was sponsored by the same NIGMS grant. If his own team did not find it necessary to acknowledge Pauling for any contribution, why are we blamed when doing the same?

In 1973 we analyzed some 200 urine samples for the Pauling Institute under a service subcontract. We were not informed what these samples were, and we were never involved in the evaluation or interpretation of the raw data provided by us to the Pauling Institute. In 1975 at the ASMS meeting, we heard for the first time, listening to a paper presented by Dr. Robinson, about the nature of the samples we analyzed two years before. This is the “another article (7) that involves the use of mass spectrometry in a way similar to that described in their article” mentioned in the Letter. Although a major part of Robinson’s presentation was devoted to describing the results generated by Gilbert St. John, Eric Kempton, Robert Dyer, and Russell Sperry of our group—researchers who spent a few months on these samples—they were never acknowledged. In other words, first Robinson cites our results without appropriate acknowledgement, and then he blames us for not giving him credit for our efforts.

We did not refer to this paper (ref. 7 of the above Letter) in our paper, since it is not customary to refer to proceedings of ASMS, which are available only to members of the society. For the same reason, we did not refer to our own paper (3) presented at the same conference (which describes some of the results included later in the Clinical Chemistry paper).

Robinson et al. are now trying to claim credit for our achievements in 1975–76 on the basis of our service work for them in 1973. There was no scientific collaboration whatsoever between the two research groups, unless submitting samples for analysis for a fee without any feedback or acknowledgment is considered as scientific collaboration.

At the stage of analyzing the data on infectious hepatitis, we have adapted some of Robinson’s computational techniques. This was done in order to allow comparison of our “diagnostic power” with that achieved by other techniques. The use of this information was fully acknowledged (ref. 12 in our paper).

This is not the first time that Dr. Robinson has tried to claim undue scientific credit. In his ASMS paper he states, “We have also been interested in the possibility that body fluids might be introduced directly into mass spectrometers for diagnostic purposes. We first proposed this possibility to Dr. Atherb of the Stanford Research Institute in 1971 as a potential use for the multipoint field ionization mass spectrometer he was designing at that time.” In his present Letter, after he has hired Dr. Atherb, in 1976, to proceed with a parallel research effort to that at SRI, he admits that it was Dr. Atherb who made the suggestion to him in 1971. We would like to emphasize here that Prof. Pauling did not coauthor the 1975 ASMS paper, and is thus not responsible for Dr. Robinson’s statements.

2. The claim of Robinson et al. that FIMS is inadequate for MCA of biological fluids because of “time dependent drift in the mass spectrometer” is utterly unfounded. We would like to emphasize that we were never consulted regarding the results of those 200 samples; in fact, we have never even been shown the results and therefore the statement that “we had jointly decided that the procedures did not yet warrant presentation of the work to the scientific community as an applicable diagnostic procedure” is untrue and misleading.

Let us quote now from Robinson’s own paper (ref. 7 of the Letter). “The diagnostic power of this chemical ionization procedure was 0.75 as compared with 0.71 for the multipoint FIMS procedure . . . we consider this a very promising method of mass spectrometry.” This statement was made on the basis of our old data, obtained with inferior instrumentation and preparation procedures, compared with those used to produce the data presented in our paper in Clinical Chemistry. Now, how does this statement of Robinson’s stand in the light of his statements in his Letter? It seems that the same experimental results are “very promising” when they appear under Robinson’s name (without giving due credit to the researchers who produced them), but they are “misleading” when he chooses to attack us. Even if the 1973 data were “drifting” and unreliable, contrary to Robinson’s own statement in 1975, what relevance does this have regarding the 1976 data, which were produced by different instrumentation and other preparatory procedures?

Robinson et al. refer in their Letter to a more recent series of measurements on melanoma urines. They are very well aware of the fact that this series of experiments was aborted. Moreover, even if FIMS/MCA would have nil diagnostic power in the case of melanoma, what relevance does this have to the case of infectious hepatitis?

