Emergency Screening of Urine, Plasma, or Gastric Contents for Barbiturates

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A procedure is described for emergency screening for barbiturates that requires no specialized equipment and can be performed in less than 10 min; thus lending itself to emergency-room use. The method is highly specific and can be used to detect as little as 1 mg/100 ml.

Additional Keyphrases barbiturate-mercury complex
• dithizone

There is an ever-increasing need for a rapid method of screening for barbiturates in the laboratory. Although the ultraviolet methods (1, 2) are the most accurate, they require 0.5 to 2 h of technician time and therefore are hardly ideal for emergency-room use. Some methods have been published which depend on the complexing reaction of barbiturates in an organic solvent with a mercury salt in an aqueous solution (3-7). However, these, too, require special equipment and have their practical limitations.

The procedure described below also depends on complexing action but requires no specialized equipment and can be performed in less than 10 min. Therefore, it lends itself to emergency-room use. The method is highly specific and is sensitive to about 1 mg/100 ml.

Materials and Method

Reagents

Phosphate buffer, 0.066 mol/liter, pH 6.95. Add 0.831 g of Na₂HPO₄·7H₂O and 0.363 g of KH₂PO₄ to distilled water and dilute to 100 ml.

Mercury reagent. Dissolve 0.5 g of mercuric chloride in 50 ml of water plus 3 drops of concentrated nitric acid;

dilute 1 ml of this solution to 50 ml with water and add 0.42 g of sodium bicarbonate.

Dithizone (diphenylthiocarbazone), about 1 mg/100 ml, in chloroform. This solution should give an absorbance of about 2.00 (check in spectrophotometer) with a 1-cm light path at 605 nm vs. chloroform as the blank. Prepare freshly every two weeks.

Method

Place 20 ml of chloroform in a 50-ml Erlenmeyer flask with a Teflon stirrer. Add 4 ml of specimen (urine, plasma, gastric contents, and a positive or negative control). Add 5 ml of phosphate buffer. Stir on a magnetic stirrer for 4 min. Aspirate the supernatant fluid with a Pasteur pipet. Add 2 ml of mercuric chloride solution, stir vigorously for 4 min, and aspirate the mercuric chloride layer. Add about 20 ml of distilled water, stir, and aspirate the supernatant (aqueous) layer as completely as possible. (A few drops of CHCl₃ may be lost.) Add 2 ml of dithizone solution under the surface of the chloroform and rotate flask very gently by hand; vigorous agitation can cause false positives. If an orange color appears immediately when dithizone is added, the sample is positive. If the blue color remains, barbiturates are reported as negative.

Experimental

This method is sensitive to 1 mg of phenobarbital, secobarbital, amobarbital, or pentobarbital per 100 ml. Since normal therapeutic concentrations in blood do not approach this, a positive reaction will indicate barbiturate poisoning as a cause of coma of undetermined origin.

The only strong positive interference known so far is from hydantoin derivatives, medication used for epilepsy. Glutethimide gives a reaction at 5 mg/100 ml and diphenylhydantoin at 8 mg/100 ml. Salicylate, even at concentrations as high as 100 mg/100 ml, does not interfere. Chlorpheniramine and methyprylon give
positive reactions only at toxic levels—greater than 4 mg/100 ml and at 150 mg/100 ml, respectively.

A series of 15 specimens tested by the screening procedure was rechecked by the ultraviolet method, and excellent correspondence as to the presence or absence of barbiturates was obtained. Only rarely did the clinician request that a quantitative test be subsequently performed.

I thank Paul Brunner, M. T., ASCP, for technical assistance.

References


Absence of Measurable Leukocyte Alkaline Phosphatase Activity from Leukocytes of Patients with Chronic Granulocytic Leukemia

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Leukocyte phosphatase activity was determined over a broad pH range. Using this procedure, we found no alkaline phosphatase activity in leukocytes from three patients with chronic granulocytic leukemia. The low values usually reported for this disease are actually attributable to acid phosphatase activity, which is slight but measurable at alkaline pH.

Additional Keyphrases sonication of cells • spectrophotometry • acid phosphatase activity of leukocytes

Decreased activities of leukocyte alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) have been reported in patients with chronic granulocytic leukemia (1, 2). Such activity measurements are useful in differentiating this disease from other leukemias (3). Such measurements are typically made at a single pH value, usually pH 10, whereas on measuring it over a broad range of pH we have demonstrated that leukocyte alkaline phosphatase activity is absent from the leukocytes of individuals with chronic granulocytic leukemia, rather than simply being decreased.

Materials and Methods

Leukocytes were isolated from both normal and leukemic blood and disrupted by sonication as previously described (4). Normal cells were obtained from apparently healthy volunteer subjects, leukemic cells from three patients with an unequivocal diagnosis of chronic granulocytic leukemia.

The phosphatase activity of the sonicates was determined as follows: 0.50 ml of substrate solution (0.015 mol of p-nitrophenylphosphate per liter) was mixed with 0.50 ml of the appropriate buffer:

(a) 2-Amino-2-methyl-1-propanol, 0.1 mol/liter, containing 0.001 mol of Mg$^{2+}$ per liter, was used over the pH range 8.5 to 11.0.

(b) Tris(hydroxymethyl)aminomethane, 0.1 mol/liter, containing 0.001 mol of Mg$^{2+}$ per liter, was used over the pH range 6.5 to 9.0.

(c) Citrate, 0.1 mol/liter, was used over the pH range 3.5 to 6.5.

De-ionized water was added to give a final incubation volume (after the addition of sonicate) of 1.50 ml. All tubes were equilibrated at 37°C for 10 min. The reaction was then started by adding sonicate. Because of the relatively greater phosphatase activity in the acid range, we used less sonicate at the lower pH values. A typical curve was obtained by incubating 0.05 ml of normal sonicate (containing 0.075 mg of protein) in the pH range of 3.5 to 6.5, 0.10 ml of sonicate (0.15 mg of protein) in the pH range of 6.5 to 9.0, and 0.20 ml of sonicate (0.30 mg of protein) in pH range of 8.5 to 11.0. The incubation was ended after 30 min by adding 10.0 ml of 0.1M sodium hydroxide. The p-nitrophenol released