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The Measurement of 3-O-Methyldopamine in Urine and Plasma by a Rapid and Specific Radioimmunoassay

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Antiserum against 3-O-methyldopamine (MD) was produced in rabbits immunized with MD hapten conjugated to hemocyanin. The antiserum was used to develop a radioimmunoassay (RIA) for MD. As little as 0.5 ng of MD in 0.1 ml can be detected. The major catecholamines and the phenolic aromatic amines (dopamine, norepinephrine, epinephrine, octopamine, and tyramine) and their metabolites (normetanephrine, metanephrine, homovanillic acid and 4-hydroxy-3-methxymandelic acid) did not bind significantly to the antibody. The RIA of MD was used to assay the endogenous level of MD in urine and plasma of hospitalized children. In children (7 mo to 13 yr), average concentration of MD in plasma was found to be 0.47 ± 0.11 ng/ml, and in urine 0.15 ± 0.05 μg/mg of creatinine (45.0 ± 16.3 μg/24 hr). In children with neuroblastoma, there was a 3- to 10-fold increase in urinary excretion and plasma level of 3-O-methyldopamine. In adults, the average urine and plasma levels were found to be 87.4 ± 3.4 μg/24 hr and 0.6 ± 0.2 ng/ml. The diagnostic applicability of the RIA of MD is discussed.

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The significant advances during the past decade in the understanding of catecholamine synthesis and metabolism in neural-crest tumors has resulted in the development of a host of biochemical tests aimed at the detection of these tumors with improved precision and greater facility (*1,2*). The neuroblastoma belongs to a group of functional tumors and is known to produce catecholamines. In contrast to the pheochromocytoma, which normally produces excess norepinephrine and/or epinephrine, the former exhibits an overproduction of dopamine and L-dopa. All these compounds undergo metabolic degradation, which is the reason for the broad pattern of metabolites these patients excrete in the urine (*3*). Dopamine is metabolized either by oxidative deamination (to yield dihydroxyphenyl acetic acid) or by 0-methylation to produce 3-0-methyldopamine (*4*). Increased urinary excretion of 3-0-methyldopamine in patients with neuroblastoma has been described by Gjessing (*5*), Robinson and Smith (*6*), La Brosse and Karon (*7*), and von Studnitz (*8*).

Several methods—including fluorometric analysis (*9,10*), phosphorimetry (*11*), ion-pair partition chromatography (*12*), and gas–liquid chromatography (*13*)—have been described for the determination of 3-0-methyldopamine in a biologic sample. Most of these methods, however, are time-consuming and, to a considerable degree, lack specificity and sensitivity.

We considered the possibility of using a radioimmunoassay for the analysis of 3-0-methyldopamine. This technique, which is based on the competition between labeled and unlabeled hapten for binding to a limited number of sites on a specific antibody, has been employed to quantitate biologically im-

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portant molecules in picogram and nanogram quantities in unprocessed serum or urine (*14*). In this study we report the production of antibodies to 3-0-methyldopamine. These antisera have been used to develop a sensitive and specific radioimmunoassay capable of detecting as little as 0.2 ng of 3.0-methyldopamine in biologic samples.

The availability of this assay allowed us to determine the plasma and urine levels of 3-0-methyldopamine in children and adults. Furthermore, the diagnostic applicability of the radioimmunoassay of 3-0-methyldopamine was evaluated by analyzing for 3-0-methyldopamine in children with suspected neuroblastoma.

MATERIALS AND METHODS

[^{3}H] 3-0-methyldopamine (sp. activity 10 Ci/millimol) was obtained commercially*. Radiochemical purity, checked by analytical thin-layer chromatography with two different solvent systems, was greater than 90%. The plate was developed to 16 cm in systems A (n-butanol:acetic acid:water; R_f = 0.7) and B (isopropyl alcohol:ammonia:water; 8:1:1; R_f = 0.68).

