Laboratory Assessment of Oxygenation in Methemoglobinemia

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Background: This case conference reviews laboratory methods for assessing oxygenation status: arterial blood gases, pulse oximetry, and CO-oximetry. Caveats of these measurements are discussed in the context of two methemoglobinemia cases.

Cases: Case 1 is a woman who presented with increased shortness of breath, productive cough, chest pain, nausea, fever, and cyanosis. CO-oximetry indicated a carboxyhemoglobin (COHb) fraction of 24.9%. She was unresponsive to O_2 therapy, and no source of carbon monoxide could be noted. Case 2 is a man who presented with syncope, chest tightness, and signs of cyanosis. His arterial blood was dark brown, and CO-oximetry showed a methemoglobin (MetHb) fraction of 23%.

Issues: Oxygen saturation (SO_2) can be measured by three approaches that are often used interchangeably, although the measured systems are quite different. Pulse oximetry is a noninvasive, spectrophotometric method to determine arterial oxygen saturation (S_aO_2). CO-oximetry is a more complex and reliable method that measures the concentration of hemoglobin derivatives in the blood from which various quantities such as hemoglobin derivative fractions, total hemoglobin, and saturation are calculated. Blood gas instruments calculate the estimated O_2 saturation from empirical equations using pH and PO_2 values. In most patients, the results from these methods will be virtually identical, but in cases of increased dyshemoglobin fractions, including methemoglobinemia, it is crucial that the distinctions and limitations of these methods be understood.

Conclusions: SO_2 calculated from pH and PO_2 should be interpreted with caution as the algorithms used assume normal O_2 affinity, normal 2,3-diphosphoglycerate concentrations, and no dyshemoglobins or hemoglobinopathies. CO-oximeter reports should include the dyshemoglobin fractions in addition to the oxyhemoglobin fraction. In cases of increased MetHb fraction, pulse oximeter values trend toward 85%, underestimating the actual oxygen saturation. Hemoglobin M variants may yield normal MetHb and increased COHb or sulfhemoglobin fractions measured by CO-oximetry.

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The purpose of this case conference is to present the limitations and distinctions of the methods used to assess oxygenation status, particularly in cases of increased dyshemoglobin fractions such as methemoglobinemias.

Cases

CASE 1

A 44-year-old female presented to the emergency department with complaints of shortness of breath, productive cough, chest pain, and subjective fever for 5 days. Pertinent findings were acral and perioral cyanosis, tachypnea, and bilateral infiltrates on the chest x-ray. Her medical history was notable for depression, recent initiation of interferon treatment for hepatitis C, and smoking. Two years before admission, she was diagnosed with familial methemoglobinemia at another hospital. Admission laboratory results (reference intervals in parentheses) were notable for a hemoglobin concentration of 160 g/L (121–154 g/L). CO-oximetry performed on a Radiometer 625 ABL showed a decreased arterial oxyhemoglobin fraction (FO_2Hb)[1] of 71.5% (90–95%), a methemoglobin (MetHb) fraction of 1.1% (<2%), an increased carboxyhemoglobin (COHb) fraction of 24.9% (0.5–1.5%), and <1% sulfhemoglobin (SHb; reference interval, <1%). At the time of presentation on room air, the arterial oxygen saturation

[1] Nonstandard abbreviations: FO_2Hb, oxyhemoglobin fraction; MetHb, methemoglobin; COHb, carboxyhemoglobin; SHb, sulfhemoglobin; S_aO_2, arterial oxygen saturation; HbM, hemoglobin M; O_2Hb, oxyhemoglobin; HHb, deoxyhemoglobin; ODC, oxygen dissociation curve; DPG, 2,3-diphosphoglycerate; and O_2sat, estimated oxygen saturation.

(S_aO_2) measured by pulse oximetry was 88% and arterial blood gas results were as follows: pH = 7.42 (7.35–7.45); P_aCO_2 = 34 mmHg (35–45 mmHg); P_aO_2 = 74 mmHg (80–105 mmHg). On 4 L of supplemental oxygen, pulse oximetry measured an oxygen saturation of 90% and arterial blood gas results were as follows: pH = 7.40 (7.35–7.45); P_aCO_2 = 36 mmHg (35–45 mmHg); P_aO_2 = 255 mmHg (80–105 mmHg).

The patient was admitted to the medical intensive care unit, treated with 100% oxygen through a rebreather face mask, and started on antibiotics (azithromycin and ceftriaxone) with diagnoses of pneumonia and carbon monoxide poisoning after confirmation from the chemistry laboratory that the MetHb and COHb results were not mistranscribed or reversed. CO-oximetry measured at 24 and 48 h after presentation showed that the COHb decreased to 16.5% and 12.0% and FO_2Hb increased to 81.4% and 77%, respectively, whereas MetHb remained <2% and SHb <1%. This slow decrease of COHb was inconsistent with a COHb half-life of 5–6 h with supplemental O_2 and of 1.5 h on 100% hyperbaric oxygen therapy. Additionally, multiple interviews with the patient and family members did not identify a likely source of accidental or intentional carbon monoxide poisoning. Consultations were requested from the clinical chemistry, toxicology, and hematology services to explore alternative explanations for the increased COHb. The clinical chemistry service recommended analyzing arterial blood on an alternative, continuous-wavelength CO-oximeter (Radiometer ABL 735), which gave a FO_2Hb of 73.6%, a COHb fraction of 3.1%, and an increased MetHb fraction of 5.0%. It is important to note that all values were flagged as unreliable according to the software rules of the instrument. On the basis of the additional CO-oximetry data and the patient's improving clinical course, a provisional diagnosis of hemoglobin M (HbM) disease was made, which was subsequently confirmed as HbM Saskatoon by a methemoglobinemia evaluation performed at Mayo Laboratories and by direct amino acid sequencing of the patient's hemoglobin at Washington University.

The patient was discharged to home on day 8 with a diagnosis of community-acquired pneumonia that responded to antibiotic therapy and congenital methemoglobinemia attributable to a HbM variant.

