70-12,658

PERPER, Timothy Pelle, 1939-
PHYSIOLOGICAL AND GENETIC STUDIES OF X-RAY RESISTANCE IN BUDDING YEAST CELLS.

The City University of New York, Ph.D., 1970
Biology-Genetics

University Microfilms, Inc., Ann Arbor, Michigan

© TIMOTHY PELLE PERPER 1970

ALL RIGHTS RESERVED

PHYSIOLOGICAL AND GENETIC STUDIES OF X-RAY RESISTANCE IN BUDDING YEAST CELLS

by

TIMOTHY PERPER

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1969

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

May 29/69
Date

Chairman of Examining Committee

May 29 1969
Date

Seymour Fogel
Executive Officer

R.K. Mortimer — Institution: University of California, Berkeley

Ben-Zion Dorfman — Institution: Albert Einstein College of Medicine

Donald D. Hurst — Institution: Brooklyn College

Seymour Fogel — Institution: Brooklyn College

Institution

The City University of New York

ACKNOWLEDGEMENTS

I would like especially to thank Dr. Carl A. Beam, my advisor for the past three years, for the continued encouragement, support and interest that he has provided throughout the course of this work. I would also like to express my appreciation to Dr. Seymour Fogel for his invaluable suggestions and criticisms. I am indebted to Dr. Donald Hurst and Dr. Norman Eaton for their interest in my research and for serving as members of my advisory committee. My thanks also to the faculty and staff of the Biology Department of Brooklyn College of the City University for considerable aid rendered to me during my tenure as a graduate student. My particular thanks go to Mrs. Jeanette Needleman of the Executive Office of the Doctoral Program in Biology.

TABLE OF CONTENTS

Acknowledgements	iii
List of Tables	vi
List of Figures	vii
Introduction	1
Materials and Methods	6
1. Strains	6
2. Media	6
3. Preparation of cells	7
4. Experimental and statistical procedures	10
5. Irradiation and incubation procedures	12
6. Non-lethal endpoints	13
Results	17
Discussion	29
1. The control of budding cell resistance	29
2. Budding cell resistance to inactivation and its relationship to induced repair	31
3. The mechanism of resistance to non-lethal damage	36
A. Mitotic recombination	37
B. Respiratory deficiency	38

TABLE OF CONTENTS (Continued)

C. Lag induction 40

D. NCT damage 41

Summary 43

Bibliography 45

Tables 53

Figures 70

LIST OF TABLES

1. Genotypes of strains of *Saccharomyces cerevisiae*.

2. Media

3. Response of singly isolated interdivisional and budding cells of strain Z2367.

4. Phenotype frequencies among auxotrophic survivors of pellet interdivisional and budding cells of Z2367.

5. Survival of haploid strain SC-7K irradiated on different media.

6. SC-7K interdivisional cell survival on YEPD, Gal and minimal medium.

7. Survival of haploid strain 15:4-4 on YEPD and on Gal.

8. Supernate interdivisional and budding cell response on YEPD and YEPGal in strain Z2367.

9. Galactose medium-induced repair in irradiated Z2367.

10. P7:3-1 interdivisional cell response on YEPD and YEPGal.

11. Interdivisional and budding cell response after overnight water storage in strain 312.

12. Response of singly isolated interdivisional and sensitive budding cells of strain Z2367.

13. Interdivisional and sensitive budding cell response on YEPD in strain Z2367.

14. Repair induced in Z2367 sensitive budding cells by pre- and post-irradiation incubation on SM medium.

LIST OF FIGURES

1. Percentages of budding cells in Z2367 supernate and pellet cell preparations incubated in liquid YEPD for various times.

2. Survival on YEPD of culture of haploid 15: 4-4 containing increasing percentages of budding cells.

3. Growth curves of slow and normal growing strains derived from Z2367.

4. Survival, lag, NCT, petite and auxotrophy induction in Z2367 pellet interdivisional and budding cells.

5. Survival, control type colony morphology and lag in Z2367 log phase culture.

6. Survival and lag induction in Z2367 log phase culture.

7. Survival of haploid SC-7K on YEPD.

8. Survival of haploid $\alpha\, hi_1 ad_2$ on YEPD.

9. Survival, sectoring and lag induction in P7:3-1 pellet cell population with different percentages of budding cells.

10. Survival, NCT and lag induction in water stored supernate interdivisional and budding cells of strain 312 irradiated and incubated on YEPD and on Gal medium.

INTRODUCTION

Budding cells of *Saccharomyces cerevisiae* are more resistant than interdivisional cells to inactivation by x-rays (12), alpha particles (22) and ultraviolet (UV) radiation (24). Budding cell resistance to x-ray inactivation cannot be accounted for by changes in the amount of DNA per nucleus, since budding cells of different ploidies are inactivated at the same rate (9), while interdivisional cells are inactivated in ploidy dependent fashion (46). Treatments which decrease pre-irradiation protein synthesis sensitize budding cells to x-rays (52), and Moustacchi (52) has proposed that budding cell resistance depends on proteins which either reduce energy transfer to DNA or which elevate post-irradiation repair. In x-ray dose fractionation experiments, budding cells receiving fractionated doses exhibit less lethal damage than those receiving the same total dose at once (7). These results suggest that post-radiation processes, absent or diminished in interdivisional cells, reduce lethal damage in budding cells. Since, however, the role of post-irradiation metabolism in budding cell resistance has not been investigated, the nature of these processes remains unknown.

The major physiological modifiers of cellular radiation response are protection and repair. Protective substances or conditions reduce radiation damage by competing with sensitive cellular targets for

active molecular species produced by primary ionizations. Protective agents include sulfhydryl-containing compounds, which scavenge free radicals (15,86), and anoxic irradiation, which reduces the formation of toxic radiochemical products (15, 36, 86).

Beam (9) found that cysteine protects interdivisional and budding cells equally, indicating that an endogenous -SH dependent process does not confer x-ray resistance to budding cells. Measurements of anoxic protection in budding cells have given conflicting results. Beam (9) has shown that anoxic x-irradiation produced equal protection in interdivisional and budding cells of haploid, diploid and tetraploid strains of S. cerevisiae. These results are expected if anoxic protection is independent of resistance conferred by budding. Alper (2), however, found that anoxic x-irradiation protected interdivisional cells somewhat more than budding cells. This difference indicates that intracellular anoxia-like protection could contribute to budding cell resistance in some, but not all, strains of yeast.

Post-irradiation treatments known to elicit repair in x-irradiated yeast include incubation on minimal medium (65), incubation in distilled water (44, 65), and UV-irradiation of x-irradiated cells (17, 23, 24, 25). Changes in susceptibility to these treatments during the cell cycle have been studied only in the case of UV-induced repair (17, 25). Survival of haploid interdivisional cells given 2537A UV immediately after x-irradiation is reduced by the UV-post-treatment. At low x-ray doses, budding cell survival is unaffected by the UV-post-

treatment. However, at x-ray doses causing exponential inactivation, budding cell survival is elevated considerably by the UV. Since UV-post-treatment lessens x-ray induced chromosome damage in Tradescantia (48, 81), the restorative effect in budding cells might result from repair of otherwise lethal chromosome damage (49). It is unknown, however, if budding cells repair more damage than inter-divisional cells in the absence of external stimulation.

Budding cells of respiratory deficient strains of S. cerevisiae are as x-ray resistant as those of respiratory competent strains (24, 45, 72). The energy producing reactions of the mitochondria are therefore not required for budding cell resistance to inactivation.

Budding cells of various yeast strains are resistant to a number of x-ray and UV induced non-lethal alterations. These include mitotic recombination (27), recessive lethality (10), post-irradiation division delay (16), lethal sectoring (11) and reversion to adenine independence (66). Budding cells are also resistant to UV induction of respiratory deficiency (24). Although resistance to inactivation and to mitotic re-combination have been shown to develop in parallel in synchronously dividing cultures, and to reach simultaneous maxima during budding (27), it is unknown if one mechanism confers resistance to all forms of damage in budding cells.

There are three general hypotheses which could explain how budding cells tolerate x-rays. These are 1) that they reorganize their

genetic material after irradiation, with the result that lethal damage is eliminated; 2) that protective processes mitigate lethal damage at the time of irradiation, and 3) that post-irradiation repair processes reduce damage to crucial targets.

These hypotheses have different consequences concerning budding cell susceptibility to non-lethal alteration. Whittinghill has pointed out (88) that somatic cells carrying lethal mutations can be rescued from death by mitotic recombination. Published results prove that budding cells are resistant to induced mitotic recombination (27), lethal sectoring (11) and lag (16). However, other non-lethal genetic alterations could confer resistance to inactivation in budding cells. Any alteration appearing only in budding cells could result from the reorganizational process of hypothesis 1. If, on the other hand, budding cells are generally protected, or if lethal and non-lethal damage share a common repair process (hypotheses 2 and 3), budding cells will be resistant to all non-lethal alterations and to inactivation.

The present investigation was undertaken to distinguish between these hypotheses, and to explore the role of post-irradiation metabolism in budding cell x-ray resistance. Furthermore, it seemed worthwhile to quantitate the induction of a number of alterations using the same yeast cells.

The results presented below indicate that budding confers resistance to both inactivation and to induction of all non-lethal alterations studied. Budding cells of both haploid and diploid strains

repair more potentially lethal damage than do interdivisional cells when grown after irradiation in new nutritional environments. Conditions abolishing budding cell resistance are described; sensitized budding cells undergo more repair than interdivisional cells when challenged appropriately. These data contradict genetic and protective models (hypotheses 1 and 2) and support repair models (hypothesis 3) for budding cell x-ray resistance in _S. cerevisiae_.

MATERIALS AND METHODS

This study requires a series of non-lethal endpoints, means for distinguishing between budding and interdivisional cell response, and methods for altering cellular metabolism after irradiation.

1. Strains

Genetically marked diploid strains of _Saccharomyces cerevisiae_ were used in all experiments measuring induction of non-lethal damage. Genotypes are shown in Table 1. Z2367, a pink, adenine and histidine requiring diploid, was obtained from Dr. D. Hurst and Dr. S. Fogel, who have described its properties extensively (29). Strain 312 is a survivor of 14 kr irradiation of Z2367. P7:3-1 is a diploid obtained by mating strain 3084-15c from Dr. D. Hurst and Dr. S. Fogel with strain $\alpha hi_1 ad_2$ from Dr. C. A. Beam. It is white, adenine independent and heterozygous for both ad_1 and ad_2.

Experiments involving shifts in post-irradiation nutritional conditions employed strains Z2367, 312 and P7:3-1 and the wild type haploid SC-7K from Dr. C. A. Beam. Several experiments used haploid 15:4-4, obtained by ascus dissection of Z2367.

2. Media

The compositions of all media are shown in Table 2. Routine growth and irradiation experiments were performed on a yeast extract -

peptone - dextrose (YEPD) medium solidified with 2% agar. It is somewhat weaker than that used in other laboratories (8, 28). Nutritional conditions during and after irradiation were altered either by 1) increasing the concentrations of yeast extract, peptone and sugar; 2) replacing glucose with galactose; or 3) using minimal medium with added adenine and histidine (SM) for experiments with Z2367 or without additions for SC-7K. Defined media follow Wickerham (89) as modified by Fogel and Hurst (unpublished), using Difco Yeast Nitrogen Base and added glucose, amino acids, adenine and uracil, solidified with 1.5% agar.

3. Preparation of cells

Cells were grown in either liquid or on solid YEPD, and for log phase experiments were harvested after 24 hours, in late log phase. Some cultures were incubated for longer periods in order to decrease the fraction of budding cells (12) and then harvested. Such aged cultures contain small interdivisional cells, large interdivisional cells and a variable but small fraction of budding cells.

Populations of interdivisional cells of uniform size were prepared by modifying the centrifugation procedure of J. Wildenberg (Ph. D. thesis) for isolating pre-existing sub-populations of cells from aged cultures. To prepare small interdivisional cells, cultures were aged in liquid YEPD for approximately two weeks, harvested

and washed in sterile distilled water. Cells were then resuspended in distilled water at a density of approximately 5 x 10^7 cells per cc, dispensed in 3 cc aliquots in 100 x 12 mm test tubes and centrifuged in an International swinging bucket centrifuge at about 600 rpm until the supernate was faintly turbid. Seen under the microscope, the supernates consisted mainly of small interdivisional cells and were pooled. If large or budding cells were present, the pooled supernates were resuspended and centrifuged longer. Final supernate preparations contain less than 0.5% budding and large interdivisional cells and are referred to as supernate interdivisional cells.

Uniform populations of large interdivisional cells were prepared from cultures which had been aged for longer periods (up to three weeks). Cells were harvested, washed and centrifuged as in the preparation of small interdivisional cells. However, after carefully decanting the supernate, 3 cc of sterile distilled water was added to the pellet with a 5 cc pipette. The mouth of the pipette was held against the inside of the tube at a slight angle, approximately one inch above the top of the pellet. The water was slowly added in such a way that it washed over the top of the pellet, swirling its uppermost layer of cells into suspension without disturbing the bulk of the pellet. The resulting cloudy supernate consisted mostly of small interdivisional cells and was discarded. Another 3 cc of water was added, the tube mixed thoroughly and centrifuged, and the pellet washed as before.

Repeated from 3 to 6 times, this procedure yields an interdivisional cell population which contains less than 0.5% of small cells and budding cells on microscopic examination. Such populations are referred to as pellet interdivisional cell preparations. Aged diploid cultures which had begun to sporulate were not used.

Budding cell populations were obtained by inoculating interdivisional cells into liquid YEPD or other medium and incubating on a roller drum at room temperature. The first bud appears very nearly synchronously (J. Wildenberg, Ph. D. thesis; Figure 1). Uniform supernate and pellet cell preparations were used either immediately or after 18 - 24 hours storage in 1 cc sterile distilled water at 6 - 8°C. Differences between populations used immediately and those stored in water will be described in the Results section.

The assumption that uniform interdivisional cell populations will become resistant when budded, upon which all experiments employing such populations depend, was tested by preparing supernate interdivisional cells of haploid 15:4-4, immediately inoculating into liquid YEPD, and determining x-ray resistance of samples taken at various times. The survival curves in Figure 2 show that the degree of resistance correlates with the microscopically observed fraction of budding cells.

For experiments requiring cells of known stage in the budding cycle, isolations were made on thin YEPD slabs using a micomanipulator.

The slabs were irradiated and transferred immediately to YEPD plates for incubation.

Budding cells in these studies were defined as those with buds less than one-half the size of the mother cell. Such budding cells have been reported to show maximal resistance (16). Interdivisional cells were defined as those lacking any signs of budding when examined using a 40x objective and 15x ocular.

4. Experimental and statistical procedures

In the basic irradiation protocol, from 5 to 15 plates were each inoculated with 0.1 ml of the appropriate cell suspension, spread and irradiated after drying. Control viability was determined using from 5 to 8 plates. Survival was calculated after correcting for any dilutions made between control and irradiated plates.

To compare interdivisional and budding cell response using synchronously dividing cultures, this procedure was used twice, once for interdivisional cells and later for budding cells. The results are given as percent response (lethal and non-lethal) before and after budding. Statistical significance of any differences was determined using the Z-test based on the standard errors of the determinations (20).

Two modifications were made in the basic protocol in order to measure effects of growth medium on irradiated interdivisional and budding cells. Firstly, all plates were inoculated from the same

cell suspension, using the same 0.1 ml pipette. Plates containing different media were then irradiated in known sequence. Statistical significance of any differences in survival was determined using the unpaired measure t-test (20). The entire procedure was repeated when the original suspension had budded.

Secondly, the number of plates containing a given medium which received interdivisional cells equalled the number inoculated with budding cells. The data from irradiated plates are then subjected to an analysis of variance (20). The principle is to partition the total variance into component variances representing the effects of medium, cell cycle and dilution, interaction and random errors on survival. Each component variance can then be compared individually with the error variance using an F test (null hypothesis: no difference). Medium effects on survival were considered significant when the medium and error variances were different at the 5% level or better.

