Table I. Falsely Positive Placidyl Values after Ingestion of Phenazopyridine Hydrochloride

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Apparent Placidyl concn, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>15.0</td>
</tr>
<tr>
<td>3</td>
<td>6.5</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*Samples were taken at unselected times after administration of phenazopyridine hydrochloride, from individuals not taking Placidyl.
*Single determinations.

Obviously, samples with low Placidyl concentrations would be diluted below the sensitivity of the method and this procedure would be unsuitable for the determination of such small amounts. However, higher concentrations of Placidyl, such as are seen in overdose situations, could easily be measured in the presence of Pyridium. The characteristic reddish-orange color was an easily observable indication that Pyridium was present in these urine samples, but was not observed in these sera.

References


Table I shows the apparent Placidyl concentrations found by this colorimetric method in urine and serum of persons who had ingested phenazopyridine hydrochloride. Urine No. 1 and serum No. 1 were collected about 8 h after a subject had ingested one 200-mg Pyridium tablet; urine No. 2 and serum No. 2 were collected about 3 h after a different subject had ingested one 200-mg Pyridium tablet. Urine No. 3 and serum No. 3 were collected from a patient who was taking four Azotrex capsules per day, which each contain 50 mg of phenazopyridine hydrochloride. None of these individuals was taking Placidyl.

The method can still be used to determine Placidyl in the presence of Pyridium by carrying the sample through the complete procedure, but substituting a blank reagent [50 ml of coned. H$_2$SO$_4$ and 100 ml of acetic acid:water (1:1, by vol)] for the Placidyl color reagent. The Placidyl concentration can then be determined from the difference between the absorbance of a sample reacted with the color reagent and that of a sample reacted with the blank reagent. For a specimen with a high concentration of Pyridium, the specimen must be diluted before a sample is taken through the procedure so that the absorbance of the chromogen can be measured.

To the Editor:

Clinical laboratories providing toxicological services, specifically analysis for drugs subject to abuse, are encountered in increasing workload. They are being required to provide analytical information on the drug content of blood and (or) urine in an attempt (a) to determine the causative agent in a comatose patient admitted to the hospital emergency department, (b) to identify drug users among employees or prospective employees in various industries and business organizations, and (c) to monitor addicts undergoing medical treatment.

The results from such testing not only have medical value but can, and in many cases do, have far-reaching social implications. The need for accuracy is paramount, to provide the physician with meaningful data to treat his patients and to eliminate the potential social repercussions resulting from the reporting of false positive results.

Recent studies by the New York State and City Departments of Health (4) demonstrate that a serious analytical problem exists in many clinical laboratories that screen urines for drug subject to abuse. Their initial studies in 1970 show that 59% of the clinical laboratories and 37% of the forensic toxicology laboratories received scores of less than 75 (out of a possible 100 points), thereby failing the proficiency test. Furthermore, additional studies indicate that 53–63% of all laboratories tested for the first time will fail! Subsequent to corrective workshops in drug screening methodology, retesting established that about 80% of all the laboratories were able to pass the proficiency exam.

These findings initiated a survey analysis of the various state health departments in an attempt to establish the existence of drug abuse-toxicology proficiency testing programs for intrastate clinical laboratories providing toxicological services. A questionnaire was sent to each of the fifty health departments as well as the District of Columbia, Puerto Rico, the Virgin Islands, and Guam. The recipients were asked:

1. Do you presently have a drug abuse and (or) toxicology proficiency-testing program?

2. If not, do you plan to establish such a program in the near future?

3. If affirmative, would you please send an outline of your testing procedure?

Follow-up letters were mailed 30 days after the initial inquiry.

Responses were received from all 54 inquiries, which we gratefully acknowledge, and their analysis indicated that only five have proficiency-testing programs—Arkansas, Florida, New York, Utah, and Wisconsin. Each conducts a blood-alcohol testing program, while New York additionally requires a urine-screening examination for drugs subject to abuse. Only New York intends to further develop their program. The remaining four stated that there were no immediate plans to expand present programs.

Four additional replies—from Connecticut, Georgia, Pennsylvania, and Guam—indicate that they have drug abuse and (or) toxicology proficiency programs in the planning stage. The Pennsylvania program is scheduled to be in operation by mid-1972 and will consist of a blood-alcohol and abusible-drug screening program. The remaining health departments have programs in various stages of development without anticipated implementation dates.

It is indeed fortunate that at least one state (New York) is presently conducting a testing program for drugs subject to abuse. The need for such a program is evident and it is hoped that other states will (a) take advantage of New York’s experience in this area.
and develop comparable regulatory programs for their state or (b) require interstate clinical laboratories providing toxicological services to participate in the toxicology proficiency program now being separately developed by the National Center for Disease Control or the College of American Pathologists. The failure to provide adequate safeguards by mandatory drug-abuse proficiency testing opens the door to unacceptable medical, as well as social, repercussions.

