TYROSINASE ACTIVITY IN MELANOCYTES OF HUMAN ALBINOS*

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Albinism is a condition that has attracted medical interest and description for many centuries. Until the past decade, however, efforts were largely confined to the publication of extensive pedigrees demonstrating the autosomal recessive inheritance of the trait and other reports illustrating the frequency of co-existing congenital anomalies—presumably also recessive. Histologic and histochemical studies were few and generally inadequate; these were based largely upon the work of Bruno Bloch who in 1917 demonstrated the formation of a pigment resembling melanin in normal epidermal melanocytes following incubation of skin sections in a solution of 1-dihydroxy-phenylalanine (1). The "dopa reaction" was an important discovery, not only in the study of the biosynthesis of melanin, but also as a useful histochemical method for the identification of melanocytes in tissues with a normal capacity for pigment formation. Albino tissues were shown, soon thereafter, not to possess dopa activity (2), and the erroneous assumption was subsequently made that melanocytes, as well as the melanogenic enzyme "dopa oxidase" which they contained, must be absent in this condition (3). In 1952, however, Becker, Jr. *et al.* (4) first announced that they had demonstrated "amelanotic" melanocytes in human albino skin using gold impregnation, and they concluded that the population density of such cells was the same as that found in normally pigmented skin. Their results have generally been accepted, although there is a considerable body of recent evidence indicating that the gold impregnated cells in question may not be melanocytes at all (5, 6). Other investigations have, however, now established the presence of melanocytes in albino tissues beyond any reasonable doubt. These cells have been identified in electron micrographs of hair follicles (7) and also of neoplasms, i.e., nevi and melanomas (8, 9).

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The finding of morphologically normal melanocytes in albinism has stimulated once more a search for the precise defect rendering these cells incapable of melanogenesis. The normal pathway of mammalian melanin synthesis is known to require, as the initial substrate, the amino acid tyrosine and the enzyme tyrosinase. Apparently, the melanocyte is the only mammalian cell in which this oxidation of tyrosine progresses to pigment formation. The specificity of this reaction provides a reliable and sensitive histochemical assay technic for "active" tyrosinase—and potential melanogenic—activity (10). Dopa is similarly oxidized but is an unsatisfactory substrate since it is oxidizable even in air.

Fitzpatrick, *et al.* (11) incubated albino skin in solutions of tyrosine, but failed to demonstrate any tyrosinase activity. It has therefore been generally assumed that the absence of pigment in albinism is due to an absence of tyrosinase, determined in humans by a single recessive gene.

As far as we have been able to determine, all previous attempts to demonstrate tyrosinase activity in albinos have been made using skin slices or homogenates. Since it is known that normal Caucasian skin contains potent tyrosinase inhibitors, it was felt that an additional effort was warranted, in which local inhibiting substances in the skin could be largely excluded and a high population density of melanocytes brought into direct contact with the substrate tyrosine. This situation was found to exist in roots of freshly epilated hairs.

MATERIALS AND METHODS

Hair from five normal Caucasian men and nine albinos† was studied. The hair color of the normal subjects included black, brown, blond, and gray (the gray hair was from a previously dark-haired individual). Two of the albino patients manifested incomplete albinism and had blond hair with light blue irides. In the seven other patients albinism was complete, and these patients had white hair, as well as other classical

† Seven of these patients were made available to the authors through the cooperation of Dr. Carl J. Witkop, Human Genetics Section, National Institute of Dental Research.

signs and symptoms of albinism (non-pigmented skin, reddish pupils and irides, photophobia, ocular nystagmus, and impaired visual acuity). Also included for investigation were hairs from albino mice, an albino rabbit, and an albino guinea pig. Animals about one week of age with a high proportion of actively growing hairs were found to be most suitable.

Hairs were epilated as described by Van Scott, *et al.* (12) and the root portions were immediately immersed in one of the following solutions:

(a) l-tyrosine, 1 mg/ml in 0.1 M phosphate buffer, pH 6.8.

(b) l-dopa, 1 mg/ml in 0.1 M phosphate buffer, pH 7.4.

(c) phosphate buffer at pH 6.8 and 7.4 respectively.

(d) sodium diethyldithiocarbamate, 0.01 M, added to solution (a).

(e) 4-chlororesorcinol, 0.01 M, added to solution (a).

