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Determination of 5-S-Cysteinyldopa in Melanomas with a Fluorimetric Method

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Differences in color of the skin and hair of animals have been the subject of much research. Such differences have been ascribed partly to differences in the relative amounts of dark eumelanins, and lighter pigments, phaeomelanins, in the pigmented tissues. The chemistry of eumelanins has been extensively discussed in the classic monograph of Nicolaus (1). The early work of Prota and Nicolaus suggested that phaeomelanins were derived from tyrosine and cysteine (2). All later studies lent support to their view. At the VIIth International Pigment Cell Conference in Seattle in 1969, Prota presented the review "Structure and biogenesis of phaeomelanins" (3), which summarizes the very important work in this field from the Department of Organic Chemistry in the University of Naples.

In 1966 two catechols giving different fluorophores when oxidized were described in human melanomas (4–6). One of them proved to be dopa, while the other remained unidentified for many years. Recent work has shown that this second catechol is 5-S-cysteinyldopa (7–9), the compound considered to be the building-stone of phaeomelanins.

This review concerns studies on 5-S-cysteinyldopa, which suggest that determination of this thiocatechol may be helpful in the definition of pigment metabolism in health and in disease.

METHODS FOR DETECTING 5-S-CYSTEINYLDOPA

Fluorimetric methods are available for detecting 5-S-cysteinyldopa in biological specimens (7, 8). 5-S-cysteinyldopa is extracted with perchloric acid and is readily adsorbed to aluminum. It can be separated from other catechols by chromatography (8).

When treated with formaldehyde 5-S-cysteinyldopa forms a fluorophore differing in fluorescence from those formed by most other catechols (7, 8). Oxidation of

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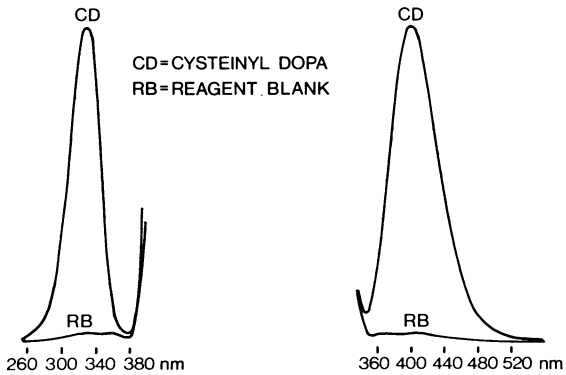

FIG. 1. Excitation and emission maxima of the fluorophore produced by iodine oxidation of 5-S-cysteinyldopa (*10*).

TABLE 1

SCHEDULE FOR DETERMINATION OF 5-S-CYSTEINYLDOPA (10)

Volumes in ml	Standard	Sample	Tissue blank	Reagent blank
Buffer, 0.2 *M* acetate buffer, pH 4.0	0.20	0.20	0.20	0.20
Eluate	—	0.20	0.20	—
Cysteinyldopa 0.5 μg/ml	0.20	—	—	—
H_2O	0.80	0.80	1.05	1.00
I. Iodine solution, 0.02 *N*[a]	0.05	0.05	—	0.05
II. 5 *N* NaOH added 2 min after I	0.20	0.20	0.20	0.20
III. 10 *N* acetic acid added 2 min after II	0.50	0.50	0.50	0.50
0.2 *M* sodium sulfite	0.20	0.20	—	0.20

[a] Dissolve 0.254 g iodine and 5 g KI in 100 ml H_2O.

5-S-cysteinyldopa according to Anton and Sayre (6) results in the formation of two different fluorophores (7–9). Extensive investigation of the conditions necessary for obtaining the two different fluorophores have led to the elaboration of methods for selectively producing the two fluorophores by the use of different oxidation procedures. The most sensitive and specific oxidation reaction results in the formation of a fluorophore with an excitation maximum at 325 and emission maximum at 405 nm (10) (see Fig. 1). The schedule for this 5-S-cysteinyldopa method is given in Table 1. Among 20 different catechol- and indolderivatives examined only a cysteinyldopa peptide was found to interfere with the 5-S-cysteinyldopa determination (10).

