0031-6997/80/3103-0179$02.00/0
PHARMACOLOGICAL REVIEWS
Vol. 31, No. 3
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Printed in U.S.A.

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The Rate Constants for the Efflux of Metabolites of Catecholamines and Phenethylamines[1]

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I. Introduction

VERY soon after radioactively labelled catecholamines and phenethylamines became available, methods were developed to separate labelled metabolites from labelled parent amines. The early methods had the disadvantage that only some of the labelled metabolites could be determined with accuracy, but recent developments of paper and column chromatographic procedures enable us to determine *all* the metabolites of a given parent amine. These methods have been refined and simplified to such an extent that they are easy to use. This methodological development coincided with the realization that determinations of labelled metabolites can help to solve many interesting problems. For instance, with this method—as well as with the use of specific inhibitors of neuronal (uptake$_1$) and extraneuronal uptake [uptake$_2$ (23)] or of the two enzymes responsible for the metabolism of catecholamines, monoaminoxidase (MAO) and catechol-O-methyltransferase (COMT)—we can now determine the neuronal and/or extraneuronal fate of exogenous or circulating amines or of amines released from adrenergic nerve endings.

It is important to realize that until the study of Levin in 1974 (32), in the large majority of investigations, the labelled metabolites were determined either in the tissue (typical of most of the earlier work) or in either the incubation medium or the perfusion fluid (especially in studies of the metabolic fate of ^{3}H-noradrenaline released from adrenergic nerve endings by electrical stimulation or other procedures). Up to 1974, virtually all such studies were interpreted on the tacit assumption that all metabolites leave the tissue in which they are formed with about equal ease; i.e., the rate constants for the efflux of the various metabolites were assumed to be very similar.

[1] In Memoriam, Walther Wilbrandt (1907–1979).

Evidence that this tacit assumption was wrong came from the first study in which the ^{3}H-metabolites of ^{3}H-noradrenaline were determined both in the tissue and in the incubation medium after rabbit aortic strips had been incubated with ^{3}H-noradrenaline for 30 min. Levin (32) calculated the "tissue content of the ^{3}H-metabolite in per cent of the total formation of the ^{3}H-metabolite during 30 min of incubation with a constant concentration of ^{3}H-noradrenaline," and he drew attention to the fact that very low percentage values for the glycol metabolites (DOPEG and MOPEG; see table 1 for abbreviations), an intermediate percentage value for normetanephrine (NMN), and very high percentage values for the acid

TABLE 1
List of abbreviations used in this review

Parent amine	Metabolite	Abbreviation
Noradrenaline	Normetanephrine	NMN
Noradrenaline plus adrenaline	Dihydroxyphenylglycol	DOPEG
	Dihydroxymandelic acid	DOMA
	3-Methoxy-4-hydroxy-phenylglycol	MOPEG
	3-Methoxy-4-hydroxy-mandelic acid	VMA
Adrenaline	Metanephrine	MN
Isoprenaline	3-Methoxyisoprenaline	OMI
Dopamine	Dihydroxyphenylethanol	DOPET
	Dihydroxyphenylacetic acid	DOPAC
	3-Methoxy-4-hydroxy-phenylethanol	MOPET
	3-Methoxy-4-hydroxy-phenylacetic acid	HVA
	3-Methoxytyramine	MT
Phenylephrine	4-Hydroxymandelic acid	HMA
	4-Hydroxyphenylglycol	HOPEG

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metabolites (DOMA and VMA) were obtained. He concluded: "In general, the neutral metabolites (DOPEG and MOPEG) passed into the incubation solution somewhat more readily than NMN. The acid metabolites (DOMA and VMA) were retained in the tissue to the greatest extent."

More recent studies have provided ample confirmation of Levin's observations. Indeed, we are now able to determine the ability of labelled metabolites to leave the tissues of their origin more accurately, namely by the determination of their rate constants for efflux. It is the aim of this review to discuss (a) the various ways in which such rate constants can be obtained, (b) the possible reasons for the pronounced differences between rate constants, and (c) the implications for the design and the interpretation of experiments in which metabolites of catecholamines and phenethylamines are determined. Catecholamines like noradrenaline and adrenaline are metabolized either by MAO (to result in the formation of DOPEG and DOMA, since the aldehyde formed by MAO is subject to further metabolism by aldehyde reductase and aldehyde dehydrogenase, respectively), or by COMT (resulting in the formation of NMN and MN from noradrenaline and adrenaline, respectively), or by both (to yield MOPEG and VMA, irrespective of the sequence of the enzymic reactions). Dopamine is metabolized by the same enzymes. Another catecholamine (isoprenaline) is a substrate of COMT only, while certain phenethylamines (e.g., phenylephrine) are substrates of MAO only. All relevant metabolites are tabulated in table 1.

For an understanding of the problems discussed here it is important to realize that both MAO and COMT are intracellular enzymes. While MAO is located in all adrenergic nerve endings and in most, but not all, extraneuronal tissues, COMT is located predominantly, possibly exclusively, in extraneuronal tissues. Special locations of enzyme activity, as for instance in liver and kidney, need not be considered in this review, since we restrict our discussion to the efflux of catecholamine and phenethylamine metabolites from *isolated organs*.

For readers not acquainted with the terminology used in the field of adrenergic mechanisms, it should perhaps be pointed out that the terms "extraneuronal uptake" and "extraneuronal tissue" refer to the translocation (uptake) of certain amines into nonneuronal cells. For a discussion of the cell types able to carry out this "extraneuronal uptake" of certain amines, see Gillespie (14).

In our discussion of the rate constants for the efflux of catecholamine and phenethylamine metabolites from the cells in which they are formed, we start from the simplest assumption, namely that they leave these cells by a process obeying first-order kinetics. Thus, we have to consider very simple (neuronal and extraneuronal) open compartments from which the metabolites leave at rates that amount to "intracellular metabolite concentration times the characteristic rate constant" (or "rate of efflux = tissue content × k"). More complex arrangements, such as multiple distribution compartments or saturable efflux, will be accepted only if the experimental evidence is incompatible with the simplest possible arrangement.

II. Procedures Used to Measure the Rate Constants for the Efflux of Metabolites

Various procedures yield estimates of rate constants. Each one has its advantages as well as its drawbacks. Ideally, several procedures should be used to determine a rate constant. In the following, a short summary of the commonly used procedures is given. In this account, experimentally determined rate constants are called apparent rate constants.

A. Rate Constant for Efflux, k = Rate of Efflux/Tissue Content

Irrespective of the experimental conditions (i.e., either during exposure of the tissue to the parent amine or during washout of tissues with amine-free solution subsequent to an initial loading with the labelled parent amine), the apparent rate constant k is obtained from "rate of efflux of metabolite/metabolite content of the tissue," provided that the two measurements relate to (roughly) the same time. However, only when the metabolite is distributed into a single compartment, does the apparent k equal the true k. If the efflux of the metabolite originates from two different compartments (characterized by two different k-values), the experimentally determined apparent k-value lies somewhere between the two true k-values. It is also possible that efflux of the metabolite originates from just one compartment, while the metabolite is firmly bound in a second compartment (without contributing to efflux). In that case, we speak of a "bound fraction," the existence of which can be verified or excluded with the procedure described in section II B.

While the determination of an apparent rate constant for the efflux of a metabolite is simple when a single metabolite is distributed into a single compartment, it is more complex when either a single metabolite distributes into two compartments (characterized by two different rate constants) or the metabolite fraction comprises two metabolites (characterized by different k-values). For instance, the column chromatographic separation of the metabolites of noradrenaline (16) yields an OMDA-fraction that contains both MOPEG and VMA. In these two cases, we determine an "apparent k_{total}", i.e., an apparent rate constant that reflects "total rate of efflux" and "total tissue content." As will be discussed in more detail in section IIB, such k_{total} lies somewhere between the two rate constants characterizing the two compartments or the two metabolites; in addition, k_{total} is influenced by the duration of the experiment.

B. Plot of "Rate of Reflux" (Ordinate) vs. "Tissue Content" (Abscissa)

Experiments have to be designed to yield pairs of values (for rate of efflux and for tissue content, both

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values determined at about the same time) that extend over a considerable range. If efflux originates from a single compartment and if it obeys first-order kinetics, the experimental values of efflux, plotted against tissue content, fall onto a straight line that intersects the abscissa at zero. The slope of the straight line then equals k. If the intercept of the straight line with the abscissa is significantly above zero, this is conclusive evidence for the existence of a "bound fraction" (10).

Since, in such measurements, both "rate of efflux" and "tissue content" are subject to error, the conventional regression analysis is invalid. However, if the scatter of the experimental points around the "ideal" straight line is not too great, the slope of the conventional regression line is a useful estimate of the apparent k-value, especially if such estimates of apparent k-values are used only to demonstrate very pronounced differences between the apparent k-values of different metabolites (table 2).

If, on the other hand, efflux of the metabolite originates from two compartments (characterized by two clearly different k-values), we face the situation illustrated by figure 1. For two different compartments (characterized by the rate constants for efflux k_1 and k_2), the broken

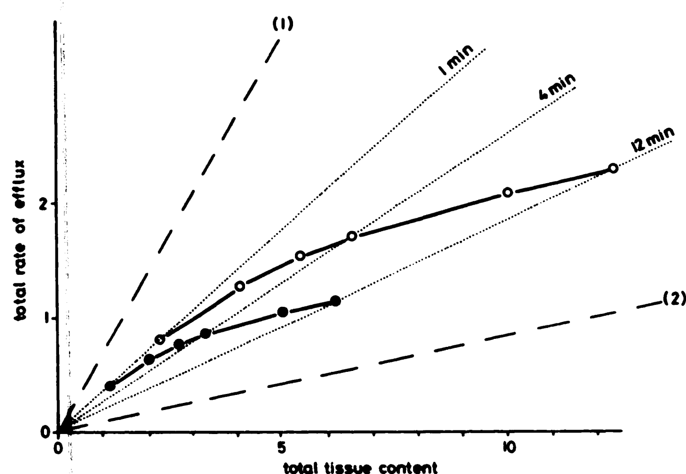

FIG. 1. Dependence of the apparent rate constant for the efflux of a metabolite on the duration of the experiment when the metabolite is formed in two kinetically differing compartments. Ordinate: total rate of efflux ($v_1 + v_2$, i.e., sum of efflux from both compartments; in arbitrary units·min^{-1}). Abscissa: total metabolite content of tissue ($C_1 + C_2$, i.e., sum of both compartment sizes; in arbitrary units). Tissues were assumed to be exposed to two different external concentrations of the parent amine: during exposure to the "high" amine concentration (○), the rate of formation of the metabolite (v_f) was 1.286 arbitrary units · min^{-1} in *each* compartment; during exposure to the "low" amine concentration (●), it was assumed to be 0.693 arbitrary units · min^{-1}. The two compartments were characterized by the rate constants for efflux of the metabolite: $k_1 = 0.693$ min^{-1} and $k_2 = 0.086625$ min^{-1}; the broken lines represent k_1 and k_2, respectively (i.e., the experimental points would have fallen onto one of the broken lines, if only *one* compartment were involved). For exposures of 1, 2, 3, 4, 8, and 12 min duration, the metabolite content of each compartment was calculated from $C_t = v_f(1 - e^{-kt})/k$, and the rate of efflux from each compartment from $v_t = v_f(1 - e^{-kt})$. The dotted straight lines were drawn through points determined after exposures of identical duration (i.e., for 1, 4, and 12 min). Their slopes represent k_{total}.

lines show where the points (for rate of efflux vs. tissue content) would have fallen if only *one* of the two compartments were involved. However, if both compartments contribute to efflux (and if we plot "total efflux" vs. "total tissue content"), the points fall between the two broken lines. As illustrated by two sets of calculations for simulation of an exposure to a low (solid circles) and to a high amine concentration (open circles), points determined for *short* exposures to the amine are closer to the line depicting k_1, while points approach that depicting k_2 when the duration of the exposure is increased. This is evident from the two curves shown in figure 1 (solid lines connecting solid or open circles), which represent points determined after 1, 2, 3, 4, 8, and 12 min of exposure to the parent amine (from left to right). In such a plot, the value for k_{total} can be visualized as the straight line connecting the point with the origin of abscissa and ordinate. The dotted lines of figure 1 illustrate the progressive decline of k_{total} with increasing duration of the exposure to the parent amine. The figure illustrates a further fact: if experiments have been carried out with two different periods of exposure to the parent amine (i.e., if only *two* of the solid points of figure 1 had been determined), the straight line drawn through these two points intercepts the abscissa at a negative value. Indeed, such a negative intercept (obtained for experiments involving varied exposures to the parent amine) is evidence for a distribution of the metabolite into more than one compartment.

If, on the other hand, the external concentration of the parent amine (instead of the duration of the experiment) is varied, it may well happen that the rate of the formation of the metabolite in the two compartments is increased by the same factor. Figure 1 illustrates that, under such conditions, apparent k_{total} (determined at a certain time) is *not* influenced by changes in the external concentration of the parent amine. The dotted straight lines (drawn for exposures of 1, 4, and 12 min) connect points determined at the same time, and these straight lines extend to the origin of abscissa and ordinate.

As pointed out above, for a graph of "rate vs. content," the experimentally determined metabolite content of tissue must cover a considerable range. The metabolite content of the tissue can be varied by variation of the duration of the experiment or by variation of the external concentration of the parent amine. In both cases, k_{total} *must* fall somewhere between "true k_1" and "true k_2." However, while variation of the external concentration of the parent amine fails to provide any evidence for or against the possibility that more than one compartment is involved, variation of the duration of the experiment provides the opportunity to test whether or not the distribution of the metabolite is restricted to one compartment.

It should be added that the results presented in figure 1 are also relevant to the analysis of a metabolite fraction consisting of two metabolites.

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C. Decline of the Efflux of the Metabolite after Sudden Inhibition of the Metabolizing Enzyme

If a perfused tissue has been in contact with the parent amine long enough to be in equilibrium, the metabolite appears in the venous effluent at a steady rate. If it is possible to inhibit the relevant metabolizing enzyme quickly and fully, the rate of efflux of the metabolite declines exponentially. If efflux originates from a single compartment, the decline should be monophasic and characterized by the apparent k. However, if efflux originated from two (or more) compartments, a biphasic (or multiphasic) exponential decline should be observed, from which apparent rate constants can be calculated with conventional procedures (peeling technique).

D. Approach of Tissue Content to Steady State

If the formation of the metabolite proceeds at a steady rate and in a single compartment (during exposure of the tissue to a constant concentration of the parent amine), the tissue content should approach steady state exponentially and with a half time of ln 2/k. If, on the other hand, two compartments are involved, the approach to steady state should be biphasic and characterized by two different k-values. However, the accuracy of apparent k-values determined in this way stands and falls with the assumption of a steady rate of formation of the metabolite throughout the exposure to the parent amine, i.e., it depends on whether the intracellular concentration of the parent amine at the locus of the metabolizing enzymes approaches a steady state quickly or slowly. If this is a slow process, the apparent rate constant reflects the slow rise of the intracellular amine concentration rather than the true rate constant for efflux of the metabolite.

The apparent rate constant is obtained from a plot of "ln (steady-state accumulation minus accumulation at time t)" against time; the slope of this line equals k (and the conventional "peeling" technique is used if there are more than one compartment).

