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Vincent DeQuattro, Albert Sjoerdsma

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Research Article

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Catecholamine Turnover in Normotensive and Hypertensive Man: Effects of Antiadrenergic Drugs

VINCENT DEQUATTRO and ALBERT SJOERDSMA

From the Experimental Therapeutics Branch, National Heart Institute, Bethesda, Maryland 20014

ABSTRACT Intravenous administration of tritium-labeled 3,4-dihydroxyphenylalanine (dopa) to human subjects resulted in the labeling of endogenous catecholamines and vanillylmandelic acid (VMA). Determination of the changes in specific activity of these compounds with time in fractional collections of urine and in cardiac biopsies from patients undergoing corrective cardiac surgery permitted estimation of apparent turnover rates. The average half-time of the exponential decline in specific activity of labeled urinary norepinephrine was about 8 hr and that of VMA was 11–16 hr in five normal subjects. No significant differences from normal were observed in eight patients with essential hypertension. The average half-life of norepinephrine was only 5 hr in cardiac patients undergoing surgery, and the levels and rate of decline of cardiac norepinephrine specific activity correlated well with the exponential phase of the urinary disappearance curve. There were significant effects of treatment with alpha-methyltyrosine, reserpine, and pargyline hydrochloride on the labeling and apparent turnover rates of norepinephrine and VMA; the effects noted were consistent with known actions of these three drugs. It is suggested that the technique used is a suitable means of assessing "over-all" catecholamine metabolism in man, particulary if combined with quantitative assay of urinary catecholamine metabolites.

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Dr. DeQuattro's present address is the University of Southern California, Los Angeles County Hospital, Department of Medicine, 1200 North State Street, Los Angeles, Calif. 90033.

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INTRODUCTION

It has been suspected that dysfunction of the sympathetic nervous system is a pathogenetic factor in essential hypertension. This supposition is tenable because of: (*1*) clinical impressions that some hypertensive patients exhibit sympathetic "hyperactivity;" (*2*) the enhanced vascular responsiveness of hypertensive subjects to injections of norepinephrine (1, 2); and (*3*) the effectiveness of treatment with antiadrenergic drugs. One would expect that gross abnormality of sympathetic nerve activity would be detected by measurements of the rate of excretion of norepinephrine and its metabolites in the urine. However, numerous studies (cf. 3) of urinary catecholamine metabolites have failed to reveal any consistent abnormalities in cases of essential hypertension. Nonetheless, a few reports of alterations in the metabolism of endogenous or exogenous norepinephrine in human (3–6) and experimental (7, 8) hypertension indicate the issue is not yet settled.

Isotopically labeled tyrosine, 3,4-dihydroxyphenylalanine (dopa), 3,4-dihydroxyphenylethylamine (dopamine), and norepinephrine have been used to determine the turnover rate of norepinephrine in tissues of intact animals and in perfused organs. These studies have contributed to our knowledge of sympathetic nerve function. Attempts to measure norepinephrine turnover rate in man have been rather limited, employing DL-norepinephrine-^{3}H (4, 9, 10) and dopamine-^{14}C (11). For this reason it was decided to carry out

further studies, particularly in regard to the question of an abnormality in norepinephrine metabolism in essential hypertension.

Dopa-^{3}H was chosen for these studies of catecholamine turnover for several reasons. Dopa is metabolized rapidly after intravenous infusion, allowing for pulse labeling of tissue catecholamines, whereas the dietary precursor, tyrosine, persists in tissues for long periods after administration (12). Unlike norepinephrine (13) and dopamine (14), L-dopa readily penetrates the blood brain barrier (14) and nonspecifically enters sympathetic neurones (15, 16) where it is converted to the neurotransmitter substance, L-norepinephrine. Thus, endogenous labeling of norepinephrine from dopa-^{3}H occurs by a more physiologic route. Of practical importance is the fact that dopa-^{3}H of high specific activity has recently become available as the levo isomer, labeled exclusively on the ring portion of the molecule.

The feasibility of endogenous labeling of catecholamines in man was shown in preliminary studies in which, after administration of dopa-^{3}H, easily measured quantities of radioactivity were recovered in urinary dopamine, norepinephrine, and vanillylmandelic acid (VMA) (17). In the present work, the rate of exponential decline of radioactivity in each urinary compound after labeling with dopa-^{3}H is utilized as an index of turnover rate. Comparative studies of turnover rates were made in normal subjects and patients with essential hypertension, the latter both in control state and during treatment with drugs known to decrease sympathetic neural activity. In several patients undergoing surgery for heart disease, the levels and rate of decline of specific activity of catecholamines in urine after dopa-^{3}H labeling were correlated directly with those in cardiac tissue.

METHODS

The subjects of study were: five normal volunteers (two males and three females, aged 19–35 yr); 10 patients (six women and four men, aged 32–54 yr) with uncomplicated essential hypertension; and eight patients (four males and four females, aged 16–42 yr) with congenital or rheumatic heart disease (functional class I-II [American Heart Association criteria], six cases; class III, one case; class IV, one case) who underwent corrective cardiac surgery. All subjects were hospitalized at the time of study. Blood pressure elevation was sustained in the hypertensive patients throughout the period of the turnover studies at approximately those values given in Table I. The mean supine blood pressure for the untreated group was 162/111. Patients with secondary complications were excluded in preliminary studies. Blood urea nitrogen and serum creatinines (of volunteers and patients alike) were normal; all patients had normal rapid sequence intravenous pyelography, and in those instances where confirmation was sought, renal arteriography was normal. The patients were not in

TABLE I
Clinical Features of the Hypertensive Patients

Patient	Age	Sex	Blood pressure*	Retinopathy grade‡	Serum creatinine	Renal evaluation tests§	Prestudy therapy‖	
							Initial	Repeat
			mm/Hg		*mg/100 ml*			
1 A. L.	32	F	134/96	I	0.8	IVP	None	Reserpine
2 E. H.	49	F	152/110	II	0.9	IVP	None	α-MPT
3 D. E.	43	F	140/112	II	1.0	IVP,A	None	α-MPT
4 R. L.	54	M	160/120	II	0.9	IVP,A	None	—
5 B. R.	52	M	250/138	II	1.0	R	None	—
6 A. T.	34	M	190/120	II	0.9	IVP,A	None	Pargyline
7 M. D.	48	F	140/100	II	1.0	IVP,A	None	α-MPT
8 H. T.	54	F	130/94	II	1.2	IVP,A	None	—
9 G. D.	33	F	146/108	II	0.9	IVP,A	Pargyline	—
10 W. J.	40	M	140/100	II	0.9	IVP	Reserpine	—

* Mean of three measurements, supine, at the time of the initial turnover study.
‡ Keith-Wagner classification. D. E. had grade IV retinopathy 1 yr before the initial turnover study.
§ Tests for screening renal arterial disease were negative in all patients: IVP, rapid sequence intravenous pyelogram; A, renal arteriogram; R, renogram in patient with iodide allergy.
‖ Patients designated as None received no medication for 4–6 wk before the study. The duration and dosage of medications of α-MPT, pargyline, and reserpine are given in both Table VI and the legend of Fig. 7.

congestive heart failure nor were they receiving digitalis glycosides. Eight patients received no drug therapy for 4–6 wk before study. Two were studied only during drug treatment, pargyline in one case and reserpine in the other. Five of these eight patients were studied again during drug therapy, three during treatment with alpha-methyl-*para*-tyrosine[1] (α-MPT) and one each during treatment with reserpine and pargyline hydrochloride (Eutonyl).

