Excretion of Nortriptyline Hydrochloride in Man I

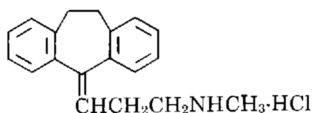
Detection and Determination of Urinary Nortriptyline

By M. E. AMUNDSON* and J. A. MANTHEY

A quantitative U.V. spectrophotometric assay procedure has been developed for the determination of urinary nortriptyline in man. A thin-layer chromatographic system is described which allows rapid detection of the presence of the drug and its metabolites in urine. The drug was found to be slowly eliminated as a mixture of free drug and metabolites.

NORTRIPTYLINE hydrochloride,[1] 5-(3-methylaminopropylidene) - 10,11 - dihydro - 5*H*-dibenzo[*a*,*d*]cycloheptene hydrochloride, has recently been introduced as an antidepressant. This communication describes an assay procedure for the quantitative determination of the drug in the urine of patients receiving therapeutic doses.

The need for urine tests for the detection and/



Nortriptyline HCl

or determination of phenothiazine and related drugs in chronic mental patients has been established (1). The assay method reported here permits a reliable evaluation of drug intake by patients treated with nortriptyline hydrochloride.

EXPERIMENTAL

Drug Administration and Urine Collection.—All subjects except the normal male used for the single dose experiments were under treatment at Central State Hospital, Indianapolis, Ind., and were on a regular oral dosage schedule which ranged from 50–150 mg./day depending upon the individual patient. Twenty-four hour urine collections were made from 7:30 a.m.–7:30 a.m. For the single-dose experiments, the subject received an oral dose of 25 or 30 mg., and urine was collected at regular intervals up to as long as 72 hr. after dosage.

Assay Method.—*Method A*.—An aliquot of the urine (25 ml. for chronic studies, 100 ml. for single dose studies) was transferred to a 250-ml. separator. The urine was made distinctly alkaline with 50% sodium hydroxide and was extracted for 3 min. with 2 successive 50-ml. portions of ether. The extracted urine was saved for *Method B*. The ether was washed with 25 ml. of 0.1 *N* sodium hydroxide and 25 ml. of water, respectively. The washes were discarded. The ether was extracted for 3 min. with 2 successive 10-ml. portions of 0.1 *N* hydrochloric acid. The acid extracts were collected in a 25-ml. volumetric flask, and the solution was made to 25 ml. with additional acid. The absorbance of this solution (solution *A*) then was determined on a suitable spectrophotometer at the $\lambda_{max.}$ of approximately 240 mμ.

Method B.—The extracted urine from *Method A* was neutralized with 6 *N* hydrochloric acid and 0.2 vol. of concentrated hydrochloric acid was added. The solution was heated on a steam bath for 1 hr. and then was allowed to cool to room temperature. The solution was made alkaline with 50% sodium hydroxide using an ice bath, if necessary, to prevent excessive warming. The solution was then extracted with ether as in *Method A*. The absorbance of the acid extract (solution *B*) was determined at the $\lambda_{max.}$ of approximately 290 mμ.

Method C.—One-tenth volume of concentrated hydrochloric acid was added to an accurately measured aliquot of solution *A*. After heating for 1 hr. on a steam bath, the solution was allowed to cool and was diluted to an appropriate volume with water. The absorbance of this solution was determined at the $\lambda_{max.}$ of approximately 290 mμ.

Methods *A*, *B*, and *C* were repeated on an equal volume of control urine to establish blank urine values.

Calculations.—All results were calculated as equivalents of nortriptyline hydrochloride.

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[1] Marketed as Aventyl HCl by Eli Lilly and Co., Indianapolis, Ind.

$$\frac{\text{abs}_{240\text{m}\mu}\,(\text{soln. }A) - \text{abs}_{240\text{m}\mu}\,(\text{blank }A)}{0.482^{2}} \times \frac{10\text{ mcg.}}{\text{ml.}} \times \frac{\text{total urine vol.}}{\text{vol. of aliquot taken}} \times \frac{1}{1000} = \text{mg. unchanged nortriptyline} + \text{"free" hydroxylated nortriptyline } (X)$$

$$\frac{\text{abs}_{290\text{m}\mu}\,(\text{soln. }B) - \text{abs}_{290\text{m}\mu}\,(\text{blank }B)}{0.433^{3}} \times \frac{10\text{ mcg.}}{\text{ml.}} \times \frac{\text{total urine vol.}}{\text{vol. of aliquot taken}} \times \frac{1}{1000} \times \frac{1}{0.85^{4}} = \text{mg. "bound" hydroxylated nortriptyline } (Y)$$

$$\frac{\text{abs}_{290\text{m}\mu}\,(\text{soln. }C) - \text{abs}_{290\text{m}\mu}\,(\text{blank }C)}{0.433^{3}} \times \text{same as for calculation of } Y = \text{mg. "free" hydroxylated nortriptyline } (Z)$$

$X - Z$ = mg. unchanged nortriptyline hydrochloride, and $X + Y$ = total mg. excreted expressed as nortriptyline hydrochloride equivalents.