CASE 2
A 52-year-old male with a past history of myocardial infarction and asthma presented to the emergency department after a syncopal episode while dancing at a nightclub. In the emergency department, he was anxious and complained of chest tightness. His current medications included aspirin, isosorbide mononitrate, albuterol, and beclomethasone inhalant. Physical examination showed that he was afebrile with a respiratory rate of 26/min, blood pressure of 75/55 mmHg, and pulse rate of 112/min. A pallor complexion with cyanotic lips, ears, hands, and feet was noted. Lungs and heart examinations and

the chest x-ray were normal. Continuous pulse oximetry indicated 87% oxygen saturation while breathing room air, increasing to 90% with 6 L of nasal oxygen. Arterial blood gas values while breathing 6 L of oxygen were as follows: pH = 7.44, P_aCO_2 = 31 mmHg; P_aO_2 = 123 mmHg. Arterial blood was noted to be dark brown, and CO-oximetry analysis showed a MetHb fraction of 23% (<2%), an FO_2Hb of 74.5% (90–95%), and COHb fraction of 0.6% (0.5–1.5%). Although he initially denied using illicit drugs, the patient confirmed that he had smoked marijuana and inhaled "amyl nitrite" before the onset of symptoms. A blood toxicology screen showed an ethanol concentration of 1520 mg/L. All other laboratory tests were normal. His chest discomfort resolved with the supplemental oxygen treatment.

Cyanosis resolved 45 min after an intravenous dose (150 mg) of methylene blue was administered over 5 min to treat the methemoglobinemia. Cardiac troponin I measurements at 0, 6, and 12 h after presentation indicated no myocardial damage. Repeat electrocardiograms remained unchanged, and CO-oximetry 12 h after admission showed a MetHb value of 0.6%. The patient was discharged on day 2 with total resolution of symptoms. At discharge, the patient described repeated inhalations of nitrite over a 30-min period before his syncopal episode and turned over a 10-mL unmarked colored bottle containing 5 mL of remaining liquid later identified as butyl nitrite by gas chromatography. The patient's NADH methemoglobin reductase activity was determined to be 15 U/g of Hb (10.1–19.4 U/g of Hb).

Discussion
HEMOGLOBIN AND OXYGEN SATURATION
A small amount of molecular oxygen (O_2) is dissolved in blood, whereas the majority (98%) is bound to hemoglobin. Hemoglobin, the O_2 transport molecule in blood, comprises four subunits: two α and two non-α (e.g., β, γ, or δ) subunits. Each subunit contains seven helices and a porphyrin heme iron moiety. It is this heme iron that reversibly binds O_2. The heme iron can exist in the Fe(II) or Fe(III) oxidation state. Only the Fe(II) ion is capable of binding O_2. Most clinically important gene mutations reduce the quantity of α- or β-chain synthesis (thalassemias) or the solubility of hemoglobin (HbS or HbC). Rarely, gene mutations alter the affinity of hemoglobin for oxygen. Hemoglobins can be divided into two classes: those capable of binding O_2 (normal hemoglobins) and hemoglobin derivatives incapable of binding O_2 (dyshemoglobins). The normal hemoglobins include oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb). The dyshemoglobins include COHb, MetHb, and SHb. COHb is produced when carbon monoxide binds to the Fe(II) in the place of O_2. SHb is a degradation product of hemoglobin and is thought to consist of sulfur bound to the pyrrol group of the porphyrin ring (1). MetHb represents the oxidized, deoxy form [Fe(III)-Hb] of hemoglobin, to which O_2 cannot bind.

Oxygen binds cooperatively to Fe(II) hemoglobin, as depicted by the oxygen dissociation curve (ODC) shown in Fig. 1. The ODC relates O_2 saturation of hemoglobin to O_2 tension (1). At high partial pressures of O_2, usually in the lungs, O_2 binds to hemoglobin to form O_2Hb. As the erythrocytes circulate through tissues, O_2Hb releases O_2 in response to the decreased O_2 partial pressure. The cooperativity of O_2 binding produces the sigmoidal shape of the ODC. Cooperativity refers to the characteristic that the remaining hemoglobin chains will have greater affinity for O_2 after one of the subunits has already bound O_2. Thus, when O_2 binds to the first subunit of HHb, it increases the affinity of the remaining subunits for O_2. O_2 is bound to the second and third subunits, and O_2 binding is further, incrementally strengthened so that at the normal O_2 tension in lung alveoli, hemoglobin is fully saturated with O_2. The same process works in reverse: once fully loaded hemoglobin releases one O_2 molecule, it releases the next more easily.

Increased fractions of COHb and MetHb are dangerous for two reasons: (a) COHb and MetHb inhibit O_2 transport by blocking heme iron-binding sites; and (b) when one or more iron atoms has bound carbon monoxide or been oxidized, the hemoglobin conformation is changed so that the O_2 affinity of the remaining heme groups is increased, thus shifting the ODC to the left, and decreasing O_2 delivery to tissues. Several other conditions, such as temperature, pH, and 2,3-diphosphoglycerate (DPG) concentration can change the oxygen affinity of hemoglobin and shift the ODC. The PO_2 at 50% oxygen saturation is

referred to as the P_{50} (Fig. 1). The P_{50} is inversely related to the O_2 affinity and is used as an indicator of the hemoglobin O_2 affinity. Decreased pH, increased DPG concentration, and increased temperature shift the ODC to the right, increasing the P_{50}, which indicates decreased O_2 affinity (1).

LABORATORY MEASUREMENTS OF OXYGENATION STATUS

Three distinct analytical approaches exist for determining oxygenation status of blood (Table 1). These are often used interchangeably by many nonlaboratorians who consider them to be equivalent measurements because in healthy individuals without dyshemoglobins, the values from all three approaches are virtually identical. It is important to consider the clinical relevance of oxygen saturation vs fractional oxyhemoglobin in cases of increased COHb or MetHb.

