Relationship of Serum Estradiol and Progesterone Concentrations to the Excretion Profiles of Their Major Urinary Metabolites as Measured by Enzyme Immunoassay and Radioimmunoassay

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Paired daily blood and urine samples were collected from 10 apparently healthy premenopausal women to compare the hormone profiles of estradiol (E2) and progesterone in serum with those of estrone conjugates (E1,Conj) and pregnanediol-3-glucuronide (PdG) in urine. Serum hormones were measured by radioimmunoassay (RIA) kits, whereas the urinary steroid metabolites were assessed by both RIA and enzyme immunoassay (EIA). RIA and EIA values for urinary E1,Conj and PdG were not different, and both methods produced urinary profiles that paralleled the profile of the parent steroid in serum. However, the simplicity, flexibility, and economy of EIA will make this method more widely applicable. Mean E1,Conj values lagged behind concentrations of serum E2 by one day or less, whereas daily urinary PdG profiles lagged behind serum progesterone by one to two days. Mean urinary profiles of E1,Conj were similar whether or not creatinine was used to adjust for urine volume; however, creatinine indexing was beneficial when urinary profiles in individual cycles were compared with changes of serum E2.

Additional Keyphrases: intermethod comparison - ovulation profile - steroid hormones

Study of the dynamics of hormone release during the ovarian cycle usually requires daily evaluations. As an alternative to daily collection of blood samples for hormone quantification, recent reports have demonstrated that the measurement of urinary metabolites of ovarian steroid hormones by direct RIA can be used to assess ovarian follicular growth and development as well as peri-implantation events (1–5).

The measurement of urinary estrone conjugates (E1,Conj) and pregnanediol-3-glucuronide (PdG) to monitor ovarian function is preferable to the measurement of steroid hormones in plasma or serum in many clinical and research settings—for example, when blood samples are difficult to obtain, when observations are required over prolonged periods, or when infrequent events can be observed only by daily assessments. The ability of subjects to collect their own urine specimens on a daily basis and store them without processing allows study designs to be carried out that involve repetitive sampling for weeks (4), months (6), and even years (7).

Although RIAs have been used effectively for measuring hormones and their metabolites, they have the disadvantages associated with use and disposal of radioisotopes. The development of nonradiometric assays, in particular enzyme immunoassays (EIAs), has eliminated the problems associated with the use of radioisotopes (8–11).

Our primary objective in this study was to develop and validate nonradiometric assays for measuring the urinary metabolites of the two major ovarian steroid hormones, estrogen and progesterone (P4), and to compare these results with those obtained by RIA. The second objective was to determine the relationship of estradiol-17β (E2) and P4 concentrations in serum to those of their primary metabolites quantified in urine collected daily throughout the menstrual cycle. Finally, we determined the effect of sample dilution on hormone concentrations by also measuring creatinine (Cr), and expressing the results in terms of mass of urinary metabolites per mass of excreted Cr.

Materials and Methods

Subjects and Sample Collection

Ten healthy women, ages 23 to 40 years, with normal reproductive histories and with menstrual cycles ranging from 26 to 32 days, were recruited for the study. The subjects used contraception methods during the study cycle (tubal ligation, barrier methods, or abstinence) and took no medications. Both urine and blood samples were collected daily, beginning on the first day of menses and continuing until the onset of the next menstrual period. Each subject collected urine (3 mL) at the first urine void each morning and froze the sample immediately (at about –20 °C) without preservatives. The samples were kept frozen until transported to the laboratory at the end of the study. Daily blood samples (5 mL) were collected between 1000 and 1300 h, and the sera were separated without delay and frozen.

Reagents

Antiserum. Estrone-3-glucuronide (E1,G; Steraloids, Inc., Wilton, NH) was conjugated to bovine serum albumin (BSA) via the carboxylic acid moiety of the glucuronide molecule by the mixed anhydride procedure described by Dawson et al. (12) and modified by Munro and Stabenfeldt (10). Polyclonal antiserum against the im-
munogen E$_2$G-BSA was produced in male New Zealand White rabbits, ages three to five months, by an immunization method similar to that of Vaitukaitis et al. (13). Pertussis triple vaccine (0.5 mL; Connaught Labs., Inc., Swiftwater, PA) was given intramuscularly with the initial immunization only.

