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SOME EFFECTS OF RADIATIONS ON CELL PROLIFERATION

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ABSTRACT

Some of the factors controlling tissue growth in animals and cell proliferation in unicellular organisms are reviewed. Radiation damage in diploid yeast cells manifests itself sometimes in delayed lethal effect and decreased cell division rate over several generations in the progeny of the irradiated cell. Recovery of the progeny of irradiated yeast cells from phenotypic expression of radiation damage can be delayed over many generations and is similar, in time rate of onset to the onset of radiation-induced animal tumors. A parallel suggests itself between the mechanism of somatic-radiation carcinogenesis and delayed recovery of unicellular organisms from radiation effect.

SOME EFFECTS OF RADIATIONS ON CELL PROLIFERATION*

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For the past several years a group at the Donner Laboratory has been engaged in a biophysical study of the effects of various radiations--including x-rays, protons, deuterons, alpha rays, and carbon particles--on the inhibition, delay, and acceleration of cell proliferation. The exact elucidation of the detailed processes by which radiation interferes with the cell-division mechanism is obviously at the root of our queries into the hazards presented to man by radiations, and into the origin of radiation-induced cancer. Paradoxically, animal experiments and studies of living tissues are not necessarily the best means by which the fundamental changes induced by radiation may be studied at the cellular level. The organization of tissues is so complicated, and so many different cell types with many relationships to each other are involved, that it seems logical to resort to systems where cells act independently of one another, as seems to be the case with some ascites tumor cells that grow akin to bacteria in ascites fluid (Klein); to culture single tumor cells (Puck); or to actually resort to unicellular organisms, which may be handled in relatively uniform populations in synthetic media.

It seems obvious that somatic cells of animal and human tissues represent a state of evolution that is much more advanced than that of yeast cells, for example, the former having much greater potentialities in differentiation. Therefore one should exercise considerable care in making deductions with respect to tissue effects from experiments on unicellular organisms. I prepared two tables in which the terminology of certain concepts concerning tissues and unicellular organisms are compared. Table I is self-explanatory; it is evident that care has to be exercised, for example, in comparing growth rates of tissue to growth rates of unicellular organisms, since the growth potential of tissues is not fully utilized owing to limiting effects by hormones and vitamins.

Table II goes farther in the comparison of tissue growth in animals and cell proliferation in unicellular organisms. In animals there is a complicated system of regulation of tissue activities, probably based on the feedback principle and involving hypothalamic and hypophyseal activity. Unicellular organisms may influence their own growth rate by virtue of their own metabolic products also, but in the laboratory one is usually able to maintain conditions under which this is not an important factor.

With the above limitations in mind, I wish to briefly summarize some of the radiobiological findings with yeast cells, and then apply these to the problem of carcinogenesis.

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†From a paper given at the 1956 meeting of the Swedish Medical Association.

Table I

Comparative terminology used in tissue and cell proliferation studies

Tissue growth	Cell division
Rate normal	Rate limited
Hormones	Limiting nutrients
Carcinogen	Mutagen
Tumor	Mutant strain
Undifferentiated growth	Mutation to independence

Table II

Some factors in the control of proliferation in animal tissues and unicellular organisms

Animal		Cell
Hormonal Feedback	———	Single Nutrient, no feedback
Genetic factor	on somatic cells on control system	——— Genetic factor
Carcinogens, Mutagens	———	Lethal Retard cell division Mutagenic Retard tumor growth

The yeast strain Saccharomyces cerevisiae is very convenient for radiation studies because its cells are available in various ploidies. There are haploids of two mating types, three diploids, two triploids, and a tetraploid cell, attributable to the efforts of many workers in the field, particularly Winge and Lindegren. Recently preparation of a quintaploid and a hexaploid strain, by mating diploids and triploids, was found possible.* All these ploidies can form vegetative colonies, and under special conditions one is able to sporulate the diploid cells, thus obtaining four haploid ascospores, and giving rise to a technique of analysis of genes and genetic linkage known as tetrad analysis.

