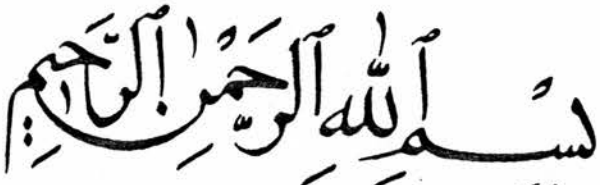
Kinetics of X-ray induced chromatid aberrations in irradiated G_2 normal human and ataxia telangiectasia cells and the influence of DNA repair inhibitors

Hossein Mozdarani, M.Sc.

Thesis submitted for the degree of Ph.D.
in Radiation Biology, to the
Department of Biology and Preclinical Medicine,
University of St. Andrews
September 1988.



In the name of God,
the Merciful, the Compassionate

Declaration

I, H. Mozdarani, hereby certify that this thesis has been composed by myself, that it is a record of my own work, and that it has not been accepted in partial or complete fulfilment of any degree or professional qualification.

Signed H. Mozdarani Date 28.9.88

I was admitted to the faculty of Science of the University of St. Andrews under Ordinance General No. 12 on Oct. 1986 and as a candidate for the degree of Ph.D. on Oct. 1987 .

Signed H. Mozdarani Date 28.9.88

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the Degree of Ph. D.

Signature of the Supervisor Date 28/9/88
Dr P.E. Bryant

Copyright

To my parents; Taymoure Agha and Rouh-Angize

To my wife and son; Miranda and Sohail

To my brothers and sisters

Contents

List of tables

Tables **Contents** **Page No.**

Figure **Page No.**

Figure **Page No.**

Abbreviations and Alternative names

AFP	Serum alpha-fetoprotein
Alu 1	Restriction endonuclease from *Arthrobacter luteus* ; recognizing and cleaving the sequence AG/CT.
APC	Aphidicolin
Ara A	9-β-D-arabinofuranosyladenine (adenine arabinoside)
Ara C	1-β- D-arabinofuranosylcytosine (cytosine arabinoside)
Ara CDP	1-β- D-arabinofuranosylcytosine diphosphate
Ara CMP	1-β-D-arabinofuranosyl cytosine mono-phosphate
Ara CTP	1-β- D-arabinofuranosylcytosine 5'-triphosphate
A-T	Ataxia-telangiectasia
AT5BI	Primary cell line of an individual displaying classical A-T syndromes
AT5BIVA	Permanent fibroblastic cell line of ataxia-telangiectasia
Bam H1	Restriction endonuclease from *Bacillus amyloliquefaciens* H; recognizing and cleaving the sequence G/GATCC.
bd	Base damage
Bq	Becquerel; Unit of radioactivity = 1 disintegration per second
BudR	5-bromodeoxyuridine
°C	Degree centigrade (Celsius)
CA	Chromosomal aberrations
C3H/ T1/2	Mouse embryo cells
CHO-K1	Chinese hamster ovary cell line
Ci	Curie; unit of radioactivity. 1 Curie=3.7×10^{10} disintegration/sec.
CO_2	Carbon dioxide
Cu	Copper
D_0	Dose of ionizing radiation required to reduce the number of surviving cells by 37% in the exponential region of the survival curve.

dATP	2'-deoxy adenosine 5'-triphosphate
dC	Deoxycytidine
dCTP	2'-deoxycytidine 5'-triphosphate
dpm	Disintegration per minute
DNA	Deoxyribonucleic acid
dsb	Double-strand breaks
E. coli	*Escherichia coli*
E. coRV	Restriction endonuclease from *Escherichia coli* J62 pLG74; recognizing and cleaving the sequence GAT/ATC.
***E. coli* K12 *rec*⁻**	A cell strain of *Escherichia coli* with *rec*- character: NH4803 *rec A*⁻, *rec B*⁻, AB2487 *rec A*⁻. These are all F⁻ and auxotrophic for thymine, arginine, histidine, leucine, proline, threonine and thiamine.
***E. coli ror* B**	X-ray sensitive mutant in *Escherichia coli*
ED_{50}	50% effective inhibitory dose
EDTA	Ethylene-diamine tetra-acetic acid, sodium salt
FCS	Foetal calf serum
FdUrd	5-fluorodeoxyuridine
$G418^R$ (neo^+)	A dominant selectable gene (in the DNA vector). Confers resistance to the neomycin analogue G418
gpt	A selectable bacterial gene (in the DNA vector) encoding for xanthine guanine phosphoribosyl transferase
g/l	Gram per litre
Gy	Gray; Unit of radiation absorbed dose = 1 J/Kg = 100 rads
^{3}H-TdR	Tritiated thymidine
HBSS	Hanks balanced salts solution
HU	Hydroxy urea
IgA	Immunoglobulin antibody
JU56	Transformed fibroblastic cells of the black-tailed Wallaby *Protemnodon bicolor*.
KBq	Kilo-becquerel = 10^3 Bq
KCl	Potassium chloride
KH_2PO_4	Potassium dihydroxygen orthophosphate
KpnI	Restriction endonuclease from *Klebsiella pneumoniae* OK8; recognizing and cleaving the sequence GGTAC/C.
KV	Kilo-volts

L5178Y	Mouse lymphoma cells
LET	Linear energy transfer
L-1210	Murine leukemic cell line
M	Mitosis
mA	Milli-ampere
μCi	Micro-Curie; 1μCi = 3.7 x 10^4 disintegration per second
MEM	Minimal essential medium (Eagle)
MEMFCS	Eagle's MEM plus 15% foetal calf serum
mm	Milli-metre
m mol/l	10^{-3} mol/l
μM	Micro mol/l = 10^{-6} mol/l
MRC5SVI	SV40 immortalized fibroblastic normal human cell line of lung origin (MRC5)
NaCl	Sodium chloride
Na_2HPO_4	di-sodium hydrogen orthophosphate
NaOH	Sodium hydroxide
NE	*Neurospora crassa* endonuclease
ng	nanogram = 10^{-12} gram
PCC	Premature chromosome condensation technique
PHA	Phytohaemagglutinin
PLD	Potentially lethal damage
PLDR	Potentially lethal damage repair
pSV2*gpt*	A recombinant plasmid containing the bacterial gene *gpt* ; (encoding for xanthine guanine phosphoribosyl transferase, XPRTase, EC.2.4.2.22).
pSV2*neo*	A recombinant plasmid containing the Tn5 Kanamycin/ neomycin/G418 gene.
Pvu II	Restriction endonuclease from *Proteus vulgaris* ; recognizing and cleaving the sequence CAG/CTG.
rad	Old unit of radiation absorbed dose = An energy absorption of 100 ergs per gram by matter; 1 centigray
***rad* 52**	X-ray sensitive mutant of Yeast deficient in dsb repair
RE	Restriction endonuclease
***rec* N**	X-ray sensitive mutant in *Escherichia coli*
RNA	Ribonucleic acid
***ror* A**	X-ray sensitive mutant in *Escherichia coli*

rpm	Revolutions per minute
ssb	Single-strand breaks
SV40	Simian virus
TBq	Tera becquerel = 10^{12} Bq
TCA	Trichloroacetic acid
UV	Ultraviolet radiation
V79	Chinese hamster lung fibroblast cell line
xrs	X-ray sensitive mutants of the Chinese hamster ovary cell line

Acknowledgements

I would like to express my most sincere gratitute to my supervisor, Dr Peter E. Bryant for his continual help, advice, encouragement and patience throughout the duration of this research work. My thanks also to all members of the radiation cytogenetics group in particular Miss A. Christie and Mr J. MacIntyre for their help and good humour in the lab.

I am deeply indebted to the Ministery of Culture and Higher Education of the Islamic Republic of Iran for providing the funds and the opportunity to make this thesis possible.

Finally, I would thank my wife, Miranda and my son, Sohail, who have been most patient and gave me every encouragement when I needed it most.

Abstract

The cytogenetic effects of X-rays alone or in combination with the nucleoside analogues, 9-β-D-arabinofuranosyladenine (ara A) and 1-β-D-arabinofuranosylcytosine (ara C), (both potent inhibitors of DNA double-strand breaks (dsb) repair), were investigated in immortalised fibroblastic cell lines of normal human (MRC5SVI) and ataxia-telangiectasia (AT5BIVA) during G_2-phase of the cell cycle. The main aims of this work were firstly to examine the relationship between DNA damage and chromatid aberrations and secondly to compare chromatid damage in A-T and normal cells following irradiation to investigate the nature of the radiation hypersensitivity of A-T cells at the chromosomal level. Thirdly to compare the influence of ara A and ara C on the radiation induced DNA lesions in G_2-phase of the cell cycle in the two cell lines.

The high radiosensitivity of this A-T line was verified by a clonogenic assay as well as chromosomal aberration studies following X-irradiation in the G_1 and G_2 phases of the cell cycle. Based on the D_0 values of the survival curves, A-T cells exhibited approximately twice the radiosensitivity of normal cells.

The average length of G_2 in these cell lines, measured by the autoradiographic labelling technique, was approximately 4.5 h for normal and 5 h for A-T cells.

A-T and normal cells X-irradiated in the presence or absence of ara

A or ara C for times up to 4 h before fixation were examined at metaphase for the presence of chromatid aberrations of both deletion and exchange types. Yields of deletions after X-rays alone in both cell lines decreased with time of post-irradiation incubation according to first-order kinetics with a half-time of approximately 2.4 to 3.1 hours which was interpreted as reflecting the underlying repair of DNA dsb. In contrast, frequencies of deletions in cells irradiated in the presence of ara A (known to inhibit repair of dsb) remained constant for all sampling times. The frequency of X-ray induced chromatid deletions in A-T cells was found to be higher by a factor of approximately 2 than that in normal cells. It was concluded that in A-T cells a higher proportion of DNA dsb are converted into chromatid deletions and that the conversion of dsb into deletions is genetically determined. In contrast to ara A, ara C was found to enhance the yields of X-ray induced deletions in both cell lines, i.e., the number of chromatid deletions doubled within two hours indicating a different mode of action of the two drugs on X-ray damage. Both drugs are shown to reduce semi-conservative DNA synthesis to 80-90 percent of control rates.

Yields of exchanges increased in normal and A-T G_2 cells as a function of time after X-ray exposure. Both ara A and ara C enhanced the frequencies of exchanges, although to a lesser extent in A-T than normal cells. The increased frequency of exchanges formed in irradiated cells in the presence of DNA synthesis inhibitors, (i.e. the absence of dsb repair) is postulated to indicate the existence of an "error-prone" mis-joining mechanism, independent of DNA synthesis and different from the mechanism of dsb repair.

1

INTRODUCTION

1. INTRODUCTION

Mechanisms of induction of chromosomal aberrations

The effect of ionizing radiation on chromosomes (the clastogenic effect) can be observed in preparations of cells blocked with colchicine or colcemid at the metaphase following exposure to X-rays. The resulting aberrations or structural rearrangements of chromosomes include breaks (deletions), gaps (achromatic lesions) and exchanges (e.g. Savage 1976, Buckton and Evans 1981).

Chromosomal aberrations are induced directly or indirectly by a wide variety of physical and chemical agents which can be classified into two broad categories, namely S-dependent and S-independent agents. S-independent agents such as ionizing radiation induce chromosome and chromatid-type aberrations. If the cell is irradiated in the unreplicated G_1 state, the broken chromatid may then duplicate as a consequence of replication, resulting in identical structural change in both chromatids of the chromosome. The aberration, therefore, will appear as a "chromosome" type at metaphase (Figure 1.1). If X-rays are delivered to the cells during G_2 phase i.e. during or after DNA replication, the structural changes will usually be seen in individual chromatids leading to "chromatid" type aberrations (Figure 1.2). Ionizing radiation usually induce a mixture of these two types of aberration in the S-phase of the cell cycle. UV and alkylating agents are termed S-dependent because the aberrations they induce can be manifested only after an intervening 'S' period (Evans 1977) after which

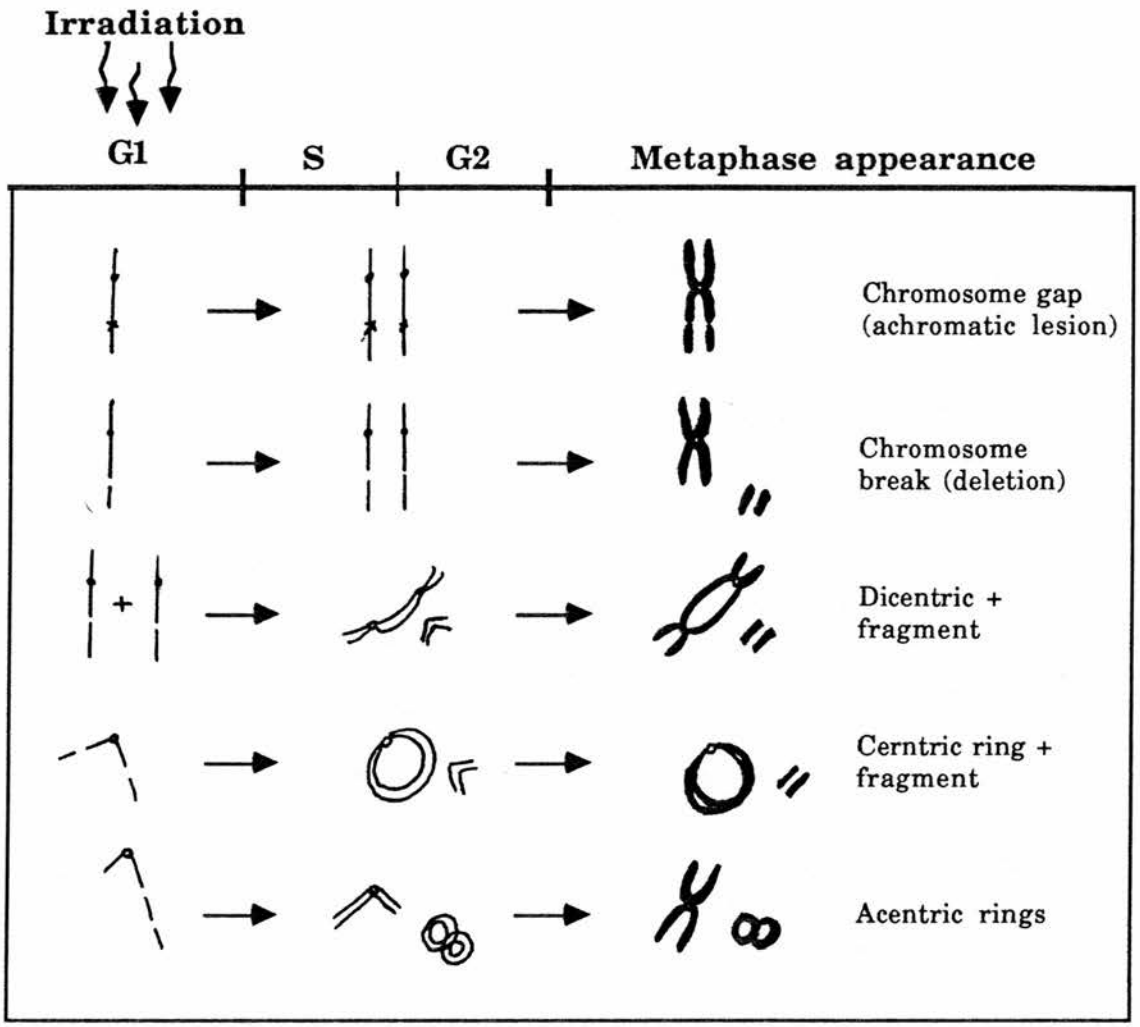


Figure 1.1: Examples of chromosome structural aberrations following irradiation of G_1 cells (Chromosome-type aberrations).

they may induce chromatid type aberrations at any cell cycle stage.

The clastogenic effect of ionizing radiation on chromosomes has been recognised since the early part of this century and is probably one of the most extensively studied clastogenic agents over the last 50 years. Sax (1938, 1940), for example, studied radiation induced chromosome aberrations in X-irradiated *Tradescantia* microspores and showed that simple terminal deletions and iso-chromatid aberrations increased approximately linearly with an increasing dose of X-rays.

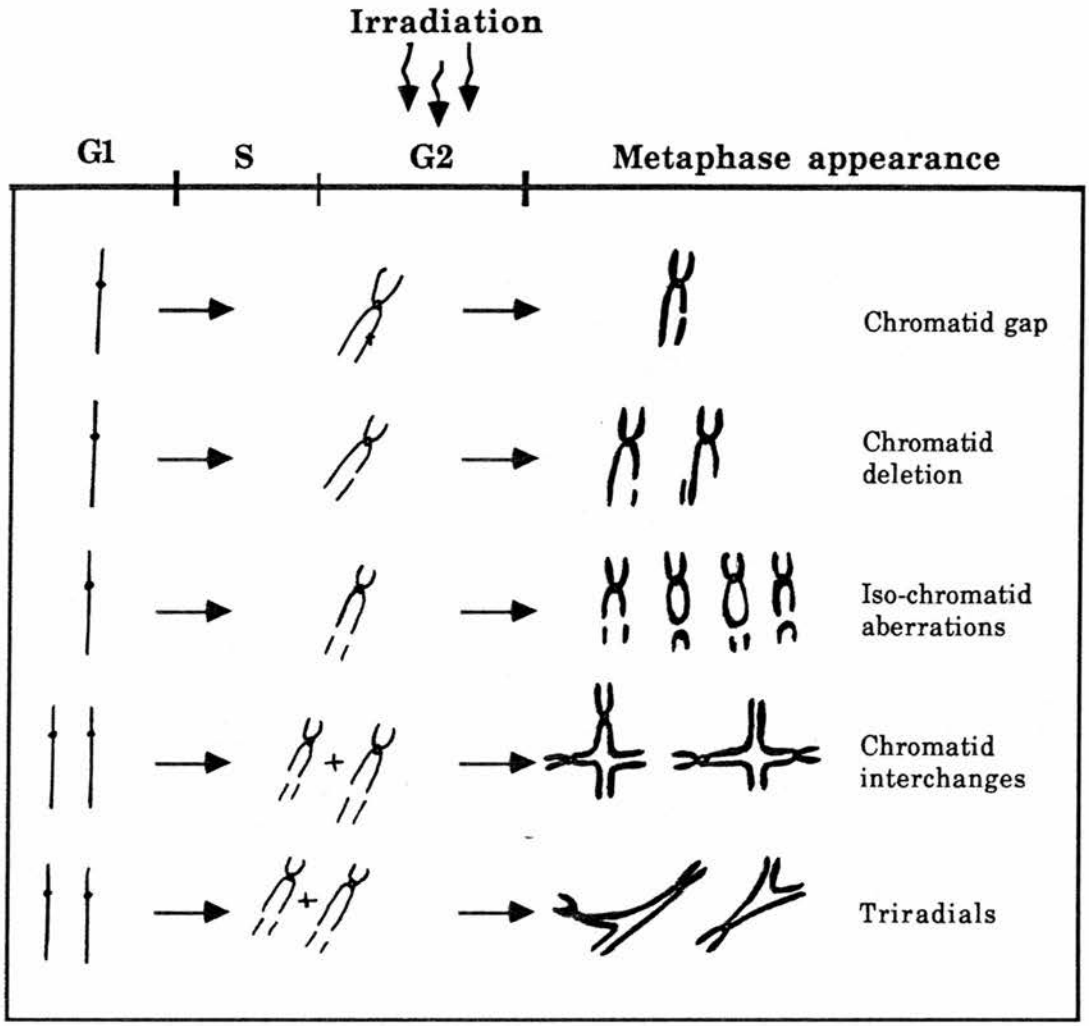


Figure 1.2: Examples of chromosome structural aberrations following irradiation of G_2 cells (Chromatid-type aberrations).

The frequency of chromosomal aberrations depends on various biological factors such as the type of cell, stage of the cell cycle, type of radiation, dose, dose rate, and the degree of physical injury produced by the radiation.

Two hypotheses were proposed for the formation of chromosomal aberrations. The first of these is the "breakage-first" hypothesis proposed by Sax (1940). This hypothesis was formulated without knowledge of the structural organisation of eukaryotic chromosomes and proposed that

X-rays induce breaks in chromosomes. According to this model most of these (induced) breaks are able to be restituted (rejoined correctly), although some may link up illegitimately (mis-join) to give rise to "exchange-type" aberrations. Those remaining unrejoined are termed "deletion-type" or simply "breaks".

Revell (1959) proposed an alternative hypothesis based on experiments with G_2 cells from the broad bean root tip (*Vicia faba*). He proposed that the breaks observed in chromatids are not formed directly, but indirectly via unspecified types of induced lesions in the chromatids. The primary events in this case are not considered to be breaks, but rather unstable lesions which usually return to the normal state (repair). Therefore, the two early stages are not chromatid breakage and reunion and the exchange itself is not formed immediately. Revell argued that these "lesions" lead to "initiation" of exchange formation. According to this exchange hypothesis of Revell, chromatid deletions that appear as simple chromatid breaks are the result of a special kind of incomplete intrachange (an exchange within the same chromosome) that occurs between two neighbouring points when they are brought into juxtaposition by a small segment of the chromosome being coiled in a single loop. Based on this interpretation, therefore, the primary lesion is not a break and all fragments as well as exchanges result from the interaction of primary lesions.

The work presented here is interpreted in terms of the former, "breakage-first" hypothesis model.

DNA is now thought to be the primary target for the induction of chromosomal aberrations (CA). Most known mutagens (DNA damaging

agents) including X-rays, are capable of inducing CA. Ionizing radiations induce a variety of damage in DNA, including directly induced single- and double-strand breaks, various types of base damage as well as DNA-DNA and DNA-protein crosslinks.

X-rays induce direct DNA single- and double-strand breaks in the ratio of about 20:1. In mammalian cells the frequency of double-strand breaks (dsb) has been shown to be approximately 40 per Gy/cell, that of single-strand breaks (ssb) approximately 1000 per Gy/cell and about the same frequency of base damage (Freifelder 1966, Corry and Cole 1968, Dean *et al.* 1969, Lehmann and Ormerod 1970, Burrell *et al.* 1971, Neary *et al.* 1972, Blöcher 1982).

All primary lesions induced in DNA are thought to be subject to cellular repair processes; however unrepaired or misrepaired lesions may give rise to chromosomal aberrations. Ssb are repaired rapidly following radiation exposure (e.g. Bryant and Blöcher 1980). Some of these are thought to be repaired simply by rejoining involving ligation, others by excision and insertion of relatively small numbers of bases; about 1-10 bases in the case of single strand breaks and base damage (Fox and Fox 1973) followed by ligation which restores the structural integrity of the DNA molecule. Dsb have been shown to be repaired more slowly (e.g. Blöcher and Pohlit 1982) with a half time of some hours. The mechanism of this repair is not fully understood, but is thought to involve recombination (Resnick 1976).

The question of which of these lesions is involved in aberration formation was the subject of controversy for many years and even now is not fully answered. Bender *et al.* (1974) postulated that chromosome

aberrations in irradiated cells arise from dsb in the G_1 phase of the cell cycle, that chromatid aberrations arise from dsb induced during G_2 phase and that a mixture of the two arise during S-phase from either directly induced dsb or dsb resulting from replication of DNA at the site of an induced single strand DNA break. Therefore this is a molecular version of the breakage-first hypothesis of Sax referred to earlier. Bender *et al.* (1974) based their model on the "mononeme" theory in which chromosomes in eukaryotes are thought to contain a single DNA double helix molecule running all through the chromosome (Bender *et al.* 1974, Evans 1977). Thus chromosomal aberrations are a consequence of breakage of this DNA strand. A directly formed break in a chromosome can be restituted to form the original (pre-break) chromosome structure, remain open to form a break or rejoin with another break to form an exchange. Such a mechanism for the formation of chromosomal aberrations from dsb is in agreement with the observation that ionizing radiation and chemical agents such as bleomycin, which cause dsb, can induce CA by treatment in the G_1, S and G_2 phase of the cell cycle. Obe *et al.* (1982), treated Chinese hamster ovary (CHO) cells with different sources of radiation (UV, X-rays, neutrons) and various chemical agents and after removal of the medium post-treated cells with *Neurospora crassa* endonuclease. From the results of these experiments, they confirmed the proposal of Bender *et al.* (1974) which in turn is in accord with the breakage-first hypothesis of Sax (1940).

It might thus be concluded that the dsb is the most probable lesion

leading to CA after ionizing radiation. The fact that densely ionizing radiations (high LET) which are efficient in the induction of DNA dsb also induce CA efficently (Obe *et al.* 1982), has provided indirect evidence for the involvement of DNA dsb in the formation of chromosomal aberrations.

Evidence supporting the view that the dsb is the DNA lesion responsible for CA was also provided by Natarajan and his colleagues (Natarajan and Obe 1978, Natarajan *et al.* 1980, Natarajan *et al.* 1982, Natarajan and Zwanenburg 1982, Natarajan and Obe 1983) who showed that incision of the DNA at single strand break sites using a single-strand specific endonuclease (*Neurospora* endonuclease) led to an increase in the number of dsb in irradiated and permeabilized cells. This also resulted in a corresponding increase in the yields of chromosome or chromatid aberrations depending upon the stage of the cell cycle at the time of irradiation.

An alternative hypothesis for the origin of the chromosomal aberrations was proposed by Preston (1980, 1982) from his studies with human lymphocytes. This author found that the frequency of chromatid breaks in G_2 cells increased with time after X-irradiation when cells were treated with cytosine arabinoside (ara C) an inhibitor of DNA repair resynthesis. Preston interpreted this as indicating that chromosomal aberrations were arising from base damaged sites in the DNA which were being enzymatically incised as a function of time, hence leading to an increase in the number of breaks. On the basis of these studies, he argued that DNA base damage, and not dsb, is responsible for the formation of aberrations. Bender and Preston (1982) extended these

studies with ara C and confirmed the earlier results of Preston (1980). Preston argued that the increase in chromosomal aberrations in X-irradiated cells treated with ara C was a consequence of accumulation of lesions due to inhibition by ara C of the polymerization steps in repair of X-ray induced DNA base damage. Double-strand base damage sites would then lead to an increase in dsb. Incision of such base damage, leading to dsb, has been experimentally verified by Ahnström and Bryant (1982).

As a result of this controversy over the molecular origin of chromosomal aberrations, a number of investigations have been made to resolve the question. For example it has been shown that "pure" dsb, induced in the DNA of cells by using restriction endonucleases, can lead to chromosomal aberrations (Bryant 1984*a*, Natarajan and Obe 1984). Using Pvu II which induces blunt-ended dsb, Bryant (1984 *a*) found that the frequency of induced CA was enzyme concentration dependent and that blunt-ended dsb were more effective in CA formation than cohesive-ended dsb. Moreover it was found that restriction endonucleases induce CA in all stages of the cell cycle (Obe and Winkel 1985) indicating that dsb are a major class of lesions leading to chromosomal aberrations (Bryant *et al.* 1987).

Characteristics of ataxia-telangiectasia

Ataxia-telangiectaia (A-T) is a human autosomal recessive multi-system syndrome which is characterised by oculocutaneous telangiectasia and progressive cerebellar ataxia; two features of classical A-T which were defined by Boder and Sedgwick (1958, 1963). The striking clinical features of A-T are neuromotor dysfunction associated with neural loss (Sedgwick 1982) and extreme immune deficiency (Waldman 1982). This syndrome manifests itself in early childhood (Miller 1982) and is accompanied by a skin condition in which permanent dilation of blood vessels (telangiectasis) occurs over the bulbar conjuctiva of eyes and faint hairlike telangiectases on the external ear and also a history of repeated infections particularly of the lungs and sinuses. Affected children are usually normal at birth, the disease commonly appearing between three and six years of age.

A-T patients show low levels of IgA, or even absence of IgA (Waldman 1982) but high levels of alphafetoprotein (AFP) in peripheral blood from 40-2800 ng/ml in contrast to the amount found in serum of normal individuals which is usually less than 20 ng/ml over age of six months (Waldman and MacIntyre 1972). Elevated levels of AFP are usually associated with hepatic carcinoma or pregnancy. AFP is synthesised primarily in the liver; especially in the immature liver.

The incidence of homozygous A-T is approximately 1 in 40,000 and is inherited as an autosomal recessive trait (O'connor *et al.* 1982). A-T is associated with an increased incidence of malignant neoplasm particularly of the lymphoproliferative type (Spector *et al.* 1982). A primary cause of death in A-T patients is progressive respiratory failure

within the first two decades of life (Boder and Sedgwick 1972) although infection and malignancies are also common causes of death.

Gotoff *et al.* (1967) first noticed the radiosensitivity of A-T patients when treating lymphosarcoma and observed a severe and unexpected reaction during conventional radiotherapy of this condition. It has been shown that this derives from an intrinsic hypersensitivity of A-T cells.

The original report of Taylor *et al.* (1975) concerning hypersensitivity of *in vitro* cultured A-T fibroblasts, derived from three A-T patients, to the lethal effects of γ-radiation has been confirmed and extended in various laboratories.

There is now general agreement that classical A-T patients are hypersensitive to the clastogenic and cytotoxic effects of both ionizing radiation and other DNA damaging agents such as bleomycin. This phenomenon has been observed clinically and demonstrated experimentally by cell survival studies on A-T fibroblasts (Taylor *et al.* 1975, Cox *et al.* 1978, Lehmann 1982, Nagasawa and Little 1983, Zampetti-Bosseler and Scott 1981, 1985) and by cytogenetic methods using lymphoid lines, lymphocytes and fibroblasts (Rary *et al.* 1974, Taylor *et al.* 1976, Natarajan and Meyers 1979, Taylor 1978, 1982, Cohen and Simpson 1982, Parshad *et al.* 1985, Bender *et al.* 1985). In the case of ionizing radiation, all A-T strains studied (approximately 30) are reported radiation sensitive and this hypersensitivity was observed irrespective of the type of radiation delivered and the conditions of irradiation; whether X-rays, γ-rays or neutrons under aerobic or hypoxic conditions (Chen *et al.* 1978, Cox *et al.* 1978, Paterson and Smith 1979, Paterson *et al.* 1979,

Weichselbaum *et al.* 1980, Arlett and Harcourt 1980). As well as showing enhanced cellular radiosensitivity, it was also found that recovery from potentially lethal damage was greatly diminished in several A-T cell strains (Weichselbaum *et al.* 1978, Cox *et al.* 1981). Recently Little and Nagasawa (1985) showed that the capacity for potentially lethal damage repair (PLDR) was almost absent in A-T cells and also found that the decrease in chromosomal breaks seen in normal cells during confluent holding, was not observed in A-T cells; consistent with the lack of PLDR.

Despite showing uniform hypersensitivity to the killing effects of X-rays and an increased number of chromosomal aberrations, A-T fibroblasts, showed neither a G_1 delay nor a G_1 block following irradiation up to 400 rad compared to measureable X-ray induced G_1 delay in normal and retinoblastoma cells (Nagasawa and Little 1983) as well as exhibiting a reduced radiation and bleomycin induced G_2 delay in A-T fibroblasts compared to normals (Zampetti-Bosseler and Scott 1981, 1985).

Inhibition of the rate of DNA synthesis following exposure to X-rays, γ-rays or bleomycin treatment is found to be greatly diminished in A-T cells (Houldsworth and Lavin 1980, Painter and Young 1980, Edwards and Taylor 1980, de Wit *et al.* 1981, Lehmann *et al.* 1982, Jaspers *et al.* 1982 *a*,*b*).

Lymphocytes from A-T patients are also shown to be radiosensitive. This was demonstrated either by measuring the ability of irradiated lymphocytes to respond to stimulation by phytohaemaglutinin (PHA) (Agarwal *et al.* 1977) or by irradiating lymphoblastoid cell lines and measuring their colony-forming ability (Chen *et al.* 1978).

Several A-T strains have been shown to respond normally to ultraviolet radiation (UV) in terms of cell killing (Lehmann *et al.* 1977, Paterson and Smith 1979, Scudiero 1980) or inhibition of DNA synthesis (de Wit *et al.* 1981).

The molecular basis of the enhanced sensitivity of A-T cells to genotoxic agents is not understood. There is as yet no general agreement concerning the defective nature of the DNA repair system in A-T. Evidence from some A-T lines suggests that there may be a defect in excision repair of base damage lesions in DNA (Paterson *et al.* 1976, Inoue *et al.* 1977, Smith and Paterson 1983) but this is not universally found in A-T lines (e.g. van der Schans *et al.* 1980). As mentioned earlier, ionizing radiation can induce DNA strand-breaks by scission of the phosphodiester backbone (e.g. van der Schans *et al.* 1982 *a*). Using the velocity sedimentation technique, it has been shown that normal human cells have the capacity to repair both single- (Epstein *et al.* 1973, Taylor *et al.* 1975, Vincent *et al.* 1975, Bradly *et al.* 1976, Paterson *et al.* 1976) and double-strand breaks (Taylor *et al.* 1975, Lehmann and Stevens 1977). It is believed that no normal human cell lacks this ability. However experiments carried out extensively with cells derived from A-T patients failed to prove the existence of a repair defect in A-T cells (Taylor *et al.* 1975, Vincent *et al.* 1975, Lehmann and Stevens 1977, Fornace and Little 1980, Thierry *et al.* 1985). These studies showed that there is no distinguishable difference in the rate of rejoining of either single- or double-strand breaks between A-T and normal cells. Fornace and Little (1980) by employing the alkaline elution technique with a resolution of approximately one single-strand break in 10^{10} daltons, suggested that a

deficiency in DNA single strand break repair is unlikely. Fluorimetric analysis of DNA unwinding and measurement of DNA strand-break induction in leukocytes from A-T and normal donors irradiated with low (0.5 - 7 Gy) and high doses (5 - 20 Gy) of X-rays also showed that A-T cells are as proficient as normal cells in repair of DNA strand-breaks (Thierry *et al.* 1985). The increased radiosensitivity of A-T cells is suggestive of a defect in some DNA repair system. A number of features of the A-T syndrome might be explained on a DNA repair defect hypothesis, for example degeneration of the central nervous system. Nerve cells are not renewable in humans so that after radiation exposure A-T nerve cells may be less able to repair than those of normal individuals. In addition, it is now evident that A-T can also be considered among the progeric, or *progeroid* (premature aging) syndromes (Boder 1985). The progeric manifestations are not limited to the cutaneous and oculocutaneous telangiectasia but are also seen in the central nervous system in A-T patients. Similarly, premature aging involving early appearance of grey hair and skin changes in A-T patients could be accounted for by a repair defect hypothesis (Waldman 1982).

Cytogenetics of A-T

Cultured cells from ataxia-telangiectasia (A-T) individuals show an increased chromosomal instability, both spontaneously and after exposure to some clastogenic and genotoxic agents. Hecht *et al.* (1966) published the first report showing spontaneous cytogenetic abnormalities of A-T cells in 1966. A year later, as a result of consistent observations of chromosome breakage and rearrangements in mitotic figures of cells

from A-T patients, A-T was classified among the chromosomal instability syndromes (Gropp and Flatz 1967). The commonly accepted disorders classified as chromosome instability syndromes which show high levels of spontaneous chromosomal abnormalities are Fanconi's anaemia and Bloom's syndrome. Although A-T patients do not show levels of chromatid type aberrations as high as either Bloom's or Fanconi's patients, they do show a higher level of rearrangements particularly involving chromosome 14. Since 1966 there have been many reports from various laboratories showing increased levels of spontaneous chromosomal breakage in A-T cells in all forms of chromosomal abnormalities; that is, chromatid breaks, gaps, chromatid interchanges, fragments and dicentrics (e.g. Hecht *et al.* 1973, Cohen *et al.* 1975, Taylor 1978, Taylor *et al.* 1981, Hansson *et al.* 1984). Cohen *et al.* (1975) showed that the level of spontaneous chromosome breakage in A-T fibroblasts was about three times greater than in normal controls. Although some A-T patients show low or normal levels of unstable chromosomal aberrations, they all appear to show high levels of cells with stable chromosome rearrangements (translocations) (Harnden 1974, Oxford *et al.* 1975, Aurias *et al.* 1980, Taylor *et al.* 1981). The most distictive feature of many A-T patients' lymphocytes is the presence of cytogenetically abnormal clones. The presence of many of these cells at the first mitosis *in vitro* suggests that they must be present *in vivo*. Their clonal nature is defined by the presence of at least three cells in a 48 h blood culture with the same stable cytogenetic rearrangement (Taylor 1982). Chromosome studies in A-T were mostly carried out on lymphocyte cultures stimulated with PHA, this might suggest that the

rearrangements are present in clones of T-lymphocytes. Several types of chromosomal rearrangements have been observed in different A-T strains. Studies have now shown that in most clones the translocation involves breakage specifically at the band 14q12 (McCaw *et al.* 1975). The deleted 14q may be translocated to one of several chromosomes including t(14;14)(q12q32) (Oxford *et al.* 1975, Rary *et al.* 1975, Al Saadi and Palutke 1976), t(X;14)(q27q12) (Oxford *et al.* 1975, Nelson *et al.*1975), t(7;14)(q36;q12) (Al Saadi and Palutke 1976) and t(14q-7q+) (McCaw *et al.* 1975). These rearrangements were particularly seen in chromosomes 7 and 14 (Aurias *et al.* 1980, Hecht and Kaiser-McCaw 1982, O'Connor *et al.* 1982). O'Connor *et al.* (1982) showed that the most frequent rearrangements involving translocations of chromosomes 7 and 14 occur in both T- and B-lymphocytes but not in a lymphoblastoid line (Epstein-Barr virus transformed B-cells) nor in skin fibroblasts from two A-T patients. However fibroblast cultures have been reported to contain random chromosome rearrangements (Aurias *et al.* 1980). The location of the break points in chromosomes 7 and 14 in A-T is thought to be highly non-random including paracentric inversion of chromosome 14 and pericentric inversion of chromosome 7. The pattern of chromosome rearrangements and break-points has been shown to be similar in A-T and normal lymphocytes (Hecht and Kaiser-McCaw 1982), but the incidence of these types of clones in A-T was estimated to be about 40 times higher than in normal cells (Taylor 1982).

The report by Higurashi and Conen (1973), who irradiated cells in G_0 phase prior to PHA stimulation, showed that chromosome breakage syndromes such as ataxia-telangiectasia, Bloom's syndrome and

Fanconi's anaemia, showed hyper-sensitivity to radiation *in vitro*. This was demonstrated by the increased frequency of rings and dicentrics following G_0 irradiation. Subsequently a remarkable increase was also observed in the frequency of chromosomal aberrations in irradiated G_2 A-T leukocytes compared to normals (Rary *et al.* 1974).

As mentioned earlier, it is well known that irradiation of cells in the G_0 phase of the cell cycle results in chromosome type aberrations whereas irradiation in S-phase or G_2 leads to induction of chromatid type aberrations (e.g. Evans 1977). It is also known that when G_1 normal cells are treated with chemical mutagens such as alkylating agents, the treated cells show only chromatid type aberrations at the next mitosis after passing through an 'S-phase'. In the case of A-T cells both chromosome and chromatid type aberrations are induced when G_0 or G_1 cells are irradiated with X-rays. Despite being efficient in repairing strand-breaks, Paterson *et al.* (1976) showed that some of the A-T strains are slow to repair DNA base damage induced by X- or γ-rays. Therefore it might be possible that some of these base damage lesions persist until the 'S-phase' and lead to chromatid aberrations via S-dependent DNA repair. The observations of Paterson *et al.* (1976) were not confirmed universally (for example, van der Schans *et al.* 1980). However, it was observed that following irradiation of A-T cells in G_0, there was a large increase in the frequency of chromatid type aberrations compared to normal cells (Taylor *et al.* 1976). This increase was found to be of the order of seven- to fifteen-fold for chromatid gaps and breaks and up to twenty-fold for

chromatid interchanges in A-T lymphocytes (Taylor *et al.* 1976, Taylor 1978). These findings are not in total agreement with those of Higurashi and Conen (1973) where, for example, there is no mention of induced chromatid damage. High levels of chromosome breakage were also found in fibroblastic cells derived from A-T patients. The level of aberrations was observed to be still higher than those arising in A-T lymphocytes (Cohen *et al.* 1975). The high level of chromosome and chromatid type aberrations following G_0 irradiation, might suggest either a higher than normal level of DNA strand breakage in A-T or a failure to rejoin damaged sites correctly.

The original report of Rary *et al.* (1974) concerning G_2 hypersensitivity of A-T lymphocytes was confirmed by Taylor (1978). Taylor (1978) found a greater than 10 fold increase in chromatid breaks and gaps following 100 rad and a 15 fold increase following 200 rad in G_2 irradiated lymphocytes was found in A-T compared to normals. Similar results were found by Natarajan and Meyers (1979) for A-T fibroblasts. Recently Bender *et al.* (1985) showed that following 50 rad X-rays delivered 1.5 hours before fixation, about four times more deletions and exchanges were observed in A-T lymphocytes compared with normals. The sensitivity of G_2 A-T cells to bleomycin was found to be two- to five-fold greater than that of normal cells (Taylor *et al.* 1979). Caffeine, which enhances the frequency of X-ray induced chromatid aberrations in G_2 irradiated normal cells, was found to have no effect on G_2 irradiated A-T fibroblasts (Hansson *et al.* 1984).

Other effects of the A-T gene

Following exposure to ionizing radiation, normal human cells do not continue their progression through S-phase but are delayed for several hours (Houldsworth and Lavin 1980, Edwards and Taylor 1980). It is thought that one DNA strand break is enough to stop initiation of DNA synthesis within a group of replicons (Povirk 1977). However, it has been shown that the rate of DNA synthesis in A-T cells is less inhibited following irradiation, bleomycin or neocarzinostatin treatment compared to normal cells, indicating that A-T cells are relatively resistant to the inhibitory effects of these clastogenic and genotoxic agents on *de novo* DNA synthesis (Houldsworth and Lavin 1980, Painter and Young 1980, Edwards and Taylor 1980, de Wit *et al.* 1980, Cramer and Painter 1981, Shiloh and Becker 1982, Smith and Paterson 1983). All these agents induce DNA-strand breaks directly. This radioresistant DNA synthesis in A-T cells was suggested to be due to completely resistant DNA chain elongation and partially resistant DNA replicon initiation (Painter 1985). This feature appeared to be consistent with the observation of Zampetti-Bosseler and Scott (1981) who showed that radiation-induced G_2 delay following exposure to 150 rad X-rays was less in primary fibroblast cells from A-T patients than in cells from normal individuals. They also reported similar findings for cells treated with bleomycin (Zampetti-Bosseler and Scott 1985). Abnormal radiogenic accumulation of cells in G_2 and M phase due to the lack of dose dependent recovery process and the expected A-T anomaly of the resistance of *de novo* DNA synthesis to radiogenic inhibition has also recently been reported for SV40

transformed A-T cells (Smith *et al.* 1985). Based on the observation of diminished inhibition of DNA synthesis following ionizing radiation, Painter and Young (1980) proposed that normal cells are able to prolong the time in which DNA-strand breaks can be repaired by delaying G_2 phase so that the chromatin structure can be returned to its normal state whereas A-T cells are deficient in this system and therefore some of the DNA-strand breaks might be left unrepaired. Therefore the high radiosensitivity in A-T cells could be a consequence of a defect in the ability of A-T cells to respond to DNA damage rather than a defect in the repair mechanism.

Because A-T cells are similar to caffeine treated normal cells in their G_2 response to ionizing radiation, i.e. A-T cells exhibit diminished inhibition of DNA synthesis (Houldsworth and Lavin 1980, Edwards and Taylor 1980), shortened mitotic delay (Zampetti-Bosseler and Scott 1981) and increased frequency of chromosomal aberrations (Taylor 1976, 1982), it was suggested (Painter and Young 1980) that their radiosensitivity is not due to their inability to repair damage but due to a failure to go through X-ray induced delays that would allow normal cells to repair damage before being expressed. Consequently it was suggested that lack of G_2 delay in A-T cells could allow cells to enter mitosis carrying unrepaired DNA strand lesions which might prove lethal. Thus A-T cells might not be hypersensitive to radiation due to defective repair but because of failure or absence of normal G_2 delay (Painter and Young 1980). The observation that caffeine has no potentiating effect on the frequency of X-ray induced chromatid aberrations in A-T fibroblasts and

that reduced inhibition of X-ray induced mitotic delay in the presence of caffeine in both normal and A-T fibroblasts, shows that these two effects are not always related (Hansson *et al.* 1984). Hansson *et al.* (1984) therefore suggested that the inability of A-T cells to go through G_2 delay that allow normal cells to repair DNA damage might not be the only explanation for the increased radiosensitivity.

Studies at a cellular level have shown that A-T cells are lacking in post-irradiation recovery processes (Cox 1982). Because chromosomal DNA seems to be the major cellular target for ionizing radiation, an association between A-T and DNA-repair deficiency seems likely. Whilst A-T cells are hyper-sensitive to ionizing radiation (X, γ-ray) they show a normal response to UV radiation (Lehmann 1982). Since UV is not capable of dsb induction, these observations as well as the speculation that there is perhaps a possible correlation between chromosome aberrations and incurred DNA damage at molecular level (e.g. Bender *et al.* 1974, Taylor *et al.* 1976, Natarajan *et al.* 1982), focused attention on the repair of DNA strand scissions in A-T since such damage is induced by ionizing radiation directly and effectively. Therefore, others have tried to explain the chromosomal radiosensitivity of A-T and its sensitivity to other mutagens in terms of the quality of DNA repair.

Recombinant DNA techniques in the study of DNA repair in A-T

An alternative approach to the study of the molecular defect in A-T has been the use of restriction endonucleases to generate dsb (Cox *et al.* 1986). In particular Cox *et al.* (1986), using a gene transfer technique,

showed that there is an apparent defect in the ability of an SV40-transformed A-T (AT5BIVA) cell line to undergo successful transfection by the plasmid pSV2*gpt* in which a dsb has been introduced into or near the *gpt* gene by the restriction enzyme *KpnI*. These authors interpreted their data in terms of an elevation in the frequency of misrepair of double-strand DNA scissions in the A-T cell line. They found that although A-T cells can repair DNA double strand scissions they do so with much lower fidelity than cells with normal radiosensitivity (MRC5SVI). Cox *et al.* (1986) postulated a dis-equilibrium between the rejoining and exonuclease digestion of DNA termini as a primary genetic defect in A-T.