The three terms, SO_2, FO_2Hb, and estimated oxygen saturation (O_2sat) have distinct definitions set by the NCCLS (2, 3). The NCCLS has recommended the use of the term "oxygen saturation" to indicate the amount of hemoglobin capable of transporting O_2 and "fractional O_2Hb" to represent the fraction of oxygenated hemoglobin. The terms "fractional saturation" and "functional saturation" refer to the FO_2Hb and SO_2, respectively. However, these latter terms can be misleading: the definition of saturation does not apply to FO_2Hb, and functional saturation is redundant. Saturation is calculated from measured parameters according to the following equations:

Total hemoglobin concentration (tHb)

$$= cO_2Hb + cHHb + cMetHb + cCOHb + cSHb$$

$$\text{Hemoglobin oxygen saturation (SO}_2\text{)} = \frac{cO_2Hb}{cO_2Hb + cHHb}$$

The hemoglobin O_2 saturation can be measured by pulse oximetry (often labeled as S_aO_2 or S_pO_2) or CO-oximetry. Regardless of the method used, oxygen saturation is a measure of the fraction of oxygenated hemoglobin in relation to the amount of hemoglobin capable of transporting O_2:

$$\text{Fractional oxyhemoglobin (FO}_2\text{Hb)} = \frac{cO_2Hb}{ctHb} \times 100\%$$

The fractional oxyhemoglobin can only be measured by a multiwavelength spectrophotometer such as a CO-oximeter. The FO_2Hb represents the fraction of oxyhemoglobin in relation to the total hemoglobin (tHb) present (including the non-oxygen-binding hemoglobins). The fraction of any of the hemoglobin derivatives may be calculated in the same manner. In healthy individuals, the S_aO_2 and FO_2Hb are approximately equal. In the presence of substantial dyshemoglobin fractions, FO_2Hb values will be considerably lower than the saturation determined by
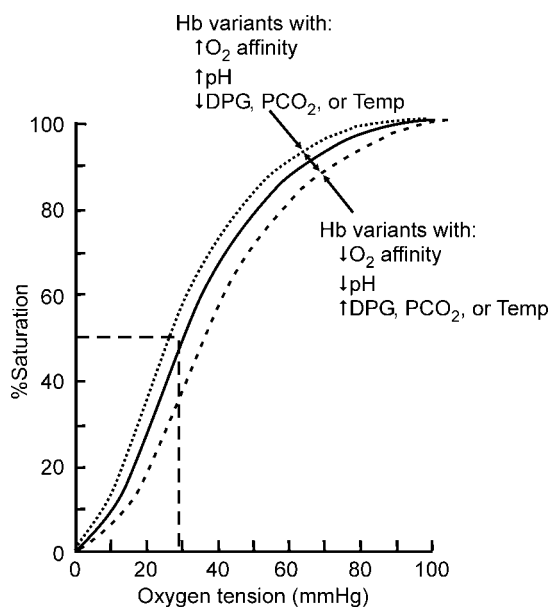

Fig. 1. ODC of hemoglobin.
The percentage saturation of hemoglobin with oxygen at different oxygen tensions is depicted by the *sigmoidal curves*. The P_{50}, indicated by the *dashed lines*, is ~27 mmHg in healthy erythrocytes. Modifications of hemoglobin function that increase oxygen affinity shift the curve to the left (*dotted curve*), whereas those that decrease oxygen affinity shift the curve to the right (*dashed curve*). *Temp*, temperature. Reprinted with permission from *Kelley's Textbook of Internal Medicine, 4th ed.* (2000, Fig. 241.2). © Lippincott Williams & Wilkins.

Table 1. Analytical methods for measuring oxygen saturation.

Device	Specimen	Measurement	Reported data	Advantages	Disadvantages	Notes
Arterial blood gas analyzer	Blood	Partial pressure of oxygen dissolved in whole blood at an electrode	Po_2 and SO_2 (O_2sat)	Also measures pH and Pco_2	Invasive, calculated SO_2 may be inaccurate in hospitalized patients and if dysHb[a] present	If the ABG and pulse oximetry are discrepant, an abnormal Hb may be present
Pulse oximeter	Transcutaneous	Absorbance at two wavelengths (660 and 940 nm) in pulsatile blood	SO_2	Noninvasive, continuous bedside monitoring	Inaccurate when interfering substances are present: MetHb, COHb, SHb, dyes	When MetHb is >25%, the pulse oximeter will read saturation of 75–85%
CO-oximeter	Blood	Absorbance of Hb derivatives by multiple wavelengths of light	SO_2, FO_2Hb, FHHb, FMetHb, FCOHb, FSHb, ctHb	Measures the concentration of Hb species	Invasive, performed in laboratory; not all instruments report SHb and total bilirubin	Most accurate method; even in cases of CO poisoning and methemoglobinemia. May be inaccurate in cases of HbM

[a] dysHb, dyshemoglobin; ABG, arterial blood gas.

pulse oximetry. Although the S_aO_2 typically remains within the normal limits in cases of carbon monoxide poisoning or methemoglobinemia, the O_2 capacity may be severely decreased, leading to fatal outcomes.

The last approach for assessing O_2 saturation is the estimated oxygen saturation (O_2sat), which is calculated by blood gas analyzers from pH, PO_2, and hemoglobin values by use of empirical equations *(4)*. These methods for measuring O_2 saturation parameters are discussed in more detail in the following sections.

SPECTROPHOTOMETRIC METHODS OF ANALYSIS

Hemoglobin molecules are colored and are thus easily measured by spectrophotometric methods. The altered molecular structure of the heme moiety in different hemoglobin derivatives gives rise to unique absorbance spectra (Fig. 2). These characteristic absorbance spectra make it possible to determine the concentration of each hemoglobin derivative present in the mixture.

Spectrophotometric methods of analysis are based on the principle that light absorbance is proportional to solute concentration. Absorbance is measured by transmitting light of a specific wavelength across a medium and measuring the intensity on the other side. Absorbance is an additive property, and it is necessary to measure absorbance at n wavelengths to obtain the concentration of n analytes. Pulse oximetry is a simple spectrophotometric method that uses only two wavelengths and measures the contribution of only two hemoglobin species, O_2Hb and HHb. More complex photometers (CO-oximeters) are available that measure absorbance at 128 wavelengths and can report *c*tHb and SO_2 in addition to FHHb, FO_2Hb, FCOHb, FHbF, FMetHb, and FSHb.
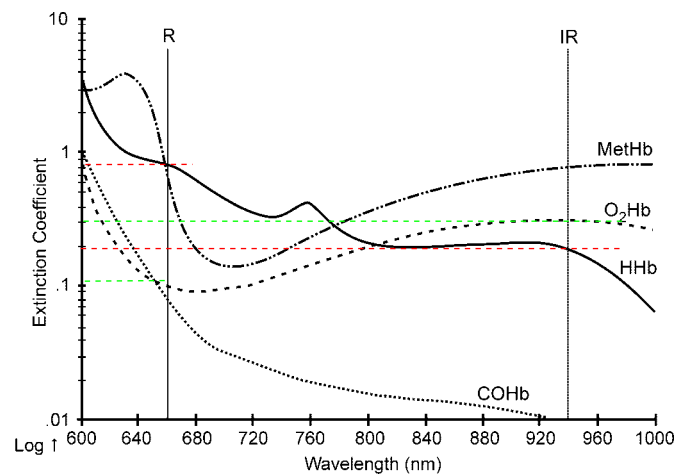

Fig. 2. Extinction curves of purified hemoglobin derivatives showing the relationship of reduced hemoglobin (*solid line*), O_2Hb (*dashed line*), COHb (*dotted line*), and MetHb (*dot-dashed line*) to light absorbance.

