We describe a case of sulfhemoglobinemia associated with toxic paint ingestion. Blood gases, oxygen content, and fractional hemoglobin derivatives were assayed with Radiometer 520 and OSM3 instruments. Although the CO-oximeters indicated the presence of sulfhemoglobin (SulfHb), the results were not quantitative. An OSM3 service software program was activated to obtain the actual concentrations of the hemoglobin fractions. Subsequently, we evaluated the performance of the OSM3 service program for the analysis of SulfHb by performing precision studies and comparing OSM3 results with those of an AVL 912 CO-oximeter. Retrospectively, we determined that the patient’s specimens contained 6% SulfHb. There was an obvious deviation between standard OSM3 oxyhemoglobin fraction measurements and those obtained by using its service program—the effect of a high SulfHb content.

INDEXING TERMS: blood gases • oxygen saturation • hemoglobin • toxicology

Sulfhemoglobinemia is the relatively uncommon condition of excess sulfhemoglobin (SulfHb) in the blood. The pigment is a greenish derivative of hemoglobin (Hb), with an absorption peak at 620 nm, but cannot be converted back to normal, functional Hb [1]. In vitro, SulHb forms when hydrogen sulfide (H₂S) is added to Hb, hence the name; in vivo, naturally occurring SulHb is postulated to be derived from H₂S produced by intestinal bacteria, but the mode of formation has not yet been elucidated [2, 3]. SulHb seldom exceeds 10% of the total Hb present.

We describe here a patient poisoned by paint ingestion who also had sulfhemoglobinemia. At admission, the patient was comatose, which limited the information available to clinicians. We report the clinical course of this patient and our efforts to identify and quantify SulfHb in his blood. We also describe our evaluation of a modified operating mode of the OSM3 CO-oximeter that could provide quantitative data in future episodes of sulfhemoglobinemia.

**Case Report**

A 35-year-old man found on a city street was apneic, cyanotic, and covered in white paint. The patient was intubated at the scene and gradually awakened after arrival at the emergency room. On admission, his axillary temperature was 33.1 °C. Physical examination showed paint covering his face, nose, and mouth. Diffuse rales showed on auscultation. The results of a heart examination were normal except for the presence of tachycardia. Mucosal burns secondary to toxic ingestion and the “odor of rotten eggs” on his breath were noted. On phlebotomy, his arterial blood was chocolate brown. The patient developed hematomas at phlebotomy sites and in the oral mucosa. The patient was treated with high-oxygen air during the first day and blood transfusions for 5 days (one unit per day), the effectiveness of which was monitored with blood gas analyses and fractionation of Hb derivatives; indeed, assays of Hb derivative fractions were ordered four times within first 2 h. The patient eventually developed peritonitis, necrosis of the stomach, hepatitis, acalculous cholecystitis, profound coagulopathy, and multiple organ failure. On day 39 he went into respiratory arrest and was not resuscitated.

The Human Subjects Review committee of the University of Washington, which subscribes to the ethical standards laid down in the Helsinki Declaration of 1975 as revised in 1983, authorized the use of patient information and specimens as described here.
Materials and Methods

Blood gas analysis and fractionation of Hb derivatives were performed with heparinized whole blood. We used an ABL 520 blood gas–CO-oximeter combination analyzer and an OSM3 CO-oximeter (both from Radiometer, Westlake, OH). For spectroscopic analysis of the same specimens, we used an HP 8452A spectrophotometer (Hewlett-Packard, Avondale, CA). Excess blood from the day of admission was frozen in a gas-tight syringe at −70 °C for retrospective studies. Heparinized venous blood from a healthy volunteer was used as an experimental control for the spectroscopic measurements.

Preparation of hemolysate. Hemolysates of the patient’s and the control’s blood were prepared by freezing the specimens at −20 °C overnight, thawing, and removing the stroma by centrifugation (900g for 15 min). Two drops of 10 g/L potassium cyanide solution were added to 1 mL of hemolysate from the patient’s specimen to eliminate interference from methemoglobin (MetHb) at wavelengths >600 nm.

