Assessment of Renal Function by Inulin Clearance: Comparison with Creatinine Clearance as Determined by Enzymatic Methods

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We compared creatinine clearances determined by enzymatic (Kodak Ektachem 700 single-slide, Boehringer Mannheim creatinine PAP) and nonenzymatic (Jaffé, HPLC) methods with glomerular filtration rate measured by inulin clearance in patients with varying degrees of renal function. The Kodak enzymatic assay gave values for creatinine 2 to 3 mg/L higher than the other methods. This resulted in significantly lower creatinine clearances than inulin clearances and creatinine clearances determined by the other methods. However, correlations between all methods for serum and urinary creatinine values and clearances were good. To avoid between-assay (enzymatic vs nonenzymatic) discrepancies, manufacturers should agree to an acceptable standard of calibration under the usual conditions used with patients.

Additional Keyphrases: multilayer film analysis • liquid-chromatographic and colorimetric assays compared

Measurement of creatinine in serum and urine is essential in evaluation of renal function (1). Values for creatinine in serum can be used to directly estimate the glomerular filtration rate (GFR). However, serum creatinine is not usually measurably increased until there is a >50% loss of renal function (2). Therefore, creatinine clearance calculated from measured creatinine concentrations in serum and urine more sensitively indicates early renal dysfunction. Although inulin clearance is considered the "gold standard" for assessment of GFR, the clearance of endogenous creatinine is widely accepted (3). Laboratory evaluation of GFR depends on accurate measurement of creatinine in serum and urine. Creatinine has traditionally been measured by variations of the alkaline picrate method, which was first described by Jaffé more than 100 years ago (4). However, numerous compounds, including ketocids, cephalosporins, and bilirubin, interfere with the Jaffé reaction. Enzymatic methods for creatinine determination that are relatively free from common interferences have recently been introduced into the clinical laboratory (5). However, an earlier report (6) indicated that creatinine clearance as measured by enzymatic methods appeared to overestimate GFR, owing to significantly lower values for serum creatinine in comparison with the Jaffé methods. To clarify these issues, we have compared the clearance of creatinine as determined by the enzymatic and Jaffé methods with the measurement of GFR by standard inulin clearance in patients with various degrees of renal function.

Materials and Methods

Patients. Twenty-four patients with various degrees of renal function were selected so as to provide six patients in each of the following groups: group A, creatinine clearance >100 mL/min; group B, creatinine clearance 50-100 mL/min; group C, creatinine clearance 25-49 mL/min; and group D, creatinine clearance <25 mL/min. These creatinine clearances were measured in ambulatory patients. All patients gave informed consent along the guidelines approved by the institutional review board.

On the day of the study, patients arrived in the clinic after an overnight fast. Oral hydration was established and an intravenous heparin-lock catheter was placed in each forearm. Baseline samples of blood and urine were obtained. An intravenous loading dose of inulin (30 mg per kilogram of body weight) was administered and, simultaneously, a maintenance infusion of inulin by means of a Harvard pump was started to maintain an inulin concentration in plasma of ~150 mg/L. After a 1-h equilibration period, three urine collections, ~30 min each, were obtained. Blood for determination of plasma inulin and serum creatinine concentra-
tions was drawn through the heparin-lock catheter at the beginning and end of each 30-min clearance interval. The mean values for consecutive serum samples and urine samples were used in calculating clearance. Final clearances were expressed as the means of three clearances measured in each of the 24 patients, corrected for body surface area. Final values for creatinine serum and urine were also expressed as the mean of four or three measurements, respectively, for each patient.

Assays. Inulin was assayed in a continuous-flow analyzer by a modified method involving resorcinol (7). Creatinine was measured by four different methods. First, creatinine was separated by isocratic cation-exchange HPLC at pH 5.8, after precipitation of proteins from serum or urine with trichloroacetic acid (8). Second, creatinine was measured by the Jaffe-based reaction in an Astra-8 analyzer as specified by the manufacturer (Beckman Instruments, Fullerton, CA). Third, creatinine was measured in the Ektachem 700 multilayer film analyzer by the single-slide creatinine assay as specified by the manufacturer (Eastman Kodak, Rochester, NY). Fourth, creatinine was analyzed by the Boehringer Mannheim (Mannheim, F.R.G.) creatinine ''PAP'' enzymatic colorimetric test in a Cobas-Bio centrifugal analyzer as specified by the manufacturer (9). Both enzymatic assays involve creatininase, creatinase, and sarcosine oxidase in their reaction schemes. By analysis of variance we identified differences in creatinine and clearance results as measured by the different methods.