In more recent work Debenham *et al.* (1987) compared the same cell lines and confirmed the earlier results of Cox *et al.* (1986). These authors also used the DNA-mediated gene transfer technique of Cox *et al.* (1984) and developed an experimental approach to examine the fidelity of repair of DNA double-strand breaks. They introduced a dsb at a unique *KpnI* or *EcoRV* site in the coding sequence of a double selection plasmid system with two dominant markers, one the dominant selectable gene *gpt* in a derived circular DNA vector containing a second dominant and selectable gene *G418* ^{R} (*neo* $^{+}$). *G418* ^{R} acted as a control for DNA transfer and gene expression so that if the dsb introduced into the non-selected (*gpt*) gene was not rejoined or was rejoined incorrectly after transfection into the recipient cell, the gene would be inactive and could be detected as a result of a lack of its expression. Using this approach Debenham *et al.* (1987) observed that the rate of misrepair in A-T cells was about 7-10 fold higher than in the normal human cell line. They explained this mis-

repair phenomenon as either over-production of an exonuclease which degraded DNA at sites of dsb or as indicating a lack of some factor which normally protects DNA termini from such degradation. However, because Debenham *et al.* (1987) did not detect elevation of exonuclease activity in crude extracts of A-T cells, some form of deficiency of protection of DNA termini was proposed as a possible explanation for mis-repair in A-T cells. Green and Lowe (1987) obtained similar results to those of Cox *et al.* (1986) but questioned the concentrations of the drugs used in the selection system. They transfected at about 5-fold lower cell density without using glycerol shock at 24 h.

The possible absence of a defect in the pathway of repair of dsb distinguishes A-T cells from other derived mutant cells that are hypersensitive to ionization radiation. For example, the *xrs* mutants of hamster cells, which are defective in the rejoining of radiation induced DNA dsb (Kemp *et al.* 1984, Bryant *et al.* 1987), the *rad* 52 series mutants in yeast (Resnick and Martin 1976), and the *E. coli* K12 *rec*$^-$ (Krasin and Hutchinson 1977), *ror* A and *rec* N mutants in *Eschrichia coli* (Glickman *et al.* 1971, Picksley *et al.* 1984). The hypersensitivity of all these mutants is related to defective DNA dsb repair. However, the *E. coli ror* B mutant appears to be similar in cellular phenotype to A-T. Despite being radiosensitive, the mutant shows normal rejoining of ionizing radiation induced DNA dsb; but low fidelity in the rejoining process may be involved (Debenham *et al.* 1987).

Properties and mode of action of ara A and ara C

Adenine arabinoside (ara A)

9-β-D arabinofuranosyladenine (ara A) was first synthesised by Lee *et al.* (1960) and later identified in *Streptomyces antibioticus* (Anderson and Schandolnik 1973). Ara A is one of the purine derivatives with a relatively selective inhibitory action on DNA synthesis (Müller 1979). Ara A is a nucleoside analogue of deoxy-adenosine. As shown in figure 1.3 it differs from the naturally occuring nucleosides only at the 2'-position of the sugar moiety (Müller and Zahn 1978).

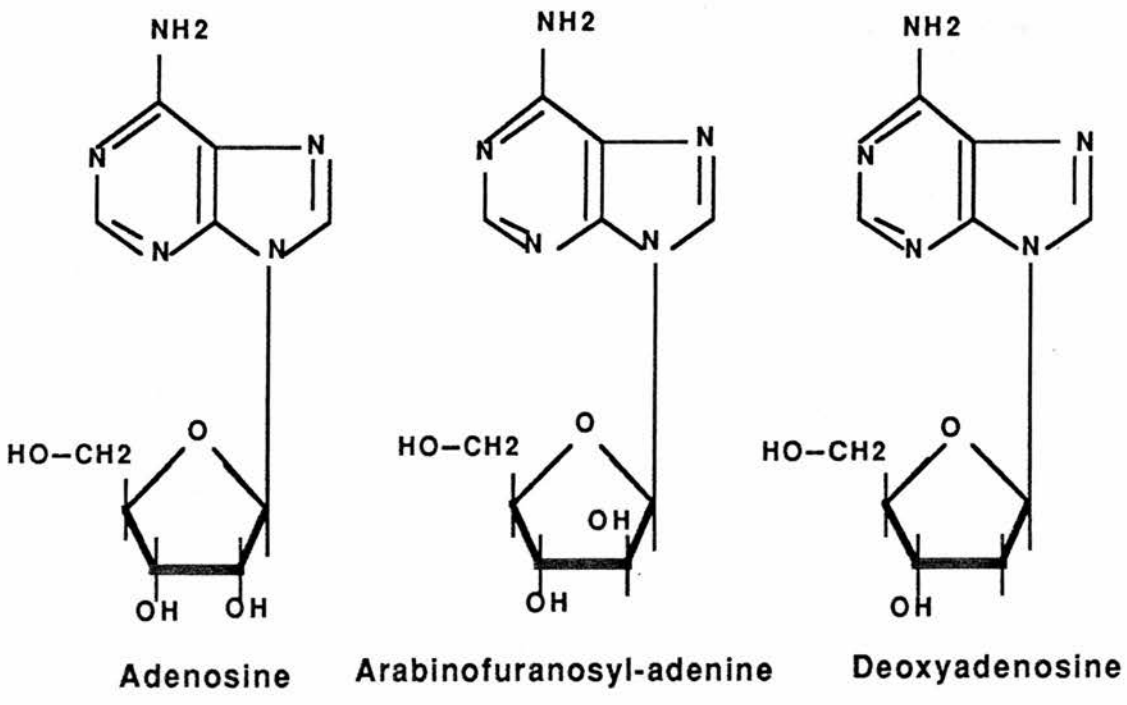


Figure 1.3. Structures of adenosine, arabinofuranosyl-adenine and deoxyadenosine.

Ara A is a promising antiherpetic nucleoside with a strong antiviral activity against DNA viruses in cell culture especially Herpes viruses (Shannon 1975) and it has been used for clinical treatment of Herpes. The inhibitory effect of ara A on DNA synthesis was known as early as 1962 (Hubert and Cohen) and it was later found that it is a potent inhibitor of mammalian DNA polymerases α and β (e.g. Furth and Cohen 1967, Müller *et al.* 1975). *In vitro* observations showed that β-ara A was more effective at inhibiting DNA β- than α- polymerase (Okura and Yoshida 1978) but this is not generally agreed. Others, for example Müller *et al.* (1975) found stronger inhibitory effect on α- than on β -polymerase. Biochemical studies have revealed that on entry into cells ara A is readily converted into corresponding 5'-mono, -di or triphosphates (Brink and Le Page 1965). The phosphorylated ara A is thought to compete with the naturally occuring nucleoside dATP at the binding sites on the DNA polymerasesα and β where it acts as a potent inhibitor of DNA synthesis (Müller *et al.* 1975, Bryant and Blöcher 1982). Iliakis (1980) reported that ara A inhibited growth in logarithmic cultures of Ehrlich ascites tumour cells by specifically inhibiting DNA synthesis and enhanced radiation induced killing in plateau-phase cells, presumably by fixing PLD. By delaying post-irradiation application of ara A at low concentrations (12μ mol/l), it was established that the ara A-sensitive steps of DNA repair take place at a rate comparable to the rate of PLD repair as measured in plateau-phase cells by delaying

plating. The inhibition of PLD repair by ara A was reduced when deoxyadenosine was added, suggesting that ara A acts competetively against dATP at the molecular level (Iliakis 1980).

Ara A acts cytostatically and increases the average volume of cells by 40% when cells are incubated with ara A (Cohen 1966, Müller *et al.* 1977). Because of its cytostatic action ara A may be useful in cancer therapy (Lee *et al.* 1960). It was also reported that the proliferation of mouse lymphoma cells (L5178Y) is reduced at low concentrations of ara A and at high concentrations the cells are lethally affected (Müller *et al.* 1975). The 50% effective inhibitory dose (ED_{50}) of ara A for L5178Y cells is found to be 2.9 μ M (Müller *et al.* 1975).

Investigations with L5178Y cells indicated that ara A inhibits DNA synthesis at a concentration of twice the ED_{50}, protein and RNA synthesis not being influenced significantly (Müller *et al.* 1975). Ara A is not incorporated into DNA (Fürth and Cohen 1968, Plunket *et al.* 1974) and chain termination does not appear to occur in mammalian DNA as has been reported for virus DNA (Müller *et al.* 1977).

Ara A has proved to be interesting from a radiobiological point of view because it was found to potentiate the killing effects of X-rays and leads to the removal of the "shoulder" region from the survival curve of X-irradiated Ehrlich ascites tumour cells. This was interpreted in terms of an inhibition of repair of potentially lethal damage (Iliakis 1980, 1981). It was found that β-ara A was more effective in causing expression of PLD when compared with α-ara A or ara C in X-irradiated cells (Iliakis

and Bryant 1983). Fixation of radiation induced PLD by ara A was also observed in plateau-phase cultures of Chinese hamster V79 cells (Iliakis 1985) and C3H mouse embryo 10T1/2 cells (Iliakis *et al.* 1987).

Ara A has also shown to enhance the chromosomal damage caused by X-rays (Bryant 1983, 1984 *b*), an effect which has been related to its strong inhibition of repair of DNA double strand breaks (Bryant and Blöcher 1982). Bryant (1983) showed that treatment of X-irradiated Ehrlich ascites tumour cells with ara A led to a large increase in the incidence of chromosomal abnormalities (anaphase bridges and fragments) suggesting that mis-repair of DNA dsb is enhanced by ara A. Ara A itself is known to induce chromatid aberrations in human leukocytes (Nichols 1964) when the cells were exposed to ara A prior or during DNA synthesis. The type of aberrations induced by ara A were described as gaps and open breaks. In this respect ara A resembles the action of cytosine arabinoside (e.g. Kihlman 1963, Kihlman *et al.* 1963, Preston 1980).

Cytosine arabinoside (ara C)

1-β-D-arabinofuranosylcytosine (ara C) is a pyrimidine nucleoside analogue of 2'-deoxycytidine in which the deoxyribose sugar is replaced by arabinose (figure 1.4) (Müller and Zahn 1978). Ara C is clinically an important cytotoxic drug which is frequently used as an antitumour and cytostatic agent (Ho and Freireich 1975) as well as a potent inhibitor of DNA synthesis of mammalian cells but with a minimal effects on other

cellular processes (Chu and Fisher 1962, Graham and Whitmore 1970 *a*, *b*, Bendict and Jones 1979). Ara C is probably one of the most widely used drugs in radiobiological and cytogenetic studies because of its biological effects and cytotoxic action in the treatment of certain leukemias (Momparler 1974). Ara C probably acts by preventing the formation of deoxycytidine diphosphate from cytidine diphosphate (Chu and Fisher 1962, Cardeilhac and Cohen 1964). Ara C potently inhibits cell proliferation, for example the concentration causing 50% inhibition (ED_{50}) in the case of L5178Y cells was found to be 0.1 μ M (Zahn *et al.* 1972). It has been shown that ara C inhibits the repair of DNA damage due to a

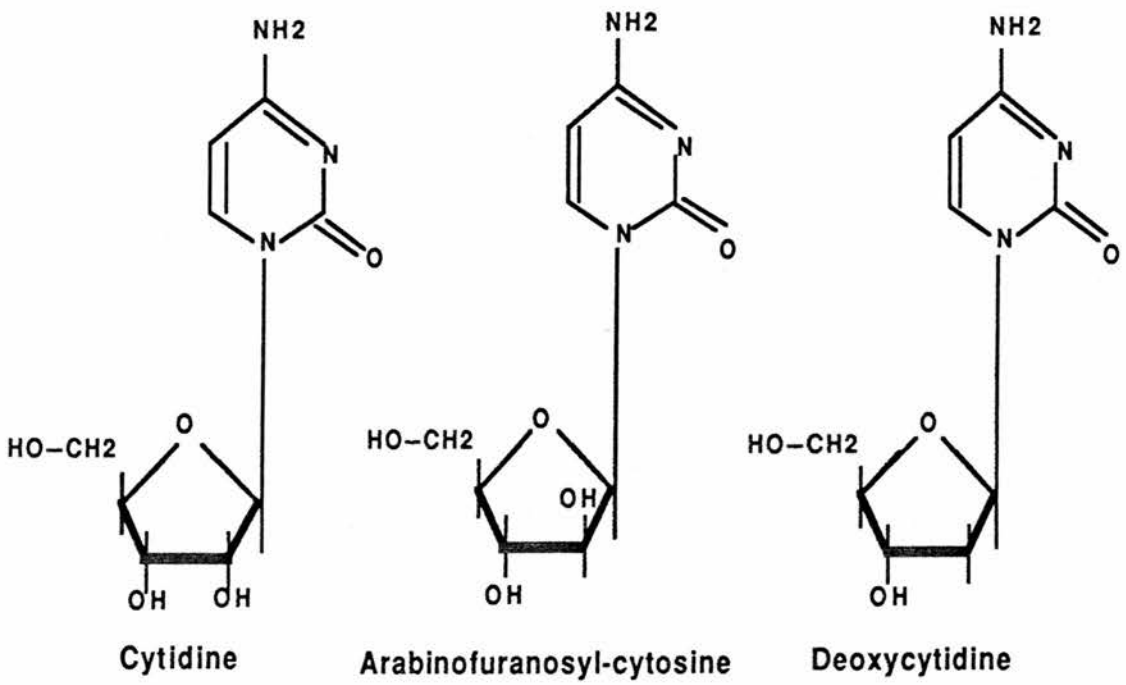


Figure 1.4: Structures of cytidine, arabinofuranosyl-cytosine and deoxycytidine.

variety of mutagens by affecting repair replication. The action of ara C can be reversed by the addition of deoxycytidine to the culture medium (Karran 1973, Hiss and Preston 1977).

Ara C is a synthetic drug (Walwick *et al.* 1959) and its structure is quite similar to the natural nucleosides, cytidine and deoxycytidine as shown in figure 1.4. It was demonstrated that ara C is rapidly transported into the cell and phosphorylated to ara CTP, the active form of the chemical in the cell, via ara CMP and ara CDP by sucessive enzymatic reactions (Kozai and Sugino 1971, Momparler *et al.* 1971). This investigation also led to the finding that arabinofuranosyl-cytosine and its phosphorylated derivatives are deoxycytidine analogues rather than cytidine analogues. The inhibition of DNA synthesis produced by ara CTP was shown to be competetive with respect to dCTP (Fürth and Cohen 1968, Graham and Whitmore 1970 *a*). Studies at the cellular level revealed that ara C is incorporated into internucleotide linkages suggesting that chain termination by itself might not be responsible for DNA synthesis inhibition (Graham and Whitmore 1970 *b*, Hunter and Franke 1975). Major *et al.* (1982) showed that the inhibition of cellular DNA synthesis by cytosine arabinoside was significantly related to the extent of ara C incorporation in DNA over a wide range of drug concentrations and time of exposure. The cumulative incorporation of the (fraudulent) nucleoside analogue into DNA might be partially responsible for the cytotoxicity and mutagenicity of ara C (Bendict and Jones 1979). It was also found that there was a direct relationship between ara C produced cytotoxicity and inhibition of DNA synthesis at high concentrations of ara C (Jones *et al.* 1976). It is believed that ara C

inhibits DNA polymerase α (Loeb *et al.* 1980, Waters *et al.* 1981) and thus inhibits DNA semiconservative replication. With an experiment using normal and excision-deficient xeroderma pigmentosum fibroblasts, Waters *et al.* (1981) studied the post-irradiation effects of ara C and aphidicolin which is a specific inhibitor of DNA polymerase α and found that ara C acts in a similar way to aphidicolin in inhibiting DNA polymerase α. Recently it was shown that cell death and chromosome aberrations are correlated with incorporation of ara C into DNA in S-phase cells and only when more than 97% of the semiconservative DNA synthesis is inhibited do these biological effects manifest themselves (Moore and Randall 1987).

In cell culture experiments, ara C has proved to be active against certain DNA viruses e.g. Pox virus and Herpes virus complex. Because of its cytostatic action ara C is frequently applied as an antitumour drug in chemotherapy (Ho and Freireich 1975). Ara C was shown to be most effective in inhibiting cell growth and repair of DNA dsb when compared to ara A but less effective in causing expression of PLD than ara A (Iliakis and Bryant 1983).

Ara C is capable of induction of chromosomal aberrations in the G_0 or G_1 and G_2 phases of the cell cycle (Kihlman *et al.* 1963, Brewen 1965, Brewen and Christie 1967). Kihlman *et al.* (1963) for example, in an early experiment found that ara C as well as FdUrd can induce chromosomal aberrations when applied for a few hours prior to mitosis. These authors suggested that chromosome breaks could be the result of inhibition of

DNA synthesis. However ara C also induces chromosomal aberrations in G_2 cells and it is believed by others to be a radiomimetic compound that induces aberrations by a mechanism un-related to the inhibition of DNA synthesis (Kihlman 1963, Brewen and Christie 1967).

It was shown that ara C inhibits the polymerization steps in the repair of damage produced by X-rays as well as DNA damaging agents such as 8-hydroxyquinoline, mitomycin C and UV in human fibroblasts and Chinese hamster cells (Hiss and Preston 1977). Combined treatment of ara C and UV resulted in an effect similar to that of X-rays in the way that both treatments were potent inducers of dicentrics and rings in G_1 normal and A-T cells, and elicited a specific hypersensitive response from A-T cells (Ejima and Sasaki 1986).

Using human peripheral lymphocytes, Preston (1980) showed that ara C causes a synergistic increase in chromosomal aberration frequencies in both G_0 (by increasing dicentrics and rings with increasing incubation time with ara C) and G_2 cells (by increasing chromatid deletions with increasing incubation time and prevention of exchange formation) cells. Preston (1980) argued that the increased frequency of chromosomal aberrations in X-irradiated cells following treatment with ara C were caused by the incision of DNA at sites of base damage. These studies with ara C were extended by Bender and Preston (1982), confirming the earlier results of Preston (1980). Preston (1980) and Natarajan and Obe (1983) found that inhibitors such as HU and ara C significantly reduce the frequency of exchange type aberrations in G_2 cells. On the other hand, recently Holmberg and Gumauskas (1986)

reported that ara C enhanced yields of X-ray induced chromosome exchanges by a factor of 2 in G_0 human lymphocytes. In experiments with G_2 cells it was shown that ara C and aphidicolin act as clastogens rather than inhibitors of DNA repair (Moore and Hodgson 1983), and recently, by means of premature chromosome condensation (PCC) of G_0 human lymphocytes, it was argued that there is no evidence that ara C inhibits the repair of X-ray induced breaks but itself acts as a clastogenic agent (Panthelias and Wolff 1985). In experiments with JU56 cells (a permanent cell line derived from the black-tailed Wallaby) it was found that ara C alone induced lesions in G_2 cells of both chromatid and chromosome types (Moore 1981). The increased frequency of chromosomal aberrations in the presence of ara C might be due to the inhibition of both single- and double-strand breaks (Natarajan *et al.* 1986).

Purpose of present investigations

It is well established that DNA constitutes the primary target for the damaging effects of ionizing radiation, and that chromosomal aberrations result from the changes induced in the DNA structure (Natarajan 1982). A better understanding of the mechanisms of formation of aberrations could be obtained by using DNA-repair inhibitors, such as ara A and ara C by studying their influence in aberration production after exposure of normal and A-T cells to X-rays.

The main aims of the work described here was largely threefold. Firstly, to investigate the kinetics of X-ray induced chromatid aberrations in G_2 cells, to test the hypothesis suggesting dsb as a major DNA lesion

leading to chromosomal aberrations (Natarajan *et al.* 1980, 1982, Natarajan and Zwanenburg 1982, Natarajan and Obe 1984, Bryant 1984 *a*, 1985, Obe and Winkel 1985, Bryant *et al.* 1987). Secondly using ara A and ara C, known as potent inhibitors of DNA dsb repair (Iliakis and Bryant 1983), proposal of Preston (1980, 1982) concerning the base-damage origin of chromosomal aberrations was examined. Thirdly to quantify the kinetics of X-ray-induced chromatid aberrations and the half time for disappearance of chromatid deletions (t1/2) in G_2 A-T cells compared to that of normal cells. This approach, was an attempt to test the hypothesis of the presence or absence of a repair defect in A-T cells at chromosomal level, in search for an explanation to the nature of the radiation hypersensitivity of A-T cells.

Use of the technique of premature chromosome condensation (PCC) (Johnson and Rao 1970) has shown that the rejoining of chromosome PCC breaks (Cornforth and Bedford 1983) parallels repair of dsb as measured by biochemical techniques (e.g. Bryant and Blöcher 1980, Blöcher and Pohlit 1982). The study of the kinetics of G_2 chromatid breaks however, provides an alternative to the PCC technique, although the observation "time window" is limited to the length of G_2 (in the case of human cells this is about 4.5 - 5 hours). By irradiating and collecting mitotic cells with colcemid at various intervals up to 3.5 hours it was possible to monitor the disappearance (rejoining) of breaks in chromatids and also the appearance of exchange type aberrations either in presence or absence of the DNA synthesis inhibitors ara A or ara C.

Other supplementary experiments presented here along with main

experiments are as follows:

1. A comparison of X-ray induced chromosome aberration yields in SV40 immortalized normal and A-T cells. This has not been done before with these cell lines.
2. Cell survival studies on normal and A-T cells: to acertain the radiation sensitivity of these two cell lines at cellular level.
3. Measurement of the duration of the G_2-phase; because most of the experiments were involved in G_2 treatment with X-rays in the presence or absence of inhibitors, a precise estimation of the average duration of the G_2-phase in each cell line was necessary.
4. Measurement of the clastogenicity of ara A and ara C: to confine the treatment time of cells with either ara A or ara C in experiments with X-rays in order to minimize the "background" of aberrations due to ara A or ara C alone. In this way it was also possible to estimate the duration of G_2-phase other than that made by autoradiography technique in these cell lines (based on the assumption that these drugs act as S-phase specific clastogens).
5. Determination of the concentration effects of ara A and ara C: to evaluate optimum concentration of these drugs for induction of similar frequency of chromosomal aberrations when administered to G_2 cells.
6. Measurement of the effects of ara A and ara C on DNA synthesis: to compare the inhibitory effects of these drugs at similar concentrations on semi-conservative DNA synthesis, judged by the reduction in incorporation of ^{3}H-TdR into DNA.

2

MATERIALS and METHODS

Cell culture

X-irradiation

Treatments with inhibitors of DNA synthesis

Preparation of chromosomes

Scoring metaphases for aberrations

- Chromatid-type aberrations
- Chromosome-type aberrations

Assay for clonogenic survival

Autoradiography for mitotic labelling

DNA synthesis assay

2. MATERIALS and METHODS

2.1. Cell Culture

All experiments were performed with asynchronus populations of human immortalized MRC5SVI and AT5BIVA cells. These are cells of fibroblastic origin transformed with Simian Virus (SV40). MRC5SVI was immortalized from the MRC5 line which was originally derived from lung tissue of a foetus with a genetically normal family history with no sign of neoplastic disease (Jacobs *et al.* 1970). AT5BIVA is a cell line derived from skin of an individual (AT5BI) displaying the classical A-T syndrome. MRC5SVI has a near-tetraploid karyotype and AT5BIVA is hypo-triploid. Both cell lines were kindly supplied by Dr. D. Scott, Paterson Institute for Cancer Research in Manchester. The doubling time for both cell lines was 22-24 hours.

These cell lines were grown and maintained under sterile conditions in Eagle's Minimal Essential Medium (MEM) (Eagle, 1955) containing 15% foetal calf serum (MEMFCS). The medium was made up by mixing the following:

100 ml/l of 10x concentrated MEM

30 ml/l of 72 g/l sodium bicarbonate

10 ml/l of 0.2 mol/l L-glutamine

10 ml/l of non-essential amino acids (100x concentrated)

10 ml/l antibiotics (penicillin and streptomycin)

840 ml/l Sterile distilled water (2x)

After mixing these components, 150 ml/l foetal calf serum (FCS) was added.

Cells were routinely grown as monolayers attached to the surface of 75 cm^2 tissue-culture grade plastic flasks (Sterilin) in 15 ml MEMFCS. Cells were passaged once a week with a change of medium after three days to keep cells in full exponential growth. For passaging, the medium was removed from the flask and the cells were detached from the surface by means of trypsinization by using trypsin/EDTA. This was prepared as follows: 10 ml/l of 5% sterile trypsin (Bacto Trypsin, Difco) was dissolved in a filter sterilised salt solution consisting of:

8 g/l NaCl; 0.2 g/l KCl; 1.15 g/l Na_2HPO_4; 0.2 g/l KH_2PO_4

0.2 g/l EDTA (ethylene-diamine tetra-acetic acid, sodium salt)

The cells were washed twice with 3-4 ml trypsin/EDTA and then placed in the incubator at 37° C for 8 minutes. After this time cells were detached from the surface of the flask and from one another. A single cell suspension was achieved by adding 5 ml of fresh MEMFCS and by pipetting up and down a few times to resuspend the cells and separate any clumps. The concentration of cells was determined by counting a sample of 0.1 ml cell suspension together with 9.9 ml Isoton in a Coulter counter (Model D) with appropriate settings; Attenuation, A=8; Aperture current, I = 0.017; and Threshold, T = 20. Cells were seeded at $5x10^4$ cells in 75 cm^2 flasks containing 15 ml MEMFCS and gassed with 5% CO_2 in air to adjust the pH of the medium. The flasks then were

incubated at 37° C until required.

2.2 . X-irradiation

Cells were irradiated in medium as a monolayer with X-rays generated by a Siemens X-ray therapy unit (Stabilipan) operating at 250 KV and 14 mA, filtered with a 0.5 mm Cu filter giving an absorbed dose-rate at the position of cells of 0.75 Gy per minute. Cells were exposed at room temprature (~20° C). The X-ray dose was monitored by a Farmer-Baldwin dosemeter (ionization chamber) and checked by ferrous sulphate dosimetry (Frankenburg, 1969). In the case of experiments with G_2 cells, cultures were kept out of incubator for less than 10 minutes. After irradiation flasks were immediately returned to the incubator.

2.3. Treatment with inhibitors of DNA synthesis

In experiments involving treatment with 9-β-D-arabinofuranosyl-adenine (Sigma; ara A) and 1-β-D-arabinofuranosyl-cytosine (Sigma; ara C) , ara A and ara C were added to the medium as a 10 m mol/l solution in Hanks balanced salts solution (HBSS). These agents were administered routinely at four hours before mitotic collection. This was therefore at least 0.5 hour before X-irradiation in the case of G_2 experiments. Both drugs were left in the culture medium until fixation; see figure 3.14 for experimental protocol. X-irradiation was carried out in presence of inhibitors. [In all experiments cells were always exposed to ara A or ara C half an hour before any treatment (e.g. ^{3}H-TdR) to allow enough time

for araA (Brink and Le Page 1965) and ara C (Momparler *et al.* 1971) to be phosphorylated in order to compete with the cellular pool of nucleotides, dATP and dCTP].

3.4. Preparation of Chromosomes

Chromosomes were prepared by a method based on that of Hsu and Kellogg (1960) and Frøland (1961) in which the cells were first brought into cell suspension using trypsin as described above. Before harvesting cells were exposed to demecolcine (colcemid) at a final concentration of 0.08 μ g/ml for 1-1.5 hours to arrest cells in metaphase. The following steps were then followed:

a) Medium was transfered to a "V" centrifuge tube.

b) The cells were trypsinized off flasks, and together with retaining trypsin washes and cells resuspended in the original medium were then collected in the "V" tube.

c) The cell suspension was centrifuged for 5 minutes at 1250 rpm in an MSE bench centrifuge.

d) The supernatant was removed by suction without disturbing the pellet.

e) The cell pellet was vortexed briefly and 5 ml of prewarmed (~37° C) "hypotonic" solution 0.075 mmol KCl was added to each tube. The cells were left in hypotonic solution for 8-10 minutes at room temperature and then centrifuged again.

f) The cells were resuspended in 0.5 ml fresh KCl using a Pasteur pipette and then freshly made Carnoy's fixative added, consisting

of methanol and glacial acetic acid (3 parts to 1). The first two ml of fixative were added drop-wise while agitating the cell suspension, the rest of fixative was then added. Cells were left in fixative for 15-20 minutes at room temperature and then centrifuged.

g) The cells were then washed by centrifugation twice more in fixative.

h) Samples were then stored in fixative at 4° C over-night.

For metaphase spreads, the cells were resuspended in a few drops of 60% acetic acid or fresh fixative, before being dropped on to chilled, wet slides. In most experiments slides were prepared by the "dry-ice" (solid CO_2) method where pre-cleaned slides were chilled on dry-ice. The cell suspension was dropped on the slide while it began to defrost from the edges. Slides then were gently dried over a spirit flame. Some other slides were made using the "cold water bath" method in which slides were chilled in ice. Chilled slides were then washed in ice-cold water before the cell suspension was dropped on. These slides were dried over a low spirit flame. Dried slides were washed in buffer solution (pH=6.4) for 5 minutes and stained in 3% Giemsa stain in buffer (pH=6.4) for 10 minutes. Stained slides were rinsed in buffer and dried at room temperature.

2.5. Scoring metaphases for aberrations

Scoring of all slides was carried out with a 16x oil immersion objective (for scanning) and a 100x oil immersion objective on a Zeiss microscope. The scoring system was based on the classifications of Savage (1976) and Buckton and Evans (1981). For MRC5SVI cells which are near-

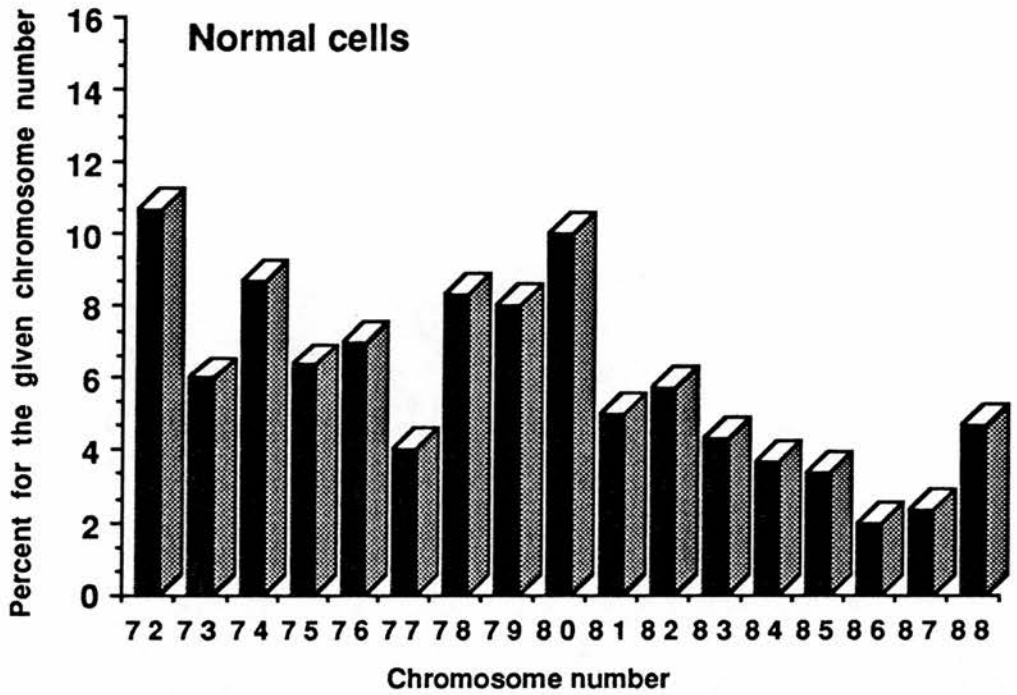


Figure 2.1: Range of chromosome number in MRC5SVI cells. Results are derived from the score of 300 cells.

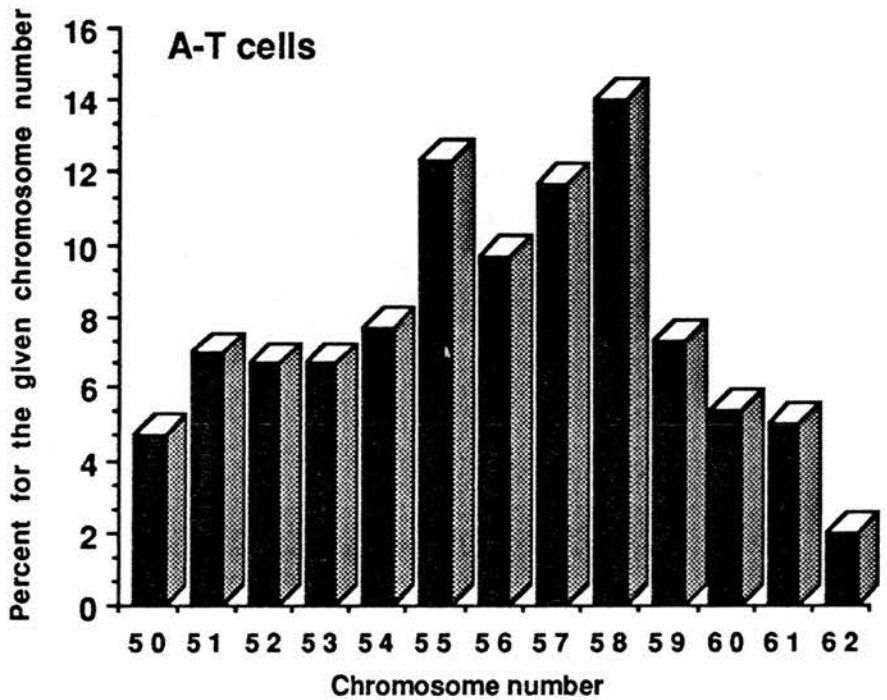


Figure 2.2: Range of chromosome number in AT5BIVA cells. Results are derived from the score of 300 cells.

tetraploid, the number of chromosomes lying between 72-88 (Figure 2.1), only metaphases with more than 70 chromosomes were scored. For AT5BIVA cells which are hypo-triploid, their chromosome number lying between 50-62 (Figure 2.2), only metaphases with more than 50 chromosomes were scored. Because most of the experiments involved short intervals between irradiation and fixation, (i.e. G_2 treatments); the type of aberrations scored were only chromatid type. A short summary of the types of chromosomal aberrations is given below.

Chromatid Type aberrations:

***Gaps or achromatic lesions*:** A chromatid gap is a non-stained region in the chromatid, involving only one chromatid of each chromosome. The non-staining region is not greater than the diameter of the chromatid (Figure 2.3, 1).

***Chromatid breaks*:** A chromatid break is similar to a gap but the terminal part of the chromatid has been displaced indicating that it is not attached to the chromosome. For a non-staining non-displaced region to be classified as a break, it should be wider than the diameter of the chromatid. Chromatid breaks were a very common type of aberration in both MRC5SVI and AT5BIVA cells after irradiation and treatment with inhibitors of DNA synthesis (Figure 2.3, 2).

***Isochromatid deletions*:** Isochromatid breaks are the results of sister chromatids which have been broken at the same points, and that often the broken ends of the sisters are observed to have rejoined. Complete rejoining of broken ends is called Sister Union (SU). Incomplete

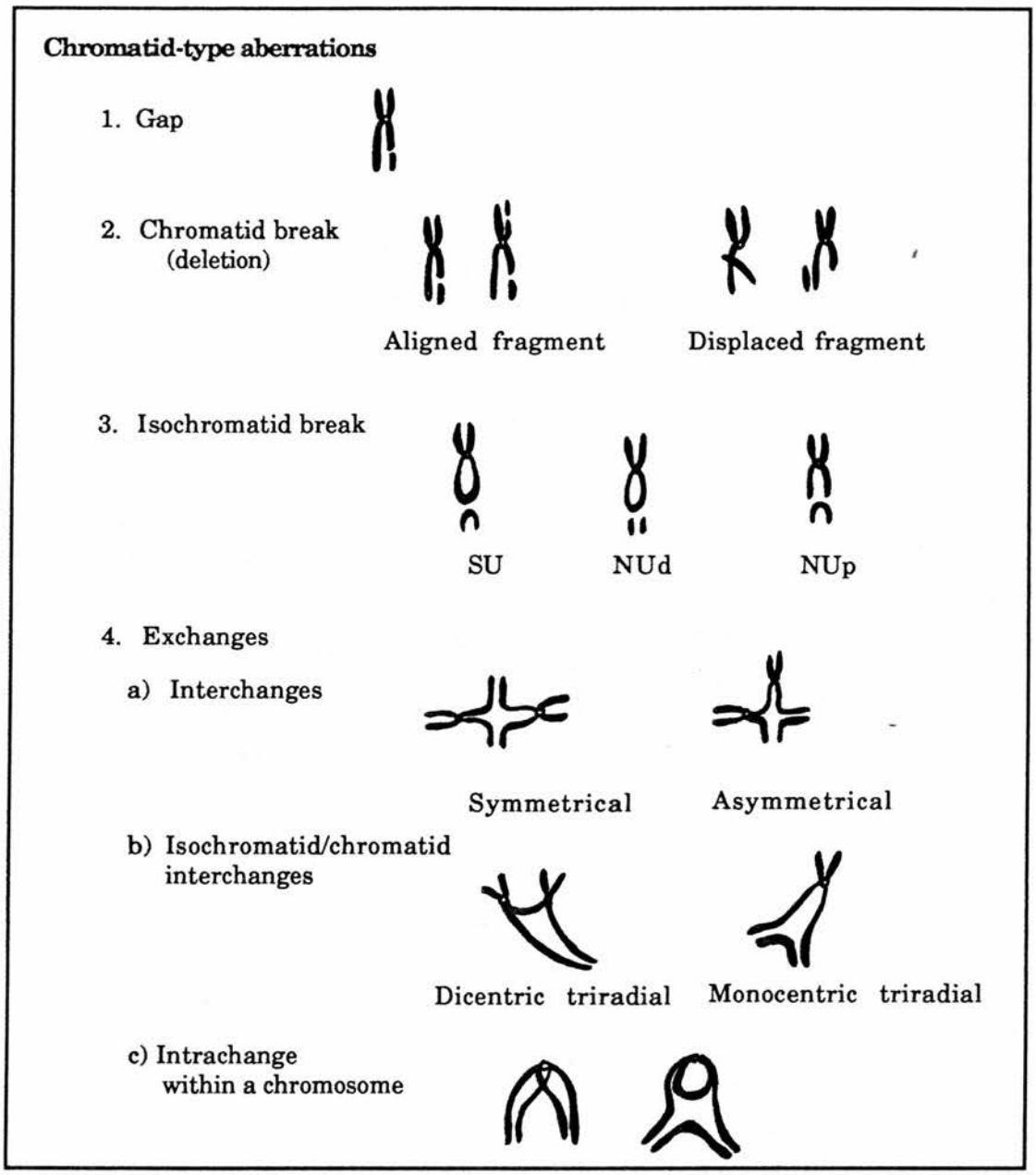


Figure 2.3. Diagrammatic presentation of the main types of chromatid aberrations which are scored.

rejoining (Non-Union, NU) occur either proximally (p) or distally (d). Fragments may be aligned or displaced. Where the type of rejoining can not be identified the fragments should be reffered to as isolocus deletions (Figure 2.3, 3).

***Chromatid exchanges*:** Damage to the genetic material may take the form of exchange between different chromosomes or chromatids. Chromatid exchanges can be either in the form of an interchange or an intrachange.

a) *Chromatid interchange*: When the interacting lesions occur in the arms of different (homologous or non-homologous) chromosomes. Simple complete chromatid interchange involves an exchange between single chromatids of two chromosomes and results in a configuration having four arms which may be referred to as a "quadriradial" (Figure 2.3, 4*a*). An exchange may also occur between an iso-chromatid break and chromatid break which leads to a "triradial" configuration (Figure 2.3, 4*b*). Exchanges can either be symmetrical, giving two new monocentric chromatids, or asymmetrical, giving a dicentric chromatid and an acentric fragment.

b) *Chromatid intrachange*: Chromatid intrachange occur when the interacting lesions are within one chromosome. Exchange within a chromatid arm is termed intra-chromatid exchange (Figure 2.3, 4*c*).

Therefore, for presenting results of the experiments with G_2 cells all types of aberrations are brought under three main categories as follows:

1- Chromatid gaps.

2- Chromatid deletions: which involve all types of breaks greater than the diameter of the chromatid and iso-chromatid breaks.

3- Exchanges: which includes all types of exchanges observed.

Chromosome type aberrations :

***Chromosome Gap (achromatic lesion)*:** Involving both chromatids of a chromosome at identical loci. Non-staining region is not greater than the diameter of a chromatid (Figure 2.4, 1).

***Chromosome break (terminal deletion)*:** Involving only one chromosome and as with all other chromosome type aberrations occuring in the unreplicated (G_1 or G_0) chromosome. A terminal deletion (acentric fragment) will result in a break larger than the width of a chromatid and without a centromere with parallel-lying paired chromatids. In most cells

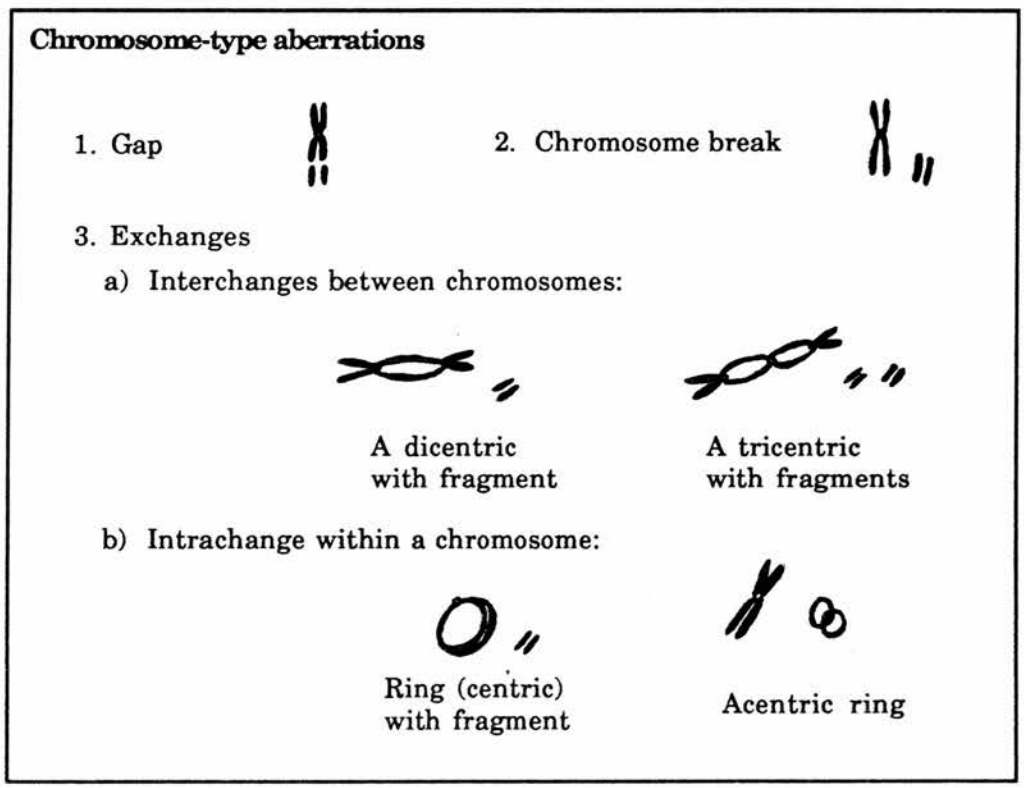


Figure 2.4. Diagrammatic presentation of the main types of chromosome aberrations which are scored.

these can not be distinguished from isochromatid breaks so both are referred to as isolocus breaks (Figure 2.4, 2).

***Chromosome exchanges*:** Exchanges involve two or more lesions in the same or different chromosomes and can either be in the form of an interchange or an intrachange (Figure 2.4, 3).

a) *Interchange between chromosomes*: The main indication of chromosome damage is the "dicentric" which results from an exchange between two chromosomes in G_1 giving a structure with two centromeres and an acentric fragment. The fragment is part of the exchange and should not be scored as a separate event. Much less frequently, a structure with three centromeres is produced which is called a "tricentic" (Figure 2.4, 3*a*).

b) *Chromosome intrachange*: Intrachange occurs within a chromosome in the forms of centric ring or acentric ring (Figure 2.4, 3*b*).

i) Centric ring: A centric ring is formed from a single chromosome which has been broken in both arms or an inter-arm intrachange. The proximal segments join to form the ring and the distal segments join to form an acentric fragment. The fragment is part of the exchange and one fragment should be allocated to each ring (or dicentric); the remaining fragments being classified as isolocus deletions (see above).

ii) Acentric ring: Acentric rings form as a result of intrachange within an arm or intra-arm intrachange. The centric part of this exchange may not be identified. Small acentric rings are called "double minutes" or "interstitial deletions".

2.6. Assay for clonogenic survival

Assay of survival after exposure to X-rays was made for MRC5SVI and AT5BIVA cells in order to determine the relative X-ray sensitivity of these cell lines.

For survival assays subcultures of 10^5 cells in 5 ml MEMFCS were set up in 25 cm^2 tissue-culture flasks (Sterilin) for both cell lines and were incubated for 48 hours at 37° C prior to irradiation. X-ray doses of up to 3 Gy and 6 Gy were delivered to monolayer (exponential) AT5BIVA and MRC5SVI cells respectively at a dose rate of 0.75 Gy/min in air and room temprature (~20° C). The cells were trypsinized off and appropriate dilution was made for each sample. These were plated out into 50 mm Petri dishes (Cel-Cult) with 5 ml fresh MEMFCS. Three Petri dishes, at each of two different cell dilutions were set up for each dose.

The MRC5SVI and AT5BIVA plates were incubated in 6% CO_2 in air, at 37° C in a humidified incubator for two and three weeks respectively. After removal of medium, colonies were fixed in methanol and stained in Giemsa.

2.7. Autoradiography for mitotic labelling

For autoradiographic experiments MRC5SVI and AT5BIVA cells were passaged in 75 cm^2 Sterilin flasks two days prior to experiments to ensure cells were in exponential growth. Then sub-cultures of cells were set up in 50 mm Petri dishes (Cel-Cult) with $2.5x10^5$ cells in 5 ml MEMFCS. Cultures were incubated for 48 hours before labelling at 37° C.

For labelling, medium was removed and replaced with 4 ml fresh

medium containing 37 KBq/ml (1 μ Ci/ml) ^{3}H-TdR with a specific activity of 1.48 TBq/mmol (40 Ci/mmol) and incubated. Cells were "continously" labelled, meaning that ^{3}H-TdR remained in the culture medium until fixation. After addition of ^{3}H-TdR medium, cells were harvested and fixed at hourly intervals up to 10 hours. The controls (i.e. without ^{3}H-TdR) were fixed immediately after incubation of treated samples. Chromosomes were prepared as described above, and slides were made by the dry ice method.

