ducibility, linearity) and specificity of antibodies cannot explain this discrepancy. After investigating several variables, we finally noticed the difference in protein concentration in the serum standards in the two assays: 49 g/L for FPIA and 66 g/L the Pharmacia RIA. We did not investigate the protein concentration of the Amerlex RIA standards.

For nine of the patients, whose serum protein concentration ranged from 62 to 75 g/L, digoxin concentrations measured by FPIA were about 20 to 30% less than those measured by the Phadebas RIA. However, when the protein concentration was low enough, as in control sera, precise values were obtained whichever assay was used (Table 1). Interference by protein can

Table 1. Digoxin as Measured by FPIA and RIA in Samples with Various Protein Concentrations

Protein concn, g/L	RIA	FPIA	FPIA[a]
	Apparent digoxin concn, nmol/L		
Patients' samples			
75	2.95	1.90	2.55
73	2.40	1.65	2.75
71	0.65	0.30	0.75
71	0.95	0.65	1.10
69	1.40	0.75	1.20
66	3.25	2.25	3.0
66	2.0	1.55	2.10
66	4.0	3.05	3.80
62	2.35	1.80	2.65
FPIA control sera[b]			
49	4.20	4.30	4.35
49	1.0	0.90	0.95
49	2.05	1.80	1.75
Mean	2.27	1.74	2.25

[a] After twofold dilution with saline (NaCl, 9 g/L).
[b] FPIA digoxin controls contain 0.96, 1.92, 4.48 nmol of digoxin per liter in normal human serum.

be suppressed by diluting serum samples with an equal volume of isotonic NaCL. When this was done, we found the mean digoxin concentration measured by the two assays was identical. We further confirmed this protein effect by investigating seven sera, all containing the same concentration of digoxin, 4.1 nmol/L, but having different protein concentrations, 5 to 100 g/L. The higher the protein concentration, the lower the apparent digoxin concentration, by 10 to 30% (Figure 2); we observed the same range of difference for the patients' samples.

Thus protein at concentrations within the normal range can substantially interfere with the FPIA procedure for digoxin. This can explain the recent finding of Erickson et al. (*Clin Chem* 29: 1239, 1983) that digoxin concentrations measured with the FPIA were statistically lower than the mean RIA values. Samples should therefore be deproteinized with trichloroacetic acid and the supernate assayed.

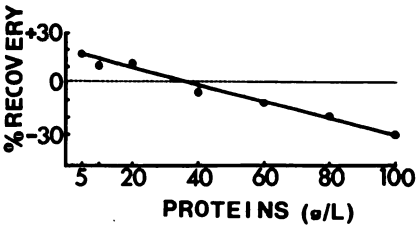

Fig. 2. Analytical recovery of serum digoxin, as a function of protein concentration (4.1 nmol/L)

We conclude that the FPIA of digoxin does not give accurate results when the protein concentration of the sample is within the normal range (60–80 g/L). This interference must be obviated, to avoid underestimation of digoxin, especially because this drug has a low therapeutic index.

J. M. Scherrmann
R. Bourdon

Université Paris V
Lab. Biochim. Toxicol.
Hôpital Fernand Widal
200, Rue du Faubourg Saint Denis
75010 Paris, Cedex 10 France

The authors of the letter in question respond:

To the Editor:

It is not uncommon to find unsatisfactory correlations between assays, including two different commercially available radioimmunoassays (RIA) for digoxin. Our comparison between the Abbott Laboratories digoxin fluorescence polarization immunoassay (FPIA) and the Amerlex RIA showed a satisfactory correlation of results (*1*). We have no such experience with any other commercially available RIA digoxin assays.

Variations in serum protein concentrations also affect the results of digoxin by RIA (*2–4*). The manufacturers of the Amerlex Digoxin RIA kit have shown it to be insensitive to changes in serum protein concentrations (package insert). Our correlation was therefore with a RIA method that was already tested for and found unaffected by changes in serum protein concentrations. Drs. Scherrmann and Bourdon did not include any data on the effects of changes in serum protein concentrations on the results by their in-house RIA for digoxin. It would be interesting to know what effect twofold dilution of patients' samples with isotonic saline would have on their RIA results.

Before one can accept the validity of a comparison method, it must be shown to be accurate and precise. Modifying the FPIA procedure to approximate one's RIA results could give invalid results unless one is certain that the RIA method is reliable in monitoring the narrow range between adequate therapy and toxicity. Obviously, if the individual laboratory has established the therapeutic range by using a particular RIA method and wishes to maintain the same reporting ranges when using another technique, it may be necessary to make adjustments in the new assay, e.g., by diluting samples for FPIA with saline to obtain comparable results. This practice may not be satisfactory for all users of the FPIA method, however.

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Naresh Rawal
F. Y. Leung
A. R. Henderson

Dept. of Clin. Biochem.
University Hosp.
London, Ontario, Canada

Rapid Quantitative Isolation and Esterification of Urinary Porphyrins for Chromatographic Analysis

To the Editor:

Determination of the pattern of urinary porphyrin excretion is useful for the differential diagnosis of disorders of porphyrin metabolism (*1*). Conversion of porphyrins present in biological samples to the corresponding methyl esters yields compounds that can be easily separated for individual quantification, either by inexpensive thin-layer chromatography (*2*) or by isocratic "high-performance" liquid chromatography (*1, 3, 4*). Current techniques for isolation and esterification of urinary porphyrins (*2*) are, however, cumbersome and time-consuming, and they can entail substantial losses of porphyrin materials.

We now report a procedure that yields quantitative isolation and esterification of porphyrins from urine samples in about a third the time required with previous methods, involving fewer manipulations and less laboratory equipment.