Thin-Layer Chromatography.—Ether extracts of several urine samples were examined chromatographically for the presence of unchanged drug and metabolites. Standard size (20 × 20 cm.) glass plates were coated with a 250-μ layer of Silica Gel G. The plates were air-dried overnight before use. The developing solvent consisted of isopropanol–water, (88:12), one-third saturated with sodium chloride. The presence of free drug and metabolites was detected by spraying the plates with 1:1 3A ethanol–sulfuric acid followed by heating in a 90–100° oven for 75 min.

Figure 1 is a representative chromatogram of the extracts from 2 urine samples from patients who had received nortriptyline hydrochloride. The urine was extracted with ether as in *Method A* of the assay procedure. After washing with alkali and water, the ether was evaporated to dryness. The residue was redissolved in a small volume of methanol and was applied to the plates. The plates were placed in the developing solvent for approximately 2.5 hr. The plates were removed, air-dried, and sprayed with the ethanolic sulfuric acid. The plate was then placed in the oven to develop the characteristic colors given in Fig. 1.

RESULTS AND DISCUSSION

McMahon *et al.* (2) investigated the metabolism of nortriptyline-N-methyl-^{14}C in rats, and N-demethylation and hydroxylation of one of the bridgehead carbons were the major metabolic changes observed. Hucker (3) reported that the 2 major metabolic reactions of amitryptyline, the dimethylamino analog of nortriptyline, were also N-demethylation and hydroxylation. The metabolism of nortriptyline in man is currently under investigation and will be the subject of a future report.

The findings of McMahon *et al.* (2) were applied to the development of this assay method. When urine from a patient who had received nortriptyline was extracted with ether, a considerable amount of material which absorbed at 240 mμ was obtained. However, only a small percentage of this material was identified as unchanged nortriptyline by thin-layer chromatography (Fig. 1). Acid hydrolysis of the extracted urine yielded upon subsequent extraction a material which exhibited 290 mμ absorbance which is characteristic of the molecule in which the dimethylene bridge of nortriptyline has been replaced by a double bond as previously reported (2, 4). Acid hydrolysis of the material which had been obtained initially by ether extrac-

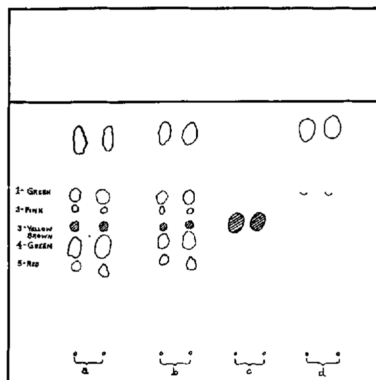

Fig. 1.—Thin-layer chromatography of urine extracts from patients receiving continued doses of nortriptyline hydrochloride. Key: a, urine extract No. 1902; b, urine extract No. 1903; c, nortriptyline; and d, control urine extract. Zone 3 identified as nortriptyline and zone 4 tentatively identified as 10-hydroxy nortriptyline. Colors shown are those observed after spraying with 1:1 ethanol–sulfuric acid and heating the plate in a 90–100° oven for 75 min.

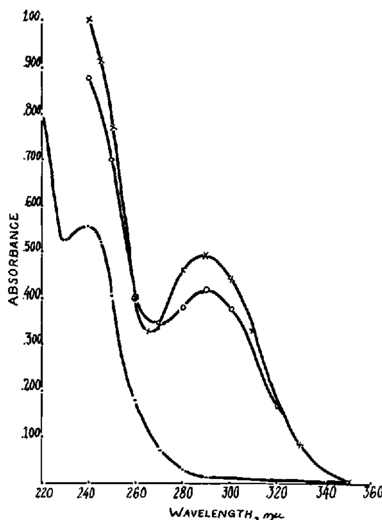

Fig. 2.—Typical U.V. absorption curves obtained on assay solutions. Key: ●, solution A = unchanged nortriptyline + "free" hydroxy derivative; ×, solution B = "bound" hydroxy derivative; and ○, solution C = "free" hydroxy derivative. Curves B and C were obtained after acid hydrolysis.

[2] 0.482 is the measured absorbance of a 10 mcg./ml. solution of nortriptyline hydrochloride in 0.1 N hydrochloric acid.
[3] 0.433 is the calculated absorbance of a 10 mcg./ml. solution of acid-hydrolyzed hydroxylated nortriptyline.
[4] 0.85 represents the recovery factor determined by adding hydroxylated nortriptyline to urine and hydrolyzing with acid as in B and C.

