CAN WE MODEL NITRIC OXIDE BIOTRANSPORT? A SURVEY OF MATHEMATICAL MODELS FOR A SIMPLE DIATOMIC MOLECULE WITH SURPRISINGLY COMPLEX BIOLOGICAL ACTIVITIES

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Abstract Nitric oxide (NO) is a remarkable free radical gas whose presence in biological systems and whose astonishing breadth of physiological and pathophysiological activities have only recently been recognized. Mathematical models for NO biotransport, just beginning to emerge in the literature, are examined in this review. Some puzzling and paradoxical properties of NO may be understood by modeling proposed mechanisms with known parameters. For example, it is not obvious how NO can survive strong scavenging by hemoglobin and still be a potent vasodilator. Recent models do not completely explain how tissue NO can reach effective levels in the vascular wall, and they point toward mechanisms that need further investigation. Models help to make sense of extremely low partial pressures of NO exhaled from the lung and may provide diagnostic information. The role of NO as a gaseous neurotransmitter is also being understood through modeling. Studies on the effects of NO on O2 transport and metabolism, also reviewed, suggest that previous mathematical models of transport of O2 to tissue need to be revised, taking the biological activity of NO into account.

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INTRODUCTION

Nitrogen monoxide, or nitric oxide (NO) as it is more commonly known, was chosen in December of 1992 as “molecule of the year” by *Science* magazine (1). Research on this remarkable gas led in 1998 to the awarding of the Nobel Prize in Physiology or Medicine to Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad. In 1980, Furchgott & Zawadzki (2) reported that endothelial cells produce a potent but unknown vasodilator, which they labeled “endothelium-derived relaxing factor.” Working independently, Khan & Furchgott (3) and Ignarro et al (4) identified the vasodilator as NO. Research from Moncada’s laboratory (5, 6) also conclusively identified NO as an endogenous nitrovasodilator produced by the endothelium. A decade earlier, Murad et al had shown that nitroglycerin and related nitrovasodilator drugs release NO and that guanylate cyclase was activated by NO (7).

*Science* magazine described NO as both “a startlingly simple molecule” and a “bizarre molecule” because it is a rapidly diffusing, free radical gas with one unpaired electron exhibiting a surprisingly diverse range of biological activity, including potentially harmful effects. This issue of *Science* featured an article on the complex biochemistry of NO by Stamler et al (8). The redox status and biochemical activity of NO can be altered by adding an electron to form the nitroxyl anion (NO−) or by removing an electron to form nitrosonium (NO+). The nitroxyl anion reacts with sulfhydryls and redox metals. The nitrosonium cation allows nitrosation reactions to occur at -S, -N, -O, and -C centers in organic molecules. The primary reactions for the uncharged NO molecule are with O2, superoxide (O2−), and redox metals, especially iron-sulfur centers in proteins.

Despite intense research activity for the past two decades, knowledge about NO is still evolving, and some aspects of NO biochemistry are not fully understood. Because the biological importance of NO was only recently recognized, quantitative mathematical models for NO biotransport are just beginning to emerge in the scientific literature. These models are reviewed and their strengths and limitations discussed, along with considerations for future modeling needs.

NITRIC OXIDE ENZYMES AND BIOCHEMISTRY

The heme-containing enzyme nitric oxide synthase (NOS) is linked to NADPH-derived electron transport via flavin adenine dinucleotide and flavin mononucleotide to catalyze oxidation of L-arginine to L-citrulline and NO (Table 1,
TABLE 1  Biochemical reactions as sources or sinks for NO biotransport modeling

Sources
1. L-Arginine + O2 → L-citrulline + NO
2. 2RSNO → RSSR + 2NO
3. SNO-Hb(Fe2+)O2 + GSH → HS-Hb(Fe2+)O2 + GSNO + O2
4. GSNO + O2− → GSH + O2 + NO
5. NO2− + H+ + NADH → NO + H2O

Sinks
1. 4NO + O2 + 2H2O → 4NO2− + 4H+
2. NO + O2− → ONOO−
3. NO + ONOO− → NO2− + NO3
4. NO + Hb(Fe2+) → Hb(Fe3+)NO
5. NO + Hb(Fe2+)O2 → Hb(Fe3+) + NO3−
6. NO + Mb(Fe2+) → Mb(Fe2+)NO
7. NO + Mb(Fe2+)O2 + H2O → Mb(Fe3+)OH + ONOO− + H+
8. 2NO + RSH + O2 → RSNO + ONOO−

source 1), with tetrahydrobipterin as an essential cofactor. It is the primary source of NO production in mammals. Several NOS isoforms are expressed by different genes. As pointed out in the brief review and recommended nomenclature for NO biochemistry by Moncada et al (9), it is an oversimplification to classify the three major NOS isoforms exclusively in terms of specific physical location. However, the conventional classification is for location, either in the endothelium (eNOS, or type III) or in certain types of neurons (nNOS, or type I), or for being induced in tissues by macrophages in response to endotoxins or cytokines (iNOS, or type II). All isoforms can be found at other locations in certain tissues. Activities for constitutive isoforms eNOS and nNOS are regulated by Ca2+ and calmodulin. Nathan (10) reviewed early studies with purified NOS, which reported 50% effective concentrations (EC50) for Ca2+ in the 200- to 400-nM range and for calmodulin in the 1- to 70-nM range. Michaelis constants (Km) for L-arginine ranged between 2 and 32 µM. Optimum activity is in the physiological pH range (11) and increases with temperature (12). O2 availability also influences NO production, discussed later in this review. The inducible isoform iNOS is not regulated by Ca2+ and can produce higher amounts of NO than can eNOS or nNOS. There is evidence, reviewed by Henry & Guissani (13), for a fourth NOS isoform that is also Ca2+ dependent and localized in mitochondrial inner membranes (mtNOS).

It is clear that the spatial location and factors that regulate NO production rates (RNO) for different NOS isoforms are necessary to develop appropriate source terms in mathematical models for NO biotransport. After it is produced, NO must
react with biologically relevant targets. There is still considerable debate about whether this occurs by simple diffusion of NO gas or whether NO combines with thiols or another carrier system for subsequent transport and release. Some of the most pertinent reactions for NO biotransport modeling are listed as sources or sinks in Table 1 and are discussed below.

Autooxidation

The reaction between NO and O$_2$ (Table 1, sink 1) to form nitrite (NO$_2^-$) is well known and has been extensively studied. In a perfectly mixed aqueous medium, the reaction is third order (first order with O$_2$ and second order with NO),

$$\frac{dC_{NO}}{dt} = -4k_{aq}C_{O_2}C_{NO}^2 = -\frac{dC_{NO^-}}{dt},$$

(1)

where the factor of 4 comes from the stoichiometry. Values for $4k_{aq}$ and the temperature at which the rate was determined by different investigators are listed in Table 2. A numerical simulation of NO disappearance in different O$_2$ concentrations using $4k_{aq}$ at 37°C (16) are shown in Figure 1. When the initial NO is low, 250 nM for this illustration, the half-life ($t_{50%}$) increases as O$_2$ decreases, and $t_{50%}$ can be hours in normal physiological tissue pO$_2$ ranges. With higher initial NO values, $t_{50%}$ would be shorter.

Liu et al (18) reported that NO autooxidation is accelerated in different hydrophobic phases, including phospholipid liposomes and biological membranes isolated from rat hepatocytes. The enhancement was approximately 300-fold (Table 2) and was attributed to higher gas solubilities for NO and O$_2$ compared with water. The net increase would be smaller for tissues because membranes comprise only a fraction of the total volume. They estimated that NO autooxidation would be eight times higher for brain or liver tissues compared with the rate in water, and that most of the reaction would take place in the membranes. On the other hand, the diffusion coefficient for NO determined by an optical method appears to be lower in liposomes and red blood cell membranes, about 50% of the value for O$_2$ (31).