In some experiments, there were significant deviations from equal viability on different media even without irradiation. Since the experimental design could not distinguish such stage -- media -- survival interactions from genuine effects of stage or media on survival in the analysis of variance, the following statistical procedure was adopted. If interactions between stage, media and survival are absent, the distribution of survivors on the two media before budding should be identical to that after budding. On the other hand, if

interactions are present, the distributions before and after budding will be different. Using the observed distribution of interdivisional cell survivors to calculate expected values a χ^2 with one degree of freedom was computed comparing the distribution of interdivisional and budding cell survivors after irradiation on the two media. The null hypothesis of no interaction was rejected if these distributions were different at the 5% confidence level.

5. Irradiation and incubation procedures

The x-ray source was a Picker x-ray machine with a Machlett OEG - 60 tube, with beryllium window, operated at 50 kv and 40 ma. Plates were irradiated 10 cm from the target, at a dose rate of 55 kr per minute, as determined using an ionization extrapolation counter. After irradiation, plates were incubated either at 30°C or at room temperature (approximately 27°C).

The number of visible colonies was recorded consecutively for at least five days after irradiation. After 24 hours incubation, pin-point colonies can be seen using an illuminated plate counter. Since such colonies can be confused with surface imperfections in the agar, counts made on the first day were corrected after 48 and 72 hours incubation. On the fifth day or later, colonies were classified with respect to colony morphology, as described below.

In some experiments, plates were replica plated for further analysis to suitable media. In these experiments, the number of

colonies never exceeded approximately 100 per plate. Phenotypes were determined on the first and fourth days after replica plating.

6. Non-lethal endpoints

Examples of heritable nuclear and cytoplasmic alterations are provided by mitotic recombination and respiratory deficiency, respectively. Survivors of x-irradiated diploids heterozygous for nutritional markers often require metabolites for which the parental strain is prototrophic. Such x-ray induced auxotrophy results from mitotic recombination (27, 50, 58). Preliminary ascus dissections have shown that both reciprocal and non-reciprocal recombination occur in x-irradiated Z2367.

Yeast cells unable to use acetate lack functional mitochondria (60). This respiratory deficiency, or petite, phenotype results from changes of either cytoplasmic determinants or of recessive nuclear genes (76). Since diploid petites do not sporulate, direct analysis of their genetic nature is not possible. However, petites obtained from related haploids can be analyzed. Ten petites resulting from 14 kr irradiation of a haploid segregant of Z2367 were mated to a respiratory competent strain, diploids obtained by prototrophy isolation (67), sporulated on KAc medium (28), and asci were dissected (41). All spore products were respiratory competent, indicating that the petite phenotype was cytoplasmically determined.

Petites induced in diploids are likely to be cytoplasmic, since their production by alteration of nuclear genes would require two events. Analogous double events conferring nutritional auxotrophy have not been observed. X-ray induced respiratory deficiency in diploids can therefore reasonable be assumed to result from cytoplasmic alteration.

In this study, petites were detected by their failure to grow after replica plating to acetate-containing medium (60). Since all replica plated colonies grew somewhat unevenly on acetate medium, sectoring for respiratory deficiency could not be detected reliably. Colonies apparently sectored for respiratory deficiency were therefore classified as respiratory competent.

X-ray induced lag was measured as delay in appearance of visible colonies on irradiated plates. In x-irradiated yeast, three known processes can delay colony formation: 1) transient division delay (16) which affects only the early divisions; 2) growth rate mutations, with slowed exponential growth rate and/or lengthened lag phase (38; Figure 3); and 3) lethal sectoring (40). The endpoint used in this study probably involves all of these processes.

Cells from diploid strains Z2367 and 312 typically form uniform colonies, but cells surviving radiation often produce morphologically altered colonies. Such altered colonies are referred to collectively as 'non-control type' or NCT colonies. They include color

variants, sectors which overgrow the remaining portion of the colony or are overgrown by it, multiple sectors, extremely small and irregular colonies and 'festonné' colonies (26, 55).

Approximately 300 NCT colonies have been picked, suspended in water and re-plated during the course of this work. Well-separated subclones from the same NCT colony almost invariably show differences in color, diameter and growth rate, unlike those from control type colonies.

Up to one-half of all NCT colonies from Z2367 are either totally white or red/white sectored. Overgrown white sectors contain petite cells, the fraction depending on the size of the sector. Small white colonies are petite. Red/white sectoring in respiratory competent colonies can be explained by the failure of ad_1 to produce pink pigment (74, 75). Preliminary ascus dissections suggest that such red/white sectoring is associated with recessive homozygosity at ad_6. Red/red and small colonies form the remainder of the NCT category. As has been reported for <u>Schizosaccharomyces pombe</u>, the fraction of small colonies increases with dose (59). Growth curves of two such colonies (Figure 3) display characteristic retardation of growth. Extensive spore death prevented the genetic analysis of these colonies. Red/red sectoring and the festonné phenotype were not further examined.

Strain P7:3-1 is heterozygous for both ad_1 and ad_2. Loss of the wild type allele of either gene would produce a totally red or red/white colony.

Colony classifications were done in reflected light, using either the magnifier of a plate counter or a binocular microscope. Unirradiated plates were at hand for reference. Since colonies on unirradiated plates variegate with age, all colony morphology studies were complete within one week of irradiation.

RESULTS

Results of preliminary experiments with log phase cultures suggested that budding cells were resistant to both lethal and non-lethal damage. To demonstrate this point unequivocally, budding and interdivisional cells were isolated from a log phase culture of Z2367 using a micromanipulator and irradiated. Table 3 shows that known budding cells are simultaneously resistant to inactivation, lag and NCT induction, unlike interdivisional cells.

A similar association between resistance to lethal and non-lethal damage was also demonstrated using uniform populations of cells. Dose response curves were obtained for a number of endpoints before and after budding a Z2357 pellet cell population. The results (Figure 4) show that budding confers resistance to inactivation (curve 1), lag induction (curve 2) and NCT induction (curve 3). The dose modifying factor* for inactivation produced by budding is approximately two. Furthermore, the fraction of petites among survivors of the budded preparation equals that of the unirradiated controls, whereas interdivisional cells are sensitive to petite induction (curve 4). Interdivisional cells are also quite sensitive to induction of auxotrophy (curve 5), the fraction of auxotrophic survivors rising abruptly to approximately 10%. However, the fraction of auxotrophic survivors

---

*The ratio of doses required to produce equal inactivation in interdivisional and budding cell preparations.

of the budded preparation never exceeds about 2%, a number which can be accounted for by the 40% residual interdivisional cells in the population.

Table 4 shows the genetic markers involved in this experiment. Auxotrophy increases with gene-centromere map distance, at each dose and in each population. Double requirements for arginine and tryptophan occur at significantly higher frequencies than double requirements for uracil and threonine. These results suggest that auxotrophic colonies arose from radiation-induced recombination, and therefore indicate that budding cells are more resistant to recombination than interdivisional cells.

NCT and lag induction curves obtained from log phase cultures of strain Z2367 show that budding confers resistance to non-lethal damage even at very high doses (Figures 5 and 6). The fraction of control type colonies among survivors (Figure 5B) declines initially, but a plateau appears at 56 kr. Approximately 20% of the survivors are resistant to NCT damage over range of doses causing extensive inactivation.

Since pellet and log phase budding cells are resistant to inactivation, and to induction of mitotic recombination, petiteness, lag and NCT damage,,none of these alterations can be a by-product of a budding cell specific process which confers resistance to inactivation. Furthermore, budding cell resistance to NCT induction makes it unlikely that relevant alterations have escaped detection.

Since budding cells are resistant to both nuclear and cytoplasmic endpoints, hypothesis 1 seems to be incorrect, and a physiological basis for budding cell resistance seems likely.

Shifts to minimal medium are reported to elicit repair in irradiated yeast (44, 65). If repair or radioprotection in budding cells depend on cellular metabolism, medium shifts might affect budding cells more than interdivisional cells. Budding and interdivisional cell response were therefore measured after irradiation on a variety of media.

A culture of haploid SC-7K containing 4% budding cells was given 168 kr on seven different media. A set of seven plates containing one plate with each type of medium was inoculated with the same 0.1 ml pipette and irradiated in known sequence. This procedure was repeated eleven times. There were five sets of seven in the control. The design permits an analysis of variance in which the total variance is partitioned into one component for pipettes, one for media and one for random error. The results are shown in Table 5.

Survival was elevated by replacing glucose with galactose in both YEPD and in Glu medium, and by irradiation on both minimal medium and on minimal medium with added adenine and histidine. Raising the concentration of yeast extract and peptone also elevated survival. This effect is apparently independent of the sugar, since survival on Glu is higher than on YEPD, and survival on Gal is higher than on YEPGal. Survival on NGlu equalled that on YEPD. Thus,

survival was raised by shifts to minimal media, to media inducing enzyme syntheses (21) and to media which contain more yeast extract and peptone. Shifts to Gal and to minimal media gave the greatest increases in survival. Since interdivisional cell survival is negligible at 168 kr (Figure 7), these results indicate that budding cells sustain potentially lethal, but reparable, damage.

To determine if haploid interdivisional cells also repair lethal damage on altered media, a supernate interdivisional cell population of strain SC-7K was given 14 kr on YEPD, Gal and on minimal medium. There was no effect of medium on survival (Table 6). Thus, the capacity to repair lethal damage on altered medium is restricted to budding cells in SC-7K.

The survival curves of haploid strains 15:4-4 and $\alpha\, hi_1ad_2$ (Figures 2 and 8, respectively) almost completely lack the budding cell shoulder. The resistance to budding cells of these strains lie between that of SC-7K budding cells and that of interdivisional cells. Nonetheless, budding cells of these strains reverse lethal damage on altered media. A population of haploid 15:4-4 containing 63% budding cells was given 28 kr on YEPD and on Gal. Survival was significantly elevated on Gal (Table 7). Preliminary experiments have shown that budding cells of strain $\alpha\, hi_1ad_2$ also repair lethal damage on Gal, and indicate that the dose modifying effect of Gal is the same as in SC-7K. The ability to repair lethal damage on altered media is independent of the presence of the mechanisms which produce the budding

cell shoulder.

Diploid strains are required in order to measure medium effects on both lethal and non-lethal damage simultaneously. Z2367 supernate interdivisional cells were prepared and immediately budded in liquid YEPD. The interdivisional and budding cell populations were given 56 kr on YEPD and on YEPGal (Table 8). Interdivisional cell survival was elevated significantly by YEPGal (30% - 41%). Budding cells were resistant to inactivation on both media, but their survival was elevated significantly further on YEPGal (44% - 54%). It can be seen that budding conferred somewhat more resistance to interdivisional cells (30% - 44%) than did YEPGal (30% - 41%). The data also indicate that YEPGal is only slightly more effective in elevating survival in budding cells than in interdivisional cells.

The fraction of NCT colonies among survivors in the interdivisional cell population was approximately 80% on both YEPD and YEPGal (Table 8). Budding reduced the fraction of NCT survivors to approximately 65% on both media. YEPGal produced a small further decrease in NCT induction in the budding cell population. Lag induction was decreased by budding, but was increased by YEPGal in both interdivisional and budding cells. Thus, in Z2367, medium effects are largely restricted to survival, but occur in both interdivisional and budding cells.

This experiment does not show whether altered medium elicits repair during the post-irradiation incubation period, or prevents damage

during irradiation. The results of the following experiment distinguish between these possibilities. The effect of Gal medium on non-lethal damage was also determined.

An aliquot of a log phase culture of Z2367 was spread heavily and evenly on a YEPD plate and irradiated with 168 kr, a dose that inactivates more than 99% interdivisional cells (compare Figures 5 and 6). The irradiated cells were then washed off the plate, and one portion immediately plated onto YEPD and onto Gal medium. Another portion was incubated in liquid Gal medium for two hours at 27°C and then plated onto YEPD and onto Gal. This procedure does not permit the calculation of survival ratios, but the relative number of colonies on YEPD and on Gal (Table 9) permits three conclusions. Firstly, Gal is effective post-radiatively in inducing an approximate doubling of survival, demonstrating that it evokes repair of lethal damage, rather than prevents it. Secondly, after two hours in liquid Gal, the cells showed higher survival when plated onto Gal than when plated onto YEPD, showing that the repair process elicited by Gal requires more than two hours for its completion. Thirdly, the frequency of NCT colonies among survivors was reduced approximately 5% on Gal, while lag induction was increased. The frequency of small white survivors (petites, in all likelihood) was unchanged by Gal. A similar experiment showed that Gal also elicits repair in SC-7K.

These experiments with Z2367 show that the major effect of a medium shift is repair of lethal damage. On the other hand, budding confers resistance to both lethal and non-lethal damage. This difference was also observed in the following experiments using diploid P7:3-1, and indicates that medium stimulated repair and budding cell resistance are not identical. Increasing the budding cell fraction from 14% to 58% in a pellet cell population of P7:3-1 elevated resistance to inactivation, lag and sectoring (Figure 9). The dose modifying factor for inactivation (curve 1) is approximately 1.7. Before budding, the lag dose response curve (curve 3) had a plateau at approximately 25%, but after budding at approximately 50%, indicating an increase in the population moiety resistant to lag. Likewise, the fraction of totally red and red/white sectored colonies (curve 2) was decreased by budding, showing that budding also confers resistance to sectoring.

However, YEPGal does not evoke repair of non-lethal damage in P7:3-1 pellet interdivisional cells (Table 10). Although survival after 56 kr was elevated by YEPGal (44% vs. 28%), neither the fraction of sectored colonies nor the fraction of auxotrophic survivors was different on the two media. Furthermore, lag induction was increased on YEPGal.

Thus, in interdivisional cells of strains Z2367 and P7:3-1, the medium stimulated process distinguishes between lethal and

non-lethal damage. In budding cells, however, medium stimulated repair of non-lethal damage might be completely masked by native resistance. Moreover, endogenous budding cell resistance might obscure quantitative differences between repair of lethal damage induced in interdivisional and budding cells. Further examination of repair evoked during budding was facilitated by discovering pre-irradiation conditions which abolish budding cell resistance.

The following experiment indicated that when budded after overnight water storage, supernate interdivisional cells produced anomalously sensitive budding cells. Supernate and pellet interdivisional cells of strain 312 were prepared from a two-week old, solid YEPD grown culture and stored overnight (18 hours) in the refrigerator in distilled water. The following day, portions of each population were budded in liquid YEPD, and interdivisional and budding cell preparations irradiated on YEPD. Supernate and pellet interdivisional cell response is indistinguishable (Table 11). When the pellet cell population was budded, the fraction of NCT survivors decreased from 63% to 29%, and the fraction of survivors visible on the first day increased from 14% to 50%. In the supernate cell population, however, budding did not confer resistance to either lethal nor non-lethal damage.

To determine if the formation of sensitive budding cells was unique to strain 312, supernate interdivisional cells of strain Z2367 were prepared from a 6-day old, solid YEPD grown culture, and stored

overnight (24 hours) in distilled water. The preparation was budded in liquid YEPD the following day, and single interdivisional and budding cells isolated with a micromanipulator and irradiated. Interdivisional and budding cell survival and NCT induction were identical (Table 12). In another experiment (Table 13), budding cells prepared from water stored supernate cells of liquid grown Z2367 failed to develop normal resistance to inactivation, NCT, lag or auxotrophy induction. These findings show that Z2367 can form sensitive budding cells, which lack the mechanisms conferring normal resistance to lethal and non-lethal damage.

The effects of pre- and post-irradiation incubation in a nutritionally demanding environment were examined in the following experment to determine if repair could be stimulated in sensitive budding cells. Supernate interdivisional cells were prepared from a three-week old, solid YEPD grown culture of Z2367 and stored overnight (18 hours) in water. The following day, portions were budded in liquid YEPD as well as in liquid minimal medium with added adenine and histidine (SM medium). The budded and unbudded preparations were then given 56 kr on YEPD and on SM.

Resistance did not develop in either the YEPD- or the SM-budded populations (Table 14). However, SM-induced repair occurred in the interdivisional cell and YEPD-budded populations. The response of these populations appears not to be the same. The ratio of

survival on SM to YEPD in the budded preparation is 37%:27% and is significantly greater than that in the interdivisional cell population (33%:27%). Thus, the sensitive budding cells responded more to SM-induced repair than did the interdivisional cells.

