The Biochemistry of Resistance to Malaria

Genes for two lethal diseases, sickle-cell anemia and thalassemia, are favored by evolution because they protect against malaria. Now the mechanisms of that protection can be studied in the laboratory

by Milton J. Friedman and William Trager

Evolution results from natural selection, operating over a range of genetic diversity that arises from the mutation and recombination of genes. Variant genes that confer some selective advantage tend to increase in frequency, whereas deleterious variants tend to be eliminated. In human populations there are very few clear examples of selection for or against specific genes in response to specific forces. The best examples are inherited diseases. Selection acts against the genes that cause such diseases, and it acts most strongly against the severest conditions.

That being the case, lethal genetic diseases should be very rare. Yet certain inherited disorders of the red blood cells, notably sickle-cell anemia and thalassemia, are observed in some populations at surprisingly high frequencies. Does that argue against natural selection? On the contrary, the sickle-cell and thalassemia genes demonstrate the force of selection in evolution. The same variant genes that cause lethal blood-cell disease in homozygous individuals (who inherit two of the abnormal genes, one from each parent) protect heterozygous individuals (who inherit one abnormal gene and one normal gene) against the lethal effects of malaria, the agent of which is a parasite that infects red blood cells. That protection maintains the high frequencies of these otherwise deleterious genes.

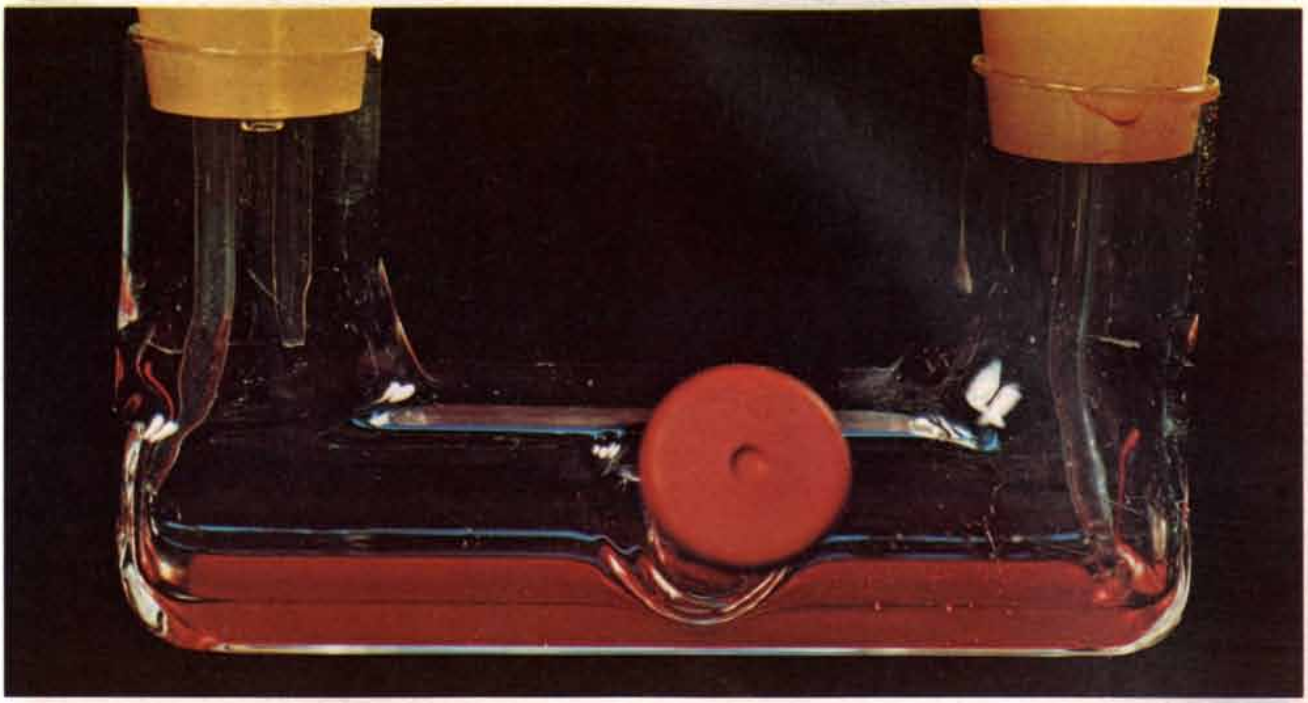
The strength of malaria as a selective force derives from the powerful effect of the parasitic disease on the health and reproductive capacity of human populations. Malaria has been a major cause of death throughout history. In Africa today malaria is endemic: it does not sweep through a population as an epidemic but rather is a constant affliction contributing to early-childhood mortality rates as high as 50 percent. It kills about 10 percent of its victims directly and contributes to the death of others by decreasing the immune system's ability to fight other infections. Because of malaria a significant number of children do not live to reproduce. Any genetic mutation that provides resistance to malaria must therefore have a high selective advantage.

It was the coincidence of the geographic range of sickle-cell disease with the range of malaria that first drew attention to the possibility that the sickle-cell gene might confer such resistance. Clinical evidence was harder to come by, but in 1954 Anthony C. Allison of the University of Oxford showed that children who were heterozygous for the sickle-cell gene had much less severe cases of the most lethal form of the disease than children who did not carry the gene. Because the parasite that causes malaria could not be maintained in a laboratory culture, however, the resistance could not be demonstrated at the cellular level, nor could its biochemical mechanism be established. Recently we have exploited a newly developed culture system to learn how the sickle-cell gene and some other variant genes that alter red-cell function confer resistance to malaria.

