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Effects of Radiations on Populations of Cells and Multicellular Organisms

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THE animal body is an assembly of cells. In considering the effects of radiation on such an assembly, the relationships between the individual cells and their assembly must be defined and, moreover, performance criteria must be found by which to characterize both the radiation effects on individual cells and the relation of these effects to the performance of the cell population.

There are three important principles which characterize the biological effects of penetrating radiations:

A. Radiation effects at the subcellular level are of a statistical nature. A single ionizing particle may lead to a decisive single radiobiological event in one of the many DNA nucleoprotein molecules or in another essential molecule. As a result, many different radiobiological events occur in the cells of an irradiated population.

B. In establishing the effect of irradiation on a cell population, the measured results are determined by the defined criterion for an experimental test. Because of the replication of genetic alterations in mitotic cell division, the criterion used for measurement frequently involves the selection of certain more-fit cells and the rejection of others, leading to end results different from what might be expected from statistical averaging of the entire population.

C. Radiobiology is an important basic tool because its fundamental interactions occur at the atomic-molecular level in essential sites of the cell, sometimes in locations not accessible to the ordinary tools of physics and chemistry. These interactions are then amplified to a level involving the entire cell—i.e., by a factor of about 10^{12}. Similarly, radiobiologic events that happened to a single cell or to a few cells may be amplified to the entire animal or human organism affecting the fate of as many as some 10^{14} cells.

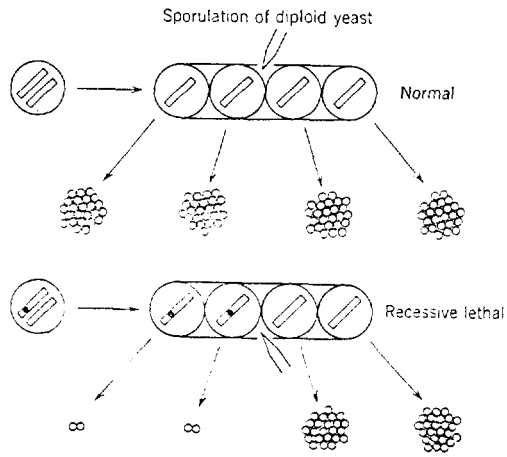

FIG. 1. Sporulation of normal and recessive lethal diploid yeast cells.

* Work at the Donner Laboratory reported in this paper was performed under the auspices of the U. S. Atomic Energy Commission.

CELL-DIVISION DELAY AND INHIBITION

Zirkle (p. 269) and Wood (p. 282) have admirably outlined the major causes of cell-division delay and inhibition in irradiated yeast cells. In order to consider population effects in detail, the discussion of the same cells is continued with the understanding that in other species many additional interesting and diverse radiobiological phenomena occur. One cannot describe in a single chapter the wealth of phenomena that have been observed.†

The following classes of genetic damage are of interest:

A. Dominant lethal damage. This leads to inhibition of colony formation in one, or in a few, cell divisions.[1]

B. Recessive lethal damage[2,3] can kill the haploid cell, but will allow survival of the diploid. In some forms of this damage, the genetic material appears to be rearranged so that progeny of such cells, for many generations, divide slower than normal cells. When the diploid which bears recessive lethal damage is sporulated, it produces two dead spores for each recessive damage. The presence of recessive lethal damage in a diploid "softens" the cell for reirradiation, already suggested by Latarjet and Ephrussi,[4] and the survival of such cells indicates greater sensitivity than the normal diploid, as shown by Tobias and Stepka[5] and by Beam.[6] Recessive lethal radiation damage is illustrated schematically in Figs. 1 and 2.

EXTENT OF DAMAGE IN NUCLEAR MATERIAL

The existence of radiation-induced auxotropic mutants of yeast cells in haploid mating types, requiring specific nutrients, makes it feasible to carry out genetic analysis of the damage induced. By mating individual cells under the microscope, one can breed diploid cells

† A good idea of the complexity of radiobiological research may be obtained by consulting the journal, Radiation Research, and the book, *Proceedings of the International Congress of Radiation Research, Vermont, 1958*, to be published by the Academic Press, Inc.

