Cancer Research 1964: Thoughts on the Contributions of Radiation Biology*

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I am deeply grateful for the honor of addressing you as the President of your Association. At the same time, I feel more humble at this moment than ever before in the awareness of my own ignorance of cancer and of the gaps in knowledge that must be filled before the members of our profession will know adequately how to prevent and control the many diverse forms of neoplasia.

Lest we become discouraged, however, it is heartening to reflect on the advances which have been made in but one of the many fields of cancer research—namely, radiation biology. If we review our progress in this field alone, we cannot help but be gratified by the insight already gained and by the promise of the explorations now in progress. There is, furthermore, scarcely a more fitting place in which to review this subject than here in Chicago, for an association between ionizing radiation and cancer was first recorded in this city. The explanation for this association lies in the following letter (38):

"E. H. Grubbé  
12 Pacific Avenue  
Dear Sir:  
This will introduce Mrs. Rose Lee, who has carcinoma of the left breast. She is willing to have you make x-ray applications. I hope you can help her.  
Yours truly,  
R. LUDLAM, M.D.

January 28, 1896."

A few days before he received the foregoing letter Grubbé, who was a manufacturer of vacuum tubes, had sought medical aid for a dermatitis on his left hand (38). Although he was unaware of Roentgen's discovery of the x-ray, which had been reported a month earlier (see 32), his physicians correctly inferred that his dermatitis had resulted from radiation emanating from the Crookes tubes to which he had exposed his left hand many times daily. Struck by the potency of the effects of radiation on Grubbé's skin, one of his physicians, Dr. Ludlam, suggested that irradiation might help a patient with inoperable carcinoma of the breast, for whom he could offer no other effective treatment. The patient, Rose Lee, was therefore referred to Grubbé to receive the first recorded radiotherapy for cancer.

During the same year Thomas A. Edison attempted to use the x-ray tube for development of a fluorescent illuminating lamp. He soon abandoned these efforts, however, explaining later (10):

"... I started to make a number of these lamps, but I soon found that the X-ray had affected poisonously my assistant, Mr. Dally, so that his hair came out and his flesh commenced to ulcerate. I then concluded it would not do, and that it would not be a very popular kind of light, so I dropped it..."

Edison's studies with the x-ray fluoroscope were continued, however, and by 1902 the degenerative changes in Dally's skin had progressed to carcinoma, necessitating amputation of the left hand above the wrist. Progression of the disease subsequently required amputation of both arms and finally led to Dally's death from mediastinal metastases in 1904. Within a decade after its discovery, therefore, the x-ray had claimed its first martyr in this country through induction of malignant growth (10).

In retrospect, the rapidity with which radiation was introduced into medical diagnosis and therapy is remarkable, even by today's technological standards. Although there were the inevitable confusion and skepticism about the medical value of the x-ray when it was first announced, within several years it became widely accepted as indispensable in diagnosis (9) and treatment (66). By 1909, more than 3000 patients with malignancies of all types had received radiotherapy (3). Before the risk of carcinogenic effects became recognized, however, a number of pioneer radiologists suffered Dally's fate (10); their neoplasms, moreover, included leukemias as well as cancers of the skin (see 31).

When the need for precautionary measures eventually became recognized and steps were taken to guard against overexposure, the earliest safety standards were based on the assumption that carcinogenesis required relatively high levels of irradiation—i.e., that there was a radiation threshold below which there was no carcinogenic risk.
Although this assumption remains to be refuted, the existence of risks has been demonstrated at progressively lower and lower dose levels by epidemiological investigations on human populations and experiments in laboratory animals. As a result, the prevailing attitude among radiation health authorities has become increasingly cautious, as is reflected by changes in their nomenclature; e.g., the "tolerance" dose of radiation is no longer referred to as such but is now called the "maximum permissible" dose, which implies that the associated risk, if any, is within acceptable limits, rather than that there is no risk at all. It is not yet possible, however, to define unambiguously the precise mathematical relationship between incidence and dose for any neoplasm in man or animals and hence to estimate confidently by extrapolation the risk of low-level irradiation (72, 74). The bulk of the data implies that the neoplastic transformation usually follows multi-hit kinetics (12, 13, 16), but the possibility of one-hit kinetics cannot be excluded in all instances (72). In the face of this uncertainty it has been recommended that the risk be assumed to vary as a linear function of radiation dose down to the lowest levels, a policy defended on the grounds that it should tend to overestimate rather than underestimate the risk and thus to provide a desirable margin of safety (72).