Reaction with Superoxide

The reaction between NO and O$_2^-$ (Table 1, sink 2) to form peroxynitrite (ONOO$^-$),

$$\frac{dC_{NO}}{dt} = -k_{O_2^-}C_{O_2^-}C_{NO},$$

(2)

was first reported by Beckman et al (32). It is unquestionably the most rapid biological reaction for NO (Table 2) and is near the maximum rate allowed by diffusion. However, the extent to which this reaction occurs is limited by the rapid reaction between O$_2^-$ and superoxide dismutase (SOD), which has an approximately one-third lower rate constant (33). Therefore, O$_2^-$ in Equation 2 represents what has escaped scavenging by SOD. Sources of O$_2^-$ include the mitochondrial electron
TABLE 2  Rate constants for NO reactions

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Temperature (°C)</th>
<th>Rate Constant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>With oxygen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In aqueous medium</td>
<td>22</td>
<td>$4k_{aq} \times 10^6$ M$^{-2}$ s$^{-1}$</td>
<td>Wink et al (14)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>$6.0 \pm 1.5$</td>
<td>Kharitonov et al (15)</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>$6.3 \pm 0.4$</td>
<td>Lewis &amp; Deen (16)</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>$8.4 \pm 1.6$</td>
<td>Lewis &amp; Deen (16)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>$9.2 \pm 1.2$</td>
<td>Lewis &amp; Deen (16)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>$8.9 \pm 1.0$</td>
<td>Caccia et al (17)</td>
</tr>
<tr>
<td>In hydrophobic phase</td>
<td>25</td>
<td>$300^*4k_{aq}$</td>
<td>Liu et al (18)</td>
</tr>
<tr>
<td>With superoxide</td>
<td>20</td>
<td>$k_{O_2^-} \times 10^6$ M$^{-1}$ s$^{-1}$</td>
<td>Huie &amp; Padmaja (19)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>$4.3 \pm 0.5$</td>
<td>Goldstein &amp; Czapski (20)</td>
</tr>
<tr>
<td>With free hemoglobin</td>
<td>20</td>
<td>$k_{Hb} \times 10^7$ M$^{-1}$ s$^{-1}$</td>
<td>Gibson &amp; Roughton (21)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.2</td>
<td>Cassoly &amp; Gibson (22)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2.5</td>
<td>Morris &amp; Gibson (23)</td>
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<td></td>
<td>20</td>
<td>1.8</td>
<td>Eich et al (24)</td>
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<tr>
<td></td>
<td>23</td>
<td>0.3–5.0</td>
<td>Rohlfs et al (25)</td>
</tr>
<tr>
<td>With intact red blood cells</td>
<td>38</td>
<td>$k_{bc} \times 10^5$ M$^{-1}$ s$^{-1}$</td>
<td>Carlsen &amp; Comroe (26)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1.7 $\pm 0.1$</td>
<td>Liu et al (27)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.52</td>
<td>Liu et al (27)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.00089$k_{Hb}$</td>
<td>Vaughn et al (28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.00126$k_{Hb}$</td>
<td></td>
</tr>
<tr>
<td>With myoglobin</td>
<td>25</td>
<td>$k_{Mb} \times 10^7$ M$^{-1}$ s$^{-1}$</td>
<td>Doyle &amp; Hoekstra (29)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.7</td>
<td>Eich et al (24)</td>
</tr>
<tr>
<td>With guanylate cyclase</td>
<td>4</td>
<td>$k_{GC} \times 10^5$ M$^{-1}$ s$^{-1}$</td>
<td>Zhao et al (30)</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td></td>
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</tbody>
</table>

transport chain for oxidative metabolism, NADPH oxidase, xanthine oxidase, and NOS itself. It is known that NOS will produce $O_2^-$ if no L-arginine is available, but that is highly unlikely in normal physiological states. There continues to be debate about the biochemical mechanisms, necessary conditions, and amount of $O_2^-$ that NOS can produce.

Because NO can also react with the product peroxynitrite (Table 1, sink 3), the net change, neglecting alternate reaction pathways for ONOO$^-$, is

$$\frac{dC_{NO}}{dt} = -(k_{O_2^-}C_{O_2^-} + k_{ONOO^-}C_{ONOO^-}C_{NO^-})$$

(3)

The complex biochemistry of peroxynitrite has received intense scrutiny in recent years.
Figure 1  Autooxidation of NO from initial value of 250 nM after instantaneous mixing is shown for different constant O₂ concentrations. Numerical solutions for the autooxidation reaction kinetics (Equation 1) are shown in a physiologically relevant O₂ range using 4kₐq at 37° C (Table 2). (Dashed line) The half-life (t₅₀%). (inset) t₅₀% as a function of O₂ or pO₂.

Heme-Protein Binding

In theory, the reaction between NO and heme-containing proteins (hP) to form ligand-bound hPNO is reversible,

\[ \frac{dC_{NO}}{dt} = -kₐC_{hP}C_{NO} + k_dC_{hPNO}, \]

although the association rate constant kₐ is several orders of magnitude higher than the dissociation rate constant k_d for both hemoglobin (Hb) and myoglobin (Mb) (21, 23, 25). Dissociation is so slow that these reactions can be considered irreversible. Only the kₐs for Hb and Mb are listed in Table 2. Following instantaneous mixing in a solution with a uniform heme-protein concentration, NO decays exponentially:

\[ C_{NO}(t) = C_{NO}(0)e^{-\lambda t} \quad \text{with} \quad t_{50\%} = \ln 2/\lambda, \]

where \( \lambda = kₐC_{hP} \) is the pseudo-first-order rate constant and \( t_{50\%} \) is the half-life. Rapid NO scavenging and very brief \( t_{50\%} \) as functions of Hb concentration...
Figure 2  Scavenging of NO from initial value of 250 nM after instantaneous mixing (Equation 5) in free hemoglobin (upper panel) or in whole blood with intact red blood cells (bottom panel) depends on Hb concentration. The rate constant for free Hb is from Cassoly & Gibson (22) and for intact red blood cells from Carlsen & Comroe (26) (see Table 2). (Dashed line) The half-life ($t_{50\%}$); (insets) $t_{50\%}$ as a function of Hb concentration. Scavenging of NO is slower in whole blood.

are illustrated in Figure 2 using $k_{\text{Hb}}$ from Cassoly & Gibson (22) (see Table 2). Although $k_{\text{Mb}}$ for Mb is similar, the reaction is slower because Mb concentrations in tissue are lower. There appears to be no difference between rate constants for NO binding to deoxygenated or oxygenated Hb and Mb (Table 1, sinks 4–7). It may be significant that in the brains of mice and humans a newly discovered “neuroglobin” has been found (34), which may play a role similar to myoglobin in muscle for facilitated transport of $O_2$ in the brain, and which could also prove to be another sink for NO.

There is a significant difference between NO reactions with free Hb and intact red blood cells (Table 2). Figure 2 illustrates NO scavenging and $t_{50\%}$ as functions of Hb concentration based on the rate determined by Carlsen & Comroe (26) for intact red blood cells. However, the reaction is still rapid, and $t_{50\%}$ is $\sim 1.8$ ms for
whole blood with 45% hematocrit. The rapid reaction between oxyhemoglobin and NO to form nitrite and methemoglobin (Table 1, sink 5) was initially observed by Kon et al (35) and is considered to be the primary biochemical pathway for deactivating NO.

The principal biological target of NO is soluble guanylate cyclase (sGC), a heme-based enzyme that converts ligand-binding free energy into protein conformational free energy for conversion of 5'-guanosine triphosphate (GTP) to cyclic 3',5'-guanosine monophosphate (cGMP). The intracellular second messenger cGMP activates protein kinases and phosphodiesterases and influences ion channels. NO increases sGC activity several hundred-fold, with a 50% maximum activity for NO of approximately 250 nM (99), although much lower values have been found (discussed below). NO binds to sGC in a two-step process. Stop flow spectrophotometric studies with recombinant sGC at 4°C show that initial binding to the heme is extremely rapid ($k > 1.4 \times 10^8 \text{ M}^{-1} \text{s}^{-1}$) and forms a six-coordinate intermediate (30). The intermediate converts to a five-coordinate state at a much slower, NO-dependent rate of $k_{GC} = 2.4 \times 10^5 \text{ M}^{-1} \text{s}^{-1}$ (Table 2). For sGC to be an effective biological sensor, NO cannot remain bound and must dissociate shortly after activation. In vivo responses of vascular smooth muscle suggest that NO dissociation must occur within 1–2 min or even faster. However, rate constants from some studies predict that deactivation could take hours. Kharitonov et al (37) found much faster dissociation rates for six-coordinate nitrosyl hemes compared with the five-coordinate state. The fastest NO dissociation rate (0.03 s$^{-1}$) for purified lung sGC was measured in the presence of GTP, cGMP, and magnesium ions. Extrapolated to 37°C, $t_{50\%}$ was ~5 s. However, in another study using sCG extracted from bovine retina, GTP and magnesium did not accelerate deactivation (38). Furthermore, Cu$^{2+}$, which is known to inhibit NO-sCG binding, prolonged deactivation. On the other hand, Bellamy et al (39) concluded that sCG behaves much differently in its natural environment. Based on in vitro studies with intact cerebellar cells, EC$_{50}$ for NO was ~20 nM, an order of magnitude lower than the 250 nM value found by Stone & Marletta (36). Another study of sGC in vascular smooth muscle also reports a low EC$_{50}$, ~10 nM (40).

