Influence of Endocrine Glands on Radiation-Induced Hyperpigmentation in Mice.* (24251)

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When pigmented mice are exposed to gamma radiation, the skin of extremities becomes progressively darkened(1). This hyperpigmentation results from increased deposition of melanin within the epidermis by epidermal melanocytes(1,2,3). Although hormones are known to play a role in regulation of mammalian pigmentation(4,5), the extent to which radiation-induced darkening may be dependent on their action is not known. The present study was designed to test the possibility of an endocrine-radiation interaction during hyperpigmentation. The hormonal balance of mice was varied either by surgical removal of specific endocrine glands prior to irradiation or by introduction of supplementary doses of hormones into intact animals concurrent with irradiation.

Materials and methods. C57BL (black) and LAF_1 (brown) mice 3 to 6 months old of both sexes were used. All mice were exposed for the duration of life to gamma rays from a Co^{60} source(6) and received total body doses of either 97 r/day or 125 r/day (measured in air without scatter). Pigmentation changes were studied in plantar surfaces of hind feet. An arbitrary grading system was used to measure pigment increase; grades ranged from 0, for no signs of darkening, to +++, the maximum grade, characterized by intense darkening of foot pads and by darkly pigmented bands on phalanges. Development of radiation-induced hyperpigmentation was studied in 5 groups of mice (approximately equally divided as to sex and strain) treated as follows: I. 50 mice, intact, irradiated (controls). II. 33 mice, castrated 3 weeks to 3 months prior to exposure to radiation. III. 20 mice, adrenalectomized 1 to 3 days prior to irradiation and maintained on 0.9% aqueous solution of NaCl following removal of adrenal glands. IV. 54 mice, given intramuscular injections of cortisone acetate (Merck) daily (0.625 mg/day to 6.0 mg/day), beginning with first day of irradiation. V. 24 mice, given subcut. injections of ACTH (Corticotropin-gel, Wilson) daily (1 to 4 U.S.P. units/day), beginning with first day of irradiation. Two additional experiments were performed to test for other possible endocrine-radiation interactions during hyperpigmentation. Six NIH Black rats of both sexes were hypophysectomized at 21 to 30 days of age. One to 2 weeks later, the animals were exposed to 200 r of 100-kvp X-rays, rats being lead-shielded so that only the right hind feet were irradiated. Thereafter they were irradiated in the same manner once a week for 7 weeks. Four intact controls were similarly exposed. Twelve mice of both strains and sexes were castrated 3 weeks prior to irradiation and exposed on plantar surfaces of feet to UV (G. E. Sunlamp: 110-125 V, 275 W, 60 Cycle, A.C.) for 2 minutes and 15 seconds on each of 16 consecutive days. Specimens of skin were obtained from feet of representative animals of all groups. These were fixed in a modified Tellyesniczky's solution. Paraffin sections 8 to 10 μ thick were made following routine histological procedures. Sections were stained with 2% aqueous solution of alum carmine. A number of whole-mount preparations of skin, prepared by the method of Smith and Lewis(7), were also examined.

Results. In control mice exposed to 97 r/day or 125 r/day of γ-rays, regardless of strain or sex, definite hyperpigmentation (+ grade) was observed in plantar surfaces of feet by ninth day of exposure; a ++ grade was evident by eleventh day and +++ grade between 19th and 25th day. Both castration

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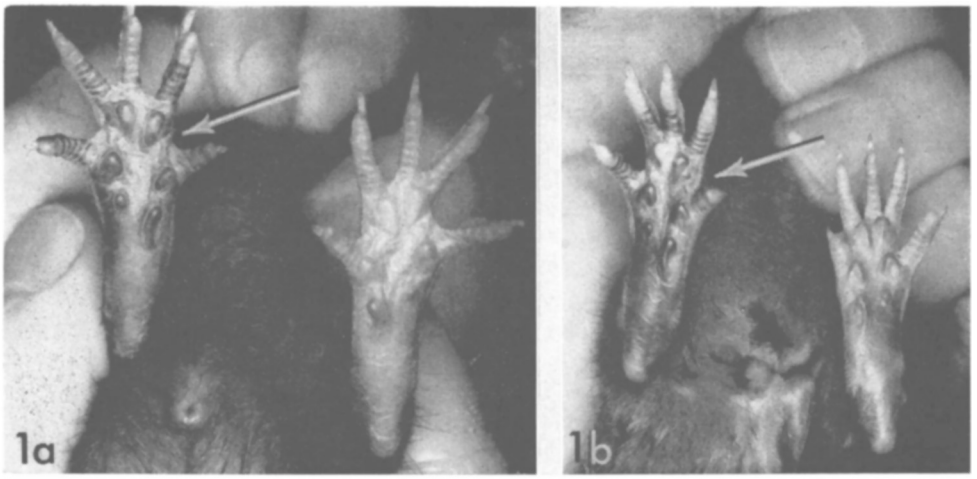