DOPA AND 5-S-CYSTEINYLDOPA IN THE NORMAL SKIN AND IN HAIRS OF DIFFERENT COLOR OF GUINEA PIGS

Black, white and yellow-red skin of multicolored guinea pigs was clipped with scissors and the hair was extracted with 0.4 *N* perchloric acid. The remaining hair was shaved off and the skin specimens were also extracted. The extracts were treated in the way described previously and dopa and 5-S-cysteinyldopa were determined fluorimetrically (6, 10).

TABLE 2

DOPA AND 5-S-CYSTEINYLDOPA IN THE SKIN AND HAIRS OF GUINEA PIGS

Tissue	Number	Dopa μg/g		5-S-cysteinyldopa μg/g	
		Mean value	Range	Mean value	Range
Red skin	4	0.10	0.05–0.15	0.25	0.19–0.41
Red hair	5	2.03	1.49–2.69	0.86	0.61–1.06
Black skin	3	0.09	0.04–0.12	0.04	0.02–0.06
Black hair	3	1.53	0.84–2.14	0.09	0.05–0.12
White skin	3	0.05	0.01–0.10	0.01	0.00–0.01
White hair	4	0.15	0.12–0.18	0.00	0.00–0.00

Table 2 gives the quantities of cysteinyldopa and dopa found extracted from the skin and hairs of the respective colors. It is evident that the amount of catechol in the white skin and the white hairs was very small. In the black skin the amounts of cysteinyldopa and dopa were rather small, but in the black hairs the dopa values were much higher than those found for 5-S-cysteinyldopa. The skin with red hair and the red hairs contained much more 5-S-cysteinyldopa than the black skin and black hair. The amounts of dopa, however, were similar to those in black skin and black hairs.

Dopa has previously been detected in the skin and in hairs of pigmented guinea pigs (11) while 5-S-cysteinyldopa has not previously been found in the normal tissues.

Dopa and cysteinyldopa are presumably present mainly in tissues containing melanin-forming organelles. The concentrations of catechols in the hairs were higher than in the skin consisting of dermis with hair follicles and epidermis. Pigment formation is minimal in the epidermal melanocytes in guinea pigs and the epidermis probably contained only a very minor portion of the catechols. The hair follicles with their rapid synthesis of pigment might be expected to contain catechols. The presence of catechols in the fully keratinized hairs may seem surprising, but it may, however, be explained by the assumption that the hair keratinocytes ingest premelanosomes together with the melanosomes.

5-S-CYSTEINYLDOPA IN HARDING–PASSEY MELANOMAS

Previous investigations have shown the presence of free dopa in Harding–Passey melanoma (12, 5). With the new fluorimetric method it has also been possible to demonstrate 5-S-cysteinyldopa in Harding–Passey tumors. The quantity of 5-S-cysteinyldopa extracted from 10 Harding–Passey melanomas was on the average 1.4 μg/g tissue, which is a concentration of the same order as that of dopa in these tumors.

CATECHOLS IN THE URINE IN MICE WITH HARDING–PASSEY MELANOMAS

Mice inoculated 3 wk previously with Harding–Passey melanomas showed a profound change in the urinary catechols (see Table 3). 5-S-cysteinyldopa was mark-

TABLE 3

DOPA, DOPAMINE (DA) AND 5-S-CYSTEINYLDOPA (CD) IN POOLED URINE OF 10 CONTROL MICE AND 10 MICE WITH HARDING–PASSEY MELANOMAS

	Dopa μg/ml	DA μg/ml	CD μg/ml
Controls	0.05	0.30	0.04
Melanoma animals	0.06	18	12

TABLE 4

5-S-CYSTEINYLDOPA (μg/g) IN MELANOMAS OF CAUCASIANS AND NEGROES (14, 15)

	Mean value	Range
Caucasians	18.5	0.6–61
Negroes	2.8	0.0–8.9

TABLE 5

DOPA, DOPAMINE AND 5-S-CYSTEINYLDOPA IN THE URINE OF HEALTHY SUBJECTS AND OF PATIENTS WITH MELANOMAS (16)

	Number	Dopa μg/ml	DA μg/ml	CD μg/ml
Controls	10	0.00–0.02	0.12–0.37	0.00–0.04
Patients with melanoma metastases	4	0.00–0.07	0.11–0.61	0.32–1.40
Patients with excised melanomas without metastases	2	0.00	0.12–0.17	0.02

edly increased and dopamine was present in very large amounts. Since Harding–Passey melanomas do not contain dopamine (5) the large amounts of dopamine in the urine of tumor-bearing animals must have been due to leakage of dopa and/or of tyrosinase from the tumors. Dopa, formed inside or outside the tumor reaching the kidney, should to a large extent be decarboxylated and excreted as dopamine or its oxidation products (13).

