PHOTOREACTIVATION: A RADIATION REPAIR MECHANISM ABSENT FROM MAMMALIAN CELLS

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Photoreactivation (PR) is a repair mechanism associated with the recovery of organisms from damage caused by ultraviolet (UV) light, and has been demonstrated in many microorganisms and higher organisms (Jagger , 1958; Blum, 1959; Setlow, 1966). However, PR has not yet been clearly and convincingly demonstrated in mammalian cells. In this report PR has been investigated in four types of mammalian cells, using an experimental system with which PR was clearly and easily demonstrated in other organisms.

Three established cell lines and one primary culture were used in these experiments. The established lines were human skin fibroblasts NCTC 2544, human amnion FL, both of which were grown in F10 medium with 20% calf serum, and mouse L grown in Eagles medium with 10% bovine serum. Explants from the hearts of 3 day old mice grown in 199 medium with 10% bovine serum were also used to obtain primary fibroblast cultures (all media and sera were obtained from Grand Island Biological Co.). Both the NCTC 2544 and the L strains originated from the skin, so that before they were cultured these cells normally experienced some visible light and PR might be expected in these cells, at least. Monolayer cultures were grown on glass coverslips in Leighton tubes, and for UV irradiations the coverslips were removed from the tubes and the cultures irradiated through a thin film of medium. The temperature was maintained at 37°C throughout the experimental treatments.

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The UV source was a General Electric germicidal lamp and a constant flux of 20 ergs/mm^2/sec at 2537A° was used throughout the experiments. After irradiation, the cultures in Leighton tubes were illuminated with PR light for periods of time up to 2 hours and the medium was always changed at the end of illumination. Conventionally, exposure to UV light is referred to as irradiation and exposure to PR light as illumination. The source of PR light was to General Electric black light lamps which gave predominantly light at 3600A°. A constant flux of 2 x 10^4 ergs/mm^2/min, filtered through 1 cm of plate glass, was used for PR. Using this experimental arrangement it was possible to demonstrate PR of the survival of E coli B and also PR of the UV-induced browning of the skin of the fruit of a higher plant, Musa sapientium (Hausser and von Oehmcke, 1933). In the case of E coli B, 5 x 10^5 ergs/mm^2 of PR light increased the number of colonies formed after irradiation with 800 ergs/mm^2 of UV by a factor of x 6. In the case of the plant, 1500 ergs/mm^2 of UV caused the skin of the fruit to brown within 8 hours of the irradiation, and this was completely prevented by 2 x 10^5 ergs/mm^2 of PR.

DNA synthesis was assayed by adding ^{3}HTdr to the medium at the following final strengths: NCTC 2544 - 2.5μc/ml, 0.065c/mmol, L strain - 2.5μc/ml, 3c/mmol, FL strain - 1.25μc/ml, 3c/mmol, mouse heart - 5μc/ml, 1.9c/mmol. After incubation in labelled medium cultures were fixed in acetic acid/alcohol (1:3) for 15 mins and air dried. Autoradiographs were prepared with Kodak NTB3 liquid emulsion and after exposure these were developed in D19 and the cultures stained through the emulsion with Ehrlich haematoxylin. Mean grain counts were determined by counting the grains over 50 labelled cells, and the fraction of labelled cells and the mitotic index determined by counting at least 500 cells.

In the first series of experiments the effect of increasing fluxes of PR light on the human skin cells was determined by irradiating cultures with 500 ergs/mm^2 UV and then illuminating with PR for periods between 1 minute and

2 hours. The ^{3}HTdr was added at the start of the illumination, and the medium changed for identical labelled medium at the end of each illumination period. All cultures were fixed after 2 hours in labelled medium. The mean grain counts and mitotic indices obtained are shown in table I. There was no stimulation of ^{3}HTdr incorporation (i.e. DNA synthesis) or of mitosis. Above 17 x 10^5 ergs/mm^2 PR light appears to have had a slight inhibitory effect additional to that caused by the UV irradiation. A flux of PR light of 2 x 10^5 ergs/mm^2 (10 minutes illumination) was chosen for all of the subsequent experiments.

Table I. Incorporation of ^{3}HTdr (2.5µc/ml, 0.065c/mmol) into DNA in Human Skin cells (NCTC 2544) in UV irradiated and PR illuminated cultures during a 2 hour labelling period (^{3}HTdr added after irradiation and present during PR illumination

UV flux (ergs/mm^2)	PR flux (ergs/mm^2)	Mean grain count	Mitotic Index
0	0	67.2	3.8%
500	0	30.8	2.8%
"	0.2 x 10^5	28.5	1.6%
"	2 x 10^5	30.4	1.8%
"	6 x 10^5	30.3	1.8%
"	11 x 10^5	29.3	1.4%
"	17 x 10^5	29.5	0.6%
"	23 x 10^5	26.5	2.6%

