concentrations of 4-aminobutyric acid in the central nervous system. It has not been released for general prescription but is available on a named patient basis for treatment of intractable convulsions. It reacts with ninhydrin and can be assayed by automated amino acid analysis (2).

High-voltage electrophoresis of urine samples from two patients receiving vigabatrin yielded bands that stained strongly with ninhydrin and did not correspond to known amino acids (Figure 1). A less-intense band was also visible on thin-layer chromatography of a sample from one of the patients, but this was not a consistent finding; however, the formulation of the ninhydrin staining solution was different for electrophoresis and for thin-layer chromatography. Amino acid analysis of these samples (Biotronik LC 5001; Wissenschaftliche Gerate GmbH, Munchen, F.R.G.) revealed several unidentified peaks. We have also observed an increase in the excretion of pyroglutamic acid in these patients, presumably owing to enzyme inhibition by vigabatrin affecting the γ-glutamyl cycle (3).

Laboratories screening for amino acid abnormalities need to be aware of drug-related and other artifacts that might interfere with the detection of genuine abnormalities. This is particularly important for drugs that are used to treat children who are likely to be undergoing investigation for metabolic disease.

We thank Dr. B. L. Priestley, Children's Hospital, Sheffield, for permission to study her patients.

References


Evaluation of an Alveolar Carbon Monoxide Monoxide Analysis Method, R. Lavoie, L. Beausoleil and E. Dewaille (1) Lab. de Biochimie, Hopital Laval, 2725 chemin Ste-Foy, Sainte-Foy, QC, Canada; and (2) DSC du Centre Hospitalier de l'Universite Laval, 2050 St-Cyrille Ouest, Ste-Foy, QC, Canada

We report here our experience with an alternative to direct measurement of carboxyhemoglobin (COHb) in blood: analysis for alveolar carbon monoxide (CO). The sampling procedure is based on that of Jones et al. (1). Subjects, standing upright, emptied their lungs, took a deep breath, and held it for 20 s. They then exhaled the first 900 mL (anatomic dead space) into a polyvinyl bag (900-mL urine-collection bags; Meditron, Montreal, Canada), and, without taking another breath, completely inflated two other 900-mL bags connected with plastic tubing (1.8 L of alveolar air in equilibrium with blood).

Within 12 s we analyzed the alveolar air samples with a CO analyzer (Model 8501 BA infrared spectrometer; BendiX, Lewisburg, WV). To obtain precise digital readings, we transformed the analog output of our CO analyzer by using a digital multimeter (Model 310B; Beckman Industrial, Brea, CA), averaging the values of two multimeter readings taken 15 s apart, 2 min after the beginning of the sample aspiration.

To study the analytical recovery, we filled 15 of the 1.8-L bags with commercial CO (Médigaz, Quebec, Canada), 57.8 μL/L, analyzing five immediately, five after 12 h, and five after 24 h. At 12 and 24 h values were respectively 98.1% and 95.2% of the first measured value (CV = 0.58% for each set of measurements).

To establish the correlation with COHb, we measured concurrently in 24 volunteers (13 smokers, 11 nonsmokers) a duplicate alveolar CO sample and a venipuncture blood sample for determination of COHb. The blood samples were analyzed by standard methods with an OSM-2 Hemoxymeter (Radiometer, Copenhagen, Denmark).

The correlation between COHb and alveolar CO is expressed by the following linear least-squares regression equation: COHb (μL/L) = 539.3 CO (μL/L) - 3.3 (n = 24), standard error of estimate = 1.904, standard error of the slope = 13.12, r² = 0.9935. Values ranged from 0.6 to 52.6 μL/L (mean 13.64 μL/L). From analyses of the duplicate samples we estimated the total CV for the method (within-run analytical variation plus variation introduced by the sampling method) to be 4.16% (2). For smokers and nonsmokers the results were respectively as follows: CV = 3.14% and 6.76%, mean = 24.8 s and 1.4 μL/L.

Repeated analyses of controls gave a within-run analytical CV = 0.4% (n = 10), a cumulative CV (total analytical imprecision) = 0.87% (n = 35). No significant drift was observed during the four-month study.

The alveolar CO analysis we used is a good method for assessing CO intoxication: it is noninvasive, inexpensive, and can be easily performed by untrained subjects. Its reproducibility was excellent, showing very low variability. This procedure can be used to document most environmental and occupational exposures to CO.