Autoradiographs were prepared by dipping slides in K2 autoradiographic emulsion (Ilford) mixed 1:1 with 1% glycerol solution (99 ml H_2O, 1 ml glycerol). Slides were left at 4° C for one week to allow enough exposure. Autoradiographs then were developed in D19b developer (Kodak) for 3.5 minutes, rinsed in distilled water for 30 seconds and finally fixed in Kodak acid fixative for 5 minutes. The slides were then stained in 3% Giemsa for 10 minutes. Slides were scored for labelled mitoses and mitotic indices. This experiment was carried out twice for both cell lines; but for the second time only labelled mitoses for samples for up to 7 hours post-labelling time was measured. Standard deviation of mean values of labelled mitoses were calculated for both experiments.

2.8. DNA Synthesis assay

For assay of DNA synthesis, suspensions of trypsinized AT5BIVA and MRC5SVI cells were prepared at about 6.5×10^5 cells per ml in 5 ml MEMFCS half an hour prior to labelling. Samples of each cell line were

treated with ara A and ara C at concentrations of 100 μ mol/l and 200 μ mol/l. Both control (untreated) and treated samples were then labelled with 3.7 KBq/ml (0.1 μ Ci/ml) ^{3}H-TdR with a specific activity of 1.48 TBq/mmol (40 Ci/mmol) and incubated in tubes in a water bath at 37° C and sampled at 5, 10, 20 and 30 minute intervals. Samples were held in "V" centrifuge tubes on ice after addition of 5 ml of ice-cold 0.15 mol/l NaCl. Samples were then centrifuged at 0° C and supernatants removed. DNA was then prepared on filters as follows:

a) Pellets were resuspended by short vortexing and 1 ml 0.03 mol/l NaOH (ice-cold) was added and cells were left for 5-10 minutes on ice.

b) 2 ml of 10% TCA (trichloroacetic acid) was added to each sample and left at 4° C over night.

c) The DNA precitipate was then filtered using fibre glass filters (Whatman, 2.5 cm diameter).

The fibre glass filters were placed in scintillation vials and 4.5 ml scintillation fluid (Packard, Insta-gel) was added. The samples were counted in a LKB 1214 Rack beta liquid scintillation counter.

3

RESULTS

Mode of action of ara A and ara C

DNA synthesis experiments

Experiments with normal cells

Experiments with A-T cells

Concentration effects of ara A and ara C

Effects of ara A on G_2 normal cells

Effects of ara C on G_2 A-T cells

Effects of combined treatment of ara A and ara C when applied to G_2 A-T cells

G_2 determination

Autoradiographic method

Measurement of G_2-phase using ara A and ara C

Treatment of A-T cells with ara C

Treatment of normal cells with ara A

Comparison of the radiation sensitivity of A-T and normal cells

Clonogenic assay

Effects of X-rays on cells irradiated 24 h before fixation

Effects of X-rays on G_2 cells

Cytogenetic studies with X-rays and ara A treated G_2 cells

Experiments with normal cells

Experiments with A-T cells

Cytogenetic studies with X-rays and ara C treated G_2 cells

Experiments with normal cells

Experiments with A-T cells

Effects of low doses of X-rays on G_2 A-T cells

3. RESULTS

3.1. Mode of action of ara A and ara C

a) DNA synthesis experiments

Experiments with normal cells

Both ara A and ara C are known as inhibitors of DNA synthesis in mammalian cells. These experiments were designed to study the effects of ara A and ara C on DNA synthetic activities of S-phase MRC5SVI and AT5BIVA cells. Samples of $6.5x10^5$ cells per ml in 5 ml MEMFCS were prepared as cell suspensions and kept in a water bath at 37° C. Two samples were treated with 100 and 200 μ mol/l ara A and two other samples with 100 and 200 μ mol/l ara C. One sample was kept as a completely untreated control. Inhibitors were administered to the cells 30 minutes prior to addition of ^{3}H-TdR radioactive label, to allow enough time for the drug to be phosphorylated. All cultures were given ^{3}H-TdR at 3.7 KBq (0.1 μ Ci) per ml and sampled at various time intervals, viz; 5, 10, 20 and 30 minutes after being exposed to ^{3}H-TdR. As shown in the histogram in figure 3.1 there was a sharp increase in ^{3}H-TdR incorporation by cells from 5 to 30 minutes in untreated samples (black bars) while the overall increase in incorporation in ara A and ara C treated samples was much lower. It can be seen that at 30 minutes post-labelling time, the overall incorporation of ^{3}H-TdR in ara A and ara C treated cells was only 10-15 percent compared to untreated controls.

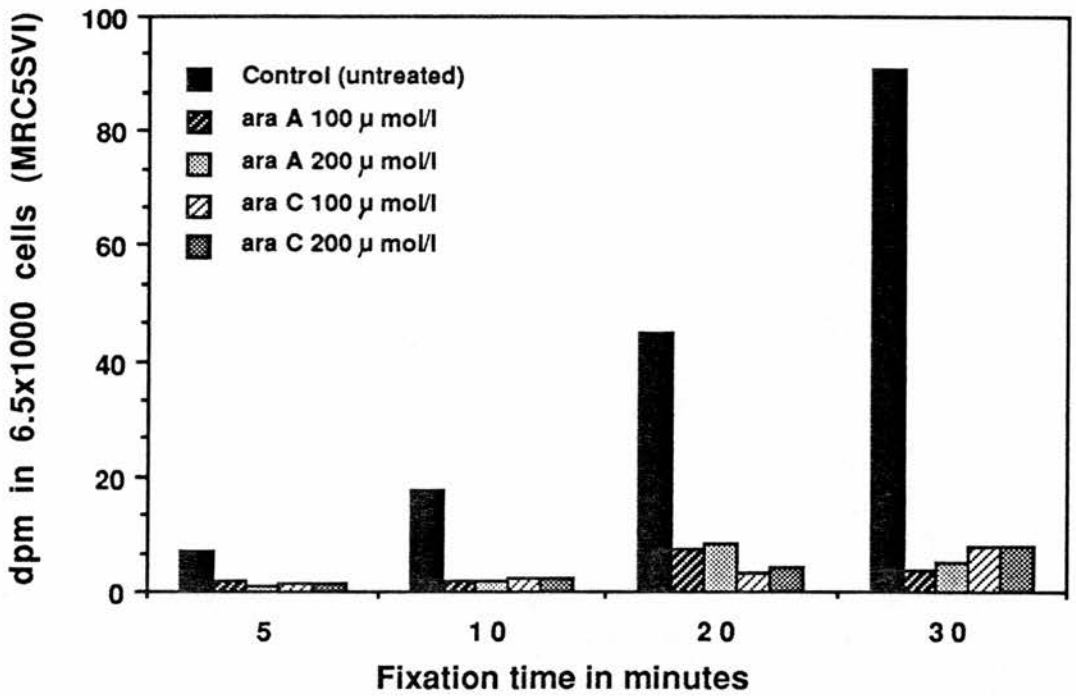


Figure 3.1. Incorporation of ^{3}H-TdR in normal cells at various periods of time in the presence or absence of ara A and ara C. Cells were treated with inhibitors 0.5 h before being exposed to ^{3}H-TdR.

Experiment with A-T cells

Figure 3.2 shows the results of ^{3}H-TdR incorporation experiment with A-T cells. In untreated samples the incorporation of ^{3}H-TdR was increased with time (black bars). This figure also show that in controls there was less DNA synthesis in A-T than in normal cells (Figure 3.1). In ara A treated samples this figure shows that the inhibitory action of ara A was concentration dependent. Incorporation of ^{3}H-TdR in the presence of 100 μ mol/l ara A was higher than that in the presence of 200 μ mol/l ara A for all samples. The overall incorporation of ^{3}H-TdR in presence of 200 μ mol/l ara A was about 10% and with 200 μ mol/l ara C

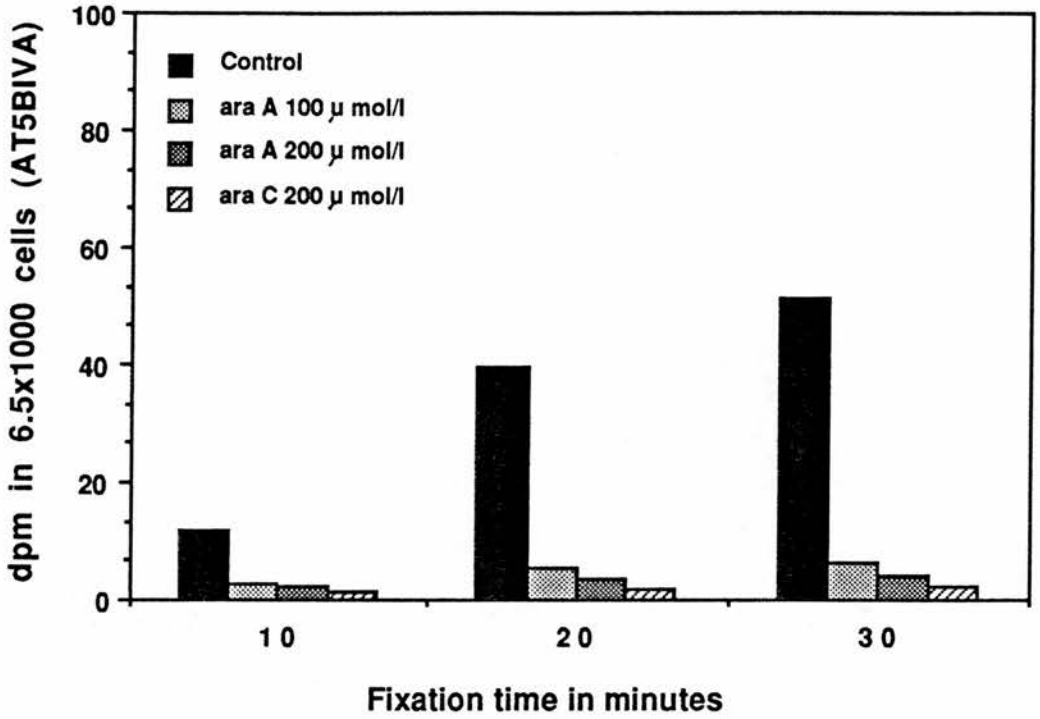


Figure 3.2. Incorporation of ^{3}H-TdR in A-T cells at various time intervals in the presence or absence of ara A and ara C. Cells were treated with ara A or ara C 0.5 h before being exposed to ^{3}H-TdR.

even less, about 5% compared to untreated control.

These experiments show that both ara A and ara C reduce incorporation of ^{3}H-TdR to 85 - 90% in both cell lines. In normal cells ara C was found to be more effective at 20 min, and ara A at 30 min post-treatment time in reducing incorporation of ^{3}H-TdR. In A-T cells ara C was found to be more effective than ara A at similar concentrations for all sampling times. Thus both ara A and ara C were found to be strong inhibitors of DNA synthesis in the cell lines used for experiments and ara C was found to be more effective than ara A at similar concentrations.

b) Concentration effects of ara A and ara C

Effects of ara A on G_2 normal cells

The results of this experiment are summarized in table 1. Figure 3.3 shows the frequency of deletions and gaps induced by various concentrations of ara A in a range of 100 to 250 μ mol/l when applied to G_2 cells. Four cultures were treated with 100, 150, 200, and 250 μ mol/l ara A four hours before fixation. The number of aberrations (deletions and gaps)

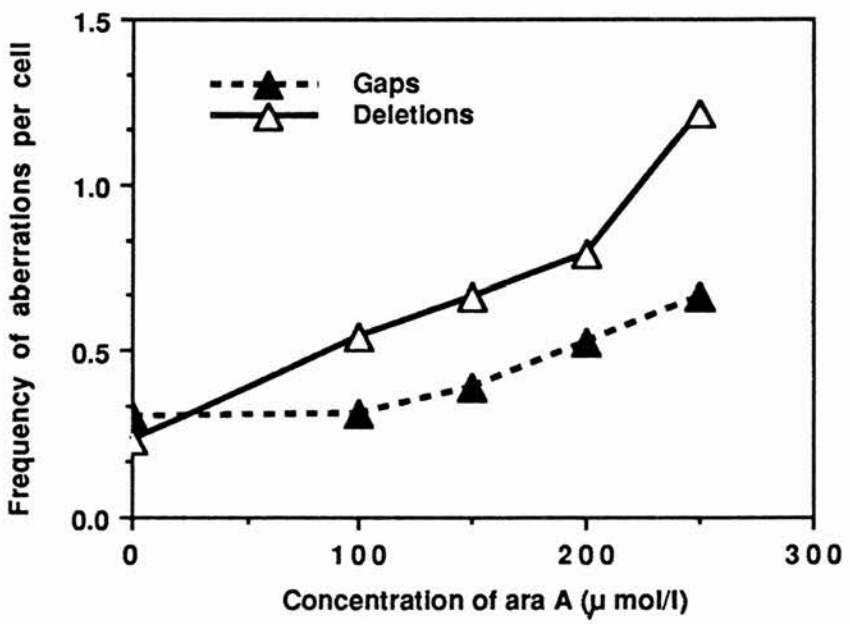


Figure 3.3. Frequency of gaps and deletions induced by various concentrations of ara A in G_2 normal cells. 150 cells were analysed for each point. The total treatment-fixation time (4 h) includes 1.5 h colcemid treatment.

increased steadily as the concentration of ara A increased in the culture medium. The frequency of deletion formation was higher than the frequency of gaps for all concentrations. Low frequency of exchanges were found for all concentrations of ara A (Table 1).

Table 1: Yields of chromatid aberrations induced by various concentrations of ara A in G_2 normal cells.

Treatment μ mol/l	No. of cells analysed	Gaps	Deletions	Exchanges
Control	150	34	45	2
Ara A 100	150	47	81	6
// 150	150	59	99	10
// 200	150	79	118	10
// 250	150	99	181	12

Effects of ara C on A-T cells

The results of this experiment are summarized in table 2. Four cultures of A-T cells were exposed to 50, 100, 150 and 200 μ mol/l ara C four hours before collection. 100 metaphases were analysed for each sample. The table (2) and figure (3.4) show that the number of deletions increased with increasing concentration of ara C. There was a sharp increase in deletions (0.84/cell) up to a concentration of 100 μ mol/l, but beyond that only a slight increase was observed (0.93/cell at 200 μ mol/l). The frequency of gaps induced by 50 μ mol/l ara C was about twice the number of gaps found in controls but there was no significant difference in gap induction with increase in ara C concentration beyond 50μ mol/l (Figure 3.4). The number of gaps induced by 50μ mol/l ara C was 38 per

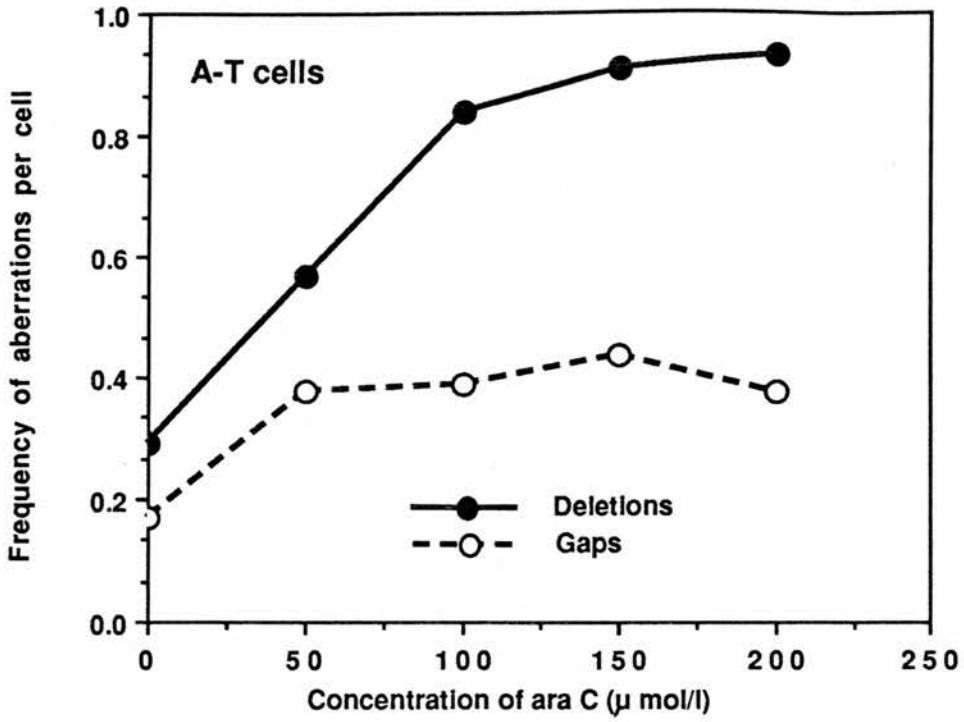


Figure 3.4. Frequency of chromatid deletions and gaps induced by various concentrations of ara C in G_2 A-T cells. Cells were treated with ara C 4 h before fixation.

100 cells and similar frequency was found for 200 μ mol/l ara C. Despite only a small increase in the frequency of deletions beyond 100 μ mol/l, the number of chromatid exchanges increased substantially and it seems to be concentration dependent (Table 2). 100 μ mol/l ara C was found to have similar effect on chromosomal aberration induction in A-T cells seen for G_2 normal cells treated with 200 μ mol/l ara A (Figure 3.3).

Table 2: Yields of chromatid aberrations using various concentrations of ara C in G_2 A-T cells.

Treatment μ mol/l	No. of cells analysed	Gaps	Deletions	Exchanges
Control	150	25	43	2
Ara C 50	100	38	57	2
// 100	100	39	84	7
// 150	100	44	91	22
// 200	100	38	93	34

c) Effects of combined treatment of ara A and ara C when applied to G_2 A-T cells

Kihlman and Anderson (1985) showed that if two inhibitors are combined and administered to cells, this might lead to the induction of chromosomal aberrations at higher frequencies than the sum of the aberrations induced by each inhibitor individually (at least by a factor of 1.5-2). It was observed that ara A acts as a powerful inhibitor of rejoining of chromatid deletions when applied to G_2 irradiated cells (experiments with normal and A-T cells, sections 3.4 & 3.5 of this chapter). Ara C proved to be inhibitor of DNA synthesis when applied to S-cells, but results obtained in G_2 experiments in combination with X-rays, imply that it might act in a similar way to radiomimetic agents rather than inhibitors. Therefore the following experiment was performed to study the combined effects of ara A and ara C on A-T cells.

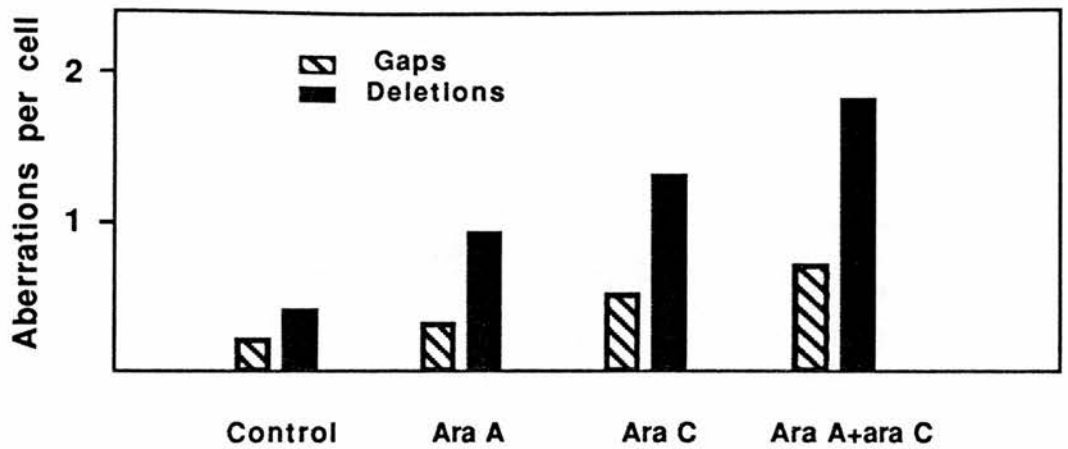


Figure 3.5. Frequencies of deletions and gaps induced by ara A (100 μ mol/l) or ara C (100 μ mol/l) alone or in combination in A-T cells exposed 4 h before fixation.

4 cultures were set up in 75 cm^2 flasks using $4x10^5$ cells in 10 ml MEMFCS. Cells were treated with ara A and ara C alone or in combination at 100 μ mol/l each at 4 h before fixation. One culture was kept as entirely untreated control. Mitotic figures were collected in each case by applying 200μ l demecolcine 1.5 hours before harvesting. 100 metaphases were scored for each sample, the result of this experiment is summarized in table 3.

Figure 3.5 shows the effects of inhibitors alone when they were applied individually or in combination. It can be seen that the number of deletions induced by 100 μ mol/l ara C alone was more than that of 100 μ mol/l ara A alone. The frequency of aberrations induced by the combined treatment of ara A and ara C was high but not more or even equal to the sum of aberrations induced by ara A and ara C individually.

This experiment, therefore show that the inhibitors did not act synergistically when applied to the cell cultures together (Table 4). Lack

of synergism might be due to competetive action of the drugs for same sites on the DNA polymerases α and β or one of the inhibitors may prevent uptake of the other (Kihlman and Anderson 1985).

Table 3. Yields of chromatid aberrations in G_2 A-T cells following exposure to 100 μmol/l ara A and ara C alone or in combination for 4 hours.

Treatment	No. of cells analysed	Gaps	Deletions	Exchanges	Total
Control	100	17	44	2	63
Ara A	100	35	89	3	127
Ara C	100	46	131	6	183
Ara A + ara C	100	65	179	19	263

Treatment	No. of cells analysed	Total aberrations (Observed)	Total aberrations (Expected)
Ara A	100	64 +	184
Ara C	100	120	
Ara A + ara C	100	200	≥ 276

Table 4. The observed and expected values of chromatid aberrations in G_2 A-T cells following exposure to ara A (100 μmol/l) and ara C (100 μmol/l) alone or in combination. Total aberrations are subtracted from the background level of aberrations.

3.2. G_2 Determination

a) Autoradiographic method

Because majority of the experiments with A-T and normal cells were performed in the G_2 phase of the cell cycle, it was necessary to determine the length of G_2 for both cell lines using an autoradiography technique (Howard and Pelc 1953). The length of G_2 was measured by means of mitotic labelling with continous incubation of cells with ^{3}H-TdR up to 10 hours and fixed at one hourly intervals. After preparation of metaphase spreads, slides were dipped into photographic emulsion and developed after one week exposure at 4° C. A mitotic figure was scored as labelled if it contained more than 10-15 (depending on the background labelling intensity) black silver grains as a result of radioactive label. 100 metaphases were scored for each sample.

The rate of passage of cells through G_2 was estimated from the percentage of labelled mitoses versus time as shown in figure 3.6 (a & b). 50% labelled mitoses defines the time showing the length of the duration of G_2 and half of the duration of mitosis ($TG_2 + TM/2$). Because, the length of mitosis for most cell lines including human cells dose not exceed 1 hour (Hall 1978), hence the length of G_2 phase for A-T cells was estimated to be about 5 hours (Figure 3.6 panel A). On the same basis G_2 duration for normal cells was estimated to be approximately 4.5 hours (Figure 3.6 panel B).

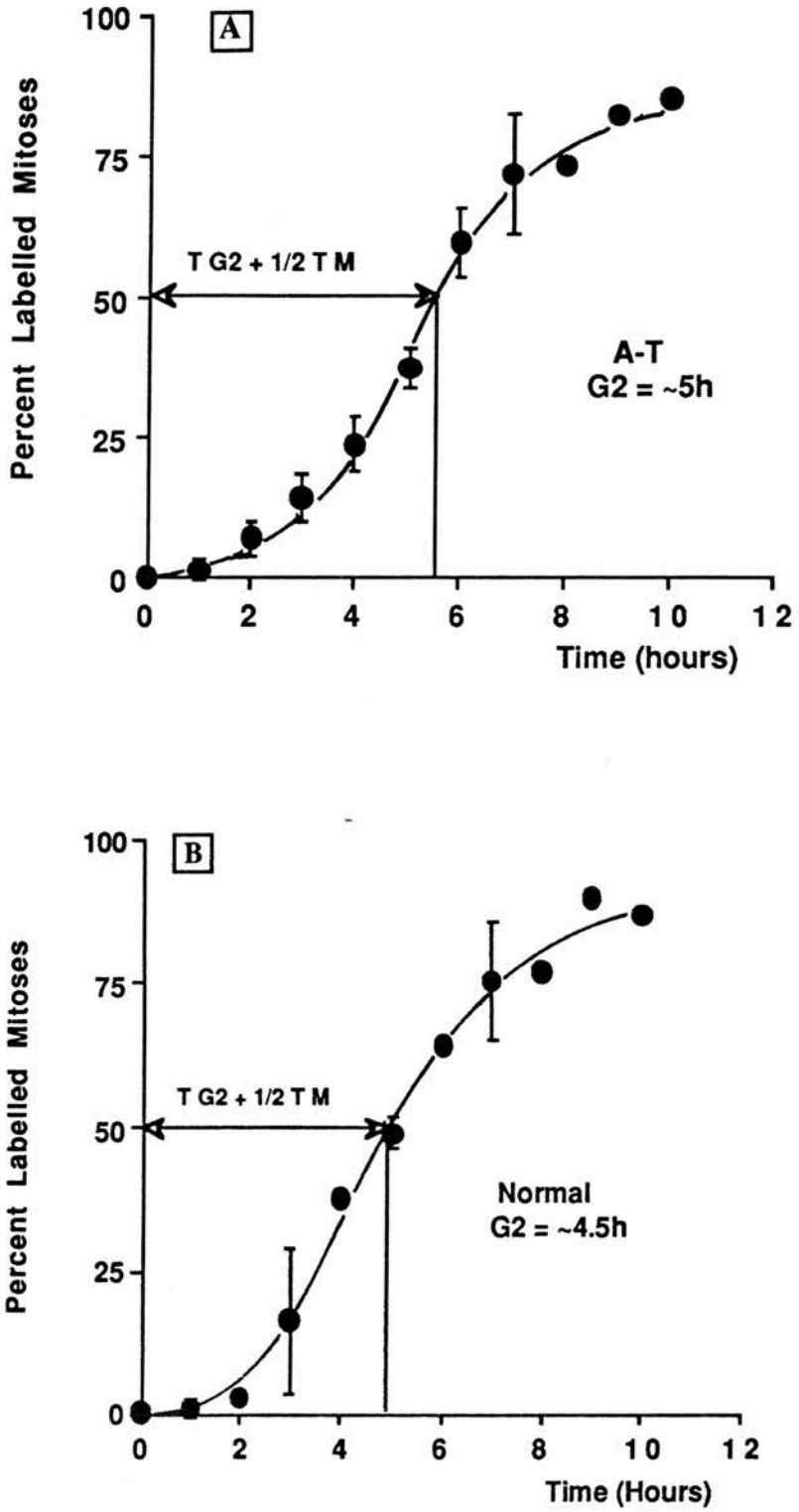


Figure 3.6. The percentage of labelled mitoses as a function of time before fixation in A-T (panel A) and normal cells (panel B). Mitotic cells were labelled following continous incubation with ^{3}H-TdR for various periods of time before fixation. The arrow and dashed line indicate the length of G_2+1/2M phases. Error bars indicate standard deviation of mean values of two independently performed experiments.

Table 5: Percentage of labelled mitoses and mitotic indices for autoradiographic experiments. Errors are standard deviation of mean values of two experiments.

Sampling time after addition of 3H-TdR in hours	MRC5SVI		AT5BIVA	
	LM%	MI%	LM%	MI%
0.0		4.19 + 1.35		3.85 + 1.20
1.0	1.0 + 1.41	2.60 + 0.92	1.50 + 2.10	1.85 + 0.92
2.0	2.5 + 0.71	3.04 + 1.22	7.00 + 2.83	2.00 + 1.27
3.0	16.0 + 12.7	2.90 + 0.59	14.00 + 4.24	2.31 + 1.33
4.0	37.5 + 0.71	2.70 + 1.63	23.50 + 4.90	2.60 + 1.40
5.0	49.0 + 2.83	2.70 + 2.38	37.50 + 3.54	2.44 + 1.75
6.0	64.0 + 0.00	2.60 + 2.23	59.50 + 6.36	1.78 + 0.80
7.0	75.5 + 10.6	2.30 + 1.28	71.50 +10.60	3.00 + 1.41
8.0	77.0	1.10	73.0	1.80
9.0	90.0	1.80	82.0	1.80
10.0	87.0	0.90	85.0	1.80

These findings are in agreement with other measurements for G_2 phase of human fibroblasts (e.g. Zampetti-Bosseler and Scott 1981). Table 5 also shows the values of percentage of labelled mitoses and percent mitotic index for each sample for both normal and A-T cell lines. These values are the mean of two experiments and mitotic index was measured by counting more than 1000 cells for each sample. The mitotic index for

both cell lines exhibited a slight delay following treatment with ^{3}H-TdR and that in both cell lines there was a further delay when the time of treatment with ^{3}H-TdR exceeded 7 hours.

b) Measurement of G_2- phase using ara A and ara C.

On the basis of the assumption that ara A and ara C act as S-phase specific clastogens, the length of the G_2-phase was determined by treatment of cells for various times before fixation with ara A or ara C. The G_2-phase of the cell cycle is only an operational definition and might not be totally free of DNA synthesis. The following experiments describe the effects of ara A on normal and ara C on A-T cells in late S and G_2 phase.

Treatment of A-T cells with ara C

100 μ mol/l ara C was applied to 5 cultures from two up to seven hours prior to harvesting. This treatment time also includes 1.5 hours demecolcine (colcemid) treatment for mitotic collection. 100 mitoses were scored for each sample. As shown in figure 3.7 the frequency of deletions induced by ara C increased slightly with time for up to 4 hours before fixation so that there was only 1.2 deletions per cell at 4 h. There was a dramatic increase beyond 4 hours reaching more than six deletions per cell at 7 hours before fixation. The frequency of gaps on the other hand up to 4 hours before fixation was at low levels, slightly higher than the background level (around 0.4 gap/cell) but it increased dramatically after

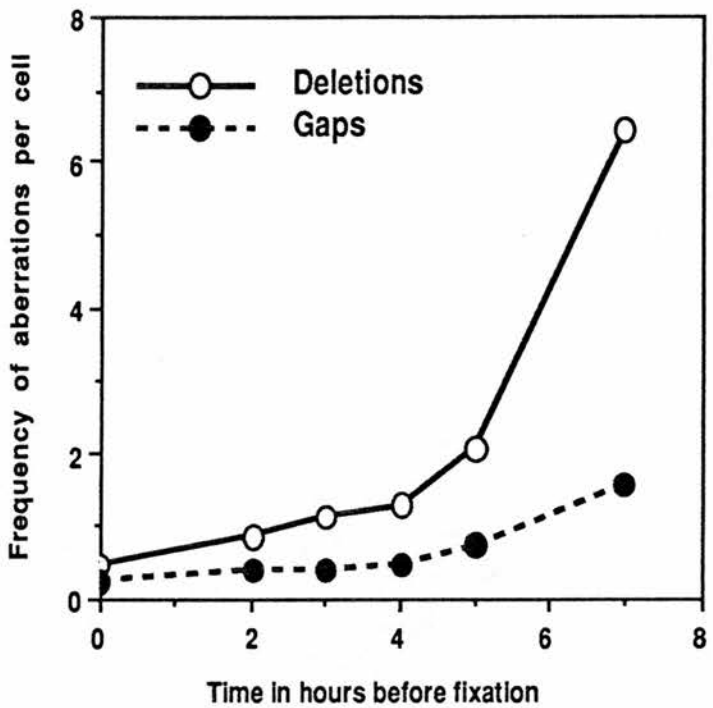


Figure 3.7. Frequencies of chromatid deletions and gaps in A-T cells when exposed to ara C (100 μ mol/l) at different time intervals before fixation.

4 h incubation with ara C; almost doubled at 5 h and reached to 1.5 gaps per cell at 7 h post-treatment incubation time (Figure 3.7). Exchanges were also observed for all treatment times. The results of this experiment are summarized in table 7.

The results of scores of chromosomal lesions were also expressed as the percentage of cells containing lesions (Table 6 and Figure 3.8). Figure 3.8 shows the net percentage of damaged A-T cells when exposed to ara C at various time intervals before fixation. It can be seen that ara C clearly produces an increase in the frequency of lesions. Because there is a very low level of synthesis or turnover of DNA in chromosomes during G_2, the effect of ara C might be limited to these cells. Ara C was

found to have a slight clastogenic effect on G_2 A-T cells during the first 3 h following treatment (Figure 3.8) since the number of damaged cells were greater than the number of labelled mitoses obtained for A-T cells (Figure 3.6 a).

Table 6: Frequency of damaged A-T cells with mitotic index following exposure to ara C (100 μ mol/l) for various time intervals before fixation.

Treatment time in hours	percentage of damaged cells	MI%
0	52	4.25
2	74	3.57
3	77	3.80
4	75	3.30
5	81	2.00
7	98	2.38

Table 7: Yields of chromatid aberrations induced by ara C (100 μ mol/l) in A-T cells when exposed at different times before fixation.

Treatment time in hours before fixation		No. of cells analysed	Gaps	Deletions	Exchanges
Control		100	23	45	8
Ara C	2.0	100	40	87	12
//	3.0	100	39	114	11
//	4.0	100	47	127	11
//	5.0	100	73	208	43
//	7.0	100	156	645	31

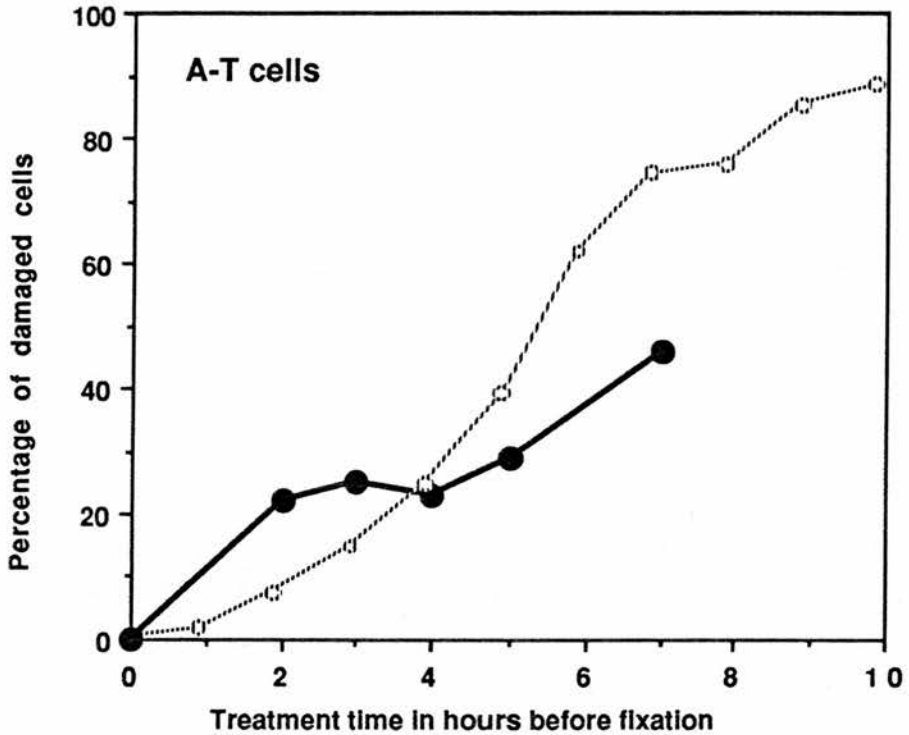


Figure 3.8. Percentage of damaged A-T cells following exposure to ara C (100 μ mol/l) for various time intervals before fixation. Values for each point are subtracted from the number of damaged cells in the controls. The dotted line shows the percentage of labelled mitoses as a function of time before fixation in A-T cells (Results from table 5)

Treatment of normal cells with ara A

Five cultures of cells were established about 44 hours before being exposed to 200 μ mol/l ara A. Ara A was applied to 4 cultures and cells were fixed at 3, 4, 5 and 7 hours after addition of ara A to the culture medium. 100 mitotic figures were analysed for each sample. The results of this experiment are summarized in table 9. Figure 3.9 shows the frequency of gaps and deletions per cell, induced by ara A as a function of treatment-fixation time.

The number of gaps induced increased with incubation time with

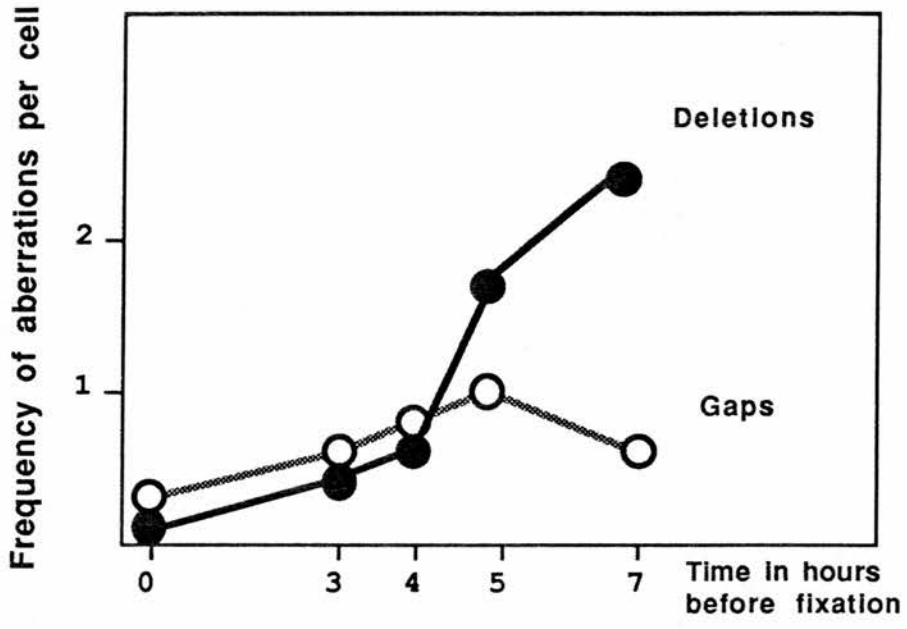


Figure 3.9. Frequency of chromatid deletions and gaps in normal cells treated with ara A (200 μ mol/l) for various periods of time before fixation.

ara A and decreased beyond 5 hours. The frequency of gaps induced up to 4 h was slightly higher than deletions but lower beyond 4 h. This shows that the damage caused by ara A in late S-phase was much more severe in terms of deletions than induction of gaps. There was also a sharp increase in deletion formation beyond 4 hours of incubation of cells with ara A so that about 2 deletions per cell were induced at 5 h and 3 deletions per cell at 7 h post-treatment incubation time (Figure 3.9). Exchanges were also seen for all treatment times though at a higher frequency for the sample treated for 7 hours (Table 9).

The results of scores of chromosomal lesions were also expressed as the percentage of cells containing aberrations for normal cells treated with 200 μ mol/l ara A (Table 8 and Figure 3.10). Figure 3.10 shows the net percentage of damaged normal cells following ara A treatment at

various time intervals before fixation. It can be seen that ara A produces an increase in the frequency of damaged cells. There was a significant increase in labelled mitoses begining at 2 hours (Figure 3.6) which suggests that some cells have a relatively short G_2 phase. These might be the cells containing aberrations induced by ara A. When comparing the frequencies of damaged cells with the values for labelled mitoses in normal cells, it seems that ara A only affected cells in DNA synthesis. This is evident by the observation that the percentage of damaged cells (Figure 3.10 solid line) are equal or lower than the values for labelled mitoses (Figure 3.10 dotted line) at corresponding time intervals.

An alternative explanation for the aberrations in G_2 cells is that there is an ongoing repair of spontaneous lesions which could lead potentially to chromosomal aberrations and that ara A and ara C are interfering with this spontaneous repair process.

As a result of these observations (effects of ara A on normal and ara C on A-T cells in the G_2 phase) these inhibitors induce a small increase in background chromosomal aberrations in both normal and A-T cells when applied in G_2. Consequently the overall treatment of cells with inhibitors for cytogenetic studies with X-rays was limited to 4 hours to minimize the contribution of ara A and ara C to the formation of chromatid aberrations.

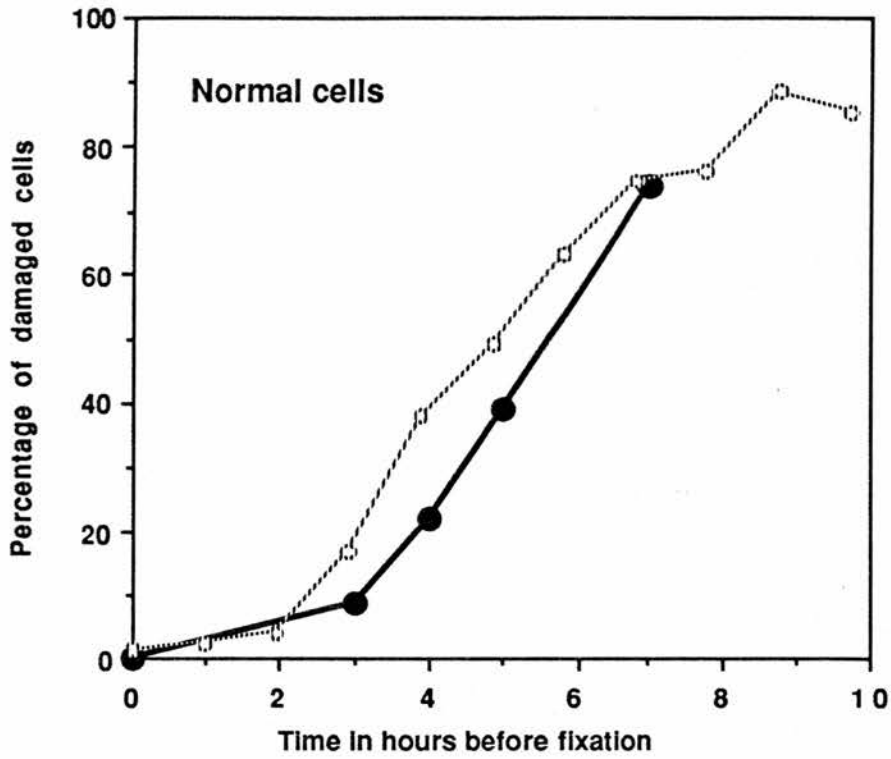


Figure 3.10. Frequency of damaged cells following exposure of normal cells to ara A (200μ mol/l) for various time intervals before fixation. Values for each point are subtracted from the number of damaged cells in the controls. The dotted line shows the percentage of labelled mitoses as a function of time before fixation in normal cells (Results from Table 5).

Table 8: Frequency of damaged normal cells with mitotic index when treated with ara A (200 μmol/l) at different time intervals before fixation.

Treatment time in hours	percentage of damaged cells	MI%
0	22	3.84
3	31	2.50
4	44	1.60
5	61	2.80
7	95.5	0.59

Table 9: Yields of chromatid aberrations induced by ara A (200 μmol/l) in normal cells following exposure at different times before fixation.

Treatment time in hours before fixation	No. of cells analysed	Gaps	Deletions	Exchanges
Control	100	30	12	2
Ara A 3.0	100	48	28	8
// 4.0	100	64	58	6
// 5.0	100	100	170	4
// 7.0	23	12	54	6

3.3. Comparison of radiation sensitivity of A-T and normal cells

Lymphocytes and fibroblast cells from A-T patients cultured *in vitro*, have been shown to be hypersensitive to ionizing radiation both in terms of loss of reproductive integrity (Taylor *et al.* 1975, Cox *et al.* 1978) and induction of chromosomal aberrations in both G_1 and G_2 phases of the cell cycle (e.g. Taylor 1982). The G_2 chromosomal hypersensitivity of A-T fibroblasts was regarded as a possible way of diagnosing this syndrome and an assay was developed in which cells from amniotic fluid was exposed to radiation (Giannelli *et al.* 1982). However, there is speculation (Cox *et al.* 1986) that simian virus (SV40) infection used to immortalize fibroblastic lines may cause genetic differences between primary lines

and the cell lines studied here, such as increased hypersensitivity to radiation and chemical mutagens and altered excision repair capacity and hence influence the results observed. The following experiments, therefore, were designed to verify the difference in radiosensitivity between AT5BIVA and MRC5SVI cells in terms of cell survival and chromosomal aberration induction.

a) *Clonogenic assay*

Figure 3.11 shows the survival curves obtained for both cell lines. In this experiment A-T and normal cells were irradiated with X-rays at various doses up to 3 and 6 Gy respectively. Each point on the graph represents the mean number of colonies counted for three dishes for each experiment, error bars represent the standard deviation of mean values calculated for two experiments. The mean values of plating efficiency obtained in two experiments for A-T and normal cells were 41.84 and 61.53 percent respectively. The survival fraction at all doses for normal cells (broken line), with D_0 of about 2 Gy was higher than those of AT5BIVA cells (solid line) which shows a D_0 of about 1 Gy. This implies that immortalized A-T cells are approximately twice as sensitive to X-rays as immortalized normal cells. This result is similar to those in previous study with MRC5SVI and AT5BIVA cells (Smith *et al.* 1985), although they showed a steeper curve (D_0 smaller) for AT5BIVA cells.