Polyclonal antibody R522 and a monoclonal antibody (4F11) specific for E$_2$G (from F. Kohan, Weizmann Institute for Science, Rehovot, Israel) were screened for use in the E$_2$G Conjug assays. A polyclonal antiserum (from S. Tilson, Syva Co., Palo Alto, CA) directed against PdG-BSA was used for the development of the EIA of PdG. A different antiserum was used in the RIA of PdG, as previously described by this laboratory (4). The polyclonal antisera were purified by ammonium sulfate precipitation and adsorbed with BSA before use in the EIA.

**Enzyme conjugate.** Horseradish peroxidase (EC 1.11.1.7) was coupled to E$_2$G and PdG (Sigma Chemical Co., St. Louis, MO) via the carboxylic acid group of the carbohydrate moiety (3-glucuronide molecule) by the mixed anhydride procedure (10). After elution of the conjugate from a Sephadex G-25 chromatographic column (Pharmacia Fine Chemicals, Piscataway, NJ) in 50 mmol/L phosphate buffer (pH 7.5), the steroid–enzyme conjugate was stored at −15 °C until used.

**Assays.** Concentrations of hormones [E$_2$, P$_0$, lutropin (LH), and follitropin (FSH)] in serum were determined by using commercial RIA kits (Diagnostic Products Corp., Los Angeles, CA). Standards for the LH and FSH assays were the World Health Organization (WHO) 1st International Reference Preparation (IRP) 68/40 and the WHO 2nd IRP-HMG, respectively. Direct RIAs for the measurement of E$_2$G Conjug and PdG in urine have been described previously (4, 14).

The competitive, microtiter plate solid-phase EIA procedure for the measurement of E$_2$G Conjug and PdG was similar in configuration to the EIA of PdG described by Munro and Stabenfeldt (10). For coating the plates, we diluted the purified antibody first in distilled water (stock solution, 10-fold dilution) and then in coating buffer solution (50 mmol/L bicarbonate buffer, pH 9.6), using 5000- and 8000-fold dilutions for the polyclonal E$_2$G Conjug and PdG antisera, respectively, and 800-fold dilution for the 4F11 monoclonal antibody. We coated flat-bottom, 96-well microtiter plates (Nunc-Immuno Plate Polyserb no. 4785094, Applied Scientific, San Francisco, CA; or Immulon 1, Dynatech Labs., Alexandria, VA) with 50 µL of the antibody coating solution per well. We sealed the plates tightly with waterproof plate-sealer covers and allowed them to incubate overnight at 4 °C, or stored them at 4 °C for as long as three weeks. Before the assay, we rinsed nonbound antisera from the wells with wash solution (0.15 mol/L NaCl solution containing 0.5 mL of Tween 20 per liter). Assay buffer (0.1 mol/L sodium phosphate buffer, pH 7.0, containing 9 g of NaCl and 1 g of BSA per liter), 50 µL, was then added without delay to each well, followed by 10 or 20 µL of diluted urine (diluted 20- to 50-fold in assay buffer), appropriate standards, or assay controls. We then added 50 µL of enzyme conjugate (diluted 15 000- and 25 000-fold in assay buffer for E$_2$G Conjug (R522) and PdG assays, respectively) to each well, covered the plates tightly, and let them incubate for 2 to 18 h at room temperature, as convenient.

To separate free from bound hormone, we emptied the plates, rinsed them five times with wash solution, and blotted them dry. Freshly prepared substrate solution (per liter, 50 mmol of citrate, 1.6 mmol of hydrogen peroxide, and 0.4 mmol of 2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid) diaminonitrogen salt, pH 4.0), 100 µL, was then added to all wells to determine the amount of conjugate bound to solid-phase antibody. The enzyme conversion of the substrate, manifested as a color change, was stopped after ~1 h by adding 100 µL of stopping solution (0.15 mol/L hydrofluoric acid, containing 6 mmol of NaOH and 1 mmol of edetic acid per liter, pH 3.3). Absorbance was measured at 405 nm with an automatic microtiter plate spectrophotometer (Dyna-tech MR600), and the data were transferred to an interfaced computer (IBM). The absorbance of the zero-concentration wells (A$_0$) was used to determine maximum binding of enzyme conjugate to the antisera. The absorbance values (A) of all standards, controls, and unknowns were divided by the average A$_0$ reading for each plate, and this ratio was multiplied by 100 to give the percentage of maximum binding. The calibration curve was constructed by using weighted least-squares linear regression according to the method of Rodbard and Lewald (15), and the raw data were reduced by logit-log transformation to yield concentrations.

