# A STUDY OF THE EFFECT OF GAMMA RADIATION ON SPORULATION AND GROWTH OF YEAST

# A STUDY OF THE EFFECT OF GAMMA RADIATION ON SPORULATION AND GROWTH OF YEAST

By

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SCOPE AND CONTENTS: The present study was initiated with the purpose of determining and comparing the effect of gamma radiation on the capacity of yeast cells to grow and sporulate. Using a new technique by which irradiated and non-irradiated yeast cells could be scored directly, it was found that sporulating yeast cells were more sensitive to radiation than growing cells, and that the inactivation of the capacity of an irradiated yeast cell to sporulate did not affect its ability to grow. Observations on irradiated sporulating cells indicated that spore-formation and reduction division of the nucleus, are closely allied phenomena .

A short discussion of a probable mechanism of action of gamma radiation on sporulating yeast is included, together with suggestions for future research.

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# I N T R O D U C T I O N

## Historical survey

Whereas qualitative radiation studies on various micro-organisms have been reported as early as 1871 by Martinand, no such reports were made concerning yeasts before 1884, when Kny studied the effects of gas-flame light on the reproductive rates of Saccharomyces cerevisiae. Lohmann (1896) reported that whereas electric light had little or no effect on yeast reproduction, diffuse day-light and strong sun-light were inhibitory. Although of historical interest these studies are of no fundamental importance.

The year 1895 was a turning point in radiation studies. This was the year when Wilhelm Konrad von Roentgen discovered X-rays. Within a year after Roentgen's discovery, Henri Becquerel in France showed that uranium ore also gave off similar penetrating radiations. Both observations were of an immediate importance to Physics and Chemistry and also to Medicine. The following years saw a great upswing in studies on the effects of radiation on living material, employing ultraviolet light, various spectral colours, radium and uranium emanations, and, of course, X-rays. While the primary interests were of a clinical nature, fundamental studies

were not neglected.

Among the early workers who studied the effects of radiation on yeast, mention should be made of Buchta (1914), who repeated the studies of Kny and Lohmann, and extended them by including ultraviolet light. He showed that yeast cells were killed by long exposures to ultraviolet light but that short exposures only inhibited the budding process. The first recorded study of the action of emanations from radioactive substances on yeast was by Jacquemin and Giurel (1914) who showed that these stimulated alcoholic fermentation and made the transformation of sugar more complete. A study on the action of radium emanation on the vitamins of yeast was undertaken by Sugiura and Benedict (1919), who demonstrated that growth-promoting factors in yeast may be partially inactivated by means of exposure to radium emanations.

An independent series of qualitative studies by the academician Nadson (after whom the yeast genus Nadsonia was named) and his collaborators in the 1920's described the effect of radium on yeast. They observed, before the discoveries of mutations due to ionizing radiations, morphological variations of a more or less permanent character in colonies of irradiated yeast. They also indicated that X-rays affect cell division of yeast more than they do the metabolic processes. Nadson's intentions were of an applied nature in baking and brewing industries, where the introduction of new strains was of prime importance to the quality of these products. At approximately the same time Wels and Osann (1925) positively demonstrated

that respiration of yeast cells, as measured by the $O_2$ consumption and $CO_2$ production suffered little or no change as the result of irradiation with X-rays, yet the reproductive rate was greatly inhibited. These latter workers concluded that the growth-inhibiting effect of the rays did not involve the energy exchange of the cell and thus definitely separated for the first time nuclear and cytoplasmic effects of radiation.

These encouraging results led Holweck and Lacassagne (1930) to quantitative studies on the survival of irradiated yeast. They determined the dose-effect relationship for such survival, and showed that 10,000 roentgens ( r ) of X-radiation delivered to yeast did not cause immediate death of the cells. Such irradiated cells divided for a time, but eventually ( i.e. at or following the next few divisions ) died. A triple dose ( i.e. 30,000 r ) caused immediate death of 50% (1) of the cells in the population so exposed. Thus it was shown that inhibition of cell division manifested itself as either "immediate death" when high radiation doses were applied, or as "delayed death" when low radiation doses were administered. These workers also observed that budding cells were more resistant to X-radiation than were resting cells of yeast. The inactivation

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(1) This is conventionally written as $LD_{50}$. This is the radiation dose required to kill 50% of the treated organisms. The reason for not using 100% for comparative purposes is that any biological community group of organisms of the same species shows variation in sensitivity. Therefore a more accurate estimate of the average sensitivity of a population is obtained by determining the $LD_{50}$ dose.

curves obtained were of a sigmoidal nature.

A year later Wyckoff and Luyet (1931) irradiated growing yeast cells with X-rays, and noticed that a large fraction of the resulting population consisted of double cells (mother cell and one daughter cell). Such double cells lived in an apparently healthy condition for days following irradiation, as indicated by eosin staining which the authors used as criterion of cell death. These workers could not explain the tendency to produce only one daughter cell. No nuclear stain was used. Survival curves were again of a sigmoidal nature.

The sigmoidal curves of both Holweck and Lacassagne (1930) and Wyckoff and Luyet (1931) were not accounted for at the time. After Winge (1935) elucidated the complete life cycle of Saccharomyces cerevisiae, showing that haploid and diploid cells occurred, which was confirmed by Lindegren (1945 ) the sigmoidal character of yeast survival curves was correlated to diploidy by Frilley and Latarjet (1944). It remained, however, for Latarjet and Ephrussi (1949) to show conclusively, that diploid cells of yeast were much more resistant to X-rays than were haploid cells (which gave exponential survival curves). Thus for the first time sensitivity of microorganisms to ionizing radiations was related to the ploidy of the cells. The fact that haploid yeast gave exponential survival curves and that diploid yeast gave sigmoidal survival curves was used by Magni (1953) for taxonomic purposes - i.e. by determining the X-ray survival curves of unknown yeasts, he was able to know whether to place them in haploid

or diploid genera. This is also of importance for the application of traditional genetic analysis, because one must know whether the organisms studied are haploid, diploid, or even polyploid.

The 1940's showed no increase with regard to yeast radiation studies as can be ascertained by the relative number of reports on this subject. It was indicated that the sensitivity of yeast cells to X-irradiation was affected by varying the oxygen tension during the irradiation period (Anderson and Turkewitz, 1941); respiration studies were taken up again (von Euler, 1942); comparison of biological effects of X-rays, alpha-particles, gamma-rays and neutrons were carried out (Gray et al, 1943; Gray, 1949), and effects of ionizing radiations on enzyme activities of yeast cells were reported (Sherman and Chase, 1949).

By 1950 it was established that the primary action of ionizing radiations on cells was genetic in nature. The work in the subsequent decade tended to emphasize the study of variation of radiation sensitivity under different physico-chemical conditions, and bio-chemical changes associated with the action of ionizing radiations on cells.

Thus Ting et al (1952) compared the effect of 0.2 Mev X-rays and 23.5 Mev X-rays on four different yeast strains. Although the kinetics of inactivation for the different strains varied, they were similar for each particular strain. In the same year Birge and Tobias (1952) showed that the growth of yeast cells under aerobic and anaerobic conditions after irradiation made no significant

difference to the outcome of the survival curve, but found that there was a definite dose-reduction factor of approximately 2 for cells irradiated in the complete absence of oxygen as compared with cells irradiated in air. Extensive studies by Wood (1953, 1954) on the influence of temperature on the survival of X-irradiated yeast showed that whereas in the temperature range of 10 - 40° C the yeast sensitivity changed insignificantly, an approximately twofold increase was noted when the suspension medium changed from the frozen to the liquid state. In the temperature range of 45 - 55° C, the X-ray sensitivity of yeast increased rapidly, indicating synergism between heat and X-ray inactivation. Investigation of the X-ray sensitivity of yeast as a function of hydrostatic pressure was ably conducted by Burns (1954), who observed no changes in the radiosensitivity of yeast cells for pressures of up to 10,000 p.s.i. during irradiation. These results were considered as evidence against the association of large molecular volume with the production of the primary lesion.

Elkind and Beam (1954) demonstrated that in yeast the relative effectiveness of X-rays and alpha-particles varied with changes in the physiological state and stage of cell division, thus emphasizing the necessity of biological as well as physico-chemical interpretations of the relative effectiveness of various radiations.

Reports on the sensitivity of metabolic systems in vivo comprise a range of extremes. Whereas Baron et al (1953) showed that respiration was unimpaired even after a dose of 380,000 r , one year later E. S. G. Barron (1954) also demonstrated that doses as small as

500 r can irreversibly inactivate some fermentative enzymes.

Such a great deal of variation in the response of metabolic mechanisms could only be attributed to differences in experimental conditions during the irradiation period and/or post-irradiation treatments. Bair and Stannard (1955) and later Barber et al (1957) showed that much of this variation depended on the time allowed to elapse between the irradiation and the testing periods.

Burns (1956) took up again the problem of inhibition of cell division in yeast. He showed that for interdivisional cells (non-budding) doses of up to 10,000 r caused little delay in the appearance of buds (2nd generation). On the other hand, a sixfold increase in delay was observed in the time of appearance of the 3rd generation. This delay time increased with an increase in dose. The 4th generation division time is little affected. A Russian worker (Meissel, 1956) observed similar delayed separation of buds, incomplete fission and nuclear division. He attributed this to the enlargement of irradiated nuclei during division and their inability to divide. Another Russian study (Korogodin, 1957) determined the survival of yeast by macro-colony counts. By making these counts after different time intervals he was able to show that radiation after-action consisted in a reduction of the average speed of growth. He proposed a mathematical equation which he considered to account for his observations. At the same time, Welch (1957) studied the effects of chronic exposure to X-rays on a steady-state population of Saccharomyces cerevisiae. He showed that continuously-grown cultures of yeast had the power to adapt to low

levels of radiation. His conclusions were based on results which he obtained w ith yeast which could live in a steady state of proliferation while being continuously exposed to X-radiation of 6150 r per generation (approximately 2,000 r/hr) . This was a dose per gene/ration equal to about 1/4 of $LD_{50}$ doses for acute exposure.

Two important reports by Bruce and Stannard (1958, 1959) have partially clarified the role played by cellular potassium after X-irradiation of yeast cells. These stud ies showed, that the permeability of yeast cell membranes was increased by relatively small doses of X-radiation. The losses of cellular potassium were greatly magnified when potassium-ion-free buffers were used. Bruce (1958) also studied this phenomenon under aerobic and anaerobic conditions. He reported that survival after X-radiation under aerobic conditions was reduced five times as much as was the potassium retentivity of the cells. On the other hand, survival of yeast cells irradiated with X-rays under anaerobic conditions was about twice as high as under aerobic conditions. The response of potassium retentivity to X-irradiation at $25^{\circ}$ C under ana erobic conditions was slight below 160,000 r , at which dose the retentivity abruptly decreased to that observed under aerobic conditions. Lowering the temperature to $0^{\circ}$ C moved the point of decline to about 300,000 r . Bruce concluded that such differential effects were indicative of interaction of radiation with the yeast cell at sites that independently controlled survival and the retention of potassium.

Rothstein (1959) has emphasized that the role of the cell membrane, particularly its permeability properties, is important in determining the cell's response to radiation. His cell membrane studies showed that the distribution of sensitive sites and of protective substances was unhomogeneous, so that the radiation sensitivity of different parts of the cell varied. Alexander (1961) suggested that although reactions due to damage to proteins and nucleic acids occurred in irradiated cells, they were not the primary reactions leading to the death of the cell. He suggested that phospholipids of the intracellular membranes were the more probable sites of primary damage.

Thus the current trend of radiobiological research concentrates on the permeability problems and properties of cell membranes.

Purpose and objectives of this study

The physical nature of radiation and the methods whereby radiant energy is absorbed by molecules are now basically understood. In contrast to this physico-chemical knowledge of the action of ionizing radiations at the molecular level, stands the incomplete and vague picture of how ionizing radiations affect living cells. The biologist, studying the effects of radiation on living organisms, must cope with the complex problem of radiation action on biological material. He can observe the end products of this action, manifested as cytoplasmic changes, inhibition of cell division or of nuclear division, mutation, or abnormal growth, and thereby obtain information bearing

on the problem. The study of the action of radiation on various potentialities of the cell should also contribute.

Saccharomyces cerevisiae offers advantages for research on the effect of radiation on living cell. One of its most important attributes is the possession of unicellular haploid, diploid, and sometimes polyploid cells. By working with unicellular rather than with multicellular organisms one avoids numerous complications that can arise owing to interactions of tissues. Thus, in multicellular organisms, the changes that follow the primary event of energy transfer and result in symptoms of radiation damage, are likely to be much more involved than would be the case with one-celled forms. Among the unicellular organisms, protozoa have the disadvantages that their motility makes observations awkward, and their growth is difficult to control, owing to their complex nutrient requirements. The minute size of bacteria makes these organisms difficult material for cytological observations. In contrast, the yeast cell is larger than bacteria and has simple nutrient requirements. Furthermore, it has a life cycle, involving alternation of diploid and haploid phases. A diploid yeast cell has the two potentialities of either reproducing vegetively by budding, or sexually by sporulation. During the former the nucleus divides by mitosis and during the latter by meiosis. (1) Both phases can be

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(1) The present state of knowledge of yeast cytology does not warrant here the use of the terms "mitosis" and "meiosis", respectively, since these expressions imply the presence of chromosomes. No absolute demonstration of these in yeast has been offered as yet. When the terms "mitosis" and "meiosis" are used in the following pages of this study, the author wishes to point out that this is done only to avoid the cumbersome expressions "equational division" and "reductional division".

obtained at will in the laboratory by changing the environment. Whereas most bacteria do not exhibit a well-defined nucleus upon staining, the yeast cell nucleus, whether haploid or diploid, can be made visible relatively easily.

