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Sequential Electron Micrography of Sickling†

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HERRICK, in 1910, first described sickle-cell anemia (1). His photomicrographs depicted sickle and crescent-shaped erythrocytes in direct blood smears. In 1917, Emmel (2) reported that a drop of blood from such a patient exhibited increasing numbers of sickle cells in a sealed preparation, thus discovering the sickling phenomenon in vitro. In 1923, Sydenstricker and his group (3) succeeded in accelerating the sickling process by adding bile or bile salts to their preparations. A few years later Hahn and Gillespie (4) showed that sickling resulted from a fall in the partial pressure of oxygen.

In 1940 Sherman (5) reported that sickle cells exhibited birefringence under the polarizing microscope. Pauling, Itano, Singer, and Wells, in 1949 (6) demonstrated that an abnormal form of hemoglobin was associated with cells capable of exhibiting the sickling phenomenon. This finding made possible the recognition of intracorpuscular hemoglobin crystallization within such erythrocytes through study of the sicklng process with the electron microscope (7). It is the purpose of this study to present sequential electron micrographs of the structural alterations undergone by both the surface ultrastructure and the intracorpuscular erythrocytic components during the sickling phenomenon.

Materials and Methods

Blood from two patients with sickle-cell anemia and from four patients with sickling trait was studied. A short clinical summary of each case is appended. The technique of Beck and Hertz was modified for electron microscopy (8,9) as follows: 0.2 ml blood was introduced into each of a series of Wassermann tubes in which had been previously placed a small amount of powdered heparin. A saline-formalin fixative was then added directly to the first two tubes of each series (0 minutes anoxia). The remaining tubes containing heparinized blood were covered with paraffin oil and allowed to stand for increasing intervals of time at room temperature. At successive timed stages in the development of anoxia in these preparations formalin-saline was added to the erythrocytes undergoing the sickling phenomenon. Thereafter the erythrocytes were allowed to stand with the fixative, under oil, for an additional 48 hours. The formalinized cells were then removed from the paraffin-oil sealed tubes and washed three times in isotonic saline and three times in distilled water. They were then spread thinly over formvar- or collodion-covered glass slides and dried.

Erythrocytes from patients with sickle-cell anemia were fixed in this way at 0; 2.5′; 17′; 30′; 50′; 1^h; $1^h10'$; $1^h50'$; $2^h30'$; 3^h; $4^h30'$; $4^h50'$; 19^h; 20^h; 21^h; 23^h; $28^h50'$; 48^h; 49^h; $66^h50'$; 72^h; $90^h50'$; and 168^h after the tubes were sealed. Cells from patients with the sickling trait were similarly fixed at 2′; 15′; $1^h2'$; $1^h5'$; 2^h; $2^h30'$; $2^h40'$; 3^h; 4^h; $4^h10'$; $6^h10'$; 20^h; 24^h; 48^h; 144^h after the tubes were sealed.

Direct specimen mounting was obtained by placing several specimen screens over suitable erythrocyte-containing areas under light microscope observation. The collodion or formvar was cut from the end of the glass slide and cellophane tape was affixed over both the specimen screens and the coated and uncoated portions of the slide. Removal of the tape then removed the erythrocyte-bearing film with it away from the glass slide. The specimen screens, between the erythrocyte-bearing film and the cellophane tape, were next cut away from the cellophane tape and studied directly in the electron microscope (10).

Figures 1-28 are electron micrographs of erythrocytes so prepared. Figures 1-10 and 24-28 are representative of 492 micrographs of erythrocytes from patients with sickle-cell anemia. Figures 11-23 are representative of 243 micrographs of erythrocytes from patients with sickle-cell trait.

Results

Formalin fixation at successive times arrested the sickling process at many stages in the transformation of the biconcave disc to sickle cells. Direct visualization of the aggregation of hemoglobin within the erythrocyte in the sickling phenomenon was obtained by the above described modification of the Beck-Hertz technique (8) which so slowed the sickling processes that sequential structural changes could be defined.

Sickle-cell anemia

Figures 1-10 depict such progressive alterations in the erythrocytes of one of our patients (Case 1) with sickle-cell anemia. The first change observed was a peripheral aggregation of hemoglobin which effected an increase in diameter of the promeniscocytes (11) over that of the normal erythrocytes. Such peripheral aggregation of the hemoglobin led to focal thinning of the disc allowing passage of electrons. In such thinned corpuscular centers, even upon immediate fixation, anisotropoid angulation of the central hemoglobin aggregates was observed (Fig. 1). Commonly (Fig. 2), there was an irregular aggregation of hemoglobin in one side of the cell with occasionally a suggestion of filamentation of the opposing surface ultrastructure from which the hemoglobin had largely withdrawn (Fig. 2). At 50′ after sealing, a few peripheral spicules were visible (Fig. 3). An hour later (Fig. 4), peripheral spiculation was more pronounced and the depth of focus permitted by electron micrography revealed that such spiculation was founded on similar underlying structural changes in the hemoglobin aggregates themselves. At approximately five hours of anoxia the intense periph-

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eral spiculation of the eccentric, hemoglobin-containing intracorpuscular masses indicated an early stage of crystallization (Figs. 5, 6, 7). This eccentric massing of the hemoglobin, offering labile but definite angulation, was the key to the structural manifestations of the sickling process.

Further sickling of the sickle-cell anemia cells of this patient (Fig. 8) at $28^h50'$ of anoxia eventuated in marked polar filamentation in which hemoglobin and surface ultrastructure both played important roles. Prolongation of the hemoglobin into the filamentous processes for great distances is apparent from an examination of Fig. 8. Less obvious is the nature of the configuration now adopted by the hemoglobin aggregates. The basic fusiform or spindle-shaped structure of the aggregates has been molded into the classical sickle or crescent shape by forces inherent within still intact, large arcs of the surface ultrastructure. At $66^h50'$ of anoxia (Fig. 9), this modified but fundamentally fusiform configuration had been accentuated. It should be observed (Fig. 9) that the sites of membranous-hemoglobiniferous bipolar filamentation were at the very regions where the forces of tactoid crystallization (12) were most exerted. At $90^h50'$ after sealing the preparations (Fig. 10), the hemoglobin aggregation was similarly well defined after its considerable withdrawal had led to near apposition of large areas of the remaining surface ultrastructure.

