Interference by Butyl Rubber Stoppers in GLC Analysis for Theophylline

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Abstract □ During a study of the pharmacokinetics of theophylline using GLC analysis, unexpectedly high values occurred in a random manner. The cause of these abnormal values was investigated, and significant interference was observed when blood samples were drawn using evacuated glass tubes sealed with butyl rubber stoppers. *In vitro* tests using distilled water showed no apparent theophylline levels due to the additives in three commonly used tubes. However, when water was allowed to remain in contact with the butyl rubber stoppers for 1 min, an apparent theophylline content of as high as 5.5 μg/ml was observed. A contact time of 60 min resulted in apparent theophylline levels of as high as 52.3 μg/ml. It was concluded that a substance leached from the butyl rubber stoppers accounted for the spurious results.

Keyphrases □ Theophylline—GLC analysis, effect of butyl rubber stoppers, blood □ Butyl rubber—stoppers, effect on GLC analysis of theophylline, blood □ GLC—analysis, theophylline in blood, effect of butyl rubber stoppers

A convenient and widely used method for collecting blood samples employs an evacuated glass tube sealed with a butyl rubber stopper[1]. The sample is drawn by vacuum into the tube by means of a multiple sampling device formed from a needle with two points: one for venipuncture and the other for penetrating the butyl rubber cap. Up to 1 min is needed to obtain one 10-ml sample.

Because of the angle at which the tubes are usually held, blood is in contact with the cap almost immediately after it is punctured. Even after this brief period, materials that have a deleterious effect upon subsequent drug analysis for theophylline can be leached from the cap.

EXPERIMENTAL

A study of the pharmacokinetics of theophylline was performed using an assay (1) involving preliminary extraction of serum samples with 5% isopropyl alcohol in chloroform, evaporation of the extract to dryness, redissolution of the residue in 50 μl of solvent, and injection of a portion of the resulting solution into a gas chromatograph[2]. The solvent system and the column packing[3] (3% OV-17 on 100–120-mesh Chromosorb W-HP) were similar to those used for the chromatographic assay of a wide variety of drugs in biological fluids. In the collection of the blood specimens, a 19-gauge butterfly scalp needle with a three-way stopcock was used to obtain blood samples when the sampling intervals were relatively short; a normal saline infusion was used to keep the lines open.

Some subjects in the study chose to have the infusion set removed when the sampling interval reached 2 hr. Subsequent samples from these individuals were obtained using disposable syringes or evacuated glass tubes. The samples were allowed to clot and then were spun down. The caps were taken off fresh tubes, which were then filled with the serum harvested by means of disposable glass pipets. These tubes were sealed with cork or, if cork was not available, with butyl rubber stoppers. The serum samples were stored in a freezer until they could be assayed. Originally, no particular precautions were taken to keep either the fresh blood or the serum from contacting the rubber closures. From observations and inquiries, it was concluded that this procedure for the drawing and subsequent handling of the specimens is not uncommon.

At this point, abnormally high assay results began to occur in an apparently random manner. To discover the source of the problem, the effects of using tubes sealed with butyl rubber stoppers were investigated. The tubes investigated were: Type A (red cap), no additives in the glass tube; Type B (orange cap), potassium oxalate and sodium fluoride in the glass tube; Type C (lavender cap), tripotassium ethylenediaminetetraacetic acid and potassium sorbate in the tube; and Type D (green cap), heparin in the tube.

Each type of tube was opened, the butyl rubber cap was discarded, and a small amount of distilled water was added (Set A). After 30 min, a sample from each tube was carried through the entire extraction and analytical procedure. A second set (Set B) of tubes was studied. The capped tubes were held vertically with the caps down. The caps were then punctured with a stainless steel needle connected to a plastic disposable syringe containing distilled water.

It had been determined previously that neither the syringe nor the needle would interfere with the assay. The tubes were allowed to fill with distilled water, and samples were taken at 1 min (approximately the time needed to obtain a 10-ml sample) and at 5, 15, and 60 min. The samples were then carried through the extraction and assay procedure.

RESULTS

The chromatograms for the tubes in Set A showed no peaks other than the one due to the internal standard used in the procedure. Thus, the chemicals in the tubes and the glass or materials leaching from the glass were not causing the problem.

The chromatograms from the tubes in Set B showed, in addition to the peak due to the internal standard, a peak occurring at 12.8 min. This peak overlapped sufficiently with the normal theophylline peak at 13.1 min so that it could be, and indeed in the initial pharmacokinetic study apparently was, interpreted as being theophylline. Table I presents the apparent theophylline content obtained from each type of tube at the indicated time. Obviously, some material was being leached quite rapidly from the butyl rubber stoppers, and this substance was substantially interfering with the assay for theophylline.

DISCUSSION

The finding of apparent theophylline levels of up to 5.5 μg/ml within a contact time of approximately 1 min is impressive in view of the fact that the serum therapeutic level of this drug is 10–20 μg/ml. Although tubes are normally kept in a vertical position during transportation and storage, longer contact times between the samples and the butyl rubber stoppers are possible. Such contact

Table I—Apparent Theophylline Content Obtained from Distilled Water in Contact with Butyl Rubber Caps (Set B Tubes)

	Apparent Theophylline Content by Tube Type, μg/ml			
Minutes	A	B	C	D
1	1.1	4.9	5.5	2.6
5	23.2	13.2	35.9	25.2
15	28.1	22.1	46.5	40.3
60	44.3	39.4	52.3	47.5

[1] Vacutainer, Becton Dickinson, Rutherford, N.J.
[2] Varian Aerograph 2400, Walnut Creek, Calif.
[3] Supelco Inc., Bellefonte, Pa.

could result in apparent theophylline levels well above the therapeutic range.