Tritium-labeled 3,4-dihydroxyphenylalanine (dopa) was obtained[2] in two forms. These were DL-DOPA-^{3}H (G, 0.256 c/mmole) and L-DOPA-^{3}H (ring 2,5,6-^{3}H, 0.5 c/mmole and 2.0 c/mmole). The purity of each batch of labeled compound was ascertained by strip counting of paper chromatograms of the material, developed in *n*-butanol saturated with 2 N HCl, and in *n*-butanol : acetic acid : water (4 : 1 : 1). In one instance it was necessary to purify the racemic compound by adsorption and elution from alumina (18). Solutions of compounds for intravenous use were prepared by dissolving or diluting in 0.25 N acetic acid or 0.01 N HCl containing 1% sodium-*meta*-bisulfite as preservative, followed by 0.45 μ Millipore filtration (filtrate pyrogen tested) and storage in dark vials at $-15°C$. Before infusion, 0.4–1.5 ml of solution was diluted to 100 ml with 5% dextrose in water.

Clinical Procedures

Intravenous infusions of dopa-^{3}H were given in the fasting state, except in some of the patients undergoing cardiac surgery who were given dopa-^{3}H 2–22 hr before cardiac biopsy (atrial, seven cases; papillary muscle, one case). All dopa-^{3}H infusions were given at a constant rate for 30 min. In the first seven studies (four normals and three hypertensives), varying doses of dopa-^{3}H were administered to establish the feasibility of endogenous labeling of catecholamines. These doses ranged from 1.02 to 4.8 mc (0.1–3.7 mg of L or DL-dopa) and were administered at a rate of 1.33–5.32 μc/kg per min for a total dose of 15–80 μc/kg. In subsequent studies L-dopa-^{3}H was infused at a rate of 0.5 μc/kg per min for 30 min for a total dose of 0.35–1.35 mc (0.14–0.53 mg). These amounts of L-dopa were found to be insufficient to alter detectably the urinary excretion of norepinephrine or VMA; the higher doses used in early studies did cause a slight increase in urinary dopamine during the 1st 3 hr after the infusion.

Beginning just before the infusion, timed urine samples were collected for 3–7 days in glass bottles containing sufficient 6 N HCl to give a final urinary pH less than 3.5 and were stored at 0°C for periods up to 1 month. In surgical patients, urine was collected by indwelling catheter during and following the operation. The atrial appendage biopsies were performed as a routine part of the surgical procedure for the purpose of cannulation of vena cavae and establishment of cardiopulmonary bypass, and not for the purpose of the present study. In one instance (C. R.) the tissue obtained was a piece of papillary muscle that was taken 10 min after institution of cardiopulmonary bypass.

Radioassay

Aliquots of urine, various eluates, and extracts were dissolved in scintillation fluid (19) and counted in a Packard Tri-carb liquid scintillation spectrometer, series 4000. Counting efficiency was in the range of 15–20% with a background of 10–20 cpm. Counts in any sample were not considered significant unless they were at least 40 cpm above background. In all instances, counting time was adjusted to assure a counting error of less than 5%.

Chemical Assays

A. Catecholamines

Dopa, dopamine, and norepinephrine were isolated from aliquots of urine by combinations of column chromatography on alumina, IRC-50 (Na^+) and Dowex-50 (Na^+), and paper chromatography (12, 18, 20). Specific separation and assay techniques were as follows.

(*1*) *Dopa.* For assay of unchanged dopa-^{3}H, 50 μg of carrier DL-dopa was added to urine aliquots. The aliquots were then subjected to alumina adsorption and elution, the eluate applied to IRC-50 columns, the effluent therefrom reapplied to alumina, and the dopa again was eluted with 0.2 N acetic acid. The final eluate was evaporated to dryness under nitrogen, redissolved in a small amount of 0.1 N HCl in ethanol, and was chromatographed on No. 1 Whatman paper in *n*-butanol : acetic acid : water (4 : 1 : 1). Paper strips were cut and the dopa was eluted with 0.01 N HCl. Aliquots of appropriate strip eluates were assayed for dopa fluorometrically (20), and a second aliquot was used for scintillation counting. In patients who had received DL-dopa-^{3}H, a third aliquot was assayed for L-dopa-^{3}H by conversion to dopamine with guinea pig kidney decarboxylase (21). Since dopa is not detectable in urine normally, the radioactive dopa in urine was assumed to have the same specific activity as that administered. Accordingly, the excretion of dopa-^{3}H could be calculated from the degree of dilution of its specific activity by the carrier dopa.

(*2*) *Dopamine and norepinephrine.* These catecholamines were adsorbed on alumina from aliquots of urine and protein-free filtrates of heart homogenates, eluted, and a portion of the eluate assayed for norepinephrine (18). In patients treated with alpha-methyltyrosine, the norepinephrine was assayed after elution from a Dowex-50 (Na^+) column as described below. The recovery of norepinephrine from urine averaged 80%. Remaining alumina eluate plus carrier norepinephrine was then applied to an IRC-50 column as for dopa (see above). Norepinephrine and dopamine were eluted with 1 N acetic acid and then chromatographed on Dowex-50 (Na^+) and eluted as separate fractions with 0.4 N HCl and 2 N HCl, as described by Bertler, Carlson, and Rosengren (20). The amines were assayed by natural fluorescence (nor-

[1] Supplied by Dr. Elmer Alpert, Merck, Sharp & Dohme, West Point, Pa.

[2] Nuclear-Chicago Corporation, Chicago, Ill.

epinephrine) or as fluorescent derivative (18, 22). Aliquots of the eluates from the Dowex-50 columns were evaporated under a N_2 jet, the amines redissolved in 0.01 N HCl, and radioassay done. Over-all recovery of norepinephrine and dopamine from urine with the "3-column" procedure averaged 70%. The aforementioned procedures permitted accurate determination of the specific activities of norepinephrine and dopamine in urine following administration of dopa-^{3}H.

The validity of determinations of the specific activities of dopamine and norepinephrine in Dowex-50 eluates was established by paper chromatography (Fig. 1). The specific activities of the amines in the Dowex eluates were similar to those of the amines after isolation from the paper. In separate studies it was also ascertained that neither the 0.4 N HCl or 2.0 N HCl Dowex eluates (fractions A and B, Fig. 1) contained significant amounts of radioactive epinephrine.

B. VMA

The specific activity of urinary VMA in patients receiving L-dopa-^{3}H was determined by conversion to vanillin and subsequent spectrophotometric and radioassay. A simple modification of the procedure of Pisano, Crout, and Abraham (23) was used in that vanillin

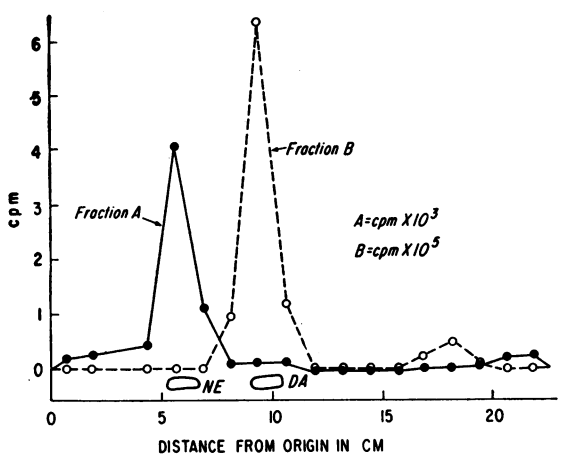

FIGURE 1 Purity of Dowex-50 eluates used for determination of specific activities of norepinephrine and dopamine. This is a semischematic presentation of results obtained when the radioactive catecholamines were isolated (see text) from the urine of a hypertensive patient (H. T.) who had received DL-dopa-^{3}H. Aliquots of the 0.4 N and 2.0 N HCl Dowex-50 eluates (fractions A and B) containing 7.28×10^3 and 9.8×10^5 cpm were chromatographed descending in a butanol: HCl system on No. 1 Whatman paper; 1.25-cm paper strips were cut, eluted (1 ml 0.01 N HCl), and counted for radioactivity. Purity of the two radioactive fractions is demonstrated with migrations of radioactivity on paper coinciding with those of cochromatographed authentic norepinephrine (NE) and dopamine (DA). The latter were located with a potassium ferricyanide spray.

was returned to 2 N NH_4OH instead of 1 M K_2CO_3 to facilitate miscibility with scintillation fluid. Specificity of the determination was assured by chromatographing the thiosemicarbazone derivative (24) of vanillin on paper in isopropanol: ammonia: water (40:1:10).[3] The appropriate region was eluted with ethanol: 0.1 N NH_4OH (1:1). One aliquot was assayed fluorometrically in 0.1 M sodium borate, pH 10, and another radioassayed in the scintillation mixture. Specific activities of the derivative after chromatography agreed closely with those of vanillin.

