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IDENTIFICATION AND QUANTIFICATION OF N-METHYLEPINEPHRINE IN HUMAN URINE

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SUMMARY

N-Methylepinephrine was analysed in human urine using a high-performance liquid chromatographic catecholamine assay. The identity of the compound was confirmed by gas chromatography–mass spectrometry. The excretion of N-methylepinephrine is slightly less than that of epinephrine: excretion values were in the range 2–65 nmol per 24 h (0.4–13 μg per 24 h) in 150 hypertensive patients. In addition to increased epinephrine excretion, increased urinary excretion of N-methylepinephrine was found in three out of five patients with histologically proven pheochromocytoma.

INTRODUCTION

In 1960, Axelrod [1] detected N-methylepinephrine (N-ME) in the adrenal glands of cows, rats, rabbits and monkeys. When N-ME was administered to a rat, large amounts of the 3-O-methylated compound, N-methylmetanephrine (N-MMN), were found in the urine [1]. In 1962, Itoh et al. [2] demonstrated the presence of N-MMN in human urine. In patients with pheochromocytoma, the urinary excretion of N-MMN appeared to parallel the excretion of metanephrine (MN). From these findings the following metabolic pathway in animals and humans was suggested [1, 2]: epinephrine (E) → N-ME → N-MMN.

We report here the identification of N-ME in human urine. Using a specific and sensitive high-performance liquid chromatographic (HPLC) assay for catecholamines, we observed a large additional peak in chromatograms of urine and plasma samples from a patient with an E-secreting pheochromocytoma. The

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compound was identified as N-ME using gas chromatography–mass spectrometry (GC–MS). Small amounts of N-ME were detected in urine samples from patients without a neural crest tumour.

EXPERIMENTAL

Subjects

The large unexpected peak was first observed in urine and plasma samples from a 29-year-old man with a five-year history of paroxysmal hypertension accompanied by headache, profuse sweating, forceful heart-beat, palpitations, pallor, dizziness and nausea. Between the paroxysms he was free of symptoms and normotensive [3]. An encapsulated pheochromocytoma was later removed from the right adrenal gland. This patient will be referred to as our patient.

In addition, we analysed urine samples from 150 hypertensive adults and from four other patients with histologically proven pheochromocytoma.

Sample collection

Urine samples (24-h collections unless otherwise stated) were collected in plastic containers containing 20 ml of 6 *M* hydrochloric acid and were stored at 4°C.

Blood samples (10 ml) were collected in chilled lithium–heparin tubes. The plasma was separated from the cells within 30 min after collection, and 50 μl of a 0.26 *M* solution of sodium metabisulphite were added. The plasma samples were stored at −20°C.

Tumour tissue was placed in ice immediately after removal and fragments from two different regions were stored at −70°C for subsequent biochemical analysis. The tissue samples were homogenized by ultrasonication in 10 vols. of 0.4 *M* perchloric acid containing 2.6 m*M* sodium metabisulphite and 1.3 m*M* disodium EDTA. The homogenate was centrifuged at 30 000 *g* for 10 min. The supernatant was diluted 1000-fold in 0.1 *M* phosphate buffer (pH 6.5) containing 2.6 m*M* sodium metabisulphite and 1.3 m*M* disodium EDTA.

Extraction of catecholamines

Free catecholamines were extracted from urine and plasma samples and from the supernatant of tissue homogenates, using a two-step sample purification scheme involving ion-exchange on a small cation-exchange column and alumina adsorption, with dihydroxybenzylamine (DBHA) as an internal standard. The detailed procedure and the chemicals used have been described previously [4].

