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Hair follicle response to the variable l.e.t. of a helium ion beam

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A homogeneous population of hair follicles in the rat tail has been used to analyse the *in vivo* response of a biological system to heavy particle irradiation. The conical configuration of the rat tail gives rise to a variable energy degradation of the beam thus yielding information on the damage elicited by the increasing l.e.t. of the helium beam at different sites on the same sample. By means of scanning electron microscopy three different zones were observed as direct evidence of helium ion penetration in the tail. Quantitative evaluation of radiation damage yielded results concerning hair follicle damage after increasing doses for different regions of the ionization curve.

Indexing terms: helium ions, hair follicles, Bragg-peak.

1. Introduction

Several models have been used to study the effects of heavy particles on biological systems. Many of the studies have been performed on cells *in vitro* (Chapman *et al.* 1977, Rajú *et al.* 1976), spheroids (Rodriguez and Alpen 1981) and tissues *in vivo* such as skin (Leith *et al.* 1973), lung (Woodruff *et al.* 1976), spinal cord (Leith *et al.* 1975) and testis (Power-Risius *et al.* 1978). In all these studies separate samples were irradiated with either the plateau region or the Bragg peak region of ionization of the beam. The results thus obtained, in terms of plateau vs Bragg-peak ratios, might be biased by the variability of radiation conditions, biological differences between samples, etc. In order to overcome these drawbacks a gelatine suspension of cells were used by Rajú *et al.* (1978) to measure cell survival at increasing depths of penetration in the same sample.

The aim of this work was to characterize a model in which the effects of the variable ionization of the Bragg curve could be analysed in the same animal, allowing a simultaneous evaluation of the biological effects of different parts of the beam trajectory in the tissue. For this, the high radiosensitivity of hair follicles was used to advantage. The rat tail was used to perform the analysis of hair follicle damage along the beam trajectory, profiting from its conical configuration that confers on the tail the properties of a variable-thickness beam energy degrader.

2. Materials and methods

In order to obtain a beam for *in vivo* biological studies, an external beam configuration was constructed at the Buenos Aires Synchrocyclotron (Bernaola *et al.*

1983). A circular beam (4 cm diameter) of 55 MeV helium ions was produced. Makrofol E foils (Bayer AG, 300 μm thick) located between the collimator and the specimen were used to detect the fluence of the beam and to evaluate the dose delivered to each animal. Makrofol foils were used as solid-state nuclear track detectors (SSNTD) and the tracks in the foils were visualized by means of light microscopy after chemical etching with PEW solution (30 g KOH, 80 g CH_3CH_2OH, 90 g H_2O) at 70°C for 40 min (Somoygi 1977). A good correlation was obtained between the area of the biological damage under study in the sample and the area for which the dosimetry was performed in the foil. The number of tracks in the foil is proportional to the beam flux, and they are produced by the products of compound nucleus decays generated by the interaction of helium ions with carbon and oxygen nuclei in the detector (Mazzei *et al.* 1984).

A detailed analysis of the dose measurements performed in different zones of the sample will be reported elsewhere taking into account the beam flux and the l.e.t. in tissue (Mazzei *et al.* unpublished observations). Briefly, the helium ions used in this work have a starting energy of 55 MeV corresponding to a range of 1820 μm in tissue. This starting energy is reduced at least to 46 MeV on reaching the first hair follicle matrices because of partial energy absorption by the SSNTD (Makrofol foils 300 μm in thickness) and by epidermal and dermal components of skin ($\sim$200 μm in thickness) (figure 1).

The portion of the curve corresponding to helium ions from 46 to 10 MeV, with l.e.t. values ranging from 16 to 55 keV/μm, was identified as the non-Bragg-peak portion of the ionization curve. Values from hair follicles affected by these energies were averaged and considered as 'non-Bragg-peak follicles' (NBP follicles).

On the other hand, helium ions with energies lower than 10 MeV constitute the Bragg-peak (BP) portion of the ionization curve (residual range 90 μm). Follicles reached by these particles are affected by 55 to 0 keV/μm l.e.t. with a maximum value of 214 keV/μm in the peak. Average values from these follicles were considered as Bragg-peak values (BP follicles) (figure 1). Values of l.e.t. for this figure were obtained by means of a computer program using the equation proposed by Benton and Henke (1969).