**Thiol Nitrosation**

There is still debate about specific mechanisms for NO reactions with thiols (RSH), and it is not clear what role nitrosothiols (RSNO) play in the transport and release of NO. It has been established that serum albumin and Hb carry some NO in the bloodstream (41–47), although the amount is in question (48, 49). Glutathione (GSH) is a low-molecular-weight thiol normally present at millimolar concentrations in tissue. Cysteine (Cys), the precursor to GSH, is also present in cells at lower concentrations. Both GSH and Cys are also present in the blood plasma at micromolar levels. In vitro electron paramagnetic resonance spectroscopy studies using physiological NO (2 µM) with 2 mM Cys or GSH under carefully controlled conditions preventing thiol interaction with iron or copper demonstrate that NO
can be trapped as nitrosated cysteine (CysNO) or nitrosated glutathione (GSNO) and later regenerated (50). The authors described the mechanism as a possible “secret passage” for NO biotransport. Trujillo et al (51) report that xanthine oxidase can catalyze decomposition of CysNO or GSNO to release NO under aerobic conditions. The involvement of superoxide was implicated for both, and the reaction for GSNO in Table 1 (source 4) was proposed, which could be totally inhibited by SOD. The reaction for decomposition of CysNO was only partially inhibited by SOD, so an alternate mechanism may also be involved. Thus, in some locations nitrosation could be a sink (Table 1, sink 8), or regeneration at other locations could be a source of NO (Table 1, sources 2–4).

Sources Other than NOS

It has also been shown that xanthine oxidase can reduce nitrite back to NO (Table 1, source 5) under anaerobic conditions and in the presence of NADH or xanthine (52, 53). The net production of NO from NO$_2^-$ decreased with increasing O$_2$, consistent with greater generation of O$_2^-$ by xanthine oxidase and reaction with NO to form ONOO-. It has been shown that blood plasma nitrite/nitrate levels increase as animals adapt to repeated periods of hypobaric hypoxia (54), but NOS activity decreases, probably because of the O$_2$ dependence of the enzyme. This suggests that another biochemical pathway is compensating for the reduced production by NOS. The amount of stored NO was estimated from relaxation of isolated rat aorta at different stages of adaptation to hypoxia, using diethyldithiocarbamate to provoke NO release from the store. The estimated size of the store increased with adaptation.

CONVECTION-DIFFUSION MODELS

The first models of NO biotransport were developed by Lancaster (55–57). Based on point sources of NO with one-dimensional diffusion and first-order reaction, these models provided some insight into the biological activity of NO. One conclusion was that NO could diffuse rapidly over fairly long distances, and that the NO concentration at any given location was determined by the sum of all NO sources and not necessarily by the closest source. Another aspect that was modeled was the strong scavenging properties of Hb, although this may have been exaggerated, because free Hb rate constants were used rather than values for intact red blood cells (Table 2). A one-dimensional simulation showed that low Hb concentrations could effectively eliminate nearly all of the NO produced from endothelial cells. The logical conclusion from the modeling was that most of the NO was “wasted” by diffusing out into the bloodstream, and this fueled speculation that the endothelial-derived relaxing factor could not be NO but perhaps some other related chemical species. This paradox has been addressed in more recent models (reviewed below).
For the ensuing discussion, we assume that NO obeys the general mass transport equation
\[
\frac{\partial C_{\text{NO}}}{\partial t} + v \cdot \nabla C_{\text{NO}} = D \nabla^2 C_{\text{NO}} \pm \sum R_{\text{NO}},
\] (6)
where convection and diffusion terms are defined as usual, using the sum of all possible reactions for NO, with a positive sign (source) for production and negative sign (sink) for loss. The mass transport equation could also be written in terms of partial pressure (pNO) using an appropriate Henry’s law solubility coefficient, but concentration (C_{\text{NO}}) is generally used in the literature. In water at 37°C, the diffusion coefficient \( D = 4.8 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} \) (58) and solubility coefficient = \( 25.8 \times 10^6 \text{ mmHg NO (mol of NO)}^{-1} \text{ per mol of H}_2\text{O} \) (59). Diffusion and solubility coefficients in different tissues are not known.

In Vitro Models

**ONE-DIMENSIONAL DIFFUSION WITH CONSTANT O_2** An in vitro model for the NO gradient as a function of time and distance in a finite layer of culture medium above cells was developed by Laurent et al (60), based on simplifications of the one-dimensional Fick diffusion equation
\[
\frac{\partial C_{\text{NO}}}{\partial t} = -D \frac{\partial^2 C_{\text{NO}}}{\partial x^2} - k' C_{\text{NO}}^2 + R_{\text{NO}},
\] (7)
where a monolayer of cells (at \( x = 0 \)) produce NO at a rate \( R_{\text{NO}} \) (in nanomoles per second per unit number of cells, or per unit volume of cells). The pseudo-second-order rate constant
\[
k' = 4k_{aq} C_{O_2},
\] (8)
for autooxidation can be used when O_2 is uniform and constant in the culture medium. If the solution is in equilibrium with room air, the O_2 concentration is 220 \( \mu \text{M} \), and using \( 4k_{aq} = 6.3 \times 10^6 \text{ M}^{-1} \text{ s}^{-1} \) (15), the pseudo-second-order rate constant \( k' \sim 10^3 \text{ M}^{-1} \text{ s}^{-1} \).

Neglecting diffusion, an upper limit for the steady state NO concentration at the surface of the monolayer (\( x = 0 \)), was estimated from
\[
C_{\text{NO}} = \sqrt{R_{\text{NO}}/k'}.
\] (9)
If the initial NO concentration is assumed to be zero, and the cells begin to produce NO when activated at \( t = 0 \), the half-time for the reaction is
\[
t_{50\%} = (\ln 3)/2\sqrt{k'R_{\text{NO}}}. \] (10)
Laurent et al (60) performed simulations for different NO production rates, and culture medium depths of either 3 mm or 6 mm, to find the steady state NO at the cell surface and predict mean nitrite (NO_2^-) levels, which were measured experimentally in the medium. For parameters used in the simulation, \( t_{50\%} = 2.2 \text{ min} \).
and the time to reach 95% of steady state was <7 min. They found that activated
murine macrophages (∼4 × 10^6 cells cultured in a 33-mm diameter Petri dish)
produced NO_2^- at an average rate of 16 µM h^{-1}, which they noted was somewhat
high compared with other studies in the literature. From the model, they estimated
that immediately adjacent to the activated macrophages, C_{NO} ∼ 4 µM and R_{NO} ∼
60 µM h^{-1} (or ∼4.2 nM s^{-1} per 10^6 cells). Assuming an average cell thickness of
10 µm, R_{NO} would be ∼490 nM s^{-1} cm^{-3}.

A limitation of this closed system modeling approach is that it does not include
any loss of NO into the air over the culture medium and, therefore, may under-
estimate the actual NO production rate. One simulation was performed with NO
at the air/fluid interface held at zero, which predicted lower NO levels at the cell
monolayer. This simulation allowed finite NO flux at the liquid/air interface, but
the amount was not discussed in detail. An open system model that includes finite
NO flux out of the culture medium would predict higher rates for NO production
because the escaping NO does not form nitrite. Laurent et al (60) also noted that
their analysis would be inaccurate if there were other NO losses, such as reactions
with O_2^-.

DIFFUSION AND REACTION IN STIRRED CELL SUSPENSIONS Chen et al (61) devel-
oped a more complicated model to simulate complex biochemical interactions in
a stirred suspension of cultured macrophages. A stagnant film model was used to
represent steady state mass transfer of NO, O_2^-, and peroxynitrite from an acti-
vated monolayer of macrophages ∼15 µm thick attached to a 175-µm-diameter
microcarrier bead into the external culture medium. The beads were assumed to be
uniformly suspended and well mixed. Because the experimental system was open
to the atmosphere, NO gas escaping from liquid to the head-space gas above the
culture medium was also included in the mass balance. This NO loss was quanti-
fied by Lewis et al (62) with a chemiluminescence detector. Production rates for
NO and O_2^- used in the model were also determined experimentally, and other
parameters were taken from the literature. The model also included nitrosation of
an amine (morpholine). Summations of steady state diffusion equations assuming
angular symmetry in spherical coordinates

\[ \sum \frac{D_i}{r^2} \frac{d}{dr} \left( r^2 \frac{dC_i}{dr} \right) \pm \sum R_i = 0 \quad \text{for} \quad r_{\text{bead}} < r < r_{\text{bead}} + \delta \quad (11) \]

were used to model diffusion and reaction of the three species (NO, O_2^-, ONOO^-)
around the beads in a film 63 µm thick. Algebraic expressions were used to char-
acterize reaction rates, based on interactions between all relevant species. Because
the nitrosation of morpholine depends on the uncharged amine, acid-base equilib-
ria was required, including CO_2/bicarbonate (H_2 CO_3). Algebraic expressions for
reaction products nitrite (NO_2^-), nitrate (NO_3^-), and nitrosated morpholine were
written. An intermediate species, nitrous anhydride (N_2 O_3), thought to be required
for nitrosation of the amine, was also included in the coupled chemical reactions,
although it was probably only present in trace amounts.
The model equations were solved numerically and compared with experimental results for different conditions. Although there was generally good correspondence between predicted and experimental results, there was a tendency to overestimate nitrate and underestimate nitrite formation, so that the predicted ratio of $\text{NO}_3^- / \text{NO}_2^-$ was higher than that found experimentally. The authors attributed this to conditions that favored ONOO$^-$ formation, which is the principle source of NO$_3^-$ formation, especially when NO flux is low.