Sickle-cell trait

The progressive structural changes of the erythrocytes from patients with sickle-cell trait were similarly initiated by peripheral migration of hemoglobin, central thinning of the disc, and an increase in corpuscular diameter as depicted in Fig. 11. There was an abortive filamentation after 15′ of anoxia (Fig. 12). More often there was, again, an irregular aggregation of the hemoglobin to one side of the cell (Fig. 13), so that at two hours of anoxia one or two (Fig. 14) bar-like masses of hemoglobin were present with near apposition of the surface ultrastructures overlying the remaining regions. In the important, early, eccentric massing of hemoglobin aggregates (Fig. 15) there was less spiculation of angulation of these aggregates, a finding which confirmed the observation of Pauling and his associates that trait cells possess an admixture of defective and normal hemoglobin. Yet the right-angled C-blocking of Fig. 16 after 2^h of anoxia bears evidence of striking complementariness of the hemoglobin mixtures.

Sequential alterations in the erythrocytes of Case 2, with the sickle-cell trait, are depicted in our Figs. 17-20, which also serve as a continuation of the series of changes shown in Figs. 11-16. When aggregation of hemoglobin about the entire corpuscular rim was accompanied by peripheral angulation of the hemoglobin aggregates (Fig. 17), the holly-wreath form of Sherman appeared ($2^h40'$ anoxia). Following the customary eccentric massing of hemoglobin (Fig. 18), a slightly spiculated bar of hemoglobin was formed (Fig. 19). Although the normal hemoglobin as well as the non-hemoglobin portion of the interior of the sickle-trait corpuscles tended to round out or lessen the degree of early crystallization of the defective hemoglobin (Fig. 15), the trait cell eventuated in complete sickling such as is depicted in Fig. 20, after 24^h anoxia. Polar filamentation, however, was inconspicuous in the sickling-trait cells (Figs. 20, 23) in contrast to its prominence (Figs. 8, 9) in the sickling-anemia cells.

Change of hemoglobin aggregation

If aggregation of hemoglobin about the corpuscular rim was incomplete at a sinlge locus, an appearance of pseudo-rim-rupture was obtained (Fig. 21), an uncommon finding in our material. Figures 22-24 are illustrative of Ponder's (11) original concept of sickling which he described using the light microscope, as a breakdown of continuity at the site of focal thinning (Fig. 22), followed by straightening of the opposite hemoglobin-containing arc (Fig. 23). Straightening of the hemoglobin-containing arc continues until contraction occurs along its entire length (Fig. 24), so that the final length of the arc is much less than the perimeter of the original disc. It is equally apparent in our electron micrographs that the form outline in sickling as depicted in Figs. 21-24 follows the change in aggregation of the hemolgobin, the fringe-like material of the sickle appearing as veil-like material within the concavity.

When the forces of crystallization were not operative early in a planoparallel direction, central thinning and anisotropoid angulation were less apparent and early crystallization was evidenced instead by peripheral angulation or by the geometric configurations shown in Figs. 25 and 26. *Unipolar* filamentation and unipolar sickling which sometimes occurred after only 17′ of anoxia (Fig. 27) substantiates the concept of underlying crystallization of hemoglobin operative, in this instance, in only a portion of the corpuscle. As further evidence for such intracorpuscular crystallization, there was the occurrence of hemoglobin aggregation in such a way as to produce the sickling phenomenon in two directions or lines of force at right angles to each other (Fig. 28).

Discussion

Hemoglobin crystallization

Insight into the gradual change from the lesser degree of hemoglobin orderliness present in the promeniscocyte to the greater degree found in the sickled erythrocyte can be gained from earlier observations with the light microscope. Herrick (1) concluded his classic paper by suggesting that "some unrecognized change in the composition of the corpuscle itself may be the determining factor" for these peculiar formations. In 1923 Sydenstricker, et al (3) observed that the cells became darker and more "brassy" and suggested that sickling was accompanied by concentration of the hemoglobin. In 1930 Graham and McCarty (13) encountered an individual in whom the cells drew out into long, needle-like bodies. In 1939 Diggs and Bibb (14) illustrated one, two, and three foci of hemoglobin condensation as sickling occurred. In addition, these authors pointed out that the erythrocytes of some patients with sickle-cell anemia retained their hemoblogin even in distilled water.

Sydenstricker, et al and Ponder (3,11) observed increased plasticity of the corpuscles early in sickling, and Murphy and Shapiro (15) reported that the early corpuscular flexibility was lost in later sickling, giving the cell an appearance "as fixed and rigid as a crystal of ice."

Although the intermediate powers of the electron microscope made apparent intracorpuscular hemoglobin crystallization as the structural basis of the sickling phenomenon (7), interpretation of

