STUDY OF THE BIOLOGICAL EFFECTS OF AMERICIUM 241 ALPHA PARTICLES ON D7 AND RAD 54.3 STRAINS OF YEAST CELLS;

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MARCH,1998

DECLARATION

I hereby declare that:

i) the report presented in this thesis is the result of my original research work

ii) this thesis has not been submitted to any other institution or body for an award of a degree other than Ahmadu Bello University - Zaria

iii) all inclusions from works other than this have been duly referenced and acknowledged.

Nsikak Paulinus Okosi

Date

CERTIFICATION

This Thesis entitled **"STUDY OF THE BIOLOGICAL EFFECTS OF AMERICIUM 241 ALPHA PARTICLES ON D7 AND RAD 54.3 STRAINS OF YEAST CELLS"** by Nsikak Paulinus Okosi meets the regulations governing the award of the degree of Master of Science(M.Sc.) in Radiation Biophysics of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This piece of work is dedicated to my dear parents Obong Paulinus Udo Okosi and Chief Mrs Sarah Paulinus Okosi whose genuine proceeds saw me through all stages of my Education, and thanks to God Almighty who is the provider of all abilities.

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ABSTRACT

Two strains of yeast cells, the D7 ***Saccharomyces cerevisiae*** and Rad 54-3 -(showing temperature repair sensitivity) were irradiated with Americium 241 alpha particles installed in two irradiation geometry. This study investigates the biological effects of Americium 241 alpha particles emerging from these two irradiation geometry on the selected strains of yeast cells with reference to the survival pattern of the two strains after alpha particle irradiation and mutation phenomenon. Mutation and survival assay for the D7 ***Saccharomyces cerevisiae*** strain and survival assay under repair permissive (23°C) and restrictive (36°C) for the Rad-54-3 strain were carried out. Mutants from the D7 yeast cell strain screened for mitotic crossing over, leading to gene conversions and reversions were selected on synthetic complete (SC) medium containing marker amino salts after three to four days post-irradiation incubation period, and the mutant frequency was calculated.. Temperature-sensitive yeast cell strain- Rad 54-3 showing temperature-dependent repair, was assayed for survival after irradiation with alpha particles from 48 MBq source and screened for survivors on full nutrient (Agar) medium under incubation in repair permissive (23°C) and restrictive (36°C) temperatures for four to five days. Results show over 90% reduction in survival fraction for the D7 yeast cells after irradiation with alpha particle fluence of 1.513 x 10^9 particles cm"^{2}. Comparative' data from the X-ray irradiations yielded a D_0 of 180.7Gy for same strain. There exist a clear distinction in the survival of this strain at the two incubation temperatures with the following inactivation cross-section:- 36°c = **1-34** urn^2; and <j23*c = **0.74 urn^2**

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CHAPTER ONE

1.0 INTRODUCTION

1.1 The research goals

Study of the biological effects of Americium 241 alpha particles from 48MBq and 185MBq alpha sources on two strains of yeast cells - the D7 Saccharomyces cerevesiae and Rad 54-3 (showing temperature dependent repair sensitivity) was carried out to provide a practical evidence on the feasibility of carrying out cell assay using the respective sources mentioned above and also to give a practical basis for quantifying cell inactivation and mutation induction in relation to irradiation geometry available and specific irradiation conditions such as the installation geometry and design and source sample distance. It was also aimed at verifying the length of irradiation times required for cell inactivation from both sources on wild type cell line and a derived mutant.

The research also had as one of its goals, generating supporting data on cell repair in relation to strand break repair and synthesis as well as giving information on the response of the cell samples to both X-rays and alpha particles in comparative terms. The goals of the research also included the generation of data for the study of cell inactivation and mutation induction trends from X-rays and alpha particles in specific strain of yeast cells used. And also to generate

supporting data for confirmation of post irradiation incubation temperature effects on the cell survival of a given cell strain mutant.

1.2 Specific Contributions Of The Research

The alpha particle sources were installed in different irradiation geometry and they also have different specific activities. Thus the irradiation times needed to reduce the survivals of test samples would vary with both the geometry of irradiation source, specific activity of the source and the cell strain used as the test sample.

Response of the cell samples to the alpha particles irradiation at two installation geometry and also the irradiation times involved in reduction of survival fraction to at least 10% are areas to which this project investigates. It also ascertains mutation frequency induced from these sources on the same strain - the D7 yeast cell strain. These information became necessary in assessing the feasibility of yeast assay with these sources.

In addition to the above, the project measured the survivals of the two cell strains obtained from X-ray irradiation and compared the results with existing data as a reference point in computing future RBE values for the alpha particle data.

1.3 Objectives Of The Project

Of recent, a yeast cell mutant - rad 54-3 showing temperature-dependent post irradiation repair sensitivity had been an important

research tool in investigating the biological effects of the radiation field in space under micro gravity conditions. With its repair permissive and restrictive temperature points at 23°C and 36°C respectively, survival patterns representing the effects from the radiation field in space had been obtained. Also a series of survival patterns for the strain obtained through irradiation using heavy ions such as Ar, Ti, and U have been generated by Kiefer and Schneider, (1991), with the objective of establishing a generalised sequence of biological action from these ionising radiation sources.

As a relatively lighter particulate radiation, alpha particles data of the survival trend for the rad 54.3 yeast mutant become important as a comparative data among the queue of other heavy ions data already generated. The question of the LET dependence of the cell response to irradiation can best be addressed with the alpha particle data for the strain under due consideration.

Biological effects of radiation on the D7-yeast cell strain had been under investigation using varying LET range of ionizing radiation and tested for survival potentials of the cell after irradiation. Also the mutation frequency test using gene conversion and reversion mutation phenomena as the biological end points had been carried out extensively using x-rays by U. Stoll, (1994) and for other series of heavy ions. This and other investigations had a view of studying some LET dependence of the biological action of ionizing radiation on biological systems. The incorporation of alpha particles

data which its generation is one of the objectives of this research will further this study.

Above all, study of the biological effects of Americium 241 alpha particle source on two selected yeast cell strains was conceived due to a desire to investigate and establish a response trend of the selected cell lines to the alpha particles using some specific biological end points such as cell inactivation and mutation phenomena, namely gene conversion and reversion. Also the experiments were designed to verify and ascertain the specificity of the gene expression conferred in these strains -as a derived mutant conveying temperature-conditional repair, (that is for Rad 54.3) and a Saccharomyces cerevisiae yeast cell line with feasible recombination, gene conversion and reversion mutation phenomena, assayed under the same test specimen and conditions, (that is with reference to D7). These confirmation objectives of the study were considered necessary since an update information on the performance of these deeply-cooled cell samples are vital as they are still in active research demand.

Specifically, the research objectives included the measurement of cell inactivation by X-rays using two cell strain - the D7 yeast cell strain and rad 54-3 strain (for comparative purposes); the measurement of mutation frequency by X-rays using the D-7 yeast cell strain (for comparative purposes); the measurement of cell inactivation by alpha particles from Americium sources of different activities and different installation geometry; the measurement of

mutation induction frequencies induced from alpha particles on the D-7 yeast cell strain and its trend; study of effect of post-irradiation incubation temperature on the survival of a temperature sensitive yeast cell rad 54-3 irradiated with X-rays and alpha particles by establishing a survival curve for its inactivation trend; measurement of absolute dose (in the case of X-rays) and fluence of alpha particles required to reduce the survival of the test samples to 37% survival and the corresponding irradiation time.

1.4 Justification Of The Work

Stable genetic alterations in cell lines leading to the derivation of mutants with known specificity is of interest when noting that they form important research tools in many areas of cell biology, biophysics (molecular and radiation studies) and genetic engineering. Cell lines so derived are necessarily preserved over a time and are constantly used in verifying prevailing problems through a short-term test systems (in laboratory assays and animal assays). An update information on the potentials/specificity of a mutant cell, response to various external factors such as chemical mutagens, ionizing radiation and temperature becomes an important way of exhaustive study on derived mutants frequently available in most bio-research laboratories.

The stability of the initial alteration induced in the genetic information of a parent DNA has every possibility of showing even further flexible alterations due to competing cellular repair

mechanisms which tend to restore the initial DNA copy in the cell system. It remains a beneficiary attempt to characterise the relatively stable mutant cells based on their response to various external conditions leading to effects such as induced and spontaneous mutation and recombination, as well as cross-sensitivity to various types of ionizing radiation Game (1980).This serves as a reference for recognising the specificity of the strains in question.

Repair processes are significant subjects of investigation in most mutant cell lines. It had been hoped that the evaluation of survival results obtained for the two cell lines under investigation would show the repair trend for these cell lines after alpha particle irradiation especially so if compared with existing data generated from other irradiation conditions.

There has been a claim that lesions produced by densely ionizing radiation are often not efficiently repaired by the cellular repair mechanisms due to the pathways required. With one of the test objects chosen in this work being a cell strain with temperature dependent repair response (the Rad 54.3), there is every possibility to contribute to this claim by studying the survival trend of this cell strain and comparing this with the study done by Kiefer et. al, (1976), for heavy ion irradiation. Their results indicated that the shoulder in the survival curve persists after heavy ion irradiation and also Schöpfer et al.(1982) confirmed a similar trend for even very heavy ions such as uranium.

CHAPTER TWO

2.0 GENERAL THEORETICAL BACKGROUND

2.1 Biological background

2.1.1 Cell survival and survival curves

Cell survival and survival curves form one section of the quantitative relationships often encountered in radiobiology. There are various other quantitative relationships formulated in radiobiology but it appears that the most prominent is the dose response curve in which the survival curve is one ICRU report (1976). Other significant relationships in the field include the dependence of effect on absorbed dose rate; the dependence of RBE on absorbed dose, and the dependence of the cellular inactivation cross-section on linear energy transfer (LET). Dose effect relationships may change with a physiological variable such as oxygen tension and presence of radio-sensitisers in a specimen. Results obtained from these conditions sometimes yield very important perspectives on basic biophysical processes that are different from those obtainable from dose-response curve.

Using some definite parameters, functional relationship between biophysical variables are shown and represented on graphs in linear and, or non-linear scales. Such graphs are fitted with more than one analytical function determined by various models and hypotheses concerning the mechanisms of the observed response. By no means however, does an agreement of a set of data with a fit function

proposed by a model confirm the accuracy of the model. Failure of fit however, disproves a background hypothesis.

Bearing in mind that radiation in sufficient amount lead to death of cells, tissues and whole animals and can also cause many kinds of functional or phenotypic changes in these animals such as mutagenic, oncogenic or tetratogenic as well as cytological changes. The effects also include non-lethal chromosome aberrations, small colony formation and other forms of heritable damages. Representation of these phenomena as a function of the applied radiation dose is an important quantitative method. Dose effect curves can take various shapes as seen in fig 2.1. The effect sometimes start out as a linear dependence on dose (curve B); with negative curvature (curve A), or with positive curvature (curve C). The curve may not even separate from the abscissa until a definite value of dose is reached as in curve D. At high doses there exist other varying possibilities in which the curve may follow, example,. continue to increase-(curve E), saturate-curve F, or due to competing processes decline as in curve G.

As mentioned earlier, curves are frequently fitted with convenient functions and when considering the survivors from a given cell population treated with radiation, it is convenient to express the dose response-curve in terms of the survival fraction of the original cell population as a function of the applied dose. For a homogeneous population of single cells (or micro-organisms) three basic forms of survival curve fits are predominant as represented in fig 2.2. In its simplest form, curve A (fig 2.2) is a fit with the function of equation 2.1 .

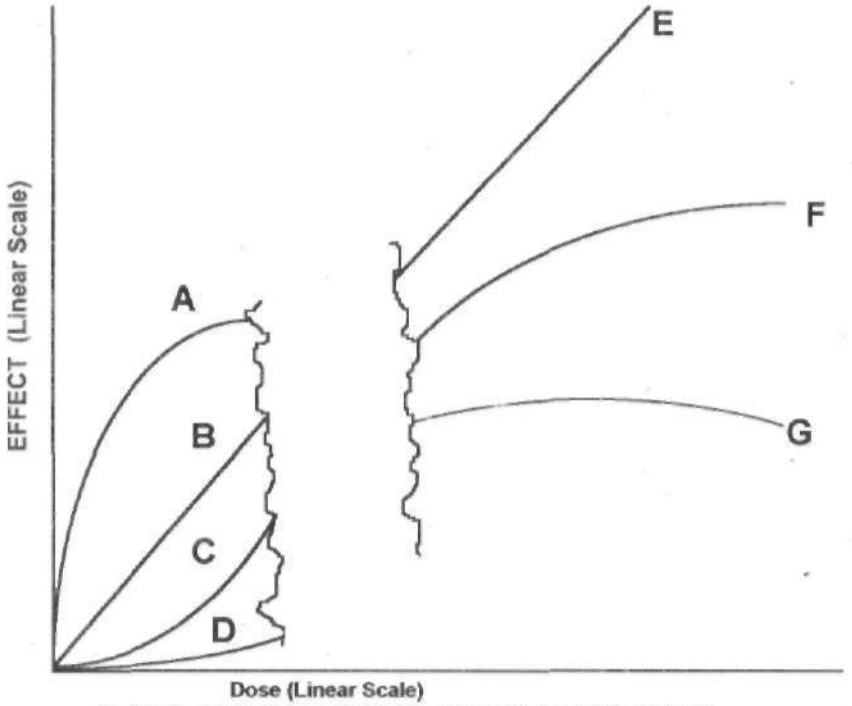


fig 2.1: Representation of various shapes of dose-effect curve
Adopted from CRU (976)

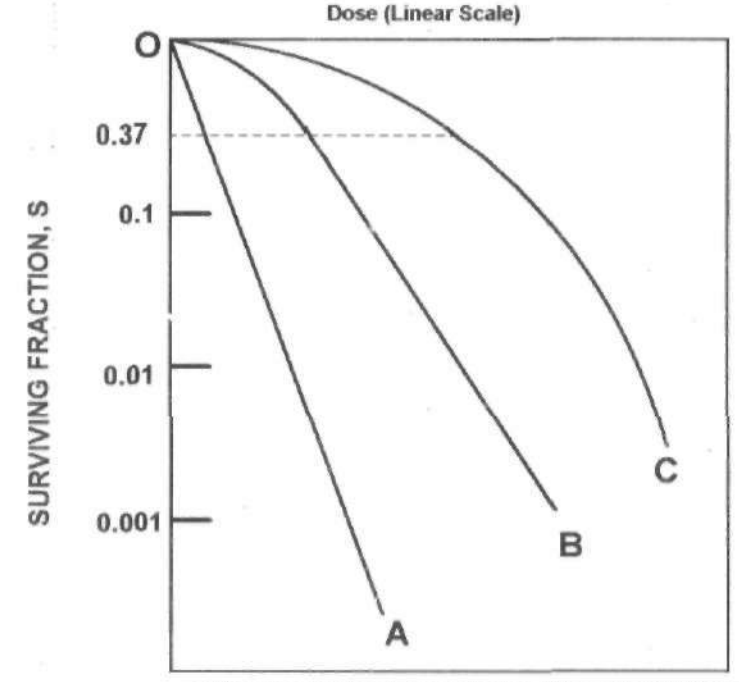


fig 2.2: Representation of various shapes of survival curve fits
Adopted from CRU (976

adopted from ICRU, 1976; where S(D) is the surviving fraction after a single absorbed dose D, and k is the slope of the curve on a semi-logarithmic plot and its inverse (1/k) as shown in equation 2.1 is

$$S(D) = e^{-kD} \quad \text{.....(2.1)}$$

known as the mean inactivation dose and is defined as the dose D required to reduce survival to 37 percent that is ~ 100(1/e) percent. This is designated D_{37} and for exponential survival curve A; $D_{37} = D_o$. In another representation(B) of the survival curve fitted with the function;

$$S(D) = 1-(1-e^{-k_n D})^n \quad \text{.....(2.2)}$$

where the inactivation constant k_n represents the sensitivity of n targets, each of which must be hit to kill a cell.

Shouldered survival curves as in fig. 2.2C which appear to have negative initial slope and which turn out to become exponential at survivals below 10 percent may be more closely fitted by a 'modified multi-target, single hit ' survival expression ICRU (1976) given as follows;

$$S(D) = e^{k_1 D}[1-(1-e^{-k_n D})^n] \quad \text{.....(2.3)}$$

The shoulder width sometimes termed quasi-threshold dose of the curve, D_q, is equal to the dose at which a back extrapolation of the exponential part of the curve crosses the level at which the surviving fraction is equal to 1.0. The back extrapolation intersects the ordinate at the extrapolation number, n which is a measure of the shoulder of

the survival curve. The parameters n, D_q, and D_o are mathematically related thus; (representations are also seen in fig 2.3).

$$\frac{Dq}{Do} = \ln (n) \quad \text{.....(2.4)}$$

where **ln (*n*)** is the normalised shoulder width i.e. the shoulder width normalised by D_o. (see fig 2.3 for the representation of the survival curve parameters)

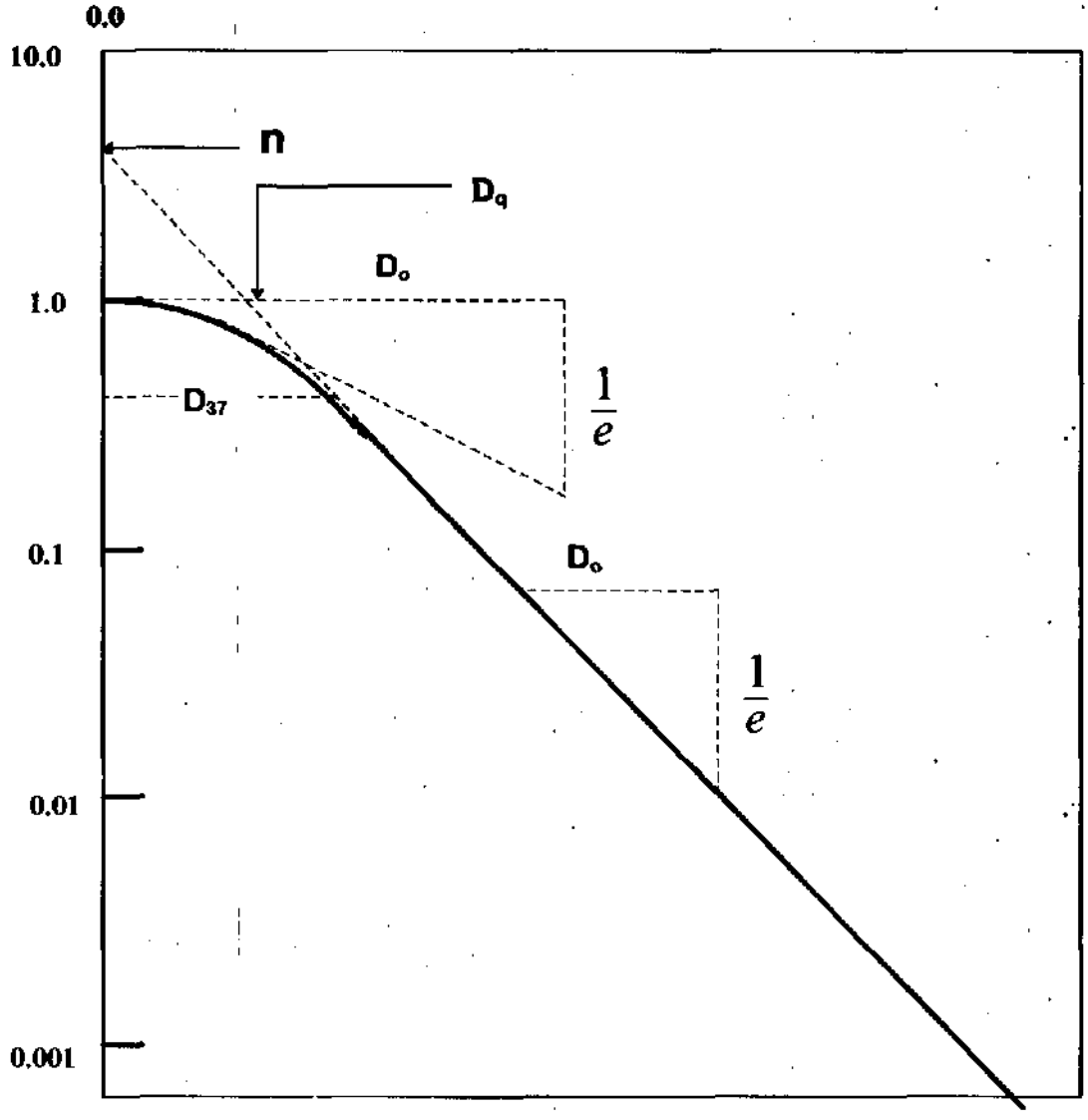


fig 2.3: Representation of survival curve parameters
Adopted from CRU (976

2.1.2 Suitability of the yeast cells for mutation studies

Saccharomyces cerevisiae is an excellent organism for studying the genetics of DNA repair processes and genomic mutations since it has a versatile and well-characterised genetic system, and its unicellular nature is convenient for studies in radiation biology and yeast mutagenesis Game, (1981). In 1967 Nakai and Matsumoto, Zakharov and Kozhina reported the first ***Saccharomyces cerevisiae*** mutants sensitive to UV radiation and in a short time many other workers reported isolation of numerous other UV-sensitive mutants. These include Snow, 1967; Cox and Parry, 1968, who isolated 96 mutants that fell into 22 separate genes. With the wealth of information about the genome of the ***Saccharomyces cerevisiae***, it is appropriate to say that the choice of the yeast cell as a test object in radiation study is quite a good option.

A standardised nomenclature for radiation-sensitive mutants was discussed at the yeast genetics meeting at Chalk River in Canada - 1970, and it was proposed that the general designation RAD, followed by a locus number, be used for genes that mutate to confer radiation sensitivity. Locus numbers from 1 to 49 were set aside for genes whose mutant alleles primarily conferred sensitivity to UV light, whereas locus numbers from 50 upwards were to be used for genes primarily controlling sensitivity to ionizing radiation. Various strains of the yeast cell have been identified and characterised for mutation-related and other forms of genomic studies. The yeast ***Saccharomyces cerevisiae*** possess at least 8 genes which are

involved in repairing one or more types of lesions induced by X-rays, Game and Mortimer,(1974). Also it has been mentioned that radiation sensitivity in the yeast ***Saccharomyces cerevisiae*** is under the influence of more than 90 genes,{Kiefer, J. (1990). An allele of an X-ray sensitive gene rad 54-3 shows a temperature conditional response to X-rays They are sensitive to X-rays at 36°C but show normal resistance at 23°C, in addition it has been shown that they also have temperature conditional double-strand break repair. Ho and Mortimer, (1975).

The D7 diploid strain was described by Zimmermann et al. (1975). It has been found out already that the strain D7 carries two non-complementing alleles, Trp5-12 and Trp5-27, which cause a requirement for tryptophan. Restoration of prototrophy is achieved in this system by intragenic recombination, mostly non-reciprocal gene conversion. There is also a homozygous condition for allele Ilvl-92 which causes a requirement for isoleucine. Restoration of prototrophy can be brought about by mutation of the mutant allele itself (reverse mutation) ***Saccharomyces cerevisiae*** strain D7 carries markers which permit simultaneous study of mitotic crossing-over, gene conversion and reverse mutation thus provide a unique opportunity to compare response of these three distinct genetic end-points in a single strain.

