On the two mechanisms of repair of ultraviolet-damaged DNA in vertebrate cells (*)

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While pyrimidine dimers can apparently be induced in the DNA of any type of vertebrate cell in culture by ultraviolet radiation, the mechanism of repair of the dimer is highly contingent upon the type of vertebrate cell involved and upon the post-irradiation conditions.

Photoreactivation.

We have examined a number of established cell lines and primary tissues representative of all the classes of vertebrates for biological evidence of photoreactivation and for the presence of photoreactivating enzyme. The assays we have employed for the presence of photoreactivation are the following (REGAN & COOK, 1967):

1. Photoreactivation of growth.

2. Photoreactivation of DNA synthesis.

3. Photoreactivation of UV irradiated *Haemophilus influenzae* transforming DNA by a crude extract of the cells in question.

4. Monomerization of UV-induced pyrimidine dimers in *E. coli* DNA by a crude extract of the cells in question.

5. *In vivo* monomerization of UV-induced pyrimidine dimers in the cell's own DNA.

Among the various vertebrate cell types we have employed in these assays there has emerged a remarkable consistency. If a cell line was positive in one assay, it was invariably positive in all; those negative in one were unequivocally negative in all.

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Our studies show that established cell lines and primary tissues from fishes, amphibians (Regan, Cook & Lee, 1968) and reptiles (Regan, Cook & Takeda, 1968) clearly possess the property of biological photoreactivation and have photoreactivating enzyme activity (Fig. 1). Chick embryo fibroblasts have photoreactivating enzyme (Cook & McGrath, 1967) and

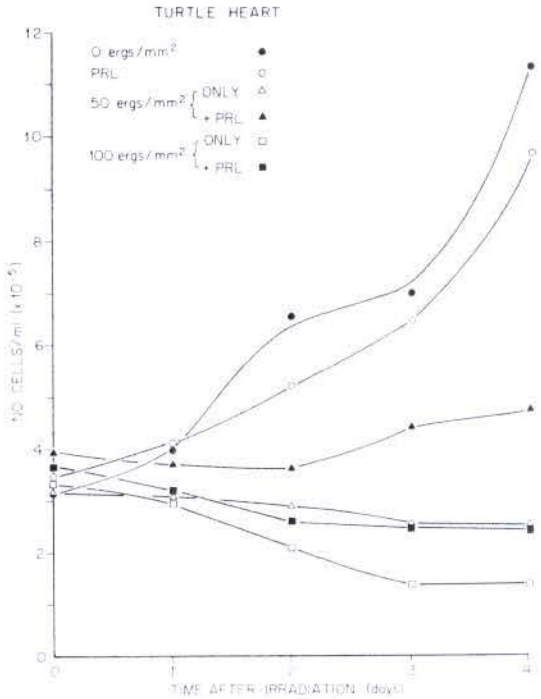

Fig. 1. — Photoreactivation of growth in reptilian cells. Effect of UV doses or 50 or 100 ergs/mm² without and with subsequent illumination with visible light.

Prefferkorn, Boyce & Coaby (1966) have shown such cells can photoreactivate UV-inactivated pseudorabies virus. Thus photoreactivation is a property of all the lower vertebrates, including birds.

Photoreactivation was demonstrable in established cell lines some of which had been cultivated *in vitro* for over nine years. This finding indicates that photoreactivation is not an organ- or tissue-specific function since such functions are generally lost from cells after prolonged *in vitro* cultivation. Rather, it would suggest that photoreactivation is a fundamental cellular function which is quite likely to be found in all cells of an animal if it is found in any of them.

Mammalian cells employed in the above assays also produced highly consistent but negative results (Fig. 2). However, the absence of photoreactivation is not characteristic of all members of the Class Mammalia

because we have recently found that marsupial cells have photoreactivating enzyme and can reverse ultraviolet damage in the presence of visible light (Cook & Regan, 1969).