The combination of all these factors, and especially the availability of haploid and diploid phases of the same organism, make Saccharomyces cerevisiae very useful for the study of the action of ionizing radiations on living cells.

Many of the contributions on the action of radiation on yeast have centred on the relative sensitivity of haploid and diploid cells. The inactivation curve for haploid cells is exponential within a certain dose range, while diploid cells show a sigmoidal inactivation curve. However, no one has compared the effect of radiation on the capacity of the yeast cell to grow and sporulate. This is the principal objective of the present investigation. An incidental part of this study consists of an investigation of the effects of ionizing radiation on nuclear division. In other words, what relation does failure of nuclear division bear to failure to grow and sporulate, or what is the likelihood of an irradiated cell dividing without a corresponding nuclear division and vice versa.

In the present study the terms SPORULATION and GROWTH will always refer to the processes of spore and bud formation, rather than the end-products of these processes, unless it is specifically indicated otherwise.

The problems of the effects of ionizing radiations on living cells is not a secluded study. The possibilities for practical applications

of the fundamental knowledge of the effects of radiation on living cells are of direct concern to everyone. There is little doubt that once the basic processes of radiation damage to cells are understood, progress in radiation therapy and in protection against the damaging action of ionizing radiations will accelerate. The study of simple forms of life in this connection may aid in the understanding of similar processes in higher forms of life.

# MATERIALS AND METHODS

## Yeast isolate

The yeast culture employed during the course of this study was an isolate from packaged yeast (Fleischman), designated as F493 and used in previous studies on sporulation and growth in the Department of Biology, McMaster University. It is a strain of Saccharomyces cerevisiae Hansen.

## Yeast stock culture medium

This medium was used to maintain the yeast stock cultures, which were transferred once a week to fresh slants. The yeast stock cultures were kept in a refrigerator between transfers.

Composition: 150 ml distilled water
3 gm Difco Agar
1.5 gm glucose
1.0 gm Difco Yeast Nitrogen Base (YNB), which is a chemically-defined medium, containing all the essential nutrilites and vitamins for the cultivation of yeast, except a source of carbohydrate. The medium was dispensed in 5 ml quantities to 16 mm test tubes, autoclaved at 15 psi pressure for 15 minutes, and then slanted.

## Buffer

In all cases, unless otherwise specified, M/20, pH 5 potassium

hydrogen phthalate (KHP) buffer was used. The pH of the buffer was always checked during preparation with a Beckman Zeromatic pH meter and standardized against commercially supplied pH 7 buffer (Beckman). Freshly prepared buffer was used for each experiment.

Presporulation medium

The presporulation medium was a chemically defined medium, containing optimal concentrations of glucose and Yeast Nitrogen Base in KHP buffer.

Composition: 36 ml M/20, pH 5 KHP buffer,

4 ml of buffer, containing 10% glucose and 1.33% YNB.

Sterilization was done by passing the solution through a Seitz filter. The medium was dispensed in 40 ml volumes in 25 mm test tubes, which had previously been plugged with cotton wool and sterilized in the oven. The cotton plugs were replaced with pieces of ultraviolet-sterilized Parafilm and the test tubes were stored in cupboard until needed. When preparing a presporulation culture, the medium in a 25 mm test tube was inoculated with yeast cells and then poured into a sterile Kolle flask, which was incubated on its flat side to insure maximum aeration. The presporulation cultures were grown for two days at 27° C , and the cells thus obtained were used for growth experiments as well as for sporulation experiments.

Millipore Filter membranes

Ten-cm square sheets of Millipore Filter (MF) membrane of type TV, 20 /u thick, and with a pore size of 50 m/u, were cut into pieces 5 mm X 10 mm, removing one corner of each as a marker. The pieces were sterilized by autoclaving in distilled water in batches of 50 per flask, and the flasks were stored in the refrigerator until needed.

The individual membranes were placed on three Whatman filter papers ( two No. 3, 9 cm circles, with one 7 cm circle on top) in a petri dish and moistened with 5.5 ml of KHP buffer. The yeast cells were seeded on the membranes using a 2 mm platinum loop for sporulation cultures and a 3 mm loop for growth cultures. The suspension liquid was drawn through the pores of the membrane by capillary forces, leaving the yeast cells on the membrane surface. The membranes were then transferred to either another petri dish (with the same number of filter papers) for staining, or to porcelain blocks partly immersed in culture media, for growth or sporulation.

Porcelain blocks

Blocks 10 mm wide, 7-10 mm thick and 40 mm long, cut from an unglazed porcelain drying plate, were used as support for the Millipore Filter membranes in the growth and in some of the sporulation cultures. When the lower part of the blocks was immersed in the respective medium, the liquid penetrated through the porcelain

to the upper surface by capillary action. The seeded membranes were placed on this upper surface of the block, thus exposing the cells to the medium. Each block accomodated six to eight MF membranes.

Since it was important that the blocks be free from impurity, the following procedure was adhered to: the blocks were washed for 24 hours in slowly-running tap water, after which they were allowed to stand in distilled water for another 24 hours. The distilled water was changed six times during this period. The blocks were then dried and heated to redness for six hours in a crucible. When cool, they were transferred to sterile petri dishes and kept in the refrigerator until needed. A set of freshly purified porcelain blocks was used in each separate experiment.

Growth cultures

Growth experiments were done using 175-ml pharmaceutical bottles lying on their flat sides. Each bottle contained seeded membranes, resting on porcelain blocks in 4 ml growth medium of the following composition.

30 ml M/20, pH 5 KHP buffer,

0.052 gm sodium acetate (anhydrous),

0.4 ml 10% $(NH_4)_2SO_4$

1.3 ml NCFV solution.

The NCFV solution was prepared by mixing two solutions, one containing the necessary minerals and trace elements, designated NCF (Nitrogen

and Carbon Free) ; and the second containing the necessary vitamins ( V ) . Essentially, the NCFV medium was the same as the Yeast Nitrogen Base, except for the omission of l-Histidine HCl, dl-Methionine, dl-Tryptophane, Folic acid, p-Aminobenzoic acid, and Riboflavin, and the nitrogen source.

The NCF solution was prepared by dissolving the following salts and compounds supplying trace elements in 1000 ml of distilled wa ter.

| | | |
|---|---|---|
| Mineral salts: | $KH_2PO_4$ | 25.00 gm |
| | $MgSO_4$ | 12.50 gm |
| | NaCl | 2.50 gm |
| | $CaCl_2$ | 2.50 gm |
| Compounds supplying trace elements: | | |
| | $CuSO_4 \cdot 5H_2O$ | 0.0010 gm |
| | $H_3BO_3$ | 0.0125 gm |
| | KI | 0.0025 gm |
| | $FeCl_3 \cdot 6H_2O$ | 0.0050 gm |
| | $MnSO_4 \cdot 1H_2O$ | 0.0100 gm |
| | $Na_2MoO_4 \cdot 2H_2O$ | 0.0050 gm |
| | $ZnSO_4 \cdot 7H_2O$ | 0.0100 gm |

The solution was sterilized by passage through a Seitz filter, and dispensed in 100 ml quantities to sterilized 175-ml pharmaceutical bottles.

A 40 ml volume of distilled water and the following components made up the vitamin solution:

| | |
|---|---|
| Biotin | 0.0002 gm |
| Calcium pantothenate | 0.040 gm |
| Inositol | 0.2000 gm |
| Niacin | 0.0400 gm |
| Pyridoxine HCl | 0.0400 gm |
| Thiamine HCl | 0.0400 gm |

This solution was also filter sterilized and then dispensed in one ml aliquots to the 100 ml quantities of the NCF medium. The resulting solution was labelled NCFV medium.

Sporulation cultures

The sporulation medium consisted of autoclaved M/20, pH 5 KHP buffer to which acetate was added. This medium was dispensed in two ml quantities into 175 ml pharmaceutical bottles, to which the yeast suspensions were added to give a total volume of four ml per bottle. The final acetate concentration was 0.02 M, the same as in the growth medium.

Nuclear stain

Membranes bearing either yeast cells and microcolonies (resulting from growth on the membranes on porcelain blocks standing in growth medium) or cells from sporulation cultures were transferred to a petri dish containing three filter papers moistened with eight ml of 0.5 % sodium azide soltuion in distilled water, and left on it for 24 hours. This fixation procedure was followed

by hydrolysis to remove most of the RNA from the cells. For this purpose the membranes were transferred to a petri dish with filter papers, moistened with eight ml of 10 % perchloric acid and placed in a refrigerator compartment where the temerature was 1-4 ° C for a period of 96 hours. The membranes were then transferred to a plate with filter papers wetted with eight ml of double-strength Gurr's pH 6.8 buffer, and left there for 30 minutes for neutralization of the acid. This step was followed by staining for 24 hours on a plate with seven ml of double-strength pH 6.8 buffer mixed with 1.5 ml of Gurr's R 66 (Giemsa) stain. The membranes were then transferred to a petri dish containing filter papers, moistened with eight ml of the same buffer. This treatment lasted for 30 minutes and served to remove excess stain from the pores of the membranes. The membranes were next pinned by insect pins to a wax plate and air-dried for 15 minutes. When dry, the membranes were put on a thin layer of Gurr's Neutral Mounting Medium on a slide, and the medium was allowed to dry in an incubator (at 27° C ) in a plastic, dust-proof box for 2 days. The membranes were then covered with a No. 1 coverslip on which a thin layer of mounting medium was evenly spread out. The coverslip was gently, but quickly, pressed down and the mount was left to dry for at least 48 hours before microscopic observation. The stained nuclei appeared blue, while the cytoplasm had a pinkish tinge. This nuclear staining technique is described in more detail elsewhere (Miller, 1961). The modification used by the writer differs in that cells were treated for 24 hours

with azide instead of two, and the mounting medium was placed on the coverslips when the latter were added, instead of on the membranes. Also only a very gentle pressure was applied.

Spore stain

Membrane Filter rectangles bearing the yeast cells or microcolonies were transferred to a petri dish with three filter papers, moistened with eight ml of Ziehl's carbol-fuchsin stain, prepared according to the emended formula given by Conn (1957). The following two solutions were mixed.

| | | |
|---|---|---|
| Solution A: | Basic fuchsin (90 % dye content) | 0.3 gm |
| | Ethyl alcohol (95 %) | 10.0 ml |
| Solution B: | Phenol | 5.0 gm |
| | Distilled water | 95.0 ml |

After 30 minutes of treatment with this stain, the membranes were transferred to a plate with filter papers wetted with eight ml of 0.1 % ruthenium red in distilled water, and left on this plate for another 30 minutes. The membranes were then taken off and arranged on a glass slide on a thin layer of Gurr's Water Mounting Medium. The slide was put in a plastic, dust-proof box and left in the incubator at 27° C for two days to dry. When dry, a coverslip of suitable size with a thin layer of the same mounting medium was placed with a gentle, squeezing motion on top of the membranes. The complete slide was then stored until required for microscopic examination. The vegetative cells appeared pinkish, whereas the

spores retained the carbol-fuchsin stain and were dark brown to dark purple. This staining method, abbreviated to CFRR (carbol-fuchsin-ruthenium red) has been described in detail elsewhere (Miller and Kingsley, 1961) .

Dosimetry

A reliable method of measuring the amount of radiation to which cells are exposed is essential for any study of their response to radiation. At first a glass dosimeter was tried (Bausch & Lomb, F-0621). Each dosimter consists of a small (15 mm by 6 mm) piece of cobalt-activated borosilicate glass, the optical density of which changes at a certain wave-length in proportion to the amount of gamma radiation it receives. However, the reproducibility of radiation dose values was found to be modified by such factors as time of storage after irradiation (fading was noticed after two hours), temperature of storage, and presence of light during storage. The recommednation as to reading time was one hour after exposure to gamma radiation (Blair, personal communication) which was not possible. Furthermore, this dosimeter was found to be inaccurate at the low ranges (i.e. below 10 Kr) . For these reasons the cobalt glass dosimeter was abandoned.

The Fricke dosimeter, on the other hand, was found to be better suited for the present requirements. This is a chemical type of dosimeter, in which the amount of chemical reaction is proportional to the dose over a wide range; it is simple and convenient

to use; it is easily prepared from shelf-reagents; it is insensitive to light or small temperature changes, and it is well suited for doses in the range of 4-50 Kr. For these reasons it was adopted for use throughout this study.