Extensive radiation experiments with cells of various ploidies have shown that diploid cells were more resistant than haploid,[1,2,3] but higher ploidies appeared to have an increased radiosensitivity. Sporulation experiments[4] and deduction of our survival curves[3,5] give one possible mechanism of lethal effect, the production of recessive lethals. When diploid cells carry recessive lethals, they themselves keep alive and multiply, but a single recessive lethal leads to two of the four haploid ascospores' being unable to divide. Part of the haploid-diploid survival of x-rays seems to be accounted for by assumption of the recessive lethal mechanism of inhibition of cell division.

Another mechanism of radiation-induced lethal effect appears to be the "dominant" lethal production, so named because it resembles production of dominant lethals in Drosophila melanogaster. Mortimer showed the existence of this mechanism by irradiating haploid cells, subsequently mating them to unirradiated haploids of the opposite mating type, and observing survival of the diploids thus produced.[6] It appears that in yeast cells with ploidies higher than two the dominant lethal effect becomes increasingly important with increasing ploidy. The dominant lethal effect, in which a single ionizing particle is capable of killing a higher-ploidy cell, is readily interpretable in terms of production of chromosomal aberrations and rejoinings. Unfortunately, yeast cell chromosomes have not been observed directly so far.

I myself have been interested in cells that are capable of surviving radiation and in the subsequent fate of such cells and their progeny. Early in the course of this investigation it became clear that diploid survivors behaved differently from haploid ones. The latter either became normal cells again, with normal cell-division periods, or died after one or two divisions. In irradiated diploid cells the situation is different: the cell and its progeny may die several generations after radiation, or they may become normal. However, there is a considerable number of irradiated diploid cells that are capable of producing colonies, which show a proliferation rate considerably below normal. These cells are sometimes, but not always, deficient in their cytochrome system in the sense of Ephrussi.[7]

A simple experiment was performed on irradiated single diploid cells with the aid of a micromanipulator. The cell was allowed to bud, and when the bud became separable from the mother cell, the two were placed far apart and allowed to bud again. The new buds were separated again. In this way "family

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*Robert K. Mortimer, UCRL, private communication.

trees" of the progeny of irradiated cells were obtained. It was found that there are many offspring (shown on Fig. 1) that will die, that is, that are incapable of producing buds. The lethal offspring can be produced several delayed generations later, and it appeared as though their production was of statistical, random nature. At the same time, some of the cells that remained capable of reproduction became healthier, as evidenced by their increased reproduction rate. One may take this experiment (to be published in more detail) as evidence for the possibility that survivors of radiation treatment may carry many defects, and that the cell possesses ability to recover by some sort of regrouping of its genetic loci and cytoplasmic constituents. Mendelian laws of inheritance, in their strict sense, do not apply to irradiated yeast cells, since offspring of the same mother show very different survival properties. Normal yeast cells do not show these phenomena, except rarely (1 in 100).

One is impressed by the lasting changes in proliferation rate that yeast cells have in the postirradiation period. Figure 2 gives a demonstration of these phenomena. In this figure, all cells have been treated similarly, and the figure shows yeast colonies on Petri dishes, photographed simultaneously. In 1 and 2 are normal single-cell isolates on potato dextrose agar, after 5 days at 20°C. Plate 5 shows single-cell irradiated isolates (50% lethal dose); note the appearance of many small colonies. In 3 and 4 are reisolates from small colonies appearing on irradiated plate No. 5. Plate 3 shows that all progeny of a single irradiated cell retained the reduced rate of growth, as shown here by the small colony size. The cells of 3 are also considerably more radiosensitive than the normal diploid cells, as one would expect from cells with a previously damaged reproductive apparatus.[6] Plate 4 again shows small colonies from another preirradiated cell, but the appearance of an occasionally larger colony. In 6, single-cell isolates from one of the large colonies of 4 are plated, giving rise to all large an healthy colonies, with more normal radioresistance than 4.

