EFFECTS OF IRRADIATED ASPARAGINE ON YEAST CELLS

By

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DECLARATION

I hereby declare that, the content of this thesis is an original work done by the author under the supervision of Professor J. Kiefer of the Institute for Biophysics, Strahlenzentrum, University of Gießen, W. Germany.

To the best of my knowledge, no part of this work has been submitted anywhere for any academic award.

Works of others consulted in the text have been accordingly referenced.

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ABSTRACT

Haploid and diplod cells of the bakers yeast, Saccharomyces cerevisiae, were grown in lOkGy and 50kGy irradiated asparagine treated Wickerham media, with an unirradiated asparagine medium as control. The method of cultivation was by inoculating about 2000 cells per mililiter from the same culture into a fresh medium and incubating for a day. This procedure was repeated daily for a period of at least eleven days,, thus bringing the the culmulative age of the cells up to 150 generations in semicontinuous logarithmic growth phase.

The growth pattern, viability and mutation on the CAN gene were monitored. The CAN mutation in the diploid cells already carrying a 'CAN-deletion' was scored as recombination. Results showed no statistically significant difference between the various treatments and the control medium cells.

It could be inferred, therefore, that irradiated asparagine iased as a sole source of nitrogen may not constitute a cytotoxic or genetic hazard to the cells. A possible, extension of this result-may be to -suggest that irradiation of nitrogen source in food may not make the food dangerous and may not constitute it into a genetic risk.

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ABBREVIATIONS

can	Canavanine (an amino acid derived from wild beans).
CAN	Canavanine locus in a yeast gene.
DNA	Deoxyribonucleic acid.
ESR	Electron spin resonance
FAO	Food and Agricultural Organization
IAEA	International Atomic Energy Agency
kGy	kilogray
pms	per million survivors
R	Röntgen
SC+Can	Synthetic Complete medium plus canavanine
SC+Arg	Synthetic Complete medium plus arginine.
UV	Ultraviolet radiation
WHO	World Health Organization.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 FOOD PRESERVATION BY IRRADIATION

Preservation of food is incapacitation or destruction of spoiling organisms or reactions in the food. Some methods used are drying, smoking, dehydration, fermentation heat treatment and chemical treatment. Radiation as a preservation technique has some advantages like: high penetration, packaging before treatment, residuelessness and cheapness. Some difficulties include: production of mutant species, colour, texture and odour changes. Some texture changes could be an advantage to the food (Goresline, 1973; Rowley and Brynjolfsson, 1980). In as much as no single preservation method is devoid of the disadvantages associated with irradiation, none has ever received the popular and legislative reluctance in its approval as irradiation has. What the people demand is that adequate information be obtained on the chemical contents of irradiated food in order that the cytotoxic and moreover the genetic consequencies of feeding a population with irradiated food could be accurately assessed (Elias, 1977). Despite these difficulties and oppositions, food irradiation for preservation is gaining ground in many parts of the world (van Kooij, 1984).

There is now an impressive wealth of information on the chemistry of irradiated food obtained from several

laboratories throughout the world and from the joint efforts of experts in the International Expert Committee on Joint Food Irradiation Projects sponsored by the WHO, FAO and IAEA. On the question of cytotoxicity, it is believed that irradiation below or up to 10kGy does not constitute any genetic or cytotoxic hazard (Committee 17, 1975). Between 10 and 25kGy had been used for high degree of inactivation of contaminants for supply of specific pathogen free diets for laboratory experimental animals. Doses between 40 and 60kGy has been used for inactivation of spoilage organisms and pathogens, in particular, Clostidium botulium for long term preservation of meat, fish and other non-acid foods (Elias, 1977). Thus, one can ask a question: could a nitrogen source given doses of about 10 or 50kGy produce harzardous substances to living cells?

1.2 THE YEAST

The yeast is a single cell eukaryotic organism capable of existing in a haploid or diploid chromatid state It is an excelent organism for examination of genetic effects of chemicals or of radiation because of its clonability, short generation time, adaptability to plating technique, well developed genetics and its easy amenability to manipulation to haploid and diploid states. It is also a better organism in terms of comparison with mammalian cells than prokaryotes. Its unicellular nature makes it convinient for studies in radiation biology.

Saccharomyces cerevisiae the common baker's yeast has a typical spherical shape, of size between 5 and 7 microns. When viewed under the microscope it is rounded in shape. It divides vegetatively by budding with average generation time between 70 and 90 minutes at 30°C culturing temperature and adequate stirring. (Matile, et al, 1969; Mortimer, et al, 1969)

1.3 GROWTH AND VIABILITY

In presence of adequate supply of growth medium and suitable culturing condition, a group of viable cells dispersed homogeneously increases its biomass and divides continuously till the nutrients are exhausted (Powell, 1956 Riccica, 1966 and Aherne, et al, 1977). As concentration of limiting nutrients decreases the growth decreases or becomes stagnant, leading perhaps to cell death or formation of spores. Thus in a culture there exist definite growth phases as shown in the sketch in fig.1. If the condition is such that all growth requirements are supplied, at all time, the cells may continue to divide indefinetly if they are viable (Matin and Gottschall, 1978; Matin et al 1978). If the culture is diluted at specific intervals and excess cells removed, the growth system would be between continuous and non-continuous or batch growth system. This system called semicontinuous growth system is described in a diagram in fig. 2. Some problems associated with the semicontinuous system are: if the time between dilutions is much, stationary effects may

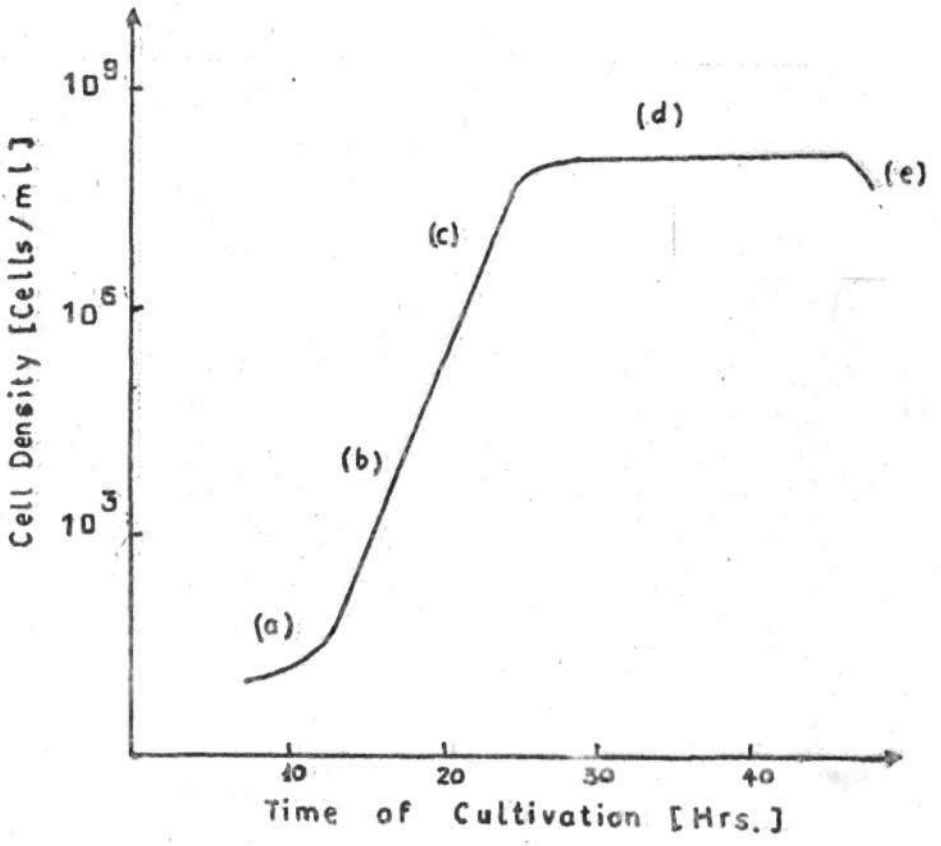


Fig 1: Sketch of theoretical batch growth characteristics.

(a) Lag growth phase (b) logarithmic growth phase (c) late log phase (d) stationary phase (e) exponential cell death phase.

(Source: Van Uden, 1971)

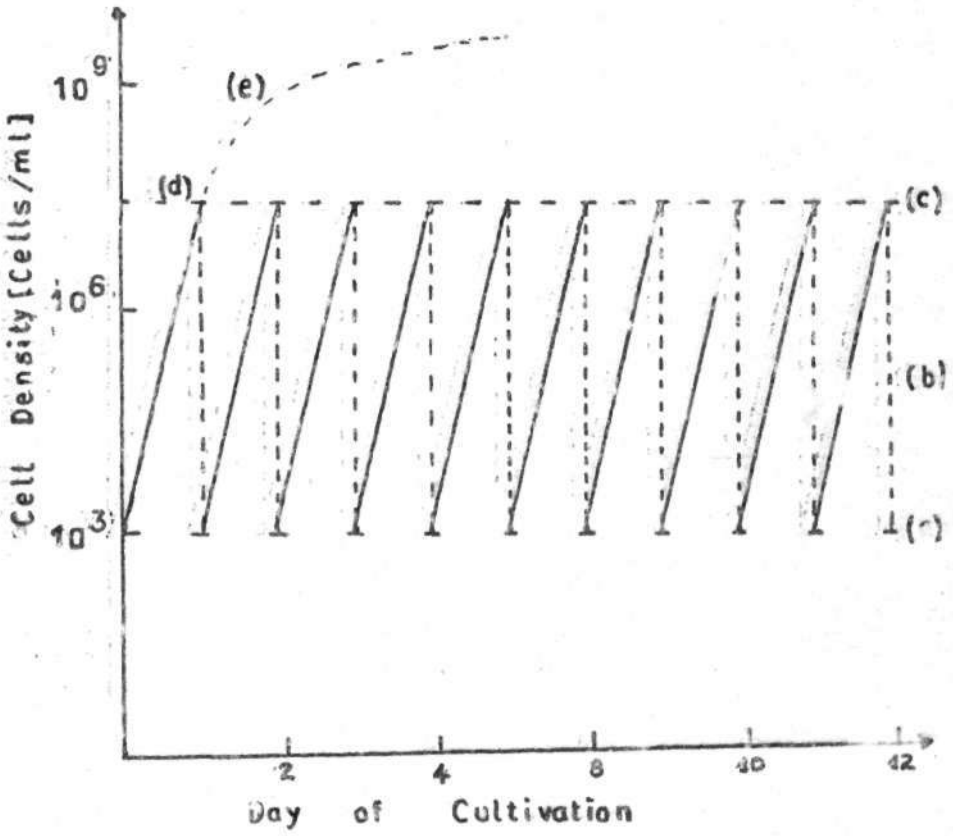


Fig. 2: Sketch of semicontinuous growth characteristics.(Daily redilution method).

(a) Daily dilution level (b) range of dilution (c) Growth interruption level (for yeast: after 24hrs. cultivation).

Note: Interruption is at (d) before the cells' growth reaches late exponential phase (e) where stationary phase effects could be felt.

(Source: Ricica, 1966).

set in, difficulties in maintaining steady state and introduction of foreign bodies may make renewal culturing system difficult (Burger, 1966; Soumalinen and Oura, 1971; Thingstad, 1974 and Shkidchenko, 1982).

1.4 GENETIC ACTIVITIES

A gene is a sequence of bases in the DNA which codes for a particular information or production of proteins that perform a specific function. Biochemical or biophysical reactions which result in changes in the structure of the bases or destroy the bases or the DNA lead to loss of function. If the cell survives such a loss of function, it becomes a mutant (Auerbach, 1962 and Drake, 1970). During cell division, an exchange of bases may occur betwee. one strand of the DNA and the other such exchange of material is called recombination. In a diploid cell recombination can occur even before cell division (Fabre, 1978). It may lead to formation of daughter cells that have differing characteristics both within themselves and between the daughters and the parent cells (gene conversion) (Holliday, 1967 and Albert et al, 1983). Cells can institute repairs of damage through various mechanisms (Howard-Flanders, 1967) Repairs can be through recombinational processes as described in Eschericia coli (Howard-Flanders et al, 1984) Sedwick (1984) supports the existence of a special repair system involving an enzyme system, like the rec-lex system. The repair system in yeast is not yet known (Kiefer, 1984).

1.5 MUTAGENICITY TESTING

Some tests devised to monitor the effects of some mutagens are: micronuclei tests, involving mutations in blood of test animals (Schmid, 1975) and host-mediated tests involving the slipping of test organism into animal's peritoneum and monitoring mutation in such organisms (Brewen, 1975). There are also other tests like the histidine reversion tests developed by Ames, et al (1973). involving Salmonella typhimurium and agar plate assay as described by Wlodkowski, et al (1975). The spot test involving the application of the test material in alcohol solution on a filter paper and laying on agar (McCalla et al, 1975). Also important is the adenine reversion tests using the so called D-strains of yeast developed by Zimmermann, (1975) and Zimmermann, et al. (1978). Sometimes the suspected mutagen is first treated with activation materials like those involved in metabolism as was done by Malling (1971) Ames et al (1973) and Mayer (1973).

Whereas the rest are reversion tests involving prokaryotes and eukaryotes, a foward mutation test is more straight foward and more frequent and is therfore, a better test than reversion tests (Kiefer, 1984). A good example which was used in this experiment is the mutation to canavanine resistance in yeast cells, involving a recessive mutation at can 1 locus monitored as defect in arginine permease activity (Grenson et al, 1966; Grenson and Hou, 1972; Lemmott, 1977a; 1977b; Whelan, 1978 and Cocke and

Mannay, 1979).

1.6 MUTAGENS AND MUTATION INDUCTION

Any agent which in the environment of the DNA influences or causes an increase of mutation rate above spontaneous level is called a mutagen. Some mutagens are chemicals (Fishbein, 1970) physical agents like UV, x-rays and other ionizing and nonionizing radiations (Kessaman, et al, 1971) and excessive temperature and pH changes (Shimazu and Tappel, 1964; Evans, et al, 1978) and loss of quality control mechanisms leading to misrepair of damages (Drake, 1970).

Mutants may be accumulated or eliminated in a semicontinuous culture depending on the growth rate of the mutants and the non-mutants. If the mutants are fast growers in the medium, dilution will eliminate the slower growers and the converse is true for the elimination of the mutants in the culture. Mutation accumulation has been found to depend on concentration of nutrilites (McAthey and Kilbey, 1977) on starvation for certain amino acids (McAthey and Kilbey, 1978) and growth limiting factors (Kubitschek and Bendigkeit, 1964).

The traditional methods employed in providing evidence of safety for irradiated food involve lengthy complicating and costly animal experiments (Ellis, 1977) The limitations in terms of excess demand on manpower and resources could be eliminated by deploying chemical investigations and making a back check using microorganisms

Here the eukaryote, yeast fits in. Consequently in trying to find out if cytotoxic or mutagenic substances exist in 10 and 50kGy irradiated asparagine, a review of the action of radiation on amino acids is important to find out possible harzardous radiolysis product on which the cytotoxic or mutagenic effect could be blamed when found. The use of semicontinuous growth process is a deviation from traditional batch and chemostat systems. It has an advantage of helping to monitor the growth directly and at the same time check for mutation and other endpoints in several cultures run concurrently.

CHAPTER TWO

RADIATION EFFECTS ON AMINO ACIDS

2.1 THEORIES OF RADIATION ACTION

The theories of radiation action on biomolecules are based on two major pathways: the biophysical theories of hit action and the biochemical theories of indirect actions. The hit theories propose that a packet of energy hits the molecule and causes ionization or excitation of the molecules and later reactions lead to observable effects like, strand breaks (Kiefer, 1984) and dimerization of pyrimidines (Chanet et al, 1975). A molecule or cell which is not hit survives, thus survival and absorbed dose are related mathematically through an exponential probability function. On the other hand, the theories of indirect action propose that chemicals in the immediated environment of the molecules can absorb energy and break up into reactive species which are capable of reacting with the molecules of interest and leading to an observable lesion. Observation of the later effect can best be determined by identifying the radiolysis products of chemicals concerned in order to predict the damages they could cause.

The action of irradiated food components on cells is mainly an indirect action of long lived reactive species produced during the irradiation of the component . Schubert (1969) and Kesavan and Swaminathan (1971) in their reviews of the effects of irradiated food substrates on cells seem to agree that some long lived reactive species could be

formed in the irradiated substrates which could lead to adverse effects on cells grown in them. This review is then an attempt to find out the radiolysis products of irradiated amino acids with view to examining their role in mutagenicity and cytotoxicity in cells.

2.2 RADIOLYSIS PRODUCTS OF AMINO ACIDS.

Ionization, excitation and breakage of bonds are the most important direct actions of radiation on amino acids. These may lead to deamination, decarboxylation, formation of sulphydryl groups, fragmentation, cross-linkage and free radical formation. The free radicals formed may persist in liquid solutions and may then react with cells. Indirect action may stem from action of radiolysis products of water in which the amino acid is dissolved.

The main reactive species produced in water by radiation are: hydrogen radical, $H^{\bullet}$, hydroxyl radical, $OH^{\bullet}$ and the aqueous electron, e_{aq}. In presence of oxygen the aqueous electron further reacts as follows:

$$e_{aq} + O_2 = O_2^{\bullet} \quad \text{(Oxygen radical)}$$

$$O_2^{\bullet} + H^{+} = HO_2$$

$$2HO_2 = H_2O_2 + O_2 \quad \text{(Hydrogen peroxide)} \qquad 2.1$$

The reaction of e_{aq} is same as that of $H^{\bullet}$, thus only the reaction of e_{aq} and $OH^{\bullet}$ are important. (Fricke and Edwin, 1966).

