CLXX. THE ACID-SOLUBLE PIGMENT OF RED HUMAN HAIR

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The pigment responsible for the distinctive colour of human red hair appears to be unknown. Text-books contain conflicting statements in regard to the question. Morse [1927] states that "red hair is a diluted black". If this is true, it becomes difficult to explain the many shades of brown which various samples of hair exhibit. On the other hand, Bodansky [1934] states: "In addition to melanin, the presence of lipochrome has been described in skin and hair, to which is attributed the characteristic red coloration which is often seen in hair." The only lipochrome discovered by Tutschku [1923] in horse hair, however, had a yellow-green colour and was present in hair of all shades except white. Neither Morse nor Bodansky quotes literature references in support of the statement contained in his text.

Zwicky & Almasy [1935] have reported that the alkali-soluble pigments of red hair, black hair and melanomas of horses cannot be distinguished spectroscopically. The present author does not agree with this conclusion, since inspection of their data shows that the ratios of the extinction coefficients of the black hair extracts and the red hair extracts are not constant. That is, the two spectral curves cannot be made to coincide at any concentrations of the pigments.

Neumann [1937] has recently made a study of the pigments of rabbit hair. Three pigments, described as yellow, brown and black, were found. The yellow pigment has the lowest molecular weight and the lowest nitrogen content while the black pigment has the highest molecular weight and nitrogen content.

Experimental

Extraction of acid-soluble pigment. Samples of red human hair were thoroughly washed with successive portions of $0{\cdot}1\,N$ NaOH, $0{\cdot}1\,N$ HCl and distilled water. The hair was then partly dried between filter papers, after which it was extracted with boiling $0{\cdot}1\,N$ HCl for periods varying from 3 to 10 days. During the first 2 days the refluxing solution remained colourless; from the third day on, however, coloration was present. The extract was concentrated by boiling and filtered to remove the hair residue and some protein which precipitated. The filtrates were clear; dilute solutions were coloured reddish yellow and more concentrated solutions were red-brown.

In order to demonstrate that the pigment extracted by this procedure was not produced by the chemical treatment, samples of black hair and of synthetic dopa-melanin (prepared by the oxidation of dopa [Arnow, 1938]) were refluxed separately with $0{\cdot}1\,N$ HCl for varying lengths of time. The red pigment was never produced in these experiments. Apparently black hair does not contain an acid-soluble pigment, and such a pigment cannot be produced from dopa-melanin by prolonged boiling with dilute HCl.

Preparation of synthetic pigment. Solutions of dopa-melanin in N NaOH were placed in 75 ml. test tubes. Air was bubbled through these solutions for several days. Excess HCl was then added and the precipitated dopa-melanin was removed by centrifuging and filtration. The resulting solution resembled in colour the extract of red hair.

The pigment produced in this manner from dopa-melanin appears to be an oxidation product. Solutions of dopa-melanin in N NaOH which are kept in containers evacuated with a water pump are stable more or less indefinitely. Similar solutions exposed to air slowly produce the acid-soluble pigment.

The experiments of Dulière & Raper [1930] indicate that melanin can be oxidized with oxygen in alkaline solution. On theoretical grounds, the production of melanin from tyrosine by tyrosinase should require 5 O per mol. of tyrosine. At pH 6 this value was found, by the above authors, but 5·23 O were used at pH 8. Moreover, "if a few drops of 30 % KOH were added to the solution in the experimental flask after all enzyme action had ceased, a further oxygen uptake was observed. This occurred outside the limits of pH at which tyrosinase is active and was probably due to production of oxidation products of melanin itself by atmospheric oxygen."

Comparison of natural and synthetic pigments. (1) Both pigments are soluble in acid, neutral and alkaline solutions. The term "acid-soluble pigment" has been used in this paper to distinguish the coloured compounds from melanin (and perhaps other hair pigments), which is insoluble in dilute mineral acid solutions.

(2) Both pigments exhibit more colour in alkaline solution than in equal concentration in acid solution. The degree of acidity or alkalinity can vary within wide ranges without affecting the colour, provided that the concentration of pigment is unaltered.

(3) The visible absorption spectra of the two pigments in either acid or alkaline solution agree qualitatively within experimental error (see Fig. 1). The absolute concentrations are not known, since neither substance has yet been isolated in pure form. It is possible, however, to adjust the solutions to equal concentrations as determined either with the spectrophotometer or with a Duboscq colorimeter. A Bausch and Lomb spectrophotometer was used in making the measurements recorded in Fig. 1. In accordance with Lambert's law, the extinction coefficient is defined by the expression,

$$\text{extinction coefficient} = \frac{1}{d} \log_{10} \frac{I_0}{I},$$

where I is the intensity of light after passage through d cm. of solution, and I_0 is the intensity of the light entering the solution. According to Beer's law, for any given wave-length this extinction coefficient is proportional to concentration, if d is maintained constant. The absorption vessels used in making these measurements were 2·5 cm. long.

