Bicarbonate Inhibits N-Nitrosation in Oxygenated Nitric Oxide Solutions

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N-Nitrosation in oxygenated nitric oxide (NO\textsuperscript{+}) solutions was previously shown to be significantly inhibited by phosphate and chloride presumably by anion scavenging of the nitrosating agent nitrous anhydride, N\textsubscript{2}O\textsubscript{3} (Lewis, R. S., Tannenbaum, S. R., and Deen, W. M. (1995) J. Am. Chem. Soc. 117, 3933–3939). Here, bicarbonate is shown to exhibit this same inhibitory effect. Rate constants for reaction of morpholine, phosphate, and bicarbonate with N\textsubscript{2}O\textsubscript{3} relative to N\textsubscript{2}O\textsubscript{3} hydrolysis at pH 8.9 were determined to be \((3.7 \pm 0.2) \times 10^{-4} \text{ M}^{-1} \cdot \text{s}^{-1}, (4.0 \pm 0.9) \times 10^{-5} \text{ M}^{-1} \cdot \text{s}^{-1},\) and \((9.3 \pm 1.5) \times 10^{-6} \text{ M}^{-1} \cdot \text{s}^{-1},\) respectively. The morpholine and phosphate rate constants at pH 8.9 are similar to those reported at pH 7.4 assuring that these results are relevant to physiological conditions. The rate constant for this previously unrecognized reaction of bicarbonate with N\textsubscript{2}O\textsubscript{3} suggests the strong scavenging ability of bicarbonate; accordingly, bicarbonate may contribute to reducing deleterious effects of N\textsubscript{2}O\textsubscript{3}. This is biologically important due to substantial bicarbonate concentrations in vivo, approximately 30 mM. Bicarbonate was previously shown to alter peroxynitrite reactivity; however, carbon dioxide is the probable reactive species. Bicarbonate is therefore potentially important in determining the fate of two reactive species generated from nitric oxide, N\textsubscript{2}O\textsubscript{3} and ONOO\textsuperscript{−}, and may thus act as a regulator of NO\textsuperscript{+}-induced toxicity.

Nitric oxide (NO\textsuperscript{+}) is an important physiological messenger that is produced by several different cell types and is involved in many processes in vivo including inhibition of platelet aggregation, blood vessel relaxation, and neurotransmission (8). An alternative to these physiologically important pathways is the formation of reactive species that may ultimately result in cytotoxic or mutagenic events by a number of possible mechanisms. Mutagenic effects may arise from the reaction of nitric oxide with superoxide (O\textsubscript{2}\textsuperscript{−}) forming peroxynitrite (ONOO\textsuperscript{−}) that can in turn oxidize many types of molecules including DNA. Alternatively, reaction of NO\textsuperscript{+} with molecular oxygen results in the formation of nitrous anhydride (N\textsubscript{2}O\textsubscript{3}) which can cause cytotoxic effects through the nitrosation of both primary and secondary amines. DNA bases containing primary amine functionalities undergo nitrosative deamination upon treatment with NO\textsuperscript{+} resulting in a modified base (9, 10). N\textsubscript{2}O\textsubscript{3} can also nitrosate secondary amines forming carcinogenic N-nitrosoamines that can damage DNA following metabolic activation. N\textsubscript{2}O\textsubscript{3} can modify other cell constituents including protein sulfhydryl groups and low molecular weight thiols such as glutathione resulting in S-nitrosothiols.

The kinetics of morpholine N-nitrosation by nitric oxide at physiological pH have recently been studied by Lewis et al. (1) using a novel reactor that allows continuous and simultaneous measurements of NO\textsuperscript{+}, nitrite (NO\textsubscript{2}\textsuperscript{−}), and N-nitrosomorpholine (NMor\textsuperscript{−}) concentrations. In this system, N\textsubscript{2}O\textsubscript{3} was identified as the key nitrosating agent (1). The measured rate constant for the reaction of morpholine with N\textsubscript{2}O\textsubscript{3} relative to N\textsubscript{2}O\textsubscript{3} hydrolysis was \(4.0 \times 10^{-4} \text{ M}^{-1} \cdot \text{s}^{-1}.\) A key finding was the inhibitory effect of phosphate and chloride on morpholine nitrosation; the rate constants for reaction of these anions with N\textsubscript{2}O\textsubscript{3} relative to N\textsubscript{2}O\textsubscript{3} hydrolysis were \(4.0 \times 10^{-5} \text{ M}^{-1} \cdot \text{s}^{-1}\) and \(9.0 \times 10^{-6} \text{ M}^{-1} \cdot \text{s}^{-1},\) respectively. Participating anions react with N\textsubscript{2}O\textsubscript{3} forming nitrosyl compounds (XNO) that can in turn react with amines or be hydrolyzed to HNO\textsubscript{2} and ultimately nitrite. At physiological pH, hydrolysis of XNO is much faster than nitrosation of amines by XNO; therefore, the anions will scavenge N\textsubscript{2}O\textsubscript{3} and lower the rate of N-nitrosation (1). Other anions including nitrate, nitrite, thiocyanate, and perchlorate have little or no effect on nitrosation.

