Calculation of the Rate of Catecholamine Synthesis from the Rate of Conversion of Tyrosine-^{14}C to Catecholamines

Effect of Adrenal Demedullation on Synthesis Rates

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SUMMARY

When tyrosine-^{14}C is infused intravenously at a constant rate into unanesthetized rats, the specific activity of plasma tyrosine increases curvilinearly with time, producing a concomitant increase in the specific activity of tissue catecholamines. By applying steady-state kinetics to this relationship, a simple model was developed from which the fractional rate constant (k) of a catecholamine compartment could be calculated. Catecholamine synthesis rates are obtained by multiplying k by the steady-state amine level. Rates calculated by this method are in good agreement with those calculated from the decline of catecholamine levels after blockade of their synthesis with α-methyltyrosine. By using the present method, it was found that adrenal demedullation results in about a 4-fold increase in the rate of synthesis of heart norepinephrine while brain catecholamine synthesis remains unchanged. It is suggested that catecholamines released from the adrenal medulla may modulate the synthesis of norepinephrine in cardiac sympathetic neurons.

INTRODUCTION

The rate of catecholamine synthesis *in vivo* can be measured by injecting radioactive norepinephrine and applying principles of steady-state kinetics to the decline of norepinephrine specific activity (1–3) or by applying the same principles to the exponential decline in the levels of norepinephrine and 3,4-dihydroxyphenylethylamine levels after the synthesis of these amines has been blocked with α-methyltyrosine (2, 3). The radioactive precursors 3,4-dihydroxyphenylalanine (4, 5) and tyrosine (6–9) have also been administered to measure catecholamine synthesis rates. Sedvall *et al.* (8) have recently presented a model for calculating the synthesis rate of catecholamines after the infusion of tyrosine-^{14}C at a constant rate. Although not stated explicitly, their mathematical system was a single closed compartment. Because newly formed norepinephrine molecules are confined to a closed compartment in this model, the rates of synthesis of norepinephrine calculated by the method of Sedvall *et al.* (8) are a function of the time of infusion. Our report concerns the calculation of catecholamine synthesis rates in single, unanesthetized animals from the conversion of tyrosine-^{14}C to radioactive catecholamines. However, our calculations, unlike those of Sedvall *et al.* (8), account for the loss of newly formed norepinephrine with time. Moreover, we show that the catechol-

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amine synthesis rates calculated with this isotopic method are comparable to those obtained from the decline of amine levels after blockade of catecholamine synthesis (3).

Because the enzymatic hydroxylation of tyrosine is probably the rate-limiting event in catecholamine formation (10), the specific activity of the NE[2] (designated $\boxed{\text{NE}}$) formed from tyrosine-^{14}C should bear a relationship to the specific activity of plasma tyrosine ($\boxed{\text{T}}$).

One possible relationship is considered in the hypothetical model of Fig. 1, where

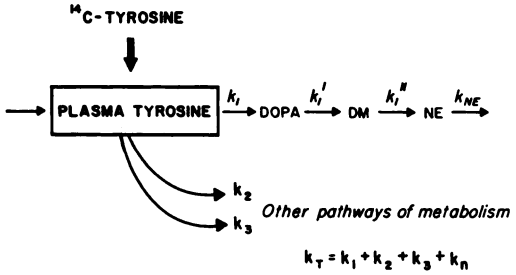

FIG. 1. *Hypothetical model representing the formation of norepinephrine from tyrosine*

DM = dopamine.

tyrosine-^{14}C is infused intravenously at a constant rate. In this model, k_1 is the fractional rate constant for the limiting event of catecholamine formation, the hydroxylation of tyrosine. It is also assumed that k_1 is smaller than k'_1, k''_1, or k_{NE}. As presented in Fig. 1, $\boxed{\text{T}}$ would be expected to change with time as shown in the following differential equation:

$$\frac{d\boxed{\text{T}}}{dt} = K - k_T\boxed{\text{T}} \qquad (1)$$

where K is the rate of change of $\boxed{\text{T}}$ in the plasma compartment. On integration, and imposing the condition that $\boxed{\text{T}} = 0$ at $t = 0$, Eq. 1 becomes

$$\boxed{\text{T}} = \frac{K}{k_T}(1 - e^{-k_T t}) \qquad (2)$$

where t = duration of infusion.

