WBCastle

From Man to Molecule and Back to Mankind

By William B. Castle

MACFARLANE BURNET, in commenting upon the value to general biology of the study of disease, wisely remarked: "It is only when something goes wrong with it that we may recognize the existence of a normal mechanism." Thus, the study in depth of sickle cell disease has led to revelations concerning the genetic control and mode of synthesis of normal hemoglobin at a molecular level. From clinical observations followed by basic research concerning a condition principally affecting some members of the black African race has come progressively better understanding of this and other biological phenomena involved in the life processes of all the races of man. For this information we owe a debt of gratitude to the victims of sickle cell disease that with further research and their collaboration can hopefully be repaid in the near future through clinical benefit to them. Because Doctor Ham was a pioneer in the study of the mechanism of red cell destruction in sickle cell disease it is perhaps appropriate for a contributor to a volume in his honor to review the historical development of our knowledge of that disorder.

FROM MAN TO MOLECULE

The history of our understanding of sickle cell disease can be likened to the opening of the proverbial Russian egg: at once another egg appears within, and inside that another and yet another and so on. Thus, in the study of sickle cell disease, first we see the ailing and anemic patient, then his deoxygenated, sickled red cell, then its abnormal hemoglobin, and finally hidden away inside the beta chain of the molecule, a single displaced aminoacid. It must be said at once that, for a condition about which so much fundamental information is known, it seems at first remarkable that prevention and therapy have only just made a beginning. This, however, is by no means a unique situation in the management of other human disease: witness the failure to prevent the vascular complications of diabetes with a sovereign remedy at hand or the indifference of the public to the well-established dangerous effects of cigarette smoking. And for sickle cell disease, although there are promising therapeutic agents, none is as yet fully effective or without some disadvantage. Again, unless healthy parents with unrecognized sickle cell trait are so informed they do not know that a child may be born to them who will shortly develop sickle cell disease.

We are indebted to Konotey-Ahulu of the Korle Bu teaching hospital for the historical perspective that sickle cell disease of "severe" and "not-so-severe" types have long been recognized in Ghana, as conditions affecting the children of seemingly normal black parents. In the Krobo tribe sickle cell disease has

---

From the Medical Service, Veterans Administration Hospital, West Roxbury, Mass., and the Department of Medicine, Harvard Medical School, Boston, Mass.

Presented in part at the First National Symposium on Sickle Cell Disease, Washington, D.C., June 27, 1974.

been traced back in one family for three centuries.[27] Definite recognition of sickle cell disease as an entity became possible in this country only after 1910 when James B. Herrick[20] of Chicago reported to the Association of American Physicians the appearance under his microscope of "peculiar elongated and sickle-shaped red blood corpuscles" in fixed and fresh films of the blood of a black West Indian student with severe anemia and a heart murmur. He cautiously suggested as a possible explanation that "some unrecognized change in the composition of the corpuscle itself may be the determining factor." The response of the medical profession to this announcement was apparently even more lethargic than to his classic description of coronary occlusion two years later.

In 1917 V. E. Emmel[12] observed what he considered to be transitional forms between disk-shaped and sickle-shaped red cells in the blood of a young anemic mulatto woman who had experienced recurrent leg ulcers. Because he saw nucleated red cells in the form of biconcave disks he assumed correctly that the sickled form of the red cells was acquired after their release from the marrow; and this transformation was observed to his satisfaction in sealed "cultures" of his patient's red cells suspended in her own or normal serum. Moreover, culture of her father's blood, though at first seemingly normal, likewise developed after some days "forms with long sharp projections and elongations." Here then, as we now know, were distinguished for the first time the hematologic features of the homozygous and heterozygous phenotypes, and of the reversible and later so-called "irreversibly sickled" forms of red cells. However, it was only with the publication of observations made upon the blood of the fourteenth case to be reported that Hahn and Gillespie[16] in 1927 clearly defined the factors involved in the acute sickling phenomenon.

