use of phencyclidine, in contrast to the experience of Niven (8), who states that there is little use of it in most of the Midwest.

The importance of drug screening has been questioned, because results are often negative and rarely alter treatment (9). The high prevalence of positive screens (66%) in our pediatric population, even with omission of ethanol and cannabinoid testing, suggests that when physician discretion is used in requesting the test, there is a substantial likelihood that one or more drugs will be found. For situations where a limited drug screen is desirable, our data indicate which choices are best for identifying the most frequently used drugs and suggest some age-related deviations from the population pattern. Acetaminophen, benzodiazepines, sympathomimetic amines, phencyclidine, salicylate, and (or) phenobarbital were present in most of the positive screens for the population. Anticonvulsants were more common in young children; illicit drugs were found mainly in older subjects. Additionally, we think it probable that ethanol and cannabinoids would have been commonly found, had they been included in our screen. Since the period covered by the data in this report, both ethanol and cannabinoid testing has become a part of our routine drug screen. Over a recent three-month interval, ethanol was detected in 7% and cannabinoids in 10% of our pediatric drug screens.

The consequences of an overdose with our most frequently identified drug, acetaminophen, and the need for early intervention with a specific treatment are well recognized. Therefore, our data underscore the significant need for acetaminophen identification. Even in the absence of comprehensive drug screening, the capability of rapidly identifying acetaminophen is essential for the entire pediatric population.

References

Effect of pH on the Absorption Spectrum of Human Oxyhemoglobin: a Potential Source of Error in Measuring the Oxygen Saturation of Hemoglobin

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Small differences in the visible absorption spectrum of human oxyhemoglobin occur with changes in pH, CO2, and 2,3-diphosphoglycerate. This may bias the spectrophotometric measurement of oxygen saturation (SO2) in blood by as much as 3%. This negative bias in SO2 at low pH cannot be explained by a lowered hemoglobin–oxygen affinity, by interference from methemoglobin, or by an increased unknown dyshemoglobin fraction at low pH. For routine clinical work, we recommend calibrating the oxygen saturation meter to read 100% SO2 for fresh blood equilibrated to a PO2 > 95 kPa, pH 7.4, and PCO2 = 5.3 kPa. Subsequent measurement of SO2 in patients' blood samples may then reflect an inaccuracy of up to 1%, which is acceptable in routine clinical practice. For research purposes, particularly where greater variations in blood pH may be encountered, the measured SO2 values should be corrected for the pH effect on the oxyhemoglobin absorption spectrum.

Additional Keyphrases: hemoglobin–oxygen affinity • spectrophotometry • variation, source of

This study arose from the observation that calibration of an oxygen saturation meter (OSM 3, Radiometer) with fresh adult blood tonometered with 100% O2 gave a measured value for hemoglobin saturation with oxygen (SO2) 0.8% higher than that for blood tonometered with a gas mixture containing 96% O2 and 4% CO2. The purposes of this study were:

1. To document the influence of blood pH on the spectrophotometric measurement of SO2 over a wide pH range, with use of two commercially available oxygen-saturation meters (OSM 2 and OSM 3, Radiometer), and to investigate possible separate effects of blood PCO2 and the concentration of 2,3-diphosphoglycerate (CPD) in erythrocytes.

2. To investigate the following possible causes of this influence:
(a) a direct effect of pH on the absorption spectrum of oxyhemoglobin and possibly deoxyhemoglobin;
(b) interference from the small amounts of methemoglobin in blood, the absorption spectrum of methemoglobin being known to change with pH;
(c) an altered oxygen affinity of hemoglobin owing to
changes in pH (Bohr effect), and also to changes in $p_{CO_2}$ and
$C_{DPG}$; and
(b) the presence of an unknown dyshemoglobin that
cannot bind oxygen, and whose fraction increases with a
decrease in pH.
3. To discuss the practical implications of the findings
with regard to measurement of $s_O2$ spectrophotometrically.

Materials and Methods

Samples

Fresh heparinized venous blood from three healthy non-
smoking adults.

Equipment

The OSM 3 oxygen saturation meter (Radiometer, Copen-
hagen, Denmark) measures absorbance at 555, 560, 577,
622, 636, and 670 nm (1) with a bandwidth of 3.2 nm.
Possible deviations from Beer’s Law are corrected automati-
cally. There is no sample dilution, hemolysis being achieved
by ultrasonnd. Another model, the OSM 2, measures absorb-
ance at wavelengths 505 and 600 nm, with a bandwidth of
6 nm (2). We also used a scanning spectrophotometer with a
bandwidth of 2 nm (not commercially available), as previ-
ously described (1), and a blood-gas analyzer (ABL 3,
Radiometer).

