antibiotics, but may have wider application. However, this application must be preceded by an appreciation (and if possible the testing) of the underlying assumptions of this relationship. The major assumption remains that the only binding that occurs outside the vascular space is to plasma proteins located there, and that the binding constants remain the same.

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Creatinine XII: Comparison of Assays of Low Serum Creatinine Levels Using High-Performance Liquid Chromatography and Two Picrate Methods

Keyphrases □ Creatinine—assay in serum using HPLC, automated picrate method, modified picrate method, comparison of methods □ High-performance liquid chromatography—creatinine assay in serum, comparison with two picrate methods

To the Editor:

Endogenous creatinine has been commonly employed to estimate the glomerular filtration rate for the study of renal function or for modifying dosages in patients with renal impairment (1–5). Clinically, the automated picrate method[1], based on a complex color reaction between creatinine and picrate in the alkaline medium, is probably most widely used to assay creatinine in plasma or serum (6–10). This method, however, is known to be nonspecific due to potential interferences by endogenous and/or exogenous substances, and often results in an overestimate of "true" creatinine levels (7–9). It appears that to date most of assay comparisons between this method and the more specific high-performance liquid chromatographic (HPLC) method were carried out in samples containing higher levels (such as >0.8 mg%) of creatinine (10, 11). Since lower levels are often found in patients, it would seem important to evaluate potential discrepancies using low-level samples. The modified picrate method of Yatzidis (12, 13) was also chosen for the present evaluation, since it has been reported to be highly specific.

A total of 30 random serum samples from patients determined by the automated picrate method in a clinical laboratory[2] and found to contain less than 0.8 mg% of creatinine, were employed in the study. The HLPC method (14) used in the present study is a slight modification of the method developed earlier in our laboratory (3). Briefly, the method involved the deproteinization of 0.1 ml of serum with 0.25 ml of acetonitrile. After vortexing and centrifugation, 50 μl of the supernatant was injected directly into the cation-exchange column[3]. The recovery in the above sample preparation is essentially 100% (3). The mobile phase with a flow rate of 3 ml/min contained 0.035 *M* monobasic ammonium phosphate adjusted to pH 4.8 with 0.01 *N* NaOH. The creatinine was monitored at 254 nm using a fixed wavelength detector[4] with a sensitivity setting of 0.005 AUFS. The retention time for creatinine was about 4.5 min. The present assay has a detection limit of 0.05 mg% (based on a signal/noise ratio of 3.0), and has a coefficient of variation for both interassay and intraassay between 1.2 and 3.0%. No interferences were found in the present and earlier (14) studies with samples obtained from patients or volunteers. It should be noted that the variable wavelength UV detector (without a noise damper) used in our earlier studies (3, 10) was much less sensitive; it had a larger base-line noise even at a previously used sensitivity setting of 0.05 AUFS. Duplicate analyses were performed using both the HPLC method and the Yatzidis method.

The results of the serum creatinine measured by the above three methods are summarized in Table I. The automated method overestimated serum creatinine by an average of 15.2% with a −16.7 to 66.7% range when compared with the HPLC method. Although the mean overestimation found in the present study is similar to the previous study (14.5%, $n = 30$) using samples with generally much higher serum levels (10), it is of interest to note (Table I) that for two samples the overestimations were >50%, and for eight samples the results were essentially identical. The above results suggest that the amount and nature of interfering substances may vary considerably with individuals.

The overestimations of serum creatinine by the modified picrate method were much higher with an average of 55.2% (ranging from −58.3 to 168%). In the previous report (10), a similar modified method (15) was found to overestimate

[1] Auto Analyzer SMA 6/60, Technicon Instruments, Tarrytown, N.Y.

[2] University of Illinois Hospital, Chicago, Ill.
[3] Partisil PXS 10/25 SCX, 30 cm, Whatman Inc., Clifton, N.J.
[4] Model 440, Waters Associates, Milford, Mass.

