DURHAM, N.C. – Science’s understanding of the evolution and role of hemoglobin, considered one of the most studied and best-understood molecules in nature, is being rewritten with the help of a common intestinal parasite that infects more than 1 billion people worldwide.

A team led by Dr. Jonathan Stamler, a Howard Hughes Medical Institute investigator at Duke University Medical Center, believes that the hemoglobin molecule found in the gut of Ascaris lumbriocoides is a remnant of a crucial evolutionary junction in which anaerobic life forms, like bacteria, separated from newly emerging aerobic organisms, such as humans. The worm, in short, reveals that hemoglobin evolved first and foremost to handle the molecule nitric oxide (NO) rather than oxygen, as scientists thought, and tells the tale of when hemoglobin ceased being a “consumer” of oxygen and became a “carrier” of oxygen, Stamler said.

In a report published in the Sept. 30 issue of the journal Nature, Stamler and collaborators from Washington University in St. Louis provide biochemical proof to support this conclusion. They show that the worm actually consumes oxygen – which it finds toxic – with the help of NO. The discovery may yield new therapies for diseases such as cancer, in which starving tumors of oxygen is a major therapeutic focus, the researchers said.

The research was supported by grants from the National Institutes of Health and the Howard Hughes Medical Institute (HHMI).

“Both structurally and functionally, Ascaris hemoglobin is a link between bacterial hemoglobin and mammalian hemoglobin,” said Washington University cell biologist Dena Minning, who, along with Duke’s Andrew Gow, are co-first authors of the paper.

“Hemoglobin in bacteria is used as an enzyme to destroy NO – the ‘primordial gas’ which evolved before there was oxygen and is toxic in high amounts – while in mammals, hemoglobin carries both oxygen and NO, using the NO to ensure oxygen delivery by dilating blood vessels,” Minning said. “But in the Ascaris worm, NO is used to remove oxygen. Thus
Ascaris hemoglobin is for the first time regulating the oxygen in its environment, although in
this case it is getting rid of it.

“From the standpoint of hemoglobin biology, this finding represents a new function and
a novel mechanism for Ascaris hemoglobin,” she said. “In bacteria, hemoglobin’s function is
detoxification, while in mammals, its function is respiration. In Ascaris, at the evolutionary
divide, we see the evolution of the ‘functional switch’ – respiratory function (control of oxygen)
and the detoxification function (removal of oxygen) are the same. And all is controlled by
NO.”

Minning’s work in Ascaris biology was conducted in the laboratory of Dr. Daniel
Goldberg, a Washington University microbiologist and HHMI investigator.

The keys to hemoglobin’s evolution, the researchers believe, are the chemical
reactions that take place between hemoglobin, oxygen and NO, a ubiquitous chemical
involved in many life processes. These findings suggest that contrary to commonly held
beliefs, hemoglobin has evolved over millions of years in response to NO, and not oxygen.

“More than a billion years ago, the atmosphere on Earth contained NO, and not
oxygen,” Stamler explained. “So the early development of hemoglobin in bacteria and other
microbes could not have been for the delivery of oxygen, but instead was for the detoxification
of NO. On the evolutionary tree, the Ascaris worm sits right at the point where bacteria
branches off one way and man in another,” he said.

“We’ve known that hemoglobin in Ascaris binds oxygen 25,000 times more tightly than
human hemoglobin – the tightest that oxygen is held by any molecule,” Minning said. “It’s
been a mystery why a hemoglobin that is supposed to deliver oxygen would bind it so tightly
that oxygen could never be released, a mystery that has baffled scientists for more than 50
years. Now we know that this oxygen can be removed through a novel enzymatic reaction
with NO.”

“In the beginning, oxygen was toxic to early plants and worms and even late bacteria,”
Stamler said. “Early hemoglobin would bind tightly to the oxygen and consume it, acting as a
detoxifier. However, in man, hemoglobin is the molecule that carries oxygen. We know what
the earliest hemoglobin did, and we know what it does now in man.

“The Ascaris hemoglobin, in the presence of NO, consumes oxygen, creating the
hypoxic, or oxygen-free environment, which it needs to live,” Stamler said. “By doing so, the
Ascaris hemoglobin is really acting like a new enzyme, or a deoxygenase, using NO to
detoxify the oxygen around it.”

It appears that structural differences have evolved in the Ascaris and human
hemoglobin molecules that account for their different capabilities. The actual location on the
molecule where the chemical reactions take place differ in the two hemoglobins, but they both employ a residue called cysteine that enables hemoglobin to actively use NO.

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“"In the *Ascaris* hemoglobin, the cysteine carrying NO is at the front, next to the oxygen, which leads to deoxygenation; and in humans, it is located at the back, away from the oxygen,” Stamler said. “Since the gases are carried on opposite sides of the molecule, they do not react with each other, allowing for the release of NO and transport of oxygen. The primordial function of detoxification has transformed into a respiratory function in man.”

While it appears that *Ascaris* hemoglobin is primarily involved in the canceling out of oxygen, the Duke and Washington University research also points to another function – protecting the worm from NO that is present in the host gut or produced by the host’s immune system.

“This ability to metabolize NO is very similar to the hemoglobin of early bacteria, but the *Ascaris* may not be able to do it quite as efficiently, and does so through a different chemical reaction,” Stamler said.

The scientific world is beginning to recognize the importance of NO in the evolution and functions of hemoglobin. In a commentary in the Aug. 31 issue of the *Proceedings of the National Academy of Sciences*, Drs. Steven Gross and Paul Lane of Cornell commented on the new scientific view brought about in large part by a series of publications over the past three years from Stamler and his colleagues. These articles have been laying the groundwork for challenging accepted beliefs about hemoglobin and have revealed entirely new functions.

“This view would be consistent with the evolutionary appearance of simple bacterial hemoglobin at a time when the Earth’s early atmosphere was anoxic, but perhaps life-threatening in its NO content,” Gross and Lane wrote. “Thus, ancestral hemoglobin may have initially functioned to detoxify NO and subsequently evolved toward a molecule that is optimized for oxygen delivery, permitting the evolution of large multicellular life forms. If so, we may owe our very existence to evolutionary pressure imposed by an NO-rich environment.”

These new insights into hemoglobin and NO could have profound effects on the development of drugs or compounds used in a wide variety of medical conditions, including, heart disease, shock, cancer and arthritis, Stamler said.

Also part of the research were, from Duke, Joseph Bonaventura, Ph.D., Rod Braun and Mark Dewhirst.

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