Introduction: congenital methemoglobinemia revisited

Quentin Gibson

Editorial note: Percy et al report the identification of the mutations in cytochrome b5 reductase responsible for congenital methemoglobinemia in a family studied by Quentin Gibson in 1943. Gibson's seminal work was a tour de force of clinical investigation. Indeed, his original experiments provided the bulk of our current understanding of the molecular pathogenesis of this disorder. Subsequently, Gibson went on to become one of the world's leading biochemists. He continues to make seminal contributions to our understanding of hemoglobin and other heme proteins. In the commentary below, Professor Gibson presents a highly personal and riveting account of the remarkable clinical, academic, and scientific circumstances that led to his discovery.—H. Franklin Bunn

Looking back over the story of the work in Belfast almost 60 years ago, it seems to me that much of it can be summed up in 2 words: serendipity and coincidence. It all started with Dr Deeny, a general practitioner in Banbridge, a town of 5000 or so, some 20 miles from Belfast. Unlike many of his peers, Deeny was alert to changes in medicine, particularly new drug treatments, prescribing them for his patients, not only as intended but in off-label experiments in other conditions. He tried ascorbic acid, which had recently become available, in several patients with congestive heart failure, presenting his positive results at clinical meetings in the Mater Infirmorum Hospital in Belfast. In the opinion of the physicians there, ascorbic acid had no effect upon the heart, and any improvement in the patients was a placebo effect. Deeny did not accept this and cast about for means to convince his colleagues of their error. As a result, he was uniquely sensitive to and interested in deeply cyanosed patients who would, he hoped, show undeniable improvement on ascorbic acid.

There cannot have been many doctors on the lookout for cyanotic patients, and these were the first cases of methemoglobinemia reported in the British Isles. Whatever the odds against the event, looking out of his window one morning, he saw an obviously blue man walking by; so at the end of his office hours, he called in at the local police station and asked who the blue man might be, learning that there were actually 2 similarly blue brothers. Presumably after clearing it with their doctor, he approached the brothers and offered to turn them pink, starting with Fred Martin and keeping Russell as a control. Fred's cyanosis was barely detectable after a month on 0.2 g ascorbic acid daily, and Deeny then presented the brothers again in Belfast. This time, although without any idea why the brothers were blue, the consultants in Belfast were agreed on one thing: there was nothing whatever the matter with their hearts. This was a commonsense conclusion, consistent with Fred's membership in his local field hockey team.

At this point, Professor Henry Barcroft, professor of Physiology in the medical school, was called in. He was just the man to investigate a disorder of gas transport for he was the son of Sir Joseph Barcroft, professor emeritus at Cambridge, who had played an important part in the fundamental work that established the properties of hemoglobin, and was also designer of an instrument, the Barcroft differential manometer, used for determining the oxygen capacity of blood. The Biochemistry Department had a dozen of these, used as microrespirometers, and almost all my experiments involved them. More immediately, the Department of Physiology possessed a Van Slyke volumetric blood gas pump, and Henry could work it, by no means an everyday skill. The Biochemistry Department had a photoelectric photometer, analogous to the Klett instrument, and Professor D. C. Harrison and I were working on the preparation of a stable inorganic standard for determining hemoglobin as alkaline hematin. These were precisely the means required to establish the gross discrepancy between oxygen capacity and total hemoglobin in Russell's blood, and the diagnosis was clinched by observing the 630-nm band of methemoglobin in neutral or slightly acid solution and its disappearance after treatment with dithionite or cyanide.

Henry then set about following Russell's treatment and a general clinical investigation of his blood and urine. Having had no part in this, I can say on rereading the resulting paper, I find it an excellent piece of work. Departments at that time were very small. In Physiology there was 1 lecturer and 2 demonstrators, the latter more or less equivalent to postdoctoral fellows. This group was responsible for lectures and practical work in physiology for 120 students and for teaching histology as well. There were 3 terms in the calendar year, and the physiology course extended over 5 terms. The result was a considerable teaching load, aggravated by the need to triplicate all practical classes because of limited accommodation, and research was almost restricted to vacations. In Biochemistry there was a professor and 2 lecturers, 1 unfortunately an alcoholic who did not contribute to the intellectual life of the department. Again, it was something of a coincidence that the apparatus and skills matched the problem.

Investigation of the mechanism of the condition fell largely into my hands by another series of unlikely circumstances. Like Henry, I was influenced by my father, a chemist and director of a local research institute. Chemistry interested me, and unlike many in my year I saw the premedical courses as an opportunity rather than as an obstacle. The importance attached to biochemistry may be judged by the fact that while the Biochemistry Department provided practical classes for medical students (mostly piss and shit, with a little bile and gastric contents thrown in), all the lectures were given by Henry Barcroft. In some ways this was a good thing because he was a first-rate lecturer, and his medical qualifications allowed him to focus on material relevant to medicine. He was also the first to confess to limited knowledge of biochemistry and did not consider starting work at the molecular level.