After considerable study of these phenomena we came to the conclusion that they represent an interesting, heretofore neglected aspect of radiation biology. Diploid cells show a depressant effect of radiation on cell division for many generations, but are capable of recovery into vigorously growing cells again without resort to sporulation or mating processes. The Berkeley group has these processes under study at present, of irradiating cells with known biochemical deficiencies.

With the full realization that animal tissues represent another level of biological complexity, one is nevertheless tempted to compare these events to the phenomena observed in the course of recovery of animal tissues from radiation effects. Somatic cells in mammals and man are mostly diploid, and in some tissues tetraploid. One frequently observes a sequence of events following exposure to sublethal radiation; these include delay in cell division, attempt of tissues to regenerate, chronic attenuation of normal growth pattern, and eventual proliferative growth, either in the form of benign-tissue rearrangement (e.g., connective tissue or keloid formation) or in eventual onset of cancer. Now it is well recognized that radiation-induced cancer has a peculiar delayed pattern of onset and that the rate of onset after the long delay is a very rapidly rising function. Such onset cannot be explained on the basis of single mutations induced by radiation. One may assume a working hypothesis that the cellular aspect of radiation tumorigenesis in its general features follows

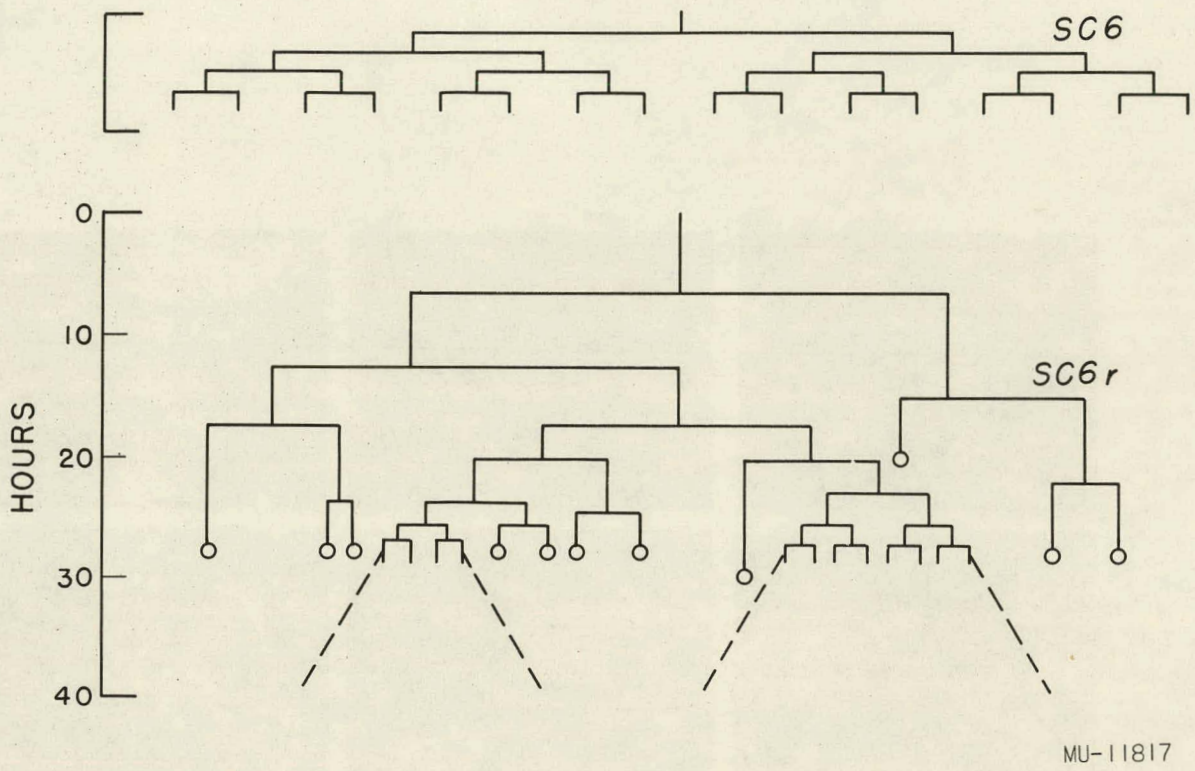

Fig. 1. Budding times of normal diploid Saccharomyces cerevisiae (top) and of the progeny of a cell irradiated by 20,000 roentgens (bottom). Small circles indicate that the particular cell has not gone on to divide further.

