may be falsely low. This could be misleading to physicians who are serially monitoring patients' hCG trends over a period of time. Sera with values for hCG exceeding 200 int. units/L should be further diluted to provide the most nearly accurate results. Elimination of the 500 int. units/L standard should be considered until the problem of nonlinearity is corrected.

References

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Modified Liquid-Chromatographic Method for Creatinine Determination: A Rebuttal

To the Editor:

Ginman and Colliss (1) reported reproducibility problems with their method for the determination of creatinine in plasma (2). We believe that their findings are a result of not exactly reproducing methods. They used Amicon membrane cones for ultrafiltration instead of the specified (2) Amicon micropartition system with YMB membranes. Some membranes retain (i.e., adsorb) certain types of compounds, and the volumes of ultrafiltrate may also vary with different membranes and techniques. It is apparent from their report (1) that the lack of reproducible analytical recovery of creatinine from plasma and the nonlinearity of the standard curves are the result of the particular membrane used rather than a failure of our method. We have shown that creatinine is not retained by the YMB membranes used in the micropartition system. We used 0.2 mL of plasma and centrifugation for 10 min at 2000 × g to produce 0.12 to 0.13 mL of ultrafiltrate.

Our results were highly reproducible over the entire concentration range of 5 to 100 mg/L and the standard curves for creatinine prepared by use of plasma filtrate were very similar to those prepared from aqueous solutions of creatinine that were not passed through the membrane. Our data show that standard curves prepared by use of aqueous solutions could validly be used to analyze for creatinine in plasma by our procedure. In agreement with the manufacturer, we find that the YMB membranes do not retain creatinine.

Next, with regard to the use of acetonicitrile for precipitating plasma proteins before liquid-chromatographic analysis: we do not agree with Ginman and Colliss (1) that the precipitation method is more reliable than the ultrafiltration method for assay of plasma creatinine when the latter is used according to our method. We have observed that the supernate, after precipitation of plasma proteins with acetonitrile, gives a high blank value for creatinine, which we believe is caused by some component in the plasma other than creatinine. Chiou et al. (3) also reported, for the same concentrations, about a 16% difference in creatinine peak heights between an aqueous solution and an aqueous-acetonitrile supernate.

Since the original report (2) we have used our method for the analysis of over 100 plasma samples; our conclusions concerning the utility and validity of that assay remain unchanged.

References

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The authors of the Letter in question respond:

To the Editor:

In the description of our modified liquid-chromatographic method for creatinine (1) we do not consider that we unjustly criticized the method of Achari et al. (2); in fact, we did state that their method was excellent for urine creatinine determinations. In our initial application of the method for the determination of creatinine in serum we admit to having used Amicon Centriflo cones rather than YMB membranes for the “clean-up” of serum samples prior to analysis by HPLC. It was not apparent to us (and neither would it be to any other reader of the paper written by Achari et al.) that the choice of membrane in this method of sample pre-treatment is so critical. In fact, if the retention of solutes is governed by molecular mass, then neither membrane should retain creatinine because the molecular-mass cutoff of the YMB and Centriflo membranes is 30 000 and 50 000, respectively. Indeed, before using them, we verified that creatinine was not retained by the Amicon cones, and it was only after continued use that we experienced inconsistent results. However, the manufacturers of the membranes have informed us that certain types of membrane can be susceptible to adsorption effects. The YMB membranes are manufactured from regenerated cellulose and have a surface area one tenth that of the cone; also, the YMB membranes have no backing, so adsorption is minimal. The Centriflo cones have a Tyvek plastic backing, which makes them more prone to adsorbing certain substances. The manufacturers have also stated that washing the membranes with saline after use gives only a superficial cleaning and it is possible that creatinine could be absorbed by the membrane, subsequent elution giving rise to the high values we encountered with various quality-control sera. More efficient washing is obtained by using 0.1 mol/L sodium hydroxide or 200 ppm free chlorine as hypochlorite followed by water; it is therefore advisable to use a fresh membrane for each separation.

There are problems associated with this method of sample pre-treatment prior to HPLC analysis, but we still maintain that precipitating the serum proteins with acetonitrile followed by ultracentrifugation at 10 000 × g for 3 h as described by Okuda et al. (3) is more reliable and rapid than to centrifuge at 2000 × g, as recommended by Achari et al. (2). We stress that the ratio of acetonitrile to serum must be at least 2 to 1 to ensure the removal of protein by precipitation, for quantitative determination of creatinine by HPLC (4). If the use of Amicon membranes is nonetheless adopted, then we recommend that such problems be emphasized in any description of the method. Moreover, the YMB membranes are now being superseded by YMT membranes, which should give