2. Doolittle MH, Lincoln K, Graves SW. Unexplained increase in serum dige
3. Critchfield GC, Wilkins DG, Loughmiller DL, Davis BW, Roll-
ina DE. Antibody-mediated interference of a homogeneous immu
14–7.

Intrasubject Variation of Creatinine and Specific Gravity Measurements in Consecutive Urine Specimens of Heroin Users, Bruce A. Goldberg,1 Barbara Loewenthal, William D. Darwin, and Edward J. Cone2 (Addiction Research Center, Natl. Inst. on Drug Abuse, P.O. Box 5180, Baltimore, MD 21224; 1 current address: Univ. of Florida College of Med., Dept. of Pathol. and Lab. Med., P.O. Box 100275, Gainesville, FL 32610-0275; 2 author for correspondence: fax 410-550-2971)

Growing concern regarding the use of illicit drugs has led to implementation of drug testing in the workplace. Unfortunately, a negative test result does not necessarily indicate that the donor has abstained from drug use (1). To reduce the incidence of false-negative drug test results obtained after intentional dilution, many toxicology laboratories rou
n tests for creatinine and specific gravity. The US Department of Transportation has recommended that a urine specimen be considered abnormally dilute if its creatinine concentration is $<1.77 \text{mmol/L}$ and its specific gravity is $<1.003$. The purpose of this study was to determine the intrasubject variability of creatinine and specific gravity in consecutive urine specimens collected from subjects over an extended period of time under controlled clinical conditions during administration of drug.

All sequential urine specimens were collected from nine male subjects who were users of heroin or cocaine (or both) and who had volunteered to participate in a controlled clinical study of the pharmacodynamics of heroin. The study was approved by the Institutional Review Board for Human Subjects Research at the Francis Scott Key Medical Center, Baltimore, MD, and was conducted under the guidelines for the protection of human subjects. Each volunteer gave informed consent. The study was conducted on the clinical ward of the Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD. Subjects were administered single doses of heroin or placebo under double-blind conditions. Aliquots of the urine samples were frozen at $-30^\circ\text{C}$ before analysis.

Creatinine measurements were performed by the Jaffé method with Boehringer Mannheim Diagnostic (Indianapolis, IN) reagents on a Hitachi 704 analyzer. Specific gravity was estimated with an Atago (Tokyo, Japan) Clinical Refractometer T2. Control samples were assayed with every batch of creatinine analyses, and all results for the controls during the study were within the expected range. The average within-run CV was 1.1% (n = 6) and the between-run CV was 3.0% (n = 10).

The mean creatinine concentration in urine specimens from the drug-using subjects was 14.1 mmol/L; individual creatinine results ranged from 1.6 to 47.0 mmol/L (Table 1). The mean specific gravity result was 1.020, with individual results ranging from 1.002 to 1.036. Intrasubject creatinine and specific gravity measurements varied in a significant manner ($r^2 = 0.593$–0.854; $P < 0.001$). The lowest creatinine value was $<1.77 \text{mmol/L}$ (n = 3 specimens), and the lowest specific gravity was $<1.003$ (n = 1 specimen). No single specimen had both a low creatinine value and a low specific gravity. Analysis of variance indicated significant differences between subjects ($P < 0.001$).

The mean urine measures obtained for specific gravity were generally within the reference interval of values reported in the literature: 1.002–1.030, according to Tietz (2). The range of creatinine values differed from those previously reported by Gowans and Fraser for random spot urine specimens from 49 men: 3.6–22.3 mmol/L (3). However, the authors noted that the reported range calculated by non-parametric measures were narrower than those determined by parametric measures.

Because opioids influence the release of antidiuretic hormone (4), we determined the effect of heroin administra-

<table>
<thead>
<tr>
<th>No. of days on study</th>
<th>No. of specimens</th>
<th>Creatinine, mmol/L</th>
<th>Specific gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (SD)</td>
<td>Range</td>
</tr>
<tr>
<td>20</td>
<td>112</td>
<td>7.9 (4.4)</td>
<td>1.7–21.5</td>
</tr>
<tr>
<td>14</td>
<td>71</td>
<td>12.3 (4.6)</td>
<td>3.2–24.9</td>
</tr>
<tr>
<td>25</td>
<td>163</td>
<td>13.9 (5.5)</td>
<td>2.4–38.1</td>
</tr>
<tr>
<td>23</td>
<td>74</td>
<td>13.4 (6.4)</td>
<td>2.1–33.1</td>
</tr>
<tr>
<td>25</td>
<td>161</td>
<td>11.6 (4.2)</td>
<td>2.1–22.5</td>
</tr>
<tr>
<td>25</td>
<td>116</td>
<td>15.1 (4.8)</td>
<td>3.2–27.5</td>
</tr>
<tr>
<td>30</td>
<td>107</td>
<td>14.8 (5.0)</td>
<td>3.8–33.4</td>
</tr>
<tr>
<td>27</td>
<td>272</td>
<td>19.4 (7.7)</td>
<td>1.6–47.0</td>
</tr>
<tr>
<td>26</td>
<td>130</td>
<td>11.7 (3.9)</td>
<td>2.6–22.9</td>
</tr>
<tr>
<td>Total</td>
<td>1206</td>
<td>14.1 (6.6)</td>
<td>1.6–47.0</td>
</tr>
</tbody>
</table>
tion on urine production and on measurements of creatinine and specific gravity. Data collected from each subject during the first 24 h after drug administration were compared with data collected during the subsequent 24 h. The differences in the creatinine and specific gravity values were not statistically significant ($P > 0.10$).