Except for hemocyanin and polyethylene glycol (6000), all chemicals were obtained commercially from the same source, listed in an article by Faraj et al. (*15*). They were of reagent grade and were used without further purification. The rabbits (males, New Zealand strain from Hiram Davis, Stockbridge, Ga.) were caged individually and were maintained on Purina rabbit chow and water *ad lib.*

Preparation of antigen. To a solution of 50 mg of hemocyanin in 5 ml of distilled water were added 50 mg of *p*-aminohippuric acid. The pH of the reaction mixture was adjusted to 6.4 with 1 N NaOH. To this suspension were added 80 mg of 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide at 25°C. The mixture was stirred at room temperature overnight and then dialyzed for 48 hr against distilled water. The (*p*-aminohippuric acid)-hemocyanin conjugate mixture was adjusted to pH 1.5 with 1 N HCl, cooled (0°C), and then a solution of 100 mg of sodium nitrite in 1 ml of water was added dropwise. The diazotized protein solution was then added dropwise, with stirring at 0°C, to 3-0-methyldopamine · HCl (100 mg) in 0.5 N NaOH (5 ml) (Fig. 1). A red color developed. The reaction mixture was stirred overnight at 4°C and then dialyzed for 48 hr against distilled water with three changes (4 l) per day. The extent of conjugation of 3-0-methyldopamine to the protein was established by carrying out the reaction with [^{3}H] 3-0-methyldopamine (2 μCi). The number of 3-0-methyldopamine residues coupled per mole of hemocyanin was determined by measuring ^{3}H concentrations in the antigen solution after dialysis. The antigen solution was lyophilized and stored at −10°C.

Immunization procedure. Rabbits were immunized with 2 mg of the antigen. It was dissolved in 0.5 ml of normal saline and emulsified with an equal volume of Freund's complete adjuvant. Each of the four rabbits received this mixture distributed among four subcutaneous injection sites at weekly intervals for 4 wk; and then every 3 wk for 1 yr. The first collection of blood was at Week 7 and later intervals. Blood was allowed to clot for 3 hr at 25°C and then centrifuged at 500 g for 20 min to separate the serum, which was then stored at −80°C.

Radioimmunoassay procedure. In 12- × 75-mm plastic tubes were placed 0.3 ml of 0.5% bovine serum albumin w/v in 0.01 *M* sodium phosphate buffer solution (pH 7.4, containing 0.03% potassium phosphate, 0.8% NaCl and 0.05% human gamma globulin). Then 0.1 ml of various dilutions of antiserum, and 0.133 ng of [^{3}H] 3-0-methyldopamine (8000 cpm) in 0.1 ml of phosphate buffer (pH 7.4) were added. The tubes were capped and incubated at 4°C overnight. Blank tubes were prepared by the addition of 0.4 ml of buffer and 0.1 ml of the labeled material. The antibody-bound [^{3}H] 3-0-methyldopamine was separated from 3-0-methyldopamine by the addition of 0.5 ml of aqueous polyethylene glycol 6000 (30% w/v) as described by Cheung and Slaunwhite (*16*). The tubes were vortex-mixed vigorously. After centrifugation (2000 g at 4°C) for 40 min, 0.2 ml of the supernates were aspirated and placed in a numbered sequence of scintillation vials. Each was then diluted with 14 ml

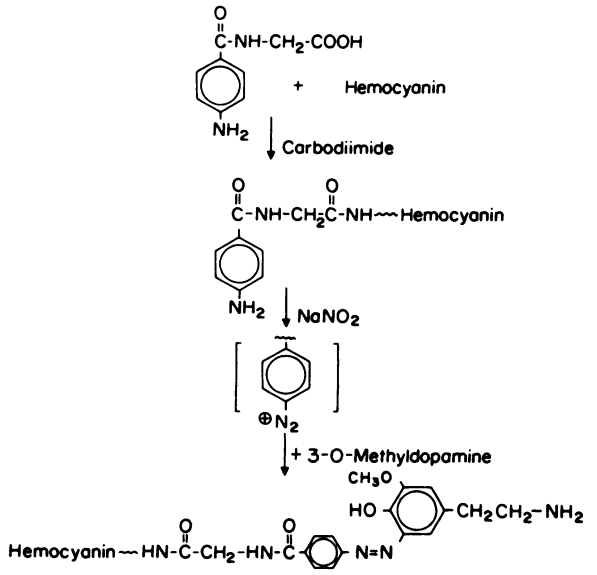

FIG. 1. Synthesis of 3-0-methyldopamine antigen.

