The Tumorigenic Action of Beta, Proton, Alpha
and Electron Radiation on Rat Skin

Progress Report

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Introduction and Summary

The purpose of the program is to achieve a better understanding of the nature of the carcinogenic hazard posed by radiation using as a model system the skin of rats and mice. Recent experiments have investigated a number of specific issues within the framework of the following four general questions. (1) Does the close proximity of unirradiated tissue affect the carcinogenic process in irradiated tissue? (2) Does the type of recovery with dose fractionation that has been observed for cell lethality occur for tumor induction? (3) Does the proliferation rate of the cells at the time of and subsequent to the irradiation affect the tumor induction process? (4) Where are the carcinogenic target cell populations in skin?

The first question relates to the "hot particle problem," i.e., the carcinogenic hazard from small volumes of irradiated tissue. We have established that the close proximity of unirradiated tissue reduces the yield of tumors in the irradiated tissue for low LET radiations, such as, electrons or X-rays (2, 16, AEC 3380-6) but the proximity effect does not occur for high LET protons (12, AEC 3380-2). With regard to the second

question, we have established that significant recovery occurs for carcinogenic damage when the radiation dose is fractionated with one (18, AEC 3380-5) or thirty-one days (10, AEC 3380-3) between fractions. The third question has been answered so far with the information that rapid proliferation at the time of irradiation does increase the carcinogenic sensitivity of the tissue but not nearly in proportion to the difference in proliferation rate (AEC 3380-8).

With regard to the fourth question, the location of the carcinogenic cells, we have established by varying the penetration of the radiation that the most likely location of the sensitive cells is at a depth of about 0.3 mm which roughly corresponds with the depth of the hair follicles in the resting stage (3). A preliminary indication is that the sensitive cells remain at about 0.3 mm even when the follicles are enlarged to a depth of about 1.0 mm in the state of hair growth (18). We were unable to induce tumors by placing the Bragg peak of an alpha particle beam at the depth of 0.3 mm in resting phase skin (7).

In a separate experiment the relative biological effectiveness (RBE) of high LET alpha particles was measured

with respect to tumor induction and found to have a value of 2.9 (9). Determinations were made of the incidence of damaged and atrophic follicles in each experiment. The ratio of damaged follicles per tumor at 80 weeks was found to be remarkably constant with a value of 2000-4000:1. When the skin was irradiated in the phase of active hair growth, tumors developed in the absence of any detectable tissue damage for shallow penetrations. A comparison of radiation carcinogenesis in mouse and rat skin showed the mouse follicles were more sensitive to the lethal action of radiation than rat follicles and hair follicle tumors occurred infrequently in mouse skin (AEC 3380-4).

Contract Activity during the Past Year

1. The 24 Hour Fractionation Experiment

An earlier experiment demonstrated that for an initial dose of 750 rads recovery equivalent to about 700 rads occurred for the carcinogenic and morphological damage. Thirty-one days was allowed to elapse between fractions and the total recovery could have included a proliferative component as well as the usual intracellular process. In the present experiment the interval between fractions was reduced to 24 hours which eliminated the possibility of substantial cell division thereby providing a

measure of primarily the intracellular component of the recovery.

The 24 hour fraction study has been completed and a report was prepared for presentation at the annual meeting of the American Association for Cancer Research (AEC 3380-5). The first dose of 1000 rads was given at 27 days of age during the resting phase of hair growth. Graded doses were given the following day, and the tumor incidence and degree of morphological damage were determined.

The shift in the ascending portions of the tumor incidence-dose curves indicated that recovery equivalent to at least 900 rads occurred. The dose shift for the hair follicle survival curves indicated the possibility of a slightly reduced recovery for lethality. The dose shift for lethality ranged from 750 rads to 950 rads since the single dose and fractionated dose curves were not exactly parallel. Another indication of a possible difference in recovery for tumor induction and follicle lethality was the fact that the height of the tumor peak was less for the fractionated exposure than for the single exposure; a result to be expected if there was less recovery for lethality

than for tumor induction.

