Title
RADIOBIOLOGICAL STUDIES ON YEAST (DISCUSSION)

Author
Mortimer, Robert K.

Publication Date
1958-09-01

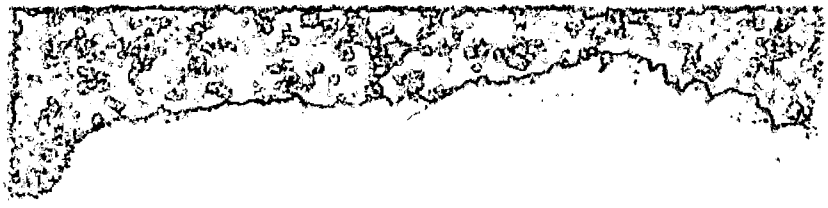
UCRL 8475

UNIVERSITY OF CALIFORNIA

Radiation Laboratory

BERKELEY, CALIFORNIA

NON STD. DIST. SPEECH



DISCLAIMER

UNIVERSITY OF CALIFORNIA

Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48

RADIOBIOLOGICAL STUDIES ON YEAST (DISCUSSION)

Robert K. Mortimer

September 1958

UCRL-[illegible]

RADIOBIOLOGICAL STUDIES ON YEAST (DISCUSSION)

Robert K. Mortimer

Donner Laboratory of Biophysics and Medical Physics
University of California, Berkeley, California

September 1958

Dr. Tobias, in his introduction, pointed out that the advantages of yeast as a test organisms for radiobiological studies have been recognized for many years. Nadson, and Holweck and Lacassagne, more than 30 years ago, performed many of the early experiments with this organism; including studies of physiological and lethal effects of radiation, and modification of these effects by changes of various biological and physical parameters. A few years later, Winge and Lawton (16) developed techniques which made possible genetic analyses in yeast including isolation of single spores and hybridization by pairing of individual spores. Still later, Lindegren (7) discovered heterothallism in certain strains of yeast and also described the first linkage maps for Saccharomyces. These experiments established a sound basis for genetic studies in yeast and at the same time made its significance as a test organism for radiobiological experiments more evident. The series of four talks which we have heard today during this symposium certainly attest to this.

In the time available to me today, I will attempt to summarize and perhaps relate at least some of the material presented in these four excellent talks, and in addition discuss some related experimental results. My discussion will be divided into two sections: I) a discussion of the various manifestations of radiation damage in yeast; and II) a consideration of modification of these damages by variations of physico-chemical and biological parameters.

I. Nature of Radiation-Induced Damage in Yeast

In Table I are summarized a number of forms of radiation damage which have been observed in yeast, and these are tentatively classified into lethal and non-lethal; genetic and non-genetic. The manifestation of radiation insult most frequently studied in yeast and also in most other microorganisms employed is cell death. That this is so is perhaps due more to the ease of assay than to a simplicity of causative factors. Magni in his talk discussed various studies aimed at describing more specifically the forms of damage which precipitate cellular death in yeast. Qualitatively it can be concluded that both recessive and dominant lethal damage are induced in x-irradiated yeast and that these are responsible for a principal fraction of cell death. However, a significant portion, at least of haploid cells, which are inactivated by x-rays do not carry any demonstrable genetic damage. The frequency of recessive lethals observed by Beam and by Magni (8) in x-irradiated non-budding diploid cells also is much lower than would be expected if all haploid inactivation were due to this form of damage. These results point to a significant portion of lethal damage in yeast cells which is non-genetic in origin. A possible candidate for at least part of this non-genetic lethal damage might be membrane destruction as described by Rothstein in his interesting paper.