High-performance liquid chromatography

Catecholamines and vanillylmandelic acid (VMA) were determined by HPLC with electrochemical detection (ED) as described previously [4]. The system included an Altex 100 A pump (Altex, Berkeley, CA, U.S.A.), a WISP 710 B autosampler (Waters Assoc., Milford, MA, U.S.A.), an electrochemical detector with a LCC 231 K thin-layer cell (Bruker, Karlsruhe, F.R.G.) and a SP 4270 integrator (Spectra-Physics, Santa Clara, CA, U.S.A.). The analytical column

was an Ultrasphere IP column, , 5 μm particle diameter, 25 cm or 15 cm×0.46 cm I.D. (Altex). The mobile phase was prepared by mixing 200 ml of HPLC-grade methanol (J.T. Baker, Deventer, The Netherlands) with 2000 ml of 0.04 *M* sodium dihydrogen phosphate, adjusted to pH 3.0 with phosphoric acid, and containing 0.13 m*M* disodium EDTA and 0.9 m*M* sodium octanesulphonate (Janssen, Beerse, Belgium). N-Methylepinephrine hydrochloride [N,N-dimethyl-1-(3,4-dihydroxyphenyl)ethanolamine hydrochloride] was obtained from Janssen as a solution in absolute ethanol.

Recovery of N-ME was determined by extracting five aqueous standard solutions (from 5 to 500 n*M*) and aliquots of four normal urine samples spiked with 50 n*M* and 500 n*M* N-ME. The urinary aliquots were extracted in triplicate to evaluate the precision of the assay.

Gas chromatography–mass spectrometry

For confirmation purposes a GC–MS analysis of the trimethylsilyl (TMS) derivatives was performed. Catecholamines were extracted from 10-ml urine samples using the two-step purification scheme. The acid alumina eluates were evaporated to dryness under a gentle stream of nitrogen. The residues were dissolved in 50 μl of pyridine, and 100 μl of N,O-bis(trimethylsilyl)trifluoroacetamide and 10 μl of trimethylchlorosilane were added. The derivatization was performed at 60°C for 10 min. GC–MS was carried out on a Finnigan 3200 gas chromatograph–mass spectrometer coupled with an INCOS data system (Finnigan, Sunnyvale, CA, U.S.A.).

The instrument was equipped with a CP-Sil 5 CB column (cross-linked methylsilicone, 25 m×0.32 mm I.D., 0.33 μm film thickness). A volume of 1 μl was injected in the split mode at an injection port temperature of 225°C. The oven temperature was set at 110°C and raised to 175°C at 8°C/min. The carrier gas was helium. Ionization was performed by electron impact (EI) at 70 eV and an emission current of 500 μA. The mass spectrometer was operated in the multiple-ion detection (MID) mode.

The full mass spectrum of the TMS derivative of N-ME was obtained after derivatization of the pure standard.

Statistical analysis

Correlation analysis was done with the non-parametric Spearman rank procedure [5].

RESULTS

Identification of N-methylepinephrine in human urine

Fig. 1 shows chromatograms of extracts from urine samples obtained from our patient. The samples were collected before surgical removal of the tumour (Fig. 1A), during the day of the operation (collection over 19 h after surgery, Fig. 1B) and one day postoperatively (Fig. 1C). The chromatogram of the extract of the urine sample collected before tumour removal (Fig. 1A) is characterized by a high E peak and a high unexpected peak between E and DHBA. Chromatograms

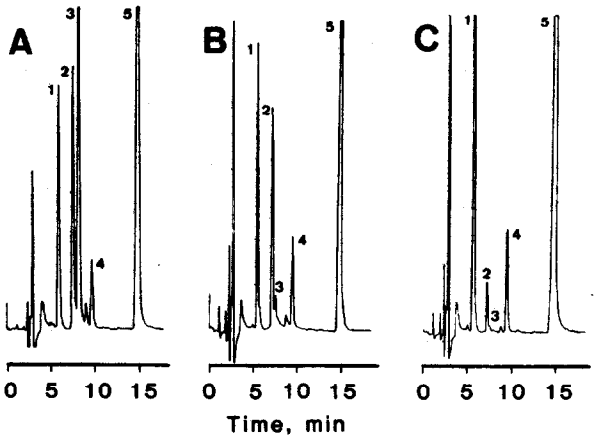

Fig. 1. Chromatograms of extracts from urine samples obtained from a patient with an epinephrine-secreting pheochromocytoma. Samples were collected before tumour resection (A), during the day of the operation (B, collection over 19 h), and one day after surgery (C). Catecholamine concentrations in A, B and C were, respectively: for norepinephrine (peak 1), 0.53, 0.41 and 1.15 μM; for epinephrine (peak 2), 0.69, 0.43 and 0.09 μM; for N-methylepinephrine (peak 3), 1.20, 0.04 and 0.005 μM; for dopamine (peak 5), 2.44, 1.14 and 2.61 μM. Peak 4 is the internal standard dihydroxybenzylamine (DHBA).

of extracts from normal urine samples showed a small peak with the same retention time. The catecholamine structure of the unknown compound was suggested by the following three observations.