Creatinine (Cr) concentrations were determined in urine samples by a microtiter plate method based on the technique of Tauskky (16). Urinary hormone concentrations were expressed as the concentration of steroid metabolite per millimole of Cr. Urinary hormone concentrations were also expressed without Cr indexing, as nanomoles (E$_2$G Conjug) per liter of urine.

**Assay Validation.** The cross-reactivity of steroids and of steroid metabolites structurally related to E$_2$G Conjug and PdG was assessed for both the polyclonal antisera (R522 and anti-PdG) and the monoclonal antibody (4F11) by the criteria of Abraham (17). Curves generated from assays of standards and serially diluted urine samples were compared for parallelism in both the E$_2$G Conjug and PdG EIAAs. Sensitivity was determined as the least amount of hormone that could be distinguished from zero concentration of standard as calculated from 95% confidence limits at the zero point of the standard curve. The precision of the assays was assessed by determination of an inplate coefficient of variation (CV) (40 aliquots of the same sample in duplicate within the same plate), an intra-assay (interplate) CV, and an interassay CV. Both inter- and intra-assay CVs were calculated by the methods of Rodbard (18).

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by each EIA was assessed by high-pressure liquid co-
chomatography (inhouse method) of urine samples
from women (n = 8) at four different phases of the
ovarian cycle. Trace amounts (2000 counts/min) of tri-
inated estrone sulfate (E1S) (specific activity 55 kCi/mol;
New England Nuclear, Boston, MA) and tritiated PdG
(specific activity 44 Ci/mol; Courtauld Institute, Middle-
sex, U.K.) were added to 500 μL of urine to monitor the
elution profile of each specific steroid conjugate. Each
supplemented urine sample was then combined with an
equal volume of ethanol/methanol (50:50, by vol), vortex-
mixed, and cooled to −5°C for 4 h. We then cen-
trifuged the cold solution, decanted the supernatant liquid,
and reduced its volume to 0.1 mL under a stream of
nitrogen. Each sample was then separated on a C18
reversed-phase column (10 μm, Ultrasphere; Altex Corp.,
Oakland, CA) with a gradient of methanol/water
(increasing methanol content from 100 mL/L to all
methanol) at a flow rate of 1 mL/min for 60 min. Forty
1-mL eluates were collected, with one-half of each sam-
ple being used for scintillation counting to monitor the
recovery of the radiolabel. The remainder of each frac-
tion was evaporated under reduced pressure, and the
residue was reconstituted in distilled water for EIA
analysis.

Results

Assay Characterization

The initial ratio of 20:1 (steroid conjugate:HRP) in the
conjugation reaction mixture gave an enzyme label with a
low molar incorporation ratio, i.e., ~1.5:1 for both
E1Conj and PdG labels, as determined by EIA and RIA.
Up to 80% of the enzyme activity in the conjugate
preparations could be bound by excess antiserum.

The polyclonal antiserum (R522) had similar specific-
ities as determined by EIA and RIA, when 1H[E1S] was
used as tracer in the RIA, E1G-HRP as tracer in the
EIA, and E1G as the standard in both EIA and RIA
(results summarized in Table 1). The main cross-reac-
tants with the E1G antibody (R522) were free estrone
(E1), E1G, and E1S. Monoclonal antibody (4F11) was
specific for E1G (100%), in that all other cross-reactivi-
ties, including those with E1 and E1S, were <0.1% (data
not shown).

Range and sensitivity of the standard curves for the
E1Conj EIA were the same for polyclonal antiserum
R522 and monoclonal antibody 4F11 when E1G was
used as standard. The range was 0.5–250.0 pg/well [50
μL of standard solutions, 0.01–5.0 μg/L (0.02–10.6 nmol/
L)]. The sensitivity of the EIAs for E1Conj was <1.0
pg/well (<10.6 nmol/L of urine) for both systems, with a
50% displacement point of the standard curve at 10–11
pg/well.

Precision of the polyclonal (R522) EIA for E1Conj was
assessed in selected, pooled urine samples containing
high, medium, or low concentrations of E1Conj. The
intraplate CV was 1.6%. The average intra-assay CVs
(intersplate CV) for the pools of high-, medium-, and
low-concentration urine samples were 4.9%, 6.6%, and

<table>
<thead>
<tr>
<th>Steroid</th>
<th>EIA % cross-reaction*</th>
<th>RIA % cross-reaction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone-3-glucuronide</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Estrone-3-sulfate</td>
<td>66.6</td>
<td>64.4</td>
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<td>Estrone</td>
<td>238.0</td>
<td>341.0</td>
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<tr>
<td>Estradiol-17β</td>
<td>7.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Estradiol-3-glucuronide</td>
<td>3.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Estradiol-3-sulfate</td>
<td>3.3</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Estradiol-17-sulfate</td>
<td>0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Estradiol-3-dihydrotestosterone</td>
<td>0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Ethyl estradiol-17β, estrogen, progesterone, pregnandiol, cortisol, estradiol-17β, estrone, testosterone</td>
<td>All &lt;0.1 by both assays</td>
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</tr>
</tbody>
</table>

*50% inhibition point of respective dose–response curves expressed as (picograms of estrone-3-glucuronide/picograms of steroid or steroid metabolite) × 100%.