Reaction of hydroxyl radical with amino acid can be summarized as in Garrison (1964) as follows:

In acidic solution

$$N^+H_3CH(R)COO^- + OH^\bullet = N^+H_3\dot{C}(R)COO^- + H_2O$$

$$N^+H_3C(R)COO^- + 2OH^\bullet = NH_3 + RCOCOOH + H_2O$$

$$RCOCOOH = RCHO + CO_2. \qquad 2.2$$

The products are ammonia, α-ketonic acids, aldehydes and carbon dioxide. In alkaline solution, the attack is on the amino group instead of the α-carbon group as in acidic solution.

$$N^+H_3CH(R)COO^- + OH^\bullet = N^+H_2\dot{C}H(R)COO^- + H_2O.$$

$$N^+H_2\dot{C}H(R)COO^- + OH^\bullet = N^+H\dot{C}H(R)COO^- + H_2O$$

2.3

This is systematic decomposition and deamination. In presence of oxygen the reaction goes on as:

$$N^+H\dot{C}H(R)COO^- + O + H_2O = NH_2OH + RCOCOOH \qquad 2.4$$

Hydroxylamine is formed instead of ammonia.

The e_{aq} reacts as follows:

$$N^+H_3CH(R)COO^- + e_{aq} = H + NH_2CH(R)COO^-$$

$$NH_2CH(R)COO^- + e_{aq} = H + NH_2\dot{C}(R)COO^-$$

2.5

The e_{aq} leads to radicalization of the amino acid which may in turn lead to formation of organic peroxides, or to polymerization.

According to Maxwell (1964) the yield for Y-irradiated glycine solution (1N) is as follows: $G(NH_3)$ = 4.3, G(C=O) = 4.5 and $G(H_2O_2)$ = 3.6. Alanine (0.1M) solution similarly irradiated gave the same results, while other amino acids gave lower yield. (Garrison, et al, 1964).

Other products of irradiating 1M solutions of various amino acids at pH = 6.5 include hydrogen, acetic acid, glyoxalic acid, aldehyde and carbon dioxide. Extremly low yield of succinic acid, aspartic acid and diamino-succinic acid were also produced (Weeks and Garrison, 1964).

ESR studies of radiolysis products indicate that in a solution of L-histidine a carboxyl anion is formed at 77K, temperature of liqiud nitrogen. On warming to room temperature, 300K, a radical is formed through addition of hydrogen to the imidazole ring (Westhof, 1975).

In methionine and adenine, the sulphonium group decomposes fast with irradiation than other parts of the molecule (Schlenk and Dianke, 1962). Adenine and methio-nine behave in the same way even when treated with an alkali or an acid solution.

The decomposition of the sulphonium group is related to the off-odour observed in irradiated meat. Also related to the off-odour are: mercaptan, hydrogen sulphide and carbonyl compounds. Dehydrogenation of amino acids

with hydroxyl group may be responsible for their higher yield of carbonyl compounds (Kolousek, et al, 1957, and Duran Tappel, 1958). Change in colour from yellow to brown is observed in hydroxyl and aromatic amino acids. Such colour changes are not reversible when treated with sodium hydrosulphite showing that the compounds formed are not simple quinones.

In terms of radiolability, Shimazu and Tappel (1964) found that in a low pH solution of insulin, cystine, tyrosine, phenylalanine, proline and histidine were the most labile. In neutral solution of cytochrome c, haemoglobin and ovalbumin, the radiolability ranks as follows: methionine, phenylalanine, histidine, cystine, serine and threonine (Ambe et al, 1961). Also, using salt free proteins, ovalbumin, catalases and chromatographically pure amino acids, the destruction of amino acids after irradiation was compared in terms of dose required to destroy 50% of the original quantity (tab.2 .1). Shimazu and Tappel (1964) postulated that the percentage of amino acid remaining was log dependent on dose.

For aliphatic and dicarboxylic amino acids radiolability increases with length of the chain. No real difference exists between the carbonyl yields of glutamic and aspartic acids. Distal amino groups have low reactivity with radiolysis products of water and guanidyl groups seem to be more labile than the distal amino groups.

In summary, the possible radiolysis products of amino acids in aqueous solutions include: ammonia, α-keto

TABLE 2.1

DOSE THAT REDUCES AMINO ACID CONCENTRATION TO FIFTY PERCENT. (D_{50})

Amino Acid	D_{50}(kGy)
Glycine	66
Aspartic Acid	56
Alanine	54
Glutamic Acid	34
Leucine	30
Valine	28
Histidine	27
Isoleucine	26
Lysine	22
Serine	21
Arginine	19
Methionine	14
Phenylalanine	11

Source: Shimazu and Tappel (1964).

acids (acetic and glyoxalic acids) aldehyes (formaldehyde) peroxides (hydrogen and organic) carbon dioxide, carbonyl compounds, nitric acids, carboxyl ions, polyglutamic acid, hydrogen sulphide, mercaptans, shorter amino acids (*e.g.* alanine from cysteine or cystine) polyglutamic acid, amines (hydroxlamine) amides and other short-lived fragments which may be difficult to identify.

The radiation chemistry of proteins is a complex subject but, it is believed that the chemistry does not differ so much from that of the amino acids from which the proteins are constituted (Kumta and Tappel, 1961).

2.3 EFFECTS OF SOME RADIOLYSIS PRODUCTS

Not all radiolysis products constitute hazard to cells, some seem to fall into categories of chemicals already classified as mutagenic or cytotoxic (Fishbein, 1970). Some of them are examined in the remaining part of this review.

Aldehyde can react with nucleotides and nucleic acids leading to formation of methyol derivatives (Auerbach *et al*, 1977). Formaldehyde, however, does not react with native DNA strands, but unwound strands denatured by formaldehyde can hardly be repaired. In lysine rich histone bodies, the formaldehyde action is more pronounced (Siomin, *et al*, 1973). In presence of oxidizing agents, it could be possible that aldehydes form peroxides, peracids and free radicals and in presence of hydrogen peroxide, polymerization of the DNA can occur (Barach and Eilon, 1969).

Formaldehyde had been found to be mutagenic in Escherichia coli (Nishioka, 1975) and Saccharomyces cerevisiae (Auerbach, et al, 1977). It has been found to induce strand breaks in exponential phase cells in a dose dependent manner. Three milimolar of formaldehyde produces 42% damage in DNA, likewise, a dose of formaldehyde which leaves 60% survival produces about 95% of the damage to the DNA (Magana-Schwenke, et al, 1978).

Peroxides had been found to be responsible for growth retardation, inactivation and mutation to respiratory deficiency in cells irradiated in liquid media (Thacker, 1975). Stationary phase cells are highly inactivated at concentrations around 100mg/ml and below this concentration, mutation is likely to occur. For transitional phase cells, inactivation is slowly dependent on dose up to 10^4mg/ml. Inactivation is dependent on the cell's ability to breakdown the peroxide and rate of permeation of the cell wall which in turn depends on population density and growth phase. Selective permeability and genetically determined differences in respiratory competences also influence a culture's ability to withstand the damaging effects of peroxides (Thacker, 1975).

Hydroxylamine is a potential inhibitor of the enzyme transaminase whose activity is important in the general metabolic machinery of the cells. It can form cytosine adduct leading to mutagenicity. However, its mutagenic effect is reduced because it is subject to degradative oxidation and reduction (Grossman and Brown,

1969).

Acidic components of radiolysis products can cause a retardation in growth and may be, mutation. It is not yet determined if the off odour products are able to cause other damages to cells apart from the loss of palatability in food meant for human consumption.

2.4 THE PURPOSE OF THE EXPERIMENT

This work is a cellular procedure for verifying the existence of long-lived radiolysis products of irradiated asparagine that can exert cytotoxic or induce genetic activities in yeast. In doing the test, the foward mutation to canavanine resistance was used in scoring for both recombination in the diploid cells and mutation in the haploid cells. The measure of cytotoxicity is the cells' growth and viability levels with respect to the control cultures.

The basic assumptions are that if cytotoxic agents which constitute hazard exist in the irradiated-asparagine medium, a definite pattern of growth would appear which would be different from that of the control. The same could be said of induced mutation and recombination. Such induced effects would be regarded as a sign that irradiated amino acids could be a cytotoxic or a genetic risk to human beings.

CHAPTER THREE

MATERIALS AND METHODS

3.1 THE IRRADIATION FACILITY

The irradiation facility consists of a set of six sources (fig. 3) arranged such that a steel ring carrying the irradiation chambers can be rotated from outside the irradiation room to a position between the sources inside. The strenght of the sources in terms of activities are given in fig. 3 and the exposure rates at various points in air inside the chamber at installation are listed in table 3.1, appendix 2.

The arrangement is such that the chamber can be pushed in and outside the irradiation room with the operator being protected by a thick concrete wall. A box directly opposite the irradiation box was designed to show the operator when the irradiation box is in position between the sources if the later is alligned with a point indicated in the sketch diagram in fig. 3 by an arrow.

3.2 DOSIMETRY

The dose delivered to the irradiation boxes by the sources in the irradiation facility was determined using the Fricke dosimeter, as described in Fricke and Edwin (1966).

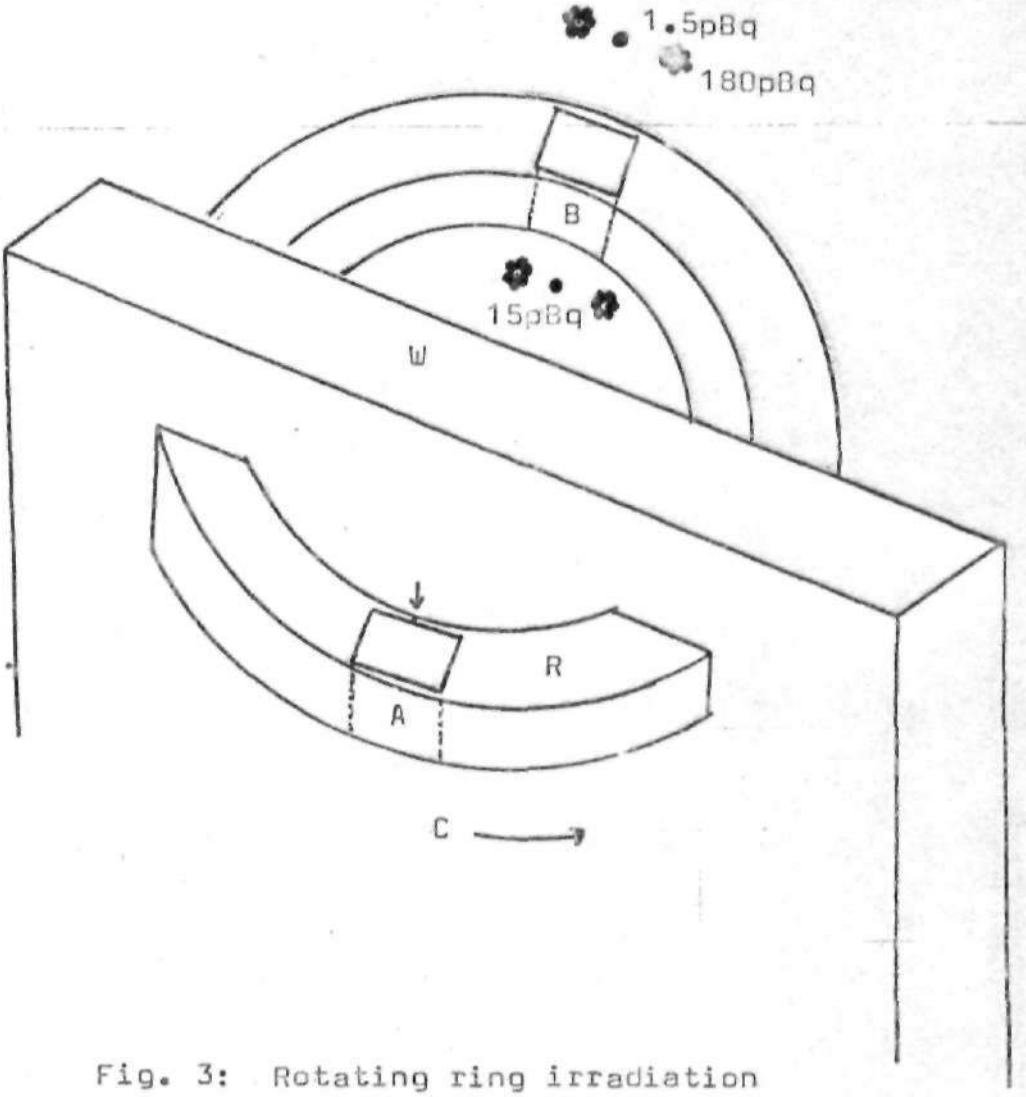


Fig. 3: Rotating ring irradiation facility (A sketch)

A and B are directly opposite boxes. When one is directly in position shown by the arrow, the other is positioned inside. within the six pack of sources.
C is the direction of rotation of the steel ring housing the boxes, R, while W is a a heavy concrete protective wall.

The main component of the dosimeter, the fricke solution, was made up by mixing 0.001M $FeSO_4$, 0.08N H_2SO_4 and 0.001M NaCl in a triply distilled ion-free water in an amber bottle under yellow light. A series of 3ml of the solution were irradiated in glass petri-dishes with cover using a Müller 150 industrial X-ray machine set to deliver at 150kV, 12mA at a distance of 12cm from the samples. Exposure was monitored using a microchamber ionization chamber and a Duplex counter set at 20 Röntgens per count. These solutions were later analysed spectroscopically using a Zeiss PMQ 11 monochromator (microcurrent -optical density change measuring system) set at 304nm wavelength (Strahlenzentrum, 1984)

The change in optical density observed was due to oxidation of Fe^{2+} by irradiation to Fe^{3+}. The oxidation is usually proportional to the dose absorbed by the solution.

The relationship between the absorbed dose and the change in optical density is given in Fricke and Edwin (1966) as follows:

$$D(Gy) = \frac{9.44 \times 10^6 \ (\Delta OD)}{\Delta \varepsilon \ . \ G(Fe^{3+})} \qquad 3.1$$

where D is for absorbed dose, (ΔOD) is for the change in optical density. ε is the extinction coefficient given by

$$\varepsilon = \frac{\log I/I_0}{g \ x} \qquad 3.2$$

I is the intensity of the monochromatic light after traversing the medium and I_o is the intensity before entering the medium. ϱ is the molar concentration of the reactive specie in the solution and x is the thickness of the measuring curvette (usually a 1cm quartz curvette as was used in this experiment).

$\epsilon(Fe^{2+})_{25^oC}$ = $1M^{-1}cm^{-1}$ at λ = 304nm.

$\epsilon(Fe^{3+})_{25^oC}$ = $2197M^{-1}cm^{-1}$ at λ = 304nm.

Thus $\Delta\epsilon = 2197M^{-1}cm^{-1}$ 3.3

Also $G(Fe^{3+})$ is the g-value giving the yield of Fe^{3+} per 100ev of energy absorbed (ev is the electron-volt a unit of energy). Its value is 15.6 at 25^oC. All constants were taken from Fricke and Edwin (1966).

Thus we have:

$D(Gy) = 275.43\ (\Delta OD)$ 3.4

A graph of change in optical density versus absorbed dose then gave a straight line with slope of $3.5 \times 10^{-3}Gy^{-1}$. This as was shown in fig. 4 is the calibration factor.

The fricke dosimeter has an upper limit at about 400Gy as shown by the second graph on fig. 4.

From the same fricke solution, 3ml of solution was placed at positions 1, 2, 3, and 4 as shown in fig 5 corresponding to positions 19, 26, 27, and 21 respectively as in diagram (Schnitt III-III) in appendix 2 which represent the bottom of the irradiation chamber where the

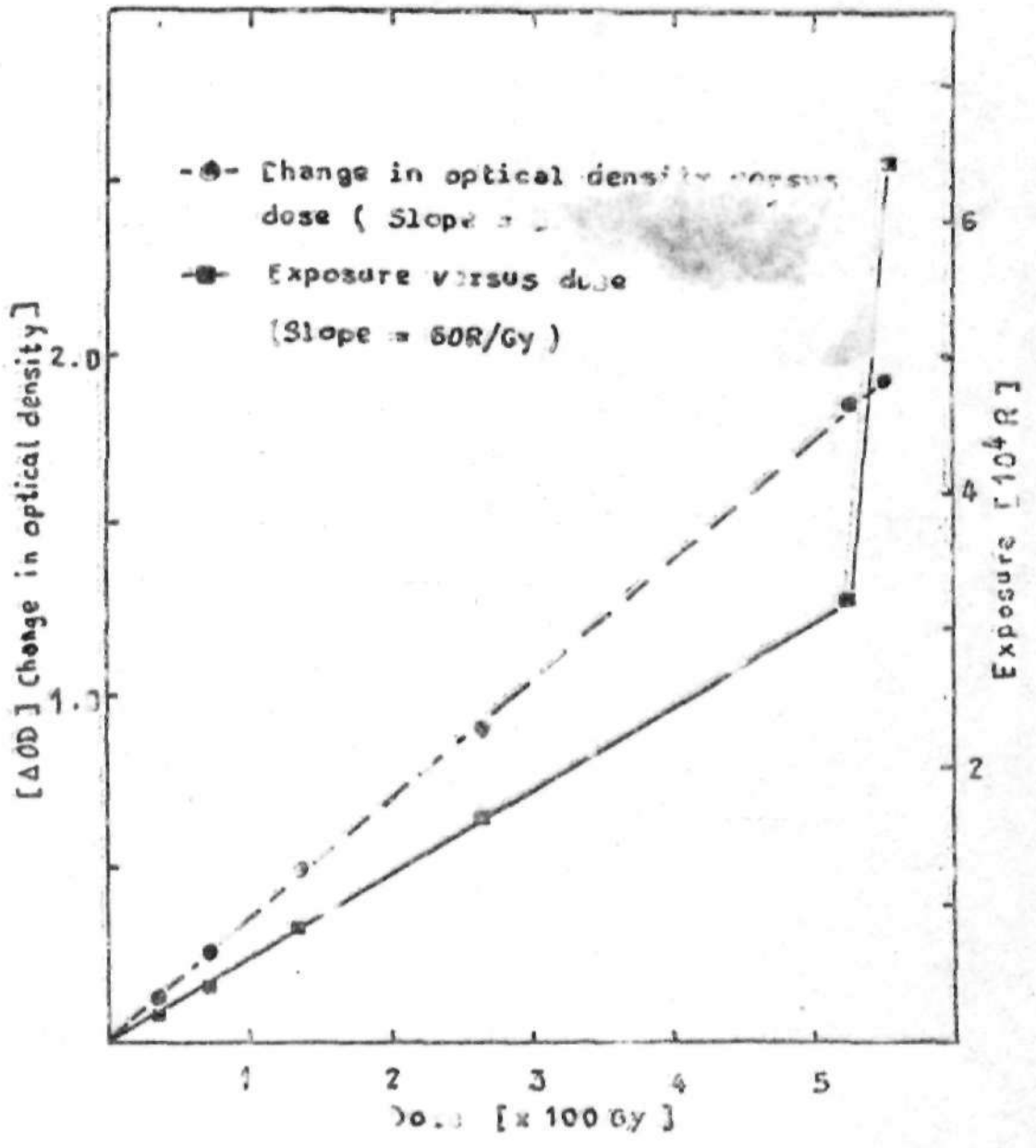


Fig. 4: Calibration curve for measurement of absorbed dose as a function of change in optical density.

