REACTIVATION OF UV-IRRADIATED AD 2 AND HSV-I IN MAMMALIAN CELLS

REACTIVATION OF UV-IRRADIATED ADENOVIRUS TYPE 2 AND HERPES SIMPLEX VIRUS TYPE I IN MAMMALIAN CELLS

by

ANN BERNICE BUESCHLEB B.SC.

A Project

Submitted to the School of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree
Master of Science
McMaster University
April, 1987

MASTER OF SCIENCE (1987) McMASTER UNIVERSITY

(HEALTH AND RADIATION PHYSICS) HAMILTON, ONTARIO

TITLE: Reactivation of UV-irradiated adenovirus type 2 and herpes simplex virus type I in mammalian cells.

AUTHOR: Ann Bernice Bueschleb, B.Sc. (University of Western Ontario)

SUPERVISOR: Professor Andrew J. Rainbow

NUMBER OF PAGES : x,96

ABSTRACT

Much research is being conducted into the causes of human cancer. A number of human autosomal recessive diseases such as Xeroderma Pigmentosum are characterized at least in part by a defect or aberration in one or more forms of DNA repair and at the same time an elevated incidence of cancer. Also, carcinogens cause mutations in DNA and the greater the carcinogenicity, the greater the mutagenicity. As a result, much attention has been focused on DNA repair and its relationship to cancer incidence.

The HCR of V antigen formation by UV-irradiated Adenovirus type 2 (Ad 2) was examined using apparently normal human fibroblasts, tumor cells (HeLa CCL2), and cells transformed by Ad 5 DNA (293, 293 N3S). A decrease in the HCR of V antigen formation was found for HeLa CCL2 cells as compared to apparently normal human fibroblasts, but not for the transformed cells. These results are discussed in terms of the characteristics of the cell types.

Herpes simplex virus type I encodes a polymerase and thymidine kinase (tk) activity which are involved in viral DNA synthesis. Paa^{r}5, an HSV-1 mutant containing one or more mutations in the polymerase gene is an antimutator. If these are also involved in viral DNA repair, then the

HSV-1 polymerase, tk activity, and mutant polymerases conferring altered mutation rates should provide excellent tools with which to probe cellular DNA repair processes and mutagenesis.

The study of the HCR of plaque forming ability of HSV-1 KOS wild type (WT), Paa^{r}5 and PTK3B (lacking thymidine kinase activity) using VERO cells revealed a decrease in the HCR of Paa^{r}5 and increase of surviving fractions of PTK3B with respect to that of HSV-1 KOS WT. Similar studies using apparently normal human fibroblasts, Xeroderma Pigmentosum and Cockayne Syndrome cells also implicated the HSV-1 polymerase in viral DNA repair.

The results are discussed in terms of the function of the HSV-1 polymerase and the DNA repair abilities of XP and CS cells.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. A.J. Rainbow for his astute guidance and advice during the course of this work. I would also like to thank Dr. M. Rathbone and Mr. David Ryan for allowing me access to their computing systems for use in the preparation of this manuscript, and Mr. J.-K. Kim for assistance in the preparation of the diagrams.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Genetic information is encoded by deoxyribonucleic acid (DNA) which is contained within the nucleus of each cell. As it is the ultimate source of instructions used in the synthesis of proteins and for the execution of cellular functions, it is essential for it to remain intact. There exist, however, several mechanisms by which the DNA may become altered. These include spontaneous mutations, DNA replication errors, and damage as the result of the exposure of the cell to a DNA damaging agent. Any of these may lead to a change in the DNA base content and, if not faithfully repaired by existing cellular DNA processes will lead to mutations.

There exist human genetic diseases with a defect in one or more forms of DNA repair accompanied by an increase in the incidence of cancer. It has therefore been suggested that there is a link between the lack of DNA repair and the incidence of cancer such that a decrease in the cellular ability to repair DNA damage results in an increase in the probability of cancer incidence. In addition, it has been found that cancer causing agents are also mutagens, and the greater the carcinogenicity the greater the mutagenicity (Newbold et al., 1980). Therefore much research is being conducted in the fields of DNA repair and the causation of cancer.

DNA Damage

The DNA double helix, as shown in figure 1, consists of two separate chains of nucleotides, each made up of a base linked to a five carbon sugar, in turn linked to a phoshate group. These chains are held together by hydrogen bonding between the bases. Adenine and thymine are joined by two hydrogen bonds, whereas three bonds link guanine and cytosine. DNA damage may result in a change in the base content and this in turn may result in a mutation.

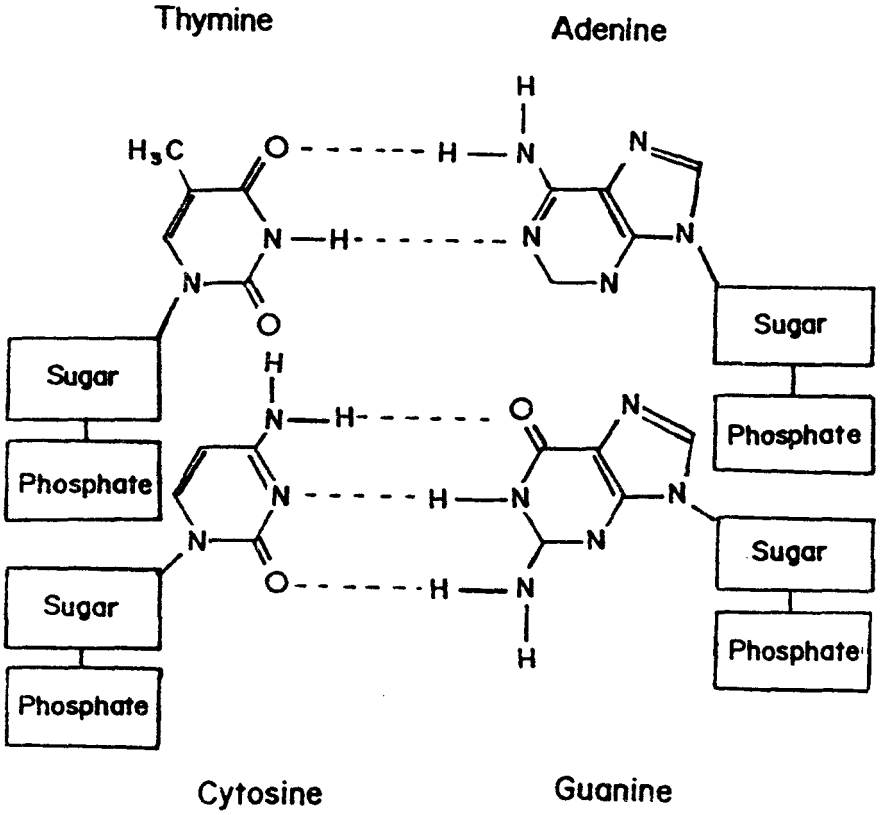
There are three main forms of DNA damage. These are spontaneous damage, damage following a replication error and damage due to the exposure of the cell to a DNA damaging agent.

Spontaneous Damage

Spontaneous damage to DNA can arise in many ways. Firstly, due to thermal disruption of the bond linking the sugar to a purine, an average of 5000 purine bases are lost per day from each human cell (Alberts, 1983). Secondly, the bases may become chemically altered either by undergoing a spontaneous structural rearrangement to form a structural isomer, or by becoming spontaneously deaminated. These altered bases have different chemical and therefore base pairing properties from the original molecules and as a result, the base content of the DNA will become altered (Topal et al., 1976). For example, deaminated cytosine will

FIGURE 1 The Structure of DNA

This figure shows the base pairing properties of the nitrogenous bases. Two hydrogen bonds join thymine adenine whereas three join cytosine and guanine.


Thymine
Adenine
Sugar
Phosphate
Sugar
Phosphate
Sugar
Phosphate
Sugar
Phosphate
Cytosine
Guanine

bond with thymine instead of guanine, thereby changing a guanine-cytosine pair to an adenine-thymine pair.

Replication Error

During DNA synthesis, the two chains become separated, and the replication machinery first chooses and inserts the correct nucleotides and then using an associated proofreading function removes incorrectly inserted nucleotides (Alberts, 1983).

Uncorrected mistakes happen with a frequency of less than 1 in 10^9 nucleotides added (Alberts, 1983), however, this allows for the possibility of the accumulation of mutations.

Exposure to a DNA Damaging Agent

DNA damaging agents exist in many forms, including ionizing radiation, ultraviolet (UV) light and chemical carcinogens. Exposure of the cell to any of these may result in damage to the DNA molecule ranging from as little as one base change to as much as gross chromosomal abnormalities.

Ionizing radiation may cause direct or indirect DNA damage. Direct damage follows the interaction of radiation with individual atoms in the DNA molecule. Indirect damage is the result of the interaction of the DNA molecule with free radicals formed as a result of the hydrolysis of the water in the cell. Typical forms of damage include the

alteration or loss of bases and single strand and double strand breaks (Niemann, 1982).

The exposure of the cell to UV light gives rise to a variety of photoproducts, the predominant one being the cyclobutane pyrimidine dimer (Setlow and Setlow, 1963). Other photoproducts include the thymine-thymine adduct (Varghese and Wang, 1968), DNA-DNA crosslinks and strand breaks.

Lastly, DNA damage may result when the cell is exposed to chemicals such as alkylating carcinogens (Newbold et al., 1980) and mustard gas (Roberts, 1978). Types of damage commonly found are single base substitutions, single strand breaks, double strand breaks and inter and intrastrand crosslinks (Roberts, 1978).

Mammalian DNA Repair Mechanisms

Each cell has at its disposal a variety of DNA repair mechanisms with which to repair DNA damage. The major processes are photoreactivation, excision repair and postreplication repair.

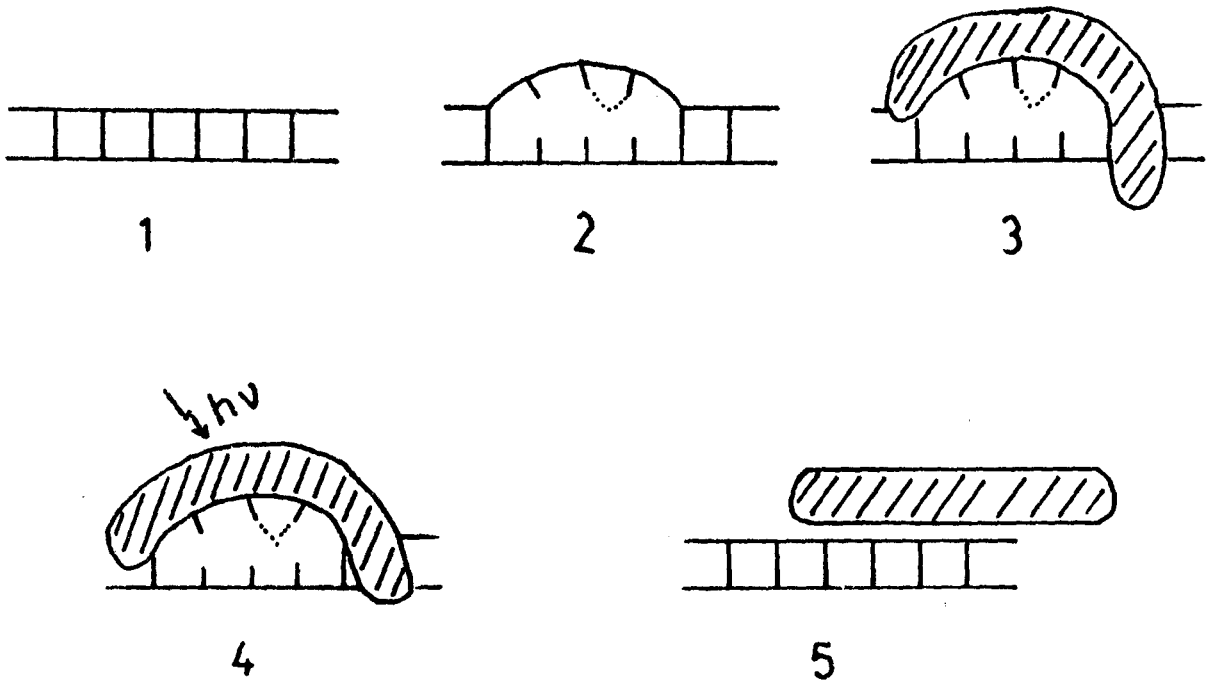
Photoreactivation

The exposure of human skin cells to ultraviolet light causes the formation of pyrimidine dimers in the DNA (Sutherland et al., 1980). A subsequent exposure to light of wavelengths between 300 and 600 nm results in a decrease in the amount of dimers present (Sutherland et al., 1980).

FIGURE 2

The Process of Photoreactivation

1. intact DNA
2. DNA with site containing pyrimidine dimer
3. photolyase enzyme binds to lesion
4. absorption of light
5. enzyme is released, free to bind to another dimer site


1
2
3
hν
4
5

This removal of dimers is due to the phenomenon of photoreactivation (Sutherland and Oliver, 1975).

Figure 2 summarizes the process of photoreactivation. Once dimers are formed, the photoreactivating enzyme becomes bound to the dimer site (Rupert, 1962). If the cell comes into contact with light of wavelengths between 300 and 600 nm, the enzyme absorbs a photon and catalyses the photolysis of the cyclobutane ring, thus restoring the two monomer pyrimidines (Sutherland et al., 1976). The enzyme is then free to bind to additional dimer sites.

Photoreactivation has been demonstrated using human skin cells (Sutherland et al., 1980), marsupial kidney cells (Cook and Regan, 1965; Lytle and Benane, 1975) and the photoreactivating enzyme has been isolated from human leukocytes (Sutherland, 1974).

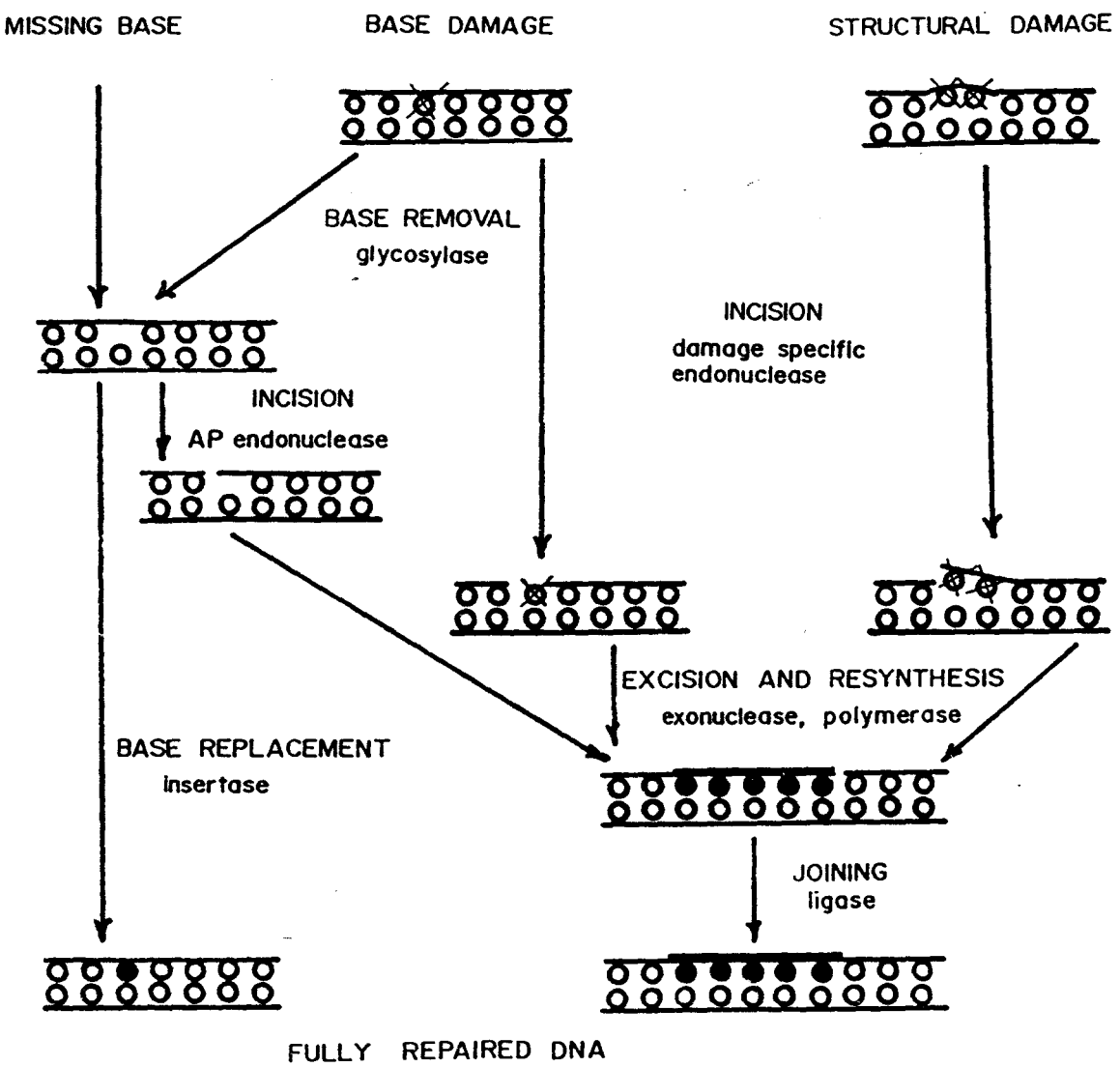
Since photoreactivation occurs only when cells are in contact with light, it is not the predominant DNA repair process.

Excision Repair

Excision repair is a repair pathway occurring before DNA replication and it responds to base and nucleotide damage. It was first demonstrated in human cells with the detection of unscheduled DNA synthesis following an exposure to UV light, or to carcinogens responsible for

FIGURE 3

Excision Repair


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

DNA damage similar to that created by UV light (Rasmussen and Painter, 1964).

The steps involved are summarized in figure 3. DNA damage is recognized by a damage specific endonuclease which makes a nick next to the damage site on the DNA molecule. This is followed by excision of the affected and surrounding region by an exonuclease and then resynthesis by a polymerase using the opposite strand as a template. The strand is then rejoined by a ligase. A single damaged base may be directly removed by a glycosylase, and the correct base may be inserted by an insertase. The number of nucleotides removed depends on the form of damage encountered. In general, either 3 - 4 or 35 - 100 nucleotides are removed, corresponding to short-patch or long-patch excision repair (Hanawalt, 1979; Grossman, 1981). Short-patch repair responds to ionizing radiation damage (Painter and Young, 1971). Long-patch repair responds to UV damage and damage by UV-like agents (Grossman, 1981) and removes 70 - 80 % of the damage sites in 12 - 24 hours (Hanawalt et al., 1979).