11.2%, respectively. The interassay CVs for these same
pools were 8.5%, 10.1%, and 13.9%.

Serial dilutions of selected urine samples produced
displacement curves parallel to that of the E1G standard
curve in the polyclonal (R522) EIA. Co-chromatography
of urine samples separated by HPLC revealed two peaks
in the E1Conj assay. The smaller (<10%) of the two
immuno-reactive peaks revealed by the E1Conj assay
was eluted with the radiolabeled E1S, and the larger peak
(>90%) was eluted immediately after, where E1G is
known to elute.

Specificity of the PdG antiserum (PdG = 100%) was
tested by EIA only, with the main cross-reaction being
20α-hydroxyprogesterone (60.7%). Cross-reactions of
the PdG antibody with other steroids and their related
metabolites are summarized in Table 2. The antiserum
used in the RIA of PdG has been described (4).

The range of the standard curve for the EIA of PdG
was 5–2500 pg/well [50 μL of 0.1–50.0 μg/L (0.2–100.7
nmol/L) standards]. Sensitivity of the assay, by the
same criteria as for the E1Conj EIA, was <10.0 pg/well
(<0.05 μmol/L of urine). The intraplate CV for the PdG

<table>
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<tr>
<th>Steroid</th>
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<tbody>
<tr>
<td>Pregnanediol-3-glucuronide</td>
<td>100.00</td>
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<tr>
<td>20α-Hydroxyprogesterone</td>
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<tr>
<td>Pregnanediol</td>
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<td>Progesterone, pregnancione, androsterose, estradiol-17β, estrogen, testosterone</td>
<td>All &lt;0.1</td>
</tr>
</tbody>
</table>

*50% inhibition point of respective dose–response curves expressed as (picograms of pregnanediol-3-glucuronide/picograms of steroid or steroid metabolite) × 100%.
EIA was 1.8% for 40 aliquots of the same sample measured in the same plate. The intra-assay (interplate) CVs for pools of high, medium, and low concentrations of PdG were 5.2%, 6.9%, and 11.0%, respectively. Interassay CVs in the same urine pools were 5.6%, 7.8%, and 13.6%.

Serial dilutions of selected urine samples were analyzed by EIA, and the dilution curves were found to parallel the PdG standard curve. Co-chromatography of urine containing tritiated PdG revealed a single immunoreactive peak concomitant with the tritiated PdG label.

Hormone Profiles

Daily serum LH and FSH concentrations, representing 10 ovarian cycles, are presented in Figure 1. We used the gonadotropin data to align the steroid data, with day 0 being the day of the gonadotropin surge (LH and FSH peak). The occurrence of ovulation in each menstrual cycle was confirmed by the measurement of serum P0, which exceeded 15 nmol/L in all cycles.

A comparison of the E1Conj profiles obtained with polyclonal antiserum R522 and monoclonal antiserum 4F11 in the 10 cycles is depicted in Figure 2. Use of the polyclonal antiserum, which cross-reacted mainly with E1 and E1S, gave a profile similar to that of the highly specific monoclonal antibody, although values were greater in the polyclonal system.

Figure 3 depicts daily mean concentrations of urinary E1Conj (nmol/mmol Cr) obtained by RIA and EIA when polyclonal antiserum (R522) was used to analyze the 10 cycles. The profiles were parallel, with a correlation coefficient of 0.94 (P < 0.01).

