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Production of Pigments Similar to Those from Hair from Amino Acid and Keratin Interaction. (19597)

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Arnow(1) found that a red pigment obtained from red human hair by continuous boiling with 0.1 N hydrochloric acid for 3 days or longer has the same absorption spectrum as solutions of oxidized (red) dopa melanin, and concluded that the red pigment is a natural oxidation product of melanin. However, Lee and Penrose(2) obtained a red pigment from the hair of a human albino by the same method and suggested that the color is not of natural origin, but is produced from some colorless chromogen in the hair.

Although soluble pigment is formed during the hydrolysis of the keratin in whole hair with strong mineral acids(3,4), neither isolated hair melanins nor solutions of hair keratin yield a soluble pigment on treatment with acid. This suggests that the soluble pigment may be the product of reactions involving intermediate keratin hydrolysis products. To determine whether keratin may be associated with pigment production from colorless chromogens in the hair, tyrosine and tryptophan were boiled in mineral acids with and without nail keratin and the resulting colors were compared. Tyrosine and tryptophan were used as chromogens since there is evidence that they may be precursors of mammalian epidermal pigments(5,6), and nail keratin because it is pigment free.

Experimental. Washed nail keratin was defatted with ether and weighed amounts were added to known concentrations of *L*-tyrosine and *L*-tryptophan in solution in 0.1 N and 6 N hydrochloric acid and 6 N sulfuric acid. The solutions were heated either continuously or intermittently under reflux for 15 hours or longer and were then allowed to stand in the light for 60 hours, during which time observations were made. Details of the treatments are summarized in Table I. Spectrophotometric readings of the red tryptophan-keratin solutions were made in a Beckman spectrophotometer. All readings were made at least 84 hours after the beginning of treatment. Chromatographic isolation of the tryptophan-keratin pigment was attempted. Adsorption on talc and Celite columns (1:1 by volume) produced sharp bands which were easily eluted with 0.2 N sodium hydroxide.

Results. The results of acid treatment are summarized in Table I. Keratin affects the color potentialities of both tyrosine and tryptophan when these are boiled with concentrated mineral acids (Exp. 2, 3, 5, and 6) and of tryptophan when boiled with dilute HCl (Exp. 2, 5, and 8). Color appears more rapidly in tryptophan solutions in HCl when keratin is present (Exp. 2, 5, and 8). There appears to be a threshold concentration for the production of color from tryptophan alone in 0.1 N HCl (Exp. 2 and 9). Black pigment formation from tyrosine is seen clearly only when tyrosine is treated with 6 N H_2SO_4 in the presence of keratin (Exp. 6) but slight color differences in 6 N HCl solutions (Exp.

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TABLE I. Differences in Pigment Production When Tyrosine and Tryptophan Are Boiled with Acid 15 Hr with and without Nail Keratin. The colors recorded are of solutions at 72 hr after beginning of treatment. Dash indicates absence of color. Numbers in parentheses indicate time in hr at which color was first observed.

Exp. No.	Material added, mg/ml solvent	Solvent		
		N/10 HCl	6 N HCl	6 N H_2SO_4
1	0	—	—	—
2	.5 mg tryptophan	—	Red-yellow, dark cast (5)	Bright yellow (2)
3	.5 mg tyrosine	—	—	—
4	2.5 mg keratin	—	Faint yellow* (5)	Faint yellow* (5)
5	.5 mg tryptophan and 2.5 mg keratin	Yellow (72)	Dark red-brown (2)	Red-brown; ppt of red-brown particles (2)
6	.5 mg tyrosine and 2.5 mg keratin	—	Faint yellow* with dark cast (5)	Yellow-brown; black ppt (2)
7	.25 mg tryptophan and .25 mg tyrosine	—	Red-yellow, darker cast than exp. 2 (5)	Red-brown, less red than exp. 5; flocculent red-brown ppt (10)
8	.25 mg tryptophan, .25 mg tyrosine and 2.5 mg keratin	Yellow (72)	Dark red-brown (2)	Red-brown with flocculent red-brown ppt; same as exp. 7 (2)
9	1 mg tryptophan	Yellow (72)	Yellow-red (4)	
10	.25 mg tryptophan and 1.25 mg hydrolyzed keratin (sol. 4)		No color change in 5 hr	No color change in 5 hr
11	.25 mg tyrosine and 1.25 mg hydrolyzed keratin (sol. 4)		Same	Same

* Yellow color due to the presence of hydrolyzed keratin in solution.

4 and 6) indicate that some pigment may be formed during this treatment. Completely hydrolyzed keratin does not induce rapid color changes as does unhydrolyzed keratin (Exp. 10 and 11).

Mixed solutions of keratin and tryptophan, both of which have been treated separately with 6 N HCl, show no distinct color changes before 9 days. Then the solutions have a purplish-blue color, which changes to red-brown within 15 minutes on heating. The bluish color resembles the transitory color which appears on the keratin particles during the first few minutes of boiling with tryptophan in 6 N HCl. This similarity, and the rapid color change in hydrolyzed keratin solutions on heating, suggest that similar reactions may occur in both solutions.