of scintillation liquid and the ^3H was measured in a liquid scintillation spectrometer; counting efficiency = 40% for H-3. Each sample was assayed in triplicate. All samples were counted to ±2% error.

In addition, after the decanting of the supernate, the precipitate was dissolved in 14 ml of scintillation fluid and ^3H was measured as above. Standard curves were obtained by adding an appropriate volume of 0.01 *M* sodium phosphate buffered saline (pH 7.4) to all tubes containing 0.1 ml of the antiserum (1:1000 dilution) and 0.1 ml of tritium-labeled 3-0-methyldopamine (8000 cpm) to make a final incubation volume of 0.5 ml. To the tubes were added various quantities ranging from 0.5 to 20 ng of 3-0-methyldopamine. The tubes were then incubated overnight at 4°C, followed by precipitation with an equal volume of 30% aqueous polyethylene glycol 6000.

Appropriate blanks containing normal rabbit serum instead of antiserum were included in each assay. Normal rabbit serum did not bind [^3H] 3-0-methyldopamine. Percentage bound was calculated as follows: let A represent the radioactivity (cpm) that was precipitated, N the radioactivity (cpm) that was nonspecifically precipitated (control tubes) and T the total radioactivity (cpm) in each incubation mixture. (The value of N was less than 5% of T, and within any run, N was consistent.) Then, percentage bound = $(A - N)/T \times 100$. Percentage free was determined by measuring the radioactivity (cpm) in the supernate. Both determinations gave the same percentage bound. The percent inhibition of the binding of [^3H] 3-0-methyldopamine to the antibody was calculated by determining the percent of counts displaced from the [^3H] 3-0-methyldopamine antibody complex by a given amount of the inhibitor. The amounts of 3-0-methyldopamine in plasma and urine were calculated by comparing its inhibition with the standard inhibition curve.

Procedure for the extraction of 3-0-methyldopamine from plasma. The following method was used for extracting 3-0-methyldopamine in plasma (usually 1 ml, but up to 2 ml). To an aliquot of plasma an equal volume of 6% w/v 5-sulfosalicylic acid was added and the mixture was shaken vigorously (vortex for 1 min). Upon centrifugation (500 g for 20 min) an aliquot of the supernate was removed and placed in a 45-ml glass-stoppered centrifuge tube. After addition of (a) 2 g of NaCl, (b) 0.3 g of anhydrous Na_2CO_3, (c) 4 ml of 0.5 *M* buffer (sodium borate, adjusted to pH 10.5 with 10 N NaOH), and (d) 30 ml of ethyl acetate, the mixture was shaken for 30 min and then centrifuged for 10 min. An aliquot (25 ml) of the organic phase was removed. To the two-phase mixture, 10 ml of ethyl acetate was added, and after shaking and centrifuging as above, 10 ml of the solvent was withdrawn. The combined fractions of ethyl acetate (35 ml) were evaporated to dryness under N_2 at 40°C. The residue was reconstituted in 0.4 ml of phosphate buffer, and an aliquot (0.3 ml) was withdrawn and analyzed for 3-0-methyldopamine by the radioimmunoassay procedure described earlier.

Patient population. Written informed consent was obtained from the patients, or from their parents, after approval of the protocol from the University Clinical Trials Committee. This study consisted of the analysis of 3-0-methyldopamine in urine and plasma in a group of hospitalized and normal children (ages 7 mo to 13 yr). Two of the hospitalized patients (Cases #21 and 22) were found to bear neuroblastomas, the diagnosis being established by histological identification of the tumor. To establish normal levels in adults, the 3-0-methyldopamine was determined in plasma and urine of adult volunteers. In the adult series, this dopamine metabolite was also analyzed in the urine and plasma of a patient with pheochromocytoma (Case #42).

