

Urinary Excretion of 5-S-Cysteinyldopa and 6-Hydroxy-5-methoxyindole-2-carboxylic Acid in Children

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5-S-Cysteinyldopa (5SCD) and 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI2C) are formed during biosynthesis of melanins. They are used as indicators of pigment formation and markers of melanoma progression in adults and could possibly be used as markers of activity, growth and even malignant transformation in large pigmented naevi in children.

We investigated the urinary excretion of these pigment precursor metabolites from 136 children, 5 to 15 years of age. The mean 5SCD excretion was 38.1 $\mu\text{mol/mol}$ creatinine. A significant age-related decrease from a mean of 60.4 $\mu\text{mol/mol}$ creatinine at 5 years of age to 28.0 $\mu\text{mol/mol}$ creatinine at age 15 was found. In a reference group (29 adults, 20–33 years of age) the mean excretion was 48.9 $\mu\text{mol/mol}$ creatinine.

The mean excretion of 6H5MI2C was 42.8 $\mu\text{mol/mol}$ creatinine at 5 years of age and 26.1 $\mu\text{mol/mol}$ creatinine at the age of 15. The mean value for the young adults was 33.4 $\mu\text{mol/mol}$ creatinine. No correlation between the mean excretion of 5SCD and 6H5MI2C was demonstrated. We suggest an upper reference level of 90 $\mu\text{mol/mol}$ creatinine for the excretion of 5SCD in the age group 5–11 years and of 60 $\mu\text{mol/mol}$ creatinine in the age group 13–15 years. Corresponding figures for the indole 6H5MI2C are 70 and 60 $\mu\text{mol/mol}$ creatinine.

The establishment of reference values in children will make it possible to use 5SCD and 6H5MI2C measurements as diagnostic tools, indicating growth or malignant transformation in giant melanocytic naevi during childhood. *Key words: pigment precursors; melanoma; pigmentary disorders.*

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The colour of human skin is mainly determined by melanins in the skin, although other pigments such as carotenes and haemoglobins are of some importance. There are two main types of melanins formed in the melanocytes of the epidermis: eumelanin, which is a black or brownish pigment, and pheomelanin, which is a yellow-red pigment. In the synthesis of melanins (Fig. 1) the amino acid tyrosine is first hydroxylated to 3,4-dihydroxyphenylalanine by tyrosinase, which also catalyzes the further oxidation to dopaquinone. In the eumelanin pathway dopaquinone is oxidized to dopachrome, which is enzymatically transformed to 5,6-dihydroxyindole-2-carboxylic acid (DHICA) or decarboxylated and transformed to 5,6-dihydroxyindole (DHI). These indoles are then oxidized to indolequinone carboxylic acid and indolequinone and polymerized to eumelanin. The primary indoles are also methylated, which in the case of DHICA among other methylated compounds gives 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI2C). In the pheomelanin pathway 5-S-cysteinyldopa (5SCD) is formed by direct nucleophilic addition of cysteine to dopaquinone or indirectly from glutathione.

Cysteinyldopa is then oxidized to bensothiazine metabolites, which are polymerized to pheomelanin (ref 1 for review).

Both 5SCD and 6H5MI2C are excreted into the urine of healthy subjects, and the excretion increases substantially in response to natural sunlight or artificial UV light (2, 3). With few exceptions, malignant melanomas are pigmented tumours, and an increased urinary 5SCD excretion is an early and sensitive sign of melanoma dissemination. Therefore, 5SCD has been measured in plasma and urine in the follow-up of adults with malignant melanoma (4). The melanin metabolite 6H5MI2C might also serve as a marker for melanoma dissemination (5–7).

Malignant melanomas in children usually emanate from large pigmented congenital naevi (8, 9). Visual evaluation and palpation of such lesions are today the only instruments for detecting malignant transformation in such a lesion.

