REACTIVATION OF UV-IRRADIATED HERPES SIMPLEX VIRUS TYPE 2 IN HUMAN CELLS

REACTIVATION OF UV-IRRADIATED HERPES SIMPLEX VIRUS TYPE 2 IN COCKAYNE'S SYNDROME AND XERODERMA PIGMENTOSUM CELLS

by

DAVID KENNETH GREGORY RYAN B. Sc.

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AUTHOR: David Kenneth Gregory Ryan, B.Sc. (University of Guelph)

SUPERVISOR: Professor Andrew J. Rainbow

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ABSTRACT

Host cell reactivation (HCR) of UV-irradiated (UV'd) herpes simplex virus type 2 (HSV-2), capacity of UV'd cells to support HSV-2 plaque formation and UV enhanced reactivation (UVER) of UV'd HSV-2 were examined in human fibroblasts. The cells were derived from four Cockayne's Syndrome (CS) patients, 5 xeroderma pigmentosum (XP) patients and 5 normal patients. Survival curves for HCR of HSV-2 plaque formation showed 2-components. HCR was not significantly different in the CS strains and an XP variant strain compared to normal, whereas all excision deficient strains showed a significant reduction in HCR. The D_{37} values for the delayed capacity curves were in the range 8.6-12.4 J/m^2 for the normal strains, 3.1-5.1 J/m^2 for the CS strains, 6.7 J/m^2 for an XP variant strain and between 0.40-1.98 J/m^2 for the XP excision deficient strains examined. UVER was also examined for HSV-2 UV-irradiated to survival levels of 10^{-2} and 10^{-3} in unirradiated cells. Maximum delayed UVER was observed in normal strains at a UV dose of 15 J/m^2 to the virus. Maximum UVER in CS cells was detected at a UV dose of 5 J/m^2 to the cells, in XP excision deficient cells maximum UVER occurred at doses ranging from 0.5-2.5 J/m^2 to the cells, and in XP variant maximum UVER occurred at 10 J/m^2 to the cells. In all cell strains the level of UVER increased with increasing UV dose to the virus.

Results are discussed in terms of the repair defects of CS and XP cells and their relationship to possible viral repair

functions. In addition, the possible existence of an inducible DNA repair response is discussed in terms of the results of this study.

INTRODUCTION

Information for a cell's structure and function is encoded in its DNA. Transcription and translation convert the information into the proteins utilized by the cell. This information is also copied by DNA replication and the identical information passed on to daughter cells. In order to maintain its hereditary and maintainence functions the information in the DNA must be stable.

Deleterious alterations are constantly occurring in the DNA of a living cell. Certain changes are spontaneous while others are induced by chemicals and radiation. The major product of ultraviolet (UV) light irradiation is the cyclobutane pyrimidine dimer (Ben Hur & Ben Ishai, 1968). Minor products include single strand breaks and DNA-protein crosslinks (Smith, 1977). Higher energy, ionizing radiations such as X or γ-rays produce single and double strand breaks (Hutchinson, 1978) as well as alterations in the bases and sugars (Ward, 1975). Chemical agents can produce a wide range of lesions such as inter- and intra-strand DNA crosslinks, DNA-protein crosslinks, base modifications (alkylation, methylation, depurination) and base substitution (Roberts, 1978).

The effect of these lesions may be to alter the coding sequence of the DNA or ability to be replicated or transcribed. The final consequence of this damage may be the death of the cell or, in the case of mammalian cells, the initiation of cancerous growth (Setlow, 1980).

It is not surprising, in view of the importance of maintaining the integrity of the DNA, that organisms have evolved

many DNA repair mechanisms. The current understanding of the enzymology of bacterial DNA repair is quite detailed (Grossman 1981, Little and Mount, 1982). Study has been aided by the isolation of many bacterial mutants and the characterization of the relevant enzymes (Hanawalt et al., 1979). In mammalian cells the understanding of the mechanisms and the genetics of DNA repair are far less advanced (Hanawalt et al., 1979; Hall & Mount, 1981). Analysis of these repair mechanisms is hampered by the relative lack of cellular mutants deficient in these processes (Hall & Mount, 1981). DNA repair processes may be particularly complex in mammalian systems due to the structure of chromatin and the nature of eukaryotic replicons (Hanawalt et al., 1979). The use of recombinant DNA technology to clone and analyse genes that are mutant in repair deficient mammalian cells may aid greatly in our understanding of mammalian DNA repair processes (Hall & Mount, 1981).

THE IMPORTANCE OF DNA REPAIR

The importance of DNA repair to the organism is more complex than the simple survival of individual cells. DNA damage has been found to be both mutagenic and carcinogenic (Setlow, 1980). The best evidence of the correlation between reduced DNA repair ability and carcinogenesis is the observation that cells from patients with xeroderma pigmentosum (XP) are deficient in the repair of DNA photoproducts. These patients also show increased incidence of sunlight induced cancer (Robbins et al., 1974; Friedberg et al., 1979). Similar correlations between defective

DNA repair and cancer proneness are seen for various other DNA repair deficient disorders including ataxia telangiectasia (Kraemer, 1977), Fanconi's anemia (see Friedberg et al., 1979) and Bloom's syndrome (German et al., 1977).

Other work correlates cellular mutagenesis with DNA repair deficiencies. Cells from patients with XP and cells from patients with Cockayne's syndrome (CS) have been shown to be hypermutable by UV light (Maher et al., 1976; Arlett, 1980).

An association between DNA repair and aging has also been suggested to exist (Williams, 1976). Progeria is a syndrome which displays markedly advanced aging (DeBusk, 1972). Cells from some patients have reduced ability to repair γ-irradiated adenovirus DNA (Rainbow & Howes, 1977). The reduced repair has not been observed in all progeria cell strains tested (Brown et al., 1980). Also, CS patients present the general appearance of premature aging (Guzzetta, 1972).

Another clinical symptom common to a number of syndromes, whose cells are DNA repair deficient, is neurological involvement. This has been described for XP (de Sanctis & Cacchione, 1932), ataxia telangiectasia (AT) (McFarlin et al., 1972) and Fanconi's anemia (FA) (Nilsson, 1960).

Involvement of the immune system is one of the major clinical symptoms of AT (Kraemer, 1977). Patients with XP have also been shown to have immunologic abnormalities (Hellman and Schuller, 1980).

The clinical symptom common to most of the syndromes mentioned is radiation or chemical sensitivity. Patients with XP,

CS or Bloom's syndrome are hypersensitive to sunlight (Robbins et al., 1974; Guzzetta, 1972; Friedberg et al., 1979) and patients with AT are hypersensitive to ionizing radiation (see Friedberg et al., 1979). In some cases DNA repair deficiencies were first suspected on the basis of these clinical sensitivities.

BACTERIAL DNA REPAIR MECHANISMS

PHOTOREACTIVATION

The most direct repair mechanism is photoenzymatic repair or photoreactivation (PR) (Figure 1). The major product of UV-irradiation is the pyrimidine dimer (Ben Hur & BenIshai, 1968). This molecule is specifically recognized by the enzyme DNA photolyase which binds to the dimer (Sutherland, 1978). The enzyme-dimer complex forms a chromophore which absorbs light in the visible range (300-600 nm). The energy absorbed by the chromophore is used to monomerize the constituent pyrimidines (Sutherland, 1978). Because PR reduces the UV-induced mutation rate in E. coli it is considered to be an error-free repair mode. (Witkin, 1976).

EXCISION REPAIR

The process of excision repair in bacteria is mediated by a large number of enzymes. A simple model of the basic process (see Figure 2) involves at least four steps: a) the incision of the DNA 5' to the damage site b) excision of the damaged site c) resynthesis in the excised region and d) ligation to restore the phosphodiester bond (from Hall and Mount, 1981).

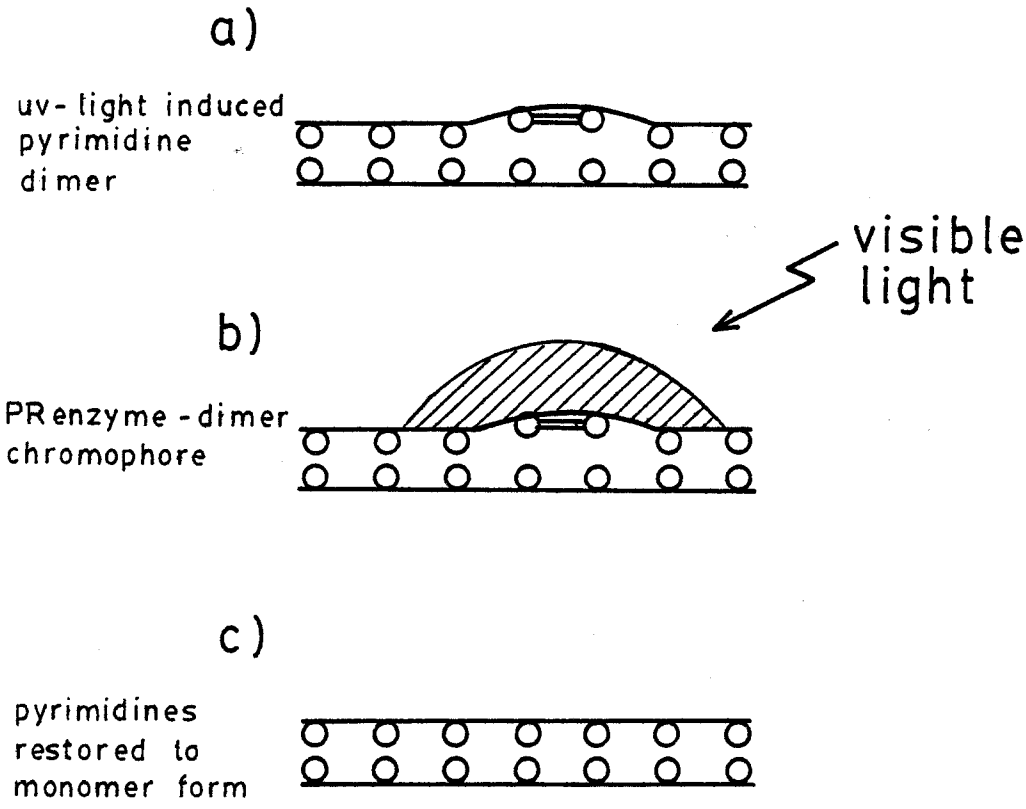
FIGURE 1

THE PROCESS OF ENZYMATIC PHOTO REACTIVATION (PR)

a) cyclobutane pyrimidine dimers produce a bulky lesion in DNA.

b) the dimer is specifically recognized by DNA photolyase (PR enzyme). The dimer-enzyme chromophore absorbs visible light.

c) the energy absorbed is utilized to monomerize the constituent pyrimidines.


a)
uv-light induced
pyrimidine
dimer
visible
light
b)
PR enzyme-dimer
chromophore
c)
pyrimidines
restored to
monomer form

The types of damage which can be removed from DNA by excision repair include small lesions such as alkylated bases and single-strand breaks. Large lesions such as pyrimidine dimers and DNA-DNA or DNA protein cross-links can also be removed by excision repair (Hanawalt *et al.*, 1979).

Two types of excision repair can occur depending upon the type of damage. Base excision repair (Figure 2A,B) occurs when base modifications are recognized by specific glycosylases which remove the altered base (Grossman, 1981). This generates an apurinic/apyrimidinic (AP) site. This site can, under some experimental conditions, be regenerated (Figure 2A) by an insertase enzyme replacing the correct base (Deutsch & Linn, 1979). The AP site is usually the target for various AP endonucleases (Lindahl, 1979) which hydrolize the phosphodiester backbone of the DNA (Figure 2B).

Nucleotide excision (Figure 2C) is used for the repair of bulky adducts such as pyrimidine dimers, ethylmethane sulphonate damage, crosslinks and chemical adducts (Grossman, 1981). A high molecular weight complex comprising the products of the UVR A, UVR B and UVR C genes recognizes the lesion and incises the DNA 5' to the lesion (Seeberg, 1978). From this point onward nucleotide and base excision repair are indistinguishable.

The excision of the lesion can be accomplished by any of a variety of exonucleolytic activities (Grossman, 1981). Some of these exonucleases are independent enzymes while

FIGURE 2

EXCISION REPAIR

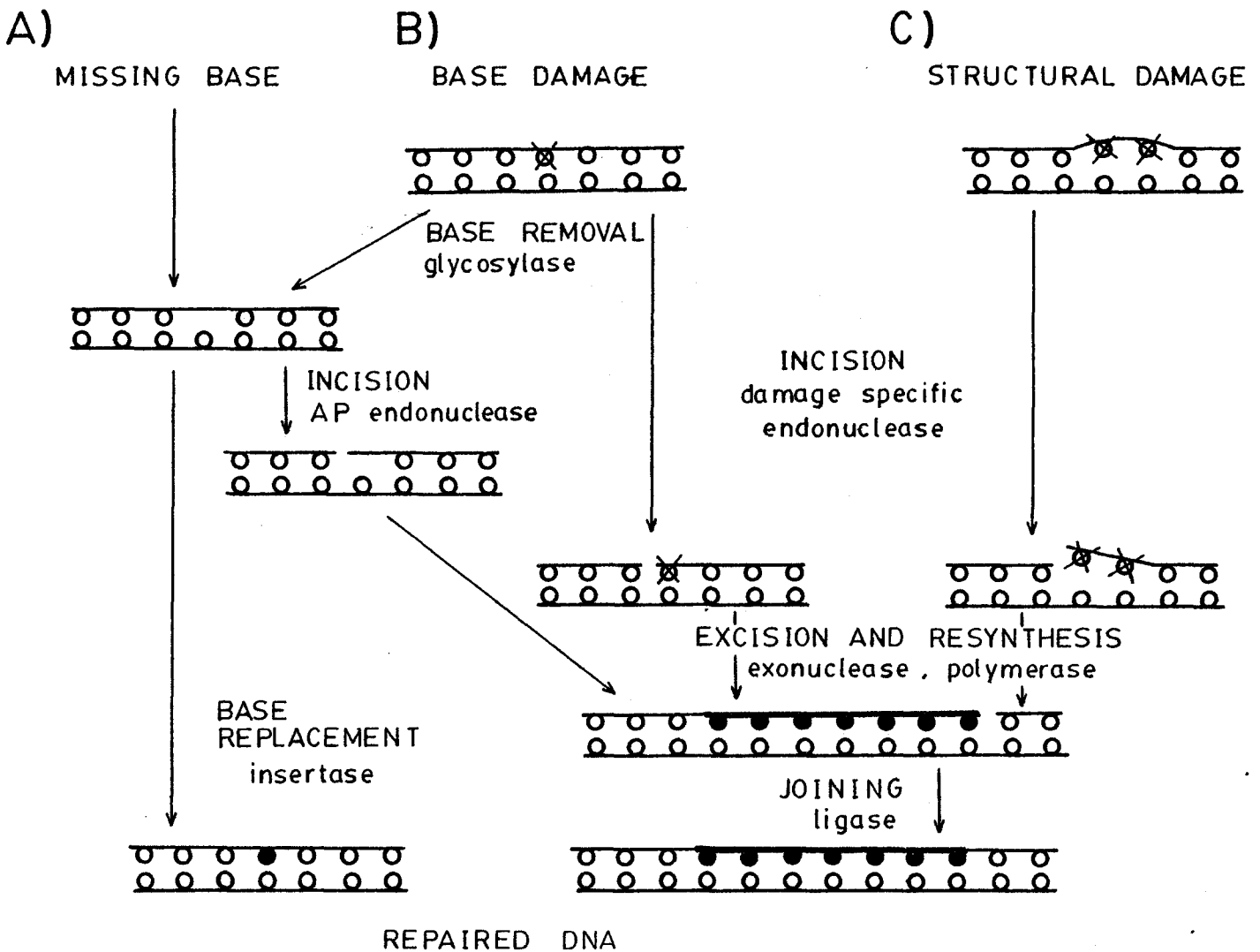
A) Direct removal of damaged base by specific glycosylase and subsequent replacement by insertase

B) Base excision repair. Damaged base is removed by specific glycosylase. Resulting AP site is target for AP endonuclease incision

C) Nucleotide excision repair. Structural defects are recognized and the DNA is incised 5' to the damage site

Excision of damage, resynthesis and ligation are performed in a similar fashion for both base and nucleotide excision repair

(Redrawn from Hanawalt et al., 1979)


A)
MISSING BASE
B)
BASE DAMAGE
C)
STRUCTURAL DAMAGE
BASE REMOVAL
glycosylase
INCISION
AP endonuclease
INCISION
damage specific
endonuclease
EXCISION AND RESYNTHESIS
exonuclease , polymerase
BASE
REPLACEMENT
insertase
JOINING
ligase
REPAIRED DNA

others are associated with polymerase activities (Grossman, 1981; Hanawalt et al., 1979).

Resynthesis of the excised patch of DNA can be performed by either of the two bacterial DNA polymerases, pol I or pol III (Grossman, 1981). Pol I is considered to be the better candidate for this function because it is able to bind at nicks in the DNA (Hanawalt et al., 1979). This would make it ideal for a coupled excision-resynthesis reaction.

Though it may account for the majority of the resynthesis, pol I is not the only polymerase involved. Mutants deficient in polymerase I (pol A) are still capable of excision repair and in fact perform more repair synthesis than pol^+ strains (Cooper & Hanawalt, 1972a). The increase in the absolute amount of repair synthesis is accounted for by the size of the repair patch. It has been shown that repair patches in E coli are heterogeneous in size. The majority of patches are short (20-30 nucleotides) while a small fraction are much larger (up to several hundred nucleotides) (Cooper & Hanawalt, 1972b). This was interpreted to mean that pol I was responsible for short repair patches and that pol III was responsible for long repair patches. Though long-patch excision repair is constitutive at low levels, it has been shown to be rec A^+, rec B^+ and lex A^+ dependent (Cooper & Hanawalt, 1972a). It is therefore an inducible function and will be discussed later. Mutants which can only perform short patch repair

(rec A^- or lex A^-) do not show UV-induced mutability (Witkin, 1976). This suggests that short patch excision repair is error-free.

The final step in excision repair is the ligation of the repaired patch to the parental DNA. This is accomplished by a single enzyme, polynucleotide ligase (Youngs & Smith, 1977).

POST REPLICATION REPAIR

Damage which has not been removed from the DNA (e.g. pyrimidine dimers, as the most thoroughly characterized lesion) can block DNA synthesis at that site. Actively replicating DNA (Figure 3A) will be interrupted and reinitiation of synthesis will occur on the other side of the damage site (Hanawalt et al., 1979). These gaps are easily detected by alkaline sucrose density gradient sedimentation (Rupp & Howard-Flanders, 1968). It is these gaps which are the target for post-replication repair (PRR) (Lehmann & Karran, 1981).

Current evidence suggests that the gaps are filled by an exchange mechanism which places DNA from the sister-strand into the gap (Rupp et al., 1971). The undamaged sister-strand is then reconstituted by repair DNA synthesis (Ley, 1973). This eliminates the gap left in the parental molecule by the strand exchange.

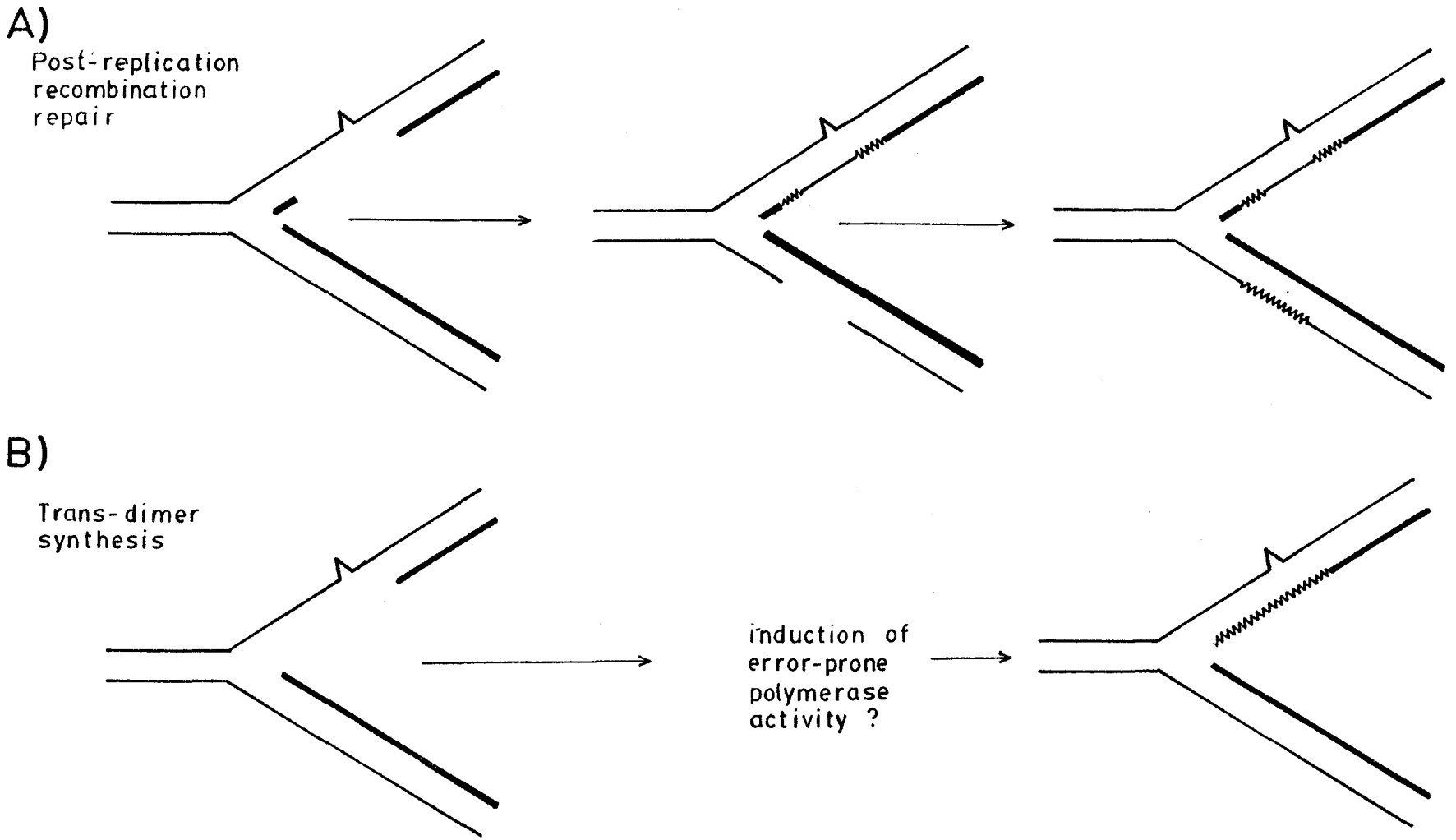
It is interesting to note that this "repair" process does not remove the damaged site. It is possible that by allowing replication to occur past damage sites the cell

FIGURE 3

Post-replication recombinational repair and trans-dimer synthesis

A) Post-replication recombinational repair or Daughter-strand gap repair

B) Trans-dimer synthesis


A)
Post-replication
recombination
repair
B)
Trans-dimer
synthesis
induction of
error-prone
polymerase
activity ?

is buying time with which to remove the damage by excision repair. Those cells which are not excision repair proficient (UVR A or UVR B) may eventually produce damage-free genomes by the gradual dilution of damaged sites.

The process of PRR is absolutely dependent upon the rec A gene (Smith & Meun, 1970). This is not unexpected considering the involvement of recombination functions (Rupp et al., 1971).

Though no direct evidence exists, PRR or daughter-strand closure is thought to be non-mutagenic (see discussion by Hanawalt et al., 1979). The observation that repair synthesis occurs on lesion-free templates (Ley, 1973) supports this conclusion.

SOS FUNCTION

A variety of treatments which damage DNA or inhibit its replication induce a coordinately controlled set of responses (Little & Mount, 1982). These responses include enhanced excision and daughter-strand gap repair capability as well as prophage induction and an increased rate of cellular mutagenesis (Little & Mount, 1982; Hanawalt et al., 1979). Also included are the phenomena of Weigle reactivation (WR) and Weigle mutagenesis (WM) (Weigle, 1953) whereby UV-irradiated bacteriophage λ produces more plaques and more mutant progeny when infected into pre-irradiated host cells.

The present model of the SOS system (Figure 4) holds that the lex A protein is a repressor molecule for the unlinked genes involved in the SOS system (Little & Mount, 1982)

FIGURE 4

A schematic of bacterial SOS induction

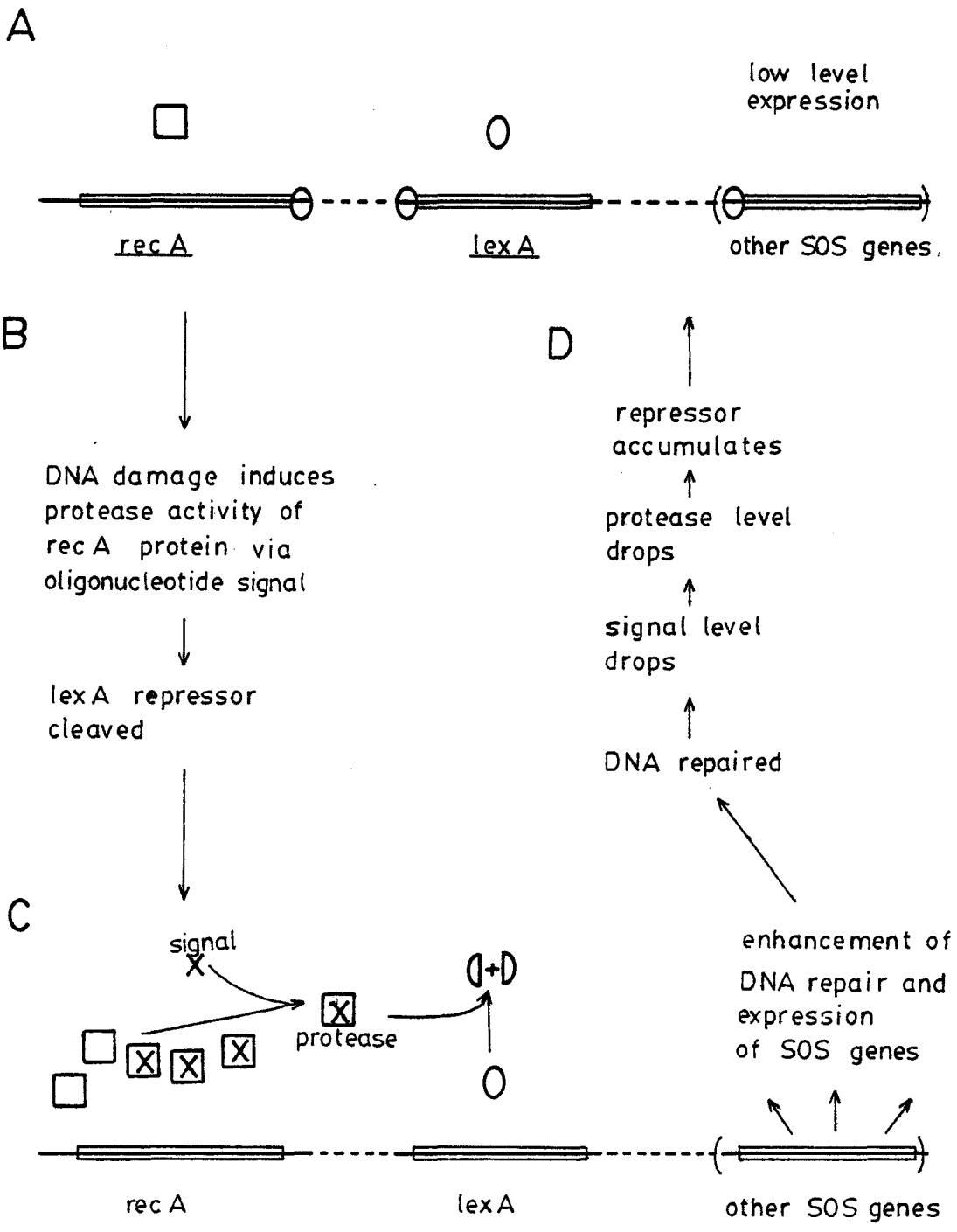
A) low level expression of lex A produces repressor molecules which bind to operator sites of various genes allowing only low level expression

B) DNA damage signal activates rec A protease which cleaves lex A repressor molecule

C) derepression of rec A, lex A and other SOS genes gives rise to SOS response

D) as DNA damage is repaired, signal level drops and rec A loses protease function, repressor molecules accumulate and low level expression is restored

(Redrawn from Little & Mount, 1982)


A
low level
expression
rec A
lex A
other SOS genes
B
DNA damage induces
protease activity of
rec A protein via
oligonucleotide signal
lex A repressor
cleaved
C
signal
protease
D
repressor
accumulates
protease level
drops
signal level
drops
DNA repaired
enhancement of
DNA repair and
expression
of SOS genes
rec A
lex A
other SOS genes

(Figure 4A). When DNA is damaged an inducing signal activates a proteolytic activity of the rec A protein (Roberts et al., 1978) which cleaves and inactivates the lex A repressor (Little et al., 1980) (Figure 4B). Derepression allows the expression of the SOS genes (Figure 4C) at higher levels and their SOS functions are expressed (Little & Mount, 1982). When the damage is repaired (Figure 4D) the protease activity of rec A disappears and the lex A repressor accumulates and represses the SOS genes (Little et al., 1980).

The best understood SOS response is prophage induction (Craig & Roberts, 1981). The viral repressor (λ repressor) protein appears to be functionally similar to the lex A protein (Little & Mount, 1982). Recent evidence (Crowl et al., 1981) indicates that activation of the rec A protease is sufficient to cause λ repressor cleavage and prophage induction.

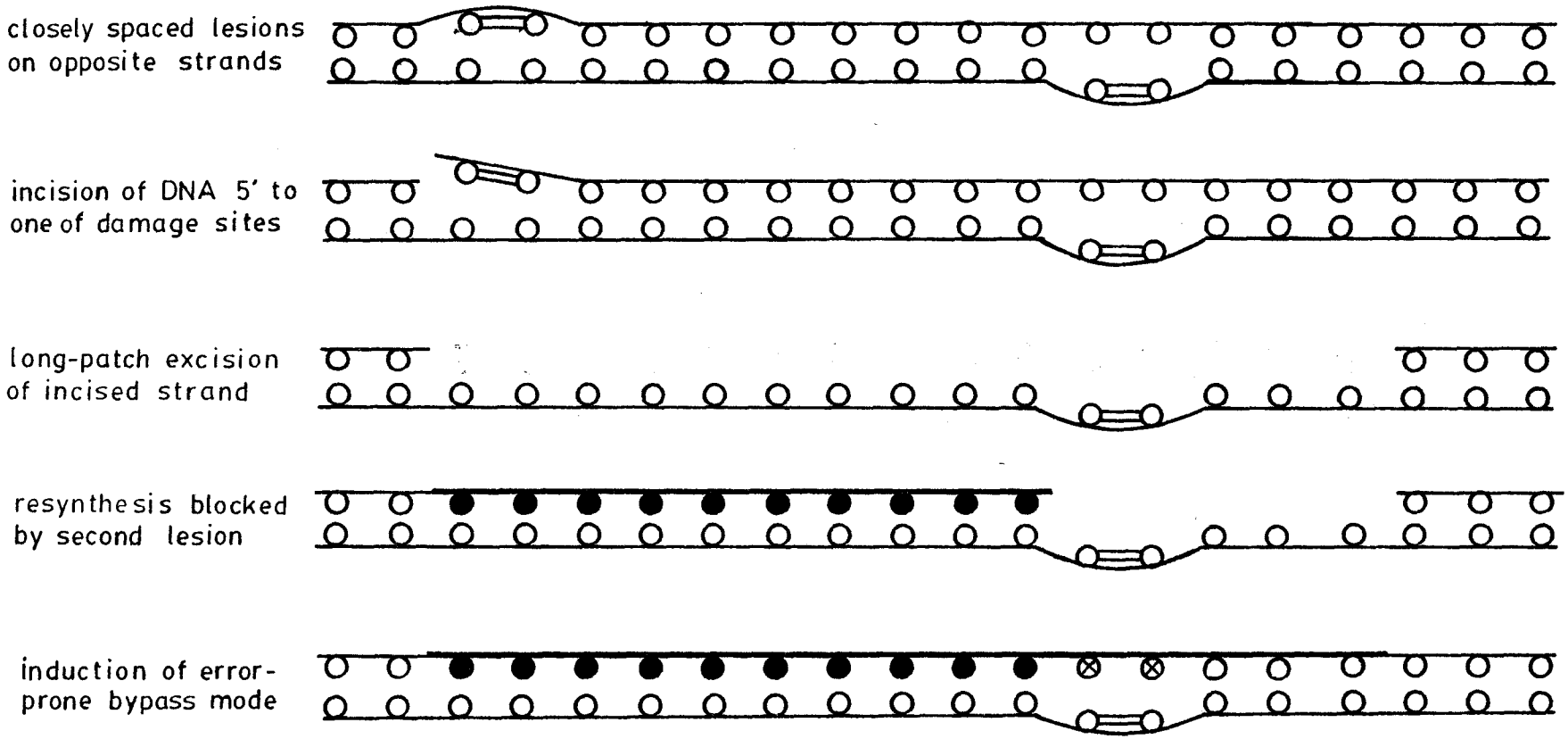
The enhancement of excision repair by the induction of the SOS system is due to two factors. The UVR A gene product (which is under lex A control) is present at higher levels and makes cells more resistant to DNA damage (Mount et al., 1975). Also, long patch excision repair is induced to higher levels as one of the SOS functions (Cooper & Hunt, 1978).

SOS error-prone excision repair is thought to arise from long-patch repair. Closely spaced lesions on opposite strands (see Figure 5) would require "transdimer synthesis" (see

FIGURE 5

The consequence of closely spaced dimer lesions

The consequence of closely spaced dimer lesions on opposite strands of the DNA molecule is the necessity of utilizing some form of by-pass repair mode, an inherently error-prone mechanism


closely spaced lesions
on opposite strands
incision of DNA 5' to
one of damage sites
long-patch excision
of incised strand
resynthesis blocked
by second lesion
induction of error-
prone bypass mode

below) to by-pass the second lesion (Hanawalt et al., 1979).

The enhancement of post-replication repair and the cause of both Weigle reactivation and Weigle mutagenesis is thought to be transdimer synthesis (Clark & Volkert, 1978). Though DNA replication is usually blocked by pyrimidine dimers, (Figure 3B) a cell which has been SOS induced is able to synthesize past the dimers (Caillet-Fauquet et al., 1977). The blockage results from the 3' - 5' editing exonuclease activity associated with bacterial DNA polymerases (Villani et al., 1978). It is possible that in induced cells the 3' - 5' editing activity is inhibited allowing random insertion of nucleotides opposite dimers (Villani et al., 1978). Indirect evidence from pol C mutants suggests the involvement of DNA polymerase III in error-prone transdimer synthesis (Bridges et al., 1976).

MAMMALIAN DNA REPAIR MECHANISMS

PHOTOREACTIVATION

Repair of pyrimidine dimers can be accomplished by a single enzyme, DNA photolyase (Sutherland, 1978b). The enzyme-dimer complex absorbs visible light and the pyrimidines of the dimer are monomerized.

This enzyme activity has been detected in different types of cells including marsupial (Lytle & Benane, 1975) and human cells (D'Ambrosio et al., 1981). The contribution of photoreactivation to other repair assays has been well established. Photoreactivating light has been shown to increase the survival of UV'd HSV in some XP excision repair deficient cell strains

(Wagner et al., 1975; Lytle et al., 1976b). Photoreactivation has also been shown to reduce the UV-light induced decrease in HSV plaque-forming ability in marsupial cells (Lytle & Benane, 1975). The same study showed that photoreactivation reduced the expression of delayed enhanced reactivation, suggesting that UV-induced pyrimidine dimers were necessary for its induction.

EXCISION REPAIR

The basic steps of excision repair in mammalian cells are thought to be similar to those in bacterial cells (Lehmann & Karran, 1981). That is, upon recognition of a damage site the initial step is the incision or nicking of the DNA. This is followed by excision and resynthesis and finally, ligation (see earlier section: Bacterial Excision Repair) (Figure 2).

As in bacterial cells excision repair can be subdivided into both base and nucleotide excision repair. An extensive battery of DNA glycosylases can recognize a wide variety of minor base alterations (Lindahl, 1979). Subsequently the base can be replaced directly (Lindahl, 1979) or the AP (apurinic/apyrimadinic) site can be the target for an AP endonuclease. A number of AP endonucleases have been identified in human cells (Linn et al., 1978) all of which incise the phosphate backbone.

For nucleotide excision repair the mechanism whereby the initial incision is made is unclear. A large body of studies using the human excision deficient mutant cell strains of

Xeroderma pigmentosum (XP) patients (see discussion by Hanawalt et al., 1979) has suggested that a complex set of gene products is required to allow the recognition of, access to and incision of DNA organized into chromatin.

Strand breaks caused by incision are difficult to detect with standard techniques and especially sensitive procedures must be used (Hanawalt et al., 1979). This may indicate that incision is a rate limiting step and that once it occurs the subsequent steps of excision, resynthesis and ligation proceed very quickly (Lehmann & Karran, 1981).

The classical demonstration of excision resynthesis in mammalian cells was the report of unscheduled DNA synthesis (UDS) in UV-irradiated cells (Rasmussen & Painter, 1964). They postulated that this low level of cell-cycle independent DNA synthesis represented the filling in of gaps left by the excision of damaged nucleotides. Subsequent experimentation has supported their hypothesis and various methods now exist for monitoring excision repair. These include measurement of repair synthesis by isopycnic sedimentation, bromouracil incorporation into repair sites followed by 313 nm-light induced hydrolysis, loss of lesion-specific-endonuclease-sensitive sites and many others which are reviewed by Paterson (1977).

A multiplicity of enzymic activities that may have some role in excision and resynthesis have been identified in mammalian cells. However, unlike E. coli - DNA polymerase I (Weissbach, 1977) none have been found which combine these

two activities (excision & resynthesis) on a single enzyme. Though not on a single enzyme, these processes may in fact be coupled in the cell. A 5' - 3' exonuclease activity purified from a human lymphoma line was stimulated by polymerase (Strauss et al., 1978). This combined activity was able to perform repair replication in vitro (Bose et al., 1978).

