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

Comprehensive
Progress Report

Fredric J. Burns and Roy E. Albert

Institute of Environmental Medicine
New York University Medical Center
New York, New York 10016

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1.0 General Summary

Rat skin has been studied for a number of years as a model of radiation carcinogenesis in a solid tissue. Accessibility of the skin enables the tumors to be detected early so that growth rate, proliferation rate, and onset times can be established accurately and, of course, the superficial location permits an accurate assessment of doses and a localization of the radiation to the tissue of interest. We have been attempting to establish as accurately as possible the nature of the dose response curve, i.e. the rate of tumor occurrence as of function of radiation dose, and the importance to tumor induction of radiologic factors, such as dose rate, fractionation, dose localization, linear energy transfer and of biologic factors, such as the proliferative state of the hair follicles and epidermis at the time of and subsequent to irradiation. The interaction of radiation and other carcinogens, especially ultraviolet light, is under study because of epidemiologic evidence suggesting a potential synergism for induction of scalp tumors.

Radiobiological recovery processes have been studied in tumor response experiments using split doses of radiation separated by various times. The recovery rate for electron induced tumors has been measured, and the oncogenic effects of high LET particles (proton, alpha, argon) are being investigated.

2.0 Progress Achieved

The major experiments in approximately the order they were performed will now be described. These descriptions are not intended to be exhaustive but are merely to indicate the general nature of the experiments and the significant findings and interpretations.

2.1 Post Irradiation Proliferation and Tumor Development

In this experiment a relationship was sought between proliferation rate, in the hair follicle and epidermal cell populations at the time of and subsequent to irradiation, and the induction rate and appearance time of skin tumors. Sustained follicular proliferation induced by repeatedly plucking the hair had little or no effect on tumor response. Also repeated stimulation of the epidermis by surface abrasion produced either no effect (at low doses) or reduced tumor appearance rate (at high doses). These results indicate that accelerated proliferation of epithelium subsequent to irradiation does not shorten the tumor appearance times.

2.2 Early Morphological Response of Growing Hair Follicles to Various Penetrations

The effect of radiation penetration depth on the morphology of the growing hair follicles has been investigated for electron radiation. Hair follicle and epidermal cell populations were studied by measuring total cellularity, total DNA content and specific activity of DNA at various times after irradiation

with several doses and skin penetrations. It is of basic importance to know whether the entire growing hair follicle, which is mitotically very active, needs to be irradiated to produce permanent morphological injury; or whether partial irradiation (shaft, bulb, stem cells) is sufficient for injury. The effect of rapid cell proliferation at the time of irradiation is also important to the injury response in terms of accelerated repopulation and repair in the follicle. These results were compared to the tumor response pattern at similar doses and penetrations.

The most efficient production of permanent morphological hair follicle damage occurred when substantial doses were delivered to the follicle matrix level at about 0.8 to 1.0 mm from the surface. Morphological hair follicle damage included atrophism, bent hair shafts, enlarged sebaceous glands and hairless follicles, several weeks after irradiation.

The rate of loss of ^{3}H-thymidine labelled DNA was found to be the same in all irradiated groups as in controls, indicating that irradiated cells were eliminated at the normal turnover rate. The rate of loss of total DNA was identical to ^{3}H-thymidine rate loss during the first 4 days post irradiation, indicating that there

was essentially no repopulation or regeneration replacement of irradiated cells during this period. Histological examination indicated a considerable loss of cells in most epidermal components at 5 to 10 days PI followed by a hyperplastic reaction in the epidermis and sebaceous gland which lasted a period of 10-60 days dependent on dose and penetration. The higher doses and deeper penetrations produced more initial cell loss and a higher degree of subsequent hyperplasia. In some follicles the hyperplasia prolonged the growing phase well beyond the control time. No correlation between acute and chronic morphological damage and tumor incidence was found.

