CLINICAL CHEMISTRY, Vol. 19, No. 4, (1973) 408–410

Improved Method for Specific Determination of Creatinine in Serum and Urine

Robert J. Mitchell

A simple method has been developed for measuring creatinine in serum and urine. Small, fast-flowing cation-exchange columns are used to separate creatinine from interfering substances. The creatinine is eluted and determined with the Jaffé reaction. The results agree well with those for the procedure in which Lloyd's reagent is used.

Additional Keyphrases: *creatinine clearance • interference removed by use of cation-exchange columns • Lloyd's reagent method compared*

The Jaffé reaction is commonly used to determine the concentration of creatinine in urine and serum by measuring the color produced when picric acid and sodium hydroxide solutions are combined with urine or protein-free filtrate from serum. As is well known, substances other than creatinine react to give positive error, the relative amount of interference usually being much greater in serum than in urine.

Many specific methods have been proposed for determining creatinine in serum and urine. In several of these, creatinine is separated from interfering substances with strongly acidic cation-exchange resin *(1–4)*. After the creatinine is adsorbed by the resin from an acid solution, it is easily eluted by increasing the pH. Most substances that reportedly interfere with the determination of creatinine by the Jaffé reaction are neutral or anionic and would not be retained on a cation-exchange resin, and so would not interfere with these methods.

In the methods of Teger-Nilsson *(1)*, Sadilek *(2)*, and Rockerbie and Rasmussen *(4)*, serum or urine is diluted with weak acid and applied to the resin, which is in the hydrogen form. After adsorption of creatinine and before it is eluted, the resin is converted to the sodium form by washing with a dilute sodium chloride solution. This is necessary because hydrogen ions in the eluate interfere with the Jaffé reaction. During conversion of the resin to the sodium form, creatinine can be eluted from the resin, causing poor recovery and incorrect results.

We have developed a method in which the creatinine is separated from serum or urine with a small cation-exchange column in the sodium form at pH 3. After a water wash, the creatinine can be directly eluted with the Jaffé reaction buffer. This rapid and accurate procedure can easily be used in the routine laboratory.

From the Ames Co., Division of Miles Laboratories, 1127 Myrtle St., Elkhart, Ind. 46514.

Received Oct. 25, 1972; accepted Jan. 29, 1973.

Materials and Methods

Reference Methods

Direct and Lloyd's reagent procedures for determining creatinine were performed on the same filtrate, essentially as described by Natelson *(5)*, except that in the Lloyd's reagent procedure the creatinine was eluted from the Lloyd's reagent and color developed with 3.0 ml of elution reagent (described below) and 0.5 ml of saturated picric acid. In the direct method the absorbance was determined 20 min after picric acid was added.

Reagents for Ion-Exchange Procedure

Adsorption buffer. The buffer contains 0.04 molar citric acid and 0.02 molar disodium phosphate (Na_2HPO_4). The buffer is adjusted to pH 3 if necessary and refrigerated when not in use, to inhibit microbial growth.

Elution buffer. The buffer (pH about 12.2) contains NaOH and disodium phosphate (Na_2HPO_4), 0.5 mol of each per liter.

Standard. Four milligrams of creatinine per 100 ml of 0.05 molar HCl. Store in an amber-colored bottle. Stable at least one month at room temperature.

NaOH solution, 2.5 mol/liter.

Saturated picric acid, about 14 g/liter.

Ion-exchange columns. Plastic columns ("Chromaflex," Cat. No. K-420160; Kontes Glass Co., Vineland, N. J. 08360) are used, with barrels cut to 10-cm length. To fill the columns, the resin (sulfonated polystyrene cation-exchange resin, AG 50W-X2, 100–200 mesh; Bio-Rad Laboratories, Richmond, Calif. 94804) is suspended in water, then drawn into a graduated pipet and allowed to settle. One-half milliliter of settled resin is used for each column. The top and bottom of the column are stabilized with porous polyethylene disks (Kontes). Before use, the disks must first be soaked in a 1 ml/100 ml solution of wetting agent such as "Brij 35" (Technicon Corp., Tarrytown, N. Y. 10594).

Procedure

Place the columns in the rack and allow them to drain, then successively wash them with about 2 ml of 2.5 molar NaOH and about 2 ml of water. Equilibrate the resin with about 4 ml of adsorption buffer.

Apply to the column 5 ml of sample [0.5 ml of serum, urine (diluted 50-fold), or standard mixed with 5 ml of adsorption buffer]. To another column add nothing; it is used as a column blank.

Wash the columns with about 4 ml water, remove the last drop from the tip of the column, then elute the creatinine into a clean tube with 3.0 ml of elution buffer.

To all tubes add 0.5 ml of saturated picric acid, mix, and after 30 min determine absorbances of unknowns and standard at 505 nm vs. blank.

Allow the columns to drain completely after each step. The same columns were used throughout this study and stored in a beaker of water when not in use. The average time required for 4 ml of solution to drain completely from the column was 2.3 min.