[2] The abbreviations used are: NE, norepinephrine; DOPA, 3,4-dihydroxyphenylalanine; DM, dopamine, 3,4-dihydroxyphenylethylamine.

Because catecholamine levels are maintained in a steady state by a balance between their rates of formation and efflux, presumably in an open, single-compartment system, the change in concentration of radioactive NE (NE*) with time would be related to tyrosine-^{14}C (T*) as follows:

$$\frac{d\text{NE}^*}{dt} = k_1\text{T}^* - k_{NE}\text{NE}^* \qquad (3)$$

and, dividing by tissue NE levels,

$$\frac{d\boxed{\text{NE}}}{dt} = \frac{k_1\text{T}^*}{\text{NE}} - k_{NE}\boxed{\text{NE}} \qquad (4)$$

Under steady-state conditions the following relationship must be true:

$$k_1\text{T} = k_{NE}\text{NE} \quad \text{or} \quad \text{NE} = \frac{k_1\text{T}}{k_{NE}} \qquad (5)$$

Substituting for NE in Eq. 4,

$$\frac{d\boxed{\text{NE}}}{dt} = k_{NE}(\boxed{\text{T}} - \boxed{\text{NE}}) \qquad (6)$$

Equation 2 can now be substituted in Eq. 6 for $\boxed{\text{T}}$:

$$\frac{d\boxed{\text{NE}}}{dt} = k_{NE}\left[\frac{K}{k_T}(1 - e^{-k_T t}) - \boxed{\text{NE}}\right] \qquad (7)$$

Since $\boxed{\text{NE}} = 0$ at $t = 0$, Eq. 7 integrates into

$$\boxed{\text{NE}} = \frac{K}{k_T}\left[1 + \frac{1}{k_{NE} - k_T}(k_T e^{-k_{NE} t} - k_{NE} e^{-k_T t})\right] \qquad (8)$$

By substituting various theoretical values of k_{NE} for 20, 40, and 60 min of infusion in Eq. 8, where $k_T = 0.047$/min and $K = 120$ dpm/mμmole/min. from Eq. 2 Fig. 2 can be constructed. The value of k_{NE} for any NE compartment is calculated by determining the specific activity of NE in the tissue and finding the corresponding value for k_{NE} in Fig. 2. The NE synthesis rate is calculated by multiplying k_{NE} by the NE level.

We have previously reported that the synthesis rate of heart NE increases fol-

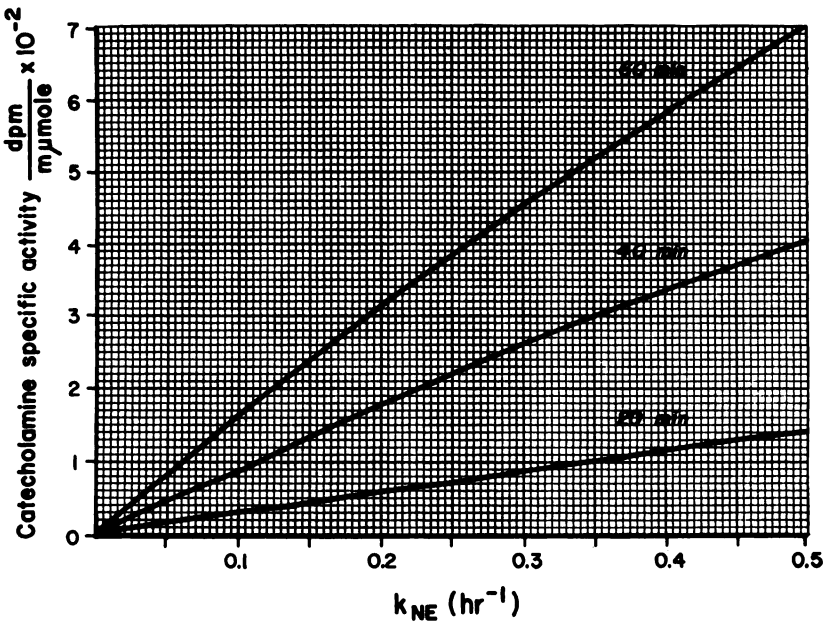

FIG. 2. *Fractional turnover rates for different values of catecholamine specific activity calculated for 20, 40, and 60 min of infusion from Eq. 8 where* $k_T = 0.047$*/min and* $K = 120$ *dpm/mμmole/min.*

lowing adrenal demedullation (11). This observation was confirmed by this new isotopic method, and the physiological implications of this finding are discussed.

