Interferences with Potentiometry of CO2 in the Ektachem 400 Analyzer

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We have investigated the Kodak Ektachem 400 Analyzer procedure for CO2 for interferences from benzyl alcohol, benzoic acid, and several compounds structurally similar to benzoic acid. Benzoic acid in plasma, at concentrations found in neonates intoxicated with benzyl alcohol, caused a large increase in the results for CO2, as did substantially above-normal concentrations of certain fatty acids and ketoads, and toxic concentrations of aspirin. We observed a correlation between increasing benzoic acid concentrations (up to 17 mmol/L) and falsely increasing CO2 values (>47 mmol/L) obtained with the Ektachem Analyzer for samples from a neonate in the intensive-care unit, who was receiving benzyl alcohol-preserved saline solutions. Although the Ektachem CO2 procedure is simple and rapid, and in most cases accurate, questionable results are occasionally encountered, as indicated by a low anion gap or a measured CO2 exceeding that calculated from blood gas measurements. Such results require the use of another method for verification.

Additional Keyphrases: benzoic acid analogs • analytical error • blood gases • multilayer film analysis

Measurement of total carbon dioxide in blood is a valuable tool in evaluating both acid–base status and electrolyte balance. Of the many methods for determining total CO2 concentration, direct potentiometry shows promise of being the most rapid, simple, and accurate (1–4). Typically, direct potentiometry involves a liquid- or polymer-membrane electrode that is selective for either carbonate or bicarbonate ions, a reference electrode, and a metering device. In the potentiometric method of the Kodak Ektachem 400 Analyzer, disposable electrode pairs contained on small multilayer slides are hydrated with 10 µL of the patient's plasma on one side and 10 µL of reference solution on the other side; the difference in potential is determined by an electrometer in the analyzer and related through standards to the total CO2 concentration. Determination of total CO2 can be combined with determinations of sodium, potassium, and chloride by similar procedures.

Recently, certain samples from patients in our neonatal intensive-care unit demonstrated discrepancies in CO2 values as measured with the Ektachem Analyzer and the Beckman Chloride/Carbon Dioxide Analyzer. The Ektachem Analyzer gave very high total CO2 values, which led to negative anion gaps (Na+ + K+)−(Cl− + CO2); with the Beckman Analyzer, values for total CO2 and calculated anion gaps were closer to the normal range and more consistent with the clinical assessment of these patients. We found that solutions containing 9 mL of benzyl alcohol per liter, as a preservative, were being used to flush umbilical artery catheters and endotracheal tubes in each of these patients. Here we describe the effects of benzyl alcohol, its metabolite benzoic acid, and other compounds similar in structure to benzoic acid on the Ektachem assay for CO2, and discuss the influence this interference might have on clinical decisions.

Materials and Methods

For the direct potentiometry of CO2 we used the Ektachem 400 Analyzer (Eastman Kodak Co., Rochester, NY 14650) and Ektachem Clinical Chemistry Slides (CO2) according to the manufacturer's specifications. Each dry, multilayered CO2 slide contains two identical electrodes, such that a complete concentration cell is formed when 10 µL of sample fluid is deposited onto one electrode and 10 µL of reference fluid is deposited onto the other electrode. Each electrode has a buffer layer (pH 8.4); an ion-selective membrane layer, which is an ion-exchange system consisting of trietylpropylammonium chloride and membrane solvents in a vinyl resin; a reference layer of sodium chloride and potassium chloride in gelatin; a silver chloride layer; and a silver layer. The analyzer measures the difference in potential between the reference and sample electrodes in response to the activity of carbonate ions, which is proportional to the total CO2 in the sample (Eastman Kodak Co. Operator's Manual).

We also used a Beckman Chloride/Carbon Dioxide Analyzer (Beckman Instruments Inc., Fullerton, CA 92634), which measures the rate of change in pH as carbon dioxide is liberated from the sample—which in turn is directly proportional to the total CO2 concentration in the sample. The compounds evaluated for possible interference (obtained from either Sigma Chemical Co., St. Louis, MO 63178, or United States Biochemical Corp., Cleveland, OH 44122) were prepared as concentrated solutions in either deionized water or Tris HCl buffer (1 mol/L, pH 8.4). We added various amounts of these solutions to pooled plasma, keeping the final sample volume constant by adding water or buffer as appropriate.

Patients' samples, collected from heel punctures into capillary tubes containing lithium heparin as anticoagulant, or by venipuncture with syringes, were temporarily stored/handled at room temperature for as long as 24 h, then stored frozen in capped 12 × 75 mm conical-bottom plastic tubes for further analysis, if necessary. We assayed samples for CO2 by both methods within 15 min of each other to minimize losses of CO2 between analyses.

We measured benzoic acid in plasma by gas chromatography as previously described (5) by a method similar to those used in other laboratories (6).

