

Effects of Methemoglobinemia on Pulse Oximetry and Mixed Venous Oximetry

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The performance of three commercially available pulse oximeters was assessed in five anesthetized dogs in which increasing levels of methemoglobin were induced. Hemoglobin oxygen saturation in each dog was monitored with three pulse oximeters (Nellcor N-100®, Ohmeda 3700®, and Novamatrix 500®) and a mixed venous saturation pulmonary artery catheter (Oximetrix Opticath®). Arterial and mixed venous blood specimens were analyzed for P_{aO_2} , P_{aCO_2} , and pH , using standard electrodes. An IL-282 Co-oximeter was used on the same specimens to determine oxyhemoglobin and methemoglobin as percentages of total hemoglobin. Methemoglobin levels of up to 60% were induced by intratracheal benzocaine. As MetHb gradually increased while the dogs were breathing 100% inspired oxygen, the pulse oximeter saturation (Sp_{O_2}) overestimated the fractional oxygen saturation (Sa_{O_2}) by an amount proportional to the concentration of methemoglobin until the latter reached approximately 35%. At this level the Sp_{O_2} values reached a plateau of 84-86% and did not decrease further. When, at fixed methemoglobin levels, additional hemoglobin desaturation was induced by reducing inspired oxygen fraction, Sp_{O_2} changed by much less than did Sa_{O_2} (regression slopes from 0.16 to 0.32). Thus, at high methemoglobin levels Sp_{O_2} tends to overestimate Sa_{O_2} by larger amounts at low hemoglobin saturations. Plots of Sp_{O_2} versus functional saturation (oxyhemoglobin/reduced hemoglobin plus oxyhemoglobin) show an improved but still poor relationship (regression slopes from 0.32 to 0.46). The Oximetrix Opticath pulmonary artery catheter behaves similarly but provides somewhat better agreement with functional saturation than do the pulse oximeters in the presence of methemoglobinemia. Pulse oximetry data (Sp_{O_2}) should be used with caution in patients with methemoglobinemia. (Key words: Blood, hemoglobin: methemoglobin; oxygen saturation. Measurement technique: pulse oximetry. Oxygen: hemoglobin saturation.)

THE ROLE of pulse oximetry as a monitor of oxygenation in the operating room, recovery room, and intensive care unit is well established.¹ The pulse oximeter estimates the oxygen saturation of arterial blood by measuring the absorbance of light transmitted through well-perfused tissue, such as the finger or ear. The light absorbance is measured at two wavelengths: 660 and 940 nm. The device calculates the ratio of the pulsatile and mean absorbances at each wavelength to create a pulse-added absorbance signal, which is presumed to reflect changes in the arterial blood volume of the tissue. The ratio (r) of the pulse-added absorbances at the two wavelengths is used to gen-

erate the oximeter's estimate of arterial saturation (Sp_{O_2}). The relationship between r and Sp_{O_2} is empirical, based upon data measured in healthy, awake volunteers breathing hypoxic gas mixtures.² (See the "Appendix" for a more detailed description of the principles of oximetry and the definitions of oxygen saturation.)

Because the pulse oximeter uses only two wavelengths of light, it can theoretically determine the concentration of only two hemoglobin species: reduced hemoglobin (Hb) and oxyhemoglobin (O_2Hb). It is not clear *a priori* how the pulse oximeter will behave in the presence of dyshemoglobins such as methemoglobin (MetHb) or carboxyhemoglobin (COHb). We have shown previously in dogs that in the presence of COHb, Sp_{O_2} overestimates fractional saturation (Sa_{O_2}) by an amount roughly proportional to COHb.³ The pulse oximeter thus "sees" COHb as though it were composed mostly of O_2Hb . For example, at COHb = 70% the Sp_{O_2} is approximately 90% when inspired oxygen fraction (FI_{O_2}) is 1.0, whereas the actual Sa_{O_2} is only 30%.

