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The determination and excretion of phenylephrine in urine

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PHENYLEPHRINE* (*m*-hydroxy-α-[(methylamino)-methyl]benzyl alcohol) has been widely used for a number of years as a sympathomimetic agent. However, methods for its determination in biological materials have apparently not been published. Cavallito *et al.*[1] studied a sustained release preparation containing phenylephrine by randomly labelling the compound with tritium. In addition, a number of methods are available for the compound alone and in various pharmaceutical preparations; however, none of these methods appeared to be practical for our studies.

Phenylephrine, being an amphoteric compound, is difficult to separate from aqueous media. This has previously been accomplished by the use of activated carbon[2] and by complexing with bromthymol blue.[3] These separations are either not quantitative or are not applicable to our case where other amines are also present.

It appeared to be logical to convert the phenylephrine into a form which could be extracted. 1,2-amino alcohols are easily oxidized by periodate to the corresponding aldehydes,[4] in this case to m-hydroxybenzaldehyde. The resulting compound could then be extracted as a phenol. In addition, the aldehyde has a high ultraviolet absorption coefficient, simplifying quantitation.

The method described below has been used to study the excretion of phenylephrine in human subjects who received formulations containing phenylephrine, phenylpropanolamine, and brompheniramine. Two formulations were compared, an elixir and a tablet. The other two compounds present do not interfere in the determination of phenylephrine by this method.

EXPERIMENTAL

Preliminary experiments were carried out to show that periodate oxidation did convert phenylephrine to *m*-hydroxybenzaldehyde. Oxidation occurred readily in saturated sodium bicarbonate solution and the resulting benzaldehyde could be extracted into ether. The benzaldehyde was then re-extracted into 0·1 N NaOH and its spectrum determined after the addition of an equal volume of ethanol. The molar extinction of this sample corresponded to that of authentic *m*-hydroxybenzaldehyde, indicating a quantitative recovery.

Samples of control urine were carried through the procedure. These showed some absorption in the region where the benzaldehyde absorbed but could be corrected for by a baseline method. Recovery studies from the addition of known amounts of phenylephrine to control urine proved to be satisfactory.

A preliminary study was conducted by analyzing the urine from a laboratory subject who received an oral dose of 10 mg phenylephrine hydrochloride. No drug could be found in the urine samples. Since phenylephrine contains two hydroxyl groups, it was suspected that it had conjugated as the glucuronide or sulfate. The urine samples were therefore hydrolyzed in 4 N HCl, adjusted to the pH of saturated $NaHCO_3$, and again analyzed. A typical absorption curve for *m*-hydroxybenzaldehyde was then found.

The following procedure was therefore adopted. A sample of 4·0 ml urine in a glass-stoppered, 50-ml centrifuge tube is heated in boiling water with 2·0 ml concentrated HCl for 30 min. To the cooled tube, 1·0 ml of 50% NaOH is added and the volume made to 10 ml with water. The solution is then saturated with solid $NaHCO_3$. The mixture is washed twice with 10-ml portions of ether. This wash effectively removes interfering materials but does not extract phenylephrine. Exactly 5·0 ml of the aqueous phase is transferred to a second 50-ml centrifuge tube, 2·0 ml sodium periodate (0·1 N in 0·215 N NaOH)[4] is added, and the solution is allowed to stand for 15 min at room temperature to oxidize the phenylephrine. The *m*-hydroxybenzaldehyde formed is extracted into 10·0 ml

* Neo-Synephrine.

ethyl ether. A 5·0-ml aliquot of the ether is dried with Na_2SO_4, the Na_2SO_4 is washed with additional ether, and the total ether is extracted with 1·5 ml of 0·1 N NaOH. An aliquot of the aqueous layer is added to an equal volume of ethanol and its absorption spectrum recorded. A baseline is drawn from 234 to 244·5 mμ and the absorbance from this line to the maximum at 240 mμ is measured. This baseline represents the nonspecific absorbance found from reagents and control urine carried through the procedure.