2.3 Penetration Effect for Tumor Induction in Growing Phase Skin

As mentionel earlier the growing stage hair follicle is much deeper in the skin and more mitotically active than the resting follicle. For this reason a variable-penetration experiment was done to determine whether a correlation existed between tumor yield and dose to different depths in skin corresponding to follicle depth. In other words, which follicle component is more important to tumor induction, the germ cells (at the level of the resting phase follicle, 0.3mm) or the follicle itself

(0.8 - 1.0 mm) in growing phase. A series of carcinogenic electron doses were given at penetrations of 2.0 mm, 1.5 mm, 1.0 mm, and 0.5 mm in rat skin.

The tumor response indicated that it was necessary to irradiate only down to the resting follicle depth, even in the presense of growing phase hair, in order to induce tumors. Therefore the sensitive site for tumor induction does not change with increased size and depth of the growing follicle. For shallow penetrations of 1.0 mm, the peak tumor yield occurred well below the ulcerating dose, suggesting that tumor sensitive cells may be killed even at doses not sufficiently penetrating to cause gross skin and hair follicle destruction.

2.4 Tumor Growth Rate and Appearance Time

A single dose of radiation in rat skin produces tumors that appear at a more or less constant rate (after an initial tumor free interval) throughout the animal's life. A question arises whether all the tumors actually started to grow as cell masses at the time of irradiation and appearance time is determined by growth rate or new tumors are constantly appearing and growth is independent of time. An initial study indicated that growth rate distributions were independent of the time of initial

appearance, but the growth rates were so variable from tumor to tumor that the conclusions could have been compromised.

Consequently, a study was launched to obtain detailed information regarding the growth rate characteristics of tumors in the size range above 1.0 mm in diameter and a special technique was developed to detect tumors as small as 0.1 mm in diameter. The growth rate analysis indicated that most of the tumors underwent retarded growth, i.e., growth rate declined with increasing size, and some reached asymptotic sizes. The search for microtumors indicated far fewer than expected on the basis of the assumption that all tumors were present from the time of irradiation. Once the tumors were large enough to detect, the incidence curves were displaced in a parallel manner for various size endpoints indicating that the major events affecting appearance time occurred at a stage of tumor development prior to detection, even as a microtumor. The results were consistent with the assumption that new tumors were forming constantly throughout the animal's life.

In spite of the necessity to irradiate to at least 0.3 mm to produce tumors, about 75% of the microtumors small enough to be assigned a definite location were in the outermost

0.15 mm, i.e., essentially in or close to the epidermis. The implications of the above finding are to be explored more fully in future work.

2.5 The Effect of Fractionated Doses of Electrons and Protons on Hair Follicles

A study comparing the effect of single and fractionated doses of electrons and protons with respect to the ability of the follicle to respond to growth stimulation, i.e., elongate and to produce hair. The technique consisted of a histological determination of the ability of the hair follicle cells to proliferate and produce a mature growing fallicle at various times after irradiation. The survival and regrowth curves of hair follicles following irradiation showed a temporary suppression caused by a delay in regrowth of new hair, which was measured as about one day of delay per 100 rads for protons. It was found that permanent growth suppression occurred at 4800 rads for electrons and at a dose greater than 1200 rads for protons. The doses required to produce 50% follicle suppression at ten days post irradiation were 700 rads and 2100 rads for protons and electrons, respectively, implying a relative biological effectiveness (RBE) of about three for the two radiations. A fractionation effect was observed for both types of radiation with about 60% recovery for protons and 100% recovery for electrons.

2.6 Growth Fraction and the G_0-Phase in the Cell Cycle (AEC 3380-15)

A paper was presented at the 13th Annual Hanford Biology Symposium which described certain theoretical aspects of the growth fraction concept and how it relates to the G_0-phase. Labeled mitosis curves for a variety of tumors and normal tissues were fitted to the curves predicted by the model with good agreement. The G_0-phase was most pronounced in slowly growing autochthonous tumors and was essentially absent in rapidly growing transplantable tumors. The growth fraction varied over wide limits essentially independent of the growth rate and type of tumor.

