

THE CHEMISTRY OF MELANIN

III. MECHANISM OF THE OXIDATION OF DIHYDROXYPHENYLALANINE BY TYROSINASE*

By HOWARD S. MASON

(From the Office of Dermatology, Industrial Hygiene Division, Bureau of State Services, United States Public Health Service, Bethesda, Maryland)

(Received for publication, May 1, 1947)

When 3,4-dihydroxyphenylalanine is oxidized in the presence of phenol oxidases (2, 3) or by silver oxide (4), a red pigment is formed. Enzymically, this pigment is converted to a melanin. In the absence of enzyme, it has been shown that the pigment may rearrange to either 2-carboxy-5,6-dihydroxyindole or to 5,6-dihydroxyindole (5). The structure of the red pigment has therefore been inferred to be either 2-carboxy-2,3-dihydroindole-5,6-quinone or the tautomeric 2-carboxy-2,3-dihydro-6-hydroxyindole-1,5-quinonimine (5-7), and on this basis Raper has proposed the accompanying hypothesis for the *enzymic* conversion of 3,4-dihydroxyphenylalanine to synthetic dopa melanin (5, 6).

This study was undertaken to determine whether or not the postulated reactions beyond the formation of the red pigment do take place in the presence of oxygen and enzyme. The enzymic oxidation of 3,4-dihydroxyphenylalanine was followed spectrophotometrically. The process was observed to proceed in three chromophoric phases, the first corresponding to the formation of the red pigment, the second to an intermediate purple pigment, and the third to the formation of melanin. By comparison of the observed spectra with those of known substances, it was possible to show that a rearrangement of the red pigment does occur during the enzymic oxidation, that synthetic dihydroxyphenylalanine melanin is probably a polymer of indole-5,6-quinone, and that the inferred *o*-quinonoid formulation of the red pigment is correct.

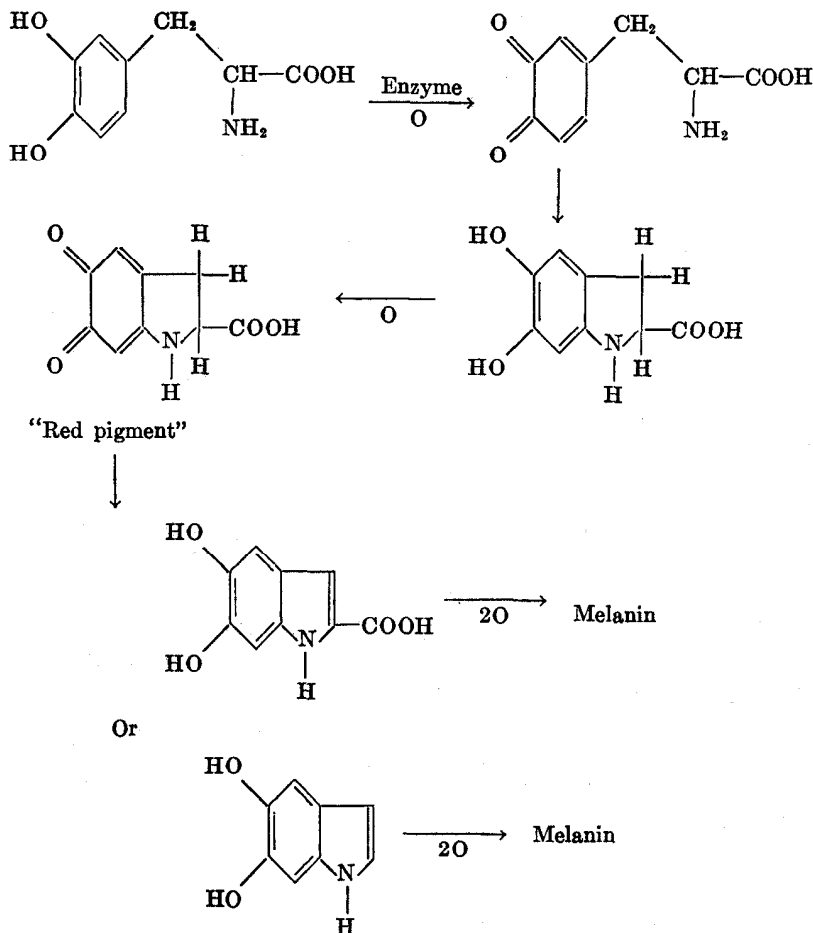
EXPERIMENTAL

The technique employed for observing the spectrophotometric course of the enzymic oxidation of dihydroxyphenylalanine has been described (1). A previously described tyrosinase preparation was used (1, *cf.* 8, 9). Silver oxide was prepared according to the directions of Helferich and Klein (10). 3,4-Dihydroxyphenyl-L-alanine was obtained from the Hoffmann-La Roche Company. It melted at 272-278° (total immersion);

* For Paper II in this series, see Mason (1).

$[\alpha]_D^{20} = -11.7^\circ$ in 1.0 N HCl (micro polarimeter tube). Buffers were prepared by adding 0.1 N NaOH to 50 ml. of 0.1 M KH_2PO_4 and diluting the mixture to 100 ml. All the experiments were conducted at temperatures of 25–28°.

Enzymic Formation of Melanin (Raper)



2-Carboxy-5,6-dimethoxyindole was synthesized from 6-nitrohomoveratrole by the procedure of Oxford and Raper (11). The product crystallized from acetone-benzene in tablets which melted at 208°. It has been reported to melt at 202–203° (11). The compound was further identified by elementary analysis.

*Analysis*¹—C₁₁H₁₁O₄N. Calculated, C 59.7, H 5.02, N 6.34
Found, “ 59.7, “ 5.24, “ 6.40

5,6-Dimethoxyindole was obtained from Professor H. S. Raper, whose kindness is acknowledged. The sample melted at 152–154°.