The composite profile of urinary E1Conj determined by EIA (see Figure 3) and the serum profile of E2 (pmol/L) determined by RIA for the same 10 cycles are shown in Figure 4. Although the composite peak values for both E1Conj and E2 occurred on the same day (day 0), four of 10 cycles had peak E2 values at day -1 (one day before the LH peak) and corresponding E1Conj peaks at day 0, and two of 10 cycles with E2 peaks at day 0 had E1Conj peaks at day +1. Serum E2 values showed a post-ovulatory nadir on day +2, and E1Conj values in urine showed a post-ovulatory nadir on day +4. Concentrations of both serum E2 and urine E1Conj increased during the luteal phase, remaining at ~50% of peak values until at least day +10 before decreasing to early follicular phase values. The mean profile of urinary E1Conj was parallel to the mean profile of serum E2 (r =

Fig. 1. Mean daily serum LH (Ⅲ) and FSH (Ⅱ) concentrations (int. units/L) for 10 menstrual cycles aligned to the day of the LH peak (day 0)

Data presented in Figs. 2–6 are from the same 10 cycles and are similarly aligned in terms of the LH peak. The bars indicate 1 SD

Fig. 2. Mean daily urinary estrone conjugate (E1Conj) concentrations (nmol/mmol Cr) for 10 menstrual cycles measured by a monoclonal and a polyclonal EIA system, with an estrone-3-glucuronide–horseradish peroxidase conjugate as label and estrone-3-glucuronide as standard (Ⅲ), results obtained by using polyclonal antibody R522; (○), results obtained by using monoclonal antibody 4F11

Fig. 3. Mean daily urinary estrone conjugate (E1Conj) concentrations (nmol/mmol Cr) for 10 menstrual cycles as determined by EIA (Ⅲ) and RIA (○)

Fig. 4. Mean daily urinary estrone conjugate concentrations (Ⅲ, nmol/mmol Cr) determined by EIA and serum estradiol concentrations (○, pmol/L) determined by RIA for paired urine and blood samples, respectively, for 10 menstrual cycles
Composite concentrations of urinary PdG (μmol/mmol Cr) obtained by EIA and RIA for the 10 cycles are shown in Figure 5. The curves are parallel, with a correlation coefficient of 0.94 (P < 0.01). PdG values by RIA were quantitatively greater than, although not significantly different (P > 0.05) from, those obtained by EIA.

Profiles of composite concentrations of serum P0 measured by RIA (nmol/L) and urinary PdG concentrations measured by EIA (μmol/mmol Cr) in the 10 cycles are depicted in Figure 6. Mean concentrations of serum P0 and urinary PdG increased by 2 SD above their baseline (day 0) concentrations on days 2 and +3, respectively [3.2 (SD 0.3) to 11.5 (SD 1.0) nmol/L vs 3.33 (SD 0.04) to 0.78 (SD 0.08) μmol/mmol Cr], indicating that the increase in PdG concentrations followed the increase in serum P0 concentrations by one day. Progesterone concentrations peaked between days +5 and +9, with values beginning to decrease at day +10. PdG concentrations tended to lag behind P0 concentrations by one to two days at the end of the luteal phase. Thus, the excretion of PdG in urine accurately reflects the serum P0 profile (r = 0.94, P < 0.01), but with a one- to two-day delay.

To establish the importance of Cr for indexing profiles of urinary steroid metabolites, we expressed the E1Conj concentrations in the 10 cycles in nmol/L of urine as well as in nmol/mmol of Cr (Figure 7). The composite profile of urinary E1Conj concentrations not indexed by Cr (nmol/L of urine) was similar to that in which concentrations were indexed by Cr (nmol/mmol of Cr), with peak values both occurring on day 0. However, when individual profiles were compared on the basis of creatinine indexing vs no indexing, the indexing resulted in individual urine patterns of E1Conj closer to the profile expected from E2 analysis in serum (Figure 8).

Discussion

Profiles of urinary E1Conj and PdG concentrations in this study were similar when determined by RIA or EIA with polyclonal antisera (Figures 3 and 5). Although we have not presented an exhaustive proof of specificity for either of the assays described here, the cross-reactions to known, structurally related compounds (Tables 1 and 2) and the parallelism of diluted urine samples to the pure standards provide evidence suggesting reasonable specificity. Co-chromatography by HPLC verified that the same compounds were detected by EIA as were previously detected by RIA (4). This co-chromatography further indicates that only compounds with identical retention times could contribute to the measurement of either E1Conj or PdG in diluted urine. Taken together, these data provide strong evidence that the assays are both quantitative and reasonably specific. Because there are no qualitative differences in the profiles obtained with the two assay systems, the choice of system depends primarily on which laboratory system is preferred. Although the data for urinary E1Conj obtained by EIA and RIA are in close agreement, the PdG data by EIA lagged behind the RIA data by one day during the developing luteal phase. One explanation for this difference could be that the same antibody was used in both E1Conj assays, whereas different antisera were used in the PdG assay systems.