Each enzyme involved in the excision repair process carries out a specific function. DNA glycosylases respond to modified bases and remove them directly by hydrolizing the N-glycosylic bond to which they are attached, thus creating an apurinic (AP) or apyrimidinic (AP_{γ}) site

(Grossman, 1981). More extensive forms of damage involve the use of a greater number of enzymes.

Structural damage, or a modified base may be recognized by one of a large number of endonucleases which makes a nick in the phosphate backbone next to the damage site. An exonuclease follows by removing terminal nucleotides (Watson, 1965). In mammals, exonucleases have been detected which work in a bidirectional or unidirectional manner (Grossman, 1981). Next, a polymerase inserts the correct sequence of bases into the gap created.

In DNA synthesis, DNA polymerases add nucleotide precursors to the DNA chain in a 5' to 3' direction (Watson, 1965). There are four known mammalian DNA polymerases, namely polymerase α, β, λ and δ (Wiessbach, 1977). Polymerase λ is the mitochondrial polymerase (Zimmermann et al., 1980) and plays no role in cellular DNA repair (Grossman, 1981). Polymerase δ is not as yet well characterized and its role in DNA repair is unclear. Polymerase α is primarily involved in DNA replication (Grossman, 1981). It is polymerase β which is responsible for the majority of the repair synthesis (Grossman, 1981).

Once repair synthesis has been completed, a ligase joins the DNA strands and repair is complete.

Herpes Virus Type I Encoded Polymerase

Herpes Simplex Virus Type I (HSV-1) encodes a

polymerase which resembles mammalian DNA polymerase α (Coen et al., 1984), is essential to viral DNA synthesis, and contains an associated 3' to 5' exonuclease activity (Knopf, 1979). The polymerase is sensitive to a number of antiviral drugs, one of which is phosphonoacetic acid (PAA), a pyrophosphate analog which inhibits viral DNA synthesis (Overby et al., 1974) by competing with natural pyrophosphate substrates. The polymerase gene comprises the region between 0.413 and 0.434 map units on the HSV physical map, and has recently been sequenced (Gibbs et al., 1985). There exist a number of polymerase (pol) mutants which encode polymerases which are resistant to PAA and the mutations responsible for this map within the region encoding the carboxy-terminal portion of the polymerase. Therefore it has been proposed that this region contains the deoxynucleoside triphosphate and pyrophosphate binding sites (Gibbs et al., 1985). The function of the N-terminal portion of the polymerase polypeptide has not as yet been established. It is however essential for viral growth and may play a role in enzyme stability, interactions with other replicative proteins or the associated 3' to 5' exonuclease activity (Gibbs et al., 1985).

It has been found that mutations in the polymerase gene affect the viral mutation rate (Hall and Almy, 1982;

Hall et al., 1984). Thus the polymerase plays a role in the control of viral mutagenesis. One pol mutant, Paa^{r}5 contains one or more mutations which map to the polymerase gene (Jofre et al., 1977) . This virus produces an altered polymerase and in addition exhibits an antimutator phenotype (Hall et al., 1984). It is not as yet certain whether the HSV-1 polymerase plays a role in DNA repair however, if it does, the HSV-1 polymerase and mutant polymerases conferring altered mutation rates will provide excellent tools with which to study cellular DNA repair processes and mutagenesis.

Herpes Virus Type I Encoded Thymidine Kinase Activity

In mammalian cells, the thymidine kinase (tk) pathway is an exogenous salvage pathway which supplies thymidine triphosphate and thus balances the deoxyribonucleoside triphosphate pools (McKenna et al., 1985). A deficiency in tk may result in an imbalance in deoxyribonucleic triphosphate pools and this may affect excision repair since incorrect nucleotides may be inserted due to a scarcity of correct ones. McKenna et al. (1985) found that some excision proficient mouse cells with a tk deficiency were more sensitive to UV light and alkylating agents than their tk proficient counterparts. This suggests that tk is indirectly involved in excision repair in mouse cells.

HSV-1 encodes a gene for a thymidine kinase activity which is distinct from that of mammalian cells (Jamieson and Subak-Sharpe, 1974). The tk pathway is involved in viral DNA replication and may or may not be involved in viral DNA repair.

Human DNA Repair Deficient Diseases

There exist a variety of rare human genetic diseases which are characterized in part by a defect in one or more DNA repair processes. Cells taken from afflicted individuals exhibit an increased sensitivity to DNA damaging agents and often the individuals themselves have an increased incidence of cancer.

Xeroderma Pigmentosum

Xeroderma Pigmentosum (XP) is an autosomal recessive human genetic disease with clinical features including high sensitivity of the skin and the eyes to sunlight, increased incidence of skin cancer, and very often neurological abnormalities.

Cells from XP patients have an increased sensitivity to UV light (Cleaver, 1970), exhibit a decreased Host Cell Reactivation of UV-irradiated herpes virus (Lytle et al., 1972; Selsky and Greer, 1978) and UV-irradiated human Ad2 (Rainbow, 1980) and decreased survival of UV-irradiated SV40 virus (Abrahams and Van der Eb, 1976). In addition, these cells have an increased

mutability following UV exposure (Maher et al., 1977).

This mutability is in part due to a defect in excision repair of photoproducts. Most XP cells have a reduced ability to excise pyrimidine dimers (Cleaver, 1978), and it is postulated the deficiency lies in a lack of or decreased amount of UV endonucleases which detect this form of damage and subsequently incise the DNA backbone. This is supported by the observation that XP cells make fewer incisions than normal cells following UV damage (Paterson et al., 1973), and that repair was increased to normal levels for XP cells complemented with a T4 endonuclease (Tanaka, 1975). It has also been observed that some XP cells held in the G_0 state, thus preventing DNA replication and thereby increasing the amount of available time for excision repair make normal numbers of incisions following UV exposure (Maher, Yang, and McCormick, personal communication). This suggests that these cells have lower levels of UV endonuclease and therefore repair UV damage at a slower rate.

It has also been observed that XP cells have a reduced survival following exposure to a variety of chemicals such as nitrogen mustard (Friedberg et al., 1979), show reduced HCR of λ-irradiated adenovirus (Rainbow and Howes, 1979) and decreased levels of photoreactivating enzyme (Sutherland and Oliver, 1975). In addition, cells

from some patients showing classic XP clinical features exhibit no deficiency in excision repair but instead a defect in post replication repair (Maher et al., 1977).

It has therefore become apparent that the cause of XP is not due to a single factor and that not all XP cases arise from the same defect. Cell fusion experiments have detected 9 complementation groups A,B,C,D,E,F,G,H and I, with varying degrees of excision repair (Fischer et al., 1985; Vermeulen et al., 1986). Cells belonging to complementation group A exhibit the lowest rate of UV induced unscheduled DNA synthesis (Friedberg et al., 1979). Those cells exhibiting normal levels of excision repair but reduced post replication repair fall into the category XP variant.

Cockayne's Syndrome

Cockayne's Syndrome (CS) is an autosomal recessive disease with clinical features including dwarfness, mental deficiency, the appearance of premature aging and an increased sensitivity of the skin to sunlight (Friedberg et al., 1979). There is however no increase in the incidence of cancer for CS patients (Schmickel et al., 1977).

Cells from CS patients show an increased sensitivity to ultraviolet light (Hoar et al, 1978; Schmickel et al, 1975; Schmickel et al., 1977), to agents which induce long-patch DNA repair (Friedberg et al., 1978)

and show a reduced HCR for UV-irradiated and γ-irradiated adenovirus (Rainbow and Howes, 1982). However, they exhibit normal levels of unscheduled DNA synthesis (Hoar et al., 1978) and normal survival when exposed to x-radiation (Schmickel et al., 1975) and to drugs inducing short-patch repair (Friedberg et al., 1979). A study (Squires and Johnson, 1983) has shown that for five CS strains tested, CS cells accumulate strand breaks following UV exposure. The provision of DNA precursors to CS cells following UV exposure results in no break accumulation. These findings suggest that CS cells have a defect in excision repair which lies in ligation and/or the coordination of DNA repair synthesis with incision and ligation (Squires and Johnson, 1983).

Host Cell Reactivation

One approach to the study of the cellular repair of DNA involves the use of viruses. By examining the fate of DNA damaged viruses after their infection of a cell, one can monitor the action of the various cellular DNA repair mechanisms.

The exposure of a cell to a DNA damaging agent may affect a variety of biochemical pathways, many of which are closely related such that damage to one may result in damage or secondary effects to another. The use of a virus, which is a simple system consisting of nucleic acid

surrounded by a protein coat overcomes this problem since the cell itself is not exposed to a DNA damaging agent.

When a virus infects a cell, the viral DNA is replicated and viral specific proteins are synthesized such that each cell produces a large number of viral progeny. If the viral DNA has been damaged before infection and the virus genome itself does not encode for any viral DNA repair enzymes, viral progeny may only be produced if the cell repairs the damage. This repair is called host cell reactivation (HCR).

Many methods employing a variety of cells and animal viruses have been used to monitor HCR. These methods include plaque formation of herpes virus (Lytle, 1971), plaque formation of SV40 (Abrahams and Van der Eb, 1976), plaque formation of adenovirus (Rainbow and Mak, 1973; Day, 1974), V antigen formation of adenovirus (Rainbow and Howes, 1979), and the repair of adenovirus lesions (Rainbow, 1974).

Transformation

There exist a number of DNA containing tumor viruses such as simian virus 40 (SV40), polyoma virus and the adenoviruses, which have the ability to transform cells into their cancerous counterparts. Resulting cellular characteristics observed in vitro include a change in cell morphology, alteration of cell surface components, a

reduction in serum requirement, the appearance of neoantigens such as tumor (T)-antigen, and the tendency to divide once confluency has been reached (Chen et al., 1976).

SV40 has a small genome of 5243 base pairs (bp) (Watson et al., 1983) which encodes for as little as ten proteins. SV40-transformed cells are consistently observed to contain a tumor (T) protein which is produced during the early phase of the SV40 life cycle and it is possible that this protein is involved in the maintenance of the transformed state.

The adenoviruses can be divided into three groups, A,B, and C corresponding to their oncogenicity in hamsters. Those viruses belonging to group C are nononcogenic in newborn hamsters. Ad 5 belongs to group C; however, it can transform cells in vitro and these cells may produce tumors following injection in animals (Graham et al., 1974). The transforming segment corresponds to the left 1% to 5 - 6% of the Ad 5 genome, and this segment has been observed to be incorporated in the genome of transformed cells (Graham and Van der Eb. 1974). This is similar to observations by Gallimore (1972) that Ad2 transformants always contain the left 14% of the Ad2 genome. The incorporation and subsequent expression of viral DNA in the infected cell seems to result in the transformation and maintenance of

the transformation state.

Human Adenovirus Transformed Cell Lines 293 and 293 N3S

Transformation of human cells by the human adenoviruses is not often observed because infection is normally followed by the lytic pathway which results in cell death. However, using the 'calcium technique' (Graham and Van der Eb. 1973) it is possible to infect cells with viral DNA fragments. In this way, the lytic pathway is avoided and transformation is possible.

Using this technique, Graham and Smiley (1977) infected human embryonic kidney cells with sheared adenovirus type 5 (Ad 5) DNA. Transformed cells were isolated and the cell line 293 was established. These cells contain the left end (transforming region) of the Ad 5 genome (Graham and Smiley, 1977) and exhibit characteristics typical of transformation by adenovirus such as the tendency to continue to divide once confluency is reached, the ability to grow in serum reduced medium and the expression of Ad 5 specific T antigens. However, unlike Ad 5 transformed hamster and rat cells which produce tumors in 90 - 100% of injected nude mice, 293 cells exhibit extremely low levels of oncogenicity (Graham and Smiley, 1977).

The passage of 293 cells through nude mice yields cells (removed from resulting tumor(s)) which are increased

in oncogenicity. The 293 N3S cell line was produced by passaging 293 cells through nude mice three times. The resulting cells exhibit an increase in oncogenicity and anchorage independence; however, they are similar to 293 cells in that they still contain the transforming region of the Ad 5 genome and continue to express antigens encoded by that region (Graham, personal communication). It is not certain what is responsible for the phenotype exhibited by 293 N3S cells.

Thus, the 293 and 293 N3S cell lines are examples of human cells transformed by Ad 5 DNA and they exhibit differing levels of oncogenicity in nude mice.

In this work, the HCR of V antigen formation of UV-irradiated Ad2 was studied using apparently normal human fibroblasts, tumor cells (Hela CCL2) and transformed cells (293, 293 N3S) in order to determine any differences in these cells' abilities to repair UV damaged DNA.

Secondly, the HCR of plaque formation by UV-irradiated HSV-1 wild type, a thymidine kinase deficient mutant (PTK3B) and Paa^{r}5, a virus specifying an altered polymerase was studied using VERO (African Green Monkey) cells, apparently normal human, XP and CS fibroblasts. The purpose of these studies was to determine any possible involvement of the HSV-1 tk activity and polymerase in viral DNA repair and also to determine, if possible the

nature of the XP and CS DNA repair defects.

Materials and Methods

Media

MEM Alpha medium (α-MEM, Gibco Catalogue #410-2000, Grand Island Biological Co., Grand Island, N.J., USA) was purchased in powder form, and made up in double glass distilled water and filter sterilized using a 0.22 micron Millipore filter (Millipore Corporation, Bedford, Massachusetts 01730, USA). This medium was used for the culture of all cell strains and lines.

Media Supplements

1. Antibiotic-Antimycotic solution : penicillin 10,000 U/ml, fungizone 25 mcg/ml and streptomycin 10,000 mcg/ml (Gibco catalogue #600-52401) was added to the medium at a 1 : 100 dilution.
2. 7.5% $NaHCO_3$ solution, autoclave sterilized, was added to the medium at a 1 : 100 dilution.
3. Fetal Bovine Serum (FBS) (Gibco catalogue #200-6140) was added to the medium at a 1 : 10 dilution. Medium with FBS, antibiotics, and $NaHCO_3$ is "complete" α-MEM , that without FBS but with antibiotics and $NaHCO_3$ is "straight" α-MEM.

Solutions

1. Phosphate-Buffered Saline (PBS) was used for the washing of cell monolayers prior to infection with virus. It consists of 8 gms NaCl, 20 gms KCl, 11.5 gms Na_2HPO_4, and 2 gms KH_2PO_4 dissolved in 1 litre double glass distilled

water and autoclave sterilized.

2. Trypsin (0.25%) (Gibco catalogue #610-5050) was used in the subculturing of Hela CCL2 and human diploid fibroblast cells.

3. Versene was used for the subculturing of 293, 293 N3S, and VERO cells. It was prepared as a 10X stock solution consisting of 2 gms EDTA, 80 gms NaCl, 2 gms KCl, 11.5 gms Na_2HPO_4, 2 gms KH_2PO_4, and 2 gms glucose dissolved in 1 litre double glass distilled water and autoclave sterilized. Stock solution was diluted 10 fold in PBS as needed.

4. Crystal Violet Stain was used for the fixing and staining of cell monolayers for plaque assays. It consists of 2 gms of crystal violet dissolved in 20 mls methanol, 144 mls PBS and 36 mls formaldehyde, and was filtered using Whatman No 2 filter paper (W. & R. Balston Ltd., England).

5. Fluorescein-conjugated anti-rabbit globulin was used in the immunofluorescent staining of cell monolayers. It consists of sheep anti-rabbit globulin conjugated with FITC, preserved with 0.01% merthiolate, and is diluted 1:20 with 1X PBS.

6. Rabbit Anti-Ad2, an antiserum against Ad2 structural proteins (Vag) was used in the immunofluorescent staining of cell monolayers. It was produced by immunizing rabbits

with purified Ad2 and harvesting the blood at six weeks. The antiserum was collected and sterilized by filtration (Rainbow, 1980). Antiserum was generally diluted 20-fold in PBS.

Cells

I. Human Diploid Fibroblasts

1. Apparently Normal Strains:

The cell strain CRL 1121 was obtained from the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852-1776.

2. Xeroderma Pigmentosum:

The cell strain GM 544B (XP complementation group A) was obtained from the NIGMS Human Genetic Mutant Cell Repository.

3. Cockayne's Syndrome:

Cell strain GM 739 was obtained from the NIGMS Human Genetic Cell Repository.

II. Other Human Cells

1. HeLa CCL2:

This cell line, originating from a human cervical carcinoma was obtained from ATCC.

2. 293:

This cell line was the generous gift of Dr. Frank Graham, Department of Biology, McMaster University, and was produced by transforming primary or early passage human

embryonic kidney cells by exposing them to sheared adenovirus type 5 (Ad5) DNA. These cells differ from the untransformed cells in cell morphology, and also in that they continue dividing once confluency has been reached (Graham and Smiley, 1977).

3. 293 N3S:

This cell line consists of 293 cells passaged through nude mice three times and forms tumors in nude mice more readily than 293 cells. It was obtained from Dr. Frank Graham, Department of Biology, McMaster University.

III. Non-Human Cells Lines

1. VERO:

This cell line, obtained from ATCC, originates from normal adult African Green Monkey kidney cells.

Growth and Passaging

Monolayers of cells were grown in plastic 'falcon' flasks obtained from Becton Dickinson & Company, 1950 Williams Dr., Oxnard, CA 93030, USA. These were kept in an incubator at 37°C with humidified atmosphere at 5% CO_2. Once confluency was reached, the medium was aspirated off the cells, and the monolayers were washed with 2-4 mls prewarmed trypsin (cell lines HeLa CCL2, CRL 1121, GM 544B, GM 739) or versene (VERO, 293, 293 N3S). The trypsin or versene was then aspirated off and 2-4 mls prewarmed trypsin or versene was added. The flasks were placed in the

incubator for 10-20 min, and then tapped to free cells. An appropriate amount of prewarmed complete α-MEM was added to the flask, and cells pipetted up and down several times to break up clumps. The suspension of cells was divided into three equal portions, each of which was placed into a flask containing an appropriate amount of complete α-MEM. These flasks were then placed in the incubator.