A fundamental implication of the excellent experimental procedures they described was, however, to remain unrecognized for over 20 years: namely, that the red cell hemoglobin itself was abnormal. Dr. Hahn and his medical student colleague employed hanging drop preparations of sickle cell blood that could be directly and continuously observed with a microscope when exposed to quick changes of known tensions of oxygen, carbon dioxide, and other gases. They noted that sickling occurred when the partial pressure of oxygen fell to 45 mm Hg, provided the hydrogen ion concentration of the blood was on the acid side of pH 7.4. More oxygen or carbon monoxide promptly restored the discoid form. Corpuscles that had lost their hemoglobin did not sickle with hypoxia. Consequently they stated:

> Sickle cell formation is a reversible phenomenon depending on the free or combined state of the hemoglobin of the susceptible corpuscles. . . . The failure of corpuscles which have lost their hemoglobin to undergo sickle cell formation is consistent with our hypothesis relating the distortion to the hemoglobin. . . . It is reasonable to suppose that sickle cells are formed in the body of an affected person wherever the oxygen tension and hydrogen ion concentration are such as to render the distorted form of the corpuscle stable.

Here then was a lucid exposition that ascribed the abnormal behavior of the contents of the sickle cell to a lowered tension of oxygen enhanced by a decrease in pH. This was clearly relating the characteristic behavior of hemoglobin toward oxygen and pH to the sickling phenomenon. Was it then because

these phenomena were so very characteristic of normal hemoglobin that the authors apparently ascribed the sickling to the anomalous behavior of normal hemoglobin rather than to the behavior of an anomalous species of hemoglobin? Indeed, the latter explanation was not proposed until 1948 when Janet Watson[41] of Downstate Medical Center, Brooklyn, N.Y., inferred from the delay in the full development of in vitro induced sickling of infant's blood until some weeks after birth that this was due to the gradual replacement of fetal hemoglobin, as she said, "... by the adult type of hemoglobin which possesses the sickling property."

Meanwhile understanding of the relation of red cell sickling to the anemia and other clinical aspects of sickle cell disease had been progressing. In 1930 Scriver and Waugh[36] confirmed the prediction of Hahn and Gillespie[16] when they found more sickled cells, less oxygen, and more carbon dioxide in samples of the venous blood of an extremity subjected to experimental venous stasis. From the improved red cell counts of their patient with sickle cell anemia following splenectomy Hahn and Gillespie had already correctly inferred that the enlarged spleen was merely performing its usual "hemoclastic function" but was "too discriminating." In 1939 Diggs and Bibb[11] demonstrated the increased mechanical fragility of deoxygenated sickled cells later confirmed by Shen et al.[37] in 1946 and carefully studied by Lange et al.[28] in 1951. Because Diggs and Bibb found in a few patients permanently sickled red cells in the pleural and ascitic fluids but not in the blood, they suggested that stagnation in the deoxygenated form might be responsible. In 1949 Shen et al.[38] produced such cells experimentally in vitro by 24-hr incubation of deoxygenated sickle cell blood. Reoxygenated, irreversibly sickled cells do not usually contain hemoglobin in the sickle state and have recently been shown by Bertles and Milner,[5] Jensen et al.,[24] and others to have permanent deformity and calcium-loaded rigidity of the cell membrane rendering them subject to rapid destruction in vivo. In 1940 Ham and Castle,[17] noting the markedly increased viscosity of samples of deoxygenated sickle cell blood in vitro, inferred that the initiation of sickling in a capillary bed, as the result of the normal reduction of oxygen tension there, would slow the local circulation, further reduce the oxygen tension of the blood, augment blood viscosity, and so initiate a "vicious cycle of erythrostasis." This cycle, describable today as a "positive feedback loop" was thought to account for the episodic incidence of the painful crises. Also in 1940 a publication by Julius Bauer[4] appeared to define the end products of the postulated "vicious cycle" in many organs as seen by the pathologist at autopsy: thrombosis, ischemia, necrosis, and fibrosis.

