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Charache S & Conley C L. Rate of sickling of red cells during deoxygenation of blood from persons with various sickling disorders. *Blood* 24:25-48, 1964.
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The rate at which sickling of red cells occurs is more closely related to the clinical severity of sickling disorders than the degree of sickling finally achieved after prolonged exposure of blood to low oxygen tensions. [The SC1® indicates that this paper has been cited in over 145 publications.]

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In medical school, I was fascinated by reports of renal disease produced in laboratory animals by immunizing them to extracts of kidney. Accordingly, when I was offered a fellowship in hematology by C. Lockard Conley, I told him that I wanted to work in immunohematology. After brief consideration, he told me I was due to learn a lot about abnormal hemoglobins, and thus ended my career as an immunologist.

Conley was interested in the relationship between what we would now call a single-point mutation in DNA and the very wide panoply of clinical illness produced by such mutations in patients with sickle-cell disease. A screening program he had introduced (which led to discovery of independent inheritance of the alpha and beta globin genes and hemoglobin Chesapeake, among other abnormalities) made available patients with clinically "severe" and "mild" disease, and I set out to find differences between them in the laboratory. In those days I thought I knew the difference between mild and severe disease, and the reviewers of the paper never even asked for a definition of our terms; today, we're all more skeptical of our ability to interpret subjective symptoms.

The research lab relied upon homemade equipment. I copied my first viscometer

from one described by Allison, using a glass tube and some steel ball bearings (which were usually given to me by bemused manufacturers). For hours, I sat in front of the tube with a stopwatch, timing the silver equator of the ball as it fell through a column of deoxygenated blood or hemoglobin solution—and gradually realized several principles that we then laid out fairly clearly. I believe those principles are still solid and guess that's why our work is still cited today. The principles (somewhat paraphrased) are: (a) the speed with which a cell becomes nondeformable as it speeds through a hypoxic microvascular bed determines whether or not it will get stuck or emerge into larger venules;¹ (b) between various forms of sickle-cell disease, patients differ in the propensity of their hemoglobin to polymerize, and that propensity in turn is related to disease "severity";² (c) within a given disease (e.g., sickle-cell anemia), we can determine only some of the factors that govern "severity";³ and (d) hematocrit plays an extremely important role in determining bulk viscosity of blood, and transfusion therapy must take that effect into account. Widespread use of modified exchange, rather than simple transfusion to reduce the proportion of sickled cells, depends upon that hypothesis.⁴

We discussed therapeutic induction of increased synthesis of fetal hemoglobin to ameliorate sickle-cell disease, but I never dreamed that I'd actually watch such an experiment.⁵ We also wrote that maintenance of the proportion of sickled cells below 60 percent by repeated transfusion could "indefinitely prevent...recurrence of symptoms of sickle cell disease." Our clinical sample was small, and I think our conclusion was probably naive, but chronic transfusion therapy is still the only generally accepted treatment for that condition.

1. Coletta M, Hofrichter J, Ferrone F A & Eaton W A. Kinetics of sickle haemoglobin polymerization in single red cells. *Nature* 300:194-7, 1982.
2. Brittenham G, Schechter A N & Noguchi C T. Hemoglobin S polymerization: primary determinant of the hemolytic and clinical severity of the sickling syndromes. *Blood* 65:183-9, 1985.
3. Powers D R, Schroeder W A, Weiss J N, Chan L S & Azen S P. Lack of influence of fetal hemoglobin levels on erythrocyte indices on the severity of sickle cell anemia. *J. Clin. Invest.* 65:732-40, 1980.
4. Charache S, Lubin B & Reid C D. Leg ulcers. *Management and therapy of sickle cell disease*. Washington, DC: National Institutes of Health, 1984. NIH Publication No. 84-2117. p. 30-1.
5. Charache S, Dover G J, Smith K D & Talbot C C. Treatment of sickle cell anemia with 5-azacytidine results in increased fetal hemoglobin production and is associated with non-random hypomethylation of DNA around the γ - δ - β globin gene complex. *Proc. Nat. Acad. Sci. US* 80:4842-6, 1983.

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