THE TUMORIGENIC ACTION OF BETA, PROTON, ALPHA
AND ELECTRON RADIATION ON THE RAT SKIN

Progress Report

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Abstract of Progress Report

The carcinogenic effect of ionizing radiation on rat skin is being studied in an attempt to learn more about radiation carcinogenesis, especially how various parameters of the irradiation, such as dose distribution within the tissue and dose rate, affect the yield of tumors. It was found that when the dose was localized to a small region of skin, the tumor yield was reduced and the magnitude of the reduction indicated that the region of reduced response might extend about 150 μ into the irradiated zone. The proliferative state of the hair follicles at the time of irradiation has relatively little effect on tumor induction although old animals are less susceptible than young or newborn animals. The penetration requirement of at least 0.3 mm for producing tumors suggests that the hair follicle germ cells could be the oncogenic targets. Comprehensive experiments are in progress to measure the rate of recovery for tumor induction in split-dose exposure protocols. Preliminary results indicate a recovery halftime of about 4 hrs. for electrons and significant recovery for protons which is used in a model to predict how recovery should affect the tumor response at low dose rates. Combinations of ionizing radiation and ultraviolet light are being applied to rat skin to determine whether they are additive for producing tumors.

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1.0 General Summary

The carcinogenic effect of ionizing radiation on rat skin is being studied in an attempt to learn more about radiation carcinogenesis, especially how various parameters of the irradiation, such as, dose distribution within the tissue and dose rate, affect the yield of tumors. Previous results have indicated that if the radiation is limited to a small region of skin the tumor yield is greatly reduced in comparison to the yield per unit area obtained when large regions of skin are irradiated with the same dose. The reduction in tumor yield for focal irradiation implies that the proximity of unirradiated tissue reduced the probability of tumors occurring in the irradiated tissue. Recent results with low energy X-rays suggest that the region of reduced tumor response extends about 150 μ into the irradiated zone.

Earlier results indicated that the radiation produces tumors most effectively if the penetration is at least 0.3 mm which corresponded to the depth of the hair germ cells which, together with histological evidence of a similarity between the tumors and hair follicles, suggested that the hair follicles, or more specifically, the hair follicle stem cells might be the targets for the oncogenic effect of radiation. Recent studies have extended the original observations to suggest that if the stem cells are the oncogenic target their proliferative state at the time of irradiation has no effect on the yield of tumors. In

other work the growth and proliferation rates of the tumors were measured in an attempt to establish whether late-appearing tumors might represent slowly growing tumors. It was concluded that the late tumors were probably new events suggesting that the radiation had not caused them directly but had created favorable conditions for their development. A comprehensive attempt to measure recovery or the fractionation effect for tumor induction is nearing completion, and the results indicate that recovery halftimes are about 4.0 hrs. A model has been developed for relating these results to dose rate effects. New work has begun to determine the interaction of ionizing radiation and ultraviolet light in producing tumors in rat and mouse skin.

2.0 Progress in the Preceding Year

The general objectives outlined in last year's proposal were achieved. Heavy emphasis was placed on measuring the recovery rate for tumor induction in split-dose experiments and one of the graduate students working on the project developed experimental and theoretical results for explaining recovery and dose rate effects as part of his doctoral dissertation. The experiment to measure the fractionation effect on tumor induction for protons which have a higher linear energy transfer (LET) than electrons used previously was completed. Somewhat surprisingly the dose-response curve for protons was nearly dose-squared, and there was

definite evidence of recovery. Experiments on the combined carcinogenic action of ionizing radiation and ultraviolet light were begun and the experiment to measure carcinogenic sensitivity as a function of age was extended to include newborn rats and year old rats.

The experiments with plutonium were deferred because of the difficulties of preventing low levels of contamination that would compromise the low level counting facilities at our Institute. An experiment to combine radiation with a carcinogenic chemical on rat skin was deferred until a new accelerator facility is available. Techniques were developed for measuring cell lethality radioautographically in the epidermis in order to compare the lethal and carcinogenic responses. Other techniques were established for measuring the angiogenesis factor in tumors as a means of assessing their status in the progression to malignancy.