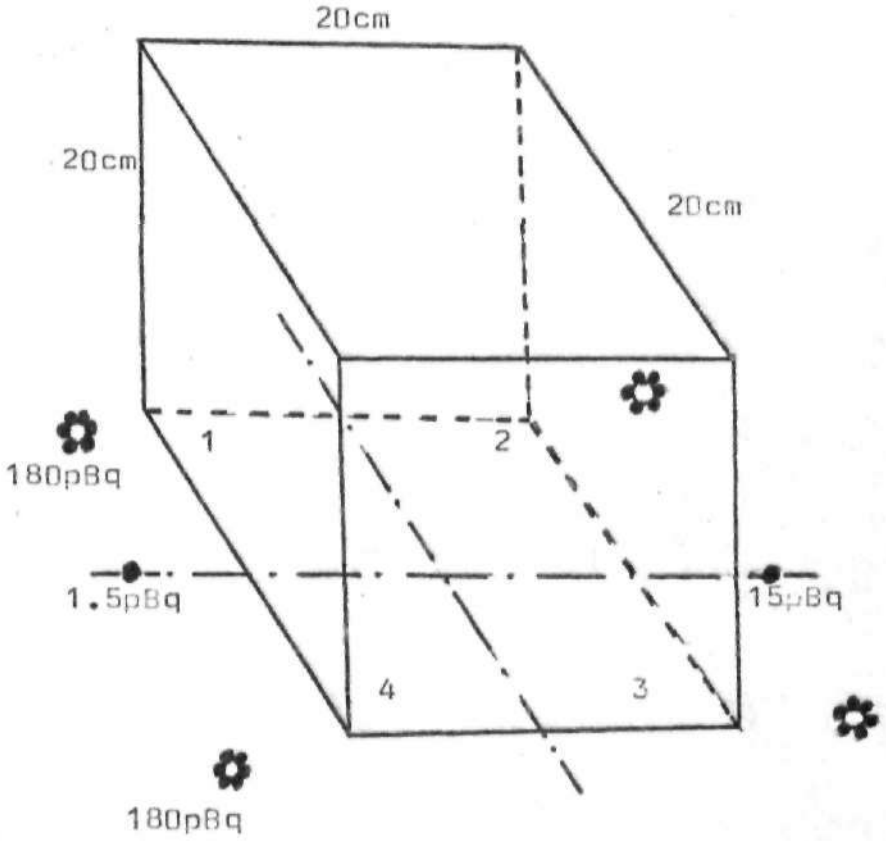


Fig. 5: Irradiation chamber showing positions of the sources and points 1 - 4 where dosimetry measurements were made.

pBq = picobecquerel (10^{12}Bq).

solutions for the experiments were irradiated. The fricke solutions in plastic containers were simultaneously irradiated for 10 minutes. The spectroscopic analysis was done as in the calibration and the results as shown in tab. 3.2. were calculated from the relationship:

$$\dot{D}(Gy/min) = \frac{1000\ (\Delta OD)}{35} = 28.57\ (\Delta OD) \qquad 3.5$$

Hence the time, t for the working solution to be irradiated such that it receive a dose, D is:

$$t(hrs) = \frac{0.58D(kGy)}{(\Delta OD)} \qquad 3.6$$

This gave for 10kGy irradiation at positions 3 and 4: t $\hat{=}$ 8hrs and for 50kGy , t $\hat{=}$ 40hrs. Positions 3 and 4 were used in all subsequent experiments reported here.

3.3 THE YEAST STRAINS

Two yeast strains, one haploid and the other diploid were used in the experiments.

The haploid, C420 38 , was originally obtained from A. P. James, Division of Biological Sciences, National Research Council Ontario, Canada. It required about 0.3g/l lysine in a minimal medium (appendix 3) to grow well i.e up to a factor of 10^8 when grown under the semicontinuous

TABLE 3.2.

DOSE RATE AT VARIOUS POSITIONS IN THE IRRADIATION CHAMBER.

Position	$\dot{D}_{air}$ (x 10^{2} Gy/min)	$\dot{D}_{Fricke}$ (Gy/min)
1	1.33	15.50
2	1.33	15.00
3	1.33	19.50
4	1.33	19.00

Note $\dot{D}_{air}$, the dose rate in air was calculated using conversion factors from exposure ratings of the various packs at the various positions immediately after the installation. (See also App. 2).

$\dot{D}_{fricke}$ was measured using the fricke dosimetry system as at the time of experimentation.

system. It did not grow well when lysine was used as a single source of nitrogen in the minimal medium.

The diploid, cd364 is a cross between a wild type haploid (carrying no CAN defect) and a mutant carrying a deletion on the CAN gene. The defect was known because it survived almost 100% with canavanine in the medium. The wild type described as A364A has the following characteristics: mating type a, ade 1, ura 1, his 7, lys 2, tyr 1 and gal 1 mutations. It was obtained from the Yeast Genetic Stock Centre, Berkeley, USA. The mutant is also described as A364A strain of mating type α, and can^{-} (position of the deletion on the gene not yet known). It was a prototroph obtained from the department of Genetics, University of Edmonton, Canada. The crossing was performed at the Strahlenzentrum of the University of Gießen, W. Germany. The cross is therefore a prototroph and survives in a minimal medium without requirements.

All stock of cells were made on complete medium containing agar plates (appendix 3). Before every experiment was done a new culture was made from the stock, to ensure that one did the work with a high percentage of viable cells (fig.6).

3.4 CELL DILUTION AND COUNTING

Dilution was made by placing 1ml of suspension in 9ml of water usually sterile bidest (fig. 7). Each dilution was then 10 times less dense than the preceding.



1. Gently scrap out a loopful of cells from stock plate. Seal the stock carefully with parafin paper and replace in refrigerator.

2. From the loopful of cells taken make a suspension of about 10ml of the cells.

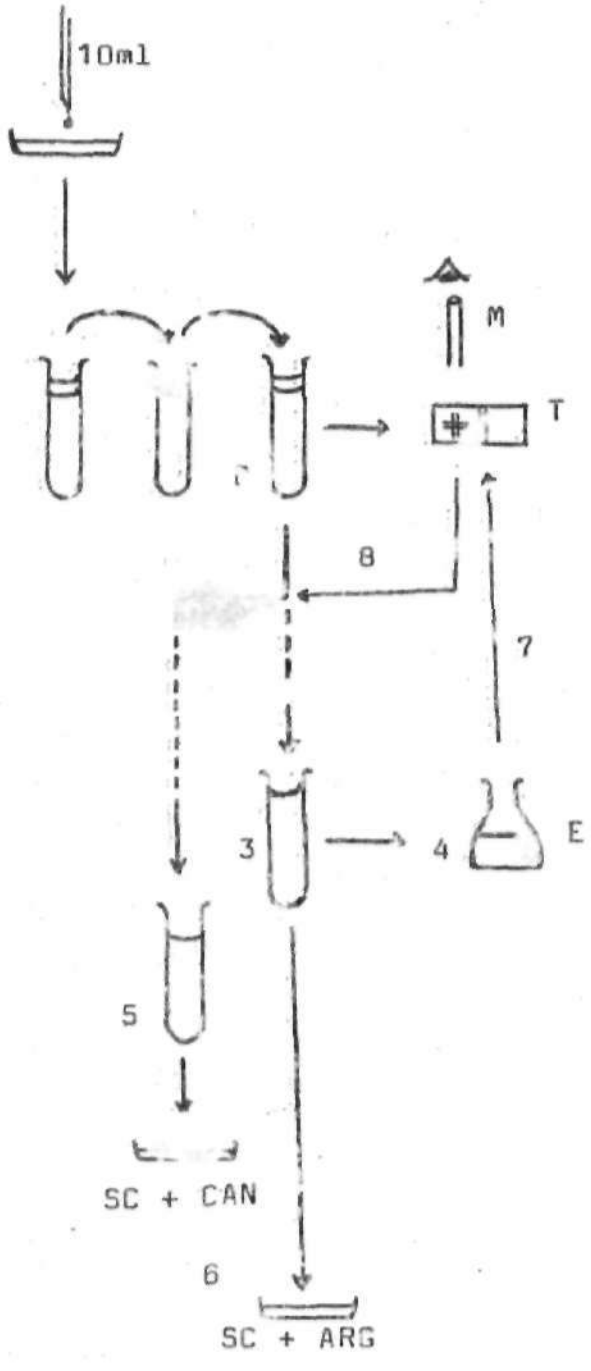
3. Pipette about 0.3ml of the suspension on the lawn of a plate containing a complete medium and spread generously using a sterile smooth loop.

4. Incubate for 3 days at 30°C.

Fig. 6: Flow diagram for preparation of working cell stock.

Note: All experiments are started using fresh stock made from the permanent stock plate.

Source: Experimental Procedures, Strahlenzentrum University of Gießen, W. Germany. (1984)



1. Wash out the cells from the plate with 10ml of sterile water and carefully pipette all into a sterile and clean test tube.

2. Make 2 dilutions and count using a microscope, M and a Thoma chamber, T.

3. Dilute to 10^3cell/ml. From that take a volume, V, that makes 10^5cells in 50ml medium. Place in Erlenmeyer conical flask, E.

4. Incubate in rotary shaker at 100rpm and 30°C.

5. From D make a dilution of 10^7 or 10^6 cells/ml and spread 0.2ml on SC + CAN and incubate in incubation room at 30°C.

6. From 3 take 0.2ml, spread on SC + ARG and incubate with 5.

Fig. 7: Daily routine flow diagram.
Dilution, counting and culturing.

Source: Strahlenzentrum of the University of of Gießen, W. Germany. (Biophysics Practicals) 1984.

The THOMA-CHAMBER was used for counting. It consists of a rectangular piece of glass with 16 tiny chambers each of volume 4×10^{-6}ml. scratched at one or the two ends. The scratched side was covered with a piece of thin glass slide with little moisturing such that a newton ring was seen at the sides. A drop of the cell suspension was carefully placed on the edge of the slide till the marked chambers are flooded. Eight out of the 16 small chambers are counted with the help of a microscope. The total volume counted was then 32×10^{-6}ml.

If N_o was the total number of cells counted in the 8 chambers, then the cell density was scored as

$$S_c = \frac{N_o}{32 \times 10^{-6}} \quad \text{cells/ml} \qquad 3.7$$

3.5 GROWTH ASSAY

For the purpose of growth assay all cells were grown in liquid Wickerham media (appendix 3). The only source of nitrogen was asparagine. Amino acids are known to satisfy the growth requirements of the yeast, Saccharomyces cerevisiae (Hinnenbusch, 1983). Asparagine being a good quality amino acid satisfies this purpose (Terrence, 1981). Other sources of nitrogen which satisfy the requirement as single sources of nitrogen are the amino acid, arginine and the salt, diammonium tetraoxosulphate IV. As far as amino acids are concerned asparagine is a better amino acid than arginine in terms of quality (Barker, 1981). It has also been shown that

asparagine accumulates during starvation giving the cells a ready pool of nitrogen source (Pauling and Jones 1980). The choice of a single nitrogen source medium was based on the fact that if multiple sources were used it would be difficult to tell which amino acid was responsible for the observed effects.

Cells that would be grown were taken from new complete medium (appendix 3) plates, diluted appropriately counted and a volume V containing a required number of cells, N was taken and inoculated into the conical flasks accordingly. This volume was determined as:

$$V = \frac{N}{S_c} \text{ ml.} \qquad 3.8$$

For batch cultivation, the cells were left in the incubator for as long as designed. To get the growth characteristics, fifteen bottles were inoculated at the same time and incubated simultaneously. Some were withdrawn at intervals and counted.

For semicontinuous cultivation, cells harvested after cultivation each day were counted and 10^3 cells per mililiter were reinoculated into the conical flasks and replaced in the incubator. It involved a circular process in fig.7 , steps 3, 4, 7 and 8. Step 7 is recounting and step 8 is redilution.

Growth was scored in terms of generations as:

$$n = \frac{\log(X/X_o)}{\log 2} \quad \text{(Ricica, 1966)} \qquad 3.9$$

where n is the number of generations per day obtained, X is the cell density at the end of the day and X_o is the inoculated cell density.

3.6 VIABILITY ASSAY

Viability of a cell line is a measure of its culture's colony forming ability. Loss of colony forming ability may be due to some cytoplasmic defects, especially leading to deformation of respiratory enzymes (Kiefer, et al, 1976) inability to metabolise (Altman and Ditter, 1968) and loss of fertility, sometimes due to increase in ploidy (Drake, 1970).

Viability assay was done by diluting the cell suspension adequately such that about 100 or 200 cells were pipetted into about 0.2ml of suspension. This amount of solution, layered generously on the lawn of an SC +ARG plate (appendix 3) in a petridish was incubated in the incubation room at 30°C for three days. This is illustrated in fig. 7 steps 3 and 5, as part of everyday process of testing the viability of the cells grown semicontinuously in the minimal medium.

It was believed that only cells which were viable formed visibly large colonies in three days. Thus if N_o cells were initially plated on the medium, and N colonies were formed, then the viability in terms of surviving fraction was scored as:

$$S.F. = \frac{N}{N_o} \qquad 3.10$$

3.7 MUTATION ASSAY

Cells damaged at the CAN locus of the gene can not produce wholesome arginine permease which is responsible for transporting arginine and its analogue canavanine into the cell (Grenson, et al, 1966). Transport of arginine into such cells can however, occur through the general amino acid permease (Hinnenbusch and Fink, 1983). This pathway can only take in structural amino acids, thus it can not take in canavanine. Thus mutated cells can survive in presence of canavanine and form colonies. Unmutated cells which take canavanine into them die.

In mutation assay, haploid cells were used, because in diploids recombination events can lead to mutation if the quality control of repairs fails, thus scored events could be a combination of mutation and recombination (Kiefer, 1984). The method involved spreading 10^6 or 10^7 cells on SC + CAN plates (appendix 3) after a suitable dilution as was described in fig 7, steps 3 and 6. The plated cells were incubated in the incubation room at 30^oC for three days.

If M_o cells were plated and M_c colonies were formed, then the mutation scored would be

$$M = \frac{M_c}{M_o} \qquad 3.11$$

It was believed that some mutated cells were perhaps not viable, and that errors in the assay methods could lead to wrong estimations, thus the mutation frequencies were recorded as the ratio between the number of mutants per plated cells and the viable fraction of cells. That is mutation frequency, M.F. is given by:

$$M.F. = \frac{M}{S.F.} \qquad 3.12$$

3.8 RECOMBINATION ASSAY

In assay for recombination, a diploid carrying a deletion in the CAN-gene of one of the DNA strands was used. Recombination repair may occur leading to the use of one strand as template for repair of the other. If the deleted strand is used, the resultant cell carries double deletion. If the intact strand was used, the resultant cell carries no deletion. On the other hand if mitotic recombination occurs, three possible configuration of the can gene for the daughters may occur: one with no deletion on both strands, another with deletion on both strands and the other with one deleted strand and one undeleted strand. In any of the cases, only cells carrying deletion on both strands survive in a medium containing canavanine.

Assay was carried out as in mutation (SC+CAN plate assay) and recombination frequency scored as:

Recombination frequency (R.F.) = $\frac{R}{S.F.}$ 3.13

Where $R = \frac{R_c}{R_o}$ is the ratio of recombinant colonies R_c over number of cells plated on the recombination plate, R_o.

Recombination and mutation assays were done together with viability assays respectively.

3.9 STATISTICAL ANALYSIS OF RESULTS

For each scored event there were at least three separate experiments. The ploted points were the average and the error bar represent the extent of standard .error in the three experiments.

Finally decisions were taken based on one way analysis of variance between results obtained from the control, the 10kGy-irradiated-asparagine-medium cultures and 50kGy-irradiated-asparagine-medium cultures. The analysis was summarized in an ANOVA-table (appendix 4.) under the table representing each experiment.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 LEVEL OF MUTATION AND RECOMBINATION IN THE USED STRAINS OF YEAST.

The level of mutation in the used strain was determined using the haploid cells irradiated under a low fluence of ultraviolet radiation (between 10 and 50 Jm^{-2}).The level of recombination was taken from Kranet (1985). He used the same diploid cells (cd364A). In both experiments, the plate assay method for determining foward mutation to canavanine resistance was used. Results (fig. 8) show that the mutation frequency was one part in a million survivors, when the same level of treatment gave up to 10 parts in a million for the recombination in the diploid. The result then justified the inclusion of a recombination test as a proper tool for identification of excess genetic activities.