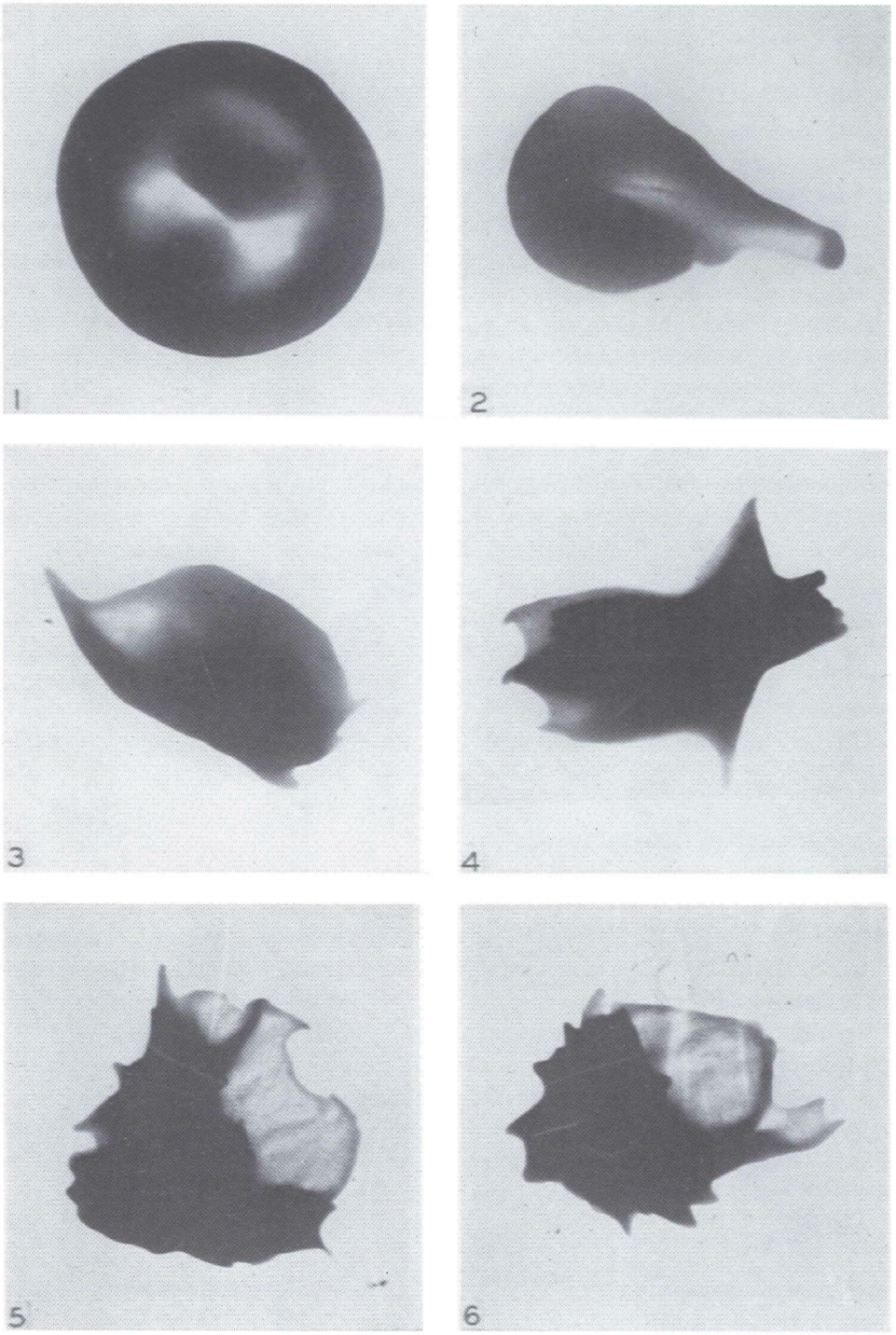


Fig. 1. RBC in sickle-cell anemia (Case 1). Peripheral aggregation and central anisotropoid angulation of hemoglobin. Immediate formalin fixation. (X 7500) **Fig. 2.** RBC in sickle-cell anemia (Case 1). Abortive filamentation. 17′ anoxia. Formalin fixation. (X 7500) **Fig. 3.** RBC in sickle-cell anemia (Case 1). Early peripheral spiculation. 50′ anoxia. Formalin fixation (X 7500) **Fig. 4.** RBC in sickle-cell anemia (Case 1). Peripheral and underlying hemoglobin spiculation. $1^{h}50'$ anoxia. Formalin fixation. (X 7500) **Fig. 5.** RBC in sickle-cell anemia (Case 1). Eccentric massing of hemoglobin with angulation of masses, evidence of hemoglobin crystallization. $4^{h}50'$ anoxia. Formalin fixation. (X 7500) **Fig. 6.** RBC in sickle-cell anemia (Case 1). Similar to Fig. 5. $4^{h}50'$ anoxia. Formalin fixation. (X 7500).