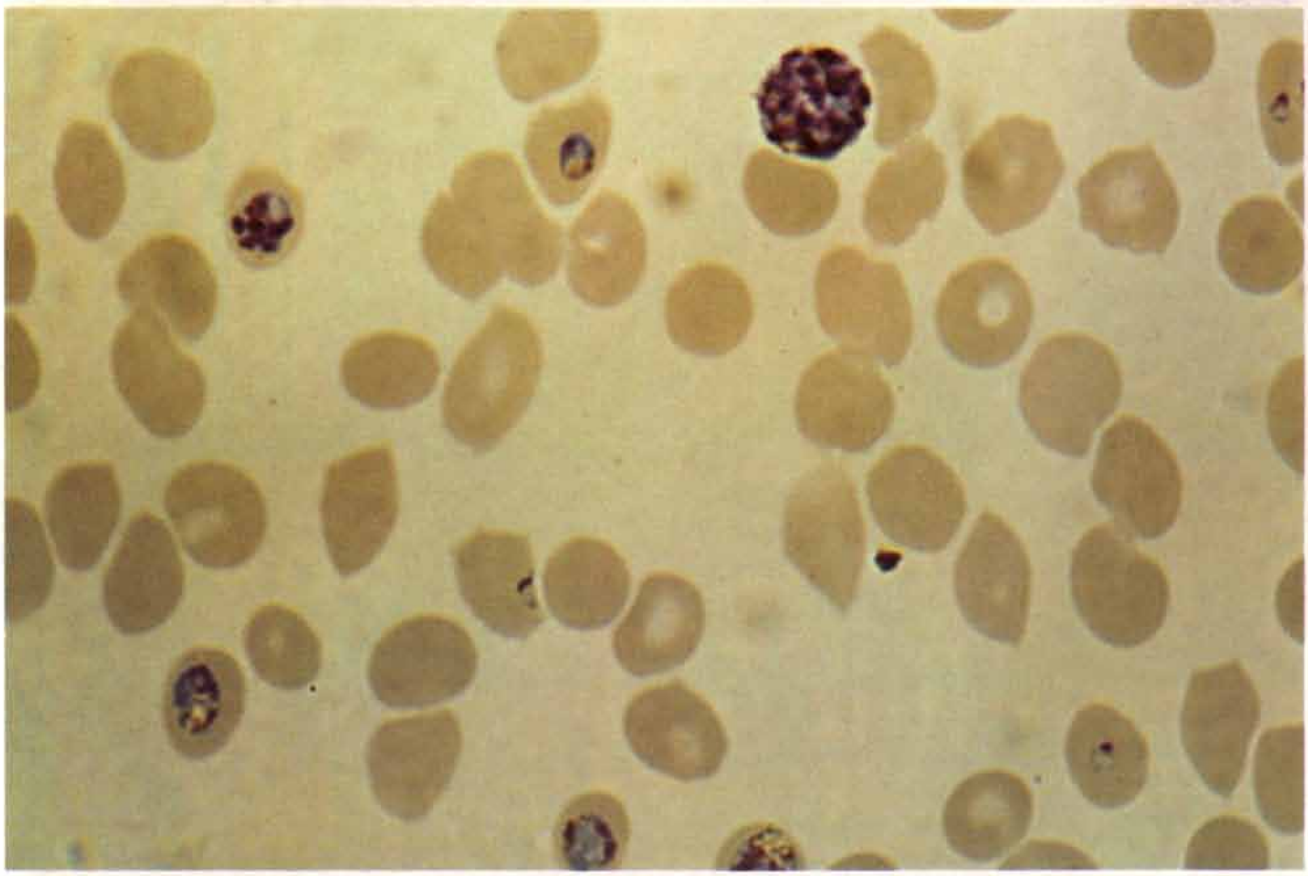
The red blood cell, where the malaria parasite encounters the altered cellular functions governed by these variant genes, is largely filled with hemoglobin: the protein that takes on oxygen in the lungs and carries it to the tissues. The other proteins of the red-cell cytoplasm are metabolic enzymes. Some of them catalyze glycolysis, whereby glucose is broken down step by step to form lactate, in the process synthesizing adenosine triphosphate (ATP). Others catalyze what is called the hexose monophosphate shunt, which maintains the coenzymes nicotinamide adenine dinucleotide phosphate (NADP) and glutathione in their reduced form. ATP is the all-purpose cellular energy carrier; reduced NADP (NADPH) and reduced glutathione are needed to prevent and repair oxidative damage. The cell membrane bounds the cell and controls its shape and deformability. It also controls the movement of ions into the cell and out of it; in particular it maintains—at the expense of ATP—a high-potassium interior against a tendency toward equilibrium with the low-potassium outside environment. On the outside of the membrane glycoproteins and glycolipids present a unique recognizable surface to the environment.

It is at this surface that the malaria parasite first interacts with the cell. The parasite is a small unicellular protozoon of the genus *Plasmodium*, four species of which cause malaria in man; the most lethal disease, responsible for a million deaths every year among African children, is caused by *Plasmodium falciparum*. A specialized form of the parasite is injected into the bloodstream by an *Anopheles* mosquito, migrates to the liver and there develops and divides to produce merozoites, the form that infects red cells. The merozoites reenter the bloodstream and recognize and bind to the red-cell membrane. A mechanism, as yet poorly understood, is activated that causes the merozoite to push in the cell membrane, which closes around it. Enclosed in a vacuole, the parasite grows, digesting hemoglobin to acquire the amino acids to make its own proteins and, we believe, utilizing red-cell glucose, ATP and reduced coenzymes in its metabolism. After a period of growth the parasite's nucleus divides several times, and then membranes enclose each nucleus and its surrounding cytoplasm. In this manner from 12 to 24 new merozoites are formed, which burst out of the cell and invade other cells.

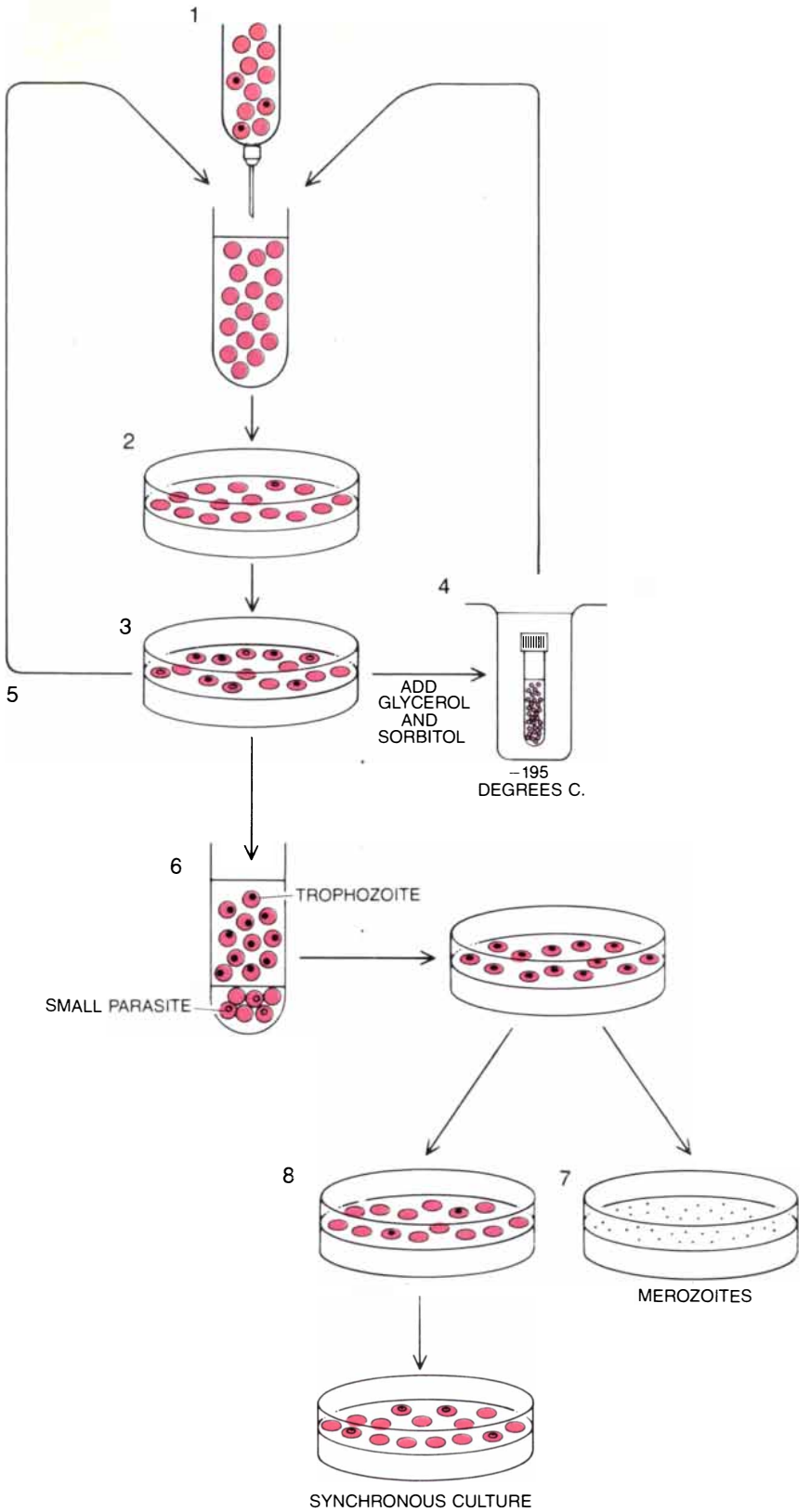
The characteristic periodic fever of malaria results from the synchronous release of merozoites, and of toxins produced by the parasite, throughout the body. In the case of *P. falciparum* this release takes place every 48 hours, the period of the parasite's developmental



CONTINUOUS CULTURE of the malaria parasite *Plasmodium falciparum* can be maintained in this apparatus. The parasites are grown in a thin layer of human red blood cells that coats the bottom of the horizontal tube and is covered with a nutritional medium. The oxygen content of the medium and other experimental conditions are manipulated by way of the vertical tubes. Cells are removed for microscopic analysis through the short tube at the front center. Until conditions for culturing the parasite were established in 1976, research on falciparum malaria, the most lethal form of the disease, had to be done with the blood of human volunteers or primate hosts.