2.7 Cell Kinetics and Tumor Induction in Rat Liver for Various Dietary Carcinogens

As a means of contrasting the tumor response in a diverse organ, such as liver, with skin, investigations have been carried out to determine the relationship of hepatic cell loss and proliferation and tumor incidence to time during and after continuous dietary levels of various carcinogens, including 2-fluorenylacetamide (2-FAA), diethylnitrosamine (DEN) and aflatoxin. For 2-FAA early cell loss was followed by apparent regeneration while at the same carcinogenic dose DEN produced hyperplasia

without significant cell loss. The enhanced proliferation and hyperplasia of the DEN response was reversible and the tissue returned to apparently normal levels when the DEN was stopped. Hepatomas appeared even when the DEN was stopped early although they were delayed in comparison to continuous DEN. These studies are being extended to include higher doses of aflatoxin.

2.8 Tumor Induction for Single and Split Doses of 10 Mev Protons

Rats were irradiated with either a single dose or two doses separated by 24 hours of 10 Mev protons produced by cyclotron at Sloan Kettering Institute for Cancer Research in New York City. 200 animals were exposed in 10 different dose groups.

Proton doses were established by calibrating an argon filled double ionization monitoring chamber at the end of the beam pipe against the current collected on a beam stop. The calibration was compared to previous results obtained for alpha particles in this chamber and agreement was found on the basis of LET ratios for protons and alpha particles. A second parallel plate ionization chamber was used to determine the relative depth-dose by measuring the ionization current after beam filtration through various thicknesses of aluminum. The anesthetized animals

were rotated through the beam at 18 rpm on a special revolving platform and a rapidly spinning disk of variable thickness aluminum absorbers was used to move the Bragg peak in and out of the skin, producing an approximately linear depth dose curve.

On the first day of exposure single doses of 75, 150, 300, 450, 600 or 750 rads were given to those animals receiving only one dose. Also 150 rads was given to those animals receiving split doses. On the second day (24 hours post-irradiation) the split dose groups received 150, 300, 450 or 600 rads, resulting in total doses equal to the single doses given on the first day.

The tumor dose-response curve for single doses increased in an approximated dose-squared fashion up to 750 rads, the highest dose used. In previous experiments with electrons, a reduction in tumor yield is seen at doses in which acute skin ulceration occurs. In the present experiment, the acute responses were relatively mild, with no ulceration, and no peak in the tumor response curve was observed. The tumor response in this experiment was more vigorous than that seen in other experiments in which rats were irradiated with either electrons or protons. When compared with electron irradiation of 28 day old rats the protons in this study have a Relative Biological Effectiveness (RBE) between 2 and 3.

When compared with rats irradiated with protons at 58 days of age, the animals in this experiment are sensitive, suggesting that age at irradiation may be an important factor in determining the tumor response.

There was a clear dose displacement between the response curves for single and fractionated exposures, which may be interpreted as resulting from recovery. The errors associated with the low tumor yiels in the 300 and 450 rad total dose groups preclude their use in making a numerical evaluation of the amount of recovery. There was, however, sufficient difference between the fractionated and single exposure groups at 600 and 750 rads to enable estimation of the amount of recovery, and the best estimate is that complete recovery occurred, although as little as 78% recovery is consistent with the upper 95% confidence limit of the observed tumor yields. These results are nearly identical to those reported previously for fractionated doses of electrons, i.e. essentially complete recovery within 24 hours.

Thus, the response of rat skin to proton irradiation shows a rapid form of recovery of both sublethal and suboncogenic injury and RBE values between 2 and 3. The combination of RBE values greater than one, and recovery indicates that while protons

induce injury more efficiently per unit absorbed dose than electrons, there is still a reversible component to the injury process.

2.9 Recovery Rate for Tumor Induction

The purpose of this study was to measure the rate of split dose recovery for skin tumor induction in rats following electron radiation and to develop mathematical formulae based on these and other results for predicting the role of dose rate in radiation carcinogenesis. Knowledge of the recovery rate is integral to designing and interpreting dose rate experiments. Recovery kinetics are also important in understanding the nature of carcinogenic transformation, and may eventually lead to a rule for the inclusion of dose rate factors in estimating human exposure limits and risks. Recovery was quantitated by comparing the tumor induction rates in rats receiving two equal dose exposures separated by various times up to six hours with the response elicited by a single exposure of the same total dose.

