Concentration changes of nitric oxide (NO) were monitored using an NO-sensitive electrode in phosphate-buffered saline (PBS) with either free oxyhemoglobin (oxyHb) or red blood cells (RBCs). In aerated PBS, the half-life of 0.9 μM NO is greater than 4 min. NO is undetectable (<50 nm) when added to a solution of oxyHb because the reaction of NO with oxyHb is rapid. The disappearance rate of NO in PBS containing RBCs is rapid, compared with PBS, but it is much slower (by a factor of approximately 650) than with an equivalent solution of free oxyHb. The half-life of NO is inversely proportional to the concentration of RBCs, independent of oxyHb concentration inside RBCs, and the disappearance rate of NO is first order in NO concentration and first order in the concentration of RBCs. After all the oxyHb reacts with NO to form methemoglobin, the disappearance rate of NO slows greatly. These data indicate that the reaction of NO with oxyhemoglobin within RBCs is limited by the diffusion of NO into the cell, which has also been shown previously for the reaction of O₂ with deoxyhemoglobin. Experimental data show that the half-life of NO in the presence of 2.1 × 10⁶ RBCs/ml is 4.2 s. From this value, we estimate that the half-life of NO in whole blood (5 × 10¹⁰ RBCs/ml) will be 1.8 ms. A simple analytical expression for the half-life of NO in PBS with RBCs was derived in this study based on a spherical diffusion model. The calculated half-life of NO from the expression is in good agreement with the experimental values.

Nitric oxide (NO)¹ is one of the 10 smallest, stable molecules of the hundreds of millions in nature (1). According to Stokes’ Law, the diffusibility of a molecule in the condensed phase is inversely proportional to its molecular radius, which thus makes NO one of the most rapidly diffusible molecules known. Its diffusion constant (D) is approximately 3300–3800 μm²/s, whether measured in aqueous solution (2) or in intact tissue (e.g. brain (3)). Membranes and other hydrophobic structures in tissue are no barrier to diffusion of NO because of its solubility in hydrophobic phases (4). The reaction of free NO with oxyhemoglobin is rapid (bimolecular rate constant k = 3.4 × 10⁷ M⁻¹ s⁻¹ (5)), and from this rate constant it can be calculated that the half-life of NO in the presence of a concentration of hemoglobin equivalent to that in the bloodstream (15 g/dl) would be very short, approximately 2 × 10⁻⁶ s. As we have pointed out previously (6, 7), the extremely rapid diffusibility of NO coupled with its rapid reaction with oxyhemoglobin apparently poses a difficulty in the postulate that free NO is the endothelium-derived relaxing factor.

Using an electrochemical method, we describe here the results of measurements of the disappearance of NO upon reaction with either oxyhemoglobin in solution or oxyhemoglobin when contained within intact erythrocytes. We find that, as reported in 1927 for the reaction of O₂ with deoxyhemoglobin (8), the NO reaction with intact RBCs is considerably slower than with an equivalent concentration of free oxyhemoglobin. We present a mathematical analysis of this phenomenon, which demonstrates that the rate of the reaction of NO with intraerythrocytic hemoglobin is limited by the rate of diffusion of NO into the cell. From our data, we estimate that in whole blood the half-life of NO will be less than 2 ms, which, although quite rapid, is considerably longer than in the presence of free hemoglobin.

**EXPERIMENTAL PROCEDURES**

Preparation of NO Solution—6 ml of phosphate-buffered saline (PBS: 15 mM phosphate (potassium) plus 0.9% NaCl pH 7.4) in a plastic vial was used in preparing saturated NO solution. The solution was bubbled with argon gas (Aldrich) for 30 min and then changed to NO gas (Aldrich) for 20 min. The NO gas was passed first through a gas-washing bottle containing 1 M deaerated KOH solution.

**RBC and Free Hemoglobin Preparation**—Blood was withdrawn from rats and centrifuged at 2300 × g for 10 min. The plasma and buffy coat were discarded, and the RBC pellet was washed 3 times with PBS (pH 7.4). The packed RBCs then were added to PBS and the solution was stirred gently. Cells were counted with a hemocytometer and were stored on ice for use. To prepare free oxyHb, 2 ml of counted RBCs was centrifuged at 2300 g for 10 min (4 °C). The packed RBCs were then added to 40 ml of 5 mM phosphate solution (pH 8), stirred and allowed to incubate for 30 min for hemolysis.