Vertical lines indicate the monitoring wavelengths used in most pulse oximeters for the red (*R*) and infrared (*IR*) at 660 and 940 nm, respectively. Modified from Wukitsch et al. Pulse oximetry: analysis of theory, technology, and practice. *J Clin Monit* 1988;4:290–301 [Fig. 2 (p. 292); with kind permission from Kluwer Academic Publishers (Courtesy of Ohmeda)].

Ratio R/IR: Abs.660nm/Abs 940nm => donneSaO2 (lecture sur une courbe calibrée)

Ex: SaO2 de 0% = 0.8/0.2 = environ 4
Ex: SaO2 de 100% = 0.12/0.3 = environ 0.4
=> Si SaO2 85% = (15%x4) + 85%x0,4= 0,6+0,34= environ 1
En cas de MetHb: on aura une **sur**estimation de la Sa02 en
dessous de 70% et une **sous**estimation de la Sa02 en dessus

Pulse oximetry. Pulse oximeters are designed to measure arterial oxygen saturation (S_aO_2) by measuring a ratio of pulsatile light transmission through a cutaneous vascular bed (e.g., digits or ear lobe) at two wavelengths (typically 660 and 940 nm) *(5, 6)*. Both O_2Hb and HHb absorb light at 660 and 940 nm; it is the ratio of the absorbance at the two wavelengths during the static and pulsatile phases from which a pulse oximeter determines S_aO_2. The ratio of the red signal (660 nm) to the near-infrared (940 nm) corresponds to an oxygen saturation from a calibration curve programmed into the device. The machines are calibrated from empiric pulse oximetry data obtained from healthy individuals after induction of hypoxemia with simultaneous CO-oximetry measurement of arterial oxygen saturation *(5)*. For example, an absorbance ratio (A_{660}/A_{940}) of 0.43 corresponds to 100% oxygen saturation, and a ratio of 3.4 corresponds to 0% oxygen saturation on most pulse oximeters *(7)*. In the absence of a dyshemoglobin, an absorbance ratio of 1.0 corresponds to an oxygen saturation of ~85% *(7)*.

The SO_2 measured by a pulse oximeter may be clinically misleading in the presence of dyshemoglobins such as COHb and MetHb. This is because the oxygen-carrying capacity of the blood will be greatly decreased when COHb or MetHb are >50% but the S_aO_2 from the pulse oximeter will be close to normal. Thus, during the initial evaluation of oxygen status in an unconscious, dyspneic, or otherwise impaired patient, it is important not to rely only on pulse oximetry but to also determine the presence of high fractions of dyshemoglobins by CO-oximetry. Fig. 3, reproduced from the study by Barker and Tremper *(8)* in dogs ventilated with carbon monoxide, shows that S_aO_2 measured by pulse oximetry clearly differs from the FO_2Hb measured by CO-oximetry with increasing COHb. O_2Hb decreases linearly with increased COHb as carbon monoxide replaces O_2 at the heme iron. Although the S_aO_2 remains >90% with increasing COHb, this value provides little information regarding the clinical picture. It is important to note that at a lethal concentration of 70%
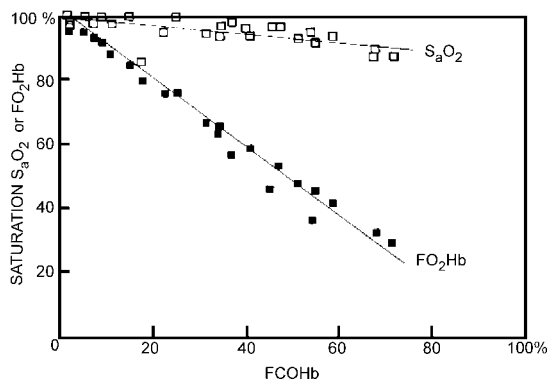
COHb, the pulse oximeter reported a saturation of 90%, although FO_2Hb by CO-oximetry had decreased to 30%, which more accurately reflects the decreased oxygen capacity. In cases of human exposure to carbon monoxide, the difference in S_aO_2 measured by pulse oximetry and FO_2Hb by CO-oximetry has been termed the pulse oximetry gap and is typically 3–5% in healthy individuals *(9–12)*.

Another study in dogs with intrathecal benzocaine-induced methemoglobinemia investigated the effect of increased MetHb on pulse oximetry values *(13)*. The comparison of S_aO_2 measured by pulse oximetry and the FO_2Hb from CO-oximetry in the presence of increased MetHb is shown in Fig. 4. There is a clear difference in the S_aO_2 and the FO_2Hb as the MetHb fraction increases. As expected, the FO_2Hb reported by CO-oximetry decreases linearly with increasing MetHb. When the MetHb concentration increases above 35%, the S_aO_2 reaches a plateau of 84–86% saturation. At this point, the pulse oximetry reading is virtually independent of the MetHb concentration. This trend toward 85% is noted in both cases presented here and in other examples of case reports of symptomatic methemoglobinemia in the literature *(14–17)*. Zijlstra et al. *(18)* reported that although the absorbance interference attributable to COHb produces an insubstantial error in S_aO_2 measured by pulse oximetry, increased MetHb gives an underestimation when S_aO_2 is >70% or an overestimation at S_aO_2 <70%.

This phenomenon may be explained by examining the spectroscopic signatures of the hemoglobin derivatives and understanding the principles of pulse oximetry. MetHb absorbs light almost equally at both 660 and 940
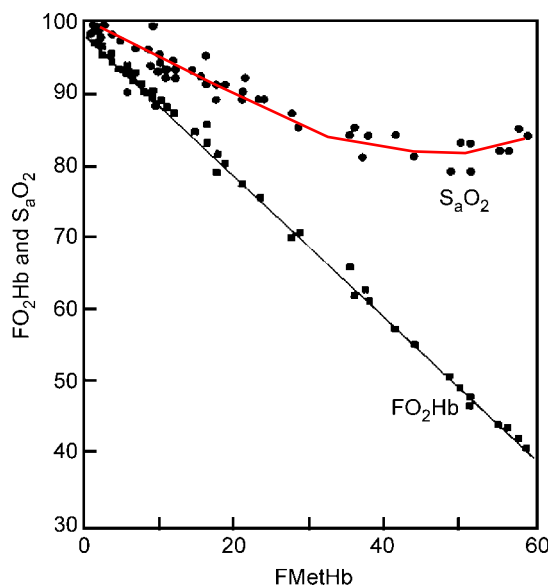

Fig. 3. S_aO_2 and FO_2Hb vs fractional FCOHb at inspired oxygen fraction (FiO_2) = 1.0.

