procedure and cannot be attributed to spectrophotometric insensitivity. (The major drawback of the Isomune-CK procedure is that it requires a sensitive spectrophotometer for the CK measurements.) Good precision is probably not mandatory for CK-MB quantitation when monitoring patients who have had acute myocardial infarcts, but we believe that it is essential following patients who have undergone open-heart surgery. At our hospital, open-heart surgery patients are discharged from the intensive-care unit as soon as their CK-MB values drop below 15 U/L. In our hands, the Isomune-CK procedure provides adequate precision to make this decision, whereas the Tandem procedure does not.

Although CK-MB results for the Tandem procedure are expressed in mass concentration units (ng/ml) and for the Isomune-CK procedure by activity concentration units (U/L), the two procedures gave similar numerical values for patients' samples. We correlated CK-MB results (range 0–80 U/L) from 92 patients' samples (suspected infarct or open heart surgery cases) for both procedures. With r = Isomune-CK results and y = Tandem-CKM results, linear regression analysis of these data gave a correlation coefficient of r = 0.9606. Results for the two procedures fitted this line well at low MB levels (less than 15 U/L by the Isomune-CK procedure). Having previously established (I) an upper limit of normal for the Isomune-CK procedure to be 6 U/L, we estimated it as 5.5 U/L by the Tandem procedure at 9 ng/ml. Above 15 (U/L) for the Isomune-CK procedure, we found that the Tandem procedure frequently gave disproportionately higher values (29 U/L vs 58 ng/ml, respectively, being the worst case). We have previously correlated results from the Isomune-CK procedure to both electrophotography and ion-exchange procedures (I) and established that the Isomune procedure was linear to at least 300 U/L relative to the latter procedure. Although more work would need to be done for verification, these results suggest that the Tandem-CKM procedure is only semiquantitative for CK-MB activities >15 U/L.

We found that turnaround times for results from the Tandem procedure were approximately double those for the Isomune-CK procedure (3 to 3.5 h for 20 patients' results by the former vs 1.5 h for the latter).

In summary, we find the Tandem procedure to be less precise, less linear, and more tedious to perform than the Isomune-CK procedure, and we feel that although it could be used to aid in the diagnosis of acute infarct, it should not be used to monitor the progress of open-heart surgery patients.

Reference

Michael Sheehan
Patricia Haythorn
Good Samaritan Hosp. and Med. Center
Portland, OR 97210

A Further Comment on Measurement of Thyrotropin

To the Editor:

The measurement of circulating concentrations of thyrotropin (TSH) has recently been the subject of much discussion, e.g., several communications that have appeared in Clinical Chemistry since 1983 (I–7). Such articles, together with information in the package inserts of commercially available reagents and kits, make it apparent that, although a high degree of sophistication has been achieved in terms of the immunochemistry of these assays, performance claims are misleading and performance is still inadequate for samples in the hyperthyroid (thyrotoxic) range. This is manifest as falsely elevated results for hyperthyroid samples and overlap with the euthyroid range. Obviously, the less sensitive the assay, the worse these problems become (8); however, even with recently described "sensitive" immunoradiometric assays (IRMA), inspection of precision profiles shows that CV of the dose estimates exceed 25% at the lower cutoff value for normal (~0.1 milli-int. unit/L). The resolution between hyperthyroid and euthyroid results being poor in this region, an unequivocal biochemical diagnosis on the basis of this single test is still not possible.

Further increases in assay sensitivity are limited by the use of 125I as the label, and recently it has been shown that a two-site assay based on the use of antibodies labeled with chemiluminescent acridinium ester has a sensitivity of detection at least 10-fold better than that attainable with 125I (9). Using this immunochrominometric assay (ICMA), we have previously shown that circulating thyrotropin concentrations in 16 hyperthyroid patients are <0.03 milli-int. unit/L. We have since confirmed this in another 80 subjects. These concentrations are at least 10-fold lower than the bottom end of the normal euthyroid range (0.4–4.0 milli-int. units/L) obtained with the same assay. Further, the information for the presence of thyrotropin measured with an appropriately sensitive immunoassay correlate with the thyrotropin response to exogenous TRH administration. The increased sensitivity attainable with an ICMA demonstrates no significant thyrotropin response to TRH in untreated, overtly thyrotoxic subjects (basal TSH <0.03 milli-int. unit/L) and also indicates some restoration of thyrotrop responsiveness in partly treated thyrotoxic patients who still have basal thyrotropin concentrations well below the normal range (Weeks et al., unpublished). Although these data are limited, they are in accord with the large volume of data published by Wide and Dahlberg, who used a less-sensitive RIAs and found that basal thyrotropin values are predictive of TRH responsiveness.

We conclude, therefore, that an appropriately sensitive thyrotropin assay (which, of necessity, is more sensitive than currently available RIAs or IRMAs) will resolve the problem of overlap resulting from assay imprecision.

References
7. White GH. Use the most appropriate and useful test(s) in evaluating thyroid function. Ibid., p 1415. Letter.