During the course of DNA deamination studies, it was found that NO\textsuperscript{+}-related deamination of calf thymus DNA at physiological pH was inhibited by sodium bicarbonate, NaHCO\textsubscript{3}. Bicarbonate therefore seems to protect biomolecules from the nitrosative effects of NO\textsuperscript{+} presumably due to an ability to scavenge N\textsubscript{2}O\textsubscript{3} and consequently inhibit nitrosation at pH 7.4. The initial evidence for bicarbonate’s inhibitory effect at pH 7.4 prompted the use of a modified reactor similar to that developed by Lewis and Deen (11) to determine the rate constant for reaction of bicarbonate with N\textsubscript{2}O\textsubscript{3}. However, bicarbonate cannot be studied reliably at pH 7.4 in this system due to extensive argon degassing resulting in a shift in the equilibrium between bicarbonate and carbon dioxide and a consequent pH increase. The rate constants were therefore determined at pH 8.9.

As shown here, bicarbonate is important in determining the fate of N\textsubscript{2}O\textsubscript{3}; the product of nitric oxide oxidation; in addition, bicarbonate has been reported to alter the rate of reactions of peroxynitrite, the product of nitric oxide reaction with superoxide (2, 3, 12). However, it has now been demonstrated that CO\textsubscript{2} is the actual species that reacts with peroxynitrite (5–7). The reactivity of bicarbonate/CO\textsubscript{2} with both N\textsubscript{2}O\textsubscript{3} and ONOO\textsuperscript{−} and the relatively high concentrations of bicarbonate in interstitial and intracellular fluids (up to 30 mM) suggest that bicarbonate is a key determinant of the fate of the reactive species generated from nitric oxide and that bicarbonate may...
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Concentrations of NMor and NO\textsubscript{2} were calculated at each cycle point for the 30-min reaction.

**Kinetic Model and Reaction Scheme**—In previous experiments with this reactor, Lewis et al. (1) showed that the principal nitrosating agent in the NO\textsuperscript{-} oxidation pathway at physiological pH is N\textsubscript{2}O\textsubscript{3} which leads primarily to NO\textsubscript{2} as summarized in reactions 1–3.

\[
k_1\]
\[2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2\]  
(Eq. 1)

\[
k_2\]
\[\text{NO} + \text{NO}_2 \rightarrow \text{N}_2\text{O}_3\]  
(Eq. 2)

\[
k_3\]
\[\text{N}_2\text{O}_3 + \text{H}_2\text{O} \rightarrow 2\text{HNO}_2 \rightarrow 2\text{NO}_2 + 2\text{H}^+\]  
(Eq. 3)

Morpholine nitrosation by N\textsubscript{2}O\textsubscript{3} and enhanced hydrolysis of N\textsubscript{2}O\textsubscript{3} by various anions (X\textsuperscript{-}) are summarized in Equations 4 and 5.

\[
k_{10}\]
\[\text{N}_2\text{O}_3 + \text{Mor} \rightarrow \text{NMor} + \text{NO}_2 + \text{H}^+\]  
(Eq. 4)

\[
k_{10}\]
\[\text{NO}_2 + \text{X} \rightarrow \text{XNO} + \text{NO}_3\]  
(Eq. 5)

Specifically, phosphate and chloride react with N\textsubscript{2}O\textsubscript{3} as shown below (1):

\[
k_{10}\]
\[\text{N}_2\text{O}_3 + \text{P}^\text{5\textsuperscript{-}} \rightarrow \text{PNO} + \text{NO}_3\]  
(Eq. 6)

\[
k_{10}\]
\[\text{N}_2\text{O}_3 + \text{Cl}^\text{1\textsuperscript{-}} \rightarrow \text{CINO} + \text{NO}_3\]  
(Eq. 7)