FATE OF MALARIA PARASITES is monitored in smears of the cultured cells on microscope slides. The stain (Giemsa's) colors the cell nuclei of the parasites dark purple and the parasite cytoplasm blue. The rate of multiplication is measured by counting the number of parasites per 100 red cells. This smear shows the parasites at various stages of their life cycle (*see bottom illustration on page 159*).



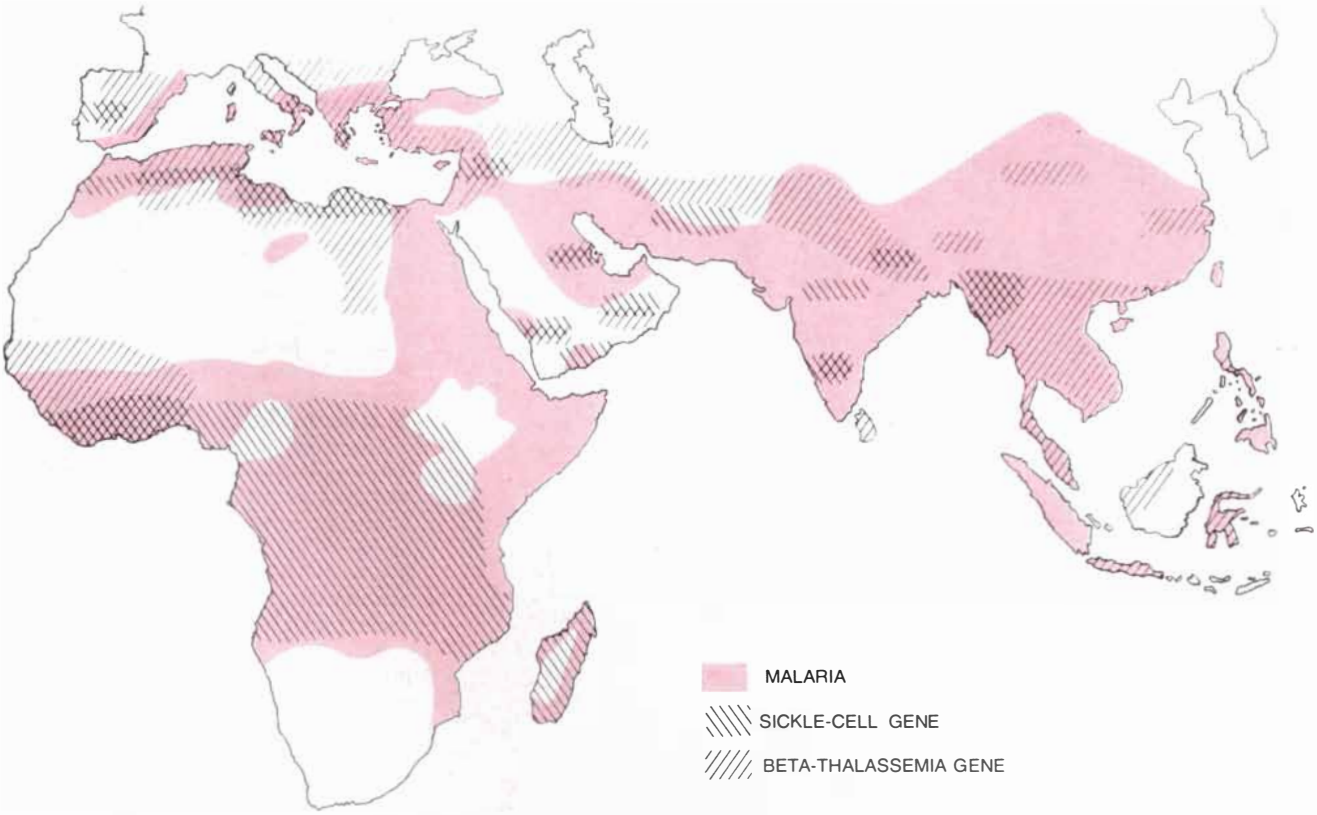
P. FALCIPARUM CULTURE is begun by inoculating fresh human red cells with parasitized cells from a patient or from a stock culture (*1*). The cells are grown at body temperature (in a culture dish, as is shown here, or in the U-tube depicted on page 155) as a thin layer covered by a nutrient medium (*2*). The culture can be sampled to measure parasite multiplication. After three or four days the cells contain a mixture of small and large parasites (*3*), which can be frozen for future use (*4*), inoculated into new cultures (*5*) or incubated in a gelatin solution to separate trophozoites (*6*); these large parasites can be grown alone to produce merozoites for further study (*7*) or can be mixed with fresh cells to produce a synchronous culture of parasites (*8*).

cycle in the red cell. The fever and the debilitation that accompany it are the major symptoms of malaria. In falciparum malaria, however, there is a more lethal effect. The infected red cell develops knobs on its surface that attach the cell to the walls of capillaries, where it lodges until the parasite is mature. When a large number of cells are thus sequestered in a vital organ such as the brain, death can result. The clearest indication of the protective effect of the sickle-cell gene is that very few carriers of the gene die from the cerebral complications of falciparum malaria.

