The Start of Something Good: The Discovery of HbA_{1c} and the American Diabetes Association Samuel Rahbar Outstanding Discovery Award

Sometimes, a scientific achievement merits its own prize. The American Diabetes Association (ADA) acknowledged Samuel Rahbar, MD, PhD, with just such an honor—the Samuel Rahbar Outstanding Discovery Award—for a contribution to the study and treatment of diabetes that resonates to this day.

Rahbar is the person who discovered that HbA_{1c} is elevated in people with diabetes. This breakthrough came in 1968 and was not immediately appreciated broadly, but over the next few decades HbA_{1c} became arguably the most important indicator of blood glucose control, enabling doctor and patient to, for the first time, critically assess the impact of lifestyle changes and medication on long-term health (Table 1).

Hemoglobin fever—Rahbar was born in 1929 in Tehran the youngest of seven children. "My mother was a teacher at a French school," he says. "My father had a small shop selling fabrics, but my mother was the pillar of our house. We were all educated." Rahbar became highly educated indeed, receiving both a medical degree and a doctorate at the University of Tehran. He stayed there for his postdoctoral studies as well.

By then, it was the 1960s, and hemoglobin was the rising star of molecular biology. Rahbar started his fellowship in 1962 with plans to study immunoglobulins. However, shortly into his fellowship, he became interested in protein structure and "discovered hemoglobin was the molecule du jour," he says.

Not only was hemoglobin easy to come by (making up 97% of erythrocytes' dry weight) but in 1960, it became one of the first proteins to have its structure solved (1). That gave researchers unprecedented insight into the connection between a protein's structure and its function. Linus Pauling, working on the protein from another angle, discovered that the hemoglobin of people with sickle cell anemia (HbS) was structurally different from that of healthy individuals. Sickle cell anemia became "one of the first examples of a genetic disease," says Anthony Cerami, PhD, who contributed to the development of HbA_{1c} as a clinical marker. The discovery of HbS set off a race to unearth other hemoglobin variants.

Rahbar caught hemoglobin-variant fever on a trip to Israel early in his postgraduate years. His brother was being treated for blood cancer at an Israeli hospital and, coincidentally, a researcher Rahbar admired, Hermann Lehmann, was visiting from Cambridge and giving a lecture.

Rahbar attended the lecture and, afterward, talked with Lehmann, who invited Rahbar to spend a few summers at Cambridge studying hemoglobin variants. Rahbar eagerly accepted and learned he was in a unique position to glean hemoglobin's secrets. Lehmann considered Iran an ideal place to study hemoglobin variants because of its ethnic populations. As early human populations passed from the Far East to Europe, Rahbar says, some stayed in Iran and "some of those tribes remained distinct. Lehmann encouraged me to establish a research unit at the University of Tehran. He believed that Iran was the best place to do genetic work." Thus, based on the advice of his mentor, Rahbar returned to Iran to establish such a research program. Rahbar hoped to find novel hemoglobin variants hidden in the blood of his compatriots.

Gearing up—The tool of choice for analyzing hemoglobin variants at the time was electrophoresis. Hemoglobin A is the most abundant type of hemoglobin, but there are hundreds of other types, says Cerami. Uncovering minor populations required separating structurally similar molecules from one another with electrophoresis. "As people developed better and better methods, they discovered that even healthy individuals have hemoglobin [subpopulations]," says Cerami.

In 1963, Graham and Grunbaum (2) introduced a new faster electrophoresis method using a cellulose acetate membrane that drew Rahbar's attention because he wanted to increase his throughput. The more blood he screened, the more new variants he could find. On the very day he read about the new technique, Rahbar built an electrophoretic cell that could run eight samples at a time, on a single 5-cm membrane, in just 20 min.

Within a few months, Rahbar and his two technicians were studying the blood of Iranians with gusto. "At six o'clock in the morning, someone would go with a motorcycle to pick up 300 small tubes of blood from the [Tehran University Hospitals]. I used to take their discarded blood samples," he recalls. "I was screening 300 blood samples a day, and the lab was running like a factory."

Another key to Rahbar's speed, he says, was a technique he developed that simplified the extraction of hemoglobin from blood. They briefly dipped Whatman 3M filter paper, cut into tapered strips, into blood samples and then allowed the paper to dry. Over ~30 min, the unwanted plasma proteins migrated away from the blood cells, which stayed on the tip of the paper. The researchers then dipped the tip into lysing reagents, freeing the hemoglobin, and applied the

Table 1—*HbA_{1c}: a history*

1966: Holmquist and Schroeder identify five subtypes of hemoglobin A, including HbA_{1c}.
1968: Rahbar recognizes that HbA_{1c} is elevated in people with diabetes.
1975: Koenig and Cerami suggest that HbA_{1c} is related to metabolic control.
1993: DCCT establishes HbA_{1c} as a valuable clinical marker in people with type 1 diabetes.
1998: UKPDS establishes HbA_{1c} as a valuable clinical marker in people with type 2 diabetes.
2010: ADA recommends using the HbA_{1c} test to diagnose diabetes and prediabetes.

sample to the cellulose acetate membrane for electrophoresis.

A mysterious band—Week after week, Rahbar scanned blood samples for novel hemoglobin variants. The researchers screened the samples under a variety of conditions, altering pH and other variables, to tease out distinct hemoglobin bands. Typically, at pH 8.6, they would see a large hemoglobin A band and a smaller and slower hemoglobin A2 band. Then, Rahbar says, they saw something interesting in the blood of a 67-year-old woman: a blurry band that appeared to travel at the helm of hemoglobin A.