Among the other enzymes known are a large number of exonucleases having somewhat different activities. All share the ability to release pyrimidine dimers from UV-irradiated DNA (Hanawalt et al., 1979; Grossman, 1981).

There are four known mammalian DNA polymerases (Weissbach, 1977) designated α, β, γ and δ. The δ polymerase is probably the mitochondrial polymerase (Grossman, 1981) and as such is the best characterized. The role of the other polymerases in DNA repair is not clear. The recently described γ polymerase (Byrnes et al., 1976) has yet to be implicated in repair. The α and β polymerases have both been inferred to be involved in DNA repair synthesis in various conflicting reports which utilize selective inhibitors of one polymerase or the other (Hanawalt et al., 1979; Lehmann & Karran, 1981). In some cases, use of the same inhibitor in different systems produces opposing conclusions. This is the case with the drug aphidicolin (Ciariocchi et al., 1979 and Pedrali-Noy & Spadari, 1980). This may reflect the differences in the experimental systems since Ciariocchi et al. (1979) used a permeable system and Pedrali-Noy & Spadari (1980) used an in vivo system. However, even with the permeable cell system, the use

of other selective inhibitors produced conflicting results (Castellot et al., 1979).

The evidence provided by aphidicolin inhibition experiments using in vivo systems (Pedrali-Noy & Spadari, 1980) has led to the belief that β polymerase plays the major role in repair synthesis (Lehmann & Karran, 1981; Spadari et al., 1982). However, the recent demonstration that aphidicolin is not absolutely specific for α polymerase but also inhibits δ polymerase over the same dose range (Goscin & Byrnes, 1982) will require a re-evaluation of the conclusions based upon the original premise of specifity.

As in bacterial cells, mammalian cells perform two types of excision repair, long-patch and short-patch. All similarities between the two systems end at that point. The mammalian short-patch repair mode operates on damage produced by ionizing radiation (e.g. X-rays) or chemicals which produce "X-ray like" damage such as alkylating agents (Regan & Setlow, 1974). The size of the repair patch has been estimated to be 3-4 nucleotides and repair is generally completed within an hour or two (Regan & Setlow, 1974). The mammalian long-patch repair mode corrects damage caused by "UV-like" agents such as ultraviolet light, N-acetoxy-acetylaminofluorine (NAAAF), aflatoxin B, and psoralen plus light (Regan & Setlow, 1974). These agents produce bulky adducts which distort the DNA helix. Their repair produces patches of 15-100 nucleotides (Regan & Setlow, 1974) and, at least at lower doses, removal of lesions occurs continually over a period of 12-24 hours

during which time 70-90% of the lesions are removed (Smith, 1978).

POST REPLICATION REPAIR OR DAUGHTER-STRAND REPAIR

An experimental design, adapted from bacterial studies has detected, in UV irradiated mammalian cells, DNA which is smaller than that from control cells. This smaller DNA can then be chased into control sized DNA by incubating the cells for 3 hours (Lehmann, 1972). This is presumably indicative of gaps left at damaged sites during replication and their subsequent repair. However, the interpretation of these results is complicated by the size of the mammalian DNA and the way in which the DNA is replicated. Multiple tandem replicating units (replicons) whose average size is 200 million daltons, must undergo daughter-strand-joining and gaps may arise in a variety of ways (Hanawalt et al., 1979).

Another attribute of PRR in bacterial cells is the presence of pyrimidine dimers in newly replicated (i.e. labelled) daughter-strands. This has also been detected in mammalian cells but different hypotheses concerning their origin exist. A replicon which was initiated before irradiation could contain dimers and become labelled during the subsequent radioactive pulse. Alternatively, dimers could be transferred, by recombination, from the parental strand into the daughter-strand. Evidence for both of these models has been presented (Meneghini & Menck, 1978; D'Ambrosio & Setlow, 1978) and neither can be ruled out at this time.

SOS FUNCTIONS IN MAMMALIAN CELLS

The observation of phenomena analogous to the SOS functions of bacteria (Little & Mount, 1982) have suggested that repair processes similar to the SOS system of *E. coli* exist in mammalian cells (Lehmann & Karran, 1981).

The enhanced survival of UV-irradiated viruses (termed UV enhanced reactivation or UVER) has been observed for Herpes virus (Bockstahler & Lytle, 1970), adenovirus (Bockstahler & Lytle, 1977) and SV40 (Sarasin, 1978) as well as the single-stranded DNA parvoviruses, minute-virus of mice (Rommelaere *et al.*, 1981) and H1 (Su *et al.*, 1981). Enhanced reactivation has been observed in human (Lytle *et al.*, 1976), monkey (Bockstahler *et al.*, 1976), marsupial (Lytle & Benane, 1975) and a variety of other mammalian cells (Lytle, 1978). The enhancement can be provoked by pretreatment of the cells with UV-light (Bockstahler & Lytle, 1970), formaldehyde (Coppey & Nocentini, 1979), a variety of chemical carcinogens (Lytle *et al.*, 1978) and ionizing radiation (Jeeves & Rainbow, 1979). The idea that UVER is an inducible function is supported by the fact that cycloheximide can inhibit this process at least for HSV in monkey cells (Lytle & Goddard, 1979) and MVM in mouse cells (Rommelaere *et al.*, 1981).

Enhanced mutagenesis has been described for UV-irradiated HSV in irradiated monkey kidney cells (Das Gupta & Summers, 1978). However, Lytle *et al.* (1980), using the same virus found that enhanced mutagenesis only occurred under conditions which also allowed for multiplicity reactivation (i.e. high

multiplicities of infection). It would seem that, for HSV, enhanced mutagenesis is dependent upon UVER and MR. These are in turn dependent upon de novo protein synthesis since they are abolished by cycloheximide (Das Gupta & Summers, 1978; Lytle, 1978).

Simian virus 40 (SV40) has also been shown to undergo increased mutagenesis when UV-irradiated virus infects irradiated cells (Sarasin & Benoit, 1980). It is interesting that the authors concluded that EM might only be detectable at low m.o.i., exactly the opposite of the conclusion that was reached concerning enhanced mutagenesis of HSV (Lytle et al., 1980).

Studies of viral mutagenesis using adenovirus have indicated that EM does not occur for this virus (Day & Ziolkowski, 1978; 1981; Craig Bennett, personal communication).

The apparent disparity of the results discussed above may be attributable to the various modes of replication employed by each of the different viruses.

In bacterial cells, one other major feature of the SOS functions is prophage induction (Little and Mount, 1982). An analogous process has been observed with mammalian retroviruses, polyoma virus and SV40 which can be induced from a latent state by treatment of the host cells with a variety of DNA damaging agents (e.g. UV-light, X-rays, mitomycin C, BrdUrd) (Fogel & Sachs, 1970; Rothschild & Black, 1970; Bockstahler & Hellman, 1979). Zamansky et al. (1980) have shown that in a cell line inducible for SV40, the UVER of

herpes virus displays a dose-response parallel to the virus induction dose response. This suggests that the two events may be related.

Final proof of SOS repair awaits the demonstration of an induced protein which is involved in the repair pathway, similar to the demonstration of the inducibility of the rec A protein in E. coli (Little et al., 1980). Recently, Miskin & Reich (1980) have demonstrated the induction of plasminogen activator, a protease, in response to DNA damage. Though initial reports are encouraging (Miskin & Ben-Ishai, 1981) a definite involvement of plasminogen activator in DNA repair has yet to be found.

DNA REPAIR DEFICIENT HUMAN DISORDERS

Just as repair deficient mutants of bacteria have been used to study the many genes involved in bacterial DNA repair, a variety of repair deficient genetic human disorders are currently being investigated to study DNA repair in human cells (Friedberg et al., 1979; Arlett & Lehmann, 1978; Lehmann & Karran, 1981). While these studies have provided insights into some aspects of DNA repair, their most significant contribution may have been to illustrate the extreme complexity of mammalian repair mechanisms.

The first human disease that was shown to be associated with reduced DNA repair was xeroderma pigmentosum (XP) (Cleaver, 1968). Consequently, the majority of work involving repair deficiency has been done with XP. Presently, a number

of other disorders have been identified which are thought to be deficient in DNA repair processes other than those deficient in XP.

XERODERMA PIGMENTOSUM (XP)

The major clinical features of XP are the severe photosensitivity it imparts and the very high incidence of skin cancers (Robbins *et al.*, 1974).

The photosensitivity of XP is reflected *in vitro* by reduced post-UV colony forming ability (CFA) (Andrews *et al.*, 1978b) and by reduced rates of unscheduled DNA synthesis (UDS) (Cleaver, 1968). The presumption that these are indicative of reduced excision repair is supported by further evidence that XP cells are unable to excise thymine dimers (Cleaver & Trosko, 1970) and that the rate of loss of dimer-specific-endonuclease sites was much reduced in XP cells (Paterson *et al.*, 1973).

The exact defect of XP is uncertain at present. However, it is known that a number of complementation groups exist (see Friedberg *et al.*, 1979 for listing) indicating that a number of genes are involved. Eight complementation groups are known at present (A-G and variant). All of the members of the complementation groups A - G have severely to moderately reduced UDS. The XP variants though reduced in post-UV CFA, show normal (100%) or near normal (80-90%) levels of UDS (Friedberg *et al.*, 1979).

Consistant with their reduced ability to perform excision repair, XP cells have been shown to be reduced in their ability

to reactivate UV damaged virus. This has been demonstrated for a number of different viruses including HSV (Lytle et al., 1972), adenovirus (Rainbow, 1980; Day, 1974) and SV40 (Abrahams & Van der Eb, 1976). The amount of host cell reactivation (HCR) for UV'd adenovirus that XP cells are capable of performing ranges from 3% to 60% and correlates very well with their relative amounts of UV-induced UDS, except for group D (Rainbow, 1981; Day, 1974, 1975b).

Reduced excision repair is not the only defect which can be identified in XP cells. Extracts from XP cells have been shown to have lower levels of photoreactivating enzyme than normal cell extracts (Sutherland et al., 1975; Wagner et al., 1975). Another defect which has been identified is the reduced rate of conversion of low molecular weight DNA to high molecular weight in UV-irradiated cells (Lehmann et al., 1977). This is presumed to indicate a defect in post-replication repair (see Lehmann, 1978 for discussion). In XP variant cells, which have normal levels of UV-induced UDS but slightly reduced HCR of UV'd adenovirus (see Day, 1975b), this is thought to be the major metabolic defect.

Just as in vitro sensitivity to DNA damaging agents has been correlated to the in vivo sun-sensitivity of XP, the cancer-proneness of XP patients may have an in vitro explanation. Maher et al. (1976) have demonstrated that all XP cells (both classical and variant) are subject to increased mutagenesis after UV-irradiation compared to normal cells.

In addition to being hypersensitive to UV light, XP cells

are hypersensitive to a variety of chemical agents such as ethylmethylsulfonate (Regan & Setlow, 1976), mitomycin C (Sasaki et al., 1977) and many others (see Friedberg et al., 1979). There are as well, a number of chemicals to which XP cells are no more sensitive than normal cells. These include both methyl- and ethyl- nitrosourea (Cleaver & Friedberg, 1976), methylmethane sulfonate and N-methyl-N^1-nitro-N-nitrosoguanadine (Cleaver, 1971). The ability of XP cells to repair some types of damage to their DNA has led some investigators (Regan & Setlow, 1974) to speculate that two forms of DNA repair exist in normal human cells, as was discussed earlier, in the section on mammalian excision repair.

COCKAYNES SYNDROME (CS)

Patients with CS are dwarfed, are usually mentally deficient and often show signs of premature aging (Guzzetta, 1972). The most interesting symptom is their extreme sun-sensitivity. The suspicion that this symptom might indicate a repair deficiency has been confirmed by the demonstration of reduced post-UV CFA (Schmickel et al., 1977). Also reduced is the ability of CS cells to perform HCR of UV-irradiated adenovirus (Day & Ziolkowski, 1978; Rainbow & Howes, 1982). The relative amount of HCR for UV'd adenovirus in CS cells ranges from 14-34% and as such is similar to the range observed for XP cells (excluding XP variant) (Rainbow & Howes, 1982).

The wide range of cellular sensitivity to UV amongst strains of CS cells suggested to some workers (Andrews et al., 1978a) that CS may be genetically heterogeneous, as is XP.

This has been confirmed by the initial demonstration of at least two complementation groups (Tanaka et al., 1981).

In trying to determine the defective repair mechanism of CS cells studies have shown them to be capable of normal removal of thymine dimers (Schmickel et al., 1977). Unscheduled DNA synthesis after UV-irradiation is normal in CS cells (Wade & Chu, 1979) as is post-replication repair (Lehmann et al., 1979). However, like XP cells (Rude & Friedberg, 1977) CS cells are unable to recover normal rates of DNA synthesis after UV-irradiation (Lehmann et al., 1979).

The mutability of CS cells is, at present, uncertain. The literature contains reports on the subject which arrive at opposite conclusions (Arlett, 1979; Wade & Chu, 1979). It may be of some importance to sort out this question since CS patients do not show an increased cancer risk, as do XP patients (Schmickel et al., 1977).

The common clinical features of XP and CS patients include sun-sensitivity (Robbins et al., 1974; Guzzetta, 1972) as well as some degree of neurological involvement. In contrast, XP patients are at high risk for skin tumours (Robbins et al., 1974) whereas CS patients do not have an increased cancer risk (Schmickel et al., 1977).

Cells from both XP and CS patients are hypersensitive to the killing effects of UV-light (Robbins et al., 1974; Andrews et al., 1978 a,b) and UV-mimetic chemicals (see Friedberg, 1979). The ability of both types of cells to repair UV-damaged adeno virus is reduced compared to normal cells (see Rainbow, 1981).

The repair defect for the majority of XP cells has been found to be an inability to perform excision repair (Cleaver & Trosko, 1970). CS cells, however, have not been found to be defective in any of the aspects of excision repair (Andrews et al., 1978a, Hoar & Waghorne, 1978; Schmickel et al., 1977; Wade & Chu, 1979), or post-replication repair (Lehmann et al., 1977) tested to date. Though various parameters related to DNA repair modes have been examined in CS cells by many laboratories, the specific repair defect associated with this disease remains obscure.

OTHER REPAIR DEFICIENT SYNDROMES

ATAXIA TELANGIECTASIA (AT)

The major clinical symptoms of AT are progressive ataxia and extensive loss of neurons. This is usually accompanied by severe immunological dysfunction (Kraemer, 1977). These patients are also at high risk for cancer (Spector, 1977).

Cells derived from AT patients are more sensitive to ionizing radiation than are normal cells (Paterson, 1978). However, their ability to repair γ -irradiated adenovirus (Rainbow, 1978) and herpes virus (Henderson & Long, 1981) is apparently normal. Pretreatment of normal human fibroblasts with γ -rays has been reported to enhance their ability to repair UV-irradiated adenovirus (Jeeves and Rainbow, 1979a). However, for UV- or γ -irradiated AT cells this enhancement of adenovirus repair does not occur (Jeeves and Rainbow, personal communication). This suggests that AT cells are

deficient in some aspect of inducible repair. ER of UV-irradiated herpes virus in X-irradiated normal and AT cells is the same whereas UVER of UV'd herpes virus was reduced in AT compared to normal cells (Hellman et al., 1981). Another report was unable to find X-ray ER of X-irradiated herpes virus in either AT or normal cells (Henderson, personal communication).

In contrast to XP cells, which are hypermutable by UV-irradiation (Maher et al., 1976), AT cells have been found to be hypomutable by ionizing radiation (Paterson, 1979; Arlett, 1980). The impairment of an error-prone repair mechanism is implied.

Studies of cellular DNA repair synthesis as measured by repair replication and unscheduled DNA synthesis have illustrated the heterogeneity of the response of AT cells to ionizing radiation (see Paterson and Smith, 1979 for review). The various AT strains can be divided into approximately equal groups designated exr^+ for those proficient in hypoxic γ-ray induced excision repair and exr^- for those deficient strains.

Evidence for additional genetic heterogeneity comes from complementation studies. Three exr^- strains have been examined and found to represent two complementation groups (Paterson et al., 1977).

Another aspect of the repair defect of AT cells is the effect of ionizing radiation on the rate of semiconservative DNA synthesis. In normal cells, ionizing (Painter and Young, 1980) and non-ionizing (Rude and Friedberg, 1977) radiation initially inhibits semi-conservative DNA synthesis but normal

rates were recovered after a short delay. In AT cells however, semi-conservative DNA synthesis is much more resistant to inhibition by ionizing radiation (Painter and Young, 1980; Painter, 1981).

FANCONI'S ANEMIA (FA)

Patients with FA are characterized as having extremely high incidence of leukemia and other malignant neoplasms (German, 1972). Cells derived from FA patients are observed to have a high frequency of spontaneous chromosome aberrations, especially chromatid-type aberrations (Friedberg et al., 1979). The frequency of these aberrations could be increased in FA cells, by a greater degree than in normal cells, by treatment with a variety of mutagenic and carcinogenic compounds (Sasaki, 1978).

FA cells have proven to be deficient in the repair of damage induced by a variety of cross-linking agents (Friedberg et al., 1979).

OTHER MAMMALIAN REPAIR DISORDERS

A number of other human disorders have been reported to be defective in the repair of DNA damage induced by some physical or chemical agent. These disorders include Bloom's syndrome, progeria, Huntington's disease, retinoblastoma and Down's syndrome (Friedberg et al., 1979).

VIRUSES AS PROBES FOR DNA REPAIR

Bacteriophages have been used extensively to probe the DNA repair capacity of bacterial cells (reviews Defais et al., 1981; Witkin, 1976). In a similar manner and using similar experimental

protocols, mammalian virus can be used to probe the DNA repair capacity of human and other mammalian cells.

The types of experiments which have been performed fall into 4 main categories. 1) Cells are infected with virus which has been treated with physical or chemical agents. The survival of the virus yields information on the constitutive ability of the cell to repair the viral lesions (host-cell reactivation or HCR). 2) Cells can be pretreated with damaging agents and subsequently infected with untreated virus. The ability of the virus to express viral functions is an indication of the amount of DNA damage a cell can tolerate and still maintain its metabolic functions (capacity). 3) The relative survival of treated virus in cells which have also been treated with radiation or chemicals. This reveals any enhancement in viral reactivation (enhanced virus reactivation or ER). 4) Determination of the mutation rate of viral genes upon infection of treated cells with treated virus. This will reveal the magnitude of inducible error-prone repair modes.

HOST CELL REACTIVATION (HCR)

In theory, when a DNA-damaged virus infects a host cell, its ability to grow (i.e. replicate) will depend upon the repair of its genome. For simple, small viruses (e.g. SV40, parvoviruses) this repair depends, at least in part, upon host mechanisms. Thus the survival of the virus is thought to reflect, in part, the inherent repair capability of the host-cell. Larger, more complex viruses (e.g. adenovirus, HSV)

may produce viral repair proteins, complicating the interpretation of some results.

Virus survival has been monitored by a variety of functions such as plaque formation (Lytle et al., 1972), viral antigen expression (Rainbow, 1978), chromosome breaks and intranuclear inclusion body formation (Rainbow and Mak, 1972) one cycle virus yield from mass culture (Coppey et al., 1978), transformation frequency (Aaronson & Lytle, 1970) and repair of adenovirus DNA lesions (Rainbow, 1974, 1977).

Using many different viruses and many different criteria for survival, the HCR ability of normal and repair deficient strains have been compared. Xeroderma pigmentosum fibroblasts are strongly impaired in their ability to reactivate damaged virus (Aaronson & Lytle, 1970; Lytle et al., 1972; Day, 1974).

The relative HCR for UV'd adenovirus in XP cells correlates well with their relative amounts of UV-induced unscheduled DNA synthesis (UDS). The only exceptions to this rule are cells of complementation group D (Day, 1975b; Rainbow, 1981). However, although cells of the XP variant group are capable of normal levels of UDS (i.e. excision repair)(Takebe, 1978) they are slightly reduced in HCR of UV'd adenovirus (Rainbow and Howes, 1979) as measured by a viral antigen (Vag) assay. This is thought to reflect their defect in post-replication repair (Lehmann, 1972). In addition, a caffeine sensitive, excision dependent repair process which contributes to HCR for UV'd adenovirus has been postulated. Day (1975a) found that HCR of plaque formation for UV'd adenovirus was partially inhibited by caffeine in normal and XP variant cells but not in classical,

excision deficient XP cells.

The HCR of UV'd herpes virus in XP cells is also reduced compared to normal cells (Lytle et al., 1972). However, the relative reduction in HCR is not as great as that observed for adenovirus suggesting that complex viruses are not as dependent upon host-cell DNA repair enzymes. The addition of caffeine during the course of infection is reported to reduce the HCR of plaque formation for UV'd herpes virus in normal human fibroblasts (Lytle, 1972) but not in XP variant cells (Selsky & Greer, 1978). Because caffeine has a greater effect on the HCR of UV'd adenovirus than on UV'd herpes virus in normal human fibroblasts it has been postulated that the caffeine sensitive repair mode, important in the repair of adenovirus, has a less significant role in the repair of herpes virus (Selsky & Greer, 1978).

HCR has also been reported for herpes virus treated with acetoxy - 2 - acetylaminofluorine (Selsky & Greer, 1978), adenovirus treated with gamma rays (Rainbow & Howes, 1979), nitrous acid (Day, 1975a) and various other chemicals (see Rainbow, 1981). For all of the treatments specified, HCR of treated virus was reduced in XP cells compared to normals. No reduction of HCR in XP cells was seen for herpes virus treated with formaldehyde (Coppey & Nocentini, 1979) or with nitrogen mustard (Selsky & Greer, 1978). Though the HCR of X-irradiated herpes virus in XP cells was slightly reduced, Lytle et al. (1972) did not consider this reduction to be significant. Zamansky and Little (1982) tested 3 XP strains and found only one that was significantly reduced in the HCR of ^{60}Co-irradiated herpes virus.

Reduced HCR of damaged virus has been reported for other types of human fibroblasts. Using the Vag assay for UV'd adenovirus, reduced HCR has been detected in Cockayne's Syndrome (CS) (Rainbow & Howes, 1982), Fanconi's anemia (FA) (Rainbow & Howes, 1977a,b), Bloom's syndrome (one cell strain only) (Krepinsky et al., 1980), ataxia telangiectasia (AT) (Rainbow, 1978) and Huntington's chorea (HD) (Rainbow, 1981). Using an adenovirus plaque assay Day et al. (1981) reported reduced HCR of UV'd herpes virus in CS cells measured by plaque assay. However, using an assay very similar to that of Day et al. (1981), Hoar & Davis (1979) found no difference between the HCR ability of normal and CS cells. Similarly, Ikenaga et al. (1979) found normal levels of HCR of herpes virus (using plaque assay) for the one CS strain tested. A recent report (Lytle et al., 1983) found that the HCR of UV'd HSV was normal in one strain and reduced in the other strain tested.

CAPACITY

The infection of cells, pre-treated with DNA damaging agents, with untreated virus will yield a measure of the cellular repair capacity. It has been suggested (Coohill, 1981) that in the study of human repair mechanisms, capacity is a better assay for cellular sensitivity than colony-forming ability since many human fibroblast strains have poor clonogenic survival.

The capacity of both human fibroblasts and monkey kidney cells to support herpes virus infection has been reported to be reduced by a variety of DNA-damaging agents including UV-light (Lytle et al., 1976; Coppey & Nocentini, 1976) and 8-methoxypsoralen plus light (Coppey et al., 1979a). Capacity

can be restored in both types of cells by delaying infection after cell treatment (Lytle et al., 1976; Coppey et al., 1979a).

XP cells, from all excision deficient strains tested, were found to be more reduced in capacity than normal cells. Unlike normal cells, delayed infection did not result in the restoration of capacity in treated XP cells (Lytle et al., 1976; Coppey et al., 1979b).

ENHANCED VIRUS REACTIVATION (ER)

The pretreatment of mammalian cells with a variety of DNA damaging agents UV (Bockstahler & Lytle, 1970), X-rays (Bockstahler & Lytle, 1977), γ -rays (Jeeves & Rainbow, 1979a,b) chemical carcinogens (Lytle et al., 1978), has been shown to lead to the increased survival of UV-irradiated nuclear replicating viruses (Bockstahler & Lytle, 1977). This enhancement occurs in a variety of host cells including monkey kidney cells (Bockstahler et al., 1976), normal, XP (Lytle et al., 1976) and CS cells (Coppey & Menezes, 1981).

As discussed earlier (see SOS Functions in Mammalian Cells) UV-light enhanced reactivation (UVER) is similar in many respects to Weigle reactivation (Weigle, 1953).

The exact mechanism of UVER is not known. It has been shown that XP cells, which are excision deficient, are capable of UVER, and that UVER therefore is not dependent upon excision repair (Lytle et al., 1976). UVER is also not dependent upon multiplicity reactivation for HSV (Coppey &

Nocentini, 1973), SV40 (Sarasin & Hanawalt, 1978) or MVM (Rommelaere et al., 1981).

It has been hypothesized that ER represents an inducible repair pathway which is induced by blockage of DNA synthesis (Sarasin & Hanawalt, 1978). Consistent with this hypothesis, drugs which block DNA synthesis (e.g. hydroxyurea, cycloheximide) enhance SV40 reactivation (Sarasin & Hanawalt, 1978). Further support is provided by the observation that a delay between cell treatment and virus infection increases virus reactivation even further (Bockstahler et al., 1976). Enhanced reactivation of HSV and MVM can be inhibited by cycloheximide (Lytle & Goddard, 1979; Rommelaere et al., 1981) implying a requirement for de novo protein synthesis.

Recently it has been shown that UV- or γ -irradiated human fibroblasts were capable of enhanced reactivation of UV- or γ -irradiated adenovirus (Jeeves & Rainbow, 1979a,b; Rainbow, 1981). Differences in the UV and γ -ray dose response of the capacity of the cells to support adenovirus V-antigen formation suggested that UVER and γ -ray ER operate via different mechanisms (Rainbow, 1981). Hellman et al. (1976) reached a similar conclusion when caffeine was found to reduce UVER in monkey kidney cells but not X-ray ER.

Though the mechanism of enhanced reactivation is not clear it seems to be related to other putative mammalian SOS responses. These include provirus induction (Zamansky et al., 1980), and enhanced mutagenesis (Lytle et al., 1980; Sarasin & Benoit, 1980). However, inhibition of ER by the protein

synthesis inhibitor, cycloheximide (Lytle & Goddard, 1979; Rommelaere et al., 1981) is not sufficient proof that ER is inducible.

ENHANCED VIRAL MUTAGENESIS (ERROR-PRONE REACTIVATION)

Determination of the viral mutation rate of viruses grown in irradiated or chemically treated host cells has been used as a measure of inducible error-prone repair in some mammalian systems.

Das Gupta and Summers (1978) first demonstrated that enhanced reactivation of HSV was accompanied be enhanced mutagenesis (EM). They also showed that cycloheximide, a protein synthesis inhibitor, prevented the enhancement of the mutation rate. Using the same virus, Lytle et al. (1980) concluded that EM only occurred during infections at high multiplicity.

More recently, Su et al. (1981) have demonstrated the simultaneous enhancement of reactivation and mutagenesis of UV-irradiated parvovirus in nitronapthofuran treated human cells. Both of these processes were inhibited by treatment with cycloheximide.

Studies using SV40 virus have established that pretreatment of CV-1 monkey kidney cells with chemical carcinogens or UV-light strongly increases the mutation frequency in surviving virus (Sarasin & Benoit, 1980). However, the reversion frequency of UV-irradiated human adenovirus type 5 ts2 was not found to be enhanced following pretreatment of normal

human fibroblasts with UV-light (Day and Ziolkowsky, 1981) shedding some doubt on the universality of the phenomenon of ER to all virus/cell systems.

The present study was undertaken to investigate the DNA repair capabilities of various human fibroblast strains using Herpes simplex virus type 2 as a probe.

Herpes viruses are large enveloped viruses which have an icosohedral capsid. The diameter of the capsid is approximately 100 nanometers and is made up of 162 capsomeres (Watson, 1973). The genome of HSV-2 is a linear double-stranded DNA molecule of molecular weight 100 x 10^6 daltons (Spear & Roizman, 1980).

During lytic infect the expression of viral genes occurs in three phases (α, β, γ) whose syntheses are coordinately regulated and sequentially ordered in a cascade fashion (Spear & Roizman, 1980).

In the present study a plaque assay was used to monitor virus function. In order to produce a plaque the initial virus must be able to produce infective progeny. In the case of DNA damaged virus this may require extensive repair. The production of late viral antigens was also monitored by indirect immuno-fluorescence. This relies upon an event much closer to the initial lesion and its repair.

The objectives of this study were:

1) to examine the levels of host-cell reactivation (HCR) capacity and UV-enhanced reactivation (UVER) of herpes virus in normal human fibroblasts;

2) to examine the levels of HCR, capacity and UVER of normal cells to those observed in Cockayne's syndrome and xeroderma pigmentosum cells;

3) to compare the levels of HCR as determined for normal, CS and XP fibroblasts by plaque assay, to the levels of HCR as determined by a viral antigen assay.

It was hoped that these experiments would help elucidate the specific DNA repair deficiencies that are present in CS and XP cells as well as the relationship of such deficiencies to the clinical symptoms of the diseases. Through these comparisons it was hoped that the role of constitutive DNA repair of viral and cellular DNA, and UV-light enhanced reactivation could be evaluated with respect to the relative cancer risks of XP and CS patients.

MATERIALS AND METHODS

MEDIA

The following media were purchased in powder form, made up in double glass distilled water and filter sterilized through a 0.22 micron Millipore filter (Millipore Corporation, Bedford, Massachusetts 01730, USA);

1) MEM Alpha medium (α-MEM, Gibco catalogue #410-2000, Grand Island Biological Co., Grand Island, N.Y., USA) for culture of all human diploid fibroblast strains
2) F15 modified MEM (Gibco catalogue #410-1500) for the growth and maintainence of all Vero cell cultures

Media Supplements

1) Penicillin-Streptomycin solution, penicillin (base) 5000 u/ml and streptomycin (base) 5000 mcg/ml (Gibco catalogue #600-5070) was added to all media to a final concentration of 1% (v/v)
2) Fungizone (Gibco catalogue #600-5295), which is lyophilized amphotericin B, was reconstituted with 20 mls of sterile double glass distilled water (concentration 250 μg/ml). This was added to all media to a final concentration of 1% (v/v)
3) 7.5% $NaHCO_3$ solution, autoclave sterilized, was added to all media to a final concentration of 1.5-2.0% (v/v)
4) Fetal Calf Serum (Gibco catalogue #200-6140) was added to α-MEM for the culture of human diploid fibroblasts. Complete α-MEM contained 10% fetal calf serum (v/v)
5) Calf Serum (Gibco catalogue #200-6170) was F15-MEM for the culture of Vero cells. Complete F15-MEM contained 5% calf serum (v/v)

SOLUTIONS

1) Phosphate Buffered Saline (PBS). This buffer was used for washing cell monolayers before irradiation. It was prepared as a 10X stock solution containing 80 gms NaCl; 20 gms KCl; 11.5 gms Na_2HPO_4; 2.0 gms KH_2PO_4 dissolved in 1 litre glass double distilled water. For use, stock was diluted 10 fold in glass double distilled water and sterilized by autoclaving.

2) Trypsin (0.25%)1X (Gibco catalogue #610-5050) was used for the subculturing of human diploid fibroblasts and Vero cells.

3) Crystal Violet Stain; this stain was used for the simultaneous fixing and staining of cell monolayers in 24-well tissue culture plates for plaque assays. It was prepared by dissolving 2 gms of crystal violet in 20 mls methanol, 144 mls 1X PBS and 36 mls of formaldehyde. This was filtered through a fluted Whatman 1 filter paper (W & R. Balston Ltd., England). This stain was reused repeatedly.

4) Fluoroscein conjugated antibody (Gibco catalogue #660-3512); sheep anti-rabbit antibody covalently linked to FITC was purchased in lyophilized form and reconstituted with distilled water. This was used as 1:10 or 1:20 dilution in 1XPBS.

5) Rabbit anti-herpes simplex virus type 2 antibody was the generous gift of Dr. Silvia Bacchetti (Department of Pathology, McMaster University, Hamilton, Ontario, Canada). Antiserum was prepared according to the method of Seth *et al.* (1974).

CELLS

HUMAN DIPLOID FIBROBLASTS

NORMAL STRAINS

Normal human diploid fibroblast cell strains GM2803 and GM969 were obtained from the Human Genetic Mutant Cell Repository, Camden, New Jersey, USA.

Cell strain CRL1221 was obtained from the American Type Culture Collection, Rockville, Maryland, USA (ATCC).

The cell strain A2 was supplied by Dr. Samuel Goldstein, Departments of Medicine and Biochemistry, McMaster University, Hamilton, Ontario, Canada.

The cell strain designated Hff was a primary human foreskin fibroblast strain, supplied by Mr. Jeff Kawamoto, Department of Microbiology, St. Joseph's Hospital, Hamilton, Ontario, Canada.

COCKAYNE'S SYNDROME STRAINS

Fibroblast strains from patients with Cockayne's Syndrome were all obtained from the Human Genetic Mutant Cell Repository. The designations of the cell strains utilized were GM1856 (CS3BE), GM1629 (CS1BE), GM2838 and GM739.

XERODERMA PIGMENTOSUM STRAINS

The cell strain GM710 (XP25RO): complementation group A was obtained from the Human Genetic Mutant Cell Repository. Cell strains CRL1376 (XP8LO): complementation group A, CRL1260 (XP4RO): complementation group C, CRL1157 (XP6BE): complementation

group D and CRL1162 (XP4BE): variant were obtained from ATCC.

GROWTH AND PASSAGING

When fibroblast cell strains reached confluency in 75 cm^2 plastic flasks (Falcon), they were subcultured by the following method. The medium was aspirated off the cells and the cell monolayer was then washed with 2 mls of either 1X trypsin (0.25%) (Gibco) or 1XPBS. The wash was aspirated off and 2 mls of prewarmed 1X trypsin was placed on the cells. The flasks were to stand at room temperature for 3 to 10 minutes. Cells were dislodged by sharply rapping the flask. The activity of the trypsin was stopped by adding an appropriate volume of prewarmed complete α-MEM. The suspension of cells was then pipetted up and down a number of times to break up clumps of cells. Typically this suspension was then aliquoted equally into 3, 75 cm^2 flasks (Falcon, Division of Becton, Dickinson and Co., Oxnard, California 93030 USA) each containing 15 mls of prewarmed complete α-MEM. These were incubated at 37°C in a humidified atmosphere with 5% CO_2. Cultures were generally confluent 5-7 days after subculture at a 1:3 ratio.

NON-HUMAN CELL LINES

VERO CELL LINE

This cell line, obtained from ATCC, was initiated from the kidney of a normal adult African green Monkey. Monolayers of this cell line were used to grow stocks of herpes simplex virus type 2 (HSV-2).

GROWTH AND PASSAGING

This cell line was grown in 150 cm^2 plastic flasks (Corning Glass Works, Corning, N.Y., 14830, USA) or in sterilized 32 oz. glass prescription bottles (Brockway Glass Co. Inc., Brown Glass and Bottle Supplies, Montreal, Quebec). Cells were grown in F15 MEM supplemented with 5% calf serum.

When a flask or bottle of cells was confluent the medium was aspirated off. The cell monolayer was washed with either prewarmed 1XPBS or prewarmed 1X trypsin (0.25%) (Gibco) which was subsequently aspirated off. The cells were released from the surface of the flask or bottle by the addition of 2 mls of 1X trypsin (0.25%) (Gibco). This was allowed to act for 3-10 minutes at room temperature, after which time a sharp rap on the edge of the flask or bottle was generally sufficient to dislodge the cells. If cells still remained attached, the flask or bottle was placed in a 37^{o} incubator for approximately 5 minutes. If the cells still remained attached they were gently removed by scraping with a sterile rubber policeman. The cell suspension was diluted with an appropriate volume of complete F15 MEM which also stopped the activity of the trypsin. This cell suspension was pipetted up and down a number of times to disrupt clumps of cells and then aliquoted equally into 5-10 sterile flasks or bottles each containing 30 mls of fresh prewarmed F15 MEM. Monolayers were confluent within 3-5 days.

ULTRAVIOLET LIGHT IRRADIATION

Irradiation of cells in all experiments was with a General Electric Germicidal Lamp G8T5 (General Electric Company, Nela Park, Cleveland, Ohio 44127, USA) emitting principly at a

wavelength of 254 nm. Dose rates ranging from 0.05 to 1 $J/m^2/sec$ were measured using a J-225 shortwave UV-meter (Ultraviolet Products, San Gabriel, California, USA). Prior to irradiation the medium was aspirated off the cells and the cell monolayer was washed with sterile 1XPBS. To irradiate, the 24-well plate (see Section Plaque Assay of Materials and Methods) was placed under the lamp, with its lid in place, at a measured distance at which the appropriate dose rate was delivered. The plastic lids were previously tested and found to be opaque to UV. The wells or chambers of cells to be exposed were uncovered for the required length of time, then re-covered. Immediately after irradiation the cells' medium was replaced with fresh prewarmed complete α-MEM and then returned to a 37^{o} incubator with a humidified 5% CO_2 atmosphere until they were to be infected.

VIRUS

HERPES SIMPLEX TYPE 2 (HSV-2)

GROWTH

To grow stocks of HSV-2, 50, 150 cm^2 tissue culture flasks (Corning) of Vero cells were prepared (see section Vero Cells, Growth and Passaging of Materials and Methods). When cell monolayers were confluent the complete F15 MEM was aspirated off and replaced with 20 mls of prewarmed F15 MEM containing only 1% calf serum (plus all other additives). After 24 hours in this medium the cells were ready to be infected. Virus for

the inoculum was HSV-2 strain 333, the generous gift of Dr. Silvia Bacchetti, Department of Pathology, McMaster University, Hamilton, Ontario. The virus was suspended in F15 MEM with no serum (but with all other additives). The multiplicity of infection was 0.1-0.5 pfu/cell. Medium was aspirated off the cells and the cells were infected with just enough inoculum to cover the cells (approximately 5 mls). Virus was allowed to adsorb for 60-90 minutes at 37° and 30 mls of F15 MEM (1% calf serum) was then aliquoted to each flask. These flasks of cells were then incubated at 37° in a humidified atmosphere with 5% CO_2 for 48 hours or until cytopathic effect (CPE) was observed for the majority of the cells (whichever occurred first). Cells were then collected by either sharply rapping the flask or by gently scraping them off with a sterile rubber policeman. Cells were then pelleted by low speed centrifugation at 4°C in an International Equipment model PR-2 refrigerated portable centrifuge (International Equipment Co., Needham Heights, Mass. USA).