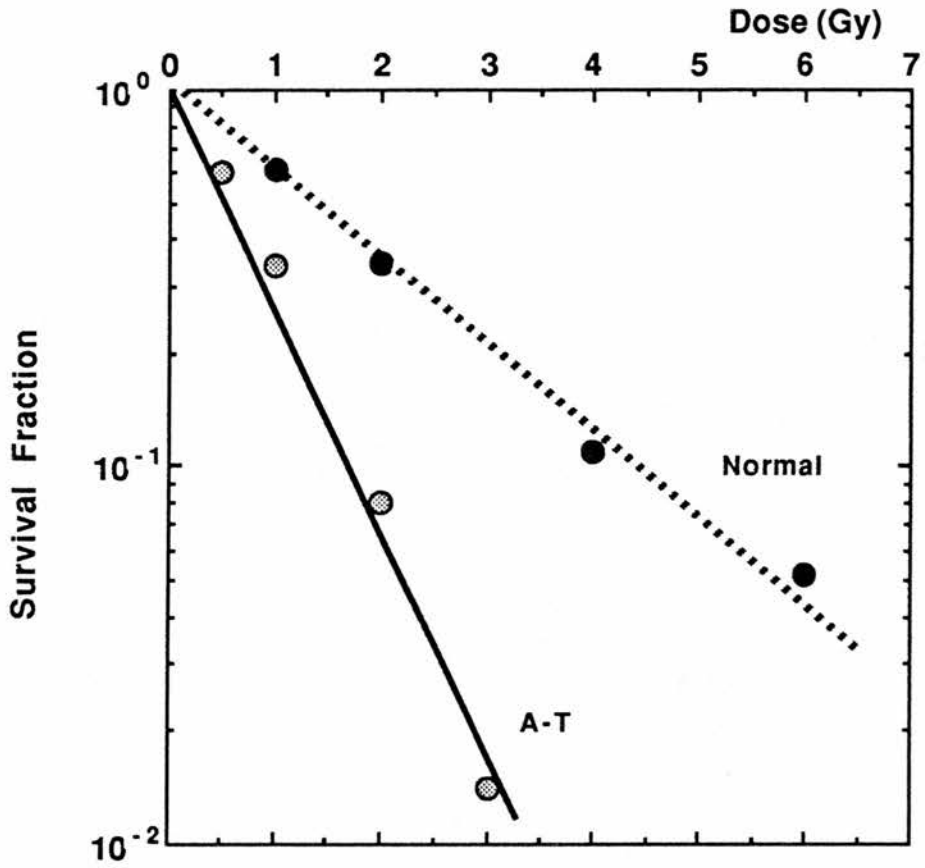


Figure 3.11. Survival responses of X-irradiated MRC5SVI (normal) and AT5BIVA (A-T) cells. Error bars represent standard deviations of mean values of two independent experiments.

b) *Effects of X-rays on normal and A-T cells irradiated at 24 h before fixation*

Four cultures of 2 x 10^5 cells (2 for each cell line) were incubated for 24 hours before X-irradiation was carried out. Cells were then irradiated at 1 and 2 Gy X-ray doses and incubated for a further 24 hours at 37° C. Colcemid at 0.08 μ g/ml was applied to the cultures for two hours before fixation. 100 metaphase figures were analysed for each sample. The results are summarized in table 10. It was found that by increasing the

Table 10: Frequencies and types of X-ray-induced chromosomal aberrations in normal and A-T cells following irradiation at 24 h before fixation.

Treatment	No. of cells analysed	Aberrations per 100 chromosomes						
		Gaps (Total)	Chromatid deletions	Chromosome deletions	Chromatid exchanges	Chromosome exchanges	Total aberrations	(- Control)
Normal cells:								
Control	250	0.38	0.38	-	0.06	-	0.86	(-)
X-rays (1 Gy)	100	0.16	0.51	0.64	0.13	0.30	1.74	(0.88)
X-rays (2 Gy)	100	0.49	0.86	1.97	0.31	1.04	4.67	(3.81)
A-T cells:								
Control	550	0.33	0.72	-	0.07	-	1.12	(-)
X-rays (1 Gy)	100	0.35	0.69	3.13	0.29	1.35	5.81	(4.69)
X-rays (2 Gy)	100	0.55	0.63	3.72	1.33	2.27	8.50	(7.38)

X-ray dose to 2 Gy, normal cells exhibited an enhanced frequency of chromosome type aberrations (approximately three times more) compared to those induced by 1 Gy X-rays. This was also true for gaps, chromatid deletions and exchanges. However in A-T cells given a dose of 1 Gy the number of induced chromosome deletions was about 1.5 times more than those of normal cells for a dose of 2 Gy X-rays. A-T cells showed about two times more chromosome type aberrations and approximately four times more chromatid type exchanges compared to normal cells at the same dose (2 Gy) (Table 10). Therefore this result indicates that A-T cells are at least twice as sensitive as normal cells in G_1 when exposed to 2 Gy X-rays and about 3 - 4 times at 1 Gy X-ray dose for induction of chromosomal aberrations. In normal cells, ionizing radiation induce chromosome-type aberrations in G_0 and G_1 and chromatid-type aberrations in G_2-phase of the cell cycle. The unusual chromatid type aberration, in particular exchange formation in G_1 irradiated A-T cells was first reported by Taylor *et al.* (1976). The results obtained with A-T and normal human lymphocytes (Taylor 1978, 1982) and fibroblasts (Natarajan and Meyers 1979) lend support to these observations.

c) *Effects of X-rays on G_2 cells*

These experiments were performed in parallel with those involving ara A and ara C treatments. The spontaneously occuring chromosomal aberrations observed in controls (entirely untreated samples) were higher in A-T than normal cells. Although the frequency of

spontaneously occured gaps and exchanges were similar in both cell lines, the frequency of chromatid deletions was higher by a factor of 2 in A-T cells. As shown in table 11, the chromosomal aberrations induced by 1 Gy dose of X-rays in A-T cells were compared to those of normal cells at the same dose level and at different irradiation-fixation time intervals. Table 11 shows that A-T cells were approximately 1.5 times more sensitive to clastogenic effects of X-ray at G_2 compared to normal cells in production of gaps and deletions as assayed per 100 chromosomes, but not twice that expected, based on G_1 results. Despite the presence of high levels of chromatid deletions in A-T cells a significant increase in the

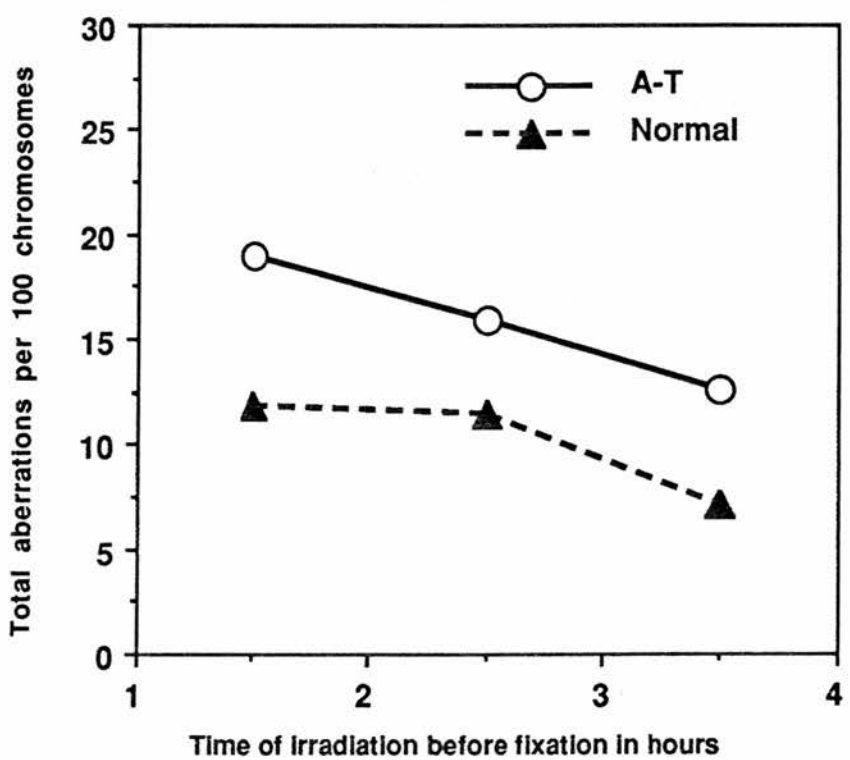


Figure 3.12. Total number of aberrations induced by X-rays (1 Gy) in G_2 A-T and normal cells irradiated at various times before fixation.

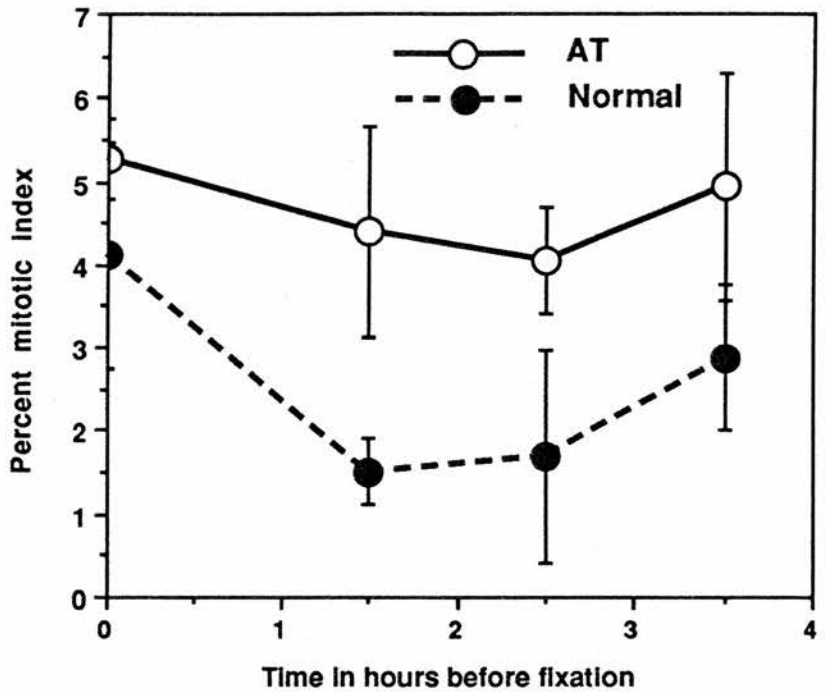


Figure 3.13. Percentage of Mitotic index in A-T and normal cells following X-irradiation (1 Gy). Error bars represent standard deviation of mean values of two experiments. 1000 cells were scored for each point.

formation of chromatid type exchanges in G_2 cells compared to normal cells was not observed (Table 11). The total number of chromatid aberrations induced by X-rays (1 Gy) in A-T and normal cells at various time intervals before fixation is depicted in figure 3.12. Background level of aberrations (in untreated controls) were subtracted from the values shown for each point. This figure (3.12) clearly shows that the frequency of X-ray induced chromatid aberrations in A-T cells were at a higher level than that of normal cells for all irradiation times before fixation. The average mitotic index values obtained from two experiments show that A-T cells suffer less radiation induced mitotic delay than normal cells (Figure 3.13). As the results of experiments described in this section

Table 11: Comparison of radiation sensitivity of normal and A-T cells when X-irradiated at a dose of 1 Gy in the G2 phase.

Treatment	Irradiation intervals (h)	No.ofcells* analysed	Aberrations per 100 chromosomes			Total aberrations (- Control)
			Gaps	Deletions	Exchanges	
Normal cells:						
Control		250	0.38 ± 0.14	0.38 ± 0.11	0.06 ± 0.01	0.86 (-)
X-rays	1.5	200	2.92 ± 0.33	9.24 ± 0.32	0.46 ± 0.10	12.62 (11.76)
X-rays	2.5	250	2.96 ± 0.41	8.70 ± 0.15	0.58 ± 0.02	12.24 (11.38)
X-ray	3.5	250	2.06 ± 0.04	5.22 ± 0.25	0.71 ± 0.09	7.99 (7.13)
A-T cells:						
Control		550	0.33 ± 0.06	0.72 ± 0.20	0.07 ± 0.07	1.12 (-)
X-ray	1.5	350	4.94 ± 1.17	14.71 ± 2.41	0.47 ± 0.27	20.12 (19.00)
X-ray	2.5	300	3.95 ± 0.94	12.33 ± 2.17	0.69 ± 0.37	16.97 (15.85)
X-ray	3.5	350	3.17 ± 0.26	9.69 ± 2.05	0.80 ± 0.31	13.66 (12.54)

* Pooled data of three experiments.

Errors are standard deviation of mean values

(Figures 3.11 & 3.12 and Tables 10 & 11) show, AT5BIVA cells exhibited at least twice the hypersensitivity, to X-rays both in terms of cell killing and chromosomal aberration yields compared to MRC5SVI cells. Thus, the suspected genetic alterations due to SV40 infection in immortalized normal and A-T cells used as systems for experiments, do not appear to play a major role in the response of these cell lines to X-rays.

3.4. Cytogenetic studies with X-rays and ara A treated G_2-cells

a) *Experiments with normal cells*

A flow diagram for the following experiments with X-rays alone or in combination with ara A and ara C is shown in figure 3.14. In this particular experiment, a dose of 2 Gy was given at 1 h intervals in the presence or absence of 200μ mol/l ara A. Cells were exposed to 0.08μ g/ml

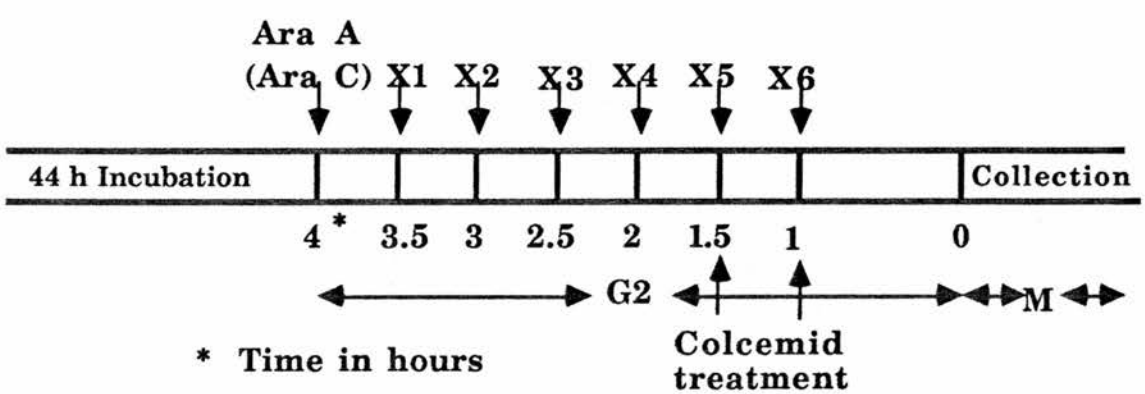


Figure 3.14. A flow diagram of the experimental protocol for G_2 normal and A-T cells. Cells were treated with ara A or ara C at least 0.5 h before irradiation and remained in the culture medium until fixation. The total treatment time (i.e. 4 h) includes 1 or 1.5 h colcemid treatment.

Table 12: Mitotic index with standard errors for normal cells exposed to X-rays (2 Gy) in the presence or absence of ara A (Results of two experiments).

Treatment time in hours before fixation	MI% - ara A	MI% +araA
Control	2.72 ± 0.22	1.60 ± 0.14
X-ray 1.0	1.62 ± 0.2	0.89 ± 0.55
X-ray 2.0	1.00 ± 0.28	0.79 ± 0.27
X-ray 3.0	1.1 ± 2.9	0.74 ± 0.05

of colcemid (4 μ g/ml) one hour before collection to arrest mitotic figures. Because of short colcemid treatment, the mitotic index of these experiments was low (Table 12). Table (13) shows summarized results of these experiments.

In X-irradiated cells the frequency of induced gaps increased slightly with post-irradiation incubation time (Figure 3.15). Figure (3.15) shows that the number of deletions (13.6 deletions per 100 chromosomes at 1 h before fixation) decreased with increasing incubation time after irradiation (8.1 deletions per 100 chromosomes at 3 h before fixation). This was interpreted as rejoining. The rejoining of chromatid deletions in this cell line followed first order (exponential) kinetics which could be fitted by the expression:

$$N = N_o \cdot e^{-\lambda t} \qquad (1)$$

where N, represents the number of breaks remaining after a repair time t; N_o, represents the number of breaks induced initially; and λ, represents the rate constant. Regression analysis of data in figure 3.15

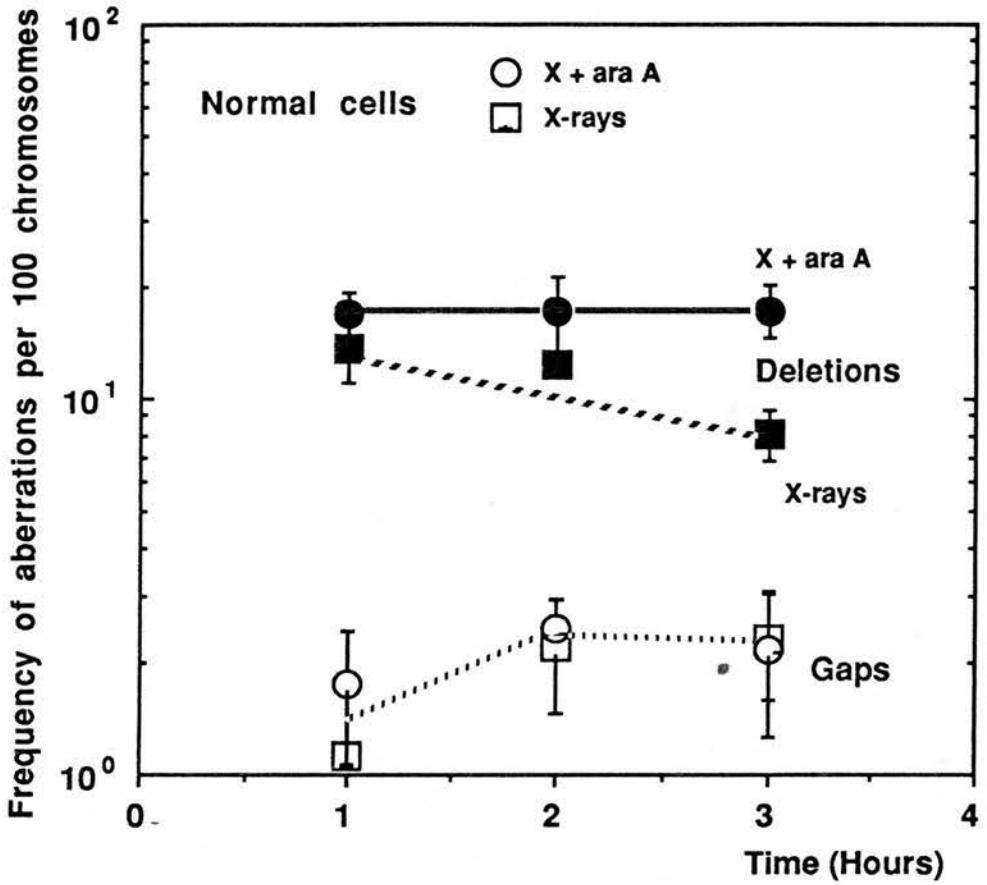


Figure 3.15. Frequencies of chromatid aberrations as a function of time in normal cells exposed to X-rays (2 Gy) alone or X-rays and ara A (200μ mol/l). The time between X-irradiation and fixation includes a 1 h treatment with colcemid. Error bars represent the standard deviation of mean values of three independently performed experiments.

for 2 Gy X-ray exposure gave values of N_o = 18.5 and λ = -0.258 with regression coefficient of 0.979.

The half-time (t 1/2) for repair of breaks was also calculated based on equation (1); i.e. if t = t 1/2 then N would be equal to N_o 1/2. By substituting these values in equation (1) we can derive the expression:

$$t_{1/2} = 0.693 / \lambda \qquad (2)$$

where t 1/2 represents the half-time for repair of breaks. This gave a

value of half-time for repair of breaks in normal cells of 2.7 h.

The frequency of exchanges in X-irradiated cells also increased with incubation time (Figure 3.16). The number of exchanges approximately doubled every hour.

The frequency of gaps induced in X-irradiated cells in the presence of ara A was quite similar to those induced by X-rays alone but at lower frequency. The average number of gaps induced in three different fixation time was about 1.9 per 100 chromosomes in X-irradiated cells and 2.1 gaps per 100 chromosomes in X-irradiated cells in the presence of ara A. The repair seen for X-irradiated cells was not observed with ara A treated samples (Figure 3.15) implying that ara A prevented rejoining of

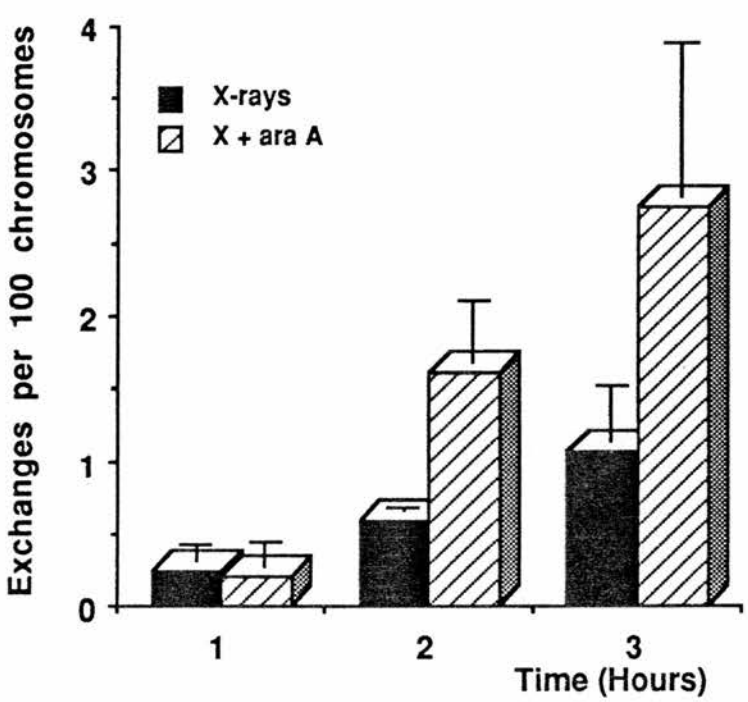


Figure 3.16. Frequency of chromatid exchanges as a function of time in normal cells exposed to X-rays alone (2 Gy) or to X-rays and 200μ mol/l ara A. Error bars represent the standard deviation of mean values of three experiments.

Table 13: Yields of chromatid aberrations in normal human (MRC5SVI) cells: mean frequencies at various times after exposure to X-rays (2 Gy) in the presence or absence of 200 μ mol/l ara A.

Treatment	Time of irradiation before fixation	No of cells analysed $	Aberration per 100 Chromosomes			
			Gaps	Deletions	Exchanges	Total
Control		250	0.46 ± 0.15	0.42 ± 0.16	0.02 ± 0.007	0.94
Ara A alone	4 h	300	0.62 ± 0.22	1.11 ± 0.01	0.13 ± 0.04	1.86
X-ray	1 h	300	1.13 ± 0.08	13.60 ± 2.59	0.24 ± 0.10	14.97
X-ray	2 h	150**	2.19 ± 0.74	12.32 ± 0.87	0.59 ± 0.02	15.10
X-ray	3 h	245	2.31 ± 0.74	8.11 ± 1.27	1.07 ± 0.38	11.49
X-ray+ara A @	1 h	300	1.73 ± 0.67	16.82 ± 2.31	0.21 ± 0.15	18.76
X-ray+ara A	2 h	150**	2.44 ± 1.66	17.10 ± 4.24	1.61 ± 0.42	21.15
X-ray+ara A	3 h	200	2.16 ± 0.91	17.27 ± 2.80	2.75 ± 1.07	22.18

** Pooled data of two experiments
@ Ara A was added to the culture medium 1 h before the first X-irradiation
$ Pooled data of three independent experiments
Errors are standard deviation of mean values.

chromatid deletions. The number of deletions observed at 1 hour before collection was more in ara A treated cells (16.8 deletions/100 chromosomes) than those after X-rays alone (13.6 deletions/100 chromosomes). This might be partially due to contribution of ara A in deletion induction and also some might have been repaired during 1 hour colcemid treatment in X-irradiated cells (Figure 3.15). But ara A by itself induced only 1.1 deletions per 100 chromosomes which is a very low frequency compared with the frequency of deletions in X-irradiated cells in the presence of ara A.

During incubation of cells after irradiation the frequency of chromatid exchanges increased in the presence of ara A. Moreover, This increase was more than a factor of two in cells irradiated 3 hours prior to collection in the presence of ara A (2.7 exchanges/100 chromosomes) compared to that of X-rays alone (1.1 exchanges/100 chromosomes) (Figure 3.16).

The average mitotic index values taken from two experiments is shown in table 12. 1000 cells were scored per point. These results indicate that ara A caused a G_2 delay in addition to that caused by X-rays alone.

b) ***Experiments with A-T cells***

The experimental protocol for study of the kinetics of chromatid aberrations for A-T cells was essentially the same as for normal cells. The difference was that X-irradiation was carried out at an earlier time (0.5 h) following addition of ara A and repeated every half hour to monitor the re-joining or mis-joining of X-ray induced aberrations in the

presence or absence of ara A in more details (Figure 3.14). The time window in this system was limited to 4 h, this is less than the duration of G_2, measured by autoradiography method which was found to be about 5 hours (Figure 3.6*a*). This restriction was made in order to minimize the incorporation (clastogenic effect) of ara A in induction of chromatid aberrations (Figures 3.9 & 3.10). However it was possible to monitor the rejoining of chromatid breaks by irradiating cells at different intervals before fixation. Ara A was applied to the cell cultures 0.5 h prior to irradiation to allow enough time for the nucleotide to be phosphorylated to the triphosphate level in order to compete with the pool of naturally occuring nucleotides (dATP) at the polymerase level. All these experiments involved multiple fixation. Four hours before collection six samples were exposed to 200 μ mol/l ara A. 30 minutes after addition of ara A to the cultures; 1st set of cultures (3.5 hours before fixation) were irradiated at 1 Gy. Control samples were also irradiated in parallel to ara A treated samples for all stages of the experiment. Ara A was present in the culture medium after irradiation until fixation. 1.5 hours before harvesting cells were exposed to 0.08μ g/ml of demecolcine. The experiment included an entirely untreated sample as well as a control for ara A alone for four hours.

Data in figure 3.17 and table 14 show that the frequency of gaps decreased with increasing incubation time. The number of gaps induced at 3.5 hours before fixation (4.95 gaps/100 chromosomes) was about 1.5 times less than the number of gaps produced at 1.5 hours before fixation (3.1 gaps /100 chromosomes).

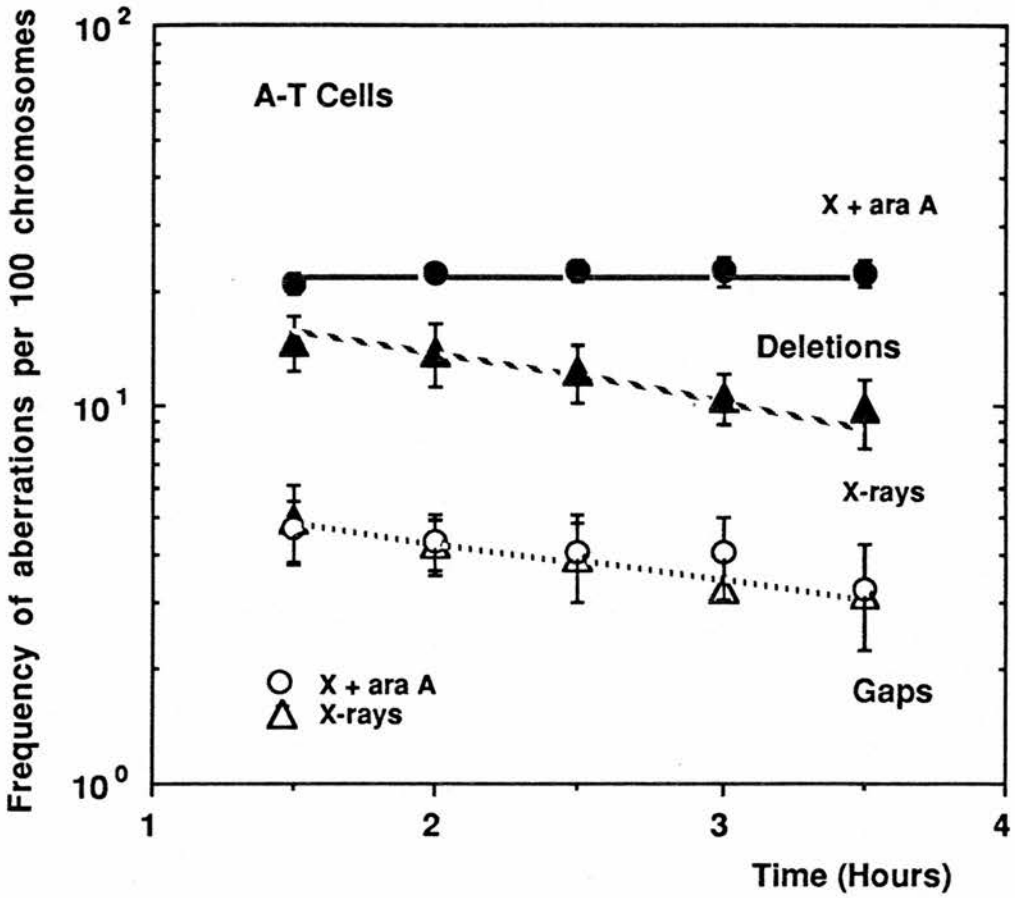


Figure 3.17. Frequency of deletions and gaps (achromatic lesions) as a function of time between exposure to X-rays (1 Gy), alone or in combination with 200 μ mol/l ara A, before fixation in A-T cells. The time between X-irradiation and fixation includes a 1.5 h treatment with colcemid. Error bars indicate standard deviation of mean values of three independently performed experiments.

Deletions were the most frequent form of aberrations observed in both X-irradiated and ara A treated cells (Figure 3.17, and Tables 14 & 15). The number of deletions in X-irradiated A-T cells at the shortest time before collection was 14.71 per 100 chromosomes. The frequency of deletions decreased with increasing post-irradiation incubation time so that in cells irradiated 3.5 hours before fixation only 9.69 deletions were produced per 100 chromosomes. This observation implies that rejoining

occurred during incubation of cells after irradiation. The kinetics of rejoining in this system also followed first order (exponential) kinetics. Regression analysis of data in figure 3.17 for 1 Gy exposure gave values of: $N_0 = 21$, and $\lambda = -0.223$ with regression coefficient of 0.977 for A-T cells. Half-time (t 1/2) for repair of breaks was calculated according to equation (2). This gave a value of t 1/2 for repair of breaks in A-T cells of 3.1 h.

The frequency of exchanges increased with increasing post-irradiation incubation time (Figure 3.18). Exchanges are thought to result from misjoining of dsb (Bender *et al.*1974, Bryant 1984*a*), therefore, the number of dsb available and incubation time are two important factors for the formation of exchanges. In spite of the presence of high frequencies of deletions in A-T cells the number of exchanges formed in these cells was not as high as expected based on the increase observed for deletions compared to normal cells.

Both A-T and normal cells showed relatively similar sensitivity to ara A alone when exposed at 4 h before fixation. Total aberrations induced by ara A in A-T (Table 14) and normal cells (Table 13) were 2.09 and 1.76 per 100 chromosomes respectively. The slight increase in aberrations seen in A-T cells was due to the high level of background aberrations in A-T cells (1.12 aberrations/100 chromosomes in A-T as against 0.9 aberrations/100 chromosomes in normal cells).

In those cells treated with 200 μ mol/l ara A and irradiated with 1 Gy X-rays in the presence of ara A, the result was similar to that of normal cells. Figure 3.17 shows that the number of deletions induced in cells irradiated 1.5 hours before fixation in the presence of ara A, was more

than that of X-rays alone (about 21 deletions with ara A versus about 15 deletions per 100 chromosomes without ara A). Ara A by itself induced very low level aberrations (1.38 deletions/100 chromosomes) compared to X-irradiated cells. This indicates that some of the chromatid deletions were rejoined during 1.5 hours demecolcine treatment in X-irradiated cells where the rejoining was inhibited when ara A was present in the culture medium. The rejoining of chromatid breaks seen in X-irradiated cells (Figure 3.17) was not seen with ara A treated cells, suggesting that ara A strongly inhibited the rejoining of chromatid breaks.

It was observed that the frequency of exchange formation after irradiation in the presence of ara A increased as the time of post-irradiation incubation of cells with ara A increased. As shown in figure (3.18 lower paner) and table 15 the number of exchanges in cells irradiated 3.5 hours before fixation in the presence of ara A (1 exchange/100 chromosomes) was slightly higher than those of induced by X-rays alone (0.8 exchanges /100 chromosomes) (Table 14). However, in the presence of ara A, the large enhancement of exchange frequencies observed in normals (Figure 3.18 upper panel) was not seen in A-T cells. The summarized results obtained with combined treatment of X-rays and ara A is shown in tables 14 & 15. The results represent pooled data of three experiments showing standard deviation of mean values.

Data for normal cells exposed to 2 Gy X-rays (Table 13) have been recalculated as frequency of aberrations per 100 chromosomes for a dose of 1 Gy, on the assumption of approximate linearly induction of chromosomal aberrations at low doses of X-rays (0.2 - 2 Gy).

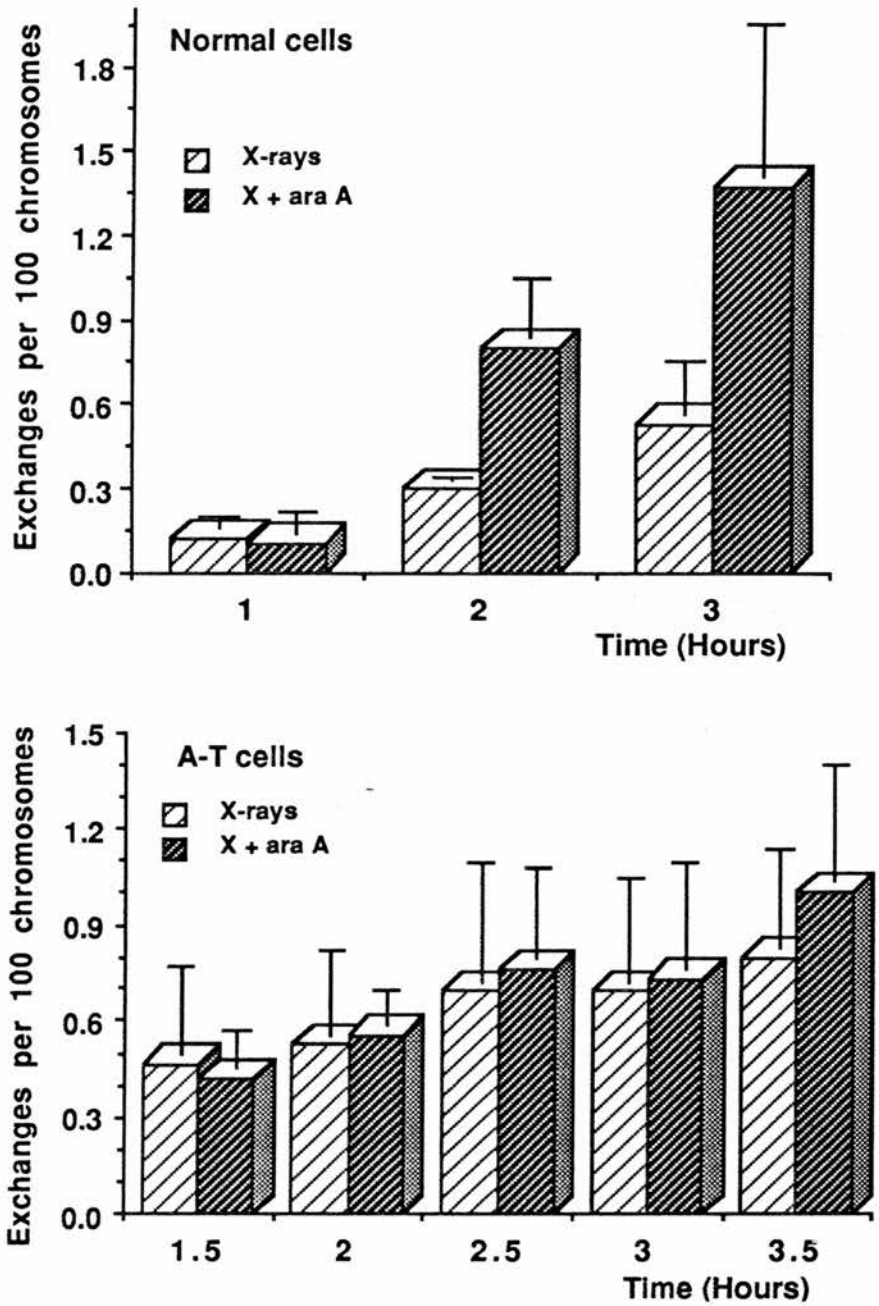


Figure 3.18. Frequency of exchanges as a function of time in normal (Upper panel) and A-T cells (Lower panel) exposed to X-rays alone (1 Gy) or in combination with 200 μ mol/l ara A at various times between X-irradiation and fixation.Top panel; data of experiments with normal cells (Table 13), originally for a 2 Gy exposure, recalculated for a dose of 1 Gy on the assumption of approximate linearity of induction in the range of 0-2 Gy. Error bars indicate standard deviation of mean values of three experiments.

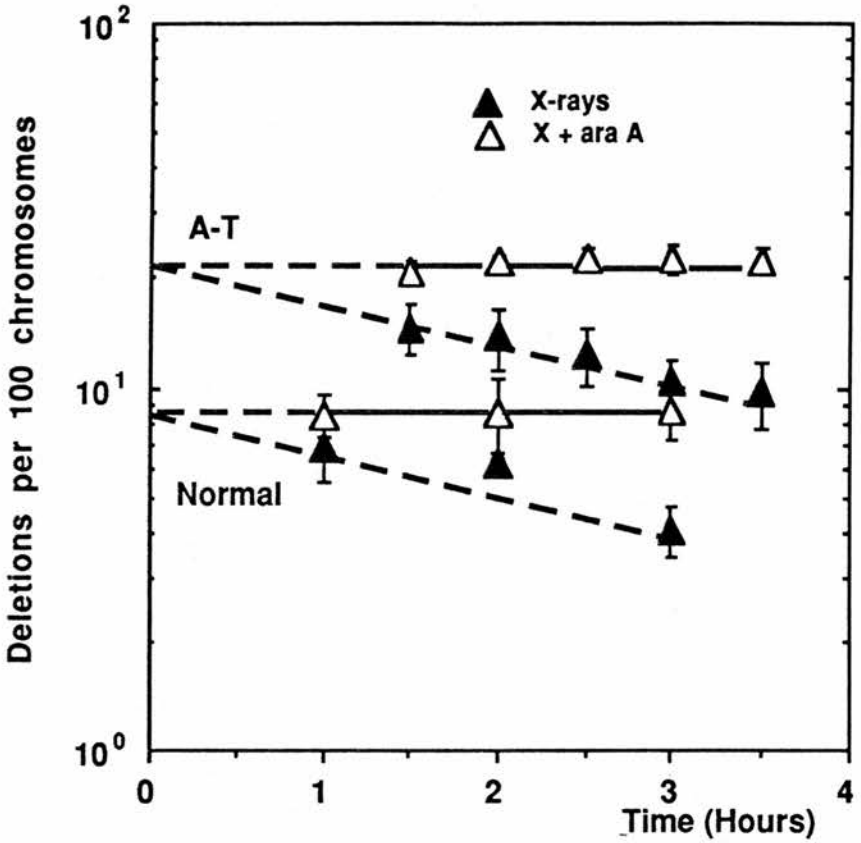


Figure 3.19. Frequencies of deletions in A-T and normal cells as a function of time between X-irradiation and fixation. Data for normal cells, originally for 2 Gy exposure has been recalculated for a dose of 1 Gy, on the assumption a linear induction over low dose range of X-rays. Error bars indicate standard deviation of mean values of three experiments.

The validity of this correction is corroborated by data for normal cells at 1 Gy obtained in a separate study (Table 19). Table 19 shows that at 1.5 h after irradiation and a dose of 1 Gy about 9 deletions/100 chromosomes were observed which is very close to the calculated value 8.5 deletions/100 chromosomes at 1 h after irradiation. A comparative diagram indicating similar exponential chromatid deletion rejoining in both A-T and normal cells and a higher level of initial (at 0 h) damage in A-T cells following X-irradiation, is shown in figure 3.19. In this figure data for deletions induced by 1 Gy of X-rays given at various intervals before fixation in the

Table 14: Yields of chromatid aberrations in A-T cells X-irradiated at a dose of 1 Gy at various times before fixation.

Treatment	Time of irradiation before fixation (h)	No. of cells * analysed	Aberrations per 100 chromosomes			
			Gaps	Deletions	Exchanges	Total
Control		550	0.33 ± 0.06	0.72 ± 0.20	0.07 ± 0.07	1.12
X-ray 1Gy	1.5	350	4.95 ± 1.17	14.71 ± 2.41	0.47 ± 0.27	20.13
X-ray 1Gy	2.0	300	4.29 ± 0.67	13.78 ± 2.65	0.53 ± 0.26	18.60
X-ray 1Gy	2.5	300	3.95 ± 0.94	12.33 ± 2.17	0.69 ± 0.37	16.97
X-ray 1Gy	3.0	300	3.23 ± 0.19	10.40 ± 1.56	0.69 ± 0.32	14.32
X-ray 1Gy	3.5	350	3.17 ± 0.26	9.69 ± 2.05	0.80 ± 0.31	13.66

* Pooled data of three independent experiments.
Errors are standard deviation of mean values.

Table 15: Effects of ara A (200 μ mol/l) on yields of chromatid aberrations in X-irradiated (1 Gy) G2 A-T cells at various intervals before fixation.

Treatment	Time of irradiation before fixation (h)	No. of cells* analysed	Aberrations per 100 chromosomes			
			Gaps	Deletions	Exchanges	Total
Ara A alone	4.0	250	0.61 ± 0.28	1.38 ± 0.22	0.10 ± 0.08	2.09
X-ray + ara A	1.5	350	4.70 ± 0.87	20.94 ± 1.41	0.42 ± 0.12	26.06
X-ray + ara A	2.0	300	4.32 ± 0.77	22.29 ± 0.73	0.56 ± 0.10	27.17
X-ray + ara A	2.5	300	4.05 ± 1.04	22.69 ± 1.55	0.76 ± 0.29	27.50
X-ray + ara A	3.0	250	4.05 ± 1.01	22.62 ± 2.00	0.73 ± 0.33	27.40
X-ray + ara A	3.5	350	3.27 ± 1.02	22.31 ± 1.91	1.00 ± 0.37	26.58

* Pooled data of three independent experiments.
Errors are standard deviation of mean values.

presence or absence of ara A to A-T and normal cells is extrapolated back to time zero. This shows that for both normal and A-T cells these lines extrapolate back to a common point; at about 22 for A-T and 8.5 deletions for normal cells.

Mitotic index (MI) of the experiments were measured and presented in table 16. 1000 cells were scored per point. Errors are the standard error of mean values of two experiments. The percentage of mitotic index in X-irradiated cells was fluctuated around control (unirradiated) level, but in ara A treated cells a decrease in MI was observed for all post-irradiation incubation intervals.

Table 16: Mitotic index with standard errors for A-T cells exposed to a dose of 1 Gy in the presence or absence of ara A (Results of two experiments).

Treatment Time in hours before fixation	MI% - ara A	MI% +ara A
Control	5 .28 ± 0.47	3.75 ± 1.76
X-rays 1.5	4.40 ± 1.27	3.10 ± 0.28
X-rays 2.0	4.68 ± 0.64	2.79 ± 0.37
X-rays 2.5	4.05 ± 0.64	3.20 ± 0.32
X-rays 3.0	4.58 ± 0.16	2.80 ± 0.42
X-rays 3.5	4.94 ± 1.35	2.65 ± 0.10

3.5. Cytogenetic studies with X-rays and ara C treated G_2-cells

a) *Experiments with normal cells*

Effects of ara C on normal cells was also studied in essentially the same way as indicated in the flow diagram shown in figure 3.14. 100 μ mol/l ara C was applied to one set of cultures four hours before collection. 30 minutes later the first set of cultures was irradiated with 1 Gy and a culture was irradiated every hour until 1.5 h before fixation. The X-ray dose was reduced to 1 Gy in order to minimise the mitotic delay and G_2 block induced as a consequence of irradiation (Table 18). 250 mitotic figures were scored for each sample.

The frequency of gaps in X-irradiated cells was approximately constant for all treatment times at a slightly higher level than that seen after a dose of 2 Gy. The average number of gaps seen after 1 Gy in X-irradiated cells was about 2.5 gaps /100 chromosomes and about 1.9 gaps /100 chromosomes in cells irradiated at 2 Gy for three different sampling times (Figure 3.15). Yields of deletions with a dose of 1 Gy was higher than half the value obtained with a dose of 2 Gy X-rays (Figure 3.19). This might be due to different times chosen for irradiation. The same trend of re-joining as seen in experiments with 2 Gy X-rays and ara A in G_2 normal cells, was observed for deletion formation. Approximately 50% of deletions were rejoined within two hours (Figure 3.20). The number of induced deletions at 1.5 h before fixation in X-irradiated cells was 9.24 /100 chromosomes which decreased to 5.22 deletions/100 chromosomes

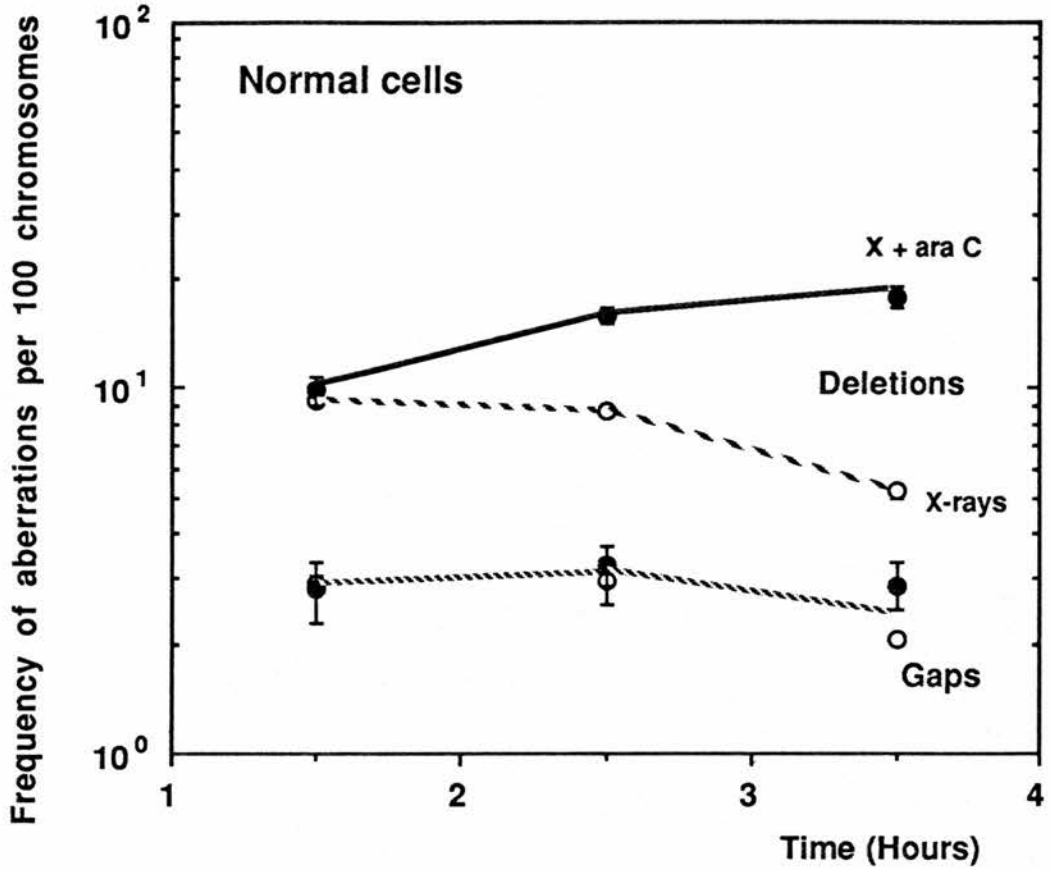


Figure 3.20. Frequency of chromatid deletions and gaps in normal cells as a function of time after a dose of 1 Gy of X-rays in the presence or absence of ara C (100μ mol/l). The time between irradiation and fixation includes a 1.5 h treatment with colcemid. Error bars represent standard deviation of mean values of three experiments.

following two hours between irradiation and fixation. Regression analysis of data in Figure 3.20 and Table 17 gave values of No = 15.28 and λ = -0.286 for a dose of 1 Gy. This gave a value of the half-time for repair of breaks of 2.42 h (equation 2) which is very close to the value of 2.7 h obtained for a dose of 2 Gy in normal cells (Table 13).