(1) The compound was recovered after an extraction procedure involving the selective adsorption of catecholamines on aluminium oxide.

(2) The compound was eluted after NE and E but before the internal standard DHBA and dopamine (DA). The order of elution was not changed when different concentrations of octylsulphonate (from 0.2 to 0.9 mM) in the mobile phase were used. The effect of the ion-pairing agent on the retention of the unidentified compound was thus similar to that on other catecholamines [4]. The order of elution indicated that the unknown was slightly less polar than E.

(3) The hydrodynamic voltammogram of the unknown was similar to that of NE and E (half-wave potential versus calomel reference electrode, +0.49 V).

These considerations and the observations of Axelrod [1] and Itoh et al. [2] suggested that the unexpected peak in our chromatograms might be N-ME. The chromatographic and electrochemical behaviour of authentic N-ME was indeed identical with that of the unknown in the urinary extract. The compounds co-eluted in mobile phases containing different concentrations of ion-pairing agent (from 0.2 to 0.9 mM) and the hydrodynamic voltammogram of the unknown matched that of the N-ME standard.

The identities of both compounds were comfirmed by GC–MS analysis of their TMS derivatives. The mass spectrum of the TMS derivative of authentic N-ME showed significant ions at *m/e* values of 58, 355 and 398 (Table I). The concentration of endogenous N-ME in urine samples was not sufficient to obtain full mass spectra, but by focusing on the characteristic fragments it was possible to

TABLE I

SIGNIFICANT IONS IN THE MASS SPECTRUM OF THE TRIMETHYLSILYL DERIVATIVE OF N-METHYLEPINEPHRINE

Fragment★	*m/e*	Relative intensity
M^+	413	0
$(M-15)^+$	398	0.4
a	58	100
b	86	0
c	355	19.8
d	324	0.5
e	325	0.1
f	267	0.5
g	281	0.5
Others	102	30
	179	2.0
	193	1.5
	308	1.3
	323	1.4

★The fragments are lettered according to ref. 6.

demonstrate the compound in the specimens analysed. GC–MS analysis of the derivatives prepared from the urinary samples revealed a chromatographic peak with the same retention time and the same characteristic *m/e* values (58 and 355) as those obtained for the TMS derivative of the N-ME standard (Fig. 2).

Pheochromocytoma and N-methylepinephrine

Table II summarizes the results of the catecholamine determinations for our patient. The molar ratio of N-ME to E ranged from 0.4 to 1.8 in the preoperative samples.

The large tumour (137 g) was located in the right adrenal gland. It contained predominantly E (45 and 49 nmol/g of tissue or 8.2 and 9.0 mg/g of tissue in two different tumour fragments) and NE (7.7 and 12.4 nmol/g of tissue or 1.3 and 2.1 mg/g of tissue). Small amounts of DA (0.18 and 0.22 nmol/g of tissue or 27 and 34 μg/g of tissue) were also identified. No N-ME or 3-O-methylated catecholamine metabolites were detected in the tumour extracts.

Fig. 3 illustrates the changes in catecholamine excretion after resection of the tumour. The excretion of N-ME decreased more rapidly than the excretion of E. In the urine collected during 19 h after surgery, the concentration of E was still elevated whereas the N-ME concentration was markedly lower than in preoperative collections (Fig. 1B). Epinephrine excretion was normal (less than 110 nmol per 24 h or less than 20 μg per 24 h) from the second day after tumour removal. Norepinepherine excretion increased after surgery and became normal (less than 590 nmol per 24 h or less than 100 μg per 24 h) after five days. In blood samples collected after tumour resection, N-ME could not be detected.