Methemoglobinemia may be congenital or induced by a large number of drugs, including local anesthetics (prilocaine, benzocaine), nitrates (nitroglycerin), nitrites, phenacetin, pyridium, primiquine, and sulfonamides. There are many case reports of potentially serious methemoglobin levels (greater than 30%) induced by topical anesthetics used in the airway.⁴⁻⁷ There are also case reports describing pulse oximeter readings during methemoglobinemia. However, the MetHb levels in these were too low (6% or less) to accurately characterize pulse oximeter behavior.^{8,9} (A case report now in press describes a MetHb level of 26%.¹⁰) The goal of the present study is to determine the behavior of three pulse oximeters (Nellcor N-100®, Novamatrix 500®, Ohmeda 3700®) in the presence of high levels of MetHb at several values of FI_{O_2} . We also present simultaneous measurements of mixed venous saturation using a fiberoptic pulmonary artery (PA) catheter. This Oximetrix Opticath® catheter employs three wavelengths (670, 700, and 800 nm) as opposed to the two wavelengths of the pulse oximeter (see "Appendix").

Methods

Five dogs weighing 15-20 kg were anesthetized with iv sodium pentobarbital (25 mg/kg). Following tracheal intubation the lungs were mechanically ventilated and normocarbica was achieved. Anesthesia was maintained

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with a continuous pentobarbital infusion, 15–20 mg · kg⁻¹ · h⁻¹. Arterial and PA catheters were inserted in each dog through a femoral incision. The PA catheter was an Oximetrix model SP-5107H[®], which measures mixed venous oxygen saturation by three-wavelength oximetry as described above. The catheter was calibrated using an external color reference standard prior to insertion, as recommended by the manufacturer.

SpO₂ in each dog was monitored by three pulse oximeters with the probes applied to the tongue: Nellcor N-100[®], Ohmeda 3700[®], and Novamatrix 500[®]. Arterial and mixed venous blood samples were obtained periodically for analysis by Radiometer ABL-2 Blood Gas Analyzer[®] and Instrumentation Laboratories IL-282 Co-oximeter[®]. The latter was electronically modified and calibrated for dog hemoglobin. Because it is often stated that pulse oximeters measure functional saturation[‡] (see "Appendix"), results will be presented in the form of SpO₂ versus both functional (FSaO₂) and fractional (SaO₂) saturation (Equations A2 and A3). FSaO₂ can be calculated from the IL-282 co-oximeter data using the relation:

$$FSaO_2 = \frac{SaO_2}{1 - \frac{MetHb}{THb}}, \quad (1)$$

which assumes that MetHb but no COHb is present in this experiment.

Monitored hemodynamic variables included arterial and PA pressures, central venous pressure, and cardiac output by thermodilution. Filling pressures were maintained near baseline throughout the experiment. Baseline values of all measured variables were established in each dog at FI_{O₂} = 1.0, 0.20, 0.15, and 0.11. Methemoglobinemia was then induced slowly and incrementally by aerosol injections of 20% benzocaine solution into the endotracheal tube. The highest MetHb% levels of 60–65% were reached after 3–5 h. At each MetHb% level a complete data set was recorded at each of the four FI_{O₂} values given above. In one dog iv prilocaine was used as an alternative method of inducing methemoglobinemia. Although this method was complicated by hypotension when the infusion rate exceeded 1 mg · kg⁻¹ · h⁻¹, a MetHb% level of 30% was achieved in 6 h with a total dose of 6 mg/kg.

In methods comparison studies such as this one, the agreement between two methods is best described by the bias and precision.¹¹ The bias is the mean difference between the values from the two methods, and the precision is the SD of the difference. A larger precision thus implies a less precise measurement. Correlation coefficient is less useful in these studies because it is dependent upon the range of the independent variable (in this case SaO₂) over