Tritium exchange

Investigations were made for evidence of tritium exchange in vivo and in vitro. When acidified urine samples were evaporated under a jet of N_2, less than 3% of the radioactivity was volatilized. It was found however that the 0.4 N HCl Dowex eluates of the initial urine specimens from a few patients underwent a 30–50% loss of radioactivity with evaporation. There was no subsequent loss with repeated acidification and evaporation. Also, it was found that several batches of L-dopa-^{3}H could be evaporated from 6 N HCl without occurrence of tritium exchange. Ring-labeled dopamine prepared by decarboxylation in vitro of a batch of L-dopa-^{3}H (500 mc/mmole) was carried through the three step procedure without evidence of tritium exchange. Therefore, the volatile radioactivity found in some Dowex eluates was probably tritiated water arising from metabolites other than dopamine or norepinephrine. In the case of such eluates the specific activity values presented are those obtained after evaporation. In the experiments with DL-dopa-^{3}H (G), no corrections were made for tritium displacement from the beta-carbon of the side chain resulting from enzymatic hydroxylation at this position.

RESULTS

Urinary catecholamine excretion in normal volunteers and hypertensive patients

The urinary excretion of the catecholamines, metanephrines, and VMA for both normal volunteers and hypertensive patients were within the range of normal values established for this laboratory (Table II). There were no significant differences between the two groups, nor were there changes after reserpine therapy. The posttherapy values of patients A. L. and W. J., respectively, were catecholamines, 29 and 34 μg/day; total methylated amines, 0.30 and 0.40 mg/day; and VMA, 4.4 and 4.3 mg/day. Changes in catecholamine metabolite excretion were noted, however, after pargyline therapy. The posttherapy

[3] Authentic vanillin thiosemicarbazone for reference purposes was supplied by Dr. Harry Goldenberg, Bio-Science Laboratories, Los Angeles, Calif.

TABLE II
Urinary Catecholamines and Metabolites in Normotensive Volunteers and Hypertensive Patients

Patients	Norepinephrine	Epinephrine	Total metanephrines	VMA
	*μg/24 hr**		*mg/24 hr**	
Normotensive				
1 D. B.	26	4	0.30	3.4
2 M. B.	42	16	0.35	4.0
3 T. R.	29	6	0.37	3.6
4 C. K.	22	5	0.27	3.2
5 L. G.	39	10	0.26	2.9
Means ± SE	32 ± 4	8 ± 2	0.31 ± .02	3.4 ± 0.2
Hypertensive				
1 A. L.	23	6	0.24	4.4
2 E. H.	53	5	–	3.9
3 D. E.	59	6	0.32	3.6
4 R. L.	17	6	–	3.8
5 B. R.	65	12	0.54	4.3
6 A. T.	54	6	0.36	4.4
7 M. D.	33	11	0.34	4.0
8 H. T.	34	10	0.25	1.6
Means ± SE	42 ± 6	8 ± 1	0.34 ± .04	3.8 ± 0.3

* Each value is the mean of two or three consecutive 24-hr urine values. The unconjugated catecholamines and VMA were determined by fluorometric (18) and photometric assay (23), respectively. See text for details. Total, conjugated and unconjugated, normetanephrine, and metanephrine were determined by photometric assay (45).

values of patients A. T. and G. D., respectively, were catecholamines, 56 and 30 μg/day; total metanephrines, 1.1 and 0.8 mg/day; and VMA, 0.8 and 1.7 mg/day. Alpha-methyltyrosine therapy resulted in decreased excretion of the catecholamines and VMA in the three patients E. H., M. D., and D. E., as given in Table VI.

Fate of dopa-^{3}H

As shown in Fig. 2 there was rapid excretion of radioactivity in the urine after the administration of radioactive dopa intravenously, with approximately 50% of the administered dose of isotope being excreted within 3 hr. The total excretion of radioactivity in 24 hr as a percentage of dose was 92% for radioactive DL-dopa and 93% for L-dopa. The percentages of radioactivity excreted as unchanged dopa and as urinary dopamine, norepinephrine, and VMA are shown in Table III. Although 0.2% of the radioactivity from DL-dopa-^{3}H and 0.6% of L-dopa-^{3}H could be recovered as unchanged L-dopa, no radioactive L-dopa was found after 3 hr. This finding was critical and indicated that there was not continuous relabeling of the catecholamines by the precursor. Following infusion of DL-dopa-^{3}H, small amounts of the D-isomer could be detected for 15–20 hr and the total as per cent of dose was about 11.5% (Table III). The excretion of radioactivity as dopamine was in the order of 10%; 1–2% was recovered as norepinephrine plus VMA. Counts appearing in these latter compounds were sufficient for determination of specific activity, not only for the first 24 hr, but for an additional 24–48 hr as well. The ratio of counts in VMA to that in norepinephrine was about 70 to 1 and approximated the ratio of endogenous levels of these compounds in the urine.

Turnover rates of norepinephrine, dopamine and VMA in normal and hypertensive subjects

While dopa-^{3}H disappeared rapidly from urine, valid information on the turnover rate of norepinephrine also required ideally that the specific ac-

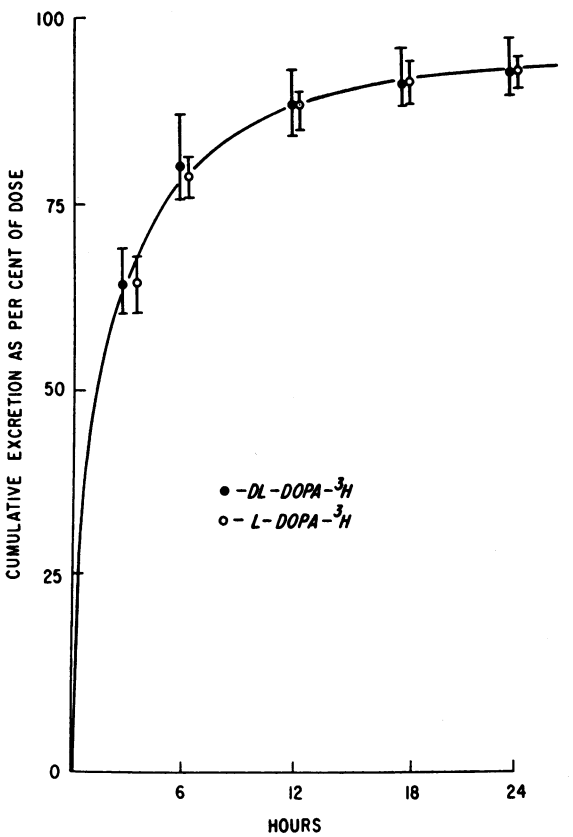

FIGURE 2 Cumulative excretion of radioactivity in the urine following intravenous administration of radioactive L-dopa and DL-dopa. Each point represents the mean of results in three subjects for each compound; the brackets denote ranges of maximum and minimum values. Individual values did not deviate from the mean by more than 10%.