Any anion that behaves in this manner will scavenger some of the N\textsubscript{2}O\textsubscript{3}, thereby decreasing the rate of N\textsubscript{-}nitrosation at neutral pH. In the above reaction scheme, all reaction rate constants are known. The overall nitrogen balance performed by Lewis et al. (1) confirms that pseudo-steady state approximations for NO\textsubscript{2}, N\textsubscript{2}O\textsubscript{3}, and XNO are valid. A detailed analysis of the conservation equations at physiological pH is given in Ref. 1. The overall reaction kinetics where N\textsubscript{2}O\textsubscript{3} is the only significant nitrosating agent are summarized by the following equations:

\[
\frac{\Delta [\text{R}_n\text{NNO}]}{\Delta [\text{NO}_3] - \Delta [\text{R}_n\text{NNO}]} = k^* [\text{RNH}]  
\]
(Eq. 8)

where the summation in Equation 9 is over all participating anions. For those anions that react with N\textsubscript{2}O\textsubscript{3}, the lumped “constant” k* will depend inversely on the anion concentration. By measuring k* in the presence of several concentrations of participating anions, the rate constants were determined for the reaction of phosphate and chloride with N\textsubscript{2}O\textsubscript{3} (1). In the presence of phosphate and one additional anion, rearrangement of Equation 9 yields:

\[
k^* = \frac{1}{2}\left(\frac{k_6}{1 + \frac{k_{6}^\text{ph}}{k_6}}\right)\left(\frac{k_6}{1 + \frac{k_6^\text{cl}}{k_6}}\right)\right)\]  
(Eq. 10)

Further rearrangement gives:

\[
\left[\frac{1}{2\sum k_6^\text{ph}{k_6^\text{cl}}}k_6^\text{ph} \frac{k_6}{k_6}\left(\frac{k_6}{k_6^\text{ph}}\right)\left(\frac{k_6}{k_6^\text{cl}}\right)\right]\]  
(Eq. 11)

Using the data for phosphate alone, linear regression of 1/(2k*P\textsubscript{5\textsuperscript{-}}) versus 1/P\textsubscript{5\textsuperscript{-}} yields k\textsubscript{6}^\text{ph}/k_6 as the intercept and slope. The values of k_6/k_6^\text{ph} for morpholine and k_6^\text{cl}/k_6 were found in this way (1). In
this work, \(k_1/k_a\) for morpholine and \(k_{10}^{\text{OH}^-}/k_a\) were determined as described above. In addition, the data for solutions containing bicarbonate and phosphate were used to calculate the rate constant for the reaction of bicarbonate with \(\text{N}_2\text{O}_3\) at pH 8.9 (\(k_{10}^{\text{HCO}_3}/k_a\)).

**RESULTS**

**Morpholine Concentration and pH**—The unprotonated form of morpholine is the substrate for nitrosation and is thus the most important form of morpholine for these experiments. Denoting total morpholine as \(\text{Mor}\) and the unprotonated form as \(\text{Mor}^0\), the respective concentrations are related by:

\[
[\text{Mor}^0] = [\text{Mor}] + 10^{pK_a-m}\]

where the \(pK\) at 25 °C is 8.5 for morpholine. The amount of morpholine available for nitrosation is 7.4% and 71.5% of the total morpholine concentration at pH 7.4 and 8.9, respectively. In phosphate buffer, the pH was nearly constant during a given experiment. In the bicarbonate reactions, the pH of the buffer rose during degassing to approximately 8.9. However, the pH during the reaction itself (i.e. after \(O_2\) addition) remained virtually constant. The concentration of unprotonated morpholine therefore did not change significantly during the reaction. In all experiments, some \(\text{NMor}\) and \(\text{NO}_2\) were present in the solution prior to introduction of \(O_2\) due to a small air leak in the flow loop that could not be eliminated.