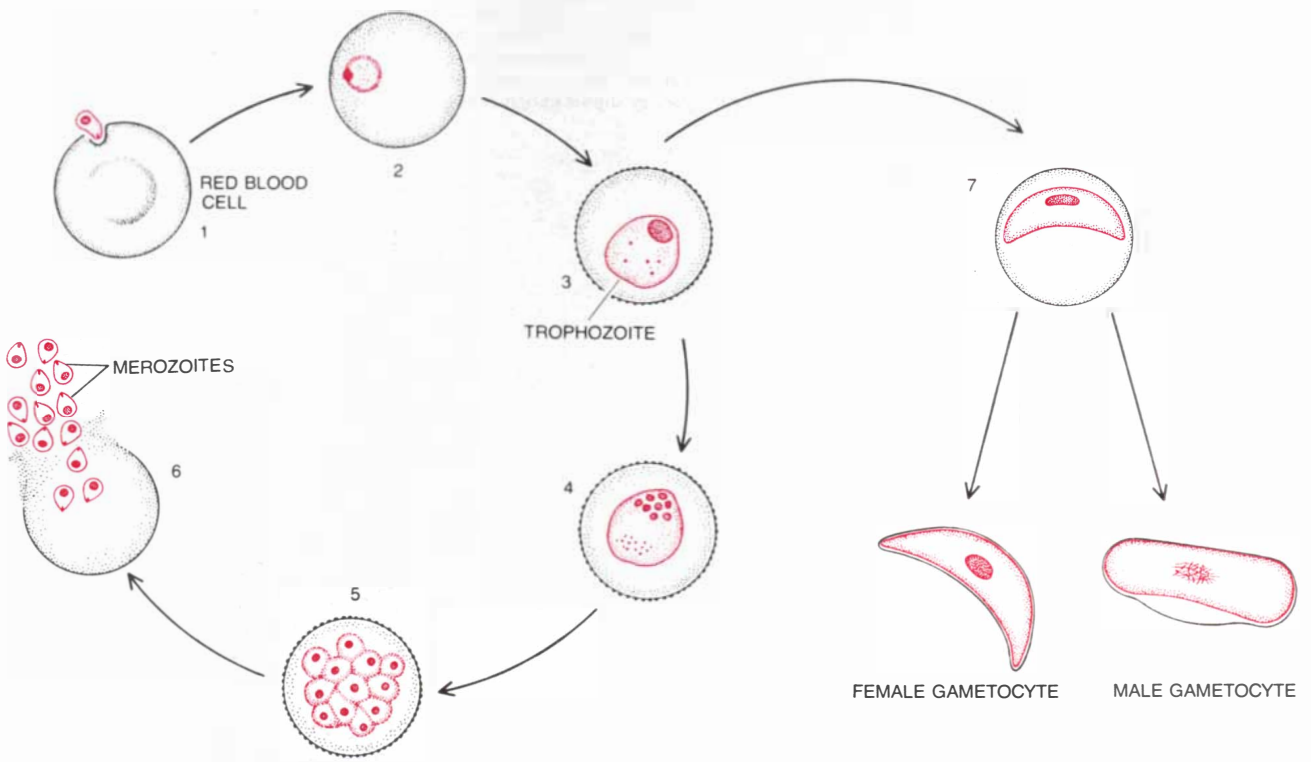
Linus Pauling and his colleagues defined a molecular disease for the first time when they demonstrated that in sickle-cell anemia the hemoglobin molecule is altered, and that people with sickle-cell disease have only the altered molecule, hemoglobin *S*, in their red cells, whereas some members of their families have about half hemoglobin *S* and half normal hemoglobin *A*. Family studies published at about the same time confirmed Pauling's findings, showing that the inheritance pattern of sickle-cell anemia could be ascribed to a single gene, with the disease appearing only in family members who are homozygous for that gene.

Adult hemoglobin is made up of two alpha chains and two beta chains, and Vernon M. Ingram of the University of Cambridge soon showed that in hemoglobin *S* only the beta chain is abnormal. The abnormality involves only one of the amino acids constituting the chain: a valine is substituted for a glutamic acid. Sickle-cell homozygotes (designated *SS*) have only hemoglobin *S* because they carry two of the mutant beta-chain genes. Heterozygotes (*AS*), who are said to have sickle-cell "trait," carry only one mutant beta-chain gene, and about 40 percent of their hemoglobin is hemoglobin *S*. If two people with sickle-cell trait have four children, the probability is, in accordance with Mendelian principles, that one child will have sickle-cell disease, one will be normal and two will have sickle-cell trait.

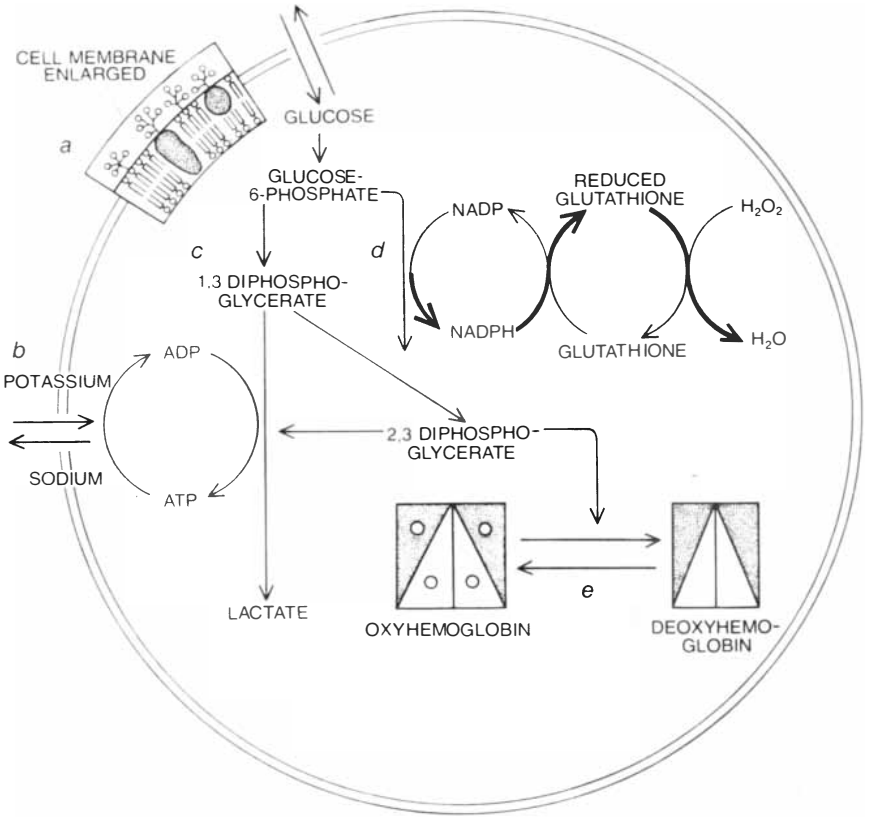
The symptoms of sickle-cell disease appear when the *SS* red blood cells lose oxygen as they circulate through the tissues. When hemoglobin *S* is deoxygenated, it tends to aggregate in long, thin fibers. The fibers distort the normally disk-shaped cells into angular forms, including the characteristic crescent shape. In a sickle-cell crisis some sickled cells block the local circulation of blood and impede the delivery of oxygen. As the oxygen level drops, more red cells sickle and the area of impaired circulation spreads, causing extensive tissue death. In the absence of advanced medical treatment the survival of hemoglobin-*S* homozygotes is very low. People with sickle-cell trait do not ordinarily suffer



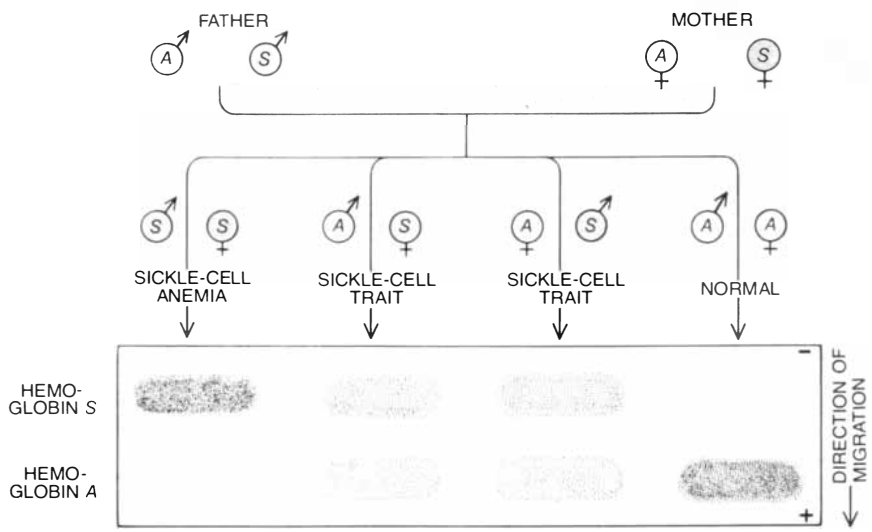
DISTRIBUTIONS of the sickle-cell gene and of the beta-thalassemia gene lie within the area where falciparum malaria was prevalent before 1930 (*color*). This geographic coincidence provided the first suggestion that resistance to malaria might be the evolutionary advantage that was tending to maintain the genes responsible for lethal blood diseases at high frequencies in certain human populations.



