Evaluation of Increased Norepinephrine Excretion in Hypertension Using L-Dopa-3H

By Vincent DeQuattro

ABSTRACT

Four hypertensive patients with features of sympathoadrenal hyperactivity similar to those of patients with pheochromocytoma had rates of excretion of norepinephrine plus epinephrine (μg/day) of 135 ± 13 (mean ± SE) compared with 441 ± 59 in five patients with pheochromocytoma and 53 ± 7 in six patients with primary hypertension. The specific activities of urinary norepinephrine and the half-life of the rate of their decline were determined for each patient for 24 hours after intraneuronal labeling of norepinephrine using L-dopa-3H. The initial specific activities of norepinephrine (dpm/mg/mole) and the half-life of the rate of their decline (hours) for the three groups were 2,920 ± 670 and 3.8; 291 ± 89 and 4.2; and 6,782 ± 1,003 and 6.0, respectively. However, the observed differences in the half-life values were not significant. The findings indicated that the rate of release and excretion of newly synthesized norepinephrine was increased in patients with hypertension and sympathoadrenal hyperactivity. Less than 1% of the infused L-dopa-3H was converted to norepinephrine in the chromaffin tumors of patients with pheochromocytoma, resulting in low specific activities of urinary norepinephrine; this may be of diagnostic value in hypertensive patients who have equivocally increased rates of norepinephrine excretion. The autonomic hyperactivity, hypertension, and excessive norepinephrine excretion in the four patients without pheochromocytoma may have been related to unidentified forms of stress.

KEY WORDS norepinephrine specific activity pheochromocytoma norepinephrine turnover rates catecholamine autonomic hyperactivity

Some patients with primary hypertension have striking features of sympathoadrenal hyperactivity, including flushing, pallor, sweating, tachycardia, and marked lability of the blood pressure. These patients are of interest for both theoretical and practical reasons. On the one hand they make credible the concept that sympathetic nerve dysfunction may cause some forms of hypertension and on the other hand they must be distinguished from patients with pheochromocytoma, a potentially curable disorder. This dual motivation led to the present study of four patients with hypertension who had clinical features of autonomic hyperactivity and increased norepinephrine excretion.

The clinical diagnosis of pheochromocytoma is confirmed by finding increased rates of urinary excretion of the catecholamines. These rates exceed 10 μg/hour in 90 to 95% of patients with pheochromocytoma, but they may be in the equivocal range of 6 to 10 μg/hour in the remaining patients (1). Norepinephrine excretion is usually normal or decreased in patients with primary hypertension (2, 3). However, 5% or more of these patients have rates of norepinephrine excretion in excess of 6 μg/hour without other evidence for pheochromocytoma (2, 4, 5). Sympathoadrenal stimulation during stress also causes increased catecholamine excretion in excess of 6 μg/hour. When this occurs in patients after extensive burns, severe physical

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NOREPINEPHRINE EXCRETION IN HYPERTENSION

exercise, major surgical procedures, or myocardial infarction and in those with brain tumors or miscellaneous neurological disorders (2, 6) there is rarely a problem in the differential diagnosis of pheochromocytoma.

During 1967 and 1968, the catecholamine content of urine specimens from 175 patients with hypertension was measured to confirm or exclude the diagnosis of pheochromocytoma. The rates of catecholamine excretion were with 2 of normal (2.5 ± 1.5 μg/hour) in 227 (90%) of these patients. The excretion rates of norepinephrine were in excess of 6 μg/hour in 15 patients; 9 had pheochromocytoma and the other 9 had increased norepinephrine excretion for unknown reasons.

This paper describes the attempt to characterize the increased norepinephrine excretion in four of the latter patients and to further distinguish them from patients with proved pheochromocytoma by comparative studies of urinary norepinephrine-3H specific activity and turnover after intravenous administration of the trium-labeled precursor L-dihydroxyphenylalanine (L-dopa-3H) (7). The specific activities of norepinephrine-3H in chromaffin tumors, adrenal glands, and spleens removed from the patients with pheochromocytoma 4 to 48 hours after L-dopa-3H infusion were useful in interpreting the urinary isotope studies. Furthermore, since L-dopa is revolutionizing therapy of some neurologic disorders, these data provide valuable knowledge regarding its metabolism in man.