Budding in SM appears to reduce the response to irradiation on SM. The X^2 comparison between the distributions of interdivisional and SM-budded cells on YEPD and on SM is not significant at the 5% level, indicating that although SM elicits repair, it elicits only as much as in interdivisional cells.

After approximately 1.5 cell divisions, the culture budded in YEPD was sampled again. This culture contained cells in their second budding, which were resistant to inactivation (Table 14), and to a lesser but significant extent, to NCT induction. Water-induced sensitization is therefore transient. Survival of budding cells in the second sample was not enhanced on SM.

Repair induced by Gal was measured in the next experiment. A population of supernate interdivisional cells of strain 312 was obtained from a ten-day old, solid YEPD grown culture, stored overnight in water, and the following day budded in liquid YEPD. Budded and unbudded populations were irradiated and incubated on YEPD and on Gal. The resulting dose response curves are shown in Figure 10, panels 1 - 4.

Before budding, the survival curve on YEPD was uninflected, and NCT and lag induction curves each showed only small shoulders

(panel 1, A, B, C). After budding, survival on YEPD increased slightly (panel 2, Al; (dose modifying factor = 1.2, as opposed to 2 for normal budding). The NCT and lag dose response curves after budding (panel 2, B, C) had small initial shoulders, but lacked the plateaus characteristic of budding cell response in log phase cultures (Figures 5 and 6). As in previous experiments, supernate cells budded after overnight water storage display very little resistance to either lethal or non-lethal damage.

Gal medium elevated the survival of both the interdivisional and budding cell populations (panels 3, A; 4, A), but the elevation of budding cell survival was greater. The dose modifying factor between Gal and YEPD in the budded preparation is 1.3, as opposed to 1.1 in the interdivisional cell population. Gal medium decreased the loss of control type colony morphology slightly in both interdivisional cells (panel 3, B) and sensitive budding cells (panel 4, B) relative to YEPD (panel 1, B; 2, B). Nonetheless, Gal did not produce the plateau characteristic of normal budding cell resistance to NCT induction (Figure 5B). No plateau appeared in the lag induction curve of sensitive budding cells on Gal (panel 4, C). Interdivisional and budding cells were each slightly more sensitive to lag induction on Gal than on YEPD (panels 1-4, C). Induced lag was greater in budding cells (panel 4, C).

These results show that in the absence of endogenous resistance, budding cell response to altered media is greater than that of interdivisional cells. The stimulated process, however, does not elevate budding cell survival to its normal level, and is almost completely restricted to lethal damage.

DISCUSSION

These results permit several conclusions concerning resistance to inactivation and non-lethal damage, and the relationship between them.

1. The control of budding cell resistance.

Resistance to both lethal and non-lethal damage develops simultaneously with budding, and is abolished simultaneously by storage of the parental interdivisional cell population in water. Similarly, the thymine analogue 5-fluorouracil completely sensitizes haploid budding cells to x-ray inactivation (52), and in diploids, 5-fluorodeoxyuridine prevents budding cells from developing their normal resistance to induced mitotic recombination (27). These results suggest that a single process, perhaps involving nucleic acid metabolism, controls the onset of resistance. This control process might be elucidated by studying mutants (13, 57, 71, 80) defective in x-ray resistance.

Other evidence also suggests that a single process controls budding cell resistance. Tauro and Halvorson (32, 83), studying the timing of α-glucosidase synthesis during synchronous growth of population of *S. cerevisiae*, found that the number of periods of peak enzyme synthesis equalled the number of functional genes for the enzyme. They proposed the generalization that if several genes control a phenotype, they express themselves at different times

during the cell cycle. Since x-ray resistance maximizes only once during the cell cycle, a single gene or set of phased genes is likely to be responsible for it. The recessive mutant gene xs-1 is known to abolish completely both budding and interdivisional cell resistance to inactivation (56,73), but studies of the sensitivity of $xs\text{-}1^-$ homozygotes to non-lethal damage have not been published. If, however, xs-1 controls resistance, $xs\text{-}1^-$ homozygotes would be expected to be sensitive to all forms of damage.

Budding cell resistance to inactivation seems nonetheless to depend on more than one process. The resistance of budding cells of haploids 15:4-4 and $\alpha\ hi_1ad_2$ is intermediate between that of SC-7K budding cells and interdivisional cells. Partial sensitization has also been obtained by treating haploid budding cells with either 5-methyltryptophan or para-fluorophenylalanine (52). Partly sensitive strains appear to lack some processes which produce the budding cell shoulder, but to retain other components of resistance (13).

Genetic analyses have shown that at least 23 genes (19) and several physiological processes (62, 73) are concerned with UV and x-ray resistance in interdivisional yeast cells. Furthermore, there are different types of x-ray lesion and repair process in interdivisional cells of diploid strain SC-6. Bachetti and co-workers found (6, 7) that more repair occurs in interdivisional cells given a fractionated x-ray dose and then stored in phosphate buffer than in cells

given the same total dose without interruption and stored in buffer. These results indicate that dose-fractionation elicits repair of lesions unaffected by liquid holding. Conversely, Patrick and Haynes (64) found that x- or UV-irradiated interdivisional cells of SC-6 stored in water did not show increased resistance to a second dose given 3-4 days later, despite significant elevation of survival induced by liquid holding. Thus, liquid holding does not evoke repair of lesions sensitizing the cells to a second irradiation. Together, these results imply that the lesions and repair processes involved in dose-fractionation and liquid holding are different. Hence it is reasonable that several processes could contribute to budding cell resistance.

2. Budding cell resistance to inactivation and its relationship to induced repair.

Results in this study suggest that budding cell resistance to inactivation depends at least in part on repair of potentially lethal damage. If budding cells possess elevated repair capacities, a balance would exist between induction and removal of damage which, at lower doses, would favor removal. Ultimately, however, more damage would be produced than could be removed. If the budding cell repair process can remove only a finite number of lesions, high doses of radiation will increasingly cause inactivation and will also increase the relative amount of reparable damage in the cell. The presence of reparable lesions in budding cells is proven by the elevation of their survival on altered medium. To account for the

sensitivity of interdivisional cells, the repair system must have a limited lifetime.

There is, however, another possible basis for the difference between interdivisional and budding cell resistance. If the proportion of lesions subject to endogenous repair maximizes during budding, budding cell survival would be greater even if repair capacities were constant during the cell cycle.

The results of repair experiments bear on these possibilities. X-irradiated haploid interdivisional cells are reported not to undergo medium stimulated repair (63, 65) (present findings confirm this observation), liquid holding repair (63, 65), UV-induced (25) and dye-sensitized photoreversal of lethal x-ray damage (31). It cannot be concluded, however, that x-ray damage in the haploid interdivisional cell is non-reparable. UV-irradiated haploid interdivisional cells undergo both medium stimulated (77) and liquid holding repair (65), and photoreactivate lethal damage (78). Since para-fluorophenylalanine post-treatments reduce UV survival (77), metabolic processes are implicated in UV resistance in haploid interdivisional cells. However, x-rays and UV do not potentiate each other in inactivating haploid interdivisional cells (25, 85). Similar absence of x-ray - UV potentiation of 'site overlap' in E. coli B_{S-1} has been interpreted by Baptist, Haynes and Uretz (5) as resulting from the absence of repair processes in E. coli B_{S-1} (33). Lack of potentiation between

gamma ray and UV induced mutagenesis in *Aspergillus nidulans* has also been interpreted as resulting from the absence of appropriate repair processes (3). If this interpretation of the absence of potentiation is correct, haploid interdivisional cells lack endogenous x-ray repair processes, although the x-ray damage itself may be reparable.

On the other hand, x-irradiated haploid budding cells undergo both medium stimulated and UV-induced repair (25), and UV-irradiated haploid budding cells photoreactivate lethal damage (25). Furthermore, there is considerable potentiation between UV and x-ray inactivation, at least at higher x-ray doses (25). These results indicate that endogenous repair processes are present in haploid budding cells.

In diploids, however, x-ray - UV potentiation occurs in both budding and interdivisional cells (24, 25, 85), and both types of cells undergo medium stimulated repair. Moreover, some UV and x-ray sensitivity genes affect both budding and interdivisional cell resistance (19, 73). These results indicate that although x-ray resistance is greatest during budding, repair occurs throughout the cell cycle. Moreover, repair in interdivisional cells and in budding cells may have steps in common.

Results with sensitive budding cells relate to the question of whether or not such repair capacities maximize during budding or remain constant during the cell cycle. The simplest interpretation for budding cell resistance is that normal budding cells have elevated endogenous repair capacities and sustain more damage subject to

repair than interdivisional cells. Sensitive budding cells would repair more damage on altered medium than interdivisional cells providing sensitization diminished endogenous repair without affecting either the frequency of reparable damage or the ability to respond to the repair evoking challenge. Such enhanced repair was observed in budding cells in both haploids and diploids.

The observation that less repair occurred in sensitive budding cells pre-adapted to SM than in unadapted cells suggests another explanation for enhanced budding cell susceptibility to evoked repair. The response of pre-adapted budding cells approached that of interdivisional cells, while interdivisional cell response seemed unaffected by pre-incubation in SM. Budding cells may therefore have undergone a unique component of medium stimulated repair.

Korogodin's group (44) has recently shown unequivocally that repair elicited by minimal medium and by water storage are identical. These workers conclude that any process extending post-irradiation lag will elevate survival in x-irradiated yeast. Since pre-adaptation would reduce the time needed for budding cells to initiate growth on SM, it would also be expected to reduce repair. Gal medium produced greater elevation of both lag and survival in sensitive budding cells than in interdivisional cells. Lag induction therefore seems to play a larger role in repair induced in budding cells than in interdivisional cells. Since the amount of exogenously stimulated repair depends not only on the ratio of reparable to non-reparable damage, but also

on the ability of the cell to respond to the challenge, these considerations suggest that repair capacities fluctuate during the cell cycle.

Repair dependent on post-irradiation metabolic processes could also vary during the cell cycle. Growth on galactose-containing media requires galactose-metabolizing enzymes (21) and on minimal media requires both amino acid and nucleic acid precursor synthesizing systems. Such enzyme systems could provide substances used for repair. Growth on Glu medium - an enriched YEPD - does not require induced enzyme synthesis, but elevates survival. Metabolites used for repair are likely to be in greater exogenous supply on Glu than on YEPD. The activities of a number of enzymes - e.g., α-glucosidase (83) and proteolytic enzymes (82) - are known to fluctuate during the cell cycle and probably others do so as well (69, 79). Repair syntheses which depend on such fluctuating enzyme systems would themselves fluctuate. The difference between budding and interdivisional cell response to altered medium could therefore result not only from the relative infrequency of reparable lesions in interdivisional cells, but also from the inability of interdivisional cells to undergo at least one component of repair.

On theoretical grounds, Powers (68) suggested that a limited number of protective molecules could elevate the back-extrapolate number e' considerably. Despite unsuccessful attempts to demonstrate sulfhydryl or anoxia-like protection in budding cells (2, 9),

budding cells might possess hitherto unidentified protective agents which elevate e'. However, in partly sensitive haploid budding cells, e' is decreased relative to SC-7K, and lesions sustained at low doses are subject to repair. If, following Powers' suggestion, it is supposed that protection is diminished in these strains, two cycles must then maximize during budding: a rise and fall in protection, and an unrelated rise and fall in the capacity to incur lesions repaired on altered medium. If, on the other hand, repair processes are partly defective in these strains, reduction of repair would simultaneously lessen e' and leave reparable lesions. Given the ability of budding cells to respond to altered media, only one cycle need be postulated: that associated with budding cell repair of lethal damage.

3. The mechanism of resistance to non-lethal damage.

There are differences between the mechanisms reducing lethal and non-lethal damage in budding cells. Budding cells remain resistant to non-lethal damage even at doses causing exponential inactivation, implying that the mechanisms differ at least in degree. Since results in this and other studies (27, 77) show that lethal and non-lethal damage is repaired differentially on altered medium, the lesions themselves are not identical. It is therefore likely that they are acted on by different processes in the budding cell.

In principle, budding cell resistance to non-lethal damage could depend either on protective or on reparative processes. Although the failure of medium shifts to induce repair of non-lethal damage suggests that resistance does not depend on repair, the following comments indicate that available evidence does not clearly favor one mechanism over the other.

A. Mitotic recombination

There is considerable evidence for a recombinational basis for radiation-induced auxotrophy in heterozygous yeast and other eukaryotes (1, 37, 39, 43, 50, 58, 91). However, events between deposition of radiation energy and recombination are not well understood. It has been suggested that radiation-induced recombination in Drosophila results from induction of translocations between homologous chromosomes (47, 53, 61, 92, 94), but studies of translocations in yeast are cytologically difficult. Radiation might produce sites of point pairing between homologous chromosomes (70) followed by exchange between them. It has also been suggested that radiation acts nonspecifically to produce a cellular pre-condition requisite for synapsis and recombination (parameiosis (34)). In the absence of unequivocal evidence validating one of these hypotheses, budding cell resistance to recombination is difficult to interpret.

The observation that medium shifts do not reduce recombination (present findings; 27) implies that the lesions responsible for recombination are independent of those causing cell death, and that

recombination, once initiated, is irreversible by medium stimulated repair. In yeast, both meiotic and mitotic recombination appear to result from excision of portions of the DNA chain followed by re-synthesis using the DNA of the homologous chromosome as a template (29, 87). Re-synthesis along the same DNA molecule may occur more often in budding cells than re-synthesis along the homologous strand. In this case, budding cell resistance to recombination depends on a kind of repair synthesis which could be independent of repair of lethal damage (33, 62, 73).

On the other hand, the enzyme systems necessary for recombination may be absent or diminished in budding cells. It must then be postulated, however, that recombination-initiating lesions are less frequent in budding cells than in interdivisional cells, for their presence in the budded products of the first post-irradiation division would be expected to induce recombination. Recombination-initiating lesions on chromosomes might be removed by processes similar to those repairing chromosome damage in higher plants (93). Alternatively, parameiosis may occur less often in budding cells than in interdivisional cells. Since metabolic dysfunctions, rather than damage to specific targets are proposed to cause parameiosis (34), resistance to it would presumably depend on protection and not repair.

B. Respiratory deficiency

The mechanism of petite induction by x-rays is also unknown. Primary ionizations outside the mitochondria seem unlikely to cause

intra-mitochondrial damage, however, since the maximum diffusion distance of free radicals in yeast is about 30A (35), the approximate thickness of the mitochondrial membranes (4). The most likely intra-mitochondrial target for petite induction is therefore mitochondrial DNA (mDNA; 76, 84). X-ray lesions in mDNA might prevent its replication, and, unless removed, could lead to the loss of mDNA associated with petiteness (76). In this view, in situ destruction of all mDNA molecules produces petiteness, and budding cell resistance depends on a protective or reparative mechanism acting at spatially well isolated mitochondrial sites.

Accumulating evidence, however, suggests that this view may be too simple. Firstly, although mitochondrial resistance maximizes during budding, and therefore shortly after synthesis of nuclear DNA (27), mitochondrial DNA and protein are probably synthesized continuously during the cell cycle (76). The elaboration of the putative intra-mitochondrial repair or protective agent would therefore be under nuclear control. Secondly, abnormalities of inner membrane structure, cytochrome and mDNA content are induced by both glucose and by anaerobiosis without inducing petiteness (42). A mechanism therefore exists which perpetuates the mitochondria under conditions of respiratory repression, independently of the integrity of mitochondrial structure, enzyme or mDNA content. Wilkie and Negrotti (76) have recently reported that the perpetuating mechanism is under the control of a

nuclear gene. When mutated, it causes glucose and anaerobiosis to induce cytoplasmically inherited respiratory deficiency. This observation suggests that radiation events in nuclei of respiratory repressed cells could lead to cytoplasmic petiteness. Wilkie (90) found that respiratory-repressed cells were in fact more sensitive to UV-induced petiteness than non-repressed cells, and suggested that the target in respiratory repressed cells might be a single template mDNA molecule. Since the present study employed glucose-grown and presumably glucose-repressed cells, budding cell resistance to petite induction might reflect a nuclear location for such mDNA templates.