LIFE CYCLE of *P. falciparum* in the red cell begins with the invasion of the cell by a merozoite (*1*). The parasite engulfs a droplet of cytoplasm, so that in section it looks like a thin ring (*2*). The ring grows and fills in to become a trophozoite (*3*); knoblike structures develop on the cell surface and attach the cell to the blood-vessel wall. There the parasite's nucleus divides repeatedly (*4*); each daughter nucleus acquires a bit of cytoplasm (*5*), and the parasite divides into from 12 to 24 merozoites, which burst the cell and begin a new cycle (*6*). Some trophozoites develop (*7*) into male and female gametocytes, which are ingested by mosquitoes to initiate the sexual phase of the cycle.



RED BLOOD CELL, the simplest cell of the body, is no more than a membrane-bounded cytoplasm. Glycolipids and glycoproteins on the surface (*a*) determine the cell's interactions with its environment and with the malaria parasite. Proteins in the membrane control the transport of substances into and out of the cell; specifically, potassium is pumped in and sodium is pumped out (*b*). This process requires energy, which is provided by ATP generated through glycolysis, whereby glucose is broken down to form lactate (*c*). The hexose monophosphate shunt (*d*) produces reduced NADP and reduced glutathione, both of which prevent and repair oxidative damage to the cell membrane. The cytoplasm is largely filled with the four-chain protein hemoglobin (*e*), which binds oxygen in the lung and delivers it to the tissues, in the process undergoing structural changes that are to some extent controlled by diphosphoglycerate.



INHERITANCE of genes for abnormal hemoglobin *S* is diagrammed for parents who are heterozygous for the abnormal gene. One child is homozygous (*SS*) for the gene, that is, he inherits two hemoglobin-*S* genes and has sickle-cell anemia. Two children inherit one gene for hemoglobin *S* and one for normal hemoglobin *A;* they are heterozygous (*AS*) like their parents and have sickle-cell "trait." One child is normal (*AA*). The hemoglobins are identified by electrophoresis (*bottom*). Hemoglobin samples placed on cellulose acetate are subjected to an electric current. Hemoglobin *S* differs from hemoglobin *A* in only one amino acid; the substituted amino acid lacks a negative charge, and so hemoglobin *S* migrates toward the positive pole less rapidly than hemoglobin *A*. *AS* heterozygotes have a mixture of the two hemoglobins.

from the disease, however. Their *AS* red cells have enough normal hemoglobin so that they sickle only under extreme conditions, such as at high altitudes.

Four years ago, after half a century of attempts to grow the malaria parasite in the laboratory, one of us (Trager) was able to define the conditions that make it possible to maintain *P. falciparum* in a continuous culture of human red blood cells in an artificial bloodlike medium. The culture system is being exploited by some workers to develop experimental vaccines containing material from various stages of the parasite's life cycle, which are being tested in animals. Other investigators are trying to purify and analyze the biochemical agents that have particular effects in the course of an infection. The system has also made it possible for us to study in detail the interactions of the malaria parasite and variant host red cells.

To learn how sickle-cell hemoglobin protects a heterozygote carrier against malaria we cultured the malaria parasite in red blood cells taken from normal donors, from individuals with sickle-cell trait and from patients with sickle-cell anemia. We did so under our standard culture conditions, in an atmosphere with an oxygen concentration of 17 percent, which created an oxygen tension in the culture medium similar to that in the lungs; the hemoglobin was fully oxygenated and the variant cells did not sickle. Under these conditions the parasites grew equally well in all three kinds of cells. This showed that there is no major alteration of red-cell metabolism in the variant cells, and that hemoglobin *S,* like hemoglobin *A,* can be digested by the plasmodium.

To test the effect of sickling on parasite growth we added a small number of infected normal cells to cultures of *SS* and *AS* cells in a 17 percent oxygen atmosphere. During the next 48 hours, the period of one growth cycle, all the parasites left their normal host cells and invaded the variant cells. When we lowered the oxygen concentration to 3 percent, the *SS* cells sickled, as did some of the *AS* cells. We monitored the parasites daily. After one day in low oxygen almost no parasites were visible in the *SS*-cell cultures; they had lysed, or disintegrated, and so had their host cells. In the *AS*-cell cultures, on the other hand, it was only on the second day that the number of live parasites decreased significantly. And rather than disintegrating, the killed parasites were still visible as shriveled masses in the cells. They looked to us like parasites that had starved, perhaps as the result of some kind of metabolic inhibition that was an indirect result of sickling.

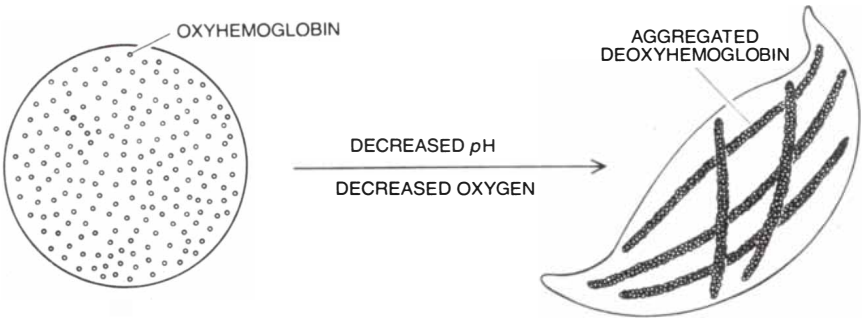
If that was the case, preventing sickling should protect the parasites. We

treated *AS* cells with cyanate, which increases the affinity of hemoglobin *S* for oxygen, making it less likely to aggregate at a given oxygen tension. After cyanate treatment and washing, the *AS* cells remained competent as hosts for *P. falciparum,* but now they did not sickle as readily. When such cells were infected and then cultured in 3 percent oxygen, the parasites survived. The inhibition we had seen in untreated cells must therefore have been due to the sickling of the cells, not simply to low oxygen. By what mechanism might sickling inhibit parasite growth?

One of the things that happen when a red cell containing hemoglobin *S* sickles is that the cell membrane becomes more permeable to potassium, which leaks out; in the low-oxygen condition the potassium level in our *AS* host cells was decreased. It had earlier been shown that parasites maintained outside red cells require a high-potassium environment, and so we hypothesized that the loss of potassium on sickling might have inhibited parasite metabolism. To test the idea we again incubated infected *AS* cells in 3 percent oxygen, but in a medium with an elevated potassium content. The cells sickled as usual in low oxygen, but now the cellular potassium level stayed high—and the parasites survived. Preventing the loss of potassium, in other words, prevented the inhibition of parasite growth in sickled *AS* cells. (Under the same conditions plasmodia in sickled *SS* cells were not protected; they died by lysis. Electron micrographs showed why. After six hours of deoxygenation needlelike bundles of aggregated hemoglobin *S* could be seen penetrating some of the plasmodia; the membranes of other parasites had been disrupted, and they were partially lysed. In other words, in the *SS* cells the parasites were killed not by metabolic inhibition but by actual physical disruption.)

The sequence of events in *AS* cells, then, seemed to be as follows: Sickling lowered the potassium level, and the low potassium level killed the parasites. This finding could not, however, fully explain the heterozygote's resistance to malaria. Because an uninfected *AS* cell has less hemoglobin *S* than an *SS* cell, it does not normally sickle in nature; it circulates through regions of low oxygen tension too quickly for sickling to take place. The progress of a parasitized cell, however, is impeded by the knobs on its surface, and the cell remains in a low-oxygen environment for many hours. Even so, fewer than 5 percent of the cells would sickle were it not for still another effect of infection.

