An acid-insoluble (trichloroacetic acid) precipitate was prepared and collected onto glass-fiber filter pads, and the radioactivity was assessed with liquid scintillation counting techniques (*5*). The *Ag-B* genotypes of the backcross progeny were identified by red cell hemagglutination with specific isoimmune reagents (*4*). Test skin grafts were prepared from the ears of each R_2 member and were transplanted to an appropriate host (*6*). Grafts were scored daily following the initial 8-day inspection.

The results of the tests conducted with animals of the two R_2 populations were as follows: (i) Significant incorporation of radioactive thymidine was always observed in the leukocyte cultures when the R_2 and the parental strain donors were incompatible at the *Ag-B* locus; when skin was grafted from these particular R_2 donors to the appropriate parental strain, host rejection was complete within 12 days (Table 1). (ii) No appreciable in vitro reactions were incited if the R_2 and parental strain donors of the leukocyte mixtures were alike at the *Ag-B* locus. Skin grafts from backcross donors compatible at this locus survived for varying periods of time (Table 1). Of particular interest was the lack of reactivity in cultures of 8 of 22 animals of this group whose skin grafts were destroyed by the 13th day (Table 1). This is illustrated in Fig. 1, a typical experiment, where the *Ag-B* compatible leukocytes of male No. 6 failed to stimulate those of Lewis origin, even though skin from this animal was rejected by a Lewis host as promptly as skin from male No. 5 and female No. 1, two *Ag-B* incompatible animals whose leukocytes did induce a marked in vitro response.

The results of these experiments indicate that the proliferative response occuring in mixed leukocyte cultures is highly selective in distinguishing between two classes of donor-host combinations which exhibit acute homograft reactions. One results from antigenic differences determined by a major histocompatibility locus—the *Ag-B*—and the other stems from a multiplicity of weaker histocompatibility factors which probably act synergistically (*7*).

These results raised the question of whether immunosuppressive procedures might prolong to different extents the survival of grafts derived from these two groups of donors on parental strain recipients. To provide an answer, three panels of Lewis rats were challenged with two skin grafts, one from an *Ag-B* incompatible and one from an *Ag-B* compatible member of the Lewis/DA × Lewis backcross population whose skin had previously been rejected by Lewis animals by the 8th day. One of these panels of bilaterally grafted animals received a single intraperitoneal inoculation of cyclophosphamide (7.5 mg/100 g) 4 days after grafting, another was inoculated with the drug at 4, 6, and 8 days (2 mg/100 g), and a third was untreated.

The results (Table 2) indicated that a difference in the two groups did exist; the grafts from the *Ag-B* compatible donor fared better than did those from the *Ag-B* incompatible donor when grafted simultaneously on the same drug-treated recipients. The fact that this difference was not more pronounced is not surprising, since the lives of skin homografts, in contrast to those of kidney, are known to be notoriously difficult to prolong with immunosuppressive measures (*8*). For this reason an even greater disparity might be expected if kidneys, rather than skin, were used.

Taken together, these results suggest that although the mixed leukocyte reaction may not be able to detect all levels of histoincompatibility, it may be useful in excluding those combinations which are least amenable to immunosuppressive therapy. In this regard it should be noted that in man there is evidence that a single locus, with multiple alleles, is also responsible for mixed leukocyte reactions (*9*).

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Microincision of Sickled Erythrocytes by a Laser Beam

Abstract. *Increased mechanical fragility of the sickled red cell is thought to be important in the genesis of the hemolytic process in sickle-cell disease. Sickled cells were observed cinematographically after microincision by a ruby-laser beam. Distortion and charring invariably occurred at the site of injury, and with small injuries there was no further cell change. With larger injuries, variably rapid retraction of spicules occurred accompanied by sphering of the cell. In some cases, progressive loss of hemoglobin accompanied and followed the changes in shape; in others the sphered cell still contained hemoglobin. Regardless of the mechanisms involved in these changes in vitro, the observations may be applicable to destruction of sickled cells in vivo. We suggest that the cells are subject to avulsion of rigid cellular processes as a result of mechanical injury incurred in normal circulation. Such injured cells may undergo either immediate hemolysis or transformation into spherocytes which are subject to erythophagocytosis.*

Erythrocytic sickling in sickle-cell disease, caused by intracellular polymerization of deoxygenated sickle hemoglobin (*1*), is considered responsible for the major features of the disease, vascular stasis, occlusion and thrombosis, and hemolytic anemia (*2–4*). Our experiments are concerned with the relationship of physical damage of the sickled cell to hemolysis. We have directly observed single sickled cells after discrete injury by a laser beam.

