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Research Article

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Tyrosinase Activity in Human Skin

INFLUENCE OF RACE AND AGE IN NEWBORNS

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ABSTRACT Tyrosinase has been measured in homogenates of foreskins from newborn babies. The tyrosine hydroxylation reaction is dependent upon 3,4-dihydroxyphenylalanine as a cosubstrate, and the K_m for tyrosine is 0.15 mM, similar to the value observed for other mammalian tyrosinases. The mean enzyme activity for black babies ($n = 169$) is about two and one-fourth times that for white babies ($n = 82$). For white babies there is a significant correlation between age at circumcision and tyrosinase activity. For black babies this correlation becomes significant when four individuals with extremely high tyrosinase activities are omitted from the series.

INTRODUCTION

Tyrosinase, present in melanosome granules within the melanocyte, catalyzes the formation of melanin, the brown or black polymeric pigment in skin, melanomas, hair, and eyes. Melanin is made by the following pathway:

$$\text{L-tyrosine} + AH_2 + O_2 \xrightarrow{1.} H_2O + A + \text{L-dopa}^1 \xrightarrow[O_2]{2.}$$

dopa quinone $\rightarrow\rightarrow$ dopachrome $\rightarrow\rightarrow$ 5,6-dihydroxyindole $\rightarrow\rightarrow\rightarrow$ melanin.

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[1] *Abbreviation used in this paper:* dopa, 3,4-dihydroxyphenylalanine.

Reactions 1 and 2 are known to be enzyme-catalyzed. The hydroxylation step requires a reducing agent (AH_2 in the above scheme) as a cosubstrate, and in animals the most effective cosubstrate is 3,4-dihydroxyphenylalanine (dopa) (1). In man, a major function of melanin is the protection of the skin and underlying tissues from the harmful effects of solar radiation, and this function is promoted by donation of melanized granules to neighboring keratinocytes.

Tyrosinase is widely distributed in nature; it has been detected or measured in the skin of many species (2) and purified to homogeneity from frog skin (3), mouse melanoma (4), and hamster melanoma (5). Measurements of tyrosinase in human skin (2, 6) have been few, and no systematic study of large numbers of individuals has been reported. In addition there are no data concerning possible differences in enzyme levels between black and white individuals.

This paper deals with (*a*) the enzymatic properties of human skin tyrosinase; (*b*) quantitative measurements of this enzyme in foreskins from newborn babies; (*c*) comparison of this enzyme's activities in black and white babies; and (*d*) an examination of changes in tyrosinase activity during the first days of life.

METHODS

Foreskins were obtained from babies circumcised in the newborn nurseries of the University Hospital, Sinai Hospital, and Mercy Hospital, all in Baltimore. Before circumcision the skins were prepared with Phisohex, Winthrop Labs, New York (University of Maryland) or Betadine, Purdue Fredrick, Norwalk, Conn. (Sinai and Mercy) followed by a saline wash. No local anesthetic was used. After

TABLE I

Time-Course, Dopa Requirement, and Proportionality with Respect to Tyrosinase Concentrations

Volume of homogenate	Dopa	Time of incubation	Tyrosinase activity
ml	*0.12 mM*	*h*	*μmol ^{3}HOH*
0.50	+	1	0.0075
0.50	+	4	0.0316
0.50	+	5	0.0405
0.50	−	4	0.0040
0.25	+	4	0.0137
1.00	+	4	0.0550

Pooled foreskins from black and white babies (2.46 g; nine skins) were homogenized in 3 vol of 0.02 M sodium pyrophosphate buffer, pH 7.4. The conditions of the assay are given in the Methods section; variations in the volume of homogenate, the absence of dopa, and incubation times are given in the Table. The results have been corrected for the blank reaction.

circumcision each skin was placed in a vial with 3–4 ml of 0.15 M NaCl and stored in a refrigerator until collected and frozen at −20°C. Skins that were pooled for kinetic studies were sometimes refrigerated for 3–4 days in saline. Of the 169 foreskins from black babies for which individual analyses are reported, 122 were frozen after 1–9 h of refrigeration while the remainder were frozen after 24–36 h of refrigeration. The mean activities of the two groups were nearly identical so they were pooled. All of the 82 foreskins from white babies for which individual analyses are reported were frozen after 1–9 h of refrigeration.