Methods

SUBJECTS

Studies of catecholamine metabolism in four groups of subjects are described in this report.

Group I.—In this group were four patients with hypertension who had sympathoadrenal hyperactivity and increased rates of norepinephrine excretion without pheochromocytoma (Table 1). Patient G.D., a black male, had sweating and bouts of tachycardia and labile hypertension for 7 years prior to this study. This patient was described previously by Brunjes et al. as a hypertensive person who gave clinical evidence of having a pheochromocytoma with increased norepinephrine and normetanephrine excretion, but normal vanillylmandelic acid excretion (8).

He had episodes characterized by intense vasomotor constriction which were relieved by the alpha-receptor-blocking agent, phentolamine. His blood pressure responded to phentolamine therapy initially. No pheochromocytoma was found at laparotomy and cystoscopy. He was treated with digoxin and hydrochlorothiazide for increasing dyspnea and edema during the 6 months prior to this study. His blood pressures remained elevated and labile. He had a pulmonary embolus one month prior to the isotope studies, and heparin was added to his therapy. He died suddenly 1 month after the isotope studies; autopsy revealed acute and chronic pulmonary emboli, severe bullous emphysema, marked cardiomegaly, and congestive heart failure. No pheochromocytoma was found.

Patient G.V., a Caucasian female, had a 10-year history of labile hypertension and facial flushing. She also had bouts of sinus tachycardia and postural hypotension. For 2½ years following these studies her hypertension and tachycardia have responded well to the beta-receptor-blocking agent, propranolol.

The specific signs of sweating, flushing, tachycardia, and lability of blood pressure mentioned in the descriptions of the individual patients were the outstanding features of the hypertensive hyperactivity in the remaining patients. Further, F.B. had "cold sweats" with "goose flesh" reminiscent of hypoglycemic reactions, and G.D. had episodes of coolness of his extremities and peripheral vasomotor instability as manifested by flushing, pallor, and tachycardia and the sweating were due to causes other than sympathoadrenal hyperactivity. How-
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>BP range</th>
<th>Fundus grade</th>
<th>Abnormal physical findings</th>
<th>ECG</th>
<th>Other diagnoses</th>
<th>Drug tests for phaeochromocytoma</th>
<th>Negative diagnostic tests*</th>
<th>BP response to therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.B.</td>
<td>34, M</td>
<td>70/80-150/110</td>
<td>I</td>
<td>facial flushing; sweating</td>
<td>normal</td>
<td>postural hypotension</td>
<td>phentolamine (—)</td>
<td>IVP, RA, laparotomy</td>
<td>postural hypotension improved with fludrocortisone</td>
</tr>
<tr>
<td>G.D.</td>
<td>45, M</td>
<td>140/80-180/120</td>
<td>II</td>
<td>venous distension; sweating; cardiac and hepato-megaly</td>
<td>ECG: P pulmonale biventricular hypertrophy</td>
<td>obstructive lung disease: chronic pulmonary emboli; class IV congestive heart failure</td>
<td>phentolamine (+)</td>
<td>IVP, RA CO₂ study</td>
<td>initial</td>
</tr>
<tr>
<td>W.D.</td>
<td>47, M</td>
<td>140/90-160/120</td>
<td>II</td>
<td>facial flushing; sweating</td>
<td>nonspecific T wave abnormalities</td>
<td>degenerative cervical arthritis</td>
<td>histamine (—)</td>
<td>IVP, RA CO₂ study</td>
<td>improvement with hydrochlorothiazide</td>
</tr>
<tr>
<td>G.V.</td>
<td>60, F</td>
<td>60/0-170/100</td>
<td>II</td>
<td>sinus tachycardia; absent pulse, left leg</td>
<td></td>
<td>postural hypotension; lumbar sympathetic block</td>
<td>phentolamine (—)</td>
<td>IVP</td>
<td>improvement with progesterone</td>
</tr>
</tbody>
</table>

*IVP = pyelogram; RA = renal arteriogram; CO₂ study = retroperitoneal CO₂ insufflation.
†Confirmation at autopsy: cause of death was acute pulmonary emboli from a right atrial mural thrombus.
‡Splenoidal enlargement of left adrenal gland not confirmed by RA or IVP.
ever, these features resembled those observed in the patients with pheochromocytoma and they were temporally related to increased norepinephrine excretion.

