curves of precipitation are shown in Figure 1.

Ampicillin, carbenicillin, amoxyccillin, procaine penicillin, cephalothin, salicylic acid, acetyl salicylic acid, and dexamethasone caused no interference. The presence of methicillin in urine may be differentiated from the turbidity attributable to protein by measuring the absorbance at different intervals during the reaction, such as 0.5 and 5.0 min. A decreasing absorbance with time indicates that the turbidity is not due to protein, which would show an increase in absorbance with time, reaching an absorbance plateau in approximately 2 to 4 min. Benzylpenicillin (1 g/L) and cloxacillin (2 g/L) may result in apparent protein concentrations of about 0.3 and 2 g/L, respectively, the curve characteristics being similar to that given by protein. If urine containing either benzylpenicillin or cloxacillin is diluted 10-fold, the curve characteristics remain unchanged, apart from lower absorbance values, while urine containing protein, similarly diluted, shows a characteristic decrease in the slope of the curve, but still reaches an absorbance plateau in 2 to 4 min.

Although these studies were done with a centrifugal analyzer, it clearly is also practicable to apply the principle of measuring absorbance at different time intervals to the manual method. In doing so, it may be possible to differentiate between turbidity owing to the presence of penicillins or other interfering drugs and turbidity owing to protein, by their different precipitation kinetics.

References

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Rapid Fluorometric Estriol Determination in Pregnancy Urine

To the Editor:

Of the several fluorometric methods for estimating total estrogens in pregnancy urine, some are fully mechanized, so that many samples can be processed per run.

However, estriol is generally considered a better index of fetal status, because total estrogens originate from both the mother's circulation and the placenta.

We report here a procedure for determining estriol specifically in less than 1 h, with good practicability and reliability.

Estriol is separated from the other principal estrogens by a simple differential extraction instead of the usual extraction of the entire estrogenic fraction, solvent evaporation, and partition between water and an organic phase (1, 2).

After acid hydrolysis of the sample at 120 °C, estrone and estradiol are extracted with a mixture of n-hexane/benzene. Estriol, subsequently extracted with diethyl ether, is estimated fluorometrically with the Kober reaction at 120 °C (3), according to the modification of Lever et al. (4).

Procedure: Add 1 mL of 2.5 mol/L HCl to a 50-μL sample of urine in a screw-capped 12-mL glass tube and heat the tube in a heating block at 120 °C for 15 min. Cool the tube in an ice bath for 5 min and add 1.5 mL of distilled water and 2.5 mL of n-hexane/benzene (equilume mixture). Vortex-mix for 30 s and aspirate the organic (upper) phase. Extract the aqueous phase twice with 4-mL portions of diethyl ether, for 30 s each time. Remove the water, transfer the extract into another tube, and evaporate it in a water bath set at 52 °C. To the residue, add 1 mL of Kober reagent (hydroquinone, 20 g/L, in H2SO4, 12 mol/L). Process standards (0.5 μg/tube) and blanks in the same way as samples. Cap the tubes, place them in a heating block at 120 °C, and mix their contents after 1 min. Continue this heating for exactly 12 min, then cool the tubes in an ice bath for 5 min, add 4 mL of fluorogenic reagent (trichloro- acetic acid and chloral hydrate, 250 g of each per liter), mix for 5 s, and cool again.

Measure the fluorescence of blanks, standards, and samples within 20 min of mixing with the fluorogenic reagent. We used 3-mL square polystyrene cells (1-cm lightpath) with a Model MPF-3 Fluorescence Spectrophotometer (Perkin Elmer Corp., Norwalk, CT) with the following settings: excitation 530 nm, emission 550 nm, band-pass slits 5 nm.

Fluorescence is linearly related to concentration over the range 0.25-50 mg/L. Sensitivity (the minimum concentration of estriol significantly differing from zero) was determined from the fluorescence measurable in urine from normal men (n = 25). The mean value was 69 μg/L (SD, 17); the least-detectable concentration was then 69 + (2 x 17) = 105 μg/L.