The first metabolic experiment was unplanned. I was around in the lab when Russell was brought in before treatment. At the time I knew that methylene blue had a potent effect in accelerating methemoglobin reduction and happened to wonder whether it would work in vitro. (Actually, I think this had been done, although with chemically induced methemoglobinemia.) I had been using the Barcroft manometers, effectively Warburg respirometers with attached thermobarometers, to determine oxygen capacity and begged a sample of blood from one of the cases to try methylene blue. It did indeed work, with very rapid oxygen uptake as the methemoglobin level became reduced followed by a continuing

slower oxygen uptake mediated by methylene blue substituting for the respiratory carriers that do not occur in blood. This showed that at least some of the enzymes in normal blood were also active in the blood of the cases. In the absence of methylene blue, little or no reduction occurred in the patients' cells regardless of whatever substrate was tried. With methylene blue and glucose as substrate, the methemoglobin level was rapidly reduced both in normal and pathologic cells. With lactate as substrate, rapid reduction was seen in normal cells, but no reduction took place in the patients' cells. When methemoglobin reduction was followed in the presence of methylene blue, oxygen was excluded from the measurements by carrying them out anerobically in the presence of carbon monoxide. Warburg had shown that the addition of glucose to washed rabbit erythrocytes speeded methemoglobin reduction; so the reactions of the glycolytic pathway were looked into. A series of comparisons showed that, in every case, blood from the patients and from healthy controls gave similar results. It seemed thatsome factor must mediate between the dehydrogenase and methemoglobin and was missing from the patients' cells.

At the time I had recently read David Green's book *Biological Oxidations* that described a 3-step scheme leading to oxygen uptake. (Serendipity, again.) In it, substrate and enzyme reduced a coenzyme (DPN or TPN). The DPNH reduced an intermediate, coenzyme factor, putatively a flavoprotein, and the flavoprotein reduced cytochrome oxidase. By analogy, if one supposed that hemoglobin was not only an oxygen carrier but also a (fantastically inefficient) oxidase, then the results could be explained by a defect in coenzyme factor activity.

It remained a problem to explain why, in the patients' cells, with methylene blue, glucose was an effective substrate while lactate was not. Dickens and others had shown that, besides undergoing glycolysis and entering the then-new Krebs cycle as pyruvate, glucose could be oxidized stepwise in what is now called the pentose shunt. The enzymes in the 2 systems have not only different substrates but different coenzyme specificity. The glycolytic enzymes require DPN while the pentose cycle enzymes require TPN. Everything could be explained if the patients' cells were deficient only in coenzyme factor 1. The 2 pathways have a different stoicheiometry of methemoglobin reduction under anerobic conditions as was later shown.

From then on, the problem was one of devising an assay and doing various control experiments to eliminate, for example, a direct reaction between DPNH and methemoglobin. At that time there was no Sigma Chemicals to turn to for reagents. DPN, for example, required a trip to a bakery to get 14 lb of yeast. This was boiled up with HCl, and the yeast filtered off on Büchner funnels with frequently changed filter papers. Precipitation with lead acetate followed; the precipitate was decomposed with H_2S from a Kipp generator, followed by precipitation with mercury, more H_2S, and then with freshly prepared cuprous chloride, and still more H_2S. The yield as I recall was about 0.3 g of 40% DPN, assayed in the Barcroft manometer by reduction with H_2 (another Kipp), catalyzed by platinized asbestos.

For me this was the end of the road, and lacking good new experiments, I turned to other things and did not even attempt to follow the field. Some final oddities: Henry found a reference to a paper from Paris in which Lian, Frumusan, and Sassier described another case and its treatment with ascorbic acid. At the end of the war, when the *Biochemische Zeitschrift* reappeared in libraries, there was a paper from Manfred Kiese describing the isolation of what he called methemoglobin reductase. His enzyme, however, was the TPN coenzyme factor 2. Working without the clues offered by the patients, he had no way of knowing that the reactions he observed in the presence and absence of methylene blue were not due to the same enzymes, except perhaps the stoichiometry, and that his enzyme did not react with methemoglobin in the absence of methylene blue.

Last, a confession: Harrison and I really fell flat on our faces in criticizing a pair of Canadian workers who described a family with a methemoglobinemic parent. They had not measured methemoglobin in the blood, and we said they must have gotten it wrong. What they had was a family with hemoglobin M. A case, not under that name, had already been described by Horlein and Weber, and we should not have taken it upon ourselves to attack Balzan and Sugarman.

I have been told that the Belfast work was the first to supply a molecular basis for an "inborn error of metabolism" and relate a specific enzyme deficiency to a disease. At the time, I did not think this particularly important but took it for granted that diseases such as alkaptonuria and phenylketonuria resulted from a specific hereditable enzyme defect, claiming only that this was the first case to involve a redox enzyme.