F.B., W.D., and G.V. received no medication for at least 1 month prior to the study and none of the four with hyperexcretion of norepinephrine received antiadrenergic drugs for at least 6 months prior to the study. The blood pressure responses to tyramine, histamine, and phenylbiguanide were normal except for a positive response to phentolamine in C.D. in 1961. Tyramine given intravenously produced systolic blood pressure elevations of 3, 15, 17, and 10 mm Hg/mg tyramine base, in F.B., G.D., W.D., and G.V., respectively. Physical examination, roentgenographic studies and laboratory evaluation including complete blood counts, serum sodium, potassium, bicarbonate, glucose tolerance test, serum urea nitrogen, creatinine, uric acid, liver function tests, and routine urinalysis were normal at the time of the L-dopa-3H study except for the abnormalities already mentioned and those listed in Table 1.

**Group II.**—In this group were five patients who had proved pheochromocytoma and increased rates of norepinephrine excretion (Table 2). The patterns of hypertension in the patients with pheochromocytoma were related to the fractional turnover rates of their tumors (Table 2). The turnover rates were obtained by dividing the 24-hour excretion of total catecholamine metabolites by the total catecholamine content of the tumors (9). The fractional turnover is an average of different values from various portions of the tumors. The patients with pheochromocytoma received oral phenoxybenzamine for 5 to 10 days prior to the isotope studies. Patient D.A. also received 30 mg propranolol daily for 4 days before the L-dopa-3H study.

**Group III.**—In this group were five normal volunteers (3 females and 2 males) aged 21 to 46, who were normotensive and had normal rates of catecholamine excretion.

**Group IV.**—There were 6 patients (3 females and 3 males) with primary hypertension without sequelae aged 32 to 54, with normal rates of catecholamine excretion who were reported in detail previously (7). The mean rates of excretion of the catecholamines and the catecholamine metabolites of these four groups, both before and after pulse labeling with L-dopa-3H, were compared with each other. In addition the rates of excretion of the catecholamine metabolites of the patients in Group I were compared with the corresponding mean values of a fifth group of 47 normal subjects and a sixth group of 73 patients with primary hypertension.
TABLE 3
Excretion of Labeled and Unlabeled Catecholamines and Vanillylmandelic Acid for 24 Hours after L-dopa-3H Infusion in Patients with Increased Norepinephrine Excretion

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hours postinfusion</th>
<th>NE (ng/hr)</th>
<th>E (ng/hr)</th>
<th>DA (ng/hr)</th>
<th>VMA (mg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-3</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
</tr>
<tr>
<td></td>
<td>3-6</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
</tr>
<tr>
<td></td>
<td>6-12</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
</tr>
<tr>
<td></td>
<td>12-24</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
<td>4.1 x 10^6</td>
</tr>
</tbody>
</table>

*Value too low to calculate.

NE, E, DA and VMA are norepinephrine, epinephrine, dopamine, and vanillylmandelic acid.

Excretion of Labeled and Unlabeled Catecholamines and Vanillylmandelic Acid for 24 Hours after L-dopa-3H Infusion in Patients with Increased Norepinephrine Excretion.

**Protocol**
Endogenous pulse labeling of the intraneuronal catecholamines was achieved by intravenous infusion of L-dopa-3H, 2, 5, 6 ring labeled, 15 μCi/kg (500 mc/m mole) in 100 ml of 5% dextrose.