C. Lag induction

Classical theories of lag induction, based on results obtained with irradiated embryonic material (18), balanced nuclear damage against repair. Burns (16) proposed that in yeast, lag is proportional to the time needed to restore damage to the division apparatus. This model implies that there is either less damage to the division apparatus in budding cells or that its repair is faster than in interdivisional cells. In x-irradiated interdivisional and budding cells, it has been found (16, 56) that very little delay occurs in either budding or in DNA synthesis during the first post-irradiation division. What delay does occur is the same in both types of cell. However, considerable delay occurs in budding and in DNA synthesis during the second post-irradiation division, and is greater in interdivisional cells than in

budding cells. Since damage to DNA would be expected to express itself immediately, these results indicate that the target for lag induction is something other than DNA, presumably replaced during the second division.

Since -SH containing compounds are formed during budding (14), resistance to lag might result from -SH protection of the division apparatus. Free radicals have been implicated in lag induction by Franz and Cole (30). However, budding in sensitive cells is presumably biochemically normal. Hence, resistance to lag is not likely to depend on removal of free radicals by the -SH compounds necessary for budding. It is more plausible that budding cell resistance to lag involves rapid repair during the second post-irradiation division.

D. NCT damage

Does response curves for NCT induction and for recessive lethality in Hansenula anomala (10) show virtually no change in slope over several decades of inactivation. Dose response curves obtained in the presence of protective agents show constant dose modification and limited efficiency, unlike the NCT and recessive lethality curves. The observation that NCT induction was decreased on Gal medium suggests that budding cells might repair at least one component of NCT damage. Repair processes have been proposed by Nasim and Auerbach (59) to reduce the frequency of sectoring. However, the analysis of the NCT category was not carried far enough to determine

if the ratio of whole to sectored colonies varied during the cell cycle or on Gal.

If the various non-lethal endpoints result from diverging radiochemical, biochemical and biological processes, and have only the initial deposition of radiation energy in common, any single process able to decrease them all is likely to act on early stages. It would then be assumed that budding cell resistance to non-lethal damage is protective. If widely distributed, such a protective agent or condition might prevent damage to mitochondria. However, no positive evidence for such a protective agent yet exists. On the other hand, the hypothesis of repair of non-lethal damage has only indirect and, as yet, inconclusive support.

SUMMARY

1. This study was undertaken to explore physiological and genetic concomitants of budding cell resistance to x-rays in *Saccharomyces cerevisiae* and to distinguish between several possible bases for budding cell resistance to x-ray inactivation. These bases are 1) that budding cells reorganize their genetic material after irradiation, thereby eliminating lethal damage; 2) that they have elevated protective capacities, and 3) that they repair lethal damage.

2. Budding cell resistance to inactivation in genetically marked diploid strains was found to be associated with resistance to mitotic recombination, respiratory deficiency, lag and colony morphology alterations in log phase, single cell and synchronized culture experiments. Thse results fail to support the first hypothesis.

3. Budding cells of both haploid and diploid strains repair more lethal damage than do interdivisional cells when irradiated and incubated in new nutritional environments. The repair process evoked by medium shifts fails to reverse non-lethal damage, unlike resistance conferred by budding.

4. Overnight refrigeration of an aged, small-cell diploid interdivisional cell population in water prevents normal resistance from developing on budding. These cells are sensitive to both lethal and non-lethal damage. The treatment is without effect on interdivisional cell response. Sensitive budding cells repair lethal but

not non-lethal damage after medium shifts, and undergo more repair than interdivisional cells.

5. The possible roles of repair and protection are discussed. The demonstration of induced repair in sensitive and resistant budding cells, the absence of positive evidence for protection and kinetic evidence all favor the hypothesis of repair of lethal and some non-lethal damage in budding cells. The basis of budding cell resistance to most forms of non-lethal damage is not understood.

BIBLIOGRAPHY

1. Abbadessa, R. and A. B. Burdick 1963 The effect of x-irradiation on somatic crossing over in Drosophila melanogaster. Genetics, 48: 1345-1356.

2. Alper, T. 1959 Variability in the oxygen effect observed with microorganisms. I. Haploid yeast: single and budding cells. Internat. J. Rad. Biol., 1: 414-419.

2. Arlett, C. F. 1966 The interaction between ultra-violet and gamma irradiation in Aspergillus nidulans. Internat. J. Rad. Biol., 11:313-320.

4. Avers, C. J., C. R. Pfeffer and M. W. Rancourt 1965 Acriflavine induction of different kinds of "petite" mitochondrial populations in Saccharomyces cerevisiae. J. Bacteriol., 90: 481-494.

5. Baptist, J. E., R. H. Haynes and R. B. Uretz 1966 Mechanism of UV-x-ray interaction in E. coli B/r: Effect of 5-bromouracil incorporation. Rad. Res., 27: 544 (Abstract).

6. Bachetti, S., M. Cassandro, R. Elli, R. Falchetti and F. Mauro 1966 Relationship between recovery from sublethal damage by dose fractionation and restoration of viability after delayed plating in diploid Saccharomyces cerevisiae. Rad. Res., 29: 295-315.

7. Bachetti, S., M. Cassandro and F. Mauro 1967 Radiosensitivity in relation to the cell cycle and recovery from x-ray sublethal damage in diploid yeast. Exp. Cell Res., 46:292-300.

8. Bachetti, S. and F. Mauro 1965 Recovery from sublethal x-ray damage in surviving yeast cells. Rad. Res., 25:103-114.

9. Beam, C. A. 1955 The influence of ploidy and division stage on the anoxic protection of Saccharomyces cerevisiae against x-ray inactivation. Proc. Nat. Acad. Sci., 48:857-861.

10. Beam, C. A. 1959 The influence of metabolism on some radiation effects. Rad. Res., Suppl. 1: 372-390.

11. Beam, C. A. 1967 The mitotic segregation of x-ray damage as a repair mechanism in budding yeast cells. Genetics, 56:544 (Abstract).

12. Beam C. A., R. K. Mortimer, R. G. Wolfe and C. A. Tobias 1954 The relation of radioresistance to budding in Saccharomyces cerevisiae. Arch. Biochem. Biophys., 49: 110-122.

13. Benathen, A. Unpublished.

14. Beran, K. 1968 Budding of yeast cells, their scars and aging. Adv. Microbial Physiol., 2:143-171.

15. Boag, J. W. 1965 The action of ionizing radiation on dilute aqueous solutions. Phys. Med. Biol., 10:457-476.

16. Burns, V. W. 1956 X-ray induced division delay in individual yeast cells. Rad. Res., 4:394-412.

17. Calkins, J. and W. Todd 1968 Evidence for a triggered or activated radiation repair system in Saccharomyces cerevisiae. Internat. J. Rad. Biol., 14:487-491.

18. Carlson, J. G. 1954 Immediate effects on division, morphology and viability of the cell. In: A. Hollaender, Editor, Radiation Biology, Volume I: High Energy Radiation. McGraw-Hill Book Co. New York. pp. 763-824.

19. Cox, B. S. and J. M. Parry 1968 The isolation, genetics and survival characteristics of ultraviolet light - sensitive mutants in yeast. Mutation Res., 6:37-55.

20. Dixon, W. J. and F. J. Massey, Jr. 1957 Introduction to Statistical Analysis. Second Edition. McGraw-Hill Book Co. New York.

21. Douglas, H. C. and D. C. Hawthorne 1966 Regulation of genes controlling synthesis of galactose pathway enzymes in yeast. Genetics, 54:911-916.

22. Elkind, M. M. and C. A. Beam 1955 Variation in the biological effectiveness of x-rays and α-particles on haploid Saccharomyces cerevisiae. Rad. Res., 3:88-104.

23. Elkind, M. M. and H. Sutton 1958 Ultraviolet mitigation of x-ray lethality in dividing yeast cells. Science, 128:1082-1083.

24. Elkind, M. M. and H. Sutton 1959 The relationship between division and x-ray sensitivity, ultraviolet sensitivity, and photoreactivation in yeast. Rad. Res., 10:283-295.

25. Elkind, M. M. and H. Sutton 1959 Sites of action of lethal irradiation: overlap in sites for x-ray, ultraviolet, photo-reactivation and ultraviolet protection and reactivation in dividing yeast cells. Rad. Res., 10:296-312.

26. Ephrussi, B., H. Jacob and S. Grandchamp 1966 Etudes sur la suppressivité des mutants a deficience respiratoire de la levure. II. Etapes de la mutation grande en petite provoquée par le facteur suppressif. Genetics, 54:1-29.

27. Esposito, R. 1966 Genetic recombination in synchronized cultures of Saccharomyces. Genetics, 59:191-210.

28. Fogel, S. and D. Hurst 1963 Coincidence relations between gene conversion and mitotic recombination in Saccharomyces. Genetics, 48:321-328.

29. Fogel, S. and D. Hurst 1967 Meiotic gene conversion in yeast tetrads and the theory of recombination. Genetics, 57:455-481.

30. Franz, J. and B. T. Cole 1962 Effects of ultraviolet-irradiated methyl linolenate on cell division and respiration in Saccharomyces cerevisiae. Arch. Biochem. Biophys., 96:382-385.

31. Freifelder, D. and R. B. Uretz 1960 Dye-sensitized photo-reactivation of x-ray damage in diploid yeast. Nature, 187:953-954.

32. Gorman, J., P. Tauro, M. LaBerge and H. Halvorson 1964 Timing of enzyme synthesis during synchronous division in yeast. Biochem. Biophys. Res. Comm., 15:43-49.

33. Howard-Flanders, P. 1968 DNA repair. Ann. Rev. Biochem., 37:175-200.

34. Hurst, D. and S. Fogel 1964 Mitotic recombination and heteroallelic repair in Saccharomyces cerevisiae. Genetics, 50:435-458.

35. Hutchinson, F. 1957 The distance that a radical formed by ionizing radiation can diffuse in a yeast cell. Rad. Res., 7:473-483.

36. Hutchinson, F. 1961 Molecular basis for actions of ionizing radiations. Science, 134:533-538.

37. James, A. P. and B. Lee-Whiting 1955 Radiation-induced genetic segregations in vegetative cells of yeast. Genetics, 40:826-831.

38. James, A. P., M. M. MacNutt and P. M. Morse 1965 The influence of dose on the spectrum of radiation-induced mutants affecting a quantitative character in yeast. Genetics, 52:21-29.

39. James. A. P. and M. M. Werner 1965 The radiobiology of yeast. Rad. Bot., 5:359-382.

40. James. A. P. and M. M. Werner 1966 Radiation-induced lethal sectoring in yeast. Rad. Res., 29:523-536.

41. Johnston, J. R. and R. K. Mortimer 1958 Use of snail digestive juice in isolation of yeast spore tetrads. J. Bacteriol., 78:292.

42. Jayaramayan, J., C. Cotman, H. R. Mahler and C. W. Sharp 1966 Biochemical correlates of respiratory deficiency VII. Glucose repression. Arch. Biochem. Biophys., 116:224-251.

43. Kafer, E. 1963 Radiation effects and mitotic recombination in diploids of Aspergillus nidulans. Genetics, 48:27-45.

44. Korogodin, V. I., Yu. G. Kapultcevich, M. N. Myasnik, A. M. Mosin and V. V. Gridnev 1968 Cellular repair processes: survival of irradiated yeast, bacteria and phages under different post-irradiation conditions. Adv. Biol. Med. Phys., 12:253-274.

45. Laser, H. 1964 Production by x-rays of petite colonies in yeast and their radiosensitivity. Nature, 203:314-315.

46. Laskowski, W. 1960 Inaktiverungsversuche mit homozygoten Hefestammen verscheidenen Ploidiegrades I. Aufbau homozygoter Stamme und Dosiseffektkurven fur ionisierende Strahlen, UV und organische Peroxyde. Z. Naturf., 15b:495-506.

47. Lucchesi, J. C. 1965 The nature of induced exchange between attached-X and Y chromosomes in Drosophila melanogaster females. Genetics, 51:209-216.

48. Merz, T., C. P. Swanson and C. N. Hemalatha 1961 Radiosensitivity and the problem of chromosome breakage and rejoining. Brookhaven Symp. Biol., 14:53-61.

49. Mortimer, R. K. 1961 Factors controlling radiosensitivity of yeast cells. Brookhaven Symp. Biol., 14:62-75.

50. Mortimer, R. K. and D. C. Hawthorne 1966 Yeast genetics. Ann. Rev. Microbiol., 20:151-168.

51. Mortimer, R. K. and D. C. Hawthorne 1966 Genetic mapping in Saccharomyces. Genetics, 53:165-173.

52. Moustacchi, E. 1963 Mécanisme de la radiorésistance des levures en bourgeonnement. Influence de certain facteurs métaboliques. Ann. Inst. Pasteur, 105:757-773.

53. Muller, H. J. 1954 The nature of the genetic effects produced by radiation. In: A. Hollaender, Editor, Radiation Biology, Volume I: High Energy Radiation. McGraw-Hill Book Co. New York. pp. 351-473.

54. Nagai, S. 1963 Methylene blue and toluidine blue interfering with the production of respiration-deficient mutants in yeast by acriflavine. Exp. Cell Res., 29:82-85.

55. Nagai, S., Y. Yanagashima and H. Nagai 1961 Advances in the study of respiration-deficient (RD) mutation in yeast and other microorganisms. Bacteriol. Rev., 25:404-425.

56. Nagy, E. J. and I. Elekes 1967 Strahlenbedingte Veranderungen in der Mitose und der Nukleinsauresynthese. Radiobiol. Radiother., 8:229-237.

57. Nakai, S. and S. Matsumoto 1967 Two types of radiation-sensitive mutant in yeast. Mutation Res., 4:129-136.

58. Nakai, S. and R. K. Mortimer 1969 Studies of the genetic mechanism of radiation-induced mitotic segregation in yeast. Molec. Gen. Genetics, 103:329-338.

59. Nasim, A. and C. Auerbach 1967 The origin of complete and mosaic mutants from mutagenic treatment of single cells. Mutation Res., 4:1-14.

60. Ogur, M., G. Lindegren and C. C. Lindegren 1954 A simple screening test for genetic studies of respiration deficiency in yeast. J. Bacteriol., 68:391-392.

61. Olivieri, G. and A. Olivieri 1964 Evidence for the two-hit nature of x-ray induced crossing-over in the centromeric region of Drosophila males. Mutation Res., 1:279-295.

62. Parry, J. M. and B. S. Cox 1968 The effects of dark holding and photoreactivation on ultraviolet light induced mitotic recombination and survival in yeast. Genet. Res., 12:187-198.

63. Patrick, M. H. and R. H. Haynes 1964 Dark recovery phenomena in yeast II. Conditions that modify the recovery process. Rad. Res., 23:564-579.

64. Patrick, M. H. and R. H. Haynes 1968 Repair-induced changes in yeast sensitivity. J. Bacteriol., 95:1350-1354.

65. Patrick, M. H., R. H. Haynes and R. B. Uretz 1964 Dark recovery phenomena in yeast I. Comparative effects with various inactivating agents. Rad. Res., 21:144-163.

66. Pittman, D. D. 1956 The relation of population heterogeneity to the plateau phenomenon in radiation dose-response curves. J. Bacteriol., 71:500-501.

67. Pomper, S., K. M. Daniels and D. W. McKee 1954 Genetic analysis of polyploid yeast. Genetics, 39:343-355.

68. Powers, E. L. 1962 Considerations of survival curves and target theory. Phys. Biol. Med., 7:3-28.

69. Prescott, D. M. 1961 The growth-duplication cycle of the cell. Int. Rev. Cytol., 11:235-282.

70. Pritchard, R. H. 1963 Mitotic recombination in fungi. In: W. J. Burdette, Editor, Methodology in Basic Genetics. Holden-Day, San Francisco, pp. 228-243.

71. Puglisi, P. P. 1967 Genetic control of radiation sensitivity in yeast. Rad. Res., 31:856-866.

72. Raut, C. and W. L. Simpson 1955 The effect of x-rays and of ultraviolet light of different wave lengths on the production of cytochrome deficient yeasts. Arch. Biochem. Biophys., 57: 218-228.