In this report optical density $D = \epsilon ml$, where l is 1.0 cm. and m is the given concentration of the absorbing substance in moles per liter. The molecular extinction coefficient ϵ corresponds to the calculated optical density of a 1 M solution.

Silver Oxide Oxidation of 3,4-Dihydroxyphenylalanine—The reaction between 50 mg. of silver oxide and 10 ml. of a solution buffered at pH 5.6 and containing 0.027 mg. (1.37×10^{-7} mole) of 3,4-dihydroxyphenylalanine per ml. was allowed to continue for periods varying from 0.5 to 10 minutes. In these experiments the reagents were shaken together for the designated period and the resultant solution filtered through No. 42 Whatman paper. Absorption spectra were then immediately measured. The results are depicted in Fig. 1, A and B. In the course of the reaction, the absorption maximum of 3,4-dihydroxyphenylalanine at 280 m μ was replaced by maxima at 305 and 475 m μ . The intensities of these maxima increased as the reaction period was increased to 3 to 4 minutes, then gradually decreased. Absorption spectra of substances intermediate between 3,4-dihydroxyphenylalanine and the red pigment did not become evident. The solvent buffer alone was shaken with silver oxide in the proportions described for 5 minutes. No change in the absorption spectrum was observed.

Rearrangement of Red Pigment—To determine the spectrochemical characteristics of the substances formed when the pigment rearranges, solutions were prepared by shaking together the reagents described above for 3 minutes, then filtering. Dissolved oxygen was rapidly removed by repeatedly exhausting the reaction vessel at an oil pump. After the solution had stood overnight, all color was discharged, although a small amount of black solid separated. This was removed on No. 42 Whatman paper and discarded. In the absorption spectrum of the filtered solution were found sharply defined maxima at 275 and 298 m μ , as are depicted in Fig. 2, A, Curve 2.

The red pigment was also observed to decolorize in the presence of acid. If a solution, prepared by the silver oxide reaction at pH 5.6, was adjusted to pH 1.3 with hydrochloric acid, decolorization was complete in 20 minutes. At pH 1.6 this process took 40 minutes and at pH 2.0 approximately 60 minutes. The absorption spectrum of the product formed at these pH

¹ I am indebted to Charles Kinser, National Institute of Health, for this microchemical analysis.

levels displayed a strong maximum at $310\text{ m}\mu$ (Fig. 2, A, Curve 3) which did not shift upon neutralization to pH 5.6. Solutions of the acid rearrangement product were also adjusted to pH 3.5 and 5.6 and allowed to stand 24 hours in the absence of oxygen. Small amounts of black pigment separated and were removed. The filtered solutions possessed the same

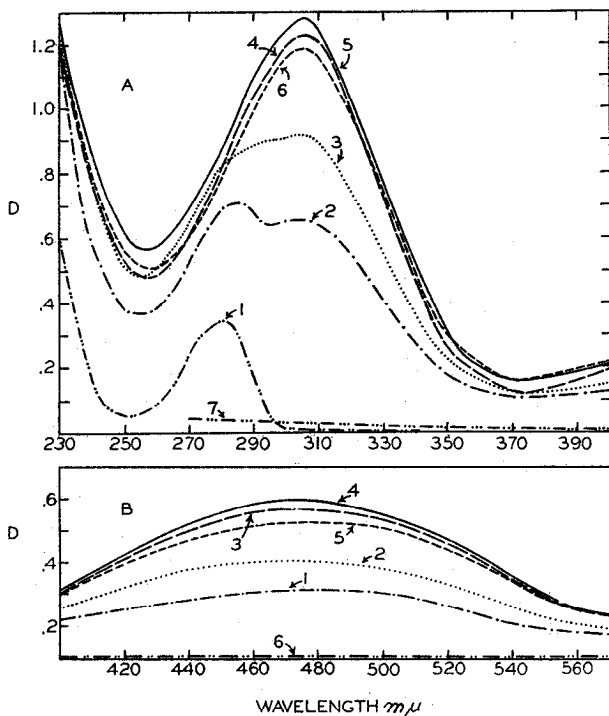


FIG. 1. The spectrophotometric course of the reaction between silver oxide and 3,4-dihydroxyphenylalanine. A, Curve 1 represents the spectrum of the original 3,4-dihydroxyphenylalanine solution, Curve 2 after 0.5 minute of reaction, Curve 3, 1 minute, Curve 4, 2 minutes, Curve 5, 3 and 4 minutes, Curve 6, 10 minutes, and Curve 7, 5 minutes of reaction between silver oxide and buffer alone. B, Curve 1 represents the spectrum observed after 0.5 minute of reaction, Curve 2, 1 minute, Curve 3, 2 minutes, Curve 4, 3 and 4 minutes, Curve 5, 10 minutes, and Curve 6, 5 minutes of reaction between silver oxide and buffer alone.

absorption spectrum as the freshly prepared rearrangement product, although the intensities of the maximum at $310\text{ m}\mu$ were lowered as much as one-third.

