Urinary Excretion of 5-S-Cysteinyldopa and 6-Hydroxy-5-Methoxyindole-2-Carboxylic Acid: Differences between Pigmented and Albino Mice

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Urinary excretion of the phaeomelanin precursor 5-S-cysteinyldopa (5-S-CD) and the eumelanin metabolite 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI-2-C) was studied in black and albino mice. The urinary concentration of 5-S-CD was 31.7 ng/ml in black and 16.1 ng/ml in albino mice. The concentration of 6H5MI-2-C was 21.0 ng/ml in the urine of black mice. The compound could not be demonstrated in the urine of albino mice.
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New sensitive methods for determining melanin precursors have proved of great value in the study of melanocyte function (1). Determination of indolic compounds has long been used in the investigation of melanoma metastasis (2). With a recently described sensitive method for determination of 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI-2-C) it will become possible to determine the normal and the pathological excretions of this melanocytic metabolite (3). During the past decade the metabolism and excretion of 5-S-cysteinyldopa (5-S-CD), originally described as a phaeomelanin precursor (4), have been extensively investigated (1). In the present study we compare the urinary excretion of 5-S-CD and 6H5MI-2-C in pigmented and albino mice.

MATERIAL AND METHODS

Adult female black (C57 B1/6J) and albino mice (NMRI) were used. To get enough urine for analysis, a pooled sample was obtained from 6 mice kept in the same metabolic cage for 24 h. Specimens were collected in plastic bottles containing 0.1 g of sodium metabisulphite and 5 ml 20% (v/v) of acetic acid. Seven groups of black mice and 7 groups of albino mice were investigated.

The concentration of 6H5MI-2-C was determined by HPLC and fluorimetry (3, 5). The mobile phase contained 2.89 g of phosphoric acid/l in 22% aqueous methanol, pH 4.0. The urinary concentration was determined by comparison with an external 6H5MI-2-C standard solution. A mixture of sample and synthetic standard solution showed a single homogeneous peak at the expected retention time. The method allowed estimation of injected amounts corresponding to less than 2.2 ng/ml.

To determine the concentration of 5-S-L-cysteinyl-L-dopa, an internal standard of 5-S-L-cysteinyl-D-dopa was added to the crude urinary specimen after the 24-h sampling was terminated (6). Proteins were precipitated by adding 1/10 volume 4 M perchloric acid, and the sample was centrifuged at 17 000 r.p.m. for 10 min. The supernatant was purified on an ion-exchange resin (AG 50W X8) followed by a phenylboronic acid gel, eluted with 0.1 M trichloroacetic acid, and then determined by HPLC and electrochemical detection (7, 8).

RESULTS

The average volume of urine from 6 animals was 9.5 ml (range 8–12), and was similar in pigmented and albino mice.

The black mice excreted 31.7 ng 5-S-cysteinyldopa/ml, and the albinos 16.1 ng/ml (Table I).

6-hydroxy-5-methoxyindole-2-carboxylic acid in the pigmented animals amounted to 21.0 ng/ml. None of the 7 specimens from albino mice contained detectable amounts of 6H-5MI-2-C. The possibility was considered that the indole metabolite could be affected by the acid pH (range 3.37–3.55) (9). Four crude 24-h samples with no added acetic acid were therefore examined. The values for black mice were similar to those obtained by the standard procedure, and no 6H5MI-2-C was found in albino animals.

DISCUSSION

Urinary 5-S-cysteinyldopa is a sensitive marker of melanin production (1). The 5-S-CD excreted in urine of albino mice in the present study may originate in the melanocytes and be the result of tyrosinase activity not leading to pigment production. In the presence of high concentrations of cysteine, almost all dopaquinone formed is rapidly metabolized to cysteinyldopa and virtually no indoles are formed (10). It is possible that tyrosinase is present at sites in the melanocyte where the concentrations of thiols are so high that indole formation is prevented by nucleophilic addition of glutathione or cysteine to dopaquinone.

Table I. *Urinary excretion of 5-S-L-cysteinyl-L-dopa (5-S-CD) and 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI-2-C) in black and albino mice*

Colour	5-S-CD (ng/ml)	6H5MI-2-C (ng/ml)
Black (n=7)		
Mean	31.7	21.0
Range	5.5–62.9	15.2–43.9
Albino (n=7)		
Mean	16.1	0
Range	7.2–20.6	0–0

However, the present findings are also consistent with total absence of tyrosinase, because 5-S-CD has been demonstrated in tissues without known tyrosinase activity (11–14). A certain proportin of 5-S-CD in urine may therefore reflect extramelanocytic "non-specific" oxidation of dopa. Such oxidation would probably result in formation of glutathionedopas, which compounds are metabolized to cysteinyldopas (15).

A recent observation on the total absence of 6H5MI-2-C, but the presence of certain quantities of 5-S-CD in the urine of an albino patient suggests that 6H5MI-2-C more specifically than 5-S-CD reflects constitutional pigment production (16). The total absence of 6H5MI-2-C in albino mice in the present study supports the opinion that the excretion of this indole is a highly sensitive marker of melanin production.

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