In the second series of experiments cultures of NCTC 2544, L strain and FL strain were irradiated with 100 or 500 ergs/mm^2, and primary cultures of mouse heart with 60 ergs/mm^2, and then illuminated with 2 x 10^5 ergs/mm^2 of PR light. Following illumination, ^{3}HTdr was added and cultures fixed at intervals thereafter. Mean grain counts were determined for each culture, and for the NCTC 2544 strain the labelled fractions and mitotic indices were also determined. The results are shown in figures 1, 2 & 3. Three effects of UV are distinguishable in the results of figure 1 : an inhibition of mitosis, an inhibition of the initiation of DNA synthesis (from the

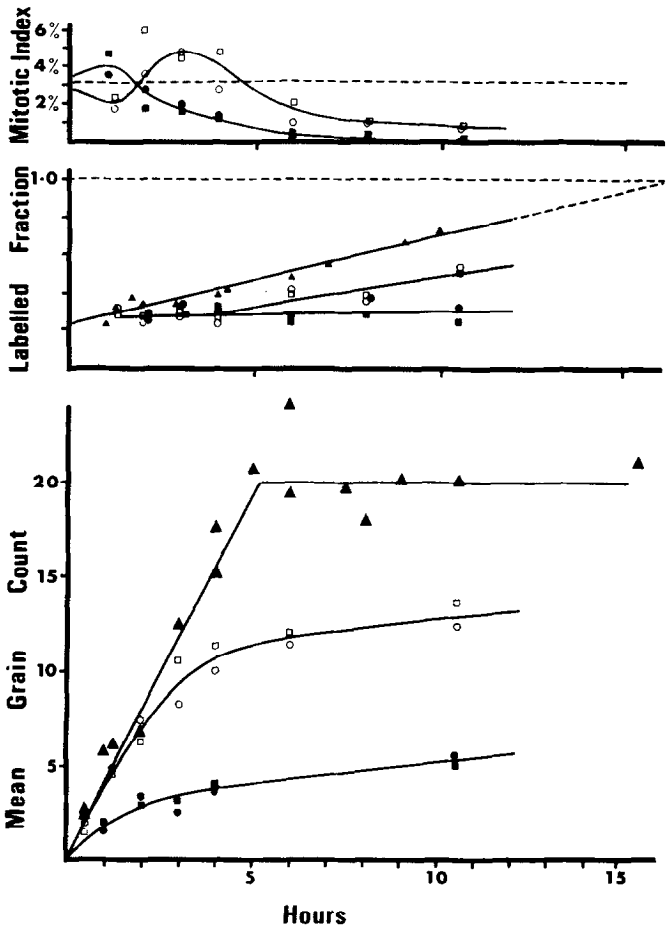

Figure 1. Mean grain counts, labelled fraction and mitotic index as a function of time in ^{3}HTdr, 2.5μc/ml, 0.065c/mmole, for human skin fibroblasts NCTC 2544. ▲-control, □-100ergs/mm^2 UV followed by 2 x 10^{5}ergs/mm^2 PR, ■-500ergs/mm^2 UV, ●-500ergs/mm^2 followed by 2 x 10^{5}ergs/mm^2 PR.

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fact that the fraction of labelled cells in irradiated cultures does not increase with incubation time in ^{3}HTdr) which is temporary after 100 erg/mm^2 and permanent after 500 ergs/mm^2, and an inhibition of DNA synthesis. An inhibition of DNA synthesis in L strain, FL strain and mouse heart fibroblasts is also shown in figures 2 & 3. Details of the radiation effects have been discussed elsewhere (Cleaver, 1965; 1966 a, b). It is clear that none of the effects of UV in these experiments are modified to any extent by illumination with PR light in either the cell strains which originated