E. Approach to Steady State for the Rate of Appearance of Metabolite in the Venous Effluent

If the time course of the rate of the efflux of the metabolite is determined in experiments with perfused tissues, virtually the same procedures (as in section II D) can be used to determine the apparent rate constant, since the approach of the rate of appearance of the metabolite in the venous effluent to steady state occurs with a half time that equals ln 2/k. Again, if the metabolite distributes into two compartments, a biphasic exponential approach should be evident, and results can be grossly distorted if the formation of the metabolite is subject to an initial delay. This point will be discussed in more detail in section II F.

F. Determination of the Two Lag Periods

This analysis proceeds in two steps: the first aims at measuring the speed with which the formation of the metabolite approaches steady state, the second aims at measuring the apparent rate constant for the efflux of the metabolite.

The cumulative appearance of the metabolite in the venous effluent is plotted against time (for experiments with perfused tissues) (fig. 2). The slope of the resulting curve first increases with time, in order to become linear when the rate of appearance in the venous effluent becomes constant. To the last experimental point (representing total efflux) the metabolite content of the tissue (analyzed at the end of the experiment) is added. This point now represents "total formation of the metabolite." Through this point a line is drawn that is parallel to the linear part of the first curve. The intercept of this parallel with the abscissa gives "$t_{lag\ system}$" (i.e., the reciprocal of that rate constant which determines the approach of the intracellular concentration of the parent amine to steady state). Figure 3 illustrates experimental results obtained by Graefe (15). In the perfused cat heart $t_{lag\ system}$ is very short for extraneuronally formed NMN, but quite long for the neuronally formed deaminated metabolites (DOPEG and DOMA) (fig. 3A). In experiments of this kind, the rates of the appearance of the neuronally formed metabolites (DOPEG and DOMA) constitute an upward concave curve at least up to the 95th min of perfusion. Hence, the necessary extrapolation is very long. However, comparison of this figure with figure 2 shows that $t_{lag\ system}$ is then easily *under*estimated (if the rate of

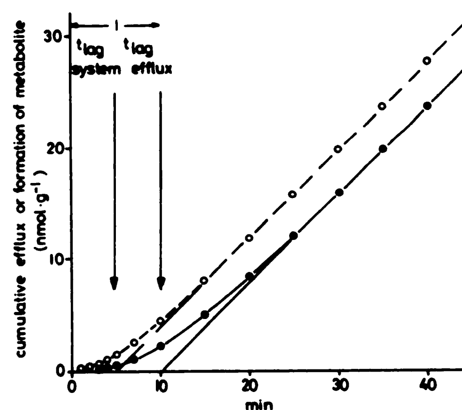

FIG. 2. Determination of $t_{lag\ system}$ and $t_{lag\ efflux}$ in a model system. Ordinate: cumulative formation (○; broken line) or cumulative efflux of metabolite (●; solid line) (in nmoles·g^{-1}); abscissa: time (in min) after onset of exposure of model compartment to a constant amine concentration. Results were calculated with the mathematical model described by Kurahashi et al. (25), which involves an uptake of the amine into the model compartment, metabolism of the amine by an intracompartmental enzyme, and an efflux of the metabolite obeying first-order kinetics. The following constants were used: $K_{m\ uptake}$, 100 μM; $V_{max\ uptake}$, 80 nmoles·g^{-1}·min^{-1}; $K_{m\ enzyme}$, 100 μM; $V_{max\ enzyme}$, 20 nmoles·g^{-1}·min^{-1}; k for metabolite, 0.2 min^{-1}; k for amine, 0. Note that extrapolation of the straight parts of the two curves yields intercepts with the abscissa at 5 min (broken line) and 10 min (solid line). The intercept of the broken line equals $t_{lag\ system}$ (5 min). The horizontal distance between the two parallels equals $t_{lag\ efflux}$ (5 min). Note that $t_{lag\ system}$ = 5 min, because $t_{lag\ system} = 1/k_{enzyme}$, where $k_{enzyme} = V_{max\ enzyme}/K_{m\ enzyme}$; and $t_{lag\ efflux}$ = 5 min, because k for metabolite = 0.2 min^{-1}; for further explanations, see Kurahashi et al. (25).

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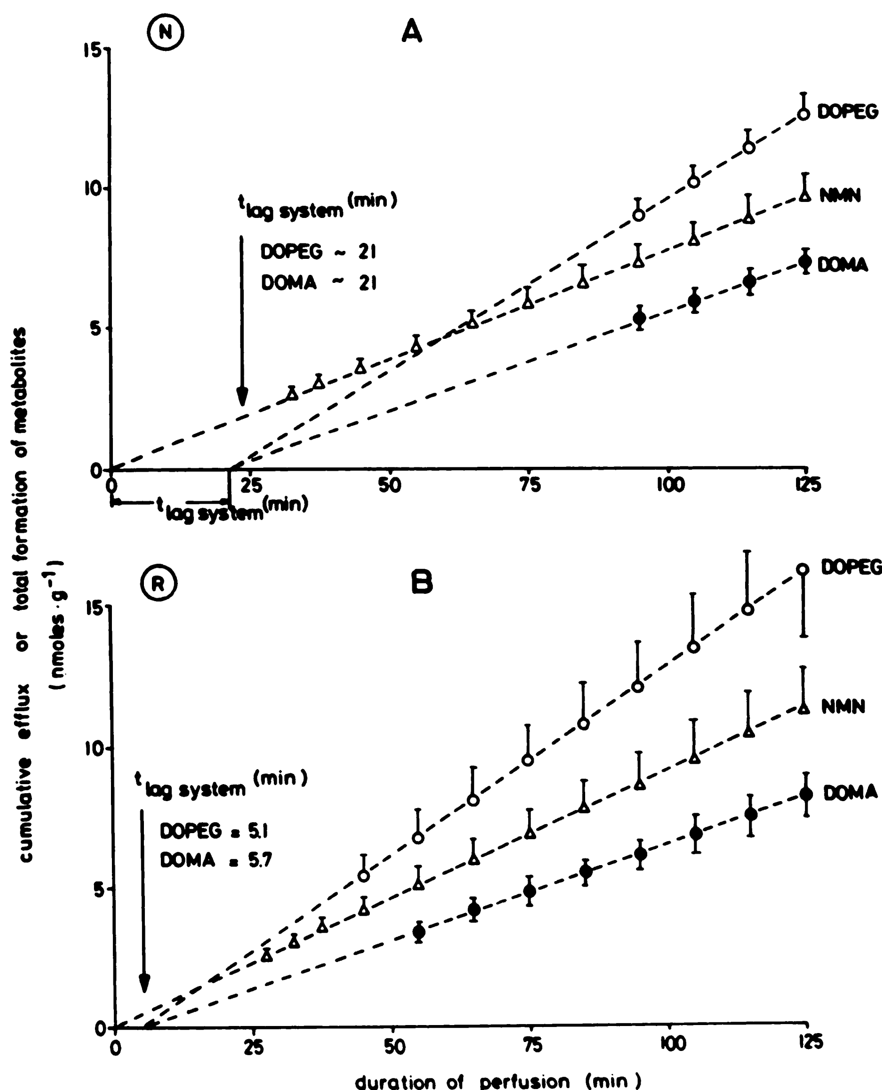

FIG. 3. $t_{lag\ system}$ for the formation of the metabolites of ^{3}H-noradrenaline in perfused cat hearts, obtained either from normal (N) or from reserpine-pretreated animals (R). Ordinates: cumulative formation of ^{3}H-DOPEG, ^{3}H-NMN, and ^{3}H-DOMA (in nmol·g^{-1}, calculated as shown in fig. 2); abscissae: time (in min) after onset of perfusion with 0.3 μM ^{3}H-noradrenaline. The symbols show "cumulative efflux plus metabolite content determined at end of experiment" for those experimental points that fell on a straight line (see fig. 2). These straight lines were extrapolated towards the abscissa. The intercepts with the abscissae show that 1) $t_{lag\ system}$ for the extraneuronally formed ^{3}H-NMN is very short in N and R, 2) $t_{lag\ system}$ for the neuronally formed ^{3}H-DOPEG and ^{3}H-DOMA is long (21 to 26 min), when the storage vesicles are intact (N), but much shorter (5.1 to 5.7 min), when vesicular storage has been impaired by pretreatment with reserpine. Thus, vesicular uptake greatly delays the approach of the intraneuronal concentration of the ^{3}H-amine to steady state. Shown are mean values from five experiments each (with S.E. as vertical bars). From Graefe (15).

formation of the metabolite has not yet reached steady state). Thus, figure 3A shows that $t_{lag\ system}$ for the formation of neuronal metabolites amounts to *at least* 21 min. On the other hand, when hearts are obtained from reserpine-pretreated cats (fig. 3B), $t_{lag\ system}$ for the deaminated metabolites is greatly reduced. Thus, vesicular uptake greatly slows the rise of the axoplasmic concentration of ^{3}H-noradrenaline, and a steady rate of metabolite formation is achieved only after a long delay. Such long delays make it virtually impossible to proceed with this analysis unless the duration of the experiment is greatly increased, but this is virtually impossible in experiments with isolated organs. However, when the delay is short in comparison with the rate constant for the efflux of the metabolite under discussion (as for extraneuronally formed metabolites or for neuronally formed metabolites after pretreatment with reserpine), the apparent rate constant for the efflux of the metabolite can then be obtained, namely by extrapolation of the linear part of the first-drawn curve (solid circles in fig. 2) to the abscissa. The horizontal distance between the two extrapolated parallels equals $t_{lag\ efflux}$; this, in turn, equals "1/k" (where k = rate constant for efflux of metabolite).

Thus, this type of analysis yields the two "lag periods" that characterize such systems: the first is determined by the rate at which the intracellular concentration of the parent amine approaches steady state, the second by the ease with which the metabolite leaves the cells.

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G. Calculation of k-Values from "Metabolite in Tissue in Per Cent of Total Formation"

As pointed out above, Levin (32) used this percentage value to demonstrate pronounced differences between the metabolites of ^{3}H-noradrenaline; metabolites with low rate constants should yield high percentage values, metabolites with high rate constants should hardly be retained at all by the tissue (and yield low percentage values). If two simplifying assumptions are made, such percentage values can be converted to apparent k-values. The two assumptions are: (a) the metabolite is formed at a steady rate throughout the incubation with the parent amine; and (b) the rate of efflux of the metabolite is constant throughout the incubation. Both assumptions are wrong. However, if we neglect this fact, we can write

$$k = (100\% - \text{Levin's percentage value})/t \cdot \text{Levin's percentage value} \quad (1)$$

(where t = duration of incubation in min). Equation 1 is equivalent to "rate/content."

As discussed earlier, the two assumptions are untenable. At least during the early part of the incubation, the rate of efflux must be below the steady-state rate of efflux, because (a) time is required for the intracellular concentration of the amine to reach steady-state, and (b) even if factor "a" did not exist, rates of efflux of the metabolite must approach steady state exponentially (in dependence on k). If this is so, we are justified in concluding that k-values calculated from equation 1 are, by necessity, *under*estimates of the true k-value. However, we are unable to specify the extent of the error (the size of which clearly depends on the magnitude of $t_{lag\ system}$ and $t_{lag\ efflux}$; see section II F). As will be shown below, useful information can be extracted from such calculations, although they are burdened with an inherent error.

H. Calculation of k-Values from "Metabolite in Tissue in Per Cent of Total Formation" When Time Course of Total Formation is Known

One error of the procedure described in section II G, namely $t_{lag\ system}$, can be avoided (a) if the compartment under study has a very short $t_{lag\ system}$ and (b) if the short $t_{lag\ system}$ can be determined accurately. Condition "a" is met by most extraneuronal systems [known to equilibrate quickly with the incubation medium or perfusion fluid (5, 18, 36)] and by neuronal systems, the storage vesicles of which have been impaired by pretreatment of the animals with reserpine (section II F). Condition "b" is met when the time course of the total formation of the metabolite is determined. We then regard $t_{lag\ system}$ as the actual beginning of the exposure to the parent amine.

The second error is due to the fact that the rate of efflux of the metabolite into the incubation medium cannot be constant throughout the incubation. However, Levin's percentage can be derived in the following way. If we deal with a one-compartment system, the metabolite content of the tissue at the time t is

$$C_t = (1 - e^{-k \cdot t})/k \quad (2)$$

provided that we assume that "rate of formation" = "steady-state rate of efflux" = unity. As pointed out above, t = duration of incubation minus $t_{lag\ system}$. Next, the total formation (TF) of the metabolite during an incubation for t minutes

$$TF = t \quad (3)$$

(again under the assumption that the rate of formation = 1). Thus, for Levin's percentage (i.e., tissue content in per cent of total formation) we can write

$$\text{Levin's percentage} = 100\,(1 - e^{-k \cdot t})/k \cdot t \quad (4)$$

This equation shows that Levin's percentage value declines (a) with increasing t and (b) with increasing k. This equation is rearranged to

$$k = 100\,(1 - e^{-k \cdot t})/t \cdot \text{Levin's percentage} \quad (5)$$

and it is solved for any experimentally determined Levin's percentage by the arbitrary selection of a likely k-value; iterative calculations are then repeated (with slightly altered k-values) until equality of the two sides of equation 5 is obtained.

I. General Comments Concerning Methods

Whenever apparent rate constants are determined as ratios (of "rate of efflux/tissue content" as in procedures described in sections II A and G), their accuracy depends critically on the accuracy of *two* measurements, rate of efflux and tissue content. If both values are so close to the methodological limits that both measurements are subject to appreciable error, the apparent rate constant becomes highly questionable. Such pairs of values should be discarded. This is the reason why, in table 2, various entries in the column "rate/content" have fewer numbers of observations than entries in the column "slope of regression line," and why, in table 3, the results obtained with O-methylated metabolites formed by the isolated adventitia (which has a very poor O-methylating capacity) are less accurate than other results. However, such pairs of values can justifiably be used in the procedure described in section II B. This is so because points determined very close to the limit of methodological error should lie close to either the ordinate or the abscissa. Thus, "suspect" points that lie close to the origin of both ordinate and abscissa confirm that the regression line intersects the abscissa very close to or at zero (even if such pairs of "suspect" values give aberrant apparent k-values, when "rate of efflux" is divided by "tissue content"). On the other hand, if such "suspect" points lie close to the abscissa but far away from the ordinate, they confirm the existence of a "bound fraction" (see above).

If only one of each pair of measurements is "too low for accurate determination," such values can yield useful information. For instance, while pairs that indicate

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"nearly zero rate of efflux associated with high tissue content" or "high rate of efflux associated with hardly measurable tissue content" cannot yield accurate apparent rate constants; two metabolites that give such results can very well be described as having a low and a high apparent rate constant, respectively. Such somewhat deficient pairs of values can be used for the procedure described in Section II B.

Finally, it should be realized that metabolites characterized by high rate constants are subject to a methodological error. If, after the end of the experiment, the weighing and the cutting of the tissue before homogenization takes time, a significant proportion of this metabolite can escape from the tissue. Hence, the rate constants of such metabolites can easily be overestimated. It should also be remembered that, during the time interval discussed here, metabolism of the parent amine may proceed after the end of the experiment, thus giving rise to overestimates of the "tissue content." Hence, for accurate determinations of apparent rate constant, it is advisable to cool the tissue immediately after the end of the experiment. However, this procedure does not solve the first-mentioned problem, since the efflux of metabolites is less temperature-sensitive than are the metabolizing enzymes.

