SOME STUDIES ON THE X-RAY EFFECTS ON RESTING HAIR CELL POPULATION

by

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DISCLAIMER

In the planning of treatment for patients with malignant disease, one of the problems the radiotherapist would like to solve concerns the responsiveness of both proliferating and non-proliferating cell populations to ionizing radiation. We have found that the hair matrix cells, which keratinize to form the hair shafts, provide a most useful test system because of the wide variations in their metabolism from states of complete proliferative inactivity (the resting or telogen hair) to states of extremely high mitotic activity (growing or anagen hair). Later in this program another paper by Fry and co-workers will present some aspects of this proliferative activity in greater detail.

We would like to discuss some of our work dealing with the resting phase of the hair cycle and its response to ionizing radiation as measured by several different techniques.

The first series of studies consisted of irradiating animals in the telogen phase of the hair cycle, following which radiation damage was measured by impaired incorporation of tritium-labelled serine into keratin (1). In all experiments, a two centimeter area on the flank of the mouse was irradiated with 45 KV x-rays with two millimeters aluminum filtration, at a focal skin distance of 11.5 centimeters. The exposure dose rate was 400 rads per minute. The technique produced a tissue penetration of 1.2 centimeters half value depth. In these experiments, the unirradiated contralateral flank served as the control site for each animal.

Mice, CF #1 female, were prepared by plucking hairs to induce anagen. They were then observed during anagen for uniformity of regrowth and allowed to continue into telogen 18 days later. On the 21st day,

graded doses of radiation were given to the right flanks of separate groups of mice. Zero to 12 days after irradiation, hairs were again plucked to induce a growing coat, and tritiated serine was injected intraperitoneally four days after plucking. When the newly growing hairs had entered the telogen phase of the cycle, samples were plucked from control and irradiated sites. The hairs were weighed and combusted in oxygen using the Schöniger technique. The tritiated water from combustion samples was then assayed for radioactivity in a liquid scintillation spectrometer. Control and irradiated samples were compared for tritium incorporation on a weight basis. The resulting data (Table I) revealed decreasing incorporation of tritiated serine with increasing doses of radiation during telogen. There appeared to be no repair of radiation injury from six hours to twelve days after irradiation, as measured by tritiated serine incorporation into newly growing hairs four days after plucking (Table II). Further studies with this hair indicator system showed that anagen hairs were more sensitive to irradiation than telogen hairs (1).

The second series of experiments on the resting hair population is an analysis of the hair density following graded doses of x-ray radiation. The animals were plucked and irradiated 22 days later when the follicles were in telogen. Three weeks afterwards, during the same telogen period, the animals were shaved on both control and irradiated sides and photographed on a Zeiss photomicroscope using ~~bright~~ dark field epi-illumination. After the initial set of photographs was taken, the mice were observed for anagen regrowth. When this first regrowth period was completed and the follicles were again in telogen (six to

nine weeks after irradiation) the animals were shaved and rephotographed.

Figure I shows a dose response curve generated by computer in a non-linear regression analysis for the expression $S = 1 - (1 - e^{-D/D_o})^n$. The parameters n and D_o were derived from this analysis and the curves which best fit these parameters were then drawn. The first count remained at 100% (survival equals 1) and showed no telogen hair loss for the first three weeks immediately following irradiation. The new cycle that began four to five weeks after irradiation may have been spontaneous or partially induced by shaving. Hair survival after this first post-irradiation growth cycle is shown in Figure I. A steady slope for the constructed curve cannot be obtained by our technique because the skin begins to ulcerate at doses above 2200 rads. Therefore, only the shoulder of the survival curve has been determined experimentally; computer analysis was necessary to evaluate the available data and project the parameters n and D_o.

Figure II presents a computer-analyzed dose response curve utilizing pooled data from several experiments to show the reaction of the 14 day anagen coat to graded doses of x-ray irradiation. By comparing this figure to the previous one, one can conclude that the telogen follicle is less sensitive to low kilovoltage x-rays than the anagen follicle.

In a third series of experiments, mouse telogen hairs were irradiated with doses of 1500 to 2500 rads, and the lengths of later generations of awl-type hairs were carefully measured (2). Three to ten months later, hairs from irradiated sites were 27% to 33% shorter than those plucked from control areas. Fifteen months after irradiation, several mice were given tritiated thymidine and biopsies from irradiated and control

sites were studied. Per cent labelled mitosis curves revealed no change in the cell generation cycle of 11 1/2 to 12 hours. On the other hand, there was a decrease in the mitotic index from 1.5 in the control sites to 0.7 in the irradiated areas.

