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RADIATION EFFECTS ON CELLS: GENETICS OR PHYSIOLOGY?

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INTRODUCTION

In the attempt to analyze biological injury by recognizing initial sites of interaction between radiation and protoplasm, many investigators have utilized homogeneous and isolated groups of cells. Even with the several advantages offered by isolated cell systems and after much recent work on this general question with these systems, progress, while steady, has been slow. For instance, it is not possible at the present time to state categorically whether certain prominent effects are brought about by nuclear as opposed to cytoplasmic, damage, or to what extent one or the other may be involved in the initial interactions that are ultimately responsible for the observed effect.[1]

In the course of investigating the physiologic and genetic effects of radiant energy and "radiomimetic" chemicals in the protozoan Paramecium

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we observed certain relationships which appear to bear upon interpretations in this area. The present paper is a description of some of the results, together with a discussion of the implications of these studies and of a few closely related ones selected from the literature on the subject.

CELL DIVISION

Cell division is one of the activities that has been examined in relation to radiation-induced chromosomal and genic damage in an effort to recognize the location of the sensitive sites. In yeast, Burns[5] shows that division delay is only slightly associated, if at all, with ploidy and concludes that cell division suppression induced by X rays is independent of damage to genic material. Sarachek et al.,[37] on the other hand, interpret their data as indicating that some, but not all, damage to the budding process is related to ploidy. The effectiveness of non-irradiated cytoplasm in the restoration of normal cell division to irradiated amebae (Pelomyxa) is clear evidence that a non-genic component can reverse this kind of injury.[7] In Tetrahymena it can be shown that cell division block induced by X rays is modified to a considerable extent by the nature of the metabolites offered the cell after irradiation,[9] either an indication of non-genic damage caused by the treatments or, improbably, of the prevention of latent mutagenesis.

Evidence derived from exposure of Paramecium aurelia to two mutagenic agents - X rays[31] and nitrogen mustard (HN2)[18, 30, 33] - is consistent with the conclusion that these agents affect cell division mainly because of an action on extra-genic material. Division delay is related to dose in such a complex manner as to make unlikely a genic interpretation. Also, this effect is temperature-dependent, in contrast to what is preponderantly a genetic effect measured in the same cell at the same time (see section on mutation production). Furthermore, the dosages of the two agents required for division delay are in the range causing cell death which, for reasons cited below, is also thought to be largely non-genic in these cells.

As shown in Fig. 1, the increase in genetic effect with X rays is very rapid in the cell compared to the suppression of fission. With HN2 the genetic effect appears more slowly in relation to suppression of fission. This is the result expected if it is postulated that X rays and HN2 disturb cell division by affecting the same cellular components, but that in getting to the micronucleus to bring about the genetic effect, high concentrations of the chemical are established in the cytoplasm. In inducing a given micronuclear change, then, HN2 may disturb the cytoplasm much more seriously, and, correspondingly, the fission mechanisms are affected to a greater extent. Although the contribution of macronuclear genic damage would not be measured by these relations, it does not seem likely that the effect is macronuclear in origin, since the micronucleus and the macronucleus should be equally sensitive and each should be equally available to HN2, as well as to X rays. If the macronucleus were involved, then for equal "genetic" effects induced by

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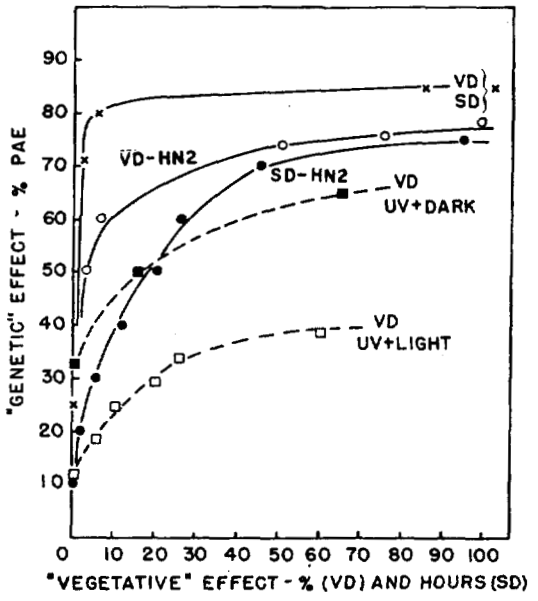

