AECL-6627

ATOMIC ENERGY OF CANADA LIMITED

L'ÉNERGIE ATOMIQUE DU CANADA LIMITÉE

DNA REPAIR, HUMAN CANCER AND ASSESSMENT OF RADIATION HAZARDS

Réparation de l'ADN, cancer chez les êtres humains et évaluation des risques d'irradiation

M.C. PATERSON and D.K. MYERS

Chalk River Nuclear Laboratories Laboratoires nucléaires de Chalk River

Chalk River, Ontario

September 1979 septembre

ATOMIC ENERGY OF CANADA LIMITED

DNA REPAIR, HUMAN CANCER AND ASSESSMENT OF RADIATION HAZARDS

by

M.C. Paterson and D.K. Myers

Health Sciences Division
Chalk River Nuclear Laboratories
Chalk River, Ontario

1979 September

AECL-6627

Réparation de l'ADN, cancer chez les êtres humains et évaluation des risques d'irradiation

par

M.C. Paterson et D.K. Myers

Résumé

On pense que les cancers, comme les anomalies génétiques, sont principalement dus à des changements se produisant dans l'ADN, matière génétique présente dans toutes les cellules vivantes. Une partie de la preuve à l'appui de cette hypothèse découle de l'étude de certains troubles héréditaires trouvés chez les êtres humains auxquels ils font courir un grand risque de cancer. Des cellules provenant de malades souffrant, en tous cas, de l'un de ces troubles appelé "ataxia telangiectasia", semblent avoir une déficience qui les empêche de réparer les dommages causés par des rayonnements et/ou par certains autres agents environnementaux. Des études relatives aux conséquences de la réparation de l'ADN permettent de penser que les évaluations courantes touchant les risques cancérogènes des rayonnements de faible radioactivité sont en grande partie correctes. Il semble y avoir une certaine marge de sécurité en jeu dans ces évaluations de risque pour la majorité de la population. Il est, cependant, déconseillé de réduire dans une large mesure les évaluations de risque couramment acceptées, par suite de l'existence des sous-groupes potentiellement radiosensibles qui forment une minorité dans l'ensemble de la population.

L'Energie Atomique du Canada, Limitée
Laboratoires nucléaires de Chalk River
Chalk River, Ontario

Septembre 1979

AECL-6627

ATOMIC ENERGY OF CANADA LIMITED

DNA REPAIR, HUMAN CANCER AND ASSESSMENT OF RADIATION HAZARDS

by

M.C. Paterson and D.K. Myers

ABSTRACT

Cancers, like genetic defects, are thought to be caused primarily by changes in DNA, the genetic material of all living cells. Part of the evidence in support of this hypothesis derives from the study of certain rare hereditary disorders in man associated with high risk of cancer. Cells derived from patients suffering from at least one of these disorders, ataxia telangiectasia, appear to be defective in their ability to repair the damage caused by radiation and/or by certain other environmental agents. Studies of the consequences of DNA repair suggest that currently accepted estimates of the carcinogenic hazards of low level radiation are substantially correct. There would appear to be some margin of safety involved in these risk estimates for the majority of the population, but any major reduction in the currently accepted risk estimates appears inadvisable in view of the existence of potentially radiosensitive subgroups forming a minority in the general population.

Chalk River Nuclear Laboratories
Chalk River, Ontario
1979 September

AECL-6627

ENVIRONMENTALLY INDUCED DNA DAMAGE, ITS DEFECTIVE REPAIR, AND HUMAN CANCER

Malcolm C. Paterson

Biology and Health Physics Division
Atomic Energy of Canada Limited
Chalk River Nuclear Laboratories

ABSTRACT

Deoxyribonucleic acid (DNA) is the repository of the genetic information indispensable to the well-being of living cells. Damage to cellular DNA by cancer-causing agents in the environment is considered to be a major factor in the development of human cancer. Evidence in support of this hypothesis derives in part from the laboratory study of ataxia telangiectasia (AT), a rare hereditary disorder in man in which afflicted patients are at high risk of cancer and respond adversely to radiotherapy. Skin cells cultured from such donors are also hypersensitive to inactivation by radiation, owing to a defect in an enzymatic mechanism for the repair of radiogenic damage to DNA. AT cells are also hypersensitive to the ultraviolet component of sunlight and certain cancer-causing chemicals suggesting that individuals unusually sensitive to irradiation may also display increased sensitivity to other environmental agents. Cells cultured from parents of AT patients are moderately radiosensitive. Persons of this genetic type are estimated to comprise ∿1% of the total population and are known to be cancer-prone. We have also observed enhanced radiosensitivity in cells cultured from three cancer-stricken members of a family with an unusually high incidence of acute myelogenous leukemia, suggesting that these family members are genetically predisposed to cancer (possibly due to hypersensitivity to irradiation). These and other clinical examples show promise as models for assessing the role of genetic factors (e.g. faulty genetic information needed for the production of DNA repair enzymes) in cancer induction by radiation and other extrinsic agents.

ABBREVIATIONS

DNA, deoxyribonucleic acid; UV, ultraviolet; D_{10}, radiation dose (in rads) reducing the survival of a population of cells to 10% of the unirradiated level; AT, ataxia telangiectasia; DRF, dose reduction factor [D_{10} (control cells)/D_{10} (indicated cells)]; ENU, ethylnitrosourea; MMS, methylmethanesulfonate; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; 4-NQO, 4 nitroquinoline-1-oxide; RTS, Rothmund-Thomson syndrome; PE, plating efficiency (percent of the unirradiated cells plated out that give rise to colonies)

INTRODUCTION

Research in radiation biology at CRNL is directed toward an improved understanding of the harmful effects of ionizing radiation on living organisms, with the aim of applying this knowledge to the assessment of the health hazards of low-level radiation to both occupational workers and society-at-large. To this end, a number of different research projects on radiation effects are being carried out, ranging from the induction of genetic changes in microorganisms to the induction of cancer in rodents. The main emphasis throughout is the enzymatic repair of radiation damage to the genetic material of living organisms (for details, see ref. 1 and the succeeding article by D.K. Myers).

THE GENETIC MATERIAL - DNA

The repository of the genetic information is DNA; it consists of two strands twisted around each other similar to two spiral staircases (see Fig. 1 in article by Myers and ref. 2, p. 208-215). Each staircase is comprised of alternating sugars and phosphates; at each sugar, one of four bases (adenine, cytosine, guanine, or thymine) projects into the stairwell and is paired by hydrogen bonding with a second base projecting from the opposite staircase. Only specific pairs of bases fit properly into the stairwell, adenine with thymine and cytosine with guanine, and hence the sequence of bases along one strand is said to be complementary to the sequence of bases along the opposite

strand. It is this sequence of bases along each strand which determines the genetic information; a unit of information is called a gene. The genetic material in living organisms contains an enormous number of base pairs. In a human, for example, there are 10^{10} base pairs per somatic cell, and there are $\sim 6 \times 10^{13}$ somatic cells in an adult, giving a total of $\sim 6 \times 10^{23}$ base pairs per individual (1,2). (Note: germ cells, the other type of cells in the human body, are involved in sexual reproduction and are numerically negligible compared to somatic cells.)

The genetic material is distributed within cells differently in different organisms. In each somatic cell in man, it is divided among 46 chromosomes in two homologous sets of 23 (thus called a diploid cell), one set inherited via a germ cell (thus called a haploid cell) from the father and the other set via a germ cell from the mother (3). Each chromosome of a homologous set contains one DNA molecule with an unique structure determined by the sequence of the four bases along each of the two complementary strands. Residing within the DNA of each set of 23 chromosomes are all the instructions needed for the somatic cell: (i) to synthesize, in a highly controlled manner, many different proteins (including enzymes) and other gene products needed to survive and carry out its specific function in the body; (ii) to eventually duplicate its 46 chromosomes and divide into two daughter cells, each containing an identical complement of genetic information accurately preserved; and (iii) to produce germ cells (sex organs only).

DNA DAMAGE AND ITS BIOLOGICAL CONSEQUENCES

Man and all other living organisms are routinely exposed to deleterious agents in the natural habitat (4,5). These include: ionizing radiation of both natural (e.g. cosmic rays) and man-made origin (e.g. radiation sources used in diagnostic medicine and fallout from nuclear weapons testing); the UV component of sunlight, and numerous chemicals. Many of these chemicals, such as benzene and 2-naphthylamine, are found in the workplace, but some, such as components of cigarette smoke and various nitrosamines, are related to life-style and dietary factors (5).

Regardless of their origin, these physical and chemical agents are believed to exert their harmful biological effects primarily as a consequence of interacting with, and structurally altering, DNA (6-8). [One possible exception is asbestos; this physical agent is chemically inert, and its mode of action is not known (8).] Let us take ionizing radiation as a case in point. At the molecular level, the alterations produced in DNA by ionizing radiation are of two major classes: (i) structural changes in individual bases, particularly thymine and cytosine, which disrupt hydrogen bonding and base stacking and thereby cause localized structural distortions in the two "spiral staircases"; and (ii) breaks arising from cleavage of the sugar-phosphate backbone of individual "staircases" (single-strand breaks) (9). These types of lesions are formed in considerable number during radiation exposure. In the case of human diploid cells cultured in the laboratory, for example, a biologically relevant dose of 385 rads (3.85 Gy) ^{60}Co γ-irradiation (i.e. the D_{10} value) will induce in the DNA of each cell $\sim$4600 single-strand breaks and $\sim$2500 base defects (10).

The presence of such lesions in DNA can result in either death of the cell or a viable cell carrying altered genetic information which would then be passed on to all subsequent generations of daughter cells. The two types of biological changes associated with chronic exposure to low levels of radiation are referred to as genetic (i.e. hereditary defects in the descendants of the irradiated person) and somatic (i.e. changes in the tissues or organs of the irradiated person presumably arising from the propagation of genetically altered somatic cells). The most serious somatic changes are those leading to the appearance of leukemia and other forms of cancer.