CRUDE VIRUS PREPARATION

The cell pellets (described in the preceding section) were resuspended in cold F15 MEM with no serum. The volume used was 0.5 mls for each flask of cells collected. This suspension was sonicated on ice for two 30 second intervals using a Biosonik III, Model BPIII40T (Bronwill Scientific, Rochester, N.Y., USA). This suspension was then centrifuged at 1500 rpm in an International Equipment Model PR-2 refrigerated portable centrifuge for 20 minutes at 4°C. The supernatent,

which contained the virus particles, was removed and aliquoted into small volumes (0.5-1.0 mls) and frozen at -70°C until use.

ULTRAVIOLET LIGHT IRRADIATION

Stock suspension of virus was diluted 1:1 with α-MEM containing no serum (but containing all other additives). Volumes of no more than 1.5 mls were placed in 35 mm plastic petri dishes (Falcon) and kept on ice. Irradiation was with a G.E. Germicidal Lamp G8T5. Dose rates were measured using a J-225 short-wave UV-meter. In general the dose rate was between 0.5 J/m^2 and 5J/m^2 depending upon the total doses required. During irradiation the viral suspension was constantly swirled to ensure even irradiation of the entire sample. To irradiate, the sample was placed directly below the centre of the lamp with the lid of the petri dish in place. The lid was then removed for the period of time required to produce the required fraction of the total dose. The lid was then replaced, to stop the irradiation. After each cumulative dose, an aliquot of the suspension was removed and used to produce a dilution series for that dose. Dilutions were in cold α-MEM with no serum.

EXPERIMENTAL PROTOCOLS

PLAQUE ASSAY

Plaque assays were carried out on 24-well tissue culture plates, either Linbro plates (Flow Laboratories Inc., Hamden, Connecticut, USA) or Nunclon plates (Nunc Inter Med, Denmark).

Two of these plates could be seeded with the cells from one 75 cm^2 flask (Falcon) of confluent fibroblasts. The procedure for this was essentially identical to the procedure for subculturing fibroblasts (see section Human Diploid Fibroblasts, Growth and Subculture of Materials and Methods). The only difference was that instead of aliquoting the cell suspension into fresh tissue culture flasks, the suspension was made up to a total volume of 48 mls with complete α-MEM. Then 1 ml aliquots of this were placed into the wells of two 24-well plates. Generally, the cells in the wells of the plates were confluent within 24 hours.

When the cells were confluent a dilution series for each of the viral suspensions was prepared (see Figure 1 for a schematic of the dilution procedure) in cold α-MEM with no serum. All medium was aspirated off of the cells and each well was inoculated with an appropriate dilution of virus (i.e. a dilution expected to produce between 1 and 30 plaques per well). For the 24-well plates the volume of the inoculum was 0.2 mls, a volume just large enough to cover the monolayer and prevent drying out. Inoculation was done in small groups so that the cell monolayers would not dry out between the time the medium was aspirated off and the time the inoculum was applied. The virus was allowed to adsorb for 60 to 90 minutes at 37° in a humidified 5% CO_2 atmosphere. After the adsorption period the cell monolayers were overlayed with approximately 1.5 mls of complete α-MEM containing 0.05% pooled immune serum globulin (human) (DIN075280 Connaught Laboratories

Limited, Willowdale, Ontario, Canada or DIN083259, Lot C5857 Cutter Laboratories Inc., Berkeley, CA. 94710 USA). The immune serum globulin (ISG) fulfilled the function that the soft agar overlay of conventional plaque assays performs (i.e. it prevents reinfection by released virus particles, allowing reinfection only by cell to cell contact). These were incubated for 48 hours at 37° in a humidified 5% CO_2 atmosphere. The medium was aspirated off after this period and the cell monolayers were fixed and stained for 5-10 minutes with a crystal violet solution containing methanol and formaldehyde (see section Solutions of Materials & Methods).

Plaques were counted and the results were plotted against the inverse of their respective dilution factors. Linear regression analysis was used to determine the titre of the original viral suspension in terms of plaque forming units per ml of original suspension volume (Figure 7).

VIRAL ANTIGEN ASSAY

Viral antigen (Vag) assays were carried out on 8-well tissue culture chamber slides (Lab-Tek Division, Miles Laboratories, Inc., Napierville, Il. 60540, USA). Eight of these slides could be seeded with the cells from one 75 cm^2 flask (Falcon) of confluent human fibroblasts. The procedure for this assay was essentially the same as for the plaque assay, with the following modifications:

a) cells from each flask were suspended in a total volume of 26 mls and 0.4 ml aliquots were placed in each well;

b) dilutions of virus were chosen to produce between 50-200 Vag positive foci per well;

c) inoculum volume was 200 µl ;

d) cells were overlayed with 0.4 mls of complete α -MEM containing 0.05% pooled immune serum globulin (human) (Connaught Labs or Cutter Labs);

e) cells were fixed at various times after infection with cold methanol: acetone (1:1) and stained for immunofluorescence (see section Immunofluorescent Staining of Materials and Methods);

f) titres were determined in terms of Vag forming units per millilitre of original viral suspension.

IMMUNOFLUORESCENT STAINING

Cells were stained for the presence of herpes simplex virus type 2 antigens by indirect immunofluorescence.

The fixed slides were rehydrated in 1X PBS for 30 minutes at 37°. Two aliquots of 25 µl of a 1:10 dilution of stock rabbit anti-herpes serum were placed on 22 x 50 mm coverslips (Corning Glass Works, Corning, New York). After draining the slides, the cell sheets on each slide were gently placed over the coverslips. Air bubbles were removed and the slides were placed, upside down, at 37° in a humid incubator for 1 hour. The coverslips were removed and the slides washed 3 times with 1X PBS, the third wash being for 30 minutes at 37°. Two aliquots of 25 µl conjugated sheep anti-rabbit globulin (Gibco) were placed on fresh coverslips. After draining the slides, the cell sheets on each slide were gently placed on the

coverslips and air bubbles were removed. Slides were incubated upside down at 37° in a humidified incubator for 1 hour. The coverslips were removed and the slides were washed 3 times in PBS, the final wash being for 30 minutes at 37°. After air-drying the slides, the cell sheets on each slide were placed on coverslips with two drops of PBS-glycerol (1:9) and air bubbles were removed. These slides were stored at -20°C until they were examined microscopically.

HOST CELL REACTIVATION OF PLAQUE FORMING ABILITY

The plaque assay described in the preceding section was used to perform this class of experiments.

The purpose of these experiments was to produce dose response curves from the virus in different human diploid fibroblast strains to ultraviolet light irradiation.

Tissue culture plates (24-well, Linbro or Nunc) were seeded with sufficient fibroblast cells, of those strains to be tested, such that the monolayers were confluent within 1-2 days. When the cell monolayers were confluent, separate groups of dilution series were prepared for each dose of ultraviolet light to the virus to be tested, including an unirradiated control series. Infections were carried out according to the protocol described previously for the plaque assay. The titre of viral suspension obtained at each dose tested was normalized to the titre of the unirradiated viral suspension. This value, expressed as a surviving fraction (SF) was plotted against the dose of ultraviolet light on semi-log graph paper.

HOST CELL REACTIVATION OF Vag FORMING ABILITY

The Vag assay described earlier was used to perform this experiment.

The purpose of this experiment was to compare the relative HCR of a different viral function (i.e. plaquing and Vag formation) in normal, CS and XP cell strains.

Chambered slides (Lab-Tek) were prepared as described earlier (see section Viral Antigen Assay of Materials and Methods). When cell monolayers were confluent, two virus dilution series' were prepared. One was of unirradiated virus and the other, virus irradiated with $50 J/m^2$ of ultraviolet light. Infections were performed as previously described. At various times after infection (10, 12, 18, 20 hours for unirradiated virus, 12, 18, 20, 24 hours for irradiated virus) cells were fixed and stained for immunofluorescence (as described earlier). The titre of the virus was determined for unirradiated and irradiated virus at each time point. The virus titres were plotted as a function of time post-infection. The surviving fraction (irradiated/unirradiated) was calculated at each time point and plotted as a function of time post-infection.

ENHANCED VIRAL REACTIVATION OF PLAQUE FORMING ABILITY

To measure the relative increase in viral reactivation that can be elicited by pre-irradiation of the host cells the following types of experiments were performed.

The parameters of this type of experiment were divided into

the following categories:

1) Time between seeding of plates and irradiation of the cells

   a) 1 day
   b) 2 days
   c) 3 days

2) Time between irradiation and infection of the cells

   a) 0 hours (immediate)
   b) 48 hours
   c) 96 hours

For each of these categories to be tested 2 groups of 24-well plates were prepared, one to be infected with unirradiated virus and the other to be infected with irradiated virus. Each group of 24-well plates represented a series of different doses of ultraviolet light to which the cells were exposed (including an unirradiated control). After a suitable recovery period (see category 2, this section) the cells were infected.

The seeding and irradiation of cells was timed such that all infections were performed at the same time, using the same sets of dilution series of the viral suspension.

Forty-eight hours after infection, the medium was aspirated off the cells and they were fixed and stained with a crystal violet solution containing methanol and formaldehyde (see section Solutions of Materials and Methods). The number of plaque forming units observed at each dose of ultraviolet light to which the cells were exposed was calculated as for the plaque assay (see section Plaque Assay of Materials and Methods). Relative plaque forming ability (RPFA) was calculated by normalizing the viral plaque forming ability on irradiated to its plaque forming ability on unirradiated cells. This produced

two sets of data, normalized RPFA of unirradiated virus and normalized RPFA of irradiated virus. Normalization was to their respective unirradiated controls. Finally, a third set of data were produced by taking the ratio of RPFA (irradiated)/RPFA (unirradiated) at each dose to the cells that was tested. This ratio was the reactivation factor (RF), indicating the relative enhancement of host-cell reactivation caused by pre-irradiating the host cells.

These three sets of data were plotted as RPFA (or RF) versus the dose of ultraviolet light to which the cells were exposed.

RESULTS

A HOST CELL REACTIVATION

The inherent ability of various cell strains to repair ultraviolet light induced DNA damage was examined using host cell reactivation of herpes virus. The end-point of the assay was the plaque forming ability of HSV-2 on the various cell strains to be tested. The relative survival of plaque forming ability of HSV-2 was obtained by comparing the pre- and post- UV irradiation plaque forming ability on fibroblast monolayers. This value was expressed as a surviving fraction for each dose given to the virus.

The ability of a cell to reactivate double stranded DNA viruses is thought to depend upon a number of different DNA repair pathways. Excision deficient XP cells have been shown to be reduced in their ability to reactivate HSV (Lytle et al., 1972).

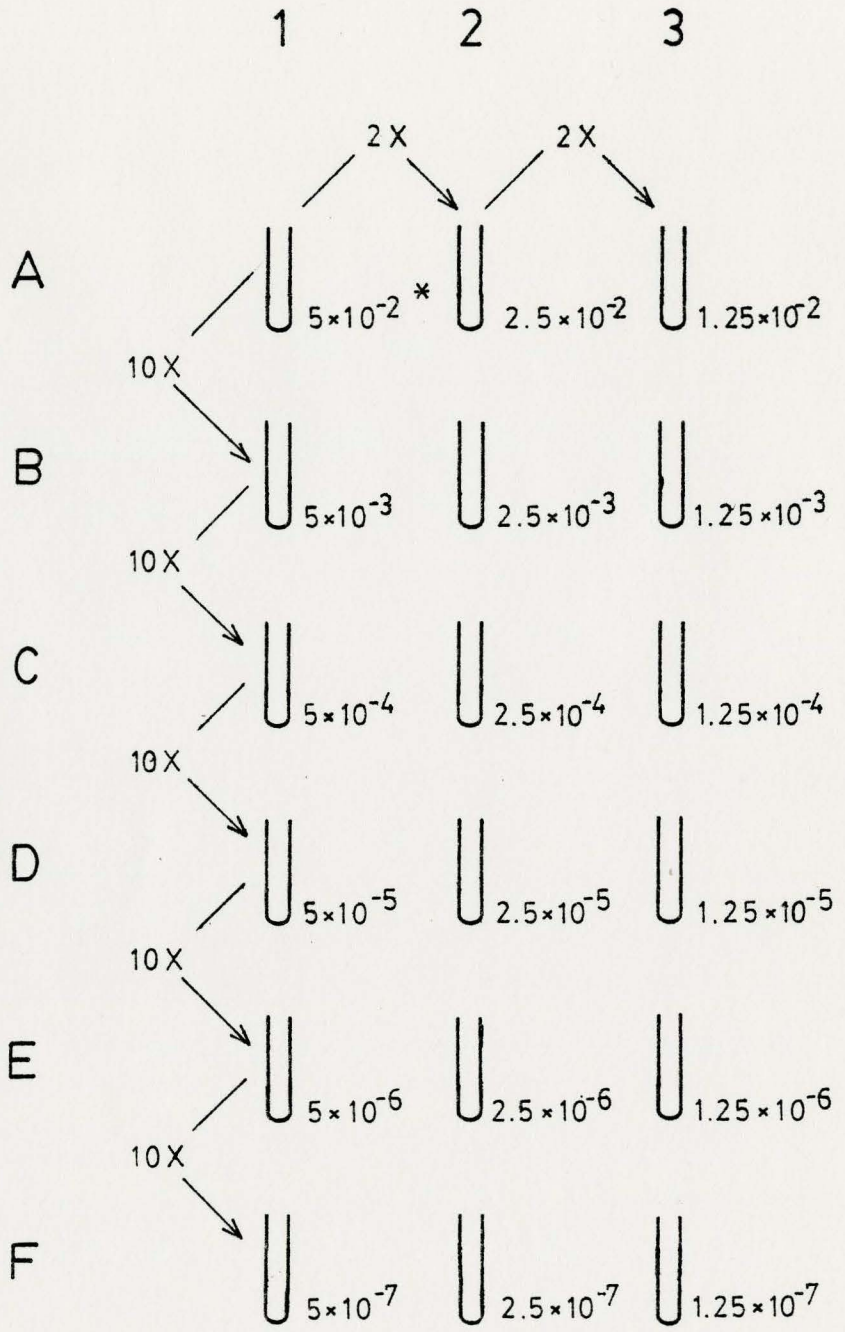
A.I EFFECT OF UV IRRADIATION ON HSV-2 PLAQUE FORMATION IN HUMAN FIBROBLASTS

The level of HCR for UV irradiated herpes virus was determined in normal, XP and CS cell strains. Cell monolayers were prepared by seeding the cells from one confluent 75 cm^2 tissue culture flask into two 24-well tissue culture plates. Cell monolayers were sufficiently confluent to be infected 24 hours later.

FIGURE 6

SCHEMATIC OF DILUTION SERIES

An aliquot of stock virus suspension was diluted in sufficient α-MEM to obtain a concentration of 5×10^{-2} millilitres of initial virus stock/millilitre total volume. A series of up to 5 ten-fold dilutions were made into α-MEM. Each of these was then twice diluted 2 fold into α-MEM.



* millilitres of virus stock / ml

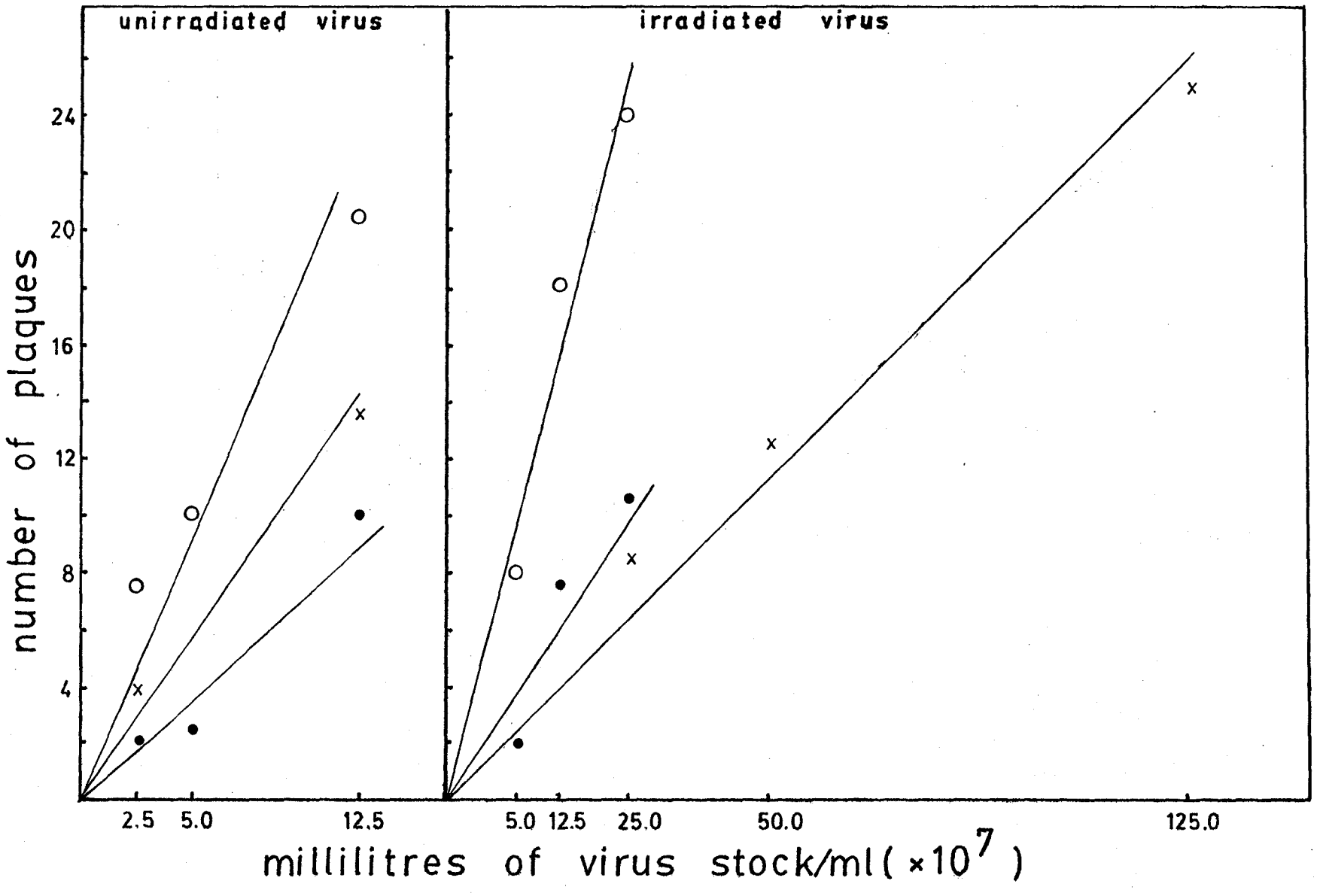
FIGURE 7

Titration of virus plaque forming ability

To determine the titre of virus the number of plaques counted is plotted as a function of the volume of virus suspension. The slope of the line through these points is the titre of the virus suspension. This is determined for unirradiated and irradiated virus.

○ CRL 1221
● CS 3BE
× XP 4RO

Each point represents the mean of duplicate determinations at that dilution. Points were fitted to straight lines by least squares analysis. The UV dose to the irradiated virus was 25 J/m^2.


unirradiated virus
irradiated virus
number of plaques
24
20
16
12
8
4
2.5
5.0
12.5
5.0
12.5
25.0
50.0
125.0
millilitres of virus stock/ml (×10^7)

Stock virus suspension was diluted 1:1 with α-MEM (no serum) and an aliquot was removed to make a dilution series (See figure 6) of unirradiated virus. After each cumulative dose of UV-light, another aliquot was taken to make a dilution series.

Duplicate wells were infected with each dilution. For each dose, 3 consecutive dilutions were used. After 1 hour of adsorption, cells were overlayed with complete α-MEM plus 0.05% immune serum to prevent reinfection by progeny virions released into the medium. Forty-eight hours later monolayers were fixed, stained and plaques were counted.

For each dose of UV to the virus the titre of virus was calculated by plotting the number of plaques versus the amount of stock suspension (i.e. the inverse of the dilution factor) (see figure 7). Points were fitted to a straight line using least squares analysis and the slope of the line through these points was used to determine the virus titre. In order to obtain the survival of HSV-2 plaque forming ability the titres of virus at each dose of UV-light were divided by the titre for the unirradiated virus. Typical UV survival curves obtained are shown in figure 8 for normal,

XP and CS strains as a function of UV dose to the virus. Error bars represent the standard error about the point. It can be seen that the HCR ability of the normal and the CS strains are not significantly different over the range of doses tested. However, the HCR ability of the XP strain is significantly lower than both the normal and CS strains. In addition it can be seen that all strains display a 2- component survival curve.

A.II UV SURVIVAL OF HSV-2 PLAQUE FORMATION

A.II.a. NORMAL HUMAN FIBROBLASTS

Figure 9 shows the pooled results for the dose response of HSV-2 plaque formation in normal cells. Five different normal strains were tested. Each point represents the logarithmically transformed mean of at least two separate determinations.

The slope of the straight line portion of the graph is given by $-1/D_o$, where D_o is the radiation dose required to reduce the survival level at any point along the straight line portion to e^{-1} or 0.37 of that level. From table 1 we can see that the D_o of the first, steeper component ranges between 26 and 36 J/m^2 for the normal strains tested. The D_o of the second component ranges from 81 to 101 J/m^2. The intercept of the second component is the point at which an extrapolation of the second component

FIGURE 8

Survival of plaque forming ability of UV-irradiated HSV-2 in normal, CS and XP fibroblasts

The figure shows typical results for the survival of plaque formation of UV-irradiated HVS-2 in normal, CS and XP fibroblasts. Monolayers of cells were prepared in 24-well tissue culture plates. Cells were infected with unirradiated or UV-irradiated HSV-2 according to the procedure in **the section** , Materials & Methods. Plaques were counted at 48 hours post-infection and used to calculate virus titre. Titres were normalized to unirradiated virus and plotted as a function of UV dose. Error bars represent one standard error.

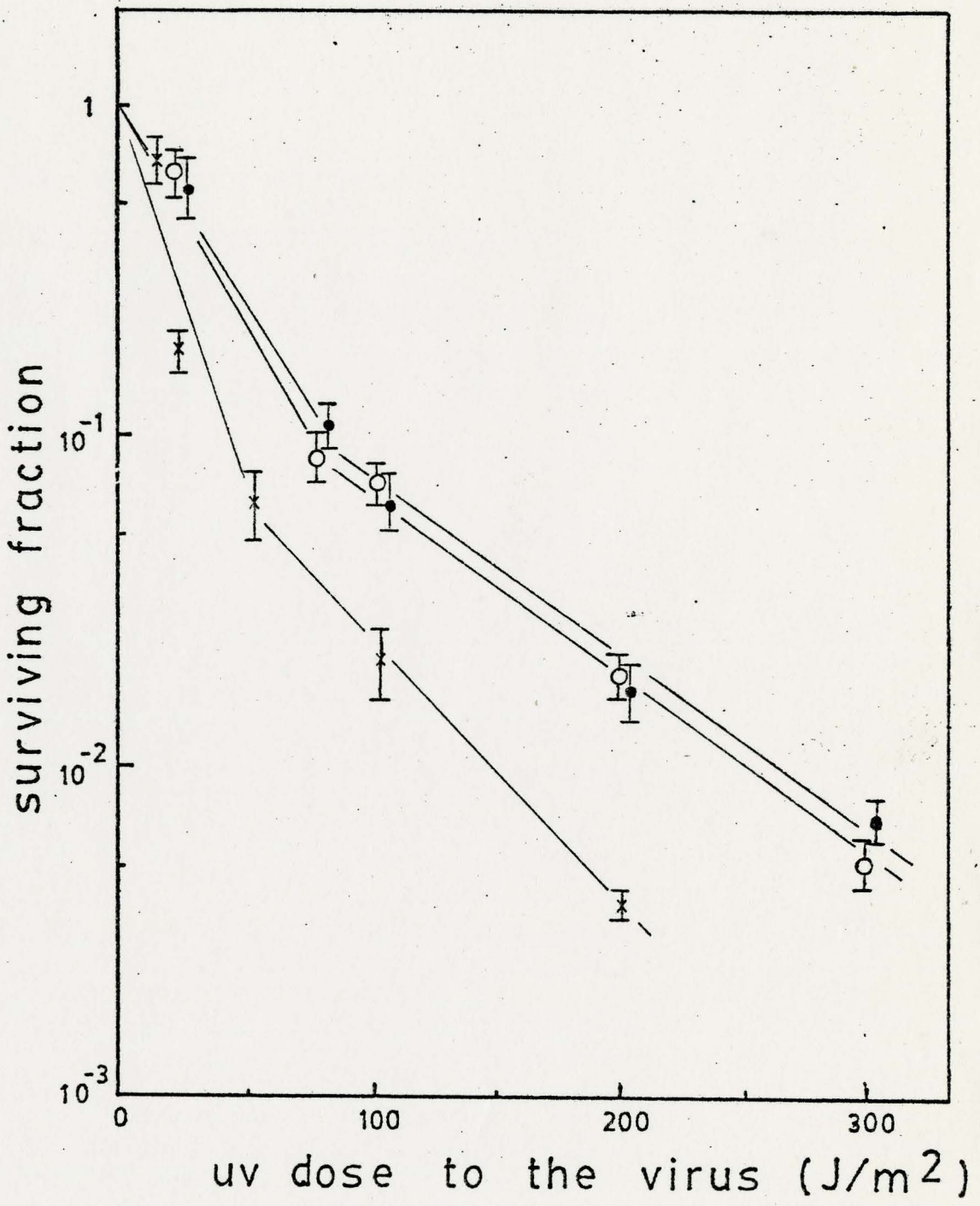

surviving fraction
1
10^{-1}
10^{-2}
10^{-3}
0
100
200
300
uv dose to the virus (J/m^2)

FIGURE 9

Survival of plaque forming ability of UV-irradiated HSV-2 in normal human fibroblasts.

# of Experiments		Cell Strain
6	△	GM 969
2	○	Hff
3	⬡	CRL 1221
2	□	GM 2803
3	◇	A2

Monolayers of normal cells were prepared in 24-well tissue culture plates. Cells were infected with unirradiated or UV-irradiated HSV-2 according to the procedure in the section Materials & Methods. Plaques were counted at 48 hours post-infection and used to calculate virus titre. Titres were normalized to that of unirradiated virus and were plotted as a function of UV dose.

Each point represents the logarithmic mean of the pooled data for 2-6 experiments performed using that UV dose to the virus. Points were fitted to two straight lines using least squares analysis.

The points used in the first and second component were determined by finding the inflection point which yielded the maximum correlation coefficients in the least squares analysis.

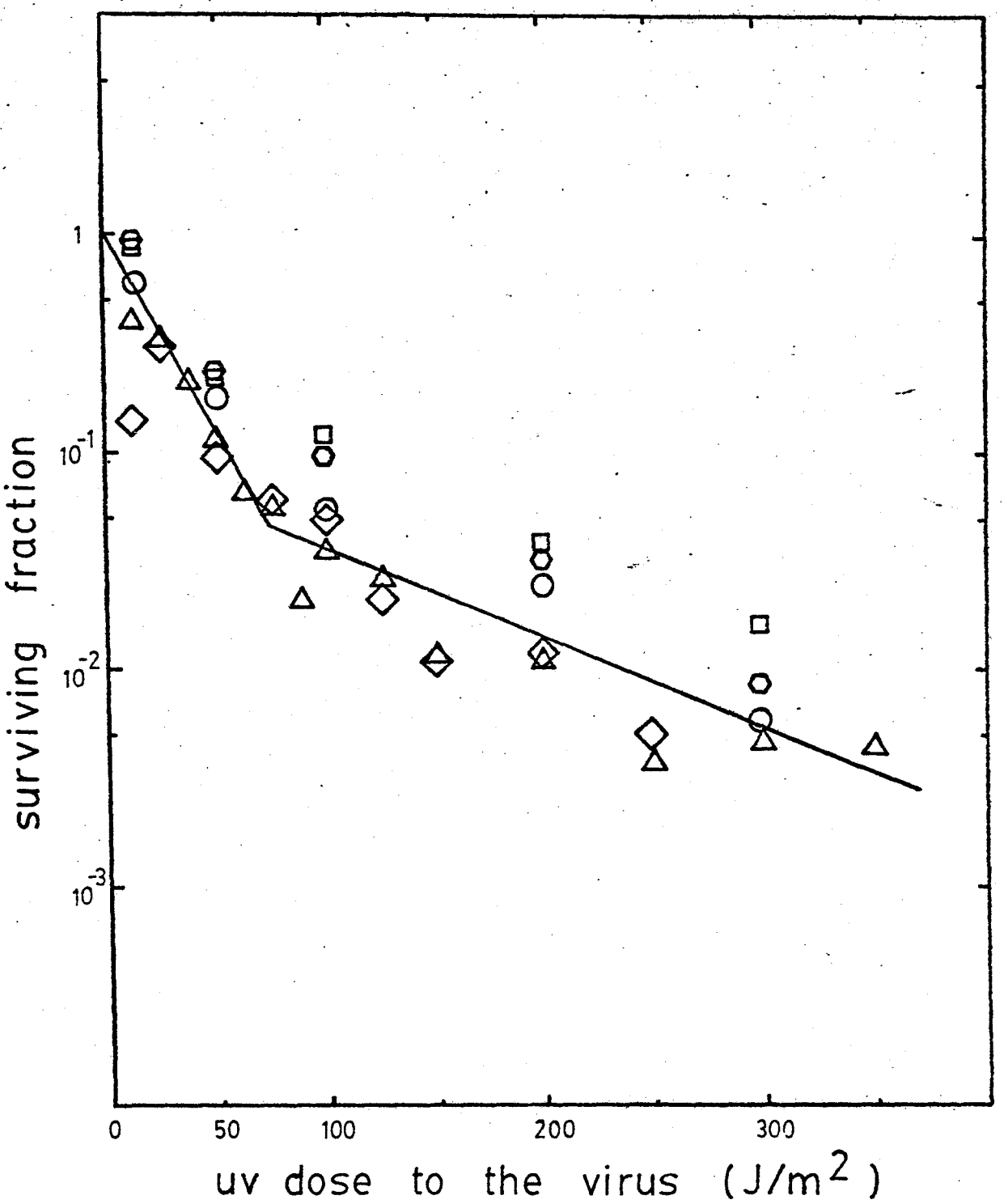

surviving fraction
1
10^{-1}
10^{-2}
10^{-3}
0
50
100
200
300
uv dose to the virus (J/m^2)

TABLE 1

PARAMETERS DESCRIBING THE SURVIVAL CURVES OF UV'd HSV-2 IN VARIOUS HUMAN FIBROBLAST STRAINS

	FIRST COMPONENT			SECOND COMPONENT			INTERCEPT (SF)
	D_o (J/m^2)	ERROR	% HCR	D_o (J/m^2)	ERROR	% HCR	SECOND COMPONENT
NORMALS GM 969	27	± 7		101	±14		0.08
CRL 1221	27	± 6		81	±13		0.34
GM 2803	26	±13		97	±35		0.32
Hff	30	± 3		96	±14		0.16
A 2	36	±28		97	±15		0.09
AVG. NORM.	29		100	94		100	
COCKAYNE'S SYNDROME							
CS 3BE	23	± 6	77	117	±27	124	0.07
GM 2838	21	± 3	72	93	±13	99	0.07
XERODERMA PIGMENTOSUM							
XP 25RO XP (A)	11	± 2	38	35	±11	37	0.03
XP 8LO XP (A)	13	--	45	37	± 4	40	0.09
XP 4RO XP (C)	14	± 9	48	51	± 4	54	0.15
XP 6BE XP (D)	12	± 1	43	26	± 4	28	0.43
XP 4BE XP (VAR)	24	±12	83	81	±12	86	0.34

intersects the zero dose axis. For the normal strains tested the second component intercept ranged between 0.08 and 0.34.

The solid line represents an average survival curve for all the normal strains tested.

A.II.b. COCKAYNE'S SYNDROME FIBROBLASTS

The survival response of HSV-2 plaque formation in two Cockayne syndrome fibroblast cell strains is shown in figure 10. Each point represents the logarithmic mean of at least two experiments.

The D_o of the first components of these two strains, CS 3BE and GM 2838, are 23 and 21 J/m^2 respectively. For the second component the D_o values are 117 and 93 J/m^2 respectively. The second component of the survival curves extrapolate to 0.07 for both strains (see table 1 for all of the above).

In table 1 the standard error of each statistic is included. It can be seen that the D_o of the first and second components of the two CS strains are not significantly different from any of the normal strains. However, the intercept of the CS strains is lower than those of the normal strains CRL 1221, GM 2803 and Hff.

In figure 10, the average survival response

of the normal strains is included. The average response of the two CS strains was calculated by linear regression analysis. The lower survival of the CS strains results primarily from a second component intercept which is lower than the intercepts of 3 of the normal strains.

The % HCR was determined by comparing the first and second component D_o of each CS strain to the average normal second component D_o. The values are 77% for CS 3BE and 72% for GM 2838 in the first component. For the second component the per cent HCR values are 124% and 99% respectively (see table 1).