Reprinted with permission from Barker and Tremper. The effect of carbon monoxide inhalation on pulse oximetry and transcutaneous Po_2. *Anesthesiology* 1987;66:677–9.

Fig. 4. S_aO_2 and FO_2Hb vs fractional methemoglobin (*FMetHb*) at inspired oxygen fraction (FiO_2) = 1.0.

The *line* is $FO_2Hb = 100 − FMetHb$. Reprinted with permission from Barker et al. Effects of methemoglobinemia on pulse oximetry and mixed venous oximetry. *Anesthesiology* 1989;70:112–7.

nm (Fig. 2). Although MetHb absorbance at 660 nm is similar to that of HHb, its absorbance at 940 nm is markedly greater than that of either HHb or O_2Hb. As a result, MetHb will contribute to the perceived absorbances of both HHb and O_2Hb in a pulse oximeter. This increases the numerator and the denominator of the 660 nm/940 nm absorbance ratio, approximating unity. As mentioned above, a ratio of 1.0 corresponds to a saturation of 85% on many pulse oximeter calibration curves.

Barker and coworkers *(8, 13)* used animal models to demonstrate that pulse oximetry measurements in the presence of increased COHb or MetHb are not representative of the clinical picture, as seen in Figs. 3 and 4. These studies demonstrate that although the S_aO_2 typically remains within the normal limits in cases of carbon monoxide poisoning or methemoglobinemia, the O_2 capacity may be severely decreased, leading to fatal outcomes. Similar observations have been described in human case reports *(6, 10, 12, 19, 20)*. The clinically relevant value in these cases is not the S_aO_2 but the FO_2Hb and COHb or MetHb fractions, which can be measured only by a CO-oximeter. Because of spectroscopic interference, increased MetHb concentrations may have substantial effects on pulse oximetry readings, whereas COHb does not spectrophotometrically alter the S_aO_2 measured by a pulse oximeter [Fig. 3 and Ref. *(18)*].

CO-oximetry. The early CO-oximeters were simplified spectrophotometers that measured light absorbance at four or more wavelengths *(21)*. The wavelengths were chosen to correspond to the specific absorbance characteristics of the hemoglobin derivatives (Fig. 2). By the mid-1980s, CO-oximeters could measure fractions of HHb, O_2Hb, COHb, MetHb, and SHb by use of six wavelengths. Current models measure absorbance at 128 wavelengths and are called continuous wave spectrophotometers. The additional wavelengths improve the accuracy of the spectrum, minimize or eliminate interfering substances, and enable reporting of other derivatives *(22)*. A peak absorbance of light near 630 nm is used to characterize MetHb. On the basis of the reporting algorithm, CO-oximeters may report the various hemoglobin fractions (i.e., O_2Hb, MetHb, COHb, and SHb) and/or the SO_2. The assumption that FO_2Hb is equivalent to S_aO_2 does not hold in cases of increased COHb or MetHb fractions; therefore, CO-oximetry should be used to assess oxygen-carrying status via FO_2Hb and the dyshemoglobin fractions until the presence of these dyshemoglobins is ruled out. Furthermore, it must be pointed out that neither S_aO_2 nor FO_2Hb reflects total hemoglobin-bound oxygen because this depends on Po_2 and total Hb concentration.

NONSPECTROPHOTOMETRIC METHODS OF ANALYSIS
Blood gas analyzers are composed of a series of electrodes that provide information regarding acid/base status (pH), respiratory function (Pco_2), and oxygenation (Po_2). High-impedance electrodes measure voltage changes to determine pH and Pco_2, whereas Po_2 is measured as current changes at a Clark electrode *(4)*. Po_2 refers to dissolved gas in blood. The concentration of dissolved oxygen (cdO_2) is linearly related to the partial pressure of oxygen in blood, according to Henry's law, where αO_2, the solubility constant of O_2, is 0.03 mL $O_2 \cdot L^{-1} \cdot mmHg^{-1}$ *(2)*:

$$cdO_2 = \alpha O_2 \cdot Po_2$$

The relationship between the calculated SO_2, often referred to as O_2sat, and Po_2 may not always be linear. SO_2 is calculated from the pH and Po_2 values and the standard ODC for oxygen saturation *(2, 4)*. Unfortunately, this approach to calculating SO_2 assumes a normal ODC. In hospitalized patients, the assumption that the ODC is normal is frequently erroneous because it relies on normal pH, temperature, DPG concentrations, and no inherited or acquired dyshemoglobins.

Salyer et al. *(23)* tested this method in 30 patients admitted to a pediatric intensive care unit. Arterial blood was analyzed by a blood gas instrument and a CO-oximeter. Comparing SO_2 as measured by CO-oximetry and as calculated from a blood gas instrument yielded a high correlation and a smaller difference for $SO_2 \geq 95\%$ and a poor correlation for $SO_2 < 95\%$ (Fig. 5). This work confirms that calculated SO_2 is not an accurate measurement of O_2 status in hospitalized patients, especially at $SO_2 < 95\%$. Although the mean SO_2 was not different between the two methods, the individual values varied as much as 6% of the measured SO_2. This last point is striking because the authors state that a 2% difference would be clinically important.

METHEMOGLOBINEMIA
Methemoglobinemia refers to the oxidation of ferrous iron [Fe(II)] to ferric iron [Fe(III)] within the hemoglobin complex *(24, 25)*. Once MetHb is formed, the hemoglobin loses its ability to transport oxygen, leading to tissue hypoxia and, in severe cases, death. Methemoglobinemia is most commonly the result of exposure to an oxidizing chemical, but it may also arise from genetic or idiopathic etiologies (Table 2). Methemoglobinemia may be misinterpreted as carbon monoxide poisoning because the presenting symptoms are similar. CO-oximetry values, blood color, and response to supplemental O_2 therapy aid in the differential diagnosis. Additionally, carbon monoxide poisoning does not produce cyanosis. MetHb >15% produces asymptomatic cyanosis that is unresponsive to supplemental O_2. MetHb fractions >20% are associated with the following symptoms: dyspnea, fatigue, nausea, dizziness, headache, and syncope (Table 3) *(24, 25)*. Symptoms worsen as the MetHb increases, with a high occurrence of mortality observed at MetHb values >70%.

