Biochemical Pharmacology, Vol. 22, pp. 2171–2181. Pergamon Press, 1973. Printed in Great Britain.

POST-MORTEM DEGRADATION KINETICS OF BRAIN NOREPINEPHRINE*

MORRIS D. FAIMAN, MICKEY B. MYERS† and RICHARD L. SCHOWEN‡

Department of Pharmacology and Toxicology, School of Pharmacy, University of Kansas, Lawrence, Kan., U.S.A.

(*Received* 15 *December* 1972; *accepted* 20 *February* 1973)

Abstract—The post-mortem degradation kinetics of norepinephrine (NE) from brain stored at 25°, 15°, 0°, −5° and −10° have been determined. The first 20 per cent of NE was lost by zero-order kinetics, while degradation of the major part of the remaining NE followed two consecutive first-order processes. Both the zero- and first-order constants showed a temperature dependency and were used to construct Arrhenius temperature plots. The zero- and first-order rate constants yielded energies of activation of 17·2 and 16·3 kcal/mole respectively. When stored at 25°, approximately 25 per cent of total NE remained in mouse brain 144 hr after death. The amount of NE remaining 144 hr post-mortem increased as the storage temperature decreased. Post-mortem NE degradation kinetics also were studied in rats. The NE degradation kinetics in rat brain were similar to those observed in mice. Although the zero-order degradation rate constant for rats was about one-third that found for mice, no difference was found in the first-order rate constants. Oxidative metabolism of NE in rat brain stopped shortly after death and metabolism proceeded non-oxidatively via normetanephrine. Monoamine oxidase (MAO) activity in mouse brain post-mortem also was found to be temperature dependent. Approximately 75 per cent of MAO activity remained in mouse brain when stored for 144 hr at 25°, while approximately 90 per cent remained when stored at −10°.

ENZYMES generally reponsible for the synthesis and metabolism of norepinephrine (NE) in brain have been found to be active after death in both man and animals.[1,2] Changes in the concentration of NE in brain tissue post-mortem can therefore be expected, and in fact occur.[1,3] The rate of loss and type of kinetics exhibited by NE in brain tissue post-mortem, however, are unknown. Such data would be valuable in helping to separate true post-mortem losses of NE from changes occurring as a result of a particular treatment. These kinetic data would allow one to predict quantitatively the loss of NE from brain post-mortem and thus would not only define losses due to storage, but would also suggest conditions to minimize losses of NE during storage. Since the turnover of brain NE varies between species, differences observed in post-mortem rates of loss between species could provide information of use in the elucidation of NE storage mechanisms in the central nervous system.

METHODS

Male Swiss Webster mice (HA/ICR-ARS Gibco) weighing 22–28 g were decapitated, their brains quickly removed, weighed and placed in glass vials the size of the brain tissue. Each vial was placed in a constant temperature bath (Neslab Instrument

* These studies were supported in part by grants from the United States Public Health Service, National Institute of Neurological Diseases and Stroke, Grant No. NS-07797, and a grant from the University of Kansas General Research Fund.

† Present address: Eli Lilly and Company, Indianapolis, Ind.

‡ Department of Chemistry, University of Kansas, Lawrence, Kan.

Company) and maintained for various periods of time at selected temperatures, hereafter called "incubation" or "incubation times". The "incubation times" in the constant temperature bath were 10, 20, 30 and 45 min and 1, 3, 6, 12, 24, 48, 96 and 144 hr. At the end of each "incubation time", the vials were removed from the constant temperature bath. At this time, control animals were decapitated and the brain was quickly removed. Separate controls were used for each time interval investigated. In all instances, whole brains from controls were analyzed for NE simultaneously with the brains maintained at the particular tempature. Differences in endogenous brain NE between controls and brains maintained at the particular temperatures were expressed as per cent of control and the data plotted to show the degradation rates. The temperatures chosen in this study were 25°, 15°, 0°, −5° and −10°.