3.0 Description of Specific Experiments

3.1 Proton Fractionation

Rat skin was irradiated with either single doses or two doses separated by 24 hrs. of 12 Mev protons produced by the cyclotron at Sloan Kettering Institute. Doses were given to groups of 20 rats.

The proton dosimetry was performed by calibrating a

monitoring ionization chamber at the end of the beam pipe by means of a Faraday cup. The ionization chamber had been calibrated previously for alpha particles, and the new calibration gave results in agreement with expectations based on the LET ratios for protons and alpha particles. A second parallel plate ionization chamber was used to measure the depth-dose curve by placing various thicknesses of aluminum on the chamber and placing it in the beam. The rats were anesthetized and rotated through the beam at 18 rpm on a special table. A rotating absorber wheel was used to remove the Bragg peak and to produce an approximately linear depth-dose curve.

On day zero single doses of 150 rads were given. These were followed 24 hrs. later by doses of 150, 300, 450 or 600 rads. On day one previously unirradiated rats were given 75, 300, 450, 600 or 750 rads. The dose-response curve for single doses was very nearly dose-squared and with the exception of one dose (450 rads) there was a clear displacement between the response curves for single and fractionated exposures indicating essentially complete recovery. The highest dose did not produce ulceration, hence, the tumor yield continued to rise with dose up to the highest dose used in the experiment. Comparison with previous proton experiments indicated good agreement and suggested the relative biological effectiveness of protons relative to electrons

was about 2.5. Hence, protons exhibit characteristics of both high and low LET radiation. The RBE is greater than one -- a high LET characteristic, -- yet the response is dose-squared and exhibits recovery -- a low LET characteristic.

3.2 Recovery Rate for Tumor Induction

The purpose of the present study was to measure the rate of split-dose recovery for skin tumor induction in rats following electron irradiation and to develop mathematical formulae based on these and other results for predicting the role of dose rate in radiation carcinogenesis. Recovery was measured by comparing the tumor response in rats receiving two exposures of equal dose separated by various times up to six hours with the response to a single exposure of the same total dose. Knowledge of the recovery rate is important because it provides a basis for designing and interpreting dose rate experiments, it adds to our understanding of the nature of the carcinogenic change, and it may eventually lead to a rule for the inclusion of dose rate factors in estimating the risks associated with human exposures.

The experimental protocol consisted of irradiating 10 cm² of dorsal skin on anesthetized 28 day old male rats to a depth of 1 mm with electrons from a Van de Graaff accelerator. The time intervals between fractions were $\frac{1}{4}$, 1.3 and 6 hrs. for total doses of 1400, 2800 and 4600 rads. There were single exposure

groups at the above doses and at 700, 1000 and 2300 rads. Each dose group consisted of between 18 and 20 rats. Observations at six week intervals, and histologic sections of all lesions taken at death provided tumor incidence data up to 64 weeks post-irradiation. All groups showed an initial latent period of about 15 weeks followed by a reasonably constant rate of new tumor appearance. This tumor appearance rate increased as approximately the square of the dose up to a peak response dose of about 2800 rads. The effects of fractionation, or splitting the dose, showed that the recovery rate was independent of dose and time, and that the recovery halftime was about 4 hrs. Hair follicle survival at 64 weeks postirradiation was scored for some of the animals as a means of estimating the lethal effect of the radiation. The recovery for follicle survival occurred with a 3 hr. halftime, which was not significantly different than the halftime observed for tumor induction.

A model is proposed for predicting the effects of dose rate on tumor induction on the basis of the recovery rate measured in the fractionation protocol. In the model it is assumed that tumors can result from either one or two dose-dependent steps. For the two-step mode the first step produces a reversible state which may either revert to the initial condition or be converted by further radiation action to an irreversible, potentially

carcinogenic state. Recovery from the reversible state is assumed to occur independently of dose and time. Mathematical equations were derived relating the production of irreversible states to dose and dose rate. The dose rate implications of the model are summarized in terms of a Dose Rate Factor (DRF), which is defined as the ratio of the acute dose to the protracted dose needed to produce the same tumor yield.