Results

Erroneous CO2 values for patients' plasma. Figure 1 shows CO2 values as measured with the Ektachem and the Beckman Analyzers for a patient in the neonatal intensive-care unit. Beginning on the day of admission (day 1) saline solutions containing benzyl alcohol were used to flush the
patient's catheter. This patient weighed less than 1 kg at birth, had severe metabolic acidosis, and was unresponsive to bicarbonate infusion. Symptoms included increased lethargy, seizures, and gasping respirations, but renal function was normal. The disparity between the CO₂ values (greater than 30 mmol/L) measured with the two analyzers was most apparent on days 6 to 8, when the benzyl alcohol intake was about 150 mg/kg of body weight per day. On day 8, the use of solutions with benzyl alcohol was discontinued, whereupon the difference between the Ektachem and Beckman CO₂ values decreased rapidly and disappeared by day 12. Although the patient improved clinically after the use of benzyl alcohol-preserved saline was discontinued, he died later of severe pulmonary interstitial emphysema. In three other patients, similar discrepancies were noted. These discrepancies also became negligible after use of benzyl alcohol-preserved saline was discontinued.

Effects of benzyl alcohol and benzoic acid. Benzyl alcohol added to pooled plasma at several concentrations had only small effects on results with the Ektachem Analyzer and no effect on those by the Beckman Analyzer (data not shown). However, benzoic acid, a metabolite of benzyl alcohol, increased the apparent CO₂ values in the Ektachem Analyzer by about 12 and 40 mmol/L at concentrations of 5 and 7.5 mmol/L, respectively (Figure 2). Concentrations of benzoic acid as great as 16 mmol/L caused no interference in the Beckman Analyzer.

Using standard gas–liquid chromatographic techniques, we assayed plasma from the neonate described above for benzoic acid. On day 6, before use of benzyl alcohol was discontinued (Figure 1), his plasma contained 17 mmol of benzoic acid per liter, and the discrepancy in CO₂ values measured with the two analyzers exceeded 47 mmol/L. By about two and one-half days later, the benzoic acid concentration decreased to 3.5 mmol/L, and the total CO₂ discrepancy had correspondingly decreased to 8 mmol/L. These results are consistent with the data shown in Figure 2; i.e., the concentrations of benzoic acid measured in the patient's plasma were sufficient to falsely increase the Ektachem Analyzer CO₂ values and completely account for the disparity in values obtained with the two analyzers.

The nature of the interference by benzoic acid. To study the nature of benzoic acid interference in the Ektachem method for CO₂, we added various concentrations of NaHCO₃ to an aqueous solution of albumin (68 g/L) and benzoic acid (4.1 mmol/L). The benzoic acid caused a proportional error (approximately 34% at this concentration of benzoic acid) rather than a set bias (Figure 3). Buffered solutions (pH 8.4) produced the same type of interference as unbuffered solutions, confirming that the interference appeared to be CO₂ dependent rather than pH dependent. Furthermore, benzoic acid in water did not produce a false reading for CO₂ until the benzoic acid concentration exceeded 10 mmol/L, indicating that the interference was not due to a direct reaction of benzoic acid with the electrode.

Effects of other compounds. Because compounds that are structurally similar to benzoic acid may reach high concentrations in plasma in certain pathological states, we tested a series of related compounds for interference with total CO₂ determinations with the Ektachem Analyzer. At a concentration of 12 mmol/L, phenylacetic acid, benzenesulfonylic acid, and cyclohexanecarboxylic acid each caused an increase in total CO₂ of at least 45 mmol/L, similar to the increase caused by benzoic acid (Figure 4). Benzamide, however, in concentrations as high as 16 mmol/L did not interfere with the Ektachem CO₂ assay.

Because concentrations of short-chain fatty acids may increase in certain disease states, we tested some for interference. Of those we tested, caproic acid caused the greatest interference (35 mmol/L increase in measured CO₂ at a caproic acid concentration of 10 mmol/L, as compared with an increase in apparent CO₂ of more than 48 mmol/L with benzoic acid in 10 mmol/L), whereas butyric and caprylic acid in concentrations up to 16 mmol/L had little effect on the Ektachem CO₂ value (data not shown).

We also investigated the effects of six compounds found in normal plasma that are increased in certain disease states (Figure 5). α-Ketoisocaprylic acid and α-keto-β-methyl-n-valeric acid caused a large positive interference in the Ektachem CO₂ assay, 10 mmol/L of either acid producing an apparent CO₂ increase of about 50 mmol/L. β-Phenylpyruvate had a small effect, increasing the Ektachem CO₂ value by 20 mmol/L at a concentration of 16.5 mmol/L. The effects of leucine, β-hydroxybutyrate, and acetooacetate were negligible at concentrations up to 16.5 mmol/L.

Aspirin, valproic acid, and drugs taken by the affected neonates were studied for possible interference. Acetylsalicylic acid, 10 mmol/L, increased the Ektachem CO₂ value by 24 mmol/L, whereas 50% greater concentrations of salicylic acid or valproic acid were needed to produce the same CO₂ increase. Spironolactone, hydrochlorothiazide, sulfathiazole, sulfisoxazole, and "Poly-vi-sol" multivitamin preparation, in
concentrations of clinical significance, did not affect the Ektachem Analyzer assay for CO₂.