The kinetics of post-mortem degradation of NE were also studied in rats. Male Sprague–Dawley rats (Holtzman) weighing 175–200 g were lightly anesthetized with ether, and 3·33 μCi ^{3}H-norepinephrine (^{3}H-NE) (5·0 Ci/m-mole, New England Nuclear) in 25 μl of Merlis's[4] solution was administered intracisternally.[5] Six min after ^{3}H-NE administration, the rats were killed by cervical fracture, decapitated and their brains quickly removed. The whole brains were placed in small glass vials. The vials were flushed with nitrogen, tightly sealed with paraffin, and placed in a water bath at 25°. At various times thereafter, the brains were removed from the bath, homogenized and assayed for endogenous NE, ^{3}H-NE, ^{3}H-dihydroxymandelic acid (^{3}H-DHMA), ^{3}H-normetanephrine (^{3}H-NMN), ^{3}H-3-methoxy-4-hydroxymandelic acid (^{3}H-VMA) and ^{3}H-glycol sulfates (^{3}H-GS). Just prior to the removal of the brains from the constant temperature bath, another group of rats were injected intracisternally with 3·33 μCi ^{3}H-NE in 25 μl of Merlis's solution and sacrificed 6 min later in the same manner. These rats were designated as controls, and their brains were analyzed for endogenous NE, ^{3}H-NE and other radioactive metabolites simultaneously with the incubated brain tissue. Each time period post-mortem had its own controls and the data were plotted as per cent of control versus time to show the degradation rates post-mortem.

The effect of temperature on the loss of MAO activity post-mortem from whole mouse brain also was studied. Mice were decapitated and their brains stored in small vials for various times as described earlier. The temperatures studied were 25°, 0° and −10°. After the appropriate time period at constant temperature, the brains were removed and the whole brain tissue was analyzed for MAO activity by the method of Wurtman and Axelrod.[6]

In order to investigate further the influence of oxidative metabolism on post-mortem degradation, pargyline, an inhibitor of MAO, was administered to one group of mice. One hr after the administration of pargyline (100 mg/kg, i.p.), the mice were sacrificed, their brains removed and placed in a constant temperature bath at 25° as described earlier. The brains were maintained at constant temperature for the time periods listed above. Just prior to removal of the brain from the bath, mice previously treated with pargyline, 100 mg/kg i.p. 1 hr earlier, and designated as controls were sacrificed. Their brains were analyzed for NE together with the brains from pargyline-treated mice whose brains had been "incubated" for the various times listed above. Changes in brain amine content as a result of storage were expressed as per cent of control as described earlier.

In all experiments no degradation of tissue was evident. Even after 6 days of incubation in the sealed vails the brain tissue was still moist and there was less than a 1 per cent loss in weight.

Rate constants for NE degradation post-mortem were calculated on a GE-635 computer using a non-linear least squares program. Energies of activation were determined by computer from a linear least squares program.

Assay of radioactive norepinephrine and metabolites. After removal of the "incubated" brain tissue from the constant temperature bath, the tissue was homogenized with an all-glass homogenizer and the total radioactivity determined with a liquid scintillation counter (Beckman LS-200). ^{3}H-NE was assayed by a modification of the aluminium-absorption method of Whitby *et al.*[7] combined with the procedure of Anton and Sayre.[8] Tritiated DHMA, NMN, GS snd VMA were assayed by the method of Kopin *et al.*[9] Internal standards of toluene-^{3}H were used to correct for counting efficiency. Recovery of intracisternally administered ^{3}H-NE averaged 39 per cent of the total ^{3}H-NE administered.

Assay of nonradioactive norepinephrine. In the post-mortem degradation studies carried out in mice, NE was extracted and determined spectrofluorometrically by the procedure of Welch and Welch.[10] Recovery for NE which was added to the homogenization and carried through the extraction procedure was 80 per cent. In the post-mortem studies in rats, a fraction of the supernatant fluid containing the radioactive NE and metabolites was retained and assayed for endogenous NE by the aluminium-oxide adsorption procedure of Anton and Sayre.[8] Recovery for NE added to the preparation and carried through the extraction procedure was 95 per cent.

Control values of NE and the standard error in the mice and rat studies averaged $0{\cdot}446 \pm 0{\cdot}016$ and $0{\cdot}413 \pm 0{\cdot}019$ μg/g respectively. In the analysis of brain tissue in all studies, the standard error for NE in the "incubated" brains, regardless of the length of "incubation", was approximately 5 per cent.

