Refinements of Assays for Low Concentrations of Inulin in Serum

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Dear Sir,

Clinicians are increasingly interested to replace the endogenous creatinine clearances because of its unreliability and isotope clearance because of irradiation by other methods of determination of glomerular filtration rate (GFR) in testing kidney function [1]. One possibility consists in calculating the clearance from the plasma disappearance curve after a single injection technique with inulin [2,3]. However, the concentrations of inulin in serum which are found with this technique at the measuring points (60–240 min after injection of inulin), are essentially lower than those obtained with the technique of continuous infusion of inulin [4]. The methods for the determination of inulin elaborated for the continuous infusion technique are not suited to measure these low concentrations of inulin reliably, because these methods are not sensitive enough. Therefore, we refined two conventional methods for inulin determination by various modifications. Consequently, we dispose on methods with high analytical sensitivity which could be of interest for nephrologists who intend to measure GFR by the single-shot technique with inulin.

We improved an enzymatic method (method I) based on the enzymatic determination of glucose/fructose [5] and a chemical method (method II) based on fructose determination after acid hydrolysis of inulin [6].

Method I: 300 μ l of serum sample or water as blank are mixed with 50 μ l of glucose-removing reagent (1.4 mg of glucose oxidase and 23 μ l of catalase in 1 ml of 100 mmol/l of triethanolamine/HCl buffer, pH 7.0; all reagents from Boehringer-Mannheim, Mannheim, FRG) and incubated at 37 °C for 4 h. Then 350 μ l of 0.54 mmol/l HClO₄ are added, mixed and incubated at 80 °C. After 15 min, this mixture is centrifuged (1,800 g, 10 min) at 4 °C and 100 μ l of the supernatant are added to 500 μ l of reagent (200 mmol/l triethanolamine/HCl buffer, pH 7.6; 4 mmol/l MgSO₄; 1.5 mmol/l NADP; 5 mmol/l ATP; 2.2 kU/hexokinase; 1.1 kU/glucose-6-phosphate dehydrogenase) into a cuvette. The absorbance is read at 340 nm (A₁) when being constant (15 min) and 4 μ l of phosphoglucose isomerase (700 kU/l) are added and the absorbance (A₂) is read again after 15 min. The differences between A₂ and A₁ for sample and blank are computed and the concentrations are calculated with the usual equations of the optical test. In order to get the true inulin concentration

in the sample, the value of serum before the inulin injection (caused by endogenous fructose in serum) is subtracted.

Method II: to remove glucose from samples, the incubation with glucose-removing reagent is performed as described in method I. Then, 350 μl of trichloroacetic acid (612 mmol/l) are added. This mixture is centrifuged after 15 min and 200 μl of the supernatant are layered on cooled (4°C) anthrone reagent (5.15 mmol/l in sulfuric acid). It is mixed thoroughly and incubated for 60 min at 37 °C. Absorbances are read at 623 nm and the concentrations are calculated with a standard (10 mg/l). Absorbance values measured in the serum samples before inulin injection are subtracted in order to eliminate the reaction of anthrone with, e.g., endogenous fructose. We optimized these reaction conditions for method II (ratio of sample in reaction mixture; deproteinization) to guarantee optimal analytical sensitivity without the precipitation of anthrone. Both methods are very well correlated (fig. 1). The detection limits are 2.5 (method I) and 1.5 (II) mg/l; the within-run precisions (60 mg/l; n = 10) indicated as variation coefficients are 1.9% (I) and 1% (II); the linearity of both methods is to about 200 mg/l. Both methods can be recommended for the determination of low concentrations of inulin in serum which occur when the mentioned single-shot technique is used. We prefer the chemical method because of its simplicity and the low reagent costs. One determination costs about 2 cents vs. about 70 cents for the enzymatic method.

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361

600

0 200 400

Inulin, mg/l (enzymatic method)

Fig. 1. Correlation between the enzymatic method and method for the determination of inulin in serum samples.

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