This tendency toward increasing caution has been encouraged further by evidence suggesting that irradiation of the fetus or embryo at diagnostic dose levels may increase the risk of cancer in childhood. The first suggestion of this, published less than a decade ago by Stewart et al. (69), provoked a succession of studies which gave ostensibly conflicting results. When the combined data from such studies were analyzed, however, with each study weighted for its contribution to the total population sample, the results supported the interpretation that intrauterine diagnostic irradiation is carcinogenic—i.e., cancer was found to occur 40 per cent more often in children with a history of antenatal irradiation than in their nonirradiated controls (Table 1). If this interpretation is borne out, then it will prompt two tentative conclusions: first, that the threshold dose for radiation carcinogenesis must be less than 1–5 rads, if a threshold exists at all; second, that susceptibility to radiation carcinogenesis is appreciably higher before birth than at any time later in life. These conclusions have far-reaching implications in terms of both mechanisms of carcinogenesis and our approach toward environmental health.

In the light of these developments, let us consider what mechanisms of radiation carcinogenesis, if any, are implicated from experimental data. Without attempting to review this subject comprehensively, I shall cite one or two examples to illustrate the kinds and complexity of the radiation effects that have been noted and the problems hampering analysis of mechanisms of radiation neoplasia.

Perhaps the most thoroughly studied radiation neoplasm is the thymic lymphomas induced by whole-body irradiation in mice. On the basis of the findings to date Kaplan (51) has postulated that radiation exerts oncogenic effects on the thymus in three ways: (a) It releases or "activates" a latent leukemia virus which is present in other tissues of the body as well as in the thymus. (b) It causes atrophy of the thymus, which is followed by stimulation of thymocytes to regenerative hyperplasia; however, atrophy and regenerative hyperplasia induced by means other than irradiation are leukemogenic in an irradiated animal, indicating that the thymus itself need not be irradiated to undergo radiation-induced neoplasia (i.e., a nonirradiated thymus is stimulated to undergo neoplasia on transplantation into an irradiated recipient). (c) It impairs regeneration of the thymus by injuring the bone marrow, thereby subjecting primitive thymocytes to prolonged maturation arrest and in this way prolonging the period during which they are maximally susceptible to the leukemia virus.

How irradiation liberates or "activates" the leukemia virus is not known, but the induction of irreversible "initiating" effects by partial-body irradiation (49) and by irradiation of individual organs in vitro, such as the brain and lung, as well as hemopoietic and lymphatic tissues (6, 7), is consistent with some type of "activation" of latent or incomplete virus. That virus may be latent before irradiation and need not enter the irradiated animal from the external environment after irradiation is further indicated by results in our laboratory with germ-free mice; i.e., the incidence of radiation-induced thymic lymphomas in germ-free RF mice (Chart 1) is as high as, or higher than, in conventionally reared RF mice, within our observation period to date (7 months). Moreover, despite the lack of any other detectable microbial agents in the germ-free leukemic mice, their tissues reveal the presence of particles morphologically indistinguishable from known leukemia viruses, suggesting that the virus has become activated in situ.1 These observations are consistent, therefore, with Gross's (34, 35, 37) hypothesis that the leukemia virus is transmitted vertically to the embryo and remains latent in vivo until "activated" by irradiation.

The process by which the oncogenic virus transforms a

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1 H. E. Walburg, Jr., A. C. Upton, G. E. Cosgrove, Jr., R. L. Tyndall, and W. W. Harris, unpublished data.

