Table I—Assay Results on Phenol and Resorcinol

Sample	Percent Results[a] on Phenol	Resorcinol
1[b]	99.6	100.2
2[b]	98.7	99.4
3[c]	100.5	0.0
Average deviation	±2.4	±1.8

[a] Average of three. [b] Phenol–resorcinol–boric acid solution. [c] Phenolated calamine lotion.

where A_a = peak area of the assay sample, and A_s = peak of the standard sample.

The results are presented in Table I, and the chromatogram is presented in Fig. 1.

DISCUSSION

The assay results (Table I) indicate that phenol and resorcinol in combination can be separated (Fig. 1) and assayed accurately. The procedure developed for the quantitative determinations of phenol and resorcinol by HPLC is very simple and rapid (no special preliminary treatment is required). No interference from other ingredients of the phenol–resorcinol–boric acid solution (acetone and boric acid) or phenolated calamine lotion USP (bentonite magma, calamine, and zinc oxide) was noted. The method provides direct results on phenol and resorcinol rather than determining one or the other by difference.

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Electron-Capture GC Determination of Subnanogram Amounts of Emepronium Bromide in Serum

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Abstract □ Barium peroxide in an acidic medium was utilized to increase the sensitivity in the benzophenone method for the determination of the quaternary ammonium compound emepronium bromide. The method is comprised of ion-pair extraction, oxidation, and quantitative determination of benzophenone by electron-capture GC. By employing small extraction and reaction volumes, the method was used in the 0.2–8-ng/ml range with a relative standard deviation of 2.5% at the 1-ng/ml level. The application of the method to human serum samples after a single oral dose demonstrated that the elimination phase for emepronium in serum had a half-life of 7–11 hr.

Keyphrases □ Emepronium bromide—electron-capture GC analysis in human serum, elimination profile described □ GC, electron capture—analysis, emepronium bromide in human serum □ Elimination profile—emepronium bromide, electron-capture GC analysis in human serum □ Anticholinergic agents—emepronium bromide, electron-capture GC analysis in human serum, elimination profile described

Emepronium bromide[1] [(3,3-diphenyl-1-methylpropyl)dimethylethylammonium bromide], a quaternary ammonium compound with anticholinergic properties, was determined by electron-capture GC in biological samples after oxidation with chromic acid to benzophenone (1). The oxidation conditions as well as the extraction of emepronium were studied in detail. Special attention was paid to the interference from metabolites. The lower limit of detection of that method was about 1 ng/ml, and down to 3 ng/ml could be determined with acceptable precision. The fate of emepronium bromide in dogs (2) and humans (3) was studied with this sensitive method.

From a pharmacokinetic point of view, there was a need for a method with a higher sensitivity to follow the fate of emepronium over a longer time. Subnanogram analysis of emepronium bromide could be realized when a study of barium peroxide oxidation in sulfuric acid revealed very low reagent blanks (4). The sensitivity was also increased by the use of small extraction and reaction volumes and by the employment of a purer internal standard (5). This paper describes the development of a barium peroxide oxidation procedure for emepronium and its application to the analysis of human serum samples for the drug after single oral doses.

[1] Cetiprin.

EXPERIMENTAL

Apparatus and Glass Equipment—*GC*—A gas chromatograph[2] equipped with a tritium electron-capture detector operating in the dc mode was used. The glass column (1.5 m × 1.8 mm) was filled with 3% DC 560 and 0.3% polyethylene glycol[3]–terephthalic acid on 100–120-mesh Gas Chrom P, acid washed and silanized. The column temperature was 139°; the flow rate of the carrier gas, nitrogen, was 30 ml/min. The detector temperature was 155°, corresponding to 138° at the detector foil.

Heating Bath—The reaction was performed in a dry bath[4] at 114°. The reaction tubes were emersed in sand so that only the water phase was covered.

Glass Equipment—The extraction was performed in 15-ml centrifuge tubes with a tapered base of 0.3 ml. The oxidation was performed in glass tubes with a height of 10 cm and an inner diameter of 0.6 cm. The tubes had a restriction at the half-height, and no condenser was necessary with this arrangement.

Reagents and Chemicals—Barium peroxide[5], anhydrous powder,

[2] Varian 1400.
[3] Carbowax, Union Carbide.
[4] Thermolyne.
[5] Matheson, Coleman and Bell.