4.2 EFFECT OF VOLUME OF IRRADIATED ASPARAGINE SOLUTION.

A preliminary experiment was performed to determine the effect of irradiated volume of solution on the scored parameters. 1g of asparagine was dissolved in various volumes of water and simultaneously given 10kGy irradiation. The range of volumes used were 0 (crystals) to 100ml of water. The growth was assessed

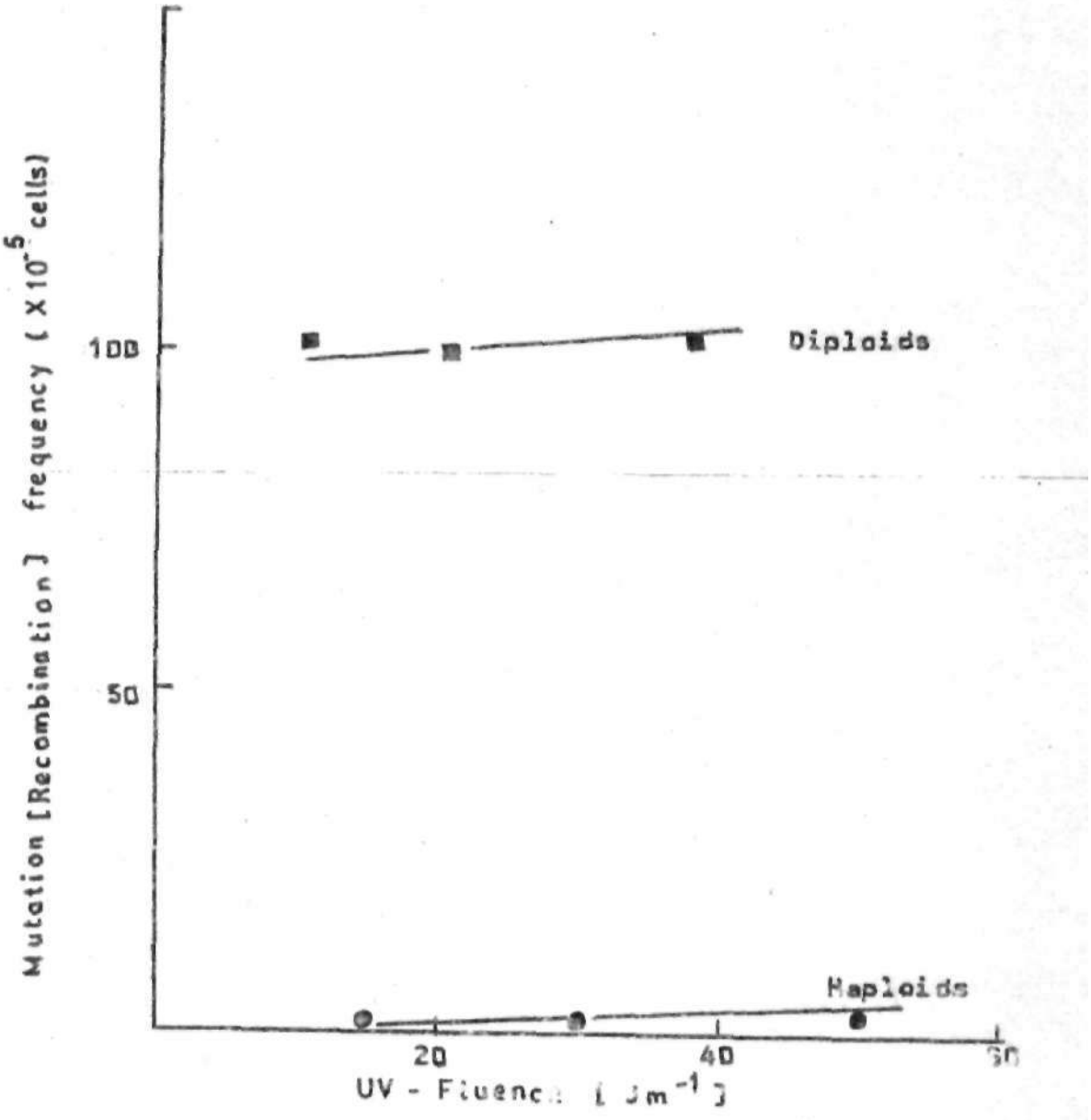


Fig. 8: Ultraviolet radiation induced mutation and recombination levels in the used strains of Saccharomyces cerevisiae

Note: The data for the diploids was adapted from experiment performed by Mr. Tilman Krankert of the Strahlenzentrum University of Gießen by his kind permission.

after cultivating the haploid cells in various Wickerham media made with the irradiated solutions for one day. Viability and mutation were assessed using the usual plate assay. Results shown in fig. 9 show no evidence of dependence of the measured parameters on the volume of irradiated solution. A close observation of the results shows that there is an inverse coincidence of mutability with viability. This means that the mutants in the culture were less viable than the non-mutants. The results also predict that the indirect effects of radiolysis products may not be the critical factorsin the experiments. The chioce of 100ml as irradiation volume for subsequent experiments was simply arbitrary.

4.3 GROWTH OF THE HAPLOID CELLS.

In the liquid batch culture, countable cell density using the THOMA-CHAMBER, was obtained after 10hrs of cultivating 1000 cells in 50ml Wickerham medium under constant stirring at 30^{0}C. The exponential phase of growth continued till nearly 40hrs. However, there was an exponential death of cells in the culture after about 80hrs. The experiment showed that at 24 hours of culturing, the cells were still in early logarithmic phase of growth. Thus, harvesting the cells at this stage would reduce the overall effects of stationary phase cells on the scored values. The results described above are shown in fig. 10.

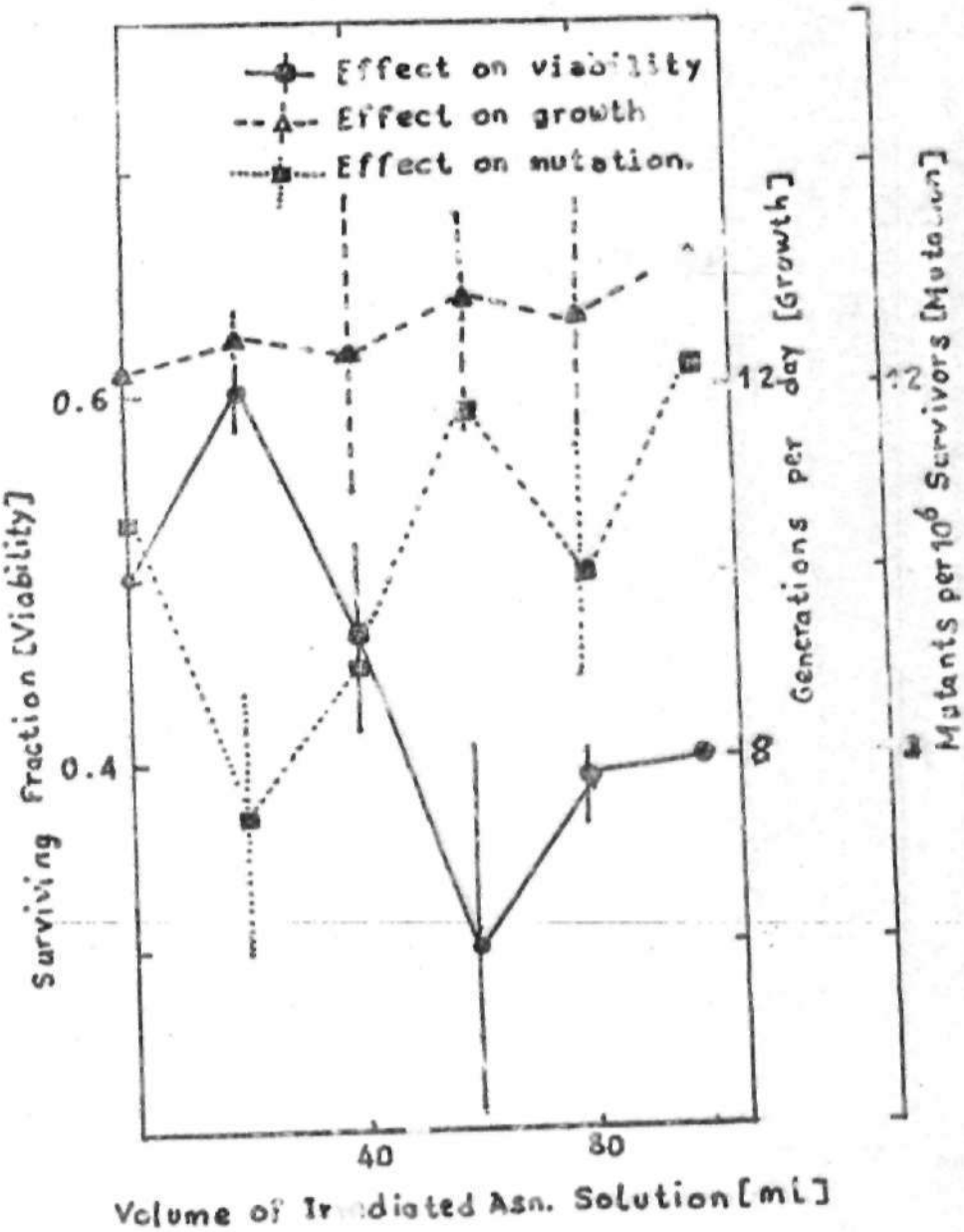


Fig 9: Graph showing the effect of the volume of irradiated solution on the various parameters. (Indirect action of radiation products).

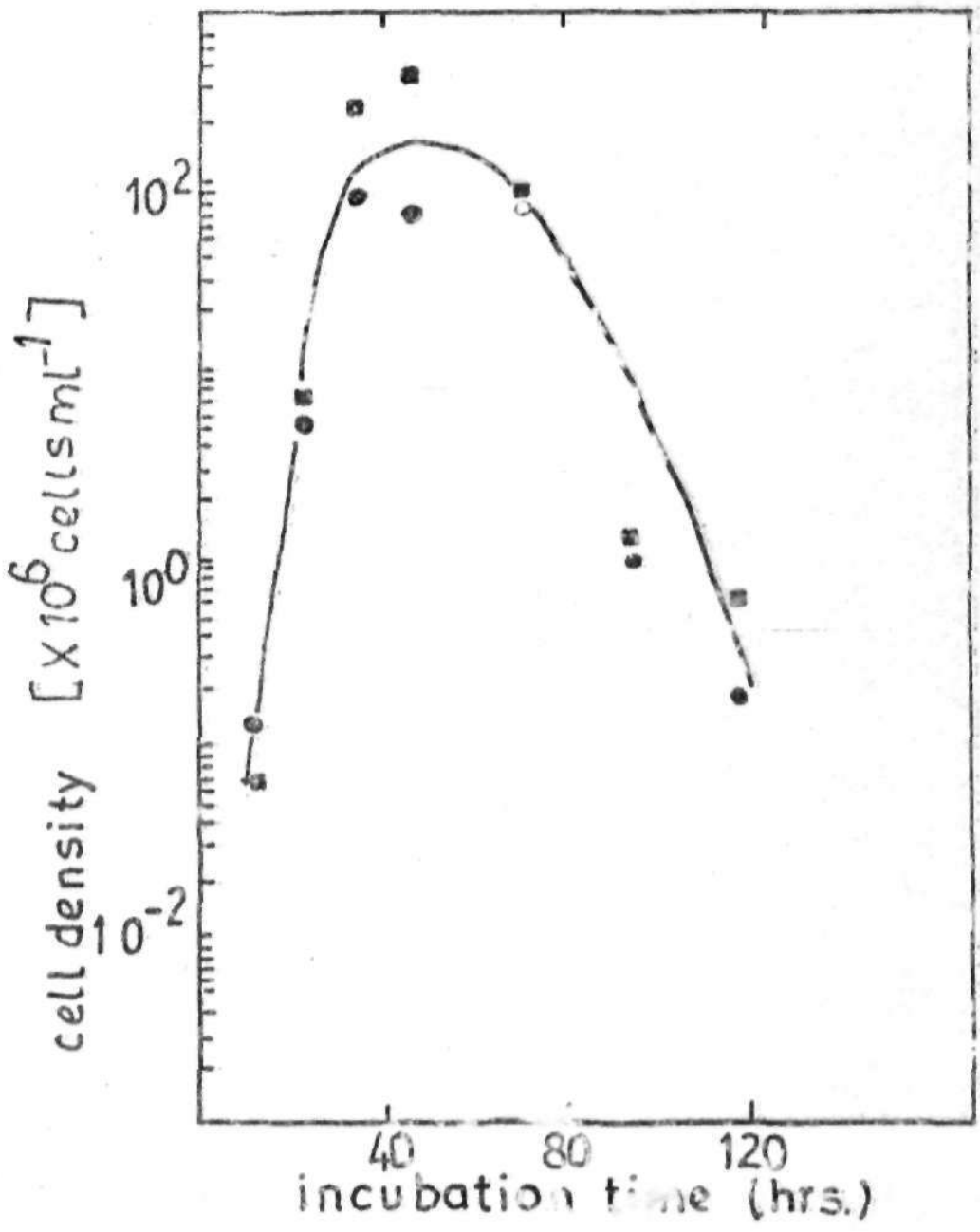


Fig. 10: Growth of 2h2 hr Deid cells in batch culture.

● Control Culture

■ 10kGy irradiated asparagine treated culture.

The average growth of the haploid cells was around 12 generations per day. From fig.11, it could be seen that apart from some scatters in the growth of the cells in the 50kGy-irradiated-asparagine culture, the growth was fairly constant. There was no clearly cut pattern of growth with regards to treatment, only scatter and mixture of points were seen. This showed that the treatment did not affect the pattern of growth.

Statistical analysis of the results indicated that there were no statistically significant differences within the results obtained for the three treatments. Tables of the above mentioned results and the subsequent analysis of variance are given in appendix 5, table 4.1.

4.4 GROWTH OF THE DIPLOID CELLS

In the liquid batch culture, countable cell density was observed after about 18 hours of cultivation. Exponential phase cell growth lasted up to around 50 hours before the stationary effect set in. No exponential cell death was observed even after 140 hours of cultivation. Thus, as with the haploids, cells harvested after a day of cultivation were still in the early exponential cell growth. In the batch culture, there was no differences seen in the growth pattern for the control culture cells and cells treated with 10kGy irradiated asparagine medium. This is shown in fig. 12.

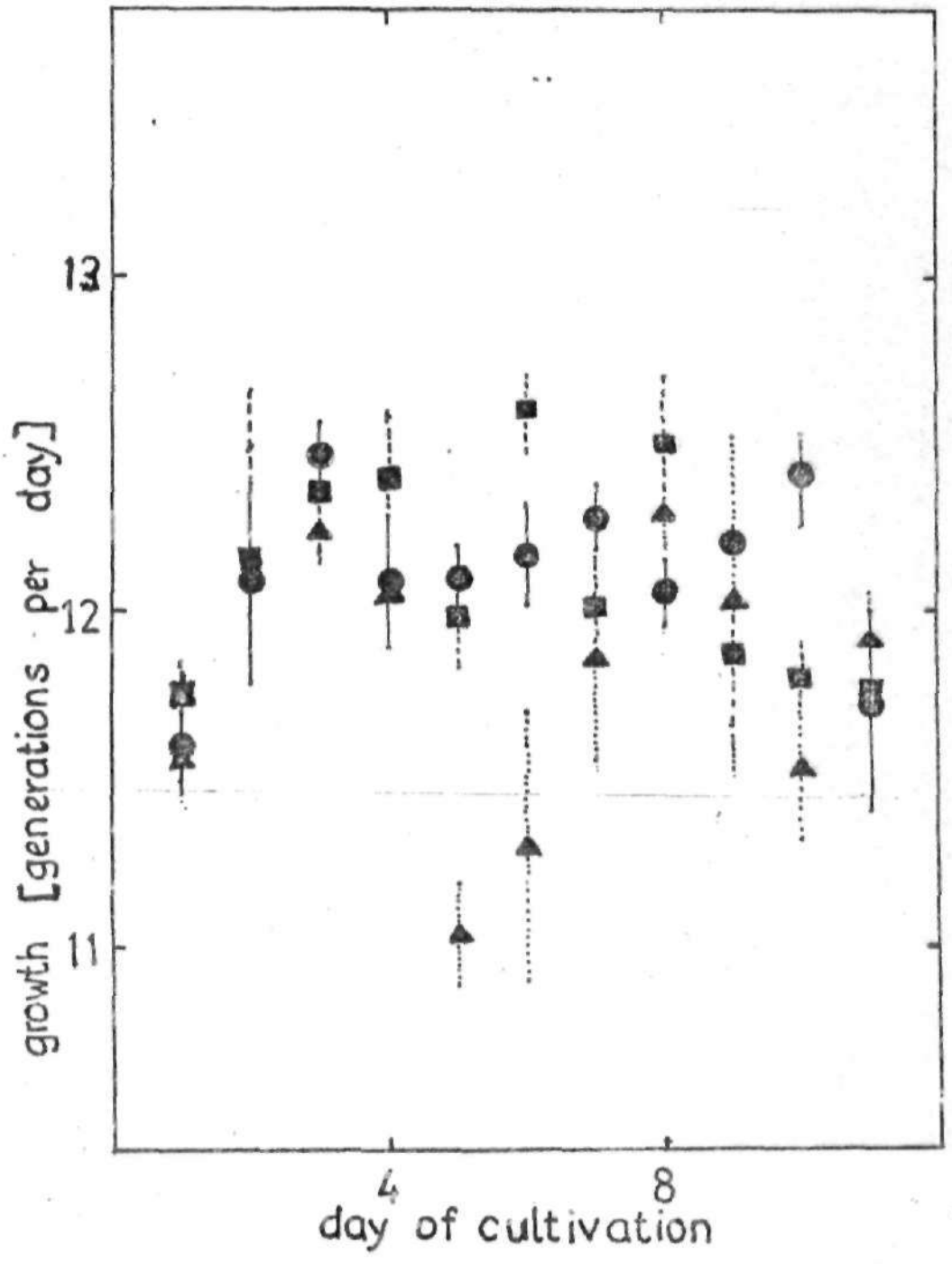


Fig. 11: Growth of the haploid cells in a daily renewal (Semicontinuous) culture.

The control (Unirradiated asparagine)

10kGy irradiated asparagine treated culture.

50kGy irradiated asparagine treated culture.