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Table I—Comparison of Serum Creatinine Levels Using Three Different Methods

HPLC, mg%	Automated Picrate Method[a], mg%	Deviation from HPLC, %	Modified Picrate Method, mg%	Deviation from HPLC, %
0.44	0.5	13.6	—	—
0.42	0.5	19.0	0.72	71.4
0.41	0.5	22.0	0.65	58.5
0.52	0.5	−3.8	0.75	44.2
0.59	0.5	−15.3	0.98	66.1
0.48	0.5	4.2	0.89	85.4
0.60	0.5	−16.7	0.88	46.7
0.47	0.5	6.4	0.80	70.2
0.57	0.6	5.3	0.91	59.6
0.47	0.6	27.7	0.95	102.1
0.48	0.6	25.0	0.83	72.9
0.48	0.6	25.0	0.20	−58.3
0.51	0.6	17.6	0.86	68.6
0.56	0.6	7.1	0.87	55.4
0.55	0.6	9.1	1.26	129.1
0.38	0.6	57.9	1.02	168.0
0.52	0.6	15.4	0.90	73.1
0.62	0.6	−3.2	0.54	−12.9
0.61	0.6	−1.6	0.84	37.7
0.49	0.6	22.4	0.86	75.5
0.64	0.7	9.4	0.92	43.8
0.62	0.7	12.9	1.11	79.0
0.51	0.7	37.3	1.10	115.7
0.61	0.7	14.8	0.88	44.3
0.61	0.7	14.8	0.46	−24.6
0.66	0.7	6.1	0.84	27.3
0.42	0.7	66.7	0.71	69.0
0.67	0.8	19.4	1.05	56.7
0.68	0.8	17.6	0.71	4.4
0.67	0.8	19.4	0.49	−26.9
Mean		15.2		55.2
±*SD*		±17.5		±47.2

[a] Only one significant figure was provided for concentrations <1.0 mg% by the printout of the instrument.

by an average of 32% in serum samples with a wider range of creatinine levels (0.62–18.5 mg% based on the HPLC method). However, for samples (n = 3) with serum levels in the range of 0.6–0.7 mg% (10) the mean overestimation was 88.2%. Thus, both of the modified picrate methods (12, 13, 15) resulted in consistently much higher creatinine values. The reason for the apparent discrepancy between their claimed specificity (12, 15) and the present as well as the previous (10, 16) findings remains to be investigated.

The results of the present study indicate that one should be cautious in using the automated and modified picrate methods for creatinine determinations. Since the HPLC method is generally considered to be more specific (3), its use should be preferred when accurate determinations are required.

It has been recently reported that creatinine might be extensively secreted and reabsorbed by renal tubules in both humans (17, 18) and animals (18, 19), and there might also be a significant nonrenal elimination in normal humans (19, 20) and normal rabbits (19). The full implications of these findings in the use of creatinine remain to be explored.

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Crystalline Anhydrous–Hydrate Phase Changes of Caffeine and Theophylline in Solvent–Water Mixtures

Keyphrases □ Phase changes—in crystals, anhydrous to hydrate, caffeine, theophylline, solubility studies □ Caffeine—crystalline phase changes, anhydrous to hydrate, solubility studies □ Theophylline—crystalline phase changes, anhydrous to hydrates solubility studies

To the Editor:

The extended Hildebrand solubility approach (1–3) was recently developed and evaluated for calculation of solubilities in solvent–water systems that are not adequately described by the regular solution theory (4). The approach is based on regression analysis of solubility data to calculate solute–activity coefficients as a function of the solubility parameter of the solvent mixture.

A problem exists with several of the solutes used in the evaluation of the extended Hildebrand approach: crystalline anhydrous–hydrate phase transformation as the water content of the solvent mixture is changed. In the dry solvent it is clear that only a nonhydrated (anhydrous or solvated) form can be present at equilibrium (5); but in aqueous solvent mixtures the equilibrium form may be anhydrous solvated or hydrated crystals. This was recently demonstrated for cholesterol in water–glyceryl-1-monooctanoate solutions (6). The transition between anhydrous and monohydrate forms occurred at 5% water (37°) and was temperature dependent. In this system the max-

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