PHARMACOKINETICS OF SODIUM NITRITE-INDUCED METHEMOGLOBINEMIA IN THE RAT

MICHAEL C. KOHN, RONALD L. MELNICK, FRANK YE, AND CHRISTOPHER J. PORTIER

Laboratory of Computational Biology and Risk Analysis, National Institute of Environmental Sciences, Research Triangle Park, North Carolina

(Received November 20, 2001; accepted March 1, 2002)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

A biologically based mathematical model was created to characterize time and dose-dependent relationships between exposure to nitrite and induction of methemoglobinemia. The model includes mass action equations for processes known to occur: oral absorption of nitrite, elimination from the plasma, partitioning between plasma and erythrocytes, binding of nitrite to hemoglobin and methemoglobin, and the free radical chain reaction for hemoglobin oxidation. The model also includes Michaelis-Menten kinetics for methemoglobin reductase-catalyzed regeneration of hemoglobin. Body weight-scaled rate constants for absorption ($k_a$) and elimination ($k_e$), the effective erythrocyte/plasma partition coefficient ($P$), and the apparent $K_m$ for methemoglobin reductase were the only parameters estimated by formal optimization to reproduce the observed time course data. Time courses of plasma nitrite concentrations and blood levels of hemoglobin and methemoglobin in male and female rats that had received single intravenous or oral doses of sodium nitrite were measured. Peak plasma levels of nitrite were achieved in both sexes approximately 30 min after oral exposure, and peak methemoglobin levels were achieved after 100 min. The model predicts that 10% of the hemoglobin is oxidized to the ferric form after oral doses of 15.9 mg/kg in male rats and 11.0 mg/kg in female rats and after intravenous doses of 8.9 and 7.1 mg/kg in male and female rats, respectively. The $t_{1/2}$ for recovery from methemoglobinemia was 60 to 120 min depending on dose and route of administration. A sensitivity analysis of the model was performed to identify to which parameters the predictions of the model were most sensitive and guide attempts to simplify the model. Replacement of the $V_{max}$ of methemoglobin reductase with a value representative of humans predicted a 10% methemoglobinemia following an intravenous dose of 5.8 mg/kg, in close agreement with an observed value of 5.7 mg/kg for humans.

Sodium nitrite is an inorganic salt used in the manufacture of dyes, treatment of textiles, and curing of meat. It is also produced from nitrate in ingested food by bacteria in the gastrointestinal tract. Mice (Smith et al., 1967) and rats (Imaizumi et al., 1980; Hirneth and Classen, 1984) exposed to sodium nitrite achieve elevated concentrations of (ferric) methemoglobin in their blood. Unlike the ferrous form of hemoglobin, methemoglobin does not bind oxygen strongly. The oxidation of oxyhemoglobin by nitrite to produce methemoglobin is a complex process that has been characterized by a lag phase followed by an autocatalytic phase (Kosaka and Tyuma, 1987; Spagnuolo et al., 1987). These phases reflect the requirement for accumulation of (ferric) methemoglobin in their blood. The goals of the modeling were to

1. Fit a biologically realistic model to the experimentally observed time courses of plasma nitrite, hemoglobin, and methemoglobin based on current knowledge of the mechanism of induction and recovery from methemoglobinemia;
2. Identify to which parameters the computed time courses are most sensitive;
3. Determine if eliminating steps in the model based on information obtained from the sensitivity analysis can still lead to a good fit to the data.

The modeling procedure itself should reveal the biological processes in which kinetics are inadequately characterized, thus providing guidance for future research.

Elevated levels of methemoglobin can lead to anemic hypoxia, a condition in which there is inadequate supply of oxygen to tissues. Hypoxia may result in cyanotic effects such as smooth muscle relax-
SODIUM NITRITE-INDUCED METHEMOGLOBINEMIA IN THE RAT

Experimental Procedures

Animal handling and dosing have been previously reported (Midwest Research Institute, 1995b). Plasma nitrite and hemoglobin were determined by previously validated methods (Midwest Research Institute, 1995a). Time course data for nitrite and methemoglobin were obtained under NIEHS contract no. N01-ES-15306 to provide data for the construction of this model. The data have been archived at NIEHS and can be retrieved from NIEHS Central Data Management (919-541-5419, voice; 919-541-3687, fax; CDM@niehs.nih.gov, e-mail) by using the contract number. Animal care and treatment were performed in compliance with the good laboratory practice regulations of the US Food and Drug Administration (21 CFR 58) and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 86–23).