Reference


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Methyl Orange as a Screening Reagent for Organic Bases

To the Editor:

Recently an improved colorimetric procedure for the assay of amphetamine in urine was reported (1). In this procedure potentially interfering drugs are removed by extracting them with chloroform at pH 5.5 and the bases remaining are extracted at alkaline pH and identified by using methyl orange (2, 5) to develop a chromogen. Only amphetamine, methamphetamine, pentazocine, and codeine purportedly produce a chromogen with the alkaline extract. We have subjected other frequently used drugs to this procedure, and suggest that the results and some additional comments are noteworthy.

Most organic bases produce chromogens with methyl orange, but several of these compounds are removed by extraction with chloroform at pH 5.5. Such compounds not included in the authors' original list are desipramine, nortriptyline, and benzphetamine.

Other drugs (in addition to methamphetamine, pentazocine, and codeine) are not removed by extraction at pH 5.5, however, but are in the alkaline extract and produce a chromogen with methyl orange. These drugs include ephedrine, methamphetamine, and phencyclidolamine. Phenylpropanolamine is a significant addition to the authors' original list because it is a common component of several proprietary preparations.

The authors adequately demonstrated that chloroform extraction at pH 5.5 effectively removed many drugs, and our results concur with their findings. This valuable step gives some degree of specificity to the otherwise non-specific methyl orange reaction. However, another important contribution of this step should be mentioned. Reacting the pH 5.5 chloroform extract with the methyl orange reagent in the same way as the alkaline extract can give valuable information concerning the presence or absence of a large number of organic bases. Thus, by analyzing the pH 5.5 chloroform extract significant additional information can be obtained with minimal additional effort. A negative result excludes numerous bases, but a positive result indicates one or more of these drugs may be present. Either result may be clinically significant.

References


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Mercury Pollution from the Laboratory: A Correctable Problem

To the Editor:

Many chemical laboratories are sympathetic to efforts by the community to avoid pollution of the atmosphere and our water systems, but have not looked critically at pollution problems generated in the clinical chemistry laboratory. A gross example is the discharge of mercuric salts into the waste water system during the analysis of serum chloride.

The most widely used automated procedure for the determination of chloride uses a saturated solution of mercuric thiocyanate as one of its reagents. The mercuric salt is in a soluble form during the entire procedure and is discarded into the sewage system after an absorbance reading in the spectrophotometer. The amount of mercury from a single day's run can be considerable. Collection of the spent chloride reagent from a single AutoAnalyzer in our Clinical Laboratory revealed that approximately 5 g of divalent mercury were discarded per week, or 260 g per year. A similar study of our SMA 12/30 revealed that it contributed 3.3 g of mercuric ion per week, or 172 g per year. These are considerable amounts for a single laboratory to discard into a city sewer system. Although the laboratory pollution with mercury is dwarfed by the total industrial contribution, it can be completely prevented by a simple procedure.

A method to take care of the situation has been described in Technicon Service Bulletin TBI-0160-00, dated October 1971. It calls for the collection in a bottle of the spent chloride reagent, followed by the addition of thioacetamide to precipitate the mercury as mercuric sulfide. The precipitate is filtered from the fluid and stored. After a period of time the mercuric sulfide can be treated chemically to convert it to metallic mercury. Thus, with little effort and low cost, mercury pollution arising from the clinical laboratory can be prevented entirely and the pollutant salvaged as metallic mercury. Clinical chemists can in this way become more responsible citizens.

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Use of p-Nitrophenyl Phosphate Dicyclohexylammonium Salt as Substrate for Alkaline Phosphatase Determination on the SMA 12/60

To the Editor:

The procedure used for determination of alkaline phosphatase activity on the SMA 12/60 (Technicon Instruments Corp., Tarrytown, N.Y. 10591) is that of Morgenstern et al. (Clin. Chem. 11, 876 (1965)), a modification of the procedure of Bessey et al. (J. Biol. Chem. 164, 321 (1946)). It is based on the enzymatic hydrolysis of p-nitrophenyl phosphate (pNPP), the color of p-nitrophenol in alkaline solution being measured. To date, the disodium salt of pNPP has been universally used as the substrate for the determination.

Recently, the dicyclohexylammonium salt of pNPP was made available to us as a possible substitute for the disodium salt (Lyne Laboratories, 750 Main St., Winchester, Mass. 01890).

The following summarizes our observations:

1. The salt is stable at room temperature and is not adversely affected.