The race of each child was taken to be the race of the mother as obtained from hospital records. For the individual skin analyses reported, the hours and dates of birth and of circumcision were computed to the nearest hour.

Fat was trimmed from each skin, and the skin was weighed to the nearest milligram (range, 40–250 mg). Each skin was minced thoroughly with a scissors and homogenized at 1-min intervals for 3 min in 1.3 ml of 0.02 M sodium pyrophosphate, pH 7.4, in a Sorvall microhomogenizer chilled in ice (Ivan Sorvall Inc., Norwalk, Conn.). Tyrosinase was measured by a modification of the tyrosine hydroxylation assay previously described (7). The incubation was for 5 h at 37°C, and the solution contained L-[3,5-^{3}H]tyrosine (1 μmol, 1 to 2×10^6 dpm); L-dopa (0.15 μmol); skin homogenate (1 ml, 20 μmol of sodium pyrophosphate, pH 7.4); in a total volume of 1.25 ml. The reaction was stopped with 0.1 ml of 20% HPO_3, and the ^{3}HOH formed was isolated by passage of the mixture through a 1.8×4-cm column of Dowex 50 (H^+) (Dow Chemical Co., Midland, Mich., 50–100 mesh). A total of 15 ml of H_2O was collected, and 0.5 ml was counted in a Packard Model 3300 liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Control studies showed that 90% of the radioactivity was collected in this volume, but the values have not been corrected for any loss. Counts were converted to disintegrations by use of external standardization. All reported values have been corrected for a blank reaction equivalent to about 0.5% of added radioactivity (0.01 μmol ^{3}HOH). To achieve this low blank the L-[3,5-^{3}H]tyrosine (purchased from Amersham/Searle Corp., Arlington Heights, Ill.) was first purified by high voltage paper electrophoresis (8), and the solution of purified tyrosine evaporated to dryness immediately before use. With few exceptions the total radioactivity measured for each sample was greater than three times that of the blank.

The data on individual skins are reported as μmol $h^{-1}g^{-1}$ skin, and the composite data are reported as means±SD. The significance of the difference in mean enzyme activities between blacks and whites was assessed by the Student *t* test. The regressions were computed by the University of Maryland Computer Center using programs from the SSP and SPSS manuals.

RESULTS

Several early experiments concerned the kinetic properties of human skin tyrosinase. The first four lines of data in Table I show that the reaction rate is constant through 5 h and that the enzyme is dependent upon dopa, as are the tyrosinases of animal skins (3, 7, 9) and melanoma (1). Dopa dependency is an important characteristic of tyrosinase and distinguishes it from