Treatment of Animals. Twelve-week old Fischer 344 rats, 55 of each sex (Charles River Laboratories Inc., Wilmington, MA), were individually housed in polycarbonate cages and maintained at 70 to 79°F on a daily 12-h light/dark cycle. After a 2-week quarantine, sodium nitrite (J. T. Baker, Phillipsburg, NJ) in deionized water was administered by lateral tail vein injection at 20 mg/kg body weight (2 ml/kg dosing volume) or by oral gavage at 40 and 80 mg/kg (5 ml/kg dosing volume). At each time point, blood (0.5 ml) was collected from three animals of each sex via the retro-orbital sinus and placed into microcentrifuge tubes containing heparin. Each animal was bled twice, under anesthesia at separate time points via alternating orbital sinuses. Rats were bled at various time points up to 120 min after an intravenous dose and up to 600 min after oral doses.

Measurement of Plasma Nitrite. Plasma nitrite concentrations were determined spectrophotometrically. Plasma was prepared by centrifugation of fresh blood samples for 10 min at 2000 rpm. Aliquots of diluted plasma, as well as sodium nitrite standards prepared in water, were mixed with potassium ferricyanide and zinc sulfate and then centrifuged and filtered. Aliquots of the filtrate were mixed with an equal volume of a solution containing 0.6% sulfanilamide and 0.12% N-(1-naphthyl)-ethylenediamine dihydrochloride in water. The absorbances of these solutions were read after 15 min at 530 nm. Nitrite concentrations in the plasma samples were estimated from a weighted regression curve of the sodium nitrite standards.

Measurement of Hemoglobin. Aliquots of fresh blood were hemolyzed in water (1:5.5, v/v). After 3 min, phosphate buffer was added, and the samples were centrifuged at 14,000 rpm for 15 min. Measurements of methemoglobin and total hemoglobin concentrations in hemolysates were based on the absorbance of methemoglobin at 630 nm. Addition of cyanide eliminated the contribution of methemoglobin to the absorbance at 630 nm. Methemoglobin standards and blood samples were treated similarly to relate absorbance values to methemoglobin concentration.

For the measurement of methemoglobin, the absorbance of the hemolysate at 630 nm was first determined in the absence of cyanide. To 100 µl of the

---

**Fig. 1.** Free radical chain reaction for methemoglobin production (Spagnuolo et al., 1987).

The chain initiation step was treated as first-order in erythrocyte nitrate concentration. Rate constant are $k_1 = 0.048$ min$^{-1}$, $k_2 = 14.1$ mM$^{-1}$ min$^{-1}$, $k_3 = 18$ mM$^{-1}$ min$^{-1}$, and $k_4 = 4.8 \times 10^4$ mM$^{-1}$ min$^{-1}$.

---

$1$ Abbreviations used are: NIEHS, National Institute of Environmental Health Sciences.
hemolysate was added 50 μl of a neutralized cyanide solution containing 5.3% w/v sodium cyanide and 5.6% v/v acetic acid, and the absorbance of the cyanide-treated sample was read at 630 nm. The absorbance in the absence of cyanide minus that in the presence of cyanide is a measure of the conversion of methemoglobin in the sample to cyanmethemoglobin. Total hemoglobin in the hemolysate was determined by oxidizing all of the heme protein to methemoglobin with potassium ferricyanide (0.1% in phosphate buffer) and recording the absorbance at 630 nm. Cyanide solution was added, and the absorbance at 630 nm was recorded again. The difference in absorbance between these samples reflects the total concentration of hemoglobin in the hemolysate.