A certain amount of physiologic MetHb formation occurs continuously as a result of endogenous oxidation. Several mechanisms exist in the erythrocytes to reduce
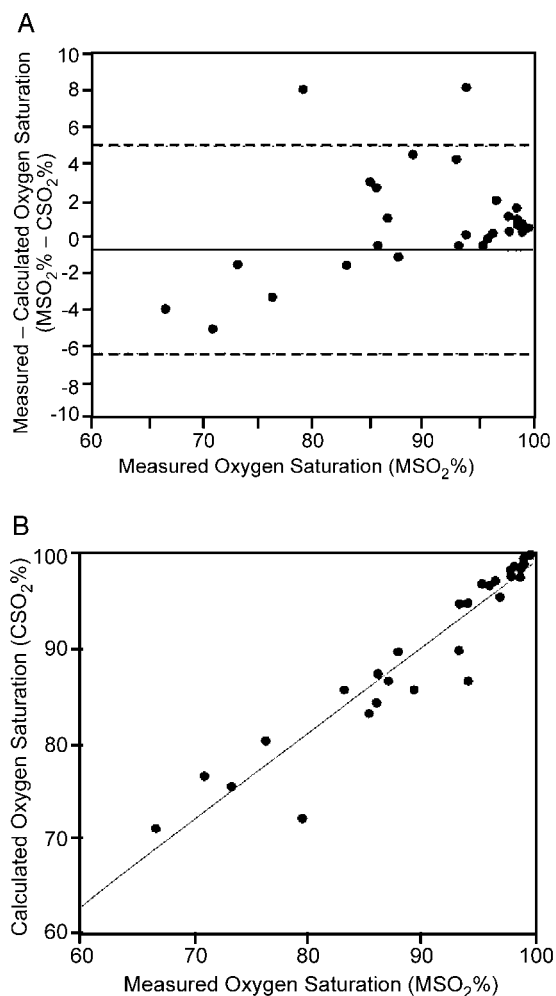
Fig. 5. Calculated (CSO$_2$%) vs measured oxygen saturation (MSO$_2$%) with regression line (A), and difference between measured and calculated (MSO$_2$% − CSO$_2$%) vs MSO$_2$% (B).

Dashed lines indicate ± 2 SD. Reprinted with permission from Salyer et al. Measured vs calculated oxygen saturation in a population of pediatric intensive care patients. *Respiratory Care* 1989;34:342–8.

Table 2. Etiologies of methemoglobinemia.

Hereditary
  HbM
  NADH-MetHb reductase deficiency
Acquired
  Medications
    Amyl nitrite
    Benzocaine
    Dapsone
    Lidocaine
    Nitroglycerin
    Nitroprusside
    Phenacetin
    Phenazopyridine
    Prilocaine
    Quinones (chloroquinone, primaquine)
    Sulfonamides (sulfanilamide, sulfathiazide, sulfapyridine, sulfamethoxazole)
  Chemical agents
    Aniline dye derivatives (shoe dyes, inks)
    Butyl nitrite
    Chlorobenzene
    Nitrate-containing foods
    Isobutyl nitrite
    Naphthalene
    Nitrophenol
    Nitrous gases
    Silver nitrate
    Trinitrotoluene
    Well water nitrates
Pediatric
  Decreased NADH-MetHb reductase activity (<4 months of age)
  Associated with low birth weight, prematurity, dehydration, acidosis, and diarrhea

MetHb to HHb; therefore, in healthy individuals, MetHb comprises only ~1% of total hemoglobin. The primary mode of MetHb reduction, accounting for 99% of daily MetHb reduction, is the cytochrome b$_5$-methemoglobin reductase (NADH methemoglobin reductase) system (26). Cytochrome b$_5$-methemoglobin reductase is a two-enzyme system comprising cytochrome b$_5$ and cytochrome b$_5$ reductase. A schematic of the enzymatic pathways for the reduction of MetHb to HHb is shown in Fig. 6 (25). Other mechanisms of MetHb reduction (ascorbic acid or glutathione reduction and NADPH dehydrogenase) are considered minor pathways under physiologic conditions (27, 28), but these minor pathways are capable of reducing large amounts of MetHb when the primary reduction mechanism is compromised. For example, patients with congenital cytochrome b$_5$-methemoglobin reductase deficiencies are able to maintain MetHb <50% (24, 25).

The majority of methemoglobinemia cases are acquired and mild, and therapy is focused on removal of the inciting toxin, oxygen administration, and observation. The half-life of MetHb is ~1 h ($t_{1/2}$ = 55 min) if reductase mechanisms are normal (29, 30). Severe methemoglobinemia is treated by the administration of a potent electron donor such as methylene blue (31–33). Therapy should be initiated at MetHb >20% in symptomatic and >30% in asymptomatic patients. The current recommended dosage of methylene blue is 1–2 mg/kg of body weight administered intravenously over a 5-min period (31, 33, 34). Generally, the MetHb concentration decreases significantly within 1–2 h after a single dose; additional doses may be necessary but should not exceed a total of 7 mg/kg. Doses of methylene blue >15 mg/kg can paradoxically incite methemoglobinemia.

During methylene blue therapy, an alternative enzyme system, NADPH dehydrogenase, becomes the primary mechanism for MetHb reduction (31). NADPH dehydrogenase reduces MetHb only in the presence of an exogenous catalyzing agent such as methylene blue and is not responsible for endogenous MetHb reduction (Fig. 6).

Table 3. Correlation of MetHb fraction and symptoms in methemoglobinemia.

FMetHb, %	Signs and symptoms
<3 (normal)	None
3–15	Possibly none
	Slate gray cutaneous coloration
15–30	Cyanosis
	Chocolate brown blood
30–50	Dyspnea
	Headache
	Fatigue
	Dizziness, syncope
	Weakness
	Pulse oximeter reads S_aO_2 ~85%
50–70	Tachypnea
	Metabolic acidosis
	Dysrhythmias
	Seizures
	CNS[a] depression
	Coma
>70	Severe hypoxic symptoms
	Death

[a] CNS, central nervous system.

