those studies the women had never taken oral contraceptives. Such difference might affect cholesterol metabolism in women.

In conclusion, we find that bile cholesterol correlates significantly and positively with serum cholesterol in patients with mixed stones and not in those with pigment stones. The role of bile triglycerides in the formation of gallstones should be studied further.

References

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DuPont acc Overestimates High Concentrations of Total Protein

To the Editor:

Different results were observed for total protein in a patient’s sample: 49 g/L with the Baxter Paramax and 57 g/L with the DuPont acc III. Both are biuret-reaction methods, and both calibrations were checked before repeat analyses. In the acc, bovine-serum-based calibrators and <4 min of incubation are used; in the Paramax, human-serum-based calibrators and a 10-min incubation are used.

We noted that the range of specimens used to verify correlation with our acc when the Paramax was recently introduced into our laboratory was 55 to 75 g/L. We made further comparisons with the acc vs the longer-used Boehringer-Mannheim (BMC) biuret reagent, which we used according to the manufacturer’s specifications in a Hitachi 705 analyzer, with human-serum-based standards and a 10-min incubation.

Several samples were analyzed for total protein in both the acc and the Hitachi 705, then analyzed by the Wisconsin Laboratory of Hygiene by the Reference Method (1), including six freshly collected human sera with total-protein concentrations ranging from 31 to 90 g/L, three solutions prepared from lyophilized protein fractions (Sigma Chemical Co, St. Louis MO 63178), human albumin (Hyland, Glendale, CA 91202), and Standard Reference Material No. 927a (National Institute of Standards and Technology, Gaithersburg, MD). Twelve additional human serum samples were analyzed by both the acc and BMC methods, to expand the range of correlation to 31 to 122 g/L (BMC).

The reference-method and BMC results agreed for all samples over the range examined. The reference acc results agreed for normal human serum and albumin samples as well as the bovine-based SRM, but disagreed for fresh serum samples with high total protein and for reconstituted human gamma globulin. The difference between acc and BMC total protein values is quite marked at high concentrations (Figure 1). A repeat correlation check between the acc and Paramax with an entirely different cohort of patients’ samples indicated similar differences at high values.

The acc overestimates total protein at high total protein concentrations.

Users of systems with non-human-based total protein calibrators and (or) short incubation times should be doubly cautious; different results from different analytical systems will confuse clinicians.

In today’s laboratory climate, evaluations frequently take the form “run 20 samples and see what it looks like.” Rather, they should have one cohort of fewer samples distributed evenly over the analytical range, for correlation, and a second near the decision-making concentrations, to evaluate testing efficiency. The acc total-protein method has survived many evaluations and been in the field for more than a decade; the differences observed here have apparently gone unnoticed. High results for total protein generated by the acc should be checked by an alternative (long incubation) method.

We thank Rita Smythe for the original observation, Jerry Fattore (Northpoint Medical Laboratory, Milwaukee) for fresh serum samples with a wide range of total protein values and BMC analyses, and David Hassenmer (Wisconsin Laboratory of Hygiene) for reference values and samples of SRM 927a.


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Digoxin-like Immunoreactive Substances in Kidney- and Liver-Transplant Patients

To the Editor:

Serum contains several digoxin-like immunoreactive substances (DLIS) that cross-react with antibodies to digoxin and also inhibit Na+ K+ -transporting ATPase (EC 3.6.1.37) activity in vitro. Increased concentrations of DLIS are encountered in patients with essential hypertension, hypertension of volume expansion, renal disease, liver disease, and preeclampsia (1, 2). DLIS have also been considered to have a potential role as a natriuretic hormone (3). Recently we reported the presence of DLIS in liver post-transplant patients with a positive fluid balance (4). We now report on the relative frequency of detectable DLIS in
kidney- and liver-transplant recipients, both as inpatients and outpatients.

Sera from digoxin-free transplant patients and healthy volunteers, as well as protein-free filtrates from these groups prepared by ultrafiltration (Centrifree Micropartition System; Amicon, Danvers, MA), were analyzed for DLIS with a fluorescence polarization immunoassay detection limit, 0.26 nmol/L (Digoxin II; Abbott Laboratories, North Chicago, IL).

We examined the potential cross-reactivity of putative DLIS materials with the digoxin antibody in this assay. Substances investigated (6) included nonesterified fatty acids (palmitic, palmitoleic, stearic, linoleic, linolenic, oleic, and arachidonic), cortisol, and lysophosphatidylcholine (Sigma Chemical Co., St. Louis, MO). The compounds, at their maximum physiological concentrations (6), were added to DLIS-negative serum.

As Table 1 shows, the DLIS positivity rate in healthy volunteers was significantly lower than in either the liver- or kidney-transplant inpatient groups and significantly lower in liver-transplant outpatients than in liver-transplant inpatients. The DLIS positivity rates in healthy volunteers and either outpatient group were not statistically different. Moreover, no differences were seen in the rates between liver- and kidney-transplant inpatients or liver- and kidney-transplant outpatients. Although no conclusive—i.e., statistically significant—difference in DLIS positivity rates was demonstrated between inpatient and outpatient kidney-transplant recipients at the 95% confidence level (P was 0.059), the results strongly suggest that detectable DLIS is found more frequently among the inpatient than the outpatient groups. The statistical significance of kidney-transplant inpatient and outpatient DLIS positivity rates will be re-examined with an increased number of kidney-transplant recipients.

None of the proposed DLIS candidates that we tested exhibited cross-reactivity in the assay. Thus, these compounds do not appear to be responsible for the DLIS activity detected in this study. The protein-free filtrates of serum from healthy volunteers as well as those from the two transplant groups were free of DLIS activity, reflecting the strongly protein-bound nature of this immunoreactive material.

These results demonstrate an increased rate of DLIS positivity in kidney- and liver-transplant inpatients vs the rate in comparable outpatient populations or healthy volunteers, as measured by our assay. Further, they suggest a possible role for this type of analysis in monitoring recovery from these transplant procedures or in correcting the underlying clinical conditions for which organ transplant was originally performed.

References

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Effect of Sex-Hormone-Binding Globulin on No-Extraction Immunoassays for Testosterone

To the Editor:

The report of Masters and Hähnel (1) concerning errors in the radioimmunometry of testosterone (T) in serum interested us. We also have investigated the influence of sex-hormone-binding globulin (SHBG) on the measurement of T in the immunchem assay (2).

We proved this effect by use of a slightly different method: Two specimens of pooled sera from women with low endogenous concentrations of T (<0.7 nmol/L) and SHBG concentrations of 253 and 10 nmol/L were used for analytical-recovery studies. A T stock solution with [1α, 2α(m)-5HT as tracer was added to both sera, giving a final total T concentration of 87 nmol/L. Then serial dilutions were performed with the same pooled sera. Beta-counting of the tritiated tracer confirmed the uniformity of the T content in the different samples. Analytical recovery of the added T was then measured by use of the Immunchem no-extraction RIA assay and an extraction RIA kit supplied by Baxter Dade.

Figure 1 (top) illustrates the influence of SHBG on the determination of T in the no-extraction assay (assay no. 1). The quantity of T measured in the serum with high SHBG content is diminished by >50% in comparison with the serum with low SHBG content. This interference can be seen throughout the whole range of T concentrations. In contrast to this, the SHBG effect cannot be detected in the extraction-assay (Figure 1, bottom). The di-