In Vivo Determination of Certain Aralkylamines

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A micro-method has been developed by which blood concentrations of certain aralkylamines can be quantitatively determined. The method makes use of thin-layer chromatography and fluorometry. Quantitative determinations are made by measuring the fluorescence exhibited by the rose bengal complex of the drug. The method utilizes 0.1-ml. samples of capillary blood from which 0.05 mcg. of drug has been detected.

In recent years a pharmaceutical need has arisen for newer and more sophisticated analytical methods. This need has been brought about by the continuous development of potent therapeutic agents, the *in vivo* distribution and the metabolic fate of which have not been completely determined. Chlorpheniramine maleate, dextromethorphan hydrobromide, phenylephrine hydrochloride, and phenylpropanolamine hydrochloride are representative of the aralkylamines that fall into the above category.

Many *in vitro* analysis methods have been reported for the quantitation of aralkylamines contained in pharmaceutical preparations. Gas chromatography has been successfully employed whereby various polyethylene glycols and silicone gum rubber have served as column coating material (1–4). The sensitivities attained, however, were not sufficient to permit the *in vivo* quantitation of the drugs.

Several paper and thin-layer chromatographic separation procedures have been recently reported to effect resolution of some aralkylamines. Cox *et al.* (5) studied 12 antihistamines. Schriftman and Shultz (6) reported a two-dimensional paper chromatographic separation procedure for a mixture of chlorpheniramine maleate, dextromethorphan hydrobromide, and phenylephrine hydrochloride. The developing media were butanol–acetic acid–water (5:1:3), and chloroform–ether (1:1).

With the advent of fluorometric techniques it was possible to quantitate lower concentrations of many compounds. It was shown by Duggan *et al.* (7) that phenylephrine fluoresces maximally at pH 1.

Brodie, Udenfriend *et al.* (8, 9) reported general methods for the estimation of basic compounds. The methods generally included extraction procedures for the isolation of the compounds from their metabolic products and from naturally occurring substances. They concluded that fluorometry was the method of choice whenever possible.

Cohen (10) found that nonfluorescent compounds, or those that were weakly fluorescent, could be converted to a highly fluorescent complex by treatment with rose bengal.

Little has been reported about the *in vivo* distribution and the excretion rate of many of the common aralkylamines. Brossi *et al.* (11) could not determine dextromethorphan blood levels. They did report, however, that from 2 to 16% of the drug was excreted in the urine.

Woods *et al.* reported that blood levels had been determined for the *levo* isomer of the drug, which had been administered to dogs and to monkeys. The isotopic technique showed the maximum concentration of the drug to be present in the plasma after 1 hr. (12).

Willner reported on the excretion of the metabolic products of dextromethorphan (13). He found the drug to be excreted both *per se* and as demethylated derivatives. These findings were made when a paper chromatographic separation procedure was used.

The excretion rate for phenylpropanolamine hydrochloride was studied by Heimlich *et al.* (14). They reported that about 90% of an orally administered 50-mg. dose of the drug was excreted, according to first-order kinetics, within 24 hr.

Excretion rates for chlorpheniramine and phenylephrine were studied by Cavallito *et al.* (15). An isotopic procedure, which required 15-ml. samples of blood or of urine, showed the excretion rates to vary widely among individuals. No apparent relationship was found to exist between the blood level and the excretion rate of tritiated phenylephrine.

Although the information cited above is extremely valuable, it is clear that there has been no general method for the quantitative, *in vivo* determination of the aralkylamines. This paper

Received August 7, 1967, from the Department of Pharmacy, Massachusetts College of Pharmacy, Boston, MA 02115

Accepted for publication September 26, 1967.

Abstracted from theses submitted by Joseph M. Theodore and Frederick J. Pruyn to the Massachusetts College of Pharmacy in partial fulfillment of Doctor of Philosophy degree requirements.

This investigation was supported by a research grant from Chesebrough-Pond's, Inc., Clinton, Conn.

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provides a practical means for performing such analyses both rapidly and accurately.