Fig. 1 shows the increase in colour in alkaline solution, since the concentration of pigment is the same in both solutions. The colour in acid solution is not qualitatively identical with that in alkaline solution. This is illustrated in Fig. 1 by a plot of the ratio of the extinction coefficients in alkaline solution to those in acid solution as a function of the wave-length. This would result in a straight line parallel with the abscissa if the colours were qualitatively identical.

(4) When acid solutions of the two pigments are shaken with n-butyl alcohol, a portion of the pigment enters the alcohol layer. If the experiment is

repeated, substituting alkaline solutions for acid solutions, no pigment enters the alcohol layer.

(5) Solutions of both pigments pass through dialysing membranes (Visking casing).

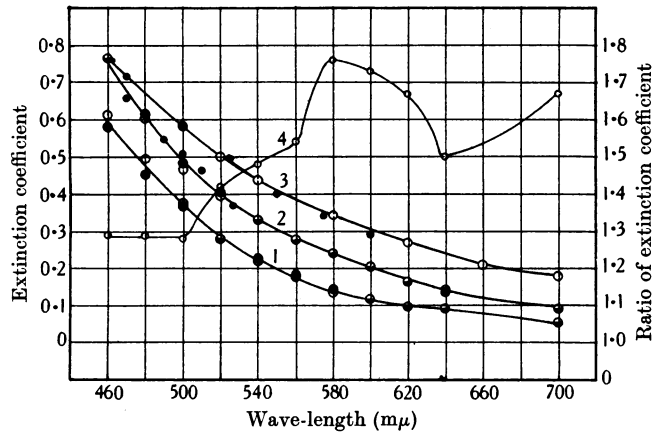

Fig. 1. Absorption spectra data.

Curve 1, pigments in hydrochloric acid solution.

- o solution of pigment extracted from red human hair.
- ● solution of synthetic pigment.
- • points calculated from data of Zwicky & Almasy [1935].

Curve 2, pigments in sodium hydroxide solution.

o, ●, • } As for curve 1.

Curve 3, dopa-melanin and black horse hair pigment in alkaline solution.

- o dopa-melanin in 0·02N NaOH (1·37 mg. per l.).
- • points calculated from data of Zwicky & Almasy [1935].

Curve 4, ratio of extinction coefficients of the pigment of red human hair (and of synthetic pigment) in alkaline solution and in acid solution.

Discussion

The evidence presented above suggests that the characteristic pigment of red human hair is an oxidation product of melanin. The strongest evidence of the identity of the synthetic and natural pigments is afforded by the spectral data. It is true that the evidence presented indicates only that the absorption spectra of the two pigments agree qualitatively with each other. It seems unlikely, however, that two chemically different substances could have the same qualitative spectra in both acid and alkaline solutions, particularly in view of the fact that the spectra are both quantitatively and qualitatively different in these solvents. In respect to the other physical properties recorded in this paper, the pigments also agree with each other.

The data of Zwicky & Almasy [1935], mentioned earlier, give some support to the present author's data. If the concentrations of the solutions studied by them are adjusted to those used in this paper by multiplying the extinction coefficients calculated from their data by a suitable constant, it is found that the spectral curve for the pigment of red horse hair agrees qualitatively with that for red human hair. This is indicated in Fig. 1, in which is also indicated the interesting fact that the spectral curve for the pigment of black horse hair agrees qualitatively with that for dopa-melanin. An inspection of these graphs will show that it is impossible to make the curve for dopa-melanin (or black horse hair pigment) coincide with that for an alkaline solution of the pigment of red hair (or oxidized dopa-melanin) by multiplication of the values of the ordinates by a constant. For this reason, the author cannot agree with the conclusion expressed by Zwicky & Almasy, that "Das schwarze Pigment der einen Art, das Pigment roter Haare...erwiesen sich im gelösten Zustand als spektroskopisch ununterscheidbar". The expression "der einen Art" is used because a pigment whose absorption spectrum was obviously different from that of melanin was found in certain samples of horse hair.

Some protein is extracted along with the acid-soluble pigment, as indicated by the fact that the hair extract gives the usual protein colour tests, whereas solutions of the pigment prepared from dopa-melanin do not.

The fact that the colour of the pigments deepens in alkaline solution suggests that the compounds are weakly acidic in character. The increased colour would then be explained by the increased ionization in alkaline solution. This view is strengthened by the finding that butyl alcohol will extract them from acid aqueous solution, but not from alkaline aqueous solution.

Summary

If red human hair is extracted with boiling $0{\cdot}1\,N$ hydrochloric acid, a red-brown pigment is obtained in solution. This pigment has the physical properties of a pigment prepared by the mild oxidation of dopa-melanin in alkaline solution. It is suggested that the distinctive colour of red hair is due to the presence in such hair of an oxidation product of melanin.

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