**Modeling Strategy.** Modeling the chemical consequences of nitrite exposure involved kinetics for absorption of an oral dose, elimination of nitrite from blood, and oxidation of hemoglobin by nitrite and by nitrogen dioxide (Fig. 1). Absorption of nitrite was represented as occurring from the stomach ($k_{abs}$) because the plasma nitrite concentration reaches its highest value within 25 min following gavage, and the time to empty a rat’s stomach is about 2 h (Encke et al., 1989). If absorption of nitrite from the stomach is a saturable process (see below), the uptake rate at concentrations below the apparent $K_m$ (in which linear kinetics are adequate) is approximated by

$$v_{uptake} = \frac{k_{transport} \cdot C_1 \cdot A}{K_m} = k_{abs} \cdot A$$

where $k_{transport}$ is the turnover of the transporter, $C_1$ is the concentration of transporter (number of transporters per stomach volume), $A$ is the anion concentration in the stomach lumen, and $K_m$ is the apparent $K_m$ for transport. If the two-dimensional density $D$ of anion transporters in the gastric epithelium is the same for males and females, the effective concentration of transporters is given by

$$C_1 = \frac{D \cdot S}{V}$$

where $S$ is the surface area, and $V$ is the stomach lumen volume. Assuming the surface area is proportional to the 2/3 power of body weight $W$, the effective concentration of transporters is inversely proportional to $W^{0.3}$. At subsaturating concentrations of the anion, the absorption rate constant is approximately

$$k_{abs} = k_j W^{0.3}$$

where the scaled rate constant for absorption, $k_j$, is independent of sex. The plasma volume, 2.97% of body weight (Delp et al., 1991; Davies and Morris, 1993), is 3.3 times the volume of the stomach lumen, 0.91% of body weight (Roth et al., 1993). Therefore, an amount of nitrite taken up from the stomach increases the plasma concentration only 0.3 times as much as it decreases the stomach lumen concentration. This was taken into account by multiplying $v_{uptake}$ in the expansion for the time derivative of the plasma nitrite concentration (see Appendix) by the ratio of the stomach lumen volume to the plasma volume.

Alternatively, in the acidic environment of the stomach, nitrite is protonated to nitrous acid, which could passively diffuse across the stomach wall. However, other anions (e.g., $SO_4^{2-}$) are absorbed from the gut by a charge-compensated (cotransport with Na$^+$) carrier (Stein and Lieb, 1986), and this mechanism does not account for the sex difference in specific absorption rate. The requirement for different parameter values for males and females was most simply attributed to differences in body weight. Males weighed about 255 g, and females weighed about 165 g. Other reasons for the sex difference are much more speculative and would have to assume the existence of unknown processes.

The major pathway for elimination of nitrite is transport into tissues in which it is oxidized to nitrate, although some nitrite is excreted in urine unchanged. This process is in addition to partitioning of nitrite into erythrocytes. If, by analogy to the uptake of other inorganic anions, the peripheral tissue transport rate is carrier-mediated and saturable, at plasma concentrations below the effective $K_m$, the rate is approximated by

$$v_{elim} = \frac{C_{total} \cdot A}{K_m} = k_{elim} \cdot A$$

where $C_{total}$ is the total carrier activity, $K_m$ is the apparent $K_m$ of the process, and $A$ is the plasma nitrite concentration. As the total carrier activity increases with the weight of the peripheral tissues, the elimination rate constant should be proportional to body weight.

$$k_{elim} = k_e \cdot W$$

where the scaled rate constant for elimination, $k_e$, is independent of sex.

To further reduce the number of adjustable parameters in the model, the equilibrium constant for dissociation of the nitrite-hemoglobin complex was estimated from literature data for the release of oxygen from erythrocyte oxyhemoglobin in the presence of sodium nitrite (Smith, 1970). The estimated value is 17.5 mM. The dissociation constant for the nitrite-methemoglobin complex at pH 7.4 was reported to be in the range 2.6 to 3.4 mM (Smith, 1967; Kosaka et al., 1979), but these values may be too large because of interfering reactions (Smith, 1967). Therefore, the lowest reported value, 2.6 mM (Smith, 1967), was used in the model. As binding of nitrite to methemoglobin (not accounted for by the partitioning into erythrocytes) does not exhibit cooperativity (Kosaka et al., 1979), the reactions at each heme were treated as independent.