But to return to progress toward the molecule. In 1940 I. J. Sherman, while working as a medical student in Wintrobe's laboratory at Johns Hopkins, studied the effects of equilibration of blood samples with air at progressively reduced barometric pressure. He confirmed the thesis of Hahn and Gillespie[16] as well as a difference between bloods that had been observed by Diggs[10] in 1932 with sealed preparations. Sherman[39] found that the red cells of nonanemic patients with sickle trait required a much greater reduction in barometric pressure before they would sickle than did those of sickle cell anemia. He also reported "without interpretation" a novel observation of great potential significance: namely, that deoxygenated sickled cells were birefringent under the polarizing

microscope, but lost this property upon unsickling with aeration. By chance the thought that this phenomenon might be due to molecular orientation associated with sickling was communicated by a medical friend to the great physical chemist, Linus Pauling, in 1946. This aroused his interest and led to the demonstration by Pauling et al.[31] in 1949 that there were clear-cut differences in the electrophoretic mobilities of normal and sickle hemoglobins. Moreover, they showed that in contrast to sickle cell anemia both types of hemoglobin were found in sickle cell trait cells in roughly equal amounts. This allowed Pauling to infer that, as J. V. Neel[30] of the University of Michigan had just published on the basis of family genetic studies, the benign clinical condition was heterozygous while the homozygous state was responsible for the anemia of the patient with sickle cell disease.

In discussing the abnormal electrophoretic property of sickle hemoglobin Pauling et al.[31] proposed that a surface region of the globin near the iron atom of the sickle hemoglobin molecule was absent in the normal molecule. This, when the blood was deoxygenated, might permit complementary interaction between sickle hemoglobin molecules causing alignment, birefringence, and distortion of the cell membrane. Consequently, Pauling called sickle cell anemia "a molecular disease." However, it remained for J. W. Harris[18] first to demonstrate the physical reality of this interpretation the following year. He showed that when cell-free solutions of sickle hemoglobin were deoxygenated they became markedly viscous and displayed under the phase microscope spindle-shaped bodies approximating red cells in size. These so-called "liquid cyrstals" or "tactoids" were birefringent under polarized light. And so the long journey from man to molecule was completed.

BACK TO MANKIND

It is clear from the benign clinical course of the individual with sickle trait, the SA hemoglobin heterozygote, whose red cells may contain up to nearly half the concentration of sickle hemoglobin found in those of sickle cell disease, that the abnormal molecule is harmless unless deoxygenation allows it to conspire with sufficient of its fellows to distort the red cell. Indeed, there is no clinical defect or laboratory finding of sickle cell disease that cannot be explained as a consequence more or less directly of that potential conspiracy. For that reason only discussion of the factors directly involved in such molecular aggregation is relevant to our journey back to mankind. Moreover, fascinating as are the details of molecular configuration and behavior and whatever their understanding may provide for the future relief of the sufferer from sickle cell disease, its clinical phenomena are the immediate result of physical changes in the intact red cell.

Suffice it to recall that the well-known "finger print" method, devised by Vernon Ingram[21] in 1957, ascribed the differences in electrophoretic mobility observed by Pauling to a charge difference caused by the substitution of a single aminoacid, valine, for the normal glutamic acid of a single trypsin-derived peptide of sickle hemoglobin. This substitution was shown to be at the sixth position from the N-terminal of the beta chain. The alpha polypeptide chain of sickle hemoglobin is unaltered. It is this single, genetically controlled, molecular

modification that permits deoxygenation in some way to cause six stacks of sickle hemoglobin molecules to become twisted around each other in a helix of high pitch as shown by the elegant work of Murayama, Bertles, Finch, Döbler, White, and others.[29]