The rearrangement product giving the absorption spectrum with maxima at 275 and 298 $m\mu$ was found to be readily oxidized either by silver oxide or in the presence of tyrosinase and oxygen. In order to obtain the absorp-

tion spectrum of an intermediate product of oxidation, it proved necessary to conduct the reaction in the presence of a limited amount of enzyme, 6.5 units in 0.1 ml. added to 3.0 ml. of solution. This reaction was carried

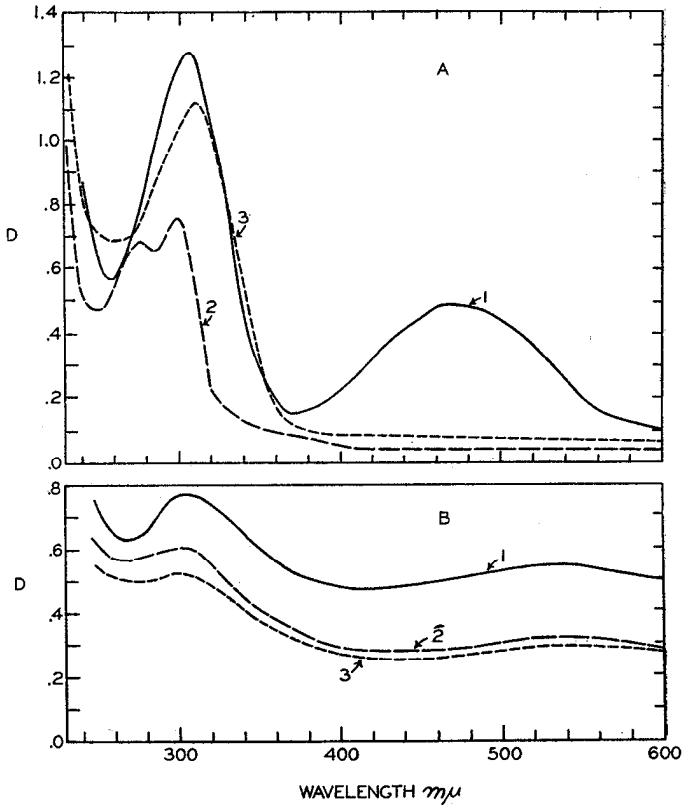


FIG. 2. Absorption spectra of intermediate oxidation products of 3,4-dihydroxyphenylalanine. A, Curve 1 represents the optimal spectrum obtained during the silver oxide-3,4-dihydroxyphenylalanine reaction, Curve 2 that observed 24 hours later, oxygen excluded, and Curve 3 that observed 20 minutes after the red pigment solution was brought to pH 1.3. B, Curve 1 represents the spectrum observed after 150 minutes of reaction between 3,4-dihydroxyphenylalanine and tyrosinase, Curve 2 that after 20 minutes of reaction between 5,6-dihydroxyindole (prepared stepwise from 3,4-dihydroxyphenylalanine) and tyrosinase at pH 5.6, and Curve 3 after 15 minutes reaction between the same reagents at pH 6.8.

out at pH 5.6 and 6.8. In each case a purple color developed and maxima appeared in the absorption spectrum at 300 and at 540 $m\mu$. These changes are illustrated in Fig. 2, B, Curves 2 and 3.

Enzymic Oxidation of 3,4-Dihydroxyphenylalanine—The concentration

of 3,4-dihydroxyphenylalanine solutions employed in this study was maintained at 0.027 mg. per ml. To 3.0 ml. of substrate solution were added

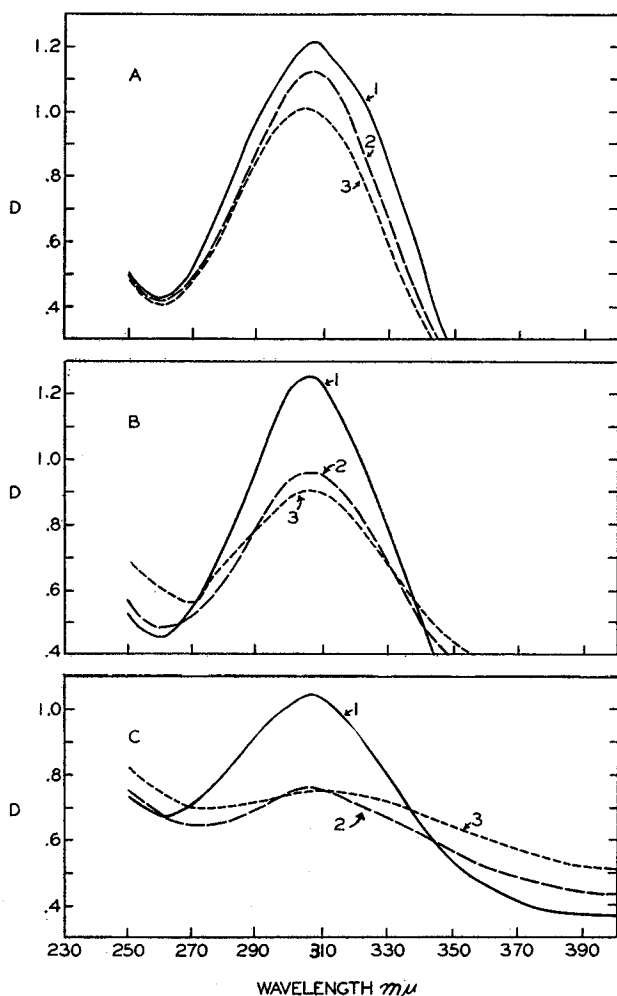


FIG. 3. The spectrophotometric course of the enzymic oxidation of 3,4-dihydroxyphenylalanine. The curves represent the spectra observed, *A*, after 10 minutes, *B*, after 25 minutes, and *C*, after 100 minutes of reaction at (Curve 1) pH 5.6, (Curve 2) pH 6.8, and (Curve 3) pH 8.4.

27 units of tyrosinase in 0.1 ml. The experiments were conducted at pH 5.6, 6.8, and 8.4. In Fig. 3, *A*, *B*, and *C* are presented the absorption spectra obtained after 10, 25, and 100 minutes of reaction. The rates of

change of density at $305\text{ m}\mu$ at the three pH values were also determined (25°) and the results are presented in Fig. 4. The absorption spectrum of the pigment obtained in this manner was the same as that prepared by the silver oxide reaction. No variation in the position of the maxima as a result of differences in pH was observed.