A 10 cm^2 area of dorsal skin on anesthetized 28 day male albino rats was irradiated to a depth of 1 mm with 0.7 Mev electrons from a Van de Graaff accelerator. The time delay between equal fractions was 0.25, 1, 3, or 6 hours for total doses 1000 (500 + 500), 1400 (700 + 700), and 2300 (1150 + 1150) rads. Single

in the above experiment. The model assumes two separate tumor induction (cell transformation) pathways by radiation. One pathway requires one dose-dependent step (event) for transformation. The other requires two dose-dependent steps, the first step being reversible and the second irreversible. Recovery from the reversible state back to the initial undamaged condition is assumed to occur independently of dose and time.

Mathematical equations were derived relating the production of irreversible states to dose and dose rate. For acute exposures the model predicts a response curve of the form $Y = A_1D + A_2D^2$. The relative magnitudes of A_1 and A_2 may be estimated for electrons from the single doses used in this experiment, and for protons from the experiment described in the presiding section. For both types of radiation, tumor incidence as a function of dose indicates an essentially dose squared response with no linear component. The large doses used preclude, however, ruling out the possibility that there may be a small linear component which may be of significance at doses much lower than those tested.

For two dose fractions, the model equations indicate that recovery of the intermediate, suboncogenic form of injury should occur in an exponential fashion. This prediction is in good agreement with the results obtained in the present experiment. The model also

exposure groups were done at 500, 700, 1150, 1400 and 2300 rads. The above doses are the doses 0.3 mm beneath the skin surface, which has been found previously to be the most relevant dose for tumor induction. Each group contained 18 to 20 animals. Tumor incidence data was recorded at 6-week observation intervals and histologic verification of all lesions was performed at death or termination of the experiment (64 weeks post irradation). The initial latent period of 15 weeks was independent of dose and fractionation interval. The subsequent reasonably constant rates of new tumor appearance were in close agreement with previous experiments, and increased at approximately the square of the dose up to a peak response dose of about 1400 rads. The effects of dose fractionation indicated that the recovery rate for tumor induction was independent of dose and time after irradiation and had a half time of about 3 hours.

Hair follicle survival was also scored in order to estimate the lethal effect of radiation on the follicular germ cells. Radiation recovery for hair follicle survival also exhibited a 3 hour halftime.

A model has been proposed for predicting the effects of dose rate on tumor induction based on the recovery rate estimated

indicates that a pronounced reduction in tumor yield should occur as a given total dose is protracted over long exposure times. This dose rate effect may be summarized in terms of a Dose Rate Factor (DRF) which is defined as the ratio of the acute dose to protracted dose which produce the same tumor yield. The usefulness of this ratio is that a given protracted dose, Dp, may be equated with an acute dose, Da., by multiplying by the DRF: Dp x DRF = Da. The DRF decreases with decreasing dose rate as long as the acute dose is on the dose squared portion of the response curve. As indicated above, it may be that there is a small linear component which becomes significant at very low doses and for Da values in the region where the linear term dominates, the reductions in dose rate would not reduce the DRF. In the model, this condition corresponds to dose rates so low that recovery effectively prevents the buildup of suboncogenic injury and all tumors are produced by the direct, dose rate independent mode, if it exists.

2.10 Kinetics of Papilloma Growth in Mouse Skin

The growth kinetics methodology normally applied to radiation induced tumors in rat skin has been used to evaluate chemically induced mouse skin tumor growth characteristics.

DMBA initiation followed by PMA promotion produced mainly

papillomas which regressed when promotion was ended. An effort was made to determine whether kinetic differences could be detected between regressing papillomas and the small proportion of non-regressing papillomas.