Predicted concentration profiles in the stagnant film were very informative. The simulation showed that superoxide was consumed rapidly, falling from an initial level of 3.32 nM to near zero only 2 $\mu$m away from the cell monolayer surface. Peroxynitrite concentration, initially at 51 nM, declined more gradually, falling to near zero $\sim 30$ $\mu$m away from the surface. The model predicted that neither species should have reached the culture medium in any appreciable amount. This was supported from experimental measurements where no $\text{O}_2^-$ could be detected, and only 0.53 pM of peroxynitrite was found. In contrast, the NO concentration was high throughout the film, dropping from 1.13 $\mu$M at the cell surface to 0.95 $\mu$M at the outer edge of the film to match the bulk solution NO concentration. The predicted bulk solution NO was a little higher than that found experimentally. The model was able to predict higher NO and lower nitrate when SOD was added to compete for the reaction between NO and $\text{O}_2^-$, although the model did not exactly match experiment values. Liu et al (27) commented that perhaps discrepancies in the model by Chen et al (61) might be smaller with a higher autooxidation rate.

In terms of the model, this would mean that most of the reaction between NO and $\text{O}_2^-$ would occur in the cell membranes, rather than in the stagnant film layer around the cells.

**In Vivo Models**

**NO PROFILE INTO VASCULAR WALL**  If NO loss in the vascular wall and surrounding tissue is due to autooxidation alone, the following steady state, one-dimensional radial diffusion equation,

$$
\frac{D}{r} \frac{d}{dr} \left( r \frac{dC_{\text{NO}}}{dr} \right) - 4k_{\text{aq}} C_{\text{O}_2} C_{\text{NO}}^2 = 0 \quad \text{for} \quad r > R_c
$$

(12)

could be used to model the NO gradient for a vessel with inner radius $R_c$. One can either assume homogeneous properties or use a multilayered model with different properties for the vascular wall and surrounding tissue. This simple model also assumes that there are no other sources of NO from nearby neurons, no induced NOS in surrounding tissue, and no interaction with NO diffusing from nearby blood vessels.

Even with these simplifying assumptions, the model is complicated by the fact that $\text{pO}_2$ varies across the vascular wall as a function of tissue properties ($\text{O}_2$ diffusion and solubility coefficients) and $\text{O}_2$ consumption rates (63, 64). The $\text{pO}_2$ profile will depend on the type of blood vessel, $\text{O}_2$ consumption rates in
the vascular wall and surrounding tissue, blood flow in the vessel lumen, blood O_2 saturation, pH, pCO_2, etc. Autooxidation of NO will also consume some O_2, although this is probably negligible compared with the metabolic use of O_2. A fully developed model incorporating all these variables would be a difficult challenge. One simplifying assumption would be to use the average O_2 concentration across the wall. For example, in 25-µm-diameter arterioles in rat mesentery, the lumen pO_2 is 43 torr (~63 µM), and there is a steep drop in pO_2 across the wall to 25 torr (~37 µM), as measured by an optical method (65). Using an average O_2 value of 50 µM, the pseudo-second-order rate constant would be 4.6 \times 10^2 M^{-1} s^{-1}, with t_{50\%} = 36.8 min (Figure 1). This would cause a negligible loss of NO, especially because the pO_2 will be even lower further out in the tissue. If autooxidation is an order of magnitude higher because of an enhanced reaction in membranes (67), t_{50\%} would be only 3.3 min, which is still slow compared with expected half-lives, on the order of a few seconds.

To simplify the task of modeling NO in tissue, Vaughn et al (66, 67) used two approaches. As a first approximation, a pseudo-first-order reaction with rate constant \( \lambda = 0.01 \text{ s}^{-1} \) was used, equivalent to \( t_{50\%} = 69.3 \text{ s} \). This permits an analytical solution, as discussed in the next paragraph. Alternately, a pseudo-second-order reaction with a rate constant of 0.05 \( \mu M^{-1} s^{-1} \) was used. Both rate constants were estimated from experimental data reported by Malinski et al (68). The profile was solved numerically, assuming zero NO flux at infinite radius, coupled to endothelial NO production and NO flux into the bloodstream, as described below.

Butler et al (69) took another approach toward modeling the NO profile in the vascular wall and surrounding tissue. They assumed that NO reacts with guanylate cyclase,

\[
\frac{D}{r} \frac{d}{dr} \left( r \frac{dC_{NO}}{dr} \right) - k_{GC} C_{GC} C_{NO} = 0 \quad \text{for} \quad r > R_a, \tag{13}
\]

where \( R_a \) is the radius on the abluminal side of the endothelium with thickness \( R_a = R_e + a \). Guanylate cyclase was assumed to be uniformly distributed in tissue at \( C_{GC} = 100 \text{ nM} \), based on unpublished data from their laboratory, with a second-order rate constant \( k_{GC} \) for the reaction. Autooxidation and dissociation of NO from sGC were not considered. In their simulation, \( \lambda = k_{GC} C_{GC} = 0.01 \text{ s}^{-1} \), which is exactly equivalent to the first-order simulation by Vaughn et al (66).

The general solution to Equation 13 is

\[
C_{NO}(r) = a_1 I_0(br) + a_2 K_0(br), \tag{14}
\]

as described by Butler et al (69), where \( I_0 \) and \( K_0 \) are zero-order modified Bessel functions of the first and second kind with the scaling factor

\[
b = \sqrt{\lambda/D}. \tag{15}
\]

The coefficients \( a_1, a_2 \) can be evaluated from the boundary conditions. Butler et al (69) assumed that the NO profile extended to infinity, requiring that \( a_1 = 0 \). Using
the NO concentration $C_{NO(R_a)}$ at the abluminal side of the endothelium to evaluate $a_2$, the NO profile is

$$ C_{NO(r)} = C_{NO(R_a)} K_0(br) / K_0(b R_a). $$  \(16\)

For a blood vessel with length $L$ and abluminal surface area $2\pi R_a L$, radial flux into the wall is

$$ J_{wall} = - (2\pi R_a L) D \frac{dC_{NO}}{dr} \bigg|_{r=R_a}. $$  \(17\)

From the derivative of Equation 16 evaluated at $r = R_a$, NO flux into the wall is

$$ J_{wall} = 2\pi R_a L C_{NO(R_a)} \sqrt{\lambda D K_1(b R_a) / K_0(b R_a)}. $$  \(18\)

where $K_1$ is the first-order modified Bessel function of the second kind.

NO PROFILE INTO BLOODSTREAM Using the first-order molar rate constant $k_{Hb}$ (per moles per second) and constant concentration $C_{Hb}$, the steady state, one-dimensional radial diffusion equation with first-order scavenging of NO by Hb can be expressed as

$$ \frac{D}{r} \frac{d}{dr} \left( r \frac{dC_{NO}}{dr} \right) - k_{Hb} C_{Hb} C_{NO} = 0 \text{ for } 0 < r < R_e, $$  \(19\)

and can also be solved analytically. Vaughn et al (66) used the analytical solution to check the convergence of their numerical simulation at steady state but did not publish details for the complete solution. As shown previously, the general solution is the sum of modified Bessel functions (Equation 16) with a different scaling factor

$$ b = \sqrt{k_{Hb} C_{Hb} / D}, $$  \(20\)

and with different boundary conditions to evaluate the coefficients $a_1, a_2$. Because the analytical solution must be finite at $r = 0$, $a_2$ is zero. For a specified NO concentration $C_{NO(R_e)}$ at the endothelial surface ($r = R_e$), the NO gradient in the bloodstream is given by

$$ C_{NO(r)} = C_{NO(R_e)} I_0(br) / I_0(b R_e). $$  \(21\)

The radial flux from the endothelium into the bloodstream is

$$ J_{blood} = 2\pi R_e L C_{NO(R_e)} \sqrt{k_{Hb} C_{Hb} D I_1(b R_e) / I_0(b R_e)}. $$  \(22\)

where $I_1$ is the first-order modified Bessel function of the first kind. In the model by Vaughn et al (66, 67), the NO source was from two concentric surfaces, one at the blood interface ($R_e$) and the other between the endothelium and vascular wall ($R_a$). Taking both fluxes into account and dividing by the volume of endothelial cells, the total rate of NO production is

$$ R_{NO} = (J_{blood} + J_{wall}) / \pi (R_a^2 - R_e^2) L. $$  \(23\)
Figure 3  NO profiles in blood and tissue predicted from models by Butler et al (69) and Vaughn et al (66) are shown for a 100-µm-diameter blood vessel with different parameters (see text and Table 2). Case a uses the Hb scavenging rate constant for free Hb from Cassoly & Gibson (22). (Inset, top left) Detail of the extremely steep gradient. Whole blood Hb scavenging rates used for cases b [Carlsen & Comroe (26)], c [Liu et al (27)], and d [Vaughn et al (28)] predict lower NO fluxes into the bloodstream. Case e is for the lowest Hb scavenging rate used in the simulations by Vaughn et al (66). The NO profile into the vascular wall and surrounding tissue for case f is for the same first-order reaction rate used by Butler et al (69) and Vaughn et al (66). Case g is for the second-order rate used by Vaughn et al (66). Case h is for a rate one order of magnitude lower, equivalent to autoxidation accelerated by hydrophobic membranes in the range predicted by Liu et al (18). (Inset, lower right) NO profiles in tissue out to a 500-µm radius, assuming uniform properties for profiles extending to an infinite radius.