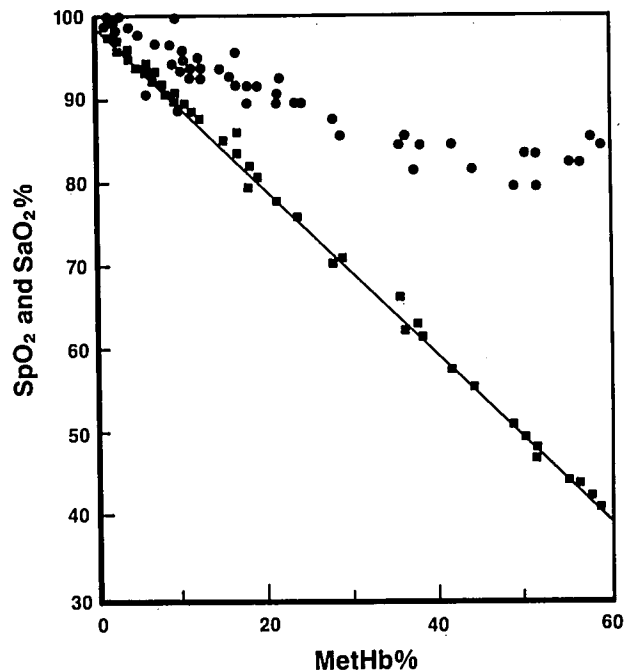


FIG. 1. Nellcor[®] SpO₂ (●) and IL-282 SaO₂ (■) versus MetHb% for FI_{O₂} = 1.0. Line shown is SaO₂ = 100 - MetHb%.

which the data are taken. Linear regression slope and intercept are also valuable when the two measured variables (SpO₂ and SaO₂) are related by a linear equation the slope of which is different from unity. The quality of the linear regression fit is indicated by the standard error of the estimate (SEE), which is the SD of the y-values with respect to the prediction of the regression equation.

Results

Figure 1 shows the effect of methemoglobinemia upon SpO₂ when FI_{O₂} is maintained at 1.0. Both SpO₂ from the Nellcor N-100[®] and SaO₂ from the IL-282 Co-oximeter[®] are plotted versus MetHb% in this figure. SaO₂ decreases linearly with increasing MetHb%, the data lying near the line SaO₂ = 100 - MetHb% (linear regression slope = -0.996; intercept = 99.6%). The SpO₂ initially decreases with rising MetHb% but with a lower slope than SaO₂. When MetHb% reaches 30–35%, SpO₂ reaches a plateau in the 82–86% range and then becomes virtually independent of MetHb%. The Novamatrix and Ohmeda instruments show similar behavior on this type of plot, except that the Ohmeda appears to reach its plateau value at slightly lower MetHb% levels. In this plot as well as the figures discussed below, the data from the prilocaine-treated animal were indistinguishable from the data from the four benzocaine-treated animals.

At each constant level of MetHb% the FI_{O₂} was varied between 0.11 and 1.0 to produce a wide range of SaO₂.

[‡] Nellcor, Inc.: Pulse oximetry reference note #2, 1987.

TABLE 1. Statistics for SpO₂ from Three Pulse Oximeters versus SaO₂ (IL-282) Grouped by MetHb Level

	Nellcor*	Ohmeda*	Novamatrix*
MetHb% = 0-15			
<i>r</i>	0.95	0.90	0.96
Slope	0.70	0.41	0.56
Intercept	30.5	56.2	42.6
Bias	5.08	4.43	4.68
Precision	4.97	6.41	7.33
MetHb% = 16-30			
<i>r</i>	0.97	0.57	0.84
Slope	0.50	0.22	0.33
Intercept	52.7	74.6	63.8
Bias	19.1	16.1	18.4
Precision	7.95	7.40	10.7
MetHb% = 31-45			
<i>r</i>	0.96	0.91	0.81
Slope	0.58	0.45	0.32
Intercept	50.3	64.3	67.2
Bias	28.4	36.1	31.2
Precision	4.41	4.94	7.07
MetHb% > 45			
<i>r</i>	0.35	0.47	0.49
Slope	0.12	0.13	0.15
Intercept	76.1	81.5	78.2
Bias	44.4	50.4	47.6
Precision	8.93	8.84	8.51

All FI_{O₂} values included.

Statistics for the relationships between SpO₂ and SaO₂ for four different ranges of MetHb% are shown in table 1. The table provides correlation coefficient *r*, linear regression slope and intercept, bias, and precision for each oximeter in each of the four MetHb% ranges. At the highest MetHb% levels we see a low correlation coefficient (0.35–0.49), reduced linear regression slope (0.12–0.15) with a large intercept, and a large positive bias (44–48%). The large positive bias indicates a consistent overestimation of SaO₂ by the pulse oximeter; the small slope and low *r* value show a weak relationship between SpO₂ and SaO₂ for high MetHb%.