Tails (approximately 4 mm across in the thickest portion) from 5-day-old Wistar rats were irradiated. The animals were placed in wax boxes and immobilized by fixing them with thin strips of adhesive tape on the abdominal region. Possible motions of the tail during exposure (that lasted only a few seconds) were checked by the etched Makrofol foil. Five animals were used for each experimental condition and they were killed 7–10 days after irradiation by neck dislocation. A longitudinal incision was performed on the tail along the caudal vein and the skin and the underlying tissues were separated from the central cartilage by soft traction. The skin was incubated in 2 N NaBr solution for 10 min at 37°C to detach the epidermis from the dermis. The epidermal samples were fixed in 3·5 per cent glutaraldehyde solution in phosphate buffer, postfixed in 2 per cent osmium tetroxide solution, dehydrated in graded concentrations of acetone and dried from CO_2 by the critical point method. Epidermal samples were mounted with the hair follicles upwards and shadowed with a carbon-platinum coating. They were photographed in a Jeol scanning electron microscope.

Measurements of follicle length were performed under the light microscope by means of a micrometer eyepiece. Approximately 1000 follicles were measured in each tail, and the mean value for five animals was considered for each irradiation

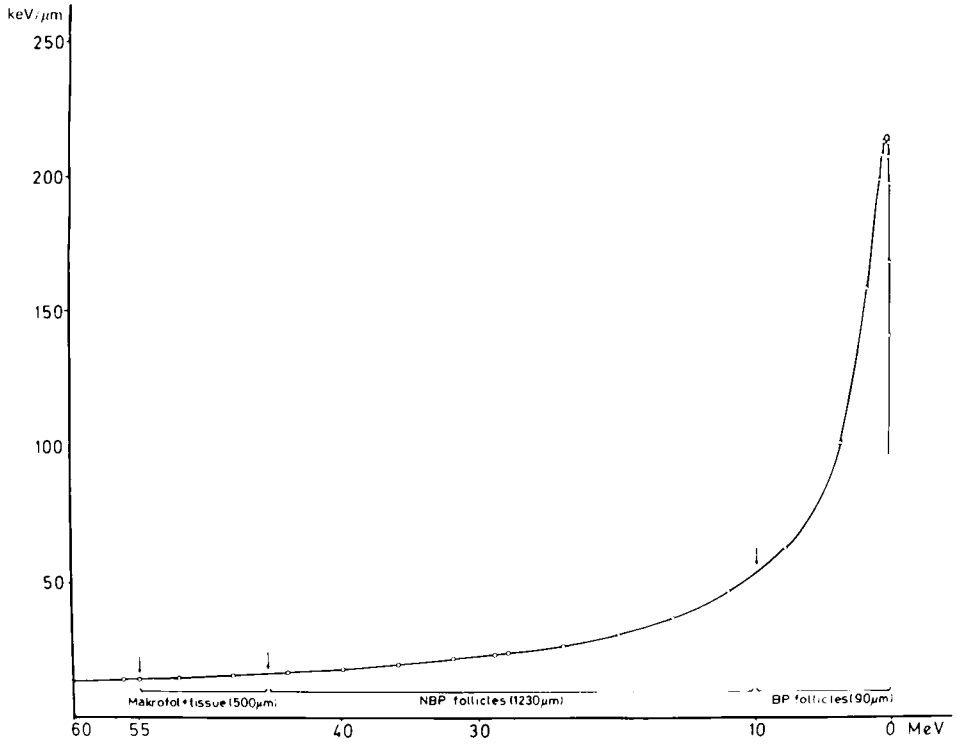

Figure 1. Bragg curve for 55 MeV helium ions (l.e.t. vs particle energy). Positions of hair follicle matrices along the alpha particle curve are shown. Arrows indicate the boundaries of the different l.e.t. regions. *Macrofol+tissue* Makrofol E detector plus epidermal and dermal tissues preceding hair follicle matrices. *NBP follicles*: hair follicles reached by particles in the non-Bragg-peak region. *BP follicles*: hair follicles reached by particles in the Bragg-peak region.

condition whenever possible. Five hundred hair follicles from the tail of each animal were measured and average values were obtained for each zone. Standard deviations of the mean values of length were calculated for non-irradiated and also for BP-irradiated follicles in each animal, and these were found to be negligible. However, NBP follicles always showed values of length that changed near the Bragg-peak zone, indicating l.e.t. spectrum variations with penetration depth. A mean of these values was assumed to represent follicles irradiated in the non-Bragg-peak region of ionization.

