A Paradox Clarified: Set-Point Values for Cholesterol, Direct and Enzymatic

To the Editor:

In my review on cholesterol (1), I questioned the set points of an SMA reference serum of Technicon Instruments Corporation because the direct value, said to be standardized by the Abell procedure (2), and the value for the enzymatic procedure, which should compare favorably to the Abell procedure, differed from each other by more than 30%. What the manufacturer apparently does is to incorporate a certain concentration of cholesterol acetate into their reference serum "to achieve a desired calibration level" (footnote of their reference serum sheet) in their reference standard, to effectively serve both procedures. This then would account for the fact that the assigned values appear strikingly, perhaps disturbingly, different.

I apologize to the manufacturer for overlooking this important point. However, in defense of my own ignorance on this matter, I would state that prior to sending in the review I had not previously seen Technicon Bulletin No. TT7-0292-10, March 1977, in which I would have encountered a brief clue to this procedure of standardization. In that bulletin is the following statement: "Due to the different set point values which are assigned to the references for the direct and enzymatic values (to compensate for the bilirubin interference and the nonenzymatic activity of cholesterol acetate, respectively . . . .) Nor had I noticed the footnote in their reference serum sheets, which I certainly should have.

References

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Analysis of Urine with the Technicon SMAC

To the Editor:

We have found a way to circumvent the problems that ordinarily preclude urine samples being analyzed with the Technicon SMAC, and now can estimate urea, creatinine, urate, and phosphate concentrations in urine in this way. We find it more convenient, with our present work load, to do these estimations with the SMAC rather than to set up separate procedures.

Sodium and potassium concentrations exceeding the range encountered in serum tend to affect the ion-selective electrodes adversely. In addition, the electrodes react with the relatively high ammonium ion concentrations of urine, aggravating this problem. Samples not containing protein in concentrations about like those in serum disturb those channels that involve dialysis, so that results are inaccurate for subsequent specimens. We have examined the possibility that urine samples might be prepared in a way that allow them to be analyzed with the SMAC without these ill effects. A diluent can be prepared containing sodium, potassium, and protein, so that urine diluted 10-fold contains sodium, potassium, protein, urea, creatinine, urate, and phosphate at concentrations that are within the range encountered in serum. Then the instrument gives useful results without ill effects. The diluent contains, per liter, 50 g of bovine serum albumin, 132 mmol of sodium, and 2.5 mmol of potassium. Specimens of 24-h urine were diluted, if necessary, to a volume of 2 litres, diluted 10-fold with the diluent, filtered through Unichem Filter Samplers, and poured into sample cups ready for analysis.

A further problem arises if more than two of these specimens are sampled consecutively. In this case, the instrument interprets the failure of a peak to appear in such channels as cholesterol and triglycerides as a breakdown in those channels, and halts the sampler. This can be avoided either by dispersing urine samples singly amongst the serum samples or by running the urine samples at the end of the batch of sera and deactivated the channels irrelevant to the urine analysis. The second approach is usually more convenient.

The urine sample had no significant effect on the results for a serum sample immediately following. This contrasts with the marked effect observed if no protein was included in the diluent.

One hundred urine samples were so analyzed on the SMAC for sodium, potassium, urea, creatinine, urate, and phosphate; on the SMA 6/60 for potassium, urea, and creatinine; and on the SMA 12/60 for urate and phosphate. Sodium values ranged from 120 to 129 mmol/liter and potassium values from 3.0 to 8.9 mmol/liter as determined by the SMAC. The difference between results for potassium by ion-selective electrode and flame photometry (due to ammonium ion) did not exceed 0.2 mmol/liter. The regression equations (concentrations in mmol/liter), with SMAC result x and SMA 12/60 or SMA 6/60 result y, were:

\[ y = 1.018x + 0.623 \]
\[ y = 0.925x + 0.010 \]
\[ y = 9.949x + 0.001 \]
\[ y = 1.015x + 0.025 \]

We believe this procedure allows urine samples to be analyzed by the SMAC without any ill effects and with acceptable accuracy.