TABLE III

Percentage of Administered Dose of Radioactivity Recovered in Various Urinary Metabolites during the 1st 24 hr after Intravenous Dopa — ^3H

Compound administered*	Subject	D-Dopa	L-Dopa	Dopamine	Norepinephrine	VMA
DL-Dopa-^{3}H	D. B.	10.0	0.2	8.6	0.023	–
	M. B.	13.0	0.2	8.0	0.023	–
	H. T.	–	–	7.4	0.014	–
	Mean	11.5	0.2	8.0	.020	
L-Dopa-^{3}H	A. L.	0.0	0.4	12.0	0.027	1.5
	E. H.	0.0	0.5	13.0	0.020	1.1
	D. E.	0.0	1.0	16.0	0.040	1.9
	R. L.	–	–	10.2	0.019	2.0
	B. R.	–	–	10.0	0.019	1.6
	Mean	0.0	0.6	12.2	0.025	1.6

* Doses administered ranged from 20 to 80 μc/kg for DL-dopa-^{3}H and were 15 μc/kg for L-dopa-^{3}H.

tivity of the intermediate compound, dopamine, also declines rapidly. A typical example of the rates of decay in specific activities of dopamine, norepinephrine, and VMA is shown in Fig. 3. The specific activity of dopamine first exceeded, then, at 12 hr became less than that of norepinephrine. Thus, the possibility exists of a small degree of relabeling of norepinephrine from dopamine during the 1st day. On the other hand, the decay curve for norepinephrine-^{3}H generally became exponential before its specific activity exceeded that of dopamine. The norepinephrine decay curve bore a precursor-product relationship with the VMA decay curve.

The norepinephrine specific activity plotted semilogarithmically against time from a representative normal subject is shown in Fig. 4. In this instance, and generally, there was an initial rapid fall, and after 6–12 hr, an exponential rate of decline with a half-time of 8 hr. This exponential decline lasted 2–3 days, the period in which there was measurable activity. In a few subjects, studies of pooled alumina eluates demonstrated the same decay rate for 4 days. Extrapolation of this exponential to zero time (y axis) affords an index of the size of the "pool.'" The height of the y intercept is inversely related to the amount of endogenous norepinephrine in the pool (dilution of the tracer). If the exponential curve is subtracted

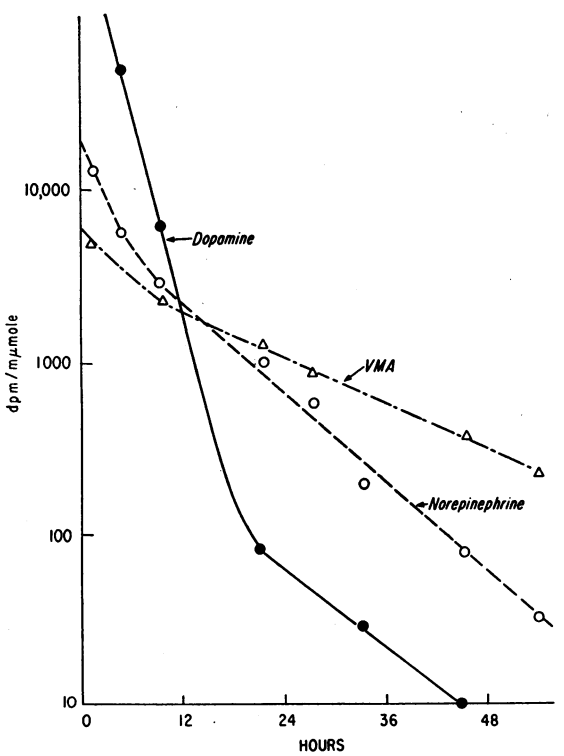

FIGURE 3 Comparison of the rate of change of specific activity of urinary dopamine, norepinephrine, and VMA after administration of L-dopa-^{3}H in a normal subject (T. R.).

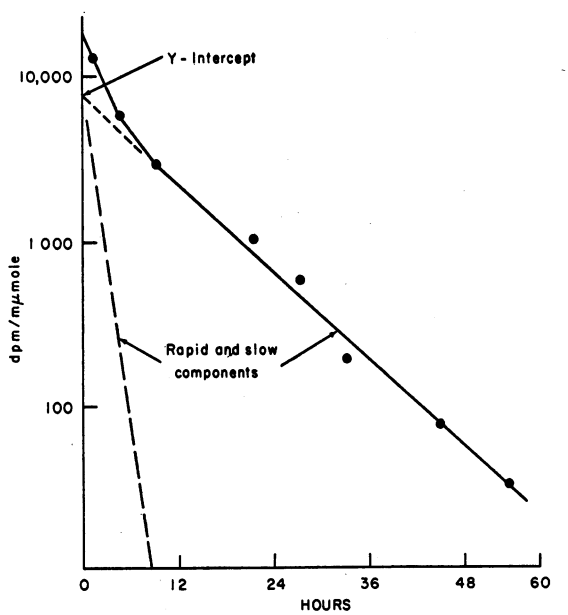

FIGURE 4 The curve of decline of norepinephrine specific activities of normal subject T. R. (cf. Fig. 3) and its two major components are illustrated. The exponential phase of decay is extended to its Y intercept and the early rapid component is derived graphically by subtracting the exponential component from the curve of decline.

from the original curve there remains an early component indicative of one or more pools of norepinephrine having a rapid turnover.

In Fig. 5, the specific activity–time curves for urinary norepinephrine in five normal subjects are compared with the average of results in six patients with essential hypertension. The curve for normal subject L. G. who received the same dose of isotope as the hypertensive subjects was almost identical to the mean curve of the hypertensives. Individual turnover rates for the three metabolites were calculated graphically from the exponential portions of curves constructed visually, and are listed in Table IV. The latter also includes results from two additional hypertensive patients (M. D. and H. T.) who received a dose

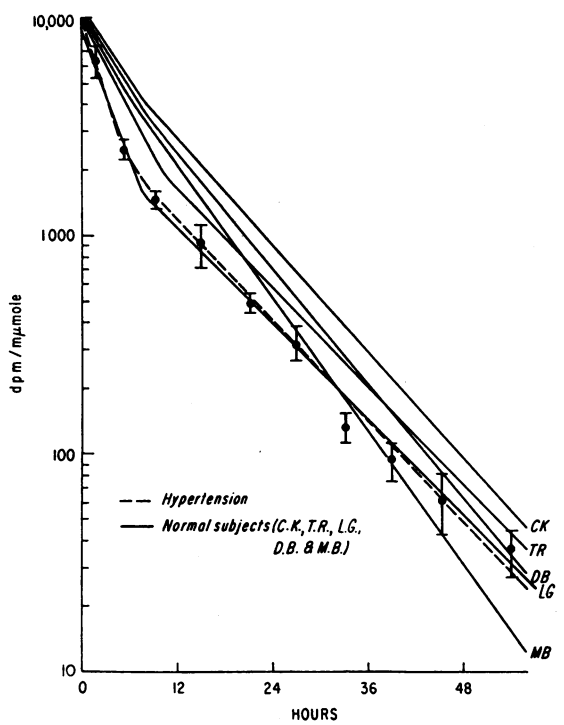

FIGURE 5 Comparison of the rate of change of specific activity of urinary norepinephrine in five normal subjects and six patients with essential hypertension. Radioactive dopa was infused over a period of 30 min beginning at zero time. Doses of precursor in the normal subjects were as follows: D. B. and M. B., 20 μc/kg of DL-dopa-^{3}H; L. G., 15 μc/kg of L-dopa-^{3}H; T. R., 25 μc/kg of L-dopa-^{3}H; and C. K., 35 μc/kg of L-dopa-^{3}H. All six hypertensive patients received the same dose of L-dopa-^{3}H, 15 μc/kg; this enabled a single curve to be drawn utilizing mean values. The brackets indicate standard errors of the means.