Fig. 1. The relationships between measures of vegetative and of genetic effects in Paramecium aurelia following treatment with X-rays (X), a nitrogen mustard compound (HN2) and ultraviolet (UV). The genetic effect is death post-autogamy (PAE) for X-ray and HN2, and reduction in vigor for UV. The vegetative effects are pre-autogamous death (VD) and suppressed division (SD). The ultra-violet plots are calculated from data in Kimball and Gaither.(17)

the two agents there should be an equal suppression of fission. This is not the case. The conclusion seems reasonable, therefore, that suppression of fission in Paramecium is unrelated to genic damage - in the micro- or macronucleus - but rather is extra-genic and, possibly, is extra-nuclear. The increasing evidence for interchange of material between the nucleus and cytoplasm in Paramecium(10) and other forms allows the alternative of extra-genic but intra-nuclear effects. An effect upon the nucleolus would be one example.

The foregoing does not imply necessarily that cell division is independent of the genic material of the nucleus. In Paramecium, it is clear that while the magnitude of the effect of X rays is dependent on dose, the rate of recovery is independent of dose; furthermore, the recovery rate is independent of the number of fission which the cell undergoes during the recovery period. These considerations indicate that the process of recovery involves structures that are not greatly affected by the dosage which produces the measured effect. Such structures may include the nucleus because of its importance in the synthetic activities of the cell. In the case of the ciliates, the convenient structure to think of in this connection is the large complex

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macronucleus with its genic material. If this structure is very highly polyploid,[38] it is conceivable that gene function is not impaired seriously at these dosage levels, and, also, that the rate of restoration of the immediate determiners of cell division is unimpaired. At extremely high dosages one might expect, on this basis, that the rate of recovery would also be affected. However, even at low dose levels such a situation may exist in cells not possessing a polyploid nucleus, as in the corneal epithelium of the rat, where recovery is also dose-independent.[12]

CELL DEATH

More complex in some ways is the question concerning the type of damage which results in death of the cell before sexual recombination. Results with microorganisms have indicated to many authors that death is a consequence of lethal genic changes induced by the radiation. This conclusion is based on inactivation kinetics which form the basis for the "target" theory of radiation effects. A single event is responsible for the death of the cell, and the most probable single event that could bring this about is the lethal gene mutation.

This theory means that a simple exponential mortality curve can be observed only in haploid cells, if most of the mutations are recessive. Furthermore, death of diploid cells derived from these haploid cells should be predictable. A number of investigators have tested this proposition in yeast cells of differing degrees of ploidy. A test of this hypothesis demonstrates that death of yeast cells of different degrees of ploidy can be explained in terms of chromosomal damage if an appropriate amount of dominant lethal production is assumed.[24] Zirkle and Tobias[44] formally fit the theory of the single event with the data obtained from haploids and diploids, and Uretz[40] presents arguments consistent with this mechanism (although not identifying the "sites") from experiments with X rays and ultraviolet radiation, alone and in combination. On the basis of these results these authors favor the view that death following irradiation is explainable by chromosomal damage in the cells.

In Neurospora similar conclusions are reached[1,28,29] from inactivation curves of uninucleate and multinucleate cells. Inactivation by ultraviolet is due to effects in the nuclei of the conidia. However, in one of these studies,[28] the problem of distinguishing between genic and nongenic changes within the nucleus is recognized.

These studies represent the kind of evidence that is presented to support the idea that death of cells after irradiation is related to changes in the genic material. There are observations, however, in these and other organisms that complicate the simplest explanation.

If there is a relationship between ploidy and resistance to radiation, then the ciliate protozoa should demonstrate very complex survival

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curves. These organisms contain a macronucleus which is thought, as mentioned above, to exert its control in the cell through a highly polyploid system of chromosomes. The survival curves in Paramecium are complex,[32] but it is only in this respect that the nuclear damage theory is satisfied.

