Evaluation of a clinically inapparent adrenal mass led to tests for pheochromocytoma (1) with findings of increased urinary metanephrine and normetanephrine but normal plasma metanephrines and catecholamines.

A 45-year-old woman presented with pulmonary embolism and thrombosis of the left arm. Her recent medical history included removal of the right breast because of cancer with postoperative locoregional radiotherapy and chemotherapy. She had a known spina bifida and an urostoma. She took pyridoxine, oxazepam, methemane, paracetamol, furosemide, and tamoxifen on a regular basis. On physical examination she had a normal blood pressure, no Cushing signs, and no evidence of tumor recurrence.

After treatment with fractionated heparin and acenocumarol the patient was further evaluated for the presence of tumor recurrence or distant metastases as an explanation for her thromboembolism. Mammography showed no signs of malignancy. The computed tomography (CT) scan of the thorax showed a pulmonary embolism but no evidence of metastases. The abdominal CT scan revealed a 2.5-cm hypodense mass in the right adrenal gland, most likely an adenoma. Because the differential diagnosis of adrenal masses with low attenuation on CT includes functional tumors, especially pheochromocytoma (2), the functionality of the adrenal mass was determined.

Cortisol was 0.09 and 0.07 μmol/L after a repeated 1-mg dexamethasone suppression test. The 24-h urinary excretion of cortisol was 37.9 nmol, which made the presence of glucocorticoid excess unlikely. Serum potassium was within reference values, and renin and aldosterone were not increased, which together with the normal blood pressure excluded primary aldosteronism. Urinary excretion of metanephrines, measured as described previously (3), was increased on repeated occasions [normetanephrine (NMN), 36 450 nmol/24 h (reference values <2900 nmol/24 h); metanephrine (MN), 10 256 nmol/24 h (reference values <250 nmol/24 h)]. Because of the patient’s impaired mobility and anxiety, no metadobenzylguanidine scan was performed.

Close inspection of the urinary chromatograms of this patient revealed, in addition to an extra peak at an unusual location, a striking decrease (10- to 15-fold) in the excretion of metanephrine and normetanephrine (3), which may be preferred for the diagnosis of pheochromocytoma (4), and plasma catecholamines were not clearly increased [NMN, 312 pmol/L (reference values <300 pmol/L); noradrenaline, 0.04 nmol/L (reference values <0.3 nmol/L); noradrenaline, 2.84 nmol/L (reference values <3.0 nmol/L)]. Because of the patient’s impaired mobility and anxiety, no metadobenzylguanidine scan was performed.

Pitfall in HPLC Assay for Urinary Metanephrines: An Unusual Type of Interference Caused by Methenamine Intake, Hanneke W.M. van Laarhoven,1 Jacques J. Willemensen,2 H. Alec Ross,2,5 Louk V.A.M. Beex,1 Jacques W.M. Lenders,4 and Fred C.G.J. Sweep2 (Departments of 1 Medical Oncology, 2 Chemical Endocrinology, 3 Endocrinology, and 4 General Internal Medicine, University Medical Centre Nijmegen, Nijmegen, The Netherlands; address correspondence to this author at: University Medical Centre Nijmegen, Department of Medical Oncology, PO Box 9101, 6500 HB Nijmegen, The Netherlands; fax 31-24-354-0788, e-mail h.vanlaarhoven@onco.umcn.nl)

DOI: 10.1373/clinchem.2003.028001
We hypothesized that interaction of a substance with the internal standard decreased the signal for the internal standard and that this substance might originate from the patient’s medications. To test this hypothesis the drugs used by the patient were dissolved in the same medium that was used for addition of the internal standard (0.1 mol/L HCl) and added to the urine of a healthy person. The quantities added to the urine sample corresponded to 200%, except for methenamine (100%), of the patient’s daily intake of furosemide (40 mg/day), acenocoumarol (2–3 mg/day), pyridoxine (20 mg/day), tamoxifen (20 mg/day), oxazepam (10 mg/day), and methenamine (3 g/day), distributed in a 24-h portion of urine, assuming complete clearance by the urine.

The resulting chromatograms are shown in Fig. 1A. We observed a decrease in the peak for the internal standard (Fig. 1A, peak IS) and the concomitant appearance of an extra, large peak (Fig. 1A, peak X) only in the portion of urine to which methenamine had been added. The other chromatograms were identical in all respects. The patient was asked to stop taking methenamine for a 2-week period. As shown in Fig. 1B, 2 weeks after she stopped methenamine intake, there was no evidence of an extra peak X, and the internal standard peak had resumed its expected height. Moreover, the MN and NMN peaks retained their previous heights. This virtually rules out high procedural losses of MN, NMN, and the internal standard as underlying these results, although the urine samples necessarily were obtained on different occasions.

These findings indicate that methenamine is responsible for the lowered signal for the internal standard, leading to false-positive values for urinary fractionated

---

**Fig. 1.** Chromatograms of urine from a healthy person to which the patient’s medications had been added (A) and the patient’s urine before and after stopping methenamine (B).

(A), pyridoxine, oxazepam, methenamine, furosemide, tamoxifen, and acenocoumarol dissolved in HCl (0.1 mol/L) were added separately to the urine. The decrease in the signal for the internal standard (IS) after addition of methenamine is indicated by the arrow. The extra peak is indicated with an X. 3-MT, 3-methoxytyramine. (B) the chromatograms are for samples taken before and 2 weeks after stopping methenamine. Two weeks after stopping methenamine, the patient’s urine had no evidence of an extra peak X, and the internal standard was increased to expected values.
metanephrines. Methenamine interferes with the determination of estriol in urine when an acid hydrolysis technique is used (5). However, to our knowledge, interaction with 4-O-methyltyramine has not been reported previously. Apart from withdrawal of methenamine medication, the use of an alternative internal standard to reliably assess urinary metanephrines in the presence of methenamine could be considered. Hydroxymethoxybenzylamine (HMBA) could be used in patients on methenamine, although methenamine affects the HMBA peak as well, but to a much lesser extent (Fig. 1B). Because small peaks are observed occasionally coeluting with HMBA (Fig. 1A), we do not recommend the use of HMBA as an internal standard.

The frequency of misleading results for urinary metanephrines may be appreciable because methenamine (Urimax, Urised, Hiprex, Uroquid-acid) is commonly used in the prevention and treatment of urinary tract infections (especially in patients with a urinary catheter or neurogenic bladder) and 4-O-methyltyramine is one of the reagents supplied by Bio-Rad for the assay of urinary metanephrines (UMET by HPLC). This problem is independent of whether the calibrators from this assay are used. Especially in patients who are on methenamine treatment, the diagnosis of pheochromocytoma should be considered only after repeated increased urinary fractionated metanephrines on measurement with different internal standards or after the patient has stopped taking methenamine. The diagnosis should be supported by other laboratory results (fractionated plasma free metanephrines and/or catecholamines in plasma or urine) and ultimately confirmed by histology. More generally, in cases in which lower recovery of the internal standard leads to increased values for urinary metanephrines, analysts should be aware of the possibility of unexpected interactions such as the one described.

References

DOI: 10.1373/clinchem.2004.032912