Simple Determination of High Urinary Excretion of 5-Hydroxyindole-3-acetic Acid with Ferric Chloride

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We describe a method for determination of urinary 5-hydroxyindole-3-acetic acid, a metabolite of serotonin and tryptophan and a useful indicator of metastasizing carcinoid tumor. The analysis is based on the color development with ferric chloride added to the residue obtained on evaporating a diethyl ether extract of the sample that has been acidified with sulfuric acid and saturated with sodium chloride. The color is measured at 510 nm. The reaction was made highly specific by the acidification so that nonspecific reactions with catecholamines and with other phenolic compounds such as salicylic acid could be eliminated.

Additional Keyphrases: screening for argentaffinoma - normal values - nitrosonaphthol method compared - carcinoid tumors

Qualitative and quantitative methods for determining 5-hydroxyindole-3-acetic acid (5-HIAA) in urine based on color development with 1-nitroso-2-naphthol (1, 2) and with dimethylaminobenzaldehyde (3) have been widely used. However, these methods require some sample purification because of their nonspecific reaction with other compounds in urine.

Ferric chloride reagent has been widely used for the detection of phenolic derivatives and catecholacetic acid (Gerhardt method) in urine, but the specificity of the reaction is low. We have modified the ferric chloride reaction and improved the specificity for 5-HIAA by adding acids such as sulfuric or hydrochloric to the extract. Substances such as salicylic acid which react sensitively with ferric chloride are nonreactive under acidic condition, but the color for 5-HIAA itself is enhanced in acidic solution.

Reagents

All compounds used for this study were from Sigma Chemical Co., St. Louis, Mo. 63178.

Ferric chloride solution. Dissolve 2.5 g of FeCl₃.6H₂O in 100 ml of an equimolar quantity of water and methanol.

Standard solution: 100 mg of 5-HIAA per liter of water.

Method

A 24-h urine sample to which 10 ml of hydrochloric acid, 6 mol per liter, is added is used. It is necessary for the patient to take no drugs for several days before the test and to avoid foods rich in serotonin.

Qualitative Method

To 0.5 ml of urine, standard, and water, add 2 ml of 0.5 mol/liter sulfuric acid and 0.2 ml of ferric chloride solution. Also, a sample blank is needed containing 0.5 ml of sample and 2.2 ml of sulfuric acid (0.5 mol/liter). Heat the tubes for 5 min at 60 °C.

Quantitative Method

(a) To a 10-ml glass-stoppered tube, add 2 ml of urine, 1 ml of 0.5 mol/liter sulfuric acid, 1 g of sodium chloride, and 4 ml of diethyl ether, mix well for 1 min, and allow the layers to separate, or centrifuge for 3 min. (b) Transfer 2 ml of the ether layer to another tube and evaporate in a water bath at 60 °C. (c) To the residue from the extracts, add 2 ml of 0.5 mol/liter sulfuric acid and 0.2 ml of ferric chloride solution, and mix well. (d) Warm at 60 °C for 5 min. (e) Measure the absorbance of standard and sample vs. a reagent blank at 510 nm.

Calculation. Calculate the daily excretion value as follows: 5-HIAA, mg/day = (A_{sample}/A_{std}) × 100 × volume of 24-h urine (liters).

Caution: If the residue from the extracts shows color, a sample blank is needed as in the qualitative method.

Results

5-HIAA and serotonin, 50 μg/tube, gave absorbances of 0.175 and 0.023, respectively. The same amount of the following compounds gave no measurable absorbance: salicylic acid, 5-aminosalicylic acid, guaiacol, phenol, α-ketoglutaric acid, pyruvic acid, oxaloacetic acid, tryptophan, phenylalanine, vanillinmandelic acid, homovanillic acid, vanillin, tyramine, and epinephrine.

Absorbance of color reaches a maximum after 5- to 10-min heating at 60 °C. After heating, it is preferable to make the reading within 20 min.

A standard curve was obtained by submitting 5-HIAA to the whole procedure. The curve was linear to at least 200 mg/liter. The maximum absorbance of standard and sample was around 510 nm. Analytical recoveries of 5-HIAA added to urine was from 80 to 87%, which is low but constant, so the data seem to be satisfactory for clinical purposes.