Obtained from Amersham/Searle, Des Plaines, Illinois.
TABLE 4
Catecholamine Excretion Rates and Norepinephrine-3H Specific Activity after L-dopa-3H in Hypertension, Hyperexcretion of Norepinephrine and Pheochromocytoma

<table>
<thead>
<tr>
<th>Condition</th>
<th>Norepinephrine ± Epinephrine (μg/24 hr)</th>
<th>Time of infusion*</th>
<th>Zero time intercept (dpm/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (5)</td>
<td>44 ± 8</td>
<td>2.9 ± .3</td>
<td>5,780</td>
</tr>
<tr>
<td>Primary hypertension (6)</td>
<td>53 ± 7</td>
<td>3.1 ± .7</td>
<td>6,022 ± 449</td>
</tr>
<tr>
<td>Norepinephrine hyperexcretion (4)</td>
<td>135 ± 13</td>
<td>14.3 ± 2.6</td>
<td>2,920 ± 670</td>
</tr>
<tr>
<td>Pheochromocytoma (5)</td>
<td>441 ± 59</td>
<td>45 ± 12</td>
<td>285</td>
</tr>
</tbody>
</table>

P values (PH vs. HEN) <.001 <.01
P values (HEN vs. Phoe) <.01 <.05

Values are means ± se. PH = primary hypertension; HEN = hyperexcretion of norepinephrine; Phoe = pheochromocytoma.

*Time of infusion is the 3/4 hour during the dopa-3H infusion and the 2½ hours after the end of infusion. Number in parentheses is number of patients.

Results
Excretion rates of the Catecholamines and Vanillylmandelic Acid in Patients with Enhanced Norepinephrine Excretion.—The excretion rates of norepinephrine, epinephrine, dopamine and vanillylmandelic acid are given for individual patients for the first 24 hours after L-dopa-3H in Table 3. They are representative of values found in two or three additional 24-hour urine samples collected before and after the dopa infusion study. Norepinephrine excretion rates were variable in patients with pheochromocytoma except G.A., who had normal values on several occasions. Epinephrine excretion rates were in the normal range except in a patient with pheochromocytoma (E.R.). The norepinephrine, epinephrine, and assays of vanillylmandelic acid used by Brunjes for Groups V and VI (3) were modifications of the methods used for Groups I-IV. However, we established that simultaneous assays of 20 urine samples by the respective methods yielded identical values.

ASSAYS
Total labeled and unlabeled catecholamines were isolated by alumina chromatography from aliquots of urine and protein-free filtrates of the tissue; norepinephrine and epinephrine were then quantified by fluorometric assay (10). Carrier norepinephrine and epinephrine were added to the alumina eluates and placed over an IRC-50 Na+ column. Norepinephrine-3H and epinephrine-3H were then separated from dopamine-3H, placing the IRC-50 Na+ eluates over Dowex 50 Na+ columns (7). The quantities of labeled norepinephrine and dopamine and unlabeled dopamine were determined from the various Dowex eluate fractions. Labeled and unlabeled vanillylmandelic acid were quantified by conversion to vanillin and subsequent photometric and radioassay (7, 11). Total free and conjugated metanephrine and normetanephrine were quantified fluorometrically (3).
patients with essential hypertension. Dopa-
mime excretion rates were above 10-20 μg/
hour in the sample bracketing the dopa
infusion because approximately 10% of the
infused dopa was excreted rapidly as dopa-
mime (7). Vanillylmandelic acid excretion
rates were increased significantly in only one
patient (F.B.) of those who had hyperexcre-
tion of norepinephrine, but they were in-
creased in all the patients with pheochromocy-
toma except T.G.

The means of total norepinephrine and
epinephrine excretion in micrograms per 24
hours, of maximum values per hour, and of
excretion rate per hour for the half hour
during and the 2% hours after L-dopa-3H
infusion are shown for each group in Table 4.
The mean rates of norepinephrine excretion of
the patients with hyperexcretion of norepi-
nephrine were significantly greater than those
of normotensive volunteers and patients with
primary hypertension, but they were signifi-
cantly less than the values in pheochromocy-
toma. Epinephrine accounted for less than 10%
of the total catecholamines in most urine
samples from the patients who excreted
excessive amounts of norepinephrine, al-
though, occasionally, samples (patients F.B.
and G.V.) contained as much as 30% epineph-
rine. Normetanephrine and metanephrine and
vanillylmandelic acid, were assayed in sam-
pies of urine from patients with increased
norepinephrine excretion which contained
both normal and elevated amounts of cate-
cholamine. With an increase in norepineph-
rine excretion there was a simultaneous rise
in normetanephrine excretion from two to ten
times the normal values while the vanillyl-
mandelic acid excretion rates were usually
normal or only slightly increased (Table 5).