RESULTS

Loss of NE in whole mouse brain exhibited multifunctional decay, with the first 20 per cent lost by a process exhibiting zero-order kinetics (Fig. 1a), while degradation of the major part of the remaining NE followed two consecutive first-order processes (Fig. 1b). The second first-order rate constant was very slow and could not be measured accurately. The rate of NE loss post-mortem in whole mouse brain decreased with decreasing temperature. As the storage temperature decreased, the per cent of NE remaining 144 hr post-mortem increased (Fig. 1b). Approximately 25 per cent of NE remained in brain 144 hr after death when stored at 25°, while at −10°, approximately 80 per cent remained. Only the degradation of NE at 25°, 0° and −10° is plotted in Fig. 1b. The decrease in NE with respect to the "incubation" time at 15° was as uniform as that found at the other temperatures and fell between the 25° and 0° data. Similarily, the data from the −5° study fell between the data obtained at 0° and −10°. Both the zero- and first-order rate constants showed a temperature dependence and were used to construct Arrhenius plots, with the zero- and first-order rate constants yielding energies of activation of 17·2 and 16·3 kcal/mole respectively (Fig. 2). In all instances the brains showed less than a 1 per cent loss in weight even after 144 hr of "incubation".

Loss of NE from whole rat brain also exhibited multifunctional decay, with the

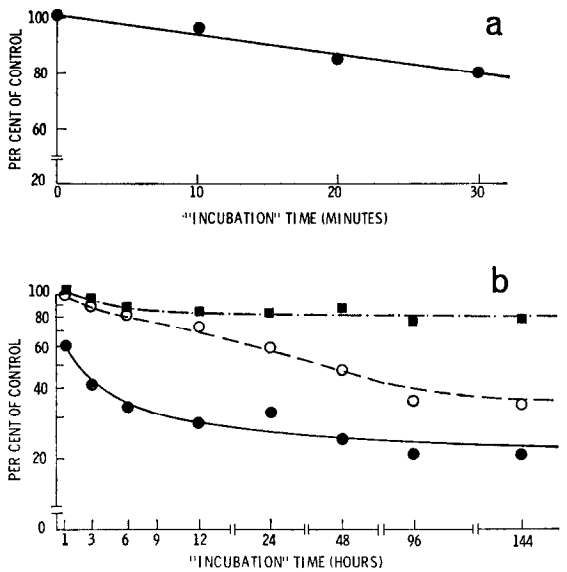

FIG. 1. (a) Zero-order NE degradation post-mortem at 25°. Each point represents the average of eight determinations. Slope drawn according to the method of least squares. (b) Effect of storage temperature on brain norepinephrine degradation post-mortem. Each point represents the average of eight determinations. Studies were carried out at 25° (●——●), 0° (○- - -○) and −10° (■——■).

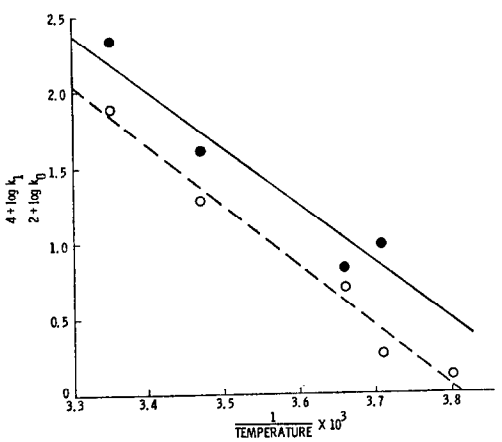

FIG. 2. Post-mortem norepinephrine Arrhenius temperature dependence. Zero-order rate constants (k_0) ●——● and first-order rate constants (k_1) ○- - -○ yielded activation energies of 17·2 ± 0·5 and 16·3 ± 1·5 kcal/mole respectively. Solid and dotted lines drawn according to the method of least squares.

first 20 per cent being lost by a zero-order process (Fig. 3a), while degradation of the remaining NE followed first-order kinetics (Fig. 3b). The fraction of NE lost by zero-order kinetics from rat brain post-mortem was significantly slower than the corresponding zero-order loss from mouse brain. However, no differences were found in the first-order NE degradation rate constants between mouse and rat brain (Table 1).