The rate constants for the autocatalytic oxidation of hemoglobin used in the model were previously determined (Spagnuolo et al., 1987) by a statistical fit to observed time courses for the oxidation of hemoglobin by nitrite in homogeneous media. These parameters include rate constants for pseudo first-order reduction of the displaced oxygen by nitrite ($k_1 = 0.048$ min$^{-1}$), oxidation of ferrous hemoglobin to methemoglobin by nitrogen dioxide ($k_2 = 14.4$ mM$^{-1}$ min$^{-1}$), oxidation of methemoglobin to ferryl hemoglobin by hydrogen peroxide ($k_3 = 18$ mM$^{-1}$ min$^{-1}$), reduction of ferryl hemoglobin to methemoglobin by nitrite and generation of nitrogen dioxide ($k_4 = 4.8 \times 10^3$ mM$^{-1}$ min$^{-1}$), and the chain termination disproportionation of nitrogen dioxide to nitrite and nitrate ($k_5 = 0.048$ min$^{-1}$). The rate constants, $k_1$ and $k_4$, characterize the kinetics of the autocatalytic phase.

When nitrite was represented as uniformly distributed between plasma and erythrocytes, the measured rate constants ($k_j$–$k_4$) greatly under-predicted methemoglobin formation. Methemoglobin formation is not limited by uptake of nitrite into the erythrocytes (Zavodink et al., 1999), suggesting that nitrite may be actively taken up into erythrocytes, perhaps in exchange for bicarbonate (Shingles et al., 1997). A first-order rate constant of 12.24 min$^{-1}$ was calculated from the measured rate of transport of nitrite in erythrocytes (Shingles et al., 1997). As the kinetic mechanism for nitrite transport across the erythrocyte membrane is unknown, this process was represented as a net partitioning between the two compartments, the effective equilibrium constant $P = C_{BACF}/C_{plasma}$ was an adjustable parameter.

Similar to the case of uptake of nitrite from the stomach, the rate of transport had to be corrected for the different volumes of plasma (55% of blood) and erythrocytes (45% of blood). That is, the time derivative of plasma nitrite entering the red blood cell had to be multiplied by the ratio of plasma volume to erythrocyte volume. The correction for nitrite exiting from the red blood cell is the inverse of this ratio (see Appendix).

Systematic deviations between the observed time course data and those predicted by a model with simplified kinetics for oxidation (see below) suggested that methemoglobin reduction is a saturable process. Therefore, a Michaelis-Menten equation was used instead of an effective first-order rate constant for methemoglobin reductase activity. The maximal velocity of methemoglobin reductase was reported as 1.8 nmole/min/mg Hb (Hagler et al., 1981). Using 158 g Hb/viter blood gives a $V_{max}$ of 0.635 mM/min as heme groups. The model treats each heme as independent, but the hemoglobin tetramer is actually bound to the reductase. Therefore, the concentration of heme units is divided by 4 in the rate equation for the reductase (see Appendix).

The apparent $K_m$ of the reductase was an adjustable parameter in the expanded model. It should be noted that of the 16 constants in the model, only four were adjustable parameters.

Equations for this model (see Appendix) were implemented in SCOP (Kootsey et al., 1986; Kohn et al., 1994). The above rate and equilibrium constants were estimated by least-squares optimization using the SCOPit program (part of the SCOP package, Simulation Resources Inc., Redlands, CA). Values for $P$, $k_j$, $k_k$, and the $K_m$ of methemoglobin reductase were estimated by least-squares optimization to reproduce simultaneously the observed time courses of plasma...
nitrite, hemoglobin, and methemoglobin in male and female rats following intravenous and gavage doses of sodium nitrite. The basal hemoglobin oxidation rate was dynamically calculated to maintain a steady state concentration of methemoglobin (1.2%) in unexposed rats.

Results

The observed and computed time courses of nitrite, hemoglobin, and methemoglobin following a single intravenous administration are given in Fig. 2. The corresponding curves for low- and high-dose gavage experiments are given in Figs. 3 and 4, respectively. Some of the deviation of the simulation results from the data are due to the fact that the sum of measured hemoglobin and methemoglobin concentrations is slightly different at several time points, whereas the model forces the maintenance of conservation of mass. Although males were approximately 60% larger than females, scaling the rate constants for absorption and elimination by body weight resulted in an accuracy of fit to the data comparable with that obtained by allowing different values for each sex.