EXPERIMENTAL

Reagents and Chemicals

The reagents and chemicals used were: acetone, spectroanalyzed, Fisher certified reagent; chloroform, Fisher certified reagent; ethyl alcohol USP; ethyl ether USP; methanol, Fisher certified reagent; ammonium hydroxide solution USP; and ethyl acetate, Mallinckrodt. Dragendorff's reagent and 0.2% aqueous ninhydrin solution were used as indicators. Rose bengal, Fisher certified reagent, was used and was purified by extraction with ethyl acetate and chloroform by the method described by Cohen (10). Chlorpheniramine maleate, chlorpheniramine free base, dextromethorphan hydrobromide, dextromethorphan free base, phenylephrine hydrochloride, and phenylpropanolamine hydrochloride were supplied by Chesebrough-Pond's, Inc.

Apparatus

The thin-layer chromatographic plates used were prepared by coating 20 cm. square glass plates with 0.5 mm. of a Silica Gel G slurry, which was prepared by mixing 1 part by weight of Silica Gel G, Brinkmann Instruments, Inc., with 2 parts by volume of distilled water. The developing chambers and the appropriate micropipets were purchased from the Fisher Scientific Co. The instrument used was a Turner fluorometer, model 110. The instrument was equipped with a 520–560 mμ green lamp, Turner No. 110-854; primary excitation filters No. 1-60 (546 mμ) and No. 58 (525 mμ); and a No. 23-A (570 mμ) secondary emission filter. The 12 × 75 mm. cells used were carefully matched and were selected for low fluorescence.

Methods

Thin-Layer Chromatographic Separation of the Aralkylamines—A thin-layer chromatographic plate, which had been stored in a desiccator, was activated for 20 min. at 110°. A 25-μl. volume of a standard ethanolic solution, containing 25 mcg., 25 mcg., and 75 mcg. of chlorpheniramine maleate, dextromethorphan hydrobromide, and phenylephrine hydrochloride, respectively, was applied to the plate 3 cm. from the lower right corner. The solvent was allowed to evaporate spontaneously at room temperature.

The chromatogram was then developed in an ammoniacal methanol system, which was attained by placing a 50-ml. beaker, containing ammonium hydroxide solution, in a corner of the developing chamber 0.5 hr. before the insertion of the plate. The chromatogram was developed for 1.5 hr., during which time the front moved a distance of 15 cm. The chromatogram was air-dried for 1 hr. at room temperature, rotated clockwise through 90°, and developed in an ammoniacal chloroform–ether (1:1) system for 1.25 hr. The front moved a distance of 15 cm.

The two-dimensional chromatogram was air-dried at room temperature, sprayed with 0.2% aqueous ninhydrin solution, and heated for 15 min. at 110°. The appearance of a purple spot indicated the presence and the location of phenylephrine hydrochloride. After cooling to room temperature, the chromatogram was sprayed with Dragendorff's reagent. The immediate appearance of 2 reddish orange spots indicated the presence and the location of chlorpheniramine maleate and of dextromethorphan hydrobromide.

The resolution of a mixture of chlorpheniramine maleate, phenylephrine hydrochloride, and phenylpropanolamine hydrochloride was effected by the above procedure also. The phenylpropanolamine hydrochloride was detected by the ninhydrin reagent.

Quantitative In Vivo Determination of Chlorpheniramine Maleate—Two 0.1-ml. samples of whole capillary blood were collected from the fingertips of the subjects. The samples were collected with a sterile microlance and a serological pipet after the fingertips had been cleansed with alcohol. One sample was used as a blank and the other as a control, which was prepared by adding 0.05 mcg. of chlorpheniramine maleate to the 0.1-ml. blood sample. One 4-mg. chlorpheniramine maleate tablet[1] was then administered orally to each subject. Capillary blood samples were collected 1, 2, 3, 4, and 5 hr. after the administration of the drug.