The number of exchanges increased slightly with post-irradiation incubation time (Figure 3.21).

In those cells treated with ara C and irradiated with X-rays, the

Table 17 :Frequencies of chromatid aberrations in X-irradiated (1 Gy) G2 normal (MRC5SVI) cells in the presence or absence of ara C (100 μ mol/l).

Treatmet	Time of irradiation before fixation (h)	No.of cells analysed *	Aberrations per 100 Chromosomes			
			Gaps	Deletions	Exchanges	Total
Control		250	0.38 ± 0.14	0.38 ± 0.11	0.06 ± 0.01	0.82
Ara C alone		250	0.67 ± 0.28	1.47 ± 0.18	0.11 ± 0.03	2.25
X-rays	1.5	200	2.92 ± 0.13	9.24 ± 0.32	0.46 ± 0.10	12.62
X-rays	2.5	250	2.96 ± 0.41	8.70 ± 0.15	0.58 ± 0.02	12.24
X-rays	3.5	250	2.06 ± 0.04	5.22 ± 0.25	0.71 ± 0.09	7.99
X-ray + ara C	1.5	250	2.82 ± 0.54	9.85 ± 0.94	0.52 ± 0.06	13.19
X-ray + ara C	2.5	250	3.27 ± 0.40	15.87 ± 0.71	0.79 ± 0.16	19.93
X-ray + ara C	3.5	250	2.89 ± 0.44	17.90 ± 1.24	1.12 ± 0.09	21.91

* Pooled data of three independent experiments

Errors are standard deviation of mean values

number of gaps induced in the presence of ara C (3 gaps/100 chromosomes average of three fixation times) was similar to those of induced by X-rays alone (2.7 gaps /100 chromosomes average of three fixation time) (Figure 3.20). The kinetics of gaps was found to be similar to that after a dose of 2 Gy X-rays, in the presence or absence of ara A.

The number of deletions increased in the presence of ara C with post-irradiation incubation time. In the sample irradiated 1.5 hours

Table 18: Mitotic index with standard errors for normal cells exposed to X-rays (1 Gy) in the presence or absence of ara C (Results of two experiments).

Treatment time in hours before fixation	MI% - ara C	MI% +ara C
Control	4.11 ± 1.37	1.97 ± 0.42
X-rays 1.5	1.51 ± 0.46	1.23 ± 0.04
X-rays 2.5	1.70 ± 1.27	1.13 ± 0.08
X-rays 3.5	2.89 ± 0.87	1.56 ± 0.01

before collection, there was no major difference in frequencies of deletions induced by either X-rays alone or X-rays in combination with ara C (9.24 as against 9.85 deletions /100 chromosomes respectively). The number of deletions induced in the presence of ara C almost doubled after two hours of post-irradiation incubation (9.85 deletions increased to 17.9 deletions /100 chromosomes). But the increase in deletion frequency in the presence of ara C was more pronounced during first hour following irradiation (Figure 3.20). The frequency of exchanges also increased in the presence of ara C with increasing incubation time after

treatment (Figure 3.21). This finding was very similar to that of ara A treated cells with 1 Gy X-rays in A-T cells.

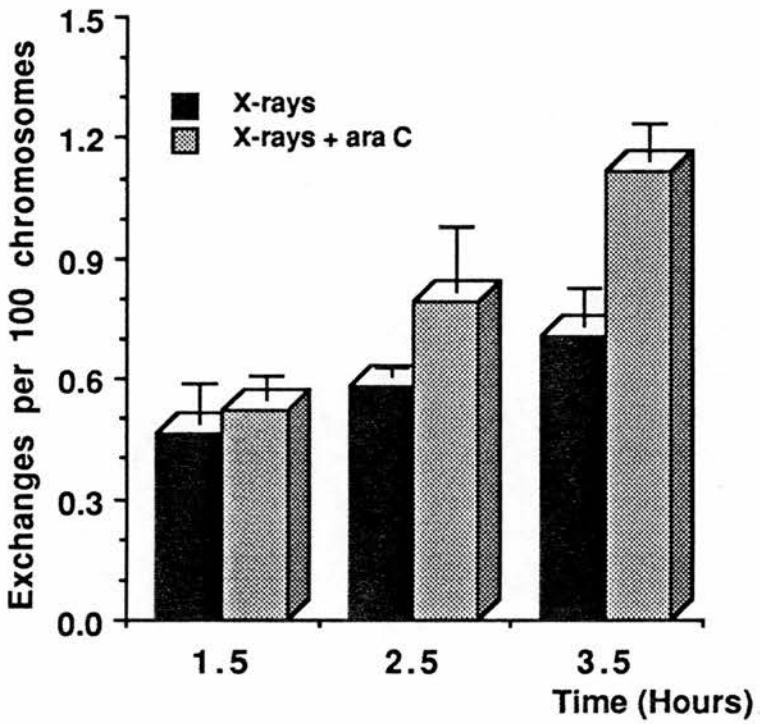


Figure 3.21. Frequency of chromatid exchanges as a function of time in normal human cells exposed to X-rays (1 Gy) in the presence or absence of 100 μ mol/l ara C. Error bars represent the standard deviation of mean values of three experiments.

b) *Experiments with A-T cells*

Experiments with ara C in X-irradiated cells were performed in parallel to those with X-rays and ara A treated cells, according to the flow diagram shown in figure 3.14. 100 μ mol/l ara C was added to some cultures, 4 hours before fixation. Irradiation was carried out at various intervals as in flow chart, starting at 30 minutes after ara C treatment.

Demecolcine was added 1.5 hours before collection and harvesting. The results of these experiments are summarized in table 20, which also indicates the standard deviation of mean values of two experiments.

In G_2 irradiated A-T cells in the presence of ara C, there was a slight decrease in the frequency of gaps between irradiation and fixation time, the trend similar to that after X-rays alone as well as to that of a combined treatment of X-rays and ara A. The number of gaps formed in cells irradiated at 1.5 hours before fixation, was however lower than those induced by X-rays alone or by X-rays and ara A (Figure 3.17).

Both A-T and normal cells showed relatively similar sensitivity to ara C (100 μ mol/l) when exposed at 4 h before fixation. Total aberrations induced by ara C alone in A-T (Table 20) and normal cells (Table 17) were 2.45 and 2.25 per 100 chromosomes respectively. Background level (in

Table 19: Mitotic index with standard errors for A-T cells exposed to X-rays (1 Gy) in the presence or absence of ara C (Results of two experiments).

Treatment Time in hours before fixation	MI% - ara C	MI% +ara C
Control	5.28 ± 0.42	3.97 ± 1.08
X-ray 1.5	4.40 ± 1.27	2.90 ± 0.73
X-ray 2.0	4.68 ± 0.64	2.85 ± 1.20
X-ray 2.5	4.05 ± 0.64	2.30 ± 1.13
X-ray 3.0	4.58 ± 0.16	2.30 ± 0.42
X-ray 3.5	4.94 ± 1.35	1.25 ± 0.10

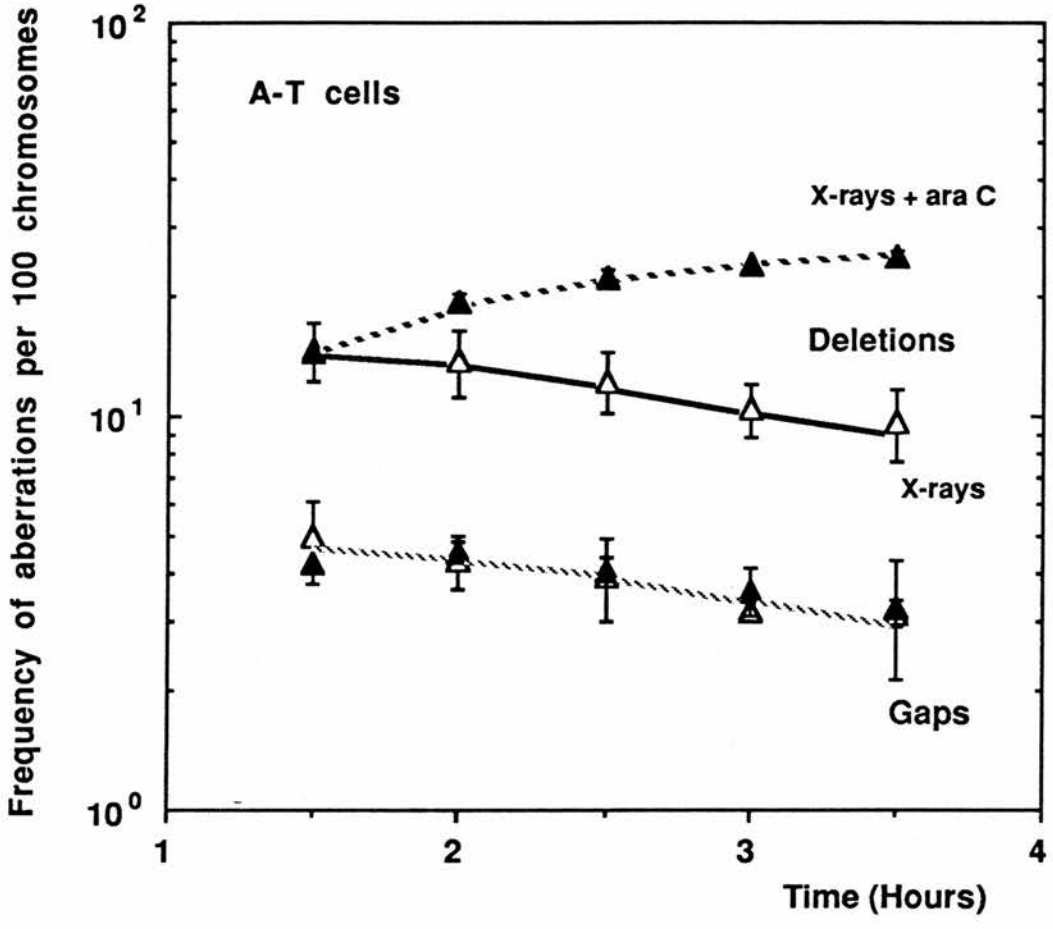


Figure 3.22. Frequency of chromatid deletions and gaps in G_2 A-T cells exposed to X-rays (1 Gy), and held in the presence or absence of ara C (100 μ mol/l) for various periods of time before fixation. Error bars represent standard deviation of mean values of two independently performed experiments.

untreated controls) of aberrations was higher in A-T cells than that of normal cells (1.12 and 0.82 per 100 chromosomes respectively).

Despite the observation of a decreasing number of chromatid deletions in X-irradiated and a constant frequency of deletions for combined treatment of ara A and X-rays, the number of deletions in G_2 irradiated A-T cells in the presence of ara C increased with post-irradiation incubation time (Figure 3.22). This increase was of the order of twice the number of deletions observed at shortest sampling time (i.e.

1.5 h before collection) within two hours. However, the increase in the frequency of deletions was most marked during the first half hour after irradiation in that the frequency of 14.57 deletions /100 chromosomes induced at 1.5 h before fixation increased to 19.50 deletions /100 chromosomes at 2 h. A slower rate of increase in deletions was found for the remaining 1.5 h after irradiation i.e. from 19.50 to 24.47 deletions /100 chromosomes. The contribution of breaks induced by ara C alone was only a fraction of total deletions induced by X-rays and ara C. Ara C alone induced only 1.59 deletions per 100 chromosomes when it was

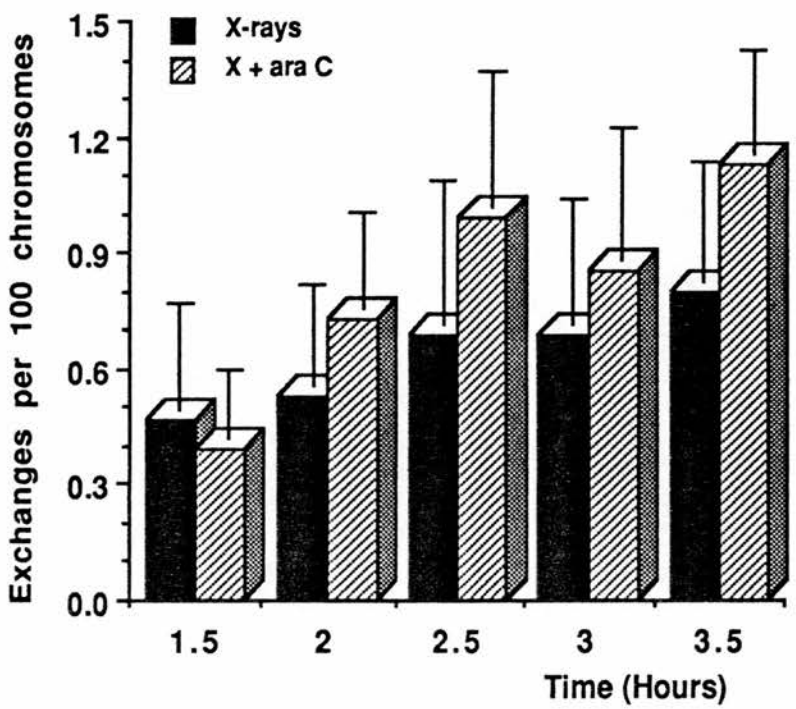


Figure 3.23. Frequency of chromatid exchanges as a function of time in A-T cells exposed to X-rays alone (1 Gy) or in combination with 100 μ mol/l ara C for various times before fixation. Error bars represent the standard deviation of mean values of two experiments.

present for four hours in culture medium.

The formation of exchanges was very similar to the pattern observed with X-rays and ara A in A-T cells; that is the frequency of exchange formation increased between irradiation and fixation. The number of exchanges observed in the presence of ara C was only slightly higher than the number of exchanges observed in cells given X-rays alone (Figure 3.23).

The mitotic index obtained in experiments with ara A and ara C and X-rays were also measured by counting more than 1000 cells for each

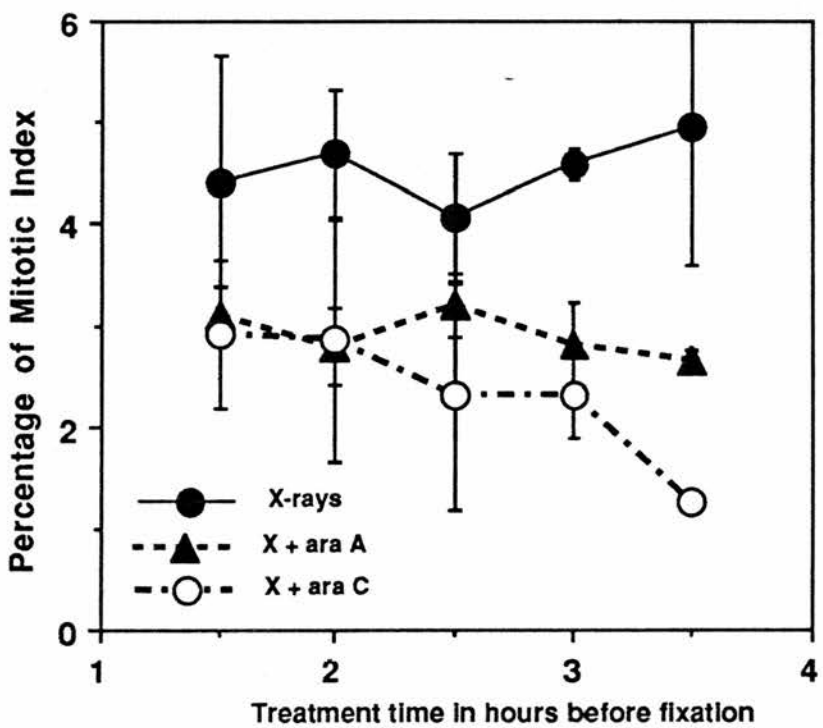


Figure 3.24. Changes in the mitotic index (MI) in A-T cells during the 3.5 h period after treatment with X-rays alone or in combination with ara A or ara C. 1000 cells were scored per point. Error bars indicate the standard error of mean values of two experiments.

Table 20: Yields of chromatid aberrations in X-irradiated (1 Gy) G2 AT5BIVA cells in the presence of ara C (100 μ mol/l).

Treatment	Time of irradiation before fixation (h)	No of cells * analysed	Aberrations per 100 chromosomes			
			Gaps	Deletions	Exchanges	Total
Ara C alone		200	0.73 ± 0.08	1.59 ± 0.03	0.13 ± 0.03	2.45
X-ray + ara C	1.5	250	4.22 ± 0.48	14.57 ± 0.22	0.39 ± 0.18	19.18
X-ray + ara C	2.0	250	4.50 ± 0.28	19.50 ± 0.98	0.73 ± 0.25	24.73
X-ray + ara C	2.5	225	4.04 ± 0.37	22.30 ± 1.36	0.99 ± 0.35	27.33
X-ray + ara C	3.0	250	3.60 ± 0.50	24.10 ± 0.32	0.85 ± 0.35	28.55
X-ray + ara C	3.5	220	3.25 ± 1.09	25.47 ± 0.69	1.13 ± 0.27	29.85

* Pooled data of two experiments.

Errors are standard deviation of mean values.

sample. The result shown in table 19 is the mean of two experiments. Mitotic index for X-irradiated samples seems to be similar for all stages of X-irradiation in G_2 phase for A-T cells (Figure 3.24). For ara A treated samples the mitotic index was lower than that seen for X-irradiated cells, and the value remained nearly constant for all samples (Figure 3.24). For ara C treated samples, the mitotic index was not only lower than for X-irradiated samples, but it decreased with increasing incubation time after irradiation (Figure 3.24).

3.6. Effects of low doses of X-rays on G_2 A-T cells

This experiment was performed to study the yield of X-ray induced chromatid aberrations induced by low doses of X-rays in the presence or absence of ara A (200 μ mol/l) at short times (1.5 hours before fixation) before mitosis or late G_2. The purpose of this study was therefore two fold; firstly to investigate the effects of low doses of X-rays in chromatid aberration induction and secondly, study of the effects of ara A on the induced chromatid aberrations. Two sets of cultures of A-T cells at 2.5×10^5 cells in 25 cm^2 flasks in 6 ml MEMFCS were incubated for 48 h. Cells then were irradiated at 0.4, 0.8 and 1.2 Gy X-rays at 1.5 hours before collection, in the presence and absence of 200 μ mol/l ara A. Ara A was applied 0.5 hours before X-irradiation. 100 mitotic figures were analysed for each sample. The result of this experiment is shown in table 21. It can be seen that the number of aberrations; gaps, deletions, and

exchanges increased with increasing X-ray dose. This increase was higher in X-irradiated cells in the presence of ara A than for X-rays alone, particularly of deletion type aberrations. The results clearly indicate that more than one third of deletions were rejoined in cells treated with X-rays alone which was inhibited by ara A during 1.5 h colcemid treatment.

Table 21: Yields of chromatid aberrations in G_2 A-T cells exposed to various doses of X-rays in the presence or absence of ara A (200 μ mol/l). Ara A was added to the culture medium 2 h before fixation and X-rays was given 0.5 h after ara A treatment.

Treatment	No of cells analysed	Gaps	Deletions	Exchanges
X-ray 0.4 Gy	100	138	264	18
X-ray 0.8 Gy	100	179	475	20
X-ray 1.2 Gy	100	260	711	33
0.4 Gy + ara A	100	169	352	13
0.8 Gy + ara A	100	261	821	20
1.2 Gy + ara A	100	235	1045	36

4

DISCUSSION

G_2-repair and formation of chromatid aberrations

Kinetics of X-ray induced chromatid breaks in G_2 human cells: The effects of ara A

The paradox of chromatid exchange formation in the presence of ara A and ara C

Chromosomal hypersensitivity of G_2 A-T cells to X-rays

Synergism of X-rays and ara C

Comparative analysis of the action of ara A and ara C

Conclusions

4. DISCUSSION

G_2-repair and formation of chromatid aberrations

Based on autoradiographic experiments, the G_2 stage begins when it is not possible to detect chromosomal DNA synthesis by incorporation of tritiated thymidine (^{3}H-TdR) into DNA. However, because this technique is not sensitive enough to detect small amounts of incorporation of the precursors, the G_2 stage in the cell cycle is only an operational definition. There is also considerable evidence for the existance of low levels of DNA synthetic activity in G_2 (Schvartzman *et al.* 1981, Moore and Hodgson 1983).

Irradiation of cells during G_2 leads to the formation of chromatid type aberrations. Palitti *et al.* (1983) proposed that three major processes operate in G_2. These are DNA repair, replicative DNA synthesis and chromatin condensation. All these may be relevant to the formation of chromatid aberrations. Based on this hypothesis, an effect on any one of these systems can affect the frequency of chromatid aberrations. G_2 has been postulated as a period of DNA repair by several authors (Waldren and Johnson 1974, Hittleman and Rao 1974 *a* & *b*, Painter 1980, Painter and Young 1980, Hartley-Asp *et al.* 1980, Zampetti-Bosseler and Scott 1981, Kihlman *et al.* 1982, Palitti *et al.* 1983). Cells irradiated or treated with chemical mutagens may be delayed in G_2 in order to repair damaged sites in DNA before entering mitosis. If the rejoining of chromatid deletions which was clearly demonstrated in figures 3.15 &

3.20 for normal and figures 3.17 & 3.22 for A-T cells represents the repair of DNA dsb, then one might conclude that the data presented here supports the idea of existence a repair mechanism in G_2 phase of the cell cycle. Blöcher *et al.* (1983) showed that repair of DNA dsb takes place in the G_2 as well as other phases of the cell cycle. Following irradiation and treatments with the inhibitor of DNA synthesis, ara A, normal cells (after 2 Gy) showed mitotic delay (Table 12). This result indicates that ara A caused a G_2 delay in addition to that caused by X-rays alone.

Although A-T cells were about twice as sensitive as normal cells when irradiated in G_2 (Table 11), there was no significant G_2 delay observed compared to the unirradiated control (Figure 3.13, Table 16). This was judged by measurement of mitotic index of the samples. This finding is in agreement with the report by Zampetti-Bosseler and Scott (1981). It is possible that this effect and the G_2 blockage effects described by Ford *et al.* (1984) could influence the frequencies and kinetics of deletions in the two cell lines. In spite of the presence and absence of a radiation induced G_2 delay in normal and A-T cells respectively (Figure 3.13), a similar rejoining rate of chromatid deletions was observed in both cell lines (Figures 3.15, 3.17, & 3.19). If the difference in G_2 delay in the two lines played a key role in the determination of the kinetics of deletions, we might expect markedly different kinetics for deletions in the two lines. This may suggest that a special class of lesions (not dsb) could be involved in causing G_2 - mitotic delay. However, following combined treatment with X-rays and ara A or ara C, A-T cells showed a significant

delay compared to those recieved X-rays alone, particularly when treated with ara C for a longer period (Figure 3.24). This observation indicates that there might be a relation between G_2 delay and repair processes during G_2 allowing more time for repair of DNA damage.

Palitti *et al.* (1983) suggested that there is a process for repair of DNA damage occuring late in G_2 which requires semi-conservative DNA synthesis. Any failure in this process will contribute to chromatid aberration formation. The existence of a repair process in G_2 phase was also shown by Lücke-Hühle *et al.* (1983).

Kinetics of X-ray induced chromatid breaks in G_2 human cells : the effects of ara A

It is evident from the kinetics of disappearance of chromatid breaks (Figures 3.15 & 3.20) that rejoining of these in normal (MRC5SVI) cells irradiated at 1 Gy and 2 Gy, occured with a half time ($t_{1/2}$) of approximately 2.4 and 2.7 hours respectively. This value is similar to the half time derived from the initial component of rejoining of chromosome breaks found in PCC experiments of Cornforth and Bedford (1983) using normal human (G_0) fibroblasts. This rejoining rate for chromosome and chromatid breaks also corresponds closely to the rate of rejoining of dsb in mouse Ehrlich ascites tumour cells (Bryant and Blöcher 1980, Blöcher and Pohlit 1982). On the assumption that each chromatid contains a single, continous DNA double helix (Bender *et al.* 1974, Evans 1977),

chromatid breaks seen in the first metaphase following G_2 irradiation would represent unrepaired DNA dsb. These dsb could arise directly or indirectly as the result of repair processes (Bender *et al.* 1974, Evans 1977, Taylor 1978, Parshad *et al.* 1980, Preston 1980). It therefore seems plausible that the rejoining of G_2 chromatid breaks, like that of G_0 chromosome breaks, reflects the underlying repair of DNA dsb although the frequencies of chromatid breaks observed are much lower than the frequencies of dsb measured by biochemical methods. For example, the frequency of breaks measured by PCC in normal G_0 human fibroblasts (Cornforth and Bedford 1983) was about 6 breaks per cell/Gy whereas measurements in mouse Ehrlich ascites tumour cells by neutral gradient velocity centrifugation yielded about 40 dsb/cell/Gy (Blöcher 1982). It seems as though only a small proportion of dsb measured by biochemical means are expressed as visible breaks in either chromosomes or chromatids. By implication, it is possible that chromatid or chromosome breaks observed in G_2 or PCC cells represent a special class of dsb or at least represent dsb in particular regions of the chromatid structure susceptible to rupture. It has been shown that the induction of "pure" dsb by restriction endonuclease (RE) in the DNA of Sendai virus permeabilized (unirradiated) mammalian cells leads to the formation of CA (Bryant 1984 *a*, Natarajan and Obe 1984). However, a comparison of the half-time of kinetics of rejoining of breaks in prematurely condensed chromosomes (Cornforth and Bedford 1983) and of breaks in chromatids (Figures 3.15 & 3.17) with biochemical data for dsb repair (e.g. Blöcher and Pohlit 1982) suggests that breaks observable at the chromosomal level

are representative of dsb in DNA.

When normal G_2 cells were exposed to ara A between irradiation and mitosis the rejoining of breaks in chromatids was strongly inhibited (Figure 3.15). This inhibition of rejoining of chromatid breaks by ara A again appears to reflect underlying inhibition of repair of X-ray induced dsb in DNA (Bryant and Blöcher 1982). Ara A alone at 200 μ mol/l caused only a low frequency of chromatid breaks unless the time of incubation of cells in ara A was increased beyond 4 h (Figure 3.9). The frequency of breaks in irradiated cells observed in the presence of ara A was higher than that in cells receiving X-rays alone. This is thought to be due partly to the small increase in breaks caused by ara A alone (Figure 3.9) and partly due to the inhibition of dsb repair between time zero (the time of X-ray exposure) and the first measurement at 1 h before fixation (Figure 3.15).

For A-T cells treated with ara A, the frequency of X-ray induced deletions was found to be approximately constant for all post-irradiation intervals. This effect was similar to the situation in normal cells but the frequency of deletions was higher in A-T cells by a factor of 1.5 - 2 for the same dose of X-rays (Figure 19).

The frequency of gaps (achromatic lesions) formed after X-rays alone or in combination with ara A was found to be much lower than that for breaks in normal cells (Figure 3.15). The frequency of gaps after both treatments was found to increase slightly during incubation of G_2 cells up to 3 h in case of normal cells (Figure 3.15). After a dose of 1 Gy , there was no increase in the frequency of gaps with increasing time between

irradiation and fiixation. The frequency of gaps obtained with 1 Gy was at a higher level compared to that of 2 Gy. For A-T cells, although yields of gaps were similar in cells exposed to X-rays alone or X-ray and ara A, the frequency slightly decreased with post-irradiation incubation time (Figure 3.17). The significance of this is not clear. Bender *et al.* (1974) suggested that gaps arise from single-strand breaks (ssb) in DNA. Conversion of single-strand base-damaged sites into ssb might thus lead to an increase in these lesions during post-irradiation incubation. One could assume that these gaps arose from ssb, as proposed by Bender *et al.* (1974), after the initial rapid repair of directly induced ssb has taken place (Bryant *et al.* 1984).

Preston (1980) suggested that chromosomal aberrations may arise specifically from base damaged sites in the DNA strand. This could occur by a mechanism in which base damage is converted into ssb; such a mechanism has been shown to occur (Ahnström and Bryant 1982). If this were the case (for gaps) then treatment with ara A, which reduces the rate of ssb repair (Bryant and Blöcher 1982), should lead to an accumulation of gaps in cells treated with this drug. Figures (3.15 & 3.17) show that for cells incubated in the presence or absence of ara A, the frequency of gaps followed similar kinetics. This indicates that a 'base-damage origin' at least for gaps is unlikely. The possibility of involvement of base damage in chromosomal aberration formation will be disscused in more detail when discussing the results of experiments with ara C.

The paradox of chromatid exchange formation in the presence of ara A and ara C

During incubation of cells after irradiation the number of chromatid exchanges increased (Figures 3.16 & 3.18). Chromatid type exchange aberrations induced by X-rays in human cells may be the result of misjoining (or illegitimate joining) of two or more chromatids before cells enter mitosis. The formation of exchanges was proposed to be the consequence of misjoining of broken chromosomes according to the "breakage-first" hypothesis (Sax 1938). In order to form an exchange, DNA lesions which give rise to exchange aberrations should be in close proximity (Natarajan and Ahnström 1968, Kihlman 1977). Although the nature of exchange formation is not yet clearly understood, Evans (1977) proposed that formation of single stranded DNA lesions induced by S-specific agents such as UV-radiation may lead to the formation of chromatid type exchange aberrations via misreplication. It was also shown that both ionizing radiation and RE induce chromosomal aberrations in the form of both breaks and exchanges when applied to G_1-S- or G_2-stages of the cell cycle (Obe and Winkel 1985, Bryant *et al.* 1987). Chadwick and Leenhouts (1978) hypothesised a mis-recombinational process for exchange formation involving interaction of a dsb in one chromosome with an undamaged homologous region of another chromosome. The proposed model was based on a model for repair of DNA double-strand breaks which relies primarily on a recombination intermediate, heteroduplex DNA (Resnick 1976). The process involves the exchange of homologous strands of chromosomal DNA and basically

requires naturally occuring DNA degradative and repair enzymes and heteroduplex recombinant structures. Based on a molecular version of the "breakage-first" hypothesis, however, it is believed that exchanges are the result of misjoining of two DNA dsb (Bender *et al.* 1974, Natarajan *et al.* 1980, Bryant 1984 *a*, Obe and Winkel 1985). Important in this context are both the number of dsb available for such exchanges to occur and the time available for exchange formation. Figures (3.16 & 3.18) clearly show the importance of the time available for exchange formation. Within two hours post irradiation the number of exchanges in X-irradiated cells increased approximately by a factor of two for both cell lines. When X-irradiated G_2 cells were treated with ara A it was found that the frequency of exchange aberrations also increased with time of treatment. Moreover the frequency was more than three times higher than the frequency obtained with X-rays alone in normal cells. This increase in exchanges with time could not be attributed to a reduction in G_2 delay as has been found for caffeine (Hansson *et al.* 1984). The mitotic index (Tables 12, 16, 18, & 19) shows that in contrast, both ara A and ara C led to a reduction in the mitotic index indicating an enhanced delay in both cell lines.

As shown in figures (3.16 & 3.18, 3.20 & 3.22) for normal and A-T cells, exchanges were formed in X-irradiated cells in the presence of either of the DNA synthesis inhibitors ara A or ara C, at higher levels than that for X-rays alone. Although the frequency of exchanges in X-irradiated A-T cells in the presence or absence of ara A increased with post-irradiation incubation time, the increase in exchanges in the

presence of ara A was less pronounced than that found for normal cells. This might be because different X-ray doses were used for normal and A-T cells. These data however, are not inconsistent with the results obtained for normal cells with ara A. Figures (3.15 & 3.17) on the other hand show that the rejoining of X-ray induced deletions in A-T and normal cells was strongly inhibited in the presence of ara A.

The increased frequency of exchanges with ara A or ara C appears paradoxical in the light of results showing an inhibition by ara A and ara C of chromatid break rejoining (Figures 3.15 & 3.17, 3.20 & 3.22) as well as a reduction of 85-90 percent of DNA synthesis by 200 μ mol/l ara A or by 100-200 μ mol/l ara C in both normal (Figure 3.1) and A-T cells (Figure 3.2) when applied in S-phase of the cell cycle and judged by ^{3}H-TdR incorporation. Because inhibition of chromatid deletion rejoining by ara A was interpreted as underlying inhibition of DNA dsb repair, the formation of exchanges would be inhibited if the same mechanism applied. Data presented here, thus does not support the proposal of Chadwick and Leenhouts (1978) for exchange formation based on a mis-recombinational process. Exchange formation based on this model would require DNA repair synthesis as is required for recombinational repair of DNA dsb, and this process (DNA synthesis) was inhibited by the presence of ara A. Furthermore, Kihlman *et al.* (1978) using*Vicia faba* roots, clearly showed that two chromatid breaks were required to form a chromatid exchange in G_2 cells and therefore mis-recombination between one damaged and one undamaged homologous chromatid is therefore unlikely to lead to the formation of chromatid exchanges. A

possible explanation for an increased frequency of exchange type aberrations in irradiated cells treated with ara A is that by holding breaks open, the inhibitor allows mis-joining of dsb to occur with a high frequency due to the presence and maintenance of a large pool of dsb (Bryant 1984 *b*). An increased yield of exchange aberrations (approximately 2 fold) was found in G_0 human lymphocytes following irradiation in the presence of ara C (Holmberg and Cumasukas 1986). These authors suggested that ara C may hold short-lived DNA lesions open so that more such breaks can interact to form exchange complexes. They therefore concluded that short-lived DNA lesions associate and form an exchange complex. The nature of short- lived DNA lesions however, is not known. Whether these lesions are simply ssb which repair rapidly or include short-lived DNA dsb is not known. Since it was shown that 50% ssb are repaired within two minutes post-irradiation (van der Schans *et al.* 1982 *b*). There might also be two classes of dsb induced by ionizing radiation; fast repair dsb with a rejoining $t_{1/2}$ of 10-20 minutes (Weibezahn and Coquerelle 1980, van der Schans *et al.* 1982 *b*) and slow repair dsb with $t_{1/2}$ of about 1.5-7 hours which was measured for the rejoining of radiation induced dsb in Ehrlich ascites tumour cells (Bryant and Blöcher 1980). Van der Schans *et al.* (1982 *b*) concluded that the dsb measured by neutral elution technique was different from the dsb measured by Bryant and Blöcher (1980) using neutral sucrose gradiant sedimentation technique. If short lived dsb are involved in exchange formation, it can be deduced that a higher frequency of short-lived dsb might be induced in normal cells than A-T cells. In other words if

X-irradiation induces more long-lived DNA dsb in A-T cells consequently a lower frequency of exchanges might be formed in the presence of ara A in A-T (Figure 3.18 lower panel) compared to normal cells (Figure 3.18 upper panel). But the question arises, however, as to why and how do mis-joining events, which by inference represent mis-repair or illegitimate joining of dsb, occur when the joining of dsb is inhibited?

A possible explanation for this paradox is that the 'mis-repair' of dsb which is evident at metaphase as the formation of chromosomal exchanges is the result of a separate independent mechanism not inhibited by ara A and thus by inference not requiring DNA repair synthesis. The fact that higher frequencies of chromosome and chromatid type exchange aberrations were found in *xrs* mutant cell lines than wild type parent CHO-K1 cells lends support to this proposal (Kemp *et al.* 1984, Kemp and Jeggo 1986, Darroudi and Natarajan 1987, Bryant *et al.* 1987). *Xrs* mutants (Jeggo *et al.* 1982) are hypersensitive to X-rays and are thought to be defective in the repair of DNA dsb (Kemp *et al.* 1984, Costa and Bryant 1988). Recently, Kemp and Jeggo (1986) showed that a 3 to 5-fold increase in chromatid gaps, breaks and exchanges occured in assynchronous populations of *xrs* cells when irradiated in late S- or G_2-phase compared to CHO-K1 cells. The high frequency of exchange formation in these cells, induced by both radiation and RE, associated with reduced rate of dsb rejoining (Kemp *et al.* 1984) clearly shows that repair of DNA dsb might not be involved in exchange formation. Therefore the presence of two mechanisms for DNA joining seems likely: the first which correctly joins dsb, is dependent on DNA repair synthesis

and might involve recombination, and the second which is independent of DNA synthesis and may misjoin dsb leading to the formation of exchange type aberrations. The notion of a two component chromosomal joining system was postulated by Countryman *et al.* (1977).

Chromosomal hypersensitivity of G_2 A-T cells to X-rays

A-T cells were shown to be hyper-sensitive to radiation and DNA damaging agents in both G_1 and G_2 phases of the cell cycle (Rary *et al.* 1974, Taylor *et al.* 1976, Taylor 1982). The high frequency of chromosome aberrations in A-T cells following radiation or bleomycin treatment suggests that unrejoined or mis-repaired breaks are one type of lesions responsible for enhanced chromosomal radiosensitivity in these cells. A-T is an autosomal recessive syndrome and shows an increased frequency of spontaneous chromosomal aberrations. The unusual finding of Taylor *et al.* (1976) which showed that A-T cells exhibit increased frequencies of chromatid type aberrations following G_0 irradiation, attracted considerable scientific interest to understand cytogenetic mechanisms underlying A-T hypersensitivity to radiation and mutagens.

The SV40 transformed A-T cell line (AT5BIVA) used in these studies retain two important characteristics of A-T derived cells. First, the A-T cells showed increased X-ray induced cell killing (Figure 3.11) (Smith *et al.* 1985). Secondly, that Smith *et al.* (1985) using flow cytofluorimetric analyses revealed that SV40 transformed A-T cells exhibited the expected A-T anomaly of the resistant *de novo* DNA synthesis to

radiogenic inhibition. These observations indicate that SV40 transformation *per se* does not affect the primary lesions responsible for radiation hypersensitivity.

A-T cells were about twice as radiosensitive as normal cells when exposed to 1 Gy X-rays in G_2-phase (Table 11). A similar effect was found previously for both lymphocytes (Rary *et al.* 1974, Taylor 1978, 1982, Bender *et al.* 1985) and fibroblasts (Natarajan and Meyers 1979, Parshad *et al.* 1985). The extent of radiosensitivity was found to be greater in primary cell lines and lymphocytes. However, in spite of the accumulation of data on the cytogenetics of A-T cells, there is still uncertainty about the nature of the chromosomal hypersensitivity of these cells when exposed to ionizing radiation and other DNA damaging agents.

Biochemical experiments carried out with A-T cells failed to prove the existance of a repair defect in these cells. The measurments of the breaks by classical sucrose centrifugation (Taylor *et al.* 1975, Vincent *et al.* 1975, Paterson *et al.* 1976, Lehmann and Stevens 1977), by alkaline filter elusion (Fornace and Little 1980, Hariharan *et al.* 1981) or by flourimetric analsyis of DNA unwinding (Thierry *et al.* 1985) implies that there is no distinguishable difference between the rate of induction or rejoining of either single- or double-strand breaks. Despite biochemical evidence for normal repair of DNA breaks in A-T cells, Taylor (1978) concluded from work based on chromosomal aberration studies, that increased chromosomal aberrations in A-T cells might be due to more unrepaired DNA dsb than in normal cells. From this hypothesis about 1 to 3 percent of DNA dsb induced in A-T cells do not rejoin in these cells

compared to only 0.3 percent in normal cells (Taylor 1978). This proposal was supported by recent data with PCC for normal and A-T lymphocytes irradiated in G_0 (Cornforth and Bedford 1985). These authors showed that X-rays induced similar frequencies of breaks in both G_0 normal and A-T cells initially. After similar initial rejoining kinetics, breaks in A-T cells plateaued out at a higher level (factor of about 10 in logarithmic scale) than in normal cells.

In the present experiments, similar kinetics (exponential) of rejoining of chromatid breaks was found for both normal (Figure 3.15 and 3.20) and A-T cells (Figure 3.17) following X-irradiation with a half time ($t_{1/2}$) of rejoining of approximately 2.4 to 3.1 hours. The results of A-T cells with X-rays may also reflect the similar rate of repair of dsb observed for A-T and normal cells (e.g. Lehmann and Stevens 1977) and parallels the initial rate of rejoining of PCC fragments in A-T and normal cells (Cornforth and Bedford 1985).

Using the "G_2 system", it was possible to demonstrate however that a higher initial frequency of chromatid deletions occurs in X-irradiated A-T cells (Table 14) than in the normal cell line (Table 13). This data confirm a report by Bender *et al.* (1985) which showed that about 4 times more deletions were observed for the same dose (50 rad) in A-T cells than in normal human lymphocytes based on deletions scored at 1.5 h following irradiation. The frequency of deletions at 1.5 h after irradiation was found to be 14.71 deletions per 100 chromosomes (approximately 7/cell) for A-T cells at 1 Gy (Table 14) whereas about 9 deletions per 100 chromosomes were found for normal cells for the same dose (1 Gy) (Table

17). Although this value was similar to the frequency of breaks measured by PCC in G_0 human normal and A-T fibroblasts where about 6 breaks/ Gy/ cell was measured (Cornforth and Bedford 1983, 1985), again this does not reflect the total number of dsb per cell per Gy as measured by biochemical methods (about 40 dsb/Gy/cell) (Blöcher 1982). The values of frequencies of deletions in G_2 cells should actually be halved when compared with G_0 or G_1 cells, since G_2 cells contain twice the amount of DNA. Assuming only about 30 percent of breaks rejoin in the first 1-1.5 h after X-irradiation, based on the data in figures 3.15 & 3.17 and equation (1) it could be concluded that A-T cells convert more dsb into chromatid breaks following X-irradiation. This is also demonstrated in figure 3.19 which shows extrapolated values of deletions at time zero (about 22 deletions per 100 chromosomes in A-T and about 8.5 deletions per 100 chromosomes in normal cells). This data is not in agreement with the report by Cornforth and Bedford (1985) for PCC fragments in A-T and normal cells where equal frequencies of breaks (about 6/cell/Gy) were observed in both cell lines at time zero.

Enhanced G_2 chromatid radiosensitivity could either reflect greater initial radiation induced damage (Parshad *et al.* 1983) or a deficiency in an aspect of DNA repair (Parshad *et al.* 1982, 1984, Gantt *et al.* 1986). But apart from showing normal repair of DNA dsb and ssb in A-T cells, biochemical techniques have also shown that a similar initial frequency of dsb is induced in both A-T and normal cells following irradiation (e.g. Lehmann and Stevens 1977, Thierry *et al.* 1985). However, the chromatid deletions which are thought to arise from dsb were at least twice as

frequent in A-T than normal cells. Therefore, based on the data shown in figure 3.19 and the results of Bender *et al.* (1985), it is possible to suggest that higher levels of dsb are converted into chromatid breaks in A-T cells than in normal cells. This high conversion of dsb into aberrations in A-T cells might be considered as one of the reasons for chromosomal, as well as cellular, hypersensitivity of A-T cells to radiation and chemical mutagens. The mechanism for this conversion of dsb into aberrations is not known. This interpretation however, is different from the proposal of Taylor (1978) and Cornforth and Bedford (1985) who postulated that residual (or unrepaired) dsb are the cause of higher yield of chromatid aberrations in A-T cells.

For X-irradiated A-T cells treated with ara A (Figure 3.17), the results were similar to those found with normal cells (Figures 3.15, 3.20) but at higher levels in A-T cells, particularly for deletions. The frequency of deletions was found to be higher than that of cells exposed to X-rays alone at 1.5 h before fixation and also was constant for all post-irradiation intervals. This was interpreted to be due to inhibition of dsb repair by ara A. Ara A by itself induced very low levels of chromatid deletions when present for 4 h in culture medium before fixation (1.38 deletions per 100 chromosomes).

At 1.5 h after irradiation, the frequency of X-ray induced deletions was higher in the presence than in the absence of ara A in A-T cells. X-rays alone induced 14.71 deletions per 100 chromosomes whereas 21-22 deletions per 100 chromosomes were induced in the presence of ara A by the same dose (1 Gy) (Figure 3.17, Table 15). This indicates that during the 1.5 h colcemid treatment prior to fixation, a fraction of induced

deletions (~30%) were rejoined in A-T cells. This value is similar to the number of deletions rejoined during 1.5-3.5 hours post-irradiation incubation in X-irradiated cells (Figure 3.17). If ara A inhibits the repair of dsb, it might be expected that irradiation of A-T cells at time zero in the presence of ara A would yield the same number of deletions (21-22) as is induced by X-rays alone. This is shown in figure 3.19. Like normal cells, data obtained for A-T cells irradiated in the presence or absence of ara A did meet at a common point when extrapolated. Ara A might be a useful tool for the study of radiation induced chromatid damage at time zero. Because of the consistent effect of ara A on X-ray induced chromatid breaks throughout the G_2 phase, it may be suggested that using ara A at any time during G_2 would represent the chromatid damage at time zero. If this could be proved in other systems such as lymphocytes, then it might be a valuable system for screening A-T heterozygote individuals.

Because disappearance of chromatid deletions in X-irradiated normal and A-T cells followed first order (exponential) kinetics between irradiation and fixation time and ara A inhibited this process (Figure 3.19), this was interpreted as reflecting rejoining of DNA dsb. Data presented here for chromatid deletions in both normal (Figures 3.15 & 3.20) and A-T cells (Figures 3.17 & 3.22) are thus in contrast to the hypothesis of base damage origin of chromosomal aberrations (Preston 1980, 1982). It does however seem entirely plausible that some aberrations might arise from dsb resulting from base-damage incision contributing to the general 'pool' of dsb in the cell. Experiments of Ahnström and Bryant (1982) suggest that the potential frequency of dsb arising from

double-stranded base damage might be 1.7 times the number of direct dsb induced. However, the kinetics and frequency of induction of these enzymatically induced dsb at the radiation dose levels employed in these cytogenetic experiments is unknown.

