found after $CCl_4$ extraction in the presence of ferricyanide possibly derives from labile conjugates of the hydroxylamine splitting during extraction. Until the presence of such a conjugate is substantiated it seems that the major part of the *N*-oxidation products of 2-naphthylamine is excreted in the form of free hydroxylamine.

There remains the interesting question whether the forced metabolic formation and excretion of the *N*-oxidation products of 2-naphthylamine by phenobarbital pretreatment might correspond to an increase in the carcinogenicity for the bladder or possibly other organs of the dog.

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# Effect of reserpine and metaraminol on excretion of homovanillic acid and 3-methoxy-4-hydroxyphenolglycol in the rat

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NOREPINEPHRINE synthesis has been studied by estimation of its turnover rate by using the rate of disappearance of the isotopically labeled catecholamine[1–3] or of the endogenous compound after inhibition of its synthesis.[4] Direct assessment of changes in the rate of norepinephrine synthesis by using isotopically labeled tyrosine *in vitro*[5–8] and *in vivo*[9, 10] is possible only if the labeled product is not destroyed or if both the labeled catecholamine and its metabolites are determined. If the synthesized catecholamine is not retained in the tissues, these methods cannot be used *in vivo*. In the

rat, homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) sulfate account for over 70 per cent of the excreted metabolites of dopamine[11] and norepinephrine[12] respectively. The excretion of these metabolites may be used as an index of the synthesis of the catecholamines, even after chronic depletion of norepinephrine stores, provided there is no major alteration in metabolic route. We have used this index to assess catecholamine formation in the intact rat during chronic administration of two drugs which deplete norepinephrine stores, reserpine and metaraminol.

Homovanillic acid-methoxy-$C^{14}$ was prepared enzymatically by using catechol-*O*-methyl transferase (COMT),[13] S-adenosylmethionine-$C^{14}$ (40 mc/m-mole; New England Nuclear Corp., Boston, Mass.) and dihydroxyphenylacetic acid (Calbiochem, Los Angeles, Calif.). The labeled compound was extracted from the acidified incubation mixture by using 4 vol. of ethyl acetate. Purity was verified by paper chromatography. MHPG-$H^3$-sulfate was isolated from the urine of rats given 1 mc DL-norepinephrine-7-$H^3$ (New England Nuclear Corp.) i.p., by using Dowex-1.[14] The two isotopically labeled compounds were mixed in a solution to contain about 60,000 cpm MHPG-$H^3$-sulfate and 40,000 cpm HVA-$C^{14}$ per ml. These compounds were found to be stable for at least 1 month at 4°.

Male Sprague–Dawley rats weighing 200–250 g were given daily i.p. injections of saline, reserpine (2·5 mg/kg) or metaraminol (3 mg/kg); urine was collected during the third day of treatment. Immediately after collection, 500 $\mu$l of the solution containing MHPG-$H^3$-sulfate and HVA-$C^{14}$ was added to each urine specimen and the urines were stored at 4° until analyzed (within 1 week of collection).

HVA was extracted from the acidified urine with ethyl acetate, and the aqueous residue was retained for assay of MHPG. The HVA was back extracted into 1 N $K_2CO_3$ and re-extracted into ethyl acetate after acidification of the aqueous phase. The ethyl acetate extract was concentrated by evaporation *in vacuo*, and the HVA was purified by paper chromatography (isopropanol:ammonia: water; 8:1:1). After elution with water, the HVA was assayed by the fluorimetric method described by Anden *et al.*[15] An aliquot of the eluate was assayed for $C^{14}$ to correct for variations in recovery of HVA from the urine.

The aqueous phase of the extracted urine containing MHPG-sulfate which had been retained was treated with 2 ml of 10% barium acetate and adjusted to pH 5·5 with 5 N sodium hydroxide. After centrifugation, an aliquot of the clear supernatant was incubated with Glusulase (Endo Products, New York, N.Y.) at 37° for 24 hr. The hydrolyzed MHPG was then assayed by the gas chromatographic method of Wilk *et al.*[16] Aliquots of the final solution containing MHPG were assayed for tritium to correct simultaneously for incomplete hydrolysis, aliquoting and partition coefficients.

In saline-treated animals, about 3 times as much MHPG as HVA was excreted (Table 1). After treatment with reserpine, there was a marked increase in excretion of HVA and a striking decrease in

TABLE 1. EFFECT OF CHRONIC DRUG TREATMENT ON URINARY EXCRETION OF MAJOR CATECHOLAMINE METABOLITES IN THE RAT*

| Drugs | (Dopamine) HVA | (Norepinephrine) MHPG | Total |
|---|---|---|---|
| Saline | 19·7 ± 2·1 | 62·3 ± 4·5 | 83·0 ± 5·0 |
| Reserpine (2·5 mg/kg/day) | 76·0† ± 8·3 | 20·2† ± 2·7 | 96·2 ± 8·4 |
| Metaraminol (3 mg/kg/day) | 31·1‡ ± 4·2 | 56·2 ± 7·8 | 87·4 ± 12·4 |

* Drugs were administered i.p. in the doses indicated, and urine was collected on the third day. Results are expressed as $\mu$g/day and are the mean excretion rates (± S.E.M.) for groups of 6 rats.