73. Resnick, M. A. 1968 Genetic control of lethality and mutation in Saccharomyces cerevisiae. Ph.D. Thesis, University of California, Berkeley. University of California Lawrence Radiation Laboratory UCRL-18404.

74. Roman, H. 1956 Studies on gene mutation in Saccharomyces. Cold Spring Harbor Symposium Quantitative Biology, 21:175-185.

75. Roman, H. 1956 A system selective for mutation affecting the synthesis of adenine in yeast. Comptes Rendues Lab. Carlsberg, Ser. Physiol., 26:299-314.

76. Roodyn, D. B. and D. Wilkie 1968 The Biogenesis of Mitochondria. Methuen and Co., London.

77. Sarachek, A. and J. T. Bish 1963 Post-irradiation protein synthesis and the inductions of cytoplasmic and genic mutations in Saccharomyces by ultraviolet radiation. Cytologia, 28:450-462.

78. Sarachek, A. 1954 A comparative study of the retardation of budding and cellular inactivation by ultraviolet radiation in polyploid Saccharomyces with special reference to photoreactivation. Cytologia, 19:77-85.

79. Scopes, A. W. and D. H. Williamson 1964 The growth and oxygen uptake of synchronously dividing cultures of Saccharomyces cerevisiae. Exp. Cell Res., 35:361-371.

80. Snow, R. 1967 Mutants of yeast sensitive to ultraviolet light. J. Bacteriol., 94:571-575.

81. Swanson, C. P. 1944 X-ray and ultraviolet studies on pollen tube chromosomes. I. The effects of ultraviolet (2537Å) on x-ray-induced chromosomal aberrations. Genetics, 29:61-68.

82. Sylven, B., C. A. Tobias, H. Malmgren, R. Ottoson and B. Thorell 1959 Cyclic variation in the peptidase and catheptic activities of yeast cultures synchronized with respect to cell multiplication. Exp. Cell Res., 16:77-87.

83. Tauro, P. and H. Halvorson 1966 Effect of gene position on timing of enzyme synthesis in synchronous cultures of yeast. J. Bacteriol., 92:652-661.

84. Tewari, K. K., W. Vötsch and H. R. Mahler 1966 Biochemical correlates of respiratory deficiency VI. Mitochondrial DNA, J. Molec. Biol., 20:453-481.

85. Uretz, R. B. 1955 Additivity of x-rays and ultraviolet light in the inactivation of haploid and diploid yeast. Rad. Res., 2:240-252.

86. Weiss, J. J. 1964 Chemical effects of ionizing radiations on nucleic acids and related compounds. Progr. Nucleic Acid Res., 3:104-144.

87. Whitehouse, H. L. K. and P. J. Hastings 1965 The analysis of genetic recombination on the polaron hybrid DNA model. Genet. Res., 6:27-92.

88. Whittinghill, M. 1955 Crossover variability and induced crossing over. J. Cell. Comp. Physiol., 45 (Suppl.):189-220.

89. Wickerham, L. J. 1951 Taxonomy of yeasts. United States Department of Agriculture Technical Bulletin, No. 1029.

90. Wilkie, D. 1963 The induction by monochromatic UV light of respiratory deficient mutants in aerobic and anaerobic cultures of yeast. J. Molec. Biol., 7:527-533.

91. Wilkie, D. and D. Lewis 1963 The effect of ultraviolet light on recombination in yeast. Genetics, 48:1701-1716.

92. Wolff, S. 1964 Are sister chromatid exchanges sister strand crossovers or radiation-induced exchanges. Mutation Res., 1:337-343.

93. Wolff, S. 1967 Radiation genetics. Ann. Rev. Genetics, 1:221-244.

94. Zimmering, S. and C. K. Wu 1964 Meiotic X-Y exchange and non-disjunction induced by irradiation in male Drosophila. Genetics, 50:633-638.

TABLE 1

Genotypes of strains of *Saccharomyces cerevisiae*.

Linkage group	I	III			V			VII		XII	
Marker and gene-centromere map distance[2]	ad_1	MT[1]	ur_3	thr_3	hi_1	ar_6	tr_2	le_1	ad_6	gal_2	ad_2
	5	21	5	34	36.4	46.4	57.2	2	26	40	–
Z2367	–/–	a/	–/+	–/+	1-315/1-1	+/–	+/–	+/–	–/+	+/–	+/+
312	–/–	a/	+/+	+/+	1[3]/1[3]	–/–	–/–	+/–	–/+	+/–	+/+
P7:3-1	+/–	a/	+/+	–/+	+ /1[3]	–/+	–/+	+/–	+/+	+/+	+/–
15:4-4	–	a	–	–	1[3]	–	–	–	+	+	+
$\alpha hi_1 ad_2$	+	α	+	+	1[3]	+	+	+	+	+	–
SC-7K	+	α	+	+	+	+	+	+	+	+	+

1) Mating type
2) Nomenclature follows Mortimer and Hawthorne (51); map distances from Fogel and Hurst (29).
3) Histidine heteroallele unknown.

TABLE 2

Media

Medium	Yeast Extract	Peptone	Sugar	Additional Components
	Final concentration, grams per litre			
YEPD	5	3.5	10, glucose	-
YEPGal	5	3.5	10, galactose	-
Glu	10	20	20, glucose	-
Gal	10	20	20, galactose	-
NGlu (54)	2	1.8	10, glucose	1.5 $(NH_4)_2SO_4$ 1.0 KH_2PO_4 0.5 $MgSO_4 \cdot 7H_2O$
KAc (28)	2.5	0	1, glucose 9.8, potassium acetate	10 mg adenine per litre

Medium	Component	Final concentration, grams per litre
Minimal medium	Yeast nitrogen base	6.7
	glucose	20
		Final concentration, milligrams per litre of minimal medium
SM medium	adenine	10
	l-histidine	10
Complete[1] and omission[2] media	adenine, uracil, l-arginine, l-histidine, l-methionine, l-tryptophan	10 each
	l-leucine, l-isoleucine	60 each
	l-lysine, l-phenylalanine, l-glutamic acid, l-serine	20 each
	l-threonine	300
	l-tyrosine	50

1) After Wickerham (88), modified according to Hurst and Fogel, unpublished.

2) Omission media lack the appropriate nutrilites.

TABLE 3

Response of singly isolated interdivisional and budding cells of strain Z2367

Dose and cell type	Cells isolated	Surviving cells	NCT survivors	Survivors visible on day 1
Interdivisional				
0 kr	40	37 .925	1 .027	35 .946
28 kr	72	34 .473	27 .794	11 .324
Budding				
0 kr	45	42 .933	1 .024	41 .976
28 kr	75	65 .867	31 .477	51 .784

X^2 against H_o: equality of response of irradiated interdivisional and budding cells, 1 d.f. each, $p < 0.01$ each.

TABLE 4

Phenotype frequencies among auxotrophic survivors of pellet interdivisional and budding cells of strain Z2367.

Dose and cell type	Colonies tested	Number auxotrophic	Number and frequency of auxotrophic colonies requiring:						
			le	ur	thr	ar	tr	ar,tr	ur,thr
Interdivisional									
0 kr	254	1	1						
28 kr	457	35	3 .086	4 .115	12 .343	22 .628	20 .572	20 .572	0 0
56 kr	476	56	8 .143	9 .161	18 .324	22 .393	28 .500	21 .375	2 .036
Budding									
0 kr	359	0							
28 kr	571	13	3 .231	4 .308	3 .231	2 .154	0 0	0 0	1 .077
56 kr	1340	23	2 .087	7 .304	7 .304	10 .435	12 .522	10 .435	2 .087
Total		127	16 .126	24 .189	40 .315	56 .441	60 .473	51 .402	5 .039

Experimental procedure as in Figure 4. Irradiated plates replica plated to omission media, and auxotrophic colonies identified and phenotypes confirmed after isolation.

TABLE 5

Survival of haploid strain SC-7K irradiated on different media.

Medium	Percent survival	
	0 kr	168 kr
Gal	100	2.32 ± 0.68[1]
Glu	100	1.97 ± 0.44
YEPGal	100	1.61 ± 0.32
YEPD	100	1.34 ± 0.39
NGlu	100	1.33 ± 0.41
SM	100	2.27 ± 0.83
Minimal	100	2.40 ± 0.73

1) 95% confidence limits (1.96 standard deviations)

Analysis of variance

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F
0 kr				
Total	1,124.60	34	33.07	
Pipettes	344.28	4	86.07	3.63*
Media	211.38	6	35.23	1.49
Error	568.94	24	23.71	

* Significant at the 5% level

Continued

TABLE 5, continued

Analysis of variance

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F
168 kr				
Total	633,985.25	76	8,341.91	
Pipettes	55,956.36	10	5,595.64	2.62*
Media	450,337.22	6	75,056.20	35.36**
Error	127,691.67	60	2,118.19	

* Significant at the 5% level
** Significant at the 1% level

t-tests against H_o: equality of average plate count after 168 kr.

Comparison	t[1]
YEPD - YEPGal	4.03**
YEPD - Gal	8.91**
Glu - Gal	5.66**
YEPD - SM	5.08**
YEPD - Minimal	7.16**
YEPD - NGlu	1.25
YEPD - Glu	4.87**
YEPGal - Gal	6.67**
SM - Minimal	1.34

1) 20 degrees of freedom

**Significant at the 1% level.

TABLE 6

SC-7K interdivisional cell survival on YEPD, Gal and minimal medium.

Medium	Percent survival	
	0 kr	14 kr
YEPD	100	2.43 ± 0.36[1]
Gal	100	2.64 ± 0.30
Minimal	100	2.80 ± 0.26

1) 95% confidence limits (± 1.96 standard deviations).

X^2 against H_o: identity of distributions on YEPD and Gal or minimal before and after irradiation = 0.5 and 2.0, respectively. 1 d.f. each, $p > 0.05$ each.

Supernate interdivisional cells prepared from 30 day old, liquid YEPD grown culture. > 1% budding cells.

TABLE 7

Survival of haploid strain 15:4-4 on YEPD and on Gal.

Medium	Percent survival	
	0 kr	28 kr
YEPD	100	31.4 ± 7.3[1]
Gal	100	41.5 ± 6.9

1) 95% confidence limits ($\pm$ 1.96 standard deviations).

X^2 against H_o: identity of distributions on YEPD and on Gal before and after irradiation = 4.8, 1 d.f., p = 0.02 - 0.05.

Procedure as in Figure 2; 63% budding cells.

TABLE 8

Supernate interdivisional and budding cell response on YEPD and YEPGal in strain Z2367.

Dose, medium and cell type	Percent survival	Percent NCT	Percent final day 2
Interdivisional			
YEPD			
0 kr	100	3.9 ± 1.8[1]	99.5 ± 0.7
56 kr	30.3 ± 5.0[2]	81.9 ± 2.2	72.9 ± 1.9
YEPGal			
0 kr	100	4.4 ± 2.1	98.8 ± 1.1
56 kr	41.2 ± 10.0	80.8 ± 1.4	67.0 ± 1.7
Budding			
YEPD			
0 kr	100	4.0 ± 2.0	99.0 ± 1.0
56 kr	43.8 ± 10.0	69.1 ± 2.2	94.2 ± 1.0
YEPGal			
0 kr	100	3.3 ± 1.8	99.3 ± 1.0
56 kr	54.4 ± 11.9	65.6 ± 2.2	90.8 ± 1.1

1) 95% confidence limits (± 1.96 standard errors of mean)
2) 95% confidence limits (∓ 1.96 standard deviations)

Supernate interdivisional cells prepared from 14 day old, liquid YEPD grown culture. 0.3% budding cells in interdivisional cell preparation; 74% budding cells in budded preparation.

Continued

TABLE 8 Continued

Analysis of variance

Source of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F
56 kr				
Total	38,901.89	43	904.70	
Between samples	16,599.51	3	5,533.17	9.9**
Media	14,376.56	1	14,376.56	25.8**
Stage, dilutions	2,520.20	1	2,520.20	4.5*
Interaction	297.25	1	297.25	0.53
Among samples (error)	22,302.38	40	557.56	

* Significant at the 5% level
** Significant at the 1% level

X^2 against H_0: identity of interdivisional and budding cell distributions on YEPD and YEPGal after irradiation = 6.0; 1 d.f.; p = 0.02 - 0.05.

TABLE 9

Galactose medium - induced repair in irradiated Z2367.

Medium and dose	Number of colonies	Percent NCT	Percent small white colonies	Percent final, day 2
0 kr				
YEPD	430/5[1]	4.7 ± 2.0[2]	0.9 ± 0.9	99.6 ± 0.2
Gal	462/5	4.1 ± 2.0	0.7 ± 0.7	99.5 ± 0.2
168 kr				
Immediate plating				
YEPD	1867/10	97.3 ± 0.8	7.8 ± 1.2	55.5 ± 2.3
Gal	3283/10	94.5 ± 0.8	8.8 ± 1.0	45.9 ± 1.7
168 kr				
Plating after two hours in Gal				
YEPD	1001/10	96.7 ± 1.2	-	56.8 ± 3.1
Gal	1376/10	96.1 ± 1.1	-	46.6 ± 2.7

1) Numerator: number of colonies; denominator: number of plates.
2) 95% confidence limits (± 1.96 standard errors of mean).

35% budding cells at time of inoculation into Gal medium; 30% budding cells after two hours in Gal.

t-tests against H_0: equality of average plate counts on YEPD and Gal.

Population	t	degrees of freedom	p
0 kr	0.80	8	0.40 - 0.50
Immediate plating	16.5	18	< 0.01
Delayed plating	9.8	8	< 0.01

TABLE 10

P7:3-1 interdivisional cell response on YEPD and YEPGal.

Medium and dose	Response, percent				
	Survival	Total red and red/white sectoring	Petite	Auxo-trophic	Final, day 2
YEPD					
0 kr	100	0	3.0 ± 3.0[1]	3.7±3.5	78.9±7.8
56 kr	28.4±11.5[2]	10.0±2.9	1	33.6±4.0	19.6±3.7
YEPGal					
0 kr	100	0	5.0 ± 4.5	4.8±4.5	62.1±10.2
56 kr	43.5±7.9	11.1±2.7	1	29.8±3.3	16.6±3.1

1) 95% confidence limits (± 1.96 standard errors of mean).
2) 95% confidence limits (∓ 1.96 standard deviations).

Comparison between response on YEPD and YEPGal at 56 kr.
H_o: identity.

Survival: $t = 3.31$, 22 degrees of freedom, $p < 0.01$
Sectoring: $Z = 0.56$, $p = 0.57$
Auxotrophy: $Z = 1.46$, $p = 0.14$

Pellet interdivisional cells prepared from 7 day old, liquid YEPD grown culture. < 0.5% budding cells.

TABLE 11

Interdivisional and budding cell response after overnight water storage in strain 312.

Dose and cell type	Percent budding cells	Percent survival	Percent NCT	Percent final, day 1
Pellet interdivisional cells				
	0.1			
0 kr		100	12.4 $\pm$ 5.1[1]	90.2 $\pm$ 4.9
28 kr		70.4$\pm$7.2[2]	62.8 $\pm$ 9.6	14.4 $\pm$ 6.6
Pellet budding cell preparation				
	74			
0 kr		100	14.0 $\pm$ 3.4	87.8 $\pm$ 3.1
28 kr		76.9$\pm$4.9	29.2 $\pm$ 4.3	50.0 $\pm$ 4.7
Supernate interdivisional cells				
	0.5			
0 kr		100	13.8 $\pm$ 5.7	74.0 $\pm$ 8.4
28 kr		76.5$\pm$7.0	53.9 $\pm$ 9.3	12.3 $\pm$ 6.2
Supernate budding cell preparation				
	83			
0 kr		100	18.5 $\pm$ 3.8	72.9 $\pm$ 4.1
28 kr		65.6$\pm$4.3	43.9 $\pm$ 5.0	25.0 $\pm$ 8.7

1) 95% confidence limits ($\pm$ 1.96 standard errors of mean).
2) 95% confidence limits ($\pm$ 1.96 standard deviations).

Cells prepared from 15 day old, solid YEPD grown culture.

TABLE 12

Response of singly isolated interdivisional and sensitive budding cells of strain Z2367.