Let us refer here to a few new experimental findings that add credibility to our published methodology. We have analyzed two groups, 15 new samples each, of normal and hepatic urines, using the 24 + 24 samples reported in our paper as the learning set, and using just the 11 most significant constituents as a diagnostic pattern. An average success score of 84% was obtained. This is a highly encouraging result in view of the fact that the new analyses were carried out six months later by another operator and on a different mass spectrometer. A variance analysis of the old data showed an average standard deviation of 16%, whereas the average difference of the “diagnostic” components was 73.4%. In other words, the average signal-to-noise ratio in the diagnostic test reported in our paper is about 4.5 to 1. Current results show a lower variance, raising further the level of confidence of the technique. We include these facts in this rebuttal since they have direct bearing on the statements of Robinson et al. regarding the reliability of the technique.

In summary, we have shown that the claims brought up against us for not giving due scientific credit to members of the Pauling Institute are utterly unfounded. Our paper in Clinical Chemistry has shown that FIMS/MCA is one of the best techniques for MCA analysis of biological fluids, and the efforts to continue and improve the sensitivity, reproducibility, and especially the speed of this technique will be highly rewarding. We understand that Robinson et al. are now proceeding with a parallel research effort on FIMS/MCA, and we welcome them as well as any other research team to join us in this highly promising field of endeavor.

References

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Intra-lot Variation in Folate Quality-Control Sera

To the Editor:

Our recent experiences in attempting to determine the cause of troubles with folate quality control led us to suspect that there were significant vial-to-vial
Table 1. Statistical Analysis of Vial-to-Vial Variation in Folate Concentrations

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Folate (μg/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Package insert value</td>
<td>12.0</td>
<td>9-15</td>
</tr>
<tr>
<td>Run of 4/27</td>
<td>20</td>
<td>11.14</td>
</tr>
<tr>
<td>10 vials assayed in duplicate</td>
<td>10</td>
<td>9.96</td>
</tr>
<tr>
<td>1 vial assayed 10 times</td>
<td>20</td>
<td>10.58</td>
</tr>
<tr>
<td>Run of 4/28</td>
<td>10</td>
<td>9.44</td>
</tr>
<tr>
<td>Digoxin (μg/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Package insert value</td>
<td>0.9</td>
<td>0.6-1.2</td>
</tr>
<tr>
<td>10 vials assayed in duplicate</td>
<td>20</td>
<td>0.93</td>
</tr>
<tr>
<td>1 vial assayed 10 times</td>
<td>10</td>
<td>0.97</td>
</tr>
<tr>
<td>Sodium (mmol/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 vials assayed once each</td>
<td>10</td>
<td>142.7</td>
</tr>
<tr>
<td>Laboratory pool for 4/76</td>
<td>50</td>
<td>148.1</td>
</tr>
<tr>
<td>Chloride (mmol/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 vials assayed once each</td>
<td>10</td>
<td>116.8</td>
</tr>
<tr>
<td>Laboratory pool for 4/76</td>
<td>50</td>
<td>118.5</td>
</tr>
</tbody>
</table>

Table 2. Comparison of Vials from the Same Lot

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>3N402</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>4N502</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Representative vial</td>
<td></td>
<td>8.08</td>
<td>0.36</td>
<td>7.6-8.6</td>
<td></td>
<td>1.12</td>
<td>0.04</td>
<td>1.1-1.2</td>
</tr>
<tr>
<td>High vial</td>
<td></td>
<td>13.00</td>
<td>1.00</td>
<td>12-14</td>
<td></td>
<td>2.54</td>
<td>0.40</td>
<td>2.1-3.2</td>
</tr>
</tbody>
</table>

Each vial was assayed five times in the same run.