Viruses

I. Adenovirus Type II

Stocks of Adenovirus Type II (Ad2) were obtained from Margaret Howes, Department of Biology, McMaster University, Hamilton, Ontario, Canada. The preparation of Ad2 has been previously described (Rainbow and Mak, 1970).

II. Herpes Simplex Virus Type I

Stocks of Herpes Simplex Virus Type I (HSV-1) were grown as follows. 7 to 10 75 cm^2 'Falcon' flasks of VERO cells were prepared as previously described. Once the monolayers were confluent, the medium was aspirated off, and the monolayers washed with 2 mls straight α-MEM. The medium was aspirated off and a 2 ml suspension of virus in straight α-MEM was added. Infectivity was at an average of .01 plaque forming units per cell (pfu/cell) as titred on VERO cells. The flasks were placed in the incubator for 90 min. with intermittent manual rocking to prevent drying out of the monolayers. 10 mls of medium containing 2% FBS was

then added to each flask, and the flasks were placed in the incubator for 48 hrs or until cytopathic effect (CPE) was observed in the majority of cells. The cells were then gently scraped into the medium using a rubber policeman, and pelletted by centrifugation at 800-1000 rpm at 4°C using a refrigerated centrifuge.

Virus Preparation

The pellet of infected cells was resuspended in an appropriate amount of cold straight α-MEM , and the suspension sonicated for two 30 second intervals separated by 60 seconds. The suspension was then centrifuged at 1500 rpm at 4°C for 20 minutes, the supernatant collected and amounts of 0.2 mls aliquoted into vials (Nunc Cryotubes, catalogue #3-40711, Nunc Intermed, Denmark) which were stored in a freezer at -70°C.

Titre of Virus

The virus was titred using VERO cells. The cells were collected in the same manner as described for subculturing, with the amount of complete α-MEM added such that the total volume was brought to 48 mls per 75 cm^2 flask. The cells were seeded into 24 well Nunclon plates (Nunc, Intermed, Denmark) by adding 1 ml of the suspension to each well, and placed in an incubator for 24 hrs to reach confluency. After this time period, the monolayers

were confluent and were ready to be infected with virus. A dilution series of virus in cold straight α-MEM with total dilution factors ranging from 10^{-4} to 10^{-9} was prepared. Next, the medium was removed from each well using a sterile pasteur pipette, and each monolayer was washed with 0.5 mls straight α-MEM. The medium was removed, and 0.2 mls of the appropriate viral dilution was added to each well. Four wells were infected per dilution. Following an incubation period of 90 min, 0.8 mls of "straight" α-MEM with 3% FBS and 0.05% human immune serum globulin (16.5% solution, Connaught, distributed by the Canadian Red Cross Society Blood Transfusion Service, DIN 07280) was added to each well. The human immune serum globulin ensured that infection occurred only as a result of cell to cell contact, and not by released viral progeny. ie. one plaque was the result of the infection of one cell by one virus particle. The plates were incubated for 48 hrs, the medium aspirated off, and the monolayers fixed and stained using a crystal violet solution.

The number of plaques in each monolayer was counted and the number of plaques was plotted as a function of the inverse of the dilution factor using a linear regression. The titre was then determined as the number of plaque forming units per ml of original suspension.

Ultraviolet Light Irradiation -Ad2 and HSV-1

Stock virus was diluted with an appropriate amount of cold straight α-MEM. One ml of this suspension was placed in a 35x10 mm style Falcon petri dish (Falcon: 1950 Williams Dr., Oxnaard, CA. 93030 U.S.A.) and kept on ice. Irradiation was carried out using a germicidal lamp (FG 596-E., General Electric Company, Nela Park, Cleveland, Ohio 44112) emitting light at predominantly 254 nm, with the petri dish lid removed and constant swirling of the viral suspension. Dose rates were generally 5-6 $J/m^2/sec$, as determined using a J-225 short wave meter (catalogue 17456-0, Will Scientific Inc. and subsidiaries Box 1050-Rochester, New York 14603), and irradiation times varied to give the desired doses. An aliquot was removed after each irradiation and a dilution series made for each dose.

Experimental Procedures

V antigen Studies

Monolayers of the cells of interest were grown, cells collected as described for subculturing and the volume of cell suspension made up to 32 mls/75 cm^2 bottle. The cells were seeded into 8-well tissue culture slides (Lab-Tek, Miles Scientific, Division of Miles Laboratories, Inc., Naperville, Il. 60566) at 0.5 ml per well and placed in the incubator for 24 hrs to allow the monolayers to reach confluency.

A dilution series was made up of irradiated and

unirradiated Ad2 as previously described. Viral UV doses were 0, 200, 400, 600, and 800 J/m^2 with dose rates of 6 $J/m^2/sec$. The chamber slides were removed from the incubator, and the medium was aspirated off. Each monolayer was washed with straight α-MEM. This was removed and 20 λ of the appropriate viral suspension added to each well. The monolayers were infected in duplicate at 3 serial dilutions per UV dose, the two remaining wells being uninfected controls. The slides were placed in the incubator for 90 min to allow the virus to adsorb. Next, each monolayer was overlaid with 0.5 mls complete α-MEM and the slides were incubated for 48 hrs.

Following the incubation period, the monolayers were fixed using the following procedure. The medium was aspirated off the monolayers, and each monolayer was carefully washed 3 times for 5 minutes each with PBS. 0.3 ml cold solution of 50:50% acetone-methanol was added to each well, and aspirated off after 10 minutes. The chamber slide gaskets were removed, and slides stained as described below or stored in a freezer until ready to be stained.

The slides were stained as follows. The fixed slides were incubated immersed in PBS for 30 minutes at 37° C and then drained well. 20-30 λ rabbit Anti-Ad2 antiserum was placed on coverslips which were then placed on the monolayers. The slides were incubated for 60 minutes at 37°

C, the coverslips removed, and slides washed well with PBS. The slides were incubated immersed in PBS for 30 minutes at 37° C and drained well. 20-30 λ conjugate was placed over coverslips which were placed on the monolayers and the slides washed well with PBS. The slides were incubated immersed in PBS for 30 minutes at 37 ° C, drained and covered with coverslips. These were stored in the refrigerator until ready to be examined.

HSV-1 Plaque Assay

Monolayers of the cells of interest were grown until confluent. The cells were collected in the same manner as described for subculturing, except that the volume was brought up to 48 mls per 75 cm^2 flask of VERO cells, or 25 mls per 75 cm^2 flask for other cell types. The cells were seeded into Nunclon plates by adding 1 ml of the cell suspension to each well. The plates were incubated for 24 hrs to allow the cell monolayers to reach confluency.

A dilution series of irradiated and unirradiated virus was made up as previously described with viral UV doses ranging from 0 to 400 J/m^2 delivered at a dose rate of 5 $J/m^2/sec$. The plates were removed from the incubator, and the medium aspirated off. The monolayers were washed with prewarmed straight α-MEM. This was aspirated off, and 0.2 mls of the appropriate viral suspension was added to each well. The wells were infected in duplicate, at three

to five serial dilutions per viral UV dose.

The plates were incubated for 90 min to allow the virus to adsorb, and 0.8 mls prewarmed complete α-MEM supplemented with 0.05% human immune serum globulin was added to each well. The plates were incubated for 48 hrs.

The monolayers were simultaneously fixed and stained by aspirating off the medium and adding 3-5 drops crystal violet solution to each well. Following a period of 5-10 minutes, the crystal violet solution was gently washed off with running water, and the plates allowed to dry. The plates were stored at room temperature until examined for plaques.

RESULTS

A. Viral Antigen Assay

The viral antigen assay was used to determine the HCR of adenovirus type 2 (Ad2) using a variety of cell types. Normal human fibroblasts, tumor cells and transformed cells were among the cell types used.

The cells of interest were seeded into tissue culture chamber slides as previously described. Following an incubation period of 24 hrs, the monolayers were infected with Ad2 at three serial dilutions for each UV dose to the virus. A higher concentration of virus was used for virus given higher UV doses.

At 48 hrs, the monolayers were fixed and stained, and the viral antigen positive cells in each monolayer were counted using a fluorescent microscope. For each dose, the number of viral antigen positive cells was plotted as a function of viral concentration and the number of viral antigen positive cells was predicted for the largest concentration using a linear regression forced through the origin (see Appendix). The surviving fraction at each viral dose was calculated by taking this predicted number of viral antigen positive cells, and dividing by that predicted for the 0 dose, and the dilution factor.

The natural logarithm was plotted as a function of viral UV dose, and a linear regression was fitted to the data points. The negative inverse of the slope, ie. D_o,

where D_o is the dose required to give an average of one lethal hit per virus and the slope is equal to $-1/D_o$, was calculated for each cell type of interest. The percent Host Cell Reactivation (%HCR) was calculated as:

$$\%HCR = \frac{D_o \text{ (cell type of interest)}}{D_o \text{ (normal human fibroblasts)}}$$

AI. Normal Human Fibroblasts

Normal human fibroblast cells were used as a control. A typical survival curve obtained is shown in figure 4. The D_o and its standard error is 205 ± 23 J/m^2. The pooled results from two experiments are shown in table 1. Other reported values for the survival curves for V antigen formation of Ad2 in normal human fibroblasts are 320 ± 40 to 370 ± 40 (Krepinsky et al., 1980) and 240 ± 20 J/m^2 to 290 ± 50 J/m^2 (Rainbow, 1984).

AII. Human Tumor Cells

The human tumor cell line used was HeLa CCL2, a cell line derived from a human cervical carcinoma. A typical survival curve obtained is shown in figure 5. The curve consists of two components and the D_o and %HCR for the first component are 75 ± 6 J/m^2 and 37 ± 7 respectively. These values are similar to those of 80 ± 1 J/m^2 and 30 % reported by Rainbow (1984), and 58 J/m^2 (as

Figure 4 Survival of V antigen formation of UV-irradiated Ad 2 in normal human fibroblasts

Surviving fraction as a function of viral UV dose

Cell type : ○ CRL 1121 D_o = 205 ± 23 J/m2

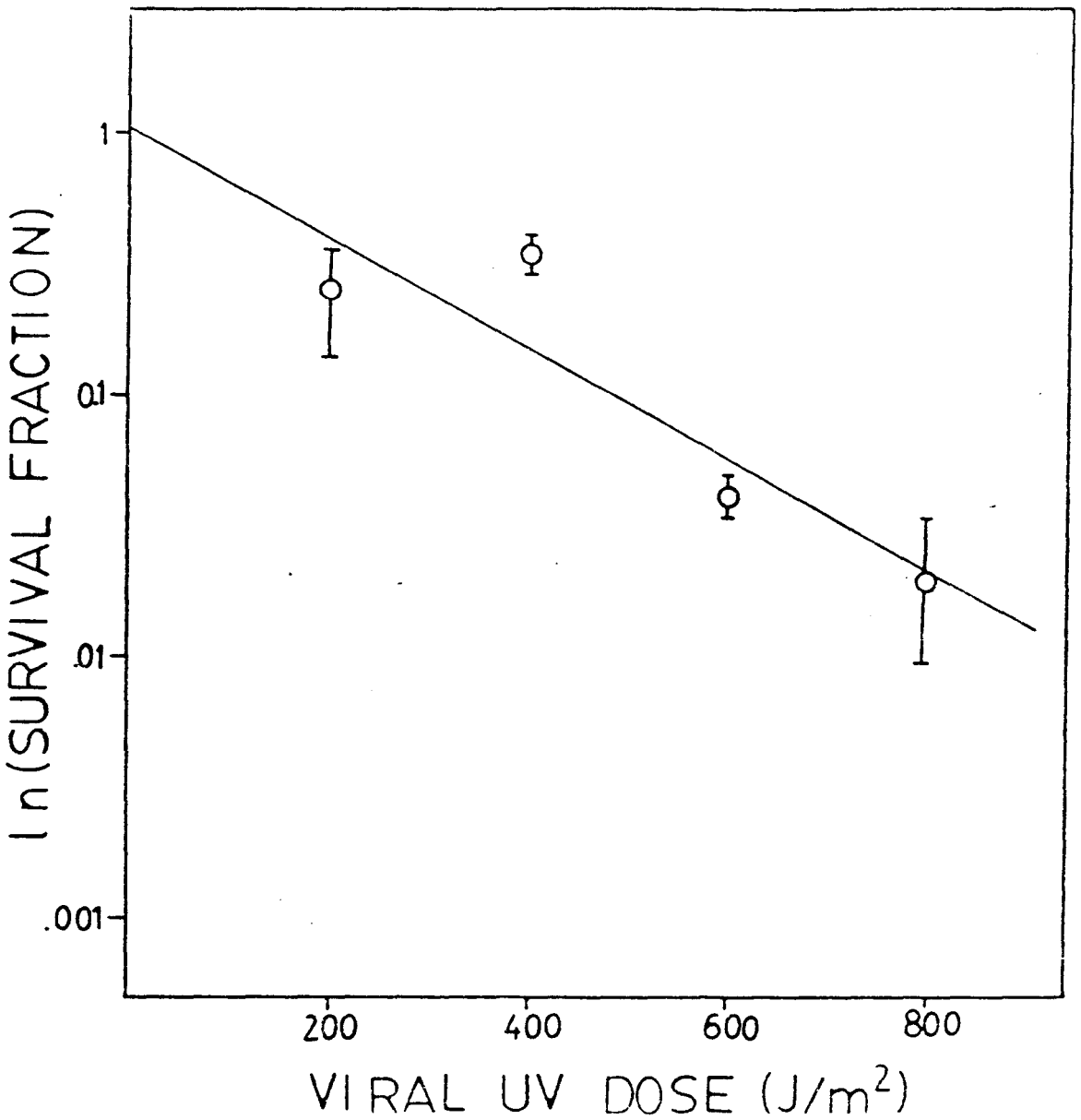

1
.01
.001
ln (SURVIVAL FRACTION)
200
400
600
800
VIRAL UV DOSE (J/m²)

Table 1 Parameters describing the survival of UV-irradiated Ad 2 in normal human fibroblasts, HeLa CCL2, 293, and 293 N3S cells

Cell Type	D_0 (J/m^2)	%HCR	No. of Expts.
CRL 1121	233 ± 27		2
HeLa CCL2	*72 ± 2 $169 ± 16	31 ± 4 73 ± 15	2
293	177 ± 24	76 ± 19	2
293 N3S	229 ± 16	98 ± 18	2

This table lists the cell types used and the D_0 values obtained for the survival curves. The virus used was Adenovirus type 2. (Pooled results)

* This D_0 value corresponds to the first component, with the curve being analysed in two components

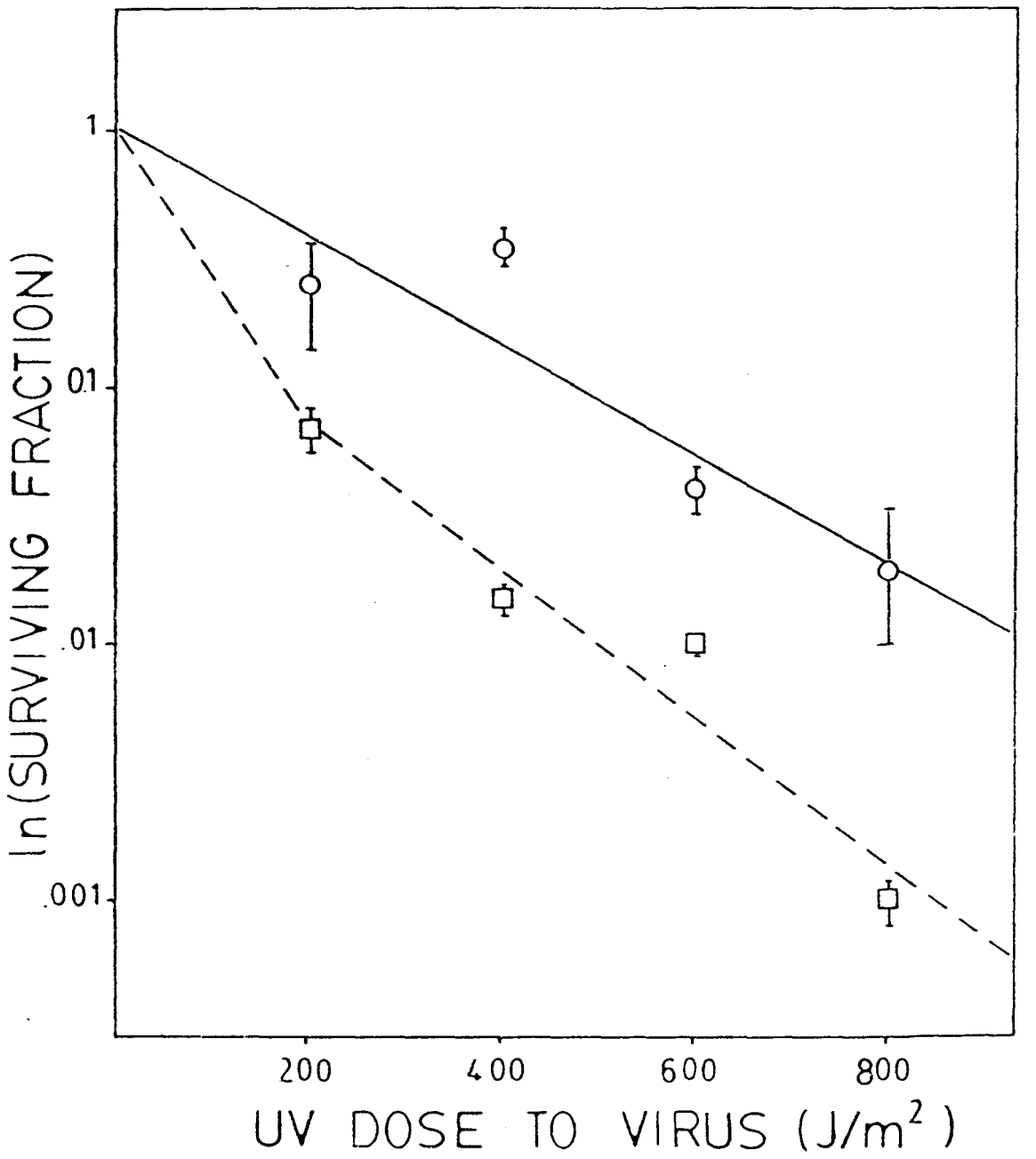
$ This D_0 value corresponds to the second component, with the curve being analysed in two components

Figure 5 Survival of V antigen formation of UV-irradiated Ad 2 in normal human fibroblasts and HeLa CCL2 cells

Surviving fraction as a function of viral UV dose

Cell type : ○ CRL 1121 D_o = 205 ± 23 J/m2

□ HeLa CCL2 D_o = 75 ± 6 J/m² (first component)


1
0.1
.01
.001
ln (SURVIVING FRACTION)
200
400
600
800
UV DOSE TO VIRUS (J/m²)

estimated from survival curve data of Johnson et al., 1986. Pooled data from two separate experiments is given in table 1.