**Electrochemical Measurements**—All electrochemical measurements were carried out at 25 ± 2 °C by a BAS 100B10 electrochemical analyzer with a PA-1 preamplifier and C2 cell stand from Bioanalytical Systems Inc. (West Lafayette, IN) (9). A platinum disc microelectrode was used as the working electrode for detecting NO. An Ag/AgCl wire and a platinum wire were used as the reference electrode and the auxiliary electrode, respectively. The electrochemical cell is a plastic vial (2 cm in diameter, 2.86 cm in height), obtained from Fisher. The working electrode, reference electrode, and auxiliary electrode were inserted in the vial containing the test solution through three holes on the cap. The sample was added through another hole (diameter less than 1 mm) using a syringe. A stirring magnet (1 cm) was placed in the vial for stirring the solution during electrochemical measurements.

Spectrophotometric Analysis—Absorption measurements were carried out using a Beckman DU-64 spectrophotometer. The absorbance was recorded immediately after adding NO to oxyHb solution. The
amount of oxyHb present during the NO titrations was followed by monitoring absorbance and using a molar extinction coefficient of 1.58 × 10⁴ M⁻¹ cm⁻¹ at 576 nm.

RESULTS

Disappearance of Free NO by Reaction with Free OxyHb and RBCs—Measurements of NO (solid tracing) and oxyHb concentrations (bar graph) after repeated additions of NO to a solution of oxyHb in an enclosed well stirred reaction vessel (conditions under which the rate of volatilization of NO is negligible, data not shown) are presented in Fig. 1. Initial additions of NO (9 µM each addition) resulted in decreased oxyHb concentration at a 1:1 molar stoichiometry, consistent with the rapid reaction of NO to produce methemoglobin (5). NO was not detectable because of its rapid disappearance (within the response time of the electrode). However, after complete reaction (after two additions of 9 µM NO to the solution that contains 18.4 µM heme), the rate of NO disappearance is dramatically slowed, and so NO becomes detectable.

Fig. 2 shows the disappearance of NO in the presence of oxyHb and of RBCs. Tracing A is a recording of NO concentration after sequential additions of 0.9 µM NO to 0.62 µM oxyHb (2.48 µM heme). Tracings B and C are recordings of NO concentration in the presence of 0.7 × 10⁶ RBCs/ml (1.05 µM total heme concentration) and 1.4 × 10⁶ RBCs/ml (2.1 µM heme), respectively. As is also true for Fig. 1, tracing A shows that the reaction of NO (added at the down arrows) with oxyHb is more rapid than the response time of the electrode. NO is detectable only after complete reaction with oxyHb (up arrow). However, tracings B and C show that the reaction of NO with oxyHb, when it is contained within RBCs, is much slower, and the time course of NO disappearance becomes measurable. Reduction of methemoglobin within the RBCs will not be significant under our conditions because this process is much slower than the changes we are observing (requiring at least 1–2 h even in the presence of reductant (11)). Comparison of B with C shows that the rate of NO disappearance is faster with higher concentrations of RBCs (0.7 × 10⁶ RBCs/ml for B and 1.4 × 10⁶ RBCs/ml for C). This is shown quantitatively in Fig. 3, where the NO decrease after addition is plotted as for a first order reaction for each cell suspension. The calculated NO half-life for the higher RBC suspension (Figs. 2B and 3A) is 12.9 ± 0.3 s, whereas it is 6.4 ± 0.1 s for the lower suspension (Figs. 2C and 3B). The fact that the half-life for the higher concentration of RBCs is approximately 50% of the half-life for the lower concentration means that the reaction is first order with RBC concentration (i.e., the rate constant is twice as large). In addition, the rates are identical for multiple additions of NO, conditions under which there is incremental loss of oxyHb (Fig. 1). This shows that the rate is independent of the internal oxyHb concentration. At the time when complete hemoglobin oxidation occurs, the disappearance of NO slows dramatically and becomes similar to the rate with free oxyHb after complete oxidation (Fig. 2A). This result indicates that oxyHb is converted to methemoglobin uniformly throughout the entire RBC population, as opposed to a rapid and complete conversion in only a fraction of the RBC population. This is because if the latter were true then...
we would not see an abrupt “switch” in the rate upon complete oxidation of all the hemoglobin but rather a gradual decrease, which would be similar in effect to gradually decreasing the number of RBCs (see Table I).