NO profiles into the bloodstream and vascular wall predicted for different parameter values are illustrated in Figure 3 for a 100-µm-diameter blood vessel. A normal hematocrit of 45% with 15 g of Hb/ml of blood was used, corresponding to a concentration of 2.25 mM, and NO was fixed at 250 nM on both sides of the endothelium. Case a is based on the free Hb value of Cassoly & Gibson (22). The NO profile drops to near zero within 1 µm. Scavenging of NO by free Hb in solution is very effective because the reaction rate is so high (Table 2). Although
this case is not appropriate for simulating normal in vivo conditions, it illustrates a potential problem for modified Hb-based synthetic blood substitutes, which could impair NO delivery to the vascular wall (70). More realistic NO profiles are shown using the lower rate constants for intact red blood cells from Carlsen & Comroe (26) (case b), Liu et al (27) (case c), and Vaughn et al (28) (case d). Case d is based on 0.00089 times the free Hb value of Cassoly & Gibson (22). NO concentrations at the lumen center are 0.1 pM, 0.15 nM, and 3.2 nM, respectively. Case e is based on the lowest rate constant used by Vaughn et al (66), equivalent to a pseudo–first-order rate $\lambda = k_{Hb}C_{Hb} = 1.5 \text{s}^{-1}$, which is $\sim 17\%$ of the value from Carlsen & Comroe (26). NO at the center of the bloodstream would be nearly 38 nM for this case, which is unreasonably high. This low scavenging rate was the only one leading to an endothelial NO level above 250 nM for the NO production rate used in the simulation (66).

The NO profile into the wall and tissue for case f is based on the value of $\lambda = 0.01 \text{s}^{-1}$ used by Butler et al (69) and Vaughn et al (66). Case g is a numerical solution for the second-order rate of $0.05 \mu \text{M}^{-1} \text{s}^{-1}$ used by Vaughn et al (66). For case h, a 10-fold lower rate was used in the numerical solution, close to the enhanced autooxidation rate with membranes (18) but still an order of magnitude higher than autooxidation in an aqueous medium. Case f has the highest NO flux into the wall.

Most of the NO diffuses out into the bloodstream. Relative to NO diffusing into the wall for case f, case a predicts that 99.8% of the total endothelial NO production would be lost to the bloodstream. With lower rate constants for intact red blood cells, NO losses to the bloodstream are 97.7%, 95.8%, and 93.6%, respectively, for cases b, c, and d. Even with the unrealistically low Hb scavenging rate for case e, 87.8% of the NO produced by the endothelium enters the bloodstream. There are possible mechanisms that might reduce this loss, including diffusional resistance from red blood cell free plasma layers near the wall. The width of this layer was unrealistically high for some of the vessel sizes simulated by Butler et al (69). Experiments by Liao et al (71) with pig coronary microvessels perfused with either free Hb or whole blood suggests that the boundary layer may be a significant factor. Another factor is the well known Fåhraeus-Lindqvist effect, in which the particulate nature of blood leads to a reduced hematocrit in small tubes. This can be less than half of the systemic hematocrit in capillaries or small arterioles and venules (diameters $\sim 15 \mu \text{m}$) and has significant effects in $\text{O}_2$ transport models (72, 73). The scavenging constant $\lambda = k_{Hb}C_{Hb}$ would be proportionally reduced in very small blood vessels. However, Vaughn et al (66) demonstrated that even when both boundary layer and reduced hematocrit were included, it was difficult to simulate NO levels in the vascular wall above 250 nM to activate guanylate cyclase, except when very low Hb scavenging constants were used. Perhaps one weakness in the modeling was that they did not predict how much of an increase in endothelial NO production would be needed over the fixed value used in the model to counteract scavenging of NO by whole blood. Of course, if sGC is sensitive to NO at much lower levels, then the model would more easily predict these values with the scavenging rates for whole blood.
INTERACTION BETWEEN NITRIC OXIDE AND O2 TRANSPORT

It is clear that vascular smooth muscle vasodilation by NO increases blood flow and O2 delivery, although it may not be the primary mechanism for coupling blood flow to metabolic requirements. There is evidence that NO directly effects O2 transport to tissue by at least four mechanisms, briefly reviewed below.

First, O2 is required for the enzymatic synthesis of NO from L-arginine as well as for its autooxidation in tissue (Table 1), and NO production rates for all three NOS isoforms are O2 dependent. If NO production follows Michaelis-Menten kinetics and other factors (such as intracellular Ca2+) remain constant, the O2-dependent properties can be represented by

\[ R_{NO} = \frac{R_{NO,\text{max}}}{O_2 + K_m} \]

(24)

where the Michaelis constant \( K_m \) is the O2 concentration at half of the maximum NO production rate \( R_{NO,\text{max}} \), as summarized in Table 3. These values are from studies with purified NOS except for that of Otto & Baumgardner (77), who obtained an estimate for \( K_m \) based on nitrite production by macrophages cultured at different O2 levels. It has been reported that the apparent \( K_m \) for O2 of nNOS is extremely high (75), well outside of the normal physiological tissue O2 range, although another group found a much lower \( K_m \) (74).

Second, NO is also transported by Hb and has an effect on O2 release. Stamler and coworkers (8, 41–45) propose that O2-dependent interaction between NO and Hb helps regulate blood flow and O2 delivery. Two cysteine sites on the Hb tetramer can react with NO or S-nitrosothiols (SNO), depending on oxyhemoglobin saturation, allowing release of NO or SNO as O2 is unloaded downstream. Thus, stored NO or SNO can contribute to blood flow regulation in addition to endothelial or neuronal NO production. NO can also shift the oxyhemoglobin equilibrium curve to the right to improve O2 delivery (80).

### Table 3

<table>
<thead>
<tr>
<th>nNOS (type I)</th>
<th>iNOS (type II)</th>
<th>eNOS (type III)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.2 ± 2.8</td>
<td>6.3 ± 0.9</td>
<td>7.7 ± 1.6</td>
<td>Rengasamy &amp; Johns (74)</td>
</tr>
<tr>
<td>~400</td>
<td></td>
<td></td>
<td>Abu-Soud et al (75)</td>
</tr>
<tr>
<td>~135</td>
<td></td>
<td></td>
<td>Dweik et al (76)</td>
</tr>
<tr>
<td>~21</td>
<td></td>
<td></td>
<td>Otto &amp; Baumgardner (77)</td>
</tr>
<tr>
<td>~88</td>
<td></td>
<td></td>
<td>Liao et al (78)</td>
</tr>
<tr>
<td>~56</td>
<td></td>
<td></td>
<td>Whorton et al (79)</td>
</tr>
</tbody>
</table>

\(^a\)Michaelis constants (\( K_m \), micromolar O2) for 50% \( R_{NO,\text{max}} \).
Third, NO has a direct effect on O$_2$ metabolism. NO competes with O$_2$ at the heme site of cytochrome oxidase to reversibly inhibit tissue O$_2$ consumption (81–84). Inhibition by NO depends on O$_2$ availability, with increasing effect as the O$_2$:NO ratio decreases (81). NO has essentially no effect on respiration when the O$_2$:NO ratio is >250:1. In a study of isolated mitochondria from rat brown adipose tissue (86), the following nonlinear relationship was found for the variation in mitochondrial respiration with O$_2$:

$$R_{O_2} = R_{O_2\text{max}} C_{O_2}^2 / (C_{O_2}^2 + K_m^2),$$  

(25)

where the apparent $K_m$ for O$_2$ increases as a linear function of NO,

$$K_m = 16 \mu\text{M}(1 + C_{NO}/27 \text{nM}).$$  

(26)

The effect of increasing amounts of NO on the O$_2$ dependence of RO$_2$ is shown in Figure 4. It should be noted that this is not the usual Michaelis-Menten kinetics used to represent O$_2$-dependent respiration, and that the $K_m$ in the absence of NO is considerably higher than others report. As shown in Figure 4, the inhibitory effect of NO is greater at low tissue O$_2$ levels. A similar linear relationship with an intercept closer to zero can be inferred from the data of Brown & Cooper (82), who found that the O$_2$ consumption of brain synaptosomes was 50% inhibited with 270 nM NO when O$_2$ was 150 µM. At lower O$_2$, closer to expected tissue levels (∼30 µM), only 60 nM NO caused 50% inhibition.

Fourth, it is possible that NO can react with thiols when O$_2$ is present (87, 88). Nitrosothiols in blood and tissue may serve as a reservoir for NO release under certain conditions, and reduction of nitrite back to NO by xanthine oxidase may occur when tissue pO$_2$ levels are very low.