Within-day precision, for 5-HIAA added to urine, was 1.45% (CV) and 0.73 of the SD (mean value, 50.16 mg/liter) with five repeated assays. Comparison of values with the present and nitrosonaphthol methods showed that sample values of 52 and 23 mg/day by the nitrosonaphthol method correspond to 83 and 50 mg/day by the present method, respectively. The normal value obtained for 38 normal individuals was 8.3 ± 3.8 mg/day (mean ± SD), but the value is higher than obtained by the nitrosonaphthol method of Pierce (4), 2 to 8 mg/day.

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The present method is intended for screening or measurement of high excretion of 5-HIAA, and it is difficult to determine the normal value because of its lesser sensitivity.

Discussion

The nitrosonaphthol test, a widely used for screening test for argentaffinoma, is highly sensitive for the detection of 5-HIAA, but the reagent must be freshly prepared before use, and even in the qualitative method undesirable pigment must be extracted with an organic solvent. The reaction with dimethylaminobenzaldehyde is also nonspecific for 5-HIAA. From these considerations, purification of the urine sample by chromatography or many steps of extraction cannot be avoided. The present method is less sensitive than the nitrosonaphthol reaction, but its specificity is better, so that it may be considered to be useful as a screening test for suspected cases of metastasizing carcinoid tumors.

The analyst must recognize that urine from patients treated with antipyrine and related drugs gives falsely positive result. Administration of drugs should be stopped several days before the day of the test, or the results checked by another method, such as the nitrosonaphthol reaction. In case of phenylketonuria, rare abnormality of phenylalanine metabolism, phenylpyruvic acid in urine reacts with ferric chloride to form a blue-green color, but it can be determined only with fresh urine because of the instability of phenylpyruvic acid, and the color fades quickly even at room temperature, so it is easily distinguishable from that of 5-HIAA.

References


Plasma Glucose Measurement with the Yellow Springs Glucose Analyzer

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The Yellow Springs Glucose Analyzer, a device for the quantitative measurement of glucose concentrations, involves the use of immobilized glucose oxidase, incorporated on a membrane covering a hydrogen peroxide sensor. Operation of the instrument is simple. After an initial calibration, 25 µl of plasma is injected into a reaction chamber. At 45 s a digital result for glucose is displayed. The within-batch coefficient of variation for the method is 1.2% or less for glucose concentrations of 0.94 to 3.98 g/liter. The between-batch coefficient of variation is 5.8% or less for glucose concentrations of 0.29 to 2.91 g/liter. Concentration and readout are linearly related to at least 4.6 g/liter. Analytical recoveries ranged from 100 to 102%. Carry-over was negligible. Values for glucose concentration obtained with the instrument compared well (r = 0.997) with those obtained with the Beckman Glucose Analyzer.

The conversion of glucose and oxygen to gluconic acid and hydrogen peroxide, catalyzed by glucose oxidase (EC 1.1.3.4), forms the basis for several widely used methods for estimating glucose. In most of them the peroxide liberated is used to oxidize precursors to dyes, which may be determined colorimetrically.

In the Beckman Glucose Analyzer technique, the dissolved oxygen in the reaction chamber is monitored electrochemically and the glucose is computed from the rate of disappearance of oxygen (1). The instrument has been evaluated and found to give results that correlate well with those obtained with methods in which hexokinase and neocuproine are used (2). Because of its small sample requirement and ease and speed of operation, the Beckman Analyzer (Model ERA-2001) has been used in our laboratory to handle both routine and urgent plasma glucose estimations.

The Yellow Springs Glucose Analyzer (Model 23A) is a newly marketed instrument (3). Similar to the Beckman instrument in that it requires only microvolumes of sample for analysis, it is easy to operate and gives quick results in the form of a digital display. It differs from the Beckman instrument in that it uses a hydrogen peroxide-sensitive instead of an oxygen-sensitive electrode. Furthermore, instead of free glucose oxidase in solution, it uses immobilized glucose oxidase. The immobilized enzyme is incorporated in a membrane that covers the tip of the hydrogen peroxide sensitive electrode. The use of immobilized enzymes is a relatively new concept (4) and its main advantage seems to be reusability.

Our purpose was to assess whether the use of immobilized enzymes offers the same speed, precision, and accuracy of analysis as those obtained with the free enzyme, and therefore

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