As reported by James and Nasim (1987), yeast, especially ***Saccharomyces cerevisiae*** are among the most frequently used tools for studying the effects of both ionizing and non-ionizing

radiation on biological systems. Much of the popularity of this species can be attributed to the fact that it is a single-celled eukaryote. Being single-celled, it can be subjected to the same finely controlled experimental conditions that are so advantageous in bacterial systems. Also, being an eukaryote, an understanding of its responses to radiation frequently has a better relevance in the interpretation of radiation effects in higher organisms, including man. Other attractions of the ***Saccharomyces cerevisiae*** to radiobiologist include the fact that it has a well-defined genetic system that is open to tetrad analysis, a simple life cycle that can be controlled in the laboratory, an ability to exist as a stable haploid, diploid or even in a state of higher ploidy, a cell size that is adequate for manipulation and microscopic study of single cells spores, a mitochondrial system that is particularly amenable to investigations, and a capability to be transformed in gene-cloning studies.

2.1.3 Mechanisms of specific cell mutation of interest

The yeast cell having been shown as a favoured test object in mutation studies has many mutant lines which are gene-specific in response to radiation and other influences. In this investigation, two yeast cell lines have been chosen as test objects for assay with Alpha particles from Americium 241. One of which confer temperature-dependent radiation-sensitivity - Rad 54.3; and the other carries markers which permit simultaneous study of mitotic crossing-over, gene conversion and reverse mutation phenomena. This area of

interest can be best explained by reviewing the concept of these mutation phenomena and their detection;

Recombination:

The occurrence of radiation-induced genetic recombination in vegetative diploid yeast cells has been recognised for many years by James and Lee-Whiting,(1955). The phenomenon is by no means restricted to yeast but is more successfully studied in this organism since it can be identified with ease and accuracy. Mitotic recombination in yeast is observed in the D7 yeast cell strain with the adenine locus. There is a pair of complementing alleles, Ade2-40 - causing an adenine requirement and forming deep red colonies and Ade2-119 -not causing a stringent requirement for adenine but causing a pink pigmentation of colonies. Both alleles being recessive, the D7 cells form therefore white colonies. However, by mitotic crossing-over and recombination processes a homoallelic marker locus can be restored in both, or in either of the loci, thereby leading to formation of pink or deep red coloured colonies from the cell strain during incubation/cell culture.

Identification of such coloured colonies is achievable by including a moderate concentration of the amino base adenine(5mg/l) in an incubation media where recombinants are screened.

Zimmerman, (1974) summarised the phenomenon of recombination, gene conversion and reversion as follows;

Gene conversion:

In the D7 strain are two non-complementing alleles at the trp-locus. These are the Trp5-12 and Trp5-27 which cause a depressive requirement for tryptophan since they are both recessive. Intragenic recombination leading to non-reciprocal gene conversion restores heteroallelic condition in the daughter cells of the mutated D7 which implies a restoration of the wild type condition -with 50 percent error free limits, and this has tryptophan proficiency. Screening for gene conversion phenomena is achievable by the use of selective media free of tryptophan for selection. Fig 2.4 shows a scheme of chromosomal exchange leading to gene conversion and restoration of the wild type in D7 yeast cell strain.

Reversion:

The isoleucin/valine-locus codes for threonin-dehydrates which is inhibited by isoleucin feedback Gundelach,(1973). The ***Saccharomyces cerevisiae*** D7 is homoallelic in this locus - ilvI-92 causing a high desire for isoleucin. Chromosomal exchange conceived as a mutation of the mutant allele itself , that is, reverse mutation, or the action of some suppresser gene -which complements the initial mutation, Zimmermann et al.(1975), thus leads to heteroallelic condition in this locus, hence terminating the high demand for isoleucin. Fig 2.5 shows a sequence of chromosomal representations illustrating real gene reversion or conceived gene suppression in ***Saccharomyces cerevisiae*** D7 yeast cell strain.

Gene Conversion in D7 Yeast Cell Strain

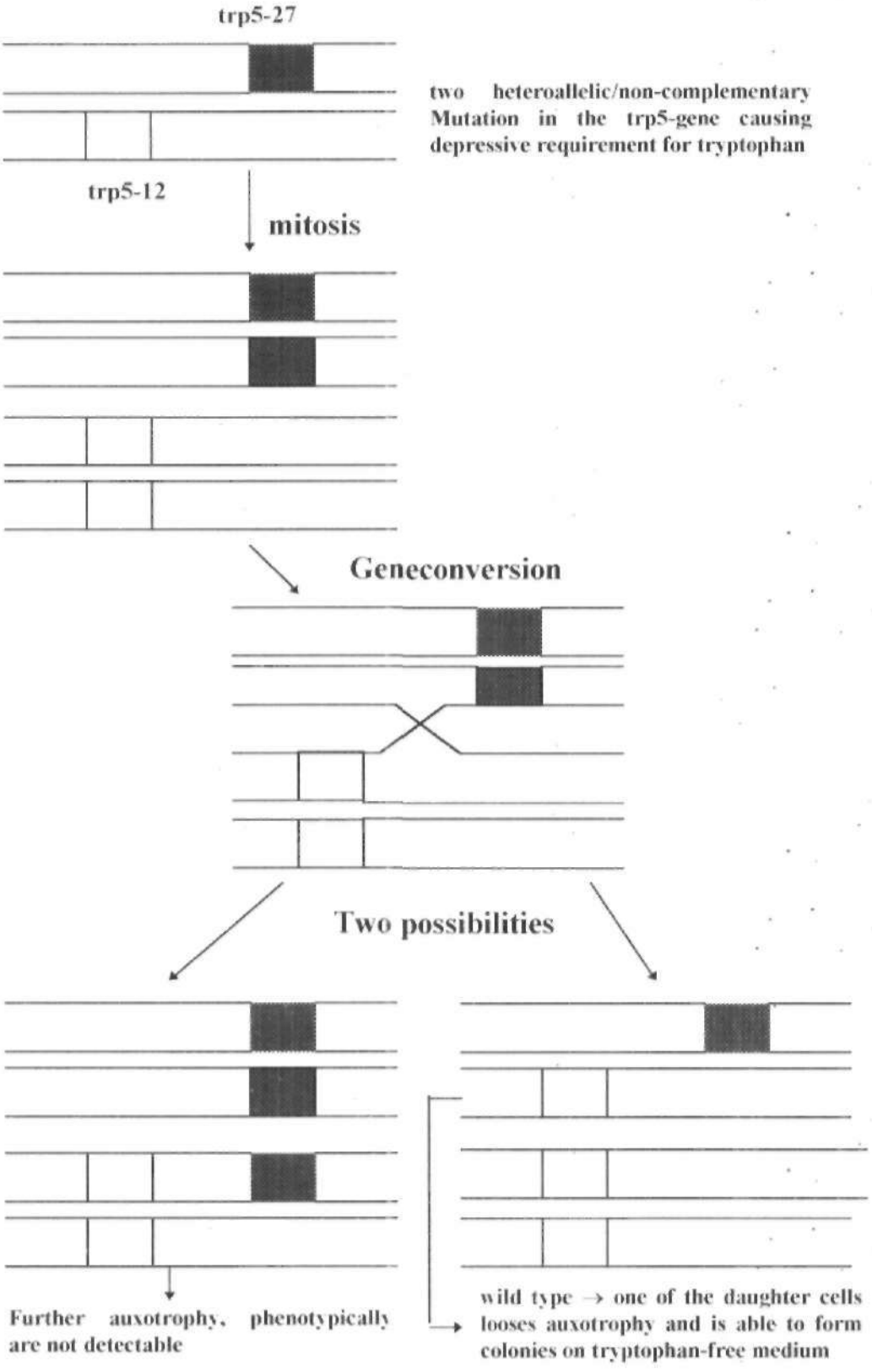


fig 2.4 Sequence of chromosomal exchange leading to gene conversion and restoration of wildtype in D7 Saccharomyces Cerevisiae.

Reversion in D7 Yeast Cell Strain

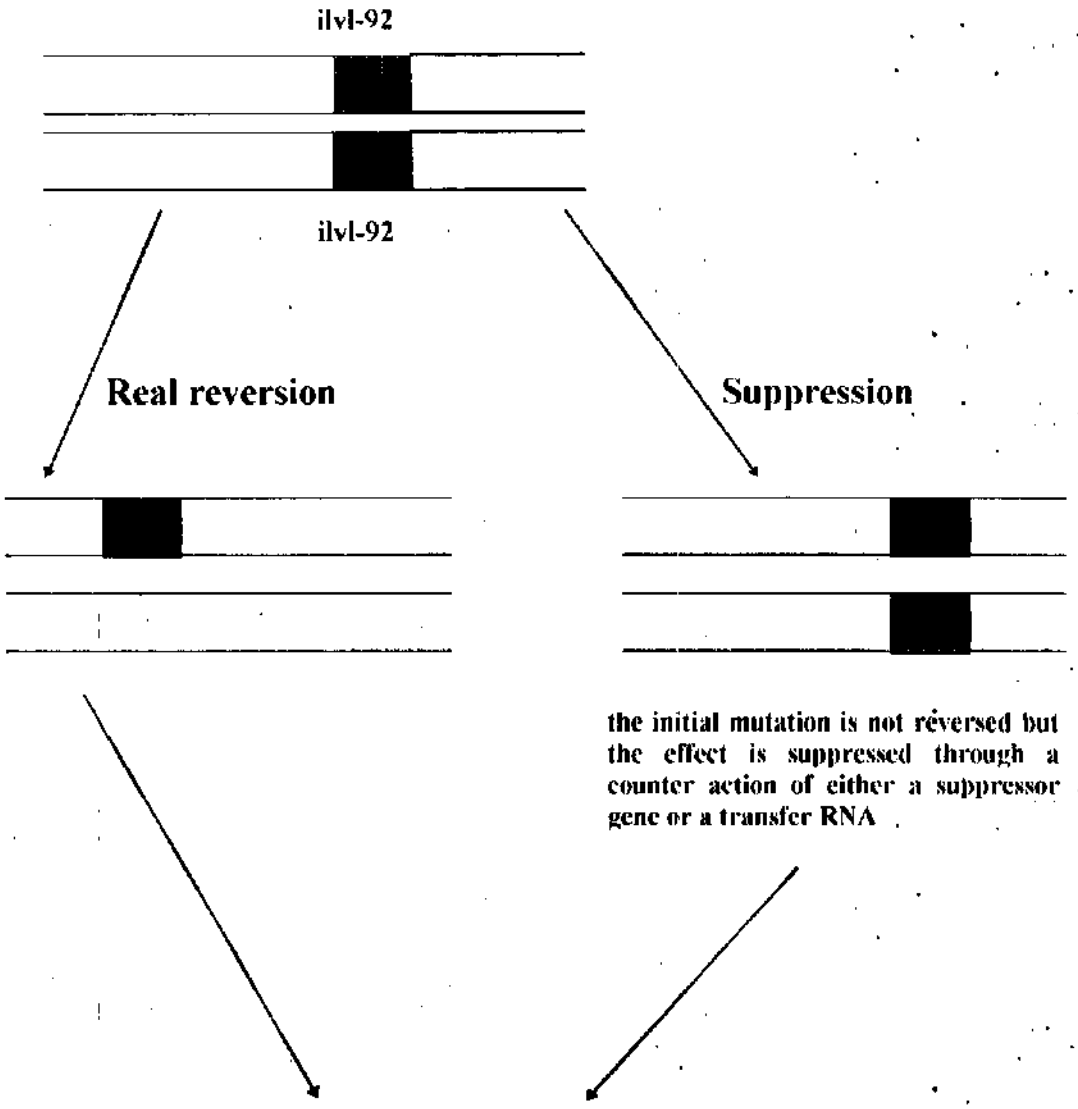


fig 2.5: Sequence of chromosomal representations illustrating gene reversion and true suppression in D7 Saccharomyces Cerevesiae

2.1.4 Time-scale radiation effects on biological systems

Energy loss from a charged particle is a characteristic parameter which is defined by the stopping power of the particle type considered, and this depends on the effective charge of the particles and on the electronic composition of the medium including factors such as electron charge density and ionization potentials. Energy absorbed in a medium accounts for the observable biological effects and this is an indication of the effectiveness of the radiation type. It is described by the linear energy transfer LET at a locally restricted values(100eV energy restriction for electrons) since it is argued that only that energy fraction is counted which leads to ionizations and, or excitations within the considered site. And this localised energy deposition is significant in biological radiation effects.

Claims of biological response to irradiation has been identified in relation to the absorbed dose and post-irradiation time-scale length. This is summarised in the scheme of fig (2.6) The time lapse between the physical interaction of radiation with the biological entity and the initiated chemical changes is remarkably short. These chemical changes in turn initiate the biological effects within the circumstance of the process and these are observable either in a short while or in some latter future, or even in latter generations.

The chemically mediated processes are seen to take place within a period of milliseconds or less after irradiation, and these processes are the initiated phase of cellular damage in biological systems.

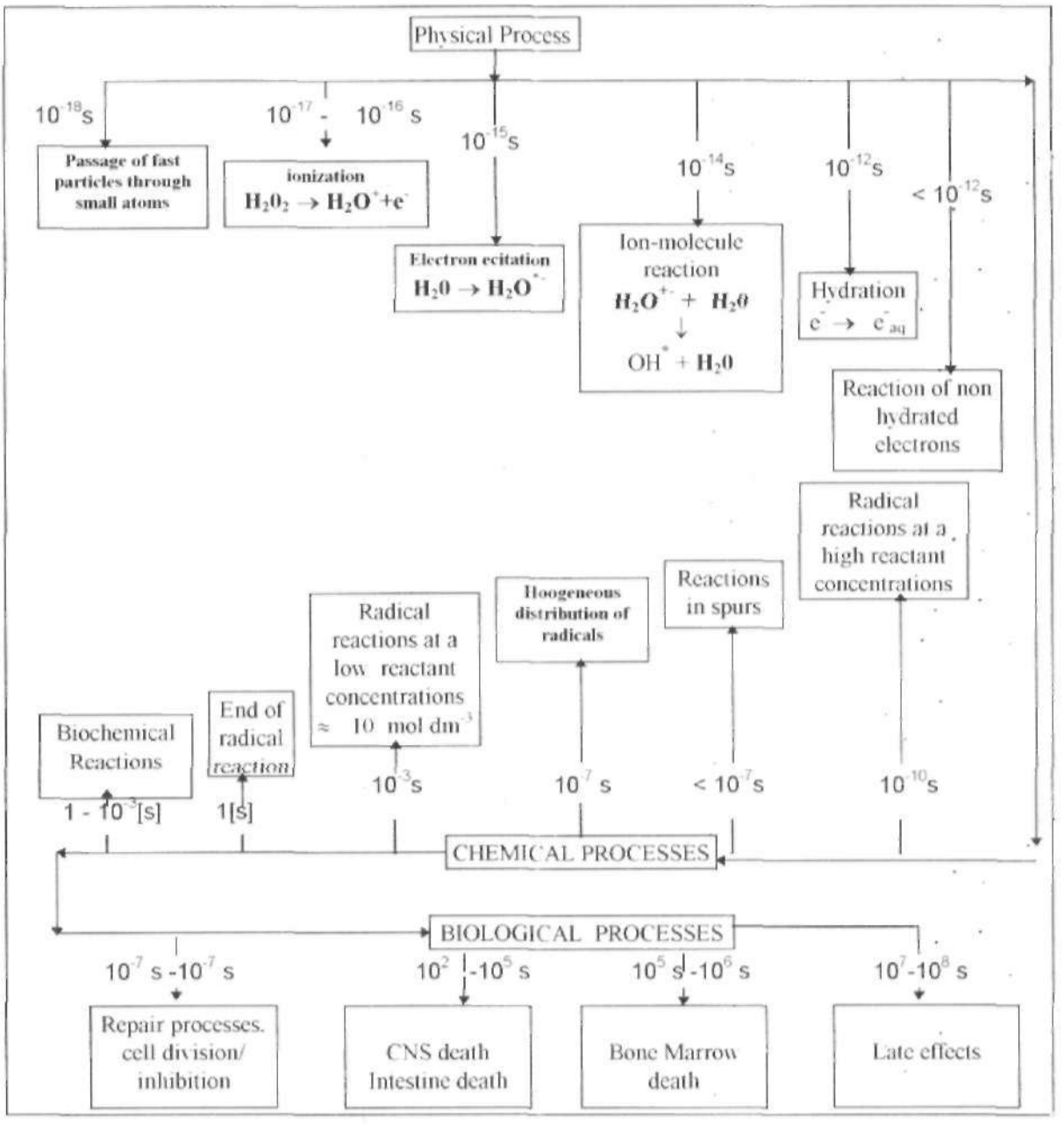


fig.2.6: Time-Scale effects of ionizing radiation showing the physical,chemical and biological processes
Modified after Kiefer, 1990.

2.1.5 Relative biological effectiveness

It can be shown that survival behaviour of cells after exposure to ionizing radiation has a dependence on radiation quality which in turn relates to the manner in which the radiation energy is deposited at the microscopic volume element of the cell, and this also depends on the density of such ionization events. Closely or sparsely ionization events characterize the radiation type in terms of track structures and the linear energy transfer, and the observable biological effects of the various radiation types are described in terms of relative biological effectiveness (RBE) of the radiation. RBE is defined as the ratio of dose which yields the same effect when the test radiation is compared with 250kV X-rays or cobalt 60 gamma radiation. Though this is not generally acceptable definition, it describes the comparative basis leading to the RBE concept itself, Kiefer,(1990).

$$RBE = \left[\frac{D(250\ KV\ X\text{-}rays)}{D(test\ radiation)}\right]_{iseffect} \qquad \text{.....(2.33)}$$

RBE factor is meant to depict a simple quantitative description relating radiation quality to observable biological radiation effects such as in cell survival. RBE values are readily deducible from dose-effect curves and seem to vary with varying geometry of the dose-effect curve. For survival curves where the dose-effect relation can change from a shouldered to an exponential type, the RBE values become larger with small doses and may even approach infinity if the slope at the origin of the shouldered curve is zero.

2.1.6 Radiation damage to dna and the repair mechanisms

There are various types of induced damages caused to the DNA of cells and various mechanisms of cellular repair of these damages. The efficiency of a repair process depends to a certain extent on the nature of the damage. It could be shown clearly that repair mechanisms are of various types and take place in a unique nature of damage.

Damage caused to the DNA by ionization is thought of as a two-fold event. Those caused through the direct absorption of radiation energy by the DNA and others caused by a secondary action of induced chemical radicals both at the aqueous DNA environment, and on the DNA itself. These effects categorized as direct and indirect effects, lead to DNA damage which range from many chemical changes in components of the DNA to the complex base destruction, single and double strand breaks of the nucleotide; liberation of nucleosides with a damaged sugar, and loss of the biological function of the DANN, that is, transcription and replication.

Directly or indirectly, by the action of chemical radicals, radiation can induce changes in the sequence of bases and therefore alter genetic code. The process which is referred to as mutation - which is a sudden random change in the nucleotide sequence of a DNA results in alteration of genetic information and as a consequence, may cause the cells and all cells derived therefrom to differ in appearance or behaviour. This is referred to as a change in phenotype. Other possible alterations include point mutation or replacement of one nucleotide by another, clastogenic mutation including insertions and deletions which

is the addition or removal of any piece of DNA from one base pair to quite extensive part, and also inversion which is the excision of a portion of the double helix followed by its re-insertion in the same position but in a reversed orientation, Gonzalez, (1994)}. Mutation is passed on from an individual cell to the progeny derived through its reproduction or replication.

Un-repaired mutation is responsible for the detrimental fate of a mutated cell, and if a mutation is not properly repaired, the outcome may result in a two-fold possibility; either the cell dies, for instance, through apoptosis, or it survives as a viable but transformed cell that may give rise to a new family of mutant cells.

2.1.7 Associated dna repair mechanisms

Excision repair

Excision repair starts with the creation of a single strand incision just before the damage position in the DNA strand involved. The lesion is subsequently removed and the resultant gap is filled by "repair replication" using the undamaged strand as the template. The remaining discontinuity is then sealed, hence a complete restitution of the originally damaged site is achieved.

This process is the best known repair mechanism and it is quite an important one. It plays a vital role in restoration of radiation-induced damage as well as in removing all kinds of lesions found in cellular DNA. It could therefore be seen as a central mechanism for maintenance of genetic information, Kiefer, (1990)

Post replication repair

Post replication repair represents another repair pathway where those damages not removed by the excision repair are taken care of. The process involves recombinational events between old and new strands and sometimes by translesion synthesis as in the case of mammalian cells. The original damage having been instituted in the old strand leads to a block of the replication polymerase at the site of the damage. A resumption of the DNA replication at a latter point beyond the damage leaves a gap in the new strand opposite the lesions. These gaps may be filled by a recombinational exchange from the complementary old strand where the respective information are available. Therefore, the gaps which are now in the old strand are filled by repair replication and the newly synthesized strand is used as a template. The limitation of this repair process is that the damage itself is not removed in the initial strand, rather, it is assured that at least the new strands are free of errors and therefore cell proliferation can proceed though not every progeny is viable.

SOS repair

SOS repair seems to be a last-resort repair process for a cell to save its viability after a fatal damage. The process attempts to repair the induced damage by every means through a combination of enzymatic activities and gene activation and inactivation. Exonuclease activity controls the exact insertion of nucleotides and if a wrong one is incorporated, this is immediately removed. Where the DNA contains a lesion in which obviously a correct base pairing can never be achieved,

DNA synthesis stops here in a dynamic manner and every attempt to fix in a wrong base is followed by a subsequent removal. Under the SOS-inducing conditions, the "proof-reading activity" of the base pairing is shut off so that nucleotides can be inserted opposite to the lesion and this is done at the expense of possible errors. The SOS-repair mechanisms appear therefore to offer just a necessary but not a sufficient pre-requisite for DNA synthesis to continue.

After translesion synthesis, the SOS-induced factors are no longer produced and the original state is re-established. This repair mechanism is a fascinating example how mutations may be produced as a consequence of "misrepair".

Mismatch repair

Under normal repair conditions, or in induced radiation damage, or even in recombinational exchanges, wrong basis may be incorporated in newly synthesized DNA strands causing a "mismatch". Cellular gene activation processes are induced to discriminate between the newly synthesized and old strand of the DNA and this is achieved by the fact that the old strand carries a methyl group at adenine base which is added to the new strand just some considerable time after replication. If a mismatch occurs between two of these sequences, the two strands are separated with the aid of some induced protein (mut U protein). A stretch of the new DNA containing the mismatch is excised by a multi-gene product and the resultant gap is filled by repair replication in the usual way. There is indirect evidence that mismatch repair exist even in higher organisms but little is known about the mechanisms.

Single-strand break repair :

Single strand break repair is considered quite a complex repair mechanism in this discussion since a number of chain events are believed to take place in about three outstanding phases to effect this repair. The first part being very fast (in milliseconds) represents purely a chemical reaction since enzyme mediated reactions are relatively slower. The second phase takes a longer time and can be shown to depend on the function of DNA polymerase. The third phase is involved with some recombinational events.

DNA single strand breaks (SSB) are one of the most frequent lesions with ionizing radiation and there exist experimental indications that they can be repaired by the cells.

Double-strand break repair:

Double strand breaks (DSB) had been considered originally to be irreversible due to its nature of drastic alterations. Recent findings of DNA super structure has brought light in support of DSB repair possibilities, though clear mechanisms are still speculative. "Misrepair" may be one of the fundamental approaches though there exist no experimental evidences. Recombinational processes have also been suggested to play a role in DSB repair mechanisms and this is still subject to prove.

DSB repair has been found in bacteria, lower eucaryotes like yeast as well as in mammalian cells. DSB are thought of as one of the major lesions responsible for cell killing. DSB repair is known not to be a fast

process, but takes a few hours for completion in mammalian cells for example, Kiefer,(1990).