**Effect of Hydroxide Ion Concentration on Nitrosation Kinetics**—It has been reported that \(OH^-\) enhances the rate of hydrolysis of \(\text{N}_2\text{O}_3\) (13). Using flash photolysis of \(\text{NO}_2^-\) ions in the presence of \(\text{NO}^-\) in the pH range 9–10, a factor for total \(\text{N}_2\text{O}_3\) hydrolysis was reported to be \(2000\ s^{-1} + 10^9 [OH^-]^{10^{-4}}\ s^{-1}\) representing terms for both water and hydroxide-induced hydrolysis of \(\text{N}_2\text{O}_3\) (13). In order to determine the rate constant for reaction of hydroxide with \(\text{N}_2\text{O}_3\) under the present conditions, morpholine nitrosation reactions were performed at several \(pH\) values in the range pH 7.4–8.9. A decrease in morpholine nitrosation at higher \(pH\) values was indeed observed indicating an increase in \(\text{N}_2\text{O}_3\) hydrolysis mediated by hydroxide. The \(k^+\) values obtained by linear regression analysis at pH 7.4, 8.0, 8.5, and 8.9 were 2600 ± 200 \(m^{-1}\), 2300 ± 90 \(m^{-1}\), 1900 ± 200 \(m^{-1}\), and 1500 ± 300 \(m^{-1}\), respectively. The rate constant for hydroxide reaction with \(\text{N}_2\text{O}_3\) was calculated using a rearrangement of Equation 10 by considering hydroxide to be an inhibitor of morpholine nitrosation analogous to the treatment of anions in previous work (1). Using this equation,

\[
\frac{1}{2k^+[\text{OH}^-]} = \frac{k_{10}^{\text{OH}^-}}{k_a} + \frac{1}{[\text{OH}]} \left( \frac{k_1 + k_{10}^{\text{OH}^-}[\text{P}]}{k_a} \right)
\]

the intercept of \(1/2k^+[\text{OH}^-] \) versus \(1/[\text{OH}^-] \) gives the value of \(k_{10}^{\text{OH}^-}/k_a\) to be \((2.3 ± 0.4) \times 10^1\). The published value for \(k_{10}^{\text{OH}^-}/k_a\) (4.0 \(\times 10^3\ \text{m}^{-1}\)) implies that \(k_{10}^{\text{OH}^-}/k_a = 9.4 \times 10^2\ \text{m}^{-1}\).

There is some ambiguity in the meaning of \(k_4\) depending on whether the hydroxide contribution is included. However, the hydroxide term does not significantly affect the \(k_4\) value at pH 7.4 due to the extremely small concentration of hydroxide at this pH. For example, if a value for \(k_4\) is assumed to be 1600 \(s^{-1}\) at pH 7.4 (1), the incremental increase for the hydroxide contribution is 150 \(s^{-1}\). Given the wide range of reported \(k_4\) values as discussed by Lewis et al. (1), this 10% difference does not seem to be very important. The rate constants here are expressed as ratios to \(k_6\).

**Effect of Phosphate on N-Nitrosation at pH 8.9**—To assess the validity of these experiments at pH 8.9, the rate constants for reaction of morpholine and phosphate with \(\text{N}_2\text{O}_3\) relative to \(\text{N}_2\text{O}_3\) hydrolysis (\(k_6/k_a\) and \(k_6^{\text{P}}/k_a\) ) were measured and compared to the published results. Various morpholine concentrations (50 \(\mu\text{M}\) to 150 \(\mu\text{M}\) ) were used in buffers of three different phosphate concentrations, 0.01 M, 0.025 M, and 0.05 M.

As seen in Fig. 2, the marked decrease in slope of \(\Delta [\text{NMor}]/\Delta [\text{NO}_2^-] \) — \(\Delta [\text{NMor}]\) versus \([\text{Mor}^0]\) with increasing phosphate concentration indicates that phosphate inhibits nitrosamine formation. When the slope was calculated by linear regression using the average data between 3 and 30 min, values of \(k^+\) at pH 8.9 for 0.01 M, 0.025 M, and 0.05 M phosphate were 1500 ± 300 \(m^{-1}\), 1100 ± 100 \(m^{-1}\), and 600 ± 100 \(m^{-1}\), respectively. The differences between these values and those previously published are due to the effect of hydroxide at pH 8.9. The rate constants for the reaction of \(\text{N}_2\text{O}_3\) with phosphate and morpholine at pH 8.9 relative to \(\text{N}_2\text{O}_3\) hydrolysis (\(k_{10}^{\text{P}}/k_a\) and \(k_{10}^{\text{OH}^-}/k_a\) ) were calculated from a plot of the equation below:

\[
\frac{1}{2k^+[\text{P}]} = \frac{k_{10}^{\text{OH}^-}}{k_a} + \frac{1}{[\text{OH}]} \left( \frac{k_1 + k_{10}^{\text{OH}^-}[\text{P}]}{k_a} \right)
\]