Ian Weeks
J. Stuart Woodhead

Dept. of Med. Biochem.
Univ. of Wales College of Med.
Heath Park
Cardiff, CF4 4XN
Wales, U.K.

More on Identifying Inherited Deficiency of Porphobilinogen Synthase

To the Editor:

Tishler and Winston recently described a screening test for delta-aminolevulinic acid in urine (1). The test is said to be useful for identification of an inherited deficiency of delta-aminolevulinic acid dehydratase (porphobilinogen synthase, EC 4.2.1.24). To complete the picture regarding this recently described inheritable biochemical lesion, we wish to note that the patients described by Doss and colleagues, as referenced by the authors, appear to be homozygotes on the basis of the fact that their enzyme activities were very subnormal and aminolevulinate excretion was manyfold normal.

We have described a similar enzyme deficiency in a family of four generations (2). However, we infer that our subjects were all heterozygotes on the basis of their enzyme activities being decreased to only about 20% of normal. At the same time, we were unable to identify any other biochemical or clinical manifestations of this disorder, including abnormal aminolevulinate excretion.

Evidently, this dehydratase is normally present in such excess that an 80% decrease in activity still allows the heme biosynthetic pathway to proceed at an adequate rate without accumulation of the substrate, aminolevulinate. The repeated clinical manifestations described by Doss and colleagues probably appear only in instances of an extreme decrease in activity such as they observed. These biochemical responses are particularly interesting in view of the possibility that the dehydratase carries a regulatory function (3).

References

2. Bird TD, Hamernyk P, Nutter JY, Labbé RF. Inherited deficiency of delta-

Robert F. Labbé
Dept. of Lab. Med.
Univ. of Washington
Seattle, WA 98195

Thomas J. Bird
Dept. of Med.
Univ. of Washington School of Med.
Seattle, WA 98195

Clearance of Pancreatic and Salivary α-Amylase in Normal Subjects

To the Editor:

Since 1977, when O'Donnell et al. (1) introduced a simple inhibitor technique for determination of pancreatic α-amylase ("P-amylase") and salivary α-amylase ("S-amylase") in serum, many investigations have been carried out on the significance of these isoenzymes of α-amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1). Few authors (2, 3) have used the method to determine the proportions of the two isoenzymes in urine, nor have optimal conditions for correct determinations been described.

We measured the urinary clearance of P-amylase and S-amylase relative to creatinine clearance in 20 healthy volunteer subjects. Results of pilot experiments made it evident that accurate measurements of both isoenzymes are only possible if the urinary pH is between 6.8 and 7.2. Therefore, sodium bicarbonate was administered orally: 4 g on the evening preceding and 2 g in the morning 2 h before urine was collected. All subjects were asked to drink at least 500 mL of tapwater to promote diuresis. A urine specimen was obtained 2 h later into 10 mL of phosphate buffer (1.0 mol/L, pH 7.0). Blood was sampled in the middle of this collection period.

We measured the catalytic activity concentrations of total α-amylase and its isoenzymes within 2 h after the urine was collected. We used the Blue Starch method (Phadebas, Pharmacia, Sweden), at 37°C. To all urine samples we added 1 mg of bovine serum albumin per milliliter. Creatinine in serum and urine was determined by the Jaffé technique (4).

In 16 of the 20 healthy volunteers we could determine the clearance of total α-amylase, P-amylase, and S-amylase, all relative to creatinine clearance. The normal values so derived were respectively 2.29% (SD 0.60), 4.06% (SD 0.98), and 0.93% (SD 0.26). Urine from the remaining four subjects contained no detectable S-amylase.

Our values for total α-amylase clearance agree well with those reported (2, 3, 5–7), but clearance of neither isoenzyme did so. We found a higher clearance for P-amylase and consequently a lower S-amylase clearance (2, 3).

In these 16 subjects P-amylase comprised 46.7% (SD 16.2%, range 22.9–72.2%) of the total α-amylase in the serum and 79.0% (SD 11.8%, range 54.3–91.6%) in the urine. These results are similar to those reported by Fridhandler et al. (7).

To test the validity of our technique we added different known amounts of P-amylase and S-amylase to each of 12 different urine samples (including a sample from one of the four volunteers whose urine showed no urinary S-amylase). We could account for 101.3% (SD 3.7) of added P-amylase and 104.2% (SD 7.3) of added S-amylase. Evidently urine contains no inhibitor of these enzymes.

The mean percentage of P-amylase relatively to total α-amylase in serum of the four subjects in whom we could not detect urinary S-amylase was significantly higher than in the serum of the other 16 volunteers (mean 80.6 ± 9.4%, range 70.8–92.2%, p < 0.01). From these values the percentage of P-amylase relatively to total α-amylase in the urine can be calculated to be more than 94%. The inhibitor method is insensitive at this high percentage of P-amylase (9).

In conclusion: We find the inhibitor method useful for determining these isoenzymes of α-amylase in urine. Urinary pH should be between 6.8 and 7.2. If the percentage of P-amylase in serum exceeds 75% of the total α-amylase, no urinary S-amylase will be detected, owing to the insensitivity of the method.

References