2.2 Physical Background

2.2.1 The Effective Charge

Ions interact with matter by way of electron capture at low energies, or collision with atomic electrons - medium and high energies, and through nuclear collisions and nuclear reactions, that is for very high energetic ions. The collision process of charged ions with the shell electrons of atoms of materials is the most prominent of the others and this is mediated by the electric field of the two partners. The energy deposition depends on the impact parameter - closest separation between the centres of the interacting particles. "Glancing" or "soft" collision with large distances leads to small energy deposition; and with smaller impact parameters implying energy transfer large compared to the electrons binding energy, the interaction situation reduces to a case similar to the Kepler's problem in which the differential interaction cross section has been shown by Kiefer, (1990) to relate as follows;

$$\frac{d\sigma}{dT_2'} = - \frac{2\Pi Z^{*2} e^4}{(4\Pi\epsilon_0)^2 . m_{eo} v_1^2} \cdot \frac{1}{T_2'^2} \quad \text{.....(2.5)}$$

where; Z^* = effective charge which is generally different from its atomic number

m_{eo} = electron rest mass

e = elementary charge

v_1 = velocity of the ion

ϵ_o = dielectric coefficient

Multiplying equation 2.5 with the electron density of the medium dN_e/dV yields a total differential cross section σ and this results in the following;

$$\frac{d\sigma}{dT_2'} = - \frac{2\Pi Z^{*2} e^4}{(4\Pi \epsilon_0)^2 . m_{co} c^2} . \frac{dNe}{dV} . \frac{Z^{*2}}{\beta^2} . \frac{1}{T_2'^2} \quad \text{.....(2.6)}$$

where β is the relative ion speed. The first term of the above equation 2.6 consist of physical constants solved to a value of 2.55 x 10^{-23} eVm^2. With the electron density in water of $3.34x10^{29}m^{-3}$ typical of a biological medium, equation 2.6 for water medium reduces to the following;

$$\frac{d\sigma}{dT_2'} = C . \frac{Z^{*2}}{\beta^2} . \frac{1}{T_2'^2} \; ; \; C = 8.5 \times 10^6 cV/m \quad \text{.....(2.7)}$$

Z^* (the effective charge depends on the ion energy and the interacting medium. It becomes smaller with low velocities because of electron capture. Models formulating expressions for calculation of effective charge has been proposed, but due to lack of experimental measurements, there are many uncertainties. However, a close approximation of effective charge calculation using water as the interaction medium is the Barkas,(1963) equation given as;

$$Z^* = [1 - \exp(-125\beta/Z^{2/3})] \quad \text{.....(2.8)}$$

Glancing collision has been said to transfer only small energy amount though the transfer probability and its occurrence is high and this fact makes the process quite important. Kiefer,(1990) has shown that average energy loss of the ion per distance travelled can be obtained from quantum mechanical solution of the following relations

$$\frac{-dT_1}{dx} = \int_{T'_{2\,min}}^{T'_{2\,max}} T'_2 \cdot d\sigma(T'_2) \quad \text{.....(2.9)}$$

Noting that $\frac{T'_{2\,max}}{T'_{2\,min}} = \left(\frac{2m_{eo}v^2}{I}\right)^2$; (I = Ionization potential);

hence equation (2.13) is given by the following approximation of Bethe-Bloch formula;

$$\frac{-dT_1}{dx} = 2C\frac{Z^{*2}}{\beta^2}\left[\ln\frac{2m_{eo}v_2^2}{I(1-\beta^2)} - 2\beta^2 - \delta - U\right] \quad \text{.....(2.10)}$$

δ and U are shell and density corrections.

2.2.2 Energy loss, linear energy transfer (LET) and range

Energy deposition events in an irradiated body is generally mediated by charged particles. These particles initiate ionization in the medium and thereby loose energy in successive steps of collision until the end of their range is exceeded. Characteristic of the radiation type is the track structure, where more or less closely spaced ionization, - that is ionization density, is an important parameter of energy deposition onto very small sites.

The energy loss of a particle per unit distance travelled in the medium is a parameter describing the linear energy transfer (LET) which is defined as the amount of locally absorbed energy per unit length. LET relates strongly to locally absorbed energy since it is considered that energy depositions which lead to ionization, and or excitations within the considered site are of significant local effect. Absolute specification of site dimensions is not possible in defining LET values, however, in the case of electrons having relatively long ranges, specification of energy limit below which energy deposition is considered local has been made and this is widely accepted for 100eV - energy restriction of LET limits. Range restrictions of LET values could also be made. The total transferred energy per unit length of a charged particle is the stopping-power (-dT/dx) and this is numerically equal to LET . There are however some differences in the two concepts. The stopping power concept deals with energy loss of the particles and the LET concept is concerned with energy deposition in the medium, Kiefer,(1990).

In the LET concept, energy deposition is viewed as a continuous process and the stochastic nature and variations of these events are not taken into account. The applicability of the LET concept to describe radiation quality in relation to observable biological effect is thus limited.

2.2.3 Radiation characterization

Biological radiation effects are typical of the radiation type, test sample and even of the test method. Sometimes it is possible to keep

the last two factors constant with the view of examining the effect of the former - radiation type on an observable biological end-point. To reach a systematic judgement in this respect a characterization of radiation types becomes an important point of view and attempts has been made to achieve this based on several perspectives and these are as follows:

Radiation quality:

Several attempts had been made in the past to use various dosimetric parameters to characterise radiation quality. Most of such parameters were valid for a few types of radiation only. These include; the peak voltage and the half value layer for 30kV; 2ma X-rays; the particle energy for neutrons, gamma rays and high energy electrons; and the parameter z^2/β^2 introduced by Butts and Katz,(1967) to describe the linear density of delta rays generated by high energy ions.

These parameters for radiation quality are partially concerned with the manner of energy deposition events and its spatial distribution as initiated by the radiation type, and on the other hand extends to other factors influencing the effectiveness of an irradiation when physical factors such as absorbed dose, absorbed dose-rate and fractionation are kept constant ICRP(1983). Radiation quality can be described either by radiometric or by dosimetric quantities. Radiometric parameters lead to explicit description of the radiation field and may not be directly related to mechanisms that determine the radiation effects. Parameters reflecting interaction of radiation with

matter are frequently related to a given dimension such as volume. This is the case with restricted values of LET and also "microdosimetry".

Generalised specification of radiation field can be performed in terms of double differential distribution of charged particles with respect to energy and direction. Though such description is inconveniently complex, ambiguous and limited in value, the distributions can be specified for neutrons, for the recoiling heavy ions, for delta rays which they generate in turn, and even for higher order electrons. Sufficiently, a detailed description of all radiation might be characterised by electron distributions though this also lacks information on the vital aspect of correlation, that is in its production in the same event. In view of these considerations, specification of radiation quality for purposes of biophysics is not in terms of the radiation as such, but of the energy which they deposit locally in individual events. The formulation of linear energy transfer-LET by Zirkle et al (1952) was a step in this direction. The LET is often used as a quantity to express radiation quality for radio-biological purposes. This parameter has its own limitations which include the fact that it does not adequately describe the energy distribution of delta rays and the connected spatial distribution of the absorbed dose; it does not consider the fluctuation of energy transfer and averages over large numbers of primary particle interactions; it is not directly measurable and has therefore a limited applicability in experimental situations.

Microdosimetry:

The concept of microdosimetry is concerned with relating some features of the absorption of ionizing radiation in matter to the size and perhaps the nature of the structures being affected in the process ICRU Report 36.(1983). This extends also to considering the number, size and distribution of energy deposits within a micro-volume of an absorber medium which perhaps takes place in such a non-uniform manner that not only the average energy from the discrete energy packets deposited per unit mass, that is, absorbed dose, but the actual number of the energy deposits, their magnitude and in fact their spatial distribution would influence the effect of the radiation on a biological system and, or other structures considered.

Track structures and relative biological effectiveness existing differently for different radiation types are evidences of interplay of *above cited factors in observable radiation effects. Microscopic* distribution of energy in irradiated matter therefore forms a central question in microdosimetry. Stochastic quantities, such as, the specific energy, z, and the lineal energy, y, have been defined and measured as they are parameters describing the energy-deposition events in microscopic structures such as small spherical volumes. Their probability distributions are also central issues in concepts of microdosimetry.

The mechanisms which leads to difference in observable effects of same dose from various radiation types can best be understood should an appropriate description of the manner in which the energy

from these radiation is initially deposited be made. Such description would require detailed information on the structure of the tracks generated in an irradiated medium. However, such a complete description of all levels of interactions taking place within an irradiated medium and the surroundings would be extremely complex and quite unmanageable for the purpose of understanding and, or interpreting radiation effects. More so, biological structures have varying composition and density and their description on the basis of uniform micro-volumes would be impractical.

Stochastic quantities such as the specific energy, z_i; the lineal energy, y_i; and their distribution functions are therefore defined to provide the convenience of associating the observed radiation effects to radiation types, their energy deposition manner and the biological entities considered at microscopic volumes. Mathematical representations of z and y are cited according to ICRU,(1976) as follows;

$$z=\frac{\epsilon}{m}\ [JKg^{-1}]\ ;\ 1Gy = 1JKg^{-1} \qquad (2.11)$$

ϵ = the energy imparted by ionizing radiation to matter of mass m;

$$\epsilon = \sum_i \epsilon_i \ \text{ and } \ \epsilon_i \equiv T_{in} - T_{out} + Q_{\Delta m} \quad [J] \qquad (2.12)$$

T_{in} = the energy of the incident ionizing particle (exclusive of rest mass

T_{out} = the sum of the energies of all ionizing particles leaving the interaction cite (inclusive of rest mass).

$Q_{\Delta m}$ = the changes of rest mass energy of the atom and all particles involved in the interaction.

{$Q_{\Delta m} > 0; \Rightarrow$ decrease of rest mass;

$Q_{\Delta m} < 0; \Rightarrow$ increase of rest mass}

m = mass of the matter(medium)

z = (the specific energy) ≡ Quotient of ϵ by m

The lineal energy y is the quotient of ϵ by $\bar{l}$, where ϵ is the energy imparted to the matter in a given volume by a single energy-deposition event and $\bar{l}$ is the mean chord length in the volume;

$$y = \frac{\epsilon}{\bar{l}} \quad \text{.....(2.13)}$$

2.2.4 Radiation Chemistry Of Water

Effects of ionizing radiation are mediated by the action of secondary electrons at the cellular levels. The ionization density and spatial distribution of the energy deposition events at the microscopic volume elements of the biological systems are determinant factors in radiation effects. The consequences of the generated delta electrons lead to chemical changes which in turn initiate the observable effects at a relatively latter future, - ranging from few seconds, minutes and hours for acute irradiation, to few days and weeks for relatively severe irradiation; and latter generations for stable genetic alterations. Such chemical changes are believed to be initiated by chemical-radicals-interaction formed in the biological systems as seen

from the yield of various biological compounds and molecules after irradiation.

Water being the main constituent of all biological system is of great significance in the evaluation of free chemical radical formations after its irradiation. This relates the dangers of chemical interactions initiated by radiation to its observable effects. Water seems not to absorb optical radiation strongly within the range from 200nm to 800nm and therefore does not constitute a great danger in photochemistry. With ionizing radiation, the situation changes since most of the energies are absorbed by water. Water is also an outstanding constituent of biological systems at about 40% to 50% composition in vegetative cells, and even up to 20% in bacterial spores.

In the absence of oxygen, water absorbs energy from ionizing radiation when it is irradiated to become either ionized or excited. Excitations lead to splitting of the water molecule according to Kiefer,(1990) as follows.

$$H_2O \rightarrow H_2O^+ + e^- \quad \text{(ionization)} \ldots\ldots\ldots\ldots\ldots\ldots (2.14)$$

$$H_2O \rightarrow H_2O^* H^\cdot + OH^\cdot \quad \text{(splitting)} \ldots\ldots\ldots\ldots\ldots\ldots (2.15)$$

The products are hydrogen atoms $H^\cdot$, $OH^\cdot$, H_2O^+ , and hydrated electron. These products possess unpaired electrons and are in effect free chemical radicals which are highly reactive. There are series of other reactions also involving the initially

produced electrons, and these yield therefore secondary products such as H atoms as shown below;

$e^- + H_3O^+ \rightarrow H^\cdot + H_2O$.....(2.16)

$H_2O^+ + H_2O \rightarrow H_3O^+ + O H^\cdot$.....(2.17)

other stable molecules could also be produced as seen from the following examples;

$e^- + H_2O^+ \rightarrow H_2O$ (recombination).....(2.18)

and, or, $H^\cdot + OH^\cdot \rightarrow H_2O$ (recombination).....(2.19)

also $H^\cdot + H^\cdot \rightarrow H_2$ .....(2.20)

$e + e + H_2O \rightarrow H_2 + 2OH^\cdot$.....(2.21)

$OH^\cdot + OH^\cdot \rightarrow H_2O_2$.....(2.22)

Radiolysis of water create some molecular hydrogen and hydrogen peroxide in addition to the short-lived radical species. Probabilities of these reactions are known to depend largely on local actions and is believed to be favoured within "spurs", "blubs" and "tracks". Interaction with solutes are only possible if the primary species are able to escape these zones.

The electron so formed is not absolutely in a free state as it interacts electrostatically with the positive end of the polar H_2O molecule. This creates a special structure shielding rapidly changing electrons from degradation. This is a dynamically feasible process in which the electron is surrounded by water dipoles at an instant, and the water shell in this situation creates

a positive electric potential at the location of the electron. Quantum-mechanically, this system can attain only certain discrete energy states just similar to the conditions in a hydrogen atom. When this is true, it could be postulated that it can be excited and can also absorb light which are all subject to measurable endpoints. Pulse radiolysis methods have been used to measure the light absorption spectrum of hydrated electron in addition to its yield measurements.

Since oxygen is an important component of biological systems, its presence is also of great significance in water radiolysis and are introduced in the treatments as follows;

$$e_{(aq)} + O_2 \rightarrow O_2^{\cdot} \quad \text{.....(2.23)}$$

and $$H^{\cdot} + O_2 \rightarrow HO^{\cdot}_2 \quad \text{.....(2.24)}$$

It must be noted that O_2 does not react with $OH^{\cdot}$ hence reactions represented in equations (2.27) and (2.28) can reach an equilibrium in the production of $HO_2^{\cdot}$ and O_2^{-} as follows;

$$HO^{\cdot}_2 \Leftrightarrow O_2^{-} + H^{\cdot} \quad \text{.....(2.25)}$$

Both of these products are quite unstable and therefore react further as shown below;

$$HO^{\cdot}_2 + HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \quad \text{.....(2.26)}$$

$$O_2^{-} + O_2^{-} \rightarrow H_2O_2 + O_2 \quad \text{.....(2.27)}$$

In the presence of oxygen in a solution, there is an increased yield of H_2O_2 while the oxygen is partly regenerated as seen

above. Under irradiation conditions, an equilibrium in the formation of these products(O_2 and H_2O_2) will be attained. A further secondary reaction observable also is the degeneration of the H_2O_2 creating hydroxyl radical which may explain the role of oxygen in radiation biology; These species are also produced in

$$e_{(aq)} + H_2O_2 \rightarrow OH^{\cdot} + OH^{-} \quad \text{.....(2.28)}$$

the absence of oxygen but with quite a low yield; These reactions are very important due to the presumed biological importance of

e.g. $$OH^{\cdot} + OH^{\cdot} \rightarrow H_2O_2 \quad \text{.....(2.29)}$$

$$H_2O_2 + OH^{\cdot} \rightarrow + HO^{\cdot}{}_2 + H_2O \quad \text{..... .(2.30)}$$

$$HO^{\cdot}{}_2 + HO^{\cdot}{}_2 \rightarrow O_2 + H_2O_2 \text{ or } HO^{\cdot}{}_2 + OH \rightarrow O_2 + H_2O \quad \text{...(2.31)}$$

$OH^{\cdot}$. However, $OH^{\cdot}$ may be scavenged by alcohol , example, ethyl alcohol, and the resultant alcohol radical is much less reactive than $OH^{\cdot}$ such that the net effect is reduced.

$$OH^{\cdot} + CH_3CH_2OH \rightarrow CH_3C^{\cdot}HOH + H_2O \quad \text{.....(2.32)}$$

2.3 Overview of related previous work

Of recent, a yeast cell mutant - Rad 54-3 showing temperature-dependent post irradiation repair sensitivity was used as an important research tool in investigating the biological effects of the radiation field in space under micro gravity conditions, Pross, (1993). With its repair permissive and restrictive temperature points at 23°C and 36°C respectively, survival patterns representing the effects from the

radiation field in space had been obtained. Also a series of survival patterns for the strain obtained through irradiations using heavy ions such as Ar, Ti, and U have been generated with a view to establish a generalised sequence of biological action from these ionising radiation sources. As a relatively lighter particulate radiation, alpha particles data of the survival trend for the Rad 54.3 yeast mutant become important as a comparative data among the other heavy ions data already generated.

Biological effects of radiation on the D7-yeast cell strain had been under investigation using varying LET range of ionizing radiation and tested for survival potentials of the cell after irradiation; and also the mutation frequency test using gene conversion and reversion mutation phenomena as the biological end points had been carried out extensively using x-ray Stoll, (1994), and for other series of heavy ions.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Media

Various types of media were prepared and used for incubation and temporary placement of the cells during experimentation. Frequently used are;

Complete Medium:

Complete medium was very often used in the "cells growing culture" experiments, and it contains the principal nutrients required for the nourishment of the cells incubated or placed on it. It sustains cell metabolism and promotes cell division processes during cell culture/incubation.

Composition

Basic nutrients of the complete medium include;

Agar	**17.0g**
Yeast Extract	**10.0g**
Peptone	**20.0g**
Glucose	**20.0g**
ion-free water	**933.0ml**
Complete Medium	**1000.0g = lkg Comp. med**

Synthetic Complete (SC) Medium

SC medium was often used as selective-media for studies related to mutation effects of radiation on cultured cells. In addition to the composition of SC medium outlined below, it also contained specific amino acids/base of interest, in varying forms and in varying

concentration - see section 3.1.3, depending on the investigation undertaken.

Composition

Basic nutrients contained in the SC medium type are given below;

Glucose- - - - -	**20.0g**	
Agar - - -	**17.0g**	
Difco Yeast Nitrogen Base--	**6.7g**	
ions-free water - - -	**956.3ml**	
SC Medium - -	**1000.0g**	**= lkg SC medium**

Salt-Agar Medium

It is important to mention that salt-Agar medium moderates the growth of cells placed on it during irradiation or storage and yet sustains the metabolism of the cells without promoting effective cell division processes.

Composition

Composition of salt Agar medium is as follows;

Agar - - -	**18.0g**	
Sodium Chloride(Nacl)- -	**7.0g**	
KH_2PO_4 -	**9.1g**	
ions-free water - -	**943.9ml**	
Salt Agar Medium - -	**1000.0g**	**= lkg Salt-Agar medium**

3.2 Amino Acid/Base Composition Of The Selective Media Used For Mutants Recovery

For the three types of mutation phenomena under investigation, the SC medium containing specific types of amino acids/base were used in their selection. The specificity of the amino acids/base composition of the

SC medium correlated to the various mutation phenomena tested for are as follows;

Amino Acid/Base Composition In SC Medium Used For The Test Of Gene Recombinations And Cell Survival:

	(base)	Adenine - - -	5mg
		Tryptophan -- -	40mg
I000ml of SC Medium	+	Isoleucin - - -	40mg
		Valin - - - -	40mg

Amino Acid/Base Composition In SC Medium Used For The Test Of Gene Reversions:

I000ml of SC Medium	+	Adenine - -- -	5mg
		Tryptophan - -	40mg

Amino Acid/Base Composition In SC Medium Used For The Test Of Gene Conversions:

	(base)	Adenine - - -	5mg
I000ml of SC Medium	+	Isoleucin - - -	40mg
		Valine - - -	40mg

3.3 Dosimetry Method

In order to describe in quantitative terms the biological effects of the radiation used during the entire experimentation, dosimetric analysis of the various sources used were conducted. Relevant dosimetry techniques which are peculiar to the individual sources worked with are outlined below.

3.3.1. The Fricke Chemical Dosimetry

Fricke chemical dosimetry was conducted with a 100kV X-ray tube source with the standard condition; 80KV ;30mA.

Composition Of Fricke Solution

Doubly distilled water (Quartz bidest) was used for the preparation of the Fricke solution used for the dosimetry. The solution had the following chemical composition;

Concentrated H_2SO_4 - - 11ml (The bidest was added first before the conc acid)

$FeSO_4 . 7H_2O$ - - - - 139mg = 0.139g

Nacl -- - - - - 29,25mg = 0.02925g

These chemicals were made into a solution of 500mls with "Quartz bidest" that is, doubly distilled water as a standard laboratory practice to safeguard against impurity

Procedure For X-Ray Dosimetry

Samples were mounted on a sample holder support positioned 13cm from the X-ray source. Samples were placed on a 3cm diameter dishes to which magnetic stirrers were placed A dosimeter - ion chamber type was used to monitor the X-rays and the X-ray tube was cooled with water during the running times. The irradiation glass dishes were washed with water, and then with ethanol and allowed to dry. In some cases the dishes were put into a blue burning flame - just before use in order to burn off the remaining ethanol which might have been present.

The Fricke solution (3mls) was irradiated at a temperature of about 20 ^{o}C and with constant stirring. The irradiation was done at 80kV, 30mA output settings of the X - Ray machine for varying time intervals (in seconds) and the following data were obtained from the photometer (SIEMEN) at a wavelength of 305 nanometre using the $1cm^2$ base area quartz kuvettes. The following data were obtained and used in the calibration curve.

	Irradiation time of the Fricke Soln [Seconds]	Extinction obtained from Photometer	Calculated doses [Gray]
	0	0.000	0.000
	10	0.291	82.895
	20	0.532	151.549
	30	0.781	222.480
	40	1.024	291.702
	50	1.329	378.586
	60	1.630	464.331

Table.3.7: Dosimetry data for 100KV X - Ray tube operated at 80kV;30mA settings.

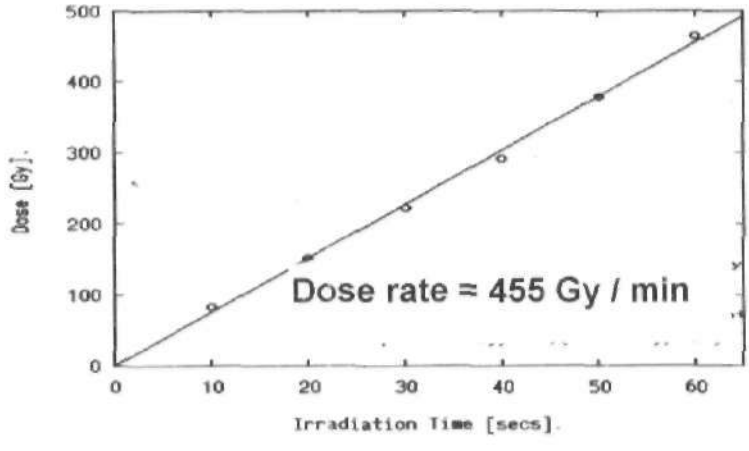


Fig. 3.8: Calibration curve for 80KV X-Rayx at 30mA - using 3mm diameter dish; 3mls of irr. Soln.

1. $D = \frac{E}{\phi T} \times 6.042 \times 10^{5}$ Gy 3.34

where:

E = Corrected extinction using blank as a reference

ϕ = base area of the kuvette used.

T = temperature coefficient (see list below)

Table 3.9: Spectrophotometric conversion factors for ferric ion absorption in the Fricke solution; after Hugo Fricke and Edwin J. Hart.