Table III lists catecholamine excretion values for other patients with histologically proven pheochromocytoma. Patient 1 was the only patient with an extra-

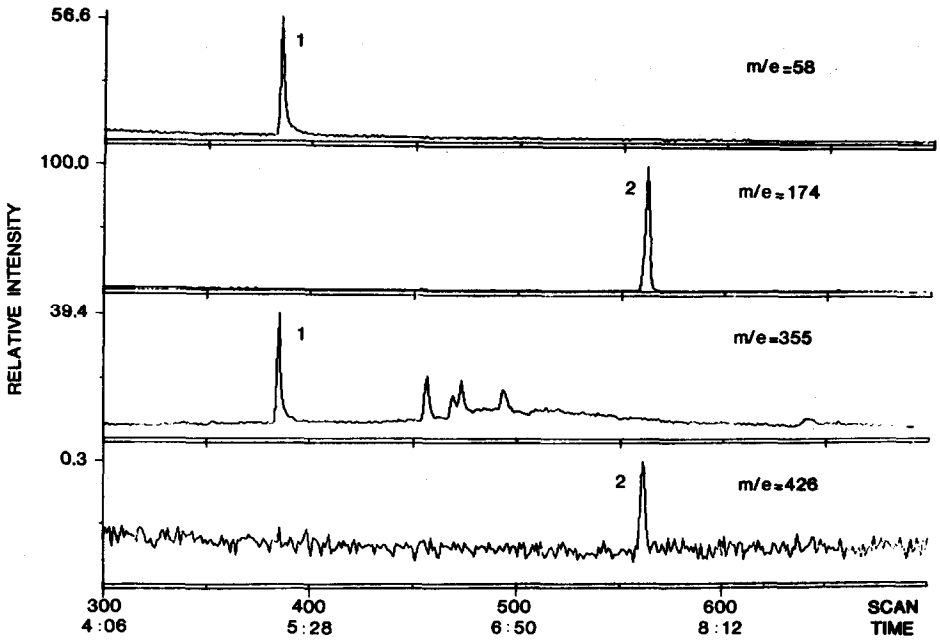

Fig. 2. GC–MS analysis using MID of the TMS derivatives of catecholamines extracted from a urine sample of our patient. The chromatographic traces were recorded at characteristic *m/e* values of the N-ME derivative (58 and 355; peak 1) and of the dopamine derivative (174 and 426; peak 2). The scan time was 0.133 s for *m/e* values 58 and 174 and 0.269 s for *m/e* values 355 and 426.

TABLE II

PLASMA CONCENTRATIONS OF CATECHOLAMINES AND URINARY EXCRETION VALUES OF CATECHOLAMINES AND VANILLYLMANDELIC ACID IN A PATIENT WITH AN EPINEPHRINE-PRODUCING PHEOCHROMOCYTOMA

Abbreviations: NE = norepinephrine; E = epinephrine; N-ME = N-methylepinephrine; VMA = vanillylmandelic acid.

	NE	E	N-ME	VMA
Plasma concentrations (nM)				
Basal ($n=4$)	2.0–2.8	1.2–3.4	1.1–2.3	
During paraoxysm	4.3	2.3	1.3	
Right adrenal vein	21.3	106	93	
Postoperatively ($n=4$)★	1.7–2.4	<0.2	<0.2	
Upper reference limit	4.7	0.9	<0.2	
Urinary excretion (μmol per 24 h)				
Preoperatively ($n=3$)	0.28–0.52	0.52–0.67	0.44–1.18	75–125
Postoperatively ($n=4$)★	0.23–0.43	0.01–0.02	0.001–0.004	19–21
Upper reference limit	0.59	0.11	0.07	35

★Postoperative samples were collected 6–11 days after tumour removal.