A recent paper (10) suggests that the serum level of 5SCD in children with giant pigmented naevi correlates with the size and the melanogenic activity in the naevus. Measuring of melanin-related metabolites might hopefully become a complementary method of monitoring activity, growth and even malignant transformation in large pigmented naevi. The normal limits for urinary melanogens have not yet been settled for children and young adults. This paper has established reference values for two of the melanin pigment precursor metabolites, 5SCD and 6H5MI2C, in the urine of children and adolescents.

MATERIAL AND METHODS

Subjects

In March 1995, an invitation to participate in this study was made to a day-care centre and to two schools in the Linköping area. The study was approved by the Ethical Committee of the Medical Faculty of the University of Linköping. Oral and written information was given to the children, and the parents gave their written consent. The drop-out was 50% in the group of 5-year-old children. Inability to give urine samples at a fixed time in the morning was the main reason for drop-outs in this group. Of the older children at least 75% participated from each age group. The reasons for drop-outs among these children were either absence from school on the sampling day or inability to deliver urine at a given time.

A total of 136 children (71 boys and 65 girls) participated. The mean age was 10.4 years (boys 10.0 years, girls 10.9 years). As a reference group 12 male and 18 female medical students were recruited (mean age: males 23.2 years, females 23.0 years). To be included in the study, subjects must not have been exposed to any strong sunlight 3 months prior to the study.

Some of the children were on asthma medication with terfenadine, β -receptor stimulators, or corticosteroids for inhalation. In the reference group, some of the females were on birth control pills.

The age, weight, height and hair colour of the subjects were recorded. Attempts to record skin type were also made. All urine samples were collected in March and April, a cold period when