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References


Improvement in the Head-Space Gas-Chromatographic Method for Determination of Three Ketone Bodies in Plasma, Masako Kimura, Naoi OgasaHarA, Kunio Kobayashi, Akira Hito, Akira Matsuoka, and Yukio Kimura (1) Dept. of Clin. Pathol. and Clin. Lab., Hyogo College of Med., 1-1, Mukogawa-cho, Nishinomiya 663, Japan; and (2) Faculty of Pharm. Sci., Mukogawa Women's Univ., Koshien Kyuban-cho, 11-68, Nishinomiya 663, Japan

We made two improvements on the procedure of the headspace gas-chromatographic method reported previously for determining the three ketone bodies in plasma (1). First, the
need for deproteinization was eliminated by diluting plasma threefold with 0.2 mol/L phosphate buffer (pH 8.0) containing 3 mg of methyl ethyl ketone per liter as the internal standard. Second, in the d-3-hydroxybutyrate (3-OHB) assay, the enzymic conversion of 3-OHB to acetone through acetoacetate (AA) was reduced from two steps to one: 3-OHB in 0.5 mL of diluted plasma sample and enzyme mixture reagent (90 μL) was converted completely to acetone in 15 min at 50 °C; the incubation time actually used was 30 min. The enzyme mixture reagent consisted of 4.6 mL of d-3-hydroxybutyrate dehydrogenase (30 kU/L), 0.7 mL of lactate dehydrogenase (LDH, 5.3 × 10⁶ U/L), 0.6 mL of β-NAD⁺ (20 mmol/L, pH 7.0), and 2.0 mL of acetoacetate decarboxylase (100 kU/L). Gas-chromatographic and reagents were the same as in the previous report, except that acetoacetate decarboxylase was purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan. In the acetone assay, we corrected the apparent acetone value by subtracting from it 3.5% of the AA concentration attributable to decarboxylation of AA by plasma albumin during the 15-min incubation at 50 °C (2–4). The actual AA concentration in plasma was calculated as 1.085 times the apparent AA concentration.

The following tabulation shows the within-day precision (CV) of three measurements of ketone bodies in plasma. The plasma components—lactate, LDH, and lipids—affect spectrophotometric results but not the results by our method.

<table>
<thead>
<tr>
<th></th>
<th>Conc. μmol/L</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>74.9</td>
<td>0.97</td>
<td>1.3</td>
</tr>
<tr>
<td>Acetoacetate</td>
<td>123.8</td>
<td>3.96</td>
<td>3.2</td>
</tr>
<tr>
<td>3-OHB</td>
<td>333.8</td>
<td>4.33</td>
<td>1.3</td>
</tr>
</tbody>
</table>

n = 20 each.

Our improved assay procedure can be used for automated analysis of the three ketone bodies in plasma. The time required for the assay, including centrifugation for plasma separation and incubation for enzymic reaction, is less than 1 h.

References


Evaluation of Sialic Acid Concentrations in Serum for the Diagnosis and Staging of Breast Cancer, W. Michael Riley,¹ Carmen Tautu,¹ Gary Verazin,² John Gregory,¹ Serene Josiah,¹ Joseph J. Prorok,² and Jack A. Alhadeff²,³ ¹Dept. of Chem., Lehigh University, Bethlehem, PA 18015; ²Lehigh Valley Hospital Center, Allentown, PA 18105; ³author for correspondence

Many glycoproteins and glycolipids from malignant cells differ in carbohydrate composition from those found in normal cells (1). Because many of these glycoconjugates contain sialic acid and can be shed into the circulation, investigators have studied total sialic acid (TSA) in serum as a marker of malignancy. Previous studies (2–6) have suggested that serum TSA concentrations are increased in breast cancer patients, but most of these studies were done on small patient groups (2–5) and without appropriate benign breast disease controls (3–5). In this current investigation, we focused on the usefulness of information on concentrations of TSA in serum for detecting and (or) staging breast cancer in a relatively large group of staged breast cancer patients and appropriate normal and benign breast disease controls.

Whole blood was drawn from 100 normal female controls, 205 patients with benign breast disease, and 164 patients with breast cancer. The breast cancer patients and patients with benign breast disease were consecutive cases from one surgical group. The blood was allowed to coagulate at 21 °C, then centrifuged at 2000 × g for 15 min. The resulting sera were stored at −20 °C until assayed for TSA as described (2). The two-tailed Student's t-test was used to determine whether the mean serum values (±SD) for TSA were significantly different in the different groups studied. Microscopic diagnosis of breast cancer was based on histologic evaluation of biopsy specimens; the final pathologic stage was determined (after definitive therapy) according to the pTNM classification (7).

The individual and mean concentrations of TSA in serum are summarized in Figure 1 and Table 1, respectively, for the normal controls, patients with benign breast disease, patients with breast cancer, and the breast-cancer patient subgroups classified from stage 1 to 4. The figure indicates considerable overlap of TSA values for stages 1, 2, and 3 and both control groups. TSA values for stage 4 patients overlapped slightly with values for normal controls but more considerably with those for benign breast disease patients. Table 1 indicates that the mean TSA concentration for breast cancer patients was significantly greater than for normal controls (P < 0.001) and patients with benign breast disease (P < 0.05). However, the mean TSA

![Fig. 1. Serum concentrations of total sialic acid (TSA) in patients in the four stages of breast cancer, in normal controls, and in patients with benign breast disease. Error bars (mean ± SD) are included for each group.](image-url)