variations in the product we were using.
Ortho Diagnostics, Inc., Raritan, N.J. 08869, kindly gave us packages of the two quality-control materials, Ortho RIA Control Sera I and II. Folate was estimated by using the 3H Kit (cat. No. CO; Diagnostic Products, Los Angeles, Calif. 90064).
Digoxin was estimated using the 3H kit (cat. No. NEA-020; New England Nuclear Corp., Boston, Mass. 02118).
Sodium and chloride were estimated with an SMA 6/60 continuous-flow analyzer (Technicon Corp., Tarrytown, N. Y. 10591). Assay methods in all cases were as specified in the package insert, except that the charcoal separation for the folate assay was performed in a nonrefrigerated centrifuge. For both radioimmunooassay methods, radioactivity in the vials was counted in a liquid scintillation counter for 10 min. Total counts were between 10 000 and 20 000. The CV for this number of counts is <1%.
The 10 vials from each package of Serum I and II were reconstituted ac-
cording to the manufacturer’s directions several hours before the first folate assay. Vials were stored at −20 °C for later assay.
Vial-to-vial variation was determined by assaying all the vials in duplicate for folate, digoxin, sodium, and chloride. Tube-to-tube variation within run was determined by assaying one vial from each package 10 times. Results are shown in Table 1.
These data show that for folate there are significant vial-to-vial differences. The standard deviation (vial to vial) in Serum I was 3.7-fold the within-vial variation. The folate concentrations in one bottle from each lot of serum appeared to be substantially higher than the mean value for that lot. These "high" vials were reassayed with a representative vial from each lot a week later. The data from the re-assay (Table 2) show that the folate concentration appears to be at least 60% greater than the representative vials. The representative and "high" vials were also assayed by using a 3H-folate kit given us by Schwarz/Mann, Orangeburg, N.Y. 10962. These data confirmed the presence of substantial apparent differences between folate concentrations in different vials.
No significant differences were found between the concentrations of sodium, chloride, and digoxin within each lot of vials.
Several other control sera were assayed for folate. The sources of these sera were Hyland Labs, Burroughs Wellcome, Bio-Reagents and Diagnostics (manufacturers of the Ortho quality-control products), and our own pooled material. We found no significant vial-to-vial variation in these materials.
We conclude that vials from the same lot appear to contain substantially different amounts of folate. The variations have always been to the high side, and are not attributable to gross manufacturing processes (such as filling errors or poor mixing of the serum pool) because the vial-to-vial variations of folate were not matched by vial-to-vial variations in sodium. This apparent increase in folate may be due to the formation of a material after bottling that interferes with the assay process.

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Ed. note: Mr. Alan Voss, Product Manager for Ortho Diagnostics Inc., says that this problem is being studied, that studies so far confirm that it is not attributable to mixing, filling, or recon-

CLINICAL CHEMISTRY, Vol. 23, No. 5, 1977 911
Bovine Serum Albumin as a Cause of Misdiagnosis in Tay-Sachs Disease

To the Editor:

In the assay for hexosaminidase A (Hex A) in leukocytes, cultured fibroblasts, and cultured amniotic fluid cells for the detection of Tay-Sachs disease and carrier identification, bovine serum albumin (BSA) is used (1,2). We find that some BSA preparations are contaminated with hexosaminidase activity. We wish to warn others of this and to show how an incorrect diagnosis could be reached by using contaminated BSA.

The 15 leukocyte samples used in the study had less than 50% Hex A when assayed with BSA (Pentex, Miles Laboratories) having no measurable Hex activity. Assay methods were as described by Kaback (2). The average total activity (Table 1) varied considerably between different BSA preparations; the average percent Hex A varied much less, although significantly.

When assayed for Hex activity, both Sigma products contained significant amounts; whereas, there was no measurable activity in the Pentex sample. Most of the BSA Hex activity was heat inactivated at pH 4.4 and 52 °C, indicating the presence of “A-like” activity. In addition, electrophoresis of the enzyme on cellulose acetate (Cellogel) resulted in a diffuse band of activity having “A-like” mobility.

Because cultured amniotic fluid cells and leukocytes utilize BSA in the assay procedures for Hex A, results could be disastrous if BSA were used that has hexosaminidase activity. A small amount of this “A-like” contaminant could result in the diagnosis of a “normal” couple when actually they were a carrier couple or in the diagnosis of a “carrier” fetus when actually there was no Hex A activity.

References


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Ed. note: A reviewer points out that this message is not new, but will bear repeating because it is mentioned more or less incidentally in the literature.

