Improved Screening Tests for Porphyrin

To the Editor:

If unambiguous results can be obtained in screening tests for porphyrin, performed on urine, blood, and stool, then quantitative analyses are rarely required for diagnosing diseases of porphyrin metabolism. Although previously described procedures (1) have proved satisfactory, a potential problem and an improvement in procedure are noteworthy.

To perform these screening tests, urinary porphyrins are extracted into acidified ethyl acetate and their fluorescence is viewed in ultraviolet light. At this point, timing can be more critical than might be expected. On continued exposure, the porphyrin-containing layer will often (and during widely varying periods of time) develop an increased fluorescence, which can be misinterpreted as elevated porphyrins. Even though caution was given concerning false-positive results after extended exposure to ultraviolet light, the possibility for error is such that this potential problem should be recalled to those that use the screening test.

To confirm that the test as described is valid, we have examined additional urine specimens that exhibited this response by quantitative porphyrin analysis (2) before and after irradiation. In no case did the porphyrin concentrations change, even though in several specimens there appeared to be a marked increase in a porphyrin-like red fluorescence. No change of fluorescence occurred in specimens remaining in room light. Therefore, the original advice remains valid: take a reading on the porphyrin fluorescence as soon as the extracted specimen is placed in the ultraviolet light, in which case normal urine will exhibit either none or a barely detectable red fluorescence. No such fluorescence changes owing to irradiation have been noted while screening stool or blood.

A limitation of the urine test lies in distinguishing coproporphyrinuria, which is relatively common, from uroporphyrinuria, which usually indicates a type of porphyria. To distinguish between these two conditions, we routinely perform a simple, quick chromatographic separation of the porphyrins by use of a procedure derived from a quantitative assay (2). To prepare the chromatographic plates, we make 3 g of silica gel-G (Brinkmann Instruments, Westbury, N. Y. 11590) into a slurry with 6.5 ml of water. One milliliter of the slurry is pipetted onto a microscope slide, which is then agitated carefully and quickly to give uniform distribution. The slide is dried either at room temperature or in an oven. These can be prepared at any time and kept in storage. The gel layer is used to accept two or three applications on one slide.

Whenever an elevated concentration of urinary porphyrin is found, 5 or 10 µl of ethyl extract of the urine is spotted onto a slide and developed in a staining jar (chloroform: methanol: ammonium hydroxide: water, 12:12:3:2, by vol) until the front has moved about halfway up the slide or the coproporphyrin layer has clearly separated from the origin (5 to 10 min). Viewed under ultraviolet light, a normal urine specimen will show a sharp coproporphyrin band at Rf 0.60-0.65, with little or no porphyrin fluorescence remaining at the origin. Specimens containing increased uroporphyrin will show a clearly visible fluorescence at the origin.

Experience has shown that simple screening tests performed on urine, blood, and stool, combined with this quick chromatographic separation of the urinary porphyrins and a Watson-Schwartz test for porphobilinogen, will almost always provide adequate laboratory information for the diagnosis of porphyras and porphyrinurias. Usually the pattern of qualitative changes found in the three specimens is more meaningful than a quantitative value obtained on a single specimen (1).

References

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At What Temperature Should Enzymes of Clinical Interest be Assayed?

To the Editor:

It is presently difficult to express a quantity of enzyme in standardized units, because what is actually measured is not an absolute quantity of substance, but a catalytic activity. This activity depends on a number of factors such as pH, temperature, concentration of substrate, etc. To define a standard unit, the Enzyme Commission of the International Union of Biochemistry (IUB) recommends use of optimal concentrations of substrate and hydrogen ions. In the case of temperature, it is not possible to define an optimal temperature; what is observed is, in fact, an empiric value of maximum velocity. Enzyme activity increases with temperature, but deactivation of the protein by heat has an opposite effect, which predominates above about 40°C.

J. King (Practical Clinical Enzymology, van Nostrand, London, 1965, Figures 1 and 2, pp 32, 33) shows that enzyme inactivation is more serious if long incubation times are used. If aldolase is incubated at 36.5°C, enzyme activity remains linear within the first hour, then begins to decrease.

The Commission of Enzymes of the IUB recommends 30°C as a standard temperature for enzyme assay. A Swedish firm offers an automatic enzyme analyzer working at a fixed temperature of 35°C. Recently, the enzyme Commission of the German Society of Clinical Chemistry issued recommendations according to which enzymes should be assayed kinetically at 25°C.

In my opinion, neither 25°C nor 30°C is satisfactory for routine assays in clinical chemical laboratories, because of the problem of time. A precise kinetic assay by colorimetry or spectrophotometry requires that a certain minimal change in absorbance (ΔA) be observed. This ΔA is much more readily obtained at 37°C than at 30°C, and more than twice as quickly as at 25°C. If, for example, transaminases are assayed, an acceptable kinetic recording is produced within 1 min at 37°C, whereas at 25°C 2 min is required. Every laboratory director will consider that more than doubling the rate of analyses by simply increasing the temperature of incubation is a big advantage.

I do not see, therefore, why incubations should not be performed at 37°C. This temperature is already widely used in biochemistry. It corresponds to the body temperature. If one measures enzymes like plasmin or renin, it is desirable to have their activity expressed in terms corresponding to physiological conditions.

The unjustified reluctance to measure at 37°C probably results from the fact that with older methods incubation times of 2 h or more were often used. In such cases, a temperature of 25°C is in-
deed preferable. But most of the enzymes presently assayed for diagnostic purposes do not require incubation times longer than a few minutes. This means that one practically measures initial velocity. This makes the effect of enzyme denaturation by heat insignificant, even at an incubation temperature of 37°C. I would therefore plead in favor of 37°C, a temperature taking account both of practical necessities and theoretical considerations.