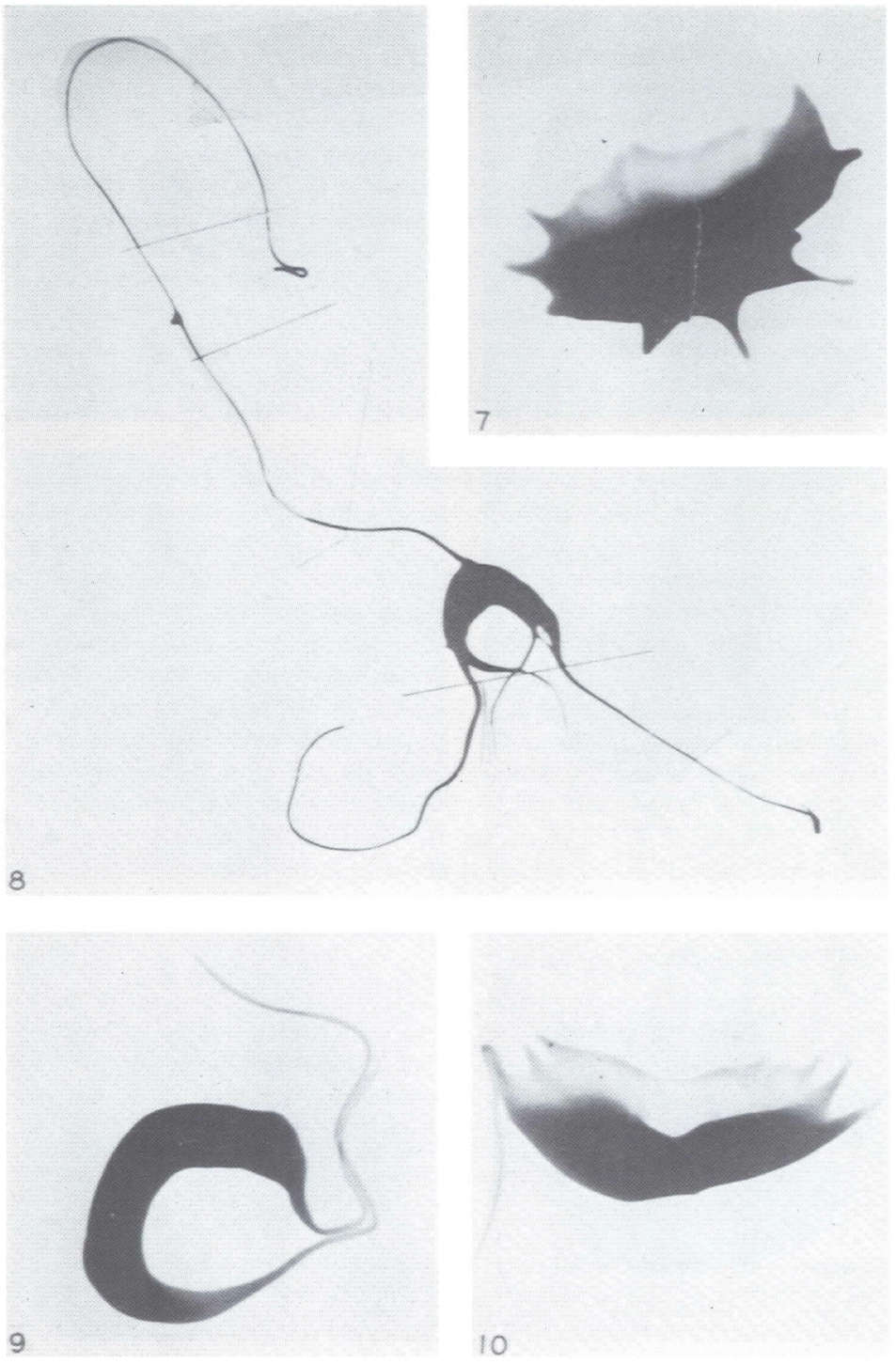


Fig. 7. RBC in sickle-cell anemia (Case 1). Similar to Fig. 5. $4^{h}50'$ anoxia. Formalin fixation. (X 7500) **Fig. 8.** RBC in sickle-cell anemia (Case 1). Extensive membranous-hemoglobiniferous bipolar filamentation with spindle-shaped corpuscular hemoglobin aggregates molded into a crescent. $28^{h}50'$ anoxia. Formalin fixation. Composite. (X 3500) **Fig. 9.** RBC in sickle-cell anemia (Case 1). Classical sickling with accentuation of the fundamentally fusiform configuration of the hemoglobin. $66^{h}50'$ of anoxia. Formalin fixation. (X 6000) **Fig. 10.** RBC in sickle-cell anemia (Case 1). Complete sickling similar to Fig. 9. $90^{h}50'$ anoxia. Formalin fixation. (X 6000)

the nature of the crystalline forms has afforded some difficulties. Some of these difficulties have been resolved by the growing awareness of a continued spectrum of structure which exists from complete disorder or amorphousness to a most ordered crystalline state of matter. In fact it has now been established that very little if any protoplasm is entirely structureless: *structura omnis e structura* (16). In other words, once the stage of amorphousness has been left, order of some degree begins. Recognition of this gamut is afforded by the numerous descriptive terms that have been introduced in this respect: fliessende Kristalle, fluessige Kristalle, tactoids, liquid crystals, partial crystals, paracrystalline state, etc. (17).

It should be emphasized that the hemoglobin within even normal erythrocytes has a definite orderliness which Perutz (18) compared to the degree of regularity to be found in a liquid metal and which Ponder (19) assessed as being "almost on the threshold of gelation." After direct visualization of the crystalline forms of hemoglobin within sickled cells, as had been predicted by Pauling, et al (6), further resolution of the problem was afforded almost at once, independently, by the studies of Harris and of Perutz and his associates (20,21).

Pauling, et al had proposed that with reduced oxygen tension, the molecules of sickle-cell hemoglobin, because of their increased complementariness, were capable of entering into chains and three-dimensional frameworks. When solutions of sickle-cell hemoglobin were in a deoxygenated and viscous state at concentrations comparable to those of intracellular hemoglobin, Harris observed spindle-shaped bodies 1-15 μ in length in sealed wet preparations. These bodies proved to be birefringent and disappeared upon rexoygenation of the hemoglobin. From such data Harris proposed that the sickled red cell was a membrane-covered tactoid (22,12).

Perutz and his associates (20,21) amplified the polarization studies of Sherman and of Harris. Furthermore, they showed that reduced sickle-cell hemoglobin was far less soluble than sickle-cell oxyhemoglobin, reduced normal hemoglobin, and normal oxyhemoglobin. They concluded that the solubility of reduced sickle-cell hemoglobin was too low for more than one seventh to be present in solution within the erythrocyte.

Abundant confirmation and extension of these concepts of the sickling process have been afforded by more recent investigations in the fields of electron microscopy (23,24) and physicochemical analysis (25).

Disturbances of surface ultrastructure

Disturbances of the surface ultrastructure, in addition to defective hemoglobin, increasingly have been implicated in sickling. Sydenstricker, Mulherin, and Houseal (3) called attention to the pronounced filamentation of sickled erythrocytes. Further they were able to produce acceleration of sickling by the addition of surface active agents, a finding confirmed in our own laboratory (26). In some cells Diggs and Bibb (14) had watched "barbs of cytoplasm gradually protrude from the surface" without visible movement or condensatory shift in hemoglobin. Erickson and her associates (27) established an abnormally high lipid content for the erythrocytes of sickle-cell anemia. In as much as filamentation (Figs 8, 9) plays such a prominent role in the sickling of erythrocytes from sickle-cell-anemia patients, further electron micrographic studies of filament formation are already under way in our laboratory.