Singer and Singer[40] in 1953 and Allison[1] in 1957 were among the first to quantitate the minimum gelling concentrations and viscosity of deoxygenated sickle hemoglobin and of mixtures with other hemoglobins. The now well-known differences in clinical severity ranging from hemoglobin SS disease through heterozygotes for $HbSO_{Arab}$, HbSD, HbSC, HbS-thalassemia, HbS-Korle Bu, HbSA, and HbS-high F correlate fairly well with the results of such gelling and with viscosity measurement in vitro. Indeed, Greenberg et al.[15] in 1957 found that the red cells of a patient with HbSA, whose cell hemoglobins were depleted because of iron deficiency, would not sickle in vitro until with iron therapy the red cell HbS concentration was above 10 gm/100 ml. It is, however, not altogether clear whether the viscosity of the deoxygenated blood as a whole or the behavior of individual cells in capillaries causing blockade is the more important. Harris et al.[19] in 1956 critically examined the direct relationship between oxygen unsaturation, sickling, increased viscosity, and mechanical fragility of the whole blood of sickle cell anemia and sickle cell trait patients. Although conducted in the presence of equal but abnormally high tensions of carbon dioxide, the experiments clearly showed that progressive changes in all these parameters began at much higher oxygen tensions in sickle cell anemia blood than in sickle trait blood. Jandl et al.[22] in 1961 were the first to study the filterability of sickle cell anemia red cells at various oxygen tensions. Using a 5-μ Millipore filter they found a 25% decrease of filterability even with oxygenated sickle cells compared to normal cells; but with decreasing oxygen tension there was a progressive and marked decrease in filterability. The initial difference noted by Jandl is now known to be due to the presence of irreversibly sickled cells, and to the increased rigidity of the membranes of other fully oxygenated red cells. Klug et al.[26] have recently critically reviewed the rheology of sickle cell disease and have made instructive video tape recordings of the microscopic appearances of human sickle cells while passing through the mesenteric capillaries of the rat during breathing of air or only 5% oxygen. This technique yields perhaps the clearest picture of the all-important events initiating sickling in vivo, unless it becomes equally possible to inspect the capillary bed of the retina in man, where the end results of occlusion and vascular damage in sickle cell disease can readily be seen during life.[3]

The implications of the in vitro and in vivo observations taken together allow us to understand to a considerable degree the clinical phenomena of sickle cell disease as they affect various organs. Sudden anemia due to the sequestration of red cells in the enlarging spleen of the child is perhaps the most dramatic event, but is part of the same process that more commonly leads to hyperplasia and later to atrophy of the spleen. Also comprehensible as a result of a similar mechanism is the stagnation, embolism, or infarction especially of vascular beds such as those of the lung or liver, that are "down stream" from any first set of systemic capillaries. The observed lag time in seconds before sickling begins, the "milking" action of the organ on its blood vessels, and the

lowered viscosity of anemic blood perhaps together explain the relative immunity of the heart to vascular crisis, despite the marked deoxygenation of the blood in the coronary veins. On the other hand, the years of increased cardiac output as a result of the anemia, sometimes increased by arterial oxygen unsaturation and further jeopardized by embolization of the pulmonary vascular bed, seem readily to explain the cardiomegaly, murmurs, and eventual congestive failure. The early reversibility of hyposthenuria or hematuria by transfusion or hydration, which is followed later by irreversible hyposthenuria, is of special interest. It is thought that these renal dysfunctions are due to decreased rate of blood flow, hypoxia, and hypertonicity operating together to cause sickling of red cells traversing the hairpin loops of the peritubular *vasa recta* of the medulla.[42]

The shortened life span of the erythrocytes in sickle cell disease has been well documented by a variety of techniques and confirmed by measurement of increased amounts of the biochemical derivatives of hemoglobin catabolism. However, in the absence of demonstrated sequestration of the erythrocytes as in the enlarged spleen of children, where the abnormal corpuscles are finally destroyed is unknown. As in some other types of hemolytic anemia there is good evidence of deterioration and of nonselective loss of phospholipids and gain of calcium by the cell membrane. These are probably, according to Jensen[23] in the case of the sickle cell, a result of repeated sickling and unsickling. The *coup de grace* is no doubt either the result of mechanical stresses on a weakened or rigid cell membrane or due to osmotic lysis when normal impermeability to cations and eventually to hemoglobin is lost. There is in this country no well-established association between painful crises and increased hemolysis, but increase of anemia within days may occur with the painless "aplastic crisis" of viral infection or of folic acid depletion.[2]