Erythrocytes suspended in plasma from patients with sickle-cell anemia were prepared with and without sodium metabisulfite. Microscopic fields

containing sickled cells were observed with phase optics and simultaneously recorded by cinematography on black and white 16-mm film. The same optical system with a constant magnification was used throughout, and the film was exposed at 16 frames per second. Approximately 16,000 frames were taken, from which two sequences containing 12 frames are presented. Selection of these few frames limited our ability to show the continuity in the changes of the cells. Prints were prepared from photographic enlargement of original negatives.

Cells were incised with a ruby laser as described by Bessis and Ter-Pogossian (*5*). The beam had a rated output of 0.5 joule/500 μsec that was focused to an area approximately 1 μ in diameter. We further limited the area exposed to irradiation by selecting for exposure a cellular process less than 1 μ in diameter.

In this system, a normal or nonsickled cell that is irradiated lyses within 10 seconds. The rapidity of visible loss of hemoglobin from the cell or coagulation of cellular hemoglobin is dependent on the surface area irradiated. Our control studies were similar to those reported by Bessis (*5*).

Figure 1 shows the transformation of a sickled cell to a spherocyte without subsequent lysis. There is a central sickled cell 20 μ long with a single superior process and two inferior processes. At the left are three acanthocytes; on the right, two holly-leaf sickled cells. Cells other than the central sickled cell remain unchanged throughout the sequence. In Fig. 1a, the light beam indicates the site at which the cell is to be incised. Figure 1b was taken 28.5 seconds after irradiation of the now beaded and coagulated process. There is no apparent injury to the subadjacent process, which is less than 2 μ distant and is indicated by the light spot. During a half-minute lapse, the shape and internal structure of the cell was unaltered. Figure 1c shows the cell 0.1 second after the second irradiation was directed to its inferior process. Coagulation of a portion of the process is evident, but the cell is otherwise unchanged. Six and one-half seconds later in Fig. 1d (26.6 seconds after the second injury) the irradiated coagulated extremities remain unchanged, but the cell body is ovoid and the internal longitudinally oriented density has broadened. The superior, nonirradiated process is attenuated but rigid. Figure 1e was taken 22.8 seconds later (49.4 seconds after injury) and shows a more rounded cell in which the internal longitudinal density is replaced by central pallor. The superior nonirradiated process has become flexible and wobbly with a terminal beaded body. The last frame (Fig. 1f) was taken 16.3 seconds later (65.7 seconds after injury), and the previously sickled cell with filamentous processes has become round and presumably spherical. The two inferior, laser-damaged processes have a constant relation to the cell, and the superior nonirradiated spicule is seen as a small, irregular attached, but remote, body. During an additional 30 minutes the cells remained unchanged, and loss of hemoglobin from the irradiated cell was not perceptible.

Figure 2 shows the order of resorption of spicules from a holly-leaf sickled cell and lysis of the cell after irradiation. In the central part of the field in Fig. 2a is a holly-leaf sickled cell with 11 spike-like projections and internal densities extending into four of the spikes. The indicator light beam and arrow in the left upper corner mark the area to be irradiated by the laser beam. Figure 2b was taken 0.1 second after irradiation and shows angulation of the injured spike. During the 13 seconds between Fig. 2b and 2c, the injured spike retracted into the cell body, and other nonirradiated spikes disap-