It was found that immune suppression exhibited no effect on regression but a non-specific irritant, ethyphenyl propiolate, inhibited regression. The rapid cell proliferation characteristics of the papillomas did not decrease after promotion ended, whereas the cell kinetics of the promoter on normal tissue were reversible. The promoter seemed to produce a dose rate effect not dissimilar to that observed for irradiation with ionizing radiation in that the regression of papillomas when painting is discontinued leads to the expectation that a reduction in the frequency of painting (Dose Rate) would correspondingly reduce the tumor yield.

2.11 The Combined Effect of Ionizing Radiation and Ultraviolet Light on Tumor Induction in Rat Skin

The promoting effect of ultraviolet light (UVL) on ionizing irradiated rat skin is being investigated. UVL and ionizing radiation are both complete carcinogens when administered separately in rat skin. Chronic UVL exposure causes edema, erythema, and scaling with subsequent eschar formation.

Comparison of tumor induction in albino rat skin were made for single doses of erythemal (275-375 um) and germicidal (254 um) UVL; and for repeated doses of erythemal UVL following single doses of electron radiation. Single exposures to erythemal UVL induce tumors of increasing incidence with dose in the range of 0.8×10^7 to 3.2×10^7 ergs/cm^2. The tumor incidence appears constant in the dose range 3.2×10^7 to 25.2×10^7 ergs/cm^2. In this dose range approximately 10 times as much germicidal UVL as erthemal UVL was required for equivalent tumor yields.

Groups of 6 or 12 male albino rats were given 690, 1380, 2060 or 3480 rads of electrons to the dorsal skin at 28 days of age followed by exposure to either 2.1×10^7 or 0.42×10^7 ergs/cm^2 erythemal UVL weekly for 12 or 20 weeks respectively. The electron induced tumors consisted primarily of squamous or basal cell types and were clearly distinguishable, both grossly and histologically, from UVL-induced tumors which were exclusively kerato-sebacous cyctic acanthomas. This marked difference in histology of the tumors may reflect differences in the oncogenic targets of the two radiations. In rats exposed to both UVL and electrons, the incidence and types of tumors indicated that the effects of the two agents were generally additive and independent

except for about an eight week delay in the appearance of electron-induced tumors during the 20 weeks of UVL exposure. For a given total dose, fractionated exposure to erythemal UVL induced fewer tumors than a single exposure, suggesting that recovery or protection occurred during the protracted exposure.

2.12 The Carcinogenic Interaction of Ionizing Radiation and a Chemical Carcinogen

The carcinogenic potential of ionizing radiation in conjunction with a chemical carcinogen is of practical importance in simulating environmental conditions. Because the tumor response for ionizing radiation is well established in rat skin, the interaction with other carcinogens can be studied with respect to equivalent doses to produce the same response. Temporal additivity can be analyzed by comparing the individual temporal incidence curves for the different carcinogens with the incidence curve obtained with combined treatment.

Arrangements have been made to use the 2.0 MeV Van de Graff accelerator at Union Carbide, Sterling Forest, New York for the initial electron exposures next month (May, 1976). The protocal consists of irradiating the dorsal skin of rats with doses of 750, 1500, 2250 and 3000 rads of electrons followed by weekly applications of 0.002%, 0.01%, or 0.05% dimethylbenz(a)anthracene (DMBA) in acetone.

At these doses, the DMBA above should produce low tumor incidences. The objective of the experiment is to determine whether prior electron irradiation alters the susceptibility of the skin to DMBA.