Temp	Coefficient
20°C	2121
21°C	2136
22°C	2152
23°C	2167
24°C	2182
25°C	2197
26°C	2212
27°C	2227

Temp	Coefficient
28°C	2242
29°C	2258
30°C	2273
31°C	2228
32°C	2303
33°C	2318
34°C	2333
35°C	2349

2. $D = \frac{dE}{dm} = \frac{N_{Fe^{3+}}}{\rho \times G} = \frac{A_v \times \Delta E \times U}{C \times \ell \times r \times G} = K \times \Delta E$ 3.35

where $K = \frac{A_v \times U}{C \times \ell \times r \times G}$

A_v = $6.02322 \times 10^{23} M^{-1}$ / Avogadro Number)

ΔE = Change in optical density (Extinction difference between the blank solution and the irradiated one)

C = Molar extinction coefficient at the working temperature (as in table 3.1)

G = G - value for the reaction = 0.156 / eV

ρ = density of the solution = 1.024 gcm^{-3}

ℓ = Optical length = 1cm

r = radius of the Kuvette used

U = 1.602×10^{-19} JeV^{-1} (conversion factor from eV to Joules)

$N_{Fe^{3+}}$ = Number of iron ions formed.

3.3.2 The Track Etching Dosimetry For Alpha Particles

To be able to quantify the number of particles emitted from the source per unit area and unit time, a dosimetry of the alpha particles was planned and carried out using the track etching methods.It is known that when an ionizing particle passes through a dielectric. the transfer of energy to electrons results in a trail of damaged molecules along the particle track. In some materials, the tracks are made visible upon etching in a solution of strong acid or base.[Knoll, G.F(1979)]. It is often noticed that upon etching of these materials in strong acid or base, the entire surface of the material is attacked with those points at which the particles made their tracks being etched at a faster rate, hence the tracks can be made to form pits on the surface which are large enough to be easily visible through a conventional microscope. Such materials used to detect particles in this manner are referred to as track-etch detectors. A list of these materials and their least detectable ion energy is given below;

Table .3.10: List of Track-etch detectors and their most appropriate radiation type. [(after Knoll,G. F. (1979)]

	Atomic Composition	Least ionizing ion seen
Inorganic Materials		
Quartz	SiO_2	100 MeV ^{40}Ar
Phlogopite Mica	$KMg_2Al_2Si_3O_{10}(OH)_2$	
Muscovite Mica	$KAl_3Si_3O_{10}(OH)_2$	2MeV ^{20}Ne
Silica Glass	SiO_2	16MeV ^{40}Ar
Flint Glass	$18SiO_2:4PbO:1.5Na_2O:K_2O$	2-4 MeV ^{20}Ne
Organic Materials		
Polyethylene Terephthalate (Cronar, Melinex)	$C_5H_4O_2$	36 MeV ^{16}O
Bisphenol A-poiycarbonate (Lexan, Makrofol)	$C_{16}H_{14}O_3$	**0.3** MeV ^4He
Polymethylmethacralate (Plexiglas, Lucite Perspex)	$C_5H_8O_2$	3 MeV ^4He
Cellulose Triacetate (Cellit, Triafol-T, Kodacel TA-401 unplasticised)	$C_3H_4O_2$	0.55 MeV ^1H
Cellulose Nitrate(CN)	$C_6H_9O_9N_2$	

Cellulose nitrate (CN) foils and CR-39 foils were used as track-etch detectors. The foils were exposed in an irradiation set-up similar to the one to be used for the cell experiments. The exposure time was varied from 30 seconds to about 2 minutes and the foils were etched in a 6N NaoH solution heated in a water bath to temperatures of 50°C - 70°C for about 2.5hrs. Various other etching conditions were also experimented for by other co-workers in the group and results compared upon track counting. This was with the view of optimizing the method to be adopted. (See figs 3.11 and 3.12)

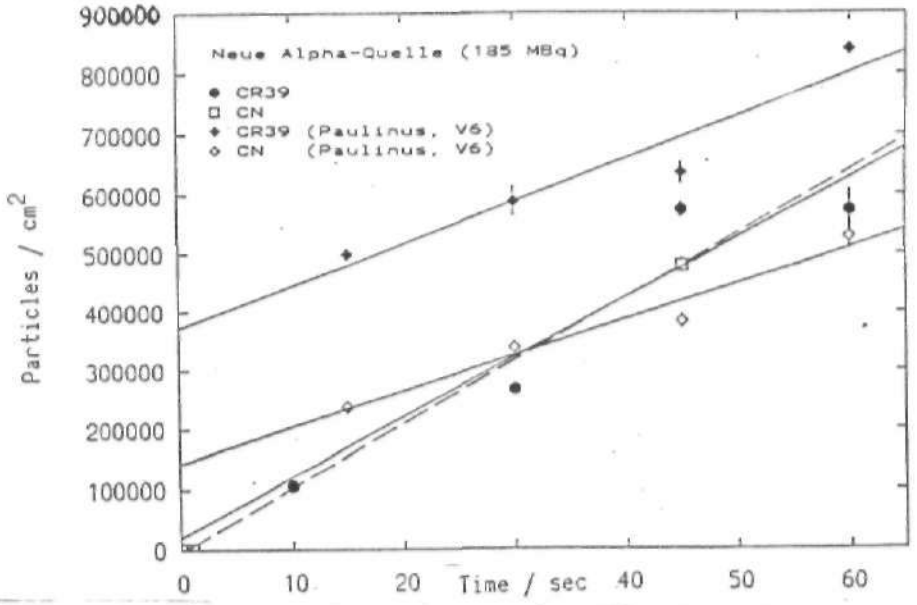


fig. 3.11 Graph of alpha particle fluence obtained from three independent track countings viewed through a CCD-camera and backed-up by a computer system; (Comparing CR-39 foils with CN foils)

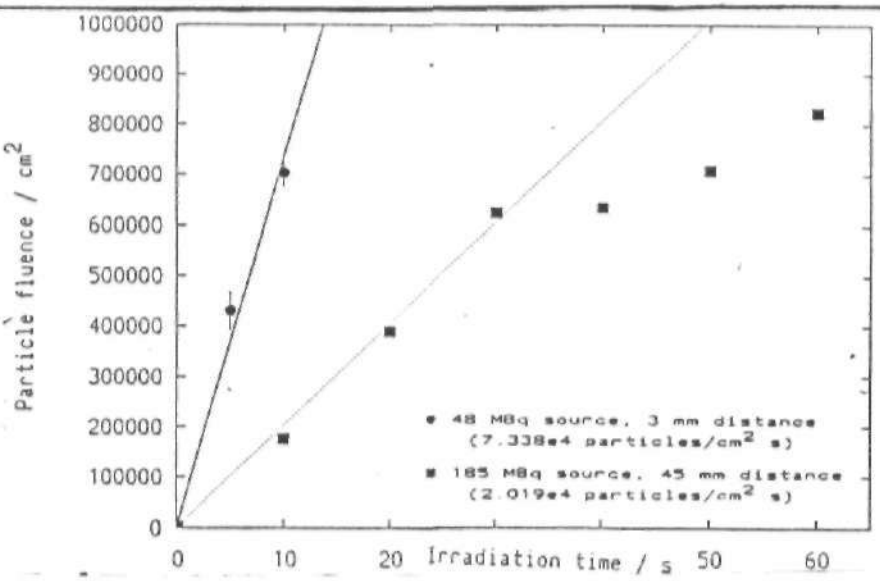


fig. 3.12 Graph of alpha particle fluence through a CCD-camera and backed-up by a computer system; (Data collected from 48MBq and 185MBq alpha sources through CR 39 foils)

Track counting was done on a light microscope system and also electron microscope. Various graphs were obtained to indicate the fluence deduced using these various techniques and varying etching conditions.

The fluence rate arrived at after series of pre-test countings are as summarised below:

Table. 3.13 Table of fluence-rates of the alpha particles sources used for the cell irradiations

Source	Set-up (See page 57-58)	Description	Fluence x 10^4 part. $Cm^{-2}s^{-1}$
48MBq	Three rings	3mm air + 2µm mylar	7.88±0.20
48MBq	Kaba	41.5mm He gas + 2µm mylar + 3mm air	3.35±0.13
185MBq	Three rings	3mm air + 2µm mylar	3.49±0.06
185MBq	Kaba	41.5mm He gas + 2µm mylar + 3mm air	17.5±0.04

3.4 Preparation Of Cells Samples

For purposes of being able to determine the approximate number of yeast cells cultured on the plates during experimentation, it was necessary that one is able to count these cells and determine their concentration per millilitre(ml) of the cell solution used either for culture or

for irradiation. Such knowledge provided the basis of analysing the number of cells grown on a medium before irradiation and the number of the cells that survived thereafter. The procedures followed in the preparation of the cell samples are outlined in fig. 3.13. The cell concentrations and counting were carried out using haematocytometer by thoma

40mls of cells solution with a concentration of 2×10^8 was required and this was to be made from the test tube S - having a cell concentration of 2.625×10^9 cells/ml, then the following calculations below yielded the volume of the concentrated yeast cells solution of concentration (2.625×10^9 cells/ml) to be diluted into 40mls with distilled water in order to result in 40mls of yeast cells solution having a required concentration of 2×10^8

$$\frac{2 \times 10^8 \text{ cells/ml} \times 40\text{ml}}{2.63 \times 10^9 \text{ cells/ml}} = \frac{2 \times 10^8 \text{ cells/ml} \times 40\text{mls}}{26.3 \times 10^8 \text{ cells/ml}}$$

$$= 3.1$$

Concentrated cell solution (3 mls in volume) from the test tube having cell concentration of 2.63×10^9 cells/ml was filled up to 40mls with distilled water to obtain 40 mls of cell solution having a concentration of 2×10^8 cells/ml.

Control experiments were made to verify the results of the concentration of yeast cells determined by this type of calculations and the following scheme summarises the procedure followed in making these controls

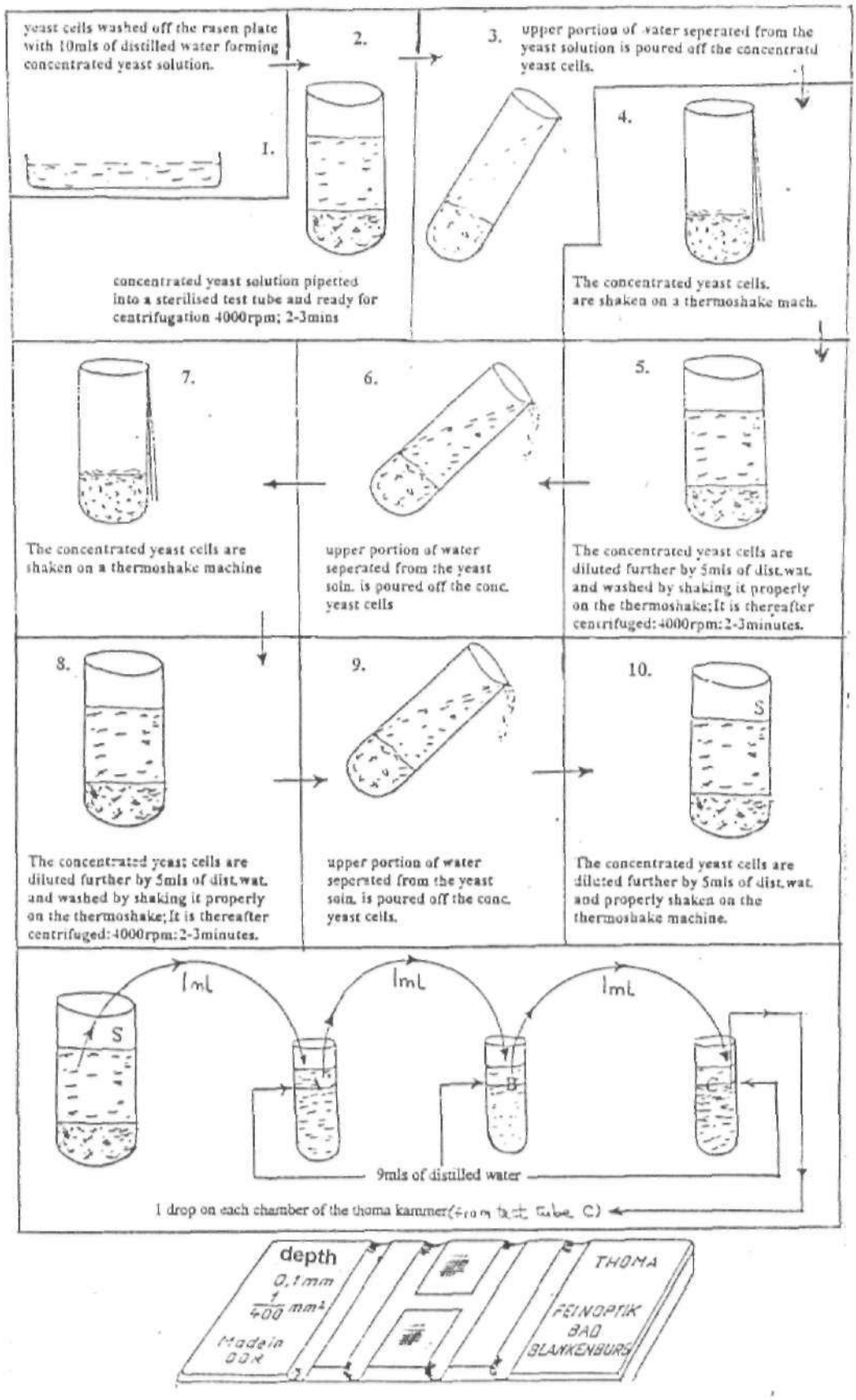


fig. 3.14 Scheme of cell washing and determination of cell concentration using a cytometer

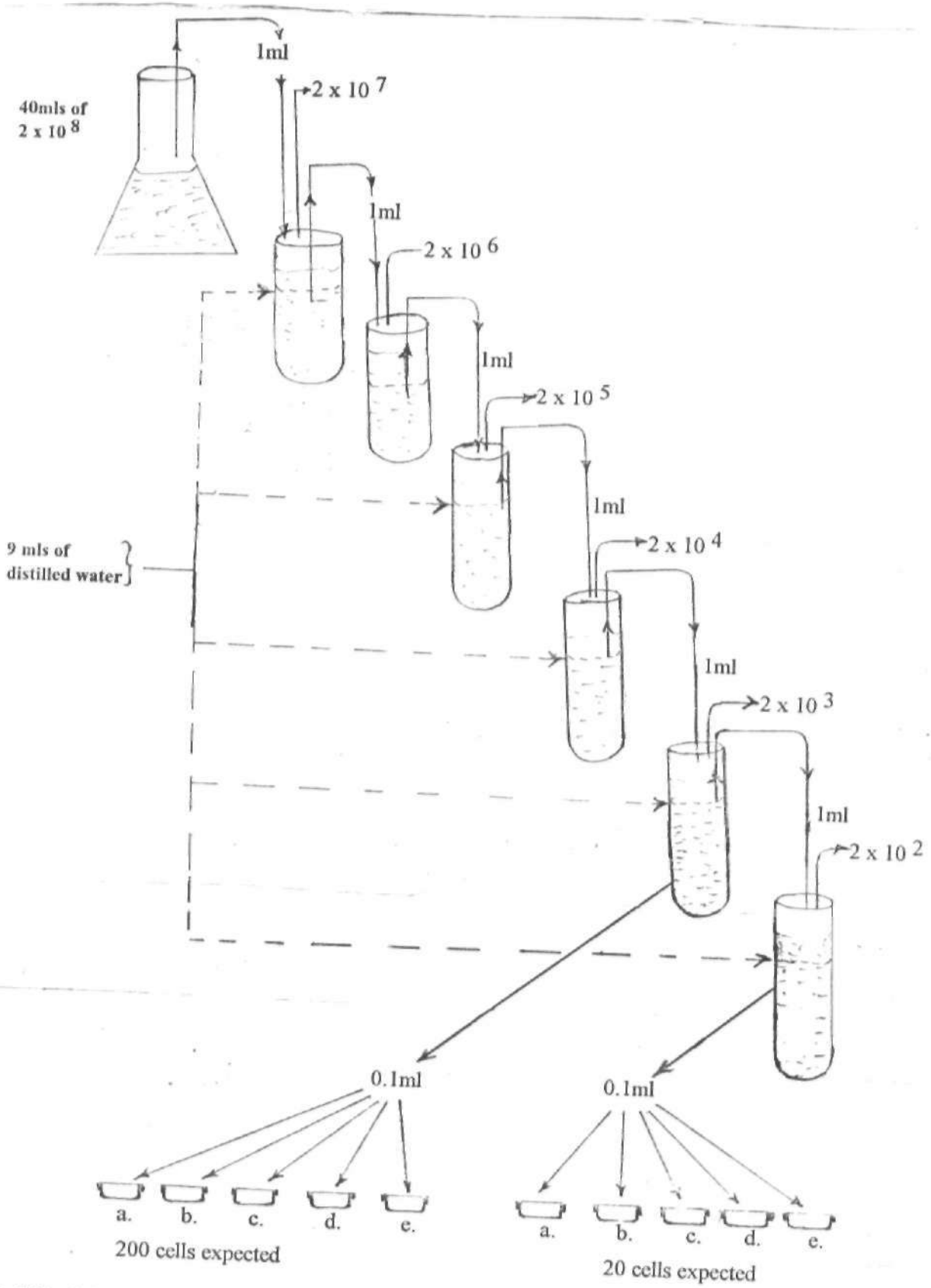


fig.3.15:: Scheme of cell solution dilutions

The results obtained for the controls made from counting the yeast cells on microscope were as follows:

Table.3.16: Table of test cells plating/incubation results

Incubation Conc.→	$2x10^2$ (0.1 ml)	$2x10^3$ (0.1 ml)
a	22	190
b	27	178
c	14	227
d	22	192
e	22	205
	107/5 = 21.9	992/5 = 198.4

3.5 Cell Irradiation And Incubation

Special filters made of fibre materials were made into circular size and sterilised under the Co-60 gamma source. Each of the filters to be used was soaked in distilled water to soften the material of the filter and to ensure that capillary draining properties of the filter were in order. In this time filters that did not allow water molecules to soak through them were discarded. Required cells number concentrated into a 1ml cell solution volume were re-diluted with 9mls of distilled water. Each of the resulting 10mls diluted-cell solution was poured through each filter held in a vacuum air pump which helped in draining the cell solution through the filter. With this system the required cells number which drained through the filter were deposited on the filter membranes of diameter 4cm .

The placement and removal of the filters on the suction pumping set-up was carried out with forceps which were sterilised in ethanol flames each time they were in use - to place or remove each filter.

The filters holding the required cells concentration/number were placed on special salt agar medium in all the times prior to experiments. As had already been said salt-Agar medium moderates the growth of cells placed on it during irradiation or storage and sustains the metabolism of the cells without promoting effective cell division processes.

Irradiated cells samples already deposited on filter membranes and placed on salt Agar medium were required for incubation in a given concentration. To achieve this, the filters holding the cells were carefully folded into two leaflets using two sterilised forceps and placed inside a test-tube containing 5mls of distilled water. This was thereafter shaken thoroughly in a vortex for at least 3 minutes to re-suspend the cells from the filters in a solution form.

Re-suspended cells were estimated to have the concentration of the cells previously deposited on the filter membrane. Without removing the filter from the re-suspension tube, 1ml of the re-suspended cells solution was always taken out from the tube and diluted with 9mls of distilled water - giving 10 mls of a cell solution which was a factor of about 10 lower in cell concentration per ml. Subsequent dilutions followed similar trend. The choice of cell dilutions to be plated was made bearing in mind the plating efficiency of the cell line used and the level of survival expected from the irradiated samples.

A volume of 0.1ml or 0.2ml of the diluted cells solution were placed on the appropriate culture media and taken for incubation in the required temperature through the required duration of time for colony formation.

Formed colonies were counted and survival fraction or number of mutants(as the case may be) were determined therefrom.

3.6 The Alpha Particle Sources And Their Properties

Mode Of Production Of The Alpha Particle Source

Americium 241 is an artificially produced radioisotope obtained by neutron activation processes using uranium and sometimes neptunium. The resulting Americium 241 has a half life of about 433 years which is quite an important justification for using the source in an irradiation experiment over a long period of time. The source decays principally by alpha of varying energies with dominated contributions from α-28 (85.2% probability; and energy of 5.486MeV); α-26 (12.8% probability, and energy of 5.443MeV). With the decay energies of other minor alpha lines existing, the maximum alpha energy stands at 5.5MeV - the decay scheme for ^{95}Am -241 is provided in appendix D5;pp 96

The daughter product of americium 241(by alpha decay) being $^{237}_{93}Np$ nucleus is reached at an excited state and also through varying spin states after alpha emission by americium. These spin states are identified and denoted as $(9/2)^-$, $(7/2)^-$, $(5/2)^-$, $(7/2)^+$, $(5/2)^+$. The excited Neptunium nucleus is de-excited by gamma emission and its principal energies are γ-9 →30KeV; γ-14 →60KeV. These gamma radiation that accompany americium 241 alpha decay are results of de-excitation of the Neptunium nucleus and this constitutes some hazard to the users of the source. Though the energy from these gamma quanta components are relatively

low, close range contact with the source over a long period of time may lead to harmful exposures due to these gamma quanta.

Manufacturer's Specification For The Sources

Source I

The source has the following specifications.

Maximum alpha energy	-	5.5Mev
Energy of the gamma component		30keV and 60keV
Half life of the source		433 years
Activity of the source		185MBq
Active area		8cm x 8cm
Activity per unit area		2.89×10^6 Bq cm^{-2}

Source II

The source has the following specifications.