The Fricke dosimeter solution was prepared according to Weiss et al. (1956) and contained 2.0 gm $FeSO_4 \cdot 7H_2O$ , 0.3 gm NaCl, and 110 ml of concentrated (95-98%) $H_2SO_4$ (analytical reagent grade) in sufficient distilled water to make 5,000 ml of solution. This solution was dispensed in 4.5 ml volumes in 16 mm Pyrex test tubes, which were sealed off with a high temperature oxygen flame. The dosimeter ampoules thus prepared were stored in a light-proof box in a cupboard until needed. For individual experiments the ampoules were firmly set into a plasticine layer of the Nalgene bottle containing the samples to be irrad iated.

After irradiation the optical density (D) of the irradiated sample was compared with that of unirradiated control solution in a Perkin-Elmer recording spectrophotometer, model Spectracord. For this examination the samples were placed in matched silica absorption cells (Beckman, 10 mm light path) and the absorption read at 305 m/u . This is the wave-length at which maximum absorption occurs.

The mechanism of the chemical reaction due to irradiation of the Fricke dosimeter solution involves the oxidation of the ferrous ion in an acidic solution, to the ferric ion.

The amount of radiation (R) is calculated from the

following formula given by Weiss et al. (1956) .

$$R(r/hr) = \frac{10^9}{e \cdot Y} \cdot \frac{D_{(sample)} - D_{(blank)}}{time\ (hr)} \qquad Eq.\ (1)$$

where e = the molar extinction coefficient taken as 2174 at 23.7° C, and Y = the ferric yield in units of micromoles per litre per 1000 r, and given as 16.4 ± 0.8 /uM per 1000 r . The yield may be taken as independent of wave-length for gamma ray applications.

Since irradiation time was 20 minutes (0.33 hr) Eq. (1) reduces to

$$R\ (r/hr) = (2.8 \cdot 10^4) \cdot \frac{D_{(sample)} - D_{(blank)}}{0.33} \qquad Eq.\ (2)$$

When total dose is required, the time factors on both sides of the equation are eliminated.

Experimental procedure

All cultural work was done under sterile conditions. A flow diagram of the experimental procedure is shown in FIG. 1 a and 1 b .

Yeast cells were transferred from slant of stock culture into a presporulation medium and incubated at 27° C for a period of 48 hours. The cell population was then separated from the presporulation medium by centrifugation, and washed three times by centrifugation with M/20, pH 5 KHP buffer. After washing, the yeast cell suspension, usually

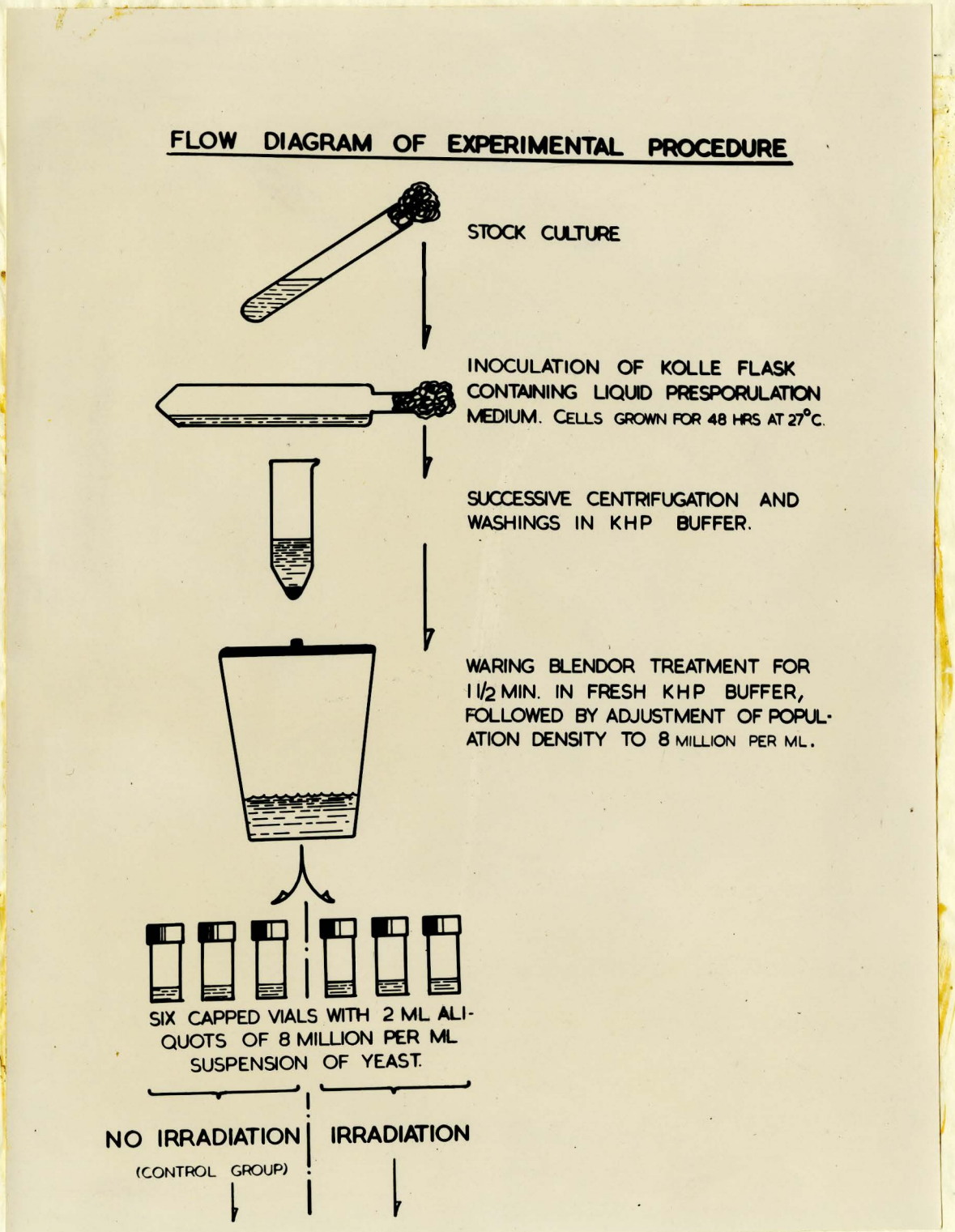


FIGURE 1 a . FLOW DIAGRAM OF EXPERIMENTAL PROCEDURE . This diagram summarizes the procedure up to and including the irradiation. See text for step by step description of the experimental procedure.

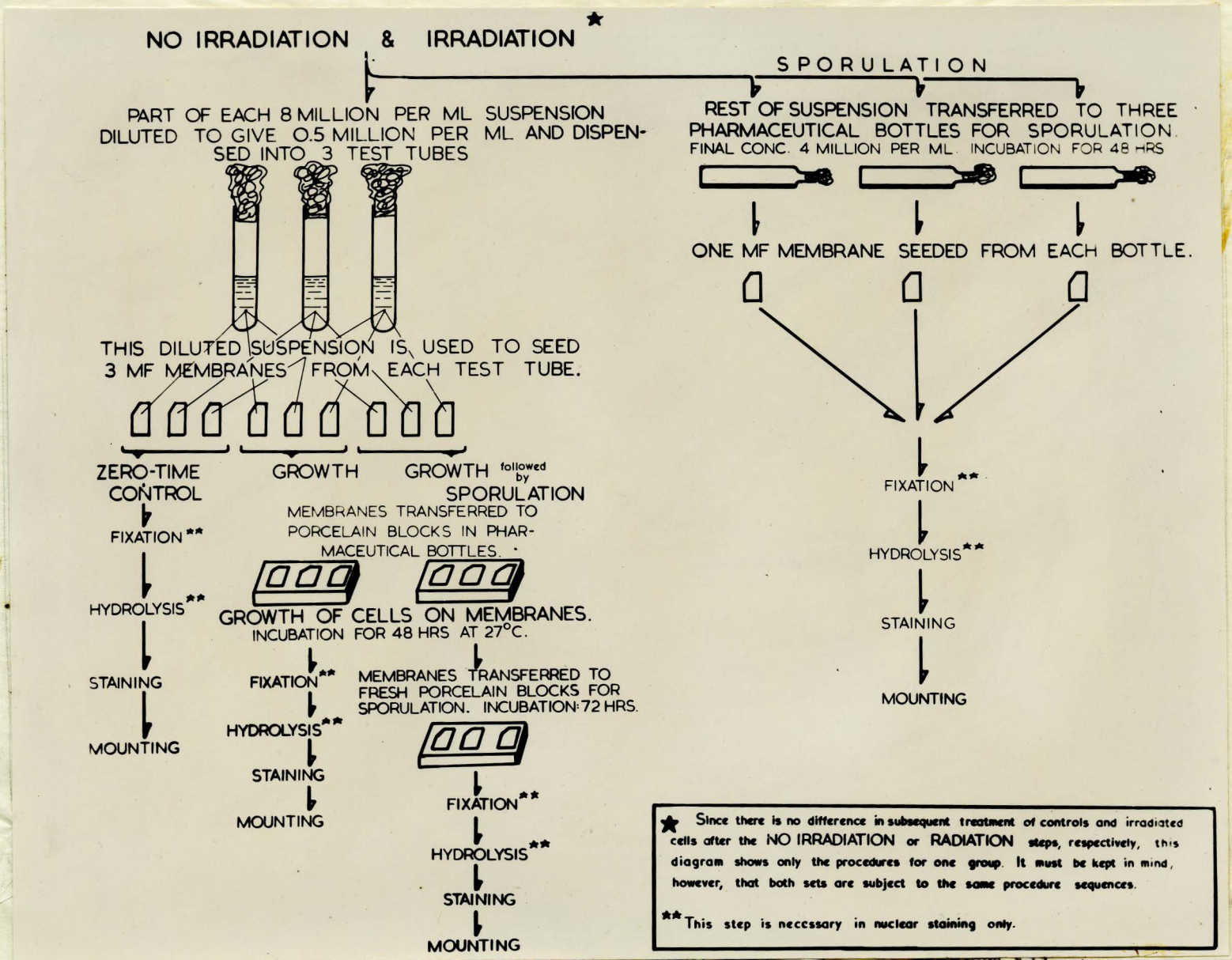


FIGURE 1 b . FLOW DIAGRAM OF
the procedure followed after
as the non-irradiated controls
cells. The irradiated cells as well
to go through the above steps.

50 ml in volume, was placed in a Waring Blendor and agitated for one-and-one-half minutes to separate the daughter cells and large buds from the parent cells. The result of this treatment gave 97 ± 3 % of single cells. The yeast cell population density was then determined using a hemacytometer counting chmaber, and the density adjusted so as to give a population of eight million per ml. Two ml of this suspension was then dispensed in each of six vials, three of which were used for irradiation, while the other three vials served as controls. For irradiation the three vials were firmly set into a 250 ml Nalgene (plastic) centrifuge bottle which had been weighted with a layer of lead shot covered with plasticine. An ampoule containing 4.5 ml of Fricke dosimeter solution was included in the Nalgene bottle, which was lowered into the Nuclear Reactor pool for irradiation. The duration of the exposure to radiation was always 20 minutes. The radiation dosage could be varied by placing the container at different distances from the reactor core face. After irradiation, 0.1 ml of the contents of each of the three vials was added to same number of test tubes, each containing 1.5 ml of M/20, pH 5 KHP buffer. This reduced the density of the yeast population to 0.5 million per ml. Millipore Filter membranes for zero-time controls as well as membranes for growth were seeded each with a 3 mm platinum loop of this suspension. All seeded zero-time control membranes were then immediately transferred to filter papers in petri dishes moistened with 0.5 % sodium azide and left there for a period of 24 hours. All growth membranes (designated as G) were transferred

to porcelain blocks in pharmaceutical bottles, each containing 4 ml liquid growth medium. An incubation period of 48 hours at 27° C followed, after which one half of the number of the G membranes were fixed immediately with azide, while the other half was put on moist filter papers (wetted with buffer) in petri dishes for 30 minutes to remove growth medium from the pores of the membranes, and then transferred to fresh, sterile porcelain blocks in pharmaceutical bottles containing 4 ml of sporulation medium. This was termed the G - S part of the experiment and its purpose was to determine whether the cells in the microcolonies that had developed on the membranes during the growth period were capable of sporulation. The incubation period on the sporulation medium was 72 hours at 27° C, after which period the membranes were stained and mounted using the CFRR procedure.

The sporulation culture was prepared as follows: the rest of the contents of each glass vial ( 1.9 ml in all ) was transferred to a 175-ml pharmaceutical bottle, containing 2 ml KHP buffer with sodium acetate giving a concentration of 0.02 M . This part of the experiment was termed the S part. The final population density was four million per ml. The pharmaceutical bottles were placed on their flat sides and incubated for 48 hours at 27° C . Then a 2 mm platinum loop of yeast cells from each bottle was seeded onto a Millipore Filter membrane, and the latter was then put through both the nuclear staining and spore staining procedures.

