PRACTICING MOLECULAR MEDICINE WITHOUT A LICENSE

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Signor Presidente della Repubblica, Presidente Antonelli, autorità, colleghi e colleghe, signore e signori, sono profondamente onorato di ricevere questo prestigioso premio Feltrinelli dall'Accademia.

As this distinguished audience knows, the Accademia Nazionale dei Lincei was founded in Rome in 1603 by Federico Cesi (a scientist and naturalist) and Johannes van Heeck (a physician) with the mission of establishing a meeting place aimed at the development of sciences. Its name refers to

the lynx, a short-tailed member of the cat family who was supposed to have sharp eyesight akin to people who dedicate themselves to science.

I will return to the lynx later in my talk.

I was initially trained as a medical doctor but have a career-long passion for molecular and evolutionary biology.

During my medical internship, I was attracted to hematology and the study of hemoglobin because I took care of Alfredo, a 14-year-old Italian American boy, who had beta thalassemia major and suffered from severe anemia and multi-organ failure due to iron overload.

This disease is caused by inherited mutations in the gene encoding beta globin, resulting in absence or markedly reduced production of this protein subunit.

During the next 40 years my clinical and research focus was on hemoglobin.

As shown on the left, hemoglobin is a protein, composed of two unlike subunits, alpha and beta, each of which is bound to heme in which an iron atom binds oxygen reversibly. Hemoglobin is present in abundance in circulating red cells and is essential for high-capacity transport of oxygen to the organs and tissues of the body. Hemoglobin, of course, is not only of prime medical importance, but also plays a central physiological role in oxygen transport in a broad range of

other animals. It is arguably the most studied of all the proteins in biology. It is the prototype of an allosteric protein in which function depends on the binding of small molecules that impact importantly on interaction between the protein's subunits.

As shown on the right-hand panel, cooperativity between hemoglobin's subunits results the oxygen binding curve having a sigmoid shape. This allows for the release of enhanced amounts of oxygen to tissues as red cells pass through the micro circulation. Thus, hemoglobin has been the prime exemplar of the broader realm of physiologic and metabolic regulation via multi-subunit proteins.

Over the last half century, a group of investigators at Sapienza University in Rome headed by Rossi-Fanelli, Antonini and Brunori have spearheaded the investigation of the biochemistry of hemoglobin and other heme proteins.

My personal adventure with hemoglobin research began at a much simpler, more clinical level. On the basis of work by the Rome group and specifically Emilia Chiancone, I showed that the binding of hemoglobin to the plasma protein, haptoglobin, and the transit of plasma hemoglobin into the urine depended in the dissociation of the tetramer into $\alpha\beta$ dimers.

My need for focused training in hemoglobin biochemistry led to a brief stay in the lab of Reinhold and Ruth Benesch at

Columbia Medical School. They were struck by the fact that in the red blood cell, the concentration of the metabolic intermediate 2,3-DPG is 1000-fold greater than that of other cells in the body, roughly the concentration of hemoglobin tetramer within the red cell. They found that the addition of DPG to a purified solution of hemoglobin resulted in a dramatic shift to the right of the oxygen binding curve and therefore a reduction in oxygen affinity. Moreover, they showed that DPG bound to deoxyhemoglobin, with 1:1 stoichiometry, but not to oxyhemoglobin. DPG along with hydrogen ions are the prime allosteric modulators of hemoglobin function.

It soon became clear that this observation had important clinical implications. Patients with anemia, irrespective of cause, were shown to have elevated levels of red cell DPG, and therefore significant additional reduction of oxygen affinity. This enables an anemic patients to have enhanced oxygen unloading to tissues, greatly compensating their deficit in red cell mass.

While working in Robin Briehl's lab at Albert Einstein in the Bronx, I made oxygen equilibrium measurements testing the impact of DPG on a variety of human hemoglobins that had identical alpha subunits, but structural differences in the beta globin. These results led me to propose that DPG binds

electrostatically to positively charged sites on the beta globin at the entrance to the central cavity of the hemoglobin tetramer. Perhaps the most gratifying surprise in my long research career was the receipt of a hand-written letter from Max Perutz, the Nobel-laureate who unveiled the 3-dimensional structure of hemoglobin. He was able to fit a model of DPG neatly into his structure of deoxyhemoglobin at my proposed binding site. This site was confirmed 24 years later by Perutz and Dobson's X-ray structure of the DPG-deoxyhemoglobin complex.