Figure 2A shows SpO₂ data from the Nellcor N-100® plotted versus fractional saturation (SaO₂) for all MetHb levels and all FI_{O₂} values of 0.2 or less. Figure 2B shows the same data plotted as SpO₂ versus functional saturation (FSaO₂). Lines of identity and linear regression are shown. Statistics for these relationships for all three pulse oximeters are given in table 2, including correlation, bias, precision, linear regression slope and intercept, and standard error of the estimate (SEE).

Mixed venous saturation from the Oximetrix Opticath® pulmonary artery catheter (SxO₂) is plotted versus fractional saturation from mixed venous blood samples measured by IL-282® (SvO₂) in figure 3A. Data for all FI_{O₂} values and all MetHb levels are shown. Four ranges of

MetHb% values are indicated by different symbols on the plot. Statistics for figure 3A (*r*, slope, intercept, bias, precision) are given in table 3. We can also use the IL-282 Co-oximeter® data to calculate a functional mixed venous saturation, FSvO₂. The Oximetrix SxO₂ values are plotted versus FSvO₂ in figure 3B. Table 3 shows statistics for SxO₂ versus FSvO₂ as well as SvO₂, providing a direct comparison of the two relationships.

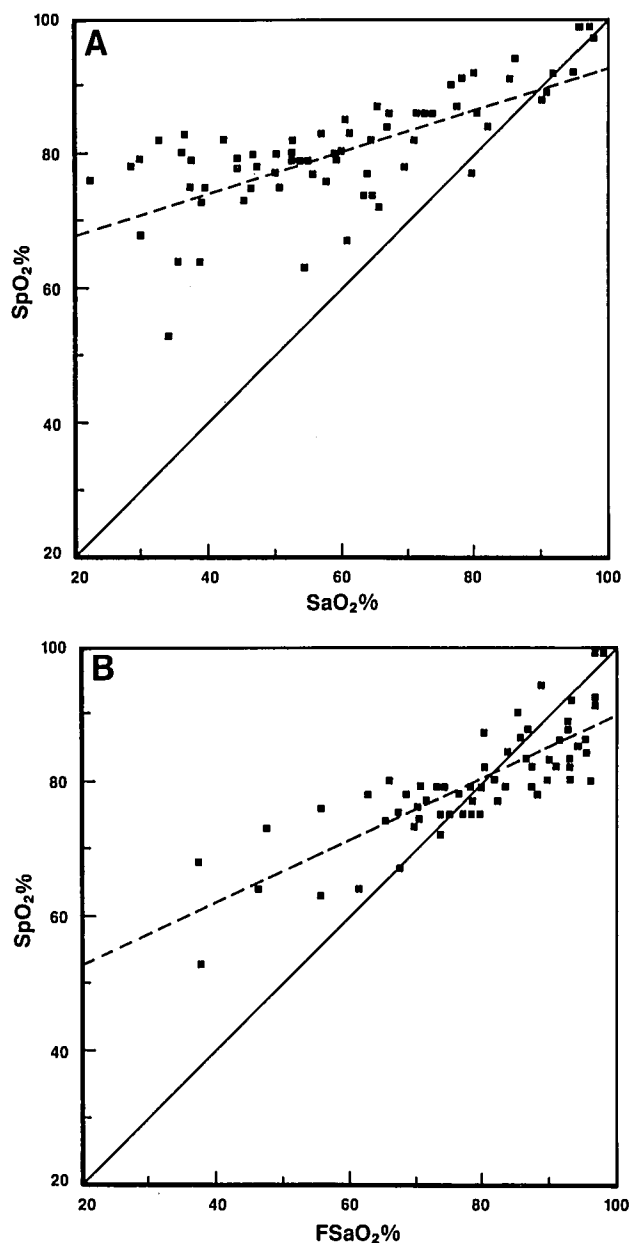


FIG. 2. Nellcor® SpO₂ (■) versus (A) SaO₂ and (B) FSaO₂. All MetHb% levels and FI_{O₂} values of 0.21 or less are included. Linear regression: - - - -, line of identity: —.

