Br. J. Pharmac. (1971), **41**, 270–277.

Effect of pretreatment with 6-hydroxydopamine on the uptake and metabolism of catecholamines by the isolated perfused rat heart

G. HELLMANN, G. HERTTING AND B. PESKAR

Institute of Pharmacology, University of Vienna, A-1090, Vienna, Austria

Summary

1. Isolated rat hearts from control and 6-hydroxydopamine pretreated animals were perfused with ^{3}H-noradrenaline or ^{3}H-dopamine, either at a low perfusion concentration ($1{\cdot}50 \times 10^{-10}$ mol/ml ^{3}H-dopamine; $1{\cdot}18 \times 10^{-10}$ mol/ml ^{3}H-noradrenaline) or a high perfusion concentration ($296{\cdot}69 \times 10^{-10}$ mol/ml ^{3}H-noradrenaline, $327{\cdot}45 \times 10^{-10}$ mol/ml ^{3}H-dopamine) for 8 minutes.

2. At the low perfusion concentration, the total activity, the radioactivity in the alumina eluates (sum of ^{3}H-dopamine, ^{3}H-noradrenaline and deaminated catechol metabolites) and the concentration of ^{3}H-dopamine, ^{3}H-noradrenaline and the deaminated catechol metabolites were decreased in the hearts of the pretreated rats as compared with the controls. The O-methylated amine metabolites were increased. The deaminated O-methylated metabolites were increased in the experiments with ^{3}H-noradrenaline and decreased in the ^{3}H-dopamine experiments.

3. Uptake of ^{3}H-dopamine and ^{3}H-noradrenaline by the hearts of 6-hydroxydopamine pretreated rats was decreased to a much smaller extent when perfused with the high concentration than with the low concentration.

4. At the high perfusion concentration there was a significant difference between control and pretreated animals with regard to the total radioactivity and the radioactivity in the alumina eluates only. The absolute and relative amounts of metabolites were not significantly changed by pretreatment with the exception of the deaminated catechol metabolites in the ^{3}H-dopamine experiments.

5. It is concluded that neuronal Uptake 1 is greatly impaired in the hearts from rats pretreated with 6-hydroxydopamine, but extraneuronal Uptake 2 remains intact.

Introduction

Catecholamines (CA) are accumulated in sympathetically innervated tissues (Axelrod, Weil-Malherbe & Tomchick, 1959; Whitby, Axelrod & Weil-Malherbe, 1961). Convincing evidence has been presented that this uptake occurs mainly into the sympathetic nerve endings (for references see Iversen, 1967). Iversen (1965) described the existence of two kinetically different uptake mechanisms for adrenaline as well as for noradrenaline (NA) in the isolated, perfused rat heart. Uptake 1 operates at low perfusion concentrations up to 1,000 ng/ml, is inhibited by cocaine

and desipramine and seems to be associated with the sympathetic neurones. Uptake 2 dominates at higher perfusion concentrations and is unaffected by cocaine, but is inhibited by metanephrine.

Iversen (1965) in experiments with immunosympathectomized rats suggested that Uptake 2 is also associated with the sympathetic nerves. Lightman & Iversen (1969) have presented evidence that Uptake 2 also operates at low perfusion concentrations, but the NA taken up by this mechanism cannot be measured because it is rapidly metabolized. Experiments using biochemical (Iversen & Langer, 1969; Eisenfeld, Krakoff, Iversen & Axelrod, 1967) and histochemical (Ehinger & Sporrong, 1968; Farnebo & Malmfors, 1969) methods indicate that the isolated, perfused rat heart can accumulate NA extraneuronally and that the extraneuronal uptake may be identical with Uptake 2. The existence of two uptake mechanisms for dopamine (DA) in the isolated, perfused rat heart with different kinetics and different patterns of metabolites is described by Hellmann, Hertting & Peskar (1970).

6-Hydroxydopamine (6-OH-DA) lowers the NA content of sympathetically innervated tissues (Porter, Totaro & Stone, 1963, Laverty, Sharman & Vogt, 1965; Thoenen & Tranzer, 1968) by causing the destruction of the sympathetic nerve endings (Tranzer & Thoenen, 1967; Malmfors & Sachs, 1968). Isolated, perfused hearts from rats pretreated with 6-OH-DA should, therefore, show no intraneuronal uptake (Uptake 1), but only an extraneuronal accumulation (Uptake 2) of catecholamines at all perfusion concentrations.