ENZYMATIC REPAIR OF DAMAGED DNA

Given the formidable array of DNA-damaging agents present in the environment and the high premium necessarily placed on maintaining the accuracy of the genetic script encoded in DNA, it is not surprising that all living organisms, ranging from bacteria to mammals, possess multiple enzymatic mechanisms whose joint actions promote the restoration of damaged sites in DNA to a normal structure (10-13).

Two repair mechanisms operating on DNA damaged by many extrinsic agents including ionizing radiation are termed excision repair and strand-rejoining; the former process acts on base defects and the latter process acts on single-strand breaks. In the excision-repair mechanism, remedial action is accomplished by the removal of a single-strand segment containing the altered base followed by replacement with a segment containing the correct sequence of bases (13-16). In the conventional model of excision repair (14), as illustrated in Fig. 1, the following four enzyme-mediated reactions are carried out in a coordinated fashion:

(i) an incision is introduced into the sugar-phosphate backbone near the altered base by an endonuclease which "recognizes" the site as having an abnormal structure;

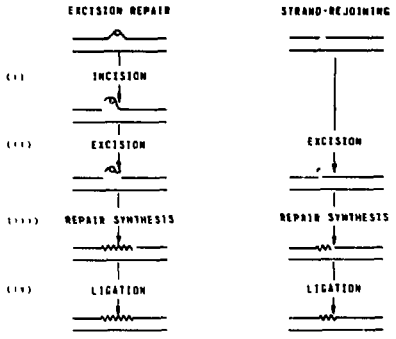

Figure 1 Schematic representation of enzymatic mechanisms for correcting base defects (left) and single-strand breaks (right) induced in DNA of human cells by ionizing radiation (see text for details)

(ii) a second single-strand nick is made on the opposite side of the defect by an exonuclease, thereby excising the damaged site from the DNA; the resulting gap is often extensively widened by additional exonucleolytic activity;

(iii) a DNA polymerase then inserts new material into the gap (a process termed repair synthesis), using the opposite intact strand for base-pairing instruction; and finally

(iv) the newly synthesized and pre-existing strand segments are joined by a DNA ligase, thus restoring strand continuity.

It has recently been shown that some classes of altered bases are released as free bases rather than within single-strand segments as in the traditional model of excision repair (15). In the newer model, the covalent bond joining the damaged base with the sugar moiety is first cleaved by a DNA glycosylase, and the modified site containing the denuded sugar is then corrected in the same fashion as a base defect in the traditional model--that is, strand incision, excision of the modified site, repair synthesis, and strand ligation. The classes of base defects acted upon by the newer mode of excision repair are believed to cause only minor localized distortions in the DNA molecule; hence the pre-incision step involving the removal of an altered base as a free entity may serve as a means of preventing abortive repair, i.e. ensure that the site, once incised, is not rejoined before the defective base is removed. It would now appear that most base defects induced by ionizing radiation are corrected by this mode of excision repair whereas base defects produced by UV light, for example, are corrected by the conventional excision-repair mode (13).

The mechanism mediating the rejoining of single-strand breaks is less well understood, although this mechanism may be assumed to be less complex than excision repair of base damage because the sugar-phosphate backbone has been severed directly by the radiation treatment, thus obviating the need to carry out the incision reaction. The restitution of a single-strand break is often accompanied by the release of nearby base and sugar moieties; thus its repair is presumably achieved by an abbreviated form of excision repair involving limited exonucleolytic digestion to "clean the frayed ends", repair synthesis and strand ligation (9, 16).

A key property of the excision-repair mechanism is its ability to correct a seemingly limitless spectrum of chemically distinct modifications in the four bases, whether induced by extrinsic agents or arising spontaneously. This versatility is given by a family of different endonucleases and DNA glycosylases (13). Whether the three types of reactions common to the excision-repair and strand-rejoining mechanisms are carried out by the same enzymes has yet to be determined.

CAUSES OF HUMAN CANCER: OUR APPROACH TO THE PROBLEM

One research project at CRNL is designed to clarify our understanding of the underlying causes of cancer in man. Two factors are thought to predominate: environmental and genetic. It is now widely held that most, if not all, human cancers are caused, at least in part, by some extrinsic factor, e.g. a cultural agent, such as cigarette smoke, or a chemical in the workplace, such as vinyl chloride (see refs. 4 and 5). Recently it has also become evident that certain genetic alterations may predispose an individual to the development of cancer. It is therefore becoming increasingly important to assess the interplay of these environmental and genetic factors in cancer induction. We are particularly interested in determining how defective genes involved in the synthesis of DNA repair enzymes may interact with ionizing radiation in cancer development.

Our basic experimental approach is to expose cultured human cells to a given cancer-causing agent (carcinogen), usually Co^{60} γ-radiation, and measure:

1. the ability of single cells to undergo many successive cell divisions (>6) and thereby form macroscopic colonies, each containing an aggregate of 100 or more cells (biological endpoint); and

2. the initial yield and subsequent fate (i.e. repair) of single-strand breaks and base defects (molecular endpoint). (Note: these cells grown in the laboratory are derived from a skin biopsy of a human donor; such cells serve as useful test material for studying the effects of ionizing radiation on man because they respond to radiation exposure in a manner similar to that of most types of somatic cells in the body.)

ATAXIA TELANGIECTASIA: HUMAN DISORDER LINKING RADIOSENSITIVITY WITH CANCER PRONENESS

We have been greatly aided in our research by the availability of skin cells derived from patients with the rare hereditary disease, ataxia telangiectasia (10, 16). This is a complex, single-gene disorder whose incidence is ∿24 per million live-births. The disease is transmitted in a recessive fashion--that is, affected individuals inherit two defective copies of an AT gene, one copy of maternal, and the other of paternal origin. Its major clinical characteristics include: (i) muscular incoordination; (ii) blood vessel dilation (eyes and skin); (iii) defective immunity; (iv) proneness to cancer (particularly lymphomas and lymphatic leukemias); and (v) hypersensitivity to radiotherapy. One in 10 AT patients develops cancer, a risk which is ∿1200 times higher than in an age-matched control population. Afflicted persons typically die before adulthood from pneumonia (due to immune deficiency) or cancer or both. In three well-documented cases, AT patients have died within six months upon receiving conventional radiotherapy for the treatment of solid malignant tumors; hence, an unusually severe reaction to ionizing radiation is observed at the clinical level (16).

Our goal has been to determine the radiosensitivity and DNA repair properties of skin cells from AT donors and thereby explain the basis of the hypersensitivity to irradiation associated with the disorder. That abnormal radiosensitivity extends to the cellular level can be seen in Fig. 2. Cells from nine unrelated AT patients all display increased sensitivity to the lethal effects of γ-irradiation compared to control cells from five unrelated normal persons; the sensitivity increase corresponds to a DRF of ∿3. This enhanced radiosensitivity of the AT strains is observed whether the radiation exposure is given under oxic or hypoxic conditions (i.e. in air or in N_2) (see Table 1). Furthermore, cells from one AT donor, while proficient in rejoining strand breaks, are ∿3 times slower in the removal (i.e. repair) of base defects than are cells from a clinically normal donor (Fig. 3). Two other AT strains, AT2BE and AT81CTO, are also defective in

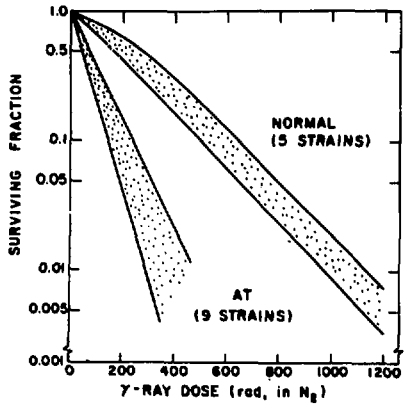

Figure 2 Range of γ-ray survival curves of cultured cells from nine AT and five clinically normal subjects Co^{60} γ-irradiation was administered under hypoxic conditions.

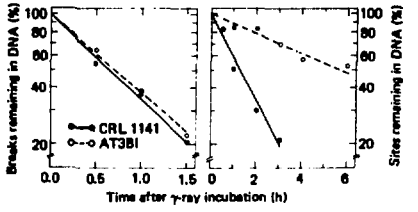

Figure 3 Time-course of the disappearance of single-strand breaks (left panel) and sites containing base defects (right panel) from the DNA of normal (CRL 1141) and AT (AT3BI) cells exposed to 50 krad (0.5 kGy) of hypoxic γ-irradiation [From Paterson *et al.* (ref. 25) with permission of MacMillan Publishing Company]

site removal but rejoin single-strand breaks at a normal rate (10,13,16). The simplest conclusion is that these AT strains lack a fully functional enzyme, presumably an endonuclease or DNA glycosylase, needed to initiate the excision of some type of base damage. Thesé combined biological and molecular data, besides offering new insight into the basic cause of the disease, provide one of the best pieces of evidence available to date for a causal relation between defective repair of DNA damage and predisposition to cancer. In short, they dramatically illustrate the importance of DNA repair

Table 1 Sensitivity of AT Strains to Cell Killing by Various Carcinogens†

Strain	Physical Agent						Chemical Agent			
	γ-rays		Neutrons		UV Light		MMS	MNNG	ENU	4-NQO
	Oxia	Hypoxia	Oxia	Hypoxia	254 nm	313 nm				
AT2BE	+++	+++	++	++	N	+	+	++	+++	++
AT3BI	+++	+++	++	++	N	N	N	++	++	N
AT4BI	+++	+++	++	++	N	++	+	N	+	+

†From ref. 16 and unpublished data (M.C. Paterson, P.J. Smith, B.P. Smith, M.V. Middlestadt, N.T. Bech-Hansen and B.M. Sell)

The following symbols are used to denote relative sensitivity: N, normal ($0.8 \leq DRF \leq 1.2$); +, slightly sensitive ($1.2 < DRF \leq 1.5$); ++, moderately sensitive ($1.5 < DRF \leq 2.0$); +++, markedly sensitive ($2.0 < DRF \leq 4.0$).

mechanisms to the well-being of man.