Sample collection and analysis. Blood samples for determination of 3-0-methyldopamine were drawn from a peripheral vein, chilled at 0°C and centrifuged immediately at 500 g for 10 min; the plasma was then removed and quickly frozen at −80°C until analysis by radioimmunoassay. Random urine samples were collected over ice and stored immediately at −80°C until analysis by radioimmunoassay. For 24-hr urine collection, the urine samples were collected in acid (pH 3) to maintain the stability of the amine. Because of the difficulty of collecting timed urine samples in most of the patients, some of the values were expressed in terms of micrograms per milligram of creatinine, which was measured by the Jaffe reaction (*17*).

RESULTS

Synthesis of antigen. After having been readily prepared, and upon dialysis, 3-0-methyldopamine-hemocyanin antigen was found to contain about 3% of nonspecifically bound 3-0-methyldopamine. When this was removed by column chromatography (*18*), the antigen was found to have 20–25 moles of the amine per mole of hemocyanin.

Sensitivity and specificity of radioimmunoassay. After five injections (Week 7), antibodies (1:50 dilution) against 3-0-methyldopamine could be detected in rabbits as determined by the binding of [^3H] 3-0-methyldopamine to the antiserum. Ouchterlony plates also indicated the presence of antibody in the sera of rabbits immunized with 3-0-meth-

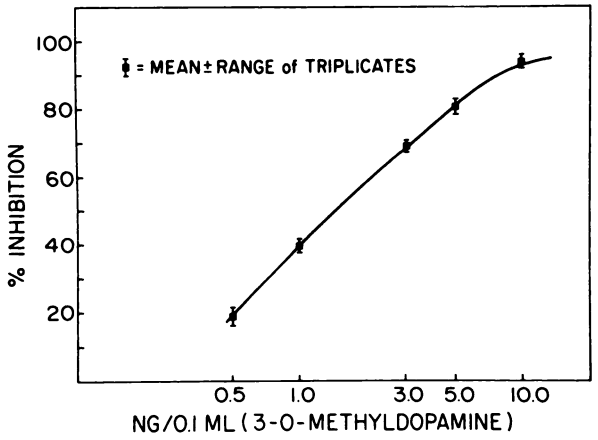

FIG. 2. Typical standard curve (semilog) showing inhibition of binding of [^{3}H] 3-O-methyldopamine to the antibody by various amounts of unlabeled 3-O-methyldopamine sample in 0.01 *M* sodium phosphate buffer. Each point represents average of three determinations. Equation of line, as determined by linear regression analysis, is $y = 0.103x + 0.2574$; correlation coefficient is 0.921.

yldopamine antigen. After 12 mo of immunization, a dilution of 1:1000 of the antiserum could bind 50% of the [^{3}H] 3-O-methyldopamine. The sensitivity of the assay is illustrated in Fig. 2. A concentration of 0.5 ng/0.1 ml of 3-O-methyldopamine can be detected by the antiserum of a rabbit immunized with the 3-O-methyldopamine-hemocyanin immunogen. On a semi-log curve, the assay approaches linearity at 50 ng/ml. 3-O-methyldopamine in urine (0.1 ml; 1:10 dilution) is detected by direct analysis without prior extraction. We found, however, that because of the low levels of this metabolite in plasma, 3-O-methyldopamine had to be extracted first from 1 ml of plasma and then analyzed by radioimmunoassay. Furthermore, when known amounts of 3-O-methyldopamine were added to plasma and urine and the radioimmunoassay was carried out, the recovery of 3-O-methyldopamine from these biologic fluids was essentially quantitative (90–95%). This indicated the absence from these samples of substances capable of interfering with the recovery of 3-O-methyldopamine. The anti-9-O-methyldopamine serum, when stored at −80°C, showed no significant changes in activity for 12 mo. The specificity of the antibody was tested with several metabolites, homologs and analogs of 3-O-methyldopamine. The results are shown in Table 1.

