tector antibodies are not able to bind to CA 125 captured by the M11 antibodies on the solid phase, resulting in a reduced assay response (Fig. 1). In the two-step version, the anti-idiotypic anti-OC125 antibodies are removed after the first incubation by an additional washing step; thus, they cannot block the OC125 detector antibodies in the second incubation step.

As reported before, the extent of reduction of recovery rate depends on the concentration of anti-idiotypic anti-OC125 antibodies. In the presence of concentrations exceeding 5000 kiloarbit units/L <20% of CA 125 was recovered (5). Because the incidence of such increased concentrations of anti-idiotypic antibodies is quite high—seen in 24% of patients after only a single infusion of OC125—we recommend that "second-generation" CA 125 assays involving M11 capture antibodies and OC125 detector antibodies in an one-step protocol not be used to monitor ovarian cancer patients who have been treated with OC125 fragments.

References

Jochen Reinserg1
Birgit Gast
Zentrum für Frauenheilkunde und Geburtshilfe
Universität Bonn
Sigmund-Freud-Straße 25
D-53127 Bonn, Germany

1 Author for correspondence.

Diurnal Rhythm in Urinary Excretion of Pyridinium Crosslinks

To the Editor:

Measurement of urinary pyridinium crosslinks provides an index of bone resorption rates (1). Crosslink excretion changes with age, but the extent of variation over 24 h is uncertain. One recent report (2) demonstrated a marked diurnal variation in crosslink excretion throughout a 24-h period in premenopausal women, with the excretion rate usually higher at night. In this and in most clinical studies, crosslinks were determined by an HPLC assay considered the "standard" method for crosslink measurement (3). Recently, simple, direct urinary immunoassays have been developed that might have many clinical applications. However, these immunoassays generally measure total pyridinium concentrations, because the antibodies produced do not adequately distinguish pyridoline from deoxypyridinoline, the bone-specific marker (3). Thus, these assays require rigorous clinical validation before final acceptance as an accurate measure of bone metabolism.

The aim of our study was to reexamine the diurnal variability in urinary excretion of pyridinium crosslinks by using one of these new commercially available immunoassays (4).

Ten apparently healthy men (ages 41.5 ± 11 years; height, 176 ± 10 cm; weight, 76.6 ± 12 kg) and seven apparently healthy premenopausal women (ages 27.4 ± 6 years; height, 165 ± 4 cm; weight, 61.3 ± 12 kg) participated in this study. The inclusion criteria were that the subjects be within 20% of ideal body weight, have no previous or present bone or connective tissue disorder, and, for women, have regular menstrual cycles and no use of hormonal contraceptives. On the day of the study, after one night of fasting and abstaining from tobacco, the participants emptied their bladders at 0700. Afterwards, urine was collected in 3-h portions until the following morning at 0700. Meals of normal composition (not controlled for gelatin content) were served at 0730, 1200–1300, and 1900–2000. Normal indoor activities and sleeping pattern were permitted.

We measured urinary pyridinium crosslinks with a commercially available ELISA kit (Collagen Crosslinks; Metra Bio-systems, Palo Alto, CA). This assay depends on an antibody that strongly reacts with free pyridoline and deoxypyridinoline and shows negligible binding (cross-reactivity <2%) to pyridoline peptides with a molecular mass >1000 Da (4).

To minimize the influence of diuresis on the final results, we corrected the pyridinium values for creatinine excretion (a creatinine assay, based on a modified Jaffé method, is included in the Metra Bio-systems kit). The overall reproducibility (CV) of the assay, including creatinine determination, was <10%. Urine aliquots were stored at −20°C until analyzed. To eliminate between-run analytical variation, we measured all samples from each participant in the same assay. To further minimize analytical variation, a single analyst performed all the assays, using a single lot of reagents.
Three statistical approaches were used to evaluate the rhythmic nature and differences in the overall pattern of the time series of results. First, an analysis of variance was performed on raw data to test whether the variations over time were merely random fluctuations. Second, Student's t-test was used to compare peak and nadir mean concentrations and to compare mean values by sex at a given time. Each subject's data were then standardized by expressing individual values as percentages of the 24-h mean. These data were fitted by a polynomial regression of the variable (crosslinks concentration) against time to construct a mathematical representation of the curves.