If genic damage is ultimately responsible for vegetative death of the cell as well as post-autogamous death, then an equivalence in effects is reasonably expected between the different kinds of chromatin damage induced by the different agents. In Paramecium this is not the case, for the result depends upon the agent used. The degree of micronuclear damage as measured by post-autogamous effects, when compared to the macronuclear (assumed as such for the purpose of this argument) damage as measured by cell survival, is different for X rays, ultraviolet light and mustard compounds. Fig. 1 demonstrates that the death of the cell occurs at a dosage which is different for X rays than it is for mustard and ultraviolet, when a given amount of micronuclear damage is used as the base-line effect. X-rays induce micronuclear damage very rapidly compared to the rate of induction of cell death, while mustard and ultraviolet induce micronuclear damage much more slowly. One understands these results if, as in the case of cell division, the homogeneous distribution of X-ray dosage throughout the cell is compared to the gradients within the cell existing in the case of nitrogen mustard and ultraviolet. As the chemical penetrates into the cell there is a decreasing concentration gradient from the periphery to the center. A similar gradient of interaction is expected from ultraviolet radiation, for the obvious reason that the cytoplasm contains many strongly absorbing compounds, such as nucleic acids and other compounds. In regard to ultraviolet effects, a restatement of the results of Kimball and Gaither[17] shows that photoreactivation of the ultraviolet effect on cell death and "genetic" events results in a relationship different from that obtained in the dark. The "genetic" effect appears to be reduced more by the light treatment than cell death, indicating the possibility of differences in sites of action. Accordingly, since we believe that most of the "genetic" effects are due to gene changes (see below), the action of nitrogen mustard, ultraviolet, and X-rays must be largely extra-genic in bringing about most of the preautogamous death observed in these experiments in Paramecium. (This conclusion implies that UV-induced gene changes are photoreversible.)

The inactivation of bacteria by radiation has been thought by some to be caused by lethal mutations. But such a conclusion does not appear to be required. In a review of recent literature Zelle[42] compares mutation production with lethal effects of various treatments and concludes that radiation inactivation is not necessarily due to lethal mutations, and in some instances may be independent of genic damage. In this analysis of the problem, the difficulties of judgments based on comparisons of several kinds of effects are pointed out.

Date from other organisms indicate that explanations other than lethal genic damage are reasonable in some cases and are required in others.

In yeast, the evidence of highly increased resistance at the time of budding[3] suggests a "physiological," non-genic component. There is other evidence that the responses of these strains are functions of their "physiological" state, including the age of the culture.[40] Lucke and Sarachek[21] interpret their evidence on inactivation of yeast strains of different ploidy as permitting involvement of sites other than genic ones, even though death and ploidy are related.

In Neurospora there is evidence of a "nongenetic" component that influences survival after X irradiation and can be measured independently of changes most probably genic in nature[41] (see also 28). This would seem to complicate the simpler explanations of inactivation in Neurospora.

In other microorganisms, there is evidence for irradiation-induced death due to non-genic causes. The fact that death in Pelomyxa can be reduced or lessened by the injection of non-irradiated cytoplasm in the irradiated cell[8] is strong evidence for the changes being non-genic in nature. In the ciliate Tillina,[4] gene mutations in the macronucleus are eliminated as causes of radiation-induced death, but for reasons other than those cited above for Paramecium.

In multicellular forms the observations are similar. In Drosophila, survival appears to be independent of ploidy.[19] Also, the earlier interpretations in Habrobracon of death in haploids and diploids have been modified recently following observations[6] that death after X irradiation of haploids and diploids from early cleavage stages through pupal stages bears no simple relation to ploidy. Indeed, at very early stages, haploids are more resistant to X irradiation than diploids, contrary to the simple hypothesis that death is due to genic or chromosomal changes.

It appears that death of cells following irradiation can not be entirely related to changes in nuclei generally. In some forms (yeasts and Neurospora) it is possible to fit the inactivation data to hit theory if proper assumptions concerning the nature of the damage under different conditions are made. Even in these organisms, however, apparently there are physiological components that contribute in an unrecognized manner to the death of the cell. In other organisms (Paramecium, Pelomyxa, Tillina and in bacteria) there is strong evidence that death of the cell results after events that are non-genic in nature. And in higher organisms (Drosophila and Habrobracon), attempts to relate radioresistance to degree of ploidy have not been successful.

MUTATION INDUCTION

One of the important effects of radiations on cells is the induction of genic lethal mutations. In organisms such as Drosophila, which possesses a well-defined sexual cycle and morphologically observable chromosomes, it can be shown that certain kinds of permanent, inherited changes brought

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about by radiations can be ascribed to changes in the material of the chromosome. The frequency of this change - a gene mutation[2] - has been shown to be linear with increasing X irradiation at lower doses at a variety of dosage rates.

The concepts developed from this kind of study have been used to explain changes induced by radiations in organisms in which rigorous tests of the Drosophila type are not possible. In these, one must attempt by other means to relate the observed changes to the genic effects. In bacteria, for instance, by both X rays and ultraviolet, permanent discontinuous changes of certain kinds are induced with low frequency; the efficiency of the ultraviolet is highest at about 2600 A, and in one strain of E. coli (K-12) a certain amount of hybridization of the changes appears to take place.[42] Consequently, interpretation of the changes as genic changes appeared reasonable.