All samples, including those to be used as blanks and as controls, were precipitated with 50 μl. of 10% aqueous trichloroacetic acid solution, mixed well with a small glass rod, and centrifuged until the supernatant liquid was clear. The supernatant liquids were applied to 0.5-mm. Silica Gel G chromatographic plates with a 100-μl. micropipet. The plates had previously been activated as has been described above. The chromatograms were developed in ammoniacal chloroform–ether (1:1) for 1 hr. and air dried at room temperature. The outermost columns of the chromatograms, to which had previously been applied 25 mcg. of chlorpheniramine maleate, were sprayed with Dragendorff's reagent to determine the exact R_f value of the drug. The areas of the chromatogram containing the drug were scraped from the plate, care being taken to insure the removal of equal areas. The samples thus removed were transferred to 18 × 150 mm. Pyrex test tubes. To each tube was added 2 ml. of ethanol. The tubes were shaken for 15 min. on a Burrell wrist action shaker. The contents of each tube were filtered through Whatman No. 1 filter paper, the filtrates being collected in 2-in. evaporating dishes. The tubes were rinsed with 2 ml. of ethanol and the rinsings passed through the appropriate filters, which were rinsed with 1 ml. of ethanol. To each filtrate was added 50 μl. of ammonium hydroxide solution.

The filtrates were evaporated to dryness on a steam bath. To each dish was added 1 ml. of an acetone–chloroform (1:1) solution containing 0.05 mcg. of rose bengal. Each sample was diluted with 3 ml. of acetone–chloroform (1:1). The contents of each dish were mixed by gentle swirling and transferred to fluorometric cells. Fluorometric analyses of the samples were carried out using the previously described filter system.

Tenth milliliter urine samples were also analyzed by the same procedure except that the urine samples were chromatographed directly.

Quantitative In Vivo Determination of Dextro-

[1] Chlortrimeton, Schering Corp.

TABLE I—R_f VALUES DETERMINED FOR CERTAIN ARALKYLAMINES[a]

Drug	Solvent A[b]	Solvent B[c]
Chlorpheniramine maleate	0.68	0.43
Dextromethorphan hydrobromide	0.51	0.53
Phenylephrine hydrochloride	0.43	0.00
Phenylpropanolamine hydrochloride	0.64	0.10

[a] All plates were coated with 0.5 mm. of Silica Gel G. [b] Ammoniacal methanol. [c] Ammoniacal chloroform–ether (1:1).

methorphan Hydrobromide—The preceding procedure for the determination of blood and of urine concentrations of chlorpheniramine maleate was used with the following exceptions. A 10-ml. dose of a dextromethorphan hydrobromide syrup,[2] containing 15 mg. of the drug in each 5 ml., was administered orally. Acetone was used to extract the drug from the Silica Gel G. Each sample was treated with 0.75 mcg. of rose bengal, and a 1% transmission filter was incorporated into the system.

In Vivo Determination of Phenylephrine Hydrochloride—The preceding procedure for the determination of blood and of urine concentrations of chlorpheniramine maleate was used with the following exceptions. A 5-ml. dose of a phenylephrine hydrochloride elixir,[3] containing 5 mg. of the drug in each 5 ml., was administered orally. Each sample was treated with 0.15 mcg. of rose bengal and 25 µl. of ethanol was added to each sample cell. The chromatogram was developed in ammoniacal methanol and 0.2% aqueous ninhydrin solution was used to indicate the R_f value of the drug.

RESULTS AND DISCUSSION

The two-dimensional thin-layer chromatographic separation procedure effected excellent resolution of the drugs. Table I shows the R_f values that were determined for each drug. It was noted that the ammoniacal chloroform–ether (1:1) solvent medium should be prepared freshly and that no more than two chromatograms be developed in such a system. The ammoniacal methanol solvent medium gave satisfactory results for up to five chromatograms.

The samples for fluorometric analysis were prepared in an acetone–chloroform (1:1) solvent medium. It was found that the acetone gave rise to an approximate tenfold fluorescence potentiation, which attained a maximum after about 20 min. Figure 1 shows the fluorescence exhibited by various concentrations of chlorpheniramine free base when complexed with rose bengal. The graph shows that accurate measurements could be made in the submicrogram concentration range.

The *in vivo* determinations were carried out using six normal males, from 23 to 34 years of age. All subjects were in a semifasting condition as only liquid nourishment was allowed during the 8 hr. preceding, and throughout the duration of, the test. All subjects had been deprived of drugs of any kind for the 72 hr. preceding the test.

[2] Romilar Syrup, Sauter Laboratories, Inc.
[3] Neo-Synephrine Elixir, Winthrop Laboratories.