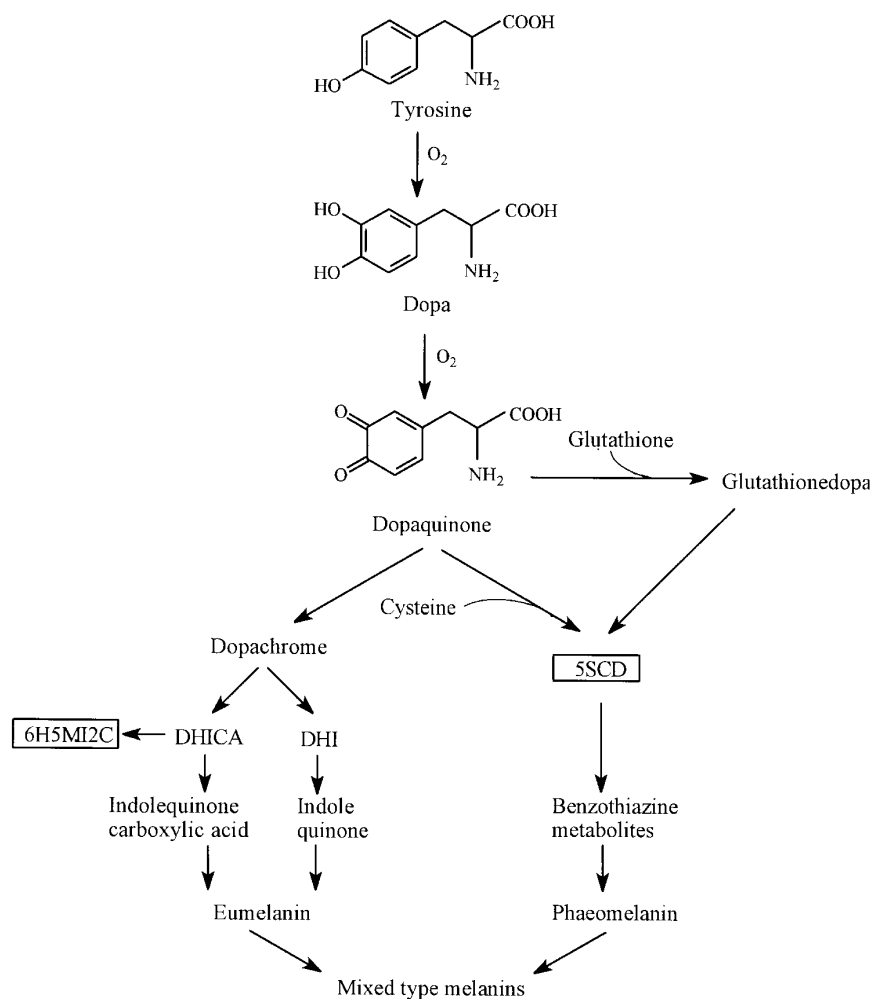


Fig. 1. Pathways of melanin synthesis. Compounds within frames were measured. *Dopa*: 3,4-dihydroxyphenylalanine, *5SCD*: 5-S-cysteinyl-dopa, *DHI*: 5,6-dihydroxyindole, *DHICA*: 5,6-dihydroxyindole-2-carboxylic acid, *6H5MI2C*: 5-hydroxy-6-methoxyindole-2-carboxylic acid.

children in Sweden are fully dressed, and also a period with at the best a few hours of weak sunshine per day.

Sampling procedure

All the samplings were made between 9 and 12 a.m. at the school nurse's office. After having been voided, the urine was directly transferred to 10-ml plastic tubes containing 0.25 ml of acetic acid, which improves the stability of the compounds, and stored at -23°C until analyzed (11). The analysis was carried out within 2 weeks.

Chemical analysis

5SCD and 6H5MI2C were determined by high-performance liquid chromatography, in the case of 5SCD with electrochemical detection (12) and in the case of 6H5MI2C with fluorometric detection (13).

Statistics

All calculations were performed using the StatView SE+graphics program. Since the distributions were not Gaussian, analysis of variance was used to evaluate differences in urinary excretion with age and sex. Simple regression tests were used to analyze possible relationships between different variables. A probability (*p*) value of 0.05 or less was considered statistically significant.

RESULTS

The age and sex distributions of the subjects are presented in Table I, together with the mean ($\pm$ SD) urinary concentrations of 5SCD, 6H5MI2C and creatinine. In Table II the excretions of 5SCD and 6H5MI2C are given per mol of creatinine. The mean concentration values of 5SCD and 6H5MI2C (Table I) did not differ much between the various groups, although the variations within the groups were substantial. With the youngest age group separate, the mean concentrations in the other age groups ranged from 0.31 to 0.44 $\mu\text{mol/l}$ for 5SCD and from 0.26 to 0.32 $\mu\text{mol/l}$ for 6H5MI2C. The concentration of creatinine increased from the lowest values in the youngest age group to a maximum at age 13 in both sexes and then declined. When the concentrations of 5SCD and 6H5MI2C were corrected for variations in water diuresis by dividing with the creatinine concentration, the mean 5SCD excretion ranged from 28.0 to 60.4 $\mu\text{mol/mol}$ creatinine and the mean 6H5MI2C from 25.9 to 44.4 $\mu\text{mol/mol}$ creatinine in the different age groups. The highest excretion values for 5SCD as well as for

Table I. Age and sex distribution of urinary concentrations of 5-S-cysteinylidopa, 6-hydroxy-5-methoxyindole-2-carboxylic acid and creatinine in young individuals

The results are given as mean $\pm$ SD.