Summary

Structural changes occurring during the sickling process were presented in sequential electron micrographs of erythrocytes obtained from two patients with sickle-cell anemia and four patients with sickle-cell trait. Direct visualization of intracorpuscular crystallization of hemoglobin in the sickling process was attained by modification of the Beck-Hertz technique which slowed the phenomenon. Formalin fixation at successive times arrested sickling at progressive stages in the transformation of the normal appearing promeniscocyte to the sickled cell. Thinning of the corpuscular center was accompanied by anisotropoid angulation of central aggregates of hemoglobin.

The next and primary structural abnormality was eccentric massing of the hemoglobin accompanied by intense peripheral spiculation and definite but labile angulation of the hemoglobin aggregates, indicative of intracorpuscular crystallization. Further evidence of this concept was presented by the geometric configurations assumed by the cells and by the phenomenon of unipolar sickling. Complete sickling of the sickle-cell anemia cells eventuated in intense polar filamentation in which hemoglobin and surface ultrastructure both played important roles.

Sickle cells are sickle-shaped because of the distortion curvature impressed upon otherwise fusiform hemoglobin tactoids by the forces inherent within the still intact portions of the surface ultrastructures. Comparison of the sickling phenomenon in trait corpuscles revealed less spiculation or angulation of the eccentric, massed hemoglobin aggregates in the early phases and inconspicuous polar filamentation in the completely sickled trait-cells.

Case Reports

Case 1

A 17-year-old black woman with sickle-cell anemia was first seen on March 13, 1948. She complained of fever (101° F) and soreness in the joints of the right shoulder, right elbow, and left ankle. There was pallor of the mucous membranes and an icteric tint to the sclerae. The heart was slightly enlarged. The affected joints were swollen and hot to the touch. Hemoglobin was 6.7 gm/100 cc, reticulocytes were 19.8%. The peripheral blood smear and sealed preparations showed marked sickling. The bone marrow presented intense normoblastic hyperplasia. There were four subsequent admissions: one for another hemolytic crisis; three for the delivery of living infants. The first two children were restudied for sickling at an age when sickling becomes manifest and were found to be positive for the sickling trait. The first child served as the second subject in this study. The hemoglobin of the mother ranged from 5.2 gm to 7 gm/100 cc in the course of her study.

Case 2

Daughter of Case 1. Sickle cell trait. Hemoglobin, 10.7 gm/100 cc.