We concluded that the recovery process for tumor induction in skin requires less than 24 hours for completion and is probably an intracellular process not involving a significant degree of cell regeneration. The histology of the tumors and the induction kinetics were approximately the same for single and fractionated exposures.

2. Sieve Protection for Tumor Induction by Grenz Rays

The results of the sieve experiment were prepared for presentation at the Radiation Research Society Meeting (AEC 3380-6). In an earlier experiment it was found that significant protection against tumor induction occurs for electrons if they are delivered in sieve or grid patterns. A similar experiment with protons, which are heavy, densely-ionizing particles, failed to show any sieve protection. The present Grenz ray experiment was intended to determine whether the apparent discrepancy in sieve protection for electrons and protons is attributable to LET differences or differences in scattering characteristics.

Grenz rays are low-voltage X-rays. They have an LET roughly comparable to the value for electrons but are similar to protons

in that they scatter very little. The rats were irradiated with sieve patterns having pore diameters of 0.5 mm and 1.6 mm.

The results of the experiment were that the tumor incidence per unit area was reduced to about 70% of the corresponding uniform pattern incidence for the 1.6 mm diameter pore pattern and to about 15% for the 0.5 mm pore pattern.

Attempts to mimic scattering by superimposing a uniform dose around the more heavily irradiated pores showed no effect on the final tumor yield. We, therefore, concluded from these data that for low LET radiation the close proximity of unirradiated tissue somehow reduces the carcinogenic action of the radiation in the irradiated tissue. With high LET radiation, proximity of unirradiated tissue has no effect on tumor induction possibly because the high LET damage is nonreparable.

3. The Effect of Dose on Growth and Induction Kinetics of Skin Tumors

A conference report was prepared for presentation at the 1973 Radiation Research Society meeting (AEC 3380-7). In rat skin after a single dose of radiation, tumors continue to appear at an approximately constant rate for at least 80 weeks which

represents a significant proportion of the rats' lifespan. If all the tumors arose at the time of irradiation then it would be logical to expect that the late-appearing tumors were simply the more slowly growing ones. However, an analysis indicated that the growth rate distribution of the tumors was independent of appearance time. This raised the possibility that the tumors were arising subsequent to the irradiation.

Growth curves were measured for 153 tumors occurring in dose groups from 1500 rads to 5000 rads. By means of a computer, Gompertz functions were fitted to the data and it was found that the growth rate at the time of initial appearance and the reduction in growth rate (retardation) were extremely variable from tumor to tumor. The concept of growth time was defined as the time required for a tumor to grow from one cell to the arbitrary minimum detectable size of 1.0 mm diameter. It was concluded from the variability in the growth rate and the retardation that the growth time distribution is probably an extremely broad function.

By fitting linear functions to the tumors per rat versus time data, values of slope and time-intercept were determined

as a function of dose. The time-intercept was found to vary slowly with dose from a maximum of about 40 weeks at doses less than 2000 rads to a minimum of about 20 weeks at doses above 4000 rads. The dose dependence of the time-intercept, while significant, is relatively minor in comparison to the dose dependence of the slope which at doses less than about 3000 rads increased as about the 1.7th power of dose.

A model was constructed where it was assumed that the tumors started growing at the time of irradiation and the appearance time depended only on the growth of the tumors. In the model the time-intercept of the incidence function represents the time required for the most rapidly growing tumor to reach visibility and the slope of the incidence function is related directly to the growth time distribution function. If the slope is actually constant, as suggested experimentally, the growth time function must also be constant. Constancy of the growth time function is suggested by the broad distributions for measured values of growth rate and retardation. While the model is useful and interesting its proof will depend on detecting microtumors and showing that they occur early and in the numbers

required to produce the macrotumors observed later.