METHODS

Male Sprague-Dawley rats weighing approximately 200 g were used. Normal animals were obtained from New York Breeding Farms, New City, New York, and adrenal demedullated and sham-operated rats, from Hormone Assay Laboratories, Chicago. Adrenal demedullated and sham-operated animals were allowed to recover from surgery for at least 14 days before the experiment. The adrenals of demedullated rats contained less than 2% of the catechols found in normal rats. All rats were fasted for 16 hr before infusion of tyrosine-^{14}C. Uniformly labeled L-tyrosine-^{14}C in 1.0 N HCl (351–368 mC/mmole) was obtained from New England Nuclear Corporation. Before infusion, tyrosine solutions were neutralized to pH 7 by titration with NaOH. Tyrosine was infused intravenously at a rate of 50 μC/hr (1.2 ml/hr) into the tail veins of unanesthetized animals confined in small plastic cylinders. At the end of the infusion the rats were decapitated and blood was collected in heparinized centrifuge tubes for the measurement of $\boxed{T}$. Brains and hearts were immediately removed, rinsed with water, blotted dry, and frozen until analyzed for radioactive catecholamines. Plasma was also frozen until analyzed.

Tissues and plasma were homogenized in 3–6 volumes of 0.4 N perchloric acid. After centrifugation, 3 ml of the supernatant fraction were brought to a pH of about 8.3 by adding 2 volumes of 0.5 M Tris buffer, pH 9. About 1 g of alumina (Woelm neutral, grade 1), prepared as described by Crout (12), was added to this solution, and catechol adsorption was effected by gently shaking the mixture by hand for 10 min.

After centrifugation, the tyrosine contained in the supernatant fraction was chromatographed on a Rexyn 102 column, 200–400 mesh (Fisher Scientific Company), buffered to pH 6.5 (50 mm × 78 mm²). A 5-ml portion of the alumina supernatant fraction was added to each Rexyn column. The initial 2.5-ml effluent was discarded; the second 2.5 ml and a 5-ml water eluate were collected and analyzed for radioactive

and stable tyrosine. Stable tyrosine was assayed by forming a fluorophor with 1-nitroso-2-naphthol, as described by Udenfriend (13). Radioactivity in the column eluate was determined by counting a 0.5-ml portion in Bray's counting mixture (14). The readiochemical purity of the eluate was assessed by freeze-drying a portion of the eluate and chromatographing the residue on Whatman No. 1 paper with 1-butanol–acetic acid–water (120:30:50) as solvent. In confirmation of a previous report (15), two radioactive compounds were found on the chromatogram of the column eluate, tyrosine (R_F = 0.45) and an unknown material (R_F = 0.21). Values for $\boxed{T}$ have been corrected to account for the presence of this material.

The catecholamine-containing alumina was washed twice with 3 ml of distilled water and then eluted with 3 ml of 0.2 N acetic acid. A 0.75-ml portion of the alumina eluate was assayed for stable norepinephrine and dopamine by a fluorescence method described previously (3). Radioactive catecholamines in the eluate were separated on a Dowex 50W-X4 column, 200–400 mesh, buffered to pH 6.0 (20 mm × 28 mm²). A separate 2-ml portion of the acetic acid eluate was passed through the column, and the effluent was discarded. Dopa and other unidentified radioactive materials were washed from the column with 5 ml of 0.1 M sodium acetate buffer, pH 6.0, followed by 5 ml of 0.4 N HCl. Norepinephrine was then eluted in 10 ml of 0.4 N HCl, and dopamine, in 5 ml of 4 N HCl. Both the norepinephrine- and the dopamine-containing fractions were freeze-dried, and the residue was dissolved in 2 ml of 0.2 N acetic acid and counted in Bray's counting mixture (14). Standards of ^{14}C-labeled tyrosine, norepinephrine, and dopamine were carried through the entire procedure to correct for extraction efficiency. The recoveries of tyrosine, norepinephrine, and dopamine were about 95, 62, and 60% respectively. Internal standards of ^{14}C-toluene were added to each sample to correct for counting efficiency. The specific activities of the catecholamines have been corrected for the loss of a carbon atom, which occurs when the uniformly labeled tyrosine is converted to a catecholamine. For comparison, the synthesis rate of brain and heart