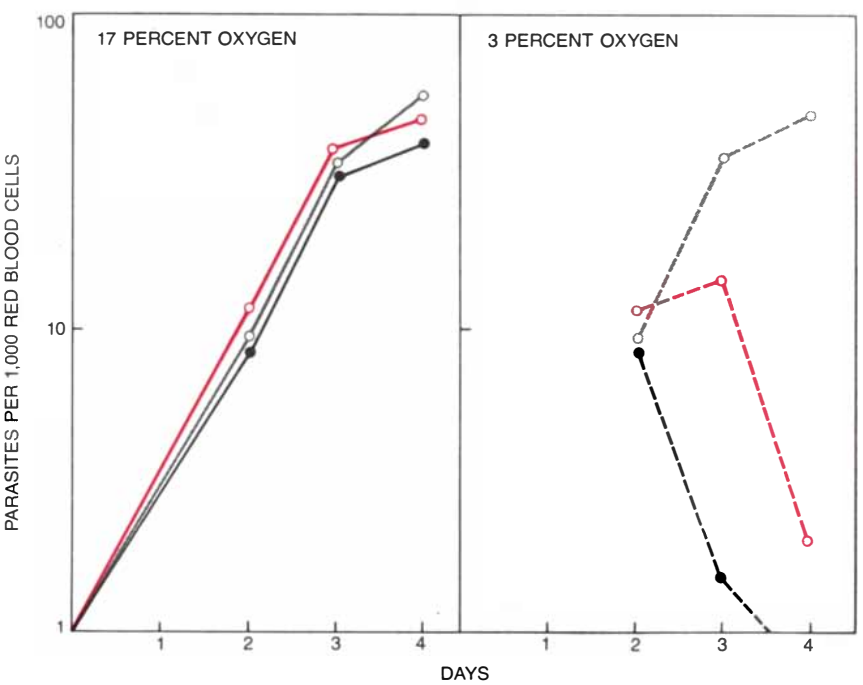
Lucio Luzzatto and his colleagues at the Istituto Internazionale di Genetica e Biofisica in Naples have shown that infected cells sickle much faster than uninfected ones. Why? We found that the intracellular environment of an infected cell is more acidic (.4 *p*H units lower) than that of an uninfected cell, so that the rate of sickling is significantly increased. We calculate, moreover, that the lower *p*H level of parasitized cells also increases the extent of sickling—up to about 40 percent.



HEMOGLOBIN S in its oxygenated state (*left*) is dispersed through the red cell, which has a normal disk shape. Unlike normal hemoglobin *A,* however, hemoglobin *S* tends to aggregate when it becomes deoxygenated in the tissues, forming needlelike quasi-crystalline structures that distort the cell into a rigid, jagged shape (*right*). Sickled cells may block capillaries, decreasing blood flow, reducing the oxygen level and thus promoting the sickling of more cells.

Taken all together these observations suggest the following mechanism of protection against malaria in sickle-cell heterozygotes. The parasite in an infected *AS* cell develops normally until the cell is sequestered in the tissues. Then, given the low-oxygen environment and the low intracellular *p*H, the host cell sickles. The potassium level drops and the parasite dies. Such a process can protect against malaria even if not all the parasites are affected, because even a reduction in the rate of multiplication of the plasmodium can give the immune system the time it needs to mount a protective response of its own. (There is an alternative hypothesis. Infected cells might, for some reason we have not discerned, sickle while circulating rather than while being sequestered, and then they might be eliminated by the filtering action of the spleen. The first hypothesis is supported, however, by evidence that heterozygotes are not protected against types of malaria in which infected cells do not develop knobs and are not sequestered in the tissues.)

In addition to sickle-cell disease and sickle-cell trait there are other inherited disorders of the red blood cells



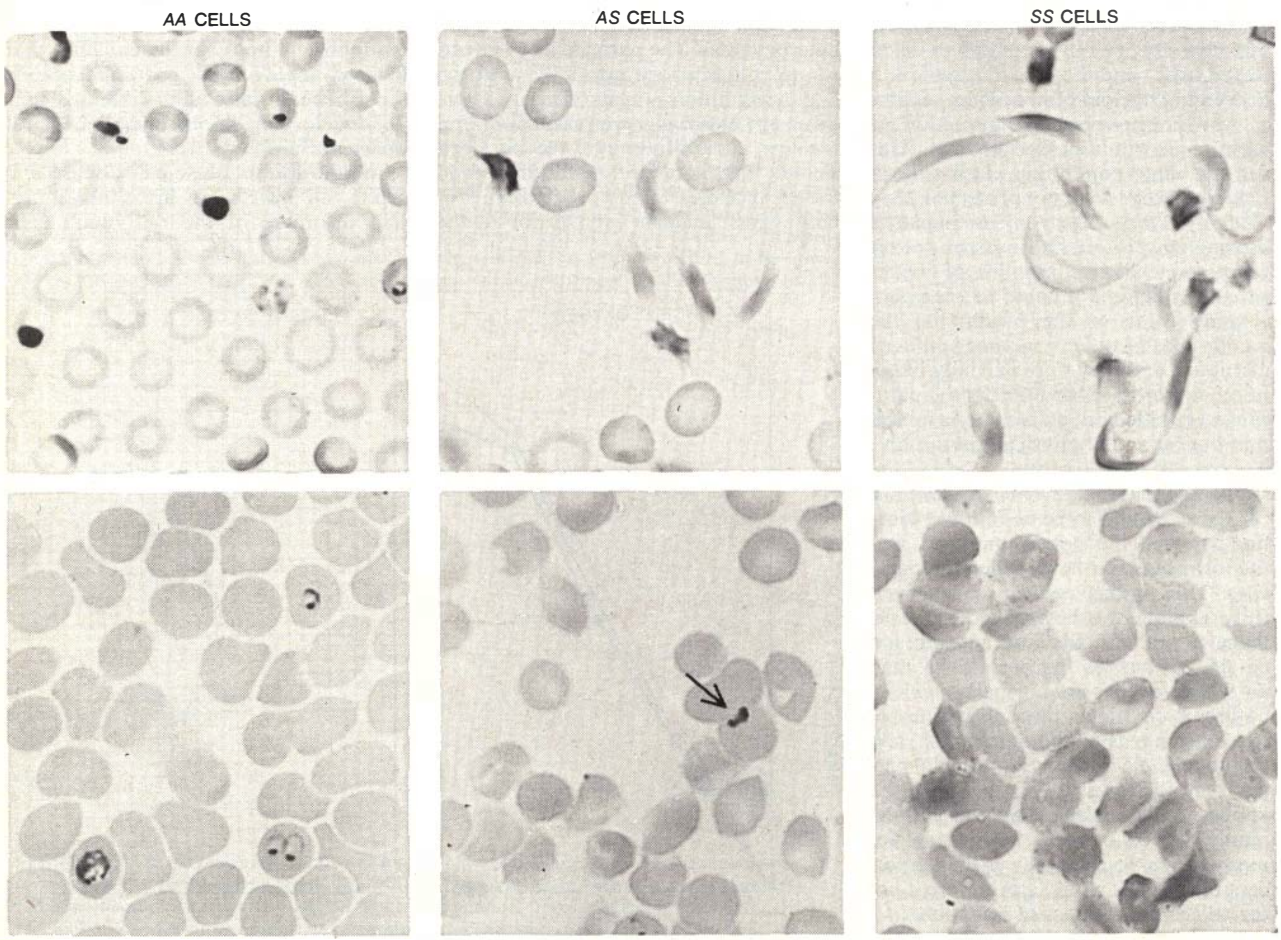
MULTIPLICATION OF P. FALCIPARUM is about the same in normal cells (*open circles*), *AS* cells (*color*) and *SS* cells (*black*) as long as the cells are in 17 percent oxygen (*left*). If after two days the oxygen level is lowered to 3 percent, however, only parasites in normal cells keep growing (*right*). The parasites in the *SS* cells die in a day, those in the *AS* cells in two days.