**Discussion**

Benzy1 alcohol is commonly used as a preservative in saline solutions and in liquid-form medications. Several papers have discussed the toxicity of benzy1 alcohol in neonates (7–9). The high concentrations of benzy1 alcohol and its metabolite benzoic acid found in plasma from premature neonates were probably from the large dose, in relation to body weight, of benzy1 alcohol contained in the “dead volume” of external catheters and to the inability of the immature liver to convert benzoic acid into hippuric acid. Benzoic acid concentrations in serum of premature neonates reportedly can reach 28.7 mmol/L (8). Benzoic acid concentrations of this magnitude in patient sera would falsely increase measured CO₂ to values exceeding 65 mmol/L on the Ektachem Analyzer.

Our limited study of the nature of the benzoic acid interference indicated that (a) the action of benzoic acid required the presence of CO₂, there being much less interference from benzoic acid in water alone; (b) the error in the measured CO₂ was a proportional error; and (c) the interference did not involve changes in pH (which would shift a greater proportion of total CO₂ to the carbonate form), shown by similar increases in CO₂ by benzoic acid in the presence or absence of strong buffers. The selective membrane in the CO₂ electrode of the Ektachem Analyzer, which is similar to those used by others (1, 2, 4), is an ion-exchange system consisting of trictylypropylammonium chloride and membrane solvents in a vinyl resin (Eastman Kodak Co. Operator’s Manual). A reasonable rationale to explain the observed interference is that benzoic acid increases the permeability/reactivity of the selective membrane of the CO₂ electrode. If so, then benzoic acid would be expected to affect any CO₂ electrode based on similar technology.

The normal concentrations of fatty acids in plasma range from 0.3 to 1.1 mmol/L (10). Although several factors can cause concentrations of fatty acids to increase, rarely will their concentrations be high enough (>7 mmol/L) significantly to affect the Ektachem Analyzer value for CO₂. Thus, interference by fatty acids is unlikely.

α-Ketoisocaproic acid and α-keto-β-methyl-n-valeric acid did not interfere with the Ektachem Analyzer CO₂ assay within their normal ranges (mean ± SD: 0.03 ± 0.014 and 0.009 ± 0.008 mmol/L, respectively) (11). However, increases in keto acids (to as much as 200-fold the normal value) can occur in cases of maple syrup urine disease; one affected individual had α-ketoisocaproic acid increased to 4.6 mmol/L and α-keto-β-methyl-n-valeric acid increased to 1.5 mmol/L (11). Combined, such concentrations would have falsely increased the apparent CO₂ as measured with the Ektachem Analyzer by about 15 mmol/L.

Therapeutic concentrations of salicylic acid (<2.2 mmol/L) do not interfere with the Ektachem Analyzer CO₂ assay in either of its commonly used forms, acetylsalicylic acid or salicylic acid. However, toxic concentrations are sufficient to interfere. A reported salicylate concentration of 1030 mg/L (7.5 mmol/L) (12) would increase the Ektachem Analyzer CO₂ value by 3 mmol/L. Although aspirin is rapidly converted to salicylic acid, approximately 30% remains as acetylsalicylic acid about 20 min after ingestion (13). Any acetylsalicylic acid still present in plasma would affect the Ektachem Analyzer CO₂ results even more, it being a more potent interferent than salicylic acid.

The valproic acid concentration needed to interfere significantly with the Ektachem Analyzer result for CO₂ was about 12 mmol/L, far in excess of its therapeutic range in
serum (0.35 to 0.70 mmol/L). Thus valproic acid is unlikely to affect the Ektachem Analyzer CO2 assay.

In 1982 the Food and Drug Administration urged all hospitals and pediatricians to discontinue the use of benzyl alcohol-preserved saline in treating premature infants (14). Since then, we have encountered many fewer samples with discrepancies between total CO2 values by the Ektachem and the Beckman Analyzers. Benzyl alcohol-preserved saline continues in widespread use with older patients in most hospitals, and users of direct potentiometric methods for CO2 should be aware of the potential for falsely increased CO2 values. Moreover, accidental or uninformed use of benzyl alcohol-preserved saline in neonates may occur.

The Ektachem Analyzer method for CO2 appears to be susceptible to interference by other compounds in patients' fluids, but only at concentrations rarely encountered. However, accurate CO2 values are important in several of the conditions in which these potentially interfering concentrations may arise (e.g., salicylate toxicity, maple syrup urine disease).

We routinely use the Ektachem Analyzer for CO2 measurement in our laboratory. In nearly all instances, the values correlate well with those obtained with other instrumentation and are undoubtedly correct. However, given the documented occurrence of benzoic acid interference and the fact that we continue to observe occasional though rare instances of interference from other (unknown) causes, we believe that users of the Ektachem Analyzer should be alert to the possibility of interference in particular individual patients. A low anion gap or a measured CO2 greater than that calculated from blood-gas measurements will especially suggest this possibility. In any suspicious instance the Ektachem value for CO2 should be confirmed by another method.

Note added in proof: We have been informed by Eastman Kodak Co. that new Ektachem CO2 slides being developed will include an increased concentration of a scavenger of carboxylic acids. In their preliminary experiments, Eastman Kodak reports substantially less interference from benzoic acid and acetylsalicylic acid.

References