III. The Rate Constants for the Efflux of Metabolites of Catecholamines and Phenethylamines

As mentioned in the INTRODUCTION, Levin (32) obtained evidence that, in rabbit aortic strips incubated with ^{3}H-noradrenaline for 30 min, the ease with which the ^{3}H-metabolites left the tissue decreased in the order MOPEG = DOPEG > NMN ≫ DOMA = VMA. Four questions arise: (a) Is the same ranking order obtained in other peripheral tissues? (b) Can this ranking order be quantified by the determination of apparent rate constants for efflux? (c) How do the values compare that are obtained with the different procedures enumerated in section II? (d) Are there quantitative differences between apparent k-values determined under different experimental conditions, and why? It should be noted that in studies in which the column chromatographic procedure of Graefe et al. (16) was used to separate the metabolites from noradrenaline, VMA and MOPEG were obtained together as the OMDA fraction. Although it is possible to separate these two metabolites by a further step of the procedure, this step was omitted in some of the studies. In that case, results for the OMDA fraction were omitted.

A. Perfused Rat Hearts

All apparent k-values in table 2 were determined during perfusion of the hearts with ^{3}H-catecholamines. The rank order of the apparent rate constants for the metabolites of noradrenaline agrees with that of Levin (32). Moreover, the differences between the extremes (i.e., between MOPEG and VMA) are very pronounced, since the apparent rate constants for these two metabolites differ by a factor of 70. Table 2 also shows that, although different procedures yield somewhat different apparent k-values, such differences rarely exceed a factor of 2.

Apparent rate constants also were determined for the metabolites of other catecholamines. The O-methylated metabolite of isoprenaline (OMI) is characterized by a high apparent rate constant. Apparent rate constants for DOPET and DOPAC were determined in hearts obtained from rats pretreated with reserpine and FLA-63 (methylhomopiperazinylthiocarbonyl disulfide, an inhibitor of the beta-hydroxylation of dopamine) and in the presence of an inhibitor of COMT (U-0521; 3′,4′-dihydroxy-2-methyl propiophenone). Again, the apparent rate constant for the ethanol metabolite (DOPET) is considerably higher than that for the acid metabolite (DOPAC). However, it should be noted that the apparent rate constant for the efflux of DOPAC is closer to that of NMN than to that of the other acid metabolites (table 2) (3).

In rat hearts perfused with 5 μM ^{3}H-phenylephrine for either 40 or 120 min, the deaminated metabolites were separated from each other by column chromatography (42). Apparent rate constants for the efflux of the glycol (HOPEG) and acid (HMA) metabolites of phenylephrine were determined from measurements of "rate of efflux" and "tissue content" (procedure described in section II A). Table 2 shows that the apparent rate constant for the efflux of the glycol metabolite (HOPEG), like that for the metabolites of noradrenaline and dopamine, is considerably higher than that for the corresponding acid metabolite (HMA).

B. Perfused Cat Hearts

Apparent rate constants were determined as described for rat hearts (15). Although differences between apparent k-values were not very pronounced, that for DOPEG was clearly higher than those for NMN and DOMA. Moreover, values for DOPEG and NMN were roughly similar to those obtained with rat hearts (table 2). The columns "approach to steady state" show for both DOPEG and DOMA that the approach to steady-state appearance of the metabolite in the venous effluent was biphasic, so that two distribution compartments were identified (with clearly differing apparent k-values). This will be discussed further in section V.

C. Perfused Rabbit Hearts

The experimental conditions were the same as described for rat and cat hearts. As for rat hearts, the apparent rate constants for the efflux of DOPEG and DOMA differed considerably. Interestingly, the apparent rate constant for the efflux of NMN was influenced by the absence or presence of cocaine in the perfusion fluid; when neuronal uptake was inhibited, the apparent rate constant for the efflux of NMN was higher than in its absence (table 2). Apparently, the neuronal uptake mech-

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TABLE 2
Apparent rate constants for the efflux of various metabolites

	Rate/Content*		Slope of Regression†		Approach to Steady State of Efflux‡				Decline after U-0521§	
	n	k (min^{-1})	n	k (min^{-1})	n	k_1 (min^{-1})	n	k_2 (min^{-1})	n	k (min^{-1})
Perfused rat hearts										
DOPET (3)[‖]	12	2.91(2.13;3.98)								
OMI (2, 4, 25)	67	0.52(0.51;0.54)	65	0.52	25	0.52(0.46;0.58)			6	0.58(0.56;0.61)
HOPEG (42)	13	0.51(0.42;0.63)								
MOPEG (13)	5	0.49(0.45;0.53)			5	0.23(0.10;0.50)				
DOPEG (13)	30	0.35(0.30;0.41)	30	0.38	9	0.17(0.14;0.22)				
NMN (13)	26	0.19(0.17;0.21)	26	0.16	9	0.13(0.10;0.17)				
DOPAC (3)	12	0.077(0.054;0.11)								
HMA (42)	13	0.025(0.020;0.031)								
DOMA (13)	30	0.016(0.008;0.032)	30	0.035						
VMA (13)	5	0.007(0.005;0.010)								
Perfused cat heart										
DOPEG (15)	40	0.54(0.45;0.64)	50	0.38	5	0.45(0.38;0.55)	5	0.059(0.038;0.093)		
NMN (15)	45	0.14(0.13;0.15)	75	0.12	19	0.20(0.17;0.23)			6	0.14(0.10;0.18)
DOMA (15)	42	0.15(0.12;0.19)	52	0.097	5	0.31(0.25;0.38)	5	0.050(0.042;0.059)		
Perfused rabbit heart										
DOPEG (15)	37	0.72(0.65;0.80)	44	0.62	4	0.57(0.27;1.19)				
NMN (15)	25	0.058(0.051;0.065)	25	0.057						
NMN COC (15)	14	0.18(0.15;0.21)	14	0.098	3	0.52(0.27;1.00)				
DOMA (15)	34	0.055(0.047;0.063)	35	0.063	4	0.52(0.28;0.94)	4	0.033(0.013;0.086)		
Rabbit aortic strips										
MOPEG (21)	18	0.035(0.023;0.053)	33	0.014						
DOPEG (21)	160	0.047(0.041;0.056)	246	0.032						
NMN (21)	52	0.029(0.024;0.036)	173	0.033						
DOMA (21)	181	0.0044(0.0040;0.0048)	250	0.0037						
VMA (21)	16	0.0042(0.0036;0.0049)	31	0.0034						
Dog saphenous vein strips[¶]										
DOPEG	9	0.037(0.024;0.059)								
NMN	3	0.014(0.003;0.068)								
MN	3	0.011(0.008;0.015)								
DOMA	9	0.0025(0.0021;0.0029)								
Cat nictitating membrane[#]										
DOPEG	21	0.062(0.045;0.077)								
NMN	21	0.0089(0.0058;0.014)								
DOMA	21	0.0021(0.0015;0.0030)								

Apparent rate constants were determined either as "rate of efflux of metabolite/metabolite content of tissue" (rate/content), or as the slope of the regression line of the plot "rate of efflux of metabolite" against "metabolite content of tissue" (slope of regression), or from the approach of the appearance of the metabolite in the venous effluent to steady state (note that *two* apparent rate constants are presented whenever the approach to steady state was biphasic), or from the decline of the rate of appearance of the metabolite in the venous effluent after inhibition of COMT by U-0521. Presented are geometric means of apparent constants (with 95% confidence limits in parentheses) together with the number of observations. Apparent k-values were determined in the following tissues and under the following experimental conditions: Rat hearts were perfused either with various concentrations of ^{3}H-isoprenaline, 1 μM ^{3}H-noradrenaline, 1 μM ^{3}H-dopamine, or 5 μM ^{3}H-phenylephrine, and rate constants were determined after perfusion for 15 to 30 min; rabbit and cat hearts were perfused with 0.3 μM ^{3}H-(−)-noradrenaline, and rate constants were determined after perfusion for 120 min; rabbit aortic strips were loaded with 1 μM H-noradrenaline for 30 min and then washed out with amine-free solution for 110 to 250 min (in most experiments the washout period was closer to 4 than to 2 hours); strips of dog saphenous vein were exposed to 0.23 μM of either ^{3}H-noradrenaline or ^{3}H-adrenaline for 60 min and then washed out with amine-free solution for 215 min (note that apparent k-values for DOPEG and DOMA obtained after loading with either catecholamine were pooled).

* Procedure described in section II A.

† Procedure described in section II B.

‡ Procedure described in section II E.

§ Procedure described in section II C.

‖ Reference numbers are in parentheses.

¶ Calculated from the raw data of Paiva and Guimaraes (41).

\# Calculated from the raw data of Luchelli-Fortis and Langer (unpublished observations).

anism is able to influence apparent rate constants for the efflux of NMN by affecting rates of efflux and/or the NMN content of the tissue.

It is interesting that such a phenomenon was not observed for cat hearts (results not shown in detail in table 2, but comparison of apparent rate constants determined in the absence or in the presence of cocaine did not reveal any difference; hence, the values appearing in table 2 stem from pooled results). In the hearts of both species, NMN is formed exclusively in the extraneuronal

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tissue; however, steady-state rates of extraneuronal formation of ³H-NMN were 10 times higher in cat hearts than in rabbit hearts, when hearts were perfused with 0.3 μM ³H-noradrenaline (in the presence of cocaine to inhibit neuronal uptake) (15).

The columns for "approach to steady state" (table 2) show that DOMA distributed into two kinetically differing compartments. This will be discussed further in section V.

D. Rabbit Aortic Strips

In these experiments strips were first loaded with ³H-noradrenaline and then washed out with amine-free solution, usually for 4 hours. At the end of the washout period, both the rate of efflux of the metabolite and the metabolite content of the tissue were determined (21). It is obvious from table 2 that the ranking order of the apparent k-values of the different metabolites is quite similar to that observed by Levin (32) for the same tissue, but all apparent k-values were considerably lower (by a factor of nearly 10) than those determined in perfused hearts. This quantitative difference will be discussed below.

E. Dog Saphenous Vein Strips

Strips were first loaded with either ³H-noradrenaline or ³H-adrenaline and then washed out with amine-free solution for 215 min. Apparent rate constants were obtained from "rate of efflux/metabolite content of tissue"; for DOPEG and DOMA results were pooled for both series of experiments. Table 2 shows that the ranking order (DOPEG > NMN > DOMA) is as expected, that the values were as low as those obtained for rabbit aortic strips, and that the apparent rate constant for MN is quite similar to that for NMN.

In other experiments, Paiva and Guimaraes (41) also determined the value for "metabolite in tissue in percent of total metabolite formation" during 30 min of incubation with either ³H-noradrenaline or ³H-adrenaline. Table 3 compares these values with those calculated by Levin (32) for the whole aorta. Although the values for the apparent rate constant of any given metabolite differ somewhat from column to column, it is evident that percentage values for glycol metabolites are lower than those for acid metabolite, and also that there are no very pronounced discrepancies between the two sets of results.

F. The Isolated Nictitating Membrane of the Cat

The isolated medial muscle was exposed to 0.5 μM ³H-(±)-noradrenaline for 30 min and then washed out with amine-free solution for 220 min. During washout the adrenergic nerves were stimulated electrically twice (after 138 and 200 min of washout, respectively), but this is immaterial for apparent rate constants for the efflux of the metabolites determined at the end of the experiment (18 min after the last period of nerve stimulation). The apparent rate constants were determined from the last 2-min sample of the efflux and the metabolite content of the tissue (procedure described in section IIA). In 10 of the 21 experiments, 0.29 μM phentolamine was present in the washout solution from the 158th min of washout onwards; in five experiments 3.12 μM phenoxybenzamine was present; in six control experiments no drug was added (M.-A. Luchelli-Fortis and S. Z. Langer, unpublished observations). Since the presence of either alpha-adrenoceptor antagonist failed to affect the apparent rate constants, all results were pooled. Table 2 shows that the ranking order of the apparent rate constants was DOPEG > NMN > DOMA. Moreover, the apparent rate constants were similar to those obtained with vascular smooth muscle (washed out for a considerable period) rather than to those determined in perfused hearts (table 2).

G. Conclusions

At the end of this section, we reach two conclusions of general importance. First, if we disregard minor differences, which may well be due to methodological errors,

TABLE 3

Metabolite content of the tissue (expressed in per cent of total metabolite formation) in rabbit aorta and dog saphenous vein

Metabolite	Rabbit Aorta			Dog Saphenous Vein
	A Isolated adventitia*	B Isolated media†	C Whole aorta	D Whole vein
MOPEG	(12.4 ± 0.9%)‡	11.4 ± 1.1%	20.8 ± 1.2%	
DOPEG	11.8 ± 0.7%	10.9 ± 1.8%	13.1 ± 0.6%	19.9 ± 1.8% (n = 8§)
NMN	(25.5 ± 6.9%)	20.4 ± 1.2%	40.2 ± 2.1%	14.7 ± 0.9% (n = 4)
MN				17.2 ± 1.0% (n = 4)
DOMA	72.6 ± 3.4%	61.1 ± 6.9%	73.8 ± 3.3%	39.8 ± 2.3% (n = 8)
VMA	(34.8 ± 4.8%)	69.9 ± 5.1%	59.6 ± 3.8%	

Strips of tissue were incubated with either 0.3 μM ³H-noradrenaline (A, B, C) or with 0.23 μM ³H-noradrenaline or 0.23 μM ³H-adrenaline (D) for 30 min. Metabolites were determined in the incubation medium and in the strips. Results (A, B, C; n = 5 or 6) were taken from Levin (32; fig. 4) or provided by Guimaraes from the results of Paiva and Guimaraes (41) 1978 (D).

* Metabolism in this preparation is predominantly neuronal.

† Metabolism in this preparation is virtually entirely extraneuronal.

‡ Since the isolated adventitia forms very small amounts of O-methylated metabolites, these percentage values are less accurate than the others.

§ Results obtained with both ³H-catecholamines were pooled.

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we find the same ranking order of the apparent rate constants for the efflux of metabolites in all peripheral organs studied thus far: glycol metabolites > NMN and MN ≫ acid metabolites. Moreover, for the rat heart, we can add that the apparent rate constant for the efflux of OMI is at least as high as that of the glycol metabolites, and that the glycol and acid products of the deamination of phenylephrine and dopamine conform with the other glycol and acid metabolites. In section IV, an attempt will be made to relate the ranking order of apparent k-values to the apparent lipophilicity of the metabolites. Second, experiments with rabbit and cat hearts provided the first evidence that at least some metabolites distribute into at least two compartments. This problem as well as the pronounced quantitative differences between the apparent k-values obtained with hearts perfused with ^{3}H-catecholamines and those obtained with strips of smooth muscle washed out for 3 to 4 hours remain to be discussed after the section dealing with apparent lipophilicity.

IV. The Apparent Lipophilicity of the Catecholamine Metabolites

A. The n-Octanol/Water Distribution Coefficient D (at pH 7.4)

Mack and Bönisch (35) determined the *n*-octanol/water distribution coefficient at pH 7.4 (D) for a variety of catecholamines and their metabolites (table 4). The O-methylation of catecholamines increased D by a factor of 2 to 3. While D is much higher for the glycol metabolites than for the parent catecholamies, the opposite is true for the acid metabolites. Thus, for most of the catecholamine metabolites, we obtain a ranking order (for D) of glycol metabolites > O-methylated catecholamines (except OMI, which has a very high D) ≫ acid metabolites.

