

DOPA AND 5-S-CYSTEINYLDOPA IN THE URINE IN HEALTHY HUMANS

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Abstract. Chromatography and fluorimetric analysis of urine extracts of healthy subjects revealed the presence of two catechol derivatives with the characteristics of dopa and 5-S-cysteinyl-dopa.

Some authors have produced evidence of dopa in urine from healthy persons (3, 9, 10), while others have been unable to confirm its presence in such urine (1, 7, 8). Recent investigations have shown that 5-S-cysteinyl-dopa, a catechol derivative excreted in large amounts in the urine of patients with melanoma (2), may interfere with the fluorimetric determination of dopa (5, 6). Evidence for the excretion of minute amounts of cysteinyl-dopa in the urine in healthy persons has also been produced, although the substance has not been identified with certainty (2). It was therefore considered of interest to study urines from healthy subjects for dopa and 5-S-cysteinyl-dopa.

MATERIAL AND METHODS

Urine was collected for 24 hours from 3 healthy Caucasians (1 male aged 43, and 2 females, aged 6 and 27) in plastic bottles containing 25 ml acetic acid and 750 mg sodium metabisulphite. 20 ml aliquots of urine were adsorbed to Al_2O_3 and eluted with 0.4 N perchloric acid. Pooled eluates were again adsorbed to Al_2O_3 and eluted with perchloric acid. After new pooling and adsorption to Al_2O_3 a last elution with 0.1 N HCl was performed. The eluate then obtained was placed on an ion exchange column, 54 x 5 mm, containing Dowex 50W-X4, mesh size 200-400, in the H^+ form. Elution was performed with 40 ml 0.5 N HCl followed by 40 ml N HCl and finally 40 ml 2 N HCl. The effluent was collected in 5 ml fractions. Dopa and 5-S-cysteinyl-dopa were determined fluorimetrically in the way described previously (1, 6). With this procedure, authentic dopa has a peak in fraction 4 and authentic 5-S-cysteinyl-dopa in fraction 18. The fractions 3-5 were chromatographed on paper and developed in phenol:0.1 N HCl (90:10) and the spots were visualized with $K_3Fe(CN)_6$.

RESULTS AND COMMENTS

All three urines examined contained a catechol derivative, which on elution from the ion exchange column showed a peak in fraction No. 4 and which had the fluorescence characteristics of dopa. On paper chromatography this substance behaved like authentic dopa.

All the urines also contained a catechol derivative with the largest amount in fraction 18. After oxidation this substance displayed the fluorescence characteristics of the 5-S-cysteinyl-dopa-fluorophore.

The finding of catechols with typical oxidation fluorophores of dopa and 5-S-cysteinyl-dopa in fractions where these substances are found to appear in model experiments, is strong evidence for the occurrence of these catechol derivatives in normal urine. The amounts of dopa and 5-S-cysteinyl-dopa found were 0.010-0.014 and 0.002-0.012 $\mu g/ml$, respectively. The recoveries of dopa and of 5-S-cysteinyl-dopa were 35 and 37%, respectively.

It is not known to what extent the excretion of dopa reflects the pigment metabolism since this amino acid is formed not only by tyrosinase, but also by tyrosinehydroxylase in the neural tissues. Tyrosinase, on the other hand, seems to be necessary for the formation of 5-S-cysteinyl-dopa and the occurrence of this catechol derivative in the urine probably reflects the synthesis of certain melanins (4).

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