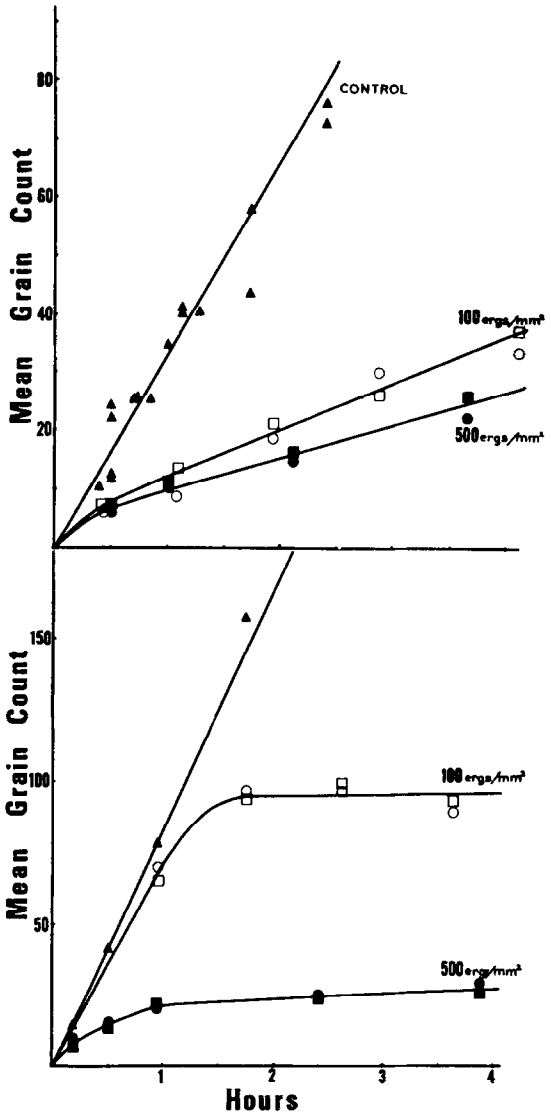

Figure 2. Mean grain counts as a function of time in ^{3}HTdr. Top - L Strain, 2.5μc/ml, 3c/mmole. Bottom - FL Strain, 1.25μc/ml, 3c/mmole. ▲ -control, □ -100ergs/mm^2, 0-100 ergs/mm^2 UV followed by 2 x 10^{5}ergs/mm^{2}PR, ■-500ergs/mm^2 UV, ●-500ergs/mm^2 followed by 2 x 10^{5}ergs/mm^2 PR.

from skin or the other cell strains. In preliminary experiments (unpublished) PR of cell killing in NCTC 2544 or L strain also could not be demonstrated. The PR repair system is consequently either absent from these mammalian cells or much more difficult to demonstrate than in other organisms.

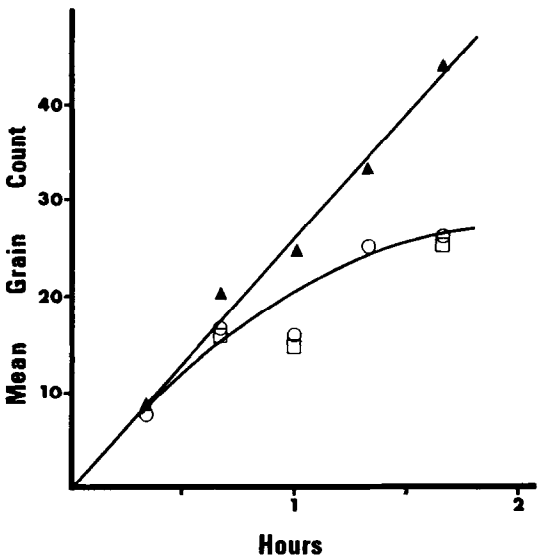

Figure 3. Mean grain counts as a function of time in ^{3}HTdr, 5μc/ml, 1.9c/mmole, for mouse heart fibroblasts in primary culture. ▲ -control, □ -60ergs/mm^2 UV, 0-66ergs/mm^2 UV followed by 2 x 10^{5}ergs/mm^2 PR.

A summary of the results of PR experiments in mammalian cells is given in table 2, and with three exceptions, every mammalian cell tested has failed to give any evidence of PR. The few cases in which positive results were obtained (Riecke & Carlson, 1955; Logan et al, 1959; Roe & Stevens, 1965) were neither as large nor as unambiguous as the PR which can be demonstrated in microorganisms. It is possible that repetition of these experiments may fail to confirm the original positive results, as occurred when an attempt was made (Chu, 1965) to confirm earlier observation of PR of chromosome aberrations (Chu, 1962). In view of the results summarised in table 2, PR is probably completely missing from mammalian cells and since PR is present in a large number of other organisms, both vertebrate and invertebrate (Jagger, 1958; Blum, 1959; Setlow, 1966), its absence from mammalian cells needs explanation. At the biochemical level PR is probably absent because mammalian cells do not possess the PR enzyme system. Since PR mainly affects UV induced thymine dimers and not other UV induced damage (Setlow,

Table 2. Summary of observations on photoreactivation in mammalian cells. (In some cases cited no experimental results were given, but the absence of PR mentioned in the text of the paper).

Cell type	Criterion	PR	Reference
Hela	cell killing	no	Montgomery et al(1964).
Chinese hamster V79	cell killing	no	Sinclair & Morton (1965)
Ascites	cell killing	yes ($in N_2$) no ($in O_2$)	Roe & Stevens (1965)
NCTC 2544 (human skin fibroblast)	DNA synthesis cell killing	no	see text
L strain	DNA synthesis cell killing	no	" "
FL strain (human amnion)	DNA synthesis	no	" "
mouse heart fibroblasts	DNA synthesis	no	" "
Chinese Hamster	DNA synthesis	no	Rasmussen & Painter (1964)
Hela	DNA synthesis	no	"
Chinese hamster	Chromosome aberrations	no	Chu (1965)
Chinese hamster	Thymine dimer cleavage	no	Trosko et al (1965)
isolated rat liver nuclei	amino acid incorporation	yes	Logan et al(1959)
mouse (whole animal)	carcinogenesis	no (PR increased carcinogenesis)	Griffen et al (1955)
" "	carcinogenesis	no	Kelner & Taft (1956)
" "	survival	yes	Rieck & Carlson (1955)

1964), dimers may not be the particular UV photoproduct that is responsible for the biological effect of UV on mammalian cells. The presence of PR as a radiation repair mechanism in most organisms with the exception of mammalian cells may eventually provide us with an insight into the evolution and significance of repair mechanisms in general, although it remains enigmatic at present.

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