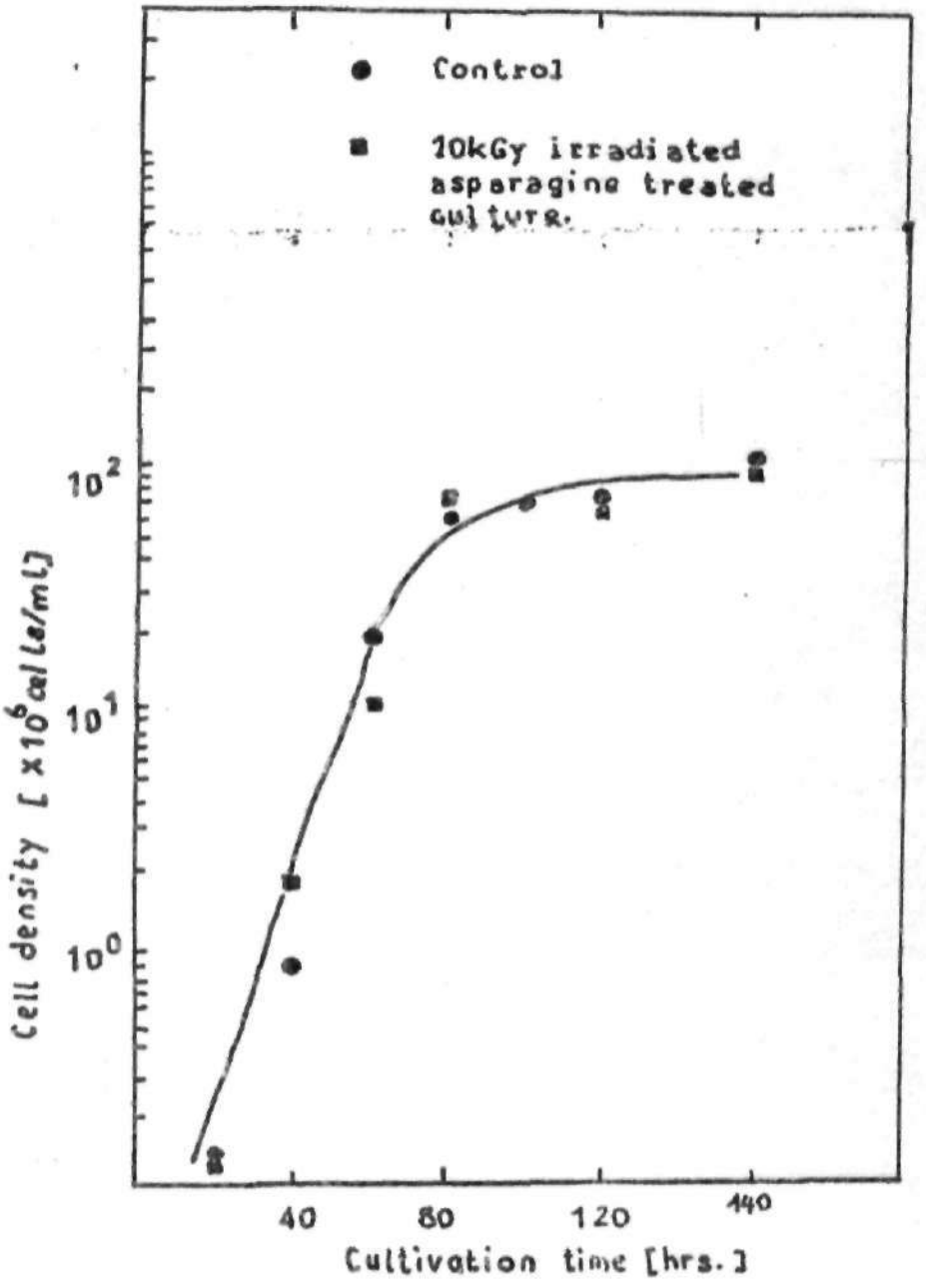


Fig. 12: Growth of the diploid cells in batch culture.

Results shown in fig. 13 indicate that growth of the diploid cells in the semicontinuous culture was increasing from around the initial 12 generations per day to up to 14 generations per day on the fifth day for irradiated asparagine fed cultures. This early rise was, however, not strictly followed by the control. After the fifth day, both the control and the 10kGy-irradiated asparagine cultures grew constantly to about 14 generations per day while the 50kGy-irradiated asparagine cultures showed a little decrease. However, the average growth remained 13 generations per day for all the three cultures.

Statistical analysis (Appendix 5, Table 4.2) showed that there were no statistically significant differences among the values scored for the three cultures.

In conclusion, therefore, it can be inferred that treatment of cells by growing them with irradiated asparagine medium did not change the growth pattern in either batch or semicontinuous systems.

4.5 VIABILITY OF THE HAPLOID CELLS.

While the control culture viability fell on the first few days of culturing, it also showed a rise on the last few days. The 10kGy-irradiated-asparagine culture showed a similar fall but no appreciable rise. The 50kGy-irradiated-asparagine culture remained constant but with a bit more scatter as shown by larger error bars. The result is shown in figure 14.

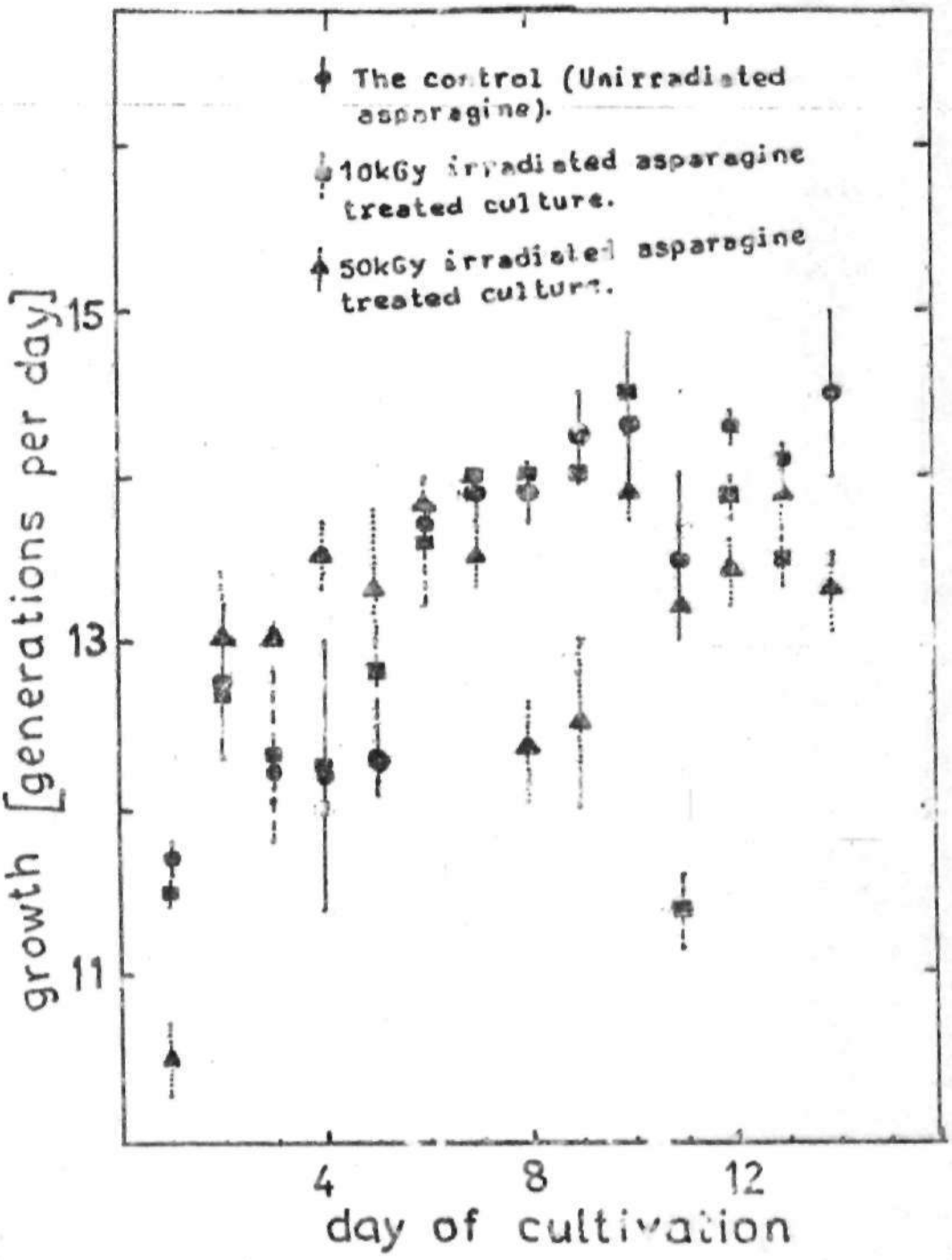


Fig 13: Growth of the diploid cells in a daily renewal (semicontinuous) culture.

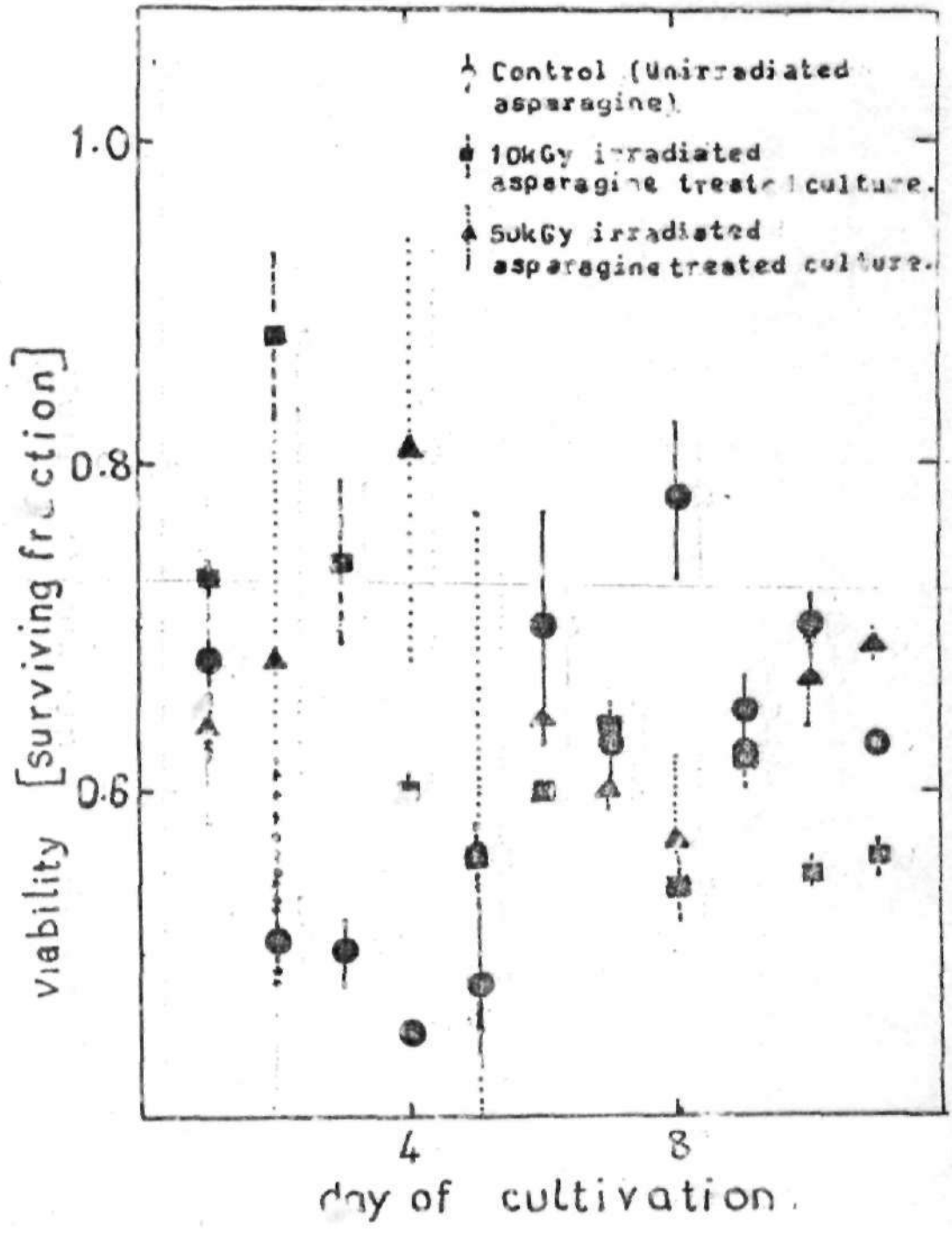


Fig. 14: Viability of the haploid cells in daily renewal (semicontinuous) cultures.

Statistical analysis showed no evidence of significant differences in the three treatments (Table 4.3, appendix 6).

4.6 VIABILITY OF THE DIPLOID CELLS

The viability level for the diploids was around 40 and 50%. This, when compared with the viability of the haploids (60%) was lower. There seem to be little variations in the viability for all the treatments (fig. 15).

Statistical analysis of variance showed no evidence of statistically significant differences (Table 4.4, appendix 6).

Thus, it can be inferred that growing of cells in irradiated-asparagine medium did not change the viability pattern.

4.7 MUTATION FREQUENCY

When cells were cultivated in the daily reinoculation (semicontinuous) method, The level of mutation fell to one part per million survivors from the recorded spontaneous level of ten mutants per million survivors. The level of mutation remained at this low level with no evidence of accumulation in all the three treatment cultures. There was a slight scatter in the recorded values for the first few days

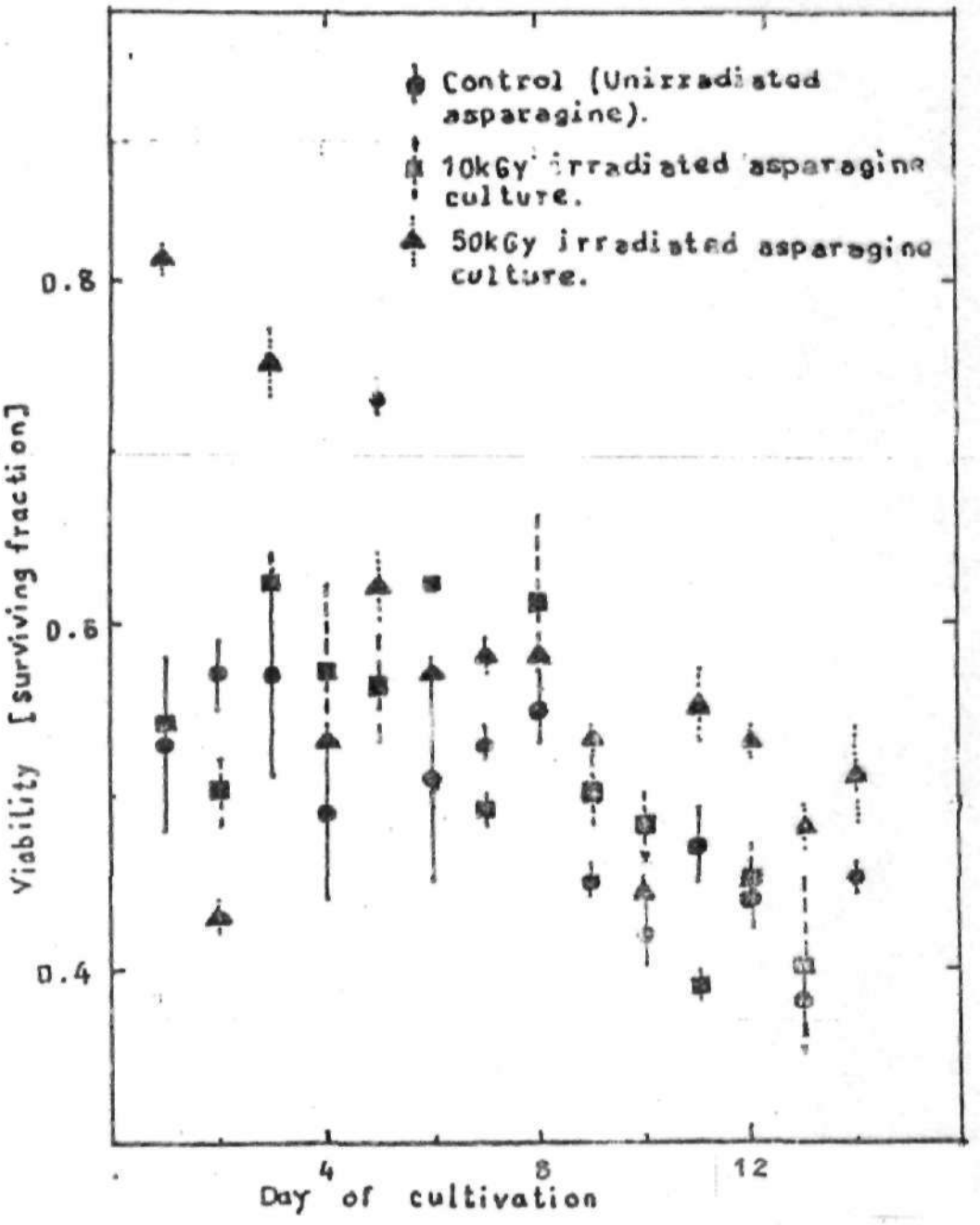


Fig. 15: Viability of the diploid cells in daily renewal (semicontinuous) cultures.

The results are shown in fig. 16.

Statistical analysis showed no statistically significant differences in all the treatment cultures (Table 4.6, appendix 7).

4.8 RECOMBINATION FREQUENCY

In the batch culture, Fig. 17, there seem to be no differences between the irradiated-asparagine treated culture and the unirradiated-asparagine culture. Both showed marked decrease in recombination frequency after about 50hrs, that was at the onset of stationary phase effects.

In the semicontinuous cultures, the results were equally consistent around one recombinant per thousand cells. This is higher than the recorded spontaneous level of about one part in ten thousand survivors (fig. 18).

Statistical analysis showed that there were no statistically significant differences in the results obtained from the three treatments.

Consequently, one can say that treatment of the yeast cells with irradiated asparagine as the only source of nitrogen did not induce mutation in the cells nor did it induce recombination in the diploid cells.