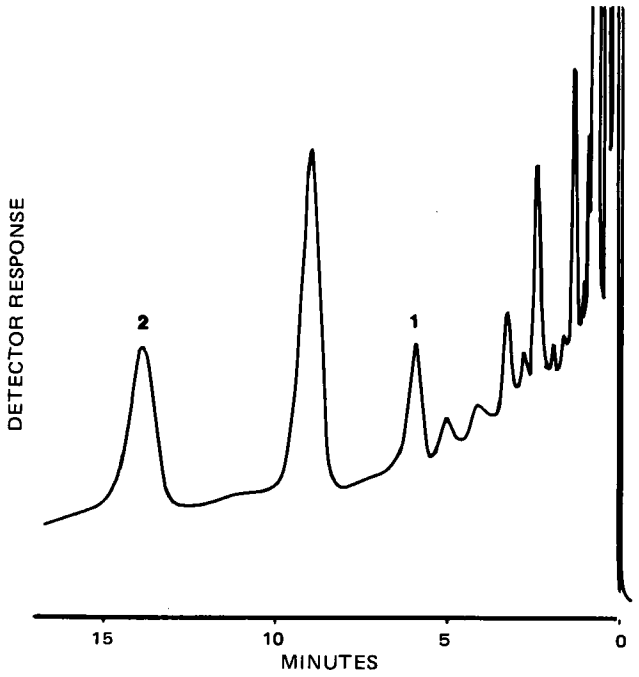

Figure 1—*Gas chromatogram of the oxidation extract from a human serum sample of emepronium. Key: 1, benzophenone from emepronium, 2.5 ng/ml; and 2,* p*-chlorobenzophenone from the internal standard, 5 ng/ml.*

was used as received. Methylene chloride[6], analytical grade, was extracted with 0.5 *M* sodium carbonate, 0.5 *M* sodium bisulfite, and water. After drying with sodium sulfate, it was distilled. Sodium perchlorate[6] solution, 0.5 *M*, was shaken with double-distilled heptane before use. Sulfuric acid solution, 3.6 *M*, was prepared from high purity sulfuric acid[7]. The sodium hydroxide[6] solution used was 1 *M*.

Internal Standard Solution—Ethyl-(3-*p*-chlorophenyl-3-phenyl-1-methylpropyl)dimethylammonium bromide was purified by a reversed-phase, ion-pair chromatographic procedure (5). It was dissolved in water and diluted to a final concentration of 9 ng/ml.

Standard Solution of Emepronium Bromide—Emepronium bromide was dissolved in water and diluted to 2 ng/ml.

Determination of Emepronium in Serum—To a serum volume containing 0.2–4 ng of emepronium in a centrifuge tube were added 1 ml of the internal standard solution, 1 ml of 0.5 *M* sodium perchlorate, water to 5 ml, and 1 ml of methylene chloride. The tube was shaken for 30 min and centrifuged. The methylene chloride phase was then transferred to an oxidation tube and evaporated to dryness in a gentle stream of nitrogen.

To the residue, 0.25 ml of 3.6 *M* sulfuric acid and about 20 mg of barium peroxide were added. Then 50 μl of heptane was added, and the tube was heated for 30 min in the heating bath. After cooling, the organic phase was shaken with 3 drops of 1 *M* sodium hydroxide. A 10–20-μl aliquot of the heptane phase was then injected into the gas chromatograph.

A standard curve was prepared by treating five samples containing 0, 0.5, 1, 2, and 4 ng of emepronium bromide from the standard solution and 1 ml of blank serum according to the described procedure.

RESULTS AND DISCUSSION

Extraction Conditions—The extraction of emepronium and the internal standard (the *p*-chloro analog) from the serum sample was as described in the chromic acid oxidation procedure (1). To increase the sensitivity of the method, the phase volumes were diminished four times compared to the previous method.

Reaction Conditions—Emepronium bromide can be converted to benzophenone by several oxidizing reagents (6–8). The oxidation yield was about 70% in all cases. However, the reagent blank was much less in the oxidation with acidic barium peroxide (4), and this reagent was chosen for a more sensitive method.

