Free Glucuronic Acid in Urine as Estimate of Tumor Load

To the Editors:

Most solid tumors show high β-glucuronidase (EC 3.2.1.31) activity as compared to normal tissue (1). Because this enzyme acts to split off glucuronic acid from glucuronides, the measurement of free glucuronic acid in urine can be used to estimate tumor load.

We have developed a method for assaying free glucuronic acid that is more accurate than the commonly used decarboxylation and resorcinol methods.

The test is based on the reaction of glucuronic acid with tetraborate in concentrated sulfuric acid. The colored complex is then treated with m-hydroxydiphenyl to make it water soluble, and measured colorimetrically. Because the test also measures bound glucuronic acid, glucuronides are precipitated in advance with excess barium hydroxide, and the excess barium hydroxide is itself removed by precipitation with concentrated sulfuric acid.

The reagents are: A, saturated solution of Ba(OH)2; B, concentrated sulfuric acid; C, 12.5 mL of sodium tetraborate in concentrated H2SO4 (stable for 100 days); and D, m-hydroxydiphenyl, 1.5 g/L in dilute (5 g/L) NaOH solution (stable for one month in refrigerator).

To 10 mL of the urine sample, add 10 mL of reagent A at room temperature and mix. After 30 min, centrifuge at 6000 rpm for 3 min; to the supernate add 10 drops of reagent B and shake. Centrifuge again (6000 rpm, 3 min), and to a test tube set in crushed ice add 0.2 mL of the supernate and 1.2 mL of reagent C; mix well. Heat the tube in boiling water for 5 min, then cool immediately in ice for about 10 min. Add 20 μL of reagent D; after 5 min measure the absorbance at 520 nm.

People without tumors excrete 200–400 mg of free glucuronic acid per 24 h (2). We find that patients with a tumor having high β-glucuronidase activity excrete as much as 7000 mg in the same period. In animals, nutritional stress may increase these values (3), but this factor is probably not significant in humans.

A few rare illnesses may also affect the amount excreted. Most glucuronidated drugs obviously increase the conjugated glucuronic acid in urine, but they have little effect on the amount of free glucuronic acid.

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Zinc Contamination of Control Serum

To the Editor:

Zinc contamination of serum samples taken in Vacutainer Tubs (Becton-Dickinson, Rutherford, NJ 07070) has been studied (1, 2) and resolved (3), either by the use of a new Vacutainer system (no. 6527) or by the use of polypropylene tubes and stoppers (the method we use in our laboratory). Nevertheless, the problem of control serum remains to be resolved, as indicated in the Letter of N. Urquhart (4).

In France, only one control serum is available for zinc assays (Biotrol Laboratories, 1 rue du Foin, 75140 Paris Cedex 03, France). We rapidly realized that reconstituted serum (with de-ionized water) could not be kept for several days, because large variations were noted on the first day. The following experiments were undertaken in the context of this instability.

Six control sera were reconstituted according to the instructions (30 min on the laboratory bench). Three tubes were capped as usual with Biotrol rubber stoppers and three were sealed with Parafilm. Zinc was then assayed (5) daily by flame atomic absorption spectrophotometry (Model 360 instrument; Perkin-Elmer Corp., Norwalk, CT 06856). The zinc concentration increased more rapidly in tubes with stoppers (initially 12.19 μmol/L, it was 16.35 μmol/L on day 4, and 21.15 μmol/L on day 10) than in tubes capped with Parafilm (12.30 μmol/L, 12.65 μmol/L on day 4, and 14.40 μmol/L on day 10). The contamination thus originated primarily from the stopper. When one of these stoppers was cut into small pieces, and placed in 10 mL of de-ionized water, it liberated almost 0.135 μmol of zinc in 10 days, which corresponds practically to the zinc content of [a] serum [sample in the tube].

In the second experiment, two reconstituted sera were placed in polypropylene tubes, were sealed with Parafilm, and were assayed daily for four days: 12.60, 13.35, 13.35, and 13.35 μmol/L. Blind zinc assays by an independent laboratory showed good stability. These results lead to the same conclusion in France as in the United States: control serum delivered with rubber stoppers cannot be trusted because the zinc content assayed depends on how long the serum has been reconstituted and stored.

References

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