Rapid Porphyrin Screening of Urine, Stool, and Blood

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Simplified chemical screening tests and an apparatus to facilitate their performance have been developed for porphyrins. The tests are applicable to urine, stool, and blood. The procedures for different specimens are essentially identical and can be performed in a few minutes with a minimum of reagents and apparatus. Results obtained are usually sufficiently definitive to permit differential diagnoses of porphyrias and porphyrinurias without resort to separation or quantitation of the individual porphyrins.

Diagnosis of the various diseases of porphyrin metabolism, the porphyrias, and porphyrinurias frequently requires knowledge of possible abnormal porphyrin levels in three specimens: urine, stool, and blood. Fortunately, quantitative information beyond the fact that porphyrin levels are significantly above normal is rarely necessary. For this reason, appropriate porphyrin screening tests will suffice in diagnosing most cases where there is a defect in porphyrin metabolism. Many screening tests have been published (1–6); however, in most cases they are applicable to a single type of specimen. Moreover, these tests are in some cases complex or cumbersome, requiring a variety of reagents, a relatively intense ultraviolet light source, and routinely must be viewed in darkened areas of the laboratory. In most routine laboratories, porphyrin assays are performed infrequently and by personnel having limited experience with this type of assay.

This report describes a basic porphyrin screening test that is applicable to urine, stool, and blood, and presumably any other biologic material that one may wish to examine. The test uses only two reagents and a minimum of apparatus. Developed concomitantly with the screen-
ing test was a source of ultraviolet irradiation enclosed in an appropriate viewing box that allows for more accurate interpretation and permits performance of the test in a normally lighted area.

Materials and Methods

Reagents
The reagents required are glacial acetic acid:ethyl acetate (HAc:EtAc) mixed in a 1:4 ratio, and 3 N HCl. The two solutions may be prepared in advance and stored until needed. Analytic reagent grade chemicals are used.

Apparatus
All tests are performed in standard glass 12- or 15-ml conical centrifuge tubes. Plastic tubes must not be used because some synthetic materials will interfere with observation of the fluorescence. The screening tests were developed and the interpretations made using a specially designed viewing box.* However, the tests can be performed using a variety of long wavelength ultraviolet light sources, providing these are of sufficient intensity, and proper evaluations with specimens of known porphyrin content have been made.

Urine Test
Five milliliters of urine plus 3 ml of HAc:EtAc are placed in a centrifuge tube and shaken thoroughly. The phases are allowed to separate by standing, or the process may be hastened by a brief centrifugation. The upper (organic) layer is observed for porphyrin fluorescence. This layer will be blue with normal urine and ordinarily appears as a bluish-pink color if significantly elevated levels of porphyrins are present.

Stool Test
A small pellet of stool, approximately the size of a 3-mm cube, is taken up on the end of a glass rod and vigorously stirred into 3 ml of HAc:EtAc in a centrifuge tube. After centrifuging, the supernatant is decanted from the residue material into a second centrifuge tube containing 0.5 ml of 3 N HCl. The solutions are shaken well and allowed to separate or are centrifuged briefly. The lower (aqueous) layer is observed for porphyrin fluorescence. Normally this layer appears almost black, while elevated amounts of porphyrin will impart an orange-red fluorescence.

*Helpful suggestions and assistance in design of the apparatus were contributed by Ultra-Violet Products, Inc., San Gabriel, Calif. This apparatus is available as the FA-2 Fluorescence Analyzer.
Blood Test

One milliliter of whole blood is added to 3 ml of HAc:EtAc in a centrifuge tube. The mixture is well-stirred, centrifuged, and the supernatant solution decanted into a second tube containing 0.5 ml or 3 N HCl. The two phases are shaken thoroughly and allowed to separate. The lower HCl layer is then observed for an orange-red porphyrin fluorescence. Using these quantities of materials, only a trace of porphyrin fluorescence is seen in normal blood.