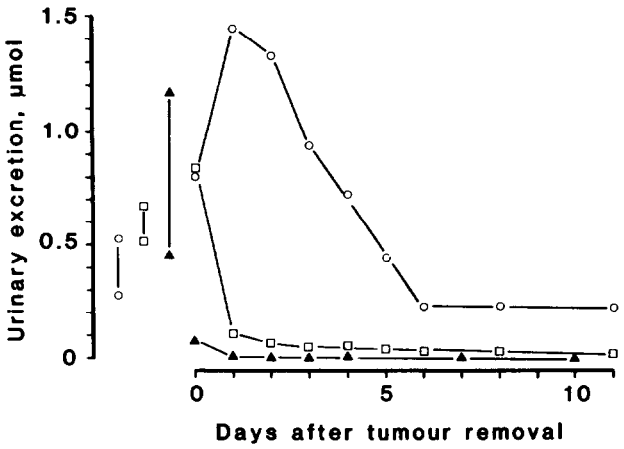

Fig. 3. Urinary excretion of norepinephrine (○), epinephrine (□) and N-methylepinephrine (▲) in our patient with an epinephrine-secreting pheochromocytoma. Urine samples were collected over 24-h periods except for the day of the operation (day 0) where the collection time was 19 h. Bars to the left of day 0 indicate the range of excretion values on three different days before tumour resection.

adrenal tumour. Norepinephrine excretion was at the upper limit of the reference values in two patients and clearly elevated in the three others. Epinephrine excretion was elevated in four patients and N-ME excretion in three. All three patients with high N-ME values also had increased excretion of E.

Assay of N-methylepinephrine

The HPLC method that was used for the assay of norepinephrine and epinephrine was suitable for the quantification of N-ME. The peak height increased linearly with the injected amount over the range studied (0.3–300 pmol). The recovery of N-ME from aqueous standard samples and spiked urinary samples averaged 67 ± 7% (mean ± S.D.). This value is the same as for other endogenous catecholamines and the internal standard DHBA [4].

The within-analysis coefficient of variation for the assay of N-ME in 1-ml

TABLE III

RANGE OF PREOPERATIVE URINARY EXCRETION VALUES IN PATIENTS WITH PHEOCHROMOCYTOMA

Abbreviations: NE = norepinephrine; E = epinephrine; N-ME = N-methylepinephrine.

Patient	Sex	Age (years)	*n*	Excretion (µmol per 24 h)		
				NE	E	N-ME
1	F	20	2	2.5–6.2	<0.02	<0.01
2	M	33	1	0.7	1.8	0.4
3	M	57	8	2.5–5.0	2.6–5.1	0.04–0.08
4	M	47	5	1.4–28.8	9.3–96.1	2.1–4.7
Upper reference limit				0.59	0.11	0.07

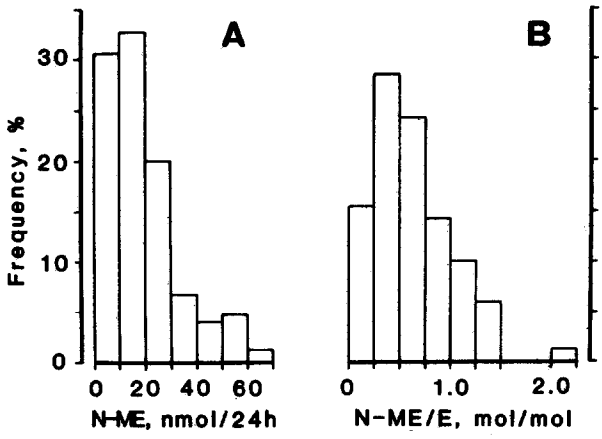

Fig. 4. Urinary excretion of N-methylepinephrine in 150 adults. (A) Distribution of excretion values; (B) distribution of the molar ratio of N-methylepinephrine to epinephrine.

samples was less than 5.6% for concentrations between 10 and 330 n*M*.

Fig. 4 shows the distribution of N-ME excretion values and of molar ratio of N-ME to E in hypertensive adults.

DISCUSSION

GC–MS is a powerful tool for the identification of catecholamine-related molecules. The mass spectra of the TMS derivatives of catecholamine-related compounds demonstrate characteristic fragments, which are obtained by benzylic cleavage and by loss of a single methyl radical from the molecular ion [6]. These characteristic fragments were also found for the TMS derivative of authentic N-ME (Table I). The intense peaks with *m/e* values of 58 and 355 correspond to the products of benzylic cleavage. The small peak with a *m/e* value of 398 corresponds to the $(M-15)^+$ ion. By selective ion monitoring (SIM) at the characteristic *m/e* values 58 and 355, we were able to demonstrate N-ME in urine samples from a patient with pheochromocytoma and from a normal subject. The results obtained by GC–MS thus confirmed the tentative identification that was suggested by the liquid chromatographic and electrochemical properties of the unexpected compound in our HPLC system.