Results

Figure 1 shows the recovery of creatinine from an abnormal serum vs. pH of adsorption buffer. Recovery of creatinine is maximum when the adsorption buffer has a pH between 3 and 4. Below pH 3 the decreased apparent recovery is probably the result of interference by hydrogen ions rather than incomplete adsorption.

Figure 2 shows that creatinine is completely eluted from the column with about 1.6 ml of elution buffer.

Figure 3 shows the color obtained with the Jaffé reaction when various elution buffers were used to elute creatinine.

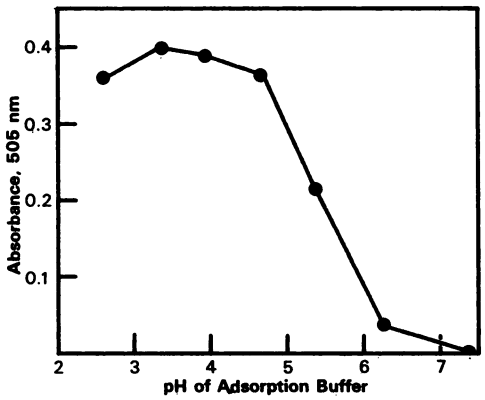

Fig. 1. Relationship between creatinine found in an abnormal serum and pH of the adsorption buffer

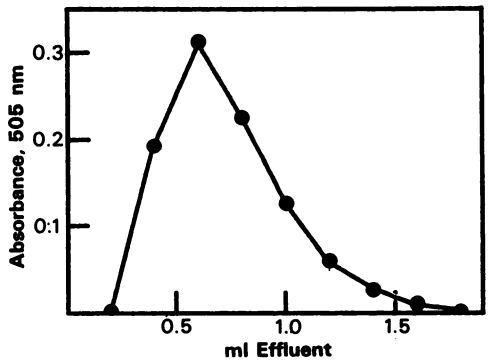

Fig. 2. Elution of 45.5 μg creatinine from ion-exchange column

Successive 0.2-ml portions of elution buffer were added to column, the eluates diluted to 3 ml with elution buffer, and color developed with 0.5 ml of saturated picric acid

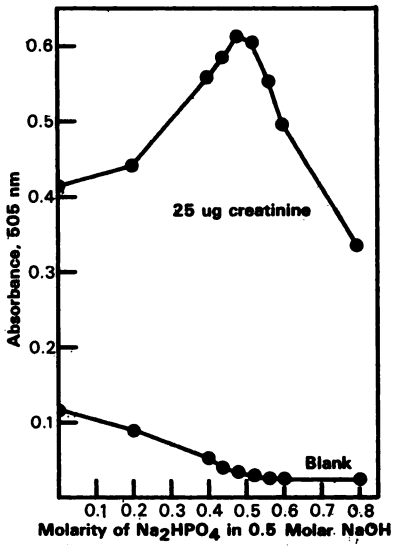

Fig. 3. Effect of composition of elution buffer on color development

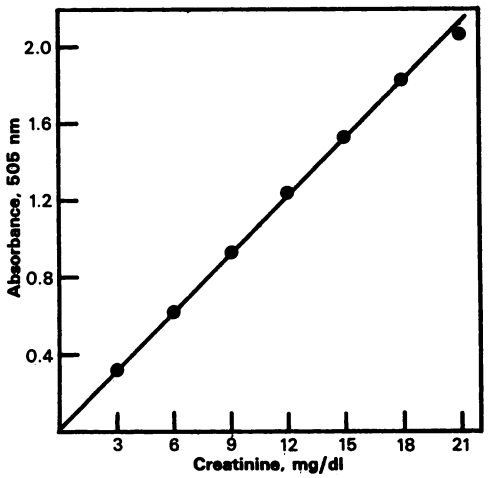

Fig. 4. Standard curve

The standard curve is linear up to 20 mg/dl with the spectrophotometer used (Model 300; Gilford Instrument Co., Oberlin, Ohio 44074). The 4 mg/dl standard gives an absorbance of about 0.410 (Figure 4).

The amount of saturated picric acid used to develop color was varied between 0.3 and 0.7 ml with less than 5% difference in final results.

Average recovery of creatinine standards from the columns was 96.7%; average recovery of creatinine added to serum was 95.1%.

When a batch of abnormal control serum was analyzed on eight successive days (a different bottle each day), the relative standard deviation of results of the direct, Lloyd's reagent, and ion-exchange methods were 2.7, 1.7, and 2.2%, respectively.

Figure 5 shows a comparison of results of the Lloyd's reagent procedure and of the ion-exchange procedure for serum creatinine.