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TABLE 1

<table>
<thead>
<tr>
<th>Study</th>
<th>Weight (percentage of total)</th>
<th>Relative risk†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ager et al. (1)</td>
<td>6.9</td>
<td>1.12 (.62–2.04)</td>
</tr>
<tr>
<td>Court-Brown et al. (19)</td>
<td>5.5</td>
<td>0.85 (.44–1.65)</td>
</tr>
<tr>
<td>Ford et al. (27)</td>
<td>11.1</td>
<td>1.71 (1.07–2.73)</td>
</tr>
<tr>
<td>Kaplan (48)</td>
<td>7.2</td>
<td>1.72 (0.96–3.08)</td>
</tr>
<tr>
<td>Kjeldsberg (54)</td>
<td>1.7</td>
<td>0.59 (0.16–2.17)</td>
</tr>
<tr>
<td>Lewis (57)</td>
<td>0.5</td>
<td>0.42 (0.01–12.4)</td>
</tr>
<tr>
<td>MacMahon (60)</td>
<td>19.1</td>
<td>1.42 (0.99–2.03)</td>
</tr>
<tr>
<td>Murray et al. (62)</td>
<td>1.4</td>
<td>0.92 (0.24–3.45)</td>
</tr>
<tr>
<td>Polhemus and Koch (65)</td>
<td>14.5</td>
<td>1.33 (0.89–2.01)</td>
</tr>
<tr>
<td>Stewart et al. (69)</td>
<td>30.5</td>
<td>1.65 (1.24–2.18)</td>
</tr>
<tr>
<td>Wells and Steer (79)</td>
<td>1.7</td>
<td>0.72 (0.22–2.40)</td>
</tr>
<tr>
<td>All studies:</td>
<td>100.1</td>
<td>1.40 (1.20–1.64)</td>
</tr>
</tbody>
</table>

* From MacMahon (59).
† Approximate 95 per cent confidence limits in parentheses.
cell to neoplastic behavior, although obscure, is a subject of intensive study at the moment (25, 50). Of interest in this connection is the consistent occurrence of an extra and marker chromosome in the marrow cells of mice with passaged radiation-induced myeloid leukemia (78). We have noted this abnormality in mice of two independent leukemia passage lines derived from different primary donors and maintained separately by serial transmission since their origin. The induction of the chromosome abnormality by cell-free ultracentrifugates and the breakage of chromosomes by viruses of other kinds (see 78) suggest that the leukemia virus may act directly on the chromosome. The leukemogenic action of the virus may thus conceivably be mediated through its chromosomal effects, recalling a cytogenetic interpretation of viral oncogenesis put forth earlier (76). Although, on the other hand, this chromosomal alteration may represent merely an epiph- enomenon, it is not an example of the random aneuploidization which is common among neoplasms. Its significance in relation to the chromosome aberration associated with the analogous leukemia of man—i.e., to the Philadelphia chromosome (39)—is a question that we hope we can resolve with further study.

Turning to the question of virus-host cell specificity, our data suggest that the same virus or viruses may induce either myeloid leukemia or thymic lymphoma in RF mice depending on the age, sex, and physiological condition of the host animal (46). This diversity of induced leukemias parallels that reported in other virus-host systems (see 36). The various leukemias and lymphomas may, however, be postulated to result from transformation of the same target cell or closely related cells. It appears, for example (Chart 2), that the stem cell responsible for populating the bone marrow, which is morphologically indistinguishable from the small lymphocyte or thymocyte (20, 21), is also responsible for renewal of the thymus (41, 42, 58). Neoplastic transformation of this cell may, thus, be envisioned to cause reticular neoplasms of various types, depending on the particular way in which differentiation of the cell is affected. In addition to offering an explanation for the diversity of neoplasms induced by the leukemia virus, this interpretation explains the action of marrow injury in the pathogenesis of radiation-induced thymic neoplasia, mentioned earlier.

Whether other radiation neoplasms involve the action of oncogenic viruses remains to be determined (e.g., activation of the mammary tumor agent in the induction of neoplasia of the breast). It is noteworthy, however, that
irradiation increases the susceptibility of mice to other viruses, including the polyoma virus (55), as well as to leukemia virus (33).

