Hemoglobin Reveals New Role As Blood Pressure Regulator

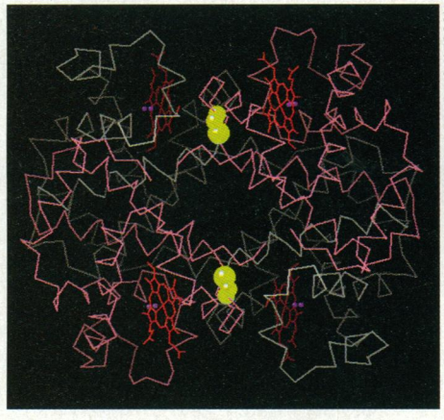
Hemoglobin may have been leading a double life, right under biochemists' noses. This familiar constituent of red blood cells, which delivers oxygen to tissues and retrieves carbon dioxide, is probably the most studied protein in existence. But a group led by Jonathan Stamler at the Duke University Medical Center reports in yesterday's issue of *Nature* that, alongside the familiar respiratory cycle, hemoglobin carries out a second cycle in which it sops up a form of nitric oxide (NO) in the lungs and releases it in blood vessels—a shuttle service that helps stabilize blood pressure. "Just when we thought we knew everything about hemoglobin, we've discovered something new," says co-author Joseph Bonaventura. "It's gratifying and exciting."

The results connect two molecules, NO and hemoglobin, that had been seen as biochemical enemies. NO, produced in the endothelial cells that line the blood vessels, relaxes the muscles surrounding the vessels and thereby controls blood pressure, among many other functions (*Science*, 18 December 1992, p. 1862). But other research had suggested that it can only do so if it avoids hemoglobin, whose iron-containing heme groups destroy it. "Everybody has looked at hemoglobin as a scavenger of NO," agrees Robert Furchgott at the Health Sciences Center of the State University of New York, Brooklyn. The new results, reported by Li Jia, Stamler, and Celia Bonaventura and Joseph Bonaventura of Duke's Nicholas School of the Environment and the medical center, "are entirely contrary to the dogma in the field," says Stamler.

The finding that hemoglobin can carry a form of NO, releasing it at crucial moments, is "provocative, interesting, and exciting," says Robert Winslow of the University of California, San Diego (UCSD), Medical School. He adds that the finding could be a boon for efforts to turn cell-free hemoglobin solutions into workable blood substitutes; in past tests, patients given such solutions often suffered dangerous rises in blood pressure. "It may be that this will open a new line of research to solve this problem," says Winslow.

The experiments build on Stamler's earlier research on compounds called S-nitrosothiols, or SNOs, which act as souped-up versions of the NO molecule, enhancing its physiological effects. SNOs form in the body when oxidized NO reacts with the highly reactive thiol group—a sulfur and a hydrogen—on the amino acid cysteine. Cysteines are common on proteins, including hemoglobin, which has a pair of them. Their function on hemoglobin "has been a mystery," says Joseph Bonaventura. But the Duke team suspected that SNOs might form on hemoglobin, perhaps when other, smaller SNOs in the bloodstream in effect hand off their NO to the pair of cysteines.

To test the idea, the team incubated hemoglobin in a bath of SNOs. Standard biochemical lore predicted that NO in any form would rapidly react with oxygenated heme groups, inactivating the NO and leaving a positive charge on the hemoglobin. But for SNO, the Duke group found, the outcome was different. SNOs rapidly formed on the hemoglobin's two cysteines, forming SNO-hemoglobin. The free SNOs "were transferring NO groups to cysteine without touching the hemes," says Stamler.



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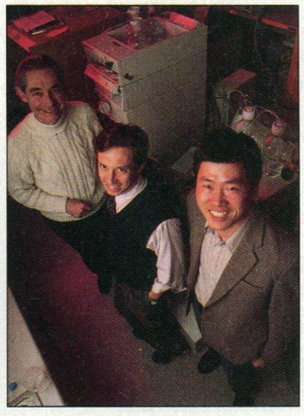

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The answer is NO. Duke researchers Joseph Bonaventura, Jonathan Stamler, and Li Jia *(left to right)* wondered what might bind to two cysteines *(yellow)* on hemoglobin.

To see whether this effect actually occurs in the blood vessels and can influence their behavior, the team placed chemical "caps" on parts of cell-free hemoglobin molecules, selectively blocking either the hemes or the cysteine thiols or leaving both sites free. They then exposed sections of rat artery to the modified hemoglobin and monitored the artery's response. The blood vessel constricted in response to all three hemoglobin preparations. But the constriction was greatest when neither the cysteines nor the heme were capped. That implied, says Stamler, that the bare hemoglobin was consuming NO, by both binding it at the cysteines and destroying it at the heme.

"The next question was," says Stamler, "If it occurred in isolated blood vessels, can it occur in the body?" The answer seemed to be yes. The team monitored the levels of SNO bound to hemoglobin in red blood cells collected at various points in rats' circulation. They found high levels of SNO-hemoglobin in red blood cells on the left side of rats' hearts—where blood has just been oxygenated in the lungs—and low values on the right side, where blood returns from the body. That suggested, says Stamler, that hemoglobin was taking on SNO in the lungs as it was oxygenated and delivering the SNO to the tissue in concert with oxygen release. To confirm that this delivery really does affect blood pressure, the team then injected rats with SNO-hemoglobin. While native hemoglobin, lacking SNO, causes rats' blood pressure to rise, it remained rock-steady when the SNO-hemoglobin went in.

Based on biochemical measurements and molecular-dynamics computations, the Duke researchers argue that this regulatory effect reflects the same kind of shape, or allosteric, changes that choreograph hemoglobin's familiar functions. Hemoglobin gives up its oxygen and sops up carbon dioxide when, upon reaching oxygen-poor tissues, it undergoes an allosteric change that reduces its affinity for oxygen. The same conformational change also releases SNO, says Stamler. The reverse effect takes place in the lungs, ensuring that hemoglobin takes on SNO at the same time as it binds oxygen and blows off carbon dioxide.

The Duke researchers also found another trigger for SNO release. Each time hemoglobin scavenges an NO in the bloodstream in the standard fashion, the positive charge left on the heme signals to the cysteine residue to release its SNO. "The moment [hemoglobin] gets hit by NO, it gives up one," maintaining a balance and keeping the vessels open, Stamler speculates.

In addition to this surprising insight into hemoglobin's regulatory roles, Vijay Sharma of UCSD notes that the work "could have a tremendous significance for blood substitutes." Simply infusing SNO-hemoglobin and not the SNO-less version, he speculates, might eliminate the blood pressure surges these preparations are notorious for causing. Still, says Sharma, "the devil is in the details," and he, like other researchers, is eager to see all the incriminating details before accepting the new picture of hemoglobin's double life.

–James Glanz