TABLE I.—URINARY EXCRETION OF NORTRIPTYLINE HYDROCHLORIDE (24-hr.)

Patient	Dose	Nortriptyline Equivalents, mg. Nortriptyline HCl, mg.	"Free" Metabolite	"Bound" Metabolite	Total	Av. % Dose
A	25 mg. bid	1.5	27.3	23.8	52.6	94.0
	25 mg. bid	2.6	18.0	20.6	41.2	
B	25 mg. qid	1.5	24.0	2.1	27.6	27.6
C	25 mg. qid	3.4	32.2	26.5	62.1	62.1
D	25 mg. qid	4.2	47.6	44.7	96.5	75.0
	25 mg. qid	2.6	28.9	22.0	53.5	
E	25 mg. qid	1.1	32.9	23.2	57.2	58.6
	25 mg. qid	3.9	43.1	21.8	68.8	
	25 mg. qid	1.9	34.5	13.5	49.9	
F	25 mg. qid	2.7	22.2	16.7	41.6	41.6
	25 mg. qid	5.3	22.0	16.3	43.6	
	25 mg. qid	4.3	30.8	10.4	45.5	
	25 mg. qid	3.0	24.6	8.3	35.9	
G	25 mg. qid	0.5	7.2	10.5	18.2	49.8
	25 mg. qid	0.8	35.7	35.3	71.8	
	25 mg. qid	1.6	37.0	35.8	74.4	
	25 mg. qid	1.0	29.5	4.5	35.0	
H	50 mg. tid	3.0	36.5	40.8	80.3	53.5
					Av.	57.8

tion of the urine exhibited the same U.V. absorption pattern. Typical curves obtained from extracts of urine before and after hydrolysis are shown in Fig. 2. It is assumed, therefore, that hydroxylation of nortriptyline does occur in man and that only part of this metabolite is subsequently conjugated.

Additional metabolites are present in the urine of man as evidenced by the chromatogram shown in Fig. 1. Only small amounts of unchanged drug were excreted (zone 3). Four metabolites were apparent in the extracts. Zone 4 has been tentatively identified as 10-hydroxynortriptyline and is present in the greatest concentration. The remaining 3 metabolites are currently under investigation. The relatively large zones near the solvent front apparently have no relationship to the metabolism of nortriptyline and represent extraneous material carried through the extraction procedure.

Triplicate assays of a urine sample by the above procedure showed a total excretion of 61.2 ± 1.8 (S.D.) mg. of nortriptyline equivalents.

Pure 10-hydroxy nortriptyline was not available to use as a standard in the assay procedure. The 0.433 absorbance value used in the calculations was calculated from the data of Villani *et al.* (4) on amitryptyline and its derivatives and represents the theoretical absorbance of a 10 mcg./ml. solution of the hydrochloride salt of the unsaturated molecule which results from the acid hydrolysis of the 10-hydroxy metabolite. Addition of a known concentration of impure 10-hydroxy material, obtained by extraction of a patient's urine to a control urine and subsequent acid hydrolysis and extraction, resulted in a recovery of 85% based on the calculated absorbance of 0.433.

Several of the patients received other drugs in addition to nortriptyline hydrochloride. Phenaglycodol[5] (1200 mg. daily), chlorothiazide[6] (500 mg. daily), perphenazine[7] (24 mg. daily), trifluoperazine[8] (45 mg. daily), and fluphenazine[9] (15 mg. daily) did not interfere with the assay procedure. Chlorpromazine[10] (300 mg. daily) did interfere in the extraction procedure, as was evidenced by the U.V. absorption curve obtained on the extract.

It was not possible to obtain control urine specimens from the patients: therefore, the results obtained from these patients are uncorrected. A 25-ml. sample of control urine from a normal female gave insignificant absorption readings when carried through methods *A* and *C*, and the blank resulting from hydrolysis of the extracted urine sample (*Method B*) was equivalent to 1.1 mcg. of nortriptyline equivalents per ml. of urine. Control urine collected from the subject, who was given a single dose of the drug, gave values of 1.5, 2.1, and 2.1 mcg. of nortriptyline equivalents per ml. of urine for methods *A*, *B*, and *C*, respectively, and the results were corrected. In this instance 100 ml. of urine was used as the aliquot for assay. Since 25-ml. aliquots were used for all assays on the chronic administration study, blank values would be relatively insignificant.