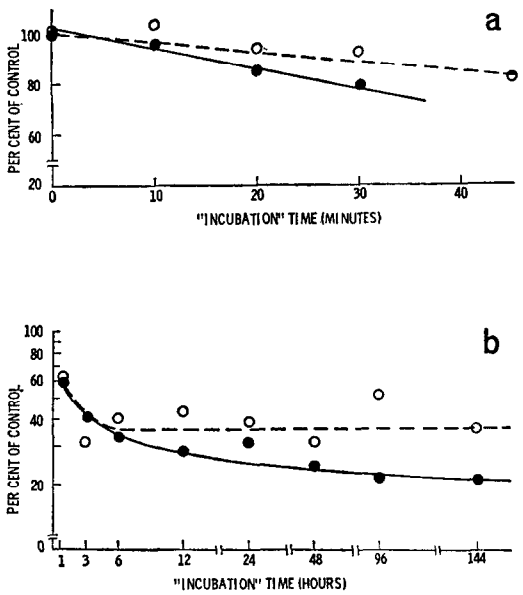

FIG. 3. (a) Zero-order NE degradation post-mortem for mouse (●——●) and rat brain (○- - -○) at 25°. Each point represents the average of eight determinations for mouse and six determinations for rat brain respectively. Slope drawn according to the method of least squares. (b) NE degradation for mouse (●——●) and rat (○- - -○) brain at 25°. Each point represents the average of eight determinations for mouse and six determinations for rat brain respectively.

After intracisternal administration of ^{3}H-NE to rats, the post-mortem loss of ^{3}H-NE paralleled that of endogenous NE. ^{3}H-NMN increased immediately after death of the animal, reaching a plateau approximately 12–24 hr later. In addition to the increase in ^{3}H-NMN, there were also increases in ^{3}H-VMA and ^{3}H-GS post-mortem, while ^{3}H-DHMA decreased only slightly after death (Figs. 4 and 5).

MAO activity post-mortem also was found to be temperature dependent. Storage of brains at 25° resulted in approximately a 25 per cent loss in MAO after 144 hr, while only approximately 10 per cent was lost after 144 hr when brains were stored at −10° (Fig. 6).

TABLE 1. EFFECT OF TEMPERATURE ON POST-MORTEM NE DEGRADATION RATE CONSTANTS IN BRAIN

Temp. (°C)	k_0* (% min^{-1} ± S.D.)		k_1† (min^{-1} ± S.D.)	
	Mouse	Rat	Mouse	Rat
25	0·772 ± 0·089‡ (47)§	0·238 ± 0·095 (29)	0·021 ± 0·005 (64)	0·015 ± 0·008 (21)
15	0·194 ± 0·070 (60)		0·004 ± 0·002 (23)	
0	0·053 ± 0·006 (64)		0·0007 ± 0·0003 (48)	
−5	0·019 ± 0·008 (32)		0·001 ± 0·001 (24)	
−10	0·014 ± 0·003 (79)			

* Zero-order rat constants.
† First-order rate constants.
‡ $P < 0·001$ (mouse vs rat).
§ Numbers in parentheses represent the number of animals used in determining the rate constants.

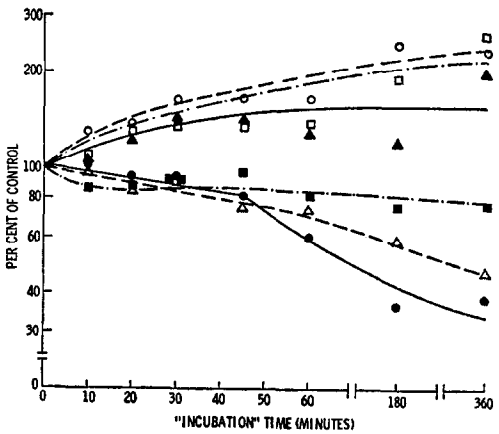

FIG. 4. Post-mortem metabolism of ^{3}H-NE in rat brain. Rats were injected with ^{3}H-NE intracisternally, sacrificed and their brains "incubated" for various periods of time at 25° (see Methods). Brain was analyzed for endogenous NE (●——●), ^{3}H-NE (△- - -△), ^{3}H-DHMA(■ - · - · ■), ^{3}H-GS (▲——▲), ^{3}H-VMA (□- · - · □) end ^{3}H-NMN (○- - -○). Each point represents the average of six brains.