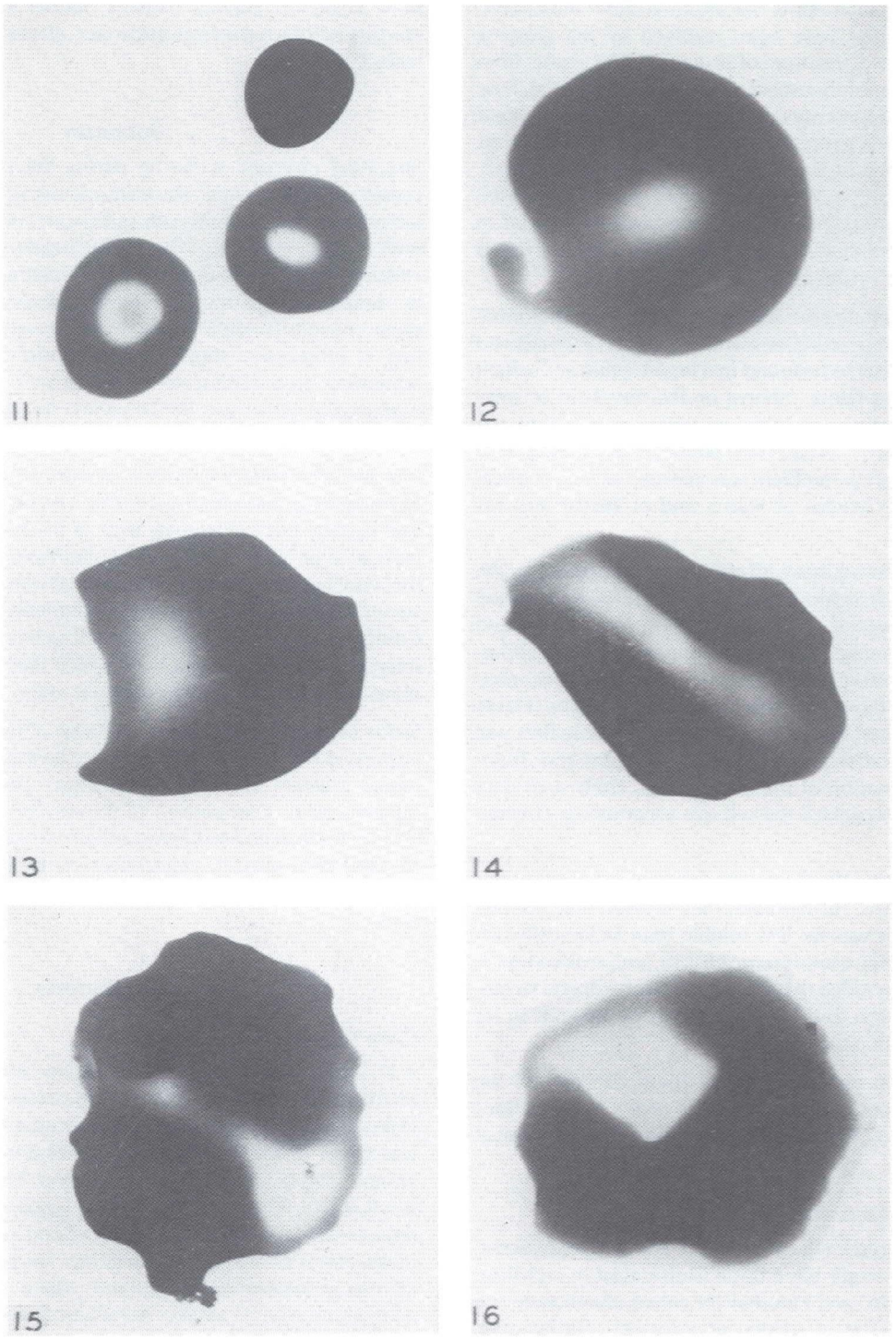


Fig. 11. RBC in sickle-cell trait (Case 4). Central thinning and increase in corpuscular diameter. 15′ anoxia. Formalin fixation. (X 3500) **Fig. 12.** RBC in sickle-cell trait (Case 4). Early filamentation. 15′ anoxia. Formalin fixation. (X 5000) **Fig. 13.** RBC in sickle-cell trait (Case 6). Irregular aggregation of hemoglobin to one side. $1^{h}5'$ anoxia. Formalin fixation (X 5000) **Fig. 14.** RBC in sickle-cell trait (Case 5). Two bar-like masses of hemoglobin. 2^{h} anoxia. Formalin fixation. (X 5000) **Fig. 15.** RBC in sickle-cell trait (Case 5). Compare with Figs. 5 and 6. Note diminished spiculation and angulation of hemoglobin aggregates in trait cell. 2^{h} anoxia. Formalin fixation. (X 5000) **Fig. 16.** RBC in sickle-cell trait (Case 4). Note right-angle blocking of complementary hemoglobin mixtures. 2^{h} anoxia. Formalin fixation. (X 5000)

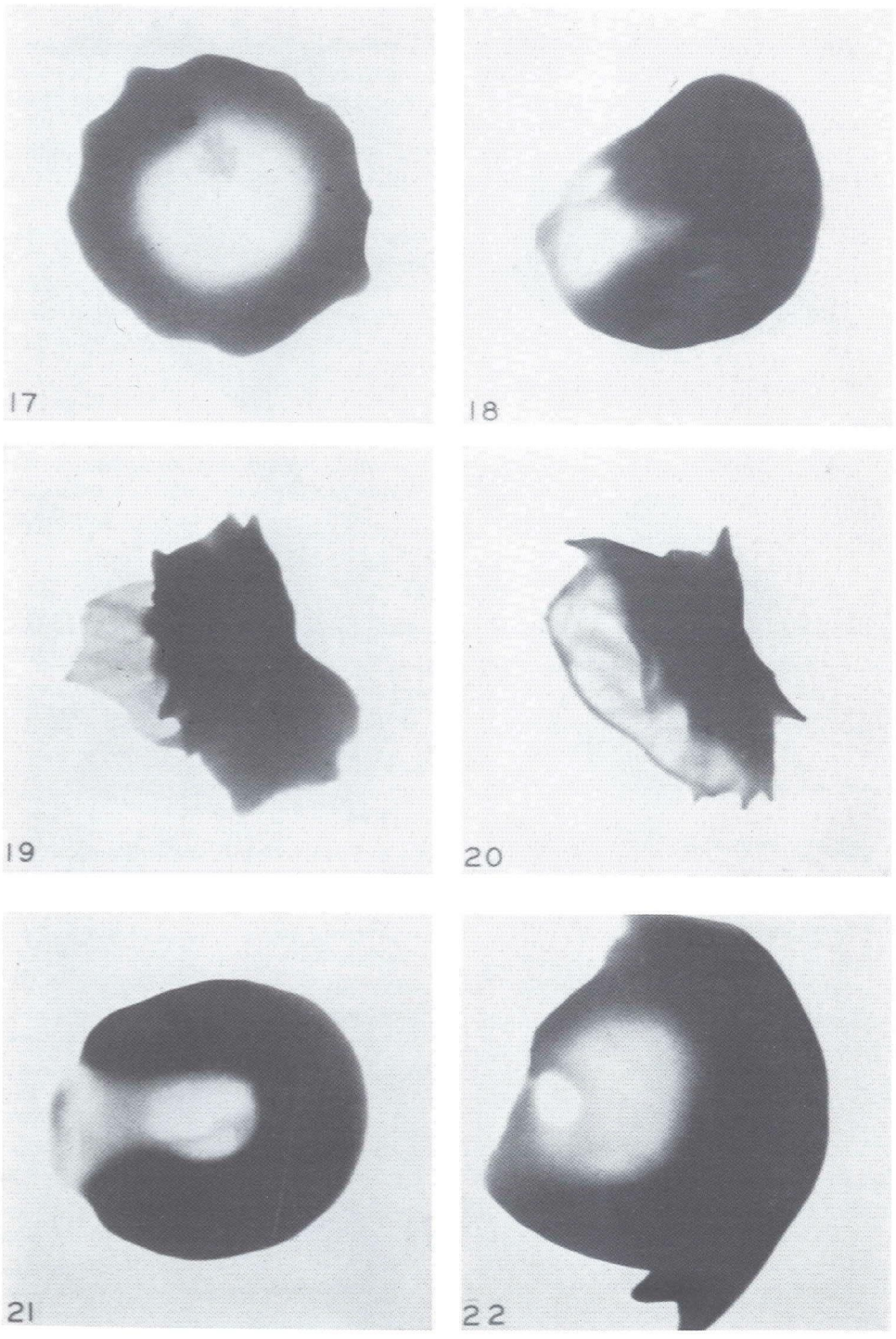
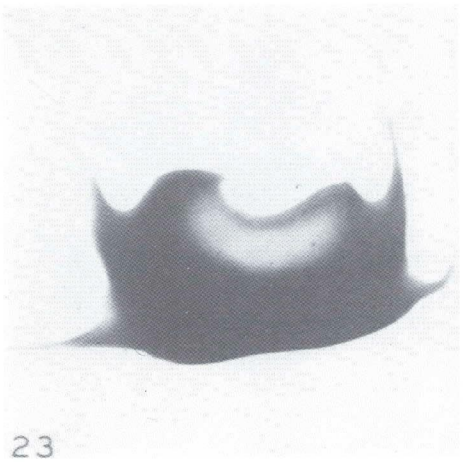
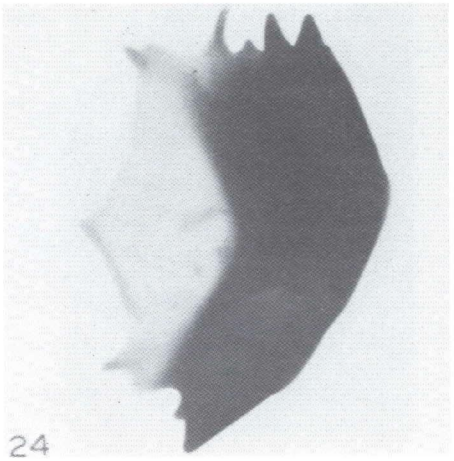
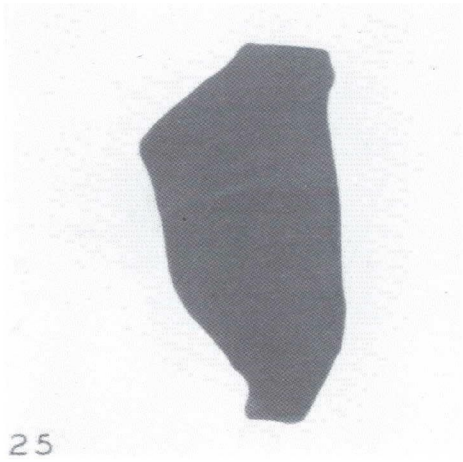
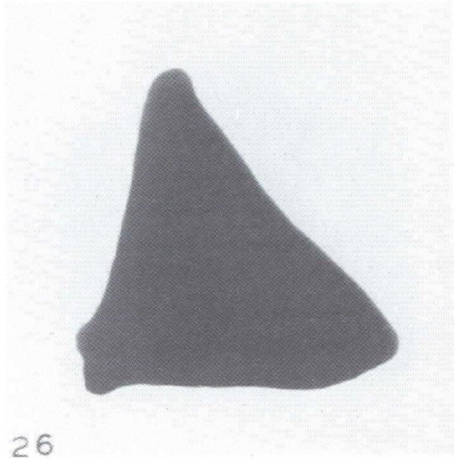