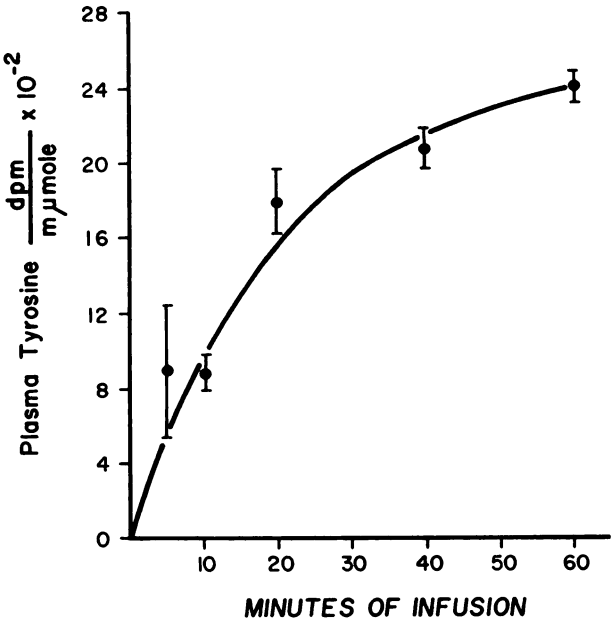

FIG. 3. *Increase in the specific activity of plasma tyrosine during an infusion of tyrosine-^{14}C (50 μC/hr)*
Each value is the mean ± standard error from at least five animals. The solid line represents the best fit values from Eq. 2.

norepinephrine in normal rats was also studied by blocking catecholamine synthesis with α-methyltyrosine and following the decline of norepinephrine levels as described previously (3).

RESULTS

When tyrosine-^{14}C is infused intravenously at the rate of 50 μC/hr, $\boxed{T}$ increases curvilinearly according to Eq. 2 (Fig. 3). The solution of this equation is

$$\boxed{T} = \frac{120 \text{ dpm/m}\mu\text{mole/min}}{0.047/\text{min}} \times [1 - e^{(0.047/\text{min})(t)}]$$

where 120 dpm/mμmole/min is the rate of change of $\boxed{T}$ in the plasma compartment and 0.047/min equals k_T. Accordingly, the average lifetime ($1/k_T$) of a plasma tyrosine molecule is approximately 21 min. During the infusion of tyrosine, $\boxed{NE}$ also increases, as predicted from Eq. 8 (Table 1).

Figure 2 presents values of catecholamine specific activity as a function of k_{NE} for 20, 40, and 60 min of infusion from Eq. 8. All the synthesis rate values (Tables 1, 2, and 3) have been calculated from Fig. 2. For example, if, after tyrosine has been infused for 60 min, the value of $\boxed{NE}$ for a brain containing 0.50 μg/g of NE were 380 dpm/mμmole, the corresponding value for k_{NE} would be 0.25/hr. The fractional rate constant (k_{NE}) found on the graph (0.25/hr), multiplied by a steady-state level (0.50 μg/g), is the synthesis rate: 0.25/hr × 0.50 μg/g = 0.13 μg/g/hr.

If neuronal NE stores behave kinetically as an open, single compartment, as proposed by our hypothetical model, the synthesis rates of the catecholamines should remain relatively constant when t changes. To test the reliability of the proposed model, values of k_{NE} and the synthesis rates of brain NE were calculated after 20, 40, and 60 min of infusion. As shown in Table 1, k_{NE} and the synthesis rate of brain NE were about equal for the three infusion periods studied. Moreover, during the infusion of tyrosine-^{14}C, plasma and tissue tyrosine levels and tissue catecholamine content did not change.