The fit to the data has excellent statistical properties. The standard error of estimate (3.59) is about 25% of the data values, comparable with the variation among replicate measurements. The optimal parameter values and their standard deviations for absorption and elimination of nitrite, the partitioning of nitrite between plasma and red blood cells, and the $K_m$ for methemoglobin reductase are given in Table 1. The standard deviations are about 1 to 6% of the parameter values. Repeated optimizations from widely different initial parameter estimates all converged to the same solution, indicating the uniqueness of these values.

The rapid decline in plasma nitrite after intravenous injection is due to transport of nitrite into erythrocytes where it can bind to hemoglobin, transport of nitrite into tissues where it may be oxidized to nitrate, and excretion of nitrite into the urine. The model predicts 10% conversion of ferrous hemoglobin to methemoglobin after intravenous doses of 8.9 and 7.1 mg/kg for male and female rats, respectively. Recovery from methemoglobinemia is dependent largely on the kinetics of methemoglobin reductase. The predicted $t_{1/2}$ for recovery is about 60 min after an intravenous dose of 20 mg/kg.

Following oral exposure, peak plasma levels of nitrite are achieved in approximately 25 min. At this time after oral doses of 40 and 80 mg/kg, plasma nitrite is predicted to be 0.11 and 0.22 mM, respectively, for males and 0.15 and 0.30 mM, respectively, for females. At this same time following the same doses, erythrocyte unbound nitrite is predicted to be 1.7 and 3.2 mM, respectively, for males and 2.3 and 45 mM, respectively, for females. The ratio of erythrocyte to plasma nitrite is 15 at all doses studied. Methemoglobin achieves its maximal concentration nearly 80 min after nitrite levels have peaked. The model predicts 10% conversion to methemoglobin after oral doses of 15.9 and 11.0 mg/kg in male and female rats, respectively. The predicted $t_{1/2}$ for recovery from methemoglobinemia in these studies is 90 to 100 (male-female) and 100 to 120 (male-female) min after oral doses of 40 and 80 mg/kg, respectively.

To identify to which parameters the behavior of the model is most sensitive, a full sensitivity analysis, a well known technique used in engineering (Frank, 1978), of the model was performed with SCoPfit. Sensitivity was expressed as the fractional deviation of a computed concentration with respect to a fractional change in a parameter value.

$$S_{ij} = \frac{\delta C_i}{\delta p_j} \bigg|_{T=T_{max}}$$

where $C_i$ is the computed concentration and $p_j$ is the parameter value. The sensitivity coefficients were evaluated when methemoglobin concentration was maximal in the simulation (i.e., at 30 and 100 min after intravenous or oral administration of sodium nitrite, respectively).

Table 2 shows the most significant relative sensitivities of methemoglobin formation to parameter variations following intravenous administration. The sensitivity to $V_{max}$ of methemoglobin reductase (not shown) is approximately the negative of the sensitivity to the $K_m$ because these two parameters are correlated. The computed concentrations of methemoglobin are most sensitive to the value of the rate constant for the free radical chain initiation reaction ($k_1$ in Fig. 1) but not to the other rate constants in the autocatalytic mechanism. Sensitivity of the computed concentrations of methemoglobin to the other parameter values followed the order $k_1 > P > K_m$. The sensitivities to
variations in other parameters are 2 orders of magnitude smaller than the values in Table 2.

Sensitivities of methemoglobin concentrations to parameter variations after oral administration followed the order $k_1 \approx k_e \approx P > k_a \approx K_m$ (Table 3). The computed plasma nitrite concentration at 30 min following intravenous administration and 100 min following oral administration is sensitive mainly to $k_e$ (Table 4). The sensitivity to $k_e$ (for oral dosing) is about 85% smaller. The sensitivities with respect to other parameters are at least an order of magnitude smaller.
TABLE 3
Relative sensitivity of predicted methemoglobin accumulation to parameter variations 100 min after oral administration of 40 and 80 mg NaNO₂/kg body weight to Fischer 344 rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>1.06</td>
<td>0.853</td>
</tr>
<tr>
<td>kₚ</td>
<td>0.352</td>
<td>0.350</td>
</tr>
<tr>
<td>kₑ</td>
<td>−1.06</td>
<td>−0.871</td>
</tr>
<tr>
<td>Kₘ</td>
<td>0.572</td>
<td>0.439</td>
</tr>
<tr>
<td>kᵢ</td>
<td>0.965</td>
<td>0.902</td>
</tr>
<tr>
<td>80 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>1.01</td>
<td>0.734</td>
</tr>
<tr>
<td>kₚ</td>
<td>0.413</td>
<td>0.354</td>
</tr>
<tr>
<td>kₑ</td>
<td>−1.03</td>
<td>−0.765</td>
</tr>
<tr>
<td>Kₘ</td>
<td>0.401</td>
<td>0.258</td>
</tr>
<tr>
<td>kᵢ</td>
<td>0.955</td>
<td>0.784</td>
</tr>
</tbody>
</table>

