THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 51, No. 1

Printed in U.S.A.

EFFECTS OF SINGLE DOSE X-IRRADIATION ON THE PIGMENT CELLS OF THE **PET** MOUSE GASTROCNEMIUS*

WILLIE M. REAMS, JR., PH.D. AND BARRY E. SCHAEFFER, M.A.†

It is generally recognized that ultraviolet and x-irradiations result in the hyperpigmentation of the mammalian epidermis. Even dermal melanocytes have been shown to respond positively to these irradiations (1–4). Only in the hair, as a component of the skin, does increased irradiation result in a decrease in pigmentation (5, 6). Prevalent postulation is that the pigment cell's response to irradiation is mediated through cues from the epidermis (7–12) or, in the case of dermal pigment cells, from the dermis (4).

A pigment cell is typically a component of an epidermal melanin unit (13, 14) or, even if dermal (3), it is still a component of the integument. Consequently, research on pigment cell behavior has been essentially limited to studies of pigment cells within their integumentary environment. Additional understanding could be gained if pigment cells could be analyzed free of direct integumentary influences.

In PET mice (15), migrating neural crest melanoblasts not only colonize the skin but also migrate from the skin and colonize underlying tissues such as the gastrocnemius muscle (16–18). The study presented here was undertaken to determine the response to X-irradiation of pigment cells resident in the gastrocnemius—a non-integumentary community.

MATERIALS AND METHODS

A single dose of X-irradiation was given the right hind leg of each of 64 PET mice. The dosage varied from 200 to 7,000r. The left hind leg was not irradiated and served as a control. Additionally, one or 2 mice of each litter were not irradiated and also served as controls. Mice were irradiated at one to 4 days of age and were sacrificed 10 days after irradiation.

Aided by NIH Grant 1T1-AM-5508-01 at MCV, and the UR Research Fund.

We are indebted to Mr. Harry Mueller, Biophysics Department, MCV, for his aid in the X-ray procedures.

Accepted for publication March 12, 1968.

* From the Division of Dermatology, Medical College of Virginia, Richmond, Virginia 23219; and the Department of Biology, University of Richmond, Virginia 23173.

† Present address: New York University.

To facilitate irradiation, the mice were mounted with adhesive strips on polystyrene platforms so that the right hind limb was extended. For accurate positioning of the limb prior to X-raying, the area to be irradiated was visibly delimited by light from an intrinsic source within the X-ray machine.

The X-ray machine was a General Electric Maxitron. At 1,000 KVP and 3 ma, the radiation intensity obtained at 1 cm distance was 500r per minute, HLV of 3.7 mm of lead. Inherent filtration of a brass-water-brass jacket allowed only hard, high-energy X-rays of practically uniform intensity to pass through the limbs.

At sacrifice, the animals were killed in ether, the hind limbs amputated and the skin was stripped back to the ankle. Following fixation in 5% formosaline, the specimens were dehydrated in alcohol and cleared in methyl salicylate. The gastrocnemius muscles of the hind legs were dissected free, teased so as to lie flat, and mounted in methyl salicylate on a slide for microscopic examination. Methyl salicylate was used as it gives the best transparency of the muscle and thus aids in visualizing the melanocytes. An ocular reticle and a tally counter were used to determine the number of melanocytes in the muscles.

In an attempt to expose latent pigment cells, the right hind leg of each of 9 mice was irradiated with a different dosage of X-rays. The gastrocnemius muscles of these mice were dissected free and treated with dopa.

As there was a few days difference in the age of the mice, it was important to determine if this difference caused a significant variation in the melanocyte counts. Therefore, the gastrocnemius muscles of 20 non-irradiated mice of equivalent ages were studied also.

RESULTS

Among the 20 non-irradiated control animals, melanocyte numbers in the left gastrocnemius ranged from 422 to 1225, and in the right gastrocnemius from 393 to 1339. Due to this wide range of variability, direct comparisons could not be made of melanocyte numbers between left muscles or right muscles of different mice of even the same age. However, a definite relationship was observed in melanocyte numbers between the left and right muscles in the same individual. In the 20 animals, the melanocyte number in the right gastrocnemius averaged only 70 more than in the left. For convenience, the comparative number of melanocytes between the right

and left muscles was represented as an index reference number. This index number was obtained by subtracting the number of melanocytes in the left gastrocnemius from the number of melanocytes in the right gastrocnemius of a given mouse. If there was a positive index number for an experimental animal, then an increase in melanocytes had been provoked in the irradiated right leg. A negative index number indicated a decrease in melanocytes in the treated muscle.