The absorption curve of the tryptophan-keratin solutions in 6 N HCl which was obtained using 6 N HCl as the standard is not appreciably different from that of either oxidized melanin or the red pigment obtained from hair by Arnow (Fig. 1). This suggests that the pigment from hair may be formed from some component, perhaps tryptophan, in the hair keratin during the prolonged acid treatment. The similarity of absorption pattern between solutions of the red pigment and of oxidized melanin may be due in part to some similarity of the light scattering particles in the 2 solutions.

Curves of both the 0.1 N and 6 N HCl solutions of tryptophan and keratin were obtained using as standards solutions containing the same concentrations of tryptophan and keratin, both of which had been treated separately. The 0.1 N acid solutions showed an absorption band with a sharp peak in the ultraviolet at 354 mμ, and the 6 N acid solutions showed a band with a maximum at 535 mμ and a minimum at 465 mμ (Fig. 2). Since solutions of keratin and tryptophan treated separately do not, when combined, show the same absorption as solutions having the same concentrations of keratin and tryptophan boiled together, an interaction product must be formed when the two are treated together which contains, in addition to the reddish brown pigment from tryptophan, some factor from keratin.

The interaction product resulting from

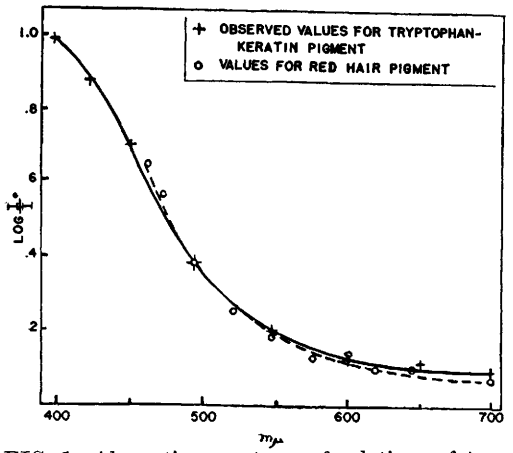

FIG. 1. Absorption spectrum of solutions of tryptophan and keratin treated with 6 N HCl obtained when read against the solvent (solid line) and the spectrum obtained by Arnow(1) for acid solutions of the red pigment extracted from red human hair (broken line).

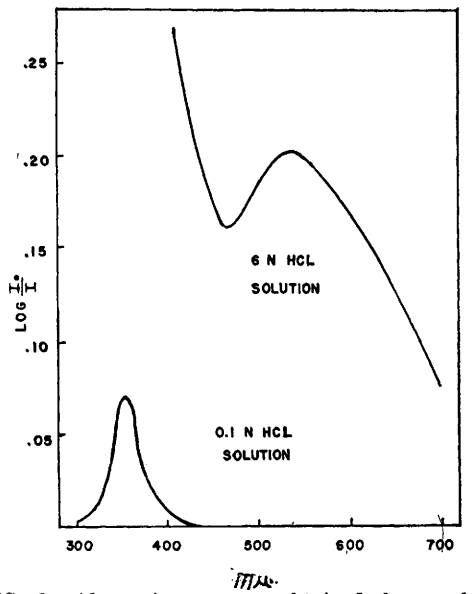

FIG. 2. Absorption spectra obtained from solutions of tryptophan and keratin treated together with .1 N and 6 N HCl when solutions containing the same concentrations of tryptophan and keratin, each of which has been treated separately with .1 N or 6 N acid, are used as standards.

treatment with 6 N acid has a spectrum showing the same points of maximum and minimum absorption as that of acid solutions of the reddish-purple phenolic iron pigment trichosiderin(7), which may be obtained from cortical granules of red human hair by heating with dilute mineral acids for short periods of time. Trichosiderin shows indicator properties and precipitates at neutrality(7,8). The tryptophan-keratin pigment contains no iron as indicated by negative thiocyanate and Prussian blue reactions, and the color is independent of pH.

The pigment isolated by chromatography has an amber color in 0.2 N NaOH. The color is independent of pH and the pigment does not precipitate at neutrality.

Summary. The soluble pigments produced when fat free nail keratin and tyrosine or tryptophan are boiled together with mineral acids differ from those produced when the keratin or amino acids are treated separately. The reaction involving tryptophan is enhanced by the simultaneous hydrolysis of keratin. Apparently keratin contributes some component(s) to the formation of pigment. The evidence favors the suggestion of Lee and Penrose that the red pigment obtained from human hair by the method of Arnow is not a natural pigment, but one produced from a colorless chromogen during the prolonged acid treatment. The pigment produced by the interaction of keratin and tryptophan in 6 N HCl shows an absorption spectrum similar to that of the pigment trichosiderin, but contains no iron. The similarity of the absorption spectra suggests that the organic portion of the natural pigment may be derived in part from tryptophan.

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