TABLE IV
Turnover Rates of Dopamine, Norepinephrine, and VMA in Normal and Hypertensive Subjects

Group	Subject	Half-lives* Dopamine	Half-lives* Norepinephrine	VMA
Normal	1 D. B.	7	8	–
	2 M. B.	6	7	–
	3 T. R.	7	7	15
	4 C. K.	6	8	16
	5 L. G.	5	8	11
	Mean	6.2	7.6	14.0
Hypertensive	1 A. L.	8	8	11
	2 E. H.	9	8	13
	3 D. E.	8	8	12
	4 R. L.	8	7	14
	5 B. R.	8	9	12
	6 A. T.	7	8	13
	7 M. D.	12	8	16
	8 H. T.	8	8	–
	Mean	8.5	8.0	13.0

*Dosages of dopa-^{3}H were as per legend, Fig. 5, and text. Values for half-lives were estimated to the nearest hr from exponential components of the decay curves.

of isotope (20 μc/kg of L-dopa-^{3}H and 80 μc/kg of DL-dopa-^{3}H) different from that given to the other hypertensive patients (15 μc/kg of L-dopa-^{3}H). We interpret the results as showing no significant differences between the two groups. This is clearly the case for norepinephrine with average half-lives being 7.6 and 8.0 hr for the normal and hypertensive groups, respectively. Possible minor differences in turnover rates of dopamine and VMA would require evaluation of a much larger series of cases. There was considerable variation in the y intercepts (cf. Fig. 4). With correction for variations in isotope dosage, the means and standard deviations of y intercept values for the normals and the hypertensive patients were 4828 ± 2407 and 3540 ± 539 dpm/mμmole, respectively.

The half-time of the rapid component (cf. Fig. 4) of the disappearance curve of radioactive norepinephrine was 1 hr for both normals and untreated hypertensives. As a further comparison of the fate of norepinephrine-^{3}H in the two groups of subjects, the ratios of counts excreted in norepinephrine within 3–6 hr to the total counts excreted in norepinephrine in 48 hr were calculated.

As shown in Table V, 50 and 54% of the total norepinephrine counts were excreted by the two groups within 3 hr, and both excreted 68% within 6 hr. Thus, there was no evidence of an abnormality in the disposition of norepinephrine-^{3}H in patients with hypertension, regardless of the time elapsing after dopa-^{3}H administration.

TABLE V

Cumulative Norepinephrine-^{3}H Excretion at 3, 6, and 48 hr after Dopa-^{3}H Infusion in Normal and Hypertensive Subjects

	Norepinephrine-^{3}H			Fraction of 48 hr norepinephrine-^{3}H recovered	
Hr...	0–3	0–6	0–48	0–3	0–6
	(*dpm* × *10*^{3})				
Normals					
1 L. G.	328	414	575	0.57	0.72
2 D. B.	414	535	821	0.51	0.66
3 M. B.	297	518	823	0.36	0.63
4 C. K.	403	586	915	0.44	0.64
5 T. R.	558	677	910	0.61	0.74
			Mean	0.50	0.68
Hypertensives					
1 A. T.	705	890	1,140	0.62	0.78
2 H. T.	646	842	1,520	0.56	0.73
3 M. D.	358	415	703	0.51	0.59
4 R. L.	287	391	551	0.52	0.71
5 A. L.	294	336	473	0.62	0.71
6 B. R.	287	405	653	0.44	0.62
7 D. E.	396	535	775	0.51	0.69
8 E. H.	260	310	500	0.52	0.62
			Mean	0.54	0.68

Effects of antiadrenergic drugs

Three drugs were selected for study because of their distinctive and fairly well documented actions on catecholamine metabolism. These were alpha-methyltyrosine (α-MPT), reserpine, and pargyline. Alpha-MPT decreases tissue levels of catecholamines in animals by inhibiting the hydroxylation of tyrosine to dopa (25). Administration of the compound to humans results in a marked decrease in the urinary excretion of catecholamine metabolites; this is presumed to be indicative of inhibition of catecholamine synthesis at the tyrosine hydroxylase step (26, 27). It is well known that reserpine depletes tissue stores of norepinephrine (28). It is generally agreed that reserpine interferes with mechanisms of storage of the amine in sympathetic neurones (29). Pargyline hydrochloride is a monoamine oxidase inhibitor. Monoamine oxidase inhibitors interfere with extra- and intraneuronal metabolism of monoamines, and in some species increase tissue levels of norepinephrine (30, 31).

A. Alpha-methyltyrosine. It was of interest to determine whether, in the presence of a decreased rate of catecholamine synthesis produced by alpha-MPT, the formation of norepinephrine and VMA from dopa-^{3}H was intact and, if so, to observe the apparent turnover rate of norepinephrine. Three patients were studied both before and during treatment with the inhibitor. The effect of alpha-MPT on the urinary excretion of norepinephrine and

TABLE VI

Effect of Alpha-Methyltyrosine on the Urinary Excretion of Norepinephrine and VMA and on the Formation of These Compounds from L-*Dopa-^{3}H*

Hypertensive patients	Norepinephrine*		VMA*		Norepinephrine-^{3}H‡		VMA-^{3}H‡	
	Control	Drug§	Control	Drug§	Control	Drug§	Control	Drug§
	(*μg/24 hr*)		(*mg/24 hr*)		(*dpm* × *10*^{3})		(*dpm* × *10*^{4})	
M. D.	32.9	11.1	4.0	1.4	703	1235	3732	2762
E. H.	52.8	15.3	3.9	2.1	500	801	2330	2920
D. E.	59.3	46.1	3.6	2.1	775	1360	2335	2355

* Values shown are means of results of determinations on three consecutive 24-hr urine collections.
‡ Values shown are dpm in 1st 48 hr after administration of L-dopa-^{3}H, 20 μc/kg in patient M. D. during control and 15 μc/kg during drug treatment, and 15 μc/kg in patients E. H. and D. E. during both control and drug treatment periods.
§ Alpha-methyltyrosine treatment was: 2.0 g/day for 14 days in patient M. D., 3.0 g for 7 days in E. H., and 2.0 g for 7 days in D. E.; equal doses (0.5 or 0.75 g) of drug were given at 6-hr intervals and treatment was continued throughout the period of study.

VMA and on the formation of these compounds from dopa-^{3}H is shown in Table VI. The decrease of norepinephrine synthesis produced by the drug was apparent from reductions in urinary excretion of the two compounds. That the pathway of synthesis from dopa to norepinephrine was intact, however, is indicated by the fact that total incorporation of radioactivity from dopa-^{3}H into urinary norepinephrine was not inhibited but increased slightly (Table VI). The values for specific activity of urinary norepinephrine after L-dopa-^{3}H and the half-life times of norepinephrine-^{3}H disappearance in the three patients are given in Table VII; specific activity–time curves for one of these patients are depicted in Fig. 6. The specific activity of norepinephrine at various times after dopa-^{3}H was increased during alpha-MPT treatment to 3–10 times that found in the control state. The turnover rate was decreased, as indicated by a 1–4 hr increase in half-life. Turnover rates of VMA were unchanged from an average half-life of about 14 hr.