A relatively large number of materials are used in the manufacture of butyl rubber stoppers, and the identification of the interfering material would be a major project. Telephone conversations with the manufacturer of the tubes led to no clues as to what the material might be. Therefore, the finding of this study indicates that any laboratories analyzing blood samples by GLC should determine whether the collection tubes will interfere with the analysis. Such an analysis can be performed quite easily by the methods used in this study.

REFERENCES

(1) F. A. Chrzanowski, P. J. Niebergall, J. G. Nikelly, E. T. Sugita, and R. L. Schnaare, *Biochem. Med.*, 11, 26(1974).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 2, 1975, from the *Department of Pharmacy, Philadelphia College of Pharmacy and Science, Philadelphia, Pa., and the ‡Pulmonary Disease Section of the Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pa.

Accepted for publication July 3, 1975.

Supported in part by National Institutes of Health Clinical Research Center Grant RR 00040.

The authors acknowledge the comments and assistance of Dr. Karl Engelman, Director of the Clinical Research Center of the Hospital at the University of Pennsylvania.

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Determination of Tinidazole in Tablets by dc Polarography

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Abstract □ A polarographic method was developed for determining tinidazole in tablets. The substance is extracted from the sample with water, pH 6.8 Britton–Robinson buffer is added to an aliquot, and the solution is polarographed at the dropping mercury electrode *versus* the saturated calomel electrode. The polarographic wave is well developed and enables a fairly precise quantitative determination. The method of standard addition is used.

Keyphrases □ Tinidazole—polarographic analysis, commercial tablets □ Polarography—analysis, tinidazole, commercial tablets □ Antitrichomonal agents—tinidazole, polarographic analysis, commercial tablets

Tinidazole[1], 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole, is an antitrichomonal agent and has a chemical structure similar to that of metronidazole, 2-methyl-5-nitro-1*H*-imidazole-1-ethanol, *i.e.*, a 2-methyl-5-nitroimidazole ring. The mechanism of the polarographic reduction of metronidazole is known (1), and from the chemical similarity it can be concluded that tinidazole undergoes the same polarographic reduction mechanism. Tinidazole and metronidazole also have similar dc polarographic behavior including a four-electron reduction wave corresponding to the reduction of the nitro group, the same slope of the $E_{1/2} = f/\text{pH}$ plot, and close values of $E_{1/2}$.

For the quantitative determination of tinidazole, the more positive wave was used. This wave is evident in a Britton–Robinson buffer of pH 6.8 and has the $E_{1/2}$ at −0.47 v *versus* the saturated calomel electrode. The more negative wave, which is not well developed, does not interfere. Tinidazole is stable at pH 6.8.

[1] Pfizer Inc.

EXPERIMENTAL

Apparatus—A recording polarograph[2] was equipped for dc polarography and had a drop timer[3]. A dropping mercury electrode with an outflow velocity of 2 mg/sec at a height of 60 cm of the mercury head was used. It was placed in a standard beaker-type cell with a saturated calomel electrode.

Reagents and Chemicals—Britton–Robinson buffer, pH 6.8, was prepared by mixing 100 ml of Solution A and 50 ml of Solution B. Solution A consisted of 0.04 *M* acetic acid, 0.04 *M* orthophosphoric acid, and 0.04 *M* boric acid. Solution B was 0.2 *M* sodium hydroxide.

For the standard solution, 60 mg of tinidazole standard was dissolved in water in a 100-ml volumetric flask (amber glass).

Procedures—Weigh a portion of finely ground sample, 100–150 mg of tinidazole, into a 250-ml volumetric flask (amber glass). Add 200 ml of water, shake well for at least 30 min, and dilute to volume with water. Filter through filter paper with fine pores, discarding the first 20 ml of filtrate. Then pipet 5 ml of the clear filtrate into a 50-ml volumetric flask, add 0.3 ml of a 0.2% aqueous solution of polyethylene glycol ether of monoisooctylphenol[4] or the same amount of a 0.2% aqueous solution of gelatin, and dilute to volume with the Britton–Robinson buffer. Pipet 25 ml of the prepared solution into a polarographic cell and purge with oxygen-free nitrogen for 10–15 min. Record the polarogram *versus* the saturated calomel electrode from 0.0 to 0.9 v applied voltage on the dropping mercury electrode with the sensitivity of 7 μamp for the full scale of the recorder. Apply a suitable damping and use a drop timer, the drop time being 2–3 sec.

After obtaining the polarogram, add 1 ml of the standard solution to the solution in the cell, deaerate with oxygen-free nitrogen for 1 min, and again record the polarogram under the same conditions. Measure the wave heights H and h (Fig. 1) and calculate:

$$\text{mg of tinidazole per tablet} = \frac{ahb \times 1000}{(1.04H - h)(w)} \qquad \text{(Eq. 1)}$$

where a = milligrams of tinidazole reference standard in 100 ml of standard solution, w = weight of sample (milligrams) taken for the

[2] Polarimeter type PO 4, Radiometer, Copenhagen, Denmark.
[3] Type DLT 1, Radiometer, Copenhagen, Denmark.
[4] Triton X-100, Rohm & Haas Co.