We are currently attempting to define further the repair defect in AT. Two aspects of this problem are under investigation: (i) identification of the repair enzyme, endonuclease or DNA glycosylase, malfunctional in AT cells; and (ii) determination of the chemical nature of the altered base acted upon by this repair enzyme. We are fortunate to have several radiation chemists and enzymologists collaborating with us on these studies, including Drs. J. Cadet (Centre D'Etudes Nucléaires de Grenoble), N.E. Gentner (CRNL), and P.V. Hariharan (Roswell Park Memorial Institute).

Strains from various AT patients are also abnormally sensitive to 14 MeV neutrons; however, the AT strains are only ∿1.8 times more sensitive to this densely ionizing radiation than are control strains from normal persons, if treated in air or in N_2 (Table 1). These results indicate that the abnormality in AT cells is less critical for the lethal effects of densely compared to sparsely ionizing radiation. The findings are also consistent with anomalous DNA repair as a basic defect in AT cells; the fraction of the total DNA damage amenable to correction by enzymatic repair is smaller for densely than for sparsely ionizing radiation (17), and therefore faulty repair should be less of a handicap for cell recovery from neutron exposure compared to γ-ray exposure.

It is of interest to determine whether AT cells are also abnormally sensitive to the lethal effects of other carcinogens in our environment. Fig. 4 shows that one strain, AT4BI, is hypersensitive to near UV light (313-nm wavelength), but exhibits a normal sensitivity to killing by far UV light (254-nm wavelength). AT2BE cells respond similarly to 313-nm and 254-nm light (Table 1). This marks the first time that human strains have been found that are hypersensitive to near but not far UV light. This

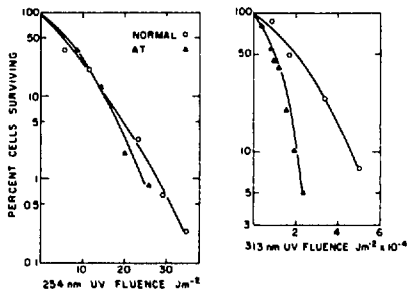

Figure 4 Survival curves of AT (AT4BI) and normal (GM38) cells upon exposure to far (left panel) and near (right panel) UV light

enhanced sensitivity to 313-nm light observed in AT4BI and AT2BE cells provides a rational explanation for why blood vessel dilation is particularly pronounced over sunlight-exposed regions of the skin in some AT patients. Furthermore, this observation implies that near UV light not absorbed by the upper atmosphere may confer a biological effect which partially mimics that produced by ionizing radiation, thus modifying the terms in which this ubiquitous carcinogen should be considered. (Note: far UV light has little practical relevance; it is absorbed by ozone in the stratosphere and thus does not penetrate to the surface of the earth. Over the years it has been extensively used in the laboratory largely because germicidal lamps are relatively cheap and readily available sources of this light, and its biological effects are for the most part similar to those produced by near UV light.)

As a general rule, AT strains also display hypersensitivity to those chemical carcinogens whose biological effects mimic those of ionizing radiation; included in this list is ENU (Fig. 5), an agent noted for its ability to induce brain tumors in rats (18). There is, however, much more variability among different AT strains in response to treatment with radiomimetic chemicals, such as MMS and MNNG, than is found for ionizing radiation (Table 1). The data are compatible with faulty DNA repair as a root cause. Radiation is known to produce a wide spectrum of lesions in DNA (9), and hence efficient repair presumably requires that a number of different repair pathways all be fully operational; conversely, a malfunction in any one of these pathways would be expected to result in the enhanced radiosensitivity observed for all AT strains. On the other hand, radiomimetic chemicals are thought to induce a relatively narrow spectrum of reaction products in DNA, and the relative yields of these products often differ greatly from one chemical agent to another (19). Consequently, if different AT strains are defective in the synthesis of repair enzymes mediating different pathways, it is not surprising that there are differences in the sensitivity of these strains to specific radiomimetic carcinogens. In brief, these findings on the response of skin cells from different AT donors to killing by various physical and chemical carcinogens serve to illustrate two general principles: (i) there are multiple genes in man which can lead to the genetic disorder AT [a possibility in keeping with the heterogeneous nature of most human genetic diseases (3)]; and (ii) persons found to be hypersensitive to ionizing radiation will in all probability also prove to be hypersensitive to certain other environmental carcinogens. A ready explanation for the second principle may lie in the likelihood that portions of the DNA damage induced by ionizing radiation and by various radiomimetic carcinogens are handled by repair pathways mediated by one or more identical enzymes (e.g. endonucleases or DNA glycosylases).

PERSONS CARRYING ONE DEFECTIVE AT GENE: RADIOSENSITIVE AND CANCER-PRONE SUBPOPULATION

Let us now turn to a matter of some practical importance. Although persons afflicted with ataxia telangiectasia (i.e. individuals inheriting two altered copies of an AT gene) are too rare to constitute a serious public health problem, it can be estimated by basic genetic principles that persons carrying one defective copy (plus one normal copy) of an AT gene comprise ~1% of the general population (20,21).

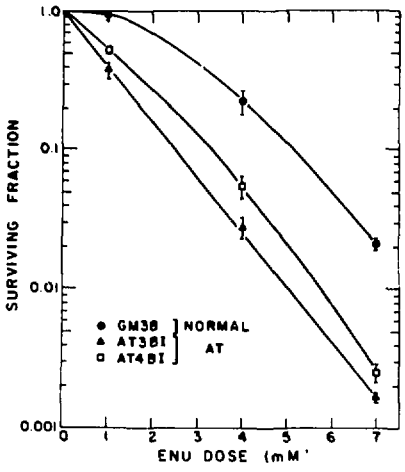

Figure 5 Survival curves of two AT strains and one normal strain upon one-hour treatment with indicated concentrations of ENU

Morover, Swift and co-workers (20) have reported that blood relatives (below the age of 45 years) of AT patients are a few times more likely to die from cancer than are members of an age-matched control population; this increased risk of cancer mortality presumably reflects the existence of a high frequency of AT carriers among these blood relatives. It is therefore of interest to measure the radiosensitivity of skin cells from presumed AT carriers--that is, the parents in AT families, each of whom presumably transmitted one defective copy of an AT gene to their children afflicted with the disease. Fig. 6 shows that cells cultured from the two parents in one AT family display γ-ray sensitivity intermediate between that exhibited by cells from their AT child and control cells from five normal subjects. These and other results (10,21) suggest that a significant fraction of the general population known to be at increased risk of cancer is also moderately radiosensitive. Clearly, continued efforts are needed to detect AT carriers in society-at-large and to evaluate the role of the interaction between this genetic state and relevant extrinsic carcinogens in the appearance of common cancers.

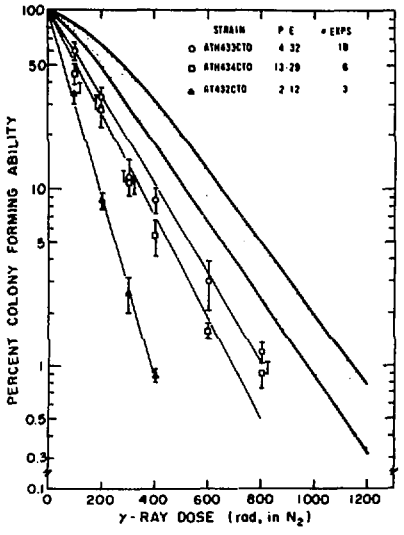

Figure 6 Hypoxic γ-ray survival curves of cell strains derived from three members [affected child (▲) plus parents (o,□)] of an AT family The shaded area represents the range of survival of control strains from five normal donors. The symbols and error bars are the means and their standard errors of multiple experiments. Range of PE and number of independent experiments conducted for each strain are indicated.

ROTHMUND-THOMSON SYNDROME: HUMAN DISORDER LINKING SUN SENSITIVITY WITH HIGH RISK OF SKIN CANCER

AT is but one of the human diseases associated with cancer predisposition that is under investigation in our laboratory. For example, Dr. P.J. Smith, a postdoctoral fellow, has been studying the Rothmund-Thomson syndrome. This is another rare genetic disease whose clinical hallmarks include: (i) sensitivity of the skin to sunlight (e.g. excessive blistering and pigmentation changes); (ii) cataract formation in both eyes at an early age; (iii) cancer-proneness (e.g. skin carcinomas); (iv) stunted growth; and (v) underdevelopment of sex organs. Skin cells from one RTS patient have been found to respond to UV light in a fashion similar to AT4BI cells, i.e. abnormally sensitive to killing by 313-nm but not 254-nm light (Fig. 7). This RTS donor had a positive clinical history of sunlight sensitivity and had developed basal cell carcinoma of the eyelid. Hence, we see here an association between hypersensitivity of cultured cells to the UV component of sunlight and the presence of skin

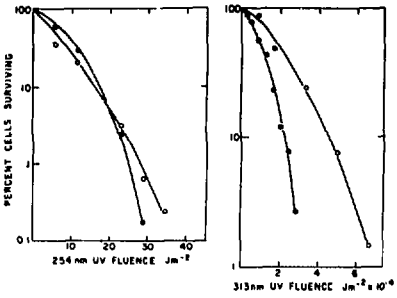

Figure 7 Survival curves of cultured cells from one RTS patient (●) and one normal person (o) upon exposure to far (left panel) and near (right panel) UV light

cancer in the donor (presumably arising from damage inflicted by solar light). Strains from other RTS patients are now under study to test the generality of this association.

