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"Dopa Oxidase" in Melanocytes of X-Irradiated Quiescent (*Telogen*) Hair Follicles. (24488)

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Melanocytes within growing hair follicles of pigmented mice contain melanin granules and active "dopa oxidase." In the presence of dihydroxyphenylalanine (dopa) *in vitro*, they form abundant dopa-melanin(1). On the other hand, melanocytes of quiescent (telogen) hair follicles in pigmented mice generally are unpigmented and are dopa negative (1). Thus, it has not been possible to identify melanocytes with certainty at resting stage of hair growth cycle. Although X-radiation has been shown, under certain conditions, to stimulate melanogenic activity within latent melanocytes of the epidermis, there is no report of a similar effect on latent melanocytes within quiescent hair follicles. Previous studies have shown that repeated partial-body exposure of mice to 200 r week of X-radiation is particularly effective in eliciting epidermal hyperpigmentation(2). Utilizing similar technics in the present study, mice with quiescent hair follicles were exposed weekly to X-radiation. The purpose was 2-fold; first, to determine whether "dopa oxidase" activity could be demonstrated in latent follicular melanocytes following X-irradiation; second, to characterize more fully the distribution of melanocytes within telogen hair follicles.

Materials and methods. Eight C3H mice (4 males, 4 females), 4 to 8 months of age, were shielded with lead, except for small rectangular area (1.5 x 2.0 cm) on the dorsum, and exposed to 200 r of X-rays once weekly for 3 or 4 weeks. The radiation factors were: 100 kv, 5 ma, 0.25-mm Al, HVL-0.55 mm Al, target distance, 15 cm, and dose rate, 580 r minute. The design of the lead shield ensured irradiation of essentially the same area of skin each week. At start of irradiation, hair follicles of all mice were quiescent (telogen stage) as indicated by light pink color and thinness of skin(3,4). Subsequently, gross examinations of skin of each mouse were made at frequent intervals to detect alterations in hair growth status. The mice were killed by cervical dislocation 24 hours after last exposure to radiation. Specimens of skin were removed from both irradiated and non-irradiated regions of the dorsum and tested for "dopa oxidase" activity according to the method of Becker(5). The specimens were then dehydrated in dioxane and imbedded in paraffin; sections were cut at 8 μ and stained with Mayer's alcoholic carmine. Skin samples from 4 non-irradiated C3H mice with quiescent hair were tested for "dopa oxidase" activity in a similar manner.

Results. Due to lack of pigmentation, melanocytes could not be identified in quiescent hair follicles of non-irradiated mice, although pigmentary debris was frequently observed. "Dopa oxidase" activity was also absent in melanocytes of non-irradiated telogen

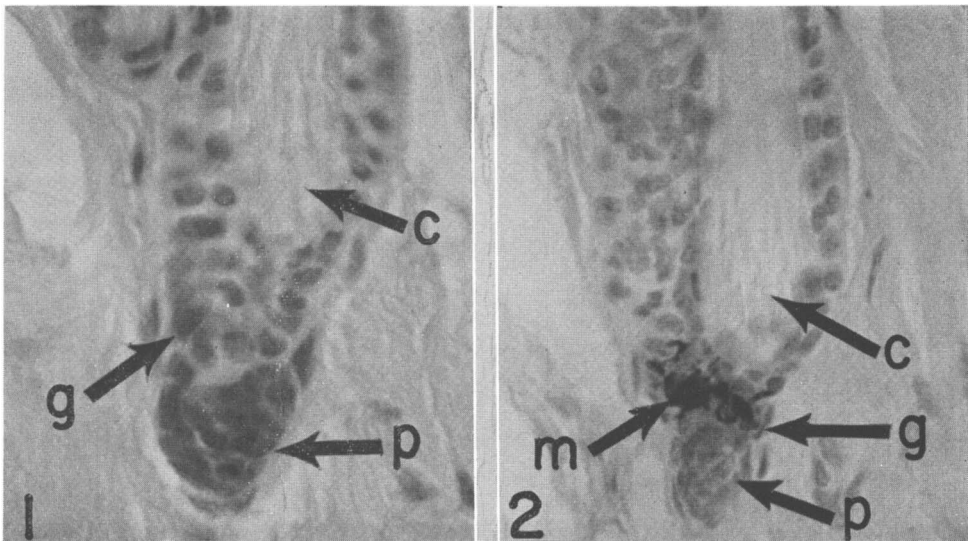

FIG. 1. Non-irradiated telogen hair follicle following treatment with dopa reagent. Note absence of dopa reactive cells. ×450.

FIG. 2. X-irradiated telogen hair follicle following incubation in dopa reagent. Two dopa reactive dendritic melanocytes are evident in the epithelial germ region. ×450.