Preparation of SulfHb-containing blood samples. Most of the sample preparation procedures for SulfHb require tonometry, centrifugation, and elimination of other Hb derivatives except oxyhemoglobin [3–7]. These complex procedures produce a nonphysiological profile for Hb fractions and cannot simulate specimens from real patients in which only the SulfHb fraction is increased. Therefore, for our method evaluation, we prepared blood samples with high proportions of SulfHb that contained other Hb derivatives as well. The preparation of SulfHb-containing samples was based on principles described by Siggaard-Andersen et al. [5] and Zwart et al. [6] but with the following modifications to prepare specimens of mixed composition:

The apparatus was constructed with a 10-mL cylindrical test tube and a 50-mL three-neck flask. About 2 mL of heparinized venous blood from healthy volunteers was placed in the test tube, 2 g of sodium sulfide was placed in the flask, and 1 mL of concentrated HCl was placed in the syringe. Glass tubing was used to connect the gas-generating flask with the blood sample in the test tube. Two to three drops (~0.08-0.12 mL) of concentrated HCl was added to the sodium sulfide, which produced H2S. A couple of minutes after the addition, the color of the fresh blood started to change from red to chocolate brown. We allowed a 30-min stabilization period for completion of the reaction and evaporation of the excess H2S. SulfHb fractions of 15–25% can be produced in blood samples by this method. Measurement of blood pH before and after the sample preparation detected no substantial pH change. Specimens with various proportions of SulfHb were obtained by diluting the above concentrate with untreated aliquots of whole blood from the same source.

Analysis of SulfHb by OSM3. The service software program on the OSM3 provides the absorbances at all six wavelengths (535, 560, 577, 622, 636, and 670 nm) and the concentration of each Hb derivative, obtained by solving linear equations. Summing the concentrations for five derivatives—oxyhemoglobin, deoxyhemoglobin, SulfHb, carboxyhemoglobin (COHb), and MetHb—yields the concentration of total Hb, and the percentage of each individual derivative present was obtained by dividing each concentration by the total Hb concentration. To compare the OSM3 service program-derived results with the results of another device, we used the Model 912 CO-oximeter from AVL Scientific Co. (Roswell, GA).

Results

Preliminary analysis. ABL results on the day of admission showed 3.5% COHb and ~3% MetHb; repeating the analysis with an OSM3 CO-oximeter yielded similar results. Flags of “SulfHb high” were displayed on both instruments. Spectroscopic analysis of hemolysate to further confirm the presence of SulfHb demonstrated an absorption band at 620 nm, not alterable by cyanide addition, thereby confirming the presence of SulfHb [8]. A repeat analysis of Hb derivatives by CO-oximeters and spectrophotometer in samples taken from the patient 7 days after admission detected no SulfHb.

Evaluation of SulfHb measurement by OSM3. In their evaluation of the performance of the OSM3, Zijlstra et al. [9] compared measurement of SulfHb by the OSM3 with that by multiwavelength measurement. Although they indicated that the fraction of SulfHb can be measured accurately, there was no detailed statistical evaluation for the SulfHb determination.

The precision of SulfHb measurements made with the service operating mode was evaluated with the SulfHb-positive aqueous dye control provided by Radiometer America (cat. no. S2160). The within-run precision study, performed by analyzing the control material 22 times, gave a CV of 0.2% for a mean SulfHb content of 8.42%. Day-to-day precision, evaluated by analyzing the control material in duplicates on 22 shifts over a 7-day period, indicated a CV of 0.4% for a mean SulfHb content of 8.43%. To evaluate the precision of the method for patients’ specimens, we analyzed in duplicate 26 specimens with SulfHb contents ranging from 0.02% to 23.4%. The mean difference between pairs was 0.013% SulfHb and the overall mean was 5.25% ± 0.05%, for a CV of 0.97%.

Finally, we compared SulfHb measurement by the OSM3 service program with measurement by AVL 912 CO-oximeter, which uses 17 wavelengths to measure the Hb derivatives and quantifies SulfHb in its routine operating mode. Twenty-seven parallel measurements were performed with both instruments. The test range was limited to 0–3.4% SulfHb because the Model 912 does not provide analysis for SulfHb proportions >3.5%. The correlation of results was good, with a slope of 1.025 and an
intercept of 0.004 ($r = 0.998$). Fig. 1 shows a Bland–Altman plot [10] of the difference between the measurements from the two instruments vs their average. The mean bias (Radiometer OSM3 – AVL 912) was 0.05% SulfHb (SD 0.06%). From these results, we conclude that measurement of SulfHb by the OSM3 service program is reproducible and agrees well with measurement by the AVL 912 at lower SulfHb values; however, the agreement deteriorates somewhat at higher SulfHb contents.