TABLE 4
Relative sensitivity of predicted plasma nitrite concentration to variation of the elimination (kₑ) and absorption (kₚ) rate constants following intravenous and oral administration of sodium nitrite to Fischer 344 rats

<table>
<thead>
<tr>
<th>Experiment, Parameter</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg/kg intravenous + 30 min, kₑ</td>
<td>−1.16</td>
<td>−0.830</td>
</tr>
<tr>
<td>40 mg/kg oral + 100 min, kₑ</td>
<td>−1.14</td>
<td>−0.938</td>
</tr>
<tr>
<td>40 mg/kg oral + 100 min, kₚ</td>
<td>−0.178</td>
<td>−0.151</td>
</tr>
<tr>
<td>80 mg/kg oral + 100 min, kₑ</td>
<td>−1.01</td>
<td>−0.798</td>
</tr>
<tr>
<td>80 mg/kg oral + 100 min, kₚ</td>
<td>−0.188</td>
<td>−0.216</td>
</tr>
</tbody>
</table>

Discussion

The disposition of sodium nitrite following intravenous and oral administration was studied by computer simulation with a model that accounts for all reactions known to be involved in production of (ferric) methemoglobin and its reduction to (ferrous) hemoglobin. Only 4 of the 16 constants in the model had to be estimated by formal optimization to enable the model to fit the experimental data. Optimizations commencing from different guesses for the parameter values all converged to the same optimum, and the estimated parameter values had small confidence limits.

The ability of the model to fit the data with absorption and elimination rate constants scaled for body weight suggests that there is no qualitative difference in disposition of nitrite between the sexes. Sensitivity analysis demonstrated that the computed behavior depends on parameters for distribution (P, kₚ, and kₑ) as much as on parameters for blood biochemistry (Kₘ and kᵢ). It is noteworthy that the behavior of the model is insensitive to variations in the dissociation constants for the nitrite complexes of hemoglobin and methemoglobin. As there is considerable uncertainty in these values, the lack of sensitivity increases confidence in the simulation. The lack of sensitivity of the computed nitrite and methemoglobin concentrations to the rate constant for transport of nitrite across the erythrocyte membrane also indicates that the model is robust, but the behavior of the model is sensitive to the apparent erythrocyte/plasma partition coefficient.

The lack of mechanistic and kinetic data on the absorption, partitioning, and elimination of nitrite required the use of first-order kinetics for these processes in the present model. The model identifies research areas where additional data are needed on critical processes that regulate nitrite disposition and induction of methemoglobinemia. For example, uptake of nitrite after oral exposure needs to be characterized. Transport of inorganic anions such as phosphate or bisulfate in the epithelia of the gastrointestinal tract is accomplished by co-transport with a sodium ion (Stein and Lieb, 1986). If nitrite is similarly transported, first-order kinetics may not be an adequate approximation. Similarly, elimination by uptake into tissues was represented as a first-order process. The partitioning of nitrite into peripheral tissues and its oxidation to nitrate is likely to be carrier-mediated as well (Stein and Lieb, 1986) and needs to be characterized. Measurement of the kinetics of methemoglobin reductase in rats would remove another parameter from the optimization and increase the reliability of the model.

Partitioning of nitrite between plasma and erythrocytes was treated as a passive equilibrium in this model, but the kinetics of nitrite transport across the erythrocyte membrane should be measured. If nitrite accumulates in red cells against the concentration gradient predicted by the present model, a more complex charge-compensated transport mechanism may be involved. The high estimated partition coefficient is consistent with an active transport process.