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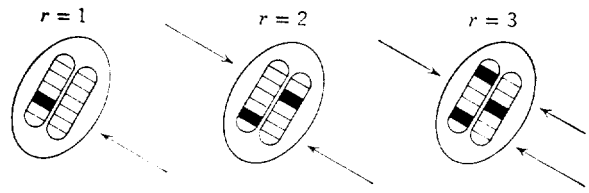

$$N = N_0(2e^{-\alpha D} - e^{-2\alpha D})^{n-r} e^{-r\alpha D}$$

N = survivors
D = dose
n = number of compartments
r = number of defects
α = constant

FIG. 2. Recessive model of radiation survival of diploid yeast cell with defects.

with recessive requirements for specific nutrients. Irradiation of such cells often makes the requirement dominant due to production of damage in allelic sites. In this fashion, it is found that damage often extends to regions greater than a single biochemical locus; moreover, the probability of damage occurring from site to site is very different. The situation reminds one of chromosome breaks, which are seen to be produced copiously in other organisms. The damage extends most of the time to chromosome parts which include more than one gene. In relatively rare events, the damage extends to a single genetic locus only or to some part of it.[7]

POSTIRRADIATION CHANGES IN NUCLEAR MATERIAL

There are gene mutations that are extremely stable and persist through very many generations. It is believed generally that these mutations persist, and do not recover, and that their production varies linearly with dose. Except perhaps in some mutations of haploid cells, this statement is not exactly true, when one regards all kinds of nuclear damage from radiation.‡ In cells of higher ploidy, the presence of genetic material in two or more sets allows postirradiation rearrangements in the course of several subsequent cell divisions. The fate of a typical diploid survivor in the postirradiation period is shown in Fig. 3, as reported by Tobias.[7] Here, the irradiated cell was placed on nutrient agar under a microscope and observed continuously. As cell divisions occurred, the mother and daughter cells were separated. It is seen from the graph that, in the course of seven subsequent cell divisions, several cells were produced that failed to divide again. The radiated cells also exhibited long cell-division delays. A typical chart showing how a normal cell gives rise to progeny is given also. The process of generation of cells lacking viability can go on for many generations so that some colonies arise where cell replication is below optimum value. For instance, by preirradiation it is easy to obtain colonies which produce one dead cell for every four normal ones.

One of the suggested mechanisms for such a postirradiation recovery effect is shown in Fig. 4. As the cell with recessive radiation damage in its nucleus divides, occasionally "chromosome crossover" occurs; i.e., segments of the chromosome exchange places with each other. The two progeny are not identical in chromosome content. In the instance shown, one of them has homozygous lethals, whereas the other will survive, having gotten rid of the damaged part. A method of experimentally demonstrating chromosome crossover uses recessive genetic markers. In the example shown, an adenineless locus was carried in the heterozygous state. When homozygous, the cells needing adenine become pink; otherwise they are ivory colored. Thus, a clone established from one irradiated diploid cell may appear color segmented, with approximately $\frac{1}{2}$, $\frac{1}{4}$, or $\frac{1}{8}$ of the cells colored, depending upon whether crossover occurred in the first, second, or third postirradiation cell division. This sort of delayed phenotypic appearance of induced chromosome changes has been known since the work with bacteria by Witkin, Demerec, and Latarjet.[8–10] Taking advantage of the detailed genetic knowledge available in yeast cells, James demonstrated ultraviolet-induced homozygosis[11] and Mortimer demonstrated the x-ray-induced phenomenon.[12]

Appearance of homozygosis, following damage, of recessive genetic traits is a defense mechanism, but it also serves to make recessive lethals existing in the population homozygous.

There are other nuclear changes suspected of occurring in the postirradiation period. In some large plant and animal cells, these are the consequences of chromosome breaks and their rejoinings. The result may be wholly- or partially-increased ploidy, euploidy, or aneuploidy.

CHANGES IN RADIORESISTANCE

In a mixed population of cells, various stages of cell division are present. It has been shown in haploid yeast cells that, in the early phase of budding, the cells are more than ten times more resistant than in inter-

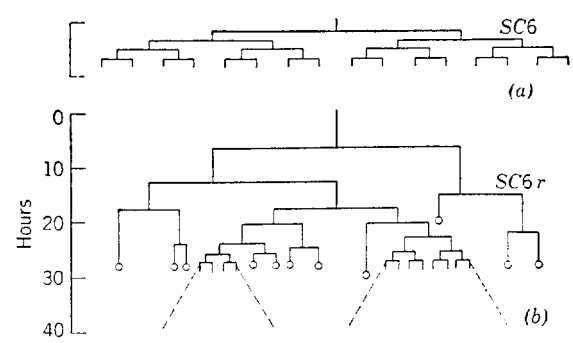

FIG. 3. Postirradiation divisions of diploid cell. (a) Nonirradiated. (b) Irradiated. Open circles indicate cells which have stopped dividing.