Table I presents the results of a series of measurements of NO disappearance with increasing numbers of RBCs and (for the highest RBC concentration suspension) two NO concentrations. As shown, the half-life of NO decreases as the number of RBCs increases, and this decrease predicts a first order dependence on RBC concentration. If the number of RBCs is fixed, repeatedly adding NO does not change the half-life of NO until the point that all the oxyHb completely reacts with NO (Figs. 2 and 3). After this point, the half-life of NO dramatically increases to a much larger value. This result means that the half-life of NO is independent of oxyHb concentration inside RBCs because oxyHb concentration inside the RBCs decreases incrementally when we repeatedly add NO to the solution (Figs. 1 and 2). Thus, the reaction of NO is first order in NO and RBC concentration but independent (zero order) of intracellular oxyHb concentration.

A Diffusion Model for Reaction of Free NO with RBCs—Hartridge and Roughton (8) reported in 1927 that reaction (binding) of O₂ with deoxyhemoglobin occurs much more slowly when the deoxyhemoglobin is contained within erythrocytes than when free in solution. Numerous studies, both experimental and theoretical, have examined this phenomenon further, and it is now believed that its origin lies in the existence of an unstirred layer surrounding the erythrocyte; the reaction of O₂ is determined by the rate at which O₂ can diffuse into the cell and become accessible to the deoxyhemoglobin (12–15). Once inside the cell, the free O₂ disappears virtually instantaneously because of the rapid reaction with the high internal concentrations of deoxyhemoglobin, making the rate of diffusion through the unstirred layer rate limiting for O₂ disappearance. Because the diffusional properties of NO are similar to O₂ (NO and O₂ differ by one atomic number) and the rate of reaction of NO with oxyHb is also rapid, it is perhaps not surprising that the same phenomenon (diffusion-limited disappearance with intact erythrocytes) should hold true.

As described for the O₂ reaction with deoxyhemoglobin within RBCs (14), the rate of NO disappearance can be described by the following empirical relationship,

\[ D \left( \frac{\partial c}{\partial x} \right)_0 = \gamma (c^b - c^s) \quad (Eq. 1) \]

where \( D \) is the diffusion constant for NO, \( \gamma \) is an empirical mass transfer coefficient, \( c^b \) is the NO concentration in the bulk solvent, and \( c^s \) is the NO concentration at the first cytoplasmic layer. We present here an analytical model that predicts a value for \( \gamma \), which is close to our experimentally determined value.

This diffusion-reaction problem is mathematically analogous to that describing current flow in a microelectrode, where the rate of the measuring event is limited by the diffusion of the detectable species (diffusion to the interior of the RBC or diffusion to the surface of the electrode). We can thus apply similar mathematical analyses.

We assume that 1) the reaction of NO with oxyHb is so fast that NO concentration inside the cell is zero (until all oxyHb inside the cell is completely reacted with NO); 2) the rate of NO diffusion through the cell membrane is so rapid that NO concentration on the surface of the cell is close to zero; 3a) a small number of RBCs exists in solution, and the average distance, \( d \), between two adjacent cells is much larger than the diameter of the cell; 3b) the transient diffusion time \( t^* \) (the time required for the diffusion to reach steady state) for NO is much shorter than the half-life of NO in the measured solution; and 4) reactions of NO with other compounds such as methemoglobin and O₂ can be ignored because they are much slower than the reaction of NO with oxyHb. Assumption 2 is based on experimental observations on oxygen uptake by RBCs (16). The transient diffusion time \( t^* \) is about 10 ms for oxygen diffusion into RBCs (14), and so we assume that it is also short for NO and assumption 3b holds.

With these assumptions, the diffusion problem can be described by the following equation,

\[ \frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2} = 0 \]

\[ c = c^s \text{ on the surface of a cell} \quad (Eq. 2) \]

\[ c = c^b \text{ as } \sqrt{x^2 + y^2 + z^2} \rightarrow \infty \]

where \( c \) is the concentration of NO at location \( x, y, z \) and \( c^b \) is NO concentration in the bulk solution. In our experiments, \( c^b \) decreases with time, but its half-life is much longer than the transient diffusion time \( t^* \) according to assumption 4.