Methylene blue itself is a powerful oxidant, and it is actually the metabolic product, leukomethylene blue, that acts as the reducing agent. Failure of methylene blue to resolve methemoglobinemia may be a result of NADPH dehydrogenase deficiency, glucose-6-phosphate dehydro-

Fig. 6. Schematic of the enzymatic reduction of MetHb to HHb.

genase deficiency (no NADPH), or a hemoglobin variant such as HbM (see below) (24). Less severe cases of congenital methemoglobinemia may be managed with an antioxidant such as ascorbic acid. Ascorbic acid is not indicated in the treatment of acquired methemoglobinemia because the rate at which it reduces methemoglobin is lower than that of the intrinsic enzymatic pathways (24, 35).

ACQUIRED METHEMOGLOBINEMIA
Toxin-induced methemoglobinemia occurs from exposure to oxidizing agents and represents the most common cause of methemoglobinemia. Case reports indicate that local topical anesthetics, dapsone, and recreationally abused nitrite drugs are the predominant causative agents (16, 20, 30, 32, 34, 36–52). Exposure to these agents can occur by ingestion, inhalation, and absorption across skin and mucous membranes. Some of the common causative agents are listed in Table 2 and include aniline, benzocaine, dapsone, phenazopyridine (pyridium), nitrites, nitrates, and naphthalene (34).

Toxins can oxidize hemoglobin to MetHb through several mechanisms: direct oxidation of hemoglobin, indirect oxidative pathways, and via metabolic activation (34). Direct oxidizers, such as benzocaine, prilocaine, and nitroaromatics, react directly with hemoglobin to form MetHb. The nitrites are powerful reducing agents that indirectly oxidize hemoglobin by reducing oxygen to the free radical superoxide or water to hydrogen peroxide. These highly reactive species oxidize hemoglobin to MetHb. Alternatively, drug metabolites and not the parent drug can be the causative agents. Aniline is metabolized to a free radical, phenylhydroxylamine, which, like nitrite, reacts with O_2 to form oxygen free radicals and then MetHb. Both dapsone and its hydroxylamine metabolite are capable of oxidizing hemoglobin. Variability in metabolism, rate of absorption, or enterohepatic recirculation among individuals may influence the severity and duration of toxin-induced methemoglobinemia.

Infants are particularly susceptible to toxin-induced methemoglobinemia because of lowered erythrocyte cytochrome b_5-methemoglobin reductase activity (50–60% of adult activity) until 4 months of age (53, 54). Case reports of infant-acquired methemoglobinemia include well-water nitrate contamination and topical teething gels containing benzocaine as the oxidizing agents (55–57).

CONGENITAL METHEMOGLOBINEMIAS
Enzyme deficiencies. Hereditary deficiencies of the erythrocyte reductive enzymatic pathways, most commonly cytochrome b_5 reductase, lead to inherited methemoglobinemia (58–61). These rare deficiencies are inherited in an autosomal recessive pattern, and patients may be deficient in either cytochrome b_5 or cytochrome b_5 reductase (26, 62). Homozygous individuals are identified at, or very shortly after, birth with unexplained cyanosis. These individuals have moderately increased MetHb concentra-
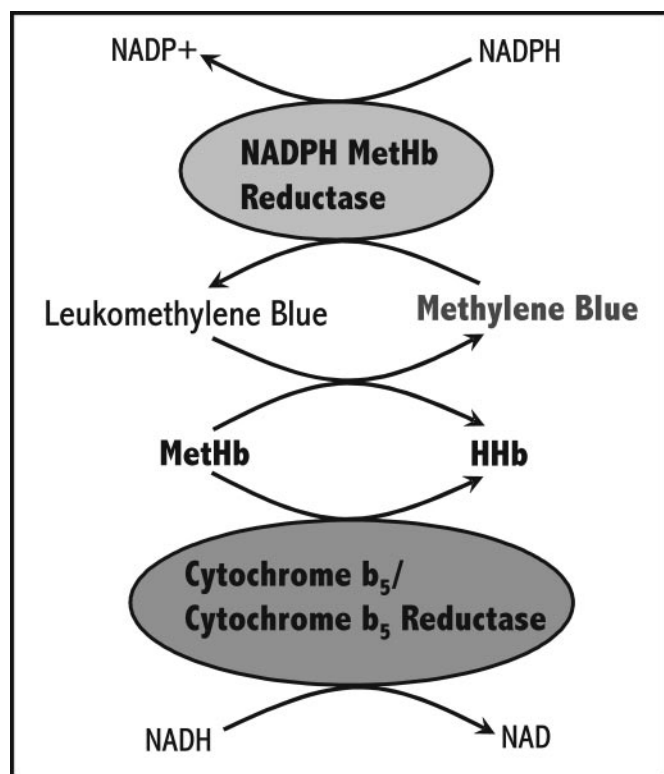
tions (10–20%), which are usually well tolerated, and mild skin discoloration (slate or chocolate gray) *(26, 62)*.

HbM variants. HbM denotes a group of abnormal hemoglobins with mutations in the globin chain that stabilize the heme iron in the oxidized form. A histidine-to-tyrosine substitution in either the α- or β-chain is identified in most HbM molecules *(26, 62)*. This aberration leads to the formation of an iron-phenolate complex that resists reduction. Several mutations have been characterized and are listed in Table 4. Depending on the mutation, the O_2 affinity may be increased or decreased. This disorder is inherited in an autosomal dominant pattern, and the homozygous form is presumed to be incompatible with life. It is important to note that the structural change in the heme iron translates into an absorbance spectrum that is significantly different from that of typical MetHb (Fig. 7) *(62)*. Several problems arise in the measurement of MetHb concentrations when a HbM variant is present. CO-oximetry may report normal fractions of MetHb, increased COHb, or increased SHb, as observed in case 1 *(1)*. The HbM spectrum lacks the characteristic MetHb peak at 630–635 nm and has a peak near 600 nm that is poorly resolved from the O_2Hb and COHb peaks. The peak absorbance of HbM may also lead to increased absorbance at 610–620 nm, which is interpreted as a contribution from SHb.

