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V. Heavy Ion and Millibeam Irradiations on Mammalian Tissue

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IN CONSIDERING the radiobiological effects of ionizing radiations, there is one area of investigation which is of considerable interest, but as yet relatively little studied, i.e., the tissue effect. In contrast to the direct cellular effect, which has been extensively studied, how do x-rays and irradiation with heavy ions affect highly organized tissues? Also, how does irradiation beam size alter the degree and nature of the tissue effect? Knowledge of these aspects when related to the more thoroughly studied intracellular and organismal effects and to the more conventional sources and methods of irradiation should increase our understanding in radiobiology.

In addition to this basic objective, i.e., a better understanding of the nature of radiation effects in biological materials, there is also a more immediately practical objective in terms of evaluating biological damage to be expected from cosmic ray heavy primary thindowns at the top of the atmosphere and in space. These thindowns from heavy ions, because of the extensive delta rays, are essentially small intense beams of radiation which can damage whole cells and columns of cells in tissues.[2,7,11,12,15,16,18,19]

CONSIDERATIONS

Tissue Effect.—Both tissue damage and organismal damage (such as death of the whole

multicellular animal following radiation) are, of course, related to radiation events occurring within the cytoplasm and chromosomes of cells. The total tissue or organismal effect, however, may become greatly amplified because of secondary effects. Tissues and organs are integrated systems of cells, and the failure of a few cells to function properly or to proliferate at a normal rate, or even to maintain normal inter-cellular continuity, can cause a tissue effect of a magnitude considerably beyond the cellular damage *per se*. For example, the bulb of a growing hair follicle is a very precisely organized arrangement of epithelial cells including cells of high mitotic activity and others maintaining the typical turgid cone arrangement. A moderate dose of 400 rads of x-rays can completely disrupt the bulb, not by directly destroying an appreciable number of cells and not by directly causing a loss of most of the mitotic activity, but rather by upsetting in some way the cellular integration. Enough cells, both structurally and mitotically competent, remain to form a hair-producing bulb, but they do not do so until several days later after a follicular reorganization has ensued. The consequence is a temporary, but only temporary, epilation of the hair growing at the time of irradiation.[1,4]

Although it has been generally considered a direct cellular effect,[3] the greying response, which is a failure of pigment to be deposited in new-growing hairs following irradiation, could also be considered a tissue response. A radiation-induced white hair results when there are no melanocytes in the bulb contributing pigment granules to the differentiating cells that form the hair; a mosaic hair with only part of

its normal pigment complement has fewer than the normal number of contributing melanocytes, often only one or two. The region in the upper bulb where the active melanogenic melanocytes transfer their granules to the epithelial recipient cells is small, and the stem cells which presumably give rise to these cells for each new hair generation occupy an area of the germ near the epithelial-dermal papilla junction of possibly 30 μ or less for most mouse hair follicles. The absence or reduction of mature melanocytes in the bulb after irradiation has indicated that the stem cells are killed directly or are indirectly made incapable of delivering pigment granules to the hair cells.[3]

A more clear example of a tissue response, still within the skin system, is that of epidermal hyperplasia. Increased mitotic activity and hyperplasia not only occur in epidermal cells damaged by the irradiation, but also in the cells immediately outside the irradiated zone.[10] A rather small amount of cellular damage can cause the hyperplastic response,[5] and ulceration occurs with larger doses. The degree of epidermal hyperplasia, as will be seen later, depends not only on the amount of epidermal tissue irradiated, but also on the amount of underlying dermis which is irradiated.

Heavy Ions.—With heavy ions of 10 Mev/nucleon from the Berkeley Heavy Ion Linear Accelerator, one can produce effects due to different LET's and also due to different depths of penetration in tissue. Carbon, oxygen, and neon nuclei, as well as alpha particles, are available with their different ion densities and calculated "useful" ranges in tissue of 280 μ for carbon, 225 μ for oxygen, and 170 μ for neon. Experiments using these particles demonstrate that the responses of skin and hair follicles to radiation are largely dependent on both LET and depth penetration.

Another source of heavy ions is not at ground level, but is the small portion of cosmic radiation at the top of the atmosphere and in space which consists of heavy primaries. The nuclei (mostly of carbon through iron, Z = 6 through

26) which terminate as thindowns at high altitudes north of 55 degrees geomagnetic latitude are the ones that have been used on black mice in capsules carried at high altitudes by Air Force balloons.