TABLE 2. Statistics for SpO₂ versus SaO₂ and FSaO₂

	Nellcor*	Ohmeda*	Novamatrix*
SpO₂ versus SaO₂			
r	0.72	0.67	0.62
Slope	0.31	0.16	0.22
Intercept	61.9%	77.4%	69.8%
SEE	5.7%	7.5%	5.4%
Bias	21.6%	28.2%	24.4%
Precision	14.0%	16.3%	15.2%
SpO₂ versus FSaO₂			
r	0.81	0.71	0.77
Slope	0.46	0.33	0.32
Intercept	43.5%	56.8%	60.4%
SEE	4.8%	4.8%	3.0%
Bias	-0.4%	-3.1%	-3.7%
Precision	9.1%	11.4%	8.9%

All MetHb% levels and FI_{O₂} values of 0.21 or less are included.

Discussion

Previous studies have shown that without dyshemoglobins pulse oximeter accuracy in dogs is similar to that found in humans.^{3,§} Data from the present study with MetHb = 0 support this finding. The present results also suggest that the pulse oximeter provides no useful measure of fractional arterial oxygen saturation at high MetHb levels (figs. 1 and 2A; tables 1 and 2). The relationship between SpO₂ and functional saturation FSaO₂ is better (fig. 2B, table 2), but it cannot be said that SpO₂ measures FSaO₂ under these conditions. If SpO₂ measured FSaO₂, then the SpO₂ values shown in figure 1 for FI_{O₂} = 1.0 would all equal 100%. Instead, SpO₂ decreases with increasing MetHb% until it reaches a plateau of approximately 85% at MetHb% levels of 30–35%. The small bias values for the SpO₂ versus FSaO₂ relationships (table 2) are misleading unless we examine figure 2B. There are roughly as many data points above the line of identity as there are below, which makes the bias small even if the relationship is poor. The precision values are near 10% for the FSaO₂ relationships, compared with roughly 15% for the SaO₂ relationships. The linear regression slopes are closer to unity for FSaO₂ than for SaO₂, and the y-intercepts are smaller. However, even for FSaO₂ these slopes are less than 0.5 and the y-intercepts are greater than 40% (table 2). This indicates that SpO₂ tends to overestimate FSaO₂ at low saturations and underestimate it at high saturations (fig. 2B). However SpO₂ overestimates fractional SaO₂ at all saturation values.

The nonlinear plateau behavior of figure 1 and the decreased slopes of the SpO₂ versus SaO₂ (or FSaO₂) relationships are somewhat predictable. Figure 4 shows light extinction coefficients versus wavelength for the four common hemoglobins: Hb, O₂Hb, MetHb, and COHb. At 660 nm the extinction coefficient of MetHb is similar to that of Hb and much greater than that of O₂Hb. At 940 nm MetHb has a greater coefficient than either Hb

