in the latest stages of the cell cycle which have a reduced repair capacity (Davies 1965) and which are unaffected by acriflavine, invariably die before division. Thus the acriflavine effects are difficult to equate with this type of D.N.A. lesion. Acriflavine greatly enhances the U.V. inhibition of R.N.A. and protein synthesis (Doudney et al. 1964, Clarke 1966). Acriflavine could act by complexing with e.g. ribosomal R.N.A. and inactivating it - the presence of a pool of products delaying death for a few generations. However, it would then be difficult to relate this to the fact that such a depletion only occurred in those cells normally dark repaired, and to the fact that PR eliminated much of the acriflavine effect.

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