The greatest mortality in sickle cell disease occurs before 10 yr of age, and a principal cause of death is infection. It is thought that the increased susceptibility to pulmonary infection, especially with pneumococci and to osteomyelitis commonly caused by salmonellae, may be due to local loss of resistance as a result of stasis or infarction in lung or marrow.[8] In initiating or propagating such infection either defect of the "properdin" system, the alternate pathway through C_3 to complement activation, or defective intracellular killing of bacteria by phagocytes may play a part.[34] In addition, as after splenectomy, especially in young children, the dysfunction or total infarction of the spleen in sickle cell disease may permit otherwise minor bacterial invasion to progress rapidly to fatal septicemia.[32]

Finally, in thinking about the future prospects for better therapy in the group of clinically adverse sickle cell syndromes, it is well to remember, as has been admirably pointed out by Konotey-Ahulu,[27] that when not in crisis the sickle cell disease patient, though anemic, is in "the steady state." And this may be much of the time. A painful crisis cannot occur without a change in his *milieu interieur*. Therefore, meticulous clinical study of possible precipitating factors just might determine a practical point of entry at which to block the vicious cycle producing crisis. As long ago as 1940 Ham and Castle[17] pointed out in theory the triggering effect of infection by causing increase in plasma viscosity

or red cell rouleaux formation. To these should obviously be added: arterial hypoxia, dehydration, acidosis, vasospasm, and congestive heart failure or other cause of venous stasis.

A priori it seems not too difficult a challenge to set aside in one way or another the effect of the mutation of a single gene, affecting somatically only half of a hemoglobin molecule, found only in the red blood cell and causing no significant alteration of that hemoglobin molecule's ability to take up and to release oxygen. Were it possible to colonize innocuously half of the patient's bone marrow with normal erythropoietic cells, benefit similar to that of repeated transfusions would be achieved. If normal genetic machinery could be inserted in half the patient's committed erythropoietic cells the trick would be turned. More hopeful is the prospect that, if nature already knows how to turn *off* fetal hemoglobin production after birth, we can learn how to turn it *on* again. All that would be needed to abolish clinical symptoms, as we know from the HbS-high F combination would be to increase the usual content of HbF hemoglobin in the patient's red cells from 5% to 30%, even possibly to only 20%. Various amounts of HbF are there already in the blood of sickle cell disease and in the HbS-thalassemia double heterozygote. Even in normal subjects acquired disease sometimes turns on HbF production a little.

Alas, these therapeutic prospects are still only the dreams of reason. Claims have been made for the beneficial effects of urea, and more recently of cyanate, in preventing molecular aggregation and increasing red cell survival, though not without undesirable side effects. Cyanate at tolerated concentrations increases the affinity of HbS or HbA for oxygen, and indicates the prospect of discovery of other drugs with similar properties. However, judging by the clinical effects of congenital hemoglobins with increased affinity for oxygen, less of this essential commodity will be delivered to the tissues.[9] The earliest attempt to combat anemia in sickle cell disease by pharmacologic means was made by Carl Moore's group in 1944. Although sickling was inhibited by breathing high concentrations of oxygen and red cell survival was prolonged,[6] the artifically increased oxygen content of the blood suppressed erythropoiesis in the bone marrow with resultant net lowering of the circulating hemoglobin level.[33] The use of partial exchange transfusion[7] avoids anemia and adds red cells that will not sickle, thus at once reducing the viscosity of the deoxygenated blood. When repeated sufficiently often transfusions provide more hemoglobin and thus automatic compensation for the suppression of the bone marrow delivery of sickle cells that is desired. Success in the treatment of an established painful crisis by intravenous hydration and alkalinization has been variable since first reported by Greenberg and Kass[14] in 1958. It would seem that systematic application of these remedies by oral administration might have greater success as prophylaxis.