Maximum alpha energy	-	5.5Mev
Energy of the gamma component		30keV and 60keV
Half life of the source		433 years
Activity of the source		48MBq
Active area		19.5cm x 8cm
Activity per unit area		3.007×10^5 Bq cm^{-2}

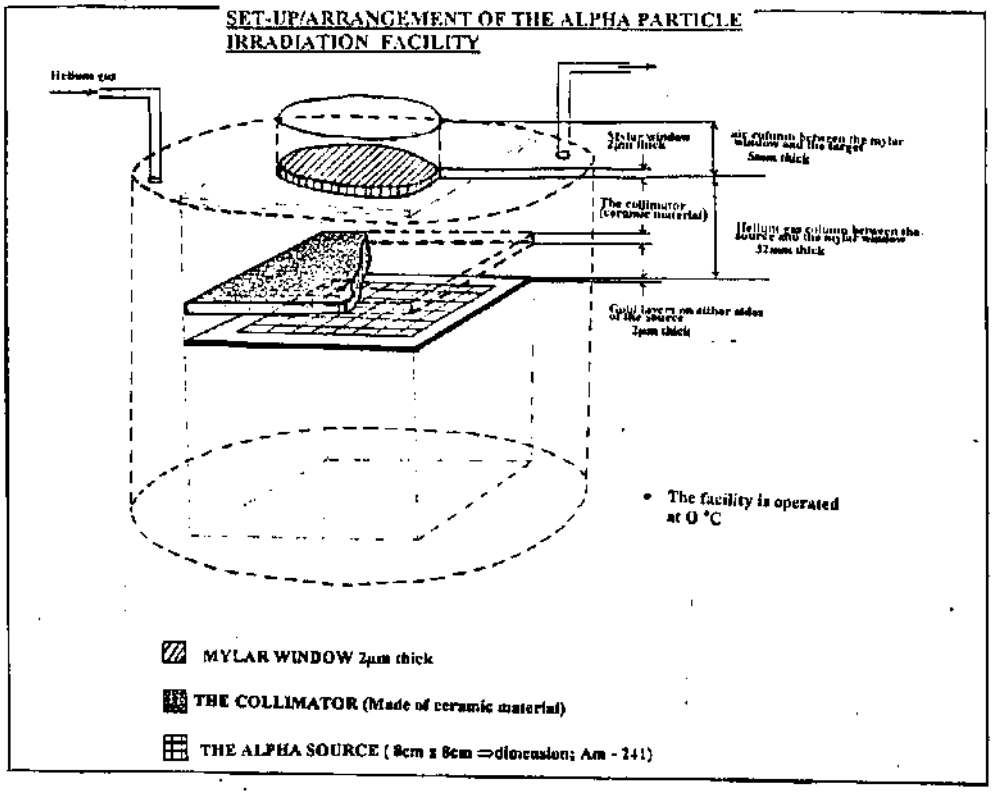


fig.3.17: Installation facility for 185 Mbq alpha particle source flooded with Helium gas

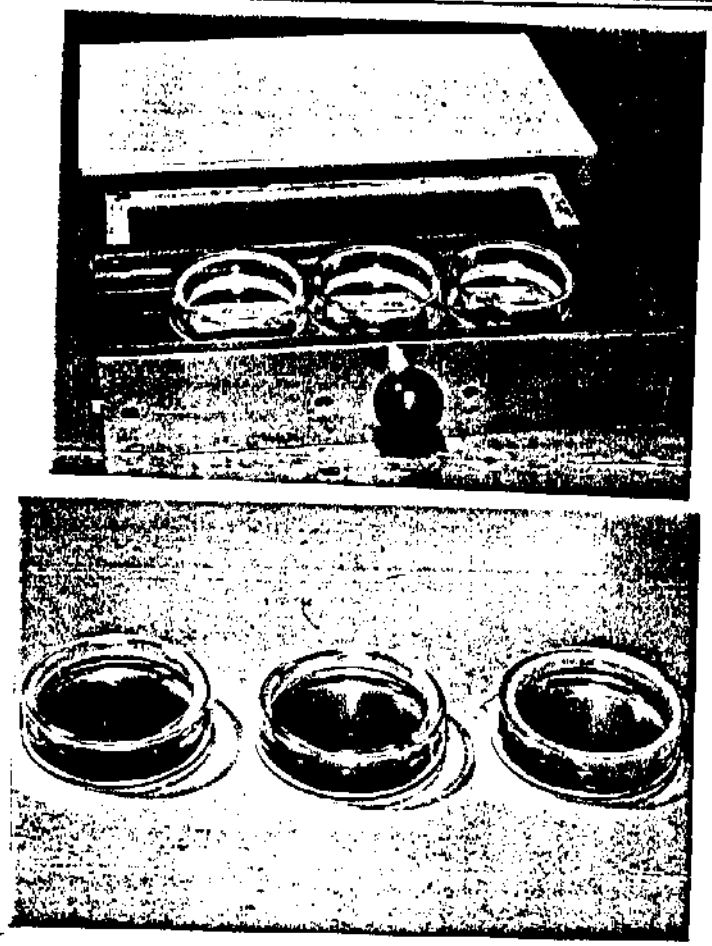


fig.3.18 Irradiation system of the old source - 48MBq alpha particle source comprising of three rings of exposure points.

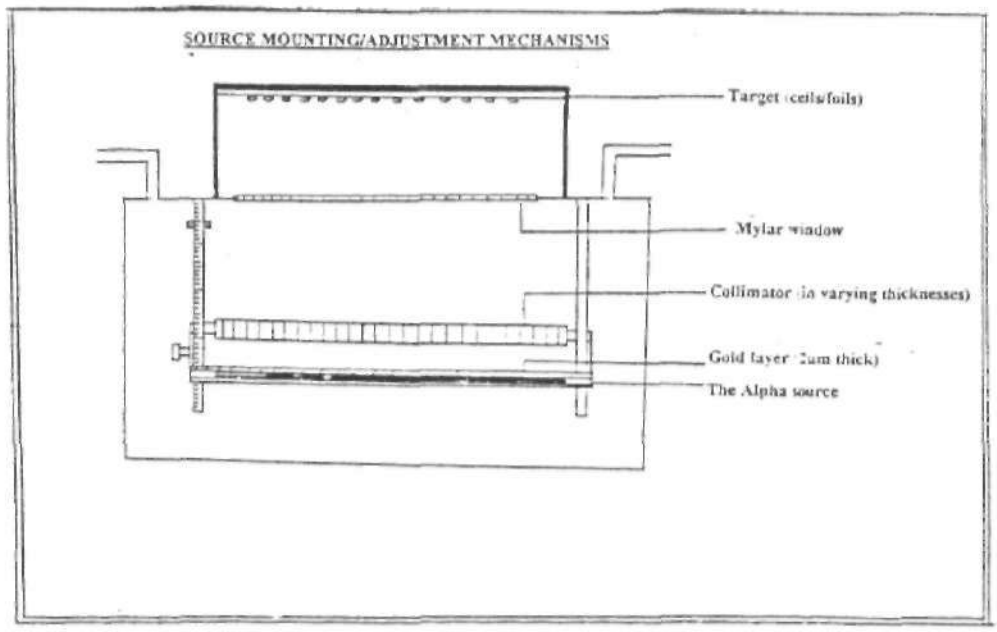


fig.3.19 Irradiation geometry of 185MBq alpha particle source in KABA set-up

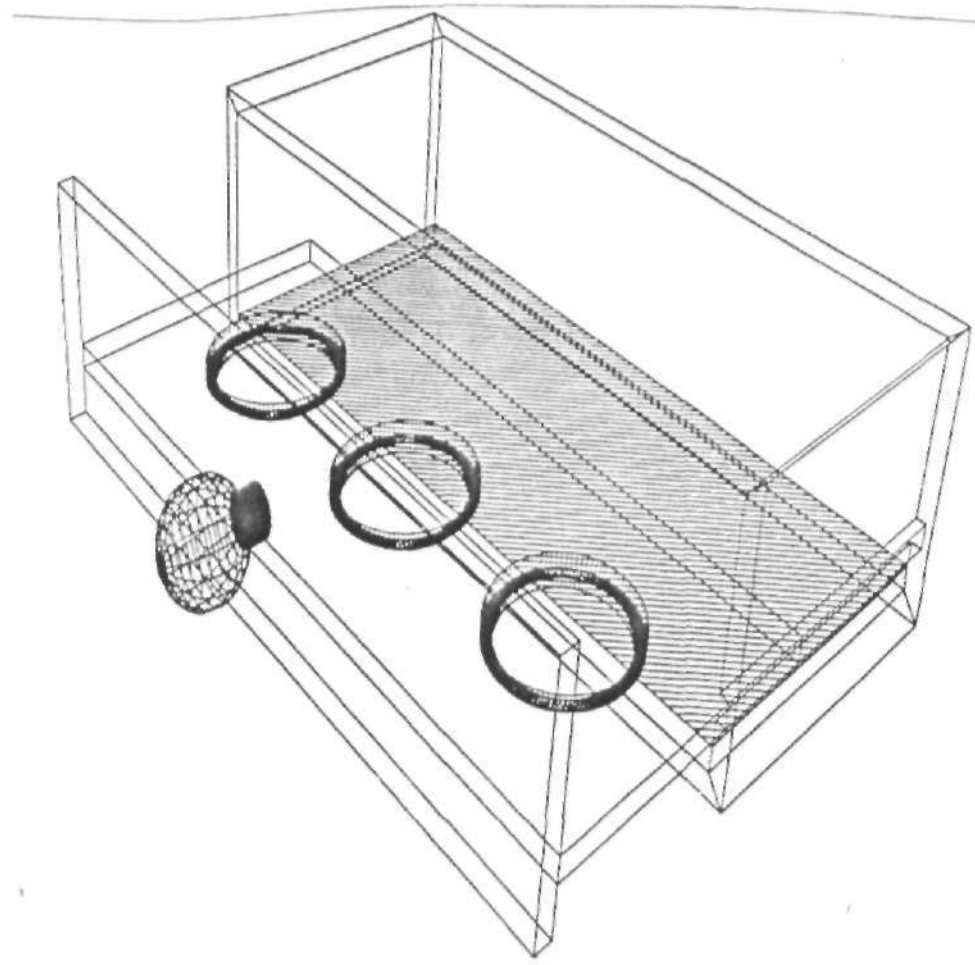


fig.3.20: Irradiation geometry of 48MBq alpha particle source in three rings exposure system.

3.7 Data Analysis And Evaluation Methods

X-Ray Doses:

Doses from X-rays were obtained by methods of Fricke chemical dosimetry general details are as presented in section 3.2.1 using spectro-photometric extinction data. Dosimetry results yielded dose-rates and the absolute X-ray doses were obtained from the relation;

$$\begin{array}{lcll} \text{Absolute} & = & \text{Doserate} \times & \text{time} \\ \text{X-ray dose} & & [\text{Dose.min}^{-1}] \text{ or } [\text{Dose.sec}^{-1}] & [\text{min}^{-1}] \text{ or } [\text{sec}^{-1}] \end{array} \quad \text{.....(3.36)}$$

Alpha Particle Fluence

Alpha particle fluences were obtained from the track-etched dosimetry methods using the track-etch detectors CN and CR foils - general principles are as presented in section 3.2.2. Results were expressed in units of number of particles per square centimetre $(P)cm^{-2}$ or number of particles per square micrometer $(P)\mu m^{-2}$. This was determined by the units on the results of other past and related investigations which were chosen as comparative basis for this work. The conversion factor between these units is given as follows;

$$\varnothing(P)\mu m^{-2} = 10^{6} \times \varnothing(P)cm^{-2} \quad \text{.....(3.37)}$$

Analysis of the fluence-rates deduced from the various irradiation configurations of the alpha particle sources used are as given in fig 3.21: The data were obtained through a computer simulated alpha particle energy spectrum, LET spectrum and fluence rate attenuation by mylar and cell layers of the irradiation systems. Self-absorption parameters for a five- layered Americium source (48MBq) was taken into account.

Experimentally determined fluence and fluence-rates were used in all calculations and in all graphical representations. It must be mentioned that the accuracy of these fluence-rates depends on the sensitivity and, the efficiency of the dosimetry method and also on the materials used.

Table. 3.21: Table of computer simulated and experimentally measured fluence-rates for various alpha particle sources used

Descriptions		Fluence-rates $\varnothing[(P)cm^{-1}]$	Average Energy E[Mev]	Average LET [KeV μm ^{1}]
Computer Simulation for (48Mbq;Three rings)				
a)with mylar foil	1. without cells	1.123×10^5	3.19	124.56
	2. with cells	9.937×10^4	2.86	131.26
b)without mylar foil	3. without cells	1.142×10^5	3.20	124.76
	4. with cells	1.004×10^5	2.89	131.47
Measured values:				
c)48MBq(three rings)	9. without mylar foil and without cells	$7.88 \pm (0.20) \times 10^4$		
fd)fluence -rate attenuated by mylar foil and cell layer.	10. resulting in 88.4% of the fluence-rate {see a(1 and 2)}	$6.972 \pm (0.18) \times 10^4$		
g)**measured** fluence-rate for 185MBq in (kaba)	11. with mylar foil but without cells	$1.75 \pm (0.04) \times 10^4$		
h)fluence-rate attenuated by cell layer.	12. resulting in 87.9% of the fluence rate {see a(3 and 4)}	$1.54 \pm (0.04) \times 10^4$		

Cell Survival Data

Cell survivals are expressed as survival fractions normalised with the controls which are set to represent a survival level of 100%. If n_o denotes average cell survival counts obtained from control plates and

$n_1, n_2, n_3, \ldots$ represent counts for dose points 1,2,3,...., then the survival fraction at these dose points denoted as sf_1, sf_2, sf_3,....., were obtained from the relations;

$$sf_1 = \frac{n_1}{n_o};\quad sf_2 = \frac{n_2}{n_o};\quad sf_3 = \frac{n_3}{n_o};\ldots\ldots sf_x = \frac{n_x}{n_o}; \qquad \ldots\ldots\ldots\ldots\ldots(3.38)$$

$$n_o \quad \frac{k_1 + k_2 + \ldots\ldots k_x}{x} \quad (k_{1\text{-}x} \equiv Controls)$$

Mutant Frequency

Mutant frequency was obtained from both the background rate indicated by the controls as well as the radiation-induced rates. The relationship used in this deductions are as follows;

$$\text{Mutant frequency} = \frac{\text{number of mutants}}{\text{number of survivors}} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3.39)$$

Mutant frequencies were normalised to a cell population of 10^6 survivors to which the analysis was based on.

4.0 RESULTS

The results obtained from the experiments were the raw data for cell survival and mutation phenomena. From these, survival fractions and mutation frequencies were computed and these are represented graphically in this section. The information presented along and deducible these graphical results include the general trend of the survival and mutation curves, the shoulder of the survival fits, the D_o , σ_r and n values for the survival curves including the fit function. The general view of the respective results are taken up more extensively under the discussion sections.

4.1 Survival And Mutation Frequency For D7 Yeast Strain After X-Ray Irradiation

The general fit of survival and mutation frequencies (gene conversions and reversions) obtained for the D7 yeast cell strain from two sets of X-ray experiments are presented hereunder. These provide comparative basis for the alpha particles action on the same strain

Cell inactivation: **X-Ray**

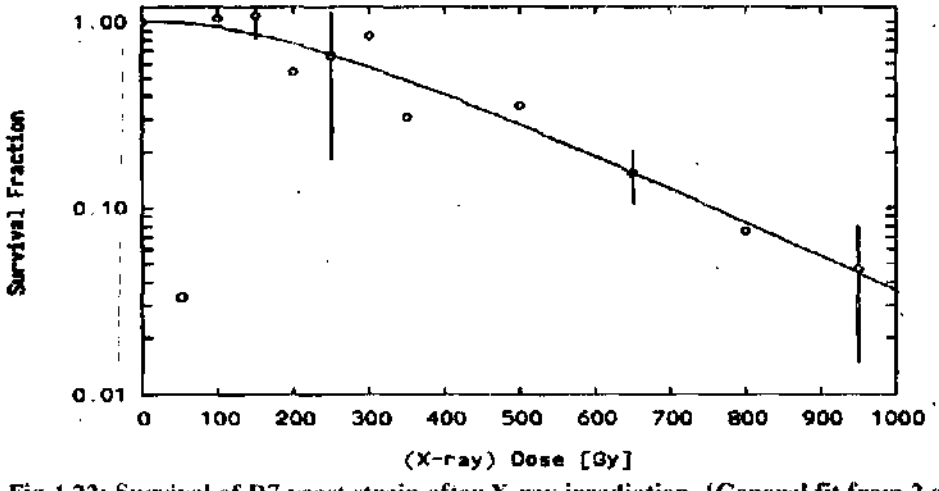


Fig 4.22: Survival of D7 yeast strain after X-ray irradiation. {General fit from 2 experiments

Equation of the curve: $\log Y = \log \{1-[1-\mathrm{Exp}\left(\frac{D}{D_o}\right)^n\}$ **Multitarget fit; D_o = 180Gy; n = 6.427**

The result presented above represents the survival of D7 yeast cells after treatment with X rays up to 900Gy. There is a reduction in the survival down to 5%

and the fit is shouldered conforming to the expected survival trend. The D_o value of 180Gy is obtained and this implies that a dose of 180Gy reduced the survival down to 37 percent and this is a significant parameter in the survival experiment.

Mutation frequency

i. gene conversion

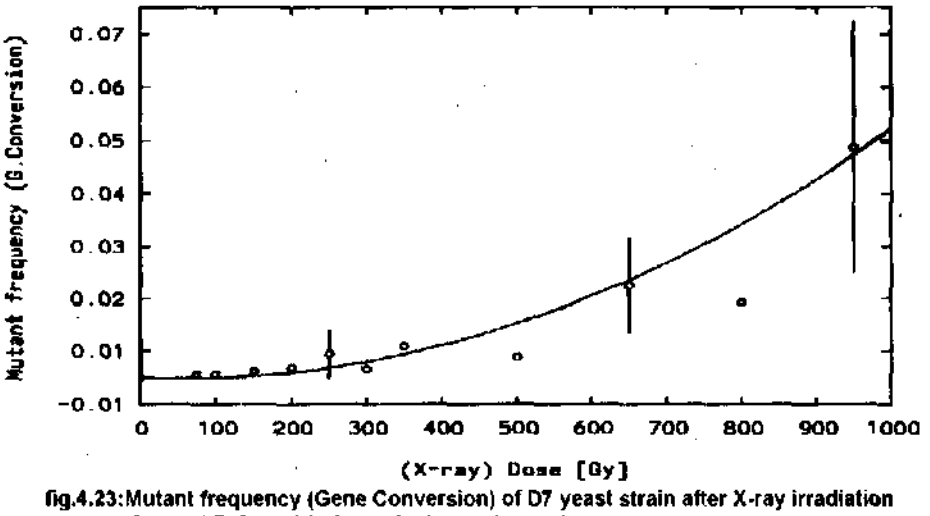
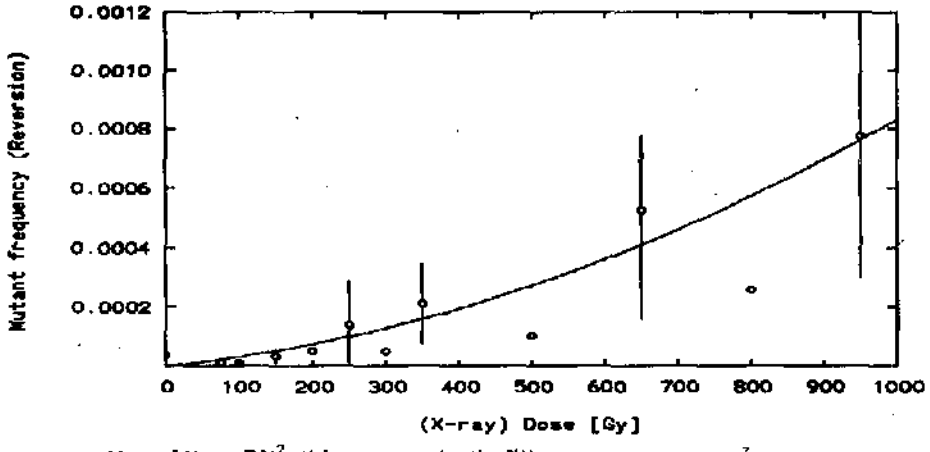


fig.4.23:Mutant frequency (Gene Conversion) of D7 yeast strain after X-ray irradiation (General fit from 2 independent experiments);

Equation of the curve: $Y = AX + BX^2$ (Linear quadratic fit); $A = -5.978 \times 10^{-6}$; $B = 5.3174 \times 10^{-9}$

ii. reversion



Equation of the curve: $Y = AX + BX^2$ (Linear quadratic fit); $A = 2.465 \times 10^{-7}$; $B = 6.4087 \times 10^{-10}$

fig.4.24:Mutant frequency (Reversion) of D7 yeast strain after X-ray irradiation (General fit from 2 independent experiments

Mutation results presented above for the action of x-rays on the D7 strain of yeast cells shows a linear quadratic fit for both cases. The mutation frequencies of

the gene conversion are however higher than those of the reversions. It is known that reversions are as a result of point mutations which are highly a chance event.

4.2 Survival And Mutation Frequency For D7 Yeast Strain After Alpha Particles Irradiation

The general fit of survival and mutation frequencies obtained for the D7 yeast cell strain from two sets of alpha particles irradiation using 185MBq and 48MBq alpha particles sources are presented hereunder. These results were obtained to compare the feasibility of survival and mutation assays from these two sources and to compute a general fit for the alpha action on the D7 yeast strain.

185 Mbq
Cell inactivation

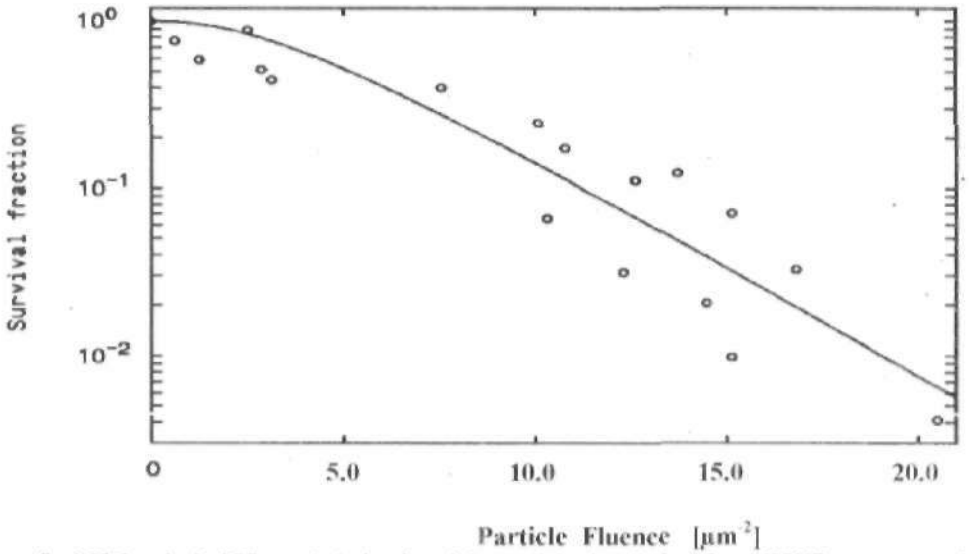


fig.4.25:Survival of D7 yeast strain after alpha particles irradiation; from 185MBq source { General fit from two independent experiments

Equation of the curve: = log Y = log {1- [1- Exp $\left(\frac{D}{D_q}\right)^n$ } Multitarget fit; σ_i = 0.297 μm^2 ; n = 2.860

The above result representing the survival of D7 yeast cells after treatment with alpha particles from a source having an activity of 185Mbq. Fluence up to 20 particles per μ^2 was applied to reduce the survival to a value less than 1 percent of the initial population. The fit is also shouldered. The inactivation cross section σ_i ,for the alpha action on the strain was obtained as 0.297 μm^2 .

Mutation frequency

i. gene conversion

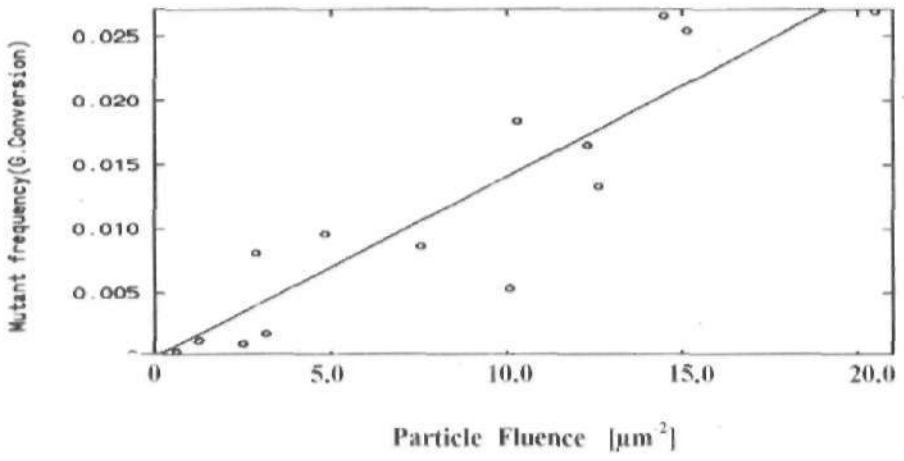


fig.4.26:Mutant frequency (Gene Conversion) of D7 yeast strain after alpha particles irradiation; from 185MBq source;{General fit from 2 independent experiments

Equation of the curve: $Y = AX + BX^2$ (Linear quadratic fit); A = 1.406×10^{-11} ; B = 4.114×10^{-23}

ii. reversion

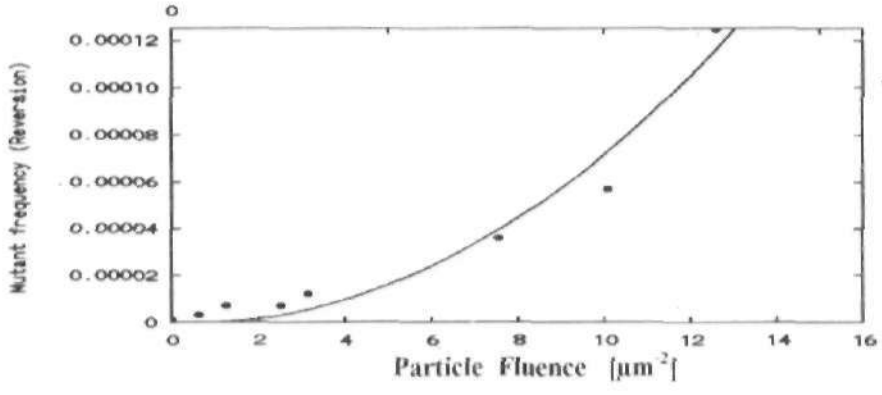


fig.4.27:Mutant frequency (Reversion) of D7 yeast strain after alpha particles irradiation; from 185MBq source;{General fit ;

Equation of the curve: $Y = AX + BX^2$ (Linear quadratic fit) : A = -7.650×10^{-15} ; B = 7.938×10^{-23}

Mutation events induced by the alpha particles on D7 yeast cell strains presented above also show linear quadratic fits for both cases - gene conversion and reversion though with different curvatures. The frequency of mutation of the gene conversions are also higher than those of the reversions just as in the case of x-ray action. Reversions are known to be point mutations and results are effected in a less frequent manner.