A.II.c. XERODERMA PIGMENTOSUM FIBROBLASTS

Figure 11 shows the survival curves for 4 different XP excision deficient cell strains. Two strains, XP 25RO and XP 8LO, both belonging to complementation group A have second component D_o's of 35 and 37 J/m^2 respectively. The major difference between the two group A XP strains is with respect to their second component intercepts. The lower survival curve of the XP 25RO strain results primarily from the lower intercept of 0.03 as compared to an intercept of 0.09 for the strain XP 8LO. The XP group D strain, XP 6BE has a second component intercept of 0.43, higher than the intercept of either of the group A strains. Although the D_o of the 2nd component of this group D

FIGURE 10

Survival of plaque forming ability of UV-irradiated HSV-2 in Cockayne Syndrome fibroblasts

# of Experiments		Cell Strain
8	△	GM 2838
8	□	GM 1856 (CS 3BE)

Each point represents the logarithmically transformed mean of the pooled data from 8 experiments using that UV dose to the virus. Points were fitted to two straight lines by least squares analysis

_____ mean CS survival curve

-------- mean normal survival curve (from figure 9)

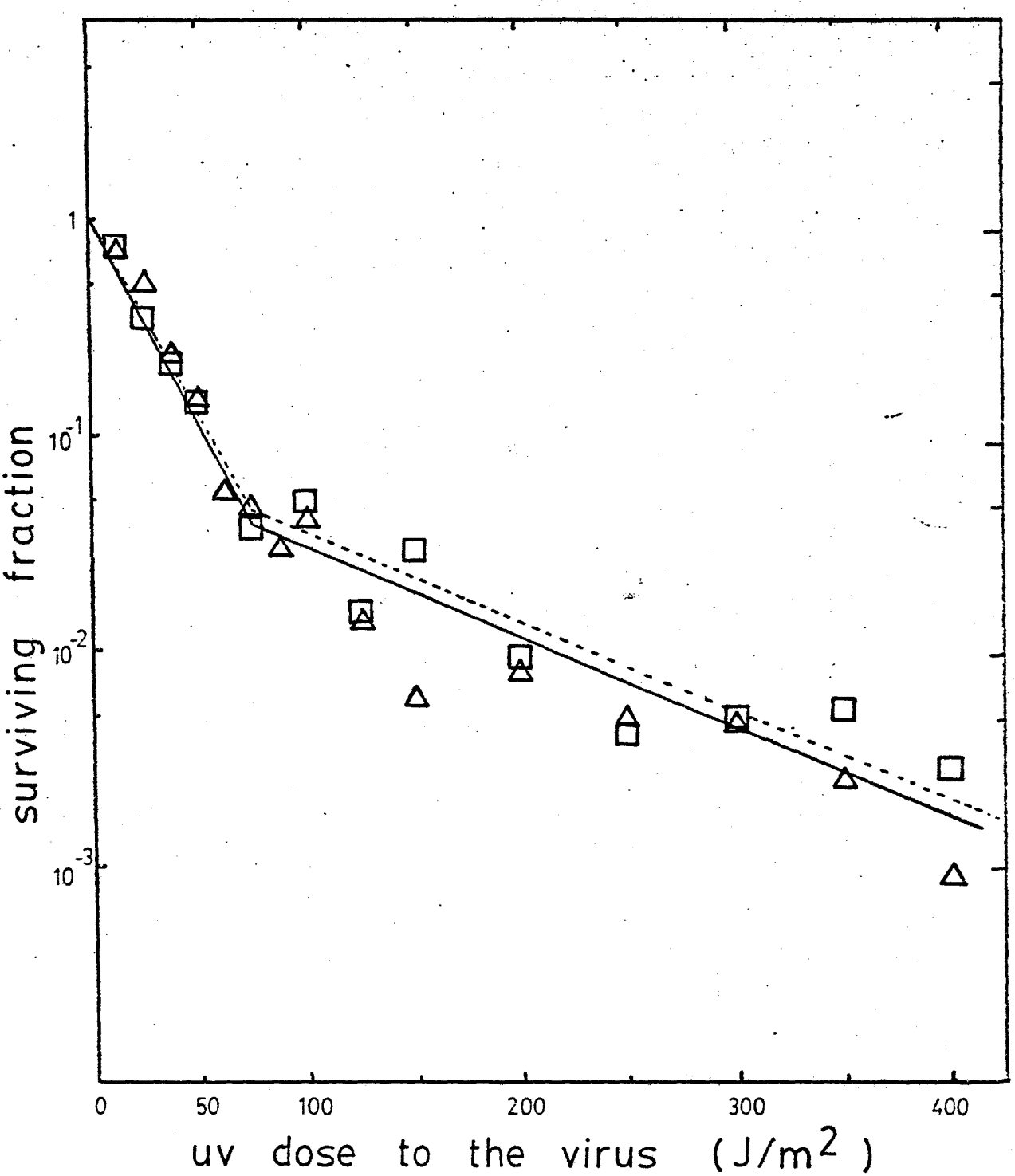

surviving fraction
1
10^{-1}
10^{-2}
10^{-3}
0
50
100
200
300
400
uv dose to the virus (J/m^2)

FIGURE 11

Survival of plaque forming ability of UV-irradiated HSV-2 in excision deficient Xeroderma Pigmentosum fibroblasts

# of Experiments		Cell Strain	
6	△	XP 25RO)	XP(A)
2	□	XP 8LO)	
2	○	XP 6BE	XP(D)
2	⬡	XP 4RO	XP(C)

Each point represents the logarithmic mean of the pooled data from experiments using that UV dose to the virus. Points were fitted to two straight lines by least squares analysis

_____ XP survival curves

-------- mean normal survival curve (from figure 9)

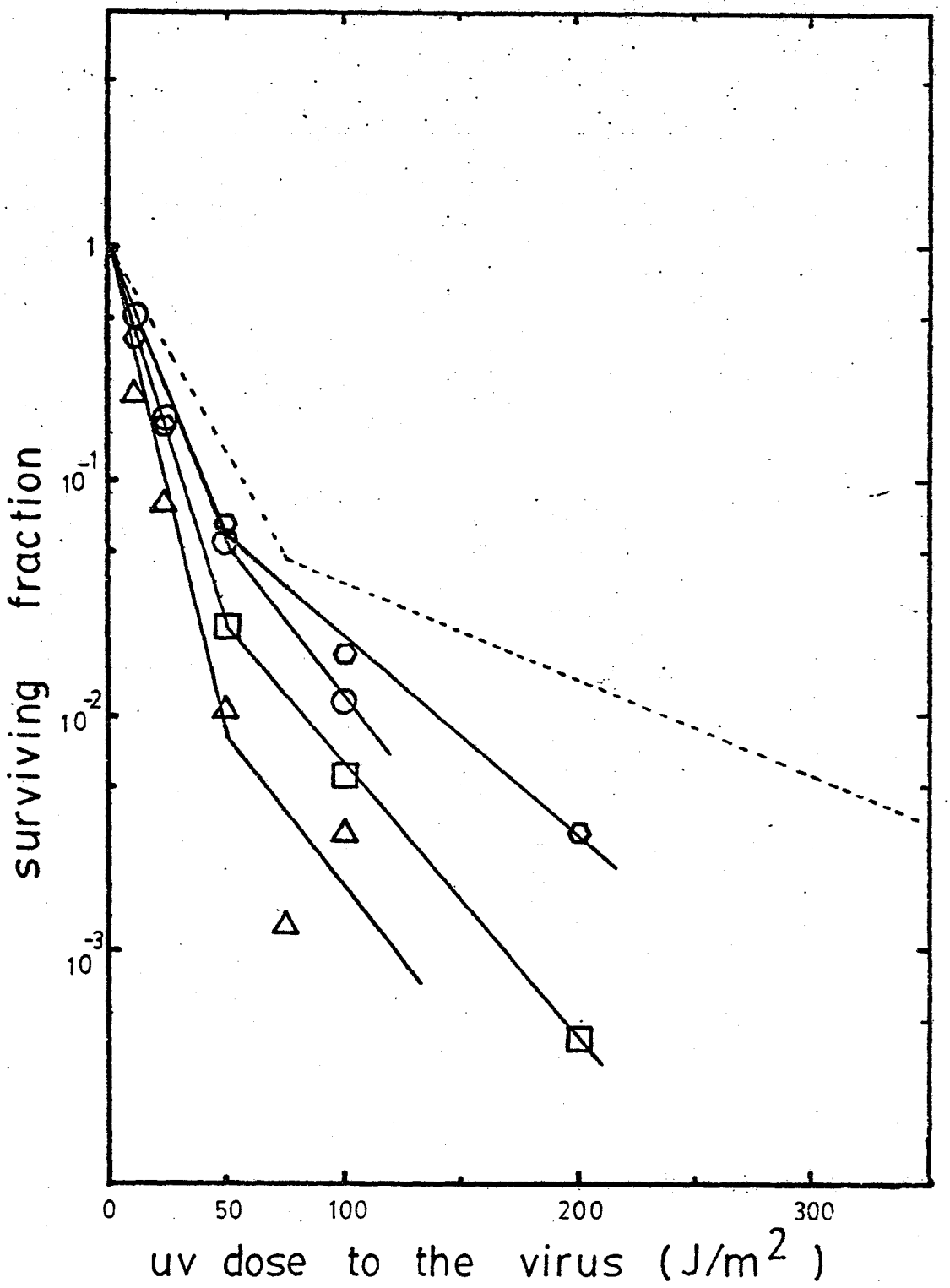
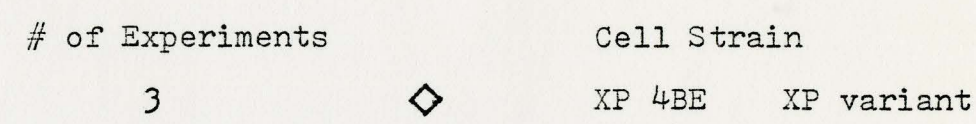

surviving fraction
1
10^{-1}
10^{-2}
10^{-3}
0
50
100
200
300
uv dose to the virus (J/m^2)

FIGURE 12

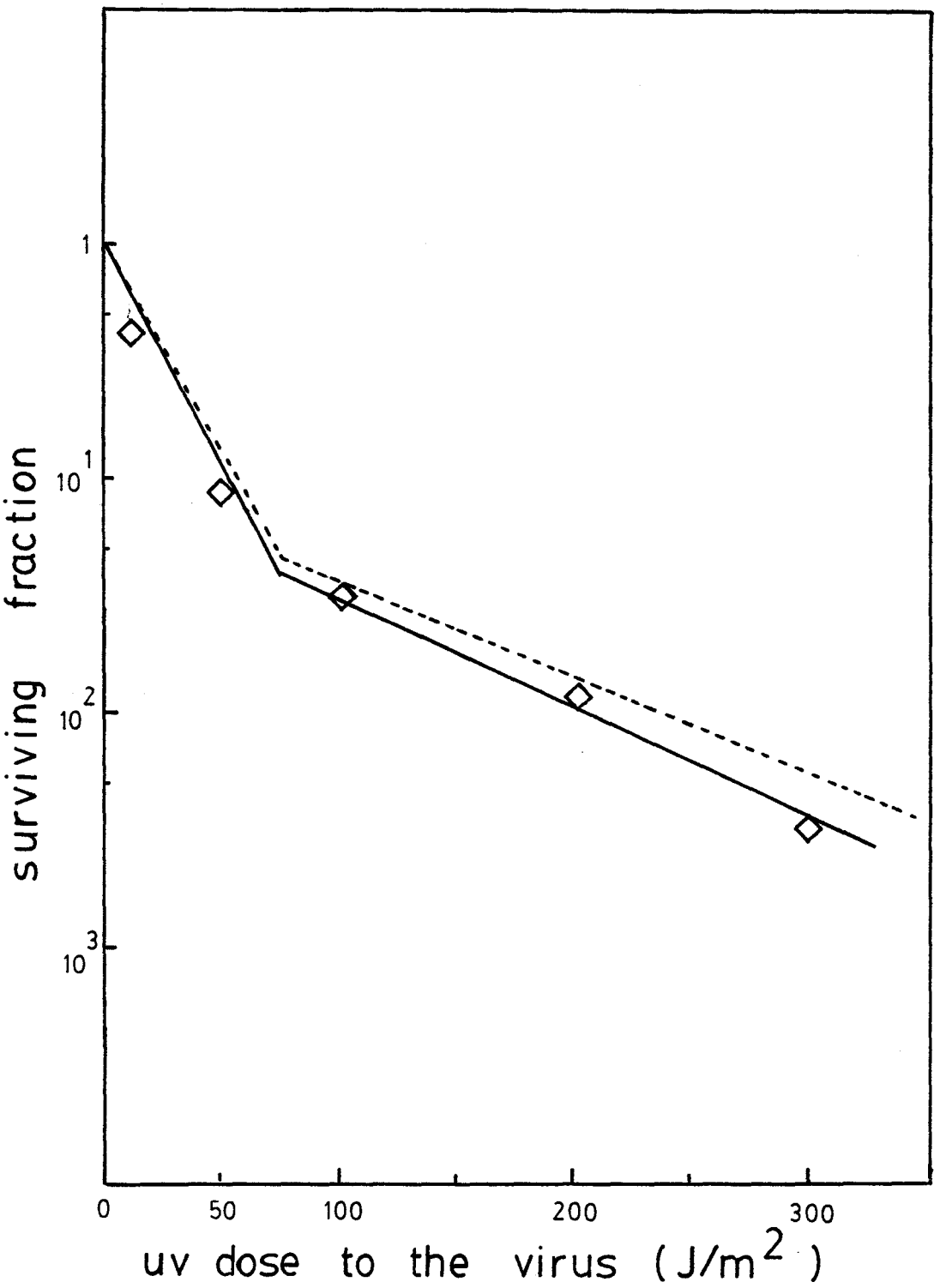
Survival of plaque-forming ability of UV-irradiated HSV-2 in excision proficient Xeroderma Pigmentosum fibroblasts



Each point represents the logarithmic mean of the pooled data from experiments using that UV dose to the virus. Points were fitted to two straight lines by least squares analysis

_____ XP variant survival curve

--------- mean normal survival curve (from figure 9)


surviving fraction
1
10^{1}
10^{2}
10^{3}
0
50
100
200
300
uv dose to the virus (J/m^2)

strain (26 J/m^2) is less than the D_o's of the second components of the XP group A strains, the overall sensitivity of the XP 6BE strain is less than the sensitivity of the group A strains. The survival curve of strain XP 4RO, complementation group C, indicates that it is the least sensitive of the excision deficient XP strains tested. The D_o of the second component is 51 J/m^2, significantly higher than for any other excision deficient XP strain tested, and the second component intercept is 0.15 (see table 1).

The survival of HSV-2 plaque formation in XP variant cells is shown in figure 12. The D_o of the second component (81 J/m^2) is lower than the average D_o of the normal strains but is not significantly different from any of the normal or CS strains. However, it is significantly higher than the D_o of all excision deficient XP strains (see table 1).

The per cent HCR of the XP excision deficient cells strains ranged from 38% (XP 25RO) to 48% (XP 4RO) for the first component. The range for the second component was 28% (XP 6BE) to 54% (XP 4RO). The XP variant strain, XP 4BE, was capable of performing 83% HCR in the first component and 86% in the second component.

For all cell strains tested the initial response to low UV doses was an exponential reduction of the plaque forming ability of the virus. As the dose

was increased (i.e. $\geqslant$ 75 J/m^2 for normal, CS and XP variant strains; $\geqslant$ 50 J/m^2 for XP excision deficient strains) the second, more resistant component of the survival curve became evident. D_o values for the first and second components (with standard errors) as well as the zero dose intercept (with upper and lower limits to 1 standard error) for the second component are listed in table 1.

B CAPACITY

B.I. CAPACITY OF UV-IRRADIATED FIBROBLASTS TO SUPPORT HSV-2 PLAQUE FORMATION

The capacity of irradiated cells to support the growth of unirradiated HSV-2 was used as a measure of the sensitivity of the cells themselves to UV-irradiation.

All monolayers were prepared in 24-well tissue culture plates. When the monolayers were confluent (at least 24 hours after subculture) the medium was removed and the cells were irradiated with UV-light. After UV exposure the medium was quickly replaced to prevent drying. Cells were then infected with an appropriate dilution of unirradiated virus either immediately or after a suitable delay. Forty-eight hours after infection the cells were fixed and stained

and plaques were counted. Virus titres were calculated as described earlier (see figure 7) and relative plaque forming ability (RPFA) was determined by normalizing the titres of virus on irradiated cells to that on unirradiated cells. These results were then plotted as the logarithm of RPFA versus the UV dose to the cells (see figure 13).

The typical results of an experiment to measure the capacity of normal, CS and XP fibroblasts for infection delayed 48 hours after irradiation are shown in figure 13. It can be seen that the capacity of the normal strain is the most resistant to UV-irradiation. The curve for normal cells is shouldered, that is to say that no significant reduction of capacity occurs for UV-doses lower than 5 J/m^2. The reduction in capacity of CS and XP cells occurs at lower doses of UV-light and no shoulder is evident.

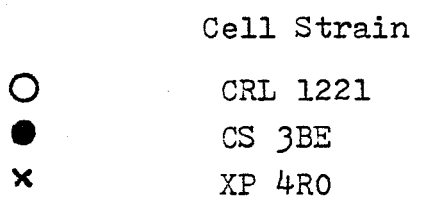
B.II. EFFECT OF DELAYED INFECTION ON CAPACITY

Because irradiation of the cells may induce certain functions similar or analogous to the SOS function of *E. coli* (see Introduction), it was considered important to determine whether a delay between irradiation of the cells and their subsequent infection had any effect upon their capacity to support HSV plaque formation.

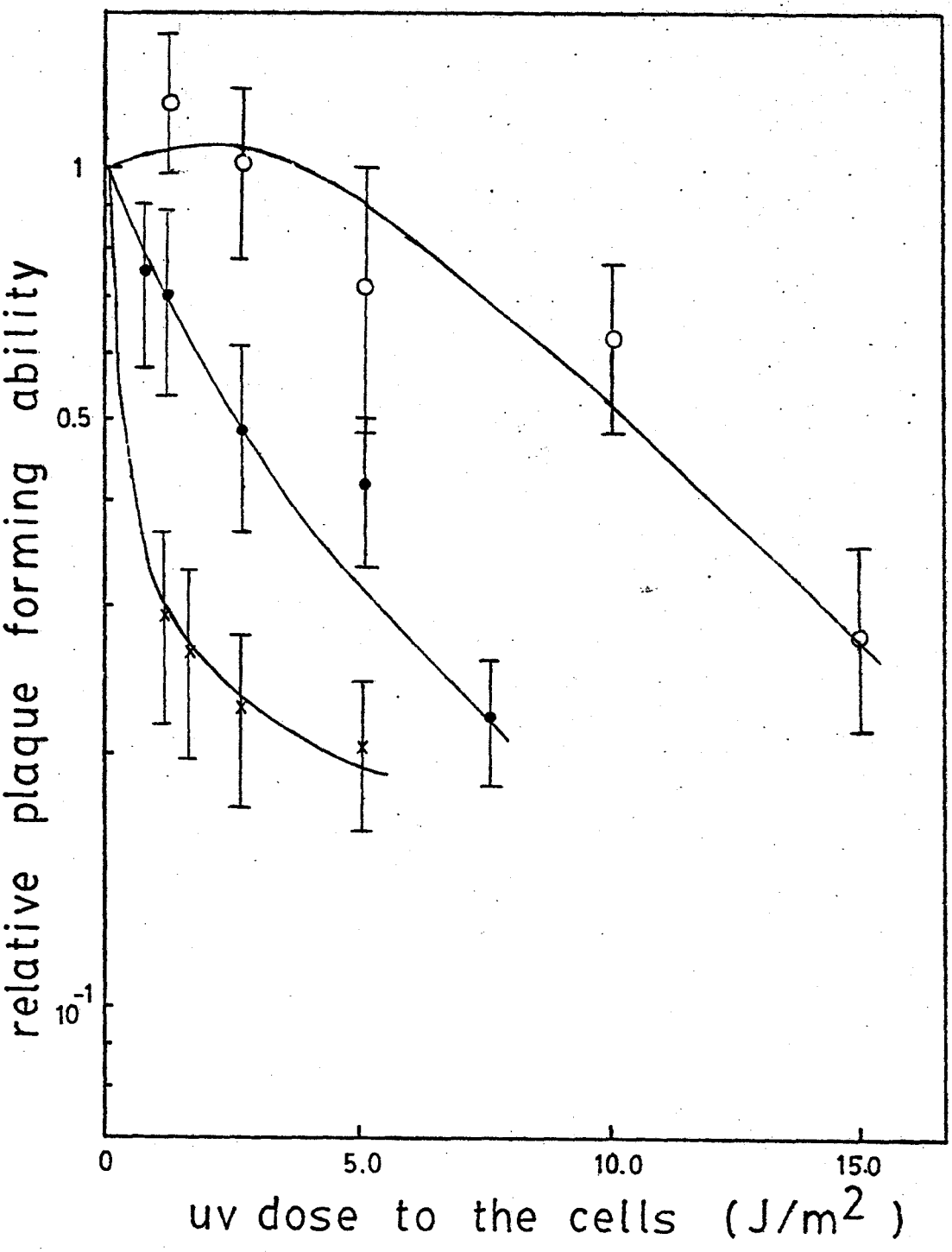
Figure 14 shows the effect of infection at 0, 48 and 96 hours after irradiation. It can be seen that

FIGURE 13

Capacity of UV-irradiated normal, CS and XP cells to support HSV-2 plaque formation



Unirradiated virus was infected into irradiated host cells and the relative-plaque forming ability of the cells was calculated as a function of UV-dose. Error bars represent the standard error for that point. The results for three cell strains from a typical experiment are shown here. Curves were fitted by eye. Infection of the cells occurred 48 hours after irradiation.


relative plaque forming ability
1
0.5
10^{-1}
0
5.0
10.0
15.0
uv dose to the cells (J/m^2)

capacity changes as infection is delayed after irradiation. Infection of the cells immediately after irradiation (figure 14A) shows that CS cells have lower capacity to support HSV plaque formation than do normal cells, at a given dose. A delay of 48 hours (figure 14B) has no apparent effect on normal cells but CS cells now display an even lower capacity. After 96 hours (figure 14C) normal cells have completely recovered their capacity to support HSV plaque formation while CS cells still display a reduced capacity over the range of doses tested.

It was therefore decided that all subsequent experiments to determine cellular capacity would include a 48 hour delay between irradiation of the cell monolayer and their infection. This was intended to maximize the differences in capacity between cells of different disease types.

B.III. CAPACITY OF UV'D HUMAN FIBROBLASTS FOR HSV-2 PLAQUE FORMATION

B.III.a. CAPACITY OF NORMAL HUMAN FIBROBLASTS

The four normal cell strains tested are plotted in figure 15. Each point represents the logarithmic mean of at least two separate experiments. The dose response curve was calculated as the best fit of the pooled data by regression analysis. The responses at doses of 5 J/m^2 and greater were assumed to be reduced exponentially. The curve was fitted by eye for doses

FIGURE 14

The relative HSV-2 plaque forming ability of UV-irradiated cells was determined when infection was delayed for various times after irradiation of the cells.

Cell Strain

Hff
GM 969

GM 2838
CS 3BE

Panel A: 0 hours between irradiation and infection
B: 48 hours between irradiation and infection
C: 96 hours between irradiation and infection

Lines are fitted by eye to each CS cell strain and to the mean value for the two normal strains.

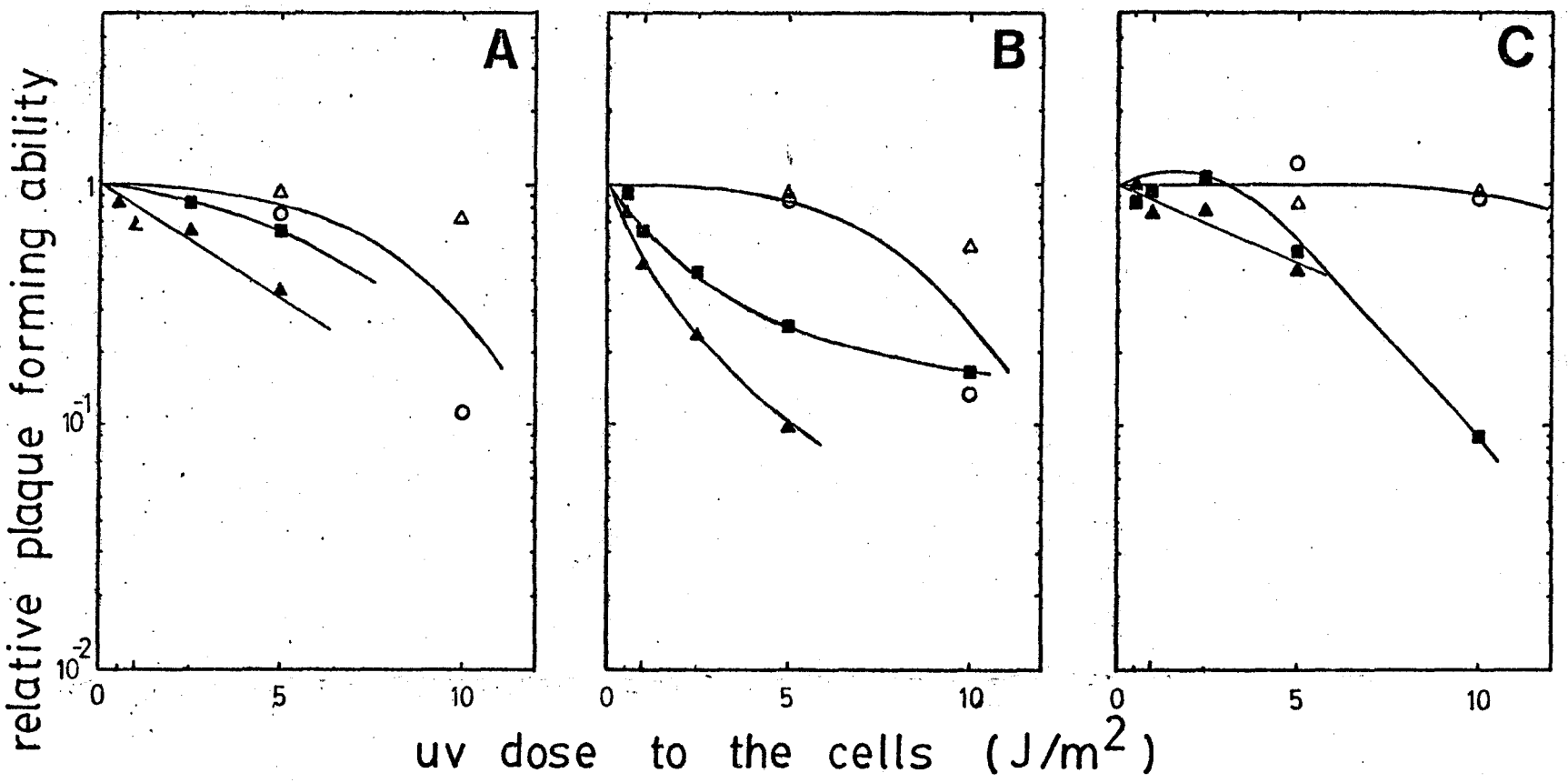
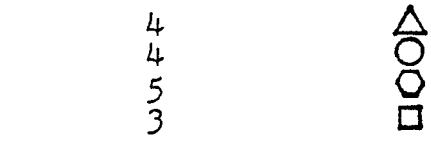
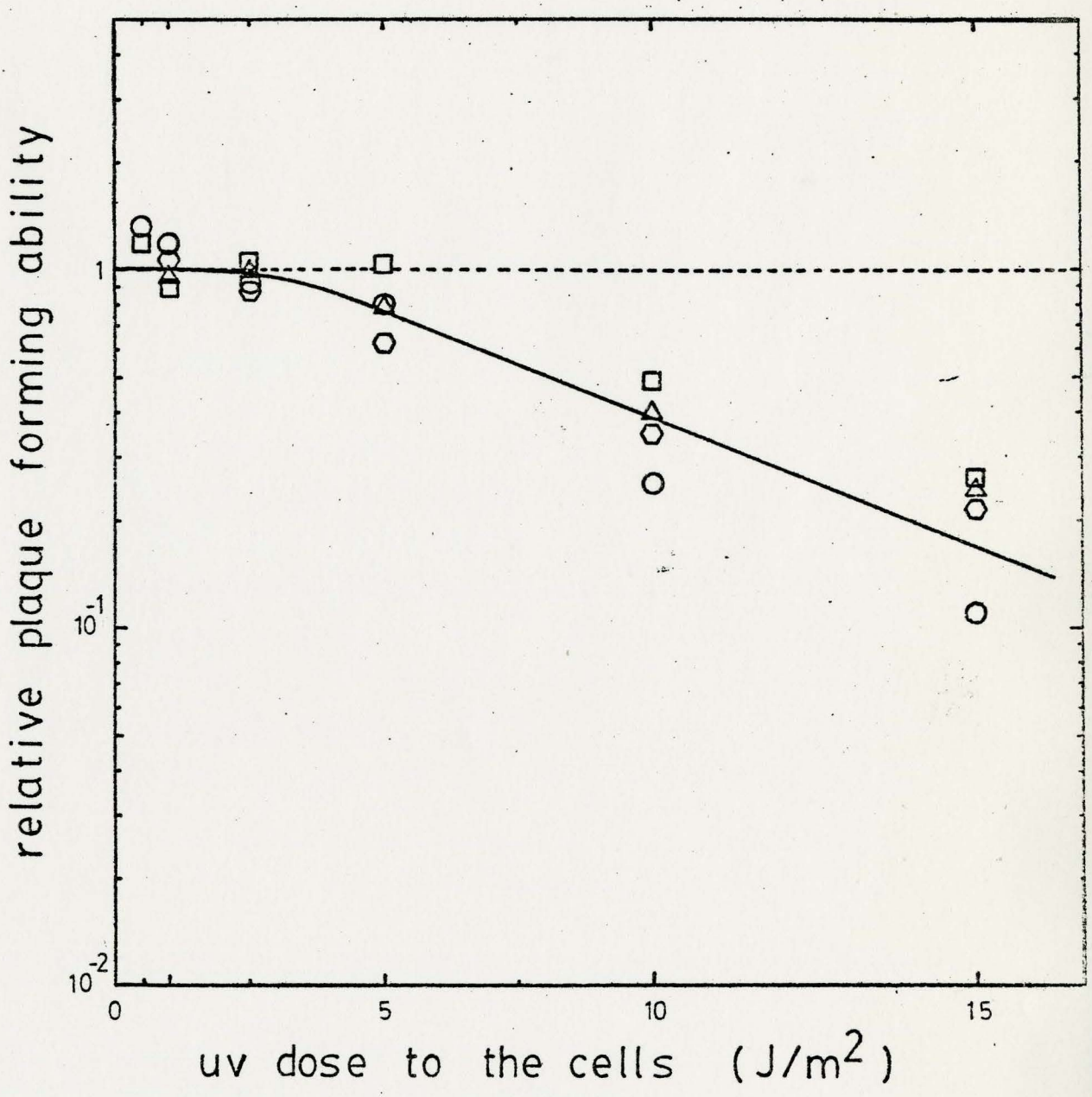

A
B
C
relative plaque forming ability
1
10^{-1}
10^{-2}
0
5
10
uv dose to the cells (J/m^2)

FIGURE 15

The capacity of UV-irradiated normal human fibroblasts to support HSV-2 plaque formation



The points on this graph represent the relative plaque-forming ability of normal human fibroblasts calculated as a function of the UV dose to the cells. Each point is the logarithmic mean of the pooled data from 3-5 separate experiments. The straight portion of the response curve was fitted by regression analysis to those points which responded to irradiation by an exponential reduction in RPFA (i.e. for doses $> 5\ J/m_2$).


relative plaque forming ability
1
10^{-1}
10^{-2}
0
5
10
15
uv dose to the cells (J/m^2)

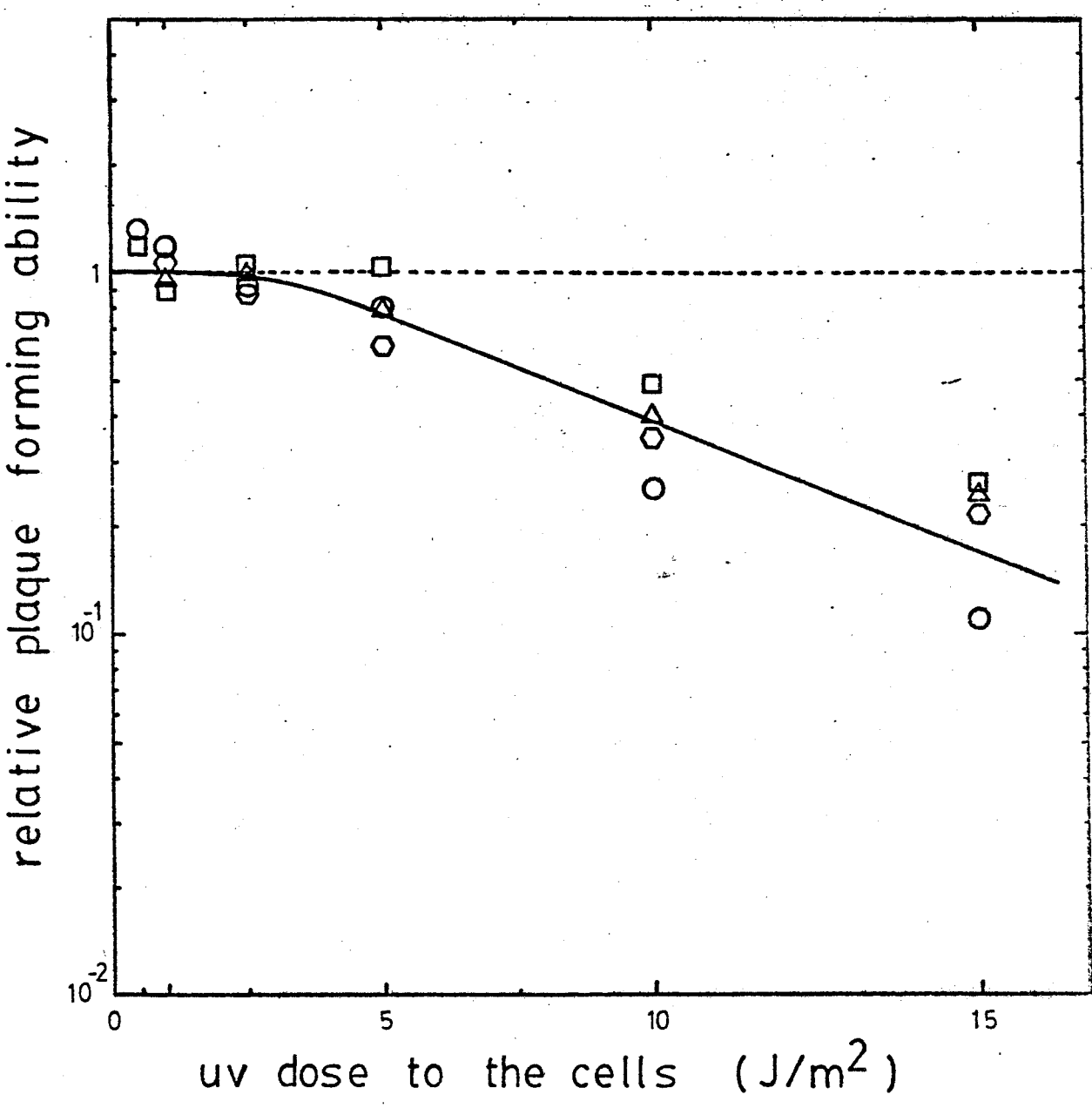

relative plaque forming ability
1
10^{-1}
10^{-2}
0
5
10
15
uv dose to the cells (J/m^2)

TABLE 2

PARAMETERS DESCRIBING THE CAPACITY OF UV'd HUMAN FIBROBLASTS TO SUPPORT HSV-2 PLAQUE FORMATION

	STRAIN	D_{37} VALUE (J/M^2)		% CAP.	MAXIMUM DOSE
NORMALS					
	GM969	11.0	± 3.4		15
	CRL1221	10.8	± 2.6		15
	GM2803	12.4	± 2.0		15
	Hff	8.6	± 1.3		15
	Avg.	10.7	SD 1.57	100.0	
COCKAYNE'S SYNDROME	CS3BE	5.1	± 1.4	47.7	10
	GM2838	3.1	± 0.4	29.0	7.5
	GM739	3.1	± 0.7	29.0	5.
	CS1BE	3.1	± 0.7	29.0	5.
XERODERMA PIGMENTOSUM	XP2540 XP (A)	0.40	± 0.01	3.7	0.5
	XP4RO XP (C)	1.98	± 0.28	18.5	5.
	XP6BE XP (D)	0.96	± 0.13	9.0	2.5
	XP4BE XP (VARIANT)	6.7	± 1.6	62.6	10

lower than 5 J/m^2.

The D_{37} is the dose of UV which reduced the relative plaque forming ability (i.e. capacity) of the cells to 37% of that in unirradiated cells. These values were interpolated by calculation from the formula which described the straight line portion of the response curve. These values are listed in table 2. The range of the D_{37} of the normal strains is 12 J/m^2 to 9 J/m^2 and the mean value is 10.7 J/m^2.

From the graph (figure 15) it can be seen that UV doses of less than 5 J/m^2 have no significant effect on the capacity of normal cells to support HSV-2 plaque formation. This results in the capacity curve displaying a shouldered response. As the UV dose is increased (i.e. > 5 J/m^2) the capacity begins to decrease exponentially. At doses of UV greater than 15 J/m^2 the cells are unable to support HSV-2 plaque formation.

B.III.b. CAPACITY OF CS FIBROBLASTS

The cells derived from CS patients display a range of responses to UV, as do normal cell strains, which can be seen in figure 16. In this diagram the solid lines represent the extremes of the range of responses for the 4 CS cell strains tested. The dashed line is the mean normal response taken from figure 15. The D_{37} values for the various CS cell strains are listed in table 2.

Over the range of doses used in these experiments

the response of CS cell capacity to UV is an exponential decrease. Unlike the normal strains used, CS cells do not display a shouldered curve even when 0.5 J/m^2 was used as the lowest dose to the cells. The maximum UV dose at which HSV plaque formation could occur in CS cells was 7.5 J/m^2. In some experiments with some strains of CS cells the monolayer of cells was able to support plaque formation for UV doses as high as 10 J/m^2. In other experiments with other strains of CS cells the monolayers were not able to support plaque-formation after doses of UV as low as 5.0 J/m^2. This may be in part related to the sensitivity of each particular CS cell strain. The least sensitive strain CS 3BE (i.e. the one with the highest D_{37} value) is the only CS strain for which data were obtained for UV fluences as high as 10 J/m^2. Conversely the most sensitive strains CS 1BE and GM 739 (with the lowest D_{37} values) have never been seen to support plaque formation after exposure to UV doses higher than 5 J/m^2 (see table 2).

The per cent capacity (compared to normal strains) of the CS strains ranged from 48% (CS 3BE) to 29% (all others tested) (see table 2).

B.III.c. CAPACITY OF XP FIBROBLASTS

In this study cells from three different complementation groups were used, groups A, C and D, plus XP variant cells. The response of XP cells of

these three groups was, like that of the CS cells, exponential over the range of doses used in these experiments. That is to say, the capacity curves displayed no shoulder (see figure 17). In this figure the solid lines represent the dose response calculated, by linear regression analysis, for each complementation group separately. It is clear that each strain or complementation group exhibits a characteristic response curve. This can also be seen by comparing the D_{37} values for each strain, which are listed in table 2.

The XP excision deficient strains tested are more sensitive to UV-irradiation than both CS and normal cells in terms of their capacity to support HSV-2 plaque formation. The D_{37} values of these XP strains range from 0.40 J/m^2 (XP25RO) to 2.0 J/m^2 (XP4RO). Expressed as per cent of capacity compared to normal strains, the range is 3.7% - 18.5% (table 2). Also, the maximum dose of UV to which these XP cells could be exposed and still support plaque formation was lower than the corresponding doses for CS and normal cells (table 2). This maximum UV-dose was lowest for the excision deficient XP strain with the lowest D_{37} (XP25RO) and highest for the strain with the highest D_{37} (XP4RO).

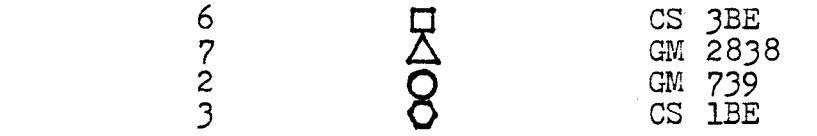
The XP variant cell strain, XP4BE, was also

tested for its capacity to support HSV-2 plaque formation after UV-irradiation. Unlike the excision deficient XP strains and the CS strains, the response curve of the XP variant cells is shouldered similar to normal strains (figure 17). Low doses of UV (> 2.5 J/m^2) do not significantly reduce the capacity of XP4BE cells to support HSV-2 plaque formation. Higher doses of UV light produce an exponential decrease in capacity. This decrease in capacity was greater than the decrease in capacity observed for normal strains but not as great as that observed for CS and excision deficient XP strains (as measured by D_{37} values). The capacity of XP variant cells to support HSV-2 plaque formation was 63% of the value for normal strains (table 2).

Plaque formation by HSV-2 did not occur on XP4BE fibroblasts which had been irradiated with more than 10 J/m^2 of UV light (table 2).

FIGURE 16

Capacity of UV irradiated Cockayne's Syndrome fibroblasts to support HSV-2 plaque formation



Each point is the logarithmic mean of the pooled data from 2-7 separate experiments. The solid lines represent the responses of the most and least UV sensitive cell strains (CS 1BE and CS 3B6 respectively). The lines were fitted by regression analysis to all points for that strain.