CELLULAR RADIOSENSITIVITY IN A LEUKEMIA-PRONE FAMILY

We have recently received a contract from the United States National Cancer Institute to expand our DNA repair studies on cultured cells derived from persons with known or suspected genetic predisposition to malignancy. Dr. N.T. Bech-Hansen, a Research Associate, and Ms. B.M. Sell, a Research Assistant, are supported by the contract. This project is beginning to yield interesting results. An example pertains to a family displaying an unusual clustering of acute myelogenous leukemia (22). The well-known ability of ionizing radiation to induce leukemia (23) prompted us to assess the radiosensitivity of skin cells from six members of this family. The results are summarized in Table 2. Strains from three family members with cancer (2649T from the mother, and 409T and 2642T from two daughters) are all significantly more radiosensitive than the control strain from a normal donor (GM38), but are not as sensitive as the strain from an AT patient (AT2BE). The radiosensitivity of the strains from the father (2650T) and from one son (2647T) are not significantly different from that of the control strain, while the strain from a second son (2648T) does exhibit increased γ-ray sensitivity. Hence, there appears to be an association between cancer proneness in an individual and enhanced radiosensitivity of his cultured cells. Given an abnormal clustering

Table 2 Sensitivity to Oxic γ-Irradiation of Skin Cells Cultured from Members of a Family with an Abnormal Clustering of Acute Myelogenous Leukemia†

Strain	Donor			No. of Experiments	D_{10}±S.E.	P
	Family Relation	Clinical Description	Age			
GM38	-	normal	9	6	407±15	
AT2BE	-	ataxia telangiectasia	7	2	169± 9	*
2649T	mother	uterine cervical cancer	41	4	301±12	*
2650T	father	normal	46	5	425±19	
409T	daughter	acute myelogenous leukemia	12	4	354±18	*
2642T	daughter	acute myelogenous leukemia	20	4	290±31	*
2647T	son	normal	16	2	417±30	
2648T	son	normal	16	3	321±25	*

†From ref. 24 and unpublished data (N.T. Bech-Hansen, B.M. Sell and M.C. Paterson)

*$P<0.05$, comparing D_{10} value to that of GM38, using the standard error of a difference as a statistical test

of malignancies in a family, studies such as radiosensitivity of cultured cells may be of value in predicting the predisposition of an individual to acute myelogenous leukemia or other cancers. Investigations are now underway to test this prediction.

It is perhaps not too surprising that one family member, although his cultured cells display abnormal γ-ray sensitivity, should be free of cancer at the present time. This person seems to possess a reduced tolerance to the harmful effects of extrinsic radiation and presumably certain radiomimetic agents. Our results have been passed on to the physician attending to the family, and this family member is now being carefully watched for the appearance of any malignancy.

SUMMARY AND CONCLUDING REMARKS

In summary, our studies have provided information on (i) who is more susceptible to cancer and why; and (ii) the role of inefficient or incomplete repair of damage to DNA in the underlying mechanism(s) of cancer induction by extrinsic agents in our biosphere.

Our work is pertinent to both the nuclear power industry and the biomedical sciences. Its significance can be summarized as follows: (i) identification of persons unusually sensitive to radiation exposure; (ii) provision of information relevant to assessment of the risk of chronic exposure to low-level radiation; (iii) clarification of the basic mechanism(s) involved in the development of cancer; and (iv) assessment of the contribution of genetic factors in cancer predisposition.

In short, our research should help explain why certain persons tend to develop certain types of cancer. This information will contribute to the development of unifying concepts concerning the origin of cancer which will in turn assist in the formulation of better strategies for the prevention and early detection of cancer.

ACKNOWLEDGMENTS

Numerous persons have been involved in the studies described here. B.P. Smith and P.A. Knight have provided expert technical help from the onset; Dr. P.J. Smith, Dr. P.H.M. Lohman, a visiting scientist from TNO, Rijswijk, Holland, and A.K. Anderson, L. Fishman, and M.V. Middlestadt, three undergraduate summer students, have contributed greatly on a temporary basis. More recently, Dr. N.T. Bech-Hansen and B.M. Sell, in addition to Drs. R.B. Miller, J.J. Mulvihill and J.M. Lamon of the Clinical Epidemiology Branch, U.S. National Cancer Institute, have made major contributions to the program. Both a multidisciplinary approach and a wide range of biological systems are being used in the research ongoing in the Biology Branch, and we have profited greatly from the expertise and encouragement freely offered by our colleagues in the Branch, particularly Dr. D.K. Myers, its Head, as well as Dr. A.M. Marko, Director of the Biology and Health Physics Division. We gratefully acknowledge partial support by U.S. NCI Contract N01-CP-81002 with the Clinical Epidemiology Branch, U.S. National Cancer Institute. Finally, I am indebted to Drs.

N.T. Bech-Hansen, N.E. Gentner, and P.J. Smith for their constructive commentary on the manuscript and to C.I. Walters for her patience and forbearance during the typing of the manuscript.

REFERENCES

1. Objectives of Research Activities in Biology Branch, Chalk River Nuclear Laboratories, 1976. Atomic Energy of Canada Limited, Report AECL-5613, 1977. See also: Myers, D.K., Radiation Biology for the Non-Biologist. Atomic Energy of Canada Limited, Report AECL-5721, 1978.
2. Watson, J.D., Molecular Biology of the Gene, 3rd edition, W.A. Benjamin, Menlo Park, Ca., 1976.
3. Levitan, M., and Montagu, A., Textbook of Human Genetics, 2nd edition (revised by Levitan, M.), Oxford University Press, New York, 1977.
4. Doll, R., Nature, Vol. 265, p. 589-596, 1977.
5. Higginson, J., Carcinogens: Identification and Mechanisms of Action (The University of Texas System Cancer Center 31st Annual Symposium on Fundamental Cancer Research, 1978), p. 187-208, Raven Press, New York, 1979.
6. Cerutti, P., DNA Repair Mechanisms (ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. 9), p. 1-14, Academic Press, New York, 1978.
7. Miller, E.C., Cancer Res., Vol. 38, p. 1479-1496, 1978.
8. Harnden, D.G., Knudson, A.G., Miller, R.W., Brookes, P., Cairns, J., Croce, C.M., Doerfler, W., Fialkow, P.J., Green, H., Magee, P.N., Muir, C.S., Paterson, M.C., Rajewsky, M.F., and Winnacker, E.L., Neoplastic Transformation: Mechanisms and Consequences, p. 247-268, Dahlem Konferenzen, Berlin, 1977.
9. Ward, J.F., Adv. Radiation Biol., Vol. 5, p. 182-239, 1975.
10. Paterson, M.C., DNA Repair Mechanisms (ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. 9), p. 637-650, Academic Press, New York, 1978.
11. Cleaver, J.E., Progress in Genetic Toxicology, p. 29-42, Elsevier/North Holland Biomedical Press, Amsterdam, 1977.
12. Bootsma, D., DNA Repair Mechanisms (ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. 9), p. 589-601, Academic Press, New York, 1978. See also: German, J., ibid., p. 625-631.
13. Paterson, M.C., Carcinogens: Identification and Mechanisms of Action (The University of Texas System Cancer Center 31st Annual Symposium on Fundamental Cancer Research, 1978), p. 251-276, Raven Press, New York, 1979. (AECL-6369).
14. Grossman, L., and Riazuddin, S., DNA Repair Mechanisms (ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. 9), p. 205-217, Academic Press, New York, 1978. See also: Waldstein, S., ibid., p. 219-224.
15. Linn, S., DNA Repair Mechanisms (ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. 9), p. 175-178, Academic Press, New York, 1978.
16. Paterson, M.C., and Smith, P.J., Annu. Rev. Genet., Vol. 13, in press.
17. Ritter, M.A., Cleaver, J.E., and Tobias, C.A., Nature, Vol. 266, p. 653-655, 1977.
18. Goth, R., and Rajewsky, M.F., Proc. Natl. Acad. Sci. USA, Vol. 71, p. 639-643, 1974.
19. Strauss, B.S., Aging, Carcinogenesis, and Radiation Biology, p. 287-314, Plenum Press, New York, 1976.
20. Swift, M., Sholman, L., Perry, M., and Chase, C., Cancer Res., Vol. 36, p. 209-215, 1976.
21. Paterson, M.C., Anderson, A.K., Smith, B.P., and Smith, P.J., Cancer Res., Vol. 39, in press.
22. Snyder, A.L., Li, F.P., Henderson, E.S., and Todaro, G.J., Lancet, Vol. 1, p. 586-589, 1970.
23. Miller, R.W., Biology of Radiation Carcinogenesis, p. 45-50, Raven Press, New York, 1976.
24. Bech-Hansen, N.T., Sell, B.M., Mulvihill, J.J., and Anderson, A.K., Proceedings Annual Joint Meeting, American Association for Cancer Research and American Society of Clinical Oncology, p. 78, 1979.
25. Paterson, M.C., Smith, B.P., Lohman, P.H.M., Anderson, A.K., and Fishman, L., Nature, Vol. 260, p. 444-447, 1976. (AECL-5410).

RISK ESTIMATES AND DNA REPAIR

D.K. Myers

Biology Branch, Chalk River Nuclear Laboratories
Atomic Energy of Canada Limited

ABSTRACT

Cancers, like genetic defects, are thought to be caused by changes in DNA, the genetic material. Different types of changes in DNA are mentioned which may be induced either by a single damaging event or by two coexistent events. In the first case, the biological effects are directly proportional to the total dose of radiation and are independent of the dose-rate. In the second case, the effects tend to disappear as the time for delivery of a given dose of γ-radiation is prolonged; this is attributed to the operation of DNA repair systems in the living organism. It seems probable that currently accepted estimates of the carcinogenic hazards of low level radiation based on the linear dose-effect model are substantially correct. There may well be some margin of safety involved in these risk estimates, but any major reduction in the currently accepted risk estimates appears inadvisable.

THE ACCEPTED RISK ESTIMATES

Data reviewed most recently by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (1) suggest that about 1 in every 200 of the fatal cancers and about 1 in every 200 of the genetic defects which occur normally in human populations might be caused by the natural background radiation level of 100 millirem (1000 μSv) per year. The International Commission on Radiological Protection (ICRP) (2) has independently published risk estimates which agree with those given in UNSCEAR and has recently reconfirmed (3) that "published information on the epidemiological and radiobiological evidence of radiation risks to man...up to May 1978 does not call for changes in the risk factors" published in 1977. Various other national agencies have from time to time issued reports with risk estimates which, by and large, agree with those suggested by UNSCEAR and ICRP (4-6).