Quantification of High-Density Lipoprotein Cholesterol in Serum by a Heparin–Mn2+ Precipitation Method

To the Editor:

We read with interest the article [Clin. Chem. 22, 1828 (1976)] by Bachorik et al., in which they compared the plasma high-density lipoprotein (HDL) cholesterol concentrations determined by heparin–Mn2+ precipitation and by preparative ultracentrifugation. They state that our recent findings (1) on the interaction between HDL and heparin in the presence of Mn2+ prompted them to re-examine the heparin–Mn2+ procedure, which under their test conditions did not precipitate HDL from whole plasma.

We have been concerned with the problem of specificity of commonly used cations, especially Ca2+, Mg2+ and Mn2+, in lipoprotein–heparin (and other glycosaminoglycans) interactions. Unlike Ca2+ or Mg2+, the Mn2+ ion produces complexes of varying insolubility between HDL and heparin. Among the subclasses of HDL, 40% of HDLd and 10% of HDLb can be precipitated in the presence of Mn2+. Therefore, the degree of insoluble complex formation is likely to be determined by the relative proportion of the HDL subclasses rather than a fixed proportion (25%) of HDL being precipitated in each case. It is likely that the magnitude of HDL–heparin interactions in whole plasma may be different from that in isolated fractions. Considering the measurement errors involved in ultracentrifugal isolation and cholesterol determination, the difference between the heparin–Mn2+ and the ultracentrifugal procedure may be reasonably small whenever the HDL concentration of the plasma is not too high and HDL 3 is the predominant HDL component or subclass.

Given the wide density ranges of lipoprotein classes and their heterogeneity under different physiological and nutritional states, it is difficult to assume that virtually “all” HDL sediments at relative density 1.07. In human plasma, HDL 3 (hydrated density 1.05) occurs in varying quantities. Individual HDL-cholesterol values, by age and sex, and pair-wise statistical analyses of the differences between heparin–Mn2+ and ultracentrifugal methods would have been helpful in this regard.

It is pertinent to mention the recent observations of Krauss et al. (3) on HDL concentration in plasma of women taking oral contraceptives. While HDL cholesterol measured after heparin–Mn2+ precipitation of whole plasma did not differ significantly from controls, the values for total HDL and HDLb measured by analytical ultracentrifugation were higher in users. In unfraccionated plasma, the heparin–Mn2+ procedure is known to result in coprecipitation of other proteins (albumin, γ-globulins, and fibrinogen), including α-lipoprotein in abetalipoproteinemic patients (4).

The authors have elegantly shown that the LDL and VLDL were satisfactorily precipitated by the heparin–Mn2+ procedure, and Mn2+ concentrations over a wide range did not cause a further decrease in cholesterol content of the HDL-containing supernatant fractions. Unfortunately, this does not prove whether or not some lipoproteins are precipitated. Analyses of the heparin–Mn2+ precipitate and supernatant from the ultracentrifugal fraction for the presence of HDL (especially for the presence of apo-AI) could have provided further evidence of the desired fractionation.

Although, the heparin–Mn2+ procedure can generally be used for estimation of HDL-cholesterol in unfraccionated plasma, certain qualitative and (or) quantitative differences among HDL subclasses in an individual’s plasma may alter the specificity of the procedure. In a continuing effort to refine the lipoprotein method used in our laboratory (5–7), we have been comparing the lipoprotein cholesterol values in different groups of individuals (children and adults) using the heparin–Mn2+, ultracentrifugal (d 1.063), heparin–Ca2+, and other procedures. The mean HDL-cholesterol indicated reasonable agreement among these methods. However, a comparison of individual values showed that there were significant discrepancies.

Table 1. Hexosaminidase Activity in Leukocytes Assayed with Various BSA Preparations

<table>
<thead>
<tr>
<th>BSA</th>
<th>% Hex A activity</th>
<th>Total Hex A activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miles “Pentex Fraction”</td>
<td>44</td>
<td>979</td>
</tr>
<tr>
<td>V</td>
<td>62</td>
<td>1667</td>
</tr>
<tr>
<td>Sigma “Fraction V”</td>
<td>47</td>
<td>1711</td>
</tr>
<tr>
<td>Sigma “Crystallized &amp; Lyophilized”</td>
<td>47</td>
<td>1711</td>
</tr>
</tbody>
</table>

*Activity = nmol 4-methylumbelliferyl released per hour per milligram of protein. Values are average of 15 samples.