B. The Correlation between Log D and Log k

To determine the correlation between these two parameters, we used the apparent rate constants of all those catecholamine metabolites for which the distribution coefficient D had been determined (35) (table 4); moreover, we used the k-values obtained with the procedure described in section IIA. Apparent k-values were divided into two groups. First, we pooled all results obtained with rat, cat, or rabbit hearts perfused with ^{3}H-catecholamines (fig. 4, upper regression line). Second, we pooled all those results obtained with strips of smooth muscle (rabbit

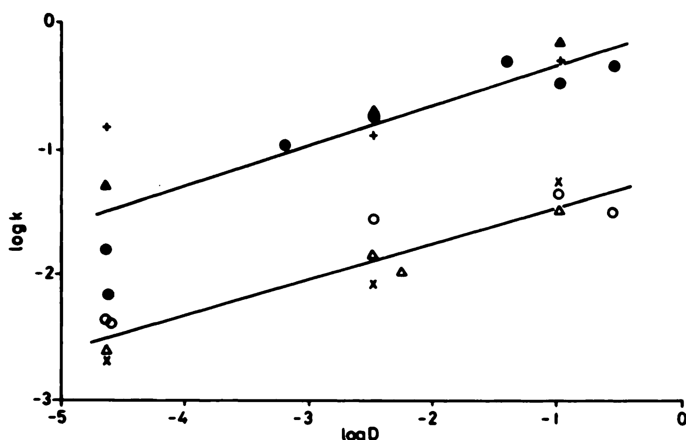

FIG. 4. Dependence of log k on log D for various catecholamine metabolites. Ordinate: log k (the k-values having been taken from table 2, column "rate/content"). Abscissa: log D (taken from table 4). Shown are results obtained with rat (●), rabbit (+), and cat hearts (▲) during perfusion with ^{3}H-catecholamines (upper regression line) and with rabbit aortic strips (○), dog saphenous vein (△), and cat nictitating membrane (×) first loaded with ^{3}H-catecholamines and then washed out with amine-free solution for 2 to 4 hours (lower regression line). For $y = a + bx$, upper regression line: $a = 0.039$, $b = 0.3299 \pm 0.0597$, $r = 0.8573$, $n = 13$, $P < .001$; lower regression line: $a = -1.097$, $b = 0.3033 \pm 0.0339$, $r = 0.9427$, $n = 12$, $P < .001$.

TABLE 4

n-Octanol/water distribution coefficients (D) of various catecholamines and catecholamine metabolites

		R₁*	R₂	R₃	R₄	log D†
Methoxyhydroxyphenylethanol	MOPET	OCH_3	OH	H	CH_2OH	0.47
Methoxyhydroxyphenylglycol	MOPEG	OCH_3	OH	OH	CH_2OH	−0.58
Dihydroxyphenylglycol	DOPEG	OH	OH	OH	CH_2OH	−1.01
O-methylisoprenaline	OMI	OCH_3	OH	OH	$CH_2—NH—CH<^{CH_3}_{CH_3}$	−1.44
Isoprenaline	ISO	OH	OH	OH	$CH_2—NH—CH<^{CH_3}_{CH_3}$	−1.88
O-methyldopamine (methoxytyramine)	MT	OCH_3	OH	H	$CH_2—NH$	−2.22
Metanephrine	MN	OCH_3	OH	OH	$CH_2—NH—CH_3$	−2.27
Dopamine	DA	OH	OH	H	$CH_2—NH_2$	−2.48
Normetanephrine	NMN	OCH_3	OH	OH	$CH_2—NH_2$	−2.49
Adrenaline	A	OH	OH	OH	$CH_2—NH—CH_3$	−2.59
Methoxyhydroxyphenylacetic acid	HVA	OCH_3	OH	H	COOH	−2.75
Noradrenaline	NA	OH	OH	OH	$CH_2—NH_2$	−3.01
Dihydroxyphenylacetic acid	DOPAC	OH	OH	H	COOH	−3.20
Methoxyhydroxymandelic acid	VMA	OCH_3	OH	OH	COOH	−4.61
Dihydroxymandelic acid	DOMA	OH	OH	OH	COOH	−4.63

* R_1, substitution in meta-position; R_2, substitution in para-position; R_3, substitution on beta-carbon; R_4, alpha-carbon with substituents.
† Log D values taken from Mack and Bönisch (35).

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aorta, dog saphenous vein, cat nictitating membrane) first exposed to ^{3}H-catecholamines and subsequently washed out with amine-free solution for 2 to 4 hours (fig. 4, lower regression line). For both groups of results the correlation was highly significant ($P < .001$); apparent rate constants increased with increasing D. Virtually the same correlation was obtained for the seven catecholamine metabolites studied in the perfused rat heart ($r = .95$; $P < .001$; $n = 7$). Moreover, when we used the apparent rate constants determined with the procedure described in section IIB, the correlation for all perfused hearts was also significant ($r = .92$; $P < .001$; $n = 10$).

Mack and Bönisch (35; see reference for details) determined not only the *n*-octanol/water distribution coefficient at pH 7.4 (D) but also the partition coefficient P (i.e., the *n*-octanol/water distribution coefficient for the unionized metabolite molecule). Comparison of pairs of values for D and P (and inspection of table 4) clearly indicates that D is determined by two factors: (a) the degree of ionization at pH 7.4, and (b) the polarity of the hydroxy-, methyl-, and methoxy-groups. In the following we shall equate D (determined at physiological pH) with "apparent lipophilicity," which is largely determined by ionization (greatly differing degrees of ionization account for pronounced differences between glycol and acid metabolites), but which is also influenced by the nature and number of the substituents on the ring or on the side chain of the molecules (see table 4 for influence of hydroxy- or methoxy-groups of glycol metabolites).

Unfortunately, the values for apparent lipophilicity of some of the metabolites that appear in table 2 are unknown, so it was impossible to include them in figure 4. Nevertheless, these metabolites also appear to conform with the correlation. For instance, the results of table 4 make it very likely that the apparent lipophilicity of the glycol and acid metabolites of both dopamine and phenylephrine should be higher than that of the corresponding metabolites of noradrenaline, since the former metabolites lack either the para-OH or the beta-OH group. Hence, it is of interest to note that the apparent rate constants for the efflux of the dopamine metabolites are higher than those for the noradrenaline metabolites, and that they are higher by about the same factor (DOPAC, 6.35 times higher than DOMA; DOPET, 7.36 times higher than DOPEG; see table 2). The same holds true for the metabolites of phenylephrine (HMA, 1.56 times higher than DOMA; HOPEG, 1.45 times higher than DOPEG). Thus, there are no obvious exceptions from the correlations depicted in figure 4.

The available evidence supports the view that apparent rate constants for the efflux of metabolite are strictly correlated with apparent lipophilicity (determined at pH 7.4); hence, efflux of such metabolites from their neuronal and/or extraneuronal cells of origin seems to be largely or totally dependent on apparent lipophilicity. If rate constants are largely determined by apparent lipophilicity, the optical rotation of stereoisomers should not influence k-values. Indeed, Henseling et al. (21) were unable to detect any differences between the rate constants determined for the metabolites of (−)-, (+)-, and (±-^{3}H-noradrenaline (rabbit aortic strips). For this reason, no attempt has been made in this review to specify the stereoisomers of amines or metabolites.

The fact that in figure 4 the slope of the regression line was not unity does not contradict this view. As pointed out by Leo et al. (31), any difference between the model system (*n*-octanol/water) and the biological membranes can result in a deviation of the slope of the regression line from unity. This argument makes it unlikely that the pronounced difference between the two nearly parallel regression lines of figure 4 is attributable solely to differences in the composition of the relevant biological membranes. For further discussion of this point, see below.

The highly significant correlations of figure 4 do not support the proposal of Muldoon et al. (40) that glycol metabolites easily leave the tissue, because they are formed from "superficially bound noradrenaline (drug-receptor interaction)." Although it will be necessary to discuss the question of compartments (see section V), the pronounced differences between the rate constants for the efflux of glycol and of acid metabolites are most easily explained by the differences in apparent lipophilicity.

V. Evidence for the Distribution of the Metabolites into Multiple Compartments

The results presented in table 2 and figure 4 provided evidence for a marked difference between the apparent rate constants for the efflux of metabolites determined in perfused hearts, on the one hand, and in strips of smooth muscle, first incubated with labelled catecholamines and then washed out for 2 to 4 hours, on the other. It was first felt that this difference might be related to the fact that the first-mentioned tissues were perfused, while the latter were incubated with catecholamines. Henseling et al. (21) considered the possibility that redistribution of the metabolites after their efflux from the cells of origin might account for this difference. However, this explanation is most unlikely, since strips should have reached a "steady state of washout" after several hours of washout with amine-free solution: redistribution should have been complete (or nearly complete) at that late time.

The most likely explanation for the differences between the two sets of results is provided by the possibility of a distribution of some (or all) metabolites into more than one compartment. If this explanation were correct, relatively short exposures to ^{3}H-catecholamines (regardless of whether the organ was perfused or incubated) should yield apparent k-values that tend to be rather close to the k-value of the compartment with the higher k-value, while experiments involving prolonged washout of (perfused or incubated) tissues should tend to yield the k-values characteristic for the compartment(s) characterized by low k-values.

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In the following subsections, the evidence for multiple distribution compartments will be presented and discussed. We shall restrict our discussion to a consideration of the number and of the neuronal and/or extraneuronal location of the distribution compartments. It is far too early for any discussion of whether the compartments are arranged in parallel or in series.

A. Approach to Steady-State Accumulation of Metabolites of Noradrenaline in Rabbit Aortic Strips

As pointed out in section II D, the rate constant for efflux should determine the half time for the approach of the metabolite content of the tissue to steady state. Moreover, if the formation of the metabolite is subject to a pronounced $t_{lag\ system}$, the apparent k-value obtained from an analysis of the approach of the tissue content to steady state should underestimate the true k-value. Figure 4 of Henseling et al. (21) shows the time course of the accumulation of the acid metabolites of ^{3}H-noradrenaline in rabbit aortic strips exposed to a constant concentration of ^{3}H-noradrenaline. As a rough guess, the half time for the approach to steady state is about 40 to 60 min for the acid metabolites. On the other hand, conversion of the apparent k-value of table 2 (for rabbit aortic strips) to a half time yields about 160 min for the acid metabolites. It is obvious from this comparison that, at least for the acid metabolites, the approach to steady state of metabolite accumulation in the tissue is much faster than expected from the apparent k-value of table 2. This discrepancy would be explained if ^{3}H-DOMA and/or ^{3}H-VMA distributed also into a compartment characterized by such a high k-value that it escaped detection in experiments involving washout for up to 4 hours (table 2).

B. Calculation of k-Values from Levin's Percentage Values

In section II G it was pointed out that "tissue content in percent of total formation of metabolite" (determined at the end of an incubation of the tissue with ^{3}H-catecholamine) can be converted to an apparent k-value, although this apparent value represents an *under*estimate of the true k-value. Table 5 shows the apparent k-values calculated (as described in section II G) from the values appearing in table 3. Comparison with table 2 clearly shows that, although the values appearing in table 5 should have *under*estimated the true k, they are all higher than the values appearing in table 2. Thus, for both rabbit aorta and dog saphenous vein, apparent k-values estimated during a 30-min exposure to ^{3}H-catecholamines tend to be higher than apparent k-values obtained during late washout. Again, such results support the view that determinations during late washout might well fail to detect distribution compartments characterized by k-values higher than those shown in table 2.

As pointed out in section II H, a reliable apparent rate constant can be calculated from Levin's percentage value only if the time course of the total formation of the metabolite has been determined. M. Henseling (unpublished observations) incubated rabbit aortic strips (obtained from reserpine-pretreated animals; MAO was inhibited by treatment with pargyline) with 0.12 μM ^{3}H-noradrenaline for 5, 10, or 30 min. ^{3}H-NMN was determined both in the incubation medium and in the tissue. The $t_{lag\ system}$ was calculated from the time course of total formation of ^{3}H-NMN, and it amounted to 1.3 min. Levin's percentage values amounted to 72%, 53%, and 27%, respectively, after 5, 10, and 30 min of incubation. From these values the apparent rate constant for the efflux of ^{3}H-NMN was calculated as described in section II H. It amounted to 0.158 min^{-1} (geometric mean of three determinations).

Comparison of this value with those appearing in table 2 and figure 4 leads to two conclusions: (a) the apparent rate constant for the efflux of NMN from rabbit aortic strips is considerably higher during relatively short incubation of the strips with ^{3}H-noradrenaline than after prolonged washout; and (b) an apparent rate constant determined under these conditions resembles the apparent rate constants determined for perfused hearts. Thus, it is safe to conclude that the two regression lines of figure 4 do not reflect differences between perfused and incubated tissues, but rather a pronounced difference between "experiments involving a relatively short exposure to the parent amine" and "experiments involving prolonged washout." It is safe to conclude from this difference that virtually all metabolites distribute into

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TABLE 5
Apparent rate constants (in "min^{-1}") for the efflux of metabolites calculated from the data of table 3 (as described in section II G)

Metabolite	Rabbit Aorta			Dog Saphenous Vein
	A Isolated adventitia	B Isolated media	C Whole aorta	D Whole vein
MOPEG	(0.270)	0.292	0.159	
DOPEG	0.283	0.306	0.254	0.166
NMN	(0.128)	0.162	0.074	0.227
MN				0.193
DOMA	(0.021)	0.036	0.020	0.075
VMA	(0.089)	0.025	0.039	

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more than one compartment. This conclusion is also borne out by the fact that, in the experiments described in the preceding paragraph, the apparent rate constant for the efflux of ^{3}H-NMN from rabbit aortic strips decreased with time. It was 0.1895 min^{-1} after 5 min of incubation, 0.1658 min^{-1} after 10 min and 0.1256 min^{-1} after 30 min. This type of dependence on the duration of the incubation must be expected if the metabolite distributes into two kinetically differing compartments.

C. *Washout Experiments with Rabbit Aortic Strips*

If an acid ^{3}H-metabolite formed during an incubation with ^{3}H-noradrenaline has distributed into a single compartment, its efflux curve (during subsequent washout with amine-free solution) should show a monophasic exponential decline. This is so, because any further formation of this metabolite during washout should have very little influence on the efflux curve (for more detailed discussion, see section VIII C). However, figure 5 shows that the efflux of the acid metabolite DOMA was biphasic; during early washout, rates of efflux of DOMA declined very rapidly before the efflux curve became as flat as expected from the apparent k-value in table 2.

Acid metabolites are known to be eliminated from the brain by a probenecid-sensitive transport mechanism. Hence, it was of interest to see whether the presence of probenecid affects efflux curves for DOMA like those shown in figure 5, especially because of the possibility that *one* of the two compartments generating the efflux of DOMA might possess a probenecid-sensitive transport system. However, the efflux of DOMA was not altered by the presence of 100 μM probenecid (M. Henseling, unpublished observations). Hence, the high apparent rate constant characterizing the very early efflux of DOMA cannot be attributed to the existence of an active, probenecid-sensitive transport mechanism in this particular compartment.

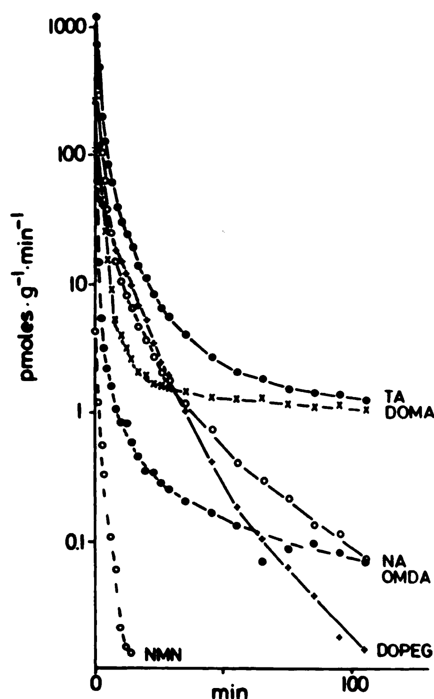

FIG. 5. Efflux of ^{3}H-noradrenaline and its ^{3}H-metabolites from nerve-free rabbit aortic strips (COMT inhibited by the presence of U-0521) initially incubated with 1.18 μM ^{3}H-noradrenaline for 30 min and then washed out with amine-free solution for 110 min. Ordinate: rate of efflux (in pmol·g^{-1}·min^{-1}; log scale); abscissa: time (in min) after onset of washout. Shown are results obtained with four strips. ●——●, total radioactivity (TA); ×- - -×, DOMA; ○——○, noradrenaline (NA); ●- - -●, OMDA-fraction; +——+ DOPEG; ○- - -○ NMN. From Henseling et al. (21).

