Identification of Barbiturates in Urine

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Abstract □ A TLC method was developed which employs a resin column for the extraction and identification of barbiturates in urine. The method is sensitive to less than 1 mcg./ml. for all of the barbiturates tested. Certain tranquilizers and sedatives appear on the chromatogram with the barbiturates, but they do not interfere with barbiturate detection. The efficacy of this method was demonstrated using urine samples obtained from both inpatients and outpatients under supervised conditions.

Keyphrases □ Barbiturates—TLC method of identification in urine, designed for drug abuse screening programs, elimination of interference from tranquilizers and sedatives □ Drug abuse screening programs—TLC method of identifying barbiturates in urine □ TLC—method of identifying barbiturates in urine, application to drug abuse screening programs

In the general area of drug abuse, much recent effort has been concentrated on the detection and treatment of narcotic addiction. However, probably due to the high price of heroin, there has been a steady increase in barbiturate abuse. For this reason, urine surveillance that includes the detection of barbiturates is important. Because of shortcomings in present methods, this laboratory has investigated and developed a new procedure for identifying barbiturates in urine.

In the last year, many different TLC procedures, solvent systems, and sprays were studied in this laboratory to detect and separate barbiturate metabolites in urine from the metabolites of other drugs such as chloral hydrate, tybamate, methyprylon, and glutethimide. These drugs, when given to drug abuse patients as part of their daily medications, were found to interfere consistently with the detection of barbiturates by existing methods. Several TLC methods were reported (1–5) but it was difficult for the present authors to distinguish barbiturates from certain sedatives and tranquilizers when following these reported procedures. Similar interference was noted using the ion-exchange paper methods of Dole *et al.* (6) and Kaistha and Jaffe (7). GLC (8), spectrophotometry (9, 10), and radioimmunoassay (11) are more sensitive than TLC for barbiturates. However, these methods indicate only one class of drugs at a time and are suitable mainly for confirmatory tests—not for routine urine surveillance performed in an average laboratory.

To avoid false-positive and false-negative results during urine surveillance of drug abuse patients who might be taking one or more interfering drugs, a reliable but simple method of identifying barbiturates was needed. This paper describes the procedure developed for detecting barbiturates in the presence of other drugs.

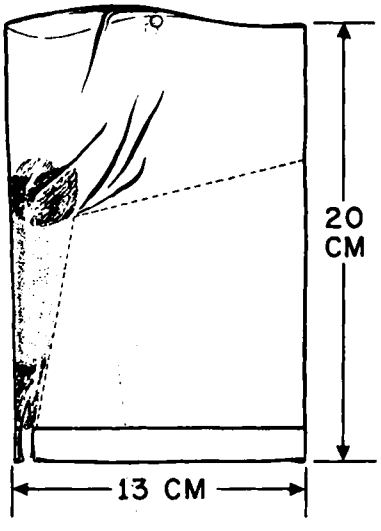

Figure 1—*A polyethylene bag is prepared and labeled for each sample. Urine (50 ml.) is poured into the upper channel of the bag, flows through the resin column, and drains out through the glass wool stopper at the bottom of the column.*

EXPERIMENTAL

Materials—Drugs were extracted from the urine using a resin column[1]. The resin is a styrene divinylbenzene copolymer which has a nonionic, macroreticular structure with high surface area capable of adsorbing many water-soluble organic compounds (12). The resin was first rinsed with 5% sodium chloride and 1% sodium bicarbonate to control bacteria and mold growth during storage. Several bed volumes of distilled water were washed over the surface of the resin until the decant became clear (13).

Columns were fabricated by heat sealing a 1.5 × 10-cm. channel on one side of a 12 × 20-cm. polyethylene bag and plugging both ends of the channel with glass wool stoppers. The columns were filled to a height of 7 cm. by gravity packing an aqueous slurry of the resin into the channel (Fig. 1). The colored portion of the eluate collected from the column was concentrated and spotted on silica gel-impregnated microfiber sheets[2].

All chemicals were of reagent grade. The solvent system consisted of chloroform–acetic acid (50:0.1) prepared fresh daily. One of the colored sprays used was diphenylcarbazone, prepared as a 0.1% solution in 95% ethanol. This reagent was stable for 2 or 3 weeks but should be refrigerated in an amber bottle. The second spray, prepared by dissolving 20 g. mercurous nitrate in 500 ml. of 0.15 *M* nitric acid, was also stable for several weeks.