No significant variation in the index numbers was found in controls of 12 to 14 days of age. There was a tendency for the total melanocyte number in both the left and right gastrocnemius to increase progressively during the period, but as the rate of increase was approximately the same in both legs, the index was not seriously affected. Therefore, the index retained its significance and could be used as a reference of comparison among animals of different ages.

Among irradiated animals there was in inverse relationship between the melanocyte index and the X-ray dosage. As shown in figure 1, as dosage increased from 200 to 7,000r, the index decreased. At 7,000r, for example, the index was —995, with 1,315 melanocytes in a non-irradiated left gastrocnemius and 320 in an irradiated right. Atypical melanocytes which showed signs of deterioration were conspicuous in the irradiated muscle (Figs. 2, 3). Many of the melanocytes appeared to have disintegrated leaving only a residue of melanin granules (Figs. 4, 5). Treatment with dopa did not reveal additional pigment cells in the muscle. Further, there was no indication of melanocyte stimulation in muscle at any dosage level tested and the surviving melanocytes showed no morphological changes when compared to controls.

For the sake of comparison, the leg skin of the experimental animals was examined also. As expected, the skin showed a progressive hypertrophy and increase of melanotic melanocytes with increasing doses of radiation. The details of the skin response are presented elsewhere (4).

DISCUSSION

The pigment cells found in the gastrocnemius of the PET mouse emigrate from the leg ectoderm and invade the developing muscle during the 14th day of gestation. Melanogenesis begins at day 17 and the number of visible melanocytes continues to increase during the first two post-natal weeks (16). Thus the intramuscular pigment cells are of the same heritage and genetic background as those resident in the integument, and their intramuscular responses can be related comparatively to responses in the integument.

The skin of the experimental legs in the present study gave the expected hyperpigmentation response to X-irradiation. Not only was the skin near the X-ray source hyperpigmented, but that on the opposite surface of the same leg gave a like response