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Interpretation of Correlation Coefficients

To the Editor:

At a session of the recent Chicago AACC meeting the performance of a new instrument was described. The general tone was favorable, but to my surprise a correlation coefficient of .830 was presented between the data obtained in one parameter with the new instrument, compared to those obtained with identical samples with an old, proven instrument.

A comment on the nature of correlation coefficients seems to be in order. A correlation coefficient of .83 implies that the values are out of control in statistical terms in 31% \((.83)^2 = 0.689\) of the cases. This does not permit any statement of clinical significance. It simply means that in almost one out of three cases no statistical prediction can be made on the basis of one set of data as to the other within the inherent repeatability of both methods. Thus, if one instrument is considered standard or reference, then surely the other is less than satisfactory.

At another session, the speaker reported that correlations were "9 or better." Here again, the powerful nature of the coefficient should be considered. While a correlation coefficient of .99 implies that the data are out of control 2% or less, at .90 this figure rises to 19%, which is only marginally acceptable. Surely, correlation coefficients must be
Volatile Organic Components in Human Urine after Ingestion of Asparagus

To the Editor:

Characteristic chromatograms or "fingerprints" associated with trace components in body liquids and effluents are termed "metabolic profiles." Much could be learned about human metabolism if these metabolic profiles could be appropriately correlated with nutritional status, physical exercise, seasonal and diurnal variations, genetic make-up, physiologic and pathologic status, and other factors (1). Some apparent controversy has appeared in the literature as to whether dietary variations for individuals cause significant differences in their respective profiles (1-4), although some foods have been known for years to cause characteristic odors in urine.

For about half of the population, asparagus produces a characteristic odor in urine (5) within a few hours after it is ingested (6). White (7) reported the responsible components to be two sulfur-containing compounds, namely, S-methyl-thioacrylate and S-methyl-3-(methylthio)-thiopropionate. This phenomenon was investigated further to determine what profile differences were attributable to asparagus in the diet.

Three subjects (all women) were chosen for the study. Subject 1 was 24 years old, a diabetic under treatment with insulin. Subject 2 was taking no drugs. Subject 3 was currently taking birth control pills (Ovulen 21). All subjects fasted 12 h before breakfast (8) samples were collected, except as noted. About 420 g of fresh boiled (for 10 min) asparagus was eaten before retiring the evening preceding urine collection. Control urines (three per subject) were collected as above, but the subjects were not allowed to eat asparagus for 72 h before urine was collected. No other dietary restrictions were imposed.

Details of the sample collection, volatile isolation, separation variables, and detection have been reported previously (3, 9). We calculated the relative retentions by the method of Novotny and McConnell (10); one peak that was consistently present in both control and test chromatograms was selected as a reference (its retention time is not presented in Table 1). Average deviations in relative retentions are reported, because the average value for relative retention of each peak in question has been calculated from at least three replications of each experiment.

Table 1 shows the effect on the urine from Subject 3 of ingestion of asparagus. Only major peaks (signal/noise >50/1) that significantly differed between the control and test runs are shown. Subjects 1 and 2 failed to produce a profile differing from control conditions; however, this was not surprising in view of the previously reported relative incidence of characteristic urine odor in the general population after ingestion of asparagus.

The experiment was duplicated, except that, in contrast to experiment 1 in which the asparagus was eaten just before retiring, in experiment 2 it was ingested for the evening meal. Prebreakfast urines were collected as in experiment 1. Subject 1 shows three major sulfur-containing components and one non-sulfur component in the urine volatiles for as long as 14 h after ingestion of asparagus. Several minor sulfur components that also were present are not reported here. This result contrasts with conclusions made by other workers, who stated that dietary effects are minimal for organic volatiles in urine. Our results also differ from those of White (7), who only determined two major sulfur-containing compounds, attributed to ingestion of asparagus. A more nearly complete study with more subjects, including qualitative gaschromatographic-mass-spectrometric analysis, will be reported later, including identification for the four components. The hydrocarbon component (Table 1) is of particular interest.

References


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