After some minutes of reaction general absorption became apparent in the spectra measured at each pH. However, at 150 minutes (in the

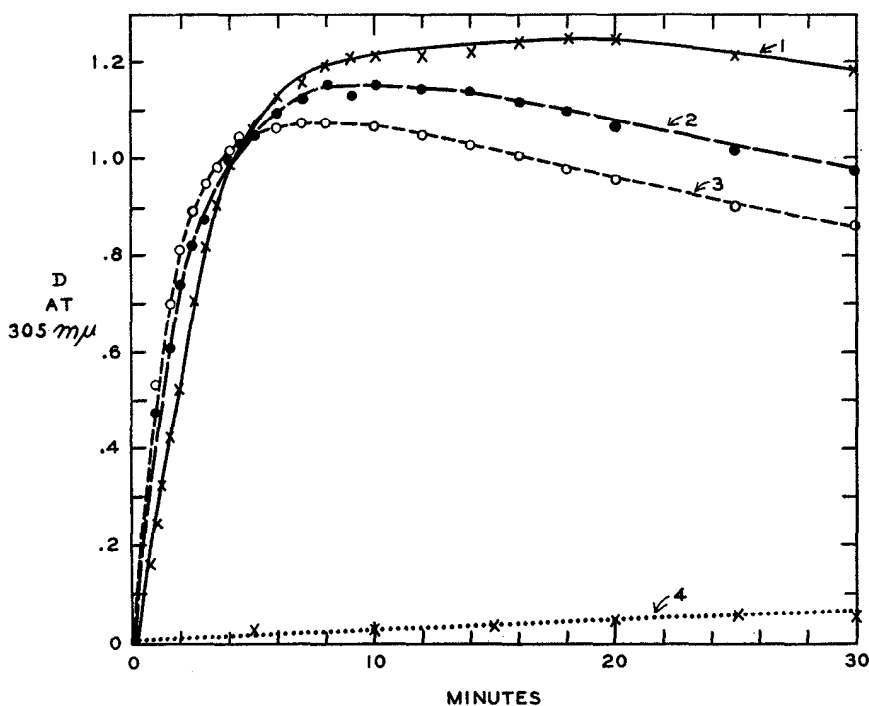


FIG. 4. The spectrophotometric course of the enzymic oxidation of 3,4-dihydroxyphenylalanine. The curves represent the optical density at $305\text{ m}\mu$ as a function of time when the reaction was conducted at (Curve 1) pH 5.6, (Curve 2) pH 6.8, and (Curve 3) pH 8.4. Curve 4 represents optical changes in the substrate solution at pH 8.4 in the absence of enzyme.

presence of tyrosinase) at pH 6.8, an intermediate phase showing maxima at 300 and at $540\text{ m}\mu$ developed. This is illustrated in Fig. 2, B, Curve 1. The absorption spectra of the reaction mixtures at each pH after 24 hours are depicted in Fig. 5.

The absorption spectra of 3,4-dihydroxyphenylalanine and the red pigment (silver oxide preparation) at pH 5.6 have been recalculated as

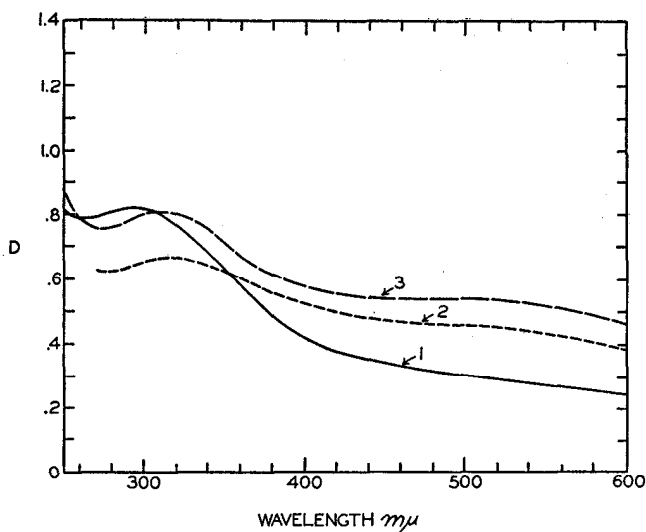


FIG. 5. Spectral curves of synthetic 3,4-dihydroxyphenylalanine melanins. The curves represent the spectra observed after 24 hours of reaction between 3,4-dihydroxyphenylalanine and tyrosinase at (Curve 1) pH 5.6, (Curve 2) pH 6.8, and (Curve 3) pH 8.4.

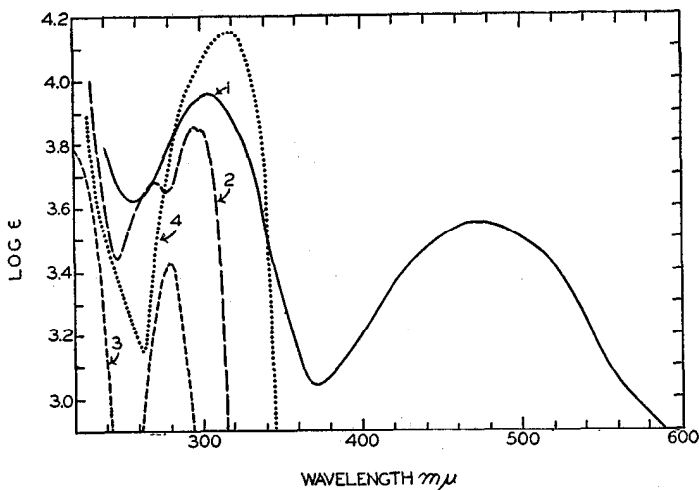


FIG. 6. Molecular extinction coefficients of (Curve 1) 2-carboxy-2,3-dihydroindole-5,6-quinone in buffer, pH 5.6 (silver oxide preparation), (Curve 2) 5,6-dimethoxyindole in ethanol, (Curve 3) 3,4-dihydroxyphenylalanine in buffer, pH 5.6, and (Curve 4) 2-carboxy-5,6-dimethoxyindole in ethanol.

molecular extinction coefficients and are presented in Fig. 6. To these have been added the absorption spectra of 5,6-dimethoxyindole and 2-carboxy-5,6-dimethoxyindole in ethanol.