Another unresolved question is the basis for the relatively long induction period characteristically preceding the appearance of radiation-induced neoplasms, which usually encompasses a considerable fraction of the life span (Table 2). In general, the induction period of such neoplasms is appreciably longer than that of neoplasms induced by viruses or chemicals. A large part of this time may be required, of course, merely for a single transformed cell to multiply into a detectable number of neoplastic daughter cells, depending on the frequency of successive doubling divisions (Table 3); e.g., any of the induction periods observed in man and other species could be explained entirely in terms of the generation times that have been documented in various tissues (see 56, 63, 64). There is evidence, however, that all neoplastic cells do not grow at the same rate within a neoplasm or at a constant rate throughout time (see 61), and that, moreover, the induction period involves a succession of qualitative changes in the cells undergoing neoplasia (see 28—30). This evidence and the occurrence of radiation-induced changes that are otherwise associated with natural aging (see 73) have prompted the speculation that neoplasia (see 16, 24) and other age-related diseases (see 22, 70) result from the gradual accumulation of multiple mutations or similar changes in somatic cells. According to this hypothesis, the age-distribution of cancer reflects the generally lengthy time required for accumulation of a carcinogenic combination of mutations in a single cell or neighboring cells, the probability of each individual mutation being low. A corollary to this theory is that the total number of mutational changes and, hence, the induction period should vary from individual to individual, depending on whether one or more of the necessary mutations is inherited (see 15). This hypothesis attributes the effects of radiation to the instantaneous induction of mutational changes that would take a long time to accumulate naturally. To this extent irradiation should cause an irradiated population to behave as if it were older, which is consistent with the frequency of induced neoplasia in relation to age at irradiation; i.e., the observed increase in the age-specific incidence at any given age corresponds to that which would result from a relatively constant increment in age rather than to a constant number of additional cases (8, 64); e.g., 10—20 times as many cases of neoplasia are induced in elderly as in young adults for a given dose of radiation (Chart 3).

Along with a presumable accumulation of somatic mutations with age, there is an observable increase in the frequency of chromosome aberrations (22, 45, 77). The induction of chromosome aberrations by irradiation, which may remain latent for years (2, 5, 14), and the association of specific chromosome abnormalities with certain

<table>
<thead>
<tr>
<th>Species</th>
<th>Life span (1000 days = 1)</th>
<th>Latent period (200 days = 1)</th>
<th>2/3 power of life span</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, rat</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>3</td>
<td>2</td>
<td>2.1</td>
</tr>
<tr>
<td>Dog</td>
<td>5</td>
<td>3</td>
<td>2.9</td>
</tr>
<tr>
<td>Man</td>
<td>35</td>
<td>8</td>
<td>10.7</td>
</tr>
</tbody>
</table>

* From Brues (11).

<table>
<thead>
<tr>
<th>Time required for formation of clone*</th>
<th>Generation, or doubling time of neoplastic cell (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 fortnight</td>
<td>0.5</td>
</tr>
<tr>
<td>1 month</td>
<td>1.0</td>
</tr>
<tr>
<td>1 year</td>
<td>12.0</td>
</tr>
<tr>
<td>1 decade</td>
<td>120.0</td>
</tr>
</tbody>
</table>

* The times shown are approximations, based on the assumption that a spherical clone 1 cm. in diameter contains 10⁹ cells and is formed by growth of all the descendants of the original neoplastic cell, multiplying at rates corresponding to the doubling times indicated.