——————— normal fibroblast capacity curve (from figure 14)

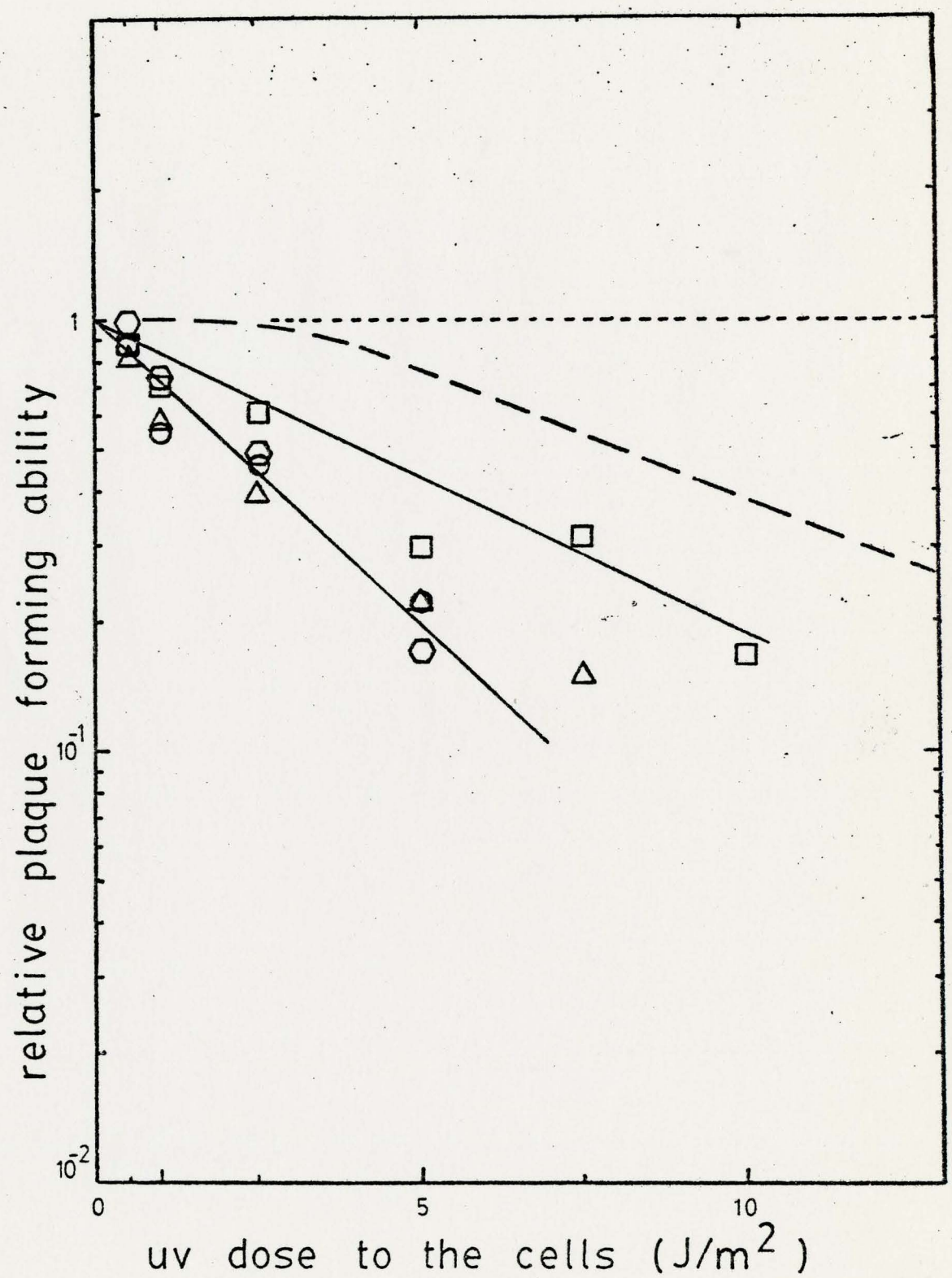
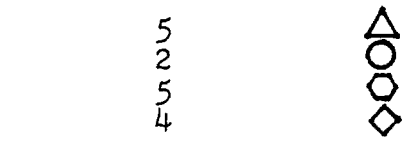

relative plaque forming ability
1
10^{-1}
10^{-2}
0
5
10
uv dose to the cells (J/m^2)

FIGURE 17

Capacity of UV irradiated Xeroderma Pigmentosum cell strains to support HSV-2 plaque formation



Each point represents the logarithmic mean of the pooled data from 2-5 separate experiments. The response curve for XP 4BE was fitted by regression analysis to those points which responded to UV-irradiation by an exponential decrease in RPFA (i.e. doses > 2.5 J/m$_2$). For all other cell strains response curves were fitted by regression analysis to all points.

— — — — normal fibroblast capacity curve (from figure 14)

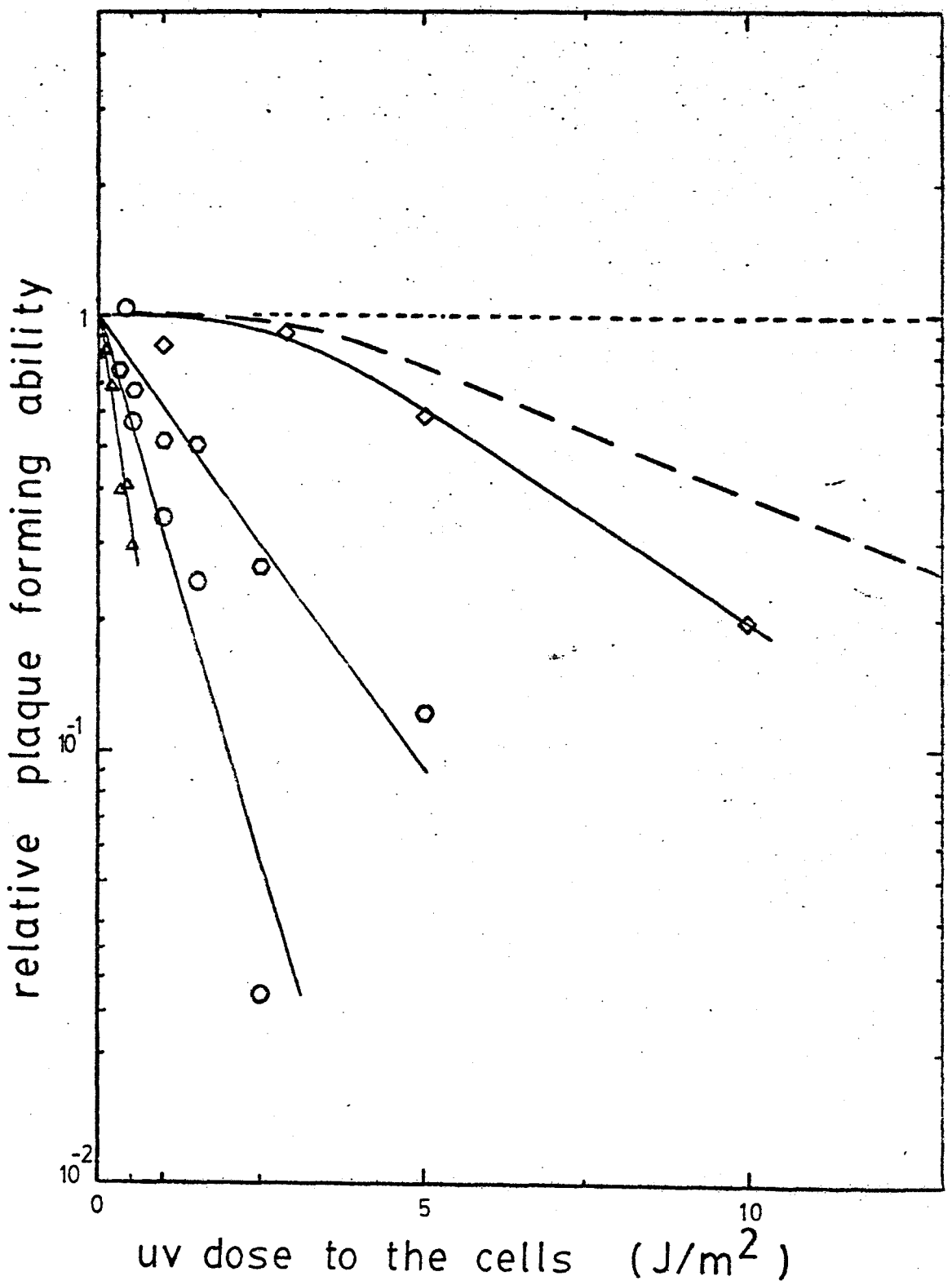
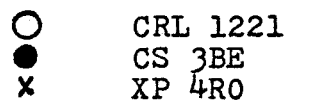
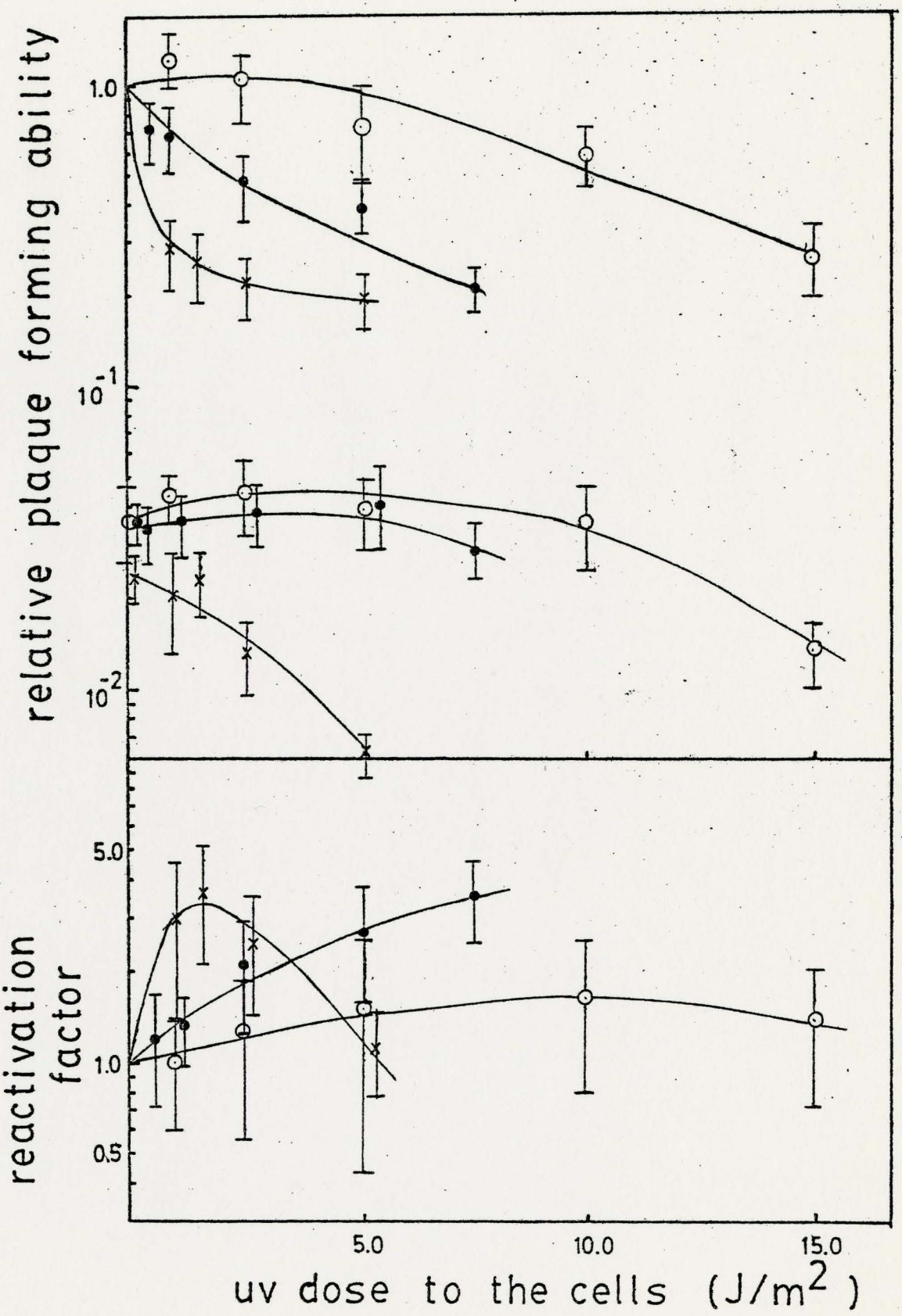

relative plaque forming ability
1
10^{-1}
10^{-2}
0
5
10
uv dose to the cells (J/m^2)

FIGURE 18

UV-enhanced reactivation of HSV-2 plaque formation in normal, CS and XP fibroblasts.



The figure shows typical results for the UVER of herpes virus plaque formation. Cells were prepared in 24-well tissue culture plates. Unirradiated and irradiated cells were infected with unirradiated virus (panel A, lower curves) at 48 hours after irradiation of the cells. Relative plaque forming ability of the virus was plotted as a function of UV dose to the cells. RF values were calculated as the ratio of irradiated virus to unirradiated virus. Values were plotted as a function of UV dose to the cells (panel B). Error bars represent the standard error for that point. Smooth curves were fitted by eye.


relative plaque forming ability
1.0
10^{-1}
10^{-2}
reactivation factor
5.0
1.0
0.5
5.0
10.0
15.0
uv dose to the cells (J/m^2)

C. UV ENHANCED REACTIVATION (UVER) OF HSV-2

PLAQUE FORMING ABILITY

Enhanced reactivation is the term used to describe the phenomenon by which irradiated virus survival is increased by pre-irradiating the host cells. The UV reactivation factor (RF) is the measure used to quantitate this phenomenon. It has been postulated (e.g. Hall and Mount, 1981; Lehmann & Karran, 1981) that UVER may be in some ways analogous to Weigle reactivation (WR) of irradiated λ phage infecting irradiated E. coli (Weigle, 1953).

Two response curves were determined in order to calculate the reactivation factor (RF). One curve (upper curves, panel A, figure 18) is the capacity of irradiated cells to support HSV-2 plaque formation. Determination of this curve was described in a preceding section (see section CAPACITY). The other curve (lower curve, panel A, figure 13) represents the ability of irradiated cells to support plaque formation of irradiated HSV-2. Pre-irradiated monolayers of cells in 24-well tissue culture plates were divided into two groups. The relative plaque forming ability of unirradiated and irradiated virus was determined on those cells. The results were plotted as a function of the UV dose to the cells (figure 18A). The enhanced survival of irradiated virus in irradiated cells is measured by the RF. The RF has been defined (Bockstahler et al., 1976) as the normalized ratio of the

infectivity (i.e. relative plaque forming ability) of irradiated to unirradiated virus at any given UV dose to the cells.

Typical results of an experiment to determine UVER of normal, CS and XP cells are presented in figure 18. In this experiment cells were infected 48 hours after irradiation. Irradiated virus was given a dose of UV sufficient to reduce its survival in unirradiated cells by approximately 100-fold. It can be seen that the RF for normal cells is never significantly different from 1. Both CS and XP cells have RF values greater than 1. The peak RF for these XP cells occurs at 1.5 J/m^2 in this experiment and at 7.5 J/m^2 for the CS cells.

C.I. FACTORS AFFECTING UVER

C.I.a. EFFECT OF DELAYED INFECTION

If UVER is an inducible function similar to Weigle reactivation then the maximal effects of pre-irradiation of the host cells may not be observed immediately. A delay which allows for the expression of the putative SOS functions may be necessary.

To determine whether a delay between irradiation and infection produces a change in UVER, the following experiment was performed.

Monolayers of CS and normal fibroblast cells were prepared in 24-well tissue culture plates. Groups of these

plates were irradiated at 96, 48 and 0 hours before infection. The UV doses to the cells were 0, 5 and 10 J/m^2 for normal fibroblasts and 0, 2.5 and 5 J/m^2 for CS fibroblasts. Two sets of virus dilutions were prepared, one for unirradiated virus and the other for irradiated. For these experiments the virus was exposed to a dose of UV sufficient to reduce its survival by a factor of 10^{-2} in unirradiated cells. RF values were calculated as described previously.

The RF values for two separate experiments were plotted as a function of the delay between irradiation and infection for each UV dose to the cells (figure 19). For both the normal and the CS cell strains it can be seen that maximum enhanced reactivation occurs 48 hours after irradiation, regardless of the UV dose to the cells.

C.I.b. EFFECT OF DELAYED IRRADIATION

It has been shown that the highly contact inhibited cells (e.g. fibroblasts) display lower levels of UVER than non-contact inhibited cells (e.g. tumour cells) (Lytle et. al., 1974, Hellman et al., 1974). Therefore it was considered important to test whether cells which had recently reached confluency expressed higher levels of UVER than cells which had been confluent for longer times.

Preparation of the 24-well tissue culture plates used in most of these experiments was such that cell monolayers were not immediately confluent. (i.e. one 75 cm^2

FIGURE 19

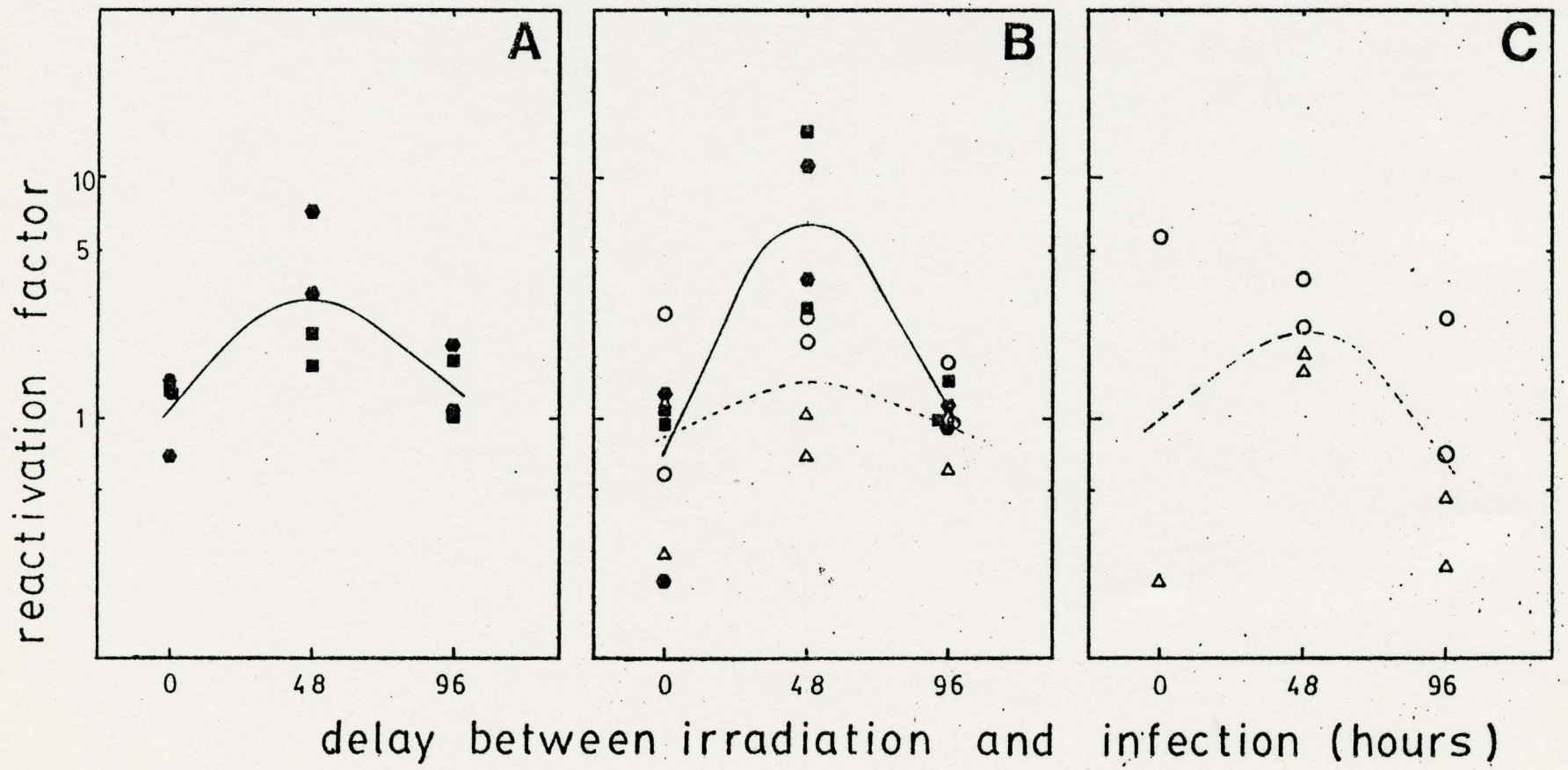
The effect on RF of a delay between irradiation and infection.

Normal Strains	△	GM 969
	○	Hff
CS strains	■	GM 1856 (CS 3BE)
	●	GM 2838

Panel A: UV dose to cells - 2.5 J/m^2
B: UV dose to cells - 5 J/m^2
C: UV dose to cells - 10 J/m^2

The data from two separate experiments is plotted as reactivation factor (RF) versus delay between irradiation and infection. Curves are drawn through the logarithmic means of all data points for each cell type (i.e. a normal curve and a CS curve).

- - - - normal strains
_____ CS strains


A
B
C
reactivation factor
10
5
1
0
48
96
delay between irradiation and infection (hours)

flask of cells was used to seed 2-24 well plates with a total area of approximately 100 cm^2). Minimum confluency was reached by 1 day after subculturing. Cells which had been subcultured (i.e. seeded into 24-well plates) 1, 2 and 3 days previously were irradiated with a range of doses of UV from 0-20 J/m^2. After 48 hours delay, cells at each dose were infected with unirradiated virus or virus irradiated to a survival level of 10^{-2} (in unirradiated cells). Cells were fixed and stained 48 hours after infection and plaques were counted. Reactivation factors were calculated as described for the previous section (EFFECT OF DELAYED INFECTION). Maximum reactivation factors were plotted as a function of the number of days between subculture (i.e. plating) and irradiation (figure 20). It is clear from this graph that as the cells remain confluent longer, they express lower levels of UVER.

In order that all subsequent determinations of reactivation factor be made when conditions are optimum, cells were subcultured and plated 1 day previous to irradiation and infection was delayed 48 hours after irradiation.

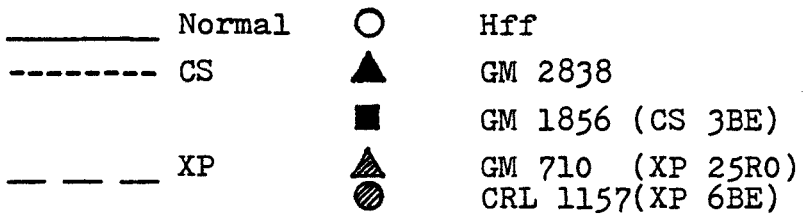
C.II. UVER OF VARIOUS HUMAN FIBROBLAST STRAINS

DETERMINATION OF RF AS A FUNCTION OF UV DOSE TO CELLS

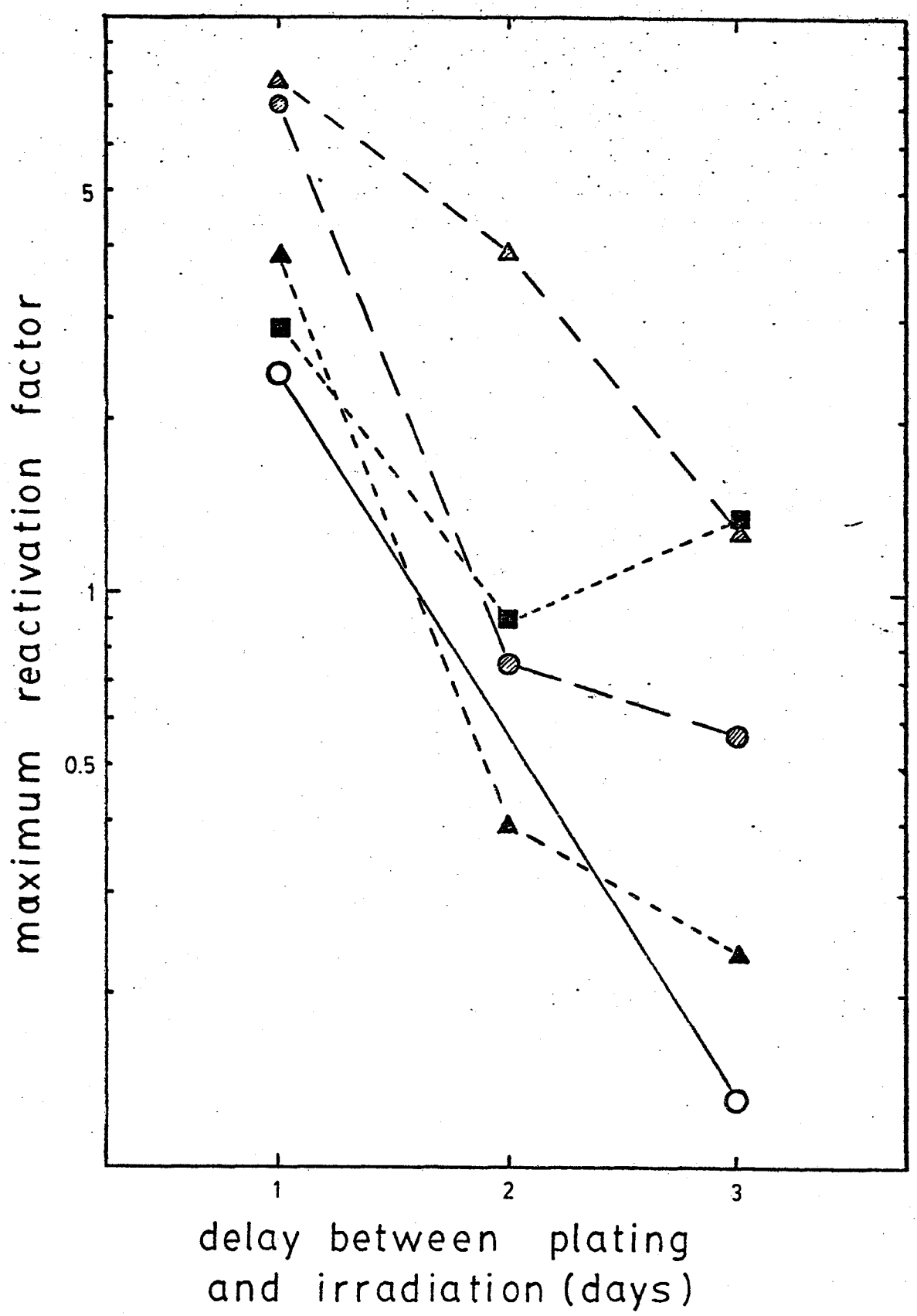
Using the optimum conditions determined above, experiments were performed to determine the UVER response of various human diploid fibroblast cell strains to

FIGURE 20

The effect of subculturing on maximum RF



Cells which had been plated at various times before irradiation were infected 48 hours later with HSV-2. Maximum RF values were plotted as a function of the time between plating and irradiation. Each point represents the maximum RF value obtained for that cell strain.


maximum reactivation factor
5
1
0.5
1
2
3
delay between plating
and irradiation (days)

UV-irradiation. The RF factors were determined as described at the beginning of this section.

As can be seen in figure 21, the RF curves for different human cell types could be characterized by a number of different parameters. Different cell types (i.e. CS cells and XP cells) are able to withstand different maximal doses to the cells (as discussed earlier for capacity). For most normal strains 15 J/m^2 is the maximum UV dose, 5 J/m^2 for CS strains, 10 J/m^2 for XP variant cells and between 0.5-2.5 J/m^2 for the various excision deficient XP cell strains (table 3). Also, different cell types display different maximal RF values and these maxima occur at different UV doses to the cells (to be discussed below). Taken together these differences can be used to characterize the response of various strains to UV irradiation.

Figure 21 summarizes the results of many determinations of RF for HSV-2 in various cell strains. Each point on this graph represents the mean of the pooled data from 2-6 separate experiments. Smooth curves were drawn by eye through the points for each XP strain. Since they displayed similar responses, all of the normal strains are represented by a single curve and all of the CS strains by another curve.

The results indicate that normal cell strains, which are the most UV resistant in terms of capacity (table 2) have very low RF factors (average of 1.3 for all normal

strains, see table 3) and relatively high doses of UV-light are required to elicit this response (15 J/m^2 for most strains, see table 3).

Cockayne's syndrome cells display higher levels of UVER, ranging from 2.15 - 3.23 (see table 3). The UV dose which elicits maximum RF values is 5 J/m^2. The RF value decreases at the next highest dose tested, 7.5 J/m^2 (see figure 21). At higher doses (i.e. 7.5 J/m^2) CS cells were unable to support plaque formation, making RF determination impossible.

The excision deficient XP cell strains display various responses to UV light depending upon their respective complementation groups. The cell strain which was most sensitive in terms of capacity, XP 25RO (table 2), was found to produce its maximum RF at a UV dose of 0.5 J/m^2 which was lower than that observed for any other strain (table 3). The other excision deficient strains, XP 6BE and XP 4RO (in order of decreasing sensitivity) yielded maximal UVER at UV doses 1 J/m^2 and 2.5 J/m^2 respectively. For all of these XP excision deficient strains the maximum RF's were very similar, ranging from 2.1 - 2.6 (table 3).

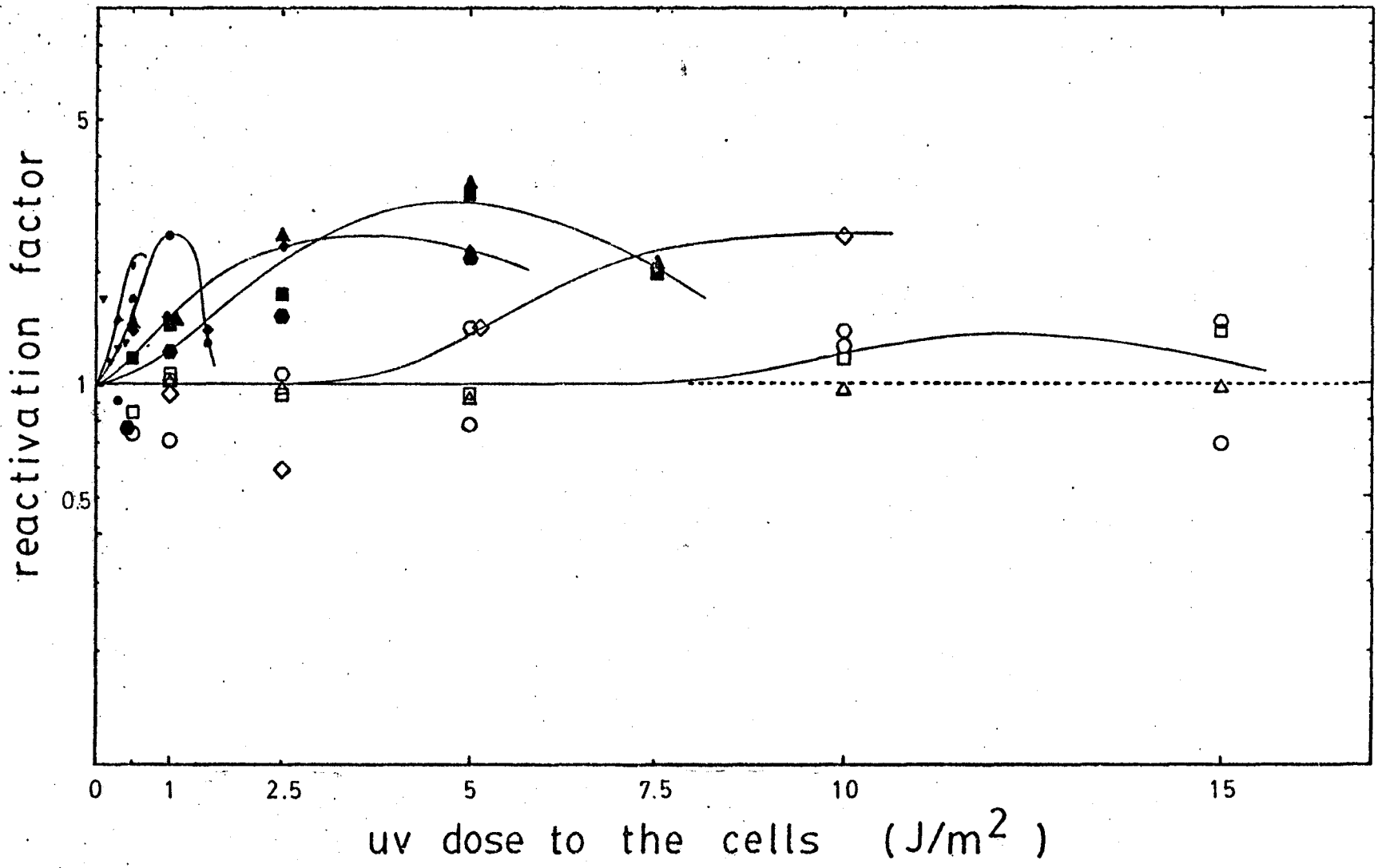
XP 4BE, an XP variant cell strain, displayed a response different from any of the other strains tested. In terms of capacity, this strain was more sensitive to UV than were normal cell strains (table 2). Maximum RF for XP 4BE was obtained at 10 J/m^2 to the cells and the RF value was 2.5 (table 3).

FIGURE 21

UVER as a function of UV dose to the cells

# of Experiments		Cell Strain
4	△	GM 969
4	○	Hff
3	□	CRL 2803
4	◇	CRL 1221
5	▲	GM 2838
6	■	CS 3BE
2	⬟	CS 1BE
3	▼	XP 25RO
2	●	XP 6BE
5	◆	XP 4RO
2	◇	XP 4BE

Each point represents the logarithmic mean of the RF from 2-6 separate experiments. Smooth curves were drawn by eye through RF value greater than one. A single curve is drawn for all normal strains and another for all CS strains. Each XP strain is represented by a different curve.


reactivation factor
5
1
0.5
0
1
2.5
5
7.5
10
15
uv dose to the cells (J/m2)

PARAMETERS DESCRIBING UVER OF UV'd HSV-Z PLAQUE FORMATION IN HUMAN FIBROBLASTS

TABLE 3

	STRAIN		DOSE TO PRODUCE MAXIMUM REACTIVATION FACTOR (J/M^2)	MAXIMUM REACTIVATION FACTOR	# OF EXPERIMENTS
NORMALS	GM969		15	0.971	4
	CRL1221		15	1.46	4
	GM2803		15	1.38	3
	Hff		10	1.26	4
COCKAYNE's SYNDROME	CS3BE		5	3.17	6
	GM2838		5	3.23	5
	GM739		5	2.40	2
	CS1BE		5	2.15	2
XERODERMA PIGMENTOSUM	XP25RO	XP(A)	0.5	2.07	3
	XP4RO	XP(C)	2.5	2.33	5
	XP6BE	XP(D)	1.	2.58	2
	XP4BE	XP(VARIANT)	10	2.45	2

C.III. UVER OF UV'D HSV-2 AS A FUNCTION OF UV DOSE TO CELLS AT LOW VIRUS SURVIVAL LEVELS

As will be seen in the following section, UVER is dependent not only upon the UV dose to the host cells but also the dose given to the virus. Experiments were therefore performed to determine the level of UVER as a function of the UV dose to the cells when the irradiated virus survival was reduced to 10^{-3}. Cells were prepared in exactly the manner described in the preceding section. Cells were infected 48 hours after irradiation and fixed and stained 2 days later. Plaque counts were used to determine titres of virus, from which RPFA and RF were determined.

This experiment was performed 3 times. In only one of these attempts was the survival of the virus reduced below 10^{-3} (see figure 22). In this experiment, the maximum RF of the XP variant strain (XP4BE) was 19.72. The maximum RF values for the other strains were 3.38, 5.66, 3.64 and 2.17 for normal cells, two CS strains (CS1BE and GM2838) and XP25RO respectively. This, therefore, suggested that the large differences in RF between normal and XP variant was strongly dependent upon the virus survival level. In order to pursue this

FIGURE 22

UV-enhanced reactivation of HSV-2 plaque formation in normal, CS, and XP fibroblasts at 10^{-3} virus survival.

GM 2151
CRL 1221
GM 2838
CS 1BE
XP 25RO
XP 4BE

The figure shows the results of a single experiment. Unirradiated and irradiated cells were infected with unirradiated (panel A, upper curves) or irradiated virus (panel A, lower curves). Relative plaque forming ability of the virus was plotted as a function of UV dose to the cells. Irradiated virus was given a dose of UV expected to reduce its survival to 10^{-3} in unirradiated cells. RF values were plotted as a function of UV dose to the cells (panel B). Curves were fitted by eye.