Age year	Number of subjects		5-S-cysteinylidopa $\mu\text{mol/l}$			6-hydroxy-5-methoxyindole- $\mu\text{mol/l}$			Creatinine mmol/l		
	Female	Male	Female	Male	Total	Female	Male	Total	Female	Male	Total
5	1	8	0.20	0.31 $\pm$ 0.20	0.30 $\pm$ 0.19	0.16	0.18 $\pm$ 0.08	0.18 $\pm$ 0.08	4.5	5.1 $\pm$ 3.6	5.0 $\pm$ 3.4
7	10	10	0.31 $\pm$ 0.18	0.46 ^a $\pm$ 0.24	0.38 $\pm$ 0.22	0.26 $\pm$ 0.15	0.32 $\pm$ 0.14	0.28 $\pm$ 0.14	6.0 $\pm$ 3.0	6.9 $\pm$ 2.0	6.3 $\pm$ 2.5
9	19	16	0.35 $\pm$ 0.15	0.37 $\pm$ 0.21	0.36 $\pm$ 0.17	0.27 $\pm$ 0.17	0.26* $\pm$ 0.17	0.26 $\pm$ 0.17	8.2 $\pm$ 3.1	7.8 $\pm$ 2.1	8.1 $\pm$ 2.7
11	10	18	0.38 $\pm$ 0.27	0.37 $\pm$ 0.17	0.37 $\pm$ 0.21	0.24 $\pm$ 0.10	0.28 $\pm$ 0.13	0.27 $\pm$ 0.12	8.8 $\pm$ 2.9	9.9 $\pm$ 4.1	9.5 $\pm$ 3.7
13	11	12	0.42 $\pm$ 0.17	0.43 $\pm$ 0.22	0.42 $\pm$ 0.19	0.35* $\pm$ 0.10	0.29 ^c $\pm$ 0.16	0.32 $\pm$ 0.13	14.4 $\pm$ 4.7	12.1 $\pm$ 2.6	13.2 $\pm$ 3.8
15	14	7	0.32 ^b $\pm$ 0.16	0.29 $\pm$ 0.17	0.31 $\pm$ 0.16	0.31 $\pm$ 0.25	0.26 $\pm$ 0.16	0.29 $\pm$ 0.22	12.2 $\pm$ 7.2	10.0 $\pm$ 5.3	11.5 $\pm$ 6.5
23	18	12	0.46 $\pm$ 0.22	0.41 $\pm$ 0.27	0.44 $\pm$ 0.24	0.27 $\pm$ 0.16	0.29 $\pm$ 0.20	0.28 $\pm$ 0.18	10.1 $\pm$ 5.6	7.7 $\pm$ 4.7	9.1 $\pm$ 5.3

Extreme values deleted from the calculations were for 5SCD, ^a 1.24, ^b 1.40 $\mu\text{mol/l}$ respectively, and for 6H5MI2C, ^c 1.99 $\mu\text{mol/l}$.

* One missing value.

Table II. Age and sex distribution of urinary excretion of 5-S-cysteinylidopa and 6-hydroxy-5-methoxyindole-2-carboxylic acid in young individuals

The results are given as mean $\pm$ SD.

Age year	Number of subjects		5-S-cysteinylidopa $\mu\text{mol/mol creatinine}$			6-hydroxy-5-methoxyindole- 2-carboxylic acid $\mu\text{mol/mol creatinine}$		
	Female	Male	Female	Male	Total	Female	Male	Total
5	1	8	44.4	62.4 $\pm$ 16.0	60.4 $\pm$ 16.0	35.6	43.7 $\pm$ 11.8	42.8 $\pm$ 11.3
7	10	10	50.8 $\pm$ 9.1	66.1 ^a $\pm$ 14.5	58.0 $\pm$ 12.2	44.1 $\pm$ 12.4	44.8 $\pm$ 14.0	44.4 $\pm$ 13.3
9	19	16	43.7 $\pm$ 7.8	46.1 $\pm$ 12.0	44.8 $\pm$ 9.7	32.7 $\pm$ 10.6	33.2* $\pm$ 12.3	32.9 $\pm$ 11.3
11	10	18	41.4 $\pm$ 10.1	37.8 $\pm$ 8.0	39.1 $\pm$ 8.8	29.1 $\pm$ 8.3	30.0 $\pm$ 9.0	29.7 $\pm$ 8.7
13	11	12	29.8 $\pm$ 7.0	37.0 $\pm$ 15.5	33.6 $\pm$ 12.3	27.4* $\pm$ 6.8	24.5 ^d $\pm$ 7.6	25.9 $\pm$ 7.6
15	14	7	27.8 ^b $\pm$ 7.4	28.2 $\pm$ 6.5	28.0 $\pm$ 7.1	25.6 $\pm$ 6.6	27.1 $\pm$ 10.1	26.1 $\pm$ 7.6
23	18	12	49.5 $\pm$ 10.2	48.3 ^c $\pm$ 12.2	49.0 $\pm$ 11.1	29.2 $\pm$ 9.4	39.7 $\pm$ 9.8	33.4 $\pm$ 10.7

Extreme values deleted from the calculations were for 5SCD, ^a 258, ^b 318, ^c 143 $\mu\text{mol/mol creatinine}$, respectively, and for 6H5MI2C, ^d 146 $\mu\text{mol/mol creatinine}$.