Key: c = hair club; g = epithelial germ; p = dermal papilla; m = melanocyte.

hair follicles; no specific cellular darkening was observed following treatment with dopa reagent (Fig. 1). In 5 of 8 irradiated mice the hair follicles in exposed areas of skin remained quiescent throughout 3 or 4 week period of irradiation. There was no indication of epilation to gross inspection. In cases where growing hair was observed in the irradiated regions it was apparently associated with normal waves of hair growth rather than elicited by the radiation process. In contrast to non-irradiated quiescent hair follicles, telogen hair follicles in exposed areas of all 5 irradiated mice contained dopa reactive dendritic cells in region of hair germ, indicative of the presence of "dopa oxidase" (Fig. 2). The dendritic processes of intensely pigmented melanocytes were insinuated between non-reactive epithelial cells of the germ region. No dopa reactive cells were observed in dermal papilla (Fig. 2). Hair follicles from shielded regions of irradiated mice contained no dopa reactive cells, with the exception of a few hair follicles close to the border of the shielded areas where slight overlapping may have occurred during the repeated exposure to radiation.

Discussion. It has been reported previously that "dopa oxidase" activity is absent in melanocytes of telogen hair follicles(1). The results of our study confirm this observation and further demonstrate that "dopa oxidase" activity may be released by X-irradiation of quiescent hair follicles. The chemical nature of radiation-sensitive anti-melanogenic factor in telogen hair follicles of mice is as yet uncharacterized. It has been shown, in other species, that sulfhydryl compounds inhibit melanogenesis in skin and that the inhibitory effect is lost following treatment with ultraviolet rays(6). Whatever its nature, during normal hair growth cycles, the inhibitory factor must be lost shortly after new hair growth starts since follicular melanocytes then synthesize pigment granules and react positively toward dopa reagent(1).

The fate of melanocytes in telogen hair follicles of mammals is controversial, in large part due to difficulty of identifying melanocytes during this stage of hair growth activity. Thus, it has been suggested that at telogen stage melanocytes are localized in the epithelial germ regions of hair follicles in mice and rats(7,8) and in dermal papillae of humans and dogs(9,10). In our study the observation that dopa reactive dendritic cells

were restricted to the epithelial germ regions of X-irradiated telogen hair follicles in mice supports the former view. Although it is quite possible that species differences exist in this regard, radiation technics utilized in our work afford a means for critical analysis of these apparently discordant viewpoints.

Summary. "Dopa oxidase" activity was absent in melanocytes of telogen hair follicles of non-irradiated mice. Following partial-body exposure to 200 r/week of X-radiation for 3 or 4 weeks, numerous dopa reactive dendritic cells were observed in the epithelial germ regions of irradiated quiescent hair follicles. Cells of the dermal papillae in irradiated and non-irradiated telogen hair follicles failed to exhibit "dopa oxidase" activity. The results thus support the view that melanocytes normally are restricted to the epithelial germ regions of hair follicles during telogen stage of hair growth cycle.

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Rabbit Endometrial Response to Intravenously Administered Progesterone. (24489)

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Spectrophotometric observations concerned with intravenously administered progesterone have revealed rapid clearance of the hormone from the ether soluble fraction of rabbit and human blood serum(1,2). The results were constant whether the vehicle in which progesterone was administered was saline, ethanol, propylene glycol or a mixture of ethyl carbamide, ethyl urethane and propylene glycol.* It has been suggested that disappearance of progesterone from the ether soluble fraction of blood serum resulted from tissue storage of this substance and continuing biological activity potential. It is, however, equally possible that rapid clearance of progesterone from blood serum resulted from rapid metabolism of the hormone. These experiments were planned to establish the biologic effectiveness of intravenous progesterone. Duration and intensity of tissue response to intravenous progesterone would indicate degree of storage and rate of destruction of the hormone.

Methods. Immature female rabbits, average weight 1200 g, were injected with 100 I.U. of estrogenic substance a day for 6 days.† Twenty-four hours after last injection of estrogen, intravenous progesterone as Proluton I. V., was administered into marginal ear vein of the rabbit. Twenty-four to 168 hours following intravenous injection, the rabbits were sacrificed. Ovaries were inspected and biopsied for evidence of activity. Uteri were removed and portions prepared for microscopic study. The Corner-Allen(3) progestational proliferation response and endometrial epithelial mitotic counts were utilized as evidence of luteoid activity within the endo-

* Progesterone dissolved in ethyl carbamide, ethyl urethane and propylene glycol was generously supplied by Schering, A. G. of Berlin, Germany as PROLUTON I.V.

† The estrogenic substance was generously supplied by Parke-Davis as THEELIN.