Interpretations

Urine

With this test, an upper-normal concentration of porphyrins, or about 200 µg/liter of urine for adults, will be just barely detectable. Occasionally, drugs, abnormal metabolites, or unknown materials will impart a fluorescence or color to the organic layer that will make interpretation of the level of porphyrins difficult. When such interfering substances are encountered or when increased sensitivity is desired, the organic layer may be removed carefully with a pipet and transferred to a second tube to which is added 0.5 ml of 3 N HCl. On shaking the solutions, porphyrins are extracted into the lower acid layer, while the interfering substances remain in the organic phase. Porphyrin fluorescence in acid is greatly intensified, and the fluorescence loses its bluish tint to become orange-red in color.

Stool

A trace of fluorescence represents about 60 µg porphyrin per gram of dry weight of stool. The amount of material used and the composition of the stool, especially its liquid content, lead to significant variations in the sampling. Nevertheless, this does not alter the usefulness of the test. In those types of porphyria with elevated stool porphyrins, the fluorescence observable in the HCl layer will be sufficiently above normal that no difficulty should be encountered in detecting a pathologic state.

The occasional presence of chlorophyll may cause confusion, since it also has a red fluorescence that is not easily distinguishable from porphyrin fluorescence. However, because chlorophyll is lipophilic, it remains in the upper (organic) layer in this test, where its usual red fluorescence may tend to have a yellowish-brown tinge. On rare occasions in which very high levels of chlorophyll prevent complete extraction from the aqueous layer, the presence of red fluorescence in this lower layer has led to a questionable or difficult interpretation. This
problem is alleviated easily by drawing off the upper organic layer containing most of the chlorophyll, adding a fresh 3-ml aliquot of HAc: EtAc to the remaining HCl layer, and shaking. This procedure will remove completely any possible chlorophyll interference from the lower HCl layer, and any remaining red fluorescence can be considered due to porphyrins.

Blood

This test gives an easily interpretable "normal," since the blood porphyrin content falls within a rather constant and narrow range. A trace of fluorescence under the test conditions is seen at a porphyrin level of 50–60 \( \mu \text{g}/100 \text{ ml} \) whole blood.

Clearly, this procedure does not distinguish between porphyrins in erythrocytes and plasma; however, such information is unnecessary to make an accurate diagnosis of any known disease of porphyrin metabolism. The test is not so sensitive or precise for detecting abnormal red cell porphyrins as viewing an unstained blood smear by fluorescence microscopy; but it is simpler and adequate for most diagnostic purposes.

Comments

While interpretation of all tests is dependent upon the porphyrin concentration, and to some extent the observer and his experience in viewing porphyrin fluorescence, each test is designed with appropriate quantities of materials to show a barely detectable porphyrin fluorescence at typically high normal concentrations. Obviously, wide fluctuations in urine volume and stool composition can make considerable differences. Nevertheless, performed as described, the screening test would unlikely fail to reveal a clinically significant defect in porphyrin metabolism. Typical test results to be expected with specimens from various diseases of porphyrin metabolism are summarized in Table 1. Of course, an accurate diagnosis must be based also upon clinical symptoms, but these findings illustrate the potential value of the screening test in differentiating the diseases listed.

There is no preferred sequence in which clinical specimens should be screened. More important, in a patient with a suspected abnormality of porphyrin metabolism, it is probably advisable to test routinely the three clinical specimens: urine, stool, and blood.