FIG. 1. Hind feet of control (a) and hypophysectomized (b) NIH Black rats after partial-body x-irradiation (200 r/wk) for 7 wk. Note intense hyperpigmentation of irradiated feet (arrows) and absence of darkening in shielded feet.

and adrenalectomy failed to modify development of radiation-induced hyperpigmentation. Similarly, irradiated mice treated with ACTH darkened at the same rate as controls. In hypophysectomzed rats, hyperpigmentation was observed in the irradiated feet by second week of exposure and increased in intensity thereafter. No obvious differences in intensity of coloration were apparent between irradiated feet of hypophysectomized and control rats (Fig. 1). The nonirradiated appendages of all animals failed to darken (Fig. 1).

In contrast, daily injections of cortisone acetate produced a striking suppression of darkening in intact, irradiated mice. In LAF_1 mice, doses of from 2.5 to 6.0 mg/day of cortisone produced the greatest inhibition of hyperpigmentation (Table I). The minimum daily dose of cortisone (0.625 mg/day) produced little to no inhibition of pigmentation. Although LAF_1 mice receiving 3 mg/day of cortisone showed no pigment increase (Table

TABLE I. Hyperpigmentation in Feet of Gamma Irradiated LAF_1 Mice Treated Daily with Cortisone.

Cortisone, mg/day	Grades of pigmentation	Duration of exposure (days) 0–8	9–10	11–15	16–18
2.5–6.0	0	100 (21)	100 (7)	100 (5)	No surv.
	+	0	0	0	
	++	0	0	0	
	+++	0	0	0	
1.25	0	100 (15)	60 (15)	0 (14)	0 (3)
	+	0	40	86	67
	++	0	0	14	33
	+++	0	0	0	0
.625	0	100 (6)	0 (6)	0 (3)	0 (2)
	+	0	100	33	0
	++	0	0	67	100
	+++	0	0	0	0
.000	0	100 (15)	0 (15)	0 (15)	0 (15)
	+	0	100	0	0
	++	0	0	100	100
	+++	0	0	0	0

Figures preceding parentheses indicate % of mice examined that exhibited grade of pigmentation shown. Figures within parentheses represent total No. of mice examined at each time interval. Decreasing numbers of observations with increasing time reflect death or sacrifice of mice in previous time intervals.

I), examination of whole-mount preparations of skin from these animals revealed that melanin formation had not been completely inhibited. The effects of cortisone on irradiated C57BL mice were quite similar to those observed in LAF_1 mice. Both adrenalectomy and treatment with cortisone (1.25 to 6.0 mg/day) brought about an early mortality of irradiated mice, the majority dying prior to nineteenth day of exposure. Only rarely did a control mouse die within this period.

Daily exposure of mice to UV brought about a darkening of plantar surfaces of feet. The response was identical in castrate and control mice however, a pronounced strain difference was evident. Darkening was observed in C57BL mice by sixth day of exposure and not until the eighth day in LAF_1 mice. In addition, LAF_1 mice did not surpass a pigmentation grade of + during the 16 day period, whereas C57BL mice achieved a grade of +++ by the twelfth day of exposure to UV.

Histological studies revealed that all instances of hyperpigmentation resulted from increased numbers of melanotic epidermal melanocytes and increased deposition by these cells of melanin within the epidermis. Only an occasional melanotic epidermal melanocyte was observed in skin from nonirradiated controls. Comparisons of pigment content in sections and whole-mounts of skin correlated well with pigmentation grades based on gross examination of plantar surfaces.

