Letters to the Editor should be typed doubled-spaced (including references) with conventional margins. The overall length is limited to five manuscript pages, including not more than one figure or one table.

Interference of Fluorescein, Used in Retinal Angiography, with Certain Clinical Laboratory Tests

To the Editor:

We would like to report the interference of fluorescein in serum, after retinal angiography, with certain laboratory methods used in the Abbott TDx, the Kodak Ektachem 700, and the Beckman Astra. During a recent evaluation of the Abbott TDx fluorescence polarization immunoassay for cortisol, we observed high background interference from a serum sample, even after a fourfold dilution. The sample had been chosen to represent a severely icteric specimen, owing to its greenish coloration. The total bilirubin concentration was subsequently determined to be within normal limits, 5 mg/L.

Suspecting a possible drug interference, the patient's medical chart was consulted, but none of the 16 medications listed would have been expected to have caused the interference. The clinical diagnosis for this patient was Type I diabetes, with renal insufficiency, deteriorated vision, and hypertension. Further investigation revealed that the patient had undergone a fluorescein angiogram in the ophthalmology clinic approximately 2 to 3 h before having blood drawn for cortisol and renal-panel determinations. The concentration of fluorescein in the patient's serum was subsequently determined by fluorometer calibration of a "spiked" blank serum pool to be about 15 mg/L (ex. 485, em 540 nm).

Because in many Abbott TDx methods, including that for cortisol, fluorescein is used as the fluorophore, we evaluated the relative occurrence of this type of specimen and the potential effect of this examination on fluorescence-based procedures and other methods used in the laboratory. Approximately 25 fluorescein retinopathy examinations are performed at Stanford University Hospital each week. Angiograms are given primarily to diabetic patients who have experienced retinal deterioration (1). An intravenous bolus of 500 mg of fluorescein is given within a few seconds, and photographs of the retinal area indicate regional blood flow and blood-ocular permeability (1–4). The examination is often repeated within two weeks, after laser treatment of the eye.

Elimination of this amount of fluorescein occurs mainly through the kidneys during some 36 to 48 h for persons with normal renal function, and the kinetics have been well defined (5–8). However, it is more slowly eliminated by patients with renal insufficiency, including many diabetics, and significant concentrations of fluorescein may remain in the blood and urine of these patients for several days. Because many of our referred outpatients have laboratory work done elsewhere, the problem of notifying clinicians and lab personnel of potential interferences is compounded.

Renes et al. (9) and McClellan et al. (10) referred to these problems in 1982 for fluorescein venography in plastic surgery, ophthalmology, cardiac service, and burn units. These investigators reported fluorescein interference over the range of 2.5 to 250 mg/L in the DuPont acc assays for magnesium, total protein, phosphorus, chloride, and neonatal bilirubin. The dye was also reported to interfere with the Beckman electrophoretic CK-MB isoenzyme method (10, 11). We found that fluorescein travels in the same position as the CK-1 band in our Corning substrate-overlay electrophoresis assay, but under an ultraviolet lamp the greenish band is easily identified visually (vs the bluish NADH fluorescence) as an interference. At 200 mg/L, fluorescein represented about 10% of the total normal CK fluorescence intensity by this method. No apparent interference was observed in the Corning electrophoretic assay for lactate dehydrogenase isoenzymes.

We evaluated cortisol and digoxin methods on the Abbott TDx for the effect of fluorescein. Complete background interferences (no results) were observed when serum controls and pools were supplemented to give a fluorescein concentration of only 1.0 mg/L. Controls and samples run after spiked samples at this concentration also showed considerable carryover effects in the TDx. For example, the blank intensity of samples and controls increased from 350 to 650 (cortisol) and from 650 to 2000 (digoxin) units when run subsequent to samples spiked at 1.0 mg of fluorescein per liter. Blank intensity interference from carryover was even more pronounced following samples containing higher concentrations of fluorescein.

Digoxin controls run on the TDx directly after samples containing 1.0 mg of fluorescein per liter were flagged "HI" and gave no result. Samples and controls immediately following a sample containing fluorescein gave high cortisol results. TDx reagents also became contaminated from the dye, due to probe-tip carryover, at fluorescein concentrations up to 200 mg/L. Other TDx methods in which fluorescein is used as the fluorophore are also expected to have these problems.

Because fluorescein might be expected to interfere by absorption, emission, or quenching in reflectance absorption methods, we also evaluated the effect of fluorescein on 28 methods that we currently use in the Kodak Ektachem. Fluorescein in the expected (7, 9) post-infusion concentration range of 1.0 to 100 mg/L interfered with serum amylase, unconjugated and conjugated bilirubins, and, therefore, the calculated results for direct and neonatal bilirubins (see Table 1). The effect on results for total bilirubin was less pronounced. Other Ektachem tests monitored at 540 nm—including glucose, cholesterol, triglycerides, and total protein—showed no significant interference at 200 mg of fluorescein per liter. However, minor increases in aspartate aminotransferase and alkaline phosphatase results were noted.