Here we report pH values as they were measured in the
study, i.e., within the plasma phase of whole blood. How-
ever, $s_O2$ was measured with hemolyzed whole blood. The
stated plasma pH ($pH(P)$) values may be converted to
erthrocyte pH [$pH(E)$] values according to the equation:
$pH(E) = 7.19 + 0.77 \cdot [pH(P) - 7.40]$ (3).

Procedures

Blood pH was adjusted within the range 6.0 to 9.0 by
adding HCl (140 mmol/L) or NaOH (140 mmol/L) after
removal of an equal volume of plasma, or by equilibrating
blood with gas mixtures containing different CO2 fractions.

2,3-Diphosphoglycerate was removed by incubating blood
at 37°C for 24 h. It is assumed that no denaturation of
hemoglobin occurred.

Methemoglobin was increased in certain samples by addi-
tion of K3Fe(CN)6 after hemolysis with Triton X-100 (4).

Full oxygenation of blood was done by tonomterizing 2 mL
of blood at room temperature with gas mixtures containing
100% O2, 96% O2/4% CO2, and 90% O2/10% CO2. Additional
experiments involved atmospheric air and air enriched with
CO2 (50 mL/L final concentration).

Full deoxygenation of blood was achieved by adding
dithionite (40 mmol/L final concentration), and adjusting
the pH to various values with Tris buffer (40 mmol/L) (4).

Hemoglobin–oxygen equilibrium curves were constructed
by measuring both a deoxygenated sample (equilibrated
with 100% argon) and a fully oxygenated sample (equili-
ibrated with 100% O2) in 10-mL glass syringes, then drawing
0.5 to 1.0 mL of atmospheric air into each syringe, rotat-
ing for 5 min, and then measuring $P_{O2}$, pH, $p_{CO_2}$ (ABL 3), and $s_O2$
(OSM 3).

Presence of an unknown dyshemoglobin. For this test, add
250 µL of HCl (140 mmol/L) anaerobically to 5 mL of blood
from a fully oxygenated sample (equilibrated with 100% O2).
Measure $P_{O2}$, pH, and $s_O2$ (OSM 3) before and after HCl addition.
If an unknown dyshemo-
globin is present only at low pH, the $P_{O2}$ should increase
upon acidification.

Results

In blood with a $P_{O2}$ >95 kPa, and over a large pH range,
$s_O2$ readings from OSM 3 may vary from 97.7% to 100.7%
depending on pH and $C_{DPG}$ (Figure 1). The OSM 2 gave
similar results, though with a separate CO2 effect. In-
creasing the methemoglobin percentage to 6% did not alter the
$s_O2$ readings with OSM 3 or affect the observed pH effect.

The precision of the measured $s_O2$, calculated in each case
from 50 duplicate measurements, was 0.1% for OSM 3 and
0.2% for OSM 2, at an $s_O2$ of 100%. Small differences in the
absorption spectrum of oxyhemoglobin caused by changes in
pH were confirmed by scanning (Figure 2 and Table 1).

No pH-related differences were seen with deoxyhemoglo-
bin, either by scanning the spectrum or by reading $s_O2$ with
the OSM 2 or OSM 3.

Figure 3 shows the abnormal form of the hemoglobin–
oxygen equilibrium curve at low pH, if $s_O2$ readings are not
Fig. 2. Spectra of hemolyzed oxygenated whole blood at pH (plasma phase) 6.75 (—) and 7.61 ( … )
Note the slightly higher absorption peaks at 535 and 577 nm and a marginally lower absorbance at 600 nm at the higher pH. These differences are more clearly
seen in the difference spectrum (upper panel, scale enlarged by 10-fold).
Because of slight differences (<1%) in the total hemoglobin concentration the two
curves have been arbitrarily normalized at a wavelength of 506 nm. See also Table 1

corrected for the pH effect on the oxyhemoglobin absorption spectrum.
Table 2 shows no increase in $p_{O_2}$ after the anaerobic addition of HCl to an oxygenated blood sample. Release of $O_2$ from 0.3 mmol of oxyhemoglobin per liter would be expected to increase $p_{O_2}$ by about 29 kPa. The small decrease in $p_{O_2}$ can be ascribed to $O_2$ consumption.