DISCUSSION

The spectral changes accompanying the transformation of tyrosine or 3,4-dihydroxyphenylalanine into synthetic melanins have previously been studied by Bloch and Schaaf (12), Florence, Enselme, and Pozzi (13), and by Ginsburg (14). Absorption spectra characteristic of intermediate substances were not reported.

The absorption spectrum of 3,4-dihydroxyphenylalanine has been determined by a number of investigators. The values reported are summarized in Table I. In the present study, the absorption spectrum of

TABLE I
Absorption Spectrum of 3,4-Dihydroxyphenylalanine

Bibliographic reference	Solvent	Maximum <i>mμ</i>	Log ϵ
Bloch and Schaaf (12)	0.1 N NaHCO ₃ , pH 8.1	280.7	4.26
Florence and Bessières (15)	pH 1.0	282	4.09
	pH 11.6	303	4.61
de Gouveia, Coelho, and Schon (16)	0.1 N HCl	279.8	3.41
	0.1 N NaOH	298.3	3.60
Abderhalden and Rossner (17)	Water	278	3.39
Marchlewski and Skarzynski (18)	0.1 N HCl	280.8	3.42
Present investigation	pH 5.6-6.8	280	3.43

3,4-dihydroxyphenylalanine at pH 5.6 and 6.8, maximum = 280 $m\mu$, log ϵ = 3.43 (Fig. 6), did not show an inherent dependence upon hydrogen ion concentration. In view of the relative agreement between the values found and those reported by de Gouveia, Abderhalden, and Marchlewski, the values of Bloch, Schaaf, and Henri, and Florence and Bessières may reflect autoxidative degradation of the solute and the presence of substances other than 3,4-dihydroxyphenylalanine in solution. Under these circumstances, reversibility of the apparent pH effect is improbable.

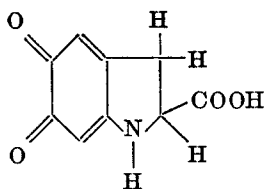
First Phase of Melanogenesis

The theory and assumptions underlying the use of kinetic spectrophotometry have been briefly discussed in another paper (19).

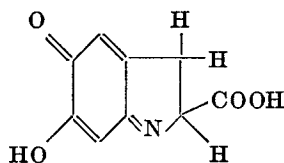
In the first spectrochemical phase of the enzymic transformation of

3,4-dihydroxyphenylalanine into a synthetic melanin, a red pigment with absorption maxima at 305 and 475 $m\mu$ accumulates. The rate of formation of this pigment increases with increasing hydroxide ion concentration (Fig. 4) within the limits investigated. The rate of formation has been shown to be dependent upon enzyme concentration ((1), and *cf.* (20)). The rate at which the pigment enters the subsequent reaction in the enzymic sequence leading to melanin also increases with increasing hydroxide ion concentration (Fig. 3, *A*, *B*, and *C*). This observation is in accord with Raper's findings (2) and suggests that at some pH lower than 5.6 (but higher than 2.0; see below) the pigment may be relatively stable. The first spectrochemical phase therefore arises because the red pigment forms more rapidly than it is consumed in the subsequent step. The failure of other intermediates to accumulate has been explained by Evans and Raper ((3) p. 2162).

The red pigment may exist in either of two postulated forms, 2-carboxy-2,3-dihydroindole-5,6-quinone (I, *a*) or 2-carboxy-2,3-dihydro-6-hydroxyindole-1,5-quinonimine (I, *b*) (5) (21). Inferences with respect to

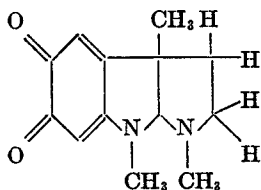


(Ia)

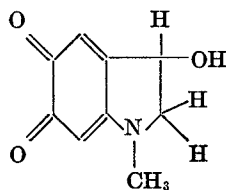


(Ib)

structure may be made by comparing the absorption spectrum of the pigment with those of substances which may be structurally related. Two such substances are rubreserine (II) and adrenochrome (III), in both of which a fixed 2,3-dihydroindole-5,6-quinone nucleus exists.



(II)



(III)

The absorption spectra of these compounds, together with those of the red pigment prepared by the silver oxide reaction and by the enzymic oxidation, and finally that of the presumably identical hallachrome (22) are presented in Table II. The positions of the maxima of the red pigment prepared by either procedure and those of rubreserine and adrenochrome

are almost identical. The intensities of absorption at these maxima of rubreserine and the red pigment are also almost identical. It is concluded that the observed spectrum of the pigment confirms the nuclear structure already proposed by Raper (5) and establishes the *o*-quinonoid tautomer as that predominating in the pH range 5.6 to 8.4.