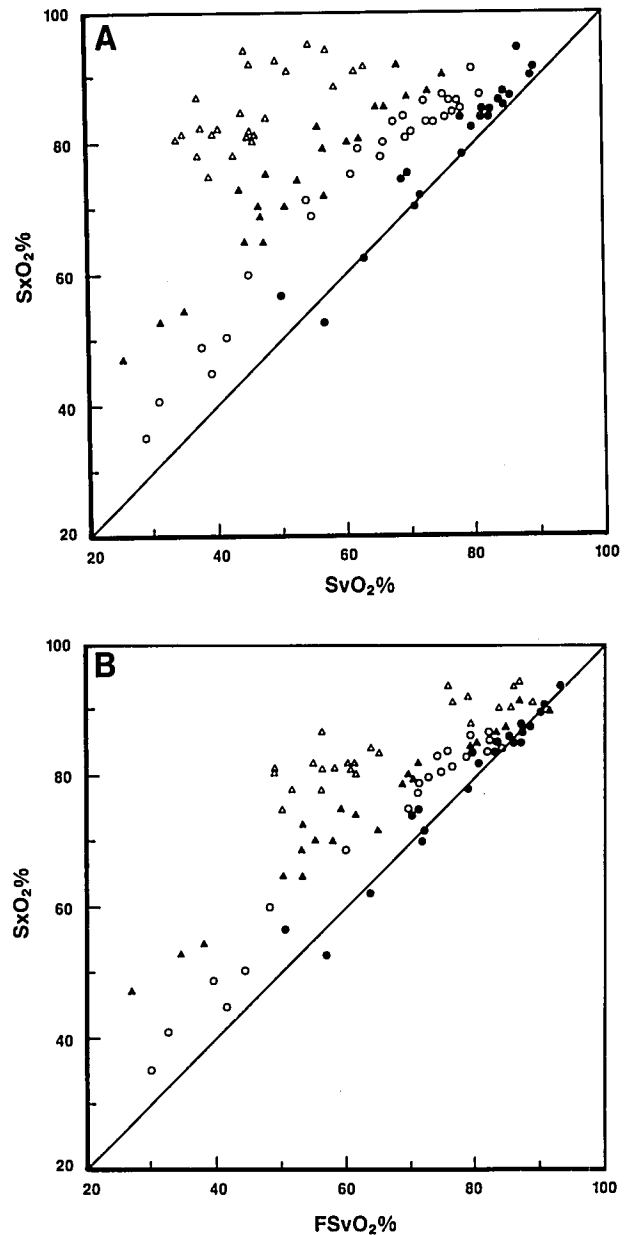


FIG. 3. Oximetry Opticath® SxO₂ versus (A) SvO₂ and (B) FSvO₂. All MetHb% levels and all FI_{O₂} values are shown. MetHb% = ● = 0–9%; ○ = 10–19%; ▲ = 20–40%; △ = above 40%.

§ Tremper KK, Hufstедler S, Zaccari J, Schaefer R, Asrani R, Sangh M, Roohk V, LaMendola R: Pulse oximetry and transcutaneous PO₂ during hemorrhagic and normotensive shock in dogs (abstract). ANESTHESIOLOGY 61:A163, 1984

TABLE 3. Statistics for Oximetric Sx_{O_2} versus Sv_{O_2} and FSv_{O_2} Grouped by MetHb% Level

	Sv_{O_2}	FSv_{O_2}
MetHb% = 0-9		
<i>r</i>	0.98	0.99
Slope	0.98	0.94
Intercept	5.3%	5.7%
Bias	3.8%	1.4%
Precision	3.2%	3.0%
MetHb% = 10-19		
<i>r</i>	0.97	0.98
Slope	0.96	0.86
Intercept	16.5%	16.1%
Bias	14.5%	7.4%
Precision	4.0%	4.5%
MetHb% = 20-40		
<i>r</i>	0.96	0.98
Slope	0.89	0.71
Intercept	31.1%	30.1%
Bias	26.4%	14.1%
Precision	4.6%	6.7%
MetHb% ≥ 40		
<i>r</i>	0.69	0.91
Slope	0.50	0.40
Intercept	66.0%	58.9%
Bias	49.9%	24.1%
Precision	7.3%	10.4%

All FI_{O_2} values included.

or O_2Hb . MetHb thus adds to the pulse-added absorbance at both wavelengths. In contrast, COHb adds significant absorbance only at the shorter wavelength, where COHb has an extinction coefficient comparable to that of O_2Hb . Sp_{O_2} is computed from the ratio *r* of the pulse-added absorbances at the two wavelengths. The presence of MetHb increases both the numerator and denominator of this ratio, which tends to drive *r* toward unity. An absorbance ratio *r* of 1.0 corresponds to an Sp_{O_2} near 85% on the pulse oximeter calibration curve.¹² This may explain the Sp_{O_2} plateau of figure 2 as well as the small slopes of the Sp_{O_2} versus Sa_{O_2} regression lines.

The Oximetrix Opticath® Sx_{O_2} overestimated Sv_{O_2} by an amount that increased in proportion to MetHb%. At the highest MetHb% levels the Sx_{O_2} error was more than 60%. The data points in figure 3A for the MetHb% range of 20-40% lie almost parallel to the line of identity (linear regression slope = 0.89). The bias for this MetHb% range is 26.4%, roughly equal to the average MetHb% value for these data. This is in contrast to the behavior of the pulse oximeters where we found a large decrease in the slope of the Sp_{O_2} versus Sa_{O_2} relationship at high MetHb% levels (tables 1 and 2). The data in figure 3A having MetHb% greater than 40% do not span a wide enough Sv_{O_2} range to provide a meaningful linear regression, but the bias for these data is 49.9%, again comparable to the average MetHb% value. The relationship between Sx_{O_2}

and FSv_{O_2} shown in figure 3B is clearly improved relative to that of figure 3A. In the highest MetHb% range (greater than 40%), the bias is 49.9% for Sv_{O_2} (fig. 3A) compared with 24.1% for FSv_{O_2} (fig. 3B). The precision values are roughly the same (7% and 10%), reflecting similar scatter in both plots for this MetHb% range. The bias and precision are small in the 0-9% MetHb% range (1.4% and 3.0%, table 3), demonstrating the accuracy of this device in the absence of MetHb.