Incubation in the solutions of tyrosine and in the control buffer was continued at 37°C for 24 hours; incubation in the dopa solutions and in the corresponding control buffer was carried out at 37°C for 5 hours. Dopa was used to determine whether hair bulbs which did not show activity with tyrosine might reveal a non-specific result with dopa. Sodium diethyldithiocarbamate and 4-chlororesorcinol are both well known tyrosinase inhibitors, the former acting to bind the copper prosthetic group of the enzyme, and the latter as a competitive inhibitor (13). Following incubation, some hairs were washed in distilled water and mounted unfixed on slides in water sealed in with stop-cock grease. Unincubated controls were similarly mounted. Preparations had a tendency to deteriorate after several weeks, but fixation before mounting could not be done since the hair bulb became opaque to light, making microscopic examination difficult. Tyrosinase activity was estimated qualitatively under a dissecting microscope by evidence of darkening of cells in the lower matrix of anagen bulbs.

RESULTS

In no instance did any hairs show evidence of darkening when incubated in the buffer solutions alone or when incubated in tyrosine solutions that also contained the tyrosinase inhibitors. All instances of pigmentation were confined to hairs incubated in solutions of either tyrosine or dopa. The results are tabulated in Tables 1 and 2.

Darkening of hair roots occurred only in the matrix of the bulb where pigment formation normally occurs (Figs. 1 and 2). Increase of pigmentation was less apparent in the hairs that were normally darkly pigmented, but was most apparent when it occurred in hairs normally devoid of pigment. Variation in the degree of darkening occurred among anagen hair bulbs from the same patient. However, no effort was made to quantitate enzyme activity. When the hair bulbs from albinos were examined under high magnification, it was possible to distinguish darkly stained fusiform cells in those bulbs which had shown darkening. No darkening of telogen (resting) roots was observed in any specimen.

The identity of the pigment containing cells seen in the roots of hairs from the albino patients after incubation in tyrosine was further ascertained by fixing these hairs in formalin-alcohol-uranium nitrate solution, imbedding in paraffin, sectioning, and staining with Gomori's trichrome stain. Dendritic cells containing pigment granules characteristic of melanocytes could easily be identified histologically.

TABLE 1

Pigmentation in hair roots of normal men and rodents

Subjects and Hair Type	No. of Specimens	Tyrosine	Dopa
Man, normal, pigmented	4	+	+
Man, normal, gray	1	−	−
Mouse, albino	4	−	−
Rabbit, albino	1	−	−
Guinea pig, albino	1	−	−

TABLE 2

Pigmentation in hair roots of albino patients

Patient	Age	Sex	Albinism	Tyrosine	Dopa
1	25	M	incomplete	+	+
2	28	M	complete	+	+
3	12	M	complete	+	+
4	9	M	complete	+	N.D.
5	5	F	complete	+	N.D.
6	40	F	complete	+	N.D.
7	15	F	complete	−	−
8	18	M	complete	−	−
9	14	F	incomplete	+	+

N.D.: not done.