In bacteria, induction of mutations by mutagenic agents results in a variety of mutation-dose relationships. Several of these can be explained only with difficulty if the changes described are genic changes in the sense described above.[42] Unexplained dose-mutation curves are observed in other microorganisms also.

In Paramecium the intracellular reorganization called autogamy has been used to test lethal mutation induction by various agents. The process results in the production of a cell with a new nuclear complex which, according to genetic information available,[38] is homozygous for all genes. If a micronucleus, after exposure to some kind of treatment, contains a lethal mutation, dominant or recessive, some of the cells derived from that single cell should not survive autogamy. Therefore, treatment of the cell, growth of clones from isolated treated cells, induction of autogamy, and observation of the number which do not survive autogamy should constitute a measure of the induction of lethal changes in the treated micronucleus.

We have used two types of mutagenic agents for the treatment of Paramecium - the nitrogen mustard HN2[30,33] and X rays.[31] Low doses of both of these induce genetic changes in the micronucleus (recognized in the way described above) and increasing dosages produce increasing effects. But, as demonstrated in Fig. 2, as time of exposure to nitrogen mustard increases, there is a definite peak through which the induced effect passes, and further treatment not only does not have more effect, but actually decreases the effect. In other words, a peaked response curve is observed. In the high dose ranges, much cell death is observed, and at the highest dosages very few survivors are available for test. Consequently, it is difficult to test higher dosages. The X-ray results are qualitatively the same (Fig. 3). There appears to be a peak through which the response passes at

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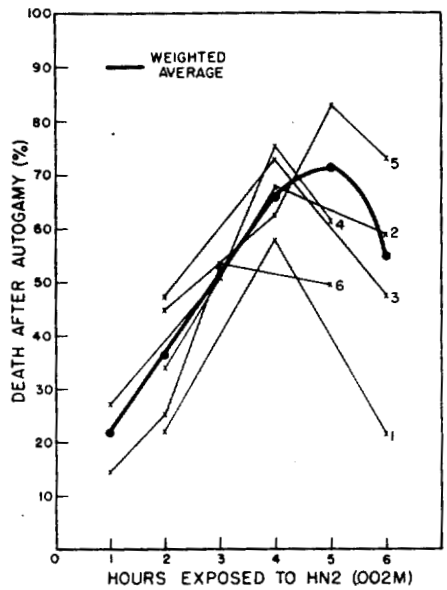

Fig. 2. The relationship between time of exposure to nitrogen mustard and genetic effect in Paramecium aurelia. The peaked response is seen in all six experiments.

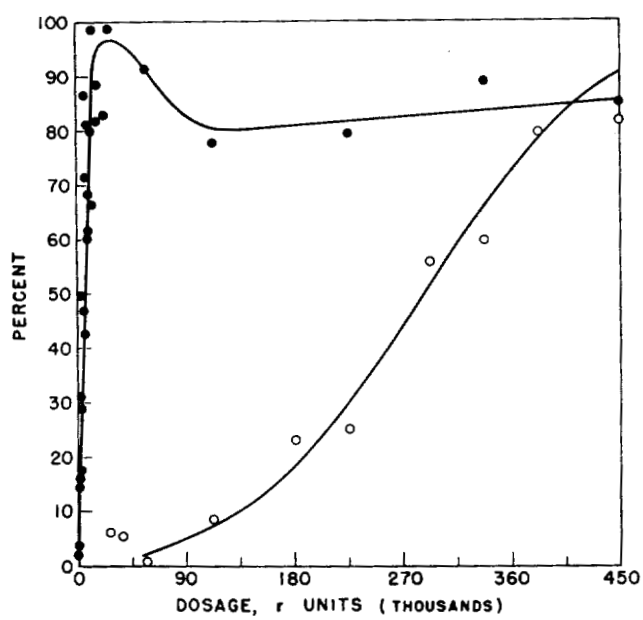

Fig. 3. The relationship between X-ray dosage and measured effect in Paramecium aurelia. The effects plotted are vegetative death (open circles) and death following autogamy (closed circles).

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approximately 40,000 r, followed by a dose range which is less efficient in bringing about this effect. As the dose is increased beyond this point, the effect again steadily increases through all dosages tested.