**Difference in oxyhemoglobin fraction.** Measurement of 61 samples with various proportions of SulfHb (0–23.4%) demonstrated a deviation between the oxyhemoglobin fractions calculated from concentrations obtained with the OSM3 service program and those originally reported by the routine analysis mode. This difference in values for the oxyhemoglobin fraction originates from the fact that SulfHb is omitted as a component of total Hb as calculated in the routine operational mode. This deviation, calculated as the difference between routine mode output and service mode output, is linearly related to percentage of SulfHb in the samples. The correlation equation is:

$$\text{difference} = -0.215 + 1.11 \times \text{SulfHb} \quad (r = 0.997).$$

**Retrospective study of the patient’s specimens.** After the OSM3 service program was evaluated, we carried out a retrospective study with the patient’s specimens from day 1. Table 1 displays the results. Because the specimen had been frozen for 2 months, no negative MetHb was present. For each analysis, the results for the operating mode were compared with the calculated values based on the service program. According to the manufacturer’s users’ handbook, a SulfHb presence of ~10% decreases the MetHb measurement by ~3.5% and increases the COHb by ~2.5%. This would explain the negative result for MetHb and the possibly increased COHb values obtained on day 1. Lack of quantitative monitoring techniques on day 1 meant that we could not determine the initial SulfHb composition. However, our retrospective data showed that a high amount of SulfHb was clearly present, in concentrations sufficient to cause the central cyanosis observed on admission.

**Discussion**

In our major metropolitan trauma care center, we have elected to make the rapid quantification of SulfHb a standard practice whenever its presence is flagged by CO-oximetry. Our data show that comparable results can be achieved with both the AVL and the Radiometer CO-oximeters if the latter are modified to function in the service mode. Both precision and accuracy are acceptable. The disadvantage of the modified OSM3 configuration is that only a company representative can turn it on and it is not part of the original claims for the device; however, unless the service mode is in use, the oxyhemoglobin fraction reported for SulfHb-containing specimens may be in error.

The ingestion of paint has not previously been described as a cause of sulfhemoglobinemia. Drug overdose is the most widely reported cause. Of 62 cases reviewed at the Mayo Clinic [11], all were associated with just four drugs, taken alone or in combination with other drugs: Bromo-Seltzer, acetanilide, sulfonamide, and phenacetin. Although constipation, which could lead to drug metabolism or modification by gut flora, was present in some of the 62 cases, its absence in others suggests that intestinal

**Table 1. Retrospective analysis with OSM3 CO-oximeter of two day 1 specimens from patient.**

<table>
<thead>
<tr>
<th>Hemoglobin derivatives</th>
<th>Specimen 1</th>
<th>Specimen 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Routine mode</td>
<td>Service mode</td>
</tr>
<tr>
<td>Total Hb, g/L</td>
<td>105</td>
<td>–</td>
</tr>
<tr>
<td>O$_2$Hb, %</td>
<td>89.3</td>
<td>83.8</td>
</tr>
<tr>
<td>COHb, %</td>
<td>4.1</td>
<td>3.9</td>
</tr>
<tr>
<td>SulfHb, %</td>
<td>High</td>
<td>6.2</td>
</tr>
<tr>
<td>MetHb, %</td>
<td>1.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

O$_2$Hb, oxyhemoglobin.
conversion of the drugs does not account for SulfHb formation in all cases. Three reports have described sulfhemoglobinemia associated with phenazopyridine ingestion [12–14], and several others report dapsonine (“DDS”) as a cause [2, 15–18]. The synthesis of phenazopyridine from diazotized aniline suggests that aniline, a documented chemical cause of SulfHb, may be a contaminant in the phenazopyridine preparations and cause illness. Metoclopramide can produce both methemoglobinemia and sulfhemoglobinemia [19]. Occupational exposure to H₂S gas is also reportedly a cause of sulfhemoglobinemia but this is controversial [20–22]. Sulfhemoglobinemia was described in four fatal cases of acute exposure to H₂S gas associated with industrial waste effluents or sewage [23]; the greenish skin color of massive SulfHb occurred in only one of these cases. A high incidence of sulfhemoglobinemia reported among the population of a city with excessive environmental pollution from volatile sulfur-containing compounds [24] may have been caused by either acute or chronic exposure to the volatiles. In our setting, SulfHb measurement is requested or a case of sulfhemoglobinemia is observed every 3–4 months.

