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Addendum
Our communication was written in reply to a communication from Dr. Thompson. His original communication was withdrawn as a result of our comments and his present communication substituted, including the Figure and Table 2 in addition to significant alterations to the text. This addendum relates to some of the changes made in Dr. Thompson’s communication.

1. We have not experienced the problems with sulphate and phosphate that occur in Dr. Thompson’s work, when we use the methods developed in our laboratories and with the stationary phases SE 30, OV1, OV 101, and OV 25. This is simply illustrated by comparing his Figure 1C with our Figure 1A and figures in our other published work. Although we agree that removal of sulphate and phosphate is desirable and could clarify their elution regions in the chromatograms, we have never experienced difficulties in detecting the metabolites that are characteristic of any of the metabolic diseases examined, including methylmalonic aciduria and branched-chain ketoaciduria. Our published work has demonstrated this clearly and we have contributed some new GC/MS identifications in some disorders studied.

Recoveries of phosphate are generally good by our methods, although in the presence of excessively high amounts some may be water washed through into the “neutral” fraction. We derivatize using BSA or BSTFA at room temperature and find that complete trimethylsilylation of phosphate occurs.

Dr. Thompson’s difficulties with his own methods can, we feel, be ascribed primarily to injection-port temperature and design problems and subsequent partial decomposition of the TMS-inorganic anions, as reported by Dr. Thompson in his original (withdrawn) communication. We have noted that in his modified communication, injection-port temperatures of 235 °C have been used.

2. The organic acid content illustrated by Dr. Thompson’s figures 1A and 1B are not identical; changes occur, for example, in peaks 22, 27, 28, and 29. Both samples in 1A and 1B have undergone barium salt precipitation and add little to the discussion on the quantitative comparison of this procedure with DEAE-Sephadex extraction alone. It is unlikely that large amounts of particular constituents would necessarily behave differently, glucaric acid for example, in our own communication, showing the same fractional losses from urine from normal subjects and from the patient with glucaric aciduria.

3. Dr. Thompson’s comments on barium salt solubility add little in support of his case. If barium sulphate, which is 170 times less soluble than barium citrate (at neutral pH), is completely removed by the barium salt precipitation procedure, and barium citrate itself shows, on Dr. Thompson’s evidence alone, some 80% losses, then a barium salt will necessarily need to be several hundred times more soluble than barium citrate to result in recoveries that could be considered adequate. Hence our own comments on this subject.

4. Organic acids in our own procedure are quantitated and detected without difficulty. Dr. Thompson attempts to refute this by reference to organic acids that are not mentioned in our original Clin. Chem. paper. However, these acids—2-methylglyceric, glyceric, and fumaric acids, for example—all elute well separated from and subsequent to phosphate on OV 101. Dr. Thompson’s comments on the dia stereoisomers of 4-deoxytricarboxylic acid are without basis: these isomers co-elute on non-polar phases such as OV 101 and we have therefore not attempted to differentiate them. They do, however, separate on OV 25, also used in our work.

5. Dr. Thompson’s last paragraph, and, particularly, last sentence, indicate clearly that he himself feels that barium salt precipitation produces results that are only qualitatively but not quantitatively reliable. Our own methods do however enable the quantitative and qualitative analysis of urinary organic acids to be made in detail, without prior removal of phosphate or sulphate.

An Improved Biuret Reagent

To the Editor:
The relatively long incubation (25–45 min) required for full color development and interference by lipemia are the two principal disadvantages of the biuret method for serum protein determination. I present here a modified biuret reagent which reacts almost instantaneously and has a clearing action on lipemic sera.

The reagent is a solution of cupric sulfate and disodium ethylenediaminetetraacetate dihydrate in a buffer of sodium hydroxide/glycine/sodium chloride (pH 13.0). This is prepared by diluting 3.8 g of CuSO4·5H2O, 6.7 g of the chelator, 17.5 g of glycine, and 14.0 g of NaCl in about 750 ml of water, adding 40.0 g of NaOH, and diluting to 1 liter. The reagent, kept in a plastic bottle, is stable at room temperature for at least a month.

When 50 µl of serum, taken from each of 200 normal subjects and patients, was mixed with 2.50 ml of reagent, the color intensity became maximum within 2–4 min at room temperature and was stable for hours. The absorbance at 545 nm was linearly related to albumin standards up to 125 g/liter, with a sensitivity equivalent to that of all biuret reagents currently in use. Apart from rapid development of color, the new reagent has a great capacity for clearing lipemic sera. This was evaluated from the absorbance values obtained for 20 lipemic sera after mixing 50 µl of sample with 2.50 ml of the present reagent, an EDTA-chelated reagent, and a tartrate-chelated reagent in 1 mol/liter NaOH, all vs. cupric sulfate blank reagents. The absorbance ranges were 0.020–0.100, 0.050–0.230, and 0.062–0.272, respectively. Consequently, only in the case of extremely lipemic sera would error be introduced with the new reagent.

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A Controversy

Ed. note: After a lengthy but, regretfully, unsuccessful attempt at reconciliation we feel obliged, in fairness, to publish the following Letters. The Journal has no independent information as to the accuracy of the statements and opinions expressed here, and can accept no responsibility for any impression in them.

To the Editor:
Some statements in the article “Diagnosis of Infectious Hepatitis by Multi component Analysis with Use of Field Ionization Mass Spectrometry” by Anbar, Dyer, and Scolnick (1) need to be corrected and amplified.

The experimental system described in this article was proposed by A. B.