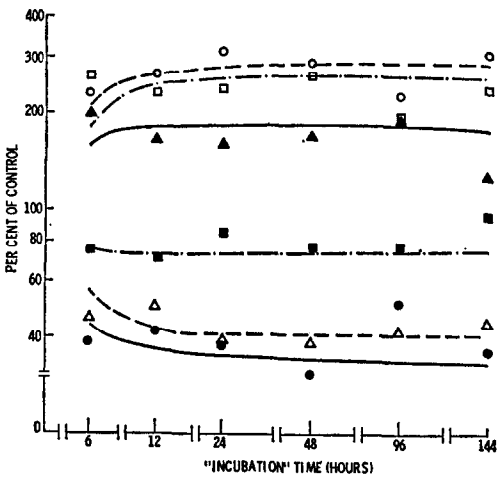

FIG. 5. Post-mortem metabolism of ^{3}H-NE in rat brain. Rats were injected with ^{3}H-NE intracisternally, sacrificed and their brains "incubated" for various periods of time at 25° (see Methods). Brain was analyzed for endogenous NE (●——●), ^{3}H-NE (△- - -△), ^{3}H-DHMA (■- · - · ■), ^{3}H-GS (▲——▲), ^{3}H-VMA (□- · - · □) and ^{3}H-NMN (○- - -○). Each point represents the average of six brains.

DISCUSSION

In both mouse and rat brain, NE was lost post-mortem with degradation occurring initially by zero-order kinetics and then by a first-order process. The rate of loss of NE was found to be temperature dependent. Utilizing the principles of kinetics and the Arrhenius temperature dependency exhibited by NE, it appears that the quantitative loss of NE post-mortem can be accurately predicted between temperatures of 25° and −10°. It also seems that the Arrhenius temperature dependency can be extended to −70° (Table 2). The concentration of NE from brains stored for 2 and 3 weeks,

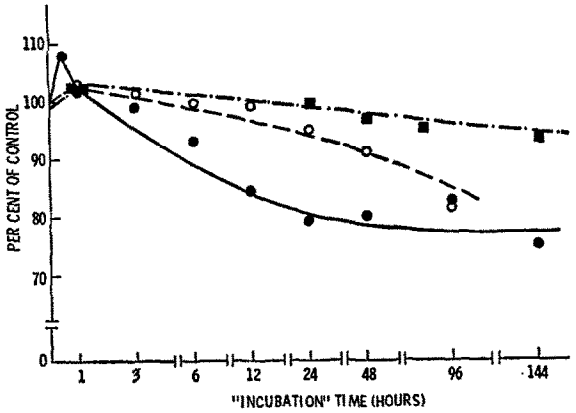

FIG. 6. Effect of storage temperature on mouse brain MAO activity post-mortem. Brains were "incubated" for the time period indicated and then analyzed for MAO. Each point represents the average of eight determinations. Studies were carried out at 25° (●——●), 0° (○- - -○) and −10° (■-·-·■).

respectively, was not found to be significantly different from control values as predicted from the kinetics. Using the energy of activation obtained with mouse brain, analysis of rat brain stored for 2 and 3 weeks similarly showed no loss in NE. This would be expected, particularly since the zero-order rate constant for NE degradation in rats is about one-third as rapid as that in mouse brain (Table 1).

TABLE 2. PREDICTION OF NE LOSS FROM BRAIN POST-MORTEM*

Species	Storage time (weeks)	Control (μg/g ± S.E.)	Conc NE predicted (μg/g)	Conc NE found (μg/g ± S.E.)
Mouse	2	0·445 ± 0·015 (8)	0·440	0·411 ± 0·007 (8)
	3	0·445 ± 0·015 (8)	0·440	0·435 ± 0·014 (8)
Rat	2	0·543 ± 0·021 (6)	0·538	0·570 ± 0·025 (6)
	3	0·543 ± 0·021 (6)	0·538	0·530 ± 0·013 (6)

* Mice and rats were sacrificed, their brains removed and stored at −70° for the time periods indicated. The activation energy (Fig. 2) was used to calculate the predicted concentration of NE at 2 and 3 weeks respectively. Controls were sacrificed just prior to analyses of stored brain, and both control and stored brains were analyzed simultaneously for NE.