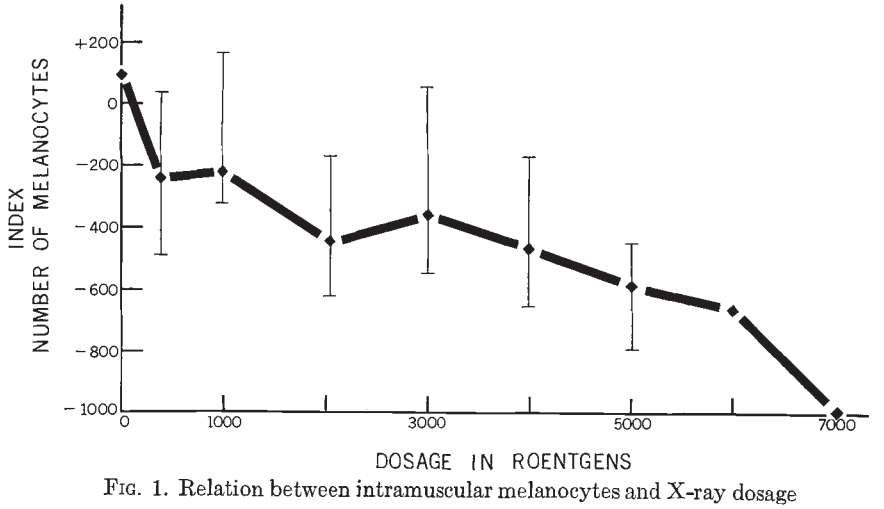

FIG. 1. Relation between intramuscular melanocytes and X-ray dosage

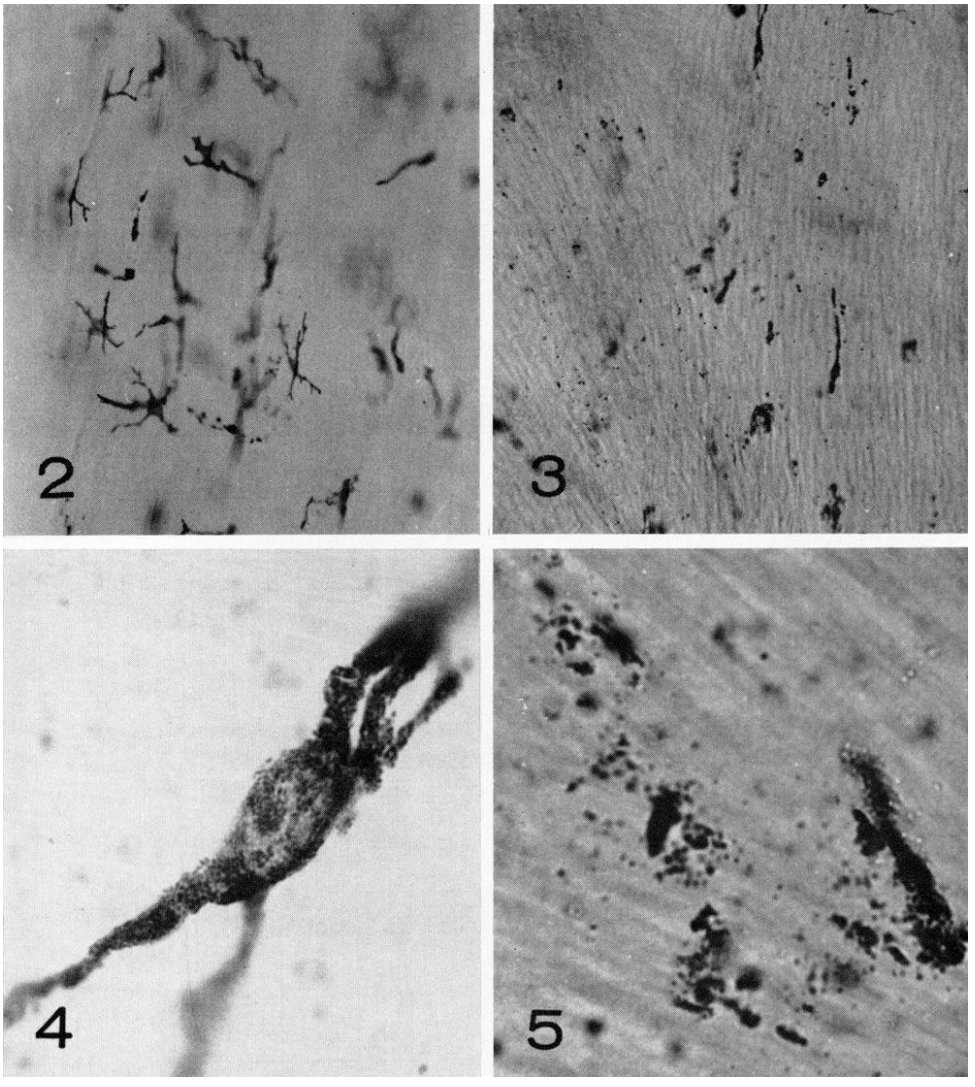

FIG. 2. Melanocytes within the gastrocnemius of an 11-day non-irradiated PET mouse. Unstained whole mount. × 100.
FIG. 3. Gastrocnemius 10 days after 4,000r X-radiation. × 100
FIG. 4. Non-irradiated intramuscular melanocyte. × 1,000
FIG. 5. Disintegration residue of irradiated melanocytes from Fig. 3. × 1,000

also (4), thus demonstrating that effective amounts of radiation for hyperpigmentation were passing through the leg muscles. Dosages of 3,000r or higher destroyed the epidermis on both sides of the leg; however, even at 7,000r the dermis and its melanocytes remained intact. The gastrocnemius showed a progressive decrease in size with increasing irradiation but, save for the melanocytes, its basic histologic integrity was relatively unaffected. The low order of response to irradiation of muscle has been partly explained on the basis of the limited mitotic rate of the muscle cells (19). It is interesting to note the evidence for the restricted mitotic activity of pigment cells in the young mouse, both in the skin (3, 4, 12, 18) and in muscle (17). Therefore, the changes in melanocyte number which resulted from irradiation probably were a function of resident pigment cells and not pigment cell proliferation.

With increasing dosages of X-irradiation,

there was a progressive decrease in the intramuscular melanocyte population—a situation not unlike that in the hair (5, 6). There was no pigment cell stimulation in muscle at any dosage level tested. As necrotic melanocytes and debris of melanin granules were evident, it appears that melanocytes outside the skin are quite radiosensitive and are damaged or destroyed by X-rays. Reams and Shervette (20, 21) made a study of the intramuscular pigment cell behavior during the days following X-irradiation of 2,000r and noted the damaging effects of the X-rays were fully expressed by the second day after irradiation. No indication of pigment cell recovery was found and it was assumed that both melanocytes and premelanocytes had been affected.