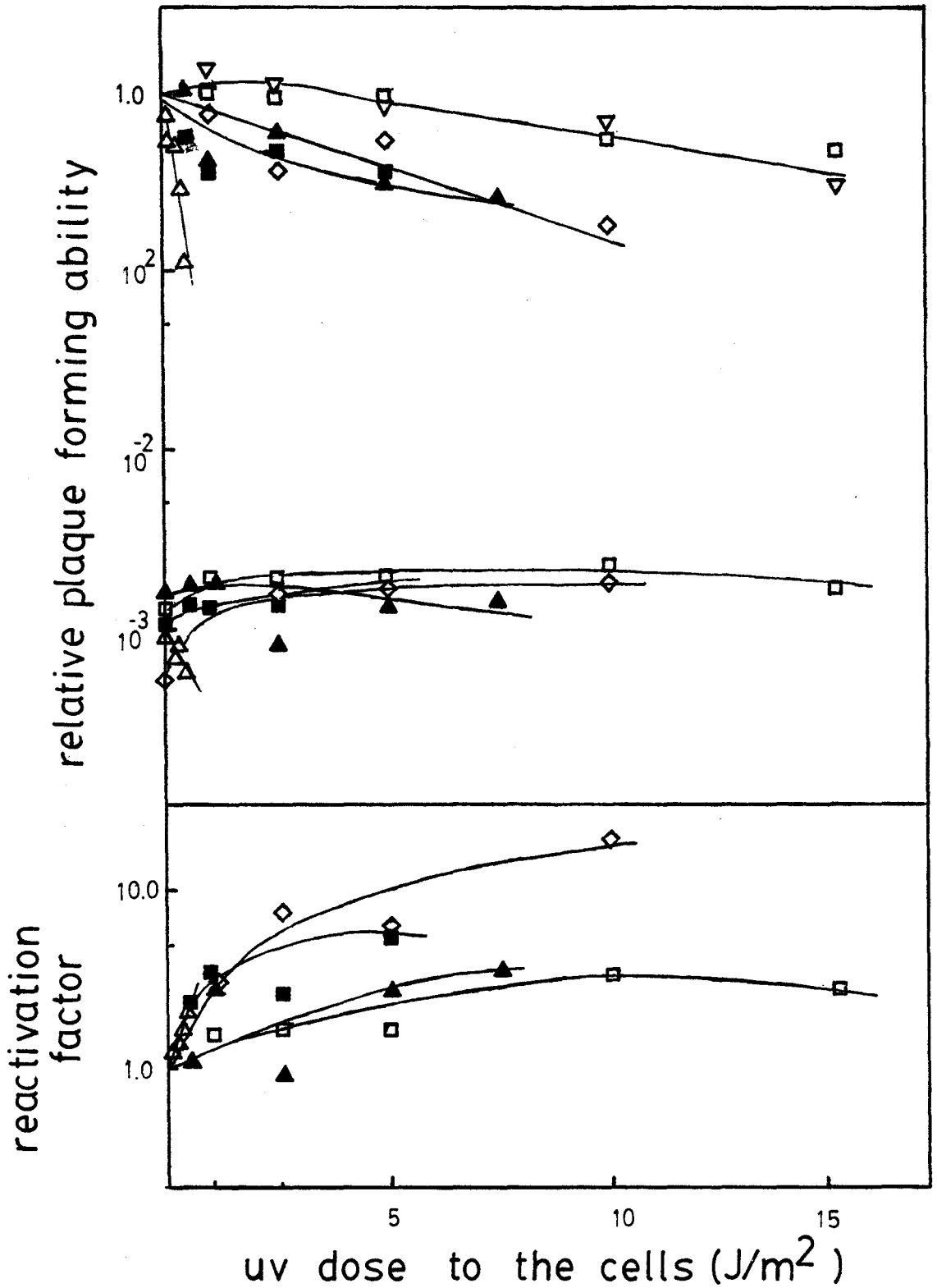
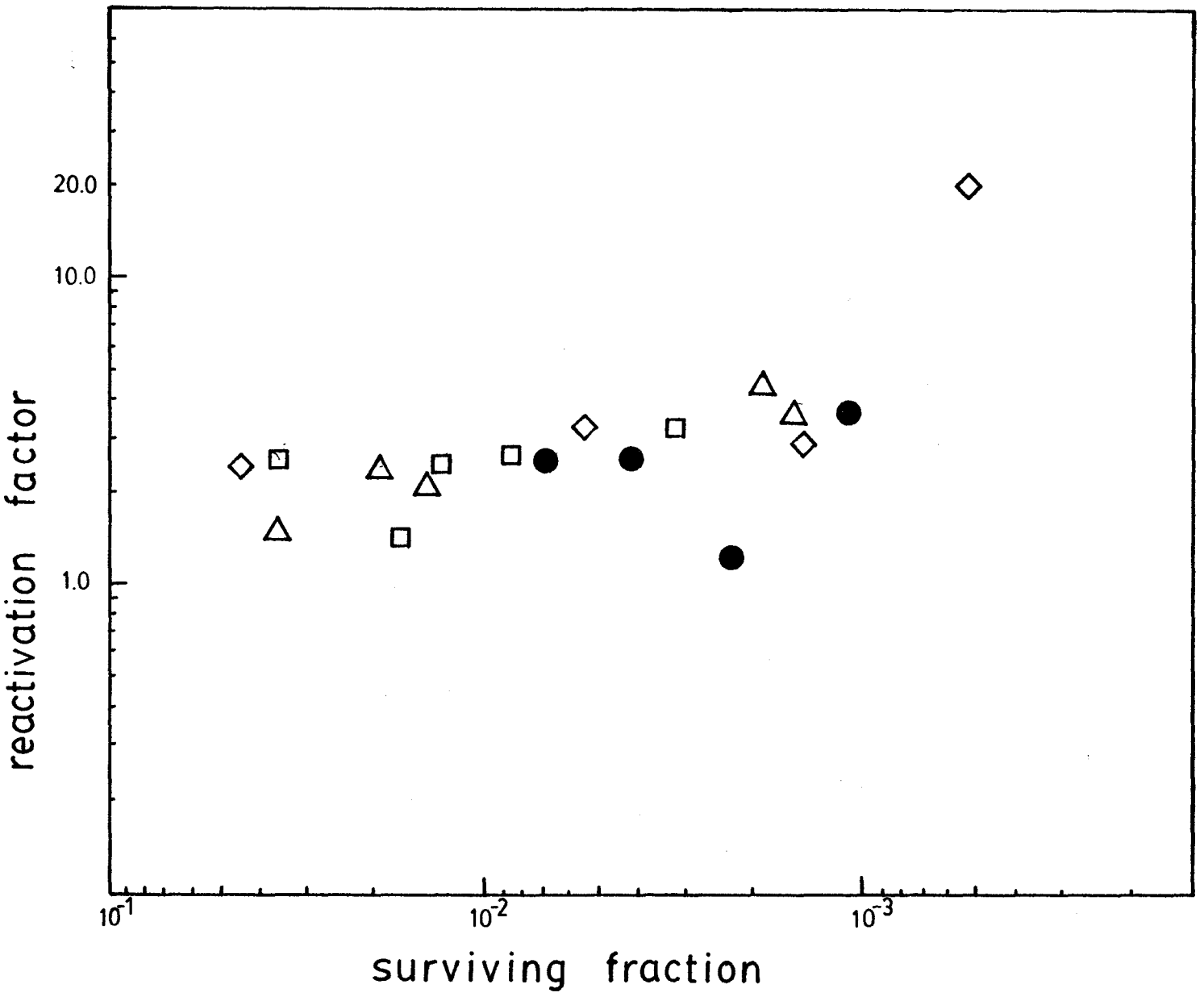

relative plaque forming ability
1.0
10^{-2}
10^{-2}
10^{-3}
reactivation factor
10.0
1.0
5
10
15
uv dose to the cells (J/m^2)

FIGURE 23

UVER of UV'd HSV-2 in human fibroblasts as a function of virus surviving fraction.

CRL 1221
GM 2838
CS 3BE
XP 4BE

The data from previous experiments was pooled and re-plotted as the reactivation factor versus the virus survival in unirradiated cells.


20.0
10.0
1.0
reactivation factor
10^{-1}
10^{-2}
10^{-3}
surviving fraction

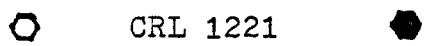
question further, results from other experiments were plotted as RF versus surviving fraction of the virus in unirradiated cells (see figure 23). Although the surviving fraction of HSV in cells other than XP variant were never reduced as low, the distribution of points in figure 23 would suggest that the RF is strongly dependent upon virus surviving fraction.

C.IV. DETERMINATION OF RF AS A FUNCTION OF UV DOSE TO THE VIRUS

To determine whether the amount of UV-induced viral DNA damage (i.e. the surviving fraction) had any effect on the level of UVER the following experiments were performed. The sets of tissue culture plates described earlier for determination of HCR (see Effect of UV on HSV Plaque Formation) were prepared in duplicate. One set was for unirradiated control cells and the other cells were irradiated with the dose of UV which induced maximum RF in that cell line (see table 3). After 48 hours the cells were infected with unirradiated virus or virus irradiated with various doses of UV (also described earlier). Cells were fixed and stained 48 hours after infection and plaques were counted. The SF of virus at the various UV doses was calculated for unirradiated cells and for irradiated cells and the ratio of SF irradiated/SF unirradiated was calculated.

FIGURE 24

UVER of UV'd HSV-2 as a function of UV dose to the virus in normal human fibroblasts.



The survival of UV'd HSV-2 was determined in normal cells which were unirradiated (panel A, solid curves) or irradiated with a dose of 15 J/m^2 (panel A, dotted curves). The ratio of virus survival in irradiated vs unirradiated cells was the reactivation factor. RF was plotted as a function of dose to virus (panel B).

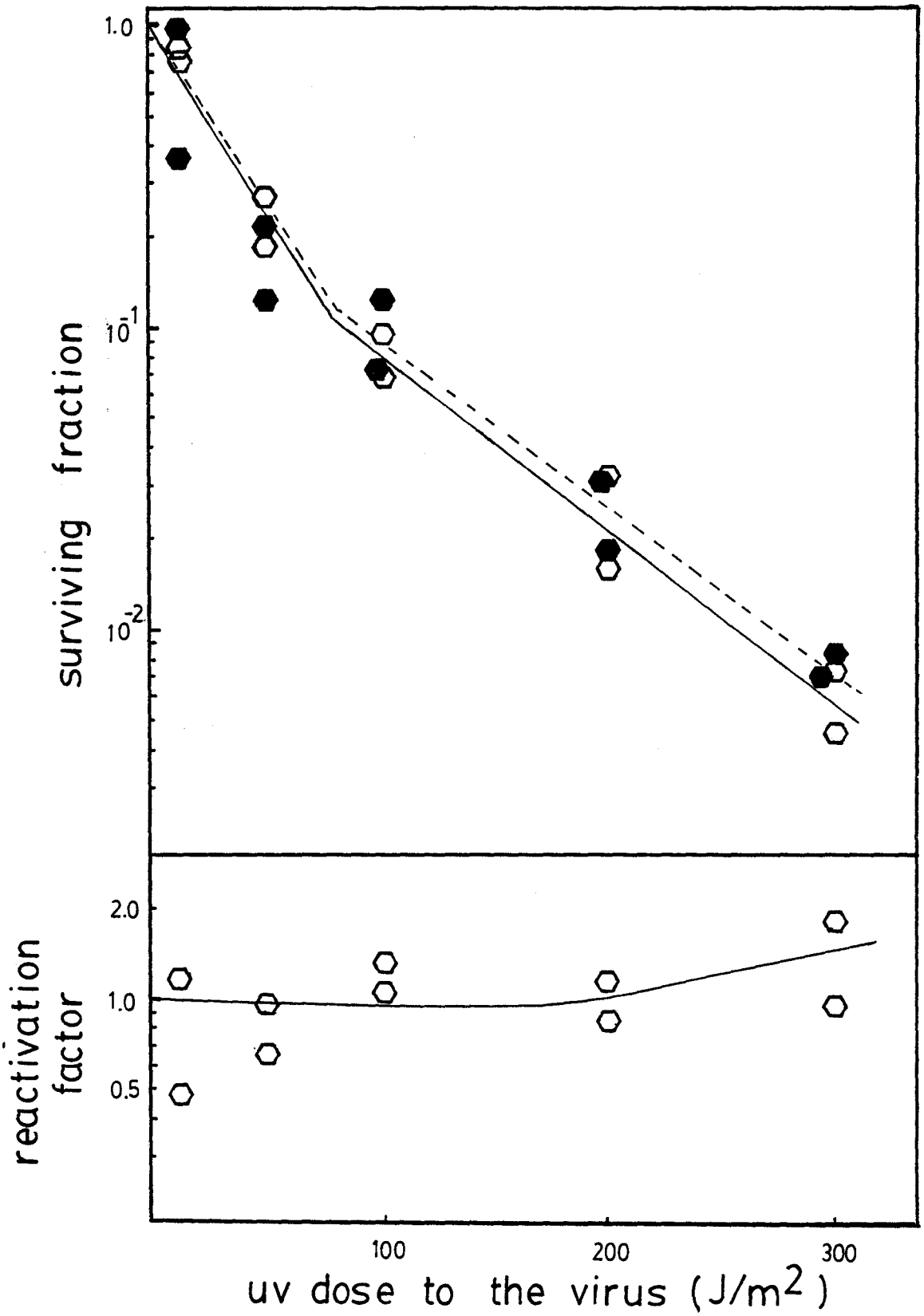
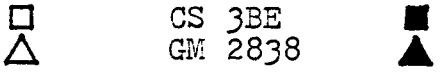

1.0
10^{-1}
10^{-2}
surviving fraction
2.0
1.0
0.5
reactivation factor
100
200
300
uv dose to the virus (J/m^2)

FIGURE 25

UVER of UV'd HSV-2 as a function of UV dose of the virus in CS fibrolasts.



The survival of UV'd HSV-2 was determined in CS cells which were unirradiated (panel A, solid curves) or irradiated with a dose of 5 J/m^2 of UV light (panel A, dotted curves). The ratio of virus survival in irradiated vs unirradiated cells was the reactivation factor. RF was plotted as a function of UV dose to the virus (panel B).

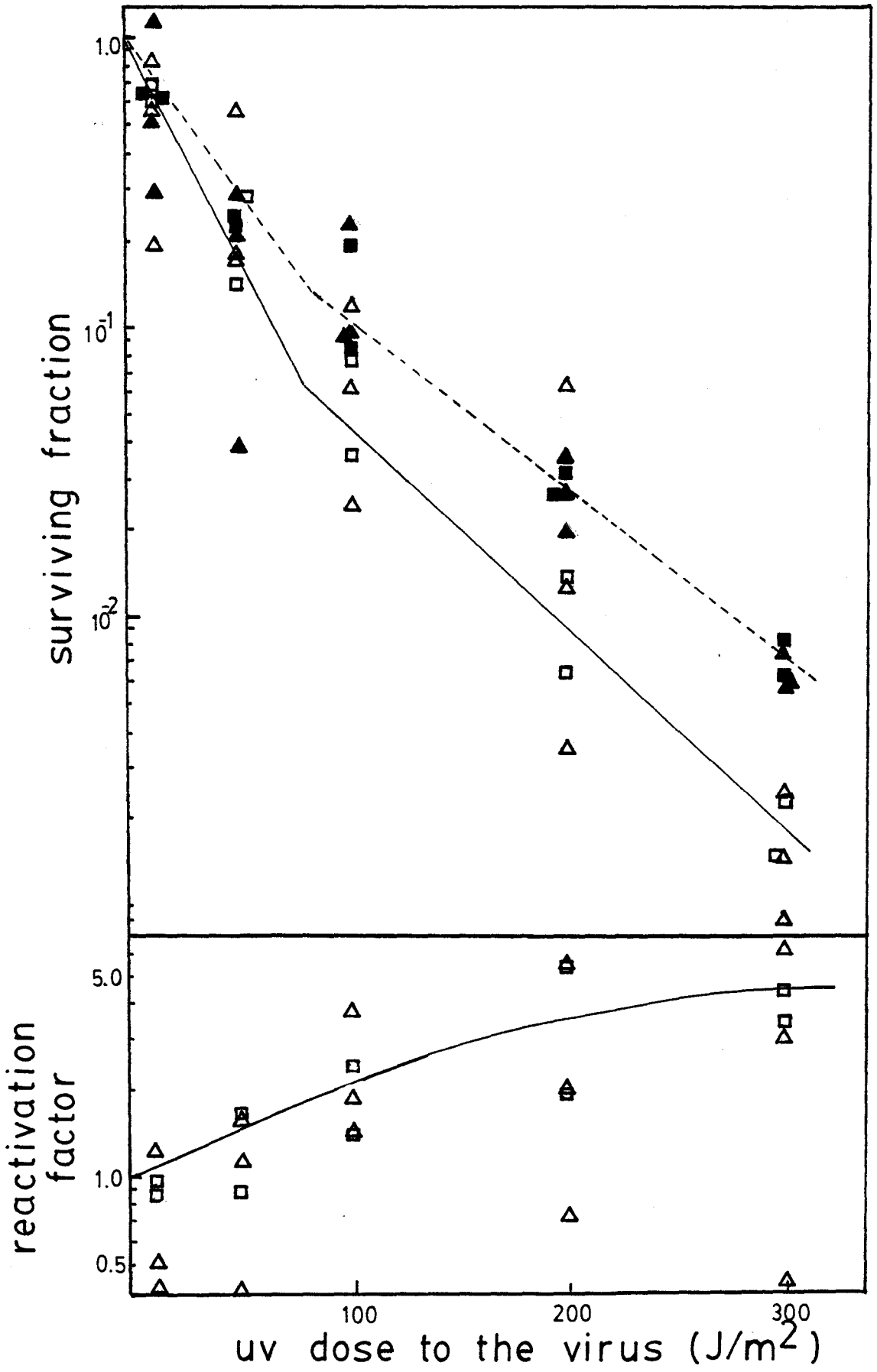

surviving fraction
1.0
10^{-1}
10^{-2}
reactivation factor
5.0
1.0
0.5
100
200
300
uv dose to the virus (J/m^2)

FIGURE 26

UVER of UV'd HSV-2 as a function of UV dose to the virus in XP excision deficient fibroblasts.

△ XP 25RO ▲

The survival of UV'd HSV-2 was determined in XP excision deficient cells which were unirradiated (panel A, solid curves) or irradiated with a UV dose of 0.5 J/m^2 (panel A, dotted curves). The ratio of virus survival in irradiated vs unirradiated cells was the reactivation factor. RF was plotted as a function of the UV dose to the virus. (panel B).

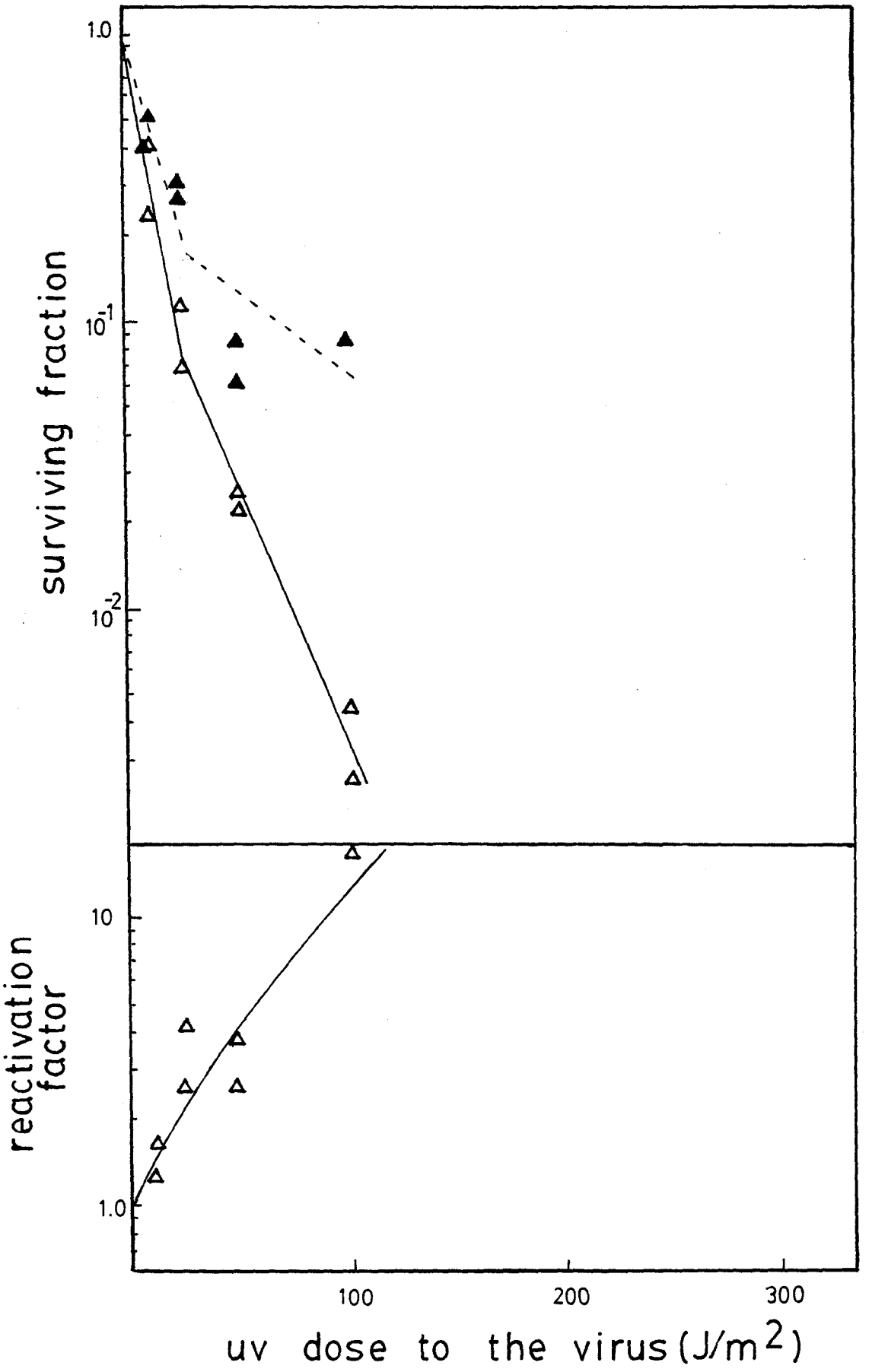
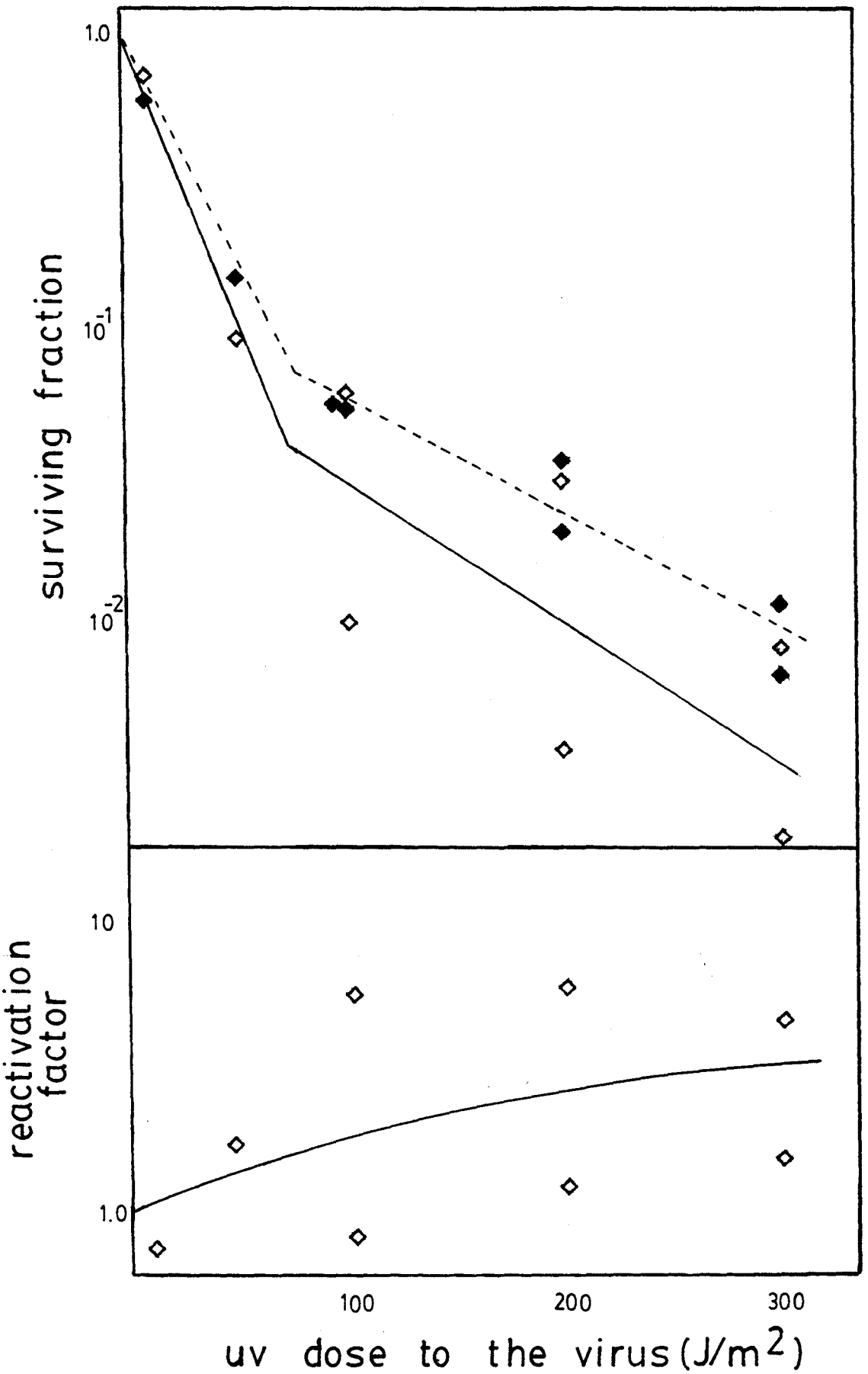

1.0
10^{-1}
10^{-2}
surviving fraction
10
1.0
reactivation factor
100
200
300
uv dose to the virus (J/m^2)

FIGURE 27

UVER of UV'd HSV-2 as a function of UV dose to the virus in XP variant fibroblasts.

◇ XP 4BE ◆

The survival of UV'd HSV -2 was determined in XP variant cells which were unirradiated (panel A, solid curve) or irradiated with a UV dose of 10 J/m^2 (panel A, dotted curve). Ratios of virus survival in irradiated vs unirradiated cells were the reactivation factors at that dose. RF was plotted as a function of UV dose to the virus (panel B).


1.0
10^{-1}
10^{-2}
surviving fraction
10
1.0
reactivation factor
100
200
300
uv dose to the virus (J/m^2)

This was the reactivation factor. In figures 24-27 the results of UVER experiments are plotted as RF versus UV dose to the virus.

For the normal strain CRL 1221 (figure 24) the RF is very low reaching a maximum value of 1.78 at 300 J/m^2 to the virus.

Two CS strains were tested, GM2838 and CS3BE. Maximum RF values of 6.56 and 5.27, respectively, were obtained at a UV dose to the virus of 300 J/m^2 for GM2838 and 200 J/m^2 for CS3BE (figure 25).

An excision deficient XP cell strain, XP25RO was tested. A maximum RF of 19.9 was obtained at a UV dose to the virus of 100 J/m^2 (figure 26). The excision proficient XP variant strain, XP4BE was also tested. Maximum RF of 5.72 was observed at a UV dose of 200 J/m^2 (figure 27).

D EFFECT OF UV IRRADIATION OF HSV-2 ON HOST CELL REACTIVATION OF VIRAL ANTIGEN FORMATION

In order to determine whether the biological endpoint measured by different assays had any effect on the measurements of relative HCR ability, an experiment was performed to assay HCR of UV'd HSV by viral antigen formation. This could then be compared to the results obtained by plaque assays.

Cells were prepared by seeding the contents of one confluent 75 cm^2 tissue culture flask into

eight 8-well chambered slides. When the monolayers were confluent, dilutions were prepared of unirradiated and irradiated (50 J/m^2) herpes virus. Four slides of each cell strain were infected with unirradiated virus and four with irradiated virus. At various times after infection (12, 18 and 20 hours) cells were fixed and were stained for immunofluorescence as described in Materials and Methods. Cell monolayers were examined under a fluorescent microscope and cells (or foci of cells) which stained positively for Vag were counted. These numbers were used to calculate the titre of the virus (as in figure 2) in terms of Vag forming units per millilitre (Vfu/ml). Vag forming units per millilitre were plotted against time (figure 28A) for both unirradiated and irradiated virus. It can be seen that Vag formation had reached a plateau at 18 hours for both unirradiated and irradiated virus. The surviving fraction was calculated (Vfu/ml irradiated/ Vfu/ml unirradiated) at each time point and was plotted in figure 28B.

In table 4 the D_o values of Vag formation at 20 hours post-infection and plaque formation are listed for 3 of the cell strains tested. The values for plaque formation were taken from table 1. The values for Vag formation were calculated by the formula

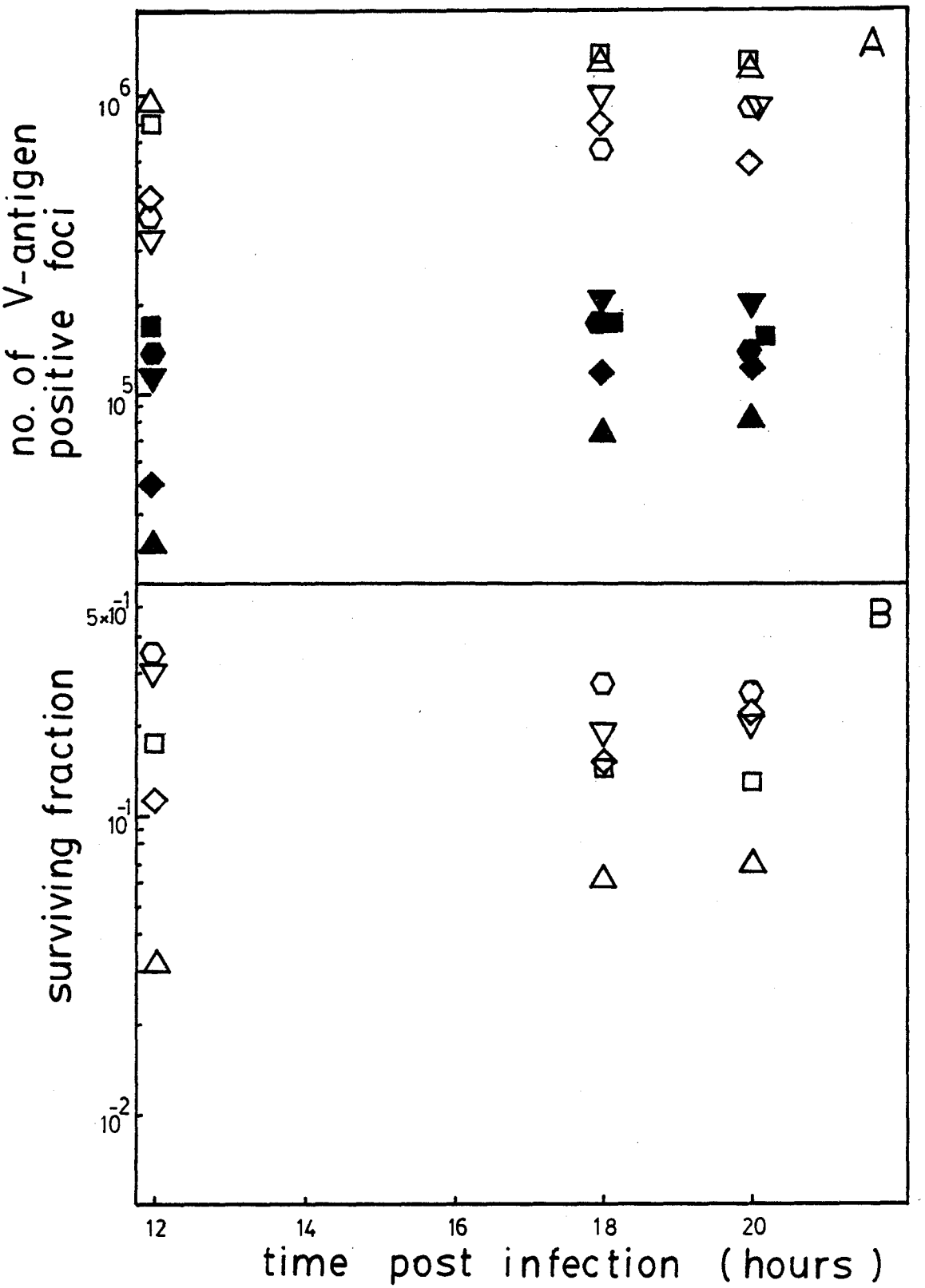
$$SF = e^{-D/D_o}$$

Figure 28

HCR of viral antigen forming ability of UV'd HSV-2 in normal, CS and XP fibroblasts.

CRL 1221
GM 2838
CS 1BE
XP 25RO
XP 4BE

The number of V-antigen positive foci were scored using a fluorescent microscope. In Panel A the open symbols represent unirradiated virus and the closed symbols represent virus irradiated with a UV-dose of 50 J/m^2. In panel B the surviving fraction was calculated as the number of V-ag positive foci for irradiated virus/unirradiated virus.


A
B
no. of V-antigen positive foci
surviving fraction
10^6
10^5
5×10^{-1}
10^{-1}
10^{-2}
12
14
16
18
20
time post infection (hours)

where D is the dose of UV given to the virus. In this case D= 50 J/m^2. It can be seen that the D_o values are higher for Vag formation than for plaque formation. This indicates that Vag formation is more resistant to UV irradiation. It can also be seen that the relative HCR ability of the two DNA repair deficient strains is the same for both assay systems.

TABLE 4

HCR OF HSV-2 VIRAL ANTIGEN FORMATION IN HUMAN FIBROBLASTS

	D_o (J/m^2)			% HCR of Normal (CRL1221)	
	XP 25RO	GM 2838 (CS)	CRL 1221 (N)	XP 25RO	GM 2838 (CS)
Vag	17.7	29.7	37.2	48	80
Plaquing *	11	21	27	41	78

* values taken from table 1

DISCUSSION

HOST CELL REACTIVATION

The ability of a DNA-damaged virus to initiate a productive infection depends to a great extent upon the nature of the host cell. Since viruses are severely limited by their small size in the number of functions they can code for, they depend upon the host to provide many functions. One such function is DNA repair. It has been shown that for very small viruses with extremely limited coding capacities (i.e. SV40) that HCR is severely decreased in cells from patients with XP as compared to normal cells (Aaronson & Lytle, 1970). These cells have been shown to have severely decreased DNA repair ability as measured by a number of criteria including unscheduled DNA synthesis (Cleaver, 1968), post-UV colony-forming ability (Andrews et al., 1978b), excision of pyrimidine dimers (Cleaver & Trosko, 1970) and loss of dimer specific endonuclease sites (Paterson et al., 1973). Together, these results imply that the reduced HCR of virus is due to the inability of the host repair mechanisms to remove DNA damage from the viral genome.

Reductions in the HCR of UV'd virus when infected into XP cells are also seen with larger viruses such as adenovirus (Day, 1974) and HSV (Lytle et al., 1972). However, as the size of the virus increases, the sensitivity to damaging agents does not increase in the manner predicted by simple target theory.

NORMAL HUMAN FIBROBLASTS

The HCR of UV'd HSV in 5 normal human fibroblast strains (see figure 9 of Results) was found to exhibit a 2-component survival curve. This is similar to results obtained by other workers for the survival curves of UV'd HSV in mammalian cells (Lytle and Benane, 1974; Albrecht et al., 1974; Hall et al., 1980). The curve consists of an initial steep component and a subsequent less steep component. The reason for the two components is not known. It has, however, been shown that multiplicity reactivation and heterogeneous virus populations are not the cause (Lytle, 1971). Some factors such as contact inhibition and depleted growth medium will decrease the zero dose intercept of the second component (see section A.II. of Results) without altering the slope (Lytle & Benane, 1974). It has also been found that differences in the HCR found at different stages of the cell cycle were not sufficient to account for the existence of a 2-component curve (Lytle and Schmidt, 1981). It should be noted that some investigators have reported the HCR of UV'd HSV to have a single component response (Takebe et al., 1974; Selsky & Greer, 1978).

The various parameters describing the HCR of the normal cell strains were calculated as described (see section A.II.a. of Results) and presented in table 1.

The D_o of the first component ranged from 26-36 J/m^2 with a mean value of 29 J/m^2. These values differ from those

obtained by Lytle and Benane (1974) which were reported to range from 10-19 J/m^2. These differences may be due to the difference in irradiation conditions. In experiments by Lytle (1971) virus was irradiated in PBS, whereas, for this study, virus was suspended in α-mem without serum for irradiation. Other differences may include the methods of dosimetry or the times at which plaques are scored. Lytle and Benane (1974) counted plaques of unirradiated virus at 3 days and irradiated virus at 4 days as compared to the 2 days employed in the present study.

The D_o of the second component ranged from 81-101 J/m^2 with a mean value of 94 J/m^2. This was again different from other reports of D_o values ranging from 32-53 J/m^2 (Lytle and Benane, 1974) and 27-32 J/m^2 (Lytle et al., 1983) for normal human fibroblasts.

The mean D_o of the second component was 3.2 X greater than the mean D_o of the first component. This agrees well with earlier reports (Lytle & Benane, 1974) in which the D_o's of the second component of the UV'd HSV survival curve in various normal human fibroblasts was 3-4 times greater than the D_o's of the first component.

In some studies of the HCR of UV'd HSV (Selsky and Greer, 1978; Takebe et al., 1978) the survival curves obtained were consistent with single component kinetics. Whether this is equivalent to one or the other of the two components seen in this study and others (Lytle and Benane, 1974; Albrecht et al., 1974; Hall et al., 1980) will be discussed below.

Selsky and Greer (1978) employed a protocol similar to that used in this study except that UV'd virus was quick-frozen and stored at -70° until use and plaque formation was enumerated at 72 hours after infection rather than 48 hours. They reported D_o values of approximately 150 J/m^2* for normal fibroblasts on survival curves with only a single component. In the work of Takebe et al. (1974), virus suspensions were made in PBS supplemented with 5% calf serum. Some experiments were performed with an agar overlay and plaques were counted at 5 days. In other experiments, a liquid overlay containing human gamma globulin was used (as in the present study) and plaques were counted at 3-5 days.

The presence of only a single component may be due to the fact that the virus survival was never reduced to less than 1%, the survival at which the second component manifests itself in normal human fibroblasts (Lytle, 1971; Lytle and Benane, 1974; Hall et al., 1980; this work). If the D_o of 150 J/m^2 is equivalent to the first component of the survival curve for the work of Selsky and Greer (1974), the reason it is so large compared to the values reported here may be due to the later time at which plaques were scored. Since the first infectious cycle of UV irradiated HSV (>200 J/m^2) is delayed from 20-24 hours to approximately 40 hours (Ross et al., 1971; Elgin et al., 1980), the later scoring time would allow for the development of plaques which would have been unnoticed at 48 hours, thereby

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* estimated by author from figures in Selsky & Greer (1978)

increasing the apparent survival, and thus the D_o, of the virus (see also Lytle, 1971). This argument also applies to the work of Takebe et al. (1974), where the D_o for normal fibroblasts was 110 J/m^2*.

It is also possible that only the second component is being seen. The similarity in D_o values (150 J/m^2 and 110 J/m^2 compared to 94 J/m^2 in this work) suggests that this is possible. How the first component is eliminated is unclear but it has been shown that cell culture conditions can effect the contribution of the first component (Lytle and Benane, 1974).

Results similar to the present work, concerning the survival of UV'd HSV in normal cells, was obtained by Coppey and Menezes (1981) using an assay for viral progeny. It was reported that a dose of 400 J/m^2 will reduce the survival of HSV-1 in normal human fibroblasts to 0.1%. This compares with an extrapolated survival of 0.2% in this study (see figure 9 of Results). The D_o of one normal fibroblast strain in that same study was approximately 100 J/m^2 for the second component.** Though it is similar to the D_o value obtained in this study it should be noted that the irradiation conditions used by Coppey and Menezes (1981) could not be determined and the assay for virus survival was virus yield. This assay is performed at relatively high m.o.i. (0.2-0.5 p.f.u./cell) which has been shown to increase the survival of UV'd HSV (Coppey and Nocentini, 1973; Hall et al., 1980).

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* calculated by author from Takebe et al. (1974)

** recalculated by author from Coppey and Menezes (1981)

Assuming that the radiosensitivity of double-stranded DNA viruses is directly proportional to their genome size then it would be expected that the D_o would vary as the inverse of the molecular weight.

One can attempt to calculate the "expected D_o" for HSV based upon the knowledge of the adenovirus D_o and the molecular weights of the two viruses. One caution that should be noted is that the UV survival curve for adenovirus is only a single component curve. Using similar irradiation conditions, UV-light sources and UV-light dosimetry, the D_o of the plaque forming ability of UV'd adenovirus type 2 (Ad 2) in human fibroblasts has been found to be 234 J/m^2 (Day and Ziolkowski, 1981). Based upon the larger molecular weight of HSV compared to Ad 2 (100×10^6 daltons vs 23×10^6 daltons (Spear and Roizman, 1980)), the expected D_o of HSV can be calculated to be 50 J/m^2. This value is obtained on the assumption that the only factor affecting the D_o is the target size (i.e. the molecular weight). Other factors which might affect the D_o include the GC content of the virus and the numbers of AT rich control sequences.