Procedure—Fifty milliliters of urine was poured through the resin column. The column was compressed to force all of the urine to pass through it, and then the compounds adsorbed by the resin were eluted with 15 ml. of methanol. The colored portion of the eluate was collected, and 10 ml. of this eluate was concentrated to about 1 ml. Forty microliters of the concentrated eluate was spotted on precoated thin-layer sheets[2] and developed in a chloroform–acetic acid solvent system for 10 min. The chromatograms were air dried and sprayed with diphenylcarbazone until a pink color became visible. The dried chromatograms were then sprayed with mercurous nitrate solution, and a blue color developed. Within 5 min., white spots appeared at approximate R_f 0.7, indicating the presence of a barbiturate.

Since R_f values may vary slightly, at least one known barbiturate urine standard must be spotted on each chromatogram. Phenobarbital produced a V-shaped spot which may be distinguished from the rectangular-shaped spot shown by the other barbiturates. The appearance and position of the white spots should be recorded immediately since they fade to a blue color, indistinguishable from

[1] Amberlite XAD-2 resin, Rohm & Haas Co., Philadelphia, PA 19105

[2] Gelman Instrument Co., Ann Arbor, MI 48106

Table I—Color Imprint and R_f Value of Drugs on Chromatogram

Generic Name	White Spot, R_f 0.7	Blue Spot, R_f 0.7	Faint Blue Spot, R_f 0.7 (or Lower)
Amobarbital	+	+	−
Pentobarbital	+	+	−
Phenobarbital	+	+	−
Secobarbital	+	+	−
Chloral hydrate	−	+	−
Chlordiazepoxide	−	−	+
Diazepam	−	−	+
Ethchlorvynol	−	−	+
Glutethimide	−	−	+
Meprobamate	−	−	+
Methyprylon	−	+	−
Tybamate	−	+	−

the background, within 15 min. Following the disappearance of the white spot, the blue background began to fade, revealing a blue spot with the same or slightly lower R_f value than the white barbiturate spot. This blue spot was characteristic of barbiturates and certain other drugs.

RESULTS AND DISCUSSION

The reliability, specificity, and sensitivity of this method were tested using urine samples from both inpatients and outpatients[3].

Reliability—To test the reliability of this method, 550 urine samples from drug abuse patients were analyzed for barbiturates and other drugs. Forty-one of these patients were taking prescribed barbiturates, and the white spot on the chromatogram revealed the presence of barbiturate in the urine of each of these. Barbiturate was also found in the urine of 15 additional patients. Since some patients admitted taking illicit barbiturates, a maximum false-positive error of <2.7% was indicated.

Specificity—Table I summarizes the color imprint and R_f value of the drugs which appeared on a chromatogram used in the present barbiturate identification system. Figure 2 illustrates the appearance of some of these drugs on the chromatogram immediately after spraying with mercurous nitrate solution and later after the background had faded. The white spot at R_f 0.7 was specific for the presence of barbiturates, and no drug listed in Table I interfered with barbiturate detection. The appearance of the blue spot at R_f 0.7 after the background faded indicated the presence of barbiturates and other drugs listed in Table I. Because of the interference of these other drugs, the blue spot obtained by using diphenylcarbazone and mercurous nitrate sprays cannot be considered as evidence specific for either barbiturates or any one of the drugs listed in Table I. However, the presence of chloral hydrate, tybamate, or methyprylon can be verified, since these drugs showed characteristic R_f values in the previously published system for the detection of opiates (12).

Sensitivity—The sensitivity of this method was determined by measuring the barbiturate concentration in the column eluate, using a modification of Broughton's procedure (14). Fifty milliliters of known barbiturate urine was poured through the resin column and eluted with 15 ml. methanol. Before concentrating 10 ml. of the colored portion of the eluate to dryness, the eluate was treated with decolorizing charcoal, which removed the pigment. Reference standards of 0.45 *N* sodium hydroxide and a combination of 0.45 *N* sodium hydroxide and boric acid solution were prepared. Each standard was compared to a solution containing 1 ml. of the standard plus 1 ml. of the decolorized eluate redissolved in 0.45 *N* sodium hydroxide. Barbiturate concentration was determined by measuring the difference in absorbance at 260 nm. The difference in the absorbance of the two test solutions when compared with the absorbance of the two standards at the same wavelength indicated the amount of barbiturate present in the urine.