From our studies of telogen or resting hairs, we can draw several conclusions concerning this non-proliferating cell population:

1. No recovery in the reduced uptake of tritiated serine was demonstrable in telogen hairs up to 12 days post-irradiation, indicating a lack of repair in the resting cell population.
2. Data on survival of hair, post-irradiation, revealed significantly lowered radiosensitivity in telogen, as compared to anagen hair populations. This was reflected in a broader shoulder and a higher D_q in the telogen survival curve.
3. Observations of the body coat made almost one year after irradiation in the telogen with 2000 to 2500 rads revealed persistently reduced hair lengths and slower hair regrowth after plucking. Per cent labelled mitosis curves showed no change in proliferative cell cycle times, but mitotic index determinations suggested permanent reductions in hair matrix cell populations.
4. We feel that the above observations may have important implications for radiation responses of non-proliferating cell populations in radiotherapeutic situations.

Our x-ray studies on non-proliferating telogen hair in mice have shown: (a) no recovery during the non-proliferating period, (b) reduced radiosensitivity with a broader shoulder on the survival curve in the resting phase, and (c) late changes with retarded regrowth and reduced hair length in telogen.

ACKNOWLEDGMENT

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REFERENCES

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TABLE I

Reduced Levels of ^{3}H-DL-Serine in Subsequent "Generation" of Mouse Hair Following Irradiation in Telogen

Dose (rads)	Animals	Intervals between irradiation and plucking	DPM/mg C (control)	DPM/mg X (Radiated)	X/C (%)	Standard error of the mean
500	8	Nil	236	192	86	7.16
1000	9	Nil	246	130	57	5.75
1500	6	Nil	268	83	39	7.07

TABLE II

Reduced Levels of ^{3}H-DL-Serine in Subsequent "Generation" of Mouse Hair Following Irradiation with 1000 Rads in Telogen

Animals	Interval between irradiation and plucking	DPM/mg C (control side)	DPM/mg X (radiated)	X/C (%)	Standard error of Mean
20	6 hours	209	149	71	3.80
20	12 hours	154	116	75	3.80
17	24 hours	76	50	66	2.33
8	4 days	117	89	76	7.16
13	8 days	286	186	65	3.46
19	12 days	239	160	66	4.01

FIGURE CAPTIONS

Figure I

Dose-Response Curve Showing Hair Density in the Second Telogen Phase Following Irradiation in Telogen Phase.

Computer derived parameters: D_o - 492 ± 54 rads

n - 34 ± 14

D_q - 1741 ± 371 rads

Figure II

Dose-Response Curve Showing Hair Density in the First Telogen Phase Following Irradiation in Anagen.