Hallachrome is the red pigment isolated from the sea worm *Halla parthenopaea* by Mazza and Stolfi (22) and characterized by them as identical to the red intermediate pigment obtained by enzymic oxidation of 3,4-dihydroxyphenylalanine. However, it is now found that the reported

TABLE II
Absorption Spectra of Red Pigment and Related Compounds

Substance	Solvent	Maximum	Log ϵ	Maximum	Log ϵ
		$m\mu$		$m\mu$	
Red pigment (silver oxide reaction)	Buffer, pH 5.6	305	3.97*	475	3.54*
Red pigment (enzymic reaction)	" " 5.6	305	3.97*	475	3.57*
Red pigment (enzymic reaction)	" " 6.8 or 8.4	305	†	475	†
Rubreserine (23)	Water	300	3.97‡	480	3.44‡
Adrenochrome (23)	"	305	§	480	§
" (24)	"	310	§	285	§
Hallachrome (22)	Buffer, pH 5.6			539	4.21

The figures in parentheses are bibliographic references.

* Calculated on the basis of the structure proposed by Raper (5).

† Decomposition was too rapid at these hydrogen ion concentrations to permit determination of optimal molecular extinction coefficients.

‡ Calculated from data originally published in graphic form.

§ Extinction coefficients not reported.

|| Recalculated from data expressed in other terms.

absorption spectrum of hallachrome differs from that of the red pigment (Table I) in that it shows a maximum in the visible region at 539 $m\mu$.

Second Phase of Melanogenesis

The second spectrochemical phase of the enzymic transformation of 3,4-dihydroxyphenylalanine into melanin was too elusive to be observed at any but pH 6.8. At this pH level the spectrum of the red pigment disappeared and two new maxima, at 300 and at 540 $m\mu$, developed (Fig. 2, B, Curve 1).

If the sequence of reactions taking place in the presence of tyrosinase involves the rearrangement of the red pigment, the second phase spec-

trum should be identical with either that of the rearrangement product itself or with that of a substance derived from this product. The latter relationship was found to obtain. The rearrangement product, prepared by maintaining the red pigment at pH 5.6 in the absence of oxygen, possessed absorption maxima at 275 and 298 $m\mu$ with no absorption in the visible region (Fig. 2, A), sharply differing from the second phase spectrum. In similar but not identical experiments Raper (5) has shown that the rearrangement of the red pigment may yield 5,6-dihydroxyindole. In the present experiments the substance formed was identified as 5,6-dihydroxyindole by the similarity of its absorption spectrum to that of 5,6-dimethoxyindole (Table III), there being in general little difference

TABLE III

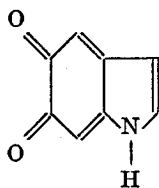
Absorption Spectra of Rearrangement Products of Red Pigment and Related Compounds

Substance	Solvent	Maximum <i>mμ</i>	Log ϵ	Maximum <i>mμ</i>	Log ϵ
5,6-Dimethoxyindole....	Ethanol	272	3.68	296	3.86
Rearrangement product (pH 5.6 reaction).....	Buffer, pH 5.6	275		298	
Rearrangement product (pH 1.3 reaction).....	" " 1.3	310			
Rearrangement product (pH 1.3 reaction).....	" " 5.6	310			
Rearrangement product (pH 1.3 reaction after standing 24 hrs. at pH 5.6).....	" " 5.6	310			
2-Carboxy-5,6-dimethoxyindole.....	Ethanol	319	4.15		

between the positions of the maxima of undissociated phenols and their methyl ethers (25, 26). These spectral curves are, furthermore, unlike those of 2-carboxy-5,6-dimethoxyindole (Fig. 6) and any of a variety of indole derivatives already reported (27-29).

However, when solutions of 5,6-dihydroxyindole, obtained by the rearrangement of 2-carboxy-2,3-dihydroindole-5,6-quinone at pH 5.6, are subjected to gentle oxidation in the presence of tyrosinase at either pH 5.6 or 6.8, a new absorption spectrum develops. It is characterized by maxima at 300 and 540 $m\mu$ (Fig. 2, B, Curves 2 and 3). The identity of these spectral curves with that characteristic of the second phase of tyrosinase oxidation of 3,4-dihydroxyphenylalanine (Curve 1) is evident. Differences in intensity are due to loss of substrate during the stepwise preparation of 5,6-dihydroxyindole.

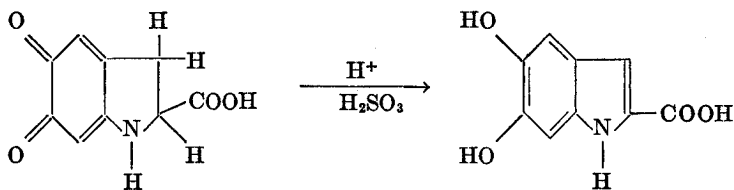
In view of the proved dehydrogenation of catechols to *o*-quinones in the presence of tyrosinase (30) and the catecholic nature of 5,6-dihydroxyindole, it may be inferred that the purple pigment possessing the absorption spectrum with maxima at 300 and 540 $m\mu$ is indole-5,6-quinone (IV) or its quinonimine tautomer.



(IV)

The second spectrochemical phase then arises because the purple pigment forms more rapidly than it is consumed in the subsequent step. In view of the instability of 2-carboxy-2,3-dihydroindole-5,6-quinone, these data also suggest that the absorption spectrum of hallachrome (Table II) measured by Mazza and Stolfi (22) was in fact that of indole-5,6-quinone. The chemical evidence in support of the structure of hallachrome which these investigators advanced remains, however, sufficient to establish the identity of hallachrome itself with 2-carboxy-2,3-dihydroindole-5,6-quinone.