That dominant lethals, as manifested by death of a zygote formed from one irradiated and one unirradiated cell, are genetic is a presumption based on findings in other biological systems. It is quite possible however, that at least a component of the dominant-lethal damage is non-genetic in origin as found in Habrobracon (1). Wood, during his talk, introduced the notion that, though a cell survives radiation exposure it need not be free of radiation effects. Indeed a number of such non-lethal effects have been described during this symposium and these are listed in Table 1 along with some additional similar effects observed in this organism. Of the non-lethal genetic changes induced by radiation are point mutations, mitotic crossing-over, and allelic recombination. The latter two occur in diploids and quite possibly also in higher ploidy cells. Point mutations are induced by both x-ray and u.v. but much more efficiently by the latter. Approximately 2% of the survivors of uv-irradiated haploid cells (1-0.01% survival) form colonies which are entirely or partially composed of cells possessing biochemical requirements not present in the unirradiated cells (10). Many other mutations affecting quantitative and other traits undoubtedly are induced but are undetected because of the screening systems employed. Examples of such mutations have recently been presented by James (5).

For diploid cells, the other forms of non-lethal genetic changes which occur include mitotic crossing-over as described by James (4) for ultra-violet irradiation and by Pittman (13) and Mortimer (see Fig. 1) for x-radiation, and allelic recombination as described by Roman and Jacob (15). As a consequence of mitotic crossing-over, a diploid cell heterozygous for a genetic character gives rise to two daughter cells, one homozygous recessive, and the other homozygous dominant. The descendants of these two cells form opposite sectors of the resultant colony. Genetic markers farther from the centromere are more susceptible to induced sectoring (Fig. 1). On the basis of results obtained for one chromosome arm (ad_2), and assuming similar effects occurring on up to 16 chromosome arms, it can be shown that a dose of 10,000 r results in 45% of the surviving cells with altered genomes, i.e., $100\left[1-(1-0.035)^{16}\right]$. Roman and Jacob (15) have described an extremely radiosensitive effect for diploid yeast that is heteroallelic for mutations at a biochemical locus. Relatively small doses of ultraviolet result in a very large increase in reversion to wild type of these cells, presumably due to intra-allelic recombination. This effect also is inducible with x-rays (see Fig. 2) and is perhaps the most radiosensitive effect known in yeast. A dose of 75 roentgens results in a doubling of the frequency of revertants.

It is important to emphasize that both mitotic crossing-over and allelic recombination occur with considerable frequency in diploid yeast cells for doses of x-ray or ultraviolet that cause relatively little cell death.

II. Modification of Radiation Damage

A. Physico-chemical Modification

Wood, in his talk has described his extensive studies of modification of radiation sensitivity by changes in phase state, temperature and degree of hydration. These studies have been concerned almost entirely with lethal effects of x-rays on haploid yeast. In general it can by summed up that changes which result in cellular dehydration reduce the sensitivity of the cell to x-rays. These results

are very fundamental to interpretation of primary radiation processes and were discussed in relation to a model based on radical action compared to one based on modifiable direct action. Obviously, as Wood stressed, more work is still necessary to define precisely which if any present model can be applied to the primary processes of cellular inactivation.

The large differences found by Hutchinson, et al. (6) for inactivation of different enzymes in wet and dry yeast are indicative of spatial effects that modify indirect action. In this light, it would be of considerable interest to undertake various physico-chemical modification experiments on a spectrum of other radiobiological damage in yeast. In this respect it can be mentioned that the oxygen effect, which was discussed by Wood, has been observed to apply in typical fashion in yeast to lethality, dominant lethality (9), mitotic crossing-over (11), allelic recombination (11), and genetic reversion (3). In all these cases, an approximate two-fold "dose reduction" has been observed when cells are x-irradiated in nitrogen as compared to air. However, there are many indications that the "dose-reduction" factor varies somewhat with the experimental conditions and also with the criterion of radiation damage. The anomolous oxygen effect discussed by Rothstein for membrane damage certainly is of interest and points again to inadequacies of our even fairly complex models.

B. Biological Factors Modifying Radiation Damage

The resistance of budding yeast to the effects of x-rays as described by Beam is of great interest to the understanding of radiation phenomena in yeast. Within a few minutes of the appearance of the bud during mitotic division, there is an abrupt and very large increase in resistance to x-ray-induced lethality. This resistance persists until the bud is of considerable size. It is of interest also that budding cells are 5 - 6 times more resistant to x-ray induced division delay (2). It would be valuable to know to what other effects in budding yeast this radiation immunity applies. Beam has shown very clearly that diploid cells in the state of budding are immune to recessive lethals and if this is applied to haploid cells can account for their extreme resistance to radiation-induced lethality. In this respect, some preliminary experiments of Mortimer (Table 2) show that budding cells are no more resistant to x-ray induced dominant lethality than are non-budding cells; in fact, most of the lethality in budding cells can be accounted for by dominant lethals.