Two enzymes, MAO and catechol-O-methyl transferase (COMT), are primarily responsible for the catabolism of NE.[11] MAO activity is considered to depend on the availability of molecular oxygen.[12] In view of a presumed deficiency of molecular oxygen after death, it would not seem unreasonable to assume that the enzymatic degradation of NE occurred non-oxidatively by way of COMT, even though sufficient MAO enzyme is available (Fig. 6). That this occurs is substantiated by the finding that ^{3}H-NMN increased post-mortem, while ^{3}H-DHMA decreased approximately 15 per cent in the first 10 min after death and remained constant thereafter. Furthermore, no significant differences in the post-mortem NE degradation rate constants were found

between pargyline-treated animals and those animals not pretreated with the MAO inhibitor (Table 3). This would appear to substantiate further the relative unimportance of the MAO pathway post-mortem. Ganrot *et al.*[13] found no appreciable formation of 3,4-dihydroxyphenylacetic acid, an oxidative deaminated metabolite, in human brain after death. Their observations would thus substantiate our findings.

TABLE 3. EFFECT OF INHIBITION OF MAO BY PARGYLINE ON NE DEGRADATION RATE CONSTANTS IN MICE POST-MORTEM*

Norepinephrine			
Control†		Pargyline	
k_0‡ ± S.D.	k_1§ ± S.D.	k_0‡ ± S.D.	k_1§ ± S.D.
0·772 ± 0·089 (47)	0·021 ± 0·005 (64)	0·967 ± 0·209 (18)	0·061 ± 0·056 (18)

* Pargyline, 100 mg/kg, was given i.p. 1 hr before the mice were sacrificed. Brain tissue was then "incubated" at 25° and the degradation rate constants were determined. Numbers in parentheses indicate the number of animals used in determining the rate constants.

† Control rate constants were calculated from non-pargyline-treated animals whose brains were "incubated" at 25° as described in Methods.

‡ Zero-order rate constants.

§ First-order rate constants.

The observation that the loss of NE post-mortem occurs initially by a zero-order process, followed by successive first-order processes, is of interest. This degradation pattern would be expected if the system exhibited Michaelis–Menten kinetics. Initially, the enzyme system responsible for NE degradation is saturated, producing a constant reaction rate (zero-order kinetics). After the concentration of the substrate has been reduced and the system is no longer saturated, the rate of the reaction becomes proportional to the concentration of the substrate (first-order kinetics). Zero- and first-order kinetics were observed in both mouse and rat brain, and in both species there was an initial loss of approximately 20 per cent before the first-order process took over. The data obtained are satisfactorily described by the integrated Michaelis–Menten equation: $K_m \ln \mathrm{NE}/\mathrm{NE}_0 + (\mathrm{NE} - \mathrm{NE}_0) + k_{cat}t = 0$, as well as by the successive zero-order and first-order functions. K_m is the Michaelis constant, NE and NE_0 are the concentrations of NE at the time t and time zero respectively, k_{cat} is the NE degradation rate at maximum velocity, and t is the time.

The loss of NE from brain tissue post-mortem appears to be due to enzymatic rather than physical factors. This is suggested from the observation that concomitant with the depletion of NE, there were increases in NE metabolites, that is, NMN, VMA and GS (Figs. 4 and 5). Formation of these metabolites would not be expected to occur if nonenzymatic processes were responsible for NE degradation post-mortem. Also, NE depletion exhibited Michaelis–Menten kinetics, a property characteristic of enzymatic processes.

When post-mortem degradation kinetics between mouse and rat brain are compared, several differences become apparent. Although there is no significant difference in the

first-order rate constants between mouse and rat brain, there is almost a 3-fold difference in the zero-order rate constants. Also, the fraction of NE remaining in rat brain after 144 hr of storage is almost twice as high as that found in mouse brain (Fig. 3b). The reason for these differences cannot be satisfactorily explained at this time. Several factors such as species differences in brain NE turnover rates, regional differences in NE distribution, and differences in the amount of NE released during sacrifice may account for the differences observed.