These results cannot be understood with the explanation applied to the low dose results, that is, that a small increase in dose causes a small increase in damage to the genic material. It may hold for the cases in which a plateau of effect is reached. For instance, lack of proportionality between X-ray dose and observed induced gene nutation in Drosophila sperm at high dosages may be explained as death of gametes caused by chromosomal damage with mutation production continuing.(25) In systems simpler than Paramecium, a saturation effect is difficult to conceive of when one considers that most of the genome is probably necessary for the continuance of life, and all parts should be radiosensitive. High dosages should continue to cause damage until 100% involvement is brought about. Apart from these considerations, however, when a definite reduction in effect occurs with increasing dose, it appears that the simple chromosomal-damage explanation is not sufficient.

Newcombe and McGregor describe results in Streptomyces which are similar - an increase in dosage of X irradiation or UV does not increase the number of mutations induced beyond a certain level, and may, under certain conditions, even reduce the number observed.(26) Non-linearity cannot be due to differential killing of spores, for preincubation reduces death but does not affect mutation induction. Newcombe suggests that "capacity for induced mutation" changes as radiation dosage to the surrounding cytoplasm increases.(27)

Other instances like these are listed in the literature. In Streptomyces flaveolus, certain mutants produced by UV and X rays increase in number with increasing dosage until a plateau or a peak is reached.(16) It probably is important that many of the mutants reverted to the "wild type" after a series of transfers. Also, the development of mutations induced in Trichophyton,(15) which bear a similar relationship to dose as those in S. flaveolus, is strongly affected by such influences as the time of incubation in salt solution after irradiation and before germination. In Glomerella the decline in the UV mutation curve at high doses is ascribable(22) to an increasing proportion of resistant pigmented spores in the populations. As in Streptomyces, phenotypic stability of the UV-induced mutants in Glomerella is poor.

Whether all these mutations also should be referred to gene changes is open to question. We recognize no reasons for believing that radiations can reverse induced chromosomal changes except at the original frequency of induction, and, therefore, reversal of gene changes is an extremely unlikely explanation of these results.

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But interpretation of the changes showing the curious dose-response relationships as strict genic changes may be allowable. The observations of McClintock[23] on mutable loci in maize suggests one general method of accomplishing it in these microorganisms. Another is suggested by the alleles in Paramecium controlling antigen type that are in turn controlled by the cytoplasm.[2] Perhaps the irradiated cytoplasm (perhaps also the nucleoplasm and structural abnormalities in the nucleus induced by high dosage) influence the expression of the mutated gene (Newcombe suggests that the mutation process itself is affected;[27] but this does not account for apparent reductions in mutation rates and only with difficulty for low stability of mutant phenotypes) to result finally in a dose-mutation curve that goes through a peak. According to this interpretation, as dosage to Paramecium increases, an environment is produced which prevents the expression of lethal genes. However, the second rise beyond the peak is difficult to explain in this system, and the general explanation appears not to fit.

There is an alternative method of accomplishing the same result which also has its counterparts in the biological literature. In yeast, mutations ("littles") can be induced which are due to losses of cytoplasmic substances as a result of the treatment.[11] In Paramecium, X-ray treatment may result in the loss of cytoplasmic particles (kappa) and a phenotypically different cell is produced.[34] After ultraviolet treatment of yeast, two types of the same cytochrome-deficient mutations have been observed - one that is inherited through a cross and is interpreted to be genic, and the other which, though permanent, is not transmitted through a cross and is interpreted as due to changes in cytoplasmic elements in the cells.[35] In Chlamydomonas, streptomycin resistance (ultraviolet-induced) may be of two types - one that is inherited in a mendelian fashion, and another that is inherited in an "extra-chromosomal" manner.[36] The former is thought to be due to genic changes, the latter not.

These studies suggest that permanent, inherited changes induced by irradiation may be due to modification of genes, or changes in non-genic sites, or to the two effects operating simultaneously. In the case of Paramecium, it is noteworthy that the peaked portion of the response curve is observed only in dosage regions of either agent in which there is a high cell death and significant suppression of fission - responses probably due to effects on non-genic entities (see above sections). In this region the cell as a whole is seriously affected by the treatment. If this cell does not die immediately, the extra-genic entities are so affected that it has a small chance of surviving autogamy. Evidence for extra-genic damage expressed after autogamy is presented by Geckler.[13]

Even at autogamy, then, death is in some part due to extra-genic damage, and the incidence of damage increases with dosage. A fraction of the population is not sensitive (such cells escape extra-genic damage for some reason), or damage is reversed in some manner and survival after high doses is found. At high doses there is little or no expression at