E + NE = total free epinephrine plus norepinephrine; MN + NMN = total free and conjuncted metanephrine
plus normetanephrine; VMA = vanillylmandelic acid. Values are in μg/hour. For F.B., 12-hour samples collected
on successive nights; for G.D., 24-hour samples collected in 1961 and 1966; for W.D., a timed sample collected im-
mediately after and 24 hours after dopa-3H; and for G.V., during and after an episode of sinus tachycardia.

*Values reported previously from this laboratory by Brunjes are means ± SD (3).
Norepinephrine excretion in hypertension

Norepinephrine-3H specific activities for the 24 hours after L-dopa-3H in the urine of hypertensive patients with normal (means ± SE for six patients) and increased norepinephrine excretion (four patients) and with pheochromocytoma (five patients) and the respective curves of decline.

Urinary norepinephrine reflects the mean plasma norepinephrine specific activity for that time period. The latter represents contributions from the various sympathetically innervated organs. The specific activities for each patient with increased norepinephrine excretion (Table 3) and the means ± SE for the six patients with primary hypertension were plotted semilogarithmically for the first 24 hours following L-dopa-3H infusion (Fig. 1). Regression lines were constructed for each group from the slopes and intercepts derived from the individual norepinephrine specific activities according to the method of least squares. The half-life of this first 24-hour phase of specific activity decline was 6.0 hours for patients with primary hypertension, 3.8 hours for those with hyperexcretion of norepinephrine, and 4.2 hours for those with pheochromocytoma; the differences were not statistically significant.

The mean values of the specific activities of norepinephrine for patients with increased norepinephrine excretion at zero time and 3 hours after L-dopa-3H were tenfold greater than those of the patients with pheochromocytoma, (Table 4). The urinary norepinephrine specific activity at zero time was calculated by extending the regression line for the rate of decline of norepinephrine specific activity to the y intercept. The lower specific activities in the patients with pheochromocytoma were a result of an increase in endogenous norepinephrine excretion and a proportional decrease in percent excretion of label as norepinephrine-3H (Table 6). However, 3 hours after L-dopa-3H, the specific activity values of one pheochromocytoma patient (D.A.) nearly overlapped values of patients with hyperexcretion of norepinephrine.

The quantities of labeled dopamine, norepinephrine and vanillylmandelic acid recovered after L-dopa-3H in cases of hyperexcretion of norepinephrine were not significantly different from those found in cases of primary hypertension or pheochromocytoma (Table 6). However, the two patients with the highest excretion rates of norepinephrine at the time of study also had the greatest percent excretion of L-dopa-3H as labeled norepinephrine. Further, the fraction of total norepinephrine-3H excreted at 6 hours was also significantly increased from 68 to 90% in cases of norepinephrine hyperexcretion and in cases of pheochromocytoma. The specific activities of dopamine and the curves of decline were similar in the three groups. The vanillylmandelic acid specific activities and the half-lives of the rate of decline of vanillylmandelic acid specific activities were decreased in the hyper-excretors of norepinephrine and the patients with pheochromocytoma compared with the values in patients with primary hypertension. The differences in vanillylmandelic acid specific activities and the rates of specific activity decline of the three groups were less than

![Graph](http://circres.ahajournals.org/figures/)

*Figure 1* Norepinephrine-3H specific activities for the 24 hours after L-dopa-3H in the urine of hypertensive patients with normal (means ± SE for six patients) and increased norepinephrine excretion (four patients) and with pheochromocytoma (five patients) and the respective curves of decline.
TABLE 6
Increased Excretion of Norepinephrine-3H after Dopa-3H in Patients with Increased Norepinephrine Excretion without Pheochromocytoma

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percent infused L-dopa-3H on</th>
<th>Percent total NE-3H recovered at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dopamine-3H</td>
<td>Norepinephrine-3H</td>
</tr>
<tr>
<td>Essential hypertension (5)</td>
<td>12.2 ± 1.1</td>
<td>0.25 ± 0.004</td>
</tr>
<tr>
<td>Hyperexcretion of norepinephrine (4)</td>
<td>10.2 ± 3.3</td>
<td>0.40 ± 0.012</td>
</tr>
<tr>
<td>Pheochromocytoma (5)</td>
<td>9.2 ± 2.5</td>
<td>0.017 ± 0.002</td>
</tr>
</tbody>
</table>

Mean ± SD.