Our findings thus indicate that N-ME is normally present in human urine and that E-secreting pheochromocytoma may be associated with increased urinary excretion of N-ME.

Textbooks and recent publications dealing with catecholamine metabolism usually do not mention the metabolic pathway suggested by Axelrod [1] and Itoh et al. [2]: E→N-ME→N-MMN. However, in vitro studies have demonstrated that the enzyme phenylethanolamine N-methyltransferase (PNMT) catalyses the N-methylation of E [7]. This enzyme is found in the adrenal medulla, and to a lesser extent in mammalian brain [7] and heart [8]. The formation of E in cells from the adrenal medulla or from pheochromocytoma apparently requires NE to leave the chromaffin granules and enter the cytoplasma where NE is N-

methylated by PNMT [9]. A fraction of the cytoplasmatic E could thus be further N-methylated to N-ME. The identification of N-ME in human urine provides additional evidence for this metabolic pathway.

The amounts of N-ME excreted by normotensive subjects or by hypertensive subjects were comparable with the amounts of E excreted. The correlation between N-ME and E excretion values was statistically significant beyond the 0.001 level, although the correlation coefficient was small ($r=0.65$): high N-ME values (50–65 nmol per 24 h) were usually associated with high E values (55–140 nmol per 24 h), but low N-ME values were found with both low and high E values.

In our patient with an E-secreting pheochromocytoma, the excretion of N-ME was in the same range as that of E. The finding of an elevated N-ME concentration in the right adrenal vein and the immediate drop of N-ME excretion after tumour resection, preceding the decrease of E excretion (Fig. 1), suggest that N-ME was released from the tumour cells, where it could have been formed by the action of PNMT on E. PNMT activity appears indeed to be present in all pheochromocytomas of adrenal origin [10].

Catecholamine overproduction in pheochromocytoma is due to increased activity of catecholamine synthesizing enzymes [11, 12]. It has been suggested that the chromaffin granules in pheochromocytoma cells may be filled to capacity and that the release of cytoplasmatic catecholamines may occur by continuous diffusion through the cell wall [12, 13]. The fact that no N-ME was found in our tumour extracts indicates that N-ME was not stored in chromaffin granules and suggests that continuous diffusion is indeed an important mechanism for the release of catecholamines from tumour cells. However, it does not exclude the release of norepinephrine and epinephrine by exocytosis of the chromaffin granules.

Increased excretion of N-ME may be a common feature of E-producing pheochromocytomas. It was observed in three of the four patients with increased E excretion (Table III).

The present evidence suggests that E formed in the tumour cells can be stored in chromaffin granules [14], or be metabolized by monoamine oxidase and/or catechol-3-O-methyltransferase if these enzymes are present [10, 11], or be N-methylated by PNMT (refs. 1 and 2 and this study). When no PNMT activity is present only NE is stored and released by the tumour [11]. At low PNMT activities only a minor fraction of the cytoplasmatic E would be converted into N-ME and the ratio of N-ME released to E released would be low. With increasing PNMT activity, the fraction of E being N-methylated should increase. As N-ME is not stored in the tumour, the ratio of N-ME released to E released should also increase. The assay of PNMT activity in the tumour tissues might confirm this theory. However, the PNMT activity in a tumour tissue extract does not necessarily represent its activity in vivo at the time when a particular urine sample was collected, since PNMT is an inducible enzyme [9] and its rate of synthesis could be affected by the drug treatment of the patient. Unfortunately, no PNMT activities were measured for the cases reported in Table III.

As the E content of pheochromocytoma is positively correlated with the PNMT activity in the tumour [15], it appears likely that the uptake of E in chromaffin

granules is not limited by the formation of N-ME at the PNMT activities found in pheochromocytomas. The rate of E diffusion through the cell wall, however, may well be limited. At very high PNMT activity, most or all of the E diffusing towards the cell wall might react with PNMT before reaching the cell wall and only small amounts of E might be released from an E-containing tumour. This could explain the lack of correlation between the amount of catecholamine in a tumour and the elevations of these amines in the plasma of some patients with pheochromatocytoma. Indeed, normal plasma E concentrations may be found in patients with an E-containing pheochromocytoma [16]. The assay of N-ME may be of value in establishing the diagnosis of pheochromocytoma in such cases.