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autogamy of the extra-genic damage incurred by the population during irradiation. Two general methods of eliminating extra-genic damage appear consistent with the data. In one, at dosages below the peak the cells expressing extra-genic contributions to death after autogamy are the very ones liable for cell death before autogamy because of the degree of this special cytoplasmic damage suffered. There is a selection against these cells, and the survivors (without such cytoplasmic damage) are used to measure the postautogamous effect. In the other, in which cell selection is not a necessary assumption, the rate of increase of extra-genic damage is overcome at the peak by a simultaneously occurring dose-dependent damage-degradation process (e.g., the simultaneous intracellular induction and destruction of anomalous extra-genic determinants). In both, genic damage increases monotonically and is responsible for all death after the peak. (Phenomenological changes, such as induced loss of micronuclei, would further complicate the picture. However, this effect appears to become serious only at dosages above 400 kr.[14]) It must be noted that this explanation is different from the "selection" ones previously considered. These propose that a cell in suffering genic damage is less able to survive than one not suffering it, and at high dosages, one measures cells with little nuclear damage. In the explanation offered here, the damage which causes the removal of cells or of cell-death determinants is non-genic, and genic damage continues regularly throughout the dose range in an irreversible manner according to expectation.

Such an explanation may apply also to results from other microorganisms. In Streptomyces and Trichophyton perhaps these two types of damage are occurring. In addition to nuclear changes, cytoplasmic damage, as shown in other microorganisms, results in the production of mutants. As this cytoplasmic damage increases, cells die, and the mutation rate drops to that determined by genic changes as these cells are removed from the population. When a plateau is observed, the cells capable of mutating for extra-genic reasons have all changed, while the contribution of genic mutation is not recognizable because it is below the observed level.

SUMMARY AND CONCLUSIONS

Certain radiation effects observed in cells have been examined for information on the general question of whether the biologically effective interaction between radiation and the cell involves the genes. At the present time there are few effects that can be definitely related to changes in genes except the particular ones that constitute the definition of the gene. However, there is indicative evidence which can be summarized as follows:

1. Cell Division: the effect of radiation on this activity of the cells is independent of degree of ploidy in yeast; cell division is strongly affected by post-treatments with non-nuclear components of the cell metabolites and by temperature changes; and cell division effects are brought about by various mutagenic agents in

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Paramecium in ways explainable by differential concentrations in the cytoplasm. This evidence indicates strongly that radiation-induced disturbance of cell division is due mainly to non-genic, reversible effects within the cell.

2. Cell Death: recognition of the causes for cell death after irradiation is more difficult. In yeast, physiological factors clearly influence the rate of cell death, yet in this organism a definite relationship between chromosomal constitution and radiation-resistance can be demonstrated, and death in different stocks can be accounted for, with remarkably few assumptions, on the basis of modified target theory. On the other hand, in bacteria there is suggestive evidence gained from comparisons of lethality studies and mutation studies that death cannot be accounted for in terms of lethal mutations. Similarly, in Paramecium and other protozoa, genic damage can be eliminated as the chief cause of radiation-induced death because of the manner in which mutagenic agents bring about the cell death response.

3. Mutation Induction: the difficulty in recognizing gene mutation as a cause of phenotype mutation is due mostly to the fact that few cells are suitable for gene-testing purposes. In several cell types in which it is possible to carry out mating procedures, discontinuous and sudden changes in phenotype can be shown to be accomplished by changes in extra-genic components of the cell as well as genic ones. Therefore, when one analyzes dose-mutation relationships, it is especially important to distinguish between the two kinds of changes possible in the cell. It can be shown that, instead of allowing inferences concerning the nature of the gene and the mutation process, certain response curves may, in fact, indicate methods different from the expected genic types.

In general conclusion, it should be pointed out that the attempts to explain mammalian radiotoxicity in terms of specific cell damage are analytically even more inadequate than such attempts in isolated cell systems. Therefore, explanations that include estimates of effect based on changes in genes and chromosomes appear to require consideration based on the evidence available from single cell systems. In these systems, the effect is not always easily related to its primary cause, even when the areas of choice are reduced to the genic on the one hand as opposed to the non-genic on the other.

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FOOTNOTES

1. We are not considering the question of "direct" vs. "indirect" action of radiations. In either case, a unitary biological event appears the same. Zirkle[43] discusses this question.

2. We accept for this presentation all changes of this type as gene changes. Lefevre[20] and Stadler[39] discuss the difficulties in this assumption.

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