When the rearrangement of 2-carboxy-2,3-dihydroindole-5,6-quinone takes place in acid solutions there is formed a product, the spectral curve of which (Fig. 2, A, Curve 3) is closely related to that of 2-carboxy-5,6-dimethoxyindole (Fig. 6, Curve 4). The formation of 2-carboxy-5,6-dihydroxyindole as a result of acid rearrangement is therefore indicated. This observation explains why reduction of a 2,3-dihydroindolequinone with sulfurous acid should yield a dihydroxyindole (5, 22):



The present evidence thus indicates that the rearrangement is subject to both hydroxide and hydrogen ion catalysis. The former mechanism is shown to lead to 5,6-dihydroxyindole and the latter to 2-carboxy-5,6-dihydroxyindole.

The identity of absorption spectra of 2-carboxy-5,6-dihydroxyindole at pH 1.3 and pH 5.6 (Table III) suggests that this substance does not

exist as a cation at either hydrogen ion concentration (26). The stability of its spectrum at pH 5.6 for 24 hours in the absence of oxygen (Table III), a condition under which the red pigment rearranges to 5,6-dihydroxyindole, proves that, during the rearrangement at pH 5.6, 2-carboxy-5,6-dihydroxyindole is not formed first, to undergo subsequent decarboxylation to 5,6-dihydroxyindole.

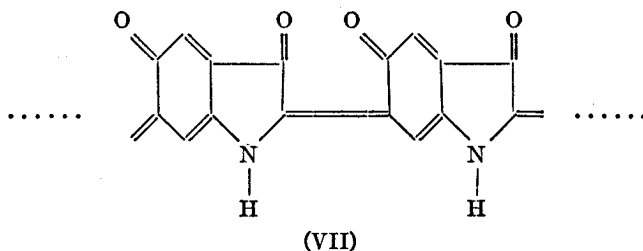
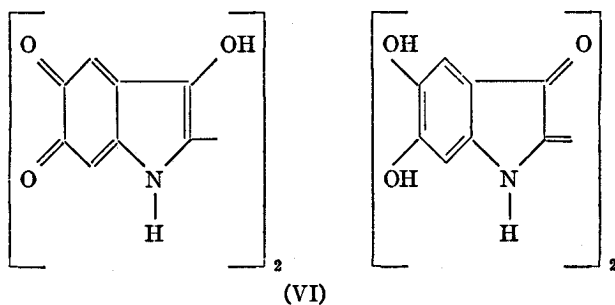
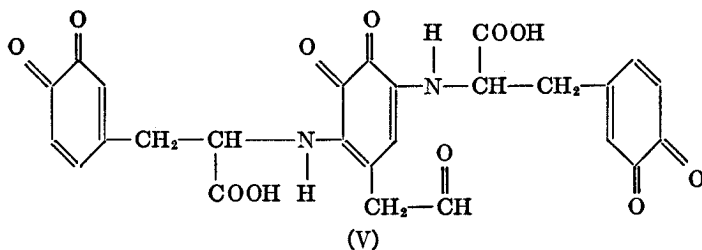
Third Phase of Melanogenesis

In the third spectrochemical phase of the enzymic formation of a melanin from 3,4-dihydroxyphenylalanine, general absorption became apparent at each of the three hydrogen ion concentrations investigated (Fig. 5). Some specific absorption was also observed in the ultraviolet region. The general absorption may best be interpreted by postulating that the resonating forms of the monomeric melanogen have coupled to produce distributed molecular sizes of a polymer containing one or more long systems of linear oscillators. Some scattering may also be present.

A variety of melanins, both synthetic and derived, have been reported to display only general absorption (12, 14, 31-35). Some positive conclusions have been drawn as a result of the rough similarities between the absorption spectra of these polymeric or high molecular weight materials (14, 34), but in view of the non-specificity of the absorption, the lack of chemical characterizations, and the colloidal nature of the absorbing substances, and, finally, because of the presence of undefined concomitant materials, it does not seem possible to refer similarities or differences in absorption spectra to similarities or differences in the structures of the pigments involved.

Several hypotheses have been advanced for the structure of the melanin formed by the enzymic oxidation of 3,4-dihydroxyphenylalanine. Bloch and Schaaf (12) suggested that the 3,4-quinone of phenylalanine undergoes intermolecular condensation with simultaneous deamination, leading to (V). This position is no longer tenable in view of the findings of Raper (5). Clemo and Weiss (36) suggest that 5,6,5',6'-tetrahydroxyindigo is formed from 5,6-dihydroxyindole by a non-enzymic oxidation of the heterocyclic ring, and that this substance, as its quinone or a molecular compound (VI), is an important constituent of melanin pigments. Cohen (37-39) has proposed that 5,6-dihydroxyindoxyl is formed from 5,6-dihydroxyindole and that melanin subsequently appears because of an indoxyl-isatin type condensation (VII). However, it has now been shown that 5,6-dihydroxyindole is readily oxidized in the presence of tyrosinase. Since the most probable product, indole-5,6-quinone, is a molecule possessing the quinone, amine, and pyrrole functions, and since there is considerable evidence establishing the reactivity of the first with the second

and third (40, 41), it now seems reasonable to regard indole-5,6-quinone as at least a bifunctional monomer capable of undergoing coupling with itself. The polymer thus arising, or its quinonoid oxidation product, would accordingly be synthetic dihydroxyphenylalanine melanin.



SUMMARY

1. The enzymic oxidation of 3,4-dihydroxyphenylalanine proceeds in three chromophoric phases which are characterized by absorption spectra with maxima at (a) 305 and 475 $m\mu$, (b) 300 and 540 $m\mu$, and by (c) general absorption.

2. The first chromophoric phase was found to correspond to the formation of 2-carboxy-2,3-dihydroindole-5,6-quinone. This tautomeric form of the substance was found to predominate in solution in the pH range investigated, 5.6 to 8.4.