The slides prepared from a typical radiation experiment are shown by a diagram in FIG. 2 . One slide accomodated the zero-time

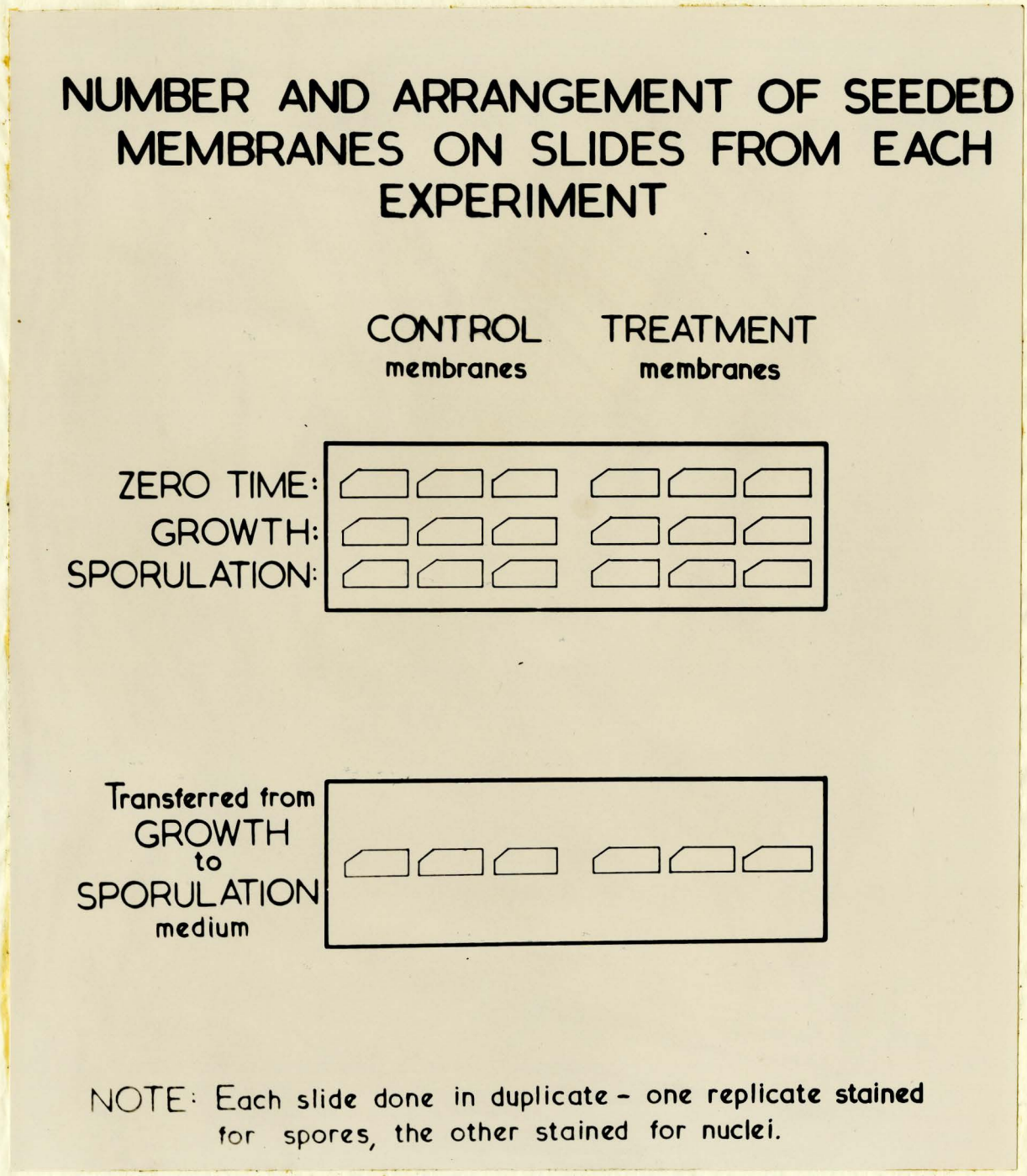


FIGURE 2 . NUMBER AND ARRANGEMENT OF SEEDED MEMBRANES ON SLIDES. This diagram shows the Millipore Filter membranes with one corner cut off as marker, and mounted on slides for microscopic examination.

controls, the G membranes, and the S membranes for both control and irradiated parts of the experiment. The second slide had the G - S part of the experiment with the respective controls. Since nuclear as well as spore stains were prepared, each experiment had to have four slides: two with nuclear stain, two with spore stain. Thus for each experiment 48 membranes were seeded with cells, which were then stained and mounted.

In the sporulation part of each experiment, 200 cells were examined on each of the six membranes (three controls and three treatments) which had been put through the spore stain, and the per cent of sporulation was thus determined for control and irradiated cells. Similarly in the growth part of each experiment 100 cells or microcolonies arising from cells were examined on each of the three control and three treatment membranes.

# R E S U L T S

A series of experiments were done in which yeast cells in suspension in glass vials were exposed to intense gamma radiation in the McMaster Research Nuclear Reactor. Following irradiation, the ability of the cells to grow and sporulate was investigated and compared.

## Effect of gamma radiation on ability of yeast cells to grow

In TABLE I are summarized the results of 16 experiments, in which the ability of irradiated yeast cells to grow was studied. A total count of individual cells was impractical in colonies containing more than 10 - 15 cells, and a means of evaluating the colony size had to be devised. For this purpose a ruled Whipple Micrometer Disc ( No. 802 ) was placed into the 10 X eye piece of a Wild microscope ( model M-20 ) and the colonies were always viewed under the same magnification, employing the 10 X ocular and a 40 X objective lenses. It was found that a small colony just fitting into a single large square of the Whipple disc consisted on the average of 15 $\pm$ 5 cells. Two such squares contained then about 30 cells, three square areas had about 45 cells, etc. The term AREA OF ONE refers to a microcolony of yeast occupying a single large square of

TABLE I

SUMMARY OF OBSERVATIONS ON THE EFFECT OF RADIATION ON ABILITY OF YEAST CELLS TO GROW

(Percentages shown are averages of 6 membrane counts)

| Radiation dose (Kr) | Expt. No. | CELL COUNT 1 | 2 | 3 | 4 | 5-10 | AREA COUNT 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 10+ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Control | 0 | 0 | 0 | 0 | 0 | 41.0 | 45.0 | 5.0 | 4.0 | 2.0 | 2.0 | 0 | 1.0 | 0 | 0 | 0 |
| 5 | 19 | 0 | 1.0 | 4.3 | 4.0 | 5.3 | 34.0 | 32.0 | 6.7 | 4.3 | 2.7 | 1.7 | 1.3 | 1.0 | 0.7 | 0.9 | 0.1 |
| 0 | Control | 0 | 0 | 0 | 0 | 0 | 60.0 | 22.0 | 5.0 | 4.0 | 4.0 | 3.0 | 0 | 0 | 0 | 2.0 | 0 |
| 10 | 17 | 1.0 | 1.0 | 3.7 | 3.3 | 10.4 | 29.7 | 25.7 | 5.7 | 11.3 | 4.3 | 1.3 | 1.0 | 0 | 0.7 | 0.5 | 0.4 |
| 0 | Control | 0 | 0 | 0 | 0 | 0 | 3.5 | 6.0 | 8.5 | 19.0 | 4.0 | 14.0 | 3.0 | 9.5 | 13.5 | 13.5 | 5.5 |
| 15 | 5 | 2.0 | 0 | 0.5 | 0.5 | 4.5 | 11.0 | 21.0 | 14.5 | 16.0 | 4.5 | 7.0 | 0 | 6.0 | 4.5 | 3.5 | 4.5 |
| 0 | Control | 0 | 0 | 0 | 0 | 0 | 5.4 | 9.8 | 8.8 | 32.8 | 7.0 | 7.8 | 4.8 | 4.0 | 7.6 | 10.2 | 1.8 |
| 16 | 13 | 1.0 | 1.0 | 0 | 0 | 2.0 | 21.0 | 24.0 | 21.0 | 15.0 | 6.0 | 2.0 | 1.0 | 3.0 | 1.0 | 1.0 | 1.0 |
| 0 | Control | 0 | 0 | 0 | 0 | 4.0 | 10.3 | 13.0 | 13.2 | 17.1 | 11.1 | 10.1 | 3.3 | 3.6 | 7.4 | 5.5 | 1.4 |
| 20 | 1 | 1.0 | 3.0 | 3.0 | 1.7 | 1.5 | 30.0 | 26.0 | 13.2 | 9.0 | 4.0 | 1.7 | 1.5 | 0.7 | 1.2 | 1.0 | 1.5 |
| 0 | Control | 0 | 0 | 0 | 0 | 20.0 | 52.0 | 16.0 | 8.0 | 4.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | 10 | 2.0 | 6.0 | 1.0 | 2.0 | 21.0 | 37.0 | 12.0 | 6.0 | 8.0 | 1.0 | 1.0 | 0 | 1.0 | 1.0 | 0.5 | 0.5 |
| 0 | Control | 0 | 0 | 0 | 0 | 58.3 | 29.7 | 10.0 | 0.7 | 0.6 | 0.3 | 0.4 | 0 | 0 | 0 | 0 | 0 |
| 27 | 12 | 1.0 | 8.0 | 3.0 | 3.3 | 29.0 | 27.0 | 18.3 | 5.0 | 3.7 | 1.4 | 0.3 | 0 | 0 | 0 | 0 | 0 |
| 0 | Control | 0 | 0 | 0 | 0 | 10.0 | 13.0 | 14.0 | 19.0 | 25.0 | 10.0 | 4.0 | 3.0 | 1.0 | 0.5 | 0.5 | 0 |
| 30 | 14 | 8.0 | 10.0 | 5.0 | 5.0 | 39.0 | 21.0 | 9.0 | 1.0 | 1.0 | 0 | 1.0 | 0 | 0 | 0 | 0 | 0 |
| 0 | Control | 0 | 0 | 0 | 0 | 9.3 | 11.3 | 6.4 | 8.8 | 12.9 | 7.9 | 8.2 | 4.5 | 8.9 | 7.3 | 6.5 | 8.0 |
| 32 | 3 | 4.0 | 9.0 | 1.7 | 2.8 | 4.7 | 19.9 | 18.6 | 11.9 | 9.6 | 4.4 | 3.3 | 1.8 | 1.8 | 1.8 | 0.7 | 4.0 |
| 0 | Control | 0 | 0 | 0 | 0 | 0 | 17.0 | 20.3 | 8.3 | 21.0 | 7.3 | 5.7 | 1.6 | 2.0 | 5.3 | 3.0 | 8.5 |
| 34 | 6 | 2.9 | 9.0 | 4.3 | 3.0 | 10.0 | 26.0 | 20.7 | 6.0 | 9.7 | 1.3 | 1.3 | 1.6 | 1.6 | 2.0 | 0.5 | 1.0 |
| 0 | Control | 0 | 0 | 0 | 0 | 0 | 3.5 | 6.0 | 8.5 | 19.0 | 4.0 | 14.0 | 3.0 | 9.5 | 13.5 | 13.5 | 5.5 |
| 35 | 4 | 2.0 | 14.0 | 3.0 | 2.0 | 11.3 | 16.7 | 16.7 | 8.3 | 11.0 | 4.3 | 6.3 | 1.3 | 1.3 | 0.7 | 0.5 | 0.6 |
| 0 | Control | 0 | 0 | 0 | 0 | 0 | 8.3 | 16.3 | 11.0 | 21.0 | 5.0 | 7.3 | 3.7 | 5.3 | 6.3 | 11.3 | 4.5 |
| 35 | 7 | 2.0 | 13.0 | 2.6 | 4.8 | 12.7 | 31.6 | 20.6 | 9.4 | 2.8 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| 0 | Control | 0 | 0 | 0 | 0 | 0 | 1.8 | 4.0 | 1.3 | 8.8 | 2.1 | 4.0 | 14.7 | 10.0 | 19.0 | 22.0 | 12.3 |
| 35 | 9 | 1.0 | 14.0 | 3.5 | 2.0 | 10.0 | 11.5 | 20.0 | 9.0 | 11.5 | 2.5 | 5.0 | 2.0 | 3.0 | 0.5 | 0.5 | 4.0 |
| 0 | Control | 0 | 0 | 0 | 0 | 0 | 45.5 | 24.0 | 7.5 | 9.0 | 4.0 | 4.5 | 2.5 | 0.5 | 0.5 | 1.5 | 0.5 |
| 41 | 16 | 17.0 | 15.0 | 3.3 | 7.3 | 21.0 | 25.4 | 5.7 | 2.0 | 2.3 | 0 | 0.7 | 0 | 0.3 | 0 | 0 | 0 |
| 0 | Control | 0 | 0 | 0 | 0 | 7.0 | 10.3 | 19.7 | 11.0 | 13.3 | 6.0 | 4.7 | 3.3 | 6.3 | 4.3 | 9.0 | 5.1 |
| 45 | 2 | 45.0 | 8.0 | 2.7 | 2.3 | 4.0 | 9.3 | 10.7 | 5.0 | 6.3 | 2.7 | 1.3 | 0 | 0.7 | 0.3 | 0.7 | 1.0 |
| 0 | Control | 0 | 0 | 0 | 0 | 0 | 26.0 | 41.0 | 17.0 | 11.0 | 2.0 | 0 | 0 | 3.0 | 0 | 0 | 0 |
| 47 | 18 | 38.0 | 9.0 | 4.3 | 3.7 | 14.3 | 19.7 | 5.3 | 1.3 | 0.3 | 1.3 | 0.7 | 0.6 | 0.3 | 0.5 | 0.3 | 0.9 |

the Whipple Micrometer Disc in the microscope viewing field. Accordingly, the colony sizes on the membranes were recorded in terms of such AREAS. However, it must be kept in mind, that not all colonies were large anough to fill out such a square. When the number of cells in such small colonies was less than 15, they were simply scored as CELLS. In other words, if for example, a microcolony had 10 cells, instead of recording it as 2/3 of ONE AREA, it was counted as 10 CELLS. Thus the sizes of the microcolonies referred to in TABLE I are expressed in terms of CELLS or of AREAS.