Results

Table 1 shows the mean serum creatinine concentrations for each patient group, as determined by the four different methods. In groups B and C (creatinine clearances 25–100 mL/min), the Ektachem-determined creatinine concentrations were significantly (P < 0.05) higher than those determined by all other methods. Figure 1 shows the correlations for a serum creatinine as measured by the Astra, Ektachem, and PAP methods vs HPLC, the comparison method. In all cases, the correlations were excellent, r = 0.99. The intercept of 0.23 for the Ektachem regression curve confirmed the bias suggested in Table 1. Mean urine creatinine concentrations for all patients' groups, separate or combined, showed no intermethod differences; the results for the combined groups were: HPLC 159 mg/L; Astra 155 mg/L; Ektachem 151 mg/L; PAP 152 mg/L. Figure 2 shows the combined mean creatinine and inulin clearances as determined by all methods. The Ektachem creatinine clearances were significantly (P < 0.05) less than all other clearances, particularly in the 25–100 mL/min clearance range. (No significant differences were observed with clearances <25 mL/min or >100 mL/min.) Although there was bias between methods, Figure 3 shows that the correlations (r) of creati-

| Table 1. Mean Creatinine Concentrations (mg/L) in Serum for Normal and Renally Impaired Patients |
|-----------------------------------------------|-------------|-------------|-------------|-------------|-------------|
| Method       | A         | B         | C         | D         |
| HPLC         | 8.5 (1.5)  | 9.8 (2.3)  | 20.2 (9.0) | 48.8 (29.0) |
| Astra        | 9.2 (1.5)  | 10.2 (2.4) | 20.3 (8.5) | 47.3 (28.3) |
| Kodak        | 9.5 (1.9)  | 12.0 (2.5) | 23.3 (10.1) | 49.0 (28.3) |
| PAP          | 8.0 (1.4)  | 10.5 (2.3) | 20.7 (9.7) | 48.8 (31.0) |

*Mean (SD). ‡Significantly different (P < 0.05) from other results for this group of patients.
nine clearances with inulin clearance ranged from 0.88 to 0.96. Neither enzymatic method was susceptible to interferences by ketocids, cephalosporins, or bilirubin (up to 100 mg/L; data not shown).

Discussion

The present study was intended to determine whether enzymatic methods for creatinine analysis could be used to calculate creatinine clearances that would be reliable for medical decision-making. An earlier report from our laboratory indicated that the Ektachem double-slide enzymatic serum creatinine method gave results 2.0 mg/L lower than those by routine Jaffé methods in normal patients (6). This bias resulted in significantly higher creatinine clearances by the double-slide Ektachem method in comparison with the Astra method. Thus, it was difficult to use both assays in our laboratory. Because we are a referral hospital for kidney disease, we discontinued use of the Ektachem double-slide method. During the course of this study, Kodak developed a new single-slide creatinine assay, based on creatininas, creatinase, and sarcosine oxidase, similar to the Boehringer Mannheim assay (9, 10). Although the single-slide assay is a true creatinine assay, free from common interferences such as ketocids and cephalosporins, the results of our present study indicate that the Ektachem enzymatic assay appears to have been calibrated to give results higher, not lower, than nonenzymatic methods.

Table 1 and Figure 1 show that the Ektachem enzymatic method gave creatinine values 2.0 to 3.0 mg/L higher than the HPLC, Astra, or PAP enzymatic methods. This resulted in creatinine clearances significantly lower than inulin clearances and creatinine clearances determined by the other methods (Figures 2 and 3). Although biased, the results by the Ektachem assay still correlated well with those by other methods for serum and urine creatinine concentration. The new Boehringer Mannheim sarcosine oxidase-based PAP assay was precise and rapid and provided analytical results for serum and urine creatinine in agreement with those by the Astra and HPLC (Table 1, Figure 1) (9). Thus enzymatic methods of creatinine should be reliable if properly calibrated. To avoid between-assay (i.e., Jaffé vs enzymatic) discrepancies, manufacturers should agree to more acceptable standards of calibration. Enzymatic creatinine measurements can be reliably utilized for the calculation of creatinine clearances if properly calibrated.

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References