Recovery was tested on aliquots of a pooled specimen of urine from men, previously fortified with estriol-16-glucuronide to reach concentrations of 5, 15, and 30 mg/L (as estriol). Percentage recoveries (± SD) were, respectively, 82.1 ± 3.1%, 79.3 ± 3.2%, and 82.7 ± 2.9% (n = 15).

We evaluated interference with estriol determination caused by incomplete separation of estrone and estradiol by adding these two steroids (20 mg/L) to different aliquots of pooled specimen of urine from men (n = 12) to give concentrations of 10 mg of each per liter and processing the samples as usual. We found about 6% cross reactivity for each steroid (estrone 6.4 ± 0.3%; estradiol 5.6 ± 0.2%). Because these two compounds together constitute no more than 20% of

<table>
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<th>Table 1. Precision Evaluation</th>
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CLINICAL CHEMISTRY. Vol. 25. No. 9. 1979 1683
avoiding time-consuming extraction steps with organic solvents.

The Kober reaction product is stable for at least an hour at 0 °C before the fluorogenic reagent has been added, but, starting with this step, fluorescence remains constant for only about 20 min, then begins to decline, even at 0 °C but more rapidly at room temperature.

Our method appears to provide reliable responses in less than 1 h, and a well-trained technician can easily process 30 samples in duplicate in half a working day. Only easily available equipment and low-cost, non-hazardous reagents are involved.

References

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Urinary Total Estrogen Methodology for Pregnancy Urine

To the Editor:
We have several comments concerning the Letter appearing in Clinical Chemistry 24, 2217 (1978) by Yang et al., who compare two techniques for extraction of the Kober chromogen during determination of total pregnancy estrogens (TPE) in urine. In their preferred technique tetrachloroethane containing trichloroacetic acid (TCA) is used, whereas the Stanbio Laboratory (1) adaptation of the method of Rouke et al. (2) involves addition of aqueous TCA followed by extraction of the color into chloroform.

Although the Royal Columbian Hospital group's suggested solvent combination does, in our hands, increase absorbance values by 25–30%, the corrected absorbance gives the same final answer. Never in the many years of our experience with repeat assays has there been a difference approaching 70%, as suggested for the aqueous TCA–chloroform. In our combined experience of almost 50,000 assays we have recorded a between-assay coefficient of variation of 5.75% as determined by our on-going computer program. This agrees with our previously published data (3). Their grossly high difference must have involved such low urinary TPE concentrations that the slightest deviation in technique would result in a vast percent discrepancy. If in fact higher estrogen concentrations were found also to differ by as much as 70% between runs, this would further suggest erratic technique or technical inexperience with the method.

Because the clinical value of urinary TPE assays depends on the trend of a series of results, it is of utmost importance that consistency of technique be maintained. We believe that this may be best accomplished by using the results of a series of urinary estrogen (E) and creatinine (C) determinations, each performed on single voided specimens, and reporting the E/C ratios. This precludes the inconvenience of 24-h urine collection and gives information more promptly to the requesting physician (4).

Yang et al. acknowledge tetrachloroethane to be more toxic than chloroform, but they imply that because of vapor-pressure differences the exposure to hazardous concentrations of the former is less. In all fairness to laboratories that may be influenced to use tetrachloroethane, we believe that reference to three toxicity statements must be made:

a. The Merck Index (5)—tetrachloroethane "is considered to be the most toxic of the common chlorinated hydrocarbons."

b. Manufacturing Chemists Assn. (MCA) warning label (6)—tetrachloroethane is "highly toxic by ingestion, inhalation and skin absorption; tolerance, 5 ppm in air."

c. NFPA Instant Warning System for Dangerous Materials (7,8)—tetrachloroethane: 4.0-0.0 (Health-Flammability-Reactivity; 4: can cause death or major injury despite medical treatment; 0: will not burn; 0: normally stable, not reactive with water).

In light of the above considerations, we think that with proper attention to technique and laboratory safety, urinary total pregnancy estrogens are most effectively determined by the method of Rouke et al. (2) or the Stanbio Laboratory adaptation (1).

References
1. Stanbio TPE Total Pregnancy Estrogen