Methods

Pretreatment with 6-OH-DA

Male rats (Wistar, 100–200 g) were used; one group received no pretreatment, whilst the second group was treated as described by Thoenen & Tranzer (1968). The latter animals received 50 mg/kg 6-OH-DA-hydrobromide intravenously twice within 24 hours. One week later these rats were again injected twice with a dose of 100 mg/kg 6-OH-DA-hydrobromide intravenously during the same time interval and were used 3 days after the last injection. The weight of both groups varied from 150 to 180 g and there was no difference in body weight and heart weight between the control and the pretreated group.

Perfusion technique of rat hearts

The perfusion technique is described in detail by Hellmann *et al.* (1970). There was no difference in the flow rates (8–10 ml/min) between the two groups. After a stabilization period of 5 min the hearts were perfused for 8 min with either DA or NA at one of the following concentrations: $1{\cdot}50 \times 10^{-10}$ mol (29·52 nCi)/ml or $327{\cdot}45 \times 10^{-10}$ mol (136·55 nCi)/ml in the case of DA and $1{\cdot}18 \times 10^{-10}$ mol (31·79 nCi)/ml or $(296{\cdot}69 \times 10^{-10}$ mol (161·97 nCi)/ml in the case of NA.

The perfusion solutions were prepared by dilution of previously prepared stock solutions of ^{3}H-DA or (±)-^{3}H-NA with oxygenated Krebs–Ringer solution to which 50 mg/ml ascorbic acid had been added. The stock solutions were prepared from 7-^{3}H-DA (NENCO 1·5 Ci/mmol) or (±)-7-^{3}H-NA (NENCO 9·71 Ci/mmol) respectively, and the appropriate amounts of unlabelled DA or (±)-NA. The hearts were homogenized in 0·4 N perchloric acid and the perfusates collected as described by Hellmann *et al.* (1970).

Determination of ^{3}H-DA, ^{3}H-NA and their metabolites

The procedures for the isolation and determination of the ^{3}H-DA and ^{3}H-DA metabolites are modifications of the isolation procedures described by Kopin, Axelrod & Gordon (1961) and Bertler, Carlsson & Rosengren (1958), as used by Hellmann *et al.* (1970). The method of Kopin *et al.* (1961) was used without modification for the isolation of the ^{3}H-NA and its metabolites.

Portions of the perfusion solutions were acidified and processed in the same way as the tissue homogenates to determine the contamination of the isolated metabolite fractions by ^{3}H-DA. Appropriate corrections were then made. A correction was made for the extracellular space using a value of 0·325 ml/g tissue (Iversen, 1963).

Determination of endogenous NA

Endogenous NA was determined fluorometrically in the alumina eluates by the trihydroxyindole method (Udenfriend, 1962). Total radioactivity (TA) was determined in the perfusates and in the supernatants of the homogenates. The following fractions were isolated and their activity determined: alumina eluates: (AL), sum of ^{3}H-DA, ^{3}H-NA and their deaminated catechol metabolites; ^{3}H-dopamine: ^{3}H-DA; ^{3}H-noradrenaline: ^{3}H-NA; deaminated catechol metabolites: ^{3}H-DH; O-methylated amine metabolites: ^{3}H-OM; and deaminated O-methylated metabolites: ^{3}H-OMDH.

Results

Effect of pretreatment with 6-OH-DA on endogenous NA

The endogenous NA concentration (mean ± S.E.M.) in the hearts of control animals ($n=5$) was $0{\cdot}742 \pm 0{\cdot}074$ μg/g; in the hearts of the 6-OH-DA treated animals ($n=5$) the NA concentration was decreased to $0{\cdot}178 \pm 0{\cdot}032$ μg/g (24% of the controls).

