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Progress Report

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Alpha and Electron Radiation on the Rat
Skin

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Progress Report

The work described here is a continuation of studies aimed at elucidating the various determinants of the dose-response relationships in radiation carcinogenesis. The general goal is to gain a better understanding of the carcinogenic hazard of radiation. Rodent skin, primarily rat and mouse skin, has been used as the experimental model for several reasons: (1) radiation carcinogenesis in skin is potentially important to man, (2) skin tumors are relatively easy to observe so that appearance times and growth rates can be determined, (3) proliferation of skin cells can be, at least partially, controlled experimentally so that the effect of cell division rates on tumor induction can be studied and (4) the skin may serve as a useful model for the carcinogenic response of other renewal or conditional renewal tissues.

In previous work under the present contract, a number of significant results have been obtained which will now be summarized (1-16). For rat skin in the resting phase of hair growth, skin tumors were produced only if the radiation penetrated at least to a depth of about 0.3 mm which roughly corresponded to the depth of the follicles (3). However, selective irradiation of the tissue

at 0.3 mm by means of an alpha particle Bragg peak did not produce more tumors than were expected from the Bragg plateau dose (5). The relative biological effectiveness (RBE) of alpha particles relative to electrons was measured and found to be about 2.9 (6). The tumor incidence per unit irradiated area was found to be less for a sieve pattern than for a uniform pattern for electron irradiation (2), but for protons which have a relatively high LET the sieve pattern did not produce a reduction of the tumor incidence (11). In all experiments a correlation was found between the incidence of atrophic follicles and the incidence of tumors with the former outnumbering the latter by a ratio of about 2000-4000:1 (4). The correlation has been sufficiently persistent to suggest that atrophic follicles actually develop or promote tumors. The epithelial tumors have histological characteristics compatible with an origin in the hair follicles (7). As the dose became increasingly pilocidal, the incidence of adnexal (hair follicle) tumors was reduced, while the incidence of epidermoid tumors remained approximately constant at doses approximately 1500 rads (12).

Results Obtained in the Past Year

1. Proton Sieve Experiment

A paper was published which described the effect of proton irradiation in a sieve pattern on the incidence of skin tumors (11). The use of protons eliminated the scattering which occurred in an earlier sieve pattern experiment with electrons but introduced a significant increase in linear energy transfer (LET). In contrast to the earlier result with electrons, protons in a sieve pattern did not show a reduction in the tumor incidence per unit irradiated area when compared to a uniform irradiation pattern.

2. Tumor Incidence in Mouse Skin

A paper has been submitted for publication which shows that mouse skin is relatively resistant compared to rat skin to tumor induction by electron irradiation (12). The epithelial tumors induced in mouse skin were predominantly epidermoid, whereas in rat skin only about 10% of the epithelial tumors were epidermoid at low doses while at high (ulcerating) doses the percentage increased to about 50%. Corresponding to the relative lack of adnexal (hair follicle) tumors in mouse skin was a relative lack of hair follicles, i.e., the mouse follicles were much more

sensitive to the lethal action of the radiation than rat follicles and had much less tendency to form atrophic, i.e., partially destroyed, follicles.

3. The Fractionation Experiment

A manuscript was prepared which described the effect of fractionation on tumor incidence and hair follicle atrophy in rat skin (14). An initial dose of 750 rads was given and followed 31 days later by graded doses. The question was whether the carcinogenically-related damage would recover equivalently to the grossly observable damage as measured by hair follicle atrophy. The results showed that recovery with respect to tumor induction was approximately the same as that associated with morphological hair follicle damage and both were in the range of 650 to 700 rads of the initial 750 rads. The interval between fractions was sufficiently long that cell proliferation could have occurred. In the second part of the fractionation experiment, the kinetics of recovery are being studied by shortening the interval between fractions to 24 hours thereby eliminating the possibility of a significant amount of cell division. The latter experiment is currently in progress.

4. Induction of Tumors during the Phase of Hair Growth

An oral presentation will be made at the Radiation Research Society meeting in May 1972 which describes the effects of dose and penetration on skin tumor incidence for irradiation during active hair growth (15). By means of plucking, the hair was induced to enter the growth phase where the mitotic activity and overall size are greatly increased relative to the resting or telogen follicle. The skin was then irradiated with various penetrations and doses of electrons.

There were several interesting findings in this experiment. The most unexpected result in view of our previous findings was that skin tumors could be induced without the occurrence of proportionate numbers of atrophic follicles. The unexpected tumors occurred at relatively shallow penetrations.