AIII. Cell Line 293

This cell line consists of human embryonic kidney cells which have been transformed using a section of Ad5 DNA (Graham and Smiley, 1977). A typical survival curve obtained is shown in figure 6. The D_o for the curve is 161 ± 23 J/m², and the %HCR is 78 ± 23 %. The pooled results from 2 experiments are shown in table 1.

AIV. Cell Line 293 N3S

This cell line consists of 293 cells which have been passaged through nude mice three times, and as a result form tumors in nude mice with an increased frequency. A typical survival curve obtained is shown in figure 7. The D_o is 200 ± 13 J/m² and the %HCR is 98 ± 18 %. The pooled results from two experiments are shown in table 1.

B. HSV-1 Plaque Assay

This plaque assay was used in order to compare the UV survival of KOS HSV-1 wild type virus and a variety of KOS HSV-1 mutants following infection of human fibroblasts and VERO cells.

The cell type of interest was seeded into 24 well Nunclon tissue culture plates as previously described. 24

Figure 6 Survival of V antigen formation of UV-irradiated Ad 2 in normal human fibroblasts and 293 cells

Surviving fraction as a function of viral UV dose

Cell type : ○ CRL 1121 D_o = 205 ± 23 J/m^2

□ 293 D_o = 161 ± 23 J/m^2

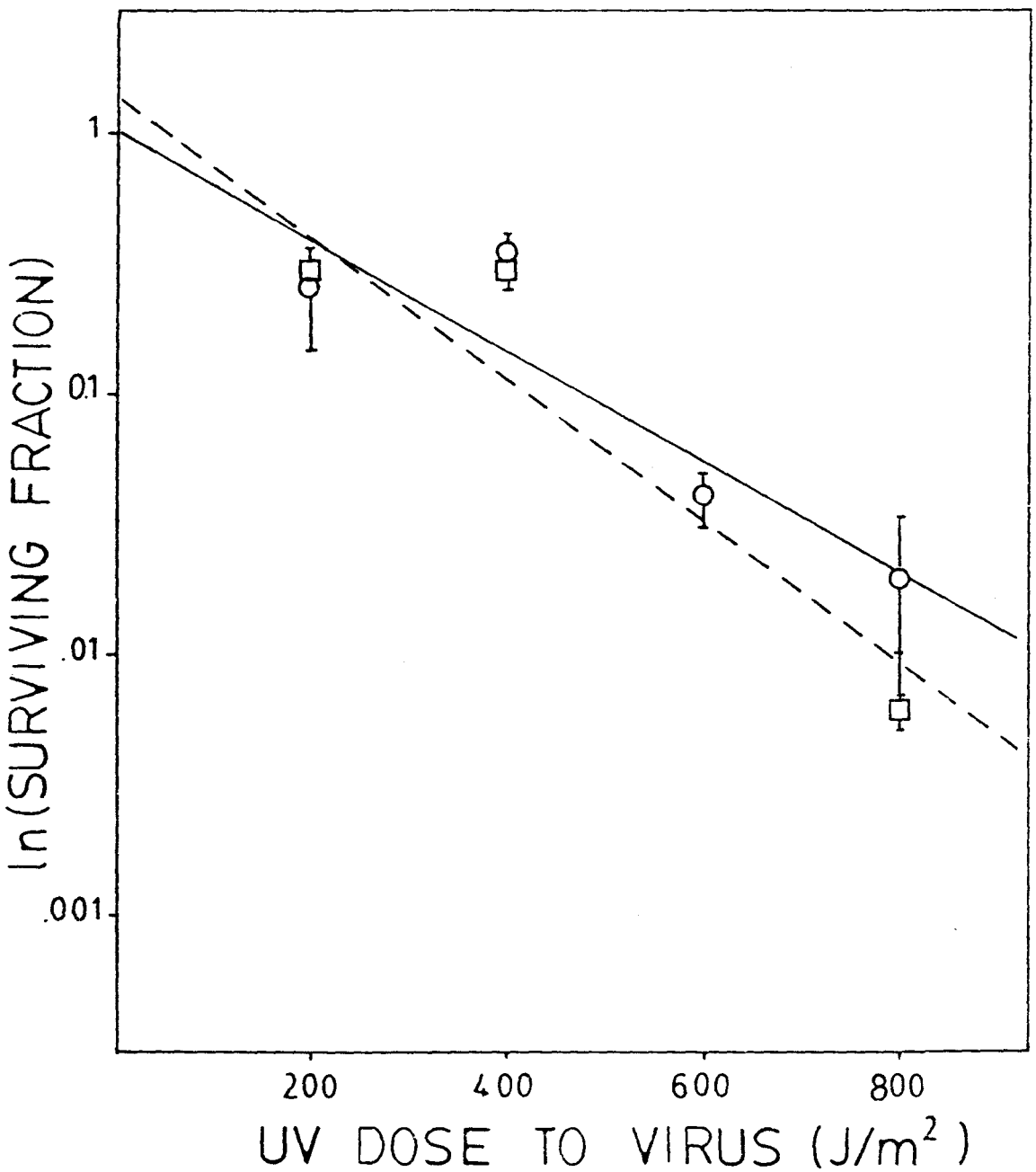
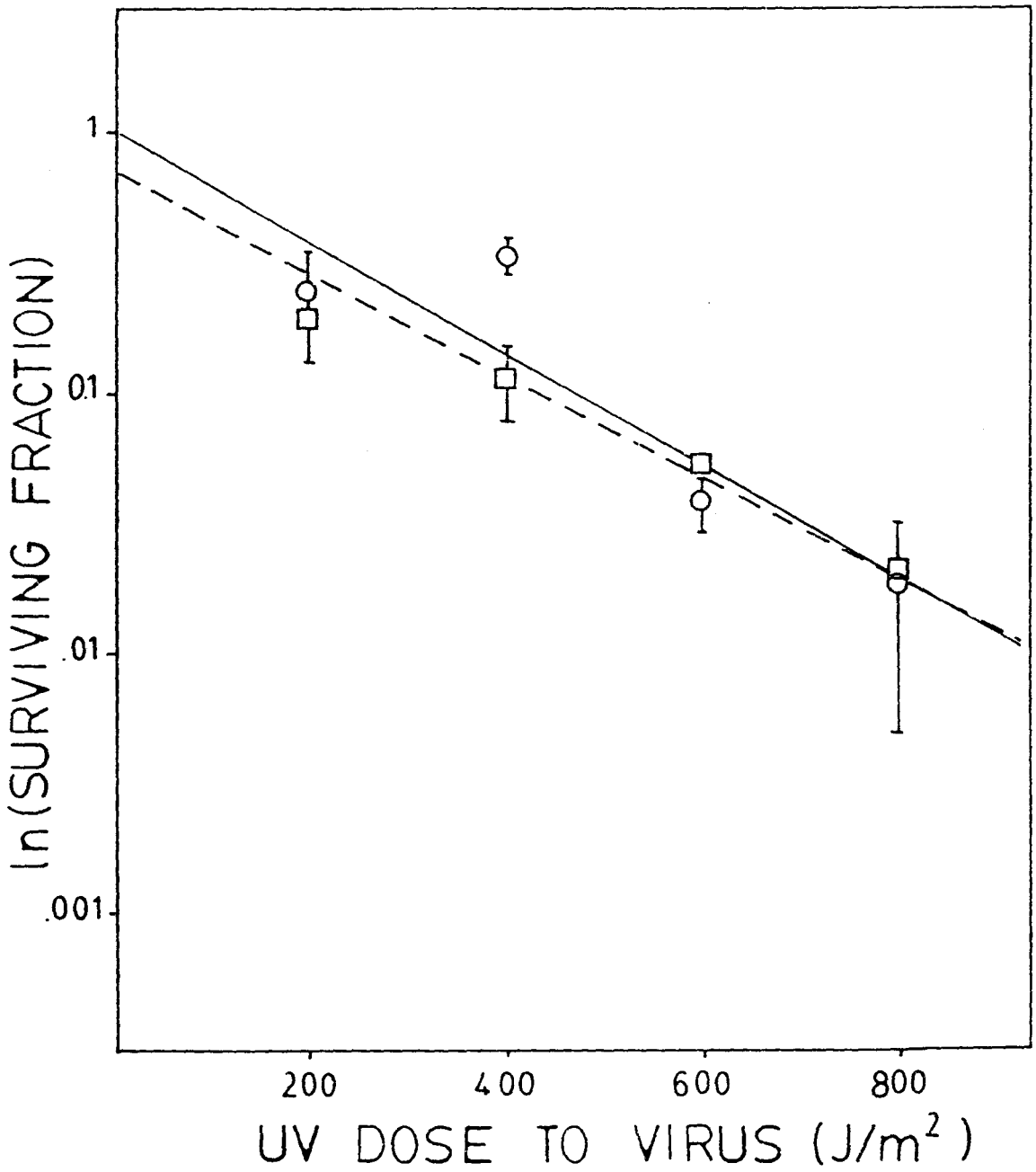

1
0.1
.01
.001
ln(SURVIVING FRACTION)
200
400
600
800
UV DOSE TO VIRUS (J/m²)

Figure 7 Survival of V antigen formation of UV-irradiated Ad2 in normal human fibroblasts and 293 N3S cells

Surviving fraction as a function of viral UV dose

Cell type : ○ CRL 1121 D_o = 205 ± 23 J/m²

□ 293 N3S D_o = 222 ± 25 J/m²


1
0.1
.01
.001
ln (SURVIVING FRACTION)
200
400
600
800
UV DOSE TO VIRUS (J/m²)

hours later, the monolayers were infected with virus at 3 to 5 serial dilutions for each UV dose. Following an incubation period of 48 hrs, the monolayers were fixed and stained, and the number of plaques on each monolayer was counted. The surviving fraction was plotted against the viral UV dose . The D_o and its standard error were calculated for each curve.

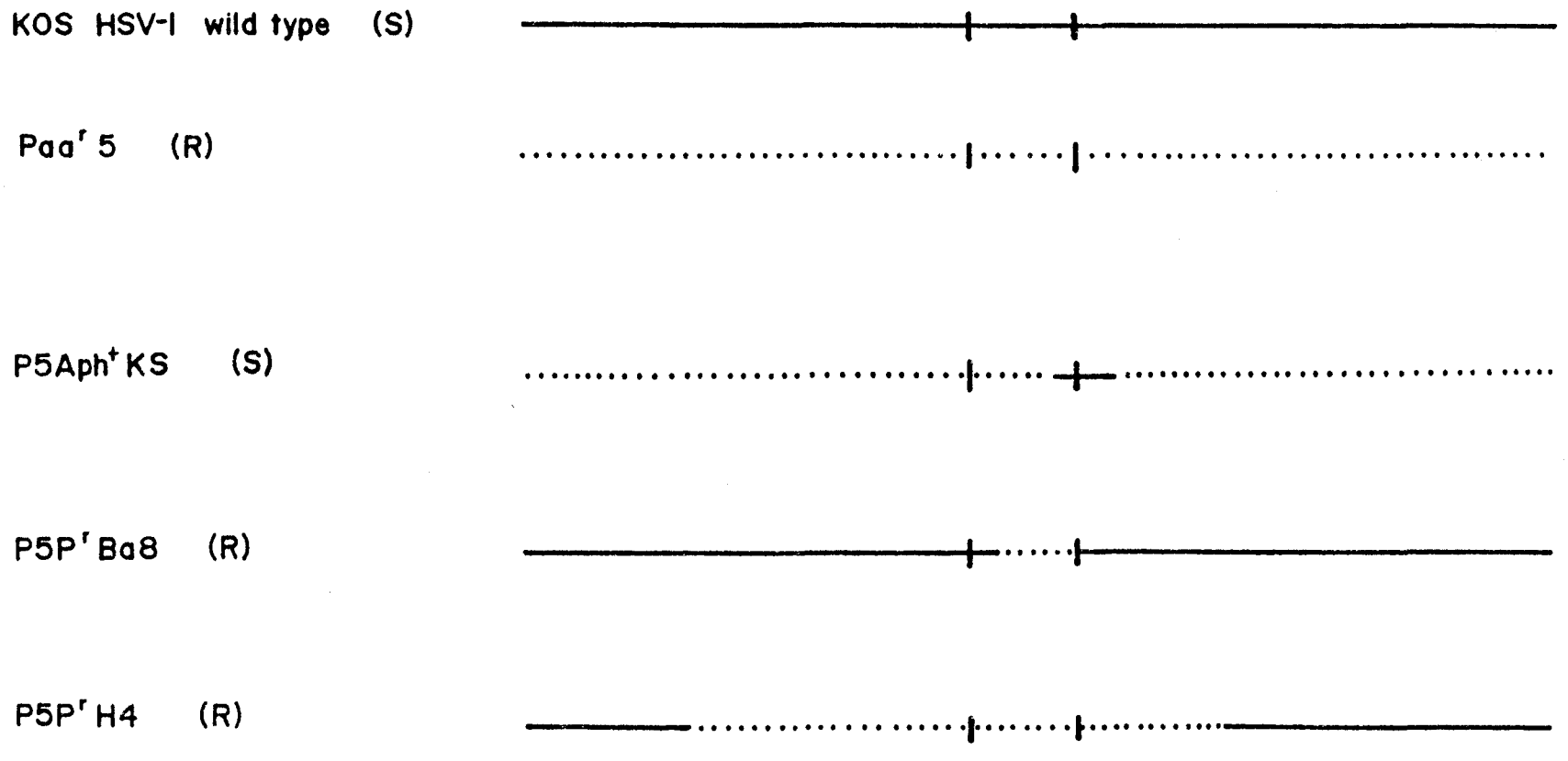
BI.VERO Cells

The plaque survival of a variety of HSV-1 viruses was determined using VERO cells following UV irradiation of the virus. The HSV-1 strains and mutants used include KOS HSV-1 wild type, an HSV-1 mutant encoding an altered polymerase (Paa^{r}5), and several recombinant viruses containing part of the Paa^{r}5 and part of the KOS genome. Details concerning these viruses are given in figure 8.

Typical survival curves obtained using HSV-1 KOS wild type and Paa^{r}5 are shown in figure 9. These curves exhibit the two component nature characteristic of infections with HSV-1 in mammalian cells (Lytle, 1971; Lytle et al., 1972; Ryan and Rainbow, 1986). The D_o values for the second components of the curves are 96 ± 12 J/m^2 for KOS wild type and 60 ± 10 J/m^2 for Paa^{r}5. The results from separate experiments are given in table 2. Average %HCR values from separate experiments and using pooled data are given in tables 3 and 4.

Figure 8 HSV-1 Recombinant Viruses

This figure shows the HSV-1 recombinants used, and the wild type or Paar5 inserts contained.



S : sensitive to phosphonoacetic acid

R : resistant to phosphonoacetic acid

Figure 9 Survival of plaque formation of UV-irradiated HSV-1 KOS WT and Paa^{r}5 in VERO cells

Surviving fraction as a function of viral UV dose

Virus : ○ HSV-1 KOS wild type D_o = 96 ± 12 J/m^2

□ Paa^{r}5 D_o = 60 ± 10 J/m^2

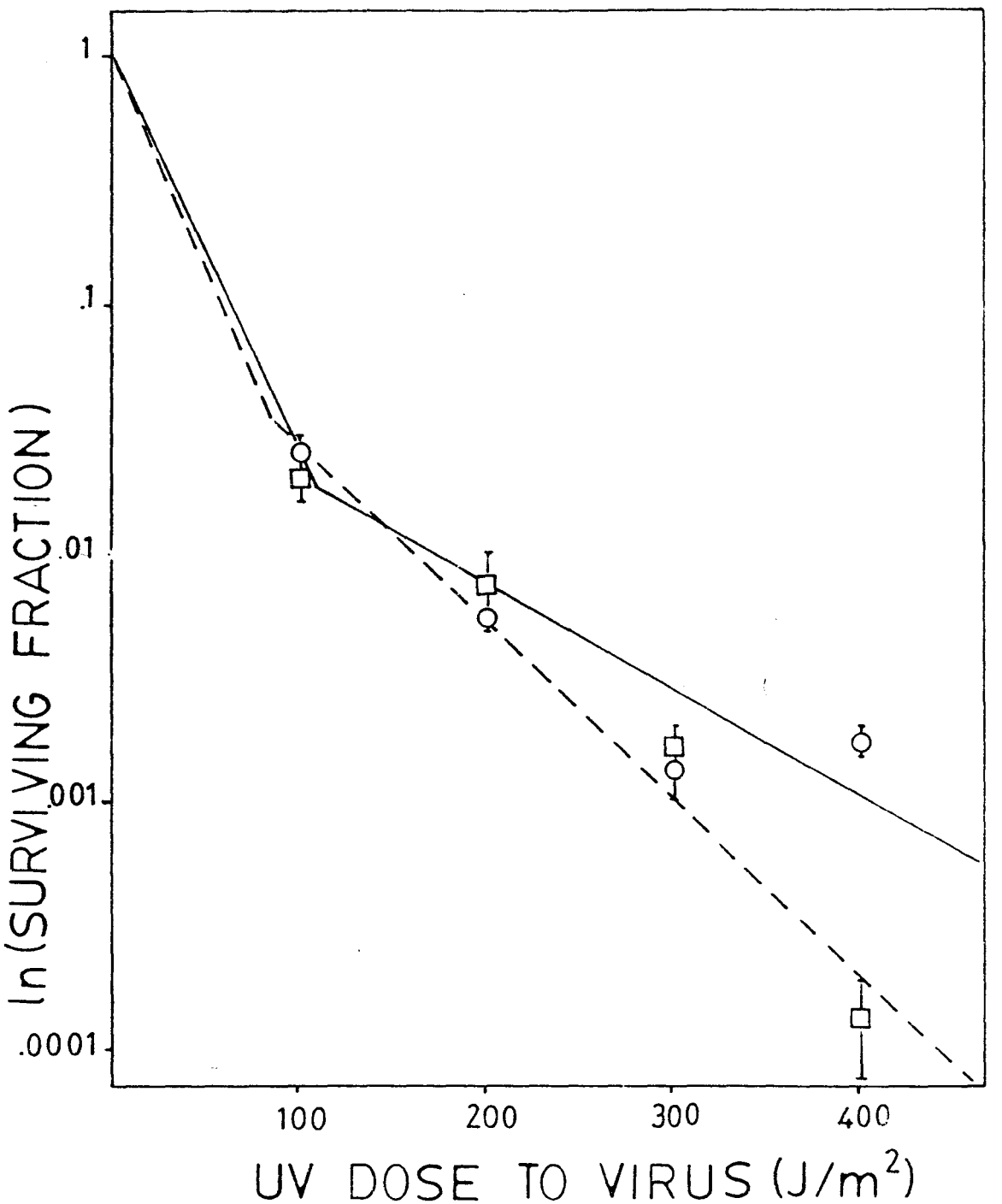

1
.1
.01
.001
.0001
ln (SURVIVING FRACTION)
100
200
300
400
UV DOSE TO VIRUS (J/m²)