Discussion
A direct effect of pH and $CO_2$ on the absorption spectrum of oxyhemoglobin has not previously been described. The present findings would have been difficult to detect except for the exceptionally high precision of the oxygen saturation meter OSM 3. It is interesting that, in 1962, Zijlstra and Mook (5) observed a positive bias when calibrating a reflectance-type oximeter with 100% $O_2$. At the time the authors speculated that this could result from changes in the erythrocyte surface, but in retrospect the data might perhaps be explained by a pH effect on the oxyhemoglobin spectrum. The effect of diphosphoglycerate is very small, which explains why it was not observed earlier by Zijlstra et al. (9).

The present study confirms a direct effect of pH, diphosphoglycerate, and $CO_2$ on the absorption spectrum of oxyhemoglobin, but no observable effect on that of deoxyhemoglobin. The differences in the oxyhemoglobin spectra at pH 6.8 and 7.6 (Figure 2) are similar to the differences recently described for adult and fetal oxyhemoglobin spectra (6, 7), and those earlier described for diphosphoglycerate (8), and presumably are ascribable to alterations in the molecular structure of the oxyhemoglobin molecule with pH, arising

| Table 1. Hemoglobin and Blood Gas Data at Low and High pH* |
|-----------------|-----------------|
| pH (plasma phase) | Low pH | High pH |
| $pCO_2$/kPa   | 6.75 | 7.61 |
| $pO_2$/kPa   | 54 | 67 |
| $sO_2$/% theoretical (10) | 99.78 | 99.92 |
| $sO_2$/% (OSM 3) | 98.5 | 100.3 |
| $sO_2$/% (OSM 2) | 99.4 | 100.5 |
| $sO_2$/% (IL 282) | 98.4 | 100.6 |
| $X_{CO_2}$/% (OSM 3) | $-0.1$ | 0.2 |
| $X_{CO_2}$/% (IL 282) | $-2.3$ | 0.6 |
| $X_{H^+}$/% (OSM 3) | 0.0 | 0.0 |
| $X_{H^+}$/% (IL 282) | $-0.3$ | 0.0 |

* With reference to the absorption spectra in Figure 2.

| Table 2. Effect of Adding HCl Anaerobically to Oxygenated Blood |
|-----------------|-----------------|
| HCl addition | NaCl addition (control) |
| pH | Before | After | Before | After |
| $pCO_2$ | 94.4 | 89.0 | 113.4 | 105.0 |
| $sO_2$ | 100.6 | 97.6 | 100.6 | 100.6 |

Total concentration of hemoglobin = 10.0 mmol/L * Measured (uncorrected).
from differences in amino acid protonation and changes in the charge of the alpha and beta chains.

The findings cannot be explained by interference from methemoglobin or by an altered affinity of hemoglobin for oxygen. Regarding the latter, according to the model of Siggaard-Andersen et al. (10), the Bohr effect will decrease $S_O_2$ by about 0.1% over the pH range studied, whereas the $S_O_2$ readings in our study decreased by 3.0% (Figure 1). Furthermore, failure to correct the $S_O_2$ readings at high $P_O_2$ values results in a marked deviation of the upper end of the hemoglobin–oxygen equilibrium curve from the theoretical curve and a decrease in the Hill slope to $<1$ (Figure 3), which is theoretically impossible.

Reports in the literature disagree as to whether normal healthy adults may contain unidentified inactive hemoglobin (dyshemoglobin) (11, 12). We therefore considered the theoretical possibility that our data could be explained by the presence of up to 3% unidentified dyshemoglobin, which was present only at low pH and had an absorption spectrum similar to deoxyhemoglobin. This possibility, however, was also excluded in our study (Table 2).

Implications of the spectrophotometric measurement of $S_O_2$. Although our studies were confined to Radiometer’s OSM 2 and OSM 3, the observed potential error in $S_O_2$ measurement will apply to other models of commercially available oxygen saturation meters. For example, calculating from the absorbance data in Figure 3 measured with the IL 282 (Instrumentation Laboratory, Lexington, MA), at wavelengths of 535, 585.2, 594.5, and 626.6 nm there is about 2% difference in $S_O_2$ and about 3% difference in $S_HBBO$ at pH 6.8 and 7.6 (Table 1).