**NITRIC OXIDE IN THE LUNG**

The ability of NO to relax smooth muscle is the principle behind using NO inhalation therapy for pulmonary hypertension, acute respiratory distress syndrome, and bronchial asthma. There is also keen interest in the diagnostic potential for measuring exhaled NO, especially as an indicator of sepsis or pulmonary inflammation. The partial pressure of NO in gas from the lower airways is normally very low, on the order of 6 ppb but is much higher in the nasal passages. Therefore, techniques for respiratory gas sampling have been designed to eliminate nasal contributions. Hyde et al (89) developed a single-compartment model to predict NO partial pressure in the lower airways ($pNO_L$) from the relationship

$$V_L \frac{dpNO_L}{dt} = \dot{V}_{NO}(P_{atm} - 47) - pNO_L [DC_{NO}(P_{atm} - 47) + \dot{V}_A],$$  

(27)

where $V_A$ is the ventilation rate (typically 12 breaths/min with tidal volume of 500 ml for a total of 6 liters/min at rest), $V_L$ is the mean volume of gas in the lower airways, $P_{atm}$ is the barometric pressure corrected for water vapor pressure (47 mmHg or torr) at 37°C, $V_{NO}$ is the rate of NO produced by lung tissue that
Figure 4  Inhibition of O\(_2\) consumption by NO predicted from modified Michaelis-Menten kinetics (Equations 25–26) reported by Koivisto et al (86) for isolated mitochondria are shown as a function of O\(_2\) or pO\(_2\) (bottom panel). (Dashed line) Half-maximal O\(_2\) consumption rates. The NO concentration required to inhibit O\(_2\) consumption by 50% predicted from these kinetics is a linear function of O\(_2\) (top panel, solid line). A similar relationship (dashed line, top panel) is inferred from the data of Brown & Cooper (82) for NO required to inhibit O\(_2\) consumption of brain synaptosomes by 50% (squares).

enters the airway gas, and DC\(_{\text{NO}}\) is the diffusing capacity for NO (DC is not a diffusion coefficient), which is 140 ml min\(^{-1}\) mmHg\(^{-1}\) at rest. The solution is given by

\[
p\text{NO}_L(t) = \frac{\dot{V}_\text{NO}(P_{\text{atm}} - 47)}{V_L} \left[ 1 - e^{-\lambda t} \right],
\]

(28)

where the first-order rate constant is

\[
\lambda = \frac{[DC_{\text{NO}}(P_{\text{atm}} - 47) + \dot{V}_\Lambda]}{V_L}.
\]

(29)

Pulmonary blood NO concentration is assumed to be zero, and \(V_L\) is considered to be constant, although it does vary as air is inhaled and exhaled. The model was also
modified to include a step change in NO, such as a deep breath of air containing NO, or a long breath hold. In the latter case, it was calculated that NO would reach steady state within $\sim 15$ s. Equations for NO exchange during inspiration or expiration were also presented.

Tsoukias & George (90) developed a more elaborate model, with the lower airways represented by a variable volume alveolar compartment, coupled to a second, fixed-volume compartment for the upper airways. The upper airway included NO production and reaction in a thin layer of tissue with bronchial blood on one side and air on the other. Because the bronchial tissue layer is very thin, a one-dimensional planar model,

$$\frac{\partial C_{\text{NO},t}}{\partial t} = D_t \frac{\partial^2 C_t}{\partial x^2} + \dot{S}_{\text{air}} - \lambda_t C_{\text{NO},t},$$

(30)

was applied, where $D_t$ is the diffusion coefficient, $\dot{S}_{\text{air}}$ is the NO flux from tissue into the upper airway gas, and $\lambda_t$ is the first-order reaction rate constant in tissue. Rates ranging between $1.38 > \lambda_t > 0.046$ s$^{-1}$ corresponding to $0.5 < t_{50\%} < 15$ s were used, and an analytical solution for the time-dependent variation in tissue concentration was described. A partition coefficient of 0.0416 (mol of NO in tissue/mol of NO in gas) based on the solubility of NO in water was used to convert $C_{\text{NO},t}$ to $p_{\text{NO}}$, assuming instantaneous mixing as NO diffused into the gas phase. The transient response to a step change in $p_{\text{NO}}$ in the gas phase showed that the NO concentration change in tissue was 90% complete in $\sim 0.6$ s, so that for most respiratory maneuvers, the steady state profile would provide sufficient accuracy in the model.

The steady state NO profile across the layer is

$$C_{\text{NO},t}(x) = C_L - \dot{S}_{\text{air}} \left( e^{-\sqrt{\lambda_t} x} - e^{-\sqrt{\lambda_t} \sqrt{L}} \right) / \lambda_t - \dot{S}_{\text{air}} \left( e^{-\sqrt{\lambda_t} \sqrt{L}} - e^{-\sqrt{\lambda_t} x} \right) / \lambda_t,$$

(31)

where the coefficient

$$\alpha = \left( C_L - \dot{S}_{\text{air}} \left( e^{-\sqrt{\lambda_t} \sqrt{L}} - e^{-\sqrt{\lambda_t} x} \right) / \lambda_t \right) / \left( e^{-\sqrt{\lambda_t} \sqrt{L}} - e^{-\sqrt{\lambda_t} x} \right),$$

(32)

where $L$ is the thickness of the layer, and $C_L$ is the NO concentration at the tissue/air interface ($x = L$). Scavenging by Hb reaction is not directly modeled, but NO concentration in blood is assumed to be negligible, so the boundary condition at the blood/tissue interface ($x = 0$) is fixed at $C_{\text{NO},t}(0) = 0$. NO flux into the bloodstream can be determined from the derivative of Equation 31. Predicted NO profiles are shown in Figure 5 for several cases, including case $a$ for parameters used by Tsoukias & George (90), with the wall concentration fixed at 0.15 nM and $\dot{S}_{\text{air}} = 0.55$ nM s$^{-1}$ cm$^{-3}$. Case $b$ is for a doubling of $\dot{S}_{\text{air}}$, and case $c$ is for 50% of $\dot{S}_{\text{air}}$. Case $d$ is for pNO one order of magnitude higher in the gas phase than in case $a$. 
Figure 5  NO profiles across a thin layer of bronchial tissue in the lower airway of the lung (Equation 31), predicted from the model by Tsoukias & George (90), are shown for different parameters. Case a is for baseline parameters with normal ventilation in their model. Case b is for twofold higher and case c is for half of the NO production rate in bronchial tissue. Case d uses baseline parameters in case a with NO partial pressure one order of magnitude higher in the gas phase.

For the upper airways, the mass balance was written as

\[
\frac{\partial C_{\text{NO}_{\text{air}}}}{\partial t} = \left[ \frac{A_s}{A_c} \right] J_{t,g,\text{air}} + V \frac{\partial C_{\text{NO}_{\text{air}}}}{\partial V},
\]

(33)

where \(A_s\) is the wall surface area, and \(A_c\) is the airway gas volume per unit axial distance, and \(J_{t,g,\text{air}}\) is NO flux from tissue into airway gas. The volumetric gas rate \(V\) represents either inspiration \(V_I\) or expiration \(V_E\). Tissue NO flux into the upper airway gas was coupled to the gas flow in and out of the alveolar compartment.

The lower alveolar compartment was assumed to be well mixed, with NO entering or leaving by convective flow and exchanging with alveolar tissue by diffusion. The mass balance for the alveolar concentration of NO was written as

\[
V_{\text{alv}}(t) \frac{dC_{\text{NO}_{\text{alv}}}}{dt} = (S_{\text{app,alv}} - DC_{\text{NO}}) + \dot{V}(C_{\text{NO}_{\text{air.end}}} - C_{\text{NO}_{\text{alv}}}),
\]

(34)
where $V_{alv}(t)$ is the time-varying alveolar gas volume, $S_{app,alv}$ is the rate of NO entering the alveolar gas produced by alveolar tissue, and $DC_{NO}$ is the diffusing capacity for NO. Analytical solutions were presented for inspiration, expiration, and breath holding and were compared with measurements from a representative human subject taken from a companion paper (91). Generally, the model was able to describe these maneuvers, with some minor discrepancies, even though no attempt was made to optimize the model parameters for the subject. One limitation discussed is the fact that the diffusing capacity for NO varies with surface area and Hb reaction rate, so it is not a simple constant.

Recently, Högman et al (92) used a simplified variation of the above model and reported that the use of three different expiratory flow rates (low, normal, and high) permitted extraction of reliable information about alveolar, lower airway, and nasal contributions from the analysis of exhaled NO data. In ten healthy humans, they reported that the average NO transfer rate of the airways was $9 \pm 2$ ml s$^{-1}$, with the airway tissue contributing $75 \pm 28$ ppb and the alveolar fraction $2 \pm 1$ ppb. Values computed from the three different flow rates were no different than values obtained from a more extensive analysis of measurements for eleven different flow rates in these ten subjects. They were also able to demonstrate significant differences between five subjects with asthma and five cigarette smokers compared with the control group.