The above risk estimates are based primarily on epidemiological studies of cancer frequency in human populations exposed to unusual levels of ionizing radiation and, in the absence of human data, on laboratory studies of genetic defects induced in mice and other organisms by ionizing radiation. However, present risk estimates are necessarily derived from results observed at high radiation doses and high dose-rates where measurable effects of radiation can be observed. The appropriateness of the standards of radiation exposure which have been set for protection of the health of radiation workers and of the general public depends upon the reliability of the estimates of biological effects of low-level radiation. There are some uncertainties involved in the extrapolation of estimates from high doses down to very low radiation doses at low dose-rates where measurable effects cannot be observed. A continuing program in basic research is therefore being carried out at the Chalk River laboratories to make certain that we know as much as possible about the long-term biological effects of low radiation doses (7). In order to understand these effects, we would like to understand the basic mechanisms responsible. For this purpose, we have concentrated on radiation damage to DNA in the living organism and the repair of this damage.

DNA AND DNA REPAIR

About 26 years ago, biologists recognized that the information for all life processes is contained in a long thread-like molecule called deoxyribonucleic acid (DNA). The DNA molecule represents the blueprint or coding tape for the construction and function of living things; it stores all the genetic information that is passed from one generation to the next. In this manner it ensures that the major characteristics of living things remain essentially constant over many generations. A small portion of the DNA molecule is illustrated in Fig. 1; other more

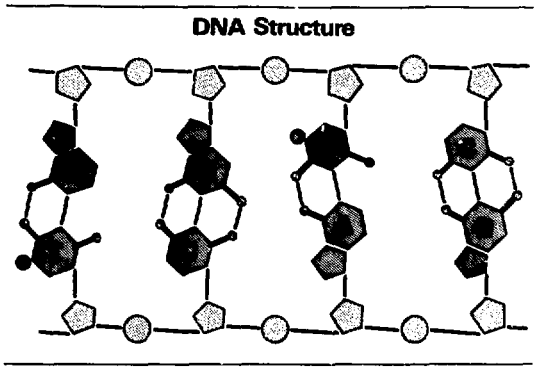

Figure 1

detailed illustrations can be found in most of the recent textbooks on molecular cell biology. On the outside of the molecule are two separate continuous strands of a particular sugar (deoxyribose) connected by alternating phosphate moieties; in the centre of the DNA molecule is a series of paired bases, whose sequence along the length of the DNA molecule provides the coded information for the intricate chemical processes carried out by a living cell. Most cells of the human body contain 46 DNA molecules of this kind, each of which is about 2 nanometres across and contains roughly one hundred million of base pairs along some 4 centimetres (4×10^7 nanometres) of length. The germ cells (sperm or ovum) each contain 23 different molecules of DNA; the 46 molecules present in most cells of the body represent 23 molecules inherited from the father plus 23 molecules with similar, but not necessarily identical, information from the mother. Thus most cells in the human body contain two non-identical but similar versions of the information for some 100,000 separate operations that are required for development and normal function.

Current data suggest that up to 50 alterations per minute (or several million a year) may occur spontaneously in the DNA of each living cell in the human body (8,9) (Table 1). Changes in DNA structure may also be caused by radiation, ultraviolet light, viruses and many environmental chemical agents.

Since the DNA coding tape, like any other organic molecule, is not inherently stable, life as we know it would probably be impossible if the living organism did not contain built-in mechanisms which repair the DNA molecule. The instructions for these repair mechanisms are also included in the DNA as part of the information necessary for life processes.

These repair systems identify and correct changes in the DNA coding tape, whether these changes arise spontaneously or as a result of exposure to environmental agents. The cancers and genetic defects which do occur naturally are believed to be the result of a small number of errors which are overlooked and therefore not repaired, or are repaired incorrectly.

Heritable genetic defects are known to be caused by changes in the genetic information, i.e., by changes in DNA. There are perhaps three basic reasons for believing that cancers are also caused by alterations in the DNA. First, any change in normal cell development, such as a cancer, presumably involves some kind of change in the genetic information controlling the normal functions of the living cell. Second, DNA-damaging agents which cause genetic defects in lower organisms usually cause cancer in mammals, and vice versa (10-12). Third, human individuals who suffer from deficiencies in their ability to repair DNA damage are also more prone than the average person to cancer development (13-16).

There are many different types of changes which can and do occur in the DNA molecule. Not all of these changes are equally likely to result in cancer. From a biochemist's point of view, radiation biologists are attempting to identify the long-term biological effects of two or three DNA defects per year due to natural radiation in a background of several million similar defects per year due to other causes (Table 1). However, it is very obvious to most radiation biologists that we still have a great deal to learn about the exact mechanisms by which DNA lesions lead to development of cancers many years later. The process of cancer development can undoubtedly be accelerated by "promoting agents" and is probably hindered by immunological factors in the healthy body.

A large part of our biology research program at Chalk River is currently concerned with an understanding of the function of DNA, with the nature of the errors introduced into the DNA by radiation, and with an understanding of the mechanisms by which this damage is repaired (7). Radiation effects are being studied with a wide variety of living organisms using advanced techniques in molecular biology, in order to be certain that we understand the effects upon other living things in our environment as well as the effects upon humans.

FACTORS WHICH AFFECT DNA REPAIR

Different types of radiation damage to DNA can be measured by a variety of physico-chemical techniques. The most prevalent types of damage appear to be single-strand breaks, various kinds of base damage including loss of a base, cross-linking of

Table 1 Estimates of the Rate at Which Defects are Introduced into the DNA of a Typical Human Cell

Cause of defects	DNA defects/cell/minute
Background radiation (0.1 rem/year)	0.000004-0.00001
Maximum occupational exposures (5 rem/ working year)	0.001-0.002
Doubling of mutation rate in male mice (10 rem/week for 10 weeks)	0.02-0.05
50 percent life shortening in mice (200 rem/week for life)	0.4-1
Spontaneous depurination	2-10
Total spontaneous degradation	10-50
Bright summer sunlight (fair skin only)	1,000-2,000
Acute exposure to 100 rem in one minute	2,200-5,000

*Data from Myers (44). Dose equivalents in rem can be converted into sieverts by dividing by 100.

strands and double-strand breaks (13). Regardless of the type of agent which caused the initial damage, most of this damage disappears from the DNA of normal organisms within a few hours as a result of active repair systems in the living organism (unless the exposure to radiation or chemical agents is so high that the repair system is unable to cope). One example of this is shown in Fig. 2. Any factor which interferes with the normal DNA repair systems might well be expected to alter the magnitude of the biological hazards of radiation. Two such factors are considered below.

(a) Hereditary deficiencies in the enzymatic DNA repair systems have been studied in microorganisms for many years. Fig. 3 illustrates the marked increase in radiation sensitivity caused by hereditary defects in two distinct repair systems in a particular radiation-resistant microorganism which is being studied at Chalk River. Much of our knowledge of the role of DNA repair mechanisms stems from this type of experiment with microorganisms.

More recently, analogous defects have been identified in humans who suffer from certain rare hereditary diseases (13-16). The clinical abnormalities associated with these hereditary diseases emphasize the importance of DNA repair systems for our normal health and development. This topic is considered in more detail in the accompanying paper by M.C. Paterson, but some further points might be noted. The data from this line of research are not expected

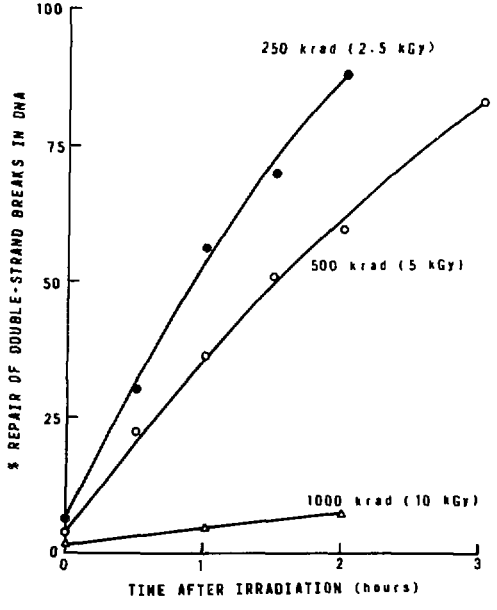

Figure 2 Repair of double strand breaks in the DNA of M. radiodurans (unpublished data from D.K. Myers and L.D. Johnson). Note that 1000 krad is lethal, 500 krad is not.

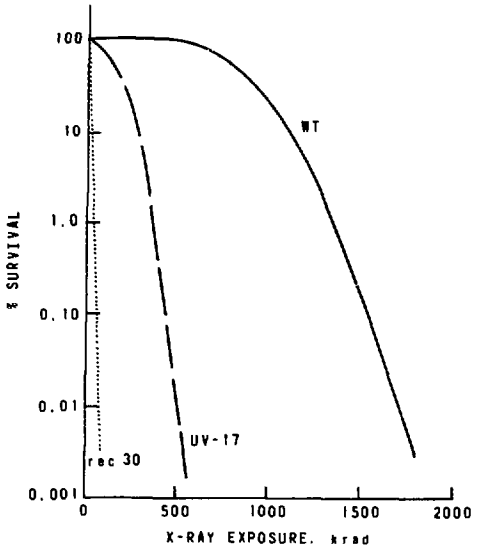

Figure 3 Radiosensitivity of normal cells (WT) of M. radiodurans and of mutant cells (UV-17, rec 30) defective in DNA repair systems (unpublished data from N.E. Gentner; note 100 krads equals 1 kGy).

to alter the risk estimates for the average persons in any way; in fact, currently accepted risk estimates are thought to be sufficiently conservative to provide some margin of safety even for a population which contains a minor proportion of radiosensitive individuals (13,17). However, it is probable that this research will help to identify those individuals in the population who are more sensitive than normal to induction of cancer by radiation and by other environmental agents (e.g., chemicals, ultraviolet light) and, even more importantly perhaps, will increase our understanding of the basic mechanisms involved in the induction of cancer by environmental agents.

(b) Chemical agents such as caffeine or acriflavine which inhibit DNA repair processes have been shown to cause an appreciable increase in the lethal effects of γ-radiation on microorganisms (Fig. 4). Synergistic interactions between γ-radiation and ultraviolet light have also been demonstrated in microorganisms (18); this appears to be due to overloading of one particular repair system with different types of DNA damage arising from exposure to these two agents. Presumably a similar synergism could occur with other carcinogenic agents or other chemicals which interfere with DNA repair.