‡ *Note added in proof.*—Most of the evidence for linearity comes from radiation studies on the sperm of the fruit fly. Very recently, Russell *et al.* [Science 128, 1546 (1958)] have furnished experimental proof of the dose-rate dependence of some mutations by irradiating male and female mice with x-rays and γ-rays.

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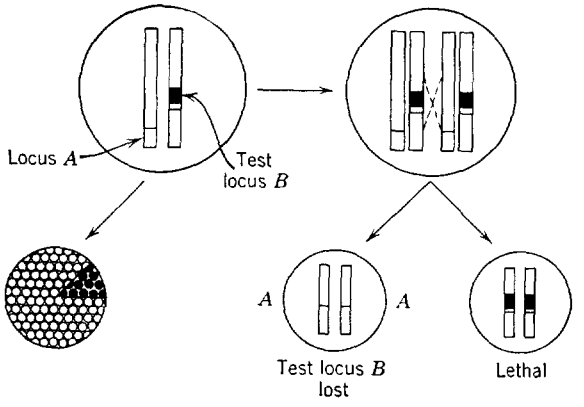

FIG. 4. Mechanism of postirradiation effect—chromosome crossover. If crossover occurs in delayed fashion, one may observe the appearance of segmented clones of cells, indicated on the left.

phase.[6,13] Moreover, kinetics of the sensitivity to radiation are different; while a single ionizing event in the proper location is sufficient for inhibition during interphase when most new DNA synthesis occurs, dividing cells require some 100 such events. Accepting DNA nucleoprotein as the site of action,§ this phenomenon is a clear indication that physical and perhaps chemical states of this substance must change radically in the course of cell division. DNA molecules undergoing synthesis are highly sensitive; existing DNA molecules exhibit decreased sensitivity. It would appear that, during mitotic division of yeast, the building blocks of the new chromosome are present in great multiplicity, after which they condense to make just two new nuclei.

RECOVERY FROM MUTATIONAL DEFICIENCIES

Point mutations induced by ultraviolet- or x-rays generally have a low but definite spontaneous reversion rate in the haploid cell. It appears that within the same biochemical locus many kinds of damage may be produced and that each of these has a characteristic reversion rate. Since many kinds of mutations may be prepared by radiation at the same locus, these eventually may serve to help in the understanding of the chemical nature of the genes and their ability to transmit information to other cell constituents.‖

When a diploid cell is made up of two haploids with heteroallelic auxotropic deficiency at the same locus, the cells cannot grow into colonies in the absence of the required nutrient. However, as Roman and Jacob have shown, ultraviolet irradiation of such a cell can bring about a striking reversion to independence of the same nutrient.[14] This occurs at unusually low doses and may be regarded as a beneficial effect of radiation, since it induces repair. The mechanism is not known. It is as though the irradiation helped the cell to make faultless replicas from the imperfect parts of the two allelic genes, a mechanism sometimes called the *copy-choice* method of replication.

CYTOPLASMIC EFFECTS

While the cell nucleus undergoes injury, cytoplasmic deficiencies also take place. There is every reason to believe that RNA enzyme molecules and proteins are equally as vulnerable as DNA. Even if such injury occurs, however, the situation for the cell usually is not assumed to be too serious, since there are many identical RNA molecules and since it is assumed that, with the aid of DNA, they may be resynthesized; on the other hand, the loss of function of an essential DNA molecule may prove fatal to the cell.

There are some autonomous cytoplasmic particles in various cells. According to the work of Ephrussi,[15] these particles in yeast are known to be able to duplicate themselves and the rate of their duplication may occur somewhat independently from that of DNA.[16] When the granules carrying the cytochromes are lost from the cells, they cannot be resynthesized. Radiation produces these small-colony mutants, with ultraviolet- being much more efficient than x-rays (as first shown by Raut[17]).

Almost any other cytoplasmic variable tested has been shown to be effected by radiation, though generally requiring higher doses than do the nuclear effects. Examples consist of disturbances in ionic balance, release of ATP and other substances from the cells, changes in activity of various enzymes, etc. These effects in yeast cells are reviewed by Rothstein.[18]

EXTRACELLULAR EFFECTS

Extracellular effects also are of importance in studying cell populations. These include the following factors:

A. Radiation effects on the nutrient medium.

B. Decrease in the autocatalytic action of cells which may synthesize important substances for other cells.

C. Release of growth-promoting and radiation-protective substances by the irradiated cells into the medium.

Interestingly enough, the last item of these seems to be of greatest importance. It first was detected in yeast cells under ultraviolet light by Loofbourow some 20 years ago, and more recently has been demonstrated for x-rays by Gunter and Kohn.[19] In mammalian tissue culture, Puck and Marcus[20] have shown such effects, while Révész[21] has worked on the problem in detail for ascites cells.