Analysis of variance revealed that the difference in pyridinium crosslink excretion among periods was statistically significant for both sexes (for men, $F = 6.64, P = 0.0001$; for women, $F = 2.35, P = 0.04$), showing an actual diurnal variation throughout the day. The excretion peak occurred at 0700 in women (mean ± SD urinary pyridinium crosslinks, 52.4 ± 14 μmol/mol creatinine) and at 1000 in men (43.1 ± 9 μmol/mol creatinine). The nadir mean value was observed at 1900 in women (34.0 ± 5 μmol/mol creatinine) and at 0100 in men (27.5 ± 5 μmol/mol creatinine). The relative differences between peak and nadir were 35% ($P = 0.013$) and 36% ($P = 0.00025$) for women and men, respectively. The mean values in men were lower at night than in women, notably between 1900 and 0700 ($P < 0.05$).

Changes in standardized mean with time were best described by a five-term polynomial model, which provided a good fit to the data (Fig. 1). Phase-displacement between the curves in two sexes was evident.

The data obtained indicate the existence of a distinct circadian rhythm of urinary pyridinium crosslink excretion in healthy people. More importantly, we found a difference between the diurnal rhythms of men and women, which was most notable during the night. Using HPLC, Schlemmer et al. (2) also reported circadian variations in the excretion rate of pyridiniums in healthy, premenopausal women, with maximum excretion between 0500 and 0600 and an amplitude of fluctuation similar to ours. However, to our knowledge, this is the first report demonstrating a significant circadian rhythm of pyridinium crosslinks in both men and women, and the first to indicate subtle sex differences in the circadian fluctuation of this marker of bone metabolism. Because the amount of pyridinium crosslinks excreted reflects the breakdown product from bone (1), knowledge of possible sex differences could provide new insight into underlying sex differences in the rate of bone remodeling.

This study also emphasizes the importance of regulating the time of urine sample collection for measuring pyridinium crosslinks in clinical practice. By limiting urine sampling to just one point along the fluctuating pattern, e.g., using a random sample during the day or a 2-h sample, important information could go undetected.

References

Franca Pagani
Mauro Pantechni

1° Lab. Analisi Chim.-Clin.
Spedali Civili
25125 Brescia, Italy

Author for correspondence.

HPLC and Drug Analysis

To the Editor:

I was amazed to read in Robert Fitzgerald's review of M. K. Ghosh's book on HPLC methods for drug analysis that "There are no texts devoted to the analysis of drugs in biological samples." We, in Europe, have, for the last 15 years, had available Clarke's Isolation and Identification of Drugs. Now in the second edition, edited by Tony Moffat of the Forensic Science Service, England, and published in London by the Pharmaceutical Press, this economical book contains not only chemical data but also references to and details of extraction procedures, HPLC, TLC, GLC, and GC/MS methods and, where appropriate, immunoassays. It may be that the book, for copyright reasons, is not available in the US; but if that is the case, then any American drug analyst worth his or her salt should hightail it over here and get a copy.

Michael J. Stewart
Inst. of Biochem.
Royal Infirmary
Glasgow, Scotland G4 0SF, UK

The author of the review responds:

To the Editor:

As pointed out by Dr. Stewart, Clarke's Isolation and Identification of Drugs is a standard reference in analytical toxicology. It is also well known in the US. Dr. Stewart has, however, taken the quoted half-sentence out of context; the sentence continues, "... often the only place to begin developing an HPLC procedure is in the library." Clarke's book is a great reference; it does not, however, contain important detail on HPLC methods, which is central to the text by Ghosh.

Robert L. Fitzgerald
Dept. of Veterans Affairs
Medical Center—113
3350 La Jolla Village Dr.
San Diego, CA 92161

Cross-Reactivity of Morphine-6-Glucuronide with Emit d.s.u.
Screening Test for Opiates

To the Editor:

Morphine is excreted in urine mostly as the inactive compound morphine-3-glucuronide (M-3-G); a small amount is excreted unchanged. The