SUMMARY

The irradiation of intramuscular pigment cells with X-rays resulted in a progressive decrease in the number of melanocytes with increasing doage of radiation. Apparently the pigment cells were damaged or destroyed by the X-rays. The conclusion is tendered that pigment cells fundamentally are radiosensitive and that the integument normally serves as a radioprotective agent. The epidermis and/or dermis, then, would be the mediators of hyperpigmentation responses of the pigment cells to radiation.

REFERENCES

1. Snell, R. S.: The effect of X-ray irradiation on melanocytes in the skin. J. Invest. Derm., *40:* 233, 1963.
2. Straile, W. E.: A comparison of X-irradiated melanocytes in the hair follicles and epidermis of black and dilute-black Dutch rabbits. J. Exp. Zool., *155:* 325, 1964.
3. Rovee, D. T. and Reams, W. M.: An experimental and descriptive analysis of the melanocyte population in the venter of the PET mouse. Anat. Rec., *149:* 181, 1964.
4. Reams, W. M. and Schaeffer, B. E.: Effects of single dose X-irradiation on the pigment cell system of the PET mouse leg skin. J. Invest. Derm. In press.
5. Chase, H. B.: Greying of hair. J. Morph., *84:* 57, 1949.
6. Chase, H. B. and Rauch, H.: Greying of hair. J. Morph., *87:* 381, 1950.
7. Chase, H. B.: Radiation damage to cells dependent on their tissue environment. Brit. J. Radiol., *31:* 65, 1958.
8. Chase, H. B., Straile, W. E. and Arsenault, C.: Evidence for indirect effects of radiations of heavy ions and electrons on hair depigmentation. Ann. N. Y. Acad. Sci., *100:* 390, 1963.
9. Cohen, J.: The pigment cell system in the light Sussex fowl. J. Emb. Exp. Morph., *7:* 361, 1959.
10. Cohen, J.: The nature of the effect of X-irradiation in depigmentation. Ann. N. Y. Acad. Sci., *100:* 400, 1963.
11. Quevedo, W. C. and McTague, C. F.: Genetic influences on the response of mouse melanocytes to ultraviolet light: The melanocyte system of hair-covered skin. J. Exp. Zool., *152:* 159, 1963.
12. Quevedo, W. C. and Smith, J. A.: Studies on radiation-induced tanning of skin. Ann. N. Y. Acad. Sci., *100:* 364, 1963.
13. Fitzpatrick, T. B. and Breathnach, A. S.: Das epidermale Melanin-Einheit-System. Derm. Wschr., *147:* 481, 1963.
14. Hadley, M. E. and Quevedo, W. C.: Vertebrate epidermal melanin unit. Nature, *209:* 1334, 1966.
15. Reams, W. M.: Registration of the PET mouse strain. Inbred Mouse Strains №5. Jackson Laboratories, Bar Harbor, 1967.
16. Mayer, T. C. and Reams, W. M.: An experimental analysis and description of the melanocytes in the leg musculature of the PET strain of mice. Anat. Rec., *142:* 431, 1962.
17. Reams, W. M.: Regulation of pigment cell colonization of PET mouse leg muscles. Anat. Rec., *155:* 89, 1966.
18. Reams, W. M.: Pigment cell population pressure within the skin and its role in the pigment cell invasion of extraepidermal tissues. Pp. 489–501, *Advances in Biology of Skin*, Vol. 8. Ed., Montagna, W., Pergamon Press, New York, 1966.
19. Bloom, W. and Bloom, M. A.: Histological changes after irradiation. Pp. 1091–1143, *Radiation Biology*, Vol. I. Ed., Hollaender, A., McGraw-Hill, New York, 1954.
20. Reams, W. M. and Shervette, R. E.: Sulfhydryl content of the normal and X-irradiated PET mouse gastrocnemius (Abs.). Amer. Zool., *7:* 782, 1967.
21. Reams, W. M. and Shervette, R. E.: X-irradiation, melanocytes and sulfhydryl content in the PET mouse gastrocnemius. In preparation.