Synergism of X-rays and ara C

Induction of chromosomal aberrations by ara C in G_2 phase of the cell cycle was reported by Brewen and Christie (1967). From the results in tables 17 & 20 showing experiments with ara C and X-rays in normal and A-T cells, it appears that ara C by itself increases the spontaneous yield of chromatid aberrations by a small proportion when cells were treated in G_2 for four hours.

Induction of chromosomal aberrations in G_2 cells following ara C treatment might either be due to inhibition of DNA synthesis (Preston 1980, 1982) or incorporation of ara C into DNA as a fraudulant nucleoside leading to the inhibition of action of a specific DNA polymerase (Waters *et al.* 1981). X-rays on the other hand, are an S-independent clastogen capable of induction of chromosomal aberrations in G_1 or G_2 phases. This is likely to be a result of its property of inducing several types of DNA damage in particular DNA dsb and ssb in both G_1 and G_2 phases of the cell cycle (van der Schans *et al.* 1982 *a*).

The frequency of X-ray induced chromatid aberrations in G_2 cells is markedly altered when irradiation is combined with ara C treatment. The synergistic action of ara C and X-rays has been shown for G_0 and G_2

irradiated human peripheral lymphocytes (Preston 1980). As shown in figures (3.20 & 3.22), a synergistic effect of ara C and X-rays was also observed with increasing post-irradiation incubation time with ara C for normal and A-T fibroblastic cells. The frequency of chromatid deletions was at a higher level for A-T cells (Figure 3.22) with a similar trend of that for normal cells (Figure 3.20). These observations thus appear to be similar to that of Preston (1980) for human lymphocytes. It seems that the appearance of X-ray induced chromatid deletions in the presence, and disappearance of deletions in the absence of ara C follow quite different and opposite trends. Disappearance of chromatid breaks occured with increasing post-irradiation incubation time indicates repair (or rejoining) of chromatid breaks which was previously shown to be parallel to DNA dsb rejoining (see above). On the other hand the frequency of aberrations induced by X-rays alone increased by a factor of 1.5-2 in the presence of ara C for both cell strains within two hours of (first) irradiation (Figures 3.20 & 3.22).

Ara C is known to inhibit the repair of UV-induced DNA lesions possibly through the inhibition of the resynthesis steps of excision repair, perhaps by incorporation into the resynthesizing sites in a competetive manner (Graham and Whitmore 1970 *a*, Hiss and Preston 1977). This inhibition was shown to lead to the accumulation of single-strand breaks (Hiss and Preston 1977, Dunn and Regan 1979). The increase in X-ray induced chromatid deletions in ara C treated cells and the increased frequency of chromosome type aberrations particularly exchanges in G_0 and G_2 treated human and mouse lymphocytes was interpreted as being

due to the inhibition of the repair of base-damaged lesions at a stage leaving single stranded gaps (Preston 1980, 1982, Bender and Preston 1982, Heartlein and Preston 1985). As a result of these observations Preston (1980, 1982) concluded that ara C enhances frequencies of chromosomal aberrations due to inhibition of repair base damage. The proposal of DNA base-damage origin for chromosomal aberrations by Preston (1980) was based on experiments using G_0 human lymphocytes. According to Hashimoto *et al.* (1975) and Michel and Laval (1982) the repair of strand breaks produced by γ-rays in G_0 human lymphocytes is monophasic and completed within approximately 4 h, leaving a certain fraction of breaks unrepaired. The mode of action of ara C might affect this slower repair process, which could result in the accumulation of strand breaks during the inhibition period of about 5 h (Fabry and Coton 1985).

As mentioned earlier, the kinetics of single- and double-strand break repair has been investigated by several authors (e.g. Blöcher and Pohlit 1982, van der Schans *et al.* 1982 *b*, 1983, Bryant *et al.* 1984). Using the neutral elution technique, it was shown that X-ray induced ssb and dsb followed similar kinetics (van der Schans *et al.* 1983), although this is not in agreement with other biochemical findings (e.g. Blöcher and Pohlit 1982). The measurement of repair kinetics using biochemical methods is still a controversial issue. The increased frequency of chromatid deletions seen in normal and A-T cells shown in figures (3.20 & 3.22), might therefore, be partly due to inhibition of the rejoining of DNA dsb by ara C through preventing the ligation steps in DNA dsb

rejoining. It might also convert some of the ssb which are repaired slowly, into dsb for example by a single strand nuclease as proposed by Natarajan and Obe (1978).

Inhibitory action of ara C on DNA synthesis is shown in figures 3.1 & 3.2 for both cell lines used for these experiments. Using 100 and 200 μ mol/l ara C caused a 85-90 percent reduction in DNA synthesis as judged by ^{3}H-TdR incorporation. Experimental evidence also exists showing that ara C blocks the rejoining of DNA dsb. For example, it was shown that ara C can block rejoining of radiation induced dsb (Iliakis and Bryant 1983) and potentiate the frequency of chromosomal aberrations induced by restriction endonucleases which induce only blunt-ended dsb (Natarajan and Obe 1984, Obe and Natarajan 1985). Recently it was proposed that increased frequencies of chromosomal aberrations occur in X-irradiated cells treated with ara C might be due to the inhibition of repair of directly induced ssb and dsb in DNA (Natarajan *et al.* 1986, Holmberg and Gumauskas 1986). The mode of action of ara C is still controversial. Using the PCC technique, Panthelias and Wolff (1985) suggested that ara C acts as a clastogenic agent with no influence on the repair of PCC fragments in unstimulated human lymphocytes. Moore and Hodgson (1983) also suggested a clastogenic property for ara C when applied to G_2 cells. Although the synergistic effect of ara C on X-ray induced breaks suggest that ara C might act as a clastogenic agent, the weight of evidence at present suggests it acts as a stronger inhibitor of DNA repair synthesis.

In view of results with ara A (Figure 19), the notion proposed by

preston (1980) that ara C is simply inhibiting repair of dsb and thus allowing incision of base damage, is not acceptable. However in the experiments reported here (Figures 3.20 & 3.22) the enhanced frequency of chromatid deletions in X-irradiated normal and A-T cells due to ara C treatment might be interpreted as an interactive action of ara C with radiation induced DNA damage leading to an increase in DNA dsb. The precise mechanism of this process, however, is not understood.

Parshad *et al.* (1980, 1982) found an enhancement in induction of gaps and breaks in G_2 irradiated normal cells of both human and mouse origin when treated with ara C or caffeine directly after irradiation but no or little effect in malignant cells. It was also shown that hypersensitivity to ionizing radiation of G_2 malignant fibroblasts was due to DNA repair deficiency (Parshad *et al.* 1984, Gantt *et al.* 1986). The data presented so far and the reports of Parshad *et al.* (1984) and Gantt *et al.* (1986), clearly shows that A-T (AT5BIVA) cells, despite being hypersensitive to radiation and derived from individuals who are cancer prone, are different from malignant cells in their response to ara C.

The frequency of gaps (achromatic lesions) in normal cells was similar for both X-irradiated and ara C treated cells and at a lower frequency compared to deletions (Figure 3.20). This was also true for A-T cells with the difference that in A-T cells the frequency of gaps decreased slightly with increasing post-irradiation incubation time (Figure 3.22). According to the mononeme theory (Bender *et al.* 1974, Evans 1977), in which a chromatid is thought to be a single continous DNA double helix, chromatid gaps may therefore represent unrepaired DNA ssb because it

is known that ssb can be directly induced by X-rays (van der Schans *et al.* 1982 *a*) or indirectly induced from incomplete nucleotide excision repair of base damage (Preston 1980). Recently it was proposed that the formation of chromatid gaps is associated with unrepaired DNA ssb, perhaps through incomplete nucleotide excision repair with initial incision of DNA without completion of the repair process (Gantt *et al.* 1986). In a series of experiments using human peripheral lymphocytes, Preston (1980, 1982) suggested that the enhanced frequency of chromosomal aberrations following G_0 or G_2 irradiation was due to the accumulation of single stranded gaps, the larger the number of gaps induced in the presence of ara C, the higher the probability of mis-joining of aberrations when the inhibitory effect of ara C is reversed by deoxycytidine. Therefore, the greater the length of ara C treatment, the more single-stranded gaps accumulate to form aberrations (Heartlein and Preston 1985). Data presented here for gaps, therefore, is different from that of Preston (1980) and Parshad *et al.* (1982, 1984). If ara C causes accumulation of single strand gaps we would therefore, expect more gaps in ara C treated cells while in fact the majority of induced breaks were deletions in both normal and A-T cells (Figures 3.20 & 3.22). This effect was similar to the situation in ara A treated A-T and normal cells (Figures 3.15 & 3.17). Thus it can be deduced that neither ara A nor ara C has a strong inhibitory effect on radiation induced ssb in both normal and A-T cells. This is in agreement with a recent proposal of Collins (1987) who recently suggested that ssb rejoining is not prevented by inhibitors of DNA polymerase α; e.g. ara A, ara C and aphidicolin (APC)

at concentrations which effectively inhibit UV excision repair (Hiss and Preston 1977). Lee *et al.* (1972) also showed that ara C at $3x10^{-5}$ mol/l had no effect on the rejoining of X-ray induced single-strand DNA breaks in L-1210 murine leukemia cells. The possible explanations for the increased number of deletions, rather than gaps, firstly may be that gaps are simply a localized uncoiling of the chromatin and are not derived from single- or double-strand breaks. Secondly, transformed and normal cells might respond differently to radiation and inhibitors. For example, SV40 transformed human cells showed increased sensitivity to radiation and DNA damaging agents (Heddle and Arlett 1980). There might also be differences in incision capacities between normal and transformed cells (Squires *et al.* 1982), as well as depressed capacities of certain repair pathways in some SV40 transformed human cells (Day *et al.* 1980, Erickson *et al.* 1980) and less dependence of replicative DNA synthesis on DNA polymerase α in transformed cells but greater involvement of polymerase β (Mattern 1980).

Ara C was found to have similar post-irradiation effect to that of APC (Waters *et al.* 1981) thus inhibiting DNA α-polymerase (Yoshida *et al.* 1977, Ikegami *et al.* 1978, Iliakis and Bryant 1983, Wist 1979). There is also evidence that DNA α polymerase is involved in repair of DNA in mammalian cells (Berger *et al.* 1979, Hanaoka *et al.* 1979, Synder *et al.* 1981, Waters *et al.* 1981). Bertazzoni *et al.* (1980) showed that β-polymerase plays a role in carrying out DNA repair because APC did not influence unscheduled DNA synthesis in UV-irradiated Hela cells.

Because of this conflicting evidence for the role of repair enzymes and the mode of action of ara C, the exact mechanism for the synergistic effect of ara C with X-rays in chromosomal aberration induction is not known yet. Further investigation is needed to resolve these problems.

The frequency of exchanges increased with increasing post-irradiation incubation time in X-irradiated normal and A-T cells (Figures 3.21 & 3.23). In the presence of ara C, this increase was enhanced in both strains (Tables 17 & 20). This observation is in agreement with the recent report of Holmberg and Gumauskas (1986) where the yield of X-ray induced chromosome exchange aberrations in G_0 cells was found to be enhanced in the presence of ara C. These authors proposed that X-ray induced exchanges are formed due to the interaction of short-lived DNA lesions. They showed that ara C does not inhibit, but causes a delay in, the repair of such DNA lesions thus resulting in a higher probability of exchange formation. These results are in conflict with the earlier report of Preston (1980) who showed that no exchanges were formed in ara C treated G_2 lymphocytes following X-irradiation; nor do they confirm the reduction of UV-induced exchanges in CHO cells in the presence of ara C (Natarajan *et al.* 1982). The enhanced frequency of exchanges in my experiments might either reflect a difference in response to ara C of lymphocyte and fibroblast cells or might be an effect of the SV40 transformation of cells.

Comparative analysis of the action of ara A and ara C

Adenosine arabinoside (ara A) and cytosine arabinoside (ara C) are synthetic nucleoside analogues (Walwick *et al.* 1959, Lee *et al.* 1960) which are frequently used as antileukemic and cytotoxic agents (Frei *et al.* 1969, Ho and Freireich 1975). Both ara A and ara C are potent inhibitors of DNA synthesis (Doering *et al.* 1966, Müller *et al.* 1975, 1978, Okura and Yoshida 1978) and probably act through inhibition of DNA polymerases α and β (Fürth and Cohen 1968). It is believed that polymerase-α acts on DNA replication (Hubermann 1981) and that β probably acts on repair function (Hübscher *et al.* 1979). *In vitro* observations suggested that ara A is more effective on β -polymerase than α -polymerase (Okura and Yoshida 1978). Ara C was shown to act specifically on α -polymerase because of its similarity with APC on X-ray induced DNA lesions (Waters *et al.* 1981) but at high concentrations it might also affect β -polymerase (Iliakis and Bryant 1983). It was shown that clastogenicity is a feature of DNA synthesis inhibitors in S-phase cells (Bender *et al.* 1974). Moore and Hodgson (1983) also proposed that inhibitors of DNA synthesis, in particular ara A and ara C, act as clastogenic agents when applied to G_2 cells. This proposal appears to be supported by the data presented in figures (3.7) and (3.9) where ara A and ara C were administered to the cells individually for a period of time before fixation. These figures show that both ara A and ara C increased the background level of aberrations during 4 h treatment. The number of

deletions induced by 100 μ mol/l ara C in A-T cells was similar to the number of deletions induced by 200 μ mol/l ara A in normal cells up to 4 h. The large increase in aberrations in ara C treated A-T cells might either be a consequence of A-T hypersensitivity to chemical mutagens or greater effectiveness of ara C than ara A.

The number of gaps induced in the presence of ara A alone in normal cells was at a higher level than deletions up to 4 h and decreased beyond 4 h while in A-T cells treated with ara C, a lower frequency of gaps was found compared to chromatid breaks.

Figures (3.3) for ara A treated cells and (3.4) for ara C treated samples clearly demonstrate that the induced chromosomal aberrations are concentration dependent. The number of deletions increased steadily between 100 and 250μ mol/l of ara A. The formation of chromatid deletions was enhanced dramatic between 50 and100 μ mol/l of ara C but there was no significant increase beyond 100 μ mol/l. More important in these studies was the observation that ara C induced similar frequencies of gaps for all concentrations used (i.e. 50-200 μ mol/l) (Figure 3.4). Results shown in figure 3.4 suggest that ara C induces chromosomal aberrations in G_2 cells by one or several different mechanisms where the inhibition of some form of DNA repair synthesis may be one of these mechanisms. It was suggested that ara C increases radiation induced DNA damage by inhibiting DNA repair (Hiss and Preston 1977, Preston 1980, Bender and Preston 1982, Heartlein and Preston 1985). Part of this

evidence was that ara C prevented exchange formation in X-irradiated cells treated in G_2 (Preston 1980, Moore 1981). This is not supported by data shown in figures (3.20 & 3.22) for A-T and normal cells treated with ara C and irradiated at a dose of 1 Gy. Exchanges were formed in both cell lines and the frequency increased with post-irradiation incubation time. A recent report by Holmberg and Gumauskas (1986), who found at least a two-fold increase in the formation of chromosome type exchanges in G_0 irradiated human peripheral lymphoctes in the presence of ara C, lends support to the data presented in figures (3.20 & 3.22).

Kihlman and Anderson (1985) using G_2 human peripheral lymphocytes showed that some inhibitors act synergistically when they are applied together. They proposed that inhibitors act synergistically when the enhancement they produce in combination is at least 1.5 times greater than the sum of the enhancement they produce separately. A similar experiment was performed for ara A with ara C. The results, summarized in tables 3 & 4 and in figure 3.5 show that ara A and ara C at 100μ mol/l each (without X-rays) did not enhance chromatid aberration induction synergistically in G_2 A-T cells. Kihlman and Anderson (1985) did not find synergism with ara C and caffeine even with X-irradiated cells. They therefore, suggested that lack of synergism between two inhibitors might be expected when two inhibitors compete for the same site on an enzyme; one inhibitor might reduce the uptake of the other; or one of them might prevent the transformation of the other to an active form; in this case for example, ara C to ara CTP. These proposals seems

true for the lack of synergism between ara A and ara C. Because both of them are nucleoside analogues and they might be incorporated into DNA synthesis in a competetive manner in the phosphorylated state.

Similarities between the action of these two drugs when applied to G_2 or S-cells and yet on the other hand, different action of ara A and ara C on X-ray induced DNA lesions in G_2 cells, suggests a peculiar and important difference in the nature of these two nucleosides. The mechanism by which ara C enhances the frequency of X-ray induced chromosomal aberrations in G_2 cells not understood.

Conclusions

Experiments have been performed in the G_2 phase of the cell cycle. G_2 is considered as a period allowing DNA strand damage repair in X-irradiated cells before entering mitosis. Data have been obtained using X-irradiated normal and A-T cells in G_2 phase in the presence or absence of the inhibitors of DNA synthesis ara A and ara C:

Firstly, my data shows first-order (exponential) kinetics for disappearance of chromatid deletions, interpreted as representing underlying DNA dsb rejoining and its inhibition by ara A. The data therefore support the proposal of Bender *et al.* (1974) where the DNA double strand break was considered as the ultimate lesion leading to the chromosomal aberration formation, and is in accord with the "breakage-first" hypothesis of Sax (1938). This is evidenced by the clear demonstration that the rejoining of chromatid deletions in X-irradiated cells parallels

the rejoining (repair) of DNA dsb measured by biochemical techniques. It is also concluded that the inhibition of DNA dsb repair by ara A underlies the inhibition of rejoining of chromatid deletions.

Secondly; based on the observation that the frequency of exchanges was enhanced in X-irradiated cells in the presence of DNA repair synthesis inhibitors ara A and ara C, two mechanisms for the joining of dsb are proposed. The first which correctly joins dsb requires the DNA repair synthesis and is an "error-free" process, and the second which is independent of DNA synthesis, may misjoin dsb leading to exchange type aberrations and thus is an "error-prone" process.

Thirdly; A-T cells were found to be hyper-sensitive to radiation both in G_1 and G_2 phases of the cell cycle compared to normals. Radio-sensitivity in G_2 phase was found to be a factor of 1.5-2 greater at 1 Gy for deletion formation. However, based on the number of chromatid deletions induced in X-irradiated cells in the presence or absence of ara A, it is suggested that A-T cells convert more DNA dsb into chromatid breaks than normal cells.

Finally; in spite of many similarities between ara A and ara C, e.g. inhibition of DNA synthesis, clastogenic action in G_2 and S-phase of the cell cycle and lack of synergism as a possible consequence of these similarities, ara A was found to have a different effect on inhibition of repair or rejoining of X-ray induced DNA lesions than that of ara C. Thus ara A and ara C appear to have a different mode of action on X-ray-induced DNA lesions.

REFRENCES

Agarwal, S.S., Brown, D.Q., Katz, E.J., and Loeb, L.A., (1977), Screening for deficits in DNA repair by the response of irradiated human lymphocytes to phytohemagglutinin. *Cancer Research*, **37**, 3594-3598.

Ahnström, G., and Bryant, P.E., (1982), DNA double-strand breaks generated by the repair of X-ray damage in Chinese hamster cells. *International Journal of Radiation Biology*, **41**, No. 6, 671-676.

Al Saadi, A., and Palutke, A., (1976), Evaluation of chromosomal aberrations in ataxia-telangiectasia. *Excerptta. Medica. Int. Cong. Ser.*, **397**, Abs 278.

Anderson, M.M., and Suhadolnik, R.J., (1973), Enzymatic epimerization of the 2' hydroxyl group of adenosine in the biosynthesis of 9-β-D-arabinofuranosyladenine by Streptomyces antibioticus. In: IUB *Ninth International Congress of Biochemistry*, Abstract Book 3r 10. Aktiebolaget Egnellska Boktryckeriet; Stokholm.

Arlett, C.F., and Harcourt, S.A., (1980), Survey of radio-sensitivity in a variety of human cell strains. *Cancer Research*, **40**, 926-932.

Aurias, A., Dutrillaux, B., Buriot, D., and Lejeune, J., (1980), High frequencies of inversions and translocations of chromosomes 7 and 14 in ataxia-telangiectasia. *Mutation Research*, **69**, 369-374.

Bender, M.A., Griggs, H.G., and Bedford, J.S., (1974), Mechanisms of chromosomal aberration production: III. Chemicals and ionizing radiation. *Mutation Research*, **23**, 197-212.

Bender, M.A., and Preston, R.J., (1982), Role of base damage in aberration formation: Interaction of aphidicolin and X-rays. In: Natarajan, A.T., Obe, G., and Altmann, H., (Eds): *"Progress in Mutation Research"*, Vol. **4**, Elsevier Biochemical Press. Amsterdam, Oxford, New York. pp 37-46.

Bender, M.A., Rary, J.M., and Kale, R.P., (1985), G_2 chromosomal radiosensitivity of ataxia telangiectasia lymphocytes. *Mutaion Research*, **152**, 39-47.

Bendict, W.F., and Jones, P.A., (1979), Mutagenic, clastogenic and oncogenic effects of 1-β-arabinofuranosylcytosine. *Mutation research*, **65**, 1-20.

Berger, N.A., Kurohara, K.K., Petzold, S.J., and Sikorski, G.W., (1979), Aphidicolin inhibits eukaryotic DNA replication and repair: Implications of DNA polymerase α in both processes. *Biochimical and Biophysical Research Communications*, **87**, 50-58.

Bertazzoni, U., Attolini, C., and Steffanini, M., (1980), DNA repair in nuclei isolated from Hela cells, in: W. Generoso, M. Shelby and F. DeSerres (Eds), "DNA repair and mutagenesis in eukaryotes", *Basic Life Science*, **15**, 357-365.

Blöcher, D., (1982), DNA double-strand breaks in Ehrlich ascites tumour cells at low doses of X-rays. I- Determination of induced breaks by centrifugation at reduced speed. *International Journal of Radiation Biology*, **42**, 317-328.

Blöcher, D. and Pohlit, W., (1982), DNA double-strand breaks in Ehrlich ascites tumour cells at low doses of X-rays: can cell death be attributed to double strand

breaks? *International Journal of Radiation Biology*, **42**, 329-338.

Blöcher, D., Nusse, M., and Bryant, P.E., (1983), Kinetics of double strand break repair in the DNA of X-irradiated synchronized mammalian cells. *International Journal of Radiation Biology*, **43**, 579-584.

Boder, E., (1985), Ataxia-telangiectasia: An overview. In: Gatti, R.A., and Swift, M. (Eds): "Ataxia-telangiectasia: Genetics, Neuropathology, and Immunology of a degenerative disease of childhood". Alan R. Liss, Inc. pp 1-63.

Boder, E., and Sedgwick, R.P., (1958), Ataxia-telangiectasia; A familial syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and frequent pulmonary infection. *Pediatrics*, **21,** 526-554.

Boder, E., and Sedgwick, R.P., (1963), Ataxia-telangiectasia. A review of 101 cases. *Little Club Clinics in Developmental Medicine*, **8,** 110-118.

Boder, E., and Sedgwick, R.P., (1972), Ataxia-telangiectasia. In: Vinken, P.J., and Bruyn, G.W., (Eds); Handbook of Clinical Neurology, vol. 14, North Holland Publishing Co, Amsterdam. pp 267-339.

Bradley, M.O., Erickson, L.C., and Kohn, K.W., (1976), Normal DNA strand rejoining and absence of DNA crosslinking in progeroid and aging in human cells. *Mutation Research*, **37**, 279-292.

Brewen, J.G., (1965), The induction of chromatid lesions by cytosine arabinoside in post-DNA synthetic human leukocytes. *Cytogenetics* , **4**, 28-36.

Brewen, J.G., and Christie, N.T., (1967), Studies on the induction of chromosomal aberrations in human leukocytes by cytosine arabinoside. *Experimental Cell Research*, **46,** 276-291.

Brink, J.J., and Le Page, G.A., (1965), 9-β-D-arabinofuranosyladenine as an inhibitor of metabolism in normal and neoplastic cells. *Canadian Journal of Biochemistry*, **43,** 1-15.

Bryant, P.E., and Blöcher , D., (1980), Measurment of the kinetics of DNA double-strand break repair in Ehrlich ascites tumour cells using the unwinding method. *International Journal of Radiation Biology*, **38,** 335-347.

Bryant, P.E., and Blöcher, D., (1982), The effects of 9-β-D arabinofuranosyladenine on the repair of DNA strand breaks in X-irradiated Ehrlich ascites tumour cells. *International Journal of Radiation Biology* , **42,** No. 4, 385-394.

Bryant, P.E., (1983), 9-β-D arabinofuranosyladenine increases the frequency of X-ray induced chromosome abnormalities in mammalian cells. *International Journal of Radiation Biology* , **43,** No. 4, 459-464.

Bryant, P.E., (1984 *a*), Enzymatic restriction of mammalian cell DNA using Pvu II and Bam H1: evidence for the double strand break origin of chromosomal aberrations. *International Journal of Radiation Biology* , **46,** No. 1, 57-65.

Bryant, P.E., (1984 *b*), Effects of ara A and fresh medium on chromosome damage and DNA double-strand break repair in X-irradiated stationary cells. *British Journal of Cancer* , **49,** suppl. VI, 61-65.

Bryant, P.E., and Iliakis, G., (1984), Possible correlation between cell killing, chromosome damage and DNA repair after X-irradiation. In Collins, A., Johnson, R.T. and Ouhnes, S.D. (Eds): *"DNA repair and its inhibition"*. IRL press , pp 291-308.

Bryant, P.E., Warring, R., and Ahnström, G., (1984), DNA repair kinetics after doses of X-rays. A comparison of results obtained by the unwinding and nucleoid sedimentation methods. *Mutation Research* , **131**, 19-26.

Bryant, P.E., (1985), Enzymatic restriction of mammalian cell DNA: evidence for double-strand breaks as potentially lethal lesions. *International Journal of Radiation Biology*, **48**, No. 1, 55-60.

Bryant, P.E., Birch, D.A., and Jeggo, P.A., (1987), High chromosomal sensitivity of Chinese hamster xrs 5 cells to restriction endonuclease induced DNA double strand breaks. *International Journal of Radiation Biology*, **52** , No. 4, 537-554.

Buckton, K.E., and Evans, H.J., (1981), Human peripheral blood lymphocyte cultures. An *in vitro* assay for the cytogenetic effects of environmental mutagens. In Hsu,T.C. (Ed), *"Cytogenetic assays of environmental mutagens "*. pp 183-199.

Burrell, A.D., Feldschreiber, P., and Dean, C.J., (1971), DNA-membrane association and the repair of double breaks in X-irradiated Micrococcus radiodurans. *Biochemica et Biophysica Acta* , **247**, 38-53.

Cardeilhac, P.T, and Cohen, S.S., (1964), Some metabolic properties of nucleotides of 1-β-D-arabinofuranosylcytosine. *Cancer Research*, **24**, 1595-1603.

Chadwick, K.H., and Leenhouts, H.P., (1978), The rejoining of DNA double-strand breaks and a model for the formation of chromosomal rearrangements. *International Journal of Radiation Biology*, **33**, No. 6, 517-529.

Chen, P.C., Lavin, M.F., Kidson, C., and Moss, D., (1978), Identification of ataxia-telangiectasia heterozygotes; a cancer prone population. *Nature* (London), **247**, 484-486.

Chu, M.Y., and Fischer, G.A., (1962), A proposed mechanism of action of 1-β-D arabinofuranosy-cytosine as an inhibitor of the growth of leukemic cells. *Biochemical Pharmacology*, **11**, 423-430.

Cohen, S.S., (1966), Introduction to the biochemistry of D-arabinosyl nucleosides. *Progress in Nucleic Acid Research*, **5**, 1-88.

Cohen, M.M., Shaham, M., Dagan, J., Shmulei, E., and Kohn, G., (1975), Cytogenetics investigations in families with ataxia-telangiectasia. *Cytogenetics and Cell Genetics*, **15**, 338-356.

Cohen, M.M., and Simpson, S.J., (1982), The use of lymphoblastoid cell lines in the study of ataxia-telangiectasia. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia-A cellular and molecular link between cancer, neuropathology and immune deficiency." New York: Wiley, pp 203-218.

Collins, A., (1987), Cellular responses to ionizing radiation: effects of interrupting DNA repair with chemical agents. *International Journal of Radiation Biology* , **51**, No. 6, 971-983.

Cornforth, M.N., and Bedford, J.S., (1983), X-ray induced breakage and rejoining of human interphase chromosomes. *Science*, **222**, 1141-1143.

Cornforth, M.N., and Bedford, J.S., (1985), On the nature of a defect in cells from individuals with ataxia-telangiectasia. *Science*, **227**, 1589-1591.

Corry, P.M., and Cole, A., (1968), Radiation-induced double-strand scissions of the DNA of mammalian methaphase chromosomes. *Radiation Research* , **36**, 528-543.

Costa, N., and Bryant, P.E., (1988), Repair of DNA single-strand and double-strand breaks in the Chinese hamster xrs 5 mutant cell line as determined by DNA unwinding. DNA repair reports, *Mutation Research* , **194**, 93-99.

Countryman, P.I., Heddle, J.A., and Crawford, E., (1977), The repair of X-ray-induced chromosome damage in trisomy 21 and normal diploid lymphocytes. *Cancer research*, **37**, 52-58.

Cox, R., Hosking, G.P., and Wilson, J., (1978), Ataxia-telangiectasia. Evaluation of radiosensitivity in cultured skin fibroblasts as a diognostic test. *Arch. Dis. Children*, **53**, 386-390.

Cox, R., Masson, W.k., Weichselbaum, R.R., Nove, J., and Little, J.B., (1981), The repair of potentially lethal damage in X-irradiated cultures of normal and ataxia telangiectasia human fibroblasts. *International Journal of Radiation Biology*, **39**, 357-365.

Cox, R., (1982), A cellular description of the repair defect in ataxia-telangiectasia in A-T. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia-A cellular and molecular link between cancer, neuropathology and immune deficiency." New York: Wiley, pp 141-153.

Cox, R., Masson, W.K., Debenham, P.G., and Webb, M.B.T., (1984), The use of recombinant DNA plasmids for the determination of DNA repair and recombination in cultured mammalian cells. *British Journal of Cancer*, **49**, Suppl. VI, 67-72.

Cox, R., Debenham, P.G., Masson, K.W., and Webb, M.B.T., (1986), Ataxia-telangiectasia: A human mutation giving high-frequency misrepair of DNA double-strand scissions. *Molecular Biology and Medicine*, **3**, 229-244.

Cramer, R., and Painter, R.B., (1981), Bleomycin-resistant DNA synthesis in ataxia-telangiectasia cells. *Nature* (London), **291**, 671-672.

Darroudi, F., and Natarajan, A.T., (1987), Cytological characterization of Chinese hamster ovary X-ray sensitive *xrs* 5 and *xrs* 6. I-Induction of chromosomal aberrations by X-irradiation and its modulation with 3-aminobenzamide and caffeine. *Mutation Research*, **177**, 133-148.

Day, R.S., Ziolkowski, C.H.J., Scudiero, D.A., Meyer, S.A., Lubiniecki, A.S., Girardi, A.J., Galloway, S.M., and Bynum, G.D., (1980), Defective repair of alkalyted DNA by human tumour and SV40-transformed human cell strains. *Nature* (London), **288**, 724-727.

Dean, C.J., Ormerod, M.G., Serianni, R.W., and Alexander, P., (1969), DNA strand breakage in cells irradiated with X-rays. *Nature* (London), **222**, 1042-1044.

Debenham, P.G., Webb, M.B.T., Jones, N.J., and Cox, R., (1987), Molecular studies on the nature of the repair defect in ataxia-telangiectasia and their implications for

cellular radiobiology. *Journal of Cell Science*, **Suppl. 6,** 177-189.

De Wit, J., Jaspers, N.G.J., and Bootsma, D., (1981), The rate of DNA synthesis in normal human and ataxia-telangiectasia cells after exposure to X-irradiation. *Mutation Research*, **80,** 221-226.

Doering, A., Keller, J., and Cohen, S.S., (1966), Some effects of D-arabinosyl nucleotides on polymer synthesis in mouse fibroblasts. *Cancer Research* , **26,** 2444-2450.

Dunn, W.C., and Regan, J.D., (1979), Inhibition of DNA excision repair in human cells by arabinofuranosylcytosine: Effect on normal and xeroderma pigmentosum cells. *Molecular Pharmacology*, **15**, 367-374.

Eagle, H., (1955), Nutrition needs of mammalian cells in tissue culture. *Science,* **122,** 501.

Edwards, M. J., and Taylor, A.M.R., (1980), Unusual levels of $(ADP\text{-}ribose)_n$ and DNA synthesis in ataxia telangiectasia cells following X-ray irradiation. *Nature* (London), **287**, 745-747.

Ejima, Y., and Sasaki, M.S., (1986), Enhanced experesion of X-ray- and UV-induced chromosome aberrations by cytosine arabinoside in ataxia-telangiectasia cells. *Mutation Research* , **159**, 117-123.

Epstein, J., Williams, J.R., and Little, J.B., (1973), Deficient DNA repair in human progeric cells. *Proceedings of the National Academic Science,* USA, **70**, 977-981.

Erickson, L.C., Bradley, M.O., Ducore, J.M., Ewig, R.A.G., and Kohn, K.W., (1980), DNA crosslinking and cytotoxicity in normal and transformed human cells treated with anti-tumour nitrosoureas. *Proceedings of the National Academic Science,* USA, **77**, 467-471.

Evans, H.J., (1977), Molecular mechanisms in the induction of chromosome aberrations. In: D. Scott, B.A. Bridges, and F.H. Sobles (Eds), *"Progress in Genetic Toxicology"* , Elsevier, Amsterdam, pp 57-74.

Fabry, L., and Coton, C., (1985), Study of the radioinduced lesions involved in the formation of chromosomal aberrations in G_0 human lymphocytes after exposure to gamma-rays and fast-neutrons. *Mutation Research*, **149**, 475-483.

Ford, M.D., Martin, L., and Lavin, M.F., (1984), The effects of ionizing radiation on cell cycle progression in ataxia-telangiectasia. *Mutation Research*, **125**, 115-122.

Fornace, A.J., and Little, J.B., (1980), Normal repair of DNA single-strand breaks in patients with ataxia telangiectasia. *Biochemica et Biophisica Acta* , **607,** 432-437.

Fox, M., and Fox, B.W., (1973), Repair replication in X-irradiated lymphoma cells in vitro. *International Journal of Radiation Biology* , **23,** No. 4, 333-338.

Frankenberg, D., (1969), A ferrous sulphate dosemeter independent of photon energy in the range from 25 Kev up to 50 Mev. *Physics in Medicine and Biology* , **14,** No. 4, 597-605.

Frei, E., III, Bickers, J.N., Hewlett, J.S., Lane, M., Leary, W.V., and Talley, R.W., (1969), Dose schedule and antitumour studies of arabinosyl cytosine (NSC 63878). *Cancer Research* , **29**, 1325-1332.

Freifelder, D., (1966), DNA strand breakage by X-irradiation. *Radiation Research*, **29**, 329-338.

Frøland, A., (1961), A simplified method for making chromosome prepration from skin biopsies. *Acta Pathologica Microbiologica Scandinavica*, **53**, 319.

Fürth, J.J., and Cohen, S.S., (1967), Inhibition of mammalian DNA polymerases by the 5'-triphosphate of 9-β-D arabinofuranosyl adenine. *Cancer Research*, **27**, 1528-1533.

Fürth, J.J., and Cohen, S.S., (1968), Inhibition of mammalian DNA polymerase by the 5'-triphosphate of 1-β-D-arabinofuranosyl cytosine and the 5'-triphosphate of 9-β-D-arabinofuranosyl adenine. *Cancer Research*, **28**, 2061-2067.

Gantt, R., Parshad, R., Price, F.M., and Sanford, K.K., (1986), Biochemical evidence for deficient DNA repair leading to enhanced G_2 chromosomal radiosensitivity and susceptibility to cancer. *Radiation Research*, **108**, 117-126.

Giannelli, F., Avery, J.A., Pembrey, M.E., and Blunt, S., (1982), Prenatal exclusion of ataxia-telangiectasia. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia--A cellular and molecular link between cancer, neuropathology and immune deficiency." New York: Wiley, pp 393-400.

Glickman, B.W., Zwenk, H., Van Sluis, C.A., and Rorsch, A., (1971), The isolation and characterisation of an X-ray sensitive ultraviolet-resistant mutant of Escherichia coli. *Biochemica et Biophisica Acta*, **254**, 144-154.

Gotoff, S.P., Amirokri, E., and Liebner, E.J., (1967), Ataxia-telangiectasia, neoplasia, untoward response to X-irradiation and tuberous sclerosis. *Am. J. Dis. Child.*, **114**, 617-625.

Graham, F.L., and Whitmore, G.F., (1970 *a*), Studies in mouse L-cells of incorporation of 1-β-D-arabinofuranosyl cytosine into DNA and on inhibition of DNA polymerase by 1-β-D-arabinofuranosylcytosine-5'-triphosphate. *Cancer Research*, **30**, 2636-2644.

Graham, F.L., and Whitmore, G.F., (1970 *b*), The effect of 1-β-D-arabinofuranosyl cytosine on growth, viability and DNA synthesis of mouse L-cells. *Cancer Research*, **30**, 2627-2635.

Green, M.H.L., and Lowe, J.E., (1987), Failure to detect a DNA repair-related defect in the transfection of ataxia-telangiectasia cells by enzymatically restricted plasmid. *International Journal of Radiation Biology*, **52**, No. 3, 437-446.

Gropp, A., and Flatz, G., (1967), Chromosome breakage and blastic transformation of lymphocytes in ataxia-telangiectasia. *Humangenetick*, **5**, 77-79.

Hall, E.J., (1978), Radiobiology for the Radiologist; Harper and Raw, Philadelphia, pp 111-128.

Hanaoka, F., Kato, H., Ikegami, S., Ohashi, M., and Yamosa, M., (1979), Aphidicolin does inhibit repair replication in Hella cells. *Biochemical and Biophysical Research Communications*, **87**, 575-580.

Hansson, K., Natarajan, A.T., and Kihlman, B.A., (1984), Effect of caffeine in G_2 on X-ray-induced chromosomal aberrations and mitotic inhibition in ataxia telangiectasia fibroblast and lymphoblastoid cells. *Human Genetics*, **67**, 329-335.

Hariharan, P.V., Eleczko, S., Smith, B.P., and Paterson, M.C., (1981), Normal rejoining of DNA strand breaks in ataxia-telangiectasia fibroblast lines after low X-ray exposure. *Radiation Research*, **86,** 589-597.

Harnden, D.G., (1974), Ataxia-telangiectasia syndrome: Cytogenetic and cancer aspects. In German, J. (Ed) " Chromosomes and Cancer". New York, Wiley, pp 619-636.

Hartley-Asp, B., Andersson, H.C., Sturelid, S., and Kihlman, B.A., (1980), G_2 repair and the formation of chromatid aberrations. I- The effect of hydroxyurea and caffeine on maleic hydrazide-induced chromosome damage in *Vicia faba*. *Environmental and Experimental Botany*, **20**, 119-129.

Hashimoto, Y., Ono, T., and Okada, S., (1975), Radiosensitivities of DNA molecules in lymphocytes from the circulating blood of man. *Blood*, **45**, 503-509.

Heartlein, M.W., and Preston, R.J., (1985), An explanation of interspecific differences in sensitivity to X-ray-induced chromosome aberrations and a consideration of dose-responce curves. *Mutation Research*, **150,** 299-305.

Hecht, F., Koler, R.D., Rigas, D.A., Dahnke, G.S., Case, M.P., Tisdale, V., and, Miller, R.W., (1966), Leukaemia and lymphocytes in ataxia-telangiectasia. *Lancet*, **2**, 1193.

Hecht, F., McCaw, B.K., and Koler, R.D., (1973), Ataxia-telangiectasia-clonal growth and translocation lymphocytes. *New England Journal of Medicine* , **289**, 286-291.

Hecht, F. and Kaiser-McCaw, B., (1982), Ataxia-telangiectasia; Chromosomes before Cancer. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia-A cellular and molecular link between cancer, neuropathology and immune-deficiency." New York: Wiley, pp 235-241.

Heddle, J.A., and Arlett, C.F., (1980), Untransformed xeroderma pigmentosum cells are not hypersensitive to sister-chromatid exchange production by ethylmethane-sulphonate; Implications for the use of transformed cell lines and for the mechanisms by which SCE arise. *Mutation Research*, **72**, 119-125.

Hieber, L., and Lucke-Huhle, C., (1983), PCC technique reveals severe chromatin lesions and repair in G_2-arrested cells after alpha-irradiation. *Experimental Cell Research,* **144**, 57-62.

Higurashi, M., and Conen, P.E., (1973), *In vitro* chromosomal radiosensitivity in "chromosome breakage" syndromes. *Cancer*, **32**, 380-383.

Hiss, E.A., and Preston, R.J., (1977), The effect of cytosine arabinoside on the frequency of single-strand breaks in DNA of mammmalian cells following irradiation or chemical treatment. *Biochemica et Biophisica Acta*, **478**, 1-8.

Hittelman, W.N., and Rao, P.N., (1974 *a*), Premature chromosome condensation. I. Visualization of X-ray induced chromosome damage in interphase cells. *Mutation Research*, **23**, 251-258.

Hittelman, W.N., and Rao, P.N., (1974 *b*), Premature chromosome condensation. II. The nature of chromosome gaps induced by alkylating agents and ultraviolet light. *Mutation Research*, **23**, 259-266.

Ho, D.H.W., and, Freireich, E.J., (1975), Clinical pharmacology of arabinofuranosylcytosine. In <Handbook of experimental pharmacology> Eds. Eichler, O., Farah, A., Herkin, H., and Welch, A.D., Springer-Verlag, Berlin-Heidlberg, New York. pp 259-271.

Holmberg, M., and, Gumauskas, E., (1986), The role of short-lived DNA lesions in the production of chromosome-exchange aberrations. *Mutation Research*, **160**, 221-229.

Houldsworth, J., and Lavin, M.F., (1980), Effect of ionizing radiation on DNA synthesis in ataxia-telangiectasia. *Nucleic Acids Research*, **8**, 3709-3720.

Howard, A., and Pelc, S.R., (1953), Synthesis of deoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. *Heredity* (Suppl) (London), **6**, 261-273.

Hsu, T.C., and Kellogg, D.S., (1960), Primary cultivation and continous propagation in *in vitro* of tissues from small biopsy specimens. *Journal of National Cancer Institute*, **25**, 221.

Hubermann, J.A., (1981), New views of the biochimstry of eukaryotic DNA replication revealed by aphidicolin, an unusual inhibitor of DNA polymerase α. *Cell*, **23**, 647-648.

Hubert-Habart, M., and Cohen, S.S., (1962), The toxicity of 9-β-D-arabinofuranosyladenine to Purine-requiring *Esherichia coli*. *Biochemica et Biophisica Acta*, **59**, 468-471.

Hübscher, U., Kuenzl, C.C., and Spadari, S., (1979), Functional roles of DNA polymerases β and γ. *Proceedings of the National Academic Science,* USA, **76**, 2316-2320.

Hunter, T., and franke, B., (1975). *In vitro* polyoma DNA synthesis: inhibition by 1-β-D-arabinofuranosyl CTP. *Journal of Virology*, **15**, 759-775.

Ikegami, S., Taguchi, T., Ohashi, M., Oguro, M., Nagano, H., and, Mano, Y., (1978), Aphidicolin prevents mitotic cell division by interfering with the activity of DNA polymerase-α. *Nature* (London), **275**, 458-460.

Iliakis, G., (1980), Effects of beta-arabinofuranosyladenine on the growth and repair of potentially lethal damage in Ehrlich ascites tumour cells. *Radiation Research*, **83**, 537-552.

Iliakis, G., (1981), Characterization and properties of repair of potentially lethal damage as measured with the help of beta-arabinofuranosyladenine in plateau-phase EAT-cells. *Radiation Research*, **86**, 77-90.

Iliakis, G., Nusse, M., and Bryant, P.B., (1982), Effects of aphidicolin on cell proliferation, repair of potentially lethal damage and repair of DNA strand breaks in Ehrlich ascites tumour cells exposed to X-rays. *International Journal of Radiation Biology*, **52**, 417-434.

Iliakis, G., and Bryant, P.B., (1983), Effects of the nucleoside analogues α–ara A, β–ara A and β–ara C on the growth and repair of both potentially lethal damage and DNA double strand breaks in mammalian cells in culture. *Anticancer Research* , **3**, 143-150.

Iliakis, G., (1985), Evidence for the induction of two types of potentially lethal damage after exposure of plateau phase Chniese hamster V79-cells to gamma-rays. *Radiation and Environmental Biophysics*, **24,** 185-202.

Iliakis, G., Wright, E., and, Nego, F.Q.H., (1987), Repair and fixation of poitentially lethal damage (PLD) as demonstrated by delayed plating or incombinationwith ara A in contact inhibited refed plateau-phase C3H mouse emberyo 10T1/2 cells grown in the presence of BrudUrd. *Radiation and Environmental Biophysics*, **26**, 47-62.

Inoue, T., Hirano, K., Yokoiyama, A., Kada, T., and Kato, H., (1977), DNA repair enzymes in ataxia telangiectasia and Bloom's syndrome fibroblasts. *Biochemica et Biophisica Acta* , **479,** 497-500.

Jacobs, J.P., Jones, C.M., and Baillie, J.P., (1970), Characterization of a human diploid cell designated MRC-5. *Nature* (London), **227**, 168-170.

Jaspers, N.G.J., de Wit, J., and Bootsma, D., (1982 *a*), The inhibition of DNA replication in ataxia-telangiectasia cells after exposure to a variety of clastogenic agents. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia-A cellular and molecular link between cancer, neuropathology and immune-deficiency." New York: Wiley, pp 193-201.

Jaspers, N.G.J., de Wit, J., and Bootsma, D., (1982 *b*), The rate of DNA synthesis in ataxia-telangiectasia fibroblasts after exposure to DNA damaging agents. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia-A cellular and molecular link between cancer, neuropathology and immune-deficiency." New York: Wiley, pp 339-345.