Precision. Within- and between-assay variation was assessed for 3-O-methyldopamine by assay of pooled plasma in the same, as well as in a different, assay ($n = 10$), according to the method of Rodbard (*19*). Intra- and interassay coefficients of variation (CV) were 11.1 and 8.9%, respectively, for 3-O-methyldopamine in plasma. The relatively high precision for replicates between assays reflects the simplicity of sampling (assays are done in plasma after extraction), the stability of the reagents, and the narrow range and steep slope of the standard curve (Fig. 2).

Studies in children. In this investigation, plasma and urine 3-O-methyldopamine were determined in a group of hospitalized and normal subjects of both sexes (7 mo to 13 yr of age). The results indicated that average plasma and urine levels of 3-O-methyldopamine were, respectively, 0.47 ± 0.11 ng/ml and 0.15 ± 0.05 μg/mg of creatinine (Tables 2 and 3).

The amounts of 3-O-methyldopamine in the urine and plasma of patients with neuroblastoma were significantly elevated ($p < 0.05$, Tables 2 and 3; Cases #21 and 22). In these subjects, an aortogram and an infusion venogram revealed a large mass adjacent to the vertebral column and inferior and medial to the right kidney. Biopsy of the mass indicated neuroblastoma.

Analysis of total catecholamines (Case #22, 276 μg/24 hr), HVA, VMA and tyramine (Cases #21 and 22: HVA 0.2 and 2.4 mg/24 hr; VMA 15 and 5 mg/24 hr; tyramine 1.5 and 4.4 mg/24 hr) in several 24-hr urine samples revealed normal HVA and VMA, but showed increased urinary excretion of total catecholamines and tyramine. Analyses of HVA, VMA, and total urinary catecholamines were performed commercially. Urinary tyramine was determined by radioimmunoassay according to the method of Faraj et al. (*15*). Average urinary tyramine excretion in children (aged 2–5 yr) is 0.5 mg/24 hr, with a range of 0.2 to 0.8 mg.

TABLE 1. INHIBITION OF BINDING OF [^{3}H] 3-O-METHYLDOPAMINE TO ANTIBODY BY 3-O-METHYLDOPAMINE ANALOGS

Compound*	I_{50}† (ng)
3-O-Methyldopamine	1.5
3,4-Dimethoxyphenethylamine	5.0
3,4-Dimethoxy-N-methylphenethylamine	10.1

* The following compounds require > 500 ng to produce 50% inhibition: dl-normetanephrine, dl-metanephrine, 4-hydroxy-3-methoxyphenylacetic acid (HVA), 3-O-methyldopa, 4-hydroxy-3-methoxyphenethanol, 4-hydroxy-3-methoxyphenylglycol, 3,4-dihydroxyphenylactic acid, 3,4-dimethoxyphenylacetic acid, 4-hydroxy-3-methoxybenzoic acid, 3,4-dimethoxyaniline, d-amphetamine, dl-p-hydroxyamphetamin, 3,4-dimethoxybenzylamine, 4-methoxy-3-hydroxyphenethylamine, 4-hydroxy-3-methoxybenzylamine, 4-hydroxy-3-methoxymandelic acid, dopamine, norepinephrine, epinephrine, tyramine, octopamine, L-dopa, L-tyrosine, 3,4-dihydroxyphenethanol, phenethylamine, and dl-3,4-dimethoxyphenylalanine.

† Nanograms to be added to 0.5 ml incubation mixture to cause a 50% inhibition of the binding of [^{3}H] 3-O-methyldopamine to anti-3-O-methyldopamine serum.

However, the 3-0-methyldopamine in the urine and plasma of one adult patient with pheochromocytoma (a norepinephrine-secreting tumor) was not significantly elevated, compared with the levels found in adult volunteers, 23–50 yr of age, of both sexes (Table 4). In this patient, diagnosis of the tumor was based on arteriographic analysis whereby a large vascular mass consistent with pheochromocytoma was found in the right adrenal gland. Furthermore, the analysis of VMA in a 24-hr urine sample indicated significant elevation of this metabolite in the urine.