"I said to myself, 'What is this? This isn't fitting with any of the known hemoglobins,'" says Rahbar. He looked at the medical record of the subject, and there on her charts it clearly stated that she had diabetes. That itself did not convince Rahbar or his colleagues that the abnormal band was linked to diabetes; it still could have been a genetic anomaly. However, it did prompt them to look at the blood of 47 additional people with diabetes. "I will always remember: It was a weekend—Friday—and I went [to the laboratory] and screened all of them. They all showed the same hemoglobin," says Rahbar. "I called it the diabetic component of hemoglobin."

To better define the band, Rahbar changed the electrophoresis conditions. By lowering the pH to 6.2 and switching to an agar gel, he was able to get a sharper diabetes band. He published his results in 1968 (3).

That same year, Rahbar came to the U.S. He wanted to "reconfirm" his findings in a different laboratory, so he joined forces with Helen Ranney, a leading scientist studying hemoglobin and sickle cell anemia who gave him access to blood samples from the neighborhoods surrounding Albert Einstein College of Medicine. Again, they found the unusual hemoglobin in 140 patients with diabetes, but it also showed up in individuals without diabetes at consistently lower concentrations (4).

From unusual to HbA_{1c}—The next step was to determine the identity of this strange hemoglobin. Rahbar looked for clues in the chromatography studies of Holmquist and Schroeder (5) that found that five minor components of hemoglobin A (HbA_{1a}, HbA_{1b}, HbA_{1c}, HbA_{1d}, and HbA_{1e}) are eluted before the major portion. Rahbar, Blumenfeld, and Ranney discovered that the electrophoretic mobility of their diabetic hemoglobin matched that of HbA_{1c} (4). Furthermore, when the researchers subjected blood samples from subjects with diabetes to Holmquist and Schroeder's chromatographic separation, the HbA_{1c} band represented between 7.5 and 10.6% of the total hemoglobin, while in normal subjects the HbA_{1c} constituted only 4–6%.

A 1968 study (6) had established that the structure of HbA_{1c} was hemoglobin plus a hexose molecule, but it still was not clear whether HbA_{1c} levels tracked with blood glucose. Rahbar returned to Iran and continued to study the problem, while other researchers slowly began to take an interest in HbA_{1c}. "For the first 5 to 6 years, no one believed this was something interesting," says Rahbar. "But it turned out to be important."

One reason people did not think that HbA_{1c} was a measure of diabetic control is because most researchers thought an enzyme was responsible for attaching glucose to hemoglobin, so HbA_{1c} should be independent of blood glucose levels. "They assumed it had to be an enzyme," Cerami says. "They hadn't thought it was nonenzymatic glycation."

The erythrocyte is unusual because it does not have a nucleus, so it makes its proteins, including hemoglobin, during development in the bone marrow. Once in the blood, total hemoglobin levels should remain about constant, but Cerami wondered whether HbA_{1c} levels would change over the life of a cell. Using erythrocytes labeled with radioactive iron to track the cells' age in mice, Cerami and colleagues found that HbA_{1c} levels increase over the lifetime of a cell. Importantly, HbA_{1c} levels increased 2.8 times faster in diabetic mice than in normal mice (7).

Later, Cerami found that HbA_{1c} levels reflected urine glucose levels in humans as well (8), offering additional evidence that the HbA_{1c} may be a helpful tool for people with diabetes. Over the next few decades, the Diabetes Control and Complications Trial (DCCT) (9) and UK Prospective Diabetes Study (UKPDS) (10) showed that blood glucose control, as assessed using HbA_{1c}, prevented the complications of diabetes. Without HbA_{1c}, this would have been nearly impossible to demonstrate.

Still at it—During the revolution in Iran, Rahbar was accused of being close to the Shah's family and was fired from his professorship at the University of Tehran. "I left everything behind, took my wife and my three daughters, and thank God I came to the United States," recalls Rahbar. "I called Helen Ranney. She was at UCSD. She came and met us and told me, 'Don't worry.'" Rahbar became a researcher at the City of Hope National Medical Center in Duarte, California, where he has been ever since—for 33 years now. Today, he is a distinguished professor of diabetes, endocrinology, and metabolism at the Center.

Rahbar has continued to study glycation and found that the reaction occurs in many types of biological molecules, including DNA and lipids. Some of his research suggests that glycation is related to the development of diabetes complications. Now, at age 83 years, Rahbar still wakes up every morning at 5:00 A.M. and heads to his laboratory by 5:30 A.M. "It's not a job for me; it's a passion," he says.

In June 2012, Rahbar accepted the Samuel Rahbar Outstanding Discovery Award from the ADA at the 72nd Scientific Sessions, Philadelphia, Pennsylvania. "I was very excited," says Rahbar, mostly because he was honored as his wife, three daughters and their husbands, and eight grandchildren watched from the front row. Once back in California, it was back to work, teasing out the secrets of life and glycation. "I know that something good is waiting for me in my lab," he says. "I enjoy every minute of it."

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Editors' note—We regret to inform the readers of *Diabetes Care* that Dr. Samuel Rahbar passed away shortly before this article went to press. He was 83 years old. The American Diabetes Association and the editors of *Diabetes Care* would like to express our sincerest condolences to the Rahbar family and Dr. Rahbar's friends and colleagues at City of Hope, Duarte, CA.

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