whose geographic incidence has been correlated with that of malaria, implying that the genes responsible for those disorders too may confer some resistance. Among those disorders are the thalassemias, which involve a deficiency in the manufacture of one or another hemoglobin chain. Beta thalassemia, for example, is a deficiency in beta-chain synthesis. Homozygous beta thalassemia, known as Cooley's anemia, is a severe disease in which little normal adult hemoglobin, if any, is synthesized; blood transfusion is usually the only means by which a patient's life can be prolonged. Yet throughout many malarial regions, and in particular around the rim of the Mediterranean, about 1 percent of all children born are homozygous for the beta-thalassemia gene and have Cooley's anemia; heterozygotes do not have the disease. Resistance to malaria has not been convincingly demonstrated for beta-thalassemia heterozygotes, but there is a very suggestive geographic correlation between the frequency of the gene and a regional history of malaria. To cite just one example, the gene frequency is much higher in the valleys of Sardinia, where malaria was for a long time endemic, than it is in the mountains, where malaria was rarer.

We set out to demonstrate a resistance effect in our culture system. We knew that one characteristic of any thalassemic cell is abnormal sensitivity of the cell membrane to damage by oxidation. When a molecule is oxidized, electrons are removed that would ordinarily have a role in the formation of chemical bonds. When lipid (fat) molecules, which are major constituents of the membrane, are oxidized, they fragment and disrupt the integrity of the membrane. The agents of oxidation in cells have not all been identified, but it is known that one such agent is hydrogen peroxide.

The malaria parasite generates hydrogen peroxide in its host cell (as has been demonstrated by N. Etkin and John W. Eaton of the University of Minnesota Medical School). Peroxides give rise in any cell to oxidative stress, challenging the cell's ability to preserve its integrity. In the more sensitive thalassemic red cell hydrogen peroxide might actually bring about damage to the membrane. We confirmed the likelihood of this effect by finding that parasites in heterozygous beta-thalassemia cells were more sensitive than parasites in normal cells to three experimental conditions. One condition was a high-oxygen environment (an oxygen concentration of from 25 to 30 percent). Another was the presence of certain chemicals that catalyze oxidation reactions. The third was the absence from the culture medium of one normal constituent: reduced glutathione, which is an intermediate in the metabolic pathway that reduces the cellular level of hydrogen peroxide.

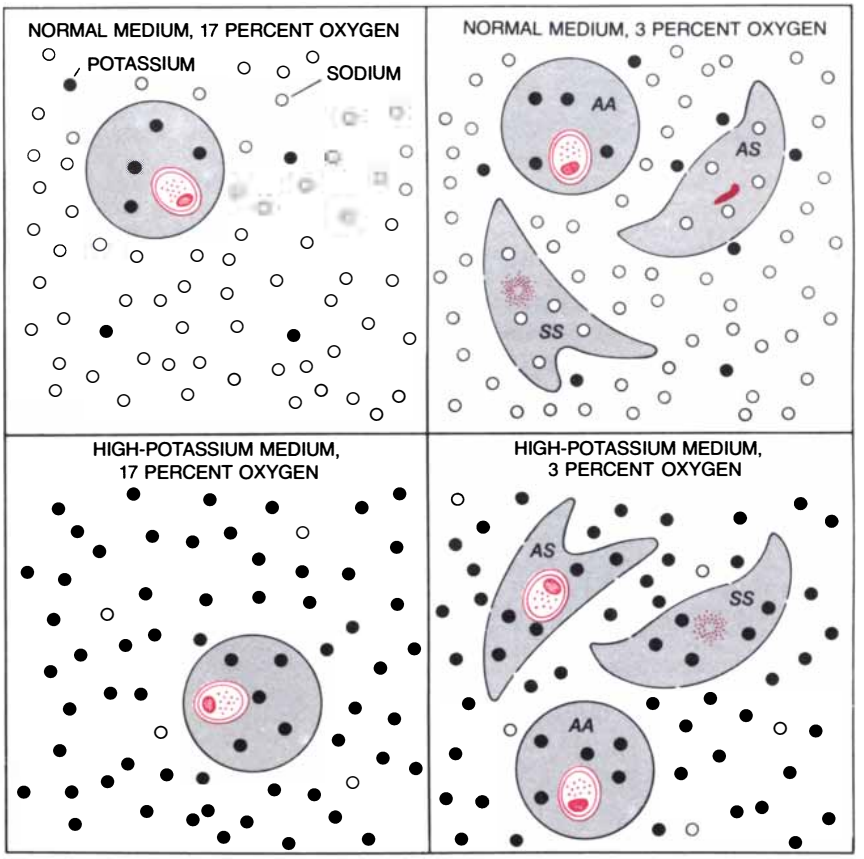
Each of these three conditions was calculated to increase the oxidative stress on the cell. Eaton found that in mice infected with malaria oxidative stress and protection against it are finely balanced. Any additional sensitivity in a thalassemic cell, therefore, might well affect the course of the infection. (Although a thalassemic cell has a low hemoglobin content, there is apparently



PARASITE-INFECTED RED CELLS that have been cultured for one day in a 3 percent oxygen atmosphere are seen in these photomicrographs either fixed in formaldehyde to preserve their shape (*top*) or smeared on a slide for better observation of the parasites (*bottom*). The normal (*AA*) cells (*left*) are still disk-shaped; some of the *AS* cells (*middle*) and all the *SS* cells (*right*) have sickled. The parasites in the *AA* cells are alive. In *AS* cells parasites are dead (*arrow*) or will die within 24 hours. In *SS* cells no parasites are visible.

enough hemoglobin to maintain the parasite; the low hemoglobin level alone did not inhibit parasite multiplication in our system.)

We found that vitamin E, which protects cell-membrane lipids against oxidative damage, prevented the death of parasites in thalassemia-trait cells under all conditions. This was supporting evidence for the idea that the membrane of a heterozygous beta-thalassemia cell is damaged by oxidation in the course of malaria infection. As for the mechanism, again potassium appears to be implicated. In a high-potassium medium the parasites in the thalassemia-trait cells developed normally under conditions that led to their death in a medium with a normal potassium concentration.



POTASSIUM LOSS is shown to be the cause of parasite death in *AS* cells. In 17 percent oxygen (*left*) the cell membrane remains intact and the potassium level is adequately maintained in either a normal physiological (low potassium) medium (*top*) or in elevated potassium (*bottom*). In a low-oxygen, low-potassium medium (*top right*) *AS* and *SS* cells sickle; their membranes are disrupted, they lose potassium and their parasites shrivel up (*AS*) or disappear (*SS*). Incubation in a high-potassium medium (*bottom right*) protects parasites in *AS* cells in spite of sickling and membrane disruption, but it does not protect parasites in *SS* cells. The cause of parasite death in *AS* cells, then, is deprivation of an essential metabolic factor, potassium. In *SS* cells, on the other hand, hemoglobin-*S* aggregates destroy parasites by physical penetration.

There were three interesting corollary results of this investigation. One was the reinforcement of a long-suspected link between malaria and an inherited deficiency in the red cell's supply of the enzyme glucose-6-phosphate dehydrogenase (G6PD). This deficiency too is prevalent in malarial regions; field studies have sometimes, but not always, demonstrated a correlation with resistance to malaria. G6PD is the first enzyme in the hexose monophosphate shunt, which regenerates NADPH, a coenzyme that is essential for protection against and repair of oxidative damage. It appeared that red cells deficient in G6PD, like thalassemia-trait cells, might be more sensitive to the hydrogen peroxide generated by the malaria parasite. We found that parasites in G6PD-deficient cells were indeed highly sensitive to stress by oxidants and were protected by antioxidant agents.