At deep penetrations, i.e., at penetrations that exceeded the depth of the follicles, the correlation between atrophic follicles and tumors was similar to what had been found previously for irradiation of resting follicles. For a shallow penetration, the tumor incidence was high in spite of the occurrence of relatively few atrophic follicles. As the dose was increased, the incidence

of atrophic follicles increased and the incidence of tumors decreased until at ulcerating doses the proportionality between atrophic follicles and tumors was restored. The results could be interpreted as a biphasic response which implies that two basically different mechanisms of induction are operating, one which involves the hair follicles and one which doesn't. Several new experiments are planned to study the biphasic nature of tumor induction in the growing phase.

5. Tumor Induction in a "Resistant" Strain of Rats (17)

There are reports in the literature that the skin of Wistar rats is relatively resistant to the carcinogenic effects of ionizing radiation. If true, it would be interesting to compare hair follicle atrophy and tumor induction in resistant and sensitive strains. Wistar rats were irradiated with graded doses, and the dose-response curves for tumor induction and gross skin damage were found to be practically identical to the comparable curves obtained in our previous experiments with Charles River (Sprague Dawley derived) rats. The Wistar rats were not resistant with respect to skin tumor induction.

6. The Carcinogenic Effect of Radiation and Methotrexate Combined

Part of the action of radiation on any population of cells involves cell death. It is impossible to know how or whether cell death is involved in the carcinogenic response. The dead cells are irrelevant per se, but they could cause an unusual regenerative stimulus which might affect the carcinogenic status of the survivors. By artificially increasing the amount of cell death at the time of irradiation, it might be possible to determine how cell death affects the tumor response. Methotrexate, a drug that kills cells in DNA synthesis (S-phase), was administered immediately prior to irradiation at doses sufficient to inhibit hair growth.

There was no effect on tumor incidence at the lower of two methotrexate doses used. However, at the higher dose, the tumor incidence was reduced by about 60% which suggested that by increasing the lethality the oncogenic cells were simply reduced in number and fewer tumors occurred. The conclusions are tentative since the experiment is still in progress and histological diagnoses of the tumors are not yet available.

7. The Effect of Tumor Growth Rate on the Tumor Incidence Curve

It is recognized in tumor induction experiments that considerable

time may be required for a tumor to grow from one cell (if it starts as one cell) to a size which is clinically observable. In practice the smallest detectable size is about 1-2 mm diameter which easily might contain 10^6 cells or more. If the growth rate is known, the time when the tumor was only a single cell can be calculated by extrapolation of Gompertz equations. The growth of several tumors from the minimum detectable size to 40 to 50 mm diameter has been measured and found to exhibit some growth retardation (a departure from true exponential growth where the growth constant decreases exponentially with time). By analysing the growth curves, the true appearance time can be calculated. Only 8 fast growing tumors have been analyzed so far and 6 of these apparently arose at time zero (the time of irradiation), although their actual appearance times varied from 32 weeks to 66 weeks. The remaining two tumors apparently started growing about 24-32 weeks after the irradiation. Further analyses are in progress to determine if growth rate and appearance time are related.

8. The Kinetics of Recovery from Carcinogenic Damage

An earlier experiment (see Section 3) showed that skin may recover as much as 650 rads to 700 rads with respect to carcinogenic

and morphologic damage if 31 days elapses between fractions. By reducing the interval between fractions to 24 hours, it should be possible to determine the relative proportion of the total recovery attributable to intracellular and proliferative recovery, since the former is a relatively rapid process occurring within a few hours while the latter is dependent on the relatively slow process of cell division.

The fractionation experiment with 24 hours between fractions was initiated during the past year. As of 22 weeks after irradiation, there is a shift of the dose-response curve which suggests that the result for 24 hours between fractions will be approximately the same as for 31 days between fractions which means that the recovery process for carcinogenesis is relatively rapid and probably does not involve cell division. This conclusion is tentative since histological diagnoses are not yet available.

9. The Critical Depth for Tumor Induction in the Growing Phase

For irradiation of skin with resting follicles, a critical depth (defined as that depth which reconciled the dose-response curves for diverse penetrations) was found at about 0.3 mm, i.e., the depth of the follicles. It was initially expected that a

similar critical depth would occur for growing follicles except at 1.0 mm corresponding to the depth of these much larger follicles. In the penetration experiment for growing phase follicles already described (Section 4), penetrations in the range of 1.0 mm to 2.0 mm had relatively little effect on the dose-response curve for tumor induction suggesting that the critical depth (if it exists) is more superficial than 1.0 mm. It would be of considerable interest to determine if the critical depth remained at 0.3 mm since it might be expected from biological considerations that that hair follicle stem cell exist permanently at this level.

Hence, an experiment was initiated where the penetration of electron radiation was reduced to 0.5 mm. At the present time (24 weeks after irradiation) no tumors have occurred for any of the 5 doses from 500 rads to 4000 rads and therefore no conclusions are as yet possible regarding the existence of a critical depth for the growing phase.