We recommend calibrating oxygen saturation meters to read $S_O_2$ 100% for a blood sample in which $P_O_2 >96$ kPa, pH = 7.4, $P_CO_2 = 5.3$ kPa, and the concentration of 2,3-diphosphoglycerate is within the normal reference interval. Practically, this can be done by using any of the gas mixtures in Table 3 and then correcting for the bias according to Figure 1. In usual clinical practice, measuring $S_O_2$ in a blood sample with an abnormal pH will then be unlikely to have a bias in $S_O_2 >1%$. For research purposes, however, where wide pH variations may be encountered and a higher degree of accuracy may be required, the measured $S_O_2$ bias may be as great as 3%, but this can be corrected by using Figure 4.

Measurement of the fetal-hemoglobin fraction with the OSM 3. Elsewhere (7) we suggested using the OSM 3 to estimate the Hb F fraction in newborn infants’ blood by utilizing the difference in absorption spectrum between Hb F and Hb A. In that situation, inaccuracy of $S_O_2$ could give large errors in the estimate of Hb F fraction. For example, with a 1% negative bias in $S_O_2$ a blood sample with 90% Hb F would be estimated by OSM 3 to contain 60% Hb F.

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Amylase in Urine as Measured by a Single-Step Chromolytic Method

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We studied a new single-step direct chromolytic method (Behring D.A.T.) for measuring urinary amylase (1,4-α-d-glucan glucoamylase, EC 3.2.1.1) activity, comparing results with those by a similar, but two-step, procedure that requires an auxiliary (coupling) enzyme. The two methods gave virtually identical relative responses to purified human pancreatic and salivary amylases. Assay of four quality-control materials to evaluate the total (day-to-day) precision of the new method yielded CVs of 4 to 7%, similar to those of the comparison method for each of the four quality-control samples. Amylase activity was measured by both methods in 110 random (i.e., untreated) urine specimens. Linear regression analysis provided a slope and y-intercept of 0.947 and 4 U/L (x = comparison method, y = direct method), respectively, and a standard error of the estimate of 25 U/L for specimens in which the amylase activities ranged from 1 to 1465 U/L (mean = 358 U/L) by the comparison method. The mean rate of amylase excretion in 2-h timed urine specimens from 95 healthy volunteers, as measured by the new method, was 7.18 (SD = 3.18) U/h, and the nonparametric (95% confidence interval) reference interval was 1.6 to 15.2 U/h. We consider the new method a promising alternative to other kinetic assays that require the use of auxiliary enzymes.

Additional Keyphrases: reference interval • two-step procedure compared

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Assay of amylase in urine is a rapid test that is often overlooked in the laboratory investigation of hyperamylasemia (1, 2). In acute pancreatitis, urinary amylase excretion reportedly (3) increases above its reference interval before serum amylase does and remains increased longer. Also, the finding of a non-elevated urinary amylase excretion rate can provide a rapid presumptive diagnosis of macroamylasemia as the source of increased serum amylase in some patients (4, 5). The clinical use of, and problems associated with, measurement of amylase activity in serum and urine have been reviewed elsewhere (6). A common deficiency of amylase assays is the lack of a normal reference interval for the urinary excretion rate of amylase as measured by the method.

Most of the methods devised for measuring amylase activity in serum or urine involve, as a first step, the action of amylase on a poly- or oligosaccharide (7–9). For example, McCroskey et al. (10) described a method in which amylase cleaves p-nitrophenyl derivatives of penta- and hexaoses to yield smaller derivatized oligosaccharides; then, in a second reaction, glucosidase (EC 3.2.1.20) liberates p-nitrophenoxide, which is then measured spectrophotometrically.

Recently, a primary substrate has been synthesized that obviates the need for the glucosidase-catalyzed reaction: maltotriose, covalently bound to 2-chloro-4-nitrophenol (CNP). The reaction scheme with this substrate is as follows:

5 CNP-α-G3 \xrightarrow{\text{amylase}} 3 CNP + 2 CNP-α-G2 + 3 G + 2 G

where G2 is maltotrioside, G3 is maltotriose, and G is glucose. The liberated CNP is measured by its absorbance at 405 nm. Because there is no requirement for a second reaction in this scheme, the method has been termed "Direct Amylase Test," or D.A.T.