sensitivity of the pH meter, and individual manual manipulations. With the bmc reagent set, the major potential source of error is improper preparation of the working solution.

As part of a monitoring program, we had access to 83 plasma samples at one time in September and another 100 in December, 1977. All but 23 of the latter samples were obtained from the same individuals as the September samples. Cholinesterase activity was determined by both procedures on all samples. Because of the difference in the procedures and manner in which the activities were calculated, we were unable to convert from ΔpH units to international enzyme units. Thus, comparing units as such was not feasible. We did determine the mean, standard deviation, and coefficient of correlation for each run and for all samples (Table 1). In a separate study, which will be reported elsewhere, in which both cattle and swine were exposed to organophosphorus compounds to produce a decrease in cholinesterase activity, the coefficient of correlation between the Radeleff and Woodward modification (3) of the Michel procedure and the bmc procedure for the individual animals (35 calves and 17 swine) was 0.90 to 0.99.

Our data indicate that the bmc procedure may be used as a replacement or as an alternative to the Michel procedure and has the advantage of a faster determination rate.

References


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Table 1. Mean, Standard Deviation, and Correlation (r) of Cholinesterase Activity Measured by Two Methods

<table>
<thead>
<tr>
<th>n</th>
<th>Mean MICHEL</th>
<th>SD</th>
<th>Mean BMC</th>
<th>SD</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>0.85</td>
<td>0.22</td>
<td>3557</td>
<td>702</td>
<td>0.96</td>
</tr>
<tr>
<td>100</td>
<td>0.93</td>
<td>0.21</td>
<td>3934</td>
<td>700</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Diurnal Variation of Serum Iron in Normal Individuals

To the Editor:

Reports of the diurnal variation in serum iron concentration vary considerably, with maximum concentrations found early in the morning (1), in the afternoon or evening (2), and at 1400 h (3).

Attempting to find a predictable trend in within-day variations in serum iron, we studied 25 apparently health adults. Duplicate serum samples were drawn between 08:00 and 09:00 h and between 15:30 and 16:00 h the same day for serum iron and total iron binding capacity (TIBC). The specimens were measured in duplicate by continuous-flow analysis (Technicon Basic AutoAnalyzer 1), with the method of Kauppinen and Gref (4); results for duplicates were averaged.

No significant change in TIBC was found. Diurnal variations in serum iron were found, but no consistent pattern was discernible. The serum iron concentrations of 18 of the 25 subjects decreased from morning to afternoon. This decrease ranged from 4 to 61%, with an average of 21%. Seven individuals showed an increase, with afternoon values ranging from 2 to 69% higher than those in morning, with an average increase of 20%.

Overall, the mean iron concentration for the 25 subjects was 990 μg/liter in the morning, and 870 μg/liter in the afternoon. It is important to note that several subjects had both normal and abnormal serum iron concentrations on the same day. There was no sex-related distinction.

We suggest that serum iron should be measured both in the morning and afternoon, to provide more meaningful information and to aid in the diagnosis of borderline cases of iron deficiency and excess.

References


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More on Gas–Liquid Chromatographic Determination of Homovanillic and Vanillylmandelic Acids in Urine from Cases of Neuroblastoma

To the Editor:

We wish to comment on the recent publication by Brewster et al. (1).

We really are astonished about the urine samples the authors show in their above-mentioned publication. We (2) and, as far as we know, other groups in other parts of the world (4–7) always find acid substances in normal urine samples other than homovanillic acid (HVA) and vanillylmandelic acid (VMA) plus the added standard. As pointed out by Jellum (4) and Bjorkman et al. (7), urine samples of adults contain an especially high amount of hippuric acid and other acids that strongly interfere with the HVA and VMA peaks. Coward and Smith (8) showed quite clearly the kind of substances that may affect the determination of HVA and VMA by means of an OV 1 column if one uses the technique described by Brewster et al. (1).

Nine years ago, Sprinkle et al. (9) published a method like the above-mentioned one. Five years later, Lanser et al. (10) commented on the impossibility of obtaining clear results for quantitative determination of HVA and VMA in normal urines because of interfering substances. Therefore we think it worthwhile promptly to warn others not to believe that things are as easy as shown by Brewster et al. (1). Additionally, the choice of p-hydroxyphenylactic acid for use as internal standard is a bad one because every normal urine sample contains various amounts of this substance.

The storage of standards like VMA in methanol can also be a source of error, because it is known that VMA can react with methanol, leading to the formation of various methylated reaction products (9).

We think that it will be good analytical practice in the quantitative deter-
mination of HVA and VMA for diagnostic purposes to recommend the use of either of two different stationary phases in gas chromatography, two different separation techniques, or gas chromatography-mass spectrometry to ensure the identity of the gas-chromatographic peaks in question.

References


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Two authors of the paper in question respond:

To the Editor:

Melchert and Hoffmeister apparently failed to appreciate the impact of silylation technique upon the solubility of hippuric acid, as emphasized by Coward and Smith (loc. cit.). The Tri-Sil TBT utilized in our procedure, containing trimethylsilylelimidazole, N,O-bis(trimethylsilyl)acetamide, and trimethylchlorosilane in pyridine solvent, precipitated nearly all the hippuric acid present in these urine samples. This variation in technique allowed the use of an OV-1 column, one which is extensively used in our laboratory for other assays.

With experience, we agree that p-hydroxyphenylactic acid is not a good internal standard for all urine samples. In our three-year experience with this method there were two patients with endogenous elevations of this substance, necessitating substitution of another internal standard (detected by internal standard peak height). Coward and Smith often varied their internal standard for individual urines, indicating the difficulty in finding a phenolic acid chemically similar to HVA and VMA (thus behaving similarly in extraction and silylation) that will never be present in human urine. We have considered adding two internal standards to all urine samples, utilizing the constancy of their peak ratios as an indicator of an endogenous compound at the internal standard location.

Although storage of VMA in methanol could yield methylation reaction products, stability of peak-height ratios to other standards indicates this most likely is not happening significantly in our system. We do not dispute that gas chromatography-mass spectrometry or gas chromatography in two different systems would be more accurate than the method we presented. We question if the additional expenditure is warranted for the purpose of providing confirmational laboratory data to clinical and surgical pathology information. In addition to the neuroblastoma data presented in our paper, we have followed the clinical course of four patients with neuroblastoma with serial VMA and HVA determinations by this method (1). The close fit of clinical and laboratory data in these patients provides evidence of the method's utility in diagnosis and prognosis of neuroblastoma, at a reasonable expense.

References


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