Technical Papers

Demonstration of Tyrosinase in Melanocytes of the Human Hair Matrix by Autoradiography

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The histochemical method of tyrosinase determination is based on the detection of melanin newly formed from tyrosine (*1*) and is not applicable to pigment cells that are already fully melanized. In order to extend this technique to the determination of tyrosinase activity in pigmented melanocytes (pigmented skin and hair, nevi, and melanomas), we have developed an autoradiographic-histochemical method that utilizes C^{14}-labeled tyrosine as the substrate.

The conversion of water-soluble tyrosine to insoluble melanin on the surface of the melanin granule presents a unique opportunity for the utilization of an *in vitro* autoradiographic technique. When the tissue is incubated in radioactive tyrosine, the melanocytes that contain an active tyrosinase convert the labeled tyrosine to labeled melanin which is fixed to the melanin granule. The unreacted, water-soluble radioactive tyrosine is washed out of the tissue, and it is then possible to record *only* the newly formed radioactive melanin on the photographic emulsion.

The autoradiographic tyrosinase method is being utilized in an extended study of the concentration and activity of tyrosinase occurring in human pigmented nevi and malignant melanomas (*2*). In this paper we present the application of this method to the demonstration of tyrosinase in melanocytes in the matrix of the human black hair bulb (*3*). In addition, it is shown that the albino and gray hair matrices do not contain tyrosinase.

Excision biopsies of human scalp skin were taken under local anesthesia from four volunteers with black hair, four with gray hair, and one albino. Tissue slices, 1 to 2 mm thick, were immersed in 2.2 ml of 0.1*M* phosphate buffer at *p*H 6.8 which contained 0.3 μc of DL-tyrosine-2-C^{14} (*4*) (0.5 μc/mM) and 3000 units of crystalline penicillin G. Incubation was carried on for 24 hr at 37°C in a Dubnoff shaker. As a control, slices from the same specimen were treated identically, except that, in addition to the substrate, a tyrosinase inhibitor, sodium diethyldithiocarbamate (0.01*M*), was present. The inhibited specimen was necessary to detect traces of unreacted radioactive tyrosine that might not have been removed from the tissue slices by washing. Following incubation, the tissue slices were fixed in 10-percent formalin for 1 hr and washed in running water for 8 hr. To prepare the tissue for autoradiography (*5*), the specimens were dehydrated, cleared in toluene, imbedded in paraffin, sectioned at 4 μ, and mounted directly on Eastman Kodak 1- by 3-in. NTB_3 (25 μ) nuclear track plates. After they were air-dried, the sections were thoroughly deparaffinized by immersion in two changes of xylol for 10 min each.

The autographic slides on which the sections were mounted were stored in black, light-tight slide boxes, sealed with photographic masking tape, and exposed for 2 wk. Following exposure, the slides were developed in Eastman D-19 developer, fixed in 20-percent sodium hyposulfite, washed, and dried in air. The tissue was then stained with lithium carmine (*5*).

Melanocytes in the hair matrix of a black hair bulb are illustrated in Fig. 1A, and the tyrosinase activity of the melanocytes is shown in Fig. 1B. The tyrosinase in the melanocytes has catalyzed the oxidation of C^{14}-labeled tyrosine to C^{14}-labeled melanin. The radioactive melanin has produced a latent image in the silver bromide crystals of the photographic emulsion. The photographic developer then reduces the grains with latent images to metallic silver. These metallic silver grains are seen outlining the hair matrix in Fig. 1B. The same melanocytes that contained tyrosinase did not catalyze the conversion of the labeled tyrosine to labeled melanin in the presence of sodium diethyldithiocarbamate (Fig. 1C). Gross examination of the hair bulbs after incubation revealed a darkening of the black hair bulb incubated in labeled tyrosine but no change in color in the hair bulbs incubated in labeled tyrosine and sodium diethyldithiocarbamate.

Examination of the epidermal-dermal junction of

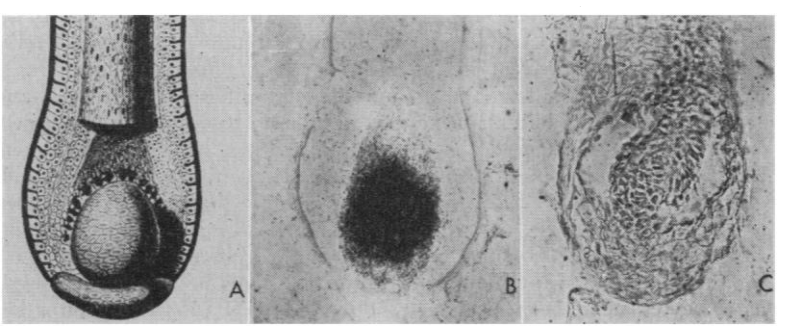

Fig. 1. Melanocytes occupy the upper portion of the bulb of a black human hair (*A*) and are arranged as an inner cone surrounding the upper part of the dermal papilla. Heavy deposition of silver grains (*B*) indicate the site of radioactive melanin formation from C^{14}-labeled tyrosine by melanocytes. Presence of sodium diethyldithiocarbamate blocks melanin formation (*C*). (×80)