4. A Comparison of Acute Injury and Tumor Induction in Skin with Growing Hair

This work was performed by one of the graduate students on the project and his Ph.D. thesis, which he successfully defended in December, 1972, is submitted as AEC Document 3380-8. Acute cell killing was quantitated by means of total cell counts, total DNA, and loss of H^3 from DNA prelabeled with H^3TdR . The acute cell killing and tumor incidence were compared for various penetrations and doses during the stage of hair growth. At a penetration of 1.0 mm, tumors occurred at 1000 rads in the absence of significant cell killing and morphologic hair follicle damage. Cell killing and follicle damage occurred only when the radiation penetrated to the full depth of the follicle. Irradiation of only the upper part or neck of the follicle with a dose sufficient to produce tumors produced no cell loss or permanent follicle dysplasia. It was concluded that while tissue injury may under certain conditions be an indicator of the generalized radiation effect, including tumor induction, the injury itself is probably not an essential element

of the tumor induction process.

At a penetration (2.0 mm) that reached the entire follicle with a substantial dose, the radiation precipitated a premature catagen which resulted in follicle involution. The radiation-induced involution occurred earlier but its rate was the same as in normal catagen. The radiation-induced involution did not necessarily involve every follicle as in the controls. Some of the follicles, especially at lower doses, were unaffected and other exhibited a regenerative capability, such that during a period when the follicles in the control rats were in a resting state, many of the irradiated follicles remained in what was probably a prolonged growing state. The result was a loss of the synchrony resulting in a mixture of resting and growing follicles. No obvious correlation could be found between the patterns of lethality and regeneration in epidermis, sebaceous glands or hair follicles and the subsequent tumor incidence.

5. Critical Depth for Tumor Induction in Growing Phase

In earlier experiments with resting phase follicles, a skin depth (critical depth) was found at 0.3 mm which reconciled the diverse tumor dose-response curves for various radiation

penetrations. The depth of 0.3 mm corresponded to the level of the follicular germ cells at the apex.

An experiment was initiated to search for a similar critical depth in the growing phase where the follicles are much larger, deeper, and mitotically more active than in the resting phase. The growing follicles extend to a depth of about 1.0 mm and if irradiation of the entire follicle were necessary to produce tumors the critical depth should be at 1.0 mm.

The experiment consisted of giving surface doses extended from 500 rads to 3000 rads in steps of 500 rads with penetrations of 0.5 mm, 1.0 mm, 1.5 mm and 2.0 mm. The results are still preliminary but indicate that the critical depth remained at about 0.3 mm in the growing phase, suggesting that the tumorigenically-sensitive cells reside in a fixed location in spite of profound changes in follicle morphology. By contrast, the critical depth for follicle lethality does correspond with follicle depth, i.e., about 0.8 mm. This result shows a clear divergence between the lethal and the carcinogenic effects of radiation.

6. A Model Concerning the Effect of Dose Rate on Tumor Induction

One of the urgent problems in radiation protection work is how to estimate the carcinogenic response for low dose rates from high dose rate results. Many of the exposure circumstances in humans for which estimations of carcinogenic hazard are required involve protraction of the dose for long periods of time. A model has been developed to improve our experimental designs and our understanding of dose rate effects in radiation carcinogenesis. The model is based on general radiobiological considerations and the results of dose fractionation experiments for tumor induction.

The basic elements of the model can be described as follows. Initially, the radiation acts on the normal state, referred to as state 1, converting it to state 2, a subcarcinogenic state capable of undergoing recovery. The action of radiation on state 2 produces state 3 which is or leads to cancer. It is assumed that the rate of conversion from state 1 to state 2 and from state 2 to state 3 occur in proportion to the dose, thereby yielding an overall dependence of the tumor incidence on the square of the dose. The actual data gave a dependence on the

dose to the 1.7th power. The reduction in the exponent of the dose could be explained either by allowing direct conversion from state 1 to state 3, thereby introducing a linear component as occurs for high LET radiation, or by introducing a cell killing component. By introducing experimental survival curves into the model, the exponent could be reduced to the experimental value without the necessity for assuming direct conversion from state 1 to state 3. It has been determined experimentally that the recovery of carcinogenic damage occurs within 24 hours. If carcinogenic recovery is similar to lethal recovery in vitro, it probably occurs mostly within 1 or 2 hours and is independent of dose.