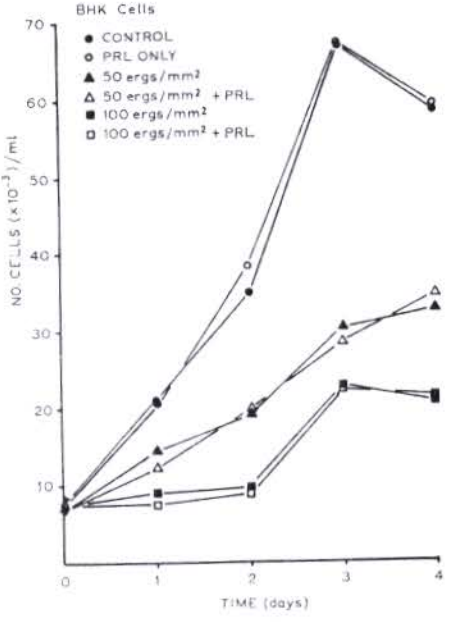

Fig. 2. — Lack of photoreactivation in cells from placental mammals. Irradiation with visible light had no rescueing effect on growth after UV irradiation (BHK cells-hamster kidney).

We applied our assays for photoreactivation to established marsupial cell lines and, where possible, to primary tissues representative of Australian South American and North American marsupial species. All of these experiments yielded positive results indicating that marsupials have photoreactivation. Thus among all the classes of vertebrates apparently only the placental mammals are devoid of the ability to photoreactivate ultraviolet damage. Why this curious phylogenetic pattern should exist is unknown, however it is conceivable that this process was lost concomitantly with the evolution of placentation, i. e., the complete development of the embryo inside the body of the mother.

Excision.

The induction of pyrimidine dimers by UV radiation in mammalian cells had been demonstrated by several authors previous to our work (Trosko, Chu & Carrier, 1965 ; Klimek, 1966). These authors studied dimers in mouse and hamster cells but found no evidence for excision (or photoreactivation) in these murine rodent cells. When we performed precisely

the same experiment with *human* cell lines, we found not only that dimers could also be induced in human cells — hardly a surprising result — but also, in contrast to the rodent cell data, that there was apparently an excision of dimers from the human cell DNA with time after irradiation. Furthermore, the dimers appeared in the TCA-soluble fraction concomitantly with their loss from the TCA-insoluble fraction (Regan, Trosko & Carrier, 1968).

To state that human cells cannot get rid of dimers by photoreactivation but can by the excision process may seem to indicate an inconsistency. However, in the excision process whether visible light is shone upon the cells subsequent to ultraviolet irradiation or not makes no difference in the outcome of the experiment. Secondly, in excision, the pyrimidine dimers is removed from the cellular DNA as a part of a dimer-rich oligonucleotide. Thus their appearance in the acid soluble cell fraction. In photoreactivation, where the dimer is monomerized *in situ*, the dimers do not appear in the acid-soluble cell fraction.

Our current studies are directed towards finding the enzymatic basis for excision and for its significance in cell repair and survival. We are also currently investigating the excision process in several human diseases involving severe ultraviolet sensitivity. Cleaver (1968) showed that the phenomenon of « repair replication » or, base insertion after UV damage to DNA, was lacking in the disease *Xeroderma pigmentosum*. Thus we exa-

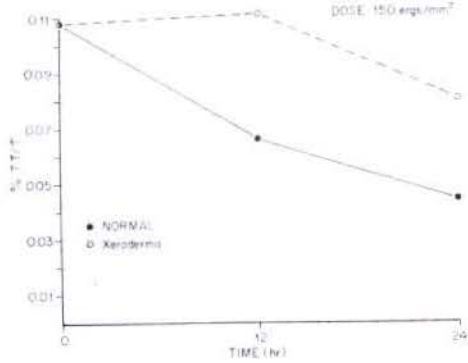

Fig. 3. — Excision of UV induced pyrimidine dimers in normal human skin cells and the lack of excision in skin cells from a patient with *Xeroderma pigmentosum*.

mined Xeroderma cells for repair at the level of dimer excision which is a more direct means of scrutinizing repair. Our results indicate that Xeroderma cells lack the ability to excise dimers while normal human skin cells readily excise a majority of their dimers within 24 hours after UV irradiation (Fig. 3).

Thus our results indicate that individuals with *Xeroderma pigmentosum* are the human analog of the excisionless, UV sensitive bacterial mutants so well-known in microbial photobiology.