Hearts perfused with $1{\cdot}50 \times 10^{-10}$ mol/ml ^{3}H-DA (low ^{3}H-DA perfusion concentration) (Table 1a)

The radioactivity in fractions TA, AL, ^{3}H-DA, ^{3}H-NA, ^{3}H-DH, and ^{3}H-OMDH was significantly lower in the hearts of the pretreated group than in the hearts of the control group. The effect was greatest in the case of AL and ^{3}H-DA and least with ^{3}H-OMDH. ^{3}H-OM, which was below the level of detection in the control group, was $0{\cdot}11 \times 10^{-10}$ mol/g in the pretreated group.

Perfusates

Only the radioactivity in fractions TA, AL and ^{3}H-DA could be determined with reasonable accuracy in the perfusates. The small amount of radioactivity in fractions ^{3}H-NA, ^{3}H-OM, ^{3}H-DH and ^{3}H-OMD cannot be determined accurately in the presence of high concentrations of ^{3}H-DA. The differences between the concentrations in fractions TA, AL and ^{3}H-DA from the perfusates of the control and the pretreated group were highly significant. The amounts of radioactivity found in fractions TA, AL and ^{3}H-DA in the hearts corresponded very well to the decrease in radioactivity of the same fractions from the perfusates. Complete quantitative agreement between the sum of radioactivity in hearts and perfusates and the radioactivity of the perfusion solution was obtained in both controls and pretreated

animals if calculated on the basis of the individual flow rates of the hearts (64–80 ml/8 min).

Hearts perfused with $327{\cdot}45 \times 10^{-10}$ mol/ml ^{3}H-DA (high ^{3}H-DA perfusion concentration) (Table 1b)

The 6-OH-DA group differed significantly from the control group only in fractions TA, AL and ^{3}H-DH. The ^{3}H-DA values were lower in the pretreated group, but the difference was not significant ($0{\cdot}1 > P > 0{\cdot}05$). The fraction of ^{3}H-DA converted to ^{3}H-NA was too small to yield exact values. The radioactivity in fractions ^{3}H-OM and ^{3}H-OMDH was approximately equal in both groups.

Perfusates

At the high ^{3}H-DA perfusion concentration there was no significant difference between the radioactivity in the perfusates of the control and the pretreated group. The amount of radioactivity taken up by the hearts represents only 1–2% of the total amount passing through the hearts during the 8 min perfusion period. This is too small an amount to be reflected by any changes in the concentration in 1 ml perfusates.

Hearts perfused with $1{\cdot}18 \times 10^{-10}$ mol/ml ^{3}H-NA (low ^{3}H-NA perfusion concentration) (Table 2a)

In the 6-OH-DA group the radioactivity in fractions TA, AL, ^{3}H-NA and ^{3}H-DH was significantly lower than in the control hearts, fraction ^{3}H-DH showing the

TABLE 1. *Distribution of total radioactivity, ^{3}H-dopamine, and its ^{3}H-metabolites in isolated rat hearts and in perfusates*

(a)

	Hearts		Perfusates	
	Control	6-OH-DA*	Control	6-OH-DA
TA	10·04±0·47	2·03±0·10*	1·34±0·01	1·47±0·02‡
AL	9·78±0·43	1·72±0·08*	1·01±0·02	1·13±0.02*
^{3}H-DA	7·30±0·44	0·98±0·06*	0·82±0.01	1·00±0·01*
^{3}H-NA	1·13±0·08	0·28±0·02*	Not determined	
^{3}H-DH	1·48±0·14	0·35±0·03*	··	
^{3}H-OMDH	0·62±0·03	0·22±0·01*	··	
^{3}H-OM	0	0·11±0·04*	··	

(b)

	Hearts		Perfusates	
	Control	6-OH-DA	Control	6-OH-DA
TA	429·08±11·50	364·25±14·38‡	314·82±2·68	309·43±1·62
AL	437·65±11·76	353·59±15·42*	312·37±3·06	312·21±3·71
^{3}H-DA	242·03±21·63	189·48±13·92	273·02±6·87	270·93±6·25
^{3}H-NA	Not determined		Not determined	
^{3}H-DH	189·74±16·27	135·29±12·42†	··	
^{3}H-OMDH	14·97± 1·18	13·79± 0·78	··	
^{3}H-OM	6·80± 0·92	8·10± 0·85	··	