Table 1 shows a comparison of serum creatinine and creatinine clearance values obtained with the di-

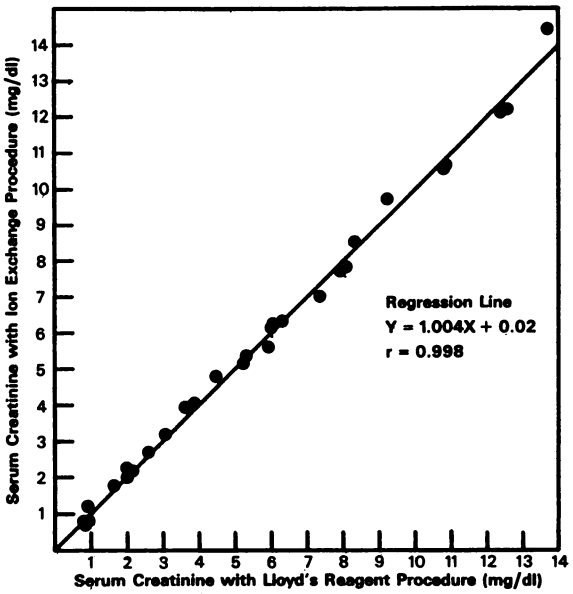

Fig. 5. Comparison of results for serum creatinine by Lloyd's reagent procedure and ion-exchange procedure

rect, Lloyd's reagent, and ion-exchange procedures on 18 apparently normal adult males. Urine collection period was 9 h.

Table 2 shows the effect on the three creatinine methods of various substances known to interfere with the Jaffé reaction. The concentration of interfering substances is far in excess of the amount that would be found in serum, but shows the relative sensitivity of the three methods to interference.

Discussion

The diagnostic value of creatinine determinations is considerably improved when a specific method is used, especially with serum having a low creatinine concentration. The effect of the variable amounts of interfering substances in serum and urine and the complexity of present specific methods for creatinine has discouraged the use of the endogenous creatinine clearance to estimate glomerular filtration rate. The new ion-exchange method presented here is much simpler to perform than the standard Lloyd's reagent procedure and does not require the precisely controlled sodium chloride wash needed in previous ion-exchange methods.

Henry *(6)* reports a median normal creatinine clearance of 120 ml/min per 1.73 m^2 of body surface when a specific method (Lloyd's reagent) was used, which is in very good agreement with the tentative normal values reported in Table 1 for the Lloyd's reagent and ion-exchange methods. Because values obtained by the new method and by the Lloyd's reagent method agree excellently (Figure 5), it should be possible to substitute the new method with no change in the normal range and a considerable savings in time necessary to do the test.

Table 1. Comparison of Results for Serum Creatinine and Creatinine Clearance for 18 Normal Men, as Determined by Three Methods

	Serum creatinine, mg/dl			Creatinine clearance, ml/min/1.73 m^2		
	Direct	Lloyd's	Ion-exchange	Direct	Lloyd's	Ion-exchange
	1.13	0.98	1.03	110	122	115
	1.20	0.99	1.04	108	126	122
	1.10	0.93	0.95	109	123	121
	1.17	1.03	1.08	110	118	115
	0.96	0.76	0.81	100	120	115
	1.13	0.95	0.97	116	131	131
	1.12	0.99	1.02	93	105	105
	1.19	1.05	1.11	114	132	125
	1.05	0.90	0.94	117	136	130
	1.08	0.93	0.99	124	144	140
	1.09	0.95	0.97	89	102	102
	0.99	0.86	0.88	103	121	120
	1.18	0.98	0.96	102	112	126
	1.14	0.98	0.95	115	130	135
	1.23	1.07	1.04	114	119	128
	1.15	0.98	0.95	106	120	128
	1.07	0.95	0.95	112	110	123
	1.05	0.90	0.91	109	115	123
Mean	1.11	0.95	0.98	108	121	122
SD	0.07	0.07	0.07	9	11	10

Table 2. Effect of Interfering Substances on Results of Three Methods for Serum Creatinine

Substances added to pooled serum	Added concn (mg/dl)	Method: Direct (mg/dl)	Method: Lloyd's (mg/dl)	Method: Ion-exch. (mg/dl)
None		1.03	0.85	0.91
α-Ketoglutaric acid	40	4.23	1.07	0.91
Sodium pyruvate	120	5.94	1.28	0.90
Oxalacetic acid	240	5.25	1.17	0.92
Acetoacetic acid	200	4.99	1.05	0.91
Ascorbic acid	360	5.40	0.92	0.94

References

1. Teger-Nilsson, A. C., Serum creatinine determination using an ion-exchange resin. *Scand. J. Clin. Lab. Invest.* **13**, 326 (1961).

2. Sadilek, L., Creatinine determination in the urine of alcaptonuric patients. *Clin Chim. Acta* **12**, 436 (1965).

3. Polar, E., and Metcoff, J., "True" creatinine chromagen determination in serum and urine by semi-automated analysis. *Clin. Chem.* **11**, 763 (1965).

4. Rockerbie, R. A., and Rasmussen, K. L., Rapid determination of serum creatinine by an ion-exchange technique. *Clin. Chim. Acta* **15**, 475 (1967).

5. Natelson, S., *Microtechniques of Clinical Chemistry for the Routine Laboratory*, C. C Thomas, Springfield, Ill., 1957, p 186.

6. Henry, R. J., *Clinical Chemistry: Principles and Techniques*, Hoeber, New York, N. Y., 1964, p 892.