The relation between radioresistance and ploidy has been discussed on many occassions previously and Magni in his excellent talk gave a very clear summary of this subject. The data available generally support the conclusion of an increase in resistance from haploid to diploid or triploid followed by a progressive decrease in radioresistance with further increases in ploidy. These findings have been explained by a decrease of recessive lethal inactivation accompanied by an increase of dominant lethal inactivation with increasing genome number. Superimposed on this general relationship of radioresistance and ploidy however are variations in resistance between strains of the same ploidy (12). Thus ploidy and division stage alone are not the only cellular parameters controlling radiosensitivity. Some of the possible explanations for this variability, including degree of heterozygosity, were discussed by Magni. The evidence which he reported for genic control over the mode of x-ray inactivation during a stage

of meiosis certainly is of great interest in this respect. Much more [illegible] genetically controlled stocks, is still necessary to fully explore this [illegible] area of research.

In closing, I would like to express the hope that more of the studies of physico-chemical and biological modification of radiation response in [illegible] will concern themselves, not only with lethality, but consider in parallel such effects as reversion, mitotic segregation, division delay and physiological effects. With such studies interrelations should be possible and our insight into the radiobiology and genetics of yeast greatly increased.

REFERENCES

1. Atwood, K.C., Von Borstel, R.C., and Whiting, A.R., Genetics 41, 804-813 (1956).

2. Burns, V.W., Radiation Research 4, 394-412 (1956).

3. Gunther, R.L., and Mortimer, R.K., unpublished.

4. James, A.P., and Lee-Whiting, B., Genetics 40, 826-831 (1955).

5. James, A.P., Proceedings 10th International Congress of Genetics, Montreal (1958).

6. Hutchinson, F., Preston, A., and Vogel, B., Radiation Research 7, 465-473 (1957).

7. Lindegren, C.C., "The Yeast Cell - Its Genetics and Cytology." Educational Publishers, St. Louis (1949).

8. Magni, G.E., Compt. rend. trav. lab. Carlsberg 26, 273-284 (1956).

9. Mortimer, R.K., Radiation Research 2, 361-368 (1955).

10. Mortimer, R.K., Lerner, R.S., and Barr, J.K., Univ. of Calif. Radiation Lab. UCRL-3746 (1957).

11. Mortimer, R.K., unpublished.

12. Mortimer, R.K., Radiation Research, in press.

13. Pittman, D.D., J. Bacteriol. 71, 500-501 (1956).

14. Roman, H., Compt. rend. lab. Carlsberg 26, 299-314 (1955).

15. Roman, H., and Jacob, F., Compt. rend. acad. sci. Paris [illegible]

16. Winge, C., and Lauritzen, C., Compt. rend. trav. lab. Carlsberg [illegible]

Table I

Manifestations of Radiation Damage in Yeast Cells

	Lethal	Non-Lethal
Genetic	1) Recessive lethal 2) Dominant lethal	1) Biochemical, morphological, etc. mutations. 2) Mitotic crossing over 3) Allelic recombination
Non-Genetic	1) Membrane destruction? 2) Lysis	1) Membrane damage 2) Division delay 3) Effects on fermentation and respiration 4) Petite production 5) Enzyme destruction

Table 2

Dominant Lethals in Budding Haploid Yeast

A. Budding cell (37.5 krad) x Non-budding cell (0 krad)

No. of zygotes = 50

No. of viable zygotes = 15

Frequency of dominant lethals in budding cells 0.70

B. Budding cell (37.5 krad) - alone

No. of cells = 53

No. of viable cells = 16

Frequency of lethals in budding cells 0.70

FIGURES

1. Upper Graph: X-ray survival curves for the three diploid cultures heterozygous for different adenine loci.

   Lower Graph: Percentage of diploid colonies exhibiting the recessive phenotype (whole and sectored) as a function of X-ray dose. The diploid cultures used were singly heterozygous for each of three morphologically identifiable loci concerned with adenine synthesis (Roman, 14). Most of the variant colonies can be shown to be a consequence of mitotic crossing-over.