3.3 Age Sensitivity in Radiation Carcinogenesis

Newborn, 28 day old, 58 day old and 350 day old rats were exposed to 20 KVP X-rays at doses of 500, 1000, 1500, 2250 and 3000 R. While the latent periods (the time from irradiation to the appearance of the first tumor) were approximately the same for the newborn, 28 day and 58 day rats and ranged from 16 to 24 weeks, the latent period of the 350 day rats was greater than 30 weeks, since no tumors had occurred by that time. The tumor yield in the newborn rats was about the same as for the 28 day rats for doses below 1500 R. However, the tumor yield dropped for doses above 1500 R in the newborns, whereas the corresponding yield for the 28 day rats continued to rise to a peak at 2250 R and then dropped at 3000 R. These results suggest that the newborn is not more sensitive to the carcinogenic effect of radiation in comparison to older rats but may be more sensitive to the lethal effects of the radiation if lethality is the basis

for the reduction in tumor response at high doses. These experiments are in progress; the rats are still living and the tumors have not yet been diagnosed histologically.

3.4 Interaction of Ultraviolet Light and Ionizing Radiation for Induction of Skin Tumors

While repeated doses of ultraviolet light is carcinogenic in mouse skin, relatively little is known about the response of rat skin or about interactions between ultraviolet light and ionizing radiation. Accordingly, an experiment was started where rat skin was irradiated with single doses of ionizing radiation (X-rays) in the dose range between 800 R and 4000 R followed by weekly exposures to 0.2×10^7 or 1.0×10^7 ergs/cm² for 5 and 3 months respectively to erythema or solar wavelengths (275-375 nm). In addition, rats were given single doses of ultraviolet radiation ranging from 0.75×10^7 to 12×10^7 ergs/cm². The destructive effect of the ultraviolet was severe for single doses of 6 and 12×10^7 ergs/cm² and there was tissue sloughing and formation of scar tissue. The repeated exposures of ultraviolet were stopped because of severe skin reactions including edema, erythema and formation of thick flakes on the skin surface. After the exposures ended healing occurred until the tissue eventually returned to normal without scar formation. A few tumors have occurred, but it is too early for a trend to have developed.

Separate groups of rats were exposed to a combination of ionizing radiation and ultraviolet light at the germicidal wavelength (254 nm). The germicidal wavelength penetrates so poorly that the epidermis is essentially the only cellular portion receiving substantial dose. In addition to determining the carcinogenic effect of the various radiations, the lethal effect on the epidermis is being determined histologically for the purpose of interpreting possible effects on tumor induction in terms of lethality and subsequent proliferation associated with regeneration.

3.5 The Carcinogenicity of Radiation and a Chemical Carcinogen Combined

An experiment to determine how rat skin responds to radiation in combination with dimethylbenz(a)anthracene (DMBA) had been scheduled to begin this month (April 1975) but a malfunction of the Van de Graaff accelerator at Union Carbide has forced a postponement. The experiment will be rescheduled as early as practicable.

The protocol of the experiment consists of irradiating rats with doses of 750, 1500, 2250 and 3000 rads then applying weekly doses of 0.002%, 0.01% or 0.05% DMBA in acetone. The DMBA is expected to produce tumors in the absence of the radiation but the basic question is whether prior irradiation

alters the susceptibility of the skin to DMBA.

3.6 Epidermal Cell Survival

The doses of radiation that are required to produce tumors in rat skin are relatively high in comparison to doses required to kill cells in culture. A series of experiments was begun to measure the degree of epidermal lethality in order to establish whether or not correlations exist with carcinogenicity especially on the descending limb of the tumor response curve at higher doses.