In any case, the magnitude of the public health problem that we face today is clear because the anomalous gene is present in about 8% of over 22 million black citizens of the United States and in the homozygous state causes disability of various and varying degrees in 35,000 of them. Education to provide all citizens with a working knowledge of the genetic background and contrasting constitutional manifestations of the trait and of the disease is urgently needed,

especially for adult blacks and the administrators of educational institutions, industrial organizations, and health and life insurance agencies. It cannot be emphasized too strongly that the individual with the trait is neither anemic nor disabled unless from some other cause. The public dissemination of essential information about sickle cell disease must be made with strict objectivity. It must be applied in the individual case with understanding and tact, if damage to individual self-esteem and racial pride are to be avoided.[35] Screening programs without adequate understanding of their purpose carry psychological hazards; and individual genetic counseling at appropriate times requires the most careful planning and application if the charge of "genocide" is to be avoided. None of this will be easy; and as pointed out by Gelfand,[13] programs of persuasion "must be directed to individuals and succeed to the extent that self-interest is evident and not too far distant." Herein lies the psychological obstacle to promoting the out-breeding of any type of transmissible genetic defect.

There is perhaps a more hopeful prospect from what David Nathan has termed "focussed genetic counseling" based on a particular pregnancy at risk, provided suitable samples of fetal or mixed placental blood can be obtained safely and reliably.[25] By this technique, because some beta chain of human hemoglobin is synthesized in the first trimester of pregnancy and the beta sickle hemoglobin chain has been detected in 6–8-cm human fetuses, parents with sickle cell trait could avoid the chance of bringing into the world a child destined to have sickle cell disease or sickle cell-thalassemia disease. All Americans can take justifiable pride in the vigorous national programs concerned with research, education, and service for sickle cell disease that are well under way. So long as these programs stimulate and provide support for individual initiative and imagination in these areas progress will be made, even in ways not presently apparent.