It can be seen that the actual D_o values are 29 J/m^2 for the first component and 94 J/m^2 for the second component. This indicates that, for the second component, HSV is less sensitive to UV-inactivation than predicted on the basis of its size. This decreased UV sensitivity may indicate that HSV DNA repair is less dependent upon host cell mechanisms than is Ad 2.

The validity of directly comparing the values used in these observations with those of other investigators is questionable since differences in experimental conditions (e.g. dosimetry, irradiation conditions, etc.) may be responsible for differences in D_o values.

The extrapolation number of the second component ranged from 0.08-0.34 with a mean value of 0.20 (table 1 of Results). These values are expressed as a surviving fraction. Lytle et al. (1983) have reported values ranging from 0.024-0.061 with a mean value of 0.042. The reason for these differences may be related to differences in cell culture conditions. Such differences have been shown to affect the extrapolation number of the second component of an HSV survival curve (Lytle and Benane, 1974).

COCKAYNE SYNDROME FIBROBLASTS

The HCR of UV'd HSV in CS cells displayed two component kinetics (see figure 10 of Results) similar to that observed in normal cells (see previous section of Discussion). Two component survival curves for UV'd HSV in CS cells has been reported by others (Lytle et al., 1983). It has also been reported that the HCR of UV'd Ad 2 in CS fibroblasts displays two-component kinetics (Day and Ziolkowski, 1981; Rainbow and Howes, 1982).

The D_o's of the first component for the two CS strains tested were 23 J/m^2 and 21 J/m^2 for CS3BE and GM2838 respectively, corresponding to relative HCR values of 77% and

72% (see table 1). These values were found to be not significantly different from those obtained for normal cells (P=0.367 and P=0.374). There are no other published values for the D_o of the first component of the UV survival curve of HSV in CS cells with which these results can be compared.

For the second component, the D_o's of the CS cells were 117 J/m^2 for CS3BE and 93 J/m^2 for GM2838. These values were not significantly different from the mean normal D_o (P=0.385 and $P > 0.40$ respectively). In terms of per cent normal HCR, the CS strains were capable of performing 124% HCR (CS3BE) and 99% HCR (GM2838).

Coppey and Menezes (1981) reported that a UV dose of 300 J/m^2 would reduce the survival of HSV in CS cells to 0.1%. This was lower than the 400 J/m^2 dose required to reduce HSV survival by the same factor in normal cells. Although a D_o value cannot be calculated from the information in their report, it can be noted that this is a lower survival than is observed in this study for the same dose (see figure 10 in Results). Differences in methodology have been discussed previously (see Normal Human Fibroblasts of Discussion).

Lytle *et al.* (1983) reported D_o values averaging 22 J/m^2 for two CS strains. The differences between the results reported here for CS cells and those of Lytle *et al.* (1983) are of approximately the same magnitude as the differences between the results for normal human fibroblasts as discussed earlier.

The important question, which these experiments have

attempted to answer, is, does a difference exist between the HCR abilities of normal and CS fibroblasts.

Other reports of the HCR ability of CS cells have differed in their conclusions. Hoar and Davis (1979) reported no difference in the adenovirus HCR ability of CS cells. Day et al. (1981), using a similar though not identical plaque assay reported that CS cells were reduced in their HCR of adenovirus. In addition, Rainbow and Howes (1982) reported reduced HCR of adenovirus V-antigen in CS cells. The reduction (expressed as % remaining HCR relative to normal cells) ranged from 14-34%. The HCR of HSV in CS cells was first assayed by Ikenaga et al. (1979) who reported no difference from normal cells. A recent report (Lytle et al.,1983) found a significant difference in the HCR of HSV between normal and one CS cell strain but not with another CS cell strain. The level of HCR for the second component was found to be 71% (CS3BE) and 83% (CS2BE) for the two CS strains tested (Lytle et al., 1983).

The HCR of UV'd HSV in CS cells is less than in normal cells. Whether they are significantly different is not yet certain (this study; Ikenaga et al., 1979; Lytle et al., 1983). The HCR of UV'd adenovirus in CS cells is significantly reduced when assayed by Vag formation (Rainbow and Howes, 1982) and by some plaque formation studies (Day and Ziolkowski, 1981) but not all (Hoar and Davis, 1979). One possible interpretation of this data is that HSV has available to it some DNA

repair function that is not available to adenovirus.

An alternative argument may be made, that differences in the biological endpoints of the two assays (i.e. Vag and plaquing) may account for the observed differences, rather than differences in the viruses. To pursue this question, HCR of UV'd HSV was determined by Vag formation. These experiments indicated that the relative level of HCR of UV'd HSV was the same for both assays (see table 4).

XERODERMA PIGMENTOSUM FIBROBLASTS

The HCR of UV-irradiated HSV in XP fibroblasts displays the same two-component kinetics observed for normal and CS cells in this study. This has also been observed by other workers (Lytle et al., 1972). The D_o's of the first component of the survival curve in excision deficient XP strains ranged from 11 J/m^2 for XP25RO (group A) to 14 J/m^2 for XP4RO (group C). This represented from 38-48% HCR as compared to normal cell strains. This is similar to the results of Lytle et al.(1972) where an XP group A cell strain was found to express 33% HCR in the first component. The first component D_o was found to be significantly different from normal for one of the XP group A strains and the group D strain (XP25RO, $P < .005$; XP6BE, $P < .0005$) but not for the group C strain.

The D_o's of the second component ranged from 26 J/m^2 (XP6BE) to 51 J/m^2 (XP4RO) for the excision deficient XP strains. The corresponding per cent HCR ranged from 28 to

54%. This is again similar to early work by Lytle et al. (1972) in which an XP cell strain was found to express 34% HCR in the second component. More recent work (Lytle et al., 1983) also reports 32% HCR in the second component of a single XP cell strain (XP2BE group C). Although relative levels of HCR in this work agree well with those of other reports, the absolute levels of UV sensitivity of HSV (i.e. D_o) are very different. Possible reasons for these differences have been discussed.

Studies of the HCR ability of XP cells have been performed using a number of different viruses. The survival of UV'd simian virus 40 (SV40) in XP cells of different complementation groups was found to range from 13% (XP group A) that of normal cells to 59% (XP group E) of normal, depending upon the complementation group (Abrahams and van der Eb, 1976). Similarly, the survival of plaque formation of UV'd Ad 2 in XP cells ranged from 3.4% (group A) to 47% (group E) of normal levels of HCR (Day, 1974). All of the work mentioned above was done using some form of a plaque assay. Other assays also detect a greatly reduced HCR of UV'd virus in XP excision deficient cells. These methods include T antigen formation and transformation frequency of SV40 (Aaronson & Lytle, 1970), detection of alkaline labile Ad 2 DNA damage (Rainbow, 1977) and Ad 2 V antigen expression (Rainbow, 1980).

The observation that UV'd Ad 2 is only reactivated to approximately 5% of normal levels in XP group A cells and that HSV is reactivated to more than 30% of normal levels in

XP group A cells has suggested to some authors that HSV may be less dependent upon host-cell DNA repair mechanisms than is Ad 2 (Selsky and Greer, 1978; Rainbow, 1981). The data presented in this work confirm the level of HCR of UV'd HSV in XP group A cells and thereby support the suggestion that HSV is less dependent upon host functions than Ad 2. This greater independence may be attributable to either a virally coded function or a virally induced function.

Reports of single component survival curves for UV'd HSV in XP cells (Takebe et al., 1974, 1978; Selsky and Greer, 1978) yield similar values for HCR ability compared to normal cells.

XERODERMA PIGMENTOSUM VARIANT FIBROBLASTS

The HCR of UV'd HSV in XP variant fibroblasts displays the same 2-component kinetics observed for all cell types in this study (see figure 12 of Results). In the work of Selsky and Greer (1978), the XP variant strain CRL1162 (XP4BE) displayed a survival curve consistent with 2-component inactivation, although the authors did not comment upon this fact.

The D_o of the first component was 24 J/m^2 (see table 1 of Results) for the one strain tested, XP4BE. This represented 83% HCR compared to normal cells. This value was not significantly different from normal (P=0.390).

The D_o of the second component was 81 J/m^2, representing 86% HCR. This value was not significantly different from normal (P=0.244). This value for per cent HCR is consistent

with the value of 80% HCR* obtained by Selsky and Greer (1978). It is, however, higher than the values obtained by Takebe et al. (1978) who used different strains, values for which, ranged from 31%-55% HCR.

Studies of the HCR ability of different viruses in XP variant cells have been performed. SV40 is reactivated to 95% that in normal cells (Abrahams and van der Eb, 1976). Using a plaque assay Day (1974) found the XP variant cells (XP4BE) could reactivate UV'd adenovirus to 69% of normal levels, a value similar to that obtained for other XP variant strains in the same study. Using the same cell strain but a different assay (i.e. Vantigen) Rainbow and Howes (1979) found that UV'd adenovirus was reactivated to 62% of normal levels.

HOST CELL REACTIVATION OF UV'D HSV-2 VIRAL ANTIGEN FORMATION

In attempting to compare the HCR of UV'd HSV-2 from this study with the HCR of adenovirus in CS cells there arises the question of whether the biological endpoint of the assays utilized modifies the results obtained. In plaque forming assays the parameter measured (namely infectivity) is far removed from the repair events. A viral antigen (Vag) assay, on the other hand, measures an event much closer to the actual repair process (Rainbow, 1978).

To address this question, a single experiment was performed to measure the HCR of UV'd HSV Vag. This was performed over a period of 12-20 hours post-infection so that any effects

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* estimated by author from figure in Selsky & Greer (1978)

caused by the UV-induced delay in the HSV replication cycle (Ross et al., 1971) could be included.

The number of Vag positive foci increased from 10-18 hours post-infection and appeared to reach a plateau for the period from 18-20 hours (see figure 25, upper panel). The surviving fraction of XP group A cells (XP25RO) at all times was lower than the CS or normal strains.

In order to compare the relative HCR of UV'd HSV in normal, CS and XP cells as assayed by plaquing and Vag formation the D_o's of Vag survival were calculated as described in Results. Using these results the per cent HCR compared to normal cells could be calculated (see table 5). Because the single UV dose used was within the range of the first component, these per cent HCR values were compared to those for the first component of plaque survival.

The D_o values for the various strains are all higher for the Vag assay than for the plaquing assay. This is in agreement with the work of Elgin et al. (1980) whose results suggested that the later in the infectious cycle a function occurs, the more sensitive it is to UV-irradiation. They related this to the relative target sizes of the different functions. The same phenomenon can be seen for adenovirus for which the D_o of the HCR of UV'd virus in normal cells is 234 ± 40 J/m^2 (Day and Ziolkowski, 1981) for plaquing and 410 ± 80 J/m^2 for Vag (Rainbow, 1980).

When the relative HCR ability of CS and XP cells is compared between Vag and plaquing assays the values obtained

for % HCR are the same for each strain (see table 5). This correlation between relative HCR levels using assays of different biological endpoints for the same virus has also been seen with adenovirus. Day (1974) reported that XP group A cells had 3.4% HCR when assayed for plaque-forming ability. Rainbow (1980) reported that another strain of XP group A cells had 6% HCR when assayed for Vag forming ability. This correlation between plaquing and Vag for HCR of UV'd Ad 2 has been seen for XP complementation groups A-F and variant (Rainbow, 1981).

CAPACITY

To determine whether a cell strain is deficient in its responses to UV-irradiation, ionizing irradiation or chemicals which produce DNA damage, the most common method is to assay for post-exposure colony-forming ability (cfa). (Lee and Puck, 1960). One of the major disadvantages of this method is that certain mammalian cell strains have inherently poor colony forming ability. Also, cfa assays require that the cell is isolated from its neighbours, a state entirely different than that found in intact tissue. Finally, the cfa assay requires the cell to divide in order to be scored.

A number of authors have used an alternative assay to determine the cellular response to DNA damaging agents (Lytle et al., 1976; Coppey & Nocentini, 1976; 1979; Coppey et al., 1979a; Coohill et al., 1979). This assay determines

the ability of treated cells to support virus infection (capacity). This can be measured in a variety of ways including one-cycle virus yields (Coppey & Nocentini, 1976) and plaque-forming ability (Lytle et al., 1976; Coohill et al., 1979). The advantages of this method include the fact that cells are in contact with their neighbours, a model more closely resembling the in vivo situation. In addition, cells are not required to divide in order to enumerate their capacity. That capacity is a DNA dependent parameter has been shown by Coohill et al.(1977) on the basis of the action spectrum of capacity inactivation.

EFFECT OF DELAYED INFECTION ON CAPACITY

Initial experiments to determine the capacity of normal and CS fibroblasts were performed with various intervals between irradiation of the cells and their infection with HSV. These experiments demonstrated that for the UV doses employed (i.e. up to 15 J/m^2) normal human fibroblasts were able to recover their ability to support HSV infection when given sufficient time (96 hours) between irradiation and infection (see figure 14 of Results). Note that no increase in capacity was evident by 48 hours. Similar recovery of capacity after UV-irradiation has also been reported for monkey-kidney cells (Bockstahler et al., 1976), and normal human fibroblasts (Lytle et al., 1976; Coppey et al., 1979b).

The capacity of CS cells (see figure 14 of Results) was lower than that of normal cells when they were

infected immediately after irradiation. This was not unexpected since CS cells have lower post-UV colony-forming ability (Andrews et al., 1978a) and cfa has been shown to respond in a manner similar to capacity for UV-irradiated XP cells (Lytle et al., 1976; Coohill et al., 1981). As the recovery time was increased to 48 hours the capacity of CS cells decreased further. Again, this was not unexpected since repair deficient XP cells had been shown to decrease in capacity with increasing time after irradiation (Lytle et al., 1976). At 96 hours after irradiation the capacity of CS cells increased to approximately the same level observed for immediate infection. The inability of CS cells to completely recover capacity correlates with the observation that UV'd CS cells do not recover their ability to undergo semi-conservative DNA replication (Tanaka et al., 1981). The fact that CS cells do not recover capacity even though they have normal rates of unscheduled DNA synthesis (UDS) (Wade and Chu, 1978, 1979; Andrews et al., 1978a) confirms the earlier observation for the various XP excision deficient cell strains (Lytle et al., 1976) that levels of capacity correlate more closely to cell survival (i.e. colony-forming ability) than to UDS. This suggests that cell death may be, in part, responsible for loss of capacity.

The cause of the decrease in capacity of CS cells at 48 hours after irradiation remains unclear. However, since the greatest difference between normal and CS cells was observed at this time, all subsequent capacity experiments

were performed at 48 hours after irradiation.

NORMAL HUMAN FIBROBLASTS

The capacity of four different strains of normal human fibroblasts to support HSV plaque-formation was tested. Capacity was measured at 48 hours after irradiation (see figure 15 of Results). Low dosesof UV (< 2.5 J/m^2) did not reduce the capacity of normal cells. In some experiments the capacity actually increased at low doses of UV. Similar responses have been observed by other workers for normal human fibroblasts (Coppey et al., 1979b). Such a response is also typical for post-UV cfa of normal human fibroblasts (Andrews et al., 1978a; Wade and Chu, 1978, 1979).

At higher UV doses (> 2.5 J/m^2) the capacity of normal fibroblasts to support HSV plaque formation decreases exponentially with increasing UV dose. This response has also been observed by others for the capacity of normal human fibroblasts (Coppey et al., 1979b) as well as their post-UV cfa (Andrews et al., 1978a; Wade and Chu, 1978, 1979).

The D_{37} (see Capacity section of Results) for the capacity of the normal strains tested ranged from 8.6 J/m^2 to 12.4 J/m^2 with a mean value of 10.7 J/m^2 (see table 2 of Results). In contrast, Coppey et al. (1979b) found that normal human fibroblasts had a D_{37} of approximately 25 J/m^2*, using a virus yield (per cell) assay and cells infected 36 hours after irradiation.

The D_{37} of colony forming ability for normal fibroblasts

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*estimated by author from figure in Coppey et al. (1979b)

ranged from 5.0-6.5 J/m^2* (Andrews et al., 1978a) and 5.0-5.5 J/m^2* (Tanaka et al., 1981).

COCKAYNE SYNDROME FIBROBLASTS

The capacity of HSV plaque formation of four strains of CS fibroblasts was determined at 48 hours after irradiation. The capacity of all CS cells decreased exponentially with increasing UV dose and no shoulder region was apparent for the response curve of any strain (see figure 16 of Results). This is similar to the cfa response of CS fibroblasts, which also does not display a shoulder (Andrews et al., 1978a; Wade and Chu, 1978, 1979; Tanaka et al., 1981).

The D_{37} of the four CS strains ranged from 3.1 J/m^2 (all strains except CS3BE) to 5.1 J/m^2 (CS3BE). The relative capacities, expressed as a per cent of the mean normal D_{37}, were 29% and 47.7% respectively. The D_{37} of CS3BE was found not to be significantly different from that of normal cells ($P=0.077$) but all other CS strains were significantly lower ($P < 0.002$) than normal. Although the D_{37} of CS3BE is not significantly lower than that of normal cells, the major difference in the shape of the capacity response curve indicates a deficiency in the capacity of CS3BE.

The D_{37} of cfa of CS3BE (1.5 J/m^2*) has been found to be slightly higher than that of some other CS strains (including CS1BE (0.7 J/m^2*), which has also been used in the present study) (Tanaka et al., 1981). The relative cfa of these CS strains was 30% for CS3BE and 14% for CS1BE. This again

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* estimated by author from figures in Andrews et al., (1978a) and Tanaka et al., (1981)

reflects the possible relationship capacity and cfa as observed for XP cells of different complementation groups (Lytle et al., 1976). In fact, Tanaka et al. (1981) showed that CS3BE and CS1BE belong to different complementation groups.

This data indicates that, for CS cells, capacity is a better indicator than HCR of cellular sensitivity to UV-irradiation.

XERODERMA PIGMENTOSUM FIBROBLASTS

The capacity of four different XP cell strains to support HSV plaque formation was determined. The four cell strains represented the excision deficient complementation group A, C and D as well as the excision proficient XP variant. Capacity was determined at 48 hours after irradiation.

The capacity of all excision deficient XP cell strains decreased exponentially with increasing UV dose (see figure 17 of Results) with no shoulder apparent for the UV doses tested. Similar responses have been noted by other workers (Lytle et al., 1976; Coppey et al., 1979b). Using lower UV doses (i.e. below 1 J/m^2) Coohill (1981) demonstrated a shoulder for some XP strains.

The capacity of the excision proficient XP variant cell strain was not affected by low (< 5 J/m^2) doses of UV light. When the UV dose was greater than 5 J/m^2 the capacity of XP4BE decreased exponentially with increasing UV-dose (see figure 17 of Results). This shoulder on the XP variant curves, similar to the normal strains was also observed by

Coppey et al. (1979b) for two different strains of XP variant fibroblasts.

The D_{37}'s of the XP strains were 0.4 J/m^2 for XP25RO (group A), 0.96 J/m^2 for XP6BE (group D), 1.98 J/m^2 for XP4RO (group C) and 6.7 J/m^2 for XP4BE (variant). The relative capacities, expressed as a per cent of the mean normal D_{37} were 3.7%, 9%, 18.5% and 62.6% respectively. Just as for CS cells, capacity is a more sensitive assay than HCR for determining cellular repair ability. All of these values were significantly different from normal ($P < 0.0005$) except that for the XP variant strain, XP4BE ($P=0.139$).

Although there were slight differences in the D_{37} values reported by Lytle et al. (1976) the relative capacities were in good agreement. The XP group A strain was the most UV sensitive in terms of capacity, next was the group D strain and next the group C strain. This is the same order of sensitivity observed in the present study as well as those of Coppey et al. (1979b). Furthermore, these results reflect the UV-sensitivity of colony-forming ability of XP cells (Andrews et al. 1978b), where the D_{37}'s* of cfa for XP(A), XP (D), XP(C), XP variant and normal cells were approximately 0.25 J/m^2, 0.5 J/m^2, 1.2 J/m^2, 2.5 J/m^2 and 6.5 J/m^2 respectively. The corresponding relative cfa values were 3.8%, 7.7%, 18.5% and 38%.

The capacity of cells to support HSV plaque formation can be reduced by UV-irradiation. Normal human fibroblasts

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* estimated by author from figure in Andrews et al. (1978b)

are able to recover capacity over the range of doses used in this study if given sufficient time after irradiation. Cells from patients with CS or XP did not recover capacity (Lytle et al., 1976; this work). This may be related to the observation that while normal fibroblasts are able to recover normal rates of semi-conservative DNA synthesis (Rude and Friedberg, 1977), neither CS cells (Tanaka et al., 1981) nor XP cells (Rude and Friedberg, 1977) possess this ability. Furthermore, AT cells, which are hypersensitive to cell killing by ionizing radiation (Paterson and Smith, 1979) are not reduced in capacity to support adenovirus Vag formation after treatment with γ-rays (Jeeves and Rainbow, 1983) and semi-conservative DNA synthesis is not inhibited (Painter, 1981). These results suggest that inhibition of viral DNA synthesis has factors in common with inhibition of cellular DNA synthesis and that the capacity of cells is a good indication of the sensitivity of DNA synthesis of the cells themselves.

The relative levels of UV-sensitivity of capacity are similar to those of post-UV colony-forming ability both for CS cells (Tanaka et al., 1981; Andrews et al., 1978a) and XP cells (Andrews et al., 1978b). This suggests that capacity and cfa may be related in that cell death is, in part, responsible for loss of capacity (Lytle et al., 1976).

UV ENHANCED REACTIVATION

The phenomenon of enhanced reactivation of virus in mammalian systems is in many ways similar to that seen in bacterial systems (Weigle, 1953). Many of the details have been presented already (see Enhanced Reactivation of Virus, Introduction).

The magnitude of the UVER response will depend upon a number of factors. Because it may be an inducible response (Radman, 1980) the ability of a culture to respond may depend upon the state of growth of the cells and the time allowed for induction to occur. Both of these possibilities were investigated. If UVER is a response to DNA damage then the amount of damage produced may affect the level of induction. Different irradiations of both the cells and the virus were performed.

EFFECT OF DELAYED IRRADIATION

It has been shown (Lytle and Benane, 1974) that the amount of HCR expressed by human cells can change with increasing time after subculture (or attainment of confluency). Contact-inhibition was also shown to be related to levels of ER (Lytle *et al.*, 1974; Hellman *et al.*, 1974).

When experiments were performed to determine the effect of delaying irradiation after plating on ER, cells were plated at a density such that they were confluent within 1 day. Cells irradiated with a range of UV doses, were infected 48 hours later with HSV and levels of ER were determined.

Maximum ER was determined each day and it was found that as the cells remained confluent longer, they expressed lower levels of UVER (see figure 20 of Results).

The results of this experiment support the suggestion that as cells become more contact-inhibited their overall repair levels decrease. This is essentially similar to the results of Lytle and Benane (1974) in which levels of HCR decreased with increasing time after confluency. In addition, work correlating the level of contact-inhibition (i.e. in tumour cells vs normal fibroblasts) with the level of UVER (Lytle et al., 1974; Hellman et al., 1974) showed that highly contact-inhibited cells such as fibroblast displayed lower levels of UVER than tumour cells.

EFFECT OF DELAYED INFECTION

It has been reported by a number of authors (Lytle et al., 1976) Coppey and Menezes, 1981; Bockstahler et al., 1976) that the observed levels of ER were maximum at a particular time after irradiation of the cells. Similar experiments were performed in this study using normal and CS fibroblasts. In this experiment cells were treated with increasing doses of UV-light and then infected at various times after irradiation. The maximal ER (see figure 19 of Results), for all doses to the cells, was found to occur at 48 hours after irradiation. This agrees very well with the work of Coppey and Menezes (1981) who found that the maximal ER of HSV-1 in human fibroblasts occurred at 36 hours.

Their results indicate little difference in ER between 36 and 48 hours. Though there have been other reports of increased ER with delayed infection, no other report determined the time course of ER in human cells.

UVER AS A FUNCTION OF DOSE TO CELLS AND VIRUS

Conditions that were considered to be optimum for the measurement of UVER were chosen as a result of the experiments described in the preceding section. Cells which had been plated 1 day previously were irradiated with various doses of UV-light and infected 48 hours later.

The level of ER in human cells depends upon both the UV dose to the cells (Lytle et al., 1976; Coppey and Menezes, 1981; Lytle, 1978) and the UV dose to the virus (Coppey and Menezes, 1981; Lytle and Knott, 1982). Experiments were of two basic types in this study. Either the dose to the virus was constant and the dose to the cells was varied or the dose to the cells was constant and the dose to the virus was varied.

The two main parameters used to describe UVER curves are the maximum RF and the UV dose at which that maximum occurs.

NORMAL HUMAN FIBROBLASTS

For normal human fibroblasts the maximum RF usually occurred at a dose of 15 J/m^2. The only exception was the cell strain Hff which had maximum RF at 10 J/m^2. Enhanced reactivation factors were determined for the normal strains,

when the virus survival was reduced approximately 100 fold. Values for RF ranged from 0.97-1.46 (table 3). Lytle et al. (1976) reported that the maximum UVER of plaque forming ability of UV'd HSV in normal human fibroblasts was approximately 2, occurring at a UV dose to the cells of 20 J/m^2 and a virus surviving fraction of 2.7×10^{-2}. These values agree very well considering the differences in the assay system (plaques of unirradiated virus scored at 3 days and irradiated virus at 4 days post-infection) and the fact that infection was delayed for four days after irradiation. Another report of UVER of UV'd HSV in normal human fibroblasts found maximal RF at a UV dose to the cells of 15 J/m^2 (Coppey and Menezes, 1981). The RF values reported ranged from 1.8-7.5. There were however, major differences in the procedure. The assay used was viral progeny, using high m.o.i. (0.5-2.0 pfu/cell). As discussed earlier (see Host Cell Reactivation of Discussion) this assay allows for multiplicity reactivation of virus. Also, the virus was given a dose of UV sufficient to reduce its survival to 10^{-3} in unirradiated cells. In the present work, when virus survival was reduced to approximately 10^{-3} greater RF values were obtained for all cell strains tested. A maximum RF value of 3.38 was obtained (see figure 22) at a UV dose to the cells of 15 J/m^2.

The efficiencies of ER were calculated for the UV dose to the cells which produced maximal RF (table 5). At virus survival levels of approximately 10^{-2}, the efficiency of ER

TABLE 5

Efficiency of UVER for plaque formation
by UV-irradiated HSV-2

VIRUS SURVIVAL LEVEL	CELL STRAIN				
	NORMALS	CS3BE	GM2838	XP4BE	XP25RO
10^{-2}	0.006-0.08[1]	0.25	0.25	0.29	0.16
10^{-3}	0.18	0.21	0.24	0.40	N.D.[2]

[1] At the UV dose to the cells eliciting maximum UVER efficiency was calculated as described by Devoret *et al.* using the formula $E = \frac{\ln RF}{\ln SF_u}$ where RF is the reactivation factor and SF_u is the surviving fraction of UV'd HSV in unirradiated cells.

[2] not determined at this survival level

ranged from 0.006-0.082. These values are much lower than that obtained by Lytle et al. (1976) from which the efficiency of UVER of UV'd HSV was calculated* to be 0.22. At lower virus survival levels (i.e. 10^{-3}), the efficiency of a single normal cell strain was 0.183. Values calculated for the efficiency of UVER of UV'd HSV from the data of Coppey and Menezes (1981) yielded an average value of 0.196 for the normal strains tested.

If the UV dose to the cells was kept constant (i.e. that eliciting maximum RF) and the dose to the virus was varied, it was found that as the UV dose to the virus increased, the level of UVER increased (figure 24), although the increase was not great. This has been found before for human cells (Lytle et al., 1976; Lytle and Knott, 1982; Coppey and Menezes, 1981).

UVER of UV'd adenovirus in normal human fibroblasts (Jeeves and Rainbow, 1983)determined as a function of UV dose to the cells, reached maximum RF levels of 3.1 ± 1.2 at a virus survival of 6.1×10^{-3} and UV dose to the cells of 10 J/m^2 - 15 J/m^2. The observation that maximal ER occurred at the same UV dose as for this study (note that dosimetry and UV source of Jeeves and Rainbow (1983) was identical to that used in the present study) indicates that this aspect of ER is a reflection of some cellular function and is independent of the virus used to assay.

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*values calculated by Jeeves (1981).

COCKAYNE'S SYNDROME FIBROBLASTS

All of the CS fibroblast strains tested in this study expressed maximum UVER at a dose of 5 J/m^2. The RF values obtained at a virus survival level of 10^{-2} ranged from 2.40 for GM739 to 3.23 for GM2838 (table 3).

The only other report of UVER of UV'd HSV in CS cells is that of Coppey and Menezes (1981) at a virus survival level of 10^{-3}. The dose of UV given to the cells to produce maximum UVER was 5.0 J/m^2 for all CS strains reported. The range of RF values was 2.0-4.0. The UVER of HSV UV'd to a survival level of 10^{-3} in CS cells in the present study was 4.57 for GM2838 and 3.39 for CS3BE (figure 22).

Determination of RF as a function of the UV dose to the virus indicated that just as for normal cells (Lytle et al., 1976; Lytle and Knott, 1982; Coppey and Menezes, 1981), the level of UVER increased with increasing UV dose to the virus (figure 25).

The efficiency of UVER of UV'd HSV in CS cells was calculated for this study (table 5 (at 10^{-2} and 10^{-3} virus survival levels)) and for the data in Coppey and Menezes (1981). The efficiency of GM2838 and CS3BE was 0.25 for both at 10^{-2} virus survival and 0.24 and 0.21 at 10^{-3} virus survival. For the four CS strains tested by Coppey and Menezes (1981) the efficiency ranged from 0.10-0.20.

The UVER of UV'd adenovirus in CS fibroblasts (Jeeves, 1981) was maximal at a UV dose to the cells of 5 J/m^2 for

one of the two strains tested (CS3BE) and 2.4 J/m^2 for the other strain (CS1BE). The RF values for the two strains fell on either side of the values obtained for normal fibroblasts.

XERODERMA PIGMENTOSUM FIBROBLASTS

The maximum UVER of XP fibroblasts depended upon the complementation group of the cell strain. Group A fibroblasts (XP25RO) had maximum UVER at 0.5 J/m^2 and the RF was 2.07. In other reports (Lytle et al., 1976) group A fibroblasts had maximum RF of 2.5 at a dose of 0.4 J/m^2 and (Coppey & Menezes, 1981) 7.0 at a dose of 0.1 J/m^2. Differences in methods between these two reports and the present study have been discussed above.

Group C fibroblasts (XP4RO) had a maximal RF value of 2.33 at 2.5 J/m^2 in this study. Others reported values were 1.5 at 5 J/m^2 (Lytle et al., 1976) and 4.8 at 2.0 J/m^2 (also strain XP4RO; Coppey and Menezes, 1981).

Fibroblasts of group D (XP6BE) expressed an RF value of 2.58 at 1.0 J/m^2 in this study. Similar values of 1.5 at 1.2 J/m^2 (Lytle et al., 1976) and 3.9 at 0.3 J/m^2 (Coppey and Menezes, 1981) have been reported from other laboratories.

Efficiencies of UVER of UV'd HSV at a virus survival of 10^{-2} in the present study were 0.16 (XP25RO), 0.21 (XP6BE) and 0.18 (XP4RO). At virus survivals of 10^{-3}, Coppey and Menezes (1981) obtained efficiencies of 0.28 (XP group A), 0.20 (XP group D) and 0.23 (XP4RO, group C).

XP variant fibroblasts (XP4BE) were found to express maximal UVER at a dose of 10 J/m^2. The RF value obtained at this dose was 2.45, when the virus survival was 10^{-2} in unirradiated cells. One other report of UVER of UV'd HSV (Coppey & Menezes, 1981) found that three XP variant strains (including XP4RO) expressed greatly increased UVER, with RF values of 17.5-26.0 at a UV dose of 15 J/m^2 and a virus survival level of 10^{-3}. Subsequent experiments using virus survival levels of 10^{-3} also demonstrated high RF values (19.72) for XP4BE, a variant strain (figure 22).

The efficiency of UVER in XP variant cells for virus survival levels of 10^{-2} was about 0.29 (table 5). At lower virus survival levels (10^{-3}) the efficiency was 0.40 for this study and 0.41 for Coppey and Menezes (1981).

The level of UVER in XP variant cells was found to increase as a function of the UV dose to the virus, both in the present study (figure 27) and in Coppey and Menezes (1981).

The UVER of UV'd adenovirus (Jeeves, 1981) in XP fibroblasts also depended upon the complementation group. Group A fibroblasts expressed maximal UVER at UV doses 0.5 J/m^2, group D at $\sim$1 J/m^2 and XP variant between 5 and 10 J/m^2.

For most of the normal and CS strains determinations of maximal RF were made at the highest UV dose at which plaque formation still occurs. Therefore, it cannot be certain if RF values were continually increasing or if they were peaking. Some strains (e.g. GM2838 and CS3BE) actually

peaked and RFs started decreasing (figure 21). The same is true for some XP strains (e.g. XP4RO and XP6BE). Different UVER assays (Coppey and Menezes, 1981; Jeeves, 1981) found that for most strains tested, RF values actually did reach a peak.

Despite minor variations in maximum RF values, determinations of the dose of UV to the cells at which that peak occurs are very similar. This similarity is found not only when using the same viral probe (this study; Lytle et al., 1976; Coppey and Menezes, 1981) but also when using a different virus (Jeeves, 1981). This argues strongly in favour of using the UVER assay as a measure of cellular response of different cell strains to irradiation.

The suggestion that ER represents the expression of a mammalian analogue of bacterial "SOS" repair (see discussion by Radman, 1980) is supported by many pieces of evidence. The coordinate expression of both ER and EM in human cells (Su et al., 1981) and the parallel expression of ER of UV'd HSV and induction of latent SV40 in a single cell line (Zamansky et al., 1980) are similar to phenomena seen in bacteria. The ability of cycloheximide to block EM (Das Gupta and Summers, 1978) and both ER and EM at the same time (Su et al., 1981) suggests that these functions require protein synthesis for their expression and that they are therefore inducible.

Recently, Miskin and Ben-Ishai (1981) have looked at

the induction of plasminogen activator (PA), a highly specific serine protease which has been closely associated with cellular transformation, neoplasia and tumor promotion (Reich, 1978). It was found (Miskin and Ben-Ishai, 1981) that normal human fibroblasts expressed very low levels of PA and that exposure to UV did not increase PA levels. However, XP cells exposed to UV showed greatly increased levels of PA. Excision deficient XP strains were maximally induced by doses of UV ranging from 1-4 J/m^2. Excision proficient XP variant strains were maximally induced for PA synthesis at doses of UV of 15 J/m^2 or greater. In addition, it was found that maximum induction of PA synthesis occurred at 48 hours after irradiation and that PA induction could be inhibited by cycloheximide.

The parallels between the expression of ER (this study) and the induction of PA (Miskin and Ben-Ishai, 1981) by UV-irradiation are very striking and suggests that the two phenomena may be different aspects of the same process, namely, mammalian SOS repair.

The ability of human fibroblasts to repair UV-light induced damage has been characterized in cells from normal and sunlight sensitive patients. The assays used have quantitated the ability of these cells to repair damaged viral and cellular DNA.

The DNA repair defect associated with XP has been well characterized. Some strains are excision deficient and unable to remove pyrimidine dimers (Cleaver & Trosko, 1970).

Others are excision proficient but hypersensitive to UV killing (Andrews et al., 1978b).

For the most part, the repair abilities of XP cells, both excision deficient and proficient, as determined in these studies has been in agreement with other published work. The ability of XP excision deficient cells to repair damaged viral DNA is greatly reduced (Lytle et al., 1972, 1983; Day, 1974; Abrahams and van der Eb, 1976; Rainbow, 1980) while XP variant cells are only slightly reduced in their ability to perform this function (Day, 1974; Selsky and Greer, 1978; Abrahams and van der Eb, 1976; Takebe et al., 1978; Rainbow and Howes, 1979). The sensitivity of XP cells to UV-irradiation has been found to be increased compared to normal. Measurements of capacity (Lytle et al., 1976; Coppey et al., 1979b) and UVER (Lytle et al., 1976; Coppey and Menezes, 1981) made in earlier studies as well as in this study correlated well with other measures of cellular DNA repair ability (Andrews et al., 1978b).

CS cells are thought to harbor some DNA repair defect. Most of the attempts to characterize the defect produce only negative answers (Schmickel et al., 1977; Wade and Chu, 1979; Lehmann et al., 1979).

The sensitivity of CS cells to UV-irradiation has been well characterized as being increased compared to normal cells. This has been accomplished using UVER (Coppey and Menezes, 1981) and post-UV colony-forming ability (Andrews

et al., 1978b; Wade and Chu, 1978, 1979; Tanaka et al., 1981). Results obtained in this study on UVER and capacity of CS cells substantiate the observation that they are hypersensitive to ultraviolet irradiation.

The ability of CS cells to repair viral DNA appears to depend upon the virus. Repair of adenovirus was markedly reduced in CS cells compared to normal cells according to most reports (Day et al., 1981; Rainbow and Howes, 1982) though some report no difference (Hoar and Davis, 1979). For the repair of HSV in CS cells a small decrease has been noted, but this difference was not significant in most studies (Ikenaga et al., 1979; Lytle et al., 1983). In this study, the repair of HSV by different CS strains was not significantly reduced compared to normal strains.

Taking into consideration the evidence that CS cells are deficient in some unknown aspect of cellular DNA repair and the observation that CS cells cannot repair adenovirus as well as they repair HSV, two possible explanations arise. One is that HSV infection may induce a host repair function not normally seen. The other is that HSV may code for its own repair function. Further evidence for an HSV induced or coded repair function comes from the observation that while the HCR of UV'd adenovirus is approximately 3-7% for XP group A cells (Day, 1974; Rainbow, 1980), the HCR of UV'd HSV is approximately 30% in XP group A cells (Lytle et al., 1972; Selsky and Greer, 1978; this work). This suggestion

has been made by other authors (Selsky and Greer, 1978; Rainbow, 1981).