The epidermal cells are labelled with a single injection of H^3 -thymidine then the incidence of doublets is determined. A doublet is two adjacent H^3 -labelled cells on radioautographs indicating that such cells (after corrections for random doublets) have undergone mitosis in the interval between H^3 -thymidine injection and the fixation of the tissue and are viable. The results for doublets at 48 hrs. after irradiation indicate the epidermal cells are much more resistant to lethality (defined as ability to divide) than most cells in culture if they are in the G_1 -phase of the cell cycle. Cells in the S-phase were considerably more sensitive than G_1 cells.

3.7 The Cell Kinetics of Rat Hair Follicles

A possible connection between morphologically-damaged hair follicles and the induction of tumors for irradiation in the

resting phase of the hair cycle provides a basis for studying the proliferation rate in damaged follicles. Morphological injury appears to be permanent but there could be increased proliferation associated with abortive repair attempts that could be correlated with tumor yields. Hence, proliferation rates were determined by stathmokinetic techniques for several doses and times but the results are not yet available.

3.8 The Kinetics of Papilloma Growth in Mouse Skin

As part of the evaluation of the growth kinetics of radiation-induced tumors in rat skin, the methodology was applied to skin tumors in mice produced by chemicals in the initiation-promotion system. The mouse skin tumors were mainly papillomas that regressed when the promoting chemical was discontinued. The regression tendency was sufficiently interesting that it was decided to determine whether kinetic differences could be detected between regressing tumors and the small proportion of tumor that did not regress.

Immune suppression had no effect on regression although the rate of regression decreased when a nonspecific irritant was applied. The tumors exhibited rapid cell proliferation which did not subside when the promoter treatments were stopped. The cell kinetic effects of the promoter on the normal tissue were reversible. The promoter seemed to produce a dose rate effect

not dissimilar to that observed for irradiation with ionizing radiation.

Bibliography

1. Burns, F. J., R. E. Albert, I. P. Sinclair and M. Vanderlaan. The Effect of a 24-Hr. Fractionation Interval on the Induction of Rat Skin Tumors by Electron Irradiation. Radiat. Res., June 1975. (In Press)
2. Burns, F. J. Theoretical Aspects of Growth Fraction in a G_0 -Model. ERDA Symposium Series CONF-731005. The Cell Cycle in Malignancy and Immunity (Ed. J. C. Hampton), 1975.
3. Albert, R. E., F. J. Burns and M. Vanderlaan. Effect of Radiation Penetration and Growth Stage of Hair Follicles on Skin Tumor Induction. Radiat. Res. 59:17-18, (1974). Abst.
4. Burns, F. J., M. Vanderlaan and R. E. Albert. Proliferation of Early Tumor Foci in Irradiated Rat Skin. Radiat. Res. 59:18, (1974). Abst.
5. Albert, R. E., M. Vanderlaan and F. J. Burns. Dose and Recovery in Rat Skin Tumor Induction. Conference Report - Presented to International Commission for Radiological Protection, Feb. 1975, COO 3380-17.
6. Burns, F. J., I. P. Sinclair, R. E. Albert and M. Vanderlaan. Radiation Penetration and the Proliferative State of the Hair Follicles in Radiation Carcinogenesis of Rat Skin.

For submission to International J. Radiat. Biol., 1975.

COO 3380-18.

7. Burns, F. J. and R. E. Albert. The Induction of Tumors in Rat Skin by Sieve Patterns of Grenz Rays. For submission to Radiat. Res., 1975.
8. Burns, F. J., M. Vanderlaan and R. E. Albert. Growth Rate and Induction Time of Radiation-Induced Skin Tumors in Rats. Conference Report - Presented at Int. Congress of Radiation Research, Seattle, Washington, 1974. COO 3380-20.
9. Burns, F. J., M. Vanderlaan, A. Sivak and R. E. Albert. The Incidence and Regression Kinetics of Mouse Skin Papillomas. For submission to Cancer Res., 1975. COO 3380-21.
10. Progress Report for year ending July 31, 1974. COO 3380-22.