3. Results

The effects produced by helium ions as they travel through the rat tail can be clearly analysed by evaluating the alterations produced in the population of hair follicles that grow beneath the epidermis. In figure 2 a schematic cross-section of a rat tail irradiated with a helium ion beam entering at the ventral side is shown. Helium ions follow a characteristic Bragg type l.e.t. distribution along their penetration path through the tissues (figure 1), whereby the tail, behaving as a variable thickness beam energy degrador, is affected at precise sites by the energy of the Bragg-peak region of ionization (from 55 to 0 keV μm). These sites are

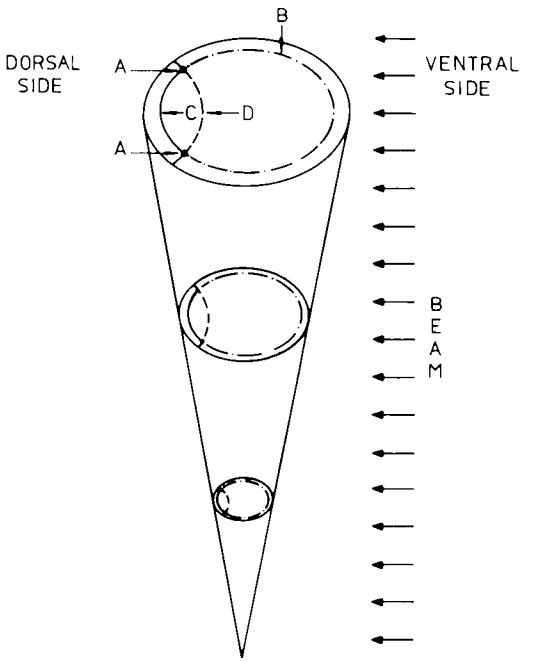

Figure 2. Schematic cross-section of a tail. Helium ion beam penetrates through the ventral side. The increasing ionization produced as it passes through the tissues determines alterations of the structures to a different extent: (A) Sites in which the Bragg-peak energy affects hair follicles; (B) region in which hair follicles are affected by the non-Bragg-peak energies; (C) zone of non-irradiated hair follicles lying beyond the alpha particle range; and (D) region affected by the Bragg-peak energy, always at the same distance from the beam entrance. At the end of the tail no Bragg-peak effect occurs.

represented as BP follicles in figure 1 and as line D in figure 2. Bragg-peak-affected follicles (BP follicles) are represented as points A in figure 2. Those follicles reached by the particles in the non-Bragg-peak region of ionization (from 46 to 10 MeV particles) are represented by NBP follicles in figure 1 and by line B in figure 2. They are affected by variable l.e.t. ranging from 16 to 55 keV/μm. Non-affected follicles lying beyond the beam range are represented by line C in figure 2.

Scanning electron microscopic images of irradiated rat tails opened longitudinally are shown in figures 3 and 4. These tails received respectively 20 and 30 Gy in the NBP region of ionization and 120 and 175 Gy in the BP region. Three distinct areas of variable damage to hair follicles are direct evidence of heavy particle action. Follicles are damaged to a different extent depending on the energy transferred by the beam along the particle trajectory (A and B in figures 3 and 4). Morphological changes in the follicles are well demonstrated from 5 to 10 days after irradiation and for a given dose they depend on the particular region of the Bragg curve which affects them: NBP follicles (B) are thin and tortuous, and the bulbs appear atrophic, whereas BP follicles (A) are atrophic and much shorter than either those described in the NBP region or the non-irradiated, morphologically intact follicles from the central area (C). The strking alterations in the follicles exposed to the Bragg-peak delineate two narrow fringes along the tail which run parallel to the borders of the cut