B. Reserpine and pargyline. A comparison of norepinephrine-^{3}H disappearance rates in hypertensive patients receiving each of these drugs with that of the six untreated hypertensive patients is shown in Fig. 7. It may be seen that the decay curves for two patients receiving long-term treatment with 0.25 mg/day of reserpine appeared to have a single component with an elevated initial specific activity (y intercept) and a markedly shortened half-life of 3 hr. The latter was much less the average half-life of 8 hr in the six untreated patients and in one of the patients (A. L.) who served as his own control. The percentage of dopa incorporated into labeled norepinephrine and VMA was unaltered in these patients. VMA half-lives were 9.5 and 10 hr compared with a mean of 13 hr in untreated hypertensive patients, and 11 hr in the patient serving as his own control.

In contrast to the effects of reserpine, treatment of two patients with pargyline resulted in a considerable slowing of norepinephrine turnover rate, as indicated by half-life values of 15 and 20 hr (Fig. 7). A control study on the patient (A. T.) with the latter value had yielded a half-time value of 8 hr, identical to the average of untreated patients. Zero time specific activity was elevated in patient A. T. and the curve in this case appeared as a single component. The VMA half-life times were 17 and 20 hr in the pargyline-treated patients.

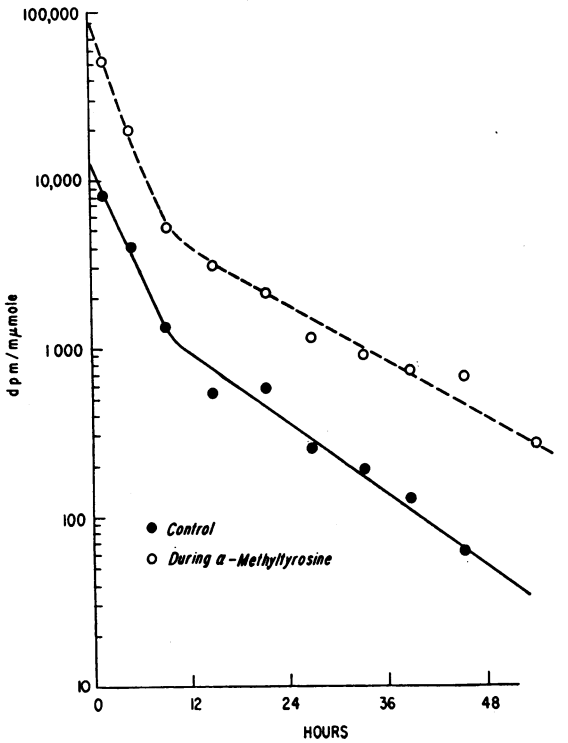

FIGURE 6 Specific activity–time curves of urinary norepinephrine in a hypertensive patient (M. D.) before and during treatment with alpha-methyltyrosine. Doses of drug and isotope were as given in Table VI.

TABLE VII

Effect of Alpha-Methyltyrosine on the Specific Activity and Disappearance Rate of Urinary Norepinephrine-^{3}H in Three Patients with Hypertension

Hours after infusion	Specific activity of norepinephrine M. D. Control	M. D. Drug*	E. H. Control	E. H. Drug*	D. E. Control	D. E. Drug*
			dpm/mμmole			
0–3	8,000	51,000	7,100	20,000	5,400	17,000
3–6	3,930	19,200	1,450	8,800	2,100	3,480
6–12	1,350	5,100	1,900	5,600	1,430	1,600
12–18	540	3,000	730	2,120	308	575
18–24	570	2,030	570	1,500	360	400
24–30	250	1,100	–	980	–	222
30–36	185	870	125	–	90	160
36–42	120	700	–		71	
36–48				450		73
42–48	60	650	82		52	
48–60	10	250	37	–	12	–
Half-life ($t_{\frac{1}{2}}$) of norepinephrine-^{3}H in hr	8	11	8	12	8	9

* Dosage of drug and dopa-^{3}H as per footnote to Table IV.

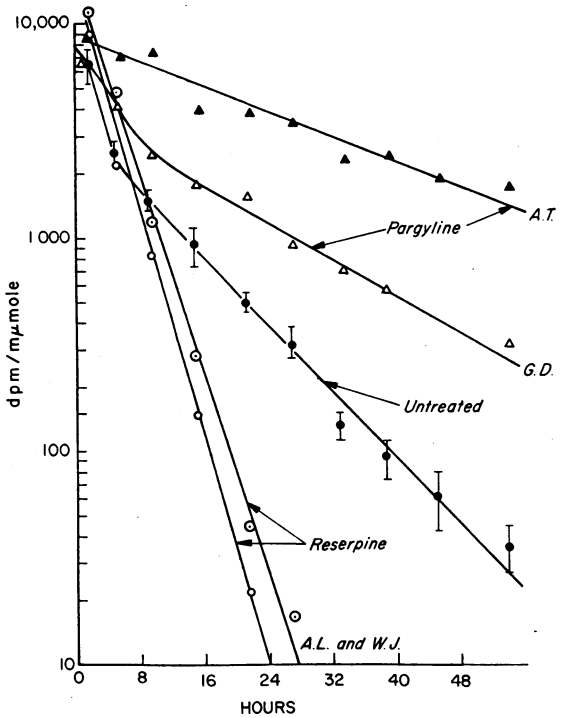

FIGURE 7 Alterations of norepinephrine turnover rate produced by reserpine and pargyline. The biological decay curves during pargyline above, and the curves during reserpine, below, are compared with the average curve of six untreated hypertensive patients (cf. Fig. 5). L-dopa-^{3}H dose in each case was 15 μc/kg. The isotope was administered to G. D. and A. T. during the 4th wk of pargyline therapy (75 mg/day orally) and to A. L. and W. J. after 5 months and 1 yr, respectively, of reserpine (0.25 mg/day orally).

Comparison of cardiac and urinary norepinephrine-^{3}H

Various stressful stimuli in animals as well as sympathetic nerve stimulation have been shown to increase greatly the rate of synthesis and turnover of norepinephrine in sympathetically innervated tissues (32, 33). It is reasonable to suppose that cardiac surgery is a considerable stimulus to the sympathetic nervous system. Also, the same combination, "stress" and overactivity of the sympathetic nerves, is frequently alluded to in relation to the pathogenesis of essential hypertension.

The patients undergoing cardiac surgery had an increased rate of turnover of norepinephrine and VMA, which was accompanied by an increased level of excretion of these compounds in the urine.[4]

[4] DeQuattro, V., and R. Kramer. Norepinephrine synthesis and turnover in man during cardiac surgery. In preparation.

The half-life values as estimated from urinary disappearance curves are listed in Table VIII. With two exceptions (W. M. and D. S.), all the norepinephrine half-lives were less than those encountered in normals or hypertensives (see also Table IV); with one exception (W. M.) this was also true of VMA. Dopamine half-lives were in the same range as in the combined normal-hypertensive group.