* One missing value.

6H5MI2C were found in pre-school children and 7-year-olds. After this age the mean excretions of both metabolites decreased, to reach the lowest values in the 13- and 15-year-old children. In the adult group, mean age 23.1 years, the excretions were again higher. The age-related differences in the excretion of 5SCD and of 6H5MI2C were statistically significant, $p=0.001$ and $p=0.008$, respectively (Table II).

The excretion of 6H5MI2C in the adult group (Table II) was significantly higher ($p=0.02$) in males than in females, while there was no gender difference ($p=0.28$) in the 5SCD excretion of this group. No significant sex differences in the excretion of the two metabolites were found in the ages 5–15 years.

In this study of children we have observed a few extreme values of both 5SCD and 6H5MI2C (Tables I and II). Individual excretion values are presented in Fig. 2. No correlation was found between the individual excretion values of 5SCD and 6H5MI2C. When individual values were tested, neither 5SCD nor 6H5MI2C excretion was correlated to height, weight, body surface, skin type or hair colour of the subjects.

DISCUSSION

Reference values have been lacking regarding the urinary excretion of the two melanin-related metabolites 5SCD and 6H5MI2C in children. One reason for this may be the practical difficulties involved in collecting 24-h samples or blood samples. In order to overcome some of these difficulties we chose to collect spot samples of urine between 9 and 12 a.m. This is not an ideal sampling technique, since the results are influenced by variations in diuresis. Expressing the excretion values as a ratio to creatinine, measured in the same sample, can partly compensate for this adversity and has traditionally been done when analyzing biochemical substances in spot samples of urine. Thus, for example, Fang-Kircher (14) recently compared the sialic acid excretion in spot urines and 24-h urines of children and adults and found a good correlation when the sialic acid excretion was expressed as $\text{mmol/mol creatinine}$ in both samples. They also demonstrated that mean sialic acid excretion increased with age, from 179 $\mu\text{mol/24h}$ in 5-year-old children to 271 $\mu\text{mol/24h}$ (+51%) in children 13–15 years of age, but obviously the creatinine excretion increased

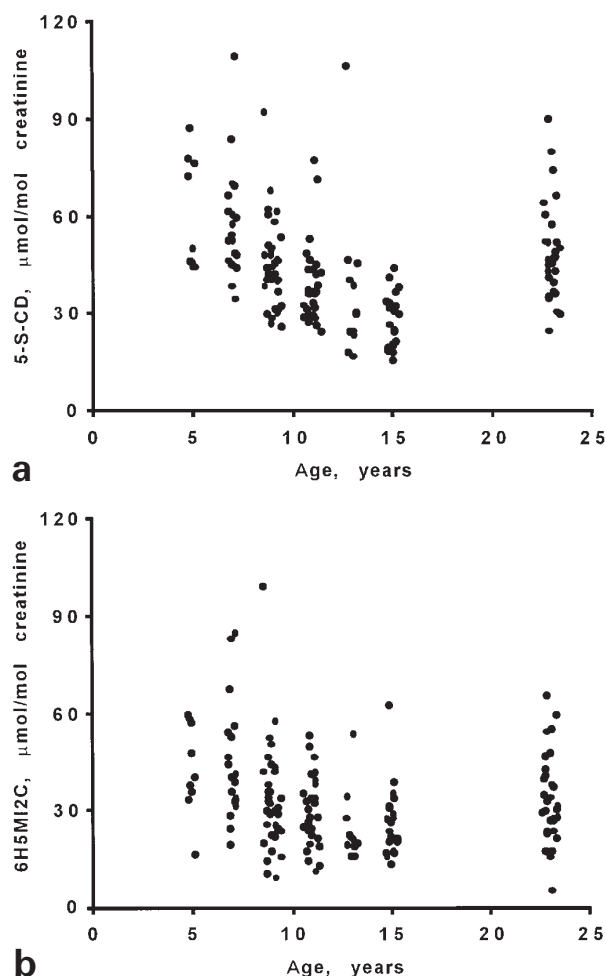


Fig. 2. Urinary excretions of (a) 5SCD and (b) 6H5MI2C in children of different ages and in a group of young adults. The extreme values (cf. Table II) are not plotted in the figure.