A second corollary implication of our results with thalassemic cells had to do with favism: a hemolytic anemia promoted by the ingestion of fava beans, which are consumed throughout the Mediterranean world. The fava bean contains a variety of substances that could increase the red cell's sensitivity to oxidants; some of the substances are related to the oxidation catalysts introduced in some of our experiments. Do those experiments mimic the consumption of fava beans by people with beta-thalassemic or G6PD-deficient red cells? If they do, the results would indicate that eating fava beans (and perhaps other foods as yet not identified) increases the level of protection against malaria in people who are heterozygous for these two red-cell disorders. Such a dietary effect could also explain the inconsistent results of studies of malaria resistance among such heterozygotes.

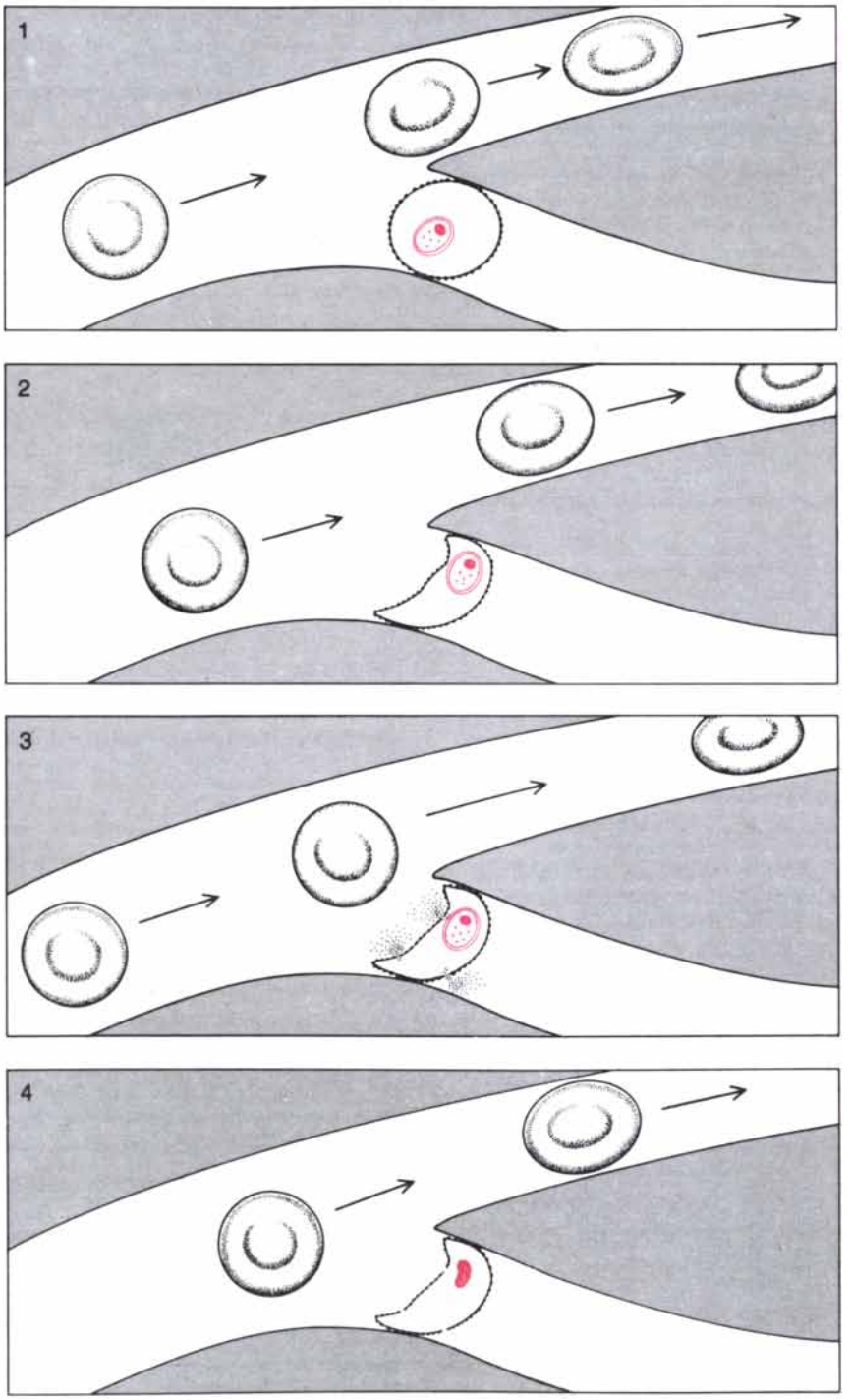
The third corollary result had to do with infants. During the first few months of life infants are almost completely protected against malaria. Geoffrey Pasvol, R. J. M. Wilson and D. J. Weatherall of the University of Oxford recently showed that fetal hemoglobin (which consists of alpha and gamma chains, persists for a time after birth and is found in some adults' red cells) may contribute to this protection; even adult red cells inhibited the growth of parasites if the cells contained fetal hemoglobin. We have found that malaria parasites in fetal red cells, like those in thalassemic cells, are highly sensitive to oxidative stress. It is not likely that malaria has been a selective force in the evolution of fetal hemoglobin, however. The fetal protein probably evolved under a different selective pressure. It has a higher affinity for oxygen than adult hemoglobin, and so it improves the delivery of oxygen to the developing fetus. The oxidant sensitivity of the fetal red cell and the resulting resistance to malaria are probably side effects of a developmental adaptation.

To sum up, the process of evolution has resulted in the selection of genetically variant red blood cells that function well enough under normal conditions but are susceptible to damage when they are infected by *P. falciparum*—damage that kills the parasite. In other words, these cells are so marginally viable that infection makes them unviable and unable to support the intracellular parasite. The genetic alteration in the case of *AS* cells is a borderline tendency to sickle, which is enhanced by the parasite's presence. The alteration in thalassemia-trait cells is increased sensitivity of the membrane lipids to hydrogen peroxide generated by the parasite. In both cases the end effect is a loss of potassium that inhibits the parasite's metabolism.

The evolutionary career of a gene can be quite complex. When a new random mutation arises, one cannot predict its effect or its potential. One could surely not have predicted that certain genes would cause lethal blood disease in a homozygote and protect the heterozygote against death from malaria. The delicately balanced contest between the selective effect of malaria on the one hand and of sickle-cell disease and beta thalassemia on the other has resulted in a balanced polymorphism: a situation

in which a heterozygote advantage coupled with a homozygote disadvantage maintains a variant gene at a low but consistent level in a population.

The mechanisms of life and death are rooted in chemistry and molecular biology. They can be explicated, to some extent, in the laboratory. Their ultimate effects on the human species are decided, however, not in the laboratory or even in the cells of individual human beings but slowly and unpredictably in evolutionary contests waged across continents and over millenniums.



CHAIN OF EVENTS depicted here may protect *AS* heterozygotes against malaria. A parasite-infected cell is characterized by knobs on its surface and by a low intracellular *p*H. An uninfected *AS* cell will pass through a short period of low oxygenation in a capillary without sickling (*1*); an infected cell, on the other hand, will be sequestered long enough so that the low oxygen level and the low *p*H cause it to sickle (*2*). Sickling causes the cell membrane to leak potassium (*3*). Deprived of potassium, the parasite dies (*4*). The death of some fraction of the infecting parasites may give the heterozygote's body time to develop its own immune response.