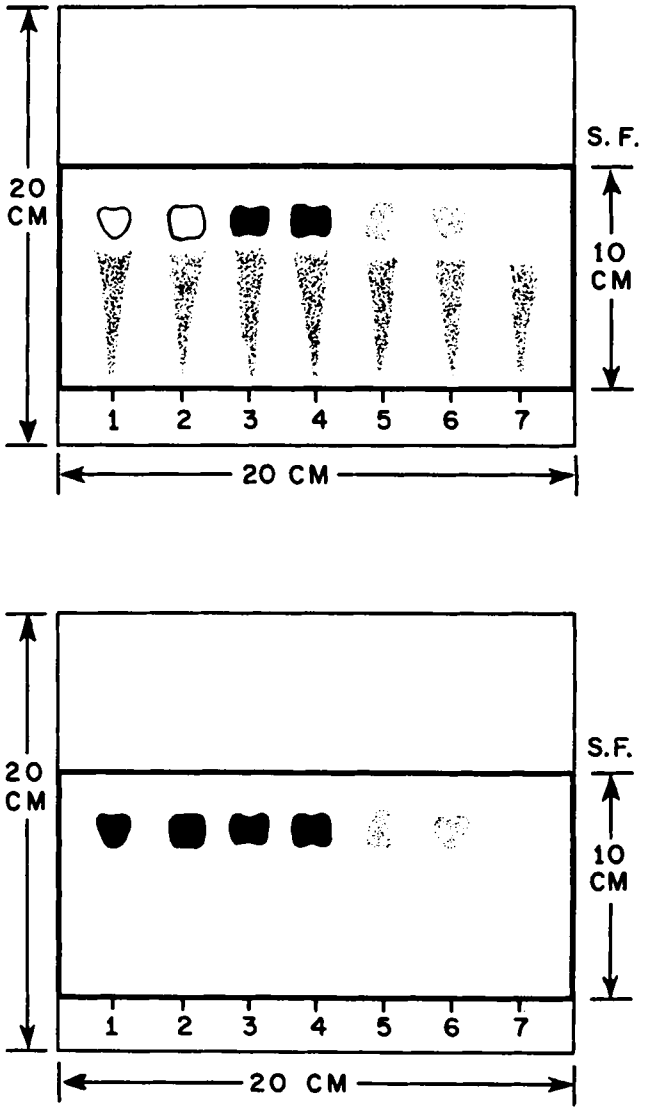

Figure 2—*(Top) Appearance of drugs on chromatogram immediately after mercurous nitrate spray. Key: 1, phenobarbital; 2, other barbiturates; 3, chloral hydrate, tybamate, and methyprylon; 4, ethinamate; 5, chlordiazepoxide, diazepam, ethchlorvynol, and glutethimide; 6, meprobamate; and 7, drug-free urine. (Bottom) Appearance of drugs on chromatogram after background has faded.*

This procedure was followed with separate urine samples containing pentobarbital, phenobarbital, and secobarbital. After the amount of barbiturate in each of the three urine samples was determined, each urine sample was diluted into smaller aliquots with normal urine to decrease the barbiturate concentration in the urine. Each diluted urine sample was assayed through the chromatographic method. The last diluted urine sample that revealed a white spot on the chromatogram represented the degree of sensitivity of the method for the detection of that barbiturate. The minimum sensitivity of the method was 0.7 mcg./ml. for patients receiving secobarbital, 0.4 mcg./ml. for patients receiving pentobarbital, and 0.02 mcg./ml. for patients receiving phenobarbital. It was also noted that barbiturate could be detected in the urine of drug abuse patients for at least 36 hr. after ingestion of 100 mg. of barbiturate. Longer periods were not checked due to the faintness of the white spots after 36 hr.