D. *Analysis of Multiple Compartments from the Approach to Steady State of the Rate of Appearance of the Metabolite in the Venous Effluent*

Additional evidence for the existence of multiple compartments was obtained in rabbit and cat hearts perfused with a constant concentration of 0.3 μM ^{3}H-noradrenaline (15). The discussion is restricted to the hearts of reserpine-pretreated animals, since, after pretreatment with reserpine, the $t_{lag\ system}$ was very short in cat hearts (see fig. 3B) and virtually nonexistent in rabbit hearts. Thus, $t_{lag\ system}$ cannot have distorted the approach to steady state. Analysis of the time course of the approach to steady state of the appearance of the various metabolites in the venous effluent (see procedure described in section II E) gave clear evidence for a biphasic approach to steady state (i.e., for a distribution into two kinetically differing compartments); this was so for DOPEG (in the cat heart) and for DOMA (in cat and rabbit heart; see table 2). The results obtained with cat hearts are illustrated in figure 6. For NMN (cat and rabbit heart) and for DOPEG (rabbit heart), on the other hand, only *one* distribution compartment was identified.

E. *Evidence for Multiple Compartments; Perfused Rat Heart*

As pointed out in section II B, an apparent rate constant (determined as "rate of efflux/tissue content") should be independent of the duration of the exposure of the tissue to the parent amine when the metabolite is distributed into a single compartment. However, if more than one distribution compartment exists, apparent rate constants should decline with increasing duration of the experiment. This point is exemplified by experiments with rat hearts perfused with 5 μM ^{3}H-phenylephrine (42). The two deaminated metabolites were separated by column chromatography, and apparent rate constants were determined from "rate of efflux/tissue content" (procedure described in section II A). As was already evident from table 2, the apparent rate constant for the glycol metabolite was much higher than that for the acid metabolite. However, at least for the glycol metabolite (HOPEG), the apparent rate constant determined after 40 min of perfusion (0.630 min^{-1} n = 7) was significantly higher than that determined after 120 min of perfusion (0.402 min^{-1}; n = 6; $P < .01$); the two rate constants

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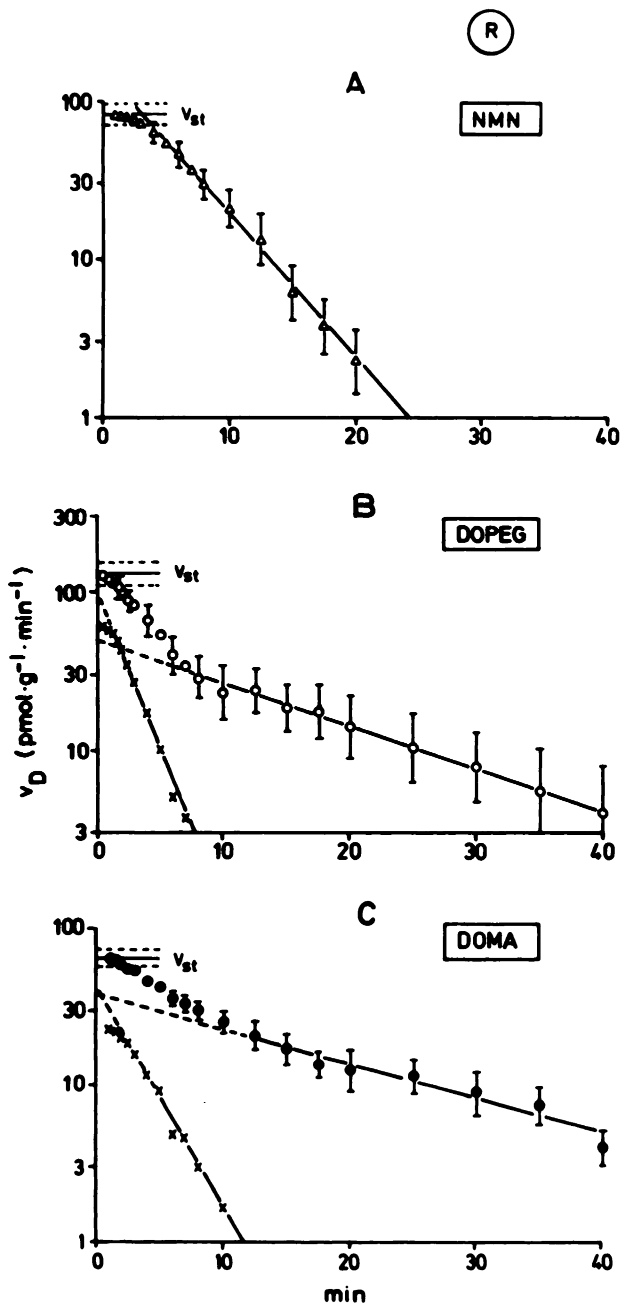

FIG. 6. Approach of rate of appearance of metabolite in venous effluent to steady state; cat hearts obtained from reserpine-pretreated animals and perfused with 0.3 μM ^{3}H-noradrenaline. Ordinates: "steady-state rate of appearance" minus "actual rate of appearance" of metabolite in venous effluent (in pmol·g^{-1}·min^{-1}; log scale); abscissae: time (in min) after onset of perfusion with ^{3}H-noradrenaline. Shown are geometric means (± S.E.) from five experiments. △, ○, and ● represent the experimental points for NMN (A), DOPEG (B), and DOMA (C), respectively, and the solid line connecting most of these points is the regression line giving the best fit when constructed from the last efflux sample. ×——× was obtained after "peeling," i.e., after subtraction of "calculated approach to steady state of the slowly equilibrating compartment" from the experimental points; these lines show the approach to steady state of the quickly equilibrating compartment. Note that the approach to steady state was monophasic for NMN and biphasic for DOPEG and DOMA. The k-values appear in table 2. This figure shows an initial delay for the approach to steady state. Most of this delay is due to the presence of a dead space in the apparatus, to the time required for the ^{3}H-amine to equilibrate the extracellular space, and to a distribution of the ^{3}H-metabolite into the fluid content of the cavities of the heart. Since a certain delay is unavoidable under these experimental conditions, no detailed analysis was carried out. From Graefe (15).

differed by a factor of 1.57. For the acid metabolite (HMA), on the other hand, a rather similar difference (by a factor of 1.44) did not reach the level of significance (0.0296 vs. 0.0205 min^{-1}, respectively; P > .05).

Since the deamination of ^{3}H-phenylephrine takes place both in the neuron and in the extraneuronal tissue, the two distribution compartments for HOPEG (see above) might well be associated with the neuronal and the extraneuronal tissue, respectively. To test this hypothesis, rat hearts were perfused with 5 μM ^{3}H-phenylephrine for 20 min in the presence of either 30 μM cocaine (to restrict the distribution of the parent amine to the extraneuronal tissue) or 87 μM corticosterone (to restrict the formation of the metabolite to the adrenergic nerve endings). Table 6 shows that the apparent rate constants for the *neuronal* efflux of HOPEG and HMA were slightly higher than those for the *extraneuronal* efflux (by factors of 1.54 and 1.09, respectively). However, in similar experiments with 1 μM ^{3}H-dopamine (3) the corresponding factors amounted to 1.15 for DOPET and to 0.70 for DOPAC (table 6). Thus, there are no consistent differences between apparent rate constants determined for neuronal and for extraneuronal efflux of metabolites. Hence, it is unlikely that the two compartments postulated above are associated with two morphologically different structures (i.e., with neuronal and extraneuronal tissues).

This view was supported by a further series of experiments in which rat hearts were again perfused with 5 μM ^{3}H-phenylephrine in the presence of 87 μM corticosterone. However, the duration of the perfusion was increased from 20 min (see above) to 120 min. Prolongation of the perfusion clearly reduced the apparent rate constants for the neuronal efflux of HOPEG and HMA to 0.354 and 0.0235 min^{-1}, respectively (i.e., by factors of 1.99 and 2.59, respectively; P < .05 for both metabolites). Thus, even when the formation of the metabolite is restricted to the adrenergic nerve endings, the metabolites distribute into at least two compartments each. Additional experiments carried out in the presence of 30 μM cocaine (to study the efflux of the metabolites from the extraneuronal tissue) also showed that the apparent rate constants for the efflux of HOPEG and HMA decreased with increasing duration of perfusion (42). Thus, the distribution of metabolites into at least two different compartments appears to be a general rule, irrespective of whether we deal with neuronally or extraneuronally formed metabolites. It might now be argued that an *extra*neuronal distribution of a neuronally formed metabolite is possible, just as is a *neuronal* distribution of an extraneuronally formed metabolite. While this might be true for the glycol metabolite (HOPEG; characterized

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TABLE 6

Comparison of apparent rate constants for the efflux of metabolites from neuronal and from extraneuronal cells of the perfused rat heart

Parent amine	Metabolite	Neuronal Efflux		Extraneuronal Efflux	
		n	k (min^{-1})	n	k (min^{-1})
Dopamine*	DOPET	6	3.117 (1.710;5.710)	6	2.719 (1.723;4.290)
	DOPAC	6	0.0645(0.0406;0.102)	6	0.0927(0.0457;0.188)
Phenylephrine†	HOPEG	6	0.703 (0.586;0.844)	7	0.456 (0.351;0.592)
	HMA	6	0.0609(0.0264;0.141)	7	0.0557(0.0400;0.0777)

Shown are geometric means (with 95% confidence limits) for apparent k-values calculated from "rate of efflux/metabolite content of tissue" (procedure described in section II A). In all experiments, "neuronal efflux" indicates that 87 μM corticosterone was present throughout the experiment (to inhibit extraneuronal uptake and deamination), while "extraneuronal efflux" indicates that 30 μM cocaine was present throughout (to inhibit neuronal uptake). All hearts were obtained from reserpine-pretreated animals.

* After perfusion with 1 μM ^{3}H-dopamine for 30 min; COMT was inhibited by the presence of U-0521 (3).

† After perfusion with 5 μM ^{3}H-phenylephrine for 20 min (42).

by a high k-value, i.e. well able to penetrate cell membranes), it is most unlikely for the acid metabolite, since the latter is nearly totally ionized at pH 7.4 (see the very low apparent rate constant; table 2). Unless there is active uptake of acid metabolites into adrenergic nerve endings and extraneuronal tissues (and there is no evidence for the existence of such a mechanism) a redistribution of the acid metabolite into a second intracellular distribution compartment must be regarded as highly unlikely.

F. Evidence for Multiple Compartments; Rabbit Aortic Strips

For rabbit aortic strips (first loaded with ^{3}H-noradrenaline and then washed out for up to 4 hours with amine-free solution) Henseling et al. (21) reported that apparent rate constants for the efflux of metabolites were not obviously affected by the presence of cocaine (to prevent any neuronal metabolite formation) or corticosterone (to prevent any extraneuronal metabolite formation). The results of a more recent attempt to determine whether apparent rate constants differ for the neuronal and extraneuronal efflux of DOPEG are shown in table 7 (M. Henseling, unpublished observations). In these strips ^{3}H-noradrenaline is deaminated both neuronally and extraneuronally; COMT was inhibited in these experiments by the presence of 100 μM U-0521 (U). When the strips (which were obtained from reserpine-pretreated animals, R) were loaded with the ^{3}H-amine in the presence of cocaine (to restrict the loading to the extraneuronal tissue, RUCOC), the apparent rate constant for the efflux of DOPEG (determined after 70 min of washout with amine-free solution) was 10 times higher than that for the efflux of DOPEG from strips loaded with ^{3}H-noradrenaline in the presence of corticosterone (to restrict the loading to the adrenergic nerve endings, RUC), irrespective of whether washout was carried out for 70 or 130 min. However, it would be erroneous to conclude that these results represent a difference between neuronal and extraneuronal efflux of DOPEG, since the neuronal efflux of DOPEG had a *high* apparent rate constant when neuronal MAO was partially inhibited by pargyline (RPUC, table 7).

TABLE 7

Apparent rate constants for the efflux of ^{3}H-DOPEG from rabbit aortic strips under a variety of experimental conditions

Experimental Condition*		n	Rate Constant (min^{-1})
RUCOC	70 min of washout	6	0.0959(0.0644;0.1428)
RUC	130 min of washout	6	0.0095(0.0066;0.0137)
RUC	70 min of washout	3	0.0140(0.0086;0.0226)
RPUC	130 min of washout	3	0.2398(0.0774;0.7429)

Strips were obtained from reserpine-pretreated animals (R) and COMT was inhibited by the presence (throughout the experiment) of 100 μM U-0521 (U).

* Experimental conditions: COC, in the presence of 30 μM cocaine throughout the experiment; C, in the presence of 87 μM corticosterone throughout the experiment; P, after pretreatment of the animal with pargyline (to inhibit MAO). All strips were incubated with 1 μM ^{3}H-noradrenaline for 30 min and then washed out with amine-free solution. Apparent rate constants were calculated from "rate of efflux of DOPEG at the end of experiment/DOPEG content of tissue." Shown are geometric means with 95% confidence limits (M. Henseling, unpublished observations).

These results must be explained in the following way. If the neuronal MAO is not inhibited (as in RUC strips), there is no accumulation of ^{3}H-noradrenaline in the axoplasm, and there is *no* formation of DOPEG during washout; during the 70 min of washout, the DOPEG originally distributed into the compartment characterized by the high k-value is virtually totally washed out, and we obtain (after 70 min of washout) a *low* apparent rate constant. However, if MAO is partially inhibited (RPUC), ^{3}H-noradrenaline accumulates in the axoplasm, and DOPEG is formed (at a low rate) throughout washout. Similarly, when COMT is inhibited (RUCOC), ^{3}H-noradrenaline accumulates in the extraneuronal tissue to such an extent that DOPEG is formed (extraneuronally) throughout washout. Irrespective of whether the formation of DOPEG is restricted to the neuron or to the extraneuronal tissue, any formation of DOPEG during washout goes hand in hand with a *high* apparent rate constant for the efflux of DOPEG. Such results indicate that at least the neuronally formed DOPEG distributes into two compartments.

The results discussed in this and the preceding section are clearly in agreement with the postulate that the

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difference between the two regression lines of figure 4 (the upper for K-values determined in hearts during perfusion with the parent ^{3}H-amine; the lower for various smooth muscle preparations subjected to 3 to 4 hours of washout with amine-free solution) must be ascribed to differences in experimental design, and not to any organ differences. We have to accept the fact that metabolites distribute into at least two kinetically different compartments (characterized by relatively high and low rate constants, respectively). It is evident from table 7 that not only the absolute length of the washout period is of importance, but also length of that washout period during which there is an appreciable formation of the ^{3}H-metabolite under study.