LABORATORY TESTS FOR METHEMOGLOBINEMIA
Oxygen saturation. Pulse oximetry and estimated O_2sat values are not recommended for the reasons discussed earlier. Recall that in methemoglobinemia, pulse oximeter values have been reported to trend toward 85% despite the actual oxygen saturation. When dyshemoglobins are present, only multiple-wavelength CO-oximetry can give accurate measurements of the true oxygen-carrying status because it assesses all hemoglobin species. However, as seen in case 1 presented here, CO-oximetry can be misleading in individuals with HbM disease.

Blood color test. A primary diagnostic consideration in a cyanotic patient is to differentiate HHb from MetHb. Blood containing high concentrations of MetHb appears chocolate brown as opposed to the dark red/violet of deoxygenated blood *(1)*. A simple bedside test is to place
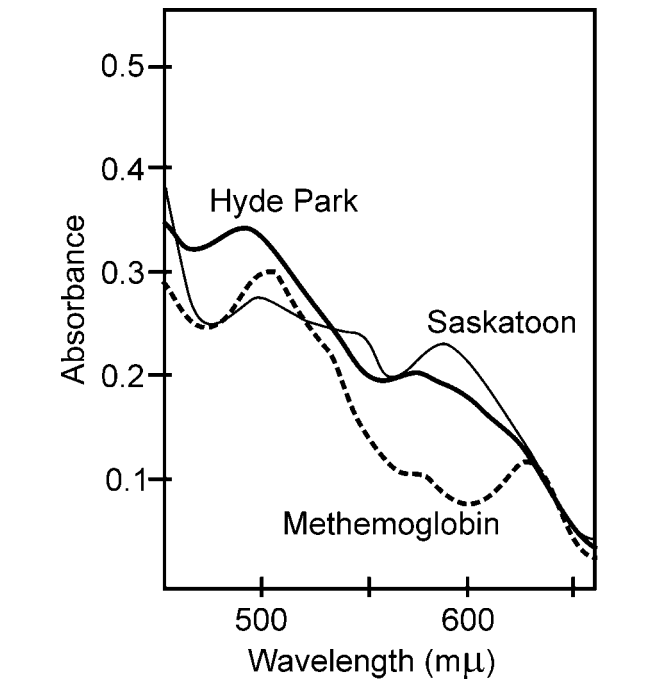

Fig. 7. Comparison of the absorbance spectra for purified MetHb protein (*dashed line*) with two HbM variants: M$_{Saskatoon}$ (*thin solid line*) and M$_{Hyde\ Park}$ (*thick solid line*).

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one or two drops of the patient's blood on white filter paper. The chocolate brown appearance of MetHb does not change with time; whereas HHb appears dark red/violet initially but brightens after exposure to air *(25)*. Gently blowing supplemental O_2 on the filter paper hastens the reaction with HHb but does not affect MetHb.

Potassium cyanide test. Reaction with Drabkin's reagent (potassium cyanide, potassium ferricyanide, and sodium bicarbonate) can distinguish between SHb and MetHb *(1)*. MetHb reacts with cyanide to form bright red cyanomethemoglobin. SHb, which like MetHb is dark brown in appearance, does not bind cyanide. After the addition of a few drops of potassium cyanide, blood containing a high concentration of MetHb turns bright red, whereas blood containing SHb remains dark brown. Quantitative results are available by measuring absorbance changes at selected wavelengths *(1)*.

Spectral characterization and ratios. CO-oximetry may be misleading in cases of HbM variants. Measurement of the absorbance at 500, 600, and 630 nm and computation of the A_{630}/A_{600} and A_{500}/A_{600} ratios can resolve such problems *(1)*. The ratios may be used to distinguish individuals with toxin-induced or congenital methemoglobin reductase deficiency methemoglobinemia from those with HbM variants. To distinguish among the different HbM variants, further spectroscopic analysis, amino acid sequence analysis, or analysis of the globin DNA is required.

Table 4. HbM mutations.

Hemoglobin	Mutation[a]
HbM$_{Boston}$	α58(E7) His→Tyr
HbM$_{Iwate, Kankakee}$	α87(F8) His→Tyr
HbM$_{Saskatoon}$	β63(E7) His→Tyr
HbM$_{Hyde\ Park}$	β92(F8) His→Tyr
HbFM$_{Osaka}$	γ63(E7) His→Tyr
HbFM$_{Fort\ Ripley}$	γ92(F8) His→Tyr

[a] Mutations are described as hemoglobin subunit, residue number, helix, and amino acid substitution.

Electrophoresis. Cellulose acetate electrophoresis is a commonly used procedure in the evaluation of patients suspected of having abnormal hemoglobins *(63)*. Some HbM variants may be identified by their relative migration times under various conditions.

Cytochrome b_5 reductase activity. Methemoglobin reductase catalyzes the NADH-linked reduction of several substrates, including ferricyanide. The activity of this enzyme is measured spectrophotometrically by monitoring the oxidation of NADH (via ferricyanide reduction) at 340 nm *(1)*. Concentrations in healthy adults range from 10.1 to 19.4 U/g of Hb. Activity in neonates (0–6 weeks) is usually 60% of that in adults; reaching adult values by 4 months of age.

Summary

CASE 1

The clinical chemistry and hematology consults noted the existence of mild polycythemia and, suspecting a high O_2 affinity HbM variant, recommended a methemoglobinemia evaluation. This was suspected because it has previously been documented that HbM will yield abnormally increased COHb and SHb but normal MetHb values when CO-oximetry is performed with a discrete six-wavelength CO-oximeter such as the Radiometer 625 ABL *(1)*. As a result, a congenital methemoglobinemia attributable to HbM may be mistakenly diagnosed as carbon monoxide poisoning or toxic sulfhemoglobinemia, as happened with this patient (despite her acknowledgement of a family history of chronic methemoglobinemia at presentation). The results confirming the HbM diagnosis were returned after the patient had been discharged.

CASE 2

The NADPH dehydrogenase in this individual was clearly functional as evidenced by his response to methylene blue. The possibility of a cytochrome b_5-methemoglobin reductase deficiency was considered because of prolonged cyanosis and because butyl nitrite had not been shown to cause such a symptomatic methemoglobinemia.

In this patient, syncope was likely a result of hypoxia attributable to MetHb and the hypotensive effects of the butyl nitrite. The MetHb value was obtained ~60 min after the patient's syncopal episode. With a half-life of 55 min, it is likely that the peak MetHb value before his arrival in the emergency department was considerably higher than 23%. Furthermore, the patient's history of isosorbide mononitrate use likely increased his susceptibility to both the vasodilatory effects and oxidizing potential of butyl nitrite.

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