By analogy with the diffusion problem for an electrode (17), the diffusion rate of NO into the RBC can be expressed by the following equation,

\[ v = D_{NO} S \left( \frac{\partial c}{\partial x} \right)_0 = g D_{NO} (c^b - c^s) S^{1/2} \quad (Eq. 3) \]

where \( v \) is the rate (in mols/s) that NO diffuses to the surface of a RBC, \( D_{NO} \) is the diffusion constant for NO, \( S \) is the surface area of the cell, \( g \) is a constant for a given cell shape (i.e., spherical, discoidal, etc.), and \( (ic/\pi a_0) \) is the concentration gradient at the cell surface. For simplicity, we assume that the shape of the RBC is spherical; we note that experimental data with measurements of NO binding to deoxyhemoglobin within RBCs (which, as described under “Discussion,” is a phenomenon similar in principle to what we measure here) show that changing the shape of the RBC from discoidal to spherical has no measurable effect on the rate of the reaction (18). The RBC radius (average radius for all cells) and surface area are \( r \) and \( S = 4 \pi r^2 \), respectively. The factor \( g \) for a spherical cell is \( (4 \pi r)^{1/2} \) (19). Substituting these values into Equation 3 gives the following equation.

\[ v = D_{NO} S \left( \frac{\partial c}{\partial x} \right)_0 = 4 \pi D_{NO} (c^b - c^s) r \quad (Eq. 4) \]

From mass conservation, the rate of NO diffusion to the surface of the cell from the bulk solution, must be equal to the rate of NO diffusion through the cell membrane,

\[ v = 4 \pi D_{NO} (c^b - c^s) r = 4 \pi r k_m c^s \quad (Eq. 5) \]

where \( k_m \) is the rate constant of the mass transfer for NO diffusion through the cell membrane. The findings from Equation 5 are as follows.

\[ c^s = \frac{D c^b}{D + r k_m} \quad (Eq. 6) \]
Substitution of Equation 6 into Equation 4 gives the following expression.

\[ v = 4\pi D_{NO}c_b \frac{r_{km}}{D_{NO} + r_{km}} \]  

(Eq. 7)

If \( k_{km} \) is large enough, or \( r_{km} \gg D_{NO} \) as we assumed in the beginning of this section, Equation 7 can be converted to the following.

\[ v = 4\pi D_{NO}c_b \]  

(Eq. 8)

Assuming the number of RBCs is \( N \) per cm\(^3\) (in units of cm\(^{-3}\)), the total disappearance rate of NO in the measured solution is as follows.

\[ dc_b/dt = -4N\pi D_{NO}c_b \]  

(Eq. 9)

Equation 9 shows that the disappearance rate of NO in the bulk solution is first order in NO concentration \( c_b \). The following is the first order rate constant.

\[ k_{NO} = 4\pi D_{NO} \]  

(Eq. 10)

Solving Equation 9, we find the follow expression.

\[ \ln(c_b/c_{b0}) = -4N\pi D_{NO}t = -k_{NO}t \]  

(Eq. 11)

The half-life, \( t_{1/2} \), which can be obtained from Equation 11.

\[ t_{1/2} = (ln2)/k_{NO} = (ln2)/4\pi D_{NO} \]  

(Eq. 12)

If \( N = 2.1 \times 10^9 \) RBCs/cm\(^3\), \( D_{NO} = 3.3 \times 10^{-5} \) cm\(^2\)/s (2), and \( r = 2.44 \times 10^{-4} \) cm (20), we have the rate constant \( k_{NO} = 0.212 \) s\(^{-1}\) and the half-life \( t_{1/2} = 3.3 \) s. The experimentally determined values were \( k_{NO} \approx 0.167 \) s\(^{-1}\) and \( t_{1/2} \approx 4.2 \) s (Table 1). Thus, the model accurately predicts the experimental results.

Combining Equation 1 with Equation 4 we have, 

\[ v = D/r \]  

(Eq. 13)

where \( r \) is the radius of the spherical cell. This is similar in form to the equation presented previously (14) for O\(_2\).

**DISCUSSION**

By using an NO-selective electrochemical sensor, we have found that the reaction of NO with oxyHb to produce methemoglobin and nitrate is slowed substantially when the hemoglobin is contained within RBCs. From Table I, we can calculate that the average first order rate constant for the reaction of NO with RBCs is \( 7.74 \times 10^{-8} \) s\(^{-1}\) on a per RBC/ml basis (i.e. the rate of NO disappearance in a volume containing 1 RBC/ml). Using our data in Figs. 2 and 3 and assuming an average rat RBC volume of 59.7 \( \mu \)m\(^3\) (21), the rate becomes \( 5.16 \times 10^4 \) s\(^{-1}\) on a per molar heme basis. Comparison of this number with that determined by Eich et al. (5) reveals that the reaction with free oxyHb is slowed by a factor of approximately 650 when the oxyHb is contained within RBCs. That the reaction with RBCs is not occurring via a small amount of hemoglobin released from a few lysed RBCs is ruled out because the concentration of free oxyHb required to yield a half-life of NO of 6–12 s (Fig. 2) would be 1.7–3.4 nM (based on the value for \( k \) reported by Eich et al. (5)), far too small an amount to react with 0.9 \( \mu \)M NO (Fig. 2). We point out that although this reaction is greatly retarded relative to free oxyHb, the value for the half-life of NO in normal blood (5 \( \times \) 10\(^8\) RBCs/ml (22)) would still be very short, less than 2 ms. This makes the blood vessel lumen a potent sink for free NO and poses a difficulty in postulating that only free NO accounts for the actions of the endothelium-derived relaxing factor, as we have pointed out previously (6, 7).