The values shown in TABLE I represent the frequency of microcolonies of different sizes, expressed as per cent of the population present on the membranes after 48 hours growth at 27° C. Each average results obtained in the treatment groups is shown together with its respective average control value placed above it.

It was noted that whereas the growing cells in the controls developed into fairly large colonies, some cells did not grow at all after irradiation; others produced one (FIG. 3), two or even more daughter cells (FIG. 4) . Some were able to produce colonies as large as the control cells. The growing control yeast population showed only one frequency maximum which was in the AREA part of the count. On the other hand, except in the first four treatments, all of the irradiated cells showed two frequency maxima. One such maximum was found to be in the double cell group and this maximum increased up to a radiation dose of 41 Kr. At higher radiation doses a sudden decrease in frequency of this particular group was noted, and

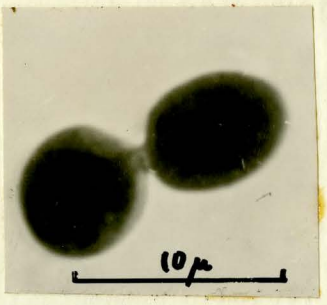


FIGURE 3 . A DOUBLE CELL PRODUCED AFTER IRRADIATION. This is a CFRR stain in which the ruthenium red step was omitted. Note that the daughter cell is as large as the mother cell yet no separation of the two has occurred, as can be seen by the narrow neck joining the two cells. Radiation dose 47 Kr.

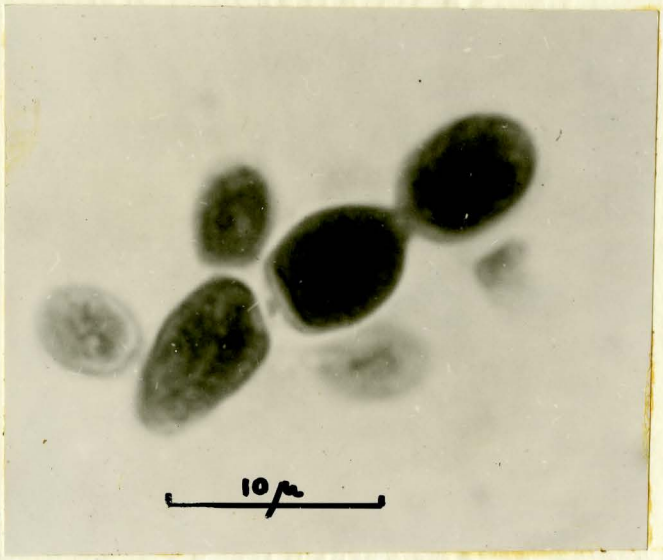


FIGURE 4 . GROWTH OF YEAST CELLS AFTER IRRADIATION. Staining is similar to FIG. 3 . The large size of the daughter cells persists after several generations as can be seen from this photomicrograph. The mother cell and the daughter cells produced altogether six cells. This group would be classified as 5-10 CELLS. Radiation dose 47 Kr.

instead the maximum occurred in the single cell group. The second frequency maximum in the treatments was found in the AREA part of the growing population. It will be noted that the AREA maxima in the irradiated populations shifted to smaller values ( i.e. toward the left of TABLE I ) when compared to their respective controls.

Effect of gamma radiation on ability of yeast cells to sporulate

When diploid cells of Saccharomyces cerevisiae sporulate they usually produce 1 - 4 spores, ( thus FIG. 5 shows a four-spore ascus and two vegetative cells that did not produce spores ). During the 14 sporulation experiments done with the yeast strain used in this investigation, the results tabulated in TABLE II in the non-irradiated control population were obtained.

Fourteen experiments were done to investigate the effect of gamma radiation on the ability of yeast cells to sporulate. FIGURE 6 a+b shows such irradiated cells, the majority of which did not produce spores. In TABLE III the results obtained in this part of the investigation are summarized. Sporulation is expressed in terms of the SPORULATION INDEX, which relates the proportion of irradiated cells that sporulated to the sporulation of the non-irradiated controls; e.g. if 60 % of the cells in the control population sporulated, and 30 % of the irradiated cells sporulated, then

$$\text{Sporulation Index} = \frac{30}{60} \cdot 100 = 50 \qquad \text{Eq. (3)}$$

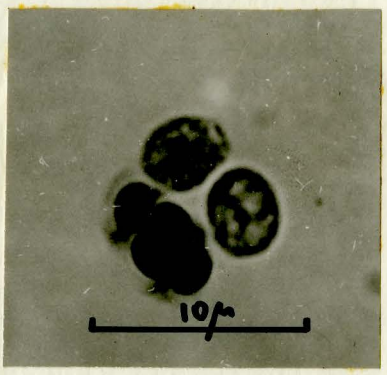


FIGURE 5 . NON-IRRADIATED SPORULATING CELLS. This figure shows a cell that has completed the sporulation process. It has four spores in its ascus. The other two cells did not form spores, although indications are that the protoplasm has concentrated in certain areas. This stain is also CFRR.

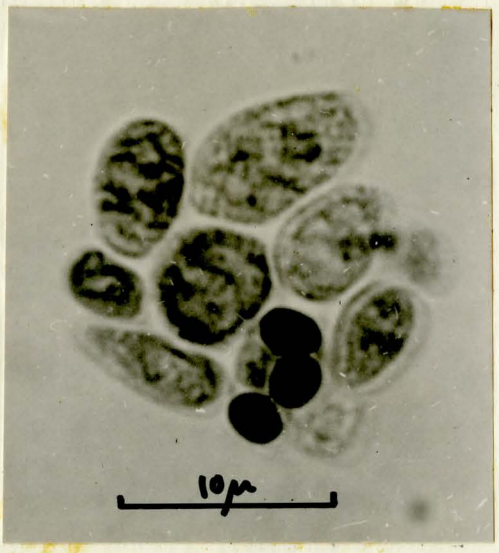


FIGURE 6 a . GFRR STAINED SPORULATING CELLS THAT WERE IRRADIATED BEFORE COMMENCEMENT OF SPORULATION PROCESS. Only three spores formed in the ascus. The rest of the irradiated cells did not form spores. Compare the size of the non-sporulated cells with cells shown in FIG. 5. Radiation dose 47 Kr.

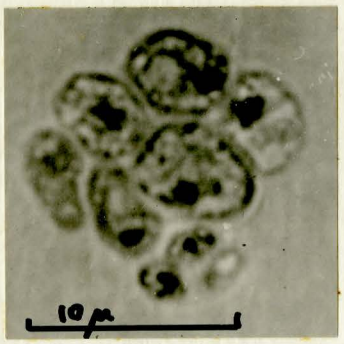


FIGURE 6 b . NUCLEAR STAIN OF IRRADIATED SPORULATING CELLS. This photomicrograph shows the nuclei of haploid spores ( at lower end of illustration) and the indistinct and diffuse nuclei of non-sporulated cells. Radiation dose 47 Kr.

TABLE II

PER CENT SPORULATION OF NON-IRRADIATED YEAST CELLS

| Non-sporulated cells | One-spore asci | Two-spore asci | Three-spore asci | Four-spore asci | Total asci |
|---|---|---|---|---|---|
| 44.1 | 2.4 | 13.9 | 27.0 | 12.6 | 55.9 |

NOTE: The standard deviation of the total sporulation was 13.5 with a probable error of 2.206

TABLE III

EFFECT OF GAMMA RADIATION ON SPORULATION ABILITY OF YEAST CELLS

| Radiation dose (Kr) | Experiment Number | Sporulation Index | Standard deviation |
|---|---|---|---|
| | C O N T R O L | 100.0 | |
| 5 | 19 | 83.6 | 7.2 |
| 15 | 5 | 48.6 | 8.1 |
| 16 | 13 | 45.4 | 7.6 |
| 20 | 1 | 39.6 | 11.8 |
| 27 | 12 | 21.3 | 6.5 |
| 30 | 14 | 27.4 | 1.4 |
| 32 | 3 | 16.3 | 8.2 |
| 34 | 6 | 15.7 | 3.2 |
| 35 | 4 | 13.7 | 2.8 |
| 35 | 7 | 10.3 | 4.1 |
| 35 | 9 | 20.0 | 6.1 |
| 36 | 15 | 16.7 | 7.9 |
| 45 | 2 | 18.9 | 3.4 |
| 47 | 18 | 18.7 | 1.2 |

It was noted that the sporulation ability of the yeast cells decreased markedly with an increase in radiation dose. A possible explanation for the low sporulation at high doses is that such cells may still be capable of forming spores, but require longer than unirradiated cells to complete the process. To test this possibility the following experiment was done. A yeast population was divided into three equal parts. One part of the population was exposed to 5 Kr radiation, the second part was exposed to 47 Kr dose, and the third part was not irradiated at all, serving as the control. After irradiation all were transferred to the sporulation medium and incubated at 27° C. Counts of sporulated cells were made at 1 day intervals for a period of 10 days, to determine how much of the population completed the spore-forming process. TAVLE IV shows the sporulation values obtained. The data are expressed as the percentage of the total population that formed spores.

The results indicate that whereas sporulation of the control population was still continuing after 10 days, no increase occurred in the 5 Kr group after 8 days, or after 2 days in the 47 Kr group. Furthermore, the 5 Kr group did not increase as much as the control from the second to the eighth day. The results indicate that if a strongly irradiated cell did not form spores within 48 hours after transfer to sporulation medium, it is unlikely, that it would complete the sporulation process after that time.

The effect of radiation on the number of spores produced per ascus was also investigated. TABLE V summarizes the results obtained in 14 experiments. The average of the controls gave a value of 2,80 spores

TABLE IV

COMPARISON OF SPORULATION OF YEAST CELLS AFTER DIFFERENT LENGTHS OF INCUBATION, AFTER 0, 5, AND 47 Kr EXPOSURE TO GAMMA RAYS.
(Values are expressed as per cent of total population)

| Radiation dose (Kr) | Days incubation (27° C) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 6 | 7 | 8 | 9 | 10 |
| 0 | 56.3 | 69.1 | 75.0 | 86.1 | 91.2 | 92.3 | 90.9 | 95.6 |
| 5 | 43.9 | 50.0 | 54.3 | 61.0 | 62.5 | 65.0 | 65.0 | 65.0 |
| 47 | 11.6 | 11.0 | 10.3 | 9.9 | 12.4 | 11.0 | 11.7 | 11.6 |

TABLE V

EFFECT OF GAMMA RADIATION ON NUMBER OF SPORES PER ASCUS

| Radiation dose (Kr) | Experiment Number | Average Number of spores/ascus | Proportion of control |
|---|---|---|---|
| 0 | C O N T R O L | 2.80 * | 1.00 |
| 5 | 19 | 2.98 | 1.07 |
| 15 | 5 | 2.76 | 0.99 |
| 16 | 13 | 2.71 | 0.97 |
| 20 | 1 | 1.13** | 0.41** |
| 27 | 12 | 2.70 | 0.96 |
| 30 | 14 | 2.74 | 0.98 |
| 32 | 3 | 2.25 | 0.81 |
| 34 | 6 | 2.68 | 0.96 |
| 35 | 4 | 2.30 | 0.82 |
| 35 | 7 | 2.75 | 0.98 |
| 35 | 9 | 2.76 | 0.98 |
| 36 | 15 | 2.48 | 0.89 |
| 45 | 2 | 2.38 | 0.85 |
| 47 | 18 | 2.42 | 0.86 |

* The standard deviation for the controls only was 0.21 with a probable error of the mean of 0.44 .

** The cell concentration in this experiment was only 1 million per ml, instead of the usual 4 million per ml, which may account for the low value obtained.

per ascus, which was then set at unity, and the sporulation of the cells that had been irradiated expressed in proportion. The results show a distinct reduction of spores per ascus with increased exposure to gamma radiation. The reduction of spores per ascus, however, was small in proportion to the reduction of sporulation, which was shown in TABLE III. Thus the maximum reduction of number of spores per ascus was down to 85 % of the control ( i.e. a reduction of 15 %) in the radiation range employed, whereas the maximum reduction of sporulation observed was to 10.3 % of the control. These results indicate that if an irradiated cell is able to sporulate it usually does so to the full extent of a non-irradiated cell.