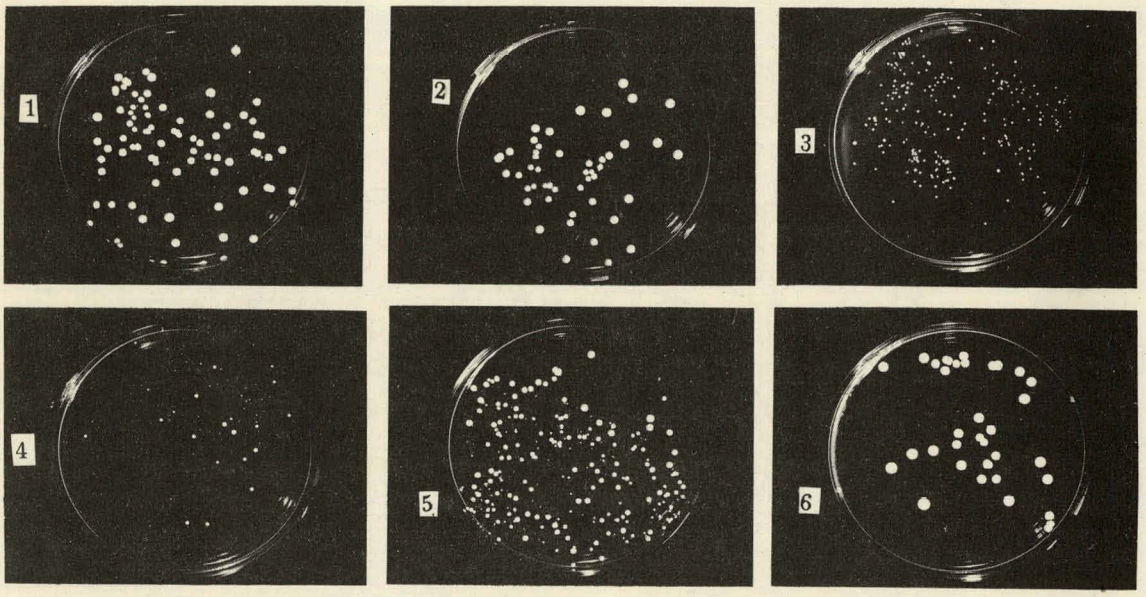

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Fig. 2. Yeast colonies from single irradiated cells. (See text.)

the pattern of injury and recovery in unicellular organisms such as the yeast cell. Thus, sublethal genetic injury to somatic cells may lead to impaired reproduction and function of the cells and, in some instances, of the organ they represent. These cells are not necessarily eliminated from the tissue, but produce progeny, forming small islands of cells with subnormal activity. As the number of such progeny increases, they form lesions that may be indentical with what is now known as a precancerous lesion in many types of cancer. Each of the cells in the group has a chance to recover via genetic rearrangements, that is, regrouping of chromosome parts by crossover, partial or full increase of ploidy, and perhaps other rearrangements. The recovery may occur in steps, and because it is a random process, may result in new, vigorously growing cells that differ in their detailed biochemical properties from the mother cell, thus becoming abnormal. Hormonal feedback may enter this mechanism of tumorigenesis, since the irradiated tissue will have subnormal activity, which elicits increased hormonal stimulation in the period of postirradiation recovery. In Fig. 3 I have plotted the parallelism between yeast cell recovery and carcinogenesis in the simplest case, a one-step recovery process, for which the rate of recovery is proportional to the number of progeny present at a given time from a given irradiated mother cell.