Dose and cell type	Cells isolated	Surviving cells	NCT survivors
Interdivisional			
0 kr	114	113 0.99	7 0.07
14 kr	122	108 0.886	20 0.208
28 kr	122	94 0.760	49 0.539
Budding			
0 kr	114	109 0.956	11 0.110
14 kr	122	100 0.820	20 0.200
28 kr	120	97 0.808	41 0.423

Differences between budding and interdivisional cell response are not significantly different at the 5% level by the X^2 criterion.

Supernate interdivisional cells prepared from 6 day old, solid YEPD grown culture. 0.5% budding cells.

TABLE 13

Interdivisional and sensitive budding cell response on YEPD in strain Z2367.

Dose and cell type	Survival	Response, percent NCT	Small white	Auxo-trophic	Final, day 1
Interdivisional					
0 kr	100	12.1±3.3[1]	8.7±2.7	8.2±2.6	92.8±2.6
28 kr	68.6±5.1[2]	65.6±5.0	13.8±3.6	12.8±3.5	30.4±4.8
Budding					
0 kr	100	12.6±3.4	6.3±2.5	3.1±2.0	96.5±1.9
28 kr	69.4±4.0	55.6±4.7	9.7±2.6	9.5±2.6	44.4±4.3

1) 95% confidence limits (± 1.96 standard errors of mean).
2) 95% confidence limits (∓ 1.96 standard deviations).

Supernate interdivisional cells prepared from 9 day old, liquid YEPD grown culture. 0.5% budding cells in interdivisional cell preparation; 70% budding cells in budded preparation.

TABLE 14

Repair induced in Z2367 sensitive budding cells by pre- and post-irradiation incubation on SM medium.

	Response, percent							
	Survival	NCT	Survival	NCT	Survival	NCT	Survival	NCT
	Interdivisional cells		4 hours in YEPD		4 hours in SM		7 hours in YEPD	
Percent budding cells	0.7		59		51		76	
Dose and post-irradiation medium								
YEPD								
0 kr	100	7.2 ±2.4[1]	100	7.2 ±1.9	100	8.8 ±3.2	100	9.6 ±3.0
56 kr	27.5 ± 7.9[2]	87.8 ±1.9	26.6 ±3.9	85.8 ±2.3	27.6 ±4.7	88.3 ±2.2	38.8 ±7.9	80.1[3] ±2.8
SM								
0 kr	100	-	100	-	100	-	100	-
56 kr	33.2 ± 7.0	-	37.2 ±6.5	-	[illegible]7.1 ±6.9	-	40.5 ±9.3	-

1) 95% confidence limits (± 1.96 standard errors of mean).
2) 95% confidence limits (± 1.96 standard deviations).
3) Comparison between interdivisional and 7 hour, budding cell %NCT; H_O: equality. $Z = 4.6$, $p < 0.01$.

Supernate interdivisional cells prepared from 20 day old, solid YEPD grown culture.

Continued

TABLE 14 Continued

Analysis of variance

Source of variation 56 kr	Sum of squares	Degrees of freedom	Mean sum of squares	F
Total	62,701.87	29	2,162.13	
Between samples	47,913.47	5	9,582.20	15.5**
Media	9,363.33	1	9,363.33	15.2**
Stage, dilutions	37,652.87	2	18,826.44	30.6**
Interaction	897.27	2	448.64	0.73
Among samples (error)	14,788.40	24	616.18	

** Significant at the 1% level.

X^2 against H_O: identity of interdivisional cell distribution on YEPD and SM with distribution of various budding cell populations on these media.

Population	Dose	X^2	Significance
4 hours in YEPD	0 kr	1.4	> 0.05
	56 kr	3.9	< 0.05
4 hours in SM	0 kr	1.3	> 0.05
	56 kr	0.1	> 0.05

Figure 1

Percentages of budding cells in Z2367 supernate and pellet cell preparations incubated in liquid YEPD for various times. Percentages were determined using a hemocytometer.

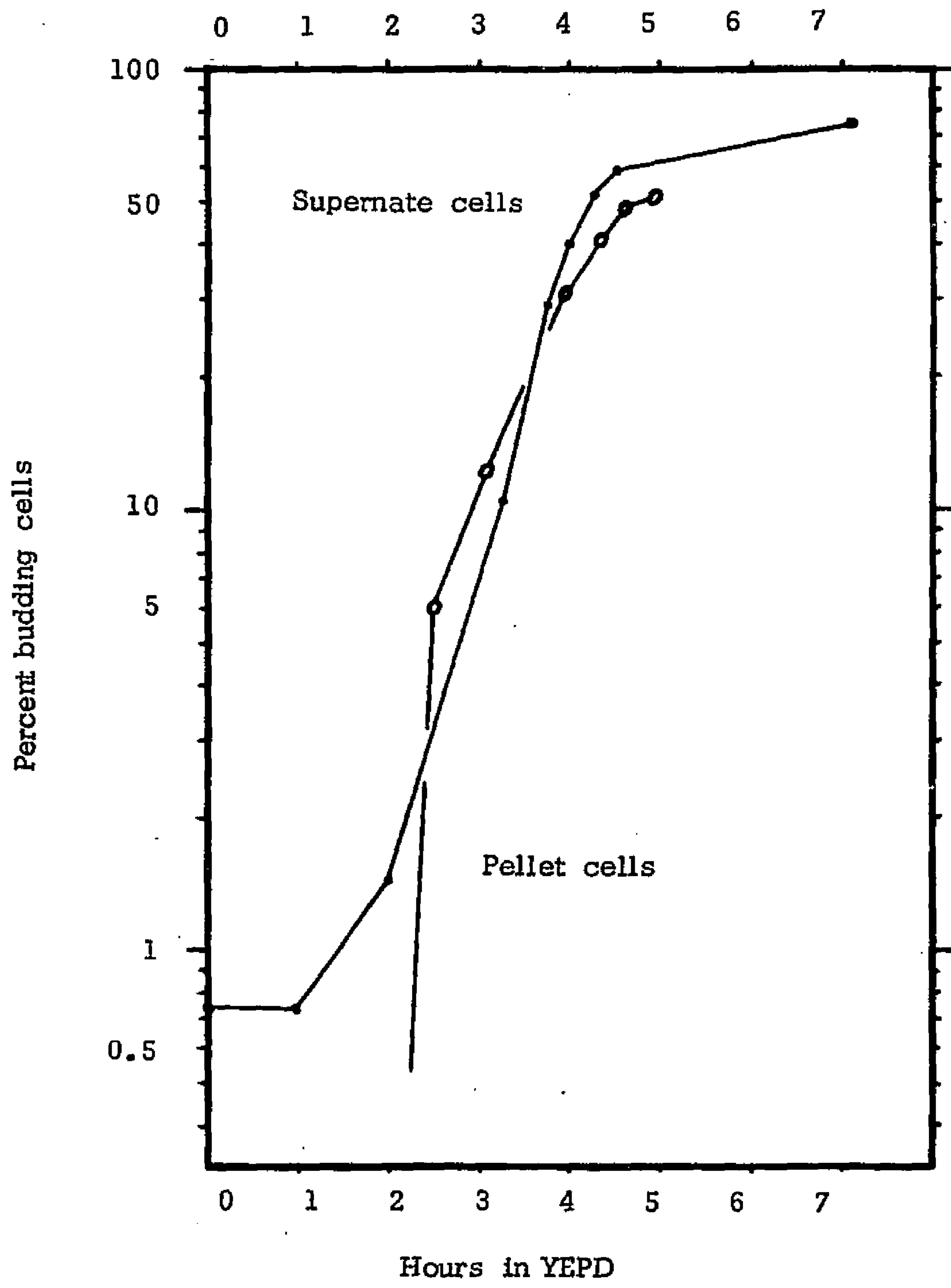

Figure 1

Figure 2

Survival on YEPD of culture of haploid 15:4-4 containing increasing percentages of budding cells. Samples were removed for irradiation after 0, 2.5, 3.5 and 4.25 hours in liquid YEPD. The percentages of budding cells at the times of irradiation are shown beneath each curve.

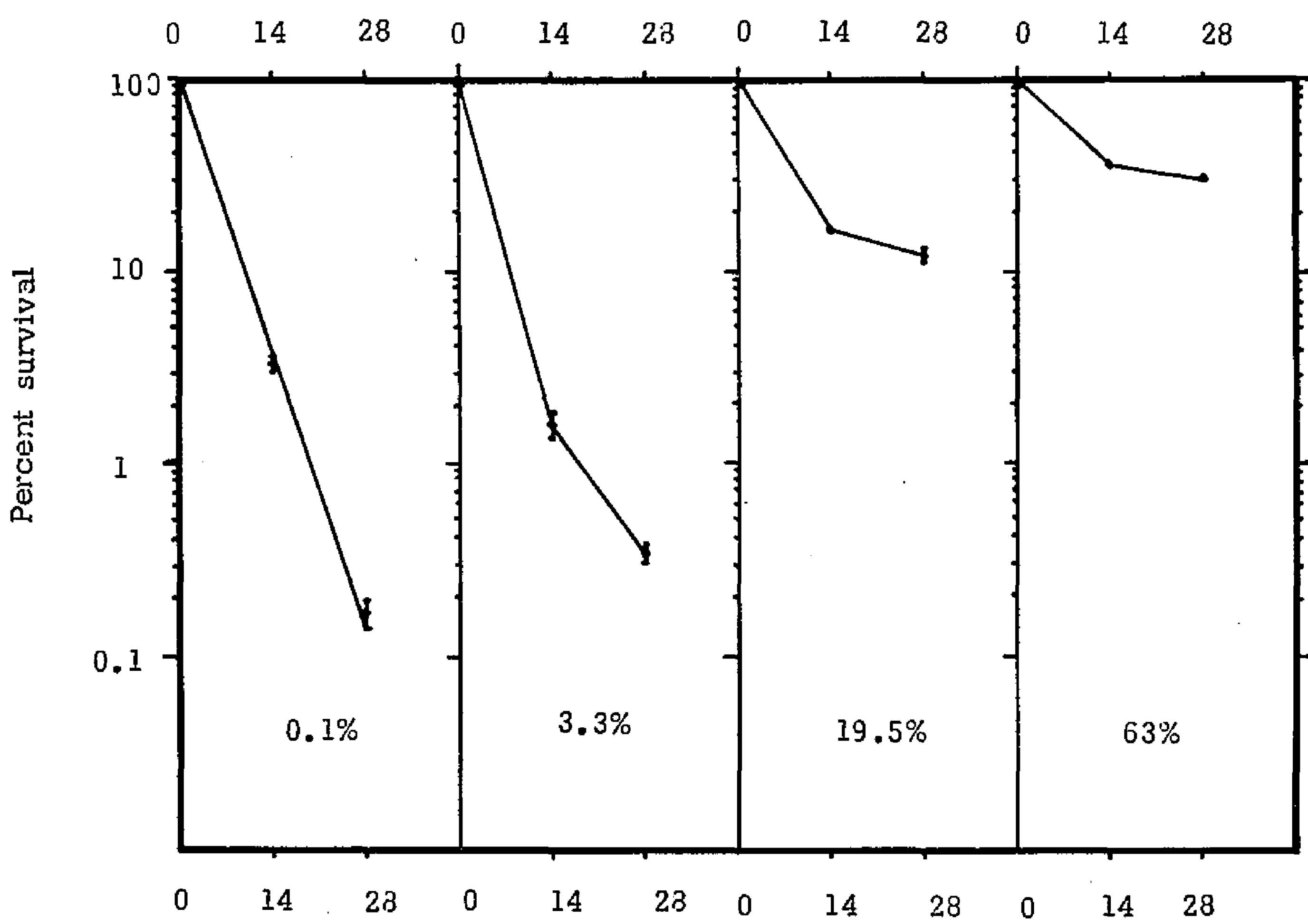

Figure 2

Figure 3

Growth curves of slow and normal growing strains derived from Z2367.

Curve 1: Respiratory competent survivor of 0 kr.

Curve 2: Respiratory deficient survivor of 0 kr.

Curve 3: Respiratory competent survivor of 336 kr.

Curve 4: Respiratory deficient survivor of 336 kr.

Approximately 1×10^5 cells were inoculated into 4 cc liquid YEPD and incubated at 30°C. Optical density at 660 mμ was determined in a Spectronic 20 spectrophotometer at various times after inoculation.

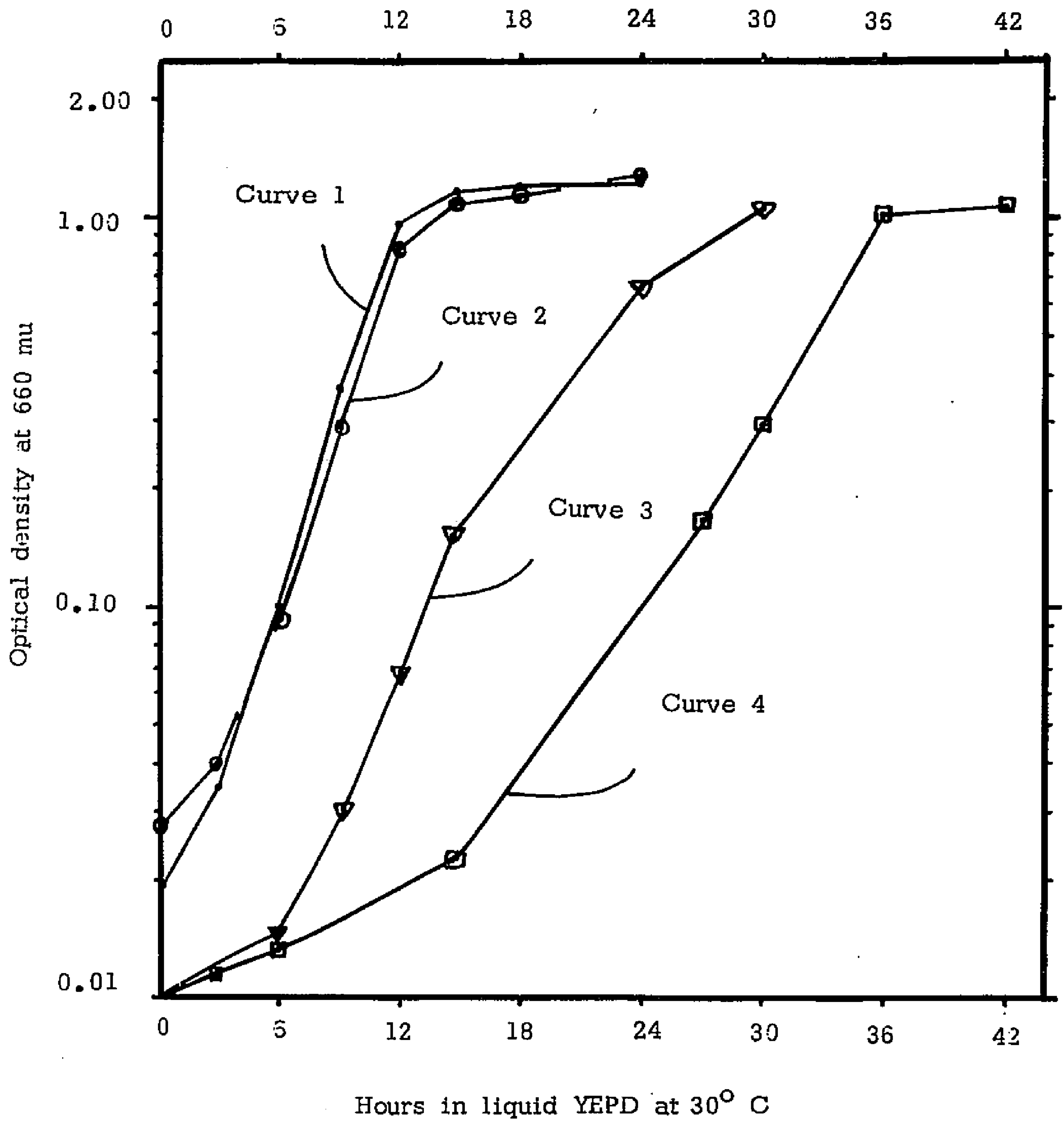

Figure 3

Figure 4

Survival, lag, NCT, petite and auxotrophy induction in Z2367 pellet interdivisional and budding cells.