Jeggo, P.A., Kemp, L.M.,and, Holliday, R., (1982), The application of the microbial 'tooth-pick' technique to somatic cell genetics and its use in the isolation of X-ray sensitive mutants of Chinese hamster ovary cells. *Biochemistry*, **64**, 713-715.

Johnson, R.T., and Rao, P.N., (1970), Mammalian cell fusion. II. Induction of premature chromosome condensation in interphase nuclei. *Nature* (London), **226**, 717-722.

Jones, P.A., Baker, M.S., and Bendict, W.F., (1976), The effect of 1-β-D-arabinofuranosylcytosine on cell viability, DNA synthesis, and chromatid breakage in synchronized hamster fibrosarcoma cells. *Cancer Research*, **36**, 3789-3797.

Karran, P., (1973), The repair of methyl methanesulphonate induced lesions in the DNA of a murine lymphoma cell. *Chemical and Biological Interactions*, **7**, 389-398.

Kemp, L.M., Sedgwick, S.G., and, Jeggo, P.A., (1984), X-ray sensitive mutants of Chinese hamster ovary cells deffective in double-strand break rejoining. *Mutation Research* , **132**, 189-196.

Kemp, L.M., and Jeggo, P.A., (1986), Radiation-induced chromosome damage in X-ray sensitive mutants (*xrs*) of the chinese hamster ovary cell line. *Mutation Research* , **166,** 255-263.

Kihlman, B.A., (1963), Deoxyadenosine as an inducer of chromosomal aberrations in Vicia faba. *Journal of Cell Comparative Physiology*, **62**, 267-272.

Kihlman, B.A., (1977), "*Caffeine and chromosomes* ". Elsevier, Amsterdam.

Kihlman, B.A., Nichols, W.W., and Levan, A., (1963), The effect of deoxyadenosine and cytosine arabinoside on the chromosomes of human leukocytes *in vitro*. *Hereditas* , **50**, 139-143.

Kihlman, B.A., Natarajan, A.T., and Anderson, H.C., (1978), Use of the 5-bromodeoxyuridine-labelling technique for exploring mechanisms involved in the formation of chromosomal aberrations. I. G_2 experiments with root-tips of *Vicia faba* . *Mutation Research* , **52**, 181-198.

Kihlman, B.A., Hansson, K., Palitti, F., Anderson, H.C., and, Hartly-Asp, B., (1982), Potentiation of induced chromatid type aberrations by hydroxyurea and caffeine. In: Natarajan, A.T., Obe, G., and Altmann, H., (Eds): *"Progress in Mutation Research"* , Vol. **4**, Elsevier Biochemical Press. Amsterdam, Oxford, New York. pp 11-24.

Kihlman, B.A., and Anderson, H.C., (1985), Synergistic enhancement of the frequency of chromatid aberrations cultured human lymphocytes by combinaions of inhibitors of DNA repair. *Mutation Research* , **150,** 313-325.

Kozai, Y., and Sugino, Y., (1971), Enzymatic phosphorylation of 1-β-D-arabino-furanosyl cytosine. *Cancer Research*, **31**, 1376-1382.

Krasin, F., and Hutchinson, F., (1977), Repair of DNA-double-strand breaks in Escherichia coli which requires rec A function and the presence of a duplicate genome. *Journal of Molecular Biology*, **116**, 81-89.

Lee, W.W., Benitez, A., Goodman, L., and Baker, B.R., (1960), Potential anticancer agents. XL. Synthesis of β-anomer of 9-(D-arabinofuranosyl)-adenine. *Journal of American Chemical Society* , **82,** 2648-2649.

Lee, Y.C., Bennett, L.R., and Byfield, J.E., (1972), Inhibition of repair of DNA single strand breaks in mouse leukemia cells by actinomycin D. *Biochemical and Biophysical Research Communications* , **49**, 758-765.

Lehmann, A.R., and Ormerod, M.G., (1970), Double-strand breaks in the DNA of a mammalian cell after X-irradiation. *Biochemica et Biophisica Acta*, **217,** 268-277.

Lehmann, A.R., and Stevens, S., (1977), The production and repair of double strand breaks in cells from normal humans and from patients with ataxia telangiectasia. *Biochemica et Biophisica Acta*, **474,** 49-60.

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

Lehmann, A.R., (1982), The cellular and molecular responses of ataxia-telangiectasia cells to DNA damage. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia--A cellular and molecular link between cancer, neuro-pathology and immune-deficiency." New York: Wiley, pp 83-101.

Lehmann, A.R., James, M.R., and Stevens, S., (1982), Miscellaneous observations on DNA repair in ataxia-telangiectasia. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia--A cellular and molecular link between cancer, neuro-pathology and immune-deficiency." New York: Wiley, pp 347-353.

Little, J.B., and Nagasawa, H., (1985), Effects of confluent holding on potentially lethal damage repair, cell cycle progression, and chromosomal aberrations in

human normal and ataxia-telangiecrtasia fibroblasts. *Radiation Research*, **101,** 81-93.

Loeb, L.A., Agarwal, S.S., Duba, D.K., Gopinthan, G.S., Shearman, C.W., Sirover, M.A., and Travaglini, E.C., (1980), Inhibitors of mammalian DNA polymerases: possible chemotherapeutic approaches; In: Sarin, P.S., and Gallo, R.C., (Eds), Inhibitors of DNA and RNA polymerases, Pergamon, Oxford, pp 27-45.

Lücke-Hühle, C., Hieber, L., and, Wegner, R., (1983), Caffeine-mediated release of alpha-radiation induced G_2 arrest increases the yield of chromosome aberrations. *International Journal of Radiation Biology*, **43,** No. 2, 123-132.

Major, P.P., Egan, E.M., Herrick, D.J., and Kufe, D.W., (1982), Effect of ara C incorporation on deoxyribonucleic acid synthesis in cells. *Biochemical pharmacology*, **31,** No. 18, 2937-2940.

Mattern, M.R., (1980), Differences between diploid and transformed human cells in the incorporation of 5-bromodeoxyuridine into DNA. *Experimental Cell Research*, **128,** 181-190.

McCaw, B.K., Hecht, F., Harnden, D.G., and Teplitz, R.L., (1975), Somatic rearrangement of chromosome 14 in human lymphocytes. *Proceedings of the National Academic Science,* USA, **72,** 2071-2075.

Michel, S., and Laval, F., (1982), Repair of γ-ray induced single-strand breaks in human lymphocytes. *Biochemistry.*, **64,** 749-751.

Miller, R.W., (1982), Highlights in clinical discoveries relating to ataxia-telangiectasia. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia -A cellular and molecular link between cancer, neuropathology and immune-deficiency." New York: Wiley, pp 13-21.

Momparler, R.L., (1974), A model for the chemotherapy of acute leukemia with 1-β-D-arabinofuranosyl-cytosine. *Cancer Research* , **34,** 1775-1787.

Momparler, R.L., Bernet, T.p., Labitan, A., and Krygier, V., (1971), Studies on the phosphorylation of cytosine arabinoside in mammalian cells. *Molecular Pharmacology*, **7,** 413-419.

Moore, R.C., (1981), Effects of 1-β-D arabinofuranosylcytosine on chromosomes, depending upon the cell cycle stage at the time of exposure. *Radiation Research* , **83,** 361-374.

Moore, R.C., and Hodgson, G.S., (1983), Clastogenic effect of DNA synthesis inhibitors in cells exposed in G_2. *Mutation Research* , **120,** 139-143.

Moore, R.C., and Randall, C., (1987), Different effects of 1-β-D arabinofuranosyl-cytosine and aphidicoline in S-phase cells, chromosome aberrations, cell cycle delay and cytotoxicity. *Radiation Research* , **178,** 73-80.

Müller, W.E.G., Rohde, H.J., Beyer, R., Maidhof, A., Lachmann, M., Taschner, H., and Zahn, R.K., (1975), Mode of action of 9-β-D-arabinofuranosyladenine on the synthesis of DNA, RNA, and Protein in vivo and in vitro. *Cancer Research* , **33,** 2160-2168.

Müller, W.E.G., Maidhof, A., Zahn, R.K., and Shannon, W.M., (1977), The effect of 9-β-D-ara A on DNA synthesis *in vivo*. *Cancer Research* , **37,** 2282-2290.

Müller, W.E.G., Zahn, R.K., Maidhof, A., Beyer, R., and Arendes, J., (1978), The effect of the cytostatic agent 9-β-D-arabinofuranosyladenine on the nucleic acid metabolism in L5178Y cells. *Biochemical Pharmacology*, **27**, 1659-1663.

Müller, W.W.G., and Zahn, R.K., (1978), Biochemistry of antivirals, mechanism of action and pharmacology. *Research in Molecular Biology*, **9**, 18-32.

Müller, W.E.G., (1979), Uptake and metabolism of purine nucleosides and purine nucleoside analogues by cells. In: *Nucleoside Analogues*; Walker, R.T., Clercq, E.D., and Eckstein, F., (Eds.). Plenum Publishing Co. pp 247-279.

Nagasawa, H., Latt, S.A., Lalande, M.E., and Little, J.B., (1985), Effects of X-irradiation on cell cycle progression, induction of chromosomal aberrations and cell killing in ataxia-telangiectasia (AT) fibroblasts. *Mutation Research* , **148,** 71-82.

Nagasawa, H., and Little, J.B., (1983), Comparison of kinetics of X-ray-induced cell killing in normal, ataxia telangiectasia and heriditary retinoblastoma fibroblasts. *Mutation Research* , **109,** 297-308.

Natarajan, A.T., and Ahnström, G., (1968), Cytogenetical effects of inorganic phosphate and 5-fluorodeoxyuridine. *Hereditas* , **59**, 229-241.

Natarajan, A.T., and Obe, G., (1978), Molecular mechanisms involved in the production of chromosomal aberrations: I-Utilization of Neurospora endonuclease for the study of aberration production in G_2 stage of the cell cycle. *Mutation Research* , **52,** 137-149.

Natarajan, A.T., and Meyers, M., (1979), Chromosomal radiosensitivity of ataxia telangiectasia cells at different cell cycle stages. *Human Genetics* , **52,** 127-132.

Natarajan, A.T., Obe, G., van Zeeland, A.A., Palitti, F., Meijers, M., and Elly Verdegaal-Immerzeel, A.M., (1980), Molecular mechanisms involved in the production of chromosomal aberrations. II- Utilization of Neurospora endonuclease for the study of aberration production by X-rays in G_1 and G_2 stages of the cell cycle. *Mutation Research* , **69**, 293-305.

Natarajan, A.T., and Zwanenburg, T.S.B., (1982), Mechanisms for chromosomal aberrations in mammalian cells. *Mutation Research* , **95**, 1-6.

Natarajan, A.T., Csukas, I., Degrassi, F,, van Zeeland, A,A., Palitti, F., Tanzarella, C., de Salvia, R., and Fior, M., (1982), Influence of inhibition of repair enzymes on the induction of chromosomal aberrations by physical and chemical agents. In: Natarajan, A.T., Obe, G., and Altmann, H., (Eds): *"Progress in Mutation Research"* , Vol. **4,** Elsevier Biochemical Press. Amsterdam, Oxford, New York. pp 47-59.

Natarajan, A.T., and Obe, G., (1983), Influence of DNA repair on radiation-induced chromosomal aberrations in human peripheral lymphocytes. In: *Radiation Induced Chromosome Damage in Man*, Ishihara, T., and Sasaki, M.S., (Eds), Alan R. Liss, New York. pp 127-140.

Natarajan, A.T., and Obe, G., (1984), Molecular mechanisms involved in the production of chromosomal aberrations: III- Restriction Endonucleases. *Chromosoma* (Berlin), **90,** 120-127.

Natarajan, A.T., Darroudi, F., Mullenders, L.H.F., and Meijers, M., (1986), The nature and repair of DNA lesions that lead to chromosomal aberrations induced by ionizing radiations. *Mutation Research*, **160,** 231-236.

Neary, G.J., Horgan, V.J., Bancr, D.A., and Stretch, A., (1972), Further data on DNA strand breakage by various radiation qualities. *International Journal of Radiation Biology*, **22,** 525-537.

Nelson, M.M., Blom, A., and Arens, L., (1975), Chromosomes in ataxia-telangiectasia. *Lancet* **i,** 518-519.

Nichols, W.W., (1964), In vitro breakage induced by arabinosyadenine in human leukocytes. *Cancer Research*, **24,** 1502-1505.

Obe, G., Natarajan, A.T., and Palitti, F., (1982), Role of DNA double-strand breaks in the formation of radiation-induced chromosomal aberrations. In: Natarajan, A.T., Obe, G., and Altmann, H., (Eds): *"Progress in Mutation Research"* , Vol. **4,** Elsevier Biochemical Press. Amsterdam, Oxford, New York. pp 1-9.

Obe, G., and Natarajan, A.T., (1985), Chromosomal aberrations induced by the restriction endonuclease Alu I in Chinese hamster ovary cells: influence of duration of treatment and potentiation by cytosine arabinoside. *Mutation Research*, **152,** 205-210.

Obe, G., and Winkel, E-U., (1985), The chromosome-breaking activity of the restriction endonuclease Alu I in CHO cells is independent of the S-phase of the cell cycle. *Mutation Research*, **152,** 25-29.

OConnor, R.D., Brown, M.G., and Francke, V., (1982), Immunologic and karyotypic studies in ataxia-telangiectasia; specificity of break points on chromosomes 7 and 14 in lymphocytes from patients and relatives. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia-A cellular and molecular link between cancer, neuropathology and immune-deficiency." New York: Wiley, pp 13-21.

Okura, A., and Yoshida, J., (1978), Differential inhibition of DNA polymerases of calf thymus by 9-β-D-arabinofuranosyladenine-5 triphosphate. *Journal of Biochemistry*, **84,** 727-732.

Oxford, J.M., Harnden, D.G., Parrington, J.M., and Delhanty, J.D.A., (1975), Specific chromosome aberrations in ataxia-telangiectasia. *Journal of Medical Genetics* , **12,** 251-262.

Painter, R.B., (1980), Effect of caffeine on DNA synthesis in irradiated and unirradiated mammalian cells. *Journal of Molecular Biology* , **143,** 289-301.

Painter, R.B., and Young, B.R., (1980), Radiosensitivity in ataxia-telangiectasia: A new explanation. *Proceeding of National Academic Science*, USA, **77,** No. 12, 7315-7317.

Painter, R.B., (1985), Altered DNA synthesis in irradiated and unirradiated ataxia-telangiectasia cells. In: Gatti,R.A., and Swift, M. (Eds): " Ataxia-telangiectasia: Genetics, Neuropathology, and Immunology of a degenerative disease of childhood". Alan R. Liss, Inc. pp 89-100.

Palitti, F., Tanzarella, C., Degrassi, F., De Salvia, R., Fiore, M., and Natarajan, A.T., (1983), Formation of chromatid-type aberrations in G_2 stage of the cell cycle. *Mutation Research*, **110,** 343-350.

Panthelias, G.E., and Wolff, S., (1985), Cytosine arabinoside is a potent clastogen and dose not affect the repair of X-ray-induced chromosome fragments in unstimulated human lymphocytes. *Mutation Research* , **151**, 65-72.

Parshad, R., Sanford, K.K., Jones, G.M., Tarone, R.E., Hoffman, H.A., and Grier, A.H., (1980), Susceptibility to fluorescent light-induced chromatid breaks associated with DNA repair defficiency and malignant trasformation in culture. *Cancer Research*, **40**, 4415-4419.

Parshad, R., Gantt, R., Sanford, K.K., Jones, G.M., and Tarone, R.E., (1982), Repair of chromosome damage induced by X-irradiation during G_2 phase in a line of normal human fibroblast and its malignant derivatives. *Journal of National Cancer Institute* , **69**, No. 2, 409-414.

Parshad, R., Sanford, K.K., and Jones, G.M., (1983), Chromatid damage after G_2 phase X-irradiation of cells from cancer-prone individuals implicates deficiency in DNA repair. *Proceedings of the National Academic Science,* USA, **80**, 5612-5616.

Parshad, R., Gantt, R., Sanford, K.K., and Jones, G.M., (1984), Chromosomal radiosensitivity of human tumour cells during the G_2 cell cycle period. *Cancer Research* , **44**, 5577-5582.

Parshad, R., Sanford, K.K., Jones, G.M., and Tarone, R.E., (1985), G_2 chromosomal radiosensitivity of ataxia-telangiectasia heterozygotes. *Cancer, Genetics and Cytogenetics*, **14**, 163-168.

Paterson, M.C., Smith, B.P., Lohman, P.H.M., Anderson, A.K., and Fishman, L., (1976), Defective excision repair of γ-ray-damaged DNA in human (ataxia-telangiectasia) fibroblasts. *Nature* (London), **260**, 444-446.

Paterson, M.C., Anderson, A.K., Smith, B.P., and Smith, P.J., (1979), Enhanced radiosensitivity of cultured fibroblasts from ataxia telangiectasia heterozygotes manifested by defective colony-forming ability and reduced DNA repair and replication after hypoxic γ-radiation. *Cancer Research* , **39**, 3724-3725.

Paterson, M.C., and Smith, P.J., (1979), Ataxia-telangiectasia: An inherited human disorder involving hypersensitivity to ionizing radiation and related DNA damaging chemicals. *Annual Revision of Genetics* , **13**, 291-318.

Picksley, S.M., Attfield, P.V., and Lloyd, R.G., (1984), Repair of DNA double-strand breaks in *Escherichia coli* K12 requires a functional rec N product. *Molecular and General Genetics*, **195**, 267-274.

Plunkett, W., Lapi, L., Ortiz, P.J., and Cohen, S.S., (1974), Penetration of mouse fibroblasts by the 5'-phosphate of 9-β-D-arabinofuranosyladenine and incorporation of the nucleotide into DNA. *Proceedings of the National Academic Science,* USA, **71**, 73-77.

Povirk, L.F., (1977), Localization of inhibition of replicon initiation to damaged regions of DNA. *Journal of Molecular Biology* , **114**, 141-151.

Preston, R.J. (1980), The effect of cytosine arabinoside on the frequency of X-ray induced chromosome aberrations in normal human leukocytes. *Mutation Research*, **69**, 71-79.

Preston, R.J. (1982), DNA repair and chromosome aberrations: interactive effects of radiation and chemicals. In: Natarajan, A.T., Obe, G., and Altmann, H., (Eds):*"Progress in Mutation Research"* , Vol. **4,** Elsevier Biochemical Press. Amsterdam, Oxford, New York. pp 25-35.

Rary, J.M., Bender, M.A., and Kelly, T.E., (1974), Cytogenetic studies of ataxia-telangiectasia. *American Journal of Human Genetics*, **26**, 70A.

Rary, J.M., Bender, M.A., and Kelly, T.E., (1975), A 14/14 marker chromosome lymphocyte clone in ataxia-telangiectasia. *Journal of Heredity*, **66**, 33-35.

Resnick, M.A., (1976), The repair of double-strand breaks in DNA: A model involving recombination. *Journal of Theoretical Biology*, **59**, 97-106.

Resnick, M.A., and Martin, P., (1976), Repair of double-strand in the nuclear DNA of Saccharomyces cerivisiae and its genetic control. *Molecular and General Genetics*, **143**, 119-129.

Revell, S.H., (1959), The accurate estimation of chromatid breakage, and its relevance to a new interpretation of chromatid aberrations induced by ionizing radiations. *Proceedings of the Royal Society of London* , **150 B**, 563-589.

Savage, J.R.K., (1976), Annotation: Classification and relationships of induced chromosomal structural changes. *Journal of Medical Genetics* , **12,** 103-122.

Sax, K., (1938), Induction of chromosome aberrations in Tradescantia Microspores. *Genetics*, **23**, 494-516.

Sax, K., (1940), An analysis of X-ray induced chromosomal aberrations in Tradescantia. *Genetics,* **25,** 41-68.

Schvartzman, J.B., Chenet, B., Bjerknes, C., and, Van't Hof, J., (1981), Nascent replicons are synchronously joined at the end of S-phase or during G_2-phase in peas. *Biochemica et Biophisica Acta* , **653,** 185-192.

Scudiero, D.A., (1980), Decreased DNA repair synthesis and defective colony-forming ability of ataxia telangiectasia fibroblast cell strains treated with N-methyl- N'-nitro-nitrosoguanidine. *Cancer Research* , **40,** 984-990.

Sedgwick, R.P., (1982), Neurological abnormalities in ataxia-telangiectasia. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia-A cellular and molecular link between cancer, neuropathology and immune deficiency." New York: Wiley, pp 23-35.

Shannon, W.M., (1975), Adenine arabinoside: Antiviral activity in vitru. In: *Adenine Arabinoside; an antiviral agent* . Pavan-Langston, D., Buchanan, R.A., and Alford, C.A., (Eds.). Ravan press, New York. pp 1-43.

Shiloh, Y., and Becker, Y., (1982), Ataxia-telangiectasia lymphoblastoid cell lines resemble non-AT cell lines in their ability to remove MNNG-induced purine methylation adducts. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia-A cellular and molecular link between cancer, neuro- pathology and immune deficiency." New York: Wiley, pp 177-187.

Smith, P.J., and Paterson, M.C., (1983), Effect of aphidicolin on *de novo* DNA synthesis, DNA repair and cytotoxicity in γ-irradiated human fibroblasts. Implications for the enhanced radiosensitivity in ataxia-telangiectasia.

Biochemica et Biophisica Acta, **739**, 17-26.

Smith, P.J., Anderson, C.O., and Watson, J.V., (1985), Abnormal retention of X-irradiated ataxia-telangiectasia fibroblasts in G_2 phase of the cell cycle: Cellular RNA content, chromatin stability and the effects of 3-aminobenzamide. *International Journal of Radiation Biology*, **47**, No. 6, 701-712.

Spector, B.D., Filipovitch, A.H., Perry, G.S., and Kerser, J.H., (1982), Epidemiology of cancer in ataxia-telangiectasia. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia--A cellular and molecular link between cancer, neuropathology and immune deficiency." New York: Wiley, pp 103-138.

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. *Mutation Research*, **95**, 389-404.

Synder, R.D., Carrier, W.L., and, Regan, J.D., (1981), Application of arabinofuranosyl cytosine in the kinetic analysis and quantitation of DNA repair in human cells after ultraviolet irradiation. *Biophysical Journal*, **35**, 339-350.

Taylor, A.M.R., Harnden, D.G., Arlett, C.F., Harcourt, S.A., Lehmann, A.R., Stevens, S., and Bridges, B.A., (1975), Ataxia-telangiectasia; a human mutation with abnormal radiation sensitivity. *Nature* (London), **258**, 427-429.

Taylor, A.M.R., Metcalfe, J.A., Oxford, J.M., and Harnden, D.G., (1976), Is chromatid-type damage in ataxia-telangiectasia after irradiation at G_0 a consequence of defective repair? *Nature* (London), **260**, 441-443.

Taylor, A.M.R., (1978), Unrepaired DNA strand breaks in irradiated ataxia-telangiectasia lymphocytes suggested from cytogenetic observations. *Mutation Research*, **50**, 407-418.

Taylor, A.M.R., Rosney, C.M., and Campbell, J.B., (1979), Unusual sensitivity of ataxia-telangiectasia cells to bleomycin. *Cancer Research*, **39**, 1046-1050.

Taylor, A.M.R., Oxford, J.M., and Metcalfe, J.A., (1981), Spontaneous cytogenetic abnormalities in lymphocytes from thirteen patients with ataxia-telangiectasia. *International Journal of Cancer*, **27**, 311-319.

Taylor, A.M.R., (1982), Cytogenetics of Ataxia-Telangiectasia. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia--A cellular and molecular link between cancer, neuropathology and immune deficiency." New York: Wiley, pp 53-81.

Thierry, D., Rigaud, O., Duranton, I., Moustacchi, E., and Magdelenate, H., (1985), Quantitative measurement of DNA strand breaks and repair in g-irradiated human leukocytes from normal and ataxia-telangiectasia donors. *Radiation Research*, **102**, 347-358.

Van der Schans, G.P., Centen, H.B., and Lohman, P.H.M., (1980), Studies on the repair defect (s) of ataxia-telangiectasia cells. In: *Proceeding to Nato advanced study institute*, Seeberg, E., and Kleppe, k., (Eds), June, 1980, Bergen, Norway.

Van der Schans, G.P., Centen, H.B., and Lohman, P.H.M., (1982 *a*), DNA lesions induced by ionizing radiation. In: Natarajan, A.T., Obe, G., and Altmann, H., (Eds): *"Progress in Mutation Research"* , Vol. **4**, Elsevier Biochemical Press. Amsterdam, Oxford, New York. pp 285-299.

Van der Schans, G.P., Centen, H.B., and Lohman, P.H.M., (1982 *b*), The induction and repair of double-strand DNA breaks in normal human and ataxia-telangiectasia cells exposed to [^{60}Co]-γ-radiation, 4-nitroquinoline-1-Oxide or bleomycin. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia-A cellular and molecular link between cancer, neuropathology and immune deficiency." New York: Wiley, pp 291-303.

Van der Schans, G.P., Paterson, M.C., and Cross, W.G., (1983), DNA strand break and rejoining in cultured human fibroblasts exposed to fast neutrons and gamma-rays. *International Journal of Radiation Biology*, **44,** 75-85.

Vincent, R.A., Sheridan, R.B., and Huang, P.C., (1975), DNA strand breakage in ataxia-telangiectasia fibroblast-like cells. *Mutation Research*, **33,** 357-366.

Waldman, T.A., (1982), Immunological abnormalities in ataxia-telangiectasia. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia-A cellular and molecular link between cancer, neuropathology and immune deficiency." New York: Wiley, pp 37-51.

Waldman, T.A., and McIntyre, K.R., (1972), Serum alpha-fetoprotein levels in patients with ataxia-telangiectasia. *Lancet* **ii,** 1112-1115.

Waldren, C.A., and Johnson, R.T., (1974), Analysis of interphase chromosome damage by means of premature chromosome condensation after X- and ultraviolet-irradiation. *Proceedings of the National Academic Science,* USA, **71,** 1137-1141.

Walwick, E.R., Roberts, W.K., and Dekker, C.A., (1959), Cyclisation during the phosphorylation of uridine and cytidine by polyphosphoric acid. *Proceeding of the Chemical Society*, p 84.

Waters, R., Crocombe, K., and Mirzayans, R., (1981), The inhibition of DNA repair by aphidicoline or cytosine arabinoside in X-irradiated normal and Xeroderma pigmentosum fibroblasts. *Mutation Research*, **94,** 229-234.

Weibezahn, K.F., and Coquerelle, T., (1980), Rejoining kinetics of DNA double-strand breaks in mammalian cells. *Radiation and Environmental Biophysics*, **17,** 351.

Weichselbaum, R.R., Nove, J.T., and Little, J.B., (1978), X-ray sensitivity of diploid fibroblasts from patients with hereditary or sporadic retinoblastoma. *Proceedings of the National Academic Science,* USA, **75,** 3962-3964.

Weichselbaum, R.R., Nove, J.T., and Little, J.B., (1980), X-ray sensitivity of fifty-three human diploid fibroblast cell strains from patients with characterized genetic disorders. *Cancer Research* , **40,** 920-925.

Wist, E., (1979), The role of DNA polymerases, α, β and γ in nuclear DNA synthesis. *Biochemica et Biophisica Acta* , **562,** 62-69.

Yoshida, S., Yamada, M., and Masaki, S., (1977), Inhibition of DNA polymerases α and β of calf thymus by 1-β-D arabinofuranosylcytosine-5'-triphosphate. *Biochemica et Biophisica Acta*, **477,** 144-150.

Zahn, R., Müller, W., Forster, W., Maidhof, A., and Beyer, R., (1972), Action of 1-β-D-arabinofuranosylcytosine on mammalian tumour cells. I. Incorporation into DNA. *European Journal of Cancer*, **8,** 391-396.

Zampetti-Bosseler, F., and Scott, D., (1981), Cell death, chromosome damage and mitotic delay in normal human, ataxia-telangiectasia and retinoblastoma fibroblasts after X-irradiation. *International Journal of Radiation Biology*, **39,** No. 5, 547-558.

Zampetti-Bosseler, F., and Scott, D., (1985), The responce of normal and ataxia-telangiectasia cells to bleomycin: Relationships between chromosome damage, cell cycle delay and cell killing. *Mutation Research*, **151**, 89-94.

Appendix I

Calculation of the Standard Deviation

The standard deviation is the most useful measure of the spread of a distribution of values about the average. It is defined as the square root of the arithmatic average of the squares of the deviations from the mean. The calculation of an unbiased estimate of the standard deviation of a frequency distribution from a sampling of observations proceeds as follows:

Call the number of measurements made n, call an individual measurement X, and call the sum of the measurements ΣX.

a. Calculate the average value, x, of the n measurments: $x = \Sigma X / n$.

b. Subtract each measurment from the mean, square the resulting difference, and sum the squares: $\Sigma\ (x - X)^2$.

c. Divide this result by one less than the number of measurements, to obtain the variance S^2: $S^2 = \Sigma\ (x - X)^2 / (n - 1)$.

d. Take the square root of the variance to obtain the standard deviation, **S**:

$$S = [\Sigma\ (x - X)^2 / (n - 1)].$$

The following method for calculation of the standard deviation is often used:

a. Sum the measurements and the squares of the measurements, ΣX and ΣX^2.

b. Determine the average value, $\Sigma X / n$.

c. Subtract from ΣX^2 the square of the sum divided by n . $\Sigma X^2 - (\Sigma X)^2 / n$. This is the sum of the squares of the deviations from the mean, or sum of squares for short.

d. Divide by one less than the number of measurements to obtain the variance, S^2.

e. Take the square root of the variance to obtain the standard deviation, **S** .

Appendix II

Some parts of this research work has been published or is currently under publication under the following titles:

1. Mozdarani, H. and Bryant, P.E., (1987), **The effect of 9-β-D-arabino furanosyladenine on the formation of X-ray induced chromatid aberrations in X-irradiated G_2 human cells.** ***Mutagenesis*** , **2** , 371-374.

2. Mozdarani, H. and Bryant, P.E., (1988), **Kinetics of chromatid aberrations in G_2 ataxia-telangiectasia cells exposed to X-rays and ara A.** ***International Journal of Radiation Biology*** , In the Press.

The indexed papers are the published one (1987) and the revised version of the second paper (1988) which is currently in the press.

Mutagenesis vol.2 no.5 pp.371–374, 1987

The effect of 9-β-D-arabinofuranosyladenine on the formation of X-ray induced chromatid aberrations in X-irradiated G_2 human cells

Hossein Mozdarani and Peter E.Bryant[1]

Department of Anatomy and Experimental Pathology, University of St Andrews, St Andrews, Fife KY16 9TS, UK

[1]To whom correspondence should be addressed

The effects of the nucleoside analogue 9-β-D-arabinofuranosyladenine (ara A) alone or in combination with X-rays on the induction of G_2-phase chromosomal damage were studied in immortalized human fibroblasts of lung origin (MRC5-SV1). Ara A is known to be an S-phase specific clastogen, a powerful inhibitor of DNA synthesis and an inhibitor of DNA double-strand break (dsb) repair. The length of the G_2-phase of this cell line could be defined as ~4 h from data for treatment of cells with ara A alone in which a sharp rise in number of chromatid aberrations was found to occur when ara A treatment times exceeded 4 h; i.e. when cells were in late S-phase at the time of treatment. The frequency of chromatid deletions in X-irradiated G_2-phase cells was found to decrease as the time between irradiation and fixation increased. This was interpreted as reflecting the underlying repair of dsb. When X-irradiated cells were treated with ara A between irradiation and fixation, the decrease in deletions found after X-rays alone was not observed. This was interpreted as reflecting the inhibition of dsb repair. Unlike deletions, the yield of exchanges increased during incubation of G_2-phase cells after X-ray exposure and the rate of this increase was found to be trebled by the addition of ara A to the medium. We postulate that the increased rate of exchange aberration formation in the absence of dsb repair indicates the existence of a second 'error-prone' misjoining mechanism which is independent of DNA synthesis.

Introduction

The technique of premature chromosome condensation (PCC) has proved valuable for studying the rejoining of breaks induced in chromosomes of cells by X-rays. Cornforth and Bedford (1983) for example showed that the kinetics of rejoining of chromosome breaks in Go human cells closely parallels those observed for repair of dsb in mammalian cells (Bryant and Blocher, 1980; Blocher and Pohlit, 1982). Study of chromatid aberrations in G_2 cells can however provide an alternative to PCC in that the induction and disappearance of chromatid aberrations can be followed as a function of time between irradiation and fixation. The time 'window' for observation of these kinetics is limited to the length of the G_2 phase.

In this paper we describe experiments in which we have investigated the kinetics of the induction and disappearance of chromatid aberrations in irradiated human G_2 cells of fibroblastic origin. We have studied the effects on irradiated G_2 cells of the purine nucleoside analogue 9-β-D-arabinofuranosyladenine (ara A). Ara A is a strong inhibitor of cellular DNA synthesis (Doering *et al.*, 1966; Furth and Cohen, 1968; Muller *et al.*, 1975) and biochemical studies have revealed that on entry into cells ara A is readily converted into the corresponding 5′-mono, di- or triphosphates (Brink and Le Page, 1965). Phosphorylated ara A is thought to compete with dATP at the binding sites on the DNA polymerases where it acts as a potent inhibitor of DNA synthesis (Muller *et al.*, 1975).

Ara A has proved to be interesting from a radiobiological point of view because it was found to potentiate the killing effect of X-rays and lead to the removal of the 'shoulder' region from the survival curve of X-irradiated Ehrlich ascites tumour cells (Iliakis, 1980, 1981). This was interpreted in terms of an inhibition of repair of potentially lethal damage. Ara A has also been shown to enhance the chromosomal damage caused by X-rays (Bryant, 1983, 1984a), an effect which has been related to its strong inhibition of repair of DNA double-strand breaks (dsb) (Bryant and Blocher, 1982). Ara A itself is known to induce chromatid aberrations in human leukocytes (Nichols, 1964) when the cells were treated during the S-phase and in this respect ara A resembles the nucleoside analogue 1-β-D-arabinofuranosylcytosine (ara C) (e.g. Kihlman, 1963; Kihlman *et al.*, 1963).

In this study we have analysed metaphase cells at various times up to 3 h after X-ray exposure so that we were able to monitor the disappearance (rejoining) of breaks in chromatids and the appearance of exchange type chromatid aberrations either in the presence or absence of ara A as a function of time during the G_2 phase.

Materials and methods

Cell culture and ara A treatment

Immortalized human cells of fibroblastic origin (MRC5-SV1) were used. Cells were grown in 75-ml culture flasks (Sterilin) in 10 ml Eagle's MEM medium supplemented with 15% FCS. Cultures of 5×10^5 cells were gassed with 5% CO_2 in air and incubated at 37°C for 48 h (total time before fixation). In the case of combined treatments with X-rays and ara A, cultures were given ara A (200 μM) at 4 h before fixation. Cultures were then exposed to X-rays (in the presence of ara A) at various intervals starting at 1 h after addition of ara A, and ara A remained in the cultures until fixation (Figure 1). Control cultures, not treated with ara A, were irradiated in parallel with ara A treated cultures. In the last hour before fixation all cultures were treated with colcemid at a final concentration of 0.08 μg/ml. Metaphase spreads were prepared by standard methods and stained in Giemsa.

X-irradiation

Irradiation was carried out with a Siemens X-ray therapy unit operating at 250 kV and 14 mA with 0.5 mm Cu filter giving an absorbed dose rate of 0.75 Gy/min. Cells were exposed as a monolayer in plastic flasks (Sterilin) in medium and at room temperature. A flow diagram of the experiment is shown in Figure 1.

Results

On the basis of the assumption that ara A acts as an S-phase specific clastogen (Kihlman *et al.*, 1963) the length of the G_2-phase was determined by treatment of cells for various times before collection with ara A at a concentration of 200 μM (Figure 2). Ara A was added to the medium at various times up to 7 h before fixation. A total of 100 metaphases were analysed for each sample except for the 7 h ara A-treated sample in which only 23 metaphases were found. After incubation with ara A for more than 4 h most of the affected cells were heavily damaged, dele-

Table I. Frequencies of chromatid aberrations per cell: mean frequencies (± standard errors of mean values) at various times after exposure to X-rays in the presence or absence of ara A[a]. Pooled data from three experiments

Treatment	Time before fixation	No. metaphases analysed	Gaps	Deletions (chromatid + isochromatid)	Exchanges
Control	1 h	250	0.31 (±0.11)	0.28 (±0.11)	0.01 (±0.002)
ara A alone[a]	4 h	300	0.54 (±0.02)	1.32 (±0.01)	0.10 (±0.03)
X-rays (2 Gy)	1 h	300	0.79 (±0.47)	9.53 (±1.81)	0.17 (±0.07)
X-rays (2 Gy)	2 h	150[b]	1.53 (±0.52)	8.63 (±0.61)	0.41 (±0.01)
X-rays (2 Gy)	3 h	245	1.62 (±0.52)	5.68 (±0.86)	0.75 (±0.27)
X-rays (2 Gy) + ara A	1 h	300	1.21 (±0.47)	11.77 (±1.62)	0.15 (±0.11)
X-rays (2 Gy) + ara A	2 h	150[b]	1.72 (±1.16)	11.98 (±2.97)	1.13 (±0.30)
X-rays (2 Gy) + ara A	3 h	200	1.52 (±0.61)	12.10 (±1.96)	1.93 (±0.75)

[a]ara A: 9-β-D-arabinofuranosyladenine at 200 μmol/l. For treatments of X-rays + ara A, ara A was added for 1 h before irradiation.
[b]Pooled data from two experiments.

ation with incubation time either in the presence or absence of ara A (Figure 3, closed symbols).

Average percentage mitotic index values taken from data of two experiments were: 2.72 (untreated cells), 1.24 (X-rays alone) and 0.81 (X-rays plus ara A). These results indicate that ara A caused a G_2 delay in addition to that caused by X-rays alone.

Discussion

From the kinetics of disappearance of chromatid breaks (Figure 3) rejoining of these in MRC5-SV1 cells occurred with a half-time of ~2 h. This value is the same as the half-time derived from the initial component of rejoining of chromosome breaks found in PCC experiments of Cornforth and Bedford (1983) using normal human (Go) fibroblasts. This rejoining rate for chromosome and chromatid breaks also corresponds closely to the rate of rejoining of dsb in mouse Ehrlich ascites tumour cells (Bryant and Blocher, 1980; Blocher and Pohlit, 1982). It therefore seems plausible that the rejoining of G_2 chromatid breaks, like that of Go chromosome breaks, reflects the underlying repair of DNA dsb although it should be borne in mind that the observed frequencies of induced chromatid or chromosome breaks are much lower than the frequencies of dsb measured by biochemical means. For example the frequency of breaks measured by PCC in normal Go human fibroblasts (Cornforth and Bedford, 1983) was 6.3 breaks/cell/Gy whereas measurements in mouse Ehrlich ascites tumour cells by neutral gradient velocity centrifugation yielded ~40 dsb/cell/Gy (Blocher, 1982). It thus seems as though only a small proportion of dsb measured by biochemical means are expressed as visible breaks in either chromosomes or chromatids. By implication, therefore, it is possible that chromatid or chromosome breaks observed in G_2 or PCC cells represent a special class of dsb or at least represent dsb in particular regions of the chromatid structure susceptible to rupture; however, a comparison of the half-time of kinetics of rejoining of breaks in prematurely condensed chromosomes (Cornforth and Bedford, 1983) and breaks in chromatids (Figure 3) with biochemical data for dsb repair (e.g. Blocher and Pohlit, 1982) suggests that breaks observable at the chromosomal level are representative of dsb in DNA. This view has recently been reinforced by work using restriction endonuclease induced dsb in DNA of Chinese hamster cells (Bryant, 1984b; Natarajan and Obe, 1984) which has shown that both chromosome and chromatid breaks can arise from specific types of dsb.

When MRC5-SV1 G_2 cells were exposed to ara A between irradiation and mitosis the rejoining of breaks in chromatids was strongly inhibited (Figure 3). The inhibition of rejoining of chromatid breaks by ara A again appears to reflect the response of X-ray induced dsb in the DNA of cells treated with ara A where a strong inhibition of repair was observed (Bryant and Blocher, 1982), and thus also supports the notion that the rejoining of chromatid breaks parallels the repair of dsb. Ara A alone at 200 μM caused only a low frequency of chromatid breaks unless the time of incubation of cells in ara A was increased beyond 4 h (Figure 2).

Our data for chromatid deletions (Figure 3) are therefore in contrast to those of Preston (1980) using human G_2 lymphocytes treated with ara C. Preston showed that the frequency of deletions in G_2 cells increased with time of incubation after irradiation, and interpreted this as indicating that the chromatid breaks were arising exclusively from base-damage in the DNA which was being enzymatically incised. This hypothesis is not supported by our data, although it seems entirely plausible that some aberrations might arise from dsb resulting from base-damage incision and contributing to the general 'pool' of dsb in the cell. Experiments of Ahnstrom and Bryant (1982) suggest that the potential frequency of dsb arising from double-stranded base damage might be 1.7 times the number of direct dsb induced. However, the kinetics and frequency of induction of these enzymatically induced dsb at the dose levels employed in these cytogenetic experiments is unknown.

The frequency of gaps formed after X-rays alone or in combination with ara A was found to be much lower than that for breaks; and the frequency of gaps after both treatments was found to increase slightly during incubation of G_2 cells up to 3 h (Figure 3). The significance of this is not clear. It has been suggested (Bender *et al.*, 1974) that gaps arise from single-strand breaks (ssb) in DNA. Conversion of single-strand base-damaged sites into ssb might thus lead to an increase in these lesions during post-irradiation incubation. If this were the case (for gaps) then treatment with ara A which inhibits DNA synthesis and reduces the rate of ssb repair (Bryant and Blocher, 1982) should lead to an accumulation of gaps in cells treated with this drug. Figure 3 shows that for cells incubated in the presence or absence of ara A the frequency of gaps followed similar kinetics. This indicates that a 'base-damage origin' for gaps is unlikely.

During incubation of cells after irradiation the number of chromatid exchanges increased (Figure 4). The formation of exchanges is thought to result from mis-joining of dsb (Bender *et al.*, 1974; Natarajan *et al.*, 1980; Bryant, 1984a). When X-irradiated G_2 cells were treated with ara A it was found that the frequency of exchange aberrations also increased with time of treatment; moreover the frequency was more than three times higher than the frequency obtained with X-rays alone. This result

Revised Version
Accepted for publication in the
International Journal of Radiation Biology

Kinetics of chromatid aberrations in G_2 ataxia-telangiectasia cells exposed to X-rays and ara A

Hossein Mozdarani and Peter E. Bryant *

Department of Biology and Preclinical Medicine, University of St. Andrews,
St. Andrews KY16 9TS, Fife UK.

* To whom correspondence should be addressed.

Running title: Chromatid aberrations in A-T cells.

Indexing terms: Ataxia telangiectasia, G_2 phase, X-ray induced chromatid aberrations, 9-β-D-arabinofuranosyladenine, DNA double-strand break repair.

Text pages: 12
Figures: 4
Tables: 4

Abstract

The cytogenetic effects of X-rays alone or in combination with 9-β-D-arabino-furanosyladenine (ara A) were studied in an immortalized fibroblastic line of ataxia telangiectasia (A-T) cells. The average length of G_2 in this cell line was determined by autoradiographic labelling (labelled mitoses) to be approximately 5 h. Samples of A-T cells treated with or without ara A, 4 h prior to fixation were irradiated at half hourly intervals, from 1.5 h to 3.5 h before fixation and then examined for the presence of metaphase chromatid aberrations. It is postulated that the kinetics of disappearance (rejoining) of chromatid deletions with post-irradiation incubation time reflects the underlying repair of dsb. This rejoining was found to be inhibited by ara A. Thus the frequency of deletions in the presence of ara A should represent the frequency of deletions in the abse nce of dsb repair. The rejoining kinetics for deletions in A-T was similar to that found in a previous study of normal human fibroblasts (Mozdarani and Bryant 1987). The number of deletions in X-irradiated A-T cells at 1.5 h before fixation was found to be higher by a factor of approximately 2 than that found previously in normal, indicating that in A-T a higher rate of conversion of dsb into chromatid deletions occurs. The frequency of exchanges induced in G_2 A-T cells was similarly enhanced but unlike the situation in normal cells, ara A was found to cause only a slight increase in this frequency.

1. Introduction

Ataxia telangiectasia (A-T) is a human autosomal recessive syndrome characterised by oculocutaneous telangiectasia and cerebellar ataxia (Boder and Sedgwick 1958), diminished inhibition of the initiation of DNA synthesis following X-irradiation (Houldsworth and Lavin 1980, Painter and Young 1980, Lehmann *et al.* 1982); less radiation induced G_2 delay in fibroblast cells compared to normals (Zampetti-Bosseler and Scott 1981, 1985); and hypersensitivity to the clastogenic and cytotoxic effects of ionizing radiation and certain other genotoxic agents such as bleomycin (Rary *et al.* 1974, Taylor *et al.* 1975, 1976, Lehmann and Stevens 1977, 1979, Natarajan and Meyers 1979, Zampetti-Bosseler and Scott 1985). Of particular importance in the present context was the finding that A-T cells exhibited an enhanced frequency of chromosomal aberrations in both G_0 and G_2 phases of the cell cycle (Taylor 1978, 1982, Bender *et al.* 1985, Parshad *et al.* 1985) as well as the unusual occurence of chromatid deletions in cells exposed to X-rays in G_0 (Taylor *et al.* 1976, Taylor 1978).

The molecular basis of the enhanced sensitivity of A-T cells to genotoxic agents is not understood and although evidence with some A-T lines suggests that there may be a defect in excision-repair of base-damaged lesions in DNA (e.g. Paterson *et al.* 1976,

Inoue *et al.* 1977, Smith and Paterson 1983), this is not universally found in A-T lines (Lehmann 1982); moreover several studies have revealed that there is no difference in the induction frequency or rejoining rate of either single- or double-strand breaks in DNA (Taylor *et al.* 1975, Lehmann and Stevens 1977, Fornace and Little 1980, Thierry *et al* 1985).

Recent studies using recombinant DNA techniques (Cox *et al.* 1986, Debenham *et al.* 1987) have indicated that the cellular sensitivity of A-T to X-rays could be caused by a dis-equilibrium between the rejoining and exonuclease degradation of the ends of DNA double-strand breaks (dsb).