Five experiments were done to determine whether by exposure of a cell to gamma radiation one can destroy the cell's ability to sporulate without affecting its ability to grow and vice versa. The procedure followed, as described in Materials and Methods, was to grow the yeast cells on membranes on porcelain blocks for two days at 27° C, and then transfer the membranes with the microcolonies to other porcelain blocks standing in a sporulation medium. After three days in the incubator, the microcolonies were examined for evidence of sporulation. The results obtained with the non-irradiated controls and the irradiated cells are shown in TABLE VI on the following page.

The microcolonies that grew on membranes that had been seeded with non-irradiated cells always produced a number of asci and the number of asci was proportional to the size of each colony. Thus the

TABLE VI

EFFECT OF RADIATION ON NUMBER OF ASCI IN COLONIES OF DIFFERENT SIZE

| Rad iation dose (Kr) | Expt. number | AVERAGE NUMBER OF ASCI PER COLONY Colony size (AREA) 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | % of colonies lacking asci |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Control | 5.1 | 10.3 | 14.5 | 20.2 | 25.7 | 29.5 | 35.4 | 39.5 | 44.5 | 0 |
| 5 | 19 | 4.0 | 9.7 | 13.2 | 17.0 | 23.0 | 26.5 | 33.9 | 36.5 | 41.5 | 0 |
| 16 | 13 | 3.0 | 5.8 | 10.7 | 14.4 | 17.8 | 22.1 | 27.3 | 31.0 | 38.0 | 4 |
| 27 | 12 | 2.5 | 4.6 | 7.4 | 10.7 | 15.3 | 18.8 | 22.1 | 26.1 | 32.1 | 6 |
| 36 | 15 | 0 | 0 | 0.7 | 1.0 | 1.0 | 1.5 | 2.5 | 3.2 | 9.0 | 23 |
| 47 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 1.7 | 2.3 | 59 |

average number of asci produced in colonies of unit size (AREA OF ONE) was found to be 5.1; in AREA OF TWO 10.3 (FIG. 7), etc. Since a colony of "unit area" contained 15 ± 5 cells, this meant that about 1/3 of the cells sporulated in the controls, and the sporulation percentage was independent of colony size.

In the colonies that grew from irradiated cells, there were fewer asci per unit area (FIG. 8), especially at the higher radiation doses. It will be also noted that some colonies that grew from cells subjected to 16,000 r or more produced no asci at all. One such colony is illustrated in FIG. 9. The per cent of such colonies was especially high (59%) after 47 Kr exposure. This included colonies that varied in size from an AREA OF ONE to an AREA OF NINE .

It is evident, that if a cell is able to grow after being exposed to gamma radiation, it is not necessarily able to sporulate. An attempt was made to determine whether the reverse was true, i.e. can a cell, that has lost its ability to grow, sporulate ? A large number of one-, two-, three-, and four-cell colonies was examined, but none of these colonies was found to contain spores. A very small percentage of the 5-10-cell colonies was found to have sporulated cells. These observations indicate that if a cell loses its ability to grow, it also loses its ability to sporulate.

Effect of gamma radiation on nuclear division in growing yeast cells

To determine whether or not any relationship exists between

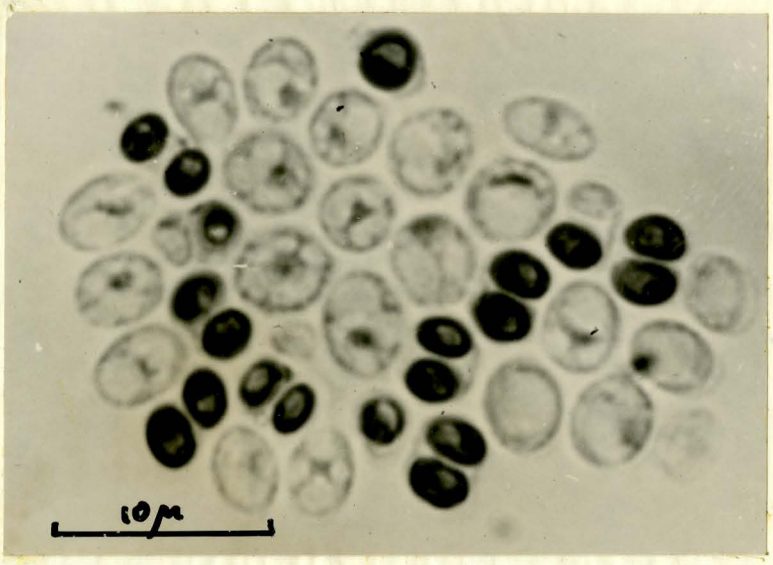


FIGURE 7. A NON-IRRADIATED GROWING COLONY (AREA OF TWO) AFTER TRANSFER TO SPORULATION MEDIUM. This is a CFRR stain of a microcolony in the G-S part of the experiment, showing two-spore asci and non-sporulated cells.

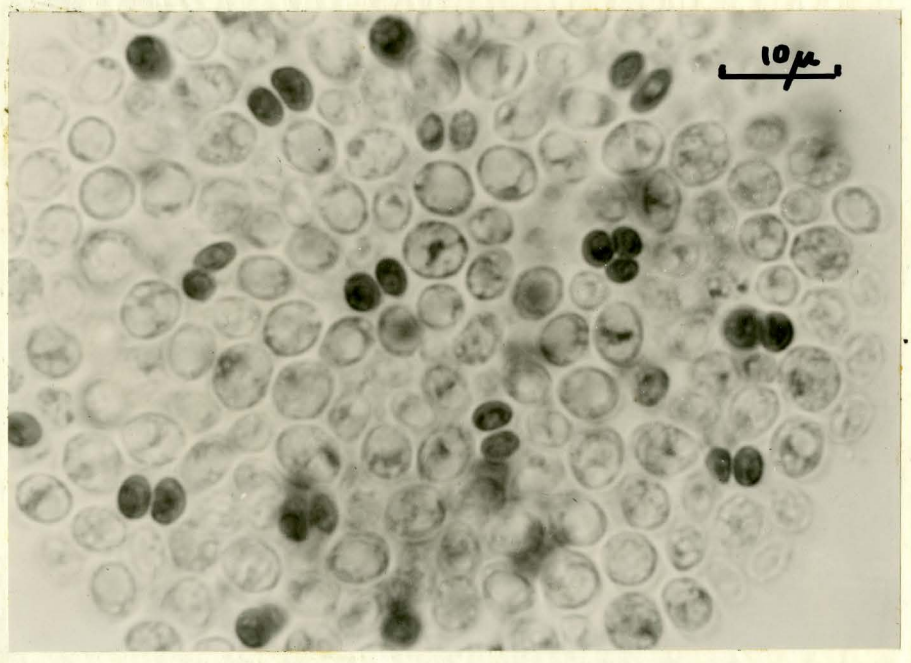


FIGURE 8. AN IRRADIATED GROWING COLONY (AREA OF SIX) AFTER TRANSFER TO SPORULATION MEDIUM. This CFRR stain shows only a few asci produced in a colony of such a large size. The magnification of this figure is ½ of Figure 7 . Some of the cells are out of focus due to the dome-shaped character of the colony. Irradiation dose 27 Kr .

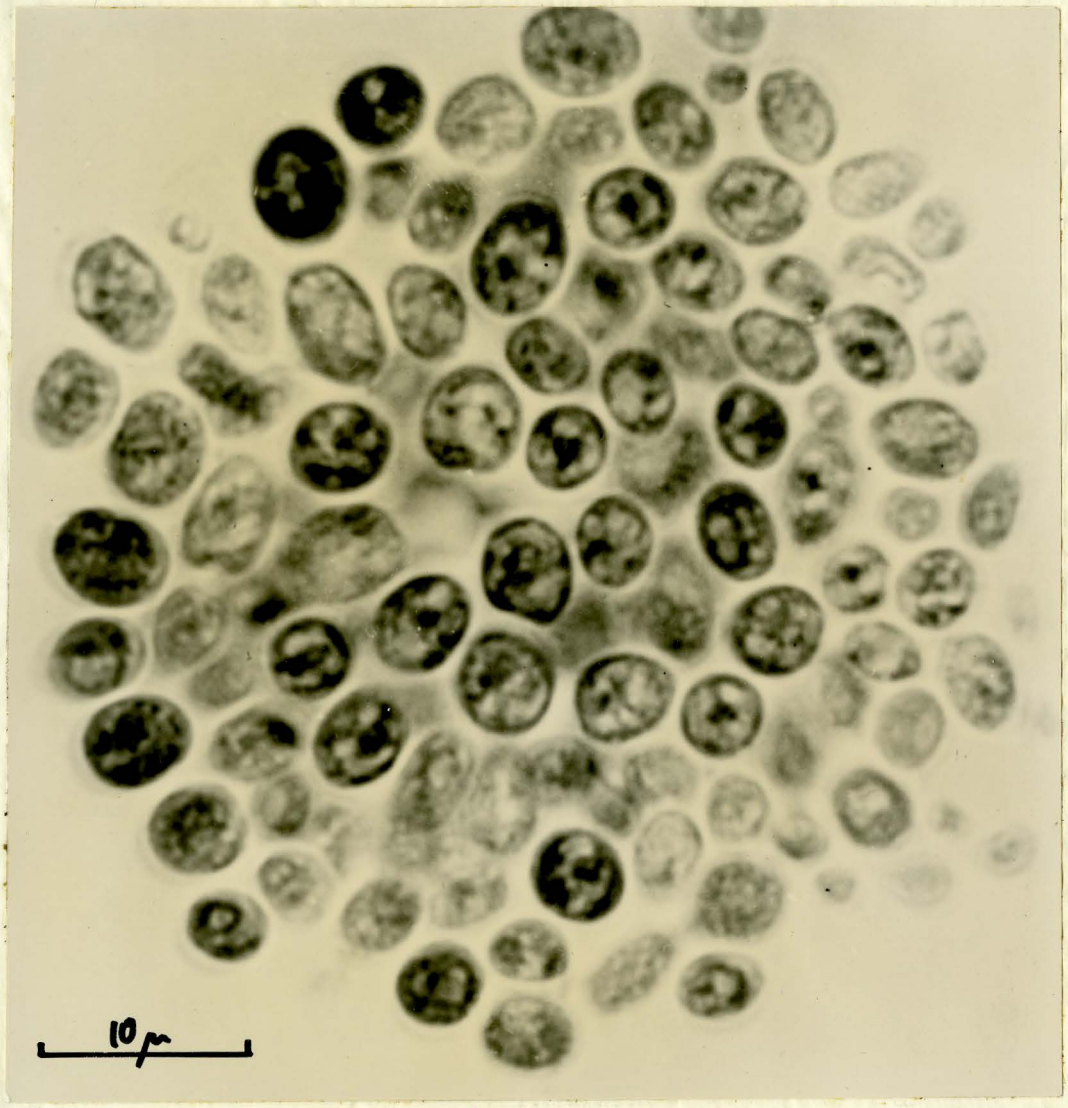


FIGURE 9 . IRRADIATED GROWING MICROCOLONY AFTER TRANSFER TO SPORULATION MEDIUM. This is a CFRR stain of a G-S microcolony which did not produce any spores. The microcolony grew to a consider able size (AREA OF FOUR) yet the cells did not form any spores. Magnification same as Figure 7 . Note the large size of the cells. Radiation dose 27 Kr.

the occurrence of double cells and the failure or abnormality of nuclear division, a sur vey mas made of such cells, using the nuclear stain. By means of this it was possible to determine whether or not the nucleus had divided, and if it had, whether one of the resulting nuclei had passed into the daughter cell.

The examination of the nuclei in the irradiated double cells showed that a proportion of these nuclei had not divided at all (FIG. 10), others appeared in the process of dividing (FIG. 11), and a third group, in which the division had been completed (FIG. 12) and each member of the pair contained a nucleus. The results of these observations, which are tabulated in TABLE VII ( page 48), indicate that at relatively low doses of radiation the number of divided nuclei predominated, whereas at the highest dose used (45 Kr), the percentage of the non-divided mother nuclei was larger. The frequency of double cell in which nuclear division was incomplete also increased at the higher radiation doses.

Effect of gamma radiation on nuclear division in sporulating cells

Observations were made on the condition of the nuclei in irradiated cells placed in sporulation medium for three days. It was found that cells in which reduction division was not completed constituted only 2% of the population. These findings indicate, that as long as reduction division of the diploid nucleus did occur after exposure to gamma radiation, then ascospores were also formed. In other words, the reduction division of the nucleus must be more

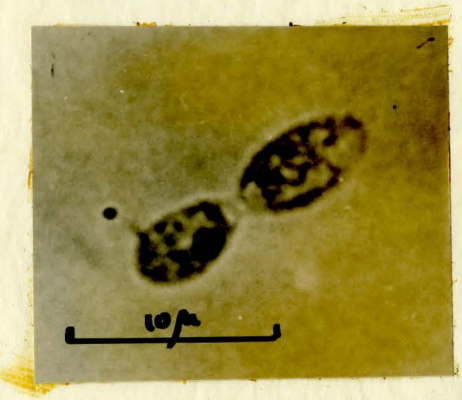


FIGURE 10. A DOUBLE-CELL WITH A NON-DIVIDED NUCLEUS. This figure illustrates an irradiated cell that produced a large daughter cell yet its nucleus did not divide. Radiation dose 27 Kr. Nuclear stain.