The model makes predictions concerning the tumor incidence in relation to the dose rate or fractionation pattern and it is our intention to test the various elements of the model, especially the recovery rate assumptions experimentally. The biological nature of state 2 is of considerable interest because within state 2 recovery is possible. High LET radiation produces a type of effect that seems to bypass state 2. It is possible that state 2 is the target for the action of various radioprotective and

radiosensitizing agents.

7. The Effect of Postirradiation Proliferation on Tumor Incidence

Carcinogenesis may require cell division and the objective of the present experiment was to determine the effect of stimulation of the cell proliferation rate subsequent to irradiation on tumor yield. Rats were irradiated with 1000 R at each of two penetrations in either the resting or growing phase of the hair growth cycle. Subsequent to irradiation hair follicle proliferation was stimulated monthly by plucking the hair and epidermal proliferation was stimulated by surface abrasion with a stiff brush also at monthly intervals for 9 months.

The final tumor histology is not yet available, yet based on visual observation neither hair plucking nor surface abrasion produced an enhancement of tumor yield. Rather, surface abrasion actually reduced the tumor yield in some groups. The data has not yet been fully analyzed, but the preliminary indication is that enhanced postirradiation proliferation has no effect on the number or appearance rate of tumors.

8. High LET Fractionation and Tumor Induction

If it is true that high-LET radiation damage can not undergo recovery, no fractionation effect is expected irrespective of the biological endpoint. An experiment was initiated during the past year to determine if this expectation can be confirmed for tumor induction in the rat skin system. A cyclotron at Sloan Kettering Institute producing 15 Mev protons is convenient and suitable for our purpose. The preliminary dosimetry has been performed and the animals irradiations are scheduled to begin shortly.

The general design of the experiment will be similar to the design of our earlier electron fractionation experiments. The doses will be adjusted to take into account the relative biological effectiveness (RBE) which we have already estimated in an earlier experiment to be approximately 3. The rats will be irradiated initially with 300 rads followed 24 hours later with graded doses, and the results will be compared to graded doses alone.

9. The Carcinogenic Action of Radiation and Methotrexate

The lethal action of radiation obviously competes with its carcinogenic action. Above, about 2500 rads, the lethal effect

of radiation dominates the carcinogenic effect, and the tumor incidence is reduced in comparison to lower doses. In skin cell death is usually compensated by regenerative division, and it was of interest to determine how increased cell death affects the tumor incidence. Methotrexate which kills cells in DNA synthesis was administered 24 hours prior to irradiation in order to artificially increase the amount of cell death at the time of irradiation. At a methotrexate dose that produced inhibition of hair growth, the tumor incidence was reduced by about 60%. An experiment was initiated to measure the magnitude of the cell death induced by the various doses in order to establish if the magnitude of the lethality was comparable to the reduction in tumor incidence.

10. Experimental Detection of Early Tumors

A technique has been developed for detecting very small tumors, i.e., about 100 μ in diameter, which is intended as a means of investigating the early events in radiation carcinogenesis. Not only would early detection shorten the duration of our experiments but also would provide the capability of confirming or refuting various tumor growth models and of assessing the

action of tumor inhibitors in early stages of growth.

The detection technique consists of staining the nuclei with a DNA specific Feulgen procedure, then clearing the full thickness skin with methylsalicylate so that cellular aggregations can be detected visually with the aid of a dissecting microscope. The lesions are then excised and sectioned serially in the conventional manner. The rate of cell proliferation in each lesion is determined by injecting H^3 -thymidine or colchicine prior to killing the animal, removing sections of skin and preparing histological sections. Several lesions in the size range from 200 μ to 500 μ have been detected and classified histologically as probable tumors.

An experiment to establish a systematic relationship between microlesions and macrotumors is currently in progress. By detecting a larger numbers of tumor events, it should be possible to extend the dose-response curves to much lower doses than is now possible.

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