REFERENCES

1. Allison AC: Properties of sickle cell haemoglobin. Biochem J 65:212–219, 1957
2. Alperin JB: Folic acid deficiency complicating sickle cell anemia. Arch Intern Med 120:298–306, 1967
3. Armaly MF: Ocular manifestations in sickle cell disease. Arch Intern Med 133:670–679, 1974
4. Bauer J: Sickle cell disease: Pathogenic, clinical and therapeutic considerations. Arch Surg 41:1344–1362, 1940
5. Bertles JF, Milner PFA: Irreversibly sickled erythrocytes: A consequence of the heterogeneous distribution of hemoglobin types in sickle cell anemia. J Clin Invest 47:1731–1741, 1968
6. Callender STE, Nickel JF, Moore CV, et al: Sickle cell disease: Studied by measuring the survival of transfused red blood cells. J Lab Clin Med 34:90–104, 1949
7. Charache S: The treatment of sickle cell anemia. Arch Intern Med 133:698–705, 1974
8. Charache S, Page DL: Infarction of bone marrow in the sickle cell disorders. Ann Intern Med 67:1195–1200, 1967
9. Charache S, Weatherall DJ, Clegg JB: Polycythemia associated with a hemoglobinopathy. J Clin Invest 45:813–822, 1966
10. Diggs LW: The sickle cell phenomenon. I. The rate of sickling in moist preparations. J Lab Clin Med 17:913–920, 1932
11. Diggs LW, Bibb J: The erythrocyte in sickle cell anemia. JAMA 112:695–701, 1939
12. Emmel VE: A study of the erythrocytes in a case of severe anemia with elongated and sickle-shaped red blood corpuscles. Arch Intern Med 20:586–599, 1917
13. Gelfand HM: Concepts of disease eradication and control. Pharos 37:13, Jan. 1974
14. Greenberg MS, Kass EH: Studies on the destruction of red blood cells. XIII. Observations on the role of pH in the pathogenesis and treatment of painful crisis in sickle cell disease. Arch Intern Med 101:355–363, 1958
15. Greenberg MS, Kass EH, Castle WB: Studies on the destruction of red blood cells. XII. Factors influencing the role of S hemoglobin in the pathologic physiology of sickle cell anemia and related disorders. J Clin Invest 36:833–843, 1957
16. Hahn EV, Gillespie EB: Sickle cell anemia: Report of a case greatly improved by splenectomy; experimental study of sickle cell formation. Arch Intern Med 39:223–254, 1927
17. Ham TH, Castle WB: Relation of increased hypotonic fragility and of erythrostasis to mechanism of hemolysis in certain anemias. Trans Assoc Am Physicians 55:127–132, 1940
18. Harris JW: Studies on the destruction of red blood cells. VIII. Molecular orientation in sickle cell hemoglobin solutions. Proc Soc Exp Biol Med 75:197–201, 1950
19. Harris JW, Brewster HH, Ham TH, et al: Studies on the destruction of red blood cells. X. The biophysics and biology of sickle cell disease. Arch Intern Med 97:145–168, 1956
20. Herrick JB: Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia. Trans Assoc Am Physicians 25: 553–561, 1910
21. Ingram VM: Gene mutations in human hemoglobin: The chemical difference between normal and sickle cell haemoglobin. Nature 180:326–328, 1957
22. Jandl JH, Simmons RL, Castle WB: Red cell filtration and the pathogenesis of certain hemolytic anemias. Blood 18:133–148, 1961
23. Jensen WN: Fragmentation and the "freakish poikilocyte." Am J Med Sci 257: 355–364, 1969
24. Jensen WN, Bromberg PA, Barefield K: Membrane deformation: A cause of the irreversibly sickled cell, abstracted, Clin Res 17: 464, 1969
25. Kan YW, Dozy AM, Alter BP, et al: Detection of the sickle gene in the human fetus: Potential for intrauterine diagnosis of sickle cell anemia. N Engl J Med 287:1–5, 1972
26. Klug PP, Lessin LS, Radice P: Rheological aspects of sickle cell disease. Arch Intern Med 133:577–590, 1974
27. Konotey-Ahulu FID: The sickle cell diseases: Clinical manifestations including the "sickle crises." Arch Intern Med 133:611–619, 1974
28. Lange RD, Minnich V, Moore CV: Effect of oxygen tension and of pH on sickling and mechanical fragility of erythrocytes from patients with sickle cell anemia and sickle cell trait. J Lab Clin Med 37:789–802, 1951
29. Magdoff-Fairchild B, Swerdlow PH, Bertles JF: Intermolecular oganization of deoxygenated sickle hemoglobin determined by X-ray diffraction. Nature 239:217–219, 1972
30. Neel JV: The inheritance of sickle cell anemia. Science 110:64–66, 1949
31. Pauling L, Itano H, Singer SJ, et al: Sickle cell anemia: A molecular disease. Science 110:543–548, 1949
32. Pearson HA, Spencer RP, Cornelius EA: Functional asplenia in sickle cell anemia. N Engl J Med 281:923–926, 1969
33. Reinhard EH, Moore CV, Dubach R, et al: Depressant effects of high concentrations of inspired oxygen on erythrocytogenesis: Observations on patients with sickle cell anemia with description of observed toxic manifestations of oxygen. J Clin Invest 23:682–698, 1944
34. Rosen FS: Sickle-cell disease and the properdin system. N Engl J Med 287:489–494, 1972
35. Rucknagel DL: The genetics of sickle cell anemia and related syndromes. Arch Intern Med 133:595–606, 1974
36. Scriver JB, Waugh TR: Studies on a case of sickle cell anemia. Can Med Assoc J 23:375–380, 1930
37. Shen SC, Castle WB, Fleming EM: Experimental and clinical observations on increased mechanical fragility of erythrocytes. Science 100:387–389, 1944
38. Shen SC, Fleming EM, Castle WB: Studies on the destruction of red blood cells. V. Irreversibly sickled erythrocytes: Their experimental production *in vitro*. Blood 4:498–504, 1949
39. Sherman LJ: The sickling phenomenon, with special reference to the differentiation of sickle cell anemia from the sickle cell trait. Bull J Hopkins Hosp 67:309–324, 1940
40. Singer K, Singer L: Studies on abnormal hemoglobins. VIII. The gelling phenomenon of sickle cell hemoglobin: Its biologic and diagnostic significance. Blood 8:1008–1023, 1953
41. Watson J: The significance of the paucity of sickle cells in newborn Negro infants. Am J Med Sci 215:419–423, 1948
42. Whitten CF: Effect of dietary protein on the renal concentrating process in sickle cell anemia. Am J Dis Child 115:262–266, 1968