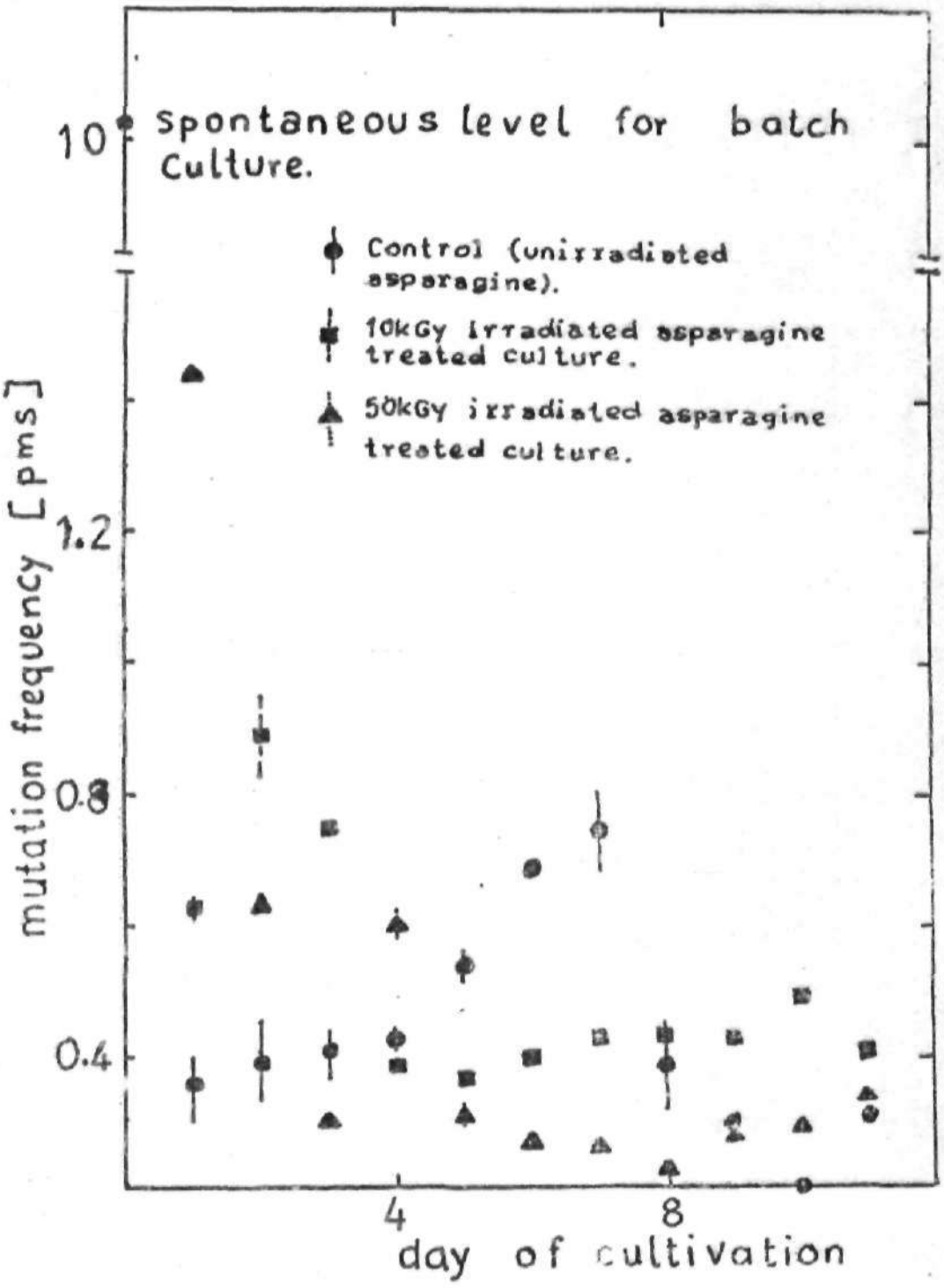


Fig. 16: Mutation frequen , in the daily renewal (semicontinuous) culture. (Haploid cells were used).

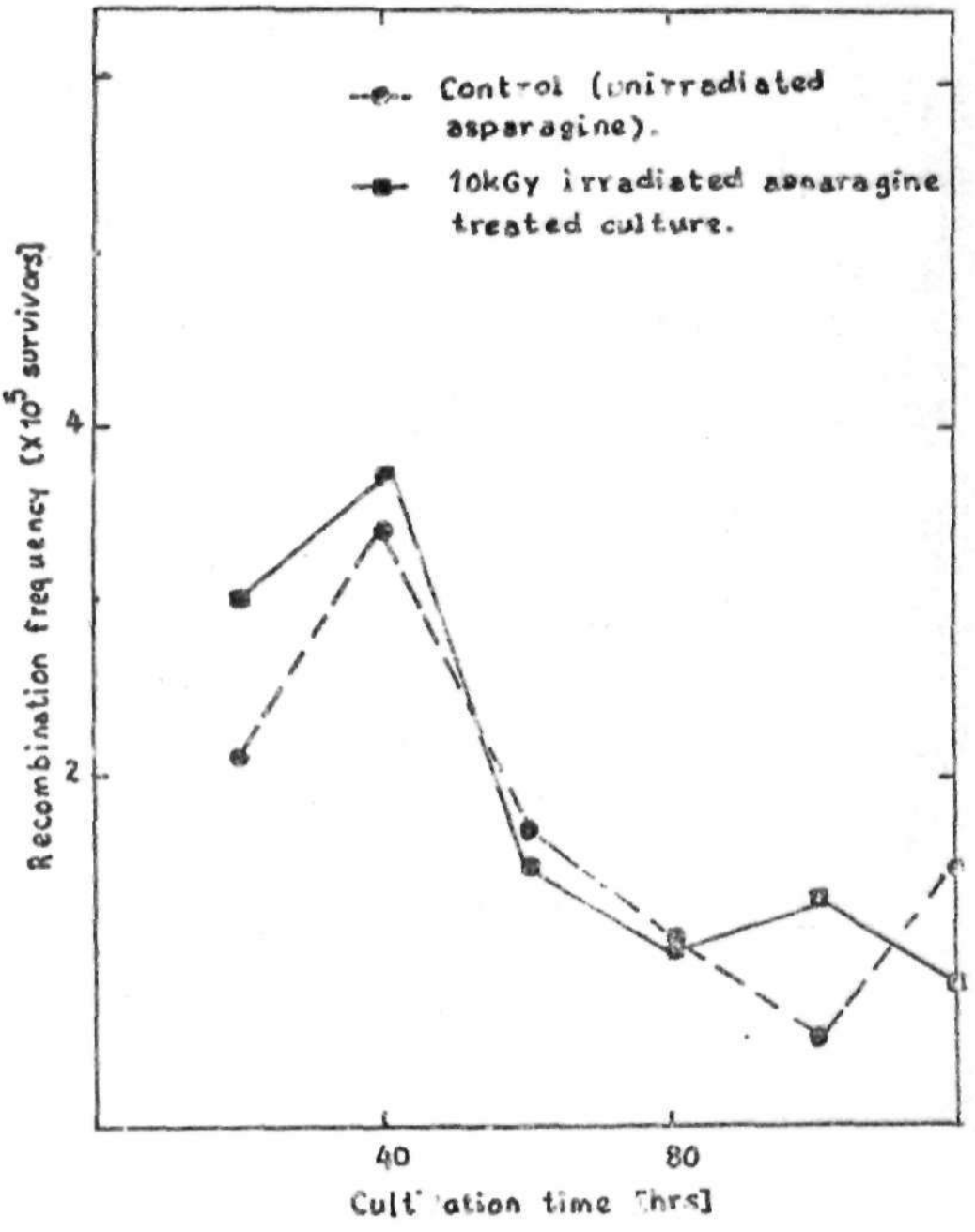


Fig. 17 Recombination frequency in cells grown in a batch culture.

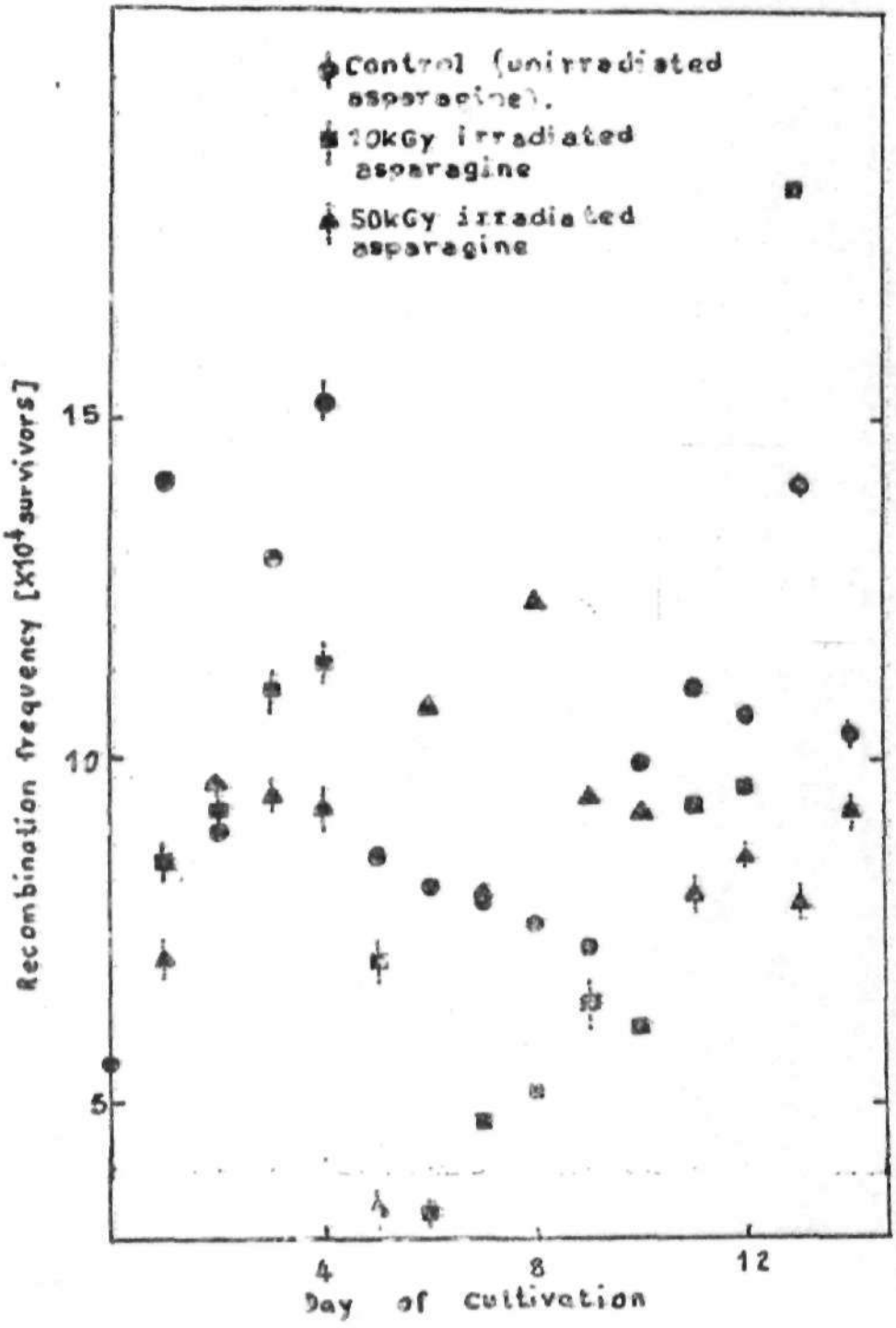


Fig. 18: Recombination frequency in the daily renewal (semicontinuous) culture.
(Diploid cells were used).

4.9 DISCUSSIONS OF RESULTS.

The results of the three major investigations in this project have shown that the treatment of the yeast cells by growing them with media containing irradiated amino acid (asparagine) as the only source of nitrogen did not change the growth or viability pattern when compared with cells grown in unirradiated asparagine media as control. No changes occured even when the cells aged up to about 150 generations in the culture. The system did not induce mutation or recombination in the cells used for the various tests.

Some remote but very important facts emerging from the results are: First, the result of the preliminary investigation showed an inverse coincidence of the cells mutability with their viability. This occured also in the controls, suggesting that the system may have some in-built mechanism that militated against accumulation of mutants in the cultures. Second, during the semicontinuous cultivation, there was a fall in the level of viability in the haploids and diploids from the usual level of around 70% and above (Kranet, 1985). The mutation level also fell too. However, there was a rise in the recombination level above the average for the recorded spontaneous recombination.

Since no attempt was made during the experimentation to go into details of finding out the root causes of the apparent antimutagenic activities in the used methods one can only list what may have been the causes.

First, if mutation and viability coincide inversely, then a fall in the visbility level must have been due to some sort of increase in mutation level, these mutants later died or did not form colonies. Second, the culturing conditions must have introduced some loss of activities in the cells, for example, feeding with starvation medium, continuous disturbance of growth during the daily renewals, washing away of viable cells which may be a small fraction and possibly the effects of other foreign bodies and cultivation conditions. Third, it could have been that mutation occured to a very low extent, due to error free repairs (Kubitschek, 1960). Whatever the case may be a comparison of the results with predicted results of mutagen treated heterogeneous culture (Oftedal, 1968) shows that a medium containing irradiated asparagine may not be a mutagen. Oftedal postulated that if the system is such that mutability coincides with viability, mutants will accumulate up to a maximum value then decline giving a plateau type characteristics. His predictions were backed up with results obtained by Stapelton (1955) Pittmans (1956) and Sinclair (1966).

Some other workers have accumulated mutants using media containing irradiated substances. For example Kuroda et al, (1961) in chinese hampster cells grown with irradiated s-iodouridine and Aiyer and Rao (1977) in S. typhimurium grown with irradiated glucose. Mutants have also been accumulated using low level chemicals in growing Saccharomyces cerevisiae, for example, Siebert and Lamperle (1974) using herbicides and Moore (1978) using bleomycin.

Some popular test methods using microorganisms for detection of mutation are those of Ames using histidine reversion in Salmonella typhimurium (Ames, et al, 1973) and Zimmerman using adenine reversion in Saccharomyces cerevisiae (Zimmerman et al, 1978). Many researchers use these methods. The problems with reversion tests are : site specificity, mutagen specificity and low frequency of occurence. It is argued that with these types of test one can score as safe agents which might be mutagens (DeSerres, 1976a). Foward mutation to canavanine resistance in yeast has been described as non-site and non-mutagen specific (Lemmontt, 1977, Gockey and Manney, 1979). The type of strain developed by Kiefer and his group (Strahlenzentrum, 1984) is excelent for determination of recombination events, at and around this canavanine locus. The canavanine locus, being large could give a better idea of genetic activities than smaller unknown sites responsible for reversion events. Since the test applied is in order, it lends its support that the irradiated asparagine may not be a mutagen.

The use of semicontinuous cultivation system is to apply low dose of the mutagen in a continual manner similar to feeding habits. It also helps to expose what could be a long time effects in future generations for accurate estimation of risks. Though accumulation of mutants is difficult in low dose chronic treatment of cells, (Srám, 1976) effective accumulation of mutants have been done by some workers in Salmonella typhimurium (Aiyer and Rao, 1977) Schizosaccharomyces pombe (Cox and Gibson,1974;

McAthey and Kilbey, 1977, 1978). All used chemostat systems. These show that semicontinuous systems do not prevent accumulation of mutants if there were any.

4.10 CONCLUSION AND POSSIBLE APPLICATIONS

It may be concluded, therefore that a media containing irradiated asparagine solution as the only source of nitrogen to growing cells of bakers' yeast, did not induce extra genetic activities and was not toxic to the cells.

A possible extension of this result, would be to suggest that irradiation of amino acid containing food may not also be cytotoxic or generate excess genetic activities in human cells. This extension can be supported by the fact that irradiation products of various amino acids are almost similar (Garrison, 1964) and the radiation chemistry of proteins does not differ so much from the radiation chemistry of constituent amino acids (Kumta and Tappel, 1961).

Some possible applications are:

1. The result would help to allay the fear that irradiated food is more dangerous than food preserved with chemicals or other preservatives.
2. A combination of yeast's foward mutation to canavanine resistance and semicontinuous culturing could be a very important substitute for multi-animal tests, thus saving costs. This has application for low level mutagens in food as well as in the environment.

Use of a submammalian system in testing for mutagens and carcinogens has three obvious advantages: All known alterations can easily be determined, metabolic activation can easily be studied in vitro and all tests can be carried out in the shortest possible time (Zimmerman, 1976). On the other hand comparison of results with mammalian systems poses some difficulties. Mammalian cells have certain unique features which include high content of repeatative DNA in their genomes which are likely to affect the frequency and spectrum of mutation. Indeed, only six percent of the mammalian DNA codes for structural genes. Also mammalian systems are known to contain some immunological mechanisms which are probably unique for multicellular organisms and are not present in micro-organisms (Freidman, 1974; Bome, 1976 and Malling, 1976).

The above arguement seem to suggest that the system being developed in this project if coupled with adequate mammalian test could be enough to test mutagenic substances and act as a handy tool in estimation of risks to future generations as discussed by Oftedal (1978).

4.11 SUGGESTIONS FOR FUTURE WORK

Some areas touched in this project which may need further investigations include:

1 Development of the foward mutation to canavanine resistance test to a standard test system for low level mutagenic activities. This should include the use, if possible of known mutagens in line with the work done

by Zimmerman for his D-series cells or by Ames and his group. Kiefer and his group are meanwhile working out the mapping of the actual point of deletion in the diploids used for these experiments.

2 Work could be done in areas involving the development of other strains of organisms that could take in some other amino acids as single nitrogen source, thereby testing the effect of such amino acids. A design using mammalian system would involve designing an automatic device for semicontinuous culturing, as mammalian cells are more susceptible to contamination than are yeast cells.

3. Work can also be done in area of using whole meals in testing for mutation involving the foward mutation and semicontinuous systems even if just for the sake of comparison.

REFERENCES

Aherne, W.A., R.S.Camplejohn and N.A. Wright.(1977). An Introduction to Cell Population Kinetics Edward Arnold Publishers, Ltd. London.

Aiyar, A.S. and V. Subba Rao (1977) Studies on Mutagenicity of Irradiated Sugar Solution in Salmonella typhimurium. Mutat. Res. 48:17.

Alberts, Bruce; Denis Bray; Julian Lewis; M.Raff; K.Roberts and J. Watson. (1983) The Molecular Biology of Cells. Garland Publishing Inc. N.Y.

Altman, P.L., and D. S. Ditter. (1968) Metabolism Fed. Amer. Soc. Exp. Biol Publ.

Ambe, K.S., U.S. Kumta and A.L.Tappel (1961) Radiation Damage to Cytochrome c and Haemoglobin. Radiat. Res. 15:709.