We also present a theoretical model for predicting the diffusion-limited reaction of NO, based on the similarity of this phenomenon to diffusion-limited reaction at an electrode (17). We predict from this model that on a per RBC/ml basis the rate of NO disappearance will be given by the following expression.

\[ k_{NO}/N = 4\pi D_{NO} \]  

(Eq. 14)

Using the values \( D_{NO} = 3.3 \times 10^{-5} \) cm\(^2\)/s (2) and \( r = 2.44 \times 10^{-4} \) cm (20), we arrive at a value for \( k_{NO}/N \) of 1.01 \( \times \) 10\(^{-7} \) s\(^{-1}\), which compares favorably to our experimental value (0.774 \( \times \) 10\(^{-7} \) s\(^{-1}\)).

Hakim et al. (23) reported that the half-life of NO in the presence of oxyHb (1.55 \( \mu \)M) is 11.5 s. They used a probe similar to the Clark-type sensor for oxygen. This probe is impressively sensitive to NO (as low as 10 nM), but its response time to concentration change of NO is slow, 2.2–3.5 s for 75% rise time at an NO concentration of 1 \( \mu \)M (24). The working electrode does not directly contact the measured solution. NO must pass through the membrane from the measured solution and then diffuse across the inner solution before it reaches the surface of the working electrode. NO is homogeneously distributed throughout the inner solution of the electrode system because of diffusion. If we add oxyHb to the measured solution containing NO, oxyHb will remove all NO in the measured solution immediately. However, oxyHb cannot directly react with NO in the inner solution because oxyHb entry into the inner solution is prevented by the membrane. NO concentration in the inner solution will still decrease with time because NO will both diffuse out of the membrane and also be oxidized at the electrode. The disappearance rate of NO in the inner solution will thus depend on the surface area of the electrode and the volume of inner solution. We feel that the curves recorded by Hakim et al. (23) in the presence of higher oxyHb concentration are not the disappearance rate of NO concentration in the measured solution, which is too fast to be recorded by their electrode, but rather the disappearance rate of NO in the inner solution after NO in the measured solution was removed by oxyHb. Our working electrode directly touches the measured solution, so its response time to a concentration change is much faster (~0.3 s) than the electrode Hakim et al. (23) used.

Our experimental data and theoretical analysis imply that if a solution contains oxyHb and we add NO to the solution, the rate of NO disappearance is dependent on the distribution of oxyHb in the solution. If oxyHb concentration is uniform in solution, the rate of NO disappearance will be greater than if the same total amount of oxyHb is contained within discrete membrane-enclosed sites in the solution, for example, located within cells. Our results explain the observation that enclosure of hemoglobin within erythrocytes attenuates its hypertensive actions (25, 26).

Scavenging of NO is believed to be the major explanation for the hypertensive effect of acellular hemoglobin, which is under active investigation as a potential artificial red blood substitute (27, 28). Indeed, this is one reason why nitrosation of a cysteine residue in hemoglobin has been proposed as an important phenomenon in endothelium-derived relaxing factor-dependent vasodilation (29, 30); by this mechanism nitrosonium equivalents can be carried by hemoglobin, thus apparently avoiding the potent scavenging activity of oxyHb for free NO (7). It is known that encapsulating Hb within liposomes greatly attenuates its hypertensive effect (as well as increasing its lifetime in the circulation) (31, 32). This effect is generally attributed to an increased scavenging of NO by free Hb resulting from tissue extravasation (33). According to our results, even without tissue extravasation, free hemoglobin will have a more than 500-fold higher scavenging ability for NO than the same amount of hemoglobin contained within RBCs. Our data
explain the observation that polymerization of diaspirin cross-linked hemoglobin (34), undertaken to prevent movement into the interstitial space, does not improve its hemodynamic properties; we postulate that hemoglobin must be enclosed within a membrane or diffusion barrier (such as in the RBC) to prevent hypertension.

