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Effects of X-irradiation on rat hair follicles

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The alterations of normal growth of hair follicles following irradiation of rat tails with X-rays have been analysed. The changes in follicle length are attributed to a series of deleterious events occurring in the hair matrix after irradiation. A dose-dependent reduction of length was demonstrated for a given post-irradiation interval. An approximate exponential response was obtained for all conditions excepting low doses for which a marked reduction of follicle length was observed a short time after irradiation and was ascribed to the depletion of highly radiosensitive cells. At later post-irradiation times this response was obscured by the resumption of follicle growth.

Indexing terms: hair follicles, dose response curve, hair cycle.

1. Introduction

Hair follicles of experimental animals offer an *in vivo* system of highly radioresponsive appendages in which radiation effects can be analysed. The alterations induced by radiation in the hair follicles are not influenced significantly by manipulations or environmental disturbances as they develop in their natural milieu.

Several reports have dealt with the study of hair follicles irradiated using various sources. They all revealed high radiosensitivity of the hair matrix depending on the hair cycle phase (Geary 1952), characterized by a marked reduction in uptake of labelled DNA precursors detected approximately 30 min after irradiation, followed by a resumption of DNA synthesis within 12 h after irradiation (Cattaneo *et al.* 1960). Concomitant alterations in protein synthesis impaired cellular functions and indirectly the reproduction of cells in anagen hair (Malkinson and Griem 1968). As a result, surface and architectural alterations as well as important modifications of hair follicle size can be observed. Changes in follicle length may be attributed to a series of deleterious events occurring in the hair matrix after irradiation leading to structural alterations. Thus a clear-cut connection between radiation damage and follicular length is suggested (Klein Szanto and de Rey 1978).

The aim of the present work was to characterize the effects of single X-ray exposures on the growth of hair follicles of the rat in order to obtain data that may permit future comparison of the effects of radiations of different qualities.

2. Materials and methods

2.1. *Animals and irradiation procedures*

Five-day-old Wistar rats were used. The animals' tails were irradiated after adequately shielding the remaining parts of the body. Irradiations and sacrifices

were performed at 10 a.m. Single X-ray doses: 0·5, 1, 2·5, 10, 12, 15, 20 and 60 Gy were delivered by means of a Philips X-ray machine used for conventional radiotherapy at 250 kV, 13 mA, 22 mm Cu and 1 mm Al filters, giving a dose-rate of 5 Gy/min. Animals were killed by cervical dislocation serially each day from 24 h to 12 days after irradiation (the 17th day of age). Some evaluations were also performed at shorter post-irradiation times.

2.2. *Removal of hair follicle samples*

The skin on the tails from irradiated, non-irradiated and sham-irradiated animals was cut open longitudinally and the skin and the underlying tissues separated from the central cartilage axis by gentle manipulation. The skin was incubated in a 2 N NaBr solution for 10 min at 37°C to detach the epidermis from the dermis. After brief fixation in formaldehyde solution, the whole epidermis of the tail was stained with 1 per cent toluidine blue solution. The epidermis was spread with the stratum corneum against a glass slide in such a way that the hair follicles could clearly be observed with the light microscope. In order to avoid parallax errors a slight pressure was exerted on the tissue with a coverslip.

2.3. *Measurements of hair follicle length*

Butcher (1934) described the growth of the hair of the albino rat beyond the age of 5 days. He found that growth was completed at about the 17th day of age at the onset of the resting period which lasts until the 31st or 32nd day of life. With the aid of a graduated eyepiece in the light microscope, daily measurements of the hair follicle size of animals from different litters were performed. A steady lengthening of the follicle, reaching a plateau approximately 12 days after birth, was recorded at a growth rate of approximately 40 μm a day. However, follicle size varied significantly from one set of litter mates to another. In order to overcome this difficulty, animals from each litter, which usually show negligible interanimal variations, were irradiated and sequential daily post-irradiation evaluations were performed. Average values from four animals were used to evaluate the response to each irradiation condition and for each post-irradiation time. Values from five litters (16–20 animals in total) were averaged to obtain the final data.

The ratio of irradiated to non-irradiated follicle length (R) was taken as an expression of radiation damage.

$$R = L_i / L_0$$

where L_i is the average length of irradiated follicles, and L_0 is the average length of control follicles (pooled from non-irradiated and sham-irradiated animals).

A two-way analysis of variance was performed with the aid of a Hewlett Packard Calculator 9810.