**NITRIC OXIDE IN THE BRAIN**

NO has at least four possible functions in the central nervous system, including (a) coupling of neuronal activity with cerebral blood flow, (b) stabilizing synaptic efficiency, (c) facilitating neurotransmitter release, and (d) directing growth of axonal arbors during development (93). The first evidence for a role of NO in brain function was from Garthwaite et al (94), who found that cerebellar neurons synthesized NO in response to N-methyl-D-aspartate release. Bredt & Snyder (95) isolated and characterized nNOS and demonstrated its widespread presence in the central nervous system. A role for NO in long-term potentiation in the hippocampus was suggested from studies by Schuman & Madison (96).

Wood & Garthwaite (97) developed a point source model for NO in the brain, similar to the approach by Lancaster (55–57). For diffusion and first-order reaction in spherical coordinates with angular symmetry, the concentration as a function of time at a fixed radial location is

$$C_{NO}(r, t) = \frac{S_{t=0}}{8(\pi D t)^{1/2}} e^{-\left(\frac{r^2}{4Dt}\right)} e^{-\lambda t} \quad (35)$$

where $S_{t=0}$ is the instantaneous strength of the point source at $r = 0$ and the pseudo-first-order reaction rate $\lambda = 0.1386$ s$^{-1}$ for $t_{50\%} = 5$ s. Shorter half-lives up to an order of magnitude faster were also modeled. NO concentration at any spatial location can be found using the principle of superposition by summing
Figure 6  Dynamic changes in brain tissue NO are shown at a location 100 µm from a point source following a single spike of NO production at \( t = 0 \) (top panel) or a 2-Hz train of five consecutive spikes separated by 500 ms (bottom panel). (Insets) Relative change in NO near the origin for both cases based on a 5-s half-life in tissue. The NO volume signaling resulting from repeated NO bursts is simulated by linear superposition of the analytical solution for a single burst in spherical coordinates (Equation 35) from the model by Wood & Garthwaite (97). The peak NO concentration decreases with distance from the source, with an increasing time lag from the initial burst.

Repeated spikes or pulses from a single source, or by summing contributions from multiple sources. An example for changes in NO at a location 100 µm away from a single point source is shown in Figure 6 for a single spike at \( t = 0 \) and for a 2-Hz train of five sequential spikes 500 ms apart. For each case, the relative change in NO at the source is also shown. Note that the increase in NO at the source lasts longer than the change in NO farther away, and that the peak is delayed in time, depending on how far away it is from the source.

The steady state profile around a single point source at \( r = 0 \) producing NO at a continuous rate would be

\[
C_{NO}(r) = \frac{S}{4\pi D F} e^{-\sqrt{\frac{r}{\lambda}}}, \tag{36}
\]
where $S = 2.1 \times 10^{-17} \text{M} \cdot \text{s}^{-1}$ causes $C_{\text{NO}}$ to be 1 $\mu\text{M}$ at $r = 0.5 \mu\text{m}$, and 1 nM at $r = 500 \mu\text{m}$ in the simulation by Wood & Garthwaite (97). Note that the solution is singular at $r = 0$, where the concentration would approach infinity. Their model suggested that a single point source of NO could influence approximately two million synapses by diffusion to a volume of brain tissue 200 $\mu\text{m}$ in diameter.

Philippides et al (98) noted that there are problems with the singular nature of an instantaneous point source, so they modeled a hollow spherical structure with NO produced in the outer shell of a sphere of 6 $\mu\text{m}$ inner diameter and 10 $\mu\text{m}$ outer diameter. They replaced the point source function with a volumetric NO production rate $R_{\text{NO}} = 1.32 \times 10^{-4} \text{M} \cdot \text{s}^{-1} \cdot \mu\text{m}^{-3}$ to generate $C_{\text{NO}} = 1 \mu\text{M}$ on the surface. They performed simulations with square wave pulses of NO production and also used arbitrary functions for a smoother rise and decay of NO pulses up to 100 ms in duration, but this made little difference in the qualitative results. One of the interesting features of the hollow sphere structure was that it caused a reservoir effect, as some of the NO diffused into the center during the pulse, then diffused outward after production ceased. They also varied the size and thickness of the hollow spheres and concluded that neurons with diameters in the 15- to 20-$\mu\text{m}$ range producing NO in 100-ms pulses will influence the largest relative volume of tissue.

They also extended the model to an irregular structure consisting of small cylindrical shapes with branches emanating from a hollow sphere. The structure used simple geometries to represent the morphology of a neuron cell body with dendrites, and the simulation included an elliptical sink to represent NO scavenging by hemoglobin in a capillary. A three-dimensional simulation at different time points was performed, showing first the initial increases in NO around the elements, then the diffusion and decay of the “NO cloud” into the space around the structures. The NO cloud could surround and move past the sink, but the NO concentrations were higher on the opposite side of the sink. Thus, contour plots at different times showing the amount of tissue around the structure with NO above the threshold for activation of guanylate cyclase were not symmetric because of the irregular shape and the distorting effect of the sink. Among the conclusions drawn from this modeling approach, Philippides et al (98) pointed out that the morphology of the source was crucial for the NO diffusion signal, but the surrounding tissue could be regarded as homogeneous. This is a marked contrast from classical synaptic neurotransmitters that diffuse more slowly over much shorter distances. The volume signaling by NO therefore does not have to address a specific target, and the target does not have to recognize the source of the signal it is receiving.

Because NO would appear to decrease synaptic specificity, it is not clear how NO can play a role in cerebellar learning through effects on long-term depression. Schweighofer & Ferriol (99) developed a neural model for adaptive movement control, incorporating NO diffusion and first-order reaction ($\lambda = 0.3 \text{s}^{-1}, t_{50\%} = 3.3 \text{s}$). The neural model consisted of two-dimensional microzones occupying an area of $315 \times 630 \mu\text{m}$, with nine columns and three rows of Purkinje cells and a distribution of synapses between Purkinje and granule cells. It was assumed that
NO was generated by spikes from inferior olive cells. The spikes were simulated by a stochastic Poisson spike generator, with the mean frequency modulated by an error signal related to an arm-reaching task. A learning rule was defined in terms of parallel fiber activity and relative change in NO at the synapses. The efficiency of learning was simulated with and without NO diffusion. It is not clear whether point sources were used to generate NO in the model, and NO concentrations were not specified. An example of relative NO levels computed for a system of four interconnected microzones during movement of shoulder and elbow joints was illustrated. High NO levels were generated when the movement error signal was large, and low levels with small errors. The model predicted that learning efficiency was improved for low-frequency inferior olive firing rates in the 0.5- to 2-Hz range. At higher frequencies, learning efficiency without NO diffusion improved because more information was received and, eventually, exceeded the efficiency computed with NO diffusion. The model suggests that NO volume signaling acts as a low-pass filter that is unable to carry high-frequency information. The model sensitivity to variations in diffusion rates was examined at fixed reaction rate \( \lambda = 0.3 \text{ s}^{-1} \), with the best learning simulated in the range \( 2000 < D < 3300 \mu \text{m}^2 \text{ s}^{-1} \).

The simulation suggests that NO can play a role in cerebellar learning, although there were some limitations. One limitation was the decrease in information with higher frequencies, and the authors suspect that they will need to include the effects of protein kinase C activation on synaptic efficiency in future models. Another limitation was cross interference due to NO diffusing from one microzone into another, which is probably a consequence of the simplified nature of the model compared with actual cerebellar morphology where distances are greater.

THE DARK SIDE OF NITRIC OXIDE

Although the models discussed above have primarily focused on NO biotransport under normal physiological conditions, there is a substantial body of literature on the pathological aspects of NO biochemistry, which is too extensive to review here. The general consensus is that excessive or unregulated NO production contributes to numerous disease states. Septic shock is an obvious example of NO overproduction. Endothelial dysfunction possibly associated with decreased NO production or increased NO degradation is thought to play a role in diabetes mellitus, hypertension, hypercholesterolemia, thrombosis, and vasospasm. Neurogenerative disorders where NO is suspected to play a role include amyotrophic lateral sclerosis and Alzheimer’s, Huntington’s, and Parkinson’s diseases. With stroke, traumatic brain injury, or cerebral ischemia, the consensus view is that eNOS can play a protective role by maintaining blood flow, while nNOS can be harmful through NO-mediated increases in glutamate neurotoxicity. Inflammation and increased NO production from iNOS can also come into play some time after the initial insult. A descriptive title from a paper by Beckman & Koppenol (100)
borrowed from a Hollywood western movie characterized the key players in NO biochemistry as “good” (NO), “bad” (O$_2^-$), and “ugly” (ONOO$^-$).

A series of kinetic modeling papers by Stanbro (101–103) suggest that “ugly” ONOO$^-$ could reach nanomolar concentrations in blood plasma and react with low-density lipoproteins. A coupled set of eight differential equations for the reactions was solved numerically for specified baseline conditions and varying input NO. Although the actual number of low-density lipoprotein particles that react might be small, this could be sufficient to initiate a chain reaction with unsaturated fatty acids, contributing to the slow buildup of atherosclerotic plaques in blood vessels. The role of antioxidants in reducing ONOO$^-$ was also investigated but was found to have little effect once it had formed. The modeling suggested that a more reasonable pharmacological strategy would be to reduce O$_2^-$ and, thus, limit lipid peroxidation by reducing the amount of ONOO$^-$ that would be formed.