Rudolph et al. (35) recently showed that liposome encapsulation of Hb decreases its vasconstrictive activity by 30–60 fold in rabbit arterial segments. Data from stopped-flow spectrophotometric measurements were interpreted as suggesting that encapsulation did not retard NO reaction with oxyHb, contrary to our conclusions here. However, under their conditions (0.96 mM NO, concentration which is in excess over the Hb concentration) it can be calculated that the half-life for this pseudo-first order reaction will be 0.02 ms ($k = 3.4 \times 10^7 \text{M}^{-1} \text{s}^{-1}$). With an instrumental dead time of 2 ms, it is highly doubtful that either this reaction or even the slower reaction with encapsulated Hb would have been observed, which is in fact what was reported (i.e. the rate in both cases was too fast for detection). A slower subsequent spectrophotometric change and then gradual decrease (presumably because of the binding of NO by the Fe(III)-heme followed by nitrosative hydrolysis (36, 37)) was detectable and was similar in rate for both acellular and free Hb. However, as we point out above, the effects of encapsulation will only be manifested if the rate of reaction within the cell or liposome is more rapid than diffusion into the cell or liposome, which will be true for reaction with oxyferro-hemoglobin but not reaction with methemoglobin. Our results here suggest that the antihypertensive effects of liposome encapsulation of Hb are because of a diminution of NO scavenging as a consequence of the diffusional limitation of NO reaction with oxyHb and lend further support to the utility of encapsulation in ongoing research to design effective hemoglobin-based blood substitutes (38).

In relatively hypoxic regions, there will be appreciable amounts of deoxyhemoglobin present within RBCs, and NO also reacts with deoxyHb, forming a heme iron-nitrosyl complex (39, 40). The rate of this reaction is very rapid, and in fact its magnitude ($k = 2.2 \times 10^7 \text{M}^{-1} \text{s}^{-1}$) is similar to the reaction of NO with oxyHb ($k = 3.4 \times 10^7 \text{M}^{-1} \text{s}^{-1}$) (5). In fact, earlier work by Carlsen and Comroe (18) showed that reaction of NO with deoxyHb within intact RBCs is, like reaction with oxyHb, so rapid that it is limited only by how rapidly the NO can become accessible to the hemoglobin within the RBC. Their rate constant for this reaction was $1.15 \times 10^9 \text{M}^{-1} \text{ s}^{-1}$, which compares favorably to the rate we report here (0.52 $\times 10^9 \text{M}^{-1} \text{ s}^{-1}$), indicating the diffusion into the cell is limiting for both reactions. This means that formation of NOHb can compete with NO reaction with oxyHb, which explains why the electron paramagnetic resonance signal from NOHb has been observed in whole blood from septic animals (41) and why its magnitude is greater in venous compared with arterial blood (42).

In the body, hemoglobin is confined within RBCs and RBCs are found within blood vessels. Thus, the half-life of NO in the spaces between blood vessels will be dependent on the distribution of blood vessels and especially on the distribution density of capillaries (7). This means that the half-life of NO in vivo cannot be specified by a single number but will be different at different locations, depending on vascularity.

REFERENCES

1. Lancaster, J. R., Jr. (1992) Am. Sci. 80, 248–259
2. Malinski, T., Taha, Z., Grunfeld, S., Patton, S., Kapturczak, M., and Tomaszewicz, P. (1993) Biochem. Biophys. Res. Commun. 193, 1076–1082
3. Meulemans, A. (1994) Neuropsy. Lett. 171, 89–93
4. Shaw, A. W., and Vesper, A. J. (1977) J. Chem. Soc. Faraday Trans. 1 8, 1239–1244
5. Kuch, R. F., Li, T., Lemon, D. D., Doherty, D. H., Curry, S. R., Alten, J. F., Mathews, A. J., Johnson, K. A., Smith, R. D., Phillips, G. N., and Olson, J. S. (1996) Biochemistry 35, 6976–6983
6. Lancaster, J. R., Jr. (1997) Nitric Oxide 1, 18–30
7. Lancaster, J. R., Jr. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8137–8141
8. Hartridge, H., and Roughton, F. J. W. (1927) J. Physiol. (Lond.) 62, 232–242
9. Sandoval, M., Liu, X., Mannick, E. E., Clark, D. A., and Miller, M. J. S. (1997) Am. J. Physiol. 272, C1400–C1405