Mean values for the specific activities of urinary norepinephrine for the eight cardiac patients and the individual cardiac norepinephrine values at various times after L-dopa-^{3}H (15 μc/kg) are shown in Fig. 8. Initially, the specific activity of

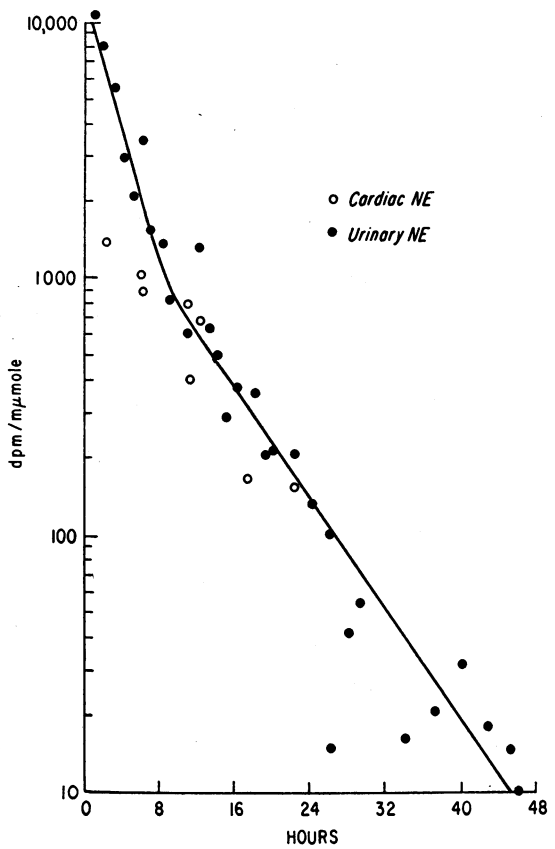

FIGURE 8 Comparison of the specific activities of norepinephrine in the urine and cardiac muscle of eight patients undergoing cardiac surgery following infusion of L-dopa-^{3}H (15 μc/kg). Each muscle point represents one value in a single patient, whereas the urinary points are means of several patients' values. Atrial muscle was obtained from 2 to 22 hr after infusion of labeled dopa. The tissue biopsy at 12 hr was specimen of papillary muscle from a patient with class IV rheumatic heart disease with mitral and tricuspid insufficiency.

TABLE VIII

Turnover Rates of Dopamine, Norepinephrine, and VMA in Patients Undergoing Cardiac Surgery with Cardiopulmonary Bypass

Patient	Cardiac lesion*	Functional class	Half-lives‡ Dopamine	Norepinephrine	VMA
				hr	
1 B. S.	ASD	I-II	10	6	10
2 D. S.	SAS	I-II	7	5	8
3 W. M.	ASD	I-II	7	7	12
4 D. B.	VSD	I-II	7	6	9
5 C. R.	MI,TI	IV	8	3	10
6 J. T.	IHSS	III	5	4	8
7 C. H.	ASD	I-II	6	4	9
8 D. S.	ASD	I-II	5	7	10
		Mean	6.9	5.2	9.5

* ASD, atrial septal defect; SAS, subaortic stenosis; MI, mitral insufficiency; TI, tricuspid insufficiency; IHSS, idiopathic hypertrophic subaortic stenosis; VSD, ventricular septal defect.

‡ Estimated from biological decay curves of specific activity following 15 μc/kg of L-dopa-^{3}H.

urinary norepinephrine exceeded that of cardiac norepinephrine, but after 10 hr the two were surprisingly similar in magnitude and apparent rate of decline. In the tissue specimens obtained at 2 and 6 hr after dopa-^{3}H infusion, 80% of catecholamine radioactivity was isolated in norepinephrine and the remainder as dopamine. None of the latter was found in cardiac tissue at 12, 17, or 24 hr after infusion. No dopa-^{3}H could be found in any of the cardiac biopsies.

DISCUSSION

Labeling technique. It is apparent that administration of dopa-^{3}H is an effective means of labeling endogenous dopamine and norepinephrine in man. Evidence of endogenous labeling of norepinephrine was also provided by isolation of radioactive urinary VMA. That suitable labeling of endogenous catecholamines was achieved is indicated by: (*1*) the rapid metabolic disposal of dopa-^{3}H; (*2*) satisfactory dilution of incorporated isotope in endogenous pools, e.g., calculated maximum specific activity of norepinephrine was about 5 mc/mmole compared with 256–2000 mc/mmole for the precursor; (*3*) absence of any increment in urinary norepinephrine or VMA excretion following dopa-^{3}H; and (*4*) absence of any pharmacologic effect with the small amounts (0.05–4.2 mg) of dopa infused. The possibility of significant relabeling of norepinephrine from dopamine exists since specific activity of the latter compound generally exceeded that of norepinephrine for the 1st 10–12 hr after injection of dopa-^{3}H. If this occurred, the specific activities of urinary norepinephrine and apparent turnover times represent maximal values. We doubt that there was significant relabeling, since the studies on heart tissue showed that dopamine disappeared rapidly from this key organ. Further, the decay of urinary norepinephrine-^{3}H became exponential within 12 hr. However, the latter does not provide complete reassurance, since large deviations from a decay curve may not be apparent on a semilogarithmic plot.

Turnover rates and pools. The assumption that changes in the specific activities of urinary dopamine, norepinephrine, and VMA mirror chemical events in sympathetically innervated organs seems most valid in the case of norepinephrine. The sympathetic nervous system is the major source of plasma norepinephrine and norepinephrine in plasma is rapidly excreted in urine independent of renal innervation (34). On the other hand, dopamine has not been detected in plasma and the origins of urinary dopamine are uncertain. Probably the changes in specific activity of urinary dopamine represent a composite of extraneuronal metabolism of dopa-^{3}H in such organs as liver and kidney, as well as turnover in sympathetic neural structures and the basal ganglia of brain (35). It is now thought that a major portion of intraneuronal norepinephrine is metabolized by monoamine oxidase and excreted predominantly as VMA (30). Thus, urinary VMA-^{3}H curves might be representative of degraded intraneuronal norepinephrine. However, VMA may also arise via *O*-methylation followed by deamination. Thus, in discussing the present work, we prefer to avoid precise interpretation of VMA specific activity curves; however, they tended to vary in the same direction as those of norepinephrine.

There is no direct experimental evidence to determine which tissue pools contribute to urinary norepinephrine and VMA. It appears that a portion of the early phase of norepinephrine-^{3}H disappearance is related to rapid turnover of the

amine in some tissues. From the results obtained in cardiac patients, synthesis and metabolism of norepinephrine in the heart appears to contribute a major share to urinary norepinephrine and VMA specific activities. In these patients, the specific activity and rate of disappearance of norepinephrine-^{3}H in the heart were similar to those in urine. If one assumes that the normal heart weighing about 300 g contains from 0.3 to 0.6 mg of norepinephrine (1–2 μg/g [28, and footnote 4]), which has a half-life of about 8 hr, then approximately 1 mg of norepinephrine or its metabolites may be released from this organ daily. This represents a considerable contribution to total urinary metabolites.

VMA pool sizes may be calculated from values for estimated zero time specific activities of this metabolite and the total amount of radioactivity incorporated from dopa-^{3}H into this end-product. In the hypertensive subjects in whom VMA turnover rates were determined, the extrapolated value at zero time averaged 2.0×10^8 dpm/mμmole. On the average in these studies 1.6% of the infused dose of dopa-^{3}H was converted to VMA, i.e., 3.7×10^7 dpm. Dividing the latter figure by the former ($[3.7 \times 10^7]/[2.0 \times 10^8]$) yields a VMA pool size of 18.5 μmoles or 3.7 mg. Based on a turnover half-time of 14 hr, about 4.4 mg of VMA should be excreted per 24 hr, which is in agreement with actual excretion values.

Catecholamine metabolism in essential hypertension. In reviewing studies on this subject before 1966, Crout (36) concluded that, if abnormalities of sympathetic function or catecholamine metabolism are present in essential hypertension, more sophisticated experimental design must be applied to find them. We would agree with this conclusion, and add that careful studies of norepinephrine and VMA turnover following dopa-^{3}H labeling also fail to reveal any abnormality in hypertension. In view of these findings and the fact that a significant degree of inhibition (up to 50%) of catecholamine synthesis may be produced by alpha-methyltyrosine in patients with essential hypertension without lowering of blood pressure, even a minor abnormality in catecholamine metabolism is unlikely to account for maintenance of essential hypertension (37).

