Reports

Anomalous Greying Effect in Mice with Higher Doses of X-rays

Abstract. Exposure of mouse hair follicles to 1200, 1300, and 1400 r of x-rays results in delay of hair growth, precocious melanogenesis, and disruption of melanocyte distribution. The diversity of greying responses obtained with 1100 to 1700 r appears to be due to inactivation of radiosensitive follicles rather than to tissue replacement or to displacement of external sheath melanocytes into the hair bulb.

Exposure of resting hair follicles of mice to x-radiation results in a graded greying effect, from a threshold response at 250 r to a 95- to 100-percent greying response at 1000 r (*1*, *2*). However, doses of x-rays of 1100 to 1700 r produce an anomalous response in which the pigment loss ranges from 60 to 100 percent (*3*).

Three hypotheses are offered as possible explanations for the variety of greying responses obtained with these relatively high doses of x-rays. First, damage to the follicle by x-radiation may necessitate regeneration of the injured follicle from the upper external sheath rather than from the hair germ (*3*). As a consequence, melanocytes from the external sheath may become incorporated into the new hair bulb, with resulting production of pigmented hair. Second, the repair of radiation wounds may occur by expansion into the injured zone of the surrounding nonirradiated tissue with its follicles and melanocytes, with resulting increase in the number of pigmented hairs in this area. Third, epilation or inactivation of follicles most sensitive to x-rays may occur, resulting in a proportional increase in less radiosensitive follicles whose melanocytes have retained their capacity for pigment production. The investigation reported here (*4*) was undertaken to ascertain the validity of these hypotheses and the effects of relatively high doses of x-rays on the distribution of melanocytes in exposed follicles.

Resting hairs were plucked from the posterior dorsum of 103 mice, male and female (strain C_{57} BL), and an area of 1 cm^2 was exposed to 1200, 1300, or 1400 r of x-rays (200 kv, 20 ma, 700 r/min, 19.5 cm from target to skin, no additional filtration). The controls were nonirradiated mice and mice exposed to 700 r of x-rays. Biopsies were taken from days 1 through 28 after treatment, and the excised tissues were incubated in dihydroxyphenylalanine (*5*) and sectioned at 20 μ.

Irradiation with 1200, 1300, or 1400 r produced alteration of both the time of initiation of hair growth and melanogenic activity. The beginning of follicle growth and differentiation was delayed for 12 to 14 days after exposure, whereas melanocytes were present in the hair germ as early as 1 day after treatment. In nonirradiated animals the onset of follicular growth and differentiation began within 1 day after plucking and was followed by melanogenesis 3 to 4 days later. After a delay of 1 to 2 days the normal sequence of follicle development and pigment cell activity occurred in the irradiated controls.

A striking effect of the doses of x-rays used in these experiments was the scattering of melanocytes throughout the hair bulb. In the maturing follicle these cells were detected in all regions of the bulb, from the distal tip to their usual position at the apex and in the upper third of the bulb. Moreover, they were not always in contact with the dermal papilla. Occasionally, however, even the dermal papilla contained active melanocytes. These experiments did not demonstrate incorporation of differentiated melanocytes from the external sheath into the new bulb. Rather, the appearance of pigment cells in the hair germ suggests that the germ itself is the source of melanocytes for future hair generations. Thus, even though treatment with relatively high doses of x-rays results in abnormal melanocyte distribution, no evidence has been found which satisfies the first hypothesis.

The second, "wound repair," hypothesis was tested by irradiating 1 cm^2 of the white belt of ruby strain mice with 1200 or 1300 r of x-rays. The irradiated area was bordered posteriorly and anteriorly by sepia hair. Growth of pigmented hair within the irradiated zone would have signified expansion into that area of the surrounding tissue, with its nonirradiated follicles and pigment cells. No pigmented hair was found within the exposed area at the end of one hair growth cycle. Therefore, tissue replacement does not offer a suitable explanation for the diversity of greying response at these levels of x-radiation.

The third hypothesis—that destruction of the more radiosensitive follicles and retention of the less sensitive follicles yields diverse greying responses—was tested statistically. Active follicles per microscopic field (100 ×) were counted in three groups of animals: mice exposed to 1300 r, mice exposed to 700 r, and nonirradiated mice. It was found that the mean number of active follicles per field in mice exposed to 1300 r was less (4.01 ± 0.33) than the number for the nonirradiated group (9.78 ± 0.10) and the number for mice exposed to 700 r (7.69 ± 0.14). Analysis of variance indicated a significant difference among treatments ($P < .001$) and among animals ($P < .001$). Furthermore, the "Student"-Fisher small-sample t test showed that loss of active follicles by exposure to 1300 r is highly significant ($P < .0001$). The difference between findings for nonirradiated animals and for mice exposed to 700 r was of borderline significance ($P = .02$).

These results, considered in the light of the demonstration by Chase and Rauch (*2*) that, of the four main hair types, the zigzags are most radiosensitive and contribute most significantly to

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

the greying response, suggest the following scheme: Exposure of resting hair follicles to relatively high doses of x-rays results in the inactivation or destruction of a number of the radiosensitive zigzag follicles, leaving a greater proportion of the larger and less sensitive monotrich, awl, and auchene follicles. This phenomenon would increase the proportion of pigmented hairs within the exposed area and would result in variable greying responses, dependent upon the number of remaining zigzag follicles. Thus, the greater variability in number of active follicles within the 1300 r group (standard error of 0.33) in comparison with the variability for the control groups probably explains the greater variability of greying responses (60 to 100 percent) in that group.