The plasma levels of an unidentified radioactive compound present in the column eluate increased from less than 1% of the tyrosine radioactivity after 5 and 10 min of infusion to about 24% after 1 hr. This radioactive material was present in greater quantities in tissue, representing about 62% of the radioactivity in heart after 1 hr of infusion. No attempt was made to identify this compound.

Table 2 presents values of $\boxed{NE}$, k_{NE}, steady-state NE levels, and the NE synthesis rates for brain and heart of normal rats found after 60 min of tyrosine-^{14}C infusion. These values were almost equal to those calculated from the decline of NE after administration of α-methyltyrosine (Table 2).

Equation 8 can also be used to calculate the synthesis rate of brain DM if it is assumed that this substance is the end product of catecholamine metabolism in those neurons that store dopamine. In the present study, the average specific activity of brain DM from four animals was 343 ± 38 dpm/mμmole, corresponding to an average k_{DM} of

TABLE 1

Calculated rate of brain norepinephrine synthesis for different infusion periods of tyrosine-^{14}C

Tyrosine-^{14}C was infused at a rate of 50 μC/hr for the time periods indicated. Values are presented as means ± standard error (number of animals).

Duration of infusion	NE specific activity	k_{NE}	Steady-state NE level	Synthesis rate
min	*dpm/mμmole*	*hr^{-1}*	*μg/g*	*μg/g/hr*
20	76 ± 9 (6)	0.26 ± 0.03	0.48 ± 0.03	0.12 ± 0.01
40	244 ± 14 (5)	0.28 ± 0.02	0.46 ± 0.02	0.13 ± 0.005
60	383 ± 29 (5)	0.25 ± 0.02	0.50 ± 0.03	0.12 ± 0.008

TABLE 2

Catecholamine synthesis rates calculated from the rate of conversion of tyrosine-^{14}C to catecholamines and from the decline of amine levels after administration of α-methyltyrosine

Tyrosine-^{14}C (50 μC) was infused at a constant rate for 1 hr, after which tyrosine and catecholamines were extracted as described in METHODS. α-Methyltyrosine (α-MT) (200 mg/kg) was administered intravenously, and rats were killed after 1, 2, 4, and 8 hr. A second dose of 100 mg/kg was administered 2 hr after the first to the animals to be killed at 4 and 8 hr. Catecholamines were extracted and synthesis was calculated as described previously (3). Values are presented as means ± standard error (number of animals).

Tissue source of NE	Method	NE specific activity	k_{NE}	Steady-state amine level	Synthesis rate
		dpm/mμmole	*hr*$^{-1}$	*μg/g*	*μg/g/hr*
Brain	Tyrosine-^{14}C	383 ± 29	0.25 ± 0.02	0.50 ± 0.03	0.12 ± 0.008 (5)
	α-MT		0.17 ± 0.04	0.56 ± 0.02	0.095 ± 0.02[a] (26)
Heart	Tyrosine-^{14}C	144 ± 19	0.089 ± 0.01	1.09 ± 0.008	0.095 ± 0.012 (11)
	α-MT		0.10 ± 0.01	0.92 ± 0.05	0.092 ± 0.009[a] (26)

[a] The standard error of the synthesis rate was calculated according to the formula

$$(A \pm a)(B \pm b) = (AB)\left[1 \pm \left(\frac{a^2}{A^2} + \frac{b^2}{B^2}\right)^{1/2}\right]$$

where A and B represent k_{NE} and the steady-state amine levels with their respective standard errors.

0.22 ± 0.03/hr (Fig. 2). By multiplying the individual k_{DM} values and the steady-state DM levels, we obtained a synthesis rate of 0.18 ± 0.03 μg/g/hr. This rate is similar to the rates calculated from the decline in DM levels after administration of α-methyltyrosine.