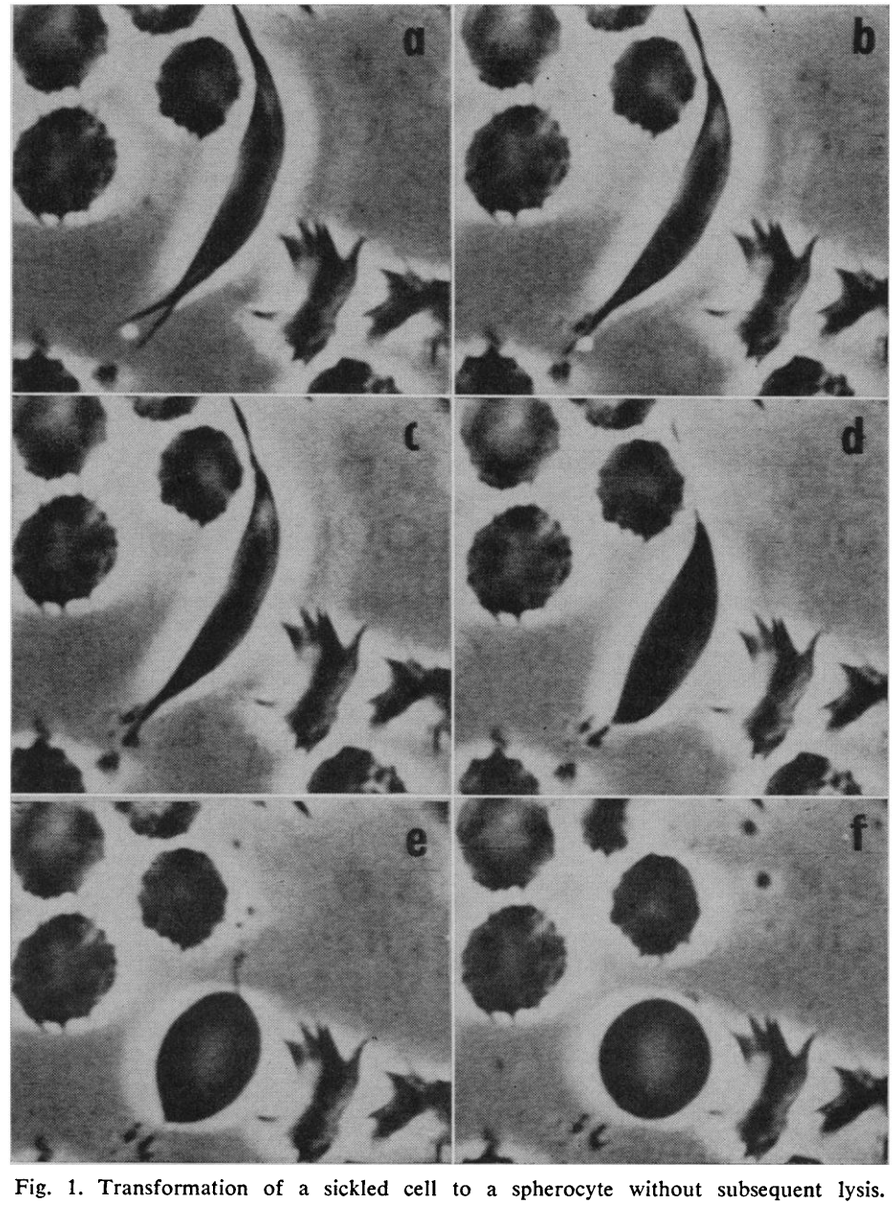

Fig. 1. Transformation of a sickled cell to a spherocyte without subsequent lysis.

peared or appeared less prominent. There was loss of definition of internal structure. In Fig. 2d, 2.3 seconds later (15.3 seconds after injury) the cell is an elongated polygonal form, and a central lucidity is present, which remained during subsequent observations. Figure 2e (18.1 seconds after injury) shows an almost completely rounded dense cell. In the interval of 30.7 seconds between Fig. 2e and 2f (48.8 seconds after injury), the cell became more rounded and progressively less dense until the red-cell ghost in Fig. 2f was seen. There is a fixed, dense, coagulated residue at the site of the original injury.

The life span of red cells in sickle-cell anemia is approximately one-tenth that of normal erythrocytes, and cells are lost from the circulation in a predominantly random fashion (*6*). Studies of sickle erythrocytes include those of membrane lipids (*7*), cellular adenosine triphosphate (*8*), energy production (*9*), electrolyte and water concentrations, and kinetics (*10*). The only significant abnormalities found depend on the hemoglobin and the sickling process. The sickle hemoglobin may accelerate cell destruction through formation of the grossly malformed sickled cell (*2, 4*). Sickle hemoglobin homogeneously distributed among cells in an amount of 20 to 45 percent (sickle-cell trait) rarely causes accelerated hemolysis (*11*). Even greater concentrations of sickle hemoglobin (70 percent), again homogeneously distributed (sickle-adult fetal hemoglobin syndrome), fail to cause accelerated lysis (*12*). The almost constant elevation of plasma hemoglobin and hemosiderinuria in sickle-cell disease (*13*) indicates that hemolysis occurs intravascularly, but the sequestration of tagged cells in liver and spleen suggests erythrophagocytosis as a prominent mechanism for loss of cells (*14*).

