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ATTEMPTED ANALYSIS AND CORRELATION OF VARIOUS RADIOBIOLOGICAL ACTIONS ON THE SAME KIND OF CELL (YEAST)

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INTERNATIONAL CONGRESS OF RADIATION RESEARCH

University of Vermont

Burlington, Vermont

August 10-16, 1958

Symposium: "Attempted Analysis and Correlation of Various
Radiobiological Actions on the Same Kind of Cell (Yeast)

Chairman's Introduction

by

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All through the ages, as far back as history records, man has made use of yeast cells and their fermentative powers sometimes knowingly, sometimes without the realization that living cells were involved. Yeast spores are found almost everywhere in the temperate zones: in the soil, on and in plants, in animals, in the air and even in the ocean. These cells supply the leaven to man's most important food: his daily bread. For thousands of years these tiny organisms have helped invisibly to keep man's spirit alive in the medicines and spirits he prepared for himself. The Roman scientist Pliny the Elder mentioned, in his "Natural History" (77 B.C.), the fermented spirit known as "bion" (from $\beta\lambda\omicron\nu$ meaning life), which was used to

cure many ills of the body. It is of interest that even in our modern days hardly a year passes without a scientific report on the effect of alcohol and of yeast extract on a number of diseases, including the radiation syndrome, and cancer.

The ancients did not know that fermentation was caused by a living organism, and when the chemical process became better known, scientific arguments ensued over some hundreds of years between those who maintained that fermentation was an ordinary chemical process, and those who believed that it may be present only in living cells. Leeuwenhoek, inventor of the microscope, described structures that we now know as yeast cells but could not settle the question whether they were alive or not. Lavoisier, the French chemist, working on the balance of a number of chemical reactions, showed that the alcohol and carbon dioxide gas formed in fermentation weighed the same as the sugar consumed; so this process became one of the cornerstones of the chemical law of "conservation of mass." For yeast cells, of course, this is not strictly true since there is synthesis of protein and of various by-products during fermentation. In 1837 Cagniard de la Tour and P. Kützing observed yeast cell division (budding) and in 1839 R. Schwann demonstrated the formation of spores. From this time on many distinguished names were to be connected with biological research on yeast cells. Perhaps the greatest light was shed by Pasteur who described the aerobic and anaerobic metabolism of these cells in detail, defined the competition between the two modes of metabolism, and showed that the cells can multiply themselves in media containing sugar, ammonia and inorganic substances. These

discoveries set the stage for the development of a new science: biochemistry. One of the early triumphs of this science was the isolation of the first pure enzyme, invertase, from yeast cells by Fischer and Buchner in 1895. Today the detailed biochemical processes occurring in fermentation compose one of the brightest chapters in biochemistry (16).

It is no wonder that yeast cells have also become of interest in radiobiology and genetics, and as the contributions to the present symposium attest, work with these tiny organisms is proving to be stimulating for the development of another new field: cellular biophysics. One should first mention the academician Madson who, in the early 20's before the discovery of mutations, had already observed profound morphological variations and permanent modifications in colonies of yeast cells following roentgen irradiation (15). The late French biophysicist, Holweck, with Lacassagne, first applied quantitative reasoning to yeast survival curves (5). Unknown to some may be the work of Koofhourew, who twenty years ago discovered many salient facts of yeast radiobiology with ultraviolet light, including the observation that cells killed by radiation can and will secrete materials into the medium which protect other cells and promote their growth and division (11). It is only in the current years that principles such as these are being applied to the survival of animal and human cells.

Radiobiologists generally use the strain Saccharomyces cerevisiae, the clones of which contain unicellular and uninucleated cells. Their size is about 5 to 7 microns and they are a simple oval in shape.

The morphology of these cells has been studied by the light microscope with various staining techniques (23), by the ultraviolet

microscope (3), and by the electron microscope (1). Also detailed studies were made by Meisel by in vivo fluorescence microscopy (13). In spite of all these efforts numerous disagreements still exist with respect to intracellular structure. Simply described, the cells have a wall perhaps 800 Å⁰ thick. The double cell membrane lies just inside the wall. Each cell has one large vacuole, a feulgen staining nucleus external to this and many mitochondria, rich in RNA. Fat inclusions may also be easily demonstrated. The cells can proliferate on simple synthetic media, they may be handled equally well in mass cultures, single cell isolates or may be manipulated individually under the microscope. When exposed to X-rays, diploid cells have proven much more resistant than haploids (8) (26). The survival curves which were obtained with unusual precision (24) seem to prove that a single event is sufficient to inhibit the haploid cells from forming colonies, while diploid inhibition requires multiple hits. Explanation of the dose effect relationship in a variety of environmental conditions as well as with radiations of different linear energy transfer became necessary. Among several theoretical models are the target theory of Lea (9), the intermediate action theory (26), the track segment theory of Wijman (22), the direct action theory of Alper and Flanders (2), the multiple ion cluster model of Flanders (6), the model of successive states of Parker (17), and the information theory model of Yockey (25). Controversial as these ideas may be, they are most helpful for furtherance of knowledge, since each model stimulates experiments and eventually leads to a better model.