Rats were divided into control animals and those pretreated with 6-hydroxydopamine (6-OH-DA) as described in **Methods**. Perfusion was carried out with either $1{\cdot}50 \times 10^{-10}$ mol/ml (a) or $327{\cdot}45 \times 10^{-10}$ mol/ml (b) ^{3}H-dopamine for 8 min (eight hearts in each group). All values (means ±S.E.M.) are expressed as 10^{-10} mol/g tissue or perfusate. TA, total radioactivity expressed as dopamine equivalents; AL, activity in the alumina eluates, sum of ^{3}H-DA, ^{3}H-NA and ^{3}H-DH; ^{3}H-DA, ^{3}H-dopamine; ^{3}H-NA, ^{3}H-noradrenaline; ^{3}H-DH, sum of the deaminated catechol metabolites; ^{3}H-OM, sum of the O-methylated amine metabolites; ^{3}H-OMDH, sum of the O-methylated-deaminated metabolites. * $P<0{\cdot}001$; ‡ $P<0{\cdot}005$; † $P<0{\cdot}02$.

smallest difference. The radioactivity in fractions ^{3}H-OM and ^{3}H-OMDH was higher in the 6-OH-DA group than in the controls.

Perfusates

As in the experiments with ^{3}H-DA it was not possible to obtain exact values for the concentration of ^{3}H-NA metabolites in the perfusate on account of their small contribution to the total radioactivity as compared with the high ^{3}H-NA activity. The differences between the pretreated group and the controls in respect of fractions TA, AL and ^{3}H-NA from the perfusates were highly significant and corresponded very well to the radioactivity found in the same fractions from the hearts.

Hearts perfused with $296{\cdot}69\times10^{-10}$ mol/ml ^{3}H-NA (high ^{3}H-NA perfusion concentration) (Table 2b)

Only the radioactivity in fractions TA and AL was significantly lower in the hearts of pretreated rats than in the controls. There was no significant difference in ^{3}H-NA ($0{\cdot}1>P>0{\cdot}05$), ^{3}H-OM, ^{3}H-OMDH and ^{3}H-DH values between the two groups.

Perfusates

As in the experiments with the high ^{3}H-DA perfusion concentration, the amount of radioactivity taken up by the hearts was too small to cause any significant change of the radioactivity of the perfusate.

Discussion

In our experiments the endogenous NA of the hearts was decreased by 6-OH-DA pretreatment to 24% of the control values. The localization of the residual NA has been discussed by Thoenen & Tranzer (1968). They assumed that some sympathetic

TABLE 2. *Distribution of total radioactivity, ^{3}H-noradrenaline and its ^{3}H-metabolites in isolated rat hearts and in perfusates*

(a)

	Hearts		Perfusates	
	Control	6-OH-DA	Control	6-OH-DA
TA	$13{\cdot}10\pm0{\cdot}72$	$2{\cdot}33\pm0{\cdot}11$*	$1{\cdot}03\pm0{\cdot}01$	$1{\cdot}14\pm0{\cdot}01$*
AL	$12{\cdot}99\pm0{\cdot}78$	$1{\cdot}47\pm0{\cdot}11$*	$0{\cdot}95\pm0{\cdot}02$	$1{\cdot}04\pm0{\cdot}01$‡
^{3}H-NA	$12{\cdot}30\pm0{\cdot}73$	$1{\cdot}24\pm0{\cdot}11$*	$0{\cdot}85\pm0{\cdot}02$	$0{\cdot}96\pm0{\cdot}01$*
^{3}H-DH	$0{\cdot}36\pm0{\cdot}03$	$0{\cdot}16\pm0{\cdot}02$*	Not determined	
^{3}H-OMDH	$0{\cdot}04\pm0{\cdot}01$	$0{\cdot}13\pm0{\cdot}01$*	,,	
^{3}H-OM	$0{\cdot}31\pm0{\cdot}06$	$0{\cdot}63\pm0{\cdot}05$*	,,	

(b)