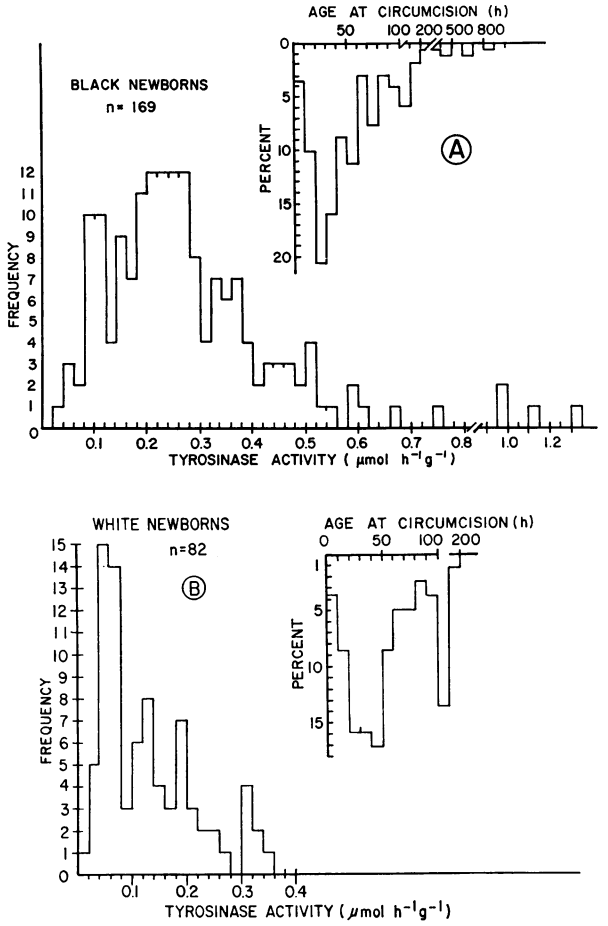

FIGURE 1 Frequency distribution of tyrosinase activities in foreskins from black (A) and white (B) newborns. The inserts show the distribution with respect to age at circumcision.

tyrosine hydroxylase which catalyzes the same conversion of tyrosine to dopa but uses a tetrahydropteridine cofactor (10). The data in the last two lines indicate that the reaction rate is dependent on enzyme concentration. The K_m for tyrosine was found to be 0.15 mM, about the same as that for melanoma (5) and hamster skin (7) enzymes.

Tyrosinase in individual foreskins was then measured as described in the Methods section, and its frequency distributions for black and white newborns are shown in Fig. 1. The mean enzyme activity for black babies (0.282±0.186) is about two and one-fourth times the mean for white babies (0.126±0.087) ($P << 0.001$). The age distributions at circumcision are also shown in these figures. Although the mean age at circumcision for black babies (68 h) is slightly older than the mean age of white babies (55 h), these differences are not significant. The peak of enzyme activity in black newborns is much broader than among white newborns.

Tyrosinase activities were analyzed further by constructing scatter diagrams of enzyme vs. age at circumcision for each race, as shown in Fig. 2; linear regressions, shown by the solid line in each graph, were also calculated for both races. The correlation coefficient between age and tyrosinase activity is 0.363 for white babies (Fig. 2B) and is highly significant ($P < 0.001$), indicating a tendency for tyrosinase activity to increase with age. However, the correlation coefficient between age and tyrosinase activity is 0.126 for black babies (Fig. 2A) and is not significant ($P = 0.1$). Inspection of Figs. 1A and 2A reveals four individuals with extremely high enzyme activities (mean = 1.106±0.184), possibly indicating membership in a distinct subset of the black population. If these are omitted from the calculation, the mean of the remaining 165 individuals is reduced to 0.263±0.134, and the regression is shown by the dashed line in Fig. 2A. The correlation coefficient between age and tyrosinase activity (0.212) is significant ($P < 0.01$) after this change.

Patel, Okun, Edelstein, and Cariglia (11) have reported data suggesting that peroxidase is responsible for the conversion of tyrosine to melanin in mouse melanoma homogenates. The possibility that the activity reported here was the result of peroxidase activity was examined by performing incubations in the presence or absence of catalase and hydrogen peroxide, as recorded in Table II. If peroxidase were responsible for the tyrosine hydroxylation reaction in skin homogenate, addition of catalase would remove endogenous hydrogen peroxide and thus inhibit the reaction, whereas addition of exogenous hydrogen peroxide might be expected to stimulate a peroxidase-catalyzed reaction. It is clear that there was no effect of catalase or hydrogen peroxide and thus no evidence that peroxidase is responsible for the tyrosine hydroxylation reaction rather than tyrosinase. This is in agreement with other reports clearly showing that tyrosinase is responsible for tyrosine hydroxylation in mouse melanoma and hair bulbs (12) and in mouse eye (13).