The existence of multiple distribution compartments complicates the analysis of the efflux of metabolites. However, it should be borne in mind that, in spite of these multiple distribution compartments, we obtain the *same* ranking order (glycol metabolites > NMN and MM >> acid metabolites) under virtually all experimental conditions, irrespective of whether they involve no washout, a short washout, or a long washout. Thus, the existence of multiple distribution compartments need not prevent us from discussing the behavior of "metabolites with high" and of "metabolites with low rate constant for their efflux."

VI. Central Nervous System

It is well known that various catecholamine metabolites are eliminated from the brain in situ by a probenecid-sensitive (and, hence, probably carrier-mediated) mechanism. This has been reported for VMA and MOPEG-sulphate (37, 38) as well as for HVA and DOPAC [although probably only after conjugation of these metabolites (43, 11, 9)]. Since most peripheral tissues seem to lack this transport system, this type of transport mechanism will not be discussed here. However, results obtained with *slices* of brain tissues are relevant to the topic of this review.

Cubeddu et al. (8) first loaded slices of rat striatum with ^{3}H-dopamine and then washed the slices with amine-free solution for 170 min. The apparent rate constants for the efflux of DOPAC and of the OMDA fraction were determined as "rate of efflux/tissue content." The results are shown in table 8. For the reasons stated in the following paragraph it is very likely that the OMDA fraction consists predominantly of HVA.

K. Starke and A. Zumstein (unpublished observations) laded slices of the head of the caudate nucleus of the rabbit with ^{3}H-dopamine and then washed them with amine-free solution for 65 min. Table 8 shows that the apparent rate constants for the efflux of DOPAC and the OMDA fraction were similar to but slightly lower than those for efflux from rat striatal slices. More important, the ratio "apparent k for DOPAC/apparent k for OMDA" was the same in both tissues. Starke and Zumstein obtained chromatographic evidence for the view that the OMDA fraction consists overwhelmingly of HVA and contains no conjugate.

Interestingly enough, both groups of workers found that probenecid did not significantly affect the apparent rate constants for the efflux of DOPAC and of the OMDA-fraction. Thus, although the available evidence concerning the efflux of metabolites from brain slices is very slender, it is in agreement with the results obtained with efflux from peripheral tissues; (a) the apparent rate constant for the efflux of DOPAC from brain slices (table 8) is of the same order of magnitude as that for efflux from the perfused rat heart (table 2); (b) just as the *n*-octanol/water distribution coefficient at pH 7.4 (D) is higher for HVA than for DOPAC (table 4), the apparent rate constant for the efflux of HVA (the predominant or exclusive metabolite of the OMDA fraction of table 8) from brain slices is higher than that for DOPAC (table 8). Just as in peripheral tissues, probenecid-sensitive transport systems do not seem to be involved in efflux from brain slices.

VII. Is the Efflux of Metabolites Carrier-Mediated?

The correlation between apparent lipophilicity of catecholamine metabolites and their apparent rate constants for efflux suggests that these metabolites leave the neuronal or extraneuronal cells (in which they are formed) by passive diffusion, the extent of which depends on apparent lipophilicity. It might be argued that the linear plots obtained with the procedure described in section II B support this view, since the linearity of such plots excludes saturability of efflux. Moreover, the apparent absence of probenecid-sensitive mechanisms in

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TABLE 8
Apparent rate constants for the efflux of some metabolites of ^{3}H-dopamine from striatal slices*

	DOPAC		OMDA-fraction	
	n	k	n	k
Rat striatal slices†	4	0.0485 ± 0.037	4	0.0737 ± 0.010
Slices of head of caudate nucleus of rabbit‡	6	0.0253 (0.0216; 0.0298)	6	0.0381 (0.0365; 0.0399)

* Calculated from "rate of efflux/tissue content" (procedure described in section II A) (presented in "min^{-1}"). For experimental details, see text.

† From Cubeddu et al. (8); presented are arithmetic means ± S.E.

‡ Provided by K. Starke; presented are geometric means with 95% confidence limits.

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the efflux of acid metabolites (see section V C) further argues against the involvement of carrier-mediated mechanisms.

On the other hand, it should be remembered that O-methylated catecholamines are not only inhibitors (24, 39, 6) but also substrates of extraneuronal uptake (44, 48). Hence, it is possible that the carrier responsible for the inward transport of the O-methylated catecholamines also effects their outward transport, provided that the K_m of the carrier for outward transport is so high that, in the plots described in section II B, the intracellular concentration of the O-methylated catecholamine did not cause saturation of efflux. In that case, the apparent linearity of the plots cannot be used as an argument against the involvement of a carrier.

First experiments with ^{3}H-NMN provided evidence for a corticosterone-sensitive extraneuronal uptake of this amine in the perfused heart, but the presence of corticosterone in the washout solution (used after an initial loading of the heart with ^{3}H-NMN in the absence of corticosterone) did not decrease the apparent rate constant for the efflux of ^{3}H-NMN (48).

More recent experiments, however, gave somewhat different results. When rat hearts were first loaded with ^{3}H-isoprenaline (in the absence of corticosterone) and then washed out with amine-free solution, the presence of corticosterone in the washout solution reduced the apparent rate constant for the efflux of ^{3}H-OMI from 0.568 min^{-1} to 0.493 min^{-7} (7). Although this effect was small, it was statistically significant ($P < .02$). Thus, at least in the perfused rat heart, OMI leaves the extraneuronal cells not only by lipophilic diffusion but also (although to a small extent) by carrier-mediated efflux.

VIII. Consequences of the Differences between the Rate Constants for the Efflux of Metabolites

Since we find the same ranking order of the apparent k-values for metabolites in all peripheral organs thus far studied in some detail, and since the same ranking order is found during "ongoing metabolism" of the parent amine and during "washout of metabolite formed earlier in the experiment," the pronounced differences, especially between the apparent rate constants for glycol and acid metabolites, must be taken into account both in the design of experiments and in the interpretation of results. In this section, some aspects of this shall be considered.

Virtually all of the subsequent subsections deal with the efflux of metabolites during *short* experiments, and we define "short" as "duration of experiment < or = 2 half-times for efflux of metabolite under study." In such *short* experiments, the pattern of the efflux of the metabolites does not mirror the pattern of their formation in the tissue (see below). However, it should be clearly understood that this statement does *not* apply to experiments in which the efflux of metabolites is studied under steady-state conditions. It is obvious that, under steady-state conditions, rate of efflux of a metabolite = rate of formation of the metabolite. However, table 2 indicates that the half-times for the efflux of DOMA and VMA ($t/2 = \ln 2/k$) are of the order of 43 to 99 min in perfused rat hearts, and between 158 and 277 min for smooth muscles washed out for 2 to 4 hours. Thus, virtually all in vitro experiments with isolated preparations (survival restricted to a few hours) are by necessity "short" experiments (at least as far as these acid metabolites are concerned). Typical "short" experiments involve, for instance, (a) exposure of the isolated preparation to ^{3}H-amines for 30, 60, or 120 min, (b) the loading of adrenergic nerve endings with labelled amine, subsequent washout of the amine, and electrical stimulation of the nerves for a few minutes, but also (c) short periods of exercise or stress in volunteers or animals. The validity of the comments offered in the subsequent subsections is restricted to such "short" experiments.

A. *The Assessment of the Relative Importance of a Metabolic Pathway*

Since glycol metabolites and O-methylated catecholamines tend to escape quickly into the incubation or perfusion fluid because of their high rate constants, analyses of the metabolites in just the tissue necessarily lead to an overestimate of the relative importance of the acid metabolites (which are well retained by the tissue). As emphasized also by Langer (27), the true picture of the total metabolism of a catecholamine is obtained only by the determination of all metabolites in tissue and incubation (or perfusion) solution. As far as peripheral tissues are concerned, this problem can be solved by an appropriate experimental design. However, in studies of the metabolism of catecholamines in the central nervous system in situ, there is always the risk that the relative importance of metabolites may be underestimated, if they have high rate constants for efflux, and if they are carried away by the circulation.

It is of interest to note that the enzymes responsible for the formation of the glycol and acid metabolites are stereoselective, since the ratio "glycol/acid metabolites" is higher when (−)-noradrenaline is deaminated than after metabolism of (+)-noradrenaline (17, 20). The metabolic fate of dopamine resembles that of (+)-noradrenaline insofar as it is preferentially deaminated to the acid metabolites.

The following example may serve to emphasize the importance of measurements of metabolites in the venous effluent of a perfused rat heart. After 30 min of perfusion with 0.95 μM ^{3}H-(−)-noradrenaline (in the presence of 30 μM cocaine to restrict the uptake and metabolism of ^{3}H-noradrenaline to the extraneuronal tissues), the heart contained 1.9 nmoles·g^{-1} of unchanged ^{3}H-amine and 2.2 nmoles·g^{-1} of total ^{3}H-metabolites (13). These values are in good agreement with those of Lightman and Iversen (33), who found about equal amounts of ^{3}H-noradrenaline and ^{3}H-metabolites after 10 min of perfusion with 3 μM ^{3}H-noradrenaline (in the presence

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of 500 μM metaraminol, to inhibit neuronal uptake). However, during these 30 min of perfusion with 0.95 μM ^{3}H-noradrenaline, the total formation of ^{3}H-metabolites (determined in the venous effluent and in the heart tissue) amounted to 14.1 nmoles·g^{-1} (13). This pronounced difference is due to the fact that only slightly more than 10% of the extraneuronally formed metabolites are acid metabolites with low rate constants. Thus, if tissues form predominantly metabolites with high rate constants, the metabolic capacity of that tissue is most easily evident from analysis of the incubation or perfusion medium, while analysis of the tissue fails to provide impressive values. However, it should be emphasized that Lightman and Iversen (33) correctly interpreted their results as indicative of a considerable metabolic capacity of the extraneuronal tissue.

B. Is MOPEG Formed in the Neuron?

After loading of the isolated nictitating membrane of the cat with ^{3}H-noradrenaline, nerve stimulation causes an overflow not only of ^{3}H-noradrenaline but also of the various ^{3}H-metabolites, including the ^{3}H-OMDA fraction consisting of ^{3}H-MOPEG and ^{3}H-VMA (29, 28, 34) see also fig. 10). Since there is a much greater overflow of ^{3}H-DOPEG than of ^{3}H-DOMA, it is very likely that ^{3}H-MOPEG accounts for most of the OMDA-fraction of the overflow. If nerve stimulation is carried out in the presence of cocaine (to inhibit the reuptake of the released transmitter), the overflow of the ^{3}H-OMDA-fraction is greatly reduced, while it is not affected by the presence of an inhibitor of extraneuronal uptake [phenoxybenzamine (29), hydrocortisone (34)]. From such results it was concluded that the ^{3}H-OMDA fraction of the overflow must have been of purely neuronal origin, formed after the neuronal reuptake of the released transmitter (29).

An alternative explanation of these results is possible, if it is remembered that DOPEG can easily penetrate cell membranes, as indicated by its high rate constant for efflux. The following sequence of events is possible: neuronal reuptake of the released transmitter leads to the formation of DOPEG, which then rapidly leaves the nerve endings; while diffusing through the extracellular space, DOPEG is able to enter extraneuronal cells without the help of the corticosterone-sensitive extraneuronal uptake mechanism, and it is then O-methylated by extraneuronal COMT. However, if we consider the very low rate constant for the efflux of DOMA, it is most unlikely that this acid metabolite can enter extraneuronal cells.

This hypothetical scheme for the formation of MOPEG is of importance for our attempts to establish whether or not adrenergic nerve endings have COMT activity, a point that is still under discussion. Evidence for a cocaine-sensitive formation of VMA would, according to this hypothesis, constitute convincing evidence *for* the existence of neuronal COMT; however, evidence for a cocaine-sensitive formation of MOPEG cannot be decisive, since it can be interpreted in two different ways. Unfortunately, evidence for or against one of the possible hypotheses is lacking.

C. The Pattern of Efflux Curves for Metabolites During Simulated Washout

In many experiments, tissues are first loaded with a labelled catecholamine, and they are then washed out with amine-free solution, sometimes for up to 3 to 4 hours.

For the interpretation of the results of such experiments, it has to be realized that the pattern of the efflux curves for the metabolites is decisively influenced by the rate constants for the efflux of the metabolites. This is illustrated in figure 7. We used the mathematical model described by Bönisch et al. (4). The model compartment is loaded with an amount of parent amine to give an initial rate of efflux of 1 (arbitrary units·min^{-1}). The intracompartmental enzyme has kinetic constants that (a) result in an initial rate of formation of the metabolite of 2 (arbitrary units·min^{-1}) and (b) ensure that the enzyme is not even partly saturated by the intracompartmental concentration of the parent amine (i.e., the K_m is substantially higher than the intracompartmental amine concentration). Both the parent amine and the metabolite escape from the model compartment by diffusional flux, i.e., efflux obeys first-order kinetics and is determined by "k for amine" and "k for metabolite," respectively. Finally, it is assumed that the initial loading of the model compartment was long enough to reach steady state; as a consequence, the metabolite content of the model compartment is high enough to ensure an initial rate of efflux of the metabolite of 2 (arbitrary units·min^{-1}). The actual constants used in these calculations appear in the legend of figure 7. If "k for amine" = 0.0693 min^{-1}, and if k_{enzyme} (= $V_{max\ enzyme}/K_{m\ enzyme}$) = 0.1386 min^{-1}, the rate constant for elimination of the parent amine from the model compartment (k_{system}) = 0.0693 + 0.1386 min^{-1} = 0.2079 min^{-1} (corresponding to a half time of 3.33 min). Calculations were repeated for metabolites with k-values varying from 0.002079 to 2.079 min^{-1}. Figure 7A shows the efflux curves for amine and metabolite. When k for metabolite >> k_{system} (2.079 min^{-1} or higher) a straight efflux curve is obtained that is parallel to that for the amine; irrespective of the magnitude of k for metabolite, the efflux curve begins at 2 (arbitrary units·min^{-1}). If k for metabolite is decreased towards k_{system} (i.e., towards 0.2079 min^{-1}), it becomes parallel to the efflux curve for the amine after an initial convexity, which becomes the more pronounced the closer k for metabolite comes to k_{system} (not shown in fig. 7A). However, when k for metabolite < k_{system}, efflux curves eventually become linear but flat, after an initial convexity, the magnitude of which is directly related to k for amine. The final slope is directly dependent on k for amine. In other words, the slope of the efflux curve for the metabolite always approaches the *lower* one of the two possible