The finding that approximately 25 and 45 per cent of NE remained in mouse and rat brain, respectively, after 144 hr of storage is especially interesting. Although COMT activity was not determined, others[1] have shown that COMT is stable after death. If COMT is available, and since sufficient MAO already is present (Fig. 6), the NE remaining 144 hr post-mortem must represent a fraction either "protected" or inaccessible to these two enzymes. Since these are post-mortem studies, the possibility exists that COMT and not MAO is the enzyme inaccessible to the NE and is therefore the enzyme of major importance in NE degradation. This is suggested from the observation that, in the rat, the loss in ^{3}H-NE levels off approximately 12 hr after death, the same time at which the increase in NMN appears to plateau (Fig. 5). In addition, if degradation of ^{3}H-NE by MAO post-mortem occurred, an increase in ^{3}H-DHMA would be expected rather than the observed decrease (Fig. 4). Since the post-mortem loss of intracisternally administered ^{3}H-NE appears to follow the post-mortem loss in endogenous NE (Fig. 5, Table 4), it can be assumed that this is what occurs *in vivo*. No significant differences were found in the endogenous and ^{3}H-NE zero-order rate constants or in the endogenous and ^{3}H-NE first-order rate constants. Also, the concentrations of endogenous NE and ^{3}H-NE 144 hr post-mortem were not statistically different from each other. It would therefore seem that intracisternally administered ^{3}H-NE mixes with the endogenous NE, as proposed by Schanberg *et al.*,[5] has ready access to the stable NE "pool", and also resists enzymatic degradation, as evidenced by the 45 per cent fraction remaining unmetabolized (Fig. 5). The physiological significance of this tightly bound fraction remains to be determined, particularly since evidence exists that newly synthesized or newly stored NE is more readily released than is older stored NE.[14–16]

A number of investigators have shown that intracisternally[17–19] or intraventricularly[20] administered ^{3}H-NE disappeared in a multiphasic fashion. In all instances it has been assumed that ^{3}H-NE disappeared according to first-order kinetics. In our post-mortem studies, both endogenous NE and intracisternally administered ^{3}H-NE

TABLE 4. ^{3}H-NE AND ENDOGENOUS NE DEGRADATION RATE CONSTANTS IN RAT BRAIN POST-MORTEM*

Endogenous NE		^{3}H-NE	
k_0† (% min^{-1} ± S D.)	k_1‡ (min^{-1} ± S.D.)	k_0† (% min^{-1} ± S.D.)	k_1‡ (min^{-1} ± S.D.)
0·238 ± 0·095 (47)	0·020 ± 0·014 (64)	0·126 ± 0·056 (28)	0·014 ± 0·004 (22)

* Studies were carried out at 25°. Numbers in parentheses indicate the number of animals used in determining the rate constants.

† Zero-order rate constants.

‡ First-order rate constants.

also disappeared in a multiphasic fashion, except that the initial disappearance of both endogenous and ^{3}H-NE was zero-order. At this time the reason for the differences in the type of kinetics between our studies and those carried out by others cannot be readily explained. Our studies involved post-mortem degradation, while the studies reported by other investigators involved administering ^{3}H-NE and sacrificing the animals at various times thereafter. This could account for some of the differences observed. Studies investigating the order by which NE is lost, however, have never been carried out. Generally most kinetic studies have not been carried out for more than two half-lives. Although zero-order data can give results suggestive of first-order kinetics, this does not necessarily mean a process proceeds in a first-order fashion.[21] In our post-mortem studies, however, we investigated the order of the degradation and found the initial loss to be truly zero-order.

It has been proposed that NE is stored in more than one "pool",[22-24] and the multiphasic disappearance of exogenously administered ^{3}H-NE is used as evidence to support this concept. Since NE also disappears in a multiphasic fashion post-mortem, it is reasonable to assume that each phase may represent a particular "pool". The "pool" bound least tightly would be considered to be lost first. Glowinski and Iversen[25] found that, in the hypothalamus, intraventricularly administered ^{3}H-NE was equally distributed between the crude pellet, synaptosomal–mitochondrial fraction, microsomal fraction, and the soluble supernatant. If whole brain is considered on the same basis as the hypothalamus, the first 20 per cent lost by the zero-order process in our studies could very well correspond to the soluble supernatant fraction, while that remaining 144 hr post-mortem, to the crude pellet fraction. This may be reasonable, since recent evidence has indicated that COMT may be present in small amounts in the cytoplasm.[26] If this is the case, this can account for the saturation phenomenon observed initially (zero-order kinetics) and the slow increase in NMN (Fig. 4). When saturation no longer exists, the rate of degradation of NE becomes concentration dependent and the process exhibits first-order kinetics. This may be reflected by the two sequential first-order processes we observed and may correspond to the synaptosomal–mitochondrial and microsomal fractions. Another possibility that should not be overlooked is that the release of catecholamines from nerve terminals presumably proceeds by exocytosis of the contents of the storage vesicles.[27] Under normal unstimulated circumstances, the rate of release is fairly constant, and as vesicles become depleted, fewer release events occur. Thus, the initial zero-order and subsequent first-order kinetics may represent different phases in the rate of secretion. Both possibilities are only speculative and further studies are needed to explain the reason for the kinetics observed.

