activity can now be explained by other terms.

The observation that the UF band appears in some samples with normal bilirubin concentrations (1–3) is confirmed in our studies. The UF band is detected at IB concentrations as low as 2 to 5 mg/L, but Figure 1 shows that the area of the peak, although small, nevertheless is correlated with IB concentrations. In the studies of others (1–3) the relationship between the intensity of the UF band and bilirubin concentrations for samples with values within the normal range for bilirubin was not investigated.

Koett et al. (3) were unable to detect the UF band in absence of alkaline phosphatase substrate, e.g., with diazo dye alone, in samples with above-normal bilirubin concentrations. In our hands, when a sample (IB concentration 33 mg/L) was run in parallel on separate plates and one was stained with alkaline phosphatase substrate and the other not, we saw no significant difference between the mean area of the UF band on the two plates: 3.84 (SD 0.36) cm² and 3.52 (SD 0.44) cm², respectively, although there was some broadening of the peak in the absence of substrate.

We conclude, therefore, that the UF band does not represent phosphatase activity and hence has little clinical significance. Rather, the UF band is an artifact caused by coupling between bilirubin and Fast Blue RR, similar to the diazotization reaction upon which bilirubin determination of serum is based (4). Recently a similar interference by bilirubin in an acid phosphatase assay has been attributed to an interaction with Fast Red diazo dye (5, 6).

References

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Diagnosing 3-Hydroxy-3-methylglutaric Aciduria

To the Editor:

We wish to alert your readers to some errors of fact and judgment contained in the paper by Buchanan and Thoene (1) on 3-hydroxy-3-methylglutaric aciduria. We question their conclusions, in the liquid chromatography-diode array technique "should allow more laboratories to establish this diagnosis." They are erroneous in stating that "this procedure affords a complete urinary carboxylic acid profile" when evidence in the same paper shows that, of all the metabolites characteristic of 3-hydroxy-3-methylglutaric aciduria (2), only 3-methylglutaconic acid is detectable (see Fig. 1, ref. 1).

We would hesitate to establish a diagnosis on the basis of an increase in the concentration of one acid, especially when that acid is not the one directly affected by the metabolic lesion. Although several other authors (3–6) have described patients with markedly increased urinary excretion of 3-methylglutaconic acid approaching the amounts reported for an infant by Buchanan and Thoene, only one of those patients had a marked 3-hydroxy-3-methylglutaric aciduria and none were proven to be 3-hydroxy-3-methylglutaryl-CoA lyase deficient (i.e., <5% of normal enzyme activity). The values reported, millimoles of 3-methylglutaconic acid excreted per gram of creatinine, were 6.0, 7.5 (3); 5.2 (4); 2.1, 1.9 (5); and 4.1 (6); Buchanan and Thoene measured 15.7 mmol/g. From these results, it is obvious that, by itself, an increased excretion of 3-methylglutaconic acid is not diagnostic of 3-hydroxy-3-methylglutaric aciduria.

In addition, their statement that "most infants die shortly after birth" is not supported by the literature (see a forthcoming review, 7). Of the 12 previously reported cases only two deaths had been recorded as of February 1986, and the original patient in whom this disorder was diagnosed is now 11 years old with no complications.

On a more technical note, we feel that it is incorrect to report concentrations to six significant figures (see Table 1, ref. 1) when the stated coefficient of variation for the assay is ~10%.

References

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The authors of the paper respond:

To the Editor:

We want to emphasize that urinary organic acid profiling by liquid chromatography complements, but does not replace, the gas chromatographic/mass spectrometric analysis of the derivatized urinary acids. We did not mean to imply that the presence of an increased urinary concentration of 3-methylglutaric acid provides a definitive diagnosis of 3-hydroxy-3-methylglutaric aciduria. Rather, such an increase indicates a potential metabolic abnormality, thus necessitating the gas chromatographic/mass-spectrometric analysis of the derivatized urinary or-

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