TABLE II

Effects of Catalase and Hydrogen Peroxide on Tyrosinase Activity

No.	Catalase	H_2O_2	Tyrosinase activity
	3.6 U	*33 μmol*	*μmol $h^{-1}g^{-1}$*
1	−	−	0.122
2	+	−	0.113
3	−	+	0.113
4	−	−	0.113

Pooled foreskins (3.0 g) from black and white babies were homogenized in 4 vol of 0.02 M sodium pyrophosphate buffer, pH 7.4. The conditions of the assay are given in the Methods section except that 0.90 ml of homogenate was used. Catalase (Worthington Biochemical Corp., Freehold, N. J.; 3.6 U) and hydrogen peroxide (33 μmol) were added where indicated. The incubation time was 6 h and the results are the averages of duplicate reactions after corrections for the blank reaction equivalent to 0.018 μmol $h^{-1}g^{-1}$.

DISCUSSION

This is the first detailed report on human skin tyrosinase. There are brief accounts of measurements in single samples of human breast and eyelid (2) and a recent measurement (6) of tyrosinase in skin from several regions of one person. The kinetic properties we observed are similar to those of mammalian tyrosinases from other sources. It is likely that there are several isoenzymes in human skin since these have been demonstrated in human (14) and animal (4, 5) melanomas and in the skin of several mouse mutants (15).

The mean tyrosinase activity for blacks is significantly higher ($P < 0.001$) than that for white newborns, and this may account partially for the greater skin pigmentation among blacks. Inasmuch as the distribution of the enzyme among blacks is very broad, however, there is a considerable overlap between the races. For the black population, the possibility exists that two or more overlapping distributions are contained within the single broad one. Additionally, the few individuals at the upper end of the enzyme distribution may be part of a group within the black population with extremely high levels of tyrosinase. It might be possible to use an additional variable, such as the intensity of pigmentation or melanin content of the skin, as an aid in further analysis. In preliminary studies we obtained light reflection measurements on many of the skin samples to obtain semiquantitative measurements of the intensity of pigmentation. However, there were several problems