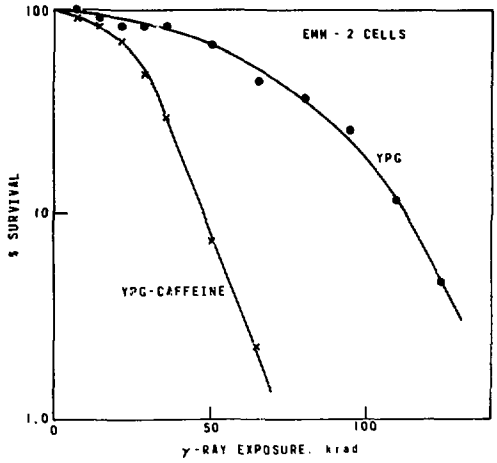

Figure 4 Radiosensitivity of yeast cells in the presence and absence of 0.1% caffeine (data from reference 45; note 100 krads equals 1 kGy).

We have therefore undertaken animal experiments to explore the induction of cancer by radiation alone and by radiation in combination with various chemical agents. To date, these studies have concentrated on caffeine (an inhibitor of DNA repair), urethane (a weak carcinogen and a promoting agent), cigarette smoke condensate (another carcinogen and promoting agent), and anthramine (a rather potent carcinogen for humans). These are long term experiments and the data are therefore still incomplete. However, it is already fairly clear that chemical agents may, under certain conditions, increase radiation effects in one system, i.e., induction of skin cancers (19), but not in another system, i.e., induction of breast cancers (13) (Fig. 5). Synergistic interactions between cigarette smoking and either alpha-radiation (from radon daughters) or asbestos have also been observed in humans (20,21).

In order to obtain some indication of the overall effects on the whole animal, we have further carried out experiments with high doses of X-radiation and of urethane (22). The results obtained with four different strains of rats indicate that the overall carcinogenic and life-shortening effects of radiation plus urethane are not greater than additive (Fig. 6).

Continued attention to the interaction of radiation and other environmental agents is obviously advisable. Any synergistic interactions occurring in human populations as they have lived over the past 30-40 years are already taken into account in the accepted estimates

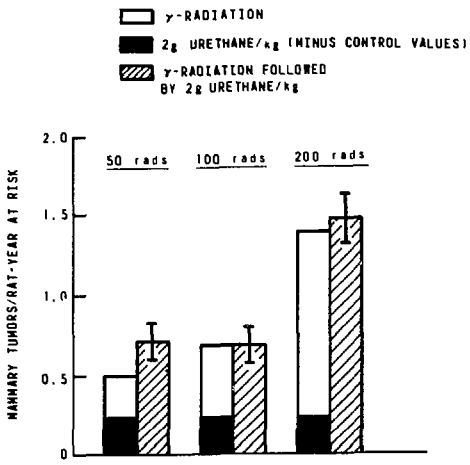

Figure 5 Induction of breast cancers in female Sprague-Dawley rats by radiation and urethane (data adapted from reference 13; note 100 rads equals 1 Gy).

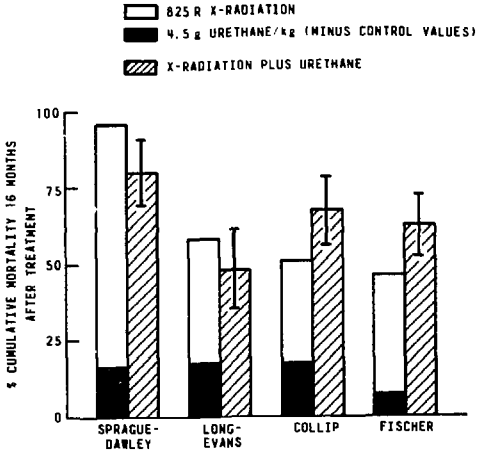

Figure 6 Cumulative mortality in four strains of rats 16 months after exposure to radiation and urethane (unpublished data from D.K. Myers; methods described in reference 22).

of the carcinogenic hazards of ionizing radiation to humans. The major problems involved appear to be, first, possible synergistic reactions between radiation and new environmental chemicals, as illustrated by data obtained at Brookhaven (with one strain of rats only and not with other strains) for cancer induction by radiation plus diethylstilbestrol (23), and, second, identification of synergistic hazards which can be minimized, as illustrated by the synergistic effects of cigarette smoking in combination with exposure to either radon daughters or asbestos in humans (20,21).

DNA REPAIR AND INDUCTION OF GENETIC DEFECTS

As noted above, most of the molecular defects in DNA that are induced by ionizing radiation, by chemicals or by ultraviolet light can no longer be detected at the molecular level after the living cells have been allowed to recuperate from the initial insult for a few hours. The disappearance of molecular defects does not necessarily mean that the DNA coding tape has, in all cases, been restored to normal. Consider, for example, what might happen during the repair of two double strand breaks which were present simultaneously and in close physical proximity to one another within the living cell. Depending upon the circumstances, repair processes could lead to (i) restoration of the normal DNA structure, (ii) inversion of a portion of the coding information within the DNA molecule, (iii) deletion of a portion of the coding tape and loss of a small fragment or its transfer to another DNA molecule, (iv) deletion of a portion of the coding tape together with the formation of a ring structure from the deleted fragment, (v) exchange of genetic information between two DNA molecules, and (vi) the formation of bridges between different DNA molecules. All of the latter radiation-induced abnormalities have been known for many years and can, indeed, frequently be seen by eye with the aid of a microscope (24,25).

Other types of incorrect repair of DNA damage are also known to occur and frequently lead to invisible minor changes or "point mutations" in the coding tape. In fact, it is suspected that most of the genetic changes which are observed after exposure of the organism to ionizing radiation, to ultraviolet light or to chemical agents are the result of incorrect repair of the initial damage produced in the DNA by these agents. There is still much to be learned about these phenomena. One of the more puzzling observations is the occurrence of "hot spots" in the DNA, i.e., certain small portions of the coding tape are more frequently altered than are other portions after exposure to a given environmental insult. There is, as yet, no known chemical difference in different portions of the DNA molecule which could account for this observation.

Some recent work at Chalk River on a particular type of genetic alteration which is induced by radiation in yeast cells may be of interest. The DNA in "diploid" yeast cells is organized into discrete, paired chromosomes which are localized within a cell nucleus; in this respect at least, yeast cells resemble human cells and differ from bacteria. However, yeast cells, in contrast to human cells, are easy to grow in a test tube and can be used for some highly sophisticated genetic experiments. Once the appropriate and rather intricate genetic manipulations have been carried out and appropriate yeast strains have been constructed, it becomes fairly simple to measure "gene conversions" induced by radiation or other agents. Gene conversion occurs when the DNA damage caused by environmental agents results in exchange of part of one DNA molecule with that of its partner; moreover, in this particular case, the exchange must occur within a given gene or coding instruction that is carried by both of the pairs of chromosomes. This exchange, it might be noted, results from the activity of one of the DNA repair systems and does not normally occur in mutant cell lines with a hereditary deficiency in this particular ("recombinational") repair system.

Radiation gives rise to this type of genetic change in yeast much more frequently than it induces the minor changes in DNA coding that are known as point mutations (13). Our interest in this particular effect stems not only from its relatively high sensitivity to environmental insults but also from the distinct possibility that this system will provide a more reliable rapid screening assay for cancer-producing agents than do the more conventional screening tests based on the ability of the agent in question to induce point mutations in microorganisms.

P. Unrau and D. Morrison have invested a considerable amount of their time recently exploring radiation effects with this particular genetic assay at the Chalk River laboratories. The main conclusions are as follows (Table 2): The number of genetic changes induced by ionizing radiation is proportional to the total dose of X- or γ-radiation over the range from one up to 10,000 rads (0.01 to 100 Gy). There is no indication of a "safe" or threshold radiation dose; there is also no evidence that effects at low doses are in any way greater than those predicted by a simple linear extrapolation from effects observed at high doses (13). The number of genetic changes induced by radiation is virtually independent of dose rate over a wide range from 0.8 up to 20,000 rads (0.008 to 200 Gy) per minute (Table 2). With this particular test system, about in every ten million "spontaneous" genetic changes could be ascribed to a background radiation level of 100 millirem (1000 μSv) per year.

A recent paper by Ito and Kobayashi (26) suggested peculiar effects of tritium β-radiation in this system. Their experiment has been repeated by Unrau and Morrison with appreciably greater precision and a wider range of tritium concentrations. The results to date do not support the preliminary report by Ito *et al.*; tritium β-radiation is not very much more effective than X-radiation and no significant dose-rate effects are observed with tritium (Table 2). The reasons for the discrepancy with the initial report by Ito *et al.* are being investigated.

DOSE-EFFECT CURVES AND DNA REPAIR

As noted above, the number of genetic changes induced by ionizing radiation can, in certain cases, be shown to be strictly proportional to the total accumulated radiation dose in microorganisms, and to be virtually independent of the dose-rate. Although the data are somewhat less precise due to the difficulty of working with large numbers of higher organisms, similar conclusions appear to be valid for the mutations that produced heritable eye-color changes in the wasp *Dahlbominus*. Previous studies by W.F. Baldwin at the Chalk River laboratories (27,28) showed that the dose-effect curve for this system was linear from 15 to 500 rads (0.15-5 Gy) and was again virtually independent of the dose-rate between 0.01 and 100 rads (0.0001-1 Gy) per minute. In both cases, therefore, the genetic change in question appears to be caused by a single damaging event in the DNA.