I was curious about the impact of DPG regulation on the hemoglobin function in other mammals. I investigated nine domestic mammal species and found that the red cells of the cat and the ruminants (sheep, cow, goat) had extremely low levels of red cell DPG but their hemoglobins had intrinsically low oxygen affinity (denoted by high P50 values, the oxygen pressure that hemoglobin is 50% bound to oxygen). Hemoglobin from these animals was totally unresponsive to DPG. In contrast other mammals (rat, guinea pig, rabbit, horse, and dog) resembled humans in having high levels of red cell DPG along with hemoglobins of intrinsically high oxygen affinity, but responsive to the addition of DPG with a decrease in oxygen affinity, and thus an increase in P50, approaching that of the cat and ruminants. Subsequently I investigated 64 other mammalian species with results fully

consistent with my much smaller survey of domestic mammals. This is an interesting example of convergent evolution wherein mammals devised separate but equally effective ways of fine-tuning oxygen affinity of red cells for optimal physiological function.

Another interesting example of convergent evolution comes from the role of hemoglobin in optimizing oxygen transport from mother to her developing fetus. Among mammals, with the exception of the cat family including the lynx, oxygen affinity of hemoglobin in fetal red cells is substantially higher than that of the maternal red cells, thus facilitating transfer of oxygen across the placenta. In humans, this is because in fetal hemoglobin β143 His, an important binding site for DPG, is replaced by γ143Ser, thereby weakening DPG binding. In most other mammals, the high oxygen affinity of fetal red cells is due to low levels of red cell DPG, which normalizes within a few days following birth.

Hb A1c is a negatively charged minor hemoglobin component that normally comprises about 5% of the total but is greatly elevated in patients with diabetes. Glucose is covalently linked to the N- terminus of the beta globin chain by a keto-amine linkage. In a perhaps foolhardy gesture of self-experimentation, I explored the biosynthesis of hemoglobin A1c, *in vivo*. I injected myself with radioactive iron, which

enters the bloodstream and is rapidly and almost fully incorporated into hemoglobin within developing red cells in the bone marrow. A few days later, the radiolabeled red cells appear in the circulation, and rise rapidly to a peak which decays slowly during the 120-day lifespan of the red cell. In contrast, I found that the appearance of radiolabeled hemoglobin A1c was slow and continuous over the lifespan of the red cell, indicating that hemoglobin A1c is formed slowly and irreversibly during the four-month lifespan of the circulating red cells. This observation strongly indicated that hemoglobin A1c provides a highly accurate and easily measured index of the average blood glucose level over a period of a couple of months and therefore is a valuable tool in monitoring the treatment of patients with diabetes. Indeed, since that time, this relatively simple lab test is in frequent clinical use world-wide. My lab also found that glycated adducts were increased in other proteins of diabetic patients, including lens crystallin and kidney basement membrane proteins. It remains unclear whether these structural modifications play a significant role in the long-term complications of diabetes.

I would like to end my talk today with a return to evolutionary biology. It is clear that non-enzymatic glycation is an unwanted and perhaps deleterious modification of protein structure. This raises the question of why, 3.7 billion years ago,

D-glucose, rather than any of the seven other D-hexose isomers, evolved as the metabolic fuel used universally by all forms of life. We determined the rate of glycation of hemoglobin by all eight isomers as well as other D-aldohexoses; and showed that D-glucose has by far the slowest rate of glycation and therefore much less prone to form unwanted protein adducts *in vivo*. This very early example of natural selection importantly minimizes risks arising from potentially dangerous non-enzymatic structural modifications of proteins.

During the time I was immersed in the study of hemoglobin, I and all others in the field were guided and motivated by Antonini and Brunori's definitive book on the molecular basis of the function of heme proteins. Several years later, Bernie Forget at Yale and I published a book which we hope would be for hematologists what the Antonini and Brunori book has been for biochemists and biophysicists.

I want to thank the Accademia for bestowing this prestigious award. I am also indebted to the many trainees and junior colleagues who worked with me on the research described in this lecture.