48 MBq
Cell inactivation:

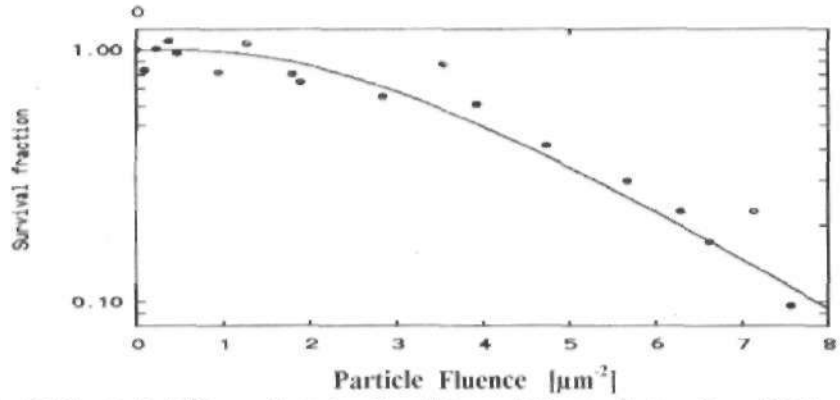


fig.4.28:Survival of D7 yeast strain after alpha particles irradiation; from 48MBq source;

Equation of the curve: = log Y = log {1- [1- Exp $\left(\frac{D}{D_o}\right)^n$ }; Multitarget fit; σ_i = 0.465 µm^2 ; n = 4.000

The survival curve above represents alpha particles action on D7 yeast cells after treatment with alpha particles source having a source activity of 48MBq. This resulted in a shorter irradiation time due to the installation geometry. Fluence up to 7 particles per µ^2 was applied to reduce the survival to a value less than 1 percent of the initial population. The curve shows a shouldered fit. The inactivation cross section σ_i ,for the alpha action on the strain in this case results in 0.465 µm^2 .

Mutation frequency
i. gene conversion

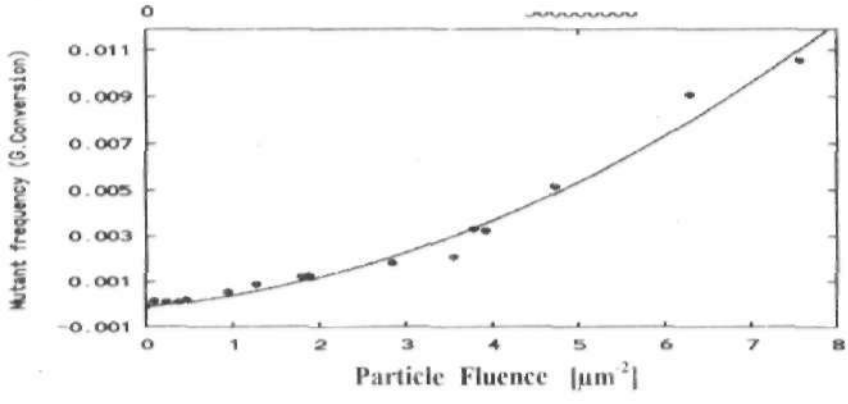


fig.4.29:Mutant frequency (Gene Conversion) of D7 yeast strain after alpha particles irradiation; from 48MBq source;(General fit from 2 independent experiments;

Equation of the curve: $Y = AX + BX^2$ *(Linear quadratic fit)* ; A = 3.483 x 10^{-12} ; B = 1.483 x 10^{-21}

ii. reversion

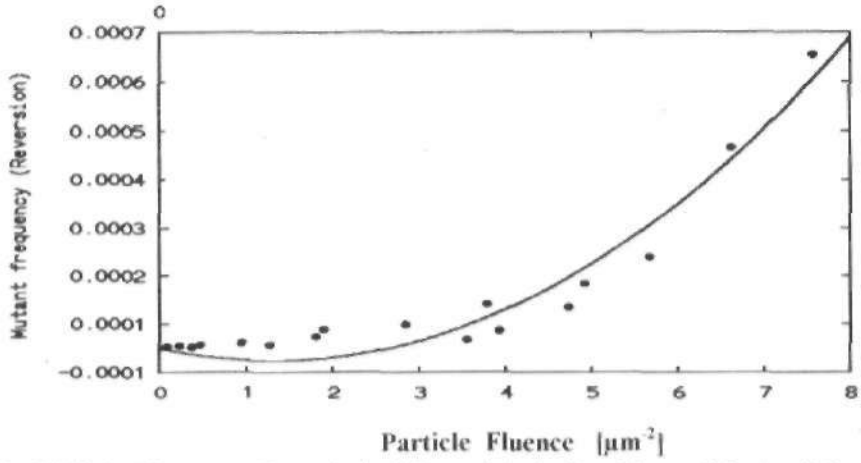


fig.4.30:Mutant frequency (Reversion) of D7 yeast strain after alpha particles irradiation; 3 from 48MBq source;{General fit from 2 independent experiments;

Equation of the curve: $Y = AX + BX^2$ (Linear quadratic fit) ; A = -3.944×10^{-13} ; B = 1.495×10^{-21}

Mutation events induced by the alpha particles on D7 yeast cell strains presented above represent that from alpha source having 48MBq as source activity. They show linear quadratic fits for both cases of gene conversion and reversion and with similar curvature though the frequency of mutation of the gene conversions are higher than those of the reversions just as in the case of x-ray action. Reversions have been noted as point mutations and show lower frequency of occurrence as compared to gene conversion.

4.3 Comparative Graphs For Survival And Mutation Frequency Of D7 Yeast Strain After Alpha Particles Irradiation From 185mbq And 48mbq Sources

The general fit of cell survival and mutation frequencies obtained for the D7 yeast cell strain from two sets of alpha particles irradiation using 185MBq and 48MBq alpha particles sources are compared hereunder. This is to show the relative range to which the individual results were obtained and to compare the effects of the

various sources on the assay. Through this comparison a judgement on whether a common fit could be achieved using the data from the two sources is easily reached.

48 Mbq And 185 Mbq Data Compared Cell Inactivation:

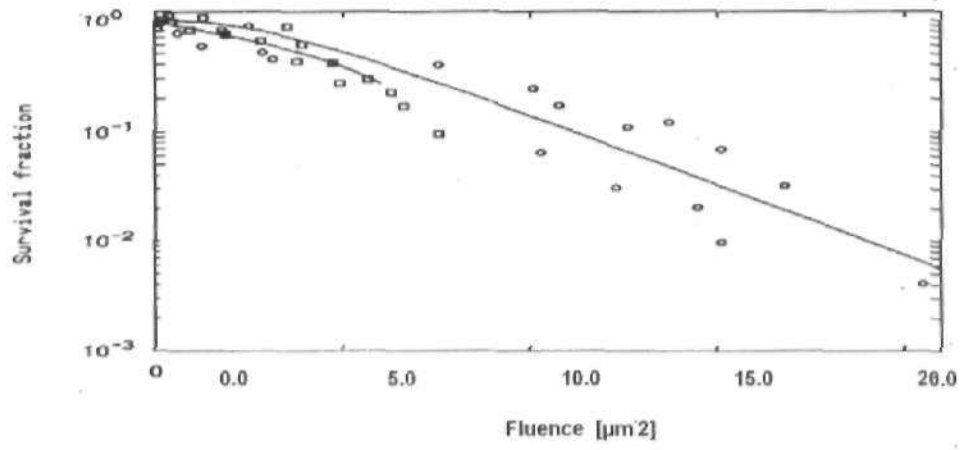


fig.4.31:Comparative fit ("SAME-SCALE FIT") for survival fractions of D7 yeast cell strain after irradiation with 185MBq and 48MBq alpha particle sources.; [Representation/comparison of the general curves]

The general fits obtained for survival assay/ cell inactivation using the two sources namely - 185MBq and the 48MBq as seen above show a close resemblance within the range of comparison. General fit of data for these two sets of results gives the next curve in fig.4.32.

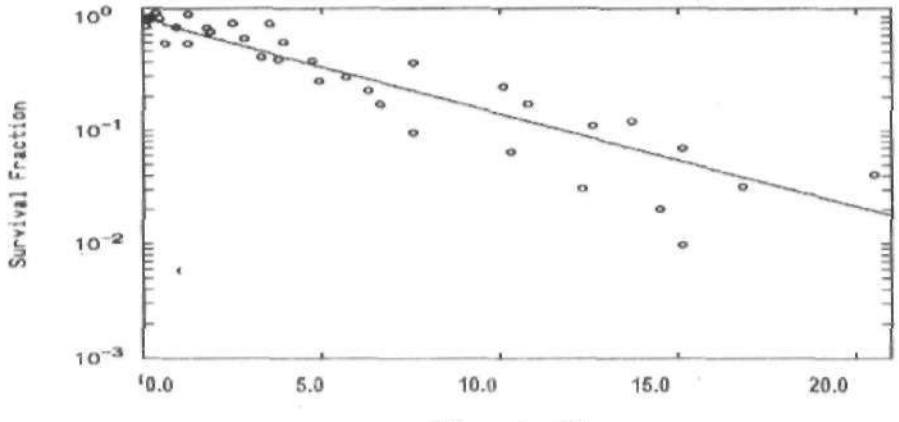


fig.4.32:Survival of D7 yeast strain after alpha particles irradiation:{Single fit of data from independent experiments involving 185MBq and 48MBq alpha particle sources.;

Equation of the curve: = log Y = log {1- [1- Exp $\left(\frac{D}{D_0}\right)^n$ }Multitarget fit; σ = 0.188 µm^2 ; n = 0.916

gene conversion:

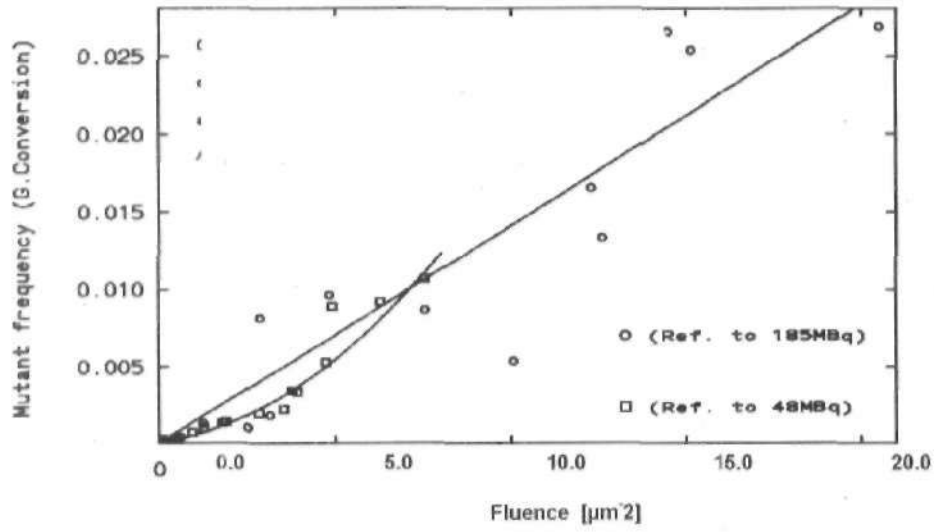


fig.4.33:Comparative fit ("SAME-SCALE FIT") for mutant frequency(gene conversion) of D7 yeast cell strain after irradiation with 185MBq and 48MBq alpha particle sources.: [Comparison of the general curves]

Mutant frequency (gene conversion) data obtained from the two sources - 185MBq and the 48MBq are also not showing a significant variation within the range of comparison. This justifies the general fit from the two sets of data which is given below;

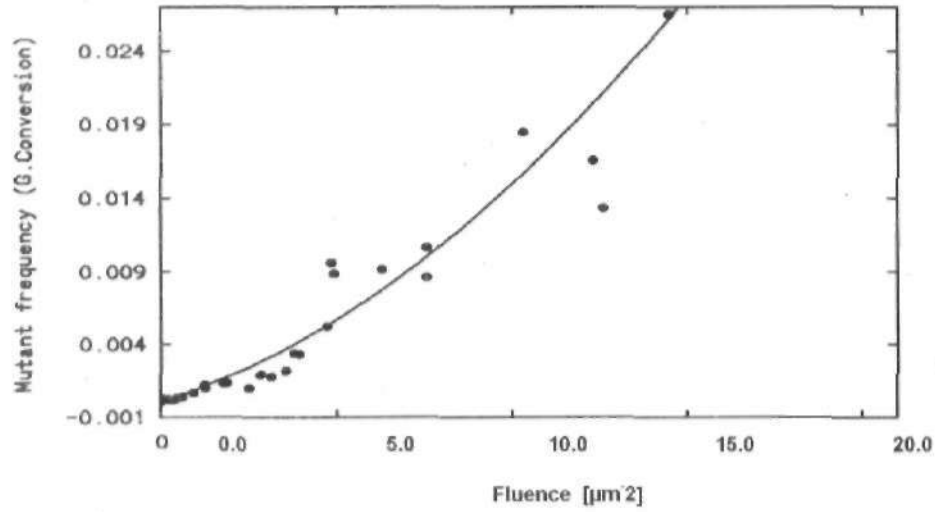


fig4.34:Mutants frequency(Gene conversion) of D7 yeast strain after alpha particles irradiation;{Single fit of data from 4 independent experiments involving 185MBq and 48MBq alpha particle sources.:-

Equation of the curve: $Y = AX + BX^2$ (Linear quadratic fit)

$A = 8.025 \times 10^{-12}$; $B = 6.974 \times 10^{-21}$

reversion:

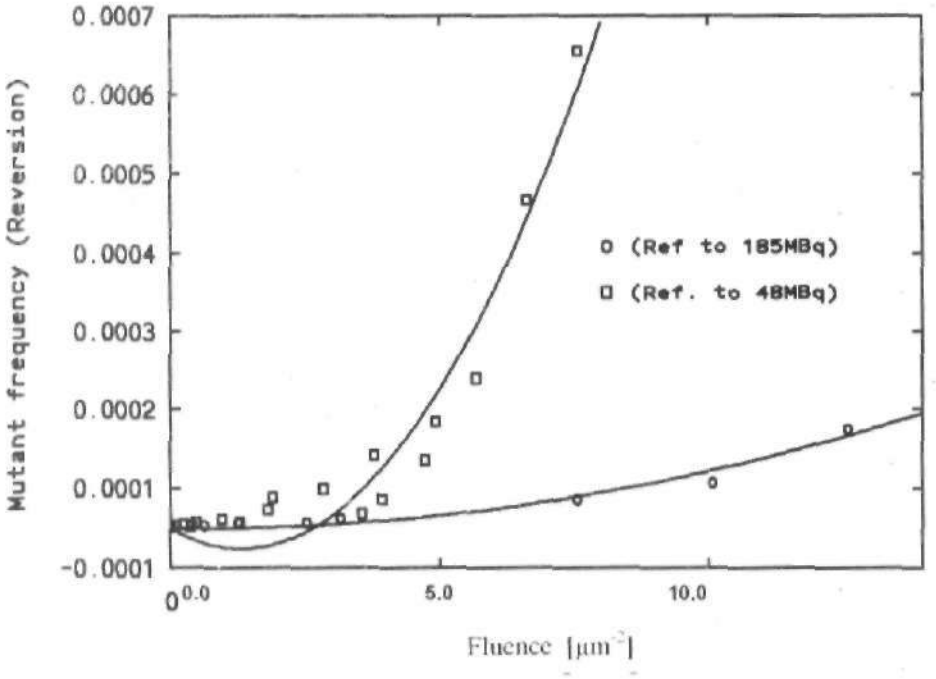


fig. 4.35:Comparative fit ("SAME-SCALE FIT") for mutant frequency(reversion) of D7 yeast cell strain afterirradiation with 185MBq and 48MBq alpha particle sources.; Comparison of the general curves;]

Mutant frequencies (reversion) obtained for the two sources in this case show a significant variation and therefore a general fit is not considered. This assay indicates a difference in the alpha effects using the two configurations which resulted in different dose-rates.

4.4 Survivals For Rad 54-3 Yeast Strain After Irradiationwith X-Rays And Incubated At Two Repair Temperature Extremes(23°C And 36°C).

The general fit of survival assays obtained for rad 54-3 yeast cell strain from two sets of X-ray experiments is presented. These results provide comparative basis for the alpha particles action on the same strain.

X-Ray Data

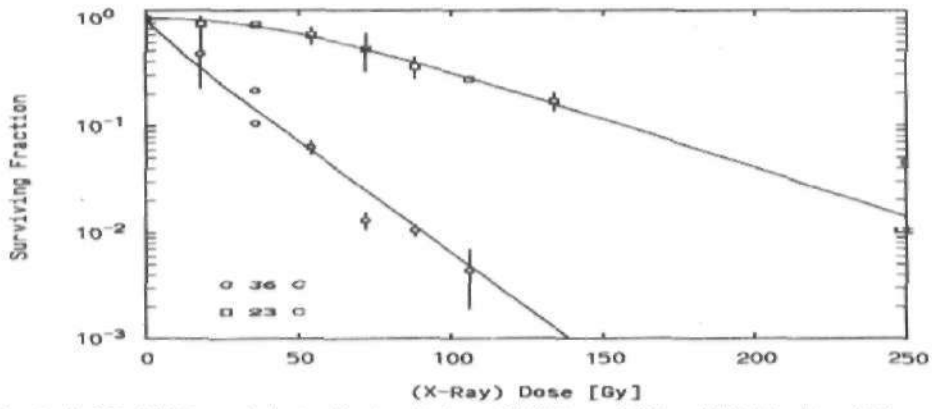


fig.4.36:Survival of Rad 54-3 yeast strain after irradiation with X-Rays;-36°C and 23°C Post irradiation.

Equation of the curve: $\log Y = \log\{1-[1-\mathrm{Exp}\left(\frac{-D}{D_o}\right)^n\}$

$D_{o\,36}$ = 20.9 Gy ; n_{36} = 1.000 ; $D_{o\,23}$ = 46.7 Gy ; n_{23} = 5.850

The result presented above represents the survival of Rad 54-3 yeast cells after treatment with X rays up to 150Gy. The strain is quite sensitive to ionizing radiation hence, there is a reduction in the survival down below 1% for cells incubated under 36°C and near 10% for cells incubated under 23oC. There is a broad distinction in the response of this strain to the x-ray treatment with respect to the incubation temperature. Those incubated under 36°C show higher sensitivity than those incubated under 23°C.

4.5 Survival Of Rad 54-3 Yeast Cell Strain After Irradiation With Alpha Particles And Incubated At Two Repair Temperature Extremes; [23°C; And 36°C

The fit of survival assays obtained for rad 54-3 yeast cell strain from six successful sets of repeated alpha particles irradiation are presented in figs. 4.37 and 4.38. These results were obtained for two incubation temperatures - 23°C and 36°C. Illustration of effect of incubation temperature which was accidentally observed in two other unsuccessful assays are also illustrated. This was obtained due to fluctuations in the temperature of the 36°C incubator.

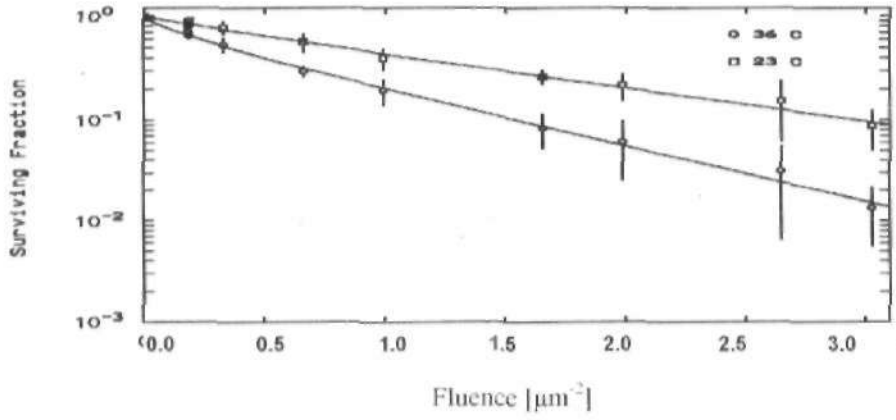


fig.4.37:Survival of Rad 54-3 yeast strain after irradiation with alpha particles from 48MBq source;-36°C and 23°C Post irradiation, incubation temperatures:

Equation of the curve: ≡ $\log Y = \log \{1 - [1 - \mathrm{Exp}\left(\frac{D}{D_o}\right)^n]\}$

$\sigma_{i\,36} = 1.2527\mu m^2$; $n_{36} = 1.000$; $\sigma_{i\,23} = 0.737\mu m^2$; $n_{23} = 0.879$

Alpha particles action on Rad 54-3 strains of yeast cells incubated at two different temperatures -23°c and 36°C are presented above. The strain being sensitive to ionizing radiation shows a dependence also on the post-irradiation incubation temperatures. The alpha action as compared with that of the x-rays shows a reduced separation between the fits representing the two temperature extremes.

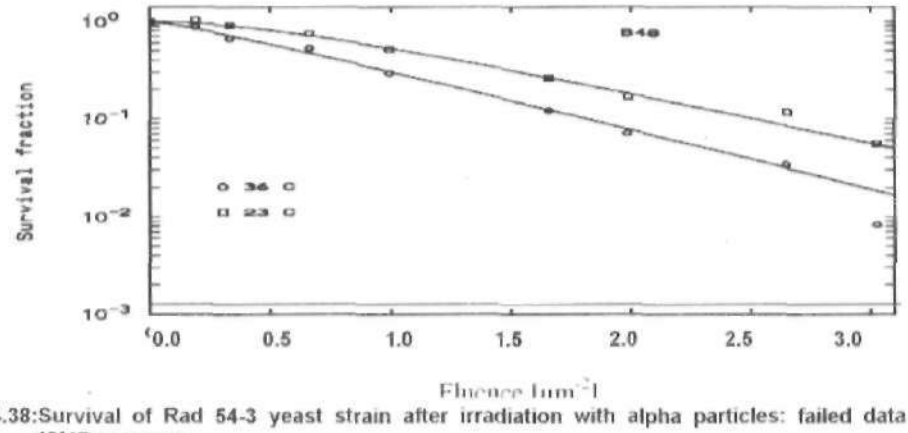


fig.4.38:Survival of Rad 54-3 yeast strain after irradiation with alpha particles: failed data from 48MBq source;

Fig 4.38 indicates a result obtained from an incubator where the 36°C temperature showed fluctuations. This result was not included in the general fit

presented above and shows a shift in the slope of the curve representing 36°C incubation temperature. This is under discussion in chapter five.

