
R. A. Chalmers
A. M. Lawson
R. W. E. Watts
Medical Research Council, Clinical Research Centre, Harrow, Middlesex, HA1 3UJ, Great Britain

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 His original communication was withdrawn as a result of our comments and his present communication substituted, including the Figure and Table 2 in addition to significant alterations to the text.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 chromatogram, 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" faction. 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 Thompson in his original (withdrawn) communication.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 21, 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 comment 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 as 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 diastereoisomers of 4-deoxytetronic 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 not only quantitatively but not qualitatively 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.

Hippocrates Yatzidis
Nephrological Center
Aretaieon University Hospital
Athens 611, Greece

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 imprecision 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.
Robinson in 1971 as a logical extension of his profiling work with L. Pauling and their co-workers (2,3) at Stanford University. He proposed this possibility to W. Aher (then) of the Stanford Research Institute as a potential use for the multipoint field ionization mass spectrometer that W. Aher was designing at that time. Pauling, Robinson, Aher, and their associates were then awarded a grant (4) for the support of this research at Stanford University and Stanford Research Institute, with L. Pauling as a principal investigator. Then, in collaboration with W. Aher and his co-workers in the Stanford Research Institute Mass Spectrometry Development Group headed by M. Anbar and with C. Spinndt, of the Physical Electronics Group at Stanford Research Institute, we have carried out some promising experiments along those lines.

Anbar, Dyer, and Scolnick acknowledge in their paper that “developments performed in a previous research project sponsored by NIGMS under grant #1-R01-GM19156-01 contributed substantially to the success of the letter.” But they fail to mention that this grant was made to Pauling and his co-workers, that the research grant proposal contained almost all of the conceptual work reported in their paper, and that the other conceptual aspects of their paper were provided to them later through their collaboration with us.

Anbar, Dyer, and Scolnick did not give any references to our recent work, including our two articles in Clinical Chemistry (5,6) and another article (7) that involves the use of mass spectrometry in a way similar to that described in their article. In addition, Anbar, Dyer, and Scolnick failed to mention in their article that experiments closely similar to those described by them were performed on the Stanford Research Institute apparatus, in collaboration with us, on multiple sclerosis, breast cancer, and fasting before the reported hepatitis experiment was done, and on melanoma after that experiment. These other studies showed a time-dependent drift in the mass spectrometer that in our opinion precluded the practical use of the patterns observed with it. It was found that the profiling strength obtained with multipoint field ionization spectrometry was not better than that obtained with chemical ionization mass spectrometry and was markedly inferior to that of existing chromatographic techniques. At each successive stage in the development of profiling by direct mass spectrometry in the Stanford University–Stanford Research Institute–Linus Pauling Institute laboratories we had jointly decided that the procedures did not yet warrant presentation of the work to the scientific community as an applicable diagnostic alternative. This conclusion was reached from experiments performed both before and after the reported hepatitis experiment. We were under the impression that there was unanimity of opinion with regard to publication between us and our collaborators, Dr. Anbar and his co-workers.

We are astonished not only at publication of this work on hepatitis without our knowledge but also at the fact that our names were not mentioned in the reference to the grant from the National Institute of General Medical Sciences. In our letter, we proposed that direct mass spectrometric profiling of body fluids would play an important role in clinical chemistry and medical diagnosis, and we are pleased that we have participated in its development. We are concerned that the readers of Clinical Chemistry may be misled by the article by Anbar, Dyer, and Scolnick.

References
4. Pauling, L., Robinson, A. B., Aher, W., Hill, R. N., Rogers, K. T., and Sharpless, R., Co-investigators, National Institute of General Medical Sciences grant GM19156-01 and 02, Medical Diagnosis by Field Ionization Spectrometry: Submitted May 1971; funded with subcontract to SRI 1972–1974; funded in renewals to Linus Pauling Institute through 1978. (This grant also provided funds to support C. A. Spinndt and his co-workers at SRI for supply of the multipoint arrays that they developed.)

Arthur B. Robinson
Linus Pauling
William Aher

Linus Pauling Institute of Science and Medicine
2700 Sand Hill Road
Menlo Park, Calif. 94025

The authors of the paper in question offer the following response:

To the Editor: The Letter of Robinson et al. raises two questions: 1. Did we deliberately avoid giving due credit to the researchers of the Pauling Institute? 2. Is field ionization mass spectrometry (FIMS) an adequate technique for the multicomponent analysis (MCA) of biological fluids?

Our answer to the first question is that none of the authors of the Letter has made any contribution to the results reported in our paper (1) beyond those explicitly acknowledged in the references of that paper. Our answer to the second question is that Robinson et al. have no objective reason whatsoever to cast any doubt on the adequacy of FIMS/MCA for the analysis of biological fluids.

1. The concept of FIMS/MCA was not new in 1971 (2), nor was the concept of MCA of biological fluids, which has been carried out in different research laboratories for over 10 years, most of which, including Pauling’s group (refs. 4, 12) were referred to in our paper. The NIGMS grant application (ref. 4 of the above Letter) was construed as part of a FIMS/MCA research program conceptualized at SRI for environmental, biological, and medical applications. The innovation in the 1971 grant application was a certain FIMS system which was proposed by Dr. Aher. This instrument, however, failed to be of practical value for MCA on a routine basis. This system, which could not produce any meaningful results from biological samples, was referred to in our paper (ref. 13), and Dr. Aher therefore got full credit for his early efforts. After his failure in 1972 on the original MCA system, he was moved to other projects in 1973. He eventually left our group by the end of 1975. The instrumentation used by us for the analysis of metabolites in urine, which was under development in 1973–76, is entirely different from that suggested by Aher, and he made no contribution to its design and construction, or evaluation and use. Prof. Pauling, who acted as Principal Investigator (PI) on the NIGMS grant, and Dr. Robinson did not make any conceptual contribution to the proposal, nor did they make any contribution whatsoever to the instrumentation development or sample preparation procedures. In spite of this insignificant contribution of Robinson et al. to our program, we courteously acknowledged the NIGMS grant, since it allowed us to gain experience in how not to perform FIMS/MCA. We did not cite the name of the PI in our acknowledgment, since this is not practiced in general. Incidentally, Robinson’s paper on MCA by MS (ref. 7 of the Letter), which carries the names of four members of the Pauling Institute, does not give any credit to Prof. Pauling, although the reported research...