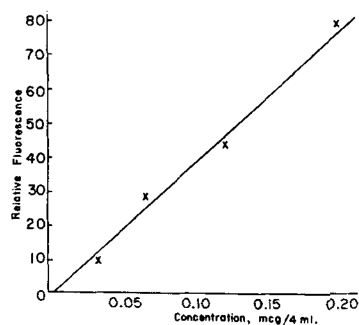

Fig. 1—Fluorescence-concentration relationship of chlorpheniramine free base when complexed with rose bengal.

TABLE II—CHLORPHENIRAMINE MALEATE BLOOD LEVELS OBTAINED FROM ORAL ADMINISTRATION OF THE DRUG[a]

Subject	(mg. % × 10²) 1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
A	0.97	1.47	3.30	2.30	1.10
B	1.87	1.77	2.80	2.20	1.40
C	1.30	1.60	1.10	0.94	...
D	2.30	2.30	2.60	2.30	1.80
E	2.50	2.90	2.50	1.70	1.40
F	1.50	1.50	2.10	...	1.30

[a] Chlortrimeton, 4 mg., Schering Corp.

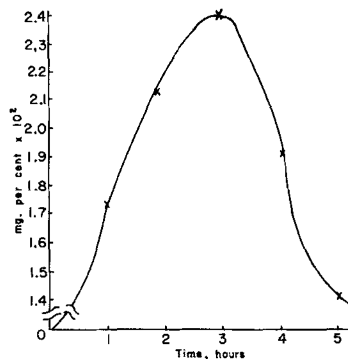

Fig. 2—Mean blood level distribution of chlorpheniramine maleate after oral ingestion of a 4-mg. dose.

The results of the analyses showed chlorpheniramine maleate to exhibit a definite absorption pattern. The maximum blood level appeared within 3 hr. after the oral administration of the drug and was followed by a rapid decline thereafter. Table II shows the chlorpheniramine maleate blood concentrations, in milligrams percent, that were determined from the subjects that were studied. Figure 2 shows the mean blood level distribution of chlorpheniramine maleate. The analysis of the urine showed that approximately 17% of the orally administered drug had been excreted unchanged within 6 hr.

A comparison of the blood concentrations that were produced from a 15-mg. and from a 30-mg. oral dose of dextromethorphan hydrobromide was made. Analyses were carried out 1, 2.5, 4.5, and 6 hr. after the ingestion of the drug. It was found that approximately 10, 25, and 16% of the orally admin-

istered 30-mg. dose was in the blood stream after 1, 2.5, and 6 hr., respectively. The 1 and the 2.5 hr. blood concentrations produced by the 15-mg. oral dose were insignificantly different from the respective concentrations produced by the 30-mg. dose, but the 4.5 and the 6 hr. concentrations were substantially lower. Approximately 8% of the orally administered drug was excreted unchanged within 6 hr.

The presence of phenylephrine hydrochloride was detected in human blood but statistical quantitative determinations have not as yet been carried out.

The *in vitro* applications of the fluorometric analysis procedure to other aralkylamines has been studied. Phenindamine required 3 times its weight of rose bengal to give the optimum degree of fluorescence. The dye–drug weight ratios were 25:1, 10:1, 1:1, and 0.5:1, respectively, for dextroamphetamine, phenylpropanolamine, phenyltoloxamine, and thonzylamine.

It should be noted that only the amine free bases respond to the fluorometric analysis procedure, thus it is necessary that the salts be converted to the free bases before analyses are carried out.

It was determined that a 99% recovery of the drugs from Silica Gel G had been effected. The recovery from blood and from urine was also nearly quantitative. The use of blanks and of controls, which were prepared from the subject's blood or urine, increased the accuracy of the procedure in that any inherent fluorescence would become nulled.

It is felt that this method can be applied to other aralkylamines and, since it has been shown that resolution of drug mixtures can be effected, that simultaneous determinations can possibly be carried out.

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Keyphrases

Aralkylamines
In vivo determination of aralkylamines
Chlorpheniramine maleate–dextromethorphan
HBr–phenylephrine HCl mixture
TLC separation
Rose bengal–aralkylamine complex
Fluorometry–analysis