We have adapted the bile acids kits marketed by Merck Diagnostics to measure total serum bile acids in the "random-access" analyzer RA 1000 (Technicon Instruments Corp., Tarrytown, NY). In this kit method, 3α-hydroxy bile acids are specifically converted to the corresponding 3-keto derivatives in the presence of NAD⁺ and with the aid of 3-α-hydroxysteroid dehydrogenase (EC 1.1.1.50). The resulting NADH reacts with nitroblue tetrazolium salt under the catalytic influence of diaphorase (EC 1.8.1.4) to give a blue formazan derivative, which is measured spectrophotometrically at 550 nm (2).

The reagents were used according to the manufacturer's instructions (3). A sample reaction solution and blank reaction solution (10 mL of each) were mixed with two drops of wetting agent, Triton X-450 (Technicon). These reagents are stable for five days at 2 to 8 °C and for 2 h in the reagent tray. The "stop reagent" was not used. The standards—equimolar amounts of sodium glycocholate, sodium glycodeoxycholate, and sodium taurocholate in a bovine serum matrix—were compared to the Merck Diagnostics. Settings for the RA 1000 system were as follows:

- Chem no. *
- Lock enter
- Name *
- Immunoassay 0
- Type 2
- Inverse 0
- %Smp vol. 50
- Filter P 5(WL 550)
- Bic chem 0
- Delay 9 30
- Default blank 1
- %Rgt vol 70
- 2nd Reagent 0
- Unit 5 µmol/L
- Unit fac 1
- Decimal 1
- RBL low 0
- RBL hi 0.080
- Range lo 0
- Range hi 160
- Cal fact *
- Std val 100
- Normal L 0
- Normal H 8.0
- Slope 1
- Intercept 0
- EP lim 0.005
- Auto lin 0

* = User-determined. Temp = 37 °C

For 15 consecutive analyses the run-to-run precision was estimated with serum quality-control material (Pathonorm L and H; Nyegaard & Co., Oslo, Norway). Values for the means (µmol/L), SDS, and CVs(%) were 31.06, 1.21, 3.91 and 77.87; 1.50, 1.93. For within-run precision, estimated with the same control material, the corresponding values were 31.06, 1.06, 3.40 and 78.89, 1.09, 1.38 (n = 20). Mean analytical recovery of the bile-acids standards added to the serum was 97% (SD 4.1%) for the three different species.

We conclude that our adaptation of the kit to use with the RA 1000 increased both precision and speed, giving results in excellent agreement with the standard procedure.

References
3. Manufacturer's insert for "Bile Acids" kit, Merck Diagnostics, Darmstadt, F.R.G.

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Diethylstilbestrol Inhibits the Estrogen-Binding Activity of Pregnancy Plasma; Possible Role in DES-Associated Pathology

To the Editor:

Administration of the synthetic estrogen diethylstilbestrol (DES) to pregnant women has been associated with development in daughters of clear-cell adenocarcinoma of the vagina and cervix (1). One factor responsible may be the lack of high-affinity interactions between the drug and the plasma proteins of mother and of fetus. Indeed, it was shown (2) that DES binds only weakly to the high-affinity protein carrier of the sex hormones, i.e., the steroid binding globulin (SBG, or tes-tosterone–estradiol binding globulin), which is considerably increased in the maternal blood during pregnancy (3).

On the other hand, DES interacts poorly with the plasma proteins of the human fetus, including alpha-fetoprotein (4). Therefore its maternal–fetal transfer and its subsequent uptake by fetal targets would meet with little opposition, the excessive impregnation of developing estrogen-sensitive tissues by the drug possibly resulting in damage later.

We wish to report an observation that indicates that DES may exert additional undesirable effects by thor-

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Serum Bile Acids Determined with an RA 1000 Analyzer

To the Editor:

Assay of bile acids in serum is used increasingly to differentiate liver diseases, especially to detect impaired hepatocellular function and hence damage to the liver (1).
oughy disturbing the equilibrium between protein-bound and free circulating estrogens in the mother. We find that, although itself a poor ligand for SBG, the nonsteroidal estrogen can, in pharmacological concentrations, almost completely inhibit the high-affinity interaction between estradiol and the carrier protein in plasma.