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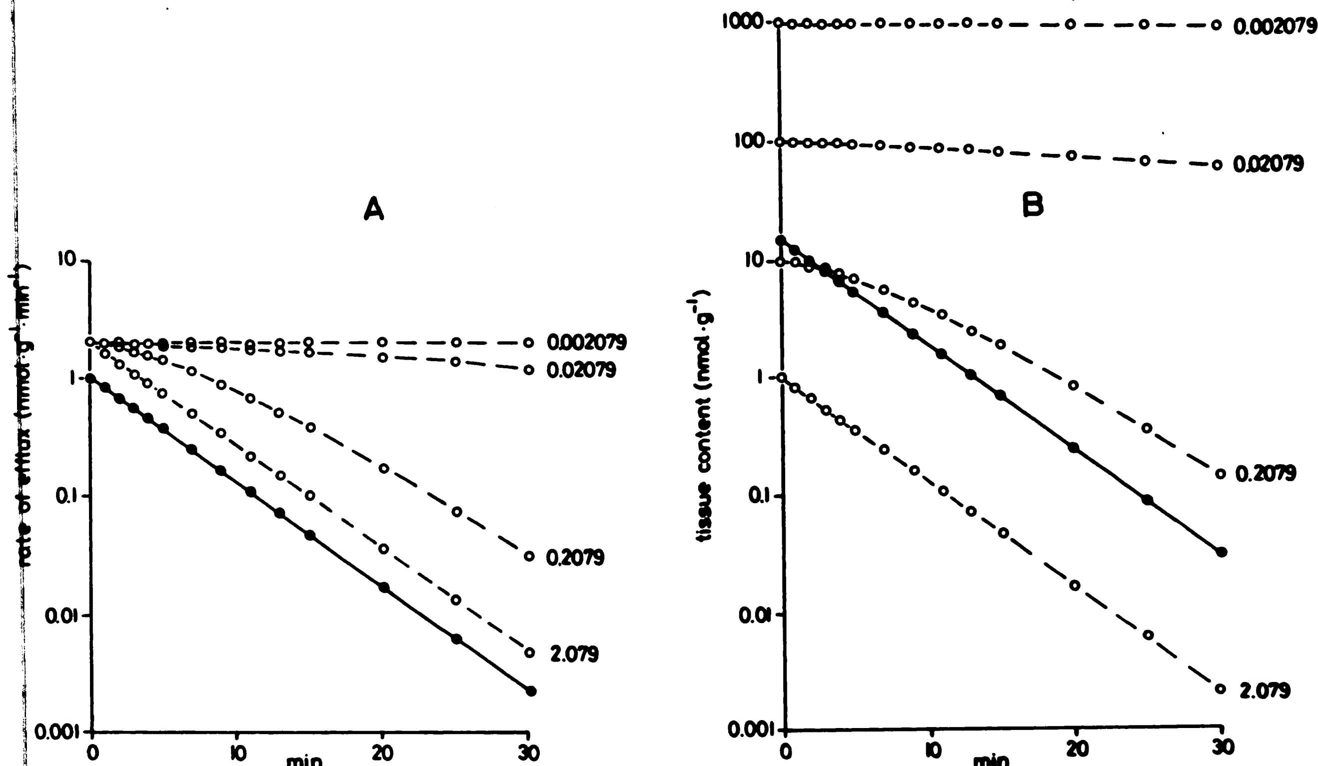

FIG. 7. Simulation of washout with a mathematical model. Ordinates: rates of efflux (in nmol·g^{-1}·min^{-1}; log scale) in A, tissue content (in nmol·g^{-1}; log scale) in B. Abscissae: time (in min) after onset of washout. Shown are curves for amine (●——●) and for metabolite (○- - -○). Results were calculated with the mathematical model described by Bönisch et al. (4), which consists of a single compartment in which the parent amine is metabolized by an enzyme; both parent amine and metabolite leave the model compartment by leakage (i.e., by a process obeying first-order kinetics). At the onset of washout the model was assumed to be in steady state. Thus, the amine content was calculated from "initial rate of efflux/k for amine" = 1/0.0693 = 14.43 nmoles·g^{-1}. The activity of the enzyme was arbitrarily selected to result in a rate of metabolism double the rate of efflux of the parent amine (i.e., $V_{max\ enzyme}/K_{m\ enzyme}$ = 0.1386 min^{-1}); $K_{m\ enzyme}$ was high enough to prevent any saturation of the enzyme ($K_{m\ enzyme}$ = 1000 μM, $V_{max\ enzyme}$ = 138.6 nmoles·g^{-1}·min^{-1}). The initial metabolite content was calculated from "initial rate of efflux of metabolite/k for metabolite." k for metabolite was varied from 0.002079 to 2.079 min^{-1} (as indicated on the graph). Calculations were carried out by numerical integration [for details, see Bönisch et al. (4)]. k_{system} (i.e., the rate constant for the disappearance of the parent amine from the model compartment by metabolism and by leakage) was 0.0693 + 0.1386 min^{-1} = 0.2079 min^{-1}. Note that the curves describing the efflux and the tissue content of the metabolites are parallel to the curves describing the parent amine, provided k for metabolite > k_{system}; however, if k for metabolite < k_{system}, the final slope of the curves is determined by k for metabolite.

determining k-values: k_{system} or k for metabolite. Figure 7B shows, for the same calculations, the amine and metabolite content of the tissue during simulated washout. Again, when k for amine > k_{system}, the curve for the metabolite became parallel to that for the parent amine (after an initial convexity if k for metabolite is close to k_{system}), while curves became linear but flat when k for amine < k_{system}. Since efflux curves are conventionally plotted with a log scale for the ordinates, the parallelism between the curves for the parent amine and those for metabolites with rate constants > k_{system} means that the ratios "rate of efflux of metabolite/rate of efflux of parent amine" (in fig. 7A) and "metabolite content/amine content of tissue" (in fig. 7B) are constant.

These model curves lead to important conclusions relevant to the interpretation of efflux curves. In the following, "high" and "low" rate constants for the efflux of a metabolite are defined as being "higher than k_{system}" and "lower than k_{system}," respectively. It follows from the results of figure 7 that, if we deal with a single compartment, the slopes of the efflux curves for metabolites with "high" rate constants directly reflect the fate of the parent amine in the compartment under discussion. Moreover, if efflux curves for amine and metabolite (with "high" rate constant) are parallel, we can conclude that amine efflux originates from that compartment in which the amine is also metabolized. For metabolites with "low" rate constants, on the other hand, efflux curves must be interpreted in a different way. Since the maximum slope of such an efflux is determined by the rate constant of the metabolite, such curves fail to reflect the fate of the amine in the compartment. Moreover, a "low" rate constant for the efflux of a metabolite can lead to the arbitrarily selected initial rate of metabolite efflux (of 2 arbitrary units·min^{-1} in fig. 7A) only after pronounced accumulation of the metabolite in the compartment during the initial loading (see the pronounced filling indicated in fig. 7B). As a consequence, efflux curves for metabolites with "low" rate constants reflect the formation of the metabolite during the initial loading much more than the formation of the metabolite during simulated washout. Hence, efflux curves for metabolites with

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"low" rate constants not only fail to reflect the fate of the amine during washout, they also fail to be greatly influenced by the formation of the metabolite during washout. Finally, figure 7B illustrates that the percentage contribution of the metabolite to the sum of parent amine plus metabolite recovered from the tissue increases with increasing duration of washout.

These considerations lead to the general conclusion that only efflux curves for metabolites with "high" rate constants permit any conclusions as to the fate of the parent amine in the single compartment under discussion. Efflux curves for metabolites with "low" rate constants cannot be interpreted in this way. In section VIII D, examples will be given from published analyses of the efflux of metabolites. In this context, it is important to understand that, as exemplified in figure 7, rate constants for metabolites are "high" or "low" relative to the k_{system} of the compartment under discussion. For instance, in "release experiments" (involving the initial loading of nerve endings with ^{3}H-noradrenaline and the subsequent determination of outflow of radioactivity from nerve endings during washout), k_{system} is very low, since it reflects the leakage of ^{3}H-noradrenaline from the storage vesicles into the axoplasm (29, 40). Under these conditions the glycol metabolites and the O-methylated catecholamines have *very* "high" rate constants. If, on the other hand, we analyse the efflux from extraneuronal compartments (usually characterized by a rather high k_{system}), k-values for the efflux of DOMA and VMA are, indeed, very "low." In short, rate constants for the efflux of metabolites have to be related to the k_{system} of the compartment under study.

It is also important to point out that the considerations of this section relate to systems that consist of a single compartment. In a two-compartment system, the efflux curve for a metabolite (characterized by a high rate constant) formed in compartment B may well be parallel to the efflux curve for the parent amine leaving compartment A, if only the efflux of the parent amine from compartment A leads to uptake of the parent amine into compartment B as the only source for metabolism. For instance, Henseling et al. (22) demonstrated for rabbit aortic strips (MAO inhibited, COMT intact; washout experiments after an initial loading) that efflux curves for ^{3}H-noradrenaline and ^{3}H-NMN were parallel; however, since the efflux of ^{3}H-NMN was corticosterone-sensitive, neuronal efflux of parent amine must have led to an extraneuronal formation of NMN. The alternative explanation, that corticosterone inhibited the efflux of ^{3}H-NMN, is highly unlikely, since Uhlig et al. (48) did not detect any significant effect of corticosterone on the efflux of ^{3}H-NMN from the extraneuronal tissue of the perfused rat heart.

D. The Influence of Rate Constants of Metabolites on Efflux Curves

Rat hearts were perfused with 0.1 μM ^{3}H-isoprenaline and 10 μM U-0521 (to inhibit COMT), so as to achieve the "filling" of a single compartment [compartment III; (4)]. On washout of the heart with amine-free solution, U-0521 was omitted from the perfusion fluid. Figure 8A shows that inhibition of COMT was rapidly reversible, since ^{3}H-OMI quickly appeared in the venous effluent. After about 10 min of washout the efflux curves for amine and OMI became parallel, as predicted in the preceding section for a metabolite characterized by a "high" rate constant for efflux. (See table 2 for apparent k-value for OMI.) It should be noted that a "filling" of compartment III with 0.1 μM ^{3}H-isoprenaline requires the presence of an inhibitor of COMT, since the enzyme activity of this compartment is so high that there is virtually no accumulation of unchanged isoprenaline when the enzyme is not inhibited. However, accumulation of unchanged amine *is* obtained (even without inhibition of COMT) when the concentration of ^{3}H-isoprenaline is increased to 0.3 μM, and when the initial perfusion lasts for 60 min. In fact, as illustrated in figure 8B, two compartments then fill with ^{3}H-isoprenaline. It is of interest to note that efflux of ^{3}H-OMI coincides with the efflux of ^{3}H-isoprenaline from the distribution compartment characterized by the higher k-value [early washout; this is "compartment III" of Bönisch et al. (6)], not with that from the distribution compartment characterized by the lower k-value [late washout; this is "compartment IV" of Bönisch et al. (6)]. Hence, such results clearly indicate that of the two distribution compartments, only compartment III has appreciable COMT activity. Finally, when rat hearts are initially perfused (for 30 min) with a very high concentration of ^{3}H-isoprenaline (23.8 μM), the efflux curve for ^{3}H-OMI remains horizontal for the first 40 min of washout (fig. 8C). Since corticosterone inhibits the efflux of ^{3}H-isoprenaline, and since it prolongs the plateau of the efflux of ^{3}H-OMI (fig 8D) such results are indicative of so pronounced a filling of compartment III that the intracompartmental concentration of ^{3}H-isoprenaline fully saturates COMT; hence, the horizontal part of the efflux curve for ^{3}H-OMI reflects a formation (and efflux) of the metabolite at the V_{max} of the enzyme, at least until the concentration of the parent amine falls enough to desaturate the enzyme (after > 40 min). These examples illustrate how efflux curves for a metabolite with a "high" rate constant permit us to draw conclusions concerning the fate of the parent amine in certain compartments.

If it is true that, as shown in figure 7A, the slope of the efflux curve for a metabolite with a "low" rate constant is determined by the rate constant, efflux curves for two metabolites with equally low rate constants (see table 2 for apparent rate constants of DOMA and VMA, rabbit aortic strips) should be parallel to each other. However, in the experiments of Henseling et al. (21, fig. 2) the efflux curve for VMA (during late washout) was clearly flatter than that for DOMA. Before the validity of the rate constants is questioned, it should be realized that the extraneuronal tissue of rabbit aortic strips contains both COMT and MAO. Hence, further metabolism of extraneuronal DOMA can take place during washout.

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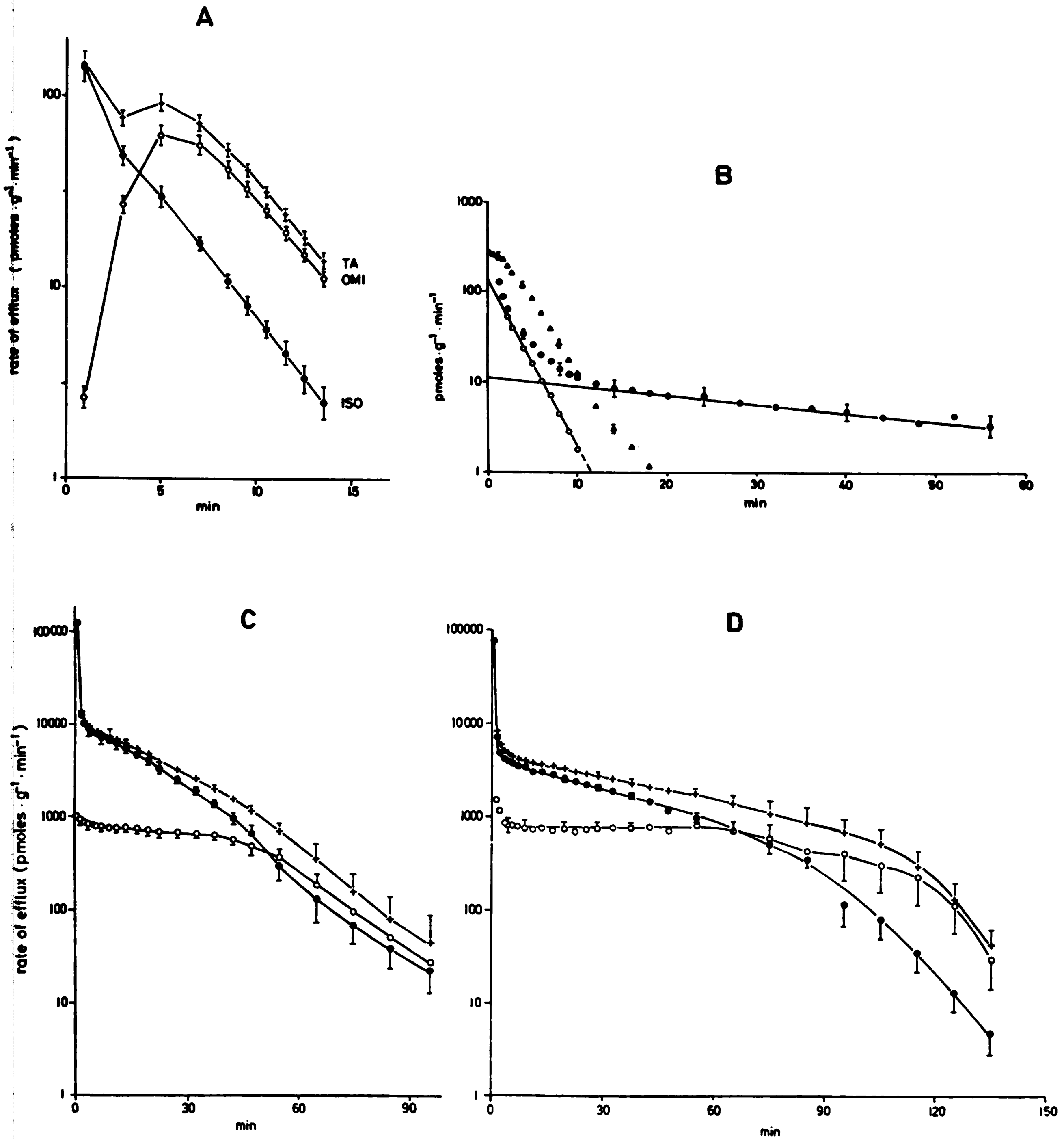

FIG. 8. Efflux curves for ^{3}H-OMI during washout (with amine-free solution) of rat hearts initially loaded with ^{3}H-isoprenaline. Ordinates: rate of efflux (in pmol·g^{-1}·min^{-1}; log scale); abscissae: time (in min) after onset of washout. A, Hearts were loaded with 0.1 μM ^{3}H-isoprenaline for 10 min in the presence of 10 μM U-0521 (to inhibit COMT). U-0521 was omitted from the washout solution. Note the quick disinhibition of the enzyme on washout, and also that efflux curve for OMI became parallel to that of ISO within about 10 min. Also shown is the efflux curve for total radioactivity (TA). Taken from Bönisch (2). B, Hearts were loaded with 0.3 μM ^{3}H-isoprenaline for 60 min (COMT intact throughout experiment). Note that the efflux of ^{3}H-ISO was biphasic (●) while the efflux of ^{3}H-OMI was monophasic (▲) and coincided with the "early" efflux of isoprenaline. The two lines (●, ○) indicate the efflux of isoprenaline from the two compartments. Taken from Bönisch (2). C, Hearts were loaded with 23.8 μM ^{3}H-isoprenaline for 30 min (COMT was intact throughout experiment). Shown are efflux curves for total radioactivity (+), ^{3}H-isoprenaline (●), and ^{3}H-OMI (○). Note that very pronounced filling of the COMT-containing compartment causes an efflux of ^{3}H-OMI that is nearly maximal for about 40 min. D, Same experimental conditions as in C, but 87 μM corticosterone was present in the washout solution. Note that corticosterone impaired the efflux of ^{3}H-isoprenaline (compare with C for 10th min of washout) and that corticosterone greatly prolonged the plateau of the efflux of ^{3}H-OMI. C and D, from Uhlig et al. (47).