10. The Dose-Response Curve for Growing Phase at 1.0 mm Penetration

In the earlier growing phase experiment (Section 4), the results for a penetration of 1.0 mm departed from what had been found for resting phase, i.e., the tumors occurred virtually in

the absence of hair follicle atrophy. In fact, the maximum tumor incidence occurred at the lowest dose used, namely 1000 rads, and raised the possibility of an even higher yield at lower doses.

An experiment was initiated to extend the dose-response curve for a penetration of 1.0 mm to determine more precisely the position of the tumor peak. Only about 24 weeks have elapsed since the irradiation and conclusions can only be tentative, but on the basis of present data, it appears that the tumor peak is between 1000 rads and 1500 rads.

11. The Effect of Postirradiation Proliferative Stimuli on Tumor Incidence

Much has been said concerning the role of cell proliferation and hyperplasia in mouse skin carcinogenesis. Since it appears likely that radiation-induced tumors in rat skin originate in the hair follicles, the possibility exists to determine whether forced proliferation of hair follicle cells affects the rate of appearance or growth rate of the tumors. The rats were irradiated at 1000 rads for various penetrations in either the resting or growing phase and were subsequently plucked or stripped (the former procedure stimulates proliferation in the hair follicles and epidermis, while the latter stimulates only the epidermis) at

4 weeks intervals. So far (about 24 weeks postirradiation) too few tumors have occurred for any but tentative conclusions to be drawn, but it appears unlikely that plucking or stripping has enhanced the tumor incidence and there may even be inhibition.

12. The Effect of a Sieve Pattern on Tumor Induction for Grenz Rays

Two earlier experiments have produced an apparent inconsistency. A sieve pattern reduced the tumor incidence per unit area for electrons but was without effect for protons. Electrons and protons differ physically in that the former are lighter and scatter more and have a much lower linear energy transfer (LET). Either differences in scattering or differences in LET or both might explain the discrepancy. An experimental determination of the correct explanation can be made through the use of low energy x-rays (Grenz rays) which have an LET comparable to electrons and scattering characteristics similar to protons.

The rats were irradiated with sieve patterns having pore diameters of 0.5 mm and 2.0 mm. In addition, an attempt was made to mimic scattering by superimposing a uniform low dose on a sieve pattern. The experiment is currently at 42 weeks postirradiation

and tumor histology is not available, but there is a significant reduction in the clinically observable tumor incidence per unit area for both pore sizes, i.e., similar to the earlier result for electrons. However, surprisingly the group where sieve and uniform patterns were superimposed is showing no reduction in tumor incidence per unit area, i.e., sieve protection was abolished by a low dose to the tissue surrounding the more heavily irradiated pore areas.

13. Cell Death and Regeneration in the Hair Follicles and Surface Epidermis

One of the graduate students engaged on the project is studying cell death and regeneration as part of his thesis project. Rats with growing phase follicles were irradiated with various doses of electrons at several penetrations corresponding to the protocol used earlier in the tumor induction experiment (Section 4). The protocols were made comparable so that the early histological response could be compared to the ultimate yield and histological type of the tumors. Cell death was measured by prelabeling dividing cells with ^3H -thymidine and determining the loss of tritium from the DNA. Regeneration was measured histologically. The results so far show that cell killing and repopulation are processes

occurring more or less independently in epidermis, hair follicles, and sebaceous glands, but too little data is now available to make correlations with the tumor incidence. This experiment will be completed soon.

14. Properties of the G₀-Phase in Rat Epidermis

Earlier experimental and theoretical work suggested the possibility that cell division in the epidermis is controlled by the release of cells from a hypothetical G₀-phase (10). If the G₀ hypothesis is correct, a stimulation of proliferation should cause an increase in the rate of release of cells from the G₀-phase. By prelabeling with C¹⁴-thymidine, the fate of G₀ cells can be determined as they pass through the S-phase. One of the assumptions made in the theoretical analysis was that the duration of the "true" G₁-phase immediately preceding the S-phase was a constant irrespective of the rate of release of G₀ cells. During the past year an experimental test of this assumption was made, and it was found that the "true" G₁-phase duration was not constant but decreased as the proliferation rate increased. The magnitude of the decrease was relatively small so that no major change in the model was necessary.

15. The Treatment of Radiation-Induced Skin Tumors with Methotrexate

Methotrexate, a chemotherapeutic drug, was administered to rats bearing radiation-induced tumors to determine if the tumors would respond. Approximately 10 tumors have been treated thus far. Five showed a temporary reduction in size within one or two weeks, two regressed completely and had not recurred by 4 weeks, and the remaining three showed no response. Studies of the response of tumors to methotrexate in relation to growth rate, size, and appearance time are continuing.

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