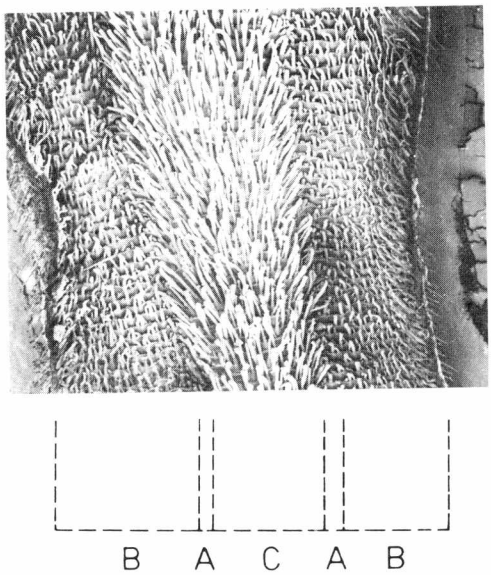

Figure 3. SEM micrograph showing damage inflicted to hair follicles of the rat tail when it behaves as a variable thickness energy degrador of the beam. The tail was longitudinally opened along the ventral side (opposite to the beam entrance side) and the skin was flattened to show in one plane the whole population of hair follicles. Three zones are observed after 20 Gy in the NBP region and 120 Gy in the BP region: (A) A narrow band consisting of markedly affected follicles reached by the Bragg-peak (A in figure 2); (B) area half divided which contains thinner and altered follicles irradiated with helium ions outside the Bragg-peak area (B in figure 2); and (C) central portion covered with normal, non-affected follicles lying beyond the beam range (C in figure 2). ×15.

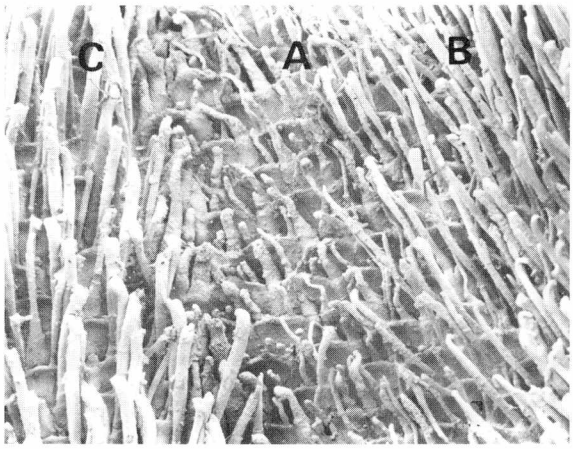

Figure 4. Higher magnification of an epidermal sample receiving 30 Gy in the NBP region and 175 Gy in the Bragg-peak region. Note at the centre the deeply altered follicles irradiated with the Bragg-peak (A) flanked by thinner follicles affected by the other region (B), and the non-affected normal follicles (C). ×45.

edges of the tail and which converge at a point near the end of the tail. Beyond this point all the follicles are irradiated by particles in the NBP region (figure 5).

A plot of follicle damage vs dose at 7 days after irradiation is shown in figure 6. The length of the follicles was measured in the NBP and in the BP areas and also in the non-irradiated region of the sample for each condition. The ratio between irradiated and non-irradiated follicle length was thus obtained. The length of irradiated hair follicles given as a percentage of the corresponding control values was used as a quantitative expression of follicle damage, and the results are shown in figure 6. The curves obtained show an increased effectiveness in producing hair follicle damage for the BP compared to the NBP particles, particularly in the 5 to

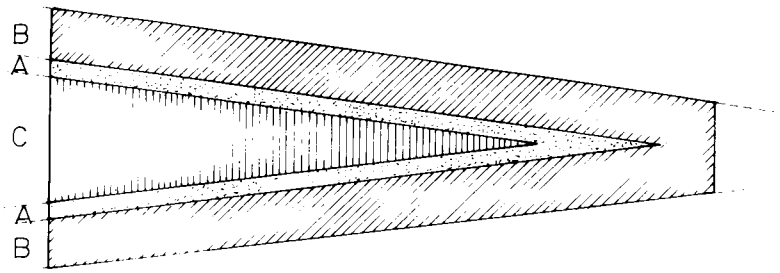

Figure 5. Schematic representation of the SEM observations corresponding to the areas covered with follicles of different length produced by helium ion irradiation. (A) Bragg-peak irradiated area; (B) non-Bragg-peak irradiated area; (C) non-affected area.