Curve 1: Percent survival ± 95% confidence limits (1.96 standard deviations)

Curve 2: Percentage of survivors visible on the first and second days after irradiation ± 95% confidence limits (1.96 standard errors of mean).

Upper curves: second day

Lower curves: first day

Curve 3A: Percentage of control type colonies among survivors.

Curve 3B: Percentage of non-control type colonies among survivors ± 95% confidence limits (1.96 standard errors of mean).

Curve 4: Percent respiratory deficient survivors ± 95% confidence limits (1.96 standard errors of mean).

Curve 5: Percent auxotrophic survivors ± 95% confidence limits (1.96 standard errors of mean).

Pellet interdivisional cells prepared from 20 day old, solid YEPD grown culture.

< 1% budding cells in interdivisional cell preparation.

60% budding cells in budded preparation.

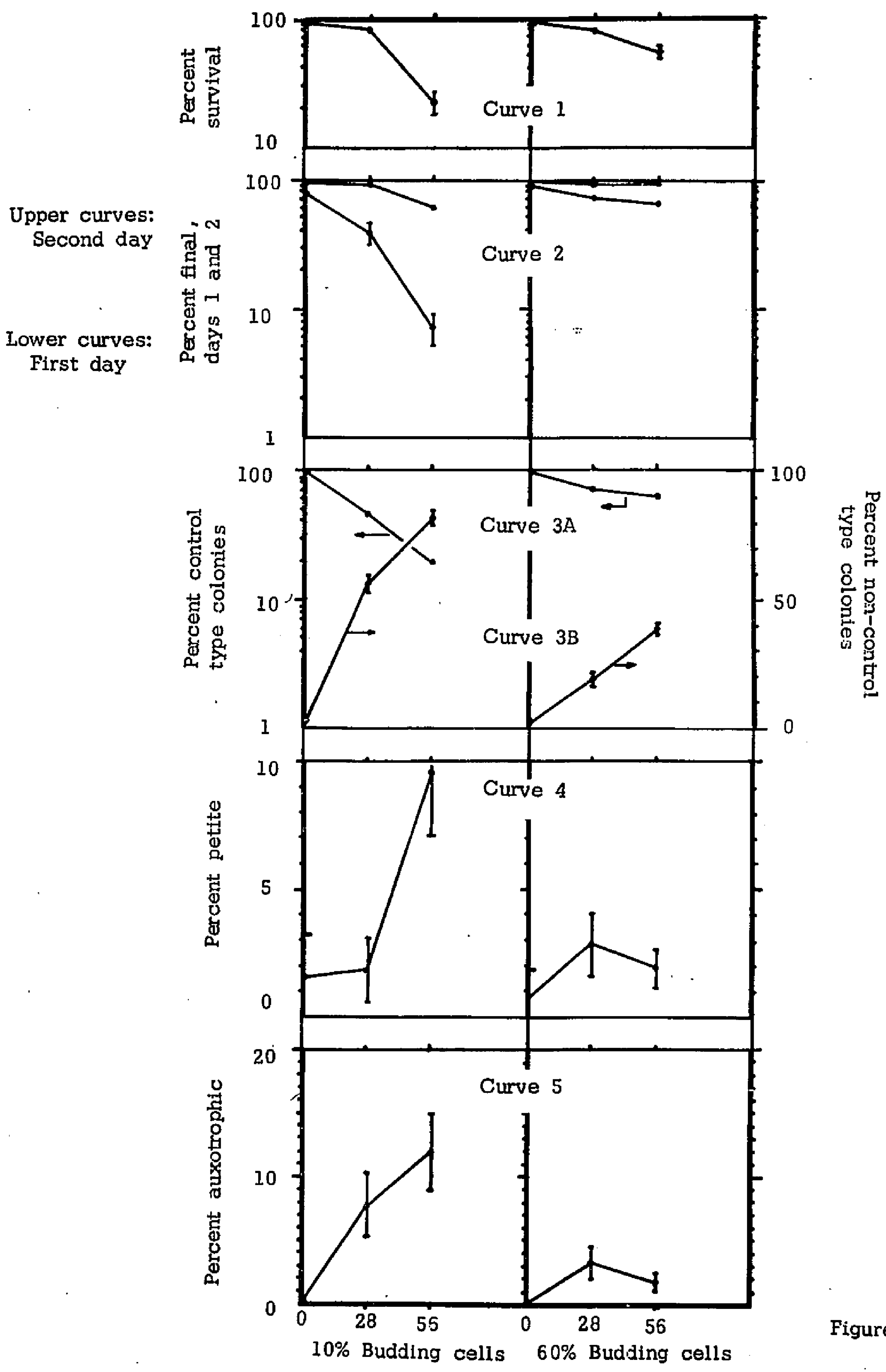

Figure 4

Figure 5

Survival, control type colony morphology and lag in Z2367 log phase culture.

Curve 5A: Percent survival ± 95% confidence limits (1.96 standard deviations).

Curve 5B: Percent control type colonies among survivors ± 95% confidence limits (1.96 standard errors of mean).

Curve 5C: Percentage of survivors visible on first day after irradiation ± 95% confidence limits (1.96 standard errors of mean).

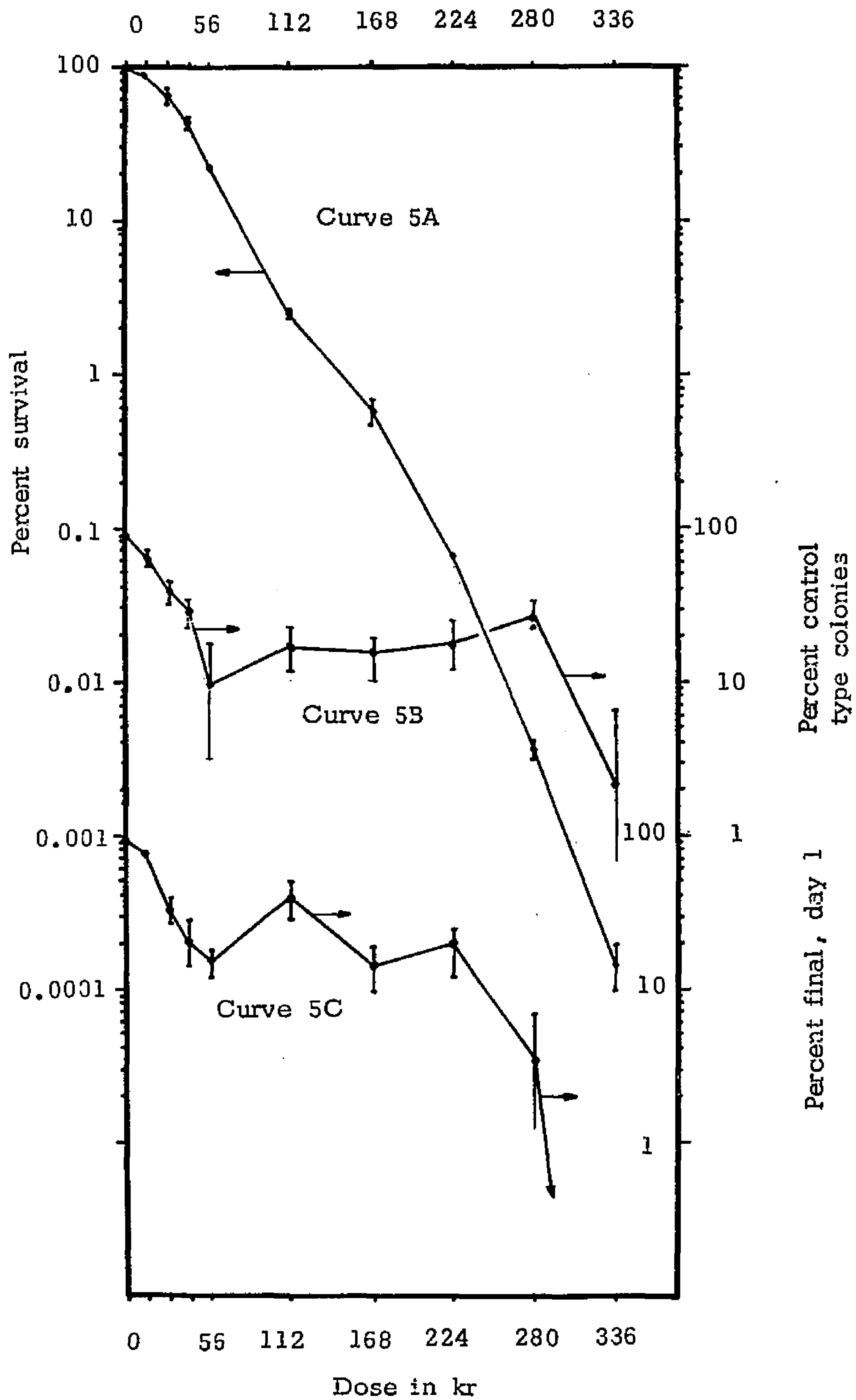

Figure 5

Figure 6

Survival and lag induction in Z2367 log phase culture.

Curve 6A: Percent survival ± 95% confidence limits (1.96 standard deviations).

Curve 6B: Percentage of survivors visible on the second day after irradiation ± 95% confidence limits (1.96 standard errors of mean).

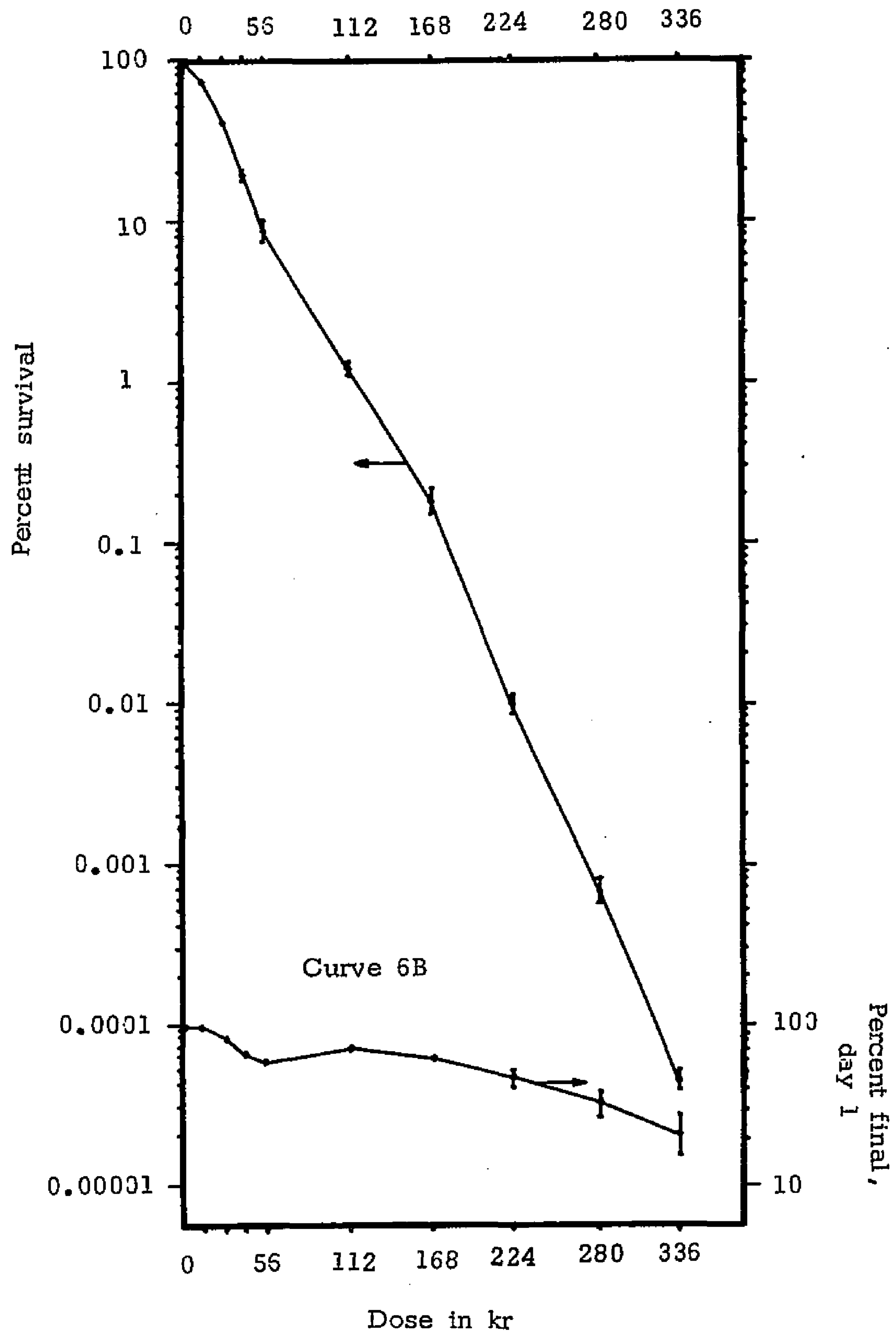

Figure 6

Figure 7

Survival of haploid SC-7K on YEPD. Percent survival ± 95% confidence limits (1.96 standard deviations).

15% budding cells. Dotted line shows continuation of interdivisional cell survival.

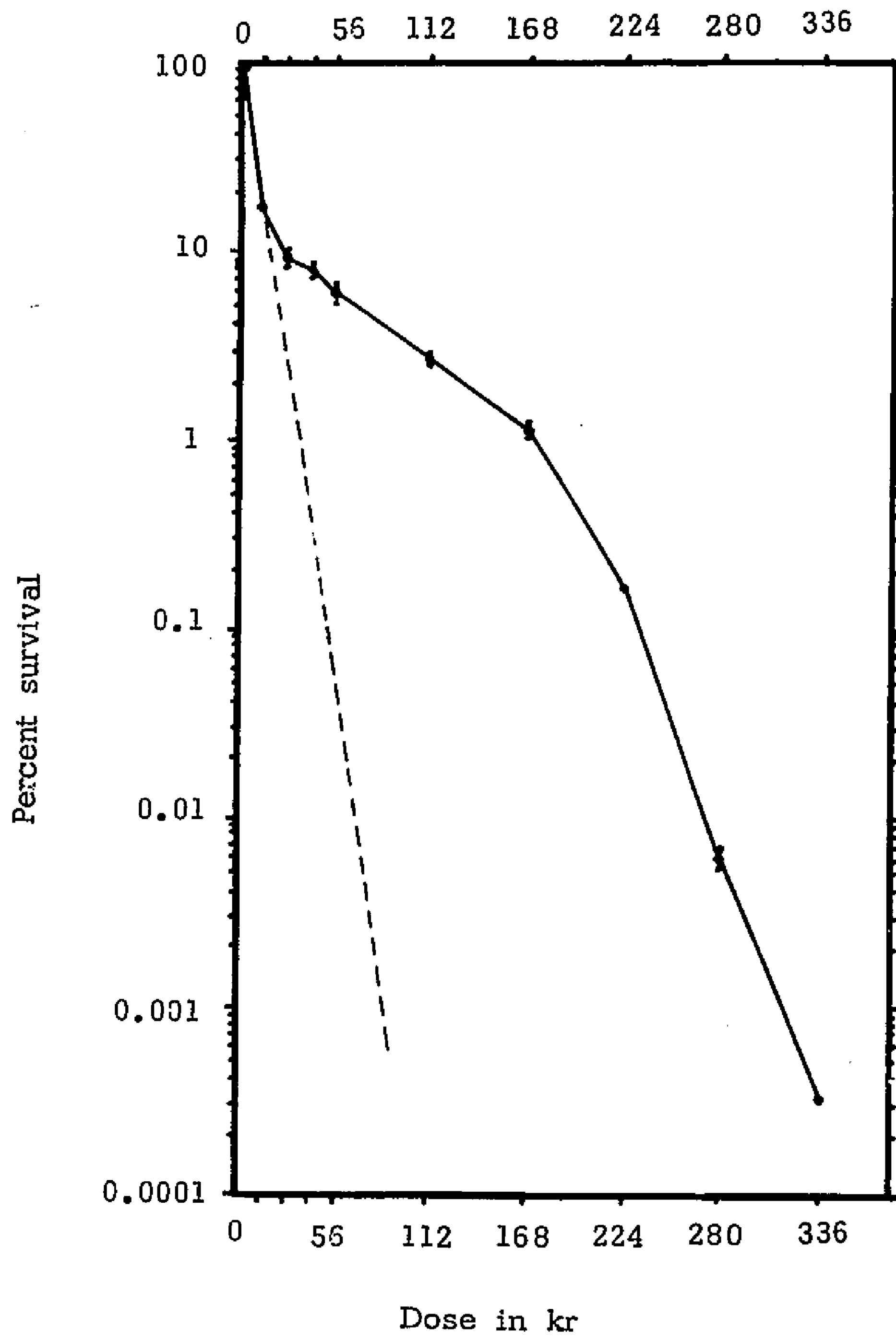

Figure 7

Figure 8

Survival of haploid $\alpha\ hi_1 ad_2$ on YEPD. Percent survival ± 95% confidence limits (1.96 standard deviations).

