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

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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 (Pheidas, 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 0.96), and 0.39% (SD 0.28). 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 11.5%, 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