Table 3 presents the specific activity, the fractional rate constant, and the synthesis rates in catecholamines in sham-operated and adrenal demedullated rats. The synthesis rate and the fractional rate constant of heart NE were increased almost 4 times as a result of adrenal demedullation. Brain NE and DM synthesis rates were not significantly affected. These results are probably not a consequence of altered tyrosine metabolism, as [T] and the specific activity of heart and brain tyrosine were essentially equal in the sham-operated and adrenal demedullated animals. An increase of heart

TABLE 3

Effect of adrenal demedullation on catecholamine synthesis rates

Tyrosine-^{14}C (50 μC) was infused at a constant rate for 1 hr, after which tyrosine and catecholamines were extracted as described in METHODS. Each value is the mean of five observations ± standard error.

	Sham-operated animals			Adrenal demedullated animals		
Tissue source of amine	Specific activity *dpm/mμmole*	Rate constant *hr*$^{-1}$	Synthesis rate *μg/g/hr*	Specific activity *dpm/mμmole*	Rate constant *hr*$^{-1}$	Synthesis rate *μg/g/hr*
Brain NE	306 ± 39	0.18 ± 0.03	0.10 ± 0.01	423 ± 75	0.28 ± 0.05	0.13 ± 0.03
Brain DM	397 ± 25	0.26 ± 0.02	0.21 ± 0.02	493 ± 91	0.33 ± 0.07	0.23 ± 0.04
Heart NE	55 ± 4	0.035 ± 0.003	0.046 ± 0.003	197 ± 36[a]	0.12 ± 0.02[a]	0.18 ± 0.02[a]
Heart NE[b]		0.06 ± 0.009	0.066		0.16 ± 0.01[a]	0.18

[a] $p < 0.01$ when compared with sham-operated rats.

[b] Values from Costa and Neff (11). Synthesis rates were calculated from the decline of heart NE after blockade of synthesis with α-methyltyrosine. The rate constant for the decline of heart NE in normal rats of the same strain and from the same sources was 0.060 ± 0.007 (16).

NE turnover rate in demedullated rats was obtained when NE synthesis was studied by blocking the formation of catecholamines with α-methyltyrosine (11) (Table 3). In these experiments, it was also found that k_{NE} in hearts of sham-operated rats was similar to that of nonoperated animals (Table 3).

DISCUSSION

Classically, the term "turnover" refers to the process of renewal of a substance in the body or in a tissue. Renewal may be accomplished in different ways: (a) a substance may be synthesized in a given tissue, or (b) a substance may be synthesized elsewhere and arrive in the tissue by means of the circulation. The term "turnover rate" implies that a steady state exists, in which the synthesis and transport of an endogenous substance into a metabolic compartment equal its breakdown and exit. For all practical purposes, the turnover rate of brain catecholamines equals its synthesis rate, as only a small quantity of amine crosses the blood-brain barrier (17). This is probably also true for the NE compartment in heart, as only 20% (18) or less (19) of the amine is thought to originate from the circulation. If a large quantity of NE did originate in the circulation, however, the turnover rate would be greater than the synthesis rate.

Spector *et al.* (18) have reported that the NE* formed from tyrosine-^{14}C in the perfused guinea pig heart had a specific activity higher than the tyrosine in heart muscle, implying that calculations of NE synthesis rates based on $\boxed{T}$, as presented here, are probably more reliable than calculations based on the specific activity of tyrosine in the entire heart.

The uptake of tyrosine into brain is a catalyzed process (20). After a pulse injection of tyrosine-^{14}C, the specific activity of brain tyrosine declines rapidly at first and then more slowly (4), denoting that brain tyrosine can be represented as being in a multicompartment system. The specific activity of NE and DM found in brain after a pulse injection of tyrosine-^{14}C declines almost in parallel with the specific activity of the rapidly turning over compartment of free tyrosine in brain (4). However, it is not possible to conclude that this compartment is in rapid equilibrium with plasma tyrosine, because the specific activity of tyrosine was not corrected for the radioactive contaminant, which changes with time. Data reported by Lewander and Jonsson (15) show that the specific activity of brain DM is significantly higher than the specific activity of purified brain tyrosine. This finding suggests that DM is formed from a precursor with a higher specific activity than that of the brain tyrosine. For this reason, brain catecholamine synthesis rates in the present experiments were calculated from the $\boxed{T}$ values rather than from the specific activity of tyrosine in the whole brain.