*Hypereexcretion of norepinephrine and pheochromocytoma values (means ± SD) are significantly increased from patients with essential hypertension, P < .02 and P < .05, respectively.

TABLE 7
Synthesis of Tumor Norepinephrine-3H Compared with Urinary Norepinephrine-3H and VMA-3H Excretion after L-dopa-3H in Pheochromocytoma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tumor removed after Dopa-3H (hr)</th>
<th>Tumor NE-3H specific activity (dpm/mg protein)</th>
<th>Converted to urine NE-3H at same time</th>
<th>Replaced from tumor at NE-3H</th>
<th>Released from NS-3H + VMA-3H</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.B.</td>
<td>25</td>
<td>0.17</td>
<td>0.02</td>
<td>0.22</td>
<td>1.2</td>
</tr>
<tr>
<td>G.A.</td>
<td>29</td>
<td>0.70</td>
<td>0.14</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>T.G.</td>
<td>46</td>
<td>0.09</td>
<td>0.07</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>D.A.</td>
<td>27</td>
<td>0.08</td>
<td>0.04</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>E.R.</td>
<td>28</td>
<td>0.82</td>
<td>0.00</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

*Zero time specific activity was calculated by constructing a line from the specific activity value of the tumor norepinephrine at the time of its removal to the Y axis, using the fractional turnover rate as the slope.
activities, as measured in atrial biopsies of patients undergoing cardiac surgery, yielded a half-life of 5.5 hours (7).

Discussion

The patients with norepinephrine hyperexcretion were a heterogeneous group whose common features were hypertension and excessive autonomic activity. They had sustained or intermittent increases in excretion of the catecholamines and their metabolites: norepinephrine, marked; normetanephrine, moderate; vanillylmandelic acid, small; and epinephrine and metanephrine, variable. Studies with L-dopa-3H in patients with norepinephrine hyperexcretion showed that they had decreased norepinephrine specific activities, an increased percent of radioactivity excreted as norepinephrine-3H, an increased fraction of labeled norepinephrine excreted within the first 6 hours after L-dopa-3H infusion, and an increased norepinephrine turnover; although the latter was not significantly different from patients with normal rates of norepinephrine excretion. These data indicate that the synthesis of norepinephrine and the release and excretion of newly synthesized norepinephrine were increased with norepinephrine hyperexcretion.

There are two major degradative pathways for the catecholamines: O-methylation via catechol-O-methyl transferase inactivates circulating catecholamines released from the adrenal medulla and nerve endings. Oxidative deamination via monamine oxidase is the principal route for the initial deactivation of norepinephrine degraded in the adrenal medulla and the sympathetic nerves (12). The ratios of the rates of excretion of the metanephrines—the products of O-methylation—to the excretion rates of vanillylmandelic acid—the product of both O-methylation and deamination—indicate which is the predominant pathway (3). Normally, in man the ratio is 1 to 10, indicating that intraneuronal degradation via monoamine oxidase is the predominant pathway. The increased ratios of urinary metanephrines to vanillylmandelic acid associated with hyperexcretion of norepinephrine suggested a relative increase in the rate of catecholamine release and correspondingly a decrease in in situ catecholamine metabolism. This evidence for increased norepinephrine release confirmed the findings of the L-dopa-3H studies. These patients had normal or subnormal pressor responses to tyramine, indicating that monoamine oxidase activity per se was not inhibited (13).

The interpretations of the specific activity data are summarized schematically in Figure 3. The patients with primary hypertension and normal norepinephrine excretion had the smallest concentration of releasable norepinephrine, and thus labeling with L-dopa-3H yielded norepinephrine with the highest specific activity. Small percents of the infused dopa-3H were incorporated into the norepinephrine of the tumors in patients with pheochromocytoma. Less than 10% of the total catecholamine radioactivity formed from the
L-dopa was synthesized in the tumors. Perhaps this was related to a small percent of the total cardiac output perfusing the tumors. The low specific activity of this tumor norepinephrine was reflected by the lowest specific activity of urinary norepinephrine in patients with pheochromocytoma.