The conditions for the barium peroxide oxidation of emepronium bromide and the internal standard in a two-phase system were studied previously (6). The sulfuric acid concentration in the oxidation solution was important, and 3.6 *M* was necessary for optimum yield in both cases. The oxidation time for emepronium bromide and the internal standard was 30 and 60 min, respectively. In this study, a somewhat higher reaction temperature, 114°, was used; the reaction was complete in 30 min for both compounds. Prolonged reaction time increased the interferences on the gas chromatograms.

Purification of Reagents—An advantage of oxidation procedures in electron-capture GC is that clean extracts are obtained because interfering materials often are completely decomposed during oxidation. However, in this low concentration range, special attention had to be paid to the reagent purity. All reagents were of the highest analytical quality but, in many cases, were not sufficiently pure for a successful analysis. For example, use of other qualities of sulfuric acid increased the control background so much that subnanogram analysis was impossible. Good results were achieved by shaking organic solvents with water followed by distillation. In some cases, the aqueous reagents were shaken with heptane before use.

Although these precautions were taken, some unidentified peaks remained in the chromatogram (Fig. 1). These peaks mainly originated from sodium perchlorate and methylene chloride. Methylene chloride has a high electron-capture response, and some impurities in that solvent remained after the evaporation procedure. The interference from serum components was low.

Reagent Blank—Due to a reagent blank in the chromic acid oxidation of emepronium, the lowest level that could be determined with acceptable precision was 3 ng/ml (1). About half of the reagent blank was due to a batch of internal standard being contaminated with emepronium bromide. By use of the reversed-phase, ion-pair liquid chromatographic procedure, this contaminant was eliminated (5). The reagent blank that originates from the oxidation procedure was much lower in acidic barium peroxide oxidation, and determinations were possible down to 0.2 ng/ml of emepronium. The blank from an aqueous sample in this procedure corresponded to approximately 100 pg of emepronium. The blank from serum varied with the individuals and added 0–200 pg to the blank from the aqueous sample.

Selectivity—The interferences from metabolites in the chromic acid oxidation procedure were discussed earlier (1–3). Barium peroxide oxidation also gives interferences from metabolites and other compounds containing the diphenylmethane moiety. Most diphenylmethane-substituted compounds gave a benzophenone yield of about 70% when oxidized in acidic barium peroxide (6). The only emepronium metabolite of importance in humans is the 4-hydroxylated one (3), and this metabolite will not interfere (1). Studies in the dog indicated that the interference from other metabolites is small (2).

Precision and Recovery—In the chromic acid oxidation procedure, the relative recovery of emepronium from serum was 100 ± 3% at the 6-ng/ml level (1). The absolute drug recoveries were studied previously with ^{14}C-labeled material in a series of experiments where quantitative recoveries were obtained. The relative recovery of the drug using the present method was 100%.

The precision of this microprocedure was good. At the 1-ng/ml level, six samples were determined with a relative standard deviation of 2.5%.

Although the interferences from peaks in the vicinity of benzophenone and 4-chlorobenzophenone peaks in the chromatograms were small (Fig. 1), an injection of a sample in the gas chromatograph could be done only at 40-min intervals. Very large peaks were eluted after the 4-chlorobenzophenone peak, and some of them could be recognized as phthalate derivatives. This problem reduced the capacity of the present method; six samples and the standard curve were processed each day.

Analysis of Emepronium Bromide in Human Serum after Oral Administration—Due to the sensitivity increase of the present method for emepronium, 10–20 times lower serum levels of the drug could be determined. The high sensitivity is of value in the determination of emepronium long after its administration and in the determination of emepronium in small sample volumes from small experimental animals.

The method was used in the establishment of serum levels of

[6] Pro analysi quality, Merck, Darmstadt, West Germany.
[7] Merck, Suprapur.