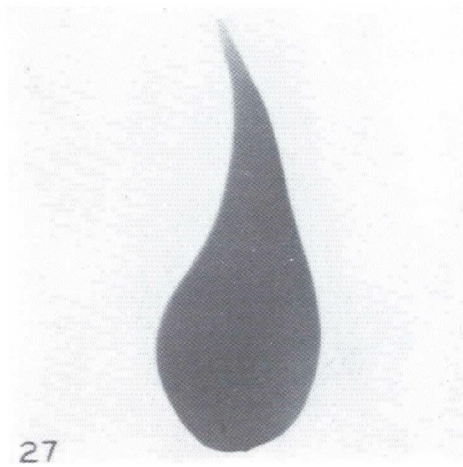
Fig. 17. RBC in sickle-cell trait (Case 2). Peripheral aggregation and angulation of hemoglobin, the holly wreath form. $2^h40'$ anoxia. Formalin fixation. (X 7500) **Fig. 18.** RBC in sickle-cell trait (Case 2). Eccentric massing of hemoglobin. $6^h10'$ anoxia. Formalin fixation. (X 7500) **Fig. 19.** RBC in sickle-cell trait (Case 2). Hemoglobin in form of slightly spiculated bar. $6^h10'$ anoxia. Formalin fixation. (X 7500) **Fig. 20.** RBC in sickle-cell trait (Case 2). Completely sickled trait cell. Note inconspicuous filament formation, compare with anemia filamentation in Figs. 8 and 9. 24^h anoxia. Formalin fixation. (X 5000) **Fig. 21.** RBC in sickle-cell trait (Case 2). Incomplete peripheral margination of hemoglobin, pseudo-rim-rupture. $4^h10'$ anoxia. Formalin fixation. (X 7500) **Fig. 22.** RBC in sickle-cell trait (Case 6). Breakdown in corpuscular continuity at site of focal thinning. 48^h anoxia. Formalin fixation. (X 5000)











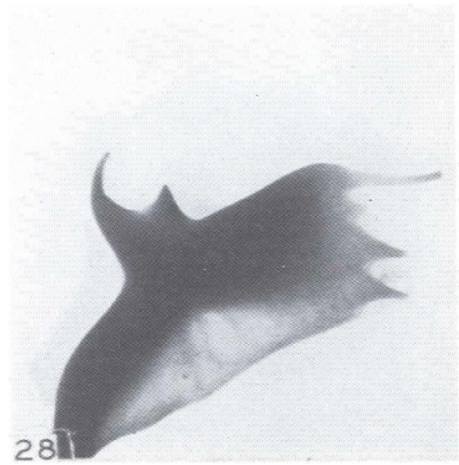


Fig. 23. RBC in sickle-cell trait (Case 5). Straightening of the hemoglobin-containing arc opposite the area of thinning. 48^{h} anoxia. Formalin fixation. (X 5000) **Fig. 24.** RBC in sickle-cell anemia (Case 3). Straightening of the hemoglobin-containing arc with contraction. $1^{h}10'$ anoxia. Formalin fixation. (X 7500) **Fig. 25.** RBC in sickle-cell anemia (Case 3). Geometric hemoglobin configuration. $2^{h}30'$ anoxia. Formalin fixation. (X 7500) **Fig. 26.** RBC in sickle-cell anemia (Case 3). Geometric hemoglobin configuration. $4^{h}30'$ anoxia. Formalin fixation. (X 7500) **Fig. 27.** RBC in sickle-cell anemia (Case 1). Unipolar sickling with remaining portion of the corpuscle as yet unaffected. 17′ anoxia. Formalin fixation. (X 6000) **Fig. 28.** RBC in sickle-cell anemia (Case 1). Hemoglobin aggregation with production of sickling in two directions at right angles to each other. See text. $90^{h}50'$ of anoxia. Formalin fixation. (X 6000)

