Editorial

One of the most exciting medical discoveries of the 1980s in the study of human physiology was the realization that nitric oxide (NO) or a derivative is the potent "endothelial-derived relaxing factor" of smooth muscle and has multifold functions in the body (1). NO is a gas with one unpaired electron and thus is a free radical ($NO^{\bullet}$) that reacts with many biological molecules. It has important roles in the function of many tissues and organs, from the cardiovascular system to the brain, and its physiology and biochemistry have been discussed in more than 10,000 papers since 1990. NO is being studied currently as a possible therapeutic agent for several disease processes. Because of the difficulties of administering a gas chronically, much attention has been focused on the use of NO donor compounds, such as L-arginine, or on the use of inhibitors of NO metabolism, such as L-NAME (N^{G}–nitro-L-arginine-methylester). More recently, the possibility of direct and chronic administration of NO gas in certain patients has been explored, generally in cooperation with anesthesiologists. Encouraging results have been obtained recently with NO inhalation therapy for hypoxic respiratory failure and pulmonary hypertension, especially in infants (2, 3).

In this issue of the *Journal*, Head et al. (4) report on the properties of red cells from sickle cell anemia patients (SS cells) equilibrated in vitro with low concentrations of NO and from patients breathing NO at 80 ppm for up to 1 h. The investigators found that NO treatment of SS cells, by either method, increased the mean oxygen affinity of the cells by about 15% (p50 is lowered about 5 mmHg, from the values around 33 mmHg characteristic of SS cells). Surprisingly, no effect was seen on the oxygen affinity of normal (AA) cells exposed to NO under the same in vitro conditions: P50 remained about 27 mmHg. The authors suggest that increasing the oxygen affinity of SS cells in sickle cell anemia patients by NO administration might be useful as a therapy for this disease.

The editorialist clearly has to deal with three questions with regard to this report: Will it work? Is it safe? and What is happening? Unfortunately we do not have full answers to any of these questions yet.

It is clear that these investigators are able to achieve a transient increase in the mean oxygen affinity of their patients' cells, an effect that generally has been thought to be of possible benefit for sickle cell anemia patients. Indeed, at any given pO_2 at equilibrium, there will be less polymer in SS erythrocytes and less cell sickling as oxygen affinity is increased (5). Such an effect was the main one seen in the extensive studies with potassium cyanate therapy two decades ago; interestingly, we are still not certain whether this increase in oxygen affinity would be beneficial. If tissues need a constant amount of oxygen for their metabolic functions, then one would expect tissue pO_2 to fall to much lower levels so that NO-treated cells could deliver the necessary oxygen. The unmodified sickle hemoglobin molecules in any population of treated SS cells, or the unmodified cells if the NO is delivered intermittently to the patient, would undergo extra desaturation to achieve the requisite oxygen delivery. This extra desaturation would cause these molecules or cells to have higher percentages of sickle hemoglobin polymer than the modified ones and would possibly increase the pathological processes in the microcirculation initiated by SS cells with intracellular polymer. Another potential difficulty of this proposed therapy may be a deleterious increased blood viscosity due to a secondary increase in red cell mass from increasing oxygen affinity and tissue hypoxia.

In other words, whereas current understanding of SS cell pathophysiology strongly suggests that reducing the intracellular concentration of sickle hemoglobin or reducing the intrinsic tendency of the hemoglobin molecules to polymerize (two effects achieved currently by hydroxyurea therapy) will be beneficial in lowering the amount of polymer at any oxygen saturation, the effect of direct changes in hemoglobin oxygen affinity are much more difficult to predict. Obviously, prospective controlled clinical studies of NO efficacy will be necessary to see if the treatment described in this report does have clinical benefit.

In addition, administration of NO raises significant safety concerns. Very high concentrations of NO lead to pulmonary edema and methemoglobinemia and may be lethal (6). Indeed, it is of some surprise that methemoglobin production from exposure of oxy-hemoglobin to NO was not more of a problem in the studies reported by Head et al. Perhaps the low concentrations of gas used and the brief administration time allowed red cell methemoglobin reductase to efficiently remove this byproduct of NO inhalation. These concerns, as well as the lack of proof of efficacy, mandate that NO inhalation be considered highly experimental at this time.