   Also included along the abscissa of the lower graph is a curve showing the absence of variant diploid colonies when only the dominant haploid parent of one of the crosses was irradiated immediately before mating.

2. X-ray induced allelic recombination (15) at the arg_4 locus in Saccharomyces. The curves show the frequency of arginine independent colonies arising from cells of each of three arginine defendent crosses. Each of the crosses contains two mutant alleles of the arg_4 locus. One is homoallelic for the arg_{4-1} allele, and one is homoallelic for the arg_{4-2} allele. The reversion frequencies of these cultures is indicated in the lower curves (interpolated from values at higher doses). The upper curve is for a culture heteroallelic for the two alleles and a much higher frequency of reversion is observed. This parallels closely the results reported for ultraviolet (15).

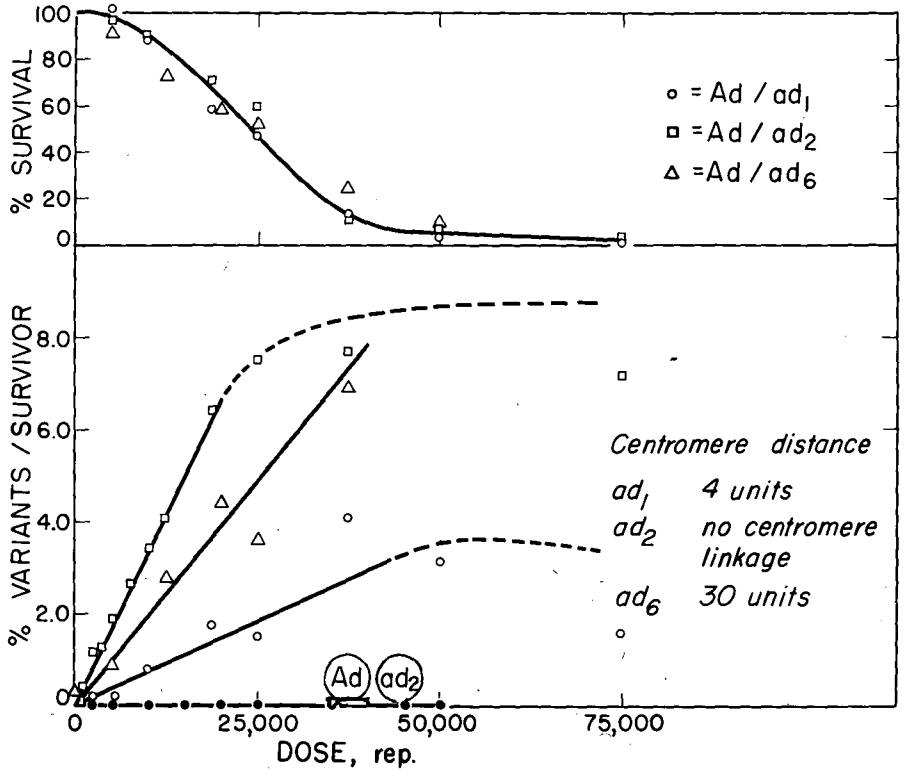

% SURVIVAL
100
80
60
40
20
0
o = Ad / ad1
□ = Ad / ad2
△ = Ad / ad6
% VARIANTS / SURVIVOR
8.0
6.0
4.0
2.0
0
Centromere distance
ad1 4 units
ad2 no centromere linkage
ad6 30 units
Ad
ad2
0
25,000
50,000
75,000
DOSE, rep.