We have investigated the high chromosomal sensitivity of A-T by studying the yields and kinetics of chromatid aberrations of both deletion and exchange types induced in the G_2 phase of the cell cycle by X-rays and the influence of the DNA synthesis inhibiting nucleoside analogue 9-β-D-arabinofuranosyladenine (ara A) on the X-ray induced chromatid damage with a view to increasing our understanding of the underlying molecular mechanisms by which chromosomal aberrations are induced.

In a previous study using "normal" human cells (Mozdarani and Bryant 1987) we showed that the number of chromatid deletions in G_2 cells decreased and the number of exchanges increased as a function of time after irradiation. In the presence of ara A the decrease in deletions with time, observed after X-rays alone, was inhibited. These kinetics were thought to reflect the underlying repair and (in the presence of ara A) inhibition of DNA dsb since the rate of disappearance (rejoining) of X-ray induced chromatid deletions paralleled the repair of dsb as measured in other cell lines by biochemical methods (Bryant and Blöcher 1980, Blöcher and Pohlit 1982) and was also similar to the kinetic of rejoining of chromosome breaks in G_0 human cells as determined by the technique of premature chromosome condensation (PCC) (Cornforth and Bedford 1983, 1985); moreover ara A has been shown to inhibit repair of DNA dsb (Bryant and Blöcher 1982). In contrast to the kinetics of disappearance of deletions, the frequency of exchanges in normal cells was found to increase with time after X-ray exposure and this increase was threefold larger in the presence of ara A than its absence. For this reason it was postulated (Mozdarani and Bryant 1987) that the molecular mechanism for mis-joining chromosomes may be different from that for the repair of dsb since as mentioned above, the latter process is known to be strongly inhibited by ara A (Bryant and Blöcher 1982).

In the present work we have extended our studies of G_2 cells to investigate the effects of X-rays alone or in combination with ara A on A-T cells at various intervals up to 3.5 hours after X-ray exposure. Using 0.5 h sampling intervals we monitored the disappearance (rejoining) of chromatid breaks and formation of exchanges.

2. Materials and Methods

2.1. Cell culture and treatment with ara A

The SV40 immortalized fibroblastic ataxia telangiectasia line AT5BIVA (A-T) was used (obtained from Dr D. Scott, Paterson Institute for Cancer Research, Manchester). Cells were maintained by weekly passage from $4x10^5$ cells/ flask (75 cm^2) in Eagle's minimal essential medium + 15% FCS (MEMFCS). For experiments, cells were set up at $4x10^5$ cells/flask (75 cm^2) and incubated for 48 hours at 37° C (total time including the treatment and incubation time before fixation). The experimental protocol was essentially the same as we have reported previously (Mozdarani and Bryant 1987). In the case of combined treatment with ara A and X-rays, cultures were treated with ara A (Sigma) at a final concentration of 200 µmol/l, 4 h before fixation. After adding ara A, cells were X-irradiated in the presence of ara A at various times from 3.5 h until 1.5 h before collection. Thus ara A remained in the cultures for 4 h; until collection. Control cultures (without ara A) were X-irradiated in parallel to those treated with ara A. Before fixation, cells were exposed to colcemid at a final concentration of 0.08 µg/ml and incubated for 1.5 h; thus for the shortest time interval (1.5 h) cells were exposed to colcemid (in the presence or absence of ara A) immediately following X-irradiation, until fixation. Collection and fixation of cells and preparation of metaphase chromosomes was carried out by standard methods and preparations were stained in Giemsa (3%).

2.2 X-irradiation

Irradiation was carried out with a Siemens X-ray therapy unit operating at 250 KV and 14 mA with 0.5 mm Cu filter giving an absorbed dose rate at the position of the samples of 0.75 Gy/min. Doses were checked using an energy independent ferrous sulphate method (Frankenberg 1969). Cells were exposed as mono-layers in flasks in medium and at room temparature to a dose of 1 Gy.

3.3 Mitotic labelling

Sub-cultures of A-T cells were set up at $2.5x10^5$ per 5 cm diameter petri dish with 5 ml MEMFCS. Cultures were incubated for 48 hours prior to labelling to ensure that cells were in exponential growth. Then medium was removed and replaced with fresh MEMFCS containing 37 KBq (1 µCi) ^{3}H-TdR per ml with specific activity of 1.48 TBq/mmol (40 Ci/mmol). Culture dishes were incubated at 37° C and one sampled every 1 h up to 10 hours. After sampling, cells were immediately trypsinised and treated with 0.075 mol/l KCl. Cells were prepared by the same methods as that for chromosome preparations. After drying, slides were dipped in K2 autoradiographic emultion (Ilford),

left at 4° C for one week and developed with D19b developer for 3.5 min and fixed in Kodak acid fixative for 5 mins. Slides were stained in 3% Giemsa. 100 metaphases were scored for each sample.

2.4 Scoring Procedure

Chromatid aberration scoring was based on the classifications of Buckton and Evans (1981) and Savage (1975). For the line of A-T cells used (AT5BIVA) which are hypo-triploid, their chromosome number lying between 50-62, only metaphases with more than 50 chromosomes were scored. Lesions smaller than the width of a chromatid were scored as gaps (achromatic lesions) and those wider than the width of a chromatid, deletions. In the data presented, the category of chromatid deletions included iso-chromatid deletions which were scored as one deletion. Exchanges included all forms of chromatid exchanges.

In order that a meaningful comparison between data for AT5BIVA (hypo-triploid) and some of our previous data (Mozdarani and Bryant 1987) for MRC5SVI (tetraploid), the results were expressed in terms of aberrations per 100 chromosomes.

3. Results

On the basis of an autoradiographic experiment (Figure 1) where labelled mitoses were scored from samples taken at intervals of 1 h, the duration of the G_2 phase was estimated to be approximately 5 h for A-T cells since the total time for G_2 + 1/2 M was measured to be about 5.5 h. This result is similar to those obtained by Zampetti-Bosseler and Scott 1981) for a primary A-T fibroblastic cell line (5 h) and similar to the value of 4 h for immortalized "normal" human fibroblasts reported previously (Mozdarani and Bryant 1987). In previous experiments involving treatment of the MRC5SVI cells with ara A alone at various times before collection (Mozdarani and Bryant 1987) it was determined that a 4 h interval before collection was the optimum time for ara A treatment in order to minimize the "background" of aberrations due to ara A alone; these aberrations originating as a result of the interference of ara A with DNA replication. In experiments with A-T cells therefore, the overall treatment time was similarly limited to 4 h before collection.

Figure 2 shows frequencies of gaps and deletions in experiments in which A-T cells were exposed to X-rays alone or to X-rays and ara A and sampled at intervals up to 3.5 h. In the cells exposed to X-rays alone the frequency of deletions decreased exponentially with increasing post-irradiation incubation time, indicating rejoining. The data for rejoining of breaks for both MRC5SVI and AT5BIVA cells (Figures 2 and 3) followed first order (exponential) kinetics which could be fitted by the expression:

$$Y = a \cdot e^{-\lambda t} \qquad (1)$$

Where Y represents the number of breaks remaining after a repair time t, a represents the number of breaks induced initially, and λ represents the rate constant. Regression analysis of data in figure 3 gave values of a = 21.0 and a = 8.5 for A-T and normal cells respectively and λ = -0.223 and λ = -0.258 for A-T and normal cells respectively. This gave a value of the half-time for repair of breaks of 2.7 h for normal and 3.1 h for A-T cells. The frequency of deletions in X-irradiated A-T cells (see also Table I) was 14.7 deletions per 100 chromosomes for 1 Gy at the shortest sampling time (1.5 h). In cells exposed to 1 Gy of X-rays in the presence of 200 µmols/l ara A (Figure 2 and Table I) the frequency of deletions remained constant at a value of approximately 22 deletions per 100 chromosomes for the various intervals between irradiation and fixation (Figure 2). In figure 3 data for deletions induced by 1 Gy of X-rays given at various intervals before fixation to A-T or normal human fibroblasts is extrapolated back to time zero on the assumption that the same kinetics pertain at shorter times. This shows that whereas for normal (MRC5SVI) cells these lines extrapolate to a common point of approximately 8 deletions per 100 chromosomes (values of ferquency of deletions for MRC5SVI cells has been halved to correct for the X-ray dose used (2 Gy) on the assumption that deletions are induced approximately linearly with dose in the low dose range), an assumption validated by data (Table III) obtained in recent studies (Mozdarani and Bryant in preparation) at a 1 Gy dose of X-rays.

In contrast to the results for deletions, the data for exchanges in the A-T cells treated with X-rays in the presence or absence of ara A (Figure 4, lower panel) showed that in both cases the frequency of exchanges gradually increased with increasing post-irradiation incubation time. In A-T cells ara A appeared to induce a small, but not statistically significant (see also table I) increase in exchanges. This increase in exchanges with time could not be attributed to a reduction in G_2 delay as has been found for caffeine (Hansson *et al.* 1984). The mitotic index (Table IV) shows that in contrast, ara A led to a reduction in the mitotic index indicating an enhanced delay in both lines. Figure 4, upper panel shows data recalculated from Mozdarani and Bryant (1987) for normal human (MRC5SVI) cells (Table II) on the assumption that the induction of exchanges at doses up to 2 Gy is an approximate linear function of dose. This assumption is validated by recent data for normal cells at 1 Gy (Table III) obtained in a separate study (Mozdarani and Bryant in preparation). On this basis the data for X-irradiated samples in the absence of ara A (Table I) suggests that a higher frequency of exchanges in A-T than in normals (Table II). However in the presence of ara A, the large enhancement of exchange frequencies observed in normals (Figure 4, upper panel) was not seen in A-T cells. In normal cells (Table II) treated with ara A, 2.7 times more exchanges were seen after 2 h between irradiation and fixation, whereas in A-T cells treated with ara A this factor was

1.06 at 2 h and the highest factor at 3.5 h was 1.25 times more than in its absence. Yields of gaps (achromatic lesions) were similar in cells exposed to X-rays alone or X + ara A (Figure 2) and decreased slightly with post-irradiation incubation time. A 3-4 fold lower frequency of gaps (after correction for dose) was observed in normal cells as compared with A-T cells, and remained almost constant for all sampling times (Table II).

4. Discussion

Despite the accumulation of data on the radiation cytogenetics of A-T cells, the nature of the chromosomal hypersensitivity of these cells to ionizing radiation and other DNA damaging agents is not yet understood. Biochemical evidence indicates no difference in the rate of induction or rejoining of double-strand breaks in normal and A-T cells. However, based on chromosomal aberration studies Taylor (1978) concluded that despite biochemical evidence for normal repair of DNA breaks in A-T cells, about 1 to 3 percent of DNA dsb induced may not rejoin in A-T cells compared to approximately 0.3 percent in normal cells. This hypothesis appears to be supported by the data for PCC fragments in normal and A-T lymphocytes irradiated in G_0 (Cornforth and Bedford 1985), where after similar initial rejoining kinetics, the frequency of breaks in A-T cells plateaued out at a higher level (factor of about 10) than in normal lymphocytes. In previous work (Mozdarani and Bryant 1987) we showed that the kinetics of rejoining of chromatid breaks were similar to those for the rejoining of dsb measured by biochemical methods using mammalian cells (e.g. Bryant and Blocher 1980, Blocher and Pohlit 1982) and also to the kinetics of rejoining of fragments in PCC experiments of Cornforth and Bedford (1983) using normal human fibroblasts. We therefore assume that the rejoining of chromatid breaks in A-T cells (Figure 2), like that of PCC fragments, reflects the underlying repair of DNA dsb. The experimental protocol for study of the kinetics of chromatid aberrations in A-T cells was similar to that reported previously for normal cells (Mozdarani and Bryant 1987) with the difference that the earliest X-irradiation was carried out at 0.5 h after addition of ara A and repeated every 0.5 h to monitor in more detail the kinetics of X-ray induced aberrations in the presence or absence of ara A (Figure 2). Ara A was applied to cells 0.5 h before irradiation to allow enough time for the nucleoside be phosphorylated to the nucleotide triphosphate level so that it could compete with the dATP at the polymerase level. Thus although the time window in this system was limited to less than the duration of G_2 which was about 5 h, it was possible by irradiating cells at different intervals before fixation to monitor the rejoining of chromatid breaks.

Measurements of mitotic index (Table IV) confirm that less G_2 delay occured in X-irradiated A-T cells than in normals in line with previous observations (Zampetti-Bosseler and Scott 1981, Hansson *et al.* 1984). It is possible that this effect

and the G_2 blockage effects described by Ford *et al.* (1984) could influence the frequencies and kinetics of deletions in the two lines however, similar kinetics of rejoining of chromatid breaks in A-T and normals were observed (Figures 2 and 3) after X-irradiation where the half time (t1/2) of rejoining was found to be 2.7 h for normal and 3.1 h for A-T cells. This presumably reflects the similar rate of repair of dsb observed for A-T and normal cells (e.g. Lehmann and Stevens 1977) and parallels the initial rate of rejoining of PCC fragments in A-T and normal cells (Cornforth and Bedford 1985). If the difference in G_2 delay in the two lines played a key role in the determination of the kinetics of deletions, we might expect markedly different kinetics for deletions in the two lines. Moreover the distributions of numbers of deletions per cell for each treatment time in the two cell lines were found to be very similar (H. Mozdarani, unpublished observations). This similarity suggests that the frequencies of deletions in the two lines were not influenced to any great extent by the differences in G_2 delay. Although the frequency of chromatid breaks in X-irradiated A-T cells was 14.7 per 100 chromosomes after 1 Gy (approximately 7/cell/Gy) this dose not reflect the total number of dsb per cell/Gy measured by neutral gradient velocity centrifugation which in murine Ehrlich ascites tumour cells was found to be about 40 dsb per Gy per cell (Blöcher 1982). Similarly, the frequency of breaks measured by PCC in G_0 human normal and A-T fibroblasts was less than that measured by biochemical methods and was found to be approximately 6 breaks/Gy/cell (Cornforth and Bedford 1983, 1985). The values of frequencies of deletions in G_2 cells should actually be halved when compared with G_0 or G_1 cells, since G_2 cells contain twice the amount of DNA.

Using the "G_2 system" in normal cells (Table II) we previously measured chromatid breaks after 2 Gy. It is clear from this data that after correction for the difference in dose, a higher level of deletions occurs per 100 chromosomes in X-irradiated A-T cells than in the "normal" line. thus the A-T line yielded 14.7 deletions per 100 chromosomes for 1 Gy (at 1.5 h after irradiation) whereas MRC5SVI (when a dose correction is made; see results section 3) yielded approximately 7 deletions/100 chromosomes for 1 Gy (at 1 h after irradiation). The validity of this correction is corroborated by recent data (Mozdarani and Bryant in preparation; see Table III) with MRC5SVI cells which shows that at 1.5 h after irradiation and a dose of 1 Gy about 9 deletions per 100 chromosomes were observed. this difference in frequencies of deletions between A-T and normals at short times after irradiation is quite different from that presented by data for PCC fragments (Cornforth and Bedford 1985) where at time zero (actually 30 min after X-ray exposure), equal number of fragments (about 6/cell/Gy) were observed in the two cell lines. Assuming not more than 30% of breaks rejoin in the first 1-1.5 h after X-ray exposure (from data in figure 1, table II, and equation 1) then it would appear that G_2 A-T cells convert more dsb into chromatid breaks soon after irradiation. this is clear also

from the extrapolated values of deletions at time zero in figure 3 (about 22 deletions/ 100 chromosomes in A-T and about 9 deletions/100 chromosomes in normal cells). This effect was also demonstrated by Bender *et al.* (1985) who showed that approximately 4 times more deletions were observed for the same dose (0.5 Gy) in A-T than in normal human lymphocytes based on deletions scored at 1.5 h.

Thus our data (and those of Bender *et al.* 1985) for G_2 are essentially different from those of Taylor (1978) and Cornforth and Bedford (1985) where it was postulated that A-T cells yield higher than normal levels of breaks or fragments as a result of residual (or unrepaired) dsb. Our data shows that despite equal levels of initially induced dsb in A-T and normal lines (Lehmann and Stevens 1977), the chromatid breaks which result from these dsb were at least twice more frequent in A-T than in normal cells. We therefore interpret our data for deletions as indicating that A-T cells convert a higher number of dsb into chromatid deletions than do normal cells. The mechanism for this conversion is at present unknown.

For X-irradiated A-T cells treated with ara A (Figure 2), the frequency of deletions was found to be approximately constant for all post-irradiation intervals, which was interpreted as the result of inhibition of dsb repair by ara A, thus probably representing the frequency of deletions at time zero. At 1.5 h interval prior to fixation, the number of X-ray induced deletions in presence of ara A was higher than that of X-rays alone: 21 deletions as against 15 deletions per 100 chromosomes respectively (Table I, Figure 2). however the data for X-rays alone and X + ara A were found to extrapolate to a common point based on first-order kinetics.

The frequency of exchanges in X-irradiated A-T cells increased with increasing post-irradiation incubation time (Figure 4) as found previously for MRC5SVI cells (Mozdarani and Bryant 1987). However the increase in exchanges caused by ara A treatment was not so striking in A-T cells as in normal MRC5SVI cells (Table II). The reasons for this are not understood. Exchanges are thought to result from misjoining of dsb (Bender *et al.* 1974, Natarajan *et al.* 1980, Bryant 1984), therefore the number of dsb available and incubation time are two important factors for formation of exchanges. It appears therefore that in A-T, ara A has a less pronounced effect than in normal cells. In normal cells Mozdarani and Bryant (1987) showed that in the presence of ara A a 3 fold increased frequency of exchanges occured in irradiated G_2 cells. Because ara A inhibits repair of dsb, the formation of exchanges would be likewise inhibited if the same mechanism applied, so that it was postulated that the mechanism for the mis-joining of dsb yielding chromosomal exchanges may be different from that for repair of dsb. The data presented here is not inconsistent with this notion, although the enhancement of yields of exchanges in A-T cells by ara A is not so pronounced as in normal cells.

In conclusion our data for these two cell lines (MRC5SVI and AT5BIVA) show that although the kinetics of rejoining of deletions is the same in G_2 A-Tcells as in normals,

A-T cells appear to convert more X-ray induced dsb into chromatid deletions. The frequency of exchanges was also increased, but in contrast to its effect in normal cells, in A-T cells ara A was found not to increase the frequency of exchanges significantly. It is possible that the high conversion of dsb into chromatid deletions could contribute to the enhanced cellular sensitivity of A-T cells to ionizing radiation.

Acknowledgements

HM is supported by a grant from the Ministry of Culture and Higher Education of The Islamic Republic of Iran. Work in the laboratory of PB is supported by funds from Cancer Research Campain.

References

BENDER, M.A., GRIGGS, H.G., and BEDFORD, J.S., 1974, Mechanisms of chromosomal aberration production: III. Chemicals and ionizing radiation. *Mutation Research,* **23,** 197-212.

BENDER, M.A., RARY, J.M., and KALE, R.P., 1985, G_2 chromosomal radiosensitivity of ataxia telangiectasia lymphocytes. *Mutaion Research,* **152,** 39-47.

BLÖCHER, D., 1982, DNA double-strand breaks in Ehrlich ascites tumour cells at low doses of X-rays. I- Determination of induced breaks by centrifugation at reduced speed. *International Journal of Radiation Biology,* **42,** 317-328.

BLÖCHER, D. and POHLIT, W., 1982, DNA double-strand breaks in Ehrlich ascites tumour cells at low doses of X-rays: can cell death be attributed to double strand breaks? *International Journal of Radiation Biology* , **42**, 329-338.

BODER, E., and SEDGWICK, R.P., 1958, Ataxia-telangiectasia; A familial syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and frequent pulmonary infection. *Pediatrics*, **21,** 526-554.

BRYANT, P.E., 1984, Enzymatic restriction of mammalian cell DNA using Pvu II and Bam H1: evidence for the double strand break origin of chromosomal aberrations. *International Journal of Radiation Biology* , **46,** No. 1, 57-65.

BRYANT, P.E., and BLÖCHER , D., 1980, Measurment of the kinetics of DNA double- strand break repair in Ehrlich ascites tumour cells using the unwinding method. *International Journal of Radiation Biology* , **38,** 335-347.

BRYANT, P.E., and BLÖCHER, D., 1982, The effects of 9-β-D arabinofuranosyladenine on the repair of DNA strand breaks in X-irradiated Ehrlich ascites tumour cells. *International Journal of Radiation Biology* , **42,** No. 4, 385-394.

BUCKTON, K.E., and EVANS, H.J., 1981, Human peripheral blood lymphocyte cultures. An *in vitro* assay for the cytogenetic effects of environmental mutagens. In Hsu,T.C. (Ed), "*Cytogenetic assays of environmental mutagens* ". pp 183-199.

CORNFORTH, M.N., and BEDFORD, J.S., 1983, High resolution measurment of breaks in prematurely condenced chromosomes by differential staining. *Chromosoma* (Berlin), **88,** 315-318.

CORNFORTH, M.N., and BEDFORD, J.S., 1985, On the nature of a defect in cells from individuals with ataxia-telangiectasia. *Science*, **227**, 1589-1591.

COX, R., DEBENHAM, P.G., MASSON, K.W., and WEBB, M.B.T., 1986, Ataxia-telangiectasia: A human mutation giving high-frequency misrepair of DNA double-strand scissions. *Molecular Biology and Medicine*, **3**, 229-244.

DEBENHAM, P.G., WEBB, M.B.T., JONES, N.J., and COX R., 1987, Molecular studies on the nature of the repair defect in ataxia-telangiectasia and their implications for cellular radiobiology. *Journal of Cell Science*, **Suppl. 6**, 177-189.

FORD, M.D., MARTIN, L., and LAVIN, M.F., 1984, The effects of ionizing radiation on cell cycle progression in ataxia-telangiectasia. *Mutation Research*, **125**, 115-122.

FORNACE, A.J., and LITTLE, J.B., 1980, Normal repair of DNA single-strand breaks in patients with ataxia telangiectasia. *Biochemica et Biophisica Acta* , **607**, 432-437.

FRANKENBERG, D., 1969, A ferrous sulphate dosemeter independent of photon energy in the range from 25 Kev up to 50 Mev. *Physics in Medicine and Biology* , **14**, No. 4, 597-605.

HANSSON, K., NATARAJAN, A.T., and KIHLMAN, B.A., 1984, Effect of caffeine in G_2 on X-ray-induced chromosomal aberrations and mitotic inhibition in ataxia telangiectasia fibroblast and lymphoblastoid cells. *Human Genetics*, **67**, 329-335.

HOULDSWORTH, J., and LAVIN M.F., 1980, Effect of ionizing radiation on DNA synthesis in ataxia-telangiectasia. *Nucleic Acids Research*, **8**, 3709-3720.

INOUE, T., HIRANO, K., YOKOIYAMA, A., KADA, T., and KATO, H., 1977, DNA repair enzymes in ataxia telangiectasia and Bloom's syndrome fibroblasts.*Biochemica et Biophisica Acta*, **479**, 497-500.

LEHMANN, A.R., 1982, The cellular and molecular responses of ataxia-telangiectasia cells to DNA damage. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia--A cellular and molecular link between cancer, neuropathology and immune-deficiency." New York: Wiley, pp 83-101.

LEHMANN, A.R., and STEVENS, S., 1977, The production and repair of double strand breaks in cells from normal humans and from patients with ataxia telangiectasia. *Biochemica et Biophisica Acta*, **474**, 49-60.

LEHMANN, A.R., and STEVENS, S., 1979, The response of ataxia-telangiectasia cells to bleomycin. *Nucleic Acids Research*, **6**, 1953-1960.

LEHMANN, A.R., JAMES, M.R., and STEVENS, S., 1982, Miscellaneous observations on DNA repair in ataxia-telangiectasia. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia--A cellular and molecular link between cancer, neuropathology and immune-deficiency." New York: Wiley, pp 347-353.

MOZDARANI, H., and BRYANT, P.B., 1987, The effect of 9-β-D-arabinofuranosyladenine on the formation of X-ray induced chromatid aberrations in X-irradiated G_2 human cells. *Mutagenesis*, **2**, 371-374.

NATARAJAN, A.T., and MEYERS, M., 1979, Chromosomal radiosensitivity of ataxia telangiectasia cells at different cell cycle stages. *Human Genetics* , **52**, 127-132.

NATARAJAN, A.T., OBE, G., van ZEELAND, A.A., PALITTI, F., MEIJERS, M., and ELLY VERDEGAAL-IMMERZEEL, A.M., 1980, Molecular mechanisms involved in the production of chromosomal aberrations. II- Utilization of Neurospora endonuclease for the study of aberration production by X-rays in G_1 and G_2 stages of the cell cycle. *Mutation Research* , **69**, 293-305.

PAINTER, R.B., and YOUNG, B.R., 1980, Radiosensitivity in ataxia-telangiectasia: A new explanation. *Proceeding of National Academic Science*, USA, **77**, No. 12, 7315-7317.

PARSHAD, R., SANFORD, K.K., JONES, G.M., and TARONE, R.E., 1985, G_2 chromosomal radiosensitivity of ataxia-telangiectasia heterozygotes. *Cancer, Genetics and Cytogenetics*, **14**, 163-168.

PATERSON, M.C., SMITH, B.P., LOHMAN, P.H.M., ANDERSON, A.K., and FISHMAN, L., 1976, Defective excision repair of g-ray-damaged DNA in human (ataxia-telangiectasia) fibroblasts. *Nature* (London), **260,** 444-446.

RARY, J.M., BENDER, M.A., and KELLY, T.E., 1974, Cytogenetic studies of ataxia-telangiectasia. *American Journal of Human Genetics*, **26**, 70A.

SAVAGE, J.R.K., 1975, Annotation: Classification and relationships of induced chromosomal structural changes. *Journal of Medical Genetics* , **12,** 103-122.

SMITH, P.J., and PATERSON, M.C., 1983, Effect of aphidicolin on *de novo* DNA synthesis, DNA repair and cytotoxicity in γ-irradiated human fibroblasts. Implications for the enhanced radiosensitivity in ataxia-telangiectasia. *Biochemica et Biophisica Acta*, **739,** 17-26.

TAYLOR, A.M.R., HARNDEN, D.G., ARLETT, C.F., HARCOURT, S.A., LEHMANN, A.R., STEVENS, S., and BRIDGES, B.A., 1975, Ataxia-telangiectasia; a human mutation with abnormal radiation sensitivity. *Nature* (London), **258,** 427-429.

TAYLOR, A.M.R., METCALFE, J.A., OXFORD, J.M., and HARNDEN, D.G., 1976, Is chromatid-type damage in ataxia-telangiectasia after irradiation at G_0 a consequence of defective repair? *Nature* (London), **260,** 441-443.

TAYLOR, A.M.R., 1978, Unrepaired DNA strand breaks in irradiated ataxia-telangiectasia lymphocytes suggested from cytogenetic observations. *Mutation Research*, **50**, 407-418.

TAYLOR, A.M.R., 1982, Cytogenetics of Ataxia-Telangiectasia. In Bridges, B.A. and Harnden, D.G. (Eds): "Ataxia-telangiectasia-A cellular and molecular link between cancer, neuropathology and immune deficiency." New York: Wiley, pp 53-81.

THIERRY, D., RIGAUD, O., DURANTON, I., MOUSTACCHI, E., and MAGDELENAT, H., 1985, Quantitative measurement of DNA strand breaks and repair in γ–irradiated human leukocytes from normal and ataxia-telangiectasia donors. *Radiation Research*, **102,** 347-358.

ZAMPETTI-BOSSELER, F., and SCOTT, D., 1981, Cell death, chromosome damage and mitotic delay in normal human, ataxia-telangiectasia and retinoblastoma fibroblasts after X-irradiation. *International Journal of Radiation Biology* , **39,** No. 5, 547-558.

ZAMPETTI-BOSSELER, F., and SCOTT, D., 1985, The response of normal and ataxia-telangiectasia cells to bleomycin: Relationships between chromosome damage, cell cycle delay and cell killing. *Mutation Research*, **151**, 89-94.

Fig. 1.

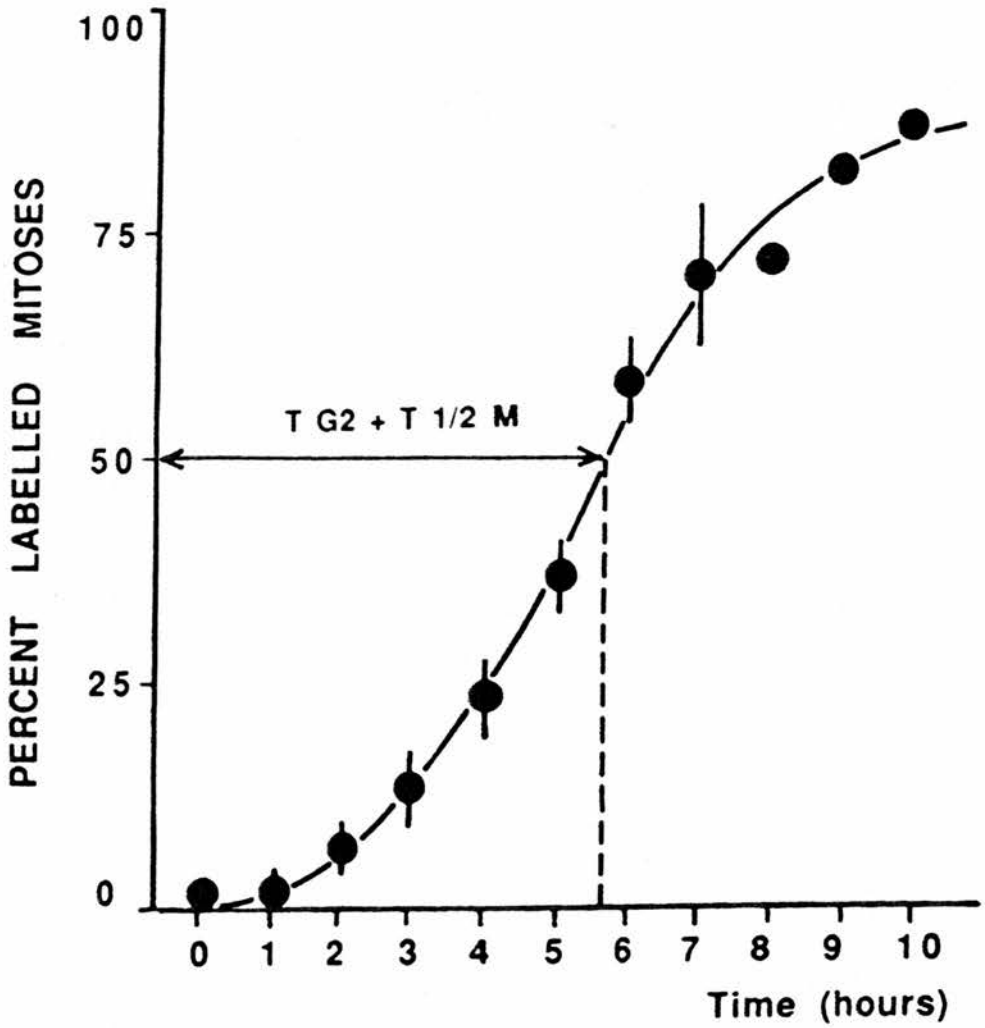


Figure 1. Percentage of labelled mitoses as a function of time before fixation in A-T cells. The arrows and dashed line indicate the length of G_2 + M phases. Error bars indicate standard errors of mean values of two independent experiments.

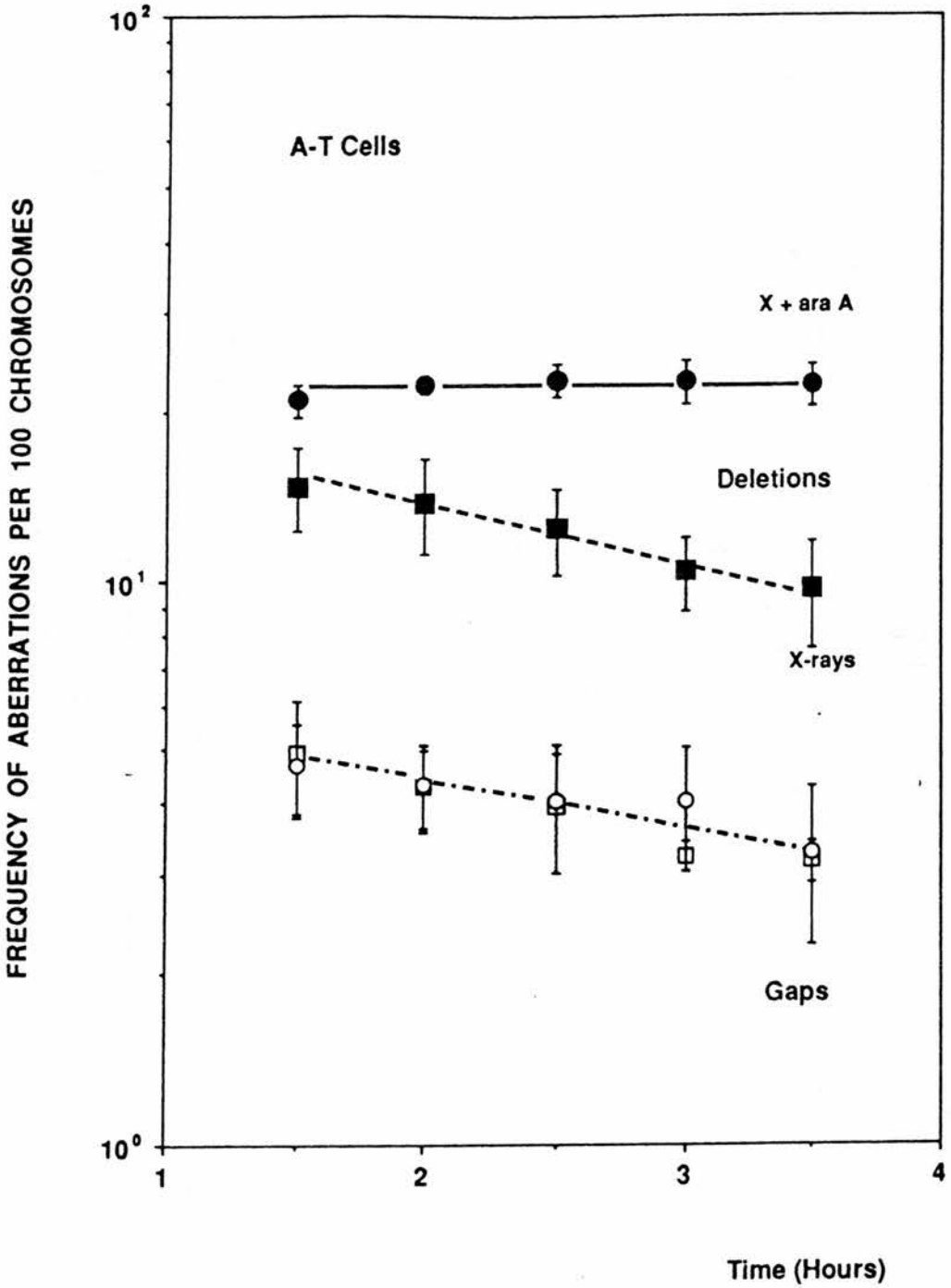


Figure 2. Frequency of deletions and gaps (achromatic lesions) as a function of time between exposure to X-rays (1 Gy), alone or in combination with 200 μmols/l ara A, before fixation in A-T cells. Error bars indicate standard error of mean values of three independent experiments.

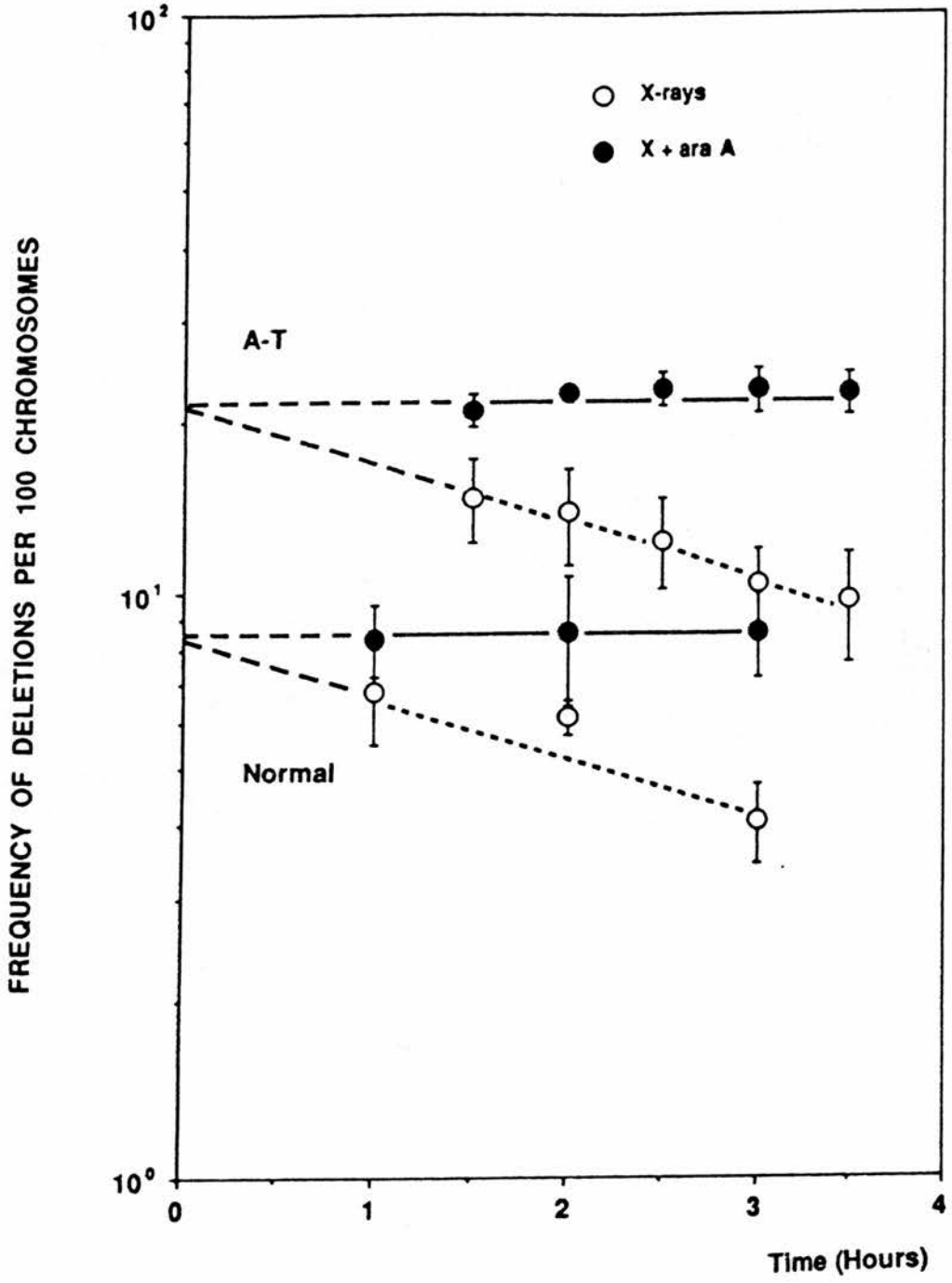


Figure 3. Frequencies of deletions in A-T and normal (MRC5SVI) cells as a function of time between X-irradiation and fixation. Open points represent data for X-rays alone; closed points represent data for X-rays + ara A (200 µmols/l). Data for MRC5SVI cells, originally for 2 Gy exposure (Mozdarani and Bryant 1987) has been recalculated as frequency of deletions per 100 chromosomes and for a dose of 1 Gy on the assumption of linear induction over the range 0-2 Gy and replotted on a semi-logarithmic scale. Error bars indicate standard errors of mean values of three independent experiments.

Fig 4.

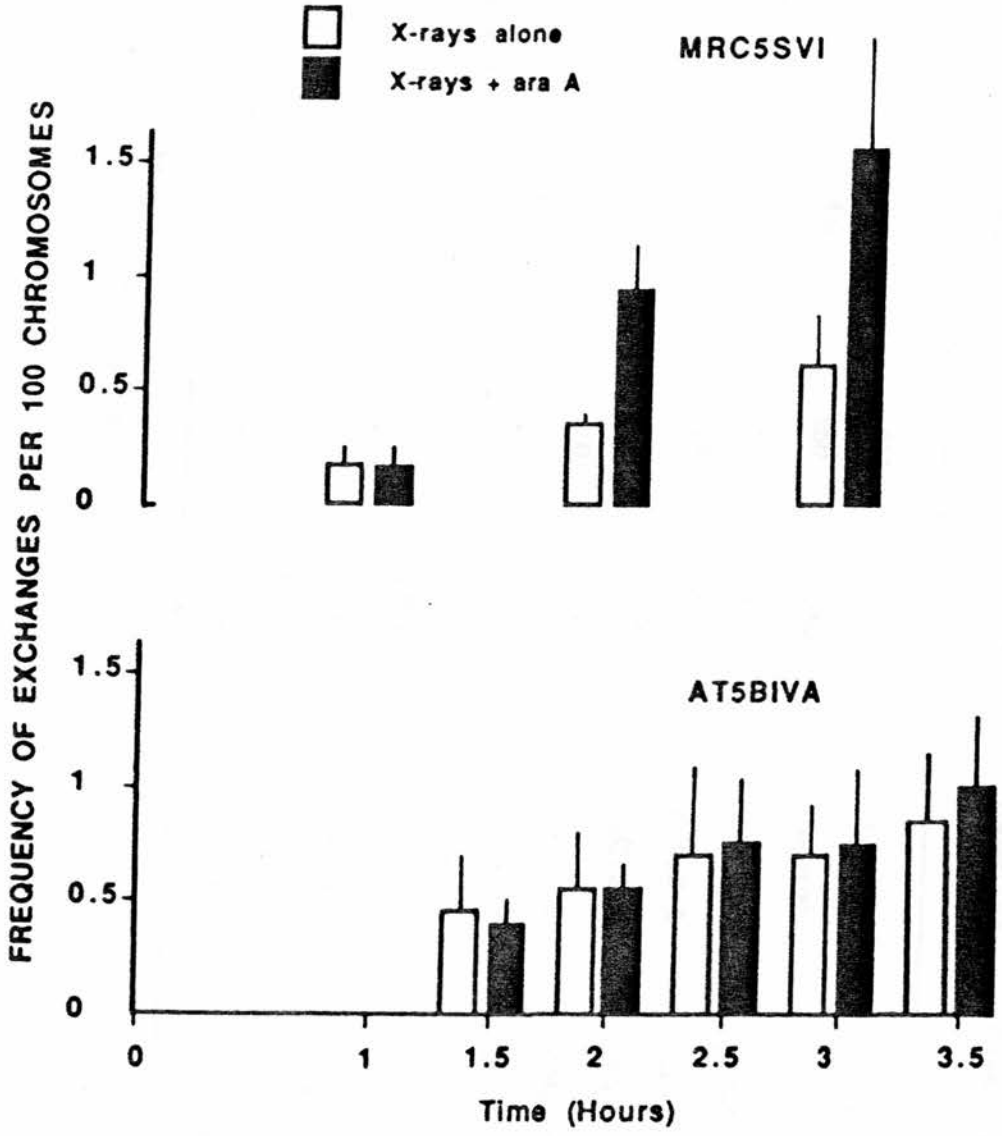


Figure 4. Frequency of chromatid exchanges in human cells at various times between irradiation and fixation. Lower panel: data for A-T cells (From table I) induced by exposure to 1 Gy of X-rays alone or in combination with ara A at 200 μmols/l. Top panel: data of Mozdarani and Bryant (1987) for normal cells, originally for a 2 Gy exposure, recalculated as frequency of exchanges per 100 chromosomes and for a dose of 1 Gy on the assumption of approximate linearity of induction in the dose range 0-2 Gy. Error bars indicate standard errors of mean values of three independent experiments.

Table II : **Yields of chromatid aberrations in X-irradiated (2Gy) normal human (MRC5SVI) cells in presence and absence of 200 μ mols/l ara A. (Data from Mozdarani and Bryant (1987) recalculated in terms of chromatid aberrations per 100 chromosomes).**

Treatment	Time of irradiation before fixation	No of cells analysed $	Aberration per 100 Chromosomes		
			Gaps	Deletions	Exchanges
Control		250	0.46±0.15	0.42±0.16	0.02±0.007
Ara A alone	4 h	300	0.62±0.22	1.11±0.01	0.13±0.04
X-ray	1 h	300	1.13±0.08	13.60±2.59	0.24±0.10
X-ray	2 h	150**	2.19±0.74	12.32±0.87	0.59±0.02
X-ray	3 h	245	2.31±0.74	8.11±1.27	1.07±0.38
X-ray+ara A @	1 h	300	1.73±0.67	16.82±2.31	0.21±0.15
X-ray+ara A	2 h	150**	2.44±1.66	17.10±4.24	1.61±0.42
X-ray+ara A	3 h	200	2.16±0.91	17.27±2.80	2.75±1.07

** Pooled data of two experiments

@ Ara A was added to the culture medium 1h before the first X-irradiation

$ Pooled data of three independent experiments

Errors are standard errors of mean values.

Table III: Yields of chromatid aberrations in X-irradiated (1 Gy) G2 normal (MRC5SVI) cells.

Treatmet	Time of irradiation before fixation (h)	No.of cells* analysed	Aberrations per 100 Chromosomes		
			Gaps	Deletions	Exchanges
Control		250	3.38 ± 0.14	0.38 ± 0.11	0.06 ± 0.01
X-rays	1.5	200	2.92 ± 0.13	9.24 ± 0.32	0.46 ± 0.10
X-rays	2.5	250	2.96 ± 0.41	8.70 ± 0.15	0.58 ± 0.02
X-rays	3.5	250	2.06 ± 0.04	5.22 ± 0.25	0.71 ± 0.09

* Pooled data of three independent experiments

Errors are standard errors of mean values

Table IV:
Mitotic index with standard errors (Results of two experiments)

Treatment time in hour before fixation	Mitotic index X-rays	Mitotic index X-ray+ara A
A-T cells (1 Gy) :		
1.5	4.40 ± 1.27	3.10 ±0.28
2.0	4.68 ± 0.64	2.79 ± 0.37
2.5	4.05 ± 0.64	3.20 ± 0.32
3.0	4.58 ± 0.16	2.80 ± 0.42
3.5	4.94 ± 1.35	2.65 ± 0.10
Normal (1 Gy):		
1.5	1.55 ± 0.6	
2.5	2.2 ± 2.1	
3.5	3.25 ± 0.9	
Normal (2 Gy):		
1.0	1.62 ± 0.2	0.89 ± 0.55
2 .0	1.00 ± 0.28	0.79 ± 0.27
3 .0	1.10 ± 2.9	0.74 ± 0.05