DISCUSSION

The recovery of phenylephrine after addition to control urine is shown in Table 1. The results

TABLE 1. RECOVERY OF KNOWN AMOUNTS OF PHENYLEPHRINE ADDED TO CONTROL URINE

Phenylephrine (μg)		Recovery (%)
Added	Found	
6·0	6·0	100
6·0	5·5	92
10·0	10·5	105
10·0	10·3	103
10·0	10·0	100
15·0	15·0	100
15·0	15·8	105

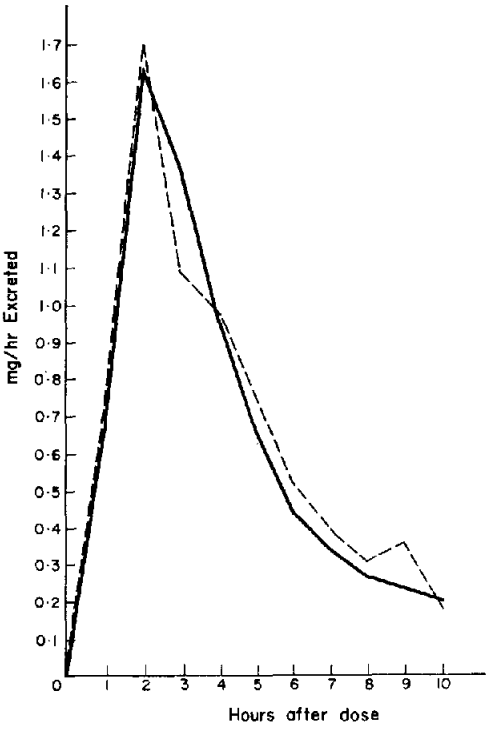

FIG. 1. This figure shows the rate of excretion of phenylephrine after oral dosage of 10 mg as tablets or elixir. The values shown are the averages from data collected on 5 male subjects. Urine pH was controlled by the administration of ammonium chloride. Elixer ———; tablet — — — —.

appear to be entirely satisfactory. The additional drugs, phenylpropanolamine and brompheniramine present in the tablets and elixir under study, do not interfere.

The initial ether wash, prior to oxidation, removes ether-soluble basic, neutral, and slightly acidic compounds that might interfere. Analysis of 20 control samples of human urine, in volumes up to 10 ml, indicates no measurable absorbance difference at the wavelengths given, indicating a zero blank value. The absorption between 234 and 250 mμ is linear in each of these samples and a straight line drawn between them is superimposed on the absorption curve. These results indicate the specificity of the method for phenylephrine. However, any compound that is not extracted *per se* by ether and is oxidized under these conditions to *m*-hydroxybenzaldehyde or to a similar compound will interfere. The method is sensitive to as little as 1 μg phenylephrine.

The absorbance of *m*-hydroxybenzaldehyde is determined in 50% ethanol–0·1 N NaOH, since the absorption coefficient has been reported to be higher in alkaline ethanol solutions.[5]

The results of an excretion study of phenylephrine are shown in Fig. 1. Five normal male subjects received 10 mg phenylephrine, on one occasion as an elixir and on the other as tablets. The acidity of the urine was maintained at pH 5·6 or below by the administration of ammonium chloride. Urine was collected at hourly intervals for 10 hr and analyzed. The average results are plotted in Fig. 1. There is no significant difference in the two curves, indicating rapid absorption from both preparations. The maximum rate of excretion (mg/hr) is at the 2-hr interval. The average total recoveries in a 24-hr period are 75 per cent for the elixir and 82 per cent for the tablet.

It is of interest to determine the form of conjugation of phenylephrine, whether as the glucuronide or the sulfate, and to what extent it is formed. Samples of urine from the above subjects were incubated with Glusulase (Endo Laboratories, Inc., Garden City, New York) or with Ketodase (Warner-Chilcott, Morris Plains, N.J.) at pH 6·2. The samples were analyzed, omitting the hydrolysis step. The results are given in Table 2. Since Glusulase contains glucuronidase and sulfatase whereas

TABLE 2. RESULTS FROM ANALYSIS OF URINE SAMPLES AFTER INCUBATION WITH GLUSULASE AND KETODASE

Sample	Phenylephrine (μg/ml)			
	Urine, not hydrolyzed	Incubated urine, not hydrolyzed	Glusulase-treated	Ketodase-treated
1	0	0	29	0·5
2	0	0·5	17	2
3	0	0	21	3

Ketodase contains only glucuronidase, it is apparent that phenylephrine is excreted almost entirely as the conjugate, principally as the sulfate, and not as free phenylephrine. A small fraction is excreted as the glucuronide.

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