Ames, B. N., W.E. Durston, E. Yamasaki and F.E. Lee. (1973) Carcinogens are Mutagens: A simple Test System Combining Liver Homogenate for Activation and Bacteria Detection. Proc. Natl. Acad. Sci. (USA) 70:2281.

Ames, B.N., F.E. Lee and W.E.Durston (1973) Construction of a set of Strains of S. typhimurium for Testing Reversion to Frameshift and Lipopoly-sachharide Mutation are Described. Proc. Natl. Acad. Sci. (USA) 70:782.

Ames, B.N., J. McCann and E. Yamasaki (1975) Carcinogens are Mutagens: A simple Test system. Mutat. Res. 33:27.

Auerbach, C. (1962) Mutation: An Introduction to Research on Mutagenesis. Oliver Boyd Ltd. Edinburg.

Auerbach, C., M. Moutschen-Dahmen and J. Moutschen (1977) Genetic and Cytotoxic Effects of Formaldehyde and Related Compounds. Mutat. Res. 39:317

Barach, U and G. Eilon (1969) Interaction of Oxidized Polyamines with DNA: Evidence for Formation of Crosslinks. Biochim. Biophys. Acta 179:473.

Barker, H. A. (1981) Amino Acid Degradation by Anerobic Bacteria. Ann. Rev. Biochem. 50:23.

Bome, H. (1976) Mutagenicity Testing 1975: Some Personal Viewpoints. Mutat. Res. 41:175

Brewen J. (1975) Host Mediated Cytogenic Assay. Mutat. Res. 31:5

Burger, M. (1966) Studies of Some Problems of Bacterial Mutation in the Chemostat. In: I. Malek and Z. Fencl (Eds) J. Liebster (tr) Theoretical and Methodological Basis of Continuous Culture of Microorganisms. Sub. Chapter 5.4. Publishing House Prague and Acad Press N.Y.

Chanet, R., R. Walters and E. Moustacchi (1975) Induction of Pyrimidine Dimers in Nuclear DNA after UV Irradiation during Synchronous Cycle of Saccharomyces cerevisiae. Int. J. Radiat. Biol. 25 (5):481.

Committee 17 (1975) J.W. Drake.(Chairman)..Environmental Mutagenic Hazards. Science, 127:503.

Cox, E. C. and T. C. Gibson (1974) Selection of High Mutation Rates in Chemostats. Genetics 77:169.

DeSerres, F.J. (1976a). The Utility of Short Term Mutagenicity Tests. Mutat. Res. 38:1

DeSerres, F. J. (1976b) Mutagenesis of Chemical Carcinogens. Mutat. Res. 41:43.

Dewey, W.C. and R.M.Humphrey (1962) Relative Radiosensitivity of Different Phases in the Life Cycle of L-P59 Mouse Fibroblast and Ascite Tumour Cells. Radiat. Res. 16:503

Drake, J.W. (1970) The Molecular Basis of Mutation Holden-Day Inc. California.

Duran, L. and A.L. Tappel. (1958) Production of Carbonyl Compounds and Sulphurnyl Compounds on Irradiation of Amino Acids. Radiat. Res. 9:498.

Evans, W. E. and J. M. Parry (1975) The Genetic Effects of Elevated Temperature in the Yeast Saccharomyces cerevisiae. Mutat. Res. 51:165

Fabre, F. (1978) Induced Intragenic Recombination in Yeast can occur during G_1 Mitotic Phase. Nature, 272:795.

Fencl, Z. (1966) Theoretical Analysis of Continuous Culture Systems. In: I. Malek and Z. Fencl (Eds) J. Liebster (tr). Theoretical and Methodological Basis of Continuous Culture of Microorganisms. Publishing House Prague and Acad. Press N.Y.

Fishbein, L., W. G. Flamm and H. C. Falk (1970) Chemical Mutagens: Environmental Effects on Biological Systems. Academic Press N.Y.

Freidmann, L. (1974) A Procedure for the Assessment of Health Hazard of Carcinogens at very low Level of Exposure. WHO Tech Rep. Series No.546:15

Fricke, Hugo and Edwin J. Hart (1966) Chemical Dosimetry. In: F. Attix and W.C.Roesch (Eds) Radiation Dosimetry Vol.2,2ed, Instrumentation 12: pp 167-189. Acad. Press N.Y.

Garrison, W. M. (1964) Action of Ionizing Radiation on Nitrogen Compounds in Aqueous Media. Radiat. Res. Suppl. 4:158.

Garrison, W. M., M.E. Jayko and W. Bennett (1962) Radiation Induced Oxidation of Protein in Aqueous Solutions. Radiat Res. 16:483.

Gocke, E. and T. R. Manney (1979) Expression of Radiation-Induced Mutations at Arginine Permease (can 1) Locus in Saccharomyces cerevisiae. Genetics 91:53.

Goresline, H.E. (1973) The Potential of Ionizing Radiation for Food Preservation. Food Irradiation Info. 2:20.

Grenson, M. and C. Hou (1972) Ammonia Inhibition of General Amino Acid Permease and Its Supression in NADP-specific Glutamate Dehydrogenase Mutants of Saccharomyces cerevisiae. Biochem. Biophys. Res. Comm. 48 (4):749.

Grenson, M., M. Mousset; J.M.Wiane and J. Bechet (1966) Multiplicity of Amino Acid Permeases in Saccharomyces cerevisiae 1: Evidence for specific Arginine Transporting System. Biochem. Biophysics Acta.127: 325

Grossman, L.and D. M. Brown (1969) The Nature and Influence of UV and Hydroxylamine Lesions in Nucleic Acid and Enzymatic Repair of the Former. In: G.E.W. Wolstenholme and M. O'Conor (Eds) Mutation as a Cellular Process . Ciba Foundation Symposium. J. A. Churchill Ltd. London.

Hannan, M. A. and A. Nasim. (1978) Genetic Activities of Bleomycin Differential Effect on Mitotic Recombination and Mutation in Yeast. Mutat. Res. 40:309.

Hinnebusch, A.G. and G.R. Fink (1983) Positive Regulation in General Amino Acid Control of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci (USA) 80:5374.

Holliday, R. (1967) Altered Recombination Frequencies in Radiation Sensitive Strains of Ustilago. Mutat. Res. 4:275.

Howard-Flanders, P. (1967) Inducible Repair of DNA. Scientific American 245 (5): 56.

Howard-Flanders, P., S.C. West and Andrzej Stavark (1984) Role of Rec. A Proteins: Spiral Filaments in Genetic Recombination. Nature 309:215.

Kesevan, P.C. and Swaminathan (1971) Cytotoxic and Mutagenic Effects of Irradiated Substrates and Food Materials. Radiat. Biol. 11: 253.

Kiefer, J. (1984) Basics of Radiation Biology Unpublished Manuscript for Lectures in Ahmadu Bello University. Prepared in Strahlenzentrum of the University of Gießen. W. Germany.

Kiefer, J., H. Koch, B. Laske and H. Walter (1976) Metabolism of Yeast Cells after UV and X-ray Irradiation. In: J. Kiefer (Ed) Radiation as a Cellular Process. Springer Verlag Berlin.

Kranert, T. (1985) Recombination Frequency in Low UV-Fluence Irradiated cd364 strain of Saccharomyces cerevisiae. Personal Communication.

Kolousek, J,, J. Liebster and A. Babicky (1957) Radiochemical degradation of DL-Methionine. Nature, 179: 521.

Kubitschek, H.E. (1969) Repair of Latent T-5 Resistant Mutants in Chemostat Cultures. In: G. E. W. Wolstenholme and M. O'Conor (Eds) Mutation as Cellular Process. Ciba Foundation Symposium, J and A. Churchill Ltd. London.

Kubitschek, H.E. (1960) The Error Hypothesis of Mutation Science 131:730.

Kubitschek, H. E. and H. E. Bendigkeit (1964) Mutation in Continuous Culture 1: Dependence of Mutation upon Growth Limiting Factors. Mutat. Res. 4:113.

Kumta, U.S. and A.L. Tappel (1961) Radiation Damage on Proteins. Nature, 191:4795.

Kuroda, Y., A. Yokoiyama and T. Kada (1975) Isolation and Characterisation of variant Clones of Chinese Hamster Cells after treatment with irradiated S-iodouridine. Mutat. Res. 33:285.

Lemontt, J. F. (1977) Mutagenicity in Yeast by Hydrazine: Dependence upon Post-Treatment Cell Division. Mutat Res. 43:165.

Lemontt, J.F. (1977a) Pathways of Ultraviolet Mutability in Saccharomyces cerevisiae III: Genetic Analysis and Properties of Mutants Resistant to Ultraviolet Radiation-Induced Foward Mutation. Mutat. Res. 43:197.

Ley, F.J. (1972) Use of Irradiation Treatment of Various Animal Feed Products. Food Irradiat. Info. 1:8.

Magana-Schwenke, N., B. Eckart and E. Moustacchi. (1978) Biochemical Analysis of Damage Induced in Yeast by Formaldehyde 1: Induction of Single Strand Breaks in DNA and their Repair. Mutat. Res. 50:181.

Maigetter, R. Z. and R. M. Pfister (1975) A mixed Bacterial Population in a Continuous Culture with and without Kaolinite. Can J. Microbiol. 21:173.

Maling, H.V. (1971) Dimethylnitrosoamines: Formation of Mutagenic Compounds by Interaction with Mouse Liver Microsomes. Mutat. Res. 13:425.

Maling, H.V. (1976) Mutagenicity Testing : Mammalian Systems. Mutat Res. 41:171

Matile, P.H., H. Moore and C.F. Robinow (1969) Yeast Cytology. In: A. Rose and J.S. Harrison (Eds) The Yeasts, Vol 1: Biology of Yeasts. Academic Press, London.

Matin, A. and J.C. Gottschall (1978) Influence of Dilution Rate on NAD(P) and NAD(P)H Concentration and Ratio in Pseudomonas sp Grown in Continuous Culture. J. gen Microbiol. 94:333.

Matin, A., Grootjans and H. Hugehius (1978) Influence of Dilution Rate on Enzyme of Intermediary Metabolism in two Freshwater Bacteria Grown in Continuous Culture. J. gen. Microbiol. 94:323.

Maxwell, C.R. (1964). Radiolysis of Aqueous Glycine Radiat. Res. Suppl. 4 Addendum: 175.

Mayer, V.M. (1973) Induction of Mitotic Crossing Over in Saccharomyces cerevisiae by Breakdown Products of Dimethylnitrosamine, Diethylnitrosamine 1-Naphthylamine and 2-Naphthylamine formed by an in vitro hydroxylation system. Genetics 74:433.

McAthey, P. and B.J. Kilbey (1977) Mutation in Continuous Culture of Schizosaccharomyces pombe 1: Dependence of the Kinetics of Mutation Accumulation upon Growth Limiting Nutrilite Mutat. Res. 44, 227.

McAthey, P. and B.J. Kilbey (1978) Mutation in Continuous Culture of Schizosaccharomyces pombe II: Effect of Amino Acid Starvation on Mutational Response and DNA Concentration. Mutat. Res. 50:175.

McCalla, D. R., D. Voutsinos and P.L. Olive (1975) Mutagen Screening with Bacteria: Nitroimidazole and Nitrifurans. Mutat. Res. 31:31.

Moore, C.W. (1978) Responses of Radiation-sensitive Mutants of Saccharomyces cerevisiae to Lethal Effects of Bleomycin. Mutat. Res. 51:165.

Mortimer, R.K. and D.C.Hawthorne (1969) Yeast Genetics In: A.H. Rose and J.S. Harrison (Eds) The Yeasts, Vol. I: Biology of Yeasts. Academic Press London.

Murthy, M.S.S. and N. Sankaranarayanan (1978) Determination of Radiation Equivalence of Ethylmethanesulphonate (EMS) for Induction of Gene Conversion in Diploid Yeast. Mutat. Res. 51:181.

Nishioka, H. (1975) Lethak and Mutagenic Action of Formaldehyde in Hcr^+ and Hcr^- Strains of Eschericia coli. Mutat.Res 17:281.

Oftedal, P. (1968) A theoretical Study of Mutant Yield and Cell Killing after Treatment of a Heterogeneous Cell Population. Hereditas 60:177.

Oftedal, P. (1976) Panel Discussion on Estimation of Risk. Mutat. Res. 41:173.

Parry, J.M. (1973) Induction of Gene Conversion in Yeast by Herbicide Preparation. Mutat. Res. 21:83.

Pauling , K.D. and G. E. Jones (1980) Asparaginase II of Saccharomyces cerevisiae: Dynamics of Accumulation and Loss in Rapidly Growing Cells. J. gen. Microbiol. 117:423.

Phillip, B.J. and P. S. Elias (1980) Short Term Mutagenicity Tests and their Application to Irradiated Foods. Food Iradiat. Info. 10:8

Pittman, D.D. (1956) The Relation of Population Heterogeneity to Plateau Phenomenon in Radiation-Dose Curves. J. Bacteriol. 71:500.

Powell, E.O. (1956) Growth Rate and Generation Time of Bacteria with Special Reference to Continuous Culture. J. gen. Microbiol. 15:492.

Ricica, J. (1966) Continuous Systems. In: I. Malek and Z. Fencl (Eds) J. Liebster (tr) Theoretical and Methodological Basis of Continuous Culture of Microorganisms. Publishing House Prague and Academic Press N.Y.

Ricica, J. (1966) Techniques of Continuous Laboratory Cultivation. In: I. Malek and Z. Fencl (Eds) J. Liebster (tr) Theoretical and Methodological Basis of Continuous Culture of Microorganisms. Publishing House Prague and Acad Press, N.Y.

Rowley, B.B. and A. Brynjolfsson (1980) Potential Uses of Irradiation in Processing of Food. Food Tech. 80, 75.

Schlenk, F and J.L. Dianko (1962) Some Effects of Ionizing Radiation on Methionine Sulphonium Compounds. Mutat. Res. 16: 327.

Schmid, W. (1975) Micronucleus Test. Mutat. Res. 31: 9.

Schubert, J. (1969) Mutagenicity and Cytotoxicity of Irradiated Food Components. Bull. Wld. Hlth. Org. 41: 873.

Sedgwick, S. (1980) Roles of Rec A Revealed. Nature 287:676.

Shkidchenko, A.N. (1982) Limitation of Growth of the Yeast, Saccharomyces cerevisiae, in Chemostat System by Carbon and Nitrogen Sources. Mikrobiologiya 53(1): 58.

Shimazu, F. and A. L. Tappel(1964) Comparative Radiolability of Amino Acids. Radiat. Res. 23:203.

Siebert, D and E. Lemperle (1974) Genetic Effects of Herbicides: Induction of Mitotic Gene Conversion in Saccharomyces cerevisiae. Mutat. Res. 22:111.

Sinclair, W.K. and R.A. Morton (1963) Variation in X-ray Response During the Division Cycle of Partially Synchronized Chinese Hamster Cells in Culture. Nature 190 (4899):1159.

Siomin, Y.A., V.V. Simonov and A. M. Poverenny (1973) The Reaction of Formaldehyde with Deoxynucleotides and DNA in Presence of Amino Acid Lysine-rich Histone. Biochim. Biophys. Acta 331:27.

Srâm, R.F. (1976) Relationship between Acute and Chronic Exposure in Mutagenicity in Mice Mutat. Res. 41:25.

Stapleton, G.E. (1955) Variation in Sensitivity of Eschericia coli to Ionizing Radiation During the Growth Cycle. J. Bact. 70:357.

Strahlenzentrum (1985) Unpublished Practical Handouts For Biophysics II Practicals, University of GieBen, W. Germany.

Soumalinen, H. and E. Oura (1971) Yeast Nutrition and Solute Uptake. In: A.H.Rose and J.S. Harrison (Eds) The Yeasts Vol.2: Physiology and Biochemistry of Yeasts. Academic Press Inc. London Ltd.

Terrance, G.C. (1981) Nitrogen Metabolism in Saccharomyces cerevisiae. In: Stratherm, et al, (Eds) Molecular Biology of Yeasts. Cold Spring Harbour Laboratories Monograph Series.

Thacker, J. (1975) Inactivation of Cells by Hydrogen Peroxide. Mutat. Res. 33:147.

Thingstad, T.F. and T.I. Langeland (1974) Dynamics of Chemostat Cultures: The Effect of Delay in Cell Response. J. Theo Boil. 46:149

van Kooij, (1984) Food Irradiation makes Progress IAEA Bull. 28 (2):17

van Uden, N. (1971) Kinetics and Energetics of Yeast Growth. In: A. H. Rose and J. S. Harrison (Eds) The Yeast Vol. 2: Physiology and Biochemistry of Yeasts. Acad. Press, London.

Weeks, B.M. and W.M. Garrison (1958) Radiolysis of Aqueous Solution of Glysine. Mutat. Res.9:291.

WHO Scientific Group Report (1974) Assessment of Carcinogenicity and Mutagenicity. Techn. Rep. Series 546.

Westhoff, E., W. Flossman and A Mueller (1975) The Action of Ionizing Radiation on Protein Radical Formation in L-Histidine Crystals. Int. J. Radiat. Biol. 27 (1):51.

Whelan, W.L., E. Gocke and T.R. Manley (1978) The CAN 1 Locus of Saccharomyces cerevisiae Mutation Rates and Fine Structure Analysis. Genetics 91:35-51

Wlodkowski, T.J. and H,S. Rosenkranz (1975) Mutagenicity of Sodium Hypochlorite for Salmonella typhimurium. Mutation Research 41: 163.

Zimmermann, F.K.(1971) Induction of Mitotic Gene Conversion by Mutagens. Mutat, Res. 11: 327

Zimmermann, F.K. (1975) Procedures Used in induction of Mitotic Recombination in Yeast, Saccharomyces cerevisiae. Mutat. Res. 31:71.

Zimmermann, F.K. (1976) Panel Discussion on Submammalian Systems. Mutat. Res. 41: 163

Zimmermann, F.K. , R. Kern and H. Rasenburger (1978) A Yeast Strain for Simultaneous Detection of Induced Mitotic Crossing Over, Mitotic Gene conversion and Reverse Mutation. Mutat. Res. 28: 381.