CYTOLOGICAL EFFECTS OF PENETRATING RADIATIONS IN TERMS OF INFORMATION THEORY

Radiations generally decrease the information content of the genetic apparatus. They also increase the

§ Although one generally assumes that chromosome damage occurs as a result of a single ionizing particle, it has never been proved that this primary interaction is with the DNA molecule. Release of an enzyme capable of acting on DNA may have the same effect, and such an assumption indeed would explain the chain reaction which results in the breaking of whole chromosomes.

‖ See chapters by Levinthal (p. 249) and by Lennox (p. 242).

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noise of the information channels—namely, extragenic components—which are instrumental in transmitting instructions for cellular functions, particularly duplication. This necessitates a lag in cell division which has a definite limit, however, and, if complete information is not transmitted in due time, the new cell is lost. Systems with redundancy in the genetic apparatus can transmit the information faster and more reliably.

Radiation damage to the coding system not only consists of the knocking out of individual symbols, but also consists of deletions of extensive parts of the code or of their rearrangement in spatial order.

The cell has mechanisms to repair the damaged genetic code to some extent. Given time, it can develop a method of more-reliable information transmission even in the presence of continuous radiation which is producing new damage at a steady rate. Recovery may occur by increasing the channel capacity and, in some instances, by correcting the code or increasing its redundance. In the course of such events, groups of cells with maximum equivocation or greatest information-transfer efficiency receive preference and may take over the colony.

POPULATIONS OF CELLS IN THE ANIMAL BODY

Radiation effects in the animal body are consequences not only of irradiation of its cells, but also of changes in functions of its organs and in their interactions with each other. For purposes of this discussion, an organ may be regarded as a heterogenous population of cells in a continuous state of activity—growth and anabolism for some cells, decay and catabolism for others. The cells are bathed in body fluids, which bring fresh nutrients and eliminate the products of cell metabolism and, at times, some of the cells themselves.

YEAST-CELL POPULATIONS IN THE STEADY STATE

To approximate conditions in an organ, Welch in our laboratory[22] worked with a population of diploid yeast cells, propagated continuously in a device resembling the chemostat of Novick and Szilard.[23] A schematic view of this device is seen in Fig. 5. When a nutrient medium flows into this system at a given rate and when a medium with cells is eliminated from it at the same rate, steady-state populations of cells are established with a constant rate of cell division. Welch has irradiated such cultures continuously with x-rays for more than one hundred consecutive cell divisions. As the radiation delayed cell division, the flow rate of the chemostat was adjusted so that a new steady state of cell population was established at the same cell density as in the controls. In a population of this kind, radiation can cause killing by the dominant lethal mechanism. The recessive lethal mechanism can also kill, either by the change of heterozygosis into homozygosis, or by the accumulation of homozygous recessive lethals. If one assumes the view that no recovery of gene-radiation damage takes place, then it follows that in each generation the population can tolerate a very small fraction of the dose required for acute killing.

Actually, the population has maintained continuous proliferation up to the highest dose rate tested, about 6000 r/generation time. The main effects of radiation were the prolongation of the time required for cell division, and the generation of some cells incapable of further cell division.

From the number of viable and nonviable cells present, it is possible to evaluate the mean lifetime of cells in the population and the average number of daughter cells a mother cell is capable of producing before her ability to undergo mitotic division is lost.

Figure 6 indicates how the number of daughter cells from each mother cell decreased rapidly with increasing dose rate. Each control cell produced about 66 daughters; this number decreased to about seven at the highest dose rate. At the same time, however, the cells appeared to acquire greater radioresistance by a factor of two than they had had prior to irradiation. The increased radioresistance appeared to develop by adaptation, rather than by mutation. A few generation times after radiation was stopped, the cells regained their normal rate of cell division. Cells taken out of the steady-state culture and irradiated in conditions of starvation showed normal radioresistance.

Although a great deal more needs to be done with continuously proliferating cellular systems, Welch has demonstrated that diploid populations can live in the presence of surprisingly large radiation levels, and that part of the radiation-induced damage recovers in the course of successive cell divisions.