One great advantage of using yeast cells is that both mitotic and meiotic division mechanisms are readily available. Figure 1 shows the life cycle of Saccharomyces cerevisiae, which we know so well due to Winge (25) and Lindgren (10). Normally the diploid cells grow well in stable fashion, forming vegetative colonies. Whenever desired, given proper nutrients, the diploid cells can be made to sporulate, giving rise to four or less haploid spores. The spores may be observed under the microscope, dissected, and individual haploid spores isolated. These then can be grown into haploid vegetative colonies. When cells of different mating types of the haploid mating type are brought together they form a zygote. Diploid cells may then be derived which will grow in vegetative colonies again. By this method, which is sometimes also known as tetrad analysis, utilizing the spore formation, accurate analysis may be made of the genetic variation in clones of cells. From the point of view of radiation damage, it is possible to carry in the diploid lethal genetic damage in the recessive form which might kill the haploid cells, but the diploids will remain alive and the damage may be reproduced any time in its unpaired form when a spore is prepared in the haploid phase (14) (19). Another equally important mode of radiation damage is the "dominant" lethal form (7) to be described in detail by the speakers.

In recent years detailed genetic and mutational mechanisms have become of interest. Yeast cells follow classical genetics. Many biochemical markers are available for mutations and many morphological markers are also known. Altogether, there is a great amount of

information available now, including some linkage maps which may soon be available to all those who wish to do detailed genetic work with the cell. It has been shown by Ephrussi that yeast cells possess cytoplasmic particles which inherit through the mechanism of cytoplasmic inheritance, whereby it is quite possible to compare genetic effects at the cytoplasmic and nuclear levels. The possibility for finding phage particles and DNA transformation is being explored in various laboratories.

Yeast cells can grow and divide both anaerobically and aerobically, whereby two basic biochemical modes and conversion from one to the other under the action of radiation may be studied. It is believed by some that the shift between anaerobic and aerobic processes may be one of the clues to the heart of the cancer problem. Several authors have drawn analogy between processes in yeast cells and carcinogenesis, either following radiation or after administration of carcinogenic chemicals (7), (4), (12), (18), (20).

In considering the papers presented at this symposium it will be apparent to the reader that a firm framework for the mechanisms of radiation damage has been erected. Water and oxygen are important modifiers of radiation action, and Thomas Wood's contribution argues clearly for the thesis that $4/5$ of the radiation effect on colony formation is indirect, mediated by the aqueous medium, whereas perhaps $1/5$ is direct, presumably on some essential part of the nucleus. There are some cytoplasmic enzyme systems, however, with a different ratio of direct to indirect radiation action.

The various types of genetic damage and methods of testing for these are clearly outlined in the contribution of G. Magni. There seems to be no reason why we should not obtain considerable quantitative information in the future with respect to the yield of each kind of damage at many different loci. Quite definite information is also available about some biological and genetic processes, to be outlined by James and Mortimer. These include mitotic cross-over, reversion, and probably other classes not yet completely understood. A puzzling chapter of radiobiology is the great increase in radioresistance of budding cells, to be outlined by Carl Bean. When we learn the reasons for the great variations in radiation sensitivity, we shall also know much more about chemical states of DNA and detailed chemical processes during its self duplication. There are impressive gains in the understanding of biochemical changes that develop in yeast cells following radiation damage interpreted by Aser Rothstein. This knowledge may increase considerably when the biochemical work can be carried out on clones of cells carrying definite minute genetic defects.

There are other fields of yeast radiobiology where much new exploration is needed. We now know that radiation damage is frequently subgenic in character: that is, the same gene may be damaged in many different ways; this leads to subgenic differences which may reflect themselves in defects in biosynthesis, in different reversion rates, and in mutual interaction of genes of higher ploidy organisms. Further exploration of radiation damage of this kind may lead to new knowledge in the field of chemical structure of genes and chromosomes. Also clues might be obtained with respect to the molecular "code" and

information transfer between genes and cell constituents.