Acknowledgements—The authors gratefully acknowledge the helpful suggestions of Dr. Ralph N. Adams, the able technical assistance of Mrs. Darlene Oosterhof, and Abbott Laboratories for the generous supply of pargyline.

REFERENCES

1. W. J. VOGEL, V. ORFEI and B. CENTURY, *J. Pharmac. exp. Ther.* **165,** 196 (1969).
2. E. G. MCGEER, P. L. MCGEER and J. A. WADA, *J. Neurochem.* **18,** 1647 (1971).
3. F. GRABARITS, R. CHESSICK and H. LAL, *Biochem. Pharmac.* **15,** 127 (1966).
4. J. K. MERLIS, *Am. J. Physiol.* **131,** 67 (1940).
5. S. M. SCHANBERG, J. SCHILDKRAUT and I. J. KOPIN, *J. Pharmac. exp. Ther.* **157,** 311 (1967).
6. R. J. WURTMAN and J. AXELROD, *Biochem. Pharmac.* **12,** 1439 (1963).

7. L. G. WHITBY, J. AXELROD and H. WEIL-MALHERBE, *J. Pharmac. exp. Ther.* **132,** 193 (1961).
8. A. H. ANTON and D. F. SAYRE, *J. Pharmac. exp. Ther.* **138,** 360 (1962).
9. I. J. KOPIN, J. AXELROD and E. GORDON, *J. biol. Chem.* **236,** 2109 (1961).
10. A. WELCH and B. WELCH, *Analyt. Biochem.* **30,** 161 (1969).
11. I. J. KOPIN and E. K. GORDON, *J. Pharmac. exp. Ther.* **140,** 207 (1963).
12. W. J. NOVICK, *Biochem. Pharmac.* **15,** 1009 (1966).
13. P. O. GANROT, E. ROSENGREN and C. G. GOTTFRIES, *Experientia* **18,** 260 (1962).
14. A. WEISSMAN, B. K. KOE and S. S. TENEN, *J. Pharmac. exp. Ther.* **151,** 339 (1966).
15. J. V. DINGELL, M. L. OWENS, M. R. NORVICH and F. SULSER, *Life Sci.* **6,** 1155 (1967).
16. J. J. SCHILDKRAUT, P. R. DRASKOCZY and P. S. LO, *Science, N.Y.* **172,** 587 (1971).
17. T. C. WESTFALL, *Life Sci.* **9,** 339 (1970).
18. E. L. BLISS, J. AILION and J. ZWANZIGER, *J. Pharmac. exp. Ther.* **164,** 122 (1968).
19. A. M. THIERRY, G. BLANC and J. GLOWINSKI, *J. Neurochem.* **18,** 449 (1971).
20. L. L. IVERSEN and J. GLOWINSKI, *J. Neurochem.* **13,** 671 (1966).
21. A. A. FROST and R. G. PEARSON, in *Kinetics and Mechanism* (Eds. A. A. FROST and R. G. PEARSON), p. 27. Wiley, New York (1963).
22. I. J. KOPIN, *Pharmac. Rev.* **16,** 179 (1964).
23. A. CARLSSON, J. JONASSON and E. ROSENGREN, *Acta physiol. scand.* **59,** 474 (1963).
24. U. TRENDELENBURG, *Anesthesiology* **25,** 259 (1964).
25. J. GLOWINSKI and L. L. IVERSEN, *Biochem. Pharmac.* **15,** 977 (1966).
26. B. JARROTT, *J. Neurochem.* **18,** 17 (1971).
27. R. M. WEINSHILBOUM, N. B. THOA, D. G. JOHNSON, I. J. KOPIN and J. AXELROD, *Science, N.Y.* **174,** 1349 (1971).