RADIATION EFFECTS ON MAMMALS AND MAN

It is impossible to do justice to the intricate and detailed research work that has been going on in radia-

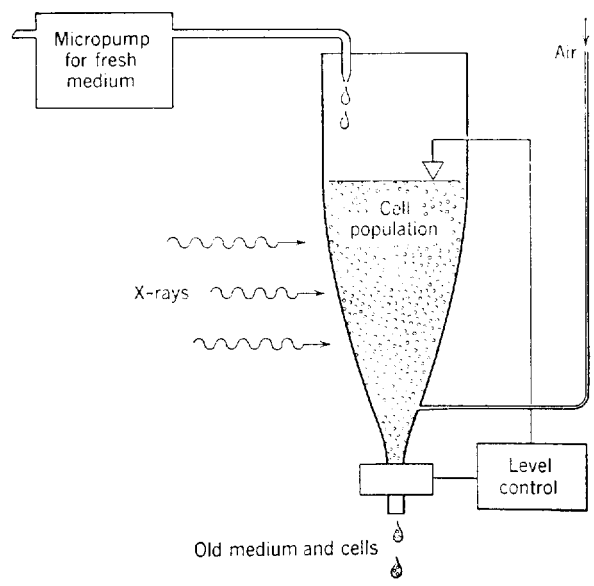

FIG. 5. Radiation of yeast-cell populations in chemostat.

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tion physiology during the past decades. The reader is referred to the excellent review articles and books on the subject.[24–26] It is important to note that bacteriological techniques of propagation recently have been applied successfully to human cells by Puck¶ and that, as a result, some information is already available on the radiosensitivity of various diploid and tetraploid human-cell strains. The mean lethal doses range from about 90–300 r, and the mechanisms of inhibition of colony formation are perhaps not too different from those for yeast cells. It is not known, however, what the radiation resistance of differentiated human cells in the body is, where it is possible that the very rapid humoral and metabolic exchange and the changes of radiosensitivity due to cell division might lead to an increase of radioresistance, as noted in the steady-state yeast-cell cultures.

RADIOSENSITIVE TISSUES

There is a 60-year-old law in radiobiology, originally proposed by Bergonnier and Tribondot; according to this law, the most radiosensitive tissues are those which have the highest mitotic index—i.e., the highest rate of cell division. One now understands that this law is only approximately true because radiation injury manifests itself mainly in the cell-division process. Radiated cells usually die when they attempt cell division; if their functions in body tissues do not require frequent cell division, the cells usually can perform ordinary metabolic and enzymatic functions for a long time post irradiation, since these functions are much less radiosensitive than cell division itself.

Correspondingly, in the acute phase, a single dose of radiation usually affects the most rapidly proliferating tissues; the bone marrow and its varied cell population and certain epithelial tissues are affected first. Early radiation deaths frequently are due to injury to the intestinal mucosa and to white-cell proliferation, while failure of the blood clotting mechanism and anemia make their appearance later. The neurons of the central nervous system of the adult are quite radioresistant, since almost no cell division occurs in them. In the developing mammalian embryo, however, as shown by Hicks,[27] nerve tissue is rapidly proliferating and has the greatest radiosensitivity.

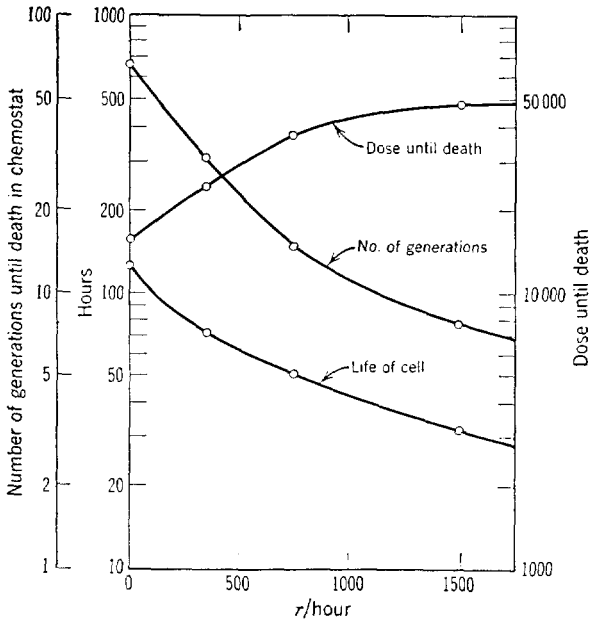

FIG. 6. Progeny decrease with dose rate.

