used excitation at 298 nm, emission at 325 nm, and 60 pg of retinol (excitation at 340 nm, emission at 460 nm) injected.

There was a linear relationship between peak area and amount injected for each compound ranging from 0.1 to 2.0 μg.

The elution profiles of retinol and tocopherols in a human plasma sample are shown in Figure 1 (right).

Mean (SE) extraction recovery was 102% (3.5%) for retinol, 95% (1.9%) for α-tocopherol, 103% (0.6%) for β-tocopherol, 100% (9.3%) for γ-tocopherol, and 96% (3.3%) for δ-tocopherol.

Retinyl acetate has been used as an internal standard in some reports (6, 8). By our method, however, retinyl acetate in human plasma samples could not be eluted as a separate peak.

Clinically, simultaneous measurement of vitamin A and vitamin E is useful and important not only to clarify the relationship between vitamin A and vitamin E but also to evaluate the effect of supplementation therapy. On the other hand, it is still unclear whether the effects of each tocopherol homolog on retinol metabolism are the same or not. Our improved method, which simultaneously separates retinol and all four tocopherol homologs within an hour, should be useful to clarify the relationship between retinol and each tocopherol homolog.

References

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Mercurochrome interference with the Abbott TDx

To the Editor:
The interference of mercurochrome (merbromin) with the Abbott TDx fluorescence polarization assay (Abbott Labs., Abbott Park, IL) described by Berne et al. (1) appears to be a rare but significant effect. The authors attribute this interference to the mercury present in the molecule. I believe that the culprit has been misidentified. The mercury atom in merbromin is bound to a dye, 2',7'-dibromofluorescein, that is structurally similar to the TDx tracer dye, fluorescein. Indeed, the Merck Index (2) states, "Very dilute solns (1:2000) of merbromin possess a yellow-green fluorescence," much like fluorescein.

I compared the fluorescence of merbromin with that of fluorescein to show the similarity in spectral pattern. A commercially obtained aqueous 20 g/L merbromin solution and an optical 20 g/L fluorescein solution from our hospital pharmacy were both diluted in saline to equimolar concentrations. I used a Perkin-Elmer (Norwalk, CT) LS-3 fluorescence spectrometer at an excitation wavelength of 485 nm and scanned the emission spectrum from 525 to 650 nm, corresponding to the respective wavelength lengths used by the Abbott TDx. At equimolar concentrations, the fluorescence of merbromin averaged 6% of that of fluorescein, with a minimum of 4.8% at 525 nm and a maximum of 6.3% at 540 nm. Fluorescein emission was highest at 525 nm, whereas the merbromin peak was shifted slightly towards longer wavelengths; otherwise, their emission patterns were similar.

I evaluated interference due to merbromin in the TDx gentamicin assay by comparing the background interference fluorescence reported by the analyzer with the measured fluorescence relative to the tracer; I found much lower background interference than that reported by Berne et al. I prepared an initial 53-fold dilution of the commercial merbromin solution in TDx buffer, giving a concentration of 375 mg/L merbromin or 100 mg/L elemental mercury (assuming the label-stated concentration). I further diluted this in TDx buffer 1000-fold and 10-fold (to 100 and 10 000 μg/L mercury) and ran these as patients' samples on the TDx, using saline as reagent. I compared the fluorescence in the completed reaction mixture, due solely to merbromin, with that of the fluorescein tag in the reaction mixture from a completed run in which I used saline as the patient's sample and TDx gentamicin reagent.

The fluorometric measurements were taken as above except that the emission was measured only at 535 nm. The saline blank and 100 μg/L mercury dilution were taken from the above run. The 10 000 μg/L solution was repeated after being prepared in patients' serum and run with gentamicin reagent to obtain a net intensity increase; otherwise, the analyzer suppressed all data for the sample. The background intensity reading, taken before the addition of the tracer, was due to merbromin plus any endogenous interferents in the sample. Table 1 compares these measurements with the background fluorescence intensity reported by the TDx at the same dilutions; the background intensities are less than would be expected from the results of Berne et al. but seem reason-

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**Table 1. Fluorescence of Merbromin as Detected by the TDx and Measured Fluorometrically Relative to TDx Tracer**

<table>
<thead>
<tr>
<th>Conc. of merbromin-bound Hg, μg/L</th>
<th>TDx background intensity, arb. units</th>
<th>Fluorescein at 535 nm relative to that of tracer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (saline blank)</td>
<td>79</td>
<td>nd</td>
</tr>
<tr>
<td>100</td>
<td>177</td>
<td>0.041</td>
</tr>
<tr>
<td>10 000</td>
<td>17 300</td>
<td>2.5</td>
</tr>
</tbody>
</table>

nd: not determined.
able when compared with the measured fluorescence due to merbromin relative to tracer.

I also directly determined the effect of inorganic mercury on the TDx gentamicin assay. Mercury atomic absorption standard (Mallinckrodt, St. Louis, MO; 1000 μg/L mercury as mercuric oxide dissolved in dilute HCl) was added to saline and to two patients' sample pools containing gentamicin to give a final concentration of mercury of 100 μg/L in each (100-fold greater than the concentration used by Berne et al.). The saline blank reported an unmeasurably low gentamicin result; the mercury-supplemented serum pools gave gentamicin concentrations of 1.9 and 6.9 mg/L (expected values 1.9 and 6.8 mg/L, respectively), showing no effect on the assay. Because of limited sample volume, the mercury concentration could be verified only in the serum pool with the lower gentamicin concentration and measured 102 μg/L by acid digestion and cold-vapor atomic absorption.

It is important to realize that the interference reported by Berne et al. is due to the fluorescence of merbromin and that serum mercury, per se, has no effect on the TDx assays for gentamicin and vancomycin up to a concentration of 100 μg/L. The effect of oral and intravenous fluorescein on fluorescence polarization assays has been well documented (3–6), because this drug is used for angiographic studies assessing optic microvascular disease, vascular viability of intestinal tissue and skin flaps, and catheter placement for localized antineoplastic drug therapy (7). However, several fluorescein derivatives have also been approved for use in cosmetics and externally applied drugs, including merbromin and fluorescein itself (2). Under unusual circumstances, these may affect fluorescence-based assays if high concentrations of the compounds are absorbed.

References

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Cholesterol Measurements in Patients’ Sera Stored at 4 or −20 °C for 24 h before Analysis with a Kodak Ektachem 700 Analyzer

To the Editor:

We report that the refrigerated storage of serum affects the subsequent measurement of cholesterol with the Kodak Ektachem 700XR Analyzer. Miller et al. (1) reported the effect of freezing on cholesterol in sera destined for accuracy transfer between analytical methods, but their experimental design could not discriminate changes of <5% to 7%. Our study involved the analyses of 41 routine patients’ samples and was carried out over 3 consecutive days. Eleven months later, the same experiment was repeated with a series of 35 routine patients’ samples. Each sample was analyzed as a freshly separated serum from a Becton Dickinson (Rutherford, NJ) SST tube. Duplicate 0.5-mL samples of each of these sera were then placed into capped 0.5-mL Kodak analyzer cups. One of each pair was stored in a refrigerator at 4 °C, the other in a −20 °C freezer. Exactly 24 h later these samples were warmed to room temperature, mixed, and then reanalyzed for cholesterol.

The paired differences data from both studies have been summarized in Figure 1. Figure 1 (top) illustrates our subjective impression that storage of the samples was associated with consistent increases in the measured value of cholesterol. This impression has been supported by statistical analysis:

Fig. 1. Distribution of the mean differences in cholesterol concentration between stored and fresh serum samples in two experiments (top and bottom panels)