Computer derived parameters: D_o - 174 ± 14 rads
for steep slope

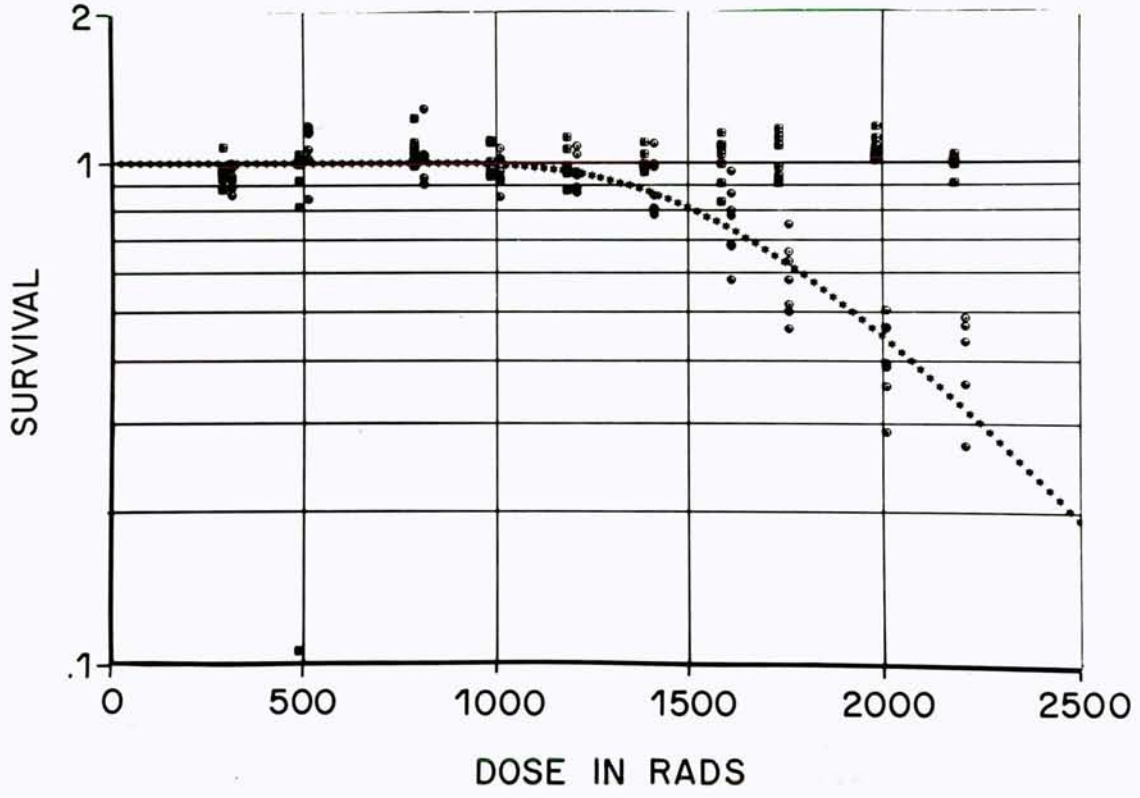
n - 20 ± 5

D_q - 516 ± 84 rads

An attempt was made to search for a 2nd population of cells:
for shallow slope

n - .6 ± 1.3

D_o - 324 ± 147 rads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1
.1
SURVIVAL
0
500
1000
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2000
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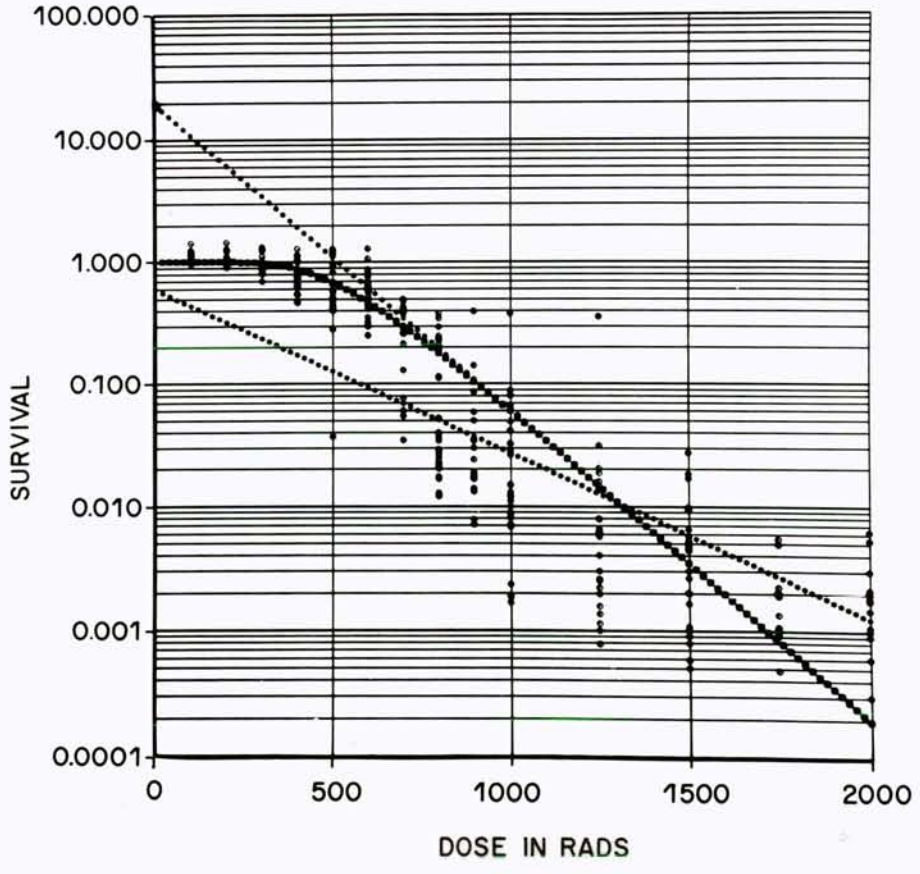
DOSE IN RADS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