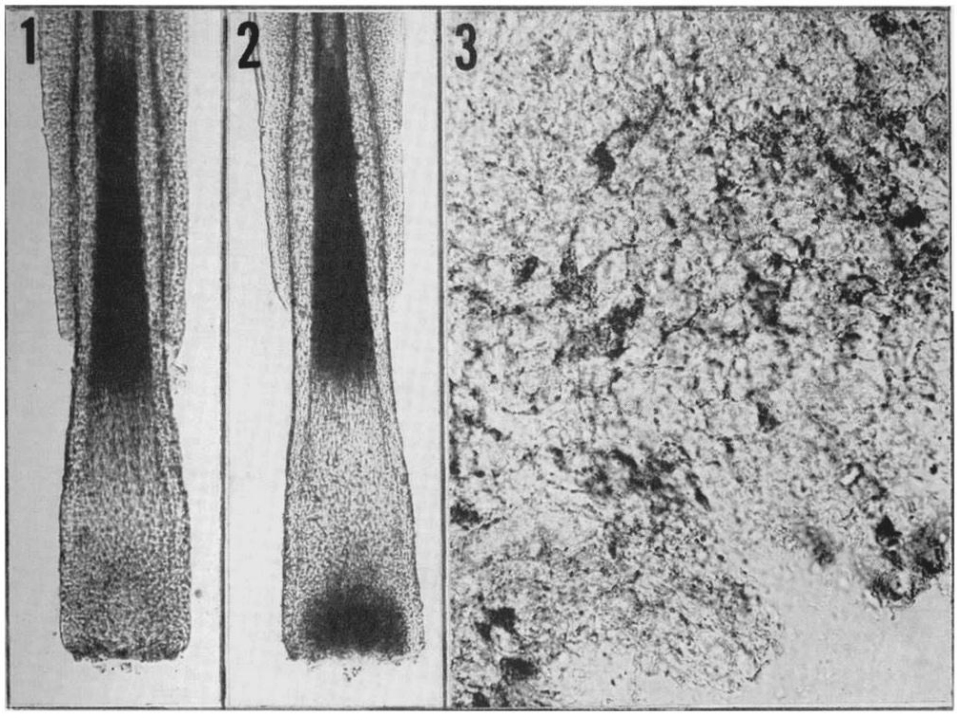

FIG. 1. Human albino hair root, control. × 56

FIG. 2. Human albino hair root, after incubation in tyrosine solution for 24 hours. Pigmentation is confined to region of lower matrix where melanocytes are normally present. × 56.

FIG. 3. Pigmented dendritic cells in unstained albino epidermis after incubation in tyrosine. × 435

Epidermis was scraped with a scalpel from the forearm of one of the albino patients (#2) and was incubated in tyrosine solution. After incubation pigment containing dendritic cells were microscopically apparent in the unstained tissue (Fig. 3).

DISCUSSION

Tyrosinase activity has never before been demonstrated in albinism, either in humans or in lower animals. The above findings are, therefore, of some significance, especially since the results in the human albino differed from those in the rodents tested.

Evidence that the observed darkening actually demonstrates the presence of tyrosinase is of necessity only presumptive, but is suggested by the following facts. First, tyrosine is known to form a black pigment only in the melanocyte in the presence of tyrosinase. Second, the pigment that we observed occurred only in those areas of the hair bulb and epidermis where melanogenesis normally occurs and was, in fact, contained only within dentritic cells indistinguishable from normal melanocytes. Third, the reaction was inhibited by known tyrosinase inhibitors.

An attempt must be made at this point to explain the failure of previous investigators, specifically Fitzpatrick and Kukita (10), to demonstrate tyrosinase activity in albino skin and hair bulbs. The technic used here differs in only one significant respect from that used by these workers—the tissues were not incubated as blocks or sections of skin. As mentioned before, it has been known for some time that normal white epidermis contains a substance which inhibits melanogenesis (14, 15). Further, extracts of white guinea pig skin, regardless of genotype, have been found to contain a substance, not found in pigmented skin, which inhibits the autoxidation of dopa (16). It is thus possible that local tissue factors inhibited tyrosinase activity in the earlier experiments, and that these may, in fact, account for the *in vivo* failure of pigment formation in albinism as well. There is no clue as yet, as to the nature of the inhibiting factor or the level at which it operates.

There is other evidence which indicates that

tyrosinase or another pigment forming enzyme system must be present in a least some types of albinism. It has been frequently observed that most human albinos, as they reach adult age, acquire the capacity to form small amounts of pigment in the skin, hair, and eyes. This is commonly spoken of as an "accumulation" of melanin with advancing age (17, 18); but since formed melanin is constantly lost in skin and hair, a continuous if slow rate of melanogenesis must be occurring in these lightly pigmented persons. Similarly, there are albino strains of lower mamals, such as the "Himalayan" rabbit, which develop pigmentation under unusual stresses.

It is of note that hair bulbs of the mice, guinea pig, rabbits, and two humans tested did not show darkening. Of the several possible explanations for this, the most likely one is that they are simply "better" albinos, *i.e.*, that the defect achieves more complete expression than in most human albinos. It may also be that albinism is not in all instances due to the same cause; in this connection, it is known that several different genes may be responsible for a white coat color in mice (19). It is very likely that the assay technic used by us is not sensitive enough to determine small degrees of enzyme activity which may be present in the hair bulbs that did not show gross darkening.

Many other implications can be drawn from the finding of a significant degree of tyrosinase activity in albinism, but these will not be further discussed here. It is clear, however, that the current theories concerning the etiology of the disease must be modified. The defect may not be a deficiency of tyrosinase, but an endogenous tissue inhibitor of this enzyme or a defect in transport of tyrosine into the melanocyte. In any event, the gene, or genes, responsible for human albinism must exhibit various degrees of expression to account for the above observations of differing capacity for melanogenesis, even among clinically "complete" albinos.

SUMMARY AND CONCLUSIONS

Pigment formation in cells indistinguishable from normal melanocytes following incubation in solutions of l-tyrosine was observed to occur in the epidermis and hair bulb matrix of human albino patients, thus implying a potential capacity for melanogenesis in human albinism.

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