This equation was fitted to the data using a nonlinear least squares fitting technique. The intercept (\(k_{10}^{\text{OH}^-}/k_a\) ) was found to be \((1.7 ± 0.2) \times 10^{-2}\) which agrees nicely with the previously reported value of \(1.0 \times 10^{-2}\) (1). The value of the rate constant for the phosphate/\(\text{N}_2\text{O}_3\) reaction relative to \(\text{N}_2\text{O}_3\) hydrolysis (\(k_{10}^{\text{P}}/k_a\) ) was then calculated to be \(4.0 \times 10^2\ \text{m}^{-1}\). The rate constant for the morpholine/\(\text{N}_2\text{O}_3\) reaction relative to \(\text{N}_2\text{O}_3\) hydrolysis (\(k_{10}^{\text{OH}^-}/k_a\) ) was determined from the slope to be \((3.7 ± 0.2) \times 10^4\ \text{m}^{-1}\) which agrees nicely with the literature value of \(4.0 \times 10^4\ \text{m}^{-1}\) (1).

**Effect of Bicarbonate on N-Nitrosation at pH 8.9**—Various morpholine concentrations (50 \(\mu\text{M}\) to 150 \(\mu\text{M}\) ) were used in a 0.01 M phosphate buffer solution containing 0.04 M sodium bicarbonate. As seen in Fig. 2, there is a significant decrease in the slope of \(\Delta [\text{NMor}]/\Delta [\text{NO}_2^-] \) — \(\Delta [\text{NMor}]\) versus \([\text{Mor}^0]\) with the
addition of 0.04 M sodium bicarbonate. Using linear regression, the value of \( k^* \) using the average data between 3 and 30 min for reactions containing 0.01 M phosphate and 0.04 M bicarbonate at 25 °C and pH 8.9 was 400 ± 60 m⁻¹ s⁻¹. The rate constant for the bicarbonate/N₂O₃ reaction relative to N₂O₃ hydrolysis (\( k_{HCO_3} / k_4 \)) was calculated from Equation 10. The resulting \( k_{HCO_3} / k_4 \) value is (9.3 ± 1.5) × 10⁵ m⁻¹ s⁻¹. The rate constants from this study are summarized in Table I, and their importance is discussed below.

### DISCUSSION

The finding that \( N \)-nitrosation of morpholine is inhibited by bicarbonate provides an additional pathway that affects the fate of N₂O₃ in vitro and in vivo. The rate constant for the bicarbonate/N₂O₃ reaction relative to N₂O₃ hydrolysis (\( k_{HCO_3} / k_4 \)) in oxygenated nitric oxide solutions is (9.3 ± 1.5) × 10⁵ m⁻¹ s⁻¹, which is greater than the rate constant for the phosphate/N₂O₃ reaction relative to N₂O₃ hydrolysis (\( k_{Pi} / k_4 \)) found to be (4.0 ± 0.9) × 10⁵ m⁻¹ s⁻¹. Inhibition by bicarbonate will be significant due to the higher rate constant for bicarbonate and higher extracellular concentrations of bicarbonate relative to phosphate. As summarized in Table I, the agreement between the published rate constants for the morpholine and phosphate reactions at pH 7.4 and the experimentally determined rate constants at pH 8.9 demonstrates the validity of performing these experiments at pH 8.9 and assures the applicability of the rate constants at pH 8.9 demonstrates the validity of performing these experiments at pH 8.9 and assures the applicability of the rate constants at pH 8.9 demonstrates the validity of performing these experiments at pH 8.9 and assures the applicability of the rate constants at pH 8.9 demonstrates the validity of performing these experiments at pH 8.9 and assures the applicability of the rate constants at pH 8.9 demonstrates the validity of performing these experiments at pH 8.9 and assures the applicability of the rate constants at pH 8.9 demonstrates the validity of performing these experiments at pH 8.9 and assures the applicability of the rate constants at pH 8.9 demonstrates the validity of performing these experiments at pH 8.9 and assures the applicability of the rate constants.
the previously reported reaction with ONOO\(^{-}\). Carbonate has long been known to alter the activity of several types of oxygen radicals including superoxide anion, hydroxyl radical, and singlet oxygen (4). In addition, peroxynitrite has been known to be unstable in carbonate buffers for many years (12). Recently, it has been demonstrated that bicarbonate does indeed affect peroxynitrite reactivity (2, 3). Bicarbonate inhibits the toxicity of peroxynitrite to Escherichia coli (2) which may be a direct result of the enhanced isomerization to nitrate leading to ONOO\(^{-}\) decomposition before it can encounter the bacteria. Extremely low levels of bicarbonate (far below physiological concentrations) are required as demonstrated by the fact that 95% protection from toxicity is observed at 5 mM bicarbonate (2). The probable mechanism involves reaction of carbon dioxide with peroxynitrite forming the nitrosoperoxycarbonate anion, O\(_5\)N-OOCO\(_2\)\(^{-}\) (3, 5, 6). Carbon dioxide increases the rate of peroxynitrite isomerization to nitrate presumably through this species (5). The result is ONOO\(^{-}\) scavenging which will be important in defining amounts of tissue injury including both oxidation and nitration products resulting from peroxynitrite (16–18).