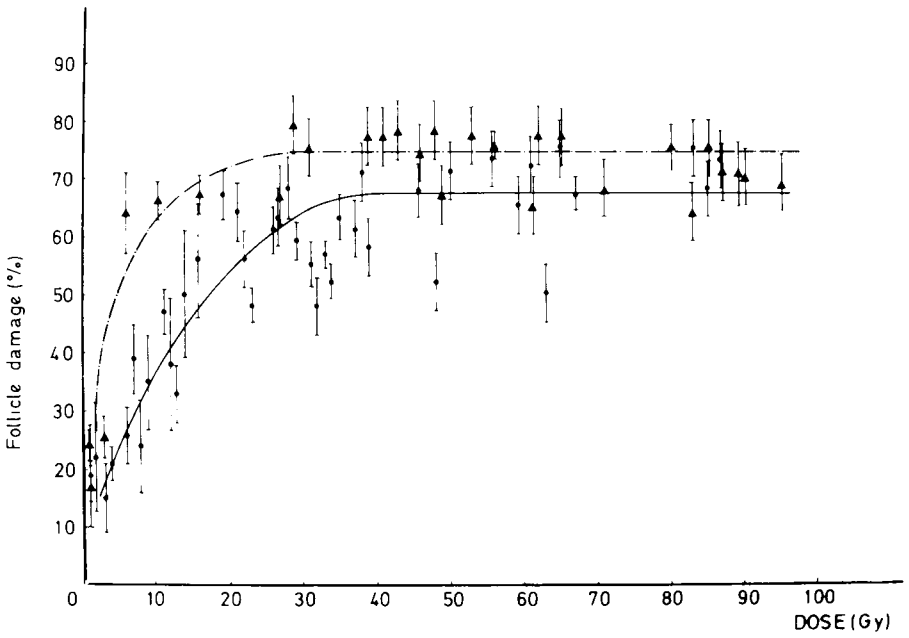

Figure 6. Follicle damage as a function of dose. Circles: values from follicles affected by the NBP region of the ionization curve. Triangles: values from follicles affected by the BP region of the ionization curve.

40 Gy dose-range. When doses higher than 40 Gy were delivered, follicle length no longer varied and the follicles appeared as minute atrophic remnants irrespective of the ionization region.

4. Discussion

The rat tail with its conical shape is an appropriate model to study *in vivo* the effects of the energy degradation of particle beams. We have selected the effects of helium ions on the hair follicles as a suitable way to analyse in the same sample biological responses with depth of penetration of these particles.

Hair follicles underwent significant changes in length and in surface architecture after irradiation (Klein Szanto and de Rey 1978). These changes are a consequence of histopathological alterations following irradiation, and are exponentially dose-dependent during the anagen phase of the hair cycle of the rat coat, i.e. from birth until the 17th day after birth (de Rey *et al.* 1986). At later times the results are markedly influenced by the follicle size variability observed at the end of the anagen phase of the cycle.

The model system used in this work allows the comparison of biological responses after heavy particle irradiation using the same sample (the tail of each rat), to evaluate radiation effects in the BP and in the NBP areas of ionization. Follicle growth was taken as a representative end point where the response was shown to vary with the dose and with the ionization region of the Bragg curve. Growth delay was increased in the BP area after doses ranging from 5 to 40 Gy. In the NBP area a marked follicle alteration was also observed but to a lesser degree. This differential effect may be attributed to the higher l.e.t. radiation in the BP region of ionization. After doses exceeding 40 Gy, hair follicle damage was no longer dose-dependent probably due to ionization saturation processes.

Thus, dose response curves displayed two portions. In the low-dose portion a dose-dependent effect was observed. This response is in agreement with previous results obtained in the same biological system after irradiation with X-rays (de Rey *et al.* 1986), and also with those reported by Leith *et al.* (1983) after skin irradiation with helium ions. After higher doses the effects were not dose dependent and complete follicle atrophy was observed. However, saturation at 100 per cent damage (i.e. size tending to zero) was never reached because non-viable remnants of follicular structures always remained visible. Comparable results were obtained after X-irradiations with doses exceeding 30 Gy (de Rey *et al.* 1986).

The dosimetry in this type of study has certain difficulties because the dose for each point in the sample is required, and there are inherent inhomogeneities in the beam. The use of SSNTD allows the complete analysis of the alpha beam in a plane perpendicular to the beam incidence axis. The dose for each point in the sample was calculated based on: beam flux distribution, energy loss as a function of ion penetration depth in tissue and the energy degradation of the beam due to the conical configuration of the tail. However, the evaluation of follicles injury as a function of the dose and l.e.t. for each point of the beam trajectory in tissue needs particular attention and methods are being developed that will permit the point to point analysis of the response along the beam trajectory.

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