CHAPTER FIVE

5.0 DISCUSSIONS/CONCLUSIONS

5.1 The Irradiation Facilities And The Physical Conditions Of The Alpha Particles Irradiation Systems

The irradiation geometry of the alpha particles sources (185MBq and 48MBq) and the physical environment housing the systems had impacts on the feasibility, success and results of the assays. With particular reference to the collimated system - (Kaba) set-up - fig 3.17,p.57 and fig.3.19,p.58, the alpha particles had to travel through helium gas and were further attenuated by the mylar foil placed between the target samples and the helium tank. The mylar foil helped in maintaining the helium gas pressure during irradiation and also protected the source from falling cell samples that would have had fallen off the target holder (salt agar plate in this case). Thus the energy of the particles was considerably reduced, and the percentage of these particles having the required energy to arrive and penetrate the cell samples was also reduced. Particle fluence detected at the target point (tables 3.13,p.49; 3.21,p.60) was correspondingly low, thus irradiation times for this system were prolonged in order to achieve the desired fluence needed for distinct effects.

The three rings system has about 3mm air column between the source and the sample and also a 2μm mylar-foil in between

which also served in protecting the source from dirt of falling cell samples (compare description in tables 3.13, .3.18, and fig.3.20, for irradiation geometry diagrams). The placement and removal of the filters from the source were mechanically achieved and the irradiation times were relatively shorter as compared to the case of the 'Kaba' systems. This was due to the fact that higher fluence-rate was detected at the target position using this set-up (compare summary given in table 3.21,p.60).

5.2 Cell Survival Of The D7 Yeast Cell Strain After Alpha Particle Irradiation

For the D7 yeast cell strain, irradiation time up to about 20 hours was required to reduce its survival to approximately 10% when irradiated with 185MBq alpha particles source in the 'Kaba' set-up. This was quite a lengthy duration as compared to approximately 3 hours required for reduction of survival of same strain to about the same level when using the 48MBq source in the three rings set-up. (Fig.3.18,p.57 and fig.3.20,p.58 show the three rings irradiation system). Results indicate about 1 percent survival of D7 yeast strain after 2hrs,40mins irradiation time using the three rings set-up and 48MBq alpha particles source. This remarkable difference due to the source geometry effects could

be technically explained to relate to attenuation of alpha particles as well as alpha particles detection and dosimetry.

All irradiation with the alpha particles were performed under 0°C so as to suppress metabolic activities of the cells during irradiation and also during post-irradiation waiting periods. This was also intended to inhibit repair mechanisms before plating and incubations at the appropriate temperature. In addition to that, the cells were deposited on filter membranes placed on salt-agar medium which is nutrient-free (composition of salt-agar medium was given in section 3.1,p.42).

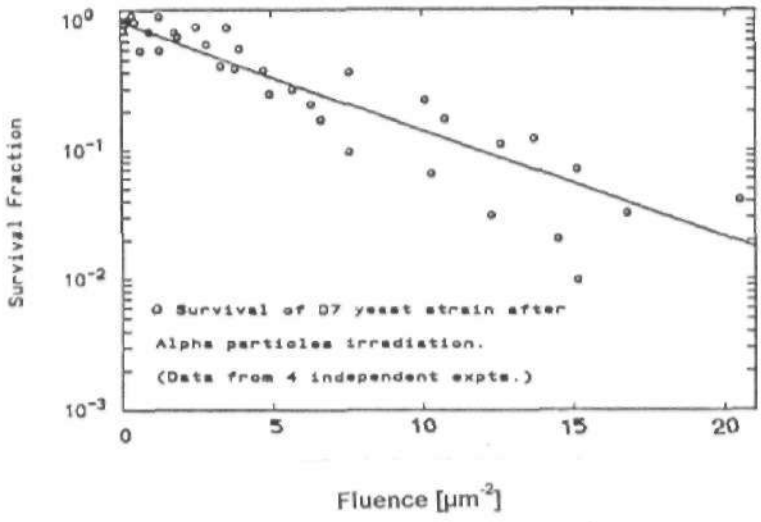
Generally, irradiation experiments using these sources - 48MBq source in three rings set-up and 185MBq in 'kaba' set-up were quite feasible both for the wild-type cell line - D7 yeast cell strain and the more sensitive mutant cell line - Rad 54-3 in which irradiation times were much more shorter than those required for the D7 strain.

Repair and effects of long irradiation times were controlled through temperature regulation and irradiation of the cell samples placed on nutrient-free (salt-agar) medium. Results of irradiation from both sources agree within error limits and can be seen in the comparative survival fits of fig.4.31, p.68. Based on such conformations, a general spectrum fit was obtained for the two sources as represented in the graphs of fig.4.32, p.68. These represents the survival of D7 after irradiation with alpha particles and the curve shows tolerable scatter points. However, extensive

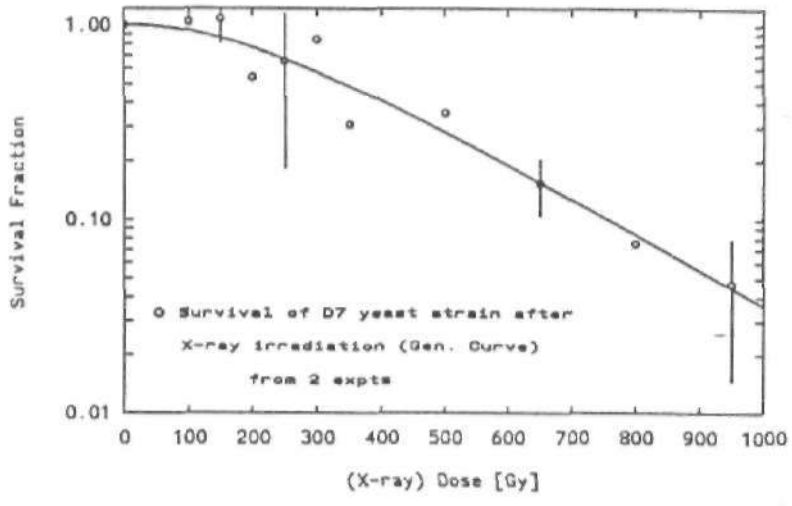
survival assays covering larger range of survival could further clarify whether or not, there exist effects of long irradiation times on the results observed.

Comparing the survival of D7 yeast strain after alpha particles irradiation (general fit at fig.4.32,p.68) with that obtained from X-ray irradiation (general fit at fig.4.22,p.62) it may be seen that the respective trends show a great deal of resemblance apart from the shoulder region which is properly defined in the case of X-ray actions and not clearly defined in the case of alpha particles results which however show cluster of points within this region. Shoulders in survival curves being indication of repair or recovery often provide knowledge of the repair of lesions produced by various types of radiation. This repair process is confirmed as being undoubtedly viable for X-ray lesions as seen from the results cited, but seem to be diminished with the alpha particles produced lesions. This point seems to be in agreement with the claim that as the LET of the radiation increases, the recovery-(which is a function of repair of sub-lethal lesions indicated by shoulders of the survival curves) is diminished. This is due to the fact that the interaction processes result mostly in single hit events.

Survival parameters - D_o-values for X-rays results and inactivation cross sections for alpha particle irradiation which were obtained from the exponential tails of the respective survival curves are summarised in table of appendix B1 and B2, pp.93-94.



Survival of D7 after X-ray irradiation
General fit from fig. 4.22, p 62



Survival of D7 after Alpha particles irradiation
General fit from fig. 4.33, p 68

5.3 Analysis Of Mutation Frequency (Gene Conversions And Reversions) Of The D7 Yeast Cell Strain After Alpha Particles Irradiation

Mutant frequencies (gene conversions and reversions) obtained for D7 yeast cell strain after alpha particles irradiation showed a consistent linear-quadratic fit - {compare fig.4.26,p.65 of the general fit for gene conversion -data from 185MBq source; and fig.4.29,p.66 of general fit for gene conversion - data from 48MBq source}; also, {compare fig 4.33,p.69 of comparative fits for 185MBq and 48MBq sources for gene conversion}; And also compare fig.4.27,p.65 of general fit for reversion - data from 185MBq source; and fig.4.30,p.67 of general fit for reversion - data from 48MBq source};and also {compare fig.4.35,p.70 of comparative fits for 185MBq and 48MBq sources for reversion.}

It is worthwhile noting that frequencies for conversion were higher than those for reversions in all the cases cited above. This is undoubtedly the expected case since reversions results are from a chance of point mutation which may either reverse the initial mutation or create an equivalent effect opposite to it. This possibility is somewhat a rare event - though feasible, and this is reflected in the low mutant frequencies observed.

Within the fluence range of investigation and comparison, there is a general conformation of results obtained from both the 185MBq and 48MBq sources despite the difference in irradiation times and other parameters. This is however not the case for all

the investigated end-points. It is noted that the conformations prevail strongly in the survival assays - fig.4.31,p.68; frequencies for gene conversions - fig.4.33,p.69; but there exist a great variation when comparing mutant frequencies obtained for reversions from both sources - fig.4.35,p.70.

Since the irradiation types (alpha particles) were the same in both instances and the cell strain was also the same in the two investigations, the only possible parameter considered to be responsible for this marked difference is the irradiation time which was significantly lengthy in the case of 185MBq source in the 'Kaba' set-up. This as explained earlier was due to low fluence-rate detected at the sample point during dosimetry

These results show that the mutations which lead to a stable reversion are more feasible with the 48MBq source in the three rings set-up having higher fluence-rate. The low fluence rate obtained from the 185MBq source placed in the "Kaba" set-up would have been due to high attenuation of the less energetic alpha particles thereby reducing the percentage of alpha particles that actually arrived to interact with the target samples. Correspondingly, mutant frequencies for reversion assays indicate a significant reduction not significantly observed in other two assays - survival and gene conversion. A general trend for alpha particles action on the D7 yeast cell strain with respect to reversions was distinctly collated with respect to the two sources as represented in the comparative graph of fig.4.35,p.70.

These two source-specific trends are also linear-quadratic and quite identical in trend.

5.4 Cell Survival Of Rad 54-3 Yeast Cell Mutant Under Repair Restricted And Permissive Temperatures [After Alpha Particles Irradiations]

Rad 54-3 yeast cell mutant being significantly sensitive to ionizing radiation shows high level sensitivity to post irradiation incubation temperature. Results obtained for this strain after a series of repeated alpha particles irradiation indicate this sensitivity in all the eight repeated assay carried out using 48MBq alpha particles source. This was also observed for X-ray irradiation of same strain for two independent assays carried out for comparative purposes.

Interesting to mention is the result of alpha particles presented in fig.4.38,p. 72 which illustrates the effect of failure in incubation temperature at 36°C. The incubator showed temperature fluctuations during incubation periods and this was correspondingly reflected as a shift in the 36°C curve towards the 23°C region (see comparison curves in fig.4.38, p.72). Stabilization of the temperature level of the incubator used however restored the "expected" difference in temperature sensitivity as seen in the general fit of six successful assays in fig 4.37,p.72 (individual fits are presented in appendix A4,p.92). The interaction cross sections for the individual fits as well as the

general fits are summarised in the table of appendix B3 and B4 including D_o values for the X-ray assays on the strain-(see pp.93-94). σ_{36} obtained from a general fit of six successful assays has a value of 1.34 μm^2 and σ_{23} = 0.74μm^2 also obtained from a general fit of six successful assay.

5.5 Conclusions

After series of experiments and observations from both the comparative and actual assays involving X-ray experiments with D7 yeast cell strain and Rad 54-3 which provided a comparative basis for alpha particles assays with same strains, the following points are raised as conclusions from the results presented and for the feasibility of the various assays carried out;

- Survival and mutation assays have been found to be feasible from both the 185MBq alpha particles source installed in the Kaba set-up and the 48MBq alpha source installed in the three rings set-up as was observed from the reduction of survival down to a fraction of less than 10 percent under 24 hours of irradiation of the D7 strain using the Kaba systems; and within approximately 3 hours using the three rings system;. It is noted therefore that these assays were successful even for the D7 strain which is also in the wild type category. Though the irradiation times seem extended for the 185MBq alpha source in the Kaba set-up, it provided a

basis for further assessment of the results obtained from the two sources as representatives of acute and chronic-type irradiation using similar radiation type-alpha particles. It was observed that cellular repair and other side effects during this long irradiation times were considerably suppressed by temperature regulation - irradiation at 0°C, and useful results were obtained from this system especially so in the cases of mutation assays.

- Helium gas flooding of the Kaba system was quite important and easy to achieve. Failure of the helium gas flow actually resulted in attenuation of the alpha particles by air which had taken over the environment of the helium tank (see Kaba set-up diagrams in fig.3.17,p.57 and fig.3.19,p.58). From the results of this first yeast assays using the D7 yeast strain in the (Kaba) set-up, it is clear that future collimated alpha beam can be obtained from this set-up to achieve a more homogenous alpha particles irradiation which could be described in terms of a homogenous alpha energy spectrum and homogeneous LET values in close approximation. This would be possible at least for the sensitive mutant cell lines and for mammalian cells as well, due to their high sensitivity to ionizing radiation.

- The D_0 value of the X-ray assay on D7 yeast strain was found to be 180.7 Gy from a pulled-data after two independent X-ray assay. Another investigator however reported a D_0 value of 175Gy

Stoll, (1989); Another feature deducible from the X-ray results includes a pronounced shoulder which is characteristic of this assay depicting repair activity.

- Mutant frequencies from the D7 yeast strain have been consistently linear-quadratic both for X-ray assays and alpha particles assay and for the two investigated end-points - gene conversions and reversions.

- Distinction in absolute values of mutant frequency (particularly reversions) had been observed between acute- high fluence-rate of alpha particles irradiation when compared to low fluence-rate of alpha particles irradiation.

- Thus, the fluence rate obtained from 48MBq source(in three rings set-up) showed a high reversion frequency than the low fluence-rate source 185MBq in "Kaba" set-up.

- The inactivation cross section obtained for alpha particles irradiation of the D7 yeast strain was found to be 0.188 μm^2. This value was obtained from a pulled-data of 4 independent experiments with the alpha particles.

- The D_0 value of the X-ray assay on rad 54-3 yeast strain was found to be 20.9 Gy at 36°C post irradiation incubation temperature, and 46.7 at 23°C post-irradiation incubation

temperature. Values were obtained from a pulled-data after two independent X-ray assays. Kiefer et. al (1990)} reported $D_{o36°C}$ = 14.4 ± 0.4 Gy and $D_{o23°C}$ = 84 ± 12 Gy. Other investigators have also reported values within this range and those obtained in this present investigation. Though the incubation medium used by most other investigators show variation in composition.

- The inactivation cross section obtained for alpha particles irradiation of the rad 54-7 yeast strain was found to be 1.2527 μm^2 at 36°C post irradiation incubation temperature, and 0.7375 μm^2 at 23°C post-irradiation incubation temperature. Values were obtained from a pulled-data after six independent alpha particles assays with rad 54-3 yeast cell strain.

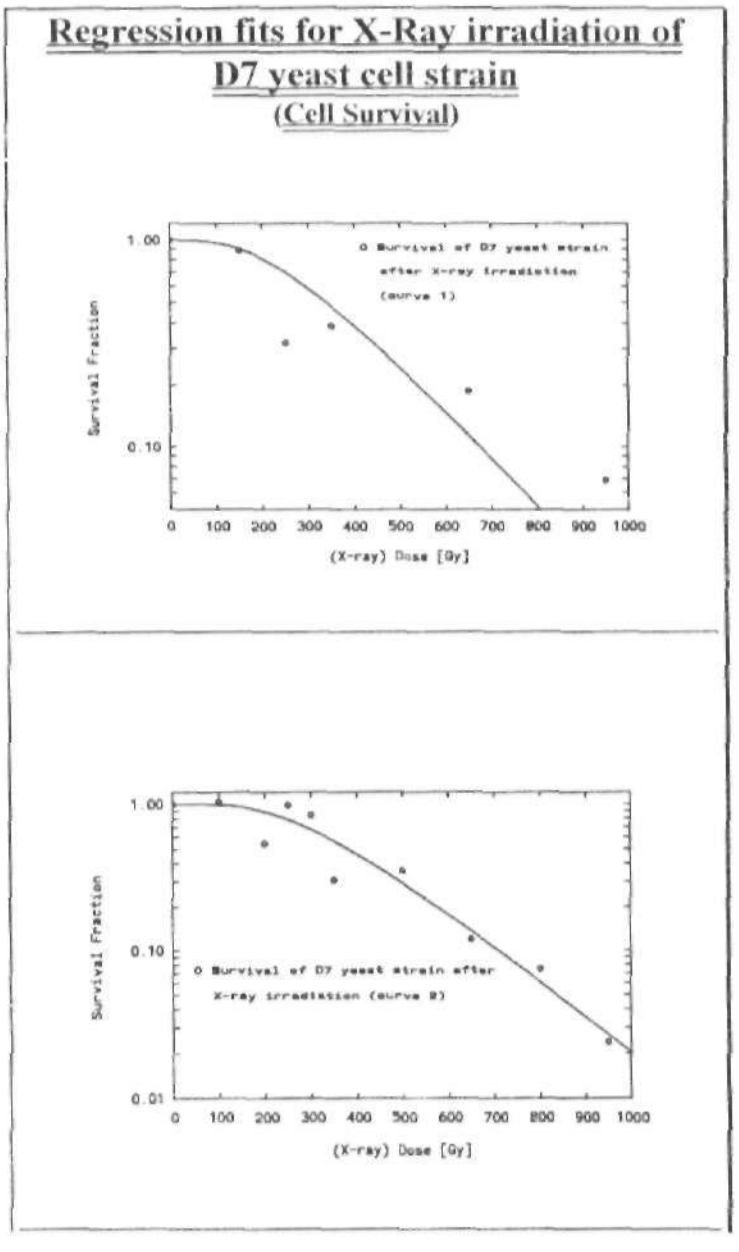
- Survival patterns induced by alpha particles in rad 54-3 and D7 yeast cell strains show reduced shoulders in the survival curve fits as compared to survival fits of same strains induced by X-rays. More especially is the separation between the curves representing the 23°C and 36°C post-irradiation incubation temperatures for rad 54-3 which shows a reduction when comparing alpha action on rad 54-3 with that of X-rays action. These are clear distinction in the response of the test samples differently to the two radiation types.

- All survival curves for alpha particles assay with rad 54-3 incubated at 36°C show identical exponential fits without any shoulder whatever. This is an indication of a reduced repair activity or recovery of this cell strain under incubation at the said temperature.

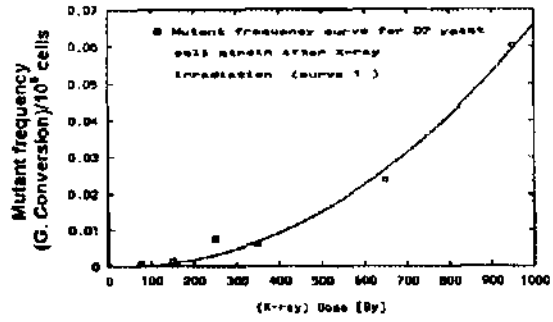
APPENDIX A1

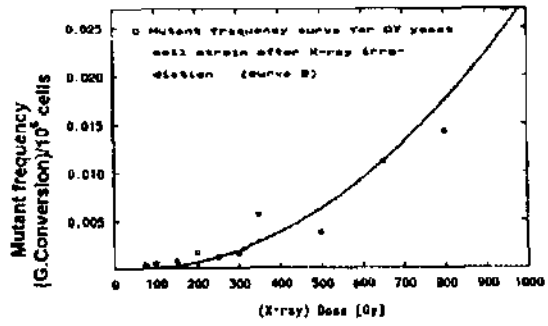
GRAPHICAL PRESENTATIONS OF SURVIVAL AND MUTATION FREQUENCY FOR D7 YEAST STRAIN AFTER X-RAY IRRADIATION

Individual fits of survival and mutation frequencies (gene conversions and reversions) obtained for the D7 yeast cell strain from two sets of X-ray experiments are presented hereunder. These provide comparative basis for the alpha particles action on the same strain and were used in computing the general fits presented in the results sections

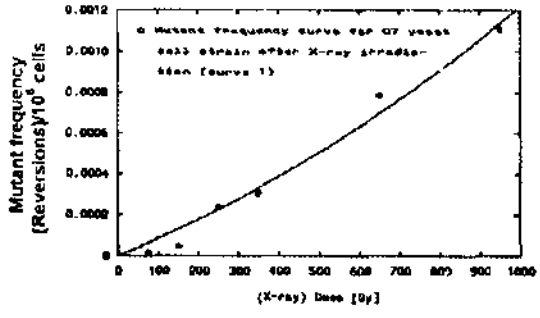


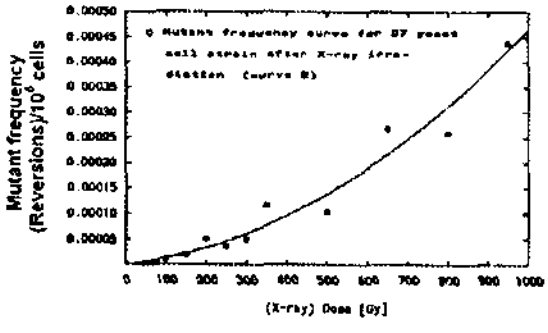
Regression fits for X-Ray irradiation of D7 yeast cell strain
(Mutation assay: G.Conversion)





Regression fits for X-Ray irradiation of D7 yeast cell strain
(Mutation assay: Reversion)



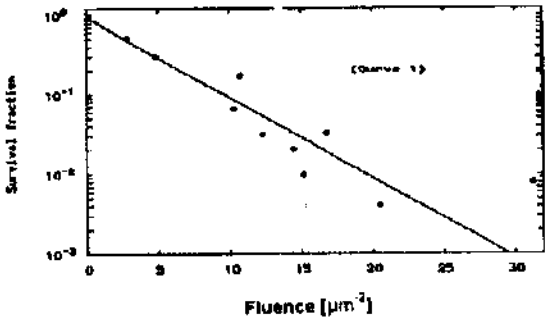


APPENDIX A2

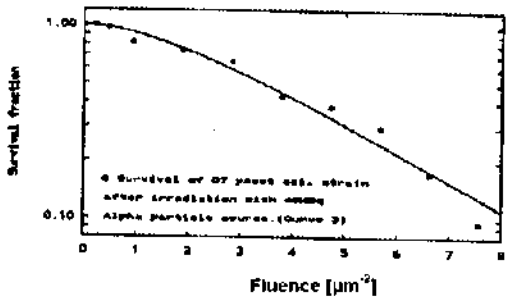
GRAPHICAL PRESENTATIONS OF SURVIVAL AND MUTATION FREQUENCY FOR D7 YEAST STRAIN AFTER ALPHA PARTICLES IRRADIATION.