Millibeams.—In the work of Zirkle and Bloom[20] the differential effects of very small microbeams on different portions of the cell have been clearly demonstrated, but a slightly larger size of beam may have more significance in terms of tissue damage. Consequently, the term millibeam is preferred here to cover the range from about 20 microns to 2000 microns, the geometric mean being 200 microns, the size most generally employed. Methods for making such apertures and a method for determining dosimetry have been published.[17] The elongate microbeam or millibeam is the most useful for skin and hair studies and could readily be used for other tissues, tissue cultures, and embryos. Besides protective effects associated with small beam irradiation, there is the possibility of irradiating localized regions of tissues without the usual systemic complications or secondary effects. An elongate electron beam, passing a 200 μ milli-slit in a brass plate, is an especially effective system because the depth in tissue is abruptly restricted, unlike x-rays. The collimated beam from the 1.5 Mev Pulsed Generator of the Radiobiology Unit at Mt. Vernon Hospital was used in this work.

Essentially, the thindowns from cosmic ray heavy primaries are also microbeams or lower order millibeams and can readily be considered as such. The damage they produce is of the whole cell or tissue type, not particularly of the intracellular type.

RESULTS

electron beam and with our milli-slit apparatus will be published with Dr. J. Boag of the B.E.C.C. Research Unit in Radiobiology, Mt. Vernon Hospital, Northwood, England.

Cosmic Ray Heavies.—Greying of mouse hair was described for the 1954 flights and agreed rather well with Schaefer's calculations.[4,13,14] The 1955 flights were less conclusive.[7] In the summer of 1960, a total of thirty-three black mice were recovered from three Air Force flights at 130,000 feet altitude from a northern latitude. Monitoring nuclear track emulsions for each mouse were also available and charts for all tracks over $Z = 19$ have been made by Brooks Air Force Base personnel. The average number of grey hairs in the prescribed 10cm^2 area in these young mice was 15.3 compared with 5 for the controls. Also of interest was a close correspondence of "streaks" of three to seven white hairs with the long tracks scored at Brooks in the emulsions. These results, with the important added track plate information, confirm the 1954 results.

Electron Beam with Crossed Milli-slits.—With crossed slits, each of which is 200 μ wide and 5 mm long, there is a greying response which is twice as great for the slit exposure parallel to the hair slope as compared with the slit exposure which is at right angles. This result is based on actual counts of white hairs, because the gross appearance alone would naturally favor the parallel slit with its overlapping affected hairs. Irradiation of the whole follicle evidently has more effect on subsequent greying than merely exposing the hair germ and its pigment stem cell region. The induced hyperplasia of the epidermis is the same for both slits. With round apertures as large as 2 mm there is more hyperplasia per rad dose than with the slits, but still no ulceration even with 5000 rads.

X-ray millibeam experiments give approximately the same results for epidermal effects as does the electron beam, but the greying difference associated with the orientation of the slits is at present inconclusive. Furthermore, it appears that the greying per dose is less than

with the electron beam and considerably less than for large beams of x-rays.[17]

HILAC.—Comparing 10 Mev/nucleon carbon ions from the Berkeley HILAC with x-ray effects[9] in relation to the hair greying response for follicles which are short and resting at the time of treatment, there is a lower threshold and a greater effect for carbon per rad. The threshold for greying is below 100 rads instead of 250 rads and the 50 per cent greying response is at about 350 rads instead of 625. When hair follicles are growing at the time of treatment, their length exceeds the range of carbon, and the populations of melanocytes in the hair bulb are far below the layer of irradiated skin. After irradiation, growing hair follicles continue to produce completely black hairs, but a pronounced greying does appear in the second hair generation several weeks later. This delayed effect indicates that damage in the upper follicle is later translated into pigment cell loss or inactivation. On resting follicles, oxygen ions produce a greying effect less than do carbon ions and more like x-rays. This may be due to the slightly shorter range of oxygen ions in tissue, which perhaps allows some of the resting germs to escape exposure.[9] Neon ions which are too short in range to expose even the resting hair germs have an effect but only at doses of 3000 rads and greater, again suggesting an indirect effect through the follicle on the pigment-producing area. This observation for neon is of particular significance because it is conceivable that some of the white hairs and streaks reported[7] for cosmic ray heavy primaries may be caused in a similar fashion.

Epidermal hyperplasia and ulceration certainly occur with these heavy ions but seem to be related to the depth of penetration into the dermis rather than to the LET difference. Actually in this respect alpha particles are most effective, with carbon, oxygen, and neon in decreasing order. An experiment using absorbers to bring all these particles down to the range of neon and thus answer conclusively the question of LET effects is still to be performed.

SUMMARY

When considered with respect to tissue responses, variations in heavy ions and an electron beam all 'with restricted ranges in tissue, variations in cosmic ray heavy particle thindowns, and variations in size of beam produce effects with certain pertinent differences. Indirect and delayed effects which can be scored in the skin tissue system are of particular interest. Even the greying response is not solely a cellular damage but reflects to some extent an effect on the upper follicle, a conclusion of possibly considerable consequence in radiobiology and in predicting damage from cosmic ray heavy ions.

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