3.8% budding cells. Dotted line shows continuation of interdivisional cell survival.

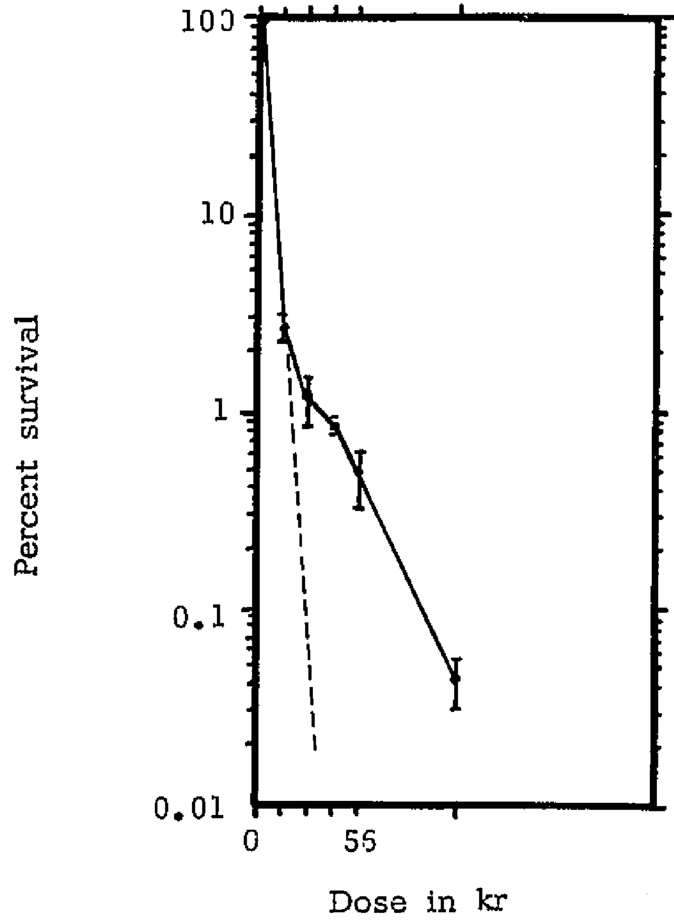

Figure 8

Figure 9

Survival, sectoring and lag induction in P7:3-1 pellet cell population with different percentages of budding cells.

Curve 1: Percent survival ± 95% confidence limits (1.96 standard deviations).

Curve 2: Percent total red and red/white sectored colonies among survivors ± 95% confidence limits (1.96 standard errors of mean).

Curve 3: Percentage of survivors visible on the first and second days after irradiation ± 95% confidence limits (1.96 standard errors of mean).

Upper curves: second day

Lower curves: first day

14% budding cells prior to incubation in liquid YEPD.
58% budding cells after incubation in liquid YEPD.

Pellet cells prepared from 48 hour, solid YEPD grown culture.

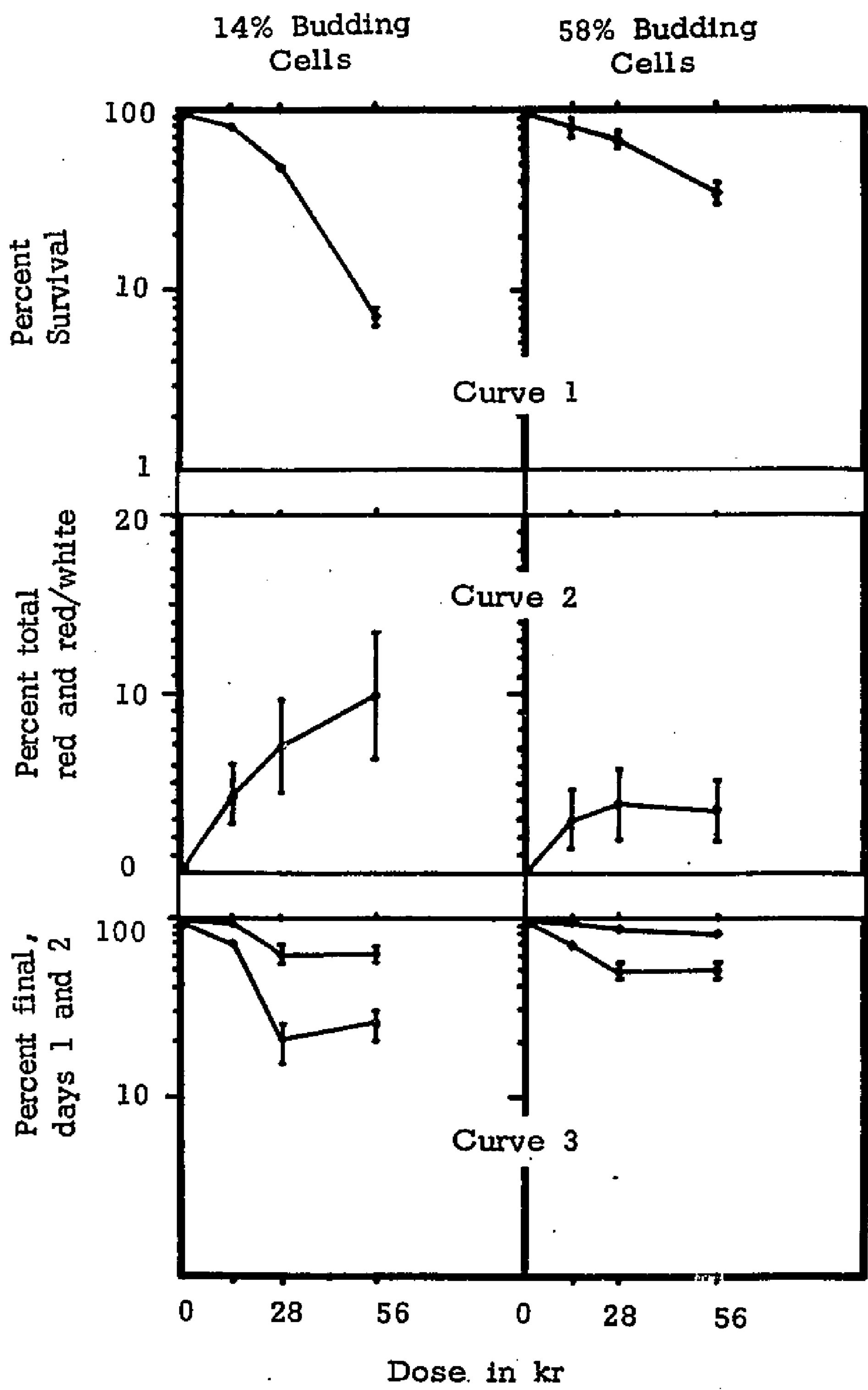

Figure 9

Figure 10

Survival, NCT and lag induction in water stored supernate interdivisional and budding cells of strain 312 irradiated and incubated on YEPD and on Gal medium.

Panel 1: Interdivisional cells irradiated on YEPD

Curve A: Percent survival

Curve B: Percent control type colonies among survivors

Curve C: Percent survivors visible on second day after irradiation

Panel 2: Budding cells irradiated on YEPD

Curve A: Percent survival

Curve B: Percent control type colonies among survivors

Curve C: Percent survivors visible on second day after irradiation

Panel 3: Interdivisional cells irradiated on Gal

Curve A: Percent survival

Curve B: Percent control type colonies among survivors

Curve C: Percent survivors visible on second day after irradiation.

Panel 4: Budding cells irradiated on Gal

Curve A: Percent survival

Curve B: Percent control type colonies among survivors

Curve C: Percent survivors visible on second day after irradiation

All points are shown ± 95% confidence limits.

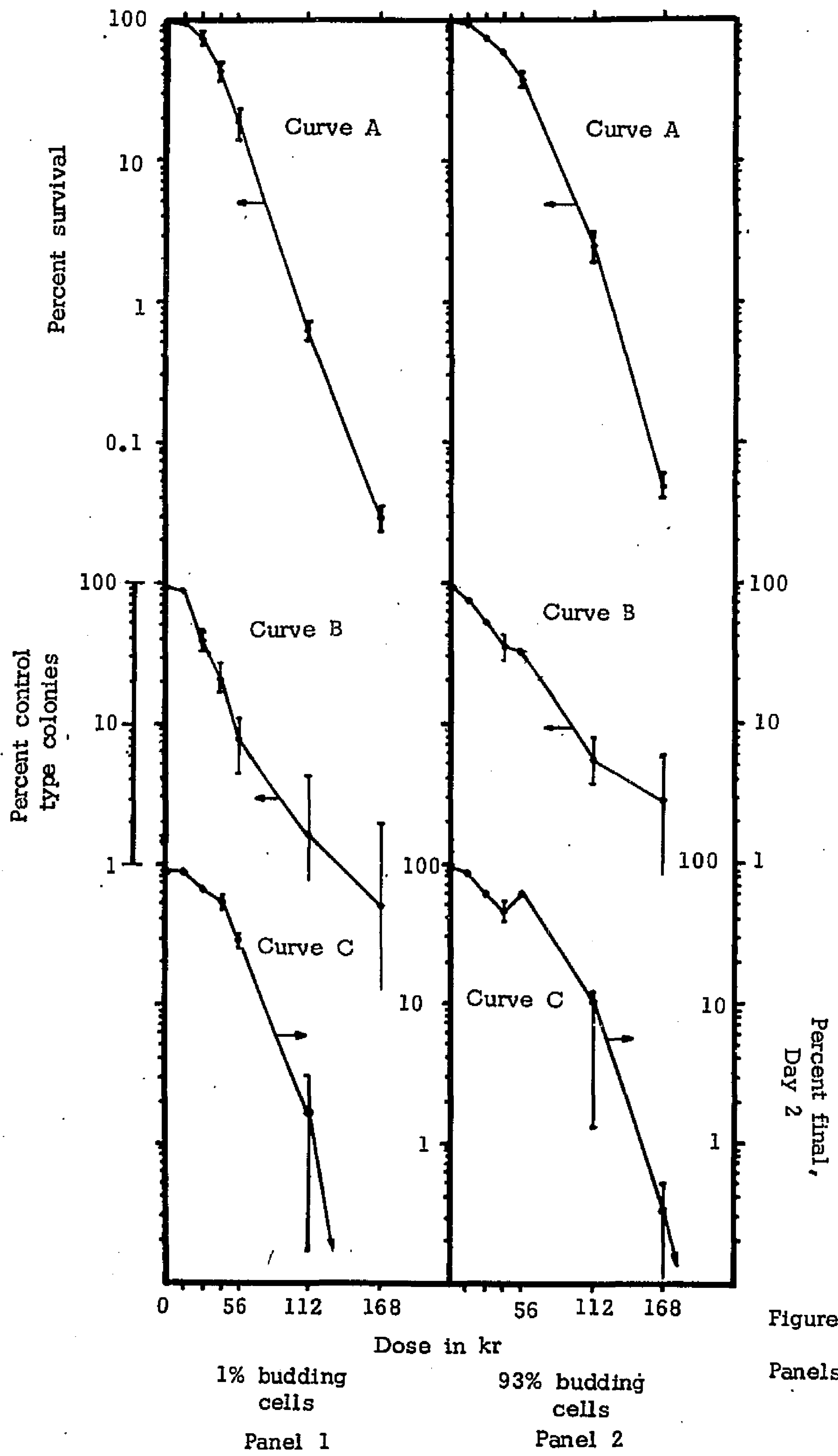

Figure 10

Panels 1 and 2

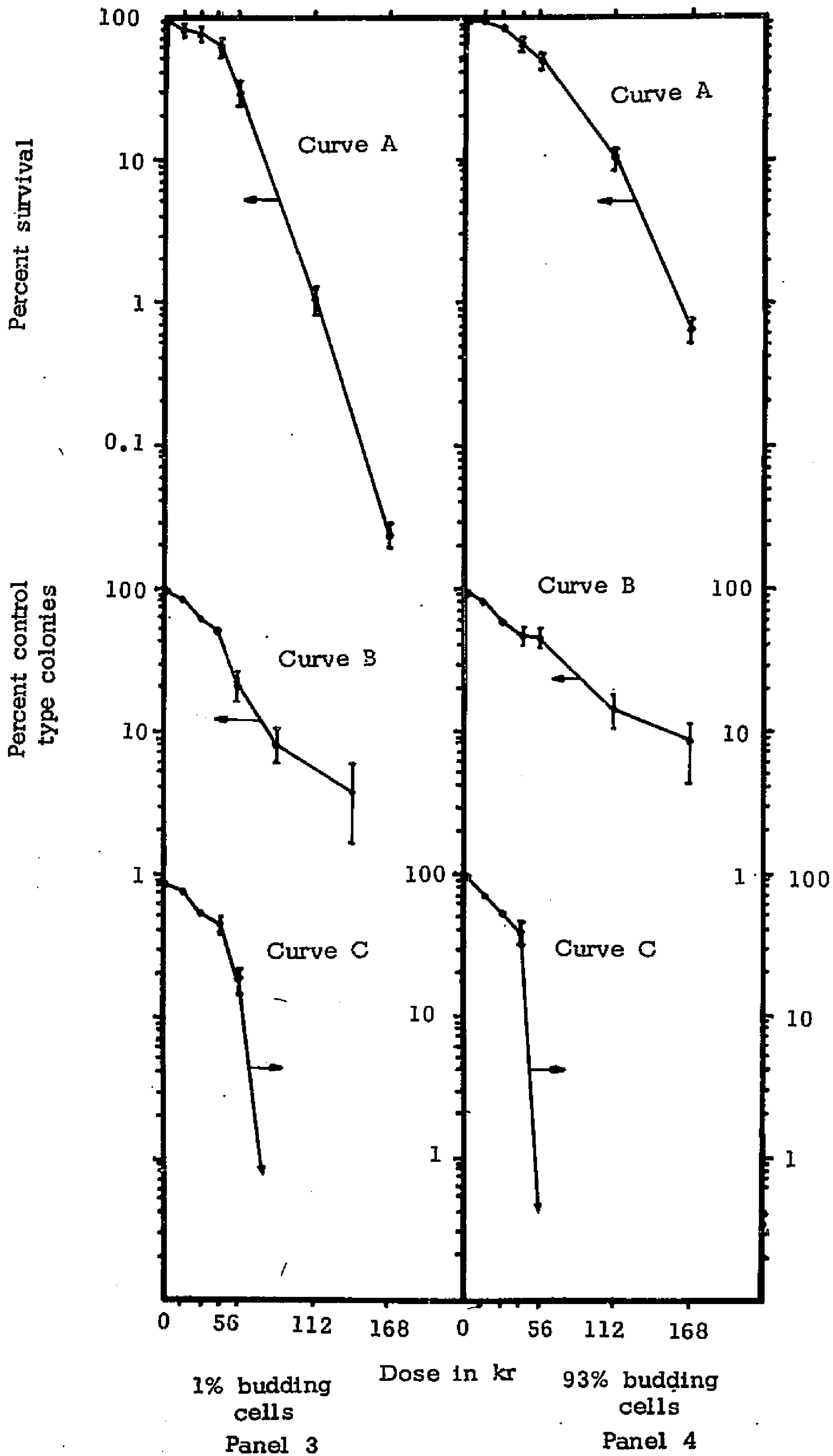

Figure 10

Panels 3 and 4