The Oximetrix® PA catheter differs from the pulse oximeter in several ways. First, the PA catheter measures reflected rather than transmitted light. Second, being immersed in blood the PA catheter has no need for the pulse-added signal analysis used by the pulse oximeter. Finally, the Opticath® uses three wavelengths (670, 700, and 800 nm) rather than the two wavelengths (660 and 940 nm) employed by pulse oximeters. This provides the Oximetrix® microprocessor with one additional Lambert-Beer equation (Equation A4), which theoretically allows it to deal with one additional hemoglobin species. Nevertheless, the PA catheter overestimates mixed venous saturation in proportion to the MetHb% level (fig. 3; table 3).

In conclusion, both pulse oximeters and mixed venous saturation PA catheters overestimate saturation in the presence of MetHb. Pulse oximeters also exhibit a decreased response to changes in saturation when MetHb% levels exceed 20%. Sp_{O_2} and Sx_{O_2} are more closely related to functional than fractional saturation during methemoglobinemia, but they do not provide clinically useful estimates of either at high MetHb levels. The three pulse oximeters used in this study performed similarly (tables 1 and 2). The data from these devices should be used with caution in patients with possible methemoglobinemia.

References

1. Eichorn JH, Cooper JB, Cullen DJ, Maier WR, Philip JH, Seeman RG: Standards for patient monitoring during anesthesia at Harvard Medical School. *JAMA* 125:1017-1020, 1986
2. Yelderman M, New W: Evaluation of the pulse oximeter. *ANESTHESIOLOGY* 59:349-352, 1983
3. Barker SJ, Tremper KK: The effect of carbon monoxide inhalation on pulse oximetry and transcutaneous PO_2 . *ANESTHESIOLOGY* 66:677-679, 1987
4. Seibert RW, Seibert JJ: Infantile methemoglobinemia induced by a topical anesthetic, Cetacaine. *Laryngoscope* 94:816-817, 1984
5. Kellet PB, Copeland CS: Methemoglobinemia associated with benzocaine-containing lubricant. *ANESTHESIOLOGY* 59:463-464, 1983
6. McGuigan MA: Benzocaine-induced methemoglobinemia. *Can Med Assoc J* 125:816, 1981
7. Sandza JG Jr, Roberts RW, Shaw RC, Connors JP: Symptomatic methemoglobinemia with a commonly used topical anesthetic, Cetacaine. *Ann Thorac Surg* 30:187-190, 1980
8. Eisenkraft JB: Pulse oximeter desaturation due to methemoglobinemia. *ANESTHESIOLOGY* 68:279-282, 1988
9. Mertzluft F, Zander R: Noninvasive oximetry using the Biox III

oximeter: Clinical evaluation and physiological aspects, Pulse Oximetry. Edited by Payne JP, Severinghaus JW. Berlin, Springer-Verlag, 1986, pp 71-77

10. Anderson ST, Hajduczek J, Barker SJ: Benzocaine-induced methemoglobinemia in an adult: Accuracy of pulse oximetry with methemoglobinemia. *Anesth Analg* (in press)
11. Altman DG: Statistics and ethics in medical research, part V. *Br Med J* 281:1473-1475, 1980
12. Polog J: Pulse oximetry: Technical aspects of machine design, International Anesthesiology Clinics: Advances in Oxygen Monitoring. Edited by Tremper KK, Barker SJ. Boston, Little, Brown, 1987, pp 137-153