2.4. *Dose–response curves*

As the phenomenon described here is characterized by a dose–response curve in which the shoulder region presents a negative slope an adequate general equation was used:

$$L_i / L_0 = \exp(-\alpha D_F)(D)$$

We have tested several functions and formulations (Elkind 1960, Dienes 1966, Gilbert 1975, Hendry 1979) in order to select the one that best fits the experimental

data and thus describes the phenomenon. Haynes' model (Haynes 1966, Burch and Chester 1981) proved to be the most appropriate.

$$I_i/I_0 = [\exp(-(a+k)D)][\exp(a/b(1-\exp(-bD)))]$$

This formula is composed of two functions that may be related to radiation responses *in vivo*, the parts being:

$\exp(-(a+k)D)$, related to the damage inflicted by radiation.

and

$\exp(a/b(1-\exp(-bD)))$, related to repair mechanisms.

where a is the coefficient related to potentially repairable damage, k is the coefficient related to non-repairable damage and b is the coefficient related to repair mechanisms.

Thus these coefficients characterize the response of this system to radiation. This model was fitted using Gilbert's algorithm (Gilbert 1969) by means of a computer in a Vax 11 from the Comisión Nacional de Energía Atómica.

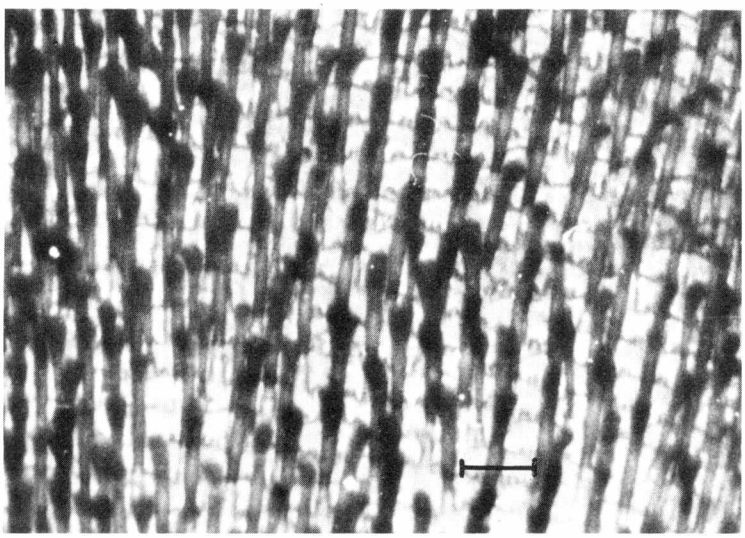
3. Results

Quantitative evaluations of hair follicle length of non-irradiated rats revealed a normal growth rate of the anagen hair follicle in the first hair cycle of the rat coat of approximately 40 μm per day. Radiation-induced alterations in this growth rate resulted in shortened hair follicles in a dose-dependent fashion. Irradiations with single doses of X-rays (0·5, 1, 2·5, 10, 12, 15, 20 and 60 Gy) showed a dose-dependent progressive reduction of growth easily recorded by averaging the size of the population of hair follicles going through the first hair cycle (figure 1).

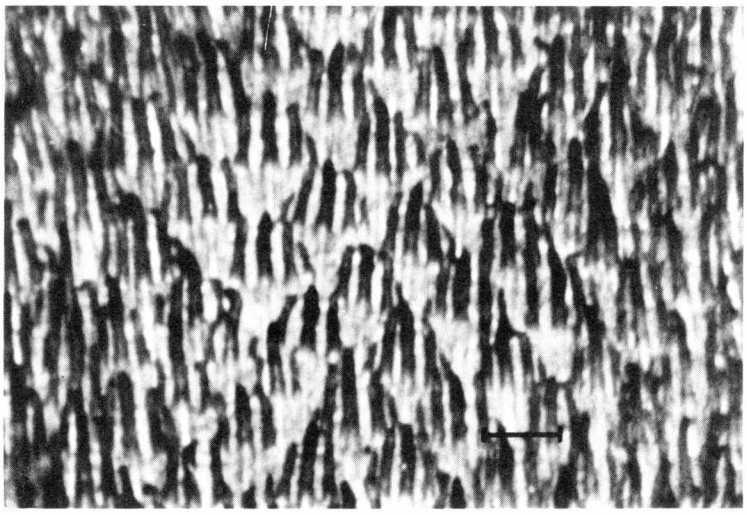
Figure 2 shows this response expressed as a fraction of the length of the non-irradiated follicles measured daily after irradiation. Effects were seen at 24 h after irradiation and even at shorter post-irradiation times not shown in the figure. However, at this time this effect is not clearly dose-dependent and the response to increasing radiation doses differ only slightly. As from the third day post-irradiation, the curves for 0·5, 1, 2·5 and 5 Gy clearly reveal the reduction in size of follicles in a dose-dependent fashion. As this effect is temporary, the re-establishment of growth is soon observed and, for lower doses (0·5 Gy), recovery of normal follicle size is demonstrated.