Figure 10 Survival of plaque formation of UV-irradiated HSV-1 KOS WT and P5P^{r}Ba8 in VERO cells

Surviving fraction as a function of viral UV dose

Virus : ○ HSV-1 KOS wild type D_o = 67 ± 4 J/m^2

□ P5P^{r}Ba8 D_o = 76 ± 1 J/m^2

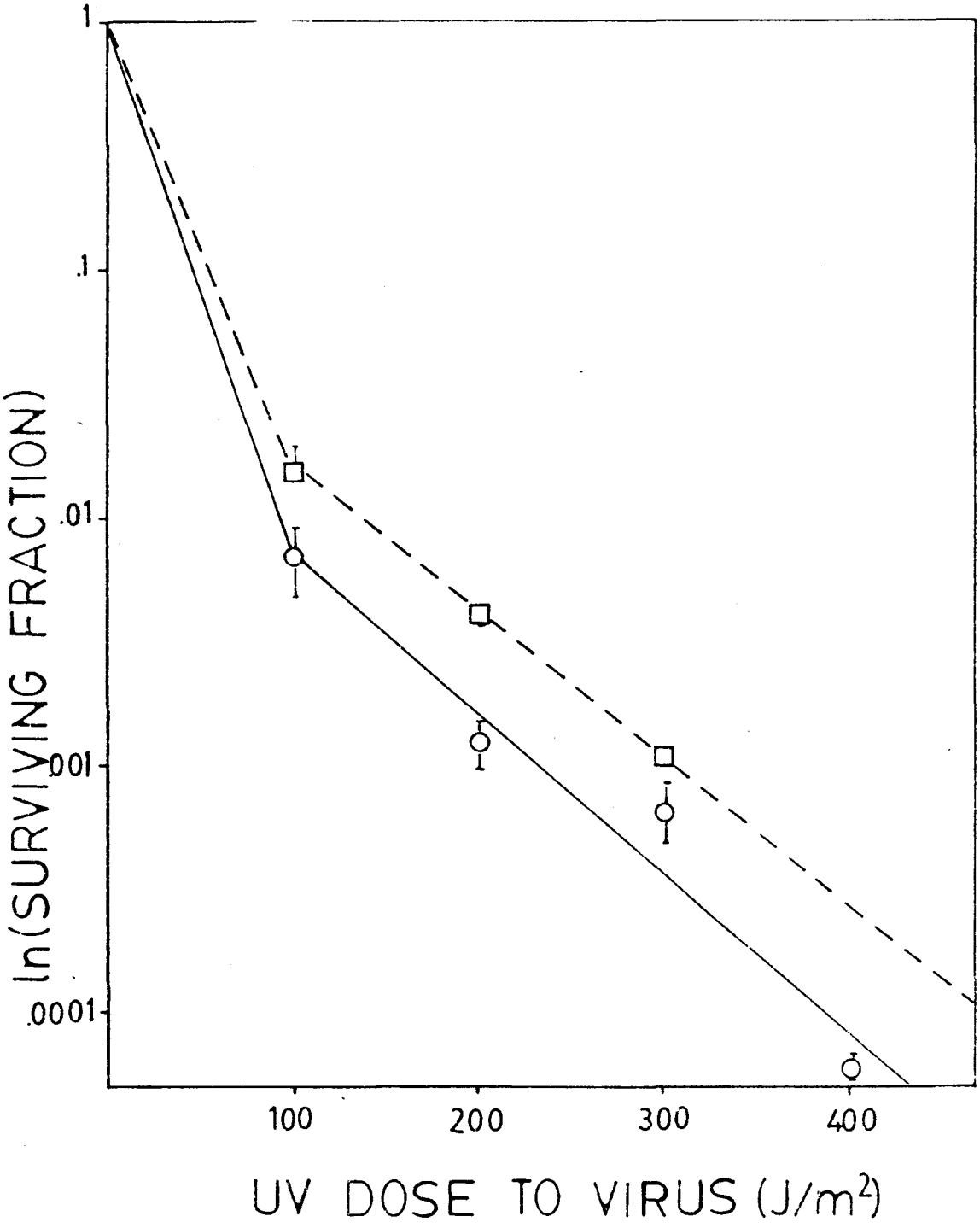

1
.1
.01
.001
.0001
ln(SURVIVING FRACTION)
100
200
300
400
UV DOSE TO VIRUS (J/m²)

Figure 11 Survival of plaque formation of UV-irradiated HSV-1 KOS WT and P5Aph⁻K2 in VERO cells

Surviving fraction as a function of viral UV dose

Virus : ○ HSV-1 KOS wild type D_o = 86 ± 9 J/m²

□ P5Aph⁻K2 D_o = 95 ± 4 J/m²

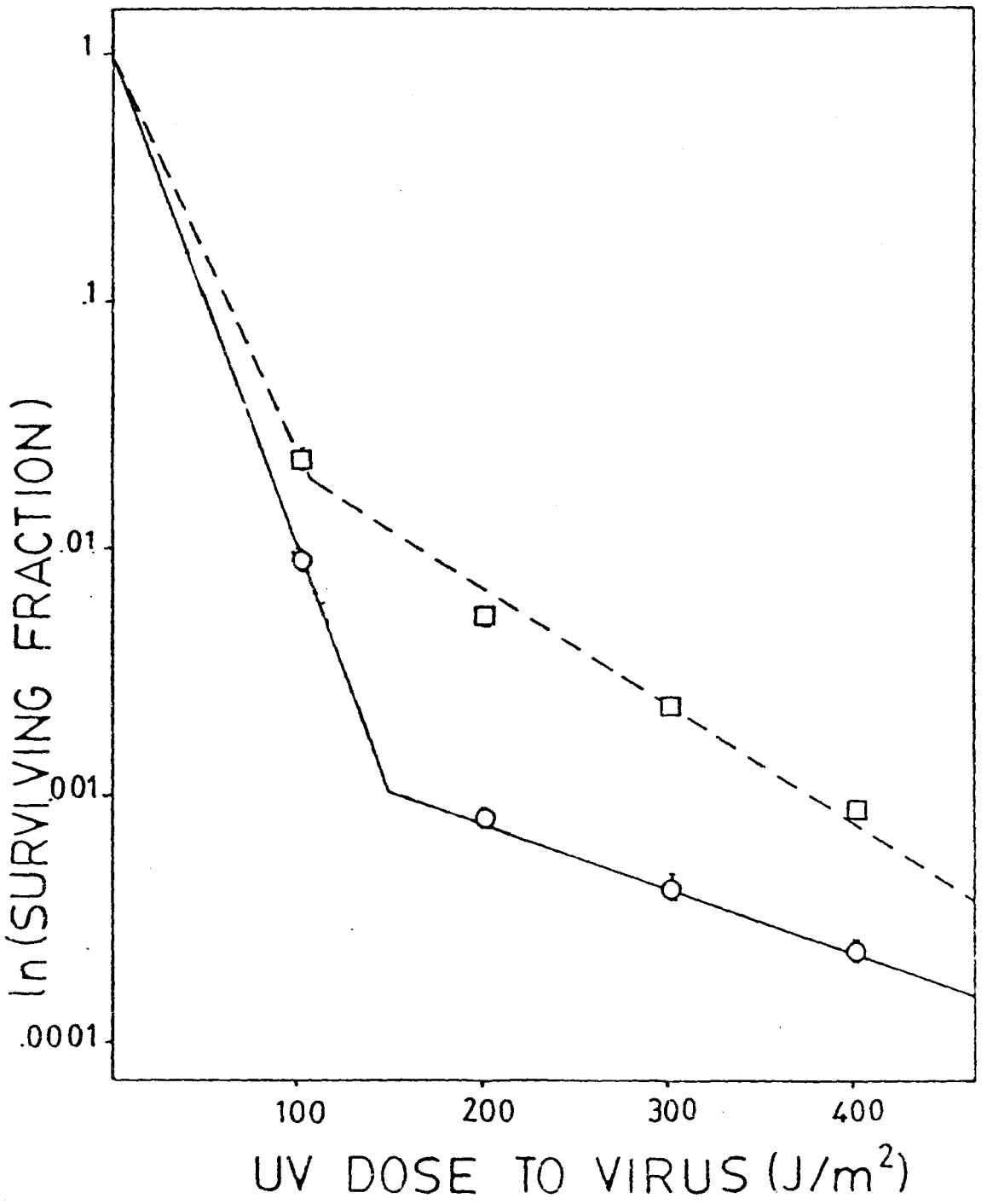

1
.1
.01
.001
.0001
ln (SURVIVING FRACTION)
100
200
300
400
UV DOSE TO VIRUS (J/m²)

Figure 12 Survival of plaque formation of UV-irradiated Paa^{r}5 and P5P^{r}H4 in VERO cells

Surviving fraction as a function of viral UV dose

Virus : ○ Paa^{r}5 D_o = 62 ± 3 J/m^2

□ P5P^{r}H4 D_o = 87 ± 4 J/m^2

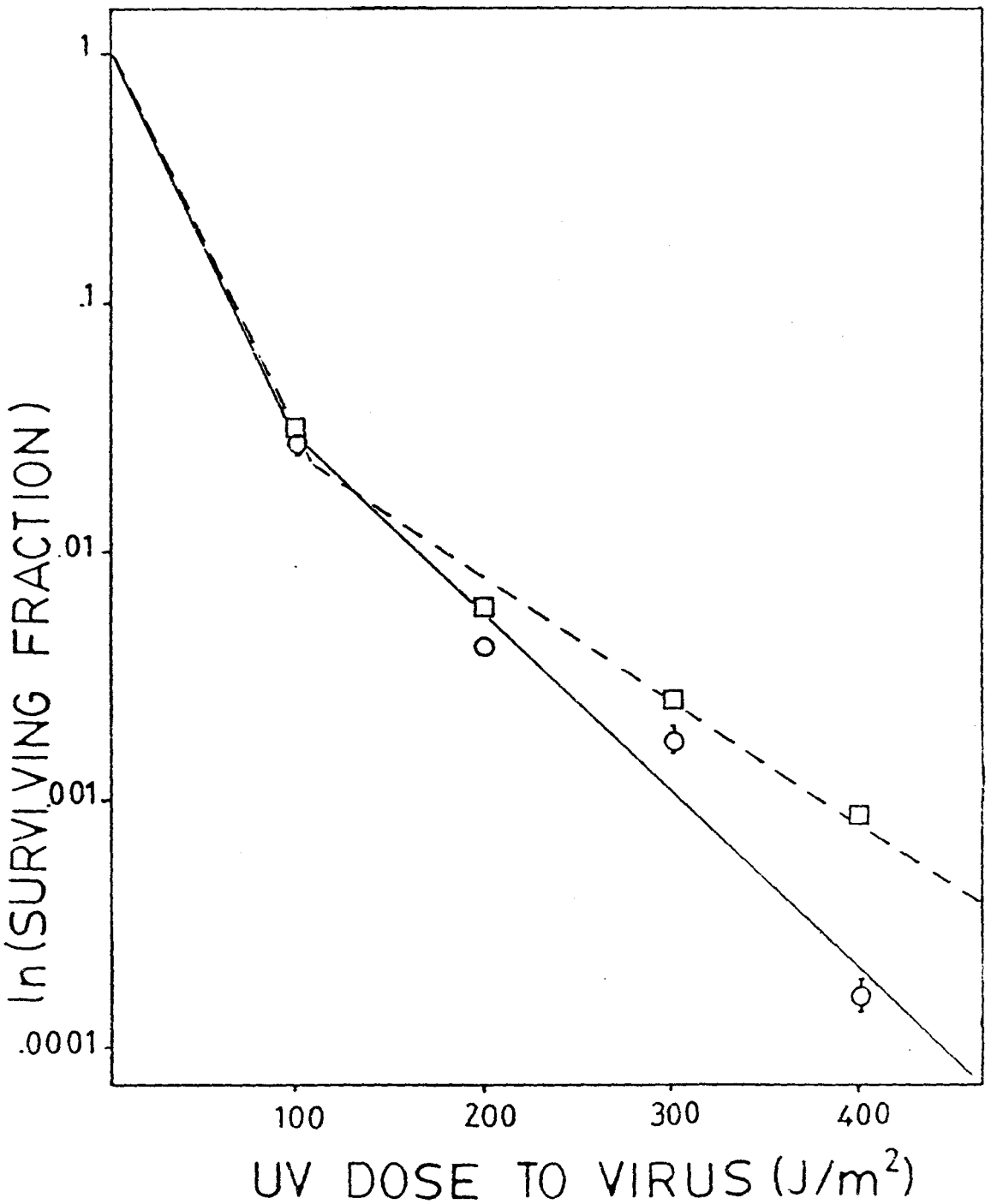

1
.1
.01
.001
.0001
ln (SURVIVING FRACTION)
100
200
300
400
UV DOSE TO VIRUS (J/m²)

Figure 13 Survival of plaque formation of UV-irradiated HSV-1 KOS WT and PTK3B in VERO cells

Surviving fraction as a function of viral UV dose

Virus : ○ HSV-1 KOS wild type D_o = 96 ± 12 J/m²

□ PTK3B D_o = 87 ± 5 J/m²

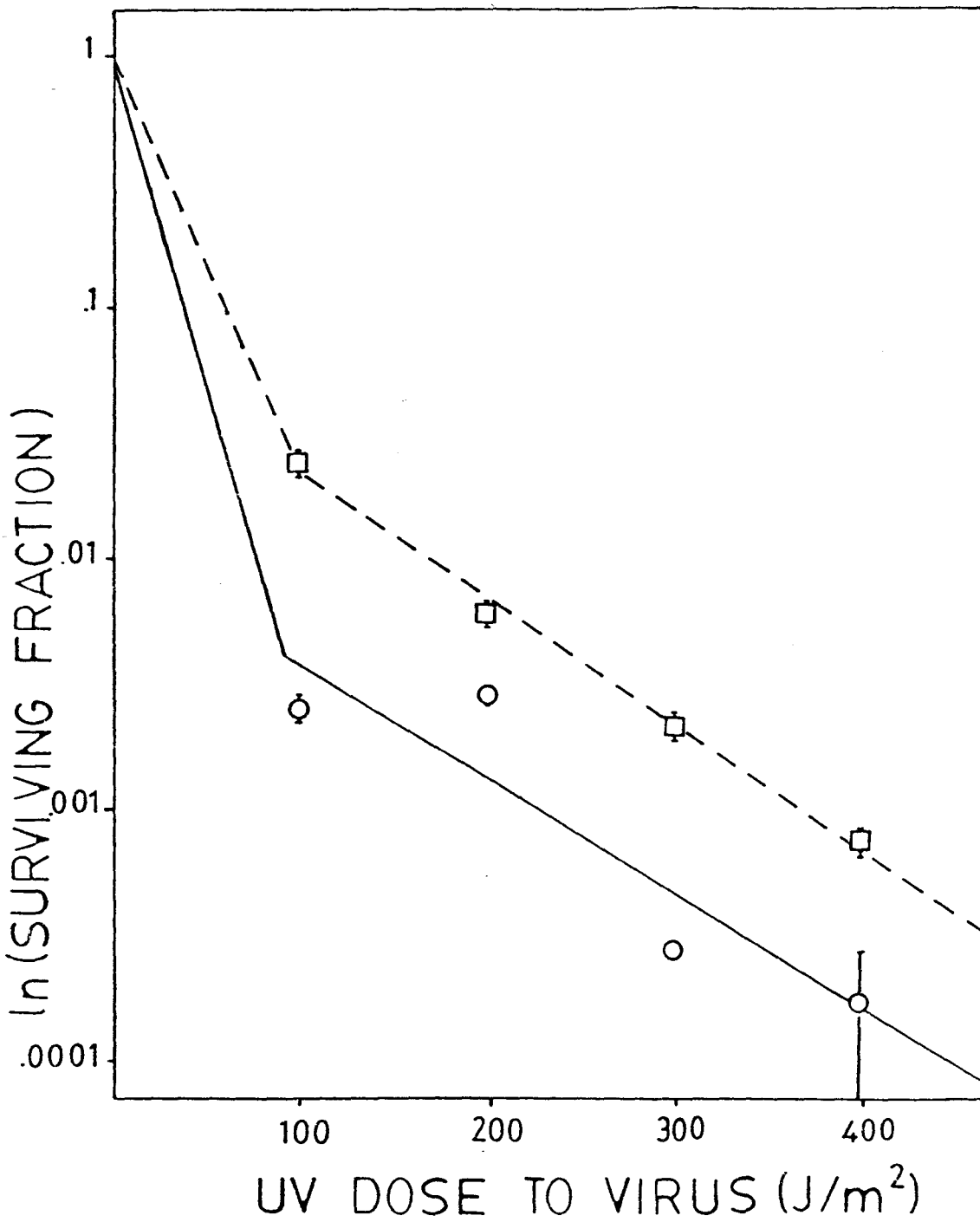

1
.1
.01
.001
.0001
ln (SURVIVING FRACTION)
100
200
300
400
UV DOSE TO VIRUS (J/m²)

Table 2 D_o values (J/m^2) obtained for several experiments for survival of plaque formation of UV-irradiated HSV-1

Expt. No.	HSV KOS WT	Paa^{r}5	PTK3B	P5P^{r}Ba8	P5Aph$^+$K2	P5P^{r}H4
1	96±12	60±10	87±5			
2		58±10	76±15			
3	103±13	77±7				
		60±4				
4		94±18				
		87±5				
5		51±2				
6	67±4			76±1		
7				85±5		
8				95±2		
9	43±1				64±4	
10	160±1				95±4	
11	84±2	70±1				110±5
12		62±3				87±4

Table 3 %HCR values (average) obtained for HSV-1 mutant viruses using VERO cells

VIRUS	Normalized to WT	No. Expt.	Normalized to Paa^{r}5	No. Expt.
Paa^{r}5	70$\pm$13	4		
PTK3B	91$\pm$17	1	138$\pm$33	2
P5P^{r}Ba8	113$\pm$8	1		
P5Aph^{r}K2	104$\pm$8	2		
P5P^{r}H4	131$\pm$9	1	149$\pm$11	2

Table 4 D_o (J/m^2) values obtained for several experiments for survival of plaque formation of UV-irradiated HSV-1 (pooled data)

Virus	1st component D_o	2nd component D_o	%HCR	No. of Expt.
HSV-1 KOS WT	21±1	85±5		6
Paa^{r}5	20±1	68±3	80±8	9
P5Aph^{r}K2	25±1	95±+2	112±9	2
P5P^{r}Ba8	23±1	87±3	102±10	3
P5P^{r}H4	29±1	97±3	114±10	2
PTK3B	24±2	91±4	107±11	2

This table lists the viruses used and the D_o values for the survival curves obtained. Cell Type : VERO.