DONALD B. GALBRAITH
HERMAN B. CHASE
Department of Biology,
Brown University,
Providence, Rhode Island

References and Notes

1. H. B. Chase, *J. Morphol.* **84**, 57 (1949).
2. —— and H. Rauch, *ibid.* **87**, 381 (1950).
3. H. B. Chase and J. W. Hunt, *Pigment Cell Biology* (Gordon, New York, 1959), p. 537.
4. This work was supported by U.S. Public Health Service grant No. C-592 and AEC contract No. AT(30-1)-2018.
5. S. W. Becker, *J. Invest. Dermatol.* **5**, 463 (1942); L. B. Russell and W. L. Russell, *Genetics* **33**, 237 (1948).

18 August 1961

Metallic Spherules in Tektites from Isabela, Philippine Islands

Abstract. Iron-nickel spherules, as much as 0.5 mm in diameter, have been found completely embedded in some philippinites. The spherules consist mainly of kamacite with unidentified pink inclusions. The meteoritic origin of these spherules seems reasonable, suggesting that the tektites containing them were formed by asteroidal or meteoritic impact.

Fresh kamacite spherules have been found in five tektites from the Isabela district of Luzon, Philippines, and they supply strong physical evidence for the meteorite impact origin of tektites.

L. J. Spencer (*1*) reported "dark spots" in indochinites and australites that show metallic luster when viewed under the microscope. Additional description is not available and these "dark spots" were not identified. Recently Vorobev (*2*) described hollow spherules, 1 to 2 mm in diameter, that consist of "magnetite" and siliceous materials on the surface of some of the philippinites. It is possible, however, that the hollow spherules are similar to bubble cavities observed by us that have been filled with reddish, silty, terrestrial material after the tektite was buried. The spherules described by Vorobev are much larger and are not iron-nickel spherules of the type reported here.

The metallic spherules, from less than 0.1 mm to as much as 0.5 mm in diameter, have an average size of about 0.16 mm. They are completely embedded in the tektites, perfectly spherical, shiny, and fresh—without the slightest trace of oxidation or alteration. Under the metallographic microscope at high magnification, the surface of the spherules is seen to be covered by a network of a pink mineral (Fig. 1). This mineral is probably the same as the round inclusions within the spherules described below. The glass adjacent to the spherules shows no more evidence of strain than the tektite glass elsewhere.

The appearance, texture, bulk specific gravity, and index of refraction of the five tektite specimens containing metallic spherules fall within the normal range for tektites from Isabela. They show flow banding and contain small round bubble cavities, similar to those in any other tektite from this locality. As in other typical tektites, inclusions of low-index glass, presumably lechatelierite, are abundant. The magnetic susceptibility and specific magnetization of specimen P_I 73 were determined by our colleague, Arthur Thorpe, as 8.60×10^{-6} electromagnetic units per gram and 0, respectively, agreeing well with philippinites which Senftle and Thorpe (*3*) have already described. These data rule out the possibility that the tektites we studied could be obsidian or any other similar material.

Under the reflecting microscope, the major mineral phase is α-iron or kamacite, silver white and isotropic in reflected light, with no visible cleavage or grain boundaries even after the specimen has been etched. The spherules contain numerous round to elongate inclusions that are less than 5 μ in diameter (Fig. 2). They are pink in reflected light and "sparkle" as the microscope stage is rotated under crossed nicols because of their strongly anisotropic character. The etched spherules show the boundaries of these inclusions more clearly.

The x-ray powder pattern obtained

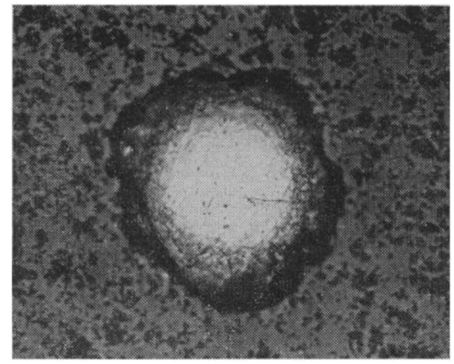

Fig. 1. Surface of a metallic spherule, 0.2 mm in diameter, with the overlying glass broken off, showing a network consisting probably of an iron phosphide.

with Fe Kα radiation shows that the metallic spherules consist of two mineral phases. The major phase is kamacite as indicated by the petrographic study, with a unit cell, $a = 2.86_2$ A. The other phase, which is estimated to be less than 5 percent, is represented by the reflections indicated in Table 1. Reflections 1, 2, 3, and 4 possibly correspond to iron phosphide, FeP (ASTM card No. 3-1066), or schreibersite, Fe_3P (*4*). Further work will be necessary to identify this phase.

The chemical composition of the metallic spherules was obtained by electron-probe microanalysis. Three spherules from specimen P_I 73 were analyzed in a mount with a piece of pure nickel, a piece of pure iron, and a previously analyzed fragment of Canyon Diablo iron meteorite as standards. The spherules contain more than 95 percent iron and 1.2 to 3.2 percent nickel. We believe that nickel content determined by our analysis is in error by no more than approximately $\pm$ 5 percent of the amount present. Electron-probe

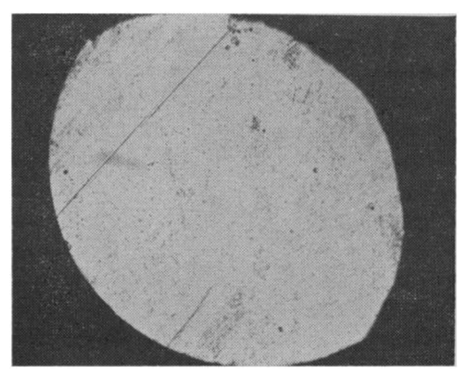

Fig. 2. Part of a metallic spherule about 0.5 mm in diameter, showing the radially distributed inclusions (possibly an iron phosphide).