The following assumptions are implicit in the model we propose for calculating catecholamine synthesis: (a) the neurons that store DM have only a small compartment of DOPA; (b) this amino acid has a specific activity about equal to that of plasma tyrosine: and (c) noradrenergic neurons have only a small compartment of DM as well as DOPA. If the specific activity of the tyrosine compartment in neurons is lower than that of $\boxed{T}$, or if the noradrenergic neurons have a large store of DOPA or DM, the synthesis rates of norepinephrine presented in this report are lower than their true values. A comparison of results obtained by other methods, as shown in Tables 2 and 3, suggests that the assumptions incorporated in our model are probably valid.

We have previously presented a method for calculating catecholamine synthesis from the rate of conversions of tyrosine-^{14}C to NE, based on the assumption that $\boxed{T}$ increases linearly with time (9). In the present study, a more appropriate equation (Eq. 2) is used to describe $\boxed{T}$. Once this equation is known, synthesis rates can be calculated from the specific activity of tissue NE or DM alone. However, $\boxed{T}$ should always be monitored to determine whether the experimental conditions alter the fractional rate constant (k_T) of plasma tyrosine. If tyrosine metabo-

lism is altered either by a drug or in response to environmental changes, new values for Eq. 2 must be obtained before the synthesis rate of NE can be calculated.

Our present understanding of the formation and storage of catecholamines in peripheral sympathetic neurons is compatible with an open, single-compartment model system (3, 21–23). This hypothetical model has been adopted to calculate the synthesis rate *in vivo* of catecholamines in the whole brain (3, 4), brain parts (2, 3, 24), and heart (3, 16, 25). Since the fractional rate constant of heart catecholamine stores calculated either by blocking tyrosine hydroxylase or by administering norepinephrine-^{3}H appears linear over many hours, one can assume that NE is continually being formed and destroyed at an almost constant rate. This contention is supported further by the present study; synthesis remained constant in brain when measured at various time intervals up to 60 min during an infusion of tyrosine-^{14}C. Although these observations provide substantial support for a single-compartment system of catecholamine synthesis and storage, they do not exclude the existence of a very small second compartment of NE.

Quite aside from the problem of different pools of norepinephrine in a single neuron, Iversen and Glowinski (2) showed that the turnover rates of NE in several brain structures are different. This finding does not conflict with the model adopted in our calculations, for the following reasons. (a) In different brain parts NE may be stored in a variable number of monoaminergic neuronal somata. It is known that the efflux rate of NE from somata is faster than from nerve endings and that this variation may not be ascribed only to a different rate of NE synthesis (2, 26). (b) The presence of (+)-NE in the racemic mixture of radioactive NE injected intraventricularly complicates the turnover measurements; Glowinski *et al.* (27) have shown that the brain metabolizes the two isomers of NE at different rates. (c) The decline of NE* levels after an intraventricular injection is multiphasic, but Iversen and Glowinski (2) failed to resolve the decline into its component parts. When the decline is resolved into its parts, one finds that the rapid phase is much faster and the slow phase much slower than the rate of decline calculated by these authors. (d) A fianl complication arises from the finding taht after an intraventricular injection, NE localizes in brain cells that normally do not contain it (28).

Recently Sedvall *et al.* (8) presented a method for calculating NE synthesis rates *in vivo* from the conversion of tyrosine-^{14}C into labeled catecholamines. They assumed that little of the newly synthesized NE was lost during a 1 hr intravenous infusion of tyrosine-^{14}C. This assumption seems incompatible with their data, because they showed that as much as 0.165 μg of NE per gram of brain could be formed in 1 hr without significant changing brain NE levels. Understeady-state conditions, a constalnt concen tration is maintained by equal influx and efflux; therefore, a quantity of NE equal to about 36% of the amine store in brain (assuming a level of 0.45 μg/g) was not accounted for in their calculations. Moreover, their method for extracting and purifying radioactive tyrosine has been questioned (15). The minimum and maximum synthesis rates presented by Sedvall *et al.* (8) are incorrect, because a radioactive metabolite of tyrosine is present in tissue and plasma samples. This metabolite increases with time and can be as much as 50–60% of the total radioactivity in brain (15). In addition, the concentration of metabolite varies with time in different tissues. Furthermore, from the data presented by Lewander and Jonsson (15), the specific activity of the catecholamines in brain may actually be higher than the specific activity of brain tyrosine.