Further novel results are expected when entire populations of yeast cells will be exposed to radiations in different states of proliferation. A modest beginning has already been made (21) giving clear indication that populations have adaptive power to radiation environment. Values with respect to the welfare of the individual appear to be at variance with welfare of a population in the course of evolution. Acute experiments should be extrapolated to conditions of chronic irradiation only with great caution.

It has been estimated that there are about 70,000 species of fungi alone, and it is very unlikely to assume that radiobiological findings applicable to a single species would in all details be applicable to all species of fungi, let alone to all plant cells or to cells of the animal world. Nevertheless, a basic similarity does exist between radiation effects on all species of cells. It is our hope that the number of possible mechanisms will be reduced to a few and that the findings of yeast radiobiology will be used as much in biology as other discoveries made in yeast at earlier times.

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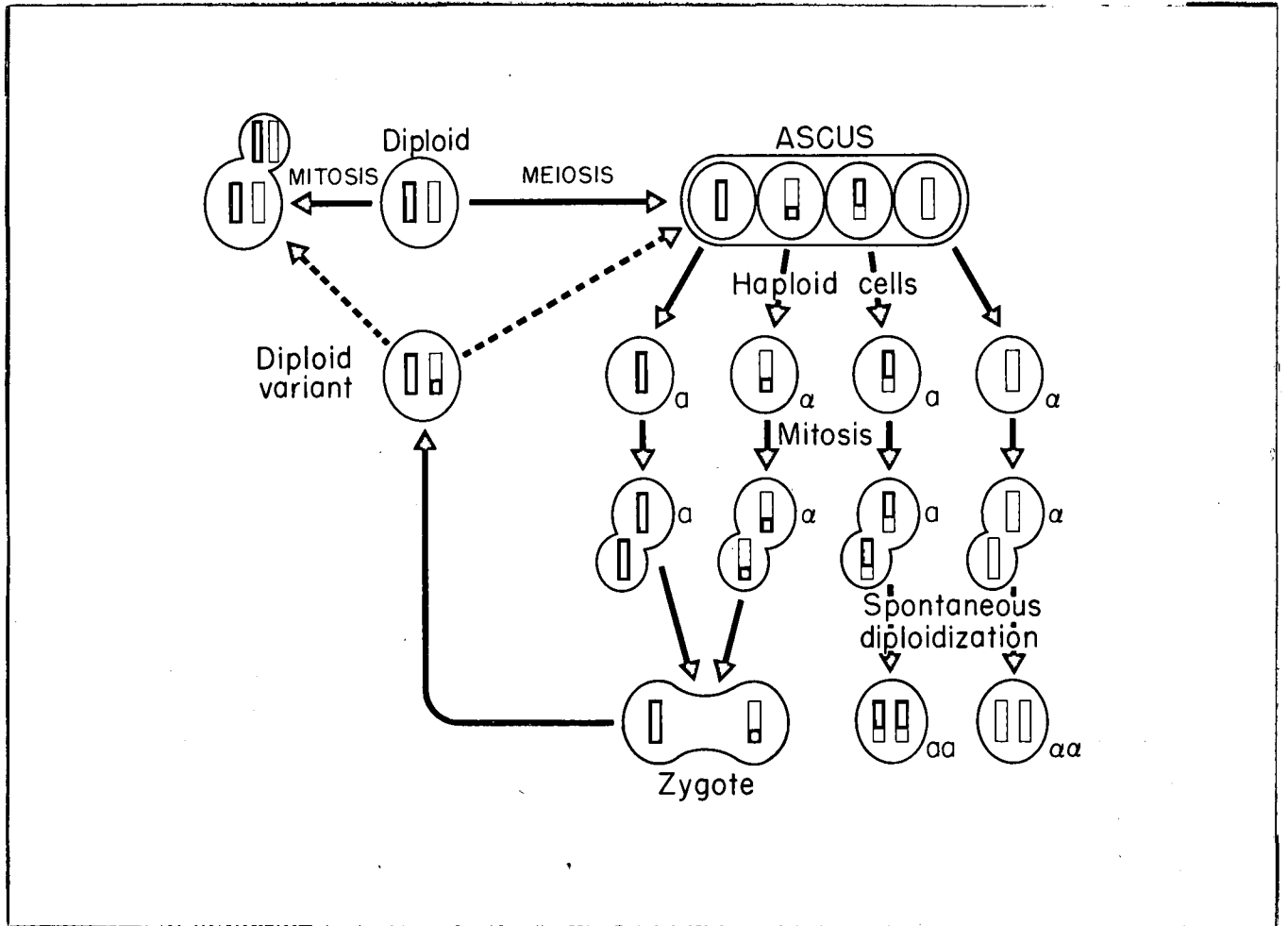


Fig. 1. Life cycle of *saccharomyces cerevisiae*