Fig. 1

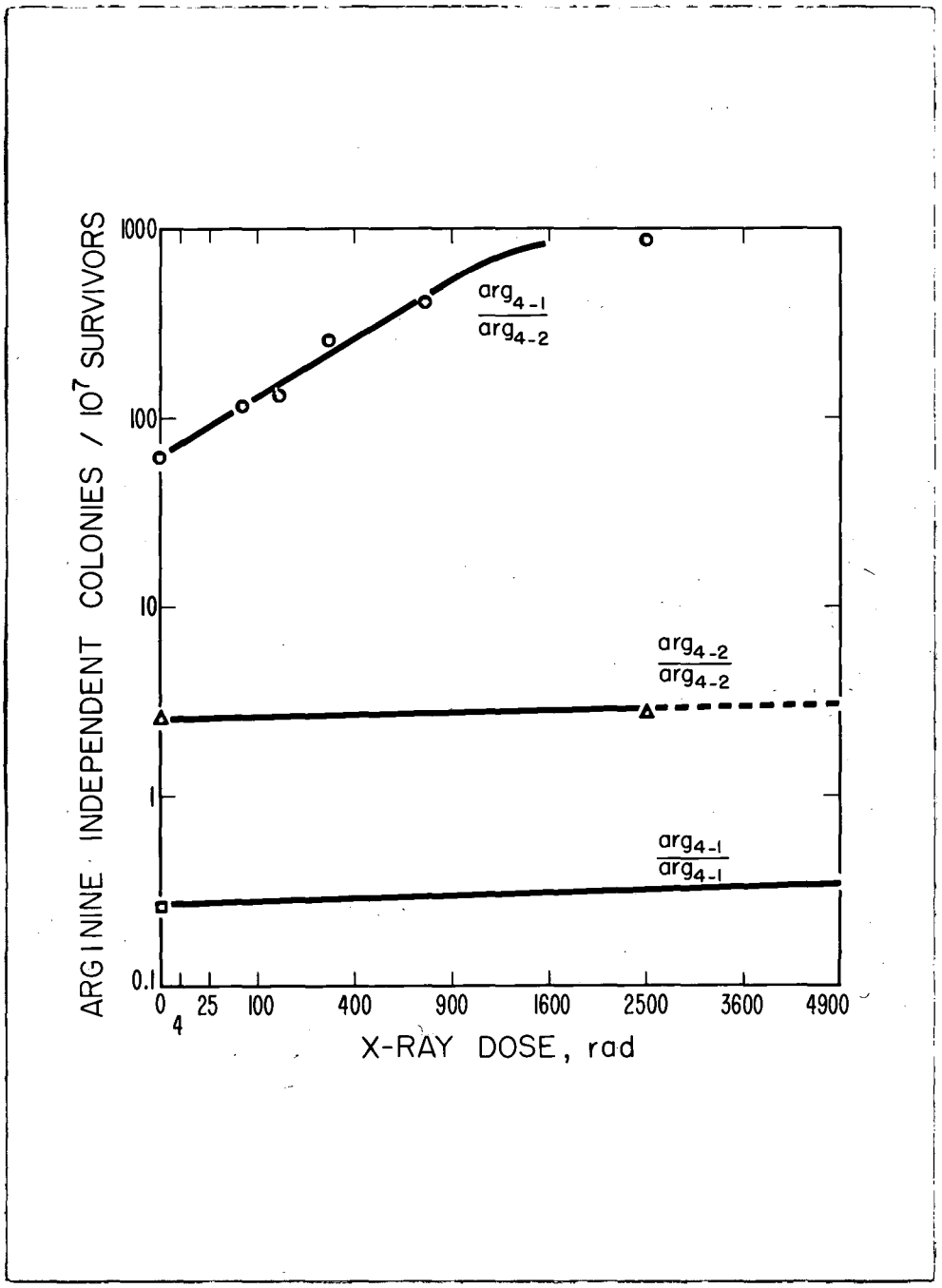

ARGININE INDEPENDENT COLONIES / 10^7 SURVIVORS
1000
100
10
1
0.1
$\frac{arg_{4-1}}{arg_{4-2}}$
$\frac{arg_{4-2}}{arg_{4-2}}$
$\frac{arg_{4-1}}{arg_{4-1}}$
0
4
25
100
400
900
1600
2500
3600
4900
X-RAY DOSE, rad

Fig. 2.