Figures 10 through 12 show typical survival curves obtained using the recombinant viruses. The D_o values for the second components range from 87 to 97 J/m^2. Results from several experiments are given in tables 2,3 and 4.

The HCR of a thymidine kinase deficient HSV-1 mutant was also examined using VERO cells. The results from a typical experiment are shown in figure 13. The D_o for the second component is 87 ± 4J/m^2. Tables 2,3 and 4 give results for several experiments.

BI. Human Fibroblasts

a) Apparently Normal Human Fibroblasts

The plaque survival of KOS HSV-1 wild type, Paa^r5 and PTK3B virus was studied using the normal human fibroblast cell type CRL 1121. Figure 14 shows survival curves obtained. The D_o values for the second components of the curves were 83 ± 16, 54 ± 5, and 67 ± 9 J/m^2 respectively.

b) Xeroderma Pigmentosum

The plaque survival of KOS HSV-1 wild type and Paa^r5 virus was studied using Xeroderma Pigmentosum type A (GM 544B) fibroblasts. Typical survival curves obtained are shown in figure 15. The D_o value for the second components are 9 ± 1 J/m^2 for KOS HSV-1 wild type and 19 ± 1 J/m^2 for Paa^r5. Pooled data from two experiments is given in table 5.

c) Cockayne's Syndrome

The cell type used was GM 739. Typical survival curves obtained are shown in figure 16. The D_o values for the second components are 19 ± 1 J/m² for KOS HSV-1 wild type and 30 ± J/m² for Paa^{r}5. Pooled results from 2 separate experiments are shown in table 5.

Figure 14 Survival of plaque formation of UV-irradiated HSV-1 KOS WT, Paa^{r}5 and PTK3B in normal human fibroblasts

Surviving fraction as a function of viral UV dose

Cell type : CRL 1121

Virus : ○ HSV-1 KOS wild type D_o = 83 ± 16 J/m^2

□ Paa^{r}5 D_o = 53 ± 5 J/m^2

△ PTK3B D_o = 64 ± 9 J/m^2

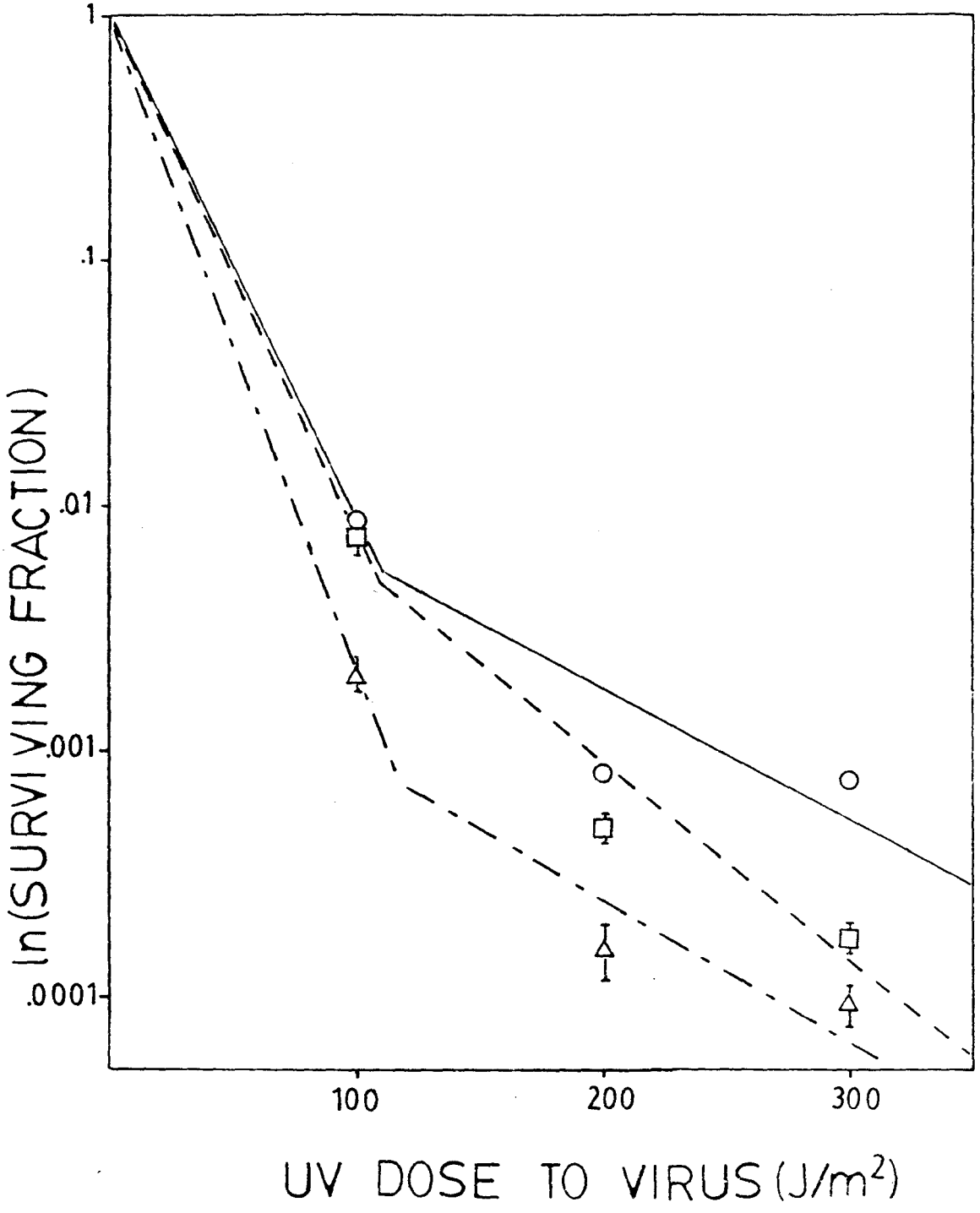

1
.1
.01
.001
.0001
ln(SURVIVING FRACTION)
100
200
300
UV DOSE TO VIRUS (J/m²)

Figure 15 Survival of plaque formation of UV-irradiated HSV-1 KOS WT and Paa^{r}5 in Xeroderma Pigmentosum cells

Surviving fraction as a function of viral UV dose

Cell type : GM 544B

Virus : ○ HSV-1 KOS wild type D_o = 9 ± 1 J/m^2

□ Paa^{r}5 D_o = 19 ± 1 J/m^2

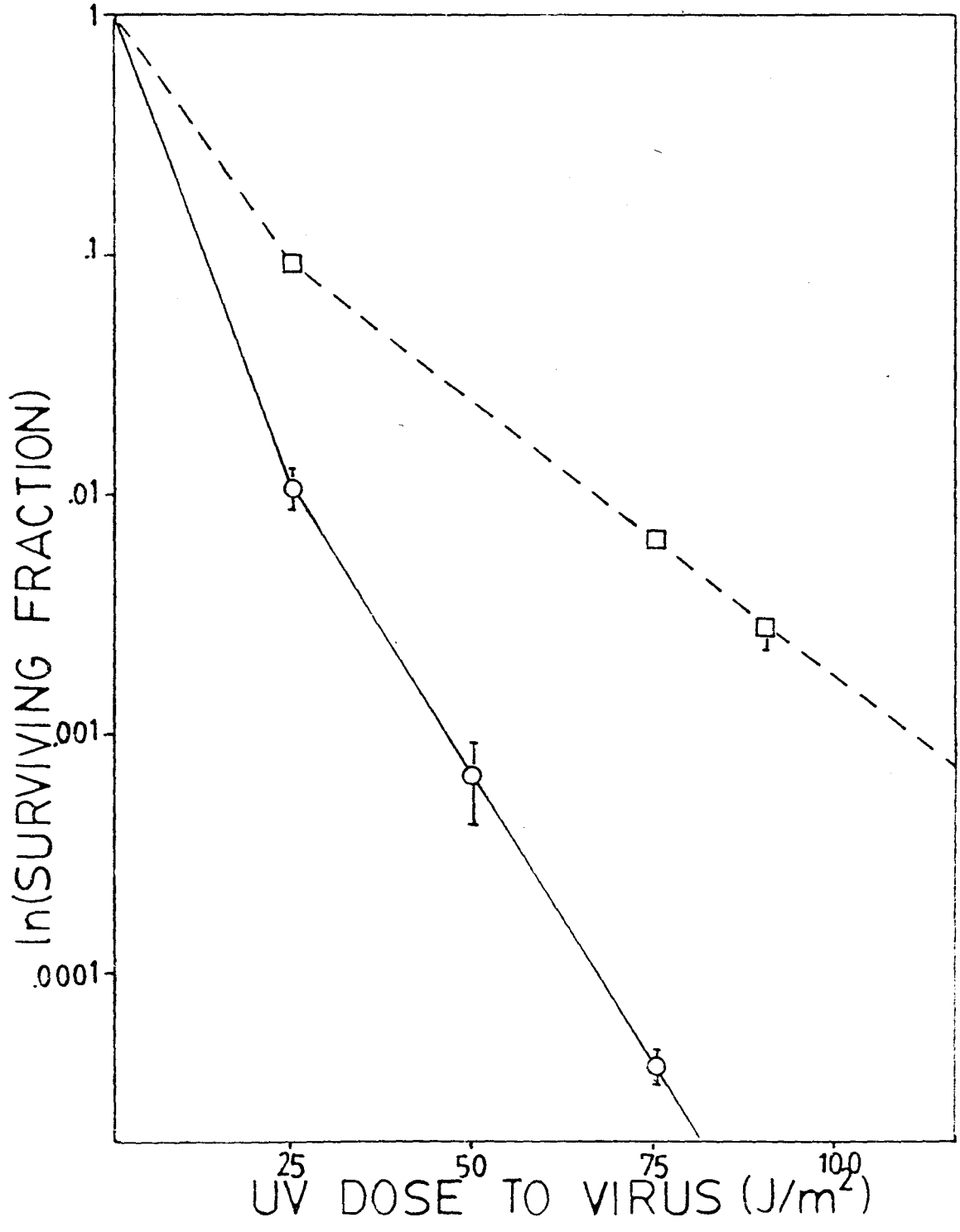

1
.1
.01
.001
.0001
ln(SURVIVING FRACTION)
25
50
75
100
UV DOSE TO VIRUS (J/m²)

Figure 16 Survival of plaque formation of UV-irradiated HSV-1 KOS WT and Paa^{r}5 in Cockayne Syndrome cells

Surviving fraction as a function of viral UV dose

Cell type : GM 739

Virus : ○ HSV-1 KOS wild type D_o = 19 ± 1 J/m^2

□ Paa^{r}5 D_o = 30 ± 1 J/m^2

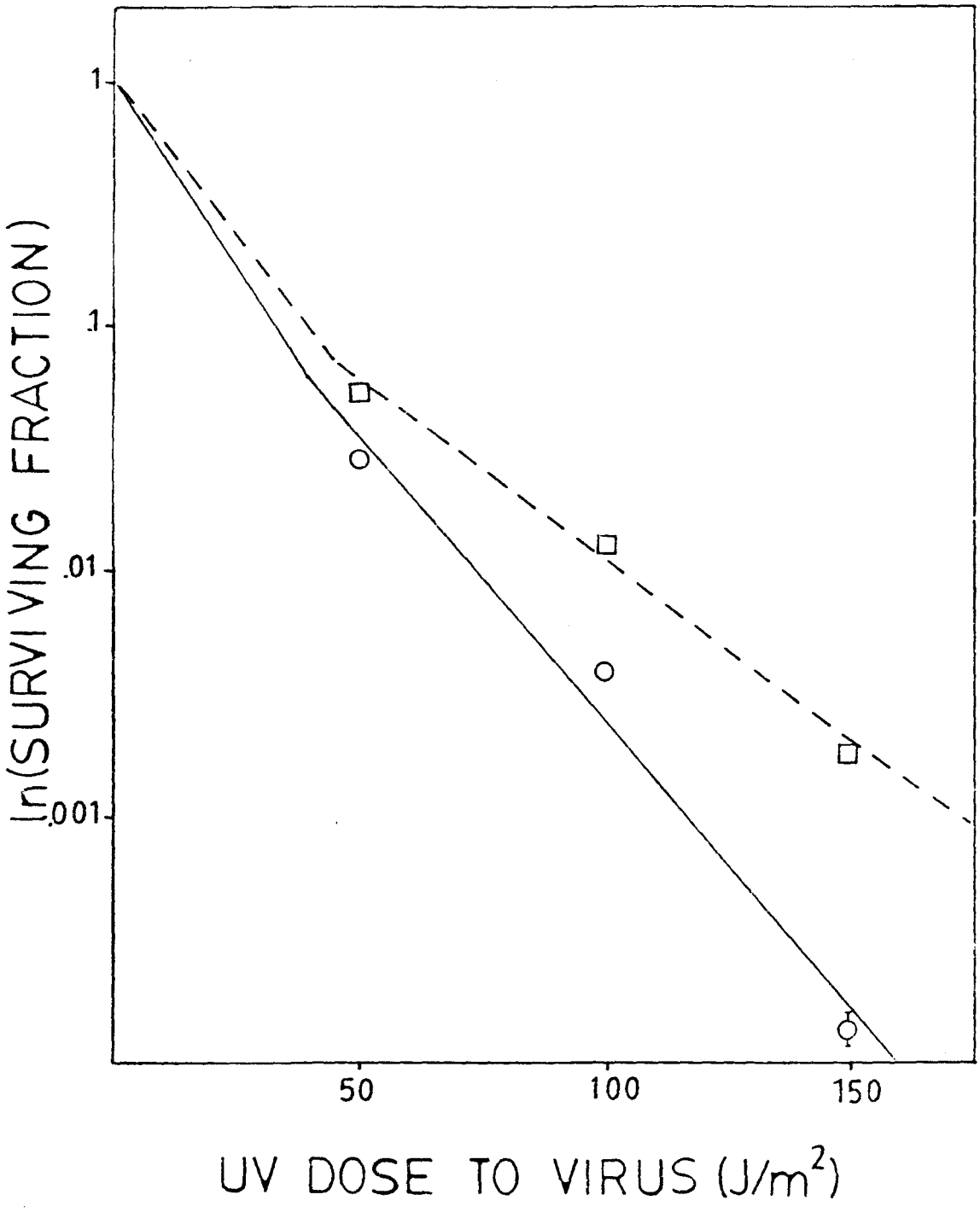

1
.1
.01
.001
ln(SURVIVING FRACTION)
50
100
150
UV DOSE TO VIRUS (J/m²)

Table 5 D_o values obtained for several experiments for survival of plaque formation of UV-irradiated HSV-1 in normal human fibroblasts, XP (A) and CS cells

Cell Type	Virus	D_o 2^{nd} comp.	%HCR	No. of Expt.
CRL 1121	HSV-1 KOS WT	83±16		1
	Paa^{r}5	54±5	65±19	1
	PTK3B	67±9	81±26	1
GM 544B	HSV-1 KOS WT	9±1	11±3	2
	Paa^{r}5	17±1	31±5	2
GM 739	HSV-1 KOS WT	19±1	23±6	2
	Paa^{r}5	43±12	80±30	2

This table shows the cell types and viruses used, and the D_o and %HCR values for the survival curves obtained.

DISCUSSION

Much research has been conducted into the causes of cancer and during the course of this research considerable attention has been focused on DNA repair and its relationship to cancer incidence. This interest in DNA repair arises from a number of sources. Firstly, carcinogens which are agents known to cause cancer also cause mutations in cellular DNA. It is these mutations, or rather the failure of the cell to correctly repair them which precipitates cancer incidence. Bouck et al. (1976) found that carcinogens induce cancer by producing somatic mutations. In addition, while studying the effects of a variety of carcinogens on cells, Newbold et al. (1980) found that carcinogens cause mutations in cellular DNA and the greater the carcinogenicity, the greater the number of mutations caused.

There also exist a number of human genetic autosomal recessive diseases which are characterized by a defect in DNA repair and at the same time an increased incidence of cancer. Xeroderma Pigmentosum (XP) is an example of these diseases. Cells taken from patients afflicted with this disease have a deficiency in DNA repair. For most XP cells, the deficiency lies in excision repair, while for XP variant it lies in postreplication repair. Maher et al (1977) found that XP cells have an increased mutability following UV exposure. XP patients

themselves are also sensitive to UV light. Accompanying these characteristics of XP is an increased elevation of cancer incidence.