	Hearts		Perfusates	
	Control	6-OH-DA	Control	6-OH-DA
TA	$452{\cdot}56\pm19{\cdot}57$	$355{\cdot}62\pm32{\cdot}98$†	$286{\cdot}66\pm0{\cdot}79$	$288{\cdot}17\pm1{\cdot}51$
AL	$404{\cdot}22\pm23{\cdot}09$	$306{\cdot}72\pm33{\cdot}96$†	$267{\cdot}32\pm1{\cdot}47$	$268{\cdot}73\pm1{\cdot}87$
^{3}H-NA	$330{\cdot}81\pm28{\cdot}46$	$240{\cdot}78\pm33{\cdot}48$	$261{\cdot}66\pm17{\cdot}88$	$266{\cdot}59\pm14{\cdot}78$
^{3}H-DH	$56{\cdot}07\pm7{\cdot}14$	$49{\cdot}71\pm4{\cdot}08$	Not determined	
^{3}H-OMDH	$7{\cdot}59\pm1{\cdot}03$	$8{\cdot}78\pm0{\cdot}77$	,,	
^{3}H-OM	$28{\cdot}15\pm0{\cdot}97$	$28{\cdot}14\pm1{\cdot}63$	,,	

Rats were divided into control animals and those pretreated with 6-hydroxydopamine (6-OH-DA) as described in **Methods**. Perfusion was carried out with either $1{\cdot}18\times10^{-10}$ mol/ml (a) or $296{\cdot}69\times10^{-10}$ mol/ml (b) ^{3}H-noradrenaline for 8 min (eight hearts in each group). All values (means ±S.E.M.) are expressed as 10^{-10} mol/g tissue or perfusate. For explanation of the abbreviations see legend to Table 1. * $P<0{\cdot}001$; ‡ $P<0{\cdot}005$; † $P<0{\cdot}05$.

nerve terminals survived the 6-OH-DA treatment, being protected by a limited blood supply or by diffusional or metabolic barriers. Another explanation offered by these authors is that the NA accumulates in the sympathetic neurons proximal to the damaged part, as is seen after nerve ligature (Dahlström & Häggendal, 1966).

Using a low perfusion concentration of ^{3}H-DA or ^{3}H-NA, the uptake of CA in the 6-OH-DA group was reduced by 80%. The diminished uptake was reflected by increased CA concentrations in the perfusates. A similar inhibition of CA uptake into isolated, perfused rat hearts was obtained by immunosympathectomy (Iversen, Glowinski & Axelrod, 1966) or by perfusion with cocaine (Iversen, 1963 ; Eisenfeld *et al.*, 1967).

In contrast, pretreatment with 6-OH-DA only slightly reduced the uptake of ^{3}H-DA or ^{3}H-NA at a high perfusion concentration. The uptake was decreased by only 15% in the chemically sympathectomized hearts. Using the kinetic data given by Hellmann *et al.* (1970), one can calculate that with a perfusion concentration of $327{\cdot}45 \times 10^{-10}$ mol/ml DA the initial rate of Uptake 1 is about 20% of the initial rate of Uptake 2.

Assuming a constant rate of uptake for both uptake mechanisms over the 8 min period, the inhibition of the intraneuronal Uptake 1 could account for a decrease in radioactivity taken up of about 20%. The decrease in the radioactivity in fractions TA, AL and ^{3}H-DA in the 6-OH-DA group corresponded fairly well to this value and may thus be explained by the diminished Uptake 1 mechanism in these hearts.

Our data present further evidence that at low perfusion concentrations CA are taken up into isolated rat hearts mainly by an uptake process which is confined to intact sympathetic nerve endings, as was concluded from experiments using histochemical methods (Farnebo & Malmfors, 1969).

A decrease in β-hydroxylation has been observed in tissues of immunosympathectomized rats by Iversen *et al.* (1966) and in surgically denervated tissues by Carlsson & Waldeck (1963) and Potter, Cooper, Willman & Wolfe (1965). The dopamine-β-hydroxylase of peripheral sympathetic nerve is associated with the intraneuronal storage granules (Potter & Axelrod, 1963). In our experiments with a low ^{3}H-DA perfusion concentration ^{3}H-NA synthesis was greatly diminished in the 6-OH-DA pretreated group. This finding can be taken as indirect evidence for a decrease in dopamine-β-hydroxylase activity after treatment with 6-OH-DA, which is in agreement with the observation of Molinoff, Weinshilboum & Axelrod (1970). That endogenous NA was still found and that β-hydroxylation could be demonstrated in the chemically denervated tissue suggest that some sympathetic nerve tissue survived the treatment with 6-OH-DA and that a part of the remaining uptake is localized in the residual sympathetic nerve tissue.