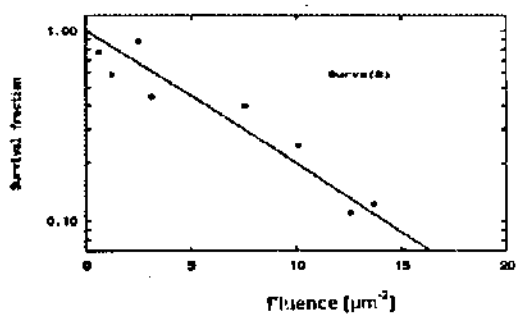
Individual fits of survival and mutation frequencies (gene conversions and reversions) obtained for the D7 yeast cell strain from two sets of alpha particles irradiations using 185MBq and 48MBq alpha particles sources are presented hereunder. These resusults were obtained to compare the feasibility of survival and mutation assays from these two sources and to compute a general fit for the alpha action on the D7 yeast strain. The general fits are presented in the results section.

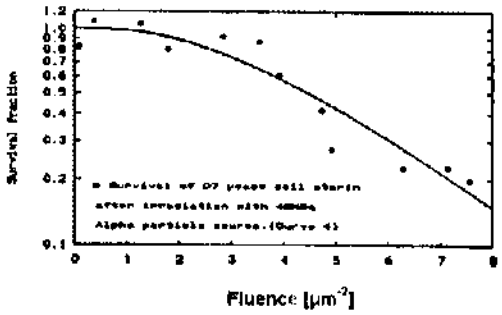
Alpha Particle Effects
on D7 yeast cell strain
Data from 185MBq Source
(Cell Survival)



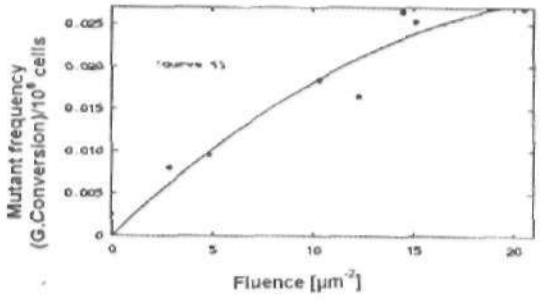
Alpha Particle Effects
on D7 yeast cell strain
Data from 48MBq Source
(Cell Survival)

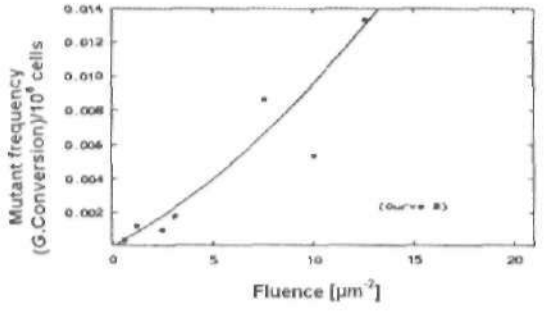




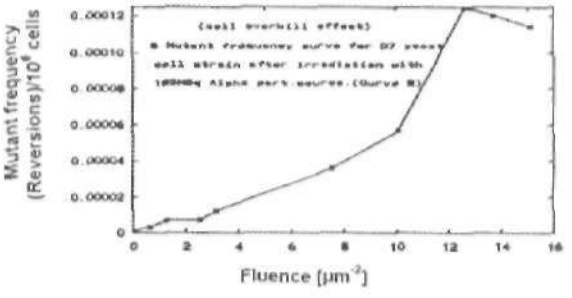


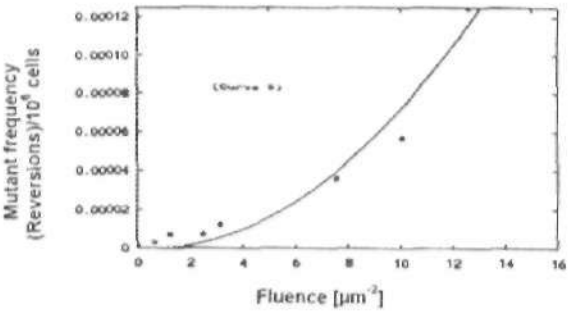
Alpha Particle Effects on D7 yeast cell strain Data from 185MBq Source (Mutation assay; G.Conversion)



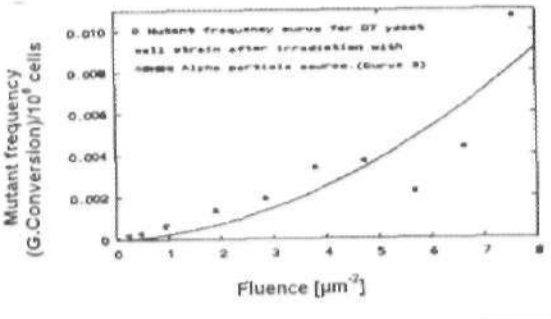


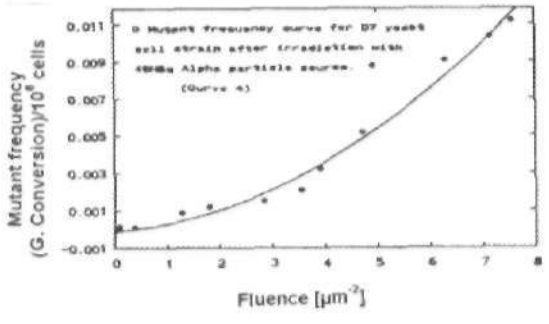
Alpha Particle Effects on D7 yeast cell strain Data from 185MBq Source (Mutation assay; Reversions)



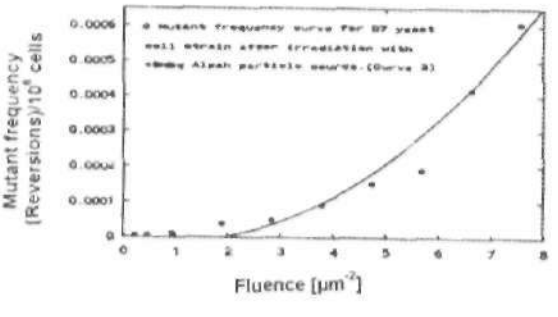


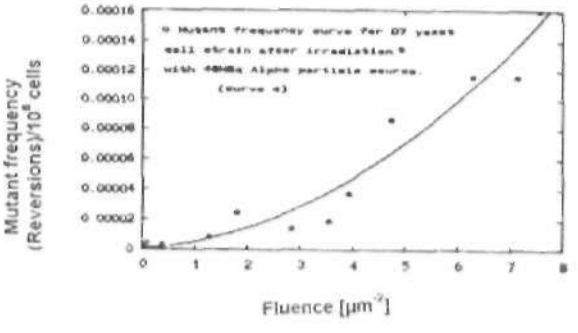
Alpha Particle Effects on D7 yeast cell strain Data from 48MBq Source (Mutation assay; G.Conversion)





Alpha Particle Effects on D7 yeast cell strain Data from 48MBq Source (Mutation assay; Reversions)

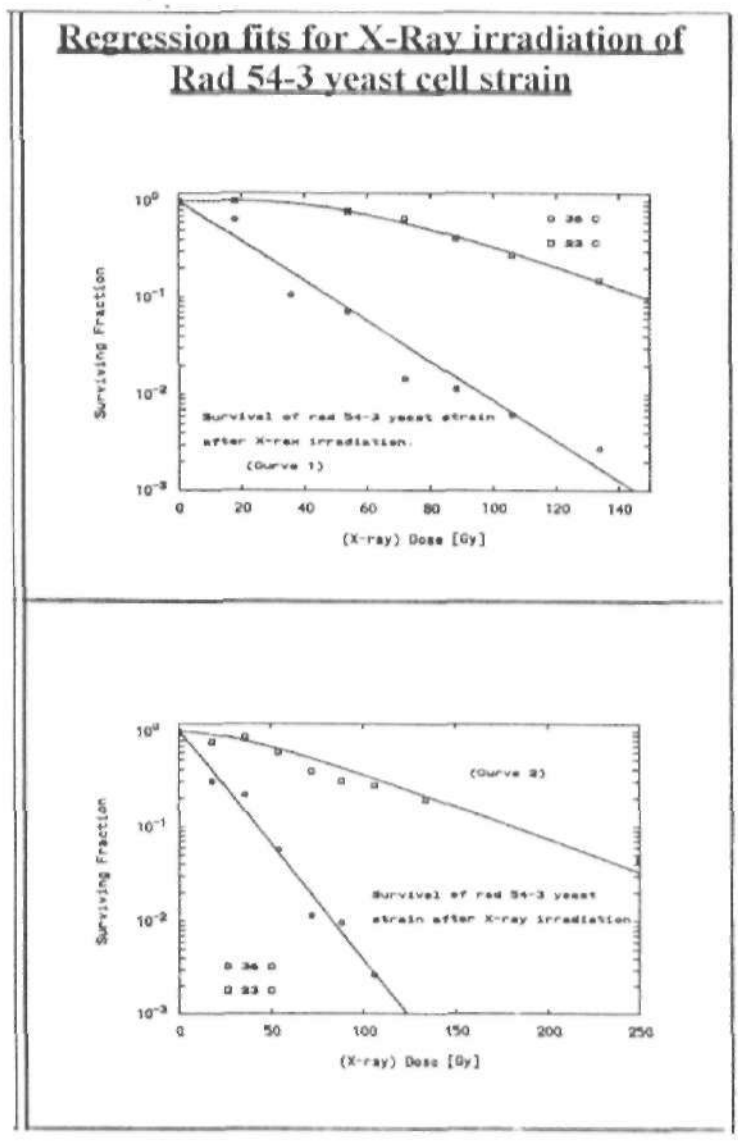




APPENDIX A3

GRAPHICAL PRESENTATIONS OF SURVIVALS FOR RAD 54-3 YEAST STRAIN AFTER IRRADIATION WITH X-RAYS AND INCUBATED AT TWO REPAIR TEMPERATURE EXTREMES(23°C AND 36°C).

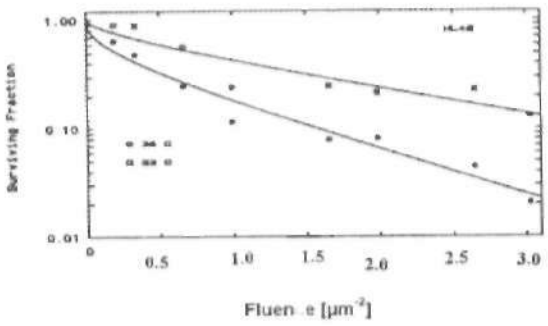
The individual fits (fits for regression) of survival assays obtained for rad 54-3 yeast cell strain from two sets of X-ray experiments are presented hereunder.

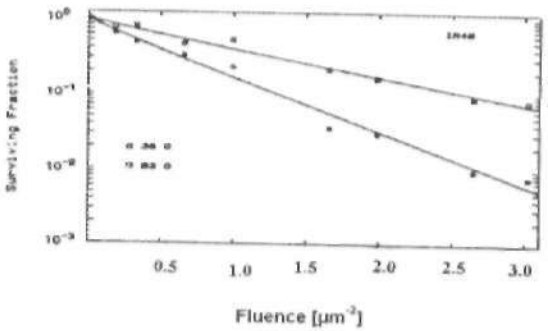


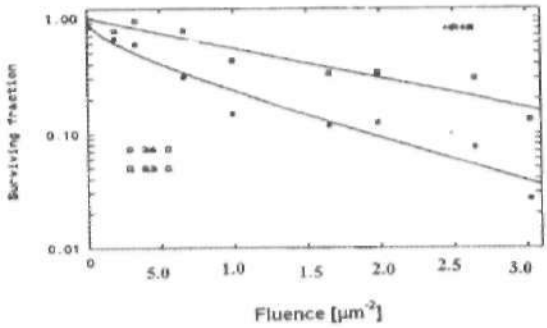
APPENDIX A4

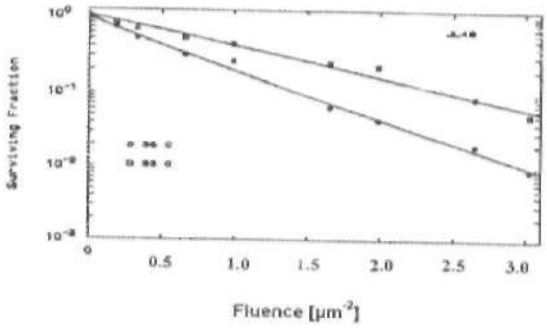
GRAPHICAL PRESENTATIONS OF SURVIVAL FOR RAD 54-3 YEAST CELL STRAIN AFTER IRRADIATION WITH ALPHA PARTICLES AND INCUBATED AT TWO REPAIR TEMPERATURE EXTREMES; [23°C; AND 36°C

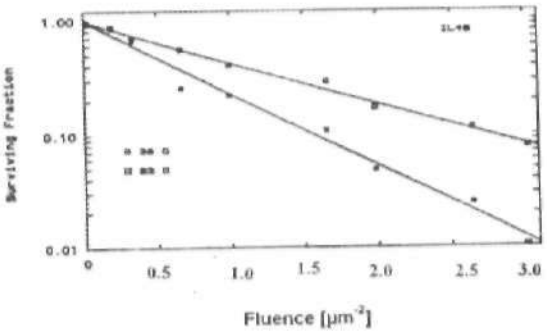
The individual fits of survival assays obtained for rad 54-3 yeast cell strain from six successful sets of independent experiments are hereunder presented. These were used in obtaining the general curve presented in the result section.

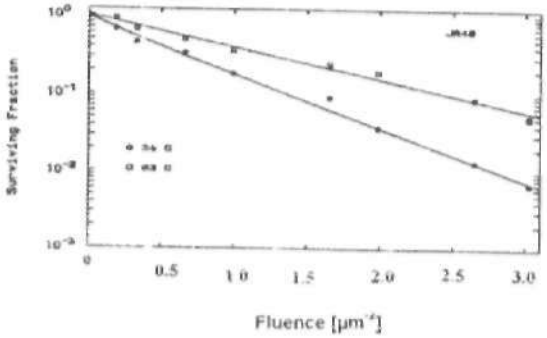












APPENDIX B → TABLES OF D_o AND σ_i FOR X-RAY AND ALPHA ASSAYS

Appdx B1

S/No	Description of assay	Specification of curves and fits	D_o values [Gy]	Average D_o[Gy]
A	Survival of D7 yeast strain after X-ray irradiation	i. curve 1;appdx A1; p.87	185.0±60.1;±32.70%	
		ii. curve 2;appdx A1; p.7	179.3±20.1;±11.21%	
		*average	→	182.15
		iii. general fit;fig.4.22; p.62	180.7	

Appdx B2

S/No	Description of assay	Specification of curves and fits	σ_i values [μm^2]	Average σ_i [μm^2]
B	Survival of D7 yeast strain after alpha particles irradiation	i. curve 1(fr;185MBq); appdx A2 p.89	0.229 ± 7.13%	
		curve 2(fr;185MBq); appdx A2 p.89	0.165 ± 17.90%	
		* average	→	0.197
		iii.general curve (185MBq); fig.4.25; p.64	0.297 ± 16.52%	
		iv. curve 3(fr;48MBq); appdx A2 p.89	0.358 ± 2.65%	
		v. curve 4 (fr;48MBq); appdx A2 p.69	0.465 ± 15.90%	
		* average	→	0.376
		vi..general curve (48MBq); fig.4.28; pp.66	0.465 ± 2.33%	

S/No	Description of assay	Specification of curves and fits	σ_i values [μm^2]	Average σ_i [μm^2]
		average of individual fits from B. i, ii, iv,and v	→	0.287
		average of general fits; B. iii, and vi.	→	0.381
		vii. general curve from 185MBq and 48MBq data; fig.4.32;p. 68	0.188 ± 11.47%	

Appdx B3

S/No	Description of assay	Specification of curves and fits	D_o values [Gy]	Average D_o[Gy]
C	Survival of rad54-3 yeast strain after X-ray irradiation	i. (36°C) appdx A3 p.91 curve 1;	21 ± 11.23%	
		curve 2;	17 ± 8.79%	
		* average: (36°C)Ci;curves 1and2	→	19.00
		i. (23°C) appdx A3 p.91 curve 1;	36.7 ± 4.46%	
		curve 2;	60.1 ± 4.21%	
		* average: (23°C)Cii;Curves 1,; 2	→	48.05
		iii. general fits; (36°C) fig.4.36; pp.71		20.9
		(23°C) fig.4.36; pp.71		46.7

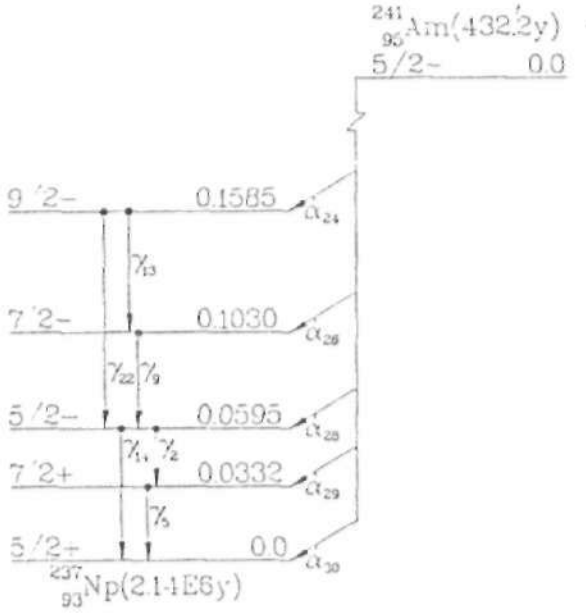
Appdx B4

S/No	Description of assay	Specification of curves and fits	Average	σ_i [μm^2]
D	Survival of rad54-3 yeast cell strain after alpha particles irradiation	i. (36°C)-48MBq appdx A4;p.92		
		curve 3;	0.947 ± 11.23%	
		curve 4;	0.857 ± 15.67%	
		curve 5;	1.457 ± 6.12%	
		curve 6;	1.616 ± 6.16%	
		curve 7;	1.464 ± 3.63%	
		curve 8;	1.563 ± 2.60%	
		* average; Curves 3,4,5,6,7,8	→	1.3171
		general fit;fig4.37;p.72	1.2527 ± 2.32%	
		ii. (23°C)-48MBq appdx A4;p.92		
		curve 3;	0.515 ± 26.85%	
		curve 4;	0.578 ± 23.83%	
		curve 5;	0.805 ± 4.65%	
		curve 6;	0.804 ± 8.41%	
		curve 7;	0.964 ± 7.65%	
		curve 8;	0.923 ± 7.33%	
		* average; Curves 3,4,5,6,7,8	→	0.7645
		general fit;fig4.37;p72	0.7375 ± 5.42%	

Appdx B5

DECAY SCHEME OF AMERICIUM 241 AND PRINCIPAL CONTRIBUTIONS

AMERICIUM



95-AMERICIUM-241

HALFLIFE = 432.2 YEARS 23-MAR-78
DECAY MODE(S): α

RADIATION	y(i) (Bq-s)$^{-1}$	E(i) (MeV)	y(i)×E(i)				
α 24	1.40E-02	5.388E 00	7.54E-02	ce-M, γ 13	2.68E-03	5.109E-02*	1.37E-04
α recoil	1.40E-02	9.100E-02	1.27E-03	ce-N*, γ 13	1.01E-03	5.552E-02*	5.60E-05
α 26	1.28E-01	5.443E 00	6.97E-01	γ 14	3.57E-01	5.954E-02	2.13E-02
α recoil	1.28E-01	9.193E-02	1.18E-02	ce-L_1, γ 14	1.09E-01	3.711E-02	4.05E-03
α 28	8.52E-01	5.486E 00	4.67E 00	ce-L_2, γ 14	9.92E-02	3.794E-02	3.77E-03
α recoil	8.52E-01	9.265E-02	7.89E-02	ce-L_3, γ 14	9.96E-02	4.196E-02	4.18E-03
α 29	2.00E-03	5.512E 00	1.10E-02	ce-M, γ 14	7.61E-02	5.510E-02*	4.19E-03
α recoil	2.00E-03	9.308E-02	1.86E-04	ce-N*, γ 14	2.67E-02	5.954E-02*	1.59E-03
α 30	3.40E-03	5.544E 00	1.88E-02	ce-L_1, γ 22	1.31E-03	7.735E-02	1.01E-04
α recoil	3.40E-03	9.364E-02	3.18E-04	ce-L_2, γ 22	8.68E-04	8.131E-02	7.06E-05
[illegible]	2.40E-02	2.634E-02	6.32E-04	ce-M, γ 22	6.24E-04	9.452E-02*	5.89E-05
[illegible] γ 2	2.34E-02	3.918E-03	1.15E-04	$L\alpha$ X-ray	2.44E-01	1.393E-02*	3.40E-03
ce-L_1, γ 2	5.61E-02	4.744E-03	2.66E-04	$L\beta$ X-ray	3.05E-01	1.751E-02*	5.34E-03
ce-L_2, γ 2	7.32E-02	8.735E-03	6.39E-04	$L\gamma$ X-ray	7.31E-02	2.093E-02*	1.53E-03
ce-M, γ 2	4.12E-02	2.191E-02*	9.03E-04	$L\eta$ X-ray	5.05E-03	1.588E-02	8.01E-05
ce-N*, γ 2	1.61E-02	2.634E-02*	4.24E-04	$L\beta'$ X-ray	1.23E-02	1.189E-02	1.46E-04
ce-L_1, γ 4	7.27E-03	9.756E-03	7.09E-05	Auger-LMM	2.94E-01	1.069E-02*	3.14E-03
ce-L_2, γ 4	2.23E-01	1.058E-02	2.36E-03	Auger-LMX	2.07E-01	1.437E-02*	2.97E-03
ce-L_3, γ 4	2.06E-01	1.457E-02	3.00E-03	Auger-LXY	3.19E-02	1.727E-02*	6.03E-04
ce-M, γ 4	1.20E-01	2.775E-02*	3.33E-03	Auger-MXY	1.59E 00	4.053E-03*	6.46E-03
ce-N*, γ 4	4.76E-02	3.218E-02*	1.53E-03	ΔE	5.07E-01	9.310E-04*	4.72E-04
γ 5	1.19E-03	3.319E-02	3.95E-05				
ce-L_1, γ 5	1.09E-01	1.077E-02	1.18E-03	LISTED X, γ AND γ= RADIATIONS			3.24E-02
ce-L_2, γ 5	3.58E-02	1.159E-02	4.15E-04	OMITTED X, γ AND γ= RADIATIONS**			1.25E-04
ce-L_3, γ 5	2.19E-02	1.558E-02	3.41E-04	LISTED β, ce AND Auger RADIATIONS			5.19E-02
ce-M, γ 5	4.20E-02	2.876E-02*	1.21E-03	OMITTED β, ce AND Auger RADIATIONS**			1.72E-04
ce-N*, γ 5	1.66E-02	3.319E-02*	5.51E-04	LISTED α AND α recoil RADIATIONS			5.57E 00
ce-L_1, γ 9	2.72E-02	2.099E-02	5.70E-04	OMITTED α AND α recoil RADIATIONS**			4.06E-03
ce-L_2, γ 9	3.47E-02	2.182E-02	7.56E-04	LISTED RADIATIONS			5.65E 00
ce-L_3, γ 9	2.76E-02	2.581E-02	7.13E-04	OMITTED RADIATIONS**			4.36E-03
ce-M, γ 9	2.38E-02	3.899E-02*	9.26E-04				
ce-N*, γ 9	9.07E-03	4.342E-02*	3.94E-04	* AVERAGE ENERGY (MeV)			
ce-L_1, γ 13	3.55E-01	3.309E-02	1.18E-04	** EACH OMITTED TRANSITION CONTRIBUTES			
ce-L_2, γ 13	3.79E-03	3.392E-02	1.29E-04	<0.100% TO Σy(i)×E(i) IN ITS CATEGORY.			
ce-L_3, γ 13	2.77E-03	3.791E-02	1.05E-04	NEPTUNIUM-237 DAUGHTER IS RADIOACTIVE.			

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