The observation that CS cells are able to repair UV'd HSV as well as normal cells (this work; Lytle et al., 1983; Ikenaga et al., 1979) but that UV'd adenovirus repair is reduced in CS cells (Day et al., 1981; Rainbow and Howes, 1982), is probably not a result of the differences in the endpoints of the assays. Two different assays, plaque-formation and Vag formation, have been used for both viruses and yielded similar results. The same observation has been made for XP cells (Rainbow, 1981), that is, that assays for plaque-formation and Vag formation produced similar estimates of relative repair ability.

Interest in mammalian inducible repair responses stems from their possible connection with mutagenesis and carcinogenesis (see Radman, 1980; Echols, 1981 for discussion). UV-enhanced reactivation (UVER) is oneof a variety of putative inducible repair responses, analogous to some aspect of bacterial SOS repair. Other possible induced responses in mammalian cells are enhanced mutagenesis (Das Gupta and Summers, 1978, 1980; Lytle et al., 1980; Sarasin and Benoit, 1980; Day and Ziolkowski, 1981; Su et al., 1981) and proviral induction (Bockstahler and Hellman, 1979; Zamansky et al., 1980).

This study has demonstrated that CS cells are capable of UVER. This report and one other (Coppey and Menezes, 1981) therefore suggest that the defect present in CS cells

does not affect their ability to be induced for UVER. The observation that CS cells are induced for maximum UVER at a UV dose lower than normal cells (this study; Coppey and Menezes, 1981) might suggest that their greater relative cellular sensitivity to UV-light induced DNA damage leads to the induction of "SOS" responses at lower UV doses. The appearance of maximal UVER at low UV doses for XP cells has been interpreted in the same way (Lytle et al., 1976).

The possible relationship between defective DNA repair associated with human diseases and carcinogenesis is a very attractive hypothesis (Echols, 1981; Setlow, 1980; Friedberg et al., 1979). Patients with XP or CS are hypersensitive to sunlight (Robbins et al., 1974; Schmickel et al, 1977) and cells from these patients are hypersensitive to UV light in vitro (Andrews et al., 1978a,b). Cells from XP patients have been shown to be hypermutable by UV-light (Maher et al., 1976) but the mutability of CS cells by UV-light has not yet been definatively demonstrated (Wade and Chu, 1979; Arlett, 1980). Characterization of the mutability of CS cells is important since it may relate the DNA repair defect of CS cells with the observed cancer incidence of CS patients. CS patients are not at increased risk for UV-induced skin cancers (Guzzetta, 1972) whereas XP patients are (Robbins et al., 1974). Therefore, differences in repair ability and fidelity may only be one aspect in the etiology of tumors. Recent evidence suggests that immune surveillance

and the induction of immune dysfunction by UV-light may be important factors in the development of UV-induced skin cancer (Kripke, 1979; Bridges, 1981).

REFERENCES

Abrahams, P.J. and van der Eb, A.J. (1976) Host cell reactivation of ultraviolet-irradiated SV40 DNA in five complementation groups of xeroderma pigmentosum. Mutat. Res. 35: 13-22.

Albrecht, T., St. Jeor, S.C., Funk, F.D. and Rapp, F. (1974) Multiplicity reactivation of human cytomegalovirus inactivated by ultraviolet light. Int. J. Radiat. Biol. 26: 445-454.

Andrews, A.D., Barrett, S.F., Yoder, F.W., Robbins, J.H. (1978a) Cockayne's Syndrome fibroblasts have increased sensitivity to ultraviolet light but normal rates of unscheduled DNA synthesis. J. Invest. Dermat. 70: 237-239.

Andrews, A.D., Barrett, S.F., Yoder, F.W. and Robbins, J.H. (1978b) XP neurological abnormalities correlate with colony forming ability after UV radiation. PNAS. 75: 1984-1988.

Arlett, C.F. (1979) In "Radiation Research", Proceedings of the sixth international congress of Radiation Research May 13-19, 1979. Tokyo, eds. S. Okada, M. Imamura, T. Terashima and H. Yamaguchi. pp. 595-602.

Arlett, C.F. (1980) Mutagenesis in repair deficient human cell strains. In: Progress in Environmental Mutagenesis. ed. M. Alacevic. Elesevier/North Holland. pp. 161-174.

Aaronson, S.A., Lytle, C.D. (1970) Decreased host cell reactivation of irradiated SV40 virus in xeroderma pigmentosum. Nature (London). 228: 359-361.

Ben-Hur, E. and Ben-Ishai, R. (1968) Trans-syn thymine dimers in ultraviolet-irradiated denatured DNA: Identification and photoreactivability. Biochem Biophys Acta. 166: 9-15.

Bockstahler, L.E., and Hellman, K.B. (1979) Induction of oncogenic viruses by light. Photochem Photobiol. 30: 743-748.

Bockstahler, L.E., and Lytle, C.D. (1970) Ultraviolet light-enhanced reactivation of a mammalian virus. Biochem Biophys Res. Comm. 41: 184-189.

Bockstahler, L.E., Lytle, C.D. (1977) Radiation enhanced reactivation of nuclear replicating mammalian viruses. Photochem. Photobiol. 25: 477-482.

Bockstahler, L.E., Lytle, C.D., Stafford, J.E., Haynes, K.F. (1976) Ultraviolet enhanced reactivation of a human virus: effect of delayed infection. Mutation Research. 35: 189-198.

Bridges, B. (1981) How important are somatic mutations and immune control in skin cancer? Reflections on xeroderma pigmentosum. Carcinogenesis. 2(5): 471-472.

Bridges, B.A., Mottershead, R.P. and Sedgewick, S.G. (1976) Mutagenic DNA repair in Escherichia coli. III. Requirement for a function of DNA polymerase III in ultraviolet-light mutagenesis. Mol gen. Genet. 144: 53-58.

Brown, W.T., Ford, J.P., and Gershey, E.L. (1980) Variation of DNA repair capacity in progeria cells unrelated to growth conditions. Biochem. Biophys. Res. Commun. 97: 347-353.

Byrnes, J.J., Downey, K.M., Black, V.L., So, A.G. (1976) A new mammalian DNA polymerase with 3' to 5' exonuclease activity: DNA polymerase 8. Biochem. 15: 2817-2823.

Caillet-Fauquet, P., Defais, M. and Radman, M. (1977) Molecular mechanism of induced mutagenesis: Replication in vivo of bacteriophage 174 single stranded, ultraviolet light-irradiated DNA in intact and irradiated host cells. J. Mol. Biol. 117: 95-112.

Castellot, J.J., Miller, M.R., Lehtomaki, D.M., Pardee, A.B. (1979) Comparison of DNA replication and repair enzymology using permiabilized baby hamster kidney cells. J. Biol. Chem. 254: 6904-6908.

Ciarrochi, G., Jose, J.G., Linn, S. (1979) Further characterization of a cell-free system for measuring replicative and repair DNA synthesis with cultured human fibroblasts and evidence for the involvement of DNA polymerase in DNA repair. Nucl. Acids Res. 7: 1205-1220.

Clark, A.J., and Volker, M.R. (1978) A new classification of pathways repairing pyrimidine dimer damage in DNA.

Clark, A.J., and Volker, M.R. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 57-72.

Cleaver, J.E. (1968) Defective repair replication of DNA in xeroderma pigmentosum. Nature. 218: 652-656.

Cleaver, J.E. (1971) In: Nucleic Acid-Protein Interactions-Nucleic Acid Synthesis in Viral Infection, eds. D.W. Ribbons, J.F. Woessner and J. Schultz, North-Holland Publ., Amsterdam. p. 87.

Cleaver, J.E. and Trosko, J.E. (1970) Absense of excision of ultraviolet induced cyclobutane dimers in xeroderma pigmentosum. Photochem. Photobiol. 11: 547-550.

Coohill, T.P. (1981) Mammalian cell viral capacity: an alternative assay for ultraviolet radiation damage. Photochem. Photobiol. 34: 525-527.

Coohill, T.P., Knauer, D.J., Fry, D.G. (1979) The effect of changes in cell geometry on the sensitivity to ultraviolet radiation of mammalian cellular capacity. Photochem. Photobiol. 30: 565-572.

Coohill, T.P., Moore, S.P., Drake, S. (1977) The wavelength dependence of ultraviolet inactivation of host capacity in a mammalian cell-virus system. Photochem. Photobiol. 26: 387-391.

Cooper, P.K. and Hanawalt, P.C. (1972a) Role of DNA polymerase I and the rec system in excision repair in E coli. PNAS. 69: 1156-1160.

Cooper, P.K. and Hanawalt, P.D. (1972b) Heterogeneity of patch size in repair replicated DNA in Escherichia coli. J. Mol. Biol. 67: 1-10.

Cooper, P.K. and Hunt, J.G. (1978) Alternative pathways for excision and resynthesis in Esherichia coli: DNA polymerase III role? In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 255-260.

Coppey, J., Averbeck, D., Moreno, G. (1979a) Herpes virus production in monkey kidney and human skin cells treated with angelicin or 8-methoxypsoralen plus 365 nm light. Photochem. Photobiol. 29: 797-801.

Coppey, J., Menezes, S. (1981) Enhanced reaction of ultraviolet - damaged herpes virus in ultraviolet pretreated skin fibroblasts of cancer prone donors. Carcinogenesis. 2: 787-793.

Coppey, J., Nocentini, S. (1973) Multiplicity effect on the survival of ultra-violet-irradiated herpes simplex virus. Int. J. Radiat. Biol. 24(6): 645-647.

Coppey, J., Nocentini, S. (1976) Herpes virus and viral DNA synthesis in ultraviolet light-irradiated cells. J. gen. Virol. 32: 1-15.

Coppey, J., Nocentini, S. (1979) Survival and herpes virus production of normal and xeroderma pigmentosum fibroblasts after treatment with formaldehyde. Mutation Research. 62: 355-361.

Coppey, J., Nocentini, S., Menezes, S., Moreno, G. (1979b) Herpes virus production as a marker of repair in ultraviolet irradiated human skin cells of different origin. Int. J. Radiat. Biol. 36(1): 1-10.

Coppey, J., Nocentini, S., Moreno, G. (1978) Herpes Virus Production by Ultraviolet-irradiated Human Skin Cells: A Marker of Repair. IARC Sci. Publ. 24(1): 407-14.

Craig, N.L. and Roberts, J.W. (1981) Function of nucleoside triphosphate and polynucleotide in Escherichia coli rec A protein-directed cleavage of phage repressor. J. Biol. Chem. 256: 8039-8044.

Crowl, R.M., Boyce, R.P. and Echols, H. (1981) Repressor cleavage as a prophage induction mechanism: Hypersensitivity of a mutant λcI protein to Rec A-mediated proteolysis. J. Mol. Biol. 152: 815-819.

D'Ambrosio, S.M. and Setlow, R.B. (1978) On the presence of UV-endonuclease sensitive sites in daughter DNA of UV-irradiated mammalian cells. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 499-504.

D'Ambrosio, S.M., Whetstone, J.W., Szinski, L., Lowney, E. (1981) Photorepair of pyrimadine dimers in human skin in vivo. Photochem. Photobiol. 34: 461-464.

Das Gupta, U.B., Summers, W.C. (1978) Ultraviolet reactivation of herpes simplex virus is mutagenic and inducible in mammalian cells. Proc. Natl. Acad. Sci. U.S.A. 75(5): 2378-2381.

Day, R.S. III. (1974) Studies on repair of adenovirus 2 by human fibroblasts using normal, xeroderma pigmentosum, and xeroderma pigmentosum heterogygous strains. Cancer Res. 34: 1965-1970.

Day, R.S. III. (1975a) Caffeine inhibition of the repair of ultraviolet-irradiated adenovirus in human cells. Mutation Res. 33: 321-326.

Day, R.S. III. (1975b) Xeroderma pigmentosum variants have decreased repair of ultraviolet-damaged DNA. Nature (Lond.) 253: 748-749.

Day, R.S., Ziolkowski, C. (1978) Studies on UV-induced viral reversion, Cockayne's syndrome and MNNG damage using adenovirus 5. DNA Repair Mechanisms pp. 535-539.

Day, R.S., Ziolkowski, C.H.J. (1981) UV-induced reversion of adenovirus 5 ts 2 infecting human cells. Photochem. Photobiol. 34: 403-406.

Day, R.S., Ziolkowski, C.H.J., DeMattina, M. (1981) Decreased host cell reactivation of UV-irradiated adenovirus 5 by fibroblasts from Cockayne's Syndrome patients. Photochem. Photobiol. 34: 603-607.

DeBusk, F.L. (1972) The Hutchinson-Gilford progeria syndrome. J. Pediat 80: 697-724.

Defais, M., Caillet-Fauquet, P., Fox, S., Radman, M. (1976) Induction kinetics of mutagenic DNA repair activity in E. coli following ultraviolet irradiation. Mol. gen. Genet. 148: 125-130.

Defais, J.J., Hanawalt, P.C., Sarasin, A.R. (1981) Viral probes for DNA repair. Adv. in Radiat. Biol. 10:

Deutsch, W.A. and Linn, S. (1979) DNA binding activity from cultured human fibroblasts that is specific for partially depurinated DNA and that inserts purines into apurinic sites. PNAS 76: 141-144.

Devoret, M., Blanco, M., George, J., Radman, M. (1975) Recovery of phage lambda from ultraviolet damage. Basic Life Sci. 5A: 155-171.

Devoret, M., Blanco, M., George, J., Radman, M. Recovery of phage lambda from ultraviolet damage. In: Molecular Mechanisms for Repair of DNA, Part A, eds. P.C. Hanawalt and R.B. Setlow, Plenum Press, New York. pp. 155-171.

Echols, H. (1981) SOS functions, Cancer and Inducible evolution. Cell. 25: 1-2.

Eglin, R.P., Gugerli, P., Wildy, P. (1980) Ultraviolet irradiation of herpes simplex virus (type 1): Delayed transcription and comparative sensitivities of virus functions. J. gen. Virology 49: 23-31.

Fogel, M. and Sachs, L. (1970) Induction and virus synthesis in polyoma transformed cells by UV light and mitomycin C. Virology. 40: 174-177.

Friedberg, E.C., Ehmann, U.K., Williams, J.I. (1979) Human diseases associated with defective DNA repair. Advances in Radiat. Biol. 8: 86-173.

German, J. (1972) Genes which increase chromosomal instability in somatic cells and predispose to cancer. Prog. Med. Genet. 8:61-101.

German, J., Bloom, D., Passarge, E. (1977) Bloom's syndrome. V. Surveillance for cancer in affected families. Clin. Genet. 12: 162-168.

Goscin, L.P. and Byrnes, J.J. (1982) DNA polymerase : One polypeptide, two activities. Biochem. 21: 2513-2518.

Grossman, L. (1981) Enzymes involved in the repair of damaged DNA. Archives of Biochem. Biophys. 211(2): 511-522.

Grossman, L., and Riazuddin, S. (1978) Enzymatic pathways of damaged nucleotide excision. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 205-217.

Guzzetta, F. (1972) Cockayne - Neill - Dingwall syndrome. In: "Handbook of Clinical Neurology" vol. 13. (P. Vinken and G.W. Bruyn eds.).

Hall, J.D., Featherston, J.D., Almy, R.E. (1980) Evidence for Repair of Ultraviolet Light Damaged Herpes Virus in Human Fibroblasts by a Recombination Mechanism. Virology. 105: 490-500.

Hall, J.D., Mount, D.W. (1981) Mechanisms of DNA replication and mutagenesis in ultraviolet-irradiated bacteria and mammalian cells. Prog. Nucl. Acid Res. Mol. Biol. 25: 54-126.

Hanawalt, P.C., Cooper, P.K., Ganesan, A.K., Smith, C.A. (1979) DNA repair in bacteria and mammalian cells. Ann. Rev. Biochem. 48: 783-836.

Hellman, K.B., Kaynes, K.F. and Bockstahler, L.E. (1974) Radiation-enhanced survival of a human virus in normal and malignant rat cells. Proc. Soc. Exp. Biol. Med. 145: 255-262.

Hellman, K.B., Lytle, C.D., Bockstahler, L.E. (1976) Radiation enhanced reactivation of herpes simplex virus: effect of caffeine. Mutation Research. 36: 249-256.

Hellman, K.B., Schuller, G.B. and Strickland, A.G. (1981) Host-cell and radiation enhanced-reactivation of herpes simplex virus in ataxia telangiectasia fibroblasts. Am. Soc. Photobiol. 9:70.

Henderson, E.E., and Long, W.K. (1981) Host cell reactivation of UV-and X-ray - damaged herpes simplex virus by Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines. Virol. 115: 237-248.

Hoar, D.I. and Davis, F. (1979) Host-cell reactivation of UV-irradiated adenovirus in Cockayne syndrome. Mutat. Res. 62: 401-405.

Hoar, D.I., Waghorne, C. (1978) DNA repair in Cockayne's Syndrome. Am. J. Hum. Genet. 30: 590-601.

Hutchinson, F. (1978) DNA strand break repair in eukaryoks: Workshop summary. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 457-463.

Hyman, R.W., Oakes, J.E., Kudler, L. (1977) In vitro Repair of the Pre-existing Nicks and Gaps in Herpes Simplex Virus DNA. Virology. 76: 286-294.

Ikenaga, M., Inoue, M., Kozuka, T., Sugita, T. (1981) The recovery of colony-forming ability and the rate of semi-conservative DNA synthesis in ultraviolet-irradiated Cockayne and normal cells. Mutation Research. 91: 87-91.

Ikenaga, M., Sugita, T., Koyuka, T., Suchara, N. and Inoue, M. (1979) Sixth International Congress of Radiation Research, Tokyo, Japan, Toppan Printing. p. 265.

Jeeves, W.P. (1981) Ph. D Thesis

Jeeves, W.P., Rainbow, A.J. (1979a) γ -ray enhanced reactivation of γ -irradiated adenovirus in human cells. Biochem. Biophys. Res. Comm. 90(2): 567-574.

Jeeves, W.P., Rainbow, A.J. (1979b) γ -ray enhanced reactivation of UV-irradiated adenovirus in normal human fibroblasts. Mutation Research. 60: 33-41.

Jeeves, W.P. and Rainbow, A.J. (1983) Int. J. Radiat. Biol. in press.

Kraeiner, K.H. (1977) Progressive degenerative diseases associated with defective DNA repair. Xeroderma pigmentosum and Ataxia telangiectasia. In: Cellular Senescence and Somatic Cell Genetics: DNA Repair Processes eds. W.W. Nichols and D.G. Murphy. Symposium Specialists, Inc, Miami, USA. pp. 37-71.

Krepinsby, A.B., Rainbow, A.J., Heddle, J.A. (1980) Studies on the ultraviolet light sensitivity of Bloom's Syndrome fibroblasts. Mutation Research. 69: 357-368.

Krypke, M.L. (1979) Immunologic Mechanisms in UV radiation carcinogenesis. Adv. Cancer Res. 34: 69-106.

Lee, H.H., Puck, T.T. (1960) The action of ultraviolet radiation on mammalian cells as studied by single-cell techniques. Radiation Research 12: 340.

Lehmann, A.R. (1972) Post-replication repair of DNA in ultraviolet-irradiated mammalian cells. J. Mol. Biol. 66: 319-337.

Lehmann, A.R. (1978) Replicative bypass mechanisms in mammalian cells: Workshop summary. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 485-488.

Lehmann, A. (1979) Genetic clues to cancer. New Scientist. 29: 686-688.

Lehmann, A.R., Karran, P. (1981) DNA Repair. Int. Rev. Cytol. 72: 101-146.

Lehmann, A.R., Kirk Bell, S., Arlett, C.F., Harcourt, S.A., deWeerd-Kastelein, E.A., Keijyer, W. and Hall-Smith, P. (1977) Repair of ultraviolet light damage in a variety of human fibroblast cell strains. Cancer Res. 37: 904-910.

Ley, R.D. (1973) Postreplication repair in an excision defective mutant of Escherichia coli: ultraviolet light-induced incorporation of bromodeoxyuridine into parental DNA. Photochem. Photobiol. 18: 87-95.

Lindahl, T. (1979) DNA glycosylas, endonucleases for apurinic/apyrimidinic sites and base excision repair. Prog. Nucleii Acids Res. Mol. Biol. 22: 135-192.

Linn, S., Kuhnlein, U. and Deutsch, W.A. (1978) Enzymes from human fibroblasts for the repair of AP DNA. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 199-204.

Little, J.W., Edmiston, S.H., Pacelli, L.Z., Mount, D.W. (1980) Cleave of the Escherichia coli. lex A protein by the rec A protease. PNAS. 77: 3225-3229.

Little, J.W. and Mount, D.W. (1982) The SOS regulatory system of Escherichia coli. Cell. 29: 11-22.

Lytle, C.D. (1971) Host cell reactivation in mammalian cells. I. Survival of ultraviolet-irradiated herpes virus in different cell lines. Int. J. Radiat. Biol. 19: 329-337.

Lytle, C.D. (1978) Radiation-enhanced virus reactivation in mammalian cells. Natl. Canc. Inst. Mono. 50: 145-149.

Lytle, C.D., Aaronson, S.A., Harvey E. (1972) Host-Cell reactivation in mammalian cells II. Survival of herpes simplex virus and vaccinia virus in normal human and xeroderma pigmentosum cells. Int. J. Radiat. Biol. 22(2): 159-165.

Lytle, C.D., Benane, S.G. (1974) Host-Cell reactivation in mammalian cells IV. Cell culture conditions affecting virus survival. Int. J. Radiat. Biol. 26(2): 133-141.

Lytle, C.D., Benane, S.G. (1975) Effect of photoreactivating light on virus infection of UV-exposed Potoroo cells. Int. J. Radiat. Biol. 27: 487-491.

Lytle, C.D., Benane, S.G. and Bockstahler, L.E. (1974) Ultraviolet-enhanced reactivation of herpes simplex virus in human tumour cells. Photochem. Photobiol. 20: 91-94.

Lytle, C.D., Benane, S.G., Stafford, J.E. (1976b) Host-cell reactivation in mammalian cells V. Photoreactivation studies with herpes virus in marsupial and human cells. Photochem. Photobiol. 23: 331-336.

Lytle, C.D., Coppey, J., Taylor, W.D. (1978) Enhanced Survival of ultraviolet-irradiated herpes simplex virus in carcinogen pre-treated cells. Nature. 272: 60-62.

Lytle, C.D., Day, R.S., Hellman, K.B., Bockstahler, L.E. (1976) Infection of UV-irradiated xeroderma pigmentosum fibroblasts by herpes simplex virus: Study of capacity and Weigle reactivation. Mutation Research. 36: 257-264.

Lytle, C.D., Goddard, J.G. (1979) UV-enhanced virus reactivation in mammalian cells: effects of metabolic inhibitors. Photochem. Photobiol. 29: 959-962..

Lytle, C.D., Goddard, J.G., Lin, C.H. (1980) Repair and mutagenesis of herpes simplex virus in UV-irradiated monkey cells. Mutation Research 70: 139-149.

Lytle, C.D. and Knott, D.C. (1982) Enhanced mutagenesis parallels enhanced reactivation of herpes virus in a human cell line. EMBO J. 1: 701-703.

Lytle, C.D., Nikaido, O., Hitchins, V.M., Jacobson, E.D. (1982) Host cell reactivation by excision repair is error-free in human cells. Mutation Res. 94: 405-412.

Lytle, C.D., Tarone, R.E., Barrett, S.F., Wirtshafter, J.D., Dupuy, J-M, and Robbins, J.H. (1983) Host cell reactivation by fibroblasts from patients with primary degeneration of the retina. Photochem. Photobiol. in press.

Lytle, C.D., Schmidt, B.J. (1981) Host cell reactivation and UV-enhanced reactivation in synchronized mammalian cells. Photochem. Photobiol. 34: 355-359.

Maher, V.M., Curren, R.D., Ouellette, L.M. et al. (1976) Effect of DNA repair on the frequency of mutations induced in human cells by ultraviolet irradiation and chemical carcinogens. Fundamentals of Cancer Prevention eds. P.N. Magee, S. Takayama, T. Sugimura, T. Matsushima. Baltimore, University Park Press. (QZ200 F982) pp. 363-382.

Maher, V.M., Ouellette, L.M., Curren, R.D., McCormick, J.J. (1976) Frequency of ultraviolet light-induced mutations is higher in xeroderma pigmentosum variant cells than in normal human cells. Nature. 261: 593-594.

McFarlin, D.E., Strober, W., Waldmann, T.A. (1972) Ataxia telangiectasia. Medicine. 51: 281-314.

Meneghini, R. and Menck, C.F.M. (1978) Pyrimidine dimers in DNA strands of mammalian cells synthesized after UV-irradiation. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 493-498.

Miskin, R., Ben-Ishai, R. (1981) Induction of plasminogen activator by UV light in normal and xeroderma pigmentosum fibroblasts. Proc. Natl. Acad. Sci. USA. 78(10): 6236-6240.

Miskin, R., Reich, E. (1980) Plasminogen Activator: Induction of synthesis of DNA damage. Cell 19: 217-224.

Mount, D.W., Walker, A.C. and Kosel, C. (1975) Effect of tsl mutations in decreasing radiation sensitivity of a recA$^-$ strain of Escherichia coli. K-12. J. Bact. 121: 1203-1207.

Nilsson, L.R. (1960) Chronic pancytopenia with multiple congenital abnormalities (Fanconi's anaemia). Acta Paediat. 49: 518-529.

Painter, R.B. (1981) Radioresistant DNA synthesis: an intrinsic feature of ataxia telangiectasia. Mutation Research. 84- 183-190.

Painter, R.B., Young, B.R. (1980) The rate of DNA synthesis in normal human and ataxia cells after exposure to X-irradiation. Radiosensitivity in ataxia-telangiectasia: a new explanation. De Wit, J., Jaspers, N.G., Bootsman, D. Mutation Res. 80: 221-226.

Paterson, M.C. (1977) Use of purified lesion recognizing enzymes to monitor DNA repair in vivo. Adv. Radiat. Biol. 7: 1-53.

Paterson, M.C. (1978) Ataxia telangiectasia: A model inherited disease linking deficient DNA repair. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 637-650.

Paterson, M.C., Lohman, P.H., Sluyter, M.L. (1973) Use of UV endonuclease from Micrococcus luteus to monitor the progress of DNA repair in UV-irradiated human cells. Mut. Res. 19: 245-256.

Paterson, M.C., Smith, P.J. (1979) Ataxia telangiectasia: an inherited human disorder involving hypersensitivity to ionizing radiation and related DNA-damaging chemicals. Ann. Rev. Genet. 13: 291-318.

Paterson, M.C., Smith, B.P., Knight, P.A. and Anderson, A.K. (1977) In: Research in Photobiology, ed. A. Castellani, Plenum, New York. pp. 207.

Pedrali-Noy, G. and Spadari, S. (1980) Aphidicolin allows a rapid and simple evaluation of DNA repair synthesis in damaged human cells. Mutation Res. 70: 389-394.

Radman, M. (1980) Is there SOS induction in mammalian cells? Photochem. Photobiol. 32: 823-830.

Rainbow, A.J. (1977) UV-induced alkaline labile DNA damage in human adenovirus and its repair after infection of human cells. Photochem. Photobiol. 25: 457-463.

Rainbow, A.J. (1974) Repair of radiation induced DNA breaks in human adenovirus. Radiat. Res. 60: 155-164.

Rainbow, A.J. (1978) Production of viral structural antigens by irradiated adenovirus as an assay for DNA repair in human fibroblasts. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 541-545.

Rainbow, A.J. (1980) Reduced capacity to repair irradiated adenovirus in fibroblasts from xeroderma pigmentosum heterogygotes. Cancer Res. 40: 3945-3949.

Rainbow, A.J. (1981) Reactivation of Viruses. from Short Term Tests for Chemical Carcinogens. Stich, H.F., RHC eds. pp. 20-35.

Rainbow, A.J., Howes, M. (1977) Decreased repair of gamma ray damaged DNA in progeria. Biochem. Biophys. Res. Comm. 74(2): 714-719.

Rainbow, A.J. and Howes, M. (1977a) Defective repair of ultraviolet and gamma-ray damaged DNA in Fanconi's anaemia. Int. J. Radiat. Biol. 31: 191-195.

Rainbow, A.J. and Howes, M. (1977b) Reduced host-cell reactivation of UV-irradiated adenovirus in Fanconi's anaemia fibroblasts. Radiat. Res. 70: 686.

Rainbow, A.J., Howes, M. (1979) Decreased repair of gamma-irradiated adenovirus in Xeroderma Pigmentosum fibroblasts. Int. J. Radiat. Biol. 36: 621-629.

Rainbow, A.J., Howes, M. (1982) A deficiency in the repair of UV and gamma-ray damaged DNA in fibroblasts from Cockayne's Syndrome. Mutation Res. 93: 235-247.

Rainbow, A.J. and Mak, S. (1972) DNA strand breakage and biological function of human adenovirus after gamma irradiation. Radiat. Res. 50: 319-333.

Rasmussen, R.E. and Painter, R.B. (1964) Evidence for repair of ultraviolet damaged deoxyribonuleic acid in cultured mammalian cells. Nature (London). 203: 1360-1362.

Regan, J.B., Setlow, R.B. (1974) Two forms of repair in the DNA of human cells damaged by chemical carcinogens and mutagens. Cancer Res. 34: 3318-3325.

Regan, J.D. and Setlow, R.B. (1976) "Repair of human DNA: radiation and chemical damage in normal and xeroderma pigmentosum cells". In: Biology of Radiation Carcinogenesis, eds. J.M. Yukas, R.W. Tennant and J.D. Regan. Raven, New York.pp. 103-113.

Reich, E. (1978) In: "Biological Markers of Neoplasia: Basic and Applied Aspects:, ed. Ruddon R.W. (Elsevier/North-Holland, Amsterdam), pp. 491-500.

Robbins, J.H., Kraemer, K.H., Lutzner, M., Festoff, B.W., Coon, H.G. (1974) Xeroderma pigmentosum: An inherited disease with sensitivity, multiple cutaneous neoplasms and abnormals DNA. Ann. Int. Med. 80: 221-248.

Roberts, J.J. (1978) The repair of DNA modified by cytotoxic, mutagenic and carcinogenic chemicals. Adv. Rad. Biol. 7:211-436.

Roberts, J.W., Roberts, C.W. and Craig, N.L. Escherichia coli. recA gene product inactivates phage λ repressor. PNAS 75: 4714-4718.

Rommelaere, J., Vos, J-M., Cornelis, J.J., Ward, D.C. (1981) UV-enhanced reactivation of minute-virus-of-mice: stimulation of a late step in the viral life cycle. Photochem. Photobiol. 33: 845-854.

Ross, L.J.N., Wildy, P., Cameron, K.R. (1971) Formation of small plaques by herpes viruses irradiated with ultraviolet light. Virology. 45: 808-812.

Rothschild, H. and Black, P.H. (1970) Analysis of SV40-induced transformation of hamster kidney tissue in vitro VII. Induction of SV40 Virus from transformed hamster cell clones by various agents. Virology. 42: 251-256.

Rude, J.M. and Friedberg, E.C. (1977) Semi-conservative deoxyribonucleic acid synthesis in unirradiated and ultraviolet-irradiated xeroderma pigmentosum and normal human skin fibroblasts. Mutat. Res. 42: 433-442.

Rupp, W.D., Wilde, C.E. III, Reno, D.L., Howard-Flanders, P. (1971) Exchanges between DNA strands in ultraviolet-irradiated Escherichia coli. J. Mol. Biol. 61: 25-44.

Rupp, W.D. and Howard-Flanders, P. (1968) Discontinuities in the DNA synthesized in an excision-defective strain of Escherichia coli. following ultraviolet irradiation. J. Mol. Biol. 31: 291-304.

Sarasin, A. (1978) Induced DNA repair processes in eukaryotic cells. Biochimie. 60: 1141-1144.

Sarasin, A., Benoit, A. (1980) Induction of an error-prone mode of DNA repair in UV-irradiated monkey kidney cells. Mutation Research. 70: 71-81.

Sarasin, A.R. and Hanawalt, P.C. (1978) Carcinogens enhance survival of UV-irradiated simian virus 40 in treated monkey kidney cells: Induction of a recovery pathway. PNAS. 75: 346-350.

Sasaki, M.S., Toda, K. and Ozawa, A. (1977) Role of DNA repair in the susceptibility to chromosome breakage and cell killing in cultured human fibroblasts. In: Biochemistry of Cutaneous Epidermal Differentiation, eds. M. Seiji and I.A. Bernstein, University Press, Tokyo. pp. 167-180.

Sasaki, M.S. (1978) Fanconi's anemia a condition possibly associated with a defective DNA repair. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 675-684.

Schmickel, L.D., Chu, E.H.Y., Trosko, J.E. and Chang, C.C. (1977) Cockayne syndrome: A cellular sensitivity to ultraviolet light. Pediatrics. 60: 135-139.

Seeberg, E. (1978) Reconstitution of an Escherichia coli. repair endonuclease activity from the separated uvrA$^+$ and uvr B$^+$/uvrC$^+$ gene products. PNAS. 75: 2569-2573.

Selsky, C.A., Greer, S. (1978) Host-cell reactivation of UV-irradiated and chemically-treated herpes simplex virus-1 by xeroderma pigmentosum, XP heterozygotes and normal skin fibroblasts. Mutation Research. 50: 395-405.

Setlow, R.B. (1980) Damages to DNA that result in neoplastic transformation. Adv. in Biol. Med. Phys. 17: 99-108.

Smith, C.A. (1978) Removal of T4 endonuclease V sensitive sites and repair replication in confluent human diploid fibroblasts. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 311-314.

Smith, K.C. (1977) Ultraviolet radiation effects on molecules and cells. In: The Science of Photobiology ed. K.C. Smith. pp. 113-142.

Smith, K.C. and Meun, D.H.C. (1970) Repair of radiation-induced damage in Escherichia coli. I. Effect of rec mutations on postreplication repair of damage due to ultraviolet radiation. J. Mol. Biol. 51: 459-472.

Spadari, S., Sala, F., Pedrali-Noy, G. (1982) Aphidicolin: a specific inhibitor of nuclear DNA replication in eukaryotes. TIBS : 29-32.

Spear, P.G. and Roizman, B. Cold Spring Harbor Monographs 10B. Molecular Biology of Tumor Viruses. ed. J. Tooze. 2nd Ed. Part 2. Cold Spring Harbor Laboratory, New York. pp. 615-746.

Spector, B.D. (1977) In: Progress in Cancer Research Therapy, eds. J.J. Mulvihill, R.W. Miller and J.F. Fraumeni, Raven, New York, vol 3, p. 339.

Straus, B., Bose, K., Altamirano, M., Sklar, R., Tatsumi, K. (1978) Response of mammalian cells to chemical damage. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 621-624.

Su, Z.Z., Cornelis, J.J. and Rommelaere, J. (1981) Mutagenesis of intact parvovirus H-1 is expressed coordinately with enhanced reactivation of ultraviolet irradiated virus in human and rat cells treated with 2-nitronapthofurans. Carcinogenesis. 2(10): 1039-1043.

Sutherland, B.M. (1978) Enzymatic photoreactivation of DNA. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 113-122.

Sutherland, B.M. (1978b) Photoreaction: evaluation of pyrimidine dimers in ultraviolet-induced cell transformation. Natl. Cancer Inst. Monogr. 50: 129-132.

Takebe, H., Fujiwara, Y., Sasaki, M.S., Sato, Y., Kozuka, T., Nikaido, O., Ishizak, K., Arase, S. and Ikenage, M. (1978) DNA Repair and clinical characteristics of 96 Xeroderma Pigmentosum patients in Japan. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 617-620.

Takebe, H., Nii, S., Ishui, M.I., Utsumi, H. (1974) Comparative studies of host-cell reactivation, colony-forming ability and excision repair after UV-irradiation of xeroderma pigmentosum, normal human and some other mammalian cells. Mutation Research. 25: 383-390.

Tanaka, K., Kawai, K., Kumahara, Y., Ikenaga, M., Okada, Y. (1981) Genetic complementation groups in Cockayne's Syndrome. Somatic Cell Genetics. 7(4): 445-455.

Villani, G., Boiteux S., and Radman, M. (1978) Mechanism of UV-induced mutagenesis: Extent and fidelity of in vitro DNA synthesis on irradiated templates. PNAS. 75: 3037-3041.

Wade, M.H. and Chu, E.H.Y. (1978) Effects of DNA damaging agents on cultured fibroblasts derived from patients with Cockayne syndrome. In: DNA Repair Mechanisms, eds. P.C. Hanawalt, E.C. Friedberg and C.F. Fox. Academic Press, New York. pp. 667-670.

Wade, M.H., Chu, E.Y.H. (1979) Effects of DNA damaging agents on cultured fibroblasts derived from patients with Cockayne's Syndrome. Mutation Research. 59: 49-60.

Wagner, E.K., Rice, M., Sutherland, B.M. (1975) Photoreactivation of herpes simplex virus in human fibroblasts. Nature. 254: 627-628.

Ward, J.F. (1975) Molecular Mechanisms of radiation-induced damage to nucleic acids. Adv. Rad. Biol. 5: 181-239.

Weigle, J.J. (1953) Induction of mutations in a bacterial virus. PNAS. 39: 628-636.

Weissbach, A. (1977) Enkaryotic DNA polymerases. Ann. Rev. Biochem. 46: 25-47.

Williams, J.R. (1976) Role of DNA repair in cell inactivation, aging, and transformation: a selective review, a speculative model. Adv. Radiat. Biol. 6: 161-210.

Witkin, E.M. (1976) Ultraviolet mutagenesis and inducible DNA repair in Escherichia coli. Bacteriol. Rev. 40: 869-907.

Youngs, D.A. and Smith, K.C. (1977) The involvement of polynucleotide ligase in the repair of UV-induced DNA damage in E coli. K-12 cells. Mol. gen. Genet. 152: 37-41.

Zamansky, G.B., Kleinman, L.F., Black, P.H., Kaplan, J.C. (1980) Reactivation of herpes simplex virus in a cell line inducible for simian virus 40 synthesis. Mutation Research 70: 1-9.

Zamansky, G.B. and Little, J.B. (1982) Survival of ^{60}Co-irradiated herpes simplex virus in 15 human diploid fibroblast cell strains. Mutation Research 94: 245-255.