The methemoglobin concentration is maximal much later than that of plasma nitrite. This condition arises because the free radical auto-catalytic mechanism continues to generate methemoglobin while nitrite levels are rapidly diminishing. This process depends on the concentration of H₂O₂, which is consumed by erythrocyte catalase (Spagnuolo et al., 1987), the activity of which was measured as 2.82 μM/min (DeMaster et al., 1986). The Kₘ of this enzyme is 25 mM (Lehninger, 1975). The model predicts a maximal H₂O₂ concentration of 70 μM 1 min after intravenous injection of nitrite and 6 to 12 μM after gavage (depending on dose and sex). As these values are far below the Kₘ, the reaction is approximately first order with a rate constant of 1.13 × 10⁻⁴ min⁻¹, orders of magnitude smaller than for the other oxidative reactions. The very small predicted rate for catalase justifies the neglect of this enzymatic activity in the model.

Because the sensitivity analysis indicated that only the chain initiation step of the hemoglobin oxidation mechanism strongly influenced the predictions of the model, the full oxidation cycle was represented by only an initiation step and a single autocatalytic step, the rate constants of which were adjustable parameters. To get a fit with a residual error comparable to that of the full model, separate first-order rate constants for absorption and elimination were required for males and females. Also, the dissociation constants for the (met) hemoglobin complexes with nitrite had to be adjustable. This increased the number of adjustable parameters (from four to eight), and the optimization was numerically unstable.

The fit of the reduced model to the experimental data exhibited several defects, especially systematic deviations between the predictions of the model and the observed blood time course data for methemoglobin, hemoglobin, and plasma nitrite concentrations. A wide range of parameter values produced similar fits to the data, indicating that the data were insufficient to uniquely identify so many parameter values. The standard deviations computed for four of the parameters were extremely large. For example, the optimal kᵢ was 0.009 ± 6.9 min⁻¹, indicating a poorly determined value for that parameter. Even worse, this simplification ignores data in the literature, which characterize the known chemistry.

The equations in this model are reasonable approximations for the kinetics of processes known to occur. Values for most of the constants in this model (75%) are fixed by independent experimental data. Use of ad hoc empirical models (Jusko and Ko, 1994) that do not represent the actual processes involved in methemoglobinemia would ignore these independent data and require the estimation of many more parameters than in the present model. Although an empirical model could summarize the relationships among the variables in an accessible way, the loss of realism would limit the insights that could be obtained from the simulation.

Parameter values for humans are required to extrapolate the predicted methemoglobinemia to humans. The kinetics of absorption,
elimination, and methemoglobin reductase would have to be measured in humans. Other parameters in the model are likely to be properties of the chemistry (oxidative rate constants, nitrite dissociation constants) and not species specific. Despite these limitations, the rat model can be adapted to predict human responses to sodium nitrite. A body weight of 70 kg was assumed. The value of $k_5$ was adjusted (0.022 min$^{-1}$ kg$^{-1}$) such that $k_{elim}$ was the same as that calculated for male rats (1.54 min$^{-1}$). The $V_{max}$ for methemoglobin reductase was reduced by 80% to match the enzymatic activity determined for humans (Stolk and Smith, 1966). When the response to an intravenous dose of 20 mg/kg sodium nitrate was tested, the resulting model reproduced the data for rats given that dose. The “human” model predicts conversion of 10% of the hemoglobin to the ferric form following intravenous injection of 7 mg NaNO$_2$/kg. Oxidation of 10% of the hemoglobin was observed in a human subject after injection of 0.4 g sodium nitrite (Chen and Rose, 1952). Assuming a body weight of 70 kg, this dose corresponds to 5.71 mg/kg, close to the predicted value. Although this estimate is based on a reported value in a single individual, this result indicates that the structure of this model is suitable for predicting methemoglobinemia in humans. Much more experimental data would be necessary to determine a range and distribution of human responses. Based on the results presented here, it appears that scaling the nitrite absorption and elimination rate constants to body weight and using measured methemoglobin reductase activity may provide reasonable estimates of the time courses of nitrite-induced methemoglobinemia in adults as well as in infants and children.