It is well known that DA is a better substrate for monoamine oxidase than NA (Blaschko, Richter & Schlossmann, 1937). It can be seen from our data that at both the low and high perfusion concentrations deaminated metabolites comprised a larger fraction of the TA in the DA experiments than in the NA experiments. In the 6-OH-DA hearts perfused with a low ^{3}H-DA or ^{3}H-NA concentration, there was a considerable decrease in deaminated metabolites as compared with the controls. This demonstrates that at substrate concentrations at which Uptake 1 predominates, most of the deamination occurs intraneuronally. On the other hand, at a high ^{3}H-NA perfusion concentration (predominantly Uptake 2) there was no significant difference in the amount of deaminated metabolites between the control

and the 6-OH-DA group. This indicates that most of the NA was deaminated extraneuronally.

At a high ^{3}H-DA perfusion concentration the radioactivity in fractions TA and AL, and also in ^{3}H-DH were lowered in the chemically sympathectomized hearts. Since DA is a better substrate for monoamine oxidase than NA, the proportion of intraneuronal deamination is far greater for DA, and hence, differences between the control and pretreated groups become of significance. ^{3}H-OM metabolites were increased in the 6-OH-DA group using a low perfusion concentration of either ^{3}H-DA or ^{3}H-NA. Similarly, an increase in O-methylation was observed under conditions in which the uptake of NA into the sympathetic nerves was inhibited by cocaine (Eisenfeld *et al.*, 1967), by immunosympathectomy (Iversen *et al.*, 1966) or by surgical denervation (Potter *et al.*, 1965). At a high perfusion concentration of ^{3}H-DA or ^{3}H-NA there was no difference in the O-methylated metabolites between controls and the 6-OH-DA group, although the relative proportion of O-methylation was increased in the latter group.

The changes in the deaminated and O-methylated metabolites of ^{3}H-NA and ^{3}H-DA observed in our experiments are compatible with the hypothesis that O-methylation occurs extraneuronally, whilst deamination takes place intra-, as well as extraneuronally. Inhibition of the uptake mechanism into the sympathetic nerve endings renders the CA subject to extraneuronal metabolic degradation. The metabolic fate of catecholamines in the isolated rat heart is therefore primarily determined by their distribution within the extra- and intraneuronal tissue compartments and not only by their affinity for enzymes. The distribution, in turn, is dependent on the perfusion concentration used and on the relative affinity of the particular CA for Uptake 1 and Uptake 2.

REFERENCES

Axelrod, J., Weil-Malherbe, H. & Tomchick, R. (1959). The physiological disposition of ^{3}H-epinephrine and its metabolite metanephrine. *J. Pharmac. exp. Ther.*, **127**, 251–256.

Bertler, A., Carlsson, A. & Rosengren, E. (1958). A method for the fluorometric determination of adrenaline and noradrenaline in tissues. *Acta physiol. scand.*, **44**, 273–292.

Blaschko, H., Ritchter, D. & Schlossmann, H. (1937). The oxidation of adrenaline and other amines. *Biochem. J.*, **31**, 2187–2196.

Carlsson, A. & Waldeck, B. (1963). β-hydroxylation of tyramine *in vivo*. *Acta pharmac. tox.*, **20**, 371–374.

Dahlström, A. & Häggendal, J. (1966). Studies on the transport and lifespan of amine storage granules in a peripheral adrenergic neuron system. *Acta physiol. scand.*, **67**, 278–288.

Ehinger, B. & Sporrong, B. (1968). Neuronal and extraneuronal localization of noradrenaline in the rat heart after perfusion at high concentration. *Experientia*, **24**, 256–266.