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Indeed, Henseling et al. (21) showed that the difference between the slopes of these two efflux curves coincided with an O-methylation of ^{3}H-DOMA to ^{3}H-VMA during late washout. Thus, while efflux curves for metabolites with "low" rate constants are virtually unrelated to the fate of the parent amine in the tissue under study, their further metabolism or formation can have a small but noticeable effect on the efflux curves.

Further examples are provided by experiments of Starke and coworkers. The adrenergic nerve endings of rabbit hearts were first loaded by perfusion of the hearts with 1 μM ^{3}H-(−)-noradrenaline for 20 min; subsequently the hearts were washed out with amine-free solution. When MAO was inhibited either by a drastic reduction of the oxygen pressure in the perfusion fluid (fig. 9A) or by the addition of 10 μM amezinium to the perfusion fluid (fig. 9B), rates of efflux of total radioactivity and of ^{3}H-DOPEG declined, while rates of efflux of ^{3}H-DOMA were very little affected. Amezinium is a compound that is taken up by adrenergic nerve endings by the cocaine-sensitive uptake mechanism (uptake$_1$) and then inhibits neuronal MAO (30, 46). It should be noted that the results of figure 9 were obtained under the conditions of "prolonged washout," i.e., after nearly 90 min of washout. It is likely that the efflux of DOMA then originates predominantly from the distribution compartment characterized by a low rate constant (see section V); hence, the rate constant may well have been as low as that for rabbit aortic strips and dog saphenous vein (table 2). This consideration is important, since results obtained *during* a perfusion with 1 μM ^{3}H-(−)-noradrenaline were different. When cocaine was added to the perfusion fluid to block the neuronal uptake of the amine, rates of efflux of ^{3}H-DOMA fell quickly towards nearly zero (13, fig. 1B). Thus, when observations are made at a time when the distribution compartment for ^{3}H-DOMA with the high rate constant (see section V) plays an important role, a quick fall of the amine concentration in the relevant compartment (after inhibition of the uptake of this amine into the compartment) does affect the rate of efflux of ^{3}H-DOMA.

The results illustrated in figure 9 throw some doubt on the interpretation by Langer and Enero (28) of some of their results obtained with isolated nictitating membranes. After loading of the adrenergic nerve endings with ^{3}H-noradrenaline and subsequent washout of the tissue for 85 min, release of ^{3}H-noradrenaline was induced by nerve stimulation. While the overflow of ^{3}H-noradrenaline went hand in hand with an overflow of ^{3}H-DOPEG of considerable proportions, there was hardly any evoked overflow of ^{3}H-DOMA (fig. 10). The authors concluded that the exocytotic release of ^{3}H-noradrenaline (and subsequent neuronal reuptake of the released amine) leads to a formation of ^{3}H-DOPEG, but not of ^{3}H-DOMA. In analogous experiments with canine saphenous vein strips Muldoon et al. (40) made similar observations and

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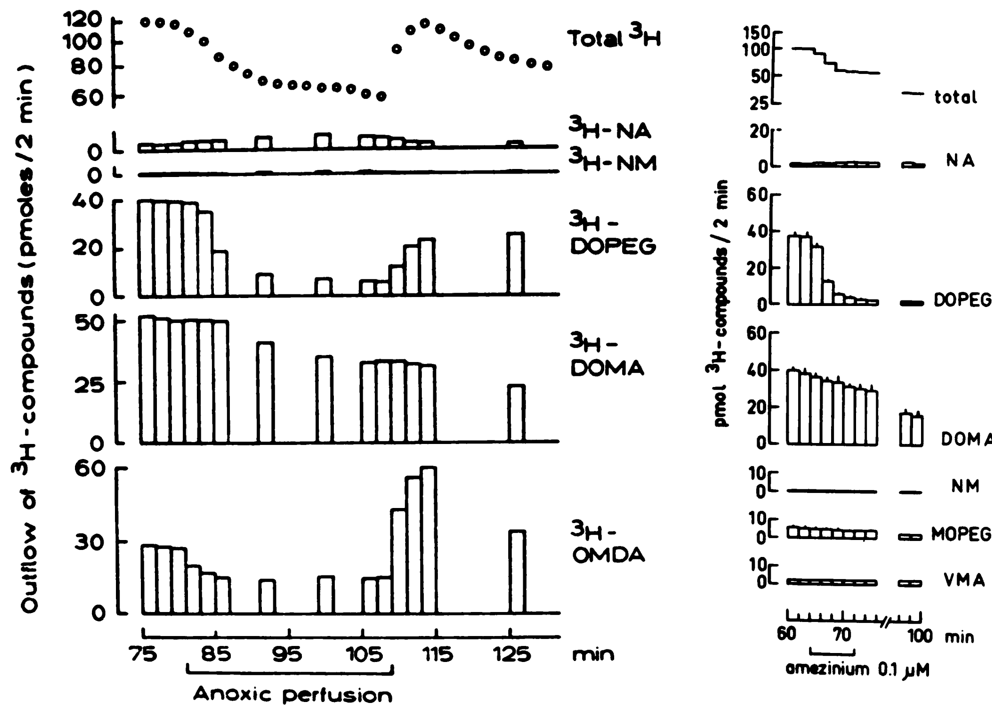

FIG. 9. Effect of inhibition of MAO on the efflux of noradrenaline metabolites from the perfused rabbit heart. Ordinates: outflow of ^{3}H-compounds (in pmol·g^{-1}·2 min^{-1}); abscissae: time (in min) after onset of washout. Hearts were initially perfused with 1 μM ^{3}H-(−)-noradrenaline for 20 min and then washed out with amine-free solution. A, From the 81st to the 109th min of washout, the perfusion medium was replaced by solution saturated with 95% N_2 + 5% CO_2. B, 10 μM amezinium was present in the washout solution from the 64th to the 72nd min. Note that inhibition of MAO caused a rapid and pronounced fall in the overflow of ^{3}H-DOPEG, while the overflow of ^{3}H-DOMA remained largely unaffected. A, from Starke (45); B, kindly provided by K. Starke and A. Steppeler (unpublished observations).

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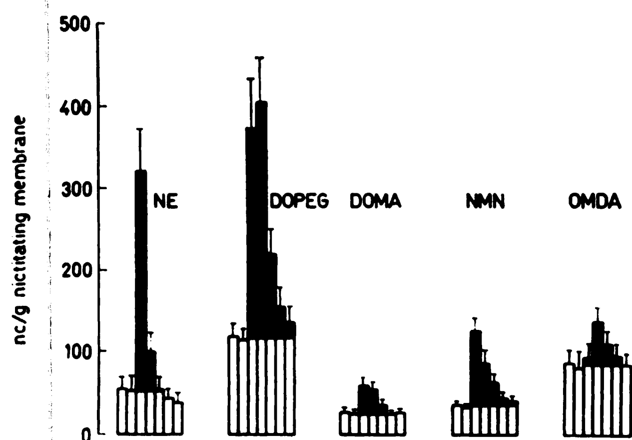

FIG. 10. The overflow of ^{3}H-noradrenaline and its metabolites during and after electrical stimulation of the adrenergic nerves of the isolated nictitating membrane of the cat. Ordinate: overflow (in nCi/g of nictitating membrane per collection period of 5 min); abscissa: columns represent consecutive collection periods of 5 min; bars indicate period of stimulation. The isolated medial muscle was first exposed to 6 μCi/ml (or 100 to 200 ng/ml) of ^{3}H-noradrenaline for 30 min and then washed out with amine-free solution. From the 100th to the 105th min of washout, the infratrochlear nerve was stimulated (10 Hz) supramaximally. Open columns represent spontaneous outflow; black columns represent stimulation-induced overflow. Note that the release of ^{3}H-noradrenaline was accompanied by a pronounced overflow of ^{3}H-DOPEG and a very small overflow of ^{3}H-DOMA. From Langer and Enero (28).

reached similar conclusions. However, if we consider the low rate constant for the efflux of DOMA as well as the results of figure 9, these conclusions can no longer be based on results such as shown in figure 10: the efflux curve for a metabolite with a low rate constant cannot be expected to reflect the changes in amine concentrations in the axoplasm. However, because of the stereoselectivity of aldehyde reductase and aldehyde dehydrogenase mentioned above, it is indeed likely that more ^{3}H-DOPEG than ^{3}H-DOMA is formed after release by nerve stimulation even if the nerve endings have been labelled with ^{3}H-(±)-noradrenaline. This is so, because the stereoselectivity of the vesicular uptake of noradrenaline (12) must necessarily lead to a preferential labelling of the storage vesicles with ^{3}H-(−)-noradrenaline. However, in order to obtain an exact value for the ratio "formation of ^{3}H-DOPEG/formation of ^{3}H-DOMA" during and after a short period of nerve stimulation, not only the overflow of the ^{3}H-metabolites should be measured, but also the ^{3}H-metabolite content of the tissue.

IX. The Ionization of Amine Metabolites and Their Renal Excretion

Figure 4 illustrated that highly ionized (i.e., acid) metabolites have difficulty in leaving tissues (i.e., have low rate constants for their efflux), while unionized metabolites (i.e., glycols) leave tissues easily (i.e., have high rate constants). For renal excretion we might well expect the opposite pattern: while the highly ionized acid metabolite should not be subject to tubular reabsorption and might possibly be actively secreted in the tubules, the unionized glycol metabolites might well be subject to very pronounced tubular reabsorption. Unfortunately, the presently available evidence to support such views is very incomplete. Nevertheless, the possibility outlined here should be borne in mind for the following reason.

Although we are unaware of any relevant publications, it is very likely that the peripheral tissues of humans resemble those of a large variety of animal species in forming either more glycol than acid metabolites from endogenous (−)-noradrenaline or about equal amounts of the two types of metabolites from racemic noradrenaline or adrenaline. If this stipulation is accepted, it is of great interest to note that acid metabolites preponderate in human urine [for noradrenaline (49), for ^{3}H-synephrine (19), for ^{3}H-phenylephrine (J. H. Hengstmann and J. Goronzy, unpublished observations)]. Apparently, little or no *free* glycol metabolites are excreted by the human kidney, although there is convincing evidence that considerable glycol formation occurs in a variety of peripheral organs of various animal species. This disparity between pronounced glycol formation in organs and poor excretion of free glycol in human urine is consistent with the view that glycol metabolites may well be subject to pronounced tubular reabsorption.

However, this disparity also leads to the question of how glycol metabolites can leave the human body. In this context, it is of interest to note that LaBrosse (26) described the conversion of labelled MOPEG to labelled VMA in a child with neuroblastoma, that Blombery et al. (1) found a similar conversion in the monkey, and that I. J. Kopin (unpublished observations) found extensive conversion of MOPEG to VMA in healthy volunteers. Thus, the free MOPEG in plasma (hand in hand with its failure to be excreted by the kidney) can be converted, probably in the liver, to the corresponding acid metabolite, which is then excreted easily.

Two conclusions can be drawn from this discussion: 1) the absence of free glycol metabolites from urine samples should *not* be interpreted as a lack of their formation in the adrenergically innervated organs of the body; and 2) more generally, it is exceedingly doubtful that the percentage contribution by the various free or conjugated metabolites to the total metabolite content of the urine can be used to assess their formation in the body. It should be realized that the "pattern of metabolite formation in the various organs" is distorted twice before the metabolites appear in the urine. After the formation of metabolites in peripheral tissues, the degree of "apparent lipophilicity" determines whether they easily or very slowly appear in the circulating blood, and further distortion of the "metabolite pattern in plasma" is inevitable if tubular reabsorption of glycol metabolites plus hepatic conversion of such metabolites to the corresponding acid metabolites take place. Thus, it is unlikely that the renal excretion of specific metabolites can serve as a

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useful index of the formation of these specific metabolites in the body.

X. Conclusions

1. The old assumption that catecholamine metabolites leave peripheral tissues with roughly similar ease is no longer tenable. The apparent rate constants for the efflux of these metabolites clearly decrease in the order: glycol metabolites > O-methylated catecholamines >>acid metabolites. This ranking order seems to be rather independent of the organ, the species, and the experimental conditions.

2. Although the evidence is by no means complete, metabolites of catecholamines appear to distribute into multiple compartments, the definition and morphological localization of which remain to be established. As a consequence, experiments that involve short exposures to ^{3}H-catecholamines tend to give higher apparent k-values for the efflux of metabolites than do experiments that involve washout of peripheral tissues for several hours.

3. Because of the highly significant correlation between apparent rate constants for efflux and apparent lipophilicity (at pH 7.4) of the metabolites, and also because of the lack of evidence for any saturability of efflux, it is likely that the efflux of metabolites is largely determined by their apparent lipophilicity at pH 7.4. The term "apparent lipophilicity at pH 7.4" is used to emphasize that the ability of the metabolites to cross cell membranes is determined both by the degree of ionization and by the presence or absence of polar substituents on the molecule. However, corticosterone-sensitive, carrier-mediated efflux of OMI has been shown to be responsible for a small proportion of total efflux.

4. The efflux of metabolites with high rate constants can be regarded as a momentary indicator of the rate of metabolism of the amine in a certain compartment. Since the rate of metabolism depends on the activity of the enzyme and on the intracompartmental concentration of the parent amine, changes in enzyme activity and/or amine concentration affect the efflux of the metabolites. If the period during which the efflux of metabolites is measured is shorter than twice the half-time for the efflux of the metabolite, this is not so for the efflux of those acid metabolites characterized by low rate constants.

5. For an analysis of the relative importance of the various metabolic pathways, it is necessary to determine metabolites in the tissue as well as in the incubation or perfusion medium.

6. It is likely that the "apparent lipophilicity" of metabolites influences not only their efflux from those cells in which they are formed but also their renal excretion. In that case, the glycol metabolites should be retained by the body while the acid metabolites should be excreted with ease.

Acknowledgments. The authors are greatly indebted to Drs. E. R. Fiebig, S. Guimaraes, S. Z. Langer, Maria A. Luchelli-Fortis, and K. Starke for their kind retrieval of raw data from old or new experiments. The authors' own experiments described here were supported by the Deutsche Forschungsgemeinschaft (Grants Bö.521, Gr.490, He.813, and Tr.96).

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