Therapeutic approaches have included NO donors, such as the early use of nitroglycerin many decades before the mechanism of its action on the cardiovascular system was known. Viagra is another well-known drug. Alternative approaches for controlling NO production have been directed toward the development of pharmacological inhibitors with specificity for the individual NOS isoforms. For example, an effective nNOS inhibitor that preserves eNOS activity may be an effective agent for treating brain injury or stroke. Highly specific iNOS inhibitors may be effective in reducing cell damage associated with inflammation. Gene therapy is also being considered. There have been several animal studies, reviewed by Chen et al (104), where successful transfer of recombinant eNOS genes to blood vessels has been achieved. Areas of clinical application could include treatment of blood vessels damaged by atherosclerosis, pretreatment of vein grafts to maintain patency, or treatment of stenosed vessels following balloon angioplasty. Gene transfer of recombinant iNOS has also been successful in animal studies and could find a clinical application for accelerating wound healing. Recombinant nNOS might be a more suitable alternative for eNOS in some applications.

PHYSIOLOGICAL MEASUREMENTS OF NITRIC OXIDE

A substantial number of landmark NO studies in the literature are based on indirect methods, such as measuring tissue or blood plasma nitrite/nitrate levels, or measuring other physiological parameters before and after inhibiting NOS. Archer (105) summarized direct and indirect methods for detecting NO and related products in biological systems. Chemiluminescence detectors provide the highest resolution and sensitivity but are limited to gas phase measurements. Electrochemical sensors for detecting NO in liquid phases are now available commercially and have been miniaturized in some laboratories. Three different NO microelectrode designs for tissue NO measurements have been developed by Shibuki (106), Malinski & Taha (107), and Buerk et al (108). The operating principle for all three microsensors is the electrochemical oxidation of NO.
The Shibuki (106) design consists of a platinum anode and silver cathode polarized at +900 mV inside a membrane-covered glass micropipette filled with acidic salt solution. Shibuki & Okada (109) used this electrode in cerebellar slices, finding increases in NO averaging 47 nM during electrical stimulation. These data provided direct evidence that NO plays a role in long-term depression of Purkinje cell neurotransmission. More recently, this type of probe measured much smaller increases in NO, averaging 380 pM in layer V of rat auditory cortex slices during electrical stimulation (110).

The second design by Malinski & Taha (107) uses a carbon fiber with a flame sharpened tip of <1 μm. The carbon fiber is sealed in a glass micropipette, leaving the tip and a small length of fiber exposed. The exposed end is coated with conducting polymer [monomeric tetrakis (3-methoxy-4-hydroxyphenyl) porphyrin] to catalyze electrochemical oxidation of NO at 640 mV relative to an external reference electrode. Negatively charged Nafion polymer was also coated over the polymer to minimize interference from NO$_2^-$ and catecholamines. However, tyrosine has been reported to be a significant interfering species for this sensor (111). In vitro and in vivo studies by Malinski et al (68, 112) have provided valuable information used for some of the NO biotransport models in this review.

The third design by Buerk et al (108) consists of a glass micropipette with a recessed gold cathode, which is polarized at +800 to +850 mV relative to an external Ag/AgCl reference electrode. Tips are typically between 5 and 10 μm, with recesses ~20–50 μm deep. The recess acts as a diffusion barrier for high-molecular-weight species, and Nafion polymer in the recess further minimizes interference from ascorbate, catecholamines, NO$_2^-$, and tyrosine. In vivo NO studies in cat optic nerve head with simultaneous blood flow measurements by laser Doppler flowmetry were reported (113, 108). We found that during stimulation of the eye by flickering light, blood flow increased by 44% and NO by an average of 88 nM. NOS inhibitors significantly attenuated the NO and blood flow responses to flicker stimulation (108) and reduced baseline NO from an initial level of ~460 nM. We also reported that NO concentration is not constant, but normally fluctuates about a mean value with a low frequency content similar to that for blood flow variations in cat optic nerve head (113). NO responses to hypoxia and hypercapnia in cat optic nerve and rat brain were compared in another study (114), and the role of NO in functional activation of the brain is under investigation. The inhibitory effect of NO on O$_2$ metabolism and the possible role of NO in O$_2$ chemoreception in the perfused cat carotid body were also studied (115). The same recessed design has been used by Bohlen (116) to measure NO on the outer walls of small arteries and veins in rat intestinal mucosa. Resting levels on the outer blood vessel wall averaged 353 nM for arteries and 401 nM for veins, well above the 250 nM target for activating sGC in the simulation by Vaughn et al (66). NO levels increased >500 nM for both arteries and veins during glucose absorption. In another study, Bohlen & Nase (117) report periarteriolar NO levels averaging 337 nM for first-order large arterioles, and 318 nM for second-order intermediate-sized arterioles in the intestinal microvasculature for resting conditions.
Experimental maneuvers that caused increases in blood flow and shear stress also increased NO.

As more physiological NO measurements are made, our knowledge base should expand and lead to significant improvements in NO biotransport models. However, there are conflicting results in the literature that need to be resolved. The brain in particular has produced some curious findings. For example, electron paramagnetic resonance studies in normal rat forebrain found basal NO levels averaging 1.6 µM, and 10-fold higher with sepsis (118). In vivo studies using microdialysis with Hb to quantify NO from metHb formation found NO levels in rat cerebellum averaging 303 nM with halothane anesthesia, and higher levels averaging 532 nM with isoflurane anesthesia (119). We found average NO levels around 1.1 µM in rat parietal cortex, increasing to 2.4 µM during recovery from a short period of severe hypoxia (114). However, Malinski et al (68) reported baseline NO levels in rat brain <10 nM, at the detection limit of the electrode. Despite the difference in basal levels, increases in NO following hypoxic insults were similar for the latter two studies. Further experimental work is needed to resolve these and many other uncertainties in the literature.

**FUTURE DIRECTIONS AND MODELING NEEDS**

It is clear that reliable models will not be developed without means to verify whether the theoretical predictions have physiological meaning, and that unreliable physiological measurements will not improve our modeling efforts. More synergistic interactions between experiment and theory need to be established to validate proposed biochemical mechanisms. In particular, the suspected role of nitrosothiols needs further modeling and appropriately designed experiments to investigate whether this “secret passage” is real.

Models need to be developed that couple NO production with O₂ transport. Recently, Clementi et al (83) commented that “the classical paradigm about the independence of oxygen consumption along a wide range of oxygen concentrations should be corrected in terms of NO metabolism.” As discussed above, these interactions include the O₂ dependence of NOS isoforms, effects of NO on O₂ metabolism, oxyhemoglobin properties, and release of NO stores when tissue pO₂ is low. Functional models need to be developed to predict how NO release controls vascular smooth muscle tone and blood flow, with feedback from wall shear stress, intracellular Ca²⁺ levels in the endothelium, and O₂ delivery and

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1A simplified one-dimensional planar model for coupled NO and O₂ diffusion from blood vessels including reversible inhibition of O₂ consumption by NO was published while this review was in proof, which demonstrates that tissue oxygenation can be enhanced, especially when metabolic requirements are high (Thomas DD, Liu X, Kantrow SP, Lancaster JR Jr. 2001. The biological lifetime of nitric oxide: implications for the perivascular dynamics of NO and O₂. *Proc. Natl. Acad. Sci. USA* 98:355–60).
NITRIC OXIDE BIOTRANSPORT MODELS

consumption in the vascular wall. Interactions with nearby blood vessels need to be incorporated into the boundary conditions, and diffusion of other substances that modify NO release should be included in the models. Further modeling work may be needed to fully interpret NO exhalation measurements from the lung, to increase the potential for clinical diagnosis of different respiratory diseases, or to evaluate effectiveness of therapeutic measures. Testing standards need to be established in the near future to evaluate the increasing database of patient information.

Modeling of the effects of NO on neurotransmission need to be extended to include Ca\textsuperscript{2+} homeostasis and control of ion channels, modulation of other neurosecretory products, and effects on other biochemical pathways. They must also be able to predict an outcome that can be verified experimentally.

The interactions between NO and the immune system have not been examined in detail in this review. However, many areas can benefit from NO biotransport modeling, including the complex behavior of leukocytes in the microcirculation, leukocyte interactions with adhesion molecules, platelet aggregation, and thrombosis in the bloodstream, and inflammatory processes in tissue. The modeling of NO biotransport will naturally be extended to pathological conditions and may be useful for evaluating therapeutic approaches directed at specific molecular or biochemical pathways.

We can anticipate that this “startlingly simple” yet “bizarre” gas and its “ugly” byproduct will continue to challenge theoreticians and mathematical modelers in the biomedical community for many more decades.

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