Comparison of the Hoesch and the Watson–Schwartz Tests for Urinary Porphobilinogen

Claus A. Plerach, Ruth Cardinal, Irene Bossenmaier, and C. J. Watson

A comparison of the Hoesch and the Watson–Schwartz tests shows that the latter, although slightly more complicated, generally yields more concise results and is superior in sensitivity and specificity for porphobilinogen. The recommendation of the Hoesch test for use as a "bedside screening" method seems unrealistic. Before the diagnosis of an "inducible" porphoria is made, a positive Hoesch test requires that indoles, indoleacetic acid, methylorosine, end-stage alcoholic malnutrition, and phenazopyridine HCl be excluded, to avoid misinterpretation.

Additional Keyphrases: diagnostic aids • interferences with the test

The modified Watson–Schwartz test for porphobilinogen (PBG) has been widely used in the diagnosis of the "inducible" hepatic porphyrias (1), especially the acute intermittent type, in which the excess of urinary PBG is most often encountered and critical in diagnosis. Recently, Lamon et al. (2) have urged that the simpler Hoesch test (3) replace the Watson–Schwartz test. They regard it as specific, and no one would deny its simplicity. If it were specific and sensitive, it clearly would warrant unqualified approval. In our experience, however, there are clinical circumstances in which the test is nonspecific for PBG and potentially misleading in diagnosis. One of these relates to the urorosein reaction, which has been known and well understood for many years, especially since the discovery that the underlying chromogen is indoleacetic acid (4). This gives a Hoesch test result that is easily mistaken for that given by PBG, while the Watson–Schwartz test is negative.

The urorosein reaction is due to the effect of strong HCl on indoleacetic acid, an effect intensified by nitrite. The presence of Ehrlich's reagent is quite unnecessary; the color reaction occurs with HCl alone. This simple method often parallels the Hoesch test in detecting indoleacetic acid by virtue of the urorosein. It is of interest that at one time this rose-red color was confused with porphyrin, in what was then designated as the "B.E.S." (Beckh, Ellinger, and Spies) test (5). It was shown clearly, however, that porphyrin is readily extracted from an ether extract of such urines with 1.5 mol/liter HCl, while the "urorosein" color is removed by 7.5 mol/liter HCl (6).

We studied 167 patients with a variety of diseases. The urine, either as a random (i.e., casual) sample or a 24-h collection, was examined by the following methods:

The Hoesch test (3) as modified by Lamon et al. (2): to 1 ml of Ehrlich's reagent [20 g of p-dimethylaminobenzaldehyde diluted to 1000 ml with HCl (6 mol/liter)], 2 drops of urine are added, and the mixture is agitated. The pink to red color reaction, if any, is timed and recorded. The test result is called positive when a pink to red color develops within 15 s.

Modified Watson–Schwartz test (7): Equal volumes (2.5 ml) of urine and H. Fischer's "modified" Ehrlich's reagent (0.7 g of p-dimethylaminobenzaldehyde, 150 ml of concentrated HCl, and 100 ml of water) are mixed, and two volumes (5 ml) of saturated sodium acetate solution is added, the mixture mixed again, and shaken with 5 ml of chloroform. If a pink to red color develops and is soluble in chloroform, this indicates the presence of urobilinogen. The aqueous phase, if still pink, is then extracted with 5 ml of butanol. As a rule, the phases separate quickly; if not, the mixture is centrifuged briefly. The test result is considered positive when a pink to red color develops that is chloroform- and butanol-insoluble.

PBG was measured quantitatively according to Mauzerall and Granick (8) in 16 urines from patients who were receiving methyldopa, had an ileus, or were end-stage alcoholics.

Thin-layer chromatography (9) was performed on urines from 10 patients, to detect indoles. Seven of these patients had an ileus, two were receiving methyldopa, one had carcinoidosis. In addition, a series of dilutions of PBG in urine ranging from 2.0 to 59.0 mg/liter, and of urobilinogen...
from 27.8 to 167.0 mg/liter, were analyzed with the Hoesch and the Watson–Schwartz tests, both graded by the same person.

Specificity

In two of 85 patients who were taking 500 and 750 mg of methyldopa daily ("Aldomet"; Merck Sharp & Dohme) for hypertension, the Hoesch test was positive, while the Watson–Schwartz test, as described previously (10), consistently gave an unusual dual color distribution between the aqueous and butanol layers, of approximately equal color intensity. This is typical of patients who are being treated with methyldopa, differing from the color distribution due to the presence of PBG, which is butanol-insoluble and remains in the aqueous phase. Examination of the urine from these two patients has thus far failed to identify the substance(s) responsible for the falsely positive Hoesch test. Conceivably, the diagnosis of an "inducible" porphyria could be missed with either test if such a patient were receiving methyldopa. This underscores the importance of a quantitative measurement of PBG in doubtful cases. In our series, PBG excretion was not above normal.