Appendix

PRINCIPLES OF OXIMETRY

Co-oximeters, such as the Instrumentation Laboratories (Lexington, Massachusetts) model IL-282[®], measure the light absorbance of blood samples at four or more discrete wavelengths. Using these data and the known extinction coefficients of all hemoglobin species shown in figure 4, the co-oximeter calculates the concentration of each species as described below. Most machines assume only four species present: oxyhemoglobin (O₂Hb), reduced hemoglobin (Hb), carboxyhemoglobin (COHb), and methemoglobin (MetHb). The data are presented as percentages of the total hemoglobin (THb), for example:

$$\text{MetHb}\% = \frac{\text{MetHb}}{\text{THb}} \times 100\%, \quad (\text{A1})$$

$$\text{SaO}_2 = \text{O}_2\text{Hb}\% = \frac{\text{O}_2\text{Hb}}{\text{THb}} \times 100\%. \quad (\text{A2})$$

The oxygen saturation as defined by Equation A2 is called fractional saturation (also oxyhemoglobin fraction), and it is the saturation measured by all laboratory co-oximeters. We also define a functional saturation (FSa_{O₂}) as the ratio of O₂Hb to the hemoglobin that takes part in O₂ transport, *i.e.*,

$$\text{FSaO}_2 = \frac{\text{O}_2\text{Hb}}{\text{Hb} + \text{O}_2\text{Hb}} \times 100\% \quad (\text{A3a})$$

$$= \frac{\text{O}_2\text{Hb}}{\text{THb} - \text{MetHb} - \text{COHb}} \times 100\%. \quad (\text{A3b})$$

Thus, the dyshemoglobins are subtracted from the denominator in calculating FSa_{O₂}.

The pulse oximeter, in contrast to the co-oximeter, obtains data at only two wavelengths and can therefore determine only two hemoglobin species: O₂Hb and Hb. It has been stated that pulse oximeter Sp_{O₂} can measure functional saturation even in the presence of MetHb or COHb because these hemoglobins do not appear in Equation A3a. Let us examine this claim by means

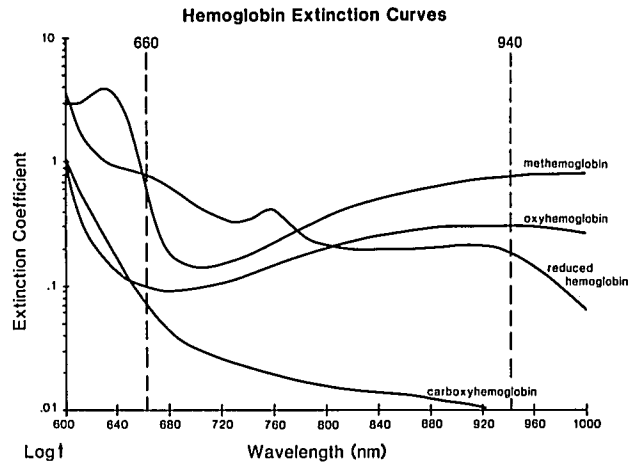


FIG. 4. Extinction coefficient versus wavelength for the four hemoglobin species: reduced Hb, O₂Hb, MetHb, COHb. Pulse oximeters use the two wavelengths 660 nm and 940 nm.

of the Lambert-Beer law, which relates the total light absorbance at a given wavelength to the extinction coefficients of the various species present in the mixture. For specific wavelength #1, this law is given by:

$$I_1 = I_{0,1}e^{-A_1}, \quad (\text{A4a})$$

where

$$A_1 = D(C_1\epsilon_{1,1} + C_2\epsilon_{2,1} + C_3\epsilon_{3,1} + C_4\epsilon_{4,1}), \quad (\text{A4b})$$

and

I_1 = transmitted light intensity at wavelength #1

$I_{0,1}$ = incident light intensity at wavelength #1

D = the light path length

A_1 = total absorbance of mixture at wavelength #1

C_1 = concentration of species #1 in mixture

$\epsilon_{1,1}$ = extinction coefficient of species #1 at wavelength #1.

Thus, if there are four species present, Equation A4b has four unknowns (C_1 , C_2 , C_3 , and C_4). The ϵ are known properties of the absorbers as given in figure 1, and E_1 is the measured absorbance. We therefore need four wavelengths and four equations like Equation A4b (one for E_2 , etc.) to solve for any of the four unknowns. In other words, with only two wavelengths (two equations) we cannot solve Equation A4b for any concentrations unless some of the C or ϵ are known to be zero. It is thus theoretically impossible for a two-wavelength oximeter to measure Sa_{O₂} or FSa_{O₂} in the presence of unknown concentrations of COHb or MetHb.