**TABLE 2**

**Relation between Minimum Latent Period and Life Span**

**TABLE 3**

**Time Required for a Neoplastic Cell To Form a Spherical Clone (Tumor) 1 Centimeter in Diameter**

**CHART 3.—Incidence of radiation-induced neoplasms in relation to age.** Legends: acute and chronic leukemia in patients irradiated therapeutically for ankylosing spondylitis, plotted against age at time of first treatment (23) (△). Acute and chronic leukemia, excluding chronic lymphatic, in general population of England and Wales 1855—1957 (18) (Δ); all malignant neoplasms in A-bomb survivors exposed within 1500 meters of the hypocenter, Hiroshima Tumor Registry 1957—1958 (40) (○); malignant neoplasms excluding leukemias and lymphomas in A-bomb survivors exposed within 1500 meters of the hypocenter, Hiroshima Tumor Registry, 1957—58 (40) (●); and all malignant neoplasms in nonexposed (beyond 10,000 meters of the hypocenter) A-bomb survivors, Hiroshima Tumor Registry, 1957—58 (40) (◇).
primary neoplasms—e.g., the Philadelphia chromosome in chronic myelogenous leukemia (39)—suggest another possible mechanism for the development of neoplasia with age and as a late sequel to irradiation.

In regard to the possible role of somatic mutations and chromosome aberrations in the pathogenesis of radiation neoplasia, it is noteworthy that irradiated cells have been found to possess a far greater capacity for homeostasis, even at the genetic level, than was suspected only a short time ago. It is estimated, for example, that over 90 per cent of chromosome breaks can be healed in some types of cells if the affected cells are metabolically active and are supplied with the energy needed for repair (see 80). The capacity for repair varies, however, depending on the type of cell irradiated and its physiological condition before, during, and after irradiation. The probability of repair of a break also appears to vary with the region of the chromosome that is broken (43). Even point mutations are thought to evolve through stages which are reparable in metabolically active cells, repair of premutational injury being credited for the relatively lower yield of mutations in mouse spermatogonia and oocytes exposed to γ-rays at low dose rates than in those exposed at high dose rates (Chart 4). The repair of premutational injury varies, moreover, by an order of magnitude or more in Paramecium, depending on the stage in the division cycle in which the cell is irradiated (Chart 5). Such phase-dependent variations may conceivably account for the capacity of a conditioning dose of x-rays to enhance the sensitivity of mouse spermatogonia to the mutagenic action of a second exposure (Chart 4); i.e., synchronization of the cells by the first dose may place them in a stage in which they are relatively poorly able to repair the premutational injury caused by the second dose, depending on the size of the first dose and the interval between doses. Such an enhancing effect of properly timed split doses can more than offset the reduction in yield of mutations that results from administration of a large dose in a single exposure (Chart 4), which has been ascribed to extensive killing of cells and selection of a radioresistant surviving subpopulation. The results imply, therefore, differential effects of split doses on mutagenesis and on lethality, which may be postulated to involve differences in the capacity of the affected cells to repair premutational, as opposed to prelethal, injury, cells recovering from sublethal radiation injury (26) likewise showing synchronization and phasic fluctuations in sensitivity to killing by a second exposure (see 47).

The basis for repair of injury from ionizing radiation is not fully known, but it is tempting to speculate that it may have mechanisms in common with repair from injury caused by ultraviolet irradiation. The latter involves elimination of radiation-induced thymine dimers (Chart 6) from the affected DNA, presumably then allowing reinsertion of normal thymines into the DNA (68). To reverse the effects of ionizing radiations, in which thymine dimers play only a minor role, if any, this process of "cutting and patching" (Chart 6) would have to correct DNA lesions of other types, a possibility which remains to...
be verified. That there are limits to the kinds and extent of repair is clear from studies with particulate radiations of high linear energy transfer, such as α particles and fission neutrons, since the effects of these radiations for certain types of injury appear relatively irreparable in comparison with the effects of x- and γ-rays (4, 44). Whatever its scope, the mere existence of error-correcting machinery which can repair injury at the level of DNA itself calls for major changes in our attitude toward mutagenesis and its possible role in carcinogenesis. It implies, for example, that the action ultimately exerted by a mutagenic or carcinogenic agent results less from its initial molecular effects than from the lesion remaining after cellular efforts at repair, the difference between initial and final lesions being potentially large, depending on how the cell modifies the initial insult.