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Repair of mammalian cell DNA: effects of drugs and mutations (*)

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Introduction.

A novel form of nonconservative DNA replication occurs in mammalian cells after irradiation with X rays (PAINTER & CLEAVER, 1967) or ultraviolet (UV) light (CLEAVER & PAINTER, 1968; CLEAVER, 1968), or treatment with chemical mutagens (ROBERTS, CRATHORN & BRENT, 1968; HAHN, YANG & PARKER, 1968). If this nonconservative replication occurs in the presence of bromouracil deoxyriboside (^{3}H or ^{14}C BrUdR), the resultant DNA has small patches of BrU and a density close to the original density of DNA in isopycnic gradients (CLEAVER & PAINTER, 1968; CLEAVER, 1968) (Fig. 1). The nonconservative replication is detected autoradiographically through the incorporation of ^{3}H thymidine ($^{3}HTdR$) into DNA during G_1, G_2, and mitosis (RASMUSSEN & PAINTER, 1964). This replication is biochemically similar to repair replication in bacteria which involves the replacement of damaged DNA bases, such as pyrimidine dimers (PETTIJOHN & HANAWALT, 1964; HANAWALT, 1968), and its characteristics are reasonably well known (Table 1). The similarity between repair replication in bacteria and mammalian cells at the biochemical level unfortunately does not allow any inferences as to the significance or importance of repair replication in mammalian cells because of the lack of radiation sensitive or resistant mutants. In an attempt to discover the role of repair replication in mammalian cells, I have used two approaches: (1) a study of the effects of drugs added after irradiation (CLEAVER, 1969*a*), and (2) a study of repair replication in hereditary human skin diseases (CLEAVER, 1968).

(*) Work performed under the auspices of the U. S. Atomic Energy Commission.

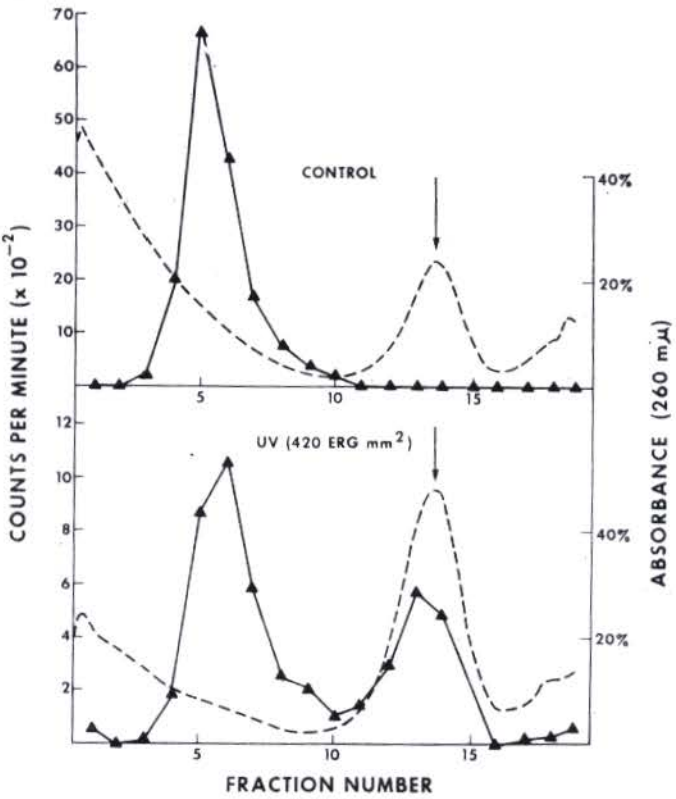

Fig. 1. — Cesium chloride equilibrium density gradient profiles of DNA from normal human fibroblasts labeled for 1 hr with BrUdR, irradiated with UV light and labeled for 4 hr in ^{3}HBrUdR (20 μCi/ml 5 μg/ml). Absorbance 260 mμ ———, ^{3}H activity —▲—, Arrows mark position of normal density DNA. Peak of ^{3}H activity at normal density from irradiated cultures is evidence for repair replication.

TABLE 1.