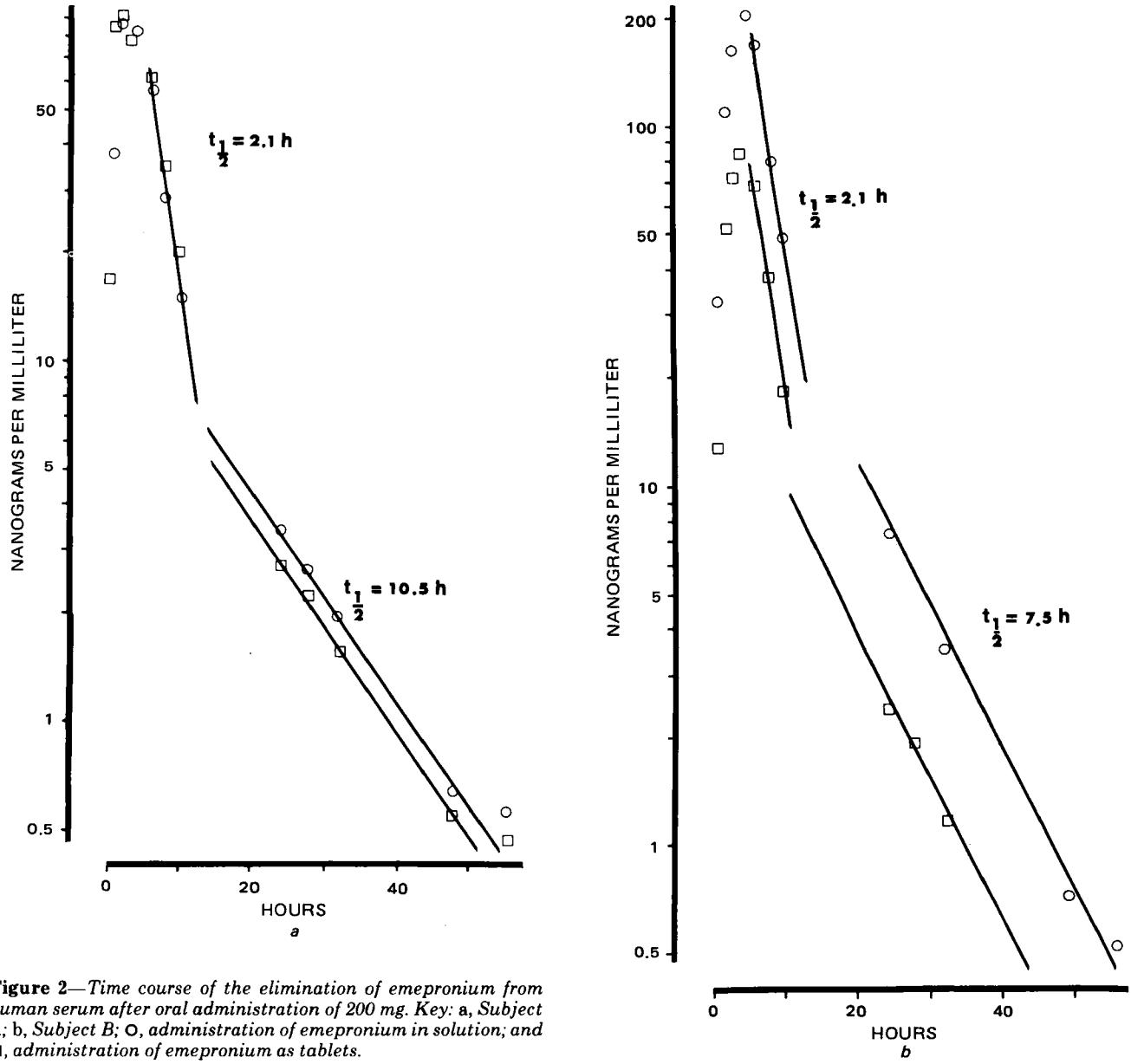

Figure 2—*Time course of the elimination of emepronium from human serum after oral administration of 200 mg. Key:* a, *Subject A;* b, *Subject B;* O, *administration of emepronium in solution; and* □, *administration of emepronium as tablets.*

emepronium in samples taken 24–56 hr after drug administration. The samples taken up to 24 hr were analyzed by the less sensitive chromic acid oxidation procedure. A few samples were analyzed with both procedures, and the results were as expected within the same order of magnitude. The time course for the concentration of emepronium in serum from two volunteers after oral administration of 200 mg in solution and as tablets is shown in Figs. 1 and 2. With this sensitivity of detection, it can be demonstrated clearly that emepronium in serum exhibits an elimination phase, the so-called β-phase, after about 24 hr. The drug half-life in serum was calculated from the slope of the curves and varied from 7.5 to 10.5 hr for these two subjects. With the earlier procedure, only the distribution phase, with a half-life of 1.5–2.5 hr, could be evaluated (1).

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