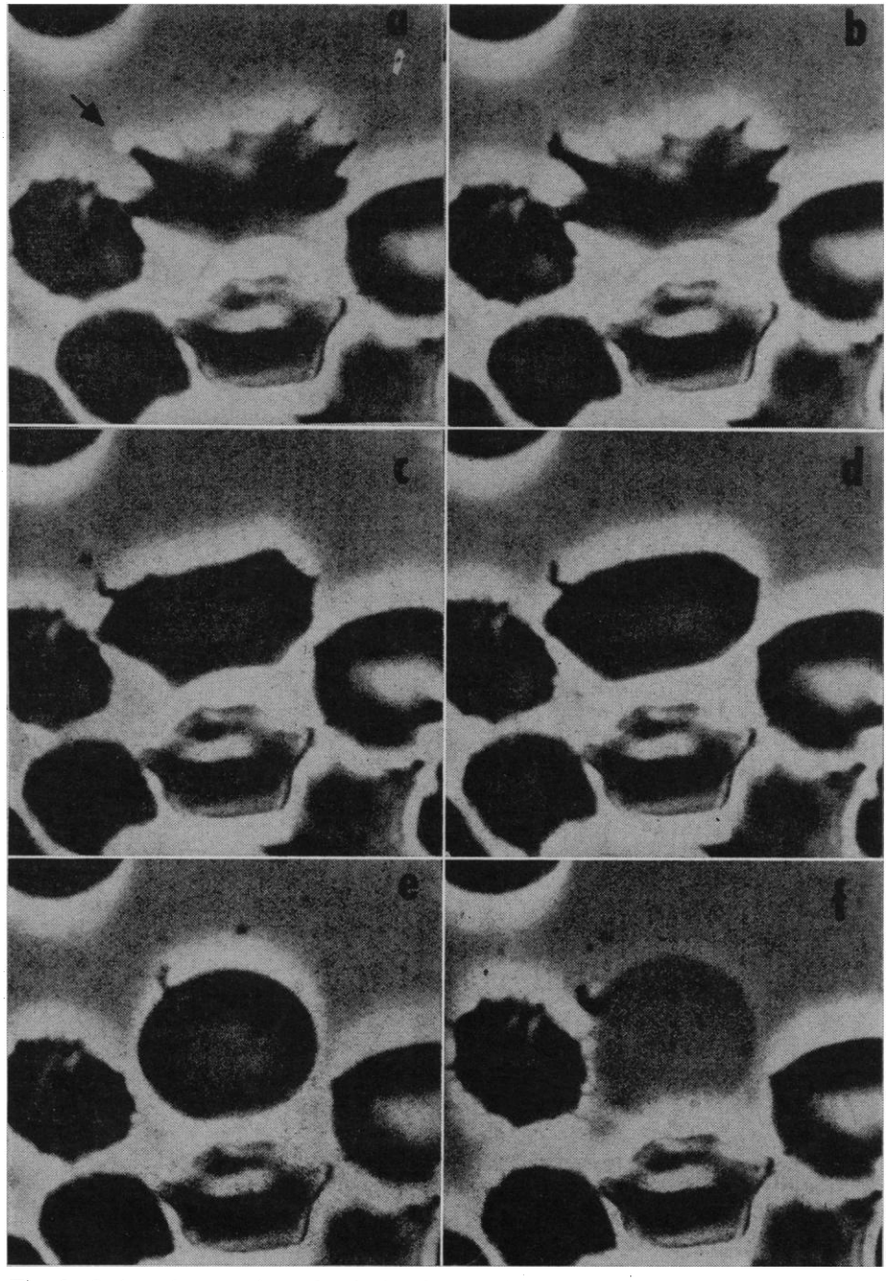

Fig. 2. Order of resorption of spicules from holly-leaf sickled cell and lysis of cell after irradiation.

There is good evidence that increased fragility of the sickled cell is an important element in the premature destruction of erythrocytes in sickle-cell disease (*4*). It has been proposed that the life span of the normal red cell may be limited by physical trauma of the buffeting of cells in the normal circulation (*15*), and recently march hemoglobinuria (*16*) and the hemolytic anemia of prosthetic heart valves and of deformed heart valves (*17*) have been cited as examples of mechanical injury leading to destruction of normal cells. Circulating sickled cells with rigid cytoplasmic projections are particularly susceptible to the buffeting action of the circulation in which avulsion of a cell process might produce large or small structural or biochemical defects leading to a "leaky cell" (*18*).