Our results are illustrated in Figure 1. We used the batchwise gel equilibration method of Pearman and Crépy (3) to measure the binding capacity of normal and pregnancy plasma for [1H]estradiol or [3H]testosterone, in the presence of increasing amounts of unlabelled DES. The concentrated solutions of DES used in these experiments (up to 10 μg per milliliter of buffer) could be effected without the help of any organic solvent by thorough vortex-mixing and subsequent vigorous shaking for 2 h at room temperature.

The drug evidently has a dramatic dose-dependent inhibitory effect on the binding of [1H]estrogen in plasma collected during the third trimester of pregnancy, plasma that is rich in SBG (curve 1). The inhibition is also seen at the lower estradiol binding of plasma from the non-pregnant woman (curve 2). On the other hand, binding of testosterone is unaffected by the tested amounts of DES, probably because the male hormone has greater affinity than does estradiol for the SBG binding sites (curve 3).

Remarkably, the inhibitory concentrations of DES fall within the range of doses reportedly administered to mothers (5).

Taken together, the former studies and the present observations suggest that when the mother is given DES the fetus becomes doubly exposed—to large amounts of easily transferred drug and to a non-regulated excess of the maternal estrogens.

Our findings might even have a wider significance. They indicate a need for reconsideration of the accepted notion that synthetic hormone analogs, while competing with natural hormones for binding to intracellular receptors, will not interfere with their binding to the specific carrier proteins in the blood (6). The competition experiments on which this notion is founded have generally involved nanomolar concentrations of steroids and analogs, i.e., much less than the usual pharmacological doses. The present observations, based on the more current micromolar therapeutic dosage, suggest that, on the contrary, hormone-like drugs can disturb normal free and bound proportions of the blood-borne hormones, and thus alter their availability to tissues and their biological impact. Possible effects of such upsetting of equilibria during therapies with hormone analogs seem worth evaluating.

References


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Self-Testing: An Emerging Component of Clinical Chemistry

To the Editor:

We were encouraged by the comments of Free and Free (Clin Chem 30:829–838, 1984) in regard to self-testing, given the considerable developmental potential for it. Of course, some informal categorization of illness has always occurred at home (what else would we lead to consultation?) so that self-testing is building on an already-established habit. One group of patients with particular experience of self-testing are those with renal disease, whether with chronic renal failure or on home hemodialysis/continuous ambulatory peritoneal dialysis (CAPD). There is an unappreciated scope for self-testing in those patients with glomerulonephritis, particularly in detecting early relapse by the appearance of hematuria, and in the monitoring of progress after renal transplantation. We cannot overemphasize that medical crises are often only a matter of the delayed recognition of progressive subclinical disturbances, which may be revealed by self-testing.

This monitoring need not be direct. We have studied 87 renal transplant patients over four years, measuring "tubular" proteinuria by various methods, including liquid chromatography. When the serum creatinine reaches or exceeds 200 μmol/L (about 20 mg/L) a tubular pattern of urinary proteins is invariable, even over several years. Of course, tubular protein excretion may increase for numerous reasons, e.g., rejection, at lower concentrations of serum creatinine.) A simple self-testing for urinary tubular proteins would detect at least any deterioration of graft function that took the serum creatinine to above 200 μmol/L, acting as an indicator of overall renal function. Such a test would provide an invaluable support for episodic hospital reviews, particularly where home-to-hospital distances were great. We are currently exploring this approach with both retinol-binding protein and α1-microglobulin as potential marker proteins.

Discovering an aberration in the course of self-testing is only part of the process of monitoring disease, however. The confidence for self-referral is an important factor. Generally the patient lacks the experience of cause and effect that trained staff have, and may fail to respond promptly to abnormal values. This has been a repeated problem in CAPD peritonitis, where, despite careful and prolonged training, patients often delay in reporting a simple "cloudiness" of the peritoneal effluent (one index of peritoneal inflammation). Without evidence, we still speculate that the more direct the consequence of the results, the more quickly will the monitoring "loop" be closed: merely understanding the basis of the test may not be enough.

The extent to which self-testing can reinforce desirable habits in diet and