2.13 Age Dependency in Radiation Carcinogenesis

Rats of various ages were exposed to low voltage x-rays in order to determine the age dependence of the dose response curves for tumor induction. Newborn, 28, 58, 100, 200 and 350 day old rats have been exposed to doses of 500, 1000, 1800, 2250, 3000, 4000 and 5000 rads. An increase in radio resistance with age was noted for the incidence of ulceration at three weeks post irradiation. Tumors were induced with latents periods (time from irradiation to appearance of first tumor) in the range of 15-24 weeks which was not strongly age dependent, with the exception of the 350 day old group which had no tumors at 30 weeks post irradiation. The subsequent tumor appearance rate was approximately constant at each dose. The typical dose response curve (tumors/rat vs. dose) ascends rapidly, reaches a peak incidence, and then descends at higher doses. Although the height of the tumor incidence peak was nearly age-independent, the position of the peak on the dose axis showed a shift to higher doses with increasing age. That is, the older rats became progressively more resistant to the oncogenic effect of the radiation. In terms of the dose at the depth of the follicular bulb, the peak tumor

incidence occurred at 1200-1800 rads in newborns, at 2200 rads in 28 day old rats (previously published data), and at about 2800 rads in the 100 day old rats based on 76, 80, and 28 weeks of observation respectively. The tumor incidence in the 200 day old animals is still too low for analysis at 16 weeks post irradiation.

An increase in radio resistance with age was observed in our previously reported studies on skin tumor in rats irradiated with electrons or protons at 28 and 58 days of age, and supports the general conclusion that several types of radiation become less oncogenic with increasing age.

2.14 Fractionation with High LET Radiation for Tumor Induction

On the basis of cell lethality data, which exhibits LET dependency, there should be little or no recovery from high LET (about 100 kev/u) radiation for the tumor induction end-point. Investigation of this point for tumor induction in rat skin is possible only by irradiation with high atomic number nuclei such as argon (atomic number 18).

These irradiations are scheduled to take place in June using the Lawrence Berkeley Laboratory heavy-ion linear accelerator (HILAC) and Bevatron in combination (BEVALAC). Preliminary dosimetric considerations and design of a suitable exposure system are underway.

A skin flap technique will be used which involves stretching

a 1 cm. wide flap of dorsal skin vertically above the back of the animal and scanning the beam horizontally through several animals in succession. The experimental protocal designed will determine if linearity exists at high LET for single doses, and also the amount and rate of recovery between split doses.

2.15 DNA Damage and Repair in Tumor Induction by Ultraviolet Light and Ionizing Radiation

Damage and subsequent repair processes of cellular DNA are believed to be related to the cellular reaction to ultraviolet light and ionizing radiation. Recently these processes have been implicated in the carcinogenic response and several experiments have been undertaken to detect DNA damage and repair following in vivo exposure.

The measurement of UVL induced excision repair DNA synthesis was attempted using the sparse labelling radioautographic technique. This involves injecting (either intradermal or intraperitoneal H^3 thymidine immediately following UVL irradiation and subsequent skin biopsies taken one hour post irradiation. Radioautographs of thin sections from the biopsies will exhibit spare labelling if DNA repair synthesis has occurred because only small segments of newly synthesized DNA will be labelled. The cells in S-phase are heavily labelled and easily distinguishable from sparsely labelled cells. In our experiments using erythemal UVL (275 - 375 nm) in the dose range of

1.58×10^7 to 25.2×10^7 ergs/cm^2 or germicidal (254 nm). UVL in the range of 0.65×10^7 to 26.0×10^7 ergs/cm^2, we found no evidence of sparse labelling above background in non S-phase cells in the dorsal skin of albino rats. This is a carcinogenic dose for both erythemal and germicidal radiations and a dose at which DNA repair synthesis has been demonstrated in hairless mouse epidermis. The erythemal UVL did produce a general decrease in H^3-thymidine uptake in both sparsely and heavily labelled background cells, indicating an inhibition of all cellular DNA synthesis. The erythemal UVL also caused a rapid depopulation of epidermal basal cells to 65% of controls at one hour post-irradiation when biopsies were taken.

The alkaline sucrose gradient technique for detection of single stranded DNA breakage in vitro developed by McGrath and Williams and refined by Elkind and other investigators is being adapted for use with epidermal cells exposed to ionizing radiation in vivo.