Nine methods were evaluated for interference in the Beckman Astra. Total bilirubin at either 20 or 140 mg/L was decreased by about 5 mg/L with increasing concentrations (up to 100 mg/L) of fluorescein, but this decrease.
Table 1. Effect of Fluorescein on Analyte Concentrations in Serum, as Measured in the Kodak Ektachem 700

<table>
<thead>
<tr>
<th>Fluorescein concn, mg/L</th>
<th>Serum amylase, U/L</th>
<th>Total bilirubin mg/L</th>
<th>Unconjugated bilirubin mg/L</th>
<th>Conjugated bilirubin mg/L</th>
<th>Direct* bilirubin mg/L</th>
<th>Neonatal* bilirubin mg/L</th>
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</thead>
<tbody>
<tr>
<td>Spiked serum:</td>
<td></td>
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<tr>
<td>0.0</td>
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<td>8</td>
<td>4</td>
<td>0*</td>
<td>4</td>
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<td>200</td>
<td>105</td>
<td>7</td>
<td>NR*</td>
<td>&lt;-5*</td>
<td>NR'</td>
<td>NR'</td>
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<td>Spiked Kodak controls:</td>
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<td>II</td>
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</table>

Values are means of duplicate results. NR means "no result." The lower limit for reporting bilirubin results in this laboratory is 2 mg/L. *Direct = total - unconjugated. **Neonatal = unconjugated + conjugated. *Outside analyzer range. Derived from flagged result. Below analyzer range. Unable to compute result.

is usually not clinically significant. The other methods were unaffected at 200 mg of fluorescein per liter.

An abnormal urine control spiked to give a fluorescein concentration of 250 mg/L was briefly evaluated for interference with urine-analyte reflectance methods in the Ektachem and the Ames Clinitek 200. The Clinitek 200 reads bichromatically at 510 to 690 nm, depending on the analyte. We saw no interference with urine amylase in the Ektachem and no false positives on the Multistix urinalysis strips.

In our opinion, the clinical laboratory has a responsibility to alert the hospital staff to the potential problem of method interference from examinations that involve the infusion of dyes. The ophthalmology clinic is rarely concerned with laboratory results on blood or urine samples. However, we feel that the medical staff should notify the patient at the time of the infusion as to the potential for interference. A short notice handed to the patient can also refer the patient's physician to the clinical chemist if there is a question regarding the interpretation of laboratory results.

We agree with others (9, 10) that, minimally, on the day of the clinic visit, laboratory specimens should be acquired before fluorescein is infused. Laboratory staff should be notified when specimens are to be drawn subsequent to the angiogram, so that, where appropriate, acceptable methods can be substituted. Additionally, technologists should be alerted to the potential for interference in sera with a greenish tint (the orange coloration of fluorescein in urine is more difficult to detect). In particular, samples which have an unusually high background intensity or carryover effect on the TDx should be checked visually under an ultraviolet lamp for endogenous fluorescence.

Receipt of specimens containing interfering amounts of fluorescein from angiograms is expected to be infrequent. However, major diabetic and ophthalmic centers perform hundreds of these examinations each month, so the potential for obtaining specimens that can interfere with their own as well as other patients' results is a practical concern. The problem of fluorescein interference and incorrect laboratory results also becomes particularly important as newer and more sensitive methods involving fluorescein and reflectance spectroscopy become more prevalent.

References
5. Maurice DM. Personal communication.
9. Renoe BW, Sanborn J, Herold DA. Fluorescein effect on magnesium and total pro-


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Errors in Estimating Urinary Protein by Use of Benzethonium Chloride

To the Editor:

We report a case of nephrotic syndrome in which a urine specimen, although containing a large amount of protein, gave a result of zero when measured by the turbidimetric method of Iwata and Nishikaze (1). The urine was strongly positive to Ames "Multis-

ix," and gave a heavy precipitate when mixed with a sulfosalicylic acid solution. Subsequent analysis with a Hitachi 704 Analyzer showed that the specimen contained 76 g of protein, 64 g of which was albumin. Electrophoresis of the undiluted urine showed the presence of alpha and beta globulins, but an absence of gamma globulins.

Investigation of the method of Iwata and Nishikaze (1) with various concentrations of serum and urine proteins showed that turbidity increases with protein concentration only up to 20 g/L. With further increases in protein concentration, the turbidity decreases to zero, such that, at a protein content of >50 g/L the benzethonium chloride solution becomes clear again (Figure 1).

We repeated this experiment with several different serum samples to determine whether different protein mixtures responded differently. We found no noticeable difference between sera,  

Fig. 1. Protein concentration vs absorbance at 660 nm

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