Grune & Stratton Journals

BLOOD—THE JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

Ernst R. Jaffé, editor
Monthly *U.S.: $45.00 per year* *Foreign: $48.50 per year*

COMPREHENSIVE PSYCHIATRY

Official Journal of the American Psychopathological Association

Fritz A. Freyhan, editor
Bimonthly *U.S.: $28.00 per year* *Foreign: $31.00 per year*

JOURNAL OF PEDIATRIC SURGERY

Official Journal of the Surgical Section of the American Academy of Pediatrics, the British Association of Paediatric Surgeons, and the American Pediatric Surgical Association

C. Everett Koop, editor
Bimonthly *U.S.: $28.00 per year* *Foreign: $31.00 per year*

JOURNAL OF SPECIAL EDUCATION

Robert L. Kalapos, editor
Quarterly *U.S.: $18.50 per year* *Foreign: $19.50 per year*

METABOLISM—CLINICAL AND EXPERIMENTAL

James B. Field, editor
Monthly *U.S.: $38.00 per year* *Foreign: $41.00 per year*

PROGRESS IN CARDIOVASCULAR DISEASES

Edmund H. Sonnenblick, editor, and Michael Lesch, co-editor
Bimonthly *U.S.: $27.50 per volume of 6 issues* *Foreign: $30.50 per volume*

SEMINARS IN ARTHRITIS AND RHEUMATISM

John H. Talbott, editor
Quarterly *U.S.: $24.50 per volume of 4 issues* *Foreign: $27.50 per volume*

SEMINARS IN HEMATOLOGY

Peter A. Miescher and Ernst R. Jaffé, editors
Quarterly *U.S.: $24.00 per year* *Foreign: $27.00 per year*

SEMINARS IN NUCLEAR MEDICINE

Leonard M. Freeman and M. Donald Blaufox, editors
Quarterly *U.S.: $25.00 per year* *Foreign: $27.00 per year*

SEMINARS IN ONCOLOGY

John W. Yarbro, editor
Quarterly *U.S.: $22.50 per year* *Foreign: $24.50 per year*

SEMINARS IN ROENTGENOLOGY

Benjamin Felson, editor
Quarterly *U.S.: $25.00 per year* *Foreign: $28.00 per year*

TRANSPLANTATION PROCEEDINGS

An Official Publication of the Transplantation Society

Felix T. Rapaport, editor
Quarterly *U.S.: $31.75 per year* *Foreign: $33.75 per year*

AUDIO JOURNAL REVIEW—GENERAL SURGERY

Robert N. McClelland, editor
Monthly *U.S.: $70.00* *Foreign: $90.00*

AUDIO JOURNAL REVIEW IN OPHTHALMOLOGY

Douglas R. Anderson, editor
Monthly *U.S.: $70.00* *Foreign: $90.00*

AUDIOLOGY: AN AUDIO JOURNAL FOR CONTINUING EDUCATION
COMMUNICATIVE DISORDERS: AN AUDIO JOURNAL FOR CONTINUING EDUCATION

Larry J. Bradford, editor
Monthlies *U.S.: $70.00 each* *Foreign: $90.00 each*