The greatest value of this report may be in focusing further attention on the role of NO binding (or liganding) in the function of normal and sickle hemoglobin. In the last two years a fascinating story has emerged from the laboratory of Stamler and his colleagues at Duke University (7, 8). They have presented evidence of the formation of S-nitrosothiols (RSNOs) in the red cell that have important roles in the regulation of vascular tone through linkage with the hemoglobin oxygenation–induced allosteric structural transitions. In their model, hemoglobin is S-nitrosylated in the lung, at the time of oxygen binding, on the evolutionarily conserved Cys β93 residues. In the tissues, as oxygen is lost from the hemoglobin tetramers, the SNO linkage is also broken and the NO group transfers to the blood vessel wall where it relaxes smooth muscle tissues to cause vasodilatation. Thus a new homeostatic mechanism is proposed in conjunction with the already subtly modulated (by protons and diphosphoglyceric acid) functions of hemoglobin. In this case, as tissue oxygen consumption increases, the hemoglobin effectively senses this and releases NO molecules that dilate the terminal arterioles, facilitating the arrival of more red cells and more oxygen.

Although there are many data that support this elegant model, it has yet to be worked out in detail. For example, it is

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likely that much of the transport and reactivity of NO is mediated by binding to low molecular weight thiol compounds, such as glutathione and cysteine, but the relative contributions of these reactions are not yet known. Further, these S-nitrosylation reactions are in competition with the long-known nitrosylation of the heme iron atoms themselves. How these two mechanisms, by which NO may affect hemoglobin function, interact is not clear; their rates, for example, depend on whether the hemoglobin is oxygenated or deoxygenated. Despite these uncertainties, it seems likely that S-nitrosohemoglobin is an important molecule in tissue oxygen delivery, and it is expected that much will be learned about its metabolism and roles in human microcirculatory physiology in the near future.

These new findings on S-nitrosohemoglobin are relevant to several aspects of the findings of Head et al. It is known that modification of the Cys β93 can directly increase the solubility of deoxy-sickle cell hemoglobin, and inhibit polymerization (9). Modifications of this residue by nitrosylation might have resulted in the increase in the oxygen affinity of the SS cells by an indirect mechanism due to the thermodynamic linkage of deoxygenation and polymerization but would not have affected the oxygen affinity of AA cells, as was observed. Increased formation of S-nitrosohemoglobin because of inhalation of NO might also cause increased vasodilatation in the peripheral tissues from increased delivery of NO as red cells lose their oxygen in the small arteries and terminal arterioles. This might be of particular benefit in sickle cell patients, where obstruction to flow in the arterioles is likely (5), and cause clinical benefit independent of direct or indirect effects on oxygen affinity. Clearly, many new questions are raised by these results of recent studies of NO on normal hemoglobin and red cell physiology. We will need to clarify how the effects on sickle hemoglobin, SS cells, and the vasculature of sickle cell patients may differ from those of individuals with AA cells.

Hemoglobin research, and much of physiology itself, has frequently been pronounced moribund or dead during the last few decades in contrast to the advances of molecular genetics. NO, which was "Molecule of the Year" of *Science* magazine in 1992, points us in new directions that may soon lead to novel therapies. The implications for better understanding hemoglobin function, including the possibility of developing more physiological blood substitutes, are enormous. Research scientists always need to remain humble in the realization that the evolutionarily-developed "wisdom of the body," in the words of E.H. Starling and W.B. Cannon (10),[1] is likely to be greater than that of our best representations of even well-studied biological mechanisms.

Alan N. Schechter
Laboratory of Chemical Biology
National Institute of Diabetes and Digestive
and Kidney Diseases
National Institutes of Health

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1. The discovery of S-nitrosohemoglobin appears to answer, at least partially, a problem posed 60 yr ago by Cannon: "The dilation of the arterioles and capillaries in active muscles is one of the most remarkable emergency adjustments . . . What causes the capillaries to dilate is not yet understood . . . However the capillaries may be opened, the great importance of their being opened should not be overlooked" (161–162).