Other types of genetic change may depend upon two coexistent events in the DNA, as suggested earlier. In these particular circumstances, the number of biological changes induced can be highly dependent on the dose-rate of sparsely-ionizing radiations such as X- or γ-rays. The distance between "damaging events" along the track of a γ-ray is usually large. Thus, more than one γ-ray track is usually required to induce two coexistent events in the DNA which are physically close enough (e.g., less than 500 nanometers apart) to interact. At low dose rates, the first site of damage produced in the DNA by one γ-ray track may have been repaired and have disappeared before the second site of damage is produced by another γ-ray, so that interaction between the two sites cannot occur at low dose-rates. Densely ionizing particles such as alpha-particles produce many damaging events within a short distance along their track; there is thus a high probability that a single alpha-particle will produce two or more physically adjacent lesions in the DNA. The dose-rate becomes largely irrelevant

Table 2 Effect of Variations in Dose and in Dose-Rate on the Induction of Genetic Changes in Yeast (unpublished data from P. Unrau and D. Morrison, using the diploid yeast strain D7-rad52a).

Radiation source	Dose rate (rads/min)	Total dose (rads)	Gene convertants /10^8 cells/rad
Co-60 γ-rays	20,000	5,000-10,000	5.1
150 kV X-rays	86	150-5,000	5.1
	32	25-500	4.4
	3.2	1.0-50	4.2*
	1.6	12.5-50	3.6
	0.8	12.5-50	3.6
Tritiated water**	5.8	44-8,630	2.6
	0.58	43-1,730	2.0
	0.058	35-176	1.8

*Average for ten measurements at different total doses, but the increments in gene conversion at 1 and 2.5 rads total dose were not statistically significant. The average for the remaining eight measurements at 5 to 50 rads total dose was 4.4±1.2 gene convertants/10^8 cells/rad.

**Data obtained with tritiated water are preliminary and should not be used to calculate precise values for relative biological effectiveness. The relative biological effectiveness of tritium β-rays is close to one when the experiments with X-rays and with tritiated water are carried out under identical conditions.

Doses in rads can be converted into greys by dividing by 100. One rad (or 0.01 Gy) of X-radiation is equivalent to one rem (or 0.01 Sv) of X-radiation.

in this latter case (Fig. 7).

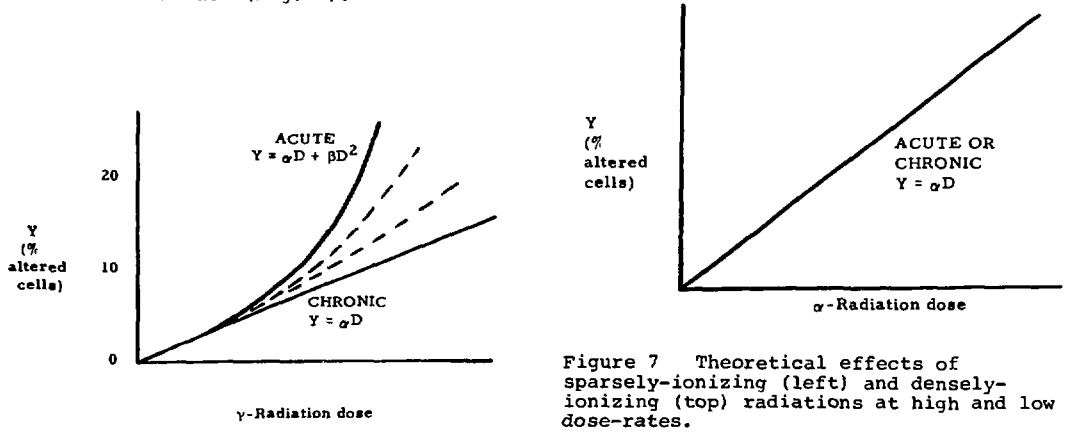

Figure 7 Theoretical effects of sparsely-ionizing (left) and densely-ionizing (top) radiations at high and low dose-rates.

However, all sparsely-ionizing radiation contains a densely-ionizing component owing to the nature of its physical interactions with matter (29,30). That is to say, occasionally a single γ-ray track may damage two sites in the DNA that are close enough to interact. A simple mathematical expression can be used to describe the observed results: $Y = aD + bD^2$, where Y is the number of induced changes, D is radiation dose and a and b are proportionality factors. The value of 'b' depends upon the biological test system used, the type of radiation, and the dose rate. In the case of sparsely ionizing radiations, e.g., γ-radiation, the 'bD^2' component will usually predominate at high doses (e.g., 100 rads or 1 Gy) and high dose rates (e.g., 100 rads or 1 Gy per minute), but only the 'aD' component remains at very low doses or at low dose rates (Fig. 7). The non-zero value of 'a' in this equation, which is caused by the densely-ionizing component of the γ-ray track, provides for a linear component in the dose-effect curve even at very low doses (down to zero) and low dose rates (e.g., 0.1 rad or 0.001 Gy per year).

Other complications need to be kept in mind when interpreting results obtained at much higher doses (e.g., 1000 rads or 10 Gy) at high dose rates. It is frequently observed that the yield of induced biological effects may decrease at these higher doses, due to the radiation-induced death or "sterilization" of the damaged cells (Fig. 8). This complication rarely affects data obtained at doses below 100 rads (1 Gy) and will, therefore, not be considered further here.

The experimental data obtained for radiation-induced somatic "mutations" (possibly small deletions) in the stamen hairs of Tradescantia (spiderwort) are in very good agreement with above considerations (31) (Fig. 8). The number of hair color changes produced by high doses (up to 100 rads or 1 Gy) of X- or γ-radiation are predominantly determined by the square of the dose at high dose rates (30-300 rad or 0.3-3 Gy per minute). As the dose-rate is decreased, the yield of induced effects at total doses of 60-100 rads (0.6-1 Gy) also decreases until it approaches a minimum value which is the same as that predicted from the 'aD' component of the dose-effect curve at high dose-rates. Rather similar effects of dose-rate are observed for induction of coat-color mutations in mice (32). An allowance for dose-rate effects is incorporated into the accepted risk estimates for the genetic hazards of low-level radiation in humans (1).

Risk estimates for induction of cancer in humans by low-level radiation are based upon the effects of radiation at high dose-rates (1) and do not make any similar allowance for a possible decrease in effects of sparsely-ionizing radiation at low

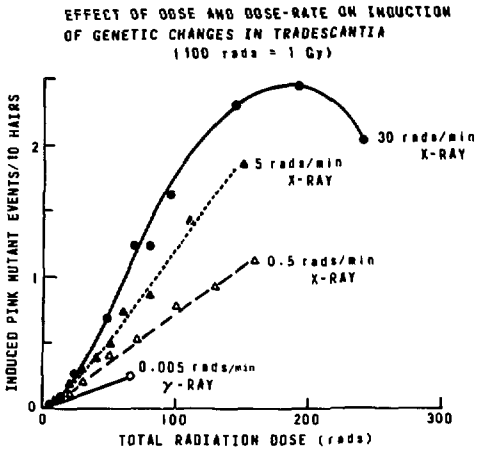

Figure 8 (data adapted from reference 31).

dose-rates. Animal experiments indicate that X- or γ-radiation at low dose-rates is frequently much less carcinogenic than at high dose-rates (Fig. 9). However, the percent decrease in efficiency at low dose-rates varies greatly from one type of cancer to another in the animal studies (33); the range extends from a decrease of a few percent in the case of breast cancer to a decrease of more than twenty-fold in the case of skin cancer. Most scientists who have considered this problem would probably agree that the accepted risk estimates for radiation-induced cancer in humans over-estimate the average risks of low levels of sparsely-ionizing radiation by perhaps two to five times. Both ICRP and UNSCEAR have in fact suggested something like this in recent publications (1,2), without putting any number on the extent of over-estimation.

One should be extremely wary of extending this conclusion to the carcinogenic hazards of radiation generally. First, as judged both from animal experiments and from the limited human data available, there is probably little or no safety factor in the accepted risk estimates for radiation-induced breast cancer in humans (1,2,4-6,33) and breast cancers do account for an appreciable portion of the total carcinogenic hazards of low level radiation. Second, the above considerations suggest that there is little or no safety factor in the accepted risk estimates for the carcinogenic hazards of low levels of densely-ionizing radiations such as alpha-particles or neutrons (34) (Figs. 7 and 9). If there are any safety factors involved in this latter case, they would have to stem from two other sources,

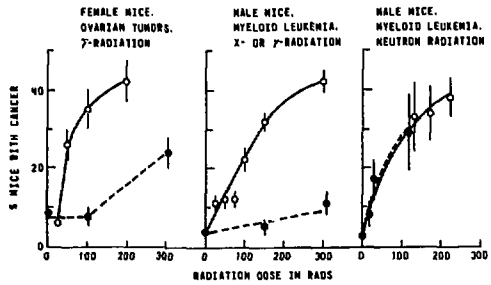

Figure 9 Numbers of cancers induced in mice by irradiation at high dose-rate (o——o) and low dose-rate (●---●) (data adapted from reference 46; note 100 rads equals 1 Gy).

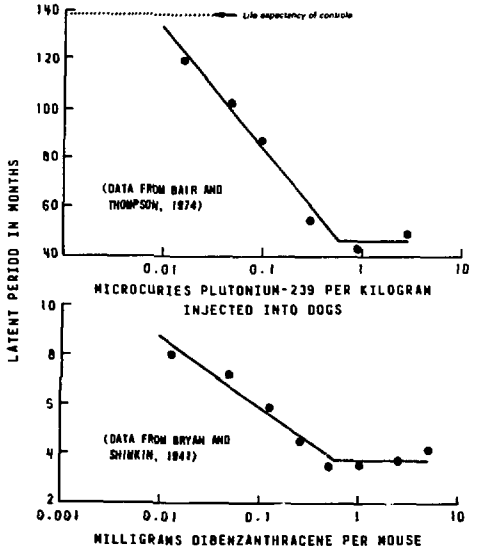

Figure 10 Variations in the latent period between exposure to a carcinogenic agent and the development of cancer (data adapted from references 47 and 48).

notably: (i) There may in some cases be an overly-generous allowance in the magnitude of the quality factors used to convert absorbed doses in rads (or grays) to dose equivalents in rems (or sieverts). (ii) As the radiation dose decreases, the latent period between exposure and cancer development may increase to a point where it exceeds the normal life span (Fig. 10); this phenomenon is poorly understood and is not well-documented,

It seems probable that estimates of the carcinogenic hazards of low levels of ionizing radiation based on the linear dose-effect model are substantially correct. There may well be some margin of safety involved in these risk estimates. However, the uncertainties involved in these risk estimates (1,32), the discovery of radiosensitive subgroups in the human population (14-16) and the possibility of synergistic interactions between radiation and new environmental agents (23) all argue against any major reduction in current risk estimates.