Occasionally a precipitate in the urine will adsorb porphyrins, especially uroporphyrin, and impart a definite orange-red fluorescence to the pellet. This fluorescence is not seen normally, and a significant elevation in urinary porphyrins can be assumed when it does appear.
Table 1. Typical Fluorescence Intensities Seen in Various Diseases of Porphyrin Metabolism

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fluorescence intensity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td>Normal</td>
<td>+</td>
</tr>
<tr>
<td>Erythropoietic protoporphria</td>
<td>+ to ++</td>
</tr>
<tr>
<td>Congenital erythropoietic porphyria</td>
<td>+++</td>
</tr>
<tr>
<td>Acute intermittent porphyria</td>
<td>+ to +++</td>
</tr>
<tr>
<td>Porphyrria cutanea tarda</td>
<td>++ to +++</td>
</tr>
<tr>
<td>South African genetic porphyria</td>
<td>+ to +++</td>
</tr>
<tr>
<td>(variegata)</td>
<td></td>
</tr>
<tr>
<td>Hereditary coproporphyria</td>
<td>+ to +++</td>
</tr>
<tr>
<td>Lead poisoning</td>
<td>+ to ++</td>
</tr>
<tr>
<td>Other porphyrinurias</td>
<td>+ to ++</td>
</tr>
</tbody>
</table>

* Fluorescence intensities: + = absent or faint; ++ = medium; +++ = vivid.

Falsely positive results have not been seen, since chlorophyll is the only known biologic compound besides porphyrins to produce a red fluorescence on illumination with long wave ultraviolet light. Chlorophyll contamination, which might rarely occur in stool specimens, can be eliminated as noted above.

The screening test does not distinguish among the three biochemically important porphyrins: uroporphyrin, coproporphyrin, and protoporphyrin; neither will it detect the porphyrinogens—ie, the reduced, colorless, nonfluorescent forms of the porphyrins. However, porphyrinogens are readily auto-oxidized, especially in acid solution, so it is most unlikely that an accurate diagnosis would be missed merely because of the presence of porphyrinogens in a specimen.

The porphyrin precursors, δ-aminolevulinic acid and porphobilinogen, are not detected by this procedure. Although δ-aminolevulinic acid excretion in the urine is especially useful in the early diagnosis of lead poisoning (7), its determination must be done by quantitative procedures, which are beyond the scope of this screening test. Porphobilinogen also can be determined either qualitatively by the Watson-Schwartz test (1) or quantitatively when desired (8).

Viewing and interpretation of the porphyrin fluorescence within a few minutes is advisable. A number of urine specimens have been found in which an initial slightly elevated fluorescence showed a definite increase either when left under ultraviolet irradiation for a period of time or when viewed 24 hr later. While this change can be confusing, no urine specimens so observed have changed from what would be considered a normal to an abnormal level of fluorescence.

Where separation of the porphyrins is desired, or quantitative in-
formation must be obtained, other analytic methods are recommended (1, 9). The described screening test is not adequate for adapting to quantitative determinations in most cases. Uroporphyrin, particularly, is incompletely extracted from biologic materials by the method used in this test. While this factor does not interfere with the value of the screening test, it does make the procedure inadequate for quantitative analysis.

The viewing apparatus mentioned was developed concomitantly with the screening tests, and all interpretations are based on its use. This apparatus has been designed to provide several advantages: (1) Specimen tubes are transilluminated to provide better detection of porphyrin fluorescence. (2) Appropriate filtering of the light source plus the inclusion of a viewing lens permits porphyrin fluorescence to be observed easily against a virtually black background, making interpretation more reliable. (3) Observations can be made under highly reproducible conditions because of the constant and controlled nature of the illumination and viewing. (4) The screening test can be carried out in a normally lighted room.

Sensitivity of the described screening test has never been a limitation; it is approximately the same as that of typical tests in current use. However, if a more or less intense illumination or a different filtering system is employed, interpretation of results would need some re-evaluation using specimens of known porphyrin content. For example, the 100-w bulb commonly found in a Wood's light will evoke a more intense fluorescence than that observed in the described viewer, and hence all specimens including normals may appear under these conditions to contain higher levels of porphyrins than described (Table 1).

Application of this procedure has eliminated largely the need for the far more difficult chromatographic or multiple extraction analytic procedure in the accurate diagnosis of the porphyrias and porphyrinurias. Moreover, the test has permitted examination of many more specimens than would otherwise be feasible and in significantly less time.

References