still more, since sialic acid excretion relative to creatinine decreased from 75 to 49 $\mu\text{mol/mol}$ creatinine (-35%) in the same age groups. This is a situation similar to ours, since the urinary creatinine concentration increased by the factor 2 from 5 to 13 years of age, while the concentrations of 5SCD and 6H5MI2C were roughly unchanged (Table I). The rather constant mean concentration of 5SCD and 6H5MI2C makes it tempting to use only the concentrations in the urine for evaluation of pigment production. However, since water diuresis varies individually, as well as with age, calculation of excretion per mol creatinine decreases the variation within groups. This can be seen if the coefficient of variation (CV) is calculated from the primary data as well as from the data relative to creatinine. Thus, in males and females taken together the range for CV was 45–63% and 41–75% for excretion of 5SCD and 6H5MI2C, respectively, in the different age groups, while expressed as $\mu\text{mol/mol}$ creatinine the corresponding ranges were 21–36 and 25–32%. Thus expressed per mol creatinine the reference ranges were narrower. However, the urinary creatinine excretion also varies between individuals

due to differences in muscle mass and different amounts of meat ingested. Therefore, the age-related variations in urinary excretion of 5SCD and 6H5MI2C, when expressed as a ratio to creatinine, might in part reflect variations in muscle mass. We are aware of the fact that our way of sampling the urine may lead to difficulties when comparing our data with the data from 24-h collections of urine in adults previously published (11). However, in spot samples from young adults 5SCD/creatinine levels were lower than 90 $\mu\text{mol/mol}$ and 6H5MI2C/creatinine levels below 70 $\mu\text{mol/mol}$, which is of the same order of magnitude as described in subjects 20–61 years of age in 24-h samplings (11). This is reassuring, and we believe that the spot sampling technique, particularly when collecting repeated urinary samples, is the most realistic one in clinical practice when dealing with children.

In this study we have observed three extreme 5SCD values and one extreme 6H5MI2C value (Table II). It is known from previous population studies that outlying values are to be found (15). By reanalysis of the samples we have excluded that analytical mistakes are responsible for the high values. Dark-skinned subjects with a high level of eumelanin formation in the skin have a higher production of 6H5MI2C than Caucasians (16), but the 5SCD excretion does not seem to be higher in dark-skinned subjects (17). In the population studied, one subject from India and one from Sri Lanka were included. One of them had a high urinary concentration of 6H5MI2C (146.3 $\mu\text{mol/mol}$ creatinine), which might be due to his dark complexion, but his 5SCD level was within the normal range (40.4 $\mu\text{mol/mol}$ creatinine). The other dark-skinned subject had normal excretions of both compounds. None of the other extreme values could be explained by recent sun exposure, red hair or any ongoing medication. The majority of the Caucasian children seemed to have a very sun-sensitive skin. With the exception of the above-mentioned 2 subjects, with skin type IV and V, a reliable skin-typing was not possible.

Hanawa et al. (10) measured serum 5SCD in young patients with giant pigmented naevi and compared their data with results from patients with small- or medium-sized congenital naevi and a control group of patients with non-melanocytic benign skin tumours. In both their comparison groups they found an inverse correlation between serum 5SCD and age. We believe that spot urinary samples can be used as well as serum samples for these purposes.

In view of our data it is reasonable to assume an upper level for the excretion of 5SCD in the age group 5–11 years of about 90 $\mu\text{mol/mol}$ creatinine and for the age range 13–15 years 60 $\mu\text{mol/mol}$ creatinine. Corresponding figures for the indole 6H5MI2C are 70 and 60 $\mu\text{mol/mol}$ creatinine. The exact upper level for a certain age is of less importance in clinical practice, since these children will be followed over a long period of time and therefore will serve as their own controls.