¶ See chapter by Puck (p. 433) for detailed discussion.

INDIRECT RADIATION EFFECTS

There are very intricate relationships between various organs of the body, and it is not surprising that remote effects of radiations have been observed. For example, if an organ receives a dose of radiation, depressive effects due to this injury manifest themselves elsewhere in the body. Starting with the work of Hevesy, it was shown that a small depression of cell division and of DNA synthesis occurs everywhere in the body if an organ, such as the spleen, is irradiated.[28] To illustrate remote effects of radiation, one may mention work done at the Berkeley cyclotron in irradiating pituitary and various regions of brain tissue by high-energy deuterons.[29] Because of their small scatter, these particles are well suited to make small radiolesions in various parts of the body. It was found that a large localized dose to the rat pituitary (size 1×2 mm) will cause progressive development of the hypophysectomized state.[30] The growth of the animal, calcification of its bones, and functions of adrenals, thyroid, and gonads are all affected; development of the remote effects has different dose-response curves for each kind of effect.

An organ that is shielded from radiation confers a degree of protection against effects from whole-body radiation to the animal. Shielding of spleen and bone marrow is of greatest benefit, sometimes increasing radiation tolerance by about a factor of two. Jacobson and his associates[31] have shown that transplantation of bone marrow, spleen, or embryo homogenate to mice which have received a dose of x-rays increases radiation tolerance. The greatest part of this effect is owing to transplantation of healthy, unirradiated bone-marrow cells, which prosper on the tissue bed of radiation-killed bone marrow. Apparently, one may allow death of all cells of the marrow by whole-body x-irradiation, and the animal may be resurrected by the administration of a sufficient quantity of serologically compatible healthy cells. This technique has given rise to valiant attempts now being made in various medical installations to save terminal leukemia patients. Patients receive a lethal dose of whole-body radiation, followed by a bone-marrow transplant obtained from healthy relatives. The radiation is supposed to kill all bone-marrow cells, including the leukemic cells. If the transplanted marrow saves the patient from the radiation effects, it

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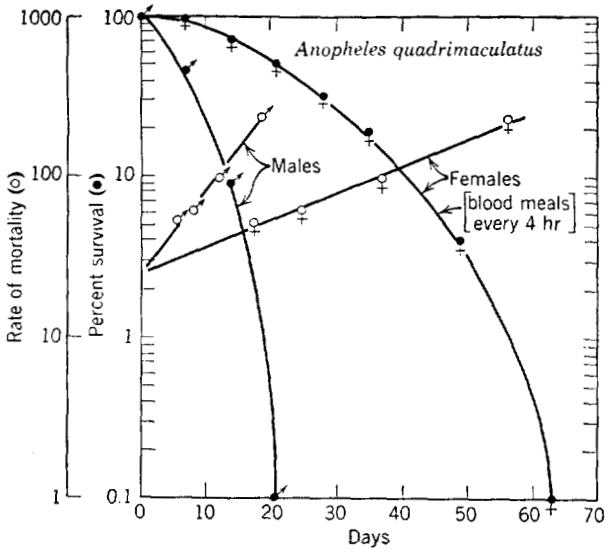

FIG. 7. Population-survival curve—mosquitoes [from W. E. Kershaw, T. A. Chalmers, and M. M. J. Lavoipierre, Ann. Trop. Med. Parasitol. 48, 442 (1954)].

is hoped that leukemia will not recur. Because of immunological reasons, much research is needed before bone-marrow transplants are completely safe for humans.[32]

LONGEVITY

In normal populations of humans, animals, invertebrates, and even unicellular organisms, there appear to be identical laws predicting the rate of death of the population. About a hundred years ago, Gompertz worked out details of this function, and at the present time Jones *et al.*[33] have applied it in detail to many problems of population survival.