DISCUSSION

The problem of generating antiserum of adequate sensitivity against small antigens has been overcome by the use of antigen that has been covalently coupled to one of a variety of larger molecules (*20*). In our laboratory, hemocyanin has been successfully used as the carrier in the development of high-affinity antibodies against a catecholamine metabolite, 3-0-methyldopamine. This approach, however, does not guarantee antibody specificity, and for 3-0-methyldopamine the problem of specificity—i.e., the ability of the 3-0-methyldopamine antiserum to discriminate between this metabolite and a series of closely related biologic amines—was a difficult one to solve.

TABLE 2. URINARY EXCRETION OF 3-0-METHYLDOPOMANE IN A GROUP OF HOSPITALIZED AND NORMAL CHILDREN

Case No.	Status or disease	3-0-Methyldopamine μg/mg of creatinine	3-0-Methyldopamine μg/24 hr
1	Urinary-tract infection	0.13	—
2	Ventricular septal defect	0.10	—
3	Alternating extropic divergence excess	0.10	—
4	Left cryptorchism	0.20	—
5	Congestive heart failure	0.16	—
6	Phimosis	0.10	—
7	Rumination syndrome	0.23	—
8	Hypospadias	0.18	—
9	Gastroenteritis	0.24	—
10	Gastroenteritis		35
11	Vomiting of unknown origin		78
12	Undifferentiated malignant neoplasms		39
13	Hypertension		30
14	Hypertension		38
15	Malignant teratoma with invasion of lung parenchyma		62
16	Normal	0.18	37
17	Normal	0.11	—
18	Normal	0.10	—
19	Normal	0.12	—
20	Normal		41
	Average ± s.d. (range)	0.15 ± 0.05 (0.0–0.24)	45.0 ± 16.3 (35.0–78.0)
21	Neuroblastoma	—	303
22	Neuroblastoma	—	825

TABLE 3. PLASMA LEVEL OF 3-0-METHYLDOPAMINE IN HOSPITALIZED CHILDREN

Case No.	Status or disease	3-0-Methyldopamine ng/ml
23	Bronchial asthma	0.39
24	Gastroenteritis	0.62
25	—	0.36
26	Enuresis	0.38
27	Hypospadias	0.60
28	Urinary-tract infection	0.50
	Average ± s.d. (range)	0.47 ± 0.11 (0.36–0.62)
22	Neuroblastoma	0.87
23	Neuroblastoma	1.23

Several investigators have indicated that the specificity of antibodies developed against small molecules can often be influenced by the introduction of a bridge agent between hapten and carrier (*21–23*). We found that the insertion of *p*-aminohippuric acid as a bridge agent between hemocyanin and 3-0-methyldopamine resulted in the formation of antibodies that were specific both to the ring and the side-chain moiety of the 3-0-methyldopamine molecule (Table 1). Moreover, there appeared to be two important regions in the molecule of 3-0-methyldopamine—the side-chain and the benzine ring—in which structural changes influence interaction with the antibody. The antibody distinguished between side-chain homologs in proportion to the closeness of their structure to the hapten. The specificity was remarkable: the antibody could readily distinguish the removal or addition of one carbon or hydroxy group on the side-chain of the 3-0-methyldopamine. From our previous work (*15,18*) and that of Cook et al. (*24*) and Smith et al. (*25*), one would expect that the introduction of a hydroxy group on the benzene ring would decrease affinity of the hapten to the antibody. This was demonstrated in the present investigation by the lack of affinity of dopamine for anti-3-0-methyldopamine serum.