A study was conducted (Squires et al., 1982) in which among others, normal, tumor and SV40 transformed cells were examined for their ability to make incisions in their DNA following UV exposure. The cells were incubated in the presence of inhibitors of DNA synthesis and this allowed the accumulation and subsequent detection of breaks made in the DNA. It was found that all tumor cells examined (HeLa S3, fibrosarcoma, colonic sarcoma, bladder sarcoma) fell in one group with break frequencies less than those of normal human fibroblasts. Similar findings resulted for SV40 transformed cells with break frequencies less than those for the tumor cells tested. Since incision of cellular DNA following an exposure to a DNA damaging agent is an integral component of excision repair, this decrease in the number of incisions made suggests a decrease in these cells' capacity to perform excision repair. Therefore it appears that the tumor cells and SV40 transformed cells studied by Squires et al. have a decrease in their DNA excision repair ability.

Thus there appears to be a link between a defect in DNA repair and the incidence of cancer. The nature of this link may be discovered through the study of cancer cells and

cells transformed by viruses into their cancerous counterparts.

However, it should be noted that a decrease in DNA repair ability accompanied by a cancer or transformed phenotype does not allow one to conclude that it is the cause of this state. Tumor and virally transformed cells exhibit a large array of characteristics which differentiate them from their normal counterparts. These include the ability to divide in vitro after confluency has been reached, changes in cell morphology and the acquirement of anchorage independence. Not all characteristics are necessary for the induction and maintenance of the cancer or transformed phenotype. As found by Shin et al. (1975) in direct reference to cells transformed by SV40, the single property which was consistently associated with tumorigenicity in nude mice was anchorage independence. Other properties of SV40 transformed cells such as expression of the SV40-Specific nuclear (T) antigen, insensitivity of cell growth to high cell density and the ability to grow in medium with reduced serum concentration were not necessary for the maintenance of tumorigenicity. By analogy with this observation, the reduction in the number of incisions made by tumor and SV40 transformed cells observed by Squires et al. (1982) may simply be a manifestation of the cancer or transformed

states of these cells. Resolution of this will involve the further study of the DNA repair abilities of tumor and virally transformed cells. If a deficiency in DNA repair is consistently associated with certain cancer or transformed cells, then it may be responsible for the altered state.

V Antigen Assay

In this work, normal human fibroblasts, tumor cells (HeLa CCL2) and transformed cells (293, 293 N3S) were examined for their ability to reactivate UV-irradiated adenovirus type 2 (Ad2). The ability of the cell to reactivate damaged virus will be indicative of its ability to repair its own DNA.

Typical survival curves obtained for these cell types are given in figures 5 to 8. All survival curves except those for V antigen formation of Ad2 in HeLa CCL2 consist of a single component, typical of single-hit inactivation kinetics. The curve for HeLa CCL2 consists of two components - an initial steep portion followed by a second less steep portion. Extrapolation of this second portion to the ordinate reveals that this represents 28 % of the total cell population.

The D_o value for pooled data for CRL 1121 is 233 $\pm$ 33 J/m^2. This D_o value was used as a control with which to compare the D_o values of other cell types. Previously reported values of the D_o for survival curves for V antigen

formation of Ad2 in normal human fibroblasts range from 320 ± 40 to 370 ± 40 J/m^2 (Krepinsky et al., 1980) and 240 ± 20 J/m^2 to 280 ± 50 J/m^2 (Rainbow, 1984). Similar experimental procedures were used in these studies , however there could be differences in dosimetry thus accounting for the differences in D_o values.

For each cell type, a %HCR value was calculated as:

$$\%HCR = \frac{D_o(\text{cell type of interest})}{D_o(\text{control cell type})}$$

D_o and %HCR values for pooled data are given in table 1. The D_o and %HCR values corresponding to the first component of the survival curve for HeLa CCL2 are 72 ± 2 J/m^2 and 31 ± 4 %. This represents the majority of the cell population, and this value agrees well with a reported observation of 30% (Rainbow, 1984). Another reported value for the D_o for the survival of Ad2 V antigen formation in HeLa cells is 58 J/m^2 (Johnson et al., 1980) (as estimated from graph by author). This D_o value differs from the previously mentionned values , and this may be due to dosimetry.

The %HCR value of 31 ± 4 % observed suggests a corresponding decrease in the cells' ability to repair UV DNA damage. This is supported by previously mentionned observations made by Squires et al. (1982) that HeLa CCL2

cells made less incisions in UV-damaged DNA than normal cells. Therefore it is possible that the cancerous phenotype of HeLa CCL2 cells results from a decrease in the cells' ability to repair cellular DNA damage. Nonetheless, one cannot conclude that this is the case since a decrease in DNA repair could be a manifestation of the cancerous state. This would be the case if it were possible to isolate HeLa CCL2 cells with normal DNA repair and yet a cancerous phenotype.

The two component nature for the V antigen survival curves observed differs from other observations of single component curves (Johnson et al., 1986) and is interpreted to be the result of the existence of a subpopulation of cells with an increased ability to repair UV damaged Ad2 as compared to the majority of the HeLa CCL2 cells. The %HCR corresponding to this subpopulation was 73 ± 15 % and this suggests a DNA repair ability between that of the majority of the HeLa CCL2 cells and normal cells. If the tumorigenicity of these cells is also between that of normal cells and HeLa CCL2 cells, then this would suggest that the decrease in DNA repair for HeLa CCL2 cells is the cause of the cancer phenotype. This could be investigated by examining the UV survival of the cells themselves. A two component survival curve consisting of a steep portion followed by a less steep portion would

indicate that there exists a subpopulation of cells with a greater repair ability of UV damaged DNA. Resistant components have been observed for survival curves of certain viruses (Drake, 1958; Barny, 1961; Abel, 1962). These components are the result of multiplicity reactivation and involve the infection of a single cell by large numbers of damaged virus. Complementation of functional and genetic information results in the formation of 'intact' viral progeny. When multiplicity reactivation occurs, there no longer exists a linear relationship between virus concentration added to the cell monolayer and number of plaques formed (Lytle, 1971), or in this case the number of V antigen positive cells. Figure 17 shows that for the studies of HCR of V antigen formation by UV irradiated Ad2 using HeLa CCL2 cells, there exists a linear relationship between V antigen positive cells and relative virus concentration for even the largest viral UV doses. Therefore, multiplicity reactivation is not the cause of the resistive component.

The %HCR values obtained using pooled data for 293 and 293 N3S cells were 76 ± 19% and 98 ± 18% respectively. Thus 293 N3S cells have either no repair deficiency for UV damaged DNA, or a deficiency so little that it is not detected in this assay. 293 cells may have a very slight repair deficiency. Squires et al. (1982) examined W138 and Bloom's Syndrome cells transformed by SV40 and found that

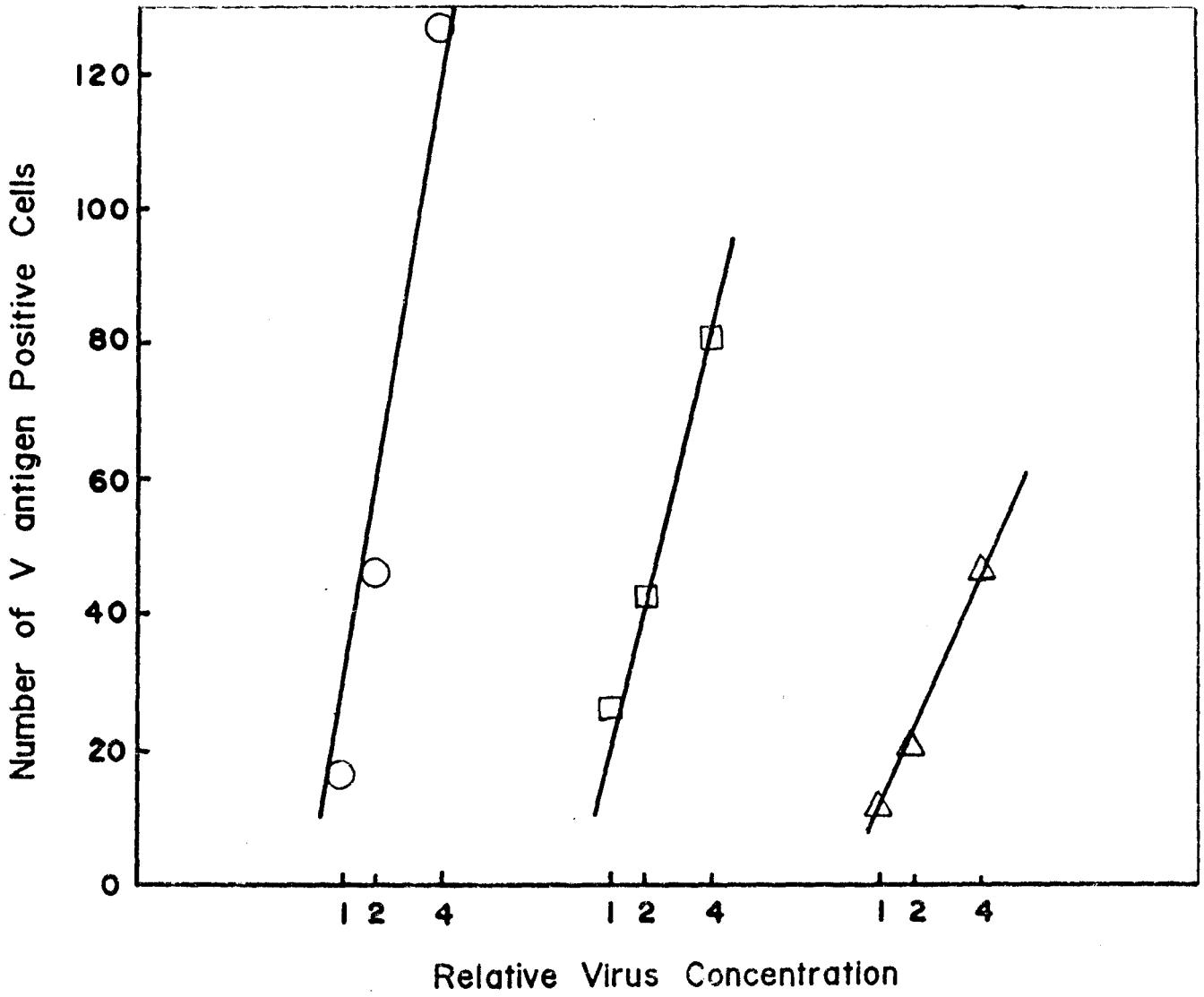
FIGURE 17 Number of V antigen positive cells scored as a function of relative virus concentration

This figure shows the relationship between the number of V antigen positive cells scorred and relative virus concentration.

○ Viral UV dose = 400 J/m^2

□ Viral UV dose = 600 J/m^2

△ Viral UV dose = 800 J/m^2


Number of V antigen Positive Cells
120
100
80
60
40
20
0
1 2 4
1 2 4
1 2 4
Relative Virus Concentration

they exhibited break frequencies less than those of tumor, normal and Bloom's Syndrome cells following UV exposure. This suggests that these SV40 transformed cells have a defect in excision repair greater than that of tumor cells. Therefore, since the 293 cells do not give rise to a decrease in HCR comparable to or larger than that of HeLa CCL2 cells, it appears that the mechanism by which Ad5 transforms these cells differs from the mechanism by which SV40 transforms the cell types studied by Squires.

293 N3S cells exhibit an increased tumorigenicity as compared to 293 cells; however, since these cells also exhibit no decrease in DNA repair ability relative to that of 293 cells, the increased tumorigenicity does not arise from a loss of DNA repair. 293 N3S cells have an increased anchorage independence and this is most likely the cause of the increased tumorigenicity.

HSV-1 Plaque Assay

HSV-1 encodes both a polymerase and a thymidine kinase activity which are involved in viral DNA synthesis (Coppey, 1977; Coen et al., 1984) and may or may not be involved in viral DNA repair. In order to determine their possible roles in viral DNA repair, the HCR of plaque formation of HSV-1 KOS wild type and mutants, PTK3B (thymidine kinase deficient) and Paa^{r}5 (encoding an altered DNA polymerase) was studied.

Since HSV-1 encodes viral DNA synthesis enzymes which might also be involved in viral DNA repair, the HCR of the virus is not solely attributable to cellular DNA repair mechanisms. However, as long as the same host cell type is used, any change in the HCR of these viruses will be due to a change in the viral DNA synthesis enzymes produced and therefore implicate them in viral DNA repair.

Figures 9 and 13 show typical survival curves obtained. These curves consist of two components, and this is typical of infections with herpes virus (Lytle, 1971; Lytle et al., 1972; Ryan and Rainbow, 1986). Both single and two component survival curves for UV irradiated HSV survival in mammalian cells have been observed (Lytle, 1971). The reason for this two component nature has not as yet been determined; however factors such as an inhomogeneous cell population and multiplicity reactivation have been ruled out. Single component viral survival curves were observed for normal mouse cells and one adult human skin fibroblast strain. The transformed counterpart of the mouse cell strain gave rise to a two component viral survival curve (Lytle and Benane, 1974) thus implicating the loss of contact inhibition as the cause of the resistant component. Table 2 shows the D_o values obtained for the second components of the viral survival curves obtained. The %HCR values were calculated

by averaging %HCR values obtained in separate experiments (refer to table 3), and also by pooling the data (refer to table 4).

The D_o values obtained using PTK3B are not different within error from that of KOS HSV WT. Therefore there is no decrease in %HCR as compared to WT. However, the survival fractions obtained using PTK3B are higher than those obtained using WT. Therefore, the lack of thymidine kinase activity has changed the survival curve and this indicates that the tk activity might be involved in repair of UV damaged HSV-1 DNA.

The D_o value for the survival curve using Paa^{r}5 is significantly less than that obtained using HSV KOS WT. This suggests that not only is the HSV-1 polymerase involved in DNA repair, but that the substitution of Paa^{r}5 for HSV WT encoded polymerase results in a decrease in viral reactivation.

Paa^{r}5 produces a polymerase which differs from that produced by HSV-1 KOS wild type in that the polymerase is an antimutator and is resistant to PAA. Since it is an antimutator, it would be expected that it would correctly repair a greater number of damage sites and that this would lead to a higher virus survival and greater D_o than that for wild type virus. However, this is the opposite of what was observed. A number of factors could be responsible for

the decrease in D_o for Paa^{r}5. Firstly, the Paa^{r}5 polymerase could repair viral DNA at a rate slower than that for the wild type polymerase. If this were true, it would repair a smaller number of damage sites in the allowed time, and this could lead to the observed decrease in viral survival. This could be verified by increasing the time from infection to staining beyond the 48 hours allowed in this assay. Increases in the time between infection and staining would allow the Paa^{r}5 encoded polymerase to repair greater numbers of damage sites. Eventually, since the number of damage sites are finite it would repair as many damage sites as the WT polymerase. Since Paa^{r}5 is an antimutator, a greater number of damage sites would be correctly repaired and this would lead to an increase in %HCR. However, since there is no evidence that it synthesizes DNA at a slower rate (Hall et al., 1984) it is unlikely that it carries out repair replication at a slower rate. Secondly, the herpes virus polymerase has an associated exonuclease activity (Knopf, 1979) and it is possible that the exonuclease participates in viral DNA repair. If the Paa^{r}5 exonuclease removes damage at a slower rate than that of wild type polymerase, or if it altogether fails to remove damage recognized by the wild type polymerase, then this would lead to a lower virus survival.

The exact reason for the observed decrease in D_o

may be discovered once the structure of the HSV-1 polymerase is more fully understood.

As mentionned earlier, Paa^{r}5 contains one or more mutations in the polymerase gene. There exist a number of recombinant viruses containing part of the wild type and part of the Paa^{r}5 genome (refer to figure 8). P5P^{r}Ba8 and P5P^{r}H4 consist of a wild type background with a Paa^{r}5 insert. As a result, these viruses produce polymerases resistant to PAA. P5P^{r}K2 consists of a Paa^{r}5 background with a wild type insert. The polymerase produced is sensitive to PAA. It should be noted that since these are recombinant viruses, the actual inserts may be less than or equal to those shown in figure 8.

The HCR of these viruses was examined using VERO cells. If P5P^{r}Ba8 and P5P^{r}H4 had given rise to HCR values similar to that of Paa^{r}5, and P5Aph^{r}K2 had given rise to an HCR value similar to wild type, then it would have been apparent that the change in D_o for Paa^{r}5 was due to mutations in the polymerase gene and not possible mutations elsewhere on the Paa^{r}5 genome.

Typical survival curves obtained using the recombinant viruses are shown in figures 11 to 13 and the D_o values for the first and second components and %HCR values for several experiments are given in tables 2,3 and 4. The D_o values obtained using P5P^{r}H4 and P5P^{r}Ba8 were not

significantly lower than that for HSV-1 KOS wild type. Therefore, although these viruses gained resistance to PAA, there was no decrease in the virus survival. The average %HCR value for P5Aph^{-}K2 is 104 ± 8 and this suggests that the wild type insert which produced a sensitivity to PAA returned the HCR to a normal level.

If the mutation responsible for the decrease in the D_o value for Paa^{r}5 was identical to or closely-linked to that which was responsible for the phenotype of resistance to PAA, then P5P^{r}H4 would be expected to produce a D_o value similar to that of Paa^{r}5. It has been suggested that Paa^{r}5 contains an additional mutation further away on the polymerase gene and which is not transferred to all the recombinants (Hall et al., 1984). The presence of this mutation in addition to that identical to or closely linked to that responsible for the phenotype of PAA resistance may be necessary for a reduction in the D_o. Thus the failure to observe a reduction in the D_o value for P5P^{r}H4 could be due to the absence of this additional mutation. The existence of a second mutation on the polymerase gene could be verified by sequencing the gene.

Normal Human Fibroblasts

The survival of HSV-1 KOS wild type, Paa^{r}5 and PTK3B was also examined using apparently normal human fibroblasts. The survival curves obtained are two component

curves and are shown in figure 15. The D_o values for the second components are given in table 5. As found using VERO cells, there was no significant difference between the D_o values for HSV-1 KOS wild type and PTK3B. The survival of PTK3B was however lower than that for WT virus. This is further support that the HSV-1 thymidine kinase activity is in some way involved in viral DNA repair. The D_o for the second component using Paa^{r}5 is significantly lower than that for HSV-1 KOS wild type. Similarly this indicates that the HSV-1 polymerase is involved in viral DNA repair.