The relative rate of death of a population of N individuals as function of time t is

$$\frac{d^2 \log N}{dt^2} = \alpha t,$$

where α is a characteristic constant of the population. To illustrate this principle, a normal survival curve of mosquitoes is reproduced in Fig. 7.[34] The relative rate of death is an exponentially increasing function. In adult humans, the death rate is described by a similar exponentially increasing function, with the rate doubling itself about every eight years. According to the theory by Jones, a single dose of radiation will modify the relative rate of death by shifting it upwards, parallel to itself. Continuous irradiation is supposed to increase the rate constant, α. Although many longevity estimations were made on the basis of this theory which can be used for daily permissible-dose determinations, rigorous experimental proof for radiation effects is not as yet available. Several other theories have been proposed.[35]

CARCINOGENESIS

It is of great interest to know something of radiation carcinogenesis. Cancer cells represent a population which is abnormal in the sense that (a) it has become independent of some of the growth-controlling factors characteristic of the tissue from which its cells originate; (b) it is frequently able to metastasize; and (c) it may have metabolic products which lead to death of the host. The rate of incidence of "spontaneous" cancer, chemical-, virus-, or radiation-induced neoplasms, usually, at least in some definite time interval, follows a law similar to that propounded for the rate of death of populations: The rate of onset increases as an exponential function of time. As an illustration, the rate of onset of bone tumors resulting from radioactive Sr^{89} incorporated in bone is shown in Fig. 8; this work was done at the Argonne National Laboratory.[36]** The rate constant of tumor incidence increases with increased radiation dose and is a more important index of carcinogenesis than is the over-all increase of tumors in a lifetime, which, in addition to the cancer-inducing factor, depends upon factors determining longevity. In practice, such an exponential rate curve means a "time lag" of varying length before the demonstrable onset of neoplastic growth.

The detailed biochemical steps that lead to cancer are not known, but it is useful to draw an analogy between diploid cells of animal tissue that have received a dose of radiation and diploid yeast cells, discussed earlier in this paper. An individual, sublethally irradiated yeast cell frequently develops an abnormal colony distinguished by its slow rate of growth in the process of "recovery." Some of the cells regain their full ability to proliferate by processes outlined in the section in which the recovery from radiation damage was dis-

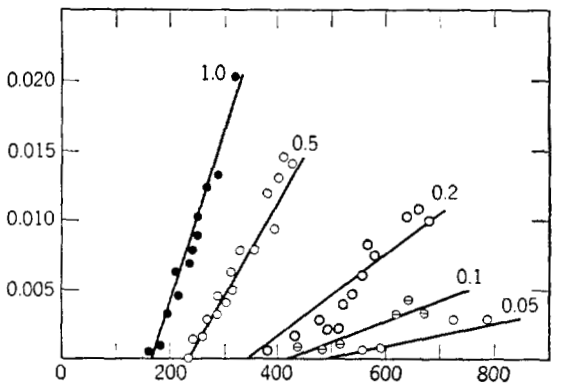

FIG. 8. Daily probability of bone-tumor development in the mouse following monthly injections of Sr^{89} (after Brues[36]). Abscissas: time after first injection (days). Ordinates: daily probability of bone-tumor development. Curves show effect of monthly doses of Sr^{89}, of 1.0, 0.5, 0.2, 0.1, and 0.05 μC/g.

** *Note added in proof.*—A. Brues and M. Finkel have published recent interpretations of their experimental work in carcinogenesis [Science 128, 637, 693 (1958)]. Examples of other views on this subject are found in the work of E. B. Lewis [Science 125, 965 (1957)] and in references 36, 40, and 42.

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cussed. This process often takes many cell generations, and in the course of it a number of changes can occur that will modify the genetic constitution of the cell. Changes resembling carcinogenesis in yeast cells also have been discussed by Lacassagne *et al.*,[37] Maisin *et al.*,[38] and Warburg.[39]

Most human cells normally are under hormonal and possibly neural control of the homeostatic apparatus and, as a result, they usually are in a state of proliferation which is below their maximum proliferative capacity. As an illustration, consider the human breast. This organ develops under elaborate hormonal influence, responding to secondary sex hormones elaborated by the ovaries and adrenals and to lactogenic hormone. In addition, the cells of this gland are influenced by growth hormone and posterior pituitary hormones. The state of this tissue and its metabolic activities are conveyed in some way to the hypothalamus and the pituitary, and as part of homeostatic control the gland may receive increased hormonal stimulation when its performance is below par.