ADDENDUM

Elias, P. S. (1977) General Introduction. In P.S. Elias and A.J. Cohen (Eds) Radiation Chemistry of Major Food Components, Its relevance to the Assessment of the Wholesomeness of Irradiated Foods. pp 1 - 4, Elsevier Scientific Publishing Company, Amsterdam.

APPENDIX I

BASIC EQUIPMENT FOR A YEAST LABORATORY

1. Autoclave (Capacity: at least 120°C temp; 1atm. pressure and for 20min. time).

2. Sterilization oven (capacity: 200°C for at least 6hrs.).

3. Rotary shaker cum incubator (capacity: 100rpm; 30°C steady temperature).

4. Incubator room (maintained at 30°C).

5. Vortex shaker

6. Microscope

7. Haematocytometer (Thoma counting chamber).

8. Mechanical counters

9. Analytical balances (sensitivity: 0.01mg).

10. Weighing balance (capacity: up to 5g).

11. pH meter

12. Refrigerator

13. Standard test tubes (300) .

14. Test tube racks.

15. 2mm-diameter glass rods.

16. Glass bottles (500, 1000ml capacities).

17. Erlemeyer flasks (50, 100, 120ml capacities).

18. Beakers (various sizes)

19. Burners (gas, electric and alcohol).

20. Pipettes (1, 2, 5, 10, 20ml sizes).

21. Pipette-holders

22. Holders for washing and drying glasswares.

23. Magnetic stirrers and magnetic pieces of various sizes covered with teflon.

24. Membrane filter pumps and membrane filters.

25. Pincers, spatulas and other holding devices

26. Petri-dishes.

APPENDIX 2

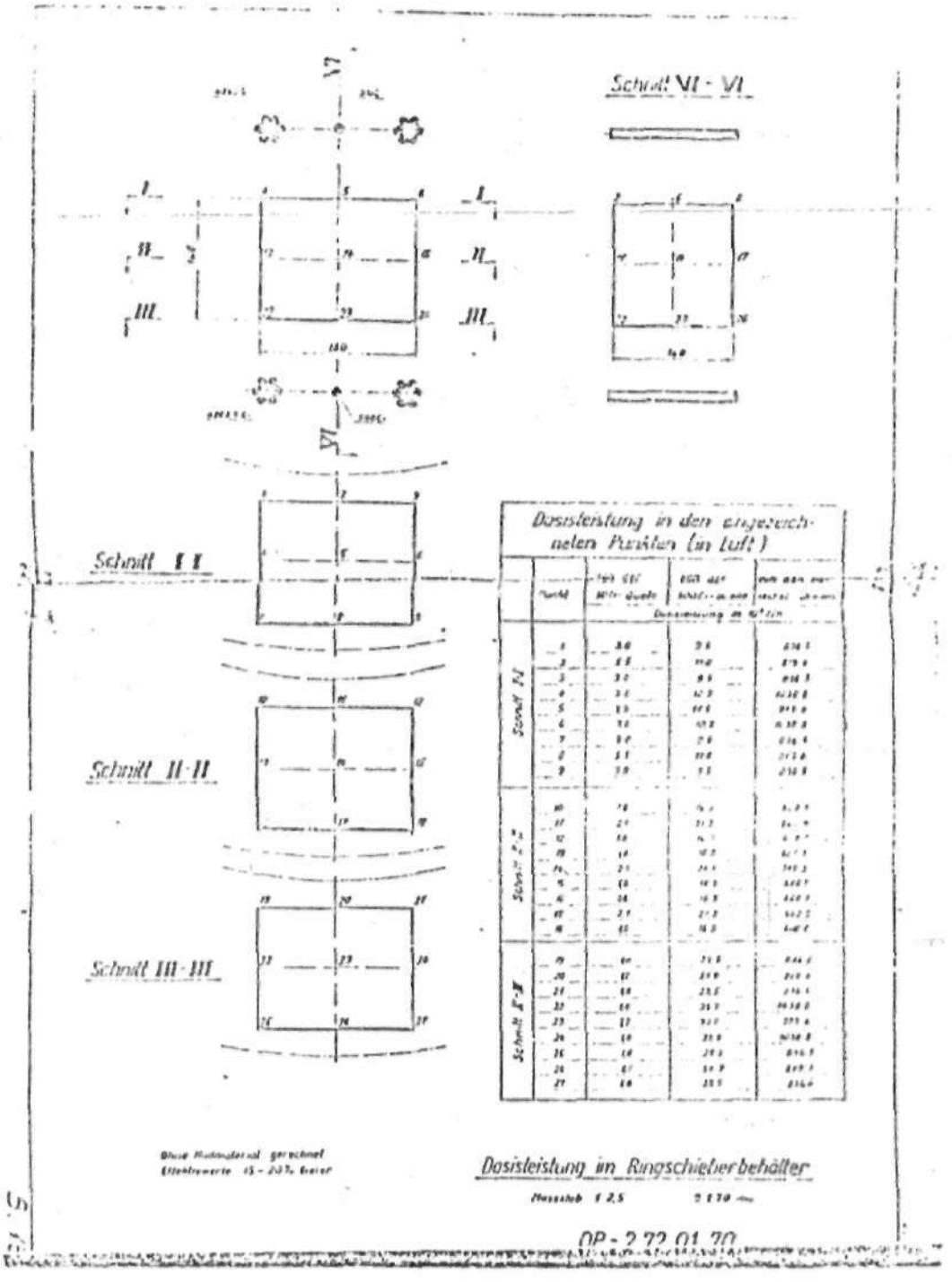


Table 3.1: Activity in the irradiation Chamber. Sector III-III (Schnitt) is the bottom of the irradiation chamber where the samples were irradiated.

APPENDIX 3

GROWTH MEDIA FOR YEAST CELLS USED.

1. THE COMPLETE MEDIUM

Glucose	20g/l
Yeast Extract	10g/l
Gelatine hydrolase peptone	20g/l

When a solid medium is required, non nutirent agar base is used.

Agar	20g/l

2. SYNTHETIC COMPLETE MEDIUM (SC).

Difco Yeast nitrogen base	6.7g/l
Glucose	20g/l

When a solid medium is required, non-nutrient agar base is added as in (1) above.

A stock solution of the following amino acids was made:

Histidine	20 mg/l
Tryptophan	20 mg/l
Adenine	20 mg/l
Methionine	20mg/l
Leucine	30mg/l

Lysine 30mg/l

A separate stock solution was made of threonine alone:

Threonine 100mg/l

2(a) SC + ARG.

As in (2) above plus an eqivalent of:

Arginine 20mg/l

made in a separate stock solution.

2(b) SC + CAN

As in (2) above plus an equivalent of:

Canavanine 10µg/l

3. WICKERHAM MEDIUM (also called the minimal medium).

Glucose 10g/l

Salts:

K_2HPO_4	0.125g/l	
KH_2PO_4	0.875g/l	
$MgSO_4$	0.5g/l	
$CaCl_2$	0.1g/l	
NaCl	0.1g/l	
$FeCl_3$	24.2g/l	(Separate solution to be made.
KI	0.1g/l	(Separate solution)

Trace element

Boric Acid	0.57mg/l
$ZnCl_2$	0.145mg/l

A stock solution of the trace elements made separately.

Vitamins

Thiamine	0.1mg/l
Pyriodoxin	0.4mg/l
Nicotine	0.4mg/l
Pantothemic acid	0.4mg/l
Inosit	0.2mg/l
Riboflavine	0.2mg/l
Paraminobenzoic acid	0.2mg/l
Biotin (Separate solution).	0.25mg/l

All solutions were made up with sterile bidest amd autoclaved at 120°C, 1atm. for 20mins. where necessary.
The equivalent volume for each solution were first calculated and pipetted into the medium before the required volume of the medium was made up using bidest.

Source: Strahlenzentrum of the University of Gießen W. Germany. Laboratory notes.

APPENDIX 4

THE ANOVA TABLE

The use of ANOVA table enables the calculation of variability within and among experimental data in a one or two way fashion. The variation can often be subdivided into various components, namely, the variability due to treatment and the variability due to error. The table is represented as follows:-

SOURCE	df	SS	MS	F(Observed)	F(table) 5%	F(table) 1%
Treatment						
Error						
Total						

df:degree of freedom = Total observation - 1.

Error df = Total df - Treatment df.

Total sum of square TSS = $\sum x_i^2 - CF$.

where $CF = \frac{(\sum x_i^2)}{GT}$; GT is the grand total.

Treatment sum of square $Tr.SS = \frac{(\sum Tr_i)^2}{R} - CF$

where R is the number of repeatations.

ESS = Error sum of square = TSS - TrSS.

Tr.MS = Treatment mean square = $\frac{Tr.SS}{Tr.df}$.

EMS = Error mean square = $\frac{ESS}{Edf}$

F(Observed) = $\frac{Tr.MS}{EMS}$.

In the use of the ANOVA in statistical analysis, the observed F-ration is compared with the table F-ratio. The results are interpreted as follows:

i. If F(observed) ▲ F(table) the results compared are said to have statistically significant differences. This is denoted by putting an asterisk below the table.

ii. If F(observed) ◭ F(table) the results compared are said to have statistically nonsignificant differences. This is denoted by writing (ns) below the table.

iii. If the table value was taken at 1% level of significant and the result is significant, it is normally described as being highly significant.

If the observed value is significant at 5% level of significant, it is simply described as significant,

To obtain an index of variability , the coefficient of variability is calculated as:

$$cv = \frac{\sqrt{EMS}}{\Sigma \bar{x}_i} \times 100\%$$

The higher the cv, the lower the reliability of the conclusion. The cv is then always accompanying the table.

APPENDIX 5

TABLES FOR CELLS' GROWTH

TABLE 4.1: GROWTH OF THE HAPLOID CELLS

Day of cultivation	Growth (Generations/day)		
	(a)	(b)	(c)
1	11.60 ±0.10	11.76 ±0.15	11.56 ±0.10
2	12.09 ±0.30	12.16 ±0.30	12.14 ±0.30
3	12.47 ±0.10	12.38 ±0.20	12.23 ±0.15
4	12.09 ±0.20	12.40 ±0.20	12.06 ±0.15
5	12.10 ±0.10	11.98 ±0.15	11.04 ±0.15
6	12.17 ±0.15	12.61 ±0.10	11.30 ±0.40
7	12.29 ±0.10	12.01 ±0.10	11.86 ±0.30
8	12.06 ±0.10	12.51 ±0.20	12.30 ±0.50
9	12.21 ±0.01	11.86 ±0.20	12.03 ±0.50
10	12.41 ±0.15	11.80 ±0.10	11.53 ±0.20
11	11.72 ±0.30	11.77 ±0.10	11.91 ±0.15

ANOVA

SOURCE	df	SS	MS	F_o	$F_{t,5\%}$
Tr	2	0.72	0.36	2.35	3.43
E.	28	3.56	0.13		
T	30	4.28			

RESULT: ns, cv = 0.27%

TABLE 4.2 GROWTH OF THE DIPLOID CELLS

Day of cultivation	Growth (Generations/day) (a)	(b)	(c)
1	11.71 ±0.10	11.50 ±0.15	10.50 ±0.20
2	12.76 ±0.45	12.68 ±0.20	13.05 ±0.40
3	12.23 ±0.10	12.32 ±0.50	13.04 ±0.10
4	12.20 ±0.80	12.26 ±0.30	13.53 ±0.20
5	12.34 ±0.15	12.83 ±0.35	13.29 ±0.50
6	13.75 ±0.15	13.60 ±0.35	13.87 ±0.10
7	13.92 ±0.30	14.04 ±0.10	13.51 ±0.20
8	13.91 ±0.20	14.01 ±0.15	12.37 ±0.30
9	14.28 ±0.20	14.04 ±0.15	12.53 ±0.50
10	14.36 ±0.50	14.54 ±0.15	13.93 ±0.20
11	13.49 ±0.50	11.39 ±0.25	13.23 ±0.15
12	14.30 ±0.10	13.87 ±0.15	13.42 ±0.20
13	14.11 ±0.50	13.48 ±0.20	13.74 ±0.50
14	14.48 ±0.50	-	13.29 ±0.25

ANOVA

SOURCE	df	SS	MS	F_o	$F_{t,5\%}$
Tr	2	23.6	0.62	0.11	4.10
E	38	34.32	0.90		
T	40	57.92			

RESULT: ns, cv = 0.51%

(a) Control culture (b) 10kGy and (c) 50kGy irradiated.

APPENDIX 6

TABLES FOR CELLS' VIABILITY

TABLE 4.3 VIABILITY OF THE HAPLOID CELLS.

Day of cultivation	Viability (surviving fraction) (a)	(b)	(c)
1	0.68 ±0.01	0.73 ±0.01	0.64 ±0.02
2	0.51 ±0.01	0.88 ±0.05	0.68 ±0.25
3	0.50 ±0.02	0.74 ±0.05	0.65 ±0.01
4	0.45 ±0.00	0.60 ±0.01	0.81 ±0.13
5	0.48 ±0.05	0.56 ±0.02	0.57 ±0.02
6	0.70 ±0.07	0.60 ±0.00	0.64 ±0.00
7	0.63 ±0.02	0.64 ±0.01	0.60 ±0.01
8	0.78 ±0.05	0.54 ±0.02	0.57 ±0.05
9	0.65 ±0.02	0.62 ±0.02	0.63 ±0.03
10	0.71 ±0.01	0.55 ±0.01	0.67 ±0.03
11	0.63 ±0.00	0.56 ±0.01	0.69 ±0.01

ANOVA

SOURCE	df	SS	MS	F_o	$F_{t,5\%}$
Tr	2	0.009	0.005	0.5	4.20
E	28	0.263	0.009		
TOTAL	30	0.272			

RESULT: ns, cv = 1.34% .

TABLE 4.4 VIABILITY OF THE DIPLOID CELLS

Day of cultivation	Viability (surviving fraction) (a)	(b)	(c)
1	0.53 ±0.05	0.54 ±0.01	0.81 ±0.01
2	0.57 ±0.02	0.50 ±0.02	0.43 ±0.01
3	0.57 ±0.06	0.62 ±0.02	0.75 ±0.02
4	0.49 ±0.05	0.57 ±0.05	0.53 ±0.01
5	0.73 ±0.01	0.56 ±0.03	0.62 ±0.02
6	0.51 ±0.06	0.62 ±0.00	0.57 ±0.01
7	0.53 ±0.01	0.49 ±0.01	0.58 ±0.01
8	0.55 ±0.02	0.61 ±0.05	0.58 ±0.02
9	0.45 ±0.01	0.50 ±0.02	0.53 ±0.01
10	0.42 ±0.02	0.48 ±0.02	0.44 ±0.01
11	0.47 ±0.02	0.39 ±0.01	0.55 ±0.02
12	0.44 ±0.02	0.45 ±0.02	0.53 ±0.01
13	0.38 ±0.02	0.40 ±0.05	0.48 ±0.01
14	0.45 ±0.02	-	0.51 ±0.03

ANOVA

SOURCE	df	SS	MS	F_o	$F_{t,5\%}$
Tr	2	0.037	0.019	2.11	4.06
E	44	0.376	0.009		
T	46	0.413			

RESULT: ns, cv = 1.29%

(a) Control, (b) 10kGy and (c) 50kGy irradiated asparagine cultures.

APPENDIX 7

MUTATION IN THE HAPLOID CELLS

TABLE 4.5 MUTATION FREQUENCY

Day of cultivation	Mutation frequency ($X 10^6$ survivors)		
	(a)	(b)	(c)
1.	0.36 ±0.65	0.63 ±0.01	1.44 ±0.00
2	0.39 ±0.06	0.89 ±0.06	0.63 ±0.00
3	0.41 ±0.04	0.75 ±0.06	0.30 ±0.00
4	0.43 ±0.01	0.39 ±0.00	0.60 ±0.02
5	0.54 ±0.02	0.37 ±0.00	0.31 ±0.02
6	0.69 ±0.00	0.40 ±0.00	0.27 ±0.00
7	0.74 ±0.06	0.43 ±0.00	0.26 ±0.01
8	0.39 ±0.06	0.43 ±0.00	0.23 ±0.00
9	0.30 ±0.00	0.43 ±0.00	0.28 ±0.00
10	0.20 ±0.00	0.49 ±0.00	0.29 ±0.00
11	0.31 ±0.00	0.41 ±0.00	0.34 ±0.00

ANOVA

SOURCE	df	SS	MS	F_o	$F_{t,5\%}$
Tr	2	0.044	0.022	0.31	3.39
E	25	1.759	0.070		
T	27	1.803			

RESULT: ns, cv = 8.02%

(a) control, (b) 10kGy and (c) 50kGy irradiated cultures.

APPENDIX 8

RECOMBINATION IN THE DIPLOID CELLS

TABLE 4.6 RECOMBINATION FREQUENCY

Day of cultivation	Recombination frequency (10^4 survivors) (a)	(b)	(c)
1	14.08 ±0.05	8.49 ±0.25	7.07 ±0.25
2	8,93 ±0.01	9.65 ±0.25	9.62 ±0.10
3	12.98 ±0.00	11.01 ±0.25	9.45 ±0.20
4	15.26 ±0.25	11.41 ±0.30	9.28 ±0.30
5	8.63 ±0.03	7.05 ±0.20	3.43 ±0.30
6	8.12 ±0.01	3.49 ±0.15	10.76 ±0.10
7	7.89 ±0.05	4.77 ±0.05	8.06 ±0.05
8	7.69 ±0.00	5.14 ±0.00	12.27 ±0.06
9	7.22 ±0.02	6.45 ±0.30	9.43 ±0.15
10	9.92 ±0.01	6.12 ±0.06	9.21 ±0.06
11	11.03 ±0.01	9.33 ±0.00	8.03 ±0.25
12	10.64 ±0.05	9.60 ±0.06	8.59 ±0,16
13	14.08 ±0.10	20.49 ±0.06	7.89 ±0.25
14	10.37 ±0.15	-	9.21 ±0.25

ANOVA

SOURCE	df	SS	MS	F_o	$F_{t,5\%}$
Tr	2	22.86	11.43	1.20	4.13
E	35	332.15	9.49		
T	37	335.01			

RESULT: ns, cv = 2.31%.