A considerable refueling of the controversy was accomplished recently by de Champlain, Krakoff, and Axelrod (7, 8) who have demonstrated a significant reduction in tissue concentrations of norepinephrine in uninephrectomized rats made hypertensive with desoxycorticosterone and NaCl. They demonstrated also a defect in the storage and retention of norepinephrine in sympathetic nerve granules of such animals; this might account for the hypertension through access of increased amounts of active catecholamine to receptor. It is unknown at the moment whether these findings are unique to the experimental model. No such abnormalities are apparent in the present studies, either from the extrapolated y intercepts of the exponential portions of the norepinephrine-^{3}H disappearance or from norepinephrine-^{3}H disappearance rates (turnover). Support for relating the findings in the rat to the clinical situation is offered, however, by the work of Gitlow et al. (9) who reported a slightly increased rate of disappearance from plasma of exogenously administered DL-norepinephrine-^{3}H and interpreted this as possibly representing a defect in tissue-binding mechanisms. Also, favoring this hypothesis are recent reports of a statistically significant increase in the urinary excretion of norepinephrine (6) and normetanephrine (5) in hypertensive as compared with control subjects.

Induced alterations of labeling and turnover. Although the techniques employed here failed to detect any abnormality of catecholamine metabolism in patients with essential hypertension, deviations from normal were easily shown in patients treated with alpha-methyltyrosine, reserpine, and pargyline, as well as during operative "stress." The mechanism of the alterations observed are interpretable if the findings are related to previous studies in animals and in man.

It is well documented in animal studies that alpha-methyltyrosine inhibits catecholamine biosynthesis at the first biosynthetic step, i.e., the the hydroxylation of tyrosine to form dopa. Thus, synthesis of norepinephrine from tyrosine-^{14}C is inhibited by the drug, whereas when dopa-^{3}H is administered to bypass tyrosine hydroxylase, incorporation of counts into norepinephrine remains intact and is actually increased in the presence of drug (25). In man, it is not considered feasible to study norepinephrine biosynthesis from tyrosine-^{14}C. However, that alpha-methyltyrosine inhibits catecholamine synthesis in man is apparent

from the marked reductions in urinary catecholamine metabolites occurring during drug treatment (26, 27), as was also observed here in three patients. The findings with dopa-^{3}H indicate that in man also, the site of inhibition by drug is at the first step. Thus, total incorporation of dopa-^{3}H into urinary norepinephrine was normal or increased. The higher specific activity of norepinephrine after dopa-^{3}H during treatment with alpha-methyltyrosine was probably due to the fact that under tyrosine hydroxylase blockade the dopa-^{3}H is not diluted as much by endogenously formed dopa (38). There was also a reduction in the rate of turnover of norepinephrine during alpha-methyltyrosine treatment, with a 1–4 hr prolongation of half-life. Apparently, relative to the already reduced tissue catecholamine stores, there was even less norepinephrine synthesized and released than under normal circumstances.

Reserpine, in small daily doses (0.25 mg), produced a marked increase in rate of norepinephrine turnover. This low level of dosage does not appear to alter catecholamine production rates in man (39). In the two patients studied here, urinary catecholamines and VMA in one patient (A. L.) during therapy were unchanged from control values of 29 μg and 4.4 mg/24 hr, respectively, while the other patient (W. J.) had levels during treatment (34 μg and 4.3 mg) which were normal by our methods. Although the rate of decline in specific activity of norepinephrine in these two reserpine-treated patients was rapid, the zero time specific activity was considerably increased. These results are consistent with the well-known inhibitory effects of reserpine on tissue catecholamine storage mechanisms (28, 29). In the reserpine-treated state, with synthesis intact, dopa-^{3}H is readily incorporated into a reduced catecholamine store that is therefore more highly labeled, but which is also more rapidly diluted by newly formed endogenous amine. The slightly increased rate of VMA turnover after reserpine is in agreement with the findings in animals by Kopin and Gordon (40) that, following the administration of reserpine, norepinephrine is released preferentially onto oxidative enzymes.

Pargyline hydrochloride produced a marked reduction in norepinephrine turnover. Although the effects of monoamine oxidase inhibitors on tissue concentrations of norepinephrine vary considerably with species, and even organ (30), it is reasonable to assume that the two patients studied had elevated tissue levels of norepinephrine. However, the degree of labeling (zero time) was increased in one patient and was unchanged in the other. Also, the percentage of label excreted as norepinephrine was increased. These results suggest enhanced incorporation from dopamine, an excellent substrate for monoamine oxidase, as seen in similar studies in animals (38, 41). Neff and Costa also demonstrated a considerable slowing of norepinephrine turnover in brain and heart of pargyline-treated rat, an animal whose tissue concentrations of norepinephrine increase after administration of the drug (42). They suggested that their results might be due to end-product inhibition of tyrosine hydroxylase with a consequent decrease in synthesis and turnover rate. A number of observations in our laboratory, including studies of incorporation of label from tyrosine and dopa into norepinephrine in animals treated with pargyline, also lend support to the hypothesis that norepinephrine synthesis is regulated by a mechanism of end-product inhibition at the tyrosine hydroxylase step (cf. 38). If this also occurs in man, then the total of urinary catecholamine metabolites should be reduced during pargyline treatment, even as during treatment with alpha-methyltyrosine. In one of the patients (A. T.) studied here, measurements of the sum of urinary catecholamines plus metanephrines plus VMA averaged 4.8 mg/24 hr during the control period and was reduced to 1.9 mg during pargyline treatment. Reduced catecholamine synthesis as a factor in the hypotensive effects of monoamine oxidase inhibitors has been considered only recently (37, 42) and will require further evaluation.

The fourth alteration of catecholamine turnover observed here was the rapid turnover of norepinephrine and VMA in patients undergoing surgery. It is well known that during a variety of stressful stimuli, of which cardiac surgery must be among the more severe, there is an outpouring of the catecholamines and their metabolites in the urine. A reduced pool size due to rapid release in the presence of a normal synthesis rate might be advanced to explain the increased turnover rate. However, Cooper et al. demonstrated that human atrial norepinephrine is not decreased during car-

diopulmonary bypass (43). Thus, our findings seem best interpreted as evidence of accelerated catecholamine biosynthesis. They are also consistent with studies in animals showing clearly that increased sympathetic activity occurring during severe stress (exercise and exposure to cold), as well as that produced by direct sympathetic nerve stimulation, is associated with increased rate of synthesis (32, 33). Thus, tissue concentrations of norepinephrine are maintained in the face of concomitant rapid release of the amine.

Assessment of catecholamine metabolism in vivo. It is doubtful that the excretion of a single substance, e.g. norepinephrine, normetanephrine, or VMA, is an adequate chemical index of sympathetic nerve dynamics. The urinary excretion of normetanephrine has been offered for this purpose, since animal data suggest that norepinephrine which leaks away from "receptors" is preferentially metabolized by *O*-methylation (44). However, no studies in man have been made to document this. Urinary norepinephrine might be acceptable, except the extent to which plasma norepinephrine represents neurohormone that has acted on receptor is unknown. Difficulties in employing a single chemical index are illustrated in the effects of treatment with a monoamine oxidase inhibitor which results in lowering of blood pressure and, therefore, a presumed decrease in release of active catecholamine. Urinary norepinephrine excretion is little changed during such treatment, whereas normetanephrine is increased and VMA is reduced. Similarly, although small doses of reserpine exert an obvious effect on norepinephrine turnover rate, and probably on sympathetic nerve function, this is not apparent from the levels of excretion of norepinephrine and its major metabolite, VMA. Until such time as direct tissue assays of the neurohormones and their biosynthetic enzymes become feasible, combined studies of turnover, as reported here, and the measurements of norepinephrine plus normetanephrine plus VMA may afford the best chemical means of detecting altered sympathetic function in man.

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