2.16 Hair Follicle Proliferation Following Irradiation

The interest in obtaining mitotic indices of irradiated hair follicles originates from the concept that the hair follicle is a possible tissue of origin for tumor cells. Thus the correlation between tumor yield and mitotic proliferation in hair follicles was examined. Four groups of CD strain rats obtained from Charles River

Laboratories, Brookline, Massachusetts, were exposed to 0.7 MeV electron radiation on their dorsal surfaces at doses of 700, 1150, 1400 and 2300 rads respectively. Animals were observed and appearance of tumors noted. At 68 weeks post irradiation, the animals were injected with vinblastine four hours prior to sacrifice. Tissue sections were made and mitotic figures counted at 1250 x magnification in resting stage hair follicles .08 mm below the epidermis.

The mitotic rate in irradiated hair follicles was significantly higher than that of the controls, although there was no clear relationship between the mitotic rate and either the radiation dose or the subsequent tumor yields. In groups receiving 700, 1150, 1400 or 2300 the rates were $0.68 \pm 0.20\ hr^{-1}$, $0.52 \pm 0.07\ hr^{-1}$, $0.41 \pm 0.07\ hr^{-1}$, and $0.57 \pm 0.09\ hr^{-1}$ respectively, while in the control group the mitotic rate was $0.09 \pm 0.09\ hr^{-1}$. The tumor yields for the various dose groups increased from 0.16 tumors/rat at 700 rads to 3.04 tumors/rat at 1400 rads, and declined to 1.28 tumors/rat at 2300 rads.

The increase in proliferation rate of follicle cells is a striking effect and further studies are being carried out to determine the effects of radiation on proliferation in the overlying epidermis. The possibility that an increased rate of cell turnover

contributes to tumor development has been long recognized for such chemical promoting agents as phorbol myristate acetate (PMA), and it may be that a similar effect is occurring in irradiated hair follicles.

2.17 Chemotherapy of Rat Skin Tumors

The present experiments were undertaken to evaluate two cytotoxic agents, methotrexate and cytosine arabinoside (Ara-C) in preventing the appearance of new rat skin tumors and in causing the regression of pre-existing tumors. Since we have previously noted a high mitotic rate in radiation-induced rat epidermal cell tumors, it was hypothesized that anti-mitotic drugs would be effective in altering the growth rates of these tumors.

Tumor bearing rats from a previous experiment were assigned to treatment groups at 76 weeks post-irradiation. In one group methotrexate was injected in alternate flanks three times weekly at a dose of 1mg/kg, while the same injection protocol was used for Ara-C (3mg/kg) or saline (2.5 ml/kg) in two other groups. The methotrexate treated animals all showed severe weight loss and died after a few injections. As such, the injection protocol with Ara-C was modified so that any animal showing 10% weight loss in two days had its treatment withheld until the weight stabilized. Survival in this group was not different from controls for the 18 week duration of therapy, and the overall weight loss for the group was

less than 10%. All grossly visible lesions were scored at the time of start of the treatments and subsequently at biweekly intervals. Ara-C did not induce tumor regression. Of 27 tumors in the treated group, 3 regressed while 2 of 38 regressed in the control group. Since we have previously observed about 10% false positives in identifying carcinomas from their gross appearance, these "regressions" may not reflect the actual disappearance of carcinomas.

For each of the groups, the number of new tumors appearing during the 18 weeks of treatment may be compared with the number of tumors appearing in the same animals for the 18 weeks prior to treatment. We have previously observed that the rate of appearance of new tumors is approximately constant and, therefore, one expects the number of tumors to be about equal in the two intervals if the chemotherepeutic agent had no effect. There were 4 new tumors in the Ara-C treated animals, compared with an expected number of 3, and in the saline treated animals, there were 14 tumors compared with 11 expected. We conclude that Ara-C did not reduce the rate of new tumor appearance.

Examination of microscope section showed that many of the tumors in the Ara-C treated animals had evidence of cell death.

Many of the tumor cells were pyknotic, and there was extensive infiltration by lymphocytes.