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REFERENCES

1. Prota G. Melanins and melanogenesis. London: Acad Press Inc; 1992.

2. Rorsman H, Agrup G, Falck B, Rosengren A-M, Rosengren E. Exposure to sunlight and urinary excretion of 5-S-cysteinyldopa. In: Riley V, ed. *Pigment cell*. Vol. 2. Basel: Karger; 1975. p. 284–289.
3. Stierner U, Rosdahl I, Augustsson A, Kågedal B. Urinary excretion of 5-S-cysteinyldopa in relation to skin type, UVB-induced erythema, and melanocyte proliferation in human skin. *J Invest Dermatol* 1988; 91: 506–510.
4. Agrup G, Agrup P, Andersson T, Hafström L, Hansson SC, Jacobsson S, et al. 5-Years' experience of 5-S-cysteinyldopa in melanoma diagnosis. *Acta Derm Venereol (Stockh)* 1979; 59: 381–388.
5. Wakamatsu K, Ito S, Fujita K. Melanin-related metabolites in urine of B16 melanoma-bearing mice. *Acta Derm Venereol (Stockh)* 1988; 68: 385–389.
6. Pavel S, Elzinga H, Muskiet FAJ, Smit JM, Mulder NH, Schraffordt Koops H. Eumelanin-related indolic compounds in the urine of treated melanoma patients. *J Clin Chem Clin Biochem* 1986; 24: 167–173.
7. Westerhof W, Pavel S, Kammeyer A, Beusenberg FD, Cormane R. Melanin-related metabolites as markers of the skin pigmentary system. *J Invest Dermatol* 1987; 89: 78–82.
8. Quaba AA, Wallace AF. The incidence of malignant melanoma (0–15 years of age) arising in "large" congenital nevocellular naevi. *Plast Reconstr Surg* 1986; 78: 174–179.
9. Swerdlow AJ, English JSC, Qiao Z. The risk of melanoma in patients with congenital naevi: A cohort study. *J Am Acad Dermatol* 1995; 32: 595–599.
10. Hanawa Y, Wakamatsu K, Ikeda S. Serum concentration of 5S-cysteinyldopa in pediatric patients with giant pigmented naevi. *J Dermatol* 1996; 23: 16–21.
11. Kågedal B, Lenner L, Årstrand K, Hansson C. The stability of 5-S-cysteinyldopa and 6-hydroxy-5-methoxyindole-2-carboxylic acid in human urine. *Pigment Cell Res* 1992; 2: 304–307.
12. Kågedal B, Källberg M, Årstrand K. Automated high-performance liquid chromatographic determination of 5-S-cysteinyl-3,4-dihydroxyphenylalanine in urine. *J Chromatogr* 1989; 473: 359–370.
13. Pavel S, Van der Slik W. Analysis of eumelanin-related indolic compounds in urine by HPLC with fluorimetric detection. *J Chromatogr* 1986; 375: 392–398.
14. Fang-Kircher SG. Comparison of sialic acids excretion in spot urines and 24-hour-urines of children and adults. *Eur J Clin Chem Clin Biochem* 1997; 35: 47–52.
15. Kågedal B, Sandström A, Tibbling G. Determination of a trivariate reference region for free thyroxine index, free triiodothyronine index, and thyrotropin from results obtained in a health survey of middle-aged women. *Clin Chem* 1978; 24: 1744–1750.
16. Wirestrand L-E, Hansson C, Rosengren E, Rorsman H. Melanocyte metabolites in the urine of people of different skin colour. *Acta Derm Venereol (Stockh)* 1985; 65: 345–348.
17. Agrup G, Hansson C, Juldorf F, Rorsman H, Rosengren A-M, Rosengren E. Excretion of 5-S-cysteinyldopa in humans with genetically dark skin. *Acta Derm Venereol (Stockh)* 1979; 59: 69–70.