Visual assessment of cyanosis is relatively unreliable. Tissue discoloration caused by SulfHb may be mistaken for that caused by reduced (deoxy-) Hb [25]. In a complex case of unknown etiology, flagging SulfHb alerts the clinician to the cause of the discoloration and the remote potential for tissue hypoxia. The clinical emergency presented by our patient is similar to many descriptions of poisonings with sulfhemoglobinemia, in that the patient was found comatose and unable to describe what had been ingested. The fact that four sets of CO-oximeter assays were ordered in 2 h probably reflects the refractoriness of the cyanosis and the clinician’s uncertainty about its origin, given that hypoxemia was not present. Central cyanosis was evident and as such required some differential evaluation in the face of toxic ingestion. Carpenter, in a recent review of cyanosis, classified sulfhemoglobinemia in the category of causes of “central cyanosis”—the blue to slate-like discoloration of the sublingual region and tongue—which “alone indicates a probable medical emergency” [26]. Shapiro et al. similarly taught that cyanosis most often is indicative of tissue hypoxia caused by the presence of reduced Hb, so that cyanosis “demands a careful and thorough clinical evaluation” [27]. A SulfHb concentration of <5 g/L can produce a skin discoloration equivalent to that produced by reduced Hb at 50 g/L, i.e., discernible cyanosis. Alternately, had the cyanosis been associated with MetHb, the more frequent type of poisoning that causes central cyanosis, administration of methylene blue might have been indicated. For our patient, oxygen administration was a first level of support after attempting to neutralize the effects of the toxic ingestion. The dysfunctional Hb decreases the fraction of Hb available to carry oxygen. Volume expansion for the management of hypotension, blood replacement, and other clinical choices could be influenced by rapid identification of a SulfHb burden in a multiply compromised patient. Finally, data from monitoring transcutaneous oxygen saturation, commonly used to survey for changes and provide alarms, are misleading in the presence of sulfhemoglobinemia.

In this case, the patient was hemorrhaging and received replacement blood daily for 5 days. This explains why SulfHb fell below the OSM3 alarm limits before the theoretical 120 days. Lim and Lower suggest exchange transfusion as a means of managing extreme sulfhemoglobinemia [13]; this would be rare, however, because in many cases the patient can tolerate high amounts of SulfHb. Reports of SulfHb tolerance display little consistency. Some describe proportions of 20% to 60% as benign for some patients [1, 12], but these lack evaluation of tissue oxygen status or of its influence on the course of patient outcome in the face of cardiac and pulmonary involvement. At least one textbook states that no treatment is needed for sulfhemoglobinemia except to remove the toxic substance that produces it [28].

In conclusion, we present a case of sulfhemoglobinemia possibly caused by paint ingestion, an association that has not been reported before. The case was sufficiently complex during the first 24 h to elicit four measurements of Hb fractions in 2 h. We also describe a method for quantifying SulfHb with an OSM3 if alternative technology is unavailable (the manufacturer makes no claims for this application). Our evaluation of SulfHb measurement by the OSM3 service program demonstrated its reproducibility and good agreement with results obtained with an AVL 912 at SulfHb fractions <3.5%. Finally, we observed a deviation in measurements of fractional oxyhemoglobin in the routine operation mode when high proportions of SulfHb are present. Rapid quantification of SulfHb in the emergency department is rarely needed in an urban trauma hospital, but we believe this case illustrates that real-time analysis is possible and may be useful when such an occasion arises.

We acknowledge the cooperation and technical assistance of the Rapid Response Laboratory staffs at the University of Washington Medical Center and at Harborview Medical Center. AVL Scientific Corp. provided the 912 CO-oximeter for this work, and Radiometer America provided technical instructions for access to the OSM3 service program.

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