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Rigorous Biochemical Criteria for the Diagnosis of Pheochromocytoma

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THE "GRAY-COLORED TUMOR" OF THE ADRENAL medulla has fascinated physicians for 80 years. The diagnosis in the past has been hazardous and error prone. The variety of diagnostic measures currently recommended suggest there has been little consensus on the best approach. The purpose of this presentation is to give our convictions on definitive diagnosis based on 6 years' experience in a highly specialized laboratory in a large medical center. By definitive diagnosis is meant sufficient confidence in the results to operate on the patient without further diagnostic investigation.

MATERIALS

From 1966 to 1972, urinary epinephrine and norepinephrine have been determined preoperatively on 22 patients subsequently demonstrated to have histologically verified pheochromocytoma. Sixteen proven cases have had preoperative metanephrine and normetanephrine urinary studies. Multiple preoperative urine studies on most patients give a total of 63 preoperative urine samples for epinephrine or norepinephrine and 29 for metanephrine and normetanephrine. Hypertensive patients not on alpha-methyl-dopa treatment and not proven to have pheochromocytoma have been the basis for "normal" values. At least two of these patients with values considered "normal" have undergone celiotomy and an abdominal pheochromocytoma was not found. Multiple follow-up studies have indicated the others not to have the tumor.

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METHODS

When a pheochromocytoma is to be ruled out, two 12-hr overnight urine specimens are collected in a tinted bottle containing 10 ml 6 *N* HCl acid to keep the pH less than 3.0. Free catecholamines (epinephrine and norepinephrine) and metanephrine metabolites (metanephrine and normetanephrine) are measured using fluorometric trihydroxyindole methods of biochemical determination [4, 12]. Alpha-methyl-dopa treatment of hypertension must be ruled out in every case because it has been found to falsely elevate both free catecholamine [6] and metanephrine metabolite values to diagnostic range for at least 2 weeks. No diet restrictions are imposed. Outpatient collections are readily made. Specimens sent by public conveyance have been acceptable as long as the urine pH remains below 3 [5].

When the definitive diagnosis has been made, attempts at localization are often done (chest X-ray, IVP, venogram, caval catheterization, arteriogram, and ^{131}I cholesterol scan) but have not been considered essential in uncomplicated cases.

Table 1. Mean Urinary Levels in Nonpheochromocytoma Hypertensives

	Normal (n = 100)	Pheochromocytoma (n = 63)
Norepinephrine	28.9[a] ± 29.0[b] ($P < 0.001$)	1250 ± 2456
Epinephrine	7.6 ± 7.4 ($P < 0.001$)	238 ± 408
	Normal (n = 37)	**Pheochromocytoma (n = 29)**
Normetanephrine	44.2 ± 38.9 ($P < 0.001$)	1127 ± 842
Metanephrine	32.9 ± 16.4 ($P < 0.001$)	1305 ± 1708

[a] All urinary values in micrograms per 24 hr.
[b] ± 1 Standard deviation.

Table 2. Basis of Criteria for Definitive Diagnosis

Urinary norepinephrine and epinephrine
Strongly positive 90% (>5 SD)
Falsely positive 2%
Urinary Normetanephrine and Metanephrine
Strongly positive 100% (>5 SD)
Falsely positive 5%

RESULTS

Table 1 shows the mean urinary levels in nonpheochromocytoma hypertensives to be norepinephrine 28.9 ± 29.0* μg/24 hr, epinephrine 7.6 ± 7.4 μg/24 hr, normetanephrine 44.2 ± 38.9 μg/24 hr, metanephrine 32.9 ± 16.4 μg/24 hr. These are compared to the mean values found in proven pheochromocytoma patients, norepinephrine 1250 ± 2456 μg/24 hr, epinephrine 238 ± 408 μg/24 hr, normetanephrine 1127 ± 842 μg/24 hr, and metanephrine 1305 ± 1708 μg/24 hr. All four group comparisons with Student's t test show $P < 0.001$. High normal limits for 95% confidence could be considered to be the mean plus 2 SD. Anything above this we consider suspicious but the diagnostic range has occurred at 5 SD in excess of the normal mean.

Using these criteria, urinary norepinephrine and epinephrine were diagnostic in 90% of all overnight urine collections in proven cases with 2% falsely positive in nonpheochromocytoma hypertensives. All 29 urine samples tested for metanephrine metabolites were diagnostic with approximately 5% falsely positive (Table 2). No patient without pheochromocytoma had false positives in both free catecholamines and metanephrine metabolite determinations. No patient with a pheochromocytoma had a falsely negative urine for metanephrine metabolites. Crisis samples have not been helpful and their interpretation is doubtful. We do not rely on them for diagnosis.

* ±1 Standard Deviation.

DISCUSSION

Multiple biochemical screening and other diagnostic tests for pheochromocytoma have been suggested that are relatively inexpensive and uncomplicated. These include the colorimetric technique for Vanillylmandelic acid (VMA) of Gitlow *et al.* [8, 9] and total metanephrine excretion by electrophoretic chromatography [15]. These are discussed in detail by Gitlow *et al.* in later publications [7, 14] with excellent results. However, most of the less specific techniques for VMA currently in use in many clinical laboratories offer only confusing diagnostic aid [1] with up to 30% falsely positive and negative results [10]. Crout [2] suggested that VMA tests are less accurate than free catecholamines and metanephrine metabolite excretion. Since savings in time and expense in our own hospital were negated by the large number of VMA tests that had to be redone, we have continued to recommend only free catecholamine and metanephrine metabolite urinary studies for pheochromocytoma evaluation. We agree with

Pertsemlidis *et al.* [14] that more than one measurement of several urinary products is sufficient for diagnosis and disagree with Mahoney *et al.* [13] that multiple methods be utilized as well as multiple determinations with each technique. The interfering medication, alpha-methyl-dopa, must be ruled out as well as severe stresses such as large thermal burns. In our laboratory we have found that alpha-methyl-dopa cannot only elevate the catecholamines but the metanephrine metabolites as well to diagnostic range.

The overnight 12-hr resting collection has been very satisfactory. Unusual stress and activity is avoided and the convenience for the patient results in more accurate collection.

Since Crout's suggestion in 1961 [3] that metanephrine metabolites be measured, it has been shown to be of excellent diagnostic support [2, 7, 10, 14]. Our results have been most encouraging. No patient with a pheochromocytoma has had a single negative urine for both metanephrine or normetanephrine.

Provocative and suppressive pharmacologic studies have no place in modern pheochromocytoma diagnosis. The definite dangers and gross inaccuracies are documented in almost every report on this subject.

Tumor localization after the diagnosis has been made is often done to help establish whether or not there is more than one lesion. Efforts at localization in this institution have been previously published [11].

SUMMARY

Based on urinary studies of patients with pheochromocytoma as compared to hypertensives considered to be without this tumor, rigorous biochemical criteria for diagnosis are proposed. Free catecholamines in 90% and metanephrine metabolites in 100% of the pheochromocytoma patients' urine samples exceeded the normal (hypertensive) values by more than 5 SD. No patient without pheochromocytoma has falsely positive values in both free catecholamine and metanephrine metabolite determinations. No patient with a pheochromocytoma had a urine sample negative for metanephrine metabolites. The combination of these studies has given excellent accuracy for definitive diagnosis.

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