The different specific activities of norepinephrine in the patients of the two groups with increased norepinephrine excretion may have diagnostic value. Only patients with pheochromocytoma had urinary norepinephrine specific activities less than 300 dpm/mumole during the first 3 hours after L-dopa-3H. On the other hand, the patients with primary hypertension had norepinephrine specific activity which exceeded 1,000 dpm/mumole. However, the norepinephrine specific activities in one patient (D.A.) with pheochromocytoma were similar to those in patients with hyperexcretion of norepinephrine. This was not merely the result of a low norepinephrine excretion rate (30 µg/hour) at the time of dopa-3H infusion, since T.G., another patient with pheochromocytoma, had low norepinephrine excretion rates (14 µg/hour) and low specific activity. D.A. was very labile emotionally, and the high specific activities may have been related to increased norepinephrine-3H secretion from the sympathetic nerves as discussed below.

The causes of the hypertension and the associated sympathetic hyperactivity in the patients with norepinephrine hyperexcretion were not identified. There may have been unidentified forms of physical, mental, or emotional stress. One patient (F.B.) displayed rage and hostility periodically, but the others were cooperative and appeared to be stable emotionally. Although patients with emotional stress usually have increased excretion of epinephrine as well as norepinephrine, active aggressive emotional displays have resulted in increased excretion of norepinephrine (14). Previous studies after L-dopa-3H demonstrated increased rates of norepinephrine turnover in humans during stress, and after reserpine therapy (7) and in patients with familial dysautonomia (15). Stress (16) and buffer nerve section (17) in animals have
resulted in increased sympathetic nerve activity and increased synthesis of norepinephrine. Direct stimulation of sympathetic nerves in animals increased the release of newly synthesized norepinephrine (18). Various stresses in man have resulted in increased norepinephrine excretion, but some forms such as physical exercise (unpublished observation in our laboratories) and surgical stress (19), while increasing norepinephrine excretion, have a less marked effect on the excretion, the catecholamine metabolites. Thus the increased rates of norepinephrine turnover (7) and the pattern of excretion of the metabolites (19) in surgical stress were similar to the respective values found in the cases of hyperexcretion of norepinephrine.

Patients who excrete excessive amounts of norepinephrine have altered sympathetic nerve function, but they differ from patients with familial dysautonomia, who have normal rates of catecholamine excretion but reduced rates of vanillylmandelic acid excretion (20). There was no clinical or laboratory evidence implicating congestive heart failure as the initial cause of the increased norepinephrine excretion. However, C.D. had congestive heart failure at the time of the isotope studies, and it is probable that compensatory sympathoadrenal hyperactivity accounted for some of the increased rate of norepinephrine excretion. Patients with congestive heart failure have increased norepinephrine excretion without alteration of norepinephrine turnover after \( L \)-dopa-\( ^3 \)H (21). Further, cardiac norepinephrine content (21) and tyrosine hydroxylase activity was decreased in patients (22) and animals (23) with congestive heart failure—evidence that norepinephrine synthesis is decreased in the heart in congestive failure. The reduced norepinephrine specific activities after \( L \)-dopa-\( ^3 \)H observed with hyperexcretion of norepinephrine indicated that the tyrosine-to-dopa pathway, and thus tyrosine hydroxylase activity, was increased. The percent of radioactivity recovered as catecholamine metabolites in norepinephrine hyperexcretion was normal; this is further evidence of intact biosynthesis of norepinephrine from \( L \)-dopa-\( ^3 \)H. The reduced norepinephrine specific activity after \( L \)-dopa-\( ^3 \)H in C.D. may have resulted in part from increased norepinephrine synthesis in extracardiac sites where there was no reduction of tyrosine hydroxylase activity and where \( L \)-dopa-\( ^3 \)H uptake was less than in the heart (Fig. 2).