We have been able to observe the sequential changes following injury of individual sickled cells with laser microincision. In the first sequence, the initial laser impact damaged the filament; this was not followed by a loss of hemoglobin or by water influx as would have been reflected by a change in shape. The second laser discharge caused additional damage to the cell membrane, resulting in a gradual change in cell conformation to a round form still filled with hemoglobin. The presence of wobbly, beaded "myelin forms" after an uninjured spicule is resorbed in the unsickling process (*19*) may represent another mechanism by which the membrane is lost, the ratio of surface to volume is reduced, and a spherocyte is formed (*20*).

The change in conformation of the sickled cell in the absence of oxygen requires consideration of the effects of laser irradiation. These effects are de-

termined by the energy and duration of the light pulse, the absorbancy properties of the irradiated substance or substances (*5*), and the amount of cellular material exposed to irradiation. The major effect of the absorbed radiation results from transformation of light energy to heat (*5, 21*). This is evident from the charring of the cells at the site of irradiation. Other less likely or less well-documented physical effects may include shock waves and secondary radiations, but these have not been described for biologic preparations of the type used. The possibility that shock waves created by laser impact caused disruption of the liquid crystalline structure of the polymerized hemoglobin molecule and reversion to the nonsickled state is not compatible with the relatively long periods of time (seconds) required for the observed changes. Whatever the exact nature of the biophysical injury to the sickled cells, it seems that subsequent events occurred as the result of interchanges between contents of the cell and its environment dictated by alteration of the intervening cell membrane. The more likely explanations seem to be those based on the size of membrane defect. Large defects allow immediate loss of hemoglobin from the cell while smaller defects preclude the direct loss of hemoglobin but allow increased exchange of electrolytes and water. The subsequent swelling of the cell may cause formation of a sphere or may proceed beyond the critical hemolytic volume resulting in osmotic lysis. These observations may have application to other types of deformed cells such as acanthocytes, burr cells, and schizocytes (*22*).

From our observations, we propose that sickled cells are subject to at least two fates following injury in vivo by normal circulatory dynamics. The first follows avulsion of processes of sickled erythrocytes allowing escape of hemoglobin and intravascular hemolysis; the second follows the loss of smaller amounts of cell membrane with creation of "leaky cells" which become spherocytes and escape immediate lysis only to undergo erythrophagocytosis.

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Fatty Acids in Eleven Species of Blue-Green Algae: Geochemical Significance

Abstract. *Analyses of the total lipids of 11 species of blue-green algae showed a simple but qualitatively variable fatty acid composition. The species can be grouped in three categories on the basis of their oleic, linoleic, and linolenic acid content. One species was unusual in that the ten-carbon acid accounts for one-half of its total fatty acid. Branched chain acids are absent in the algae, but are major components of marine bacteria. The geochemical significance of the data is discussed.*

Algae are often pointed to as possible major source materials for the organic matter found in sediments (*1*). Blue-green algae are especially interesting because of their ability to grow in environments which favor the preservation of organic matter, such as hypersaline and reducing environments. The total fatty acid composition of 11 species (8 marine) of blue-green algae were measured in the hope of learning something about organism-sediment interactions. Very little data on the fatty acid composition of blue-green algae is available (*2*). The fatty acid composition of four marine bacteria were also measured for comparison with the blue-green algae. The simple and qualitatively variable fatty acid patterns found in this study are geochemically useful and it is felt they may be of interest to others.

The conditions used to grow the pure cultures of algae are given in Table 1. *Trichodesmium* was collected from large unialgal surface blooms which occurred near Port Aransas in the Gulf of Mexico. The results in Table 2 were obtained on samples collected in August and October 1965; the same results were found with a sample collected in July 1966.

The bacteria were isolated from sediment collected in Baffin Bay, about 30 miles (48 km) south of Corpus Christi, Texas. The isolation medium was aged 80 percent sea water with 0.1 g of yeast extract, Trypticase, and Soytone per liter, and 1 μg of vitamin B_{12} per liter, solidified with 1 percent Difco agar. The cells used for analysis were grown in shake cultures on 80 percent sea water containing 0.5 g of yeast extract, Soytone, and Trypticase per liter, and 1 μg of vitamin B_{12} per liter. The four organisms were Gram-negative small rods.

The analytical procedure was designed to detect fatty acids containing from 10 to 20 carbon atoms. The moist sample was treated with methanol for 15 minutes while being stirred and subjected to ultrasonic vibrations. The methanol was recovered by filtration. The sample was treated with chloroform and again stirred and subjected to ultrasonic vibrations. The recovered chloroform was combined with the methanol, and the mixture was almost completely dried with a rotating evaporator. This residue was taken up in chloroform and washed with 1*N* HCl to remove inorganic salts. The chloroform was evaporated under a