Among the analogs that were employed to determine the specificity of the antibody (Table 1), 3,4-dimethoxyphenethylamine and its N-methyl derivative were the only ones that exhibited affinity for the antibody comparable to that of 3-0-methyldopamine. However, much controversy has surrounded the

TABLE 4. URINARY AND PLASMA LEVELS OF 3-O-METHYLDOPAMINE IN ADULTS

Case No.	Status or disease	3-O-Methyldopamine Urine μg/24 hr	3-O-Methyldopamine Plasma ng/ml
29	Normal		0.93
30	Normal		0.76
31	Normal		0.34
32	Normal		0.75
33	Normal		0.81
34	Normal		0.60
35	Normal		0.75
36	Normal		0.65
37	Normal		0.51
38	Normal	85.1	0.31
39	Normal	84.0	
40	Normal	91.2	
41	Normal	89.2	
	Average ± s.d.	87.4 ± 3.4	0.64 ± 0.2
	(range)	(84.0–91.2)	(0.31–0.93)
42	Pheochromocytoma	100.0	0.50

identity of this compound in biologic fluids. Using gas-, paper-, and thin-layer chromatography, Jones et al. (*26*) could not find this amine in the urine from either normal or schizophrenic subjects.

The antibody against 3-0-methyldopamine was used to develop a rapid, accurate, reproducible, and sensitive radioimmunoassay (Fig. 2) for the quantitation of 3-0-methyldopamine. With this simple procedure, it was possible to determine as little as 5 ng/ml of 3-0-methyldopamine in urine by direct analysis. Below this level, the biologic fluid (such as plasma) would have to be extracted and concentrated for analysis. However, the ease and simplicity of the extraction method facilitated the processing of multiple plasma samples.

The availability of the radioimmunoassay of 3-0-methyldopamine allowed us to use this technique to determine the endogenous level of this metabolite in urine and plasma in a group of adults and children. In this respect, only limited results of quantitative urinary 3-0-methyldopamine analysis have been published. A comparison of our data with those reported in the literature demonstrates that our values for free 3-0-methyldopamine in urine are similar to those reported by Kaser and Thomke (*27*), Armstrong (*28*), and Gjessing (*5*), whereas those indicated by Smellie and Sandler (*29*) are several times higher. In view of the data of the present investigation, the high concentration of 3-0-methyldopamine obtained by Smellie and Sandler must be seriously questioned. It is possible that there are highly fluorescent compounds in urine that are likely to interfere with their fluorimetric measurements of 3-0-methyldopamine.

Neuroblastoma, one of the most common solid malignancies of childhood (*30*), produces one or more of the catecholamines and their metabolites in excessive quantities. However, the enormous variability in both the quantitative and qualitative spectra of these compounds makes it difficult to decide which metabolite should be selected for diagnostic, prognostic, or investigative studies. Despite the fact that the catecholamine metabolites, VMA and homovanillic acid (HVA), are relatively simple to measure and are among the most frequently elevated in neuroblastoma (*2*), there are cases of "nonsecreting" tumors (*31,32*) in which metabolites less commonly assayed might be helpful in establishing a diagnosis or assessing the progress of the disease. In this respect, the radioimmunoassay of 3-0-methyldopamine was used to ascertain the diagnosis in two patients with neuroblastoma (proven by biopsy). Both had a significant elevation of plasma and urinary 3-0-methyldopamine (Table 2). However, VMA and HVA excretion in 24-hr urine specimens were found to be normal. In contrast, the plasma and urinary levels of 3-0-methyldopamine in a patient with pheochromocytoma were found to be normal. It is therefore apparent from this preliminary investigation that the diagnosis of neural-crest lesions requires a detailed understanding of the synthesis, storage, and metabolism of the catecholamines, their precursors, and their by-products.

The radioimmunoassay of 3-0-methyldopamine, with its high degree of specificity and sensitivity, may provide a quantitative test for the differential diagnosis of neural crest tumors. Furthermore, the levels of 3-0-methyldopamine in biologic fluids could reflect upon the in vivo metabolism of catecholamines by catechol-0-methyltransferase, since abnormalities in the metabolism of catecholamines are associated with many other pathologic conditions such as hypertension and Parkinsonism (*33*).

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FOOTNOTE

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