5-S-CYSTEINYLDOPA IN HUMAN MELANOMAS

5-S-cysteinyldopa has been demonstrated in melanomas from persons with red hair as well as in melanomas from persons with other complexions (9, 14). It is remarkable that this sulfur-containing catechol is present in melanomas in many Negroes (15), indicating the existence of a phaeomelanin pathway also in very dark subjects, at least under pathologic conditions. The mean values and ranges of 5-S-cysteinyldopa in melanomas of Caucasians and Negroes are given in Table 4. The highest concentrations were observed in Caucasians with red hair. Some melanomas in Negroes contained more 5-S-cysteinyldopa than did Caucasian melanomas, but in other Negro melanomas no cysteinyldopa was found.

5-S-CYSTEINYLDOPA IN THE URINE IN PATIENTS WITH MELANOMA METASTASES

The new fluorimetric method for determining 5-S-cysteinyldopa has made it possible to quantitate this substance in the urine. Table 5 gives the amounts of dopa, dopamine and cysteinyldopa in the urine of controls and of melanoma patients. The excretion of 5-S-cysteinyldopa is pronounced in patients with known melanoma metastases, but normal in those without clinical signs of metastases (16). Since the urinary excretion of 5-S-cysteinyldopa may be normal in the patients with amelanotic melanoma metastases, determination of 5-S-cysteinyldopa seems to be informative only in those cases where it is excreted in increased amounts.

Dopa is a substance, which, if present in large amount in the urine, may be a sign of the existence of malignant melanocytes, forming eumelanin. Increased urinary excretion of dopa (17–20) and dopamine (19, 20), the decarboxylation product of dopa, has been reported in patients with melanoma, but determination of 5-S-cysteinyldopa seems to be the most sensitive method for demonstrating the presence of melanoma in Caucasians, since the excretion of dopa and dopamine was normal in many of our patients with increased excretion of 5-S-cysteinyldopa.

DISCUSSION

The pigment chemists of Naples have produced convincing evidence that 5-S-cysteinyldopa plays an important role in the formation of phaeomelanin. The occurrence of this catechol in the body has now also been demonstrated.

The new oxidation method for determining 5-S-cysteinyldopa makes it possible to define the distribution of this catechol in normal and diseased pigment-forming tissues.

The method will certainly find many applications in pigment research. It will be helpful in analysing the pigment-forming system and then, not by studies of the pigmented end products which are often very difficult to decompose, but by quantitation of one of the important molecules forming the pigment. Classification of human and experimental melanomas on the basis of their relative amounts of dopa and 5-S-cysteinyldopa and investigations for any correlation with histologic and clinical findings would be welcome.

For problems related to pigment-cell genetics the method will open up new approaches to the analysis of the simultaneous formation of eumelanin and phaeomelanin. The possibilities of determining 5-S-cysteinyldopa in urine may be helpful in defining the pigment formation in a given person. The preliminary results in patients with melanoma metastases suggest that the analysis of 5-S-cysteinyldopa in the urine may be of practical clinical value in the diagnosis and follow-up of patients with melanoma.

SUMMARY

A sensitive fluorometric method for determination of 5-S-cysteinyldopa has been used in investigations of pigment formation.

The results indicate that this thiocatechol is an important intermediate substance in many types of pigment synthesis. Thus 5-S-cysteinyldopa is found in the hair

follicles and in the keratinized red and black hairs of guinea pigs. 5-S-cysteinyldopa occurs in Harding–Passey melanomas of mice as well as in the urine in tumor-bearing animals.

5-S-cysteinyldopa has been detected in all Caucasian melanomas examined. The highest concentrations have been found in melanomas in patients with red hair. Also many melanomas in Negroes contain 5-S-cysteinyldopa.

Determination of catechols in urine in patients with melanoma metastases has shown that 5-S-cysteinyldopa may be present in large amounts in spite of normal excretion of dopa and dopamine.

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