The ideas presented here provide a framework for detailed investigation of carcinogenic mechanism. They give plausible clues for explaining why sublethal doses of radiation are more effective for carcinogenesis than a single heavy dose; a greater number of viable subnormally active cells result from the former. A study of such cells may also be useful in arriving at a qualitative understanding of the action of chemical carcinogens. However, such studies should be pursued with the full realization that induction of tumors by radiation has, in some instances, been shown to have complicated dependence on humoral mechanisms and irradiation of another part of the body (e.g., see Ref. 11).

Depression of cell division rate in yeast cells goes along with various forms of depression of aerobic metabolism. This aspect of the problem and its relation to carcinogenesis has been under study for many years by Lacassagne and co-workers[8] and Maisin et al.[9] In view of the many years of effort of the Warburg school,[10] there is very little doubt that there is a shift toward anaerobic metabolism in cancer. On the other hand, recent demonstrations of abnormal chromosomes and abnormally large chromosome numbers also add to the view that carcinogenesis has both cytoplasmic and nuclear aspects. Yeast cells, with their well-known cytochrome system and excellent genetics, are very fine organisms for basic studies of these relationships.

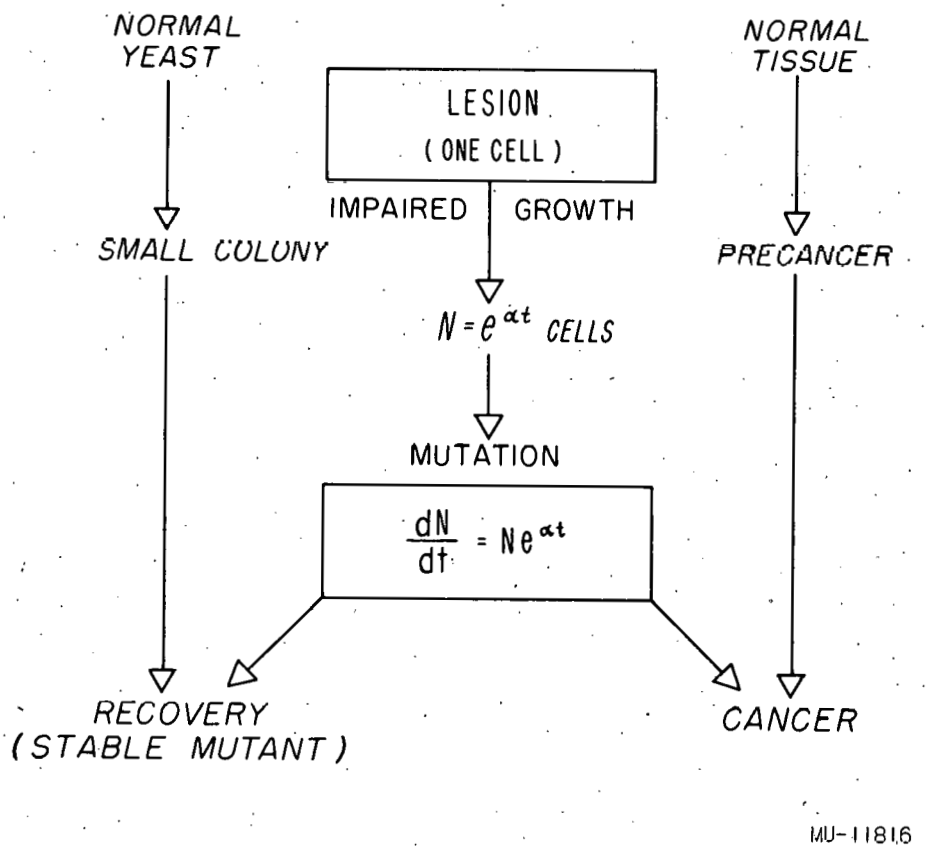

Fig. 3. Parallelism betwen (a) radiation effect on diploid yeast cells followed by recovery, and (b) possible development of cancerous lesions in animals.

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