A few words concerning the theory of "supra-linearity" may be useful in conclusion. It has been alleged on the basis of some very shaky epidemiological data on radiation workers at Hanford that low doses of radiation cause more cancers in humans than would be predicted on the basis of the linear dose-effect model (35, 36). However, it is generally agreed that radiation workers, whether at Windscale (U.K.) (37), Hanford (U.S.A.) (38), or Pickering (Canada) (39), appear to be healthier than the average person of the same age in the general population. As noted in a recent publication, the original proponents of the above theory "did not claim that cancer was a major hazard of the nuclear industry or even that the cancer mortality of Hanford workers was significantly raised" (40). The original statistical analysis of the Hanford data (35) has been severely criticized by other scientists (38,41) and scientific discussion of these data is still continuing. The epidemiology of radiation workers is considered in more detail in the accompanying paper by T.W. Anderson.

Meanwhile, it might be noted that, with one possible exception (42), data on induction of cancer in animals by radiation do not provide any support for the concept of "supra-linearity". Data on induction of genetic changes in the DNA coding by radiation fail to support this concept. Our understanding of DNA repair mechanisms provides no reason to believe that low level radiation would be more effective per unit dose than higher radiation levels. This does not mean that "supra-linearity" is therefore impossible; research on possible alternative mechanisms is in fact supported by AECL at the Whiteshell Nuclear Research Establishment (43). A more correct conclusion from the accumulated results of thirty years of research in radiation biology would be that the concept of "supra-linearity" is high improbable.

ACKNOWLEDGEMENT

The author is indebted to J.D. Childs, N.E. Gentner, G.C. Hanna, R.V. Osborne, A. Petkau, and P. Unrau for valuable advice, and to Colleen Walters for preparation of this manuscript.

REFERENCES

1. United Nations Scientific Committee on the Effects of Atomic Radiation, Sources and Effects of Ionizing Radiation, United Nations, New York, 1977.
2. ICRP Publication 26, Annals of the ICRP, Vol. 1, No. 3, 1977.
3. ICRP Publication 28, Annals of the ICRP, Vol. 2, No. 1, 1978.
4. Medical Research Council, The Hazards to Man of Nuclear and Allied Radiations, H.M.S.O., London, 1960.
5. Advisory Committee on the Biological Effects of Ionizing Radiations, The Effects on Populations of Exposure to Low Levels of Ionizing Radiation, National Academy of Sciences, Washington, 1972.
6. Final Reports of the National Cancer Institute Ad Hoc Working Groups on Mammography in Screening for Breast Cancer and a Summary Report of Their Joint Findings and Recommendations, J. Natl. Cancer Inst., Vol. 59, p. 496-541, 1977.
7. Objectives of Research Activities in Biology Branch, Chalk River Nuclear Laboratories 1976. Atomic Energy of Canada Limited, Report AECL-5613, 1977. See also: Radiation Biology for the Non-Biologist. Atomic Energy of Canada Limited, Report AECL-5721, 1978.
8. Lindhal, T., and Nyberg, B., Biochemistry, Vol. 11, p. 3610-3618 (1972).
9. Crine, P., and Verly, W.G., Biochim. Biophys. Acta, Vol. 442, p. 50-57, 1976.
10. Ames, B.N., McCann, J., and Yamasaki, E., Mutation Res., Vol. 31, p. 347-364, 1975.
11. Bridges, B.A., Nature, Vol. 261, p. 195-200, 1976.
12. Meselson, M., and Russell, K., Origins of Human Cancer, Cold Spring Harbor Conferences on Cell Proliferation, p. 1473-1482, 1977.
13. Myers, D.K., Paterson, M.C., Gentner, N.E., Unrau, P., and Zimmermann, F.K., Late Biological Effects of Ionizing Radiation, Vol. 2, p. 447-459, International Atomic Energy Agency, Vienna, 1978.
14. Paterson, M.C., Smith, B.P., Knight, P.A., and Anderson, A.K., Research in Photobiology, p. 207-218, Plenum Press, New York, 1977.
15. Cleaver, J.E., Progress in Genetic Toxicology, p. 29-42, Elsevier/North Holland Press, Amsterdam, 1977.
16. Paterson, M.C., Carcinogens: Identification and Mechanisms of Action, p. 251-276, Raven Press, New York, 1979.
17. Myers, D.K., Summary and Proceedings of a Biology Workshop on Biological Repair Mechanisms and Exposure Standards, p. 106-144, Institute for Energy Analysis, Oak Ridge, 1978.
18. Gentner, N.E., and Werner, M.M., Molec. gen. Genetics, Vol. 164, p. 31-37, 1978.
19. McGregor, J.F., J. Natl. Cancer Inst., Vol. 56, p. 429-430, 1976.
20. Archer, V.E., Gillam, J.D., and Wagoner, J.K., Annals N.Y. Acad. Sciences, Vol. 271, p. 280-293, 1976.
21. Selikoff, I.J., Hammond, E.C., and Churg, J., J. Amer. Med. Assoc., Vol. 204, p. 106-112, 1968.
22. Myers, D.K., Radiation Res., Vol. 65, p. 292-303, 1976.
23. Shellabarger, C.J., Stone, J.P., and Holtzman, S., Cancer Res., Vol. 36, p. 1019-1022, 1976.
24. Newcombe, H.B., Encyclopedia of Medical Radiology, Vol. 2, part 1, p. 487-632, Springer-Verlag, 1966.
25. Grosch, D.S., Biological Effects of Radiations, Blaisdell Publishing Company, New York, 1965.
26. Ito, T., and Kobayashi, K., Radiation Res., Vol. 76, p. 139-144, 1978.
27. Baldwin, W.F., Radiation Res., Vol. 49, p. 190-196, 1972.
28. Baldwin, W.F., and Knight, P.A., Radiation Res., Vol. 63, p. 320-325, 1975.
29. Bacq, Z.M., and Alexander, P., Fundamentals of Radiobiology, 2nd edition, Pergamon Press, New York, 1961.
30. Johns, H.E., The Physics of Radiology, revised 2nd edition, Charles C. Thomas Publisher, Springfield, 1964.
31. Nauman, C.H., Underbrink, A.G., and Sparrow, A.H., Radiation Res., Vol. 62, p. 79-96, 1975.
32. United Nations Scientific Committee on the Effects of Atomic Radiation, Ionizing Radiation: Levels and Effects, United Nations, New York, 1972.
33. Mays, C.W., Lloyd, R.D., and Marshall, J.H., Proc. Third Intl. Congr., p. 417-428, International Radiation Protection Association, 1973.
34. Rossi, H.H., and Mays, C.W., Health Physics, Vol. 34, p. 353-360, 1978.
35. Mancuso, T.F., Stewart, A., and Kneale, G., Health Physics, Vol. 33, p. 369-385, 1977.
36. Kneale, G.W., Stewart, A.M., and Mancuso, T.F., Late Biological Effects of Ionizing Radiation, Vol. 1, p. 387-412, International Atomic

Energy Agency, Vienna, 1978.
37. Dolphin, G.W., Technical Report No. NRPB-R54, National Radiological Protection Board, Harwell, 1976.
38. Marks, S., Gilbert, E.S., and Breitenstein, R.D., Late Biological Effects of Ionizing Radiation, Vol. 1, p. 369-386, International Atomic Energy Agency, Vienna, 1978.
39. Anderson, T.W., Ontario Hydro Mortality 1970-1977 (4th annual report), Ontario Hydro, Toronto, 1978.
40. Kneale, G.W., Stewart, A.M., and Mancuso, T.F., Health Physics, Vol. 36, p. 87, 1979.
41. See, for example, the following: Mole, R.H., Lancet, Vol. 1, p. 1155-1156, 1978. Sanders, B.S., Lancet, Vol. 2, p. 840, 1978. Sanders, B.S., Health Physics, Vol. 34, p. 521-538, 1978. Anderson, T.W., Health Physics, Vol. 35, p. 743-750, 1978. Gertz, S.M., Health Physics, Vol. 35, p. 723-724, 1978. Cohen, B.L., Health Physics, Vol. 35, p. 582-584, 1978. Reissland, J.A., Technical Report No. NRPB-R79, National Radiological Protection Board, Harwell, 1978.
42. Rossi, H.H., Biological and Environmental Effects of Low-Level Radiation, Vol. 1, p. 245-251, International Atomic Energy Agency, Vienna, 1976.
43. Petkau, A., and Chelack, W.S., Biochim. Biophys. Acta, Vol. 433, p. 445-456, 1976.
44. Myers, D.K., Atomic Energy of Canada Limited, Report AECL-5715, Chalk River, 1977.
45. Gentner, N.E., Molec. gen. Genetics, Vol. 154, p. 129-133, 1977.
46. Upton, A.C., Randolph, M.L., and Conklin, J.W., Radiation Res., Vol. 41, p. 467-491, 1970.
47. Bair, W.J., and Thompson, R.C., Science, Vol. 183, p. 715-722, 1974.
48. Bryan, W.R., and Shimkin, M.B., J. Natl. Cancer Inst., Vol. 1, p. 807-833, 1941.

ISSN 0067 - 0367

To identify individual documents in the series we have assigned an AECL- number to each.

Please refer to the AECL- number when requesting additional copies of this document

from

Scientific Document Distribution Office
Atomic Energy of Canada Limited
Chalk River, Ontario, Canada
K0J 1J0

Price $3.00 per copy

ISSN 0067 - 0367

Pour identifier les rapports individuels faisant partie de cette série nous avons assigné un numéro AECL- à chacun.

Veuillez faire mention du numéro AECL- si vous demandez d'autres exemplaires de ce rapport

au

Service de Distribution des Documents Officiels
L'Energie Atomique du Canada Limitée
Chalk River, Ontario, Canada
K0J 1J0

Prix $3.00 par exemplaire