Characteristics of repair replication in mammalian cells.

1. The amount of repair replication corresponds to the amount of base damage, UV > X rays (RASSMUSSEN & PAINTER, 1966).
2. The amount of repair varies markedly between different cell types ; some primary human cell lines show the most and some Chinese hamster (DFAF) and mouse (L) lines show the least (PAINTER & CLEAVER, 1966).
3. Repair occurs throughout the cell cycle, over all chromosomes, and in proliferating and differentiated cells (RASMUSSEN, 1968 ; PAINTER & CLEAVER, 1969).
4. Repair saturates above 200 to 400 erg/mm^2 UV and at low doses (one to two D_0's) most of the repair is complete within 2 hr (RASMUSSEN & PAINTER, 1966 ; CLEAVER, 1969 *b*, *c* ; RASMUSSEN, 1968).

5. At saturation, the maximum replacement is approximately 0.02 to 0.1 per cent of the genome.
6. Repair and semiconservative replication show no preference for TdR over BrUdR (CLEAVER, 1970).
7. The repair « patch » is indistinguishable from the native polynucleotide chain (PAINTER, 1969).

Drugs and repair replication.

The influence of numerous drugs on repair replication in HeLa cells is summarized in Table 2. None of these drugs has any specific effect on repair replication although some inhibit only semiconservative replication (Group I). The compounds in Group I all reduce the supply of precursors for DNA synthesis and may inhibit semiconservative replication because large quan-

TABLE 2.

Effects of drugs on repair replication of UV damage in mammalian (HeLa) cells.

	Semiconservative	Repair
Group I — Drugs limiting supply of DNA precursors:		
hydroxyurea	+	0
cytosine arabinoside	+	0
5-aminouracil	+	0
fluorodeoxyuridine	+	0
amethopterin	+	0
Group II — Drugs binding to DNA:		
acriflavine	+	+
crystal violet	+	+
actinomycin D	+	+
phleomycin	+	+
Group III — Miscellaneous modes of action:		
caffeine	+	0
iodoacetate	+	+
cycloheximide	+	+
phenethyl alcohol	+	+

+ Means significant inhibition; 0 means no effect. For experimental details see CLEAVER (1969*a*).

tities of precursors required for this in contrast to very small amounts required for repair replication (Table 1). The compounds in Group II all bind to DNA and may then inhibit both repair and semiconservative replication. Although no compound has a preferential effect on repair, such binding to DNA could be part of the mechanism whereby some of these compounds kill irradiated bacteria (HARM, 1967 ; REITER *et al.*, 1966) and mammalian cells (ELKIND, WHITMORE & ALESCIO, 1964) and enhance UV mutagenesis (WITKIN, 1961). In Group III, caffeine has no effect on repair even though it does kill UV-irradiated mammalian cells (RAUTH, 1967). Caffeine, however, is lethal only during the first post-irradiation S phase (RAUTH, 1967) and probably interferes with post-irradiation semiconservative replication rather than repair (CLEAVER & THOMAS, 1969).

Repair replication in human skin diseases.

The only current evidence for the functional significance of repair replication has been found in *Xeroderma pigmentosum*, a human skin disease. The cells of this disease cannot perform repair replication after exposure to ultraviolet light (CLEAVER, 1968 ; CLEAVER 1969*b*) (Table 3). *Xeroderma pigmentosum* is the only hereditary disease yet discovered in which the cells cannot perform repair replication. Others that have been studied include light-sensitive (Rothmund-Thomson, *Lupus erythematosus*) and premature aging diseases (progeria). *Xeroderma pigmentosum* cells cannot repair damage to DNA bases but can repair damage which involves a chain break (e. g. X-ray damage) (CLEAVER, 1969*b*). The defective stage in the disease is probably at an initial step of repair at which an endonuclease starts the excision process. If pyrimidine dimers are the important photoproducts involved in UV damage to human cells, then *Xeroderma pigmentosum* cells should not excise dimers.

Significance or importance of repair replication.