The foregoing information from studies on radiation effects has broad significance for carcinogenesis in general, not to mention cancer therapy. It is particularly relevant to chemical carcinogenesis, since the effects of radiation result from chemical disturbances within the cell and since the carcinogenic effects of radiation can be reproduced by chemicals of a variety of types, particularly by the radiomimetic alkylating agents (Table 4). The relatively high organ-specificity of chemical carcinogens has overshadowed their systemic effects until relatively recently (71), with the result that too little attention has been given to long-term physiological effects of these agents other than neoplasia. Studies with radiation support the need for broader inquiry into the biological effects of these agents and point to directions in which such explorations may be fruitful.

The combined effects of chemical carcinogens and radiation also need to be systematically investigated, particularly in the light of modern concepts of mutagenesis and carcinogenesis. The implications of possible interactions between radiation and chemicals in initiating, promoting, and co-carcinogenic effects are obvious. Study of the interactions between both types of agents as concerns additive, synergistic, and inhibitory effects in carcinogenesis should be coordinated with study of interactions in effects on genes, chromosomes, mitosis, and cell survival. Recent developments in experimental biology promise to provide the tools and approaches with which such correlative studies may soon be meaningfully conceived and carried out within one and the same cell population.

On the practical side, it is increasingly urgent that we improve our knowledge of the risks associated with exposure to radiation and other carcinogenic agents in our environment. The explosive development of technology

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**TABLE 4**

<table>
<thead>
<tr>
<th>AGENT</th>
<th>DOSE†</th>
<th>NO. MICE</th>
<th>MEAN SURVIVAL TIME (DAYS)</th>
<th>INCIDENCE OF NEOPLASMS PER CENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thymic lymphomas</td>
<td>Myeloid leukemia</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>3.7-4.5 mg/kg (X1)</td>
<td>158</td>
<td>561</td>
<td>5</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>2.4 mg/kg (X4)</td>
<td>104</td>
<td>490</td>
<td>21</td>
</tr>
<tr>
<td>Triethylene melamine</td>
<td>3.0-4.0 mg/kg (X1)</td>
<td>146</td>
<td>508</td>
<td>16</td>
</tr>
<tr>
<td>Triethylene melamine</td>
<td>1.5 mg/kg (X4)</td>
<td>99</td>
<td>427</td>
<td>33</td>
</tr>
<tr>
<td>Myleran</td>
<td>12 mg/kg (X4)</td>
<td>109</td>
<td>511</td>
<td>35</td>
</tr>
<tr>
<td>X-rays</td>
<td>500-600 r (X1)</td>
<td>242</td>
<td>396</td>
<td>26</td>
</tr>
<tr>
<td>X-rays</td>
<td>300 r (X4)</td>
<td>109</td>
<td>355</td>
<td>33</td>
</tr>
<tr>
<td>Controls</td>
<td>No treatment</td>
<td>227</td>
<td>635</td>
<td>8</td>
</tr>
</tbody>
</table>

* From Conklin et al. (17; also J. W. Conklin, A. C. Upton, and K. W. Christenberry, "Further Observations of Late Somatic Effects of Radiomimetic Chemicals and X-Rays in Mice," to be published).
† Figures in parentheses denote number of treatments; other figures denote dose per treatment.
‡ Long-term survivors; mice dying within first 30 days after treatment are excluded.
is changing the world at a dizzying pace. Already, for example, the atom has been harnessed in certain parts of our country to light homes, hospitals, and factories, and it is likely to become a major source of our power within the century ahead. Likewise, the use of toxic chemicals is being woven ever more inextricably into the complex fabric of modern science, industry, agriculture, and medicine. The crowning paradox of our time is that the very fruits of science which can liberate us from poverty, pestilence, and hunger can also destroy us. Our crucial task now, therefore, is to recognize this challenge, to accept it, and to meet it successfully.

In closing, I can think of no more fitting remarks to address to the members of this Association than the late President John F. Kennedy's (52) concluding words to the members of the Academy:

"It reminds us of what the great French Marshal Lyautey once said to his gardener:

'Plant a tree tomorrow.' And the gardener said, 'It won't bear fruit for a hundred years.' 'In that case,' Lyautey said to the gardener, 'plant it this afternoon.' That is how I feel about your work."

REFERENCES


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