Eisenfeld, A. J., Krakoff, L., Iversen, L. L. & Axelrod, J. (1967). Inhibition of the extraneuronal metabolism of noradrenaline in the isolated heart by adrenergic blocking agents. *Nature, Lond.*, **213**, 297–298.

Farnebo, L. O. & Malmfors, T. (1969). Histochemical studies on the uptake of noradrenaline and α-methyl-noradrenaline in the perfused rat heart. *Eur. J. Pharmac.*, **5**, 313–320.

Hellmann, G., Hertting, G. & Peskar, B. (1970). Uptake kinetics and metabolism of 7-^{3}H-dopamine in the isolated perfused rat heart. *Br. J. Pharmac.*, **41**, 256–269.

Iversen, L. L. (1963). The uptake of noradrenaline by the isolated perfused rat heart. *Br. J. Pharmac. Chemother.*, **21**, 523–537.

Iversen, L. L. (1965). The uptake of catecholamines at high perfusion concentration in the rat isolated heart: a novel catecholamine uptake process. *Br. J. Pharmac. Chemother.*, **25**, 18–33.

Iversen, L. L. (1967). *The Uptake and Storage of Noradrenaline in Sympathetic Nerves*. pp. 108–147. Cambridge University Press.

Iversen, L. L., Glowinski, J. & Axelrod, J. (1966). The physiologic disposition and metabolism of norepinephrine in immunosympathectomized animals. *J. Pharmac. exp. Ther.*, **151**, 273–284.

Iversen, L. L. & Langer, S. Z. (1969). Effects of phenoxybenzamine on the uptake and metabolism of noradrenaline in the rat heart and vas deferens. *Br. J. Pharmac.*, **37**, 627–637.

KOPIN, I. J., AXELROD, J. & GORDON, E. (1961). The metabolic fate of ^{3}H-epinephrine and ^{14}C-metanephrine in the rat. *J. biol. Chem.*, **236**, 2109–2113.

LAVERTY, R., SHARMAN, D. F. & VOGT, M. (1965). Action of 2,4,5,-trihydroxyphenylethylamine on the storage and release of noradrenaline. *Br. J. Pharmac. Chemother.*, **24**, 549–560.

LIGHTMAN, S. L. & IVERSEN, L. L. (1969). The role of Uptake 2 in the extraneuronal metabolism of catecholamines in the isolated rat heart. *Br. J. Pharmac.*, **37**, 638–649.

MALMFORS, T. & SACHS, C. (1968). Degeneration of adrenergic nerves produced by 6-hydroxydopamine. *Eur. J. Pharmac.*, **3**, 89–92.

MOLINOFF, P. B., WEINSHILBOUM, R. & AXELROD, J. (1970). The effect of reserpine and 6-hydroxydopamine on dopamine β-hydroxylase activity. *Fedn Proc.*, **29**, 278.

PORTER, C. C., TOTARO, J. A. & STONE, C. A. (1963). Effect of 6-hydroxydopamine and some other compounds on the concentration of norepinephrine in the hearts of mice. *J. Pharmac. exp. Ther.*, **140**, 308–316.

POTTER, L. T. & AXELROD, J. (1963). Properties of norepinephrine storage particles of the rat heart. *J. Pharmac. exp. Ther.*, **142**, 299–305.

POTTER, L. T., COOPER, T., WILLMAN, V. L. & WOLFE, D. E. (1965). Synthesis, binding, release and metabolism of norepinephrine in normal and transplanted dog hearts. *Circulation Res.*, **16**, 468–481.

THOENEN, H. & TRANZER, J. P. (1968). Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **261**, 271–288.

TRANZER, J. P. & THOENEN, H. (1967). Ultramorphologische Veränderungen der sympathischen Nervenedigungen der Katze nach Vorbehandlung mit 5-und 6-Hydroxydopamin. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **257**, 257, 343.

UDENFRIEND, S. (1962). Fluorescence Assay in Biology and Medicine. London: Academic Press.

WHITBY, L. G., AXELROD, J. & WEIL-MALHERBE, H. (1961). The fate of ^{3}H-norepinephrine in animals *J. Pharmac. exp. Ther.*, **132**, 193–201.

(*Received July* 21, 1970)