The role of repair replication in mammalian cells is not clearly revealed by the experiments hitherto described. Unfortunately, there is, as yet, no definite correlation between deficiencies in repair replication and sensitivity to killing by UV light. In *Xeroderma pigmentosum* the criteria of sensitivity are clinical, such as erythema and high incidence of cutaneous cancers. A correlation has, however, been established between the amount of repair replication and the level of cell survival after treatment with methyl and ethyl methane sulphonates (HAHN, YANG & PARKER, 1968). Excision of damaged regions of DNA is a prerequisite for repair replication (SETLOW, 1966 ; HANAWALT, 1968). Although pyrimidine dimers have been identified

TABLE 3.

Amount of repair replication after 4 hr labelling in ^{3}HBrUdR (20 μCi/ml 1.2 Ci/mmole) after irradiation.

Cell type	Dose *	Amount of repair (dpm/μg DNA, 2000 dpm/μg = .1 per cent replacement)
Human fibroblasts :		
Normal (WI38)	420 erg/mm^2	1310
Diseased + – *Xeroderma pigmentosum* .	420 erg/mm^2	below background
Diseased + – *Xeroderma pigmentosum* . (de Sanctis Cacchione Symdrome)	420 erg/mm^2	below background
Diseased + – Rothmund Thomson . .	420 erg/mm^2	3380
Diseased + – Progeria	420 erg/mm^2	2070
Diseased + – Blooms	420 erg/mm^2	1110
Diseased + – Granulomatous	420 erg/mm^2	2030
Diseased + – *Lupus erythematosus* . . .	420 erg/mm^2	2000
Chinese hamster (V79)	70 erg/mm^2	844
Chinese hamster (V79)	105 erg/mm^2	1780
Chinese hamster (V79)	700 erg/mm^2	2040
Normal human fibroblasts (WI38) . .	20 kr X rays	302

* The UV dose is on the plateau of the dose response and corresponds to the maximum amount of repair which occurs.

+ These diseases all affect the skin and most are presumed to have a genetic cause. The precision of these measurements is low and the specific activities of the human cell types are not regarded as significantly different, except for *Xeroderma pigmentosum*.

as the particular UV photoproduct excised from bacteria (SETLOW, 1966), such identification is not yet established for mammalian cells. Human cells can excise half of the pyrimidine dimers from DNA (REGAN, TROSKO & CARRIER, 1968), one mouse ascites cell line excises 30 percent (HORIKAWA, NIKAIDO & SUGAHARA, 1968), but many rodent cell lines apparently excise none at all (TROSKO, CHU & CARRIER, 1965 ; KLIMEK 1966 ; CLEAVER & TROSKO, 1969). Even excision of half the dimers leaves a very large number

of dimers in the DNA of surviving cells. The role of pyrimidine dimers in UV damage to mammalian cells is therefore difficult to evaluate.

There are at least three possibilities concerning the fate of pyrimidine dimers in mammalian cells: (1) pyrimidine dimers are irrelevant to mammalian UV damage, (2) excision produces large acid insoluble oligonucleotides, (3) excision occurs, but in many cells at an extent below resolution in chromatograms. In view of the importance of dimers in most biological systems, alternative (1) is unlikely. Alternative (2) is probably incorrect because large patches to replace oligonucleotides would produce repaired molecules denser than normal in isopycnic gradients (CLEAVER & PAINTER, 1968; CLEAVER, 1968). If alternative (3) were correct, we would then have to conclude that excision repair, though present, is relatively unimportant in mammalian cells. It is conceivable that mammalian cells have other repair systems which do not require the replacement of damaged regions to facilitate cell survival. One such repair system, distinct from excision repair, has been identified in an excision-deficient *Escherichia coli* (RUPP & HOWARD-FLANDERS, 1968), and some evidence for a similar system has recently been obtained in Chinese hamster cells (CLEAVER & THOMAS, 1969).

Addendum. — In a survey of ten separate *Xeroderma pigmentosum* patients including both clinical forms I now find (with improved methods of detection) that the majority (seven out of ten) do in fact perform detectable levels of repair replication. These levels are all below normal, and hence do not invalidate the foregoing conclusions about the disease, and there is no simple correlation between levels of repair in the two clinical forms of the disease as appeared initially (CLEAVER, 1968).

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