Table I is a summary of the data obtained on 24-hr. urine collections from patients on a chronic dosage regimen of nortriptyline hydrochloride. All patients had been receiving the drug for several months. In the limited number of patients studied there appears to be a correlation between the dose administered and the amount of drug recovered in the urine. Individual fluctuations in the rate of excretion of the drug are quite apparent, however. It is evident that only small amounts of unchanged nortriptyline are excreted and that a somewhat larger amount of the hydroxy metabolite is excreted in the "free" than in the "bound" or conjugated form. In the 8 patients studied, an average of about 58% of the administered dose was excreted in the urine in a 24-hr. period.

Single doses of 25 and 30 mg. were given to a normal, healthy male to determine the excretion pattern after a single dose. These data are given in Table II. In this subject the maximum excretion

[5] Marketed as Ultran by Eli Lilly and Co.
[6] Marketed as Diuril by Merck Sharp and Dohme.
[7] Marketed as Trilafon by Schering Corp.
[8] Marketed as Stelazine by Smith Kline & French.
[9] Marketed as Prolixin by E. R. Squibb & Sons.

[10] Marketed as Thorazine by Smith Kline & French.

TABLE II.—NORTRIPTYLINE HYDROCHLORIDE EXCRETION AFTER SINGLE DOSES

Dose, mg.	Collection Interval, hr.	Nortriptyline HCl	"Free" Metabolite	"Bound" Metabolite	Total	% of Dose
25	0–24	Negligible	4.6	4.9	9.5	38.0
30	0–24	1.0	3.2	3.4	7.6	25.3
	24–48	0.8	2.1	2.0	4.9	16.3
	48–72	0.8	0.7	1.7	3.2	10.7
	Totals	2.6	6.0	7.1	15.7	52.3

TABLE III.—NORTRIPTYLINE HYDROCHLORIDE EXCRETION AFTER 3 SUCCESSIVE DAILY 10-mg. DOSES

Day	Dose, mg.	Nortriptyline HCl	Nortriptyline Equivalents, mg.[a] "Free" Metabolite	"Bound" Metabolite	Total
1	10	0.96	0.98	1.91	3.85
2	10	0.95	0.84	2.35	4.14
3	10	0.90	2.36	2.52	5.78
4	None	0.93	2.03	3.22	6.18

[a] Urine was collected during the 24-hr. interval between doses and during the 24-hr. interval following the final dose.

rate was found to occur in the 4–8-hr. interval after dosage. The drug is slowly excreted in the urine and substantial levels were found 2–3 days after dosage. Within the 0–24-hr. interval a lower percentage of the administered dose is recovered in the urine than from those patients on chronic administration of the drug.

The data in Table III show the gradual increase in the amount of drug excreted daily as the dose is maintained over a period of 3 days. These data were obtained on the same subject as was used in the single dose study.

From the data presented, it is evident that the assay method described is capable of permitting a reliable evaluation of nortriptyline hydrochloride ingestion. If one is interested only in whether a patient has taken his medication and is not interested in the total amount of nortryptyline present in the urine, it would be necessary to carry out only *Method A* of the assay procedure. The presence of the drug can also be detected by the TLC system described.

SUMMARY

1. A quantitative U.V. spectrophotometric assay method has been developed for the determination of nortriptyline hydrochloride and its principal metabolites in the urine of man.

2. The drug is slowly excreted in the urine primarily as conjugated and unconjugated 10-hydroxy nortriptyline. Little unchanged nortriptyline is excreted.

3. An average of 58% of the daily dose was recovered in 24-hr. urine samples from patients on a continuing dosage regimen of the drug. An average of 34% of the dose was recovered from the 24-hr. urine of a subject given single doses.

4. A thin-layer chromatographic system is described which permits rapid identification of the presence of the drug and/or metabolites in the urine.

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Adrenolytic Activity of Atropine, (+)-Hyoscyamine, Atroscine, Homatropine, and Related Compounds

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The adrenolytic activity of atropine, (+) and (−) hyoscyamine, (−)-hyoscine, atroscine, and related compounds was determined by their ability to antagonize the lethal effect of epinephrine in rats. (+)-Hyoscyamine was found to be more active than atropine, and atropine was slightly more active than atroscine (racemic hyoscine). The *levo*-isomers, (−)-hyoscyamine and (−)-hyoscine, were inactive. These results indicate that (+)-hyoscyamine and (+)-hyoscine are responsible for the effect of atropine and atroscine, respectively. Homatropine and benztropine, but not tropine itself, were active. Benztropine was approximately one-fiftieth as active as phentolamine. A pair of esters of tropine had weak adrenolytic activity, but their pseudotropine isomers were inactive. Atropine aminoxide (genatropin) and atropine methyl nitrate had no adrenolytic activity.

IT HAS long been known that atropine, in high doses, inhibited or reduced the effect of epinephrine and sympathetic stimulation on various arterial beds (1). Those results included the effect of epinephrine on arterial strips from horses, oxen, and rabbits and perfused arterial

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