Other investigators have studied the use of creatinine analyses to detect abnormal dilution of urine. An uncontrolled fluid ingestion study conducted by Needleman et al. (5) revealed that ingestion of large amounts of liquid produced a decrease in random urinary creatinine values. Several hours were required to produce a fall in creatinine concentration to $< 4.4$ mmol/L. These authors concluded that creatinine output was a sensitive measure to the amount of fluid ingested but that the relationship was neither linear nor immediate.

In a study of urine specimens collected from former heroin abusers participating in an outpatient methadone-maintenance treatment program, Lafolie et al. (6) recommended that urinary creatinine concentrations be measured routinely, having observed false-negative drug test results in some specimens when creatinine results were $< 4.3$ mmol/L. Lafolie et al. also noted a significant decrease in measured creatinine ($< 3.8$ mmol/L) in urine specimens collected from healthy volunteers who drank 0.5 L of water; the urinary creatinine content did not return to normal for several hours.

To develop quantitative measures for creatinine, specific gravity, and pH for use in the identification of adulterated specimens, Edwards et al. (7) studied 144 specimens with abnormal color, smell, sedimentation, or response by immunoassay. Utilizing reference intervals of 1.007–1.035 for specific gravity and $< 4.0$ mmol/L for creatinine, they found $48\%$ of the specimens acceptable. When they used creatinine and specific gravity measures independently, $68\%$ and $55\%$ of the specimens, respectively, were also acceptable. Measurement of pH helped them identify six unacceptable specimens.

In conclusion, the data collected during our study supports the widely used values for creatinine and specific gravity measurements for evidence of dilution.

References


**Spuriously High Concentration of Serum Free Thyroxine due to Anti-Triliodothyronine Antibodies, Rémy Sapin,1,2 Françoise Gasser,1 Andreas Boehn,3 and Muriel Rondeau2 [1 Inst. de Phys. Biol. (Dir. Pr. J. Chambron), Faculté de Médecine, F-67085 Strasbourg Cedex, France; 2 Méd. Interne A (Pr. D. Christmann), Clin. Méd. A, Hôpital Central, CHRU, F-67091 Strasbourg Cedex, France; 3 Author for correspondence: Fax Int + 33 88 37 14 97; E-mail sapin@alsace.u-strasbg.fr]

Free thyroxine (FT4) is now frequently measured in serum by one-step labeled antibody assays based on a Solid-Phase Antigen-Linked Technique (SPALT) (1). In this assay the serum sample is incubated with a large excess of triiodothyronine (T3)-coupled solid phase and with a limited amount of labeled anti-T4 antibody. Because the solid phase acts as a ligand of low affinity for the anti-T4 antibody, interference from circulating anti-thyroid hormone (anti-T4 or anti-T3) autoantibodies (THAA) is theoretically possible. However, until now, to our knowledge, this assay was considered to be only slightly affected by THAA (2–4). The highest measured FT4 values (up to 35 pmol/L) could be related to therapy with T4 (4).

Nevertheless, recently we observed a very high FT4 value (131 pmol/L) measured by a SPALT assay (Amerlex-MAB4; Kodak Clinical Diagnostics, Amersham, UK) in the serum of a hospitalized patient with Crohn disease. This 35-year-old man was euthyroid by clinical evaluation and by his normal thyrotrpin serum concentration (0.59 mIU/L, normal range 0.15–4.5 mIU/L) determined with Berilux kit (Behring, Marburg, Germany). The biological evaluation showed a moderate hypergammaglobulinemia (17 g/L, normal range <14 g/L) with increased IgG concentration (21.8 g/L, normal range <17 g/L). The followed procedures were in accordance with the Helsinki Declaration of 1975 as revised in 1983.

This patient's serum contained anti-T3 but no anti-T4 antibodies, as identified with the technique of Allan et al. (5) by using the analog T3 or T4 radioactive tracer of the Amerlex-M FT3 or FT4 kits, respectively (Kodak Clinical Diagnostics). The percentage of radioactive T3 or T4 analog tracer precipitated by polyethylene glycol, 12%, was above normal (5% in absence of THAA) but still quite low. The measurement of Amerlex-M FT3 remained normal (4.1 pmol/L, reference range 3.7–9.2 pmol/L). Fixation of a radioactive [125I]-labeled native T3 tracer on the fraction precipitated by polyethylene glycol was also not very high: 7.1% (vs normal of <5% in absence of THAA). That the free thyroid hormone concentrations were normal was confirmed by two-step RIAs known to be unaffected by the presence of THAA: FT4, 24.8 pmol/L (normal range 9–25.7 pmol/L by Gammacoat®; Instar, Stillwater, MN); FT3 3.7 pmol/L (normal range 3.1–6.1 pmol/L by Ria-gnost®; Behring).

This very marked interference in the FT4 Amerlex-MAB assay shows an interaction of anti-T3 autoantibodies with the T3 immobilized on the solid phase. This interaction can occur in the presence of THAA of high affinity for the T3 fixed on the solid phase; perhaps the serum of this patient contains an antibody with a high affinity towards the T3-bridge that is used to link the solid phase to T3. This interaction can also occur with THAA of low affinity but of high capacity (i.e., the ligand is present in high concentration) (4).

The prevalence of THAA seems to be low (1/2360) (2) but the frequency of THAA is much higher in hypothyroid (7%), hyperthyroid (1.5%), and nontyroid autoimmune (7.5%) patients (6). Compared with other one-step FT4 kits.