Xeroderma Pigmentosum

The survival of HSV-1 KOS wild type and Paa^{r}5 virus was examined using Xeroderma Pigmentosum complementation group A (GM544B) cells. Typical survival curves obtained are shown in figure 16. Pooled data from two separate experiments was used to determine the D_o values for the second components of the survival curves (refer to table 5). The D_o value obtained for HSV-1 KOS wild type virus is 9 $\pm$ 1 J/m^2 and this value differs from reported values of 23.5 J/m^2, 38.2 J/m^2 (Ryan and Rainbow, 1986) and 5.5 $\pm$ 1.2 J/m^2 (Lytle et al., 1982) for other XP(A) strains. The differences between these values could be due to the differences between these strains and also to dosimetry and the fact that Ryan and Rainbow used HSV-2 virus and Lytle et al. used the HSV-1 macro plaque strain.

virus is 17 ± 1 J/m² and this is significantly greater than that of 9 ± 1 J/m² obtained using HSV-1 KOS wild type virus. As described in the previous section, the D_o value for Paa^{r}5 virus is significantly less than that for HSV-1 KOS wild type for infection of normal human fibroblasts or VERO cells. Since the only variable changed between the experiments with normal human fibroblasts and those with Xeroderma Pigmentosum cells is the cell type, these different observations must be due to a difference in these cells' ability to repair HSV-1 DNA.

Nonetheless, the reason for this observation is uncertain. It has been suggested that XP cells lack one or more enzymes which together participate in the DNA repair process. It is possible that the HSV-1 virus polymerase complements this deficiency .

If Paa^{r}5 had acquired an associated endonuclease activity recognizing UV damage, then a higher virus survival would be anticipated since a greater number of damage sites would be repaired. However, since neither HSV-1 polymerase or human polymerase contain an associated endonuclease activity (Ostrander and Chang, 1980), this is unlikely.

XP A cells have a deficiency in excision repair and make few to no incisions following UV DNA damage (Paterson et al., 1973). This deficiency may be the result of a lack

et al., 1973). This deficiency may be the result of a lack of UV endonucleases or enzymes necessary to the recognition of the more predominant forms of UV damage such as pyrimidine dimers (Selsky and Greer, 1978). It is also possible that those damage sites which are recognized by an endonuclease are sites which the Paa^{r}5 and KOS WT polymerases' associated exonuclease activities may remove. In this case, the HSV-1 KOS wild type and Paa^{r}5 polymerases would repair the same number of damage sites. Since the Paa^{r}5 is an antimutator, it would make fewer mistakes than the wild type polymerase and this would result in the observed increase in Paa^{r}5 virus survival.

Cockayne's Syndrome

The survival of HSV-1 KOS wild type and Paa^{r}r5 virus was also examined using Cockayne's Syndrome (CS) (GM739) cells. Figure 17 shows typical survival curves obtained. Using pooled data from two separate experiments, the D_o for the second component was 19 ± 1 J/m^2 for HSV-1 KOS wild type virus. This value is lower than reported values of 64.6 and 71.1 J/m^2 (Ryan and Rainbow. 1986) for other CS strains.

The D_o for the second component, using pooled data was 43 ± 12 J/m^2 for Paa^{r}5, and as observed earlier using XP cells is significantly greater than that obtained for HSV-1 KOS wild type.

determined. It has been suggested that CS could have a decreased fidelity of unscheduled DNA synthesis (Andrews et al., 1978). Since the polymerase produced by Paa^{r}5 is an antimutator, survival of plaque formation by Paa^{r}5 could be increased above that for HSV-1 KOS wild type because it complements the deficiency to a greater extent than the wild type virus does.

In summary, The HCR of V antigen formation of UV-irradiated Ad2 and of the plaque formation of UV-irradiated HSV-1 was measured. This allowed the comparison of the abilities of a variety of cell types to repair UV-damaged Ad 2 and HSV-1 DNA. It was determined that HeLa CCL2 cells have a reduced ability to repair UV damaged DNA, whereas 293 and 293 N3S cells, which have been transformed using Ad 5 DNA have little to no deficiency.

The results for HCR of plaque formation by UV-irradiated HSV-1 suggest that the HSV-1 polymerase and tk activity play a role in the repair of UV damaged viral DNA. The mutant Paa^{r}5 contains one or more mutations in the polymerase gene which confer an altered polymerase which is resistant to PAA and is an antimutator. It is not certain whether the mutation responsible for the resistance to PAA is solely responsible for the decrease in HCR observed using Paa^{r}5 in place of HSV-1 KOS WT.

The HCR observed using CS and XP cells suggests

that the Paa^{r}5 polymerase may complement the CS and XP DNA repair deficiencies to a greater extent than the WT polymerase. The exact nature of any complementation may be discovered when the structure and function of the HSV-1 polymerase is better understood.

APPENDIX

Each assay required the infection of cell monolayers with virus at a minimum of three serial dilutions per UV dose to the virus. The number of viral antigen positive cells or the number of plaques in each monolayer was plotted as function of virus concentration and the value for the highest virus concentration was predicted using the following linear regresion.

Given that for zero virus concentration, the number of viral antigen positive cells or the number of plaques in each monolayer is zero, a linear regression was fitted through the origin. For the regression, the number of viral antigen positive cells or the number of plaques in each monolayer, and the virus concentrations were represented by Y and X respectively. The regression gives the following relationship:

$Y = \beta X + \epsilon$ (Snedecor and Cochran, 1980)

where : β is the slope

ϵ is a random variable drawn from $(0, \sigma^2)$

The estimate of Y ie. Y for X_o, a previously measured X value is:

$Y = bX_i$ where $b = \Sigma X_i Y_i / \Sigma X^2$ and the standard error is given by

$S_y = X_o S_{y,x} / \Sigma X_i^2$

where $S_{y,x} = (\Sigma Y_i^2 - (\Sigma X_i Y_i)^2)/(n-1)$

and n is the sample size.

Using these calculated values, and taking the dilution factors into account, the surviving fraction for each UV dose to the virus was calculated.

BIBLIOGRAPHY

Abel, P. (1962) Multiplicity and marker rescue with Vaccinia virus. Virology 17, 511-519.

Abrahams, P.J., 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.

Alberts,B., Bray,D., Lewis, J., Raff, M., Roberts, K., and Watson, J.D. 1983. Molecular Biology of the cell. New York: Garland.

Andrews, A.D., Barrett, S.F., Yoder, F.W., and Robbins, J.M. (1978) Cockayne's syndrome fibroblasts have increase of sensitivity to ultraviolet light but normal rates of unscheduled DNA synthesis. J. Invest. Derm. 70, 237-239.

Barry, R.D. (1961) The multiplication of Influenza virus II. Multiplicity reactivation of ultraviolet irradiated virus. Virology 14, 398-405.

Chen, L.B., Gallimore, P.H., and McDougall, J.K. (1976) Correlation between tumor induction and the large external transformation sensitive protein on the large external transformation sensitive protein on the cell surface. P.N.A.S. 73, 3570-3574.

Cleaver, J.E. (1978) DNA repair and its coupling to DNA replication in eukaryotic cells. Biochem. Biophys. Acta 516, 489 - 516.

Cleaver, J.E. (1970) DNA repair and radiation sensitivity in human (xeroderma pigmentosum) cells. Int. J. Rad. Biol. 18, 557 - 565.

Coen, D.M., Aschman, D.P., Gelep, P.T., Retardo, M.J., Weller, J.K., and Schaffer, P.A. (1984) Fine mapping and molecular cloning of mutations in the herpes simplex virus DNA polymerase locus. Virology 49, 236 - 247.

Cook, J.S., Regan, J.D. (1969) Photoreactivation and photoreactivating enzyme activity in an order of mammals (Marsupialia). Nature 223, 1066 - 1067.

Coppey, J. (1977) Common precursor pathways of herpes DNA and of repair synthesis in ultraviolet irradiated cells. Nature 265, 260 - 267.

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

Drake,J.W. (1958) Interference and multiplicity reactivation in Poliovirus. Virology 6, 244-264.

Fischer,E., Keijzer,W., Thielmann, H.W., Popanda,D., Bohnert,E., Edler,L., Jung,E.G., and Bootsma,D. (1985) A ninth complementation group in xeroderma pigmentosum, XP I. Mutat. Res. 145,217-225.

Friedberg, E.C., Ehman, U.K., Williams, J.I. (1979) Human diseases associated with defective DNA repair. Adv. Rad. Biol. 8, 86 - 174.

Gallimore, (1972) Tumour production in immunosuppressed rats with cells transformed in vitro by Adenovirus type 2. J. Gen. Virol. 16, 99-102.

Gibbs, J.S., Chiou, H.C., Hall,J. D., Mount, D.W., Retondo, M.J., Weller, J.K., and Coen, D.M. (1985) Sequence and mapping analyses of the herpes simplex virus DNA polymerase gene predict a C-terminal substrate binding domain. P.N.A.S. 82, 7969-7973.

Graham, F.L. and Smiley, J. (1977) Characterization of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. 36, 59 - 72.

Graham, F.L., Van der Eb, A.J., and Heijneker, H.L. (1974) Size and location of the transforming region in human adenovirus type 5 DNA. Nature 251, 687-691.

Graham, F.L., Veldhuisen, G., and Wilkie, N.M. (1973) Infectious herpesvirus DNA. Nature New Biology 245, 265-266.

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

Hall, J.D., Almy, R.E. 1982) Evidence for the control of herpes simplex virus mutagenesis by the viral DNA polymerase. Virology 116, 535 - 543.

Hall, J., Coen, D., Fisher, B., Weisslitz, M., Randall, S., Almy, R., Gelep, P., and Schaffer, P. (1984) Generation of genetic diversity in herpes simplex virus: an antimutator phenotype maps to the DNA polymerase locus. Virology 132, 26 - 37.

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.

Hoar, D.I., and Waghorne, C. (1978) DNA repair in Cockayne syndrome. Am. J. Hum. Genet. 30, 590-601.

Jofre, J.T., Schaffer, P.A., and Parris, D.S. (1977) Genetics of resistance to phosphonoacetic acid in strain KOS of herpes simplex virus type 1. J. Virol. 23, 833-836.

Johnson, R.T., Squires, S., Elliott, G.C., Rainbow, A.J., Koch, G.L.E., and Smith, M. (1986) Analysis of DNA repair in XP-HeLa hybrids; lack of correlation between excision repair of UV damage and adenovirus reactivation in an XP(D) - like cell line. Carcinogenesis 7, 1733-1738.

Knopf, K.W. (1979) Properties of herpes simplex virus DNA polymerase and characterization of its associated exonuclease activity. Eur. J. Biochem. 98, 231 - 244.

Krepinsky,A.B., Rainbow, A.J., and Heddle, J.A. (1980) Studies on the ultraviolet light sensitivity of Bloom's Syndrome fibroblasts. Mutat. Res. 69, 357-368.

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

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

Lytle, C.D., Benane, S.G. (1975) Effects of photoreactivating light on virus infection of UV-exposed potoroo cells. Int. J. of Rad. Biol. 27, 487 - 491.

Lytle, C., Ray, R. III., Hellman, K., and Bockstahler, L. (1976) Infection of UV-irradiated xeroderma pigmentosum fibroblasts by herpes simplex virus: study of capacity and Weigle Reactivation. Mutat. Res. 36, 257 - 264.

Lytle, C., Aaronson, H. (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. Rad. Biol. 22, 159 - 165.

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

Lytle, C. Nikaido, O., Hitchins, V. and Jacobsen, E. (1982) Host cell reactivation by excision repair is error - free in human cells. Mutat. Res. 94, 405 - 412.

Maher, V.M., McCormick, J.J., Grover, P.L., Sims, P. (1977) Effect of DNA repair on the cytotoxicity and mutagenicity of polycyclic hydrocarbon derivatives in normal and xeroderma pigmentosum human fibroblasts. Mutat. Res. 43, 117 - 138.

McKenna, P.G., Yassen, A.A., and McKelvey, V.J. (1985) Evidence for the indirect involvement of thymidine kinase in excision repair processes in mouse cell lines. Som. Cell. Mol. Gen. 11, 239-246.

Newbold, R.F., Warren, W., Medcalf, A.S.C., Amos, J. (1980) Mutagenicity of carcinogenic methylating agents is associated with a specific DNA modification. Nature 283, 596 - 599.

Niemann, E.G. (1982) Radiation Biophysics, In Biophysics, eds. W. Hoppe, W. Lohmann, H. Markl, H. Ziegler. Springer Verlag, New York. pp. 289 - 300.

Ostrander, M., Cheng, Y. (1980) Properties of herpes simplex virus type I and type 2 DNA polymerase. Biochem. Biophs. Acta 609, 232 - 245.

Overby, L.R., Robishaw, E.E., Scheicher, J.B., Skipkowitz, A.R., Mao, J.C.-H. (1974) Inhibition of herpes simplex virus replication by phosphonoacetic acid. Antimicrobial agents and chemotherapy 6, 360-365.

Painter, R.B., Young, B.R. (1971) Repair replication in mammalian cells after X-irradiation. Mutat. Res. 14, 225 - 235.

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

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

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

Rainbow, A.J. (1981) Reactivation of Viruses. in Short - Term Tests for Carcinogens. edited by H.F. Stich and R.H.C. San pp. 19 - 35 Springer Verlag.

Rainbow, A.J. (1984) Reduced capacity of tumor derived and SV40- transformed human cells to repair UV-irradiated adenovirus. Proceedings of the NATO advanced study institute on radiation carcinogenesis and DNA alterations.

Rainbow, A.J., Howes, H. (1979) Decreased repair of gamma - irradiated adenovirus in xeroderma pigmentosum fibroblasts. Int. J. Rad. Biol. 36, 621 - 629.

Rainbow, A.J., Howes, M. (1982) A deficiency in the repair of UV and T-ray damaged DNA in fibroblasts from Cockayne's syndrome. Mutat. Res. 93, 235 - 247.

Rainbow, A.J. and Mak, S. (1970) Functional heterogeneity of virions in human adenovirus types 2 and 12. J. Virol. 5, 188 - 193.

Rainbow, A.J. Mak, S. (1972) DNA strand breakage and iological functions of human adenovirus after gamma irradiation. Rad. Res. 50, 319 - 333.

Rainbow, A.J., Mak, S. (1973) DNA damage and biological function of human adenovirus after U.V. irradiation. Int. J. Rad. Biol. 24, 59 - 72.

Rassmussen, R.E., Painter, R.B. (1964) Evidence for repair of ultra-violet damaged deoxyribonucleic acid in cultured mammalian cells. Nature 203, 1360 - 1362.

Regan, J.D., 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.

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

Rupert, C.S. (1962) Photoenzymatic repair of ultraviolet damage in DNA. II Formation of an enzyme - substrate complex. J. Gen. Physiol. 45, 725 - 741.

Ryan, D., Rainbow, A. (1986) Comparative studies of host - cell reactivation, cellular capacity and enhanced reactivation of herpes simplex virus in normal, xeroderma pigmentosum and Cockayne syndrome fibroblasts. Mutat. Res. 166, 99 - 111.

Setlow, J. and Setlow, R. (1963) Nature of photoreactivable ultra - violet lesion in deoxyribonucleic acid. Nature 197, 560 - 562.

Schmickel, R.D., Chu, E.H., Trosko, J. (1975) The definition of a cellular defect in two patients with Cockayne Syndrome. Pediat. Res. 9, 317.

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

Selsky, C.A. and 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. Mutat. Res. 50, 395 - 405.

Snedecor, G.W., and Cochran, W.G. 1980, Regression In: Statistical methods, The Iowa State University Press, Ames, Iowa, USA, pp. 149-174.

Squires.S., Johnson, R.T., and Collins, A.R.S. (1982) Initial rates of DNA incision in UV-irradiated human cells. Differences between normal, xeroderma pigmentosum and tumour cells. Mutat. Res. 95, 389- 404.

Squires, S., and Johnson, R.T. (1983) U.V. induces long-lived DNA breaks in Cockayne's Syndrome and cells from an immunodeficient individual (46BR) : defects and disturbance in post - incision steps of excision repair. Carcinogenesis 4, 565-572.

Stich, H.F., Stich, W., Lam, P. (1974) Susceptibility of xeroderma pigmentosum cells to chromosome breakage by adenovirus type 12. Nature 250, 599 - 601.

Sutherland, B.M. (1974) Photoreactivating enzyme from human leukocytes. Nature 248, 109 - 112.

Sutherland, B.M., Oliver, R. (1975) Low levels of photoreactivating enzyme in xeroderma pigmentosum variants. Nature 257, 132 - 134.

Sutherland, B., Oliver, R., Fuselin, C.O., Sutherland, J.C. (1976) Photoreactivation of pyrimidine dimers in the DNA of normal and xeroderma pigmentosum cells. Biochemistry 15, 402 - 406.

Sutherland, B.M., Harber, L.C. Kochevar, I.E. (1980) Pyrimidine dimer formation and repair in human skin. Cancer Res. 40,3181 - 3185.

Tanaka, K., Sekiguchi, M., and Okada, Y. (1975) Restoration of ultraviolet - induced unscheduled DNA synthesis of xeroderma pigmentosum cells by the concomitant treatment with bacteriophage T4 endonuclease V and HVJ (Sendai virus). P.N.A.S. 72, 4071 - 4075.

Topal, M.D., Fresco, J.R. (1976) Complementary base pairing and the origin of substitution mutations. Nature 263, 285 - 289.

Varghese, A.J. and Wang, S.Y. (1968) Thymine - thymine adduct as a photoproduct of thymine. Science. 160, 186 - 187.

Vermeulen,W., Osseweijer, P., de Jonge, A.J.R. and Hoejmakers, J.H.J. (1986) Transient correction of excision repair defects in fibroblasts of 9 xeroderma pigmentosum complementation groups by microinjection of crude human cell extracts. Mutat. Res. 165, 199-206.

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

Watson,J.D. Molecular Biology of the gene. 1965 WA Benjamin, Inc. Don Mills, Ontario.

Watsom, Tooze, Kurtz 1983 Recombinant DNA Scientific American Books, W.H. Freeman and Company, New York, pp.130

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

Zimmermann, W., Cheng, S., Bolden, A., Weissbach, A. (1980) Mitochondrial DNA replication does not involve polymerase . J. Biol. Chem. 255, 11847 - 11852.