Note that N-ME would not be measured with the various fluorometric and radioenzymic methods that are extensively used for the assay of catecholamines. The fluorometric trihydroxyindole method involves oxidation of the amines to the corresponding adenochromes [17]. This reaction requires a primary or secondary amine function and is thus not expected to occur with N-ME. In radioenzymic methods based on the 3-O-methylation of catecholamines catalysed by catechol-O-methyltransferase [18, 19], a concentrated solution of the non-radioactive methylated derivatives of the "common" catecholamines (epinephrine, norepinephrine and dopamine) is added after the enzymic conversion to localize the 3-O-methylated compounds after thin-layer chromatography. N-Methylmetanephrine formed from N-ME would remain undetected as no appropriate carrier was added, unless it had the same migration as one of the other 3-O-methylated compounds. These fluorometric and radioenzymic methods might fail to provide evidence of catecholamine overproduction in cases of pheochromocytoma releasing predominantly N-ME. In metanephrine assays based on the periodate oxidation to vanillin [20], N-MMN, the 3-O-methylated catabolite of N-ME, would be oxidized and thus would contribute to the total metanephrine result. This could be a reason why some authors [21, 22] have found the determination of urinary metanephrines to be a more reliable test for screening and identifying patients with pheochromocytoma than the assay of urinary free catecholamines.

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REFERENCES

1 J. Axelrod, Biochim. Biophys. Acta, 45 (1960) 614.
2 C. Itoh, K. Yoshinaga, T. Sato, N. Ishida and Y. Wada, Nature, 193 (1962) 477.

3 A. Dupont, P. Vanderniepen, E. Gerlo, G. Somers, L. Vanhaelst, P. in 't Veld, W. Gepts, R. Sacré and R. Six, J. Endocr. Invest., 8 (1985) 153.
4 E. Gerlo and R. Malfait, J. Chromatogr., 343 (1985) 9.
5 W.C. Schefler, Statistics for the Biological Sciences, Addison-Wesley, Reading, 1980, p. 216.
6 S.E. Hattox and R.C. Murphy, Biomed. Mass Spectrom., 5 (1978) 338.
7 J. Axelrod, J. Biol. Chem., 237 (1962) 1657
8 L. Pohorecky, M. Zigmond, H. Karten and R. Wurtman, J. Pharmacol. Exp. Ther., 165 (1969) 190.
9 R. Perlman and M. Chalfie, Clin. Endocr. Metab., 6 (1977) 551.
10 J. Feldman, J. Blalock, R. Zern and S. Wells, J. Clin. Endocrinol. Metab., 49 (1979) 445.
11 B. Jarrot and W. Louis, Clin. Sci. Molec. Med., 53 (1977) 529.
12 M. Goldstein, N. Prochoroff and S. Sirlin, Experientia, 21 (1965) 592.
13 H. Winkler and A. Smith, Lancet, i (1968) 793.
14 H. Blaschko, D. Jerrome, A. Robb-Smith, A. Smith and H. Winkler, Clin. Sci., 34 (1968) 453.
15 J. Feldman, Res. Commun. Chem. Path. Pharm., 34 (1981) 389.
16 W.M. Manger and R.W. Gifford, Jr., Pheochromocytoma, Springer-Verlag, New York, 1977, p. 297.
17 S.C. Chattoraj, in N.W. Tietz (Editor), Fundamentals of Clinical Chemistry, W.B. Saunders, Philadelphia, 2nd ed., 1976, Ch. 13, p. 812.
18 K. Engelman and B. Portnoy, Circ. Res., 26 (1970) 53.
19 M. Da Prada and G. Zürcher, Life Sci., 19 (1976) 1161.
20 J. Pisano, Clin. Chim. Acta, 5 (1960) 406.
21 W.M. Manger and R.W. Gifford, Jr., Pheochromocytoma, Springer-Verlag, New York, 1977, p. 214.
22 E. Bravo and R.W. Gifford, N. Engl. J. Med., 311 (1984) 1298.