Doses larger than 10 Gy promptly halted hair follicle growth with no signs of recovery. They appeared shorter, normal development became impaired and a gradual dose-dependent drastic shortening of follicles was observed. In the region beyond 20 Gy only small remnants of follicular structures remained visible. These structures were considered as non-viable and their length was assumed to later tend to zero.

By plotting follicle damage (follicle-length ratio R between irradiated and non-irradiated follicles) versus dose at different post-irradiation days, dose–response curves were obtained from the 3rd day onwards. Figure 3 shows dose–response curves corresponding to the measurements performed 3, 5 and 7 days after irradiation. All the curves display similar patterns: they show an exponential response for all conditions but, after low doses (1–5 Gy), they reveal an important length reduction which confers a particular pattern to the dose–response curve. At



(*a*)



(*b*)

Figure 1. Hair follicles from a rat tail following detachment of dermis from epidermis: (*a*) non-irradiated hair follicles (scale bar: 300 μm); (*b*) hair follicles exposed to 15 Gy X-rays (scale bar: 200 μm).

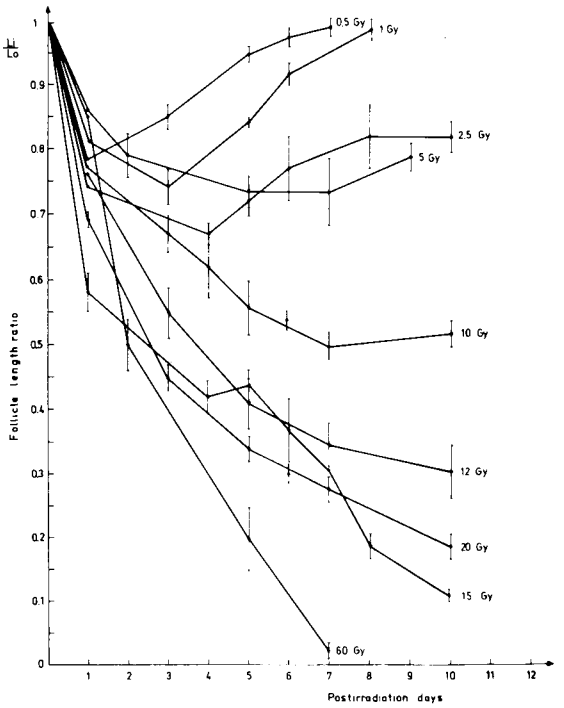


Figure 2. Radiation effect on hair follicle growth analysed during the first hair cycle of the albino rat. The ratio of irradiated to non-irradiated hair follicle length was plotted as a function of the time elapsed after exposure to varying doses of X-rays. Bars indicate standard errors.

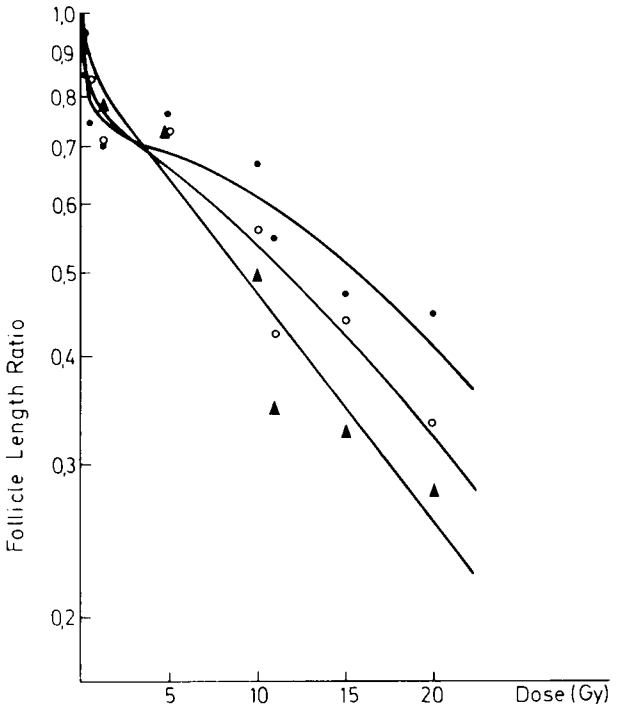


Figure 3. Dose-response curve on a semilogarithmic scale. Data points are taken directly from figure 2. Interpolation of the lines drawn in figure 2 are also used. Data from animals sacrificed 3 (●), 5 (○) and 7 (▲) days after irradiation.

longer post-irradiation times (up to the 12th day, because measurements at later times are markedly influenced by the end of the anagen portion of the hair cycle) this responsiveness is modified by the resumption of follicle growth (not shown in figure 3).