There is increasing evidence available to show that hormonal balance is an important factor in radiation carcinogenesis. The intricate pattern of the hormonal factors has been studied for more than 80 years; here a few recent experiments only are noted, with mammary cancer as a restrictive example. Lacassagne has shown clearly that mammary cancer in mice may be induced by estrogen in females, as well as in castrated males. More recently, it appears that the joint application of estrogen and lactogenic hormone accelerates the onset of cancer. Bond *et al.*[40] found that a sublethal dose of whole-body irradiation in a strain of rats induced mammary cancer. However, the frequency of mammary cancer was decreased greatly if the ovaries of the animals were removed, thus eliminating most of the endogenous, secondary sex hormones. It is known that, following subtotal I^{131} thyroidectomy in mice, thyroid tumors as well as pituitary tumors result. Furth *et al.*[41] found that some rats recovering from whole-body radiation developed pituitary tumors, apparently in response to hormonal demands from endocrine target organs. When such tumors are transplanted into other rats of the same inbred strain, hormonal stimulation from the graft includes adrenal, thyroid, and mammary tumors in the new hosts. Apparently, whenever some tissue in the hypothalamic-pituitary-endocrine organ system develops sublethal tissue damage involving chromosome derangements, so that the tissue is unable to perform its functions satisfactorily, there is strong hormonal and/or nervous stimulation of these tissues causing increased rate of proliferation. Under such conditions, genetic rearrangements may lead to neoplastic tissues. An interesting illustration is furnished by localized deuteron irradiation of the rat pituitary at Berkeley.[29] Here 945 rep to the rat pituitary led within one year to the development in every irradiated animal of pituitary tumors, with 3.5 tumors per gland. Higher doses led to lessened incidences of tumors, and when most pituitary cells were killed, none of the animals developed pituitary tumors.

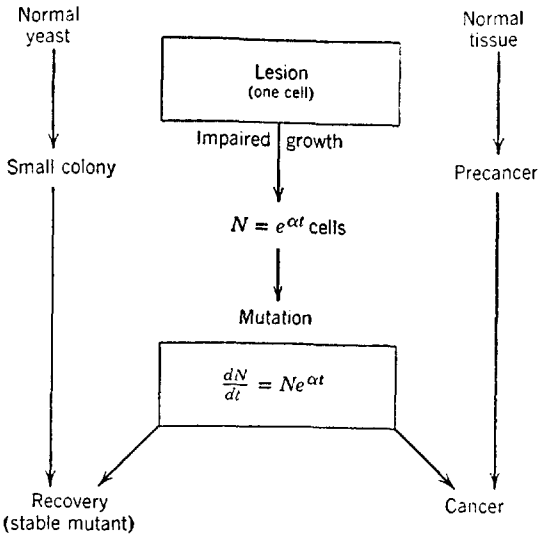

FIG. 9. Radiation carcinogenesis.

There are many other instances of hormonal influence over carcinogenesis. Perhaps the most complete investigations of this kind were made by Kaplan and his group.[42]

It would appear that a better understanding of homeostatic control mechanisms may lead to an understanding of the etiology of cancer and possibly to some methods of prevention or delay of its onset. In the meanwhile, it has been apparent that cancer cells that do not become completely undifferentiated in the carcinogenic process still retain some measure of dependence on hormonal control. Work is in progress in many countries to hypophysectomize patients with advanced and progressing metastatic carcinoma, in the hope that the cancer cells will stop growing and the tumors will regress.

The proton- and α-particle beams of the Berkeley 184-in cyclotron have proved to be promising tools for research on hypothalamic functions and for therapeutic investigations involving radiation hypophysectomy. The techniques and initial results have been described.[43] While hypophysectomy is not the final answer to cancer therapy, there are some patients who appear to have benefited from the pituitary irradiation. Detailed biochemical study of induced tumor regressions may bring additional clues with respect to the nature of the hormones which control normal or abnormal proliferation.

In conclusion, radiobiology has been useful as an aid to understanding not only the complex physiological changes following irradiation exposure, but also, the mechanism of carcinogenesis. Basic research on molecules and cells has helped in the understanding of some phenomena of cell division and of the nature of some

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genetic transformations. In Fig. 9, the present status of understanding of radiation carcinogenesis is shown in simplified fashion. It is apparent from the figure that there is a qualitative similarity between postirradiation events in diploid yeast cells and in somatic tissue cells. Many of the diploid yeast survivors develop small-colony mutants, some of them cytochrome deficient, that proliferate slowly and revert to rapid cell division many generations later. This process may be analogous to the slow development of benign precancerous lesions and the subsequent somatic mutation to cancerous cells. The time rate of occurrence of both sets of phenomena is quite similar. It is clear, then, that more work is needed to ascertain the genetic and biochemical features of delayed recovery in irradiated unicellular organisms and changes in the aerobic-anaerobic metabolism which, according to Warburg,[39] are known to occur in carcinogenesis. Perhaps such work may furnish some day direct clues to the detailed etiology and inhibition of cancer.

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