Discussion. The present results indicate that radiation-induced hyperpigmentation may occur in the absence of hormonal contributions by hypophysis, gonads, and adrenals. This is of interest in view of previous observations that hormones derived from these glands play an important role in regulation of mammalian pigmentation. It has been reported that melanophore-stimulating hormone (MSH) derived from the hypophysis elicits hyperpigmentation in humans(4), and that adrenocortical hormones suppress pigmentation in humans(4), rats(8) and hamsters(9), and enhance pigmentation in the guinea pig(10). It has been suggested that hormones of the adrenal cortex suppress pigmentation by inhibiting release of MSH from the hypophysis(4). In the present study, the inhibition of pigment formation observed in irradiated mice treated with cortisone might have resulted from direct action on the melanocytes or indirect action by suppressing release of essential pituitary hormones. The demonstration of abundant hyperpigmentation in irradiated hypophysectomized rats would appear to support the former interpretation. Failure of ACTH to inhibit pigment formation is surprising since ACTH promotes release of cortisone-like compounds from the adrenal cortices. Possibly the employed doses of ACTH were insufficint to promote release of amounts of cortical hormones adequate to produce an inhibition. It is also possible that rapid inactivation of ACTH(11) or contamination of ACTH preparations by other hormones(12) would account for its ineffectiveness in suppressing pigment formation. Failure of castration to influence development of hyperpigmentation in irradiated mice indicates that species differences may exist in this regard, since male and female human castrates have been reported to tan but poorly when exposed to ultraviolet light(5). No definitive explanation can be given at present for failure of LAF_1 mice to tan significantly on exposure to UV in contrast to the marked hyperpigmentation elicited by gamma irradiation. It is possible in both cases that the differences in quality of the radiations may account for the differences in results.

It is of interest to note that in preliminary experiments diethylstilbestrol suppressed radiation-induced hyperpigmentation in LAF_1 female mice as did cortisone. This finding is in contrast to previous reports of enhanced pigmentation in humans(13) and guinea pigs (10) treated with this compound. It is quite possible that the high daily dose of diethylstilbestrol (1 mg/day) employed in the present experiments, would account for this difference in response.

Our findings support the view that radiation-induced hyperpigmentation is largely a local phenomenon since pigmentation increase was restricted to regions directly exposed to radiation. In addition, removal of endocrine

glands known to influence pigmentation did not significantly alter the occurrence of hyperpigmentation in irradiated mice. However, that this process is not entirely refractory to hormones is evidenced by inhibition of melanin formation in irradiated mice treated with large doses of exogenous cortisone or of diethylstilbestrol.

Summary. C57BL and LAF_1 mice became hyperpigmented in the extremities when exposed daily to gamma radiation. This process was not significantly altered by adrenalectomy, castration or daily treatment with ACTH. Hypophysectomized black rats, X-irradiated weekly, developed hyperpigmentation at the same rate as controls. Although it would appear that induction of hyperpigmentation by radiations is largely free of endocrine control, daily treatment of gamma irradiated mice with large doses of cortisone will suppress pigment formation.

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Effect of Various Adrenal Steroids on Internal Fluid and Electrolyte Shifts of Fasted Adrenalectomized Dogs.* (24252)

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Glucocorticoids readily restore to normal the markedly disturbed fluid and electrolyte equilibria of adrenalectomized dogs exhibitings severe insufficiency. This is accomplished in animals deprived of food and water, by hormonal mobilization and shift of fluid and electrolytes from cells and certain tissues to the dehydrated extracellular (and intravascular) compartment. Experiments reported here indicated that aldosterone and DOC are incapable of relieving severe symptoms of adrenal insufficiency or of inducing internal redistribution of fluid and electrolytes in the *fasted* dog. The data suggest tentative assignment of certain functional roles for mineralo- and glucocorticoids in regulatory control of salt and water balance. The materials and methods used in studies on adrenalectomized dogs have been adequately described (1,2).

Results. Effect of 2-methyl-9α-fluorohydrocortisone, Aldosterone and Desoxycorticosterone on plasma and urine electrolytes and plasma volume of fasted adrenalectomized dogs recovering from insufficiency. A. 2-methyl-9α-fluorohydrocortisone (2-methyl FF free alcohol). Five dogs were tested and to-

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