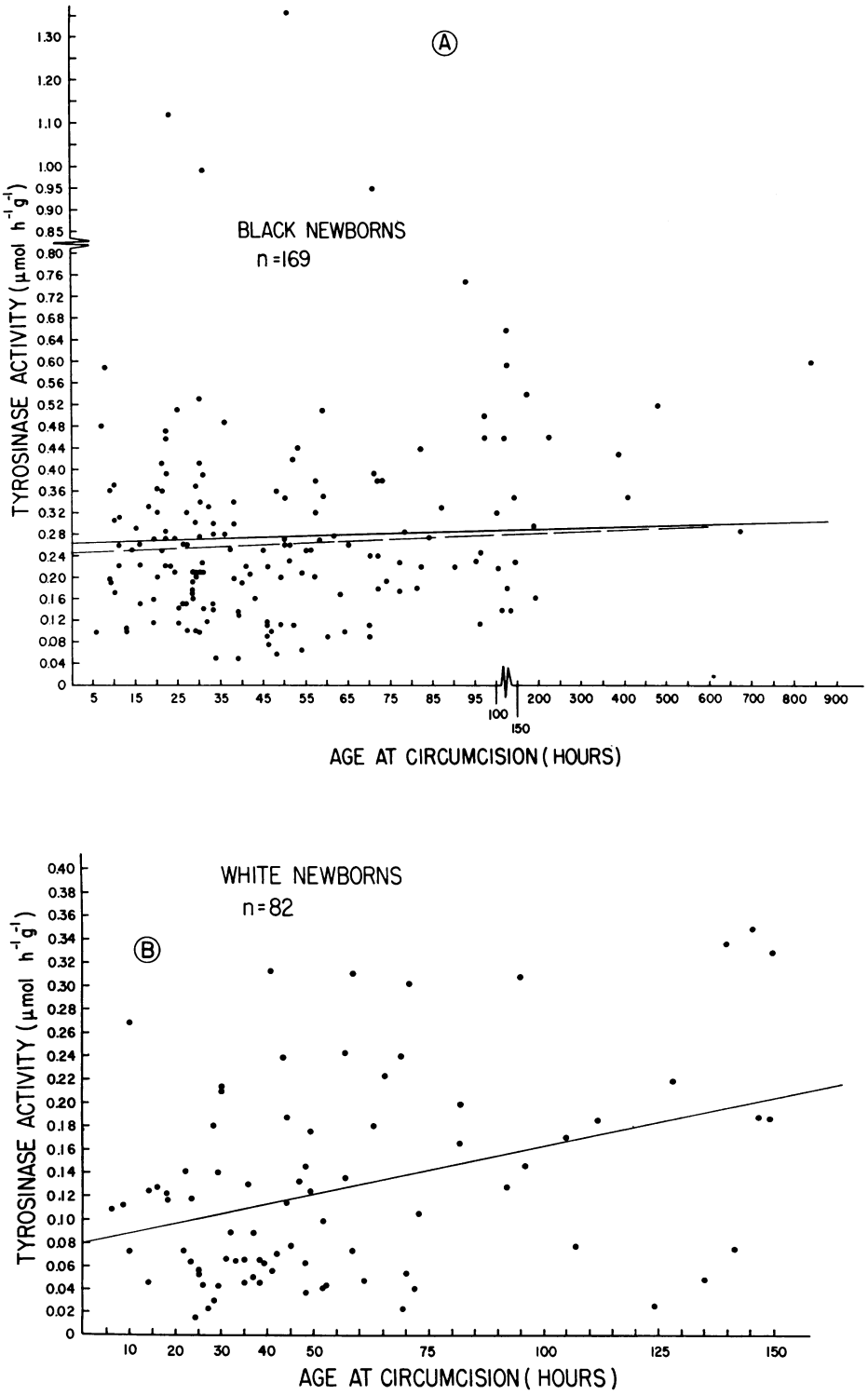

FIGURE 2 Scatter diagrams of tyrosinase activity vs. age at circumcision for black (A) and white (B) newborns. The regressions are shown as solid lines. The dashed line in (A) was the regression line obtained after omission of four individuals with tyrosine activities of 0.951, 0.991, 1.12, and 1.36 μmol $h^{-1}g^{-1}$.

with this approach: the lack of uniform pigmentation, interference by blood which could not be removed easily, and differences in the size of the foreskin.

There are factors other than the amount of tyrosinase that may account for some of the greater pigmentation among blacks. Szabo (16) has shown that melanosomes which have been transferred to keratinocytes occur singly in blacks, while in whites and orientals these melanosomes occur in groups enclosed by a membrane. Differences in fur color among mice are related in part to the size and shape of melanosomes (17, 18). A recent report (19) indicates that dark-skinned blacks have significantly larger melanosomes than whites or light-skinned blacks. Although there are no significant differences in melanocyte distribution between the different races, blacks have more melanosomes than whites (16). The latter correlates with the elevated levels of tyrosinase found in blacks.

The positive correlation between age and tyrosinase activity found for white and black newborns must be interpreted cautiously. Tyrosinase does increase dramatically in the skin of newborn hamsters (7) and mice (9), and this increase is correlated with the first burst of hair growth, the skin itself being largely unpigmented. Hair follicles are absent from foreskin so that increases in tyrosinase would be related to increases within the basal layer melanocytes. It is not known whether the increase in tyrosinase correlated with an increase in pigmentation.

Injection of β-MSH (melanocyte-stimulating hormone) into newborn hamsters and mice (9) increases tyrosinase levels significantly. Recently it was shown (20) that β-MSH levels are progressively elevated in the blood of women during pregnancy and that high levels of β-MSH are present in fetal chord blood and in amniotic fluid. Although it is uncertain whether β-MSH plays a physiological role in regulating tyrosinase, it is possible that the elevated levels of β-MSH in the fetus serve to induce fetal tyrosinase.