Elucidation of the exact reaction pathways and rate constants for the reaction of bicarbonate/CO\(_2\) with peroxynitrite will provide additional information about the fate of NO\(^{\cdot}\) in specific systems. Then, reactions of NO\(^{\cdot}\) with oxygen and superoxide can be modeled in detail. The newly determined rate constant for the bicarbonate/N\(_2\)O\(_3\) reaction will contribute to kinetic modeling ultimately enhancing the understanding of the numerous biological roles of NO\(^{\cdot}\) both as a messenger and as a cytotoxic or mutagenic agent.

REFERENCES
1. Lewis, R. S., Tannenbaum, S. R., and Deen, W. M. (1995) J. Am. Chem. Soc. 117, 3933–3939
2. Zhu, L., Gunn, C., and Beckman, J. S. (1992) Arch. Biochem. Biophys. 298, 452–457
3. Radi, R., Cosgrove, T. P., Beckman, J. S., and Freeman, B. A. (1993) Biochem. J. 299, 51–57
4. Michelsen, A. M., and Maral, J. (1983) Biochimie 65, 95–104
5. Uppu, R. M., Squadrato, G. L., and Pryor, W. A. (1996) Arch. Biochem. Biophys 327, 335–343
6. Lymar, S. V., and Hurst, J. K. (1995) J. Am. Chem. Soc. 117, 8867–8868
7. Lymar, S. V., Jiang, Q., and Hurst, J. K. (1996) Biochemistry 35, 7855–7861
8. Mencada, S., Palmer, R. M. J., and Higgs, E. A. (1991) Pharmacol. Rev. 43, 109–142
9. Nguyen, T., Brunson, D., Crespi, C. L., Penman, B. W., Wishnok, J. S., and Tannenbaum, S. R. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 3030–3034
10. deRojas-Walker, T., Tamir, S., Ji, H., Wishnok, J. S., and Tannenbaum, S. R. (1995) Chem. Res. Toxicol. 8, 473–477
11. Lewis, R. S., and Deen, W. M. (1994) Chem. Res. Toxicol. 7, 568–574
12. Keith, W. G., and Powell, R. E. (1969) J. Chem. Soc. A 80
13. Treinin, A. and Hayon, E. (1970) J. Am. Chem. Soc. 92, 5821–5828
14. Lewis, R. S., Tamir, S., Tannenbaum, S. R., and Deen, W. M. (1995) J. Biol. Chem. 270, 29350–29355
15. Carela, R., Harley, J. P., and Noback, C. R. (1990) Human Anatomy & Physiology, McGraw Hill, New York
16. Radi, R., Beckman, J. S., Bush, K. M., and Freeman, B. A. (1991) Arch. Biochem. Biophys 298, 481–487
17. Radi, R., Beckman, J. S., Bush, K. M., and Freeman, B. A. (1991) J. Biol. Chem. 266, 4244–4250
18. Ischiropoulos, H., Zhu, L., Chen, J., Tsai, M., Martin, J. C., Smith, C. D., and Beckman, J. S. (1992) Arch. Biochem. Biophys. 298, 431–437