Case 3

A 25-year-old black woman had been discharged from the Armed Services because of sickle-cell anemia. First seen by us on July 1, 1947, she was admitted to the hospital because of severe right lower quadrant pain of two days' duration. Physical examination was negative except for marked pallor. Sealed preparations as well as direct smears of the peripheral blood showed marked sickling. Hemoglobin was 7.5 gm/100 cc. The patient's symptoms disappeared on symptomatic therapy. There were four subsequent admissions: for another abdominal crisis; twice for the delivery of normal living infants without difficulty; and for a tonsillectomy. Hemoglobin ranged from 6.0 gm to 7.7 gm/100 cc; reticulocytes from 1.0 to 27.6%.

Case 4

A 25-year-old black woman was first admitted in November, 1946 with sickle-cell trait. Two admissions with normal deliveries. Sealed sickling test positive but no findings to suggest sickle-cell anemia. Hemoglobin ranged from 11.5 gm to 13.4 gm/100 cc.

Case 5

A 51-year-old black man was first admitted in October, 1944 with a fracture of his left tibia. Sealed sickling test was positive. There was nothing in his history to suggest sickle-cell anemia. He was readmitted in February, 1949, in uremia with a diagnosis of carcinoma of the prostate. Hemoglobin values ranged from an original 14.9 gm down to 12.2gm/100 cc.

Case 6

A 48-year-old black man with sickle-cell trait was observed seven years including six hospitalizations for the treatment of hypertension and hypertensive heart disease. Sealed sickling test was positive, but there was no history to suggest sickle-cell disease. Numerous blood counts were normal.

Acknowledgments

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References

1. Herrick JB. Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia. Arch Int Med 1910;6:517.
2. Emmel VE. A study of the erythrocytes in a case of severe anemia with elongated and sickle-shaped red blood corpuscles. Arch Int Med 1917;20:586.
3. Sydenstricker VP, Mulherin WA, Houseal RW. Sickle cell anemia. Report of two cases in children with necropsy in one case. Am J Dis Child 1923;26:132.
4. Hahn EV, Gillespie EB. Sickle cell anemia: Report of a case greatly improved by splenectomy: Experimental study of sickle cell formation. Arch Int Med 1927;29:233.
5. Sherman IJ. The sickling phenomenon with special reference to the differentiation of sickle cell anemia from sickle cell trait. Johns Hopkins Hosp Bull 1940;67:309.
6. Pauling L, Itano HA, Singer SJ, Wells IC. Sickle cell anemia, a molecular disease. Science 1949;110:543.
7. Rebuck JW, Sturrock RM, Monaghan EA. Sickling processes in anemia and trait erythrocytes with the electron microscopy of their incipient crystallization. Fed Proc 1950;9:340.
8. Beck JSP, Hertz CS. Standardizing sickle cell method and evidence of sickle cell trait. 1935;5:325-32.
9. Rebuck JW, Woods HL, Monaghan EA. Electron microscopy of sickle cells. Proc Soc Exper Biol Med 1948;68:220.
10. Rebuck JW. Structural changes in sensitized human erythrocytes observed with the electron microscope. Anat Rec 1953;115:591-614.
11. Ponder E. The sickling phenomenon and its bearing on the problem of red cell structure. J Exper Biol 1945;21:77.
12. Bernal JD, Fankuchen I. X-ray and crystallographic studies of plant virus preparations. J Gen Physiol 1941-42;25:111.
13. Graham GS, McCarty SH. Sickle cell (meniscocytic) anemia. South Med J 1930;23:598.
14. Diggs LW, Bibb J. Erythrocyte in sickle cell anemia; morphology, size, hemoglobin content, fragility and sedimentation rate. JAMA 1939;112:695.
15. Murphy RC, Shapiro S. Sickle cell disease. Arch Int Med 1944;74:28.
16. Frey-Wyssling A. Submicroscopic morphology of protoplasm. New York: Elsevier, 1953.
17. Hober R. Physical chemistry of cells and tissues. Philadelphia: Blakiston, 1945.
18. Perutz MF. Submicroscopic structure of the red cell. Nature 1948;161:204.
19. Ponder E. Hemolysis and related phenomena. New York: Grune and Stratton, 1948.
20. Perutz MF, Liquori AM, Eirich F. X-ray and solubility studies of the haemoglobin of sickle-cell anemia patients. Nature 1951;167:929.
21. Perutz MF, Mitchison JM. State of hemoglobin in sickle-cell anemia. Nature 1950;166:677.
22. Harris JW. Studies on the destruction of red blood cells. VIII. Molecular orientation in sickle cell hemoglobin solutions. Proc Soc Exper Biol Med 1950;75:192.
23. Bessis M, Bricka M, Breton-Gorius J. Different aspects de la surface des erythrocytes falciformes observés au microscope électronique. Rev Hemat 1953;8:222.
24. Bessis M, Bricka M, Breton-Gorius J, Tabuis J. New observations on sickle cells with special reference to their agglutinability. Blood 1954;9:39.
25. Singer K, Singer L. Studies on abnormal hemoglobins: VIII. The gelling phenomenon of sickle cell hemoblobin: Its biologic and diagnostic significance. Blood 1953;8:1008.
26. Rebuck JW, Monto RW, Sturrock RM. The structural basis of sickling and its electron microscopy. Am J Path 1952;28:530.
27. Erickson BN, Williams HH, Hummell FC, Lee P, Macy IG. The lipid and mineral distribution of the serum and erythrocytes in the hemolytic and hypochromic anemias of childhood. J Biol Chem 1937;118:569.