† $P < 0{\cdot}01$.

‡ $P < 0{\cdot}05$.

urinary MHPG; but the sum of these metabolites did not differ significantly from that in saline-treated animals. Metaraminol administration resulted in a significant increase in HVA excretion. No significant changes in MHPG excretion were apparent, and there was no alteration in the sum of these metabolites.

It is likely that almost all dopa formed from tyrosine in the adrenergic neurone is converted to dopamine. Some of the dopamine is destroyed by monoamine oxidase (MAO) or by COMT and is finally excreted as HVA. Since the excretion rate of MHPG exceeds that of HVA, it is likely that most of the dopamine formed is converted to norepinephrine, which is subsequently metabolized to MHPG.

After administration of reserpine, norepinephrine stores are rapidly depleted[17] and the rate of MHPG excretion is accelerated.[18] One hr after the treatment with reserpine, conversion of labeled tyrosine to norepinephrine is found to be decreased in the isolated rabbit heart.[19] Since conversion of dopamine to norepinephrine (and its metabolites) is markedly decreased by reserpine pretreatment,[7, 19] it was concluded that diminished norepinephrine synthesis is a consequence of inhibition of conversion of dopamine to norepinephrine, presumably because of inhibition of dopamine uptake by the dopamine-$\beta$-hydroxylase-containing storage vesicles.[20, 21]

In patients treated with reserpine, there is an elevated HVA excretion and a decrease in urinary VMA.[22] In rats treated with large doses of reserpine, the increase in HVA is more marked and the decrease in MHPG more striking than the corresponding changes in patients; but there is no significant change in the sum of MHPG and HVA (which have similar molecular weights). Since reserpine does not appear to alter the ratio of *O*-methylated to catechol-deaminated products of catecholamines,[18] these results suggest that, in spite of the marked diminution in tissue levels of norepinephrine, there is no increase in hydroxylation of tyrosine.

Similarly, metaraminol, in doses which markedly diminish tissue levels of norepinephrine by displacing the catecholamine,[23] does not alter total catecholamine production (Table 1). There appears to be some interference with conversion of dopamine to norepinephrine, since HVA excretion is elevated in the metaraminol-treated rats, but the variability in urinary MHPG may have obscured the significance of the slight decrease in mean levels of this metabolite. The total tissue content of norepinephrine does not appear to influence hydroxylation of tyrosine. This reaction has been considered to be the rate-limiting step in norepinephrine biosynthesis,[24] and levels of free catecholamines are thought to affect the reaction by a negative feedback.[6] Since catecholamine formation is not accelerated when norepinephrine synthesis is diminished and its stores depleted, it appears that dopamine, which probably is present mainly in an unbound form, may be the catecholamine that is mainly responsible, at least in these circumstances, for feedback inhibition of tyrosine hydroxylase.

When low amounts of dopa-$H^3$ are injected, there is no increase in norepinephrine-$H^3$ formation during nerve stimulation.[9, 10] If dopa-$C^{14}$ is present in excess, however, formation of norepinephrine-$C^{14}$ is accelerated by sympathetic nerve stimulation.[25] This apparent discrepancy may be explained if dopamine uptake by vesicles is accelerated during nerve stimulation. Dopa-$C^{14}$ is rapidly decarboxylated and the excess dopamine-$C^{14}$ cannot be converted to norepinephrine-$C^{14}$ as rapidly as it is formed. Under these circumstances the rate-limiting step is uptake of dopamine and conversion to norepinephrine.

When trace amounts of dopamine-$H^3$ are formed, the rate of decarboxylation does not exceed the capacity for uptake and $\beta$-hydroxylation, so that conversion of dopa-$H^3$ to dopamine-$H^3$ is rate-limiting. Increased conversion of tyrosine to norepinephrine during stimulation is therefore not reflected in increased norepinephrine-$H^3$ formation from trace amounts of dopa-$H^3$. The mechanism of increased tyrosine hydroxylation during stimulation might involve increased uptake by emptied vesicles of cytoplasmic dopamine, which is converted to norepinephrine to replace the lost catecholamine. The consequent decrease in cytoplasmic dopamine and decreased feedback inhibition of tyrosine hydroxylase may be the stimulus for increased dopa formation.

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## Effects of desipramine on noradrenaline uptake into isolated nerve granules

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DESIPRAMINE (DMI) is one of the most potent inhibitors of noradrenaline (NA) uptake into sympathetically innervated tissues.[1] Considerable evidence indicates that the inhibitory effect of this drug on the uptake process is exerted on a NA transport mechanism located in the axonal membrane.[2,3] However, the concept that DMI may, in addition, affect amine uptake into the intraaxonal storage particles ("nerve granules ') has recently been proposed.[4]

The present experiments were prompted by the observation that DMI causes some inhibition of NA uptake into isolated whole bovine splenic nerves *in vitro*,[5] and were designed to determine to what extent the observed effect of DMI could be due to interference with NA uptake into the granules from such nerves.

### MATERIAL AND METHODS

Bovine splenic nerves were obtained at the slaughter house within 15–30 min post mortem, and were immediately chilled on ice. After careful removal of contaminating tissue, the nerves were