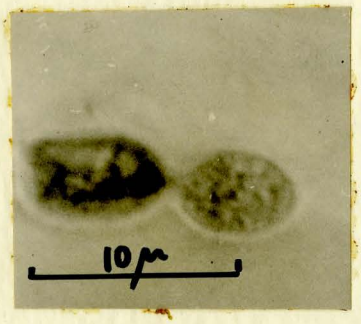


FIGURE 11. AN IRRADIATED DOUBLE-CELL SHOWING INCOMPLETE NUCLEAR DIVISION. Note the nucleus in the neck of the mother cell. This stage is one step further than the stage illustrated in Figure 10. Radiation dose 27 Kr. Nuclear stain.

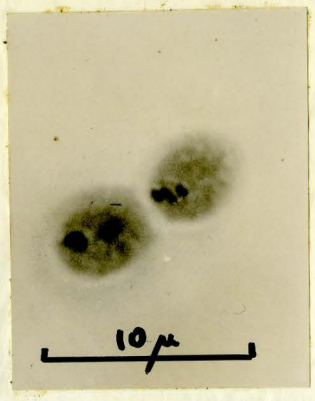


FIGURE 12 . AN IRRADIATED DOUBLE-CELL SHOWING COMPLETED NUCLEAR DIVISION. This mother cell not only produced a daughter cell, but the nucleus of the mother cell also divided successfully. Radiation dose 16 Kr. Nuclear stain.

TABLE VII

EFFECT OF RADIATION ON NUCLEAR DIVISION IN TWO-CELL GROUPS *

| Radiation dose (Kr) : | 5 | 10 | 16 | 20 | 27 | 30 | 35 | 45 |
|---|---|---|---|---|---|---|---|---|
| Per cent nuclear divisions completed | 98** | 96** | 100.0 | 91.0 | 85.0 | 59.3 | 39.0 | 20.0 |
| Per cent nuclear divisions not completed | 2 | 0 | 0 | 0 | 5.1 | 20.7 | 40.0 | 21.6 |
| Per cent with no evidence of nuclear division | 0 | 4 | 0 | 9.0 | 9.9 | 20.0 | 21.0 | 58.4 |

* Since none of the controls had any two-cell groups the controls are not included.

** The experimental error in these two counts is large, since these values were obtained from only 6 and 8 double cells, respectively. Such cells were very infrequent at low radiation doses.

sensitive to radiation than the stage of sporulation in which the spore walls form around haploid nuclei.

Effect of gamma radiation on morphological and cytological changes in yeast

The most conspicuous change of yeast cells exposed to radiation was an increase in size of these cells. Thus irradiated cells were 2-3 times larger (FIG. 13) than the non-irradiated controls (FIG.14), and the protoplasm of the irradiated cells appeared granulated and finely vacuolated (FIG. 13 and FIG. 15) . At higher doses of radiation some cells lost their characteristically oval shape and were distorted, as shown in FIG. 16 .

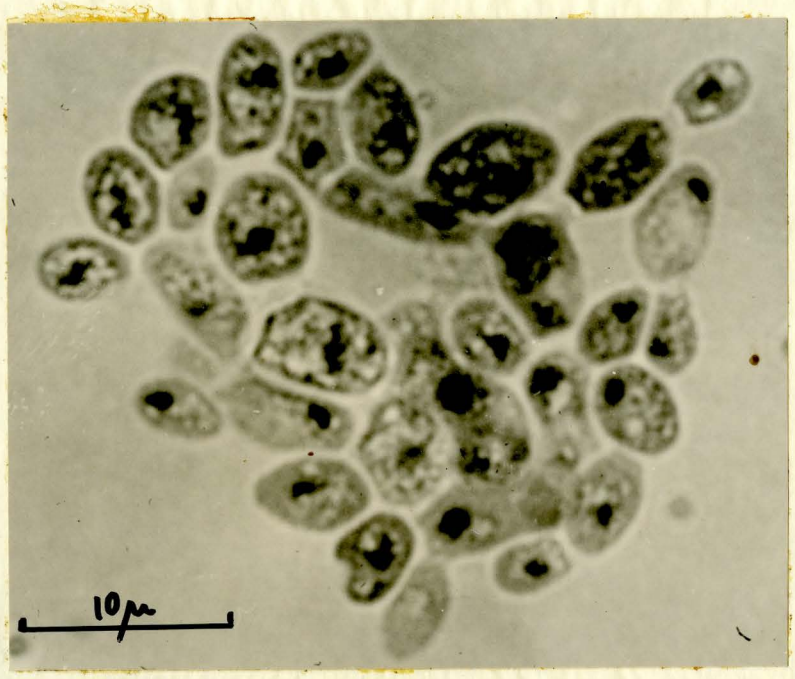


FIGURE 13 . NUCLEAR STAIN OF IRRADIATED CELLS IN A COLONY. Note the large size of the irradiated cells, and compare it with the non-irradiated cells of Figure 14. Magnification of both figures is same. Radiation dose 16 Kr. Nuclear stain.

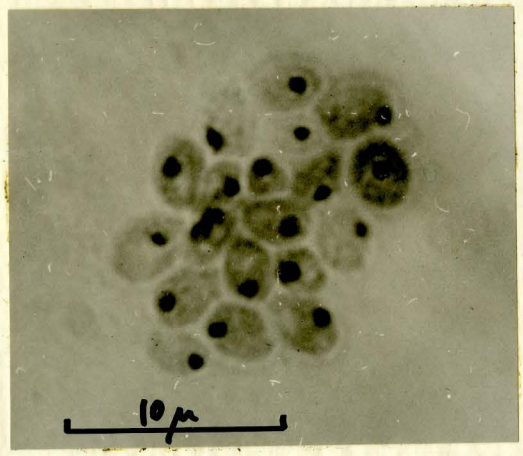


FIGURE 14 . NUCLEAR STAIN OF NON-IRRADIATED CELLS IN A MICROCOLONY. Note the difference in the appearance of the protoplasm which, in contrast to irradiated cells, is quite homogeneous in appearance. Also note smaller size of these control cells. Nuclear stain.

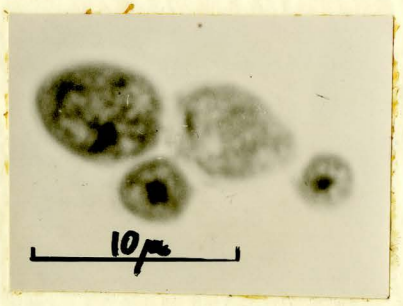


FIGURE 15 . NUCLEAR STAIN OF IRRADIATED CELLS THAT ARE GRANULATED AND VACUOLATED IN APPEARANCE. This is a group of four cells that grew after irradiation. Each cell has one nucleus and can be said to have the ability to multiply. Again, note the granulated appearance of the protoplasm of the cells. The original mother and daughter cells are much larger in size than the two subsequent generations. Radiation dose 16 Kr. Nuclear stain.

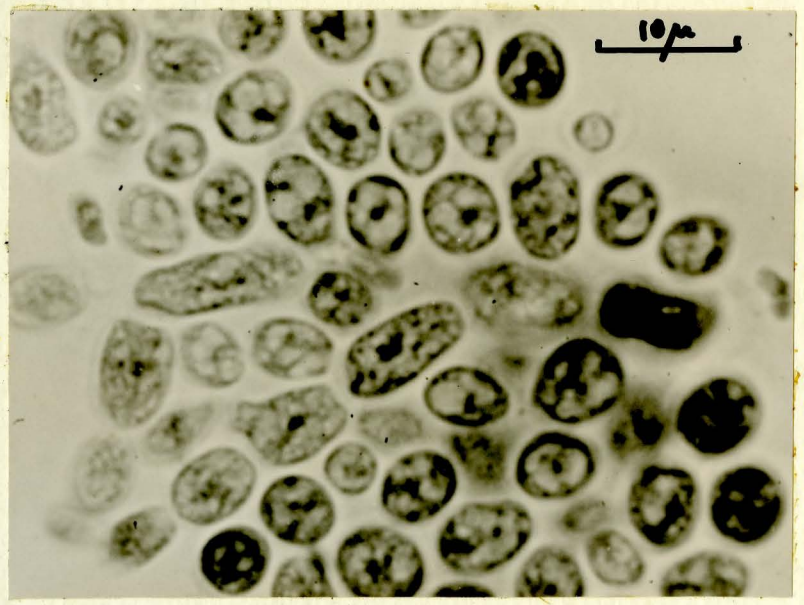


FIGURE 16 . DISTORTED IRRADIATED CELLS IN A COLONY. This is a CFRR stain illustrating the malshaped cells that occured in some of the colonies that grew from cells after exposure to radiation. The originally irradiated cells are much larger than the later generations and also are changed morphologically. Radiation dose 16 Kr.

# D I S C U S S I O N

This study was undertaken primarily to determine and compare the effect of gamma radiation on the growth and sporulation processes in yeast, and also to compare the effect of radiation on nuclear divisions that occur during growth and sporulation. Before any comparison between the two processes can be made, a basis for such comparison must be established.

An irradiated cell that has produced more than 5-10 cells could be counted as having the ability to grow. Or cells could be counted that had produced four, three, two, or only one daughter cell. Conversely, the criterion of cell inactivation could be taken as loss of ability to produce a daughter cell, or more than one, two, three, or even perhaps up to 10 daughter cells. Thus in FIG. 17 the data of TABLE I are plotted using as criteria of inactivation the inability of cells to produce any, or one, two, etc., daughter cells. The values plotted were obtained in the following manner: if, for instance, the population on a membrane consisted of 3 % single cells, (and the one-cell criterion of growth was used), then the rest of the population (97 %) was considered to have grown. Similarly, if the four-cell criterion was used, then all cells that produced more t han three daughter cells were considered as having the ability to grow after irradiation. As is expected, when the less demanding criteria

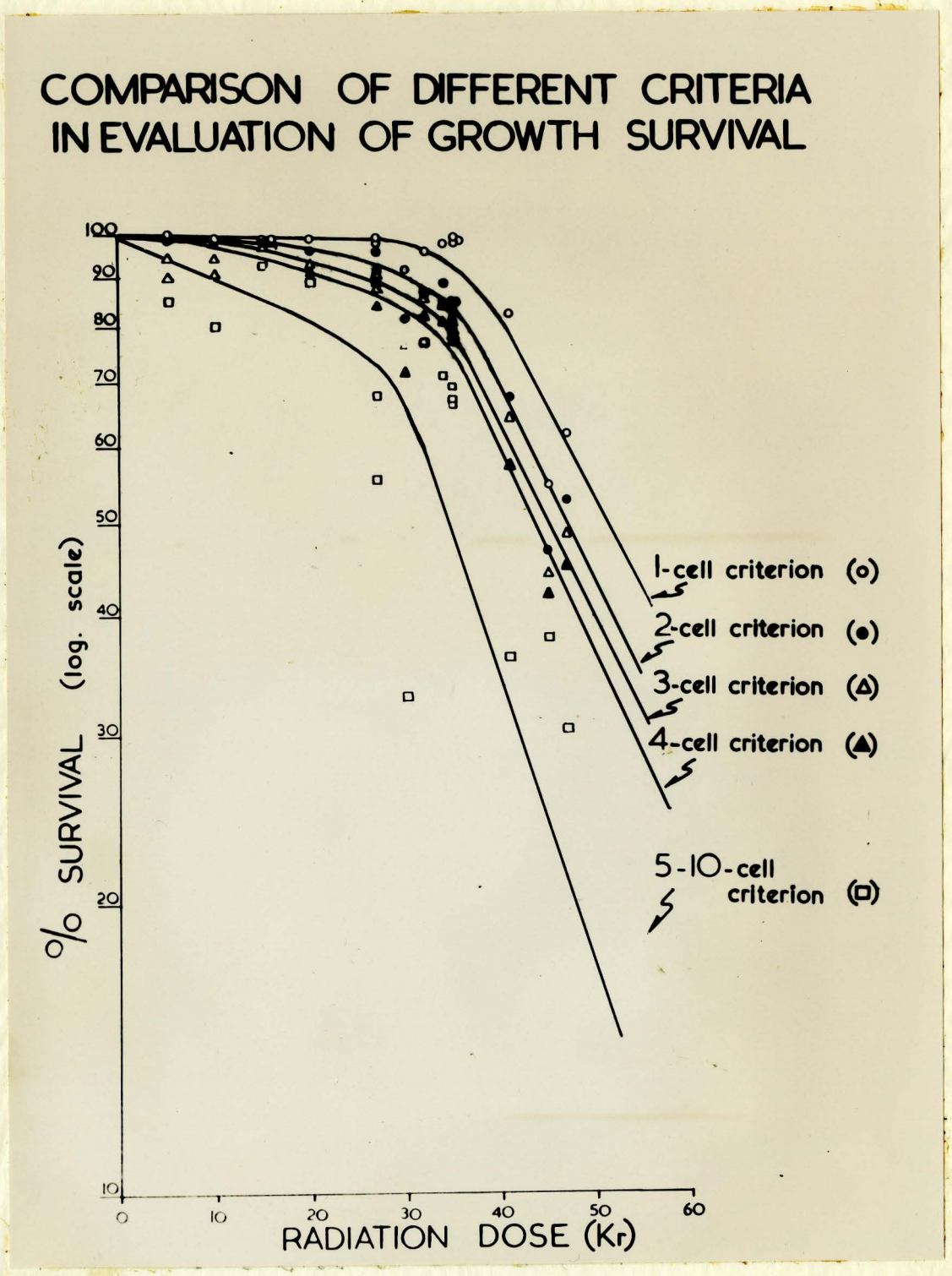


FIGURE 17 . COMPARISON OF DIFFERENT CRITERIA IN THE EVALUATION OF GROWTH SURVIVAL. Note that the shape of the curves remains essentially the same no matter what criterion of survival is used. See text for explanation of the method of scoring survival.

of growth are used (such as the ability to produce one or two daughter cells), the survival curve falls off less steeply, than when more demanding criteria are used. With none of the criteria used in preparing FIG. 17 does the survival curve approximate a straight line. The shape of these curves is sigmoid and is similar to that of curves obtained by other invsetigators ( Wyckoff and Lyuet, 1931, Frilley and Latarjet, 1944, Latarjet and Ephrussi, 1949) in studies of the survival of irradiated diploid yeast cells using other methods.