Confirmation—The identity of the white spot as barbiturate was confirmed using ^{14}C-labeled phenobarbital. A 48-hr. urine sample was collected from a rat given one injection of phenobarbital (25 mg./kg.) containing 2 mcg. of ^{14}C-phenobarbital labeled in the 2-

[3] At the Drug Abuse Treatment and Rehabilitation wards and clinics, Wood Veterans Administration Center. There were approximately 30 drug abuse patients in the wards and about 80 outpatients at any given time. Periodically, the staff also participated in studies to provide control and standard barbiturate urine samples.

position. The urine was processed by the described method, and the developed chromatogram was placed on X-ray film for 48 hr. The resulting radioactive spot on the X-ray film had the same shape and position as the white spot on the chromatogram.

CONCLUSIONS

Urine surveillance is an integral part of every successful drug abuse treatment and rehabilitation program. For urine surveillance to be effective, samples must be collected, labeled, and transported under controlled conditions. Urine must be analyzed accurately, and results must be reported promptly. A reliable laboratory method, specific for abuse drugs, is required to avoid both false-positive results, which would damage a patient's confidence and morale, and false-negative data, which could permit the undetected use of abuse drugs to continue.The laboratory method must also be inexpensive and efficient enough to evaluate a high volume of samples rapidly on a continuing basis.

The barbiturate identification procedure developed by the present investigators satisfies these requirements. It is particularly significant that interference from sedatives and tranquilizers has been eliminated. The method described in this paper avoids false-positive results and provides reliable identification of barbiturate metabolites in the urine of patients taking various drugs.

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Precursor-Type Insect Repellents: Kinetics of Hydrolysis

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Abstract □ The rates of ester hydrolysis of representative dihydroxyacetone monoesters were determined by titrimetric analysis. The esters were designed to elicit prolonged insect repellency by gradual hydrolysis in dermal tissue. No correlation was found between the agents' repellent activity and their susceptibility to hydrolysis. The repellency elicited by the esters appears to be due to the intact molecules rather than a hydrolytic product.

Keyphrases □ Dihydroxyacetone monoesters—rates of hydrolysis, effect on insect repellency □ Insect repellents, dihydroxyacetone monoesters—rates of hydrolysis, compared to repellent activity □ Hydrolysis kinetics—dihydroxyacetone monoesters, attempted correlation of hydrolysis rates and repellent activity

In previous articles from these laboratories, the syntheses and insect repellent data of 1,3-dihydroxy-2-propanone (dihydroxyacetone) monoesters were reported (1–4). The activity of these precursor-type repellents was explained, in part, by their ability to hydrolyze, subsequent to topical application, and thereby release the insectifugal acids. The rationale of this approach was previously discussed in detail (2, 5). Compounds I, III, IV, VI, XI, and XIII (Table I) were studied because of their similarities in structure and physical properties and also because of their wide range in repellent activity. Due to the many physical parameters which reportedly play a role in the insect repellency of a compound (6), the objective was to ascertain if the hydrolysis of the dihydroxyacetone esters was responsible for their activity or if some other factors were involved.

EXPERIMENTAL

Materials—Compounds I, III, IV, VI, XI, and XIII were previously prepared in these labotatories (1–4). However, since these agents can exist in both monomer and dimer (a cyclic hemiketal) forms, the structure and purity of these moieties had to be confirmed. Heating the dimeric dihydroxyacetone monoesters in ethanol converts the dimer to the corresponding monomeric form (7). Solutions of the esters in either acetone or ethanol (the ethanol solution had been previously heated) elicited identical chromatographic behavior, that is, a single spot with the same R_f values[1]. Therefore, in an acetone–water (3:1) solution, the medium selected to conduct the hydrolysis studies, the esters would appear to be exclusively in the monomeric state. IR spectra of the samples were equivalent to those of analytically pure compounds; NMR spectra were consistent with their respective structures[2].

[1] The TLC procedure utilized Mallinckrodt SilicAR TLC-7GF as the sorbent and acetone–water (3:1) as the solvent system. The compounds were applied to the plates and, after development, the spots were visualized with iodine vapor.

[2] Spectra (IR and NMR) were obtained with the Beckman model IR-33 and the Hitachi Perkin-Elmer model R-24 spectrophotometers, respectively.