The antimitotic agent Ara-C was neither able to reduce the new tumor appearance rate, or induce regression in preexisting lesions. The implication of these observations is that tumors of both large (microscopically visible) and small (subvisible) size are able to grow in spite of what appears microscopically to be a large lethal stress. There is no prophylactic value to the antimitotic agent in preventing tumor development under the conditions of this experimental test.

2.18 Epidermal Cell Survival

Radiation has both toxic and carcinogenic effects on tissue, and it has been suggested that cell lethality at high doses may be responsible for the reduction in tumor yield. The objective of these studies is to evaluate the radiation cell killing in rat skin, for not only single doses, but for split doses of radiation where recovery of both sublethal and suboncogenic injury is likely to be occurring. The assay developed uses the ability of cells to complete the first post-irradiation mitosis as an index of viability.

The experimental procedure involves the pulse labelling of cells with H^3-Thymidine, allowing 24 hours to elapse, and then making whole mount preparations of the epidermis. During the

24 hour interval, the labelled (S-phase) cells will progress through mitosis and appear as adjacent labelled cells (labelled doublets) in the sheet of epidermal cells.

Irradiation increases the number of cells which are incapable of completing mitosis, and thus reduces the fraction of labelled cells appearing as labelled doublets. Variation in cell cycle sensitivity to radiation may be quantitated by varying the timing between the irradiation and ^{3}H-Tdr injection. For example, concurrent irradiation and labelling assays the survival of S-phase cells, while irradiation several days prior to labelling assays the survival of cells that were in G_1 at the time of irradiation.

Preliminary results indicate that S-phase cells irradiated with either electrons or Grenz Rays have a broad shoulder on which little cell killing occurs. 400R reduced the number of labelled doublets only slightly (90 % survival), while doses larger than 800R prevented mitosis in nearly all cells. Results for late G_1 cells (24 hours between irradiation and labelling) indicate the absence of any ^{3}H-Tdr incorporation, which may be the result of DNA synthesis suppression.

Current plans are to more carefully define the shape of the survival function for S-phase cells, and to extend the

interval between irradiation and labelling to longer time periods to overcome the suppression of thymidine incorporation. Plans also include the measurement of split dose recovery for this lethality endpoint, once the survival curves are established.

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Opinion about state of knowledge in field of radiation carcinogenesis

Radiation carcinogenesis is still a field of scientific concern because it is likely that radiation exposures, whether medical, occupational or experimental, will continue to occur among large populations of people. A better understanding of the risks associated with these exposures is essential for rational management of radiation. The shape of the dose response curve must be known and, for extrapolating to low doses, the basis or mechanism that determines the shape must be known. Recovery is an important but puzzling radiobiological occurrence and the finding that recovery is operative for oncogenesis makes it imperative to obtain a better understanding of the process and the conditions under which it occurs.

The rat skin may serve as a useful model for solid tumors in providing answers to such questions that arise in connection with the interaction of radiation with other oncogens in the environment. The mechanism of oncogenesis itself is obviously an active area of scientific inquiry and radiation may provide certain clues to the mechanism that cannot be determined in other ways. In other words the study of radiologic and biologic factors in radiation oncogenesis is likely to provide information that could be useful in the interpretation of mechanisms and could also be of practical value in providing a basis for controlling risks because of inadvertant or unavoidable exposure to ionizing radiation.

GRADUATES TRAINED

NAME	DEGREE	THESIS TITLE
Martin Vanderlaan	Ph.D.	Experimental and theoretical aspects of recovery rate for tumor induction in rat skin by electron radiation.
Paul Strickland	M.S.	The effect of fractionated doses of electrons and protons on hair follicles in the albino rat.
Ian P. Sinclair	Ph.D.	A comparison between the early cellular response to electron radiation and the production of tumors.

Federal Support for Overall Research Program

1. E(11-1)2737 ERDA Temporal Aspects of Tumor Response

   $256,140 6/1/75 - 5/31/76

2. E(11-1)3380 ERDA Present Study

   $50,234 8/1/75 - 7/31/76

3. Proposed: NIH Environmental Carcinogens in Experimental Atherogenesis

   $80,380 one year