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REFERENCES

1. Pomerantz, S. H. 1966. The tyrosine hydroxylase activity of mammalian tyrosinase. *J. Biol. Chem.* **241**: 161–168.
2. Chen, Y. M., and W. Chavin. 1967. Comparative biochemical aspects of integumental and tumor tyrosinase activity in vertebrate melanogenesis. *In* The Pigmentary System. W. Montagna and F. Hu, editors. Pergamon Press, New York. 253–268.
3. Barisas, B. G., and J. S. McGuire. 1974. A proteolytically activated tyrosinase from frog epidermis. *J. Biol. Chem.* **249**: 3151–3156.
4. Burnett, J. B. 1971. The tyrosinases of mouse melanoma: isolation and molecular properties. *J. Biol. Chem.* **246**: 3079–3091.
5. Pomerantz, S. H., and J. P-C. Li. 1973. Purification and properties of tyrosinase isoenzymes from hamster melanoma. *Yale J. Biol. Med.* **46**: 541–552.
6. Chen, Y. M., and W. Chavin. 1974. Tyrosinase activity in a highly pigmented human melanoma and in Negro skin. *Proc. Soc. Exp. Biol. Med.* **145**: 695–698.
7. Pomerantz, S. H. 1969. L-Tyrosine-3,5-^{3}H assay for tyrosinase development in skin of newborn hamsters. *Science (Wash. D. C.).* **164**: 838–839.
8. Atfield, G. N., and C. J. O. R. Morris. 1961. Analytical separations by high-voltage paper electrophoresis. Amino acids in protein hydrolysates. *Biochem. J.* **81**: 606–614.
9. Pomerantz, S. H., and L. Chuang. 1970. Effects of β-MSH, cortisol, and ACTH on tyrosinase in the skin of newborn hamsters and mice. *Endocrinology.* **87**: 302–310.
10. Nagatsu, T., M. Levitt, and S. Udenfriend. 1964. Tyrosine hydroxylase: the initial step in norepinephrine biosynthesis. *J. Biol. Chem.* **239**: 2910–2917.
11. Patel, R. P., M. R. Okun, L. M. Edelstein, and N. Cariglia. 1974. Peroxidatic oxidation of tyrosine to melanin in supernatant of crude mouse melanoma homogenates. *Biochem. J.* **142**: 441–443.
12. Holstein, T. J., C. P. Stowell, W. C. Quevedo, Jr., R. M. Zarcaro, and T. C. Bienicki. 1973. Peroxidase, "protyrosinase," and the multiple forms of tyrosinase in mice. *Yale J. Biol. Med.* **46**: 560–571.
13. Hearing, V. J. 1973. Mammalian melanogenesis: tyrosinase versus peroxidase involvement and activation mechanism. *Arch. Biochem. Biophys.* **158**: 720–725.
14. Burnett, J. B., and H. Seiler. 1969. Multiple forms of tyrosinase from human melanoma. *J. Invest. Dermatol.* **52**: 199–203.
15. Holstein, T. J., W. C. Quevedo, Jr., and J. B. Burnett. 1971. Multiple forms of tyrosinase in rodents and lagomorphs with special reference to their genetic control in mice. *J. Exp. Zool.* **177**: 173–184.
16. Szabo, G. 1969. The biology of the pigment cell. *In* The Biological Basis of Medicine. E. E. Bittar and N. Bittar, editors. Academic Press, Inc., New York. 59–91.
17. Rittenhouse, E. 1968. Genetic effects on fine structure and development of pigment granules in mouse hair bulb melanocytes. I. The *b* and *d* loci. *Dev. Biol.* **17**: 351–365.
18. Rittenhouse, E. 1968. Genetic effects on fine structure and development of pigment granules in mouse hair bulb melanocytes. II. The *c* and *p* loci, and *ddpp* interaction. *Dev. Biol.* **17**: 366–381.
19. Olson, R. L., J. Gaylor, and M. A. Everett. 1973. Skin color, melanin, and erythema. *Arch. Dermatol.* **108**: 541–544.
20. Ances, I. G., and S. H. Pomerantz. 1974. Serum concentrations of β-melanocyte-stimulating hormone in human pregnancy. *Amer. J. Obstet. Gynecol.* **119**: 1062–1068.