In our calculations, Eq. 4 presupposes that the $\boxed{\text{NE}}$ lost from the tissue is equal to the $\boxed{\text{NE}}$ present in tissue. In a recent report (29) it was suggested that protracted electrical stimulation (30 cps) of the cat splenic nerve causes a selective release of newly synthesized NE. Therefore, one might conclude that the $\boxed{\text{NE}}$ leaving the tissue is greater than the $\boxed{\text{NE}}$ present in tissues. However, at this "supraphysiological" frequency of stimulation, the normal reup-

take of neurally released NE is greatly inhibited (30, 31) whereas at physiological rates of stimulation (6–8 cps) 80% of the released NE is retrieved into storage (31). Therefore, the loss of newly synthesized NE during physiological activity must be minimal. Moreover, the present understanding of the physiology of sympathetic nerve terminals in the cat spleen does not elucidate whether a rapid rate of nerve stimulation (29) unmasks multiple compartments for storage and synthesis of NE in a single neuron or a variation in the excitability characteristics of sympathetic neurons within the spleen.

The catecholamines that are continuously released from the adrenal medulla into the circulation supposedly contribute to the maintenance of sympathetic tone in most peripheral tissues. In contrast, NE released by peripheral sympathetic nerve terminals is probably responsible for producing phasic changes of sympathetic tone in a limited population of postsynaptic cells surrounding a given nerve ending. If this general view is accepted, homeostasis could be maintained after removal of the adrenal medulla by increasing catecholamine synthesis in peripheral sympathetic neurons to compensate for the loss of circulating catecholamines. Synthesis could be accelerated by increasing sympathetic nerve activity or by altering amine steady-state levels. As shown in Table 3, adrenal demedullation produces about a 4-fold increase in heart NE synthesis without significantly changing the rates of synthesis of NE and DM in brain. From these results one might infer that the blood-brain barrier prevents circulating catecholamines from modulating the function of central catecholaminergic neurons. Although many other experimental variables could cause an increase in NE specific activity in the hearts of demedullated rats, we believe that an increase in its rate of synthesis in heart plays a substantial role. This belief is supported by the finding that the efflux of NE from the hearts of demedullated rats is increased after blockade of synthesis with α-methyltyrosine (Table 3). It is possible that demedullation impairs adrenal cortical function and this impairment reflects itself in the rates of NE synthesis. This seems improbable, however, as adrenal demedullation does not significantly alter plasma corticosterone levels in the rat (32), suggesting that cortical function is essentially normal.

An interesting difference in heart NE turnover rates is documented in Tables 2 and 3. These tables show that the synthesis rates of heart NE in Sprague-Dawley rats from two commercial sources are different. Similar observations have already been reported for brain catecholamine turnover rates (3).

In support of a relationship between adrenal medulla and the function of sympathetic neurons, Pouliot (33) found that adrenal demedullated rats exposed to 4° maintained their thermal equilibrium and excreted greater quantities of NE than normal rats exposed to cold. After treatment with guanethidine, demedullated rats failed to excrete increased amounts of NE and to maintain their equilibrium. Pouliot concluded that, on exposure to cold, demedullated rats utilize greater amounts of peripheral NE than control rats. This compensating mechanism is apparently important for the survival of demedullated rats exposed to cold. Additional support for the physiological interaction between adrenal and peripheral catecholamines is provided by reports that hypophysectomy reduces catecholamine synthesis in the adrenal (34) and increases synthesis of NE in the heart (35). Moreover, Harrison and Seaton (36) have shown that adrenal demedullation in monkeys results in a 3-fold increase in the urinary excretion of NE and a 90% reduction in epinephrine.

The proposed method for calculating catecholamine synthesis is simple and requires only a single sample for measurement, and the results are readily reproducible. The close agreement between this and other methods for calculating synthesis rates tends to support a simple model system rather than a more complex model as representative of catecholamine storage and synthesis. The present report elucidates the usefulness of this method for studying the physiological factors that control catecholamine synthesis.

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