No similar difficulties are encountered in obtaining a satisfactory criterion of loss of sporulation ability on irradiation. One need only express the sporulation of the irradiated cells as per cent of the non-irradiated controls. The data already summarized in TABLE III are plotted in FIG. 18 for comparison with the growth results shown in FIG. 17. An unexpected finding is, that the points in FIG. 18 appear to lie on a straight line when semi-logarithmic paper is used, and an attempt must be made to account for the difference observed in the shape of the curves.

Wyckoff and Luyet, (1931), Frilley and Latarjet, (1944), and Latarjet and Ephrussi (1949) demonstrated that the shape of the inactivation curve for diploid yeast cells was sigmoid. This was found also in the present investigation. Latarjet and Ephrussi (1949), on the other hand, found that the radiation inactivation of haploid yeast cells gave an exponential curve, which when plotted on semi-logarithmic paper gave a straight line. This was subsequently confirmed by Zirkle and Tobias (1953) .

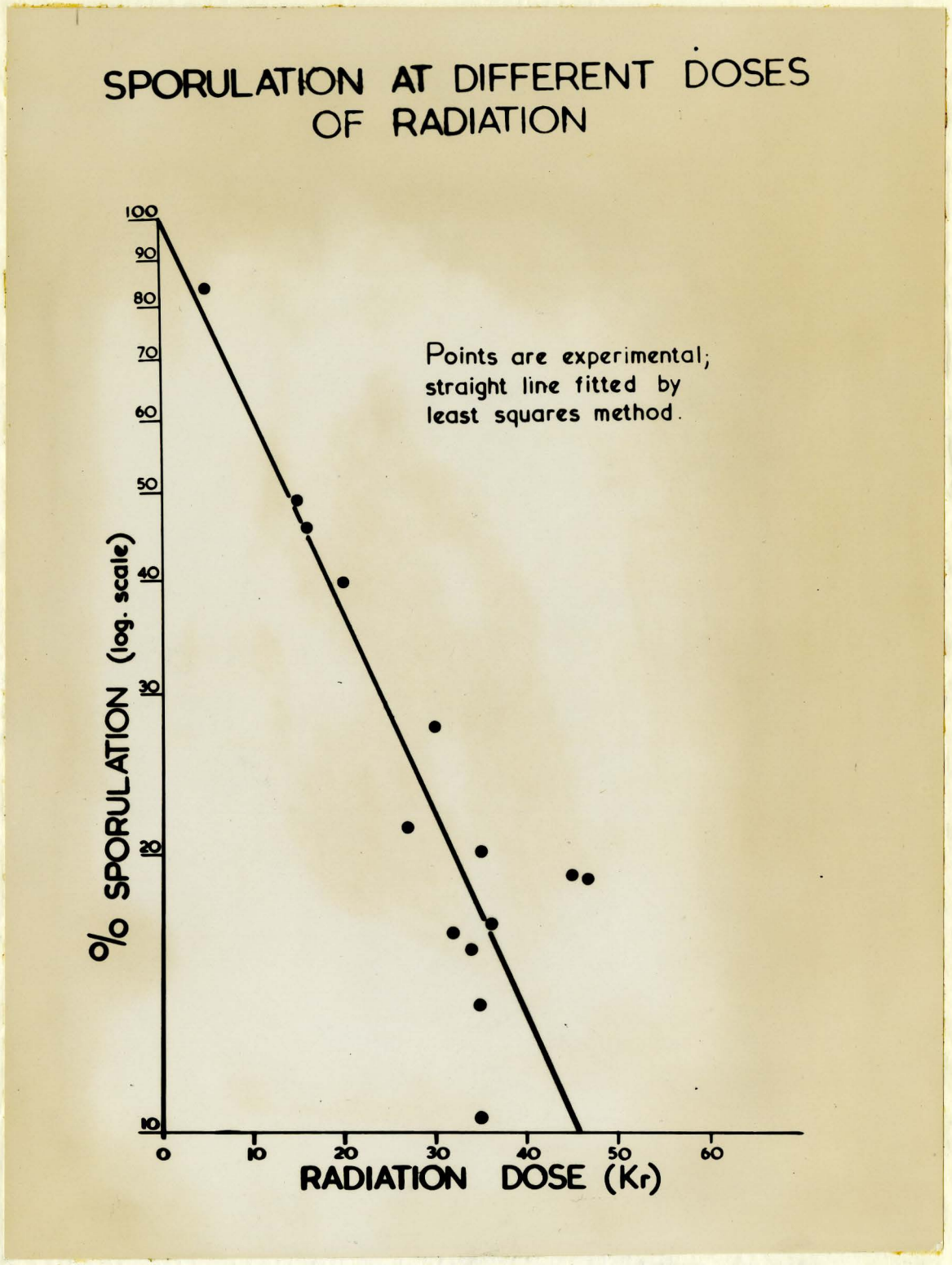


FIGURE 18 . SPORULATION AT DIFFERENT DOSES OF RADIATION. Compare the straight line sporulation curve of irradiated cells with the sigmoid curves of growing cells exposed to radiation illustrated in Figure 17 . Note that the ordinate is expressed in terms of a logarithmic scale.

The present investigation indicates that an exponential inactivation curve is obtained with sporulating diploid cells, when inability to sporulate is taken as the criterion of inactivation. This observation has never before been made.

The finding of a sigmoid type of inactivation curve in diploid cells has been interpreted by Latarjet and Ephrussi (1944) and later by Tobias (1952) to mean that at least two sensitive sites must be affected by radiation, before any reduction of growth can be observed. These workers considered these two units to be homologous genes. In the haploid cell only one gene of each kind will be present, and therefore, a single ionization causing mutation of one gene may suffice to bring about the inactivation of the cell. This would be expected to give a straight-line relationship when plotted on semi-logarithmic paper.

In contrast to growth, the ability of an irradiated cell to sporulate is destroyed by much less radiation. It can be therefore speculated, that for successful sporulation to occur, a large number of genes is necessary, whereas a smaller number of genes is required for growth. Thus, if for instance, 1000 genes were necessary for sporulation and only 200 genes sufficed for growth to proceed, then the greater sensitivity of the sporulation process could be accounted for. It must be kept in mind, however, that if the inactivation of sporulation and growth is brought about by mutation of both members of at least one pair of genes to recessive alleles, then the inactivation curves of both growth and sporulation should be sigmoid. Since

this is not the case, (the sporulation curve being a straight line), the hypothesis that more genes are required for sporulation than for growth seems improbable. An alternative explanation is that, whereas growth ability is inactivated by the radiation action on at least two homologous genes, the loss of sporulating ability may result from mutation of only one member of certain gene pairs.

Other possible explanations of the action of radiation on growing and sporulating yeast cells could include the following:

a) Breakage of chromosomes: broken chromosomes could during mitosis and meiosis interfere with the mechanics of successful completion of division. Since, however, little is known about the details of meiotic and mitotic divisions in yeast, no successful interpretation of the action of radiation by chromosomes breakage in yeast can be brought about.

b) Cytoplasmic effects: whereas non-genetic effects of radiation on yeast have been known to occur (Sarachek et al, 1954) it is nevertheless difficult to imagine a single vital molecule, other than a gene, which would inactivate a cell to such a degree. Cytoplasmic effects will likely never result in a straight line.

c) Destruction of centriole: Lindegren et al. (1959) claimed that the centriole was the primary site on which radiation acted. According to these workers a haploid yeast cell has one centriole, a diploid yeast cell has two centrioles, etc. Inactivation of yeast cells by exposure to radiation is brought about by the

the inactivation of these centrioles. All centrioles must be destroyed before a yeast cell is inactivated. Since no centrioles were observed in the course of this investigation, Lindegren's claim is not confirmed by the present study.

In conclusion it can be said that the present study has found that the sporulation process in yeast is more sensitive to radiation than growth. The greater sensitivity of sporulation as compared with growth is also reflected in the observation that gamma radiation frequently destroyed the ability of a cell to sporulate without affecting its capacity to grow. Furthermore, since only successful meiosis brought about the formation of spores, the sporulation inactivation curve probably closely approximates the meiotic inactivation curve. These are the main contributions of this investigation.

Since sporulation is preparatory to sexual multiplication, the present investigation has shown that in effect sterility can be produced in yeast by exposing them to gamma radiation. Practical applications of being able to make yeast sterile, can be introduced into the baking and brewing industries, when sexual recombinations of a satisfactorily performing strain must be prevented. Thus, exposure of vegetative cells of such a strain to low doses of gamma radiation could make the yeast sterile.

A satisfactory criterion of growth must be established in order to compare the $LD_{50}$ doses of growing and sporulating cells. Thus a double cell is not a satisfactory criterion of growth, because some

of the nuclei did divide and others did not. On the other hand, it can be said, that a triple cell did grow, because examination of nuclei showed that the nuclear division was always complete in such cell groups. Accordingly, a satisfactory criterion of growth in this connection can be the production of two or more daughter cells after irradiation. When the $LD_{50}$ dose of such cell group is compared with the $LD_{50}$ dose of sporulated yeast cells (47 Kr and 13Kr, respectively), then it is seen that sporulation is approximately 3.5 times as sensitive to radiation as growth.

One contribution of general significance is the usefulness of the Millipore Filter membrane technique described in the present study. The usual method of studying the action of radiations on yeast cells involves the plating out of irradiated cells on a nutrient agar medium. After a suitable time of incubation, the number of visible colonies that have appeared are counted. The effect of radiation is then expressed in terms of the number of cells plated out that failed to produce visible colonies. As the cells that do not grow are not counted, it is necessary to estimate their abundance by comparing the treated populations with unirradiated control populations. Other techniques include the observation of cells isolated by micromanipulation and grown on agar after exposure to radiation. The technique used in the present investigation combines attributes of both of the above methods. Cells that do not grow can be counted directly; all degrees of growth activity, between failure to bud at all and production of microcolonies containing several hundred cells, are evident. By using one or the

other stain one can demonstrate the nuclei or the spores inside the cells. Cells can be irradiated either dry or moist. Radiations of low penetration can be successfully used. Moreover, permanent slides are obtained. This is of great advantage, since slides can be filed away and reexamined as desired. During the course of this investigation, the method of scoring results chnaged several times, yet there was no need to repeat experiments. Probably, the technique used and described in the present investigation could be easily adapted to other types of radiation experiments, especially using microorganisms.

This preliminary study suggests that future research should concentrate on the detailed study of nuclear division processes in yeast.

# SUMMARY

1) The comparison of radiation action on growing and sporulating yeast cells, has shown, that of the two processes, sporulation is the more sensitive.

2) The inability of yeast cells to sporulate shows an exponential relationship to the radiation dose to which the cells were exposed. This is in contrast to growing yeast cells, where the relationship was expressed as a characteristic sigmoidal inactivation curve.

3) It was shown that an irradiated cell may grow, forming colonies of considerable size, but composed of cells, that do not sporulate when transferred to a sporulation medium. Thus it is possible to inactivate by exposure to radiation the capacity of a cell to sporulate, without affecting its ability to grow.

4) Observations indicated, that as long as the nucleus of an irradiated cell can undergo meiosis, it will also complete the formation of spores. This implies that the radiation-sensitive stage of sporulation is the nuclear division.

5) The Millipore Filter membrane culture and staining methods employed, were shown to be of value in research on the effect of radiation on microorganisms.

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