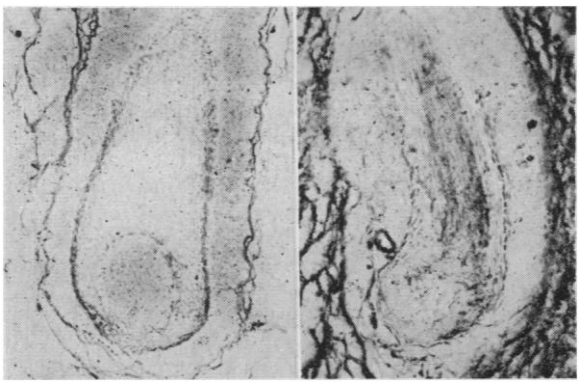

Fig. 2. (Left) Autoradiograph of a human albino hair bulb that has been incubated in C^{14}-labeled tyrosine. No evidence of radioactive melanin formation is seen in the area of the matrix. (×89) Fig. 2. (Right) Autoradiograph of the hair bulb of a 56-year old male with gray hair. No tyrosinase activity is noted in the area of the matrix. (×155)

the same autoradiograph of human white scalp skin containing tyrosinase-active melanocytes in the black hair bulb revealed that the *epidermal* melanocytes did not convert labeled tyrosine to labeled melanin.

Melanocytes located in the albino hair bulb failed to convert labeled tyrosine to labeled melanin, as is evidenced by the absence of silver grains in the area of the matrix (Fig. 2, left). Thus, albino melanocytes in the hair matrix lack the enzyme tyrosinase necessary for melanin synthesis. In a previous study (*6*), melanocytes were identified in human albino epidermis with gold impregnation. The absence of melanin in albino epidermal and hair matrix melanocytes is, therefore, the result of a genetically transmitted tyrosinase deficiency and cannot be attributed to lack of melanocytes.

Figure 2 (right) is a tyrosine-C^{14} autoradiograph of the hair matrix of a gray hair bulb. The absence of silver grains in the area of the matrix indicates a lack of tyrosinase in the human gray hair matrix.

The autoradiographic tyrosinase method has revealed that an important difference exists between the state of the tyrosinase system located within melanocytes of the human epidermis and the melanocytes of the human hair matrix. Previous histochemical studies (*1*) with irradiated and unirradiated human white skin established the existence of an *inhibited* tyrosinase system in epidermal melanocytes. The autoradiographic studies reported here have shown an uninhibited tyrosinase system in the melanocytes of the human black hair matrix and an inhibited tyrosinase system in the epidermal melanocytes. These basic facts are in agreement with the clinical findings of heavily melanized (black) hair and coexistent white skin. It appears that the large amount of melanin that is required to pigment rapidly growing black hair is supplied by the active-functioning tyrosinase system in the hair matrix melanocytes. Tyrosinase-active melanocytes have been previously observed only in irradiated skin and in malignant melanomas (*7*). To repeat, the tyrosinase system in *epidermal* melanocytes remains in a relatively dormant state unless it is activated by ionizing radiation.

References and Notes

1. T. B. Fitzpatrick *et al.*, *Science* **112**, 223 (1950).
2. T. B. Fitzpatrick and A. Kukita, report in preparation.

3. This investigation was supported in part by the Sloan Foundation, the Damon Runyon Fund, and U.S. Public Health Service grant C2408.

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Effect of Feeding Dogs the Flesh of Lethally Irradiated Cows and Sheep

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To our knowledge, there have been no reports of studies concerning the possible toxicity of the ingested flesh of animals receiving lethal doses of gamma radiation. Based on biophysical conceptions of the interaction of radiation and matter, it was the consensus that the flesh of these animals would not prove harmful if it were consumed (*1*). The present experiments were designed to test these opinions (*2*).

Lewis *et al.* (*3*) and Witt *et al.* (*4*) have shown that sterilization by high levels of irradiation does not impart toxic properties to foods. This would indicate that, if deleterious compounds occurred in the flesh of irradiated animals, they would be the result of the effects of ionizing radiation on living systems. Several workers (*5*) have implicated toxic humoral factors as a constituent of the total-body irradiation syndrome. Thus, the possibility existed that the ingestion of flesh from irradiated animals might prove injurious.

Cobalt-60 sources of gamma radiation, arranged at a site described by Wilding *et al.* (*6*), were used for the exposures. The rate of irradiation was approximately 40 r/hr. One cow received 6400 r and another 7000 r of continuous total-body radiation, and they were destroyed *in extremis*. Sheep died from exposures of 90 hr (3600 r) to 132 hr (5280 r). An initial hyperirritability followed by complete physical collapse were the only gross symptoms noted in the irradiated animals. The cattle had a slight increase in erythrocytes, together with the near annihilation of leukocytes and a 70-percent reduction in platelets *ante mortem*. The flesh of the boned carcasses of the two irradiated and two control cows and the nine irradiated and nine control sheep was ground twice and maintained in a frozen state until used.

Fifteen weanling pure-bred male beagle pups were used in the irradiated cow study. In trials I and II, seven and eight pups, respectively, were divided into two groups by weight and litter (Table 1). The basal