The patients with pheochromocytoma had slower fractional turnover rates of tumor norepinephrine than the turnover rates determined by the \( L \)-dopa-\( ^3 \)H method. Fractional turnover indicated the rate of replacement of all norepinephrine synthesized in the tumor. Presumably, norepinephrine stored prior to its in situ degradation has a longer half-life than norepinephrine released and excreted unchanged. The rates of turnover after \( L \)-dopa-\( ^3 \)H were more representative of norepinephrine released from the tumor and excreted unmetabolized than norepinephrine degraded in situ and excreted as a catecholamine metabolite. The increased fraction of norepinephrine-\( ^3 \)H excreted at 6 hours in pheochromocytoma compared with the values in the patients with essential hypertension may have been related to the displacement of norepinephrine-\( ^3 \)H newly synthesized in sympathetic nerves by the norepinephrine circulating from the tumor. Although phenoxybenzamine increased norepinephrine turnover rates in animals (24), it is unlikely that this drug produced the differences found in pheochromocytoma. The norepinephrine specific activities and the curve of their decline after \( L \)-dopa-\( ^3 \)H in a patient with hypertension were not altered after 2 weeks of phenoxybenzamine, 30 mg/day orally (unpublished observation).

The pattern of hypertension in the patients with pheochromocytoma correlated well with tumor size and fractional norepinephrine turnover rates: small tumors with rapid fractional turnover were found in patients with sustained hypertension, whereas large tumors with slow fractional turnover rates were present in patients with labile blood pressure elevation (9). It may be that patients who have tumors with rapid fractional turn-
over also have earlier symptoms and seek medical help when the tumors are small.

The estimated turnover rates of tissue norepinephrine for spleen and heart were similar to those described previously in animals. The half-life of the adrenal norepinephrine turnover rate was 27 hours, although it has been described as 7 days in the rat adrenal (25). There is additional evidence that adrenal catecholamine turnover may be rapid in man. The fractional turnover rate of epinephrine in the human adrenal is 0.028 \( \mu \text{g} / \text{hour} \) when calculated from the epinephrine secretion rate of 0.01 \( \mu \text{g} / \text{kg} \times \text{min}^{-1} \) (26) and the content of adrenal epinephrine (approximately 1.5 mg). This yields an epinephrine turnover with a half-life of 25 hours, similar to that found for norepinephrine in this study. Interestingly, little epinephrine-\(^3\)H was formed from dopa-\(^3\)H in the adrenal glands; of the adrenal catecholamine radioactivity, more than 90% was norepinephrine-\(^3\)H and less than 5% was epinephrine-\(^3\)H. Of the infused L-dopa-\(^3\)H, 0.41% was converted to norepinephrine in the heart, 0.01% in the spleen, and 0.026 in the adrenals. Since the total excretion of labeled catecholamine metabolites was approximately 2% of the infused dopa-\(^3\)H, these measurements confirmed earlier findings in man which indicated that the heart contributed a large proportion of the total urinary norepinephrine-\(^3\)H and vanillylmandelic acid-\(^3\)H (7).

Patients with equivocal or marked elevations of norepinephrine and catecholamine metabolite excretion require other diagnostic measures to exclude the diagnosis of pheochromocytoma. The relatively normal rate of excretion of vanillylmandelic acid may have been the most important clinical laboratory clue to ruling out a diagnosis of pheochromocytoma. The dopa-\(^3\)H infusion and the determination of norepinephrine specific activity was of additional diagnostic value in this group of patients. The hypertension appeared to be causally related to the excessive sympathetic activity and increased norepinephrine synthesis and turnover, an association which has been described in some animal models with hypertension (17, 27).

Hyperexcretion of norepinephrine is rare, but 5% of a group of patients with hypertension who were being evaluated for possible pheochromocytoma had this abnormality. Two of 47 normal subjects studied by Brunjes (3), whose mean values are given in Table 4, had catecholamine excretion rates in excess of 6 \( \mu \text{g} / \text{hour} \), although the pattern of excretion of the catecholamine metabolites were normal. One may properly ask whether these patients who excrete excessive amounts of norepinephrine represent a subset of patients with hypertension due to sympathetic nerve dysfunction or if the increased excretion merely represents one end of the normal bell-shaped curve.

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