Adult male humans exposed to the above intravenous doses were predicted to achieve a near steady state of methemoglobin in less than 25 min. The predicted half-time for recovery exceeds 6 h. The slower recovery in humans compared with rats is due to lower methemoglobin reductase activity and slower elimination of nitrite from plasma.

This exercise demonstrates how two common fallacies can easily mislead a modeler. The first fallacy is that mathematically simplified models are always preferred over more complex models. Although such simplifications may be necessary when data are lacking, when results of independent experiments are available to fix certain equations and parameters (as is the case in this work), excessive simplification can lead to inadequate models, the parameter values of which cannot be reliably estimated because of ill conditioning. The other fallacy is that, given enough parameters, you can make any model fit. The properties of the reduced model demonstrate that this commonly held notion is simply untrue.

### Appendix

\[
\frac{d[NO_2^-]}{dt} = k_{\text{oxid}} \cdot [NO_2^-]_{\text{plasma}}
\]

\[
\frac{d[NO_2^-]}{dt} = k_{\text{uptake}} \cdot \frac{Vol_{\text{stomach}}}{Vol_{\text{plasma}}} \cdot V_{\text{plasma}} - k_{\text{elim}} \cdot [NO_2^-]_{\text{plasma}}
\]

\[
\frac{d[NO_2^-]}{dt} = k_{\text{transport}} \cdot \frac{Vol_{\text{plasma}}}{Vol_{\text{RBC}}} \cdot V_{\text{plasma}} - k_{\text{elim}} \cdot [NO_2^-]_{\text{plasma}}
\]

\[
\frac{d[NO_2^-]}{dt} = k_{\text{transport}} \cdot [NO_2^-]_{\text{plasma}} \cdot \frac{Vol_{\text{plasma}}}{Vol_{\text{RBC}}} - [NO_2^-]_{\text{plasma}} - \frac{[NO_2^-]_{\text{RBC}}[Hb_{3^+}^4]}{K_{\text{RBC}}} + [Hb_{3^+}NO_2^-]_{\text{KmetHb}} - v_1^{\text{oxid}} + v_2^{\text{oxid}} + v_4^{\text{oxid}} + 0.5 \cdot v_5^{\text{oxid}}
\]

\[
\frac{d[Hb_{3^+}NO_2^-]}{dt} = [NO_2^-]_{\text{RBC}}[Hb_{3^+}^4] - [Hb_{3^+}NO_2^-]_{K_{\text{metHb}}} - v_5^{\text{oxid}}
\]

\[
\frac{d[Hb_{3^+}]}{dt} = V_{\text{basal oxid}} - v_{\text{reductase}} - [NO_2^-]_{\text{RBC}}[Hb_{3^+}^4] + [Hb_{3^+}NO_2^-]_{K_{\text{metHb}}} - v_3^{\text{oxid}} + v_4^{\text{oxid}}
\]

\[
\frac{d[Hb_{3^+}NO_2^-]}{dt} = [NO_2^-]_{\text{RBC}}[Hb_{3^+}^4] - [Hb_{3^+}NO_2^-]_{K_{\text{metHb}}} + \frac{v_2^{\text{oxid}}}{5}
\]

\[
\frac{d[NO_2^-]}{dt} = [NO_2^-]_{\text{RBC}}[Hb_{3^+}^4] - [Hb_{3^+}NO_2^-]_{K_{\text{metHb}}}/2
\]

\[
\frac{d[Hb_{3^+}]}{dt} = [NO_2^-]_{\text{RBC}}[Hb_{3^+}^4] - [Hb_{3^+}NO_2^-]_{K_{\text{metHb}}} - v_3^{\text{oxid}} - v_4^{\text{oxid}}
\]

### References


Midwest Research Institute (1995b) Toxicokinetics of Sodium Nitrite in Rodents. Midwest Research Institute, Kansas City, MO.

National Toxicology Program (2001) Toxicology and carcinogenesis studies of sodium nitrite (CAS no. 7632–00-0) in F344/N rats and B6C3F1 mice (drinking water studies). Technical Report Series No. TR-495, NIH publication No. 01–3954, National Institutes of Environmental Health Sciences, Research Triangle Park, NC.