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Abbreviations for Enzyme Names

To the Editor:

I was stunned by the editorial "Abbreviations and Names of Enzymes" that appeared on page 319 of CLINICAL CHEMISTRY, Vol. 18, No. 4, 1972.

As the Secretary since 1964 of the IUPAC-IUB Commission on Biochemical Nomenclature (CBN), which has taken over Enzyme Nomenclature for IUB from the original commission, I know that no such action has been taken. Moreover, abbreviations for the names of enzymes are strongly disfavored by CBN and by its associated body, the IUB Commission of Editors of Biochemical Journals (CEBJ), which is reflected in many Instructions to Authors of CEBJ journals. More than that, the Chairman of the Commission on Quantities and Units of the IUPAC Section on Clinical Chemistry has often expressed to me his commission's abhorrence of any such move. And finally, this Office of Biochemical Nomenclature of the NAS-NRC has not made any move in this direction.

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I erred; Dr. Cohn is entirely correct. As plainly stated in the article cited in the editorial, the recommendations were contrary to CBN policy and in fact originated elsewhere: these were the provisional and personal recommendations of a "joint working party" of the Association of Clinical Biochemists, the Association of Clinical Pathologists, and the Royal College of Pathologists.

Although CBN et al. are against the vain proliferation of abbreviations, for good and obvious reasons, my expressed opinion that these particular recommendations are prudent and useful ones is unchanged. We shall continue to allow authors to use a single abbreviation for an enzyme, and we shall continue to spell out such nonstandard abbreviations in a footnote. Our "Information for Au-

thors" (CLIN. CHEM. 18, 1, 1972) is very specific on this point.

Finally, in both the article cited and the editorial, glucosephosphate isomerase should have been spelled as two words, not three.

J. S. KING, JR.
Executive Editor
CLINICAL CHEMISTRY

Comparison of Two Radioassay Methods for Vitamin B₁₂

To the Editor:

We have compared two different radioassay methods for determining vitamin B₁₂ in serum. The methods primarily differ in the nature of their B₁₂ binders and in the way that free and bound B₁₂ are separated. Frenkel et al. (1) use pooled, Millipore-filtered, normal human sera for binding and DEAE-cellulose powder for the separation of free and bound B₁₂. Wide and Killander (2) use intrinsic factor coupled to polystyrene particles (Sephadex), and this method is currently available in kit form (Pharmacia Labs. Inc., Piscataway, N. J. 08854).

Both methods involve extraction of the B₁₂ from endogenous binding proteins, incubation with [¹⁵³Co]-cyanocobalamin and binder, and repetitive washing steps to separate free and bound B₁₂.

Our data (Table 1) indicate that results are essentially the same for both methods and that they give similar normal ranges (300-1000 pg/ml). Precision data (Table 2) suggest that the intrinsic factor-Sephadex method is the better.

In our laboratory, a routine B₁₂ assay run usually numbers eight patients, one control, and six standards, all of which are measured in duplicate. With the serum binder-DEAE-cellulose method about 10-12 h of technician time is required for such a run, from initial set-up (with reagents already prepared) to completion of the assay (excluding gamma counting and calculations). One hour of that time is devoted to incuba-

Table 1. Values for Serum Vitamin B₁₂ Obtained on the Same Samples by Different Radioassay Methods

<table>
<thead>
<tr>
<th>Serum binder-DEAE-cellulose method</th>
<th>Intrinsic factor-Sephadex method</th>
<th>Serum binder-DEAE-cellulose method</th>
<th>Intrinsic factor-Sephadex method</th>
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</thead>
<tbody>
<tr>
<td>pg/ml</td>
<td>pg/ml</td>
<td>pg/ml</td>
<td>pg/ml</td>
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<tr>
<td>1750 1800</td>
<td>1048 1000</td>
<td>1380 134</td>
<td>1160 156</td>
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<tr>
<td>1100 1160</td>
<td>294 410</td>
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<td>926 770</td>
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<tr>
<td>250 340</td>
<td>444 420</td>
<td>500 590</td>
<td>642 600</td>
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<tr>
<td>1000 1160</td>
<td>842 860</td>
<td>510 520</td>
<td>192 110</td>
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<tr>
<td>890 840</td>
<td>704 730</td>
<td>34 0</td>
<td>798 920</td>
</tr>
</tbody>
</table>

*All results are the average of duplicate samples. Results of the two radioassay methods had a correlation coefficient (r) of 0.9801. The equation of the regression line was $y = 1.0482x + 2.2588$ (y being the intrinsic factor-Sephadex method, and x being the serum binder-DEAE-cellulose method).

Table 2. Precision Data Compared for Two Vitamin B₁₂ Radioassay Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Mean</th>
<th>Range</th>
<th>SD</th>
<th>RSD, %</th>
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<tr>
<td>Within-day</td>
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<td></td>
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<tr>
<td>Transcobalamin-DEAE-cellulose</td>
<td>16</td>
<td>528</td>
<td>432-613</td>
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<td>Intrinsic factor-Sephadex</td>
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<td>713</td>
<td>630-750</td>
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<td>Day-to-day</td>
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</tr>
<tr>
<td>Transcobalamin-DEAE-cellulose</td>
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<td>232-379</td>
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<td>14.8</td>
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<tr>
<td>Intrinsic factor-Sephadex</td>
<td>8 d</td>
<td>359</td>
<td>315-400</td>
<td>30</td>
<td>8.3</td>
</tr>
</tbody>
</table>

*All results are the average of duplicate samples from different serum pools.