Haynes' model proved to fit these experimental data adequately. From this, values for the parameters in the equation were obtained and are shown in the table. A two-way analysis of variance showed a significant change in follicular length due to radiation exposure. Probability ($F \geqslant 11{\cdot}34$) is smaller than 0·01. No significant differences were obtained as post-irradiation times varied.

Survival parameters of follicles.

L_i/L_0	Parameter	Post-irradiation time	
		5 days	6 days
$[\exp(-(a+k)D][\exp(a/b(1-\exp(-bD)))]$	a^{-1}	52 Gy	23 Gy
	b^{-1}	3 Gy	2 Gy
	k^{-1}	37 Gy	44 Gy

4. Discussion

We have presented a new assay to analyse the response to ionizing radiation *in vivo*. This model was chosen for the practical advantage it affords of analysing, by means of rather simple techniques, a large number of appendages that are composed of several subpopulations of cells of varying radiosensitivity. The well-known deleterious effects induced by X-rays on rat hair follicles alter the continuous reproduction of matrix cells during the anagen period of the hair cycle (Geary 1952, Cattaneo *et al.* 1969, Dienes 1966). The marked reduction in hair follicle length referred to in this work can be associated with a reduction in tissue mass as a consequence of differentiation and maturation of cells without normal cell replacement from the highly radiosensitive stem cell compartment (Potten 1981). The effects described may be progressive and irreversible leading to follicular atrophy when no recovery and repopulation is achieved (after doses larger than 12 Gy). As Potten (1983) reported, the cells progressively exhausted their division potential and the cellular mass diminishes as is revealed by the presence of atrophic remnants of hair follicles receiving doses exceeding 15 Gy. However, a steady recovery of follicle function takes place after doses lower than 10 Gy. It is of interest to note that a marked reduction of follicle length was measured a few hours after irradiation whatever the dose received, in agreement with data revealing a drastic decrease in the mitotic index of matrix cells 2–12 h after irradiation (Cattaneo *et al.* 1960, and unpublished own data). Nevertheless, radiation-induced impairment of hair bulb nourishment due to alterations in the vascular supply should also be considered but only for doses larger than 10 Gy and more than five days after irradiation (Schwint *et al.* 1984).

An interesting result yielded by this study was that all follicles in an irradiated area were similarly affected in such a way that small deviations from mean values were observed. This is in contrast with results from other authors who plucked the skin as a way to stimulate resting follicles to start a new cycle. They found that affected and non-affected follicles coexisted in the experimental area after irradiation. However, the uniformity of data recorded in the present investigation is

relevant and can be attributed to the homogeneous and synchronous population of hair follicles in the first hair cycle of the rat coat (from birth until the 17th day after birth; Butcher 1934). In this way the same hair follicle stage was guaranteed and as a consequence an equal response to radiation was expected. Hair follicle growth is the result of proliferation and differentiation of matrix cells, so alteration in hair follicle growth after irradiation could be caused by impairment of the normal contribution of matrix cells to the pool of differentiating cells.

Dose–response curves exhibited roughly an exponential shape. However, values for low doses although somewhat dispersed, could indicate a particular response of hair follicles to low radiation. The marked length reduction of about 30 per cent for some conditions could be ascribed to the depletion of a highly radiosensitive group of cells that are deeply affected even by low doses. These results would suggest that two components representing two cell populations, characterized by different responses to increasing doses of ionizing radiation, may exist and that they may represent follicle clonogenic cells (Potten 1983). Their location and morphological features have not yet been established. Other authors (Malkinson and Griem 1968) reported results in connection with these findings, i.e. a reduction of as much as 50 per cent in the incorporation of tritiated thymidine into DNA of matrix cells with doses much lower (2 Gy) than those needed to affect the remaining 50 per cent, coupled with the appearance of many dead cells. Al-Barwari and Potten (1979) considered the existence of two types of epidermal basal cells: a clonogenic stem cell class and a majority class capable of proliferation but no clonogenicity and relatively unaffected by X-rays. Further experimental work is required to analyse in detail the response of hair follicles to very low doses of X-rays.

Concerning dose–response curves, Haynes' formula was used to fit experimental data and a, b and k coefficients were calculated. The coefficients of k (Gy) were approximately the same at 5 or 6 days after irradiation. This result is in agreement with the expected findings taking into account that k is related to non-repairable damage. The inter-relationship between a and b calculated for 6 days after irradiation is almost half that calculated for 5 days after irradiation. This could be interpreted as a protracted action of repair mechanisms. Regarding the b coefficient related to repair mechanisms, the extremely low values calculated for the reciprocal would indicate the high radiosensitivity of the repair mechanism.

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