3. 2-Carboxy-2,3-dihydroindole-5,6-quinone rearranges to 5,6-dihy-

droxyindole upon standing at pH 5.6 to 6.8. When 5,6-dihydroxyindole is enzymically oxidized, a product is obtained, the spectrum of which corresponds to that of the second phase of the enzymic oxidation of 3,4-dihydroxyphenylalanine.

4. 2-Carboxy-2,3-dihydroindole-5,6-quinone rearranges to 2-carboxy-5,6-dihydroxyindole upon standing at pH 1.3 to 2.0.

5. The spectral curves of melanins formed by the enzymic oxidation of 3,4-dihydroxyphenylalanine at pH 5.6, 6.8, and 8.4 display general absorption.

BIBLIOGRAPHY

1. Mason, H. S., *J. Biol. Chem.*, **168**, 433 (1947).
2. Raper, H. S., and Speakman, H. B., *Biochem. J.*, **20**, 69 (1926).
3. Evans, C. E., and Raper, H. S., *Biochem. J.*, **31**, 2155, 2162 (1937).
4. Duliere, W. L., and Raper, H. S., *Biochem. J.*, **24**, 239 (1930).
5. Raper, H. S., *Biochem. J.*, **21**, 89 (1927).
6. Raper, H. S., *J. Chem. Soc.*, 125 (1938).
7. Veer, W. L. C., *Rec. trav. chim. Pays-Bas*, **58**, 949 (1939).
8. Ludwig, B. J., and Nelson, J. M., *J. Am. Chem. Soc.*, **61**, 2601 (1939).
9. Miller, W. H., Mallette, M. F., Roth, L. J., and Dawson, C. R., *J. Am. Chem. Soc.*, **66**, 514 (1944).
10. Helferich, B., and Klein, W., *Ann. Chem.*, **450**, 225 (1926).
11. Oxford, A. E., and Raper, H. S., *J. Chem. Soc.*, 417 (1927).
12. Bloch, B., and Schaaf, F., *Biochem. Z.*, **162**, 181 (1925).
13. Florence, G., Enselme, J., and Pozzi, M., *Bull. Soc. chim. biol.*, **17**, 268 (1935).
14. Ginsburg, B., *Genetics*, **29**, 176 (1944).
15. Florence, G., and Bessières, S., *Bull. Soc. chim. biol.*, **28**, 657 (1946).
16. de Gouveia, A. J. A., Coelho, F. P., and Schon, K., *Rev. fac. cien., Univ. Coimbra*, **6**, 391 (1936-38).
17. Abderhalden, E., and Rossner, E., *Z. physiol. Chem.*, **176**, 249 (1928).
18. Marchlewski, L., and Skarzynski, B., *Bull. internat. Acad. polon. sc., Série A*, 241 (1929).
19. Mason, H. S., Schwartz, L., and Peterson, D. C., *J. Am. Chem. Soc.*, **67**, 1233 (1945).
20. Wright, C. I., and Mason, H. S., *J. Biol. Chem.*, **165**, 45 (1946).
21. Fieser, L., *J. Am. Chem. Soc.*, **50**, 439 (1928).
22. Mazza, F. P., and Stolfi, G., *Arch. Sc. Biol.*, **16**, 183 (1931).
23. Ellis, S., and Jones, R. N., *J. Pharmacol. and Exp. Therap.*, **79**, 364 (1943).
24. Braconier, F., le Bihan, H., and Beaudet, C., *Arch. internat. pharmacod.*, **69**, 181 (1943).
25. Anderson, L. C., and Geiger, M. B., *J. Am. Chem. Soc.*, **54**, 3064 (1932).
26. Jones, R. N., *J. Am. Chem. Soc.*, **67**, 2127 (1945).
27. Holiday, E. R., *Biochem. J.*, **30**, 1795 (1936).
28. Grinbaum, R., and Marchlewski, L., *Bull. internat. Acad. polon. sc., Série A*, 171 (1937).
29. Boryniec, A., and Marchlewski, L., *Bull. internat. Acad. polon. sc., Série A*, 392 (1931).

30. Pugh, C. E. M., and Raper, H. S., *Biochem. J.*, **21**, 1370 (1927).
31. Daniel, J., *J. Genetics*, **36**, 139 (1938).
32. Young, W. J., *Biochem. J.*, **8**, 460 (1914).
33. Baker, M. R., and Andrews, A. C., *Genetics*, **29**, 104 (1944).
34. Arnow, L. E., *Biochem. J.*, **32**, 1281 (1938).
35. Zwicky, H., and Almasy, F., *Biochem. Z.*, **281**, 103 (1935).
36. Clemo, G. R., and Weiss, J., *J. Chem. Soc.*, 702 (1945).
37. Cohen, G. N., *Bull. Soc. chim. biol.*, **28**, 104 (1946).
38. Cohen, G. N., *Bull. Soc. chim. biol.*, **28**, 107 (1946).
39. Cohen, G. N., *Bull. Soc. chim. biol.*, **28**, 354 (1946).
40. Gulland, J. M., *Biochem. J.*, **26**, 32 (1932).
41. Schmidt, J., and Sigwart, A., *Ber. chem. Ges.*, **46**, 1497 (1913).

**THE CHEMISTRY OF MELANIN: III.
MECHANISM OF THE OXIDATION OF
DIHYDROXYPHENYLALANINE BY
TYROSINASE**

Howard S. Mason

J. Biol. Chem. 1948, 172:83-99.

Access the most updated version of this article at
<http://www.jbc.org/content/172/1/83.citation>

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
<http://www.jbc.org/content/172/1/83.citation.full.html#ref-list-1>