In a search for urines containing high amounts of indoles, we tested 35 patients with ileus from a variety of causes. In 11, questionably positive Hoesch tests of various intensities were recorded, while the Watson–Schwartz test was negative in all patients with ileus. The color reaction in the Hoesch test in this group commenced between 2 and 10 s, slightly slower than with PBG, reached maximal intensity between 2 and 45 s, and faded somewhat during the following hours. The intensity of the questionably positive Hoesch test in these patients appeared to be closely related to their general condition; the longer the ileus lasted, the greater the likelihood of a false-positive test. Urine giving this color reaction did so even if kept at 4 °C for 77 days. PBG, when measured, was not found to be above normal.

Further analysis by thin-layer chromatography (9) showed that indoles were present in excess in urine from all seven patients having an ileus who were examined with this method. Normal urine gave barely visible spots related to indoles. When the Hoesch test was faintly positive and the color developed slowly, a moderately high amount of indoles was seen on chromatograms, as compared to those patients for whom the Hoesch test became positive faster and the indole-related spots were much more intense.

A study of end-stage ethanolics (in progress and to be reported later) shows that urine from nine of 39 of these patients gave an instantaneously positive Hoesch test, unrelated to the urorosein reaction (4), whereas the Watson–Schwartz test had been negative in all 39 patients. Quantitative measurements of PBG in four of the nine false-positive urines gave results within normal limits.

When urobilinogen was dissolved in normal urine, the Hoesch test became positive at a concentration of 167 mg/liter, while the Watson–Schwartz test was still negative, because all of the color was completely chloroform soluble.

The urine of eight patients, each of whom was ingesting 150–600 mg of phenazopyridine HCl ("Pyridium"; Warner/Chilcott) daily because of urinary tract symptoms, showed a red color reaction within 2 s when examined with the Hoesch test. A pink-red color was also obtained when two drops of each of these urines were added to 1 ml of 6 mol/liter HCl alone. In the Watson–Schwartz test, a deep-red color developed when the "modified" Ehrlich's reagent was added to urine containing phenazopyridine HCl, but the addition of sodium acetate changed the color to orange, thus presenting a clear distinction from PBG.

Sensitivity

Four urines containing 23, 43, 48, and 59 mg of PBG per liter, respectively, as determined with the method of Mauzerall–Granick (8), were diluted with normal urine to concentrations ranging down to 2.0 mg of PBG per liter. The result of the Hoesch test was unequivocally positive for PBG concentrations exceeding 11 mg/liter, while the Watson–Schwartz test results were positive for PBG concentrations exceeding 6 mg/liter.

Discussion

Specificity

Contrary to statements by Lamon et al. (2), we find that the Hoesch test lacks specificity. The interpretation of the test, especially in end-stage alcoholics or in patients having an ileus, can be misleading. This is unfortunate, because an acute porphyria of the "inducible" group (1) would have to be considered in differential diagnosis. The color reaction develops slightly faster when due to PBG, as compared with other Ehrlich reagents. However, a clear-cut distinction does not seem possible on this basis, as we have observed the appearance of a red color unrelated to PBG in as short a time as 2 s, which could be called "instantaneous." The description of the color as "cherry-red" or "bright pink" is in our experience not specific enough to permit distinction from non-PBG reactions. The Hoesch test would be improved by simultaneous exclusion of Pyridium with 6 mol/liter HCl, which also, preferably after ether extraction, detects the presence of indoles (see above). When we could compare the reaction of a questionable Hoesch test with that of a urine known to contain at least 11 mg of PBG per liter, only minor problems were encountered in interpretation. However, this is difficult because PBG is not very stable in urine. It is preferable to use a quantitative method when there is doubt on this score.

Falsely positive results due to urobilinogen are indeed only rarely to be expected in clinical practice, as the amount of urobilinogen required for a color reaction in the Hoesch test is excessive.

Sensitivity

We agree with Lamon et al. (2) that the Hoesch test, during an attack of an "inducible" porphyria, will in all
probability be unequivocally positive. Also, we note that the Watson–Schwartz test sometimes easily permits the diagnosis of an "inducible" porphyria interictally at PBG concentrations that are insufficient to give a reaction in the Hoesch test. If the appearance or solubility of a color in either the Hoesch or the Watson–Schwartz test is questionable, we recommend quantitative measurement of PBG.

Finally, we would like to mention that we prefer to perform the Hoesch and the Watson–Schwartz tests in a laboratory rather than at the bedside, because we want not only the convenience of having all required materials on hand but more importantly for the consistent and good lighting, because color interpretation is highly important. A small ward laboratory, properly equipped, is ideal for use in the study of patients admitted for diagnosis and treatment of an "inducible" hepatic porphyria.

References