The relationship between concentrations of serum E2 and urinary E1Conj is similar to that reported by Wright et al. (1) in humans and Monfort et al. (19) in
laboratory macaques. Comparison of the paired hormone values in the late follicular phase indicates that estrogen metabolites reach the urine 12–24 h after free estrogen appears in blood. The peak concentration of E1Conj in urine occurred approximately one day after the serum E2 peak in 60% of the cycles, with 40% of the cycles demonstrating concomitant peaks. Peak serum E2 values (927 (SD 50) and 963 (SD 108) nmol/L, on days −1 and 0, respectively) represented a 3.5-fold increase over early follicular phase concentrations. The urinary E1Conj peak on day 0 (37 (SD 3) nmol/mmol Cr) represented a 2.5-fold increase in concentration over the same time interval. This relationship supports the concept that estrogen production by the ovaries can be monitored accurately through evaluation of urine as well as blood samples. Increased estrogen excretion in the late follicular phase accurately reflects the pre-ovulatory estrogen secretion, showing an easily discernible increase on the same day or one day earlier than the day of the serum LH surge. Because increased estrogen excretion can be detected as early as three days before ovulation, this information may be useful in predicting ovulation.

The correspondence between concentrations of serum P0 and urinary PdG was also parallel. Urinary PdG concentrations increased six- to eightfold from the follicular phase of concentrations to the midluteal peak, compared with a 15- to 20-fold increase for serum P0 values. The excretion of PdG in urine tended to parallel the increasing serum P0 concentrations with a one-day lag; at the end of the luteal phase, decreasing serum P0 concentrations were reflected by urinary PdG about one to two days later. Nevertheless, the urinary PdG and blood P0 concentrations provide comparable information for confirming the occurrence of ovulation and the presence of a functional corpus luteum.

Although polyclonal antisera to steroids and steroid conjugates have been shown to be useful in the development of sensitive EIA systems (10, 11), the use of monoclonal antibodies has advantages in terms of specificity and long-term availability. Thus we compared assays involving monoclonal and polyclonal antibodies developed against E1G. In our hands, the E1Conj EIA system developed by using a monoclonal antibody was not as effective as the polyclonal system. Monoclonal antibody (4F11) bound considerably less enzyme conjugate than did polyclonal antiserum (R522), even when a greater concentration (800- vs 5000-fold dilution) was used for coating the wells. Although 4F11 was very specific for E1G, the relatively small change in absorbance associated with the standard displacement curve limited the accuracy of the assay. Another, less-specific monoclonal antibody assessed was found to bind enzyme conjugate effectively (20 000-fold coating dilution) and to produce good displacement curves. However, this antibody was relatively insensitive (50% displacement was 30.5 pg) compared with the other antibodies tested and it lacked specificity, producing values 10-fold greater than any values obtained with polyclonal systems. Moreover, the ovarian cycle profiles generated with this antibody showed little resemblance to the profiles obtained with measurements of circulating E2.

At least one report has suggested that changes in concentrations of steroid hormone metabolites in urine provide accurate physiological information without being indexed by Cr (20). The results from our study indicate that mean hormone profiles may be similar whether or not Cr is used to index individual hormone values. However, comparisons of blood and urine hormone profiles from individual subjects indicate that paired values of blood and urine may vary significantly if Cr indexing is omitted. The occurrence of false values at or near a critical point in an individual profile could change the interpretation of the entire profile, as illustrated in Figure 8. Thus, the present data indicate that the correspondence of the urinary profile to the serum profile may be significantly improved in some individual cases if Cr indexing is used.

Kits for detecting LH and human choriongonadotropin in urine are widely available as self-administered assays to detect the fertile period and early pregnancy, respectively. There is little doubt that this technology will be extended to include measurements of steroid hormone metabolites for assessing ovarian function. As a foundation for these future developments, our report provides a comparison of steroid metabolite profiles obtained by using both a radiometric assay format and an enzyme immunoassay format, as well as a comparison of these measurements with the steroid hormone profiles in blood. The data indicate that similar information regarding ovarian steroid production can be
obtained by the analysis of their concentrations in either daily blood or urine. The use of urine samples simplifies the collection technique and is equally applicable to cross-sectional or longitudinal studies of ovarian function. The EIA system simplifies the laboratory procedure because the assays are rapid, the counting of radioactivity is not required, and the need for expensive equipment is reduced. The increased flexibility and cost savings inherent in this approach will make these assays more widely available—initially to the clinical laboratory, and ultimately to the patient at home.

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References

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